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EFFECTIVENESS OF SYMBIOSIS BETWEEN FODDER GALEGA (*Galega orientalis* LAM.) AND *Rhizobium galegae* ON FALLOW LAND

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ABSTRACT

A three-year field experiment investigated the effectiveness of symbiosis between extremely extensively used fodder galega (*Galega orientalis* Lam.) plants and *Rhizobium galegae*. A bacterial culture was obtained from root nodule bacteria and applied onto seeds prior to sowing. In summer each year, over the maximal biomass development as well as in autumn the weight of the underground and over-the-ground plant parts was measured, including the content and accumulation of basic elements. The effectiveness of symbiosis between inoculated and non-inoculated plants was visible with a more rapid development, twice higher biomass increase over the

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sowing year, eleven-fold higher over the second research year and fifteen-fold – over the third year. Inoculated plants contained on average by 1% of nitrogen more than non-inoculated, and over the third research year, over the greatest biomass development, accumulated from a few to a few dozen-fold more phosphorus, potassium, magnesium, calcium and nitrogen. The differences in the amount of nitrogen accumulated by inoculated and non-inoculated plants showed that over 90% of nitrogen was fixed.

Key words: *Galega orientalis*, *Rhizobium galegae*, inoculation, symbiosis effectiveness, biomass, nutrients, fallow land

INTRODUCTION

Biological nitrogen fixation is second to photosynthesis most important biochemical process on earth and amounts to an annual of about 140 m tonnes, out of which 80% is fixed by symbiotic bacteria, while the other 20% by free-living microorganisms. The most active organisms fixing N_2 include *Rhizobium* bacteria which live in symbiosis with papilionaceous plants. The greatest effectiveness of fixing N_2 is recorded for papilonaceous perennials; e.g. alfalfa fixes over the vegetation period from 100 to 350 kg ha^{-1} of N [18]. The plant group is represented by fodder galega (*Galega orientalis* Lam.) which, especially in Estonia, has been applied as a fodder crop, also due to its early spring re-growth, lush growth and persistence [6, 14, 16, 19]. A relatively short period of fodder galega breeding has not been long enough to eliminate some characteristics which impede its agricultural usage, e.g. lodging, rapid lignification, seed hardiness, slow initial growth of over-the-ground plant parts [4, 5]. Recently it has been suggested to use this species to manage soils temporarily excluded from agricultural use. Such application of fodder galega is favourable thanks to considerably low habitat requirements, lush plant development, especially over 2–3 use year, high weight of underground parts and no plant spreading to the environment as a weed. The characteristics allow fodder galega for an effective competition with the natural flora (keeping the field free from weed infestation) and enhance soil fertility [4, 5]. Similarly results of research into the application of galega to devastated land management remain promising [1, 17].

Fodder galega lives in an effective symbiosis with nitrogen-fixing bacteria *Rhizobium galegae* [8]. The bacteria are not present in soils on which galega has not been cultivated and therefore plant inoculation is necessary. The early research was reported by Hauke-Pacewiczowa [3] who applied bacteria isolated not only from roots of that plant but also from vetch and broad bean roots to inoculate galega. Further more detailed reports did not confirm, however, any relation between *Rhizobium* isolated from galega and other species of nitrogen fixing bacteria, which was observed only in respect to bacteria living in symbiosis with clover [2, 12]. The research, which also drew on hybridisation of DNA, nodulation tests and phage typing, made it possible to show a unique character of *Rhizobium* nodulating with *Galega orientalis* Lam. and *Galega officinalis* L. not related to other familiar strains of *Rhizobium* and *Bradyrhizobium* [9, 20]. Strains of these bacteria are so specific that being active towards *Galega officinalis*, they form ineffective nodules on *Galega orientalis* and vice versa. Strains of bacteria proper for both species of the *Galega* genus differ also in their maximum growth temperature [10]. The research into the symbiosis of fodder galega carried out in Finland by Lindström et al. [11] showed also that *Rhizobium galegae* bacteria are tolerant to cultivable soil acidity. Harsh winters can lower their population considerably. According to Liu et al. [13] *Rhizobium galegae*, just like other strains of *Rhizobiaceae* family can cause a decomposition of glyphosate and can use it as the only source of phosphorus.

The symbiosis effectiveness can be estimated with indirect or direct methods. The activity of that process is estimated with acetylene to ethylene reduction measurements, which reflects a reduction of N_2 to NH_3 by nitrogenase. The method applied in field conditions is quite difficult to carry out and requires a conversion of repeated measurements into an annual effect [7, 18]. Another method, the isotope method, requires highly specialised equipment, which makes it expensive, however, as reported by Vance et al. [18] citing other authors, it is more accurate. To evaluate the symbiosis effectiveness one can also make use of non-nodulating forms of the same plant species or form on which ineffective nodules are formed [15]. The symbiosis effectiveness can also be evaluated quite easily using soil in which nitrogen-fixing bacterial populations corresponding to the plant species researched do not occur [18]. According to Liu et al. [13] *Rhizobium galegae*, just like other strains of *Rhizobiaceae* family can cause a decomposition of glyphosate and can use it as the only source of phosphorus.

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The most essential aim of research into the effectiveness of symbiosis is usually evaluating an increase in plant dry matter yield and in their content and accumulation of nitrogen due to inoculation with adequate bacterial strains. Similarly, an effect of this process on the content and accumulation of other elements in plants can be also interesting. So far the research into symbiosis of fodder galega with nitrogen fixing bacteria were carried out in labs or short-term field experiments and concerned, first of all, the year of sowing. Lindström [7] only carried out the investigations over two years, showing amongst others that a seasonal activity of fodder galega nitrogenase reminds that of alfalfa rather than of red clover.

The aim of the present research was to evaluate the effectiveness of symbiosis between fodder galega (*Galega orientalis* Lam.) and *Rhizobium galegae* in field conditions over the year of sowing and over two years of extensive use, expressed as a weight and dynamics of over-the-ground and underground plant parts and accumulation of essential macroelements in plants due to inoculation.

The working hypothesis assumed that inoculation of fodder galega for extremely extensive use as fallow land preserving virgin soil will be very effective for plants, which will enhance accumulation of biomass rich in nutrients. Additionally, inoculation should also facilitate estimating the amount of nitrogen fixed in symbiosis.

MATERIAL AND METHODS

The experiment was carried out over 1997 – 1999 at the Minikowo Agricultural Experiment Station in the Kujawy and Pomorze Province. Set up as a single-factor experiment, it included two objects: non-inoculated (NIN) and inoculated (IN) in three reps. Thirty-six 20 l bottomless plastic bags were dug down into the soil to make water infiltration possible and to provide plants with conditions close to natural as well as to facilitate root weight measurements. The soil, whose pH was neutral (7.2 in H2O and 6.8 in 1 M KCl), showed a very high content of available phosphorus forms (24.3 mg of P_2O_5 in 100 g), medium of potassium (13.5 mg K_2O) and high of magnesium (8.3 mg of Mg in 100 g). No N-mineral fertilisation was applied.

MOR 198 population seeds, obtained locally were sown by hand, 20 each time, on April, 28 1997 into pots, 2 cm deep. Seeds in 18 pots were inoculated with *Rhizobium galegae* culture obtained from fodder galega root nodules. The number of *Rhizobium galegae* bacteria amounted to about $80 \cdot 10^3$ per one seed. After full emergence (4 weeks off the planting) 10 plants per pot were left.

Marking and measurements were taken twice over each vegetation season:

- in summer – on August 6 of the sowing year at full vegetative plant development, June 18 in 1998 and June 4 in 1999 at full flowering;

- in autumn – following the end of vegetation period: November 5 in 1997, October 24 in 1998 and October 28 in 1999.

Before detailed observations no over-the-ground plant parts were mown to simulate extremely extensive plant use farming – fallow land. Giving up the harvest of over-the-ground plant parts was also important in eliminating its temporary effects on symbiosis. Three pots were dug out from each of the two objects. The length of cut stems and dry matter weight of over-the-ground parts (stems, leaves, inflorescences or infructescences) were measured. The 35 cm underground plant parts (pot depth) were rinsed in sieve, nodulation was being observed on roots and underground dry matter weight (roots, runners and root nodules). The contents of elements were determined separately in over-the-ground and underground plant parts: total N with Parnas-Wagner distiller, phosphorus with vanadium-molibdenium method, potassium and calcium with flame photometry and magnesium with colorimetric method. The chemical analysis results were variance-analysed and the differences were verified with t-Student test at p=95%.

The weather conditions of the sowing year over most of the vegetation period were favourable for seed germination, plant emergence and further plant development. Late in the summer a rainfall shortage was recorded, however it had no considerable effect on well-rotted and advanced fodder galega plants. Over successive years both in vegetation periods and in winters there were noted no weather phenomena which could affect plant development essentially.

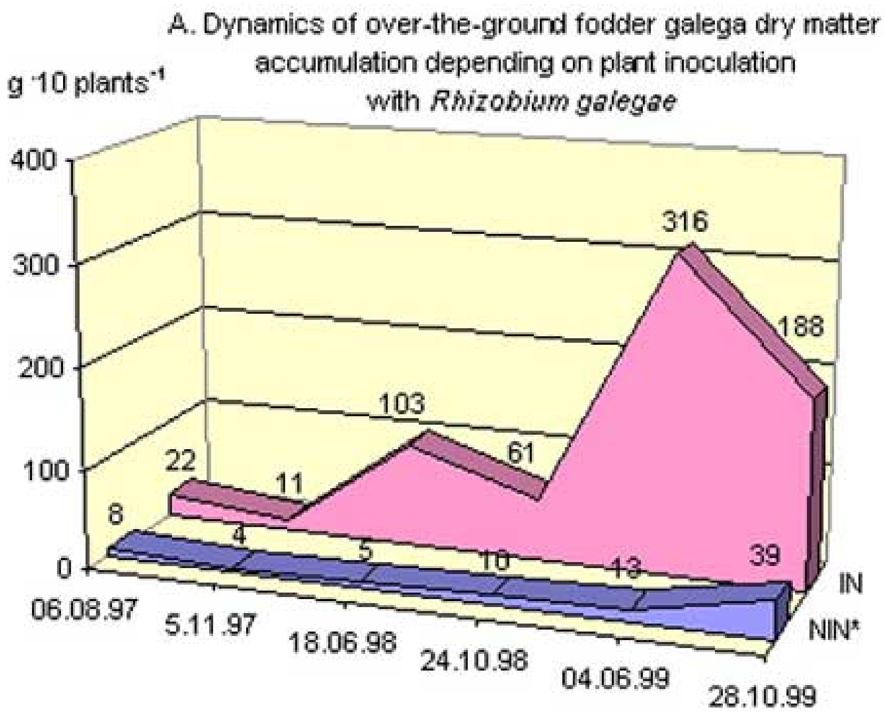
RESULTS AND DISCUSSION

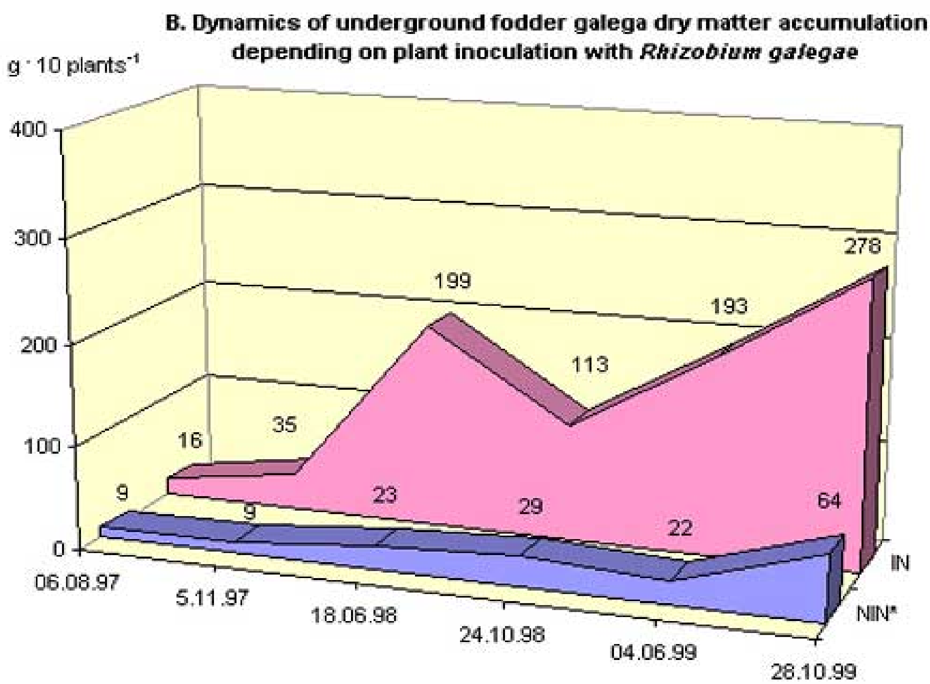
Growth and development of fodder galega in the year of planting are slow, especially of over-the-ground parts. Similarly, over the first several months nitrogenase activity is low, which shows a relatively slow symbiosis of galega with Rhizobium galegae [7]. Frydova et al. [2], studying Galega officinalis, a relative species of fodder galega, did not find any differences in inoculated and non-inoculated dry matter weight of plants even after 188 days. The present research revealed nodulation effect as soon as 6 weeks after planting, which was shown by visible galega plant development differences. Inoculated plants had 2-3 fully-developed leaves, while non-inoculated 1-2 leaves, only. At the beginning of August runners length of inoculated plants amounted to 3-6 cm and non-inoculated – 1-3 cm. Similarly 35-cm long roots developed, while dry matter yield of underground parts of inoculated plants exceeded twice the yield of non-inoculated ones (Fig. 1).

Non-inoculated fodder galega plants showed N want symptoms, i.e. were short with pale green stems and leaves, non-flowering and non-fruiting. In 1999 roots of these plants were observed to have a few nodules (in June – 4-5 per plant roots, and in October – 25-30), which means that non-inoculated plants were inoculated with symbiotic bacteria transferred from a neighbouring galega plantation. Finally after 2 years of study also non-inoculated galega plants showed effective symbiosis, which was confirmed by 1999 autumn observations. As compared with the corresponding period of the previous year, plants were 27 cm higher, over-the-ground dry matter weight more than doubled and the underground dry matter weight – tripled (Fig. 1).

Over the research period there was no effect of inoculation on galega plant winterhardiness and health status. Plant overwintering was good on both objects and no disease symptoms were noted.

Fig. 1. Fodder galega dry matter and stem length dynamics





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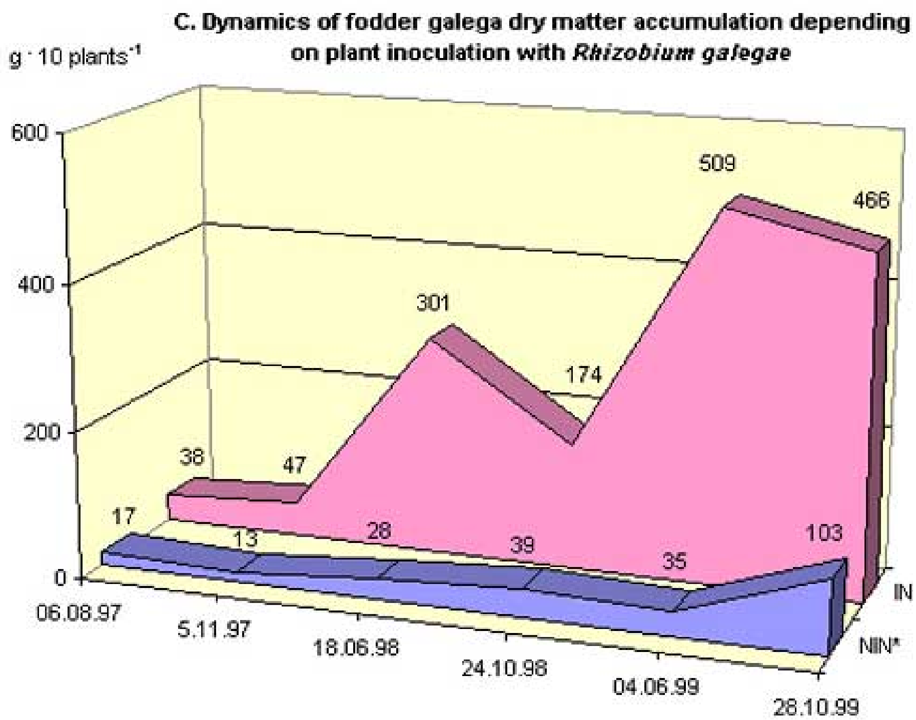

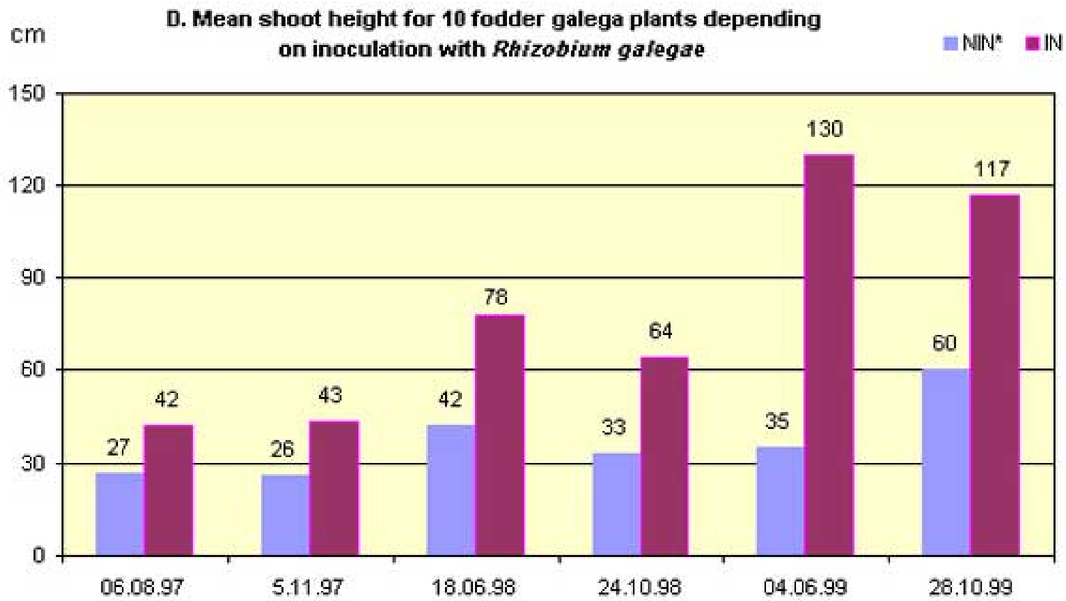

*** NIN non-inoculated; IN - inoculated**

Due to inoculation, summer plant biomass was more than doubled in the sowing year, 11-fold higher in the second research year and was almost 15-fold higher in the third year, as compared with the non-inoculated plants. In autumn the increase was almost three times higher in the sowing year and more than four times in the two successive years. In summer the over-the-ground and total plant biomass were higher than in autumn even though plants were not mown (Fig. 1C), which was due to a relatively quick loss and desiccation of leaves, both typical for galega, whose share in the over-the-ground yield amounts to 50-70% [4, 14, 16]. Desiccation of plants made them incapable of symbiosis. Lindström [7] comparing seasonal nitrogenase activity in Finland showed that N_2 fixation by fodder galega ended as

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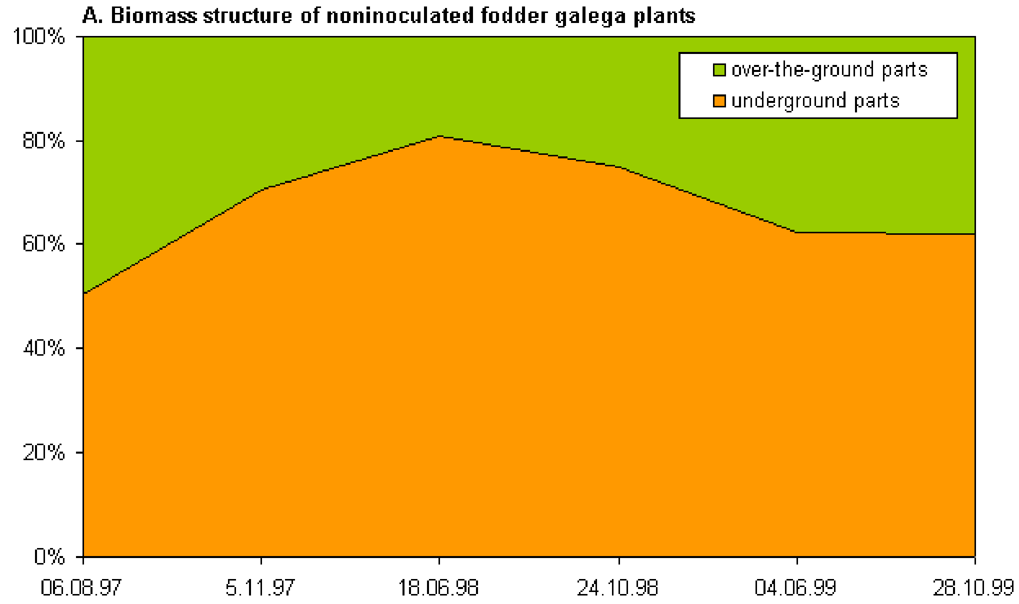
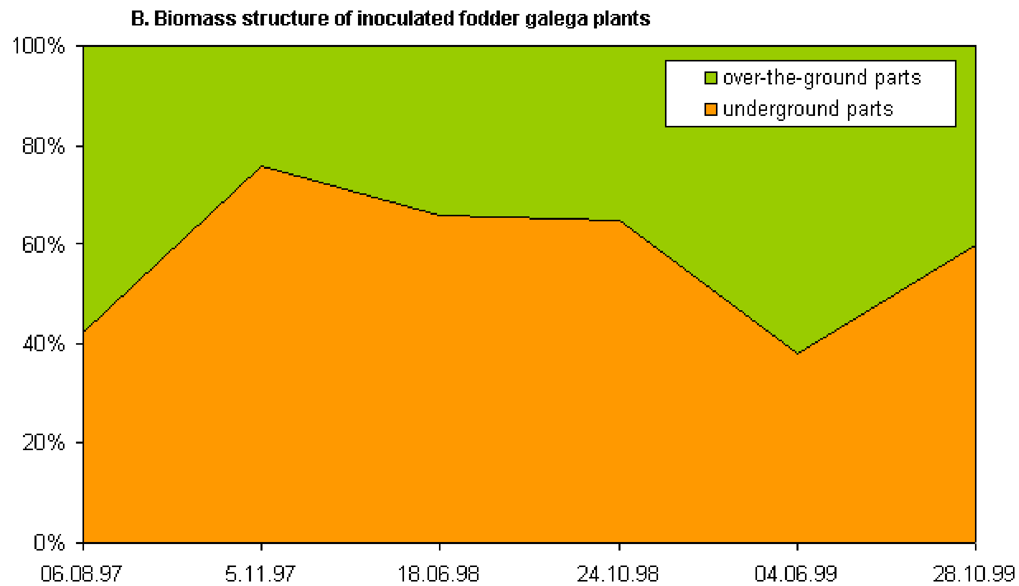
early as in September, while red clover and alfalfa nitrogenase activity continued to the beginning of November. In the present research galega dry matter underground weight was higher in autumn than in summer, unlike the over-the-ground weight, except for 1998.

In the second research year the underground weight doubled the over-the-ground weight on both objects. In the third year a further intensive increase was observed both in the over-the-ground (over summer) and the underground (over autumn) biomass. These data, especially for inoculated plants, confirm the galega plant development reported in literature [6, 16, 19].

Fig. 2. Fodder galega biomass structure





The share of underground parts in the biomass of inoculated plants ranged from 40 to 75%, while that of non-inoculated plants was higher and ranged from 50% to 80% (Fig. 2A and B). The differences in underground plant biomass between the objects were slightly distorted by lush underground biomass of inoculated plants, especially in the third year, due to a limited pot volume.

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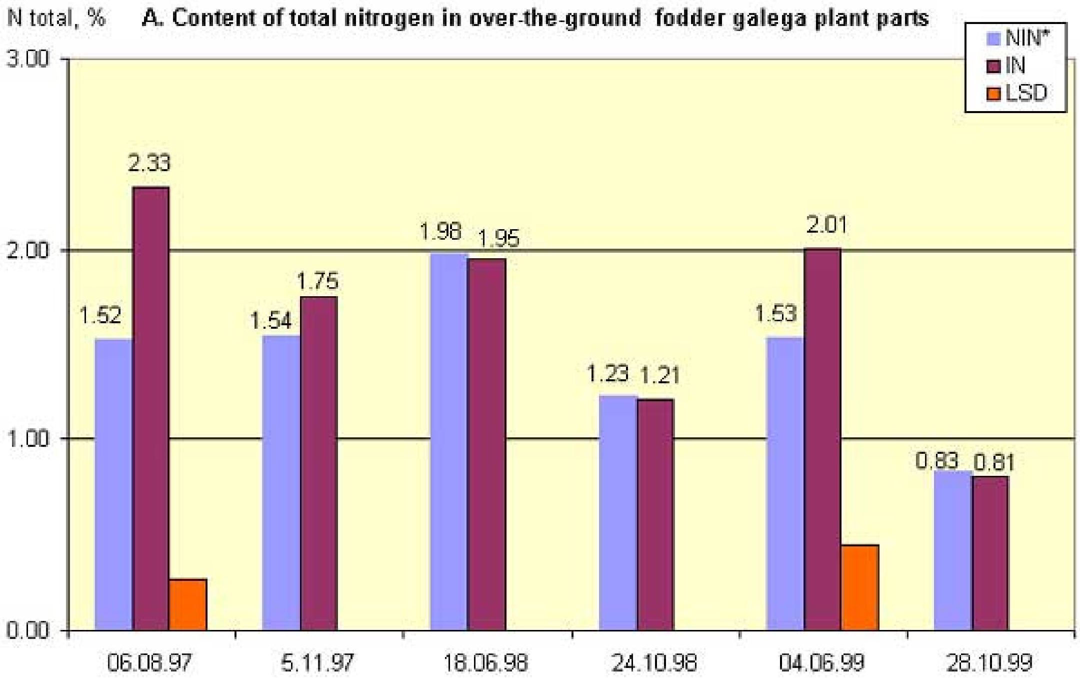
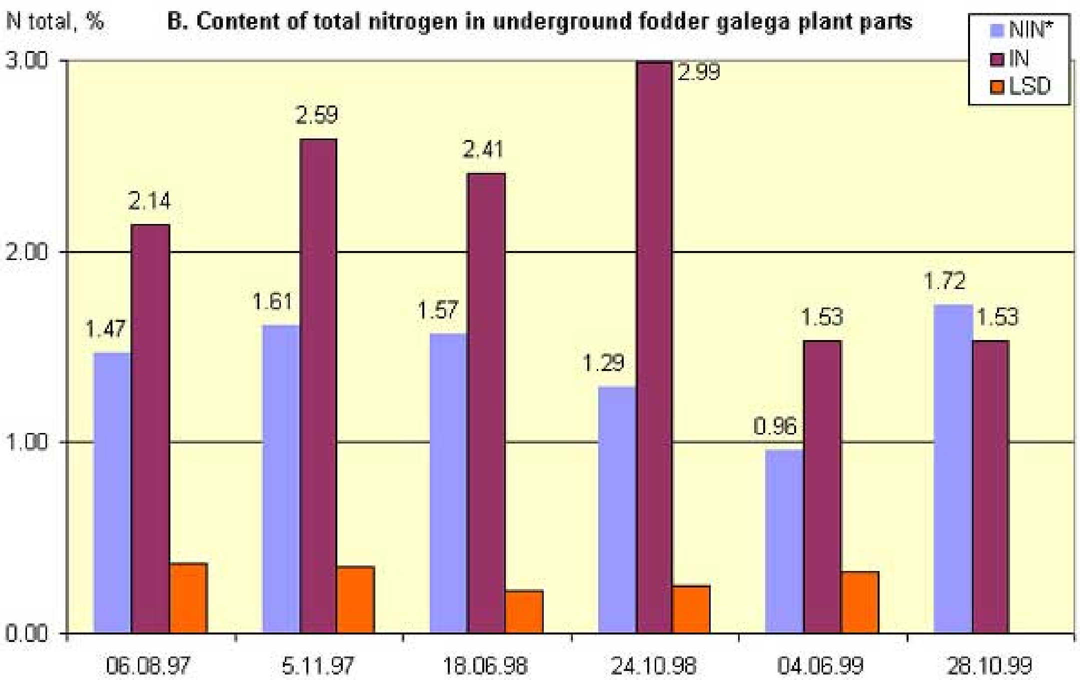
The over-the-ground total nitrogen content ranged from 0.83 to 2.33% of dry matter and in summer it was considerably, 0.7% higher, than in autumn (Fig. 3A). The data in Figure 3 showing small differences between the objects can suggest that plant inoculation and symbiosis effectiveness affected N content slightly, only. Over the most research period there was observed no significant variation in plant N content (except for data obtained in the summers of 1997 and 1999). However, the chemical analysis included non-inoculated plants in their vegetative phase and inoculated ones which reached full flowering in summer or full maturity in autumn. According to others [4, 14, 16], the N content in fodder galega harvested prior to flowering ranges from 2.5 to 3.5 % and drops quickly later on.

Fig. 3. Total N content and accumulation





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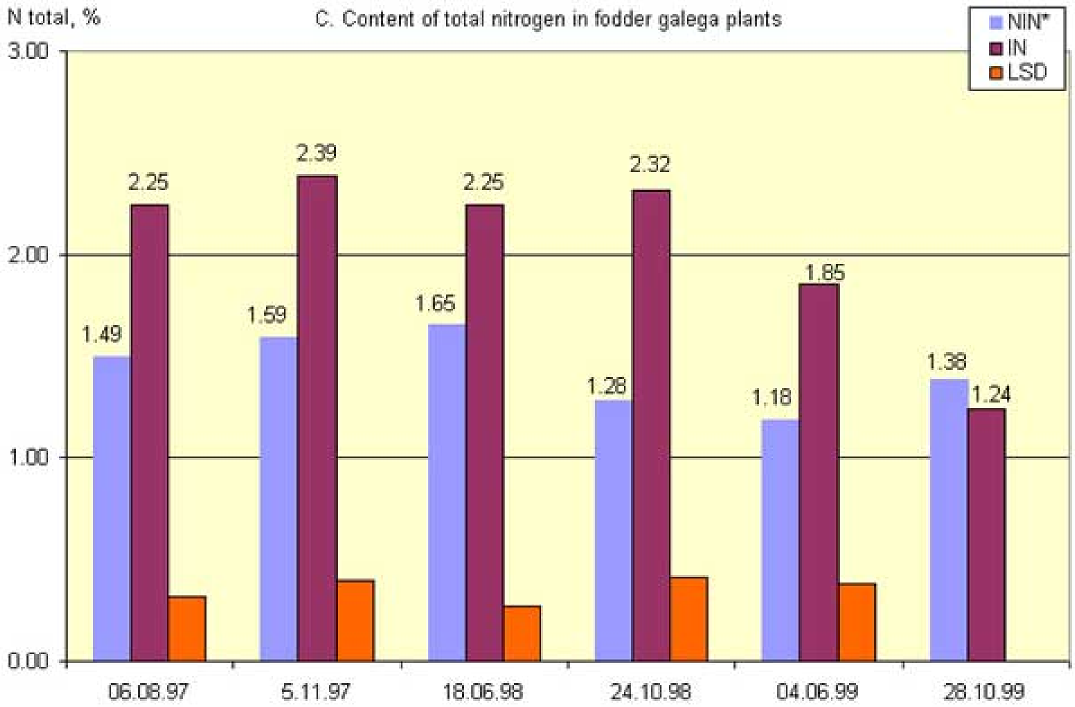

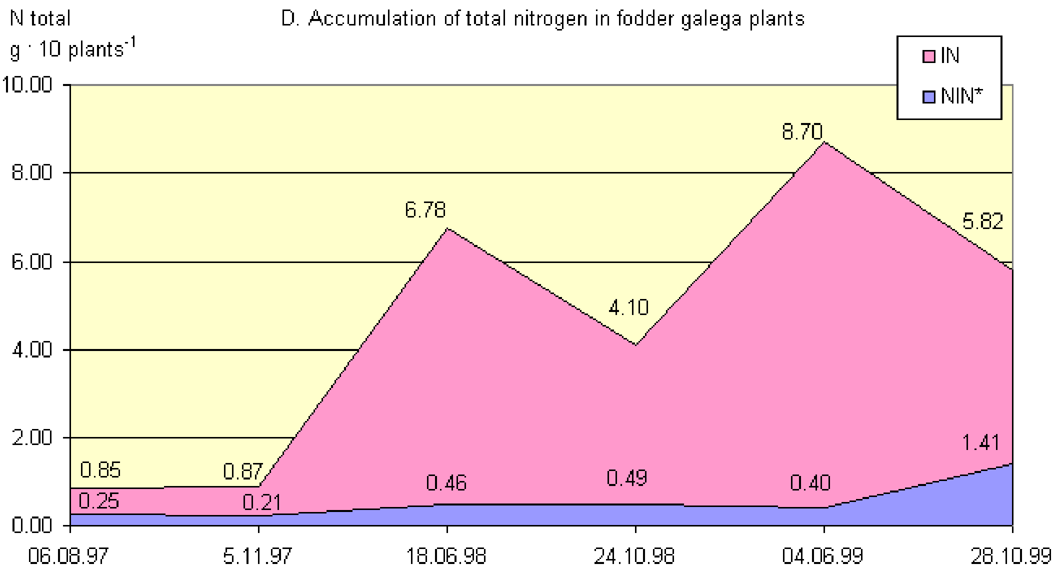

*** NIN non-inoculated; IN - inoculated**

The inoculation resulted in clear and mostly significant differentiation in N content in underground plant parts (Fig. 3B). In inoculated plants the N content ranged from 1.53% to 2.99%, while in non-inoculated – from 0.96 to 1.57%. In the autumn of 1999 there was observed an increase in N content in non-inoculated underground plant parts, due to starting symbiosis. The average N content in inoculated plants, except the final measurement date, was about 1% higher than in non-inoculated plants (Fig. 3C).

Investigating symbiosis it is essential to show how much N accumulated in plant has been fixed and how much has been taken up from soil. In the present research mineral N fertiliser was not applied therefore non-inoculated plants could use only soil N, while plants living in symbiosis – from soil N and N_2. Comparing the amounts of N accumulated by inoculated and non-inoculated plants (Fig. 3D) makes it possible to calculate that plants fixed 73% of N in

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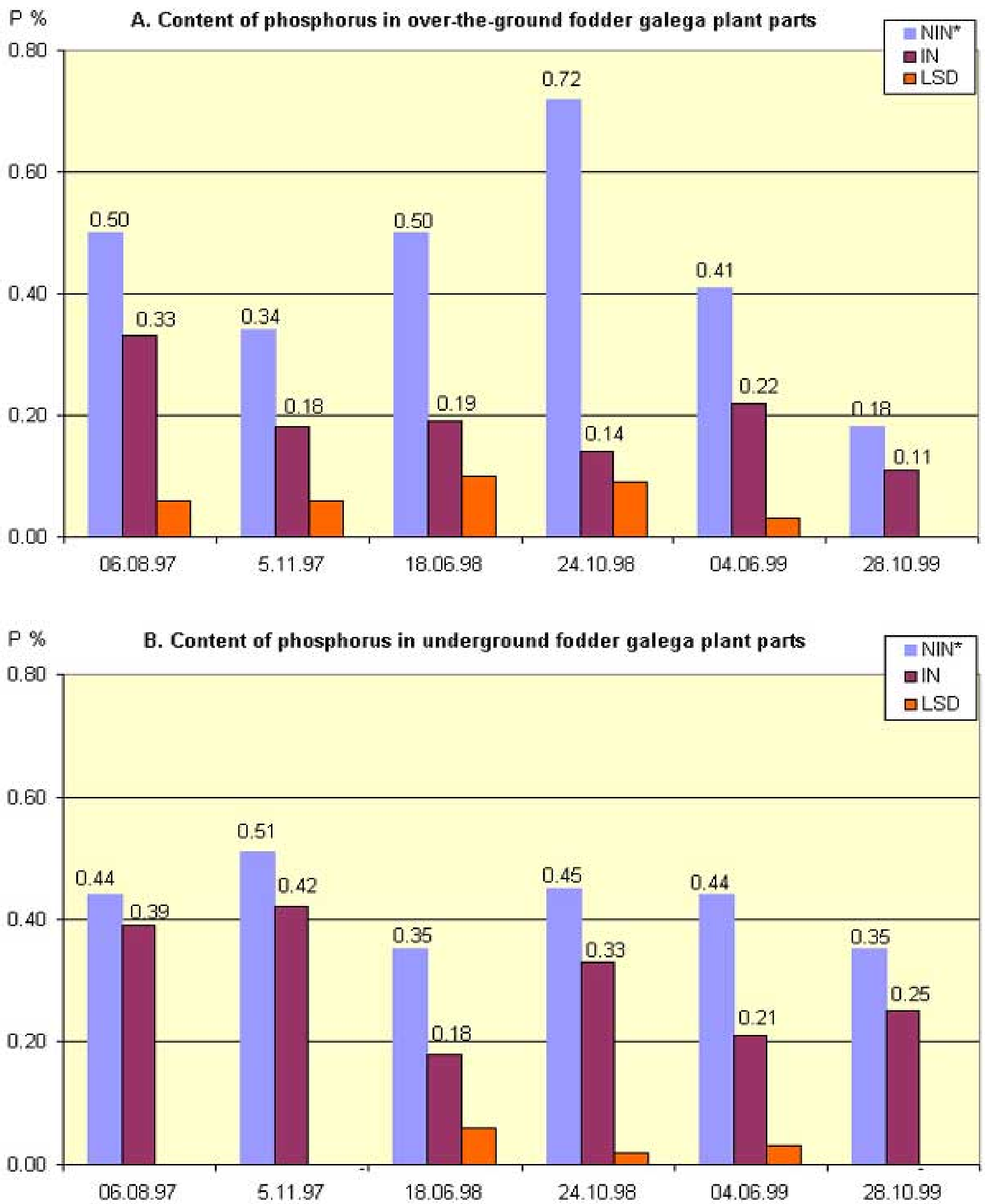
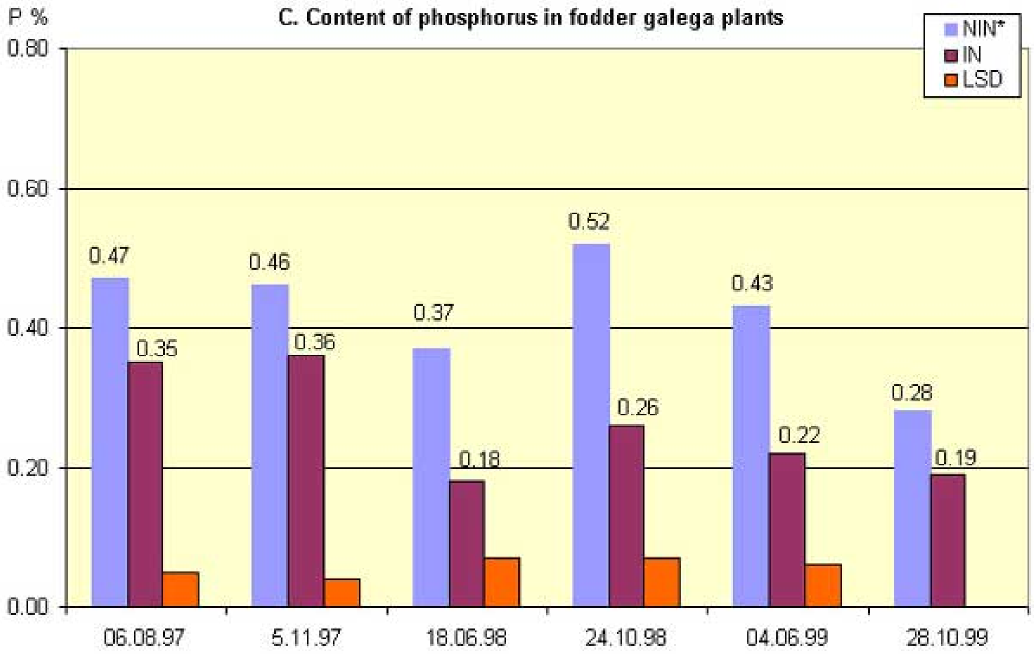
the year of sowing, 93 – 95% in the second and third year, while in autumn of the second year when no symbiosis symptoms were observed – 88%. Analysing the results obtained by the authors and by others [4, 5, 14, 16], one can conclude that over the years of full use the fodder galega underground and over-the-ground dry matter yields are similar and ranges from 8 to 10 t per ha. At 2.5% N content in over-the-ground parts and 2% in underground parts, total N accumulation range from 360 to 450 kg per ha including 90%, i.e. 324-405 kg of fixed N.

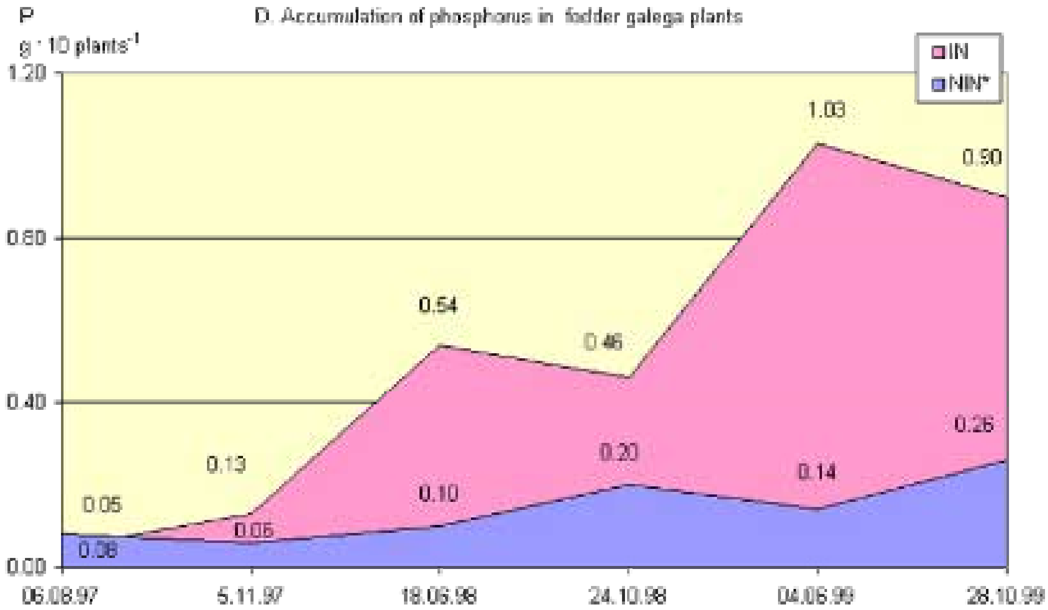
As the yield of underground parts can be higher than that of the over-the-ground parts [16], which was confirmed also in the present research, the accumulated amount of N fixed by fodder galega in its total biomass can considerably exceed the accumulated amount of N_2 fixed by alfalfa and other perennial papilionaceous plants [18].

The present research showed that active symbiosis of fodder galega with *Rhizobium galegae* affects not only the yield and N content but also – indirectly contents and accumulation of the other macroelements (Figs. 4-7).

Fig. 4. Phosphorus content and accumulation







*** NIN non-inoculated; IN - inoculated**

The non-inoculated plants contained mostly significantly more phosphorus, and over some measurement periods - also more potassium than inoculated plants (Figs. 4-5). Over the 3-year study the content of both elements, especially in over-the-ground parts of non-inoculated plants ranged greatly from season to season. The potassium content in summer was by about 1% higher than in autumn, while phosphorus content changes were irregular. In the last research year, mainly in autumn when also nodules were identified on non-inoculated plant roots, a clear decrease in the contents of both elements was recorded both in over-the-ground and underground plant parts to their contents in inoculated plants. Irrespective of plant inoculation, the contents of phosphorus and potassium decreased with age. Over the final research stage mean potassium content in total plants was about 1% lower and phosphorus – about 0.2% lower as compared with the values obtained in the sowing year. Although the contents of those components in soil under the plants studied were not analysed, the above

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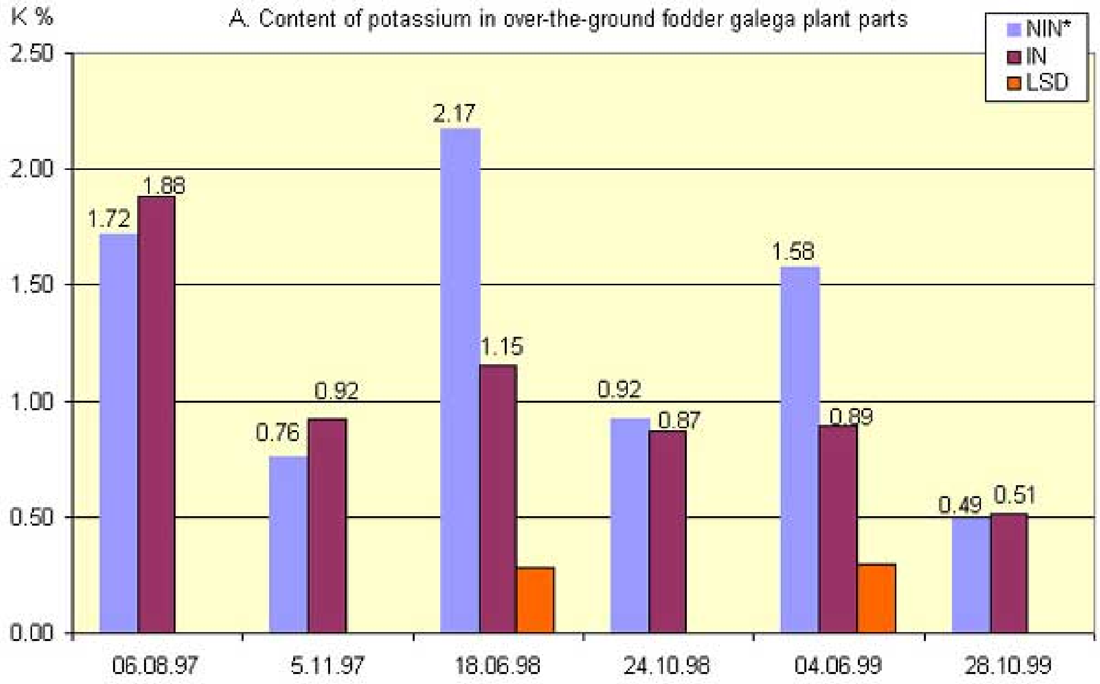
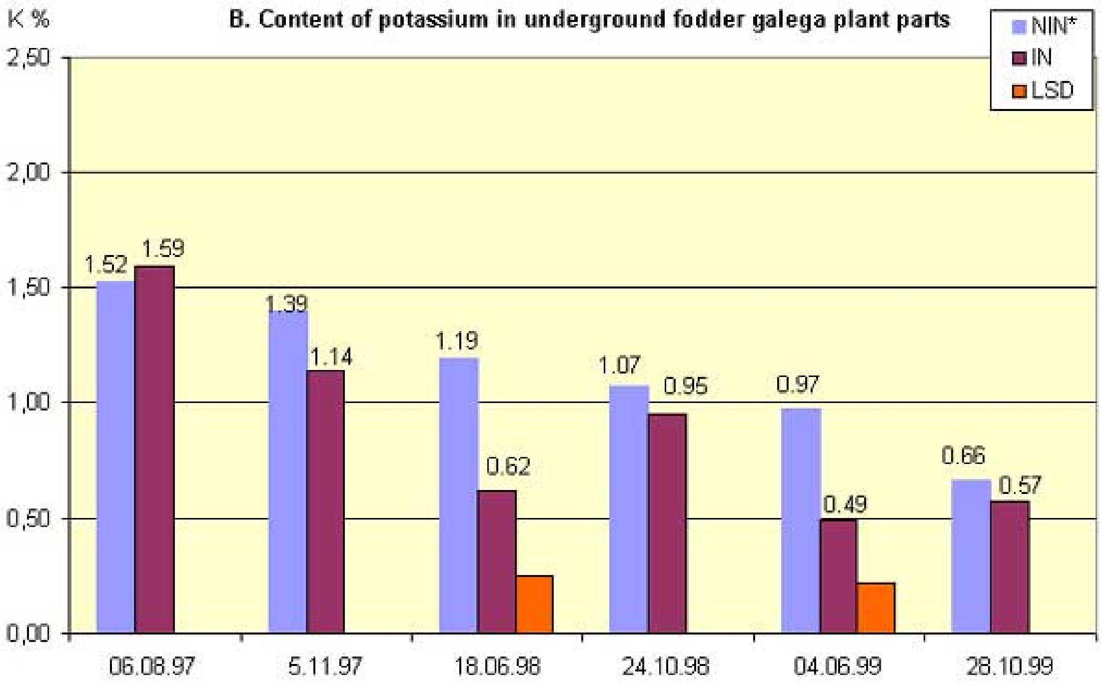
relationships must have been related to the availability and then to the degree of gradual soil depletion increasing with an increase in the over-the-ground and underground fodder galega plant weight. Analysing the parallel fallow land field experiment, also a gradual and progressive with years decrease in potassium, phosphorus and magnesium contents was recorded in soil under lush fodder galega (data unpublished).

Fig. 5. Potassium content and accumulation





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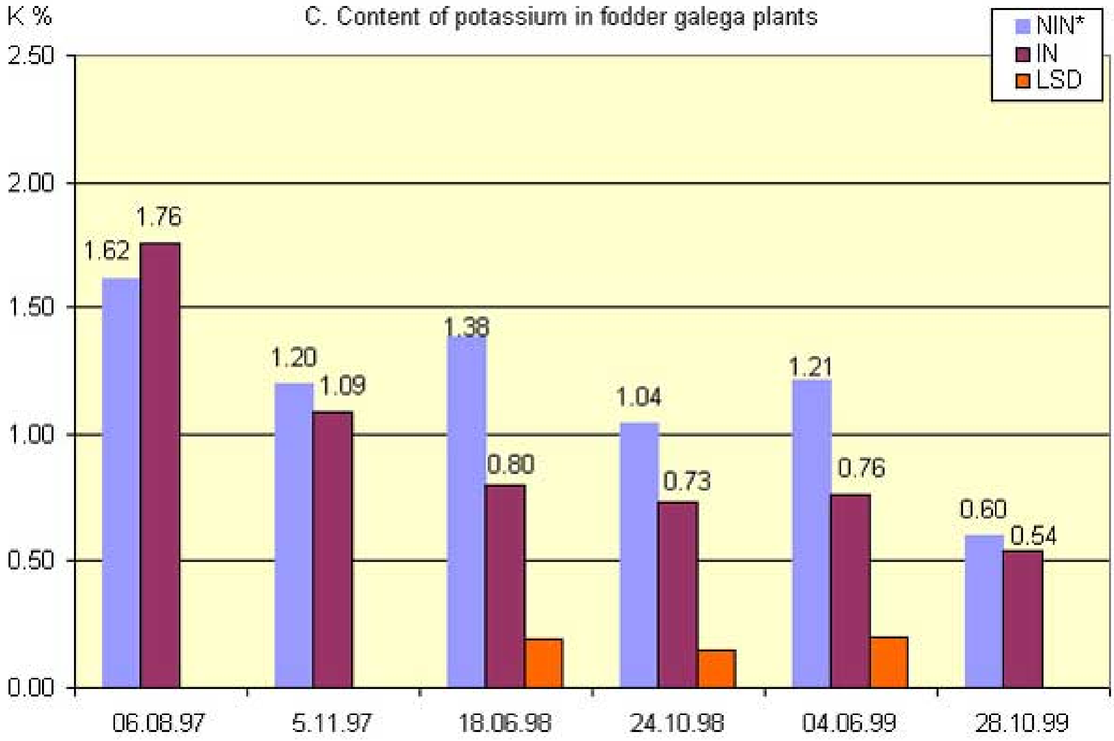

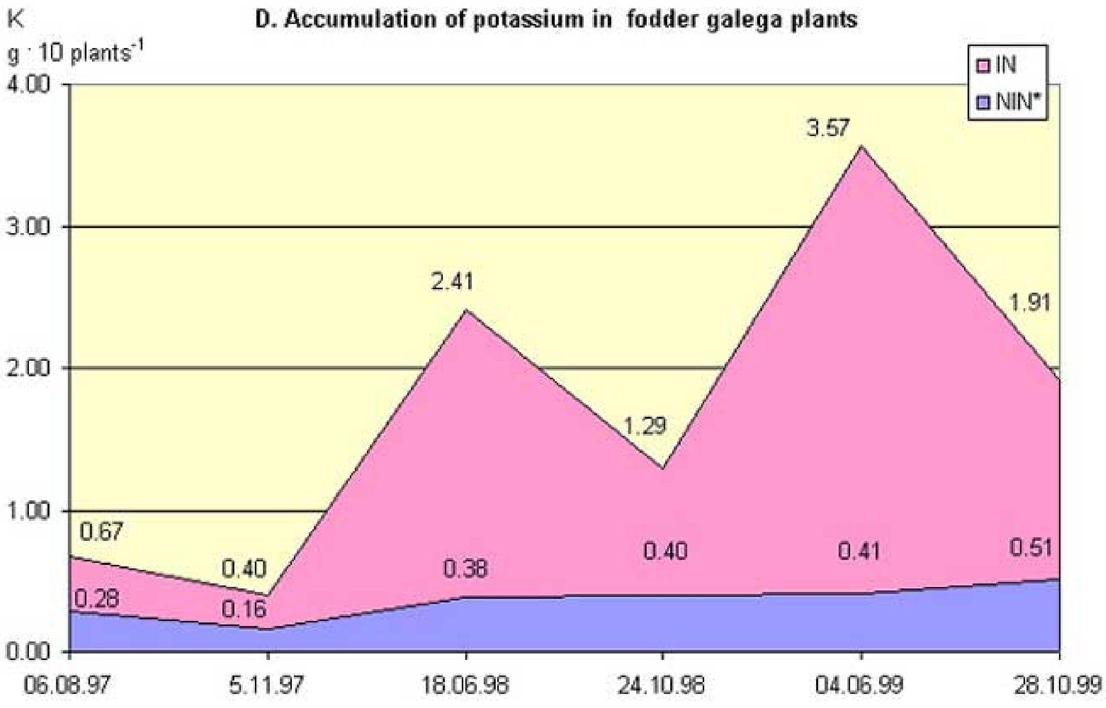

*** NIN non-inoculated; IN - inoculated**

The pots used limited root penetration, especially of inoculated plants at the end of study when a lack of room for root development was noted. It could have also lowered the content of potassium whose high amounts are taken up by papilionaceous plants, including fodder galega [4, 14].

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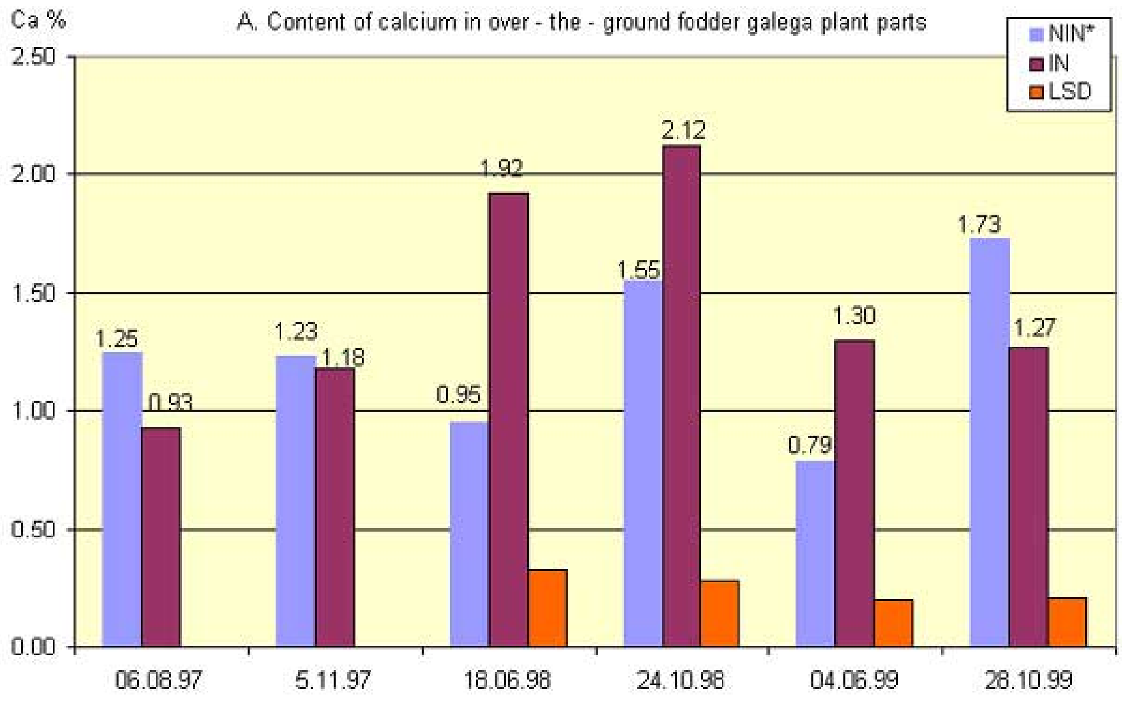
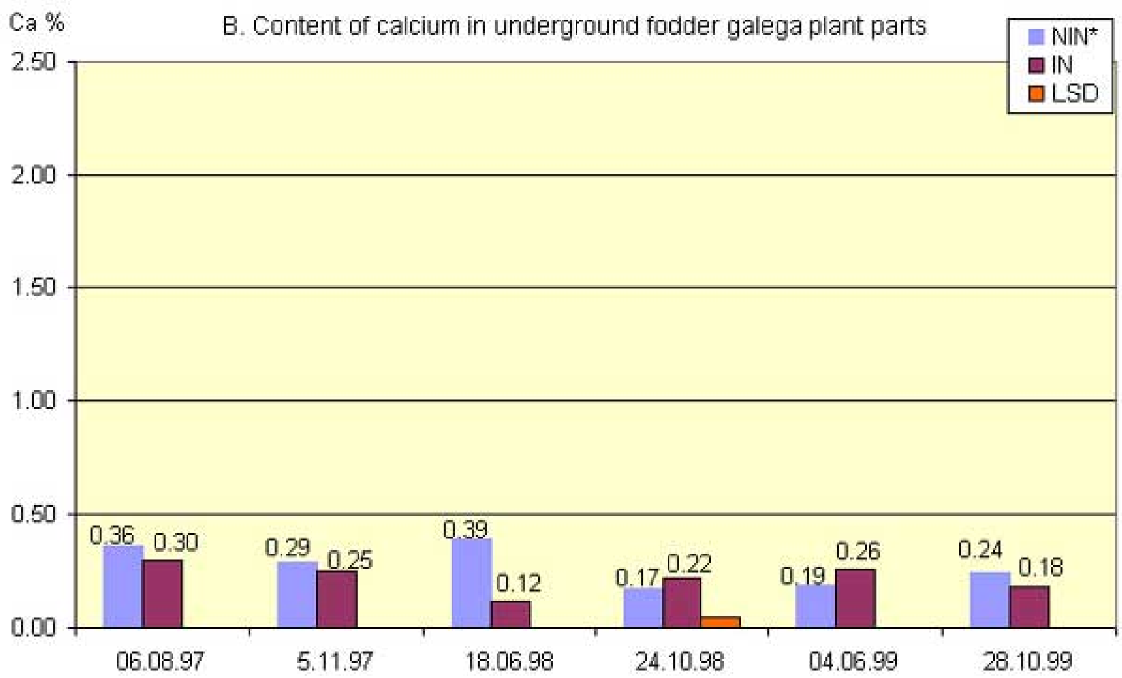
The content of calcium in over-the-ground part, in specific, and in plant as a whole over full-use years was predominantly higher in inoculated than in non-inoculated plants. In the underground parts, except for markings of June 1998, the content of calcium in both objects was similar (Fig. 6A – C).

Fig. 6. Calcium content and accumulation





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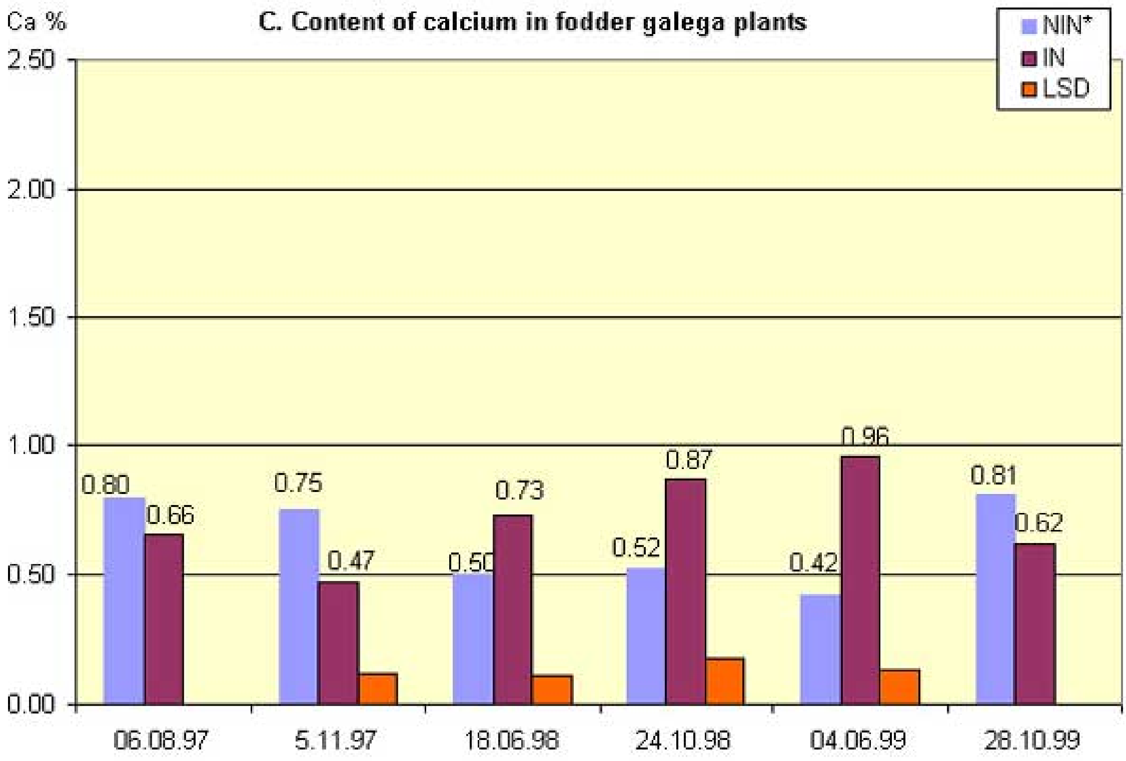

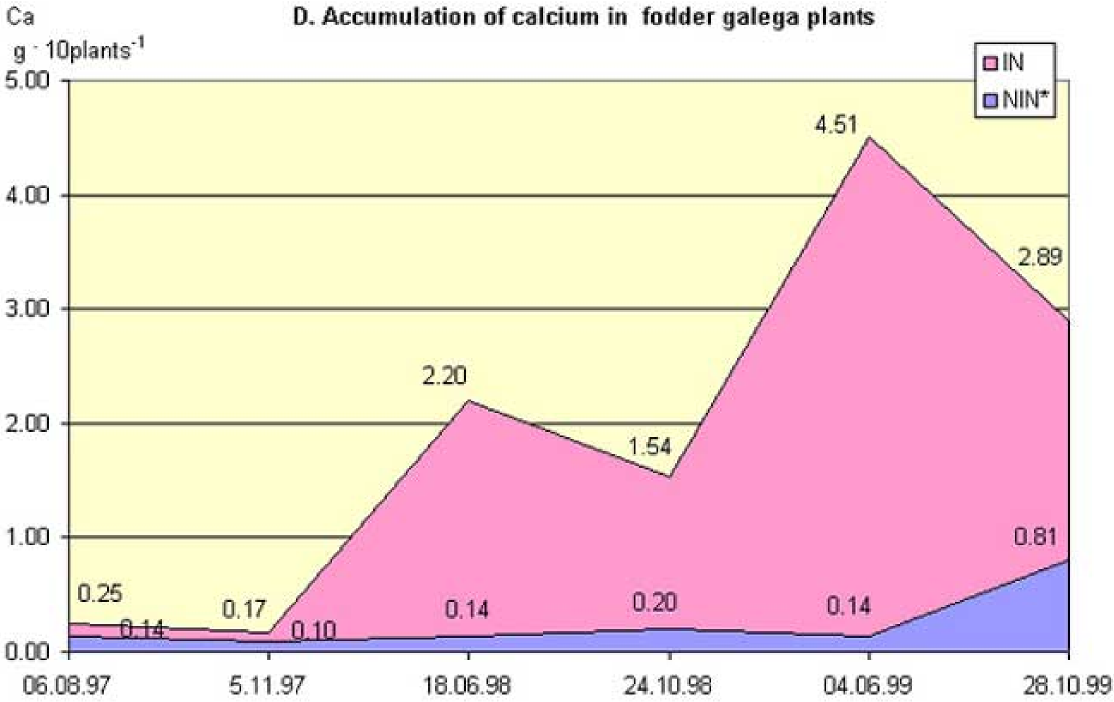

*** NIN non-inoculated; IN - inoculated**

The content of magnesium both in over-the-ground and underground parts of fodder galega most frequently depended on neither the plant inoculation nor the measurement date. Neither did the content change with successive research years (Fig. 7A - C).

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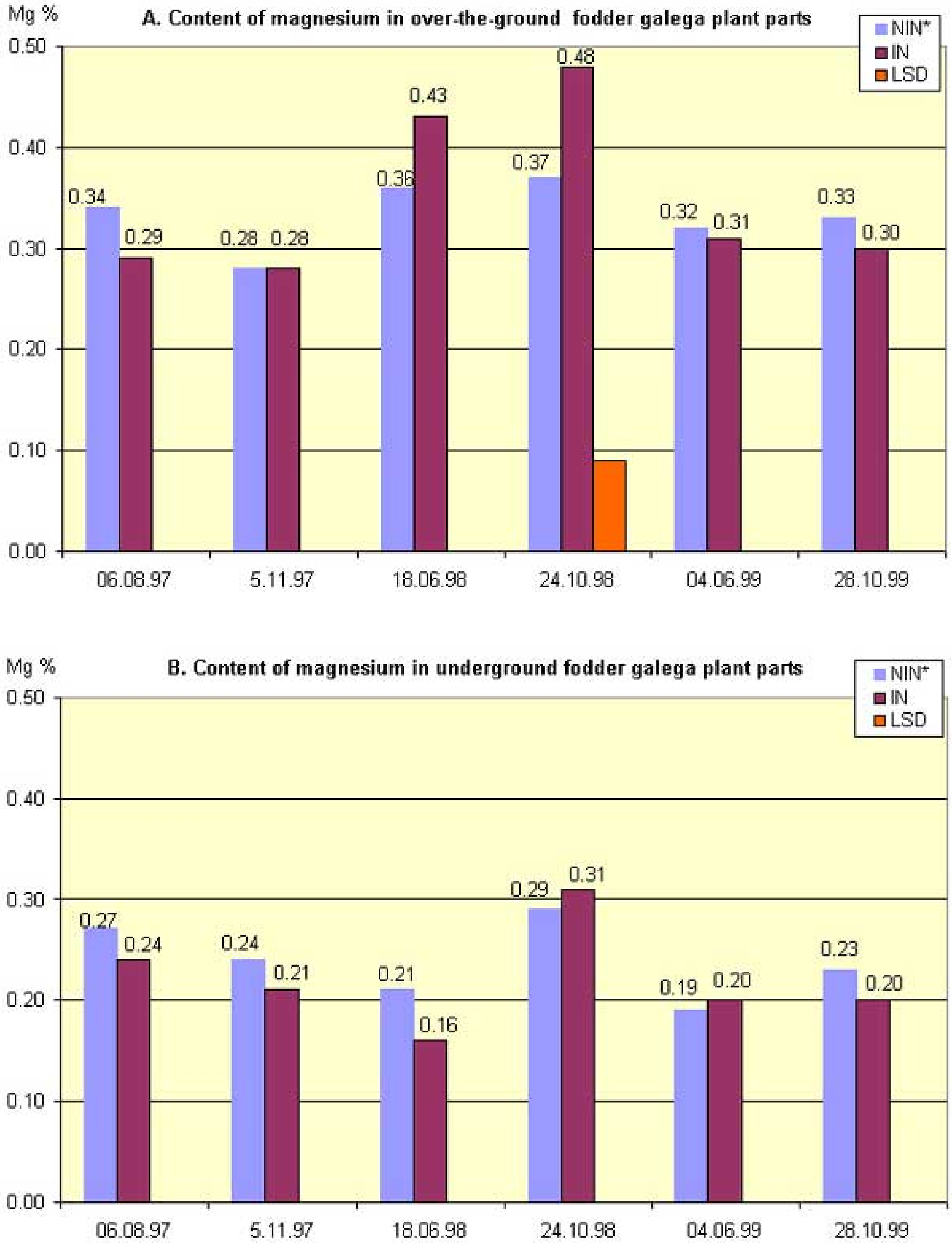
Fig. 7. Magnesium content and accumulation



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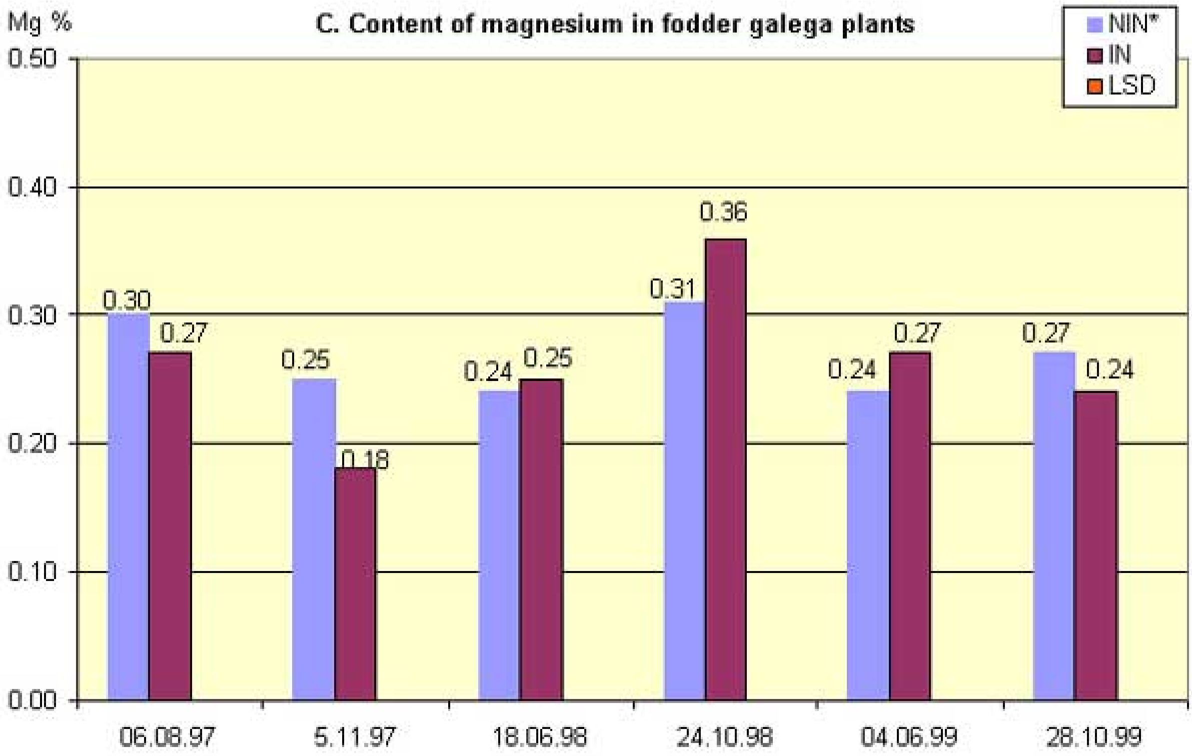

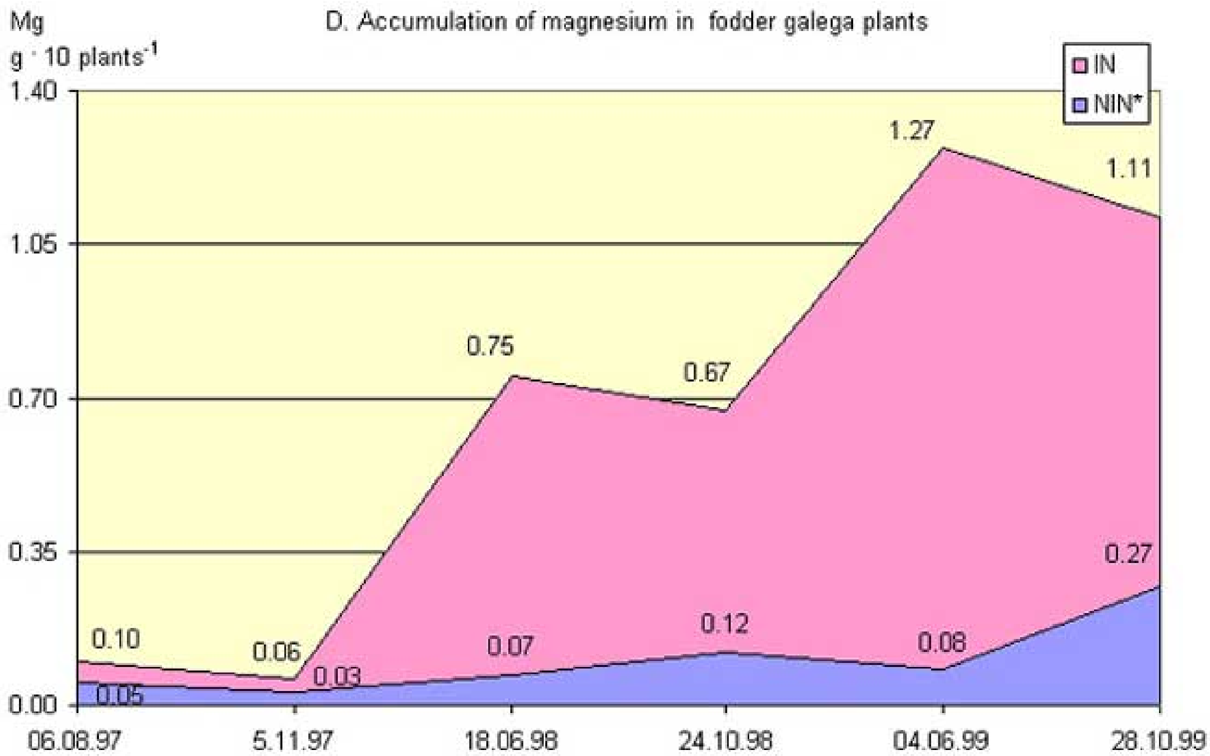

*** NIN non-inoculated; IN - inoculated**

Out of all the elements taken up from soil, fodder galega plants accumulated most potassium and calcium and then magnesium and phosphorus (Figs. 4D - 7D). The highest accumulation of all the macroelements was noted in summer of the third year of the study when in

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inoculated plants the content of calcium was 32-fold higher, magnesium - 16-fold higher, potassium – 9-fold higher and phosphorus – 7-fold higher than in non-inoculated plants.

CONCLUSIONS

1. The effectiveness of symbiosis between inoculated fodder galega (*Galega orientalis* Lam.) plants used extremely extensively and *Rhizobium galegae* was shown in an increase in biomass: a doubled biomass in the sowing year, 11-fold in the second and 15-fold in the third year, as well as a 1% higher total nitrogen content in the dry matter.
2. The highest accumulation of all macroelements was noted in the summer of the third year of the study. In inoculated plants the accumulation of phosphorus, potassium, magnesium, calcium and nitrogen was several and a few dozen higher than in non-inoculated plants.
3. Out of the total content of nitrogen accumulated in fodder galega biomass over intensive plant development – in the second and third year of the study, more than 90% was fixed.

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