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ORIGINAL PAPER

INFLUENCE OF FERTILIZATION AND MYCORRHIZA ON GROWTH AND DEVELOPMENT OF RHODODENDRON (*RHODODENDRON HYBRIDUM*) IN A NURSERY*

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Abstract

In recent years, a dynamic growth in the cultivation of plants from the *Ericaceae* family, including rhododendrons, has been observed. This study on rhododendron plants was carried out in containers under controlled conditions in 2011-2013. The aim was to show the influence of fertilization and mycorrhizal inoculation on the growth and development of rhododendron cv. Anneke in the first three years of cultivation. This cultivar belongs to the group of Knap-Hill--Exbury hybrids. A two-factor experiment was established in a complete randomization system. The following factors were investigated: different methods of fertilization and use of mycorrhizal vaccine. The starting material in the experiment were *in vitro* propagated seedlings. A significant effect of a fertilization method on the length and number of rhododendron shoots has been demonstrated. The longest shoots of plants were recorded after the application of slow-release fertilizer (SRF) - Hortiform pH, and the shortest ones were grown after the application of fertigation. There was higher frequency of ERM on the roots of plants fertilized with slow-release fertilizer (SRF) than those fertilized with the use of fertigation and individual mineral fertilizers. The mycorrhizal frequency (ERM) on inoculated rhododendron's roots ranged from 36% to 65%, while without inoculation it fell down to 4% to 9%. It was found that the slow-release fertilizers (SRF) applied satisifed the nutritional requirements of rhododendron for macronutrients, except phosphorus, while fertigation requires further improvement in terms of the nutrient composition.

Keywords: rhododendron, ericoid mycorrhizae, Slow Release Fertilizer, nursery crop.

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INTRODUCTION

In recent years, a dynamic growth in the cultivation of plants from the *Ericaceae* family, including rhododendron, has been observed in Poland (Pacholczak et al. 2016). Rhododendron has specific habitat requirements, it is sensitive to abiotic stresses and susceptible to dangerous infectious diseases, hence cultivation of this species can cause some problems. Plants grown in a nursery are often exposed to changes in the nutrient content in the substrate, e.g. after heavy rainfall. The use of excessive fertilizers doses on a single occasion results in an excessive concentration of ions in the plant's rhizosphere. Therefore, fertilization of *Ericaceae* plants should take into account both their sensitivity to salinity and specific requirements for the soil pH. The basic method of fertilization consists of application of single-nutrient mineral fertilizers. However, more and more often fertilizers with controlled release of nutrients (CRF – Controlled Release Fertilizer) or slower release of nutrients (SRF – Slow Release Fertilizer) as well as fertigation (Rutten 1980, Nowak et al. 1995) are applied. Fertigation has previously been used primarily to grow crops under cover, but nowadays it is more and more often supplementary to plant nutrition with mineral fertilizers in a nursery, or it completely replaces the latter (Bi et al. 2007, Michałojć, Koter 2012, 2015). The *Ericaceae* plants, in their natural habitats, form symbiosis with mycorrhizal fungi. This type of mycorrhiza is called ericoid mycorrhizae (ERM). The ericoid mycorrhizae grow between root hairs of *Ericaceae* plants and the hyphae of fungus *Hymenoscyphus ericae* and of the *Oidiodendron* genus such as *O. griseum, O. maius, O. cerealia* and *O. rhodogenum* belonging to the *Ascomycotina* division (Read 1983, Douglas et al. 1989, Perotto et al. 1995, Kerley, Read 1998). The roots of *Ericaceae* plants do not develop root hairs and it is believed that the mycorrhizal mycelium replaces their functions. Mycorrhizae stimulate the growth of host plants through a better supply of nutrients such as nitrogen, phosphorus and microelements (Stribley, Read 1980, Bidartondo et al. 2003, Konieczny, Kowalska 2017). ERM fungi have the ability to decompose cellulose, hemicellulose, pectins and lignins. Owing to their ability to produce proteases, they can release and utilize proteins as the only source of nitrogen (Cairney et al. 2000). This feature is of great importance for maintaining vegetation in areas poor in nutrients. Despite the growing popularity of *Ericaceae* plants, there is no precise information on the effects of fertilization and mycorrhiza on the growth and development of plants in the nursery.

The aim of the study was to show the influence of fertilization and mycorrhizae on the growth and development of rhododendron of cv. Anneke during the first three years of cultivation in containers.

MATERIAL AND METHODS

Studies with rhododendron of cv. Anneke were carried out under controlled conditions in the Experimental Farm Lublin-Felin (51°13′36.9″N, 22°37′56.8″E), in 2011-2013. The analytical part of the research was carried out at the Laboratory of Department of Plant Cultivation and Nutrition, University of Life Sciences in Lublin, and at the Department of Agricultural Microbiology at the IUNG PIB in Puławy.

The research material was rhododendron (*Rhododendron hybridum*) of cv. Anneke. This cultivar belongs to the group of Knap-Hill-Exbury hybrids.

The experiment was established in a complete randomization system as a bifactorial trial. One replicate consisted of a single plant growing in a 2.0 dm^3 pot (first year of study) and a 4.0 dm^3 one (second and third years of the study). Each combination included eight replicates.

The starting material in the experiment were *in vitro* propagated seedlings. Plants were grown in high peat substrate containing 3.2 mg dm^{-3} N-min, 5.3 mg dm^{-3} P, 2.8 mg dm^{-3} K and 6.1 mg dm^{-3} Mg, and with EC 0.09, pH 4.2.

The following factors were investigated:

I. Different methods of fertilization.

II. Mycorrhizal vaccine.

Three methods of fertilization were used in the research:

Traditional fertilization. Single-nutrient fertilizers: ammonium nitrate (NH_4NO_3 – 34% N), monopotassium phosphate (KH_2PO_4 – 22.9% P and 28% K), potassium sulfate (K_2SO_4 – 41.5% K), magnesium sulfate ($MgSO_4$ – 17.4% Mg) were used in the initial period of plant growth, applied in three doses. The micronutrients were used only once (mg dm^{-3}): Fe – 8.0 (iron citrate), Cu – 26.0 ($CuSO_4 \cdot 5\ H_2O$), Zn – 1.7 ($ZnSO_4 \cdot 7\ H_2O$), Mn – 8.0 ($MnSO_4 \cdot H_2O$), B – 3.4 (H_3BO_3), Mo – 7.0 ($(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$). Before planting the plants into containers, 1/3 N, P, K, Mg and whole doses of micronutrients were used in each year, while the remaining amount of nitrogen, phosphorus, potassium and magnesium was divided into two doses, of which one was used in the mid of May, and the other one in mid-June. This method of fertilization was considered the control.

1. Slow release fertilizer (SRF) - Hortiform pH – composed of: N – 17%, P – 3.5%, K – 12.5%, Mg – 1.8%, S – 10%, B – 0.01%, Co – 0.002%, Cu – 0.01%, Fe – 0.5%, Mn – 0.10%, Mo – 0.001%, Zn – 0.01%. Nutrients contained in this fertilizer occur in forms with an extended period of up to 6 months for their access to plants. A dose of 3 g dm^{-3} Hortiform pH was used in its entirety before starting the vegetation in each year of study.

2. Fertigation – fertilization combined with irrigation. Nutrient solution of the composition (mg · dm^{-3}): 14 N-NH_4, 56 N-NO_3, 15 P, 58 K, 60 Ca, 12 Mg, 16 S-SO_4, 0.4 Fe, 0.3 Mn, 0.2 Zn, 0.06 Cu, 0.1 B, 0.01 Mo, 5.5 pH and 1.4 was applied every other day from the start of plant growth (end of April / beginning of May) to mid-September in each year of the study.

With each of the above fertilization methods in each year of research, the same amount of nutrients was provided to plants (g dm^{-3} by year): 0.51 N, 0.10 P, 0.38 K, 0.05 Mg, 0.90 S.

The second factor investigated was mycorrhization of plants:

1. Plants inoculated with mycelium (M+).
2. Plants without inoculation – control (M-).

In the first year of testing before planting into pots, half of each treatment was inoculated with a mycorrhizal vaccine. The roots of plants were soaked in an aqueous solution of the vaccine, which contained fungi of the genus *Oidiodendron* and *Hymenoscyphus* sp. (M+), while the remaining plants were planted without inoculation (M-). The mycorrhizal vaccine came from the Mycorrhizal Mycology Laboratory Mykoflor.

The research was carried out outdoors, from April 2011 to November 2013. Soil under the plants was padded with a black nursery mat.

Biometric measurements

In each year of the study, biometric measurements were carried out, including: length (cm) and the number of one-year shoots per plant (pcs) as well as the number of inflorescence buds (pcs).

Chemical analyses

Substrate samples for chemical analyses in each study year were collected on two dates: I – in the first ten days of August; II – at the end of October. They were obtained from the pots using a shortened Egner's probe. A 0.5 dm^{-3} sample was taken from each treatment. In the 0.03 M CH_3COOH extract of the substrate, the following were determined: N-NH_4 and N-NO_3 by Bremner distillation method with modification by Starck, P-PO_4 colorimetrically by the vanadomolybdate method, K, Ca and Mg by means of the AAS technique (Perkin-Elmer, Analyst 300), salt concentration – via conductometry, expressed as the level of electrical conductivity (EC) .

Statistical analysis of the results was carried out by the method of variance analysis for a two-factor experiment; each year was analyzed separately using the Tukey`s test for assessment of differences at the significance level $\alpha = 0.05$.

Mycological analyses

The colonization of the roots of cv. Anneke rhododendron by ERM mycorrhizal fungi was determined after three years of growing plants in pots. The assay was carried out using the modified method of PHILIPS and HAYMAN (1970). After completing the growing season in October 2013, root samples were taken along with the substrate from each treatment. The roots were separated from the substrate by washing under running water, and were then cut into 2-5 mm fragments. Colonization of rhododendron roots by mycorrhizal fungi was determined by microscopy. In 50 separate observation fields of a preparation, ERM fungal structures were recorded at 100× magnification. The presence of any morphological form of the ericoidal fungus in the field of view was considered to signify colonized root. The percentage of colonization was calculated according to the following formula: number of colonized fragments / 50 pieces of root × 100.

Weather conditions

The course of climatic conditions in the years 2011-2013 has been illustrated on the basis of data obtained from the Meteorological Station of the Department of Agrometeorology, University of Life Sciences in Lublin, which is located in the Experimental Farm Felin (Figure 1).

The year 2011 was characterized by an average air temperature of 8.5°C, which was higher than the long-term average by 1.2°C. Monthly average air temp. ranged from -4.7°C (December) to 18.8°C (August). The year 2012 was characterized by an average air temp. of 8.4°C, which was warmer than the long-term average by 1.1°C. Average monthly air temp. ranged from -7.4°C (February) to 21.4°C (July). The year 2013 was warmer than the long-term average by 1.2°C, and the average temp. was 8.5°C. The average monthly temp. in 2013 ranged from -3.8°C (January) to 19.2°C (August).

Total rainfall in 2011 was 555.4 mm and it was close to the long-term average (551.4 mm). However, the rainfall was unevenly distributed (Figure 1). The sum of rainfall in 2012 was 510 mm, which was lower by 41 mm compared to the long-term average. The most rainfall was recorded in October, which indicates its uneven distribution (Figure 1). In 2013, the annual rainfall totaled 715.7 mm and it was higher by 164.1 mm compared to the long-term average. Most rainfall was recorded in July, when it was by 42.5 mm higher than the long-term average, and the least was in October, by 35 mm less than the average long-term. The weather data from 2011-2013 indicate uneven distribution of rainfall and milder climate characterized by higher air temp. of 1.2°C, on average.

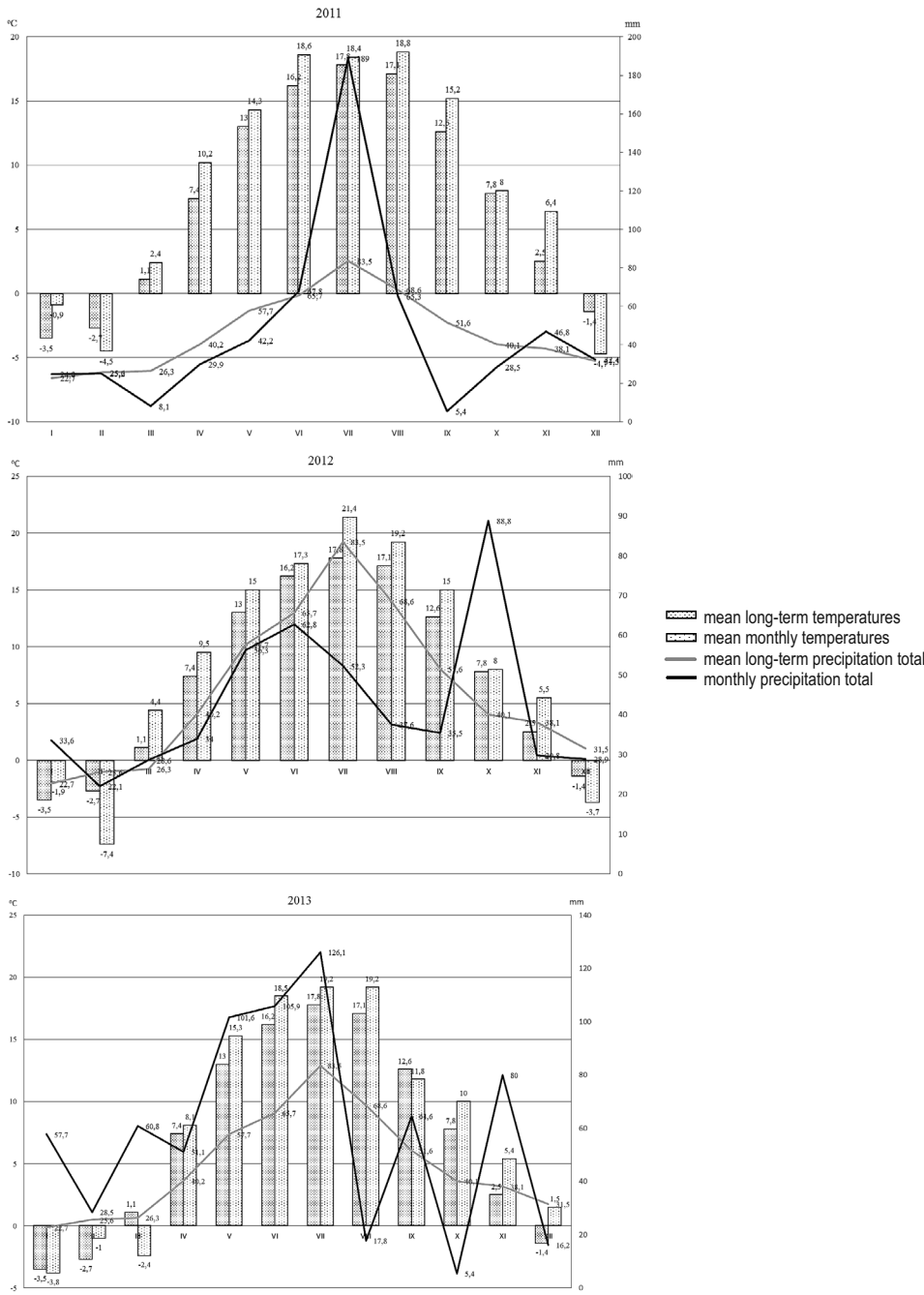

Fig. 1. Monthly rainfall (mm) and mean monthly air temperature (°C) in 2011-2013. Data recorded at the weather station of the Department of Agrometeorology, University of Life Sciences in Lublin.

RESULTS AND DISCUSSION

The results regarding the growth and development of rhododendron from particular years are presented in Table 1. The influence of a fertilization method on the length of rhododendron shoots has been demonstrated. The longest shoots of plants were recorded after the use of a slow-release fertilizer (Hortiform pH), whereas the shortest ones grew after the application of fertigation, but the latter became noticable in the second and third year of research, which indicates the need to improve the composition of a medium for this species. At the same time, it should be emphasized that the annual growth rate that characterized annual plants was the highest – the average length of their shoots was 15.0 cm, while two-year-old ones grew by 9.8 cm, and three-year-old plants – by 9.0 cm. There is no clear evidence in the literature regarding the effect of a fertilization method on the growth and development of *Ericaceae* plants in container cultivation. In the present research, it was shown that among the three fertilization methods applied, the most beneficial effects were obtained after the use of the fertilizer with slow release of nutrients. This was also confirmed by Bosiacki et al. (2009, 2011), who demonstrated the high suitability of CRF, SRF fertilizers for fertilizing ornamental bushes in nurseries.

Results on the number of developed annual rhododendron long shoots show significantly more of these in plants after applying Hortiform pH compared to the other fertilization methods, although the biggest differences were observed in three-year-old plants. The average number of shoots in annual plants was 6.1, two-year 15.4, and three-year 16.3 shoots per one plant (Table 1).

Observations concerning the number of developed inflorescence buds showed their presence after the first year of cultivation. However, a significant effect of the fertilization methods on the number of inflorescences has been demonstrated in two and three-year-old plants. On average, the number of inflorescence buds on annual plants was 1.6, increasing to 10.0 on two--year-old plants and reaching 18.1 per plant on three-year-old ones (Table 1).

When assessing the impact of the applied mycorrhization of plants on the biometric traits studied, a significant effect of this treatment was demonstrated in the third year of the study, although a beneficial effect of ERM was also noted in the first and second cropping year (Table 1). The results show that the significant impact of the beneficial symbiosis of the higher plant with the fungus was visible after two years of study, therefore this period should be defined as the necessary time for the development of mycelium on the roots of plants.

The results are confirmed by studies carried out on rhododendron and other *Ericaceae* plants, which have shown that plants coexisting with mycorrhizal fungi grow much faster and have longer annual gains compared

Table 1

Influence of the type of fertilization and mycorrhiza inoculation on selected biometric traits of large-flowered rhododendron cv. Anneke

Mycorrhizae (A)	Type of fertilization (B)	Length of shoots (cm)			Number of long shoots (number per plant)			Number of inflorescence buds (number per plant)		
		2011	2012	2013	2011	2012	2013	2011	2012	2013
M-	control	11.3	9.7	7.4	5.1	14.1	12.9	2.1	4.9	14.9
	Hortiform pH	16.8	11.1	10.4	6.3	16.8	21.0	1.1	12.1	29.1
	fertigation	15.2	8.1	6.3	7.0	13.2	9.9	0.9	1.8	5.2
M+	control	12.1	9.4	9.3	6.2	16.1	15.9	2.1	10.1	20.8
	Hortiform pH	19.9	11.9	13.2	6.4	16.3	25.1	2.2	16.8	35.1
	fertigation	14.7	8.2	7.2	5.8	15.8	12.9	0.9	14.1	3.2
Mean for A	M-	14.4	9.6	8.0	6.1	15.2	14.6	1.4	6.3	16.4
	M+	15.6	9.8	9.9	6.1	16.6	18.0	1.7	13.7	19.7
Mean for B	control	11.7	9.6	8.4	5.6	15.1	14.4	2.1	7.5	17.9
	Hortiform pH	18.4	11.5	11.8	6.4	16.6	23.1	1.7	14.5	32.1
	fertigation	14.9	8.2	6.8	6.4	14.5	11.4	0.9	8.0	4.2
Total average		15.0	9.7	9.0	6.1	15.4	16.3	1.6	10.0	18.1
$NIR_{0,05}$	for A	n.s.	n.s.	0.7	n.s.	n.s.	2.6	n.s.	1.7	2,0
	for B	3.3	2.5	1.1	n.s.	1.5	3.8	n.s.	2.5	3,0
	AxB	2.7	2.2	3.2	n.s.	3.2	4.1	n.s.	3.7	6.1

to uninoculated plants (Księżniak et al. 2010). A similar relationship was noted in studies carried out on olive trees (Porras-Soriano 2009), and pine trees (Kubiak 2007*a*). According to Nowak (2004) and Falkowski et al. (2009), mycorrhiza accelerates and increases the abundance of flowering in many ornamental plants. However, Matysiak (2009) showed that the stimulation of flower buds is caused by the production of plant growth regulators from the gibberellin group by mycorrhizal fungi. In the present studies, the rhododendron of cv. Anneke showed a larger number of inflorescence buds in plants inoculated than in control plants. The research carried out by Kubiak (2007*b*) clearly indicates the benefits of using mycorrhiza in nursery plants, where inoculated plants are much better prepared for growth and development after planting into a permanent place, the more so because this treatment is performed only once in a lifetime of a plant.

The extent of rhododendron root colonization by ERM fungi was analyzed in the third year of cultivation after the growing season. A significantly higher degree of the growth of mycelium populations was observed in plants inoculated rather than in uninoculated ones (Table 2). In the mycelium-inoculated combinations, the highest degree of root colonization with mycorrhizal fungi (60-65%) was found after the fertilization with the slow-release fertilizer

Table 2

Influence of the fertilization methods on the degree of ERM colonization of roots of large-flowered rhododendron cv. Anneke

Type of fertilization	M+ (%)	M- (%)
Control	36-40	5-6
Hortiform pH	60-60	7-9
Fertigation	52-56	4-5

(Hortiform pH). A slightly smaller degree of colonization was recorded on the roots of plants fertilized by fertigation (52-56%). The smallest one was found on the roots of control plants (36-40%). However, the degree of mycelium colonization on the roots of plants without inoculation should be assessed as low, ranging from 4% to 9% (Table 2). Symbiosis of plant roots with ERM depends on many factors, including the nutrient content in the root environment (Bidartondo et al. 2002, 2003, Hajiboland et al. 2010). Confirmation of this dependence arises from the present results. The largest amount of mycelium was recorded on roots of plants fertilized with the slow-release fertilizer (SRF), where their availability of nutrients to plants was evenly distributed during the growing season.

The content of nutrients and the concentration of salt in the substrate used for the cultivation of rhododendron were determined twice in each study year: at the beginning of August and at the end of October. Large variation in the nutrient content in the subsoil was found on both the first and the second date of sampling (Figure 2). The average nitrogen content varied within 30-266 mg dm^{-3} N-min. The highest nitrogen content appeared after traditional fertilization, and the lowest one was determined after fertigation. Importantly, after the plant growing period ended, the amounts of nitrogen remaining in the substrate varied from 30 to 122 mg dm^{-3} N-min., depending on a fertilization method of (Figure 2). The content of phosphorus in the middle of the growing season ranged from 46 to 79 mg dm^{-3} of $P\text{-}PO_4$, and after its termination it varied from 1 to 8 mg dm^{-3} of $P\text{-}PO_{4,}$ suggesting the complete use of phosphorus by plants. The potassium content in the substrate ranged from 40-103 mg dm^{-3} K on the first sampling date and from 2-49 mg dm^{-3} K on the second one. After the end of the growing season, most potassium was recorded in the treatment where single-nutrient mineral fertilizers (control) were used. The calcium content in the substrate varied less; on the first date its content was from 489-577 mg dm^{-3} Ca, while on the second sampling date it fell down to between 273-322 mg dm^{-3}. The magnesium content in the first period ranged from 108 to 154 mg dm^{-3} Mg, and in the second one it varied from 76 to 115 mg dm^{-3} of Mg. The results show that nitrogen, phosphorus and potassium are taken up by plants during the entire growing season, as evidenced by their low content in the substrate on the second date of sampling. However, it seems jus-

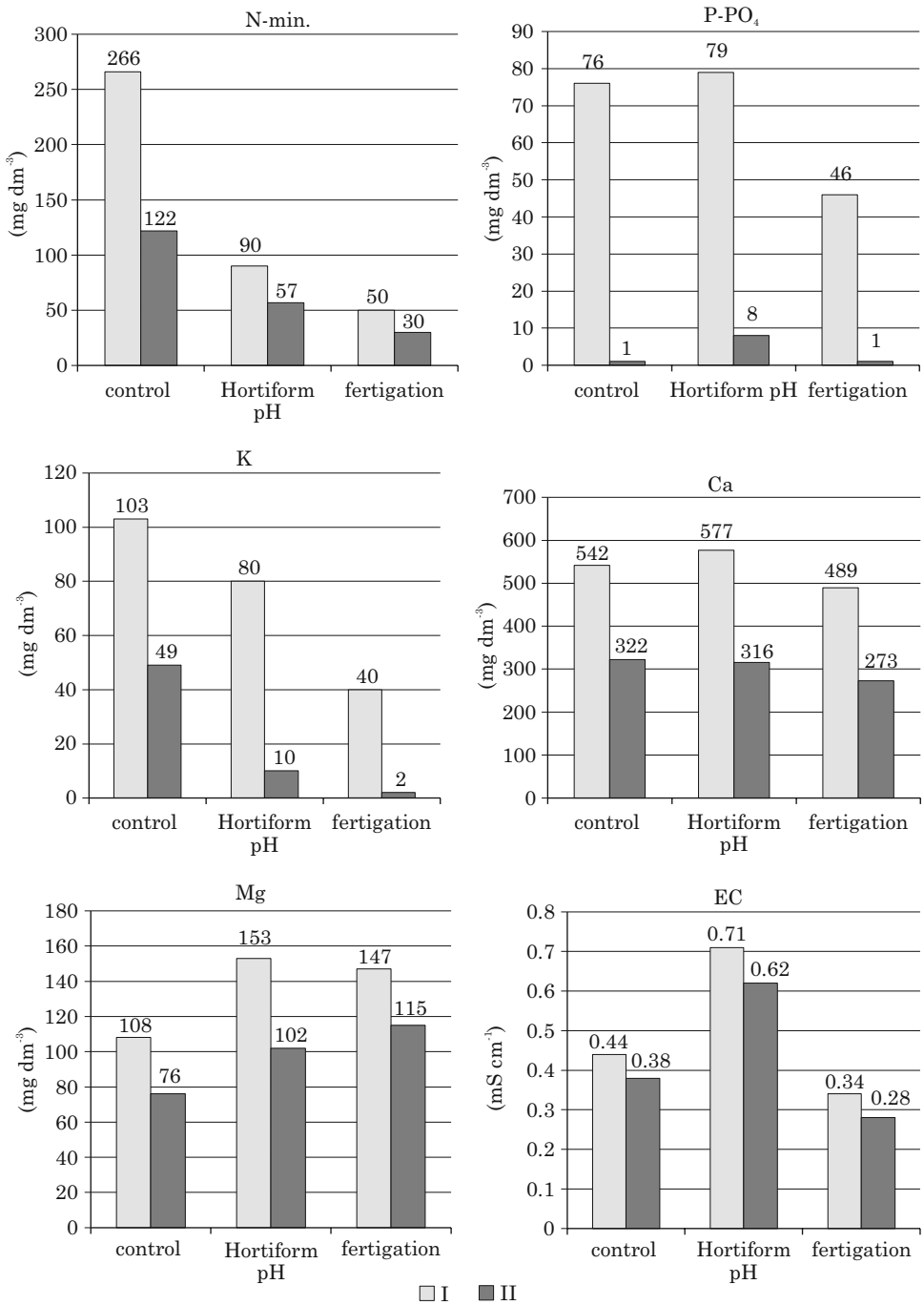

Fig. 2. Content of N-min., P, K, Ca, Mg (mg dm^{-3}) and EC (mS cm^{-1}) in the substrate. Average from 2011-2013. I – first ten days of of August, II – third ten days of October

tified to use fertilizers with slow release of nutrients because the results achieved in this treatment show less variation in the content of components in the substrate. In addition, during the growing season, there was a decrease in salt concentration, which confirms the uptake of nutrients from the substrate by plants. According to Aendekerk (1997), Matysiak et al. (2001), Michałjć, Koter 2012), the standard content of macronutrients as well as pH and salinity (EC) of high peat substrate for growing ornamental shrub plants with low nutritional requirements, including rhododendron, amounts to (mg dm^{-3}): N-min. 110-130 ($N\text{-}NH_4$:$N\text{-}NO_3$ at 1:10), 30-50 P, 120-140 K, 80-140 Mg, 30-50 $S\text{-}SO_4$, pH 4.5-5.8, EC<0.8 mS cm^{-1}. In the present research, the pH of the rhizosphere varied significantly depending on a treatment and year of research, although the fluctuations observed were ambiguous and difficult to interpret. In all the treatments, the pH of the rhizosphere was within the recommended range (Aendekerk 1997, Matysiak et al. 2001, Michałjć, Koter 2012).

In the present study, nutrient content in the substrate close to the standard values was demonstrated on the first sampling date, which proves that the optimal supply of plants with these components was ensured.

Considering the size of the biomass obtained and the optimal range of plant nutrition, it should be emphasized that slow-release fertilizers fulfill the nutritional requirements of rhododendron for all macronutrients except phosphorus, while fertigation requires further improvement of the nutrient composition and the method of its application.

CONCLUSIONS

1. Significant effects of the fertilization methods on the length and number of rhododendron shoots have been demonstrated. The longest shoots of plants were recorded after the use of the slow-release fertilizer (SRF) Hortiform pH, and the shortest ones grew after the application of fertigation.

2. Mycorrhizal frequency on ERM inoculated roots of rhododendron ranged from 36% to 65%, and without inoculation it varied from 4% to 9%.

3. There was a higher frequency of ERM on the roots of plants fertilized with the slow-release fertilizer (SRF) than nourished by fertigation and with the mono-component fertilizers.

4. It was found that the slow-release fertilizer (SRF) meets the nutritional requirements of rhododendron in terms of all macronutrients except phosphorus, while fertigation requires further improvement of the nutrient composition.

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