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Quantification of consumption of corn pollen by the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield

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Abstract

1 *Coleomegilla maculata* DeGeer feeds on corn pollen in the field, but the degree to which this predator relies on corn pollen as part of its diet is not well understood. We quantified the amount of pollen consumed by *C. maculata* second, third and fourth instars and adults in the field.

2 Laboratory experiments were conducted to determine the digestion rate and duration of different stadia or stages using temperature regimens that reflected field conditions during anthesis. *Coleomegilla maculata* larvae and adults were collected from the field and the amount of pollen in their digestive tracts was determined gravimetrically. The rate of digestion, duration of each life stage and the field observations were used to estimate the amount of pollen consumed by second, third and fourth instars and adults.

3 Our models estimate that larvae consume 0.66, 1.67 and 3.30 mg of pollen during the second, third and fourth stadia, respectively. Adults consumed an estimated 13.15 mg during anthesis.

4 The relevance of our results to ecological risk assessment of transgenic insecticidal corn and predator life history strategies is discussed. The results presented here are a first attempt to quantify pollen consumption by a predator, and future areas of research are suggested.

Keywords Biological control, Bt, facultative phytophagy, ladybeetles, pollinivory, risk assessment, transgenic crops, *Zea mays*.

Introduction

Coleomegilla maculata DeGeer has historically been one of the most abundant predators in cornfields of eastern North America (Udayagiri *et al.*, 1997; Wright & DeVries, 2000; Musser & Shelton, 2003; Lundgren *et al.*, 2004). In addition to being an important source of mortality for several corn pests (i.e. aphids, and eggs of *Ostrinia nubilalis* and *Helicoverpa zea*) (Wright & Laing, 1980; Cottrell & Yeargan, 1998; Phoofolo *et al.*, 2001), this predator feeds on corn pollen in the field (Ostrom *et al.*, 1997; Lundgren *et al.*, 2004). In laboratory tests, a large proportion of *C. maculata* can complete larval development on a diet consisting solely of corn pollen (Smith, 1965; Pilcher *et al.*, 1997; Lundgren & Wiedenmann, 2004). Eggs of *C. maculata* are more abundant during anthesis (Cottrell & Yeargan, 1998; Pfannenstiel & Yeargan, 2002; Lundgren *et al.*, 2004), and the majority of *C. maculata* larvae and adults that occur in corn during anthesis feed on corn pollen (Lundgren *et al.*, 2004). Although *C. maculata* feeds on corn pollen in the field, the amount of pollen that it consumes over its life or during specific life stages is unknown. Quantification of corn pollen consumption by *C. maculata* would be useful not only to scientists interested in life history strategies of natural enemies and biological control, but also to regulators and scientists who must assess the risk of transgenic insecticidal corn hybrids.

Entomophagous insects feed on nonprey foods, including nectar, fungal spores, prey products such as honeydew,

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and pollen (Forbes, 1880, 1883; Allen, 1979; Alomar & Wiedenmann, 1996; Canard, 2001; Lundgren *et al.*, 2004). Predators feed on these foods to sustain themselves when prey becomes scarce, to enhance their growth and development when only nutritionally suboptimal prey are available, and to accumulate critical nutrients for overwintering, dispersal and egg maturation (Schneider, 1969; Hagen, 1986; Jervis & Kidd, 1996; Eubanks & Denno, 1999; Coll & Guershon, 2002; Patt *et al.*, 2003). Nonprey foods such as pollen can influence the feeding behaviour of omnivores in many ways, either increasing or decreasing predation (Coll & Guershon, 2002). Within agricultural habitats, providing high-quality nonprey foods could attract or arrest biological control agents that may suppress pest populations (Eubanks & Denno, 2000; Harmon *et al.*, 2000), and may decrease intraguild predation and cannibalism (Schellhorn & Andow, 1999; Janssen *et al.*, 2003). Conversely, abundant nonprey food such as pollen may actually reduce predation of certain key pests (as suggested by Cottrell & Yeargan, 1998; Pfannenstiel & Yeargan, 2002). Furthermore, plant materials that possess insect-resistance properties may adversely affect omnivorous predators that feed on them (Overney *et al.*, 1998; Weiser & Stamp, 1998). By quantifying the amount of pollen ingested by predators, we can begin to uncover the relative roles of phytophagy and predation by omnivorous predators.

Quantification of insect feeding under field conditions is often a difficult process, particularly when dealing with discrete packages of food such as prey (Sunderland, 1996). When quantifying predation, it becomes necessary to distinguish between predation rates and consumption rates (Sunderland, 1996). The predation rate is the number of prey that is consumed by a predator per unit time, and quantification of this phenomenon is complicated by a number of factors, such as the heterogeneity in the physiology of prey populations and the feeding behaviour of predators (Sunderland, 1996). A much easier value to estimate is the consumption rate, or the biomass of food ingested by a predator. The concept of consumption rate is easily applied to pollinivory, where the variability in the size and structure of pollen grains is overwhelmed by the small size and high number of grains ingested. Although quantifying pollen consumption is feasible using any one of a variety of techniques developed for studying predation, we could find no reports that quantified the amount of pollen consumed by predatory arthropods under field conditions.

Here, we present a quantification of the amount of transgenic corn pollen fed on by *C. maculata* under field conditions. Using a combination of laboratory experiments and field observations designed to estimate the digestion rate of corn pollen by *C. maculata*, the duration of larval development under temperatures that reflected field conditions, and the amount of pollen ingested by larvae and adults in the field, we estimate pollinivory by this species during corn anthesis. These data can be used to examine the life history strategies of this predator in the corn system, and will be of value to risk assessments aimed at evaluating the risk of transgenic insecticidal corn pollen to this omnivorous predator.

Methods

Laboratory experiments

Pollen for laboratory experiments was produced by a transgenic corn hybrid (parents: Fr390YG × FR9661; seed donated by Illinois Foundation Seed, Champaign, Illinois) that expresses the Cry1Ab toxin from the Yieldgard™ gene (Monsanto) for resistance to lepidopteran pests. This was not the same transgenic hybrid as the one planted in the field (see below), but expressed the same transgenic toxin (but probably a different amount). Thus, antibiosis or differential digestion efficiencies resulting from Cry1Ab would be observed in the field-collected beetles and beetles in the laboratory assays. Corn plants, grown two per 3.8-L pot in a greenhouse room, were watered twice daily with trickle irrigation, and each pot was fertilized weekly with 2.2 L of aqueous Peters Professional® General Purpose Fertilizer (20N, 10K, 20P; Scotts®, Allentown, Pennsylvania) at a concentration of 250 p.p.m. Tassels were contained in 2.3-kg paper bags, which were stapled shut before pollen was shed. Pollen was sifted through a sieve with a 0.063-mm pore size (sieve #230, Dual Manufacturing Co., Chicago, Illinois) and examined under a dissecting microscope at × 50 magnification to confirm that there was no contamination by anther fragments and other plant tissue. Pollen was placed in 36-mL sealed plastic vials (Bioquip Products, Gardena, California) and kept at −10 °C until use.

Larval staging

We determined experimentally that head capsule width was a consistent representation of the different larval stages of *C. maculata*. A laboratory colony of *C. maculata*, which originated from field-collected individuals, was reared on artificial diet #7 of Atallah & Newsom (1966), without tetracycline, continuously for approximately 2 years with annual additions of field-collected individuals. Rearing conditions for the colony were an LD 16 : 8 h photoperiod at 28–30 °C and approximately 70% RH. Between four and 10 individuals were examined when they reached the ages: first instar, 2-day-old; first instar, 3-day-old; second instar, 1-day-old; second instar, 3-day-old; third instar, 1-day-old; third instar, 3-day-old; fourth instar, 1-day-old; and fourth instar, 3-day-old. The durations of stadia were determined by observing the larvae daily and recording each larval moult. The head capsules of the larvae were measured dorsally with a micrometer at × 50 magnification. The head capsule widths were compared among the different aged larvae with analysis of variance (ANOVA) and significantly different means were separated using the Tukey–Kramer means comparison (Jmp 3.2.6, SAS Institute, Inc., Cary, North Carolina).

Larval development rates under simulated field conditions

The development rate of pollen-fed *C. maculata* larvae under a temperature regimen similar to that observed in the field during anthesis was recorded in the laboratory. Growth chamber conditions were an LD 16 : 8 h photoperiod; 28 °C during the day and 17 °C at night; and approximately 70% RH. Larvae from the colony were reared on artificial diet until a range of ages was present. Groups of larvae that were nearing the end of the first ($n = 10$), second ($n = 17$) and third ($n = 23$) stadia were placed individually into 32-mL plastic cups that contained a saturated cotton wick and were covered with plastic lids. Each day, 15 mg of greenhouse-produced corn pollen was given to each larva, and the cups and lids were changed every 48 h. The cups were checked daily for shed skins until pupation, and the mean durations of the different stadia were compared among the different stadia with ANOVA and significantly different means were separated using the Tukey–Kramer means comparison (Jmp 3.2.6).

Weights of empty guts

In preliminary observations, we determined that 12 h was sufficient for *C. maculata* adults and larvae to entirely purge their guts of pollen and diet. Groups of 10 second, third, and fourth instars, and adults, were isolated from the laboratory colony and provided with only water for 12 h. The digestive tracts of starved individuals were dissected, dried at approximately 25 °C for > 2 h, and weighed to the nearest 0.01 mg on an electronic balance. In this way, the mean weights of the empty digestive tracts were determined for each instar and the adult stage. The sensitivity of our scale did not allow us to reliably measure the weight of the guts or gut contents of first-instar *C. maculata*, and these individuals were not included in any of the analyses.

Digestion rate of corn pollen

A series of experiments were conducted in the laboratory to determine the rate of corn pollen digestion in different aged *C. maculata* in the laboratory. Preliminary research showed that unfed larvae and adults of *C. maculata* could fill their midguts with corn pollen in < 2 h (J.G.L., unpublished data). Second, third, and fourth instars from the colony were staged based on head capsule width. Larvae and adults were isolated by their developmental stages in 32-mL plastic cups with plastic lids (product #9051 and 9053, Bio-Serv, Inc, Frenchtown, New Jersey). These individuals were provided with only water, in the form of a saturated cotton wick, for 12 h to clear their gut contents. Before 09.00 h on the morning of the experiment, groups of each stadium or stage were provided with 0.5 g of corn pollen, which was more than they could eat during a 3-h period. Rearing conditions for the experiment were an LD 16 : 8 h photoperiod at 28 °C (the same as the maximum daily temperature observed during our field experiment; see below) and approximately 70% RH. After feeding for approximately 3 h, the individuals were transferred to cups without food but with a water wick. To determine how long the pollen was retained in the digestive tracts, individuals were frozen at −10 °C to stop digestion at a range of time intervals beginning when feeding was ceased. These time intervals varied among the different life stages: second instar: 0, 1, 2, 3, 4 and 6 h; third instar: 0, 1.5, 3, 4.5, 6, 8 and 8.67 h; fourth instar: 0, 2, 4, 6.25, 8.67 and 10.5 h; adult: 0, 1, 2, 3, 4.5 and 6 h. At each time interval, four to eleven individuals were frozen. The experiment was continued until the guts were clear of any yellow grains or pigments for a particular stage. It was assumed that pollen consumption during the 3-h feeding period was similar for groups killed at the different time intervals.

After they were frozen for 1–3 h, the digestive tracts were dissected out of each individual at room temperature. After drying at approximately 25 °C for 3–24 h (at least 3 h were required to completely dry the digestive tracts), the digestive tracts and their contents were weighed to the nearest 0.01 mg on an electronic balance. A mean weight for the digestive tract with pollen was determined for each time interval for each stage. From this value, we subtracted the mean weight of the empty digestive tracts for each respective stadium or stage to arrive at a mean pollen weight per time interval for each stadium or stage. A scatterplot with the mean pollen weights for each time interval was created for each stadium or stage, and a linear regression was fitted to each plot (Jmp 3.2.6). ANOVAs were used to determine whether the weights of pollen decreased significantly over time in the different instars and stages.

Field experiment

Research was conducted between 24 and 25 July 2003 at Champaign, Illinois (40.062°N, 88.190°W) in a 3.6-ha (187 × 192 m, N to S × E to W) cornfield. The corn hybrid planted at the site was Pioneer 34B24, and it expressed Cry1Ab from the Yieldgard™ gene for resistance to lepidopteran pests. Rows were spaced 0.71 m apart and plants were spaced 0.20 m apart. Four 100-m^2 (10 × 10 m) replicate plots were established near the corners of the field; two plots were nine rows from the western edge of the field and two plots were 17 rows from the eastern edge of the field. The duration of anthesis was monitored (Lundgren *et al*., 2004), and research was conducted on the third or fourth day of anthesis (depending on the plot) when a random sample of 40 plants per plot revealed that > 75% of the plants were shedding pollen. Pollen was found in the guts of field-collected beetles and larvae on each of the 11 sample days after the onset of anthesis (Lundgren *et al*., 2004), and the daily temperatures during this 11-day period were recorded: the average daily temperature over anthesis was 21.9 °C [(maximum air temperature + minimum air temperature)/2]; average maximum daily temperature was 27.3 °C and average minimum daily temperature was 16.5 °C (Water and Atmospheric Resources Monitoring Program, Illinois Climate Network 2003; Illinois State Water Survey, Champaign, Illinois).

On 23 July, we starved larvae in the *C. maculata* laboratory colony for approximately 16 h, but provided them with a saturated cotton wick in a 60-mm plastic Petri dish (Becton Dickinson Labware, Franklin Lakes, New Jersey). Larval stages were estimated based on size. On 24 July, we placed 100 second, 90 third and 32 fourth instars in each of the plots; 10 second instars were placed on each of 10 plants, 10 third instars were placed on each of nine plants and eight fourth instars were placed on each of four plants per plot. The plants on which the larvae were placed were randomly selected and marked with pink flagging tape, and were located similarly within each of the plots. The opened Petri dishes that contained the larvae were lodged securely in the collar of the third leaf from the base of the plant. On 25 July, all *C. maculata* adults and larvae that could be found were collected from the plots. Searches for larvae were localized around the release plants, but no effort was made to distinguish between laboratory-reared and endemic populations of larvae. Larvae and adults were stored on dry ice pending return to the laboratory, where they were stored in liquid nitrogen until they could be processed.

Larvae collected from the field were staged in the laboratory based on head capsule width. The digestive tracts of larvae and adults were dissected at room temperature, and the proportion of the gut contents that was corn pollen was estimated visually under $\times$ 50 magnification. The guts were dried for 3–24 h, and weighed to the nearest 0.01 mg on an electronic balance. Thus, a weight for the digestive tract plus its contents was determined for each individual. From these values, the mean weight of empty digestive tract for the respective stadium or stage was subtracted to arrive at the weight of the contents for each individual. The mass of corn pollen in the gut of each individual was determined by multiplying the weight of total gut contents by the proportion of gut contents visually estimated to be corn pollen. From these data, a mean weight of corn pollen per gut was determined for second–fourth instars and adults for each replicate plot. Only a single adult was captured in one of the plots; the data from this replicate were omitted from analyses because of the low sample size. The mean weights of pollen in the guts were compared among the different stadia and stages with ANOVA and significant differences in the means were separated using the Tukey–Kramer means comparison. In addition, the mean proportion of individuals in each instar or stage that contained corn pollen was calculated from the four replicates. Finally, the adult numbers from all plots were pooled because the sex ratios were inconsistent in different plots, and the amount of pollen in the guts of male and females were compared with a *t*-test (Jmp 3.2.6).

Calculation of pollen consumption under field conditions

Digestion rates observed in the laboratory, durations of each stadium as determined in the laboratory, and the field observations of gut contents were used to calculate the amount of corn pollen ingested by *C. maculata* over the duration of potential exposure. The mean weights of pollen observed in the guts of field-collected individuals for each plot were substituted into the respective digestion rate equations as the y-intercept (b). We assumed that *C. maculata* replenished the pollen at a constant rate, such that the rate of ingestion was the inverse of the rate of digestion that we observed in the laboratory (m). To calculate the estimated mean amount of pollen consumed by the sampled population under field conditions, we used the equations presented in Table 1 and included the mean duration of each stadium or stage that we observed in the laboratory as the (x) variable. The results for each stadium were then compiled into a mean consumption rate for the entire larval stage. The duration of the adult exposure was assumed to be 11 days, which was the duration of the sample period during which pollen was observed in the guts of *C. maculata* adults collected from the experimental cornfield (Lundgren *et al.*, 2004). To provide a potential range in the amount of pollen consumed during each stadium or stage, we substituted the high and low standard deviations for the mean duration of each stadium or stage (Table 1).

Table 1 Estimated rate of corn pollen consumption by field-collected *Coleomegilla maculata*

Stadium/stage	Duration, mean ± SD[1] (h)	Weight of pollen in guts of field-collected beetles, mean ± SD (1×10^{-5} g)	Rate of pollen consumption $y = b + m(x)$ [2]
Second	60.00 ± 20.40^{a}	5.05 ± 3.40^{a}	$y = 5.05 + 1.01(x)$
Third	87.60 ± 20.68^{b}	17.08 ± 8.17^{a}	$y = 17.08 + 1.71(x)$
Fourth	116.88 ± 23.23^{c}	41.00 ± 5.82^{b}	$y = 41.0 + 2.47(x)$
Adult	264	24.63 ± 13.91^{ab}	$y = 24.63 + 4.89(x)$

Results are based on the amounts of pollen observed in field-collected guts and the rates of digestion for the different life stages as determined in the laboratory (Figs. 1–4). Values within columns followed by different superscript letters are significantly different (Tukey–Kramer means comparison, $\alpha = 0.05$)

[1]The duration of each stadium was determined in the laboratory under a temperature that reflected the conditions observed during anthesis in the field. The duration of exposure for the adult stage is based on the duration that field-collected individuals could be encountered with pollen in their stomachs. See text for further details.

[2]y is the amount of pollen consumed; x is the duration of each life stage (from preceding column); b is the mean amount of pollen (1×10^{-5} g) observed in the guts of field-collected individuals (see text for further details); m is the rate of consumption (1×10^{-5} g/h) required to maintain pollen at a constant level as determined in the laboratory (Figs. 1–4).

Table 2 Head capsule widths for *Coleomegilla maculata* larvae of different ages

Instar	Age within stadium (days)	Head capsule width (mm) (mean ± SEM)
First	2	0.39 ± 0.0033^{a}
First	3	0.38 ± 0.0049^{a}
Second	1	0.51 ± 0.0059^{b}
Second	3	0.51 ± 0.0056^{b}
Third	1	0.68 ± 0.0080^{c}
Third	3	0.68 ± 0.0098^{c}
Fourth	1	0.89 ± 0.015^{d}
Fourth	3	0.89 ± 0.0031^{d}

Values followed by different superscript letters are significantly different (Tukey–Kramer means comparison, $\alpha = 0.05$).

Results

Head capsule width was significantly different among instars, but not different within instars ($F_{7,57} = 754.49$, $P < 0.0001$; Table 2). The duration of the fourth stadium was significantly longer than the second and third stadia ($F_{2,47} = 25.31$, $P < 0.0001$) (Table 1). As measured in the laboratory, the mean ± SEM weight of empty guts for second, third and fourth instars, and adult *C. maculata* were 0.074 ± 0.02 ($n = 8$), 0.19 ± 0.04 ($n = 8$), 0.26 ± 0.03 ($n = 10$) and 0.32 ± 0.03 mg ($n = 10$), respectively. The weights of empty adult male and female digestive tracts were similar ($t_8 = 0.53$, $P = 0.61$).

Weights of pollen found in the guts of dissected larvae and adults were significantly correlated with the amount of time elapsed after feeding. There was a significant linear relationship between the weights of pollen in the guts and the time elapsed after feeding for adults ($F_{1,4} = 18.87$, $P = 0.012$; Fig. 1), second instars ($F_{1,4} = 22.07$, $P = 0.0093$; Fig. 2), third instars ($F_{1,5} = 10.17$, $P = 0.024$; Fig. 3) and fourth instars ($F_{1,5} = 26.74$, $P = 0.0036$; Fig. 4). In the laboratory, pollen was retained in the guts of adults, second, third and fourth instars for 6.73, 7.18, 8.01 and 13.64 h, respectively (Figs. 1–4). Also in the laboratory, the guts of adults, second, third and fourth instars contained a mean maximum of 0.33, 0.07, 0.14 and 0.34 mg of corn pollen, respectively (Figs. 1–4).

In the different plots, 25, 11, 3 and 1 adults were recovered; the plot with only a single observation was excluded from the analyses. We recovered a maximum mean ± SEM of 11.25 ± 2.32, 10 ± 1.98 and $70.63 \pm 15.39\%$ of the second, third and fourth instars that were placed in the field, respectively. The guts of fourth instars contained significantly more pollen than adults or other instars ($F_{3,11} = 13.68$, $P = 0.0005$) (Table 1). For third and fourth instars, the mean amount of pollen observed in the guts of field-collected individuals was greater than the estimated maximum observed in our laboratory studies, although we did not apply statistics to these observations (Table 1,

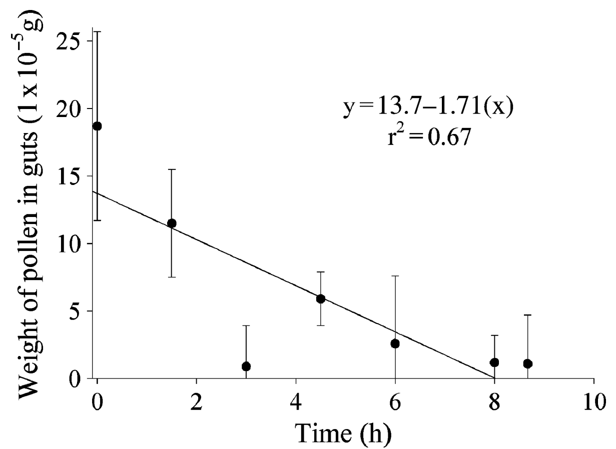

Figure 2 Digestion rate of corn pollen for second-instar *Coleomegilla maculata* after feeding for 3 h, determined gravimetrically. Each data point represents a sample of 4–9 individuals, with error bars indicating SEM.

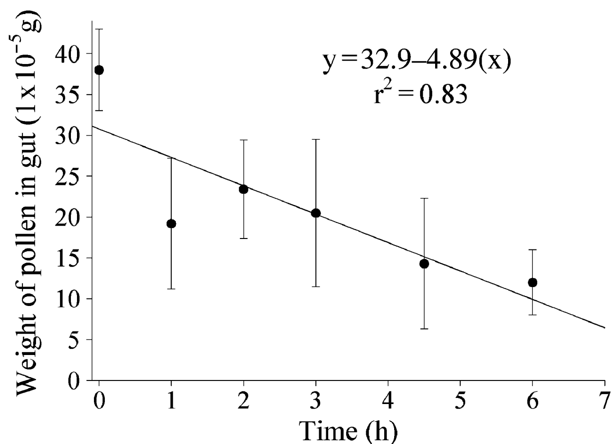

Figure 1 Digestion rate of corn pollen for adult *Coleomegilla maculata* after feeding for 3 h, determined gravimetrically. Each data point represents a sample of 7–10 individuals, with error bars indicating SEM.

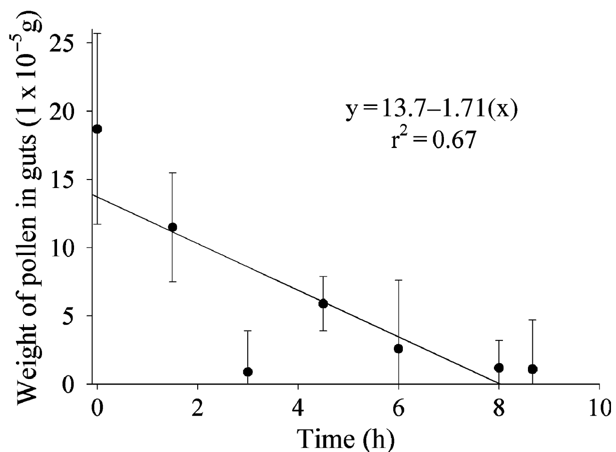

Figure 3 Digestion rate of corn pollen for third-instar *Coleomegilla maculata* after feeding for 3 h, determined gravimetrically. Each data point represents a sample of 5–10 individuals, with error bars indicating SEM.

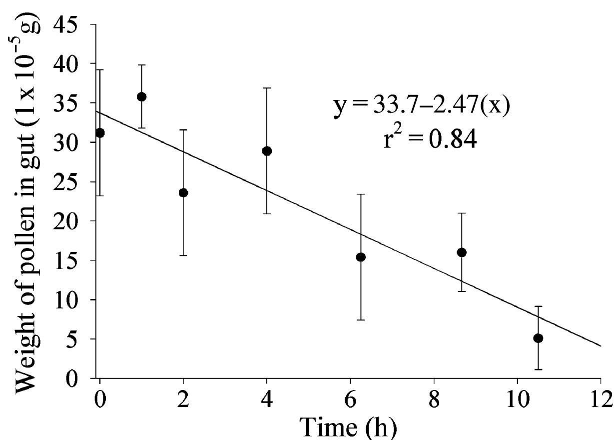

Figure 4 Digestion rate of corn pollen for fourth-instar *Coleomegilla maculata* after feeding for 3 h, determined gravimetrically. Each data point represents a sample of 9–11 individuals, with error bars indicating SEM.

Figs. 3 and 4). Field-collected adults are predicted to consume more pollen than larvae over a similar duration of exposure (Table 3). The high and low estimates of pollen consumption deviated from the mean by 32, 21 and 17% for second, third and fourth instars. The guts of females contained significantly more corn pollen than males (mean ± SEM; 0.43 ± 0.09 and 0.04 ± 0.02 mg, respectively; $t_{38} = 4.19$, $P = 0.0002$); all females and 81% of males had pollen in their guts. The proportions (mean ± SEM) of field-collected second (0.86 ± 0.05), third (0.60 ± 0.11) and fourth (0.80 ± 0.05) instars that had pollen in their guts were not statistically different ($F_{2,9} = 3.08$, $P = 0.096$), and the proportion of larvae with pollen in their guts (0.76 ± 0.03) was significantly lower than the proportion of adults with pollen in their guts (0.91 ± 0.05; $t_5 = 2.74$, $P = 0.041$).

Discussion

We found a high incidence of pollinivory by *C. maculata* larvae and adults during anthesis, but the amount of pollen consumed by larvae and adults in the field was less than in the laboratory. Research focusing on how alternative foods affect pollinivory, the duration that *C. maculata* is actually exposed to pollen in the field, and how digestion rates of pollen differ under field and laboratory conditions will help to explain the low intensity of pollinivory that we observed and to refine models that quantify pollinivory under field conditions. The results of this research will serve as the basis for future research concerning risk assessment of transgenic insecticidal corn pollen, and the life history strategies of *C. maculata* in the field.

Table 3 Amount of corn pollen consumed by *Coleomegilla maculata* in the field

	Pollen consumed (mg)		
Instar/stage	Low estimate[a]	Mean estimate	High estimate
Second instar	0.45 ± 0.02	0.66 ± 0.02	0.87 ± 0.02
Third instar	1.32 ± 0.04	1.67 ± 0.04	2.02 ± 0.04
Fourth instar	2.73 ± 0.03	3.30 ± 0.03	3.87 ± 0.03
Larval stage	4.50	5.63	6.76
Adult	NA	13.15 ± 0.08	NA

[a]The low and high estimates are based on ± 1 SD for the duration of each stage (*x*), for additional information see Table 1 and text. NA, Not applicable.

Even though we observed a high incidence of pollinivory in *C. maculata* larvae and adults, the amount of pollen consumed by *C. maculata* was lower than expected from laboratory studies. Between 60 and 80% of field-collected *C. maculata* larvae and adults had corn pollen in their digestive systems. In one recent study, Lundgren *et al.* (2004) found that a similar proportion of naturally occurring larvae and adults contained pollen in their guts (more than 75%). The current research supports the notion that the majority of larvae and adults that occur in corn habitat consume corn pollen during anthesis, but the estimates of pollen consumption (Table 3) suggest that *C. maculata* does not consume as much pollen in the field as they do under laboratory conditions. Under laboratory conditions, fourth instars can consume more than 10 mg of pollen per day (Lundgren & Wiedenmann, 2004). Pollen is very abundant during anthesis and its availability is not likely to be a limiting factor in consumption. The fact that *C. maculata* larvae consumed so little corn pollen in the field suggests that they must rely on other foods as components of their diet, or that their digestion capabilities are different under field conditions than in the laboratory. This other food includes prey, other pollen and fungal spores (Forbes, 1880; Smith, 1960; Lundgren *et al.*, 2004). The relative importance of pollen and alternative foods to the feeding behaviour of *C. maculata* should be explored further.

Research into the duration that *C. maculata* is exposed to corn pollen in the field will help to refine future pollen consumption estimates. Although we attempted to replicate the temperature regimen observed during anthesis in our laboratory experiments, one area that should be revisited is how biotic (i.e. presence of alternate foods) and abiotic factors (i.e. humidity; Hodek & Honěk, 1996), and microclimates within cornfields (Orr *et al.*, 1997) affect the duration of each life stage. Furthermore, additional research should investigate how long corn pollen remains in the field and how long it is palatable to *C. maculata*. Lundgren *et al.* (2004) found that anthesis lasts for approximately 8 days, but *C. maculata* larvae and adults fed on corn pollen for at least 11 days, when sampling was ceased. Although larvae in all stadia were observed in cornfields during anthesis, the majority of *C. maculata* larvae were first instars, owing to the fact that the number of eggs was associated with pollen shed. If the duration of exposure to pollen is increased beyond the 11 days that we observed, then adults and a greater number of larvae will probably ingest more pollen than is reported here.

The digestion rate of corn pollen by *C. maculata* under field conditions is another area that merits further research. In our laboratory studies, *C. maculata* was starved after the initial feeding period, and this may

have affected the digestion rates that we observed. For example, starvation before a feeding episode led to increased digestion rates of prey in the predator *Poecilus cupreus* (Lövei *et al*., 1985). By contrast, feeding alternative food or no food after an aphid meal did not significantly affect the rate of aphid digestion with other carabids and a staphylinid (Lövei *et al*., 1990). In addition, we observed that field-collected third and fourth instars contained more pollen in their guts than was ingested during the 3-h feeding period in our laboratory study. Although initially using lower amounts of pollen in the laboratory experiments may have adversely affected the digestion rates, we feel that these effects were minimal because the relationship between time and the amount of pollen in the guts was linear for all tested larvae and adults, even early in the monitoring period. Finally, we assumed that *C. maculata* replenishes the pollen in its gut at a constant rate under field conditions. Additional research on how the feeding behaviour of *C. maculata* in the presence of other foods affects the pollen consumption rate would help to refine this portion of our models.

Transgenic plants that express insecticidal substances in their pollen potentially pose risk to predators that rely on pollen as food. Risk assessment is often defined as having two components: (i) hazard, such as the toxicity of transgenic corn pollen to specific predators and (ii) exposure, or the likelihood that these predators will ingest a toxic dose of transgenic corn pollen in the field (Sears *et al*., 2001). Risk cannot be adequately assessed if one of these components is lacking. Previous research on the risk of transgenic corn pollen to *C. maculata* has focused on the hazard posed by the insecticidal proteins expressed in individual events (Pilcher *et al*., 1997; Duan *et al*., 2002; Lundgren & Wiedenmann, 2002). The exposure estimates presented in Table 3 will help to complete the risk assessment equation for the interaction between *C. maculata* and corn pollen, but it is important to define exactly what the numbers presented here represent. For example, the value of 0.66 represents the mean pollen ingestion for second instars that occur in cornfields and, if laboratory toxicity assays determine that feeding 0.66 mg of pollen to second instars is fatal, then half of the second instars are at risk of ingesting a lethal dose of pollen under field conditions. Additional information that addresses how reductions in *C. maculata* numbers affect the levels of pest control in corn, and what level of reductions in the different life stages lead to compromised pest suppression, will help to refine the models presented here.

One interesting outcome of this research is that, on average, females consumed 10-fold more pollen than males in the field, and all females contained pollen in their guts. Even though females reared on aphids had higher fecundity than pollen-reared females in the laboratory (Lundgren & Wiedenmann, 2004), it may be that pollen is still an important source of food for female *C. maculata*. For example, female coccinellids often require more and different nutrition compared with males to produce eggs (Hodek & Honěk, 1996), and therefore may rely on other foods such as corn pollen to a greater degree when it becomes abundant. Insects are known to self-select dietary constituents to optimize their nutrition (Greenstone, 1979; Waldbauer & Friedman, 1991). Single dietary items are seldom nutritionally optimal, and several different foods or prey are often necessary components of an optimal diet for predators (Greenstone, 1979; Coll & Guershon, 2002; Patt *et al*., 2003). It is not clear whether *C. maculata* ingests corn pollen because it contains some critical nutrient that it cannot obtain from its other food, or whether it feeds on the pollen because it is very abundant and easily acquired.

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