Diacyl, Alkylacyl, and Alkenylacyl Phospholipids of Meloidogyne javanica Females[1]

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Abstract: The phospholipid composition and acyl, alkyl, and alkenyl group compositions of diacyl, alkylacyl, and alkenylacyl phosphoglycerides of *M. javanica* were investigated. Phospholipid was comprised of 61.7% choline phosphoglyceride, 22.0% ethanolamine phosphoglyceride, and smaller quantities of six other lipids. Phospholipid fatty acid was more unsaturated than neutral lipid fatty acid and contained 61.3% octadecenoic (18:1) acid. Fatty acid at the 1-position of diacyl phospholipids was shorter and more saturated than that at the 2-position. Compared to choline phosphoglyceride, ethanolamine phosphoglyceride contained less 18:1 and 20:5 and more 18:0 and 20:0 acid. Alkenylacyl and alkylacyl compounds comprised 34.6% and 9.3%, respectively, of the ethanolamine phosphoglyceride but only 0.5% and 0.6% of the choline phosphoglyceride. Alkenylacyl and alkylacyl ethanolamine phosphoglycerides contained a smaller percentage of 20-carbon polyunsaturated acid at their 2-positions than did their diacyl analogue. At least 95% of the alkenyl and alkyl groups were 18:0 compounds. Tomato roots did not contain alkenylacyl or alkylacyl phosphoglycerides; their occurrence in *M. javanica* is a significant biochemical difference between the nematode and its host. *Key words:* lipids, ether lipids, plasmalogens, fatty acids, tomato, root-knot nematode.

Even though plant-parasitic nematodes are rich sources of lipid (7,16,18,22,23), knowledge of their phospholipid composition is limited to the polar lipid (i.e., phospholipid plus glycolipid) or phospholipid fatty acid composition of *Meloidogyne arenaria, M. incognita,* and *Globodera solanacearum* (18,22). More information about nematode phospholipids is needed because phospholipid molecular species composition is an important variable among animal species, tissues, and organelles (10). Furthermore, in addition to the more commonly studied diacyl phosphoglycerides, alkenylacyl (or plasmalogen) and alkylacyl phosphoglycerides (Fig. 1) occur in many animals, but presence of these ether-containing lipids in plant-parasitic nematodes is unknown. The purpose of this study was to quantify the phospholipid composition, investigate the occurrence of ether-containing phospholipids, and determine the positional distribution of acyl, alkyl, and alkenyl groups in individual phospholipids from females of *M. javanica*. For comparative purposes, the neutral lipid fatty acid composition was also investigated.

Received for publication 20 October 1980.

[1]Scientific Article No. A2806, Contribution No. 5856 of the Maryland Agricultural Experiment Station. Adapted from a portion of a Ph.D. dissertation by the senior author. This investigation was supported in part by Regional Research Project NE-101, Cooperative State Research Service, U.S. Department of Agriculture. The authors thank Dr. R. S. Hussey for the culture of *M. javanica*.

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MATERIALS AND METHODS

M. javanica was propagated on greenhouse cultures of *Lycopersicon esculentum* 'Rutgers,' and females from seven-week-old cultures were obtained by maceration of root sections (12). Lipid from lyophilized nematodes was extracted by the method of Folch et al. (9). Total lipid was fractionated into neutral lipid, glycolipid, and phospholipid with a column of 100–200 mesh Unisil (Clarkson Chemical Co., Williamsport, Pennsylvania) (24).

Fatty acid methyl esters were prepared from neutral lipid and phospholipid by transesterification with BCl_3-methanol and purified by thin-layer chromatography (TLC) on glass plates coated with Silica Gel G (E. Merck, Darmstadt, West Germany) with benzene as the developing solvent (20). Methyl esters were identified and quantified as previously described (15, 17).

Individual phospholipids in the phospholipid fraction were identified, quantified, and purified for subsequent analysis by several chromatographic procedures and chemical tests (6). Fatty acid methyl esters were prepared from purified choline and ethanolamine phosphoglycerides as above.

Because diacyl, alkylacyl, and alkenylacyl phospholipids have not yet been separated from each other in the intact state, they were quantified and analyzed as their glyceride acetate derivatives. Therefore, 1,2-diacyl-, 1,3-diacyl-, 1-alkyl-2-acyl-, and 1-alkenyl-2-acylglycerol acetates were pre-

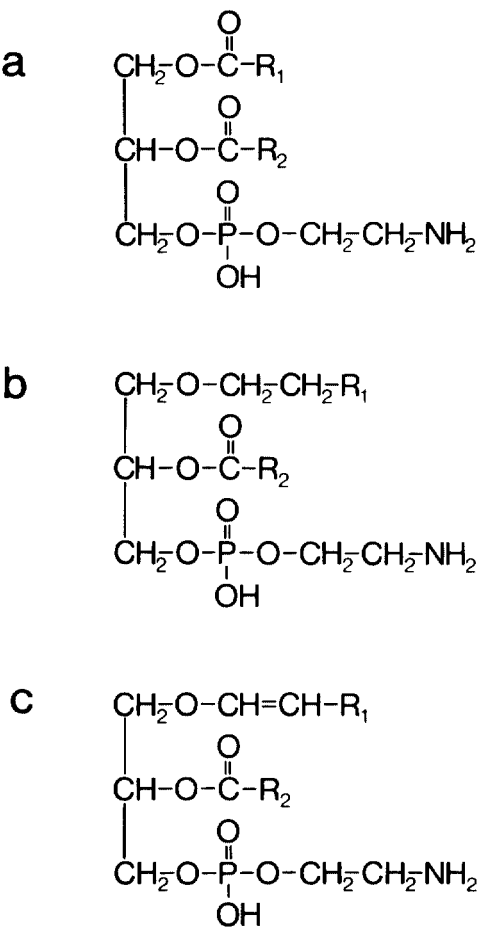

Fig. 1. Ethanolamine phosphoglycerides: 1,2-diacyl ethanolamine phosphoglyceride, or phosphatidylethanolamine (a); 1-alkyl-2-acyl ethanolamine phosphoglyceride (b); and 1-alkenyl-2-acyl ethanolamine phosphoglyceride, or ethanolamine plasmalogen (c).

pared from choline and ethanolamine phosphoglycerides, separated by three TLC systems, and quantified by photodensitometry (6). We assumed that the small amounts of 1,3-diacylglycerol acetates were artifacts, resulting from acyl migration in 1,2-diacyl compounds, and we discarded them. Positional distribution of fatty acids in 1,2-diacylglycerol acetates, acyl and alkyl group compositions of 1-alkyl-2-acylglycerol acetates, and acyl and alkenyl group compositions of 1-alkenyl-2-acylglycerol acetates were determined as previously described (6).

The presence of plasmalogens and alkyl-acyl phosphoglycerides in tomato roots was determined. Lipid was extracted from lyophilized roots by the method of Nichols (21), and phospholipid was isolated from total lipid as described above. Presence of alkenylacyl phospholipids was determined by HCl-reaction TLC (25), which detects aldehydes derived from alkenyl groups. Presence of alkylacyl phosphoglycerides was determined by saponification of phospholipase C hydrolysis products and subsequent TLC (4), which detects liberated alkylglycerols.

Results presented are the means of three separate experiments with separate harvests of nematodes, except that alkyl groups were analyzed only twice.

RESULTS

Lipid content of *M. javanica* females was 40.5% of the dry weight. Weight determinations of the three major lipid fractions are presented in Table 1. Neutral lipid was the major lipid class, followed in abundance by phospholipid and glycolipid.

Table 1. Lipid composition of *Meloidogyne javanica*.

Lipid class	% of total lipid	% of nematode dry weight
Neutral lipid	84.2	34.1
Glycolipid	2.5	1.0
Phospholipid	13.3	5.4

Choline phosphoglyceride was the most abundant phospholipid followed by ethanolamine phosphoglyceride and six other compounds (Table 2).

Table 2. Phospholipid composition of *Meloidogyne javanica*.

Phospholipid	% of total
Choline phosphoglyceride	61.7
Ethanolamine phosphoglyceride	22.0
Serine phosphoglyceride	7.0
Sphingomyelin	4.1
Lysophosphatidylcholine	2.6
Inositol phosphoglyceride	2.4
Diphosphatidylglycerol	0.2
Phosphatidic acid	Trace

The fatty acid compositions of neutral lipid, phospholipid, and choline and ethanolamine phosphoglycerides are shown in Table 3. Over half of the fatty acid in each

Table 3. Fatty acid composition of neutral lipid (NL), phospholipid (PL), choline phosphoglyceride (CP), and ethanolamine phosphoglyceride (EP) from *Meloidogyne javanica*. Results are expressed as relative percentage of total fatty acid from each lipid.

Fatty acid*	NL	PL	CP	EP
10:0	0.01	0.00	0.00	0.00
12:0	0.11	0.00	0.00	0.00
iso-13:0	0.01	0.00	0.00	0.00
iso-14:0	0.00	0.01	0.03	0.02
14:0	1.9	0.10	0.08	0.36
iso-15:0	1.2	0.24	0.16	0.58
15:0	0.00	T†	T	0.03
iso-16:0	T	T	0.01	0.02
16:0	7.8	1.7	1.8	2.0
16:1	2.5	1.0	0.94	2.9
iso-17:0	0.64	0.62	0.47	0.55
17:0	0.00	T	0.01	T
iso-18:0	0.00	0.24	0.01	0.46
18:0	6.2	8.0	4.9	16.0
18:1	74.6	61.3	68.0	54.6
18:2	0.48	3.7	3.1	5.6
18:3	0.08	0.18	0.30	0.20
20:0	0.24	4.0	1.3	3.8
20:1	1.9	4.1	4.3	2.9
20:2	0.05	0.07	0.22	0.11
20:3	0.39	1.3	0.90	1.7
$20{:}4\omega^6$	0.07	1.8	0.83	1.3
$20{:}4\omega^3$	0.36	1.4	0.87	1.3
20:5	0.77	9.5	11.4	4.4
22:0	0.11	0.22	0.18	0.50
22:1	0.15	0.48	0.32	0.74
24:0	0.49	0.00	0.00	0.00

*Fatty acids are represented by a system in which the first number represents the number of carbon atoms and the second represents the number of double bonds.

†Trace amounts detected.

was 18:1 acid. Neutral lipid contained proportionally more 16:0 and 18:1 acids than phospholipid, which contained greater percentages of polyunsaturated 20-carbon acids. Ethanolamine phosphoglyceride contained larger proportions of 18:0 and 20:0 acids and smaller percentages of 18:1 and 20:5 acids than did choline phosphoglyceride.

Results of photodensitometric quantification of TLC plates upon which diacyl-, alkenylacyl-, and alkylacylglycerol acetates had been separated are presented in Table 4. The majority of the glyceride acetates, and hence the parent phospholipids, were diacyl compounds. Alkenylacyl and alkylacyl phosphoglycerides comprised nearly half of the ethanolamine phosphoglyceride

Table 4. Classes of choline phosphoglyceride (CP) and ethanolamine phosphoglyceride (EP) from *Meloidogyne javanica*. Results are expressed as relative percentage of total CP or EP.

Phosphoglyceride class	CP	EP
1,2-diacyl	95.5	54.9
1,3-diacyl	3.4	1.3
1-alkenyl-2-acyl	0.5	34.6
1-alkyl-2-acyl	0.6	9.3

but only about 1% of the choline phosphoglyceride.

The 1-position of diacyl ethanolamine phosphoglyceride contained more 18:0, 20:0, 22:0, 20:1, and 22:1 acids than the 2-position, which contained more *iso*-15:0, 16:1, 18:1, 18:2, 18:3, 20:3, 20:4, and 20:5 acids (Table 5). The fatty acid compositions of the alkenylacyl and alkylacyl compounds resembled that of the 2-position of their diacyl analogue; however, the ether-containing lipids contained smaller percentages of 20:3, $20{:}4\omega^6$, and 20:5 acids (Table 5). Alkenylacyl ethanolamine phosphoglyceride contained greater proportions of *iso*-15:0, 16:1, 18:1, and 18:2 fatty acids and lesser percentages of 14:0, 16:0, 18:0, and $20{:}4\omega^3$ acids than its alkylacyl analogue.

Greater quantities of *iso*-15:0, 16:0, *iso*-17:0, 18:0, 20:0, 22:0, 18:1, 20:1, and 22:1 fatty acids were at the 1-position of diacyl choline phosphoglyceride than at the 2-position, where larger amounts of 16:1, 18:2, 20:2, 20:3, 20:4, and 20:5 acids occurred (Table 6). Insufficient quantities of alkenylacyl and alkylacyl choline phosphoglycerides were obtained for satisfactory acyl, alkenyl, or alkyl group analysis.

Alkenyl and alkyl group compositions of alkenylacyl and alkylacyl ethanolamine phosphoglycerides resembled each other, as 95% of the total alkenyl or alkyl groups were 18:0 compounds (Table 7). Unsaturated alkenyl or alkyl groups were not detected.

Neither aldehydes nor alkylglycerols could be derived from tomato root phospholipids.

DISCUSSION

The lipid content of females of *M. javanica* (40.5% of dry weight) was similar

Table 5. Fatty acid composition of the 1-position of 1,2-diacyl ethanolamine phosphoglyceride and the 2-positions of 1,2-diacyl, 1-alkenyl-2-acyl, and 1-alkyl-2-acyl ethanolamine phosphoglycerides from *Meloidogyne javanica*. Results are expressed as relative percentage of total fatty acid at each position of each phosphoglyceride.

Fatty acid	Diacyl 1-position	Diacyl 2-position	Alkenylacyl 2-position	Alkylacyl 2-position
iso-14:0	T*	0.03	0.00	0.00
14:0	0.27	0.41	0.22	2.0
iso-15:0	0.13	0.69	1.0	0.49
15:0	0.08	0.09	0.02	0.08
iso-16:0	0.03	0.01	0.01	0.09
16:0	4.0	3.0	2.0	13.4
16:1	0.81	3.3	5.0	2.5
iso-17:0	0.89	0.34	0.51	3.3
17:0	0.04	T	T	0.03
iso-18:0	0.13	0.15	0.03	0.08
18:0	38.4	2.1	1.1	6.0
18:1	31.0	63.4	75.1	62.9
18:2	0.74	8.9	11.0	2.2
18:3	0.00	1.0	0.43	0.53
20:0	10.7	0.00	0.50	0.09
20:1	6.4	0.53	0.50	0.28
20:2	0.44	0.09	0.09	0.13
20:3	0.00	2.9	0.19	0.00
20.4_{ω^6}	0.00	1.8	0.28	0.30
$20{:}4_{\omega^3}$	0.00	2.7	1.1	4.7
20:5	0.00	8.7	0.84	0.85
22:0	3.0	0.00	0.00	0.00
22:1	2.9	0.00	0.00	0.00

*Trace amounts detected.

to that of *M. arenaria* (39.6%) and *M. incognita* (46.3%) (18). Larvae of *M. javanica* have been reported to contain 30–40% lipid (23,30). The phospholipid content of *M. javanica* (5.4% of dry weight) was slightly higher than the polar lipid content of *M. arenaria* (4.4%) and *M. incognita* (3.9%) (18). Although richer in total lipid, *Meloidogyne* is a poorer source of phospholipid than the free-living nematodes *Turbatrix aceti* (10.2%) (6) and *Panagrellus redivivus* (7.9%) (26). As in *T. aceti* (6) and the animal parasite *Trichinella spiralis* (5), glycolipid comprised about 1% of the dry weight of *M. javanica*.

Phospholipid from *M. javanica* consisted largely of choline and ethanolamine phosphoglycerides, which are also the most abundant phospholipids in *T. aceti* (6), *P. redivivus* (26), and several animal-parasitic nematodes (1,2,5,13,14,28,29). However, the ratio of choline to ethanolamine phosphoglyceride varies greatly among these genera.

Although previously unknown in plant-parasitic nematodes, alkenylacyl and alkylacyl phospholipids comprised 34.6% and 9.3%, respectively, of the ethanolamine phosphoglyceride. However, 99% of the choline phosphoglyceride was of the diacyl type. In *T. aceti*, ether lipids were found to comprise 53.7% of the ethanolamine phosphoglyceride and 10.8% of the choline phosphoglyceride (6). Similarly, mammalian alkenylacyl and alkylacyl phospholipids are predominantly ethanolamine phosphoglycerides (11).

The plasmalogen content of muscle from *Ascaris lumbricoides* females is about twice that of reproductive tissue (2). An interesting coincidence is the occurrence of greater levels of plasmalogens in *T. aceti*, a migratory nematode, than in the sedentary females of *M. javanica*, which contain a smaller percentage of muscle. The plasmalogen content of larvae of *M. javanica* would be of interest and would likely be higher on a dry weight basis than that of adult females.

Alkenylacyl and alkylacyl phospholipids were not detected in tomato roots. These

Table 6. Fatty acid composition of the 1- and 2-positions of 1,2-diacyl choline phosphoglyceride from *Meloidogyne javanica*. Results are expressed as relative percentage of total fatty acid at each position.

Fatty acid	1-position	2-position
iso-14:0	0.01	0.02
14:0	0.13	0.11
iso-15:0	0.37	0.06
15:0	0.02	0.03
iso-16:0	T*	T
16:0	4.0	0.99
16:1	0.63	1.9
iso-17:0	0.95	0.05
17:0	0.02	T
iso-18:0	T	T
18:0	7.6	1.0
18:1	77.6	57.6
18:2	0.57	7.1
18:3	0.00	0.84
20:0	1.4	0.00
20:1	6.0	1.3
20:2	0.14	0.44
20:3	T	2.9
$20:4\omega^6$	0.00	2.2
$20:4\omega^3$	0.00	2.6
20:5	0.00	20.7
22:0	0.24	0.00
22:1	0.38	0.00

*Trace amounts detected.

lipids are usually considered to be absent in higher plants (3), although small amounts of plasmalogens may occur in developing tissues of a few species (19). In any event, abundance of plasmalogens and alkylacyl phosphoglycerides in *M. javanica* is a significant biochemical difference between the nematode and its host and could be exploited by an appropriate chemical control agent.

Table 7. Alkenyl and alkyl group compositions of 1-alkenyl-2-acyl and 1-alkyl-2-acyl ethanolamine phosphoglycerides from *Meloidogyne javanica*. Results are expressed as relative percentage of total alkenyl or alkyl group.

Compound	Alkenyl groups	Alkyl groups
14:0	0.02	0.00
16:0	0.09	1.4
17:0	4.0	0.00
18:0	95.8	95.2
19:0	0.10	2.3
Unknown*	0.00	1.2

*Emerged shortly before 19:0 on both polar (ethylene glycol succinate) and nonpolar (methyl silicone) gas-liquid chromatographic columns.

Polyunsaturated fatty acids were concentrated in the phospholipid of *M. javanica*, rather than in the neutral lipid. A similar abundance of polyunsaturated acids in phospholipid or polar lipid occurs in *M. arenaria* (18), *M. incognita* (18), *T. aceti* (8), *P. redivivus* (26), and *T. spiralis* (5), but not in *Globodera solanacearum*, in which triglycerides and free fatty acids are more unsaturated than polar lipid (22).

The major phospholipid fatty acid of *M. javanica* was 18:1 acid, as it is in *M. arenaria* and *M. incognita* (18). The proportions of a few phospholipid fatty acids differ slightly among the three species, but the reproducibility of such differences is unknown. An apparently conspicuous difference is the report of *iso*-18:0 content of 7–10% in polar lipid from *M. arenaria* and *M. incognita*, whereas the value obtained in the present investigation for *M. javanica* is 0.2%. Although it is possible that such a difference occurs, it is more likely that the *iso*-18:0 fatty acid methyl ester chromatographed in the previous study predominantly consisted of 18:0 dimethylacetal, which had been produced from alkenyl groups during transesterification and had not been separated from methyl esters during purification on an alumina column (6).

Choline phosphoglyceride from *M. javanica* was more unsaturated than ethanolamine phosphoglyceride. A similar situation exists in *T. aceti* (6). Lack of data prevents comparison to other nematodes, but the situation is reversed in most other organisms (27). Association of saturated fatty acids with the 1-position and unsaturated acids with the 2-position of phosphoglycerides from *M. javanica* is a feature shared with *T. aceti* (6) as well as most other organisms (10).

Alkenylacyl and alkylacyl ethanolamine phosphoglycerides from *M. javanica* contained fatty acids similar in structure and quantity to those at the 2-position of their diacyl analogue, but the latter contained a greater percentage of polyunsaturated acid. A similar situation exists in *T. aceti* (6). Such comparisons have not been frequently performed in other organisms, but mammalian plasmalogens generally contain a

larger percentage of unsaturated fatty acid than the 2-positions of the corresponding diacyl compounds (10). As in *T. aceti* (6), ethanolamine plasmalogen from *M. javanica* contained larger proportions of 18:1 and 18:2 acids and smaller percentages of 16:0 and 18:0 acids than did alkylacyl ethanolamine phosphoglyceride.

Approximately 95% of the alkenyl and alkyl groups from *M. javanica* were 18:0 compounds. Unsaturated alkenyl and alkyl groups were not detected, whereas they occur in small amounts in *T. aceti* (6) and comprise over half of the alkenyl groups of the animal parasites *A. lumbricoides* (2) and *Dirofilaria immitis* (13). The phospholipid alkyl and alkenyl group compositions of other nematodes would be of interest, as would studies of nematode phospholipid metabolism.

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