| And a second |  |         |         |              |
|--|--|---------|---------|--------------|
| EKOLOGIA POLSKA<br>(Ekol. pol.)  | 24   | 2       | 263-271 | 1976         |
|  | and the second | daraa . |         | anna la bail |

## Ewa MIANOWSKA

Department of Agrocenology, Institute of Ecology, Polish Academy of Sciences, Dziekanów Leśny near Warsaw

# RESEARCH ON THE BIOLOGY AND ECOLOGY OF *PANAGROLAIMUS RIGIDUS* (SCHNEIDER) THORNE

## IV. EFFECT OF TEMPERATURE AND SOIL MOISTURE ON THE GROWTH AND STRUCTURE OF A POPULATION

ABSTRACT: Two populations of *Panagrolaimus rigidus* (Schneider 1866) Thorne 1937, of different origin were investigated. Population growth was found to be affected by 30°C temperature and high soil moisture (100% of maximum soil water capacity). Agar culture nematodes appeared to be more stenobiotic, and more strongly reacting to adverse environmental conditions than soil culture nematodes. This was indicated by the numbers and age distribution of both populations cultured under different temperature and moisture conditions. Soil culture nematodes colonized the soil medium more abundantly than the agar culture nematodes did. The latter, however, were found to be more abundant in decomposing seed grain.

Contents

- 1. Introduction
- 2. Material and methods
- 3. Results
  - 3.1. Numbers
  - 3.2. Distribution of nematodes in microhabitats
  - 3.3. Population structure
  - 3.3.1. Age distribution
  - 3.3.2. Sex ratio
- 4. Summary
- 5. Polish summary (Streszczenie)
- 6. References

#### **1. INTRODUCTION**

Temperature and moisture belong to the more important abiotic factors exerting an influence on the development of the nematodes. Obviously, the individual nematode species vary in the degree of their tolerance to these factors.

[263]

In the literature concerned with the nematodes many papers can be found dealing with the effect of temperature on the development of nematodes representing different species. The respective studies usually aimed at establishing optimum temperature and the limits of thermic tolerance of the particular species. G o w e n (1970) carried out studies on the nematodes of the species *Tylenchus emarginatus* Cobb 1893. He has found that at 5°C the larvae of this species do not grow. For *Aphelenchus avenae* Bastian 1865 the growth limiting factor is the temperature of 35°C (F i s c h e r 1969). The results reported by M a m i y a (1971) indicate that the nematodes *Pratylenchus penetrans* Chitwood and Oteifa 1952 grow much more slowly at 15°C than at: 20°, 25° and 30°C. It has appeared that 25°C is the optimum temperature for this species. The optimum temperature for *Acrobeles complexus* Thorne 1937 is 34°C, while temperatures of 10° and 37°C have an adverse effect on the growth of these nematodes (T h o m a s 1965). *Aphelenchoides besseyi* Christe 1942, as indicated by the studies carried out by H u a n g, H u a n g and L i n (1972), does not reproduce at 35°C. For *Ditylenchus myceliophagus* J. B. Goodey 1958 25°C appeared to be the optimum growth temperature, while 30°C already had an inhibitory effect (E v a n s and F i s c h e r 1969).

One of the species exceptionally resistant to high temperatures is *Dorylaimus granuliferus* Cobb 1893 found in hot springs with a temperature of  $51^{\circ}$ C (B r u e s 1939). Individuals of the species *Plectus granulosus* Bastian 1865, kept in a dry environment at  $66^{\circ}$ C survived for 4 days. As an example of an exceptionally high resistance to low temperatures, *Anquillula silusiae* De Man 1913 can be mentioned, a species that can survive a temperature of  $-192^{\circ}$ C (D e C o n i n c k 1951).

There is much less information on the effect of moisture on the development of nematodes. Most of the studies are concerned with the effect of soil moisture on the nematodes of the genera *Heterodera* and *Meloidogyne*. P a welsk a-K o z ińsk a (1971) has found that the nematodes of the species *Heterodera schachtii* Schmidt 1871 develop best at a moisture level of 40-60% of capillary capacity of the soil. The results of the studies carried out by J o h n s t o n (1958) indicate similar moisture requirements for *Tylenchorhynchus martini* Fiedling 1956. As reported by W a 1 1 a c e (1963), the optimum moisture level for the development of most species ranges from 40 to 80% of field soil water capacity.

Many papers deal with the effect of both abiotic and biotic factors on the sex ratio in the nematodes. In this case, too, most frequent are studies dealing with the representatives of the genera: *Heterodera* and *Meloidogyne*.

The effect of the trophic conditions of the host plant, and of overcrowding on the numeric ratio of females to males in *Meloidogyne incognita* Chitwood 1949 was studied by Mc Clure and Vighiercho (1965). They have found that in a medium poor in nitrogen there are more males than there are females. The amount of vitamins and macroelements in the medium did not affect the sex ratio, whereas overcrowding caused a decrease in the number of females. As indicated by the studies of Davide and Triantaphyllou (1967a, 1967b), neither the age of the plants, nor the temperature affected the sex ratio in *Meloidogyne incognita* or *M. javanica* Chitwood 1949. The effect of temperature on the sex ratio in *Ditylenchus myceliophagus* was studied by Evans and Fischer (1969). The results reported by these authors seem not to be true, because the material they are based on is too small. In their researches into the effect of temperature on the sex ratio in *Panagrellus redivivus* T. Goodey 1945, Hansen and Cryan (1966) found that at 28°C males constituted 90% of the population.

The aim of the present work was to study the development and structure of two Panagrolaimus rigidus (Schneider 1866) Thorne 1937 populations reared under different

264

temperature and moisture conditions. *P. rigidus*, which K o zło w sk a and D o m u r at (in press) include in the group of parasaprobiotic nematodes, is characterized by an exceptionally high resistance to high temperatures, being one of the three nematode species, side by side with *Diploscapter coronatus* Cobb 1913 and *Aphelenchoides parietinus* Bastian 1865, occurring in hot springs at  $58-61^{\circ}C$  (W i n slow 1960).

#### 2. MATERIAL AND METHODS

<sup>6</sup>In the study two populations of the nematode *Panagrolaimus rigidus*, of different origin, were used. One of them, hereafter designated by A, was reared for five years on an agar medium with an addition of chicken egg yolk. Individuals of the second population, hereafter referred to as population B, were newly extracted from soil. The animals were kept in 25 ml beakers filled with sterile sand. In each beaker five wheat seedlings were planted. The growth of the nematodes was studied at the following temperatures:  $10^{\circ}$ ,  $20^{\circ}$  and  $30^{\circ}$ C, and at three different moisture levels: 25, 50 and 100% maximum soil water capacity. The maximum water capacity of the soil was determined by the desiccator method (B o r o w i e c et al. 1967). As soon as the seedlings had attained 1 cm in length, each beaker was inoculated with 50 nematode females and 25 males. The moisture was maintained at an appropriate level by daily replacement of the missing water. A total of 4 up to 8 replications of each experiment were used. After 26 days the nematodes were extracted by using a modification of Baerman's method. The nematodes were extracted separately from each plant part: the leaves, roots, the remains of the seed grain, and from the soil. After 48-hours' extraction the nematodes were counted, females, males and larvae separately.

## 3. RESULTS

## 3.1. Numbers

In each experimental combination population B was found to be more abundant than population A (Fig. 1). The nematodes in the agar culture (A) were found to grow best at 20°C and a moisture level of 25%. A slightly lower density of *Panagrolaimus rigidus* was recorded at 10°C at the same moisture level. The lowest density in this population was observed at 30°C at moisture levels of 25 and 50%. The soil nematode population (B) was most numerous at 10° and 20°C at moisture levels of 25 and 50%. The fact that for the individuals of population Athe most favourable temperature was 25% can be attributed to the frequent drying up of the agar, which may have led to the breeding of a line of nematodes adapted to a low moisture.

A statistical analysis based on the use of a modification of Student's test made it possible to check the differences between individual experiments for significance. In the case of population A both at  $10^{\circ}$  and at  $20^{\circ}$ C the difference<sup>1</sup> between the density of the nematodes reared at 25 and 50% moisture was found to be statistically significant. However, this difference did not occur in the comparison of moisture levels 50 and 100%.

<sup>&</sup>lt;sup>1</sup> All the data have been calculated for the significance level = 0.05.



Ewa Mianowska

Fig. 1. Densities of two populations of *Panagrolaimus rigidus* (Schneider 1866) Thorne 1937 under different conditions of temperature (in <sup>o</sup>C) and soil moisture (in % of maximum water capacity of the soil) *I* – population derived from an agar culture (*A*), 2 – population derived from a soil culture (*B*)

In population B at  $10^{\circ}$  and  $20^{\circ}$ C no significant differences could be found between the density of nematodes at 25 and 50% moisture levels, but significant differences in numbers occurred between the nematodes reared at 50 and 100% moisture. This confirms the earlier finding that the agar culture nematodes clearly preferred low moisture levels. The nematodes of the soil population showed less marked moisture requirements, population B nematodes at 25 and 50% not differing much numerically.

The 100% moisture was found to be clearly unfavourable for both populations. At this level of soil moisture all the spaces among the soil particles become filled with water, and the resultant lack of air in the soil spaces causes suffocation of the nematodes. This probably is the cause of the small numbers of the nematodes at 100% moisture. No statistically significant differences could be seen between the numbers of the nematodes reared at the same moisture level at  $10^{\circ}$  and  $20^{\circ}$ C. Only at  $30^{\circ}$ C were small numbers of individuals found in hoth populations, which indicated an inhibitory effect of temperature on the growth of the nematodes under study.

## 3.2. Distribution of nematodes in microhabitats

The two populations differed in their occurrence in three separate microhabitats: the soil, plant, and the decomposing remains of the seed grain (Table I). In population A the largest number of nematodes was extracted from the soil, and considerably smaller numbers from the plants and from grains. In population B slightly different ratios were found: by comparison with population A, more P. rigidus were found in the soil, in the plants – about the same number as in the case of population A, while much fewer were found in the grain. A similar distribution of the nematodes was found in all the experimental combinations, except at  $20^{\circ}$ C and 100% moisture. Such a distribution of the individuals of the populations under study should perhaps be attributed to their adaptation to the conditions under which they had been previously reared. For instance, in the agar medium containing chicken egg yolk the nematodes which originally had fed on the microorganisms decomposing the organic matter, grew well and multiplied in the remains of the decomposing grain. The nematodes reared in the soil continued to grow best in soil.

 Table I. The percentage of Panagrolaimus rigidus (Schneider 1866) Thorne 1937 in the soil, plants and grain (experiment at 20°C)

A – population originated from agar culture, B – population originated from soil culture

| Percentage of maxi-<br>mal water capacity of<br>soil | Soil |      | Plant |      | Grain |     |
|--|------|------|-------|------|-------|-----|
|  | A    | B    | A     | В    | A     | В   |
| 25   | 53.8 | 77.8 | 19.4  | 16.7 | 26.8  | 5.5 |
|  | 41.5 | 43.5 | 38.4  | 55.3 | 20.1  | 1.2 |

In the above mentioned experiments  $(20^{\circ}C \text{ at } 100\% \text{ moisture})$  an exceptionally high percentage of nematodes was found in the plants (mainly in the leaves) -38.4% in population A, and 55.3\% in population B. At this temperature and at this moisture level the leaves of the seedlings became a very favourable medium for the growth of nematodes, because they began to decompose thus providing a substrate for the microorganisms which are the basic food item of the nematodes under study.

The presence of a certain proportion of *P. rigidus* in normal plant tissues has once again confirmed the earlier findings (K o złowska and Mianowska 1971, K o złowska and Domurat 1971a, 1971b) that the nematodes of this species, although they are not included in the group of true parasites of plants, possess the capability of penetrating into the tissues of higher plants.

#### 3.3. Population structure

#### 3.3.1. Age distribution

The age structure of both populations was closely related to the density of the nematodes. Higher abundance levels of *P. rigidus* were accompanied by a much higher percentage of larval stages than of adult forms. Therefore in all the experiments with population A (less abundant) the percentage of adult individuals was higher than in population B (Figs. 2, 3).

An analysis of the age distributions of both the populations in the particular temperature and moisture combinations confirms the assumption that there exists a relationship between the age distribution and density. Under conditions favourable to the growth of the population the percentage of adult individuals was lower, while under the unfavourable conditions of temperature, 30°C, and moisture, 100%, the percentage of adult forms was high.

Similar relationships have been reported by Evans and Fischer (1969), who investigated, among other things, the age distribution of *Ditylenchus myceliophagus* population reared under various temperatures.







Ewa Mianowska

## 3.3.2. Sex ratio

Under conditions which were most favourable for the growth of the populations, females and males occurred in equal proportions. In population A a slight predominance of females could be seen. At the unfavourable temperature of  $30^{\circ}$ C, in population B there were more males than females (Fig. 4).





In the case of population A at 30 °C no predominance of males could be seen (at both moisture levels there were 50% females and males).

At 100% moisture under less favourable temperature conditions for both populations, there were more females than males. Possibly, the females are more resistant to high moistures.

#### 4. SUMMARY

The effect was studied of temperature (10, 20 and  $30^{\circ}$ C), and of three moisture levels (25, 50 and 100% of maximum soil water capacity) on the growth and structure of two populations of *Panagrolaimus rigidus* (Schneider 1866) Thorne 1937 of different origin. Individuals of one of the populations came from an agar culture continued for many years, those of the other population – from a soil culture.

The population of the nematodes derived from the soil culture was found to be more abundant in all experimental combinations (Fig. 1). The least favourable conditions of growth were:  $30^{\circ}$ C temperature and 100% moisture. The population of laboratory origin grew best at  $20^{\circ}$ C and at 25% moisture. Temperatures of  $10^{\circ}$ , or  $20^{\circ}$ C, and moisture levels 25, or 50% provided favourable conditions for the growth of the population of nematodes from the soil culture.

Studies of the occurrence of the nematodes in three different environments: the soil, plants, and seed grain remains indicated that the laboratory culture nematodes preferred the decomposing grain, whereas the soil culture nematodes preferred the soil medium (Table I).

The percentage of larval stages was found to be much higher in the population derived from the soil (much more numerous) than in the laboratory population (Figs. 2, 3). This is indicative of a higher dynamics of the soil population.

Under conditions favourable to the growth of the population, the ratio of females to males was 1:1, whereas at the unfavourable temperature  $30^{\circ}$ C males predominated numerically over females, at 100% moisture a converse situation – numerical predominance of females was seen (Fig. 4).

By comparison to the laboratory nematodes, the soil culture nematodes appeared to be more viable and possess a wider range of tolerance. The latter reacted more weakly to the adverse conditions of the environment. A conclusion may, therefore, be drawn, that the agar culture nematodes had become more stenobiotic.

The processes observed during the present investigations suggest that in any comparative studies it is necessary to take into account the origin of the populations investigated, and the differences in their capability to adapt themselves to the culture conditions.

#### 5. POLISH SUMMARY (STRESZCZENIE)

Przeprowadzono badania nad wpływem temperatury (10, 20 i 30°C) oraz trzech poziomów wilgotności (25, 50 i 100% maksymalnej pojemności wodnej gleby) na rozwój i strukturę dwóch populacji *Panagrolaimus rigidus* (Schneider 1866) Thorne 1937, różniących się pochodzeniem. Przedstawiciele jednej z nich pochodzili z wieloletniej hodowli agarowej, drugiej – z hodowli glebowej.

Populacja nicieni hodowanych dotychczas w glebie była znacznie liczebniejsza we wszystkich wariantach doświadczenia (fig. 1). Dla obu populacji najmniej korzystne warunki rozwoju stwarzały: temperatura 30°C i wilgotność 100%. Najkorzystniejszymi warunkami dla rozwoju populacji pochodzenia laboratoryjnego była temperatura 20°C i wilgotność 25%. Dla nicieni z hodowli glebowej korzystne warunki rozwoju stwarzały temperatura 10 lub 20°C i wilgotność 25 lub 50%.

Wyniki badań nad występowaniem nicieni w trzech mikrośrodowiskach: glebie, roślinie i pozostałościach ziarna siewnego wskazują, że nicienie z hodowli laboratoryjnej preferowały rozkładające się ziarno, a nicienie z hodowli glebowej – środowisko glebowe (tab. I).

Stwierdzono, że w populacji pochodzenia glebowego (znacznie liczniejszej) udział procentowy osobników w stadiach larwalnych był znacznie wyższy niż w populacji laboratoryjnej (fig. 2, 3). Świadczy to o większej dynamice populacji glebowej.

Stosunek samic do samców w warunkach korzystnych dla rozwoju populacji wynosił 1:1, natomiast w niekorzystnej temperaturze 30°C zaobserwowano liczebną przewagę samców nad samicami, a przy 100% poziomie wilgotności odwrotnie – liczebną przewagę samic nad samcami (fig. 4).

Nicienie z hodowli glebowej okazały się bardziej żywotne i bardziej plastyczne niż nicienie z hodowli laboratoryjnej. Te ostatnie o wiele silniej reagowały na niekorzystne warunki środowiska. Nasuwa się wniosek, iż nicienie z hodowli laboratoryjnej stały się bardziej stenobiotyczne.

Zaobserwowane zjawiska sugerują, że przy wszelkich badaniach porównawczych należałoby uwzględnić pochodzenie badanych populacji oraz liczyć się z róźnymi możliwościami ich adaptacji do warunków hodowlanych.

#### 6. REFERENCES

- 1. Borowiec J., Gliński J., Turski R., Wondrausch J. 1967 Analiza gleby w laboratorium i w polu – WSR, Lublin, 150 pp.
- Brues C. T. 1939 Studies on the fauna of some thermal springs in Dutch East Indies Proc. Am. Acad. Arts Sci. 73: 71-95.
- 3. D a vide R. G., Triantaphyllou A. C. 1967a Influence of the environment on development and sex differentiation of root-knot nematode. I. Effect of infection density, age of the host plant and soil temperature – Nematologica, 13: 102–110.
- 4. Davide R. G., Triantaphyllou A. C. 1967b Influence on the environment on development and sex differentiation of root-knot nematode. II. Effect of host nutrition – Nematologica, 13: 111–118.
- 5. De Coninck L. A. P. 1951 On the resistance of the free living nematode Anguillula silusiae to low temperature Biodynamica, 7: 77–84.
- E v a n s A. A. F., F i s c h e r J. M. 1969 Development and structure of populations of Ditylenchus myceliophagus as affected by temperature – Nematologica, 15: 395-402.
- 7. Fischer J. M. 1969 Investigations on fecundity of Aphelenchus avenae Nematologica, 15: 22-28.
- G o w e n S. R. 1970 Observations on fecundity and longevity of *Tylenchus emarginatus* on Sitka Spruce seedlings at different temperatures – Nematologica, 16: 267–273.
- 9. Hansen E. L., Cryan W. S. 1966 Variations in sex ratio of *Panagrellus redivivus* in response to nutritional and heat stress Nematologica, 12: 355–358.
- 10. Huang C. S., Huang S. P., Lin L. P. 1972 The effect of temperature on development and generation periods Aphelenchoides besseyi Nematologica, 18: 432–438.
- 11. J o h n s t o n T. 1958 The effect of soil moisture on Tylenchorhynchus martini and other nematodes Proc. La Acad. Sci. 20: 52–55.
- Kozłowska J., Mianowska E. 1971 Research on the biology and ecology of *Panagrolaimus rigidus* (Schneider) Thorne. I. The influence of food changes of morphometric characteristics of *P. rigidus* Ekol. pol. 19: 701-714.
- 13. K o złowska J., D o murat K. 1971a Research on the biology and ecology of *Panagrolaimus rigidus* (Schneider) Thorne. II. The influence of the initial density and of interspecies competition on the development of populations of saprophagous nematodes Ekol. pol. 19: 715–723.
- 14. Kozłowska J., Domurat K. 1971b Research on the biology and ecology of Panagrolaimus rigidus (Schneider) Thorne. III. Plant influence on development of P. rigidus populations Ekol. pol. 19: 723-726.
- 15. Kozłowska J., Domurat K. (in press) Der Einfluss der Sticktstoffdüngung auf die Bodennematodengemeinschaften (In: International Symposium of Crop Protection) – Gent.
- 16. Mamiya Y. 1971 Effect of temperature on the life cycle of Pratylenchus penetrans on Cryptomeria seedlings and observations on its reproduction Nematologica, 17: 82–92.
- 17. Mc Clure M. A., Vighiercho N. 1965 The influence of host nutrition and intensity of infection on the sex ratio and development of *Meloidogyne incognita* in sterile agar cultures of excised cucumber roots Nematologica, 12: 248–258.
- 18. P a w e l s k a-K o z i ń s k a K. 1971 Wzrost populacji mątwika burakowego (Heterodera schachtii Schm.) przy różnych poziomach wilgotności gleby – Zesz. probl. Postęp. Nauk roln. 121: 67–75.
- Thomas P. 1965 Biology of Acrobeles' complexus Thorne, cultivated on agar Nematologica, 11: 395-408.
- 20. Wallace H. R. 1963 The biology of plant parasitic nematodes Edward Arnold LTD, London, 288 pp.
- 21. W i n s l o w R. D. 1960 Some aspects of the ecology of free living and plant-parasitic nematodes (In: Nematology fundaments and recent advances with emphasis on plant parasitic and soil form. Eds. J. N. Sasser and W. R. Jenkins) – The University of North Carolina Press, Chapel Hill, 341-419.

Paper prepared by J. Stachowiak

AUTHOR'S ADDRESS: Mgr Ewa Mianowska Instytut Ekologii PAN Dziekanów Leśny k. Warszawy 05–150 Łomianki Poland