biotic titre was obtained at 120 hr. The results of various aeration levels (table 1) showed that maximum antibiotic level was obtained with 0.25 VVM aeration at 120 hr.

The control flask (shake flask on rotary shaker) always gave the maximum yield of 7200 units/ml at 144 hr.

The above data indicate that the provision of adequate aeration increased the antibiotic titre in the fermentor and maximum titre obtained in a shorter period when compared to the shake flasks. Although the difference in the antibiotic titre run at 0.5, 0.75 and 1.0 VVM aeration levels was not significant, the antibiotic yield was maximum at 0.25 VVM aeration level. The data at 0.25 VVM indicates that reduction of air supply towards the end of the growth phase does not significantly affect neomycin titre. Further, it indicates that 0.25 VVM may be the critical level.

16 June 1986; Revised 22 August 1986

- 1. Bartholomew, W. H. Karow, E. O., Sfat, M. R. and Wilhelm, K. H., *Ind. Eng. Chem.*, 1950, **42**, 1801.
- 2. Strohm, J., Dale, R. F. and Peppler, H. J., Appl. Microbiol., 1959, 7, 235.
- 3. Bartholomew, W. H., Adv. Appl. Microbiol., 1960, 2, 294.
- 4. Virgilio, A., Marcelli, E. and Agrimino, A., Biotech. Bioeng., 1964, 6, 271.
- 5. Feren, C. J. and Squires, R. W., Biotech. Bioeng., 1969, 11, 583.
- 6. Robinson, C. W. and Wilke, C. R., Proceedings of the 4th International Fermentation Symposium, 1972, p. 73.
- 7. Lockhart, W. R. and Squires R. W., Adv. Appl. Microbiol., 1963, 5, 157.
- 8. Pertti, H., Markkanen and Michael J. Bailey, J. Appl. Chem. Biotechnol., 1975, 25, 863.
- 9. Sambamurthy, K. and Ellaiah, P., Hind. Antibiot. Bull., 1974, 17, 24 and 41.
- 10. Grove, D. C. and Randall, W. A., Assay methods of antibiotics: A laboratory manual, Medical Encyclopaedia, Inc., New York, 1955.
- 11. Pharmacopoeia of India, Supplement, Government of India, 1975.

NITROGEN FIXATION AND TRANSFER OF FIXED NITROGEN TO ASSOCIATED CEREAL IN A MAIZE-COWPEA MIXED CROPPING SYSTEM

D. D. PATRA* and B. V. SUBBIAH
Nuclear Research Laboratory, Indian Agricultural
Research Institute, New Delhi 110 012, India.

* Present address: Department of Soil Science and
Agricultural Chemistry, SKN College of Agriculture,
Johner 303 329, India.

In mixed cropping systems there are a number of possible pathways by which nitrogen enters the cereal and legume components of the cereal-legume association. The contribution from soil and fertilizer is directly evaluated by labelling soil or fertilizer N. The contribution from atmospheric source in the case of legume and legume fixed source in the case of cereal is extremely difficult to estimate by using simple isotopic techniques. The use of ¹⁵N enriched materials to label the organic N in soil provides a method to estimate the amount of N fixed by a legume. This technique depends on the use of ¹⁵N enriched materials incorporated into the soil and pre-incubation of the soil to immobilize the 15N, thus bringing a rapid equilibration of the added N availability ratio. Several workers have compared this technique with other methods. Good agreement was observed between ¹⁵N dilution¹ and difference methods² but estimates of acetylene reduction method³ was lower. In the present investigation soil and fertilizer N labelling technique4 has been adopted for a quantitative estimation of the N fixed by cowpea and transferred to companion maize in a mixed cropping system.

A field study was conducted at IARI, New Delhi in 1981–82. The soil of the experimental site had a pH 8.6, organic C, 0.64%, total N, 0.07%, CEC, 8.2 me/100 g soil and the texture sandy loam. Available P and exchange K content were 0.07% and 1.8 me/100 g soil respectively. The soil belonged to Mehrauli series and had been classified as non-acid hypothermic family of Ustochrepts.

Maize (Zea mays L) and cowpea (Vigna sinensis L) were grown together and separately to assess the amount of N fixed by cowpea and possible transfer of N to the companion maize. Crops were grown in three combinations viz (i) maize alone, (ii) maize-cowpea mixed cropping and (iii) cowpea alone. The mixed cropping had the same maize plant population as was in pure maize and cowpea was grown in

between two rows of maize. Fertilizer as well as soil ¹⁵N labelling technique was adopted to differentiate the uptake of N from soil and fertilizer. Soil N was labelled by applying ¹⁵N labelled urea (2.000 at.% excess ¹⁵N) @ 100 kg N ha⁻¹ to half of the plots and incubated for six months prior to sowing. The remaining half of the plots received nonlabelled urea at the same time and rate to maintain uniform N status. During sowing labelled urea (1.928 at.% excess ¹⁵N) was applied to nonlabelled plots and nonlabelled urea to labelled plots @ 120 and 60 kg N ha⁻¹ to pure cropped maize and mixed cropping

respectively. Cowpea alone in either case received no fertilizer N. The treatment combinations thus consisted of three cropping systems and replicated thrice for two labelled N sources i.e. (a) soil N labelled with ¹⁵N and (b) fertilizer N labelled with ¹⁵N. A basal dose of P₂O₅ and K₂O at the rate of 60 kg ha⁻¹ was applied to all treatment combinations. The plant samples were taken at 30 and 60 days after sowing and at maturity stage. The oven-dried (70°C) plant samples were analyzed for total N following Kjeldahl method⁵. The ¹⁵N/¹⁴N ratios in the concentrated distillates were deter-

Table 1 Relative uptake (mg/plant) of nitrogen by plants from soil, fertilizer and atmosphere at different stages of growth (mean of three replications)

Crops	Total uptake	Uptake from ferti- lizer	Uptake from soil	Fixed by cowpea/contribution to maize
30 days after sowing				
Maize alone	55.95	21.83 (39.02)	31.66 (56.59)	2.46 (4 40)
Maize cropped with cowpea	55.45	19.85 (35.79)	32.35 (58.34)	3.25 (5.86)
Cowpea alone	113.60		67.22 (59.17)	46.38 (40.83)
Cowpea cropped with maize	96.36	27.15 (28.17)	42.51 (44.12)	26.70 (27.71)
60 days after sowing				
Maize alone	286.80	142.50 (49.69)	135 60 (47.31)	8.70 (3.03)
Maize cropped with cowpea	252.20	100.80 (40.00)	122.40 (48.50)	28.97 (11.50)
Cowpea alone	254.15	_	128.80 (50.70)	125.30 (49.30)
Cowpea cropped with maize	218.10	40.77 (18.73)	77.10 (35.40)	100.20 (45.95)
At maturity				
Maize alone	1927.50	1090.20 (56.60)	818.60 (42.50)	18.70 (0.97)
Maize cropped with cowpea	1856.60	640.30 (34.50)	706.40 (38.05)	509.90 (27.46)
Cowpea alone	844.47	-	393.80 (46.64)	450.60 (53.40)
Cowpea cropped with maize	892.00	196.20 (22.10)	308.60 (34.60)	386.40 (43.32)

mined by an emission analyzer⁶ as emission spectrometry has some advantages compared to mass spectrometry⁷ and its accuracy can also be improved to ± 0.0002 at.% in 0.366–1.37 at.% ¹⁵N.

The data (table 1) reveal that at first sampling (30) days after sowing) the total N uptake was similar in maize grown as sole crop and that under mixed cropping; but in case of cowpea although no N has been applied to sole cropping, its total uptake of N (113.6 mg plant⁻¹) was higher than that under mixed cropping system (96.4 mg plant⁻¹). Maize took up 36-39% of the total N from fertilizer and the rest from the soil with no significant difference between pure and mixed cropping. Cowpea as a sole crop fixed about 41% of its total N uptake while as mixed crop fixed only 28% from the atmosphere. Similarly, the contribution of soil N was less in mixed cropped cowpea as compared to that under pure cropping, as a part of the total N came from the fertilizer source. In the second stage (60 days after sowing), the pure cropped maize took almost an equal amount of N from fertilizer and soil; however, the mixed cropped maize depended much on soil N. About 29 mg N plant⁻¹ of the mixed cropped maize came from the source other than soil and fertilizer. Similarly pure cropped maize received 8.7 mg N plant⁻¹ from some unknown source. The increased amount of N in mixed cropped maize was presumed to be transferred from the companion cowpea either through underground transfer of organic N compunds or nodule decay. The cowpea under pure cropping fixed about 49% of its total N uptake; the intercropped cowpea on the other hand fixed about 46% of its total N uptake and took 19 and 35% from fertilizer and soil respectively.

At the maturity stage, the contribution from fertilizer and soil towards the total N uptake of pure cropped maize was 57 and 43% respectively. The intercropped maize took 35 and 38% of its total N from fertilizer and soil respectively; the remaining from legume and other sources. The pure cropped cowpea fixed significantly higher percentage of its N (53%) from the atmosphere as compared to that as mixed crop (43%). It was observed that cowpea fixed 451 mg N plant⁻¹ when grown alone and 386 mg N plant⁻¹ when grown with maize. The reduction in N fixation by the mixed cropped cowpea was attributed to shading by the companion maize causing retardation of photosynthesis 10. Furthermore, application of 60 kg N ha⁻¹ to the mixed cropping met a part of the N demand of cowpea and thereby reduced its potentials for N

fixation. Lower N fixation by legume in high fertility conditions was explained by Eaglesham et al¹¹.

It is evident from the foregoing results that the contribution of soil N towards total N uptake of maize decreased with maturity of the crop; this was more pronounced in the case of mixed cropped maize since it had an additional source of legume fixed N. Pure cropped maize received 2.5, 8.7 and 18.7 mg N plant⁻¹ at 30, 60 days and at maturity stage respectively, from sources other than fertilizer and soil. These amounts may be attributed to the contribution from resistant fractions of soil organic N which was not labelled with ¹⁵N during incubation. Thus, if the role of seed N is considered to be insignificant, the accurate estimate of the N fixed by legume and that transferred to the associated cereal would be the apparent estimate less the contribution from the unknown sources, which is a very small quantity. At maturity, cowpea contributed about 27% of the total N uptake by maize. In greenhouse conditions cowpea contributed about 32% of the N uptake of maize¹².

4 September 1986

- 1. Legg, J. O. and Slogger, C., In: *Proc. Second Int. Conf. Stable Isotopes*, Chicago, Illinois, October. 1975.
- 2. Ruschel, A. P., Vose, P. B., Victoria, R. L. and Salati, E., Pl. Soil, 1979, 53, 513.
- 3. Hardy, R. W. F., Burns, R. C. and Holsten, R. D., Soil Biol. Biochem., 1973, 5, 47.
- 4. Patra, D. D., Sachdev, M. S. and Subbiah, B. V., J. Nucl. Agric. Biol., 1985, 14, 152.
- 5. Jackson, M. L., In: Soil chemical analysis, Prentice Hall, New Delhi. 1967.
- 6. Fiedler, R. and Proksch, G., Anal. Chim. Acta., 1975, 78, 1.
- 7. Yamamuro, S., Soil Sci. Pl. Nutr., 1981, 27, 405.
- 8. Ruschel, A. P., Salati, E. and Vose, P. B., Pl. Soil., 1979, 51, 425.
- 9. Finlay, R. C., In: Soyabean production, protection and utilization, University of Illinois, Urbana, USA, 1975, 78.
- 10. Haynes, R. J., Adv. Agron., 1980, 33, 227.
- 11. Eaglesham, A. R. J., Ayanaba, A., Ranga'Rao and Eskew, D. L., Soil Biol. Biochem., 1981, 13, 169.
- 12. Patra, D. D., Sachdev, M. S. and Subbiah, B. V., Biol. Fert. Soils, 1986, 2, 165.