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DISEASE COMPLEX INVOLVING *ROTYLENCHULUS RENIFORMIS* AND *RHIZOCTONIA SOLANI* IN OKRA

by
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The association of *Rotylenchulus reniformis* with soil inhabiting plant pathogenic fungi is well known (Jenkins and Taylor, 1967; Khader *et al.*, 1972). A disease complex involving *Rhizoctonia solani* and root-knot nematodes in Okra has been investigated by Golden and Van Gundy (1975) and Chhabra *et al.* (1977).

We have studied the interaction of *R. reniformis* Linford and Oliveira and *R. solani* Kuehn, in Okra (*Abelmoschus esculentus* L.) with reference to time of infection and level of inoculum of the two pathogens.

Materials and Methods

The nematodes were maintained as a monoculture on cotton (*Gossypium hirsutum* L.) cv MCU.5. They were hatched from egg masses by incubation in the laboratory for 15 days at room temperature (Muralidharan and Sivakumar, 1975). The fungus was isolated from a diseased cotton plant and multiplied in potato-dextrose agar (PDA) (Ricker and Ricker, 1936) or a sand-maize medium (SMM).

In a first experiment seeds of Okra cv Pusa Sawani were surface sterilized by immersion in 0.1% mercuric chloride for one minute, then washed in sterile water and sown in autoclaved loamy soil in 250 g plastic containers. Six plants were grown in each pot. The nematode and the fungus were inoculated alone or in combination with either of the pathogens preceding the other by five days or

simultaneously. The first inoculation was carried out on the 10th day after germination (DAG). The pathogens were inoculated at a depth of 1 cm, close to the plant, with 250 nematodes/plant (approximately 50 percent of this population consisted of females) and/or one disc of the fungus in PDA of 1 cm diameter cut with a sterilized cork borer one week after incubation. The experiment was conducted in glasshouse at 20-36 °C. When wilt symptoms appeared the roots were removed and stained in acid fuchsin-lactophenol and examined for the presence of the nematode.

In a second experiment the interaction of two levels of nematode population (200 and 1000/pot with approximately 50 percent consisting of immature females) and two levels of fungal inoculum (1 and 5 cm diameter of agar) inoculated at 15 or 30 days after germination of okra seeds was studied. The three variables were arranged in all possible combinations consisting of 25 treatments, including an uninoculated control. The treatments were randomised and replicated three times and the pots were placed on a glasshouse bench.

Results and Discussion

In the first experiment plants wilted 17 DAG when the nematode preceded the fungus and 22 DAG when the fungus preceded the nematode (Table I). The fungus when present alone caused wilting 30 DAG. In concomitant inoculations wilt appeared 20 and 27 DAG for infections on the 10th and 15th DAG, respectively. The nematode did not cause wilt in the absence of the fungus. The roots in all treatments with the nematode were infested by females of *R. reniformis*.

In the second experiment, the plants in all treatments developed wilt symptoms except those infected with nematodes alone and the uninoculated controls. The plants wilted in three to four days after the onset of the first symptoms (Table II).

Early inoculation (15 DAG) with low fungal inoculum (LF) caused wilt 54 DAG and late inoculation (30 DAG) with LF, 65 DAG. Plants wilted earlier i.e., 45 DAG, for early inoculation and 57 DAG for late inoculation, when the fungal inoculum was high (HF) in the absence of the nematode.

Table I - *Interaction between the reniform nematode* R. reniformis *and the fungus* R. solani *on okra (Mean of six replicates).*

Treatment	First appearance of wilt (DAG)	100% wilt (DAG)	Nematode population/plant at the time of wilting (1)
Nematode only 10 DAG (2)	—	—	12.5 (1.16) f
Fungus only 10 DAG	25	30	— (0.30) a
Nematode + Fungus 10 DAG	15	20	10.2 (1.09) d
Nematode 10 DAG + Fungus 15 DAG	13	17	11.7 (1.14) e
Fungus 10 DAG + Nematode 15 DAG	17	22	9.3 (1.05) c
Nematode + Fungus 15 DAG	22	27	8.2 (1.01) b
Control	—	—	— (0.30) a

(1) Figures in parentheses are log (x+2) transformed values used in statistical analysis. Column figures followed by different letters are significantly different from each other at P = 0.01 level according to Duncan's new multiple range test (Steel and Torrie, 1960).

(2) Days after germination of seeds.

When both the pathogens were inoculated concomitantly, plants wilted earlier when inoculated 15 DAG and when either one of the pathogens was at a high density. Thus, when both the pathogens were at low densities, wilting was observed on the 28th and 61st DAG and at high level on the 23rd and 44th DAG, for early and late inoculations, respectively. Inoculation with high densities of the nematode (HN) and LF concomitantly caused wilt 34 and 46 DAG for early and late inoculations, respectively. Under concomitant inoculation of low densities of the nematode (LN) and HF plants wilted 25 and 51 DAG for early and late inoculations, respectively. HN inoculated at 15 DAG followed by HF or LF 30 DAG caused wilt 34 and 38 DAG, while LN at 15 DAG and LF or HF 30 DAG caused wilt 43 and 37 DAG, respectively.

When HF preceded the nematode, wilting was observed 42 DAG under HN and 51 DAG under LN. LF when succeeded by HN caused wilt in 44 DAG and by LN in 54 DAG (Table II).

The results clearly showed that the process of wilting was hastened in the presence of the nematode and was dependent on the age of the plant and the level of inoculum of the pathogens.

The reniform nematode multiplied well in the presence or absence of *R. solani*. The root population showed large variations between the treatments. The variations are believed to be mainly

Table II - *Influence of age of plant and inoculum densities of* R. reniformis *and* R. solani *in disease complex (Mean of three replicates).*

Treatment (1)	Shoot (2)		Root		Root population/plant	First appearance of wilt (DAG)	Appearance of 100% wilt (DAG)
	Fresh weight (g)	% decrease over control	Fresh weight (g)	% decrease over control			
LN 15 DAG	3.7 j	41.1	1.3 k	40.8	11.3 (1.12) e	—	—
LN 30 DAG	2.8 g	55.2	1.0 gh	55.0	11.0 (1.11) de	—	—
HN 15 DAG	3.4 i	45.9	1.1 j	46.0	17.7 (1.29) i	—	—
HN 30 DAG	4.7 k	23.9	1.6 l	23.7	13.7 (1.20) fg	—	—
LF 15 DAG	3.5 ij	44.9	1.8 m	15.6	0 (0.30) a	50	54
LF 30 DAG	3.5 j	44.0	1.2 jk	44.1	0 (0.30) a	60	65
HF 15 DAG	3.0 g	52.0	1.0 h	51.7	0 (0.30) a	42	45
HF 30 DAG	1.9 cd	70.1	1.0 gh	55.0	0 (0.30) a	54	57
(LN + LF) 15 DAG	3.1 h	50.7	1.0 i	50.7	9.7 (1.07) c	25	28
(LN + HF) 15 DAG	3.0 gh	51.7	1.0 j	51.7	9.7 (1.07) c	22	25
(HN + LF) 15 DAG	1.5 ab	75.6	0.5 b	75.4	12.7 (1.18) f	31	34
(HN + HF) 15 DAG	1.2 a	80.7	0.4 a	80.6	10.7 (1.10) d	20	23
(LN + LF) 30 DAG	2.6 f	59.1	0.6 bc	73.5	7.3 (0.97) b	55	61
(LN + HF) 30 DAG	2.2 e	64.0	0.8 e	64.0	9.0 (1 04) bc	47	51
(HN + LF) 30 DAG	1.4 a	77.4	0.5 ab	77.3	16.7 (1.27) hi	43	46
(HN + HF) 30 DAG	1.1 a	78.2	0.5 a	78.2	15.7 (1.25) h	42	44
LN 15 DAG + LF 30 DAG	2.5 f	60.5	0.9 fg	58.8	11.0 (1.11) de	39	43
LN 15 DAG + HF 30 DAG	2.3 e	63.4	0.8 e	64.7	11.0 (1.11 de	34	37
HN 15 DAG + LF 30 DAG	2.3 e	63.8	0.8 e	63.5	9.3 (1.05) c	33	38
HN 15 DAG + HF 30 DAG	2.1 de	67.1	0.7 de	66.8	10.3 (1.09) cd	31	34
LF 15 DAG + LN 30 DAG	2.3 e	63.4	0.8 ef	63.0	9.7 (1.07) c	47	54
LF 15 DAG + HN 30 DAG	2.3 f	62 8	0.8 f	62.6	15.0 (1.23) gh	42	44
HF 15 DAG + LN 30 DAG	1.7 b	73.4	0.6 bc	73.5	9.3 (1.05) c	45	51
HF 15 DAG + HN 30 DAG	1.8 c	71.3	0.6 cd	71.1	12.3 (1.20) ef	39	42
Uninoculated control	6.2	—	2.1 n	—	0 (0.30) a	—	—

(1) LN = Nematode low inoculum, HN = Nematode high inoculum, LF = Fungus low inoculum, HF = Fungus high inoculum, DAG = Days after germination.

(2) Figures in parentheses are log (x+2) transformed values.
Column figures followed by different letters are different at P = 0.01 level according to Duncan's new multiple range test (Steel and Torrie, 1960).

due to the difference in the time of wilting between the treatments. The fungus appears to have no antagonistic or synergistic action on the nematode (Tables I, II).

Pre-emergence damping-off due to *R. solani* is common in Okra under field conditions. The investigations show that the presence of *R. reniformis* determines the expression of wilt at later stages. Okra cultivars generally have a duration of 90 days under Indian conditions and reach maturity in about forty five days after sowing. The presence of both the pathogens would cause patchiness in the field at the production stage, thereby affecting the yield.

SUMMARY

Glasshouse studies showed that when the reniform nematode, *Rotylenchulus reniformis* and the fungus *Rhizoctonia solani* were present Okra plants succumbed to wilt at an early stage. When the nematode inoculation preceded the fungus wilting occurred earlier than when the plants were inoculated, first by the fungus. The fungus caused wilt 45-60 days after germination (DAG) in the absence of the nematode, depending upon the level of inoculum and the age of the plant, while the initial injury by the nematode shortened the period to 34-43 DAG. When both pathogens were inoculated at high densities 15 DAG, wilt occurred 23 DAG. A low initial nematode inoculum preceding the fungal inoculum also caused early wilting irrespective of the density of the latter.

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