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Studies on Lipids in Some Homopterous Insects

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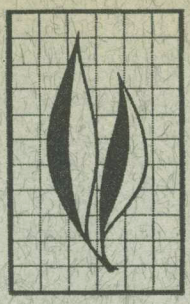

There is little information available on the occurrence of lipids in aphids or other sucking insects, and little is known of the role of lipids in aphid nutrition. The purpose of the present investigation was to obtain preliminary information on several phases of these problems. The classes of lipids extracted from one aphid, *Macrosiphum barri* Essig, were separated by column chromatography. The fatty acids in each of these classes as well as in the crude lipid extracts from 20 other species of aphids and six species of leafhoppers were separated by gas-liquid chromatography. Nearly all of the fatty acids in *M. barri* were in the form of triglycerides, as in other insects that have been studied by previous investigators. The free fatty acids accounted for only a small percentage of the total quantity of lipids extracted from *M. barri.*

In general, aphids had a high proportion of the fatty acids with a carbon chain length of 14 or less and a low proportion of fatty acids with 18 carbon atoms. The reverse was found for the leafhoppers analyzed. The fatty acid composition of an aphid appears to be a species characteristic, not appreciably influenced by the host plant. It changed with the stage of development in some species but not in others. Its possible usefulness in aphid taxonomy is uncertain at present.

Fatty acids were found in the honeydew of two species of aphids. Their relative proportions in the honeydew of the green peach aphid and in the juice of its host suggest that linoleic acid may be required in the diet of that aphid.

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Studies on Lipids in Some Homopterous Insects[1]

MOST OF THE STUDIES of lipids extracted by organic solvents from various insects have been made on leaf- or stem-feeding Lepidoptera, Coleoptera, or Diptera. The values of the iodine numbers used to characterize the crude lipids from these insects—ranging approximately from 100 to 140—indicate that, although the variation is great, the degree of unsaturation is, in general, high.

Gilmour (1961) and Hilditch (1956) have reviewed the occurrence of lipids in insects. The first report on the lipid composition of an aphid was that of Timon-David (1928), who demonstrated that lipids from gall-forming aphids of the genus *Pemphigus* have the remarkably low iodine number of 1.5 and contain a high proportion of the volatile, short-chain fatty acids.

Lipids in insects usually occur in the form of glycerides. Giral (1946), however, noted the virtual absence of glycerides in an orthopteron, *Sphenarium purpurascens* Charpentier. Wren and Mitchell (1959) made a definitive study on the separation of various lipid classes in *Drosophila* spp. and identified several phosphatides and other compound lipids.

Friend (1958) and House (1961) reviewed the literature on the nutritional requirements of insects for specific lipids. To date all insects studied showed a requirement for a sterol (Gilmour, 1961), though not necessarily for the same specific sterol (Levinson and Bergmann, 1957). When critically studied, many insects showed also a requirement for a polyunsaturated fatty acid. Thus, Dadd (1960) reported that linoleic acid was essential for the development of *Locusta migratoria* Linnaeus and *Schistocerca gregaria* (Forsk.). Fraenkel and Blewett (1946) found that either linoleic acid or arachidonic acid could satisfy the requirement of three species of *Ephestia* for a polyunsaturated fatty acid. Classically, the inability of an animal to synthesize, either *in vitro* or *in vivo*, a compound found in its body carries the connotation that this compound is necessary in the animal's diet. Thus, the inability of *Locusta migratoria* to synthesize *in vitro* appreciable amounts of polyunsaturated fatty acids (Tietz, 1961) supports Dadd's findings. Zebe and McShan (1959) reported that *Prodenia eridania* (Cramer) failed to incorporate C^{14}-acetate in significant amounts into polyunsaturated fatty acids *in vitro*. Van Handel and Lum (1961) obtained the same results *in vivo* with *Aedes sollicitans* (Walker) and *A. taeniorhynchus* (Wiedemann).

No reports on the lipid requirements of aphids have been found. Until Mittler and Dadd (1962) developed a method for feeding aphids through a membrane, no one succeeded in feeding aphids artificially for extended periods.

[1] Received for publication July 13, 1962.

The direct analysis of phloem sap would provide some information on an aphid's diet—depending, of course, on the completeness of the analysis. Mittler (1953 and 1958) and von Dehn (1961) identified free amino acids and some sugars in the phloem sap exuded through aphid stylets severed *in situ* by the method of Kennedy and Mittler (1953). However, the minute volumes of sap collected by this method do not permit chemical manipulations other than direct chromatographic analysis. It is often assumed that aphids feed exclusively on phloem sap, but Esau, Namba, and Rasa (1961) demonstrated that this is not the case for *Myzus persicae* (Sulzer), the green peach aphid. Therefore, the determination of an aphid's diet would be somewhat tentative even if a complete analysis of phloem sap were possible.

In an attempt to circumvent the lack of information on the food of aphids, Maltais and Auclair (1952), Mittler (1953), and Lamb (1959) analyzed honeydew; Mittler (1953 and 1958) and Waterhouse (1957) compared such analyses with the sap of the host plant. If such comparisons are valid, even in part, then a compound present in greater abundance in the sap than in the honeydew might be regarded as important if not essential in the aphid's diet.

None of these partial chemical analyses of honeydew indicated the presence of lipids. This circumstance, together with the paucity of information on the occurrence of lipids in aphids, suggested the need for a study on the subject. The purpose of the present investigation is simply to obtain facts which may be used for further, more definitive studies.

MATERIALS AND METHODS

Unless otherwise stated, the aphids analyzed were apterae in various stages. Winged adults, parasitized individuals, unwanted species, debris, etc., were removed from the collections by hand. The remaining aphids were placed in vials containing chloroform and methanol (1:1 v/v). The lipids were extracted with this solvent mixture, after the method of Folch, Lees, and Sloane-Stanley (1957). In some cases the aphids were collected dry, lyophilized, and stored at –10° F under a nitrogen atmosphere until analyzed. With leafhoppers, only the adults were analyzed. Generally, 50 mg of the insects were sufficient for an analysis.

Fractionation. Both a silicic acid column[2] and a Florisil[3] column were used to fractionate the crude lipid extract from one aphid, *Macrosiphum barri* Essig. First a portion of the crude lipid extract was placed on the silicic acid column and the neutral lipids were eluted with chloroform. The phospholipids retained on the column were then eluted with dry methanol (Van Handel, 1959). The phospholipids were weighed but not analyzed further. A second portion of the crude lipid extract was placed on a 12-gm deactivated Florisil column, 1.2 cm × 40 cm, where the lipids were eluted by the method of Carroll (1961) with the solvents indicated in figure 1. Eighty-three 5-ml fractions were collected, the solvents were evaporated *in vacuo*, and the eluted solutes were determined gravimetrically.

Preparation of Methyl Esters. For analyses by gas-liquid chromatography (GLC), the fatty acids extracted from

[2] A slurry in chloroform of 0.5 gm silicic acid (suitable for chromatographic purposes, Mallinckrodt Chemical Works) plus 0.5 gm Hyflo Super-Cell (Johns-Manville Corp.) made a column 0.75 cm × 10 cm.

[3] Florisil is the trade name of a white, granular mixture of magnesium oxide 15.5 per cent, silicon dioxide 84.0 per cent, and sodium sulfate 0.5 per cent, made by the Floridin Company, Tallahassee, Florida.

TABLE 1

CALIBRATION OF THE HYDROGEN FLAME IONIZATION DETECTOR: RESPONSE OF INSTRUMENT TO FATTY ACID METHYL ESTERS IN STANDARD MIXTURE *C*

(Metabolism Study Section, N.I.H.)

Fatty acid	Composition of Mixture *C*		Response of instrument
	As published	As determined by instrument*	
	per cent by weight	*per cent*	*per cent*
C_8	1.55	0.78	50.32
C_{10}	2.99	2.08	69.56
C_{12}	5.96	5.03	84.39
C_{14}	11.96	11.29	94.40
C_{16}	19.41	19.42	100.05
C_{18}	24.91	26.20	105.18
C_{20}	33.19	35.08	105.69

* Calculated from the relative retention times and the heights of the peaks.

all aphids were first converted to their methyl esters, as follows: The crude lipid extract was washed twice with water to remove the methanol and the water-soluble impurities. The chloroform layer was dried over $MgSO_4$, filtered, and evaporated to dryness at 60°C in a rotating evaporator. Two ml of dry methanol, redistilled over KOH and zinc dust, were added and brought to a gentle boil, to dissolve the lipid material. Next, 0.2 ml of 0.1 N sodium methylate in dry methanol was added, the mixture was gently boiled for one minute, after which 15 ml of water were added, plus sufficient 0.1 N HCl to adjust the pH to about 6.8. Esters were extracted three times from the aqueous solution with 5-ml portions of diethyl ether. The ether extracts were combined, dried for 30 minutes over $MgSO_4$, and filtered directly into a Kadurna-Danish flask equipped with a Schneider column. Ether was removed at 45–50° C until the volume was reduced to about 100 μl, and 0.3- to 1.2-μl aliquots of this were injected onto the GLC column, to separate the methyl esters.

Gas-Liquid Chromatography. The liquid phase of the GLC column consisted of polyethylene glycol adipate[4] plus 85 per cent phosphoric acid and was coated on 60/80-mesh Chromosorb W[5]. This was packed into eighth-inch stainless steel tubing, 5 feet long. The carrier gas was water-pumped nitrogen, and the instrument was fitted with a hydrogen flame ionization detector. Originally the polyethylene glycol adipate was 25 per cent of the column, by weight, and the phosphoric acid was 2 per cent, but as the column was operated for several months at 203–213° C, or near the upper limits of the liquid phase, the percentage of the liquid phase decreased appreciably with time. As a result, the exact percentage of the liquid phase for any given analysis was not known.

The GLC apparatus was calibrated with standard mixtures of fatty acid methyl esters.[6] Standard Mixture *C* contains a homologous series of seven methyl esters, from C_8[7] to C_{20}, and was used to test the mass sensitivity of the detector. The response was curvilinear

[4] LAC-2R-446, Cambridge Industries Company, Inc., Cambridge, Mass.

[5] Johns-Mansville Corp.

[6] Standard Mixtures *C* and *D* were obtained from Dr. W. H. Goldwater, Metabolism Study Section, National Institutes of Health.

[7] A shorthand designation is used to indicate the carbon chain length and the degree of unsaturation: C_8 is caprylic acid, $C_{16:1}$ is palmitoleic, and so forth.

for the short-chain fatty acids and approximately linear for fatty acids with chain lengths of C_{14} and longer (table 1). The figures in tables 2 through 10 reflect this nonlinear response. For example, in any given analysis the true amount of C_8 was actually 50 per cent greater than that reported. Standard Mixture *D* was used to test the ability of the column to separate two closely related fatty acid methyl esters. It showed that the resolving power of the column was sufficiently high to give a clean separation of C_{16} from $C_{16:1}$ and of C_{18} from $C_{18:1}$.

The fatty acids present in the lipid extracts were identified by comparing their relative retention times with those of the known standards. The relative amount of any given fatty acid was estimated from the product of the retention time and the peak height. The actual amount could then be calculated on the basis of the percentage response of the detector for that acid, as determined by calibration of the instrument with Standard Mixture *C*.

Samples. Duplicate samples of each extract were analyzed on the GLC column but, for most of the insect collections, duplicate extracts were not prepared. To test the biological variation between collections, duplicate collections of three aphid species were analyzed and the variations obtained for the separate collections of a species were estimated as standard deviations.

Honeydew samples were collected each 24 hours on tared aluminum foil and washed from the foil with tepid water. The aqueous solution was acidified to pH 6.0 and the lipid components were extracted three times with a chloroform:ethanol (5:2 v/v) mixture. Acidification and ethanol helped prevent the formation of troublesome emulsions. The chloroform layer was washed twice with water and dried over $MgSO_4$, and the methyl esters were prepared as described above.

Juice expressed at 5,000 pounds per square inch from freshly frozen sugar beet leaves (*Beta vulgaris*) was centrifuged at 2,000 *g* for 30 minutes. Lipids were extracted from the slightly cloudy supernatant, as described for honeydew, and the methyl esters were prepared.

TABLE 2

FATTY ACID COMPOSITION OF APTERAE OF THREE SPECIES OF APHIDS, WITH THE VARIATIONS BETWEEN DUPLICATE OR TRIPLICATE COLLECTIONS EXPRESSED AS STANDARD DEVIATIONS

Fatty acid	*Myzus persicae* (Sulzer) from tomato	*Macrosiphum euphorbiae* (Thomas). Pink form from oxalis, green and pink forms from myrtle	*Pemphigus bursarius* (Linnaeus) from lettuce roots and a black poplar leaf gall
	per cent	*per cent*	*per cent*
C_4	.....	0.68 ± 0.15	0.65 ± 0.51
C_6	4.37 ± 0.89	3.99 ± 0.61	0.30 ± 0.02
C_8	N.D.*	0.26 ± 0.15	9.37 ± 3.10
C_9	N.D.	1.59 ± 0.14	N.D.
C_{10}	1.41 ± 0.59	0.26 ± 0.09	0.24 ± 0.14
C_{12}	1.91 ± 0.20	5.64 ± 1.06	3.99 ± 0.47
C_{14}	79.03 ± 3.09	71.59 ± 2.10	49.39 ± 4.62
C_{16}	4.05 ± 1.32	3.34 ± 0.67	21.87 ± 3.47
$C_{16:1}$	0.33 ± 0.16	0.59 ± 0.21	0.19 ± 0.11
C_{18}	3.42 ± 1.25	1.66 ± 0.23	4.26 ± 0.97
$C_{18:1}$	2.70 ± 0.34	2.79 ± 0.45	2.22 ± 0.22
$C_{18:2}$	2.38 ± 1.08	0.74 ± 0.07	1.23 ± 0.76
$C_{18:3}$	.....	0.25 ± 0.10	.....
$C_{20:0}$	.....	6.12 ± 2.51	.....

* N.D. = no detectable amount.

RESULTS

Biological Variation. Table 2 gives the analyses of two collections of *Myzus persicae* from tomato (*Lycopersicon esculentum*); three of *Macrosiphum euphorbiae* (Thomas)—the pink form from *Oxalis* sp. and both the green and the pink forms from myrtle (*Vinca* sp.); and two of *Pemphigus bursarius* (Linnaeus)—one from lettuce roots (*Lactuca sativa*) and the other from a leaf gall on black poplar (*Populus nigra*). In general, when a given fatty acid averaged only 1 to 4 per cent of the total sample, the C.V. (coefficient of variation = the standard deviation expressed as a percentage of the mean) ranged from 20 to 50 per cent; when a fatty acid made up 5 to 15 per cent of the sample, the C.V. ranged from 10 to 30 per cent; and when a fatty acid made up more than 20 per cent of the sample, the C.V. ranged from 2.5 to 9 per cent. As the C.V. for any constituent was inversely proportional to its mean, it would require large numbers of samples to show significant differences between those fatty acids that occur in low concentrations. The present investigation was not designed to test whether or not the amounts of the individual fatty acids differed significantly between different aphid species; standard deviations were not determined for each species, and only gross differences are considered significant. From the data in table 2, for example, it is questionable whether the amounts of C_{14} in *Myzus persicae* and *Macrosiphum euphorbiae* differ significantly. However, *Pemphigus bursarius* is considered to have significantly less C_{14} and significantly more C_8 and C_{16} than either of the other two species.

Classes of Lipids in Macrosiphum barri. A collection of *M. barri* from lettuce yielded approximately 173 mg of crude lipid extract. From 16.71 mg of this extract, placed on a silicic acid column, 12.58 mg were recovered with chloroform and only 0.25 mg with methanol. Thus, phospholipids made up about 1.5 per cent of the crude lipid extract. No further studies were made on them.

When 115.5 mg of the crude lipid extract were run on the Florisil column (fig. 1), no significant amounts of sterol esters were eluted, but 80.8 per cent of the crude extract (93.4 mg) was recovered as triglycerides. Approximately 1 mg of sterols was recovered, but the diglycerides were barely detectable and there was little more of the monoglycerides than of the sterols. Free fatty acids made up only 2.8 per cent of the crude extract, as 3.3 mg were recovered. The uneluted material, 12.8 mg by difference, was soluble in 1:1 solution of methanol and water.

The triglycerides were transesterified by the methods described above, and the free fatty acids were esterified with diazomethane. The GLC analyses of these two fractions are shown in figure 2, below an analysis of the unfractionated crude lipid extract. Table 3 gives the percentage of each fatty acid obtained in each of the three analyses. It is of special interest that the analysis of the free fatty acids showed a combined total of 56 per cent for oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$), whereas neither the glycerides nor the unfractionated lipids contained more than 7 or 8 per cent of the two acids combined. Although free fatty acids composed only 2.8 per cent of the crude lipid extract on an actual weight basis, 58 per cent of the linoleic acid and 16 per cent of the oleic acid in the crude extract occurred in the free form.

Host-Plant Influence on Fatty Acid Composition. Table 4 contains the results of analyses performed on apterae of *Myzus persicae* and *Macrosiphum euphorbiae* from various hosts. Considering the normal biological variation, there is no indication that the host plant appreciably affected the fatty acid composition of either of these

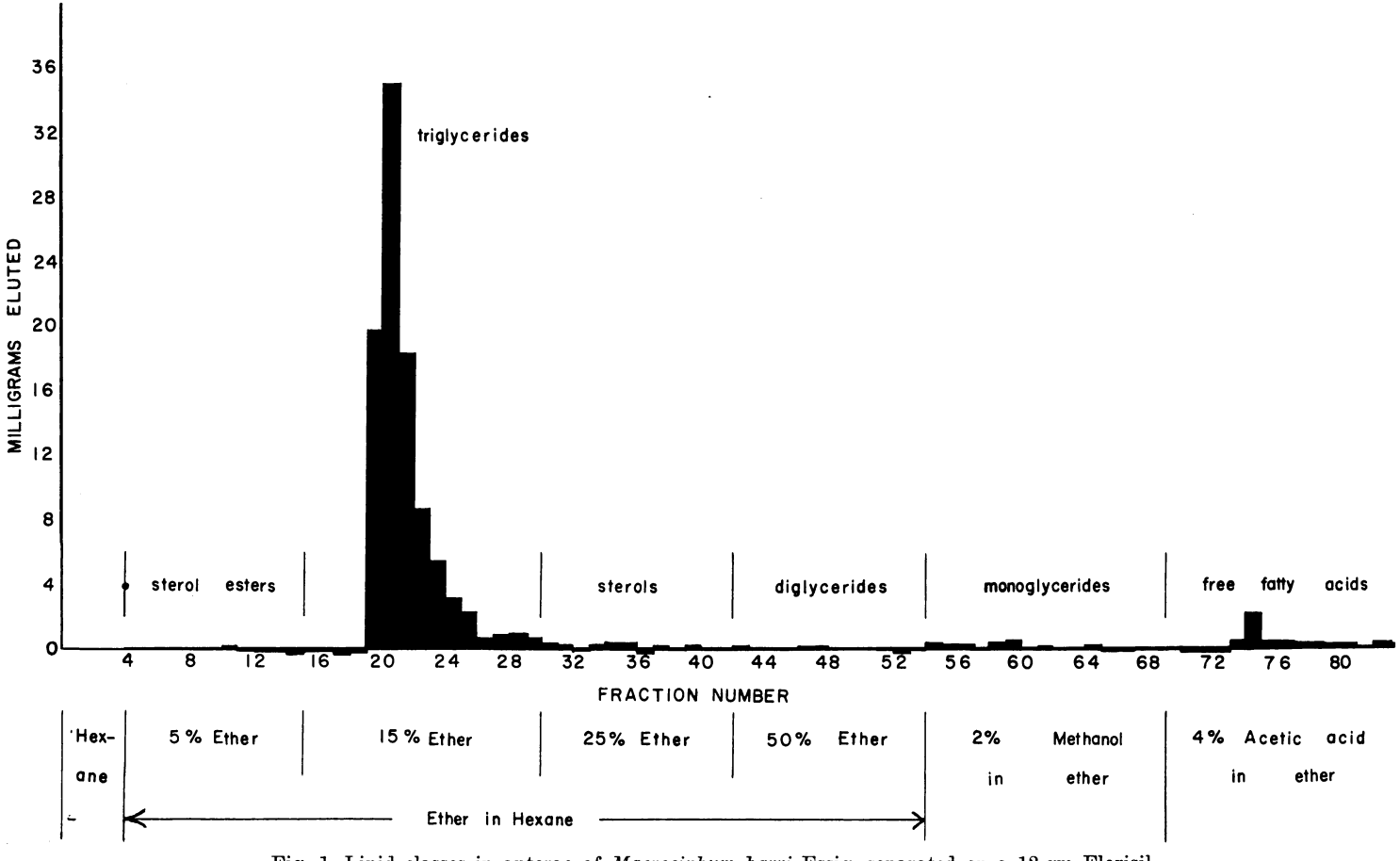

Fig. 1. Lipid classes in apterae of *Macrosiphum barri* Essig, separated on a 12-gm Florisil column; 5-ml fractions were collected. Total load was 115.5 mg of lipid extract, and total recovery was 102.7 mg.

insects. Similarly, apterae of *Pemphigus bursarius* collected from lettuce roots did not differ appreciably in fatty acid composition from those collected from the black poplar galls. Although the figures are not shown in the table, apterae of *Rhopalosiphum maidis* (Fitch) from sorghum (*Sorghum vulgare*) had the same fatty acid composition, within the limits of biological variation, as those from johnsongrass (*Sorghum halepense*). Thus, it appears that the fatty acid composition of aphids may be a characteristic of the species rather than a manifestation of diet.

Physiological Age and Fatty Acid Composition. In some insects, the fat composition changes during the life cycle (Gilmour, 1961). To test aphids for such changes, *Myzus persicae* was analyzed at three different stages and three other species at two stages each. Table 5 shows that there was no difference between apterous adults of *M. persicae* and first- or second-instar nymphs. Nymphs were not separated from apterous adults in any of the other analyses. There was no significant difference between alatae and apterae of either *Macrosiphum barri* or *M. granarium* (Kirby). However, in *Rhopalosiphum fitchii* (Sanderson) and in *Myzus persicae,* the fatty acid composition of alatae was appreciably different from that of the apterae analyzed. Alatae of *R. fitchii* had more C_{14} and less C_{16} than apterae. The reverse occurred in *M. persicae:* alatae had less C_{14} and more C_{16} than either nymphs or apterous adults. Thus, it appears that the various fat changes during aphid life cycles are perhaps characteristic for certain species rather than for aphids in general. These changes were probably not related to the host plant, for both *Macrosiphum granarium* and *R. fitchii* were reared on barley (*Hordeum vulgare*) and all three stages of *Myzus persicae* were collected from the same sugar beet plant.

Fatty Acid Compositions of Different Aphids and Leafhoppers. Fatty acid compositions were determined for 21 species of aphids (tables 2, 6, 7, and 8) and six species of leafhoppers (table 9). The percentages of particular fatty acids varied widely between aphids and leafhoppers and between different species within each group. However, some patterns are evident. Aphids have relatively large amounts of C_{14}—from 33 to 87 per cent of the total fatty acid content—and small amounts of the C_{18} series—rarely more than 5 to 10 per cent of C_{18}, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ together. These statements apply to all aphid species analyzed except *Rhopalosiphum nymphaeae* (Linnaeus) in table 7 and *Therioaphis maculata* (Buckton) in table 8. All four species of *Aphis* in table 6 had relatively large amounts of C_9, and so did *Macrosiphum granarium* and *M. rosae* (Linnaeus) in table 6 and *Eriosoma lanigerum* (Hausmann) in table 8. Four of the five species of *Rhopalosiphum* in table 7 had nearly as much C_{16} as C_{14}, or more—a condition not observed in the other aphids analyzed. This might be a characteristic of the genus.

In contrast to aphids, leafhoppers (table 9) had only a small amount of C_{14} (usually less than 2 per cent) and a large amount of the C_{18} series—especially of $C_{18:1}$, which ranged from 29 to 63 per cent. Although not many species of leafhoppers were analyzed, there were some striking differences in fatty acid patterns. For example, *Erythroneura elegantula* Osborne, taken from grape (*Vitis vinifera*), had nearly 33 per cent $C_{18:3}$ and *Empoasca filamenta* DeLong, collected from gaillardia (*Gaillardia pulchella*), had 29 per cent. In contrast to this, four species, viz., *Circulifer tenellus* (Baker) from sugar beet, *Hordnia circellata* (Baker) from yeddo-hawthorn (*Rhaphiolepis umbellata*), *Colladonus montanus* (Van Duzee) from *Brassica* sp., and *Macrosteles fascifrons* (Stål) from celery (*Apium graveolens*), had little or no $C_{18:3}$. Figure 3 illustrates graphically the fatty

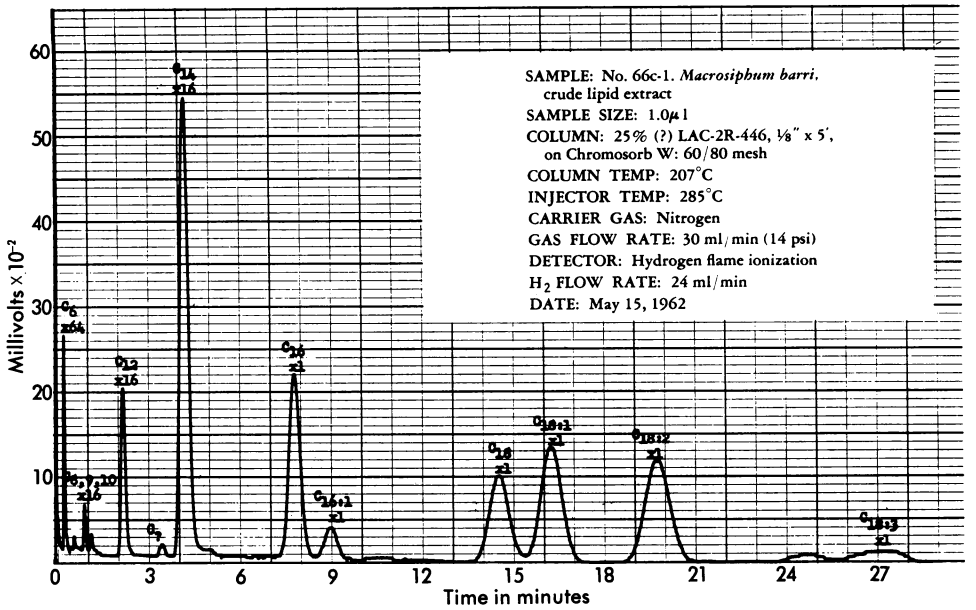

Fig. 2*A*. GLC chromatogram of lipids extracted from apterae of *Macrosiphum barri* Essig: crude lipid extract.

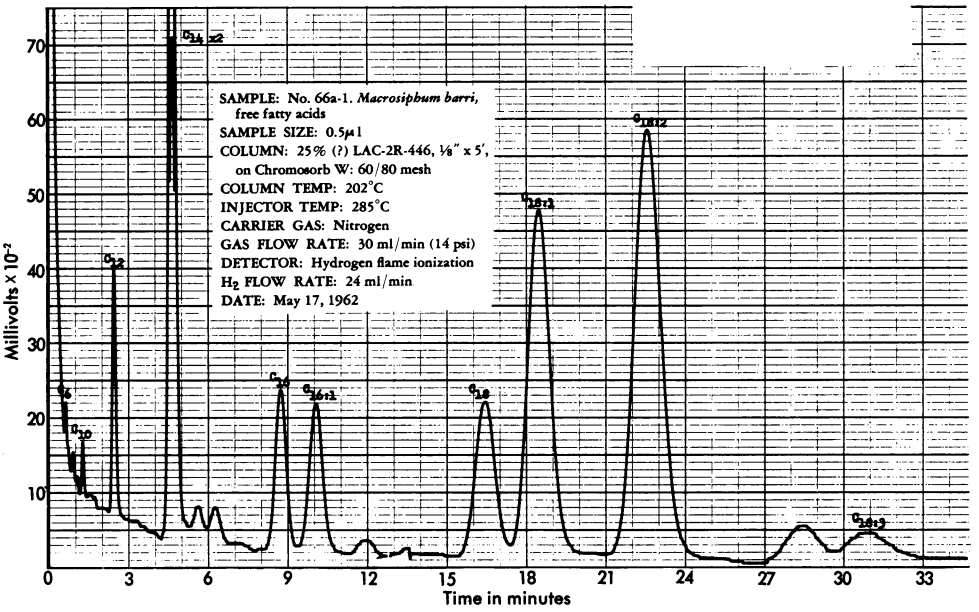

Fig. 2*B*. GLC chromatogram of lipids extracted from apterae of *M. barri:* free fatty acid fraction from Florisil column.

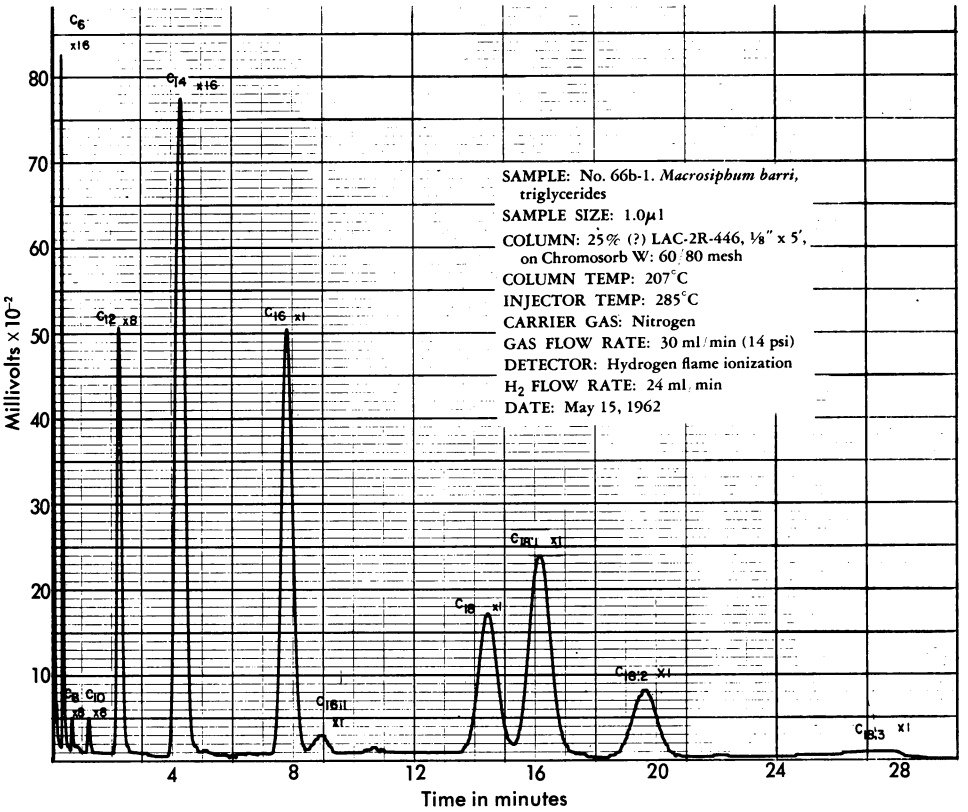

Fig. 2C. GLC chromatogram of lipids extracted from apterae of *M. barri:* triglyceride fraction from Florisil column.

TABLE 3

FATTY ACID COMPOSITION OF CRUDE AND FRACTIONATED LIPIDS FROM APTERAE OF *Macrosiphum barri* ESSIG REARED ON LETTUCE

Fatty acid	Lipid fraction		Unfractionated lipid extract
	Free fatty acids	Triglycerides	
	per cent	*per cent*	*per cent*
C_6	Tr*	5.62	4.27
C_8	Tr	0.25	0.31
C_9	0.51	Tr	1.42
C_{10}	0.28	0.48	0.68
C_{12}	2.56	11.15	12.54
C_{14}	18.52	67.34	65.61
C_{16}	4.42	4.95	2.97
$C_{16:1}$	5.25	0.26	0.64
C_{18}	7.74	3.04	2.58
$C_{18:1}$	22.85	4.92	3.89
$C_{18:2}$	33.20	1.99	4.13
$C_{18:3}$	2.78	Tr	0.35
$C_{20:0}$	1.87	Tr	0.57

* Tr = trace: not more than 0.15 per cent.

TABLE 4

FATTY ACID COMPOSITION OF APTERAE OF *Myzus persicae* (SULZER) AND *Macrosiphum euphorbiae* (THOMAS) FROM VARIOUS HOSTS

Fatty acid	Hosts of *Myzus persicae*							Hosts of *Macrosiphum euphorbiae*	
	Tomato, 8 leaves	*Hibiscus* sp., mature	Cabbage, senescing	Sugar beet leaves				Myrtle, blooming	Oxalis, blooming
				Young	Mature	Senescing	57° F*		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
C_4	....	....	....	....	....	....	....	0.79	0.94
C_6	4.88	2.89	6.05	2.85	3.16	1.85	2.18	3.64	4.58
C_8	N.D.†	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Tr‡	0.47
C_9	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.65	1.22
C_{10}	1.54	1.00	1.96	1.54	1.28	2.17	0.60	0.31	Tr
C_{12}	1.81	2.35	2.20	1.13	2.03	1.29	2.18	6.30	4.23
C_{14}	80.86	86.56	82.94	84.88	78.74	84.60	85.97	71.21	74.65
C_{16}	3.31	3.20	3.12	2.52	3.48	2.65	3.80	3.40	3.02
$C_{16:1}$	0.45	0.11	Tr	Tr	0.97	0.32	0.97	0.70	0.33
C?	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.38	0.32
C_{18}	2.72	1.21	2.31	2.31	2.58	2.29	1.56	1.71	1.51
$C_{18:1}$	2.57	1.27	1.39	2.55	3.56	2.43	1.97	3.01	2.33
$C_{18:2}$	1.78	1.11	Tr	2.15	4.17	2.39	0.54	0.73	0.68
$C_{18:3}$	....	....	....	....	....	....	....	0.25	0.32
$C_{20:0}$	....	....	....	....	....	....	....	6.30	5.36

* For the eight days before the analysis the plant with aphids was held at 57°F under 14 hours of light daily.
† N.D. = no detectable amount.
‡ Tr = trace: not more than 0.15 per cent.

TABLE 5

FATTY ACID COMPOSITION IN FOUR APHID SPECIES, COMPARING DIFFERENT STAGES OF THE LIFE CYCLE

Fatty acid	*Macrosiphum granarium* (Kirby) from barley		*Rhopalosiphum fitchii* (Sanderson) from barley		*Macrosiphum barri* Essig from lettuce		*Myzus persicae* (Sulzer) from sugar beet leaves		
	Apterae	Alatae	Apterae	Alatae	Apterae	Alatae	Nymphae*	Adult apterae	Alatae
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
C_4	N.D.†	1.96	....	....	Tr‡	0.29	....	....	....
C_6	5.51	2.58	1.98	1.55	3.81	2.89	2.15	3.16	1.74
C_8	Tr	Tr	Tr	N.D.	Tr	Tr	N.D.	N.D.	N.D.
C_9	5.61	2.58	1.57	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
C_{10}	Tr	Tr	N.D.	0.57	0.42	0.58	2.08	1.28	0.65
C_{12}	2.16	1.25	0.58	2.69	10.83	14.80	1.73	2.03	1.73
C?	Tr	Tr	9.15	5.91	N.D.	N.D.	N.D.	N.D.	N.D.
C_{14}	79.73	82.41	32.86	48.56	66.10	67.91	78.10	78.74	58.86
C_{16}	3.26	3.60	42.67	22.18	4.07	3.72	3.41	3.48	9.03
$C_{16:1}$	0.29	0.37	Tr	0.71	0.71	0.83	1.62	0.97	1.88
$C_{16:2}$§	N.D.	Tr	0.95	0.34	Tr	Tr	Tr	Tr	Tr
C_{18}	1.01	1.47	3.41	3.31	2.74	1.74	2.26	2.58	7.68
$C_{18:1}$	0.87	1.25	3.12	6.56	5.78	5.58	3.55	3.56	8.57
$C_{18:2}$	0.53	1.30	3.76	7.89	4.07	1.25	5.07	4.17	9.84
$C_{18:3}$	1.01	1.20	....	....	....	....	....	....	....

* First or second instar.
† N.D. = no detectable amount.
‡ Tr = trace: not more than 0.15 per cent.
§ Identification not certain.

TABLE 6

FATTY ACID COMPOSITION OF APTERAE OF FIVE SPECIES OF *Macrosiphum* AND FOUR OF *Aphis*

Fatty acid	*M. granarium* (Kirby) from barley	*M. barri* Essig from lettuce	*M. pisi* (Harris) from alfalfa	*M. euphorbiae* (Thomas) from myrtle	*M. rosae* (Linnaeus) from *Rosa* sp.	*A. nerii* Fonscolombe from *Nerium oleander*	*A. fabae* Scopoli from *Chrysanthemum* sp.	*A. gossypii* Glover from *Viburnum japonicum*	*A. helichrysi* Kaltenbach from *Vinca* sp.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
C_4	N.D.*	Tr†	0.72	0.79	0.11	Tr	N.D.	0.39	Tr
C_{iso-6}	N.D.	N.D.	0.84	N.D.	0.80	N.D.	N.D.	N.D.	0.90
C_6	5.51	4.27	3.37	3.64	2.99	Tr	1.79	18.27	3.29
C_8	Tr	0.31	Tr	Tr	Tr	N.D.	N.D.	N.D.	N.D.
C_9	5.61	1.42	0.33	1.65	5.23	12.97	6.40	7.62	11.18
C_{10}	Tr	0.68	1.44	0.31	Tr	Tr	Tr	N.D.	Tr
C_{12}	2.16	12.54	12.41	6.30	1.87	3.98	3.11	1.69	2.60
C_{14}	79.73	65.61	57.25	71.21	78.79	68.19	70.16	34.60	76.59
$C?_{-1}$‡	Tr	....	2.72	Tr	0.17	N.D.	N.D.	N.D.	Tr
C_{16}	3.26	2.97	4.01	3.40	3.59	9.50	13.03	24.71	2.21
$C_{16:1}$	0.29	0.64	0.85	0.70	1.56	N.D.	N.D.	0.27	1.02
$C?_{-2}$	N.D.	....	Tr	0.38	0.29	N.D.	N.D.	0.21	0.17
C_{18}	1.01	2.58	2.35	1.71	0.75	0.95	1.44	0.71	0.43
$C_{18:1}$	0.87	3.89	5.49	3.01	1.61	2.21	1.83	0.86	1.11
$C_{18:2}$	0.53	4.13	3.45	0.73	0.78	1.50	2.24	6.40	0.46
$C_{18:3}$	1.01	0.35	0.36	0.25	0.39	....	....	1.23	Tr
$C_{20:0}$	N.D.	0.57	4.01	6.30	1.05	....	....	N.D.	N.D.

* N.D. = no detectable amount.
† Tr = trace: not more than 0.15 per cent.
‡ $C?_{-1}$, represents unknown peak number 1; $C?_{-2}$, unknown peak number 2.

TABLE 7

FATTY ACID COMPOSITION OF APTERAE OF FIVE SPECIES OF *Rhopalosiphum*

Fatty acid	*R. fitchii* (Sanderson) from barley	*R. pseudo-brassicae* (Davis) from mustard (*Brassica* sp.)	*R. maidis* (Fitch) from sorghum	*R. nymphaeae* (Linnaeus) from duckweed (*Lemna* sp.)	*R. padi** (Linnaeus) from barley
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
C_4	N.D.†	Tr‡	N.D.	N.D.	1.43
C_6	1.98	2.40	4.74	1.27	3.28
C_8	Tr	Tr	Tr	Tr	Tr
C_9	1.57	2.19	N.D.	N.D.	1.68
C_{10}	N.D.	Tr	Tr	Tr	0.41
C_{12}	0.58	1.01	1.65	0.41	9.37
$C?_{-1}$§	9.15	N.D.	N.D.	7.94	N.D.
C_{14}	32.86	32.72	45.18	18.70	54.00
C_{16}	42.67	54.70	37.60	63.43	11.75
$C_{16:1}$	Tr	Tr	Tr	Tr	0.40
$C?_{-2}$	0.95	N.D.	N.D.	Tr	0.95
C_{18}	3.41	4.08	3.83	4.93	1.28
$C_{18:1}$	3.12	2.13	4.00	1.92	3.32
$C_{18:2}$	3.76	0.75	2.98	1.38	1.04
$C_{18:3}$	....	Tr	....	....	Tr
$C_{20:0}$	....	N.D.	....	....	10.10

* Identification not certain; specimens are apparently in a complex composed of *R. fitchii* and *R. padi* (Richards, 1960).
† N.D. = no detectable amount.
‡ Tr = trace: not more than 0.15 per cent.
§ $C?_{-1}$ represents unknown peak number 1; $C?_{-2}$, unknown peak number 2.

TABLE 8

FATTY ACID COMPOSITION OF APTERAE OF FIVE SPECIES OF APHIDS

Fatty acid	*Eriosoma lanigerum* (Hausmann) from flowering plum (*Prunus* sp.)	*Toxoptera aurantii* (Fonscolombe) from *Camellia* sp.	*Brevicoryne brassicae* (Linnaeus) from radish (*Raphanus sativus*)	*Hyalopterus arundinis* (Fabricius) from apricot (*Prunus armeniaca*)	*Therioaphis maculata* (Buckton) from alfalfa
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
C_4	0.91	Tr*	....	N.D.†	1.71
C_{iso-6}	0.24	2.04	N.D.	N.D.	N.D.
C_6	1.39	0.55	1.23	4.68	9.29
C_8	Tr	Tr	0.14	N.D.	0.51
C_9	12.38	2.27	0.23	N.D.	1.84
C_{10}	Tr	Tr	2.67	Tr	1.12
C_{12}	10.81	4.81	8.08	3.33	9.61
C_{14}	56.36	62.39	60.00	50.85	38.31
C_{16}	13.62	22.08	19.44	36.82	11.74
$C_{16:1}$	0.58	Tr	0.13	0.28	0.74
C_{18}	1.61	1.26	2.49	1.47	6.40
$C_{18:1}$	1.15	1.84	2.13	1.07	5.37
$C_{18:2}$	0.48	0.74	1.31	1.50	8.59
$C_{18:3}$	0.42	Tr	1.25	N.D.	1.68
$C_{20:0}$	N.D.	2.25	0.87	N.D.	N.D.

* Tr = trace: not more than 0.15 per cent.
† N.D. = no detectable amount.

TABLE 9

FATTY ACID COMPOSITION OF ADULTS OF SIX SPECIES OF LEAFHOPPERS*

Fatty acid	*Erythroneura elegantula* Osborne from grape	*Empoasca filamenta* DeLong from gaillardia	*Circulifer tenellus* (Baker) from sugar beet	*Hordnia circellata* (Baker) from yeddo-hawthorn	*Colladonus montanus* (Van Duzee) from *Brassica* sp.	*Macrosteles fascifrons* (Stål) from celery	
						Short-winged	Long-winged
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
C_4	Tr†	....	0.13	....	0.97	Tr	4.31
C_6	Tr	0.02	Tr	0.19	Tr	Tr	Tr
C_8	N.D.‡	N.D.	Tr	Tr	Tr	Tr	N.D.
C_{10}	Tr	N.D.	0.13	Tr	Tr	0.02	N.D.
C_{12}	0.07	0.25	0.36	1.25	0.26	0.31	0.31
C_{14}	0.79	0.73	1.48	8.30	1.18	2.13	2.08
C_{16}	14.73	23.11	18.52	36.13	16.50	20.59	17.73
$C_{16:1}$	4.62	3.37	2.72	2.11	0.89	1.73	1.76
C_{18}	1.81	1.47	3.14	1.84	2.40	3.59	3.50
$C_{18:1}$	40.27	28.80	51.93	32.70	63.01	61.67	56.39
$C_{18:2}$	3.88	12.69	20.94	16.52	14.79	10.32	13.73
$C_{18:3}$	32.64	28.70	0.56	0.96	0.00	0.00	0.00

* All species except *Hordnia circellata* are phloem feeders.
† Tr = trace: not more than 0.15 per cent
‡ N.D. = no detectable amount.

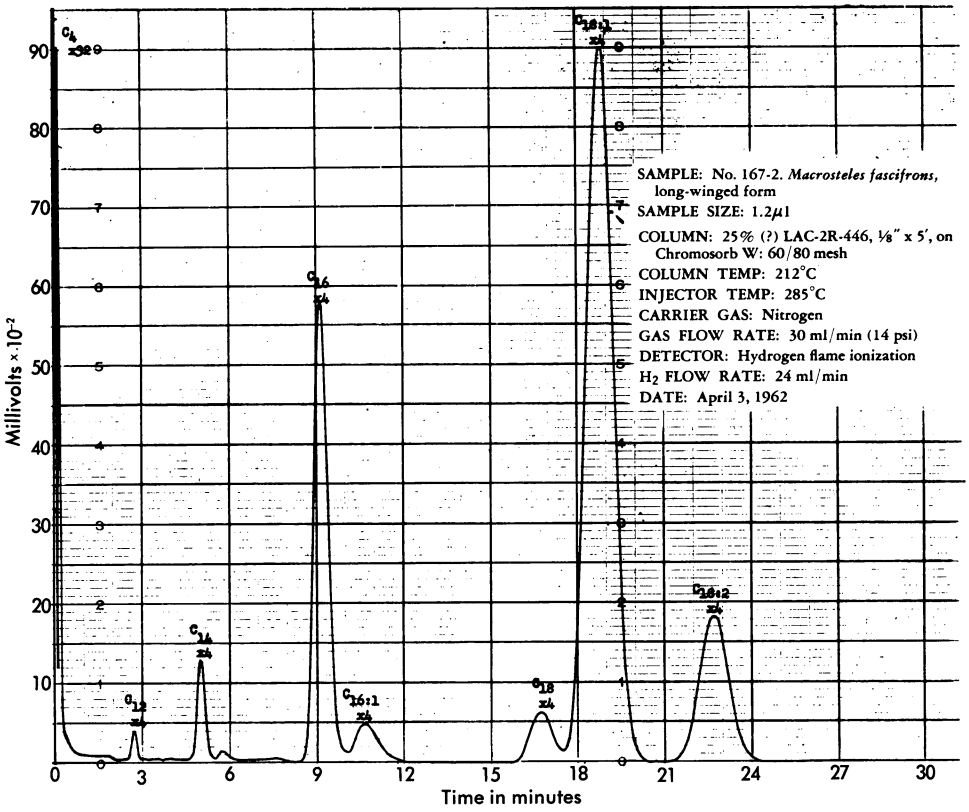

Fig. 3. GLC chromatogram of fatty acids extracted from adults of a typical leafhopper, *Macrosteles fascifrons* (Stål), long-winged form. Compare the peaks representing C_{14} and the C_{18} series with those in fig. 2*A*.

acid pattern in the leafhopper *M. fascifrons.*

Occurrence of Fatty Acids in Honeydew and in Plant Juice. Airdried honeydew from *Myzus persicae* yielded 9.10 mg of material extractable with chloroform:ethanol. This was 12.55 per cent of the 72.59 mg collected over a three-day period. Honeydew of *Therioaphis maculata* yielded 10.7 mg of ether-extractable material—15.88 per cent of the 67.5 mg of honeydew. The pressed sugar beet leaves yielded 7.5 mg of ether-extractable material—0.042 per cent by weight of the 18 ml of fresh juice.

Table 10 and figure 4 show the analyses of these extracts. Several unidentified peaks—*a, b, c, d, e,* and *f*—appear in the same locations in the three chromatograms and probably represent the same materials, presumably fatty acids. The most conspicuous difference in the three analyses was that honeydew of *Myzus persicae* contained very little $C_{18:2}$, although that fatty acid was the third most abundant in the beet-leaf juice and fourth in the honeydew of *Therioaphis maculata.* However, the total C_{18} series in *M. persicae* honeydew had a percentage midway between that in the other two analyses and higher than that in the plant juice.

TABLE 10
FATTY ACID COMPOSITION OF HONEYDEW AND OF SUGAR BEET LEAF JUICE

Fatty acid	Honeydew from		Sugar beet leaf juice
	Therioaphis maculata (Buckton) on alfalfa	*Myzus persicae* (Sulzer) on sugar beet	
	per cent	*per cent*	*per cent*
C_4	....	N.D.*	Tr†
C_6	Tr	Tr	Tr
C_8	0.12	1.01	Tr
C_9	0.12	0.86	0.99
C_{10}	0.28	1.12	0.67
C?-a‡	0.23	0.23	0.72
C?-b	0.51	0.46	0.97
C_{12}	0.64	3.12	1.57
C?-c	0.43	0.76	1.22
C_{14}	11.38	18.20	25.01
C?-d	4.64	2.65	1.95
C?-e	2.29	0.67	1.80
C_{16}	18.52	18.97	18.60
$C_{16:1}$	8.63	7.79	3.67
C?-f	1.85	0.63	3.16
C_{18}	4.13	15.29	5.56
$C_{18:1}$	35.67	16.33	10.11
$C_{18:2}$	9.32	1.03	11.66
$C_{18:3}$	....	9.70	9.14
$C_{20:0}$	....	1.16	3.17

* N.D. = no detectable amount.
† Tr = trace: not more than 0.15 per cent.
‡ The unidentified peaks are indicated as C?-a, C?-b, and so forth. They had the same relative retention times in each of the three analyses.

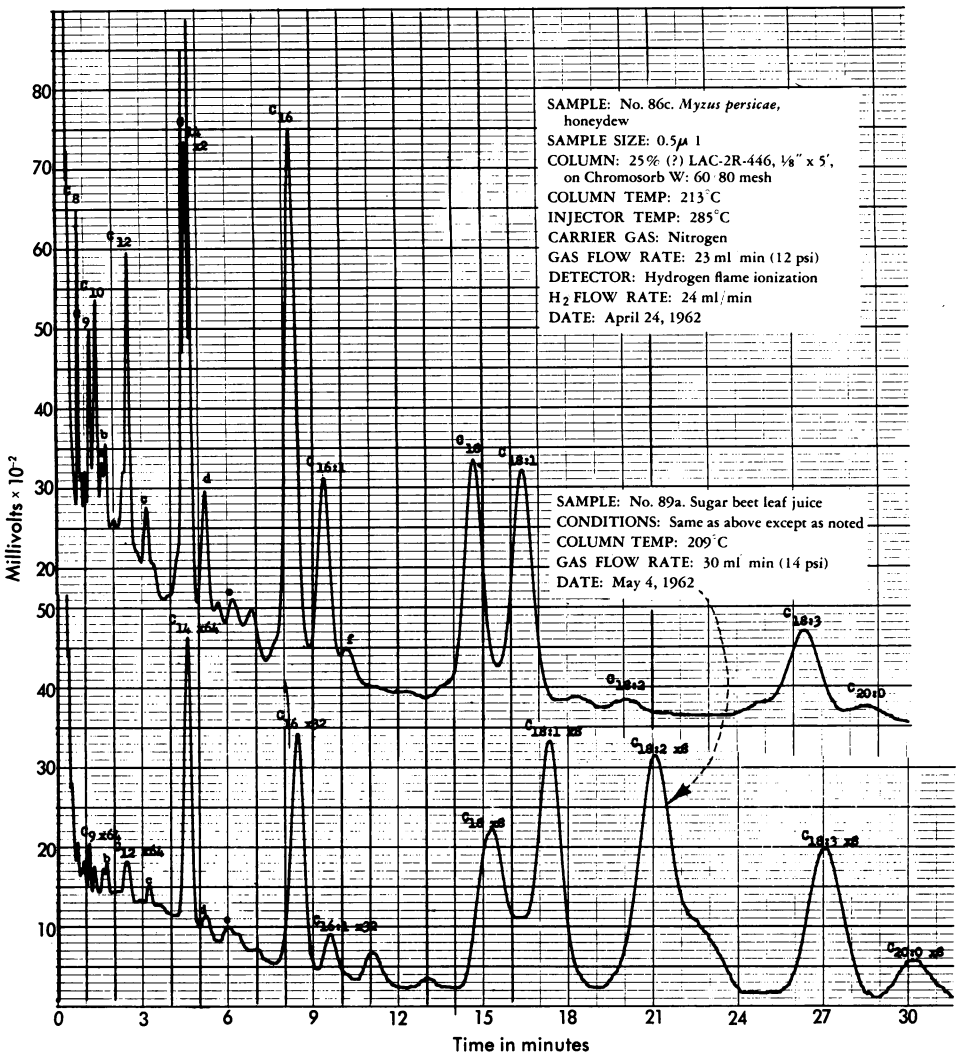

Fig. 4. GLC chromatograms showing the relationship of the fatty acids in green peach aphid honeydew and those in expressed sugar beet leaf juice. The relative retention times do not match exactly, as the column conditions were slightly different.

DISCUSSION

The possible taxonomic value of lipid studies such as these has been the subject of much debate in this laboratory. Although no great numbers of aphid species were analyzed, the data suggest evident patterns in the lipid relationships for certain species. The taxonomic value of such relationships cannot be assessed properly without more analyses. Meanwhile, these lipid relationships may have a limited taxonomic usefulness. For example, aphids collected from barley in the Salinas Valley, California, were tentatively identified as *Rhopalosiphum fitchii,* but an error in identification was suspected because the fatty acid analysis of this collection was in such disharmony with analyses of other collections of *R. fitchii.* Winged adults of the Salinas

collection identified by the California State Department of Agriculture were returned with the label *Rhopalosiphum padi* (Linnaeus) complex. Richards' (1960) discussion of this complex corroborates the evidence of the lipid studies that two species are involved.

Gilmour (1961) cited several examples to illustrate that the composition of fats in insects is influenced to a certain extent by diet. In general, high-fat diets influence body-fat composition to a greater extent than do low-fat diets. The approximate fat composition of most leafy vegetables is only 0.2 to 0.4 per cent of the fresh weight (Peterson and Strong, 1953). Such a low percentage implies that the fatty acid compositions even of unrelated hosts cannot differ so much that they would alter the body fat of aphids appreciably—even if one assumes that an aphid's body-fat composition is dependent on its diet. Moreover, a comparison of the analyses for two aphids from the same host shows that such an assumption is not completely true: *Therioaphis maculata* in table 8 and *Macrosiphum pisi* (Harris) in table 6, both collected from Vernal alfalfa (*Medicago sativa*), had different fatty acid patterns.

The question whether aphids require any lipid materials in their diets cannot be answered definitely until techniques for artificial feeding of aphids are perfected. However, the present study suggests that aphids require at least some lipids. *Myzus persicae* may require some linoleic acid in its diet, because this fatty acid was abundant in sugar beet leaf juice but virtually absent from the honeydew emitted by aphids fed on sugar beet leaves. The close qualitative agreement in other respects between the two chromatograms of figure 4 indicates that the plant juice analyzed was fairly representative of the aphids' diet, though leaf juice was analyzed instead of phloem sap. Only three of 11 extracts from *M. persicae* apterae (tables 2, 4, and 5) contained as much as 4 per cent linoleic acid, but in extracts from alatae (table 5), *M. persicae* had 9.84 per cent linoleic acid and *Rhopalosiphum fitchii* 7.89 per cent. The highest percentage of linoleic acid in any extract from apterae was 8.59 per cent in *Therioaphis maculata* (table 8), whose honeydew also had a high content—9.23 per cent.

If, indeed, the green peach aphid requires $C_{18:2}$, the means by which it acquires this material should be of great interest. Zimmermann's (1960) review of the transport of organic substances in phloem does not mention lipids. Ziegler (1956) reported phosphatides in sieve tube exudates of several trees in concentrations below 0.1 mg/ml but considered these nonmobile. Further studies are needed on the lipids in honeydew and on the form in which lipids occur in plant juice, especially in sieve tube exudates.

SUMMARY

The fatty acid composition was determined for 21 species of aphids and six species of leafhoppers. In addition, the classes of lipids extracted from the aphid *Macrosiphum barri* were separated by column chromatography. Nearly all of the fatty acids in *M. barri* were in the form of triglycerides, and the free fatty acids made up less than 3 per cent of the total lipid content. In general, aphids had a high proportion of C_{14} and a low proportion of the C_{18} series, and the reverse was found for leafhoppers. The fatty acid composition of an aphid appears to be a species characteristic, not appreciably influenced by the host plant. It changed with the stage of development in some instances but not in others. Fatty acids were found in the honeydew analyzed for two species of aphids. Their relative proportions in the honeydew of the green peach aphid and in the juice of its host plant suggest that linoleic acid may be required in the diet of that aphid.

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