
5

Effect of nitrogen source on nodule population

The early development of a legume - Rhizobium endosymbiosis, manifested by the formation of root nodules, is vital for successful establishment and growth of legumes in nitrogen deficient soils. The symbiosis results in a nutritional complementation where the legume can be considered as a carbon-rich, nitrogen-deficient phototroph and the rhizobia as a carbon-deficient, nitrogen-fixing heterotroph (Verma & Nadler 1985). The resulting symbiosis makes the plant autotrophic with respect to the availability of reduced N, a limiting factor in plant nutrition. However, increased N input via fixation is self-limiting as high soil N levels inhibit nodulation. This is because legumes use combined N in preference to fixing atmospheric dinitrogen (N_2) (Allos & Bartholomew 1955). Substrate N has been shown to have an inhibitory effect on (i) the infection of legume root hairs by the endosymbiont,

(ii) the nodule mass per plant, although the reduction in nodule mass may sometimes be compensated for by nodule number per plant, which may not be seriously affected, and (iii) nitrogenase activity of the nodules, expressed as N_2 fixation rate of nodulated plants (Vincent 1965, Gibson 1974, Vallis 1978, Streeter 1988). On the contrary, nil or very low amounts of combined nitrogen may produce N deficiency symptoms and minimize nodulation and N_2 fixing capabilities due to reduced growth and vigour of the host plants. The detrimental effects of combined nitrogen also vary depending upon the form of N to which the root system is exposed i.e. NO_3^- -N or NH_4^+ -N.

The two leaf morph populations of T. repens seem to differ in their N requirement (Pradhan & Tripathi 1984). This study was therefore, undertaken to investigate the nodulation pattern and N_2 fixing abilities of the two clover populations as affected by varying levels of combined N, and to obtain data which would indicate the source and level of N that may result in maximum yield, nodulation and subsequent N_2 fixation. In order to achieve these objectives, two experiments were performed. Experiment 1 was carried out using NO_3^- -N (supplied as KNO_3) and experiment 2 was performed using NH_4^+ -N (supplied as NH_4Cl).

MATERIALS AND METHODS

These have been described in Chapter 3

RESULTS

Experiment 1

Plant growth

Increasing additions of KNO_3 resulted in an increase in shoot weight in both the marked and unmarked leaf morph populations of white clover (Fig. 5.1a). The difference in shoot weights between the two leaf morph populations and among the different levels of KNO_3 was not significant at the first harvest (H1). However, at the second and final harvest (H2), there was a significant variation in shoot dry weights both between the leaf morph populations and amongst KNO_3 levels ($P < 0.01$), the unmarked population exhibiting higher values. The large increase in shoot dry weights at H2 was also highly significant ($P < 0.01$) as compared to weights at H1 (Tables 5.1, 5.2).

There was a corresponding increase in root dry weight with increasing levels of KNO_3 in both the clover populations (Fig. 5.1b). The root dry weights were significantly higher ($P < 0.05$) in the marked population as compared to the unmarked at H2. This is contradictory to that of shoot dry weights which were significantly higher in the unmarked population.

Though there was an increase ($P < 0.01$) in plant weights with addition of KNO_3 at both harvests, there was no significant difference in weights between the two leaf morph populations (Tables 5.1, 5.2).

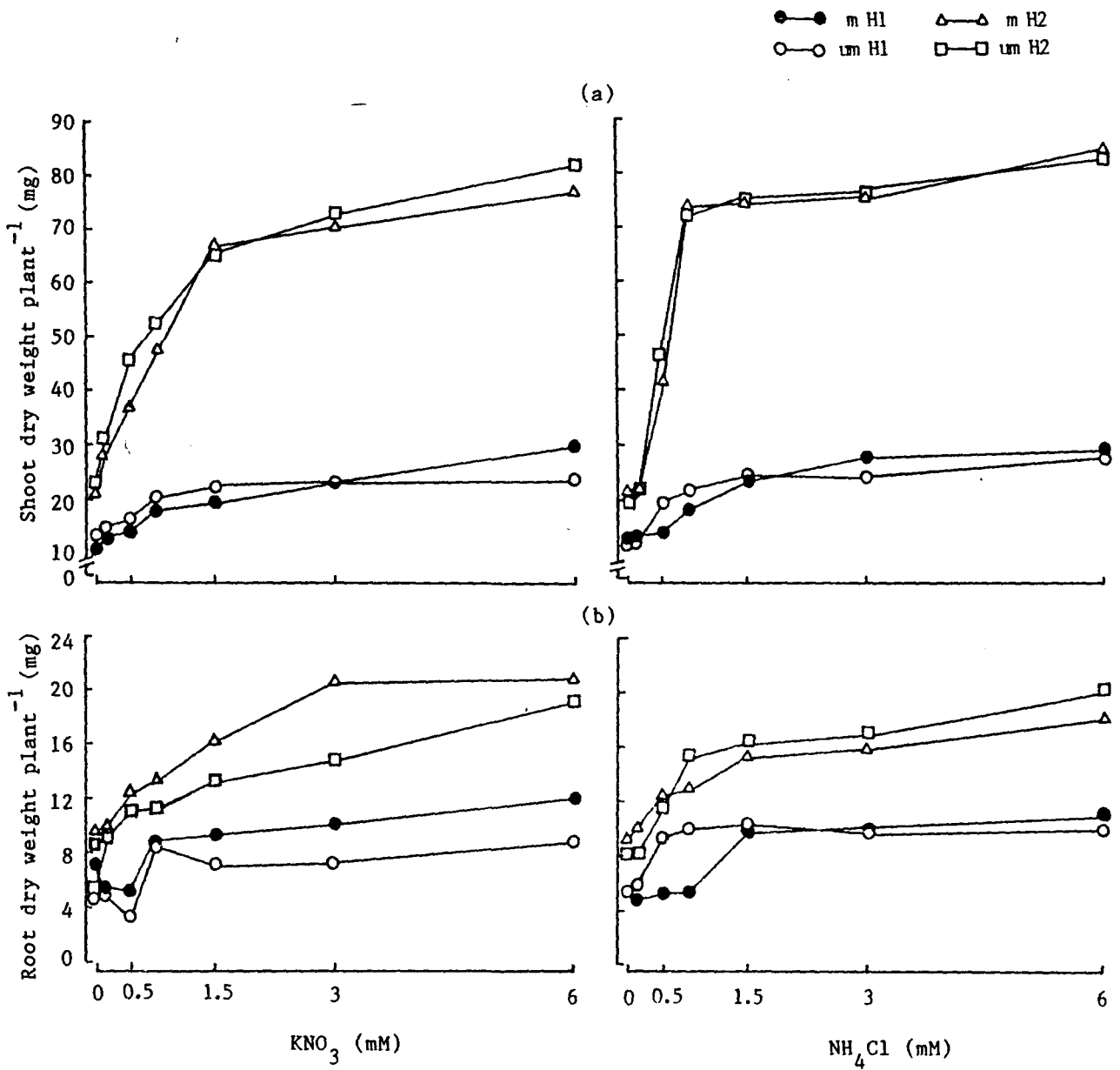


Fig.5.1. Effect of increasing concentrations of NO_3^- and NH_4^+ on (a) shoot dry weight and (b) root dry weight per plant in the two leaf morph populations of the clover at the two harvests. H1, harvest 1; H2, harvest 2; m, marked population um, unmarked population.

Table 5.1. Analysis of variance of legume parameters as influenced by the two leaf morph populations, different N levels and the two forms of N at harvest 1.

Plant parameters	Source of variation	df	F value	Level of significance
Shoot dry weight	Leaf morphs	1	0.32	ns
	N levels	6	0.21	ns
	N forms	1	2.75	ns
	L.morph x form	1	0.30	ns
	L.morph x level	6	0.78	ns
	Form x level	6	2.20	ns
Root dry weight	Leaf morphs	1	2.57	ns
	N levels	6	31.89	P < 0.01
	N forms	1	7.81	P < 0.05
	L.morph x form	1	32.59	P < 0.01
	L.morph x level	6	4.27	ns
	Form x level	6	4.52	P < 0.05
Plant dry weight	Leaf morphs	1	0.07	ns
	N levels	6	66.56	P < 0.01
	N forms	1	6.78	P < 0.05
	L.morph x form	1	3.29	ns
	L.morph x level	6	1.78	ns
	Form x level	6	6.41	P < 0.05
PSA	Leaf morphs	1	7.49	P < 0.05
	N levels	6	260.62	P < 0.01
	N forms	1	32.50	P < 0.01
	L.morph x form	1	4.88	ns
	L.morph x level	6	2.13	ns
	Form x level	6	6.41	P < 0.05

ns = not significant

Table 5.2. Analysis of variance of legume parameters as influenced by two leaf morph populations, different N levels and the two forms of N at harvest 2.

Plant parameters	Source of variation	df	F value	Level of significance
Shoot dry weight	Leaf morphs	1	17.76	P < 0.01
	N levels	6	1169.58	P < 0.01
	N forms	1	83.34	P < 0.01
	L.morph x form	1	7.96	P < 0.05
	L.morph x level	6	48.23	P < 0.01
	Form x level	6	3.30	ns
Root dry weight	Leaf morphs	1	7.96	P < 0.05
	N levels	6	47.35	P < 0.01
	N forms	1	0.56	ns
	L.morph x form	1	11.64	P < 0.05
	L.morph x level	6	1.26	ns
	Form x level	6	1.19	ns
Plant dry weight	Leaf morphs	1	1.05	ns
	N levels	6	971.99	P < 0.01
	N forms	1	60.07	P < 0.01
	L.morph x form	1	0.03	ns
	L.morph x level	6	32.15	P < 0.01
	Form x level	6	3.64	ns
PSA	Leaf morphs	1	11.29	P < 0.05
	N levels	6	162.27	P < 0.01
	N forms	1	7.19	P < 0.05
	L.morph x form	1	0.59	ns
	L.morph x level	6	0.62	ns
	Form x level	6	1.69	ns

ns = not significant

Photosynthetic area

Increase in plant with addition of KNO_3 was associated with significant ($P < 0.01$) increase in the photosynthetic area (PSA) per plant (Table 5.3), which was significantly ($P < 0.05$) greater in the unmarked leaf morph population as compared to the marked one (Tables 5.1, 5.2).

Nodule number

The mean nodule number per plant did not show any definite correlation with addition of KNO_3 in either of the leaf morph populations. At H1, the marked population exhibited an increase in the nodule number with increasing KNO_3 levels whereas the unmarked population showed an initial increase after which the number decreased sharply with no nodules being formed at higher exposures to KNO_3 . There was a significant ($P < 0.01$) difference in the nodule number per plant between the two leaf morph populations, the marked population exhibiting higher values at both harvests. Nodule number per plant was significantly higher at H2 as compared to that at H1. At H2, the nodule number showed an initial increase with additions of KNO_3 after which the number sharply decreased with no nodules being formed at higher concentrations of KNO_3 . The marked population, which nodulated even at higher concentrations of KNO_3 at H1, showed a suppression in nodulation at similar concentrations at H2. The nodule number per plant varied considerably amongst KNO_3 concentrations and the variations were significant (Tables 5.4, 5.5) at both harvests (Fig. 5.2).

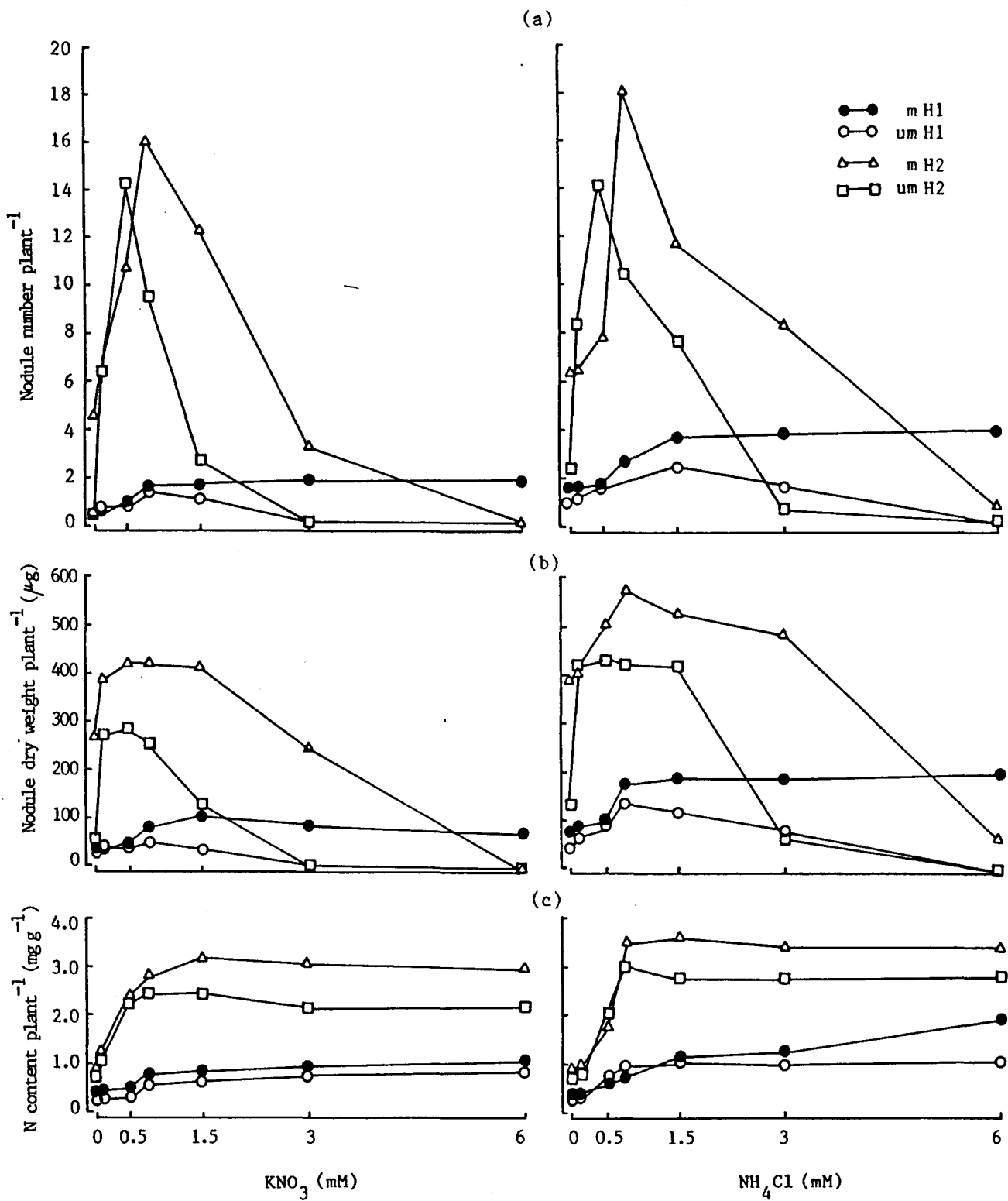


Fig.5.2. Effect of increasing concentrations of NO₃⁻ and NH₄⁺ on (a) nodule number (b) nodule dry weight, and (c) N content per plant in the two leaf morph populations of the clover at the two harvests.

Table 5.3. Effect of increasing concentrations of KNO_3 on the nodule dry weight, plant N concentration and photosynthetic area (PSA) per plant in the two leaf morph populations of the clover at the two harvests.

KNO_3 concentration (mM)	Harvest 1						Harvest 2					
	Marked population			Unmarked population			Marked population			Unmarked population		
	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)
0	48.6	23.85	143.64	47.3	16.73	140.40	58.7	28.79	129.32	70.9	26.51	131.93
0.1	52.2	25.01	148.96	49.7	18.08	191.10	59.3	33.90	160.93	43.2	27.09	459.00
0.5	51.0	25.13	198.72	50.3	18.63	229.50	39.6	35.46	291.18	20.1	30.12	507.00
0.75	49.3	27.98	588.00	33.6	18.79	576.00	26.3	40.09	491.30	26.7	31.19	553.84
1.5	60.3	28.03	585.01	34.9	21.01	625.11	34.7	38.67	814.46	49.3	30.74	910.65
3.0	47.3	28.76	615.78	-	17.31	782.46	78.9	33.51	1186.18	-	26.03	1349.64
6.0	45.0	26.00	666.54	-	18.63	809.19	-	30.06	1418.10	-	22.14	1378.98

- absence

Table 5.4. Analysis of variance of nodule characteristics of the clover as influenced by two leaf morph populations, different N levels and the two forms of N at harvest 1.

Nodule characteristics	Source of variation	df	F value	Level of significance
Nodule number plant ⁻¹	Leaf morphs	1	50.32	P < 0.01
	N levels	6	7.02	P < 0.05
	N forms	1	68.62	P < 0.01
	L.morph x form	1	4.74	ns
	L.morph x level	6	1.48	ns
	Form x level	6	6.52	P < 0.05
Nodule weight per plant	Leaf morphs	1	29.02	P < 0.01
	N levels	6	6.94	P < 0.05
	N forms	1	3.22	ns
	L.morph x form	1	1.61	ns
	L.morph x level	6	1.40	ns
	Form x level	6	4.69	P < 0.05

ns = not significant

Table 5.5. Analysis of variance of nodule characteristics of the clover as influenced by two leaf morph populations, different N levels and the two forms of N at harvest 2.

Nodule characteristics	Source of variation	df	F value	Level of significance
Nodule number per plant	Leaf morphs	1	52.59	P < 0.01
	N levels	6	25.35	P < 0.01
	N forms	1	27.75	P < 0.01
	L.morph x form	1	0.09	ns
	L.morph x level	6	0.88	ns
	Form x level	6	3.40	P < 0.05
Nodule weight per plant	Leaf morphs	1	1.71	ns
	N levels	6	1.24	ns
	N forms	1	2.33	ns
	L.morph x form	1	0.01	ns
	L.morph x level	6	0.34	ns
	Form x level	6	0.59	ns

ns = not significant

Mean nodule weight

Although the number of nodules per plant showed a significant ($P < 0.05$) increase with the addition of KNO_3 in both the leaf morph populations at H1, there was no correlation between nodule number and mean nodule weight in either population. At H2, however, there was a strong negative correlation between nodule number per plant and mean nodule weight (Fig. 5.3).

Nodule weight per plant

The total nodule mass per plant showed a distinct trend, being proportional to that of nodule number per plant (Fig. 5.2b). The difference in the nodule weights was significant between the two leaf morph populations ($P < 0.01$) and NO_3^- levels ($P < 0.05$) at H1 with the marked population exhibiting greater nodule weight per plant. Between harvests, the variation in nodule mass per plant was highly significant ($P < 0.01$) with the weights being much higher at H2 than at H1.

Total N per plant

Figure 5.2c gives the corresponding N content per plant with increasing NO_3 levels. The N content showed an increase following NO_3 additions. The N content varied significantly between NO_3 levels ($P < 0.01$) at both H1 and H2. The marked population showed a higher N content ($P < 0.01$) as compared to the unmarked population at both harvests (Tables 5.6, 5.7).

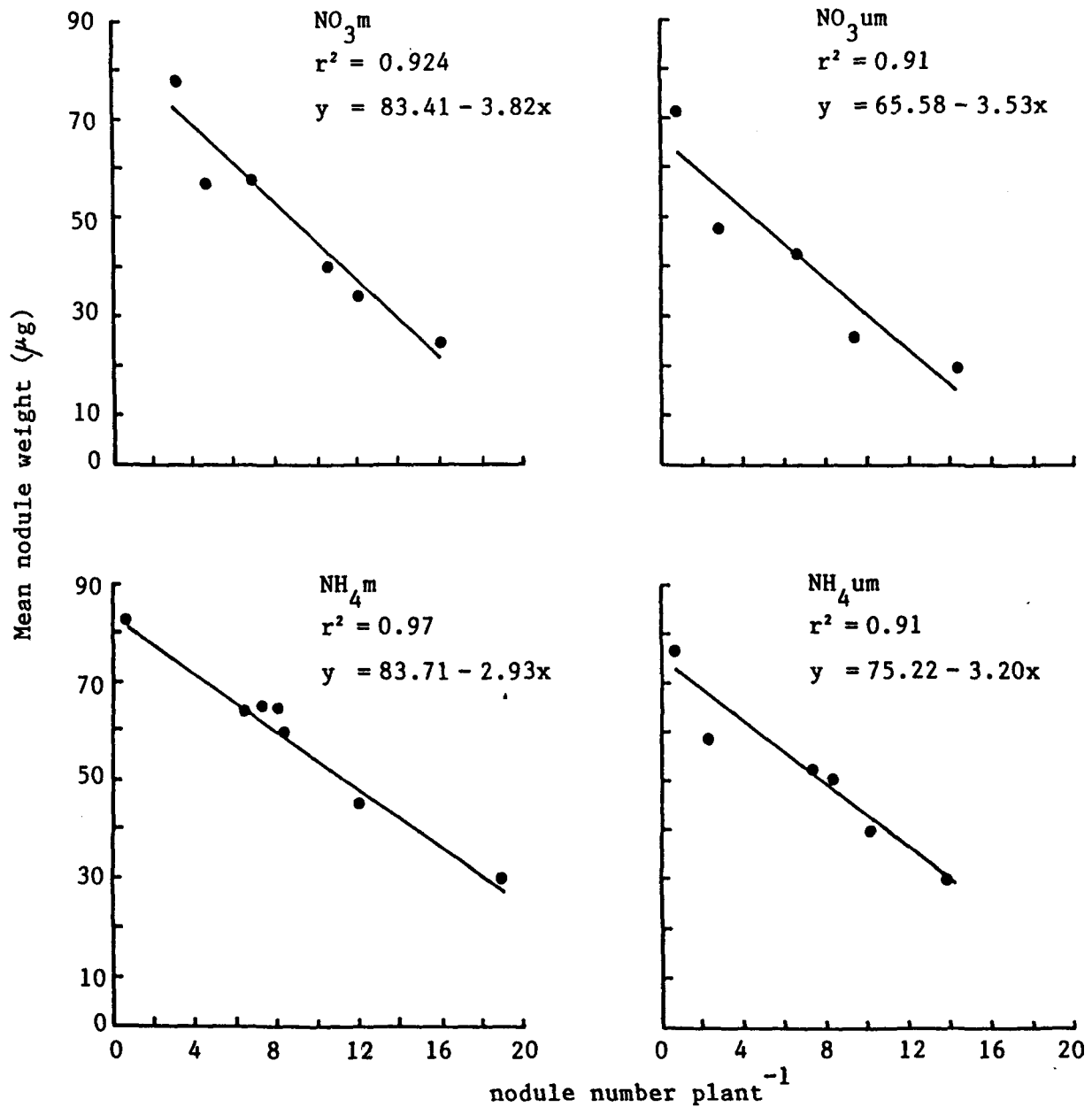


Fig.5.3. Regression showing the relationship between nodule number plant⁻¹ and mean nodule weight in the marked (m) and unmarked (um) leaf morph populations of clover supplied with NO₃⁻ and NH₄⁺.

Table 5.6. Analysis of variance of growth parameters of the legume as influenced by two leaf morph populations, different N levels and the two forms of N at harvest 1.

Growth parameters	Source of variation	df	F value	Level of significance
R_W	Leaf morphs	1	0.07	ns
	N levels	6	65.39	$P < 0.01$
	N forms	1	4.75	ns
	L.morph x form	1	2.68	ns
	L.morph x level	6	3.01	ns
	Form x level	6	1.99	ns
R_N	Leaf morphs	1	7.14	$P < 0.05$
	N levels	6	13.42	$P < 0.01$
	N forms	1	11.75	$P < 0.05$
	L.morph x form	1	0.71	ns
	L.morph x level	6	0.41	ns
	Form x level	6	0.53	ns
Total N per plant	Leaf morphs	1	19.71	$P < 0.01$
	N levels	6	52.59	$P < 0.01$
	N forms	1	49.52	$P < 0.01$
	L.morph x form	1	5.55	ns
	L.morph x level	6	2.33	ns
	Form x level	6	3.26	ns

ns = not significant

Table 5.7. Analysis of variance of growth parameters of the legume as influenced by two leaf morph populations, different N levels and the two forms of N at harvest 2.

Growth parameters	Source of variation	df	F value	Level of significance
R_W	Leaf morphs	1	0.08	ns
	N levels	6	22.96	$P < 0.01$
	N forms	1	2.77	ns
	L.morph x form	1	1.24	ns
	L.morph x level	6	0.80	ns
	Form x level	1	4.49	$P < 0.05$
R_N	Leaf morphs	1	0.92	ns
	N levels	6	7.42	$P < 0.05$
	N forms	1	4.66	ns
	L.morphs x form	1	0.40	ns
	L.morph x level	6	1.03	ns
	Form x level	1	1.71	ns
Total N per plant	Leaf morphs	1	199.78	$P < 0.01$
	N levels	6	521.20	$P < 0.01$
	N forms	1	34.66	$P < 0.01$
	L.morph x form	1	1.81	ns
	L.morph x level	6	18.58	$P < 0.01$
	Form x level	1	28.25	$P < 0.01$

ns = not significant

Relative growth rate and Relative N accumulation rate

The relative growth rates (R_W) (Table 5.8) showed a significant ($P < 0.01$) positive relationship with increasing KNO_3 levels at H1 for both leaf morph populations. At H2, the R_W values seemed to follow the trend exhibited by nodule number, nodule mass and N content of the clover populations, showing a sharp initial rise after which the R_W values either decreased (as in the marked population) or did not show any particular trend (as in the unmarked population). The variations in the R_W values were, however, significant at H2 ($P < 0.01$) as in H1.

The relative N accumulation rates (R_N) showed a similar trend to that of R_W and increased significantly ($P < 0.01$) with the addition of KNO_3 at H1 for both the leaf morph populations. Values were significantly ($P < 0.05$) higher for the marked population as compared to the unmarked one. At H2, the R_N values varied significantly ($P < 0.05$) showing a trend similar to that of R_W , reaching a maximum at 0.5 mM KNO_3 in both leaf morph populations, following which there was a gradual decline at higher concentrations (Tables 5.6, 5.7).

Experiment 2

Plant growth

Figure 5.1a shows the relationship between NH_4^+ levels and corresponding shoot weights in the two clover populations. The shoot

Table 5.8. Effect of increasing concentrations of KNO_3 on relative growth rate (R_W) ($\mu\text{g d}^{-1}$) and relative N accumulation rate (R_N) ($\mu\text{g d}^{-1}$) in the two leaf morph populations of the clover at the two harvests.

KNO_3 concentration (mM)	Harvest 1				Harvest 2			
	Marked population		Unmarked population		Marked population		Unmarked population	
	R_W	R_N	R_W	R_N	R_W	R_N	R_W	R_N
0	3.96	10.10	3.34	4.01	3.80	5.27	3.02	6.58
0.1	4.08	10.98	4.11	5.98	5.46	7.82	5.57	8.70
0.5	4.23	11.21	4.43	6.72	7.35	12.38	10.80	14.55
0.75	9.67	18.31	10.68	13.14	7.39	10.18	7.58	11.51
1.5	10.61	19.27	10.78	14.96	8.18	10.68	7.70	10.23
3.0	12.72	21.78	11.39	16.59	7.78	8.97	8.14	8.85
6.0	16.17	18.79	12.64	18.71	6.70	6.62	8.68	7.46

weights increased ($P < 0.01$) with NH_4 increments at both harvests. At H1, there was no significant difference in shoot dry weight either between the two leaf morph populations or between the two N sources (NO_3 and NH_4). At H2, not only did the shoot weights show a significant ($P < 0.01$) increase with additions of NH_4Cl but there was also a significant ($P < 0.01$) variation between the two leaf morph populations, the unmarked plants exhibiting greater shoot weights. The plants exposed to NH_4 too showed better shoot growth as compared to the NO_3 treated plants in both leaf morph populations. There was a marked increment in the shoot weights at H2 as compared to H1 ($P < 0.01$) for both the leaf morph populations in the NH_4 treated plants.

A corresponding increase in root biomass with additions of NH_4Cl was observed ($P < 0.01$) for both leaf morph populations at H1, though there was no significant difference in the root weights between the populations. Plants supplied with NH_4 also exhibited greater root weights ($P < 0.05$) than those supplied with NO_3 . At H2, the root weight in the unmarked population was significantly ($P < 0.01$) higher compared to the marked population. Root growth was enhanced with the addition of NH_4 and there was a significant variation between NH_4 levels. Between harvests, the root weight showed a significant ($P < 0.01$) increase at H2 compared to H1 for both leaf morph populations.

The total plant weights did not show any significant variation between the two leaf morph populations at either harvest although

the increase in weight associated with NH_4 additions was significant at both harvests. The NH_4 treated plants also showed better growth, expressed as greater plant weight, as compared to NO_3 treated plants, both at H1 ($P < 0.05$) and at H2 ($P < 0.01$).

Photosynthetic area

Table 5.9 gives the PSA per plant corresponding to increasing levels of NH_4 . PSA showed a significant ($P < 0.01$) increase between NH_4 levels for both leaf morph populations at both harvests. Of the two leaf morph populations, PSA was greater in the unmarked population ($P < 0.05$) both at H1 and H2. Plants supplied with NH_4 also showed greater PSA as compared to NO_3 treated plants, both at H1 ($P < 0.01$) and H2 ($P < 0.05$).

Nodule number

As in experiment 1, the nodule number per plant (Fig. 5.2a) did not show any definite relation with the addition of NH_4Cl . At H1, the increase in NH_4 levels caused an increase in nodule number per plant in the marked population, while in the unmarked population, there was an initial increase followed by a decline with no nodules being formed at 6 mM NH_4 . The marked population showed a significantly ($P < 0.01$) better nodulation with a larger number of nodules being formed per plant as compared to the unmarked population at both H1 and H2. The variation in nodule number per plant with increasing NH_4 levels was significant, both at H1 ($P < 0.05$) and at H2 ($P < 0.01$). In general, the clover populations had a significantly ($P < 0.01$) larger number of nodules per plant at H2 as compared

Table 5.9. Effect of increasing concentrations of NH_4Cl on the nodule dry weight, plant N concentration and photosynthetic area (PSA) per plant in the two leaf morph populations of the clover at the two harvests.

NH_4Cl concentrations (mM)	Harvest 1						Harvest 2					
	Marked population			Unmarked population			Marked population			Unmarked population		
	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)	Nodule dry weight (μg)	Plant N concentration (mg g^{-1})	PSA per plant (mm^2)
0	50.3	25.11	129.01	46.3	21.36	138.04	63.8	29.31	143.64	59.3	26.03	165.36
0.1	50.9	25.07	144.97	46.7	22.01	146.83	64.0	30.01	163.59	50.9	26.91	188.71
0.5	52.7	28.03	153.44	53.1	23.71	181.76	64.1	33.13	294.68	30.1	34.18	401.71
0.75	62.0	30.18	353.06	50.0	30.11	329.31	30.3	41.01	499.97	40.2	34.16	566.72
1.5	48.9	33.07	582.01	42.1	31.70	543.00	45.1	40.03	735.21	53.7	30.03	880.08
3.0	50.0	33.16	631.12	41.0	30.80	629.01	59.8	37.01	1008.72	78.6	29.85	1016.89
6.0	49.8	35.01	665.60	-	28.17	734.16	83.4	33.16	1274.91	-	26.51	1403.43

- absence

to numbers at H1.

Plants supplied with NH_4^+ developed a larger number of nodules per plant as compared to NO_3^- treated plants. There was a complete inhibition of nodulation in the unmarked population occurring at the highest level of $\text{NH}_4\text{-N}$ i.e. 6 mM at both harvests (Table 5.10).

Mean nodule weight

At H1, there was no correlation between nodule number and mass per nodule in either leaf morph population. Figure 5.3 gives the regression model of nodule number per plant plotted against mean nodule weight at H2. Of the two leaf morph populations, the correlation was stronger in the marked ($r^2 = 0.97$) than in the unmarked ($r^2 = 0.91$) population. Between the harvests, there was no significant difference in mean nodule weight in either leaf morph population.

Nodule weight per plant

The total nodule mass per plant (Fig. 5.2b) showed an initial increase with addition of NH_4 for both leaf morph populations. At higher levels of NH_4 , the nodule weight per plant did not increase much in the marked population, and in the unmarked population there was a gradual decline associated with increased levels of NH_4 from 1.5 mM onwards. At H2, the nodule mass per plant was significantly ($P < 0.01$) greater than at H1 for both leaf morph populations. Between the two leaf morph populations, there was a significant ($P < 0.01$) difference in the nodule mass per plant

Table 5.10. Effect of increasing concentrations of KNO₃ and NH₄Cl on the nodule number per unit (100 mg) plant dry weight.

Nitrogen levels (mM)	KNO ₃				NH ₄ Cl			
	Harvest 1		Harvest 2		Harvest 1		Harvest 2	
	marked	unmarked	marked	unmarked	marked	unmarked	marked	unmarked
0	2.97	3.09	14.81	2.09	8.21	5.57	19.66	8.03
0.1	2.94	3.46	17.34	16.52	8.62	6.77	19.77	26.91
0.5	4.64	4.01	21.55	24.19	8.71	5.40	14.66	24.01
0.75	5.73	4.95	26.38	14.92	11.62	9.06	21.76	11.74
1.5	5.77	3.62	14.57	3.38	10.98	8.10	12.95	8.40
3.0	5.21	-	3.49	-	10.04	4.61	9.02	0.84
6.0	4.17	-	-	-	7.93	-	0.66	-

- absence

with the marked population exhibiting higher values (Tables 5.4, 5.5).

Total N per plant

Addition of NH_4Cl resulted in a steady increase in the total N content per plant (Fig. 5.2c) in both marked and unmarked leaf morph populations at H1. Total plant N at H2 showed a steep rise with increasing NH_4^+ concentration up to 0.5 mM level but at higher concentrations, the values did not increase. Plants of the marked population had significantly ($P < 0.01$) greater amounts of total N than those of the unmarked population, at both harvests. Total N increments associated with additions of NH_4Cl were significant ($P < 0.01$) at both harvests.

Relative growth rate and Relative N accumulation rate

Table 5.11 gives the corresponding values of R_W with the additions of NH_4Cl . The R_W values increased steadily up to 0.75 mM NH_4 following which there was either no definite trend (marked population) or only a marginal increase (unmarked population) on further additions of NH_4Cl . Variations in corresponding values of R_W was significant ($P < 0.01$) among NH_4 levels for both leaf morph populations. The R_W values at H1 showed a rapid increase with additions of NH_4Cl , especially in the marked population, with the 6 mM NH_4 treatment recording about 2.5 times the value in the case of the N free control. The unmarked population showed a sharper increase in the R_N values with a three fold increase as compared to the N free control. At H1, the R_N values were generally higher for the marked population

Table 5.11. Effect of increasing concentrations of NH_4Cl on relative growth rate (R_W) ($\mu\text{g d}^{-1}$) and relative N accumulation rate (R_N) ($\mu\text{g d}^{-1}$) in the two leaf morph populations of the clover at the two harvests.

NH_4Cl concentration (mM)	Harvest 1				Harvest 2			
	Marked population		Unmarked population		Marked population		Unmarked population	
	R_W	R_N	R_W	R_N	R_W	R_N	R_W	R_N
0	4.07	11.01	3.18	7.60	3.95	5.16	3.73	5.48
0.1	3.31	10.24	3.25	8.16	4.43	5.82	3.99	11.09
0.5	4.76	13.42	10.56	16.63	7.73	9.03	5.51	8.35
0.75	7.65	17.25	11.23	21.01	10.04	12.52	8.31	9.29
1.5	12.87	24.10	12.97	23.54	7.67	9.15	7.73	7.31
3.0	14.72	25.98	13.03	23.15	6.84	7.69	7.75	7.52
6.0	15.65	27.76	14.45	16.88	7.19	6.78	7.98	8.35

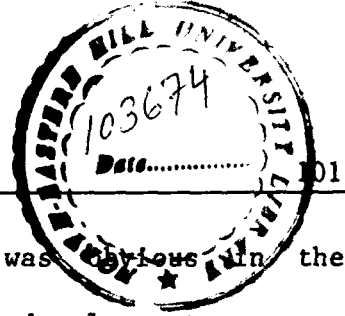
($P < 0.05$) than the unmarked, and varied significantly ($P < 0.01$) between the NH_4 levels.

At H2, R_N values in both leaf morph populations increased up to 0.75 mM NH_4 level after which the values gradually decreased with further increments of NH_4Cl . Plants exposed to NH_4 (experiment 2) recorded higher values ($P < 0.05$) for R_N than plants exposed to NO_3 (experiment 1) at H1. At H2, however, this trend was reversed with the NO_3 treated plants recording R_N values marginally higher than NH_4 treated plants (Tables 5.6, 5.7).

DISCUSSION

Addition of KNO_3 or NH_4Cl led to a steady increase in the weights of both shoot and root of the two clover populations at H1. Though shoot weights were greater than corresponding root weights at respective N levels, this difference was markedly pronounced at H2 when shoot weights were up to four times that of the root, especially at higher N levels (Fig. 5.1a). This trend suggests that most of the photosynthates produced as a result of augmented plant growth at higher N levels was used for shoot development which also functions as a sink for assimilates (Batra 1978, Chapman 1986).

Although the total plant weights did not differ significantly between the two leaf morph populations, the allocation pattern showed a conspicuous variation with the marked plants exhibiting better root development as compared to the shoot. This trend,



though insignificant in NH_4 treated plants, was obvious in the plants exposed to NO_3 . On the contrary, shoot development appears to be better in the unmarked population. The development of nodules at nil N in both the leaf morph populations, indicates that nodule formation is not N dependent but the initial increase in nodule number per plant following N addition, clearly indicates that low levels of N in soil enhance nodule formation and growth. There is a time lag between nodule formation and active N_2 fixation by the newly formed nodules and during this lag period the host plant is dependent on N present in the soil to tide over the temporary N crisis. Therefore, such plants show signs of N deficiency, even if only temporarily, when soil N levels were low (Streeter 1988). Plants of the N free controls, though nodulated, faced initial N deprivation and exhibited poor growth as indicated by low plant biomass (Figs. 5.1a, b). The nodule numbers per plant at H1 at 0.1 mM and 0.5 mM nitrogen were only marginally higher than at nil N but at H2 there was almost a four fold increase in nodule number and a sharp corresponding increase in weights of nodules and host plants.

Plants from the N free controls at H2 exhibited stunted growth and had low plant and nodule biomass. They were pale and occasionally had chlorotic leaves thereby exhibiting the classical symptoms of N deficiency. This indicates that initially some N is required by the legume to tide over the temporary period of crisis prior to commencement of N_2 fixation. Thus, the plants grown at nil N exhibited poor growth in spite of nodulating considerably. This is in

conformity with the findings of Harper (1974), Pankhurst & Jones (1979), Das (1982) and Rawsthorne et al. (1985).

In both experiments, the sudden drop in nodule number following the sharp initial increase at H2, ultimately culminating in the total inhibition of nodulation, is interesting. Of the two forms of N available to a nodulated legume - combined N and molecular dinitrogen (N_2) - the first form is usually the preferred form (Wery et al. 1978). Nodule development and function are energy demanding processes requiring a continuous supply of photosynthates from the aboveground sinks (Bergersen 1974, Chapman 1983) and the initial development of nodules is dependent on the growth of the host plant which is enhanced with the addition of combined N. After the plants have grown considerably as was the case at H2, they require larger quantities of N which is mostly provided by the increased number of root nodules. However, the nodule number per plant which showed a sharp increase at lower N levels, starts decreasing rapidly with N additions in both leaf morph populations, indicating that the legume probably switches over to the more preferred source of N i.e. combined N (Stone & Buttery 1986). The combined N inhibits nodulation by affecting a broad range of infection events which include a decrease in root hair deformations (Truchet & Dazzo 1982), a decrease in the binding of rhizobia to root hairs (Dazzo & Brill 1979), a decrease in the number of infection threads (Munns 1977) and an increase in the number of aborted infections (Munns 1977). According to Dazzo & Brill (1979) higher levels of combined N hamper nodulation which is mediated via inhibition of infection

of root hairs by the rhizobia. They have reported that with an increase in N levels, the accumulation of the host lectin on root hair surfaces is drastically reduced even though there may not be any inhibition of its synthesis by the host. This in turn prevents R. trifolii from accumulating on and adhering to root hair surfaces, thereby reducing infection and subsequent nodule formation.

Between harvests, the marked population actually showed a reduction in nodule number at H2 as compared to H1 at 6 mM NO₃ or NH₄ (Fig. 5.2). The plants harvested at H1 were exposed to the high N level only for a short duration and therefore, the detrimental effects of increased levels of soil N on nodulation were not obvious. However, after prolonged exposure to the high nitrogen, the plants substituted combined N for atmospheric dinitrogen (N₂) and do not need to develop any more nodules afresh. Existing nodules are also shed as they are temporarily denominated as non-functional and probably become expensive for the host to maintain due to channeling of photosynthates (Wedderburn 1983). In both experiments the marked population showed an increased root growth which contributes to an increase in infection sites resulting in greater number of nodules being formed. Greater nodule number per plant in the marked population was also exhibited under field conditions (Chapter 4). According to Pradhan & Tripathi (1984) the marked population has a greater N requirement than the unmarked population and the better nodulating ability of the former is probably a means of fulfilling its higher N requirement. The newly formed nodules also act as sinks for photosynthates from the shoot and

therefore, any increase in number of nodules would probably contribute to increase in nodule weight (Wedderburn 1983). This seems to be true in the present study. Though nodule number per plant drops sharply with increasing N levels, there is an increase in mean nodule weight (Fig. 5.3) indicating that higher levels of N may be detrimental to nodule initiation and formation but not to nodule growth. Thus the host plant compensates for the fewer successful infections by an increase in mass per nodule - an adaptation to sparse nodulation. The strong negative correlation between the two parameters exhibited by the marked population in both the experiments, indicates the greater tendency of the N demanding marked population to compensate for the curtailed number of nodules by augmented nodule mass. Between the two experiments, the coefficient of regression (r) was lower in the NO_3 treated plants indicating that in comparison to NH_4 , not only was NO_3 a stronger inhibitor of nodulation but also of nodule growth. The probable causes for this are discussed after the following section.

Though mean nodule weight did not vary significantly between the two leaf morph populations in either experiment, the values tended to be higher in the marked plants which could be considered an important strategy to meet the higher N requirement of the marked plants, as N_2 fixation is a function of nodule weight.

The growth response of the nodules, measured as total nodule mass per plant, shows a sharp rise with low N increments which can be attributed both to a steep increase in nodule number and a

simultaneous rapid growth of newly formed nodules triggered by a flush in shoot growth stimulated by N additions. The decrease in the total nodule mass following a drop in nodule number is lessened due to compensation for fewer successful infections with an increase in mass per nodule. Though the drop in nodule number is associated with an increase in mean nodule weight, the number of nodules was too low to maintain the total nodule mass per plant. Compensation thus, does not preclude the importance of nodule number and in case of sparse nodulation, increased numbers will be required to bring about an increase in nodule biomass.

Results of the present investigation indicate that of the two forms of N supplied, NO_3 is a more potent inhibitor of nodulation in clover. This holds good for inhibition of infection as well as inhibition of nodule growth and activity. According to Dazzo & Brill (1978), the infection process is more sensitive to NH_4 than to NO_3 , but the findings of the present study are in conformity with those of the majority of workers on a wide range of legumes, who hold the opposite view (Darbyshire 1966, Dixon 1969, Streeter 1981, Ursino et al. 1982, Eardly et al. 1984, Sawhney et al. 1985). Wong (pers. commn.) also holds the latter view. Nodule growth has also been reported to be more sensitive to NO_3 than NH_4 (Imsande 1986). Our findings are in conformity with those of Streeter (1981), Ursino et al. (1982) and Sawhney et al. (1985) who also reported that several weeks exposure to 4-8 mM NO_3 leads to a reduction in nodule growth. In soybean, long term NO_3 supply was, however, found

not to influence the number of infected, uninfected or cortical cells (Streeter 1988). Thus the decline in nodule size in response to N supply could probably be ascribed to a decrease in cell size.

When NO_3^- is supplied, its reduction and assimilation via nitrate reductase requires considerable reducing power and as such the amount of photosynthates, that could have been otherwise available for distribution to nodules is reduced. Studies on clover (Small & Leonard 1969), cowpea (Kahn & Kahn 1981) and soybean (Truchet & Dazzo 1982, Kouchi & Yoneyama 1984) showing a decrease in ^{14}C -labelled photosynthates to nodules in the presence of NO_3^- , lends support to this view. Nitrate is also partially reduced to nitrite which, besides being toxic to all living organisms including Rhizobium, is known to destroy IAA which is required for infection. This view has been confirmed by Dixon (1969) and Munns (1977) who reported that IAA supplied with NO_3^- could mitigate the inhibitory effects to some extent. Nitrite also oxidises leghaemoglobin to its inactive ferric form (Rigaud & Puppo 1977) which could affect the nitrogenase enzyme. Temperate legumes reduce a greater proportion of their nitrate in roots than tropical legumes (Andrews 1986). The inhibitory effects of NO_3^- on nodulation in white clover, a temperate legume, are probably mediated via the above-mentioned mechanisms.

Latimore et al. (1977) and Rabie et al. (1980) suggest that $\text{NH}_4\text{-N}$ also acts in a similar way, but the inhibitory effects are less severe due to its reduced state. $\text{NH}_4\text{-N}$ -treated plants also exhibited increased root growth leading to a greater number of infection

sites which probably contributes to the increase in nodule number and weight (Wedderburn 1983) as discussed earlier.

Results indicated that NO_3^- -treated plants failed to nodulate at higher N levels (3 mM for unmarked population and 6 mM for marked population) vis-a-vis to NH_4^+ -treated plants where nodulation was totally inhibited only at 6 mM for the unmarked population (the marked population still forming some nodules). This confirms that not only is NO_3^- a stronger inhibitor of nodulation but also the marked population is more resistant to the inhibitory effects of combined N as indicated by its greater nodule number as compared to the other population.

Between the two leaf morph populations, the marked population exhibited a marginally higher N content per plant in both the experiments. This could be attributed to the greater nodule number per plant of the marked population coupled with its higher N requirement which could be fulfilled by the uptake of combined N by the root system if the amount of symbiotically fixed N_2 proved inadequate. The higher N contents per plant in the NH_4^+ treated ones (experiment 2) compared to the NO_3^- treated ones is probably the outcome of the greater number of nodules which develop in response to NH_4^+ addition. Nodule mass per plant was also higher in NH_4^+ treatments and thus the greater amount of potential N_2 fixing tissue could be responsible for the increased levels of total plant N in experiment 2.

At nil N level, the corresponding increment in total N of the legume is a measure of the amount of N_2 fixed by the root nodules. The total N content of the unmarked plants is comparable to that of the marked plants in spite of their lower nodule number and nodule mass per plant. This implies that the nitrogenase activity and subsequent fixation rates are considerably higher in the unmarked population probably fuelled by larger amounts of photosynthates from significantly greater aboveground biomass and photosynthetic area. However, the marked population has an edge over the unmarked population in total N content per plant, which is probably indicative of its higher N requirement, fulfilled by a much greater nodule number and nodule mass per plant (Fig. 5.2). With the addition of N to the substratum, corresponding estimates of fixation are, however, difficult to assess as the legume prefers to take up the readily available combined N in lieu of fixing atmospheric N_2 . According to Ladha et al. (1988) contribution of N by fixation varies from 50-80% of the total N content of a legume, the relative proportion of fixed N_2 increasing with a corresponding decrease in the amounts of combined N in the substratum. A more conservative estimate of contribution to the total N content in legumes by the symbiotic process has been put at 50% (Pate et al. 1979). In the present study, as the amount of added N was increased, corresponding contribution of N via fixation decreased along with nodule number and biomass. Since this is not obvious due to total plant N remaining somewhat constant, the process may more specifically be referred to as N_2 assimilation in lieu of N_2 fixation. This

symbiosis is thus of immense ecological significance in the N economy of the habitat, especially in N deficient environments.

Whether growth and nodulation of the two leaf morph populations of white clover was stimulated or depressed, was dependent on the form and concentration of the applied N. In this study, both NO_3^- and NH_4^+ supplied at 0.75 mM had the greatest beneficial effect on nodule number and nodule mass per plant in both leaf morph populations. The better-nodulating marked population developed nodules at 3 mM and 6 mM NO_3^- and NH_4^+ respectively, even after the 12 week period of exposure to N. However, it is possible that N levels not inhibitory in adequately watered small container experiments could be inhibitory in the field where lower soil moisture levels might make N nonlimiting and also restrict the development of an adequate soil population of the appropriate microsymbiont. The marked leaf morph population of T. repens thus tended to overcome the self limitation imposed due to high soil N levels and can be expected to nodulate and continue to fix N_2 under such stress. The shallow rooting local clover populations with nodulation and N_2 fixation occurring within a 20 cm depth, is therefore vital in the N economy of grasslands and for vegetation establishment in N-deficient situations.