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Effects of Temperature and Dietary Lipids on Phospholipid Fatty Acids and Membrane Fluidity in *Steinernema carpocapsae*[1]

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Abstract: The phospholipid composition of *Steinernema carpocapsae* was studied in relation to diet and culture temperature. When reared at 18 and 27.5 C on *Galleria mellonella* or on an artificial diet supplemented with lard, linseed oil, or fish oil as lipid sources, nematode phospholipids contained an abundance of 20-carbon polyunsaturated fatty acids, with eicosapentaenoic acid (20:5(n − 3)) predominant, regardless of the fatty acid composition of the diet. Because the level of linolenic acid (18:3(n − 3)) in nematode phospholipids was very low and because eicosapentaenoic acid was present even when its precursor (linolenic acid) was undetectable in the diet, *S. carpocapsae* likely produces n − 3 polyunsaturated fatty acids by de novo biosynthesis, a pathway seldom reported in eukaryotic animals. Reduction of growth temperature from 25 to 18 C increased the proportion of 20:5(n − 3) but not other polyunsaturated fatty acids. A fluorescence polarization technique revealed that vesicles produced from phospholipids of nematodes reared at 18 C were less ordered than those from nematodes reared at 27.5 C, especially in the outermost region of the bilayer. Dietary fish oil increased fluidity in the outermost region but increased rigidity in deeper regions. Therefore, *S. carpocapsae* appears to modify its membrane physical state in response to temperature, and eicosapentaenoic acid may be involved in this response. The results also indicate that nematode membrane physical state can be modified dietarily, possibly to the benefit of host-finding or survival of *S. carpocapsae* at low temperatures.

Key words: bacterium, diet, entomopathogenic nematode, fatty acid, fluidity, fluorescence anisotropy, *Galleria mellonella,* insect, lipid, membrane, nematode, phospholipid, *Steinernema carpocapsae,* temperature, *Xenorhabdus nematophilus.*

Commercialization of entomopathogenic nematodes as biological control agents of insects would be accelerated by improvements in nematode persistence after field application and by increasing the shelf life of products containing entomopathogenic nematodes (17). Little research, however, has focused upon the biochemical, biophysical and physiological mechanisms involved in the sensitivity of entomopathogenic nematodes to low humidity, extremes of temperature, or extended periods of storage. Based upon a recent examination of the lipid and fatty acid compositions of *Steinernema* spp. and *Heterorhabditis* spp. during storage, Selvan et al. (27,28) proposed that the higher relative percentage of unsaturated fatty acids found in *H. bacteriophora* than in *S. carpocapsae* or *S. glaseri* resulted in poorer survival.

The phylum Nematoda includes species that are among the most adaptive animals, surviving under very extreme environmental conditions. In any organism exposed to fluctuating environmental conditions, maintenance of the functional integrity and proper physicochemical properties of biological membranes is a prerequisite for survival. Prevention of the bilayer to non-bilayer transition of membranes in desiccating animals greatly contributes to their resumption of activity upon rehydration (6,7). Furthermore, ability to adjust membrane fluidity to prevailing temperature is an important factor determining the activity, life cycle and distribution of cold-blooded animals (13,14). Lipids, especially phospholipids, are essential structural components of biological membranes and regulate membrane fluidity; substantial evidence indicates that membrane lipids change in thermally acclimating uni- and multicellular poikilo-

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therms (5,30). Two major factors that affect the phospholipid fatty acid composition of poikilothermic animals are diet and temperature (5,10,11,31).

Whether entomopathogenic nematodes respond to changes in environmental temperature by adjusting the physicochemical properties of their membranes is unknown. Therefore, the purpose of this investigation was to determine the combined effect of dietary fat and growth temperature on the chemical composition and physical properties of membrane phospholipids of the entomopathogenic nematode *S. carpocapsae*.

Materials and Methods

Nematode culture: Steinernema carpocapsae (Mexican strain) was kindly provided by Dr. Ramon Georgis (Biosys, Palo Alto, CA) and then cultured in last-instar larvae of the wax moth *Galleria mellonella* (24) or in mini-Bedding cultures (1), as modified by researchers at Biosys. Twenty-four hours before addition of nematodes, the Bedding cultures had been inoculated with primary cells of the N_2 strain of *Xenorhabdus nematophilus,* obtained from E. Szállás and A. Szentirmai (Department of Microbiology, Kossuth Lajos University, Debrecen, Hungary) and cultured in liquid medium (24). Three incubators at 18, 25, and 27.5 C ensured proper ambient temperature. After extraction from the Bedding cultures, the mixed life stages of *S. carpocapsae* were rinsed five times in M9 buffer (29).

Experimental treatments: Three replicates of each experiment were performed. Nematodes were grown on media containing different fatty acid compositions at two tolerable temperature extremes (18 and 25 C) to determine the biosynthetic origin of their fatty acids and to learn whether *S. carpocapsae* adjusts the composition of membrane lipids to temperature. Unmodified Bedding cultures contained beef lard previously extracted with chloroform–methanol 2:1 (16) and purified on a silicic acid column with chloroform. Dietary fatty acids were modified by replacement of lard with cold-pressed linseed oil (Plant Oil Research Institute, Budapest, Hungary) or fish oil (Activepa 30, Biocare Co., Bergen, Norway). Linseed oil provided linolenic acid (18:3(n − 3)), the precursor of n − 3 eicosapolyenoic acids; fish oil provided 20:4(n − 3), 20:5(n − 3) and 22:6(n − 3) fatty acids; and lard provided palmitic (16:0), stearic (18:0) and oleic (18:1) acids. (Fatty acids are described by a system in which the first number represents the chain length, the second number indicates the number of double bonds, and the position of the first double bond from the methyl end of the fatty acid is in parentheses). In other experiments, the polar head group compositions of phospholipids from *S. carpocapsae* grown in lard-supplemented Bedding cultures at 18 and 27.5 C were compared.

Extraction and analysis of lipids: Lipids were extracted with chloroform–methanol 2:1 (v/v; nematode weight/solvent volume = 1/20) in an Ultraturrax (Heidelberg, Germany) homogenizer and purified according to Folch et al. (16). The total lipid extract was fractionated by silicic acid column chromatography using chloroform to elute the neutral lipids and methanol to elute the polar lipids (predominantly phospholipids). The total lipids and subfractions were stored in benzene containing 0.05% butylated hydroxytoluene at −20 C until subsequent analysis. Phospholipids were fractionated by thin-layer chromatography (TLC) on silica gel G chromatoplates (E. Merck, Darmstadt, Germany) (15). Compounds were identified by comparison to authentic standards (Serdary Research Laboratory, London, Ontario, Canada). Spots were sprayed with 0.5% 8-anilino-1-naphthalenesulfonic acid (Sigma Chemical Co., St. Louis, MO) in methanol, visualized under UV light, transferred into glass tubes, and quantified by lipid phosphorus analysis (25).

Total phospholipid was transmethylated in 5.0% HCl in methanol at 80 C for 2.5 hours. Fatty acid methyl esters were separated by gas-liquid chromatography in a

Hitachi model 280-60 instrument containing a 2-meter column (3.0 mm i.d.) of 80–100 mesh Supelcoport (Supelco, Bellefonte, PA) coated with 10% FFA liquid phase. The oven was programmed from 140 to 185 C with a temperature increase of 1.0 C/minute, and the chromatograph was fitted with a flame ionization detector connected to a data processor. Methyl esters were identified by comparison to authentic standards and by plotting the relative retention times of the unknowns against the number of carbon atoms present.

The fatty acid compositions of *X. nematophilus* N_2 cultured on LB broth, last-instar *G. mellonella* raised at 25 C, and the *X. nematophilus* N_2-inoculated Bedding diets supplemented with lard, linseed oil, or fish oil were determined by homogenization with chloroform–methanol and purification of the total lipids by procedures identical to those used with nematodes. The total lipids were not subsequently fractionated but were directly transesterified in methanolic HCl.

Effects of temperature and diet on membrane structural order: Three different anthroyloxy fatty acid probes were incorporated in phospholipid vesicles obtained from nematodes grown at 18 or 27.5 C in Bedding cultures supplemented with lard or fish oil. The anthroyloxy fatty acids were 2-(9-anthroyloxy)stearic acid (2-AS), 12-(9-anthroyloxy)stearic acid (12-AS), and 16-(9-anthroyloxy)palmitic acid (16-AP). Fluorescence anisotropy (R_{ss}) was measured at 7, 17, and 27 C. Use of these fatty acid labels provides information about fluidity relationships along the fatty acyl chains in the bilayer, in this case at the C-2, C-12 and C-16 carbon atoms. Lower anisotropy values represent less ordered, more fluid structures.

To 250 μg of phospholipids in chloroform was added 2.5 μg of 2-AS, 12-AS, or 16-AP in 10 μl tetrahydrofuran. The solvent was carefully removed under high vacuum, and the phospholipids were vortexed in 4.0 ml Tris · HCl (pH 7.4) for 5 minutes at room temperature to form multibilayer vesicles. A Hitachi MPF 2A computer-controlled, thermostatted spectrophotofluorimeter was used for determination of fluorescence anisotropy. The sample was excited with vertically polarized light at 366 nm, and vertically and horizontally polarized light was measured at 430 nm. Ten readings were made per observation, with three replicates per probe.

Results

Polar head group composition of phospholipids: When phospholipids obtained from *S. carpocapsae* maintained in Bedding cultures supplemented with lard were subfractionated by thin layer chromatography, choline and ethanolamine phosphoglycerides were the two major components, each amounting to 38–44% of the total phospholipid (Table 1). Phosphatidylinositol, sphingomyelin, lysophosphatidylcholine, diphosphatidylglycerol (cardiolipin) and phosphatidic acid each composed only 1–5% of the total phospholipid. Growth temperature did not affect the relative distribution of the phospholipid head group composition (Table 1).

Fatty acids of phospholipids in S. carpocapsae, G. mellonella, and culture media: Bedding diets supplemented with linseed oil and fish oil had greater proportions of 18- or 20-carbon $n - 3$ polyenoic fatty acids, respectively, than lard-supplemented diet

Table 1. Polar head group composition of phospholipids of *Steinernema carpocapsae* grown at two different temperatures on Bedding cultures containing lard.

Phospholipid	18 C	27.5 C
Cardiolipin	3.1	3.0
Phosphatidic acid	0.3	0.2
Phosphatidylglycerol	0.3	0.7
Phosphatidylethanolamine	40.1	42.8
Phosphatidylserine	4.6	4.0
Phosphatidylinositol	2.1	2.4
Phosphatidylcholine	38.7	40.7
Sphingomyelin	5.5	4.6
Lysophosphatidylcholine	5.3	1.6

† No effort was made to separate diacyl from alkylacyl and alkenylacyl phosphoglycerides; percentages include all forms of the respective phosphoglyceride.

(Table 2). The 20-carbon $n - 3$ fatty acids were not detected in *Galleria mellonella* last-instar larvae; instead, the insects were characterized by the presence of 16:0, 18:0, 18:1($n - 9$), and some 18:2($n - 6$) (Table 2). Long chain polyunsaturated fatty acids were also absent in the bacterial symbiont of *S. carpocapsae, X. nematophilus.*

At least 25 fatty acids were detected in the total phospholipid of *S. carpocapsae;* the major components were 14:0, 16:0, 16:1, 18:0, 18:1($n - 9$), 18:2($n - 6$), 20:4($n - 3$), and 20:5($n - 3$) (Table 3). The fatty acid composition of the triacylglycerols was qualitatively identical to that of the phospholipids, but the relative percentages of individual fatty acids were different (Table 4).

Effects of diet and temperature on phospholipid fatty acids: At 25 C, *S. carpocapsae* propagated in Bedding cultures supplemented with linseed oil contained greater proportions of linolenic (18:3($n - 3$)) and docosahexaenoic (22:6($n - 3$)) acids than nematodes propagated in *G. mellonella;* fish oil supplementation resulted in nematode accumulation of eicosapentaenoic (20:5($n - 3$)) and 22:6($n - 3$) acids (Table 3). Reduction of growth temperature from 25 to 18 C increased ($P < 0.05$) the percentage of 20:5($n - 3$) fatty acid in all cultures (Table 3). The levels of the other long chain polyunsaturated fatty acids, 20:4($n - 6$), 20:4($n - 3$) or 22:6($n - 3$), did not increase with decreasing growth temperature, with the exception of linoleic acid (18:2($n - 6$)). The percentage of oleic acid was lower ($P < 0.05$) at 18 C in Bedding cultures supplemented with linseed oil or fish oil than in corresponding cultures at 25 C.

Effect of temperature and diet on structural order of phospholipid vesicles: Phospholipid vesicles obtained from nematodes grown at 18 C were less ordered at each segment of the bilayer than those from nematodes grown at 27.5 C (Table 5). The effect of temperature was most pronounced in the outermost segment of the bilayer, as indicated by results with the 2-AS probe, whereas in the deeper layers (at C-12 and C-16), this increased fluidization was less drastic. Addition of fish oil to the diet resulted in a decrease of the anisotropy parameter in the C-2 segment of the bilayer at each reading temperature, but the deeper layers became more rigid.

Discussion

Our results confirm and extend earlier observations (3,4,23,26) that polar lipids in

Table 2. Relative percentages of fatty acids in total lipids of mini-Bedding diets and other foods consumed by *Steinernema carpocapsae.*

Fatty acid	Lard	Bedding + lard	Bedding + linseed oil	Bedding + fish oil	*Galleria mellonella*†	*Xenorhabdus nematophilus*
12:0	0.0	0.0	0.0	0.0	0.0	1.5
14:0	2.1	3.4	0.9	8.2	0.5	4.2
14:1	0.0	0.0	0.0	0.0	0.0	8.9
16:0	23.3	42.0	17.0	18.7	31.0	30.6
16:1	3.0	0.5	1.6	9.2	1.7	12.4
17:0	0.0	0.0	0.0	0.0	0.0	17.7
18:0	19.6	15.8	14.5	4.5	2.3	0.4
18:1	43.6	23.6	28.1	15.2	52.4	13.9
18:2($n - 6$)	3.3	7.9	10.0	2.7	6.5	0.8
18:3($n - 3$)	0.0	0.0	9.5	0.6	0.6	0.0
20:0	0.0	0.0	0.0	0.0	0.0	1.7
20:5($n - 3$)	0.0	0.0	0.0	16.0	0.0	0.0
22:1	0.0	0.0	0.0	0.0	0.0	0.5
22:6	0.0	0.0	0.0	10.7	0.0	0.0
Others	5.1	6.8	18.4	14.2	5.0	7.4

Fatty acids are described by a binumeric system in which the first number represents the chain length and the second number indicates the number of double bonds.

† Last-instar larvae reared at 25 C.

TABLE 3. Combined effects of growth temperature and different growth media containing various dietary fatty acids on relative percentages of fatty acids in phospholipids of *Steinernema carpocapsae*.

Fatty acid in *S. carpocapsae* phospholipids	*Galleria mellonella*		Bedding culture + linseed oil		Bedding culture + fish oil	
	25 C (N = 5)	18 C (N = 4)	25 C (N = 5)	18 C (N = 3)	25 C (N = 5)	18 C (N = 5)
14:0	0.3 ± 0.1	1.9 ± 0.6	1.4 ± 0.3	1.2 ± 0.6	3.7 ± 0.3	2.7 ± 1.1
16:0	9.2 ± 0.5	11.0 ± 0.7	6.0 ± 1.9	4.1 ± 1.8	13.0 ± 4.2	12.1 ± 3.5
16:1	0.7 ± 0.2	1.3 ± 0.4	†	†	†	†
18:0	7.0 ± 0.9	5.5 ± 0.6	8.9 ± 1.4 a	9.8 ± 0.6 b	8.2 ± 1.4	8.0 ± 1.5
18:1	17.6 ± 1.2	16.4 ± 1.6	28.2 ± 1.6 a	23.4 ± 1.9 b	22.3 ± 2.8 a	18.3 ± 0.6 b
18:2(n − 6)	22.1 ± 2.3	25.7 ± 2.4	9.4 ± 0.8 a	11.0 ± 1.2 b	6.1 ± 1.6 a	7.8 ± 1.1 b
18:3(n − 3)	0.3 ± 0.1	0.7 ± 0.3	3.9 ± 1.1	5.7 ± 4.0	0.0	1.0 ± 0.7
20:2(n − 6)	1.0 ± 0.3	1.2 ± 0.4	1.6 ± 0.5	1.7 ± 0.7	0.5 ± 0.2	0.7 ± 0.4
20:3(n − 6)	7.0 ± 1.4	3.6 ± 0.9	5.8 ± 0.7 a	4.7 ± 0.4 b	2.6 ± 1.3	1.8 ± 0.1
20:4(n − 6)	2.9 ± 0.6	2.1 ± 0.7	5.8 ± 0.7 a	4.1 ± 0.4 b	2.3 ± 0.7	2.3 ± 0.7
20:4(n − 3)	8.4 ± 1.3	5.4 ± 2.1	7.1 ± 0.8	8.4 ± 2.5	6.3 ± 2.7	7.5 ± 2.2
20:5(n − 3)	14.5 ± 1.4 a	17.5 ± 1.0 b	15.7 ± 1.2 a	19.8 ± 1.0 b	21.3 ± 1.2 a	26.0 ± 1.6 b
22:6(n − 3)	0.0	0.0	0.6	0.4	2.5 ± 1.2	3.3 ± 0.4
Others	9.0	8.7	5.7	5.7	11.8	8.5

Within a given growth medium, values for a specific fatty acid at two different temperatures followed by different letters are significantly different ($P < 0.05$) by Student's t-test.

Fatty acids are described by a system in which the first number represents the chain length, the second number indicates the number of double bonds, and the position of the first double bond from the methyl end of the fatty acid is in parentheses.

† Trace quantity (<0.1% of total fatty acid) detected.

nematodes are rich in n − 6 and n − 3 polyunsaturated fatty acids. In contrast, Selvan et al. (27) found only low percentages of linoleic and linolenic acids and no C-20 polyunsaturated fatty acids in the total lipids from infective juveniles of *Heterorhabditis bacteriophora* and the All strain of *S. carpocapsae*. This difference cannot be explained by the fact that we investigated phospholipids but Selvan et al. (27) analyzed total lipid, because we also found 20:4(n − 6), 20:5(n − 3), and 20:4(n − 3) fatty acids in the total lipids of the Mexican strain of *S. carpocapsae*. It is possible that strains of entomopathogenic nematodes have striking differences in their fatty acid biosynthetic machinery, or that methodological differences (e.g., gas chromatography) could account for the disparity.

Saturated fatty acids are synthesized in eukaryotes by an enzyme complex (fatty acid synthetase), and the saturated acids

TABLE 4. Fatty acid composition of total lipids and major lipid fractions from *Steinernema carpocapsae* grown in mini-Bedding cultures supplemented with lard at 27.5 C.

Fatty acid	Total lipid	Phospholipid	Triglyceride	Free fatty acid	Steryl ester
14:0	1.2	0.3	1.1	30.2	4.9
16:0	8.5	10.4	9.7	27.6	24.0
16:1	2.9	1.0	3.4	1.2	0.0
18:0	15.4	15.8	21.2	11.9	14.7
18:1	36.1	16.9	34.6	13.2	23.0
18:2(n − 6)	12.8	16.2	12.4	6.2	22.0
18:3(n − 3)	0.0	0.0	0.0	0.0	0.0
20:1(n − 9)	2.1	1.8	1.3	0.0	0.0
20:2(n − 6)	1.1	0.8	1.0	0.0	0.0
20:3(n − 6)	3.4	7.7	2.3	0.0	0.0
20:4(n − 6)	2.4	4.8	1.6	0.0	0.0
20:4(n − 3)	2.2	7.7	0.8	0.0	0.0
20:5(n − 3)	4.2	13.7	2.5	0.0	0.0
Others	7.7	2.9	8.1	9.7	11.4

TABLE 5. Fluorescence anisotropy (anisotropy parameter R_{ss}) of three anthroyloxy fatty acids at three different temperatures in phospholipid vesicles obtained from *Steinernema carpocapsae* grown at two different temperatures in modified Bedding cultures supplemented with lard or fish oil.

Fatty acid label†	Growth temperature	7C	17 C	27 C
	Lard supplementation			
2-AS	27.5	0.213	0.211	0.211
2-AS	18	0.151	0.138	0.130
12-AS	27.5	0.120	0.126	0.107
12-AS	18	0.103	0.094	0.089
16-AP	27.5	0.101	0.092	0.100
16-AP	18	0.086	0.085	0.081
	Fish oil supplementation			
2-AS	27.5	0.154	0.155	0.149
12-AS	27.5	0.158	0.152	0.151
16-AP	27.5	0.145	0.122	0.123

† 2-AS = 2-(9-anthroyloxy)stearic acid; 12-AS = 12-(9-anthroyloxy)stearic acid; 16-AP = 16-(9-anthroyloxy)palmitic acid.

are desaturated to form the corresponding monoenoic fatty acids (18). With the exception of some insect species (2,8), most animals do not synthesize long chain polyunsaturated fatty acids de novo; instead, they elongate and further desaturate nutritionally required fatty acids such as linoleic and linolenic acids (18). Our experiments with *S. carpocapsae* further support the hypothesis that de novo polyunsaturated fatty acid biosynthesis occurs in some species of nematodes, as has been proposed in microbivorous nematode species (20–22,26). *Steinernema carpocapsae* contained appreciable amounts of C-20 polyunsaturated fatty acids when grown in *Galleria mellonella* larvae or in Bedding medium containing lard as the dietary fat. The level of linolenic acid is rather low in *Galleria mellonella* caterpillars (0.6% of total fatty acid), absent in *X. nematophilus,* and absent in *S. carpocapsae* propagated in Bedding cultures supplemented with lard. Although dietary linolenic acid could have been converted to longer polyunsaturated $n - 3$ derivatives, de novo biosynthesis in *S. carpocapsae* appears likely.

Evidence of the ability of *S. carpocapsae* to elongate and desaturate dietary linolenic acid towards the carboxyl group was provided by experiments in which the Bedding medium was supplemented with linseed oil, which is rich in linolenic acid. In these nematodes, the proportion of 20:5($n - 3$) was higher than in those fed lard as a lipid supplement. In contrast, the high level of this fatty acid in nematodes from medium supplemented with fish oil at 25 C could have resulted from selective absorption or deposition of this acid. Therefore, the amounts of C-20 polyunsaturated fatty acids could be regulated by at least three mechanisms: chain elongation and desaturation of linolenic acid, selective absorption or deposition, and de novo biosynthesis. Experiments with radiolabeled fatty acids and precursors are needed to measure the relative contribution of each mechanism.

Accumulation of 20:5($n - 3$) was enhanced in *S. carpocapsae* by reduction of growth temperature from 25 C to 18 C, regardless of the nematode food source being *Galleria melonella* or Bedding medium enriched with linseed oil or fish oil. The absence of 22:6($n - 3$) in *S. carpocapsae* grown in *G. mellonella* or in Bedding cultures supplemented with lard suggests that this nematode cannot elongate and further desaturate the 20:5($n - 3$) acid. In contrast to deposition of this acid, the deposition of 22:6($n - 3$) from fish oil was not enhanced by low temperature. Further investigations are needed to learn whether the low temperature-induced reduction in the level of oleic acid (18:1($n - 9$)) and increase in linoleic acid (18:2($n - 6$)) results from desaturation of oleic acid towards the methyl end.

Choline and ethanolamine phosphoglycerides were the two major phospholipids of *S. carpocapsae,* each amounting to 35–40% of the total phospholipid. Although ethanolamine phosphoglycerides were not further fractionated in this study, substantial proportions (ca. 30–50%) of alkylacyl and alkenylacyl ethanolamine phosphoglycerides occur in the total ethanolamine phosphoglycerides of some nematode species (3,4,26).

Temperature-induced changes in phos-

pholipid fatty acid compositions were reflected in the ordering state of phospholipid vesicles. The outermost segment of the bilayer, as measured by 2-AS, showed the most sensitive response to changes in temperature. In this respect, vesicles from *S. carpocapsae* resemble phospholipid vesicles from fish and shrimp adapted to different temperatures (12). Model studies on synthetic and native phospholipids from fish and shrimp demonstrated that this ordering is controlled by the amounts of specific phosphatidylethanolamine molecular species, such as 18:1/22:6, 18:1/20:5, or 18:1/20:4 (position 1/position 2 fatty acids) (9,12). Similar structural alterations in phospholipids might control membrane ordering in *S. carpocapsae*. Decreased order in the outermost region of the bilayer may facilitate membrane permeability and the accommodation of proteins involved in thermal adaptation. Administration of fish oil to nematodes at 27.5 C disordered even further the C-2 region of the bilayer; the 2-AS anisotropy parameter measured at 7 C in phospholipid vesicles from these nematodes was identical with that measured at 7 C in vesicles from nematodes administered lard at 18 C. Because unsaturated fatty acids directly or indirectly affect survival and activity of many organisms at reduced temperatures (19), entomopathogenic nematodes with thermally or dietarily increased membrane fluidity may be more active at lower temperatures and thus more effective as biological control agents. Experiments are being performed to determine if the host-finding activity of *S. carpocapsae* is related to the physical properties of phospholipid vesicles.

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