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Variation in *Rhizobium leguminosarum* response to short term application of NH_4NO_3 to nodulated *Pisum sativum* L.

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Summary This study was conducted to determine the effect of short term application of NH_4NO_3 on nodule function and to determine whether the rhizobial isolate used was a significant factor in this effect. Pea plants were inoculated with 10 different *Rhizobium leguminosarum* isolates and grown for 3 weeks in N-free medium before addition of 0, 1, 2 or 5 m*M* NH_4NO_3 for 2 to 7 days. Acetylene reduction and leghemoglobin content decreased with increasing exposure time to NH_4NO_3 and with increasing concentration of NH_4NO_3. NH_4^+ and NO_3^- depletion from the nutrient medium were assayed in plants exposed to 5 m*M* NH_4NO_3 and mean uptake rates were similar for each ion. There were significant differences among isolates in the rate of decrease of C_2H_2 reduction with increasing NH_4NO_3 concentration (C_2H_2 reduction responsiveness to NH_4NO_3) 4 and 7 days after addition of NH_4NO_3 but no differences after 2 days of exposure to NH_4NO_3. There were significant differences among isolates in NH_4^+ depletion from the nutrient medium but these differences were not correlated with the differences observed in C_2H_2 reduction. Ranking of the isolates for C_2H_2 reduction responsiveness to NH_4NO_3 applied to plants with nodules was different from that obtained when NH_4NO_3 was applied at seeding. Isolates with varying sensitivity to NH_4NO_3 may be useful tools for determining the mechanisms responsible for inhibition of symbiotic N_2 fixation by combined nitrogen.

Introduction

Combined nitrogen has complex inhibitory effects on symbiotic N_2 fixation in legumes. The addition of combined nitrogen, in the form of NH_4^+ or NO_3^- can lead to inhibition of infection, nodule development and nodule function as well as promoting nodule senescence[5,13,19]. In order to differentiate between the effects of combined nitrogen on the infection-nodule development process and on nodule function (N_2 fixation) combined nitrogen is applied at planting in the first case[7,17,20] or as a short term application after nodules have formed in the latter case[1,4,9,21].

The mechanisms responsible for the inhibitory effect of combined nitrogen on nodulation and symbiotic N_2 fixation have not been resolved but there is good evidence that both the host plant and the rhizobial strain play important roles. Variation in NO_3^- inhibition of nodulation has been reported among legume species[7] and among cultivars within a

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species[6]. Recently, mutants of pea[10] and soybean[2,3] which nodulate in the presence of high concentrations of NO_3^- have been described. The rhizobial strain is also a source of variation with respect to nodulation ability in the presence of low levels of combined nitrogen[8,12,15,18].

Variability within plant and rhizobial species in inhibition of nodule function by combined nitrogen has been less well studied. Differences among three cowpea rhizobia were reported when NO_3^- was applied 27 days after planting[11] but no significant differences were found among 16 *R. japonicum* strains subjected to similar treatment[12]. As the mechanism responsible for combined nitrogen inhibition of nodule function may differ from that involved in the early stages of infection and nodule development, responses within plant cultivars or rhizobial strains may also differ.

In an earlier study, ten isolates of *R. leguminosarum* were found to vary in symbiotic effectiveness when NH_4NO_3 was applied at planting[15]. This suggests differences among the isolates in nodule initiation and development. In this study the response of these same isolates was assessed after NH_4NO_3 was applied to peas with established nodules in order to determine the effect on nodule function.

Materials and methods

Cultures

Rhizobium leguminosarum isolates 128C23, 128C30, 128C40, 128C52, 128C54, 128C70, 128C79, 175G3 and 175G16 were obtained from Dr J Burton, Nitragin Co., Milwaukee, WI. *R. leguminosarum* isolate NA502 was provided by Dr R Roughley, Australian Inoculation Control Service, Narara, N.S.W., Australia. The cultures were grown in modified yeast-extract mannitol (0.5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 0.5 g yeast extract, 0.5 g $CaCO_3$, and 10 g mannitol in 1 liter of distilled water, pH 7.3) at 30°C for 3 days prior to seed inoculation.

Plant inoculation and growth

Seeds of *Pisum sativum* L. cv. Homesteader were surface sterilized, planted in sterilized, modified Leonard jar assemblies containing Turface (Wyandotte Chemicals Ltd., Wyandotte, MI) and N-free nutrient solution, and inoculated with the appropriate isolate as previously described[15]. The plants were grown in a controlled environment chamber (Enconaire, Winnipeg, Manitoba) with a 16-h photoperiod, day/night temperatures of 20°C/15°C, and an irradiance level corresponding to photon flux densities (400–700 nm) of 400 $\mu Em^{-2}s^{-1}$ using a mixture of fluorescent and incandescent lights. After 3 weeks the nutrient solution was replaced with fresh N-free medium or N-free medium supplemented with NH_4NO_3 to give a final concentration of 1, 2 or 5 m*M*. Four replicate plants of each treatment were harvested at 0, 2, 4, and 7 days after application of NH_4NO_3 and assayed for characters associated with N_2 fixation and plant growth.

In a second parallel experiment three replicate plants of each *R. leguminosarum* isolate — pea combination were grown for 3 weeks in N-free medium, followed by supplementation with 5 m*M* NH_4NO_3 for 1 week. Samples of the nutrient solution were taken 1, 3, 5, and 7 days after addition of NH_4NO_3 for determination of NH_4^+ and NO_3^-. To compensate for the water taken up by the plants the growth medium was replenished to the original level with N-free nutrient medium and mixed prior to sampling for NH_4^+ and NO_3^-.

Assays

Uptake hydrogenase activity of a washed segment of excised lateral root with attached nodules

was determined by the $^3H_2$ uptake method as described previously[16]. The remainder of the root was assayed for H_2 evolution, C_2H_2 reduction and leghemoglobin content as previously described[15]. Data are expressed per gram fresh weight (gfw) of root. Plant shoots were dried for 48 h at 70°C and weighed. Shoot N content was determined by automated Kjeldahl (A/SN. Foss Electric, Hillerd, Denmark). NH_4^+ and NO_3^- were measured colorimetrically using an autoanalyser (Chemlab Instruments Ltd., Hornchurch, England).

Statistical analyses

Acetylene reduction data obtained for the 10 isolates were logarithmically transformed prior to analysis to homogenize the variances. The transformed data were analyzed by factorial analysis of variance and the means presented are transformed back into linear scale. The transformed means for C_2H_2 reduction were regressed against NH_4NO_3 concentration at each harvest in order to obtain a more sensitive measure of combined N effects on C_2H_2 reduction[15]. The slopes for each isolate, defined as the C_2H_2 reduction responsiveness to NH_4NO_3, were compared by analysis of variance and differences between slopes determined by the Duncan multiple range test at the 5% probability level. Similar analyses were applied to the leghemoglobin data. Significance of rank correlations was determined from Kendall's coefficient of rank correlation.

Results

Response to NH_4NO_3 application

Mean C_2H_2 reduction rates decreased when NH_4NO_3 was applied at three concentration to 10 *R. leguminosarum* — pea combinations with established nodules (Fig. 1). Analysis of variance indicated that NH_4NO_3 concentration and time of exposure to NH_4NO_3 were significant sources of variation. Plants exposed to NH_4NO_3 for 7 days showed more inhibition of C_2H_2 reduction than those exposed for 2 days. The other symbiotic characters assayed also showed the greatest changes in response to NH_4NO_3 after 7 days of exposure, so only the 7-day data are presented for these characters (Fig. 2). Mean H_2 evolution rates declined at a rate similar to that observed for C_2H_2 reduction and as a result, relative efficiency of N_2 fixation [1-(H_2 evolved in air/C_2H_2 reduced)] was not significantly affected by NH_4NO_3 treatment. Five of the isolates had uptake hydrogenase activity and the mean H_2 uptake rates of these isolates tended to decrease in the presence of NH_4NO_3. The leghemoglobin content of the nodules decreased with increasing NH_4NO_3 but at a slower rate than did C_2H_2 reduction (in the 5 m*M* NH_4NO_3 treatment, leghemoglobin content was 58% of the N-free control while C_2H_2 reduction rate was only 35% of the control after 7 days). Shoot dry weight and shoot N content increased with increasing NH_4NO_3.

In a parallel experiment, using only 5 m*M* NH_4NO_3, the uptake of NH_4^+ and NO_3^- from the Leonard jar reservoir was assayed over the 7-day period (Fig. 3). The mean uptake of each ion by plants inoculated with the 10 isolates was a linear function of time and mean rates for each ion were similar (0.252 mmol NO_3^- plant^{-1} d^{-1} and 0.251 mmol NH_4^+ - plant^{-1} d^{-1}).

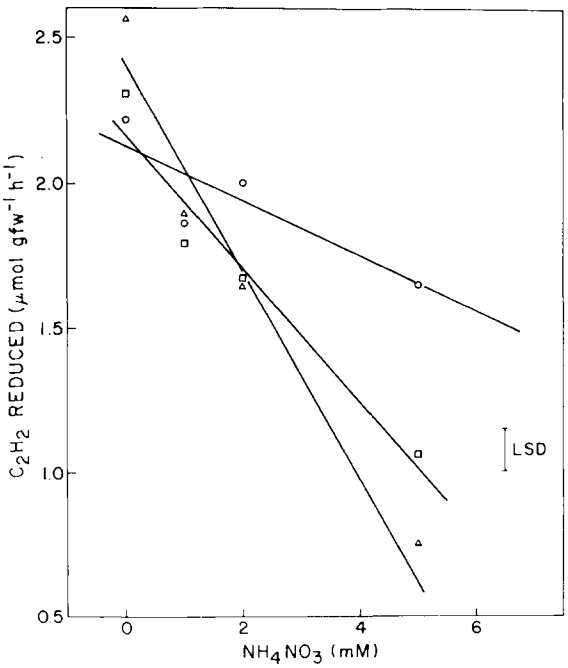

Fig. 1. Effect of NH_4NO_3 concentration and time after NH_4NO_3 addition on C_2H_2 reduction in peas nodulated by ten isolates of *R. leguminosarum*. Values are the means for ten isolates. Plants were harvested 2 (○), 4 (□) and 7 (△) days after 1, 2 or 5 m*M* NH_4NO_3 was added.

Variation in isolate response to NH_4NO_3 application

To determine whether isolates varied significantly in their response to NH_4NO_3 applied to established nodules, C_2H_2 reduction data for each isolate were subjected to analyses of variance. There were significant differences among isolates exposed to NH_4NO_3 for 7 days in C_2H_2 reduction rates and in C_2H_2 reduction responsiveness to NH_4NO_3 (Table 1). The latter responsiveness term is probably a better measure of an isolate's sensitivity to combined nitrogen than C_2H_2 reduction alone because it measures the change in C_2H_2 reduction over a range of NH_4NO_3 concentrations[15]. The C_2H_2 reduction responsiveness at day 7 was significantly correlated with mean C_2H_2 reduction rate ($p < 0.05$) only when isolates 128C30 and NA502 were excluded from the comparison. These isolates had low rates of C_2H_2 reduction and low sensitivity to NH_4NO_3 addition (4 fold less than the most sensitive isolates, 175G16 and 128C23).

There were no significant differences among isolates in C_2H_2 reduction responsiveness 2 days after exposure to NH_4NO_3 but significant differences were apparent after 4 days (data not presented). The ranking of the

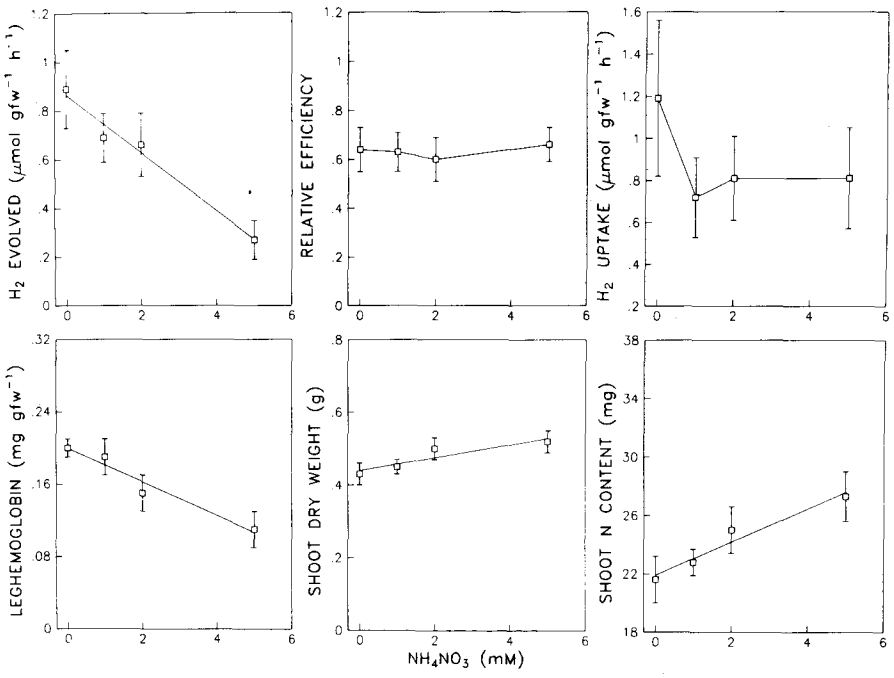

Fig. 2. Effect of NH_4NO_3 concentration on H_2 evolution, relative efficiency of N_2 fixation, H_2 uptake, leghemoglobin content, shoot dry weight and shoot nitrogen content of nodulated peas exposed to NH_4NO_3 for 7 days before harvesting. Values are the means for 10 *R. leguminosarum* isolates except for H_2 uptake, where means are for the 5 Hup^+ isolates (128C23, 128C30, 128C40, 128C52, 128C54). *Bars* represent standard errors.

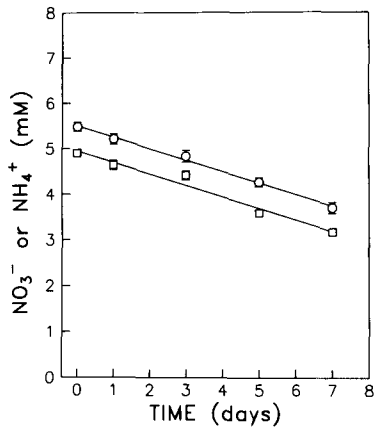

Fig. 3. Concentration of NH_4^+ (○) and NO_3^- (□) in the nutrient medium of nodulated peas exposed to 5 m*M* NH_4NO_3 for 7 days. Values are the means for 10 *R. leguminosarum* isolates; *bars* represent standard errors.

Table 1. Mean C_2H_2 reduction rates, C_2H_2 reduction responsiveness to NH_4NO_3 and leghemoglobin content 7 days after application of 3 concentrations of NH_4NO_3 to peas inoculated with 10 isolates of *R. leguminosarum*

Isolate	C_2H_2 reduction rate[a] (μmol. gfw^{-1}. h^{-1})	C_2H_2 reduction responsiveness to NH_4NO_3[b]	Leghemoglobin content[a] (mg gfw root^{-1})
175G3	3.32 a[c]	−0.208 bc	0.218 ab
128C54	2.66 ab	−0.160 ab	0.240 a
128C52	2.48 ab	−0.163 ab	0.165 abc
128C40	1.92 abc	−0.167 ab	0.112 cd
128C79	1.71 bc	−0.289 c	0.154 abcd
128C30	1.30 cd	−0.097 a	0.116 cd
175G16	1.13 cd	−0.496 d	0.144 abcd
128C23	1.80 cd	−0.425 d	0.138 bcd
128C70	1.08 cd	−0.305 c	0.091 d
NA502	0.75 d	−0.111 ab	0.106 cd

[a] Each value is the mean calculated over all NH_4NO_3 treatments.
[b] Slope of regression of C_2H_2 reduction *vs* NH_4NO_3 concentration.
[c] Numbers followed by the same letter in a column are not significantly different at the 5% level by Duncan's multiple range test.

isolates with respect to C_2H_2 reduction responsiveness on day 4 was similar to that observed on day 7 with the exception of isolates 128C40 and 128C23 which were proportionately more inhibited by NH_4NO_3 on day 7 than on day 4 (321 and 417%, respectively) when compared with the other isolates (138 $\pm$ 14%).

There were significant differences among isolates in leghemoglobin content of the roots after 7 days of exposure to NH_4NO_3 (Table 1) but no significant differences in leghemoglobin responsiveness to NH_4NO_3 (data not presented). There was a significant correlation ($p < 0.01$) between mean leghemoglobin content and mean C_2H_2 reduction rate on day 7. It is possible to obtain a measure of specific nitrogenase activity (SNA) by expressing N_2 fixation as C_2H_2 reduced per mg leghemoglobin per hour[16]. On day 7 SNA of isolates NA502 and 128C30 increased from a mean of 7.5 $\pm$ 0.4 μmol mg^{-1} h^{-1} in the absence of NH_4NO_3 to 10.4 $\pm$ 2.7 μmol mg^{-1} h^{-1} in the presence of 5 m*M* NH_4NO_3. In the eight other isolates tested, SNA decreased in the presence of NH_4NO_3 (from a mean of 16.4 $\pm$ 1.5 to 8.0 $\pm$ 1.4 μmol mg^{-1} h^{-1} at 0 and 5 m*M* NH_4NO_3, respectively).

Analyses of NH_4^+ and NO_3^- uptake by plants exposed to 5 m*M* NH_4NO_3 for 7 days showed significant differences among isolates in total NH_4^+ taken up (Table 2) but no significant differences in total NO_3^- taken up or in the rate of N uptake. Total NO_3^- taken up in 7 days was significantly correlated ($p < 0.05$) with plant dry weight after 7 days of exposure to NH_4NO_3 but there was no correlation with C_2H_2 reduction.

Table 2. Total uptake of NH_4^+ by peas nodulated with 10 isolates of *R. leguminosarum* and exposed to 5 m*M* NH_4NO_3 for 7 days

Isolate	NH_4^+ taken up in 7 days (mmol plant^{-1})
128C30	2.74 a[a]
128C52	2.26 ab
175G16	2.13 abc
175G3	1.82 bc
128C79	1.69 bc
128C70	1.60 bc
128C40	1.60 bc
128C23	1.59 bc
128C54	1.47 c
NA502	1.45 c

[a] Numbers followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Total NH_4^+ taken up in 7 days was not significantly correlated with plant dry weight or C_2H_2 reduction.

Discussion

Addition of NH_4NO_3 to the nutrient solution of nodulated *Pisum sativum* L. plants led to decreases in nitrogenase activity (as measured by C_2H_2 reduction and H_2 evolution) and in leghemoglobin content. Similar results have been reported in peas exposed to higher concentrations of NO_3^- or NH_4^+[1,3,9]. In one study, heme concentration decreased at a rate equal to that of acetylene reduction in peas exposed to 10 or 20 m*M* NH_4NO_3 while nitrogenase synthesis was not affected[1]. This led to the postulate that the observed decrease in nitrogen fixing capacity was caused primarily by a decrease in leghemoglobin. Others, however, observed that leghemoglobin content declined more slowly than C_2H_2 reduction[3], results which agree with the data presented here (Fig. 1 and 2).

Carbohydrate deprivation has been proposed as a mechanism responsible for reduced nitrogenase activity in nodules exposed to NO_3^-[5,9,19]. Consistent with this theory would be increases in relative efficiency of N_2 fixation and in H_2 uptake as occurs when plants are grown at low irradiance[14]. Such increases in relative efficiency and H_2 uptake were observed in plants exposed to NH_4NO_3 from seeding[15] but did not occur during short term exposure of nodulated plants to NH_4NO_3 (Fig. 2).

Ten isolates of *R. leguminosarum* which displayed variability in their response to NH_4NO_3 applied at seeding[15] also varied in their response to NH_4NO_3 when it was applied to plants with established nodules (Table

1). This isolate effect was not evident 2 days after addition of NH_4NO_3 but significant differences could be demonstrated after 4 or 7 days. Relative to the other isolates, two isolates were significantly more sensitive to NH_4NO_3 on day 7 than on day 4. This temporal variability may be related to differences in initial uptake rates or partitioning of N within plants. Nitrogen uptake measurements, however, did not show any significant deviations from linearity for these two isolates (data not shown).

The rate of depletion of NH_4^+ and NO_3^- from the nutrient solution was examined as a factor which might relate to the variable response of isolate C_2H_2 reduction to NH_4NO_3 application, but no correlation was apparent. Although the mean rates of uptake for each ion were similar, plants nodulated by different isolates did show some variability in NH_4^+ uptake (Table 2). No clear relationship was found between NO_3^- uptake and delay in nodule appearance when NO_3^- was applied at planting of several legume species in a hydroponic system[7]. It should be noted that the clay support system in the Leonard jar was capable of adsorbing 0.74 mmol NH_4^+ and 0.29 mmol NO_3^- when the nutrient solution contained 5 m*M* NH_4NO_3. Further experiments designed to measure the actual amount of nitrogen taken up by the roots and the partitioning of that nitrogen within the plant are planned. These may clarify whether a relationship exists between nitrogen ion uptake/partitioning and inhibition of N_2 fixation in plants nodulated by the isolates.

The ranking of the 10 isolates with respect to C_2H_2 reduction responsiveness to NH_4NO_3 applied at seeding[15] was compared to that when NH_4NO_3 was applied to nodulated plants (Table 3). A rank correlation was not significant, due primarily to differences in the rankings of isolates NA502 and 128C30. If these isolates were omitted from the analysis a highly significant correlation ($p < 0.01$) was obtained. For the majority of isolates examined here, this suggests that performance with respect to nodule initiation and development in the presence of combined nitrogen will also be a good indicator of performance for nodule function and *vice versa*. For example, isolates which were strongly inhibited by NH_4NO_3 applied at seeding (128C70, 175G16 and 128C23) were also the most sensitive when NH_4NO_3 was added to 3-week-old plants. The exceptions to this generalization, isolates NA502 and 128C30, are also of interest. Nodule initiation and development in plants inoculated with these isolates were quite sensitive to addition of combined nitrogen, but when established nodules containing these isolates were exposed to similar concentrations of combined nitrogen, C_2H_2 reduction was only slightly inhibited (Table 1) and SNA increased. Isolates such as these may be helpful in establishing which factors are important in the inhibition of nodule function by combined nitrogen.

Table 3. Ranking of *R. leguminosarum* isolates for C_2H_2 reduction responsiveness to NH_4NO_3 applied at seeding or to 3 week-old plants

Isolate	Rank	
	NH_4NO_3 applied at seeding	NH_4NO_3 applied to 3 wk-old Plants
128C54	1[a]	3
128C52	2	4
175G3	3	6
128C40	4	5
128C79	5	7
NA502	6	2
128C30	7	1
128C70	8	8
175G16	9	10
128C23	10	9

[a] Ranking according to increasing sensitivity to NH_4NO_3.

They may also be of value in future studies with legume mutants capable of nodulating in the presence of high concentrations of NO_3^-[2,3,10]. Nodulation inhibition has been overcome in these plants but specific nitrogenase activity was markedly decreased in the mutant and in the parent in the presence of NO_3^-. Isolates such as NA502 and 128C30 should permit optimal N_2 fixation under these conditions.

These data indicate that nodule function is affected by short term application of NH_4NO_3 to pea plants and that *R. leguminosarum* isolates vary in repsonse to this treatment. Identification of isolates or cultivars with differing sensitivities to NH_4NO_3 at the nodule initiation, nodule development and nodule function stages may be useful for elucidating the mechanisms involved in inhibition of symbiotic N_2 fixation by combined nitrogen at each stage.

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References

1 Bisseling T, Van den Bos R C and Van Kammen A. 1978 The effect of ammonium nitrate on the synthesis of nitrogenase and the concentration of leghemoglobin in pea root nodules induced by *Rhizobium leguminosarum*. Biochim. Biophys. Acta 539, 1–11.

2 Carroll B J, McNeil D L and Gresshoff P M 1985 Isolation and properties of soybean [*Glycine max* (L.) Merr.] mutants that nodulate in the presence of high nitrate concentrations. Proc. Natl. Acad. Sci., USA 82, 4162–4166.

3 Carroll B J, McNeil D L and Gresshoff P M 1985 A supernodulation and nitrate-tolerant symbiotic (nts) soybean mutant. Plant Physiol. 78, 34–40.

4 Chen P-C and Phillips D A 1977 Induction of root nodule senescence by combined nitrogen in *Pisum sativum* L. Plant Physiol. 59, 440–442.

5 Gibson A H and Jordan D C 1983 Ecophysiology of nitrogen-fixing systems *In* Encyclopedia of Plant Physiology New Series, Vol. 12C, pp 301–390. Eds. O L Lange, P S Nobel, C B Osmond and H Ziegler, Springer Verlag, Berlin.
6 Gibson A H and Harper J E 1985 Nitrate effect on nodulation of soybean by *Bradyrhizobium japonicum*. Crop Sci. 25, 497–501.
7 Harper J E and Gibson A H 1984 Differential nodulation tolerance to nitrate among legume species. Crop Sci. 24, 797–801.
8 Heichel G H and Vance C P 1979 Nitrate-N and Rhizobium strain roles in alfalfa seedling nodulation and growth. Crop Sci. 19, 512–518.
9 Houwaard F 1980 Influence of ammonium and nitrate nitrogen on nitrogenase activity of pea plants as affected by light intensity and sugar addition. Plant and Soil 54, 271–282.
10 Jacobsen E and Feenstra W J 1984 A new pea mutant with efficient nodulation in the presence of nitrate. Plant Sci. Letters 33, 337–344.
11 Manhart J R and Wong P P 1980 Nitrate effect on nitrogen fixation (acetylene reduction). Activities of legume root nodules induced by rhizobia with varied nitrate reductase activities. Plant Physiol. 65, 502–505.
12 McNeil D L 1982 Variation in ability of *Rhizobium japonicum* strains to nodulate soybean and maintain fixation in the presence of nitrate. Appl. Environ. Microbiol. 44, 647–652.
13 Munns D N 1977 Mineral nutrition and the legume symbiosis. *In* A Treatise on Dinitrogen Fixation. Section IV. Agronomy and ecology, pp 353–391. Eds R W F Hardy and A H Gibson. John Wiley and Sons, New York.
14 Nelson L M 1983 Hydrogen recycling by *Rhizobium leguminosarum* isolates and growth and nitrogen contents of pea plants (*Pisum sativum* L.) Appl. Environ. Microbiol. 45, 856–861.
15 Nelson L M 1983 Variation in ability of *Rhizobium leguminosarum* isolates to fix dinitrogen symbiotically in the presence of ammonium nitrate. Can. J. Microbiol. 29, 1626–1633.
16 Nelson L M and Child J J 1981 Nitrogen fixation and hydrogen metabolism by *Rhizobium leguminosarum* isolates in pea root nodules. Can. J. Microbiol. 27, 1028–1034.
17 Oghoghorie C G O and Pate J S 1971 The nitrate stress syndrome of the nodulated field pea (*Pisum arvense* L.). Techniques for measurement and evaluation in physiological terms. *In* Biological Nitrogen Fixation in Natural and Agricultural Habitats. Plant and Soil Spec. Vol. pp 185–202. Eds. T A Lie and E G Mulder. Martinus Nijhoff, Dordrecht, The Netherlands.
18 Pate J S and Dart P J 1961 Nodulation studies in legumes. IV. The influence of inoculum strain and time of application of ammonium nitrate on symbiotic response. Plant and Soil 15, 329–346.
19 Rigaud J 1981 Comparison of the efficiency of nitrate and nitrogen fixation in crop yield. *In* Nitrogen and Carbon Metabolism. pp 17–48. Ed. J D Bewley. Martinus Nijhoff, Dordrecht, The Netherlands.
20 Streeter J G 1985 Nitrate inhibition of legume nodule growth and activity. I. Long term studies with a continuous supply of nitrate. Plant Physiol. 77, 321–324.
21 Streeter J G 1985 Nitrate inhibition of legume nodule growth and activity. II. Short term studies with high nitrate supply. Plant Physiol. 77, 325–328.