BEHAVIOR

Larval Feeding on *Bt* Hybrid and Non-*Bt* Corn Seedlings by *Harmonia axyridis* (Coleoptera: Coccinellidae) and *Coleomegilla maculata* (Coleoptera: Coccinellidae)

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ABSTRACT Zoophytophagy is an omnivorous activity that occurs when a primarily carnivorous species feeds on plant material. Plant feeding by beneficial predators may have negative consequences if the plant material has been chemically treated, contains toxins, or was transgenically altered. Although common in predaceous Hemiptera, zoophytophagy has been rarely studied in aphidophagous coccinellids. This study examined the likelihood of feeding on *Bt* and non-*Bt* corn seedlings by third- and fourth-instar coccinellid larvae, the regularity of feeding events by fourth instars, and the effect of leaf feeding on development time and adult size. Both third- and fourth-instar *Harmonia axyridis* Pallas and *Coleomegilla maculata* DeGeer consume leaf tissue, with fourth instars being significantly more likely to feed on corn seedlings. *C. maculata* larvae ingested leaf tissue more frequently than *H. axyridis*. Furthermore, when given access to corn seedlings daily, development time of fourth-instar *C. maculata* increased after *Bt* hybrid corn treatments compared with non-*Bt* corn treatments. Zoophytophagous feeding behavior is thought to sustain predators during times of low prey availability, and leaf tissue feeding by coccinellids has typically been attributed to their need for water. However, in this study, tissue feeding regularly occurred even though coccinellid larvae had constant access to water and a daily ad libitum supply of aphids. We suggest that, in addition to environmental conditions, the physiological state of the zoophytophagous species will influence the probability of plant feeding.

KEY WORDS *Coleomegilla maculata*, Cry1Ab endotoxin, dietary mixing, *Harmonia axyridis*, zoophytophagy

Facultative zoophytophagy occurs when a carnivorous species feeds on plant material (Coll and Guershon 2002). Plants are typically less nutritious than animal prey because they are harder to digest and have low levels of essential nutrients (Coll and Guershon 2002). Therefore, plant feeding by zoophytophagous species is often attributed to both reduced prey availability and a need for water (Hagen 1962, Hodek and Honěk 1996, Naranjo and Gibson 1996, Eubanks and Denno 1999, Coll and Guershon 2002). Another potential explanation for zoophytophagy is dietary self-selection, when organisms select alternative food resources in addition to their typical diet, which results in a beneficial nutritive mix (Waldbauer and Friedman 1991). For dietary self-selection to take place, the food selected must be nonrandom, and the selecting organisms must fare equally or better compared with those without the additional resource (Waldbauer and Friedman 1991). Plant feeding can, in some zoophytophagous species, increase survival, fecundity, longevity, reduce development time, and reduce cannibalism (Coll 1998, Eubanks and Denno 1999, Gillespie and McGregor 2000, Patt et al. 2003, Leon-Beck and Coll 2007).

Plant feeding by predators may be harmful if the plant material ingested has been chemically treated, contains toxins, or was transgenically altered (Coll 1998, Pilcher et al. 1997). Concern regarding potential nontarget effects of transgenic crops has led to several studies examining effects of direct and indirect consumption of *Bt* hybrid corn expressing the Cry1Ab endotoxin by predators (Obrycki et al. 2004, Harwood et al. 2005, O'Callaghan et al. 2005), and many have shown effects of transgenically altered plants on beneficial predators (Hilbeck et al. 1998, Bai et al. 2005, Guo et al. 2005, Zhang et al. 2006). For example, the coccinellid, *Propylea japonica*, has been shown to have several life history characteristics, e.g., development time, altered because of direct feeding on Cry1Ab-expressing *Bt* rice pollen (Bai et al. 2005) and feeding on aphid prey containing Cry1Ac/Cry1Ab-expressing *Bt* endotoxins (Zhang et al. 2006).

Coccinellidae is a predominately predaceous insect family that includes several beneficial zoophytophagous species that are present in corn (Hodek 1973, Lundgren and Wiedenmann 2004). The coccinellids,

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Harmonia axyridis Pallas (Coleoptera: Coccinellidae) and *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), which are used in these studies, are abundant in transgenic and nontransgenic corn in North America (Lundgren et al. 2004, Koch et al. 2006). Both adults and immatures are important predators of many corn pest species (Hodek and Honěk 1996, Dixon 2000). *H. axyridis* is an invasive species in the United States that feeds on numerous prey types (Snyder and Evans 2006); the larvae feed on corn pollen and other coccinellids, and adults feed on several types of fruit (Koch et al. 2004, Lundgren et al. 2004, Snyder and Evans 2006). *C. maculata* is native to North America and is one of the most euryphagous coccinellid species known, feeding on numerous arthropod species, pollen, and nectar (Hodek 1973, Hodek and Honěk 1996). *C. maculata* larvae can complete development to the adult stage when fed a pollen-only diet (Smith 1960, Lundgren and Wiedenmann 2004). Prior studies have found that there are no fitness effects after the ingestion of *Bt*-containing arthropod prey or transgenically altered corn pollen by *C. maculata* (Giroux et al. 1994, Duan et al. 2002, Lundgren and Wiedenmann 2002, 2005); however, there are no studies that have examined the effect of leaf tissue consumption.

Although often reported in hemipterans (Naranjo and Gibson 1996, Gillespie and McGregor 2000), leaf tissue feeding by primarily aphidophagous coccinellids is considered uncommon and has been predominately attributed to accidental ingestion and the need for water by adults, and to our knowledge, has not been reported for immatures (Hodek 1973). Because satiated predators are considered more selective in their prey choice than starved predators (Withers and Browne 2004), our first objective was to determine whether satiated coccinellid larvae, with constant access to water, will feed on leaf material and to quantify the amount of tissue consumed. Carnivores often ingest inappropriate and potentially harmful prey items (Scriber and Slansky 1981, Hodek and Honěk 1996, Strohmeyer et al. 1998) and despite the possible explanations for the occurrence of zoophytophagy, plant feeding may be accidental. Inappropriate food items may be avoided after the initial consumption (van Driesche and Murray 2004); therefore, our second objective examined the regularity of leaf feeding during the immature stage. If larvae deem the plant to be an unsuitable food source, no future feedings are hypothesized to occur. The final objective tested the hypothesis that consumption of leaf tissue from *Bt* hybrid corn would have no effect on larval development time, survival, adult weight, and adult elytral length compared with consumption of non-*Bt* hybrid corn.

Materials and Methods

Three laboratory studies were performed to determine the likelihood of feeding on leaf tissue, the frequency of feeding by immature coccinellids, and to quantify potential effects on various life history traits (survival, adult size, and development time) caused by feeding on *Bt* hybrid plants.

Model Organisms. Corn seedlings, *Zea mays* L. (Poales: Poaceae), expressing Cry1Ab endotoxin for resistance to lepidopteran pests (Yieldgard, *Bt* corn hybrid NX 6503, event MON 810; Monsanto, St. Louis, MO) and a non-*Bt* corn hybrid (N65-M7; Monsanto) were used in these studies. The Cry1Ab toxin is specific to Lepidoptera (Pilcher et al. 1997) and is unlikely to cause mortality to coccinellids, thus allowing us to test leaf tissue consumption by the same individuals over time. In addition, field-collected *C. maculata* and *H. axyridis* adults have screened positive for Cry 1Ab toxins before anthesis (Harwood et al. 2007). Seedlings, rather than mature plants, were used to test for leaf tissue feeding because *Bt* endotoxins are present in seedlings (Harwood et al. 2006), and newly vegetative growth is more vulnerable to herbivory (Bing et al. 1991). *C. maculata* and *H. axyridis* have been documented to occur in young corn (Musser and Shelton 2003, Nault and Kennedy 2003), and *C. maculata* immatures will disperse from nearby weeds, across bare ground, to colonize corn plants (Cottrell and Yeargan 1999). *C. maculata* and *H. axyridis* feed on corn leaf aphids (*Rhopalosiphum maidis* Homoptera: Aphidiae) (Musser and Shelton 2003), and *R. maidis* is attracted to and colonizes seedling corn (Dicke and Sehgal 1990, Bing et al. 1991). *Rhopalosiphum padi* (Homoptera: Aphidiae) also will colonize young corn plants (Lumbierres et al. 2004).

Plant and Insect Cultures. *Zea mays* seeds (10–15) were planted in 110-mm-diameter pots (Kord Products, Toronto, Canada) with Promix BX soil (Premier Horticulture, Quakertown, PA) and watered daily; seedlings were grown under greenhouse conditions (25–29°C, L:D 14:10 h). Seedlings used in these studies were a mean height of 6.30 ± 0.15 (SE) cm and between the vegetative emergence (VE) and vegetative first leaf stage (V1). Plants were positioned vertically in all experimental arenas, because plant feeding is more likely to occur if plants are upright as under natural growing conditions, rather than horizontally within a dish (S.E.M., unpublished data).

Adult *C. maculata* and *H. axyridis* were collected from overwintering sites during fall 2006 from the bases of silver maple trees, *Acer saccharinum* L. (Sapindales: Sapindaceae), at the University of Kentucky Spindletop Research Farm, Lexington, KY. Egg-producing *H. axyridis* and *C. maculata* females were separated into individual petri dishes (Fisherbrand, polystyrene, 100 by 15 mm; Fisher Company, Pittsburgh, PA) and were provided cotton soaked with 10% sucrose water. Females were also provided with an ad libitum supply of *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), a suitable prey item for *C. maculata* and *H. axyridis* (Hodek 1973). *A. pisum* were reared on faba bean, *Vicia faba* L. (Fabales: Fabaceae). Larvae used in this study were first-generation offspring from these field-collected females. Twenty-four hours after egg eclosion, larvae were separated and placed individually within plastic petri dishes (Fisherbrand, polystyrene, 60 by 15 mm) and provided with an ad libitum supply of *A. pisum* daily. Cotton soaked with 10% sucrose water was also provided within all rearing dishes. Adults and larvae were maintained at 20.0 ± 1.0°C with a photoperiod of L:D 16:8 h. Voucher specimens of *C. maculata* and *H. axyridis* were deposited in the University of Kentucky Insect Museum, Department of Entomology, University of Kentucky, Lexington, KY.

Feeding Rate and Damage. Observations were made on individual larvae within 24 h after their larval molt to third and fourth instar. Each treatment (non-*Bt* or *Bt* seedling) and instar were replicated 10 times for each species, and treatments were randomly assigned. Larvae were transferred into individual experimental arenas with a small paint brush and carefully placed on the cotton at the base of the seedling. Before the start of the bioassay (≈5 min), seedlings were excised at their soil base, and cotton was wrapped around the base of each stem. The cotton was soaked with water to ensure that larvae had access to water immediately before seedling contact. Each plant was placed vertically into a 500-ml glass flask (Pyrex, 2-cm-diameter opening), such that the cotton-wrapped stem was lodged into the opening of the flask. Timing of each trial began once a larva walked onto the surface of the plant and ended when the larva left the plant or after 30 min. The time limit of 30 min was used because preliminary observations determined that cut seedlings lose turgidity ≈45 min after excision from their soil base. To be considered an event, a larva must remain on the plant surface for at least 2 s. A new seedling and container were used for each event.

Visual observations of the location and the number of feeding sites were documented. The amount of tissue consumed was calculated by estimating the tissue volume (length × width × depth; mm^3) missing from the plant using a stage micrometer within a Leica MZ95 stereomicroscope at ×60 magnification. If a larva fed at multiple sites on the plant, the total volume consumed was calculated.

Feeding Frequency. Data were collected on the incidence of successive plant feeding events during the fourth instar until pupation. Third instars were not used because of their low rate of plant feeding (see Results). Treatments (non-*Bt* or *Bt* seedling) were randomly assigned within 15 h of the fourth instar molt, and each treatment (plant type) was replicated five times for each species. Rather than excising seedlings, intact *Bt* hybrid and non-*Bt* corn seedlings were individually transplanted into Reynolds Del-Pak plastic containers (diameter 10.5 cm, height 6 cm; Reynolds Metal Co., Richmond, VA) containing Promix BX soil. An additional plastic container with a central opening within the base (2.5 cm diameter) was placed over the seedling and rested on the soil surface. Water-soaked cotton was placed around the base of the seedling to provide moisture and to prevent larval escape. Plants were randomly assigned to individual larva, and each larva was exposed to that same plant twice (2 consecutive d) before the plant was replaced with a new seedling. Seedlings were replaced every second day to prevent plant-induced responses to insect herbivory and to keep plant height approximately the same for each encounter.

Larvae were carefully transferred with a small paint brush from their rearing dishes, placed individually on the cotton-wrapped stem, and allowed to move freely onto the plant. Timing of each trial began once the larva walked onto the surface of the plant and ended when the larva left the plant or after 30 min. Observations and timing only began if the larva remained on the plant for at least 2 s. After each plant exposure, larvae were returned to their rearing dishes, fed an ad libitum supply of *A. pisum*, and maintained at 20.0 ± 1.0°C, L:D 16:8 h. Data were recorded on the number of times fourth instars fed on the seedlings, and a stereomicroscopic analysis of the plant surface confirmed the occurrence of plant feeding after each event.

Effects on Life History Traits. To determine whether plant exposure influences different life history traits (survival, adult size, development time), measurements taken from larvae exposed (treatment) or not exposed (control) to non-*Bt* or *Bt* seedlings for 4 h daily during their fourth instar were compared. Treatments were randomly assigned to 15 larvae after their molt to fourth instar and were replicated five times for each species. Seedlings were transplanted into Reynolds Del-Pak plastic containers (as above), and larvae were placed individually within these containers. Arena design was as previously described in the feeding frequency methods, except that a lid was added to prevent larvae from leaving the dish during the exposure time period. A lid was created by inverting an additional Reynolds Del-Pak plastic container and loosely sealing it over the container. Water-soaked cotton was placed around the base of the plants, and only water-soaked cotton was provided to fourth instars in the control containers. Larvae were placed in the same container each day, and seedlings were replaced every second day. Containers were maintained under fluorescent lighting during the daily 4-h plant exposure interval. The time period of 4 h was chosen based on the assumption that it may be common for larvae in the field to encounter time periods in which they do not encounter prey. Support for this assumption comes from the ability of *C. maculata* and *H. axyridis* larvae to complete development when fed only two *A. pisum* per day (Moser 2003). After each plant exposure, larvae were returned to their rearing dishes, fed an ad libitum supply of *A. pisum*, and maintained at 20.0 ± 1.0°C, L:D 16:8 h.

Data were recorded on survival, development time (fourth instar and pupal stage), sex, and adult body size (dry weight and elytral length). Duration of the fourth instar was measured in days between the fourth instar molt and the pupal molt; duration of the pupal stage was measured in days between the pupal molt and adult eclosion. A digital image of each adult was taken and elytral length was determined by digital imaging each adult and lengths were calculated in tpsDIG32 software (http://life.bio.sunysb.edu/morph) to the nearest 0.001 mm. Sex was subsequently determined by dissection, in which the tip of the abdomen was removed to reveal the spermatheca or the aedeagus. Adult dry weight was obtained by placing the adult and abdominal tip within a Boekel oven (Philadelphia, PA) at 50.0 ± 5.0°C for 4 d to ensure a consistent moisture level when comparing weights (S.E.M., unpublished data). Dried adults were weighed to the nearest 0.01 mg using a Mettler AE 100 analytical balance (Mettler-Toledo, Columbus, OH).

Statistical Analysis. Likelihood-ratio χ^2 analyses were performed to determine whether there were differences in the rate of plant feeding based on corn type, species, and instar (JMP 5.1.2; SAS Institute 2005). A two-way analysis of variance (ANOVA) was used to compare the amount of plant material consumed by the factors of instar and plant type for *C. maculata* and *H. axyridis* (JMP 5.1.2). The interaction between plant type and instar was not analyzed for *H. axyridis* because each instar was not represented for each plant type because of a lack of feeding by third instars. One-way ANOVA was used to compare differential consumption of plant material by *C. maculata* and *H. axyridis* (fourth instar only; JMP 5.1.2). Before analysis, the volume of plant material consumed was $\log_e$ transformed to obtain a normal distribution.

Feeding frequency, defined as the number of days an individual fed on corn divided by the total number of days exposed to the plant, was analyzed with a two-way ANOVA to test the effects of coccinellid species, plant type, and their interaction (JMP 5.1.2). Frequency data were arcsine-root transformed before the analysis (JMP 5.1.2).

Two-way ANOVA was used to compare the fixed effect of treatment, the random effect of sex, and their interaction on elytral length and adult weight for each species (JMP 5.1.2). The development time did not meet assumptions of normality and was therefore analyzed using a likelihood-ratio χ^2 analysis (JMP 5.1.2). All tests were examined at the $P < 0.05$ level of significance, and normal distributions were confirmed with a Shapiro-Wilk W test on the residuals (JMP 5.1.2).

Results

Feeding Rate and Damage. *Coleomegilla maculata* and *H. axyridis* third and fourth instars fed on leaf tissue of *Bt* and non-*Bt* corn seedlings. Feeding events were concentrated on the leaf blade (29%), coleoptile (28%), leaf edges (25%), and on the leaf whorl (18%; Fig. 1).

The feeding rate, defined as the percent of coccinellids observed to consume plant material during the 30-min trial, of *C. maculata* was significantly greater than *H. axyridis* (χ^2 test; $\chi^2 = 10.48$; df = 1,78; $n = 80$; $P = 0.0012$; Fig. 2a). However, a similar amount of plant material was ingested by both species ($F = 0.04$; df = 1,22; $P = 0.84$). Within species, corn type did not influence feeding rate for *C. maculata* (χ^2 test; $\chi^2 = 0.10$; df = 1,38; $P = 0.75$; Fig. 2b) or *H. axyridis* (χ^2 test; $\chi^2 = 1.31$; df = 1,38; $P = 0.25$; Fig. 2c). Larval age was a significant factor in the rate of plant feeding; fourth instars fed on plants more often than third instars for both species (χ^2 test: *C. maculata;* $\chi^2 = 8.63$; df = 1,38; $P = 0.0033$; *H. axyridis;* $\chi^2 = 7.79$; df = 1,38; $P = 0.0052$; Fig. 3, a and c).

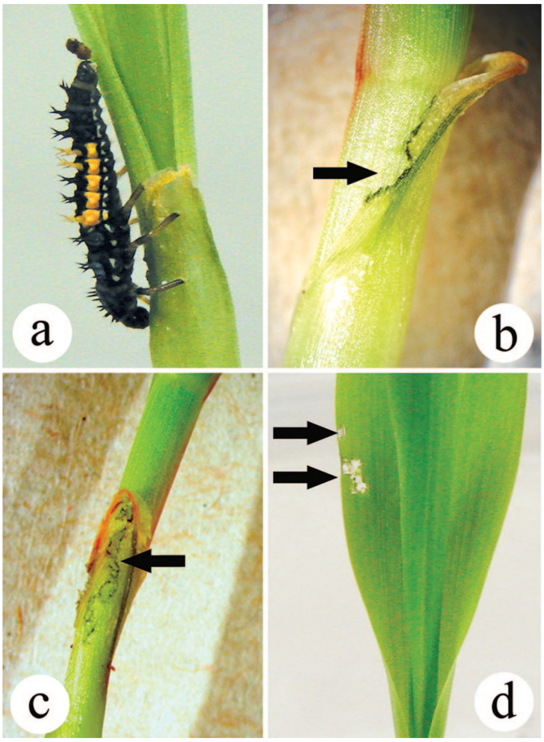

Fig. 1. *Harmonia axyridis* fourth instar feeding on the edge of a corn seedling's coleoptile (a) and its subsequent damage (b), and *C. maculata* fourth instar larval feeding damage on a corn seedling's coleoptile (c) and leaf blade (d). Feeding damage is indicated by an arrow.

The volume of plant material consumed by *C. maculata* did not differ by instar or corn type (*Bt* versus non-*Bt;* instar: $F = 1.91$; df = 1,19; $P = 0.18$; plant type: $F = 0.12$; df = 1,19; $P = 0.73$; Fig. 3b), and the interaction was also not significant ($F = 0.42$; df = 1,19; $P = 0.53$). The volume of plant material consumed by *H. axyridis* also did not differ between corn types ($F = 3.94$; df = 1,6; $P = 0.09$). However, there was a significant difference in the volume consumed between instars; fourth instars ingested more plant material than third instars ($F = 14.00$; df = 1,6; $P = 0.0096$; Fig. 3d).

Feeding Frequency. Each individual was observed to ingest leaf tissue at least once during its fourth instar, and the majority (100% *C. maculata;* 70% *H. axyridis*) fed on seedlings on ≥2 d. Feeding frequency (number of days fed/total number of days × 100) was not affected by plant type ($F = 0.39$; df = 1,16; $P = 0.55$; Table 1), and the interaction between plant type and coccinellid species was not significant ($F = 0.32$; df = 1,16; $P = 0.32$). However, the feeding frequency of *C. maculata* (average 79%) was significantly greater compared with *H. axyridis* (average 43%; $F = 14.05$; df = 1,16; $P = 0.0018$; Table 1). Furthermore, 30% of *C.*

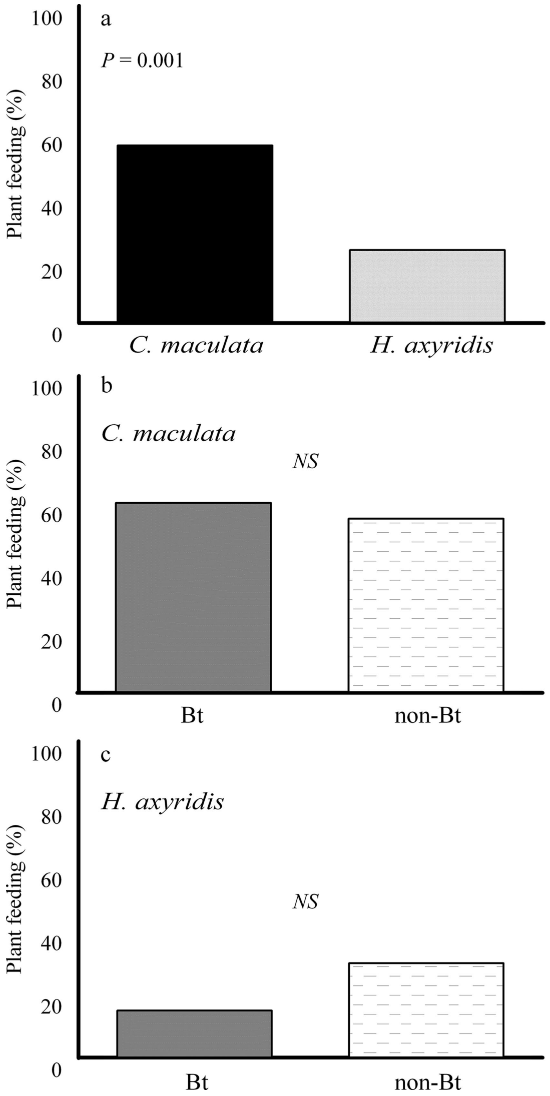

Fig. 2. Percentage of third and fourth instar feeding on corn seedlings (a); the percent of feeding based on plant type (*Bt* hybrid and non-*Bt* corn seedlings) by *C. maculata* (b) and *H. axyridis* (c).

maculata larvae were observed to feed on *Bt* hybrid and non-*Bt* corn plants every day until pupation.

Effects on Life History Traits. No larvae of either species died during these studies. Sex did not affect fourth instar or pupal stage development time for either species (χ^2 test: *C. maculata;* fourth instar: χ^2 = 2.52; df = 3,69; P = 0.47; pupal stage: χ^2 = 0.50; df = 2,71; P = 0.78; *H. axyridis;* fourth instar: χ^2 = 1.46; df = 3,68; P = 0.69; pupal stage: χ^2 = 1.41; df = 3,68; P = 0.70). However, the development time of fourth-instar *C. maculata* varied significantly between individuals fed *Bt* hybrid corn compared with those exposed to the non-*Bt* seedling (χ^2 test; χ^2 = 19.27; df = 6,66; P = 0.0037). Development time of fourth instars in the *Bt* hybrid treatment was 4% longer than those in the control, and development time was 8% longer (≈0.5 d at 20°C) compared with the non-*Bt* treatment (Table 2). Pupal development was similar between treatments for *C. maculata* (χ^2 test; χ^2 = 7.22; df = 4,69; P = 0.12; Table 2). *H. axyridis* fourth instar and pupal stage development time did not differ between treatments (χ^2 test: fourth instar: χ^2 = 10.66; df = 6,66; P = 0.10; pupal stage: χ^2 = 10.08; df = 8,63; P = 0.26; Table 2).

Plant exposure did not influence adult weight or elytral length of *C. maculata* (weight: F = 3.56; df = 2,69; P = 0.22; length: F = 9.70; df = 2,69; P = 0.09; Table 2) or *H. axyridis* (weight: F = 13.64; df = 2,68; P = 0.07; length: F = 1.47; df = 2,68; P = 0.40; Table 2). Size was significantly influenced by sex; females were larger than males. The elytral length was longer for females compared with males for both species (mean ± SE; *C. maculata:* female 4.32 ± 0.04 mm; male 3.84 ± 0.04 mm; F = 2934.43; df = 1,69; P = 0.0003; *H. axyridis:* female 5.38 ± 0.05 mm; male 4.89 ± 0.04 mm; F = 37.28; df = 1,68; P = 0.0257), as was the adult weight (*C. maculata:* female 2.24 ± 0.07 mm; male 1.88 ± 0.04 mm; F = 46.19; df = 1,69; P = 0.0209; *H. axyridis:* female 8.15 ± 0.17 mm; male 7.40 ± 0.16 mm; F = 42.86; df = 1, 68; P = 0.0223). The interaction between plant type and sex on size was not significant for *C. maculata* (elytral length: F = 0.03; df = 2,69; P = 0.97; weight: F = 0.51; df = 2,69; P = 0.60) or *H. axyridis* (elytral length: F = 0.51; df = 2,68; P = 0.60; weight: F = 0.31; df = 2,68; P = 0.73).

Discussion

A common explanation for the seemingly suboptimal behavior of zoophytophagy is that it only occurs if arthropod prey availability is low (Hagen 1962, Naranjo and Gibson 1996, Eubanks and Denno 1999, Coll and Guershon 2002). However, reduced prey and water availability do not explain the observations made in this study; larvae had constant access to water and, although prey were not on the plant substrate, larvae were never restricted to the plant. Despite this, the majority of third- and fourth-instar *C. maculata* (58%) and a lower percentage of third- and fourth-instar *H. axyridis* (23%) remained and ingested leaf tissue. In addition, most larvae fed on the seedlings repeatedly during their fourth instar.

Although plant feeding did not produce any observed benefit, there was a cost based on plant type consumed. Development time of fourth-instar *C. maculata* was longer if larvae ingested *Bt* hybrid corn compared with the non-*Bt* isoline leaf tissue. *H. axyridis* ingested a similar amount of leaf tissue as *C. maculata,* yet, unlike *C. maculata,* there was no difference in development time between larvae that consumed *Bt* and non-*Bt* corn. This difference between species may be related to differences in the relative amount consumed. A gut-dissection analysis revealed that field-collected *C. maculata* ingested significantly more corn pollen than *H. axyridis* (Lundgren et al. 2004). In this study, the average fourth-instar *H. axyridis* is twice as large as the average fourth-instar *C. maculata* (wet weight within 24 h of molt to fourth instar: *H. axyridis,*

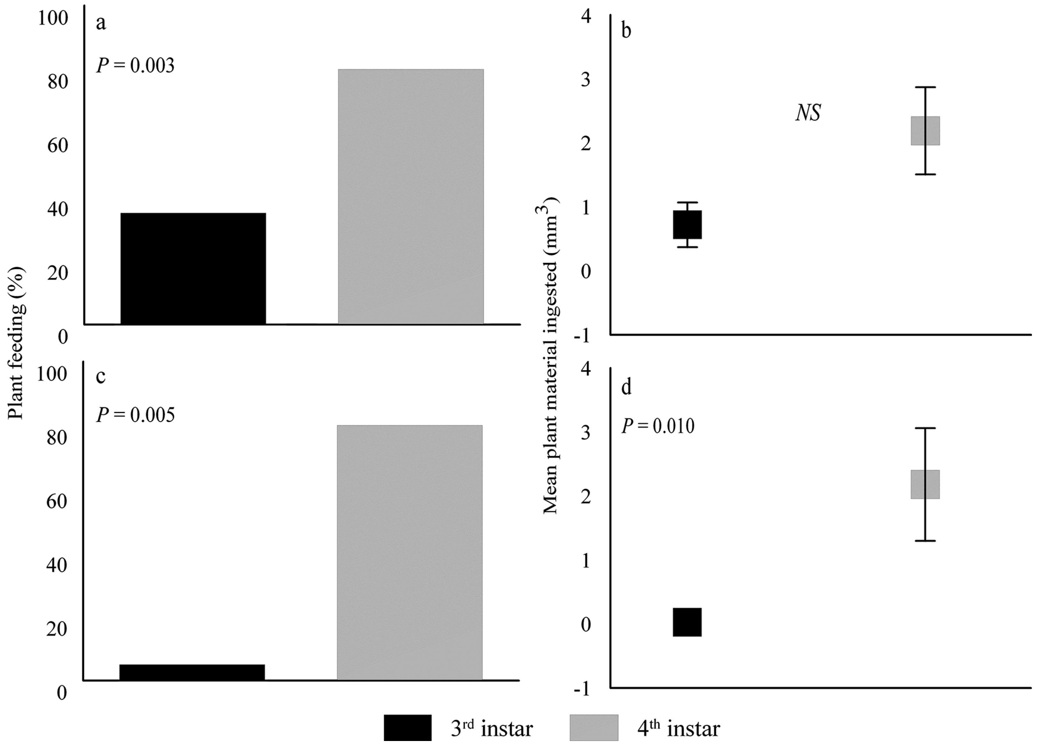

Fig. 3. Differences in the percent of plant-feeding and volume of leaf tissue (mean ± SE; mm^3) ingestion between third- and fourth-instar *C. maculata* (a and b) and *H. axyridis* (c and d). Plant feeding was analyzed with a likelihood-ratio χ^2 analysis and the volume consumed was log-transformed and analyzed with a two-factor ANOVA.

18.01 ± 0.86 mg; *C. maculata*, 7.66 ± 0.79 mg), thus on a per volume basis, *C. maculata* acquired more leaf tissue. *C. maculata* also fed on seedlings more frequently than *H. axyridis.* Therefore, it is likely that *C. maculata* ingested more plant material, relative to body size and feeding frequency, than *H. axyridis.* Corn byproducts containing Cry1Ab have a similar effect on a leaf-feeding trichopteran, *Lepidostoma liba* (Rosi-Marshall et al. 2007). *Bt* corn litter consumption by *L. liba* did not result in additional mortality, but growth rates decreased by >50% compared with non-*Bt* corn litter (Rosi-Marshall et al. 2007).

Cry1Ab *Bt* endotoxins are present in leaves of seedling corn (Harwood et al. 2006), and the increased development time observed in this study may be caused by the Cry1Ab toxin or other plant characteristics caused by the transgenic event. For example, higher lignin content had been reported in *Bt* crops and because lignin can decrease plant digestibility (Scriber and Slansky 1981, Saxena and Stotzky 2001, Poerschmann et al. 2005), it could result in increased development times. Increased development times are related to decreased survival in many coccinellid species (Dixon 2000). However, because there were only minor differences in the life history traits between treatments, it is unknown if larval coccinellid feeding on *Bt* hybrid corn seedlings will have negative effects on *C. maculata* or *H. axyridis* populations. Seedlings used in this study were not chemically or structurally analyzed, and further studies are needed to determine the cause of the increased development time.

In generalist herbivores, dietary mixing is often attributed to nutrient balancing (Bernays and Minkenberg 1997), and dilution of toxic substances found in food sources (Freeland and Janzen 1974, Bernays and Minkenberg 1997, Waldbauer and Friedman 1991). These hypotheses are applicable to the coccinellid species used in this study. *C. maculata* and *H. axyridis* are generalist feeders that occur in many agroecosystems, such as alfalfa, wheat, soybean, corn, and orchards (Hodek and Honěk 1996), and larvae are likely to encounter numerous potential prey sources that not only vary in their nutritious value, but also in their level of chemical defense (Hodek and Honěk 1996). This diversity in habitat and prey types may prevent adaptation to any one species-specific defense mechanism (Sloggett and Majerus 2000, Rana et al. 2002). However, diet diversification is a strategy that may

Table 1. Feeding frequency (mean ± SE) on *Bt* hybrid and non-*Bt* corn seedlings by fourth-instar *C. maculata* and *H. axyridis*

Species	*Bt* (*n* = 5)	Non-*Bt* (*n* = 5)	Total (*n* = 10)
C. maculata	0.79 ± 0.11	0.79 ± 0.08	0.79 ± 0.06
H. axyridis	0.33 ± 0.07	0.52 ± 0.10	0.43 ± 0.06

Feeding frequency is the number of days an individual fed on corn divided by the total no. of days during its fourth instar.

Table 2. Life history measurements (±SE) for *C. maculata* and *H. axyridis* when given daily access to *Bt* hybrid corn seedlings, non-*Bt* corn seedlings, or no plant (control) as fourth instars

Species	Treatment	Elytral length (mm)	Adult dry weight (mg)	Fourth instar (d)	Pupal stage (d)
C. maculata		$P = 0.09$, $n = 75$	$P = 0.22$, $n = 75$	$P < 0.01$, $n = 75$	$P = 0.12$, $n = 75$
	Control	4.11 ± 0.008	2.06 ± 0.048	**5.7 ± 0.1**	5.8 ± 0.1
	Bt	4.04 ± 0.008	2.12 ± 0.049	**6.0 ± 0.1**	6.0 ± 0.1
	Non-*Bt*	4.07 ± 0.008	1.99 ± 0.048	**5.5 ± 0.2**	6.0 ± 0.1
H. axyridis		$P = 0.40$, $n = 74$	$P = 0.07$, $n = 74$	$P = 0.10$, $n = 75$	$P = 0.26$, $n = 75$
	Control	5.21 ± 0.066	8.20 ± 0.112	5.8 ± 0.1	7.3 ± 0.1
	Bt	5.03 ± 0.066	7.69 ± 0.112	5.8 ± 0.1	7.2 ± 0.1
	Non-*Bt*	5.24 ± 0.069	7.57 ± 0.116	5.9 ± 0.1	7.4 ± 0.1

Bold values indicate statistical significance. Elytral length and adult weight were analyzed with a two-factor ANOVA and development times with a likelihood-ratio χ^2 analysis.

allow these species to overcome chemical defenses by diluting the amount of toxin consumed from any single prey item. In invertebrate predators, prey may also be selected based on the predators requirement for specific nutrients (Mayntz et al. 2005), and coccinellid larvae may have also consumed leaf tissue to obtain a specific nutrient that aphid predation alone does not provide (Giles et al. 2001).

The frequency of feeding on leaf tissue by coccinellid larvae was likely influenced by both their physiological state and environmental effects. Late-instar coccinellids will consume more prey than is required for development (Hodek 1973) and larvae used in this study had access to aphids during their entire development except when exposed to corn seedlings. Therefore, their energy reserves were likely high and their need for highly nutritious prey was not an immediate requirement for survival. By remaining on the plant, larvae may have reduced costs associated with foraging, such as risk of increased predation (Leon-Beck and Coll 2007). The lack of arthropod prey on seedlings may have further increased the probability of plant feeding. Assuming that leaf tissue provides an energetic gain, opportunistic feeding on leaf tissue increases a larva's net energy intake rate by maximizing the amount of energy obtained during the time spent on the plant (Krebs and Davies 1997).

The ability to ingest and digest both plant and animal material has major consequences in terms of an organisms' ecological role, as well as its behavioral and physiological capabilities (Coll and Guershon 2002). Despite the added complexity, omnivory is likely more common than has currently been reported and the nutritive benefit of plant feeding by zoophytophagous species is still largely unknown (Coll and Guershon 2002). Although we did not document a positive effect for larvae exposed to plants, potential benefits should not be disregarded because any nutritive gain resulting from plant feeding may have been masked by the high levels of aphids that were provided. Alternative prey is of particular importance in biological control programs because feeding on alternative food sources often sustains predator populations during times of low prey abundance (Murdoch et al. 1985). Zoophytophagous feeding behavior may also be significant if the effectiveness of the natural enemy is reduced because of feeding on chemically treated or transgenically altered plants. For example, if coccinellid immatures feed on genetically-modified corn leaves expressing the Cry3 gene, which targets Coleopterans (Duan et al. 2002), coccinellid colonization of corn may be disrupted and reduced aphid control may result. Furthermore, additional studies are needed to address the underlying mechanisms associated with plant feeding, such as satiation and prey encounter rate, the frequency of feeding in the field, and the consequences of leaf tissue ingestion by coccinellids.

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