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Seasonal dynamics of histological parameters of the needles of Scots pine (*Pinus sylvestris* L.) growing in conditions of excess ammonia

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Ammonia is one of the most important aerial pollutants which might affect morphology of conifers. The purpose of our study was to assess morphological and histological parameters of the needles of Scots pine (*Pinus sylvestris* L.) growing under elevated concentration (up to 17.5 μg m^{-3}) ammonia (near JSC *Achema*, Lithuania) during the vegetation season (June–August, 2009). Current-year needles from the young age stand with higher concentration of air ammonia (17.5 μg m^{-3}) were wider ($p < 0.05$; 1.11 times) when compared to the needles of Kačerginė site (3.9 μg m^{-3}). The investigation of the seasonal dynamics of the needle morphology (length, width, thickness) and the thickness of the needle tissues in the cross-section (epidermis, hypodermis, mesophyll and central cylinder tissues – transfusion parenchyma and sclerenchyma) in most other cases did not reveal statistically significant differences between the trees growing in ammonia-polluted and relatively clean sites. It was true for both middle-aged and young age stands. Under the elevated concentration of air-ammonia, only some tendencies were observed for thicker needles (up to 1.05 times), thicker adaxial mesophyll (up to 1.10 times) and adaxial transfusion parenchyma (up to 1.06 times) of the needles. For middle-aged stands significant ($p < 0.01$) needle age differences were observed according to the abaxial and adaxial mesophyll thickness (up to 1.21 times thicker in 1-year-old needles), the same was true for the needles of the young age stand (up to 1.25 times). At the end of August, the average length of 1-year-old needles was 76 mm, width 1.7 mm and thickness 0.76 mm. For some histological parameters statistically significant ($p < 0.05$) seasonal differences were observed: the thickness of adaxial transfusion parenchyma was decreasing and the sclerenchyma width was increasing within the June–August period.

Key words: needle tissues, needle morphology, seasonal dynamics, conifers, sclerenchyma, transfusion parenchyma

INTRODUCTION

Scots pine is well adapted to acidic and low nutritional value soils, very sensitive to air pollution. Various changes in the needle structural and functional properties were reported for the conifers affected by the acid rain or ozone injury (Kivimaenpaa et al., 2005; Haberer, 2006; Fares et al., 2010). Worldwide field studies and experiments have shown numerous adverse effects of nitrogen

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pollution. Exposure to elevated NO_x (annual mean concentrations ranged from 77 nl l^{-1} to 98 nl NO_x l^{-1}) of herbaceous species resulted in species-specific changes in growth and phenology, with a consistent trend for accelerated senescence and delayed flowering (Honour et al., 2009).

Ammonium deposition correlates with the ammonification rate in the soil (Lorz et al., 2010). Ammonia deposits to the soil turn into ammonia ions and nitrates and might be absorbed by plants through the roots (Jokela et al., 1995). Nutrition through the roots is essential, because rhizosphere of plants has a large surface area. Simulated nitrogen deposition might cause adverse effect on mycorrhyza development and decreased frequency of mycorrhizal roots in the humus layer (Skeffington, Wilson, 1988; Heinsdorf, 1991). Chronic N additions to the stands have affected red pine (*Pinus resinosa*) needle morphology, nitrogen metabolism (or partitioning), photosynthetic capacity and foliage productivity. Pine stand exposure to higher concentrations of air ammonia might increase nitrogen concentration (Pérez-Soba, Van der Eerden, 1993; Pérez-Soba et al., 1994). Under high N deposition red pine foliar N concentration has significantly increased, but this increase was accompanied by a decoupling of the photosynthesis–N relationships (Bauer et al., 2004). The other negative impact of ammonia to the plants is related to the disturbances in nutritional balance (Van Dijk, Roelofs, 1988; Kaupenjohann et al., 1989; Ferm et al., 1990; Dueck et al., 1991; Pearson, Stewart, 1993; Fangmaier et al., 1994; Kupčinskienė, 2001). One-year exposure of young saplings of Scots pine to gaseous NH_3 at 53 or 105 μg m^{-3} in open-top chambers has caused an increase in the needle biomass and N concentration, and decrease of the element ratios (K/N and P/N), showing that fumigation disrupted the nutrient balance (Pérez-Soba, Van der Eerden, 1993), increased the amount of arginine (Ferm et al., 1990; Pérez-Soba et al., 1994; Kupčinskienė, 2006). Due to elevated ammonia the xeromorphic structure of the needles of Scots pine might undergo changes and become more mesomorphic (Holopainen et al., 1992; Palatova, 2002).

Pine stand exposure to higher concentrations of air ammonia might change essential oil chemical composition (Momen et al., 2002; Judzentiene et al., 2006; Maciag et al., 2007; Kupčinskienė et al., 2008; Mateus et al., 2010), decrease frost resistance (De Kam et al., 1991; Dueck et al., 1991) and increase drought sensitivity in plants.

Scots pine (*Pinus sylvestris* L.) is the most widely spread and naturally growing in Lithuania forest tree species. Since early 90s forest tree conditions near stationary pollution sources in Lithuania have been in monitoring process. Various components of the forest ecosystem have been taken into account including the soil (Armolaitis, 1998; Vaicys, 1999; Armolaitis et al., 1999), herbaceous layer (Stiklienė, 2008) and trees in particular (Skuodienė, Kairiūkštis, 1996; Juknys et al., 2003; Stravinskienė, Erlickyte, 2003; Stiklienė, 2008).

Numerous studies have described changes of Scots pine stands growing near the nitrogen fertilizer factory in Lithuania (JSC *Achema*) (Kupčinskienė, Huttunen, 2005; Judzentiene et al., 2006; Kupčinskienė, 2006). Among the investigated morphophysiological parameters of the pines histological features of the needles have not drawn wider attention (Stiklienė, 2008).

The present study aims at evaluation of the effects of elevated air ammonia on the morphology and histological parameters of the needles during the vegetation season.

MATERIALS AND METHODS

The study area selected was in the vicinity of JSC *Achema* annually (2008–2009) emitting over 2 000 kg of various pollutants. The sampling was performed in two different age (middle-aged and young age) stands of Scots pine growing around the factory in 0.5 km distance (west, north and north-east direction) and at the control site (Kačerginė) in 39 km distance from the factory in the south-west direction (unprevailing wind). Near the nitrogen fertilizer factory, the summer concentration of air ammonia (2000–2006) was respectively 16.1 μg m^{-3}, 17.2 μg m^{-3} and 17.5 μg m^{-3}, and at the control site it was 3.7 μg m^{-3} [25]. The age of the middle-aged stands ranged within 28–35 years, and the age of the young age stands ranged within 15–20 years. Forest sites on arenosols of *Vaccinio-myrtillo-Pinetum* were examined. During the vegetation period, tree sampling was performed three times (26 June, 30 July, 30 August 2009). Eight dominant trees were selected in each site. From every tree one shoot needles of 2 age classes (current-year and 1-year-old) were taken. Needle morphology was estimated using 10 needles from the shoot of each age class. The thickness and width of the needle were measured by micrometer (Moore & Wright, England).

The following parameters from the needle cross-section were taken: thickness of abaxial and adaxial cuticle, epidermis, hypodermis (further cuticle + epidermis + hypodermis are called under surface tissues), mesophyll, transfusion parenchyma, sclerenchyma thickness, also sclerenchyma length as described by Jokela (1998). Needle tissues were measured under the light microscope. In the present study 384 histological samples were assessed.

The mean values of the pine sites were calculated using each tree summarized data. Factorial analyses were accomplished to evaluate the site, stand age-related, sampling time-related and needle age-related differences. Dispersive, correlation analyses (Spearman's correlation) were accomplished by SPSS and MS Excel packages.

RESULTS

For the middle-aged stands the thickness of abaxial surface tissues (cuticle + epidermis + hypodermis) in the cross-section of the current-year needles ranged in the interval 25.5–26.6 μm, for 1-year-old needles the interval was similar 26.0–26.9 μm (Fig. 1a). For the young age stands, the thickness of abaxial surface tissues (cuticle + epidermis + hypodermis) in the current-year needles was 25.7–26.3 μm and in 1-year-old needles it was 25.5–26.4 μm. There were no significant site-related, stand age-related or needle age-related differences. Within the vegetation season, abaxial tissues covering needles remained of nearly the same thickness.

For the middle-aged stands, the thickness of adaxial cuticle + epidermis + hypodermis in the cross-section of the current-year needles was 25.8–26.3, in 1-year-old needles it was 25.9–26.5 (Fig. 1b). For the young age stands, the thickness of adaxial surface tissues (cuticle + epidermis + hypodermis) of the current-year needles ranged in the interval 25.9–26.7 μm, for 1-year-old needles the interval was similar 26.0–27.1 μm. There were no significant site-related, stand age-related or needle age-related differences. Within the vegetation season, covering tissues of the needles remained of nearly the same thickness. Comparison of the needle abaxial and adaxial sides showed that surface tissues were of very similar thickness. According to all tissues, the thickness in the cross section of 1-year-old needles covering tissues of both sides comprised 7%.

For the middle-aged stands, the sclerenchyma thickness in the current-year needles was 100–133 μm and in 1-year-old needles it was 119–133 μm (Fig. 1c). For the young age stands, the sclerenchyma thickness for the current-year needles was of wider range 96.4–137.1 μm and in 1-year-old needles it was 116–144 μm. For the mechanical tissues, there were also no significant site-related, stand age-related differences. Within the vegetation season, tissues covering needles remained of nearly the same size while the thickness of sclerenchyma was significantly ($p < 0.05$) increasing up to 1.25 times for the current-year needles of the young age stands. More intensive growth in this tissue occurred between June and July compared to that of July and August. The sclerenchyma thickness did not undergo greater seasonal changes among 1-year-old needles. According to all tissues, the thickness of sclerenchyma in the cross section of 1-year-old needles comprised 18.5 %.

The sclerenchyma width of the needles was several times bigger compared to the sclerenchyma thickness. In the middle-aged stands, the sclerenchyma width in the current-year needles was 520–698 μm and in 1-year-old needles it was 625–768 μm (Fig. 1d). In the young age stands, the sclerenchyma width of the current-year needles was in the range 548–713 μm and in 1-year-old needles it was 639–786 μm. Within the vegetation season, sclerenchyma was significantly ($p < 0.05$) increasing up to 1.20 times for the current-year needles of the middle-aged stands. For young age stands, the sclerenchyma width in August was up to 1.18 times higher. For both age stands, variation in the sclerenchyma of 1-year-old needles was much smaller and depended on the site.

For the middle-aged stands, the thickness of abaxial mesophyll in the current-year needles was 175–190 μm, and in 1-year-old needles it was 195–204 μm (Fig. 2a). In the young age stands, the abaxial mesophyll thickness in the current-year needles was 176–192 μm, in 1-year-old needles it was 195.2–206 μm. For the middle-aged stands, the thickness of adaxial mesophyll in the current-year needles was 167–189 μm and in the 1-year-old needles it was 199–219 μm (Fig. 2b).

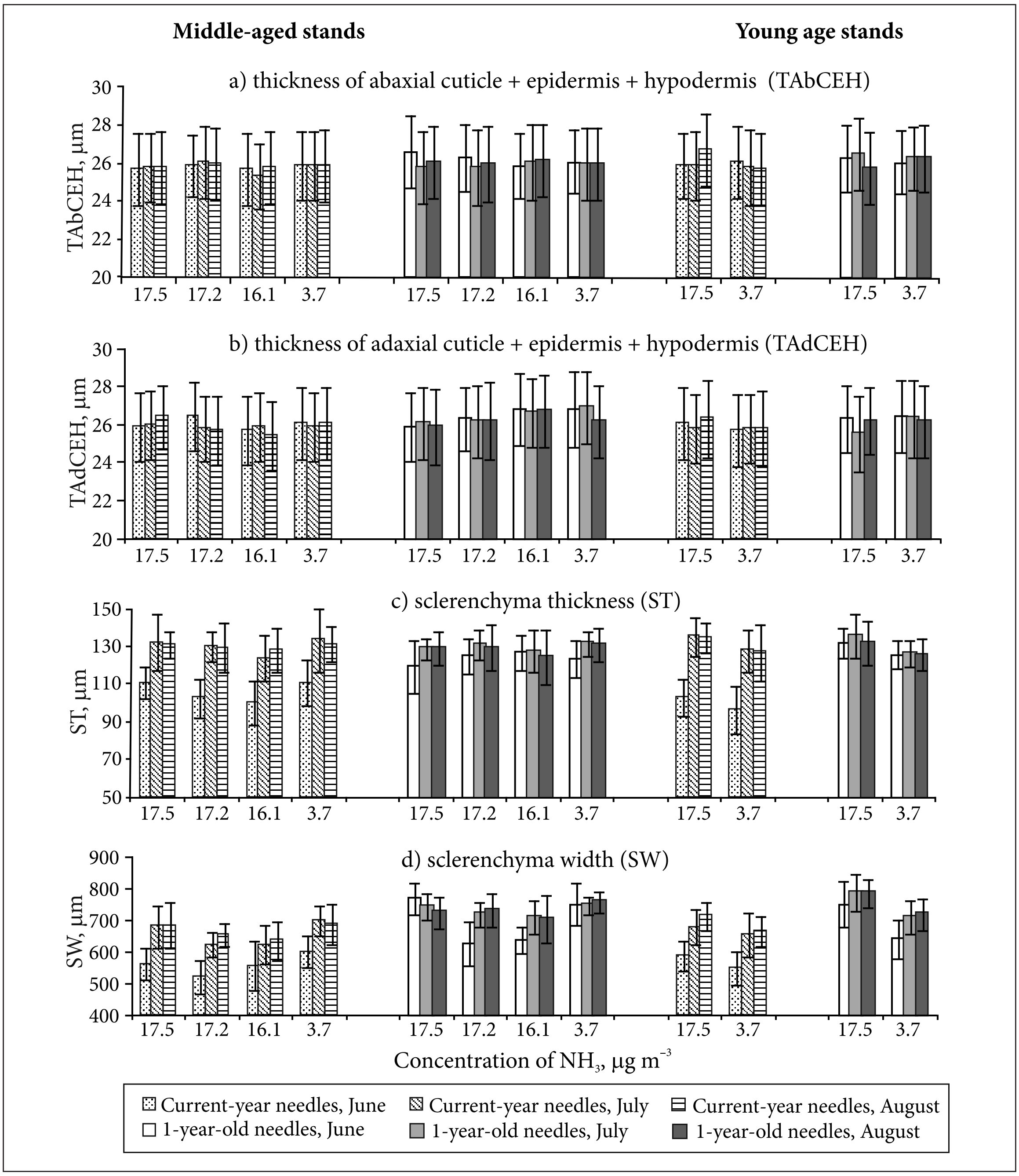

Fig. 1. Seasonal dynamics of the tissue size (thickness of abaxial cuticle + epidermis + hypodermis and sclerenchyma) in the cross sections of current-year and 1-year-old needles of Scots pine (*Pinus sylvestris* L.) young age and middle-aged stands growing in different concentrations of aerial ammonia (June–August, 2009)

For the young age stands, the adaxial mesophyll thickness in the current-year needles was 164–193 µm, in 1-year-old needles it was 204–221 µm (Fig. 2b). For both age stands adaxial mesophyll in the current-year needles was larger up to 1.10 times in sites with higher concentration of air ammonia (17.5–16.1 µg m^{-3}) compared to the control site although differences were not significant. For middle-aged stands, significant ($p < 0.01$) differences were observed between the current-year and 1-year-old needles according to the abaxial and adaxial mesophyll thickness. Abaxial mesophyll in 1-year-old needles was larger by 1.05–1.12 times compared to the current-year needles, while adaxial mesophyll differences were smaller by 1.16–1.21 times. For young age stands, the

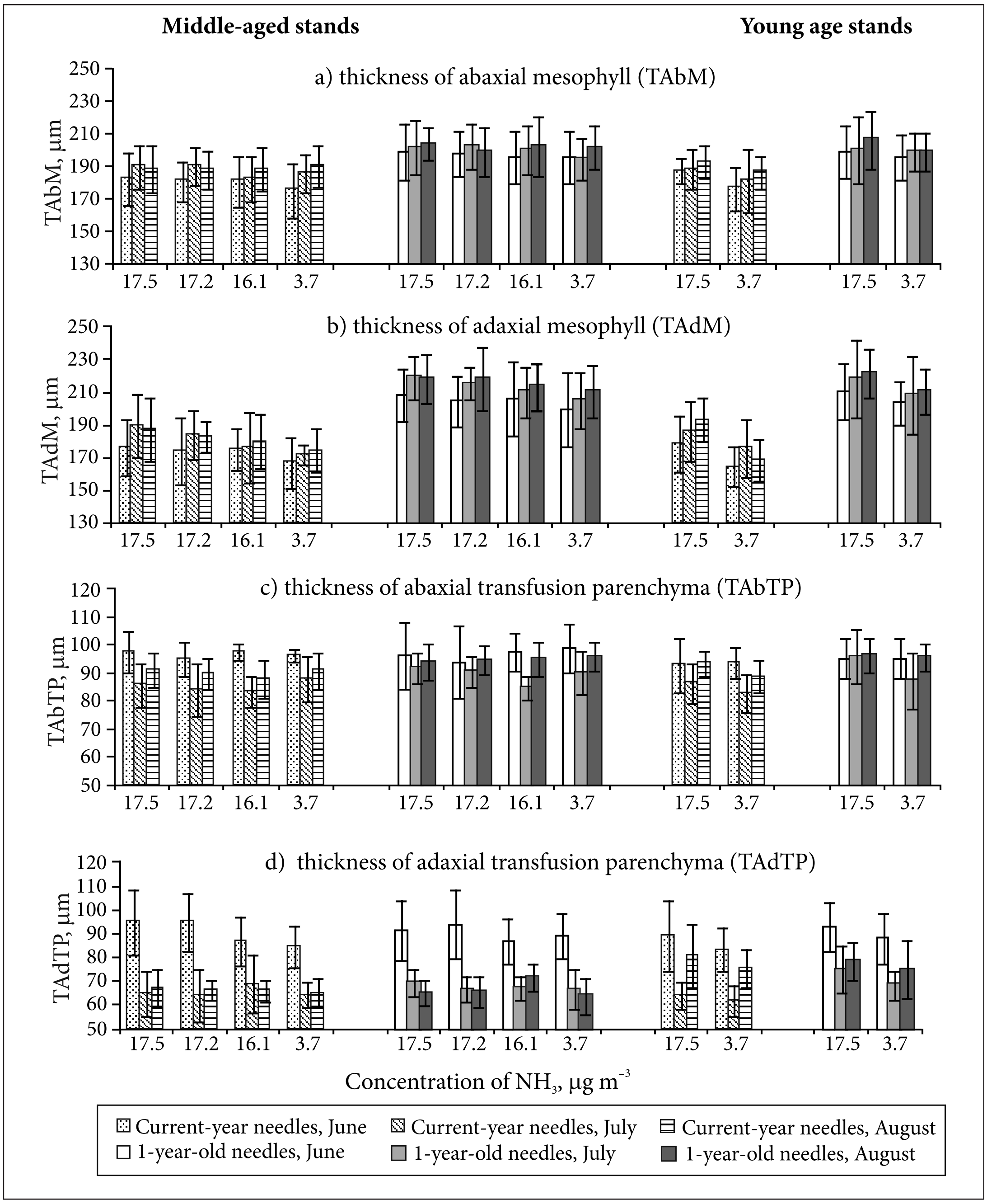

Fig. 2. Seasonal dynamics of the tissue size (thickness of abaxial and adaxial mesophyll, abaxial and adaxial transfusion parenchyma) in the cross sections of current-year and 1-year-old needles of Scots pine (*Pinus sylvestris* L.) young age and middle-aged stands growing in different concentration of aerial ammonia (June–August, 2009)

adaxial mesophyll thickness was up to 1.25 times bigger ($p < 0.01$) in 1-year-old needles compared to the current-year needles. Within the vegetation season, needle abaxial and adaxial mesophyll were slightly increasing (insignificant changes) for the current-year needles. According to all tissue thickness in the cross section of 1-year-old needles mesophyll tissue comprised 54.8%.

For the middle-aged stands, the abaxial transfusion parenchyma thickness in the current-year needles was 83.3–97.6 µm, and in 1-year-old needles it was 84.5–98.8 µm (Fig. 2c). For the young age stands, the abaxial transfusion parenchyma thickness in the current-year needles was 82.7–94 µm, and in 1-year-old needles it was 87.5–96.4 µm. For the middle-aged stands, the thickness

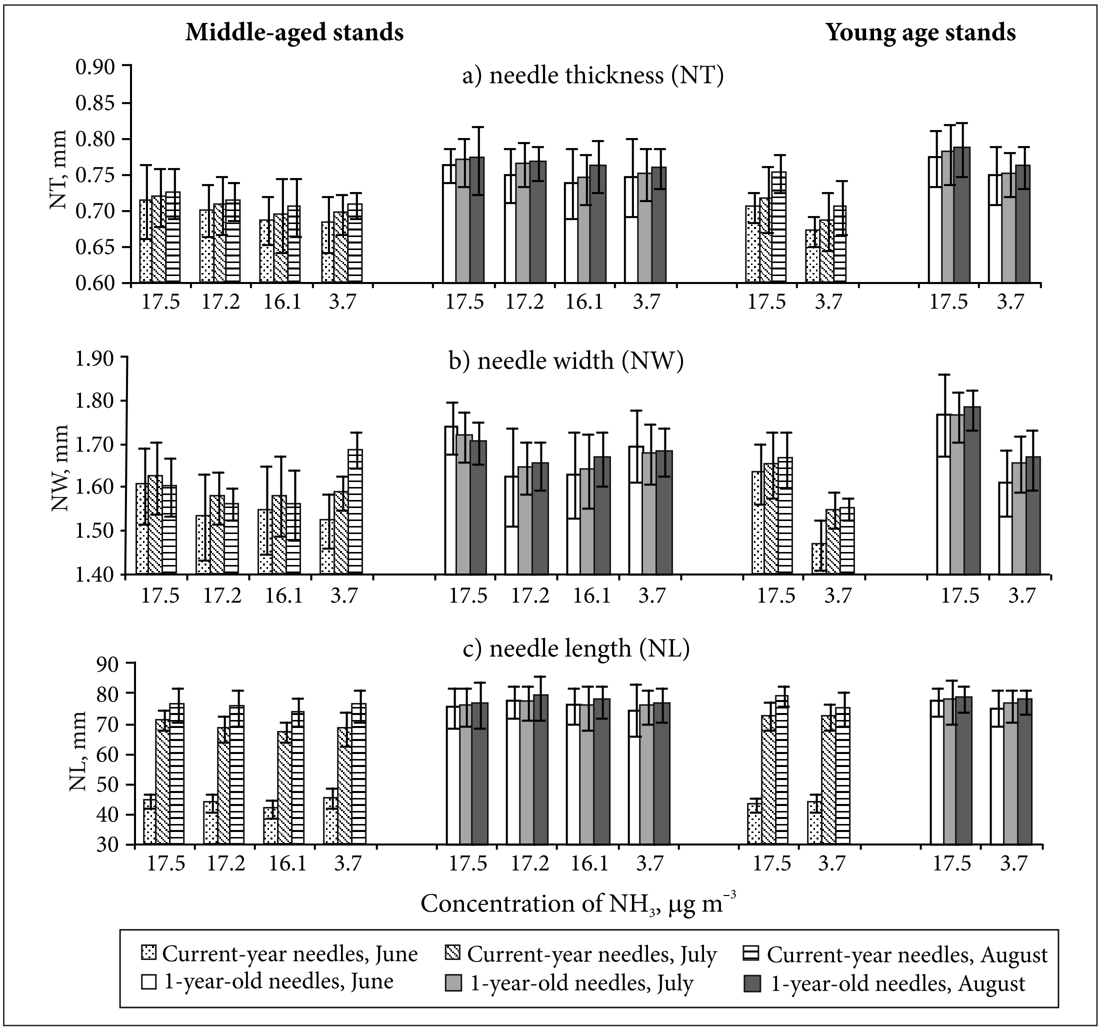

Fig. 3. Seasonal dynamics of the needle thickness, width and length of current-year and 1-year-old needles of Scots pine (*Pinus sylvestris* L.) young age and middle-aged stands growing in different concentrations of aerial ammonia (June–August, 2009)

of adaxial transfusion parenchyma in the current-year needles was 64.3–95.2 µm and in 1-year-old needles it was 64.9–94 µm (Fig. 2d). For the young age stands, the adaxial transfusion parenchyma thickness in the current-year needles was 61.9–89.3 µm, in 1-year-old needles it was 68.5–92.9 µm. In all cases site-related differencies were not significant. Stand age-related differences in transfusion parenchyma were also unexpressed. The adaxial transfusion parenchyma thickness was slightly larger in 1-year-old needles compared with the current-year needles. For young age stands, the adaxial transfusion parenchyma thickness in the current-year needles was significantly ($p < 0.05$) larger (up to 1.17 times) in June when compared to that in July–August. The same was true for middle-aged stands although seasonal differences were larger (up to 1.48 times). According to the tissue thickness in the cross section of 1-year-old needles, transfusion parenchyma comprised 22.6%.

For the middle-aged stands, the thickness of the current-year needles was 0.68–0.72 mm, and the thickness of 1-year-old needles was 0.74–0.77 mm (Fig. 3a). For the young age stands, the thickness of the current-year needles was 0.67–0.75 mm and the thickness of 1-year-old needles was 0.75–0.79 mm. For the middle-aged stands, the width of the current-year needles was 1.52–1.62 mm and the width of 1-year-old needles was 1.64–1.74 mm (Fig. 3b). For the young age stands, the needle width of the current-year needles was 1.47–1.66 mm and the width of 1-year-old needles was 1.61–1.78 mm.

For the middle-aged stands, the needle length in the current year was 76.3–42 mm, the length of 1-year-old needles was 78.7–74.5 mm (Fig. 3c). For the young age stands, the needle length in the current year was 43.2–79.2 mm, the length of 1-year-old needles was 75.3–78.2 mm. Current-year needles from the young age stand with higher concentration of air ammonia (17.5 μg m^{-3}) were wider ($p < 0.05$) up to 1.11 times compared to the needles of Kačerginė site (3.9 μg m^{-3}). For the middle-age stands in June, 1-year-old needles were up to 1.10 times ($p < 0.01$) thicker, up to 1.11 times ($p < 0.01$) wider and up to 1.82 times ($p < 0.01$) longer compared with the current-year needles. For the young age stands in June, 1-year-old needles were up to 1.12 times ($p < 0.01$) thicker, up to 1.10 times ($p < 0.01$) wider and up to 1.79 times ($p < 0.01$) longer compared with the current-year needles. Stand age-related differences in needle morphology were not significant. Within 26 June – 30 August, the length of the current-year needles was increasing up to 1.76 times, changes in the needle width and thickness were not as great as in the length.

Some of the one-year-old needle morphological and tissue parameters were interrelated. The abaxial mesophyll thickness correlated with the adaxial mesophyll thickness ($r = 0.650$, $p < 0.001$), abaxial transfusion parenchyma thickness ($r = 0.553$, $p < 0.001$). The adaxial mesophyll thickness correlated with the adaxial transfusion parenchyma thickness ($r = 0.287$, $p < 0.05$). The needle width correlated with the adaxial and abaxial mesophyll tissues ($r = 0.622$, $p < 0.001$; $r = 0.736$, $p < 0.001$), abaxial transfusion parenchyma thickness ($r = 0.278$, $p < 0.05$), needle thickness ($r = 0.738$, $p < 0.001$). The needle thickness correlated with the adaxial and abaxial mesophyll tissues (respectively, $r = 0.684$, $p < 0.001$; $r = 0.795$, $p < 0.001$), abaxial and adaxial transfusion parenchyma thickness (respectively, $r = 0.440$, $p < 0.01$; $r = 0.227$, $p < 0.05$).

DISCUSSION

Scots pine usually grows in cold climate coupled with the low level of nutrients in the soil. Conifers are very sensitive to elevated ammonia flux (Pearson, Stewart, 1993). Surplus of this gas to Scots pine might result in detrimental changes of the physiological processes which finally lead to decreased resistance of the trees to drought or frosts (Jokela, 1998).

Needles with thicker cuticle, smaller intercellular space in the mesophyll and wider sclerenchyma tissues of the central cylinder are typical for pines in natural habitats. In such conditions conifer needles are of xeromorphic structure. Xeromorphic features play protection role against environmental stresses (drought, freeze, pathogenic organisms) (Huttunen, 1973; Jokela, 1998). On the contrary, mesomorphic needle features are related to thicker and longer needles and also larger width of adaxial mesophyll (Jokela et al., 1995; Jimenez, 2000).

Application of calcium, ammonium and nitrate containing fertilizers with total nitrogen concentrations corresponding to 0, 75, 150, 250, 500 and 1 000 kg ha^{-1} to the stands of Scots pine resulted in changes of nitrogen concentration in the needles and also natural structure transformation to the mesomorphic one (Jokela et al., 1995). Fertilization with high level nitrogen (250, 500, and 1 000 kg N ha^{-1}) caused changes in the current-year needle thickness and width. Tissues of adaxial mesophyll, central cylinder, especially sclerenchyma were the main targets of the elevated nitrogen deposition. Larger cells of transfusion parenchyma could be an indicative for the mesomorphic needles.

In the ultrastructural level mesomorphic features of the needles are distinguished by a larger area of parenchyma and bigger cells of this tissue, thinner cuticle and hypodermis tissues (Murtaza, Paul, 1989).

Despite better growth in terms of higher mass and length of the needles under high nitrogen deposition, simultaneously adverse effects might occur including decrease in the area of sclerenchyma. In the case of N excess, the cell walls of sclerenchyma might become thinner due to deficiency of certain nutrients, for example, boron (Jokela et al., 1995; Jokela, 1998).

According to former decade data, Scots pine growing at higher ammonia concentration near the factory has longer needles (Kupčinskienė, Huttunen, 2005; Kupčinskienė, 2006). Fertilization with 200 kg N ha^{-1} increased the needle length, but larger than 400 and 600 kg N ha^{-1} deposition did not influence the morphology of needles (Kellomaki et al., 1982).

Before the present assessment, pine needle histology has not been investigated in the pine stands close to the factory. In 2005–2006, needle tissue parameters were taken from the middle-aged stands growing in areas less polluted by ammonia (gradient interval only 5.0–13.2 μg m^{-3}) in 2.5–22 km distance from the factory. Length of the needles decreased with growing distance from the factory, presumably due to lower concentrations of air ammonia (Stiklienė, 2008). According to our study, the length, thickness and width of the needles did not reflect pollution influence at the elevated concentration of air ammonia near the JSC *Achema*. Despite of it, documented in the present investigation needle length was quite big compared with other industrial regions of the country (Kupčinskienė, 2006).

Absence of distinctions in the needle morphology and tissue structure between two different stands (middle-aged and young age) might be explained by small-range stand age differences (15 or less years) compared to the entire pine tree age.

The fact that adaxial mesophyll was up to 1.25 times bigger in 1-year-old needles shows that September, also May of the next year might be important months for needle physiological processes. The same could be said about the needle thickness and width. In August, for most other tissue parameters needle age-related peculiarities were not documented. It is in agreement with the absence of climate extremes within the 2008 and 2009 summer period.

Significant differences in the needle tissue structure reflected seasonal needs of the trees: in June requirements for water and nutrient transport are much higher compared to the end of August, it has got reflection in the thickest transfusion parenchyma at the beginning of summer. Contrariwise, the proportion of sclerenchyma tissue in the needle was increasing within the growing season, it corresponded to the increasing current-year needle length.

The present level of industrial ammonia pollution is lower than the threshold for effects at the level of needle tissue parameters assessed by light microscopy.

CONCLUSIONS

At the level of the present industrial ammonia pollution, significant changes in morphology and histological parameters of the needles of Scots pine were not observed. Investigation of the size of various tissues in the cross-section of the needles sampled from the stands growing in the elevated aerial ammonia site did not reveal mesomorphic features of the needles.

Stand age significant differences were not observed according to needle morphology and histological parameters.

Statistically significant differences were observed between current-year and 1-year-old needles according to sclerenchyma width and mesophyll thickness, these tissues were increasing within age.

Seasonal differences were most expressed according to the current-year needle morphology and some histological parameters: sclerenchyma and width were increasing and adaxial transfusion parenchyma decreasing within June–August.

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DIDESNĖS AMONIAKO TARŠOS APLINKOJE AUGANČIOS PAPRASTOSIOS PUŠIES (*PINUS SYLVESTRIS* L.) SPYGLIŲ HISTOLOGINIŲ RODIKLIŲ KAITA PER AUGIMO SEZONĄ

Santrauka

Amoniakas yra vienas labiausiai spygliuočių spyglių audinių sandarą žalojančių teršalų. Tyrimu siekta nustatyti, ar dėl AB „Achema“ išmetamo amoniako (vidutinės amoniako koncentracijos ore prie gamyklos vasaros mėnesiais siekia 17,5 μg m^{-3}) vegetacijos metu (birželį–rugpjūtį) kinta paprastosios pušies (*Pinus sylvestris* L.) spyglių morfologiniai ir histologiniai rodikliai. Ištyrus spyglių ilgį, storį, plotį ir audinių storį pagal spyglio skerspjūvį, adaksialinės ir abaksialinės spyglio dalies audinių – kutikulos, epidermio, hipodermos, mezofilo, transfuzinės parenchimos, sklerenchimos – storį, pakitimų dėl taršos nenustatyta. Tokia išvada prieita ištyrus tiek pušų jaunuolynus, tiek pusamžius medynus, augančius prie AB „Achema“, ir palyginus juos su atitinkamo amžiaus medynais Kačerginėje. Didesnės amoniako koncentracijos aplinkoje buvo pastebėtos tik spyglių storėjimo (iki 1,05 karto), adaksialinės pusės mezofilo storėjimo (iki 1,10 karto) ir adaksialinės pusės transfuzinės parenchimos storėjimo (iki 1,06 karto) tendencijos. Rugpjūčio pabaigoje spyglių ilgis siekė 75–79 mm, plotis – 1,6–1,7 mm ir storis – 0,74–0,77 mm. Nustatyti statistiškai reikšmingi ($p < 0,05$) sezoniniai spyglių audinių pakitimai: transfuzinės parenchimos storis mažėjo, o sklerenchimos audinio storis didėjo nuo birželio iki rugpjūčio mėnesio.

Raktažodžiai: spyglių audiniai, spyglių morfologija, spygliuočiai, sezoninė kaita, sklerenchima, laidžioji parenchima