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# Sterol limitation in a pollen-fed omnivorous lady beetle (Coleoptera: Coccinellidae)

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ABSTRACT

Nutritional constraints of non-prey foods for entomophagous arthropods are seldom investigated, yet are crucial to understanding their nutritional ecology and function within natural and managed environments. We investigated whether pollen from five maize hybrids was of variable quality for the lady beetle, *Coleomegilla maculata*, whether suitability of these pollens was related with their sterol profiles, and how augmenting sterols (β-sitosterol, cholesterol, or ergosterol) affected the fitness and performance of *C. maculata*. Preimaginal survival, development rates, the duration of the pre-oviposition period, post-mortem adult dry weight, adult hind tibial length, sex ratio, fecundity, cohort generation time ($T_c$), net replacement rate ($R_0$) and intrinsic rate of increase ($r$) were measured. Individual sterols in the pollens were quantified using GC–MS. Pollens were of variable suitability for *C. maculata*; the development rate was positively correlated with the amount of 24-methylene-cholesterol and r was positively correlated with episterol and 24-methylene-lophenol found in the pollens. Performance of *C. maculata* was entirely unaffected by augmenting pollen meals with sterols. This research shows that pollens clearly vary in their sterol contents intraspecifically, which affects their suitability for omnivores that rely on pollen. However, sterols appear to be only one of the limiting nutrients in pollens.

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## 1. Introduction

Although best appreciated for their ability to consume insect prey, most entomophagous insects regularly consume non-prey foods and are often best described as omnivores (Coll and Guershon, 2002; Wäckers, 2005; Hunter, 2009; Lundgren, 2009b). Indeed, non-prey foods such as nectar, pollen, seeds, and fungus can be nutritionally competitive with prey in terms of their energetic contents, and gross content of carbohydrates, protein, and lipids (Lundgren, 2009b). Although some entomophagous insects can survive and reproduce entirely in the absence of prey, most species require some balance of prey and non-prey foods for optimal performance (Bernays, 1992; Behmer and Nes, 2003; Toft, 2005; Lundgren, 2009a). The nutritional constraints of plant-based foods for entomophagous insects remain poorly understood, but identifying specific nutritional deficiencies in prey and non-prey foods is critical to understanding the underlying nutritional ecology of omnivory.

Pollen is an abundant, pervasive, and nutritious food consumed by a broad range of insects (Stanley and Linskins, 1974; Wäckers, 2005; Lundgren, 2009b). Pollen is a rich source of lipids, proteins (amino acids), and carbohydrates, but interspecifically it varies widely in its nutrition, both taxonomically and according to plant life history traits (e.g., anemophilous versus zoophilous pollens) (Petanidou and Vokou, 1990; Petanidou, 2005; Petanidou et al., 2006; Praz et al., 2008). Indeed, even within a plant species, genetics and environment can produce differences in the nutritional suitability of pollen for insects (Lundgren and Wiedenmann, 2004; Karise et al., 2006; Obrist et al., 2006; Lundgren, 2009b). One nutrient that is potentially limiting in pollen is sterol, a requisite component of insect diets since most cannot synthesize the sterols essential for hormone synthesis, gene expression, and cell membrane function (Behmer and Nes, 2003). Although many pollens contain phytosterols, this nutrient could still be limiting for pollen-fed insects like predators, which may not be well-adapted to dealkylating phytosterols or adjusting their Δ5, Δ7, Δ22, and Δ24 double bonds into a form usable for the predator's physiological processes (Thompson et al., 1973; Svoboda and Robbins, 1979; Keiser and Yazlovetsky, 1988; Bernays, 1992; Svoboda et al., 1994; Behmer and Nes, 2003).

*Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) is a widespread predator throughout North American agroecosystems. In addition to its contributions to managing cropland pests such as aphids and Colorado potato beetles (Wright and Laing, 1980;

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Hazzard and Ferro, 1991; Weber and Lundgren, 2009), *C. maculata* consumes a range of pollens. Their omnivorous habits are well documented (Lundgren et al., 2004, 2005; Michaud and Grant, 2005; Moser et al., 2008), and they can complete development and reproduce on a diet composed solely of pollen and water (Lundgren and Wiedenmann, 2004). Omnivory on maize plants and pollen by this species has made it a model organism for studying the ecological impacts of GM crops on natural enemies (Pilcher et al., 1997; Duan et al., 2002; Lundgren and Wiedenmann, 2002; Moser et al., 2008). One outcome of research on this study system is that maize pollens from different hybrids vary in their nutrition, and that *C. maculata* survival is positively correlated with organic matter (and negatively correlated with ash content) in the different pollens (Lundgren and Wiedenmann, 2004). Amino acid and quercetin (a bitter flavonoid pigment responsible for maize pollen's yellow color) levels in these different pollens were not responsible for their relative suitabilities (Lundgren and Wiedenmann, 2004). In this paper, we (1) establish intraspecific variability in the suitability of different maize pollens for performance (e.g., development, survival, weight, etc.) and fitness (e.g., reproductive capacity and population growth) of *C. maculata*, (2) correlate the performance and fitness of *C. maculata* with the sterol contents of the pollens in their diets, and (3) determine whether augmenting sterols in pollen-only diets improves their suitability for *C. maculata*.

## 2. Methods

### 2.1. Insects and plants

*C. maculata* were collected in Beltsville, MD and were reared continuously for at least four months on a mixture of pollen substitute (Bee-Pro®, Mann Lake LTD, MN, USA), eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae), dried freshwater amphipods (*Gammarus lacustris* G.O. Sars, Tetra Holding, Inc, Blacksburg, VA), and occasional aphid meals as they were available (*Aphis glycines* Matsumura and *Rhopalosiphum rufiabdominalis* [Sos]; Hemiptera: Aphididae). Unpublished research has shown that *E. kuehniella*, *A. glycines*, and *R. rufiabdominalis* were nutritionally equivalent for performance and fitness of *C. maculata* (Pilorget, unpubl. data). The colony and all experiments described below were maintained at 28 °C, 16:8 h light:dark, and 35–45% relative humidity.

Five non-transgenic, commercially competitive maize hybrids were grown concurrently in a greenhouse: NB4703 (Monsanto Company, St. Louis, MO), NK4242 (Northrup King Company, Golden Valley, MN), DKC57-30 (DeKalb Seeds, Monsanto Company, St. Louis, MO), Pioneer 3730 (Pioneer Hi-bred International, Johnston, IA), and LH330 × LH273 (Illinois Foundation Seeds, Champaign, IL). Two plantings of LH330 × LH273 plants were made (one in 2006, one in 2007) because the LH330 × LH273 (2006) was completely used in the pollen suitability experiment. All maize plants were grown in 3.8 L pots (two plants per pot), watered twice daily, and fertilized weekly with 1.88 ml of 20–20–20 (N–P–K; Plantex, Plant Product Co., Brampton, Ontario, Canada) fertilizer and 0.63 ml of chelated Fe (Sequestrene 330 Fe, Becker-Underwood, Inc, Ames, IA, USA) in 0.5 L of water. As tassels were exposed, they were covered in a paper bag to collect the pollen. Bags were changed each morning during anthesis, and contaminants were separated from the pollen with a sieve (180 μm, U.S.A. Standard Testing Sieve No. 80, Fisher Scientific, Pittsburgh, PA, USA) and stored in a sealed plastic vial at −80 °C until use.

### 2.2. Lady beetle performance on pollens

Intraspecific variability in pollen suitability for *C. maculata* fitness and performance was experimentally assessed. Neonates ($N$ = 140; distributed randomly from clutches of approximately 30 mothers) were allowed to consume their chorions, and aside from a water-saturated wick, were otherwise starved for 24 h prior to being randomly assigned to one of seven dietary treatments. Larvae in the dietary treatments ($n$ = 20 per treatment) were fed one of five maize pollens, LH330 × LH273 (2006) pollen and *E. kuehniella* eggs (70:30, pollen:eggs by weight), or were given only water. Larvae were reared individually in covered plastic cups (33 ml volume, Georgia Pacific, Dixie Business, Norwalk, CT) which were checked daily for survival and development. Cups were changed (along with food) approximately every 48 h, and water was offered continuously to all larvae in the form of a water-saturated cotton wick. Upon eclosion, all adults were transferred to a uniform diet of pollen substitute and *E. kuehniella* eggs (70:30 by weight), and water. This was done to mimic the adult's ability to disperse from maize fields in order to self-select optimal diets under field conditions. Adults were transferred to a mating chamber after all females had eclosed, and were allowed to mate with males of their treatment for 24 h. Females were then separated and allowed to oviposit for 4 d after their first clutch before being frozen. Previous experience with this study system suggested that not all matings are successful (J.G.L., pers. observation), and we sacrificed the ability to associate individual females with their larval data in favor of increasing the likelihood of equitable mating compared to that sometimes experienced with individual pairings (e.g., if a female is unintentionally provided with a sterile or unenthusiastic male). Post-mortem, the adults were dried at 60 °C in an oven. The duration of each larval stadium, durations of the entire larval and pupal stages, preimaginal survival rate, hind tibial length (of the adult stage), pre-oviposition period, 5 d fecundity, and post-mortem adult dry weight were recorded for each individual.

From these life history parameters, we estimated population growth metrics for each treatment using the model parameters presented by Price (1975). Specifically, the age specific survivorship at reproduction ($l_x$), and the proportion female ($m_x$) was calculated for each treatment. The net replacement rate ($R_0$) for each treatment was calculated as $l_x \times m_x \times 5$ d fecundity for each female; it was assumed that all eggs were fertilized. Total cohort generation time ($T_c$) was calculated for each treatment by adding the mean pre-oviposition time to the mean development period for each treatment. From this information, the instantaneous rate of population growth rate ($r$) was calculated as $\mathrm{Ln}\, R_0/T_c$.

### 2.3. Sterol contents of the maize pollens

The mean sterol types and levels were identified in each pollen produced. For each hybrid, the pollen from 8 to 10 plants was consolidated and mixed thoroughly. Sterols were extracted independently from three randomly collected samples (0.1 g each) of each pollen stock. The pollen was germinated in 10 ml of a solution (10% sucrose, 0.1% boric acid; Linskins and Schrauwen, 1969) until 90% of pollen had germinated (approximately 2.5 h). The germination solution was dried under $N_2$, and lipids were extracted in 50 ml of chloroform:methanol (80:20) for 10 h. The chloroform:methanol was evaporated off, and the resulting lipids were saponified in 14 ml of 45% ethanolic KOH solution for 3 h. Water (20 ml) was added to the mixture, which was then rinsed with hexane repeatedly until the organic phase of the mixture was clear. The organic phase of the solution was then rinsed in 10% ethanol until the pH had stabilized (approximately 8.5–9.5). The solution was evaporated, and the remaining saponified lipids were subjected to GC–MS.

Pollen samples were analyzed for sterol content by GC–MS. Germinated and saponified maize pollen extracts were dissolved in a known volume of chloroform and 1 μl portions were injected into a Hewlett Packard Model 5890A gas chromatograph equipped with a temperature- and pressure-programmable on-column

injector (Agilent Technologies, Palo Alto, CA, USA) and a 1 m retention gap, connected to a J&W Scientific DB-1MS capillary column (0.2 mm × 12.5 m, 0.33 μm phase thickness) (Folson, California). The GC was coupled to an HP 5970B quadrupole mass selective detector (Agilent Technologies, Palo Alto, CA, USA). The helium carrier gas flow was a constant 0.75 ml/min. The column temperature was initially held at 150 °C for 4 min, programmed to 320 °C at 4 °C/min with a final hold of 15 min. The quantification data was calculated using standard curves, with $C_{28}$ n-alkane as authentic standard. The data from 0 to 6.25ng was a straight line, 6.25 to 100 ng was a first order polynomial, and above 100 ng was a straight line. Standards were run after the samples each day.

Structural identification of pollen sterols was confirmed by analyzing sterol constituents as their tri-methyl silyl ester derivatives. A portion of each pollen extract was transferred to a cone shaped GC–MS vial with chloroform and the solvent evaporated with $N_2$ gas. Dry benzene was added, followed by N,O-*bis*(trimethylsilyl)-acetamide (BSA), and dimethylformamide at a volume ratio of 20:20:60, respectively. The mixture was heated at 72 °C for 20 min, cooled and 1 μl injected into the GC/MS using the same column and conditions listed above.

### 2.4. Sterol augmentation and lady beetle performance

The ability of commercially available sterols to improve the suitability of two pollens (NK4242 and LH330 × LH273 [2007]) for *C. maculata* was assessed. Neonate *C. maculata* ($N = 330$; assigned randomly from clutches of approximately 30 mothers) were allowed to consume their chorions, but were otherwise starved (aside from water) for 24 h prior to the assay. Larvae were then randomly assigned to one of 11 dietary treatments ($n = 30$ per treatment). In addition to a starved treatment that received only water, a series of five treatments was devoted to each of the two pollens. These treatments were pollen alone, pollen + β-sitosterol (S1270, Sigma–Aldrich, St. Louis, MO), pollen + cholesterol (C8867, Sigma–Aldrich), pollen + ergosterol (45480, Sigma–Aldrich), and pollen + *E. kuehniella* eggs (70:30 by weight). For each of the pollen + sterol treatments, a mixture of 0.1% sterol by weight was mixed with the pollen (a commonly reported required level in insect diets; Bernays, 1992). Larvae were reared individually in 33 ml covered plastic cups, and were checked daily for survival and development. Rearing cups and food were changed approximately every 48 h, and upon eclosion the adults were fed 70:30 pollen substitute: *E. kuehniella* eggs. The same life history parameters were measured as described for the previous assay.

### 2.5. Data analysis

In the pollen suitability assay, the percentages of larvae that survived to adulthood were compared among treatments using contingency table analysis (Systat 11); a separate analysis was performed on the pollen-only treatments. The mean durations of each stadium, combined preimaginal stages, pre-oviposition periods, cohort generation times ($T_c$), 5 d fecundities, net replacement rates ($R_0$), and intrinsic rates of increase ($r$) were compared among treatments using the Kruskal–Wallis non-parametric ANOVA (means were separated with non-parametric Mann–Whitney $U$ pairwise comparisons) (SYSTAT Software, 2004). Mean post-mortem dry weights of females were compared among treatments using ANOVA; a two-factor ANOVA (treatment and sex as factors) was used to compare hind tibial lengths of males and females among treatments; means were separated using LSD comparisons (SYSTAT Software, 2004). The mean quantities of total sterols, 24-Methylene cholesterol (22,23 Methylene Cholestene-3β-ol), episterol (Cholesta-7,24-dien-3-ol), and 24-Methylene lophenol in the five pollens were related to *C. maculata* larval duration, preimaginal survival, pre-oviposition period, and fecundity using regression analyses of general linear models (independent for each sterol-life history parameter contrast).

In the sterol augmentation assay, similar approaches were used to compare treatments, with some exceptions. Preimaginal survivorship rates and sex ratios were compared among NK4242 and LH330 × LH273 (2007) treatments using independent two-way contingency table analyses. For the NK4242 treatments, an additional analysis compared the no-prey treatments. Mean duration of preimaginal stages, pre-oviposition periods, cohort generation times ($T_c$), net replacement rates ($R_0$), and intrinsic rates of increase ($r$) were compared among the treatments using a two-factor ANOVA (with pollen and treatment as factors). The durations of each stadia in treatments fed NK4242 pollen were compared using independent Kruskal–Wallis non-parametric ANOVAs. The effect of treatment on hind tibial lengths of male and females were compared with ANOVA (with pollen, treatment, and sex as factors). Mean female post-mortem dry weights were compared among treatments using two-factor ANOVA (with pollen and treatment as factors).

## 3. Results

### 3.1. Lady beetle performance on pollens

The percentage of larvae that survived to adulthood were 63 (NB4703), 85 (NK4242), 74 (DKC57-30), 58 (Pioneer 3730), 60 (LH330 × LH273; 2006), 100 (prey + pollen treatment), and 0% (starved treatment) (Pearson $\chi^2_6 = 50.32$, $P < 0.001$). When the prey + pollen and unfed treatments were removed from analysis (leaving the pollen treatments), the proportions of individuals that survived until adulthood were statistically similar (Pearson $\chi^2_4 = 4.63$, $P = 0.32$).

The durations of development varied substantially among the treatments, with larvae fed prey + pollen developing significantly faster than the pollen-fed treatments (Table 1). The 4th stadia in the no-prey treatments varied significantly in duration ($\chi^2_4 = 12.09$,

**Table 1**
Life history parameters of *Coleomegilla maculata* reared on pollens from five different maize hybrids or maize pollen (LH330 × LH273 [2006]) + *Ephestia kuehniella* eggs (pollen suitability experiment). All values represent mean ± SEM; sample size is indicated in parentheses. Values within columns followed by different letters are significantly different from one another ($\alpha = 0.05$).

| Treatment | Development time (d) | Pre-oviposition period[a] | Cohort generation time ($T_c$) | 5 d fecundity (eggs) | $R_0$ | $r$ (×100) |
|---|---|---|---|---|---|---|
| Prey + pollen | 11.95 ± 0.18 (20)a | 15.50 ± 1.33b (8) | 27.45 ± 1.33 | 31.00 ± 5.70 | 248.00 ± 45.56 | 19.92 ± 1.39a |
| NB4703 | 15.50 ± 0.65 (10)bc | 12.00 ± NAab (1) | 27.50 ± NA | 50.00 ± NA | 50.10 ± NA | 14.23 ± NA ab |
| NK4242 | 14.93 ± 0.35 (14)bc | 16.83 ± 1.59b (6) | 31.76 ± 1.59 | 28.50 ± 7.50 | 171.01 ± 45.00 | 15.15 ± 1.62b |
| DKC 57-30 | 14.31 ± 0.31 (13)b | 14.78 ± 3.18ab (4) | 25.39 ± 4.32 | 26.67 ± 10.49 | 80.01 ± 46.08 | 15.92 ± 2.10ab |
| Pioneer 3730 | 15.36 ± 0.24 (11)c | 10.80 ± 0.41a (4) | 26.16 ± 0.41 | 33.25 ± 6.33 | 116.37 ± 22.15 | 18.01 ± 0.90ab |
| LH330 × 273 (2006) | 15.09 ± 0.48 (11)bc | 16.25 ± 1.50ab (2) | 31.34 ± 1.50 | 31.50 ± 3.50 | 62.94 ± 6.99 | 13.21 ± 0.28ab |
| | $\chi^2_5 = 45.21$, $P < 0.001$ | $\chi^2_5 = 12.38$, $P = 0.03$ | $\chi^2_5 = 9.47$, $P = 0.09$ | $\chi^2_5 = 2.72$, $P = 0.74$ | $\chi^2{}_5 = 9.19$, $P = 0.10$ | $\chi^2{}_5 = 12.26$, $P = 0.03$ |

[a] Sample sizes for all columns to the right of this column are indicated here in parentheses.

**Table 2**
Weight and size for *Coleomegilla maculata* reared on different maize pollen treatments. Eggs of *Ephestia kuehniella* were used as prey in the indicated treatments. Letters within a column indicate significantly different means ($\alpha = 0.05$), and comparisons are segregated by experiment, and by pollen within the sterol augmentation experiment.

| Treatment | Female dry wt (mg) | Female tibial length (mm) | Male tibial length (mm) |
|---|---|---|---|
| *Pollen suitability experiment* | | | |
| Prey + pollen | 10.01 ± 0.29 (8)a | 1.82 ± 0.02 (8)a | 1.74 ± 0.02 (12)a |
| NB4703 | 7.60 ± NA (1)ab | 1.79 ± 0.07 (3)ab | 1.69 ± 0.04 (7)ab |
| NK4242 | 6.23 ± 0.34 (6)c | 1.71 ± 0.07 (8)ac | 1.68 ± 0.02 (9)ab |
| DKC 57-30 | 6.08 ± 0.55 (4)c | 1.74 ± 0.02 (10)ac | 1.69 ± 0.05 (4)ab |
| Pioneer 3730 | 5.88 ± 0.31 (4)c | 1.68 ± 0.05 (5)bc | 1.61 ± 0.04 (6)b |
| LH330 × LH273 (2006) | 6.85 ± 1.35 (2)bc | 1.61 ± 0.05 (5)c | 1.62 ± 0.04 (7)b |
| *Sterol augmentation experiment* | | | |
| NK4242 (pollen only) | 6.11 ± 0.61 (6) | 1.78 ± 0.07 (5)ab | 1.74 ± 0.07 (8)a |
| NK4242 + β-sitosterol | 6.20 ± 0.45 (9) | 1.77 ± 0.31 (9)ab | 1.69 ± 0.07 (6)a |
| NK4242 + cholesterol | 7.06 ± 0.51(9) | 1.75 ± 0.04 (9)a | 1.63 ± 0.04 (6)a |
| NK4242 + ergosterol | 6.83 ± 0.46(10) | 1.79 ± 0.05 (10)ab | 1.74 ± 0.04 (4)a |
| NK4242 + prey | 7.23 ± 0.53(11) | 1.87 ± 0.02 (12)b | 1.75 ± 0.03 (13)a |
| LH330 × LH273 (2007) (pollen only) | 6.04 ± 0.44 (12) | 1.78 ± 0.04 (12)a | 1.76 ± 0.02 (9)b |
| LH330 × LH273 + β-sitosterol | 6.43 ± 0.53 (7) | 1.74 ± 0.07 (7)a | 1.66 ± 0.02 (13)a |
| LH330 × LH273 + cholesterol | 6.64 ± 0.52 (9) | 1.76 ± 0.04 (9)a | 1.68 ± 0.02 (10)a |
| LH330 × LH273 + ergosterol | 7.03 ± 0.45(9) | 1.81 ± 0.04 (12)a | 1.69 ± 0.04 (9)ab |
| LH330 × LH273 + prey | 7.41 ± 0.52(9) | 1.82 ± 0.02 (11)a | 1.77 ± 0.02 (9)b |

$P = 0.02$); 1st through 3rd instars fed the different pollens developed equally fast (1st instar: $\chi^2_4 = 1.58$, $P = 0.81$; 2nd instar: $\chi^2_4 = 2.41$, $P = 0.66$; 3rd instar: $\chi^2_4 = 7.56$, $P = 0.11$). Specifically, 4th instars fed pollens of NK4242 and C57-30 had significantly shorter development than those fed pollens of LH330 × LH273 (2006) and NB4703 (those fed Pioneer 3730 developed at an intermediate rate). Among the no-prey treatments, larvae fed DKC57-30 developed significantly faster than those fed Pioneer 3730 (Table 1).

Size was significantly different among the treatments, and some pollen-fed adults were similarly sized to the prey + pollen-fed larvae (Table 2). Adult female dry weights were significantly affected by treatment (treatment: $F_{5,\ 19} = 18.92$, $P < 0.001$) (Table 2). Specifically, females reared on prey + pollen were heavier than females fed pollen only, except those fed NB4703 pollen. Females reared on NB4703 were heavier than all other pollen-fed treatments except those fed LH330 × LH273 (2006). Tibial length of males and females were affected similarly by dietary treatment, and males were smaller than females (treatment: $F_{5,\ 50} = 5.06$, $P < 0.001$; sex: $F_{1,\ 50} = 5.18$, $P = 0.03$; treatment × sex: $F_{5,\ 50} = 0.47$, $P = 0.80$) (Table 2). Male and female hind tibial lengths were statistically similar within the pollen-only treatments. Beetles fed pollen + prey had longer hind tibiae than those fed Pioneer 3730 or LH330 × LH273 (2006); all other no-prey treatments were similar to the treatment fed prey + pollen. Sex ratios were equivalent in the treatments, and can be derived for each treatment from Table 2 (Pearson $\chi^2_5 = 0.83$, $P = 0.98$).

Reproduction was also affected by larval diet. Pre-oviposition period differed significantly among the treatments (Table 1). Females fed Pioneer 3730 pollen had a shorter pre-oviposition period than those fed NK4242. There was no effect of treatment on the 5 d fecundity, the cohort generation time ($T$) or on the populations' net reproduction rate ($R_0$) (Table 1). There was an effect of dietary treatment on the intrinsic rates of increase in the different cohorts, with *C. maculata* populations fed prey + pollen having a significantly higher growth rate than pollen-only treatments, except the population fed NK4242 pollen (Table 1).

### 3.2. Correlations between lady beetle performance and sterol contents

Three dominant sterols were consistently identified in the six maize pollens: 24-methylene cholesterol (22, 23-Methylenecholestene-3β-ol; major mass spectral fragment ion at $m/z$ 314, other diagnostic ions at $m/z$ 229, 271 and 293), episterol (Cholesta-7, 24-dien-3-ol; major ion at $m/z$ 271, others at $m/z$ 299, 314, 398, 412), and 24-methylene lophenol (major ion at $m/z$ 285, others at $m/z$ 328, 397, and 412) (Table 3). Structural identifications of the three major sterols were confirmed by GC–MS analyses of their trimethyl silyl (TMS) derivatives. Mass spectra for 24-methylene cholesterol and episterol showed a predicted molecular ion ($M^+$) at $m/z$ 470 and prominent M-15 ions at 455, with diagnostic fragment ions at $m/z$ 296, 341, 386 for 24-methylene cholesterol and $m/z$ 343 and 386 for episterol. The mass spectrum of the TMS derivative for 24-methylene lophenol was characterized by $M^+$ ion at 484, a M-15 ion at $m/z$ 469 and diagnostic ions at $m/z$ 357 and 400.

Larval development times and intrinsic rates of population increase ($r$) of the different treatments were strongly correlated with the sterol contents of the pollens. Larval development times were significantly positively associated with the levels of 24-methylene cholesterol in the pollens (Fig. 1a; Table 4). The intrinsic rates of population increase ($r$) were strongly and positively correlated with both episterol and 24-methylene lophenol contents in the pollens (Fig. 1b; Table 4). The total sterol contents

**Table 3**
Major sterol contents (μg sterol $g^{-1}$ pollen ± SEM)[a] of the six pollens used in the two experiments, as determined by GC–MS.

| | Sample size | 24-Methylene cholesterol | Episterol | 24-Methylene lophenol | Total Sterols |
|---|---|---|---|---|---|
| NB4703 | 3 | 0.8 ± 0.2 | 2.6 ± 0.2 | 3.2 ± 0.1 | 8.3 ± 0.6 |
| NK4242 | 2 | 1.2 ± 0.6 | 3.9 ± 0.9 | 4.5 ± 0.6 | 11.6 ± 2.6 |
| DKC 57-30 | 3 | 1.3 ± 0.3 | 3.6 ± 0.5 | 4.0 ± 0.6 | 10.5 ± 0.2 |
| Pioneer 3730 | 3 | 0.7 ± 0.2 | 4.5 ± 0.9 | 5.5 ± 1.2 | 12.0 ± 1.6 |
| LH330 × LH273 (2006) | 2 | 0.5 ± 0.0 | 1.9 ± 0.1 | 1.9 ± 0.2 | 5.1 ± 0.3 |
| LH330 × LH273 (2007) | 2 | 1.3 ± 0.3 | 4.4 ± 0.7 | 4.2 ± 0.6 | 11.7 ± 2.1 |

[a] Microgram values were calculated from the quantities of sterols as determined from the GC–MS integrated peak area for each component and standard curves for the authentic standard as described in Section 2.

**Table 4**
Regression analyses of sterol contents of the five maize pollens, and means of the various indicated life history parameters of *Coleomegilla maculata* raised on the pollens. Statistically significant relationships are indicated in bold italics.

| | 24-Methylene cholesterol | Episterol | 24-Methylene lophenol | Total sterols |
|---|---|---|---|---|
| Larval duration | $\mathbf{F_{1,\ 3} = 14.82;\ P = 0.03}$ | $F_{1,\ 3} = 0.44;\ P = 0.56$ | $F_{1,\ 3} = 0.15;\ P = 0.72$ | $F_{1,\ 3} = 0.70;\ P = 0.46$ |
| $T_c$ | $F_{1,\ 3} = 0.18;\ P = 0.70$ | $F_{1,\ 3} = 0.67;\ P = 0.47$ | $F_{1,\ 3} = 0.79;\ P = 0.44$ | $F_{1,\ 3} = 0.56;\ P = 0.51$ |
| Larval survival | $F_{1,\ 3} = 7.05;\ P = 0.07$ | $F_{1,\ 3} = 0.34;\ P = 0.60$ | $F_{1,\ 3} = 0.14;\ P = 0.73$ | $F_{1,\ 3} = 0.74;\ P = 0.45$ |
| Total survival | $F_{1,\ 3} = 2.49;\ P = 0.21$ | $F_{1,\ 3} = 1.07;\ P = 0.38$ | $F_{1,\ 3} = 0.70;\ P = 0.47$ | $F_{1,\ 3} = 1.70;\ P = 0.28$ |
| Tibial length | $F_{1,\ 3} = 1.67;\ P = 0.29$ | $F_{1,\ 3} = 0.17;\ P = 0.71$ | $F_{1,\ 3} = 0.23;\ P = 0.67$ | $F_{1,\ 3} = 0.52;\ P = 0.52$ |
| 5 d fecundity | $F_{1,\ 3} = 0.40;\ P = 0.57$ | $F_{1,\ 3} = 0.43;\ P = 0.56$ | $F_{1,\ 3} = 0.16;\ P = 0.72$ | $F_{1,\ 3} = 0.25;\ P = 0.65$ |
| $r$ | $F_{1,\ 3} = 0.20;\ P = 0.69$ | $\mathbf{F_{1,\ 3} = 17.22;\ P = 0.03}$ | $\mathbf{F_{1,\ 3} = 22.26;\ P = 0.02}$ | $F_{1,\ 3} = 7.58;\ P = 0.07$ |

of the pollens were marginally correlated with $r$. $T_c$, survival, female hind tibial length, and fecundity of the five pollen-fed cohorts were unassociated with pollen sterol content (Table 4).

### 3.3. Sterol augmentation and lady beetle performance

There were significant differences in the preimaginal survivorship of treatments fed NK4242 pollen when the pollen + prey treatment was included in the analysis (Pearson $\chi^2_4 = 12.10$, $P = 0.02$); there were no differences in the survival of the four treatments that received NK4242 pollen but no prey (Pearson $\chi^2_3 = 1.01$, $P = 0.80$). There were no differences in the preimaginal survival rates of treatments fed pollen of LH330 × LH273 (2007) (Pearson $\chi^2_4 = 0.14$, $P = 0.99$). Specifically, percent of individuals that survived to eclosion were 45 (NK4242 pollen only), 50 (NK4242 pollen + β-sitosterol), 57 (NK4242 pollen + cholesterol), 47 (NK4242 pollen + ergosterol) and 83% (NK4242 pollen + prey). Preimaginal survival rates for those fed pollen of LH330 × LH273 (2007) were 70.00 (pollen only), 67 (pollen + β-sitosterol), 69 (pollen + cholesterol), 67 (pollen + ergosterol), and 67% (pollen + prey).

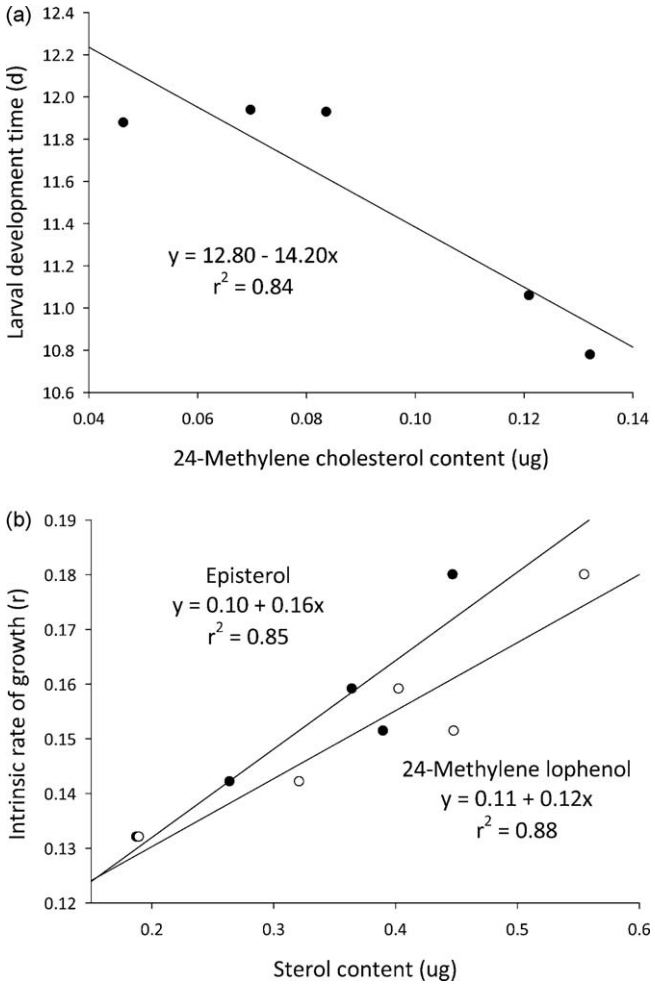


**Fig. 1.** Linear associations of mean *Coleomegilla maculata* development (a) and population growth rates (b) fed one of five different maize pollens and the mean sterol contents of each pollen. Each symbol represents the treatment mean of *C. maculata* fed a different maize pollen, and the respective sterol contents of that pollen. Supporting statistical analyses can be found in Table 4.

There was a consistent and significant effect of treatment on development rate, largely driven by the relatively fast development of the treatment offered both pollen and *E. kuehniella* eggs (pollen: $F_{1,\ 236} = 0.16$, $P = 0.69$; treatment: $F_{4,\ 236} = 10.01$, $P < 0.001$, pollen × treatment: $F_{4,\ 236} = 16.61$, $P = 0.06$) (Table 5). Of the treatments fed NK4242 pollen, all but 4th instars developed at significantly different rates (1st instar: $\chi^2_4 = 9.37$, $P = 0.05$; 2nd instar: $\chi^2_4 = 19.45$, $P = 0.001$; 3rd instar: $\chi^2_4 = 21.67$, $P < 0.001$; 4th instar: $\chi^2_4 = 6.20$, $P = 0.19$). In the 1st stadium, the larvae in the pollen-only treatment developed significantly slower than the prey + pollen and pollen + cholesterol treatments. Similarly, 2nd instars in the pollen-only treatment took longer to develop than those fed pollen + β-sitosterol, pollen + ergosterol, and pollen + prey; 2nd instars fed pollen + prey also developed significantly faster than those fed pollen + cholesterol. 3rd instars reared on prey + pollen developed significantly faster than 3rd instars in all of the no-prey treatments. There were no differences among development rates of individual instars fed the LH330 × LH273 (2007) pollen treatments with no prey. Pre-oviposition period was statistically similar amongst all of the treatments (pollen: $F_{1,\ 66} = 0.55$, $P = 0.46$; treatment: $F_{4,\ 66} = 0.73$, $P = 0.58$, pollen × treatment: $F_{4,\ 66} = 0.36$, $P = 0.84$) (Table 5). There also were no differences among treatments in $T_c$ (pollen: $F_{1,\ 66} = 0.73$, $P = 0.40$; treatment: $F_{4,\ 66} = 0.69$, $P = 0.60$, pollen × treatment: $F_{4,\ 66} = 0.88$, $P = 0.48$), $R_0$ (pollen: $F_{1,\ 64} = 0.36$, $P = 0.55$; treatment: $F_{4,\ 64} = 0.29$, $P = 0.89$, pollen × treatment: $F_{4,\ 64} = 1.47$, $P = 0.22$), or r (pollen: $F_{1,\ 65} = 0.41$, $P = 0.52$; treatment: $F_{4,\ 65} = 0.35$, $P = 0.84$, pollen × treatment: $F_{4,\ 65} = 0.38$, $P = 0.82$) (Table 5).

Hind tibial lengths were significantly affected by treatment and sex, and the two pollens produced beetles with similar hind tibial lengths (pollen: $F_{1,\ 63} = 0.09$, $P = 0.77$; treatment: $F_{4,\ 163} = 4.26$, $P = 0.003$; sex: $F_{1,\ 163} = 16.91$, $P < 0.001$; pollen × treatment: $F_{4,\ 163} = 0.36$, $P = 0.84$; pollen × sex: $F_{1,\ 163} = 0.21$, $P = 0.65$; treatment × sex: $F_{4,\ 163} = 0.37$, $P = 0.83$; pollen × treatment × sex: $F_{4,\ 163} = 0.46$, $P = 0.77$). Of the treatments fed NK4242 pollen, those fed pollen + cholesterol had significantly shorter hind tibiae than those fed pollen + prey (all other treatments were equivalent). Of the treatments fed LH330 × LH273 (2007) pollen, those fed pollen only and pollen + prey had significantly longer tibiae than those fed pollen + β-sitosterol or pollen + cholesterol. Females had significantly longer hind tibiae than males. There were no significant effects of pollen or treatment on the adult dry weights of females (pollen: $F_{1,\ 81} = 0.01$, $P = 0.94$; treatment: $F_{4,\ 81} = 1.90$, $P = 0.11$; pollen × treatment: $F_{4,\ 81} = 0.15$, $P = 0.96$) (Table 2). Sex ratios of treatments fed NK4242 pollen were equivalent (Pearson $\chi^2_4 = 3.76$, $P = 0.44$), as were those of treatments fed

**Table 5**
The effects of augmenting sterols of two maize pollens on life history parameters of *Coleomegilla maculata* (sterol augmentation experiment). Eggs of *Ephestia kuehniella* were used as prey in the indicated treatments. Values represent mean ± SEM; numbers in parentheses indicate sample sizes. Statistically different means in a column within a pollen are followed by different letters ($\alpha = 0.05$).

| Pollen | Treatment | Development time (d) | Pre-oviposition period (d) | Cohort generation time ($T_c$) | 5-d fecundity (eggs)[a] | $R_0$ | $r$ (×100) |
|---|---|---|---|---|---|---|---|
| NK4242 | Pollen only | 15.62 ± 0.63(13)b | 5.67 ± 0.76 (6) | 22.20 ± 0.24 (5) | 38.00 ± 6.40 (5) | 190.00 ± 31.98 | 23.38 ± 0.83 |
| | Pollen + β-sitosterol | 14.53 ± 0.29(15)b | 6.43 ± 0.72 (7) | 20.87 ± 0.72 (7) | 36.00 ± 9.45 (7) | 252.00 ± 66.13 | 25.30 ± 2.18 |
| | Pollen + cholesterol | 15.31 ± 0.44(16)b | 11.33 ± 3.60 (9) | 26.22 ± 3.60 (9) | 33.11 ± 0.47 (9) | 298.00 ± 52.25 | 23.83 ± 2.73 |
| | Pollen + ergosterol | 14.86 ± 0.33(14)b | 9.33 ± 2.20 (9) | 24.23 ± 2.20 (9) | 35.33 ± 7.20 (9) | 318.00 ± 64.83 | 24.22 ± 2.61 |
| | Pollen + prey | 13.52 ± 0.10(25)a | 6.44 ± 1.59 (9) | 20.02 ± 1.59 (9) | 37.22 ± 5.40 (9) | 335.00 ± 48.55 | 30.08 ± 2.72 |
| LH330 × LH273 (2007) | Pollen only | 15.10 ± 0.24(21)b | 7.78 ± 1.80 (9) | 23.11 ± 1.81 (9) | 30.89 ± 5.33 (9) | 278.00 ± 47.99 | 24.06 ± 1.83 |
| | Pollen + β-sitosterol | 15.25 ± 0.36(20)b | 8.57 ± 2.72 (7) | 24.00 ± 2.72 (7) | 47.71 ± 7.30 (7) | 334.00 ± 51.09 | 25.57 ± 2.97 |
| | Pollen + cholesterol | 14.65 ± 0.25(20)b | 9.43 ± 2.43 (7) | 23.83 ± 2.86 (6) | 38.17 ± 7.16 (6) | 229.00 ± 42.97 | 24.05 ± 2.86 |
| | Pollen + ergosterol | 15.10 ± 0.31(21)b | 9.38 ± 2.85 (8) | 24.79 ± 2.85 (8) | 30.75 ± 8.82 (8) | 246.00 ± 70.60 | 22.74 ± 2.30 |
| | Pollen + prey | 13.50 ± 0.11(20)a | 9.80 ± 2.99 (5) | 23.44 ± 2.99 (5) | 39.20 ± 8.15 (5) | 196.00 ± 40.72 | 23.42 ± 3.36 |

[a] Sample sizes in columns to the right of this column are indicated here.

LH330 × LH273 (2007) (Pearson $\chi^2_4 = 2.90$, $P = 0.58$); sex ratios for specific treatments can be derived from Table 2.

## 4. Discussion

This research reveals a clear correlation between the type and quantity of pollen sterols and the intrinsic rate of increase and larval development rate of lady beetle larvae reared on them. Low quantities of sterols were found in maize pollens, and the most abundant sterols were different from those that have previously been reported from this species. In spite of apparent sterol limitation in maize pollen, augmenting sterol quantities did not consistently improve *C. maculata* performance. Thus, sterols are likely only one of the nutritional limitations facing entomophagous insects when they rely on pollen to support larval development, and this knowledge has implications for understanding the nutritional ecology underlying omnivory.

Maize pollen was found to be very low in sterols; maximum total sterols were 0.0012% by weight and most insects require at least 0.1% of their diet to be comprised of sterols (Bernays, 1992; Cohen, 2004). Clearly, an exclusive diet of maize pollen is a poor source of dietary sterols. Other research has uncovered primarily 24-methylene-cholesterol in pollen from *Z. mays* (Standifer et al., 1968; Knights and Smith, 1976). In our study, 24-methylene-cholesterol was the third most abundant sterol in maize pollen, comprising <10% of total sterol content. Rather, episterol and 24-methylene-lophenol were more abundant. Differences in our findings from previous research may be related to the methods we used to germinate the pollen, thereby releasing its internal nutrients as would happen within an insect's stomach (Haslett, 1983; Human and Nicolson, 2003). Other studies (Standifer et al., 1968; Knights and Smith, 1976) did not explicitly state that they germinated the pollen prior to saponification of the lipids, and may have isolated primarily surface sterols from the possibly unbroken pollen exine.

Because maize pollen was low in sterols, small variations in sterol content among pollens were found to be strongly correlated with *C. maculata* performance; these relationships were only fully apparent after simultaneously examining developmental time, survival, and reproduction in the different treatments. The development rate of *C. maculata* was strongly correlated with the amount of 24-methylene-cholesterol present in the pollens, and the intrinsic rates of increase measured in the different treatments were strongly correlated with the amount of episterol and 24-methylene-lophenol present in the pollens (Table 4, Fig. 1). This suggests that *C. maculata* is able to use phytosterols in its life processes either directly or by converting them to cholesterol, as has been observed in some other phytophagous and omnivorous insects (Bernays, 1992; Keiser and Yazlovetsky, 1988; Thompson et al., 1973). The only other coccinellid to have been studied in respect to sterol utilization is the omnivorous *Coccinella septempunctata* L. (Svoboda and Robbins, 1979); this insect presumably did not convert dietary sterols, and was largely composed of sterols that were directly derived from prey. A similar pattern was seen in the predatory larvae of lacewings (Keiser and Yazlovetsky, 1988). Patterns in the relationships found in the present study were not easily seen when examining individual life history parameters (e.g. development rates, adult weight and size, fecundity, etc.; aside from the 24-methylene-cholesterol/development rate relationship). While measuring treatment effects on numerous individual life history parameters often is a useful process, the present study makes a strong case for conducting laboratory assays whose aim is to measure the relative quality or toxicity of different diets within the overall context of comparing comprehensive life history parameters (such as $r$) of the various treatments. A final point worth making is that the sample sizes in some of the life history parameters were small, and this may have reduced the precision of our estimates; it is possible that other life history parameters are also correlated with sterol contents of the pollens.

In spite of maize pollen's clear sterol limitation, augmenting pollen-only diets with one of three sterol sources did not improve *C. maculata* performance. Mixing prey with pollen diets improved developmental rates over the no-prey diets for both NK4242 and LH330 × 273 (2007) pollen-fed treatments, suggesting nutrient limitation in the no-prey treatments that was not fully compensated by sterols augmentation. The most likely reason is that sterols are not the only limiting nutrient in pollen. Other nutrient deficiencies could be of direct importance to *C. maculata*, or indirectly affect the uptake of the critical sterol nutrients. Lipid contents of a food are known to influence sterol uptake (Bernays, 1992; Behmer and Nes, 2003), and if additional lipid limitations are present in maize pollens (see Lundgren and Wiedenmann, 2004), than this could inhibit sterol uptake in spite of augmentation. Although other nutrients (i.e., protein, specific amino acids, carbohydrate, lipid contents) in maize pollens were not correlated with *C. maculata* performance on different maize pollens, small differences in organic matter were strongly and positively correlated with survival (Lundgren and Wiedenmann, 2004), suggesting a nutrient limitation. Alternatively, many pollens are strongly defended from pollinivory structurally and chemically (Lundgren, 2009b), and ash content was previously found to be negatively correlated with *C. maculata* performance (Lundgren and Wiedenmann, 2004). Thus, there may be some inorganic defensive property present in pollen that restricts its suitability for this omnivore. Additionally, entomophagous insects may not be able to sense sterols in a food item (Behmer and Nes, 2003), and thus

*C. maculata* may not have increased feeding to compensate for sterol limitation in the pollen + sterol diets. Finally, insects undertake sterol metabolism in a staggering number of ways, and additional research on sterol nutrition in *C. maculata* may reveal that ergosterol, β-sitosterol, and cholesterol are not easily used by this omnivorous insect, whereas 24-methylene-cholesterol, episterol, and 24-methylene-lophenol would have been more appropriate (if not easier to obtain).

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