

PATHOGENICITY OF *TYLENCHORHYNCHUS ZAMBIENSIS* TO MAIZE[†]

María Eugenia Venditti¹ and Gregory R. Noel²

Center for Economic Entomology,¹ and U.S. Department of Agriculture, Agricultural Research Service, Crop Protection Research Unit, Department of Plant Pathology,² University of Illinois at Urbana-Champaign, Urbana, IL 61801, U.S.A.

ABSTRACT

Venditti, M. E., and G. R. Noel. 1995. Pathogenicity of *Tylenchorhynchus zambiensis* to maize. *Nematropica* 25:7-13.

Two greenhouse experiments were conducted to determine the effect of *Tylenchorhynchus zambiensis* on growth of maize (*Zea mays* L.) hybrid MO17 × A634 and to determine the population levels required to adversely affect maize. Initial nematode population levels (Pi) evaluated were 0.0, 0.4, 4.0, or 42 nematodes/cm³ of soil (respectively, 0, 500, 5 000, or 50 000/pot). An additional treatment evaluated the effect of nematode associated microorganisms (AM) which accompanied the highest level of nematode inoculum. After 60 days, plant height, fresh and dry foliar and root weights and numbers of nematodes were determined. In both experiments plant height, fresh and dry foliar weight, root weight, and total plant weight were reduced significantly ($P \leq 0.05$) by *T. zambiensis* at the highest inoculum level. In both experiments when Pi = 500 or 5 000 nematodes, effects of *T. zambiensis* on maize growth were inconsistent. Although roots appeared healthy, the AM treatment had a significant negative effect on root weight in experiment 1 and was numerically less in the second experiment. The highest final population (Pf) of *T. zambiensis* (Pf = 1 825 000 nematodes/pot) was obtained when Pi = 5 000 (4.0 nematodes/cm³ of soil). The reproductive factor ($R = Pf/Pi$) was 1 184.0, 365.8, and 25.2, respectively for Pi = 500, 5 000, or 50 000. These data indicate that *T. zambiensis* is a weak pathogen and requires a large population to affect maize growth.

Key words: maize, nematode, pathogenicity, *Tylenchorhynchus zambiensis*, *Zea mays*.

RESUMEN

Venditti, M. E. y G. R. Noel. 1995. Patogenicidad de *Tylenchorhynchus zambiensis* en maíz. *Nematropica* 25:7-13.

Se condujeron dos experimentos en invernadero para determinar el efecto de *Tylenchorhynchus zambiensis* en el desarrollo del maíz híbrido MO17 × A634 y para determinar los niveles poblacionales requeridos para afectar adversamente al maíz. Los niveles de población iniciales que se evaluaron fueron 0.0, 0.4, 4.0, o 42 nematodos/cm³ de suelo (respectivamente, 0, 500, 5 000, o 50 000/maceta). Un tratamiento adicional evaluó el efecto de los microorganismos asociados (MA) al nemátodo en el tratamiento con mayor nivel de inóculo. Después de 60 días, la altura de las plantas, el peso fresco y seco del follaje y el peso fresco y seco de las raíces fueron determinados. En ambos experimentos la altura de las plantas, el peso fresco y seco del follaje, de las raíces y el peso total de las plantas fueron reducidos significativamente ($P \leq 0.05$) por *T. zambiensis* en el tratamiento con mayor nivel de inóculo. En ambos experimentos cuando Pi = 500 o 5 000 nematodos, el efecto de *T. zambiensis* en el desarrollo del maíz fue inconsistente. A pesar de que las raíces parecían sanas, el tratamiento con MA tuvo un efecto significativo en el peso de las raíces en el experimento 1 y fue numéricamente menor en la segunda prueba. La mayor población final de *T. zambiensis* (Pf= 1 825 000 nematodos/maceta) fue ob-

[†]A portion of an M.S. Thesis submitted by the first author to the University of Illinois. Mention of a trademark of proprietary product does not constitute a guarantee of warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that also may be suitable.

tenida cuando $P_i = 5\,000$ (4.0 nematodos/cm³ de suelo). El factor reproductivo ($R = P_f/P_i$) fue $1\,184.0$, 365.8 , y 25.2 respectivamente para $P_i = 500$, $5\,000$ o $50\,000$. Los datos indican que *T. zambiensis* es un patógeno débil que requiere grandes poblaciones para afectar el desarrollo del maíz.

Palabras clave: maíz, nematodo, patogenicidad, *Tylenchorhynchus zambiensis*, *Zea mays*.

INTRODUCTION

Maize (*Zea mays* L.), the staple cereal of the majority of Zambians, is the most widely cultivated crop in the Republic of Zambia (4), and most of the 633 000 ha planted annually is grown in monoculture (1). Nematodes are important pests of maize world wide (18). In Southern Africa, species of 23 nematode genera that reproduce on maize have been identified (9). Walters (26) found 14 nematode genera associated with maize in the major production areas of South Africa. Species of eight nematode genera were recorded on maize in Zimbabwe (12). Martin (15) listed 13 genera of plant-parasitic nematodes in the Federation of Rhodesia and Nyasaland (Zimbabwe, Zambia, and Malawi). A survey of plant-parasitic nematode species associated with maize was done in production areas in the Republic of Zambia (G. R. Noel, unpubl.). Field studies in Zambia demonstrated that maize 'MM603' is a good host of *Helicotylenchus pseudorobustus* (Steiner) Golden, *Paratrichodorus christiei* (Allen) Siddiqi, *Pratylenchus zeae* Graham, *Scutellonema brachyurus* (Steiner) Andrassy, *Meloidogyne javanica* (Treub) Chitwood and an undescribed species of *Tylenchorhynchus* (10). Lawn *et al.* (10) documented the occurrence and population dynamics of a polyspecific community of plant-parasitic nematodes, including a *Tylenchorhynchus* sp. [subsequently described as *Tylenchorhynchus zambiensis* n. sp. (25)], on maize. The study was concerned primarily with reduction in yield associated with the nematode community and did not involve proof of pathogenicity. Some species of *Tylencho-*

rhynchus and related genera are associated with plant disease (2,5,20,24), but little is known concerning their pathogenicity and economic importance on maize. In the mid-western and southern U.S.A., *Tylenchorhynchus maximus* Allen and *Tylenchorhynchus claytoni* Steiner were associated with yield loss on maize (6,16,17). Mahapatra and Das (13) obtained significant stunting and reduction in plant weight of maize when infected with *Tylenchorhynchus mashhoodi* Siddiqi & Basir.

Limited information is available concerning the host-parasite relationship of *T. zambiensis* and maize. The objectives of this study were to determine whether *T. zambiensis* is a pathogen of maize and to provide data on the population levels required to effect a reduction in growth of maize.

MATERIALS AND METHODS

A polyspecific population of nematodes obtained from a maize field in Magoye, Zambia, was brought into the U.S. under quarantine (10). A monospecific culture of *T. zambiensis* was established in a quarantine greenhouse on maize hybrid MO17 × A634.

Two greenhouse experiments were conducted from June to August 1992 and from April to June 1993. Soil temperature was recorded each morning and afternoon during the experiments. The five treatments utilized in both experiments were a nontreated control, 500, 5 000, or 50 000 nematodes/pot (respectively 0.0, 0.4, 4.0, or 42 *T. zambiensis*/cm³ of soil) and nematode-associated microorganisms (AM) without nematodes. The AM treatment was

included to determine growth responses from microorganisms associated with the nematode. Nematode and AM inocula were prepared by extracting nematodes from soil using centrifugal-flotation (7). The supernatant containing AM was obtained from a suspension equivalent to the highest nematode inoculum level by allowing nematodes to settle for 90 minutes in a beaker. The suspension of AM without nematodes was poured into a second beaker and used for inoculum. In the second experiment the supernatant also was poured through a 500 mesh screen (25- μ m openings) to remove nematodes that may have remained suspended.

Maize seed were germinated on moist filter paper. When the radicles were 3-4 cm long, one seedling was planted in each of 17-cm-diam clay pots, containing 1 200 cm³ of autoclaved soil (3:1 sand/sandy loam mixture). When pots were filled with soil, three 1.5-cm-diam centrifuge tubes were inserted into the soil in each pot to a depth of 3 cm. Pots were watered daily for several days. On the same day that seedlings were transplanted, the soil was infested by removing the centrifuge tubes and pipetting equal volumes of the required nematode suspension into the holes. The holes were filled with 50 cm³ of autoclaved soil. Treatments were arranged on a greenhouse bench in a completely randomized design with eight replications.

Every week each pot received 200 ml of the soluble fertilizer Hyponex[®] (1.2% ammoniacal nitrogen, 5.8% nitrate nitrogen, 6% phosphorus, and 19% potassium). At 27 and 41 days after planting, 400 ml of Rapid Gro[®] (5.3% ammoniacal nitrogen, 5.1% nitrate nitrogen, 12.6% urea nitrogen, 19% phosphorus and 17% potassium) also were applied. Plants were sprayed when necessary with dienochlor to control mites and with malathion to control aphids.

Sixty days after inoculation, plants were excised at the soil line and height and fresh weight were obtained. The tops were placed in a drying oven at 55°C for 4 days to obtain dry weights. Roots were washed free of soil and fresh weights were determined. After roots were dried at 55°C for 4 days, dry weights were recorded. Nematodes were extracted from soil using centrifugal-flotation (7). Nematode populations were determined by counting nematodes in three 1-ml aliquants and multiplying the mean by the extract volume. Reproduction and survival of the nematode was determined by using the reproduction factor (R) where R is defined as the ratio of final population density (Pf) to initial population density (Pi) (19).

Statistical analysis (11) utilized analysis of variance and Fisher's least significant difference ($P \leq 0.05$) to compare means. Data of both experiments were compared using analysis of combined experiments (14) to determine whether the data could be combined and analyzed as one experiment.

RESULTS

When compared by combined experiment analysis (14), data for Pf and R did not differ between the two experiments; however, significant differences were found for the data concerning the influence of *T. zambiensis* on maize growth. Population and growth data from both experiments were analyzed separately.

Experiment 1: Plant height was significantly lower in pots infested with 50 000 nematodes compared to those infested with 500 nematodes and the noninfected controls (Table 1). However, significant differences were not observed among the treatments with 50 000 nematodes/pot, 5 000/pot, and AM.

Table 1. Influence of inoculum density of *Tylenchorhynchus zambiensis* and associated microorganisms (AM) on growth of 'MO17 × A634' maize 60 days after inoculation, experiment 1.^w

Treatment	Foliage			Root		Plant	
	Ht (cm) ^x	Fwt (g) ^y	Dwt (g) ^z	Fwt (g) ^y	Dwt (g) ^z	Fwt (g) ^y	Dwt (g) ^z
Control	204.5 a	289.8 a	86.2 a	116.6 a	15.8 a	406.4 a	102.0 a
AM	201.5 ab	274.4 a	87.5 a	97.9 cb	12.9 b	372.3 b	100.4 a
500	203.8 a	286.5 a	81.6 a	108.5 ab	14.1 ab	394.9 ab	95.8 a
5 000	200.8 ab	282.4 a	76.9 a	103.1 ab	13.6 ab	385.5 ab	90.5 a
50 000	192.1 b	251.5 b	61.3 b	81.8 c	10.1 c	333.4 c	71.4 b

^wData are means of eight replications. Means followed by the same letter in columns are not different ($P \leq 0.05$) according to Fisher's LSD mean separation.

^xHeight.

^yFresh weight.

^zDry weight.

Fresh and dry foliar weights were reduced when the Pi was 50 000 nematodes compared to all other treatments (Table 1). No differences were observed among the other treatments. A significant reduction in fresh root weight occurred when the Pi = 50 000 treatment was compared to Pi = 500, Pi = 5 000 and control, but Pi = 50 000 did differ from the AM treatment. A reduction in dry root weight occurred at the highest inoculum level and the AM treatment, but dry root weight of the plants inoculated with 50 000 nematodes were smaller than for plants which received the AM treatment.

Final population densities and R of *T. zambiensis* differed significantly among the three inoculum levels evaluated in this study (Table 2). Based on population densities at termination of the experiment, the greatest nematode population increase was obtained with Pi = 5 000 nematodes/pot for which Pf was 1 829 000. The lowest Pf (592 000) of *T. zambiensis* was recovered from pots infested with 500 nematodes. When Pi was 50 000 nematodes Pf was 1 261 000. Nematode reproductive indices

were 1 184.0 for Pi = 500, 365.8 for Pi = 5 000, and 25.2 for Pi = 50 000. An average of 7 100 (range 400-31 000) *T. zambiensis* were detected in the experimental units of the AM treatment.

Experiment 2: Inoculation of maize with 50 000 nematodes resulted in significant reductions in plant height, fresh foliar weight, and dry foliar weight when compared to all other treatments (Table 3).

Table 2. Final populations of *Tylenchorhynchus zambiensis* in soil in relation to initial populations on 'MO17 × A634' maize 60 days after inoculation, experiment 1.^w

Pi ^x	Pf ^y	R ^z
500	592 000 c	1 184.0 a
5 000	1 829 000 a	365.8 b
50 000	1 261 000 b	25.2 c

^wData are means of eight replications. Means followed by the same letter in columns are not different ($P \leq 0.05$) according to Fisher's LSD mean separation.

^xPi = Initial inoculum/pot.

^yPf = Final population/pot.

^zR = Reproduction factor, Pf/Pi.

Table 3. Influence of inoculum density of *Tylenchorhynchus zambiensis* and associated microorganisms (AM) on growth of 'MO17 × A634' maize 60 days after inoculation, experiment 2.^w

Treatment	Foliage			Root		Plant	
	Ht (cm) ^x	Fwt (g) ^y	Dwt (g) ^z	Fwt (g) ^y	Dwt (g) ^z	Fwt (g) ^y	Dwt (g) ^z
Control	174.9 a	296.8 a	86.3 a	197.7 a	43.1 a	494.5 a	129.5 a
AM	168.5 ab	275.9 ab	77.1 a	178.9 a	39.1 ab	454.8 ab	116.2 ab
500	174.9 a	260.2 b	75.3 a	171.5 ab	36.8 bc	431.7 b	110.2 b
5 000	160.0 b	257.5 b	73.3 a	144.8 b	32.8 c	402.3 b	108.1 b
50 000	135.5 c	214.1 c	53.2 b	91.9 c	17.5 d	306.0 c	70.7 c

^wData are means of eight replications. Means followed by the same letter in columns are not different ($P \leq 0.05$) according to Fisher's LSD mean separation.

^xHeight.

^yFresh weight.

^zDry weight.

Significant differences were observed between Pi = 5 000 and the control for plant height and fresh foliar weight, but not dry foliar weight. No significant differences were observed between Pi = 5 000 and AM. Similarly, Pi = 500 did not differ from AM in plant height, fresh foliar weight, or dry foliar weight. Fresh foliar weight was the only parameter for which Pi = 500 differed significantly from the control. None of the plant growth variables were affected significantly by the AM treatment.

In the second experiment, trends similar to the first experiment were obtained for Pf and R (Table 4). The highest final population of 1 875 000 was recorded for Pi = 5 000 and the lowest 522 000 for Pi = 500. When Pi = 50 000, the final population was 1 386 000. Values of R were 1 044.0 for Pi = 500, 375.0 for Pi = 5 000, and 27.7 for Pi = 50 000 nematodes. Low populations of *T. zambiensis* were recovered from pots of the AM treatment. Populations ranged from 20 to 1 700 with an average of 390. *Tylenchorhynchus zambiensis* was not recovered from any control pots in either experiment.

During the first experiment soil temperatures ranged from 19 to 36°C ($\bar{x} = 26.1^\circ\text{C}$) and from 20 to 33°C ($\bar{x} = 25.5^\circ\text{C}$) during the second experiment.

DISCUSSION

Tylenchorhynchus zambiensis was reported from maize fields in Zambia (10), but its role as a pathogen was not defined. Greenhouse studies demonstrated that 'MO17 ×

Table 4. Final populations of *Tylenchorhynchus zambiensis* in soil in relation to initial populations on 'MO17 × A634' maize 60 days after inoculation, experiment 2.^w

Pi ^x	Pf ^y	R ^z
500	522 000 c	1 044.0 a
5 000	1 875 000 a	375.0 b
50 000	1 386 000 b	27.7 c

^wData are means of eight replications. Means followed by the same letter in columns are not different ($P \leq 0.05$) according to Fisher's LSD mean separation.

^xPi = Initial inoculum/pot.

^yPf = Final population/pot.

^zR = Reproduction factor, Pf/Pi.

A634' maize was an excellent host for *T. zambiensis* and that the nematode can reduce foliar and root growth of maize. *Tylenchorhynchus zambiensis* reached a maximum population density at medium inoculum levels (5 000 nematodes/plant), and nematode reproductive indices decreased as inoculum density increased. Rates of nematode population increase are generally inversely proportional to Pi (22). These differences in nematode reproduction at the different inoculum levels may be due to differences in availability of food supply, with a greater root mass per nematode occurring at the lower initial population density. The reduction of root growth at inoculum levels of 50 000 nematodes may have adversely affected nematode reproduction. However, Seinhorst (22) suggested that the lower multiplication rates obtained at higher inoculum levels probably result from competition for feeding sites within the nematode population rather than reduction of the available amount of food.

Stunting of the root system, which is associated with retardation of plant growth, is the most characteristic symptom of parasitism by species of *Tylenchorhynchus* (8). In both experiments foliar and root growth of 'MO17 × A634' maize was reduced by *T. zambiensis* at Pi = 50 000 nematodes (42 nematodes/cm³ of soil). However, in the second experiment, the nematode reduced some measures of plant growth at Pi = 5 000 nematodes (4.0 nematodes/cm³ of soil). Differences in results between the two experiments may be due to different environmental factors during the time of the year during which the experiments were conducted. It is possible that temperature may have influenced the pathogenic ability of *T. zambiensis*. Nematodes are more susceptible to extremes of temperature than are plants (21). Although not measured, differences in natural light intensity and pho-

toperiod also may have influenced plant growth. Under the more optimum growing conditions during the first experiment, the plants may have attained more growth before nematode populations reached damaging levels.

The only significant difference between the control and the AM treatment was root weight in the first experiment. Roots of plants that received the AM treatment and different Pi's of *T. zambiensis* exhibited no symptoms of root rot. Davis *et al.* (3) noted that the microorganisms associated with 240 *T. nudus*/cm³ of soil significantly reduced root length of bentgrass and annual bluegrass, whereas the microorganisms associated with 120 nematodes/cm³ of soil did not produce any effect on root length of either grass.

Damage to 'MO17 × A634' by *T. zambiensis* occurred at Pi > 5 000 nematodes (4.0 nematodes/cm³ of soil). Seinhorst (23) and Rhode (21) pointed out that only when the population density of the nematode in question exceeds the tolerance limit of the host plant, the yield will be affected or symptoms of disease will be apparent. In addition to the preplant or initial nematode density, environmental influences also are important (19). Reductions in growth of maize in the presence of *T. zambiensis* demonstrated the potential importance of this nematode. However, it is unknown how the environment or concomitant plant-parasitic species such as *H. pseudorobustus*, *P. christiei*, *P. zae* and *S. brachyurus* would affect the pathogenicity of this species. More precise damage thresholds are needed before developing management strategies for *T. zambiensis*. Therefore, additional field experimentation and studies on the interrelationships of *T. zambiensis* and other plant-parasitic species are needed to determine the role of *T. zambiensis* in disease of maize under field conditions in Zambia.

LITERATURE CITED

1. ANONYMOUS. 1993. Final crop forecasts 1992/93 crop season. Ministry of Agriculture, Food and Fisheries and Central Statistical Office, Republic of Zambia, 13 pp.
2. BIRCHFIELD, W., and W. J. MARTIN. 1956. Pathogenicity on sugarcane and host plant studies of a species of *Tylenchorhynchus*. *Phytopathology* 46:277-280.
3. DAVIS, R. F., G. R. NOEL, and H. T. WILKINSON. 1994. Pathogenicity of *Tylenchorhynchus nudus* to creeping bentgrass and annual bluegrass. *Plant Disease* 78:169-173.
4. DODGE, D. J. 1977. *Agricultural Policy and Performance in Zambia*. Institute of International Studies, University of California, Berkeley, California, U.S.A., 285 pp.
5. GRAHAM, T. W. 1954. The tobacco stunt nematode in South Carolina. *Phytopathology* 44:332.
6. GRIFFIN, G. D. 1964. Association of nematodes with corn in Wisconsin. *Plant Disease Reporter* 48:458-461.
7. JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.
8. JENKINS, W. R., and D. P. TAYLOR. 1967. *Plant Nematology*. Reinhold Publishing Corporation, New York, New York, U.S.A., 270 pp.
9. KEETCH, D. P. and N. H. BUCKLEY. 1984. A Check-list of the Plant-Parasitic Nematodes of Southern Africa. Technical Communication, Department of Agriculture, Republic of South Africa, Pretoria, South Africa, No. 195, 213 pp.
10. LAWN, D. A., G. R. NOEL, and J. B. SINCLAIR. 1988. Plant-parasitic nematodes associated with sunflower and maize in the Republic of Zambia. *Nematropica* 18:143-154.
11. LITTELL, R. C., R. J. FREUND, and P. C. SPECTOR. 1991. *SAS® System for Linear Models*. Third Edition. SAS Institute Inc., Cary, North Carolina, U.S.A., 329 pp.
12. LOUW, I. W. 1982. Nematode pests of maize. Pp. 69-72 in D. P. Keetch and J. Heyns, eds. *Nematology in Southern Africa*. Science Bulletin, Department of Agriculture and Fisheries, Pretoria, Republic of South Africa, No. 400, 170 pp.
13. MAHAPATRA, S. N., and S. N. DAS. 1984. Pathogenicity of *Tylenchorhynchus mashhoodi* on maize (*Zea mays*). *Assam Agricultural University Journal of Research* 5:119-121.
14. MCINTOSH, M. S. 1983. Analysis of combined experiments. *Agronomy Journal* 75:153-155.
15. MARTIN, G. C. 1961. Frequency of occurrence of genera of plant-parasitic nematodes in soil samples from the Federation of Rhodesia and Nyasaland. *Rhodesia Agricultural Journal* 58:322-323.
16. NELSON, R. R. 1955. Nematode parasites of corn in the coastal plain of North Carolina. *Plant Disease Reporter* 39:818-819.
17. NELSON, R. R. 1956. Resistance to the stunt nematode in corn. *Plant Disease Reporter* 40:635-639.
18. NORTON, D. C. 1984. Nematodes parasites of corn. Pp. 61-94 in W. R. Nickle, ed. *Plant and Insect Nematodes*. Marcel Dekker, Inc., New York, New York, U.S.A.
19. OOSTENBRINK, M. 1966. Major characteristic of the relation between nematodes and plants. *Mededelingen Landbouwhogeschool Wageningen* 66-4, 46 pp.
20. REYNOLDS, H. W., and M. M. EVANS. 1953. The stylet nematode, *Tylenchorhynchus dubius*, a root parasite of economic importance in the Southwest. *Plant Disease Reporter* 37:540-544.
21. RHODE, R. A. 1965. The nature of resistance in plants to nematodes. *Phytopathology* 55:1159-1162.
22. SEINHORST, J. W. 1961. Plant nematode interrelationships. *Annual Review of Microbiology* 15:177-196.
23. SEINHORST, J. W. 1965. The relation between nematode density and damage to plants. *Nematologica* 11:137-154.
24. SMOLIK, J. D., and R. B. MALEK. 1973. Effect of *Tylenchorhynchus nudus* on growth of Kentucky bluegrass. *Journal of Nematology* 5:272-274.
25. VENDITTI, M. E., and G. R. NOEL. 1995. Description of *Tylenchorhynchus zambiensis* n. sp. (Nemata: Tylenchidae) from Zambia. *Nematropica* 25:1-6.
26. WALTERS, M. C. 1979. The possible status of parasitic nematodes as limiting factors in maize production in South Africa. *Proceeding of the Second South Africa Maize Breeding Symposium, Pietermaritzburg, 1976*. Technical Communication, Department of Agriculture Technical Services, Pretoria, Republic of South Africa. No. 142:112-118.

Received:

25.IX.1994

Accepted for publication:

1.XI.1994

Recibido:

Aceptado para publicación: