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CHARACTERIZATION, DISTRIBUTION, CATABOLISM, AND SYNTHESIS OF THE FATTY ACIDS OF THE TWO-SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE**

M. V. WALLING, DAVID C. WHITE, and J. G. RODRIGUEZ

University of Kentucky, Lexington

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Abstract—A method is described that permits limited culture of *Tetranychus urticae* Koch in a chemically defined diet. The fatty acids of the mite *T. urticae* consist of normal saturated and monoenoic acids of from 12 to 20 carbon atoms. Polyunsaturated linoleic and linolenic acids are also found. The C_{18} series of fatty acids comprise over 80 per cent of the total fatty acids. There is an increase in linolenic and a decrease in palmitic and palmitoleic acids during progression through the protonymphal, deutonymphal, and adult growth stages. The proportions of the other fatty acids remain nearly constant during the growth phases. A higher proportion of stearic and a lower proportion of linolenic acid are contained in the phospholipids than in the neutral lipids. Starvation of the mites leads to a rapid loss of linolenic acid with lesser decreases of the other fatty acids. Palmitic acid is rapidly utilized by starving adult mites in some experiments. Adult mites maintained on a liquid diet containing tritiated acetate incorporate the isotope into saturated monoenoic and polyenoic fatty acids.

INTRODUCTION

THE PHYTOPHAGOUS mite *Tetranychus urticae* Koch can be reared from the protonymphal to the adult state in limited numbers using a chemically defined diet and the technique described herein. Including lipids in the diet seems prudent since rates of ingestion studies show that mites favour this type of diet over one lacking the lipids (Rodriguez, unpublished). The host plant, and especially the leaf cells that are the food for the mite, contain various lipids. The elucidation of fatty acids in *T. urticae* was of paramount interest in endeavouring to improve a chemically defined diet. We were guided generally by the work on insect lipids (FAST, 1964; GILBY, 1965).

Because metabolism of the lipid components in mites was unknown, a study to elucidate them was deemed a worthy objective. Other objectives included determining the distribution between neutral lipids and phospholipids as well as catabolism determination during starvation. Still another objective was to study *in vivo* synthesis of fatty acids from tritiated acetate administered in the diet.

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MATERIALS AND METHODS

Mites reared from plants

To do this work, it was necessary to utilize *T. urticae* reared on plants as well as mites reared on artificial diet. *T. urticae* were reared on detached dwarf bean leaves by the leaf disk culture method (RODRIGUEZ *et al.*, 1967). Mites were harvested from growing plants as 'tear-drop' colonies for some studies. Mites were starved by transferring them to waxed weighing papers encircled with Stickem, a polymerized butane, isobuten, and butane product (Michel and Pelton Company, Emerville, California) placed with the waxed side up on water-soaked cotton pads. The paper disks were enclosed in small Petri dishes. Mites were collected with a fine sable brush and transferred to the saponification mixture.

Mites were weighed with a Cahn electrobalance. For each growth stage fifty mites were weighed with the following results: Protonymphs 2·58 μg (standard deviation = 0·71); deutonymphs, 5·76 μg (S = 1·94); and teneral female adults 9·90 μg (S = 1·32).

Artificial culture of mites

(1) *Feeding chambers.* The mite enclosure was adapted from polypropylene Nalgene (Nalge Company, Rochester, N.Y.) tube closures. The bottom was a No. 29 closure and was designed to serve as a friction-fit closure for a Nalgene centrifuge tube 29 mm wide: this piece was left intact. The top was a No. 32 tube closure which was adapted with a hole 20 mm in diameter. Four 1·5 mm holes were drilled equidistant around the observation window which was covered with a glass cover slip (Fig. 1).

The nutrient pad was made of green Scott Industrial Foam (Scott Paper Company, Chester, Penn.), a porous sponge-like material of polyurethane foam having 80 pores/lineal in. for holding the liquid diet. The reticulated foam pad, $\frac{1}{8}$ in. thick, was punched out in disks 23 mm wide and it also served as a platform for the membrane. Three feeding chambers fit in a standard Petri dish (Fig. 1).

All of the apparatus described thus far was autoclavable.

(2) *Membrane and sticky barrier.* The membrane was a flexible film made from Butvar B-76 (Monsanto Company, St. Louis, Mo.). This resin membrane was made from 1·5% polyvinyl butyral dissolved in a 9 : 1 (v/v) mixture of ethyl acetate and amyl acetate. The membrane was formed by placing 0·15 ml (3 drops) of the solution onto the surface of sterile distilled water in a sterile glass Petri dish bottom, 50 mm wide. This procedure produced a membrane approximately 45 mm wide and approximately 87 mμ thick that permitted all forms of *T. urticae* to feed well. This material was not autoclavable. A barrier was essential to restrict the mites on the membrane and Stickem was used. This was applied with a disposable hypodermic syringe minus the needle. This material is apparently not repellent to the mites, but they seldom get stuck in it if conditions are to their liking.

(3) *Glove box and asepsis.* The experiments were housed in a custom-made plywood glove box with a slanted glass top, sloping to the front. The box was enamelled

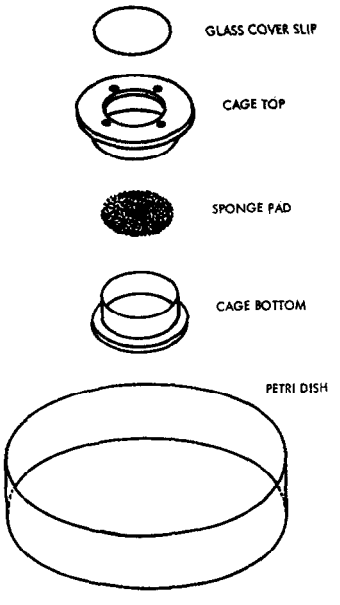

FIG. 1. Autoclavable components of feeding chamber. Three cells fit into a standard Petri dish.

white and measured 24 × 36 in. and was 17 in. high at the rear. The front panel was 10 in. high and had two 6 in. arm ports fitted with iris-diaphragms (Air Control, Inc., Morristown, Penn.). Filtered air is provided by a rotary blower, capable of 140 ft^3/min, which is attached to an 8 × 8 in. glass-asbestos absolute filter (Cambridge Filter Corporation, Syracuse, N. Y., Model 1A-25–1) whose minimum efficiency removes 0·3 μ particles. The filter housing was attached to the side of the glove box. The air flow into the glove box may be regulated by: (1) holes bored into the housing between the blower and the filter, and (2) by closing the iris-diaphragm to create back-pressure of air inside the box. Mites about three-quarters of the way into the deutonymphal quiescent stage were sterilized in 5% formalin, rinsed, and transferred to the feeding chamber.

The glove box was mist sprayed with a 1 : 1000 solution of benzalkonium chloride (12·8%) and the box was closed with the air filter system operating, but with the iris-diaphragms opened slightly. The experimental dishes were placed in the glove box about 1 hr after benzalkonium chloride application and the iris-diaphragms were closed. There was, however, a small amount of air flowing (about 1 ft^3/min) through the box. A green filter (535 mμ) was then placed over the glass top of the box (RODRIGUEZ *et al.*, 1967) but with all of the light coming from outside the box. The light source was standard cool-white fluorescent at 97 ft-c. Temperature and humidity of the laboratory were maintained at 80 ± 2°F (27°C ± 1°C), and 55 to 60 per cent r.h. The r.h. of the mite micro-environment was presumed to be near saturation since the membrane allowed some water loss.

(4) *Diet.* The amino acid fraction of the diet reflects improvements made as a result of studies in amino acid utilization (RODRIGUEZ and HAMPTON, 1966). This diet (Table 1) permits us to conduct certain experiments. To prepare the diet the amino acids and RNA were weighed and added to 40 ml of sterile distilled water using a magneto mixer with slight warming. This solution was cooled, titrated to a pH of 6·0 with 4 N KOH, and brought up to 40 ml volume and again mixed.

TABLE 1—THIS DIET MINUS THE FATTY ACIDS OF THE LIPID FRACTION WAS USED IN THIS STUDY

Compounds	mg/100 ml	Compounds	mg/100 ml
L-Alanine	150	Palmitic acid	2·80
L-Arginine	200	Oleic acid	6·30
L-Aspartic acid	200	Linoleic acid	1·90
L-Cysteine	50	Linolenic acid	0·63
L-Glutamic acid	180	Vitamin A	4·0
Glycine	200	Vitamin E	3·0
L-Histidine	100	Tween 80	10·00
L-Isoleucine	100		
L-Leucine	160	Choline chloride	10·00
L-Lysine	120	Inositol	6·00
L-Methionine	60	Biotin	0·0001
L-Phenylalanine	60	Thiamine Hcl	0·20
L-Proline	60	Riboflavin	0·20
L-Serine	80	Nicotinic acid	0·30
L-Threonine	120	Pyridoxine HCl	0·10
L-Tryptophan	40	*p*-Amino benzoic acid	0·50
L-Tyrosine	50	Folic acid	0·34
L-Valine	120	Ascorbic acid	25·00
		Ca-pantothenate	0·50
Ribonucleic acid	100		
		K_2HPO_4	37·50
Sucrose	2000	NaFe*	0·50
Levulose	500	$Na_2HPO_4.12H_2O$	10·60
Dextrose	500	$MgSO_4.7H_2O$	15·60
		NaMn*	0·25
Cholesterol	0·63	$CoCl_2.6H_2O$	0·04
B-Sitosterol	0·31	$CaCl_22H_2O$	0·06
Stigmasterol	0·31	NaCu*	0·04
Stearic acid	1·25	NaZn*	0·06

* Chelates.

The complete diet will permit surface-sterilized quiescent larvae to develop to the adult stage.

The lipids normally were made up in stock solutions in ethanol and pipetted into a 50 ml beaker. Water (5 ml) was added until the mixture became cloudy. This solution was evaporated to dryness by a nitrogen stream and then made up to 40 ml volume with sterile distilled water. A Tween 80 solution 10 mg in 1 ml was added

from a stock solution and the lipid fraction was then sonicated. For this study, however, the fatty acids were omitted. Since the mites had been reared through the deutonymphal stage on natural food (bean leaf disks) it was deemed prudent to create a 'demand' for fatty acids during the term of the fatty acid synthesis experiment.

Some water-soluble vitamins were in stock solutions: the others were weighed out as are the sugars. These were dissolved in sterile distilled water and brought up to a final volume of 10 ml.

The mineral fraction was made up of salts in stock solutions and made up to 10 ml vol.

The diet was mixed in the sequence given above. The amino acids and the sugar and water-soluble vitamin fractions were sterilized by microfiltration, while the lipid and mineral fractions were autoclaved. The possible utilization and incorporation of tritiated acetate in fatty acid synthesis in the mites were studied. These mites were reared on bean leaf disks until they reached the deutonymphal quiescent stage. At this point about 200 female mites were surface sterilized with 5% formalin and then were transferred to four feeding chambers containing the synthetic fatty-acid-free diet (Fig. 1). A total of 1·25 mc of tritiated acetate (sp. act. 49 mc/mM) was added to 10 ml of diet, and the mites were allowed to feed for 6 days, at the end of which time 154 adult female mites were harvested.

Isolation of the fatty acids

Intact mites (about 2 mg) were saponified under nitrogen in 1 ml of 3 M KOH in 50% (v/v) ethyl alcohol at 100°C for 2 hr in screw-cap tubes with Teflon-lined caps. The cooled saponification mixture was then extracted with 2 vol. of petroleum ether and the ether discarded This extraction was repeated until all the green pigment was removed. The aqueous phase was then acidified to pH 1·0 with HCl, the fatty acids recovered in petroleum ether, dried and dehydrated, and methylated in acidified methyl alcohol as described (WHITE and COX, 1967). The methyl esters were dried in a stream of nitrogen at 30°C and dissolved in a small volume of petroleum ether containing methyl nonadecanoic acid (Applied Science Company, State College, Penn.) as a concentration of 0·5 mμmoles/μl. The methyl esters with the methyl nonadecanoate as the internal standard were then injected into the gas–liquid chromatograph.

Hydrogenation of the fatty acids

Fatty acid methyl esters were hydrogenated in methyl alcohol in the presence of a 5%platinum catalyst on charcoal as described by KANESHIRO and MARR (1961).

Separation of the neutral and phospholipids

After subjecting the mites suspended in chloroform to ultrasonic vibration for a few minutes the lipids were extracted by the procedure of BLIGH and DYER (1959). The lipids were then separated on an 11 by 300 mm column of silicic acid (Unicil, 200–400 mesh, Clarkson Chemical Company, Williamsport, Penn.) using the

procedure described by WHITE and COX (1967). The phosphate of the phsopholipids was quantitively recovered in the phospholipid fraction. Phosphate was determined by the procedure of BARTLETT (1959) modified for the Technicon Auto-analyzer.

Gas–liquid chromatography

Methyl esters of the fatty acids were separated, using polar columns packed with 15% ethylene glycol succinate (EGS) on Gas Chrom P 60/80 mesh prepared by the Applied Science Company. The glass columns were 3 mm i.d. and 1·8 m long. The non-polar columns were packed with 3·8% General Electric silicone oil SE–30 on 80–100 mesh Diatoport S prepared by the Hewlett-Packard Co., Avondale, Penn. Columns were placed in a model 402 dual-column gas chromatograph made by the F & M Division of Hewlett-Packard Co., Avondale, Penn. The operating parameters were described by WHITE and COX (1967).

Fatty acid methyl esters were collected in capillary tubes after separation by gas–liquid chromatography with the apparatus described previously (WHITE and COX, 1967). The fatty acid methyl esters were washed from the capillaries with the scintillation mixture described below.

Determination of radioactivity

Radioactivity was determined in a Packard scintillation spectrophotometer model 3003. The fatty acid methyl esters were dissolved in 9·28 mM 2,5 (2,5-tertbutyl benzoxazol)-thiophene (BBOT) in toluene. The efficiency of tritium counting was 31·4 per cent.

Designation of the fatty acids

The response for each fatty acid methyl ester was calculated as the product of the peak height and the width at half-peak height. The molar response of the hydrogen flame detector was essentially equal for fatty acid methyl esters of between 12 and 20 carbon atoms as determined with known mixtures of fatty acid methyl esters. In this paper the subscript of the C indicates the number of carbon atoms in the fatty acid. The superscript $C^{=}$ indicates the fatty acid is unsaturated. The polyunsaturated fatty acid methyl esters are indicated with multiple superscripts.

RESULTS

Identification of the fatty acids

Fatty acid methyl esters derived from adult mites were subjected to gas–liquid chromatography. The relative retention times of the esters were compared with those of authentic fatty acid methyl esters. The logarithm of the retention time was plotted against the number of carbon atoms from analyses performed with polar and non-polar columns (JAMES, 1960). The retention times of normal saturated fatty acid methyl esters when plotted in this manner lie on a straight line. The retention times of monoenoic fatty acid methyl esters when plotted this way lie on lines of slightly different slopes that are above the saturated esters when examined

with the polar column and below the saturated esters when examined with the non-polar column. This is illustrated in Fig. 2. If the identifications are correct then the proportions of the fatty acid methyl esters in the same sample should be identical when analysed with either the polar or non-polar column. This is the case, as is illustrated in Table 2. The unsaturated fatty acid methyl esters were hydrogenated, forming the expected saturated esters (Fig. 2) in the predicted amounts (Table 3).

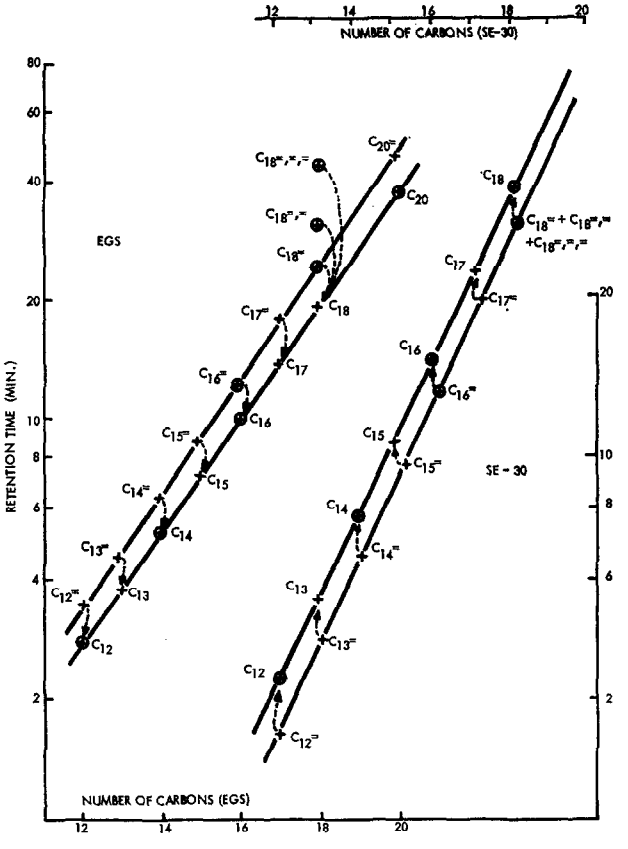

FIG. 2. Logarithm of the retention time vs. the number of carbon atoms in the gas–liquid chromatographic analysis of fatty acid methyl esters of *T. urticae*. Liquid phases represented are the non-polar silicon oil on the right side of the graph and polar EGS on the left side of the graph. Authentic fatty acid methyl esters are marked as ○; fatty acids found in the mites are indicated as ×. The arrows and broken lines indicate the change in retention time after hydrogenation of the sample in methanol.

Fatty acid composition of the mites

The data of Table 4 indicate the fatty acid composition of the mites taken from dwarf bean leaf disk cultures at the protonymphal, deutonymphal, and adult growth stages. In every stage analysed, linolenic acid was found to be the most abundant

TABLE 2—COMPOSITION OF THE FATTY ACIDS OF *T. urticae* AS DETERMINED BY GAS–LIQUID CHROMATOGRAPHY WITH COLUMNS OF ETHYLENE GLYCOL SUCCINATE (EGS) AND SILICONE OIL (SE-30)

Acid*	EGS	SE-30
C_{12}	3·0	3·5
$C_{12}^{=}$	0·8	0·8
C_{13}	2·0	1·5
$C_{13}^{=}$	1·6	3·1
C_{14}	27·7	25·8
$C_{14}^{=}$	6·4	6·3
C_{15}	8·8	9·1
$C_{15}^{=}$	9·0	11·1
C_{16}	100·0	100·0
$C_{16}^{=}$	47·1	46·6
C_{17}	18·5	18·5
$C_{17}^{=}$	15·2	13·9
C_{18}	305·4	313·0
$C_{18}^{=}$	104·5	—
$C_{18}^{=,=}$	81·7	958·6
$C_{18}^{=,=,=} + C_{20}$	806·1	—
C_{20}	33·9	—
$C_{20}^{=}$	—	—
$C_{18}^{=} + C_{18}^{=,=} + C_{18}^{=,=,=} + C_{20}$	992·0	974·2

* Data for the fatty acid methyl esters were taken from analyses like those illustrated in Fig. 2. The esters were identified by their retention times before and after hydrogenation in methanol. The relative areas were calculated as the peak height times the width at half-peak height and are expressed in mm^2. The responses from each column were normalized so that the responses of C_{16} were equal to 100.

acid. The major acids were found to be, apart from linolenic, palmitic, palmitoleic, stearic, oleic, and linoleic acids. There is a remarkable similarity in the fatty acid composition between the growth stages. A progressive increase in the proportion of $C_{18}^{=,=,=}$ and a progressive decrease in the proportion of C_{14}, C_{16}, and $C_{16}^{=}$ is seen. The amount of fatty acid corresponds to from 0·5 to 1·5 per cent of the wet weight of the mites. Starvation for half the growth stage induces changes in the proportions of fatty acids. The consistent change during starvation is the decrease in the proportion of $C_{18}^{=,=,=}$.

Starvation of the adult mites

To determine if fatty acid utilization represented a significant portion of the metabolism of the starving mite, the proportions of fatty acids were determined with female mites that had been on bean leaf disks in the adult stage for 5 hr, and these were compared with analyses of teneral female mites which were transferred from the leaf culture to wax paper disks and allowed only water for periods of 5, 10, and 15 hr. Fatty acid methyl esters were isolated from fifty mites in each of these

TABLE 3—CONVERSION OF UNSATURATED FATTY ACID METHYL ESTER FROM *T. urticae* BY HYDROGENATION IN NEUTRAL SOLVENT TO THE CORRESPONDING SATURATED METHYL ESTER*

Acid	Before hydrogenation	After hydrogenation	Per cent recovered†
C_{14}	98·0	112·0	—
C_{14}^{-}	12·5	0·0	101
C_{15}	18·0	53·3	—
C_{15}^{-}	32·0	0·0	109
C_{16}	486·5	560·0	—
C_{16}^{-}	104·0	0·0	95
C_{17}	40·0	54·0	—
C_{17}^{-}	18·0	0·0	93
C_{18}	1050·0	5018·0	—
C_{18}^{-}	518·5	0·0	—
$C_{18}^{-,-}$	542·5	0·0	—
$C_{18}^{-,-,-}$	3051·0	0·0	98
C_{20}	80·0	80·0	—

* Amount of each fatty acid methyl ester is expressed in mm^2 as determined from chromatograms like Fig. 2. Adult female mites. An EGS column was used and the chromatograms were normalized to equal areas of C_{20}.

† The per cent recovered represents the percentage of saturated fatty acid methyl ester found after hydrogenation compared with that expected if 100 per cent unsaturated fatty acid methyl esters were saturated.

TABLE 4—COMPOSITION OF THE FATTY ACIDS IN FEMALE *T. urticae* IN LIFE STAGES BEFORE AND AFTER STARVATION FOR THE DURATION OF THE LIFE STAGE*

	Protonymph		Deutonymph		Adult	
Acid	Fed	Starved	Fed	Starved	Fed	Starved
C_{12}	—	1·11	1·19	7·07	0·70	—
C_{12}^{-}	0·16	0·82	1·06	—	—	—
C_{13}	0·41	—	—	0·29	0·35	—
C_{13}^{-}	—	—	—	—	0·06	—
C_{14}	4·41	4·00	3·46	3·16	1·41	3·13
C_{14}^{-}	1·20	1·81	0·13	1·18	0·16	1·16
C_{15}	2·60	2·41	0·44	1·63	0·30	1·24
C_{15}^{-}	0·98	1·55	0·79	1·18	0·48	—
C_{16}	17·67	14·51	9·41	15·40	7·82	7·07
C_{16}^{-}	9·81	7·39	4·32	5·04	1·71	3·02
C_{17}	0·98	0·96	1·86	0·98	0·74	—
C_{17}^{-}	0·41	0·95	2·39	0·37	0·28	—
C_{18}	13·50	17·52	13·85	20·25	16·52	18·02
C_{18}^{-}	6·87	10·97	5·68	8·70	8·34	8·53
$C_{18}^{-,-}$	6·54	5·12	6·83	4·45	7·53	5·23
$C_{18}^{-,-,-} + C_{20}^{-}$	34·36	28·03	45·01	30·22	51·59	48·07
C_{20}	1·80	1·12	3·55	—	1·46	4·48

* Expressed as per cent of the total fatty acids.

conditions. Fig. 3 indicates the amount of each fatty acids/mite in the starvation period. The C_{18} series of fatty acids comprise about 84 per cent of the fatty acids of the mites. The more unsaturated the C_{18} fatty acid the more rapid is its utilization during starvation. The other significant fatty acids C_{12}, C_{14}, $C_{16}^{=}$ are utilized during starvation at a rate comparable with that for C_{18}. In this experiment palmitic acid disappeared rapidly from the starving mites.

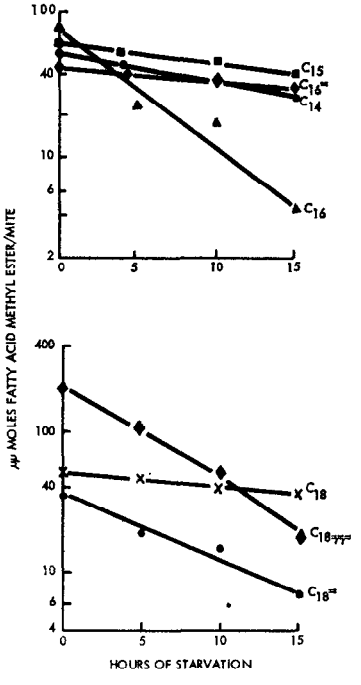

FIG. 3. Utilization of fatty acids by *T. urticae* during starvation. Fatty acid methyl esters were analysed from adult female mites that fed on bean leaves for 5 hr, or were starved for 5, 10, or 15 hr and supplied only water. Each sample represents the fatty acids in fifty mites.

Fatty acid composition of the neutral and phospholipids

The rapid utilization of the $C_{18}^{=,=,=}$ prompted examination of the lipids to see if there was an unusual distribution of this fatty acid. The lipids were extracted from mites subjected to ultrasonic vibration in chloroform by the BLIGH and DYER (1959) procedure, and the neutral and phospholipids were separated by silicic acid chromatography (WHITE and COX, 1967). About 30 per cent of the fatty acids were associated with the neutral lipids. (The tritium was not localized in the fatty acids methyl esters, but it seems reasonable that *T. urticae* can form fatty acids from acetate.) The principal difference between the neutral and phospholipid fatty acids appears to be in the higher proportions of C_{18} and the lower proportion of $C_{18}^{=,=,=}$ in the phospholipids when compared with the neutral lipids. The female

TABLE 5—FATTY ACIDS OF THE NEUTRAL LIPIDS AND PHOSPHOLIPIDS OF *T. urticae**

Fatty acid	Neutral lipid	Phospholipid
C_{12}	1·4	0·50
C_{14}	12·3	1·30
$C_{14}^{=}$	0·7	0·01
C_{15}	1·4	0·70
$C_{15}^{=}$	1·7	0·90
C_{16}	8·2	5·70
$C_{16}^{=}$	4·1	1·60
C_{17}	0·9	1·00
$C_{17}^{=}$	0·1	0·90
C_{18}	18·4	35·60
$C_{18}^{=}$	14·5	12·20
$C_{18}^{=,=}$	8·2	13·10
$C_{18}^{=,=,=}$	35·3	24·40
C_{20}	2·3	3·90

* Percentages of fatty acids calculated in Table 1 after analyses with EGS column.

TABLE 6—INCORPORATION OF TRITIATED ACETATE INTO THE FATTY ACIDS OF *T. urticae**

Acids	Counts/min	mμmoles	Specific activity (counts/min per mμmole)
C_{12} and $C_{14}^{=}$	35·5	0·05	718·0
$C_{16}+C_{16}^{=}$	34·0	0·12	283·0
$C_{18}+C_{18}^{=}$	250·6	0·28	895·0
$C_{18}^{=,=}+C_{18}^{=,=,=}$	54·5	0·24	227·0
Total	375·0	0·69	543·5

* Fatty acid methyl esters were separated by gas–liquid chromatography and collected. The radioactivity was determined as described in the text.

mites introduced to the tritiated diet as quiescent deutonymphs emerged as adults, and under these conditions the mites increased approximately 500 per cent in mass. After saponification and isolation of the fatty acids, a total of 3240 counts/min were detected in the fatty acids. This represents an incorporation of 2×10^{-5} per cent of the added tritium. Methyl esters of the fatty acids were prepared and separated by gas–liquid chromatography. The column was attached to a stream splitter and half the sample collected in capillary tubes, as described previously (WHITE and Cox, 1967). The capillary tubes were washed repeatedly with the toluene-containing scintillation fluid, and the specific activity of the isolated methyl esters was determined. Results of this experiment are illustrated in Table 6. This experiment suggests that the mites are capable of incorporating acetate into the saturated and unsaturated fatty acids. The specific activity of the acetate in the

growth medium was 49 mc/mM. The average specific activity of the isolated fatty acid methyl esters was 104 μc/mM. The tritium was not localized in the fatty acid methyl esters, but it seems reasonable that *T. urticae* can form fatty acids from acetate.

DISCUSSION

Characterization

The fatty acids identified in *T. urticae* show a make-up comparable to that of the insects in general but resemble especially members of the Lepidoptera in having an abundance of the trienoic C_{18}, linolenic acid. To our knowledge, linolenic acid has not been characterized as the most abundant fatty acid in insect orders other than the Lepidoptera, with the possible singular exception of an orthopteran (SCOGGIN and TAUBER, 1950). On the basis of omnivorous food habits and general physiological characteristics of the mites, we might expect the similarity, if any, to be greatest with respect to some aphids; however, this is apparently not the case. BARLOW (1964) has shown the composition of the Aphidae to be heavily weighted toward the shorter-chain fatty acids, the major acid being myristic most consistently. One species analyzed by Barlow shows as much as 99 per cent of the fatty acids as myristic acid. It should be noted that *T. urticae* normally feeds from leaf cells while aphids are phloem tube feeders.

The one report of mite fatty acid composition available concerns not a phytophagous mite but rather a trombidiid (KAHN *et al.*, 1961), and shows a common fatty acid composition, but with palmitic and palmitoleic acids predominating, while the unsaturated C_{18} acids, oleic, linoleic, and linolenic, total only 13 per cent. This shows less similarity to the phytophagous species reported here than to insect species, although it is closer taxonomically. Two other arachnids in the literature, spiders of the families Pisauridae and Theridiidae (BARLOW, 1964), show an abundance of the C_{18} mono- and di-enoic acids, oleic, and linoleic acids, however.

Catabolism

On the basis of our findings, we infer that the two-spotted spider mite may use the fatty acids for sustenance during the non-feeding quiescent stages, and in addition for the development of reproductive tissues during the deutonymphal (pre-adult) quiescent stage. In this connexion, we have shown decreases in the significant acids during starvation. The C_{18} series depicts a trend: the more unsaturated the acid, the more rapid is its utilization.

In general, the lipids are an important energy source of higher animals, but little is known in respect to insects, and virtually nothing in respect to mites, of the mechanisms involved in the conversions of lipids which yield energy. Various researchers have postulated and/or shown the use of lipids in insects as the primary source of energy for many processes, especially in reproduction and flight (FULTON and ROMNEY, 1940: BEALL, 1948; KROGH and WEIS-FOGH, 1951). It has also been well established that the insect stores lipids prior to diapause. In this connexion, LAMBREMONT and BLUM (1963) suggest that the location of metabolic weak points

in lipid storage during transition into diapause may have significant agricultural application in control of pest species. A similar approach has been suggested by BARLOW (1964) in the use of antimetabolites in control programmes.

Biosynthesis

Since the organisms involved in this study were not reared aseptically throughout their life cycle, it cannot be said what synthesis capabilities apparently attributable to the organism are in fact due to possible microbial activity.

Our results indicate synthesis did occur for both saturated and unsaturated even-numbered carbon chains from C_{12} to C_{18}. This is somewhat unusual in the case of linoleic and linolenic acids, since only trace quantities of polyenoic fatty acids have been found to be synthesized from ^{14}C-acetate in insects (BADE, 1964; STRONG, 1963; LAMBREMONT, 1965).

CONCLUSIONS

The polyenoic fatty acid, linolenic acid, is apparently of prime importance in the metabolism of the two-spotted spider mite, *Tetranychus urticae* Koch. This fatty acid forms a significant proportion of the total fatty acids of both neutral and phospholipids. The proportion of linolenic acid increases during the protonymphal to adult stages. Linolenic acid is rapidly utilized during starvation, and it can be synthesized from the acetate in the adults. In these respects the fatty acid metabolism of *T. urticae* differs significantly from that of phytophagous insects in the few studies that have been reported.

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