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Measurement of N_2-fixation in field-grown pigeonpea [*Cajanus cajan* (L.) Millsp.] using ^{15}N-labelled fertilizer

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Abstract

Two experiments were carried out from 1981 to 1983 in Vertisol fields at ICRISAT Center, Patancheru, India to measure N_2-fixation of pigeonpea [*Cajanus cajan* (L.) Millsp.] using the ^{15}N isotope dilution technique. One experiment examined the effect of control of a nodule-eating insect on fixation while another investigated the effect of intercroping with cereals on fixation and the residual effect of pigeonpea on a succeeding cereal crop. Although both experiments indicated that at least 88% of the N in pigeonpea was fixed from the atmosphere, one result is considered fortuitous in view of the differential rates of growth of the legume and the control, sorghum [*Sorghum bicolor* (L.) Moench]. The difference method of calculation indicated negative fixation and the results emphasized the problem of finding a suitable nonfixing control. In a second experiment, when all plants were confined to a known volume of soil to which ^{15}N fertilizer was added in the field, these problems were overcome, and isotope dilution and difference methods gave similar results of N_2-fixation of about 90%. In intercropped pigeonpea 96% of the total N was derived from the atmosphere. This estimate might be an artifact. There was no evidence of benefit from N fixed by pigeonpea to intercropped sorghum plants. Plant tissue ^{15}N enrichments of cereal crops grown after pigeonpea indicated that the cereal derived some N fixed by the previous pigeonpea. Thus residual benefits to cereals are not only an effect of 'sparing' of soil N.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is one of the major grain legumes of the semi-arid tropics. It is sometimes grown as a pure crop, but more typically it is grown in relatively complex systems where it is intercropped or mixed with other cereal or pulse crops (Willey *et al.*, 1981). Measurement of N_2 fixation is necessary to evaluate the contribution of N from pigeonpea in these cropping systems. Using the difference method (*i.e.* the difference in the total N content of the legume and a nonfixing control plant), with sorghum as a nonfixing control, Kumar Rao *et al.* (1981) estimated that the amount of N_2-fixed by pigeonpea genotypes of different maturity groups ranged between 6 and 69 $kg\,N\,ha^{-1}$. These estimates can only be approximate. The assumption was made that no differences existed in soil N uptake between plants of pigeonpea and sorghum, and also the losses due to denitrification and leaching were not accounted for.

Use of the stable isotope ^{15}N allows quantification of symbiotically fixed N_2, soil- and fertilizer-derived N in legumes by application of either the isotope dilution technique (Legg and Sloger, 1975; Rennie *et al.*, 1978) or the modified 'A' value method (Fried and Broeshart, 1975). A nonfixing reference plant similar to the legume in rooting pattern and timing of soil N assimilation gives the

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most accurate results (Witty, 1983). La Favre and Focht (1983) reported that 91 to 94% plant N of pigeonpea, grown in a greenhouse in soil columns containing ^{15}N-enriched organic matter, was derived from N_2 fixation. No such studies have been reported with pigeonpea growing in the field

In 1980 an attempt was made to measure N_2-fixation by two pigeonpea cultivars in a field sowing in Alfisol soil at ICRISAT Center, Patancheru, India. Castor bean (*Ricinus communis* L.) was used as the nonfixing control due to its suitability to this soil and environment but plant growth and nodulation of pigeonpeas were poor, apparently due to damage by the nematode Hoplolaimus, and no N_2-fixation was detected (JVDK Kumar Rao and PJ Dart, unpublished data).

Between 1981 and 1983 two field experiments were conducted on Vertisols at ICRISAT Center, Patancheru, India, to measure N_2-fixation of sole pigeonpea using the isotope dilution technique. In addition one experiment examined the effect of attempted control of a nodule-eating insect (*Rivellia angulata*) on N_2-fixation, while the other investigated the effect of intercropping with cereals on N_2-fixation and the residual effect of pigeonpea on a succeeding cereal.

Materials and methods

For isotope dilution studies with pigeonpea grown in Alfisols castor bean is considered to be a suitable nonfixing control plant because of its similarity to pigeonpea in duration and ability to grow on marginal lands. However, the castor bean does not grow well in Vertisols. Hence a long-duration sorghum [*Sorghum bicolor* (L.) Moench] was chosen as a suitable control for use in Vertisol although it was difficult to choose a cultivar with a maturity similar to pigeonpea cultivars used.

The two Vertisol fields used in the studies had the following characteristics

Factor	Experiment 1[a]	Experiment 2[b]
pH	8.3	8.1
Soluble salts (mmhos cm^{-1})	0.23	0.28
Organic matter (%)	0.78	n.d.+
Inorganic N(mg kg^{-1})	15.0	6.5
Olsen's P(mg kg^{-1})	5.5	3.0

[a] Measurement of N_2-fixation and the effects of attempted control of nodule eating insects

[b] Measurement of N_2-fixation by pigeonpea as a sole crop and intercropped with sorghum and the residual effect of pigeonpea

\+ n.d. not determined

Experiment 1. Measurement of N_2-fixation and the effects of attempted control of nodule eating insects

The following treatments were randomized in a randomized block design with three replicates: 1) Aldrin spray on soil surface at 2 kg active ingredient ha^{-1} per application; 2) Carbofuran granules placed at a depth of about 7 cm at 40 kg of 3% granules ha^{-1} per application; and 3) No insecticide (control). Aldrin was sprayed on the soil surface at sowing , 25 and 55 days after sowing to prevent the egg laying of *R. angulata.* Carbofuran (a systemic insecticide) applied only in treatment 2, was mixed with the top 8 cm of soil at sowing and applied in a furrow 8 cm deep and 15 cm away from plant row at 25 and 55 days after sowing to give protection against all soil-borne insects, including *R. angulata.*

Pigeonpea cv. ICP 1 (maturity 6 months) and sorghum cv. IS 19747 were sown in 10 × 10 m plots with 50 cm from row to row and 20 cm from plant to plant within the row. Within each plot there were 16 rows of pigeonpea and 4 rows of sorghum in the middle of the plot. A basal dressing of 17 kg P ha^{-1} as single superphosphate was broadcast and mixed with the top 5 cm soil in all the plots. Nitrogen-15 was applied to the aldrin and control treatments only on a subplot of 3 m^2, which consisted of a 1.2 m length of 3 rows of pigeonpea and an adjacent 2 rows of sorghum. Labelled nitrogen ($(^{15}NH_4)_2SO_4$ at the rate of 20 kg N ha^{-1} containing 2.428 atom % excess ^{15}N) was dissolved in tap water and sprayed on the soil surface at the rate of 200 ml m^{-2}, and the same amount of unlabelled N-fertilizer was similarly applied to the remainder of each plot. Pigeonpea seeds were inoculated with peat inoculant of an effective Rhizobium strain IHP 195 (10^5 cells $seed^{-1}$).

Pigeonpea plants were sampled from the unlabelled areas for nodulation, acetylene reduction activity (ARA), and dry matter at 47 and 104 days after sowing. Sorghum was not sampled for ARA and dry matter along with pigeonpea. All fallen plant parts of pigeonpea were collected from 3 m^2

each of labelled and unlabelled areas for analysis. All ^{15}N subplots were harvested 140 days after sowing.

Only results from aldrin and control treatments are presented.

Experiment 2. Measurement of N_2-fixation by pigeonpea as a sole crop and intercropped with sorghum and the residual effect of pigeonpea

Plants were grown in plots measuring 3.85 × 3.85 m in the center of which a 75 cm diameter cylinder was buried to its full depth of 60 cm. To place the cylinder, soil was excavated from the center of the plot in 15 cm layers. The bottom of each cylinder was closed with polyamide cloth, preventing roots from penetrating but allowing water to drain away, and the original soil was used to fill the cylinders in the same sequence. A mixture of ^{15}N labelled fertilizer ($K^{15}NO_3$ 40 atom % excess) at the rate of 2 kg ^{15}N ha^{-1} and sucrose solution was applied on each 15 cm layer. The C:N ratio of the fertilizer:sucrose mixture was 10:1, to promote microbial incorporation of inorganic N into organic forms so as to maintain the ^{15}N availability at a fairly constant rate to both the legume and the nonfixing control throughout the growth period. The cylinders were left for 15 days before sowing. A basal dressing of 17 kg P ha^{-1} as single superphosphate was banded along the rows 10 cm deep in all plots.

In addition to sole pigeonpea, treatments were chosen to examine the pigeonpea-sorghum intercrop. The normal combination of pigeonpea with a hybrid sorghum (in this case cv. ICP 1–6 and CSH 6, respectively) was examined and the two crops finally harvested at their respective maturity dates. The pigeonpea was flowering at the time of harvest of the sorghum hybrid. The sorghum cultivar, IS 3003, having similar maturity to the pigeonpea, was used as a nonfixing control for the isotope dilution calculation. It was also included in an intercrop with pigeonpea to examine the possibility of N transfer from pigeonpea after flowering. The treatments also included a sole crop of IS 3003 supplied with dried, fallen plant parts of pigeonpea (equivalent to about 40 kg N ha^{-1}) mixed with the top soil at sowing. This rate of leafy material is commonly deposited under a pure pigeonpea crop.

Sole cropped pigeonpea was planted in rows 75 cm apart, while sole sorghum and the intercrop of sorghum and pigeonpea (2 rows of sorghum followed by 1 row of pigeonpea) were planted in 37.5 cm rows. Plant-to-plant spacing within rows was 25 cm in all treatments. Pigeonpea seeds were inoculated with peat inoculant of Rhizobium strain IHP 195 before sowing.

At 38 days after sowing, 10 pigeonpea plants from sole and intercropped plots (outside the cylinders) were examined for nodulation, ARA, and dry matter production. The hybrid sorghum was harvested 112 days after sowing and the pigeonpea and sorghum cultivars 160 days after sowing.

In the following year, 1983 rainy season, the plots were cultivated taking care to minimize carryover of soil between plots, while soil in the cylinders (top 15 cm) was manually loosened. Maize cv Deccan Hybrid 101 was sown on 22 June 1983 at a spacing of 75 cm between rows and 20 cm within a row. A total of 17 kg P ha^{-1} as single superphosphate was uniformly applied as a band 20 days after sowing. The crop was harvested 83 days after sowing.

Analytical methods

Plant parts collected from the ^{15}N-labelled area were analysed for total N and ^{15}N content. Total N was estimated by a semi-micro-Kjeldahl method (Anonymous, 1978) and ^{15}N measurements were made using a Micromass M622 mass spectrometer on samples prepared from the digest by a Conway micro-diffusion procedure (Conway, 1939) at the Rothamsted Experimental Station, England.

Results

Experiment 1

Both insecticides were effective in reducing the proportion of nodules damaged after 47 days, but nodule dry weight was only slightly affected and the effect had disappeared by 104 days when the insecticide-treated plots had fewer nodules than the control. There were no significant differences in any other parameter and by 140 days aldrin-treated and control plots of pigeonpea yielded the same (Table

Table 1. Total dry matter, nitrogen yields, atom % ^{15}N excess (weighted means) of pigeonpea and sorghum and N_2 fixation by pigeonpea as affected by Aldrin at 140 days after sowing, ICRISAT Center, rainy season, 1981

Treatment	Dry matter (kg ha^{-1})		N yield (kg ha^{-1})		Atom % ^{15}N excess		% NdfF[a]		% NdfS[b]	% FUE[c]		% N fixed by pp	N_2 fixed by pp (kg ha^{-1})
	PP	S	PP	S	PP	S	PP	S	S	PP	S		
Aldrin	5540	11660	78	68	0.0243	0.2047	1.0	8.4	91.6	3.9	27.8	88	69
No Aldrin	5630	17530	77	92	0.0194	0.1547	0.8	6.4	93.6	3.1	29.2	88	68
SE ±	510	1865	5	10	0.0060	0.0307	0.1	1.5	1.5	0.3	3	3	4
CV (%)	16	22	10	22	48	30	30	35	3	25	19	6	9

PP = Pigeonpea; S = Sorghum

[a] % Nitrogen derived from fertilizer

[b] % Nitrogen derived from soil

[c] % Fertilizer use efficiency

1). However, sorghum growth was adversely affected by aldrin.

Throughout the growing period the sorghum produced more dry matter than the pigeonpea, an effect particularly obvious in the first 60 days (data not presented). At harvest, at 140 days, pigeonpea was still in the mid-podfilling stage while sorghum was mature. Total dry matter of sorghum was greater than that of pigeonpea (Table 1) but the differences in N uptake were minimal, suggesting that factors other than N were controlling growth. Nevertheless, ^{15}N enrichment was higher in sorghum than pigeonpea with both treatments (Table 1) and the calculated proportion of N derived from fixation in pigeonpea was 88%, amounting to 68–69 kg N ha^{-1}.

Experiment 2

Pigeonpea was well nodulated at 38 days, and no significant treatment differences were found for the nodulation, nitrogenase activity, or dry matter (data not presented). At final harvest pigeonpea and sorghum each grew better as sole crops than in intercrops. In contrast to Experiment 1, where availability of N did not appear to be limiting (Table 1), N yield in sorghum in this experiment (Table 2) was much less than by pigeonpea. Again it was found that 88% of the N in pigeonpea grown as a sole crop was derived from N_2 fixation while intercropped pigeonpea derived 96% of its N from symbiosis (Table 2). This presumably happened because the sorghum crop depleted most of the

Table 2. Grain, total dry matter and N yields and atom % ^{15}N excess of pigeonpea ICP 1–6 and sorghum cultivars CSH 6 and IS 3003 at maturity, and N_2 fixation by pigeonpea grown in cylinders[a], ICRISAT Center, rainy season 1982

Treatment	Grain yield (g cylinder^{-1})	Dry matter (g crop cylinder^{-1})	N yield (g crop cylinder^{-1})	Atom % ^{15}N excess	% N derived from fixation by pigeonpea
1. Pigeonpea (PP)	52.5	352	4.41	0.058	88
2. Sorghum hybrid CSH 6 (SH)	19.4	143	0.71	0.984	
3. Sorghum cv IS 3003 (SC)	4.7	252	0.84	0.498	
4. Pigeonpea intercropped with CSH 6	PP 35.5	259	3.44	0.017	96
	SH 18.6	116	0.59	1.032	
5. Pigeonpea intercropped with IS 3003	PP 20.1	231	2.52	0.019	96
	SC 0.3	146	0.55	0.796	
6. IS 3003 with fallen plant parts	3.8	242	0.77	0.291	
SE ±	3.8	32.9	0.312		NS[b]

[a] Area of cylinder-enclosed soil surface = 0.44 m^2.

[b] The difference in the magnitude of % N fixed by pigeonpea under Treatment 1 and Treatment 4 or Treatment 5 was detected to be insignificant at $P = 0.05$.

Table 3. Total dry matter, N and ^{15}N enrichment of maize cv Deccan Hybrid 101 at maturity grown in concrete cylinders in rainy season, 1983

Previous treatment (in rainy season 1982)	Dry matter g cylinder^{-1}	N yield mg cylinder^{-1}	^{15}N atom excess (%)
Pigeonpea	221	960	0.077
Sorghum hybrid CSH 6	227	925	0.132
Sorghum cv IS 3003	75	356	0.118
Pigeonpea intercropped with CSH 6	162	687	0.081
Pigeonpea intercropped with IS 3003	135	617	0.098
IS 3003 with fallen plant parts	102	469	0.118
SE±	34.4	156.7	0.0195

available N, thereby making pigeonpea more dependent on symbiosis for its N requirements. By the difference calculation using cv. IS 3003 as the nonfixing reference 3.57 g N was fixed cylinder^{-1} (Table 2) or 81 kg N ha^{-1}.

If there were transfer from the legume to the cereal, the ^{15}N enrichment of sorghum grown as an intercrop would be less than as a sole crop, but this was not found (Table 2). The reasons for the lower enrichment of the sorghum cv. IS 3003 than the hybrid are not clear, however, it might be due to different fertilizer utilization efficiency (FUE) of different genotypes.

Residual effect of pigeonpea

Pigeonpea grown as sole crop did not significantly benefit maize yield over that of the sorghum hybrid CSH 6 (Table 3) but the low ^{15}N enrichment of maize following pigeonpea either as sole or intercrop compared with the sorghums suggested that a greater part of the available soil N was from residual fixed N.

Discussion

Choice of nonfixing reference plants

In Experiment 1 the sorghum cultivar grew faster and matured earlier than pigeonpea during early growth. Generally pigeonpeas grow slowly in the early stages at a rate of 1.3–3.0 kg ha^{-1} day^{-1} for the period from 7 to 35 days after sowing; 8–16 kg ha^{-1} day^{-1} in the 35 to 63-day period. Although pigeonpea could grow as fast as sorghum in the later stages (as high as 170 kg ha^{-1} day^{-1}) (Sheldrake and Narayanan, 1979) comparable early figures for sorghum hybrid CSH 6 were about 70 and 220 kg ha^{-1} day^{-1} (M Natarajan, personal communication). Such differences in growth rate could well have differentially affected the uptake of applied ^{15}N as shown in % NdfF and % FUE in Table 1. Not only could sorghum have had more opportunity to rapidly utilize the available ^{15}N because of more complete and rapid growth in the soil, but this utilization could have occurred before the decline of ^{15}N either from mineralization of ^{14}N from organic matter or from leaching and other losses (*cf.* Witty, 1983). If the sorghum carried on artificially high level of ^{15}N, the N_2 fixed by pigeonpea would have been overestimated.

The problem encountered with an unsuitable reference plant is exacerbated when applying the difference method to these results. In the control plots, with best sorghum growth, N uptake by sorghum was greater than by pigeonpea, apparently as a result of exploitation of a greater volume of soil.

The problem of differential growth rates, however, was successfully overcome in Experiment 2, when the roots of all crops were confined to the same volume by the cylinder and the root-proof cloth. Thus in spite of the lower yields of the sole sorghum crops compared with pigeonpea (Table 2) total dry matter production for the intercrops were essentially the same as the pure pigeonpea, suggesting equal utilization of the nutrients and moisture in the cylinders. Using the long-duration sorghum cv IS 3003 as the nonfixing reference, pigeonpea was estimated by isotope dilution to have fixed 3.89 g N cylinder^{-1} (=88 kg N ha^{-1}) and a similar figure of 81 kg N ha^{-1} by the difference method as the requirements of a nonfixing control are similar in both the methods.

Thus the apparent unsuitability of the nonfixing control, as in the first experiment, can be overcome by this method of restriction of the roots, even though the different crops may complete their growth at a different rate. We therefore conclude that the estimates of fixation obtained in the second experiment are likely to be accurate and they are certainly of the same order as those reported by La Favre and Focht (1983), who found in greenhouse studies that pigeonpea fixed 91–94% of its total N. However, the estimate of fixation by intercropped pigeonpea might be an artifact because the percent utilization of fertilizer N by the legume in intercropping was less compared to that of sole crop. This reduced percent utilization of fertilizer N by intercropped pigeonpea may be attributed to the competition for available nutients, including ^{15}N, from the associated sorghum crop. The finding in Experiment 1 of fixation of 88% of the total N must be considered as fortuitous in view of the visual evidence of differential growth rates between pigeonpea and the sorghum control and the overall differences between N uptake.

Without root restriction the choice of a suitable nonfixing control certainly poses problems with pigeonpea, because of its slow initial growth. To date, no non-nodulating lines or isolines are available, although even such desirable controls are not necessarily the most suitable (Rennie, 1982). Of the agricultural crops currently grown in India only sorghum and cotton (*Gossypium* spp.) in heavy Vertisols and castor bean in Alfisols have maturities comparable to that of pigeonpea. The suitability of castor needs to be evaluated in the Alfisols.

Availability of N_2-fixed by pigeonpea to intercropped or succeeding cereal crops

The fact that a component of mixed cropping systems in India is commonly a legume has focussed attention on the possibility that N_2 fixed in the root nodules may be utilized by the associated crop. Eaglesham *et al.* (1981) reported transfer of fixed nitrogen from cowpea [*Vigna unguiculata* (L.) Walp.] to maize based on significant dilution of ^{15}N in intercropped maize compared to sole cropped maize, but no such dilution was detected in Experiment 2 (Table 2).

Kumar Rao *et al.* (1983) reported that fallen leaf material could be a factor contributing to the beneficial effect of pigeonpea on a succeeding cereal crop. When fallen leaves were incorporated into the soil at a similar rate to that normally lost by pigeonpea (40 kg N ha^{-1}), a slight but non-signficant dilution of ^{15}N enrichment of plant tissues in sorghum was found (Table 2, treatments 3 and 6). But there was no increase in total N uptake by sorghum, suggesting that factors other than N were limiting. The lack of an increase in growth of sorghum plants with fallen leaves was probably due to the immobilization of the available N in the soil.

In the 1983 maize crop, sown following Experiment 2, the ^{15}N enrichment of the maize was lower in all treatments in which pigeonpea was grown previously (Table 3), indicating that some of the N absorbed came from N_2 fixed by the previous crop. Total N yield was much less where long duration sorghum had been grown previously, indicating that this crop depleted more soil-N than pigeonpea. The shorter duration sorghum hybrid, however, did not have the same effect of reducing growth of the subsequent maize, perhaps because it had been harvested much earlier and mineralization was allowed to increase the amount of plant available N and other nutrients.

Using the plant tissue enrichments of maize to calculate the contribution of N_2 fixed by the legume to the N in the maize it is indicated that 35% of the N in maize came from N_2 fixed by the pigeonpea. However, it is not clear whether the same contribution would be made in open soil where the fixed N in underground plant parts of pigeonpea might have been more dispersed. It is clearly demonstrated that some N in the underground plant parts of pigeonpea is available to growth of a succeeding crop, and any beneficial effect is not confined to sparing of soil N.

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