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EFFECT OF SINGLE AND CONCOMITANT INOCULATIONS OF *HETERODERA CAJANI* AND *FUSARIUM SOLANI* ON THE ACTIVITIES OF NITRATE REDUCTASE AND GLUTAMINE SYNTHETASE IN COWPEA

by
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Summary. Two hundred juveniles of *Heterodera cajani* and 2 g of *Fusarium solani* mycelial pieces in single inoculations significantly reduced nitrate reductase and glutamine synthetase activity in cowpea cv. Pusa Dophasli, 70 days after planting in a pot experiment. However, concomitant inoculations of nematode and fungus mitigated their individual effects on enzymes activity which is also evident from the reduction in nematode populations in presence of the fungus.

The pigeonpea cyst nematode, *Heterodera cajani* Koshy, is highly pathogenic to cowpea (Koshy and Swarup, 1972; Sharma and Sethi, 1975). In nature, nematodes interact with a variety of organisms including root rot/wilt fungi, which affect the growth of the host plant. However, knowledge of the mechanism by which nematodes alone or in association with fungi reduce the growth of host plants is far from complete. Nutrient imbalances observed in nematode infested plants may be a major factor contributing to pathogenicity.

Nitrate assimilation and biological nitrogen fixation represent the major sources of reduced nitrogen for plant growth and seed yield in legume crops. The end product of both the processes is ammonia which is incorporated into the amino acids (Miflin and Lea, 1980). Nitrate reductase (NR) and glutamine synthetase (GS) are the key enzymes responsible for the assimilation of nitrate and ammonia, respectively (Abrol *et al.*, 1984).

We investigated the effect of infection by the pigeonpea cyst nematode (*Heterodera cajani*) and *Fusarium solani* (Mart.) Sacc. both separately and in combination on the growth of the cowpea host and on the activities of NR and GS in the shoots and roots.

Materials and methods

Plants of cowpea, *Vigna unguiculata* (L.) Walp, var. Pusa Dophasli were grown in 15 cm diameter clay pots filled with sandy loam soil pre-sterilised at 15 psi for 3 hours. Ten day old cowpea seedlings were inoculated with 200 freshly hatched second stage juveniles of *H. cajani* and two grams of mycelial pieces of *F. solani* cowpea isolate grown on potato dextrose agar medium either singly or in combination to make three treatments. An additional untreated control was also maintained. All the treatments were replicated six times. The pots were randomised in a screen house (22.9 — 31.5° C). Inoculations were made simultaneously to the exposed roots of the plants which were then immediately covered with soil. Seventy days after inoculation, the plants were uprooted and washed free of soil. Total leaf area was measured using a Denko model AAM7 leaf area met^{er} (Hayashi Denko Co.Ltd, Tokyo, Japan). Leaves were pooled and cut into small sections. NR activity in vivo was assayed according to the method of Klepper *et al.*, (1971) and GS activity by that of Elliott (1955).

Nematodes were extracted from six pots of each treatment by a modified Cobb's sieving and gravitation method using a set of sieves of mesh size 20, 60, 325 and 400 (Schindler, 1961). Final nematode densities given in Table I include the soil population extracted as explained above, number of eggs in cysts and the number of females on roots.

Results and discussion

Shoot weight was significantly decreased by the nematode and fungal inoculations when made separately but not in combination (Table I). However, leaf area was drasti-

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cally decreased in each of the three treatments. The presence of *F. solani* affected the multiplication of the pigeonpea cyst nematode and the final population was considerably reduced compared with the treatment without fungus. A similar effect was found with *H. glycines* and *Phytophthora megaspora* on soybean (Campos and Schmitt, 1981) and *H. zeae* and *F. solani* on maize (Lal *et al.*, 1982). Nigh *et al.*, (1980) reported up to 46% parasitism of eggs of *Heterodera schachtii* by *F. oxysporum*.

NR activity in shoots and roots and GS activity in shoots was considerably reduced by both the nematode and the fungus when they were inoculated separately. However, in the roots there were non significant differences between GS activity of the control and treated plants. Knypl and Janas (1976) also observed decreased NR activity in roots of carrot infested with *Meloidogyne hapla*. Further, roots of nematode infested plants produced less cytokinins (Brueske and Bergeson, 1972) and this factor may be responsible for decreased NR activity. The increased NR and GS activity in combined inoculations compared to single inoculations may indicate that fungal activity is diverted to nematode parasitism and consequently their individual effects on the host also are altered.

The mechanism of host growth reduction either in single or combined inoculation compared to control may be due to the effect of these pathogens on two key enzymes, namely NR and GS, consequently affecting assimilation of ammonia and in turn plant growth.

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TABLE I - *Effect of single and concomitant inoculation of* Heterodera cajani *and* Fusarium solani *in cowpea.*

Treatments	Shoot weight (g)	Leaf area (cm^2)	NR activity µ mol No_3-reduced 9^{-1}fr. wt. hr^{-1}		GS activity µ mol GHA formed 9^{-1}fr. wt. min^{-1}		Total nematode population
			Shoot	Root	Shoot	Root	
Control	12.6	374	1.83	0.95	2.33	0.63	
Nematode	7.3	271	0.36	0.34	1.90	0.62	1560
Fungus	6.1	177	0.90	0.12	1.69	0.70	
Nematode + Fungus	10.8	266	1.11	0.31	2.40	0.56	340
SEM	1.66	16	0.06	0.03	0.03	0.03	
CD 5%	4.07	40	0.14	0.07	0.08	0.07	

Literature cited

ABROL Y.P., KUMAR P.A. and NAIR T.V.R., 1984 - Nitrate assimilation and grain nitrogen accumulation. *Adv. Cereal Sci. Tech.*, *6*: 1-48.

BRUESKE C.H. and BERGESON G.B., 1972 - Investigation of growth hormones in xylem exudate and root tissue of tomato infected with root-knot nematode. *J.Exp. Bot.*, *23*: 14-22.

CAMPOS V.P. and SCHMITT D.P., 1981 - Nematode population, soybean emergence and development as a result of simultaneous inoculation with *Heterodera glycines* and *Phytophthora megasperma* var.rojac. *Fitopatol. Bras.*, *6*: 489-496.

ELLIOTT W.H. 1955 - Glutamin synthetase. In: *Methods in Enzymology* (Eds S.P. Colowick and N.O. Kaplan) Academic Press, New York Vol. 2, pp. 337-342.

KLEPPER L., FLESHER D. and HAGERMAN R.H., 1971 - Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. *Plant Physiol.*, *48*: 580-590.

KNYPL J.S. and JANAS K.M., 1976 - Is decreased activity of nitrate reductase in roots of carrot infested with northern root-knot nematode related to the increased level of chlorogenic acid? *Biochem.Physiol.Pflanzen.*, *169*: 607-615.

KOSHY P.K. and SWARUP G., 1972 - Susceptibility of plants to pigeonpea cyst nematode, *Heterodera cajani*. *Indian J.Nematol.*, *2*: 1-6.

LAL A., MATHUR V.K. and AGARWAL P.C., 1982 - Studies on the effect of *Fusarium solani* parasitism on *Heterodera zeae*. *Nematologica*, *28*: 447-450.

MIFLIN B.J. and LEAD P.J., 1980 - Ammonia assimilation. In: *The Biochemistry of Plants* (Ed. B.J. Miflin). Academic Press, New York, pp. 169- 202.

SCHINDLER A.F., 1961 - A simple substitute for a Baermann funnel. *Plant. Dis. Reptr.*, *45*: 747-748.

SHARMA N.K. and SETHI C.L., 1975 - Effects of initial inoculum levels of *Meloidogyne incognita* and *Heterodera cajani* on cowpea and their population development. *Indian J.Nematol.*, *5*: *148-154.*

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