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Effect of temperature on the *in vitro* multiplication of seven *Radopholus similis* isolates from different banana producing zones of the world

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Summary – A study was carried out to compare the multiplication rate as a function of temperature of several isolates of the burrowing nematode *Radopholus similis* cultured monoxenically on carrot discs. These isolates were collected on banana roots in seven production areas of the world (Costa Rica, Martinique, Guadeloupe, Guinea, Ivory Coast, Sri Lanka, and Queensland). The study was divided into two parts. In the first part, each isolate was studied at four temperatures regimes : 21, 24, 27 and 30 °C. In the second part, the seven isolates were studied concurrently at two temperatures : 30 and 33 °C. All the isolates showed a similar pattern of behaviour in relation to the temperature. Multiplication rate was very low at 21 °C, increased rapidly in relation to the temperature to reach a maximum level at 30 °C, falling sharply at 33 °C. On the other hand, the different isolates had very different intrinsic multiplication rates at all temperatures. The isolates from Ivory Coast, Costa Rica and Guinea showed the highest multiplication rate, whereas the isolates from Martinique and Queensland had the lowest. These results confirm the wide range of intraspecific biological diversity of *Radopholus similis*.

Résumé – ***Effet de la température sur la croissance* in vitro *de sept isolats de* Radopholus similis *provenant de différentes zones de production bananière du monde.*** – Une étude a été réalisée afin de comparer l'effet de la température sur le taux de multiplication de divers isolats du nématode *Radopholus similis* en élevage monoxénique sur rondelles de carottes. Ces isolats ont été collectés sur racines de bananiers dans sept zones productrices du monde (Costa Rica, Martinique, Guadeloupe, Guinée, Côte d'Ivoire, Sri Lanka, et Queensland). Cette étude a été divisée en deux parties. Dans la première, les différents isolats ont été étudiés séparément, à quatre températures : 21, 24, 27 et 30 °C. Dans la seconde, les sept isolats ont été étudiés en même temps à deux temperatures : 30 et 33 °C. Les différents isolats se comportent de la même manière vis-à-vis de la température. La croissance des populations est très faible à 21 °C et augmente fortement avec la température jusqu'à un maximum à 30 °C pour chuter brusquement à 33 °C. En revanche ces isolats se différencient par leur taux intrinsèque de multiplication, ceux de Côte d'Ivoire, du Costa Rica et de Guinée présentant les plus fort taux de multiplication. A l'opposé, les isolats de Martinique et du Queensland ont les taux les plus faibles. Ces résultats confirment la très forte diversité biologique au sein de l'espèce *Radopholus similis*.

Key-words : banana, biological diversity, multiplication rate, *Radopholus similis,* temperature.

Abundant information is available on the biological and physiological diversity of nematodes (Sturhan, 1971; Dropkin, 1988). The knowledge of the intraspecific diversity, especially in relation to pathogenicity, is essential for research concerning plant-nematode interactions leading towards successful plant breeding for resistance and effective integrated nematode control through cultural practices, such as crop rotation (Wallace, 1963; Sturhan, 1971; Dropkin, 1988).

The biological diversity of *Radopholus similis* was first studied on isolates from Central America and the Caribbean (Edwards & Wehunt, 1971; Pinochet, 1979, 1988; Tarté *et al.,* 1981; Rivas & Román, 1985). These studies revealed a large diversity in pathogenicity to banana plants, in direct relation to the multiplication rate. Recently, it has been shown that the diversity of this species extends to other areas of the world (Sarah *et al.,* 1992, 1993). *R. similis* diversity is apparently due to divergent evolution under different environmental and host conditions. Of the environmental conditions, temperature might play an important role.

Studies on influence of temperature on sub-specific biological diversity performed on nematodes species are relatively rare (Fagbenle *et al.,* 1989; Jaehn & Lordello, 1990; Rutherford *et al.,* 1992). To our knowledge, no studies have been carried out to date on *R. similis.*

Materials and methods

The study was performed with seven *R. similis* isolates from different banana growing areas of the world. Five of these, Martinique (Morne Rouge), Guadeloupe (Neufchâteau), Costa Rica (Talamanca), Ivory Coast (Anguédédou) and Sri Lanka (Hantane), had been previously studied in relation to their pathogenicity on banana plants (Sarah *et al.,* 1993). Two further isolates, one from Guinea (Balikouré) and the other from Aus-

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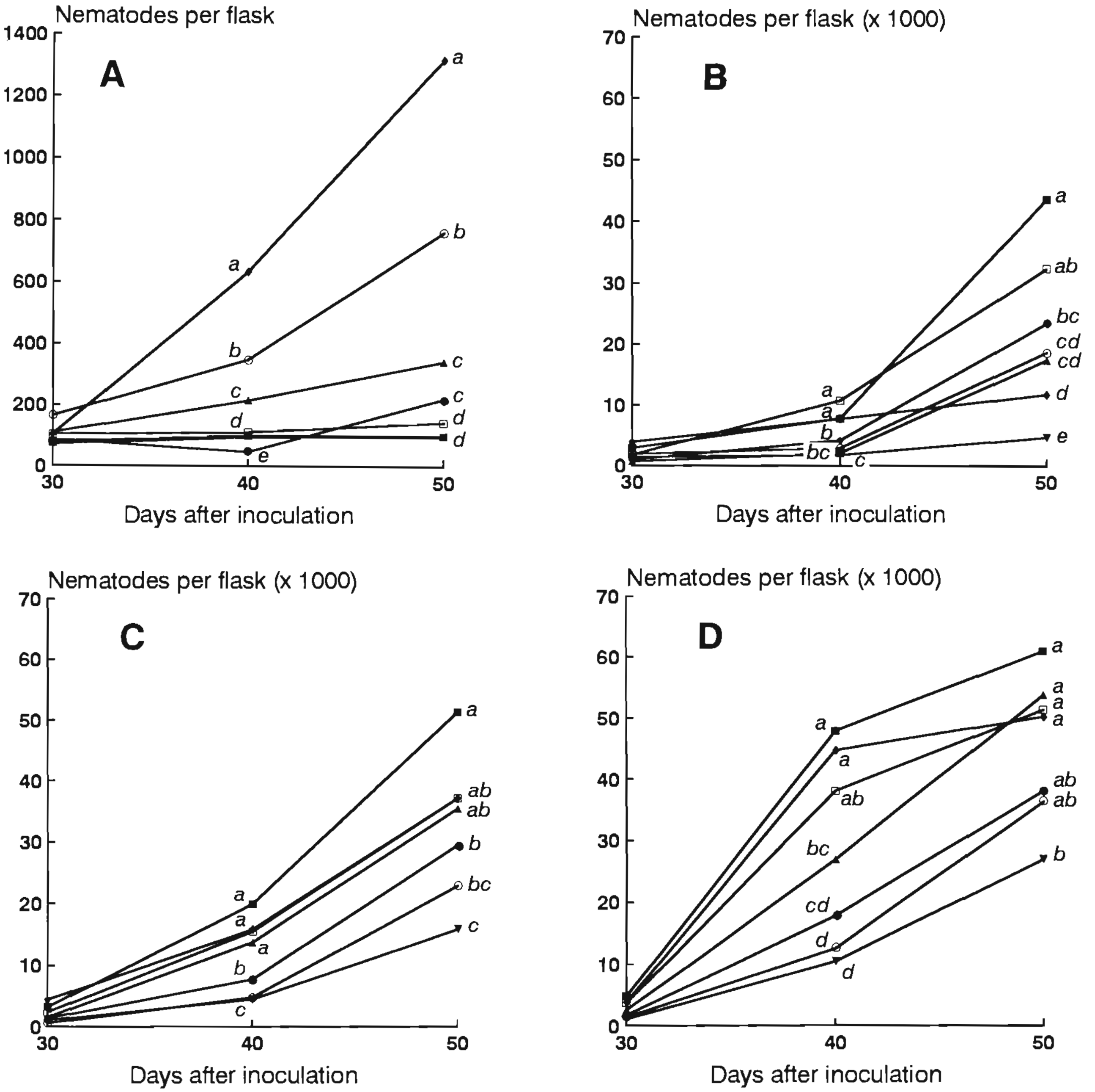

Fig. 1. *Population build-up of seven* Radopholus similis *isolates at different temperatures on carrot discs.* A : 21 °C; B : 24 °C; C : 27 °C; D : 30 °C. (■ : Ivory Coast; □ : Guinea; ♦ : Costa Rica; ▲ : Guadeloupe; ▼ : Martinique; ● : Sri Lanka; ○ : Queensland). *Means of five replicates. Actual data are presented, but data were transformed to* $\log_{10}$ (x + 1) *for analysis. Means at the same date with the same letter are not different according to Newman and Keuls test (*P ≤ *0.05).*

inoculation date. At 30 °C, the multiplication rates were very high and similar to those observed in the first study 30 and 40 days after inoculation whichever isolate was considered. This shows that the results obtained here are reliable and reproducible. Therefore, the results of the two studies may be combined to draw graphs of population levels observed at 40 days as a function of temperature, for the different isolates (Fig. 2). At 40 days no carrot tissue degradation was observed and the data obtained reflect the reproduction rate of each isolate. The graphs are similar in appearance for all isolates, showing 40-day nematode number increasing with temperature to a maximum at 30 °C and falling back sharply at 33 °C.

The pooling of data from the two studies allowed good separation, by Newman and Keuls test, of the levels observed at 30 °C 40 days after inoculation (Fig. 2). The Ivory Coast and Costa Rica isolates reached the highest population levels. The Guinea isolate showed population levels lower than those of the Ivory Coast isolate, but not significantly different from those of the Costa Rica isolate. These were followed in order of decreasing levels by the Guadeloupe isolate, then the Sri Lanka isolate. The Queensland and Martinique isolates presented similar population levels, which were the lowest observed here.

Discussion

The general pattern of population build-up as influenced by temperature was roughly uniform among the different isolates of *R. similis* studied here. All the isolates reached highest numbers at 30 °C. The minimum temperature limit for multiplication was probably not far below 21 °C and the maximum temperature limit was close to 33 °C. This maximum temperature was not a lethal temperature, since the nematodes recovered were alive, but there was little or no reproduction at this temperature whatever the considered isolate. One should make the hypothesis that this homogeneity among isolates should have been induced by the common breeding temperature at which all the isolates were maintained for a period ranging from 3 years (Martinique, Ivory Coast) to 1 year (Queensland). Thermal acclimatization is widespread among invertebrates, and thermal preferendum for moves of some nematode species has been proved to be influenced by temperature of storage (Croll, 1967; Robinson, 1989; Robinson & Heald, 1989, 1993). However, the observed optimal temperature (30 °C) was higher than the breeding temperature (27 °C), and there was a good homogeneity of relative multiplication rate as a function of temperature for all isolates. Therefore this homogeneity would appear to be a character inherent to the species whose biological unity seems to have resisted modifications despite divergent evolution under different environmental conditions.

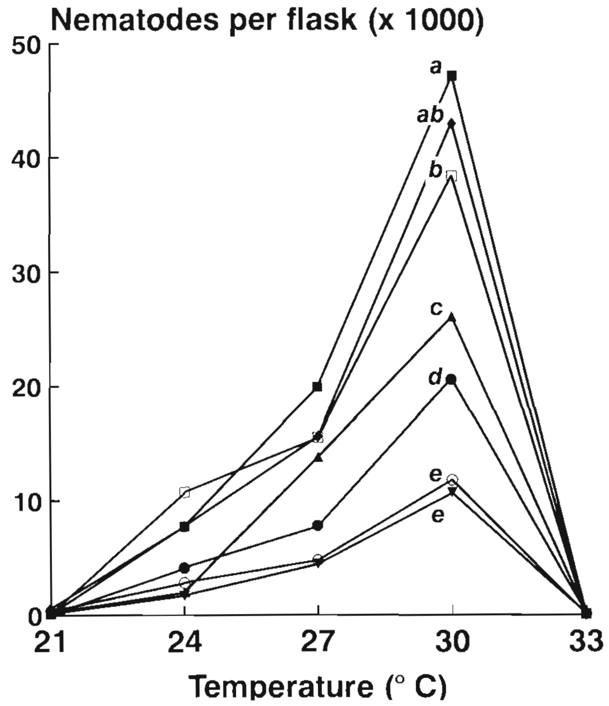

Fig. 2. *Final population of seven* Radopholus similis *isolates at different temperatures, 40 days after inoculation on carrot discs.* (■ : Ivory Coast; □ : Guinea; ♦ : Costa Rica; ▲ : Guadeloupe; ▼ : Martinique; ● : Sri Lanka; ○ : Queensland). *Data are means of five replicates for 21, 24, 27 and 33 °C and ten replicates for 30 °C. Actual data are presented, but data were transformed to* $\log_{10}(x + 1)$ *for analysis. At 30 °C, means with the same letter are not different according to Newman and Keuls test* ($P \leq 0.05$).

Although the general pattern of final population as a function of temperature is similar for all isolates, the intrinsic reproduction rate was highly variable depending on the geographical origin. There was a fairly low isolate temperature interaction between 24 and 30 °C which are the temperatures allowing high rates of multiplication. In this temperature interval, isolates ranked in almost the same order in terms of population build-up, variation being generally non-significant. The only exceptions were the number of nematodes of the Costa Rica isolate at 24 °C 50 days after inoculation and the reduction of multiplication rate of the isolates from Ivory Coast, Guinea and Costa Rica between 40 and 50 days related with degradation of carrot tissue at 30 °C. In any other case isolate ranking agrees with the relative degree of pathogenicity in banana plants observed in previous experiments (Sarah *et al.*, 1993). The five isolates common to these previous experiments (Ivory Coast, Costa Rica, Guadeloupe, Sri Lanka and Martinique) were ranked in the same order considering decreasing multiplication rate which is strongly associated with pathogenicity. However, there was one exception. The Martinique isolate showed a higher reproduction rate than the Sri Lanka isolate in banana plants roots at 27 °C (Sarah *et al.*, 1993). This correlation between multiplication rate *in vitro* on rarrot discs and pathogenicity to banana roots indicates that the pathogenic diversity of *R. similis* is associated with intrinsic factors that point towards important genetic differences between the different isolates.

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