Effects of Cyclopropenoid-Containing Cotton Oil on the Life Cycle and Lipid Metabolism of the Blowfly *Lucilia sericata*[1]

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ABSTRACT

Purified oil of the Java Olive Bush, *Sterculia foetida* L. (Malvales: Sterculiaceae), fed to developing larvae resulted in deleterious effects on the life cycle of *Lucilia sericata* (Meigen). There was an inverse correlation between the concentration of oil fed to the larvae and the resulting pupal weight. Pupae obtained from oil-fed larvae were misshapen, and few adults emerged from pupae of larvae fed 0.5–1% oil. In addition, no eggs were obtained from these adults.

Feeding *S. foetida* oil caused alterations in the fatty acid composition of the blowfly that resulted in changes in the ratio of saturated to unsaturated fatty acids in both the neutral lipids and phospholipids. Specifically the relative levels of the saturates — myristic, palmitic and stearic acids—increased, and their corresponding monounsaturates—myristoleic, palmitoleic and oleic acids—decreased, thereby altering the physical consistency of the fatty acid mixture.

The same correlation existed between the level of oil fed and the effects both on life cycle and fatty acid composition; therefore it appears that the action of *S. foetida* oil on the fatty acid composition of the blowfly is responsible for the effects on its life cycle. In addition, it is likely that the cyclopropenoid group of sterculic acid is the active component, since supplements of oleic acid did not adversely affect *L. sericata*.

For many years it has been known that disorders occur in animals fed oil extracted from plants of the Order Malvales; e.g., discoloration in hens' eggs (Sherwood 1928), and hardening of the fats of other animals (Deuel 1955). The cyclopropenoid compounds, sterculic acid (8-2 octyl-1-cyclopropenyl octanoate, the cyclopropene derivative of oleic acid), and malvalic acid (7-2 octyl-1-cyclopropenyl heptanoate) have been identified as the constituents of cottonseed oil responsible (Masson et al. 1957). The amount of these acids present in the oils varies with the species of plant. For example, the seed oil of the commercial cotton plant, *Gossypium hirsutum* L., contains 0.5–1% of these acids, and the seed oil of the Java Olive bush, *Sterculia foetida* L., contains 55% sterculic and 7% malvalic acid (Phelps et al. 1965).

What effects do such compounds have on insects which might attack plants of the Order Malvales? Hargraves (1948) reported that dipterous insects comprise 0.6% of harmful insects on cotton, whereas Evans (1952) reported an average of 5.1% dipterous pests distributed over all commonwealth crops. The insect pests of other orders were almost the same for cotton as for all other commonwealth crops. Pearson (1958) also noted this discrepancy, and at that time he stated "The general nature of the insects that attack cultivated cotton is determined by the character of the plant and its end products." Recently, Beroza and LaBreque (1967) and Lang and Treece (1971) demonstrated that the oil of *S. foetida* fed to adults of the house fly, *Musca domestica* L., and the face fly, *Musca autumnalis* De Geer, acted as a low-order female chemosterilant. They concluded that the chemosterilizing activity of the oil was due to the action of sterculic acid as an antimetabolite of some fatty acid. Since Barlow (1963) had previously demonstrated that high levels of the monounsaturated fatty acid, palmitoleic acid, were characteristic of dipterous insects, this study was undertaken to determine the effect of *S. foetida* oil on the level of palmitoleic acid and other fatty acids in the blowfly *Lucilia sericata* (Meigen). Perhaps these insects with relatively high concentrations of palmitoleic acid are particularly sensitive to cyclopropene fatty acids.

METHODS AND MATERIALS

Purified oil from the Java Olive bush was obtained as a gift from the Southern Regional Research Laboratory of the USDA. Groups of three 25-g diets of fresh calves' liver containing 0.01, 0.5, 1.0, 7.5, and 15% *S. foetida* oil were placed in 250-ml beakers. A small amount of sawdust was mixed with the diet to prevent its drying out. Thirty-six 1st-stage *L. sericata* larvae were placed on these diets and allowed to develop. Control diets with (a) no supplement and (b) 1.0% oleic acid supplement were treated similarly. The insects were reared as described by Williams and Smith (1970). Reproductive capacity was measured as the number of pupae formed/female from the eggs laid for 15 days after the 1st egg lay within a group. One-way analyses of variance and Duncan's multiple range test were applied to the results.

Lipid analyses of control insects and those reared on 0.01, 0.5, and 1% *S. foetida* oil-supplemented diets were made on 3rd-stage larvae, removed from the diet 24 h after they stopped feeding and prior to pupation. Analyses of insects reared on diets supplemented with 7.5 and 15% *S. foetida* oil were made on 2nd instars 24 h after feeding stopped and prior to death. The total lipids were extracted by the method of Bligh and Dyer (1959). Neutral lipids and phospholipids were separated by the method of Borgström (1962), saponified (Lepper 1950) and esterified with boron trifloride-methanol (Morrison and Smith 1964).

Analysis of the fatty acid methyl esters was carried out by GLC (Thompson and Barlow 1971). The liquid phase was 15% diethylene glycol succinate on chromosorb W (AW), mesh 60/80, and the carrier gas was helium. Fatty acid standards including myristic (C14:0), myristoleic (C14:1), palmitic

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(C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids were run for qualitative and quantitative detemminations.

RESULTS AND DISCUSSION

Effects on Development and Reproduction.—Table 1 shows the effects of feeding *S. foetida* oil on the development of *L. sericata*. No statistically significant differences existed between any of the groups in the numbers of larvae which pupated. There is an inverse correlation between the concentration of *S. foetida* oil supplements and pupal weight. Insects reared on the oleic acid-supplemented diets are the largest, while those reared on the *S. foetida* oil-supplemented diets were the smallest. In addition, pupae from the *S. foetida* oil-supplemented diets were variable in size with rather bizarre shapes (Fig. 1). No statistically significant differences were evident in the percent of pupae from which adults emerged in the control, oleic acid, and 0.01% *S. foetida* oil-supplemented groups, but significantly fewer flies emerged in the 0.5 and 1% supplemented diets than in the other 3 diets (Table 1). Dissections were made of several pupae reared on *S. foetida* oil diets from which adults did not emerge. On the basis of external morphology they yielded apparent adults of small size.

Flies reared on the oleic acid-supplemented diets yielded the greatest number of pupae/female, almost twice as many as the control. However, this was not a statistically significant difference (Table 1). *S. foetida* oil fed at 0.01% had no significant effect, but the flies reared on 0.5 and 1% *S. foetida* oil-supplemented diets laid no eggs.

When larvae were reared on 7.5 and 15% *S. foetida* oil-supplemented diets, they stopped feeding in the second stadium and died.

Effects on Fatty Acid Composition.—It is evident that feeding *S. foetida* oil causes changes in the ratio of saturated to unsaturated fatty acids, thereby altering the physical character of both neutral fats and phospholipids (Table 2). Specific alterations are most apparent when *S. foetida* oil is fed at lethal levels, 7.5 and 15% (Table 3). The relative levels of the saturates, myristic, palmitic, and stearic acids increase, and their corresponding monounsaturates, myristoleic, palmitoleic, and oleic acids decrease (Table 3). These results support those of Evans

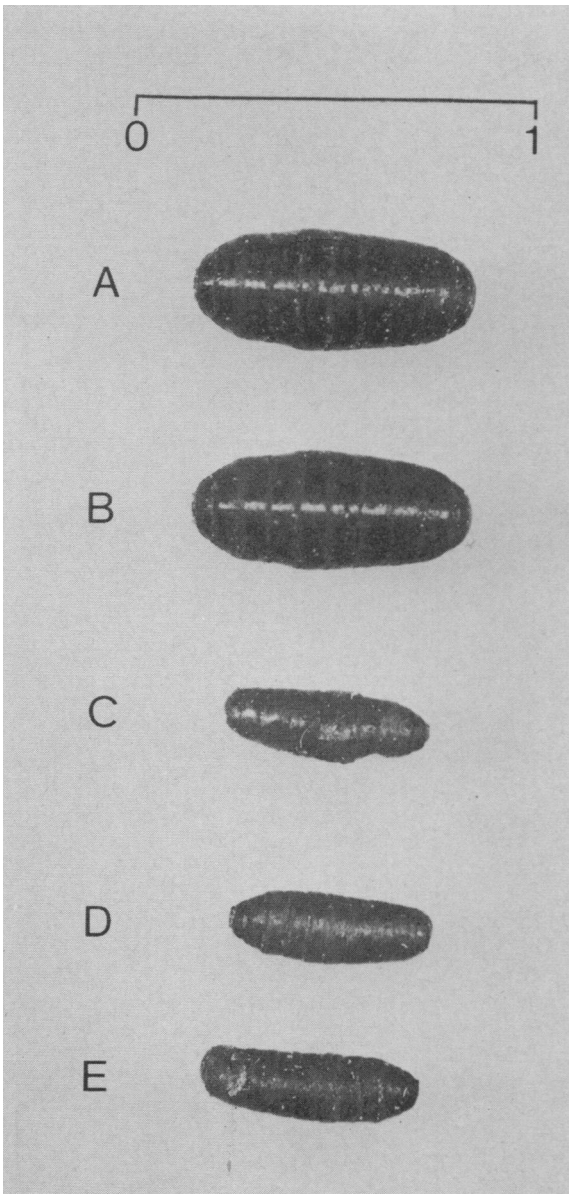

FIG. 1.—Pupae of *L. sericata* reared on *S. foetida* oil-supplemented diets. A, Control; B, 1% oleic acid supplement; C, 0.5% *S. foetida* oil; D and E, 1.0% *S. foetida* oil (scale in centimeters).

Table 1.—Effects of feeding *S. foetida* oil on the fecundity and life cycle of *L. sericata*.

Dietary supplement	% pupation	Pupal weight (mg)	% emergence	Number of adults ♂ : ♀	Reproductive capacity
1.0% oleic acid	91± 2 a[a]	31±2 a	75±13 a	25±4 a 1.2	453±116 a
None (control)	85±10 a	30±2 a	69±14 a	25±5 a 1.7	229± 97 a
0.01% *S. foetida* oil	89± 8 a	28±3 b	67±24 a	24±9 a 1.3	209± 28 a
0.5% *S. foetida* oil	78±10 a	20±1 c	9± 5 b	3±2 b ?	0 b
1.0% *S. foetida* oil	71±10 a	15±1 d	8± 6 b	2±1 b 1.0	0 b

[a] Standard derivatives are shown; values followed by the same letter are not significantly different at 95%.

Table 2.—Effects of feeding *Sterculia foetida* oil on the fatty acid composition of the lipids of 3rd-stage *L. sericata* larvae.

Lipid class	Dietary supplement	Fatty acid composition[a]							Ratio of saturated: unsaturated fatty acids
		C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	
Neutral lipids	none (control)	8	3	27	16	10	28	8	0.82
	1.0% oleic acid	6	2	25	16	10	32	9	0.69
	0.01% *S. foetida* oil	5	Trace	27	18	13	26	11	0.82
	0.5% *S. foetida* oil	6	Trace	30	17	15	23	9	1.04
	1.0% *S. foetida* oil	3	Trace	36	8	21	21	11	1.50
Phospholipids	none (control)	9	4	22	21	6	30	8	0.59
	1.0% oleic acid	5	1	26	17	2	38	10	0.50
	0.01% *S. foetida* oil	4	Trace	28	21	3	34	11	0.53
	0.5% *S. foetida* oil	7	Trace	27	24	5	32	6	0.63
	1.0% *S. foetida* oil	4	Trace	37	12	5	28	15	0.84

[a] The first number represents the carbon chain length, the second, the number of double bonds (see Methods and Materials).

et al. (1963), who demonstrated that cottonseed oil fed to poultry resulted in higher levels of stearic acid and lower levels of oleic acid; and Johnson et al. (1967) who demonstrated the inhibition of desaturation of stearic acid to oleic acid by sterculic acid. The level of oleic acid in insects fed 1% oleic acid-supplemented diets was somewhat greater than the controls, and the ratio of saturated to unsaturated fatty acids was slightly lower. There was little difference between the controls and the 0.01% *S. foetida* oil-supplemented insects.

It is apparent from the present study that the effects of *S. foetida* oil are not specific for 18 carbon unit acids alone, but for 16 and 14 carbon unit acids as well. It should be noted here that insects are unable to synthesize polyunsaturated fatty acids de novo, including C18:2, and these are nutritional requirements.

The alterations in fatty acid composition that occur in *L. sericata* as a result of feeding *S. foetida* oil to larvae persist through pupation and eclosion, and the fatty acid composition does not revert back to that of the control group after feeding stops (Table 4). However, in the adult the ratio of saturates to unsaturates decreases as a result of increased levels of C18:2. It appears that the insect is using less C18:2 than other fatty acids during pupation; a fat mixture of a physical character more like the control group results.

Several studies have resulted in the elucidation of the metabolism of cyclopropenes in rats. Shenstone and Vickery (1961) showed that the cyclopropene group is hydrogenated to the corresponding cyclopropane, and Wood and Reiser (1964) demonstrated that β oxidation of the resultant cyclopropanes is halted at the ring. The *cis* and *trans* isomers of the cyclopropane metabolic products of sterculic acid were not detected in any of the insects analyzed, but they may have been present in very minor and undetectable amounts, since the cyclopropene was fed at such low levels.

In general, the significance of the fatty acid composition appears to be the requirement for a lipid mixture of a certain physical character to fulfill specific structural and/or functional requirements at the molecular and cellular level. The degree of saturation is, therefore, most important. For example, such relations have been well established in membranes and phospholipids (Demel et al. 1967). The present study demonstrates that the degree of saturation, and therefore the physical nature of the fatty acid composition of the neutral lipids and phospholipids in *L. sericata*, is altered by feeding *S. foetida* oil. There is also the same correlation between the level of *S. foetida* oil fed and both the effects on the fatty acid composition and the effects on the life cycle. It appears likely that the action of *S. foetida* oil on the fatty acid composition of the blowfly is responsible for the effects on its life cycle. In addition, it appears likely that the cyclopropenoid group of sterculic acid is the active component, since supplements of oleic acid did not adversely affect *L. sericata*. However, in addition to these effects, the possibility that sterculic acid may

Table 3.—Effects of feeding *Sterculia foetida* oil at 7.5 and 15% levels on the total fatty acid composition of 2nd-stage *Lucilia sericata* larvae.

Dietary supplement	Fatty acid composition							Ratio of total saturated: total unsaturated fatty acids	Ratio	
	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2		C16:0 / C16:1	C18:0 / C18:1
None (control)	2	Trace	28	25	6	28	11	0.56	1.12	0.21
7.5% *S. foetida* oil	5	Trace	33	18	13	20	11	1.04	1.83	0.65
15% *S. foetida* oil	12	Trace	35	13	10	19	10	1.33	2.70	0.53

Table 4.—The total fatty acid composition of *Lucilia sericata* pupae and adults fed 1.0% *Sterculia foetida* oil as larvae.

	Fatty acid composition							Ratio of saturated: unsaturated fatty acids
	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	
Pupal age: 2 days	2	Trace	39	8	11	24	16	1.08
Pupal age: 4 days	3	Trace	42	8	10	25	12	1.22
Pupal age: 6 days	4	Trace	43	6	11	22	14	1.38
Adults	5	Trace	32	8	11	22	22	0.92

be acting to affect other aspects of metabolism, such as the inhibition of desaturation of C18:2 or other unsaturates to yield as yet unknown prostanoic acid-like derivatives analogous to prostaglandins in mammals, has not been ruled out.

The possible use of fatty acid derivatives as anti-metabolites and possible taxa-specific insecticides was suggested by Barlow (1964), and Lang and Treece (1971) pointed out that *S. foetida* oil may still merit further testing to this end. However, the quantification of dosage-response relations in some previous studies may be in question, because the effects of the inhibition of oleic acid synthesis may be largely offset by the direct incorporation of dietary oleic acid into the insect's fats. Oleic acid is generally present in large amounts in diets (48% of the total fatty acids in our liver diet). At the present time, we are carrying out preliminary studies to organically synthesize the cyclopropene derivative of the 16-carbon monounsaturate, palmitoleic acid, which is consistently present at high levels in dipterous insects and is present in only minor quantities in most diets (not detected in our liver diet). We hope to measure and compare the action of the 16-carbon cyclopropene to sterculic acid.

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