9 General Discussion

The growth and establishment of a legume plays a pivotal role in the N economy of the habitat due to the immense N_2 fixing capability within its root nodules. However, the development and functioning of the nodules depend not only on the presence of a compatible rhizobial strain in the rhizosphere of the legume roots, but also on conducive micro-environmental condiions. The nodules in Trifolium being of the indeterminate type are further important as they can continue to grow and fix N_2 over prolonged periods of time.

As the young nodules belonging to category 'a' mature, they increase both in size as well as in the number of lobes. Thus they become larger and heavier so much so that they could be put under categories 'd' or 'e'. Though the nodule number in Trifolium during the later part of the year (September-October) increases only marginally, there is a sharp increase in the nodule dry weights during this period, which may be attributed to the greater proportion of older and larger nodules in the nodule population.

White clover has a high water content (Hayward 1953) and is susceptible to drought. It therefore reacts to the dry winter spells by shedding its larger root nodules which help it overcome the decreasing ratio of fixed N_2/absorbed CO_2 with increasing soil water tensions and low temperatures (Kuo & Boersma 1971). Field studies revealed that the nodule number per plant increased sharply following rains. Loss of soil nutrients and drop in NO_3 levels caused by heavy rains could trigger the formation of a greater number of nodules as a compensatory mechanism. The increased soil moisture levels would result in greater movement of rhizobia in soil (Hamdi 1971), increase the rate of infection of root hairs (Worrall & Roughley 1976) and enhance the supply of photosynthates from the flush of newly formed leaves - which would all lead to development of a greater number of 'juvenile' nodules.

The unmarked population had a greater proportion of their nodules belonging to the younger category whilst the reverse was true for the marked population, which indicates that the longevity of nodules is greater in the latter population. The marked population can therefore be expected to be more efficient in N_2 fixation compared to the unmarked population which bears a greater percentage of 'juvenile' nodules. Under stress, the unmarked population probably sheds a greater percentage of its larger nodules which results in an increase in the proportion of 'juvenile' nodules.

Trifolium repens exhibited a decrease in the nodule number per plant when supplied with increasing levels of NO_3 or NH_4, but the reduction in nodule number was compensated by an increase in the weight of the remaining nodules. This indicates that increased levels of N are detrimental to nodule initiation but not to nodule growth, and increased nodule mass may be seen as an adaptation to sparse nodulation under such conditions. Of the two leaf morph populations, the marked one shows stronger negative correlation between nodule number and mean nodule weight, which indicates that the marked population is better adapted to compensation of reduced number by increased weight of nodules.

Of the two forms of N, NO_3 was a stronger inhibitor to both nodule initiation and nodule growth compared to NH_4. The reduction and assimilation of NO_3 requires considerable energy which leads to a reduction in photosynthates otherwise available to the nodules. This has been confirmed using ^{14}C on a wide range of legumes including clover, by several workers (Small & Leonard 1969, Kahn & Kahn 1981, Truchet & Dazzo 1982). Also, nitrite reduced from nitrate besides being toxic to living organisms, destroys IAA which is required for infection (Dixon 1969, Munns 1977). The inhibitory effects of NH_4 are less severe due to its reduced state (Rabie et al. 1980). The increase in nodule number in the NH_4 treated plants may be attributed to the increased root growth of these plants which in turn, causes an increase in the number of infection sites (Wedderburn 1983) as discussed in Chapter 4. Between the two forms of nitrogen, the N content per plant was consistently higher in the NH_4^+ treated plants than in the NO_3^- treated ones, which is the outcome of a greater number of nodules which develop in response to NH_4^+ addition. Increased nodule mass per plant in the NH_4^+ treatments also indicated that NH_4^+ is not as severe an inhibitor to nodulation as NO_3^-.

As the clover plants grow, their stoloniferous habit leads to the plants encountering a wide mosaic of edaphic variables including variations in the soil pH. The pH of the microenvironment may be reduced following heavy rains which would result in leaching of soluble bases. When edaphic conditions are strongly acidic (pH 4.5), survival of the R. trifolii population in the soil is doubtful which is reflected in the failure of nodulation of the clover, whereas at pH 5.0-6.0 there was a steady increase in both nodule number per plant as well as in the nodule mass. Nodule growth was more sensitive to low pH than the nodule number. At reduced pH levels, decrease in nodule population coupled with low N_2 fixation rates would lead to N deficiency and suppressed growth. Strongly acidic conditions also severely affect root growth due to which the clover may fail to utilize sub-soil moisture leading to apparent moisture stress.

R. trifolii is also susceptible to dryness (Davey et al. 1989) and the reduced nodule number exhibited by both the leaf morph populations at 10% soil moisture could be attributed to the sharp decrease in the number of infection threads (Worrall & Roughley 1976). Moisture stress may also result in shedding of pre-formed nodules from the host root system leading to a further decrease in the nodule population. Results of the present investigation reveal that intermediate levels of soil moisture (10-20%) are most conducive for nodulation as this range also ensures adequate aeration.

In the grasslands, *T. repens* is commonly subjected to defoliation along with companion grasses during grazing by cattle. Removal of the leaves would result in the loss of the photosynthesizing tissue of the legume which acts as the 'source' of fixed carbon for the other plant parts. Defoliation may result in the export of stored photosynthates from 'sinks' like stolons, adventitious roots and nodules to the defoliated shoot apices. This causes a decrease in the number of nodules in the severely defoliated plants. Low root biomass resulting in such situations leads to a decrease in the number of infection sites (Wedderburn 1983) and an increase in the number of aborted infections in the absence of a carbon source.

Nodules are 'sheltered places' (Bergersen 1971) for the endophytes within, which pay back the host by providing nitrogenous compounds. For efficient N_2 fixation, homeostasis in terms of a steady supply of energy, constant removal of fixed products and the maintenence of a precisely poised oxygen concentration, are all important for sustained activity. Senescing nodules may also provide a survival mechanism for the endophyte. As they are shed, the husk provides first, a source of nutrients, then a shelter, thereby minimizing the effects of desiccation and other adverse soil conditions.

Long term exposures to higher concentrations ($>$1.5 mM) of N were severely inhibitory to nodule initiation. Though low concentrations (0.1 0.75 mM) proved greatly beneficial, nil N strongly inhibited nodule initiation. Compared to NH_4^+, NO_3^- was a stronger inhibitor - both to nodule initiation and nodule growth. Nodulation was totally inhibited at pH 4.5, thereafter there was a sharp rise in the nodule population up to pH 5.5-6.0, following which the nodule population dropped as conditions approached neutrality. Although short-term exposures to high soil moisture levels (up to 30%) led to an increase in nodule population, in the long term, 10-20% soil moisture proved to be optimum. High defoliation severely affected nodule initiation and low defoliation proved less detrimental. Incorporation of NPK did little to alleviate the negative effects due to the imposed stress. Only N-supplied plants exhibited a significant negative correlation between nodule number per plant and mean nodule weight, which indicates that higher levels of N was the only stress that was inhibitory to nodule initiation but not to nodule growth.

Management practices to ensure the growth of a well-established clover population bearing an appreciably high number of nodules, would include minimizing grazing during the dry winter and spring when the clover is particularly susceptible to defoliation. Soil N levels, prevailing in the swards, do not seem to be inhibitory to nodulation and growth of the clover as is evident from Chapter 4. Liming of the swards would not be beneficial as results of the present study indicate that the growth and nodulation of the clover is best at pH 5.5-6.0, which is the usual range of soil pH in the swards around Shillong.

Of the two leaf morph populations, the marked one consistently exhibited greater number of nodules per plant as well as greater nodule mass. Increased number of potential sites of N_2 fixation coupled with greater amount of N_2 fixing tissue in this population indicates that this population is of greater significance in determining the N status of the habitat. Prevailing environmental stresses in winter - low soil moisture and temperature - are inhibitory to N_2 fixation and therefore, the clover nodules in winter serve more as an overwintering population (than as potential sites of N_2 fixation) which can resume growth and function the following spring with the return of favourable conditions.