Effects of ageing and starvation on respiration and food reserve content in adult *Hirschmanniella spinicaudata*

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SUMMARY

Females and males of *Hirschmanniella spinicaudata*, freshly extracted from infested rice roots, were kept *in vitro* at 28° in the dark. Just after extraction and then every three weeks, batches of animals of the same sex were submitted to physiological measurements: survival, respiration and body content of lipids and carbohydrates. Numbers of survivors decreased with time to zero at 33 weeks for females and 36 weeks for males. Initial food reserves were 1.5 µg of lipids and 0.7 µg of carbohydrates per female and 0.9 µg of lipids and 0.5 µg of carbohydrates per male. After nine weeks of storage 86% of lipids and 65% of carbohydrates were consumed in females and 88% of lipids and 71% of carbohydrates in males. Initial hourly rates of O_2 uptake were 5.4 nl per female and 2.1 nl per male. After twelve weeks of storage these rates dropped to 0.7 nl per female and 0.5 nl per male.

RÉSUMÉ

*Effets du vieillissement et du jeûne sur la respiration et la teneur en réserves nutritives chez les adultes d'*Hirschmanniella spinicaudata

Des femelles et des mâles d'*Hirschmanniella spinicaudata*, fraîchement extraits de racines infestées de riz, sont gardés in vitro à 28° à l'obscurité. Juste après l'extraction, puis à des intervalles de temps de trois semaines, des lots d'animaux de chaque sexe sont testés pour plusieurs paramètres physiologiques : survie, respiration et teneurs en lipides et glucides. Le nombre des animaux survivants décroît avec le temps et devient nul à 33 semaines pour les femelles et 36 semaines pour les mâles. Les teneurs initiales de réserves nutritives sont de 1,5 µg de lipides et 0,7 µg de glucides par femelle et de 0,9 µg de lipides et 0,5 µg de glucides par mâle. Après neuf semaines de stockage, 86% des lipides et 65% des glucides sont consommés chez les femelles et 88% des lipides et 71% des glucides chez les mâles. La consommation horaire initiale d'oxygène est de 5.4 nl par femelle et de 2,1 nl par mâle. Après douze semaines de stockage, ces consommations chutent à 0,7 nl par femelle et 0,5 nl par mâle.

Until the present time, quantitative studies concerning the effects of starvation on the chemical composition of infective stages in plant parasitic nematodes have been confined to second stage juveniles of species fitted with egg-masses (Van Gundy, Bird & Wallace, 1967 ; Ogunfowora, 1978 ; Reversat, 1980, 1981). The lack of information about other infective stages is probably due to difficulties in obtaining sufficient amounts of biological material needed for chemical determinations. Adults of the nematode *Hirschmanniella spinicaudata* are infective to rice roots (Merny, 1972) and characterized by their rather large size (Luc & Fortuner, 1978). A female of *H. spinicaudata* is equivalent to 100 and a male to 60 juveniles of *Meloidogyne javanica*, according to the calculation of the wet weight by Andrássy (1956). Thus sampling of these animals for chemical determination is possible by individual handling.

The present study was initiated to determine some effects of ageing and starvation on females

and males of *H. spinicaudata*. Survival, respiration and food reserves content were measured after different times of storage *in vitro*.

Materials and methods

Nematode production

Populations of *H. spinicaudata* were increased in the greenhouse on rice *(Oryza sativa L.)* cv. Morobérékan. Pots containing 2.5 liters of steam sterilized sandy soil were sowed with six seeds and watered daily until the appearence of the fourth leaf (three weeks). At this date pots were placed in containers with sealed bottom, flooded with water and then mass inoculated with 2 000 individuals of mixed stages. Every two weeks from sowing to the yield, each pot was fertilized with 215 mg of urea and 175 mg of $K H_2 PO_4$. Once a week pots were taken out of the containers, drained for 48 hours and then replaced and flooded in the containers. These conditions of alternative flooding and drainage prevented the formation of toxic soluble sulphurs and favoured the development of *H. spinicaudata* populations (Fortuner, 1976). Infested roots were harvested eight to twelve weeks after inoculation, carefully cleaned and then macerated in an aqueous solution. At the beginning of this work animals stored in water were attacked by an unidentifieded fungus, which killed them all in a few weeks. Agallol ®, which is a fungicide prevented this infection. Thus maceration of the roots and further storage of animals were carried out in a solution of 125 ppm of Agallol ® in 4 mM sodium phosphate buffer, pH 7 (Gomori, 1955), called AP solution. Roots in AP solution were aerated by diffusion in a shallow tray or by bubbling in a jar, and kept at 28° in the dark. Every three days, roots were placed in fresh AP solution and nematodes leaving the roots were recovered in the solution. Nematodes were allowed to pass through two layers of kleenex ® paper mounted on a coarse plastic sieve (aperture 1 mm) and clean nematodes were collected 24 hours later. At this date, nematodes were considered at the zero time of the experiment whereas their trophic contact with the plant had been interrupted since 2.5 ± 1.5 days. Nematodes were then stored in AP solution in 1 liter Roux bottles with the deepness of the medium not exceeding 4 mm at 28° in the dark.

Survival

At the zero time, 300 females and 300 males were divided in lots of 25 individuals of the same sex. Each lot was placed in a perspex round box with a diameter of 3.5 cm with 2.5 ml of AP solution. Boxes with a device preventing evaporation were stored under the preceding conditions. Every three weeks each lot was tranfered in fresh AP solution to a new box, by hand picking each individual. When handling, individuals were tested for survival by checking activity, either spontaneous or when stimulated with a probe. Inactive animals were isolated and examined for the possible alteration of the body content due to death. Dead individuals were discarded and survivors were counted.

Chemical determinations

The day before the times 0, three, six and nine weeks the stored nematodes were placed on kleenex ® paper in AP solution as before for selection of the motile individuals. After one day the suspension was distributed in perspex counting slides (Merny & Luc, 1969) and females and males were individually hand picked under the dissecting microscope and deposited in special centrifuge tubes (Reversat, 1976) filled with de-ionized water. For each of the storage times six tubes with 75 females and six tubes with 100 males were prepared. In order to achieve a random sample each adult was picked following the rows of the slide and deposited in a different tube until all the tubes received the required number of individuals. Nematodes were settled by centrifugation and the excess water was discarded. Tubes with nematodes in about 20 µl of water were stored at —20° until use.

For each of the carbohydrates and lipids determinations, three tubes were used. General procedures for chemical determinations (Reversat, 1976) were modified as follows. For carbohydrates, 0.25 ml of KOH 30 % was added to each tube and then tubes were kept in a boiling

water bath for 1/2 hour during which nematodes were completely digested. After cooling, the content of tubes was made up to 1 ml with de-ionized water. Carbohydrate content of the potassic solution was spectrophotometrically determined with the anthron reagent against a standard of glucose (Seifter *et al.*, 1950). For lipids, 2.5 ml of concentrated H_2SO_4 was added to each tube and then tubes were kept in a boiling water bath for ten minutes during which animals were completely digested. After cooling, lipid content of the sulfuric solution was spectrophotometrically determined with the sulpho-phospho-vanillic reagent (Drevon & Schmit, 1964) against a standard of olive oil dissolved in concentrated H_2SO_4. Results are expressed in microgram per individual (µg/ind.).

Respiration

Respiration rate was determined using glass stoppered Cartesian divers (Reversat, 1975). Animals in the divers were kept in a solution containing 7.5 ppm of 2-methoxyethyl mercury chloride (active ingredient of Agallol) in 20 mM sodium phosphate buffer, pH 7 (Gomori, 1955). Measurements commenced two hours after the introduction of animals into the divers and were made at 28° during six hours. From four to ten individuals were introduced in the 10 µl divers according to previous experiments. Results are expressed in nanoliter of oxygen per individual per hour (nl/ind./hr.). Respiration was measured on eight replications for each sex after 0, three, six, nine and twelve weeks of storage.

Results

Survival

Stored animals exhibited four survival states : *a*) active, *b*) inactive with a twisted habitus, reacting well to a light touch, *c*) inactive with a straight habitus, internal structure unchanged, reacting poorly even to intense touch, generally, by bending at the neck level, *d*) inactive with a straight habitus, internal structures altered, never reacting to touch. When animals were stored individually, stages *b* and *c* were reversible to stage *a* but stage *d* was not reversible. Thus stages *a*, *b* and *c* were counted as survivors throughout the experiment. The percentage of survivors decreased steadily in both sexes and became nil at the end of 33 weeks in females and 36 weeks in males (Fig. 1).

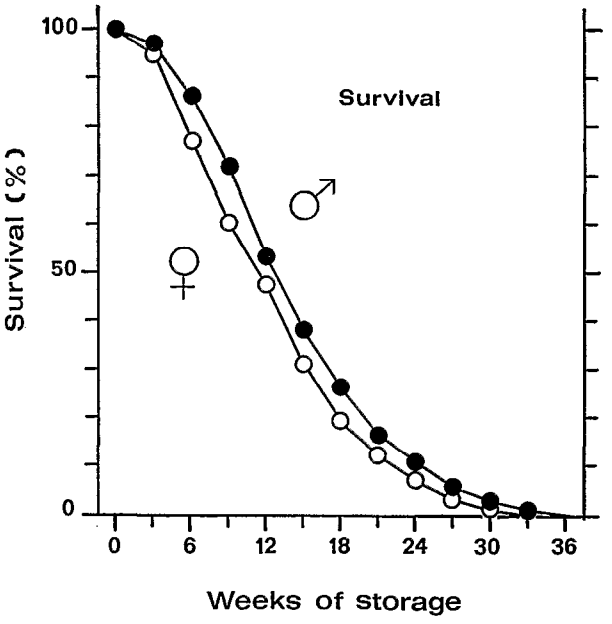

Fig. 1 : Survival curves of adult *Hirschmanniella spinicaudata* (Each curve was determined on 300 individuals, stored at 28° and examined individually every three weeks).

Chemical composition

Lipid content decreased rapidly in both sexes during the first three weeks and then the rate of decrease slowed (Fig. 2). At the end of nine weeks, consumption of lipids expressed as a percentage of initial content was 86% in females and 88% in males. In both sexes, carbohydrate content remained constant during the first three weeks interval and then decreased (Fig. 3). At the end of nine weeks, consumption of carbohydrate was 65% in females and 71% in males.

Respiration

In both sexes, respiration decreased rapidly during the first three weeks and then remained almost constant (Fig. 4).

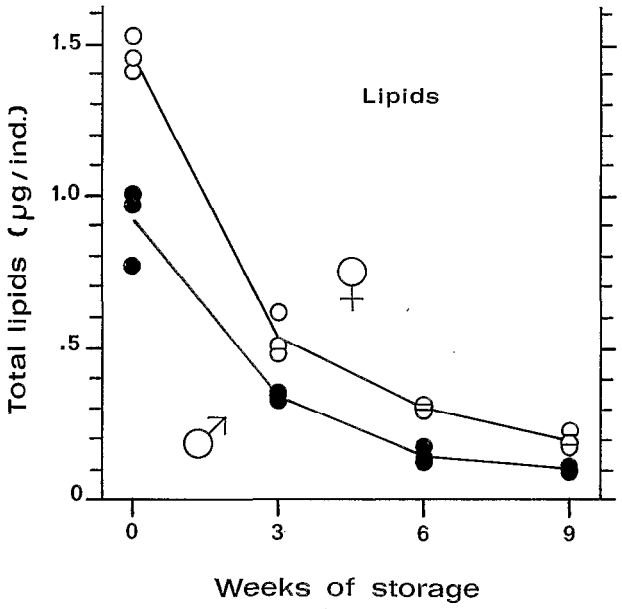

Fig. 2 : Effect of storage time on the lipid content of adult *Hirschmanniella spinicaudata* (For each storage time, three replicates were determined for each sex, females, open circles and males, black circles. Straight lines are joining means, which are not figured).

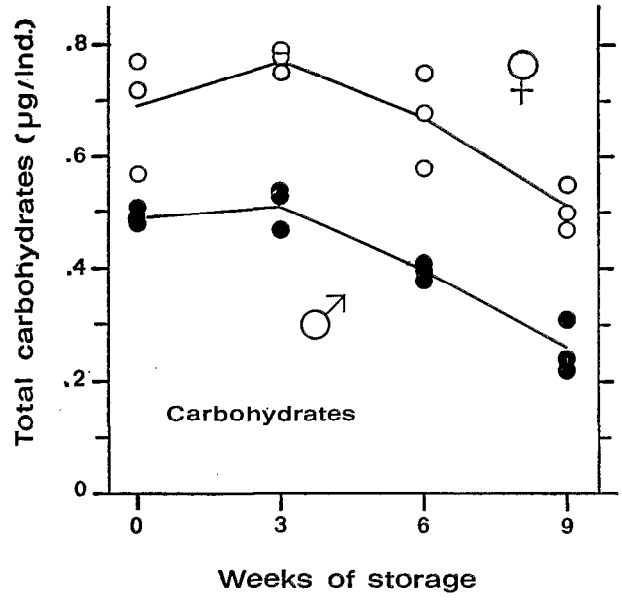

Fig. 3 : Effect of storage time on the carbohydrate content of adult *Hirschmanniella spinicaudata* (specifications as in Fig. 2).

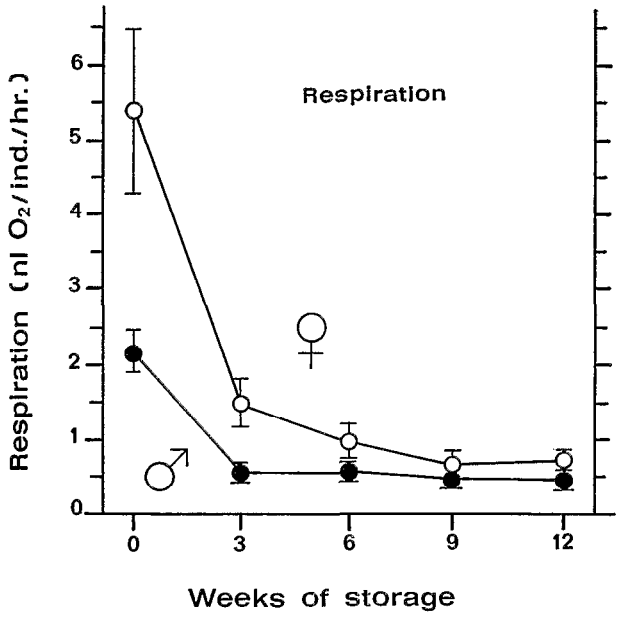

Fig. 4 : Effect of storage time on the respiration (rate of O_2 consumption) of adult *Hirschmanniella spinicaudata* (For each storage time, eight replications were determined at 28° and the vertical line equals the confidence interval at 95%).

Discussion

Animals for study were selected from a mass rearing and their actual age was heterogeneous when the experiment started. Synchronous rearing obtained by inoculating only second stage juveniles may provide a more precise approach. Stages a, b, c and d might be presumably the sequence of natural events leading animals to death. Assessment of activity, either spontaneous or stimalated with a probe, has been used by some authors (Golden & Shafer, 1960 ; Slack, Riggs & Hamblen, 1972) for survival determination. However, activity, could be reversely interrupted by resting phases (Barrett, 1969). The demonstration of degenerate body content as used by Davies and Fisher (1976) and in the present study appears a more reliable test to ascertain nematode death.

Lipid content was greater than carbohydrate content in freshly extracted nematodes and in the course of ageing, lipids were consumed before carbohydrates (Fig. 2 & 3). This has been observed in juveniles of *Helerodera oryzae* (Reversat, 1980), *Meloidogyne javanica* (Rever-

sat, 1981) and *Strongyloides ratti* (Barrett, 1969). Since the rate of lipid consumption was very high during the first three weeks (Fig. 2) it is suggested that feeding occured constantly when animals were inside the roots. This could be correlated also with the interruption of egg-laying in stored females.

A decreasing rate of respiration with age has been observed in several plant parasitic nematodes (Rohde, 1960 ; Wallace & Greet, 1964 ; Bhatt & Rohde, 1970 ; Van Gundy, Bird & Wallace, 1967 ; Reversat, 1980). Atkinson (1976) suggested that in animals experiencing normally prolonged absence of food, which is true for *H. spinicaudata*, the observed decline of respiration rate may have an adaptative significance. An answer to this question could be obtained by refeeding animals after some weeks of starvation.

Equivalence between results of respirometry and results of chemical determinations may be tested by 1) integrating the curves of respiration rates (Fig. 4) for the period 0-9 weeks 2) calculating the required quantity of oxygen for a complete oxidation of lipids (Fig. 2) and carbohydrates (Fig. 3) consumed during the period 0-9 weeks. Suitable coefficients are 2 019 ml/g for lipids and 829 ml/g for carbohydrates (Polonowki *et al.;* 1966). There is a good correlation for females : animals consumed 2 760 nl of oxygen and the oxidation of reserves needed 2 720 nl of oxygen. However males consumed less oxygen (1 250 nl) than required for oxidation of reserves (1 830 nl). This suggests that the metabolism could be different in both sexes : a complete oxidation of reserves in females and an incomplete oxidation of reserves probably balanced by an excretion of organic substances in males.

ACKNOWLEDGEMENTS

The author thanks Bayer AG, Leverkusen (West Germany) for kindly supplying Agallol ® and 2 methoxymethyl-mercury chloride and T. Maiga for assistance in the making of illustrations.

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Accepté pour publication le 25 octobre 1980.