Ife Journal of Agriculture, Vol. 12, Nos. 1 & 2 1990

The effect of time and sequence of introduction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp *tracheiphilum* race 1 on four cowpea cultivars.

B.N. OKOLE and J.O. AMOSU
Department of Plant Science
Obafemi Awolowo University
Ile-Ife, Nigeria.

Abstract

The effect of time and sequence of introduction of *Fusarium oxysporum* f. sp. *tracheiphilum* race 1 and *Meloidogyne incognita* was studied on four cowpea cultivars showing different levels of resistance to either or both pathogens. Cowpea cultivars used were Ife Brown which is susceptible to both pathogens, TVu 990 which is resistant to both pathogens, TVu 1560 which is resistant to the nematode and susceptible to the fungus and TVu 347 which is resistant to the fungus and susceptible to the nematode. When the plants were inoculated with the fungus one week before the nematode, and simultaneously two weeks after planting, there were significant reductions ($P = 0.05$) in fresh shoot and root weights of cowpea cultivars, Ife Brown and TVu 347. For TVu 347 the lowest fresh shoot and root weights were recorded when both pathogens were inoculated one and two weeks after planting. But the lowest fresh shoot and root weights were recorded for TVu 1560 when the plants were inoculated with fungus one week before the nematode. Time and sequence of introduction of the nematode and fungus on TVu 990 had no significant difference from the control. The highest gall indices on the roots occurred on Ife Brown and TVu 347 when the nematode was inoculated two weeks before the fungus. TVu 1560 and TVu 990 roots had small galls in all the timing and sequences used. Vascular discolouration and leaf epinasty ratings were significantly higher ($P = 0.05$) than the control for cowpea cultivars, Ife Brown, TVu 1560 and TVu 347 when the nematode was inoculated two weeks before the fungus while TVu 990 had no significant difference from the control for the two traits measured. These studies revealed that the nematodes have to enter and become established in the root of resistant cowpea cultivars for certain periods of time before wilt symptoms become evident.

Introduction

The root-knot nematode-fusarium wilt disease complex caused by the organisms *Fusarium oxysporum* f. sp. *tracheiphilum* (E.F. Smith) Synder and Hansen and *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 has been known to be a serious disease of cowpea (Thomason, et al., 1959; Okole and Amosu, 1990). Studies involving root-knot nematode and Fusarium wilt interactions in other crops such as cotton, tobatto and tomato have shown that nematode infection enchances subsequent disease development. Mechanical injuries resulting from nematode larval penetration has been reported to furnish avenues of ingress for fungus pathogen (Armstrong and Armstrong, 1958). By varying the time intervals between inoculation of tobacco plant with nematode and the fungus pathogen, Porter and Powell (1967) were able to show the role played by the root-knot nematodes in such

complexes. When inoculation of *M. incognita* preceeded *F. oxysporum* f. sp. *nicotianae* or any of the following species of soil inhabiting fungi, *Pythium, Curvularia, Botrytis, Aspergillus Penicullium* and *Trichoderma* by several weeks, wilt or necrosis were more severe than in Plants inoculated simultaneously with both fungus and nematode (Porter and Powell, 1967; Powell, 1971).

There is paucity of information on the effect of sequence timing of infestation on Fusarium wilt-root-knot nematode interaction in cowpea (Thomason, *et al.*, 1959). The present study was designed to determine the effect of sequence and timing of the introduction of fusarium and root-knot nematode on wilt development in cowpea.

Materials and Methods

Sources of material and preparation of inocula

Cultivars of cowpea, *Vigna unguiculata* s. sp. *unguiculata* (L.) Walp, used were Ife Brown, susceptible to both pathogens; TVu 990 resistant to both pathogens; TVu 1560, resistant to the nematode but susceptible to the fungus and TVu 347 resistant to the fungus but susceptible to the nematode (Amosu, 1974; Oyekan, 1977; Singh and Allen, 1979). 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 50ml of stock mineral solution consisting of (29.16g $Ca(NO_3)_2.4H_2O$, 2.19g KNO_3, 31.18g $Mg(NO_3)_2.6H_2O$, 5.75g KH_2PO_4, 3.03g $MgSO_4.4H_2O$ 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 distilled water and final concentration were determined.

Roots of six-weeks old cowpea plants inoculated with *M. incognita* in plastic buckets were harvested, washed and placed in an aqueous solution of Phloxine B (0.15g/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 standardized by dilution method and counted under the dissecting microscope.

Experimental procedure

A randomised complete block design in a factorial arrangement was used for the experiment. There were two factors. Factor A comprised of four cowpea cultivars: Ife Brown, TVu 990, TVu 1560 and TVu 347. Factor B comprised the seven sequences and timing of introduction of pathogens to the host. These were (i) Nematode inoculation one week after the fungus (FIN), (ii) Nematode inoculation two weeks after the fungus (F2N), (iii) Fusarium inoculation one week after the nematode (NIF), (iv) Fusarium inoculation two weeks after the nematode (N2F), (v) Simultaneous inoculation one week after planting (1N + F), (vi) Simultaneous inoculation two weeks after planting (2N + F) and (vii) control. For the control, only sterile distilled water was added to each of the cultivars at the different sequences

and timing of inoculations. Nematode population used was 5×10^3 eggs 1700g. soil while the fungal spore concentration was 0.5×10^6 spores/700g soil. Inoculations were made through three pieces of plastic drinking straws. In cases where the second pathogen was to be introduced, the straws were left until the second inoculation (Okole and Amosu, 1990). Each treatment was replicated five times. The 140 cups were arranged in a factorial manner on a bench in the screenhouse.

Data Collection

Eight weeks after planting, plants were carefully removed from the soil to minimise root damage. Roots were washed and rated for disease severity. The roots and shoots were weighed separately to determine the fresh weight. Other traits determined eight weeks after planting were root-knot galling using Hartman and Sasser's (1978) rating of 0-5, degree of leaf epinasty using Yang, *et al.*, (1976) classification of 0-5, and vascular discolouration estimated visually through the cotyledonary nodes using Little and Hills (1978) pretransformed scale of 0-5. The data were subjected to analysis of variance and means were separated using LSD.

Results

Effect on fresh shoot and root weights

The data from the fresh shoot and root weights of the four cowpea cultivars showed that some sequence and timing treatments had significant effects ($P = 0.05$) on cowpea cultivars Ife Brown, TVu 1560 and TVu 347 but not on TVu 990 (Table 1). When the fungus was inoculated one week before the nematode (F1N) and when both inocula were present simultaneously two weeks after planting (2N + F) fresh shoot and root weights of the susceptible cowpea cultivar Ife Brown were significantly lower than those of the control at $P = 0.05$ (Table 1). There was no significant difference in the fresh shoot and root weights with the different sequence and timing treatments for the resistant cowpea cultivar TVu 990 at $P = 0.05$ (Table 1) TVu 1560 had its lowest fresh shoot and root weights when the fungus was inoculated one week before the nematode (F1N), (Table 1). TVu 347, which is resistant to the fungus and susceptible to the nematode had the lowest fresh shoot and root weights when both pathogens were inoculated one and two weeks after planting (1N + F and 2N + F), (Table 1).

Effect on root-knot galling

The data on root-knot galling showed that highest root gall indices were observed for root-knot susceptible Ife Brown and TVu 347 cowpea cultivars when the nematode was inoculated two weeks before the fungus (N2F) (Table 2). Galls were more pronounced and heavy on roots of Ife Brown cowpea than on the other three cowpea cultivars. Galling indices on TVu 990 and TVu 1560 were tiny and were not significantly ($P = 0.05$) different from the control plants (Table 2).

Effect on wilt development traits

Generally, wilt was more severe on Ife Brown than on the other three cowpea cultivars. To a greater extent, Ife Brown exhibited symptoms of all the traits

TABLE 1: EFFECTS OF SEQUENCE AND TIMING OF INTRODUCTION OF *MELOIDOGYNE INCOGNITA* AND *FUSARIUM OXYSPORUM* f. sp. *TRACHEIPHILUM* ON FRESH SHOOT AND ROOT WEIGHTS OF FOUR COWPEA CULTIVARS

Time and sequence of inoculation	*IFE BROWN Susceptible to nematode and fungus*		*TVu 990 Resistant to nametode and fungus*		*TVu 1560 Resistant to nematode and susceptible to fungus*		*TVu 347 Resistant to fungus and susceptible to nematode*	
	Fresh Shoot Wt. (g)	*Fresh Shoot Wt. (g)*	*Fresh Shoot Wt. (g)*	*Fresh Shoot Wt. (g)*	*Fresh Shoot Wt. (g)*	*Fresh Shoot Wt. (g)*	*Fresh Shoot Wt. (g)*	*Fresh Shoot Wt. (g)*
F1N	0.44*	0.28	11.41	3.21	4.56	1.01	9.49	2.37
F2N	4.42	1.76	13.00	3.46	8.45	2.04	8.38	2.64
N1F	4.38	1.81	11.60	3.18	7.76	1.85	8.60	2.40
1 N+F	4.48	2.50	12.00	3.21	8.90	1.70	5.47	1.82
2 N+F	0.49	0.31	12.96	3.46	9.86	2.75	4.31	0.95
Control	11.30	2.69	13.17	3.59	10.78	2.47	12.94	2.98
LSD (0.05)	1.76	0.61	1.76	0.61	1.76	0.61	1.76	0.61

* Each value is the mean of ten plants.

TABLE 2: EFFECTS OF SEQUENCE AND TIMING OF THE INTRODUCTION OF *MELOIDOGYNE INCOGNITA* AND *FUSARIUM OXYSPORUM* f. sp. *TRACHEIPHILUM* ON ROOT KNOT GALLING OF FOUR COWPEA CULTIVARS

Time and sequence of inoculation	Root-knot galling (a)			
	Cowpea cultivars			
	IFE BROWN	TVu 990	TVu 1560	TVu 347
F1N	3.7*	0.2	0.7	0.3
F2N	3.0	0.2	0.8	0.7
N1F	4.0	0.2	0.9	2.6
N2F	4.8	0.4	0.8	2.8
1 N + F	3.6	0.2	0.6	1.8
2 N + F	3.8	0.3	0.8	2.2
Control	0.0	0.0	0.0	0.0
LSD = 0.05	1.01	1.01	1.01	1.01

*Each value is the mean of ten plants

(a) 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 5 = over 100 galls or egg masses.

TABLE 3: EFFECTS OF SEQUENCE AND TIMING OF THE INTRODUCTION OF *MELOIDOGYNE INCOGNITA* AND *FUSARIUM OXYSPORUM* f. sp. *TRACHEIPHILUM* ON ROOT KNOT GALLING OF FOUR COWPEA CULTIVARS

Time and sequence of inoculation	*IFE BROWN Susceptible to nematode and fungus*			*TVu 990 Resistant to nematode and fungus*			*TVu 1560 Resistant to nematode and susceptible to fungus*			*TVu 347 Resistant to fungus and susceptible to nematode*		
	VD[1] (0-5)[a]	*LP[2] (0-5)[b]*	*DW[3]*	*VD (0-5)*	*LP (0-5)*	*DW*	*VD (0-5)*	*LP (O-5)*	*DW*	*VD (0-5)*	*LP (0-5)*	*DW*
F1N	4.00*	4.60	21	0.20	0.10	NW[4]	2.40	2.40	31	0.80	0.40	NW
F2N	3.80	3.50	29	0.10	0.00	NW	2.20	2.20	35	1.00	0.80	NW
N1F	4.00	4.20	22	0.20	0.00	NW	2.80	3.00	32	1.80	2.20	38
N2F	5.00	4.80	18	0.40	0.20	NW	3.40	3.80	29	3.80	3.20	38
1 N + F	4.00	4.00	29	0.00	0.00	NW	2.60	2.20	34	1.60	1.20	41
2 N + F	4.20	4.60	24	0.20	0.00	NW	3.00	3.50	32	3.40	2.40	39
Control	0.00	0.00	NW	0.00	0.00	NW	0.00	0.00	NW	0.00	0.00	NW
LSD = 0.05	1.39*	1.33	2.00	1.39	1.33	2.00	1.39	1.33	2.00	1.39	1.33	2.00

[1]VD = Vascular discolouration; [2]LP = Leaf Epinasty; [3]DW = Days to wilt; [4]NW = No plant wilted 42 days after planting.

*Each value is the mean of ten plants.

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

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

measured earlier than on the other three cowpea cultivars. Data for vascular discolouration and leaf epinasty for all treatments were significantly higher than the control ($P = 0.05$). It took 18 days for wilt symptoms to appear when the nematode was inoculated two weeks before the fungus (N2F), (Table 3). The different time and sequence treatments were not significantly different from the control for the resistant cultivar TVu 990 for all the three traits measured (Table 3). Wilt traits data for TVu 1560 which is resistant to *M. incognita* showed no significant difference with the treatments for both vascular discolouration and leaf epinasty. However, where plants were inoculated with the nematode two weeks before the fungus (N2F) leaf epinasty and vascular discolouration readings were highest and plants wilted 29 days after inoculation (Table 3) TVu 347 which is resistant to Fusarium wilt had moderate to severe ratings of vascular discolouration and leaf epinasty and wilted on the 38th day where nematodes were inoculated one and two weeks before the fungus (Table 3). Also where nematode and fungus were inoculated simultaneously one and two weeks after planting, severe ratings for vascular discolouration and leaf epinasty were recorded and the plants wilted between 39 and 41 days after inoculation (Table 3).

Discussion

When the fungus was inoculated one week before the nematode (F1N) fresh shoot and root weights of Fusarium susceptible cultivars, Ife Brown and TVu 1560, were significantly lower than the control. These results are different from those reported by Mani and Sethi (1987) on chickpea. They reported that the treatments receiving nematode inoculum prior to either of the two fungi used recorded higher reduction in growth parameters than the treatments receiving either of the fungi prior to the nematode inoculation. However, they did not state whether or not the chickpea cultivars used were susceptible or resistant to the pathogens.

Root-knot galling indices were highest in situations when the nematode preceeded the fungus by two weeks for cowpea cultivars Ife Brown and TVu 347. Wilt development was also highest on cowpea cultivars Ife Brown, TVu 1560 and TVu 347 when the nematode was inoculated two weeks before the fungus (N2F). These results are similar to those reported on tobacco (Porter and Powell, 1967), cotton (Melakeberhan and Evans, 1981) and chickpea (Mani and Sethi, 1987). The results show that plants were maximally predisposed to Fusarium wilt by *M. incognita* only after the nematodes had been in contact with the plants for two or more weeks.

TVu 347 which is resistant to Fusarium wilt was attacked by the fungus in the presence of the nematode and wilted on the 38th day. In breaking resistance to Fusarium wilt, it is possible that the nematode has a double role, not only that of providing entry for the fungus, but also of causing some host response that lowers the natural resistance. Jenkins and Coursen (1957) had also shown that mechanical wounding of the roots of the wilt-resistant tomato variety Rutgers did not increase the incidence of Fusarium wilt, but that *M. hapla* or *M. incognita acrita* on the roots increased the severity of Fusarium wilt.

With cowpea cultivar TVu 990, which is resistant to both pathogens, it was observed that there was no significant difference from the control plants for all the traits measured. No plants of the TVu 990 cultivar wilted as was reported in TVu 347

plants which are resistant only to the fungus. Therefore, the most practical long-range root-knot-fusarium disease complex control programme should be based on the use of resistant cultivars. However, only cultivars resistant to root-knot and Fusarium wilt can withstand both pathogens, thus reducing losses from complexes that may exist between them.

Difference in wilt development between the treatments in nematode resistant but fungus susceptible cultivar TVu 1560 and fungus resistant but nematode susceptible TVu 347 are significant. Wilt traits were more severe in TVu 1560 than in TVu 347 especially where nematodes were added before the fungus inoculation. The mechanism by which root-knot nematodes increase the incidence of Fusarium wilt may be by causing root injuries resulting from larval penetration which provide portals of entry for fungi (Gill, 1958; Armstrong and Armstrong, 1958) or by morphological changes (Fattah and Webster 1983) or by physiological alterations (Sidhu and Webster 1977) in the plants. Some or all of the processes stated may play some role in predisposing plants to *Fusarium* wilt development. These aspects need to be carefully investigated.

References

Amosu, J.O. 1974. The reaction of cowpea *(Vigna unguiculata* (L.) Walp) to root-knot nematode *(Meloidogyne incognita)* in Western Nigeria. *Niger. Agric. J. 11:* 165-169.

Armstrong, G.M. and Armstrong, J.K. 1958. Effect of cutting roots on the incidence of Fusarium wilt of cotton, tomatoes, cowpeas and other plants. *Phytopathology 48:* 347 Abstr.

Fattah, F.M. and Webster, J.M. 1983. Ultrastructural changes caused by *Fusarium oxysporum* f. sp. *lycopersici* in *Meloidogyne javanica* induced giant cells in *Fusarium* resistant and susceptible tomato cultivars. *J. Nematol 15:* 128-135.

Gill, D.L. 1958. Effect of root-knot nematodes on *Fusarium* wilt on mimosa. *Plant Dis. Reptr. 42:* 587-590.

Hartman, K.M. and Sasser, J.N. 1978. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. pp. 67-77. *In* Barker K.R., Carter C.C. and Sasser, J.N. (Eds.) *An Advanced treatise on Meloidogyne* Vol. II. *Methodology.* North Carolina State University, Raleigh, 223p.

Hussey, R.S. and Barker, K.R. 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. *Plant Dis. Reptr. 57:* 1025-1028.

Jenkins, W.R. and Coursen, B.W. 1957. The effect of root-knot nematodes, *Meloidogyne incognita acrita* and *M. hapla* on Fusaium wilt of tomato. *Plant Dis. Reptr. 41:* 182-186.

Little, T.M. and Hills, F.J. 1978. *Agricultural Experimentation, Design and Analysis.* John Wiley and Sons, New York, 350pp.

Mani, A. and Sethi, C.L. 1987. Interaction of root-knot nematode, *Meloidogyne incognita* with *Fusarium oxysporum* f. sp. *ciceri* and *F. solani* on chickpea. *Indian J. Nematol 17:* 1-6.

Melakeberhan, H. and Evans, A.F. 1981. Interaction of root-knot nematode and vascular wilt fungi in selected Tanzanian cotton varieties. *J. Nematol 13:* 450 Abstr.

Okole, B.N. and Amosu, J.O. 1990. Interaction of different population levels of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *tracheiphilum* race 1 on cowpea. *Ife J. Agric.* (In Press)

Oyekan, P:O. 1977. Reaction of some cowpea varieties to *Fusarium oxysporum* f. sp. *tracheiphilum* in Nigeria. *Tropical Grain Legume Bulletin 8:* 47-49.

Porter, D.M. and Powell, N.T. 1967. Influence of certain *Meloidogyne* species on *Fusarium* wilt development in flue cured tobacco. *Phytopathology 57:* 282-285.

Powell, N.T. 1971. Interactions between nematodes and fungi in disease complexes. *Ann. Rev. Phytopath. 9:* 253-274.

Sidhu, G. and Webster, J.M. 1977. Predisposition of tomato to the wilt fungus *(Fusarium oxysporum lycopersici)* by root-knot nematode *(Meloidogyne incognita). Nematologica 23:* 436-442.

Singh, S.R. and Allen D.J. 1979. *Cowpea pests and diseases.* I.I.T.A. Ibadan, Manual Series No. 2, 113p.

Thomason, I.J., Erwin, D.C. and Garber, M.J. 1959. The relationship of root-knot nematode *Meloidogyne javanica* to *Fusarium* wilt of cowpea. *Phytopathology 49:* 602-606.

Yang, H., Powell, N.T. and Barker, K.R. 1976. Interactions of concomitant species of nematodes and *Fusarium oxysporum* f. sp. *vasinfectum* on cotton. *J. Nematol 8:* 74-80.