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# Effect of *Heterodera cajani* on biomass and grain yield of pigeon pea on vertisol in pot and field experiments

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Glasshouse and field trials were conducted to determine the effects of the cyst nematode, *Heterodera cajani* on biomass and grain yield of pigeon pea, *Cajanus cajan.* Shoot length, fresh and dry shoot masses, leaf area and pod yields of pigeon pea were significantly reduced by *H. cajani*. In glasshouse pot experiments, an initial density of 1.0 juveniles per cm<sup>3</sup> soil caused 14 to 24% reduction in plant height, root and shoot mass and leaf area. Application of carbofuran 3G (1.5, 3.0 and 6.0 kg a.i./ha) in autoclaved soil in pots did not improve growth of pigeon pea; however, its application (6 kg a.i./ha) in *H. cajani*-infested fields reduced the nematode density (P < 0.05) and improved plant growth and yield. The densities of eggs and juveniles of *H. cajani* were 72 and 48% lower in the carbofuran-treated plots than in the control plots 35 and 52 days, respectively, after sowing. No such differences were observed at 70 days after sowing. The tolerance limit for pod yield in field experiments was 2.6 eggs and juveniles of *H. cajani* per cm<sup>3</sup> soil at sowing time. Grain yield was 20 to 25% higher in the carbofuran-treated plots than in the control plots. Application of carbofuran protected the roots from nematode damage during the early stages of plant growth and resulted in good plant growth and yield.

# INTRODUCTION

Pigeon pea (*Cajanus cajan*) is cultivated extensively on vertisols (black cotton soils) in India. The pigeon pea cyst nematode, *Heterodera cajani*, is an important nematode pest of pigeon pea in India (Sharma & McDonald, 1990). High population densities of *H. cajani* were associated with stunted pigeon pea plants in vertisols, and infection reduced the growth of pigeon pea in glasshouse experiments (Sharma *et al.*, 1985, Sharma & Nene, 1988). The objective of the present study was to determine the influence of *H. cajani* densities on plant growth and yield of pigeon pea grown on vertisols.

# MATERIALS AND METHODS

Trials were conducted between 1984 and 1990 in a glasshouse (maximum temperature 25–30°C, minimum temperature 20–22°C), and ICRISAT's research farm (17°N, 78°E, 545 m above sea level. For glasshouse pot experiments, the *H. cajani* population was collected from pigeon pea roots in the field and was cultured on susceptible pigeon pea genotypes ICP 2376 and ICPL 227 (medium duration) and ICPL 87 (short duration). These genotypes were used in different trials conducted in the glasshouse and field.

#### Pot experiments

#### Effect of H. cajani on pigeon pea biomass

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Seeds of ICP 2376 were sown in 10-cm-diameter pots containing 500 cm<sup>3</sup> autoclaved mixture of sand, farmyard manure and vertisol soil (5:3:4). Ten days after sowing in March 1984, 0, 5, 50, 500, 1000 and 5000 second-stage juveniles in water suspension were added to the soil in each pot. Each treatment (nematode population density) was replicated 10 times. The pots were irrigated regularly with 100 ml tap water per pot. Data on plant height, fresh and dry shoot and root masses, leaf area and nematode population densities per pot were recorded after 68 days. Maximum and minimum temperatures in the glasshouse during this experiment ranged between 30 and 35°C and 25 and 28°C, respectively. Similarly, effects of two nematode population densities (0 and 5000 second-stage juveniles) of H. cajani on ICPL 87 were studied in 15-cmdiameter pots in June 1988. The treatments were replicated 14 times and data on plant growth were recorded at the time of podding after 105 days. Maximum and minimum temperatures ranged between 25 and 30°C and 20 and 23°C, respectively.

# Effect of carbofuran on pigeon pea biomass

Five seeds of ICPL 227 were sown in each pot of 22.5-cm-diameter containing autoclaved vertisol. Four doses of carbofuran 3G (2,3-dihydro-2, 2-dimethyl-7-benzofurenyl-*N*-methylcarbamate) equivalent to 0, 1.5, 3.0 and 6.0 kg a.i./ha were added to the soil at a depth of 5–6 cm. The seeds were sown after covering the chemical with a thin layer of soil. The treatments were replicated six times. Data on plant growth and biomass were recorded 100 days after sowing.

# Two field experiments

An H. cajani-infested vertisol (silty clay loam, very fine montmorillonitic, calcareous, typic pellustert) field where pigeon pea had been cultivated in the rainy season (June December) for the last 10 years was selected at the research farm of ICRISAT. Rotylenchulus reniformis, Helicotylenchus retusus, Tylenchorhynchus vulgaris and Praty*lenchus zeae* were other plant-parasitic nematodes present in relatively small numbers in this field. An area of 240  $m^2$  was divided into 20 plots of 9.6 m<sup>2</sup> each. Seeds of ICPL 227 were sown in these plots on ridges in four rows in July 1987; interrow distance was 60 cm. Ten plots were treated with carbofuran 3G at the rate of 6 kg a.i./ha at the time of sowing, 5-6 cm depth below the surface of the ridges, and was covered with a thin layer of soil. The treatments were assigned to plots in a randomized block design. This trial was repeated again in the rainy season of 1988 with 15 replications (plots) and six rows per plot at another site on the research farm.

Nematode population densities were estimated by assaying a composited soil sample. Each sample consisted of six random cores of soil collected with a 2.5-cm-diameter  $\times 45$ -cm-long tube auger. Samples were collected to a depth of 20 cm from individual plots at the time of sowing, two to four times during the crop growth period and at harvest.

Soil samples collected for nematode assay were also examined for soil insects 30-40 days after the application of carbofuran. Eleven sprays of insecticides were used to control the foliar insect pests, particularly *Helicoverpa armigera*. Plant growth and vigour was rated on a 1-5 scale (1 = excellent growth, 5 = poor growth), 6-8 weeks after sowing. Ten plants in each plot were randomly selected and plant height was recorded at 45, 75, 105, and 175 days after sowing. Data on plant biomass, grain yield and nematode population densities were recorded at harvest in January. The nematode populations were extracted from 100-cm<sup>3</sup> samples by suspending the nematodes in water, pouring the suspension through nested sieves (850, 180 and 38  $\mu$ m pore), and placing the residues from the 38  $\mu$ m pore sieve on modified Baermann funnels (Schindler, 1961). Cysts from soil and roots were collected on a 180  $\mu$ m pore sieve (Sharma & Nene, 1986). Eggs and juveniles in the cysts were counted after gently crushing the cysts on a glass slide. The roots were gently washed with tap water, females were counted with the unaided cyc, and cggs were extracted from the cgg sacs on the root systems (Sharma & Nene, 1987).

Nematode counts were transformed to  $\log_{(x+1)}$ for statistical analysis. Reduction in nematode densities in the carbofuran-treated plots was calculated as [(A - B)/A]100, where A = nematode population density in control plot, and B = nematode density in carbofuran-treated plot. Loss in yield due to *II. cajani* was calculated as: [(X - Y)/X]100, where X = yield in carbofuran treated plots and Y = yield in untreated plots. The tolerance limit of pigeon pea to H. cajani was estimated in a pot experiment (with six inoculum levels), and in untreated plots in 1987and 1988-season trials using Seinhorst's model:  $Y = m + (1 - m)^{A^{P-D}}$ , where Y = yield on a scale of 0.1, m = minimum yield on a scale of 0.1, T = tolerance limit, P = nematode population level, and z = a constant less than one. This model is based on that described by Ferris et al. (1981).

# RESULTS

# Pot experiments

# Effect of H. cajani on pigeon pea biomass

Shoot length, shoot and root masses and leaf area were significantly reduced by *H*. *cajani* (P < 0.05) (Table 1). Plants in the nematode-inoculated treatments were stunted and their foliage was lighter green than that of plants growing in H. *cajani*-free soil. Nematode density was negatively correlated with plant height and shoot and root masses. The tolerance limit for plant height, fresh and dry shoot masses, dry root mass and leaf area was 0.01 juveniles per cm<sup>3</sup> soil; for fresh root mass, the tolerance limit was 0.02 juveniles per cm<sup>3</sup> soil. An initial density of one juvenile per cm<sup>3</sup> soil caused a 14 to 24% reduction in shoot length. shoot and root masses and leaf area, whereas an initial density of five juveniles per cm<sup>3</sup> soil caused 42 to 79% reduction in these parameters.

Initial nematode density/500 cm <sup>3</sup> soil	Shoot length (cm)	Shoot mass (g)		Root mass (g)		Leaf area
		Fresh	Dry	Fresh	Dry	(cm <sup>2</sup> )
0	37.3	5.8	1.9	9.6	1.4	221-2
5	35.4	4.9	1.7	10.2	1.3	207.9
50	32.9	4.8	1.6	9.6	1.2	188-2
500	31.7	4.4	1.4	7.5	1.0	174.0
1000	29.8	4.0	1.3	6.5	0.7	152.9
5000	21-4	1.8	0.6	4.3	0.3	77.6
LSD ( $P < 0.05$ )	1.40	0.44	0.13	0.69	0.12	15.52

 Table 1. Effect of Heterodera cajani on pigeon pea (cv. ICP 2376) biomass in a glasshouse pot experiment

The number of cysts per g root and number of eggs and juveniles at harvest were greater (P < 0.05) in pots with inoculum densities of 500 and more juveniles than those pots with inoculum density of 50 juveniles. The average cyst number per g root was 30, 51, 54, and 47 in pots inoculated with 50, 500, 1000 and 5000 juveniles, and the average egg and juvenile densities were 2655,

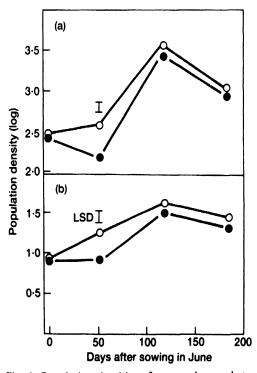


Fig. 1. Population densities of eggs and second-stage juveniles, and cysts of *Heterodera cajani* in carbofuran-treated and control plots. (A) eggs and second-stage juveniles; (B) cysts.  $\bigcirc -\_ \bigcirc = \bigcirc = \bigcirc = \bigcirc = \bigcirc = \bigcirc$ 

18016, 23909 and 24539, respectively, in these treatments.

The effect of *H. cajani* infection on growth of ICPL 87 was similar to that on ICP 2376. Plant vigour was reduced and flowering in ICPL 87 was delayed by 10 days in nematode-infested soil. Plants height and dry shoot mass were reduced (P < 0.05) by 14 and  $63\%_0$ , respectively, by the nematodes. Plant height was 29.0 cm in control and 25.0 cm in nematode-infested soil; dry shoot mass per plant was 4.0 and 1.5, respectively. Flower number, leaf mass and leaf number were significantly reduced by the nematode infection (P < 0.05).

#### Effect of carbofuran on pigeon pea biomass

Application of carbofuran 3G in autoclaved soil did not significantly affect the growth of ICPL 227. Plant height measured 4, 8 and 12 weeks after sowing in carbofuran-treated soil was not significantly different from that in soil without carbofuran (control). Average plant height ranged between 19·2 and 22·7 cm in four treatments. The number of nodes per plant ranged between 10·5 and 11·9. The number of leaves, number of nodes, fresh and dry root and shoot masses were not affected by any dose of carbofuran. Plants in soil containing 6 kg a.i./ha of carbofuran initially showed burning of leaf margins.

#### **Field experiments**

At sowing time, population densities of eggs and juveniles of *H. cajani* in 1987 and 1988 ranged between 90 and 575 per 100 cm<sup>3</sup> soil. The average nematode population densities assessed before application of carbofuran did not differ signifi-

	Plant height (cm)				
Treatment	45 <sup>b</sup>	75	105	175	
Untreated (control) Carbofuran 3G (6 kg a.i./ha) CV (%)	28-9 49-1** 3-8	77·4 114·5** 9·0	116·2 36·4** 5·5	146·9 171·0** 5·4	

 
 Table 2. Plant height of pigeon pca genotype cv. ICPL 227 in untreated and carbofuran-treated vertisol plots<sup>a</sup>, 1987/8

**\*\*** Significantly different (P < 0.01) from control.

<sup>a</sup> Heterodera cajani-infested soil.

<sup>b</sup> Days after sowing.

cantly (P < 0.05) between the control plots and plots to be treated with carbofuran. Application of carbofuran reduced the population densities of H. cajani (P < 0.05) (Fig. 1). Cyst, egg and juvenile densities were significantly lower in the carbofuran treated plots. Trends in reduction in nematode densities and increase in plant biomass were similar in the 1987 and 1988 experiments. Average population densities of eggs and secondstage juveniles of *H. cajani* estimated 35 days after sowing in 1987 were 72% lower in the carbofurantreated plots than in the control plots. The nematode density 52 days after sowing in 1988 was 48% lower in carbofuran-treated plots than in the control plots. Average density of eggs per g root was 875 in the treated plots and 1795 in the control plots. Nematode densities in carbofurantreated and control plots did not differ 70 days after sowing.

Plant height, shoot mass, pod mass and grain yield were significantly greater (P < 0.05) in the carbofuran-treated plots than in control plots

Table 3. Shoot pod and grain masses (kg/ha) of pigeonpea genotype ICPL 227 in untreated and carbofuran-<br/>treated vertisol plots<sup>a</sup> 1988/9

Treatment <sup>b</sup>	Dry shoot mass	Pod mass	Grain mass
Untreated (control)	4899	2200	1357
Carbofuran 3G (6 kg a.i./ha)	6079*	2704*	1701*
CV (%)	18.5	15.6	17.6

\* Significantly different (P < 0.05) from control.

\* Heterodera cajani-infested soil.

<sup>b</sup> Fifteen replications (plots) for each treatment.

(Tables 2 and 3). Flowering was later by 5-12 days in the control plots than in the treated plots. Differences in plant height were clearly visible at 45 days after sowing and were maintained throughout the crop growth period. Crop growth was more uniform (1.5 rating on a 1-5 scale) in the carbofuran-treated plots than in the control plots (4.2 rating). In 1987, application of carbofuran resulted in 32.7 and 23.7% increase in fresh and dry shoot masses, 18.7% increase in pod yield and 25.0% increase in grain yield. In 1988, percentage increases in these parameters in carbofuran-treated plots were 19.0, 19:3, 18.6 and 20.2%, respectively. Pod number increased significantly by 17%. The tolerance limit for pod mass based on 2 years' field trial data was 2.6 eggs and juveniles per cm<sup>3</sup> soil.

# DISCUSSION

We have considered yield of pigeon pea as a measurable product of economic value. Foliage, dry stems and grains are all of value to the farmer in the semi-arid tropics, and it is clear that H. cajani reduces pigeon pea biomass and grain vields, Saxena and Reddy (1987) also reported loss in yield of pigeon pea due to H. cajani. Glasshouse trials revealed the pathogenic nature of the nematode. The nematode takes less than 3 weeks to become adult and produce eggs. Many eggs hatch within a week of completing their development and a large number of juveniles attack the roots which are growing in a limited soil volume in pots. Therefore, the tolerance limit estimated for a nematode-plant system may differ depending on the volume of soil, the size of container used for the experiment and on the watering and fertilizer regime. Zaki & Bhatti

(1986) reported that an inoculum level of 0.1 juveniles per g soil caused significant reduction in pigeon pea biomass in pots containing 1000 g soil. Application of carbofuran 3G to the autoclaved soil did not result in better plant growth. In the field trials, populations of soil insect pests were low, and foliar pests were controlled by repeated toliar applications of insecticides. Therefore, we conclude that increased yields of pigeon pea in carbofuran-treated plots were attributable to the control of H. cajani during the initial plant growth period. Pigeon pea is vulnerable to nematode damage when the root system is small and the nematode population large. Application of carbofuran protects the roots during the early stages of plant growth, when roots of mediumduration pigeon pea grow slowly, resulting in better crop growth and yield. Heavy soil texture and high soil pH accelerates the degradation of carbofuran (Carso et al., 1973) and this may be the reason for the limited period of control of nematodes in the carbofuran-treated vertisol plots. Crop management practices that could keep the nematode population under control for the entire season might result in further yield improvements. Alternatively, protection of pigeon pea roots from H. cajani infection for 8 weeks after sowing is beneficial in reducing losses caused by the nematode.

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# REFERENCES

- Caro J.H., Freeman H.P., Glotfelty D.E., Turner B.C. & Edward W.M. (1973) Dissipation of soil-incorporated carbofuran in the field. *Journal of Agricultural* and Food Chemistry 21, 1110-1015.
- Ferris H., Turner W.D. & Duncan L.W. (1981) An algorithm for fitting Seinhorst curves to the relationship between plant growth and preplant nematode densities. *Journal of Nematology* 13, 300-304.
- Saxena, R. & Reddy D.D.R. (1987) Crop losses in pigeon pea and mungbean by pigeon pea cyst nematode. *Heterodera cajani. Indian Journal of Nematology* 17, 91-94.
- Schindler A.F. (1961) A simple substitute for Baermann funnel. *Plant Disease Reporter* 45, 747-748.
- Sharma S.B. & McDonald D. (1990) Global status of nematode problems of groundnut, pigeonpea, chickpea, sorghum and pearl millet and suggestions for future work. Crop Protection 9, 453-458.
- Sharma S.B. & Nene Y.L. (1986) Extraction of *Hetero*dera cajani cysts using modified sieving technique. *Indian Journal of Nematology* 16, 123-124.
- Sharma S.B. & Nene Y.L. (1987) A technique to extract Heterodera cajani eggs from eggsacs. International Pigeonpoea Newsletter 6, 54-55.
- Sharma S.B. & Nene Y.L. (1988) Effect of Heterodera cajani. Rotylenchulus reniformis, and Hoplolaimus seinhorsti on pigeonpea biomass. Indian Journal of Nematology 18, 273–278.
- Sharma S.B., Nene Y.L. & Saka V.K. (1985) Plant parasite nematodes in pigeonpea fields at ICRASAT Centre. International Pigeonpea Newsletter 4, 41-42.
- Zaki F.A. & Bhatti D.S. (1986) Pathogenicity of pigeonpea cyst nematode. *Heterodera cajani* Koshy, 1967 on some pulse crops. *Indian Journal of Nemato*logy 16, 30–35.