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Role of *Pratylenchus sudanensis*, A Root-Lesion Nematode in the Syndrome of Cotton Wilt in Gezira Area of Sudan

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Abstract: Interaction between root-lesion nematode *Pratylenchus sudanensis* and the fungus *Fusarium oxysporum* f.sp. *vasinfectum* on cotton was studied under controlled glass-house conditions. The growth of the plants was reduced significantly when plants were inoculated with both pathogens after sequential and simultaneous treatment. Reduction in length and weight of shoot and root was maximum in simultaneous inoculations with Barakat cotton cultivar. The rate of *P. sudanensis* multiplication was adversely affected when fungus was inoculated earlier than nematode. Although the three tested *Gossypium barbadense* cotton cultivars are differed in their susceptibility to nematode attack, they invariably showed faster infection and the number of wilted plants was greater when the fungal infection occurred in the presence of the nematode. Such differences were most apparent with fungal isolates obtained from Gezira Research Station Farm on Barakat cultivar as compared to GS (81) and Giza (70) cotton cultivars.

Key words: *Pratylenchus sudanensis*, *Fusarium oxysporum* f.sp. *vasinfectum*, root-lesion development, interaction, cotton, Gezira, Sudan

INTRODUCTION

A number of nematode-fungus disease complexes have been recognized and discussed by several authors (Powell, 1963, 1971; Fielding, 1969; Bergeson, 1972; Back *et al.*, 2002; Abd-El-Alim *et al.*, 1999). The association between *Pratylenchus* sp. and other organisms was probably first noted by Zimmermann, Cobb and Steiner and others (Taylor, 1990). The first detailed information, however, was that of Godfrey (1929) who observed unidentified fungi and bacteria in lesions produced by *Pratylenchus brachyurus*. Hilderbrand and Koch (1936) also emphasized the importance of the genus *Pratylenchus* in the root-rot complex of cotton and strawberry in Canada. The mechanism of such association has not yet been fully elucidated and seems to be quite complex. However, in certain instances, when root-lesion nematodes produced large lesions in the plant root, other organisms seemed to have a better access, at least physically (Kerr, 1956; Seinhorst, 1961). This mechanism may not always happen, however, as Agu and Ogbuji (2000) pointed out. Such interaction between nematodes and other organisms are fortuitous but there may be a closer relationship between a nematode species and a fungus or bacterium (Francl and Wheeler, 1993).

The role of the Sudanese root-lesion nematode *Pratylenchus sudanensis* (Loof and Yassin, 1970) in the syndrome of cotton wilt caused by *Fusarium oxysporum* f.sp. *vasinfectum* was investigated early by Yassin and El-Nur (1969) and Yassin (1974). Their preliminary reports showed that *Fusarium* wilt occurred earlier and with a higher percentage of wilted plants in the presence of the nematode. Yassin (1974) also added that both fungus and nematode ramified better when they coexisted. Recently, there is a severe infection with *Fusarium oxysporum* f.sp. *vasinfectum* in cotton plants and always associated with the presence of the root-lesion nematode *Pratylenchus sudanensis* and was still not clear whether there was some other causal relationship between the two pathogens in the disease syndrome. Therefore, with this aim in view, it was considered desirable to study the effect of *Fusarium oxysporum* f.sp. *vasinfectum* on the host infection, nematode population density, root-lesion formation and their combined effects on plant growth parameters.

MATERIALS AND METHODS

Glass-house experiments were conducted at the Gezira Research Station (GRS), Sudan, during 2003-2004. A susceptible cotton, *Gossypium barbadense* L. cv. Barakat was included in all tests.Other cultivars, namely

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Table 1: Inoculation of cotton plants (cv.Barakat) with *Pratylenchus sudanensis* and *Fusarium oxysporum* f.sp. *vasinfectum* separately and together and effects on growth of plants and wilt development*

	Length (cm)			Weight (g)			
Inoculation schedule	Shoot	Root	Total	Shoot	Root	Total	Nematode population in soil and root
Nematode alone (N)	50.00	20.30	70.30 (24.17)	100.10	75.12	175.22 (26.55)	10575
Fungus alone (F)	41.22	15.10	56.32 (78.20)	76.80	51.60	128.40 (80.00)	-
N+F simultaneously	22.40	11.13	33.53 (86.12)	46.11	21.00	67.11 (87.00)	7860
N 7 days prior to F	20.00	9.00	29.00 (35.00)	40.00	20.00	60.00 (84.00)	6330
F 7 days prior to N	21.60	10.00	31.60 (29.00)	41.00	22.20	63.20 (76.00)	4200
No pathogen (control)	55.20	28.46	83.66	120.30	100.30	220.60	-
C.D (p = 0.05)			3.362			2.990	
C.D (p = 0.01)			4.500			3.111	

*Values are means of three replicates. In parenthesis is given the percent reduction over control

GS (81) and Giza (70) of *G.barbadense* and Barac (67) B of *Gossypium hirsutum* were also included for comparison. Seeds of these cultivars were surface-sterilized by treatment for 5 min with 0.1% mercuric chloride solution in water, followed by several rinsing in sterilized distilled water. The sterilized seeds were then planted directly in sterilized sand and river silt (1:1, v/v) in 3-inch clay pots. Immediately after planting, the pots were inoculated either with 100 nematodes (composed of a mixture of males, females and larval stages) 7 day prior to fungus inoculation, or with fungus 7 day prior to nematode inoculation. Individuals of nematodes were obtained from the stock cultures maintained on cotton plants in large containers containing naturally infested field soil.

Four different isolates of *Fusarium oxysporum* f.sp. *vasinfectum* were used. These were obtained from the roots of susceptible cotton cv. Barakat grown at GRS (Plot 1 and 82), Gamousi and Sidera areas . The isolates were prepared in pure cultures, using Potato Dextrose Agar medium (PDA). Prior to inoculations, a one- to two-week-old culture of each isolate was prepared in suspension in tap water and the inoculum density was assessed (50-80 thousands chlamydospores/cc). Aliquots of 15 cc of the spore suspension were then poured onto each of the test pots.

For interaction studies, pots were inoculated with both organisms simultaneously or as the case may be according to the scheme shown in Table 1-4. All treatments were arranged in complete randomized block design with 3 replicates in each treatment and un-inoculated pots served as control. The experiments were terminated after 75 days and the plants were up-rooted and gently washed. Cobb's sieving and decanting method followed by Baermann's funnel technique (Southy, 1970) was used for nematode recovery from soil. Length and fresh weight of shoot and root were measured. Other observations were also undertaken to monitor the progress of wilt in the same manner as reported by Yassin and Dafalla (1982).

Table 2: Interaction between *Fusarium oxysporum* f.sp. *vasinfectum* and *Pratylenchus sudanensis* in cotton cv.Barakat*

Weeks after inoculation	N alone	F alone	N+F	None (control)
1	0/42	0/47	2/45	0/46
2	0/42	8/47	13/45	0/46
3	0/42	11/47	15/45	0/46
4	0/42	12/47	16/45	0/46

* Values refer to mean number of plants showing typical symptoms of vascular wilt/total;N= Nematode;F= Fungus; N+F= Nematode plus Fungus

Table 3: Interaction between *Fusarium oxysporum* f.sp. *vasinfectum* isolates and *Pratylenchus sudanensis* in cotton(*Gossypium barbedense*) cv.Barakat and cv.GS(81)

	Plants with vascular wilt symptoms(%)			
	cv. Barakat		cv. GS(81)	
Fusarium isolate(area)	Fungus alone	Fungus+ nematode	Fungus alone	Fungus+ nematode
Gamousi	0	5.5	2.7	5.5
GRSF (Plot 1 and 82 mixture)	34	39.5	12.5	22.8
Sidera	2.3	13.5	3.3	10
Control	0	0	0	0

*Values are means of three replicates

Table 4: Interaction between *Fusarium oxysporum* f.sp. *vasinfectum* and *Pratylenchus sudanensis* in three cotton cultivars*

	Plants with vascular wilt symptoms (%)					
	Barakat		Barac (67) B		Giza (70)	
Fusarium isolate	Fungus alone	Fungus+ nematode	Fungus alone	Fungus+ nematode	Fungus alone	Fungus+ nematode
GRSF (Plot 1)	28.0	46.8**	0	0	0	3.9
GRSF (Plot 82)	26.9	35.0**	0	0**	1.8	6.1

*Values are means of three replicates, **Barakat and Giza (70) are *Gossypium barbedense* cultivars;Barac (67) B is a *Gossypium hirsutum* cultivar, ***Root necrosis

RESULTS

Perusal of the data presented in Table 1 reveals that both *P. sudanensis* and *F. oxysporum* f.sp. *vasinfectum* caused significant (p = 0.01) damage to cotton cv. Barakat. The fungus infection caused more reduction in the length and weight of shoot and root of the plants than

Fig. 1: A typical symptoms of vein-clearing due to the fungus *Fusarium oxysporum* f.sp. *vasinfectum*, in cotton cv. Barakat, some 15-20 days following inoculation (right); More advanced symptoms showing the blackening of the apical part of the plant, some 4 weeks following inoculation (left)

the nematode. Sequential inoculation of both the pathogens, irrespective of time interval, caused much plant reduction than by either pathogens. Inoculation with root-lesion nematode followed by *F. oxysporum* f.sp. *vasinfectum* 7 days later caused significant ($p = 0.01$) damage than inoculation with *F. oxysporum* f.sp. *vasinfectum* followed by root-lesion nematode 7 days later. However, the maximum reduction in plant length and weight was recorded when *P. sudanensis* and *F. oxysporum* f.sp. *vasinfectum* were inoculated simultaneously (Table 1-4). All of the *G.barbadense* cultivars showed a typical symptoms of *Fusarium* wilt attack (Fig. 1) and the degree of infection varied with the cultivar used. The percent infection was 46.8 in Barakat cultivar as compared to 22.8 and 3.9 in GS (81) and Giza (70) cultivars, respectively (Table 3 and 4). *G. hirsutum* cv. Barac (67) B showed no symptoms of *Fusarium* infection (Table 4). Although the three tested *G.barbadense* cotton cultivars were differed in their susceptibility to nematode attack, they invariably showed greater and faster infection and the number of wilted plants was higher when the fungal infection occurred in the presence of the nematode. Such differences were most apparent with fungal isolates obtained from the Gezira Research Station Farm (GRSF) on Barakat cultivar (Table 4) and of the four *Fusarium* isolates, the GRSF cultivar group was the most infective.

The multiplication of *P. sudanensis* nematode and root-lesion formation in plants were greater when nematode inoculated singly and further reduced significantly in all treatments whenever the fungus was present. The minimum nematode population in soil and roots was found to be 4200 nematodes and the poorest root-lesion formation judged by percent reduction (76%) in length of the shoot and weight of the roots have been found where fungus was inoculated earlier to nematode. The number of nematode population in soil and roots was built-up rapidly (10575 nematodes) and root-lesion formation was recorded distinctly in case where nematode was applied singly (Table 1).

DISCUSSION

The results obtained in the present investigation suggested that the *Fusarium* wilt of cotton plants was more pronounced wherever P. *sudanensis* was inoculated in combination with *F. oxysporum* f.sp. *vasinfectum* resulting in poor growth of plants (Table 1).The intensity of disease was maximum when nematode and fungus were inoculated simultaneously. Our results also confirm the early finding of Yassin (1974) who worked with *P. sudanensis* from the same locality using the same Barakat cotton cultivar. *P. sudanensis* like many other plant-parasitic nematodes, e.g., root-knot in association with vascular wilt of chilli (Zaidi and Tiyagi, 1989) and other *Pratylenchus* species in association with black shank of tobacco (Inagaki and Powell, 1969) seems to pave the way for fungus invasion. Such a mechanism is not purely mechanical since the immunity of cotton cultivar Barac (67) B to *Fusarium* was not broken in the presence of *P. sudanensis*, even though necrotic lesions developed on the roots of this immune variety. In the present study, nematode inoculated 7 days prior to fungus and fungus 7 days prior to nematode did not cause much reduction in plant growth in comparison to the plants inoculated simultaneously. These findings are in close agreement with the results obtained by other workers the w orld over (Francl and Wheeler, 1993;

Back *et al*., 2002). Other workers in their studies demonstrated that the infection by root-lesion nematodes increased the incidence or severity of *Fusarium* wilt on susceptible cotton cultivars. However, those previously published results were not confirmed in the other plant-nematode-fungus combination results (Hutton *et al*., 1973). Thus, it appears that modification of *Fusarium* wilt incidence or severity may be related to the specific nematode-fungus combination. Furthermore, these controversial results indicate that interaction between soil-borne fungi and root-lesion nematodes are biological and physiological rather than physical in nature (Mai and Abawi, 1987). In our study, the subsequent enhancement of nematode multiplication and fungus colonization in *Fusarium* wilt- susceptible cultivar Barakat is a significant observation, because of higher inoculum production of both pathogens in soil and roots and warrants further investigations.

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