

# The Effects of Nitrate and Asparagine on Nitrogen Fixation (The Acetylene Reduction Activity) in Inoculated Faba Bean

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## Abstract

The response of nitrogen fixation (as assessed by the acetylene reduction assay) in *Vicia faba* cv. Fiord, inoculated with three different strains of *Rhizobium leguminosarum* bv *viciae* (NA 533, SU 391 and CC 305) to two sources of combined nitrogen, nitrate and the amino-acid asparagine, applied exogenously, were investigated in a pot experiment. Inoculated seedlings were established under sterile conditions in sterilised coarse river sand and transferred to pots covered with sterilised polypropylene lids in a laminar flow cabinet. These were later transferred to a growth room. Thirty-five days after sowing (DAS), the plant roots were flushed daily with nutrient solutions containing 0, 2.5 and 10 mM nitrate or asparagine using a 'pour through' system. Dry matter, nodule weight, N accumulation and acetylene reduction (ARA) were monitored for a further 8 days. Inoculation with strain NA 533 gave highest dry matter yield, nodulation and ARA compared to inoculations with the other two strains, indicating that the symbiosis with strain NA 533 was highly effective. Both nitrate and asparagine were apparently taken up by the plants and contributed to total N, but plant growth was not significantly affected by nitrate or asparagine in all symbioses during the experimental period and there were no significant interactions between treatments. While inoculation with strain NA 533 appeared to supply all the N needed for growth, plants inoculated by strains SU 391 and CC 305 took up more N from both nitrate and asparagine, the uptake being highest in plants inoculated by strain SU 391, indicating this symbiosis to be less effective. The contribution to total plant N was significantly greater with asparagine than with nitrate. The higher contribution of N to plants from the 'less effective' symbioses did not lead to rapid increases in dry weight since differences in plant performance had already been established by the time treatments were imposed. Nodule dry weight and ARA were significantly depressed by nitrate and asparagine in all symbioses and in proportion to the concentrations applied. Also, the decline in ARA induced by asparagine was always greater than that with nitrate. This experiment suggests that plant size and the efficiency of the symbiosis may indirectly contribute to making a particular symbiosis less sensitive to combined N.

*Key words:* Acetylene reduction activity, asparagine, faba bean, nitrate, nitrogen fixation, nodules

## Introduction

Nitrate is the most common form of N found in agricultural soils and the most potent inhibitor of N<sub>2</sub> fixation (Hageman, 1979). It is usually supplied at sowing so that the responses to NO<sub>3</sub><sup>-</sup> usually attributed to effects on nodule functioning represent an interaction between many processes. Strains of *Rhizobium* are also known to vary in their capacity to nodulate the same host in the presence or absence of combined N (Pate & Dart 1961; Hoglund, 1973; Munns, 1977;

Harper & Gibson 1984). However, little appears to be known about the differential responses to mineral N by mature symbioses established by different strains of *Rhizobium*, i.e. the direct effects of NO<sub>3</sub><sup>-</sup> on N<sub>2</sub> fixation. The basis for these effects is also not well known.

One of the hypotheses advanced to explain combined N effects on N<sub>2</sub> fixation is the diversion of assimilates from nodule functioning to enzymes associated with the reduction of combined N when it is supplied to an ac-