EFFECTS OF HIGH CONCENTRATIONS OF CALCIUM SALTS IN THE SUBSTRATE AND ITS pH ON THE GROWTH OF SELECTED RHODODENDRON CULTIVARS

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ABSTRACT

For proper growth and development, rhododendrons need acidic soils, whereas calcium carbonate ($CaCO_3$) in the substrate markedly limits their growth. In this study, we analysed the reactions of rhododendrons to high concentrations of calcium salts and pH in the substrate. We used 4-month-old seedlings of *Rhododendron* 'Cunningham's White' and 1.5-year-old seedlings and rooted cuttings of *R.* 'Cunningham's White' and *R.* 'Catawbiense Grandiflorum'. Their reactions depended mostly on calcium salt type added to the substrate (sulphate or carbonate). An increase in concentrations of phenolic compounds was detected mostly in roots of the plants grown in a substrate with a high calcium carbonate content. Addition of calcium salts to the substrate caused a significant rise in total nonstructural carbohydrates in leaves and roots of the studied plants. As compared to the control, an increase in substrate pH in the variant with calcium carbonate limited the activity of acid phosphatase, while lowering of substrate pH in the variant with calcium sulphate, significantly increased its activity. Along with the rise in substrate pH, a remarkable increase was observed in the activity of nonspecific dehydrogenase (DHA) in the substrate with $CaCO_3$, as compared to the control. Unfavourable soil conditions (high calcium content and alkaline pH) caused a decrease in assimilation of minerals by the studied plants (mostly phosphorus and manganese). Our results show that the major factor limiting rhododendron growth is an increase in substrate pH, rather than an increase in the concentration of calcium ions.

KEY WORDS: root development, shoot development, carbohydrates, phenols, acid phosphatase, nonspecific dehydrogenase, pH, minerals.

INTRODUCTION

For proper growth and development, rhododendrons need acidic soils, with pH 4.0-5.5 (Czekalski 1991; Tiwari and Chauhan 2005). The presence of calcium carbonate ($CaCO_3$) in the substrate, via alkalization of the rhizosphere, markedly limits their growth. Excessive calcium uptake by a plant may lead to disturbances in ion balance, to the disadvantage of other nutrients (such as potassium and magnesium), or to changes in cytosol pH and a decrease in solubility of some ions, e.g. of iron (Chaanin and Preil 1992; Balakrishnan et al. 2000). It is very important in what form the calcium salts are supplied and if are soluble in the substrate. All changes in environmental factors deviating from optimum growth conditions are more or less stressful to plants. Plants can use the same or similar defence mechanisms in response to various stress factors. Plant growth in extreme conditions (e.g. for rhododendrons: in substrates with a high calcium content and pH) causes changes in their morphology and in numerous metabolic processes. A majority of studies of the influence of calcium on rhododendrons are concerned with observations of external symptoms and changes, which are reflected mostly in leaf chlorosis and poor plant growth. A major aim of this study was to describe the changes that take place in rhododendrons under the influence of high concentrations of calcium carbonate or sulphate on selected metabolic processes.

In view of the important role played by phenolic compounds in plant protection, mostly against infection by pathogens and pests, they are stored in cells in some strategic sites, where they are involved in signalling or directly in defence responses to stress conditions (Beckman 2000).

Sugars are important metabolites, used by plants in defence responses under the influence of various stress factors. Environmental stresses induce changes in concentrations of soluble carbohydrates and starch, which is reflected in modified activity of the enzymes participating in carbohydrate metabolism (Szadel and Lorenc-Plucińska 2002).

An analysis of changes in the activity of acid phosphatase and nonspecific dehydrogenase in the substrate helps to explain the changes in plant metabolism observed after the addition of calcium salts to the substrate (Bojarczuk et al. 2002).

Specific requirements of ericaceous plants, including rhododendrons, in relation to substrate, markedly limit the possibilities of their cultivation. This study was aimed to improve our understanding of the mechanism of plant sensitivity to unfavourable soil conditions. Our results can aid in selection of rhododendrons tolerant to high concentrations of calcium salts in the substrate and a high substrate pH.

MATERIALS AND METHODS

Plant material and growth conditions

In the first part of the study, we used 4-month-old seedlings of *Rhododendron* 'Cunningham's White', grown in controlled environmental conditions (in a growth room at 22-23°C and fluorescent light intensity of 7.5 Wm^2 for 16 hours a day). They developed from seeds collected from 25-year-old shrubs growing in the Kórnik Arboretum (52°15'N and 17°04'E). The seeds were sown in early June, to a substrate placed in plastic containers (20×30 cm). The substrate was a mixture of peat (pH 4.0), soil from under rhododendron bushes growing in the Arboretum (pH 4.5), and perlite (pH 6.0) (v/v/v, 1:1:1). The substrate was supplemented with calcium sulphate $CaSO_4$ or calcium carbonate $CaSO_3$ (0.01, 0.05, or 0.1 mol · dm^{-3} of substrate). Control seedlings were grown in a substrate without addition of calcium salts. Four moths after sowing, seedling growth was assessed on the basis of root length, root area, as well as dry weight of the shoot and roots. Next the root : shoot dry weight ratio was calculated. Each variant was represented by three replications, and biometric data for each replication were means for 10 seedlings each. The measurements were made with the use of WinRhizo software (Regent Instruments Inc., Quebec, Canada). The data were subjected to analysis of variance, based on the Tukey test ($P = 0.05$).

In the second part of the study, we used 1.5-year-old seedlings of *R.* 'Cunningham's White' and 1.5-year-old rooted cuttings of *R.* 'Cunningham's White' and *R.* 'Catawbiense Grandiflorum'. At the beginning of June the seedlings and rooted cuttings were planted in pots (0.7 dm^3). After potting, the fully developed leaves from the previous growing season were removed, and only the young leaves from the current year were left. Each experimental variant was in five replicate and represented by 10 plants. The substrate was a mixture of peat (pH 4.0), soil from under rhododendron bushes (pH 4.5), and perlite pH 6.0 (v/v/v, 1:1:1). Depending on experimental variant, the substrate was mixed with calcium sulphate $CaSO_4$ (S) or calcium carbonate $CaCO_3$ (C), at a concentration of 0.05 mol · dm^{-3} of substrate. The control (K) was composed of rooted cuttings grown in a substrate without addition of calcium salts. The plants were kept in a plastic tunnel. Substrate moisture was maintained at a stable level (60-80% content of the water). Plant material from each experimental variant was collected for analyses 4 months after planting in the substrate. At the end of the experiment (October), we measured pH (in H_2O) of substrates and concentration of soluble forms of macro- and micronutrients. Plant material collected from 22-months-old seedlings and cuttings was used for physiological analyses of plant reactions to the increased concentration of calcium salts in the substrate.

Assays of phenolic compounds in leaves and roots

The concentration of total soluble phenolics (SP) was measured using Johnson and Schaal's (1957) method modified by Singleton and Rossi (1965), with Folin-Ciocalteu's Phenol Reagent. Phenolics from 0.1 g of dry plant material were extracted with 96% and 80% ethanol. Absorbance after the colour reaction was measured on a spectrophotometer (SECOMAM S 750, France) at $\lambda = 660$ nm. SP was calculated per 1 μM of chlorogenic acid · g^{-1} DW. Each experimental variant was represented by five replications.

Assays of carbohydrates in leaves and roots

Concentrations of sugars were measured colorimetrically: total nonstructural carbohydrates (TNC) with the use of anthrone (Haissig and Dickson 1979), while starch with dianisidine (Hansen and Møller 1975). A sample (0.02 g) of dry matter was extracted 3 times in 5 ml of methanol-chloroform-water (v/v/v, 1:1:1). The extract was used to assay TNC after a colour reaction with anthrone ($\lambda = 625$ nm). TNC content was expressed as % of dry weight (DW). After hydrolysis by means of amyloglucosidase, and oxidation by means of the peroxidase-glutathione oxidase complex, the precipitate was used to measure starch content. For this purpose, a colour reaction with O-dianisidine dihydrochloride was applied ($\lambda = 450$ nm). Colorimetric measurements were made by a DU 640 spectrophotometer (Beckman 2000). Glucose was used as a standard for TNC and starch assays. Each experimental variant was represented by five replications.

Assays of acid phosphatase activity

The substrate was placed on Petri dishes and dried at room temperature for 1 h. Depending on experimental variant, the substrate was supplemented with: 0.2 M phosphate buffer (pH 6.5), 0.115 M *p*-nitrophenyl phosphate, and toluene. The substrate was incubated for 1 h at 37°C. To stop the reaction, we added to each variant 2 ml of 0.5 M NaOH, and 0.5 ml of 0.5 M $CaCl_2$. Next, the mixture was filtered through blotting paper and the filtrate was. The amount of released *p*-nitrophenol, used as a measure of the activity of acid phosphatase diluted 10-fold, was measured spectrophotometrically at $\lambda = 400$ nm. Extinction values for the control and the sample were determined with respect to a blank. The control value was subtracted from the value for the sample. The difference was used to estimate *p*-nitrophenol content on the basis of the standard curve for *p*-nitrophenol, and expressed in mg of nitrophenol · g^{-1} DW · h^{-1} (Tabatabai and Bremner 1969). The analyses were performed in 3 replications.

Activity of nonspecific dehydrogenase (DHA) in the substrate

Nonspecific dehydrogenase activity was assayed using the tetrazolium method (Thalmann 1968) modified by Rossel et al. (1997). Depending on experimental variant, the substrate was supplemented with 0.5 M Tris buffer pH 8.0 and 1% TTC (2,3,5-triphenyltetrazolium chloride). The mixture was incubated for 24 h at 30°C. To stop the reac-

tion, in each variant 30 ml of 96% ethanol was added, and shaken for 2 min on a rotary shaker. Next the mixture was filtered through blotting paper. The concentration of TTF (triphenyltetrazolium formazan), as an indicator of activity of nonspecific dehydrogenase, was measured spectrophotometrically at $\lambda = 480$ nm. The control value was subtracted from the value for the sample. The difference was used to estimate formazan content on the basis of the standard curve for INT-formazan, and expressed in mg of formazan $\cdot$ g^{-1} DW $\cdot$ $(24\ h)^{-1}$. The analyses were performed in 3 replications.

Quantitative analysis of nutrients in the substrate

In the substrate, concentrations of bioavailable ions were estimated (Ca, Mg, S-SO_4, P, Na, Fe, and Mn). The nutrients were assayed in 0.03 N acetic acid extract, using atomic emission spectroscopy. Additionally, we estimated in 1 N HCl extract the concentration of total Fe and Mn in the substrate. Also substrate pH was measured in each variant. The analyses were performed at the Central Analytic Laboratory, Research Institute of Pomology and Floriculture, Skierniewice, Poland.

Assays of nutrients in leaves and roots

The leaves and roots were dried at 65°C for 48 h. The total concentrations of selected elements were measured in leaves and roots as follows: Ca, Mg, S, P, Na, Fe, Mn – wet digestion at room pressure (open system in an extract of nitric acid with chlorous acid 4:1) using inductively coupled plasma atomic emission spectroscopy (ICP-AES) and C without pre-treatment using C/N Analyzer (Vario-Max).These analyses were preformed in the Laboratory of Environmental Chemistry, Forest Research Institute, Warsaw, Poland.

Statistical analysis

The data were subjected to one-way analysis of variance. The Tukey test was used for pair-wise comparisons ($P = 0.05$).

RESULTS AND DISCUSSION

Calcium plays a key role in many physiological processes in plants. For example, it controls the activity of many enzymes as a secondary mediator of environmental information, and participates in mechanisms of water and nutrient uptake (Starck 2002). A small amount of calcium salts in the substrate stimulates seedling growth and rooting of rhododendron cuttings (Czekalski 1991; Bojarczuk 1995). An excessively high calcium content of the substrate limits their growth and development. The reactions of rhododendrons to high concentrations of calcium ions in the substrate depend on the taxon and chemical form in which they are supplied to plants (Giel and Bojarczuk 2002).

In this study, an increase in calcium content of the substrate caused significant changes in seedling growth of *R.* 'Cunnigham's White'. An increase in $CaCO_3$ content of the substrate, in contrast to $CaSO_4$, changed its pH from acidic to alkaline (Table 1). The presence of $CaCO_3$ in the substrate (at a concentration of 0.05 mol $\cdot$ dm^{-3} of substrate) caused a significant inhibition of root and shoot growth, as compared to the control (Fig. 1). An increase in $CaCO_3$ content resulted also in an increased root : shoot dry weight ratio (Fig. 1E). This is a characteristic reaction of plants growing in stressful conditions. Calcium sulphate added to the substrate, in contrast to calcium carbonate, did not limit plant growth, while in low concentrations (0.01 mol dm^{-3} of substrate) it even stimulated root growth (Fig. 1A-C). Calcium carbonate concentrations of 0.05-0.10 mol $\cdot$ dm^{-3} of substrate proved to be more toxic to rhododendron seedlings, although the concentration of bioavailable calcium ions in the substrate was nearly 2-fold lower than in the variant with calcium sulphate (Table 1). An increase in calcium carbonate content contributes to an increase in HCO_3^- ions in the substrate, which limits the uptake of iron ions by plants, or delays iron transport from roots to shoots. This may result in poorer plant growth and chlorosis of leaves (Drehmal and Preil 1992). Calcium sulphate, because of the acidifying effect of group SO_4^{-2}, does not increase the substrate pH, so it does not exceed the optimum values for rhododendron seedlings. This result confirms earlier findings about a low toxicity of gypsum to ericaceous plants (Drehmel and Preil 1992; Borkowska 1996).

The second part of this study was aimed to explain how the high calcium content and pH of the substrate affects some metabolic processes, e.g. synthesis of phenolic compounds, biological activity of the soil or bioavailability of nutrients to 1.5-year-old rhododendron cuttings and seedlings.

Assays of phenolic compounds in plant tissues are used to analyse effects of biotic and abiotic factors on the condition of the studied plants. Reactions of rhododendrons to calcium salts in the substrate varied depending on the applied salt. An increase in concentrations of phenolic compounds was detected mostly in roots of the plants grown in a substrate supplemented with calcium carbonate (Fig. 2). An increase in concentrations of phenolic compounds was recorded also in leaves of cuttings of *R.* 'Catawbiense Grandiflorum' in the variants with calcium sulphate and calcium carbonate (Fig. 3). Rhododendron cuttings with a very high rooting potential are characterized by a high concentration of phenolic compounds (total phenols, mono- and orthodiphenols), especially after auxin treatment (Bojarczuk

TABLE 1. Effects of calcium salts on Ca^{2+} content and pH of the substrate. Means marked with the same letter do not differ significantly ($P < 0.05$).

Salt	Ion content [mg $\cdot$ dm^{-3} of substrate]	Salt content [mol $\cdot$ dm^{-3} of substrate]			
		0	0.01	0.05	0.10
$CaSO_4$	Ca^{2+}	237±4.5 a	473±3.1 b	1807±62.1 c	3453±59.9 d
	pH	4.67	4.22	4.1	4.41
$CaCO_3$	Ca^{2+}	237±4.5 a	343±3.1 b	903±13.5 c	1932±33.2 d
	pH	4.67	4.95	6.24	7.66

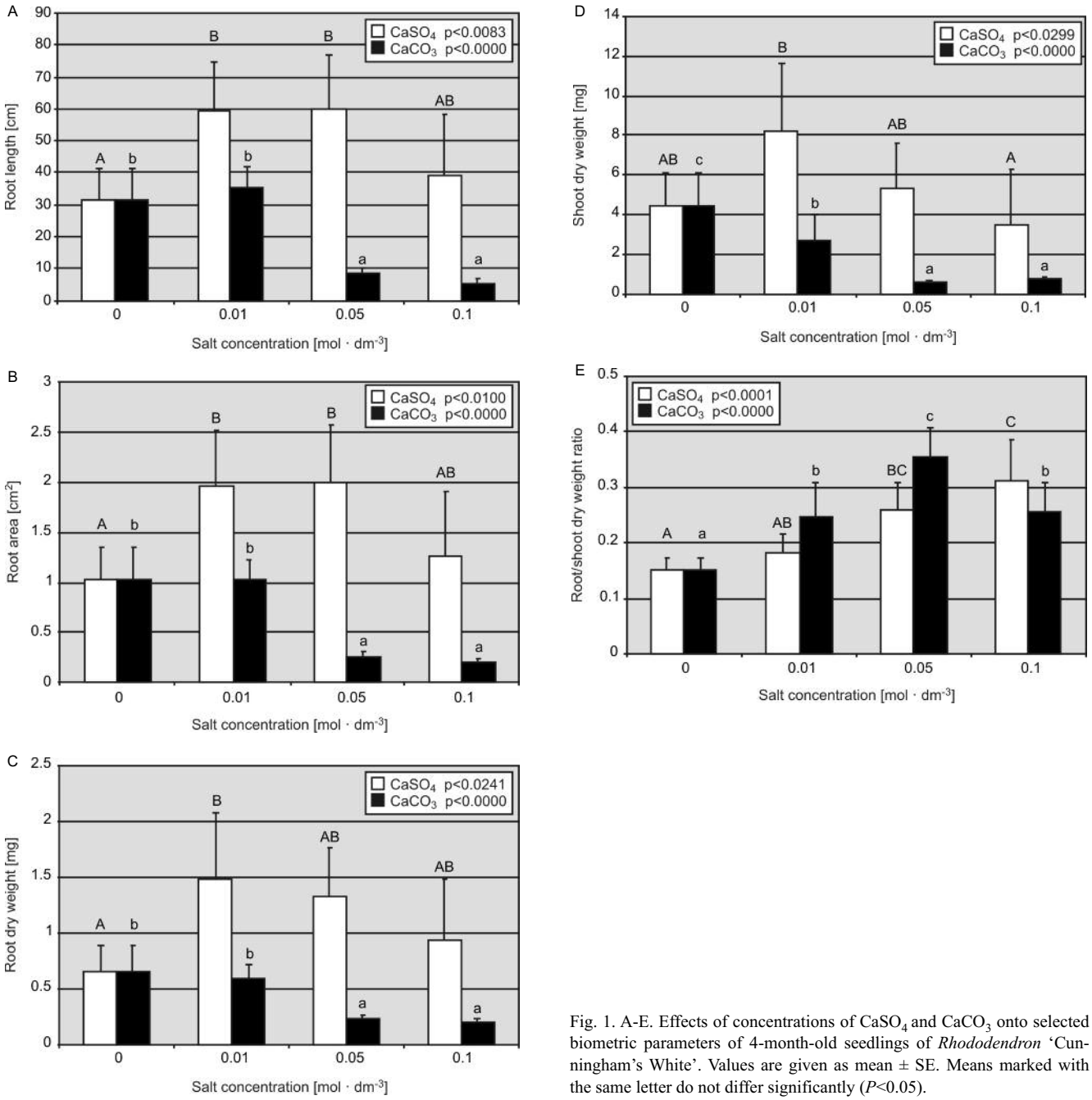

Fig. 1. A-E. Effects of concentrations of $CaSO_4$ and $CaCO_3$ onto selected biometric parameters of 4-month-old seedlings of *Rhododendron* 'Cunningham's White'. Values are given as mean ± SE. Means marked with the same letter do not differ significantly (P<0.05).

1995). In view of the protective function, performed by phenolic compounds in plants, an increase in their concentration in the studied rhododendron cuttings and seedlings grown in a substrate with calcium carbonate may suggest that the plants are stressed by the increase in $CaCO_3$ content of the substrate and the resultant increase in substrate

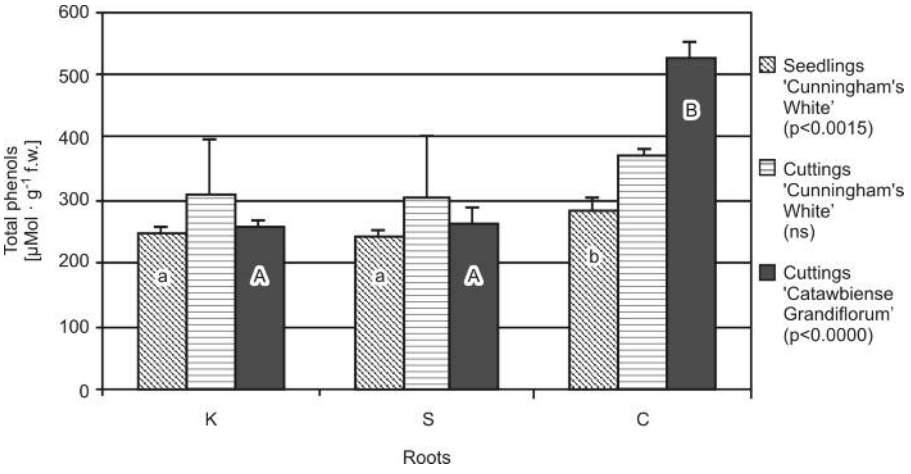

Fig. 2. Effects of calcium sulphate (S) and calcium carbonate (C) on concentrations of soluble phenolics (SP) in rhododendron roots. Salt concentration: 0.05 mol · dm^{-3} of substrate. (K = control). Other data as in Figure 1.

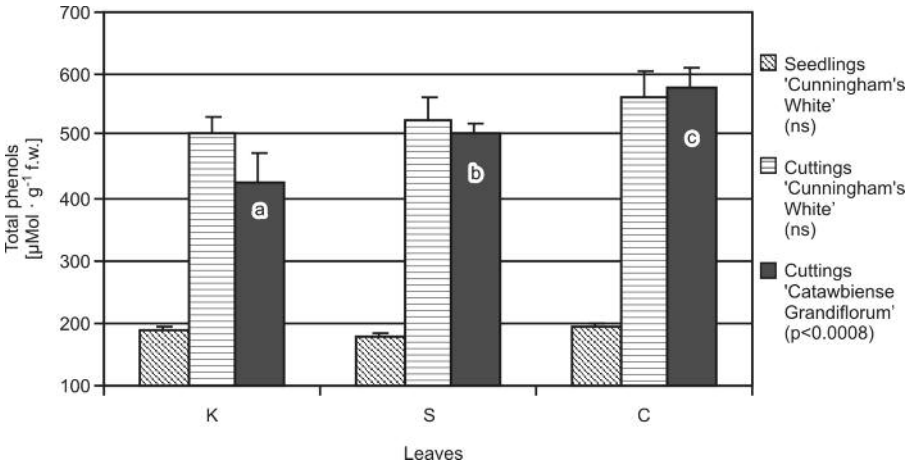

Fig. 3. Effects of calcium sulphate (S) and calcium carbonate (C) on concentrations of soluble phenolics (SP) in rhododendron leaves. Salt concentration: 0.05 mol · dm^{-3} of substrate. (K = control). Other data as in Figure 1.

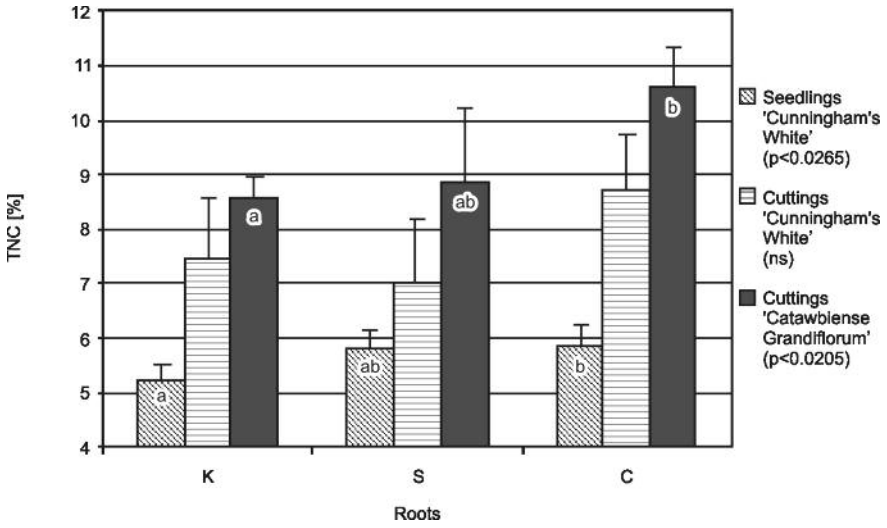

Fig. 4. Effects of calcium sulphate (S) and calcium carbonate (C) on the percentage of total nonstructural carbohydrates (TNC) in dry weight of rhododendron roots. Salt concentration: 0.05 mol · dm^{-3} of substrate. (K = control). Other data as in Figure 1.

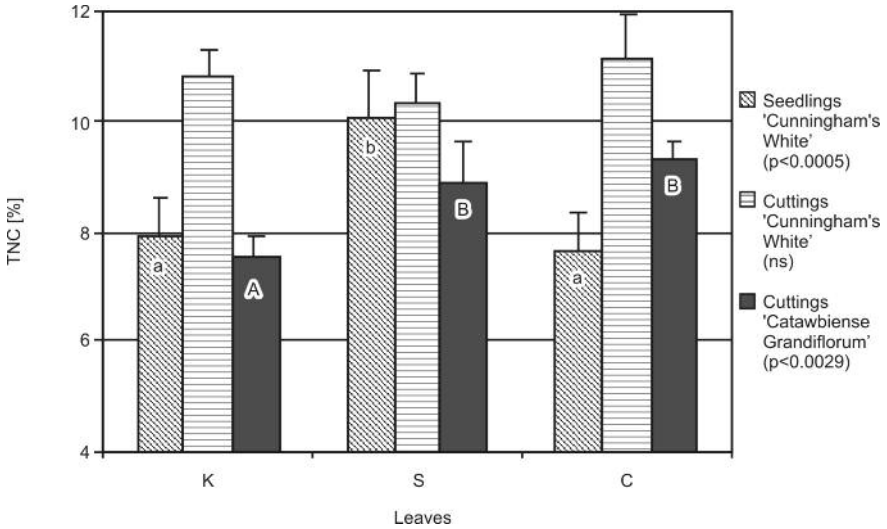

Fig. 5. Effects of calcium sulphate (S) and calcium carbonate (C) on the percentage of total nonstructural carbohydrates (TNC) in dry weight of rhododendron leaves. Salt concentration: 0.05 mol · dm^{-3} of substrate. (K = control). Other data as in Figure 1.

pH. Omokolo and Boudjeko (2005) detected an increase in concentrations of phenolic compounds in roots of *Xanthosoma sagittifolium,* infected by a fungal pathogen, *Pythium myriotylum*. Werner and Karolewski (2004), who studied pine seedlings growing on a substrate contaminated with heavy metals, reported a decrease in concentrations of phenolic compounds in roots of these plants. Similar effects were observed in roots of seedlings of *Betula pendula*, grown in a substrate with a high aluminium content, while in leaves, which were indirectly exposed to the negative influence of toxic ions, concentrations of phenolic compounds increased, as compared to the control (Bojarczuk et al. 2002, 2006). The low concentrations of phenolic compounds in roots of the plants growing in the polluted environment, can be the reason for the greater sensitivity of roots to infection with fungal pathogens (Smith 1987; Bojarczuk and Przybył 2005).

The addition of calcium salts to the substrate caused a significant increase in total nonstructural carbohydrates (TNC) in roots and leaves of the studied plants (Figs 4 and

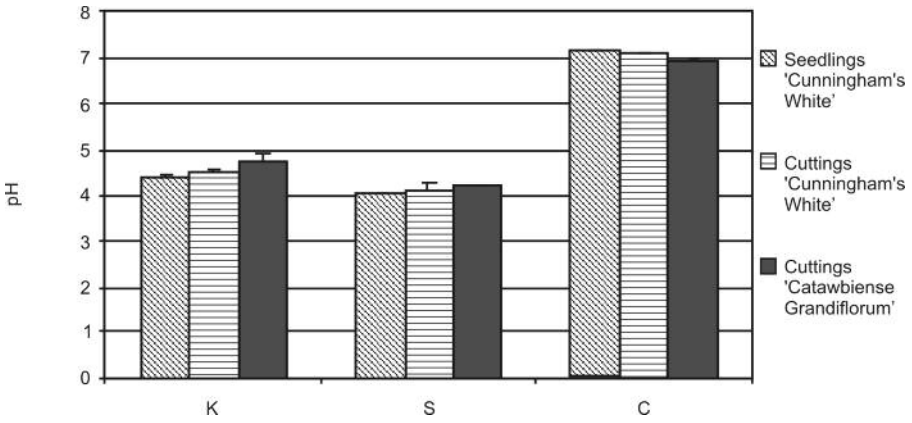

Fig. 6. Effects of calcium sulphate (S) and calcium carbonate (C) on substrate pH, depending on rhododendron cultivar. Salt concentration: 0.05 mol · dm^{-3} of substrate. (K = control). Values are given as mean ± SE.

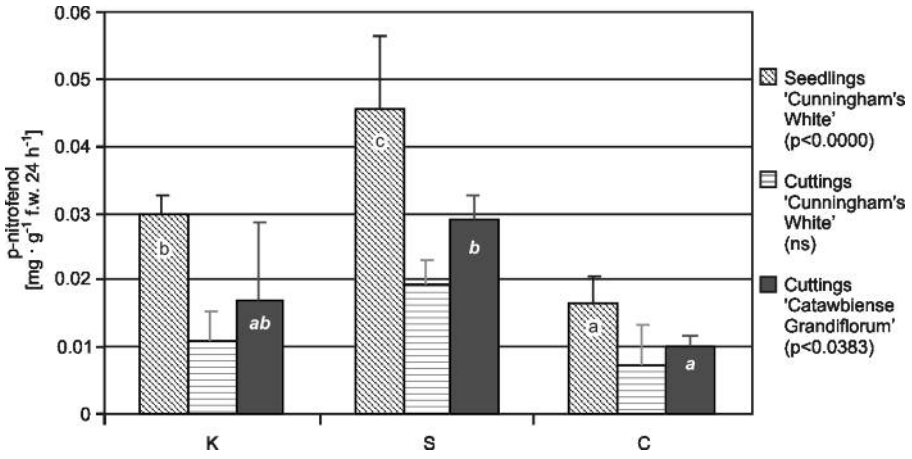

Fig. 7. Effects of calcium sulphate (S) and calcium carbonate (C) on acid phosphatase activity in the substrate, depending on rhododendron cultivar. Salt concentration: 0.05 mol · dm^{-3} of substrate. (K = control). Other data as in Figure 1.

5). The increase could be a result of osmotic stress, caused by the addition of calcium salts to the substrate, as well as the limited utilization of photosynthetic products, because of growth inhibition. Greger and Bertell (1992) in *Beta vulgaris* L 'Monohill' observed an increase in sugar content in a variant with a higher Cd concentration, as compared to a variant with a 4-fold lower concentration of Cd ions in the substrate. One of the possible reasons for the increase in carbohydrates could be the limited utilization of photosynthetic products, as a result of the reduced growth rate of plants in the variant with a higher Cd content of the substrate. Young et al. (1999) studied the influence of replanting on seedling development in *Picea mariana*, under water stress. Those authors also reported an increase in TNC in needles and roots of the replanted seedlings, as compared to the control. The increased amount of nonstructural carbohydrates could be due to some damage in the root system and limited penetration of the substrate by roots as a result of replanting, which significantly reduced the uptake of water and mineral salts. Lorenc-Plucińska and Stobrawa (2005) observed an increase in TNC in roots of *Populus deltoides*, grown on a substrate contaminated with heavy metals. According to those authors, the increased concentration of sugars may result from inhibition of starch hydrolysis as well as disturbances in carbohydrate metabolism in roots of the plants grown in the polluted environment.

Acid phosphatase activity in the substrate is directly related to substrate pH. In comparison with the control (K), the increase in substrate pH in the variant with calcium carbonate (C) limited the activity of the enzyme, while the lowering of substrate pH in the variant with calcium sulphate (S) significantly increased the activity of acid phosphatase (Figs 6 and 7). Differences in the activity of the enzyme between the two variants (S and C), caused by differences in substrate pH, affected the assimilation of phosphorus by plants and led to a significant decline in its concentration in dry weight of leaves and roots of rhododendrons in variant C (Table 2). Ciereszko and Barbachowska (2000) showed that elimination of phosphorus from the culture medium caused a significant increase in sugar content of roots and both young and older leaves of beans (*Phaseolus vulgaris* L.), as compared to the control, grown on a complete medium. Probably the increased concentration of sugars, in response to water deficit, can determine the carbon reserves in plants, which are used for construction of new tissues when phosphorus is available, but can also lower the osmotic potential in root cells, and in this way it can also affect the ability to take up some mineral salts form the substrate (Zohlen and Tyler 2004; Shane et al. 2008). Such conclusions seem to be confirmed by a significant increase in carbon content (%) of roots of *R.* 'Cunningham's White' and *R.* 'Catawbiense Grandiflorum' cuttings, grown in the substrate with $CaCO_3$ (Table 3).

Changes in substrate pH, caused by the addition of calcium salts, affected also the activity of nonspecific dehydrogenase (DHA) (Fig. 8). Along with the increase in substrate pH (Fig. 6), DHA activity clearly increased in the substrate with $CaCO_3$, as compared to the control, and the substrate with $CaSO_4$. Activity of nonspecific dehydrogenase is a measure of microbial activity in the soil. Changes in microbial activity can depend on physical factors, such as temperature, and soil moisture content (Arnold et al. 1999; Chaurasia et al. 2005). Also the kinds of substances produced by plants and released by the root system to the

TABLE 2. Effects of calcium sulphate (S) and calcium carbonate (C) on concentrations of Ca, Mg, S, and P in rhododendron leaves and roots. Salt concentration: 0.05 mol · dm^{-3} of substrate. (K = control). Other data as in Table 1.

Cultivar	Experimental variant	Ca			Mg		
		Plant		Substrate (bioavailable) [mg · dm^{-3}]	Plant		Substrate (bioavailable) [mg · dm^{-3}]
		Leaves [g · kg^{-1}]	Roots [g · kg^{-1}]		Leaves [g · kg^{-1}]	Roots [g · kg^{-1}]	
Seedlings of 'Cunningham's White'	K	9.0 b	3.9 a	152 a	4.5 a	1.4 b	72 a
	S	8.2 a	7.8 b	1537 b	4.7 b	1.4 b	141 c
	C	7.9 a	7.7 b	1362 b	4.4 a	1.3 a	119 b
		p<0.0001	p<0.0001	p<0.0000	p<0.0107	p<0.0074	p<0.0000
Cuttings of 'Cunningham's White'	K	5.2	2.9 a	389 a	2.7 b	1.7 b	74 a
	S	5.4	3.0 a	4330 c	2.7 b	2.0 c	134 c
	C	5.4	4.2 b	2016 b	2.2 a	1.4 a	103 b
		ns*	p<0.0005	p<0.0000	p<0.0019	p<0.0005	p<0.0000
Cuttings of 'Catawbiense Grandiflorum'	K	6.7 ab	2.7 a	366 a	2.7 b	1.8 b	69 a
	S	6.2 a	2.7 a	3775 c	2.9 b	1.8 b	135 c
	C	7.1 b	3.4 b	1943 b	2.0 a	1.6 a	106 b
		p<0.0346	p<0.0001	p<0.0000	p<0.0001	p<0.0019	p<0.0000

Cultivar	Experimental variant	S			P		
		Plant		Substrate (bioavailable) [mg · dm^{-3}]	Plant		Substrate (bioavailable) [mg · dm^{-3}]
		Leaves [g · kg^{-1}]	Roots [g · kg^{-1}]		Leaves [g · kg^{-1}]	Roots [g · kg^{-1}]	
Seedlings of 'Cunningham's White'	K	1.8 a	5.2 b	208 a	1.1	1.1 b	<3.0
	S	4.5 b	10.1 c	4625 b	1.1	1.1 b	
	C	1.6 a	3.4 a	254 a	1.0	0.9 a	
		p<0.0000	p<0.0001	p<0.0000	ns	p<0.000	–
Cuttings of 'Cunningham's White'	K	1.5 b	2.4 a	89 a	2.2 c	4.0 b	nd**
	S	2.4 c	3.2 b	18563 b	1.9 b	4.1 b	
	C	1.3 a	2.5 a	133 a	1.5 a	2.3 a	
		p<0.0000	p<0.0001	p<0.0000	p<0.0000	p<0.000	–
Cuttings of 'Catawbiense Grandiflorum'	K	1.9 b	2.4 b	76 a	2.0 c	3.7 c	nd
	S	2.4 c	3.0 c	15590 b	1.8 b	3.1 b	
	C	1.6 a	1.5 a	135 a	1.5 a	1.7 a	
		p<0.0000	p<0.0000	p<0.0000	p<0.0000	p<0.000	–

* ns – not significant; ** nd – not determined

soil (root exudates) may affect the species composition of microbial communities, and influence their activity within the rhizosphere (Kieliszewska et al. 2003; Ström et al. 2005). Quilchano and Marañón (2002) revealed a significant positive correlation between DHA activity and soil moisture, pH, as well as concentrations of calcium, magnesium and potassium in the soil. Pereira et al. (2006), who studied microbial activity in a soil polluted with heavy metals, also found a positive correlation between DHA activity and soil pH. Those authors suggested that low soil pH causes an increase in mobility of heavy metals, which has a negative effect on microbial growth.

Changes in substrate composition, caused by the addition of calcium salts, significantly affected the concentrations of macro- and micronutrients in leaves and roots of the studied plants. In spite of the greater availability of calcium ions in the substrate in variant S, as compared to C, resulting from the higher solubility of $CaSO_4$ than of $CaCO_3$, calcium concentration in dry weight of roots was the highest in cuttings grown in the substrate with $CaCO_3$ (variant C) (Table 2). The increase in calcium concentration caused a decrease in concentrations of Mg ions in roots of the plants grown in the substrate with $CaCO_3$ (variant C), as compared to the control (K), which can result from antagonism between Ca and Mg ions (Table 2). The increase in substrate pH in variant C can be also the cause of the low Mn content in the studied plants (Table 3). According to Henriques (2004), Mn deficit has a negative effect on photosynthesis as a result of a reduced number of chloroplasts in leaf mesophyll of plants with symptoms of Mn deficit. Spiers (1984) suggests that Mn deficit in plants that need acid soils for proper growth is an important factor limiting

TABLE 3. Effects of calcium sulphate (S) and calcium carbonate (C) on concentrations of Mn, Fe, Na, and C in rhododendron leaves and roots. Salt concentration: 0.05 mol · dm^{-3} of substrate. (K = control). Other data as in Table 1.

Cultivar	Experimental variant	Mn			Fe		
		Plant		Substrate (bioavailable) [mg · dm^{-3}]	Plant		Substrate (bioavailable) [mg · dm^{-3}]
		Leaves [g · kg^{-1}]	Roots [g · kg^{-1}]		Leaves [g · kg^{-1}]	Roots [g · kg^{-1}]	
Seedlings of 'Cunningham's White'	K	617 b	188 b	5.6 b	111 b	186	0.7 b
	S	566 b	179 b	15.9 c	85 a	181	1.1 c
	C	327 a	103 a	3.5 a	91 ab	231	0.5 a
		p<0.0000	p<0.0005	p<0.0000	p<0.0274	ns*	p<0.0000
Cuttings of 'Cunningham's White'	K	474 a	189 b	5.2 b	136	157 a	0.7 c
	S	634 b	200 b	13.9 c	108	146 a	0.3 a
	C	495 a	77 a	1.0 a	144	194 b	0.5 b
		p<0.0004	p<0.0000	p<0.0000	ns	p<0.0023	p<0.0000
Cuttings of 'Catawbiense Grandiflorum'	K	560 b	147 b	4.8 b	124	149 b	0.7 b
	S	539 b	141 b	14.8 c	130	172 c	0.3 a
	C	395 a	18 a	1.0 a	138	132 a	0.7 b
		p<0.0000	p<0.0006	p<0.0000	ns	p<0.0001	p<0.0000

Cultivar	Experimental variant	Na			C	
		Plant		Substrate (bioavailable) [mg · dm^{-3}]	Leaves [% DW]	Roots [% DW]
		Leaves [g · kg^{-1}]	Roots [g · kg^{-1}]			
Seedlings of 'Cunningham's White'	K	6.5	4.4 a	254 a	45.3	46.8
	S	5.5	4.8 ab	318 b	45.1	46.4
	C	6.6	5.8 b	304 b	44.9	45.8
		ns	p<0.0161	p<0.0005	ns	ns
Cuttings of 'Cunningham's White'	K	0.5 a	2.5 a	154	49.8 a	49.1 a
	S	1.1 c	3.1 b	147	51.5 b	49.0 a
	C	0.7 b	2.3 a	155	50.7 ab	51.0 b
		p<0.0000	p<0.0040	ns	p<0.0268	p<0.0129
Cuttings of 'Catawbiense Grandiflorum'	K	0.11 a	3.5 b	137	49.2	48.4 b
	S	0.20 b	5.2 c	144	48.0	47.2 a
	C	0.11 a	2.3 a	150	48.9	50.1 c
		p<0.0092	p<0.0000	ns	ns	p<0.0001

* ns – not significant

their development in a substrate with a higher pH. That author found also that an increase in soil pH causes additionally a more intensive assimilation of sodium ions by plants, which has a negative effect on their growth. However, in this study, an increased amount of assimilated Na ions, as compared to the control, in plants grown in a substrate with a high pH (variant C), was observed only in roots of seedlings and leaves of cuttings of *R.* 'Cunningham's White' (Table 3).

The increase in calcium sulphate content of the substrate had a significant effect on sulphur content of leaves and roots of the studied rhododendrons (Table 2). Nevertheless, our results suggest that the increased pH in the substrate with $CaCO_3$, had a more unfavourable effect on plant growth than the increased amount of sulphur in leaves of cuttings and seedlings of the analysed rhododendrons. Rennenberg (1984) suggests that plants can control the uptake of SO_4^{-2} anions from the soil (in contrast to Cl^- and HCO_3^- ions), so that their content does not exceed the levels toxic to plants.

The application of calcium carbonate leads to an increase in the concentration of HCO_3^- ions in the soil solution. This lowers the uptake of iron or limits the transport of iron ions from roots to shoots, which is reflected in chlorotic patches between leaf veins (Chaanin and Preil 1992; Hell and Stephan 2003). The higher concentration of iron ions in roots of seedlings and cuttings of *R.* 'Cunningham's White', grown in a substrate supplemented with calcium carbonate (pH 7.1), as compared to the control (pH 4.6), in relation to the lower Fe level in roots of cuttings of *R.* 'Catawbiense Grandiflorum', grown also in the substrate with $CaCO_3$, suggests a greater tolerance of *R.* 'Cunningham's

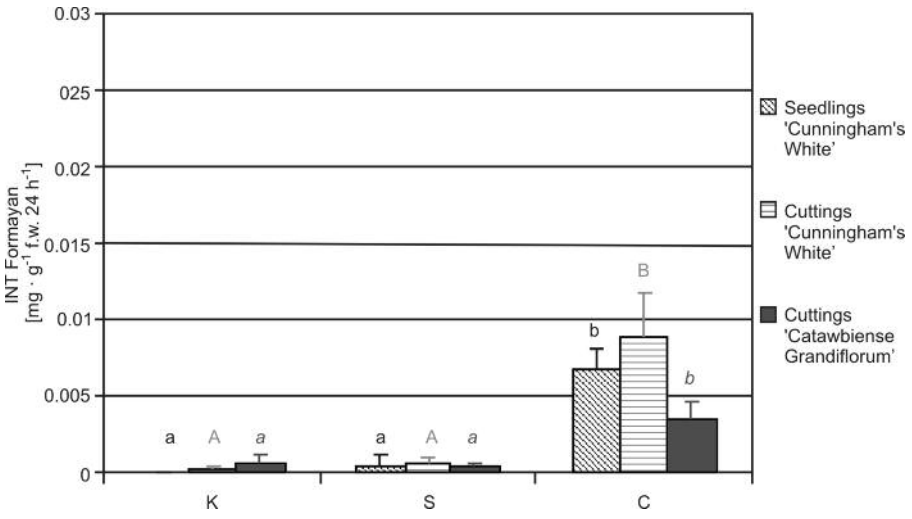

Fig. 8. Effects of calcium sulphate (S) and calcium carbonate (C) on nonspecific dehydrogenase activity in the substrate, depending on rhododendron cultivar. Salt concentration: 0.05 mol · dm^{-3} of substrate. (K = control). Other data as in Figure 1.

White' to an increase in substrate pH as compared to *R.* 'Catawbiense Grandiflorum' (Table 3). The results for seedlings and cuttings of *R.* 'Cunningham's White' varied in respect of the other parameters (concentration of phenols and carbohydrates). The influence of calcium salts on plant growth was significantly lower in 1.5-year-old rooted cuttings of *R.* 'Cunningham's White', than in seedlings of *R.* 'Cunningham's White', in relation to the control. This may indicate a significant effect of the greater genetic variation the studied seedlings, as compared to cuttings, on results of this study.

CONCLUSIONS

Our results suggest that an increase in substrate pH is the major factor limiting the growth of rhododendrons. The influence of calcium salts on plant growth does not depend on the amount of assimilated Ca ions, but on the type of anion present in the added salt.

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