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Journal of Insect Physiology 50 (2004) 567–575

Journal of Insect Physiology

www.elsevier.com/locate/jinsphys

Nutritional suitability of corn pollen for the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae)

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Received 5 December 2003; received in revised form 5 April 2004; accepted 6 April 2004

Abstract

The nutritional suitability of corn pollen for the facultatively phytophagous predator *Coleomegilla maculata* was studied in the laboratory. Dry matter, organic matter, ash, crude protein, amino acid, and quercetin contents of pollen from 10 hybrids of field corn were determined. *C. maculata* were reared on pollen or aphids + artificial diet for their entire lives; larval duration, post-mortem adult dry weights, fecundity within 7 days of mating, and mortality rates were compared among the treatments. In another experiment, *C. maculata* larvae were reared on pollen; weight gained, pollen ingested, and frass produced were compared among instars. Also, consumption relative to increases in larval biomass and the efficiency with which larvae converted corn pollen into biomass were compared among instars. Beetles reared on aphids had greater weights and fecundity and a shorter larval duration relative to the pollen-fed beetles. The percentages of organic matter and ash in corn pollen were significantly correlated with *C. maculata* mortality, and we hypothesize that some micronutrient or phytochemical is at sub-optimal levels for *C. maculata* development in some of the pollens. We observed an increase in the conversion efficiency of pollen and a decrease in the consumption relative to biomass of *C. maculata* as the larvae aged, which suggests a physiological or behavioral alteration in the feeding behavior of *C. maculata* during the larval stage.

Keywords: *Rhopalosiphum* spp.; Conversion efficiency; Biomass; Quercetin; Anthesis; Biological control; Facultative phytophagy; Transgenic crops; *Zea mays*

1. Introduction

Pollen is a food for numerous insects, including many species of natural enemies. As examples, species of Syrphidae (Diptera) (Schneider, 1969; Hagen, 1986), Carabidae and Coccinellidae (Coleoptera) (Allen, 1979; Hodek and Honěk, 1996), Chrysopidae (Neuroptera) (Pilcher et al., 1997; McEwen et al., 2001), predatory Heteroptera such as *Orius* spp. (Anthocoridae) and *Podisus* spp. (Pentatomidae) (Kiman and Yeargan, 1985; Alomar and Wiedenmann, 1996; Pilcher et al., 1997; Corey et al., 1998), and parasitic Hymenoptera (Hagen, 1986) feed on pollen to varying degrees. Facultative phytophagy by entomophagous insects may allow them to survive periods of low prey densities (Benton and Crump, 1981; Hemptinne and Desprets, 1986; Hagen, 1986; Hodek and Honěk, 1996; Wiedenmann et al., 1996), or provide them with critical or extra nutrients necessary for egg production or overwintering (Hagen, 1962; Schneider, 1969; Jervis and Kidd, 1986; Hodek and Honěk, 1996). Many species of Coccinellidae feed on pollen, but it is seldom nutritionally sufficient to sustain larval development (Smith, 1960b; Hemptinne and Desprets, 1986); an exception to this rule is *Coleomegilla maculata* (Coleoptera: Coccinellidae), which can successfully develop on a diet consisting solely of corn pollen (Smith, 1960a,b; Hodek et al., 1978).

Corn pollen is abundant during anthesis, and a number of natural enemies feed on corn pollen during this period (Pilcher et al., 1997). Each corn tassel produces

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0022-1910/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2004.04.003

2–5 million pollen grains over approximately 8 days (Hoeft et al., 2000), although higher values have been reported (Goss, 1968). Much of this pollen stays within the field margins (Raynor et al., 1972; Jesse and Obrycki, 2000; Pleasants et al., 2001), presumably because the pollen grains are each 90–100 μm in diameter and they are not well suspended in the air (Raynor et al., 1972). The nutrition of corn pollen as food for insects has been studied primarily with *Apis mellifera* (Hymenoptera: Apidae) (Standifer, 1966, 1967; Standifer et al., 1968; Stanley and Linskins, 1974). Corn pollen contains members of all of the major classes of organic and inorganic nutrients. For instance, corn pollen contains sugars and relatively high levels of starch (primarily amylopectin) (Goss, 1968; Stanley and Linskins, 1974; Roulston and Buchmann, 2000), amino acids and proteins such as adenine and choline (Goss, 1968; Stanley and Linskins, 1974), lipids and phytosterols (Goss, 1968; Standifer et al., 1968), and phosphorus, potassium and other inorganic minerals (Goss, 1968; Stanley and Linskins, 1974). Corn pollen contains almost no carotenoid pigments (J.G.L. unpublished data); this is a biochemical feature that is relatively unique to corn pollen, and corn pollen is yellow because of the presence of the flavonoid pigment quercetin and its derivatives (Goss, 1968).

C. maculata is one of the most prevalent chewing predators in Midwestern corn (Udayagiri et al., 1997; Wright and DeVries, 2000) and is a source of mortality to several pests of corn, including aphids, eggs of *Ostrinia nubilalis* (Lepidoptera: Crambidae), and *Helicoverpa zea* (Lepidoptera: Noctuidae) (Wright and Laing, 1980; Cottrell and Yeargan, 1998; Phoofolo et al., 2001; Pfannenstiel and Yeargan, 2002). In addition to being an important predator, *C. maculata* is also facultatively phytophagous on corn pollen under field conditions (Lundgren et al., 2004). Fluctuations in the population of *C. maculata* have been correlated with anthesis of sweet and field corn (Benton and Crump, 1981; Cottrell and Yeargan, 1998; Lundgren et al., 2004). In fact, corn pollen may be preferred over prey as food (Smith, 1965), and corn pollen may temporarily detract from predation of target pests (Cottrell and Yeargan, 1998; Pfannenstiel and Yeargan, 2002; Lundgren et al., 2004). Several aspects of pollinivory by *C. maculata*, such as the effects of intraspecific nutritional variation of corn pollen on *C. maculata* fitness and fecundity of pollen-reared individuals, have not been explored thoroughly.

Although it is well documented that insect natural enemies feed on corn pollen, the nutritional suitability of corn pollen (or any other pollen, for that matter) for natural enemies is not well understood (Nordlund et al., 2001). Commercial hybrids of transgenic insecticidal corn hybrids express the delta-endotoxins of the entomopathogen *Bacillus thuringiensis* (*Bt*) in their pollens, and there is concern over potential adverse effects of transgenic pollen to natural enemies, including the predator *C. maculata* (Pilcher et al., 1997; Lundgren and Wiedenmann, 2002). Understanding the feeding behavior of natural enemies that ingest corn pollen is necessary to designing and interpreting laboratory assays that quantify the toxicity of pollen from transgenic insecticidal corn hybrids to the non-target species, *C. maculata*. The current research addresses the intraspecific nutritional variability of corn pollen, its effect on the fitness of pollen-fed *C. maculata* relative to prey-fed individuals, and the feeding efficiency of *C. maculata* larvae reared on corn pollen.

2. Materials and methods

2.1. Corn and insects

Seeds from nine hybrids of field corn were provided by Illinois Foundation Seeds (Champaign, IL), and seed of one hybrid was provided by Pioneer Hi-Bred International (Johnston, IA). All hybrids were commercially competitive, non-transgenic hybrids except for one, which was transgenic and contained the Yieldgard™ gene that expresses the Cry1Ab insecticidal protein from *Bt* for control of Lepidoptera. Parents for the hybrids were: (1) Fr390YG × FR9661 (transgenic), (2) LH213 × FR9671, (3) FR3113 × FR2108 BmA022, (4) FR9612 × LH185, (5) FR1909 × FR9661 BmA051, (6) FR3484 × FR4901, (7) FR3361 × FR9661, (8) FR1064 × FR9642, (9) FR3484 × LH185, and (10) Pioneer hybrid 34G81. Two corn plants per 3.8-l pot were grown in a single greenhouse, the plants 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, PA) at a concentration of 250 ppm. Tassels were contained in 2.3-kg paper bags that were stapled shut before pollen was shed. Pollen was sifted through a sieve (sieve no. 230, Dual Manufacturing Co., Chicago, IL) with a 0.06-mm pore size and examined under a dissecting microscope at 50× 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, CA) and kept at −10 °C until use.

The *C. maculata* colony originated from field-collected individuals and had been reared on artificial diet no. 7 of Atallah and Newsom (1966), without tetracycline, continuously for approximately two years, with annual additions of field-collected individuals. Newly hatched first instars remained unfed with their chorions and siblings for 24 h before being assigned to experimental treatments. *Rhopalosiphum maidis* (Fitch) and *R. padi* (L.) (Homoptera: Aphidae) were collected from infested non-transgenic corn plants grown in a

separate greenhouse from the pollen-producing plants. We did not distinguish between aphid species when offering them as food for *C. maculata*, but most of the aphid population was comprised of *R. maidis*.

2.2. Nutritional profiles of pollen

We quantified the levels of dry matter, organic matter/ash, crude protein, amino acid contents, and quercetin in pollens from all hybrids. Dry matter determinations and dry-ashing procedures were conducted according to the AOAC (2003). Organic matter was calculated by subtracting the ash percent from dry matter percent. Crude protein content (total nitrogen) of the pollens was determined on a combustion-based determinator (LECO FP-2000, LECO Corporation, St. Joseph, MI) (AOAC, 2003). Amino acid profiles (percentage of dry matter) were created using an amino acid analyzer with ion exchange chromatography (Beckman 126AA amino acid analyzer, Beckman Coulter Inc., Fullerton, CA) using citrate buffers, and ninhydrin was used for detection; analyses were conducted according to Spitz (1973) by Dr. George Fahey's laboratory (University of Illinois, Urbana, IL). High-performance liquid chromatography (HPLC) was used to determine quercetin content of the pollens (HPLC conducted by Craft Technologies, Wilson, NC). A Betasil C_{18} column (4.6 × 150 mm, 5 μm; Thermo Hypersil-Keystone, PA) was used to analyze the samples. The mobile phase consisted of a gradient of 0.05% TFA in water and 0.05% TFA in acetonitrile, with a flow rate of 1.0 ml/min. The UV spectra were recorded at 280 nm and electrochemically at 150 and 660 mV. Pollen from hybrid 1 was macerated in SEB4 extraction buffer (product no. ACC01958, Agdia Inc., Elkhart, IN) and subjected to an immunostrip test specific for Cry1Ab/c, δ-endotoxins from *Bt* (product no. STX06200, Agdia Inc.)

2.3. Fitness assays of C. maculata reared on the pollens

C. maculata ($n = 30$ per treatment) were reared until 10 days after adult eclosion on one of five dietary treatments: pollen from hybrids 1–4, or aphids + artificial diet. First-instar *C. maculata* were randomly assigned to the treatments and were reared in 30-ml plastic cups with plastic lids (product nos. 9051 and 9053, Bio-Serv, Inc, Frenchtown, NJ) under experimental conditions of 27 °C, 14:10 L/D, and 25–35% r.h. *C. maculata* in the aphid + diet treatment received aphids during the larval stage and artificial diet during the adult stage, and food was supplied in excess and replaced daily. Each day, approximately 10 mg of pollen was added to each cup in the pollen treatments. For all treatments, water was supplied as a saturated cotton wick. Cups were replaced every other day, and water wicks were replaced when they became soiled.

The duration of larval development, post-mortem adult dry weight, fecundity, and mortality rates during the larval stage, pupal stage, and first 10 days of adulthood were compared among treatments. The proportions of larvae that pupated, eclosed, and survived for 10 days post-eclosion were recorded, and cumulative mortality rates for different treatments were compared using likelihood ratio tests (JMP 3.2.6, 1999). Ten days after eclosion, individual female beetles were exposed to two males from the *C. maculata* colony over 24 h for mating, and then each female was isolated in a 30-ml plastic cup with a plastic lid. The number of eggs laid per female was counted each day for 7 days, and cups were replaced within 24 h of female oviposition. At the end of the oviposition period, male and female experimental beetles were frozen at −10 °C for 24 h, dried at 105 °C for 24 h, and weighed to the nearest 0.01 mg. Because the numbers of females in the pollen-fed treatments were low and oviposition rates did not differ among the pollen treatments, oviposition data from females in all pollen treatments were pooled and compared with the data from females in the aphid + diet treatment with a *t*-test. The weights and duration of larval development were compared among the pollen-fed and aphids + diet-fed beetles using ANOVAs, and significantly different means ($P < 0.05$) were separated using the Tukey-Kramer means comparison (JMP 3.2.6, 1999).

2.4. Feeding efficiency assays

Newly hatched *C. maculata* ($N = 17$) were reared solely on pollen from hybrid 10 in 30-ml plastic cups with plastic lids at approximately 22 °C, 14:10 L/D, and 20–30% r.h., and water was provided as a saturated cotton wick. Each day, larvae and frass were weighed separately to the nearest 0.01 mg. Each day, we also recorded larval molts. The weights of the cast skins were always <0.01 mg and were not considered in the biomass studies. Each larva was provided each day with 20 mg of corn pollen, and the cups containing pollen and the water wicks were measured to the nearest 0.01 mg before feeding and again after feeding for 24 h. The difference between the starting and ending cup + pollen + water weights was recorded each day. Because water evaporated from the cotton water wick and was absorbed by the pollen, the disparity between starting and ending weights for each 24-h period was considerably larger than the actual mass of pollen consumed each day. To remedy this water bias, the pollen consumption weights were adjusted each day by subtracting the minimum weight difference each day from the rest of the observations. The daily pollen consumption for each individual was summed during each

stadium, and divided by the respective durations of each stadium for each individual. Thus, variation in daily consumption rates within instars is not considered in this analysis. Furthermore, the calculated mean daily pollen consumption rate was often more than the amount of pollen given each day because the dried pollen readily absorbed atmospheric moisture; thus daily pollen consumption rates are only estimates for comparing among the different stadia. We also determined the daily biomass accumulation of *C. maculata* larvae during each stadium by summing the daily biomass accumulation over each stadium, and dividing by the respective number of days in each stadium. Similarly, we determined the daily frass accumulation for each stadium.

The consumption of corn pollen relative to increases in body mass was examined for *C. maculata* in the different stadia using the consumption index (CI) formula presented by Waldbauer (1968). This index is:

$$\mathrm{CI} = \frac{\text{weight of pollen consumed}}{\text{biomass accumulated}}$$

Also, the efficiency at which *C. maculata* larvae converted pollen into biomass was calculated for each instar using an adaptation of the efficiency of converted digested material (ECD) formula of Waldbauer (1968). The formula used was:

$$\mathrm{ECD} = \frac{\text{biomass accumulated}}{(\text{amount of food consumed} - \text{amount of frass produced})}$$

The consumption indices, conversion efficiencies, daily and instar biomass accumulations, pollen consumption rates, and frass production rates were compared among the stadia using ANOVAs, and means were separated with the Tukey-Kramer means comparison when $P < 0.05$.

3. Results

Variation among the hybrids was more pronounced as nutritional constituents of corn pollen became less abundant (Tables 1 and 2). The range of variation (maximum−minimum values) represented 4.8, 0.8, 0.8, 17.2, and 60.1% of the mean for dry matter, organic matter, ash, crude protein, and quercetin, respectively. Proline was the most abundant amino acid in corn pollen, and variation in the total amino acid content in the different pollens ranged from 12.5 to 68.1% of the mean (mean ± SEM was 23.5 ± 3.45%) (Table 2). Cry1Ab was detected serologically in the pollen from hybrid 1.

C. maculata fed aphids + diet developed faster, weighed more, had higher fecundity, and had higher survivorship than the pollen-fed beetles (Table 3, Figs. 1 and 2). The larval duration was shorter for *C. maculata* fed the aphid + diet treatment than those fed pollen ($F_{4,\,119} = 14.24$, $P < 0.01$) (Table 3). Adult weight was significantly greater for *C. maculata* fed aphids + diet than for those fed pollens 1, 2, and 3 ($F_{4,\,94} = 3.76$, $P = 0.01$) (Table 3, Fig. 1). Larval duration, adult weight, and fecundity of *C. maculata* did not vary among the pollen treatments (Table 3 for larval duration and adult weight). There were no differences in

Table 2
Mean (±SEM) amino acid profiles for field corn pollen ($N = 10$)

Amino acid	Mean (% of dry matter)	Range (% of dry matter)
Alanine	1.69 ± 0.038	1.49–1.89
Arginine	1.14 ± 0.019	1.03–1.22
Aspartic acid	2.48 ± 0.049	2.17–2.65
Glutamic acid	2.54 ± 0.076	2.21–3.02
Glycine	1.30 ± 0.023	1.16–1.39
Histidine	0.54 ± 0.011	0.48–0.58
Isoleucine	1.15 ± 0.020	1.03–1.24
Leucine	1.77 ± 0.032	1.57–1.90
Lysine	1.84 ± 0.033	1.72–1.95
Phenylalanine	1.05 ± 0.018	0.93–1.12
Proline	3.01 ± 0.19	2.19–4.24
Serine	1.29 ± 0.035	1.11–1.51
Threonine	1.21 ± 0.024	1.05–1.30
Tyrosine	0.69 ± 0.011	0.61–0.73
Valine	1.48 ± 0.028	1.32–1.61

Table 3
Larval duration and post-mortem adult dry weights for *C. maculata* reared on different diets. Values within columns followed by different letters are significantly different (Tukey-Kramer means comparison, $\alpha = 0.05$)

Treatment (n)	Larval duration (SEM) in days	Adult weight (SEM) in mg
Aphids + diet (21)	9.24 (0.26) A	5.41 (0.41) A
Pollen 1 (24)	10.83 (0.21) B	3.59 (0.40) B
Pollen 2 (26)	11.00 (0.17) B	3.80 (0.43) B
Pollen 3 (26)	11.12 (0.16) B	3.61 (0.42) B
Pollen 4 (27)	11.19 (0.22) B	4.53 (0.34) AB

Table 1
Nutritional indices of field corn pollen ($N = 10$)

	Dry matter (%)	Organic matter (%)	Ash (%)	Crude protein (%)	Quercetin (μg/g)
Mean (SEM)	92.08 (0.45)	97.27 (0.07)	2.73 (0.07)	24.39 (0.36)	324.60 (19.49)
Range	89.95–94.45	96.80–97.58	2.42–3.20	22.73–26.88	233–428

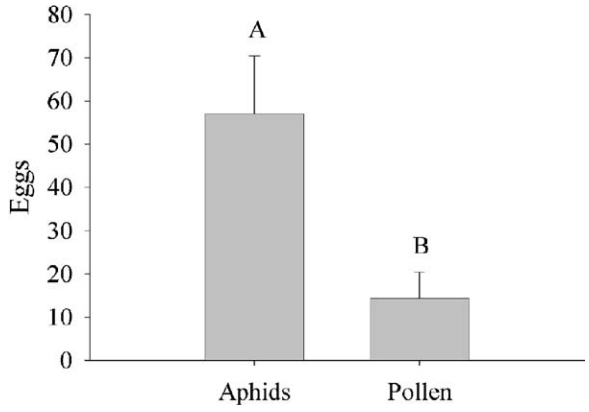

Fig. 1. Comparison of the fecundity (mean ± SEM number of eggs per female) of *C. maculata* reared on aphids + diet or pollen for 7 days after mating. Bars with different letters are significantly different from each other (*t*-test; $\alpha = 0.05$).

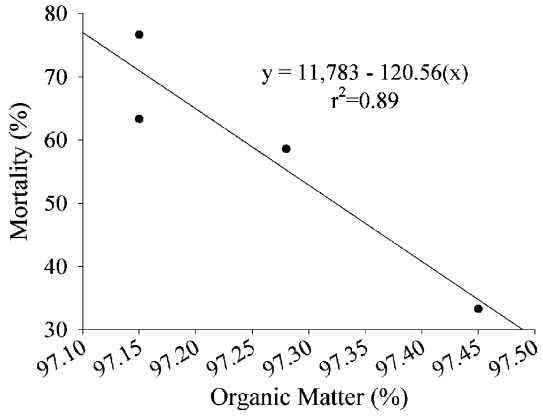

Fig. 3. Comparison of the organic matter (%) of corn pollens and the mortality levels at 10 days post-eclosion of *C. maculata* fed the different pollens.

fecundity among females fed the different pollens ($F_{3,\,13} = 0.77$, $P = 0.53$). Females reared on aphids+ diet laid significantly more eggs than those reared on corn pollen, when the pollen treatments were pooled ($t_{25} = 10.64$, $P < 0.01$) (Fig. 1). Mortality in larvae and pupae was not significantly different among the treatments (larval stage: $\chi^2_{4,\,144} = 5.77$, $P = 0.22$; pupal stage: $\chi^2_{4,\,144} = 3.38$, $P = 0.50$) (Fig. 2). Adult mortality differed significantly among the treatments when the aphid + diet treatment was included ($\chi^2_{4,\,144} = 20.02$, $P < 0.01$) and excluded from the analysis (i.e., when only the pollen treatments were considered) ($\chi^2_{3,\,115} = 12.37$, $P = 0.01$) (Fig. 2).

The amount of organic matter/ash of the corn pollen was correlated with the mortality 10 days after adult eclosion ($F_{1,\,2} = 20.03$, $P = 0.047$) (Fig. 3).

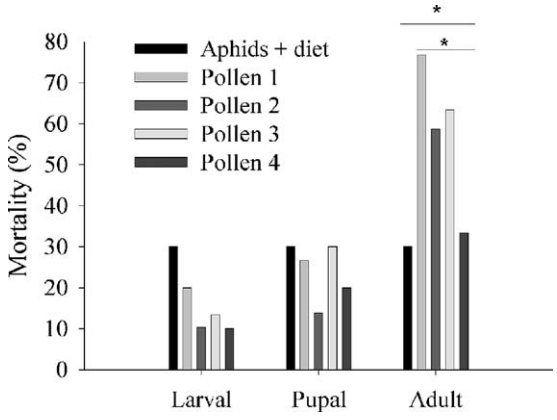

Fig. 2. Comparison of the cumulative mortality levels during the larval stage, pupal stage, and 10 days after adult eclosion for *C. maculata* fed different diets. Significant variation (indicated by ∗) was observed in early adulthood among all diet treatments, and when the aphids + diet treatment was removed from the analyses (likelihood ratio test, $\alpha = 0.05$).

Organic matter and ash are perfectly, inversely related to each other, such that the equation that compares ash content with mortality level is:

$$\text{Ash content (\%)} = 2.3123 + 0.0074 * \text{Mortality (\%)}$$

Dry matter, crude protein, and quercetin contents for the pollens were not significantly correlated with the mortality level of *C. maculata* fed the different pollens (dry matter: $F_{1,\,2} = 1.56$, $P = 0.34$; crude protein: $F_{1,\,2} = 0.50$, $P = 0.55$; quercetin: $F_{1,\,2} = 0.64$, $P = 0.51$). Similarly, mortality was not correlated with the levels of any of the amino acids ($P > 0.35$ and $r^2 < 0.42$ for amino acids).

Different instars consumed different amounts of pollen and converted pollen into biomass at significantly different rates. The mean ± SEM durations of the first, second, third, and fourth stadia were 2.12 ± 0.13, 2.71 ± 0.19, 4.56 ± 0.16, and 6.36 ± 0.23 days, respectively. Fourth instars accumulated biomass at a greater rate per day than did third instars, which accumulated biomass at a greater rate per day than did first and second instars ($F_{3,\,59} = 210.15$, $P < 0.0001$); mean± SEM daily biomass accumulations were 0.20 ± 0.02, 0.34 ± 0.02, 0.63 ± 0.03, and 1.53 ± 0.07 mg/day for first, second, third, and fourth instars, respectively. Cumulative biomasses accumulated over the stadia were also significantly different ($F_{3,\,61} = 419.64$, $P < 0.0001$) (Fig. 4). On average, third instars consumed significantly more pollen per day than the other instars ($F_{3,\,60} = 5.45$, $P = 0.002$); mean ± SEM daily consumption for first, second, third, and fourth instars was 17.43 ± 3.08, 18.96 ± 2.48, 30.64 ± 2.82, and 21.84 ± 1.33 mg/day, respectively. Also, cumulative pollen consumption was significantly greater by the third and fourth instars than by first and second instars ($F_{3,\,60} = 33.54$, $P < 0.0001$) (Fig. 5). There was significant variation within the daily and instar frass

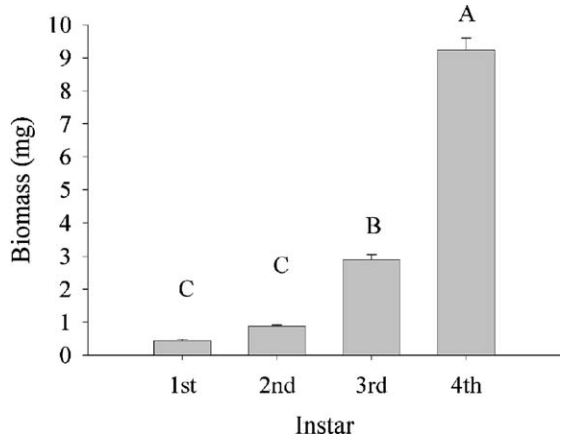

Fig. 4. Biomass accumulated (mean ± SEM) during each stadium for pollen-fed *C. maculata*. Bars with different letters are significantly different from each other (Tukey-Kramer means comparison; $\alpha = 0.05$).

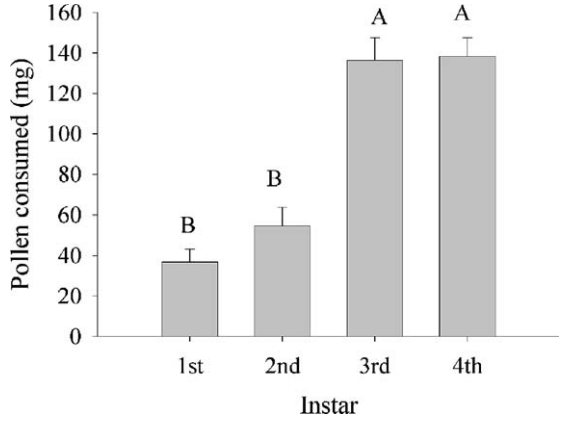

Fig. 5. Pollen consumed (mean ± SEM) during each stadium for *C. maculata*. Bars with different letters are significantly different from each other (Tukey-Kramer means comparison; $\alpha = 0.05$).

production rates. Mean ± SEM daily frass production for first, second, third, and fourth instars was 0.04± 0.01, 0.11 ± 0.01, 0.22 ± 0.02, and 0.76 ± 0.04 mg/day, respectively ($F_{3,\,59} = 175.91$, $P < 0.001$). Cumulative frass production for the first, second, third, and fourth instars was 0.09 ± 0.02, 0.32 ± 0.08, 0.97 ± 0.11, and 4.78 ± 0.22 mg/instar, respectively ($F_{3,\,61} = 301.20$, $P < 0.001$). Frass production was greatest during the fourth stadium, at an intermediate level during the third stadium, and statistically indistinguishable during the first and second stadia. The CI decreased significantly as the larva aged ($F_{3,\,59} = 7.36$, $P < 0.001$) (Fig. 6A). The ECD during the fourth stadium was significantly greater than the ECDs for the other stadia ($F_{3,\,59} = 25.96$, $P < 0.0001$) (Fig. 6B).

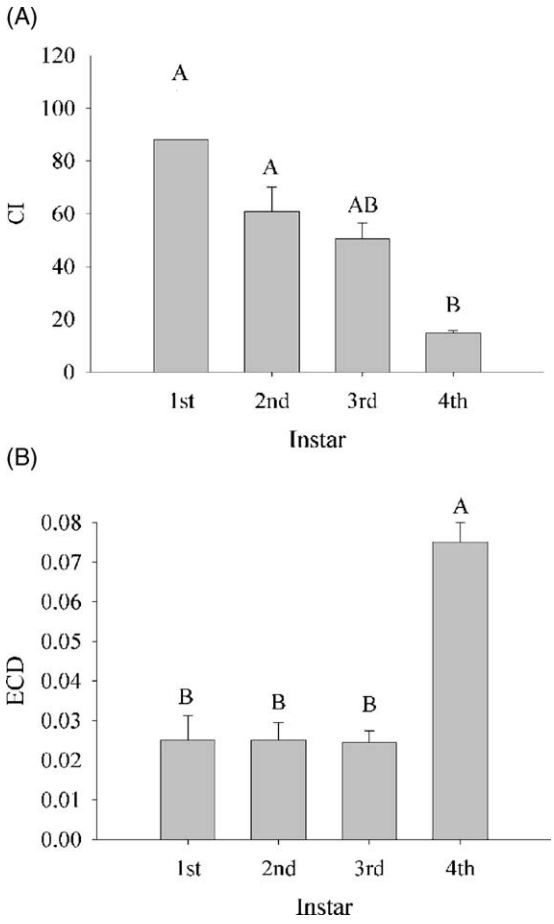

Fig. 6. The CI (g pollen eaten/g of weight gained) and ECD (g of biomass gained/g pollen assimilated) for each instar of *C. maculata*. Bars with different letters are significantly different from each other (Tukey-Kramer means comparison; $\alpha = 0.05$).

4. Discussion

We found that aphids + artificial diet were superior to corn pollen for the development of *C. maculata*. There were significant increases in fecundity and weight, significant decreases in larval duration, and generally lower mortality rates for aphid-fed *C. maculata* relative to pollen-fed *C. maculata*. Our research supports previous findings that corn pollen-fed *C. maculata* larvae did not survive as well as aphid-fed individuals (Smith, 1960a); however, we found much higher levels of pre-imaginal survival in pollen-fed individuals than has been reported in some studies (<66% survival, Smith, 1960a; <48% survival, Pilcher et al., 1997) (Fig. 2). Our results suggest that although *C. maculata* can complete development on corn pollen (Fig. 2), other foods are likely important in improving survivorship of this species. Previous research supports this assertion; *C. maculata* is generally regarded as one of the more polyphagous coccinellid species (Hodek and Honěk, 1996). Gut dissections of *C. maculata*

adults have revealed that they feed on fungal spores and various insect prey in addition to pollen (Forbes, 1880; Lundgren et al., 2004).

The levels of the different nutritional components of corn pollen that we measured in our research are similar to those reported in the literature. Pollen was dried in our experiment, hence the high percentage of dry matter in our pollens. The ratio of organic matter: ash observed in our research falls within the range of that observed in other research. Ash content of corn pollen in one study ranged between 0.83 and 3.13% (Goss, 1968), but other reports generally have indicated ash content of corn pollen between 2 and 4% (Stanley and Linskins, 1974, and references therein). Similarly, crude protein levels in our study were similar to crude protein levels indicated in other research, although there is considerable variation in the literature. One study revealed protein levels as low as 15.8% in sweet corn pollen (Standifer, 1967), although other studies have generally reported protein to be 23–27% of the dry weight of corn pollen (Goss, 1968; Roulston et al., 2000, and references therein). Proline has previously been found at relatively high levels in corn pollen, though our values of proline content surpass those shown in the literature (Stanley and Linskins, 1974). Storage of corn pollen results in alterations of amino acid profiles (Linskins and Pfahler, 1973), and because the pollen used in our study was stored for several days before analyses, the results shown here should be interpreted with care. In general, we did not observe the same ratios of amino acids in corn pollen that have been observed in previous research (Linskins and Pfahler, 1973; Stanley and Linskins, 1974). It should be noted that the low level of variation that we observed in the amounts of macronutrients (e.g., organic matter) does not necessarily mean that micronutrient and phytochemical constituents (e.g., proline and quercetin) are not highly variable among hybrids.

Although quercetin has been identified as being present in corn pollen (Goss, 1968), we are not aware of previous efforts to quantify this phytochemical in corn pollen. The level of quercetin in corn pollen (324 μg/g) is high relative to other plants (Wiermann, 1968, as presented in Stanley and Linskins, 1974). For comparison, quercetin contents in broccoli, apple peel, onion, and tea are 68, 230, 348, and 16 μg/g, respectively (Justesen et al., 1998), and the mean ± SD quercetin content in bee-collected pollens were 6.6 ± 1.96 μg/g (Bonvehí et al., 2001). The bitter taste of several flavonoids such as quercetin is speculated to defend pollen from herbivory (Stanley and Linskins, 1974; Harborne, 1979). It would be interesting to elucidate with further experimentation whether wind pollinated plants defend their pollens from herbivory with elevated levels of anti-feedant substances like quercetin. Finally, the variation in the abundance of this phytochemical gives credence to the hypothesis that other micronutrients more critical to the nutrition of *C. maculata* could have significant variation among the hybrids used in our study and could potentially lead to the low eclosion rates that we observed.

The slight variation in organic matter and ash content in the different pollens was strongly correlated with mortality rates in pollen-reared *C. maculata*, suggesting that some critical nutrient was at sub-optimal levels in some of the pollens. Most of the mortality in pollen-fed *C. maculata* occurred during early adulthood (Fig. 2), and more specifically during eclosion to adulthood. From our data, it is impossible to discern whether the mortality is related to critical organic constituents that are lacking in some of the pollen hybrids or to inorganic toxic substances that are more prevalent in certain hybrids. Because crude protein and amino acid levels in the pollens were not highly correlated with mortality, variation in lipid or carbohydrate components of the organic matter may be responsible for the poor eclosion rates. Sterols are organic components that could be investigated further. Insects cannot synthesize sterols, so they must rely on dietary sterols to produce critical hormones needed for ecdysis and other physiological functions (Chapman, 1998). The primary sterol in corn pollen is 24-methylene-cholesterol (59–64.5% of sterol isolates), although a number of other phytosterols are present at lower levels in corn pollen (Goss, 1968; Standifer et al., 1968). Lipid levels in corn pollen are among the lowest observed in any pollens, and sterols compose only 0.1% of the organic constituents of corn pollen (Standifer, 1966; Stanley and Linskins, 1974). Variation in major or minor sterol components may have led to the high rate of mortality during eclosion in our study. Also, it should be noted that our results support those of Pilcher et al. (1997), who found that pollen from lepidopteran-specific transgenic insecticidal corn had no deleterious effects on the fitness of pollen-fed *C. maculata* larvae. Although *C. maculata* mortality was generally the highest for larvae reared on hybrid 1, the difference was not significantly greater than for beetles fed the other hybrids; further, mortality was strongly correlated with the nutritional constituency of the pollens, rather than with potential toxicity from the insecticidal protein (Fig. 3).

We found that the consumption index decreased and the efficiency that *C. maculata* larvae convert pollen into biomass increased as larvae aged (Fig. 6). Both of these results can be explained by the fact that *C. maculata* consumed similar amounts of pollen on a daily basis over the duration of the larval stage (except during the third stadium), and gained weight at a greater rate as larvae aged. Because each stadium became progressively longer as larvae aged, the larvae had higher total pollen consumption rates during the third and fourth stadia (Fig. 5). Holometabolous insects often

grow at a faster rate in later stadia, but the observation that the ECD of *C. maculata* larvae is higher and the CI is lower during later stadia is contrary to many reports in the literature (see Scriber and Slansky, 1981, for a review). Our research suggests that there is a physiological or behavioral alteration that occurs in late-instar *C. maculata* larvae, which allows them to derive more nutrition from pollen, such that late instars are able to produce more biomass from the same amount of corn pollen. These observations do not translate to other foods for *C. maculata*; *C. maculata* larvae consume considerably more aphids as they age (up to 15 and 50 aphids per day during the first and fourth stadia, respectively; Lundgren, 2004). It is possible that poor nutrition of corn pollen relative to prey may have affected the ECD and CI. Also, in our study, within-instar variability in daily pollen consumption might reveal different consumption rates over the larval stage. For instance, fourth instars ingested a large amount of pollen on some days and no pollen on others, such that the mean daily pollen consumption over the instar was similar to the mean daily pollen consumption by the first and second instars. Our dataset unfortunately did not allow us to study within-instar variability in pollen consumption with enough precision to examine these relationships.

The results of this research are applicable to designing assays that quantify the toxicity of transgenic corn pollen to *C. maculata*. First, our research demonstrates that not all corn pollen is nutritionally equal. Future pollen-feeding assays should use only nutritionally equivalent hybrids of corn to reduce nutritional variation that could potentially lead to spurious differences in toxicity between treatments and the control. Also, it seems that there is some physiological or behavioral alteration to pollen-feeding that occurs during the larval stage of *C. maculata*. Toxicity assays should address potential alterations in susceptibility or dose ingestion over the larval stage by exposing *C. maculata* larvae to the toxic pollen for the duration of larval development. Finally, although corn pollen is a suitable food source for the larval development of *C. maculata*, it is not ideal, and under field conditions this species likely relies on corn pollen as only one component of a wider diet. Consequently, pollen-only toxicity assays should be interpreted as a worst-case scenario, with the understanding that toxic effects discovered in the laboratory should not be extrapolated to field conditions.

Acknowledgements

We thank Kristine Cruz, Russell Heinrichs, Alison Huber, Carlos Pavon, and Anthony Razzak for technical assistance; Illinois Foundation Seeds and Pioneer Hybrid International for providing the corn seed; Elizabeth Flickinger and Dan Grunloh for running the nutritional profiles of corn pollen; Craft Technologies for quantifying the quercetin in the different pollens; and Susan Fahrbach, Lee Solter, and Kevin Steffey for reviewing earlier drafts of the manuscript.

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