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CONSUMPTION OF FOOD RESERVES BY STARVED SECOND-STAGE JUVENILES OF *MELOIDOGYNE JAVANICA* UNDER CONDITIONS INDUCING OSMOBIOSIS

BY

GEORGES REVERSAT

Laboratoire de Nématologie, ORSTOM, B.P. 1 386, Dakar, Sénégal

Freshly hatched juveniles of *Meloidogyne javanica* were stored at 28° either in dilute medium (4 mM phosphate buffer pH 7) or in concentrated medium (0.3 M Na Cl in dilute medium). At weekly for the former and biweekly intervals for the latter, juveniles were assayed for motility, infectivity and food reserves (carbohydrates, lipids and proteins). Motility and infectivity of juveniles stored in dilute medium decreased to zero in 5 weeks during which 60% of the food reserves were consumed. The rate at which food reserves were consumed by juveniles stored in concentrated medium, which induced a state of quiescence, was 7.5 times slower: motility and infectivity remained unchanged during 10 weeks. The rate of reproduction of juveniles stored in dilute medium was unaffected.

Soil nematodes may experience high osmotic pressures in drying soils or after application of fertilizers (Wallace, 1971) which induces quiescence and enables the nematodes to survive long periods (Dropkin *et al.*, 1958; Reversat, 1975). The reduction of metabolic rate by high osmotic pressures was investigated by respirometry (Wallace & Greet, 1964; Bhatt & Rohde, 1970; Reversat, 1977) but quantitative data on the long-term effects of these conditions on food reserves are few (Cooper & Van Gundy, 1971). Previous work on the effect of storage on second-stage juveniles of *Meloidogyne* spp. showed that lipid reserves were consumed but provided only qualitative information about the fate of carbohydrates and proteins (Chitwood, 1951; Van Gundy *et al.*, 1967; Ogunfowora, 1979). I studied the consumption of food reserves (lipid, carbohydrate and protein) by second-stage juveniles of *Meloidogyne javanica* stored in a dilute and a concentrated medium and examined the effect of concentrated medium on motility and infectivity, and the effect of storage time in dilute medium on dry weight and ability to reproduce.

MATERIALS AND METHODS

Juveniles: A clone of *Meloidogyne javanica*, established from a single egg-mass, was propagated on kenaf (*Hibiscus cannabinus* L.) in 2.5 l pots of steam sterilized sandy soil in the greenhouse. Four weeks after sowing (10 seeds/pot), each pot was inoculated with 15,000 freshly hatched juveniles. One wk after sowing and then every 2 wks, 215 mg of urea and 175 mg of $KH_2 PO_4$ were added per pot.

Pots were buried in sand at 28°. Five wks after inoculation, roots were harvested, washed and placed in a mist chamber. The first nematodes, collected after 24 hrs, were discarded and then juveniles were collected every 48 hrs. Juveniles were placed on sieves lined with four layers of Kleenex tissue and active juveniles, which passed through the tissue, were collected 24 hrs later. This was zero storage time: the actual age of the juveniles was 2 ± 1 day.

Storage: Juveniles were stored in 1 l Roux bottles (surface area: 220 cm²), 1 million/100 ml storage medium. The dilute medium was 4 mM sodium phosphate buffer, pH 7 (Gomori, 1955). The concentrated medium was obtained by adding 3 M Na Cl to the dilute medium, in six equal steps at intervals of 1 hr, to give a final concentration of 0.3 M Na Cl. Samples of juveniles in dilute medium were taken at 0, 1, 2, 3 and 4 wks and in concentrated medium at 2, 4, 6, 8 and 10 wks. The transfer of juveniles from concentrated to dilute medium was made in two equal steps at intervals of 2 hr. Active juveniles were selected as before. Five aliquots counted before and after passing through 'Kleenex tissues' gave estimates of motility.

Infectivity and reproduction: Seeds of kenaf were surface sterilized in 0.2% Agallol for 30 min. and germinated on wet sterile sand in Petri dishes for 2 days in the dark at 28°. Seedlings were transplanted to 10 ml beakers of wet sterile sand. A day later, 100 motile juveniles, collected individually in a micropipette, were deposited on the sand. Beakers were kept at 28°, illuminated with fluorescent tubes (2,000 lux at leaf level) and watered as needed. Six days later, seedlings were harvested and their roots washed and stained with the cold cotton blue lactophenol (de Guiran, 1966). Later, stained juveniles were counted in the roots. This procedure was repeated for every storage time in dilute and in concentrated medium, 16 replications on each occasion. For juveniles in dilute medium, twenty five additional replications were divided into groups of five at harvest. Each group of inoculated seedlings was inserted in the stopper of 2 l jars filled with Hoagland's nutritive solution diluted to 1/4 of its normal strength: aeration was provided by bubbling. Nutrient solution was renewed after 5 wks and then every wk until 8 wks. Juveniles released in the solution were counted.

Chemical determinations: Motile juveniles were divided into twelve samples with equal numbers of individuals (Reversat, 1976, 1980a). Numbers of juveniles were determined in three samples, and other batches of three samples were used to determine carbohydrate with anthrone reagent (Seifter *et al.*, 1950), lipid with sulpho-phospho-vanillic reagent (Crehange & Metais, 1972) and total nitrogen with Nessler reagent (Umbreit *et al.*, 1964), as previously described by Reversat (1976). Dry weight of juveniles stored in dilute medium was determined with a quartz microfibre balance (Reversat, 1976) on three additional samples.
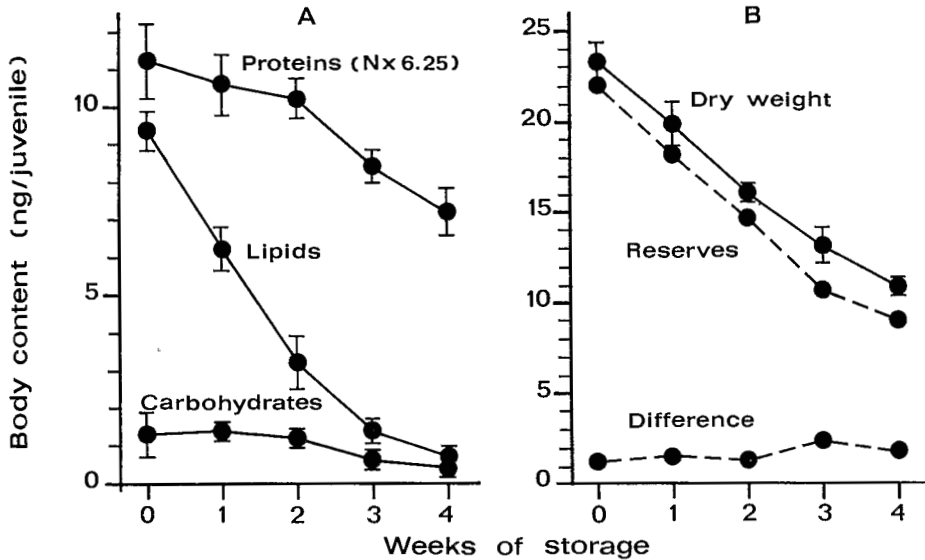
Fig. 1. Changes in dry weight and chemical composition of *Meloidogyne javanica* juveniles stored in dilute medium. A: carbohydrates, lipids and proteins (Each point is the mean of three replicates and the vertical line equals the confidence interval at 95%). B: dry weight (Each point is the mean of three replicates and the vertical line equals the confidence interval at 95%). Calculated parameters: reserves (Sum of carbohydrates, lipids and proteins) and difference between dry weight and reserves are represented by broken lines.

RESULTS

Changes of chemical composition. Carbohydrates, lipids and proteins (Total N × 6.25) decreased with storage time in dilute medium (Fig. 1A), lipids by as much as 92%. Dry weight and reserves (sum of carbohydrate, lipid and protein) both decreased with time at the same rate (Fig. 1B). The difference between dry weight and reserves did not change with time during the experiment (correlation coefficient 0.69 with a R limit of 0.87).

In both media, lipid and nitrogen content decreased (Fig. 2), but more slowly in the concentrated medium than in the dilute. The carbohydrate content of juveniles stored in concentrated medium followed a different pattern: it increased during the first 2 wks (values at 0 and 2 wks were significantly different at the P = 0.01) and then remained the same.

Motility and infectivity. Motility of juveniles decreased sharply in dilute medium, ceasing after 5 wks of storage (Fig. 3). During storage in concentrated medium, juveniles lay motionless and straight; on return to dilute medium they always recovered and became highly mobile (Fig. 3).

Infectivity was calculated for each storage time by multiplying the rate of motility by the percentage of juveniles invading roots. Infectivity dropped abruptly during the 2nd wk of storage in dilute medium but remained constant in concentrated medium (Fig. 3).
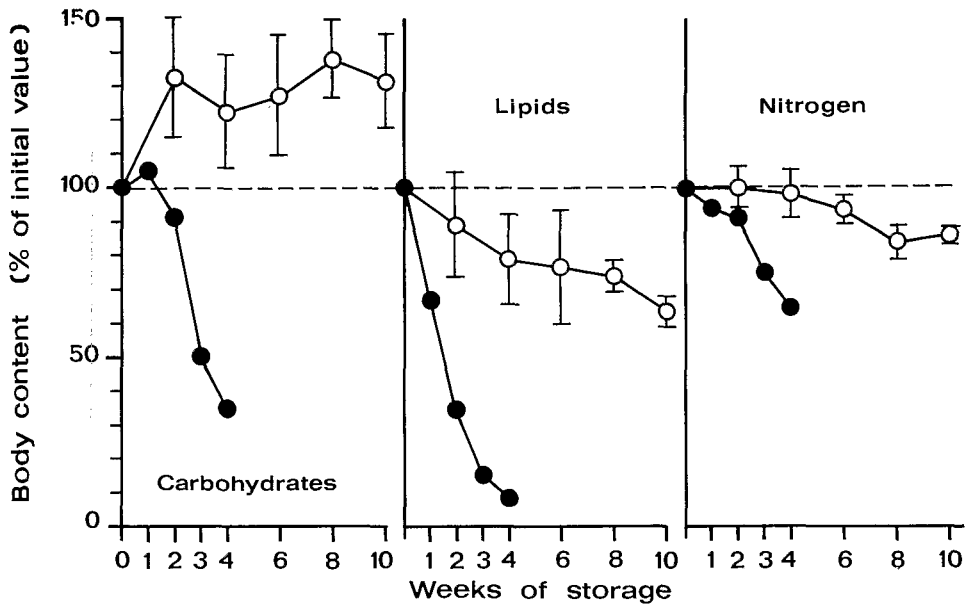
Fig. 2. Changes of carbohydrates, lipid and nitrogen content of *Meloidogyne javanica* juveniles dur-
ing storage: ●, in dilute medium; ○, in concentrated medium. (Each point is the mean of three
replicates and the vertical line equals the confidence interval at 95%; confidence intervals for
dilute medium are given in fig. 1A).

Reproduction. P_i, the initial population density of juveniles in the roots of five
kenaf seedlings, was estimated by counting numbers of stained juveniles dur-
ing the determination of infectivity. P_f was the number of juveniles released by
females that developed from P_i. The ratio P_f/P_i for each storage time was
almost constant throughout the experiment, indicating that reproduction was
unaffected by the age of the inoculum (Fig. 4).

DISCUSSION

Osmobiosis and quiescence. Since all the food reserves studied were affected by
storage, physiological age may be characterized by the Total Reserves Index
(TRI) defined as the sum of carbohydrates, lipids and proteins contained
expressed as a percent of its initial value. Time related changes of TRI for
juveniles in both media are in Fig. 5. TRI decreased linearly with time
($P < 0.01$) and the slope for juveniles stored in dilute medium was 7.5 times
that for juveniles stored in concentrated medium. Thus, TRI followed the
theoretical pattern of quiescence defined by Cooper & Gundy (1971). Infectivi-
ty was closely related to TRI: after 1 wk juveniles stored in dilute medium had
a TRI of 83% and an infectivity of 46% and the corresponding values after 8
wks for juveniles stored in concentrated medium were 85% and 46% respec-
tively. Only after 30 wks would juveniles stored in concentrated medium reach
the physiological state of juveniles after 4 wks of storage in dilute medium.
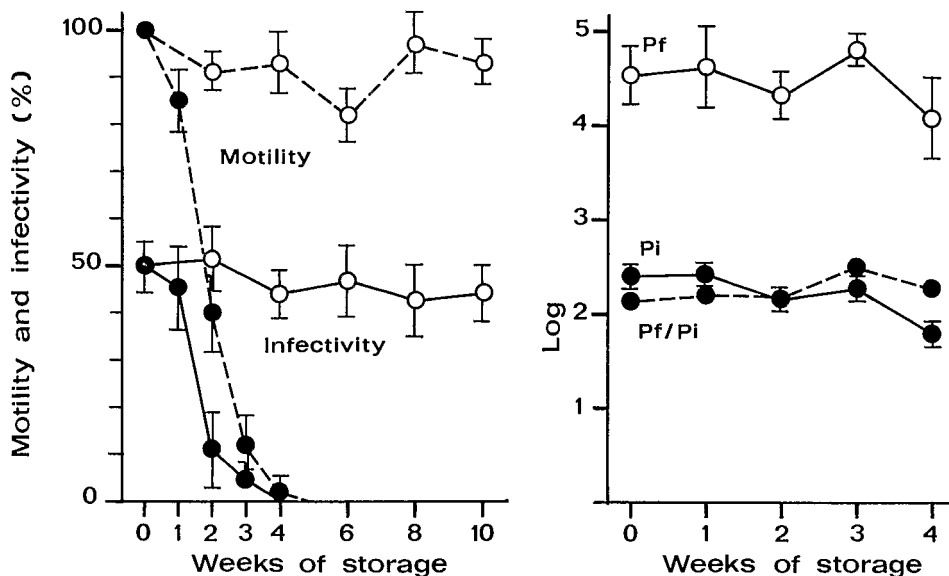
Fig. 3. Motility (- - -) and infectivity (———) of *Meloidogyne javanica* juveniles after storage: ●, in dilute medium; ○, in concentrated medium. (Each point is the mean of five replicates for motility and 16 replicates for infectivity and the vertical line equals the confidence interval at 95%).

Fig. 4. Effect of a pre-inoculation storage in dilute medium of *Meloidogyne javanica* juveniles on their subsequent multiplication on kenaf. Pi, initial numbers of invading juveniles in roots of five kenaf seedlings; Pf, final numbers of juveniles released from the same five plants during 8 wks in hydroponic culture. (Each point is the mean of five replicates and the vertical line equals the confidence interval at 95%). Calculated parameter: the ratio Pf/Pi is represented by a broken line.
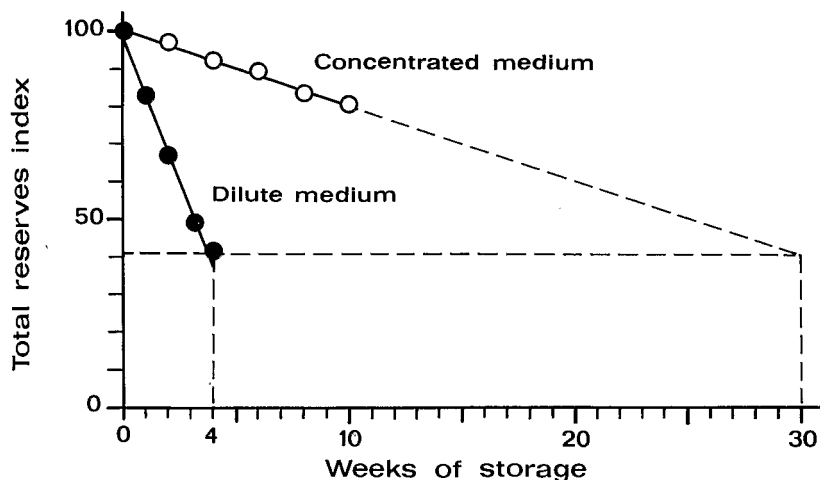

Fig. 5. Variation of the Total Reserves Index of *Meloidogyne javanica* juveniles (see text) during storage: ●, in dilute medium; ○, in concentrated medium (slopes of regression lines: −15% in dilute medium and −2% in concentrated medium). The horizontal broken line represents the lowest value of TRI in juveniles, observed after 4 wks of storage in dilute medium. The extrapolated regression line of TRI for concentrated medium intersects the horizontal broken line at 30 wks.

Thus, osmotic pressure induced an osmobiotic state in juveniles of *M. javanica* by decreasing the rate of consumption of food reserves. High osmotic pressures depress the rate of metabolism of plant parasitic nematodes (Wallace & Greet, 1964; Bhatt & Rohde, 1970; Reversat, 1977) but not to the extent I observed in the present study. Since the above workers measured respiration during the day following application of osmotic stress, it is suggested that metabolism is reduced to its lowest rate only after one or several days.

Effects of storage in dilute medium. The consumption of lipid reserves in juveniles of *M. javanica*, as % of initial content, was estimated at 60% after 10 days at 27° (Van Gundy *et al.*, 1967), 65% after 7 days at 28° (Reversat *et al.*, 1980) and 65% after 14 days in the present study. These differences may be attributed to the use of different clones of the same species and to the different storage conditions, especially to the density of nematodes per unit of surface area in contact with free air in the storage vessels. Lowered oxygen concentration slowed the consumption of reserves by *Meloidogyne* juveniles (Van Gundy *et al.*, 1967; Ogunfowora, 1978): excretory products released in the storage medium may also influence the rate of metabolism. At the end of my experiment, before the last juveniles became immobile, 8% of the lipid reserves were unconsumed (Fig. 1A). These remaining lipids may have a structural rather than a metabolic function.

The consumption of granular proteins and glycogen was demonstrated histochemically in *Meloidogyne* juveniles (Chitwood, 1951; Van Gundy *et al.*, 1967; Dropkin & Acedo, 1974). Our work quantifies consumption for the first time. The agreement between the change in dry weight and the change in reserves (Fig. 1B) showed that major substances involved in the metabolism of starved juveniles were accurately determined by the methods used.

Storage time did not influence the overall rate of reproduction of *M. javanica* juveniles (Fig. 4). Although egg hatching and egg laying may not have been completed when the experiment was stopped, the result suggests that juveniles without food reserves were able to establish themselves in the roots and develop into adults. This contradicts the findings of Dropkin & Acedo (1974), who showed the importance of endogenous food reserves in the development of invading *Meloidogyne* juveniles, before giant cells become functional. Possibly only few of the invading juveniles developed into adults but they had an enhanced rate of reproduction. Thus, an investigation of the rate of reproduction of individual females, which have developed from young and old juveniles is needed.

Food reserve interconversions. In juveniles immersed in concentrated medium, during the first 2 wks, the constant protein content and the significant increase of carbohydrates and decrease of lipids (Fig. 2) demonstrates the conversion of lipids to carbohydrates as in other nematodes (Passey & Fairbairn, 1957; Cooper & Van Gundy, 1970; Rubin & Trelease, 1975; Reversat, 1980b). In juveniles stored in dilute medium, the conversion would be obscured by the rapid consumption of reserves.

RÉSUMÉ

Consommation des réserves nutritives au cours du jeûne chez les juvéniles du second stade de Meloidogyne javanica, *dans des conditions induisant l'osmobiose.*

Des juvéniles fraîchement éclos de *Meloidogyne javanica* sont stockés à 28°, soit dans un milieu dilué (tampon phosphate pH 7-4 mM), soit dans un milieu concentré (NaCl à 0,3 M dans le milieu dilué). À des intervalles de temps d'une semaine pour le premier milieu et de deux semaines pour le second, on a mesuré l'activité, le pouvoir infestant et la teneur en réserves nutritives des juvéniles. L'activité et le pouvoir infestant des juvéniles maintenus en milieu dilué décroissent rapidement jusqu'à zéro en 5 semaines, tandis que 60% des réserves nutritives sont consommées. Chez les juvéniles maintenus en milieu concentré, la vitesse de consommation de ces réserves est 7,5 fois plus faible, révélant ainsi un état de quiescence, et l'activité et le pouvoir infestant restent constants durant 10 semaines. La durée du stockage des juvéniles en milieu dilué n'affecte pas leur taux global de reproduction.

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