Effect of temperature on the *in vitro* reproduction of *Ditylenchus destructor* isolated from groundnut

Dirk De WAELE* and Ria WILKEN

Grain Crops Research Institute, Private Bag X1251, Potchefstroom 2520, Republic of South Africa.

SUMMARY

The effect of temperature on egg production, hatching and the life cycle of *Ditylenchus destructor* isolated from groundnut in South Africa was investigated *in vitro*. The optimum temperature for the development of *D. destructor* was 28 °C. At this temperature, most eggs were produced, egg hatch started from 2 days onwards, mean time required for hatching was 4.4 days, egg viability was higher than 90 % and the length of the life cycle from adult to adult was between 6 and 7 days. 34 °C was also favorable for the development of *D. destructor*. As the temperature decreased below 28 °C, fewer eggs were produced, egg hatch started later, the time required for hatching increased and egg viability decreased. At 16 °C, the length of the life cycle from adult to adult was 12 days.

RÉSUMÉ

Effet de la température sur la reproduction in vitro *de* Ditylenchus destructor *isolé de l'arachide*

L'effet de la température sur la production des œufs, leur éclosion et le cycle biologique de *Ditylenchus destructor* isolé de l'arachide en Afrique du Sud a été étudié *in vitro*. La température optimale pour le développement de *D. destructor* est de 28 °C. C'est à cette température que le maximum d'œufs est produit; l'éclosion commence après deux jours, le temps moyen nécessaire pour celle-ci étant de 4,4 jours; la viabilité des œufs est d'au moins 90 % et la durée du cycle, d'adulte à adulte, est de 6 à 7 jours. La température de 34 °C est également favorable au développement de *D. destructor*. Lorsque la température est inférieure à 28 °C, le nombre d'œufs produits est plus faible, leur éclosion est plus tardive, le temps nécessaire pour l'éclosion augmente et la viabilité des œufs diminue. A 16 °C, la durée du cycle, d'adulte à adulte, est de 12 jours.

Recently, *Ditylenchus destructor* Thorne was for the first time reported in hulls and seeds of groundnut (*Arachis hypogaea* L.) in the Republic of South Africa, causing severe damage (Jones & De Waele, 1988; De Waele *et al.*, 1989). Until then *D. destructor* was mainly known as a pathogen of potato tubers and bulbs of flowers in Europe and the USSR (Hooper, 1973). In the United States, its spread on potatoes was stopped by a fumigation-quarantine program (Darling, Adams & Norgren, 1983).

Inoculation of groundnut callus tissue with 50 juveniles and adults of *D. destructor* resulted in a 600-fold increase in nematode numbers within five weeks (Van der Walt & De Waele, 1989). On callus tissues of carrot, clover, potato and tobacco, *D. destructor* increased 4 000-fold within 4 months (Faulkner & Darling, 1961). One of the many factors determining the increase of a nematode population is the length of the life cycle which can vary from a few days to a year or more (Norton, 1978). Temperature is important in regulating the time required for completing the life cycle. The embryology and reproduction of *D. destructor* have been studied but the length of the life cycle was not reported (Anderson & Darling, 1964). The objectives of our study were to investigate *in vitro* the effect of temperature on *i)* egg production, *ii)* hatching, and *iii)* life cycle from adult to adult of *D. destructor*.

Materials and methods

The *D. destructor* population used in our study was maintained on groundnut callus tissue and was originally obtained from naturally infected groundnut (Van der Walt & De Waele, 1989).

EFFECT OF TEMPERATURE ON EGG PRODUCTION

Twenty-five males and 25 females of *D. destructor* were hand picked at random from the population maintained on groundnut callus tissue and transferred

* *Present address : Plant Genetic Systems NV, Jozef Plateaustraat 22, 9000 Gent, Belgium.*

to 3.5-cm-diameter Petri dishes containing 5 ml of tap water. The Petri dishes were then incubated at 16°, 22°, 25°, 28° and 34 °C for 24 h after which the number of eggs was counted in each Petri dish. There was one Petri dish for each temperature. The experiment was performed twice.

EFFECT OF TEMPERATURE ON HATCHING

One hundred males and 100 females of *D. destructor* were hand picked at random from the population maintained on groundnut callus tissue and transferred to a 3.5-cm-diameter Petri dish containing 5 ml of tap water. The Petri dish was then incubated at 25 °C for 4 h. Thirty eggs, not older than 4 h, were collected at random with a Pasteur pipette and transferred to 3.5-cm-diameter Petri dishes containing 5 ml of tap water. The Petri dishes were then incubated at 10°, 16°, 22°, 25°, 34° and 40 °C for 3 weeks during which period the larval hatch was counted daily in each Petri dish. There was one Petri dish for each temperature. The experiment was performed twice. The effect of temperature on the number of days required for egg hatch was subjected to regression analysis. The equation for the multiplicative regression was $y = ax^b$, where a and b are regression coefficients.

EFFECT OF TEMPERATURE ON THE LIFE CYCLE FROM ADULT TO ADULT

Groundnut cv. Sellie callus tissue cultures were established on a modified MS medium (Van der Walt & De Waele, 1989) and incubated at 25 °C for 3 weeks. One hundred and fifty cultures were aseptically inoculated with 200 ± 15 eggs, 60 ± 5 juveniles and 60 ± 5 adults of *D. destructor* obtained from the population maintained on groundnut callus tissue. The infected cultures were then incubated at 16°, 25° and 34 °C for 20, 16 and 14 days, respectively. Eggs, juveniles and adults were counted daily in three cultures of each temperature from 3, 5 and 11 days onwards for cultures incubated at 16°, 25° and 34 °C, respectively. The callus tissues were first soaked in tap water for 5 min, sectioned with a scalpel and forced through a 710 µm-pore sieve. As a result the cells were separated and it was possible to count the different nematode stages. The life cycle from adult to adult was considered complete when 320 adults were recovered.

Results

EFFECT OF TEMPERATURE ON EGG PRODUCTION

In both experiments, the highest number of eggs was produced at 28 °C and the lowest number of eggs at 16 °C (Fig. 1). Egg production at 22°, 25° and 34 °C was similar. At 28 °C, five to ten times more eggs were produced than at 16 °C and 1.5 to 2.5 times more eggs than at 22°, 25° or 34 °C.

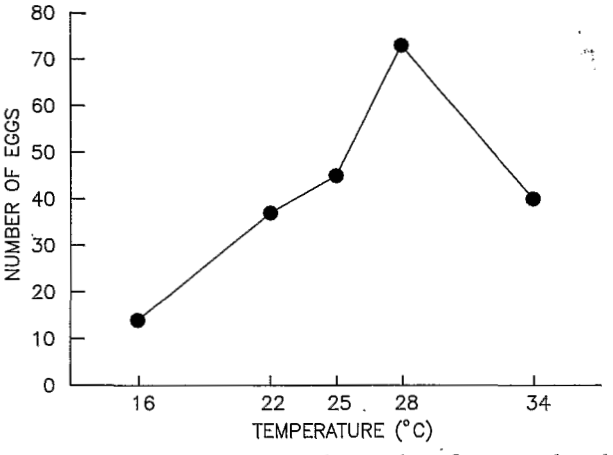

Fig. 1. Effect of temperature on the number of eggs produced by 25 females of *Ditylenchus destructor*.

EFFECT OF TEMPERATURE ON HATCHING

Egg hatch started from 8, 4, 4, 2 and 2 days onwards at 16°, 22°, 25°, 28° and 34 °C, respectively (Fig. 2). No egg hatch was observed at 10° and 40 °C. At 16°, 22°, 25°, 28° and 34 °C, 50 % or more of the eggs had hatched after 11, 7, 5, 4 and 3 days, respectively. At 16° and 22 °C about 75 % of the eggs hatched compared with more than 90 % at 25°, 28° and 34 °C. Eggs which had not hatched after 3 weeks at 10°, 16°, 22° or 40 °C were incubated at 25 °C but no additional egg hatch was observed. From data given in Fig. 2, one can calculate, with the help of integrals, that the mean time required for egg hatch was 11.1, 6.8, 5.5, 4.4 and 3.8 days at 16°, 22°, 25°, 28° and 34 °C, respectively (Fig. 3). The multi-

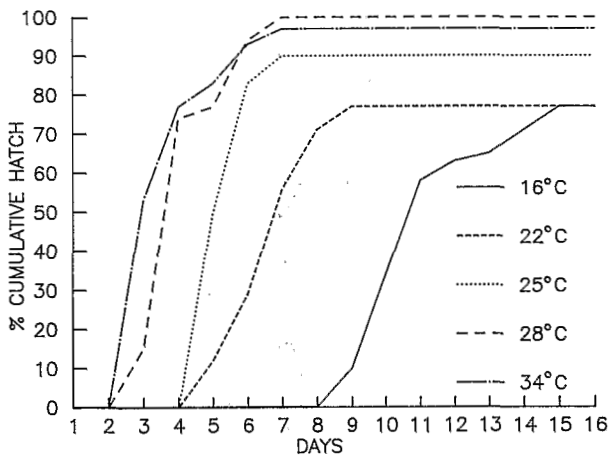

Fig. 2. Effect of temperature on the percent of cumulative egg hatch of *Ditylenchus destructor*.

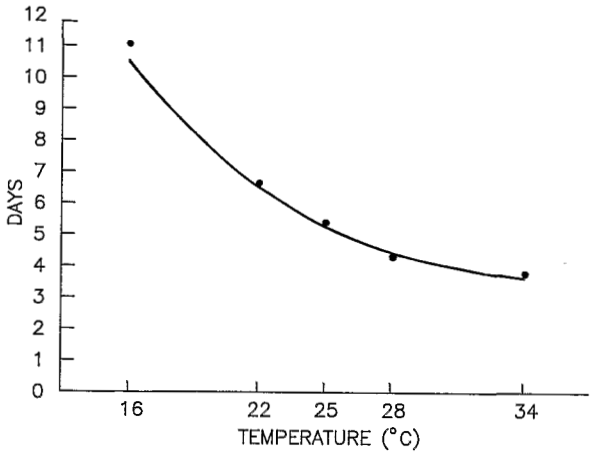

Fig. 3. Correlation between temperature and number of days required for egg hatch of *Ditylenchus destructor* (multiplicative regression coefficient $r = -0.87$, $y = e^{6.518-1.496(\log e^{x})}$. Calculated from data of Fig. 2 with the help of integrals.

plicative regression correlation coefficient ($r = -0.87$, $a = 6.518$, $b = -1.496$) for temperature and number of days required for egg hatch was highly significant ($P = 0.001$) (Fig. 3).

EFFECT OF TEMPERATURE ON THE LIFE CYCLE FROM ADULT TO ADULT

D. destructor completed its life cycle from adult to adult on groundnut callus tissue in 12 and 8 days at 16° and 25 °C, respectively. At 34 °C, the growth of the callus tissue was adversely affected : after a few days part of the callus tissue showed brown discoloration. At this temperature, *D. destructor* completed its life cycle from adult to adult in 13 days. The change in number of eggs, juveniles and adults at 16°, 25° and 34 °C are shown in Fig. 4. Two weeks after inoculation, the total populations (including all stages) had increased 1.4, 27.4 and 13.2 times at 16°, 25° and 34 °C, respectively. During this period, number of eggs and juveniles increased 29.1 and 38.6 times, respectively, at 25 °C compared with 11.3 and 14.3 times, respectively, at 34 °C; the number of adults increased 19.7 times at 34 °C compared with 10.7 times at 25 °C. After the same period, a decrease in number of eggs was observed at 16 °C while the number of juveniles and adults increased 3 and 2.7 times, respectively. At 25 °C, the number of eggs increased rapidly during the first 15 days after inoculation while the number of adults increased slowly. At 34 °C, the rate of increase of all stages was slow until 12 days after inoculation. Thereafter the number of eggs, juveniles and adults increased rapidly. At 16 °C, the number of all stages fluctuated. Twenty days after inoculation, about the same number of eggs, juveniles and adults were

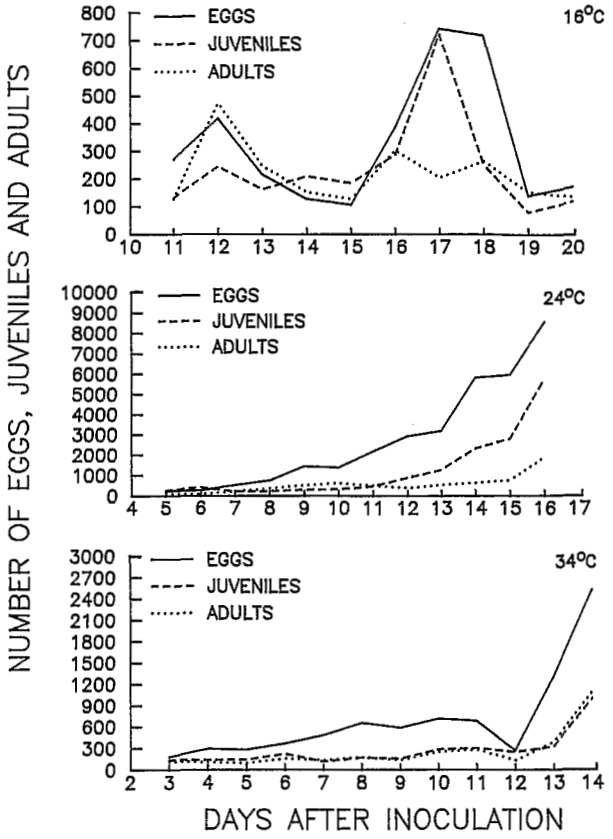

Fig. 4. Mean number of eggs, juveniles and adults of *Ditylenchus destructor* per callus tissue culture inoculated with 200 ± 15 eggs, 60 ± 5 juveniles and 60 ± 5 adults, incubated at 16 °C (upper), 25 °C (middle) and 34 °C (lower).

observed compared with 11 and 14 to 15 days after inoculation.

Discussion

The results indicate that temperature has a great effect on egg production, hatching and the length of the life cycle from adult to adult of *D. destructor*. The optimum temperature for the development of *D. destructor* isolated from groundnut in South Africa was 28 °C. At this temperature, most eggs were produced, egg hatch started from 2 days onwards, mean time required for hatching was 4.4 days and egg viability was higher than 90 %. Since at 25 °C the length of the life cycle from adult to adult was 8 days and the mean time required for hatching was 1.1 days longer than at 28 °C, the life cycle from adult to adult at 28 °C takes between 6 and 7 days. Higher temperatures, however, are also favorable for the development of *D. destructor*. At 34 °C, fewer eggs were produced but the start of egg hatch and

egg viability were similar to those at 28 °C while the mean time required for hatching was only 3.8 days. The length of the life cycle from adult to adult at 34 °C could not be determined due to the direct, adverse effect of the temperature on the callus tissue but it probably also takes between 6 and 7 days. As the temperature decreased below 28 °C, fewer eggs were produced, egg hatch started later, the time required for hatching increased and egg viability decreased. At 16 °C, the length of the life cycle from adult to adult was 12 days. However, at this temperature *D. destructor* failed to increase its population much higher than the initial population. Observations made during the present study showed that variations in number of eggs laid by individual females were considerable. The highest number of eggs produced by a single female within 24 hours, incubated at 28 °C, was 13.

Few plant-parasitic nematode species complete their life cycle within less than 10 days (Norton, 1978). The optimum temperature for the development of most species is also lower than 28 °C (Norton, 1978). Although no information is available on the optimum temperature for the development of *D. destructor* populations occurring in the moderate climates of the northern hemisphere, it is unlikely that their optimum temperature is 28 °C. Distinct differences in physiology between populations of the same species indicate the existence of ecotypes who may or may not be manifest as distinct pathotypes separated by their reproduction on differential sets of hosts (Dropkin, 1988). Although *D. destructor* is widespread on groundnut in South Africa, no damage to potatoes has been reported (De Waele *et al.*, 1989).

Soil temperatures in the groundnut producing areas of South Africa often exceed 25 °C at 0-30 cm deep. The short life cycle of the local *D. destructor* populations and the favorable temperatures will result in numerous generations of this nematode during the growing season. Even a small population of *D. destructor* present at planting could result in a large population towards harvest.

Accepté pour publication le 24 juillet 1989.

ACKNOWLEDGEMENTS

The authors thank Miss. A. de Jongh for preparing the illustrations.

REFERENCES

ANDERSON, R. V. & DARLING, H. M. (1964). Embryology and reproduction of *Ditylenchus destructor* Thorne, with emphasis on gonad development. *Proc. helminth. Soc. Wash.*, 31 : 240-256.

DARLING, H. M., ADAMS, D. & NORGREN, R. L. (1983). Field eradication of the potato rot nematode, *Ditylenchus destructor*. A 29-year history. *Pl. Dis.*, 67 : 422-423.

DE WAELE, D., JONES, B. L., BOLTON, C. & VAN DEN BERG, E. (1989). *Ditylenchus destructor* in hulls and seeds of peanut. *J. Nematol.*, 21 : 10-15.

DROPKIN, V. H. (1988). The concept of race in phytonematology. *A. Rev. Phytopathol.*, 26 : 145-161.

FAULKNER, L. R. & DARLING, H. M. (1961). Pathological histology, hosts and culture of the potato rot nematode. *Phytopathology*, 51 : 778-786.

HOOPER, D. J. (1973). *Ditylenchus destructor. C.I.H. Descript. Pl.-paras. Nematodes*, Set 2, No. 21 : 3 p.

JONES, B. L. & DE WAELE, D. (1988). First report of *Ditylenchus destructor* in pods and seeds of peanut. *Pl. Dis.*, 72 : 453.

NORTON, D. C. (1978). *Ecology of plant-parasitic nematodes.* New York, John Wiley & Sons : 268 p.

VAN DER WALT, P. C. W. & DE WAELE, D. (1989). Mass culture of the potato rot nematode *Ditylenchus destructor* on groundnut callus tissue. *Phytophylactica*, 21 : 79-80.