Effect of Nitrogen Compounds on Nitrogen Fixation in Legume-Rhizobium Symbiosis

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One of the most important problems of symbiotic relationships in the "legume plant - Rhizobium" system is the regulation of the functioning of the nitrogenase complex, localized in the cells of microsymbiont-bacteroids. The main pathways for the regulation of nitrogenase activity are determined by the presence of oxygen, carbon substrates and combined nitrogen compounds /HARDY, 1977; SHEEHY et al., 1983/.

The inhibiting effect of ammonium and other easily assimilable nitrogenous compounds on the nitrogenase ability of nitrogen fixers is caused first of all by the repression of ATP synthesis and the process of oxidative phosphorylation /BERGERSEN and TURNER, 1973; TUBB and POSTGATE, 1973/.

In symbiotic systems, unlike free-living diazotrophs, the regulation of nitrogenase activity by means of the presence or absence of combined nitrogen compounds will be exercised by the host-plant, which is assumed to be responsible for the assimilation of fixed nitrogen. The effects of nitrogenous compounds on nitrogen fixation are exerted through their influence on the physiology of the plant, i.e. in processes involving the distribution of photosynthates, the synthesis of leghaemoglobin and the assimilation of ammonia /BISSELING et al., 1978; ROMANOV and TIKHONOVICH, 1987/.

When studying the nitrate influence on the efficiency of symbiosis, the depression of nodule growth was shown to be a local external effect. This finding is based on experiments using the split root technique, with local applications of nitrate /HARPER and COOPER, 1971/.

The adverse effect of added nitrate may be due to nitrite, formed by dissimilatory nitrate reduction. The nitrogenase activity of an already established symbiotic system could also be inhibited by nitrite, if this compound formed a complex with leghaemoglobin, oxidized leghaemoglobin or inactivated nitrogenase /RIGAUD et al., 1973/.

It was interesting to study the extent of the ammonium and nitrate effects on the nitrogenase complex in legume-rhizobial symbiosis from the point of view of the stage of infection and the relationships formed between the symbionts when the nitrogen-containing compounds were applied.

This paper contains the results of a number of comparative microvegetative experiments on the effect of ammonium and nitrate on different stages of nodule formation and on the formation of nitrogenase complexes in the legume-rhizobial symbiosis Rhizobium meliloti - Medicago sativa.

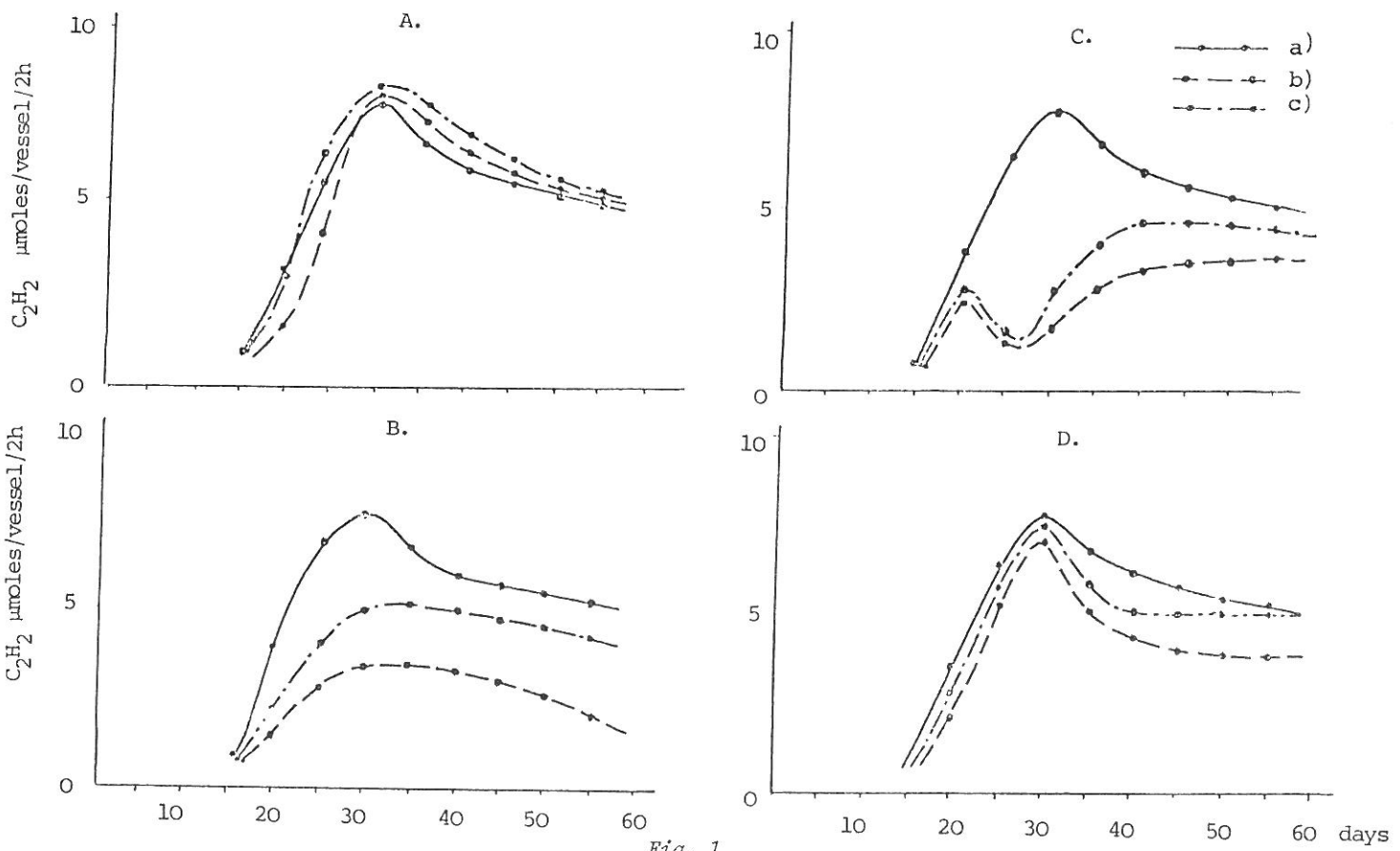

Fig. 1

Influence of ammonium and nitrates on nitrogenase activity of lucerne root nodules. Treatments with ammonium or nitrate /17 ppm N/ were performed in different stages of infection and nodule development: A. before vegetation; B. 10 days, C. 20 days, D. 30 days after sowing of plants. a/ Control; b/ NH_4 treatment; c/ NO_3 treatment

Lucerne plants, inoculated by an active /425a/ strain of R. meliloti, were grown under sterile microvegetative conditions at 20-22 °C in a luminostat regime with 16 hours of light and 8 hours of dark, where the light intensity was 16000 lux. The vegetation period continued for 60 days after inoculation. The lucerne was grown in 500 ml Erlenmeyer flasks /5 plants per flask/, using quartz sand as a substrate, with the addition of nitrogen-free Tornton medium and microelements. Ammonium /$(NH_4)_2SO_4$/ and nitrate /$NaNO_3$/ treatments, with a dose of 17 ppm N, were carried out at different stages of inoculation and nodule growth /at the beginning of the experiment and 10, 20 and 30 days after inoculation/.

In investigations carried out earlier /MISHUSTIN and VERNICHENKO, 1987/ ammonium and nitrate doses ranging from 17 to 136 ppm N were studied; the minimum mineral nitrogen dose applied before the inoculation of the plants, proved to have no influence on the appearance of legume-rhizobial symbiosis and its nitrogen-fixing activity. In this paper studies were made on the effect of this minimum ammonium or nitrate dose /17 ppm N/ when added in the initial stages of nodule formation and at the stage when the nitrogenase system actively fixed nitrogen.

The nitrogenase activity of intact nodules of lucerne was determined by the acetylene reduction method with a flame-ionizing detector on a Chrom-3 gas chromatograph. The gas phase was 10% acetylene in air and the incubating time was 2 hours. The results of the experiments are presented in Figure 1.

The addition of ammonium before lucerne vegetation did not depress the nitrogenase activity of lucerne, in fact, the nitrate stimulated the nitrogen-fixing activity of the nodules. A small dose of nitrogen compounds applied before the inoculation of the plants, did not disrupt the process of inoculation /Fig. 1A/ and by the time the nitrogen-fixing activity began part of the applied mineral nitrogen proved to be assimilated by the plants, thus excluding any possible inhibition of nitrogenase. Nitrogen compounds were found to have a depressing effect when applied 10 or 20 days after the sowing and inoculation of the plants /Figs 1/B and 1/C/ i.e. during the period of bacteroid formation and the initial stages of nitrogen fixation. Both the growth and specific activity of the nodules were inhibited.

Mineral nitrogen had no substantial depressing effect once the symbiosis was completely developed /30 days after the inoculation of the plants /Fig. 1/D/. In this case there was a slight decrease in nitrogenase activity and the inhibition proved to be irreversible.

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