AN ANALYSIS OF NODULE POPULATION DYNAMICS IN WHITE CLOVER (*TRIFOLIUM REPENS* L.) UNDER DIFFERENT ECOLOGICAL CONDITIONS

by

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I certify that the thesis entitled "**An analysis of nodule population dynamics in white clover (*Trifolium repens* L.) under different ecological conditions**" submitted by Debashis Dutta, for the degree of **Doctor of Philosophy** of the North-Eastern Hill University, Shillong, embodies the record of original investigation by him under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the **Ph.D. Degree.** The work has not been submitted for any degree of any other University.

Shillong
The 12th December 1994.

Supervisor

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1 Introduction

Nitrogen is indispensable to plant life as it is an essential constituent of chlorophyll, proteins and protoplasm. This element is thus required in considerable amounts by the plants for growth, especially of the shoot system. Among the major elements, nitrogen is also the one which is readily lost from the ecosystem (Jefferies *et al*. 1981) as the rate of its uptake by the growing plants generally exceeds the rate at which it becomes available by decomposition of complex organic molecules into simpler inorganic forms. Consequently, soils rarely contain enough nitrogen for maximal plant growth. This deficit is usually compensated in forest ecosystems by nitrogen input through litter and in agro-ecosystems by the addition of nitrogenous fertilizers. However, in grasslands this compensation is rarely brought about as the rate of litter accumulation or input is minimal owing to the low above-ground biomass and its frequent partial removal due to grazing. This may result in the sward becoming moribund due to poor growth and decreasing ground cover thereby enhancing leaching and erosion losses of soil (Skeffington & Bradshaw 1980, Jefferies et al. 1981).

Of the large number of living organisms on the earth, only a few prokaryotic microorganisms, the diazotrophs, have the capacity to utilize atmospheric nitrogen for their growth by a process called biological nitrogen fixation. They are responsible for more than 60% of the earth's newly fixed nitrogen (Postgate 1982). Nitrogen fixation contributes substantially to the nitrogen status of arable lands and productivity of agroecosystems. In future agriculture, nitrogen fixation is expected to be of still greater importance since it offers an alternative to the energy-demanding production of nitrogenous fertilizers. Biological nitrogen fixation is only matched by industrial processes like the Haber-Bosch process where the nitrogen is reduced in the presence of powerful catalysts at very high temperature and pressure, for the production of ammonia. The microorganisms which fix nitrogen may be either free living or those which enter into symbiotic association with higher plants.

Symbiotic nitrogen fixation is one of the most important biological processes affecting plant production and soil fertility due to the large amounts of elementary nitrogen fixed by this system. These amounts, when calculated per unit of nitrogen-fixing cell material, are 100-200 times higher than those calculated for free living nitrogen fixers.

Among higher plants, two groups of nitrogen-fixing systems are known: (i) bacteria belonging to the genus Rhizobium living in symbiosis with leguminous plants like Trifolium, and (ii) micro-organisms belonging to the Actinomycetes (Frankia) which enter into symbiotic associations with non leguminous plants like Alnus, Myrica and Hippophae (Burns & Hardy 1975). These symbiotic associations are expressed morphologically by the formation of specialized galls called nodules on the host plant. Nodulation is, thus, a symptom of colonization by an endophyte and the formation of a diazotrophic symbiosis. These nodules are usually formed on the roots of the host, as in the above-mentioned symbiosis, but may be formed on the leaf (as in Psychotria) or on the stem (as in Sesbania). This symbiotic association between the two organisms involved, results in the development of an elaborate system which leads to the host plants becoming independent of soil nitrogen.

Leguminosae (Fabaceae) is one of the largest families of the higher plants comprising 3 sub-families (Papilionaceae, Mimosaceae and Caesalpinaceae) (Allen & Allen 1981). The family comprises about 640 genera with 12,000 species. The family is probably of tropical origin but now members of this family can be found in the tropical, sub-tropical, temperate and even in arctic regions (Allen et al. 1964). They range in habit from small forbs like Trifolium to trees like Acacia. Of the large number of legume species recorded, only ca. 10% of them have been examined for nodulation. Nodulation is widespread in Papilionaceae (85% of the examined species nodulate), less common among Mimosaceae (25%) and rare among Caesalpinaceae.

The nodule bacterium, *Rhizobium* belonging to the family Rhizobiaceae, includes a number of species which are soil saprophytes (Bergey 1984). They are capable of nodulating certain legumes and symbiotically fixing atmospheric nitrogen. Rhizobia are microaerobic and were, for long, thought to be obligate symbiotic diazotrophs, unable to fix nitrogen in the absence of the host plant. However, later some strains were shown to be capable of microaerobic diazotrophy *ex planta* (Gibson *et al*. 1977).

Each *Rhizobium* species comprises a number of different strains which may differ in their nodulating and nitrogen fixing ability. However, effectiveness of the symbiosis depends not only on the bacterium but also on the host legume and prevailing environmental conditions.

Symbiosis is attained after a series of successful events for which a synchronized co-operation between the legume and the endophyte is required. To begin with, a well established population of the bacteria in the rhizosphere of the potential host is a prerequisite for nodulation. The bacteria are attached to the root hair surfaces by linkages between specific polysaccharides secreted by the rhizobia and lectins present on the plant roots (Dazzo & Hubbell 1975). Adhesion of bacteria to root hairs leads to curling or deformation of the root hairs (Martensson 1980). This curling, commonly referred to as the shepherd's crook, entraps some of the bacteria. At this site an infection thread - an invaginated tube of root hair cell wall within which the entrapped rhizobia multiply and are confined in a chain along the lumen of the tube - develops (Callaham & Torrey 1981, Bhuvaneshwari 1984). As it grows, the 'thread' penetrates the basal cell of the root hair, grows between the cortical cells and ramifies, initiating some cortical cells to divide (Newcomb 1980) and finally a young nodule is formed containing the bacteria which are initially rod shaped but gradually become irregular bacteroids (Bergersen 1974). Leghemoglobin, an oxygen buffering protein, is synthesized within the nodules and serves as an oxygen barrier for the nitrogenase enzyme (which is sensitive to oxygen and is damaged by it). As the host plant supplies energy to the nodules in the form of photosynthates, nitrogenase is activated and begins the function of nitrogen fixation.

Nodulation is thus of immense ecological significance as the nodulated legumes contribute substantially to soil fertility by virtue of their remarkable nitrogen fixing capability. The nitrogen fixation rates associated with legumes like T. repens are at least ten times greater than those of non leguminous species (Skeffington & Bradshaw 1980) and have been reported to be as high as 620 Kg N $ha^{-1} yr^{-1}$ (Nutman 1974). This makes nodulated legumes the largest single contributors to fixed nitrogen in the biosphere. When grown in association with grasses, a considerably large part of this fixed nitrogen is released into the soil during decomposition of legume litter. Also, there is conclusive evidence to show that in a mixed population of Trifolium repens and Paspalum dilatatum grown at nil soil nitrogen level, some amount of nitrogen fixed by the legume is passed on to the grass (Pradhan & Tripathi 1985) even much before the decomposition of the legume litter takes place. Introduction of legumes into pastures or to degraded ecosystems is of much advantage as (a) the legumes use elementary atmospheric dinitrogen and thus an unending reserve of the element can be utilised, (b) the legume residues accumulating in the soil contain larger amounts of decomposable nitrogenous compounds than the grass residues, and (c) the use of expensive nitrogenous fertilizers can be done away with as the legume growth contributes nitrogen to the soil system and the energy cost of using legumes to maintain a sward has been estimated to be considerably less than using fertilizers (Laidlaw & Wright 1980). The use of legumes as a source of nitrogen supply in derelict ecosystems is an attractive proposition and an actively fixing legume can supply nitrogen to the root zone of a sward continuously in a way no fertilizer can (William & Cooper 1976).

T. repens forms an important pasture component of the grasslands in and around Shillong where it plays a significant role in enriching the nitrogen status of the swards. The clover is composed of two distinct leaf morph populations - one characterised by the presence of distinct 'V'-shaped white markings on the leaflets (marked population) and the other devoid of such leaf markings (unmarked population) (Plate 1). Two linked groups of genes control the leaf marking in *T. repens* (Corkill 1971), which is due to the presence of air spaces within the palisade tissue, and is controlled by multiple alleles at a locus in one of these groups (Carnahan

Plate 1. A view of the study site showing profuse growth of *Trifolium repens*

et al. 1955). Views on the adaptive significance of the leaf markings in *T. repens* are contradictory. Charles (1968) is of the view that the marked leaves of clover might help grazing animals like sheep to form an image, so as to select it from the grass mixture because of its palatability, while Cahn & Harper (1976) have demonstrated that sheep avoid *T. repens* with marked leaves (Plate 2).

Though considerable work has been carried out on the *T. repens* - *R.trifolii* symbiosis, nodulation patterns in the two leaf morph populations have not been studied. A detailed analysis of the various ecological factors on nodulation of this legume has also not been carried out in spite of the fact that white clover is an important component of the Indian grasslands at higher elevations. The high annual rainfall received in the north-eastern states causes severe leaching of nutrients from the top soil, leaving it porous and infertile and therefore, a luxuriant growth of *T. repens* in the local grasslands assumes special significance in the maintenance of soil fertility. Even though white clover plays a pivotal role in the ecology of these swards, its importance has been grossly undermined. Consequently, this is a neglected field of research, except for studies by Pradhan (1984) and Pradhan & Tripathi (1985) on its competitive ability.

The present study was conducted in swards at Shillong as well as in the green house at the North-Eastern Hill University during 1990-1993. The major objectives of the study were as follows: (i) To determine the variations in the nodule population of *T. repens*

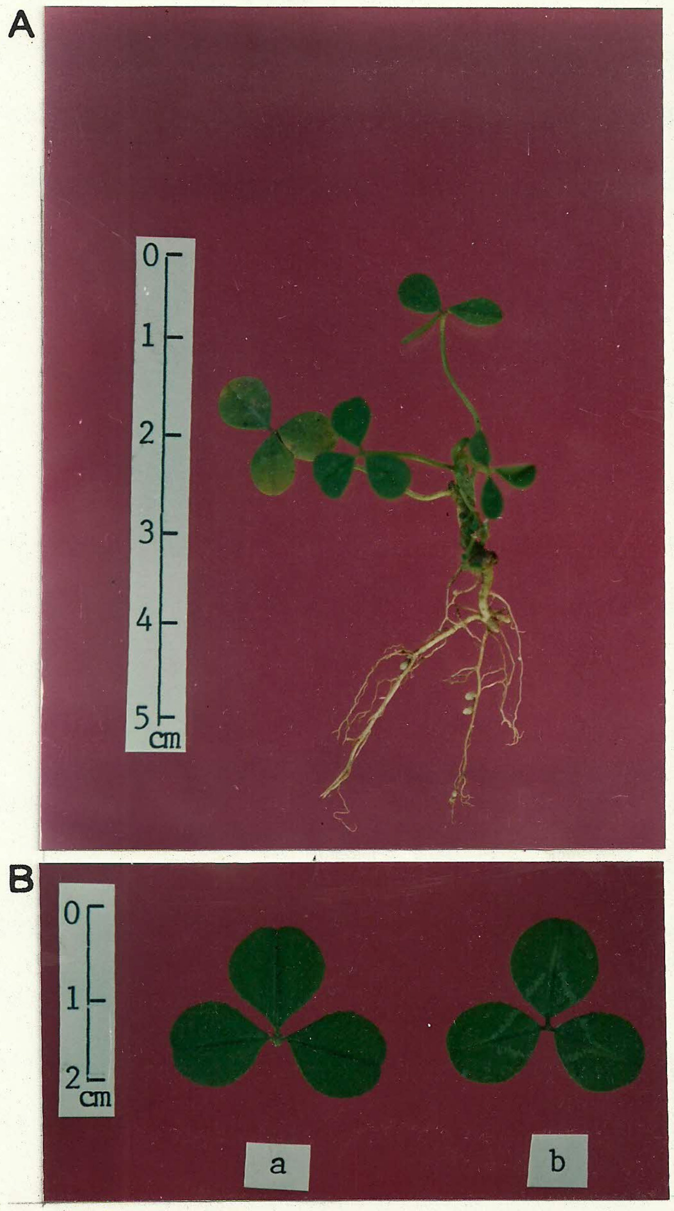

Plate 2. (A) T. repens plant bearing root nodules.
(B) Leaves of the (a) unmarked and (b) marked leaf morph populations.

under different ecological conditions, (ii) To determine the differences, if any, in the nodulation pattern in the marked and unmarked and unmarked leaf morph populations of T. repens, (iii) To determine whether nitrogen accumulation rates varied in the two leaf morph populations, and (iv) To suggest ecological conditions and management practices that could augment nodulation and consequent nitrogen fixation.

The thesis has been divided into ten chapters. The subject matter of the thesis has been introduced in Chapter 1 (the present chapter). A brief outline of the legume-rhizobium symbiosis and the main objectives of the study are also included in this chapter. Chapter 2 (Review of Literature) presents a review of the selected and important studies carried out on the subject of the thesis and related areas. Chapter 3 dealing with 'Materials and Methods' gives a brief description of the protocols followed for the different experiments and the materials used. Seasonal changes in nodule population and corresponding changes in soil nitrogen levels in field conditions have been presented in Chapter 4. The results of the greenhouse experiments are dealt with in Chapters 5-8. Chapter 5 embodies the results pertaining to the effects of substrate nitrogen on the nodule population. Chapter 6 deals with the nodulation pattern in relation to varying soil pH, and the effects of soil moisture content on nodule population have been presented in the following chapter (Chapter 7). Chapter 8 contains the results on the nodulation pattern in relation to defoliation and NPK levels. The results presented in Chapters 4-8 have been discussed in detail in each of these chapters. However, the findings of the studies on the various aspects have been briefly discussed in an integrated manner in Chapter 9 (General Discussion) and a summary of the entire study has been presented in Chapter 10.