Soil Mot Biochem. Vol. 21, No. I, pp. 155-159, 1989
Printed in Great Britain

0034-0717/89 $3.00 + 0.00

^{15}N-UPTAKE, N2-FIXATION AND RHIZOBIAL. INTERSTRAIN COMPE T'ITION IN SOYBEAN AND BEAN, INTERCROPPED WITH MAIZE

ROBERT C. ABAIDOO

University of Hawaii NifTAL Project, 1000 Holomua Avenue, Paia, Maui, HI 96779-9744, U.S.A.

and

CHRISTOPHER VAN KESSEL

Department of Soil Science, University of Saskatchewan, Saskatoon, Sask., Canada S7N OWO

(Accepted 29 June 1988)

Summary-N_2-fixing activity of soybean and beans monocropped or intercropped with maize was measured by the ^{15}N dilution method. Intercropped bean and soybean, planted in the same hole with maize , or in two separate planting holes, showed a decrease in atom % ^{15}N as compared with monocropped bean and soybean. However, compared to when monocropped, intercropped non-nodulating soybean isoline and unincoulated bean showed a highly significant decrease in atom % ^{15}N. Whereas the total amount of ^{15}N-label accumulated in monocrop and intercrop systems remained constant, intercrraped maize accumulated more and intercropped legumes less ^{15}N compared with monocropped maize or legume, respectively. The decrease in atom % ^{15}N excess of intercropped N_2-fixing legumes could be explained by higher N_2-fixing activity, a different ^{15}N-uptake pattern or a difference in competition for soil N between intercropped legumes and maize.

Intercropping bean or soybeans did not alter the competition pattern of the introduced strains of rhizobia. TAL *102* (USDA 110) and TAL *182* were the best competitors for soybeans and beans, respectively, and formed the greatest number of nodules, independent of cropping systems tested.

INTRODUCTION

Intercropping legumes with non-legumes has proved to be beneficial for subsistence farmers of the tropics and subtropics where they are limited by low crop productivity and also inflexible land tenure systems. Finlay (1975) outlined advantages of iptercropping systems such as profit and resources maximization, built-in balanced nutritional supply of energy and also an improvement in soil fertilty. Evans (1960) presented the system as one that has a general trend toward higher yield while Willey (1979) claimed the system provided greater stability. Intercropped systems have revealed variations in terms of gains to the non-legume components as well as legume components due to different competitive situations which arise. Willey (1979) reviewed the situations and classified them into three main categories: (1) mutual inhibition; (2) mutual cooperation; and (3) compensation. It is clear that whatever the type is, there is always the initial competitibn for growth resources between the component plants.

It is reasonable, to suggest that non-legume associations will create competition since the two plants have to utilize resources from the same environment. Higher soil nutrient removal in intercropped systems has been reported (Leihner, 1983) and soil fertility may decrease more quickly (Mason *et al.,* 1986). The possibility that lower soil N availability causes an increase in N_2-fixing activity by legumes when intercropped with non-legumes has been suggested by Danso *et al.* (1987), Morris and Weaver (1987) and Ta and Faris (1937). Ofori *et al.* (1987) found no apparent increases and Vasilas and Ham (1985) reported a decrease in N_2-fixing activity.

We have evaluated and quantified the proportion of N derived from N_2-fixation in soybeans and beans when cropped in association with maize. Legumes and maize seeds were planted in the same or in different holes. The former is an old practice still in use in Central America whereas the latter is the more commonly used form of planting in intercropping practices. We have also determined the effect of maize cropping on the nodule-forming potentials of the component strains of inoculum used.

MATERIALS AND METHODS

Soil (Humoxic tropohumult) was obtained from Haiku on the island of Maui, Hawaii. The top 2.0 cm of the site was removed and soil dug to a depth of 15.0 cm and sieved (< 6.5 mm).

Soil amendments

The initial pH of 4.0 of the soil was brought up to 5.8 through the supplementation of 3.0 g $Ca(OH)_2$ kg$^{-'}$ of soil (oven-dried basis). Nutrients (mg kg$^{-'}$) were added as: Mg as $MgS0_4$, 25; K as K_2S0_4, 100 and P as KH_2PO_4, 400. Soil and nutrients were thoroughly mixed and distributed into 6.01. pots and 4.5 kg amounts. Micronutrients were provided by liquid micronutrient concentrate (Monterey Chemical Company) at 0.5 ml kg$^{-'}$ of soil. This supplied kg$^{-'}$ soil 7.5 mg Fe; 2.5 mg Zn; 1.75 mg B; 0.15 mg Co; 0.2 mg Mo; 0.75 mg Cu; and 2.3 mg Mn.

Table 1. Shoot dry matter, shoot N accumulation and ^{15}N-uptake in monocropping and mixed cropping systems

Cropping system	^{15}N uptake: Legume (μg ^{15}N plant^{-1})	^{15}N uptake: Maize (μg ^{15}N plant^{-1})	^{15}N uptake: Total (μg ^{15}N pot^{-1})	Shoot dry matter accumulation (g pot^{-1})	Total N accumulation (mg N pot^{-1})
Soybean, inoculated	36	—[c]	144	7.7	227.2
Soybean + maize, inoculated[a]	10	59	138	8.7	176.5
Soybean maize, inoculated[b]	10	57	134	8.7	156.6
Non-nodulating soybean + maize[a]	5	51	112	7.5	65.3
Non-nodulating soybean	37	—	148	5.3	82.2
Bean, inoculated	38	—	152	10.4	276.2
Bean + maize, inoculated[a]	22	51	146	9.9	197.4
Bean, maize, inoculated[b]	31	40	142	9.1	180.9
Bean + maize, uninoculated[a]	21	46	134	7.2	94.1
Bean, uninoculated	32	—	128	6.8	121.8
Maize	—	32	128	6.9	48.9
LSD ($P \leq 0.05$)	6		22	0.9	27.9

[a]Legume and maize seeds placed in the same planting hole.
[b]Legume and maize seeds planted separately in two planting holes.
[c]No value present.

Tracer N was supplied as ^{15}N-enriched $(NH_4)_2SO_4$ at 0.19 mg N kg-' soil (79.9 atom % ^{15}N) in an aqueous solution to finally bring the moisture content of the soil to 37% which corresponds to 0.01 mPa. Soil was allowed to equilibrate for 3 days before planting. Moisture content was maintained through careful daily watering until harvest.

Planting and inoculation

Glycine max (L.) Merr. cv. Clark, its nonnodulating isoline, *Phaseolus vulgaris* (L.) cv. Kentucky and *Zea mays* (L.) cv. Mexicana were surface sterilized in 3% H_20_2 before use. Monocropped treatments were planted initially with eight seeds while intercropping sets received four pairs of legume and maize seeds. Peat cultures of *Bradyrhizobium* strains recommended for soybeans, i.e. TAL 102 (USDA 110), TAL 377 (USDA 138), TAL 379 (USDA 136) and *Rhizobium* strains recommended for beans, i.e. TAL 182; TAL 1383 (CIAT 632), TAL 1797 (CIAT 899) were obtained from the NifTAL Project's Rhizobium germplasm. Liquid inocula of the strains were prepared to constitute a mixture of approximately equal numbers of the three component strains with a final population of $1 \cdot 10^7$ cells ml^{-1} solution applied to each legume seed at planting. Pots were arranged in a completely randomized block design, replicated four times, and placed in a greenhouse whose temperature ranged from 22-28°C. Seedlings were thinned to a total of four plants per pot 7 days after planting.

Harvest and sample preparation

Plants were harvested after 7 weeks. Shoots, roots and nodules were dried tit 60°C to constant weight. Shoot samples were ground (< 0.2 mm), processed for total N, including $N0_3$ and NO_2 (Bremner and Mulvaney, 1982) and atom % ^{15}N using a Micromass 602E automatic mass spectrometer. N_2-fixation was calculated using the formulas described by Rennie and Rennie (1983).

Strain identification

The proportion of nodules occupied by each strain was determined by the immunofluorescence technique. Twenty-four nodules from each replicate treatment were examined. Nodule smears were made from oven-dried nodule samples by the method of Somasegaran *et al.* (1983). Gelatine-rhodamine isothiocyanate was used to suppress background nonspecific strain reactions (Bohlool and Schmidt, 1968) before staining with stain-specific fluorescent antibody using the procedure of Schmidt *et al.* (1968). A Zeiss Standard Microscope 14, with incident light fluorescence illuminator equipped with an Osram HBO 50 w mercury vapour light source was used for the microscopic examination of the stained cells.

RESULTS AND DISCUSSION

Dry matter

Total above ground dry matter accumulation varied with patterns of cropping and inoculation (Table 1). Combined shoot dry matter accumulation was greater in inoculated soybean intercropping treatments than inoculated and uninoculated monocropping systems. In bean, however, the inoculated monocropping treatment accumulated higher dry matter than the inoculated intercropping treatments. These results suggest that competition between species (C. K. Hiebsch, unpublished Ph.D. thesis, North Carolina State University, 1980) for resources, which may have occurred in the intercropping systems, was greater in bean than soybean intercropping systems due to the latter's high potential for biological N_2-fixation and the former's greater dependence on soil N. In evaluating the benefits to maize, it is clear that maize grown with inoculated soybean accumulated higher dry matter and total N (Table 2) than monocrop maize, while differences did not exist between monocrop maize and maize intercropped with beans. Maize when planted in the same hole or different holes with soybean accumulated the same amount of dry matter. However, total N-uptake by maize planted in different holes was significantly higher compared with maize and soybean planted in the same hole. No such effect was found with beans. Allen and Obura (1983) observed 23-26% reduction in dry matter yield of maize when intercropped with inoculated soybean. Eagleshalm *et al.* (1981) established dry matter and N gains to maize when intercropped with inoculated cowpea while Graham and Rosas (1978) showed that the benefits to maize in associated plantings with bean depends on the bean

Table 2. Atom % ^{15}N excess, dry matter accumulation and total N-uptake by maize as affected by cropping system

Cropping system[a]	Atom % ^{15}N excess (%)	Dry matter accumulation (g plant^{-1})	Total N uptake (mg plant^{-1})
Maize	0.2096	1.73	12.2
Maize + soybean, inoculated	0.2083	2.42	22.6
Maize, soybean, inoculated	0.2415	2.37	18.9
Maize + non-nodulating soybean	0.2564	2.99	24.7
Maize + bean, inoculated	0.2065	1.77	15.7
Maize, bean, inoculated	0.1667	1.68	15.3
Maize + bean, uninoculated	0.2409	1.84	15.1
LSD ($P \leqslant 0.05$)	0.0359	0.43	3.6

[a]See Table 1 for explanation.

cultivar. The above suggest that the N benefits to the non-legume component of any intercropping system depends largely on legume efficiency at utilizing soil N as well as its potential in biological N_2-fixation.

Nodulation and N_2*-fixation*

Intercropped beans planted in the same hole produced more nodules than when planted in different holes or grown as sole crops (Table 3). We suggest that the increase in nodulation might be due to the formation of more nodules through more secondary infections in beans as a response to N stress generated by the maize's utilization of the available soil N.

Atom % ^{15}N excess of soybeans and beans was greatly influenced by cropping system (Table 3). Intercropped soybean and bean showed a significant decrease in atom % ^{15}N excess as compared with the monocrop. However, intercropped non-nodulating soybean isoline also showed a highly significant decrease in atom % ^{15}N excess as compared with the monocropped non-nodulating soybean. Obviously, this reduction in atom % ^{15}N excess cannot be explained by an increase in N_2-fixation of the legume but rather by a difference in competition for labeled ^{15}N between the two species and where maize is more competitive than the non-nodulating soybean or by inherent differences in the N-uptake pattern of the two species. A similar decrease in atom % ^{15}N also occurred in the uninoculated intercropped bean as compared with the uninoculated monocropped bean. However, the uninoculated bean did form nodules (Table 3). Therefore, an increase in N_2-fixing activity may also have contributed to the decrease in atom ^{15}N. Total ^{15}N-uptake by maize increased significantly when competing with a legume for labeled N as compared with maize competing against maize (monocropped maize). For legumes, the opposite occurred and the highest accumulation of ^{15}N-label occurred when the legume was competing against a legume. However, the total amount of ^{15}N-label accumulated in the different cropping systems remained fairly constant (Table 1).

After application of ^{15}N-labelled inorganic N the atom % ^{15}N of the available soil N pool will decrease (Witty, 1983). If a non-nodulating soybean is competing against maize and a delayed uptake or a difference in competition for ^{15}N occurs, a lower atom %^{15}N value in the non-nodulating soybean would be found. Based upon the results obtained from the non-nodulating soybean isoline, the consistent decrease in atom %^{15}N of inoculated intercropped bean and soybean as compared with the inoculated monocropped beans and soybeans can be explained as follows: (1) a higher N_2-fixing activity by the inter-

Table 3. Atom % ^{15}N excess, nodulation and N_2-fixation in soybean and bean as affected by cropping system

		Nodule		N derived from N_2-fixation		Total N fixed	
Cropping system[a]	Atom % ^{15}N excess	Number (per plant)	Weight (mg plant^{-1})	Soybean[b] (%)	Maize[c]	Soybean[b] (mg plant^{-1})	Maize[c]
Non-nodulating soybean	0.1444	—	—	—	—	—	—
Non-nodulating soybean + maize	0.0465[d]	—	—	—	—	—	—
Bean, uninoculated	0.0911	72	ND[e]	—	—	—	—
Bean + maize, uninoculated	0.0545[d]	67	ND	—	—	—	—
Soybean, inoculated	0.0506	32	116	65.0	75.9	37.1	43.2
Soybean + maize, inoculated	0.0132	42	145	67.9	93.7	40.0	54.3
Soybean, maize, inoculated	0.0147	33	142	65.7	93.0	39.2	55.3
LSD ($P \leqslant 0.01$)[f]	0.0103	NS[g]	15	NS	3.5	NS	11.5
Bean, inoculated	0.0442	182	271	69.8	78.9	48.3	54.5
Bean + maize, inoculated	0.0212	251	336	54.5	90.0	45.1	74.5
Bean, maize, inoculated	0.0325	196	275	30.2	84.4	23.1	63.7
LSD ($P \leqslant 0.01$)[f]	0.0070	16	33	14.8	3.8	16.5	17.8

[a]See Table 1 for explanation.
[b]Non-nodulating monocropped and non-nodulating intercropped soybean used as reference plant for monocropped and intercropped soybean and bean, respectively.
[c]Monocropped and intercropped maize used as reference plant for monocropped and intercropped soybean and bean, respectively.
[d]Non-nodulating soybean and uninoculated bean pairs are significantly different according to a t-test at $\alpha = 0.01$ and $\alpha = 0.05$, respectively.
[e]ND = not determined.
[f]LSD for inoculated monocropped and intercropped soybeans or beans.
[g]NS = not significant.

Table 4. Effect of maize–soybean cropping systems on the interstrain competition pattern of *Bradyrhizobium japonicum*

	Nodule occupancy[b]		
Cropping system[a]	TAL 102 (%)	TAL 377 (%)	TAL 379 (%)
Soybean	60.2	38.4	18.7
Soybean + maize	60.8	38.5	23.2
Soybean, maize	62.1	31.7	15.5
LSD ($P \leq 0.05$)		10.7	

[a]See Table 1 for explanation.
[b]Values represent means of arc sine transformations of percentage of nodules formed by a particular strain on the basis of nodules solely occupied by the strain or co-inhabited with other strain(s)

cropped legume as compared with the monocropped legume; (2) a different uptake pattern or competition for labeled soil N-uptake by the legume as compared to maize; or (3) a combination of 1 and 2. If explanation 2 or 3 is correct, calculating the percentage of N derived from N_2-fixation by isotope dilution method using maize as a reference crop would overestimate N_2-fixation.

Attributing the change in atom % ^{15}N in the intercropped legume solely to a change in N_2 -fixation rates can be calculated (Table 3). Using maize as the reference crop, significant increases in N_2 -fixing activity and total mg N fixed in intercropped bean and soybean were found as compared with the monocropped bean or soybean. Using non-nodulating intercroppped soybean as a reference crop for intercropped soybean, no such increases in N_2-fixing activity and total mg N fixed were found. Intercropped beans showed a highly significant decrease in N_2-fixing activity and total mg N fixed. In this study a suitable choice for a reference crop for intercropped soybean systems could be the intercropped nonnodulating soybean. For intercropped beans, this choice of the reference crop becomes less clear. Overall, it is apparent from the above that the choice of the non-N_2-fixing reference crop is crucial for measuring N_2-fixation in intercropping studies.

No apparent effect of maize cropping on the competition patterns of the introduced strains of rhizobia on nodulating soybean (Table 4) or bean (Table 5) was found. There was no preferential section or inhibition of any of the component strains of the inoculum by the presence of maize roots in the rooting medium of the legumes. TAL 102 (USDA 110) was the best competitor for soybeans in all treatments while TAL 182 (NifTAL original) was the best for the beans. The competitive ability of USDA 110 is well documented (Kosslak *et al.*, 1983; Kosslak and Bohlool, 1985; George *et al.*, 1987). To

Table 5. Effect of maize-bean cropping systems on the interstrain competition pattern of *Rhizobium leguminosarum* biovar *Phaseoli*

	Nodule occupancy[b]		
Cropping system[a]	TAL 182 (%)	TAL 1383 (%)	TAL 1797 (%)
Bean	72.83	19.53	20.35
Bean + maize	76.05	16.35	17.53
Bean, maize	78.93	12.08	17.90
LSD ($P \leq 0.05$)		7.92	

[a]See Table 1 for explanation.
[b]Values represent means of arc sine transformations of percentage of nodules formed by a particular strain on the basis of nodules solely occupied by the strain or co-inhabited with other strain(s).

our knowledge, little is documented on the competitive ability of TAL 182 even though it is known as an effective N fixer when associated with beans (Pacovsky *et al.*, 1984).

Although a consistent decrease in atom % ^{15}N excess in intercropped legumes as compared with monocropped legumes was observed. we were not able to attribute this decrease solely to higher N_2-fixing activity. Highly labeled ^{15}N-$(NH_4)_2SO_4$ was applied, creating a highly enriched soil N pool for a short period of time. By intercropping a nonnodulating soybean with maize, a possible different ^{15}N-labelled soil N-uptake pattern or a difference in competition for ^{15}N between non-nodulating soybeans and maize occurred, resulting in lower atom ^{15}N values for the non-nodulating soybean. The same phenomenon may also have occurred with inoculated bean or soybean competing against maize for ^{15}N. The combined effect of the decline in atom % ^{15}N of the available soil N pool, differences in N-uptake and competition for available soil N on the crop can be controlled by incorporating the ^{15}N-label into the soil N organic fraction, whereby the release of ^{15}N over time will be more stable and constant (Legg and Sloger, 1975; Witty, 1983). The possible changes as described above by cropping legumes and nonlegumes together and the subsequent effect on their atom % ^{15}N would become marginal.

Acknowledgements-*We thank* Drs B. B. Bohlool, P. W. Singleton and J. P. Roskoski for reviewing the manuscript; Kevin Keane and George Swerhone for technical support. The preparation of the manuscript by Susan Hiraoka and Elaine Farkas is highly appreciated. Robert C. Abaidoo was supported by a fellowship of the International Atomic Energy Agency, Vienna and U.S. Agency for International Development Cooperative Agreement. Saskatchewan Institute of Pedology Contribution No. 8580.

REFERENCES

Allen J. R. and Obura R. K. (1983) Yield of corn, cowpea and soybean under different intercropping systems. *Agronomy Journal 75, 1005-1009.*

Bohlool B. B. and Schmidt E. L. (1968) Nonspecific staining: its control in immunofluorescence examination of soil. *Science 162, 1012-1014.*

Bremner J. M. and Mulvaney C. S. (1982) Nitrogen-total. In *Methods of Soil Analysis* (A. L. Page, R. H. Miller and D. R. Keeney, Eds), pp. 595-624. American Society of Agronomy, Madison.

Danso S. K. A., Zapata F., Hardarson G. and Fried M. (1987) Nitrogen fixation in fababeans as affected by plant population density in sole or intercropped systems with barley. *Soil Biology & Biochemistry 19, 411-415.*

Eagleshatn A. R. J., Ayanaba A., Ranga Rao V. and Eskew D. L. (1981) Improving the nitrogen nutrition of maize by intercropping with cowpea. Soil *Biology & Biochemistry* 13, 169-171.

Evans A. C. (1960) Studies in intercropping. 1. Maize or sorghum with groundnuts. *East African Agriculture* 27, 7-8.

Finlay R. C. (1975) Intercropping soybeans with cereals. In *Soybean Production, Protection and Utilization (D. K.* Whigham, Ed.), pp. 77-85. International Soybean Program (INTSOY) Series No. 6, University of Illinois, Urbana.

George T., Bohlool B. B. and Singleton P. W. (1987) *Bradyrhizobium japonicum*-environmental interactions: nodulation and interstrain competition along an elevation transect. *Applied & Environmental Microbiology 53,* 1113-1117.

Graham P. H. and Rosas J. C. (1978) Plant and nodule development and nitrogen fixation in climbing cultivars of *Phaseolus vulgaris* L. grown in monoculture, or associated with *Zea mays* L. Journal *of* Agricultural *Science Cambridge 90,* 311-317.

Kosslak R. M. and Bohlool B. B. (1985) Influence of environmental factors on interstrain competition in *Rhiz*obium japonicum. *Applied & Environmental Microbiology 49,* 1128-1133.

Kosslak R. M., Bohlool B. B., Dowdle S. and Sadowsky M. (1983) Competition of *Rhizobium japonicum* strains in early stages of soybean nodulation. *Applied & Environmental Microbiology 46,* 870-873.

Legg J. O. and Sloger C. (1975) A tracer method for determining symbiotic nitrogen fixation in field studies. In *Proceedings of the* 2nd International Conference on Stable Isotopes (E. R. Klein and P. D. Klein, Eds), pp. 661-666. Oak Brook, Illinois.

Leihner D. E. (1983) Management and evaluation of intercropping systems with Cassava. Centro Internacional de Agricultura Tropical (CIAT), Cali, Columbia.

Mason S. C., Leihner D. E. and Vorst J. J. (1986) Cassavacowpea and cassava-peanut intercropping. II. Nutrient concentrations and removal. *Agronomy* Journal *78,* 441-444.

Morris D. R. and Weaver R. W. (1987) Competition for nitrogen-15 depleted ammonium nitrate and nitrogen fixation in arrowleaf clover-gulf ryegrass mixtures. Soil *Science Society of* America Journal *51,* 115-119.

Ofori F., Pate J. S. and Stern W. R. (1987) Evaluation of N,-fixation and nitrogen economy of a maize-cowpea intercrop system using 15N dilution methods. Plant and *Soil 102,* 149-160.

Pacovsky R. S., Bayne H. G. and Bethlenfalvay G. J. (1984) Symbiotic interactions between strains of *Rhizobium phaseoli and* cultivars of *Phaseolus* vulgaris L. Crop *Science 24,* 101-105.

Rennie R. J. and Rennie D. A. (1983) Techniques for quantifying N2-fixation in association with non-legumes under field and greenhouse conditions. Canadian Journal *of Microbiology 29,* 1022-1035.

Schmidt E. L., Bankole R. O. and Bohlool B. B. (1968) Fluorescent antibody approach to the study of rhizobia in soil. Journal *of Bacteriology 38,* 1987-1992.

Somasegaran P., Woolfenden R. and Halliday J. (1983) Suitability of oven-dried root nodules for *Rhizobium* strain identification by immunofiuorescence and agglutination. Journal *of Applied Bacteriology 55,* 253-261.

Ta T. C. and Faris M. A. (1987) Effect of alfalfa proportions and clipping frequencies on timothy-alfalfa mixtures. II. Nitrogen fixation and transfer. Agronomy Journal *79,* 820-824.

Vasilas B. L. and Ham G. E. (1985) Intercropping modulating and non-modulating soybeans: effects on seed charactersitics and dinitrogen fixation estimates. Soil *Biology & Biochemistry 17,* 581-582.

Willey R. W. (1979) Intercropping-its importance and research needs. Part I. Competition and yield advantages. *Field Crop* Abstracts *32, 1-10.*

Witty J. F. (1983) Estimating N2-fixation in the field using 15N-labelled fertilizer: some problems and solutions. Soil *Biology & Biochemistry 15,* 631-639.