

Dynamics Over Many Years for Nodule Bacteria in Food Legume Grasses with and without the Presence of the Host Plant

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The increase in the number of nodule bacteria in the soil under legume host plants and its decrease under other cultures were registered by many scientists /KRASILNIKOV, 1944; KORENYAKO and KRASILNIKOV, 1948; KALNINSH, 1957, 1958, 1969; KALNISH et al., 1958/. There are also data on the decrease in the potential nitrogen fixing activity of *Rhizobium trifolii* in the presence of clover /EGOROVA, 1955/. However, a more detailed determination of the life period of nodule bacteria in soil where there is no opportunity for symbiosis and the period during which they retain the ability to fix atmospheric nitrogen is of interest for practical purposes. The survival of lucerne and clover nodule bacteria in the absence of *Leguminosae*, the decrease in their potential nitrogen fixing activity, and the change in the rhizobia number after many years of lucerne and clover cultivation in the same field followed by a long interval will be reported in this paper. The population of *Rhizobium meliloti* in leached chernozem, and sod-podzolic soil and the population of *Rhizobium trifolii* in sod-podzolic soil were analyzed.

Methods

The fields from which the soil samples were taken differed only in the time since the last tilling at all geographical sites. By determining the number of bacteria and the potential nitrogen fixing activity, an idea could be formed on the changes taking place in these figures during a period of more than 20 years. To assess the extent to which seasonal dynamics superpose on the dynamics of many years, samples were taken twice a year /October-November and May-June/.

Samples of leached chernozem were collected from fields at the Lukyanenko P. P. Krasnodarsky Institute of Scientific Research on Agriculture.

For the purpose of isolating *R. meliloti* and *R. trifolii*, samples of sod-podzolic soil were collected from experimental fields at the Institute of Agriculture of the Lithuanian SSR and the Viliams All-Union Institute of Scientific Research on Feeds /in the Moscow region/.

The determination of the number of nodule bacteria was carried out by the method of VILSON, modified by KRASILNIKOV and KORENYAKO /1940/. The method consists of the infection of indicator legume plants with different di-

lutions of soil suspension and the subsequent recording of the nodules formed. The nitrogen fixing activity of rhizobia in symbiosis with indicator plants was determined by the acetylene reduction method /SHEMAKHANOVA et al., 1976/ three weeks after inoculation. The values of acetylene reduction in different variants, corresponding to various dilutions of a suspension from one sample where nodulation took place, were averaged.

Results and discussion

Under sowings of legume grasses the number of rhizobia reached quite high values. The *R. meliloti* content in leached chernozem under 3-4-year-old lucerne was especially high - 6.00×10^7 - 1.53×10^8 cells/g /Fig. 1A/.

The process whereby this number decreases when lucerne is succeeded in crop rotation by other cultures includes at least two stages. The first is characterized by considerable deviations in the data on the content of *R. meliloti* cells in one g of soil: between 3.20×10^4 and 1.28×10^6 cells/g in samples taken in autumn, and between 1.47×10^3 and 4.50×10^5 cells/g in summer. Six years after the last lucerne cultivation, the differences between the summer and autumn number increased even more. If, in samples taken in autumn, the *R. meliloti* content stabilized at a level of 1×10^3 - 10^4 cells/g, then in samples taken in summer a sharp decrease took place in the nodule bacterial titre /Fig. 1A/. This fact contributes to a more precise determination of the period for which the values of *R. meliloti* in the soil remain high in comparison with the background level. This period is connected with the after-effects of legume cultures on the population of its microsymbionts.

The decrease in the potential nitrogen fixing activity reflects a qualitative reduction in the nodule bacterial population and a diminution in the proportion of active strains in the population. Comparing data obtained for chernozem samples in autumn and summer, it can be said that for *R. meliloti* high values of acetylene reducing activity - 153.7-214.0 ethylene nm/plant/day - were retained in the absence of lucerne during 2-3 years, after which they decreased to 35.1-51.6 ethylene nm/plant/day /Fig. 1A/.

In sod-podzolic soil under 2-year-old lucerne the *R. meliloti* content was lower than in chernozem /under 3-4-year-old lucerne/, as a consequence of which its subsequent decrease was not so sharp.

In general, it can be said that the process by which the number of *R. meliloti* changes over many years in the absence of the host plant in sod-podzolic soil goes through approximately the same stages as in chernozem, but at each stage the rhizobial number is about an order of magnitude less than in chernozem /Fig. 1B/. Here, the stabilization period for higher values of around 1×10^4 - 10^5 cells/g/ can be put at 6-10 years. After 22-23 years, the lucerne nodule bacterial content decreased to 1.10×10^2 cells/g in autumn samples and to 4.5×10^2 cells/g in summer samples.

The nitrogen fixing activity of rhizobia in sod-podzolic soil decreased by the second year after the replacement of lucerne by other cultures from 590.1 to 109.7 ethylene nm/plant/day /Fig. 1B/.

The number of *R. trifolii* found under lucerne in a sod-podzolic soil in the Moscow region was less than the number of *R. meliloti* under lucerne in a sod-podzolic soil in Lithuania, being 6.00×10^5 cells/g.

The first year after the replacement of clover by cereal grasses the content of its microsymbionts decreased by 3.5 orders of magnitude and became restabilized at the 2.31 - 3.20×10^2 cells/g level /Fig. 1C/. In this case no increase was observed in the nodule bacterial number.

The potential nitrogen fixing activity of *R. trifolii* decreased in the first year after clover from 741.6 to 130.1 ethylene nm/plant/day.

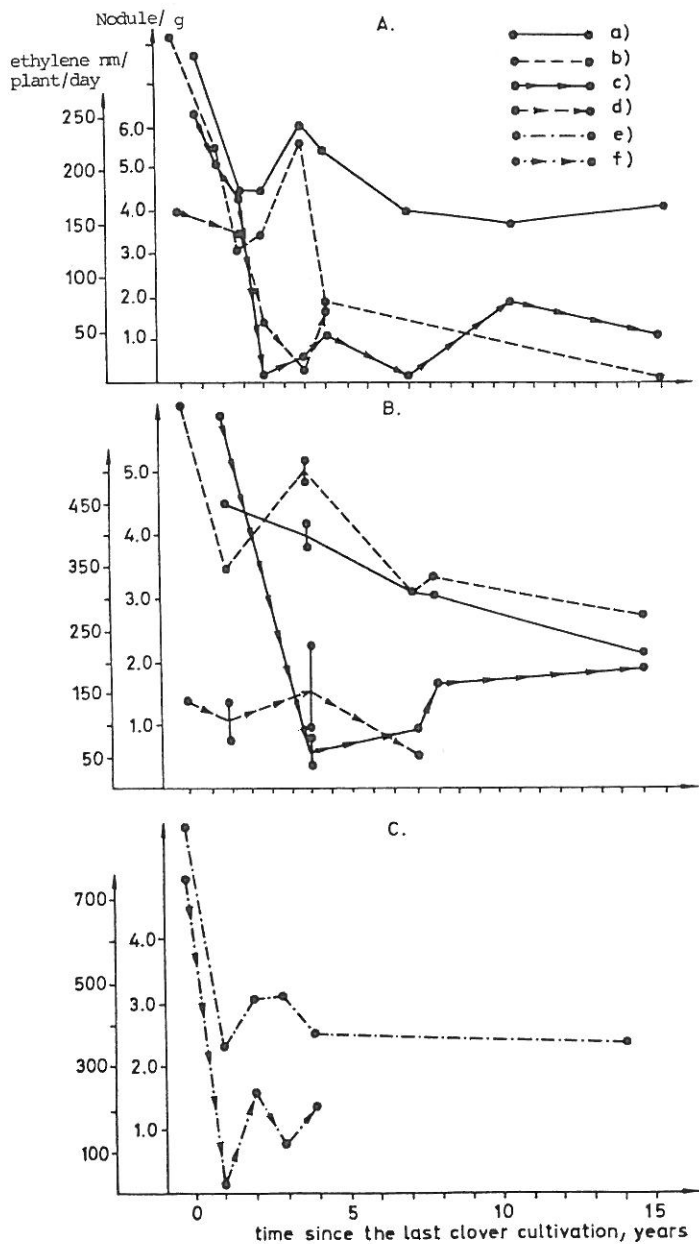


Fig. 1
 The dynamics over many years for *Rhizotium meliloti* in leached chernozems /A/ and sod-podzolic soil /B/ and for *Rhizobium trifolii* in sod-podzolic soil /C/.
 a/ Autumn number; b/ summer number; c/ autumn acetylene reduction; d/ summer acetylene reduction; e/ number; f/ acetylene reduction

Studies were also made on the increase in the nodule bacterial number in the soil after long cultivation of the host plant. The data obtained are presented in Fig. 2. The most substantial increase in *R. trifolii* occurs during the first year of clover /from 2.31×10^2 to 1.00×10^4 cells/g/, while *R. meliloti* number increases both in chernozem and sod-podzolic soil during the first and second years of lucerne production. A sharp increase in lucerne nodule bacteria from 3.20×10^5 to 1.58×10^8 cells/g was noted in the fourth year.

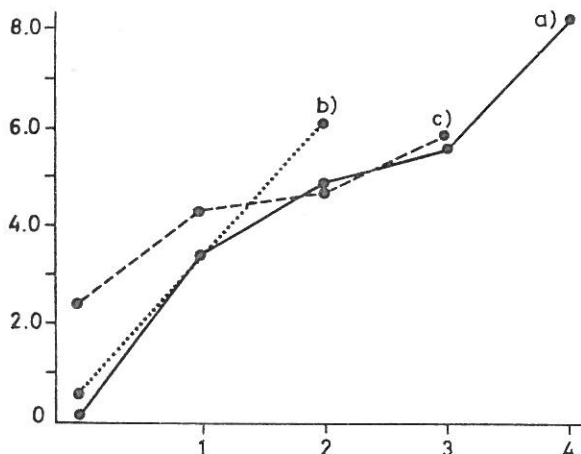


Fig. 2

Increase in nodule bacteria number after long cultivation of the host plant. Horizontal axis: Years of cultivation of host plant. Vertical axis: Nodule/g. a/ Number of *R. meliloti* in chernozem; b/ number of *R. meliloti* in sod-podzolic soil; c/ number of *R. trifolii* in sod-podzolic soil

Conclusions

The number of nodule bacteria reaches high values under legume plants; it decreases under other cultures and increases again if Leguminosae are included in the crop rotation.

The content of *Rhizobium meliloti* cells in the soil is determined by the time since the last lucerne cultivation. The dynamics over many years are superposed by seasonal dynamics. For *Rhizobium trifolii* such regularities were not noted.

In the absence of the host plant the population is reduced not only in quantity but also in quality, with the decrease in the share of active strains in its composition. This process is more rapid for *R. trifolii* than for *R. meliloti*. A rapid decrease in nitrogen fixing activity is observed due to the nitrogenization of legume grass stands even when the number of nodule bacteria in the soil is high.

The decrease in the number of nodule bacteria and in their potential nitrogen fixing activity has different characteristics in the two soils studied /in the leached chernozem and sod-podzolic soil/.

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