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Impact of nodule damage by *Rivellia angulata* on N_2-fixation, growth, and yield of pigeonpea (*Cajanus cajan* L. Millsp.) grown in a Vertisol *

J. V. D. K. Kumar Rao and S. Sithanantham

Legumes Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, A. P. 502324, India

Summary. Damage caused by *Rivellia angulata* larvae to pigeonpea root nodules at the ICRISAT center in India was greater in the crop grown on Vertisols (up to 86%) compared to that on Alfisols (20%). Attempts to quantify the field effects of nodule damage on growth and yield of pigeonpea in a Vertisol, involving many heavy applications of soil insecticides (aldrin and hexachlorocyclohexane) failed because the insecticides did not control the pest and adversely affected the growth of the pigeonpea and the subsequent crop of sorghum (*Sorgorum bicolor* L. Moench). The impact of nodule damage on pigeonpea growth, yield and nutrient uptake was successfully studied in greenhouse-grown plants at three N levels. In this pot study, artificial inoculation with *Rivellia* sp. led to substantial nodule damage (70%). The results of this damage were a significant overall reduction in nodule dry weight (46%), acetylene reduction activity (31%), total leaf area (36%), chlorophyll content of leaves (39%) and shoot dry weight (23%) 68 days after sowing. At maturity, *Rivellia* sp. infestation caused significant reductions in top dry weight (22%), root and nodule dry weight (27%), seed dry weight (14%), and total N (29%) and P uptake (19%). The problems and prospects of manipulating nodule damage so as to reduce N losses in pigeonpea are discussed.

Key words: Nodule damage – *Rivellia angulata* – Nitrogen fixation – *Cajanus cajan* – Pigeonpea – Vertisol

Increasing the plant availability of biologically fixed N is one of the important ways of improving crop yields (Hardy and Havelka 1975). However, nodule damage by insects can seriously affect the N_2-fixing ability of nodules in crop legumes. Extensive damage to the root nodules of pigeonpea [*Cajanus cajan* (L.) Millsp.] were observed not only at the ICRISAT center but also in farmers' fields in Andhra Pradesh and Maharashtra. Sithanantham et al. (1981) concluded that this damage was caused by the larvae of *Rivellia angulata* (Diptera: Platystomatidae). The larva of this insect bores into the core of the nodule, causing a substantial reduction in nodule mass. The extent of nodule damage is greater in pigeonpea grown in Vertisols than in Alfisols (Sithanantham et al. 1981). However, its impact on N_2 -fixing ability and plant growth has not been evaluated. In the present investigation we attempted to quantify the impact of nodule damage by *Rivellia angulata* on acetylene reduction activity (as a measure of potential N_2-fixing ability), plant dry matter, and N uptake, first in a Vertisol field trial and later in pot experiments.

Materials and methods

Soil used. Two types of soil, Vertisol and Alfisol, were used at the ICRISAT center, Patancheru, India. A detailed description of their characteristics has been given by El-Swaify et al. (1985).

Field experiment (study 1). During the rainy season of 1984 – 1985 two high-yielding pigeonpea genotypes, ICPL 87 (short duration) and ICPL 270 (medium duration), were grown in a Vertisol and Alfisol field at the ICRISAT center to determine the response to *Rhizobium* sp. inoculation. The cultivars were grown in plots of 4×4.5 m, and the trial was laid out in a split-plot design with cultivars in the main plot and *Rhizobium* sp. in subplots in three replications. The crop was grown with protective irrigation. Forty days after sowing, 10 plants per plot were carefully dug out with a

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Offprint requests to: J. V. D. K. Kumar Rao

crowbar. The nodules were collected, washed in tap water and grouped as healthy or damaged based on the presence of a pin hole or large hole with or without a larva.

Field experiment (study 2). This study was conducted during the 1985–1986 rainy season in order to estimate the loss in N_2-fixing ability, growth and yield of pigeonpea in Vertisols due to nodule damage, and also to compare the yields of pigeonpea plants that were dependent either on symbiotically fixed N or fertilizer N. Accordingly, the field experiment consisted of four treatments, replicated six times and laid out in a randomized block design as detailed below:
(1) No soil insecticides, no N fertilizer. (2) With soil insecticides, no N fertilizer. (3) No soil insecticides, with N. (4) With soil insecticides, with N.

Pigeonpea genotype ICPL 87, inoculated with *Rhizobium* sp. strain IC 3195 to provide about 10^5 rhizobia seed^{-1}, was sown on 26 June 1985 on either side of 60-cm ridges at a spacing of 30×10 cm. Each plot comprised 20 rows 4 m in length (4×6 m). A basal dose of P at 17 kg ha^{-1}, as single superphosphate, and $ZnSO_4$ at 40 kg ha^{-1} was applied as the land was prepared. N in the form of urea was banded in the furrows of treatments 3 and 4. The soil insecticide, aldrin (30% emulsifiable concentrate), at 4 or 2 kg active ingredient (a.i.) ha^{-1}, was mixed with water at 2000 l ha^{-1} and applied uniformly on the soil surface using rose cans. For treatments 1 and 3, an equivalent quantity of water was applied to the soil surface. Hexachlorocyclohexane (10% dust at 4 kg a.i. ha^{-1}) was applied uniformly to the surface. The higher doses of these insecticides were applied to prevent the flies from laying eggs, and no noticeable phytotoxicity symptoms were observed. Care was taken to prevent the drift of insecticides to neighboring plots by holding a cloth screen around the plots during insecticide application. The details of insecticides and fertilizer N applied are given in Table 1.

The crop was sprayed during flowering/podding with endosulfan (0.07%) to control pod borer (*Heliothis armigera*). Irrigation was provided 133 and 168 days after sowing. Five well-bordered plants in a row were sampled for nodulation, acetylene-reducing activity and dry matter 20, 40, 68, and 97 days after sowing. At each sampling the roots and nodules were separated from adhering soil and the nodules were categorized as healthy or damaged. The plant material was oven-dried at 70 °C and weighed. The gas samples, collected 30 min after incubation of root nodules in air-tight glass containers containing 10% acetylene, were analysed for acetylene and ethylene using a gas chromatograph (Kumar Rao and Dart 1987). Three flushes of pods were harvested, the first 130 days after sowing, the second 173 days after sowing and the third and final flush was harvested 220 days after sowing. At each harvest of pods, the above-ground plant biomass was harvested from a 4.2-m^2 area, dried and weighed.

In order to detect the residual effect of the treatments in the following rainy season, i.e. starting in June 1986, the land was plowed and ridged at 60-cm intervals, thus ensuring little movement of soil between plots. The sorghum hybrid CSH 6 was sown on 28 June 1986 and harvested on 1 October 1986. The plants were dried and the above-ground biomass was weighed.

Table 1. Insecticide and fertilizer applications

Insecticide name and rate (kg a.i. ha^{-1})	Time of application (days after sowing)	N as urea (kg ha^{-1})	Time of application (days after sowing)
Aldrin (4)	11	20	0
Aldrin (2)	16	50	37
Aldrin (2)	30	50	70
Aldrin (2)+	43, 51		
HCH[a] (4)	57, 65 and 88		

[a] HCH, hexachlorocyclohexane

Pot experiment (study 3). This greenhouse study involved a comparison of pot-grown pigeonpea plants that were artificially infested by the adult insect (*Rivellia angulata*) with those not infested. To determine whether the impact of nodule damage on plant growth and yield was influenced by the N status of the soil, we studied the same soil at three levels of N – low (depleted) N, normal N and added (fertilizer) N – by suitable manipulations. The experiment thus consisted of six treatments (three N levels each with and without *Rivellia* sp. infestation) and was laid out as a factorial randomized block design with six replications.

Plastic pots (25 cm diameter at the top) were filled with unsterilized Vertisol soil at 6.9 kg pot^{-1}. The total N, available N and available P (Olsen) contents of the soil were 617, 23 and 7 ppm, respectively. Single superphosphate (1.43 g pot^{-1}, equivalent to 20 kg P ha^{-1}) was mixed uniformly through the soil in each pot.

In the low-N treatment, sorghum was grown for 2 months, and then pigeonpea was sown to deplete the soil N. In addition, to immobilize any remaining available N, rice straw at 10 t ha^{-1} was incorporated into the soil in each pot before the pigeonpea was sown. In the added-N treatment, urea was mixed into the soil at 20 kg N ha^{-1}. Pigeonpea ICPL 87 was sown in the pots on 4 July 1986 at 10 seeds per pot. At sowing, the seeds were inoculated with peat culture of an effective *Rhizobium* sp. strain, IC 3195, suspended in water, at 10^7 rhizobia seed^{-1}. After emergence, the seedlings were thinned uniformly to four per pot.

The plants were infested with *Rivellia* sp. larvae by confining field-collected adults (10 per pot) in wire-mesh cages covering the plants in each pot. Eggs were laid on the plants and soil, and the larvae then moved to the nodules. This confinement of adults was carried out at weekly intervals from 4 to 7 weeks after sowing. The pots in the non-infested treatments were also covered by wire-mesh cages, but without *Rivellia* sp., for the appropriate duration, to avoid any confounding effect of caging *per se* on plant growth. All the pots were watered to maintain field capacity.

The required pots for the six treatments and six replications were kept in duplicate sets to permit destructive sampling at two stages of plant growth. Sixty-eight days after sowing, one set was sampled and the number of nodules (total and damaged), the acetylene reduction activity of available nodules, and leaf area and plant dry matter were recorded. Leaf area was measured by an Automatic Leaf Area Meter (Delta-T Devices Ltd., Burevell, Cambridge, England). In addition, the youngest fully open trifoliate leaf was assayed for total chlorophyll content, according to the method of Hiscox and Israelstam (1979).

The second sampling was caried out on mature plants (124 days after sowing) in the other set of pots. Fresh and dry weights of the above-ground (shoots + pods) and below-ground plant parts (roots + nodules) were recorded. Leaves, flowers, and pods shed before this sampling were also retained and added to the shoot weight. Dry plant powder was used to estimate N and P contents.

Results

Field experiment (study 1)

The overall percentage of nodules damaged by *Rivellia* sp. was greater in the Vertisols (86%) than in the Alfisols (20%). For ICPL 87, mean nodule damage in the Vertisols and the Alfisols was 88% (range 23–100) and 25% (0–50), respectively. For ICPL 270, the respective means were 90% (57 – 100) and 15% (4 – 94). However, there was significant variation, and a high coefficient of variation in the percentage of nodules damaged among replicate plots of the same genotype.

Field experiment (study 2)

Nodule damage was observed as early as 20 days after sowing, even though the nodules were still small (about 1 mm in diameter). Forty days after sowing, nodule damage (range 39% – 65%) was observed in all treatments, including the soil-insecticide-treated plots (Table 2). Aldrin treatment led to a significant reduction in the proportion of nodules damaged, with a concurrent increase in acetylene reduction activity of the nodules compared to the unprotected plants. The plants in treatments receiving aldrin, with or without urea, looked greener and slightly bigger than the plants grown in plots without the soil insecticide. In an attempt to ensure complete control of nodule damage, aldrin was supplemented with hexachlorocyclohexane at weekly intervals, starting 43 days after sowing. Substantial nodule damage (up to 39%) was observed in the insecticide-treated plots 68 days after sowing in spite of such an intensive use of insecticides. Up to this stage, the acetylene reduction activity of the nodules in plots protected by insecticides was higher than in the control plots. The shoot dry matter was little affected by insecticide application. Even 97 days after sowing the nodule damage persisted in the insecticide-treated plots (up to 40%). Further, there was an apparent reduction in shoot dry matter in these plots.

A grain yield of 2.9 t ha^{-1} (total of three harvests) was obtained in plots without either soil insecticides or urea (Table 2). The grain yield was significantly lower in the plots treated with soil insecticides, particularly in the absence of fertilizer N. At the first harvest, shoot dry matter tended to be low in the insecticide treated plots while at the final harvest it was greater in plots with fertilizer N than in those without fertilizer N. Overall, the shoot dry matter declined with successive harvests, due to loss of leaves and plant senescence.

The residual effect of pigeonpea grown with and without insecticides and fertilizer N during the rainy season of 1985 was studied on the yields of a sorghum hybrid CSH 6 grown in the subsequent rainy season 1986 (Table 2). Pigeonpea grown with fertilizer N increased the sorghum yields compared to pigeonpea without N. Further, the sorghum yields were consistently low in plots that had been treated with insecticide. The effect of nodule damage on plant growth could not be assessed despite the heavy insecticide applications because the insecticides did not effectively control the damage.

Table 2. Effect of soil insecticides (aldrin and hexachlorocyclohexane), applied at two levels of N, on nodule damage, acetylene reduction activity, and dry-matter yields of pigeonpea genotype ICPL 87 grown on Vertisols during rainy season 1985 – 1986 and the yields of sorghum subsequently grown during rainy season 1986 – 1987 at ICRISAT center

Effects	No N, no insecticide	No N, + insecticide	With N, no insecticide	With N, + insecticide	± SE[a]
A. Effect on pigeonpea crop, 1985 – 1986					
Nodules damaged (%)					
40 DAS[b]	62	46	65	39	4.5**
68 DAS[b]	44	39	34	33	4.7 NS
Acetylene reducing activity (μM C_2H_4 plant^{-1}h^{-1})					
40 DAS[b]	0.9	2.6	0.7	1.9	0.37**
68 DAS[b,c]	1.68	2.13	1.26	1.52	0.186*
Shoot dry matter at harvest (kg ha^{-1})					
Harvest 1	4070	3450	4040	3730	166.6 NS
Harvest 2	2870	2900	3090	3470	209.7**
Harvest 3	2690	2610	2870	2990	84.6*
Total grain yield (kg ha^{-1})					
Pooled for 3 harvests	2850	2350	2750	2610	60.7**
B. Effect on subsequent sorghum crop, 1986 – 1987					
Top dry matter (kg ha^{-1})					
At harvest	4450	4100	5160	4880	181**
Grain yield (kg ha^{-1})					
	1680	1490	1960	1800	68**

[a] * Significant at $P<0.05$; ** significant at $P<0.01$

[b] Days after sowing

[c] Values analysed after square root transformation

Pot experiment (study 3)

Observations made 68 days after sowing, showed that artifical infestation by *Rivellia* sp. reduced the total number of nodules at the low-N level but increased the number of nodules in the normal or added-N treatments (Table 3). The success of the artificial infestation as a means of creating substantial nodule damage (mean 70%) was clearly seen at all three N levels. Some damage (mean 16%) also occurred in control plants, probably due to lack of complete control over escape of *Rivellia* sp. in the experiment. Plants infested with *Rivellia* sp. showed a significant reduction in nodule dry weight (mean 46%), N_2-fixing ability (acetylene reduction activity; mean 31%), and in total leaf area (mean 36%) across the three N levels. The reduction in leaf chlorophyll content (mean 39%) was also significant, but differed in extent among the N levels. At this stage of the crop (68 days after sowing) there was a significant reduction (mean 23%) in shoot mass in the infested pots across the three N levels.

At maturity the impact of *Rivellia* sp. infestation persisted, as evidenced by a significant reduction in top dry mass (mean 22%), root and nodule dry mass (mean 27%), and seed dry mass (mean 14%) across the three N levels (Table 4). The yield of total N and P from the plants harvested was also significantly less in the pots infested with *Rivellia* sp. (mean reduction by 29% and 19%, respectively) but the extent of reduction differed significantly between N levels.

Discussion

In the first study, a wide range of variability in the percentage of damaged nodules was observed among replications (plots) in the same field. This result indicated that *Rivellia* sp. infestations can be highly heterogeneous, and so a large number of samples is required to represent a location. Nevertheless, the overall incidence of damage was much greater in the Vertisols than in the Alfisols. This trend has also been observed at the ICRISAT center in subsequent years. Probably, the soil type *per se* influences the preference for or survival of *Rivellia* sp., Vertisols being more attractive than Alfisols for infestation. Unpublished data on trap catches of *Rivellia* sp. adults at the ICRISAT center during 1986–1987 suggest that more adults were intercepted in Vertisols than in Alfisols. Studies on damage by *Metopina* sp. to chickpea nodules (Rupela et al. in preparation) have also shown that soil moisture and aeration tend to influence nodule damage levels. The exact reasons for the greater damage in fields of Vertisols still needs to be clarified.

The next experiment (study 2), designed to assess the impact of nodule damage under field conditions, was not successful, since the plots that were to be kept free from damage for comparison suffered substantial

Table 3. Effect of artificial infestation with *Rivellia* sp. under three levels of soil N on nodule damage, acetylene reduction activity, leaf area, leaf chlorophyll, and shoot dry weight of pigeonpea genotype ICPL 87 grown in pots containing a Vertisol, 68 days after sowing in rainy season 1986–1987, ICRISAT center

N level	*Rivellia* sp. infestation	Total root nodules (no.)	Nodules (%) damaged by *Rivellia* sp.	Nodule dry weight (mg pot^{-1})	Acetylene reduction activity (μM C_2H_4 pot^{-1}h^{-1})	Leaf area per pot (cm^2)	Leaf chlorophyll content (mg per g fresh weight)	Shoot dry weight (g per pot)
Low	Yes	193	91	61	0.7	85	0.85	2.2
	No	250	28	324	12.1	691	3.97	5.0
	Mean	221	60	193	6.4	388	2.41	3.6
Normal	Yes	447	64	590	28.4	1470	3.37	13.1
	No	212	10	929	25.3	2129	3.79	16.0
	Mean	330	37	760	26.9	1799	3.58	14.6
Added	Yes	258	55	459	16.4	1586	2.99	13.2
	No	213	9	806	28.5	2062	4.09	16.3
	Mean	236	32	632	22.5	1824	3.54	14.7
Overall	Yes	299	70	370	15.1	1047	2.40	9.5
	No	225	16	686	22.0	1627	3.95	12.4
	Mean	262	43	528	18.6	1337	3.18	11.0
Effect of N levels[a] ±SE		21.2**	4.7**	54.1**	2.33**	82.1*	0.139*	0.47*
Effect of *Rivellia* sp. infestation[a] ±SE		17.3**	3.8**	44.2**	1.91**	67.0*	0.113*	0.38*
Interaction[a] ±SE		29.9**	6.6	76.5	3.30	116.1	0.196**	0.66

[a] For significance see Table 2

nodule damage, in spite of heavy doses and repeated application of the two contact insecticides, aldrin and hexachlorocyclohexane. Sidderame Gowda and Siddappaji (1981) have recommended soil application of a systemic insecticide such as aldicarb for control of *Rivellia* sp. damage to nodules of several legumes. However, the effect of these insecticides on plant growth tends to confound the comparison with normal (control) plants. Insecticide use therefore appears to be an unsatisfactory way of assessing the impact of nodule damage in the field. The limited suppression of nodule damage in insecticide-treated plots seen in the early stages was reflected in an increase in the N_2-fixing ability (as assayed by acetylene reduction activity) of the plants. This was indirect evidence of the potential harm to the N economy of the plant from nodule damage.

The heavy dose and intensity of the insecticide applications in this trial – 18 kg of aldrin and 20 kg hexachlorocyclohexane (a.i. ha^{-1} in eight split doses) – seems to have adversely affected the soil environment. The total grain yield and shoot dry matter of pigeonpea, as well as the growth of a succeeding sorghum crop, were lower in these plots compared to plots without insecticide. This result indicated that these insecticides are of little use in studies aimed at assessing the impact of nodule damage on pigeonpea under field conditions.

The pot-culture study proved useful in assessing the impact of *Rivellia* sp. damage. A method of caging adult *Rivellia* sp. on pot-grown plants to create differing levels of nodule damage was developed only recently (Sithanantham et al. 1987). The increase in total nodules observed when plants were infested by *Rivellia* sp. in soils with normal or added N suggests that plants try to compensate for nodule damage by developing additional nodules. These additional nodules, however, were apparently not produced under low-N conditions. Substantial reductions in nodule dry weight and acetylene reduction activity were found in plants with damaged nodules, especially when the soil was low in N. This suggests that the plant response in increasing the number of nodules as compensation for damage does not really contribute to a better N balance. In soybean, Newsom et al. (1978) reported that insect injury to nodules led to reduced N_2 fixation, and in *Amphicarpa bracteta*, Foote (1985) has shown that nodule damage by *R. pallida* reduced N_2 fixation. The present results agree with these reports but are the first estimates in pigeonpea.

The other effects of nodule damage were the reductions seen in the leaf area and in the chlorophyll content, which reduce the photosynthetic ability of the plants; the effects are exacerbated by a diversion of photosynthates towards nodule repair in infested plants. The ultimate effect of substantial nodule damage in pigeonpea plants was seen as a reduction in shoot and root dry matter and in the mass of seeds produced. The quantity of N and P in the plants was also reduced as a consequence of nodule damage.

Table 4. Effect of artificial infestation of pot-grown pigeonpea (ICPL 87) plants with *Rivellia* sp. at three levels of soil N on plant growth at crop maturity, rainy season, 1986 – 1987, ICRISAT center

N level	*Rivellia* sp. infestation	Plant top dry weight (g pot^{-1})	Plant root plus nodule dry weight (g pot^{-1})	Seed dry weight (g pot^{-1})	Yield of total N (mg pot^{-1})	Yield of total P (mg pot^{-1})
Low	Yes	2.8	0.65	0.02	40	6.1
	No	10.6	3.12	0.27	264	21.9
	Mean	6.7	1.88	0.15	152	14.0
Normal	Yes	25.6	6.95	3.20	538	48.4
	No	30.2	8.44	4.03	636	53.6
	Mean	27.9	7.69	3.62	587	51.0
Added	Yes	26.8	6.80	3.82	504	49.6
	No	29.7	8.16	3.90	615	51.9
	Mean	28.3	7.48	3.86	559	50.8
Overall	Yes	18.4	4.80	2.35	361	34.7
	No	23.5	6.57	2.73	505	42.5
	Mean	21.0	5.69	2.54	433	38.6
Effect of N levels[a]						
±SE		0.87**	0.234**	0.421**	18.0**	1.47
Effect of *Rivellia* sp. infestation[a]						
±SE		0.71**	0.191**	0.344**	14.7**	1.20
Interaction[a]						
±SE		1.22	0.331	0.596	25.5*	2.07

[a] For significance see Table 2

Similar indirect effects of nodule damage on plant growth and yield have been reported earlier in soybean (Koethe and van Duyn 1984).

The adverse effects of nodule damage were relatively greater in soils low in available N, a common observation in the semi-arid tropics.

Management approaches to nodule damage include the selection of pigeonpea genotypes that can resist or tolerate the attack by *Rivellia* sp. or such cultural practices as irrigation, as observed for *Metopina* damage in chickpea (ICRISAT, unpublished data 1986). Another way of controlling nodule damage may be to develop pigeonpea *Rhizobium* sp. strains with the toxin-producing gene of *Bacillus thuringiensis* that is effective against Lepidopteran larvae. These *Rhizobium* sp. strains can probably be developed through genetic engineering. Another possibility is to construct transgenic plants of pigeonpea with insect resistance, for instance through the introduction of the gene for the *B. thuringiensis* insecticidal protein and its expression in the adult plant. The latter approach may be possible, since Vaeck et al. (1987) have reported on the feasibility of engineering tobacco plants that defend themselves against some Lepidopteran insects which are sensitive to the *B. thuringiensis* insect toxin.

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References

El-Swaify SA, Pathak P, Rego TJ, Singh S (1985) Soil management for optimized productivity under rainfed conditions in the semi-arid tropics. Adv Soil Sci 1:1 – 64

Foote BA (1985) Biology of *Rivellia pallida* (Diptera: Platystomatidae), a consumer of the nitrogen fixing root nodules of *Amphicarpa bracteata* (Leguminosae). J Kansas Entomol Soc 58:27 – 35

Hardy RWF, Havelka UD (1975) Nitrogen fixation research: A key to world food? Science 188:633 – 643

Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot 57:1332 – 1334

Koethe RW, van Duyn JW (1984) Aspects of larval and host relations of the soybean nodule fly, *Rivellia quadrifasciata* (Diptera: Platystomatidae). Environ Entomol 13:945 – 947

Kumar Rao JVDK, Dart PJ (1987) Nodulation, nitrogen fixation and nitrogen uptake in pigeonpeas (*Cajanus cajan* (L.) Millsp) of different maturity groups. Plant and Soil 99:255 – 266

Newsom LD, Dunigan EP, Eastman CE, Hatchison RL, McPherson RM (1978) Insect injury reduces nitrogen fixation in soybeans. Louisiana Agric 21:15 – 16

Sidderame Gowda TK, Siddappaji C (1981) *Rhizobium* nodule damage by insect fauna and their control. In: GK Veerash (ed) Progress in soil biology and ecology in India. Univ Agric Sci, Bangalore, Tech Ser No. 37, pp 228 – 230

Sithananthan S, Kumar Rao JVDK, Reed W, Dart PJ (1981) Studies on nodule damage in pigeonpea. In: Nene YL (ed) Proc Int Workshop on Pigeonpeas, vol. 2, 15 – 19 Dec 1980, ICRISAT, Patancheru, India, pp 409 – 415

Sithananthan S, Kumar Rao JVDK, Rameshwar Rao V (1987) A pot culture method for infesting pigeonpea root nodules with *Rivellia angulata*. Int Pigeonpea Newslett 6:68 – 69

Vaeck M, Reynarts A, Hofte H, Jansens S, Benckeleer MD, Dean C, Zabean M, Montagu MV, Leemans J (1987) Transgenic plants protected from insect attack. Nature (Lond) 328:33 – 37

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The other effects of nodule damage were the reductions seen in the leaf area and in the chlorophyll content, which reduce the photosynthetic ability of the plants; the effects are exacerbated by a diversion of photosynthates towards nodule repair in infested plants. The ultimate effect of substantial nodule damage in pigeonpea plants was seen as a reduction in shoot and root dry matter and in the mass of seeds produced. The quantity of N and P in the plants was also reduced as a consequence of nodule damage.

Table 4. Effect of artificial infestation of pot-grown pigeonpea (ICPL 87) plants with *Rivellia* sp. at three levels of soil N on plant growth at crop maturity, rainy season, 1986 – 1987, ICRISAT center

N level	*Rivellia* sp. infestation	Plant top dry weight (g pot^{-1})	Plant root plus nodule dry weight (g pot^{-1})	Seed dry weight (g pot^{-1})	Yield of total N (mg pot^{-1})	Yield of total P (mg pot^{-1})
Low	Yes	2.8	0.65	0.02	40	6.1
	No	10.6	3.12	0.27	264	21.9
	Mean	6.7	1.88	0.15	152	14.0
Normal	Yes	25.6	6.95	3.20	538	48.4
	No	30.2	8.44	4.03	636	53.6
	Mean	27.9	7.69	3.62	587	51.0
Added	Yes	26.8	6.80	3.82	504	49.6
	No	29.7	8.16	3.90	615	51.9
	Mean	28.3	7.48	3.86	559	50.8
Overall	Yes	18.4	4.80	2.35	361	34.7
	No	23.5	6.57	2.73	505	42.5
	Mean	21.0	5.69	2.54	433	38.6
Effect of N levels[a]						
± SE		0.87**	0.234**	0.421**	18.0**	1.47**
Effect of *Rivellia* sp. infestation[a]						
± SE		0.71**	0.191**	0.344**	14.7**	1.20*
Interaction[a]						
± SE		1.22	0.331	0.596	25.5*	2.07**

[a] For significance see Table 2

Similar indirect effects of nodule damage on plant growth and yield have been reported earlier in soybean (Koethe and van Duyn 1984).

The adverse effects of nodule damage were relatively greater in soils low in available N, a common observation in the semi-arid tropics.

Management approaches to nodule damage include the selection of pigeonpea genotypes that can resist or tolerate the attack by *Rivellia* sp. or such cultural practices as irrigation, as observed for *Metopina* damage in chickpea (ICRISAT, unpublished data 1986). Another way of controlling nodule damage may be to develop pigeonpea *Rhizobium* sp. strains with the toxin-producing gene of *Bacillus thuringiensis* that is effective against Lepidopteran larvae. These *Rhizobium* sp. strains can probably be developed through genetic engineering. Another possibility is to construct transgenic plants of pigeonpea with insect resistance, for instance through the introduction of the gene for the *B. thuringiensis* insecticidal protein and its expression in the adult plant. The latter approach may be possible, since Vaeck et al. (1987) have reported on the feasibility of engineering tobacco plants that defend themselves against some Lepidopteran insects which are sensitive to the *B. thuringiensis* insect toxin.

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References

El-Swaify SA, Pathak P, Rego TJ, Singh S (1985) Soil management for optimized productivity under rainfed conditions in the semi-arid tropics. Adv Soil Sci 1:1–64

Foote BA (1985) Biology of *Rivellia pallida* (Diptera: Platystomatidae), a consumer of the nitrogen fixing root nodules of *Amphicarpa bracteata* (Leguminosae). J Kansas Entomol Soc 58:27–35

Hardy RWF, Havelka UD (1975) Nitrogen fixation research: A key to world food? Science 188:633–643

Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot 57:1332–1334

Koethe RW, van Duyn JW (1984) Aspects of larval and host relations of the soybean nodule fly, *Rivellia quadrifasciata* (Diptera: Platystomatidae). Environ Entomol 13:945–947

Kumar Rao JVDK, Dart PJ (1987) Nodulation, nitrogen fixation and nitrogen uptake in pigeonpeas (*Cajanus cajan* (L.) Millsp) of different maturity groups. Plant and Soil 99:255–266

Newsom LD, Dunigan EP, Eastman CE, Hatchison RL, McPherson RM (1978) Insect injury reduces nitrogen fixation in soybeans. Louisiana Agric 21:15–16

Sidderame Gowda TK, Siddappaji C (1981) *Rhizobium* nodule damage by insect fauna and their control. In: GK Veerash (ed) Progress in soil biology and ecology in India. Univ Agric Sci, Bangalore, Tech Ser No. 37, pp 228–230

Sithanantham S, Kumar Rao JVDK, Reed W, Dart PJ (1981) Studies on nodule damage in pigeonpea. In: Nene YL (ed) Proc Int Workshop on Pigeonpeas, vol. 2, 15–19 Dec 1980, ICRISAT, Patancheru, India, pp 409–415

Sithanantham S, Kumar Rao JVDK, Rameshwar Rao V (1987) A pot culture method for infesting pigeonpea root nodules with *Rivellia angulata*. Int Pigeonpea Newslett 6:68–69

Vaeck M, Reynarts A, Hofte H, Jansens S, Benckeleer MD, Dean C, Zabean M, Montagu MV, Leemans J (1987) Transgenic plants protected from insect attack. Nature (Lond) 328:33–37

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