Dynamics of symbiotic nitrogen fixation by meadow legumes at various development phases in the conditions of the Novgorod region of the North-West of Russia

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Abstract. The data on the study of the influence of the developmental phase of meadow legumes on their symbiotic nitrogen fixation are presented. It has been experimentally proved that the mowing time significantly affects the formation of the symbiotic apparatus and the accumulation of biological nitrogen. Due to symbiotic fixation on medium-acid soils of heavy texture in the first two years of life, Carmine meadow clover cultivated from the beginning of budding phase to the beginning of flowering, about 80–90 kg of nitrogen per 1 ha was accumulated, which corresponds to 57–67% of the total removal with the harvest. Such a symbiotic apparatus provided high protein feed from the main cut for hay without additional mineral nitrogen fertilizers in the range of 4.4–5.4 tons of dry weight or 19.3–22.2.2 tons of green mass per hectare. Later mowing of the grass stand led to a significant reduction in the use of biological nitrogen. The removal of biological nitrogen decreased by 1.8 times and amounted to only 45 kg per 1 ha.

1. Introduction
In crop science, the use of biological nitrogen sources is of great practical importance. Nitrogen-fixing microorganisms are in symbiosis with plants of the “Legume” family. Nitrogen-fixing microorganisms can absorb from 2 to 5 kg of air nitrogen. In favorable growing conditions, legumes can fix up to 120 kg of nitrogen per 1 ha. Most of the air nitrogen acquired by legumes is removed from the field with the harvest, the other part remains in the root and crop residues, enriching the soil with nitrogen.

In the process of growth and development, legumes under favorable symbiotic conditions satisfy their needs for nitrogen and transfer part of the fixed nitrogen to another culture with which they are cultivated together (4). The introduction of legumes into the composition of meadow grass stands allows for increasing their productivity, increasing the production of vegetable protein both due to the harvest of these crops, and due to their aftereffect. Therefore, the expansion of meadow legume-grass grass stands is a significant solution in the resource conservation of forage production, increasing the productivity of the forage hectare and maintaining soil fertility.

The symbiotic activity of legumes and the productivity of meadow grass stands are greatly influenced by the phase of plant vegetation, during which they are harvested.

The aim of the research was to study the influence of the development phase of meadow legume plants on their symbiotic nitrogen fixation in the conditions of the Novgorod region.

The research objectives included:
1) analysis of the formation of nodules on the roots of meadow legumes plants in various phases of development;
2) determination of the productivity of legume-grass grass stands depending on the mowing period;
3) the study of the dynamics of the accumulation of biological nitrogen by legumes in the phases of development.

2. Methods and materials
The experimental part of the study of the influence of the development phase of meadow legume plants on their symbiotic nitrogen fixation in the Novgorod region was carried out on legume-grass meadow grass stands of the 2nd and 3rd years of life during 2017–2018.

In legume-grass agrophytocenosises, the following meadow clover varieties were included: Sedum and Carmine, hybrid clover of Lujanin variety.

The control and background in the experiment was a mixture of meadow fescue grass of Suydinskaya variety, timothy grass of Leningradskaya-204 variety and awnless brome of Dragon variety.

The experiment was carried out on a medium acid derno-podzolic soil, of heavy mechanical composition, highly provided with mobile forms of phosphorus and potassium (table 1). That is, the soil of the experimental field in all respects belonged to the category of increasedly cultivated.

| Humus, % | pH of the salt extract | Mg per 100 g of soil |
|---------|------------------------|---------------------|
| 2.6     | 5.6                    | 23.7                |

Observations, yield accounting were carried out according to the methods of the All-Russian Feed Research Institute named after V.R. Williams (1971, 1987). Before laying the experiments, soil analysis was carried out at the station of the “Novgorodskaya” agrochemical service. The determination of saline PH was carried out using the electrometric method; mobile phosphorus according to Kirsanov; exchange potassium on a flame photometer.

Accounting for productivity in the experiments was carried out by the cutting method in three phases of clover vegetation: the beginning of budding, full budding - initial blossom and full bloom. The data on the productivity of meadow grass stands were processed according to B.A. Armor (2011). The analysis of nodules on the roots of legumes and the accumulation of biological nitrogen were determined by the method of P. Vavilov. and G.S. Posypanova (1983).

3. Results and discussion
The accumulation of biological nitrogen directly depends on the activity of symbiotic bacteria. The more nodules are formed on the roots of legumes, the more actively they work, the higher the efficiency of symbiotic nitrogen fixation.

The symbiotic apparatus is greatly affected by the phase of plant development. When studying the dynamics of the formation of nodules on the roots of clovers in various phases, it was found that as the phenological phases of development pass, the number of nodules increases (table 2).

In the budding phase on one clover plant, depending on the type and variety of clover, 28 to 76 nodules were formed. Upon transition to the onset of flowering, the number of nodules increased by 21-70%, compared to the previous phase. A particularly sharp increase in this indicator was observed in hybrid clover. By the beginning of flowering phase, the number of nodules increased by 1.7 times, compared with the previous phase.

By the full bloom phase, even more nodules have formed on one clover plant – by 1.5-1.8 times more, compared with the beginning of initial blossom phase. The largest number of nodules in this phase of development was formed by Carmine meadow clover. More than 160 nodules were formed on one plant.
Table 2. Formation of the symbiotic apparatus and the accumulation of biological nitrogen, depending on the phase of vegetation, average 2017-2018.

| Grass stands | Vegetative phase | The number of nodules per 1 plant, pcs. | The number of nodules per 1 g of root, pcs. | Removal of symbiotic nitrogen with the harvest, kg from 1 ha |
|--------------|------------------|----------------------------------------|---------------------------------------------|-------------------------------------------------------------|
| Background + meadow clover | Beginning of budding | 57.4 | 95.7 | 51.5 |
| Sedum | Full budding – initial blossom | 69.5 | 49.6 | 41.9 |
| | Full bloom | 126.8 | 63.4 | 15.7 |
| Background + meadow clover | Beginning of budding | 76.6 | 109.4 | 89.2 |
| Carmine | Full budding – initial blossom | 102.7 | 68.5 | 76.9 |
| | Full bloom | 166.4 | 83.2 | 45.4 |
| Background + hybrid clover | Beginning of budding | 28.1 | 40.1 | 53.9 |
| Full budding – initial blossom | 47.8 | 31.9 | 34.4 |
| | Full bloom | 72.7 | 34.6 | 14.7 |

With the growth of clovers, the power of the root system and its weight also increased. When recalculating the number of nodules per 1 g of root, it was found that a more powerful symbiotic apparatus in all types and varieties of clovers was formed in the phase of the beginning of budding. For 1 g of root, from 40 to 109 nodules were formed. In this phase, the accumulation of biological nitrogen proceeded more intensively. Such a symbiotic apparatus made it possible to obtain from 1 ha of clover-legume grass stands from 3.0 to 4.4 tons of dry weight or from 15.1 to 19.3 tons of green mass, with a share of the legume component from 37.5 to 55.8% of total mass of the harvest. The protein content in the forage at the beginning of budding phase varied from 161 to 180 kg of crude protein per 1 ton of dry weight, depending on the type and variety of clover. The priority in productivity was legume-grass grass stands with meadow clover of Carmine variety. Due to symbiotic fixation, it accumulated about 90 kg of biological nitrogen per 1 ha, which corresponds to 63% of the total yield with the harvest (figure 1). For meadow clover of Sedum variety and for hybrid clover, the yield of symbiotic nitrogen was only slightly more than half of the total yield with the harvest, that is 51.5 – 53.9 kg per 1 ha.

By the beginning of the blossom phase, there was a decrease in the operability of the symbiotic apparatus by 1.3 – 1.9 times. At the same time, the accumulation of symbiotic nitrogen decreased by 14 – 36% or by 9.6 – 19.5 kg of nitrogen per 1 ha. The protein content in the forage decreased by 6 – 10%, compared to the beginning of budding phase.

By the full bloom phase of clovers, the apparatus operation again increased by 8 – 28%, compared to the previous phenological phase. However, an increase in the number of nodules per 1 g of root did not affect the accumulation of biological nitrogen. In the phase of full bloom, the yield of symbiotic nitrogen in all species and varieties of clover was minimal and varied from 14.7 to 45.4 kg per 1 ha. In this case, the protein content of the forage decreased by 17 – 21%, compared with the previous phase, and varied from 121 to 140 g per 1 kg of dry weight.
Figure 1. Dynamics of the symbiotic nitrogen fixation by legume-grass grass stands in the phases of development of clover.

As in the previous phases of development, the meadow clover of Carmine variety stood out here. In the full bloom phase of this clover, more than 90 nodules per root were formed and about 50 kg from 1 ha of biological nitrogen was accumulated.

4. Conclusion
Thus, it has been experimentally proven that the functioning of the symbiotic apparatus of meadow legumes and the accumulation of biological nitrogen are greatly influenced by plant species and varieties, their growing conditions, and also the phenological phases of development of legumes. As the phenological phases of the development of clovers pass, the accumulation of biological nitrogen decreases.

The greatest accumulation of biological nitrogen on medium-acid soils of heavy mechanical composition in the first two years of life was noted during the period from the beginning of budding to the beginning of flowering on legume-grass stands with the participation of meadow clover Carmine. On one plant and 1 g of the root of this clover, an average of about 90 nodules were formed. The symbiotic nitrogen yield with the harvest amounted to 80–90 kg per 1 ha, which corresponds to 57–67% of the total yield from the harvest. Such a symbiotic apparatus provided high protein feed from the main cut for hay without additional mineral nitrogen fertilizers in the range of 4.4–5.4 tons of dry weight or 19.3–22.2 tons of green mass per hectare. Later mowing of the grass stand led to a significant reduction in the use of biological nitrogen. The removal of biological nitrogen decreased by 1.8 times and amounted to only 45 kg per 1 ha.

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