



Article Influence of Fertilization and Mycorrhizae on the Nutritional Status of Rhododendron (*Rhododendron hybridum*) in a Nursery

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Abstract: Background: This study of the large-flowered azalea cv. 'Anneke' investigated the impact of two factors, i.e., methods of fertilization and mycorrhization, on the nutritional status of plants during three years of nursery cultivation. Methods: Single mineral fertilizers, a slow-release fertilizer Hortiform pH (SRF), and fertigation in combination with mycorrhization of plants, were applied. Plant roots were inoculated with fungi from the genera Oidiodendron and Hymenoscyphus sp. The nutritional status of the large-flowered azalea in the first three years of cultivation was assessed based on macroelements. Results: The analyses revealed significantly higher content of nitrogen, phosphorus and calcium in the leaves of plants inoculated with fungal mycelium. A beneficial effect of plant mycorrhization on plant nutritional status, i.e., higher levels of nitrogen, phosphorus, potassium, calcium, and magnesium, was noted in the second and third years of azalea cultivation. Conclusions: Significant amounts of the nutrients were utilized in the middle of the growing season and almost fully utilized after the season. Hence, the necessity to supplement nutrients in each subsequent year of plant vegetation was postulated. Fertigation was shown to require further improvement of the nutrient solution.

Keywords: ericoid mycorrhizae; macroelements; rhododendron; SRF

1. Introduction

Azaleas are permanent elements of gardens and parks. Their flowers provide a wonderful color palette in late May and early June. Noteworthy is the high frost resistance of azaleas, which are, therefore, fully suitable for cultivation in temperate climate conditions. Plants from the family Ericaceae: Rhododendron, Andromeda, Calluna, Erica, Kalmia, and Pieris have low nutritional requirements [1–3], as nutrient-poor soils are their natural habitat. The plants take up nutrients from raw humus, which contains poorly decomposed plant parts with a small amount of mineral substances [4]. Although nutrient-poor localities are natural habitats for Ericaceae plants, nursery practice and investigations indicate that supplementation with appropriate amounts of nutrients stimulates these plants to grow intensively [5]. The substrate used in an ornamental plant nursery is garden peat containing trace amounts of nutrients, which should therefore be supplied to plants in sufficient amounts for proper growth and development. In practice, single mineral fertilizers, multicomponent fertilizers and slow-release fertilizers are used for fertilization. In recent years there has been dynamic growth in the cultivation of Ericaceae plants, including the large-flowered azalea [6]. Fertilization of Ericaceae plants should meet the specific soil pH requirements of these plants. The basic fertilization method involves the use of single mineral fertilizers. However, fertilizers with controlled release of nutrients (CRF = Controlled Release Fertilizers) or slow release of nutrients (SRF = Slow Release Fertilizer) together with fertigation, are used more frequently [7]. Although fertigation has been applied primarily in the cultivation of plants under cover to date, this treatment is increasingly being used as supplementation or replacement of mineral fertilization [8]. In their natural habitats,



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Ericaceae plants establish symbiosis with mycorrhizal fungi. This type of mycorrhiza is referred to as ericoid mycorrhiza (ERM) [9]. Ericoid mycorrhizae are formed between the root hairs of Ericaceae plants and hyphae of Hymenoscyphus ericae and fungi from the genus Oidiodendron, e.g., O. griseum, O. maius, O. cerealia, and O. rhodogenum from the subclass Ascomycotina [10–13]. The roots of Ericaceae plants do not develop hairs, and the mycorrhizal mycelium is believed to take over their functions. The mycorrhiza stimulates the growth of the host plant via a better supply of such nutrients as nitrogen, phosphorus, and microelements like zinc and copper, and increases the resistance of plants to stress factors, increasing the intensity of photosynthesis [14–19]. Tested mycorrhizal vaccines stimulated the growth and number of leaves of the rhododendron plants [20]. Mycorrhizae influence better uptake of compounds unavailable to plants, making them available, especially of phosphorus [21–23]. It is assumed that mycorrhizal fungi supply 80% of plants with nitrogen and phosphorus. There are approx. 50,000 species of fungi forming mycorrhizal communities from approx. 250,000 plant species [24]. Significant increases in P, N, Fe, Zn and Cu concentrations have been noted in the mycorrhizal roots, even when plants were grown in a medium with a high concentration of CaCO₃ [25]. ERM fungi have the ability to decompose cellulose, hemicellulose, pectins and lignins. Due to their ability to produce proteases, they can release and utilize proteins as the only nitrogen source [26]. This trait is highly important for sustenance of vegetation in nutrient-poor habitats. Despite the growing popularity of Ericaceae plants, there is no specific information about the impact of fertilization and mycorrhizae on the growth and development of Ericaceae plants cultivated in nurseries.

The aim of the study was to show the effect of the fertilization method and plant mycorrhization on the nutritional status of large-flowered azalea cv. 'Anneke' during the first three years of cultivation in containers in a nursery. The presented results are a continuation of the previously published work on the influence of fertilization and mycorrhizae on growth and development of rhododendron [27].

2. Materials and Methods

The investigations of the large-flowered azalea were carried out in controlled conditions at the Lublin-Felin Experimental Station (51°13′36.9′′ N, λ = 22°37′56.8′′ E).

The research material was the azalea (*Rhododendron hybridum*) cv. 'Anneke' representing the group of Knap-Hill-Exbury hybrids.

The experiment was carried out in a two-factor design. The plants were grown in a 2.0 L container (1st study year) and in a 4.0 L container (2nd and 3rd study years). Each combination had eight replications.

In vitro propagated cuttings were the starting experimental material. The plants were grown in a high-peat substrate with contents of N-min., P, K and Mg below 10 mg \cdot L, an EC of 0.09 and pH 4.2.

The effects of the following factors were assessed:

- I. Different fertilization modes.
- II. Mycorrhizal inoculation.

Three fertilization methods were used:

1. Traditional fertilization. Single fertilizers: ammonium nitrate (NH₄NO₃, 34% N), monopotassium phosphate (KH₂PO₄, 22.9% P and 28% K), and potassium sulfate (MgSO₄, 17.4% Mg) were applied in three doses at the beginning of the initial growing season. Microelements were used once (mg·L⁻¹): Fe, 8.0 (iron citrate), Cu, 26.0 (CuSO₄·5H₂O), Zn, 1.7 (ZnSO₄·7H₂O), Mn, 8.0 (MnSO₄·H₂O), B, 3.4 (H₃BO₃), and Mo, 7.0 ((NH₄)₆Mo₇O₂₄·4H₂O). Before the plants were transferred to containers, a 1/3 dose of N, P, K and Mg, as well as total microelements, were applied in each study year. Other amounts of nitrogen, phosphorus, potassium and magnesium were divided into two doses. A subsequent dose was supplied in the second ten days of May and in the last ten days of June. This fertilization method was regarded as the control.

- 2. Slow-Release Fertilizer (SRF) Hortiform pH with the following composition: 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% and Zn, 0.01% was used. The nutrients contained in this fertilizer have an extended 6-month period of availability for plants. A total 3 g·L⁻¹ dose of Hortiform pH was applied before the start of vegetation in each study year.
- 3. Fertigation is fertilization combined with irrigation. A nutrient medium with the composition 14 mg·L⁻¹ N-NH₄, 56 mg·L⁻¹ N-NO₃, 15 mg·L⁻¹ P, 58 mg·L⁻¹ K, 60 mg·L⁻¹ Ca, 12 mg·L⁻¹ Mg, 16 mg·L⁻¹ S-SO₄, 0.4 mg·L⁻¹ Fe, 0.3 mg·L⁻¹ Mn, 0.2 mg·L⁻¹ Zn, 0.06 mg·L⁻¹ Cu, 0.1 mg·L⁻¹ B and 0.01 mg·L⁻¹ Mo was applied every other day from the start of vegetation (end of April/beginning of May) to mid-September in each study year.

Each of these fertilization methods provided the plants with the same amounts of nutrients: 0.52 g/L/year N, 0.10 g/L/year P, 0.38 g/L/year K, 0.05 g/L/year Mg and 0.90 g/L/year S.

The other factor studied was the mycorrhization of the plants:

- 1. mycelium-inoculated plants (M+),
- 2. mycelium-uninoculated plants as control (M-).

In the first study year, half of each combination was treated with the mycorrhizal inoculation before the transfer of the plants into containers. The roots of these plants were soaked in an aqueous inoculum solution containing Oidiodendron fungi and Hymenoscyphus sp. (M+), and the other plants were not inoculated (M-).

The investigations were carried out in an open area from April to November. The soil under the plants was covered with black nursery mat.

Plant material samples (fully developed leaves from the middle part of a one-year shoot) were collected in the first ten days of August in each study year. The plant material was dried at 50 °C and ground. The following parameters were determined:

Total N after combustion in concentrated H₂SO₄ using the Kjeldahl method with the Kjeltec System 2002 Distilling Unit apparatus

After incineration of the plant mass at 200 $^{\circ}$ C followed by dry combustion at 450 $^{\circ}$ C and cooling, the ash was supplemented with diluted hydrochloric acid in a ratio of 1:2. In this extract, the following parameters were determined:

Phosphorus (P), colorimetrically with the vanadomolybdate method

K, Ca, and Mg, with the ASA method (Perkin-Elmer, Analyst 300) [28]

Substrate samples intended for chemical analyses were collected with a shortened Egner sampler from the containers in each study year in two terms: (I) in the first ten days of August, and (II) in the second ten days of October.

The following parameters were determined in the $0.03 \text{ M CH}_3\text{COOH}$ extract at the solution-to-substrate ratio of 1:10:

- N-NH₄ and N-NO₃ (N-min.) with the Bremner microdistillation method modified by Starck,
- P-PO₄ with the vanadomolybdate method,
- S-SO₄ with the nephelometric method with BaCl₂
- K, Ca, and Mg with the atomic absorption spectrophotometry ASA method (Abalyst 300 Perkin Elmer)
- pH (H₂O) with the potentiometric method
- salt concentration (EC) with the conductometric method, expressed as the level of electrical conductivity.

The results were analyzed statistically using analysis of variance for a two-factor experiment. Each year was analyzed separately with Tukey's test at the significance level of $\alpha = 0.05$.

3. Results and Discussion

The assessment of the nutritional status of the large-flowered azalea in the first three years of cultivation was based on the content of total nitrogen, phosphorus, potassium, magnesium and calcium in plant leaves.

The analyses detected average nitrogen content in the range of 12.5–23.5 g N-tot.·kg⁻¹ d.m.) (Table 1). Its lowest content was determined in plants subjected to fertigation, and the highest level was found in the slow-release fertilizer Hortiform pH (SRF) variant (Figure 1). Moreover, there was a significant effect of the mycelium inoculation on nitrogen content, which was significantly higher in the mycelium-inoculated plants than in the uninoculated ones (Figure 2). This correlation was confirmed in studies conducted by [29]. The available literature demonstrates a wide range of nitrogen nutrition of azaleas: 1.96–2.24% N [30], and 1.64% N [1]. In a study carried out by [31], a level of 1.88–2.20% N was shown to be the optimal range of nitrogen fertilization of azaleas. Aendekerk [1] reported symptoms of nitrogen deficiency only at a content of 0.67% N. The present study showed that fertilization with Hortiform pH ensured the optimal nitrogen status of the plants, with the content of the element in the range of 19.1–23.5 g N·kg⁻¹ d.m. In turn, plants fertilized through fertigation contained lower levels of nitrogen (12.5–17.5 g N·kg⁻¹ d.m.). Nevertheless, no symptoms of nitrogen deficiency were observed in the plant leaves.



Figure 1. Influence type of fertilization on concentrations of N-total, P, K, Ca, Mg ($g \cdot kg^{-1} d.m.$) in leaves of azalea cv. 'Anneke'. Mean from years. Results marked with the same letters are not significantly different.

The phosphorus content in the azalea leaves was on average 2.0–3.6 g $P \cdot kg^{-1}$ d.m. (Table 1). Significantly higher contents of this element were detected in the leaves of plants receiving single fertilizers (control) compared to those fertilized with SRF and through fertigation. A similar significant effect of mycelium inoculation on phosphorus content was demonstrated (Figure 2) As reported by Aendekerk [1], the optimal phosphorus content is 0.37% P, whereas Kreij et al. [30] found 0.30–0.50% P in azalea leaves. Li et al. [32] found 0.08% P in plants fertilized without N increasing to 0.14% P in plants fertilized with 20 mm N, and the values depended on the type of container. All types of fertilization ensured optimal plant nutrition with phosphorus. Noteworthy is the gradual increase in the phosphorus content of plants with years of cultivation. In the available literature, phosphorus content below 0.15% P is considered critical. No such content was recorded in these studies, and no such value was recorded in the present study. Ristvey et al. [33] suggested that only very low constant levels of phosphorus are needed to support the

growth of young azaleas. In many fertilizers, the N: P ratio significantly exceeds plant requirements, and the optimal ratio of nitrogen to potassium in the substrate should be 20:1.

The potassium content in the leaves of the large-flowered azalea cv. 'Anneke' was on average 5.7–8.8 g K·kg⁻¹ d.m. in the mycelium-uninoculated variants and 6.7–7.4 g K·kg⁻¹ d.m. in the inoculated plants. The highest potassium level was determined in the leaves of control plants and the lowest content was detected in plants fertilized through fertigation. Noteworthy are the lower differences in the potassium content in the inoculated plants (Table 1). The standard potassium content in azalea leaves was estimated at 0.37% by Aendekerk [1] and 0.78–0.98% by Kreij et al. [30]. These results suggest a highly varied optimal range of potassium nutrition in these plants. The plants in the present study did not show symptoms of potassium deficiency, and the content of this element in the range of 5.0–9.0 g K·kg⁻¹ d.m. should be regarded as optimal.

The calcium content in the leaves of the large-flowered azalea cv. 'Anneke' ranged from 7.5 to 10.9 g Ca·kg⁻¹ d.m. (Table 1). Its highest level was determined in plants fertilized with the slow-acting fertilizer (Hortiform pH). It was slightly lower in the fertigation variant, and lowest in the single-fertilizer treatment (control) (Figure 1). Moreover, mycelium inoculation contributed to a significant increase in the calcium content in the plants (Figure 2). The standard calcium content in azalea leaves was shown to be 1.60–2.00% by Kreij et al. [30], 0.72–1.39% by Aendekerka [1] and 0.60–1.20% by Michałojć and Koter [31]. These data indicate a very wide optimal range of calcium nutrition in plants. Our observations of the plants carried out during the growing season did not reveal any symptoms of calcium deficiency.

The content of magnesium in the leaves of the large-flowered azalea cv. 'Anneke' was in the range of 1.5-2.6 g Mg·kg⁻¹ d.m. There was a slight variation in the magnesium content between the study years (Table 1). The fertilization methods and mycelium inoculation had no significant effect on the magnesium content in the plants (Figures 1 and 2). The standard magnesium content in azalea leaves were estimated at 0.60% by Aendekerk [1], 0.17–0.33% by Kreij et al. [30] and 0.14–0.25% by Michałojć and Koter [31]. In turn, the critical magnesium contents in Ericaceae plants were 0.07% and 0.09%, as reported by Kreij et al. [30] and Aendekerka [1], respectively. The fertilization applied in the present study ensured optimal magnesium level in the plants.

The assessment of the effect of the mycorrhization inoculum with Oidiodendron and Hynemoscyphus sp. fungi on the nutritional status of the large-flowered azalea showed similar levels of the analyzed nutrients in the inoculated and uninoculated plants in the first cultivation year. As shown in earlier studies, Michałojć et al. [27] mycorrhizal frequency on ERM inoculated roots of rhododendron ranged from 36% to 65%, and without inoculation it varied from 4% to 9%. 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 monocomponent fertilizers. A beneficial effect reflected in higher nitrogen, phosphorus, potassium, calcium and magnesium content was noted in the second and third years of cultivation of the 'Anneke' plants. Moreover, significantly higher levels of nitrogen, phosphorus and calcium were determined in the leaves of the mycelium-inoculated plants, whereas there was no clear effect on the content of potassium and magnesium (Table 1).

A study conducted by Nowak [34] reported no significant effect of mycorrhizae on the content of nitrogen, phosphorus, potassium, and calcium, but a higher level of magnesium was noted. In turn, Smith and Read [14] and Konieczny and Kowalska [18] confirmed the beneficial effect of mycorrhizae on the phosphorus and nitrogen content in plants.

The same amounts of nutrients were supplied to the plant substrate in all combinations $(g/plant^{-1}/year)$: 1.02 N, 0.48 P, 0.9 K, and 0.18 Mg. A similar dose was used in the fertigation treatment, i.e., (in mg·L⁻¹): 14 N-NH₄, 56 N-NO₃, 15 P; 58 K, 60 Ca, 12 Mg, 16 S-SO₄, 0.4 Fe, 0.3 Mn, 0.2 Zn, 0.06 Cu, 0.1 B, and 0.01 Mo. The medium with this composition was used every other day from the beginning of May to mid-September in each study year. As shown by Aendekerk [1] and Davidson et al. [35] the standard content of macronutrients and the values of pH and salinity (EC) in high-peat substrates used for

cultivation of ornamental shrubs with low nutritional requirements, such as the azalea, are (mg·L⁻¹): N-min. 110–130 (N-NH₄:N-NO₃ in a ratio of 1:10), 30–50 P, 120–140 K, 80–140 Mg, 30–50 S-SO₄, pH 4.5–5.8 and EC < 0.8 mS·cm⁻¹.

The analyses of the substrate were conducted to determine nutrient richness in the middle of the growing season (1st ten days of August) and after the growing season (second ten days of October).

Table 1. Effect of type of fertilization and mycorrhization on content of N-total, P, K, Ca and Mg ($g \cdot kg^{-1} d.m.$) in leaves of azalea cv. 'Anneke'.

Mycorrhizae (A)	Type of Fertilization (B)	Years	N-Total	Р	K	Ca	Mg
Without ERM (M-)	Control	Ι	17.8	2.50	7.40	7.50	1.60
		II	19.4	3.00	7.40	7.80	1.50
		III	20.3	3.60	8.80	7.80	1.80
	Mean for years		19.2 b	3.00 a	7.90 a	7.70 d	1.60
		Ι	20.5	2.30	6.30	9.80	2.10
	Hortiform pH (SRF)	II	20.3	2.40	6.80	9.20	1.90
		III	20.8	2.60	6.90	8.80	1.50
	Mean for years		20.5 a	2.40 bc	6.70	9.30 b	1.80
	Fertigation	Ι	13.9	2.00	5.70	8.80	2.60
		Π	13.1	2.20	6.80	8.00	1.80
		III	16.1	2.30	7.80	8.80	1.70
	Mean for years		14.4 d	2.22 c	6.80	8.50 c	2.00
	Control	Ι	17.5	2.50	7.30	8.10	1.80
		Π	19.6	3.00	6.90	8.00	1.70
		III	20.5	3.30	7.20	9.10	1.60
	Mean for years		19.2 b	2.90 a	7.10 b	8.40 cd	1.70
MAL EDM (M.)	Hortiform pH (SRF)	Ι	19.1	2.60	6.90	10.9	2.30
With EKM (M+)		II	20.9	2.90	7.00	9.60	1.80
		III	23.5	3.00	7.40	9.80	1.60
	Mean for years		21.2 a	2.80 ab	7.10 b	10.1 a	1.90
	Fertigation	Ι	12.5	2.10	6.70	10.6	2.10
		II	16.0	2.30	7.00	9.20	1.50
		III	17.2	2.50	7.20	10.4	1.50
		15.2 c	2.30 c	7.00 b	10.0 ab	1.70	
General mean			18.3	2.60	7.10	9.00	1.80
LSD _{0.05}							
for $A \times B$			1.00	0.40	0.50	0.70	n.s.

Without ERM (M-) = nonmycorrhizal plants; with ERM (M+) = mycorrhizal plants; n.s. = nonsignificant. Results marked with the same letters are not significantly different.

The present results demonstrated high nitrogen utilization by plants already in the middle of the growing season, and traces of this element after its end (Figure 3). In the analyzed period, the reduced content of mineral nitrogen was determined in the substrate of azaleas with mycorrhizal-inoculated roots (Figure 4). This proves that the inoculated plants took up nitrogen from the substrate more efficiently, as evidenced by the higher nitrogen content in the inoculated plants. Numerous studies indicate a beneficial effect of mycorrhizal fungi on nitrogen uptake from the substrate [36–39]. The highest content of N-min. in the substrate was found in the slow-release fertilizer Hortiform pH variant, whereas the lowest value was determined in the fertigation combination. These results indicate that slow-release fertilizers successfully supply nitrogen and ensured an appropriate degree of plant nutrition with this element. The fertilization of azaleas through fertigation requires



further research. A study conducted by Michałojć et al. [27] demonstrated the lowest growth and development of shoots after application of fertigation, whereas the present study showed the lowest content of nutrients in plant leaves.

Figure 2. Influence of mycorrhization on concentration N-total, P, K, Ca and Mg ($g \cdot kg^{-1} d.m.$) in leaves of azalea cv. 'Anneke'. Mean from years. Without ERM (M-) = nonmycorrhizal plants; with ERM (M+) = mycorrhizal plants. Results marked with the same letters are not significantly different.

In the substrate used for cultivation of azaleas, higher content of available phosphorus was noted after the application of single fertilizers (control), which indicates that the plants absorbed it at a slower rate (Figure 3). Additionally, its lower content was determined in the substrate of plants treated with the mycorrhizal inoculum, compared with the uninoculated substrate, which indicates that the plants utilized the element more efficiently (Figure 4). Noteworthy is that trace amounts of this component were found in the first and second analysis terms. This proved phosphorus uptake from the substrate by plants, as confirmed by its higher content in the plant leaves. Mycorrhizal fungi have been reported to enhance phosphorus uptake by plants [37].

A higher potassium level in the substrate was recorded in both periods after the application of single mineral fertilizers, whereas the lowest content was found in the fertigation variant (Figure 3). Moreover, mycorrhization resulted in higher utilization of potassium by the plants; hence, its lower content in the substrate from the cultivation of the inoculated plants (Figure 4). The application of mycorrhizae has a positive effect on the uptake of macroelements, including potassium, by plants [40]. This was fully confirmed in the present study.

Calcium is an element regulating soil/substrate pH in addition to its physiological functions in plants. In the first analysis term in the present study, the highest calcium content in the azalea substrate was shown for the slow-release fertilizer Hortiform pH combinations. Slightly lower levels were determined after the application of single fertilizers, and the lowest values were detected in the fertigation variant. In turn, the calcium content in the substrate in the second analysis term was more homogeneous (Figure 3). As in the case of the nutrients discussed above, a lower level of the element was found in the substrate of the mycorrhiza-inoculated plants compared to the uninoculated combinations (Figure 4). This proves the beneficial effect of mycorrhizal fungi on calcium uptake from the substrate. Similar findings have been reported in other studies [40].

Regardless of the term of the analyses, the highest magnesium content in the substrate was recorded in the fertigation combinations and the lowest value was noted in control variants (Figure 3). In turn, in the middle of the growing season (term I), the content of magnesium in the substrate was similar, whereas lower amounts of the element were determined in the substrate of the inoculated plants (Figure 4). Studies conducted by [37,40] indicated a beneficial effect of mycorrhizal fungi on the uptake of magnesium by plants.

The presence of sulfur in the substrate of the analyzed plants derived from the sulfate fertilizers applied. Throughout the study, the content of the element in the middle of the growing season ranged on average from 80 mg to 138 mg S-SO₄·L⁻¹. After the growing season (term II), its content was substantially lower (18–62 mg S-SO₄·L⁻¹). The highest sulfur level was demonstrated in the Hortiform pH-fertilized combinations, and the lowest amount was noted in the fertigation variant (Figure 3). Higher efficiency of sulfur utilization was noted in the mycorrhizal-inoculated combinations, as evidenced by its lower content in the substrate (Figure 4). As specified by Komosa [41], the standard sulfur content in the substrate for ornamental plants with low nutritional requirements was established at 30 mg to 50 mg·S-SO₄ L⁻¹. Studies conducted by [37,40] also confirmed the beneficial effect of mycorrhiza on the uptake of this nutrient by plants.



Figure 3. Cont.



Figure 3. Concentration of N-min., P, K, Ca, Mg and S-SO₄ (mg·L⁻¹) dependent of type of fertilization and analysis term in the azalea substrate. Average from years. I = first decade of August; II = third decade of October.



Figure 4. Concentration of N-min., P, K, Ca, Mg S-SO₄ (mg·L⁻¹) dependent of micorrhization and analysis term in azalea substrate. Average from years. I = first decade of August; II = third decade of October; without ERM (M-) = nonmycorrhizal plants; with ERM (M+) = mycorrhizal plants.

Azaleas have specific requirements in terms of soil/substrate reaction. Peat with a pH value of 4.6 was used as a substrate in the study. During the vegetation period, the reaction of the substrate fluctuated from 4.09 to 5.79 in the individual study years (Table 2). The optimal reaction of the substrate is in the range of pH 4.5–5.8 [35]. The fertilization methods applied in the study ensured the optimum pH of the substrate in the azalea cultivation.

Cultivation of plants in containers is associated with a risk of excessive salt concentration in the substrate. The present experiments showed that the doses and methods for application of the fertilizers did not elevate salt concentrations in the substrate. The salt concentration in the azalea substrate during the study period was on average 0.47 mS·cm⁻¹ in the middle of the growing season and 0.38 mS·cm⁻¹ at the end (Table 2). The acceptable salinity (EC) for ornamental plants with low and medium nutritional requirements ranges from 0.80 to 1.10 mS·cm⁻¹ [41] Furthermore, the presence of mycelium in the plant root zone contributes to elimination of excessive salt concentration in the substrate and, consequently, its negative impact on plants [42].

	Type of Fertilization (B)	pH						EC ***	
Mycorrhizae (A)		1st Year		2nd Year		3rd Year		mS·cm ^{−1}	
		I *	II **	Ι	II	Ι	II	Ι	II
Without ERM (M-)	Control	4.51	4.94	4.29	5.77	5.00	5.15	0.47	0.31
	Hortiform pH	4.28	5.11	4.19	5.57	4.28	4.52	0.65	0.48
	Fertigation	4.25	5.08	4.45	5.28	4.27	5.25	0.31	0.24
With ERM (M+)	Control	4.23	5.03	4.30	5.69	4.46	4.63	0.45	0.42
	Hortiform pH	4.31	4.68	4.25	5.79	4.04	4.31	0.67	0.57
	Fertigation	4.09	4.52	4.70	5.28	4.20	5.38	0.28	0.24
Total range		4.09–5.11		4.19–5.79		4.04–5.38		0.47	0.38

Table 2. Effect of fertilization and mycorrhization on reaction pH $_{(H2O)}$ and salinity EC (mS·cm⁻¹) in azalea substrate.

I * = analysis first decade of August, II ** = analysis third decade of October. EC *** = average in years.

4. Conclusions

- 1. As indicated in the first three years of azalea cultivation in containers, single mineral fertilizers and Hortiform pH (SRF) ensured an appropriate plant nutritional status in terms of the content of nitrogen, phosphorus, potassium, calcium and magnesium, while fertigation required further improvement of the nutrient solution.
- 2. Plant mycorrhization exerted a beneficial effect on plant nutrition, as higher contents of macro elements in plants were demonstrated in the second and third years of azalea cultivation.
- 3. The nutrients applied were largely utilized by the plants by the middle of the growing season, and almost complete utilization of the elements was noted at the end of the growing season. Therefore, the nutrients need to be supplemented in each subsequent year of vegetation.

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References

- 1. Aendekerk, T. Fertilization Guide for Nursery Crops; Boomteelt, Praktijkonderzoek: Boskoop, The Netherlands, 1997.
- 2. Hamim, A.; Miché, L.; Douaik, A.; Mrabet, R.; Ouhammou, A.; Duponnois, R.; Hafidi, M. Diversity of fungal assemblages in roots of Ericaceae in two Mediterranean contrasting ecosystems. *C. R. Biol.* **2017**, *340*, 226–237. [CrossRef] [PubMed]
- Vohník, M.; Sadowsky, J.J.; Kohout, P.; Lhotáková, Z.; Nestby, R.; Kolařík, M. Novel Root-Fungus Symbiosis in Ericaceae: Sheathed Ericoid Mycorrhiza Formed by a Hitherto Undescribed Basidiomycete with Affinities to Trechisporales. *PLoS ONE* 2012, 7, e39524. [CrossRef] [PubMed]
- 4. Douds, D.D., Jr.; Johnson, C.R.; Koch, K.E. Carbon cost of the fungal symbiont relative to net leaf P accumulation in a split-root VA mycorrhizal symbiosis. *Plant Physiol.* **1988**, *86*, 491–496. [CrossRef]
- 5. Bi, G.; Scagel, C.F.; Fuchigami, L.H.; Regan, R.P. Differences in growth, and nitrogen uptake and storage between two containergrown cultivars of Rhododendron. *J. Environ. Hortic.* 2007, 25, 13–20. [CrossRef]
- 6. Marczyński, A. Kierunki Rozwoju Szkółkarstwa Ozdobnego w Polsce. Monografia: Ogrodnictwo Ozdobne Sektorem Gospodarki Narodowej Red; Rabiza-Świder, J., Skutnik, E., Eds.; Wyd. SGGW: Warszawa, Poland, 2013; pp. 109–114. (In Polish)
- Bosiacki, M.; Golcz-Polaszewska, M.; Kozik, E. Slow-release fertilizers in the production of horticultural plants. Part I. Effect of Osmocote exact standard fertilizer on the growth and condition of nourishing of selected taxons of ornamental trees and shrubs. J. Res. Appl. Agric. Eng. 2009, 54, 29–35.
- 8. Michałojć, Z.; Koter, M. Effect of fertilization and mycorrhizal on growth and nutritional status of cranberry (*Vacinnium macrocarpon* Ait.) in the nursery. *J. Hortic. Res.* **2015**, *23*, 49–56. [CrossRef]

- 9. Wei, X.; Chen, J.; Zhang, C.; Pan, D. A new *Oidiodendron maius* strain isolated from *Rhododendron fortunei* and its effects on nitrogen uptake and plant growth. *Front. Microbiol.* **2016**, *7*, 1327. [CrossRef]
- Douglas, G.C.; Heslin, M.C.; Reid, C. Isolation of *Oidiodendron maius* from Rhododendron and ultrastructural characterization of synthesized mycorrhizas. *Can. J. Bot.* 1989, 67, 2206–2212. [CrossRef]
- 11. Perotto, S.; Perotto, R.; Faccio, A.; Schubert, A.; Varma, A.; Bonfante, P. Ericoid mycorrhizal fungi: Cellular and molecular bases of their interaction whit the host plant. *Can. J. Bot.* **1995**, *73*, 557–568. [CrossRef]
- 12. Kerley, S.J.; Read, D.J. The biology of mycorrhiza in the Ericaceae XX. Plant and mycorrhizal necromass as nitrogenous substrates for the ericoid mycorrhizal fungus *Hymenoscyphus ericae* and its host. *New Phytol.* **1998**, *139*, 353–360. [CrossRef]
- Casarrubia, S.; Daghino, S.; Kohler, A.; Morin, E.; Khouja, H.R.; Daguerre, Y.; Vigneault-Fourrey, C.; Martin, F.M.; Perotto, S.; Martino, E. The hydrophobin-like mSSP1 may be an effector in the ericoid mycorrhizal symbiosis. *Front. Plant Sci.* 2018, *9*, 546. [CrossRef]
- 14. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*; Elsevier: Amsterdam, The Netherlands, 2008.
- 15. Kubiak, J. Mikoryzacja roślin i aplikacja szczepionek mikoryzowych. Probl. Inż. Rol. 2005, 2, 25–32. (In Polish)
- Hardoim, P.R.; van Overbeek, L.S.; Berg, G.; Pirttilä, A.M.; Compant, S.; Campisano, A.; Döring, M.; Sessitsch, A. The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiol. Mol. Biol. Rev.* 2015, *79*, 293–320. [CrossRef] [PubMed]
- 17. Lo Presti, L.; Lanver, D.; Schweizer, G.; Tanaka, S.; Liang, L.; Tollot, M.; Zuccaro, A.; Reissmann, S.; Kahmann, R. Fungal effectors and plant susceptibility. *Annu. Rev. Plant Biol.* **2015**, *66*, 513–545. [CrossRef]
- Konieczny, A.; Kowalska, I. Arbuscular Mycorrhiza—Partner in communication. Acta Sci. Pol. Hortorum Cultus 2017, 16, 73–78.
 [CrossRef]
- Casarrubia, S.; Martino, E.; Daghino, S.; Kohler, A.; Morin, E.; Khouja, H.R.; Murat, C.; Barry, K.W.; Lindquist, E.A.; Martin, F.M.; et al. Modulation of Plant and Fungal Gene Expression upon Cd Exposure and Symbiosis in Ericoid Mycorrhizal *Vaccinium myrtillus*. *Front. Microbiol.* 2020, *9*, 341. [CrossRef] [PubMed]
- Księżniak, A.; Orlikowski, L.B.; Szałański, W.; Wróblewska, B. Effect of ericoid mycorrhizal inoculants on the growth, development and healthiness of rhododendronvariety Nova Zembla and Northern highbush blueberry variety Bluecrop. *Prog. Plant Prot.* 2013, 53, 190–195. (In Polish)
- 21. Krupa, A. Mikoryzy i ich wielofunkcyjna rola w środowisku. Chem. Environ. Biotechnol. 2010, 14, 175–182.
- 22. Koide, R.T.; Mosse, B. A history of research on arbuscular mycorrhiza. Mycorrhiza 2004, 14, 145–163. [CrossRef]
- Almario, J.; Jeena, G.; Wunder, J.; Langen, G.; Zuccaro, A.; Coupland, G.; Bucher, M. Root-associated fungal microbiota of nonmycorrhizal Arabis alpinaand its contribution to plant phosphorus nutrition. *Proc. Natl. Acad. Sci. USA* 2017, *114*, E9403–E9412. [CrossRef]
- 24. Van der Heijden, M.G.A.; Martin, F.M.; Selosse, M.A.; Sanders, I.R. Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytol.* 2015, 205, 1406–1423. [CrossRef] [PubMed]
- Labidi, S.; Ben Jeddi, F.; Tisserant, B.; Debiane, D.; Rezgui, S.; Grandmougin-Ferjani, A.; Lounès-Hadj Sahraoui, A. Role of arbuscular mycorrhizal symbiosis in root mineral uptake under CaCO₃ stress. *Mycorrhiza* 2012, 22, 337–345. [CrossRef]
- 26. Cairney, J.W.G.; Sawyer, N.A.; Sharples, J.M.; Meharg, A.A. Intraspecific variation in nitrogen source utilization by isolates of the ericoid mycorrhizal fungus *Hymenoscyphus ericae* (Read) Korf and Kernan. *Soil Biol. Biochem.* **2000**, *32*, 1319–1322. [CrossRef]
- 27. Michałojć, Z.; Koter, M.; Dzida, K.; Jarosz, Z.; Pitura, K.; Jamiołkowska, A.; Księżniak, A. Influence of fertilization and mycorrhiza on growth and development of rhododendron (Rhododendron hybridum) in a nursery. J. Elem. 2019, 24, 1349–1361. [CrossRef]
- 28. Ostrowska, A.; Gawliński, S.; Szczubiałka, Z. Metody Analizy i Oceny Właściwości Gleb i Roślin; Instytut Ochrony Środowiska: Warszawa, Poland, 1991.
- 29. Yin, L.J.; Zhang, C.Y.; Yang, B. Characteristics of nitrogen absorbed by ericoid mycorrhizal fungi and impact on growth of Rhododendron fortunei. *Sci. Agric. Sin.* **2010**, *43*, 868–872.
- 30. De Kreij, C.; Sonneveld, C.; Warmenhoven, M.G.; Straver, N. Normen Voor Gehalten Aan Voedingselementen Van Groenten En Bloemen Onder Glas. *Ser. Voedingsplossingen Glas.-Bouv* **1990**, *15*, 23.
- 31. Michałojć, Z.; Koter, M. Effect of different fertilization on growth and nutrition of azalea (*Rhododendron* L.). Acta Agrobot. 2012, 65, 123–132. [CrossRef]
- 32. Li, T.; Bi, G.; Harkess, R.L.; Blythe, E.K. Mineral nutrient uptake of Encore Azalea 'Chiffon' affected by nitrogen, container, and irrigation frequency. *HortScience* **2019**, *54*, 2240–2248. [CrossRef]
- 33. Ristvey, A.G.; Lea-Cox, J.D.; Ross, D.S. Nitrogen and phosphorus uptake efficiency and partitioning of container-grown azalea during spring growth. *J. Am. Soc. Hortic. Sci.* 2007, 132, 563–571. [CrossRef]
- 34. Nowak, J. Effects of mycorrhization and phosphorus nutrition on nutrient uptake, growth and flowering of China aster (*Callistephus chinensis* L. Nees) cultivated on ebb-and-flow benches. *Acta Agrobot.* **2009**, *62*, 77–81. [CrossRef]
- Davidson, H.; Meclenburg, R.; Peterson, C. Nursery Management; Administration and Culture, 4th ed.; Prentice-Hall, Inc.: Upper Saddle River, NJ, USA, 2000; p. 524.
- 36. Jakobsen, I.; Abbott, L.K.; Robson, A.D. External Hyphae of Vesicular-Arbuscular Mycorrhizal Fungi Associated with *Trifolium subterraneum* L. I. Spread of Hyphae and Phosphorus Inflow into Roots. *New Phytol.* **1992**, *120*, 371–380. [CrossRef]
- 37. Hawkins, H.J.; Johansen, A.; George, E. Uptake and Transport of Organic and Inorganic Nitrogen by Arbuscular Mycorrhizal Fungi. *Plant Soil* **2000**, *226*, 275–285. [CrossRef]

- Jin, H.; Pfeffer, P.E.; Douds, D.D.; Piotrowski, E.; Lammers, P.J.; Shachar-Hill, Y. The Uptake, Metabolism, Transport and Transfer of Nitrogen in an Arbuscular Mycorrhizal Symbiosis. New Phytol. 2005, 168, 687–696. [CrossRef]
- Püschel, D.; Janoušková, M.; Hujslová, M.; Slavíková, R.; Gryndlerová, H.; Jansa, J. Plant-fungus competition for nitrogen erases mycorrhizal growth benefits of Andropogon gerardii under limited nitrogen supply. *Ecol. Evol.* 2016, *6*, 4332–4346. [CrossRef]
 Marschner, H.; Dell, B. Nutrient uptake in mycorrhizai symbiosis. *Plant Soil* 1994, *159*, 89–102. [CrossRef]
- Komosa, A. Żywienie roślin ozdobnych. In *Rabiza-Świder. Biologia Roślin Ozdobnych. Wybrane Zagadnienia;* Starc, Z., Ed.; Wyd. SGGW: Warszawa, Poland, 2015; pp. 59–123.
- 42. Porras-Soriano, A.; Soriano-Martin, L.A.; Porras-Piedra, A.; Azcon, R. Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *J. Plant Physiol.* **2009**, *166*, 1350–1359. [CrossRef]