

Impact of dietary sterols on life-history traits of a caterpillar

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Abstract. Although lepidopteran larvae have been shown to metabolise a number of different phytosterols, their life-history traits in response to these different phytosterols may vary with the species. Using the specialist moth, *Plutella xylostella* (Linnaeus) (Lepidoptera), growth, development and fecundity on artificial diets containing host and non-host phytosterols were measured over two generations. Because of the nature of the experimental design, maternal effects linked to dietary sterol could be assessed. In both generations, survival was highest on diets containing sitosterol as the primary phytosterol. A significant decrease in survival on stigmasterol, a non-host phytosterol, was observed between generations. As expected, survival on a control diet with only trace-sterols decreased between generations. Larval duration and pupal duration in both generations was longest on the diets containing the non-host phytosterols, stigmasterol and spinasterol, and on the trace-sterol diet. Finally, fecundity was affected by dietary sterol. In the first generation, fewer viable eggs were produced on the stigmasterol and trace-sterol diets than on wheatgerm control diet. In both generations, dietary sterol significantly influenced percentage egg viability. Overall, these results indicate that differences in phytosterol structure can have pronounced effects on life-history traits, especially when they are projected to the population level. The implications of these results are discussed with respect to phytosterol use by phytophagous insects and their host plant affiliations.

Key words. Insect nutrition, life-history traits, Lepidoptera, *Plutella xylostella*, phytosterol, sitosterol, spinasterol, stigmasterol.

Introduction

Insects, like most other organisms, require sterols for the production of cellular membranes and as precursors to steroid hormones (Clayton, 1964; Svoboda & Thompson, 1985). Unlike vertebrates, however, insects cannot synthesise sterols *de novo* from isoprenoid precursors and must acquire these essential nutrients from their diets (Rees, 1985; Svoboda & Thompson, 1985).

Cholesterol, a Δ^5 sterol (Fig. 1), is the most common sterol found in insects and other animals. Thus, carnivorous insects, or those parasitic on other animals, have a ready supply of an appropriate sterol. Phytophagous insects, however, cannot rely on a dietary source of cholesterol because only trace amounts

are found in plants (Nes & McKean, 1977; Salt *et al.*, 1991; but see Garg *et al.*, 1987). Phytophagous insects either use the sterols present in their host plants directly or metabolize them to other sterols to meet the requirements for proper growth and development (Svoboda & Thompson, 1985).

It has been noted that among the Lepidoptera examined to date, none differ significantly from what are considered to be the 'normal' pathways of dealkylation and the production of cholesterol (Svoboda *et al.*, 1994). Despite this uniformity with respect to the metabolism of dietary phytosterols, physiological responses (e.g. developmental time and growth rate) to different phytosterols vary. For example, the generalist caterpillar, *Heliothis zea*, metabolises both sitosterol and campesterol to cholesterol (Ritter, 1984), but develops faster when reared on a diet containing sitosterol (Ritter & Nes, 1981). Among specialist moths, *Bombyx mori* metabolises both sitosterol and stigmasterol to cholesterol (Ikekava *et al.*, 1966), but develops faster and weighs more on a sitosterol diet (Ito, 1961). Likewise, *Manduca sexta*, a specialist on plants in the Solanaceae, metabolises sitosterol, campesterol, stigmasterol, brassicasterol,

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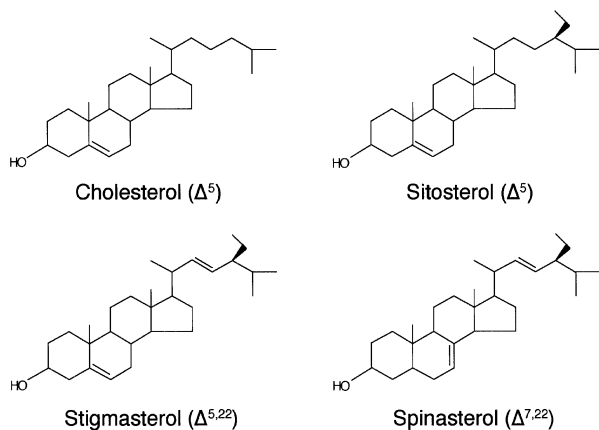


Fig. 1. Sterol structures found in living systems. Cholesterol is the dominant animal sterol. Sitosterol is the dominant phytosterol found in the Brassicaceae, the host plants of *P. xylostella*. Stigmasterol is only found in low levels in the Brassicaceae; spinasterol has yet to be identified in the Brassicaceae.

dihydrobrassicasterol, 24-methylenecholesterol and fucosterol to cholesterol but grows relatively slower on the dihydrobrassicasterol and 24-methylenecholesterol diets (Svoboda *et al.*, 1967; Svoboda & Robbins, 1968).

Life-history studies of phytosterol use by caterpillars, unfortunately, have not always been performed at the same level of detail as metabolic studies. These studies often report survival and weight gain through an arbitrary timepoint and, in many instances, statistical comparisons between treatments have not been performed. Additionally, information on developmental time between pupation and eclosion is lacking and, to our knowledge, no data on reproduction with respect to sterol structure have been reported. This study reports detailed analysis of life-history traits associated with using novel dietary phytosterols throughout the entire life-cycle of the specialist moth, *Plutella xylostella* (Linnaeus) (Lepidoptera). We show that *P. xylostella* exhibits reduced survival and slower growth on artificial diets containing novel phytosterols relative to the normal, dominant dietary sterol. It documents, for the first time, how dietary phytosterol affects reproductive output in a lepidopteran insect. We also show that the maternal diet of *P. xylostella* influences survival between generations. Finally, using our data, we estimate the effects of using different dietary phytosterols at the population level.

Materials and Methods

Insects

The insects used in this experiment came from a 1988 Geneva, NY strain of *P. xylostella* and were maintained on a sitosterol-based artificial diet (Shelton *et al.*, 1991). Adult insects were kept in LD 16:8 h at 28°C in 30 × 30 × 30 cm screen cages and provided *ad libitum* with 10% sucrose solution. Eggs used as a source for first generation neonates were collected by placing several 10-cm² pieces of wrinkled

aluminium foil previously coated with a boiled homogenate of cabbage leaf tissue into each cage of several hundred newly eclosed adults.

Sterols

The diamondback moth, *P. xylostella*, is a specialist on plants in the Brassicaceae, a family with a sterol profile dominated by the Δ^5 phytosterols, sitosterol and campesterol (Nes *et al.*, 1976). Stigmasterol comprises a very small fraction of the total phytosterol pool of plants in this family. In our study, we used these phytosterols as well as the phytosterol spinasterol. Spinasterol has yet to be identified in the Brassicaceae and has double bonds in positions 7 and 22.

Sitosterol (Δ^5) and stigmasterol ($\Delta^{5,22}$) were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Sitosterol, derived from soybean, was a mixture of 60% sitosterol, 27% campesterol (Δ^5) and 13% dihydrobrassicasterol (Δ^5); the profile and ratio of sterols from soybean are similar to those found in the Brassicaceae (Nes *et al.*, 1976). Stigmasterol was \approx 99% pure. Spinasterol ($\Delta^{7,22}$) was extracted from store-bought spinach, *Spinacia oleracea*, by methods previously described (Heupel, 1989), purified by thin layer chromatography (TLC) to 99% pure as determined by gas chromatography–mass spectroscopy (GC-MS) analysis and recrystallized. Solvents used for spinasterol extraction and purification were redistilled reagent grade, purchased from Sigma Chemical.

Insect diet

Because the wheatgerm and flax oil used in the diet contained sterols, it was necessary to extract them. Phytosterols were removed from raw wheatgerm (basal material used to construct the diet) by column- and TLC. Raw wheatgerm was ground using a mortar and pestle and extracted (3 ×) with 500 ml of methanol that was subsequently evaporated to dryness. The methanol residue was resuspended in 70% methanol-water and partitioned against *n*-hexane (counter-saturated). The *n*-hexane residue was re-suspended in a minimal volume of *n*-hexane and saponified (5% potassium hydroxide in methanol). The sterols were separated by silica column chromatography (Heupel, 1989). The column fractions containing sterols were pooled and separated further by TLC (Salt & Adler, 1985). Compounds which co-migrated with authentic cholesterol and lanosterol standards were scraped and eluted with methanol for further analysis by gas chromatography (GC) and confirmed by GC-MS. All column chromatography and TLC fractions not containing sterols were recombined with the extracted raw wheatgerm.

Flax oil sterols were removed by column chromatography. Flax oil (0.1 ml) was suspended in 2 ml of *n*-hexane. The *n*-hexane oil suspension was placed on a silica column and separated (Heupel, 1989). Sterols, which eluted with the ether and ether-methanol washes were further characterized by GC-MS. The column fractions not containing sterols were

recombined and concentrated to produce the reconstituted flax oil.

Despite solvent extraction of the wheatgerm and flax oil, $2 \times 10^{-5}\%$ sterol (mostly sitosterol and cholesterol) was detectable in the reconstituted diet. Over 40% of this residual sterol was due to the sterol content of casein (1.3 μg cholesterol/g casein). As test sterols were added at a concentration of 0.05% (dry weight), a 2500-fold excess of the test sterol over contaminating sterols existed.

We tested *P. xylostella* survival and performance on a wheatgerm-based artificial diet (Shelton *et al.*, 1991) with one of five different sterol treatments: (1) the culture wheatgerm-based diet (control), (2) a wheatgerm-based diet with sterols extracted (trace sterol), (3) a wheatgerm-based diet with sterols extracted and sitosterol added back ('soybean' sitosterol), (4) a wheatgerm-based diet with sterols extracted and spinasterol added, and (5) a wheatgerm-based diet with sterols extracted and stigmasterol added. Sterols were added to the diets at a 0.05% wet weight concentration. The wheatgerm-based diet (control) was used as a standard to measure normal growth, development and reproduction. The trace-sterol diet served two purposes: it measured performance with very low levels of dietary sterols, and allowed us to measure the importance of sterol allocation to eggs between generations by comparing performance of individuals from culture-reared females with individuals from females reared on trace-sterol diets. The 'soybean' sitosterol, spinasterol and stigmasterol diets allowed us to compare the effect of host plant and novel sterols on development, growth and fecundity.

Experimental design

The different sterol treatments were tested over two successive generations, using eggs from culture-reared females in the first generation and eggs from experimental females mated with normal culture males in the second generation. Larval and pupal (time from pupation to eclosion) developmental time, pupal weight and fecundity were recorded for both generations. Neonates used in the first generation experiments came from eggs laid by culture-reared females in their second day after eclosion [these appear to have the highest viability (personal observation)]. The eggs were sterilized in 10% formalin, rinsed with deionized water, placed in a 25-cm Petri dish and incubated at room temperature ($\approx 22^\circ\text{C}$) for 2–3 days. Within a few hours of hatching, neonates were transferred individually to 1.9-ml Eppendorf tubes containing one of the five sterol treatments. Each Eppendorf tube contained one larva and its cap contained multiple pin holes for ventilation. Approximately fifty individuals were assigned to each treatment. We reared the larvae in a Percival incubator at 28°C in LD 16:8 h and observed them daily to record mortality and pupation. At pupation, the sex and weight of each individual was recorded. Male pupae were transferred to new Eppendorf tubes and monitored daily for eclosion. Female pupae were transferred to mating cups that contained a 10% sucrose solution in an Eppendorf tube plugged with a cotton wick, an oviposition substrate (a $\approx 10\text{-cm}^2$ wrinkled aluminium foil

strip coated with cabbage juice homogenate) suspended from the top of the container and two similarly aged male pupa from the laboratory culture. We monitored individual mating cups twice daily to determine days to eclosion of the experimental females and ensure that culture-reared males were available for mating. Sucrose solution levels were checked daily and egg sheets were collected for counting. We removed the first egg sheet after an initial deposition of \approx thirty eggs (the number chosen as a minimum clutch size) and collected new egg sheets over the next 3 days. We placed individual egg sheets in Petri dishes and returned them to the incubator. After the neonates had hatched from eggs, we recorded the total number of eggs and the number of viable eggs. Viable eggs were translucent and had small emergence holes; non-viable eggs were opaque in appearance.

To determine whether dietary sterols had any performance effect on successive generations of *P. xylostella*, we reared second generation *P. xylostella* on the same diet as their maternal parent. The rearing procedures and data collection were identical to those previously described for the first generation. We used five neonates from each of ten maternal matings for each diet, producing a second generation of \approx fifty individuals per sterol treatment.

Statistical analysis

Survival both within and between generations was analysed using the Ryan's test for multiple comparisons (Ryan, 1960). The test allows for comparison of proportions, and as it is a non-parametric test, makes no assumption about the underlying distribution of the data. The test calculates a required difference (RD_k) that is then contrasted to an observed difference between two proportions. If the observed difference is greater than the required difference it is concluded that the samples being compared differ statistically.

We analysed larval duration, pupal weight and pupal duration using ANOVA, and growth rate using ANCOVA. Fecundity, including total egg production, viable egg production and percentage viability, was analysed using repeated measures analysis of variance because observations were repeatedly made over successive days on single individuals. All the data were normally distributed and met the underlying assumptions of normality required for ANOVA and ANCOVA analyses (Steele & Torre, 1980). Post-hoc comparisons for all main effects were performed using the Tukey–Kramer test with α set at 0.05 (Steele & Torre, 1980). Post-hoc comparisons of interactions within sterol treatments were analysed using Least Square Means with α set at $0.05/n$ (protected α , where n = the number of possible comparisons). Males and females were analysed separately. Between-generation comparisons of performance within sterol treatments, and insect sex where appropriate, were made using *t*-tests with α set at 0.05.

Results

Survival

There was no statistically significant difference in survival to eclosion on any of the diets except for stigmasterol in the

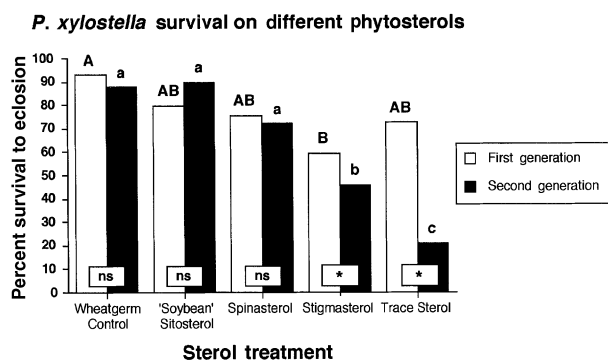


Fig. 2. Percent survival to eclosion by *P. xylostella* in both the first and second generation when reared on one of five sterol treatments. Letters above the bars indicate statistically significant differences (Ryan's multiple comparison test for proportions; $\alpha = 0.05$). Capital letters are used for the first generation and lower case letters are used for the second generation. * = significant difference between generations within a sterol treatment.

first generation ($RD_k = 0.240$, Obs. diff. = 0.340). Survival on the stigmasterol diet was only 55% (Fig. 2a). Survival to eclosion by *P. xylostella* was highest on the wheatgerm diet (92%).

Survival on the stigmasterol diet was again low in the second generation (45%). It was significantly lower than survival on the wheatgerm, 'soybean' sitosterol or spinasterol diets ($RD_k = 0.246$, Obs. diff. = 0.440), but significantly higher than survival on the trace-sterol diet ($RD_k = 0.224$, Obs. diff. = 0.250). Survival was equally high on the wheatgerm and 'soybean' sitosterol diets (88 and 90%, respectively). Although survival on the spinasterol diet was lower than either the wheatgerm or 'soybean' sitosterol diets, the difference was not statistically significant ($RD_k = 0.192$, Obs. diff. = 0.180). In contrast to the first generation, survival on the trace-sterol diet was significantly lower than on any other treatment ($RD_k = 0.224$, Obs. diff. = 0.250), with only 21% surviving to eclosion.

Between generations, the proportion of individuals surviving on the wheatgerm ($RD_k = 0.148$, Obs. diff. = 0.054), 'soybean' sitosterol ($RD_k = 0.171$, Obs. diff. = 0.0100) and spinasterol ($RD_k = 0.202$, Obs. diff. = 0.033) diets did not differ significantly. However, the 13.3% decrease in survival on the stigmasterol diet and the 51.8% decrease in survival on the trace-sterol diet was statistically significant ($RD_k = 0.102$, Obs. diff. = 0.133 and $RD_k = 0.100$, Obs. diff. = 0.518, respectively). In both generations, mortality occurred most frequently during the larval stage.

Larval and pupal performance

In the first generation, sterol treatment significantly affected larval and pupal performance. Larval development was significantly faster on the wheatgerm and 'soybean' sitosterol diet (Fig. 3a). Larval duration on the spinasterol, stigmasterol and trace-sterol diet was 116, 122 and 137% that of the wheatgerm diet. No difference in larval duration between males and females was detected (Table 1a). Female pupal weight in

all treatments was always significantly higher than that of males (Table 1b, Fig. 3b). Females on the wheatgerm and 'soybean' sitosterol diet had significantly higher growth rates than females on the other three diets; males had the highest growth rate on the wheatgerm control and the lowest growth rate on the trace-sterol diet (Table 1c, 2). For both males and females, pupal period was significantly shorter on the wheatgerm and 'soybean' sitosterol diet than on the spinasterol, stigmasterol and trace-sterol diet (Table 1d, Fig. 3c). However, a significant sex by sterol interaction was observed (Table 3d). Males spent significantly more time as pupae than females on the spinasterol and trace-sterol diet than on the other three diets (Fig. 3c).

In the second generation, significant differences in larval duration, pupal weight, growth rate and pupal duration were again observed, and they were similar to those seen in the first generation. Larval development was again significantly faster on the wheatgerm and 'soybean' sitosterol diet (Table 1a). Relative to the wheatgerm diet, larval duration on the trace-sterol, spinasterol and stigmasterol diets was 123, 136 and 136% longer, respectively (Fig. 3a). No difference in larval duration was detected between males and females (Table 1a). For pupal weight, females always weighed significantly more than males, regardless of sterol treatment (Table 1b, Fig. 3b). Male pupae on the 'soybean' sitosterol diet were smaller than male pupae on the wheatgerm diet, but no other differences between sterol treatments were observed. Females had higher growth rates on the wheatgerm and 'soybean' sitosterol diet compared to the spinasterol and stigmasterol diet, whereas males had the highest growth rate on the wheatgerm diet (Tables 1c and 2). Finally, a significant sterol by sex interaction was observed for pupal duration (Table 1d). Males spent more days as pupae on the stigmasterol and trace-sterol diet compared to the 'soybean' sitosterol and spinasterol diet; females spent more time as pupae on the trace-sterol diet compared to the wheatgerm and 'soybean' sitosterol diet (Fig. 3c). Males took longer as pupa than females on the stigmasterol diet, but otherwise there were no differences in days as pupa between males and females.

When performance of neonates obtained from females reared on a sitosterol-rich diet (first generation) was compared to neonates obtained from females reared on the different sterol diets (second generation), differences were observed. Larval duration on the wheatgerm (d.f. = 74, $t = 9.980$, $P < 0.01$), 'soybean' sitosterol (d.f. = 79, $t = 15.335$, $P < 0.01$) and trace-sterol (d.f. = 47, $t = 5.347$, $P < 0.01$) diets was significantly shorter in the second generation than the first generation; no difference was observed across generations on the spinasterol or stigmasterol diet. Except for a decrease in the pupal weight of females reared on the 'soybean' sitosterol diet (d.f. = 40, $t = 3.543$, $P < 0.01$), no difference in pupal weight across generations was recorded. Over all treatments, a significant increase in growth rate was observed in the second generation (d.f. = 306, $t = 12.985$, $P < 0.01$). Pupal duration was longer on the wheatgerm and 'soybean' sitosterol diet in the second generation for both males (d.f. = 28, $t = 10.091$, $P < 0.01$ and d.f. = 33, $t = 5.941$, $P < 0.01$, respectively) and females (d.f. = 40, $t = 4.379$, $P < 0.01$ and d.f. = 38, $t = 4.415$,

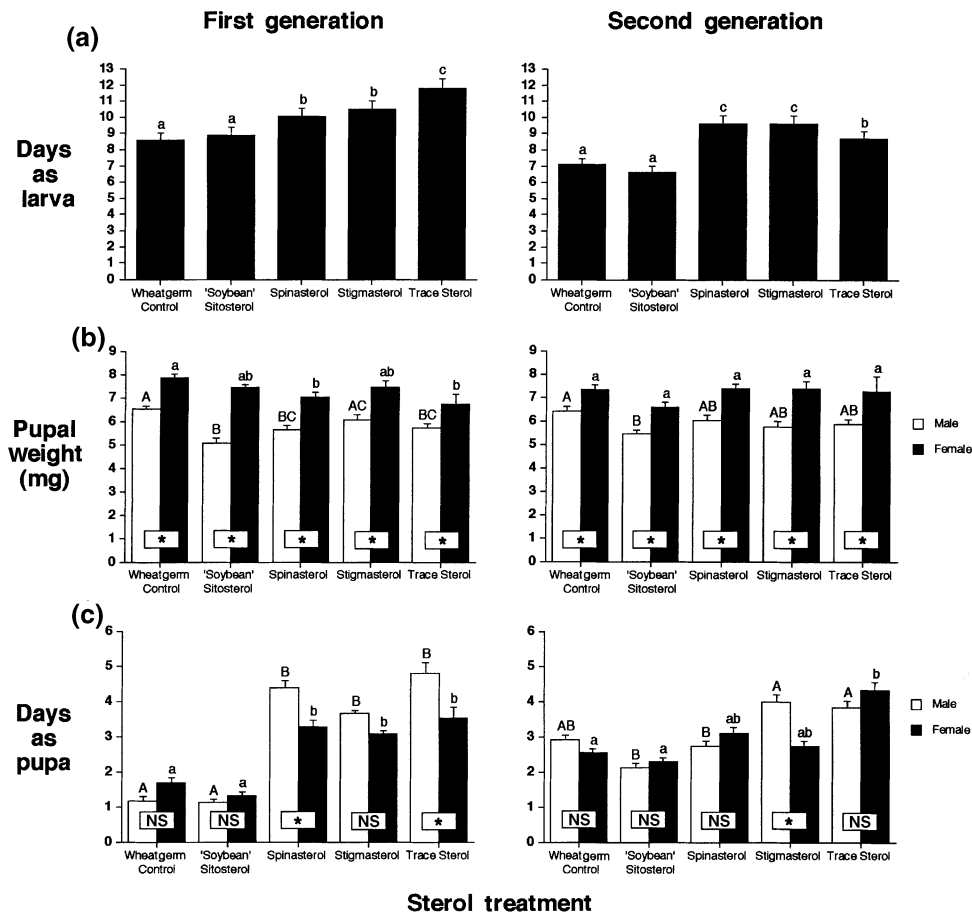


Fig. 3. Larval and pupal performance of *P. xylostella* in both the first and second generation when reared on one of five sterol treatments: (a) amount of time (days \pm SEM) between hatching and pupation, (b) pupal weight (mg \pm SEM) immediately following pupation and (c) amount of time (days \pm SEM) between pupation and eclosion. For days as larvae, letters above the bars indicate statistically significant differences between treatment means (Tukey–Kramer post-hoc comparison; $\alpha = 0.05$). For pupal weight and days as pupa, capital letters indicate significant differences between males and lower case letters indicate significant differences between females (LSMeans post-hoc comparison; $\alpha = 0.05/10$ (the number of possible comparisons)). * = statistically significant difference within a sterol treatment between males and females (Least Square Means comparison; $\alpha = 0.01$ [NS = not significant]).

$P < 0.01$, respectively). Pupal duration for males on the spinasterol diet decreased in the second generation (d.f. = 38, $t = 6.242$, $P < 0.01$). For all other treatments and sexes, pupal duration did not differ significantly between the generations.

Fecundity

Total egg production did not differ between treatments in either generation (Table 3a,b), although fewer eggs were produced on the wheatgerm control in the second generation (d.f. = 18, $t = 2.107$, $P < 0.05$). The number of eggs/day decreased in a significant linear fashion over successive days in both generations (Table 3a).

The wheatgerm diet produced the greatest number of viable eggs in the first generation whereas the spinasterol and trace-sterol diet produced the fewest (Table 3b, 4). There was no difference in viable egg production between sterol treatments in the second generation, excluding the trace-sterol treatment

(Table 3b). However, a significant day by sterol treatment interaction was observed in the second generation. In contrast to the pattern of viable egg production on the wheatgerm and 'soybean' sitosterol diet, spinasterol-reared females produced the same number of viable eggs on days one and two, whereas the stigmasterol-reared females produced the same number of viable eggs over the first 3 days. For both generations, overall viable egg production decreased in a significant linear fashion over successive days (Table 3b). Across generations, there was no difference in the number of viable eggs produced (d.f. = 97, $t = 0.325$, $P = 0.746$).

When egg production and egg viability were combined and analysed as percentage egg viability, a significant difference between the sterol treatments was observed in both generations (Table 3c). In the first generation, the highest percentage egg viability occurred on the wheatgerm diet and lowest percentage egg viability on the stigmasterol and trace-sterol diets (Table 4). Although egg viability in the first generation did not vary significantly between days, a significant linear decrease from



Fig. 4. Estimated population size and developmental time of *P. xylostella* reared on the different sterol treatments after two complete generations. These projections assume a starting population of 100 individuals in a 1:1 sex ratio and no mortality other than that related to the sterol treatment.

the first to fourth day was observed (Table 3c). In the second generation, percentage egg viability varied significantly over the 4 days that eggs were collected, and decreased in a linear fashion (Table 3c). No difference in percentage egg viability between generations was observed for any sterol treatment, but averaged across all treatments, percentage egg viability was higher in the second generation (d.f. = 101, $t = 2.516$, $P < 0.05$).

Discussion

Survival on the trace-sterol diet

The literature suggests that survival of Lepidoptera on trace-sterol diets can be highly variable. Partial survival of the grain moth, *Sitotroga cerealella*, was reported on a trace-sterol diet (larval establishment of 57%), although none of these larvae became adults (Chippendale, 1971). In contrast, the corn borer, *Diatraea grandiosella*, died as first instars when reared on a trace-sterol diet (Chippendale & Reddy, 1972). The corn earworm, *H. zea*, completed no more than one moult, despite continual feeding and frass production, when it was moved from a cholesterol diet to a trace-sterol diet (Ritter & Nes, 1981). Although trace-levels of contaminating sterols remained in all of these diets, growth was always limited and development incomplete.

In our experiment, sterol allocation to the eggs probably explains the high survival on the trace-sterol diet in the first generation. A similar interpretation was used to explain full development of the cabbage fly, *Hylemya brassicae*, on a meridic diet without added sterols (Dambre-Raes, 1976). The possibility that high survival of *P. xylostella* was linked to carry-over of egg reserves (most likely phytosterols) derived from the previous generation is supported by the low survival

on the trace-sterol diet in the second generation. Second generation neonates probably had little sterol allocated to their eggs since they originated from females reared on trace-sterol diets. It is intriguing, however, that some individuals in the second generation survived to eclosion. That these individuals survived to eclosion, and in the case of two females, produced eggs, suggests that they: (1) were highly efficient at absorbing the trace amounts of cholesterol and sitosterol contaminants present in the diet, and/or (2) had more sterols allocated to their eggs than non-surviving individuals.

Survival on novel phytosterols

Differential survival on stigmasterol, relative to sitosterol, has been reported in other Lepidoptera. *Sitotroga cerealella* had lower survival on diets containing stigmasterol (70%) relative to sitosterol (93%) (Chippendale, 1971). Likewise, *D. grandiosella* had lower survival on a stigmasterol diet ($\approx 80\%$) compared to a sitosterol diet ($\approx 100\%$) (Chippendale & Reddy, 1972). In both of these studies, however, differences in survival were not compared statistically. It is surprising that survival of *P. xylostella* on the spinasterol diet was: (1) not significantly different from survival on the wheatgerm or 'soybean' sitosterol diet, and (2) significantly higher than on the stigmasterol diet in the second generation.

Our results suggest that the requirement for specific sterols in *P. xylostella* may not be absolute; there is flexibility that permits the use of novel phytosterols. Sparing, or substituting other sterols for, cholesterol may be one possible mechanism. This substitution can be very dramatic, as demonstrated in the house fly, *Musca domestica*, where the 'essential' cholesterol requirement was found to be only about 0.5% of the total sterol requirement, or less than 100 ng per insect (Robbins, 1963). In our experiment, sterol contamination in

Table 1. Results from analysis of variance on (a) larval duration, (b) pupal weight, (c) larval growth rate and (d) pupal duration of male and female *P. xylostella* in response to different sterol treatments. Sterol treatments include: (1) a wheatgerm control, (2) 'soybean' sitosterol, (3) spinasterol, (4) stigmasterol, and (5) a trace-sterol control.

Source	First generation			Second generation		
	d.f.	F-ratio	P-value	d.f.	F-ratio	P-value
(a) Days as larva						
Sterol	4	38.99	***	4	90.29	***
Sex	1	2.42	ns	1	0.07	NS
Sterol × sex	4	0.88	ns	4	1.50	NS
Error term	177			132		
(b) Pupal weight						
Sterol	4	8.49	***	4	5.21	***
Sex	1	131.17	***	1	58.65	***
Sterol × sex	4	3.21	*	4	0.68	NS
Error term	177			132		
(c) Larval growth rate						
Sterol	4	32.57	*	4	30.71	***
Sex	1	61.87	***	1	37.56	***
Sterol × sex	4	3.02	***	4	1.71	NS
Weight (covariate)	1	3.60	***	1	7.33	***
Error term	175			131		
(d) Days as pupa						
Sterol	4	49.59	***	4	11.03	***
Sex	1	6.08	*	1	0.44	NS
Sterol × Sex	4	4.23	***	4	3.21	*
Error term	156			123		

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = not significant

Table 2. Growth rates (mg pupal weight/days as larvae \pm SEM) of male and female *P. xylostella* on the different sterol treatments. Growth rates were compared using LSMeans tests with $\alpha = 0.05/10$ (the number of possible comparisons). Significant differences between sterol treatments are indicated by different letters.

Sterol treatment	First generation growth rate		Second generation growth rate	
	Male mg/day (\pm SEM)	Female mg/day (\pm SEM)	Male mg/day (\pm SEM)	Female mg/day (\pm SEM)
WG Control ¹	772 (\pm 20) a	902 (\pm 18) a	946 (\pm 20) a	1008 (\pm 32) a
Sitosterol ²	581 (\pm 26) bc	831 (\pm 15) a	810 (\pm 22) b	1032 (\pm 31) a
Spinasterol	588 (\pm 21) b	694 (\pm 26) b	602 (\pm 39) bc	778 (\pm 31) b
Stigmasterol	608 (\pm 26) b	706 (\pm 48) b	614 (\pm 44) bc	771 (\pm 45) b
Trace sterol	504 (\pm 28) c	596 (\pm 48) b	677 (\pm 45) bc	858 (\pm 85) ab

¹Wheatgerm control diet

²'Soybean' sitosterol diet

the diet (3 μ g/g dry weight of diet), coupled with cholesterol allocation to the eggs, could have met a minimal essential requirement for cholesterol. Based on rates of food consumption for other lepidopterans, we estimate that *P. xylostella* could ingest as much as 10 mg of diet (dry weight) during larval development. If we make a generous assumption that all the sterol in the ingested diet is absorbed, a maximum of 30 ng of cholesterol and sitosterol could have been sequestered per insect. Spinasterol and stigmasterol could have been used as a substitute to meet the total sterol

requirements where cholesterol is not essential (Clark & Bloch, 1959; Clayton & Bloch, 1963).

Performance on novel phytosterols

Whatever the mechanism that permits survival on the stigmasterol and spinasterol diets, the decreased performance indicates that there are problems in substituting either of these phytosterols for sitosterol. Larval and pupal development on

Table 3. Results from repeated measures analysis of variance on (a) egg production, (b) viable egg production and (c) percentage viability of *P. xylostella* in response to different sterol treatments. The trace-sterol treatment was omitted from the analysis in the second generation because only two females produced eggs. Sterol treatments include: (1) a wheatgerm control, (2) 'soybean' sitosterol, (3) spinasterol, (4) stigmasterol, and (5) a trace-sterol control.

Source	First generation			Second generation		
	d.f.	F-ratio	P-value	d.f.	F-ratio	P-value
(a) Egg production						
Sterol	4	1.56	NS	3	0.40	NS
Subject (Group)	48			46		
Day	3	60.02	***	3	38.06	***
Contrast (Linear)	1	178.53	***	1	49.01	***
Day × sterol	12	0.51	NS	9	1.62	NS
Day × subject	143			137		
(b) Viable egg production						
Sterol	4	3.47	*	3	1.63	NS
Subject (Group)	46			42		
Day	3	44.32	***	3	33.84	***
Contrast (Linear)	1	131.95	***	1	40.78	***
Day × sterol	12	0.66	NS	9	2.28	*
Day × subject	137			125		
(c) Percent egg viability						
Sterol	4	3.52	*	3	3.01	*
Subject (Group)	46			42		
Day	3	2.45	NS	3	3.67	*
Contrast (Linear)	1	6.41	*	1	7.78	***
Day × sterol	12	0.89	NS	9	0.86	NS
Day × subject	137			125		

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = not significant

Table 4. Viable egg production (\pm SEM) and percentage egg viability (No. viable eggs/No. total eggs \pm SEM) of *P. xylostella* on the different sterol treatments in (a) the first generation and (b) the second generation. Eggs were collected over the first four days of reproduction, but only the totals are presented. The number of females used to collect eggs is indicated in parentheses. Treatment differences were detected using Tukey–Kramer multiple comparison tests with $\alpha = 0.05$. Significant differences between sterol treatments are indicated by different letters.

Sterol treatment	Viable eggs Total (\pm SEM)	Percent egg viability Total (\pm SEM)
(a) First generation		
WG control ¹ (7)	202.7 (21.3) a	82.0 (3.9) a
Sitosterol ² (9)	158.3 (16.7) ab	73.1 (3.7) ab
Spinasterol (16)	135.3 (11.5) b	64.1 (2.7) ab
Stigmasterol (11)	138.3 (17.2) ab	58.1 (4.9) b
Trace sterol (10)	118.5 (14.2) b	56.9 (4.2) b
(b) Second generation		
WG control ¹ (13)	153.9 (17.2) a	80.5 (1.5) ab
Sitosterol ² (16)	171.6 (13.7) a	83.3 (1.4) a
Spinasterol (15)	124.9 (16.8) a	66.5 (3.2) b
Stigmasterol (6)	156.3 (18.9) a	77.0 (2.2) ab
Trace sterol	omitted because of small sample size	

¹Wheat germ control diet.

²'Soybean' sitosterol diet

the spinasterol and stigmasterol diets was extended relative to the wheatgerm and 'soybean' sitosterol diets. We suggest the problem may be associated with removal of the C-24 alkyl group on stigmasterol and spinasterol.

The ability to dealkylate is widespread among phytophagous and omnivorous insects and is the accepted mechanism by which sitosterol, the most common phytosterol, is converted to cholesterol (Svoboda & Thompson, 1985; Ikekawa *et al.*, 1993). However, metabolic studies have revealed that dietary sterols other than sitosterol are not always metabolised efficiently to cholesterol in Lepidoptera. Although *Spodoptera littoralis* reared on a stigmasterol diet contained a significant proportion of cholesterol, over 40% of the recovered tissue sterol contained an alkyl group at the 22-position (Svoboda *et al.*, 1989). Likewise, *H. zea* does not metabolize spinasterol to cholesterol efficiently; 62% of its tissue sterol was lathosterol, whereas 33% was recovered as unmetabolized spinasterol (Ritter, 1984).

If the inability to dealkylate phytosterols does not prevent absorption across the midgut, as is suggested in a number of phytophagous insects (Ritter, 1984; Svoboda & Thompson, 1985; Behmer, Elias & Grebenok, unpublished data), at least two possible mechanisms may explain the reduced performance of *P. xylostella* on diets containing spinasterol and stigmasterol. First, 'leaky' membranes may occur if alkylated phytosterols are incorporated in a cell's phospholipid bilayer (Stein, 1981). Once alkylated sterols have been incorporated into new cells,

the alkyl group on the cholestane side chain can prevent phospholipids from packing tightly around sterol molecules. This could result in a decreased ability to regulate the movement of important ions and molecules across the cell membrane which may lead to reduced weight gain and/or prolonged development. Second, the inability to efficiently metabolise sterols other than sitosterol to cholesterol could influence the production of 20-OH ecdysone, the insect molting hormone. A low titer of cholesterol, the required precursor for 20-OH ecdysone, could reduce the amount of molting hormone produced or limit the rate at which it is produced. If this occurs, development may be slowed. This may be the case with *H. zea* that had tissue sterol concentrations of only 5% cholesterol when reared on a spinasterol diet (Ritter, 1984); they always took longer to develop than individuals reared on diets containing sterols (e.g. sitosterol) that could be efficiently dealkylated to cholesterol (Ritter & Nes, 1981).

Maternal effects associated with novel phytosterols

Interesting patterns in survival and performance on the different sterol diets also were observed between generations. First, survival on stigmaterol was significantly lower in the second generation compared to the first generation. Second, larval duration on all diets except the spinasterol and stigmaterol diet was shorter in the second generation than in the first generation. The reduced survival on stigmaterol between generations and a failure to observe a decrease in larval duration on either the spinasterol or stigmaterol diets might be tied to sterol allocation to eggs. In the grasshopper, *Locusta migratoria*, cholesterol and ecdysteroid titers of eggs from females fed diets with sterols that could not be metabolized to cholesterol were half those of females fed diets with sterols that could be converted to cholesterol (Corio-Costet *et al.*, 1989). If *P. xylostella* had difficulty converting stigmaterol or spinasterol to cholesterol, as is suggested by the developmental data, cholesterol and ecdysteroid egg levels may have been reduced. An inability by first generation females to efficiently convert either spinasterol or stigmaterol to cholesterol for allocation to eggs, either for storage or as the precursor for 20-OH ecdysone, may have consequences for embryonic and larval development.

Fecundity on novel phytosterols

This is the first paper to present results detailing the effect of dietary phytosterols on fecundity in Lepidoptera. Sterol treatment affected both the number of viable eggs and the percentage of the total eggs that were viable. Sterol treatment also affected patterns in egg production over successive clutches and between generations. Typically, *P. xylostella* lay the largest number of eggs on the first day they oviposit and produce fewer eggs on each successive day (Abro *et al.*, 1992). Both egg production and viable egg production followed this pattern in the first generation. In the second generation, however, the pattern of egg and viable egg production on the stigmaterol

diet was different from the other treatments. Both egg and viable egg production remained level over the first 3 days before decreasing on the fourth day.

Cholesterol and ecdysteroids are common components of most insect eggs and are essential for embryonic development (Hoffman & Lagueux, 1985). In *L. migratoria*, ecdysteroid titers in the eggs were reduced by up to 80% when females were reared on plants containing sterols that could not be metabolized to cholesterol (Costet *et al.*, 1987). Reduced titers of ecdysteroids had demonstrable effects on egg viability; eggs with ecdysteroid levels of less than or half control eggs failed to complete embryonic development. A decreased efficiency in the conversion of stigmaterol to cholesterol by *P. xylostella*, for use during embryonic development or as a precursor for 20-OH ecdysone, could explain decreased egg viability or a shift in the pattern of egg production.

It is worth noting that the lack of dramatic differences in egg production, egg viability and the pattern of egg production between the different sterol diets may be a result of sterol contribution by males to females during copulation. Because we were most interested in the female contribution to reproduction, we mated females reared on the different sterol diets with males that had been reared on the sitosterol-rich culture diet. Nuptial gifts have been reported in a number of lepidopteran species (Dussourd *et al.*, 1991; Wiklund *et al.*, 1993; Sculley & Boggs, 1996), but whether sterols are part of this contribution has not been reported. Pilot experiments to test for male transfer of sterols to females during reproduction were inconclusive, so the question of whether males contribute sterols to females during reproduction remains unresolved.

Impact at the population level

The effects of novel phytosterols on *P. xylostella* are even more pronounced when they are projected at the population level. We used our data for percentage survival, total egg production and percentage viability to estimate how large a population of *P. xylostella* could grow if it was maintained on one of the different sterol treatments. Each treatment had a starting population of 100 individuals in a 1:1 sex-ratio, and for simplicity, we assumed no mortality from either biotic or abiotic factors. Large differences between the populations were observed after only two generations; populations maintained on the diets containing novel sterols were less than half compared to those maintained on the 'soybean' sitosterol diets.

We also estimated developmental time for each population, using our data on larval and pupal development. In this case, we assumed it takes 2 days to produce eggs following eclosion and three days for the first clutch of eggs to hatch. On average, it took spinasterol- and stigmaterol-reared *P. xylostella* one week longer to reach the third generation than those reared on 'soybean' sitosterol. In some regions of the world, *P. xylostella* can produce up to six generations per season. In our scenario, and under ideal conditions, for every six generations produced on spinasterol or stigmaterol, seven generations could be produced on 'soybean' sitosterol. Besides losing one generation, slower development also increases exposure to predators and

abiotic mortality factors. The impact of dietary sterols is even more dramatic when these additional components are considered.

Implications for host-range

The observed differential survival, development and reproductive output of *P. xylostella* on diets that vary in sterol profile is consistent with the premise that not all sterol structures can be used efficiently by all insects (Bernays, 1992). For specialist insects like *P. xylostella* that have a long-standing affiliation with certain host plants, their ability to use phytosterols they rarely encounter may be compromised. Although many factors influence host plant use in phytophagous insects, it is possible that sterol metabolic restrictions may play an important role in determining host plant affiliations or, at the least, in restricting use of novel hosts; especially considering that the phytosterol profile often reflects plant taxonomy. For example, the Cucurbitaceae and Theaceae contain almost exclusively Δ^7 sterols, whereas the Poaceae and Asteraceae have almost exclusively Δ^5 sterols (Nes, 1977; Nes & McKean, 1977). The Amaranthaceae and Chenopodiaceae are characterized by the presence of Δ^5 , Δ^7 and Δ^{22} sterols in various ratios (Salt *et al.*, 1991). The biochemical deficiency for sterol production in insects represents a unique and extremely important biochemical difference between insects and many other taxa (e.g. plants and vertebrates). Understanding how this deficiency and metabolic restrictions limits phytosterol use in phytophagous insects may help us to better understand both insect feeding behaviour and patterns of host plant use in the field.

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