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Short note

INFLUENCE OF TEMPERATURE ON *IN VITRO* REPRODUCTION OF *PRATYLENCHUS COFFEAE, P. GOODEYI,* AND *RADOPHOLUS SIMILIS*

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The migratory endoparasitic nematodes *Pratylenchus coffeae, P. goodeyi,* and *Radopholus similis* are the species of major concern in banana (*Musa* AAA), and plantain (*Musa* AAB and ABB) production throughout the world (Tarte & Pinochet, 1981; Gowen & Quénéhervé, 1990). The burrowing nematode, *R. similis,* is considered the most damaging of the three species; it is widespread in the hot and humid tropics. *Pratylenchus coffeae* is found in a wider range of hosts and environments, whereas *P. goodeyi* appears to be common in cooler subtropical and highland environments, mainly East and Central Africa (Bridge, 1988). *P. goodeyi* is also considered an important nematode pest of bananas in the Canary Islands, Cyprus, Crete and Taiwan (Stover & Simmonds, 1991). The purpose of the present investigation was to measure the reproduction of three important *Musa* attacking nematodes at a low, medium and high temperature regime under monoxenic conditions.

Material and methods

Pratylenchus goodeyi and *R. similis* were obtained from banana *Musa* AAA in Tenerife, Canary Island, and Anguédédou, Ivory Coast, respectively. *P. coffeae* was originally isolated from *Coffea arabica* roots in Retalhuleu, Guatemala. The isolates were reared moxenically on carrot *(Daucus carota)* disc cultures (Moody *et al.,* 1973) and incubated at 23-24 °C for two to three generations. Nematode inoculum was recovered from cultures, surface sterilized with 2000 ppm of streptomycin sulfate for 3 h followed by three rinses in sterile water prior to the inoculation of carrot sections cv. Nantesa. Each carrot section weighing approximately 2.5 g was placed in a 5 cm-diam. Petri dish. Ten gravid females from each isolate were individually collected with a micropipette and delivered to the surface of a carrot section in 0.1 ml water suspension. Three sets of ten cultures per isolate were prepared and kept in three incubators at 16, 21 and 25 (± 0.5) °C for 75 days. At the end of this period, both nematodes and carrot culture were recovered from the Petri dish and macerated in a blender at 14 500 rpm for 30 s (three 10 s periods separated by 5 s intervals). The suspension was passed through 150 µm-pore sieves (100 mesh) and twice through the 25 µm sieve (500 mesh). Carrot root tissue and debris collected on the 150 µm sieve were discarded. The different developmental stages of each nematode species were counted under the compound microscope based on 1 ml aliquots in a low volume of suspension. Final nematode populations were analyzed by an ANOVA. Data were log_{10} transformed $(x + 1)$ for analysis. Means were compared by Tukey's multiple range test ($P \leq 0.05$ and 0.01).

Results and discussion

Pratylenchus goodeyi reproduced significantly more at 21 °C than at 16 °C, although no differences were found between 21 °C and 25 °C (Table 1). Reproduction of *P. coffeae* differed significantly at each of the three established temperatures. At 16 °C this species barely multiplied. Optimum population increase was recorded at the high temperature regime. A similar pattern was observed with *R. similis,* although in this case no eggs, juveniles or males were found at 16 °C suggesting that reproduction did not occur. The females of *R. similis* recovered (five specimens) seem to be survivors from the initial inoculum. This indicates that this temperature is within the limits of nematode survival and insufficient for nematode reproduction. Highest population build-up for *R. similis* was obtained at 25 °C. Comparative reproduction between the three species clearly indicates *P. goodeyi* multiplies significantly more at a lower temperature and considerably less at 25 °C than *P. coffeae* and *R. similis* (Table 1). There were no differences in the reproduction between the three nematodes at 21 °C. In relative terms, the population build-up of *P. goodeyi* was far below those of the two other species. An explanation could be that *P. goodeyi*'s intrinsic multiplication rate is really lower than the other species, or that *P. goodeyi* has a very sharp thermal optimum that was not measured in the experiment (temperatures between 17 to 20 °C, and 22 to 24 °C). On the other hand, carrot

Table 1. *Reproduction of* Pratylenchus goodeyi, P. coffeae, *and* Radopholus similis *at 16, 21 and 25 °C in monoxenic carrot cultures 75 days after inoculation with ten gravid females.*

Nematode	Temp. (°C)	Nematode developmental stages (%)				Final population
		Eggs	Juveniles	Males	Females	
P. goodeyi	16	34 (19)*	77 (42)	4 (2)	68 (37)	183 *a* C**
	21	75 (9)	638 (74)	63 (7)	88 (10)	864 *b* A
	25	138 (32)	250 (59)	0 (0)	38 (9)	426 *ab* A
P. coffeae	16	15 (27)*	12 (22)	3 (5)	25 (46)	55 *a* B
	21	71 (15)	163 (34)	99 (20)	152 (31)	485 *b* A
	25	3 830 (31)	5 530 (45)	1 312 (10)	1 728 (14)	12 400 *c* B
R. similis	16	0 (0)*	0 (0)	0 (0)	5 (100)	5 *a* A
	21	547 (21)	1 469 (55)	116 (4)	536 (20)	2 668 *b* A
	25	15 250 (36)	19 700 (46)	1 558 (4)	5 907 (14)	42 415 *cb* B

* Values in parentheses are percentages of the total population.

** Means in column for each isolate followed by the same letter do not differ according to Tukey's test ($P < 0.01$). Low case letters : differences between temperature for the same nematode; upper case letters : differences between nematodes at the same temperature : left column : 16 °C; central column : 21 °C; right column : 25 °C.

disc might not have been an ideal medium for multiplication, as it is for *R. similis* and *P. coffeae.*

The low and high temperature requirements for *P. goodeyi* and *R. similis,* respectively, have been documented mainly based on monitoring populations at different altitudes in Rwanda (Sarah, 1989) and Cameroon (Bridge *et al.,* 1995). However, the temperature requirements, especially the minimum levels, for the development of each of these nematodes species were unknown. These results substantiate the previous observations and indicate that *R. similis* does not persist in temperatures below 16 °C or perhaps, even slightly higher than 16 °C. On the other hand, both *Pratylenchus* species appear to adapt to a wider range of temperatures in which *P. coffeae* shows a preference for temperatures around 25 °C similar to that required by *R. similis.* Optimum temperature for *P. coffeae* development appears to be well over 25 °C. On citrus *(Citrus jambhiri),* optimum reproduction occurred at 29.5 °C (Radewald *et al.,* 1971). In this study, nematode reproduction was not measured above 25 ± 0.5 °C.

It is noteworthy that *R. similis* is not present in the Canary Islands where *P. goodeyi* is widespread on bananas. It is likely that the burrowing nematode has been introduced through infested rhizomes (seed material) brought in from Central America or Africa this century, but the cooler temperatures that prevail in the Canary Islands, especially from December to April, have probably prevented its survival. *P. coffeae* is also present in the Canary Islands, although its occurrence is rare. It could have been introduced from Central America where it is commonly found on bananas together with *R. similis.* The tolerance of *P. coffeae* to a wider range of temperatures, specially in the lower range (16 to around 20 °C) would explain its survival in the Canary Islands.

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