PLANT-PARASITIC NEMATODES AND *NEOCOSMOSPORA VASINFETCA* VAR. *AFRICANA* ASSOCIATED WITH SOYBEANS IN THE REPUBLIC OF ZAMBIA

Dennis A. Lawn, Gregory R. Noel, and J. B. Sinclair

Respectively, Centro Internacional de Mejoramiento de Maize y Trigo, AP 6-641, Delegación Cuauhtémoc, 06600 México, D.F.; USDA, ARS and Department of Plant Pathology, University of Illinois at Urbana-Champaign. Urbana, IL 61801, U.S.A.

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ABSTRACT

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Field trials were established during the 1985–86 cropping season at Mkushi in the Central Province and at Magoye in the Southern Province of Zambia. Soybean cvs. Bragg, Jupiter, Santa Rosa, and Hernon 147 were planted in soil either fumigated with ethylene dibromide or not fumigated. Soil was infested with *Pratylenchus zeae, Helicotylenchus pseudorobustus, Paratrichodorus christiei* and *Scutellonema brachyurum* at the Central Province location and with *S. brachyurum, Meloidogyne javanica* and *Tylenchorhynchus* sp. (undescribed) at the Southern Province site. Nematode populations were not significantly different among cultivars in either fumigated or nonfumigated plots. Populations of all species except *P. christiei* were significantly lower ($P < 0.05$) following fumigation. At Mkushi significantly higher populations of *P. christiei* were recovered from fumigated plots and were associated with 16%, 10% and 6% yield decrease of 'Bragg', 'Santa Rosa' and 'Hernon 147', respectively. Yields of 'Jupiter' were increased 6% by fumigation. Infection of soybean roots with *Neocosmospora vasinfecta* var. *africana* was observed for the first time in the Republic of Zambia in nonfumigated plots at Magoye infested with *S. brachyurum, M. javanica,* and *Tylenchorhynchus* sp.

Key words: control, EDB, *Glycine max, Helicotylenchus pseudorobustus, Meloidogyne javanica, Neocosmospora vasinfecta, Paratrichodorus christiei,* population dynamics, *Pratylenchus zeae, Scutellonema brachyururm,* soybean, *Tylenchorhynchus.*

RESUMEN

Lawn D. A., G. R. Noel, y J. B. Sinclair. 1988. Presencia de nematodos fitoparásitos y de *Neocosmospora vasinfecta* var. *africana* en la soya de República de Zambia. Nematrópica 18: 33-43.

Se efectuaron varios ensayos de campo en la campaña agrícola de 1985–1986 en Mkushi en la Provincia Central y en Magoye en la Meridional de la República de Zambia. En los ensayos se plantaron los siguientes cultivares de soya en suelos fumigados con dibromo de etileno asi como en otros sin el tratamiento: 'Bragg', 'Jupiter', 'Santa Rosa', y 'Hernon 147'. El suelo de la Provincia Central estaba infestado con *Pratylenchus zeae, Helicotylenchus pseudorobustus, Paratrichodorus christiei,* y *Scutellonema brachyurum* y el de la Provincia Meridional con *S. brachyurum, Meloidogyne javanica,* y *Tylenchorhynchus* sp. (no descrita). Los cultivares no ejercieron influencia alguna sobre las problaciones de nematodos tanto en las parcelas fumigadas como in las sin tratamiento. Las poblaciones de *P. christiei* resultaron ser más elevadas en las parcelas fumigadas en Mkushi que en las no fumigadas y se observó que la presencia de este parásito resultó en bajas en los rendimientos de 'Bragg', 'Santa Rosa', y 'Hernon 147' de 16, 10, y 6%, respectivamente. Los rendimientos de 'Jupiter' aumentaron en un 6% con la fumigación. Se observó infección por *Neocosmospora vasinfecta* var. *africana* de las raíces de soya en parcelas sin fumigación en Magoye las que también contenían *S. brachyurum, M. javanica*, y *Tylenchorhynchus* sp. lo que constituye el primer registro de este hongo fitopatógeno en la República de Zambia.

Palabras claves: BDE, combate químico, combate de nematodos, dinámica poblacional, *Glycine max*, fitomejoramiento, *Meloidoyne javanica, Neocosmospora vasinfecta*, nuevos registros, *Paratrichodorus christiei, Pratylenchus zeae, Scutellonema brachyurum*, soya, *Tylenchorhynchus*.

INTRODUCTION

Advances in the science of nematology preceded Zambian independence in 1964, with research that emphasized surveys, identification and control of *Meloidogyne* spp. The most comprehensive of these surveys, published in 1958 by G. C. Martin (10), included a host list of plant species attacked by *Meloidoyne* spp. in the Federation of Rhodesia and Nyasaland (Zimbabwe, Zambia, and Malawi). The four most common *Meloidogyne* spp., *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, *M. hapla* Chitwood, and *M. arenaria* (Neal) Chitwood and races of some of these species inhabit soils in south central Africa. Little research has been published on nematode pathogenicity to soybean (*Glycine max* (L.) Merr.) in southern Africa, although species in 10 genera have been associated with soybean in the region (1).

Zambia is a land-locked country within south central Africa which lies at latitude 15° South and approximately 1 500 m elevation. Although diverse rainfall accumulations throughout the country affect agricultural production, the relative stability of the soil temperature is important in the occurrence of soil-borne plant-pathological problems. The Zambian soil moisture regime, typified by the locations in this study, has been classified as a Ustic moisture regime (14). Mean annual soil temperature is 22 C or higher and annual fluctuations in soil temperatures are less than 5 C at a depth of 50 cm. Mean annual rainfall varies, but there is an absence of ground water or water held at a tension $<$1500 kPa in the soil for at least 90 cumulative days in most years.

The objectives of this study were to identify the important plant-parasitic nematodes and associated etiological agents in representative soybean production areas, to determine nematode population dynamics during the growing season, and to determine yield loss caused by the most prevalent nematode species.

MATERIALS AND METHODS

Experimental plots were established in 1985 in the Central Province on a private farm 20 km south of Mkushi and in the Southern Province at Magoye Regional Research Station. Average annual rainfall at Mkushi and Magoye is 930 mm and 791 mm, respectively. Soil types were an Oxic Paleustult (sandy loam/loamy sand topsoil and a sandy clay/clay subsoil) at Mkushi (4) and a Typic (oxic) Palestalf (sandy clay loam topsoil and a clay subsoil) at Magoye (7).

Land preparation included conventional tillage, application of lime at 200 kg/ha and fertilization with 300 kg/ha of 10-20-10-10 (N-P-K-S). Soybean cvs. Bragg, Santa Rosa, Hernon 147 and Jupiter were planted either in nonfumigated soil or in soil fumigated with ethylene dibromide (EDB, MO-Shell Chemicals (Zambia) Ltd.) using a Shell® fumigun 14 days prior to planting, at the rate of 75 L a.i./ha broadcast. EDB was diluted in three parts water, and 3 ml of this emulsion was injected to a depth of 30–35 cm at points 30 cm apart. Treatments were applied to six-row plots, 4-m-long with 50-cm-row spacing. On 11 and 13 December 1985 at Mkushi and Magoye respectively, seeds were inoculated with *Bradyrhizobium japonicum* (Buchanan) Jordan, and 150 seeds/4-m row were sown by hand 2–3 cm deep. Plots were hand-weeded as necessary. The experimental design was a split plot with four replications. Nematode population data were analyzed as a split-split plot with cultivars as main plots, fumigation as sub plots and sampling dates as sub-sub plots. Yield was determined by harvesting 3.5 m of the two center rows in each plot. 'Bragg', 'Santa Rosa', 'Hernon 147', and 'Jupiter' were harvested at 15, 17, 17, and 20 weeks after planting (WAP) at Mkushi. Seeds were not harvested at Magoye. Seeds were dried, weighed and seed moisture was adjusted to 10%. In addition, lots containing 300 seeds from each plot were weighed to determine effects of treatments on seed size.

Soil was sampled at planting, 4 and 8 WAP, and at harvest. Soil samples were collected with a 2-cm-d probe to a depth of 30–35 cm. For each plot, 12–15 cores were randomly collected from the two center rows, approximately 3 cm from the base of the plants. After thoroughly mixing the soil, a 250 cm^3 aliquant was processed to extract nematodes. After suspending the sample in 3 L of water, nematodes were extracted by Cobb's gravity sieving technique (6) using 850- and 38-μm-pore sieves. Residue with nematodes was collected on the 38-μm-pore sieve and separated using the rapid centrifugal-flotation technique (9). Nematodes extracted from each sample were suspended in 25 ml of water and populations were estimated by counting two 1-ml aliquots at planting and harvest and one 1-ml aliquot at 4 and 8 WAP.

Roots were sampled at 4 and 8 WAP by digging and compositing five root systems from the two border rows of each plot. After rinsing, all roots were examined microscopically and galls and nodules were counted. Roots were then cut into 2- to 3-cm-long sections and placed on a Baermann funnel (2) for 7 days. Populations of nematodes were recovered from a 26-μm-pore sieve and counted. Roots were dried for 3 days at 75 C and weighed to estimate the number of nematodes/g dry root. Additional root samples from 8 WAP at the Magoye location were surface sterilized with 0.5% sodium hypochlorite and plated on potato-dextrose agar amended with 100 ppm streptomycin sulfate.

RESULTS

Plant-parasitic nematodes present at the Mkushi location were *Pratylenchus zeae* Graham, *Helicotylenchus pseudorobustus* (Steiner) Golden, *Paratrichodrus christiei* (Allen) Siddiqi, *Scutellonema brachyurum* (Steiner) Andrassy, and *Xiphinema* sp., whereas those at Magoye were *S. brachyurum, M. javanica, Tylenchorhynchus* sp., and *Xiphinema* sp. The *Tylenchorhynchus* sp. is undescribed (A. M. Golden, pers. comm.). Populations of each species within EDB-fumigated or nonfumigated test plots were never significantly different ($P < 0.05$) among the four cultivars, nor were any cultivar × EDB interactions significant. Therefore, data presented in Figures 1–3 depict average populations of each nematode associated with all four cultivars at each location.

At Mkushi, initial population levels of *P. zeae* of 140 and 30/250 cm^3 of soil in nonfumigated and fumigated plots, respectively, declined to negligible levels of 11 and 5/250 cm^3 of soil by harvest. Low populations recovered from roots verified this trend. Populations of *H. pseudorobustus* also declined from initial levels of 110 to 45/250 cm^3 of soil by harvest in nonfumigated plots and from 64 to 25/250 cm^3 of soil in fumigated plots. *P. christiei* populations increased from low initial levels in both treated and nontreated soil (Fig. 1) and were significantly higher in fumigated plots at 8 WAP and harvest (Fig. 1 and Table 1). Analysis of variance indicated a significant ($P < 0.05$) EDB, sample date, and EDB × sample date interaction (Table 1). *S. brachyurum* populations increased in nonfumigated plots at both locations but were supressed by fumigation (Fig. 2 and Table 2). The interactions between this species and EDB, sample date, and EDB × sample date were significant ($P < 0.05$) at both locations. At Magoye, initial populations of *Tylenchorhynchus* sp. were maintained on soybean since soil and root populations did not increase significantly (Fig. 3). Analysis of variance demonstrated an EDB, sample date, and cultivar × sample date interaction (Table 1), in spite of the large sampling error as indicated by the large CV%. Both *M. javanica* and *Xiphinema* sp. were also present in the Magoye trial, but low populations and large variability precluded determination of significant differences among treatments.

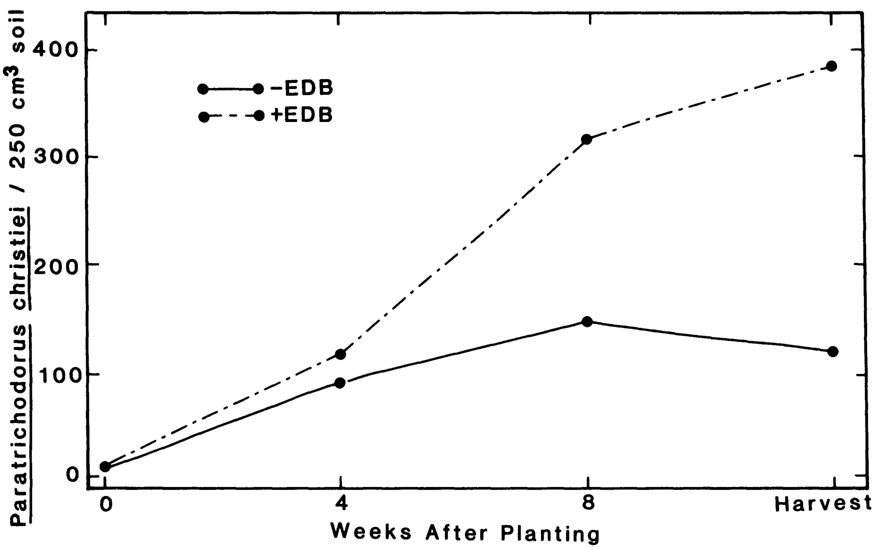

Fig. 1. Population dynamics of *Paratrichodorus christiei* at Mkushi in soil either fumigated with EDB or not fumigated and planted with soybean cvs. Bragg, Santa Rosa, Hernon 147, and Jupiter. Harvest occurred at 15 weeks after planting (WAP) for 'Bragg', 17 WAP for 'Santa Rosa' and 'Hernon 147', and 20 WAP for 'Jupiter'.

At both locations, *B. japonicum* nodules ranged from one to five per root system on all cultivars regardless of fumigation, except 'Hernon 147' in which six to ten were observed. Root galling by *M. javanica*

Table 1. Analysis of variance of *Paratrichodrus christiei* populations at Mkushi and *Tylenchorhynchus* sp. populations at Magoye on four soybean cultivars grown in soil either fumigated with EDB or not fumigated.

		Mean squares	
Source of variation	df	*P. christiei*	*Tylenchorhynchus* sp.
Block	3	16 445.31	2 220.05
Cultivar (CU)	3	27 695.31	3 001.30
Error A	9	19 913.19	4 889.32
EDB	1	416 328.12*	46 894.53*
CU × EDB	3	9 466.15	2 011.72
Error B	12	16 806.64	3 857.42
Sample date (SD)	3	391 601.53*	6 516.93*
SD × CU	9	24 513.89	4 707.03*
SD × EDB	3	127 252.60*	4 121.09
SD × CU × EDB	9	9 991.32	3 352.86
Error C	72	13 060.98	1 800.13
CV%		77.4	141.1

$^*P < 0.05$.

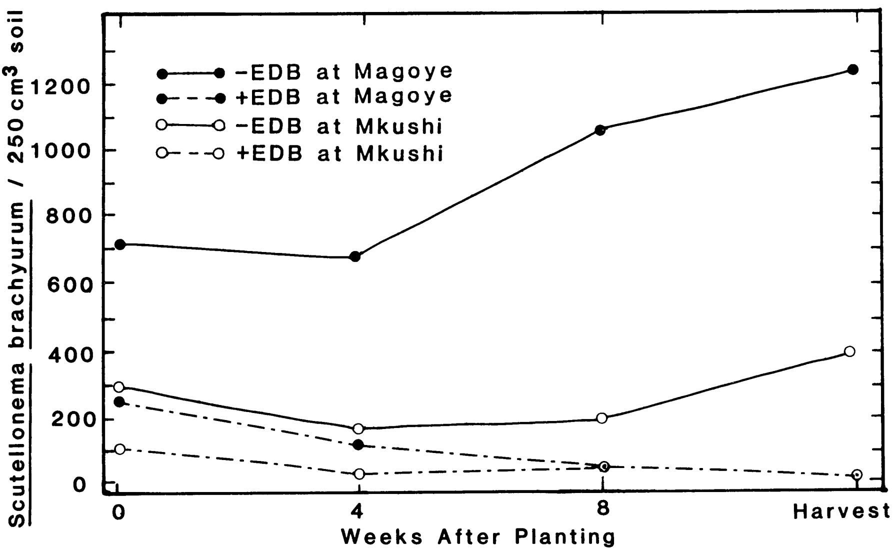

Fig. 2. Population dynamics of *Scutellonema brachyurum* at two locations in Zambia in soil fumigated with EDB or not fumigated and planted with soybean cvs. Bragg, Santa Rosa, Hernon 147, and Jupiter. Harvest occurred at 15 weeks after planting (WAP) for 'Bragg', 17 WAP for 'Santa Rosa' and 'Hernon 147', and 20 WAP for 'Jupiter'.

occurred at Magoye, but numbers of galls indicated low nematode populations and nonuniform distribution. Severe stunting and nitrogen deficiencies were evident by 8 WAP at the Magoye location. Roots from

Table 2. Analysis of variance of *Scutellonema brachyurum* populations four cultivars of soybean grown in soil either fumigated with EDB or not fumigated at two locations in Zambia.

Source of variation	df	Mean squares: Mkushi	Mean squares: Magoye
Block	3	24 379.88	401 958.00
Cultivar (CU)	3	41 215.82	487 530.90
Error A	9	38 234.05	444 518.77
EBD	1	1 439 692.38*	18 738 676.76*
CU × EDB	3	29 744.45	278 442.38
Error B	12	29 627.28	458 930.66
Sample date (SD)	3	99 809.57*	156 658.53*
SD × CU	9	25 174.15	43 316.51
SD × EDB	3	107 973.63*	643 611.63*
SD × CU × EDB	9	5 004.88	39 158.53
Error C	72	16 712.78	52 494.03
CV%		80.0	46.1

*$P < 0.05$.

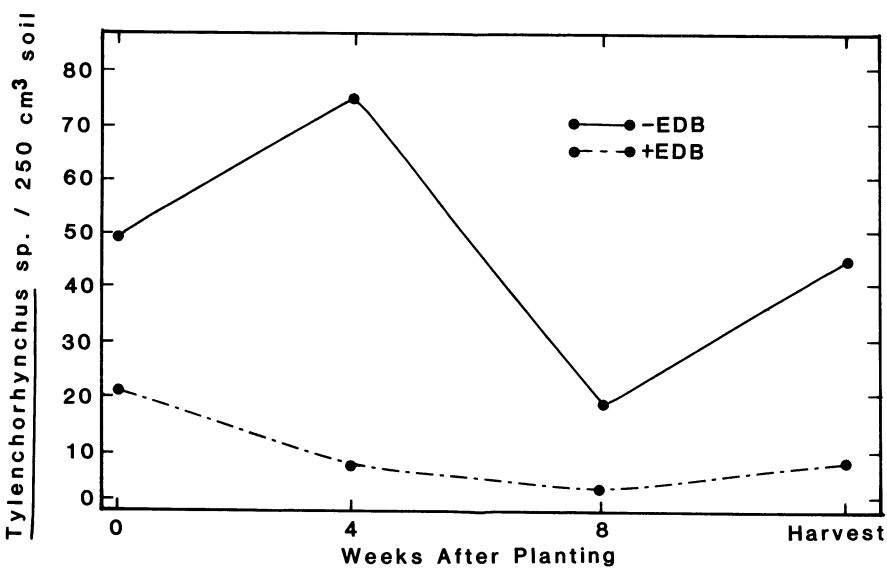

Fig. 3. Population dynamics of *Tylenchorhynchus* sp. at Magoye in soil either fumigated with EDB or not fumigated and planted with soybean cvs. Bragg, Santa Rosa, Hernon 147, and Jupiter. Harvest occurred at 15 weeks after planting (WAP) for 'Bragg', 17 WAP for 'Santa Rosa' and 'Hernon 147', and 20 WAP for 'Jupiter'.

plants grown in nonfumigated soil were discolored and severely rotted, whereas roots from plants grown in fumigated soil were not (Fig. 4a). *Fusarium chlamydosporum* Wollenw. & Reinking and *Neocosmospora vasinfecta* var. *africana* (v. Arx) P. Cannon & D. Hawksw. were isolated from diseased roots (Fig. 4b).

At Mkushi, fumigation resulted in yield decreases of 16, 10 and 6% in 'Bragg', 'Santa Rosa', and 'Hernon 147', respectively (Table 3). In contrast, fumigation resulted in a 6% yield increase of 'Jupiter'. Analysis of variance revealed significant ($P < 0.05$) cultivar and EDB treatment differences in yield (Table 4). An FLSD comparison showed that 'Jupiter' yielded significantly ($P < 0.05$) lower than 'Bragg', 'Santa Rosa' or 'Hernon 147' in both fumigated and nonfumigated plots. Yields were significantly ($P < 0.05$) lower in fumigated plots than in nonfumigated ones. Fumigation did not affect 300-seed weights ($P < 0.05$). Reliable yield data were not obtained from the Magoye location due to poor plant growth and severe termite damage which occurred as termites fed on stems and pods when the soybeans reached maturity.

DISCUSSION

Soybean is rated as a good host for *P. christiei* (5). With an adequate and suitable food source, populations of this nematode have reportedly

Fig. 4. A) Roots of plants grown in soil either fumigated with EDB (left) or not fumigated (right) and B) perithecia of *Neocosmospora vasinfecta* var. *africana* emerging from surface sterilized roots excised from field-grown soybean and plated on potato-dextrose agar amended with 100 ppm streptomycin sulfate.

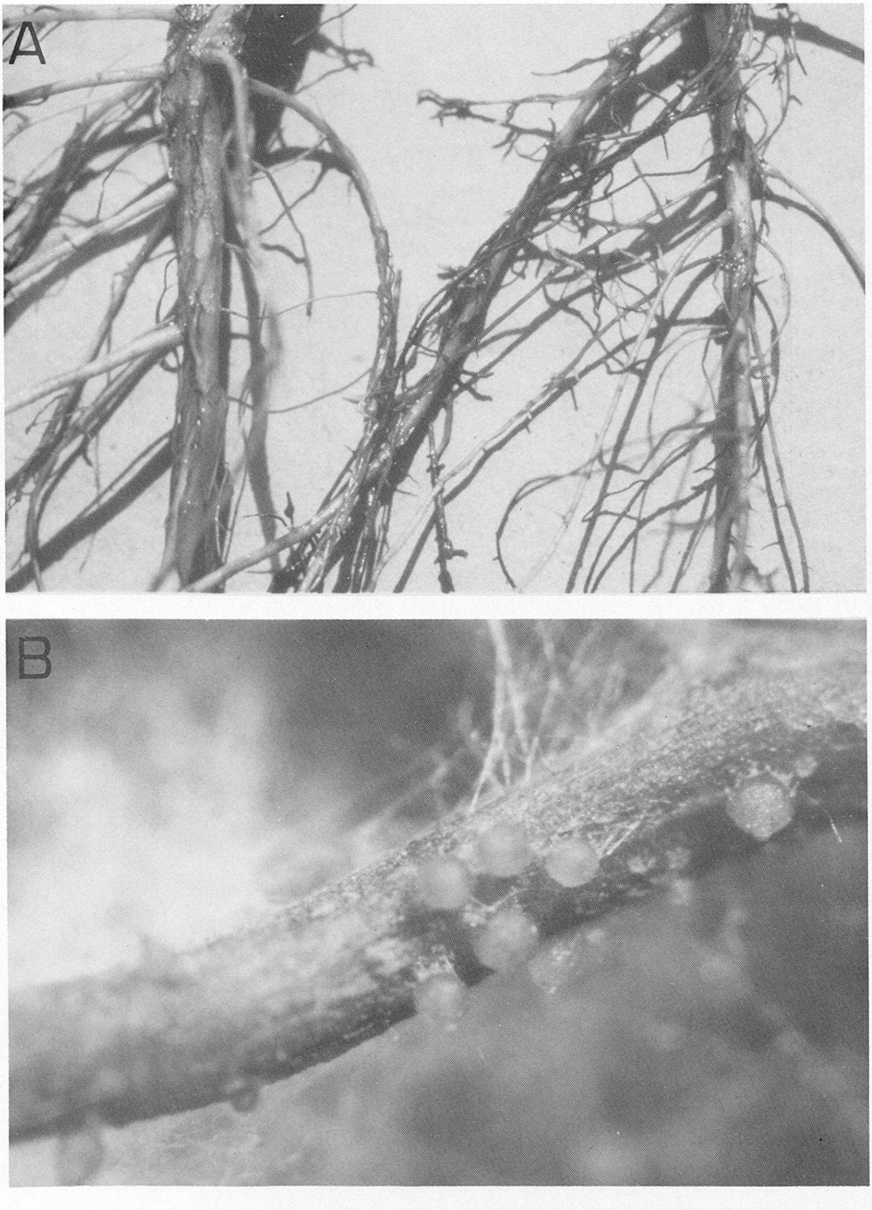

Fig. 4. A) Roots of plants grown in soil either fumigated with EDB (left) or not fumigated (right) and B) perithecia of *Neocosmospora vasinfecta* var. *africana* emerging from surface sterilized roots excised from field-grown soybean and plated on potato-dextrose agar amended with 100 ppm streptomycin sulfate.

Table 3. Yield of soybean cvs. Bragg, Santa Rosa, Hernon 147, and Jupiter grown in soil either fumigated with EDB or not fumigated.

Cultivar	EDB treatment	kg/ha
Bragg	-[z]	2 840
Bragg	+	2 397
Santa Rosa	-	2 608
Santa Rosa	+	2 351
Hernon 147	-	2 814
Hernon 147	+	2 654
Jupiter	-	1 762
Jupiter	+	1 874
CV%		9.8

[z]+ = EBD applied broadcast at 75 L a.i./ha; - = no EBD application.

increased by three or four times in fumigated plots compared to non-fumigated controls (5,13). Similar increases were observed at the Mkushi location. Suppression of *P. zeae, H. pseudorobustus* and *S. brachyurum* population development in fumigated plots at Mkushi suggested that the lack of nematode competition in fumigated plots together with control of other soil-borne microorganisms, influenced *P. christiei* population dynamics. Significantly lower yields of 'Bragg', 'Santa Rosa', and 'Hernon 147' in fumigated plots, indicated that *P. christiei* influenced soybean yields. The opposite effect observed with 'Jupiter' may have been due to indirect effects of lower yield potential and later maturity. Preplant levels of 20–40 *P. christiei* per 100 cm^3 of soil have been reported as threshold levels for damage to soybean (3). However, preplant levels of this nematode in moisture regimes such as those in Zambia may not provide an accurate assessment of post-plant population development potential. Nematode survival during the dry season is likely dependent upon the thick-walled eggs, which were not quantified at planting. Thus, the damage potential would be underestimated using the extraction techniques utilized in this study. The cosmopolitan nature of this nema-

Table 4. Analysis of variance for yield of four soybean cultivars grown in soil either fumigated with EBD or not fumigated.

Source of variation	df	Mean squares
Block	3	0.04460
Cultivar (CU)	3	0.16424*
Error A	9	0.02094
EDB	1	0.03754*
CU × EDB	3	0.01340
Error B	12	0.00680
CV%		9.8

$*P < 0.05$.

tode throughout Zambia (B. K. Patel, Ministry of Agriculture and Water Development, pers. comm.) and the potential to underestimate at planting population levels of *P. christiei*, warrants further studies into its biology and effects on soybean yields in Zambia.

In addition to *P. christiei*, soybean cvs. Bragg, Santa Rosa, Hernon 147, and Jupiter were good hosts for *S. brachyurum*, supporting large populations. Parasitism of soybean by *S. brachyurum* has been reported previously (12), but detailed reports of its population dynamics and damage to soybean in southern Africa were lacking. Populations of *Tylenchorhynchus* sp. did not vary from those recorded at planting, indicating that soybean is not a good host for this species. Nonuniform root-knot galling on these cultivars was probably due to the severe root rot observed at 8 WAP which adversly affected development of *M. javanica*. The greater nodulation observed with 'Hernon 147' is likely a result of its ability to nodulate more efficiently with indigenous strains of *B. japonicum*.

The occurrence of *N. vasinfecta* var. *africana* infecting soybean was the first observation of this disease in the Republic of Zambia. Previous reports from other countries associated *N. vasinfecta* with a soybean stem rot (8,11), and nematodes were associated with symptoms in the field (8). The earlier papers reported browning of pith and xylem tissue which was not observed in Zambia, although stem lesions extended 2–3 cm up the stem from the soil surface. The soil pH at mid-season was 4.8, which is typical of Zambian soils. Differences in symptoms observed in Zambia and other locations may have resulted from different fungal isolates and/or nematode species or the additional stress of acidic soil conditions. Since production of soybean is increasing rapidly in southern Africa and *M. javanica* and *S. brachyurum* are distributed widely in the region, *N. vasinfecta* var. *africana* infections may be observed with increasing frequency. Additional studies are required in Zambia to determine the role of these nematode species in disease and possible interactions with *N. vasinfecta* var. *africana* on soybean.

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