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Nodulation and Nitrogenase Activity of *Vicia faba* and *Glycine max* in Relation to Rhizobia Strain, Form and Level of Combined Nitrogen

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With 2 Figures

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Summary

ABDEL WAHAB A. M. & ABD-ALLAH M. 1995. Nodulation and nitrogenase activity of *Vicia faba* and *Glycine max* in relation to Rhizobia strain, form and level of combined nitrogen. – Phyton (Horn, Austria) 35 (2): 177–187, 2 figures. – English with German summary.

Differences in nodulation and nitrogen-fixing activity among strains of *Rhizobium leguminosarum* and *Bradyrhizobium japonicum* could be explained by differences in response of the symbiotic system to the form or level of nitrogen supply. We compared the nodulation and nitrogenase activity of two legumes (faba bean and soybean, one cultivar each) inoculated with two strains of *Rhizobium leguminosarum*

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biovar *viceae* RCR 1001 and RCR 1044 or *Bradyrhizobium japonicum* (RCR 3442 and 3407) and supplied with different rates of ammonium chloride or potassium nitrate. Strain RCR 1001 with broad bean Giza 3 and RCR 3442 with the soybean cv. Clark were more efficient and showed higher rates of nodulation and acetylene reduction at 4 and 8 *mM* N in comparison with the less efficient combinations strain RCR 1044 with broad bean and RCR 3407 with soybean.

Zusammenfassung

ABDEL WAHAB A. M. & ABD-ALLAH M. H. 1995. Knöllchenbildung und Nitrogenaseaktivität von *Vicia faba* und *Glycine max*. in Abhängigkeit von *Rhizobium*-Stamm sowie Art und Menge der Stickstoffverbindungen. – Phyton (Horn, Austria) 35 (2): 177–187, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Unterschiede in der Knöllchenbildung und in der Aktivität in der Stickstoffbindung zwischen Stämmen von *Rhizobium leguminosarum* und *Bradyrhizobium japonicum* konnten durch unterschiedliche Reaktionen des Symbiosesystems auf Art oder Menge der Stickstoffgaben erklärt werden. Wir verglichen die Knöllchenbildung und die Nitrogenaseaktivität zweier Leguminosen (jeweils Kulturformen von *Vicia faba* und Sojabohne), welche mit zwei Stämmen von *Rhizobium leguminosarum var. viceae* RCR 1001 und RCR 1044 oder *Bradyrhizobium japonicum* (RCR 3442 und 3407) infiziert und mit verschiedenen Mengen von Ammoniumchlorid und Kaliumnitrat versorgt wurden. Stamm RCR 1001 mit *Vicia* Giza III und RCR 3442 mit der Sojabohne cv. Clark waren am leistungsfähigsten und zeigten stärkere Knöllchenbildung und Acetylenreduktion bei 4 und 8 mM N im Vergleich zu den weniger leistungsfähigen Kombinationen mit dem Stämmen RCR 1044 mit *Vicia* und RCR 3407 mit Sojabohne.

Introduction

Broad bean (*Vicia faba* L.) and soybean (*Glycine max* L. Merr.) are important protein food and feed constituents in many countries. Despite their ability to form N_2-fixing root nodules with Rhizobia, legumes have the potential to grow well upon the application of low levels of nitrogen fertilizer (HANSEN & al. 1989). It has long been recognized that strains of *Rhizobium* or *Bradyrhizobium* vary in the extent to which they can form symbiotic association with legumes and fix nitrogen (RUSCHEL & al. 1979). It follows that, the selection of *Rhizobium* strains for high seed-production via maximization of the N_2-fixation activity in broad bean and soybean is desirable (ABD-ALLA 1994).

Little, however, is known concerning the rhizobial strain effect on the potential of their symbiotic combinations in presence of combined N. NELSON 1987 suggested that the differences between strains for NO_3^- and NH_4^+ tolerance were correlated to the developments and functioning of nodules rather the initiation of nodules.

It is widely accepted that the capacity for N_2 fixation by nodulated legumes is influenced by the mineral nitrogen in soils in at least two ways. Firstly, the process of nodulation may be promoted by relatively low levels of available nitrogen (nitrate or ammonium), higher concentrations of which are almost or always depressive to nodulation (STREETER 1988). Secondly, the rate of N_2 fixation by an actively growing, well-nodulated plant (pre-existing nodules) is always suppressed by higher rates of combined nitrogen (HERDINA & SILSBURY 1989).

The objective of the present study was to select for *Rhizobium*-legume combinations which could nodulate and fix N_2 better following the applications of different levels of KNO_3 or NH_4 Cl. Two commonly used cultivars (*V. faba* cv. Giza 3 and *G. max* cv. Clark) inoculated separately with the two strains of either *R. leguminosarum* bv. *viceae* (RCR 1001 or 1044) or *B. Japonicum* (RCR 3442 or 3407). The parameters used to evaluate the symbiotic efficiency were nodule number and mass as well as acetylene reduction rates.

Materials and Methods

Bacterial Cultures:

Rhizobium leguminosarum bv. *viceae* RCR 1001 and 1044 and *Bradyrhizobium japonicum* RCR 3407 and 3442 obtained previously from Rothamsted Experimental Station, U. K. were used. Bacteria were grown in 250 ml Erlenmeyer flasks containing 40 ml yeast extract mannitol broth (SOMASEGARAN & HOBEN 1985).

Plant material:

Seeds of broad bean cv. Giza 3 and soybean cv. Clark were surface sterilized and germinated in sterile Petri dishes containing damp sterile filter paper. Seeds were maintained wet and incubated at 30° C for 2–3 days. Three seedlings were planted into plastic pots containing 3 kg autoclaved clay soil and immediately inoculated with 10^6 cells ml^{-1} of appropriate rhizobia or bradyrhizobia. Plants were grown in a greenhouse under natural day light. Temperature during the experiments averaged (day/night) 21/6° C for faba bean (winter crop) and 37/18° C for soybean (summer crop).

Combined nitrogen treatments:

Two sources of combined nitrogen, KNO_3 and NH_4 Cl were used at four concentrations. Pots were classified into three groups (a) control pots i. e. seedlings inoculated with specific bacteria but without combined notrogen. (b) plants inoculated with specific rhizobia or bradyrhizobia and subjected to four levels of KNO_3 (c). The same as (b) but supplied with four rates of NH_4 Cl. Nitrogen sources applied after 19 or 27 days of planting *Vicia faba* or *Glycine max*, respectively. The rates of applied nitrogen were 4, 8, 16 und 32 mM (referred to as T_1, T_2, T_3 and T_4, respectively). The controls were symbolized as To. The pots were arranged in completely randomized block design with three replicates and rerandomized every week.

Analyses:

Nitrogenase activity was determined using a closed system on detached root system and the acetylene reduction assay (HARDY & al. 1968) was employed. The closed system assay has been criticized by MINCHIN & al. 1983, 1986. However, this technique is still being employed in several research laboratories (e. g. DEVRIES & al. 1989, WU & HARPER 1990) dealing with large number of analyses, provided that precautions to avoid substantial errors, should be considered. For C_2H_2 reduction assays, undisturbed roots, cut-off at cotyledonary nodes, were placed in 556 ml mannitol bottles and sealed with a rubber septum, immediately injected with C_2H_2 to give 10% final concentration. The bottles were then incubated at 28° C for 1 h and the reaction was terminated using 6 M HCl. A 500 µl gas sample was injected into a Pye Unicam FID 104 gas chromatograph fitted with 4 ft coiled glass column packed with activated alumina at 150° C. The carrier gas was pure nitrogen at 40 ml/min. Two controls, to check indigenous production of ethylene were assayed. Subsequently nodules of individual roots were counted and their total weight per plant was recorded.

Results

Nodulation

Data in Tables 1 and 2 show that nodule number of both faba bean and soybean increased significantly on plants supplied with 4 and 8 mM N (T_1 and T_2). Nodulation at low and moderate levels of NH_4 Cl was slightly greater than with KNO_3 treatments. On the other hand, higher rates of either salts, 16 (T_3) and 32 mM N (T_4), greatly reduced the number of nodules.

Similary, nodule mass (the same Tables) exhibited the same pattern with the application of two nitrogen forms i. e. increased at low N levels and significantly decreased with higher N rates. Data of Table 1 also show that faba bean cv. Giza 3 plants inoculated with *R. leguminosarum* bv.

Table 1

Effect of form and level of combined nitrogen on nodule number and nodule fresh weight of *Vicia faba* cv. Giza 3 inoculated either with *R. leguminosarum* biovar *viceae* RCR 1001 (R_1) or RCR 1044 (R_2)[a].

Treatment (mM N)	Days after treatment: 14		28		42		49	
	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
0	25 (0.57)	18 (0.41)	45 (0.75)	32 (0.56)	53 (0.93)	43 (0.63)	43.7 (0.93)	39.7 (0.56)
KNO_3								
4	32 (0.98)	23 (0.53)	55 (0.86)	38 (0.62)	57 (1.13)	46 (0.88)	46.0 (0.96)	42.0 (0.52)
8	29 (0.67)	17 (0.32)	52 (0.86)	31 (0.60)	52 (0.98)	43 (0.67)	46.0 (0.96)	40.7 (0.31)
16	21 (0.56)	12 (0.30)	31 (0.68)	25 (0.47)	40 (0.80)	34 (0.58)	34.0 (0.77)	29.7 (0.47)
32	17 (0.44)	11 (0.22)	26 (0.51)	22 (0.31)	37 (0.70)	26 (0.39)	30.0 (0.67)	23.0 (0.46)
NH_4 Cl								
4	37 (0.73)	23 (0.63)	58 (0.93)	40 (0.71)	55 (1.21)	46 (0.72)	49.3 (1.09)	46.7 (0.65)
8	32 (0.52)	14 (0.29)	54 (0.63)	36 (0.53)	52 (0.94)	37 (0.65)	46.3 (0.88)	33.0 (0.48)
16	19 (0.49)	11 (0.25)	29 (0.63)	23 (0.34)	38 (0.79)	29 (0.46)	34.3 (0.77)	24.3 (0.43)
32	13 (0.32)	10 (0.26)	25 (0.48)	21 (0.29)	35 (0.69)	26 (0.45)	29.0 (0.67)	24.0 (0.33)
LSD P = 5%	5.1 (0.027)	3 (0.022)	4.4 (0.025)	3.9 (0.057)	3.6 (0.045)	3.2 (0.034)	5.90 (0.06)	2.1 (0.03)

[a] Values in parentheses indicate the fresh weight of nodules (g/plant).

Table 2

Effect of form and level of combined nitrogen on nodule number and nodule fresh weight of *Glycine max* cv. Clark inoculated either with *B. japonicum* RCR 3407 (R_1) or RCR 3442 (R_2)[a].

Treatment (mM N)	Days after treatment 14		21		28		35		42	
	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
0	15 (0.36)	17 (0.38)	24 (0.54)	27 (0.54)	34 (0.64)	37 (0.71)	46 (0.72)	50 (0.75)	40.0 (0.61)	39.7 (0.56)
KNO_3										
4	20 (0.41)	22 (0.44)	28 (0.59)	31 (0.61)	40 (0.83)	43 (0.86)	54 (0.86)	60 (0.92)	45.7 (0.71)	49.0 (0.67)
8	16 (0.40)	19 (0.42)	29 (0.55)	31 (0.58)	37 (0.79)	40 (0.80)	50 (0.87)	51 (0.88)	40.7 (0.72)	44.7 (0.64)
16	10 (0.24)	11 (0.29)	14 (0.30)	17 (0.33)	21 (0.37)	24 (0.42)	29 (0.43)	35 (0.48)	25.0 (0.34)	30.6 (0.30)
32	9 (0.24)	11 (0.26)	11 (0.28)	13 (0.30)	19 (0.35)	20 (0.37)	26 (0.42)	30 (0.42)	22.67 (0.33)	28.0 (0.27)
NH_4 Cl										
4	22 (0.43)	23 (0.47)	29 (0.62)	30 (0.62)	42 (0.84)	42 (0.83)	56 (0.90)	55 (0.92)	48.3 (0.69)	52.0 (0.63)
8	17 (0.38)	20 (0.46)	28 (0.57)	31 (0.60)	39 (0.81)	39 (0.88)	51 (0.86)	50 (0.88)	43.0 (0.71)	41.0 (0.62)
16	9 (0.25)	11 (0.27)	14 (0.29)	18 (0.35)	24 (0.39)	25 (0.44)	32 (0.42)	38 (0.49)	26.7 (0.32)	26.3 (0.27)
32	9 (0.24)	11 (0.25)	13 (0.28)	14 (0.32)	21 (0.37)	22 (0.42)	30 (0.41)	33 (0.44)	23.7 (0.31)	26.0 (0.29)
LSD	3 (0.02)	2.1 (0.03)	2.4 (0.05)	3.2 (0.03)	5.1 (0.02)	3.2 (0.06)	3.7 (0.09)	5 (0.05)	4.1 (0.02)	03.5 (0.03)
P = 5%										

[a] Values in parentheses indicate the fresh weight of nodules (g/plant).

viceae RCR 1001 produced more nodules and greater mass than plants inoculated with strain RCR 1044. Also in soybean cv. Clark, *B. japonicum* strain RCR 3442 induced better nodulation on this cultivar compared to the second strain, RCR 3407 (Table 2).

Nodule activity

Figs 1 and 2 illustrate the C_2H_2-reducing activity of both legumes nodulated by the tested rhizobia strains. Again, the application of 4 and 8 mM N markedly stimulated absolute nitrogenase activity of both rhizobia-host combinations.

Analysis of variance revealed that the two sources of combined N at 4 mM had similar promotive effect on *Vicia faba*. Plants supplied with 8 mM N (T_2) as NH_4Cl recorded insignificant increases in N_2-ase activity than values obtained with KNO_3. This was maintained until the third harvest 28 days after treatment (Fig. 1). Soybean, however responded differently to the combined N treatments. with both forms and the two N levels (T_1 and T_2, Fig. 2). Data of the strain effect revealed that *V. faba* nodulated by *R. leguminosarum* RCR 1001 exhibited higher rates of ARA compared to plants inoculated with *R. leguminosarum* RCR 1044. Also, *G. max* plants nodulated by *B. japonicum* RCR 3442 yielded a higher potential of N_2-ase activitiy than strain RCR 3407.

The application of higher rates (16 and 32 mM) of both N forms significantly depressed ARA. Our results (Figs. 1 und 2) thus indicate that *V. faba*-RCR 1001 and *G. max*-RCR 3442 combinations performed better with low and moderate rates of combined N.

Discussion

Results obtained in the present study suggest a positive effect of low and moderate NO_3^- – and NH_4^+-N supply on nodulation and nitrogen fixation of the two legumes inoculated with the tested rhizobial strains with better performance of strain RCR 1001 – *V. faba* and RCR 1044 – *G. max* combinations. More nodule number with greater mass and higher ARA were obtained with these symbioses than with the other two strains used here. The response of both legumes to NO_3^- or NH_4^+-N supply during nodulation and early growth stages (Tables 1 and 2) conform with the established patterns of the response of nodulating legumes to exogenous nitrogen. Stimulatory effects of low and moderate levels of combined N on nodule development and on nitrogenase activity have frequently been described (Hill-Cottingham & Lioyds-Jones 1980, Streeter 1988).

Our results thus agree with previous investigations that plant response to applications of combined N may vary according to its form and level. Our findings also agree with earlier reports that NH_4^+ is the preferred N-source (Houwaard 1980, Rys & Phung 1984).

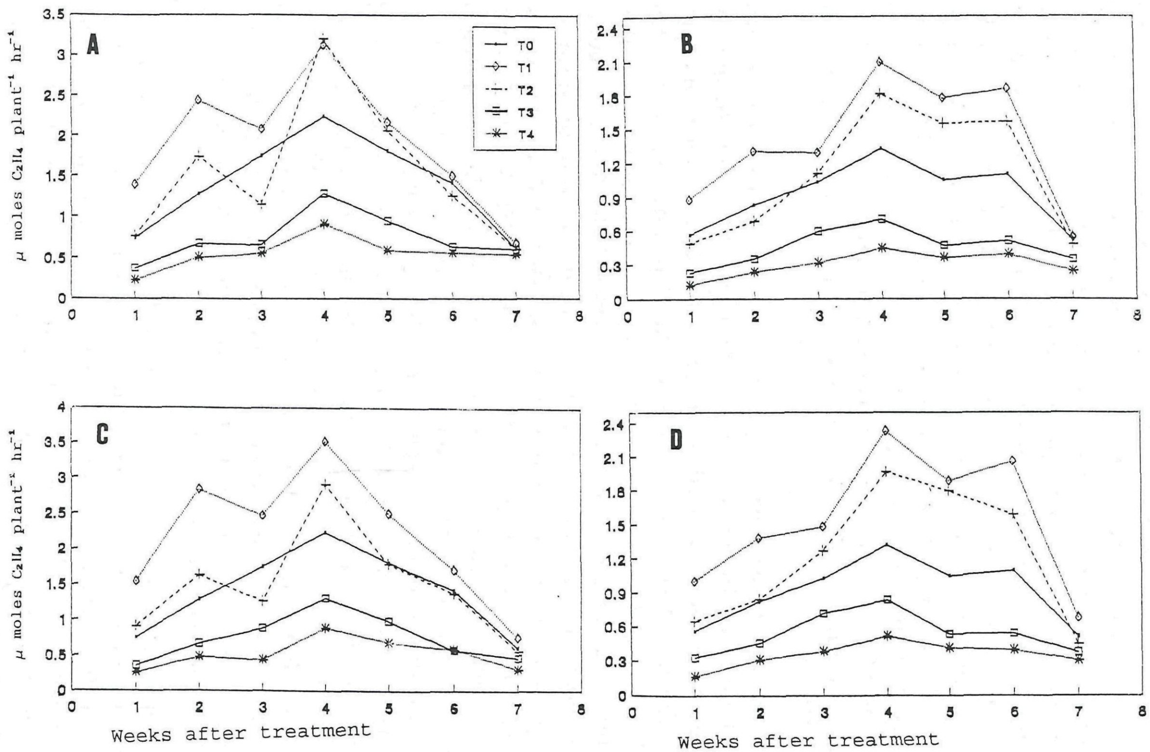

Fig. 1. Effect of KNO_3 (A and B) and NH_4 Cl (C and D) supplied to 19-day old faba bean cv. Giza 3 inoculated with *R. leguminosarum* RCR 1001 (A and C) or RCR 1044 (B and D) on absolute nitrogenase activity. – Treatments: T_0 = 0 mM N, T_1 = 4 mM N, T_2 = 8 mM N, T_3 = 16 mM N, T_4 = 32 mM N.

Strains of rhizobia vary in their ability to nodulate and produce N_2-ases activity when combined N is supplied (MCNEIL 1982, NELSON 1983, NELSON & EEDIE 1988). GIBSON & HARPER 1985 observed limited nodulation of soybean cv. Williams when inoculated with 46 strains of *B. japonicum* followed by NO_3^- treatment. Of these, *B. japonicum* strains CB 1809 and USDA 110 produced the same nodule mass with the same cultivar in the absence of nitrate. But when supplied with the low levels of NO_3^-, strain CB 1809 produced 1.75 times as much nodule mass as the second strain. Similar results were published by STREETER 1986 who found that nodules formed by *R. phaseoli* 127 K 14 had a higher ARA than nodules formed by strain 127 K 12 in the presence or absence of combined N. TAYLOR & al. 1983 reported similar findings by using different *Rhizobium-Phaseolus* combinations. Moreover, NELSON 1987 concluded that two strains of *R. leguminosarum* exhibited optimal N_2-fixation in the presence of inorganic nitrogen fertilizers.

Results obtained here evidently suggest that application of higher rates of combined N (16 and 32 mM) to established *Rhizobium/Bradyrhizobium*-legume symbioses caused reduction of nodule occupancy and mass coupled with severe loss in N_2-ase activity. The depressive effect of higher levels of KNO_3 and HN_4 Cl on nodule function was also reported by other authors (ROUGHLY & al. 1983, DAVIDSON & ROBSON 1986, ABDEL WAHAB & ABD-ALLA 1988). This inhibitory effect is partly attributed to the destruction of bacteroids and their membranes and secondly due to interference with normal nodule physiology (BECANA & SPRENT 1987). ABDEL WAHAB & ABD-ALLAH 1993 reported that high levels of combined nitrogen significantly increased nitrite, ammonia and nitrate as well as nitrate reductase in nodule cytosol and bacteroids. Nitrate reductase is known to compete with nitrogenase for ATP, thus resulting in the decrease of nitrogenase activity. Despite earlier suggestions that the inhibitory effect of N on nodulation is probably plant-mediated (MUNNS 1977), it appears, however, that the sensitivity of N_2-ase activity to combined N was also dependent upon the rhizobial strain and form of N. Strain RCR 1001-faba bean and RCR 3442-soybean combinations were more tolerant to combined N under our experimental conditions.

It can be concluded form results obtained here that the rhizobial-legume combinations could be successfully manipulated by the selection of *Rhizobium/Bradyrhizobium* strains with better performance in the presence of convenient levels of N fertilizers, regardless of the host cultivar. However, many more strains and cultivars need to be tested to substantiate this conclusion. Moreover, the sensitivity or tolerance of rhizobia strains to combined N was dependent on the form and level of nitrogen as well as the partners with which they establish their symbiotic associations.

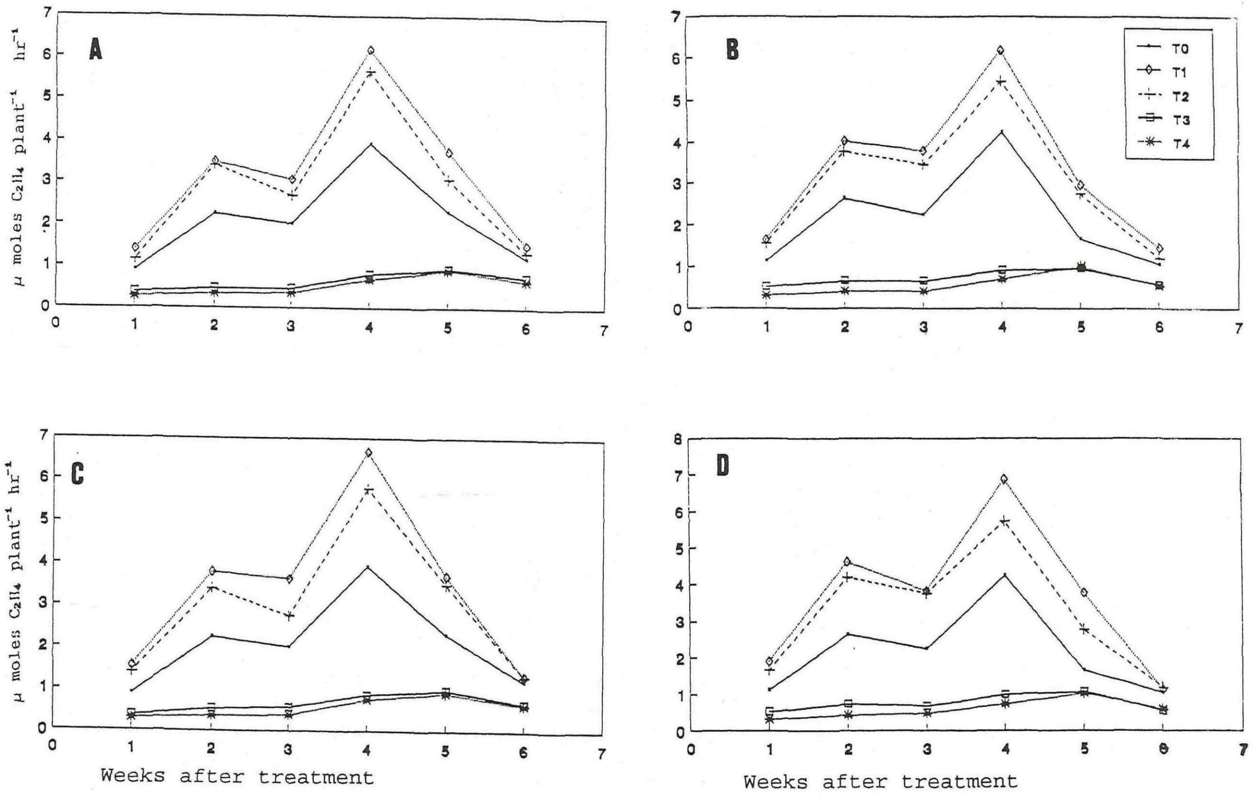

Fig. 2. Effect of KNO_3 (A und B) and NH_4Cl (C and D) supplied to 27-day old soybean cv. Clark inoculated with *B. japonicum* RCR 3407 (A – Treatments: T_0 = 0 mM N, T_1 = 4 mM N, T_2 = 8 mM N, T_3 = 16 mM N, T_4 = 32 mM N.

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