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Effects of Sex, Age, and Diet on the Triacylglycerol Fatty Acid Composition of Subtropical Boll Weevils, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae)

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Six fatty acids accounted for ca. 96% of the total triacylglycerol acids of subtropical adult boll weevils (*Anthonomus grandis* Boheman). In general, females had larger amounts of the major acids, but proportions of each were similar in both sexes. Oleic and palmitic acids were the most abundant in weevils, regardless of sex, age, and diet. Oleic/palmitic acid ratios were consistently greater in boll-fed than in square-fed weevils, regardless of sex or age. Of the C_{18} fatty acids, the more saturated ones (stearic and oleic) were more abundant in boll-fed than square-fed weevils, and the less saturated ones (linoleic and linolenic) were more abundant in square-fed than boll-fed weevils. Total fatty acid amounts were small the first 4 days after pupal eclosion and then increased with age in square-fed weevils; amounts fluctuated greatly with age in boll-fed weevils. Few qualitative differences between subtropical weevils and those of more northern latitudes were proven.

Triacylglycerols (triglycerides) function in storage and liberation of metabolic energy (Downer, 1985) and are the dominant lipid class in animal fat. Lambremont and Blum (1963) found 23 fatty acids in the body fat of boll weevils, *Anthonomus grandis* Boheman, from Louisiana. Lambremont et al. (1964) reported that eight major acids from the triacylglycerol fraction account for ca. 98% of the total fatty acids in these weevils. However, it has been reported that adult boll weevils from the Rio Grande Valley of Texas are biochemically (Keeley et al., 1977; Guerra et al., 1983) and physiologically (Guerra et al., 1982) different from weevils of more northern latitudes. Guerra and Garcia (1982), Guerra et al. (1984), and Guerra (1986) also concluded that subtropical (Brownsville, TX) and tropical (Tapachula, Mexico) weevils can remain physiologically active and reproductive throughout the year as long as temperatures are mild and host plants are available. Alternatively, they may remain in a "quiescent" metabolic state encapsulated inside dry cotton bolls. For these reasons, we hypothesized that the composition of triacylglycerols from subtropical boll weevils may also be different from that of weevils of more northern origins. In this work, we investigated the effects of sex, diet, and age on the triacylglycerol fatty acids of adult boll weevils from the Lower Rio Grande Valley of Texas. This if the first report of triacylglycerol fatty acids from subtropical boll weevils reared on cotton squares and bolls.

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MATERIALS AND METHODS

The effects of sex, diet, and age on the fatty acid composition of subtropical boll weevils were determined by analyzing the triacylglycerol fatty acids of male and females 1, 2, 3, 4, 7, 14, 21, and 28 days after pupal emergence and fed as adults either cotton squares or bolls.

Weevils from infested cotton fruit were collected during the normal cotton season (June–July) in a field near Mercedes, TX. Squares (buds) and bolls were kept separately in outdoor screen-covered 30 × 30 × 30 cm wooden cages protected from rain. After emergence, adults were put in mixed-sex, same-age groups (ca. 40 insects each) in wide-mouth 1000-mL plastic containers with cheesecloth covers. Adults emerging from squares or bolls were fed squares or bolls, respectively, and were kept outside the laboratory but protected from rain. Weevils reaching their test age were sexed (Little and Martin, 1942; Agee, 1964), weighed (wet), and individually triturated in a 2-mL Teflon capsule containing a plastic cylindrical pestle and 0.5 mL of a 2:1 chloroform–methanol extracting solvent (Folch et al., 1957). The trituration materials and procedures were the same as those described by Albach and Guerra (1984), except that the capsule was shaken only once for 30 s and the contents were decanted into a glass vial without centrifugation. The capsule was rinsed twice with 0.5 mL of the extracting solvent. Extracts were stored at −20 °C until analysis. Triacylglycerols were isolated by a two-step procedure. First, they were separated from more polar lipids with a silica SEP-PAK cartridge (Waters Associates, Milford, MA). Extract was loaded onto a hexane-solvated cartridge, and nonpolar and moderately polar lipids including triacylglycerols were eluted with 10 mL of 1% isopropyl alcohol in hexane. Eluant was filtered through a 0.5-μm Millex-SR filter

(Millipore Corp., Bedford, MA). The solvent was evaporated under a stream of nitrogen at low heat. The extract was redissolved in 1 mL of hexane and concentrated to ca. 200 μL under nitrogen. The purpose of this first cleanup step was to remove polar lipids that would precipitate in the high-pressure liquid chromatograph (HPLC) used as the second isolation step.

HPLC was used for the second isolation step (Waters Associates). The 200 μL of extract was injected onto a Resolve silica Radial-PAK cartridge (5-μm particle size) in an RCM-100 module. The mobile phase was 2 mL/min of the following: 100% hexane for 2 min; 0.5% isopropyl alcohol in hexane for 4 min; and 1% isopropyl alcohol in hexane for 14 min. Detection was at 210 nm. The triacylglycerols eluted as a single base-line-resolved, 1-min-wide peak after ca. 13 min (total elution time). This was verified by elution of standard tricaproin, tripalmitin, and trilinolenin (Sigma Chemical Co., St. Louis, MO). The triaclyglycerol fraction was collected from ca. 1 min before until 2 min after the apex of the peak.

The triacylglycerols were transesterified to make fatty acid methyl esters as follows. The solvent from the HPLC collection was evaporated under nitrogen, and the triacylglycerols were redissolved in 1 mL of benzene in a 10-mL vial. About 10 mg of anhydrous sodium sulfate was added to the vial followed by 5 mL of sodium methoxide/methanol reagent (Alltech Associates, Deerfield, IL). The reaction mixture was swirled and then allowed to sit at room temperature for 10 min. The mixture was decanted into a separatory funnel containing 3 mL of water, and the fatty acid methyl esters were extracted with successive aliquots of 6, 3, and 3 mL of hexane. The resulting solution was concentrated to 1 mL under nitrogen.

The fatty acid methyl esters were analyzed by gas–liquid chromatography (GLC) (Shimadzu Scientific Instruments, Columbia, MD) with a flame ionization detector. Analyses were done on a DB 225 Megabore column (J&W Scientific, Inc., Rancho Cordova, CA) (30 m, fused silica, 0.53-mm i.d., 1-μm film). A splitless injection (0.5-min split valve closed) of 1 μL was used. The column oven conditions were as follows: 50 °C for 5 min; 3 °C increase/min until 170 °C; 1 °C increase/min until 200 °C; 200 °C isothermal. Data were quantitated by comparisons of peak heights of standards and unknowns. The standard solution contained valeric, caproic, heptanoic, caprylic, nonanoic, capric, lauric, tridecanoic, myristic, myristoleic, pentadecanoic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, linolenic, arachidic, and arachidonic methyl esters (Sigma) in quantities from 20 to 50 ng increasing with chain length.

The statistical design was a split plot with sex and diet in the main plot and ages in subplots since weevils of each age were removed from the main plot at different times. Three weevils of each sex/diet/age treatment were tested. Analyses of variance were conducted on quantities and percentages of the six most abundant fatty acids (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic). Each of the two analyses used the model: Response (quantity or percentage) is a function of sex, diet, age, fatty acid, and interactions. The replication × sex × diet interaction was used to test all treatments' effects except those involving age. Age effects were tested by using the residual mean square. Individual mean differences were tested by LSD.

Control tests were conducted to determine yields of fatty acid methyl esters from known amounts of tripalmitin and trilinolenin added concurrently to the trituration capsule. These triacylglycerols were chosen to test polar and nonpolar and saturated and unsaturated fatty acid yields. Eight replications were conducted. The results were used to adjust the quantities obtained from weevils.

RESULTS AND DISCUSSION

Control tests indicated that 72.7% (±SE = 4.3%) of palmitic acid and 73.5% (±4.4%) of linolenic acid added to the trituration capsule as tripalmitin and trilinolenin were recovered as methyl esters at the end of the analysis. Further tests with tripalmitin showed that 50–60% of the losses occurred during transesterification with sodium methoxide. Transesterification at temperatures higher than room temperature (up to 80 °C) and/or for longer periods (up to 48 h) yielded lower recoveries than conditions of 10 min at room temperature that we used in this work.

Most of the variability in the control test recoveries occurred among replications rather than between acids within replications, possibly due to inconsistent quality of the sodium methoxide reagent that appeared cloudy on some occassions. The mean difference between recoveries of palmitic and linolenic acids was 0.8% (±0.5%). The consistently small differences in recoveries of these two very different long-chain acids indicate that other long-chain acids should behave similarly. Data in Tables I and

Table I. Quantities and Percentages of the Total of the Six Most Abundant Fatty Acids in Male and Female Subtropical Boll Weevils[a]

	quantity, μg/weevil		% total	
acid	male	female	male	female
palmitic	152 x d	193 y c	27 x	26 x
palmitoleic	34 x ab	41 x a	6 x	6 x
stearic	28 x a	42 x a	5 x	6 x
oleic	198 x e	257 y d	35 x	35 x
linoleic	71 x bc	91 x b	13 x	12 x
linolenic	75 x c	107 y b	13 x	15 x

[a] Means are composites of weevils of both diets and all ages. Percentages are percentages of the total of these six acids. Means in the same horizontal row and in the same group (quantity or percentage) followed by the same letter (x, y) and means in the same vertical column followed by the same letter (a–e) are not significantly different from each other at the 5% level by Fisher's LSD.

Table II. Quantities, Percentages, and Ratios of the Six Most Abundant Fatty Acids in Subtropical Boll Weevils Fed Two Diets[a]

	quantity, μg/weevil		% total		ratio (oleic/x)	
acid	squares	bolls	squares	bolls	squares	bolls
palmitic	226 x c	119 y b	29 x	23 y	1.0	1.9
palmitoleic	39 x a	35 x a	5 x	7 x	6.0	6.3
stearic	30 x a	41 x a	4 x	8 y	7.5	5.5
oleic	230 x c	225 x c	30 x	44 y		
linoleic	114 x b	47 y a	15 x	9 y	2.0	4.9
linolenic	137 x b	46 y a	18 x	9 y	1.7	4.9

[a] Means are composites of weevils of both sexes and all ages. Percentages are percentages of the total of these six acids. Means in the same horizontal row and in the same group (quantity or percentage) followed by the same letter (x, y) and means in the same vertical column followed by the same letter (a–c) are not significantly different from each other at the 5% level by Fisher's LSD.

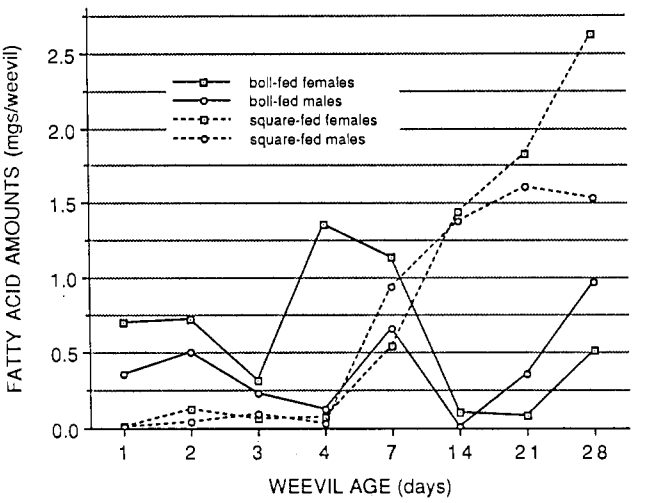

Figure 1. Fatty acid amounts in adult weevils of different ages fed daily with fresh cotton squares (buds) or bolls.

II and in Figure 1 reflect correction for all sample-handling losses based on the mean of the recoveries of palmitic and linolenic acids.

Six fatty acids accounted for ca. 96% of the total triacylglycerol fatty acids of our subtropical boll weevils (Table I). These fatty acids in order of abundance were oleic ($C_{18:1}$), palmitic (C_{16}), linolenic ($C_{18:3}$), linoleic ($C_{18:2}$), palmitoleic ($C_{16:1}$), and stearic (C_{18}) acids. We also found smaller amounts of the following: ca. 2% of myristic (C_{14}), ca. 0.5% of pentadecanoic (C_{15}), ca. 1% of heptadecanoic (C_{17}), and ca. 1% of arachidic (C_{20}).

Lambremont and Blum (1963) working with diapausing and nondiapausing weevils reported 23 fatty acids in weevils from Louisiana, including those we found and many trace components. We made no attempt to detect the trace fatty acids. These authors also reported that oleic and palmitic acids were the most abundant fatty acids in weevils. However, they reported arachidic acid present in smaller amounts (<0.1%) than we consistently found here (ca. 1%). According to recent reports (Cripps et al., 1986; Downer, 1985), arachidic acid must be incorporated from insect diets. On this basis, if we assume arachidic acid is present in larger amounts in subtropical cotton plants, then there is a possibility of using this particular fatty acid as a location marker for long-range migration studies with boll weevils. More research is needed to evaluate this potential marker.

In general, female weevils had larger amounts of the major fatty acids than males, but the proportions of these acids (expressed as percentages of the six fatty acids) did not differ between sexes (Table I). However, percentages of the six major acids varied greatly between square- and boll-fed weevils (Table II). Oleic and palmitic acids were the most abundant, regardless of the cotton diet. Oleic/palmitic ratios were consistently greater in boll-fed than in the square-fed weevils, regardless of sex or age, according to analysis of variance ($p < 0.01$). The results for oleic and palmitic acids are consistent with those reported by Lambremont et al. (1964) for a strain of weevils from Louisiana; therefore, we suggest that at least this biochemical characteristic may be constant for weevils from all latitudes, regardless of their physiological state. We found in our study that ratios of oleic to the rest of the major fatty acids except palmitoleic also were unique for each diet (Table II). This finding suggests that any of these ratios could be used to distinguish boll-fed from square-fed weevils in the field.

Of the C_{18} acids, the more saturated ones, stearic and oleic, made up greater percentages of the total in boll-fed than square-fed weevils (Table II). Similar results were obtained with laboratory-reared boll weevils from Louisiana and Mexico (Lambremont et al., 1964). These authors showed that fatty acid ratios in weevils do not appear to be entirely due to the fatty acid composition of squares and bolls. Another factor for consideration is environmental temperature. Robb et al. (1972) showed that *Musca domestica* reared under colder conditions contained higher percentages of unsaturated fatty acids than saturated acids in their phospholipids, compared to flies reared at warmer temperatures. In the present work, environmental temperatures do not account for the differences in ratios of saturated and unsaturated triacylglycerol fatty acids in square-fed and boll-fed weevils, since both square-fed and boll-fed weevils were reared during warm summer conditions. Possibly, specific chemicals from cotton squares and bolls trigger differential incorporation of fatty acids, or conversion from one oxidation state to the other. In any case triacylglyceorls from boll-fed weevils contain more saturated than unsaturated acids, and it appears this characteristic makes them more able to survive overwintering (a prolonged host-free period). K. R. Summy (personal communication) reported that boll-reared weevils survived longer starvation periods.

Effects of age on triacylglycerol fatty acids were complex. Oleic and palmitic acids were the most abundant acids in weevils of all ages, although the effects were not significant at each individual age. Curves relating the individual acids to age showed that amounts of six acids varied with age similarly. Therefore, individual acids were combined for further analyses of sex × diet × age effects.

In square-reared male and female weevils, the total amounts of the six fatty acids were very small and about the same for the first 4 days, and then fatty acid amounts increased steadily and dramatically with weevil age (Figure 1). These low triacylglycerol levels during the first days after adult eclosion have also been reported by Lambremont et al. (1964, 1966), who found that most extracted lipids in newly emerged weevils were in the phospholipid fraction. They also observed that after the weevils fed for a few days, the triacylglycerol levels increased in the body fat.

In boll-reared weevils, the total amounts of the six major acids fluctuated greatly with age in both sexes. The fluctuations between 3 and 4 days for females, 7 and 14 days for both sexes, and 14 and 28 days for females were significant ($p < 0.05$). Also the age curves for square-fed vs boll-fed weevils differed significantly according to the age × diet interaction ($p < 0.01$). The reason for this fluctuation is yet unknown, but we speculate that it may be a metabolic cycling of lipid utilization. Alternatively, seasonal changes in the fatty acid titers in cotton bolls or one or more environmental factors may have been the cause.

Lambremont et al. (1964) reported that boll-fed adults from Louisiana incorporated more triacylglycerols than square-fed weevils. Our results showed that in general more fatty acids were incorporated in the square-fed weevils ($p < 0.01$) although the effect was age dependent (Figure 1). As discussed above, the levels of fatty acids in boll-fed weevils fluctuated greatly with age. Since Lambremont et al. (1964) did not specify age in their analyses, differences between our results and theirs may be due to these cyclic age effects on fatty acid amounts in square- vs boll-fed weevils. It was interesting to note that although amounts of the six major acids in boll-fed weevils fluctuated with age, the wet weights of these weevils changed only slightly throughout the 28 days of observation. Mean wet weights ± SE ranged from 19.2 ± 0.9 mg for 1-day-old adults to 20.8 ± 0.5 mg for 28-day-old weevils. Also, boll-fed weevils (19.7 ± 0.3 mg) were consistently heavier than square-fed weevils (14.3 ± 0.6 mg) ($p < 0.05$) despite the greater accumulation of acids by the latter. The sex × diet × age interaction was also significant ($p < 0.05$) because fatty acid amounts of male and female boll-fed weevils did not change in the same way from 3 to 4 days of age (Figure 1). While amounts in males decreased, amounts in females increased significantly ($p < 0.05$). The same effect occurred in square-fed weevils, but overall amounts were much smaller. Whether or not these sex × age effects are inherent or environmentally caused cannot be determined from our data.

It is logical that metabolic differences exist between weevils from northern (temperate) and tropical latitudes due to the weevil's response (or adaptation) to different overwintering environments. Subtropical weevils exhibit strong migratory flights after the cotton harvest to seek

new feeding and breeding sites and remain physilogically active and reproductive throughout the mild winters due to ample availability of regrowth cotton (Guerra and Garcia, 1982). On the other hand, weevils from temperate latitudes are faced with subfreezing temperatures soon after cotton harvest, and as a result regrowth from cotton plant residues rarely occurs. These weevils must seek overwintering shelters and enter into a diapauselike physiological state to slow down their metabolic rate to maintenance level. Further, overwintering weevils from subtropical latitudes (north latitude 25°55′) have been described as biochemically "unique", because their fat content and abdominal succinate-cytochrome reductase activity were much lower than those of other diapausing insects (Keeley et al., 1977). In conclusion, while subtropical weevils differ in many respects from those of more northern latitudes, we found only a few differences (e.g., arachidic acid) in triacylglycerol fatty acids in summer-reared weevils of the two groups. Further research is needed to determine whether lipid differences exist between overwintering weevils of the two groups.

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Registry No. Oleic acid, 112-80-1; palmitic acid, 57-10-3; stearic acid, 57-11-4; palmitoleic acid, 373-49-9; linoleic acid, 60-33-3; linolenic acid, 463-40-1; myristic acid, 544-63-8; pentadecanoic acid, 1002-84-2; heptadecanoic acid, 506-12-7; arachidic acid, 506-30-9.

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