



Effect of defoliation and NPK treatments on nodule population

White clover is an important pasture component of the grasslands in and around the Shillong plateau. Besides being a significant contributor of fixed nitrogen to these ecosystems via its root nodules, it serves as a forage legume for cattle. This results in clover leaves being frequently defoliated, along with companion grasses, during grazing. During periods of peak vegetative growth, the clover leaves are less accessible due to greater grass growth. However, during the drier periods of the year the growth of grasses is less vigorous and clover leaves are more accessible to grazers (Clark et al. 1984). This would lead to mild and severe defoliation of clover in the former and latter situations, respectively. Moreover, the high defoliation risk for clover arises because leaves are nearly always completely removed, whereas in grasses leaves are only partially grazed (Chapman et al. 1990).

In a prostrate, stoloniferous plant like T. repens, the trifoliate compound leaves thus serve as the primary photosynthesizing tissue from where the photosynthates are channelized to different plant parts. Partial removal of the leaves would, therefore, disturb the primary carbon 'source' and affect partitioning of photosynthates to the different 'sink' tissues and affect the growth of the clover population. According to Suckling (1976) and Lambert et al. (1982, 1986), growth of T. repens is also affected if deficiencies of nutrients, particularly of P, exist.

Several studies have been made to investigate the effects of defoliation in white clover by Chapman and his co-workers (Chapman 1983, Chapman et al. 1984, Clark et al. 1984, Chapman et al. 1990, Chapman et al. 1991, Chapman et al. 1992). However, these studies deal with the pathways of carbon following removal of the source tissue. N_2 fixation being an energy-intensive, root nodules of legumes consequently have a high energy demand (Ryle et al. 1985, Gordon et al. 1985). It would therefore be interesting to determine how the number, growth and activity of nodules in the two leaf morph populations of white clover were affected following removal of the leaf (source tissue) and addition of NPK, to alleviate nutrient deficiencies, if any.

MATERIALS AND METHODS

These have been described in Chapter 3

RESULTS

Plant growth

Figure 8.1a represents the mean shoot dry weights of the two leaf morph populations exposed to the two defoliation and NPK levels, compared to the controls at the two harvests. The shoot dry weights varied significantly ($P < 0.01$) between treatments with the weights being highest in the plants of both populations exposed to 'low defoliation - high NPK (LH)' treatments at H1. At H2, however, the control plants of both populations had the highest shoot dry weights. At both harvests, 'high defoliation - low NPK (HL)' treatments resulted in minimum shoot dry weights. Between harvests, there was a significant difference in the shoot dry weights with values recorded at H2 being almost twice that at H1 for all treatments including the 'control' plants. However, there was no significant difference in the shoot dry weights between the marked and unmarked plants (Table 8.1).

Root dry weights also varied significantly ($P < 0.01$) between the treatments. The highest values were recorded under the LH treatments and lowest under the HL treatment for both the leaf morph populations. At H2, root dry weights were significantly ($P < 0.01$) higher compared to the values recorded at H1. Of the two leaf morph populations, the marked one exhibited better root growth and had significantly ($P < 0.01$) greater dry weights at both harvests (Fig. 8.1b).

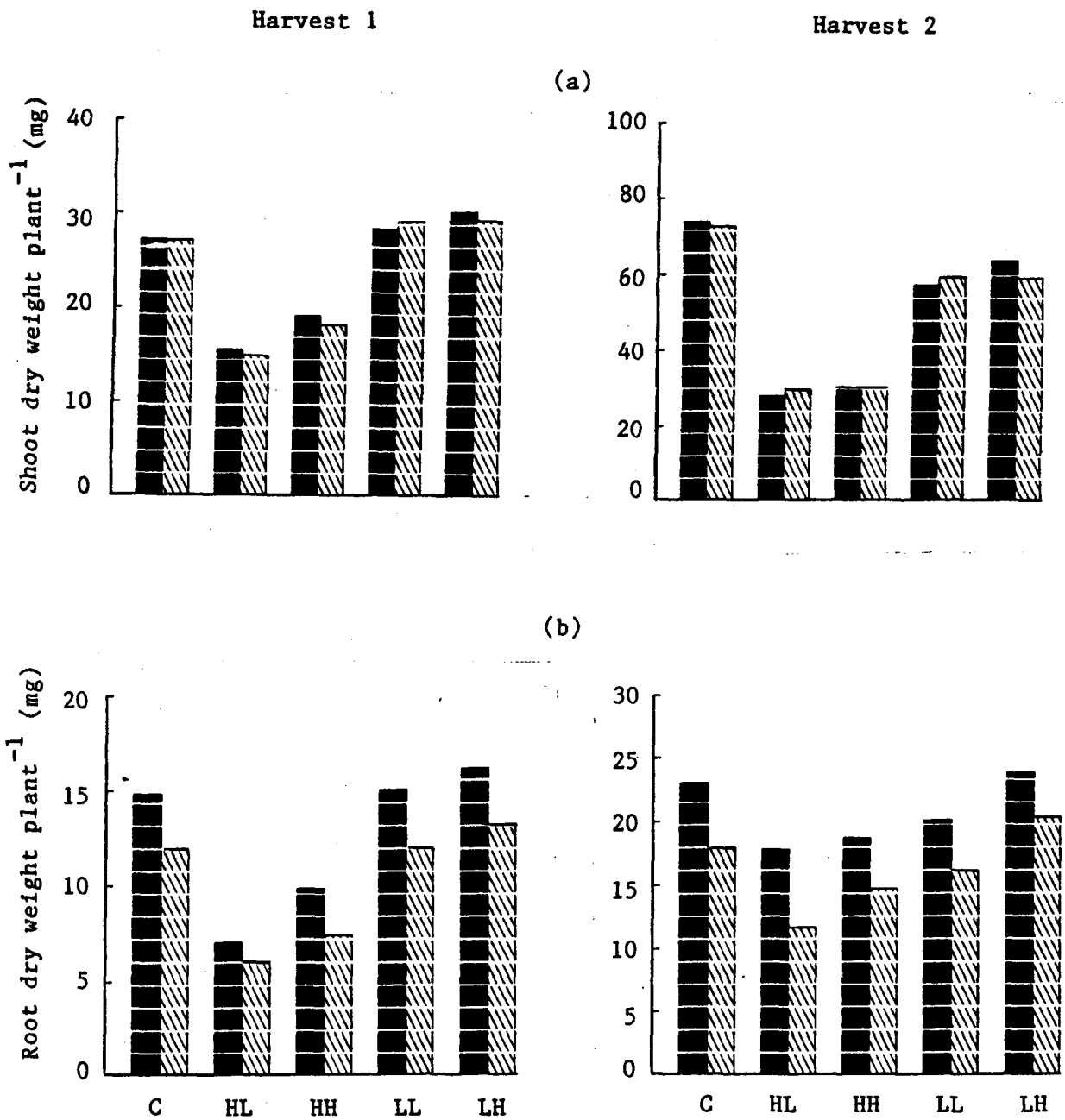


Fig.8.1. Effect of defoliation and NPK treatments on (a) shoot dry weight and (b) root dry weight per plant in the marked (■) and unmarked (▨) leaf morph populations of the clover at the two harvests. C = control, HL = high defoliation-low NPK, HH = high defoliation-high NPK, LL = low defoliation-low NPK, LH = low defoliation-high NPK.

Table 8.1. Analysis of variance of the legume growth parameters as influenced by harvests, leaf morph populations and defoliation and NPK treatments.

Plant parameters	Source of variation	df	F value	Level of significance
Shoot dry weight	Harvests	1	2164.99	P < 0.01
	Leaf morphs	1	0.28	ns
	Defoln. treat	4	403.29	P < 0.01
	Harv x Morph	1	0.01	ns
	Harv x defoln	4	120.71	P < 0.01
	Morphs x defol	4	1.74	ns
Root dry weight	Harvests	1	75.01	P < 0.01
	Leaf morphs	1	28.75	P < 0.01
	Defoln. treat	4	109.13	P < 0.01
	Harv x Morph	1	2.04	ns
	Harv x defoln	4	22.24	P < 0.01
	Morphs x defol	4	4.45	ns
Plant dry weight	Harvests	1	5724.39	P < 0.01
	Leaf morphs	1	77.38	P < 0.01
	Defoln. treat	4	1037.00	P < 0.01
	Harv x Morph	1	5.59	ns
	Harv x defoln	4	188.75	P < 0.01
	Morphs x defol	4	2.62	ns
PSA	Harvests	1	272.06	P < 0.01
	Leaf morphs	1	15.18	P < 0.05
	Defoln. treat	4	1105.13	P < 0.01
	Harv x Morph	1	3.76	ns
	Harv x defoln	4	0.98	ns
	Morphs x defol	4	7.60	ns

ns = not significant

Table 8.2. Effect of defoliation treatments 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.

Defoliation treatments	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)
Control	47.8	31.07	763.00	40.8	27.63	818.77	70.1	39.7	1291.08	66.3	32.6	1380.50
HL	30.4	20.79	105.46	30.7	20.00	145.12	30.1	26.3	150.90	29.7	23.1	160.51
HH	31.7	22.86	113.16	32.9	21.36	164.05	30.7	27.0	169.54	29.8	24.6	166.51
LL	39.3	30.36	681.00	28.7	27.89	690.30	56.2	33.5	1040.01	48.1	30.0	1138.63
LH	42.6	32.11	1065.03	39.0	29.01	1129.65	57.9	34.7	1118.26	52.0	30.9	1228.36

Total plant dry weights also varied significantly ($P < 0.01$) between the treatments with the largest weights recorded for the Lh treatments at H1 and for the control plants at H2 in both leaf morph populations. Minimum weights were recorded for the HL treatments at both harvests. The increment in plant weights at H2 was highly significant ($P < 0.01$). Of the two leaf morph populations, the marked one invariably recorded significantly ($P < 0.01$) greater values for plant dry weights at both harvests (Table 8.1).

Photosynthetic area

The mean PSA per plant showed wide variation ($P < 0.01$) between the treatments. At H1, maximum PSA was recorded for the plants exposed to LH treatments and at H2 for the control plants. The minimum PSA was recorded under H1 treatments at both harvests. Between harvests, the PSA varied significantly ($P < 0.01$) especially with respect to the controls where values at H2 were almost twice that at H1. Between the two leaf morph populations, the unmarked plants exhibited significantly ($P < 0.01$) greater PSA at both harvests (Tables 8.1, 8.2).

Nodule number

The nodule number per plant of the two leaf morph populations exposed to different treatments at the two harvests are presented in Figure 8.2a. The nodule number varied significantly ($P < 0.01$) between the treatments with the maximum number of nodules per plant formed for the plants exposed to LH treatment at H1 and for the 'controls' at H2. Minimum number of nodules was formed under

HL treatment in both leaf morph populations at either harvest. Plants of both populations at H2 developed significantly ($P < 0.01$) greater number of nodules; of the two leaf morph populations, the marked plants had significantly ($P < 0.01$) greater number of nodules at both harvests (Table 8.3).

Mean nodule weight

The mean weight per nodule showed significant ($P < 0.05$) variation between the treatments. The 'controls' of both the leaf morph populations formed the largest nodules and the plants under H1 treatment formed the smallest nodules. However, there was no significant difference in nodule weight either between the marked and unmarked plants or between the harvests (Table 8.2).

Nodule weight per plant

The nodule weight per plant showed a significant ($P < 0.01$) variation between the treatments. At H1 the maximum values were obtained either under LH treatment (marked population) or under 'control' (unmarked population) whereas at H2, the maximum values were recorded for the 'controls' in both leaf morph populations. Lowest nodule mass per plant was recorded for the plants under HL treatment at both H1 and H2. At H2, both leaf morph populations exhibited a significant ($P < 0.01$) increase in nodule mass per plant. At both harvests, nodule mass per plant was significantly ($P < 0.01$) more in the plants of the marked population than in the unmarked ones (Fig. 8.2b)(Table 8.4).

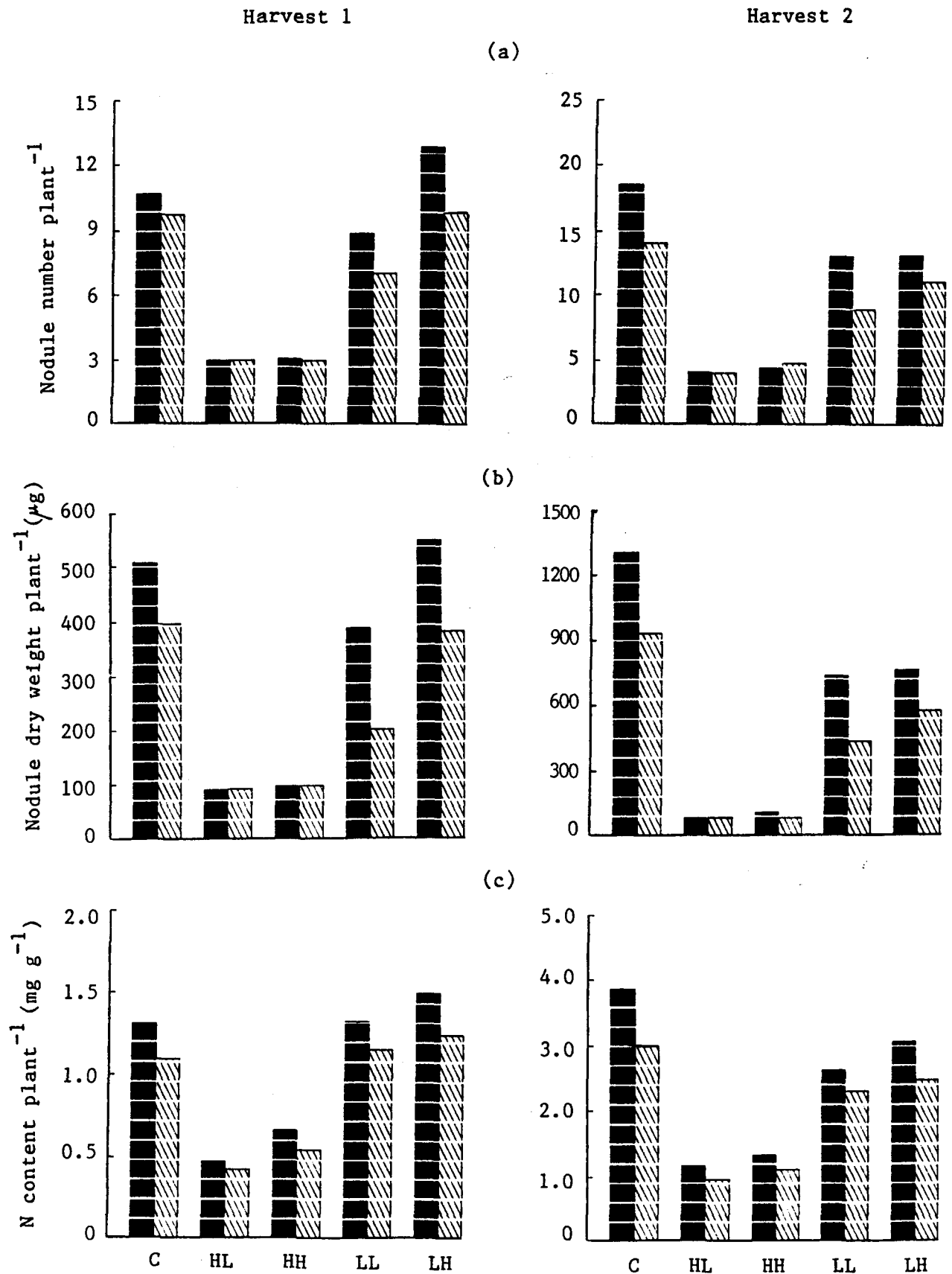


Fig.8.2. Effect of defoliation treatments on (a) nodule number (b) nodule dry weight and (c) N content per plant in the marked (■) and unmarked (▨) leaf morph populations of the clover at the two harvests.

Table 8.3. Effect of defoliation treatments on the nodule number per unit (100 mg) plant dry weight.

Defoliation treatments	Harvest 1		Harvest 2	
	marked population	unmarked population	marked population	unmarked population
Control	25.14	24.69	18.90	15.28
HL	13.31	14.37	8.84	9.58
HH	10.59	11.78	9.14	10.64
LL	20.37	17.01	16.66	11.80
LH	27.58	23.01	14.80	13.86

Table 8.4. Analysis of variance of nodule characteristics of the clover as influenced by harvests, leaf morph populations and defoliation and NPK treatments.

Nodule characteristics	Source of variation	df	F value	Level of significance
Nodule number	Harvests	1	24.05	P < 0.05
	Leaf morphs	1	22.03	P < 0.01
	Defoln. treat	4	122.93	P < 0.01
	Harv x Morph	1	1.29	ns
	Harv x defoln	4	8.62	P < 0.05
	Morph x defol	4	3.05	ns
Mean nodule weight	Harvests	1	4.83	ns
	Leaf morphs	1	1.07	ns
	Defoln. treat	4	20.13	P < 0.05
	Harv x Morph	1	2.79	ns
	Harv x defoln	4	4.03	ns
	Morph x defoln	4	0.95	ns
Nodule weight per plant	Harvests	1	91.15	P < 0.01
	Leaf morph	1	31.22	P < 0.01
	Defoln. treat	4	126.10	P < 0.01
	Harv x Morph	1	0.65	ns
	Harv x defoln	4	3.21	ns
	Morph x defoln	4	2.80	ns

ns = not significant

Total N per plant

Total N per plant varied widely between treatments with values varying significantly ($P < 0.01$). At H1, maximum values were obtained under LH treatment, whereas at H2, peak values were recorded for the 'controls' for both leaf morph populations. The minimum values were recorded under HL treatment at both harvests. Although there was no significant variation in the total N per plant between the two leaf morph populations, values were significantly ($P < 0.01$) higher at H2 as compared to H1 (Fig. 8.2c).

Relative growth rate and Relative N accumulation rate

Relative growth rates (R_W) varied significantly ($P < 0.01$) between treatments with R_W being highest for the LH plants at H1 and for the 'controls' at H2. Lowest R_W was recorded under HL treatments at H1 and under HL treatments at H2. Variation in R_W was also significant ($P < 0.01$) between the two leaf morph populations with the marked one showing higher values for R_W , and between harvests ($P < 0.01$) with R_W being much greater at H1 as compared to H2.

Relative N accumulation rates (R_N) too differed widely between the treatments, populations and harvests (all $P < 0.01$) and generally followed the same trend as R_W (Tables 8.5, 8.6).

DISCUSSION

In a wide range of plant forms including T. repens, it has been well established that the leaves by virtue of being the most

Table 8.5. Effect of defoliation treatments 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.

Defolia- tion treatment	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
Control	16.53	26.79	15.39	23.83	6.51	8.41	6.54	7.83
HL	6.72	10.75	5.59	8.99	5.53	7.36	5.37	6.49
HH	10.63	16.13	8.62	13.05	4.12	5.41	4.43	5.53
LL	16.95	26.85	16.09	24.68	4.53	5.29	4.77	5.37
LH	18.04	28.81	16.63	25.82	4.93	5.53	4.86	5.35

Table 8.6. Analysis of variance of growth parameters of the clover as influenced by harvests, leaf morph populations and defoliation and NPK treatments.

Growth parameters	Source of variation	df	F value	Level of significance
Total N plant ⁻¹	Harvests	1	194.97	P < 0.01
	Leaf morphs	1	5.82	ns
	Defoln. treat	4	27.99	P < 0.01
	Harv x Morph	1	5.80	ns
	Harv x defoln	4	27.90	P < 0.01
	Morph x defoln	4	1.00	ns
R _W	Harvests	1	4268.64	P < 0.01
	Leaf morphs	1	25.82	P < 0.01
	Defoln. treat	4	354.72	P < 0.01
	Harv x Morph	1	32.07	P < 0.01
	Harv x defoln	4	326.43	P < 0.01
	Morph x defoln	4	0.59	ns
R _N	Harvests	1	5344.91	P < 0.01
	Leaf morphs	1	53.92	P < 0.01
	Defoln. treat	4	317.57	P < 0.01
	Harv x Morph	1	0.54	ns
	Harv x defoln	4	3.75	ns
	Morph x defoln	4	0.54	ns

ns = not significant

important photosynthetic tissue, are the major exporters of fixed carbon (C) in the form of carbohydrates, to the other plant parts on a priority basis (Hoshino et al. 1964, Chapman 1983, Robin et al. 1987, Chapman et al. 1991). Thus in T. repens the leaves normally act as the 'source' from where C is transported into compartments of shoot apices, stolons, branches, adventitious roots and nodules (which function as 'sinks') depending on prevailing nutritional requirements of these compartments. Conversely, grazing by herbivores, which causes partial defoliation, may lead to carbohydrates being translocated from previous sinks like stolon tissue towards the damaged part of the plant to compensate for the lack of photosynthate production in previously existing sources (Chapman et al. 1991). The marked reduced growth of T. repens, exhibited by both leaf morph populations following severe defoliation could therefore be attributed to a restriction in the size of the C source which determines growth of the defoliated plants (Chapman et al. 1991). Though the absence of mature leaves in the severely defoliated plants could partially explain their greatly reduced weights, this was more strongly related to the restricted development of the stolon tissue which accounts for the major part of the biomass of this legume. Addition of NPK nutrients could hardly help alleviate the detrimental effects of severe defoliation (Fig. 8.1a).

Though defoliation in T. repens has been reported to result in a marginal increase in the size of the remaining leaves within a 3-4 day period (Chapman et al. 1990), severe defoliation can have

more serious and far-reaching consequences. Compared to companion grasses, white clover is known to be more sensitive to defoliation (Clark et al. 1984) and up to 30% of all stolon death and 30% of leaf loss have been reported to be related to the occurrence of severe defoliation (Chapman et al. 1984).

The nodulation pattern of the defoliated and 'control' T. repens plants is interesting. During the initial period of growth, light defoliation coupled with the addition of NPK (LH treatment) resulted in a flush of new leaves developing to compensate for the partial loss of source tissue. This might have led the plants under 'LH' treatments to produce a large number of leaves and consequently higher PSA as compared to the controls, in both the leaf morph populations at H1. This could explain the development of the largest number of nodules per plant in this treatment for both leaf morph populations at the end of the 4 week period (H1). Conversely, better root growth in this treatment (Fig. 8.1b), probably fuelled by the added NPK, would per se result in increased sites of infection by Rhizobium leading to the enhanced number of symbiotic root nodules formed. However, at the end of the 12 week period (H2), the nodule number in the 'controls' superseded that of the LH treatments. The formation of a greater number of nodules in plants exposed to LH treatments probably leads to the imposition of severe demands of the nodulated root system for carbohydrates from the source resulting in periods of crisis since the young leaves are not able to cope up with such demands (King 1978). The control plants not being subjected to any such imposed stress show better growth

producing a larger number of leaves and greater PSA, and are thus able to form and sustain a larger number of nodules.

Nodulated roots of legumes require a continuous supply of C drawn from the source tissue for their growth and development. In addition, roots often act as reserves for deposition of carbohydrates (Danckwerts & Gordon 1989) and it has been estimated in T. repens, using ^{14}C , that of the total amount of C moving from the source to different sinks, the major portion (37-47%) is channelized to the root system. N_2 fixation is an energy-intensive process and nodules and nodules, consequently, have high energy demands (Ryle et al. 1985, Gordon et al. 1985). Respiratory losses associated with N_2 fixation are also high at ca. 9 moles of CO_2 / mole N_2 fixed as measured by the acetylene reduction assay (Ryle et al. 1989), accounting for nearly half of the C respired by whole plants (Gordon et al. 1987). The low number of nodules that develop in the severely defoliated plants could thus be attributed to the removal of the source tissue in these plants resulting in their becoming deficient in carbon. Starvation of sinks results in their poor growth (as indicated by the low root biomass), which not only drastically reduces sites of infection by Rhizobium but probably also leads to aborted infections in the absence of C for sustenance.

According to Chapman et al. (1990), the leaves of white clover attain an autotrophic state with respect to C supply when they have expanded to about 35% of their maximum surface area and reached about 50% of their final dry weight. Thereafter, they export an

increasing percentage of the C they fix to the rest of the plant as they develop further. However, prior to attaining autotrophy young leaves also act as significant C sinks importing C from stolons. In the present study, severe defoliation resulted in removal of all mature leaves and the newly emerging leaves were possibly acting as sinks instead of a source, and thereby the flow of carbon to roots may decrease. Thus severe defoliation, which restricted the development of nodules (via its detrimental effects on root growth) also affected nodule growth as C would be channelized in the opposite direction towards young emerging sink leaves. This explains the low nodule weights associated with reduced nodule number per plant. As a result, the nodule mass per plant was proportional to the nodule number (Fig. 8.2c).

N_2 fixation being an energy demanding process, the 'low defoliation' and 'control' plants, which developed both the highest nodule number as well as nodule mass per plant, were the most efficient N_2 fixers fuelled by a continuous supply of C from the aboveground source tissue. This explains the high concentration of Kjeldahl N and total plant N in the plants of these treatments (Tables 8.2, 8.4).

Severe defoliation of T. repens plants, which would retard the transition of emerging leaves from C sink to C source, would therefore be the major attribute restricting nodule number as well as nodule growth.

Of the two leaf morph populations, the marked one consistently

developed more nodules per plant (significant at P 0.01). This was true for the defoliation treatments as well as the 'control' at both harvests. The marked population is known to have a greater requirement for N (Pradhan & Tripathi 1984) and the larger number of nodules borne by this population is a probable attempt to fulfil its higher N requirement via symbiotic N₂ fixation. Results indicate that the root dry weights of the marked plants are significantly (P 0.01) greater as compared to that of the unmarked plants at both harvests. This indicates that the marked plants have a larger zone of their root system accessible to infection by the Rhizobium and this would aid increasing the number of nodules formed, as discussed earlier. Though there was no significant difference in mean nodule weights between the two populations, the marked plants by virtue of their greater nodule number, had a significantly (P 0.01) greater nodule mass per plant. However, the concentrations of Kjeldahl N were significantly higher (P 0.01) in the marked population although their PSA was considerably (P 0.01) lower. This reveals that the marked population, despite a significantly smaller C source, is a more efficient N₂ fixer.

The results of this experiment indicate that the stolon tissue plays an important role in facilitating a high degree of physiological plasticity with respect to C distribution and utilization in T. repens. The shoot meristems also took priority over sites of C utilization or storage, such as the nodulated roots and stolons, in response to defoliation and appear to act as the major sinks

of carbon. Stolons being a rich source of stored carbohydrates (Baur-Hoch et al. 1990) are used in restoration of leaf tissue of defoliated plants (Danckwerts & Gordon 1989) at the expense of other sinks like nodulated roots. Though alleviation of nutrient deficiencies, particularly of P, is known to enhance the clover growth (Suckling 1976, Lambert et al. 1982, 1986), the present results indicate that under defoliation stress, alleviation of nutrient deficiencies does little to remedy the detrimental effects of drastic reductions in the C source of nodulated legumes.