The sterols of the khapra beetle, *Trogoderma granarium* Everts

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Summary. The khapra beetle, *Trogoderma granarium* Everts, does not dealkylate and convert dietary C_{28}- or C_{29}-phytosterols to C_{27}-sterols such as cholesterol. There is, however, an increase in the concentration of cholesterol and campesterol in its tissues relative to the dietary concentrations of these sterols, presumably as a result of selective uptake.

Until recently, phytophagous insects generally have been considered to be capable of dealkylation and conversion of dietary C_{28}- and C_{29}-Δ^5-phytosterols to cholesterol to satisfy their need for an exogenous source of essential sterol[2]. However, studies with 2 phytophagous insects, the Mexican bean beetle, *Epilachna varivestis* Mulsant[3], and the milkweed bug, *Oncopeltus fasciatus* (Dallas)[4], have revealed unusual variations in the utilization and metabolism of dietary phytosterols by these 2 species: The Mexican bean beetle dealkylates C_{28}- and C_{29}-phytosterols to form C_{27}-sterols, but produces primarily saturated sterols as well as significant amounts of lathosterol (Δ^7-cholestenol) rather than cholesterol. The milkweed bug, however, appears to utilize dietary C_{28}- or C_{29}-plant sterols without alteration before they are incorporated into the larval or adult tissues or into the egg, and apparently is able to utilize certain of these plant sterols as precursors for their molting hormones (e.g. the C_{28}-molting hormone, makisterone A)[5]. We have now found that the khapra beetle, *Trogoderma granarium* Everts, is also unique in the manner that it utilizes dietary phytosterols. This stored-product insect does not appear to dealkylate and convert C_{28}- or C_{29}-dietary plant sterols to C_{27}-sterols to any significant extent; instead it selectively incorporates certain of the ingested sterols into its tissues.

The test insects were reared on a diet consisting of a mixture of wheat and brewer's yeast (95:5). Pupae and adults were weighed and stored in methanol, under nitrogen, until extraction. The insects were extracted in $CHCl_3$-MeOH (2:1) by homogenizing in a Ten Broeck homogenizer. The diet was similarly extracted after thorough grinding with a mortar and pestle. After saponification of the crude lipids, the sterols were purified as previously described[6] by column chromatography on alumina (Woelm, ICN Pharmaceuticals, Cleveland)[7], and the column fractions were monitored by TLC. UV-spectra of these samples, taken in MeOH, indicated the presence of a very small amount of a $\Delta^{5,7}$-diene. The sterols were identified by comparing relative retention times (RRTs) of the insect sterols with those of authentic sterol standards by GLC analysis on a 1% OV-17[8] and an 0.75% SE-30[9] system; they were also quantified by GLC on the SE-30 system.

After GLC analysis, the sterols were acetylated (in pyridine-acetic anhydride, 2:1) and chromatographed on 3-g columns of 20% $AgNO_3$-impregnated Unisil as previously described[6]. The column fractions were monitored on 20% $AgNO_3$-impregnated silica gel H chromatoplates developed in benzene-hexane (1:1) to determine whether significant levels of other than Δ^5-sterols were present in the sterol fractions.

The relative percentages of the sterols present in the khapra beetle pupae and adults, and the percentages of the sterols of the diet are listed in the table. It is notable that only Δ^5-sterols were identified in this insect and that no 7-dehydrocholesterol could be identified by GLC as the free sterol. Even after acetylation and examination of fractions from $AgNO_3$-Unisil column chromatography on $AgNO_3$-TLC, we could not positively detect any 7-dehydrocholesterol. The bulk of the sterol acetates behaved as Δ^5-sterol acetates, but there was a noticeable amount of material from the most polar column fraction (benzene) that migrated in almost the same way as 7-dehydrocholesterol acetate. Also when this material was heated after spraying with 50% H_2SO_4, it developed a color that was similar to that of 7-dehydrocholesterol acetate. However, GLC did not confirm that it was 7-dehydrocholesterol acetate, nor was there sufficient UV-absorption to correlate with the mass, as indicated by TLC-analysis. Furthermore, GC-mass spectral (GC-MS) analysis indicated that the material in this fraction was not steroidal in nature. We must, therefore, conclude that if the UV-absorption which indicated the presence of a $\Delta^{5,7}$-diene was caused by the presence of 7-dehydrocholesterol or other related sterols (e.g. ergosterol), we were unable to detect them by other means even though the samples were handled with caution to protect them from exposure to light or oxygen. However, only a very small amount ($<<1\%$) of a Δ^7-containing sterol would be enough to satisfy the requirement for precursors of the molting hormones.

The very low levels of cholesterol that occur in the pupae and adults of *T. granarium* could be accounted for through selective uptake of the small percentage of cholesterol in the diet. This process is probably also responsible for the relative increase in campesterol in the insect tissues. Such selective uptake and/or retention of dietary sterols has been elegantly demonstrated in studies with the house fly, *Musca domestica* L.[10].

Trogoderma granarium appears to be unable to dealkylate C_{28}- or C_{29}-phytosterols to produce C_{27}-sterols such as cholesterol, and in this regard it is similar to the milkweed bug[4]. If phytophagy arose secondarily in dermestids such as the phytophagous khapra beetle, then these insects may not have developed the mechanism(s) to dealkylate phytosterols. This situation may be analogous to that of the Mexican bean beetle, which is a member of the predominantly predacious coccinellids, and is unable to convert C_{28}- and C_{29}-plant sterols to cholesterol[3]. However, the relative percentages of both cholesterol and campesterol in the tissues of *T. granarium* are greater than those found in the diet; in the milkweed bug, only the percentage of cholesterol is greater than that in the diet. It is noteworthy that such a small quantity of a $\Delta^{5,7}$-diene containing sterol was detected compared with that found in certain other insects[11], particularly another stored-products insect, *Tribolium confusum* Jacquelin duVal[8], in which 7-dehydrocholesterol comprises about 50% of the total sterols when phytosterols are the dietary sterols.

Studies are underway to determine the fate of several radiolabeled sterols (including ^{3}H-sitosterol and ^{3}H-stig-

Sterols of pupae, adults and diet of *Trogoderma granarium*

Sterol	Pupae	Adults	Diet
Cholesterol	1.2*	1.3	0.5
Campesterol	67.0	59.4	25.0
Stigmasterol	2.7	2.6	5.1
Sitosterol	29.1	36.7	69.4

* Relative percent of total sterols.

masterol) fed to *T. granarium* in semidefined diets. These should permit us to determine unequivocally whether this insect dealkylates or metabolizes phytosterols to any significant extent. Our findings further emphasize the diversity that exists in the physiological and biochemical mechanisms involved in the utilization of sterols as essential nutrients by insects.

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Influence of photoinduction on aminotransferase activity in biloxi soybean, *Glycine max.* L., Merr.

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Summary. Increasing number of photoinductive cycles markedly influenced the activity of 5 different aminotransferase systems as compared to non-inductive cycles. The activity was related to the requirement of the corresponding aminoacid synthesised during the process of photoinduction in the leaves.

Aminotransferases are related to the growth and development of plants, and this activity was also found to be influenced by photoperiodic treatments[1-7]. However, the activity of glutamate-pyruvate and glutamate-oxaloacetate aminotransferases were not related to photoinduction in a long-day plant *Lolium temulantum*[6]. But the activity of glycine-aspartate system markedly increased due to 7 short-day cycles in biloxi soybean, a short-day plant[1].

An attempt was therefore made to study the changes in the activity of 5 different aminotransferase systems with the increasing number of short-day cycles in the leaves of biloxi soybean.

Materials and methods. Plants of biloxi soybean (*Glycine max.* L., Merr.) were grown under continuous light. After completion of 1 month, plants were divided into 4 groups and exposed to 3, 7 and 15 photoinductive cycles (8 h light and 16 h darkness). Control plants (0 photoinductive cycles) were kept under continuous light. More details about the experimental methods are described elsewhere[3]. Fully expanded green leaves were chopped and homogenated in phosphate buffer (pH 7.2) and centrifuged at 3000× g at −2 °C. The supernatant was dialysed against distilled water for 12 h with continuous stirring at 5 °C. The dialysed extract was used as a source of the enzyme and assayed for its activity using ^{14}C labelled amino acid as one of the substrates. The activity of 5 different aminotransferase systems, i.e. glutamate-oxaloacetate, glutamate-pyruvate, glutamate-glyoxylate, glycine-pyruvate and glycine-ketoglutrate, were assayed by taking a known amount of ^{14}C labelled glutamate/glycine along with ketoacids depending upon the aminotransferase system mixture, as detailed by Cossins and Sinha[8]. The reaction mixture was incubated for 1 h at 30 °C to allow the amino acid to be transaminated. For separation of end products, a similar method as described earlier by Sengupta et al.[9] was used. The ^{14}C activity was assayed in a tracerlab liquid scintillation counter. Enzyme protein after digestion of the enzyme precipitate, obtained by trichloroacetic acid, with sulphuric acid and hydrogen peroxide was estimated by nesserlization.

Results and discussion. The table shows that the increasing number of short-day cycles markedly enhanced the enzyme activity as compared to control, except at 3 cycles treatment where glycine was taken as a donor. 3 systems, i.e. glutamate-oxaloacetate, glutamate-pyruvate and glycine-ketoglutrate, showed the maximum activity at the stage of 7 inductive cycles, whereas other 2 systems, i.e. glutamate-glyoxylate and glycine-pyruvate, it was at 15 cycles. Number of flowers produced per plant also significantly increased with the increasing number of short-day cycles. Decrease and increase in some of the other biochemical constitutents were also observed with the increasing number of photoinductive cycles[3,7]. Endogenous concentrations of Cu and Fe micronutrients showed continuous increase up to 15 inductive cycles, while Zn and Mn increased only up to 7 cycles, and GA-like substances decreased up to 7 cycles in biloxi soybean[10,11].

It may be expected that the activity of 5 different aminotransferases investigated here differentially changed when

Effect of photo-induction on the aminotransferase activity of five different systems in biloxi soybean (*Glycine max.* L. Merr.)

Transaminase systems	Number of photoinductive cycles given (cpm/mg protein)			
	0	3	7	15
Glutamate-oxaloacetate	5500	7164	8595	7316
Glutamate-pyruvate	6774	7549	7610	6957
Glutamate-glyoxylate	7417	7862	8519	8679
Glycine-pyruvate	4039	4036	4119	6267
Glycine-ketoglutrate	3289	3263	3661	3582
Mean	5404	5975	6501	6560
Number of flowers/plant	0.0	1.63	15.9	34.6

CD 5% P for transaminase systems = 270.19; for flowering = 7.5.