INTERRELATIONSHIP BETWEEN *HETERODERA CAJANI* AND *FUSARIUM UDUM* IN PIGEONPEA[1]

S. B. Sharma and Y. L. Nene

Legumes Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India.

Accepted: *24.I.1989*
Aceptado:

ABSTRACT

Sharma, S. B., and Y. L. Nene. 1989. Interrelationship between *Heterodera cajani* and *Fusarium udum* in pigeonpea. Nematrópica 19:21–28.

In a greenhouse pot culture experiment, *Heterodera cajani* enhanced *Fusarium udum* pathogenicity in the wilt-susceptible pigeonpea (*Cajanus cajan*) genotype 'ICP 2376', but reactions of the wilt-tolerant ('BDN 1') and wilt-resistant ('ICP 8863') genotypes to the fungus were not altered by the nematode. Plants of 'ICP 8863' exhibited better ($P = 0.05$) growth than 'ICP 2376' and 'BDN 1' when inoculated with the fungus. Nematode reproduction was reduced on all three lines when plants also were inoculated with *F. udum*.

Key words: Cajanus cajan, Fusarium udum, Fusarium wilt, *Heterodera cajani,* interactions, pigeonpea cyst nematode.

RESUMEN

Sharma, S. B., y Y. L. Nene. 1989. Interacción entre *Heterodera cajani* y *Fusarium udum* en grandul. Nematrópica 19:21–28.

Es un experimento de invernadero se encontró que *Heterodera cajani* aumentó la patogenicidad de *Fusarium udum* en el genotipo silvestre-susceptible 'ICP 2376' de gandul (*Cajanus cajan*), pero no la afectó en el genotipo silvestre-tolerante ('BDN 1') ni en el silvestre-resistente ('ICP 8863'). Las plantas 'ICP 8863' crecieron mucho mejor ($P = 0.05$) que 'ICP 2376' y que 'BDN 1' cuando se inocularon con el hongo. La reproducción del nematodo se redujo en las tres líneas cuando las plantas se inocularon con *F. udum*.

Palabras claves: Cajanus cajan, Fusarium udum, Heterodera cajani, interacción, marchitez de *Fusarium,* nematodo de quiste del grandul.

INTRODUCTION

Fusarium wilt, caused by *Fusarium udum* Butler, is one of the most serious soilborne diseases of pigeonpea (*Cajanus cajan* (L.) Millsp.) in India and other production areas (6,9). Pigeonpea cyst nematode (*Heterodera cajani* Koshy), considered as one of the important nematode pathogens of pigeonpea, is widely distributed in pigeonpea-growing areas of India (7,12). During surveys of pigeonpea fields at ICRISAT Center, we found this nematode in high numbers in the fusarium wilt

[1]Submitted as ICRISAT Journal Article No. 774 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

screening nursery located in the Vertisol area (11). Because cyst nematodes (*Heterodera* spp.) influence several soilborne fungal diseases (1), and since a brief report by Edward and Singh (3) indicated that *H. cajani* caused more damage to pigeonpea in association with *F. udum*, we investigated the interrelationship between *H. cajani* and *F. udum* on wilt-resistant ('ICP 8863'), wilt-tolerant ('BDN 1'), and wilt-susceptible ('ICP 2376') pigeonpea genotypes. This paper reports the effects of the two pathogens on plant growth and influence of the pigeonpea genotypes and *F. udum* on nematode reproduction.

MATERIALS AND METHODS

Nematode inoculum: Heterodera cajani was isolated from the pigeonpea wilt nursery located on a Vertisol at ICRISAT Center and increased on pigeonpea genotype 'ICP 2376', grown in 30-cm-diam pots. Nematode inoculum was obtained by processing soil and roots using Cobb's decanting and sieving technique, followed by the modified Baermann funnel method (2, 10). Second-stage juveniles (J2) of *H. cajani* were collected the day prior to use and stored at 20 C.

Fungus inoculum: *Fusarium udum* was isolated from the pigeonpea wilt nursery and cultured on sand pigeonpea flour meal in 250-ml flasks using a method described by Nene et al. (9). Thirty flasks were inoculated with the fungus and incubated at room temperature (25–30 C) for 15 days and 50 g/pot (38 760 propagules/g of dry soil) of this inoculum was used (8).

Interaction experiment: Surface-sterilized seeds of the pigeonpea genotypes, 'ICP 2376' (wilt-susceptible), 'ICP 8863' (wilt-resistant), and 'BDN 1' (wilt-tolerant) were germinated in autoclaved riverbed sand and 10-day-old seedlings of uniform size were transplanted into 10-cm-diam earthen pots containing autoclaved sand and Vertisol mixture (3:1). There were 120 pots (40 pots per pigeonpea genotype) each containing two seedlings. For each pigeonpea line, the treatments included (1) control; (2) inoculation with *H. cajani* alone; (3) inoculation with *F. udum* alone; and (4) inoculation with *H. cajani* + *F. udum*. On the day prior to transplanting seedlings, *F. udum* inoculum (50 g/pot) was thoroughly mixed into the soil in each of 60 pots. On the next day 1 000 J2/pot were added to the rhizosphere of the transplanted seedlings using a 20-ml pipette. There was a factorial arrangement of pots in a completely randomized design with 10 replications. Plants were sprayed with 0.3% demeton-S-methyl when needed, to control mealy bugs and mites. Fifteen days after inoculation plants were fertilized with 50 mg of diammonium phosphate. The effect of the pathogens on the shoot length was recorded 19, 30, 45, and 55 days after inoculation. Wilt incidence (number of dead plants due to *F. udum* infection) was recorded every 2 to 3 days. Data on fresh and dry shoot weight, fresh root weight, number of nodes on the main stem, nodule (*Rhizobium* sp., cowpea group) number, and nematode population were recorded at 64 days. *Rhizobium* inoculum was derived from water that was used for irrigation and we assume that all plants received the same amount of inoculum.

The experiment was repeated using wilt-resistant 'ICP 8863'. The second experiment was different from the first in that 50 g of sand pigeonpea meal (9:1) also was added to pots receiving the nematode alone and to the control treatments in order to avoid any nutritional differences resulting from the presence of pigeonpea meal in the treament with *F. udum* alone. There were 15 replications (pots) for each treatment. Data were recorded 60 days after inoculation.

The first experiment was conducted during September–November 1984 and the second during April–June 1985 in a greenhouse at ICRISAT Center. Ambient air temperature ranged from 25 to 30 C.

Nematode population estimation: The nematode population was assessed by processing the pot soil using Cobb's sieving and decanting technique (2). Cysts were collected on a 180-μm-pore sieve (80 mesh) and J2 on a 38-μm-pore sieve (400 mesh). Nematodes and residue were placed on a modified Baermann funnel (10) to estimate the J2 population.

Data on plant growth parameters, number of nodules, and nematode populations were analyzed using the analysis of variance. Data on nematode populations were $\log_e$ transformed before statistical analysis.

RESULTS

Effect of H. cajani on plant growth: Nematode infection reduced plant growth; however, these adverse effects varied among the pigeonpea genotypes. In the case of 'ICP 8863', dry shoot weight was reduced ($P = 0.05$), whereas with 'BDN 1' and 'ICP 2376' the adverse effect was on fresh root weight ($P = 0.01$) (Table 1). The number of nodules formed on 'ICP 2376' also was reduced ($P = 0.01$) by the nematode (Table 1). We observed that shoot length (Fig. 1) and the number of nodes on the main stem of the genotypes were suppressed during the initial stages of infection, with the effect gradually becoming more pronounced as the experiment progressed.

Effect of F. udum on wilt incidence and plant growth: All 'ICP 2376' plants were dead (wilted) within 30 days (Table 2). The wilt incidence was 40% (8 of the 20 plants were dead due to *F. udum* infection) in 'BDN 1'. Shoot length and number of nodules were reduced ($P = 0.05$) in the surviving plants. There was no wilt incidence in 'ICP 8863'; however, the number of nodules was reduced significantly ($P = 0.05$). Growth was suppressed up to 30 days, but the plants regained vigor thereafter

Table 1. Effects of *Heterodera cajani* and *Fusarium udum* alone and combined on growth of pigeonpea 'ICP 8863', 'BDN 1', and 'ICP 2376' and number of nodules 64 days after inoculation.

	Fresh root weight (g)			Fresh shoot weight (g)			Dry shoot weight (g)			No. nodules		
Treatment	ICP 8863	BDN 1	ICP 2376[z]	ICP 8863	BDN 1	ICP 2376[z]	ICP 8863	BDN 1	ICP 2376[z]	ICP 8863	BDN 1	ICP 2376[z]
Control	2.9	2.9	2.4	2.0	1.4	1.0	0.8	0.5	0.4	36.6	27.6	36.1
F. udum	4.8	2.0	—	3.9	1.8	—	1.3	0.6	—	14.5	5.5	—
H. cajani	2.7	1.8	1.3	1.6	1.1	0.7	0.5	0.4	0.2	27.4	20.5	14.5
F. udum + *H. cajani*	4.2	1.7	—	3.5	1.3	—	1.0	0.4	—	11.4	7.8	—
SE	0.36	0.25	0.12	0.34	0.21	0.09	0.11	0.07	0.03	3.41	2.79	3.31

[z]All plants of wilt susceptible genotype 'ICP 2376' were dead in *F. udum*, and *F. udum* + *H. cajani* treatments.

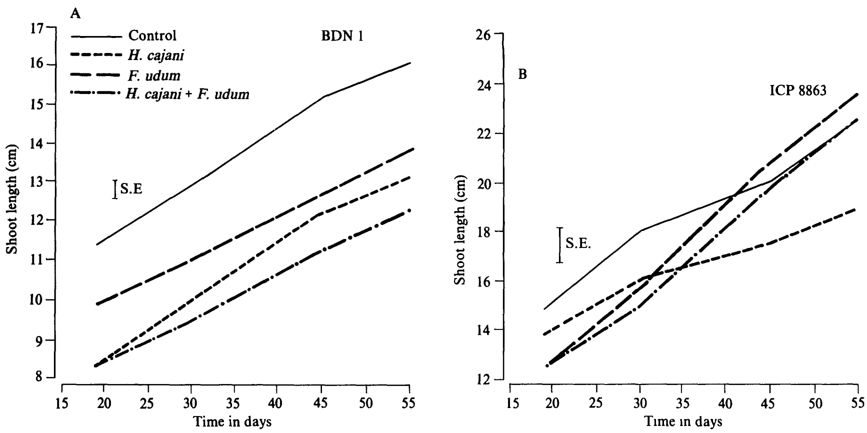

Fig. 1. Effect of *Heterodera cajani* and *Fusarium udum* on shoot length of pigeonpea genotypes. A) Wilt-tolerant 'BDN 1'. B) Wilt-resistant 'ICP 8863'.

(Fig. 1). This was supported by significant ($P = 0.01$) increases in fresh and dry shoot weights and fresh root weight (Table 1). The fungus reduced the root weight of 'BDN 1' ($P = 0.05$).

Combined effect of H. cajani and F. udum: All 'ICP 2376' plants died as was the case with the *F. udum* alone treatment. In the presence of the nematode, wilt incidence was enhanced. Twenty-three days after inoculation, 45% of the plants wilted in the fungus-alone treatment, whereas pots treated with *H. cajani* showed 80% wilt incidence. Wilting was 100% within 25 days when plants were infected with both pathogens. The *F. udum* alone treatment resulted in 70% wilting within 25 days (Table 2). The influence of nematode and fungus varied with the genotype. Wilting of 'BDN 1' was not affected by presence of the nematode; eight plants wilted in the fungus-alone treatment compared to seven in the

Table 2. Effect of *Heterodera cajani* on *Fusarium udum* wilt incidence in the pigeonpea genotypes 'ICP 2376' and 'BDN 1'.

		Wilt incidence (% dead plants)	
Pigeonpea genotypes	Days after inoculation	*F. udum*	*F. udum* + *H. cajani*
ICP 2376	20	10	30
	25	70	100
	30	100	100
BDN 1	20	5	5
	25	10	15
	30	25	20
	64	40	35

nematode + fungus treatment. *Fusarium udum* appeared to have inhibited the effect of the nematode on shoot length (Fig. 1). Growth reduction caused by the nematode was compensated by an increase in plant growth in the presence of the fungus. Plant growth of 'ICP 8863' in pots inoculated with nematode + fungus was greater ($P = 0.01$) than that in the control pots (Table 1). Interactions between *H. cajani* and *F. udum* on 'ICP 8863' and 'BDN 1' were not significant.

The results obtained during April–July 1985 confirmed that plant growth of 'ICP 8863' in fungus alone, and in the nematode + fungus treatment was significantly better ($P = 0.01$) than that of the controls.

Effect of pigeonpea genotypes on H. cajani reproduction: Nematode reproduction was significantly lower ($P = 0.01$) on 'ICP 8863'. Numbers of eggs + J2/cyst were not affected adversely, but nematode development and cyst formation was reduced ($P = 0.01$) on 'ICP 8863'. Nematode reproduction on 'ICP 2376' and 'BDN 1' was similar (Table 3).

Influence of F. udum on reproduction of H. cajani: Heterodera cajani reproduction was suppressed ($P = 0.05$) on 'BDN 1' and 'ICP 8863' by the presence of *F. udum*. Final cyst population and total nematode population produced on the two pigeonpea genotypes were significantly ($P = 0.05$) lower. Numbers of eggs + J2/cyst were not affected (Table 3).

DISCUSSION

It is evident from the data that the presence of *H. cajani* did not break the resistance of 'ICP 8863' to *F. udum*. The reaction of wilt-tolerant pigeonpea 'BDN 1' to *F. udum* was not altered by the nematode; in the wilt-susceptible genotype death was more rapid when the nematode and fungus were present together. Increase in wilt incidence in a local pigeonpea cultivar was observed (4) in the presence of the *H. cajani*. Perhaps the cultivar was susceptible to *F. udum*. Nematode reproduction

Table 3. Effect of *Fusarium udum* on the reproduction of *Heterodera cajani* on pigeonpea genotypes in pot experiments, 64 days after inoculations with 1 000 second-stage juveniles (J2).

	Cysts/pot		Eggs + J2/cyst		Total population/pot[y]	
Pigeonpea genotypes	*H. cajani*	*H. cajani* + *F. udum*[z]	*H. cajani*	*H. cajani* + *F. udum*[z]	*H. cajani*	*H. cajani* + *F. udum*
ICP 8863	308.0	54.6	50.9	48.4	16 647	2 697
BDN 1	518.0	58.6	40.9	49.9	25 591	2 951
ICP 2376	544.6	—	44.7	—	24 890	—
SE	63.9	8.5	5.0	4.9	2 238	395

Data were $\log_e$ transformed for analysis.
[y]Includes eggs + J2 in cysts + J2 in soil.
[z]All 'ICP 2376' plants were dead in *H. cajani* + *F. udum* treatment and data on *H. cajani* populations were not recorded.

was significantly lower on 'ICP 8863' and its root system suffered less damage. There are no known sources of pigeonpea resistance to *H. cajani*, but 'ICP 8863' appears to be less susceptible to *H. cajani*. Studies on losses in yield of this genotype by *H. cajani* would be desirable. Presence of the *F. udum* reduced the reproduction of *H. cajani*, however, the fecundity (production of eggs/female) was not affected adversely. Reduced nematode reproduction in the presence of *F. udum* also was observed in another study (4). Similarly, *Fusarium oxysporum* Schlect. emend. Synd. & Hans. reduced the reproduction of *H. schachtii* Schmidt (5). Reasons for reduced reproduction of *H. cajani* in presence of *F. udum* were not determined. Studies on the influence of *F. udum* on penetration of roots by *H. cajani* and on feeding sites of the nematode, parasitism of nematode J2 by the fungus, and production of toxins by the fungus that are detrimental to *H. cajani* might be helpful in understanding the mechanism(s) for reduction in nematode reproduction. The fungus reduced numbers of nodules, but the reduction in nodule number did not affect the plant growth, particularly 'ICP 8863.'

The increased growth response of 'ICP 8863' in the presence of *F. udum* may be from the increased availability of nutrients from the pigeonpea meal that may have been hydrolyzed by the fungus; it also is possible that the plants produced more vegetative growth to compensate for the effect of the fungal infection. The increased growth in the presence of the fungus may thus also be a defense reaction. More detailed investigations are required to better understand the mechanism of resistance of 'ICP 8863' to *F. udum*. It would be interesting to determine whether the increased vegetative growth in the presence of the fungus results in increased yield.

LITERATURE CITED

1. ARMSTRONG, M., P. JATALA, and H. JENSEN. 1976. Bibliography of nematode interactions with other organisms in plant disease complexes. Station Bulletin 623. Agricultural Experimental Station, Oregon State University, Corvallis, Oregon, U.S.A.
2. COBB, N. A. 1918. Estimating the nema population of soil. U.S. Department of Agriculture Technology Circular 1. U.S. Government Printing Office: Washington, D.C.
3. EDWARD, J. C., and K. P. SINGH. 1979. Interaction between *Heterodera cajani* and *Fusarium udum* on pigeonpea. Allahabad Farmer 50:23–24.
4. HASAN, A. 1984. Synergism between *Heterodera cajani* and *Fusarium udum* attacking *Cajanus cajan*. Nematologia Mediterranea 12:159–162.
5. JORGENSON, E. C. 1970. Antagonistic interactions of *Heterodera schachtii* Schmidt and *Fusarium oxysporum* (Woll.) on Sugarbeets. Journal of Nematology 2:393–398.
6. KANNAIYAN, J., Y. L. NENE, M. V. REDDY, J. RYAN, and T. N. RAJU. 1984. Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa and the Americas. Tropical Pest Management 30:62–71.
7. KOSHY, P. K., and G. SWARUP. 1971. Distribution of *Heterodera avenae, H. zeae,* and *Anguina tritici* in India. Indian Journal of Nematology 1:106–111.

8. NENE, Y. L., J. KANNAIYAN, and M. V. REDDY. 1981. Pigeonpea diseases: resistance-screening techniques. Information Bulletin No. 9. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India.
9. NENE, Y. L., V. K. SHEILA, and S. B. SHARMA. 1984. A world list of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* (L.) Millsp.) pathogens. Pulse Pathology Progress Report 32. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India.
10. SCHINDLER, A. F. 1961. A simple substitute for a Baermann funnel. Plant Disease Reporter 45:747–748.
11. SHARMA, S. B., V. W. SAKA, and Y. L. NENE. 1985. Plant-parasitic nematodes in pigeonpea fields at ICRISAT Center: International Pigeonpea Newsletter 6: 54–55.
12. SHARMA, S. B., and G. SWARUP. 1984. Cyst Forming Nematodes of India. Cosmo Publications: New Delhi.

Received for publication: *28.IV.1988*
Recibido para publicar: