NODULATION STUDIES IN LEGUMES

IV. THE INFLUENCE OF INOCULUM STRAIN AND TIME OF APPLICATION OF AMMONIUM NITRATE ON SYMBIOTIC RESPONSE

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INTRODUCTION

Numerous investigations of the effects of combined nitrogen on the nodulation of legumes have demonstrated that maximum symbiotic performance is associated with growth media deficient in combined nitrogen (see Fred, Baldwin, and McCoy; Wilson; Nutman; van Schreven).

High levels of combined nitrogen have been shown to reduce nodule numbers, and inhibit nodule growth and fixation; the reduction in these quantities varying with the species of legume; with the amount and form of combined nitrogen applied; and with the time of its application to the host plant. Stimulation of symbiosis by small amounts of combined nitrogen has been recorded by several workers (e.g. Giobel; Fred and Wilson; MacConnell and Bond).

Recent work would also suggest that the site of application of combined nitrogen to the host plant, the pH of the medium, growth temperatures, and general climatic conditions, may also influence the competitive assimilation of molecular and combined nitrogen by the nodulated legume.

In this paper evidence is presented of differences between various bacterial symbionts of the one host plant in both their infection and fixation responses to added combined nitrogen. A change with time in the sensitivity of a given association to com-

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bined nitrogen is reported. Examples of stimulation or injury of symbiosis by added nitrogen are described and it is suggested how these may be compounded to produce long-term effects on plant growth and nitrogen fixation.

GENERAL METHODS

The effects of combined nitrogen on the symbiosis of four legumes, purple vetch (Vicia atropurpurea Desf.), common vetch (Vicia sativa L. var. Golden Tares), cowpea (Vigna sinensis Endl. var. Poona) and barrel medic (Medicago tribuloides Desr. strain 173) were examined using the sand-culture technique previously described.

Ammonium nitrate was used as the source of combined nitrogen, and, in all but one experiment, was applied at sowing. Treatments were compared on the basis of the amounts of combined nitrogen initially applied to each pot and subsequently available to each developing plant. Plants were grown in natural daylight in glasshouses in Sydney (Experiments 1, 2, 3, 5) and Belfast (Experiment 4).

Washed, sterilised river sand was used for all experiments. The original moisture status of the sand was maintained with regular addition of distilled water and nitrogen-free mineral nutrients. Treatment replicates were randomly located on the glasshouse benches and the pots were spaced and the contained plants thinned so that no obvious restrictions of growth were imposed in any treatment. Further details of cultural conditions are given in descriptions of the individual experiments.

Inoculation at sowing gave initial bacterial populations well in excess of those suggested as necessary for maximum nodulation. Nodule bacteria with code prefixes NA and SU were strains obtained from the School of Agriculture collection, University of Sydney; those with prefixes V are part of a collection of Irish and Australian strains held in the Botany Departments of the University of Sydney and Queen's University, Belfast.

In short-term experiments (1–3) primary-root nodulation (nodule number, size, and colour) was used to record the treatment responses. This was considered a reliable measure of symbiotic reaction to the initial levels of combined nitrogen since in the legumes studied infection of the primary root is completed in the first ten days of seedling growth – before appreciable amounts of nitrogen have been absorbed by the host plant. (Similar results on nitrogen uptake by legumes in agar-tube culture have been recorded by Gibson.) In long-term experiments (4 and 5) symbiotic responses are only indirectly related to the initial concentration of combined nitrogen since later infections of the lateral and minor root systems take place after a portion of the nitrogen supplement has been assimilated by the host plant.

In such experiments nodule tissue production, nodule efficiency, and total nitrogen fixation in the host plant were determined to evaluate symbiotic activity. The relevance of these and other recorded quantities is discussed in an earlier paper.

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