

The nutritional significance of sterol metabolic constraints in the generalist grasshopper *Schistocerca americana*

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Received 5 May 1998; accepted 21 July 1998

Abstract

Sterols are essential nutrients for grasshoppers, as well as all other insects, but metabolic constraints can limit which phytosterols support normal growth and development. In the current study, the generalist grasshopper *Schistocerca americana* was used to address two questions related to grasshopper sterol nutrition: (1) how does sterol quantity influence growth and survival, and (2) how do mixtures of suitable and unsuitable sterols at different concentrations influence growth and survival? Results from the first experiment indicated that this grasshopper species had a minimum sterol requirement of 0.05% dry weight; as sterol quantity increased above this concentration, however, survival and performance were not enhanced. Results from the second experiment revealed two novel aspects of sterol nutrition in grasshoppers: (1) when suitable sterols were limiting, most individuals could not use unsuitable sterols to meet the minimum sterol requirement (i.e. no sparing occurred), and (2) above a certain threshold, unsuitable sterols were deleterious even when suitable sterols were present at a concentration that alone permits normal growth and development. We discuss these physiological findings in terms of how sterol metabolic constraints in grasshoppers might influence foraging. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Acrididae; Feeding behavior; Insect–plant interactions; Metabolic constraints; Orthoptera; Sterols

1. Introduction

Grasshoppers, like all other arthropods, are unable to biosynthesize sterols and must acquire these essential nutrients from their food (Clayton, 1964; Svoboda and Thompson, 1985). Cholesterol is the common tissue sterol in grasshoppers, but since plants have little or no cholesterol (Nes and McKean, 1977; Salt et al., 1991; but see Garg et al., 1987), grasshoppers must metabolize plant sterols to cholesterol (Svoboda et al., 1994). Biochemical constraints, however, can limit which phytosterols are metabolized to cholesterol. Among the acridids, constraints on sterol metabolism appear to be a shared characteristic; eight species, representing four different subfamilies, have now been studied and none is capable of completing development on diets that contain sterols with a double bond at position 7 (Δ^7) and/or at

position 22 (Δ^{22}) (Dadd, 1960; Behmer, 1998). Biochemical studies indicate that grasshoppers cannot isomerize Δ^7 -sterols to Δ^5 -sterols and that a double bond at position 22 prevents dealkylation of any alkyl group at C-24 (Charlet et al., 1988; Behmer, 1998).

Grasshoppers tend to be generalists at the individual level (Fox and Morrow, 1981), often feeding on plants from many different families (Joern, 1979, 1985; Bernays and Chapman, 1994). Different plants, however, contain diverse sterols, many of which grasshoppers cannot metabolize to cholesterol. Hundreds of plants have been analyzed for sterols, from which some patterns are now beginning to emerge. First, there are two general variations in phytosterol structure (Fig. 1): (1) in the sterol nucleus, the presence of a double bond at position 5 or position 7; and (2) on the side chain, the addition of a methyl or ethyl group at C-24 and/or the presence or absence of a double bond at position 22. Second, plant species in a given family and especially a given genus often have very similar sterol profiles (Patterson, 1994). For example, the Poaceae and Asteraceae have almost exclusively Δ^5 -sterols while the Cucurbitaceae and

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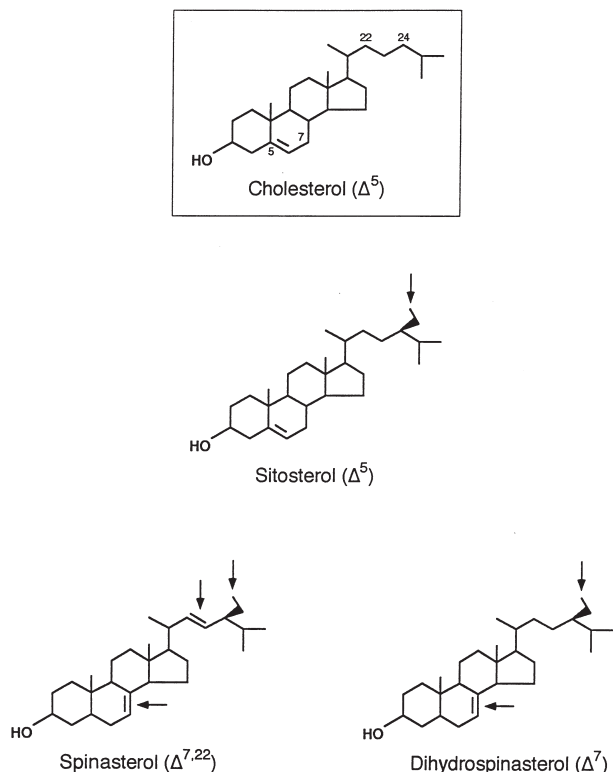


Fig. 1. In phytosterols, double bonds often occur in the nucleus at position 5 or 7 and/or on the side chain at position 22. Alkyl groups, either methyl or ethyl, are common at C-24. Sitosterol, spinasterol and dihydrospinasterol are plant sterols used in this study. The arrows indicate structural differences from cholesterol.

Theaceae have almost exclusively Δ^7 -sterols (Bergmann, 1953). In the order Caryophyllales, seven of the 12 families possess major and sometimes dominant quantities of Δ^7 -sterols; the other five families possess dominant quantities of Δ^5 -sterols (Salt et al., 1991). Lastly, most plants contain a mixture of sterols. Where Δ^5 -sterols are present, sitosterol (Δ^5), campesterol (Δ^5) and stigmasterol ($\Delta^{5,22}$) often co-occur, although sitosterol is usually most abundant (Bergmann, 1953; Nes and McKean, 1977). Stigmasterol is, however, sometimes present to upwards of 40% of the total sterol profile (Willuhn and Kostens, 1974; Jewers et al., 1976). Where Δ^7 -sterols are dominant, $\Delta^{7,22}$ -sterols are also usually present (Nes and McKean, 1977; Salt et al., 1991).

In all eukaryotic organisms, sterols play a major role in two important physiological processes: (1) they are used as structural components in cell membranes, and (2) they serve as precursors for steroid hormones. For the latter process, specific sterols are sometimes required. In grasshoppers, as well as many other insects, cholesterol is a required precursor in the pathway to the production of the molting hormone 20-OH ecdysone (Grieneisen, 1994). Whether grasshoppers have an equally specific requirement for cholesterol in cell membranes, though, is not known. Studies with other insects suggest that when

cholesterol is in limited supply, different sterols can be substituted into membranes to meet the minimum sterol requirement without any apparent negative effect on development or survival. This mechanism has been termed 'sparing' (Robbins, 1963). In the house fly, *Musca domestica*, this sparing mechanism appears to be quite dramatic; the 'essential' cholesterol requirement was found to be only 0.5% of its total sterol requirement (Robbins, 1963). Sparing mechanisms have also been shown in the hide beetle, *Dermestes vulpinus* (Clark and Bloch, 1959) and the fruit fly, *Drosophila melanogaster* (Kircher and Gray, 1978).

It has generally been assumed that phytophagous insects that have cholesterol as the dominant tissue sterol can spare it when it is in limited supply (Svoboda and Thompson, 1985). In this paper, we report a detailed analysis of growth and survival of the generalist grasshopper *Schistocerca americana* (Drury) when it is reared on diets containing: (1) different quantities of sitosterol (a suitable sterol), and (2) mixtures of sitosterol and spinach sterols (the latter are all unsuitable). Results from the first experiment indicate that, above a minimum threshold, performance and survival are not enhanced as quantities of suitable sterol increase. Results from the second experiment show that sparing mechanisms are only of temporary value in grasshoppers. Furthermore, these also indicate that unsuitable sterols may prevent development even when sufficient amounts of suitable sterols are available.

2. Materials and methods

2.1. Experimental insect

The polyphagous grasshopper *S. americana* (Drury) occurs throughout the south and eastern United States and Mexico (Harvey, 1981); it has been recorded as feeding on a wide range of cultivated and naturally occurring plant species (Kuitert and Connin, 1952). Insects used in the following experiments were taken from a laboratory colony reared on a diet of Romaine lettuce, 7–10-day-old seedling wheat and wheat bran. The colony was maintained under standard laboratory conditions with photophase LD 16:8h and radiant heat LD 24–35:19–22°C. The radiant heat was supplied by a 150 W incandescent bulb during photophase which allowed the grasshoppers to self-regulate their body temperature.

2.2. The sterols

The sterols used in this study are shown in Fig. 1. Sitosterol is the most common sterol in plants (Nes and McKean, 1977) and differs from cholesterol, the dominant animal sterol, only by the presence of an ethyl

group at C-24. Sitosterol (purchased from Sigma Chemical, St. Louis, MO) was derived from soybean and was a mixture of 60% sitosterol, 27% campesterol and (Δ^5) and 13% dihydrobrassicasterol (Δ^5); all of these are suitable sterols for the closely related species *S. gregaria* (Dadd, 1960). We refer to the sitosterol mixture throughout the rest of the paper as ‘soybean sitosterol.’ Spinasterol ($\Delta^{7,22}$) and dihydropinasterol (Δ^7) have a methyl group at C-24 and are the dominant sterols found in spinach; they typically occur in a 2:1 ratio (Salt et al., 1991). These two sterols, which we collectively refer to as the spinach sterols, were isolated from spinach, *Spinacia oleracea*, using standard lipid extraction techniques and thin layer chromatography; the amount collected was quantified using HPLC (Heupel, 1989).

2.3. The diet

Grasshoppers were reared on an artificial diet similar to the one used by Simpson et al. (1988). All diets contained a 14% protein (a 3:1:1 mixture of low fat, vitamin-free casein, bacteriological peptone and egg albumen); 14% digestible carbohydrate (a 1:1 mixture of sucrose and white dextrin); ~ 68.0% cellulose; 2.4% Wesson’s salts; 0.5% linoleic acid; 0.3% ascorbic acid; 0.2% vitamin cocktail (Dadd, 1961). The type and amount of sterol added to the diet varied depending on the treatment (see experimental protocols below).

2.4. Quantitative effects of sitosterol (experiment 1)

For this experiment, grasshoppers were raised under standard culture conditions until they molted to the fourth stadium. Upon molting to the fourth stadium, nymphs were weighed and transferred individually to cylindrical plexiglass cages (15 cm diameter \times 40 cm high) with a wire mesh top; a Petri dish 17 cm wide was used as the bottom. Fifteen nymphs were started on each of four diets containing different concentrations of ‘soybean sitosterol.’ The concentrations, as a % dry weight, were: (1) 0.025%, (2) 0.05%, (3) 0.1%, and (4) 0.2%. These concentrations were selected based on work previously done by Dadd (1960) and were selected to determine the sterol requirement of *S. americana*. The diet was placed in a small 5 cm Petri dish and presented to the grasshoppers dry (a mixture of powder and clumps). Each cage also contained a capped plastic vial of water, with a moist cotton wick extended out of the cap, for drinking. Finally, a wire mesh strip, approximately 25 cm long and 2 cm wide, was placed in each cage to facilitate molting. The grasshoppers were reared in a Percival incubator at 35°C day/28°C night with a photophase of L:D 16:8 and monitored daily to record the stadium duration. At each molt, grasshoppers were weighed and the old food was replaced; the water vials were refilled as necessary. Experiments ran until all the

grasshoppers had died or, alternatively, molted to the adult stage. To ensure that grasshoppers had entered the adult stage in a satisfactory manner (i.e. they were robust and healthy) they were monitored for one additional day after this final molt.

2.5. Mixture effects (experiment 2)

For this second experiment, eggs were collected from culture reared females and incubated at 35°C until they hatched. Upon hatching, nymphs were weighed and transferred individually to 30 ml plastic cups (Solo Cup, Urbana, IL) that contained ventilation holes on both the sides and lids. Approximately 20 nymphs were started on each of the six different sterol treatments (Table 1). These included: (1) a 0.05% ‘soybean sitosterol’ diet (high sitosterol), (2) a 0.025% ‘soybean sitosterol’ diet (low sitosterol), (3) a 0.05% spinach sterol diet (spinach sterols), (4) a mixture of 0.05% ‘soybean sitosterol’ and 0.05% spinach sterol to give a total sterol pool of 0.1% (high mixture), (5) a mixture of 0.025% ‘soybean sitosterol’ and 0.025% spinach sterol to give a total sterol pool of 0.05% (low mixture), and (6) a diet containing only trace sterol levels; the albumen (16.6 μ g cholesterol/g) and casein (1.3 μ g cholesterol/g) were the source of the sterol contamination, but it was very minor ~ 0.0018% dry weight. For this experiment, the artificial diet was suspended in a 1% agar solution in a 1:4 dry ingredients:water ratio and presented to grasshoppers as small cubes; the diet was replaced daily. In contrast to the previous experiment, these grasshoppers were given the diet cubes because they otherwise would not grow successfully on the dry granular diet.

As in experiment 1, grasshoppers were reared in a Percival incubator at 35°C day/28°C night with a photophase of L:D 16:8 and monitored daily to record the stadium duration; the weight of the grasshopper was recorded at each molt. When grasshoppers reached the fourth stadium, they were transferred to 180 ml plastic cups (Southwest Glassware, Tucson, AZ) that had ventilation holes on both the sides and lids. A small strip of wire mesh, which provided a perch to facilitate molting, was affixed to the underside of each lid. Experiments ran until all the grasshoppers had died or were 1 day into the adult stage.

2.6. Statistical analysis

For both experiments, survival among the different treatments was compared using the nonparametric Lifereg Procedure (SAS). When significant differences among treatments were identified, a Tukey-type multiple comparison was employed (Fox, 1993). Performance on the different sterol treatments, which included developmental time and weight gain, was compared using the nonparametric Kruskal–Wallis test. When significant

Table 1
Sterol treatments used in the second experiment

Treatment	Sterol composition and amounts		
	'Soybean sitosterol' (mg/g)	Spinach sterols (mg/g)	Total sterol (mg/g)
<i>Single sterol treatments</i>			
High sitosterol	0.50	—	0.518
Low sitosterol	0.25	—	0.268
Spinach sterol	—	0.50	0.518
Trace sterol ^a	—	—	0.018
<i>Mixed sterol treatments</i>			
Low mixture	0.25	0.25	0.518
High mixture	0.50	0.50	1.018

The mixed sterol treatments tested for: (1) a sparing mechanism (the low mixture), and (2) competitive interactions between suitable and unsuitable sterols (the high mixture). The single sterol treatments were the controls.

^aTrace levels of cholesterol were identified in both the albumen and peptone.

differences were detected, a Tukey-type comparison on medians was performed (Zar, 1996). In the mixture experiment, a Mann–Whitney U-test was used to analyze performance of grasshoppers in the last stadium since there were only two treatments on which grasshoppers completed development.

3. Results

3.1. Quantitative effects of sitosterol (experiment 1)

Results from the first experiment indicate the minimum quantitative need for sterols in *S. americana* is approximately 0.05% dry weight (Table 2(a); Fig. 2(a)). When the concentration of 'soybean sitosterol' was 0.025%, survival was below 50% by the beginning of the sixth stadium. Survival on this diet was significantly lower than on diets with 'soybean sitosterol' concentrations of 0.05% or more. In all cases, other than

0.025% 'soybean sitosterol' diet, survival was greater than 85% at the beginning of the adult stage.

The concentration of 'soybean sitosterol' in the diet also affected developmental time and weight gain. The cumulative amount of time required to reach the sixth stadium and adulthood was significantly longer on the 0.025% 'soybean sitosterol' diet than on the other diets (Table 3(a); Fig. 2(b)); there was, however, no difference in the cumulative developmental time to any stadium among the diets with concentrations of at least 0.05%. During the fourth stadium, grasshoppers on diets with at least 0.05% 'soybean sitosterol' gained significantly more weight than grasshoppers on the 0.025% diets; no difference in weight gain was detected during this stadium among the diets with concentrations of at least 0.05%. In the fifth and sixth stadia there was no difference in weight gain among insects that survived on any of the different diets.

Table 2
Results from survival analysis using the Lifereg Procedure (SAS)

(A) Lifereg Procedure				
	<i>df</i>	χ^2	<i>p</i> -value	
	3	167.43	<i>p</i> < 0.001	
(B) Multiple comparisons				
	0.25 mg/g	0.50 mg/g	1.00 mg/g	2.00 mg/g
0.25 mg/g	—	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05
0.50 mg/g		—	ns	ns
1.00 mg/g			—	ns
2.00 mg/g				—

Grasshoppers were reared from the start of the fourth stadium on artificial diets containing different concentrations of 'soybean sitosterol.' Multiple comparisons among the different treatments were made using a one-tailed Tukey-type test.

ns = Not significantly different.

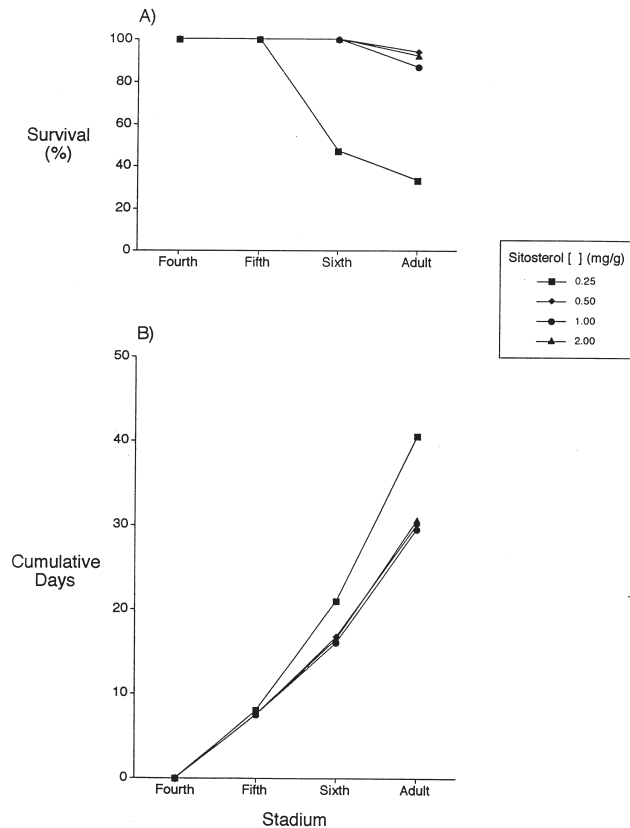


Fig. 2. Performance of *S. americana* reared on artificial diets containing different concentrations of 'soybean sitosterol': (A) points on the graph indicate the percent of the total individuals that were still alive at the beginning of a given stadium, and (B) cumulative development time on the different treatments is plotted using individuals that survived through a given stadium are represented (data are expressed as medians).

3.2. Mixture effects (experiment 2)

Significant differences in survival were detected among the different sterol treatments. Overall, survival on the high sitosterol diet was best compared to all the other diets (Table 4; Fig. 3); it was 100% at the start of the fifth stadium and 85% at the start of the adult stage. Survival on the high and low mixture diets did not differ significantly from each other despite a difference in the concentration of suitable sterols present; the low mixture treatment was, however, the only diet besides the high sitosterol diet to produce adult grasshoppers. Survival on both of these diets was significantly better than on the spinach sterol, low sitosterol or trace sterol diet. On the spinach sterol diet, which contained only unsuitable sterols, survival was significantly better than on the low sitosterol or trace sterol diet. Finally, survival on the low sitosterol diet was slightly, but significantly better than on the trace sterol diet.

Results from the second experiment indicate that sterol profile also significantly influenced development and growth. The cumulative number of days to each sta-

dium was significantly less through each of the first three stadia on the high mixture diet compared to the low mixture and spinach sterol diets (total sterol pools of 0.1%, 0.05% and 0.05%, respectively) (Table 5; Fig. 3). Developmental time on the high sitosterol diet (total sterol pool of 0.05%) was intermediate among these treatments through each of the first three stadia; it was not significantly longer than the high mixture diet, nor was it significantly shorter than either the low mixture or spinach sterol diets. Cumulative developmental time through the fourth stadium on the high mixture diet was shorter than on the high sitosterol, low mixture or spinach sterol diet. The difference in cumulative developmental time through the fifth stadium was, however, only significant between the high mixture and spinach sterol diets. Finally, the difference in cumulative developmental time through the sixth (and final) stadium on the high sitosterol and low mixture diets, which were the only treatments that produced adults, was not significant. Interestingly, sterol profile did not significantly affect grasshopper weight until the final stadium; in this stadium, the median weight of grasshoppers on the high sitosterol diet was significantly greater than on the low mixture diet.

4. Discussion

In the first experiment, survival and performance of the generalist grasshopper *S. americana* was higher when dietary sterols were at a concentration of 0.05% dry weight (dw) or more. Similar results have also been reported for the generalist grasshopper *S. gregaria* (Forsk.) and the oligophagous grasshopper *L. migratoria* L. (Dadd, 1960). Among plants, leaf sterol concentrations range from 0.01% to approximately 0.3% dw, with occasional values of 1% in meristematic tissue (Nes, 1977). Some plant species may, therefore, be nutritionally suboptimal for grasshoppers with respect to sterols. Interestingly, grasshopper performance and survival was not enhanced as the concentration of sterol in the diet increased above 0.05% dw, although further work is required to examine effects on fecundity. The bulk of dietary sterols in insects is allocated for structural purposes and presumably when this has been met there is no further benefit to ingesting larger quantities. Although not observed in this study, it is worth noting that dietary sterols at very high concentrations can sometimes be deleterious in grasshoppers; when *L. migratoria* was reared on diets with cholesterol concentrations of 1.4% from hatch, mortality exceeded 90% after only 24 days (Dadd, 1960).

The importance of obtaining a certain minimum threshold of dietary sterol was also demonstrated in the second experiment. Grasshoppers reared on diets with total sterol pools of at least 0.05% dry weight, regardless

Table 3

Results from Kruskal–Wallis tests measuring cumulative developmental time (days) and cumulative weight gain (mg wet mass) for *S. americana* over the last three stadia when reared on diets containing different concentrations of ‘soybean sitosterol’

	Stadium		
	Fourth	Fifth	Sixth
(A) Cumulative developmental time (days)			
<i>df</i>	3	3	3
<i>p</i> -value	ns	$p < 0.01$	$p < 0.01$
Sitosterol (mg/g)			
0.25	a	a	a
0.50	a	b	b
1.00	a	b	b
2.00	a	b	b
(B) Cumulative weight gain (mg)			
<i>df</i>	3	3	3
<i>p</i> -value	$p < 0.05$	ns	ns
Sitosterol (mg/g)			
0.25	118 (± 8) a	345 (± 27) a	703 (± 187) a
0.50	147 (± 11) b	378 (± 36) a	769 (± 83) a
1.00	146 (± 10) b	391 (± 54) a	821 (± 174) a
2.00	135 (± 23) b	379 (± 44) a	751 (± 97) a

All data are expressed as medians (± MAD). Multiple comparisons among the different treatments were made using a Tukey-type test for medians. Different letters indicate significant differences among the treatments. ns = Not significantly different.

Table 4

Results from survival analysis using the Lifereg Procedure (SAS)

(A) Lifereg Procedure						
	<i>df</i>	χ^2			<i>p</i> -value	
	5	125.37			$p < 0.001$	
(B) Multiple comparisons						
	High mixture	High sitosterol	Low mixture	Spinach sterols	Low sitosterol	Trace sterol
High mixture ^a	—	$p < 0.001$	ns	$p < 0.05$	$p < 0.001$	$p < 0.001$
High sitosterol		—	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Low mixture ^b				$p < 0.05$	$p < 0.001$	$p < 0.001$
Spinach sterol				—	$p < 0.001$	—
Low sitosterol						
Trace sterol						

Grasshoppers were reared from the start of the first stadium on artificial diets containing different concentrations/combinations of ‘soybean sitosterol’ and spinach sterols. Multiple comparisons among the different treatments were made using a Tukey-type test. Where a priori predictions about the direction of the response were made, one-tailed tests were employed.

ns = Not significantly different.

^aContained 0.5 mg/g ‘soybean sitosterol’ and 0.5 mg/g spinach sterols.

^bContained 0.25 mg/g ‘soybean sitosterol’ and 0.25 mg/g spinach sterols.

of whether or not they can be metabolized to cholesterol, lived longer than grasshoppers reared on diets with total sterol pools of less than 0.05%. It was particularly interesting that grasshoppers on the spinach sterol diet (Δ^7 - and/or Δ^{22} -sterols that cannot be metabolized to cholesterol) lived longer than those on the low ‘soybean sitosterol’ diet. The failure to survive to the adult stage on the spinach sterol diet, however, is consistent with what has been shown in a number of other grasshoppers

(Dadd, 1960; Behmer, 1998). That greater than 60% of the individuals on this treatment survived through the third stadium and almost 40% survived through the fifth stadium, though, suggests that some type of temporary sparing mechanism may have been operating. Perhaps contamination from the albumen and peptone could have provided some of the ‘essential’ cholesterol required as a precursor at least in the pathway to production of the insect molting hormone 20-OH ecdysone; this contami-

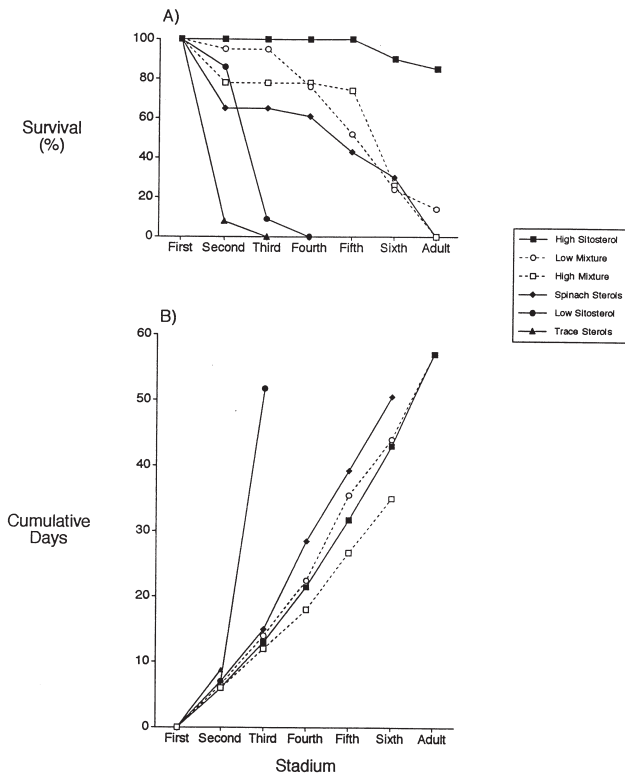


Fig. 3. Performance of *S. americana* reared on artificial diets containing different combinations and concentrations of 'soybean sitosterol' and spinach sterols: (A) points on the graph indicate the percent of the total individuals that were still alive at the beginning of a given stadium, and (B) cumulative development time on the different treatments is plotted using individuals that survived through a given stadium are represented (data are expressed as medians). Closed points and solid lines indicate single sterol treatments while open points and dashed lines indicate treatments with both suitable and unsuitable sterols.

nation was, on a dry weight basis, equivalent to 4% of the minimum total sterol requirement. It is also possible that cholesterol allocated maternally to grasshopper embryos may have still been present and provided some of the 'essential' cholesterol required to produce molting hormone (Hoffman and Lagueux, 1985; Behmer, 1998).

It has been assumed that most phytophagous insects can employ a sterol sparing mechanisms that will allow development to the adults stage when 'essential' sterols are limiting (Svoboda and Thompson, 1985). Results from the low mixture diet in this study suggest that this is generally not the case for grasshoppers. Most of the individual grasshoppers failed to complete development to the adult stage despite the fact that: (1) the total sterol pool was equal to that of the high sitosterol diet, and (2) half of this sterol pool was a suitable sterol. There was, however, variation among individuals in their ability to tolerate Δ^7 - and/or Δ^{22} -sterols. First, three of the 20 grasshoppers started on the low mixture diet completed development to the adult stage. Second, the performance of grasshoppers that did survive through the various sta-

dia, as measured by the cumulative developmental time and weight gain, was never significantly different from the high sitosterol treatment.

The absolute failure of grasshoppers reared on the high mixture diet to complete development to the adult stage was perhaps the most intriguing outcome from the second experiment, especially since these diets had concentrations of 'soybean sitosterol' that alone was sufficient to allow good survival and development. This result, when combined with those on the spinach sterol diet and low mixture diet, strongly suggests that the unsuitable sterols used in these experiments were not just poor or unusable but were positively deleterious. Interestingly, there was no statistical difference in survival between the high and low mixture diets, but grasshoppers on the high mixture diet did develop faster. It is difficult to know, however, whether this is a function of higher concentrations of suitable sterols or total sterols. The lack of a significant difference in developmental time on the high sitosterol and low mixture diets might imply that a higher concentration of total sterol in the diet is responsible for this result.

In contrast to the results with 'soybean sitosterol' and low mixture diets, mortality in the first stadium was high on diets with spinach sterol concentrations of at least 0.05%; deaths typically occurred within the first 2 days. Over the next two stadia, however, survival remained constant. The early mortality may have been a result of the grasshoppers refusing to eat these diets. Previous studies have shown that the grasshopper *S. americana* develops aversions to foods containing sterols that are unsuitable for normal growth and development (Lee and Bernays, 1988; Champagne and Bernays, 1991; Behmer, 1998). In these experiments, rejections were particular strong in response to foods containing sterols with a Δ^{22} -configuration and occurred at concentrations of only 0.2% dry weight. That many individuals in the current study did feed on the diets with high concentrations of spinach sterols is not surprising considering they were dependent on these diets for all their nutrients, including water. When grasshoppers have no other food options, an initial aversion will often be overridden by general hunger (Simpson and Ludlow, 1986).

Several studies with grasshoppers, as well as other insects, indicate that constraints on sterol metabolism do not prevent the absorption of unsuitable sterols across the midgut (Ritter, 1984; Svoboda and Thompson, 1985; Behmer, 1998). It is therefore likely that Δ^7 - and/or Δ^{22} -sterols are negatively impacting grasshoppers at the cellular and/or hormonal level. First, if unsuitable sterols are incorporated into a cell's membrane, it's structural integrity may be compromised, causing it to become 'leaky' (Stein, 1981). For example, any C-24 alkyl group on the cholestane side chain can prevent phospholipids from packing tightly around sterol molecules. This may result in a reduced ability to regulate the movement of

Table 5

Results from Kruskal–Wallis tests measuring cumulative developmental time (days) and cumulative weight gain (mg wet mass) for *S. americana* through the first five stadia when reared on diets with different concentrations/combinations of 'soybean sitosterol' and spinach sterols

	Stadium					
	First	Second	Third	Fourth	Fifth	Sixth
(A) Cumulative developmental time (days)						
<i>df</i>	4	3	3	3	3	1
<i>p</i> -value	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	ns
<i>Treatments</i>						
High mixture	a	a	a	a	a	
High sitosterol	a	ab	ab	b	ab	a
Low mixture	ab	b	b	bc	ab	a
Spinach sterols	ab	b	b	c	b	
Low sitosterol	b					
(B) Cumulative weight gain (mg)						
<i>df</i>	5	4	3	3	3	1
<i>p</i> -value	ns	ns	ns	ns	ns	$p < 0.05$
<i>Treatments</i>						
High mixture	11 (± 1)	38 (± 3)	91 (± 6)	189 (± 11)	391 (± 43)	
High sitosterol	11 (± 1)	41 (± 5)	85 (± 12)	167 (± 34)	365 (± 81)	773 (± 160) a
Low mixture	11 (± 2)	39 (± 4)	82 (± 7)	172 (± 16)	347 (± 33)	489 (± 16) b
Spinach sterols	11 (± 2)	38 (± 3)	85 (± 12)	193 (± 12)	402 (± 23)	
Low sitosterol	12 (± 1)	22 (± 5)				
Trace sterol	9 (± 2)					

All data are expressed as medians (± MAD). Multiple comparisons among the different treatments were made using a Tukey-type test for medians. For the sixth stadium, the data were analyzed using the Mann–Whitney U-test. Different letters indicate significant differences among the treatments (letters in the columns are omitted when differences are not significant). ns = Not significantly different.

important ions and molecules across the cell membrane. In mammalian systems, changes in the cholesterol content of the membrane are known to affect the activities of many membrane-bound enzymes and cell functions, including: the (Na⁺ + K⁺)-ATPase (Kimmelberg, 1975), adenylate cyclase (Whetton et al., 1983), endocytosis (Heiniger et al., 1976), receptor binding (Heron et al., 1983), transport processes (Chen et al., 1978; Saito and Silbert, 1979; Grunze et al., 1980) and thermal sensitivity (Cress and Gerner, 1980). If arthropods are similarly affected by changes in a cell's cholesterol content, detrimental effects on development may be expected. Second, unsuitable phytosterols may interfere with the production of the insect molting hormone, 20-OH ecdysone (Katz et al., 1971). Insects must generate a particular level of 20-OH ecdysone to molt (Rees, 1985) and a failure to exceed this threshold may extend development or, more likely, prevent it from occurring at all.

Grasshoppers are highly mobile relative to other phytophagous insects and diet mixing by individuals appears to be a common phenomenon (reviewed by Strong et al., 1984; Bernays and Chapman, 1994). In grasshoppers, generally, diet mixing also leads to enhanced growth rate (e.g. Kaufmann, 1965; MacFarlane and Thorsteinson, 1980). In both mammals (Freeland and Janzen, 1974) and birds (Guglielmo et al., 1996) an additional benefit

of diet mixing has been proposed; it may be an effective tactic to dilute potentially noxious plant chemicals. Perhaps grasshoppers use diet mixing as a way to limit the intake of unsuitable sterols. Among different plant species grasshoppers may encounter in the field, concentrations of unsuitable sterols range from being only a minor to sometimes dominant proportion of the total sterol profile (Bergmann, 1953; Salt et al., 1991). In the current study, however, even low concentrations of unsuitable sterols had severe negative effects in grasshoppers. Food–plant selection of grasshoppers in the field as it relates to plant sterol profile has yet to be critically examined, but behavioral studies in the lab indicate that learned aversions to plants with unsuitable sterols can develop quite rapidly (Champagne and Bernays, 1991; Behmer, 1998). Although plant primary compounds have often been viewed as relatively unimportant factors influencing food selection in phytophagous insects (reviewed by Berenbaum, 1996), a plant's sterol profile may play an important role in determining patterns of host plant affiliations in grasshoppers, especially considering the widespread nature of sterol metabolic constraints in grasshoppers and their inability to tolerate even low concentrations of unsuitable sterols.

Acknowledgements

We gratefully acknowledge E.A. Bernays for providing logistical support and thank E.A. Bernays, R.F. Chapman, and M. Singer (Arizona) for thoughtful criticisms and suggestions on earlier versions of the manuscript. R. Grebenok and E. Schmelz aided in the collection and quantification of the spinach sterols. G.A. Fox and S. Smith provided statistical advice and assistance. This work was partially supported by grants from Sigma Xi and the Orthopterists' Society awarded to S.T.B. Support was also provided through the interdisciplinary Training Group on Plant–Insect Interactions (NSF BIR-9220332) at the University of Arizona. An NIH undergraduate research training grant from the University of Arizona (T32-A107475) and additional funds from the Interdisciplinary Training Group on Plant–Insect Interactions provided partial support for D.O.E.

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