EFFECT OF STORAGE TEMPERATURE ON THE *IN VITRO* REPRODUCTION OF *RADOPHOLUS SIMILIS*

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RESUMEN

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Se estudió la reproducción de *Radopholus similis* en cultivos monoxénicos de zanahoria almacenados a 15 y 20°C durante varios períodos de tiempo. El estudio se dividió en dos partes. En la primera se utilizaron 3 grupos de 6 frascos cada uno inoculados con 160 *R. similis.* Las extracciones se realizaron 50, 70 y 90 días después de la inoculación. En la segunda, se inocularon 100 *R. similis* sobre 4 grupos de 5 frascos cada uno almacenados a 15°C durante 50, 90, 135 y 260 días, respectivamente. Seguidamente, cada grupo se trasladó a una temperatura de 27°C. Las extracciones se realizaron en el momento en que se observó un deterioro de los discos de zanahoria en alguno de los frascos de cada grupo. Los resultados demuestran que *R. similis* no se reproduce sobre discos de zanahoria a 15°C y que la población inicial desaparece entre el día 135 y 260 después de la inoculación. Por el contrario, a 20°C se observó un crecimiento de la población hasta los 90 días. A partir de este momento el deterioro de las zanahorias comienza a ser un factor limitante para la reproducción del nematodo. Se demuestra que el uso de una temperatura de almacenamiento de 15°C seguida de un período de incubación a 27°C, puede ser una alternativa para aumentar la longevidad de los cultivos monoxénicos.

Palabras claves: cultivos monoxénicos, mantenimiento, *Radopholus similis,* reproducción, temperatura.

Monoxenic cultures on carrot disks are widely used for rearing migratory endoparasitic nematodes, which include the burrowing nematode *Radopholus similis* Cobb, 1913 (3-5,7). This method is quite simple and allows the production of a large amount of nematodes in a relatively short time. However, due to the degradation of carrot tissues, the cultures need to be transferred to fresh carrot disks frequently. Longer term storage methods would be highly desirable to reduce the number of transfers needed to maintain collections.

Previous experiments showed that reproduction of *R. similis,* as well as degradation of carrot tissues, was very low at 21°C (2). Therefore, temperatures below 21°C could allow longer storage of *R. similis* cultures when mass production of inoculum is not urgently needed. The aim of this work was to determine *R. similis* reproduction and maximum storage time in cultures maintained at <21°C.

Nematodes were extracted from banana roots collected at Anguédédou (Ivory Coast), then reared on carrot disks for three years at 27°C (±0.5°C). Monoxenic cultures were established in 100-ml flasks containing 5 ml of 1% agar solution and 4 or 5 carrot disks (1-cm-diam, 5-7 g total weight). A sterile water suspension of surface-sterilized (500 ppm dihydrostreptomycin and 0.01% mercuric chloride) nematodes (89-90% females and 10-11% males and juveniles) was poured on the surface of the carrot disks.

Two experiments were conducted. In the first experiment, 160 (±20) nematodes per flask were inoculated in three series of six flasks each, which were stored at 20°C

(±0.5°C). Nematode extractions were performed 50, 70, and 90 days after inoculation for each series respectively. In the second experiment, 100 (±16) nematodes per flask were inoculated in four series of five flasks each. These were maintained at 15°C (±0.5°C) for 50, 90, 135, and 260 days for each series, respectively. After each storage period, cultures were transferred and incubated at 27°C (±0.5°C) to accelerate nematode reproduction. Nematodes were extracted when degradation of carrot tissues became clearly visible in any flask of the series, i.e. 45 days for the first two series, 75 days for the third series, and 70 days for the fourth series. For nematode extraction, the contents of the flasks were washed out and macerated in a blender (2). The suspension was poured on a sieve column (250, 80, 50, and 32 µm, respectively). Carrot tissue and debris collected on the 250-µm sieve were discarded. Nematodes and tissue debris collected from the other three sieves were separated using the centrifugation-flotation technique (1).

At 20°C, nematode numbers per flask increased in relation to the storage time. At 50 days after inoculation, a mean standard deviation of 930 + 119 nematodes were recovered (Pf/Pi = 5.8); at 70 days, 9 380 + 1 183 (Pf/Pi = 58.6); and at 90 days, 14 650 + 653 (Pf/Pi = 91.6) were recovered. The degradation of carrot tissue did not allow the culture to be maintained after 90 days.

At 15°C, the longer the cultures were stored, the lower the amount of nematodes that were recovered (Table 1). After a 50-day storage at 15°C, cultures transferred and incubated at 27°C for 45 days produced an average of 29 000 nematodes per flask (Pf/Pi = 290), but only 1/3 of this number after a 90-day storage at 15°C and 45 days at 27°C. When the storage at 15°C lasted for 135 days, carrot tissue degradation became visible 75 days after transfer of cultures at 27°C, and an average of 7 700 nematodes were recovered (Pf/Pi = 77). After a 260-day storage at 15°C, no nematodes were recovered. These results indicate that *R. similis* was not able to reproduce on carrot disks at 15°C and that the initial population disappeared between 135 and 260 days. Very recent studies confirm that *R. similis* does not reproduce at 16°C (6). In contrast, population build-up occurred in our test at 20°C. Therefore, the thermal minimum for *R. similis* reproduction and survival would be between 16 and 20°C.

In terms of storage for maintaining collections, moderate temperatures (15-20°C) prolong the viability and reduce degradation of monoxenic cultures of *R. similis*. At 20°C, culture storage may be twice as long as at 27°C (90 days, rather than 40-50

Table 1. Final population of *Radopholus similis* after inoculation of 100 nematodes in monoxenic carrot disk cultures stored at 15°C for 50, 90, 135, and 260 days, and then transferred and incubated at 27°C for 45, 45, 75, and 70 days, respectively.

Storage time at 15°C (days)	Incubation time at 27°C (days)	Nematodes/culture[z]
50	45	29 090 a ± 4 660
90	45	10 820 b ± 1 132
135	75	7 740 b ± 2 745
260	70	0 c

[z]Data are mean ± standard deviations of five replications. Means followed by the same letter do not differ according to the Newman & Keuls test ($P \leq 0.05$).

days). *Radopholus similis* can survive at 15°C for a limited time, and our findings indicate that cultures can be stored at this temperature for at least 135 days (4.5 months) when 100 nematodes are inoculated on carrot disks. In that case, the total duration of storage, following transfer at 27°C to obtain a significant amount of nematodes before carrot degradation, was 210 days (7 months).

LITERATURE CITED

1. COOLEN, W. A., and C. J. D'HERDE. 1972. A method for the quantitative extraction of nematodes from plant tissue. State Agricultural Research Center, Ghent, Belgium. 77 pp.
2. FALLAS, G. A., and J. L. SARAH. 1995. Effect of the temperature on the *in vitro* reproduction of seven *Radopholus similis* isolates from different banana producing zones of the world. Fundamental and Applied Nematology 18:(in press).
3. HUETTEL, R. N. 1985. Carrot disk culture. Pp. 153-154 *in* B. M. Zuckerman, W. F. Mai, and M. B. Harrison. Plant Nematology Laboratory Manual. University of Massachusetts Agricultural Experiment Station, Amherst, Massachusetts, U.S.A.
4. KAPLAN, D. T., and E. L. DAVIS. 1990. Improved nematode extraction from carrot disk culture. Journal of Nematology 22:399-406.
5. O'BANNON, J. H., and A. L. TAYLOR. 1968. Migratory endoparasitic nematodes reared on carrot disks. Phytopathology 58:385.
6. PINOCHET, J., C. FERNANDEZ, and J. L. SARAH. 1995. Influence of temperature on *in vitro* reproduction of *Pratylenchus coffeae, P. goodeyi,* and *Radopholus similis.* Fundamental and Applied Nematology 18:(in press).
7. SARAH, J. L., C. SABATINI, and M. BOISSEAU. 1993. Differences in pathogenicity to banana (*Musa* sp., cv. Poyo) among isolates of *Radopholus similis* from different production areas of the world. Nematropica 23:75-79.

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