
6

Effect of pH on nodule population

Soils exhibiting acidic reactions are of common occurrence worldwide as also in this part of our country. This is mainly caused due to leaching of bases by percolating water which becomes more pronounced following heavy rainfall resulting in lowered pH of the soil leaving it porous and eroded, with subsequent low levels of organic matter and reduced fertility. Low pH (≈ 5) is associated with aluminium and manganese toxicities (Munns 1977). Low pH also results in limited availability of calcium, molybdenum and phosphate (Coventry & Evans 1989). A shift in pH also reduces or increases the availability of many other elements and nutrients in the soil. These effects may occur either by biological or chemical mechanisms or by both.

Soil pH and the associated factors affect the growth and activity of the root nodule bacteria, the host legume and the symbiosis as such. Availability of calcium, which is closely linked

to pH, affects the nodulation process, besides the metabolic processes of the endophyte. The local populations of T. repens and the associated strains of symbiotic R. trifolii, found in this part of the country are also strongly influenced by the abovementioned factors as far as colonization and nodulation of the legume by the endophyte is concerned. Both leaf morph populations of T. repens seem to prefer slightly acidic soils whereas still lower pH values though apparently not a severe stress, limit the nodulation process resulting in low N₂ fixation. Poor productivity of pastures has occasionally been attributed to nodulation failure in acid soils (Foy 1984, Coventry et al. 1985). This chapter attempts to analyse the nodulation pattern over an acidic pH range in the two legume populations.

MATERIALS AND METHODS

These have been described in Chapter 3

RESULTS

Plant growth

There was a significant ($P < 0.05$) increase in shoot weights of the clover populations with increasing pH levels (Fig. 6.1a). Between the two leaf morph populations, there was a significant ($P < 0.01$) variation in the shoot weights with the unmarked population exhibiting higher values for shoot dry weights at both harvests. The increase in shoot biomass from H1 to H2 was also highly signifi-

cant ($P < 0.01$) (Table 6.1).

Along with increasing values of shoot dry weight, there was a corresponding increase ($P < 0.05$) in root dry weight in both marked and unmarked clover populations (Fig. 6.1b). The root biomass of the marked population showed a distinct edge over the unmarked at H1, though at H2 the difference in dry weights between the two populations disappeared. Unlike the shoot dry weight, the root dry weight of the marked population was generally greater than the unmarked population, though the difference was not significant. The root dry weights at H2 were significantly ($P < 0.01$) greater.

Increasing above- and below-ground biomass values, associated with increasing pH levels, led to simultaneous increments ($P < 0.01$) in the total plant dry weights. However, the difference between the two leaf morph populations was not significant. Total plant biomass exhibited a near three fold increase at H2 as compared to H1 (Table 6.1).

Photosynthetic area

The PSA showed a significant ($P < 0.01$) increase with increasing pH showing almost a four fold increase at pH 6.5 as compared to pH 4.5. Between the two leaf morph populations there was no significant variation in PSA although values were marginally higher for the marked population at both the harvests. There was a more than two fold increase in the PSA from H1 to H2 (Tables 6.1, 6.2).

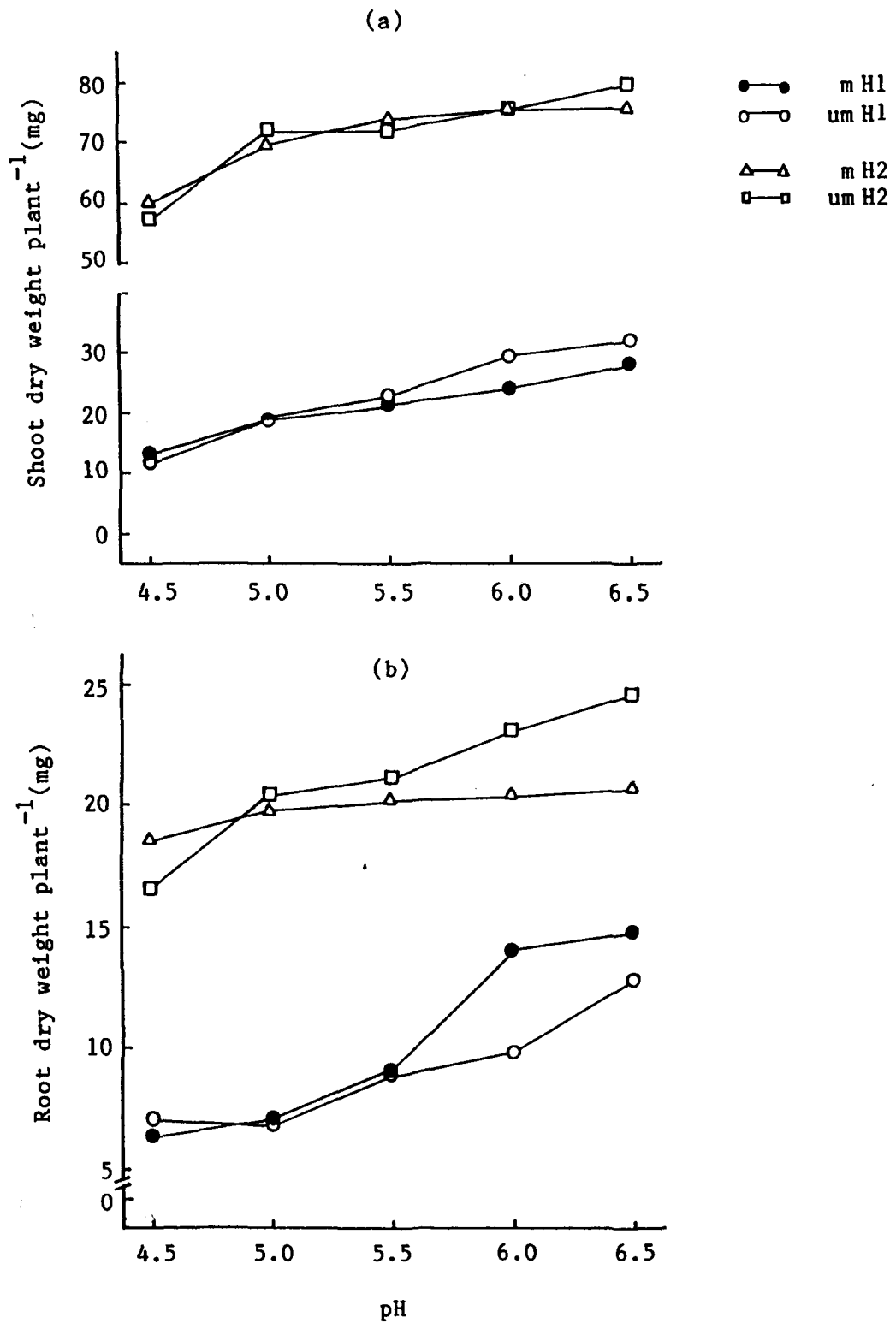


Fig.6.1. Effect of pH on (a) shoot dry weight and (b) root dry weight per plant in the two leaf morph populations of the clover at the two harvests.

Table 6.1. Analysis of variance of the legume growth parameters as influenced by harvests, leaf morph populations and varying pH.

Plant parameters	Source of variation	df	F value	Level of significance
Shoot dry weight	Harvests	1	2171.50	P < 0.01
	Leaf morphs	1	15.82	P < 0.05
	pH levels	4	12.01	P < 0.05
	Harv x morph	1	18.89	P < 0.05
	Harv x pH	4	0.79	ns
	Morph x pH	4	0.74	ns
Root dry weight	Harvests	1	147.28	P < 0.01
	Leaf morphs	1	0.00	ns
	pH levels	4	6.67	P < 0.05
	Harv x morph	1	1.42	ns
	Harv x pH	4	0.50	ns
	Morph x pH	4	0.06	ns
Plant dry weight	Harvests	1	6937.90	P < 0.01
	Leaf morphs	1	2.14	ns
	pH levels	4	139.00	P < 0.01
	Harv x morph	1	0.12	ns
	Harv x pH	4	4.75	ns
	Morph x pH	4	1.32	ns
PSA	Harvests	1	205.02	P < 0.01
	Leaf morphs	1	0.09	ns
	pH levels	4	87.52	P < 0.01
	Harv x morph	1	0.99	ns
	Harv x pH	4	1.58	ns
	Morph x pH	4	0.99	ns

ns = not significant

Table 6.2. Effect of pH 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.

pH levels	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)
4.5	-	18.02	142.31	-	17.61	140.61	-	18.75	383.76	-	18.48	357.00
5.0	47.0	24.16	264.32	43.7	23.99	270.40	78.1	31.12	456.21	76.3	26.79	447.58
5.5	45.3	28.64	490.00	40.1	24.87	501.31	39.2	37.00	952.60	40.7	28.29	1078.77
6.0	40.8	31.49	578.24	39.7	27.08	518.63	40.1	40.79	1080.72	38.9	30.08	1128.08
6.5	40.1	33.00	646.28	37.0	24.97	654.31	64.6	39.00	1281.36	45.3	29.53	1092.78

- absence

Nodule number

The mean nodule number per plant increased ($P < 0.01$) with increasing pH (up to pH 6.0) in both leaf morph populations. However there was a drop in nodule number at pH 6.5. At pH 4.5, plants of either leaf morph population failed to nodulate even after 12 weeks of growth. Plants of the marked population nodulated better and exhibited a significantly ($P < 0.01$) higher nodule number per plant as compared to unmarked plants at both harvests. Except at pH 4.5 when the legume failed to nodulate, at all other pH levels, nodule number per plant showed almost a four fold increase ($P < 0.01$) at H2 as compared with numbers recorded at H1 (Tables 6.3, 6.4).

Mean nodule weight

The mean nodule weight showed an inverse relationship with nodule number and with increasing pH levels (Table 6.2) at both harvests. Variation in mean nodule weight with pH was significant ($P < 0.01$). At both harvests, the mean weight per nodule was greater in the marked population than in the unmarked, though the difference was not significant. The mean nodule weights were significantly ($P < 0.01$) greater at H2 than at H1 (Table 6.3).

Nodule weight per plant

Figure 6.2b shows the variation in total nodule weight per plant with pH. This parameter showed a trend similar to that of nodule number per plant (Fig. 6.2a). Except at pH 4.5, the total nodule weight per plant showed a gradual increase up to pH 6.0 at H1. At

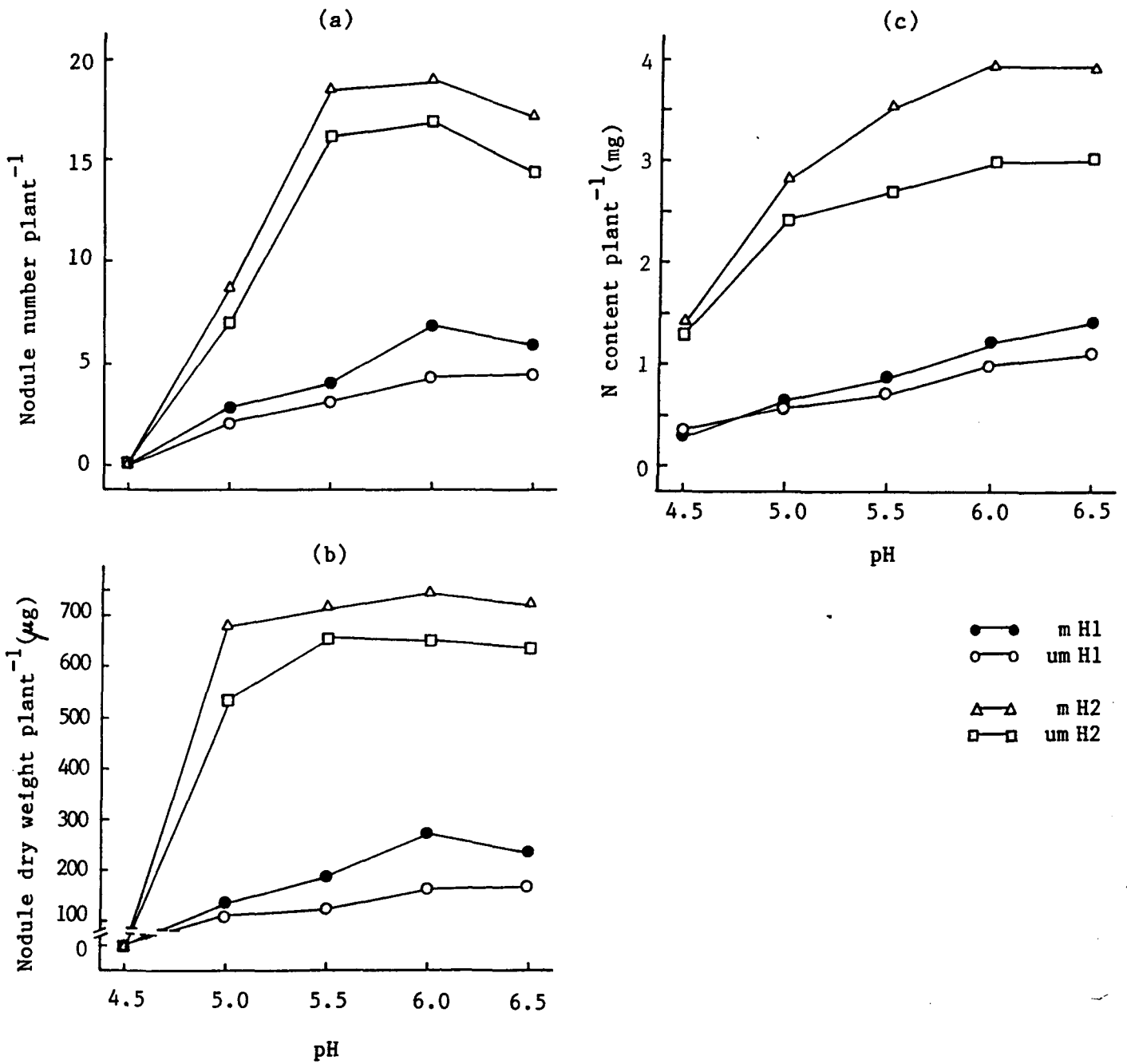


Fig.6.2. Effect of pH on (a) nodule number (b) nodule dry weight, and (c) N content per plant in the two leaf morph populations of the clover at the two harvests.

Table 6.3. Analysis of variance of nodule characteristics of the clover as influenced by harvests, leaf morph populations and varying pH.

Nodule characteristics	Source of variation	df	F value	Level of significance
Nodule number	Harvests.	1	1667.34	P < 0.01
	Leaf morphs	1	43.11	P < 0.01
	pH levels	4	457.50	P < 0.01
	Harv. x morph	1	2.46	ns
	Harv. x pH	4	154.47	P < 0.01
	Morph x pH	4	3.54	ns
Mean nodule weight	Harvests	1	21.42	P < 0.01
	Leaf morphs	1	2.99	ns
	pH levels	4	111.22	P < 0.01
	Harv. x morph	1	0.18	ns
	Harv. x pH	4	11.92	P < 0.05
	Morph x pH	4	1.07	ns
Nodule weight per plant	Harvests	1	1158.12	P < 0.01
	Leaf morphs	1	29.39	P < 0.01
	pH levels	4	217.02	P < 0.01
	Harv. x morph	1	1.07	ns
	Harv. x pH	4	73.07	P < 0.01
	Morph x pH	4	2.18	ns

ns = not significant

Table 6.4. Effect of soil pH on the nodule number per unit (100 mg) plant dry weight.

Soil pH	Harvest 1		Harvest 2	
	marked population	unmarked population	marked population	unmarked population
4.5	-	-	-	-
5.0	11.04	9.38	9.69	7.60
5.5	13.23	9.92	19.24	16.99
6.0	17.36	10.80	19.15	16.76
6.5	13.59	10.10	17.12	13.72

- absence

H2, there was a steep rise at pH 5.0 from the initial nil value at pH 4.5, thereafter a gradual increase was observed up to pH 6.0 after which the values either increased marginally or decreased at pH 6.5. Variations in total nodule mass per plant were significant ($P < 0.01$) between pH levels. Of the two leaf morph populations, the marked one consistently exhibited greater nodule mass per plant ($P < 0.01$) at both harvests. At H2 the total nodule mass per plant recorded a three to four fold increase ($P < 0.01$) as compared to the values obtained at H1 (Table 6.3).

Total N per plant

The total N content per plant showed a steady significant ($P < 0.01$) increase with increasing pH at both harvests. Between the two leaf morph populations, the marked population exhibited higher ($P < 0.01$) N content per plant as compared to the unmarked population at both harvests (Table 6.5).

Relative growth rate and relative N accumulation rate

Table 6.6 presents the relative growth rates (R_W) and relative N accumulation rates (R_N) corresponding to the different pH levels for the two leaf morph populations at H1 and H2. There was a significant ($P < 0.01$) positive correlation between R_W and pH levels. However, at H2, the R_W values exhibited a reverse trend, decreasing gradually with increasing pH levels in both leaf morph populations. The difference in R_W values was significant ($P < 0.01$) amongst pH levels though the variation in R_W between the two leaf morph

Table 6.5. Analysis of variance of growth parameters of the clover as influenced by harvests, leaf morph populations and varying pH.

Growth parameters	Source of variation	df	F value	Level of significance
Total N plant ⁻¹	Harvests	1	948.03	P < 0.01
	Leaf morphs	1	29.95	P < 0.01
	pH levels	4	70.99	P < 0.01
	Harv. x morph	1	14.31	P < 0.05
	Harv. x pH	4	13.18	P < 0.05
	Morph x pH	4	2.59	ns
	R _W	Harvests	1	472.88
Leaf morph		1	1.09	ns
pH levels		4	124.37	P < 0.01
Harv. x morph		1	3.39	ns
Harv. x pH		4	499.53	P < 0.01
Morph x pH		4	0.27	ns
R _N	Harvests	1	447.48	P < 0.01
	Leaf morph	1	7.61	ns
	pH levels	4	124.37	P < 0.01
	Harv. x morph	1	0.86	ns
	Harv. x pH	4	105.76	P < 0.01
	Morph x pH	4	0.60	ns

ns = not significant

populations was not significant.

The relative N accumulation rates (R_N) showed a trend similar to that of R_W (Table 6.6).

DISCUSSION

Soil acidity at pH 5.0 and below results in toxicity due to increased uptake of Al and Mn and limited availability of Ca in particular (Foy 1984, Coventry & Evans 1989). There was a steady increase in shoot dry weight in both leaf morph populations with increasing pH at both harvests. In legumes, shoot dry weights are known to decrease sharply with increased H^+ ion concentration (Cline & Kaul 1990). Thus the low shoot dry weights recorded at lower levels of pH could be attributed to toxicity resulting primarily from increased accumulation of H^+ besides Al and Mn. As the pH increases, there is an enhancement of shoot weights following partial alleviation of the prevailing toxic condition. Results of this study indicate that toxicities due to H^+ accumulation and related factors also affect root development which is reflected in reduced dry weight. This is in conformity with the findings of Kehoe & Curnow (1963) who reported that in acid soils, root development in subterranean clover was restricted to such an extent that the plants could not utilize subsoil moisture as the soil dried. Al toxicity similarly results in inhibition of root growth and proliferation (Foy 1984) which restricts ability of plants to fully exploit soil moisture (Bromfield *et al.* 1983).

Table 6.6. Effect of pH 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.

pH	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
4.5	4.24	6.06	4.78	6.24	10.96	11.27	10.28	10.66
5.0	9.04	15.41	8.95	15.21	9.56	11.52	9.79	10.65
5.5	11.29	20.35	11.83	18.61	8.88	10.84	8.63	9.63
6.0	15.00	25.48	15.26	23.37	7.23	9.23	7.35	8.18
6.5	16.81	28.02	17.50	24.38	6.45	7.75	6.48	7.79

Both nodule number and nodule mass per plant showed a steady increase at pH >4.5 up to pH 6.0 following which there was a slight decrease in both. Low soil pH, especially below pH 5.0, primarily results in toxicity due to Al (Foy 1984, Coventry & Evans 1989). Al is an abundant element in the soil occurring in a wide variety of mineral forms which are largely inert at neutral pH (Flis et al. 1993). As the pH decreases, Al is mobilized into the soil solution and may become toxic to plants and soil organisms. Distribution of various ionic species of Al is pH-dependent (Martin 1991) and slight changes in pH may significantly affect the relative concentration of various charged Al species and hence, the toxicity of Al. Small changes in pH can therefore significantly affect the growth of rhizobia in soil (Thornton & Davey 1983, Richardson & Simpson 1989). Cultures of root nodule bacteria have been reported to grow after a large initial decline in viability following exposure to Al (Keyser & Munns 1979) and it has been suggested that this probably involves some physiological adaptation of the rhizobia to the presence of Al. This trend has also been confirmed for R. trifolii (Whelan & Alexander 1986). Such an adaptation may require some time to develop. This may explain the initial time lag between inoculation by Rhizobium and nodule formation, and perhaps the wide variation in the nodule number per plant between the two harvests might have been caused on account of this. Though the local populations of T. repens and the associated R. trifolii usually grow in slightly acidic conditions which prevail over most of the areas in the hill region of north-east India, tolerance to acidity does

not necessarily confer on plants the tolerance to Al (Thornton & Davey 1983).

In the pH range of 4.3-5.0, toxicity due to Al and related factors severely affects both the legume and the endosymbiont. Recent studies have revealed the adverse effects of Al on the legume-Rhizobium symbiosis at pH < 5.0 (de Carvalho et al. 1981, 1982, Franco & Munns 1982) when the Al remains in solution. Al was reported to affect growth and survival and hence the number of root nodule bacteria in soil (Cooper et al. 1983, Coventry & Evans 1989), nodule initiation (de Carvalho et al. 1982, Murphy et al. 1984, Brady et al. 1990) and root hair formation of the host (Hecht-Buchholz et al. 1990). At pH 4.3-4.7, prevailing Al concentrations are high enough to be severely toxic to T. repens (Wood et al. 1983) leading to stunted growth of roots and total inhibition of root hair development. Results of the present investigation reveal that nodule formation in T. repens was totally inhibited at pH 4.5 for both leaf morph populations. It is not clear whether the lack of nodulation was directly due to inhibition of Rhizobium multiplication in the rhizosphere or due to the inhibition of root hair development of the legume, but it is probable that both the endosymbiont as well as the legume were affected. The critical pH for R. trifolii survival in soil was reported to be 4.5-4.7 (Bryan 1923) and for growth in laboratory media pH 4.5-5.0 (Graham & Parker 1964). Thus at pH 4.5, the presence of a considerably large rhizospheric population of R. trifolii is doubtful. The increase in nodule number and nodule mass per plant at pH 5.0 and 5.5 in both leaf morph

populations could be attributed to the decrease in H^+ ion concentration and reduced toxicity levels of Al and Mn. At pH 5.0 the nodule number had still not increased appreciably although the nodule mass showed a marked increase, especially at H2. This could be because Al suppresses nodule initiation at concentrations which do not affect nodule growth and function (Robson & Bottomley 1991). At pH 5.5 Al is no longer toxic as it precipitates out of solution (Flis et al. 1993) and this is probably expressed by the large increase in nodule number at H2 in both the leaf morph populations.

Mn toxicity resulting from increased solubility at low pH is also known to inhibit nodulation. Dobereiner (1966) has suggested that the sensitivity of legumes to acid soils is due to the specific effect of Mn on the legume - Rhizobium symbiosis. Transitory waterlogged conditions are known to increase the levels of exchangeable divalent Mn. It has been reported that at low pH, Mn toxicity retards the growth of R. trifolii (Holding & Lowe 1971) and reduces the number of nodules formed on several varieties of white clover (Vose & Jones 1963). In the present study, though development of waterlogged conditions in the pots even transitorily, is improbable, Mn toxicity resulting from low pH could well have played a part in restricting nodulation. Besides, acidic conditions are usually associated with Ca deficiency (Munns 1977) and Ca being a macro-nutrient, its deficiency could be a critical factor in determining growth of the legume.

Temperate legumes like T. repens, generally require higher levels

of Ca, especially when depending on symbiotic N₂ fixation. However, more Ca is needed for nodule formation than for either N₂ fixation or plant growth (Lowther & Loneragan 1968, Lie 1974, Andrews 1976) and thus the time of nodule initiation is the most Ca demanding and acid sensitive step (Munns 1968). According to Smit et al. (1987), a Ca-binding protein Rhicadhesin, identified in Rhizobium, is involved in the initial attachment of the diazotroph to the host root hairs and thus Ca is required for successful initiation of the symbiosis. The infection threads which develop are also composed of Ca-rich material (Sethi & Reporter 1981) and the non availability of Ca could lead to an increase in the number of aborted infections thereby drastically reducing the nodule number.

Ca plays a variety of roles in root nodule bacteria (O'Hara et al. 1988, Robson & Bottomley 1991), many of them apparently involved with the stabilization of the bacterial lipopolysaccharide. Though Ca can slightly alleviate the Al-mediated inhibitions in the legume - Rhizobium symbiosis, this is only partial (Richardson et al. 1988) and Keyser & Munns (1979) conclude that Ca offers too little protection against Al to be biologically significant. The results of the present investigation indicated by the positive response of the symbiosis to the addition of Ca is in conformity with those of Coventry et al. (1985) and Richardson et al. (1988). It is probable that the beneficial effects of Ca are mainly mediated via its neutralizing effect and not due to the presence of the element itself as suggested by Richardson et al. (1988) and Pijnenborg et al. (1990).

The marginal drop in nodule number and nodule mass per plant at pH 6.5 is interesting. The soils exhibit slightly acidic reaction in this part of the country and it may be assumed that the local population of white clover and associated *R. trifolii* are adapted to such conditions. The root nodule bacteria are known to be extremely sensitive to small changes in pH which can significantly affect its growth (Thornton & Davey 1983, Richardson & Simpson 1989) and it is probable that at pH 6.5, when the soil reaction tends towards neutrality, the growth of the rhizospheric population of rhizobia is affected leading to reduced numbers and, subsequently, low infectivity of host roots. This could explain the marginal drop in nodule number at pH 6.5. It is also likely that senescence and abscission of nodules may have increased under these conditions (Davey *et al.* 1989) as a reaction of the host plant to pH conditions approaching neutrality.

Studies by Evans *et al.* (1980) and Wolff *et al.* (1993) have revealed that nodule growth (expressed as nodule weight) is more sensitive to changes in soil pH than nodule number. This marginal drop in nodule mass per plant following a decrease in nodule number could have been further caused by the reduction in nodule growth under prevailing conditions. Between the two leaf morph populations, the marked one exhibited significantly ($P < 0.01$) higher nodule number per plant as compared to the unmarked population at both harvests (Fig. 6.2a). This could be attributed to the higher N requirement of the marked population (Pradhan & Tripathi 1984) which forms more root nodules as a means of fulfilling this require-

ment by symbiotic N_2 fixation as discussed in Chapter 5.

There was no significant variation in mean nodule weight between the two leaf morph populations and the higher nodule mass per plant exhibited by the marked population could be attributed to a greater number of nodules that develop per plant in the marked population.

However, it is interesting to note that a reduction in nodule number per plant was not compensated by an increase in mean nodule weight. This indicates that low pH is not only detrimental to nodule initiation (infection response) but also to nodule growth (growth response). This is in conformity with the findings of Wolff et al. (1993) who observed that the nodule growth was more sensitive to changes in soil pH than nodule number.

H^+ ion toxicity arising at low pH, besides hampering nodulation, is also deleterious to N_2 fixation of the nodulated legumes. Cline & Kaul (1990) reported that such conditions affect N_2 fixation more than plant growth since legumes grew well if supplied with N but showed deficiency if dependent on N_2 fixation. Thus at low pH, reduced nodulation coupled with inhibited N_2 fixation (expressed by R_N) would lead to N deficiency and could be responsible for the suppressed growth of the inoculated plants (Mengel & Kamprath 1978, Alva et al. 1987) as the present results suggest.

The recommended pH for the growth of white clover in hill pastures is 5.2-5.8 (HFRO 1979). The results of the present investigation suggest that nodule formation and symbiotic function can

be considerably reduced at the lower end of this pH range. Though soils in and around Shillong have a pH range of 5.5-6.0, it is probable that this value may drop due to leaching of soluble bases following heavy rains. Transitory waterlogged conditions may also lead to Mn toxicity and both these factors would severely hamper nodulation and N_2 fixation under natural conditions.

Of the two leaf morph populations, the marked one exhibits better nodulation over the acidic pH range in terms of production of greater number and mass of nodules per plant, and thus its role in the N economy of the swards would be more important under prevailing conditions of low pH.