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The lipid content and fatty acid composition of hatched second stage juveniles of *Globodera rostochiensis* and *G. pallida*

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Summary – *Globodera rostochiensis* and *G. pallida* hatched (1-day-old in potato root diffusate) second stage juveniles (J2) contained 29.2 % and 27.2 % lipid (of dry weight), respectively. *Globodera rostochiensis* J2 lipid consisted of 72.8 % neutral lipid, 11.3 % free fatty acids and 15.9 % non-acetic phospholipid. The neutral lipid fraction comprised 95.5 % triacylglycerides, 1.8 % diacylglycerides, 2.3 % monoacylglycerides and 0.4 % cholesterol ester. *Globodera pallida* J2 lipid consisted of 73.0 % neutral lipid, 13.2 % free fatty acids and 13.8 % n.-a. phospholipid. Total unsaturated fatty acids comprised 77.4 % and 80.6 % of total lipid in *G. rostochiensis* and *G. pallida*, respectively. The two species showed similar fatty acid profiles. Twenty fatty acids were identified, ranging from C14 to C22; the major lipid classes were composed predominantly of C20 (50-60 %) and C18 (30-35 %) fatty acids. The three most abundant fatty acids found in both species were C20:4, C20:1 and C18:1, making up more than 60 % of the total. *Globodera rostochiensis* J2, which had hatched over 24 h periods during the first 2 weeks exposure to potato root diffusate, had the same fatty acid composition as those which hatched over 96 h periods in weeks 3 and 4.

Résumé – ***Contenu lipidique et composition en acides gras des juvéniles de deuxième stade de* Globodera rostochiensis *et* G. pallida** – Les juvéniles de deuxième stade (J2) de *Globodera rostochiensis* âgés d'un jour (éclos dans les diffusats de racines de pomme de terre) contiennent en moyenne 29,2 % de lipides (poids sec), tandis que ceux de *G. pallida* en contiennent en moyenne 27,2 %. Les lipides des J2 de *G. rostochiensis* J2 sont composés de 72,8 % de lipides neutres, 11,3 % d'acides gras libres et 15,9 % de phospholipides. La fraction lipidique neutre comprend elle-même 95,5 % de triacylglycérides, 1,8 % de diacylglycérides, 2,3 % de monoacylglycérides et 0,4 % d'ester de cholestérol. Les lipides de *G. pallida* J2 sont composés de 73,0 % de lipides neutres, 13,2 % d'acides gras libres et 13,8 % de phospholipides. 77,4 % des acides gras totaux de *G. rostochiensis* et 80,6 % de ceux de *G. pallida* sont instaurés. Les deux espèces présentent un profil d'acides gras similaire. Vingt acides gras ont été identifiés, allant de C14 à C22; les principales classes de lipides sont surtout composées d'acides gras en C20 (50-60 %) et C18 (30-35 %). Les trois acides gras les plus abondants caractérisés chez les deux espèces sont C20:4, C20:1 et C18:1, représentant à eux seuls plus de 60 % du total. Les J2 de *G. rostochiensis* récoltés toutes les 24 heures au cours des première et seconde semaines après l'exposition aux PRD, présentent la même composition en acides gras que les J2 récoltés toutes les 96 heures pendant les troisième et quatrième semaines.

Key-words : Fatty acids; neutral lipids; phospholipids; potato cyst nematode.

The lipid content of animal-parasitic, plant-parasitic and free-living nematodes appears to vary between different species and even between different stages within one species. Plant-parasitic nematodes have a high lipid content (24-67 %) compared with most free-living nematodes (20-29 %) and nematode parasites of vertebrates (10-25 %) (Tracey, 1958; Roberts & Fairbairn, 1965; Wilson, 1965; Sivapalan & Jenkins, 1966; Van Gundy *et al.*, 1967; Barrett, 1968; Barrett *et al.*, 1971; Krusberg, 1972; Krusberg *et al.*, 1973; Reversat, 1976, 1980; Womersley *et al.*, 1982; Kapur & Sood, 1985). However, some entomopathogenic nematodes also appear to contain relatively large amounts of lipids (12-38 %) (Gordon & Cornect, 1987; Selvan *et al.*, 1993*b*; Wijbenga & Rodgers, 1994; Stolinski, 1994).

Lipids are the predominant long term energy reserves in nematodes from aerobic habitats, presumably because upon oxidation they generate a greater number of ATP molecules per mole of substrate compared with carbohydrates (Lehninger, 1975). Thus, most plant-parasitic nematodes have large proportions of neutral lipids (the primary form of stored lipid) and only small proportions of the more structural phospholipids; the majority of neutral lipids are in the form of triacylglycerides, with smaller amounts of other neutral lipid classes such as diacylglycerides, monoacylglycerides, free cholesterol and cholesterol esters (Krusberg *et al.*, 1973; Chitwood & Krusberg, 1981; Womersley *et al.*, 1982; Stolinski, 1994). In most plant-parasitic nematodes, C18 fatty acids predominate (Krusberg, 1967; Krusberg *et al.*, 1973; Chitwood & Krusberg, 1981; Womersley *et al.*, 1982) but in *Globodera solanacearum* a high C20 content has been reported, which distinguishes *Globodera* from other genera of plant-parasitic nema-

todes (Orcutt *et al.*, 1978). Saturated fatty acids provide more energy than unsaturated fatty acids and the longer the carbon chain the more energy can be obtained by beta-oxidation. Long chain unsaturated fatty acids also have higher melting points than shorter chain fatty acids and so have saturated in comparison to unsaturated fatty acids of the same chain length (Stryer, 1988). The ratio of unsaturated fatty acids to saturated fatty acids differs between species but most plant-parasitic nematodes contain a high percentage of unsaturated fatty acids (75-92 %) (Krusberg, 1967; Krusberg *et al.*, 1973; Orcutt *et al.*, 1978; Chitwood & Krusberg, 1981; Womersley *et al.*, 1982).

Oil Red O has been used to quantify the neutral lipid reserves of second stage juveniles (J2) of the potato cyst nematodes (PCN), *G. rostochiensis* and *G. pallida*, and their rate of depletion under various environmental conditions (Storey, 1983, 1984; Robinson *et al.*, 1987*a, b*) but there is no published information on the different lipid classes of these two species. As part of a programme to examine the lipid metabolism of PCN, this paper presents results of a comparison of the total lipid, free fatty acid and non-acetic phospholipid contents of hatched J2. The neutral lipid fraction of *G. rostochiensis* was analysed further to determine the amounts of triacylglycerides, diacylglycerides, monoacylglycerides and cholesterol esters.

Material and methods

Potato root diffusate (PRD) was obtained by the method of Fenwick (1949), stored in plastic bottles at 4 °C and diluted 1 in 4 (v/v) with distilled water (DW) before use. In 1993, single generation cysts of *G. rostochiensis* Ro1 and *G. pallida* Pa1, both grown on cv. Désirée in glasshouse pot cultures, were extracted and stored at 4 °C. In 1994, about 500 mg (dry weight) of these cysts were soaked in DW for 7 days at 18°C. The cysts were then surface sterilized (Sanft & Wyss, 1990), firstly in 100 ml of a mixture of 1 ml NaOCl, 0.01 % (v/v) Tween 20 and 99 ml double distilled water (DDW) for 20 min, and then in 100 ml of a mixture of 10 ml H_2O_2 (30 % w/v), 50 ml 96 % (v/v) ethanol and 40 ml DDW for 10 min. The cysts were then washed in autoclaved DW (3 × 10 min) and placed in PRD at 18 °C. Hatched J2 of both *Globodera* species were collected daily (from day 2 to day 13) and J2 of *G. rostochiensis* every 96 h (from day 17 to day 29) and surface sterilized for 2 min in 0.02 % (w/v) $HgCl_2$ / 1 % (w/v) streptomycin sulphate, followed by 3 min in 1 % chlorohexidine digluconate. The J2 were then washed in DDW (3 × 3 min) and stored in liquid N_2 until further use.

Following storage, the J2 were homogenized with a microhomogeniser on dry ice, put into a small screw capped vial (4 ml volume) and weighed (wet weight) and then freeze dried for 48 h. After re-weighing (dry weight = 1 volume), nematode lipids were extracted using a modified (Bailey, 1970) method of Folch *et al.* (1957). Nineteen volumes of chloroform-methanol (2:1 v/v) were added to each vial, which was purged with nitrogen, sealed with parafilm and kept at 5 °C for 48 h. After equilibration at room temperature, the solvent was removed from each vial using a Pasteur pipette and put into pre-weighed Quickfit tubes. A further 20 volumes of chloroform-methanol were then added to the vials which were left to stand for 30 min before the solvent was removed and added to the first 19 volumes in the Quickfit tubes. The equivalent of 22 % of the volume in the tube (= 40 fold the dry weight) of 0.04 % (w/v) $CaCl_2$ was added to the Quickfit tubes. The tubes were shaken vigorously, then centrifuged for 15 min at 2000 *g*. This gave two clearly separated phases. The upper phase was removed using a Pasteur pipette to leave the lower phase and another 22 % of the volume of solvent containing chloroform, methanol and 0.04 % (w/v) calcium chloride (3:48:47) was added. After shaking the tubes vigorously they were centrifuged for 15 min at 2000 *g* and the upper phase was discarded. Absolute ethanol (0.4 ml) was added to each tube and the solvent was then evaporated using a rotating vacuum evaporator. After determining its weight, the lipid in each tube was dissolved in 0.4 ml of chloroform and transferred into a small vial, purged with nitrogen, sealed with parafilm and stored at – 40 °C.

Solid phase chromatography

About 10 % of each sample was retained for total lipid (TL) analysis and the rest was separated into different lipid classes by solid phase chromatography (Figlewicz *et al.*, 1985; Kaluzny *et al.*, 1985). Aminopropyl-bonded columns (Bond Elut, Varian Associates, Inc.; 500 mg) and several solvents with different polarity were used to separate the sample into neutral lipids (NL)(4 ml of chloroform/2-propanol 2:1 v/v), free fatty acids (FFA) (4 ml of 2 % [v/v] acetic acid in diethyl ether) and non-acetic phospholipids (PL) (4 ml of methanol). Acetic phospholipids could not be eluted by this method (Christie, 1992). A part of the NL fraction was retained and the rest was split into triacylglycerides (TG) (6 ml of 1 % [v/v] diethyl ether, 10 % [v/v] methylene chloride in hexane), diacylglycerides (DG) (4 ml of 5 % [v/v] ethyl acetate in hexane), monoacylglycerides (MG) (4 ml of chloroform/methanol 2:1 [v/v], and cholesterol ester [CE] 4 ml of hexane). The compositions of the various fractions were checked by TLC. These samples were then dried, reweighed, dissolved in chloroform and stored at – 20 °C in glass vials purged with nitrogen.

Gas chromatography

Lipids (except the FFA fraction) were hydrolysed with 0.5 M NaOH in methanol (1 ml) under reflux in a water bath at 100 °C (15 min for NL, TG, DG, MG;

45 min for CE; 60 min for TL, PL). Fatty acid methyl esters (FAMEs) were obtained by first incubating with 1 ml boron trifluoride (under reflux) for 3 min; hexane (1 ml) was then added and the samples left under reflux for 1 min (Morrison & Smith, 1964). After allowing the mixture to cool to room temperature, a saturated sodium chloride solution (0.6 ml) was added to each tube, which was mixed gently and left for 60 min to allow the phases to separate. The upper phase, containing hexane and FAMEs, was then transferred into pre-weighed Quickfit tubes. Anhydrous sodium sulphate (100 mg) was added to remove any remaining water and the samples dried in a rotating vacuum evaporator. The tubes were then re-weighed to determine the FAMEs weight and hexane (0.05-0.1 ml) was added.

FAMEs were analyzed using a Varian Vista 401 GC equipped with a polar Carbowax column (30 m length, 0.32 mm internal diameter, 0.25 mm film). Column conditions were as follows : injector temperature 250 °C; detector temperature 270 °C; split injection 1:50. The temperature programme started at 100 °C isotherm for 2 min, increasing linearly to 160 °C at a rate of 10 °C/min, then linearly up to 235 °C at 2 °C/min and isotherm at 235 °C for 1 min. Retention times of FAMEs were compared with authentic standards (Restek Corporation, Maidenhead, England; Matreya, Pleasant Gap, PA, USA).

The general linear modelling package GLIM (© 1985 Royal Statistical Society, London, V 3.77) was used for statistical analysis (Crawley, 1993). Significance was tested at the 5 % level. Percentage data were arcsine transformed and analysed by ANOVA.

Results

The total lipid concentration of freshly hatched J2 of *G. rostochiensis* Ro1 (29.2 %) and *G. pallida* Pa1 (27.2 %) and the percentage composition of their constituent major lipid classes was found to be similar (Table 1). There was no significant difference between the two species and interactions were not significant. The neutral lipid fraction in *G. rostochiensis* consisted of 95.5 % triacylglycerides, 1.8 % diacylglycerides, 2.3 % monoacylglycerides and 0.4 % cholesterol ester.

A high degree of unsaturation was found in all lipid classes in both species (Table 2). The two species showed similar fatty acid profiles of total lipid, neutral lipid and phospholipid (Table 2; Figs 1, 2). In total, twenty fatty acids were identified in both species. Each lipid class consisted mainly of C20 and to a lesser extent of C18 fatty acids with the exception of the cholesterol ester fraction, which showed a completely different pattern, with C16 > C18 > C20 for both *G. pallida* and *G. rostochiensis* (Table 2). Differences between the cholesterol ester fraction and all other fractions were significant for both species. Compared with the other lipid classes, the cholesterol ester fractions contained significantly greater amounts of C14:0 and C15:0 (Figs 1, 2).

Table 1. *Mean weight and percentage of total of different lipid classes of freshly hatched second stage juveniles of* Globodera rostochiensis *Ro1 and* G. pallida *Pa1 (as Fatty Acid Methyl Esters; FAMEs).*

	Globodera rostochiensis		*Globodera pallida*	
Lipid class	% of total	µg FAME/mg dry weight	% of total	µg FAME/mg dry weight
Free fatty acid	11.3	33 (29-39)	13.2	36 (29-45)
Phospholipid	15.9	46 (40-56)	13.8	38 (35-55)
Neutral lipid	72.8	213 (198-228)	73	199 (181-205)
- Acylglycerides	72.5	212 (197-227)	72.8	198 (180-203)
- Cholesterol esters	0.3	1 (1-2)	0.2	1 (1-2)
Total lipid	100	292 (273-309)	100	272 (265-292)

n = three replicates; the dry matter of *G. rostochiensis* and *G. pallida* J2 was found to be 21.7 and 20.8 % respectively.

In all classes where C20 and C18 predominated, C20:4, C20:1 and C18:1 accounted for approximately 60-70 % of the total, with C20:4 being the greatest in most fractions (C20:1 > C20:4 in phospholipids). C18:2 was found in consistently greater amounts than C18:3, and there was more C20:3 than C20:2 in both species. Other unsaturated fatty acids detected were C20:5 and C22:1 (Figs 1-2). The main saturated fatty acids were C18:0 > C20:0 > C16:0 in both *G. pallida* and *G. rostochiensis*. Other saturated fatty acids included C14:0 and C22:0. The only odd numbered fatty acid detected was C15:0 which was found in the free fatty acid fraction and in relatively large amounts in the cholesterol ester fraction of both *Globodera* species. Two branched-chain fatty acids, *iso*-C15:0 and *iso*-C17:0, were found particularly in the phospholipid fraction; *G. rostochiensis* containing higher levels than *G. pallida* (Figs 1, 2).

The fatty acid composition and degree of saturation of two samples of hatched J2 of *G. rostochiensis* (Ro1 1993), the first collected daily from day 2 to day 13 and the second every 96 h from day 17 to day 29 after putting the cysts into PRD, were not significantly different except for C20:1 (Fig. 3).

Discussion

The lipid content of *G. pallida* and *G. rostochiensis* J2 was within the range previously observed for cyst nematode species (Orcutt *et al.*, 1978; Reversat, 1980). In

Table 2. *Fatty acid composition (%) of different lipid classes of freshly hatched second stage juveniles of* Globodera rostochiensis *Ro1 and* G. pallida *Pa1 (mean ± standard deviation).*

	Total lipid		Free fatty acid		Phospholipid		Neutral lipid					
							Total		Acylglyceride		Cholesterol ester	
	Ro1	Pa1	Ro1	Pa1	Ro1	Pa1	Ro1	Pa1	Ro1	Pa1	Ro1	Pa1
C14	0.6 ± 0.2	0.7 ± 0.1	1.1 ± 0.1	1.4 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.3	0.3 ± 0.1	0.8 ± 0.2	0.4 ± 0.0	3.6 ± 0.2	4.2 ± 0.6
C15	0	0	0.6 ± 0.1	1.1 ± 0.5	0	0	0	0	0.3 ± 0.1	0	3.8 ± 0.2	3.5 ± 0.1
C16	5.6 ± 0.6	5.1 ± 0.3	6.8 ± 0.3	11.1 ± 2.3	7.1 ± 0.3	5.4 ± 0.7	5.8 ± 0.1	8.4 ± 0.2	6.3 ± 0.5	6.3 ± 0.1	45.6 ± 1.7	48.3 ± 1.2
C18	32.2 ± 2.0	32.9 ± 0.7	27.2 ± 0.7	34.8 ± 0.8	40.8 ± 0.9	36.0 ± 2.0	32.0 ± 1.0	36.4 ± 1.1	32.3 ± 1.6	35.8 ± 0.6	26.1 ± 0.3	24.8 ± 0.3
C20	54.8 ± 1.0	59.1 ± 1.2	57.1 ± 1.4	46.5 ± 2.5	47.0 ± 1.5	54.1 ± 1.4	58.1 ± 0.8	53.6 ± 1.3	55.0 ± 1.3	56.0 ± 0.8	19.4 ± 0.7	17.3 ± 0.3
C22	1.5 ± 0.0	1.7 ± 0.1	3.4 ± 0.1	2.1 ± 0.2	1.3 ± 0.1	1.2 ± 0.0	1.9 ± 0.2	1.2 ± 0.4	1.5 ± 0.1	1.3 ± 0.2	0	0
Saturated	17.3 ± 0.1	18.9 ± 0.4	15.3 ± 0.7	19.8 ± 0.9	22.4 ± 0.5	16.4 ± 1.4	18.5 ± 0.9	20.0 ± 0.3	18.3 ± 0.5	18.6 ± 0.2	30.2 ± 1.0	28.7 ± 1.6
Unsaturated	77.4 ± 0.4	80.6 ± 0.3	80.9 ± 0.3	77.2 ± 1.0	74.4 ± 1.0	80.9 ± 1.3	80.0 ± 1.1	79.9 ± 0.3	77.9 ± 0.9	81.2 ± 0.1	68.3 ± 1.5	69.4 ± 0.2
Total	94.7 ± 0.5	99.5 ± 0.6	96.2 ± 0.7	97.0 ± 1.0	96.8 ± 1.4	97.3 ± 2.6	98.5 ± 1.9	99.9 ± 0.1	96.2 ± 1.3	99.8 ± 0.1	98.5 ± 0.9	98.1 ± 1.5

n = three replicates.

contrast, values reported for *Meloidogyne* species were considerably higher (Krusberg *et al.,* 1973). However, when individual classes of lipids are compared differences between cyst and root-knot nematodes are less consistent.

The high and stable levels of free fatty acids found in both species of PCN are much greater than would be expected as they normally constitute less than 1 % of the total lipids in animal tissues (Barrett, 1981). However, high levels of free fatty acids have been reported in numerous nematode species (Roberts & Fairbairn, 1965; Wilson, 1965; Barrett, 1968; Womersley *et al.,* 1982), but Barrett (1981) notes that high levels of free fatty acids reported in helminths may, in part, be an artefact due to the hydrolysis of more complex lipids during extraction and analysis.

The phospholipid content, only non-acetic phospholipids were investigated, of J2 of *G. pallida* and *G. rostochiensis* (*c.* 14-16 %) was greater than that reported in *G. solanacearum* females (three-fold), *M. incognita* juveniles (two-fold), and *M. arenaria* eggs (three-fold), but similar to that in females of *M. javanica* (13.3 %) (Krusberg *et al.,* 1973; Orcutt *et al.,* 1978; Chitwood & Krusberg, 1981; Stolinski, 1994). Triacylglycerides (95-96 %) form the major part of the neutral lipid fraction in *G. rostochiensis* as in *G. solanacearum* females (Orcutt *et al.,* 1978), therefore being the major storage form of energy.

The relative amounts of the twenty saturated and unsaturated fatty acids (C14-C22) found in the TL fraction of *G. rostochiensis* and *G. pallida* were similar to values reported for many other plant-parasitic nematodes (Krusberg, 1967; Krusberg *et al.,* 1973; Orcutt *et al.,* 1978). For example, Krusberg *et al.* (1973) found nineteen fatty acids in *M. arenaria* and *M. incognita,* ranging from C14 to C20 plus traces of C10:0 and C12:0, whilst fatty acids in *G. solanacearum* females ranged from C14 to C22, although only fourteen fatty acids were reported to be present (Orcutt *et al.,* 1978). The fatty acid composition in both *Globodera* species in the present investigation was dominated by C20 (primarly C20:4 and C20:1) and to a lesser extent by C18 (primarly C18:1) fatty acids. This agrees with previous work on *G. solanacearum* females where the same three fatty acids contributed at least 60 % of the total lipid (Orcutt *et al.,* 1978). In contrast, in most other plant-parasitic nematodes studied, lipids were dominated by C18:1 alone (Krusberg, 1967; Krusberg *et al.,* 1973; Chitwood & Krusberg, 1981; Womersley *et al.,* 1982; Stolinski, 1994). The presence of large amounts of C20:4 and C20:1 is a major difference between the *Heterodera/Globodera* group and other plant-parasitic nematodes studied but the significance of the difference is unclear.

Diet is known to influence the fatty acid composition of entomopathogenic nematodes (Wijbenga & Rodgers, 1994; Fodor *et al.,* 1994). Sivapalan and Jenkins (1966) suggested that *Panagrellus redivivus* may have the ability to synthesize C20 fatty acids from C18:1 or C18:2. Lipids in potato roots consist mainly of C16:0, C18:2 and C18:3 fatty acids (Holz, unpubl.) and *Globodera* species may also have the ability to synthesize C20 but the ability to saturate C18:2 and C18:3 to C18:1 may be limited, thus explaining the large amounts of C20:1 and C20:4 in *Globodera* species. Both C20:4 and C20:5 are known to be phytoalexin elicitors for potato (Bostock *et al.,* 1981). The presence of large amounts of C20:4 in J2s suggests that it might play a role in the parasite/host plant interactions. It has also been suggested (Thompson, 1973) that polyunsaturated fatty acids or their de-

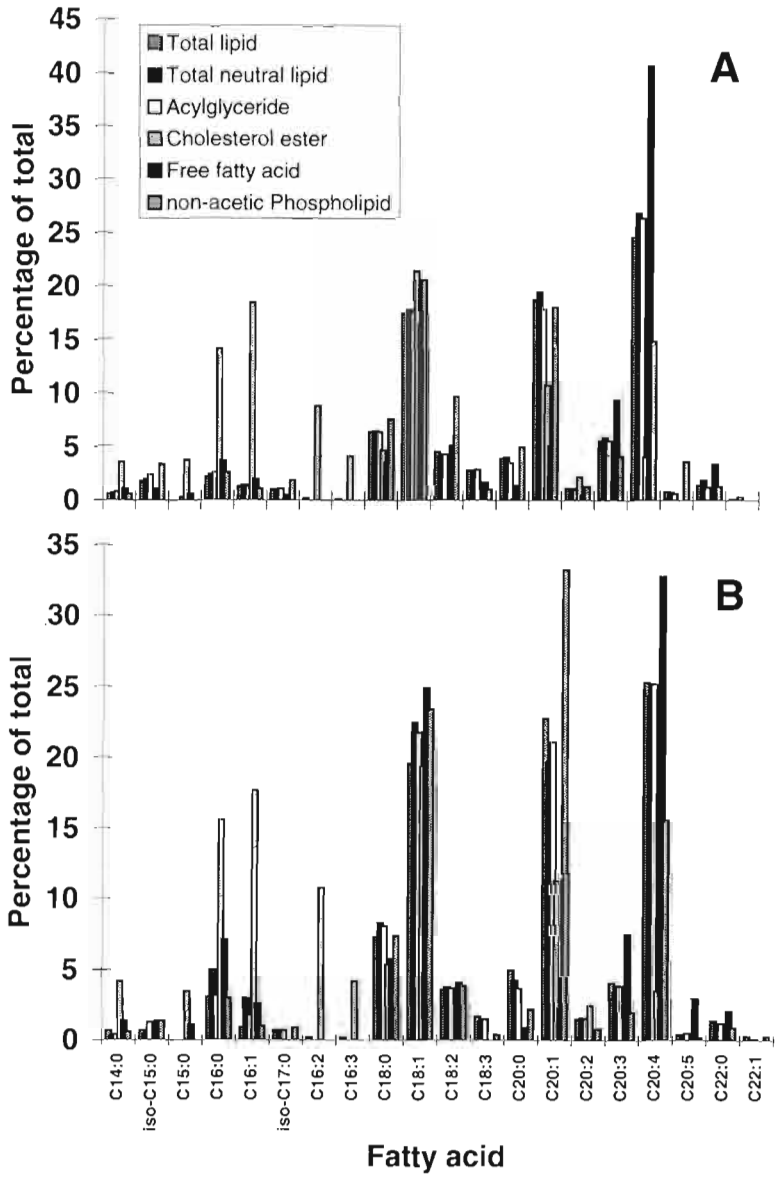

Fig. 1. *Fatty acid composition of different lipid classes in freshly hatched second stage juveniles (1993 single generation). A :* Globodera rostochiensis *Ro1; B :* Globodera pallida *Pa1.*

rivatives have specific metabolic functions in nematodes.

Only one odd-numbered fatty acid (C15:0) was identified in *G. rostochiensis* and *G. pallida* and this has also been found in *M. javanica* females (Chitwood & Krusberg, 1981), in the animal-parasitic nematodes, *Foleyella agamae* and *Haemonchus contortus* (Kapur & Sood, 1985; Aisien *et al.,* 1986), and in the free-living nematode *Turbatrix aceti* (Fletcher & Krusberg, 1973). The two branched-chain fatty acids, *iso*-C15:0 and *iso*-C17:0, found in *G. rostochiensis* and *G. pallida* have also been reported in *M. arenaria, M. incognita* and *M. javanica* together with *iso*-C18:0 (Krusberg *et al.,* 1973).

Overall, the lipid content of the two species of PCN was very similar. Although this might have been expected because of their close phylogenetic relationship, differences have been found in their physiology which might have been reflected in their fatty acid composition. For example, Robinson *et al.* (1987*a*) showed that *G. rostochiensis* J2s utilized their lipid reserves more rapidly than *G. pallida* during storage at different sand moisture contents while results suggested that *G. pallida*

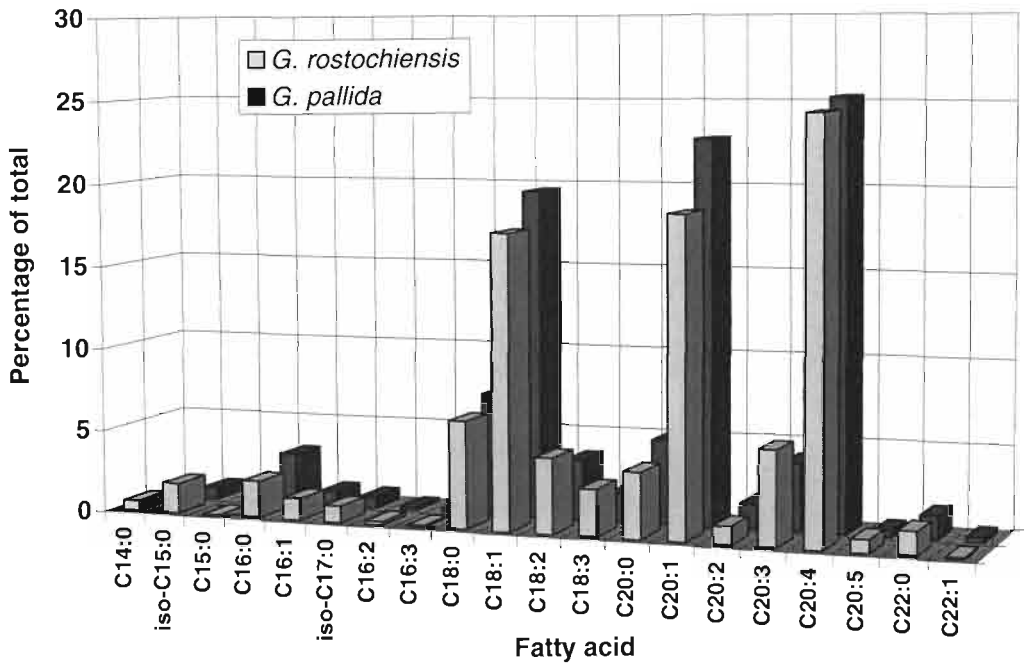

Fig. 2. *Comparison of the fatty acid composition of the total lipids of freshly hatched second stage juveniles of* Globodera rostochiensis *Ro1 (1993 single generation) and* G. pallida *Pa1 (1993 single generation).*

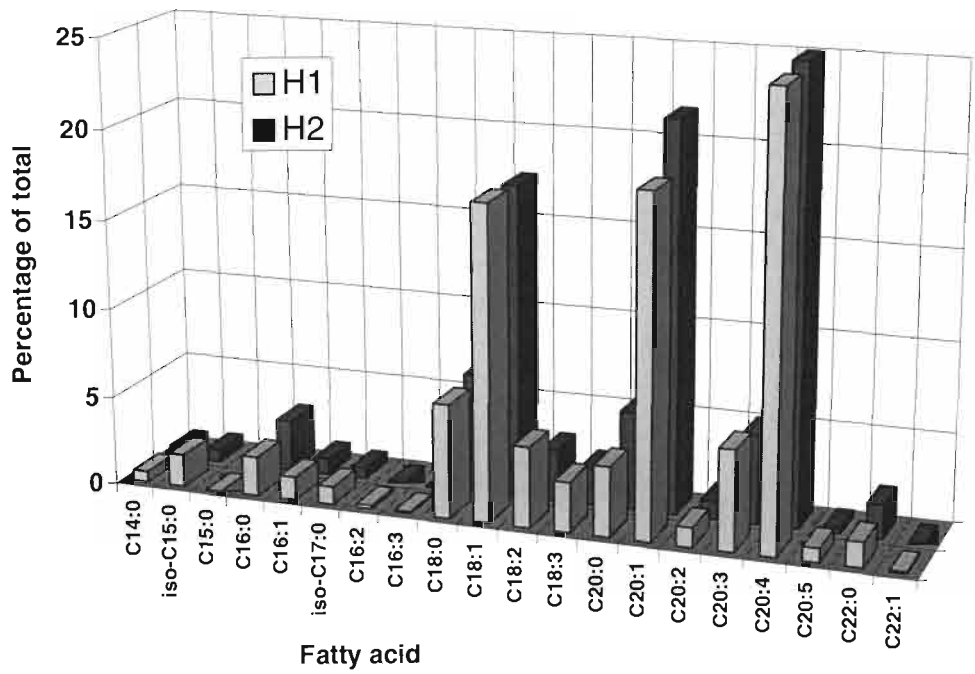

Fig. 3. *Comparison of the fatty acid composition of two samples of* Globodera rostochiensis *Ro1 second stage juveniles (1993 single generation) (H1 = collected daily from day 2 to day 13 after first exposure to potato root diffusate; H2 = collected every 96 h from day 17 to day 29).*

has a lower temperature optimum for hatching than *G. rostochiensis* (Robinson *et al.*, 1987*b*). Hatched J2 of *G. rostochiensis* collected every 24 h during the first two weeks of exposure to PRD showed a similar fatty acid composition to hatched J2 collected every 96 h during weeks three and four. Robinson *et al.* (1985) showed that unhatched and hatched J2, stimulated by PRD, used their lipid reserves more rapidly than unstimulated J2. Results obtained in the present work indicated that J2 were able to retain the same fatty acid profile even after 3-4 weeks exposure to PRD. These findings are in contrast to work on some other nematodes. For example, in the entomopathogenic nematode, *Steinernema feltiae,* infective juveniles showed a decline in the proportion of total polyunsaturated fatty acids relative to those of monounsaturated and saturated fatty acids after four weeks at 22°C (Wijbenga & Rodgers, 1994). In contrast, when freshly emerged infective juveniles of *S. glaseri, S. carpocapsae* and another entomopathogenic species, *Heterorhabditis bacteriophora,* were stored at 25°C, the percentage of unsaturated fatty acids increased (Selvan *et al.*, 1993*a*). Fresh and aged (8 days) infective juveniles of the animal parasite *Strongyloides ratti,* stored at 24 °C, also contained different fatty acid profiles, with C18:1 being metabolized most rapidly (Barrett, 1969).

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References

Aisien, S. O., Opute, F. I., Ali, S. N. & Obiamiwe, B. A. (1986). Lipid composition of adult *Foleyella agamae. Int. J. Parasit.,* 16 : 655-657.

Bailey, H. H. (1970). Isolation and purification of helminth total lipids. *In :* MacInnis, A. J. & Voge, M. (Eds). *Experiments and techniques in parasitology.* San Francisco, USA, W. H. Freeman & Co. : 174-175.

Barrett, J. (1968). Lipids of the infective and parasitic stages of some nematodes. *Nature,* 218 : 1267-1268.

Barrett, J. (1969). The effect of ageing on the metabolism of the infective larvae of *Strongyloides ratti* Sandground, 1925. *J. Parasit.,* 59 : 3-17.

Barrett, J. (1981). *Biochemistry of parasitic helminths.* London, UK, Macmillan Publishers Ltd., 308 p.

Barrett, J., Ward, C. W. & Fairbairn, D. (1971). Lipid metabolism in the free-living nematodes *Panagrellus redivivus* and *Turbatrix aceti. Comp. Biochem. Physiol.,* 38 : 279-284.

Bostock, R. M., Kuc, J. A. & Laine, R. A. (1981). Eicosapentaenoic and arachidonic acids from *Phytophthora infestans* elicit fungitoxic sesquiterpenes in potato. *Science,* 212 : 67-69.

Chitwood, D. J. & Krusberg, L. R. (1981). Diacyl, alkylacyl, and alkenylacyl phospholipids of *Meloidogyne javanica* females. *J. Nematol.,* 13 : 105-111.

Christie, W. W. (1992). Solid-phase extraction columns in the analysis of lipids. *In :* Christie, W. W. (Ed.). *Advances in lipid methology - one.* Dundee, UK, The Oily Press : 1-17.

Crawley, M. J. (1993). *GLIM for ecologists. Methods in ecology.* Oxford, UK, Blackwell Scientific Publications, 379 p.

Fenwick, D. (1949). Investigations on the emergence of larvae from cysts of the potato-root eelworm *Heterodera rostochiensis.* I. Technique and variability. *J. Helminth.,* 23 : 157-170.

Figlewicz, D. A., Nolan, C. E., Singh, I. N. & Jungalwala, F. B. (1985). Pre-packed reverse phase columns for isolation of complex lipids synthesized from radioactive precursors. *J. Lipid Res.,* 26 : 140-144.

Fletcher, C. L. & Krusberg, L. R. (1973). Investigation of some lipids from *Turbatrix aceti. Comp. Biochem. Physiol.,* 45 : 159-165.

Fodor, F., Dey, I., Farkas, T. & Chitwood, D. J. (1994). Effects of temperature and dietary lipids on phospholipid fatty acids and membrane fluidity in *Steinernema carpocapsae. J. Nematol.,* 26 : 278-285.

Folch, J., Lees, M. & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.,* 226 : 497-509.

Gordon, R. & Cornect, M. (1987). Nutrient composition of *Romanomermis culicivorax* in relation to egg production and metabolism. *J. Nematol.,* 19 : 487-494.

Kaluzny, M. A., Duncan, L. A., Merritt, M. V. & Epps, D. E. (1985). Rapid separation of lipid classes in high yield and purity using bonded phase columns. *J. Lipid Res.,* 26 : 135-140.

Kapur, J. & Sood, M. L. (1985). *Haemonchus contortus :* Qualitative and quantitative analysis of lipids. *Zentbl. VetMed.,* 32 : 345-353.

Krusberg, L. R. (1967). Analyses of total lipids and fatty acids of plant-parasitic nematodes and host tissues. *Comp. Biochem. Physiol.,* 21 : 83-90.

Krusberg, L. R. (1972). Fatty acid composition of *Turbatrix aceti* and its culture medium. *Comp. Biochem. Physiol.,* 41 : 89-98.

Krusberg, L. R., Hussey, R. S. & Fletcher, C. L. (1973). Lipid and fatty acid composition of females and eggs of *Meloidogyne incognita* and *M. arenaria. Comp. Biochem. Physiol.,* 45 : 335-341.

Lehninger, A. L. (1975). *Biochemistry : The molecular basis of structure and function; 2nd Edition.* New York, USA, Worth, 1104 p.

Morrison, W. R. & Smith, L. M. (1964). Preparation of fatty acid methyl esters and dimethylacetates from lipids with boron fluoride-methanol. *J. Lipid Res.,* 5 : 600-608.

Orcutt, D. M., Fox, J. A. & Jake, C. A. (1978). The sterol, fatty acid and hydrocarbon composition of *Globodera solanacearum. J. Nematol.,* 10 : 264-269.

REVERSAT, G. (1976). Étude de la composition biochimique globale des juvéniles des nématodes. *Cahiers ORSTOM, Sér. Biol.,* 11 : 225-234.

REVERSAT, G. (1980). Effect of *in vitro* storage on the physiology of second stage juveniles of *Heterodera oryzae*. *Revue Nématol.,* 3 : 233-241.

ROBERTS, L. S. & FAIRBAIRN, D. (1965). Metabolic studies on adult *Nippostrongylus brasiliensis* (Nematoda : Trichostrongyloidea). *J. Parasit.,* 51 : 129-138.

ROBINSON, M. P., ATKINSON, H. J. & PERRY, R. N. (1985). The effect of delayed emergence on infectivity of juveniles of the potato cyst nematode *Globodera rostochiensis*. *Nematologica,* 31 : 171-178.

ROBINSON, M. P., ATKINSON, H. J. & PERRY, R. N. (1987*a*). The influence of soil moisture and storage time on the motility, infectivity and lipid utilization of second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Revue Nématol.,* 10 : 343-348.

ROBINSON, M. P., ATKINSON, H. J. & PERRY, R. N. (1987*b*). The influence of temperature on the hatching, activity and lipid utilization of second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Revue Nématol.,* 10 : 349-354.

SANFT, U. & WYSS, U. (1990). Monoxenischer Resistenztest für Kartoffelgenotypen gegenüber dem Zystennematoden *Globodera pallida*. 2. Durchführung des Tests mit Nematoden. *Potato Res.,* 33 : 55-66.

SELVAN, S., GAUGLER, R. & GREWAL, P. S. (1993*a*). Water content and fatty acid composition of infective juvenile entomopathogenic nematodes during storage. *J. Parasit.,* 79 : 510-516.

SELVAN, S., GAUGLER, R. & LEWIS, E. E. (1993*b*). Biochemical energy reserves of entomopathogenic nematodes. *J. Parasit.,* 79 : 167-172.

SIVAPALAN, P. & JENKINS, W. R. (1966). Phospholipid and long-chain fatty acid composition of the nematode *Panagrellus redivivus*. *Proc. helminth. Soc. Wash.,* 33 : 149-157.

STOLINSKI, M. (1994). *Lipid utilization in free-living stages of soil nematodes*. Ph. D. Thesis, Imperial College, London, UK, 215 p.

STOREY, R. M. J. (1983). The initial neutral lipid reserves of juveniles of *Globodera* spp. *Nematologica,* 29 : 144-150.

STOREY, R. M. J. (1984). The relationship between neutral lipid reserves and infectivity for hatched and dormant juveniles of *Globodera* spp. *A. appl. Biol.,* 104 : 511-520.

STRYER, I. (1988). *Biochemistry*. New York, USA, Freeman, 473 p.

THOMPSON, S. N. (1973). A review and comparative characterization of the fatty acid compositions of seven insect orders. *Comp. Biochem. Physiol.,* 45 B : 467-482.

TRACEY, M. V. (1958). Cellulase and chitinase in plant nematodes. *Nematologica,* 3 : 179-183.

VAN GUNDY, S. D., BIRD, A. F. & WALLACE, H. R. (1967). Aging and starvation in larvae of *Meloidogyne javanica* and *Tylenchulus semipenetrans*. *Phytopathology,* 57 : 559-571.

WIJBENGA, J. & RODGERS, P. B. (1994). Lipid content of insect parasitic nematodes. *IOBC/WPRS Bull.,* 17 : 155-158.

WILSON, P. A. G. (1965). Changes in lipid and nitrogen content of *Nippostrongylus brasiliensis* infective larvae aged at constant temperature. *Exp. Parasit.,* 16 : 190-194.

WOMERSLEY, C., THOMPSON, S. N. & SMITH, L. (1982). Anhydrobiosis in nematodes II : Carbohydrate and lipid analysis in undesiccated and desiccated nematodes. *J. Nematol.,* 14 : 145-153.