BIOLOGICAL NITROGEN FIXATION BY INOCULATED SOYA BEANS (*GLYCINE MAX*) IN PURE AND MIXED CULTURE WITH MAIZE AS ESTIMATED BY THE ACETYLENE REDUCTION ASSAY

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ABSTRACT

Biological Nitrogen Fixation (BNF) by soya beans (*Glycine max*) was estimated using the acetylene reduction assay (ARA) for varieties Davis, Kudu, Impala, Hardee, Geduld, and an unidentified variety, grown in pure and mixed cultures with maize (*Zea mays*) over two seasons. All varieties had higher levels of BNF when planted early in the growing season than when planted late. Davis had consistently low BNF. Low BNF levels correlated with small but significant reduction in grain protein percentages. At high BNF levels, plants in mixed culture fixed about half as much N as plants in pure cultures. Soya beans can fix up to 50 kg of N per hectare. When BNF levels are high, over one third of the N in the beans at harvest is derived from the atmosphere.

INTRODUCTION

Geduld and Hardee are the main recommended varieties of soya beans (*Glycine max*) grown in Malaŵi. The latter is preferred in the Southern Region. Davis performs well, and Kudu, Impala and other unidentified varieties are also grown (Malaŵi Government, 1979).

Recommendations for cultivating soya beans include inoculation with *Rhizobium* bacteria to take advantage of Biological Nitrogen Fixation (BNF). However, many factors influence the actual levels of BNF (Lie, 1974; Vincent, 1974). For example, cropping patterns do not affect the BNF of un-inoculated beans (*Phaseolus vulgaris*) or groundnuts (*Arachis hypogea*) (Edwards *et al.*, unpublished; Edwards, 1988).

This study was undertaken to determine BNF under different of cropping patterns, and planting dates by different varieties of soya beans.

MATERIALS AND METHODS

The soya bean variety trials were conducted in Zomba district, and included four replicates with two rows on each of four ridges in the plots. An unidentified variety from

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Mzimba was grown only in pure culture plots, but varieties Davis, Kudu, Impala, Geduld, and Hardee were grown in both pure and mixed culture plots with maize (*Zea mays*). Early planting was done in December (5/12/86 and 17/12/88) and the late planting about 6 to 8 weeks later (29/1/87 and 1/2/88). Single superphosphate (19% P_2O_5) was applied at 300 kg/ha to all trials except the 1987/88 late planting. All of the soya bean plots were inoculated with *Rhizobium* type SBG obtained from the Chitedze Agricultural Research Station. As plants could not be removed from the centre ridges (reserved for yield data), samples of two to three plants were taken from the inside row of the border ridges. Sampling was done at 5-week intervals except in the 1987/88 when it was done at 3-week time intervals.

Details of the Acetylene Reduction Assay (ARA) for estimating BNF and a discussion of the empirical validity of the method have been given (Edwards *et al.*, 1988). The theoretical conversion factor of N_2 reduction used throughout this study was 1/3 the rate at which C_2H_4 is produced. This falls between values determined experimentally using ^{15}N, which are 1/2.0 (Mague and Burris, 1972) and 1/3.9 (Stewart *et al.*, 1967). Total N was determined in duplicate by the Kjeldahl method on 1.00g or 2.00g samples of ground bean flour. The percentages N were calculated to 12% moisture and the protein percentages from 5.7 x N% (Tkachuk, 1969). One sampling of Davis was done on whole plants with their tops scaled through the top of a flask (Mague and Burris, 1972), to show that excised roots give the same BNF as whole plants. Integrated BNF values were used in this experiment.

RESULTS

Whole plant samples of Davis gave the same rate as root system samples. The results presented are based on integrated samples. The integrated BNF values of the six varieties for both early and late plantings are shown in Table 1. They ranged from 22 to 530 mg per plant per week or 2.2 to 54 kg per ha.

There were no differences in BNF between the 1986/87 and 1987/88 growing seasons. However, there was a significant drop ($p < 0.05$) in BNF between the early and late planted soyabeans in all varieties. BNF was particularly and consistently low (range 22 - 64 mg/plant) for the late planted soya in 1986/87 growing season.

The mixed cultures always had less BNF than pure cultures of the same variety. These were were consistently in early planted (both seasons) and late planted 1987/88 seasons (when BNF was high). The percentage decrease in mixed cultures on a per plant basis averaged 48 $\pm$ 19% for the 11 comparisons for which BNF exceeded 100 mg per plant. This pattern was not obvious with the 1986/87 late planted crop (when BNF was low) where, for example, Geduld had higher BNF in mixed than pure culture. Thus performance of individual varieties was not consistent between mixed and pure culture when BNF was low.

Table 1. Acetylene Reduction Assay BNF estimates in Early and Late Planted Soya Beans.

Soya Bean Variety	Cropping pattern	Early Planting Dates				Late Planting Dates			
		5/12/86		17/12/88		29/1/87		1/2/88	
		mg/plant	kg/ha	mg/plant	kg/ha	mg/plant	kg/ha	mg/plant	kg/ha
Davis	pure	190	26.0	260	8.9	22	2.6	58	2.7
	mixed	100	14.0	175	3.8	26	2.6	55	2.6
Kudu	pure	490	45.0	290	12.0	50	8.5	210	19.0
	mixed	170	15.0	190	12.0	43	6.1	46	4.0
Impala	pure	370	54.0	305	20.0	64	8.7	57	3.7
	mixed	250	31.0	145	7.2	57	6.8	110*	8.3*
Hardee	pure	560*	20.0*	530	49.0	37	2.7	250	6.2
	mixed	490*	24.0*	385	22.0	36	2.2	68	1.8
Geduld	pure	520	27.0	450	36.0	37	2.9	200	17.0
	mixed	360*	15.0*	330	14.0	51	3.6	71	4.1
Unidentified	pure	520*	78.0*	350	13.0	44	7.7	215	21.0

* technical problems with gas chromatography in taking these measurement

Although there were no varietal differences, Hardee, Geduld, Mzimba (un-identified), Kudu and Impala gave high BNF in descending order. Variety Davis in pure culture gave the lowest BNF of all the varieties on average for both seasons in the early planted crop. In mixed culture Davis was among those with low BNF levels. Hardee and Geduld grown in pure and mixed culture consistently gave high BNF on a per plant basis followed by Mzimba (un-identified), Kudu and Impala. Since Hardee and Geduld had poor germination, Kudu and Impala gave higher BNF on a per hectare basis in the first season. In the second season Hardee and Geduld gave the highest BNF per hectare.

Samples of Davis in 1986/87 and 1987/88 and Kudu in 1987/88 showed diurnal variations. Samples taken at approximately 9:30 am gave higher reduction rates than those taken at 1:30 pm and 5:30 pm. The lowest reduction rates were observed in samples taken at 5:30 am.

Figure 1 shows the rates of biological nitrogen fixation for three soya bean varieties (Kudu, Geduld, and Impala) over the 1986/87 season.

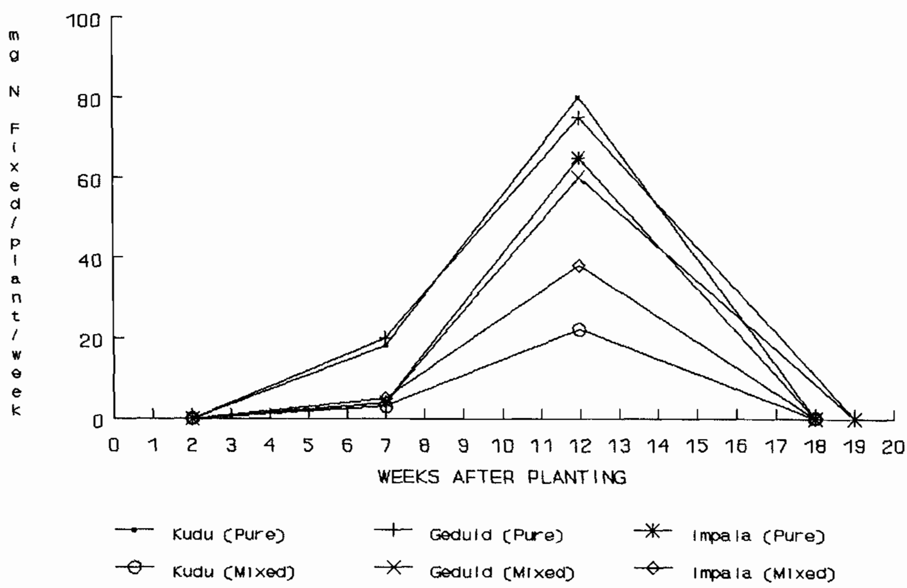

Figure 1 Biological nitrogen fixation by soya beans at Zomba over the 1986/97 growing season as estimated by Acetylene reduction assay (ARA).

The BNF rates were very low in very young plants, but rose significantly by the 7th week and reached peak by the 12th week after planting. After this they delined to very low levels by the 18 or 19th week after planting. These patterns were representative of the results for all varieties tried. The 1987/88 samples (taken at 3-week intervals), however, did not exhibit a sharp peak. The maximal rates of acetylene reduction for pure culture soyabeans ranged from 22 to 52 μmol C_2H_2 per hour per plant.

Kudu had the highest difference in maximal BNF rates between soya grown in pure (> 80 mg/plant/week) and mixed (< 25 mg/plant/week). Geduld had the smallest difference while Impala was intermediate.

Table 2 shows the percentages of N and protein in the 1986/87 season. Differences between late and early planted soya beans were also reflected in the N and protein percentages.

Table 2. Nitrogen and protein percentages for the early planting and late planting of soya beans for 1986/87.

Soya Bean Variety	Cropping pattern	Early Planting Dates		Late Planting Dates	
		% N	% Protein	% N	% Protein
Davis	pure	5.99	34.1	5.26	30.0
	mixed	5.49	31.3	5.52	31.5
Kudu	pure	5.88	33.5	5.46	31.1
	mixed	5.56	31.7	5.38	30.7
Impala	pure	6.05	34.5	5.34	30.4
	mixed	5.77	32.9	5.33	30.6
Hardee	pure	5.91	33.7	5.40	30.9
	mixed	5.90	33.6	5.68	32.4
Geduld	pure	5.82	33.2	5.47	31.1
	mixed	5.81	33.1	5.72	32.6
Unidentified	pure	5.90	33.6	5.61	32.0
Average	pure	5.92	33.8	5.42	30.9
	mixed	5.71	32.5	5.53	31.5
Std. Dev.	pure	0.08	0.5	0.12	0.7
	mixed	0.17	1.0	0.17	1.0

There were also differences between pure and mixed cultures in percentage N and protein. This tended to occur when BNF was high (>100). The early planted soya averaged 5.92% and 5.71% N and 33.8% and 32.5% protein in pure and mixed culture. The late planted soya averaged 5.42% and 5.53% N and 30.9% and 31.5% protein in pure and mixed culture. The differences were, however, not significant. When BNF was low, as in many of the late planting values in Table 1, the mixed and pure cultures gave about the same BNF. This also led to the similar protein percentages (late planted in Table 2) between pure and mixed cultures of the late planted trial.

From the N percentages of Table 2 and the yield results of soyabean, the quantity (in mg) of N found in the grain of each variety per plant at harvest in 1986/87 was calculated. Comparing these to the BNF per plant revealed that 19% to 43% and only 6 to 20% of the N in the grain was derived from the atmosphere in the early planting and late planting, respectively.

DISCUSSION

The rates of Acetylene reduction found in this study for soyabean are about the same as those reported for soyabean in Canada by Rennie and Dubetz (1984a) and are comparable to the maximal rates for groundnuts in Malaŵi (Edwards *et al.*, 1988).

The diurnal variations contrast with those of Mague and Burris (1972) who observed maximum rates at 5:00 pm for soya beans in the USA.

Uneven germination and different plant densities on some varieties led to great differences in BNF between the early and late planting and between the two seasons on a per hectare basis. Water stress could also have led to lower soyabean yields as well as BNF on the late planted plots.

The highest average protein percentage of 33.8% (38.4% on a dry weight basis) reported for the early planting 1986/87 is less than is reported for four varieties of soya beans in Zimbabwe. In fact, the variety Roan in Zimbabwe as a cross between Kudu and 196/6/22 contained 41.8% protein, yields higher than, and is agronomically as good as Kudu under low altitude conditions (Tichagwa, 1986). Rennie and Dubetz (1984b) found 34.2 to 42.7% protein for inoculated soya beans in Canada and 30 to 34% protein for un-inoculated soya beans which did not fix N because their soil had no indigenous *Rhizobia* bacteria.

Our findings in pure and mixed stands were slightly lower (at 32.5 - 33.8 and 30.9% - 31.5% respectively). When BNF is high the pure cultures have always higher BNF than mixed cultures, whereas when BNF is low, there are no differences between the pure and mixed stands.

Acetylene may inhibit enzymatic processes which make reducing equivalents available for BNF so that the ARA underestimates BNF. The average conversion factor of 0.5 determined by Mague and Burris (1972) may be the best value. However, some of the underestimation of BNF due to the use of 0.333 as the conversion factor in this study is offset by the fact that routine sampling was done near the time of the diurnal peak in BNF.

Our ARA estimations show rates similar to those obtained by Rennie and Dubetz (1984a). One of their trials on which the ARA was conducted was also monitored by the isotope dilution technique using ^{15}N. Accurate percentages N derived from the atmosphere (Ndfa) were determined to fall between 50 and 60%. The less accurate estimations of this study give a range of 6 to 43%; These may overestimate grain Ndfa because they are calculated as if all of the N fixed has gone to the grain, since the vegetative portions of the plants were left in the field where their N could return to the soil. A more comprehensive study of Rennie and Dubetz (1984b) using only the isotope dilution technique gave values from 18.8 to 55.3% Ndfa depending on inoculant type and soya bean variety.

Variety Davis consistently gave lower BNF levels when compared to Kudu, and Hardee which in pure culture consistently gave highest BNF on a per plant basis. This poor BNF performance was also observed for Davis in a third intermediate planting (Edwards, 1988) and at Makoka in 1986/87 pure culture (Edwards, unpublished). Strains of *Rhizobia* which are more effective with this variety should be sought in southern Malaŵi. The high BNF found in Hardee and Geduld is probably why they have come to be the main recommended varieties in Malaŵi.

Up to 50 kg of N may be fixed per hectare by soya beans. Biological Nitrogen Fixation can replace a large amount of N removed in the crop which might otherwise need to be replaced by artificial fertilizers. Comprehensive data including yields under different agronomic and cultural conditions are required before recommending varieties or cropping practices for soyabean. However, this study on BNF implies that both mixed cropping and late planting reduce the advantages expected from soya beans fixing their own N. Farmers in many areas in Malaŵi also prefer mixed cropping for different reasons.

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REFERENCES

Edwards, R.A., Jere, R., Chauluka, D (1988). Biological nitrogen fixation by un-inoculated beans and groundnuts in pure and mixed cultures with maize at Zomba as estimated by the acetylene reduction assay. *Luso Journal of Science and Technology*, **9** : 11 - 21.

Edwards, R.A. (1988). Biological nitrogen fixation as estimated using the acetylene reduction assay to demonstrate effective *Rhizobium* bacteria are indigenous to Malaŵi near Zomba. Chemical Society of Malaŵi Proceedings of Conference 1988.

Lie, T.A. (1974). Environmental effects on nodulation and symbiotic nitrogen fixation. In: *The Biology of Nitrogen Fixation*. North-Holland Publishing Company, Amsterdam, 55 - 82.

Mague, T.H. and Burris, R.H. (1972). Reduction of acetylene and nitrogen by field-grown soybean. *New Phytology* **71** : 275 - 286.

Malaŵi Government (1979). *Recommendations for soya bean cultivation*. Ministry of Agriculture and Natural Resources, Extension Aids Circular No. 2/79, Lilongwe, Malaŵi, JN 113-4M-X1-79.

Rennie, R.J. and Dubetz, S. (1984a). Effect of fungicides and herbicides on nodulation and N_2 fixation in soybean fields lacking indigenous *Rhizobium japonicum*. *Journal of Agronomy* **76** : 451 - 454.

Rennie, R.J. and Dubetz, S. (1984b). Multistrain vs. single strain *Rhizobium japonicum* inoculant for early maturing (00 and 000) soy bean cultivars: N_2 fixation quantified by ^{15}N isotope dilution. *Journal of Agronomy* **76** : 498 - 502.

Stewart, W.D.P., Fitzgerald, G.P. and Burris, R.H. (1967). *In situ* studies of N_2 fixation, using the acetylene reduction technique. *Proceedings of the National Academy of Science, U.S.A.* **58** : 2071 - 2078.

Tichagwa, J.S. (1986). Crop variety release notice: soya bean variety roan. *Zimbabwe Agricultural Journal* **83** : 23.

Tkachuk, R. (1969). Nitrogen-to-protein conversion factors for cereals and oilseed meals. *Cereal Chemistry* **46** : 419-423.

Vincent, J.M (1974). Root-nodule symbiosis with *Rhizobium*. In: *The Biology of Nitrogen Fixation*. North-Holland Pub., Co., Amsterdam, 265-341.