The Influence of Temperature on Development and Sex Differentiation of *Meloidogyne graminis*[1]

C. W. LAUGHLIN, A. S. WILLIAMS AND J. A. FOX[2]

Abstract: *Meloidogyne graminis* (Sledge and Golden) Whitehead on *Cynodon* sp. (var. 'Tifgreen' bermudagrass) was studied at four temperatures; 16, 21, 27, and 32 C. Both mode and rate of development were temperature dependent. Females developed more rapidly and in greater numbers at 27 C: saccate females exuding matrices were present 14 days following inoculation, eggs were laid after 21 days and newly-hatched larvae were present in the matrix at 25 days. Sex differentiation to males was 80% at 32 C and 4% at 27 C. No males were observed at 21 or 16 C. Developing males were present 14 days following inoculation and emerged from roots after 21 days at 32 C. In populations pre-exposed to 27 C then transferred to 32 C, the percentage of males ranged from 0 for 1 day exposure at the initial temperature to 45.5% after 5 days. After 11 days pre-exposure the recovery of males was 4.3%. Individuals interpreted to be male sex reversals and male intersexes were noted. Pre-exposure at 32 C for 1 or 2 days followed by 27 C produced 1–2% males, while exposure for 3 or more days at 32 C followed by 27 C produced 90% males.

The influence of various environmental factors upon development and sex differentiation of members of the family Heteroderidae has been extensively reported. Among these factors were: (i) crowding of the infective nematodes (2, 5, 8, 9), (ii) physiological age of the host plant (2, 8), (iii) nutritional condition of the host plant (1, 3, 6) and (iv) application of growth regulators to the host plant (4). It has been speculated that temperature, which is known to influence nematode infectivity, rate of development and reproduction (7, 10), may also influence sex differentiation of root-knot nematodes (2).

The objective of the present study was to investigate the influence of temperature on the rate of development and sex differentiation of *Meloidogyne graminis* (Sledge and Golden) Whitehead.

Received for publication 15 November 1968.

[1] Contribution No. 146, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute, Blacksburg, Virginia.

[2] Assistant Professor of Nematology, Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32601; Professor of Plant Pathology, Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40506; and Assistant Professor of Plant Pathology, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute, Blacksburg, Virginia 24061, respectively.

MATERIALS AND METHODS

A population of *M. graminis* collected from a 'Tifgreen' bermudagrass golf green in Franklin, Virginia, was maintained in the greenhouse on 'Tifgreen' bermudagrass. Cuttings of 'Tifgreen' bermudagrass were rooted in distilled water. Plants with roots greater than 1 cm but less than 2 cm in length were selected and planted in 100 cc polypropylene centrifuge tubes having basal drainage apertures and containing a methyl bromide fumigated soil mixture. The prepared soil mixture contained 3 parts loam, 2 parts washed sand and 2 parts 'Weblite'® (Weblite is an expanded shale by-product). One hundred larvae in 1 ml distilled water were pipetted directly onto roots, and covered with a thin layer of sterilized soil. Inoculated plants were placed in water-proof crocks in each of four constant temperature tanks at 16, 21, 27, and 32 ± 1 C. Nematode development was estimated by examining two plants from each treatment at 0.5, 1, 3, 7, 14, 21, 25, 28, and 35 days after inoculation. Roots were washed in water and stained with acid fuchsin in 1 : 1 mixture of absolute alcohol and glacial acetic acid for 24 hr. Stained roots were cleared and stored

in saturated chloral hydrate solution. Nematodes were dissected from the roots and the various developmental stages and sex ratios determined.

The effect of temperature upon sex ratio was measured on four single-plant replicates at each treatment temperature. To equalize initial infection, all were inoculated in soil, incubated 48 hr at 27 C, then washed free of soil and transferred to 50 ml tap water in separate 100 ml beakers in temperature treatment groups. Water from each beaker was examined for males and changed at 3-day intervals. At 26 days the roots were stained and cleared, and embedded males, females and larvae were counted.

To define temperature-versus-time effects and whether sex differentiation is reversible, 14 each of 3-replicate groups of inoculated plants were prepared (as described above) and distributed half to 27 C and half to 32 C. Replicate groups were interchanged between the two temperatures at 1, 2, 3, 4, 5, 8, and 11 days after the infection period. Starting 17 days after inoculation and continuing for an additional 9 days at 3-day intervals, nematodes were removed and counted. To permit observation of gonad development, males were stained 2 min at 80 C with acid fuchsin in 1 : 1 absolute alcohol and glacial acetic acid. At the end of the trial, roots were fixed in FAA at least 48 hr, washed in tap water for 5 min, stained 2 min in boiling acid-fuchsin-lactophenol and stored in clear lactophenol. The nematodes were removed from the roots and mounted in clear lactophenol for identification of developmental stage and sex based on the characteristics described by Triantaphyllou (8).

RESULTS

Temperature influenced both the development rate and sex ratio of *M. graminis*. Larvae penetrated bermudagrass roots at 27 and 32 C within 12 hr and those at 16 and 21 C within 24 hr. At 16 and 21 C, only larvae were present in roots up to 14 days after inoculation. Nevertheless, females with matrices were found 21 days after inoculation at the lower temperatures. Eggs and larvae were evident after 28 days at 21 C. At 16 C eggs were observed after 28 days but newly hatched larvae were not found until 35 days after inoculation. At 27 and 32 C larvae had molted and appeared swollen 7 days after the plants were inoculated. Saccate females exuding matrices were apparent 14 days after inoculation. Females at these temperatures laid eggs 21 days after inoculation, and larvae were present in the matrix at 25 days.

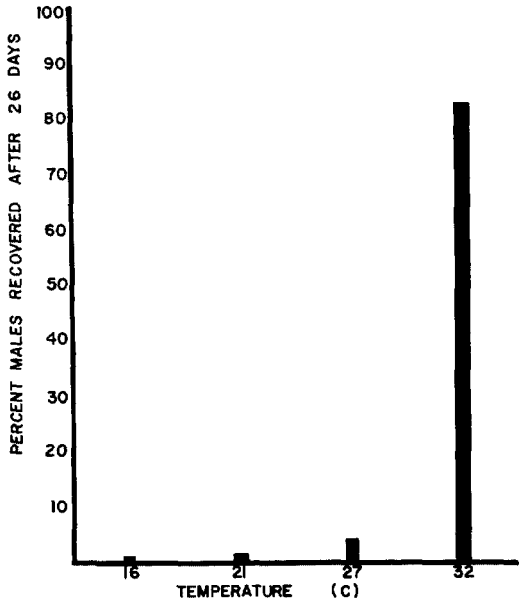

FIG. 1. Effect of continuous temperature treatments on sex differentiation of *Meloidogyne Graminis* in 'Tifgreen' bermudagrass roots. A 2-day infection period at 27 C preceded all treatments.

Males predominated in plants incubated at 32 C, and few were observed at lower soil temperatures. Developing males were present 14 days after inoculation at 32 C and emerged from roots 21 days after inoculation.

In further studies temperature influenced

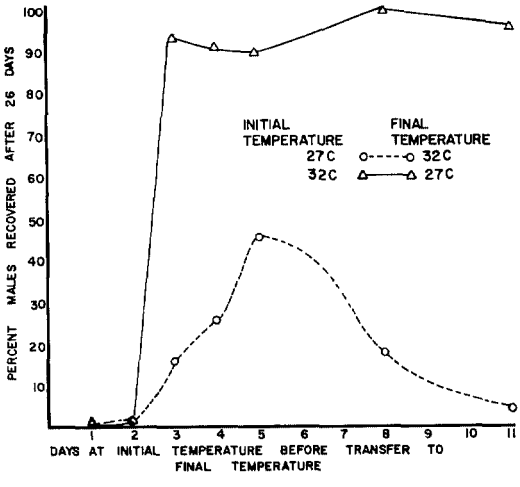

FIG. 2. Effect of temperature—time treatments on sex differentiation of *Meloidogyne graminis*. A 2-day infection period at 27 C preceded all treatments.

sex differentiation and subsequent sex ratios (Fig. 1). At 32 C males emerged from bermudagrass roots in beakers of tap water 20 days after inoculation. At 32 C the frequency of males was above 80% but only 4% at 27 C. No males were detected at 16 or 21 C.

The duration of initial periods at 27 or 32 C influences sex differentiation in *M. graminis* (Fig. 2). Males in populations maintained first at 27 C then at 32 C increased from 0% after one day of incubation at the initial temperature to a maximum of 45.5% after a 5 day incubation period. After 11 days at the lower temperature, the percentage of males had decreased to 4.3%. Individual nematodes interpreted to be male intersexes or male sex reversals were observed among nematodes initially incubated 4, 5, 8, and 11 days at 27 C. Male intersexes and male sex reversals possessed two testes rather than one. In addition male intersexes had slightly thickened tissue and an interruption of cuticular annulation in the region corresponding to the vulvar region of females. The spicule and gubernaculum were lacking in a single specimen.

In populations held at 32 C, then transferred to 27 C, the frequency of males developing after incubation for 1 or 2 days was less than 3% while after 3 days or longer it exceeded 90%. No intersexes or morphological abnormalities were observed.

DISCUSSION

Temperature affected rate of development and sex differentiation of *M. graminis* larvae in roots of 'Tifgreen' bermudagrass. Results of the present studies generally support the hypothesis presented by Davide and Triantaphyllou (4) on the mechanism of sex differentiation in root-knot nematodes. The effect of environment on biochemical and physiological processes in roots may alter the host-parasite relationship producing physiological changes which influence sex differentiation. Investigators (3, 9) have associated maleness with food stresses, but little is known about nematode nutritional requirements and how development and differentiation may be influenced by qualitative and quantitative food supply differences. However, one could expect relatively minor changes in *Cynodon* sp. when shifted from 27 C to 32 C for 3 days and then back to 27 C. The effect, therefore, may be directly upon the nematode during a critical period in its life cycle.

The biochemical bases for environmental effects on sex differentiation of nematodes is an unexplored area. If nematode sex differentiation involves sex hormones and differentiation depends upon hormonal imbalance, possibly the environmental conditions affect the relative concentrations of these hormones. The definite requirement for, and irreversibility of, the initial 2–3 days at 32 C for maleness seems to support the hypothesis of a controlling hormonal system which is developing during the first few days of gonad

development. The definite reaction to an initial temperature of 27 C, which is only partially reversed by exposure to 32 C (if it comes soon enough), also seems to support this speculation.

LITERATURE CITED

1. BIRD, A. F. 1960. The effect of some single element deficiencies on the growth of *Meloidogyne javanica*. Nematologica 5:78–85.
2. DAVIDE, R. G., and A. C. TRIANTAPHYLLOU. 1967. Influence of the environment on development and sex differentiation of root-knot nematodes. I. Effect of infection density, age of the host plant and soil temperature. Nematologica 13:102–110.
3. DAVIDE, R. G., and A. C. TRIANTAPHYLLOU. 1967. Influence of the environment on development and sex differentiation of root-knot nematodes. II. Effect of host nutrition. Nematologica 13:111–117.
4. DAVIDE, R. G., and A. C. TRIANTAPHYLLOU. 1968. Influence of the environment on development and sex differentiation of root-knot nematodes. III. Effect of foliar application of maleic hydrazide. Nematologica 14:37–46.
5. ELLENBY, C. 1954. Environmental determination of the sex ratio of a plant parasitic nematode. Nature, (London) 174:1016–1017.
6. OTEIFA, B. A. 1953. Development of the root-knot nematode, *Meloidogyne incognita*, as affected by potassium nutrition of the host. Phytopathology 43:171–174.
7. THOMASON, I. J., and B. LEAR. 1961. Rate of reproduction of *Meloidogyne* spp. as influenced by soil temperature. Phytopathology 51:520–524.
8. TRIANTAPHYLLOU, A. C. 1960. Sex determination in *Meloidogyne incognita* Chitwood, 1949, and intersexuality in *M. javanica* (Treub, 1885). Ann. Inst. Phytopathol. Benaki, N. S. 3:12–31.
9. TYLER, J. 1933. Reproduction without males in aseptic root cultures of the root-knot nematode. Hilgardia 7:373–388.
10. WALLACE, H. R. 1966. Factors influencing the infectivity of plant parasitic nematodes. Proc. Roy. Soc., Series B., Biol. Sci. 164: 592–614.