Chapter 4

RESULTS

4.1 Experiment I

Ureide determination and calibration from screenhouse experiment

Degree of nodulation in screenhouse experiment was determined by visual observation ten days after sowing. Table 3 shows the description of nodulation in rice bean plants under three levels of nitrate solution.

Table 3. Degree of nodulation in rice bean from screenhouse experiment.

NO3 level (mM)	Degree of nodulation*
0	very prolific
8	a few, non-working nodules**
12	none

* By visual observation 10 days after sowing
** Determined by pale, greenish coloration of nodules (Peoples et al., 1989)
mM milliMolar

The relative ureide abundance (RU %) in xylem sap was determined for each nitrate treatment and was subsequently calculated for molar concentrations using the formula mentioned before (Formula 2, see section 3.1.3).

The RU indeces from 0mM NO3 at two sampling stages, R1 (start of flowering) and R6 (seed set), revealed high values of 90% and 88%, respectively; 12mM NO3 had 7% at R1 and 13% at R6; 8mM NO3 showed similar results with 12 mM, therefore results were not included in the calibration. The calibration curve relates xylem solute composition to rice bean reliance on N2 fixation in the presence of soil N. It is assumed that with no application of nitrate solution in the rooting medium, rice bean will have 100% Pfix or it derives its N requirements fully from N2 fixation, whereas, for nutrient solution containing 8 mM or 12 mM nitrate, the plants will have no N2 fixation, 0% Pfix.

The relationship between RU and Pfix is linear so only two points of N nutrition were considered, 0mM and 12mM. Plotting the two points together, the resultant line (Fig.2) followed the same calibration curve made by Rerkasem and Rerkasem (1990). Results from two sampling stages (R1 and R6) were combined considering that the results were similar which were also the same findings of Peoples et al. (1989). From the experiment, it was observed that at 0 mM NO3 level rice bean attained an average of 89% RU and at 12 mM NO3 level, an average of 10%. The plants still produced little amount of ureide but did not actually fix N because there were no nodules.

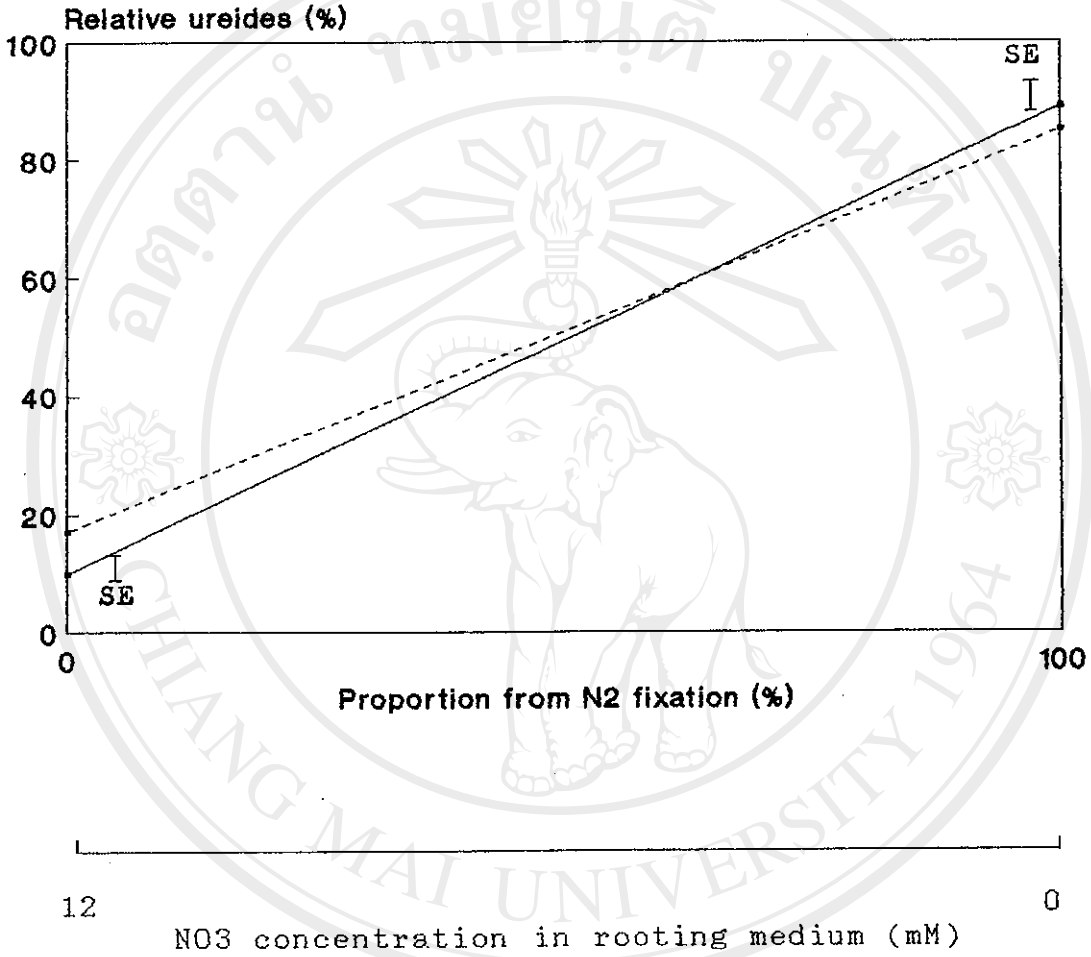

Figure 2. Relationship between the relative abundance of ureides in root-bleeding sap and the proportion of N derived from fixation of rice bean. Calibration obtained from screenhouse experiment conducted (——); SE is the standard error of the mean relative values (± 3%). Reference calibration for rice bean obtained in glasshouse study (---) by Rerkasem and Rerkasem (1990).

The Pfix value was then determined from the calibration curve and was used in the Pfix calculations of field experiment both for vegetative and reproductive stages, where:

$$Pfix = 1.33 (RU - 10)$$

4.2 Experiment II

Intercropping system with delayed sowing of rice bean into upland rice

When intercropped with simultaneously sown rice bean, rice had disappeared at 80 days after sowing and were completely gone at harvest time; while rice in the intercrop treatment with intermediate sowing of rice bean disappeared by day 100, and only one replication yielded 94 kg/ha of dry matter at harvest time.

Therefore, the results for rice are primarily from only two treatments, monoculture rice and intercrop rice with late sowing of rice bean. The two other intercrop combinations were then dropped out in the analysis of variance.

Rice bean, on the other hand, was attacked by insects at pod development stage, thus sampling was made only up to R3. Inspite of that, the plants later recovered and

produced new flowers and pods.

4.2.1 Dry matter yield

Rice

Total dry matter (straw and seed) accumulation of rice is shown in Fig. 3.a. Dry matter accumulation was comparable in monorice and intercropped rice with late bean sowing. However, towards the end of the growing season, the monocropped rice accumulated 5 t/ha of dry matter by 120 days, whereas, intercropped rice accumulated only 3.6 t/ha.

Rice bean

In general, rice bean intercrop accumulated a lot less dry matter than in monocrop up to stage V12. From V20 onwards the differences disappeared (Table 4).

4.2.2 Grain yield and yield components

Rice

Monocropped rice yielded an average of 2,750 kg of grains/ha, intercropping depressed grain yield of rice by 40% (Table 5).

The yield reduction was associated with a decrease in the number of seeds per panicle and seed weight per panicle.

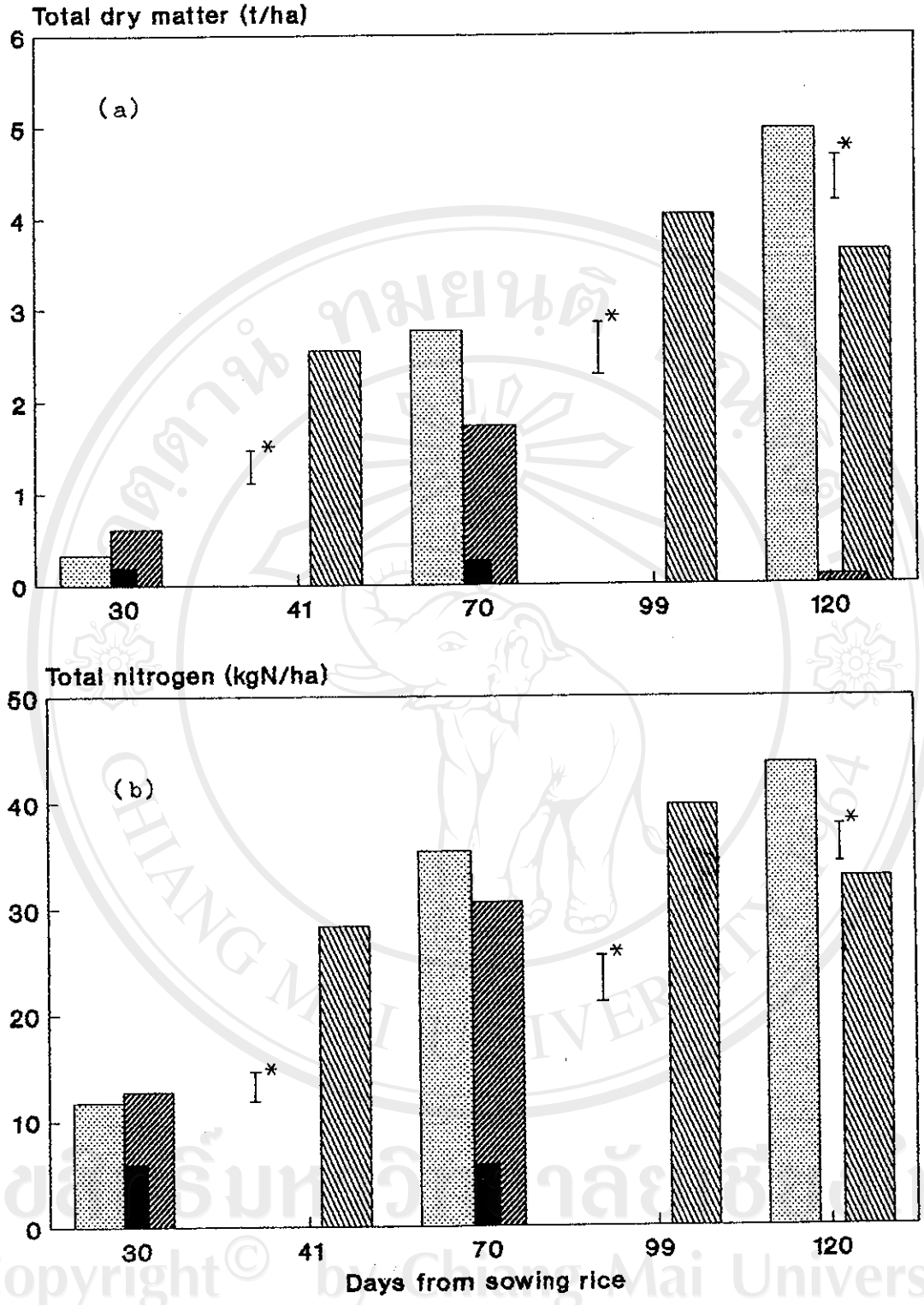

Figure 3. Effects of intercropping on dry matter accumulation (a) and total nitrogen yield (b) in rice. * is the standard error at each harvest. Monocropped rice; Intercropped rice with rice bean sown simultaneously; Intercropped rice with intermediate bean sowing; Intercropped rice with late bean sowing.

Monocropped rice produced higher number of seeds per panicle as well as seed weight per panicle than the intercropped rice ($P<0.05$).

Table 4. Rice bean shoot dry matter (kg/ha) over time.

Cropping system	Growth stage				
	V6	V12	V20	R1	R3
Sole					
Simultaneous	1467	4235	6862	5968	5503
Intermediate	1379	4610	5855	5745	5676
Late	1639	4946	6944	5917	5123
Intercrop					
Simultaneous	1233	4040	7217	5187	4535
Intermediate	1223	3350	6192	5080	5015
Late	730	1431	4721	4236	3850
LSD 0.05	549 *	1643 **	NS	NS	NS

NS Not significant
** Indicate significance at 0.01 level of probability

Rice bean

The grain yield of rice bean among the treatments did not differ significantly where it ranged from 518 kg/ha to 632 kg/ha (Table 6). The number of seeds per pod and 100 seed weight did not significantly affect yield while seed weight per pod contributed to the variations in bean yield. The time to grain maturity of the photosensitive rice bean which flowered towards the end of October did not differ as to monocrop or intercrop treatments, but the differences were related with the time of bean sowing. Either in

monocrop or intercrop treatments, simultaneous sowing took 237 days to mature; and 19 and 34 days earlier than simmultaneous sowing for intermediate and late sowing, respectively.

Table 5. Effects of intercropping on grain yield and yield components of rice.

Cropping system	Grain yld[a] (kg/ha)	Straw yld (kg/ha)	TDM (kg/ha)	Panicles/ hill	Seeds/ panicle	Seed wt./ panicle[a]	1000 seed wt.[a] (g)	% Unfilled seeds
Sole	2750	2222	4972	7.8	126	2.2	44.4	20
Intercrop								
Sole	0	0	0	0	0	0	0	ND
Intermediate	(31)	(63)	(94)	(1)	(13)	(.1)	(37)	(79)
Late	1668	1966	3634	6.9	77	1.4	43.8	33
LSD 0.05	995 *	NS	1003 *	NS	46 *	.06 *	NS	NS

a. Determined at 14% MC
ND Not determined
NS Not significant
TDM Total dry matter (straw + seed)
( ) Data in parentheses are taken from one rep. only, other reps zero yield
*, ** Indicate significance at the 0.05 & 0.01 levels of probability, respectively.

4.2.3 Total nitrogen yield

Total nitrogen (shoot N + seed N) yields both for rice and rice bean followed a similar trend to that of dry matter accumulation.

At 120 days after sowing, the intercropped rice (with late bean sowing) had accumulated significantly less N than monocropped rice (Figure 3.b).

Table 6. Effects of intercropping on grain yield and yield components of rice bean.

Cropping system	Grain yld[a] (kg/ha)	SDM (kg/ha)	TDM (t/ha)	Seeds/ pod	Seed wt[a] pod (g)	100 seed wt[a] (g)
Sole						
Simultaneous	632	5503	6135	8.16	.90	10.3
Intermediate	620	5676	6296	8.01	.88	10.9
Late	607	5123	5730	8.34	.93	10.7
Intercrop						
Simultaneous	557	4536	5092	7.96	.88	10.3
Intermediate	577	5016	5592	8.24	.93	10.7
Late	518	3850	4368	8.34	.92	10.1
LSD 0.05	NS	NS	1004 *	NS	.02 **	NS

a. Determined at 12% MC
SDM Shoot dry matter
TDM Total dry matter (shoot + seed)

In rice bean, late sowing of the bean in the monocrop treatment yielded the highest amount of total nitrogen at stage V6 (67.5 kgN/ha) while the other treatments ranged from 17.8 kgN/ha to 46.9 kgN/ha. At stage V20, late sowing of the bean in the intercrop yielded the lowest amount of 29 kgN/ha. From the following sampling stages, V20, R1, and R3, the treatments did not vary significantly ($P<0.05$) (Table 7).

4.2.4 Land Equivalent Ratio (LER)

The LER for grain yield of the intercrop is 1.49 (SE ± 0.20), 1.66 (SE ± 0.09) for shoot dry matter, and 1.51 (SE ± 0.40) for total N yield.

4.2.5 Nodulation

By ten days after sowing, rice bean became well nodulated with an average of 10 nodules per plant. During the early vegetative stages, V6 and V12, nodule formation was most abundant in the monocrop treatments. Intercropping in all sowing dates reduced nodule number and weight in most of the sampling stages. At late vegetative stage (V20) late bean sowing in both monocrop and intercrop treatments showed the highest nodule number and nodule weight ($P<0.05$) (Table 8). Differences in nodule number and nodule dry weight per plant were more pronounced during the vegetative stages than in the reproductive stages.

4.2.6 Nitrogen fixation

The reliance of plant growth on fixed nitrogen was determined by measuring the relative ureide contents of rice bean xylem sap.

The relative abundance of ureides (RU %)

The relative ureide (RU) index is used as a measure of the proportion of total xylem sap or extract N in the form of allantoin or allantoic acid, when relating ureide

Table 7. Total nitrogen (kgN/ha) in rice bean shoot dry matter as influenced by intercropping.

Cropping system	Growth stage				
	V6	V12	V20	R1	R3
Sole					
Simultaneous	46.9	81.5	113.1	111.3	100.7
Intermediate	45.1	103.1	137.8	122.4	101.3
Late	67.5	100.6	144.7	119.7	98.0
Intercrop					
Simultaneous	38.6	72.2	115.5	106.3	89.8
Intermediate	33.7	70.6	131.7	117.7	103.3
Late	17.8	29.1	91.6	93.5	72.2
LSD 0.05	15.9 **	37.6 **	NS	NS	NS

Table 8. Differences in rice bean nodulation during late vegetative stage (V20) as influenced by intercropping

Cropping system	Late vegetative stage (V20)	
	Nodule/plant	Nodule drywt. (mg/plant)
Sole		
Simultaneous	57	31
Intermediate	81	39
Late	136	103
Intercrop		
Simultaneous	59	23
Intermediate	62	24
Late	146	91
LSD 0.05	43 **	57 *

contents to symbiotic reliance in calibration curves (See section 3.1.3) (Peoples et al., 1989). It estimates the proportion of N compound in xylem sap that is in the form of ureides.

All cropping systems showed high RU values at stage V20 (Fig. 4.a) and declined gradually at the reproductive stages. At stage V20, the intercrop treatment where rice bean was sown late into rice showed the highest value of 90.6%, while the monocrop rice bean treatments at all sowing times did not differ significantly in their RU values ($P<0.05$). The values ranged from 71.3% to 72.4%. The ureide indeces of the intercrop treatments were higher than the monocrop treatments throughout the growing period.

The proportion of total plant nitrogen derived from N2 fixation (Pfix)

The function describing the relationship between Pfix and the relative abundance of ureides in root-bleeding exudate is: Pfix = 1.33 (RU - 10), which is based from the calibration curve of screenhouse experiment conducted (See section 4.1).

Both in the monocrop and intercrop treatments, the proportion of total nitrogen derived from nitrogen fixation

(Fig. 4.b) was at its peak at stage V20. Late sowing of the bean in intercropping system favored higher Pfix value. The highest Pfix value was 100% from the intercrop treatment with rice bean sown one month after rice. The monocrop bean treatments did not differ significantly, 79.7% to 81.1% ($P<0.05$). The reliance on fixation gradually declined in later growth stages. The same trend could also be observed with the relative abundance of ureides.

The amount of nitrogen fixed

Nitrogen fixation inputs were calculated by multiplying the amount of plant nitrogen accumulated between harvests with the mean proportions of nitrogen fixed between the two stages.

The seasonal patterns of nitrogen fixation were similar in all treatments. Amount of nitrogen fixed was the least at V6 and increased sharply until V20 where it was at its maximum amount. From stage V6 to V20, the increase was very much pronounced in the intercrop treatment where rice bean was sown two weeks after rice, 81.7 kgN/ha. The monocrop treatment with rice bean sown at the same time with rice showed the least increase (Table 9).

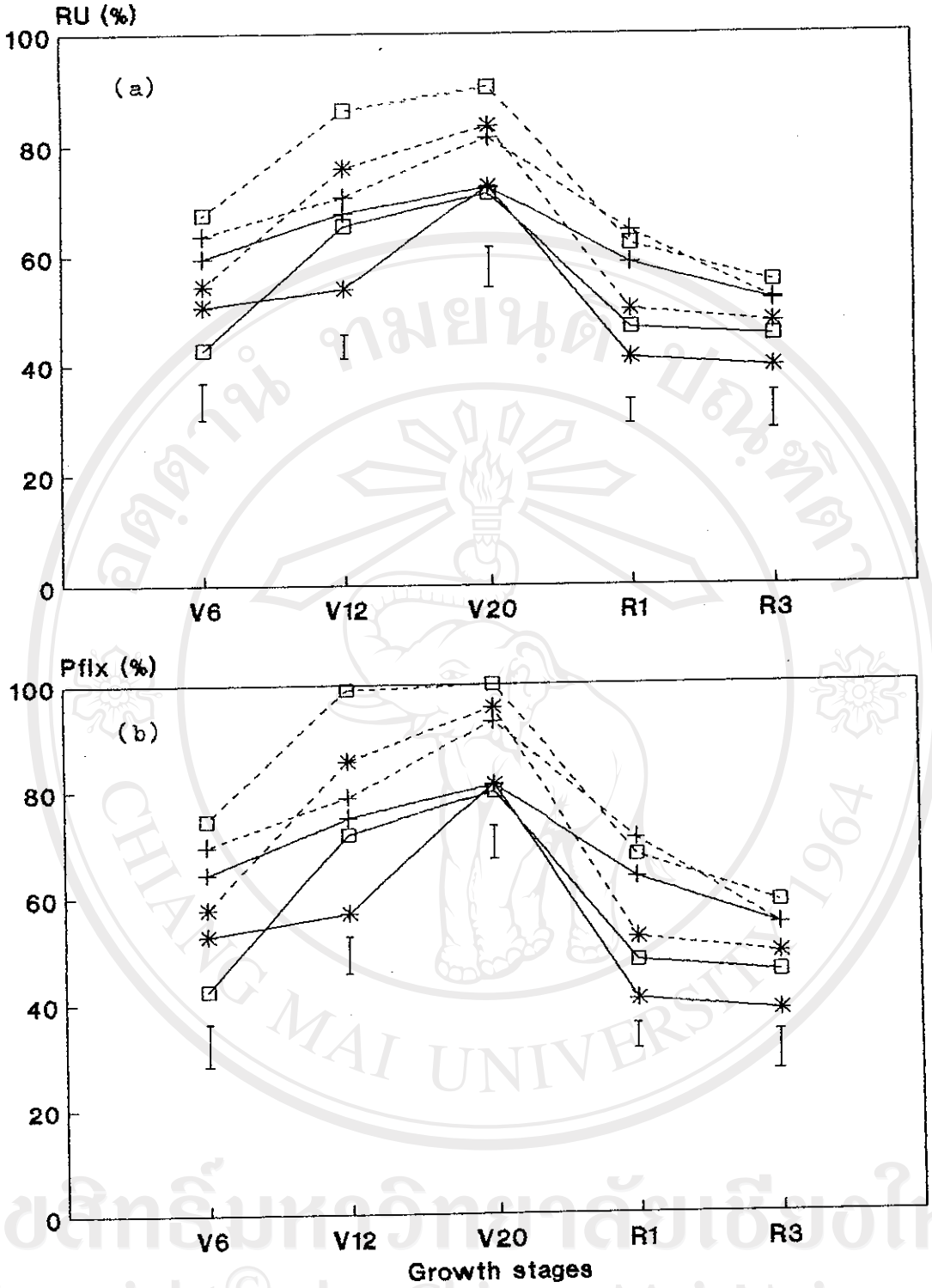

Figure 4. Effects of intercropping on the relative ureide (a) and proportion of nitrogen fixed (b) over time. Bars represent standard error for RU(a) and Pfix (b) values.(——) Represents monocrop treatments; (---) Intercrop treatments; (+) Simultaneous sowing; (*) Intermediate sowing; and, (□) Late sowing.

4.3 Experiment III

Effects of rice bean cropping system on a subsequent corn crop

Time of bean sowing either in monocrop or intercrop treatments did not differ significantly ($P<0.05$) in their effects on the following corn crop.

4.3.1 Dry matter yield

Within three sampling stages, one month after seed sowing (30 DAS), at silking (55 DAS) and at physiological maturity (88 DAS), shoot dry matter of corn following rice bean, in monocrop or intercrop treatments, had significantly higher yield ($P<0.05$) than the preceding monorice treatment (Table 10). The difference was about the same throughout the growing season where there was an increase of about 30% in the preceding bean treatments.

4.3.2 Grain yield and yield components

Corn following rice bean, in monocrop or intercrop, yielded 7.3 t/ha to 8.5 t/ha while that from monorice was 5.4 t/ha (Table 10). Yield variations were associated with the differences in the number of seeds per ear. Number of ears per plant and 100 seed weight were not related to the differences in yield.

Table 9. Accumulated amount of N2 fixed (kgN/ha), total N and % N fixed over time.

Cropping system	Fixed N (kgN/ha)			Total N	N from fixation
Growth stage ==>	V6	V12	V20	(kg/ha)	(%)
Sole					
Simultaneous	14.1	38.2	62.8	113.1	55.4
Intermediate	11.1	42.8	66.6	137.8	48.5
Late	13.7	32.6	65.9	144.7	45.6
Intercrop					
Simultaneous	12.4	37.3	74.4	115.5	64.5
Intermediate	8.9	35.3	90.6	131.7	68.8
Late	5.6	15.3	78.0	91.6	84.5
LSD 0.05	5.0	8.4	10.6	NS	8.5
	*	**	**		**

Table 10. Dry matter, grain yield and yield components of corn grown after pure crops and mixtures of upland rice and rice bean.

Preceding cropping system	Dry matter (t/ha)			Grain yld[a]	Ears/ plant	Seeds/ ear	100 seed[a] weight
	30 DAS	55 DAS	88 DAS	(t/ha)			(g)
Monorice	.64	3.5	8.7	5.4	1.0	371	29.9
Sole rice bean							
Simultaneous	.99	5.8	12.1	8.5	1.09	450	33.9
Intermediate	.82	5.1	14.6	7.3	1.0	481	33.8
Late	.84	6.3	15.3	7.5	1.09	424	34.1
Intercrop							
Simultaneous	.80	5.6	12.1	8.3	1.0	488	32.9
Intermediate	.82	5.4	13.5	7.6	1.0	464	33.5
Late	.78	5.1	14.0	7.3	1.09	449	33.1
LSD 0.05	.18	1	2.2	1.5	NS	60	NS
	*	**	**	**		*	

a. Determined at 14% Moisture Content
DAS Days after sowing

4.3.3 Crop total N

Grain nitrogen and shoot nitrogen in corn did not differ significantly among the preceding bean treatments and, all were higher than the rice monocrop ($P<0.05$) (Table 11).

Table 11. Total nitrogen (kgN/ha) in dry matter and grain of corn.

Preceding cropping system	Days from sowing			Grain N
	30	55	88	(kgN/ha)
Sole rice	14.8	39.5	67.2	61.6
Sole rice bean				
Simultaneous	23.9	74.2	115.4	125.2
Intermediate	20.1	58.7	137.8	106.9
Late	21.0	79.2	138.4	108.7
Intercrop				
Simultaneous	20.2	71.1	112.5	119.2
Intermediate	21.2	66.3	133.8	101.5
Late	19.0	62.4	138.5	98.0
LSD 0.05	4.9 *	15.6 **	32.3 **	29.0 **

4.4 Simple nitrogen balance of rice-corn, and rice/rice bean-corn cropping systems

A simple nitrogen balance of the system was calculated by considering the amount of nitrogen fixed as the input and the outputs are the seed nitrogen yields of rice, rice bean, and corn.

The balance is said to be simple because all other external inputs or sources of N that may contribute to the soil N pool, like rainwater, animal droppings, soil mineral N and others, and natural processes of nitrogen losses from the system, like denitrification, volatilization, leaching and others are not considered.

Nitrogen balance for rice bean at stage V20 (Table 12) ranged from 42.6 kgN/ha to 46.7 kgN/ha from the monocrop treatments; and 56.4 kgN/ha to 72 kgN/ha from the intercrop treatments. Intermediate sowing of rice bean in the intercrop yielded the highest value and monocrop rice bean sown at the same time with rice was the lowest ($P<0.05$).

All the bean treatments had a positive nitrogen balance after rice grain harvest, ranging from 42.6 kgN/ha to 72 kgN/ha and monorice had -30.6 kgN/ha nitrogen balance (Table 13). Summing-up the seed nitrogen yields of rice and corn then subtracting the total from the amount of nitrogen fixed, the highest negative balance was from rice monocrop (-92.2 kgN/ha), followed by monocrop bean sown at the same time with rice (-82.7 kgN/ha). The least negative balance of 29.5 kgN/ha was from the bean intercrop sown two weeks after rice.

Table 12. Effects of intercropping on rice bean nitrogen balance at stage V20[a]

Cropping system	Grain yld (kg/ha)	Total N (kg/ha)	N seed (%)	N seed (kg/ha)	N fixed (kg/ha)	N fixed[b] (%)	N balance[c] (kg/ha)
Sole							
Simultaneous	632	113.1	3.20	20.2	62.8	55.4	42.6
Intermediate	620	137.8	3.22	20.0	66.6	48.5	46.7
Late	607	144.7	3.20	19.4	65.9	45.6	46.5
Intercrop							
Simultaneous	557	115.5	3.24	18.1	74.4	64.5	56.4
Intermediate	577	131.7	3.22	18.6	90.6	68.8	72.0
Late	518	91.6	3.28	17.0	77.5	84.5	60.5
LSD 0.05	NS	NS	NS	NS	10.5 **	8.5 **	10.5 **

a. Data means of four replicates
b. Calculated as N fixed % = (N fixed/Total N) x 100
c. Calculated as N balance = N fixed (kg/ha) - N seed (kg/ha)

Table 13. Simple nitrogen balance (NB) of rice-corn, and rice/rice bean-corn cropping systems.

Cropping system	N fixed	Rice seed N	NB[1]	Corn seed N	NB[2]
	(kgN/ha)				
Sole rice	-	30.6	-30.6	61.6	-92.2
Sole rice bean					
Simultaneous	42.6	-	42.6	125.2	-82.7
Intermediate	46.7	-	46.7	106.9	-60.3
Late	46.5	-	46.5	108.7	-62.2
Intercrop rice bean					
Simultaneous	56.4	0	56.4	119.2	-62.8
Intermediate	72.0	0	72.0	101.5	-29.5
Late	60.5	20.5	40.0	98.0	-58.0
LSD 0.05	10.5 **	8.4 *	9.9 **	29. **	9.8 **

1. Nitrogen balance after rice-rice bean cropping, calculated as N-fixed - rice seed N.
2. Nitrogen balance after rice-rice bean and corn cropping, calculated as N-fixed - rice seed N + corn seed N.