

Interaction of different population levels of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *tracheiphilum* race 1 on cowpea

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Abstract

Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *tracheiphilum* race 1 was investigated in the screenhouse on cowpea cultivars Ife Brown and TVu 990 which are respectively susceptible and resistant to both pathogens. Ife Brown was infected earlier and more severely than TVu 990 by both pathogens acting either singly or in various combinations and at all concentrations tested.

When both pathogens were present together, there were decreases in fresh root and shoot weights of both TVu 990 and Ife Brown cowpea cultivars as the inocula increased. Generally, increases in fungal concentrations resulted in decrease in galling especially in the Ife Brown cowpea cultivar.

Wilting was more pronounced on Ife Brown than on TVu 990. Infection occurred on TVu 990 only at very high nematode population and fungal concentrations. TVu 990 plants did not show any external signs of wilting 42 days after inoculation, but it took just 16 days for the Ife Brown cowpea cultivar to wilt at the highest combined inocula levels. Increasing the nematode population significantly increased the possibility of *Fusarium* colonizing and producing symptoms on the resistant cowpea cultivar.

Introduction

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *tracheiphilum* race 1 (E.F. Smith) Synder and Hansen and root-knot caused by *Meloidogyne incognita* (Kofoid and White 1919) Chitwood, 1949 are two important soil-borne pathogens of cowpea in Nigeria (Oyekan, 1977; Caveness, 1979; Odunfa, 1980). The race 1 of the fungus was first reported in Nigeria by Oyekan (1975).

Studies have been made to determine the effect of co-habitation between root-knot nematode and *Fusarium* wilt of cotton (Martin *et al.*, 1956; Perry 1963; Minton and Minton, 1966; Yang, *et al.* 1976; Jorgenson, *et al.*, 1978; Garber *et al.*, 1979; Hyer *et al.*, 1979) and tomato (Jenkins and Coursen, 1957; Bowman and Bloom, 1966; Hirano, 1975; Sidhu and Webster 1977, 1981). Information on the interaction of root-knot nematode and *Fusarium* wilt on cowpea is scanty. The first and perhaps the only report on this vital relationship was given by Thomason *et al.*, (1959). They reported that when soil was infested with *Fusarium* and *M. javanica* the *Fusarium* wilt value was higher on the wilt-tolerant Grant variety than on the wilt-susceptible Chino 3 variety.

This study therefore compares the reaction of two cowpea cultivars, that are varied in resistance, to the different egg population and spore concentration levels of

M. incognita and *F. oxysporum* f. sp. *tracheiphilum* race 1 separately and together.

Materials and Methods

Nematode and fungal inocula

The culture of *M. incognita* was started with a single egg mass, and maintained in the screenhouse on cowpea cultivar TVu 43. The fungus isolate, *F. oxysporum* f. sp. *tracheiphilum* was supplied by Dr. Therberge of the International Institute of Tropical Agriculture (I.I.T.A.), Ibadan. It was grown in a liquid medium containing 10g of glucose and 50 ml of stock mineral solution consisting of (29.16g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 2.19g KNO_3 , 31.18g $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 5.7g KH_2PO_4 , 3.03g $\text{MgSO}_4 \cdot 4\text{H}_2\text{O}$ and 0.56g H_3BO_4 per litre of distilled water. After the flasks were shaken on a rotary shaker for seven days, the culture medium was filtered through four layers of cheesecloth to remove the mycelium (Yang, *et al.*, 1976). The spores were counted with a hemacytometer. Dilutions were made with sterile distilled water and final concentrations was determined. The different concentrations of fungal inoculum levels were 0, 0.5×10^6 , 1×10^6 , 10×10^6 , and 100×10^6 spores per 700g soil.

Roots of six week old cowpea plants inoculated with *M. incognita* in plastic buckets were harvested, washed, and placed in an aqueous solution of Phloxine B (0.15 g/litre of tap water) for 15-20 minutes to stain the egg masses. Stained egg masses were picked and the eggs were released using the method of Hussey and Barker (1973). The eggs were standardised by dilution method and counted under the dissecting microscope. The different nematode population levels used were 0, 0.5×10^1 , 5×10^2 , 5×10^3 and 5×10^4 eggs per 700g. soil.

Culture of Plants and Experimental procedure

Cowpea (*Vigna unguiculata* s.sp *unguiculata* (L.) Walp) cultivars used for this study were Ife Brown and TVu 990 (Iron) which are susceptible and resistant to both pathogens respectively (Amosu, 1974; Oyekan, 1977; Singh and Allen, 1979).

Seeds of Ife Brown and TVu 990 were surface sterilized by immersion in 0.5% sodium hypochlorite solution for five minutes before planting. Three surface sterilized seeds of each cultivar were planted in 500 ml plastic cups containing 700g. of steam pasteurized potting medium made up of top forest soil and sand in the ratio 2:1 (V/V). The plants were thinned one week later to two per cup.

The fungal and nematode inocula as indicated above were introduced separately and in various combinations on the two cowpea cultivars. Inoculations were made through three pieces of plastic drinking straws measuring 10 cm in length and 5 mm in diameter spaced at equal distances around the one-week old seedlings. Each treatment was replicated five times. The 250 cups were arranged in a randomised complete block design in a screenhouse. Foliage diseases and insect attack were controlled with Dithane M-45 at 0.264% a.i and Dimethoate (Rogor 40) at 0.198% a.i per litre of water respectively. The plants were sprayed with the chemical weekly throughout the duration of the experiment.

Data collection

Data recorded during and six weeks after inoculation included number of days to wilt, leaf epinasty, fresh shoot and root weights, root-knot galling, extent of colonization and vascular discolouration by *F. oxysporum* f. sp. *tracheiphilum*.

Wilt development (leaf epinasty) was rated using Yang *et al.* (1976) class rating of 0 (no wilt symptom) to 5 (plant completely wilted or dead). Six weeks after inoculation, each plant was cut at the soil level, roots were carefully removed from the soil, washed and evaluated for root-knot galling and weighted thereafter. Root-knot galling of each plant was rated using Hartman and Sasser's (1978) scale of 0 (no galls) to 5 (heavily galled). The shoots were weighed and the cut stems evaluated for discolouration through the cotyledonary nodes using Little and Hills (1978) pretransformed scale of 0-5. Data were subjected to analysis of variance, and means were separated using LSD.

Results

Effect of Fungus/Nematode pathogens singly and in combination on fresh shoot and root weights of cowpea

Fresh shoots of the resistant cowpea cultivar constantly weighed more than the susceptible cultivar at each nematode population level (Table 1). The shoot weights of TVu 990 and Ife Brown gradually declined as the nematode population levels increased from 0 to 5×10^4 eggs/700g. soil. These reductions were statistically significant ($P = 0.05$) for Ife Brown but not for TVu 990 cowpea (Table 1). In both cultivars, the root weight increased slightly as the nematode population levels increased. However, these increases were not statistically significant (Table 2).

The fresh shoot and root weights of both TVu 990 and Ife Brown cowpea cultivars decreased with increases in *Fusarium* spore concentrations. Fresh shoot and root weights of *Fusarium*-treated plants were significantly lower than those from the control ($P = 0.05$) for Ife Brown, but not TVu 990 cowpea (Tables 1 and 2). When both pathogens were present together, there were decreases in fresh shoot weights of both TVu 990 and Ife Brown cowpea cultivars as the inocula increased (Table 1). Fresh shoot weights were significantly lower under combined *Fusarium*/*Nematode* treatments than under individual pathogen treatments at $P = 0.05$ (Table 1). Fresh shoot weights of both cowpea cultivars were significantly lower at the two highest nematode and fungal populations levels than at their lower levels at $P = 0.05$ (Table 1).

The fresh root weights of both the susceptible and resistant cultivars also declined with increases in the Fungus/*Nematode* inocula levels (Table 2). At the two highest inocula levels, the roots of the susceptible Ife Brown cowpea cultivar were scanty, rotten and necrotic, whereas those of the resistant TVu 990 cowpea cultivar appeared healthy.

Effect of the Fungus/Nematode pathogens singly and in combination on the root-knot index

The root galling index of the Ife Brown cowpea cultivar increased with nematode population (Table 3). Except at the highest nematode population level where tiny

TABLE 1: EFFECT OF VARIOUS SPORE CONCENTRATIONS OF *F. OXYSPORUM* F. SP. TRACHEIPHILUM RACE 1 AND *M. INCOGNITA* EGG POPULATION LEVELS ON FRESH SHOOT WEIGHT OF IFE BROWN AND TVu 990 COWPEA CULTIVARS

<i>FURARIUM</i>	<i>FRESH SHOOT WEIGHT (G)</i> <i>NEMATODE EGGS/700 G SOIL</i>									
	0		5×10		5×10^2		5×10^3		5×10^4	
	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.
<i>Spores/700 g soil</i>	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990
0	15.21*	16.98	13.78	16.45	12.54	16.38	11.16	16.00	10.98	15.99
0.5×10^6	13.85	16.81	11.49	12.91	11.00	13.03	9.10	12.05	9.08	11.96
1×10^6	12.88	16.70	10.86	13.07	11.21	13.00	10.18	12.15	8.91	13.17
$10. \times 10^6$	11.71	16.67	10.00	12.23	9.86	11.88	8.79	10.86	1 8.05	11.04
100×10^6	11.26	16.00	10.10	12.00	8.80	11.01	6.41	10.01	1.67	10.00

LSD 0.05 = 1.004 for individual inoculum.

LSD 0.05 = 2.25 for combined inocula.

* EACH VALUE IS THE MEAN OF 10 PLANTS

TABLE 2: EFFECT OF VARIOUS SPORE CONCENTRATIONS OF *F. OXYSPORUM* F. SP. TRACHEIPHILUM RACE 1 AND *M. INCOGNITA* EGG POPULATION LEVELS ON FRESH ROOT WEIGHTS OF IFE BROWN AND TVu 990 COWPEA CULTIVARS

<i>FURARIUM</i>	<i>FRESH SHOOT WEIGHT (G)</i> <i>NEMATODE EGGS/700 (g) SOIL</i>									
	0		5×10		5×10^2		5×10^3		5×10^4	
	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.
<i>Spores/700 g Soil</i>	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990
0	3.16*	3.49	3.41	3.53	3.65	3.63	3.64	3.60	3.80	3.68
0.5×10^6	2.78	3.47	3.28	3.48	2.94	2.99	2.74	2.96	2.12	2.35
1×10^6	2.80	3.40	3.00	3.42	2.84	2.93	2.74	2.96	2.12	2.35
$10. \times 10^6$	2.50	3.36	2.53	2.81	2.51	2.62	2.09	2.93	2.00	2.10
100×10^6	2.12	3.00	2.330	2.75	2.15	2.19	1.83	2.06	0.84	1.89

LSD 0.05 = 0.27 for individual inoculum

LSD 0.05 = 0.60 for combined inocula.

Each figure is the mean of 10 observations.

galls were observed, no root galls were found on the TVu 990 cowpea cultivar (Table 3). The presence of both pathogens affected root galling on the two cowpea cultivars to varying degrees. Generally increased fungal concentration suppressed the galling especially on the Ife Brown cowpea cultivar (Table 3).

Effect of the Fungus/Nematode pathogens singly and in combination on wilt development

Data on wilt traits (leaf epinasty, vascular discolouration and days to wilt) ratings for both cowpea cultivars showed that wilt development was more rapid and severe on the susceptible Ife Brown cultivar than on the TVu 990 resistant cultivar (Tables 4, 5 & 6). Vascular discolouration, leaf epinasty and numbers of days to wilt readings of the Ife Brown cultivar were reduced by the combined pathogens at their various inocula levels ($P = 0.05$, but not the resistant TVu 990 cowpea cultivar (Tables 4, 5 and 6). Moreover, the TVu 990 plants showed no external signs of wilting 42 days after inoculation, although the susceptible Ife Brown plants wilted 16 days after inoculation under the highest inocula levels of 100×10^6 spores and 5×10^4 egg/700g. soil (Table 6).

TABLE 3: EFFECT OF VARIOUS SPORE CONCENTRATIONS OF *F. OXYSPORUM* F. SP. TRACHEIPHILUM AND *M. INCOGNITA* EGG POPULATION LEVELS ON FRESH ROOT-KNOT GALLING OF IFE BROWN AND TVu 990 COWPEA CULTIVARS

FURARIUM Spores/700 g soil	ROOT KNOT GALLING (0-5 ^b) NEMATODE EGGS/700g SOIL									
	0		5 x 10		5 x 10 ²		5 x 10 ³		5 x 10 ⁴	
	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.
	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990
0	0.0*	0.0	1.9	0.0	2.4	0.0	3.2	0.0	5.0	0.2
0.5 x 10 ⁶	0.0	0.0	1.8	0.0	3.1	0.8	3.0	0.8	4.0	1.2
1 x 10 ⁶	0.0	0.0	1.5	0.3	2.8	0.6	2.8	0.4	2.0	1.2
10. x 10 ⁶	0.0	0.0	1.3	0.4	0.6	2.8	0.5	1.5	1.15	1.4
100 x 10 ⁶	0.0	0.0	1.0	0.1	2.3	0.2	2.2	0.6	1.8	0.6

LSD 0.05 = 0.42 for individual inoculum
LSD 0.05 = 1.40 for combined inocula.

- * Each value is the mean of 10 plants.
(0-5): 0 = No gall or egg mass; 1 = 1 - 2 galls or eggs masses; 2 = 3-10; 3 = 11-30; 4 = 31-100 and over 5 over 100 galls or egg masses.

TABLE 4: EFFECT OF VARIOUS SPORE CONCENTRATIONS OF *F. OXYSPORUM* F. SP. TRACHEIPHILUM AND *M. INCOGNITA* EGG POPULATION LEVELS ON VASCULAR DISCOLOURATION OF IFE BROWN AND TVu 990 COWPEA CULTIVARS

FURARIUM Spores/700 g soil	VASCULAR DISCOLOURATION (Cotyledonary node): 0-5 (b) Nematode eggs/700g soil									
	0		5 x 10		5 x 10 ²		5 x 10 ³		5 x 10 ⁴	
	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.
	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990
0	0.0*	0.0	0.0	0.0	0.4	0.0	0.0	0.00	0.00	0.0
0.5 x 10 ⁶	1.2	0.0	2.4	0.0	2.4	0.8	2.8	0.8	2.9	0.8
1 x 10 ⁶	1.4	0.0	2.5	0.3	2.6	1.6	2.8	0.4	3.1	1.0
10. x 10 ⁶	2.0	0.0	2.2	0.3	2.6	1.0	2.6	1.0	3.5	1.2
100 x 10 ⁶	2.2	0.0	2.8	0.3	3.0	1.0	3.6	1.8	4.0	1.8

LSD 0.05 = 0.58 for individual inoculum

LSD 0.05 = 1.83 for combined inocula.

* Each figure is the mean of 10 plants

(b) 0-5 = Extent of vascular discolouration seen in cross section through the cotyledonary node, scored on a scale pre-transformed to arc sine where 0=0, 1=10, 2=35, 3=65, 4=90 and 5=100%.

TABLE 5: EFFECT OF VARIOUS SPORE CONCENTRATIONS OF *F. OXYSPORUM* F. SP. TRACHEIPHILUM AND *M. INCOGNITA* EGG POPULATION LEVELS ON LEAF EPINASTY OF IFE BROWN AND TVu 990 COWPEA CULTIVARS

FURARIUM Spores/700 g soil	LEAF EPINASTY (0-5 ^b) NEMATODE EGGS/700g SOIL									
	0		5 x 10		5 x 10 ²		5 x 10 ³		5 x 10 ⁴	
	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.
	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990
0	0.0*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5 x 10 ⁶	1.2	0.0	1.6	0.0	1.8	1.0	1.8	0.8	2.6	0.0
1 x 10 ⁶	1.4	0.0	2.0	0.2	1.7	1.0	2.0	0.4	3.2	0.62
10. x 10 ⁶	1.6	0.0	2.0	0.2	1.8	1.2	2.2	0.8	4.85	1.0
100 x 10 ⁶	2.0	0.0	2.0	0.4	2.3	1.2	2.3	1.0	4.8	1.0

LSD 0.05 = 0.59 for individual inoculum

LSD 0.05 = 1.87 for combined inocula.

* Each figure is the mean of 10 plants

(b) (0-5) 0 = no wilt symptom; 1 = trace with 1 or 2 leaves showing epinasty; 2 = half of the leaves with epinasty and beginning to yellow; 3 = three-quarters of the leaves on plants with epinasty and yellowing portion of the leaf beginning to dry; 4 = All the leaves showing epinasty, lower beginning to fall off; 5 = plants dead.

TABLE 6: EFFECT OF VARIOUS SPORE CONCENTRATIONS OF *F. OXYSPORUM TRACHEIPHILUM* AND *M. INCOGNITA* EGG POPULATION LEVELS ON WILT DEVELOPMENT 42 DAYS AFTER INOCULATION

FURARIUM Spores/700 g soil	NUMBER OF DAYS TO WILT) NEMATODE EGGS/700g OF SOIL									
	0		5 x 10		5 x 10 ²		5 x 10 ³		5 x 10 ⁴	
	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.
	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990	Ife Brown	TVu 990
0	NW	NW**	NW	NW	NW	NW	NW	NW	NW	NW
0.5 x 10 ⁶	40*	NW	40	NW	39	NW	29	NW	29	NW
1 x 10 ⁶	38	NW	34	NW	35	NW	30	NW	24	NW
10. x 10 ⁶	35	NW	29	NW	27	NW	19	NW	19	NW
100 x 10 ⁶	30	NW	28	NW	25	NW	20	NW	16	NW

LSD 0.05 = 3 for individual inoculum

LSD 0.05 = 5 for combined inocula.

* Each value is the mean of 10 plants.

** NW = No plant wilted 42 days after inoculation

Discussion

Evidences presented above show that the nematode or the fungus, when inoculated individually, was able to cause appreciable damage to the susceptible cowpea cultivar, Ife Brown, but not the resistant TVu 990 cultivar. The highest galling index was on roots inoculated with nematode alone. This result could be attributed to lack of competition pressure in the sole nematode treatment. This results in a favourable environment for nematode multiplication without interference from the fungus. But when both pathogens were inoculated together, increased damage due to the pathogens was observed in both Ife Brown and TVu 990 cowpea cultivars indicating that *F. oxysporum* f. sp. *tracheiphilum* and *M. incognita* have an interacting influence on Fusarium wilt in cowpea, especially at high concentrations of the pathogens. This result was similar to those reported for *Fusarium* wilt fungus and root-knot nematode interaction on other crops such as cotton (Martin *et al.*, 1956; Garber *et al.*, 1979) tobacco (Powell, 1963, 1971) tomato (Hirano, 1975; Sidhu and Webster, 1977, 1981) and chickpea (Mani and Sethi, 1987). With combined inocula, synergistic interaction was observed for vascular discolouration, leaf epinasty, days to wilt and fresh shoot weight while antagonistic interaction was observed for root-knot galling. At high nematode and fungus population levels, there were significant reductions in fresh shoot and root weights. Galling indices were also reduced on the susceptible cultivar, Ife Brown. The reduction in root weights and galling indices could have resulted from root necrosis and rotting which were very pronounced at very high nematode and fungus population levels and especially on Ife Brown plants. Decayed and necrotic tissue are usually unfavourable for nematode development. Root injury in form of destruction and blockage of the transport vessels by these pathogens could also

account for reduced foliage growth.

In the resistant cowpea cultivar TVu 990, no significant difference was observed between the control and the pathogen-treated plants for all the wilt traits measured. It had been stated that root-knot nematode infections might not always result in break down of resistance to *Fusarium* wilt (Hirano, 1975; Jones *et al.*, 1976; Beckman, 1987). However, this study shows that at very high nematode population levels and *Fusarium* concentrations, there was pressure that tended to lead to some infections on TVu 990. This indicates that TVu 990 cultivar is not immune to the fusarium pathogen but only moderately resistant (Oyekan, 1977). In order to reduce the prevalence of root-knot and fusarium, it will be advisable to plant cowpea cultivars resistant to both pathogens such as TVu 990. Since TVu 990 is not a commercially acceptable cultivar in Nigeria, it could be crossed with commercially acceptable cultivars susceptible to both *Fusarium* and root-knot nematode such as Ife Brown, Mala, Kwara, Local White and Local Brown.

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