The Effect of Wood Ash Fertilisation on the Anatomy and Localisation of Lignin in Scots Pine (*Pinus sylvestris* L.) Needles

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Abstract

The effect of wood ash application to the soil on the anatomy and lignin accumulation in the needles of 20-year-old Scots pine trees growing on nutrient poor sandy soil (Arenosol) was investigated. Comparative-analytical studies were conducted in seven sample plots treated with different doses of wood ash (0.25, 0.5 and 1.0 kg m^{-2}) and in a control plot without treatment. For anatomical analyses cross-sections of needles of one and two-year-old were made. These were stained, photographed under microscope and measured. Analysis showed that, in general, the current-year needles had the largest average value of anatomical characteristics in all sample plots. At the same time the xylem of the needles of pines from the sample plot with wood ash application of 1.0 kg m^{-2} and from the control plot increased with age. Significant differences ($p < 0.05$) in the anatomical characteristic between the needles from trees growing on sample plots treated with 0.5 and 1.0 kg m^{-2} and the control plot were detected. The greatest anatomical differences between these needles were detected in the oldest needles. Histochemical analysis showed a greater degree of lignification of cellular walls in older needles and more intensive accumulation of lignin in the needles from sample plots treated with 0.5 and 1.0 kg m^{-2}. Morphological measurements showed the positive trends in the length and mass of needles at 0.25 kg m^{-2} wood ash doses.

Key words: *Pinus sylvestris*, needle anatomy, lignification, wood ash

Introduction

Main environmental conditions affecting the growth and bioproduction of trees are the content of nutrients and their availability to trees in the soil and the pH level, temperature, water and light. Optimal uptake and concentration of nutrients in tissues ensure balanced physiological processes (including photosynthesis, respiration and lignification) and bioproduction (Aerts and Chapin 2000, Niinemets and Kull 2003, Niinemets and Lukjanova 2003a, 2003b). Recycling of nutrients should be a fundamental principle in sustainable forestry (Steenari and Lindqvist 1997). Needle analysis has often been used as a diagnostic method to describe the nutritional status of trees (Linder 1995, Brække 1996, Makoto and Koike 2007) as well as biomass increment and growth conditions of trees (Niinemets et al. 2002, Niinemets and Kull 2003) and to indicate to both natural and human made disturbances in ecosystems (Pallardy 2008). So the various possible forms of natural disturbances, from floods to forest fires, and anthropogenic factors from agricultural practices to environmental pollution may cause very different degrees and scales of change accompanied with disbalanced nutrient cycling in ecosystems and physiological, anatomical and morphological alterations in plants (Waring and Running 2007). Fertilisation can improve the growth of evergreen species and cause changes in anatomical features depending on the fertiliser character (Thomas and Packham 2007).

As wood ash from forest biomass burning contains nutrients needed by plants in almost the same proportions they occur in growing trees, wood ash may be used for forest fertilisation. To improve growth conditions enrichment of forest soils with nutrients (Rehfuess et al. 1991, Mandre and Korsjukov 2004) and compensatory fertilisation with wood ash on mineral soils (Ohno and Erich 1990, Ohno 1992, Nilsson 2001, Perucci et al. 2006) and on tropical acid soils (Voundi Nkana et al. 1998) have been used. Clearly positive results in increasing the increment of trees were obtained in the case of acid bog soils (Mälkönen and Kukkola 1991). It is generally accepted that wood ash has a positive effect on the growth and yield of agricultural crops (Naylor and Schmidt 1989, Kärblane 1996, Demeyer et al. 2001). On drained peatlands in Finland considerable increases in the growth of Scots pine were achieved (Lukkala 1951, Silfverberg and Huikari 1985, Moilanen et al. 2002, 2004). Nitrogen is a most important element limiting the growth of trees on mineral soils (Hånell and Magnusson 2005). Irrespective of the fact that wood ash does not contain N, its application may promote mineralisation of considerable reserves of soil organic N, thus improve over the long-term the availability of N for tree growth (Moilanen and Silfverberg 2004). Numerous studies have indicated positive effects of fertilisation with wood ash (Mandre et al. 2006, Ozolinčius et al. 2007a) and with wood ash combined with other nutrients (McDonald et al. 1994), but the effects of wood ash on forest soils and on the physiological state of forest trees are not fully understood or quantified as yet. However, no clear stem growth response to ash application on mineral soils has been reported (Jacobson 2003). The trees often show no growth response at all, or only a slight decrease or increase in growth observed (Ohno and Erich 1990). In general, researchers agree that the fertilising effect of wood ash on mineral soils manifests itself over a long period and that the main aim of its use should be mitigation of the deficit of nutrients (Haveraaen 1994, Saarsalmi et al. 2001).

Information on the use of wood ash can be found in several papers (Hånell and Magnusson 2005, Nieminen et al. 2005, Park et al. 2005); however, their results are often antagonistic on the description of the fertilising effect and neutralising properties of wood ash. There is sufficient information showing the influence of wood ash on the concentration of nutrient elements (Jacobson 2003, Saarsalmi et al. 2006, Ozolinčius et al. 2007a), on carbohydrate concentration (Klõšeiko and Mandre 2002, Mandre 2006), on pigment synthesis and lignin concentration in the needles (Mandre et al. 2004, 2006). The data on the effect of wood ash on the anatomy and lignification of needles depending on the amount of wood ash applied to the soil are scarcer. Thus, the aim of the present work was to determine the influence of wood ash on the anatomy of pine needles and the allocation of lignin into needles. We put forward the hypothesis that due to the application of wood ash changes affecting their morphology may occur in the anatomy of needles. Allocation of lignin in tissues and the intensity of lignification depend on the doses of wood ash applied to soil.

Materials and methods

Study area

The needles were collected in 2005 from Scots pine trees growing on sites located in a Scots pine stand established by sowing in northern Estonia (59°18’26'' N; 24°43’28'' E) in 1982. The sandy soils (Arenosol by FAO classification at 1990) of the study area are characterised by low concentrations of K and Mg compared to other regions of Estonia (Petersell et al. 1997). In the summer of 2000 and 2002 wood ash was applied on plots in different treatments. All together seven 1000 m^2 experimental sample plots were established: control without wood ash (2); treatments with 0.25 (2), 0.5 (2) and 1.0 kg (1) wood ash per m^2.

For morphological and anatomical investigations current year and one-year-old needles (n = 45) were collected randomly from the middle horizon of the crowns of trees from each sampling plot.

Wood ash characteristics

The wood ash used in our field experiment had an average pH_{H2O} 12.1–12.6 and its chemical composition was as follows: N – 250 mg kg^{-1}, P – 15 500 mg kg^{-1}, S – 10 150 mg kg^{-1}, Al – 3300 mg kg^{-1}, Ba – 1560 mg kg^{-1}, Ca – 123 000 mg kg^{-1}, Cu – 197 mg kg^{-1}, Fe – 10 400 mg kg^{-1}, K – 48 000 mg kg^{-1}, Mg – 19 400 mg kg^{-1}, Na – 17 900 mg kg^{-1}, Mn – 9850 mg kg^{-1}, Pb – 76 mg kg^{-1}, Zn – 4340 mg kg^{-1} (Mandre 2004). The concentration of chemical elements in wood ash was determined with an ICP-spectrometer and atomic adsorption spectrometer in the Central Laboratory of the Estonian Environmental Research Centre Ltd., where standard ISO methods are used.

Needle morphology, anatomy and measurements

To characterise the state of Scots pines in the sample plots measurements of needle length (cm) and fresh and dry mass (mg) were made. For dry mass determination the needles were stored in an oven at 70ŗC until they reached a constant weight.

For anatomical measurements cross-sections were made from middle part of the needles. The needles were pre-fixed with 3% glutaraldehyde in 0.1 mol L^{-1} phosphate buffer, pH = 7.3, and fixed in 1% solution of OsO_4 according to a widely known technique (Bozzola and Russell 1992, Ruzin 1999). Then tissues were dehydrated with ethanol and xylol, and embedded in paraffin. Cross-sections of needles (10–20 µm) were cut with the microtome (Leitz, Germany) and mounted on glass. For lignin visualisation the slices were stained with 5% safranin O previously removing paraffin using the widely known histochemical method (Grimstone and Skaer 1972). The lignified cell walls stained red. The cross-sections were viewed in the bright field at ×100 magnification with a microscope (Micros MC400A, Austria) and photographed with a Nikon Coolpix 5400 camera (Nikon, Tokyo, Japan).

The MapInfo Professional for Windows 4.0 (MapInfo Corp. Inc., Troy, NY) was used to measure the thickness, width and tissue areas of the current year and one-year-old needles from the images. The tissues separated were epidermis, mesophyll, xylem, phloem and sclerenchyma. Average values of anatomical characteristics were calculated for all these tissues (Table 1). The stained cross-sections revealed the localisation of lignin. The degree of lignification was determined visually by the intensity of staining.

Table 1. Means anatomical characteristics of needles of Scots pine growing on control plot (Control) without treatment and sample plots treated with different doses of wood ash (0.25; 0.5; 1.0 kg m^{-2}) (±SE; n = 45–100) Significance of differences between characteristics of the needles in control (untreated) and treated sample plots determined by Kruskal-Wallis test $*p < 0.05$, $**p < 0.01$

Sampling site	Control	0.25 kg m^{-2}	0.5 kg m^{-2}	1.0 kg m^{-2}
Needles length, cm	2.17±0.10	2.63±0.30	2.14±0.10	2.12±0.10
Needles fresh mass, mg	47.46±4.00	69.95±8.47	39.31±5.00	38.3±5.01
Needles dry mass, mg	24.90±4.78	30.15±5.25	17.39±3.05*	17.00±2.75*
Width of needle cross-section, mm	1.04±0.07	1.15±0.05	1.28±0.02*	1.23±0.02*
Thickness of needle cross-section, mm	0.55±0.02	0.59±0.02	0.54±0.02	0.60±0.01
Total needle cross-section area, mm^2	127.01±6.85	129.58±3.25	125.77±3.65	145.75±3.25
Epidermis, mm^2	10.96±0.66	10.92±0.25	13.67±0.59*	13.37±0.49*
Mesophyll, mm^2	72.34±4.97	80.34±2.98	76.42±2.23	94.16±2.68*
Lignified tissues, mm^2	12.71±0.52	12.30±0.30	14.47±0.53	15.60±0.40**
Vascular bundles, mm^2	3.72±0.54	3.36±0.43	2.86±0.21	3.31±0.19
Xylem, mm^2	1.75±0.31	1.38±0.11	1.61±0.19	2.23±0.18

Statistical analyses

The significance (p) of differences between the mean attribute values of needles in the control and treatment sample plots was estimated by multiple comparison of means by the nonparametric Kruskal-Wallis test. For statistical calculations the Statistica 7.0 software (StatSoft, Inc. Tulsa, OK) was used.

Results

The study showed an increase in the length growth of needles in case of the treatment 0.25 kg m^{-2} and no statistical differences between the control and other variants were found (Table 1). Analyses of needles weights revealed differences in the fresh and dry mass of needles and decreased dry mass of needles at the treatment 0.5 and 1.0 kg m^{-2} was statistically significant (Table 1)

Results of the analyses of anatomical characteristics showed that the needles of Scots pine growing on the sample plots treated with wood ash had mostly larger average values of the parameters compared to the control (Table 1). Differences from the control were observed in the average of all treatments in width (23%), thickness (10%), total cross-sectional area (15%), epidermis (25%), mesophyll (30%) and xylem (27%). Especially stand out the anatomical characteristics of needles of pines growing on the sample plots treated with 1.0 kg m^{-2} wood ash. Visual observations of the stained cross-sections of needles showed accumulation of lignin in all studied needles. The tissues accumulating lignin make up on average 10% of the total area of control needles. For the sample plots treated with wood ash 0.25, 0.5 and 1.0 kg m^{-2} these characteristics were respectively 9%, 11% and 12%. Besides, measurements of tissues revealed a 23% larger area of lignifying tissues in the case of the treatment 1.0 kg m^{-2} compared to the control (Table 1).

The average value of anatomical characteristics of needles depending on the age of the needles and the dose of wood ash applied are presented in Figures 1 and 2. Analysis of anatomical characteristics revealed that the average value of measured characteristics of the cross-sections of the current year needles were mostly larger than those of the one-year-old needles both in the control plot and the wood-ash treated plots (Figures 1 and 2). In the plot treated with 0.25 kg m^{-2} wood ash, the width and the average area of the vascular bundle of the current year needles were largest and these figures decreased with the age of needles. The one-year-old needles had larger xylem and vascular bundle areas on the control plot and the needle width on the sample plot treated with 0.5 kg m^{-2} wood ash and epidermis, mesophyll and lignifying tissues on the sample plot treated with 1.0 kg m^{-2} ($p < 0.05$). Except the needles from the sample plot treated with a wood ash dose of 0.5 kg m^{-2}, the proportion of the area of lignified tissue of the total area

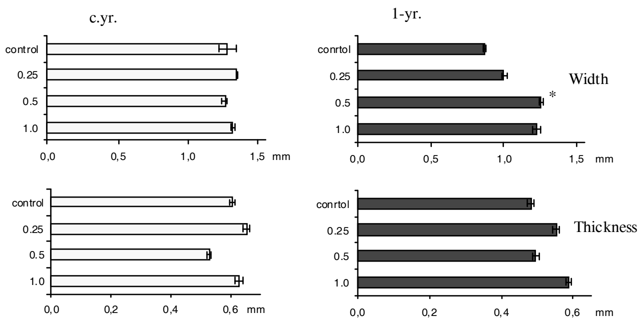

Figure 1. Mean anatomical characteristics of cross-sections of Scots pine needles depending on needle age and the amount of wood ash applied on the sample plots (±SE; n = 45). Variants of wood ash doses: control, untreated sample plot; 0.25; 0.5; 1.0 kg m^{-2}. c.yr. – current year needles; 1-yr. – one-year-old needles

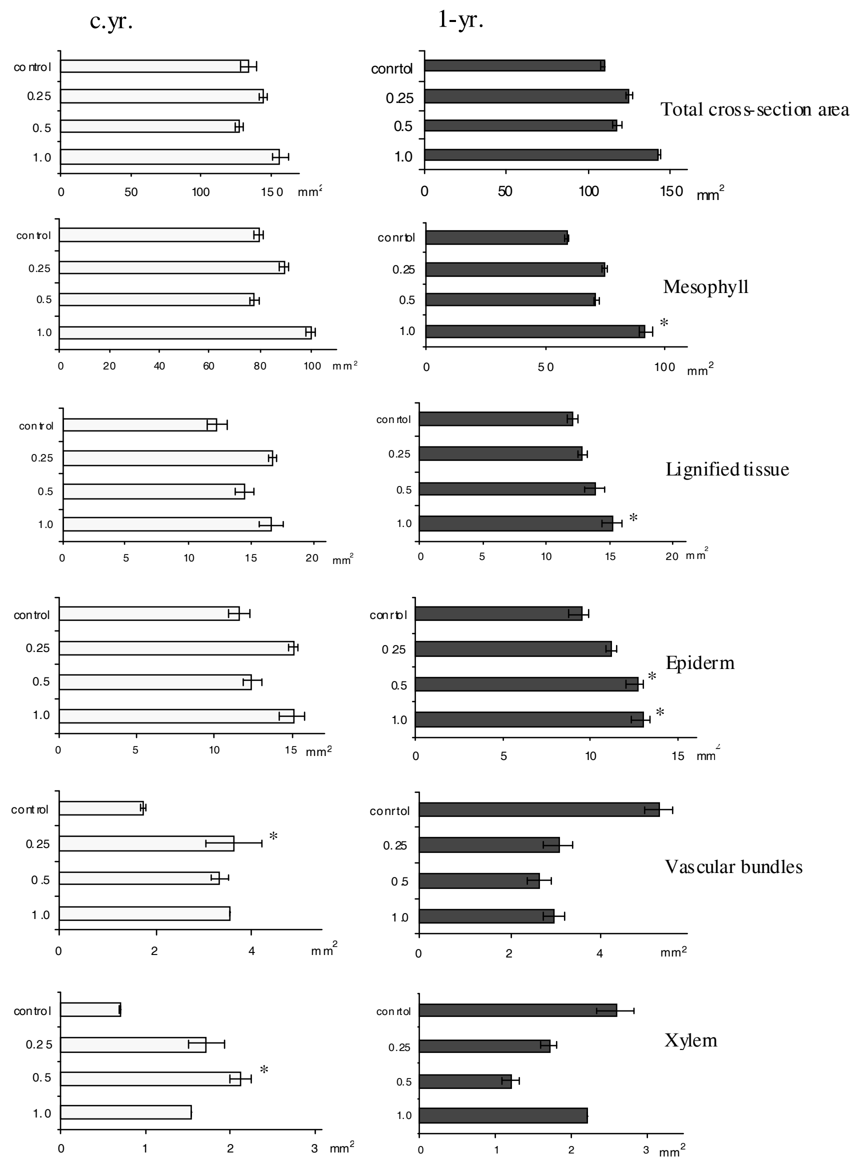

Figure 2. The distribution of tissues in Scots pine needles depending on needle age and the amount of wood ash applied on the sample plots (±SE; *n* = 45). Variants of wood ash doses: control, untreated sample plot; 0.25; 0.5; 1.0 kg m^{-2}. c.yr. – current year needles; 1-yr. – one-year-old needles

of the cross-section increased in the one-year-old needles. Statistically significant differences ($p < 0.05$) in the vascular bundle, xylem, mesophyll, the total area of cross-section and the needle width were observed between the current year and one-year-old control needles. Visual observation of cross-sections of the needles of different age showed that in older needles the staining was more intensive than in the current year needles (Figure 3).

In the sample plot treated with 0.5 kg m^{-2} wood ash, the needles had the smallest total cross-sectional and mesophyll areas. However, the percentage of mesophyll of the total cross-section was larger in needles from the plot fertilised with 1.0 kg m^{-2} wood ash while the mesophyll percentage was smallest in the control plot. In general, the one-year-old needles collected from the control plot had a smaller total area and mesophyll area than the needles from treated plots. On the control plot the area of the vascular bundle of the one-year-old needles was larger than in the treated plots. Increasing area of the xylem with age was characteristic of both the control plot and the treated

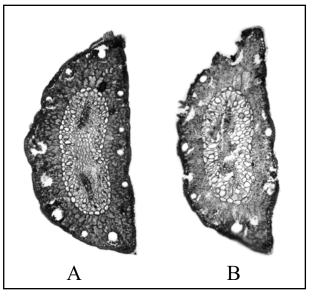

Figure 3. Anatomy and lignification of Scots pine needles of different age. General view of needle cross-section at×100 magnification. A – current year needle and B – one-year-old needle

(1.0 kg m^{-2}) plot. On the sample plots treated with 0.25 and 0.5 kg m^{-2} wood ash a decrease in the xylem area of needles was observed, but the decrease was not significant statistically.

Visual observations of stained preparations did not reveal notable differences in lignin accumulation between treatments. The cell walls of the xylem and epidermis of the current year needles were stained and in the one-year-old needles the staining of these tissues was even more intensive, except in the needles from the plot treated with 0.5 kg m^{-2} wood ash, in which the staining of the xylem of the current year needles was more intensive than that of one-year-old needles (Figure 4).

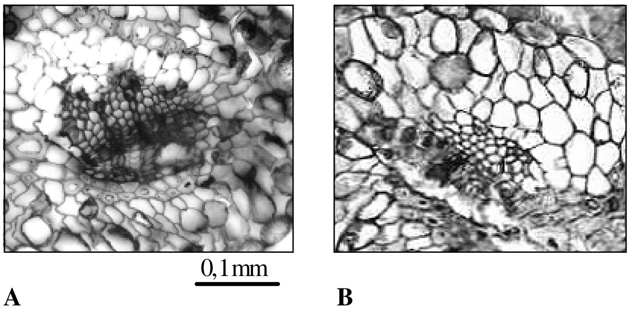

Figure 4. Vascular bundles of current year needle (A) and one-year-old needle (B) of Scots pine growing on sample plots treated with wood ash in doses of 0.5 kg m^{-2}. View at ×400 magnification. Lignified cell walls are more intensively stained in the current year needle

Discussion

The wood ash applied on nutrient poor forest sandy soils affected the morphological, anatomical characteristics and lignification in Scots pine needles. Sandy soils are characterised by nutrient deficiency as compared to optimal conditions and compensatory fertilisation may be recommended for successful biomass production (Ohno and Erich 1990, Ohno 1992, Nilsson 2001, Perucci et al. 2006). Also it is known that any changes in growth conditions may bring about disturbances in organisms compared to their previous state. Fertilisation with wood ash may cause both positive and negative disturbances in the functioning of forest ecosystems.

The application of wood ash had an increasing effect on the pH and the concentrations of exchangeable base cations in the soil (Waring and Running 2007, Mandre 2005). In the case of acidic soils, the soil pH rises and mineralisation of organic N takes place (Saarsalmi et al. 2001). The effects of wood ash were dose-dependent and responses of trees to the wood ash application varied at different doses. Although Saarsalmi et al. (2004) stated that wood ash application had no effect on Scots pine volume growth (Saarsalmi et al. 2004), the positive effect on the relative diameter and height increments at doses 0.25 kg m^{-2} was found by Pärn (2005). Also at the wood ash doses 0.25 kg m^{-2} was established the increase of needle length, fresh and dry mass. Generally morphological parameters at 0.25 kg m^{-2} of wood ash application exceeded those of the control plot and on other experimental plots tested with higher doses of wood ash.

There were found differences between anatomical and morphological responses of needles to different doses of wood ash application, while the increase of anatomical characteristics was found just at the highest doses of treatment. Usually, the widest and thickest needles with the largest total cross-sectional area grow on pines in nutrient-rich soil (Niinemets and Lukjanova 2003a, Lukjanova and Mandre 2006). The study results obtained in this work show that the anatomical characteristics of pine needles, except the vascular bundles, were in general larger in plots treated with wood ash than in the control plot. A statistically significant difference from the control ($p < 0.05$) was observed in the average area of mesophyll and of lignifying tissues of the needles of pines growing on the sample plots treated by doses of 1.0 kg m^{-2} of wood ash (Table 1). The total area of the cross-section of these needles was also largest although the difference was not significant statistically. Larger needle cross-sectional area further results in larger needle dry mass and greater needle-area-based photosynthetic capacity (Palmroth and Hari 2001, Han et al. 2003). However, the results of Mandre et al. (2004) show that application of wood ash provoke a decrease in the fresh and dry mass of the needles of Norway spruce seedlings. At the same time, stimulation of biomass formation of Scots pine was observed on experimental plots treated with doses of 0.25 kg m^{-2} (Ots and Haugas 2004, Mandre et al. 2006). Enhanced allocation to foliage increases the photosynthetic productive surface, but also increases requirements for mechanical support and water and nutrient uptake (Mencuccini et al. 1997, Sutinen and Saarsalmi 2008). Our results showed that after the application of wood ash the measured anatomical characteristics of the needles of Scots pine had higher mean values, indicating improvement of the nutrient status for pines.

Histochemical analysis showed accumulation of lignin in all the investigated needles and the percentage of lignin accumulating tissues of the total average cross-sectional area was the smallest in the control needles. An earlier study showed that in the middle and lower layers of the crown of the pine the lignin concentration is lower than the respective figure for the control trees and the needles from upper layers of the crown growing on soil treated with wood ash have 10–20% more lignin than control (Mandre 2004). Analysis of preparations did not reveal any significant differences between the amounts of lignin accumulated in the needles depending on the plots although the percentage of the area of lignified tissues of the total cross-sectional area was largest in the plots treated with the largest dose of wood ash.

Our results revealed that the anatomy and lignification of needles depended on needles age and on the doses of the wood ash applied for soil treatment. The greatest values of anatomical characteristics (total cross-sectional area, needle cross-section width and thickness, area of epidermis and mesophyll), independent of the plot, were observed in the youngest needles (Figures 1 and 2). In current-year needles, also intensive photosynthesis is taking place, testified by the larger mesophyll area and percentage of the total cross-sectional area as compared to the one-year-old needles. Visual observations of cross-sections of the needles of different age showed that in older needles the staining was more intensive than in the current year needles. In the current-year needles, only some cell walls of the xylem became stained although in some of them the proportion of xylem in the conducting tissues was larger than in the one-year-old needles, or to put it differently, the lignification process was just starting. In the one-year-old needles, the xylem area had decreased, but the staining of lignin-accumulating tissues was intensive. The area of the lignin-accumulating tissues decreased rather than increased, but their staining intensified, which means that the amount of lignin increases, the structure of lignin became more compact or its accumulation tightened. The obtained results agree with the data of the studies carried out with spruce and pine needles (Hanisch and Kilz 1990, Lukjanova and Mandre 2006).

In the one-year-old control needles, the xylem and vascular bundle areas and their proportion in the total area were larger than in the current-year needles. In the plots treated with 0.25 kg m^{-2} wood ash, the vascular bundle's average area of the current year needles was greater and it decreased with the age of the needles. In the ash-treated plots (0.25 and 0.5 kg m^{-2}), also a decrease in the needle xylem area could be observed. These study results suggest that in the control plot the tissue through which nutrients are transported was still at the growth stage while in the treated plots this process had terminated, which verification needs the further investigations.

The one-year-old needles collected from the control plot had the smallest total cross-sectional and mesophyll areas than the needles collected from the ash-treated plots. Among the one-year-old needles the needles from the sample plots treated with 1.0 kg m^{-2} had the largest mesophyll area and its percentage of the total area. We can assume that during the first two years the needles from the treated sample plots developed an optimum amount of vascular tissues for efficient transport of mineral nutrients and water. The same results were obtained by Lukjanova and Mandre (2006, 2008) when studying needles of Scots pine growing on dunes. Jokela et al. (1997) observed that the mesophyll area was greater in needles with a high or intermediate K concentration than in needles with a low K concentration, possibly indicating greater production of photoassimilates in these trees. Lignifying tissues and the proportion of the area of the lignified tissue of the total area of cross-section were also larger in the two-year-old needles from the plot treated with 1.0 kg m^{-2} wood ash, which is characteristic of the ageing of plant organisms (Miidla 1989, Polle et al. 1997).

Generally the staining of lignified tissues of the one-year-old needles was more intensive than in the current-year needles. At the same time the staining of the xylem of the current-year needles from the treated (0.5 kg m^{-2}) area was more intensive than that of the one-year-old needles. Earlier larger amounts of lignin were found in needles of Norway spruce and Scots pine growing in areas treated with wood ash doses of 0.25 and 0.5 kg m^{-2} (Mandre 2005, Mandre et al. 2006).

To sum up, it was earlier shown that wood ash fertilisation enriches forest soil (Mandre et al. 2004, Ozolinčius et al. 2007c), affecting thus the anatomy of needles and their lignification. We established that larger amounts of wood ash (1.0 and 0.5 kg m^{-2}) affected the needle anatomy more than smaller amounts, which agrees with earlier findings. According to Mandre et al. (2004), on nutrient-poor sandy soil tree biomass formation and pigment synthesis are stimulated in the case of treatment with 0.5 kg m^{-2} in the third year of the experiment. Significant increases of the P, Ca and Mg concentrations were observed in the ground vegetation biomass after the application of 5.0 t wood ash per ha by Ozolinčius et al. (2007b). Studies by Perucci et al. (2008) showed that wood ash affects the chemical composition and pH of agricultural soils, microbial biomass and microbial C/N ratio only during one year after application. However, other findings show that the fertilising effect of wood ash may last for years (Demeyer et al. 2001, Saarsalmi et al. 2006).

Conclusions

This research revealed that wood ash fertilisation affected the morphology, anatomy and lignification of

Scots pine needles, while the responses in anatomy and morphology are strongly dose-dependent and often contrary. Increase in the needle mass and length was found at lower doses of wood ash, while the areas of the mesophyll, epidermis and lignified tissue in the pine needles from sample plots treated with higher doses of wood ash application. The influence of wood ash on the physiology and anatomy of *Pinus sylvestris* is not well understood. As our results did not support all standpoints on the doses and duration of the effect of wood ash shown by some authors in the literature (Ots and Haugas 2004, Mandre et al. 2006), additional investigations are needed to confirm the effect of wood ash on the anatomical structure of conifer needles and tree physiology as a whole.

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ВЛИЯНИЕ УДОБРЕНИЯ ДРЕВЕСНОЙ ЗОЛОЙ НА АНАТОМИЧЕСКОЕ СТРОЕНИЕ И ЛИГНИФИКАЦИЮ ХВОИ СОСНЫ ОБЫКНОВЕННОЙ (*PINUS SYLVESTRIS* L.)

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Резюме

Исследовалось влияние древесной золы, используемой в качестве удобрения для повышения плодородности почвы, на анатомическое строение и локализацию лигнина в хвое сосны обыкновенной (*Pinus sylvestris* L.). Исследовалась хвоя двадцатилетних сосен, произрастающих на бедной минеральными элементами песчаной почве. Был проведен сравнительный анализ хвои сосен, растущих на экспериментальных участках, обработанных различными дозами древесной золы (0,25; 0,5 и 1,0 кг/м^2), и на контрольных участках, не обработанных золой. Для этого были приготовлены микропрепараты из хвои текущего и первого годов, которые были окрашены сафранином и затем сфотографированы под микроскопом. Измерения проводили на полученных таким образом дигитальных фотографиях поперечных срезов иголок. Результаты анализа показали, что в основном наибольшие средние анатомические показатели имеет хвоя текущего года. Средняя площадь ксилемы хвои сосен с участков, контрольного и обработанного 1,0 кг м$^{-2}$ древесной золы, увеличивается со временем. Были найдены существенные изменения ($p < 0,05$) анатомических показателей (площадь мезофилла и тканей, накапливающих лигнин) между хвоёй с контрольного участка и участков, обработанных древесной золой в дозах 0,5 и 1,0 кг/м^2. Особенно значительные различия в анатомических параметрах с этих участков у хвои первого года. В основном, гистохимический анализ показал большую степень лигнификации у хвои первого года и интенсивную лигнификацию клеточных стенок хвои деревьев, растущих на участках, обработанных древесной золой в дозах 0,5 и 1,0 кг/м^2.

Ключевые слова: *Pinus sylvestris*, анатомическое строение, хвоя, лигнификация