

MONITORING THE DAMAGE TO EPICUTICULAR WAXES AT SILVER BIRCH (*BETULA PENDULA* ROTH.) IN THE CHANGING AIR POLLUTION SPECTRUM OF THE ORE MOUNTAINS

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Abstract

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The paper deals with the study of damage to the assimilatory apparatus of silver birch (*Betula pendula* Roth) using the study of changes of epicuticular waxes in the period 1997 to 2009. During the period, changes in the air pollution spectrum occurred in the area of the Ore Mountains. At the beginning of the monitoring, above-limit concentrations of sulphur oxides were the main anthropogenic factor. These concentrations resulted in the degradation of epicuticular waxes. Statistical evaluations demonstrated the high dependence of damage to epicuticular waxes on high concentrations of sulphur oxides. Damage to epicuticular waxes by air pollution increased with the increasing altitude of a forest stand. Since 2001, measured values of SO₂ decreased to a minimum but a new phenomenon appeared damaging the assimilatory apparatus of forest stands, namely above-limit concentrations of ground ozone. These above-limit concentrations of ground ozone damage the assimilatory apparatus including epicuticular waxes at all silver birch stands occurring at altitudes above 800 m. This finding was also proved by the high coefficient of determination.

birch (*Betula pendula* Roth.), air pollution, epicuticular waxes, altitude, damage, sulphur oxides, ground ozone

In the 80s of the 20th century, considerable damage to forest stands occurred by high concentrations of sulphur dioxide in the Ore Mountains. After 1989, a marked decrease of air-pollution load occurred but at the highest locations of the Ore Mountains, concentrations of sulphur oxides reached always above-limit values for forest trees, which became evident on the health condition of silver birch. In case of damage to the assimilatory apparatus by air pollution, functional disturbances and finally also abnormalities in the morphology of leaves occur first. These disturbances reflect largely physiological processes (Hoad *et al.*, 1998). The plant epidermis with cuticle and waxes are damaged as the first due to the influence of external factors on the plant (Tuomisto and Neuvonen, 1993). Due to the effect of air pollution, the loss of waxes occurs together with

the change of structures of epicuticular waxes (Hoad *et al.*, 1992). The cuticle with wax surface increases the resistance of leaves against the penetration of gases such as SO₂, O₃ and NO_x. Properties of cuticle also largely affect the accumulation and subsequent behaviour of pesticides, growth regulators, leaf nutrients and other chemical preparations (Ylimartino *et al.*, 1994). In recent ten years, the change in the air pollution spectrum occurred at high-altitude areas of the Czech Republic. Sulphur oxides decreased to minimum values but above-limit concentrations were measured of ground ozone for forest ecosystems. At present, ozone is considered to be the most important gaseous harmful substance affecting the health condition of forests in Europe (Ashmore, 2003). The harmfulness of ozone depends on the detoxication capacity of

a plant differing at particular tree species (Ditmar *et al.*, 2004). In various experiments, relatively high susceptibility of birch was demonstrated (Skelly *et al.*, 1998, Pääkkönen *et al.*, 1998, Karnosky *et al.*, 2002, 2003). At the necrosis of cells of upper mesophyll, damage caused by chlorosis and necrotic spots on leaves becomes visibly evident. In addition to leaf damage, ozone also affects unfavourably the total energy balance of trees, which results in the reduction of resistance to other stress factors (Jones *et al.*, 2004).

MATERIAL AND METHODS

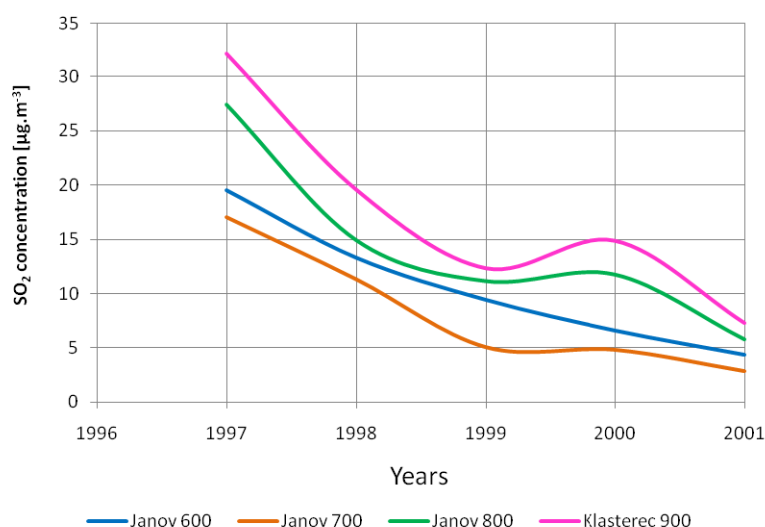
In the period 1997 to 2009, damage to the assimilatory apparatus of silver birch (*Betula pendula* Roth.) was evaluated by the method of changes in epicuticular waxes in the altitudinal gradient from 600 to 1000 m of the Ore Mountains, namely on ten research plots of the second age class in relation to the changing air pollution spectrum and environment parameters. Sampling the plant material was carried out on the first week in September, always from three trees of each of the monitored localities. The amount of epicuticular waxes on the leaf surface was determined in the laboratory according to the method of Günthardt *et al.* (1994). Measured values of SO_2 concentrations in 1997 to 2001 were obtained from stations of the ČHMU (Czech Hydrometeorological Institute) occurring in the vicinity of the monitored silver birch stands. From 2003, concentrations of ground ozone were measured directly in the evaluated stands using passive dosimeters of Gradko Co. UK. Every-year installation of passive dosimeters occurred in the phenological stage "foliage beginning" 100%. The passive dosimeters were tested compared to AIM ozonometers (Czech Hydrometeorological Institute) with very good results (Šrámek *et al.*, 2007). At all

research plots, air temperatures were continually measured in the lower part of crowns where also the passive dosimeters were placed in four weeks intervals. Solar radiation and precipitation were monitored in the open area.

RESULTS AND DISCUSSION

At the beginning of our monitoring in 1997–1999, above-limit concentrations of sulphur oxides for forest trees were measured in the Ore Mountains also in the summer season (SO_2 concentrations exceeding $20 \mu\text{g} \cdot \text{m}^{-3}$ (Fig. 1).

Mean monthly maxima of SO_2 reached $49 \mu\text{g} \cdot \text{m}^{-3}$ at these localities in summer months, which proved negatively in the quantity and quality of epicuticular waxes of silver birch (Bednářová, 2001). The development of epicuticular waxes at silver birch in the Ore Mountains in the period 1997 to 2009 is characterized by Figs. 2 and 3. Literature sources (Turunen *et al.*, 1995) mention that even short-term exceeding above-limit concentrations of sulphur oxides shows irreversible negative impacts on the degradation of epicuticular waxes in forest trees. In 1998, lower concentrations of sulphur oxides were measured at all localities as against 1997. At lower altitudes, the decline of sulphur oxide concentrations by 32% occurred and at higher altitudes, the air pollution load decreased even by 55% as compared to values from the previous year. This change showed itself favourable in the condition of epicuticular waxes. In 1997, $53 \text{ mg} \cdot \text{g}^{-1} \text{DM}$ (dry mass) were detected on leaves from the lowest (alt. 600 m) research plot and in silver birch stands at the highest altitude (1000 m) only $34 \text{ mg} \cdot \text{g}^{-1}$. Quantitative analyses of the plant material in 1998 demonstrated the higher amount of epicuticular waxes on silver birch leaves at all altitudes of monitored stands as compared to the previous



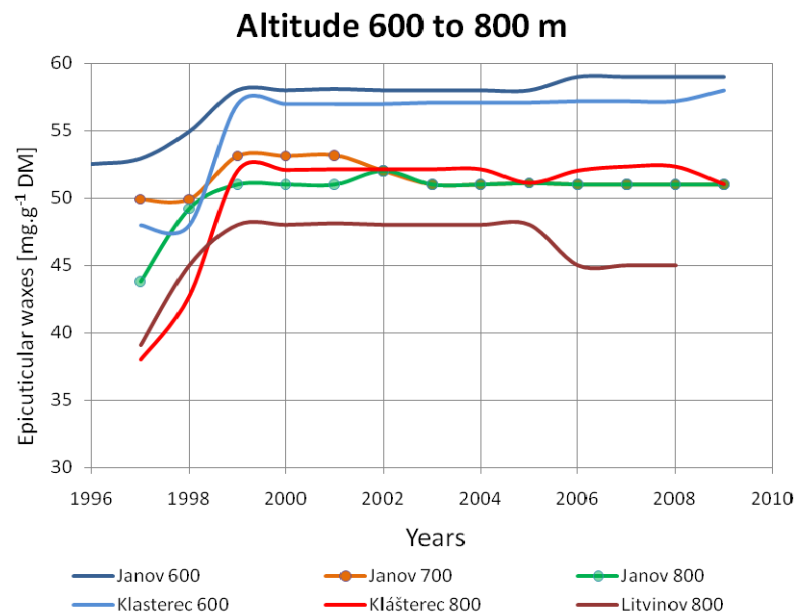
1: Mean monthly concentrations of $\text{SO}_2 \mu\text{g} \cdot \text{m}^{-3}$ in monitored areas of the Ore Mountains in the period May to August

year. At an altitude 600m, 55 mg.g⁻¹DM (dry matter content) leaves were measured and in stands at an altitude of 1000m some 35 mg. g⁻¹DM leaves were found. This situation is characterized in Figs. 1 and 3. In 1999, another decline the SO₂ air pollution load occurred, which resulted in the repeated increasing the amount of epicuticular waxes on birch leaves in the period of leaf sampling (at the beginning of September).

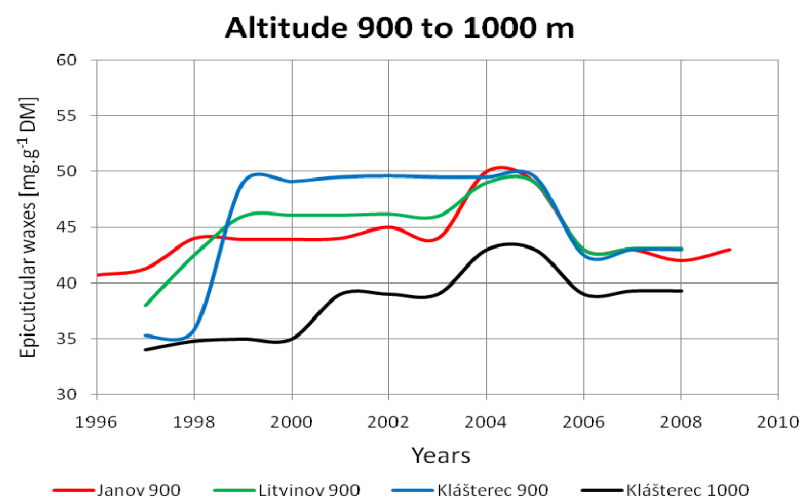
In monitored stands at higher altitudes, the increased amount of epicuticular waxes on birch leaves occurred in 1999, namely as many as by 27% as against 1998. An increase in the total amount of epicuticular waxes on birch leaves became mostly evident at stands growing at higher altitudes together with the marked decline in the concentration of

sulphur oxides in this year. Since 1999, the amount of epicuticular waxes from stands at lower altitudes (600–800m) ranges nearly at the same level. Only at the Litvínov stand at an alt. of 800m, the higher loss of epicuticular waxes occurred in 2006, which could be possible to explain by high concentrations of ground ozone at altitudes exceeding 800m in this year.

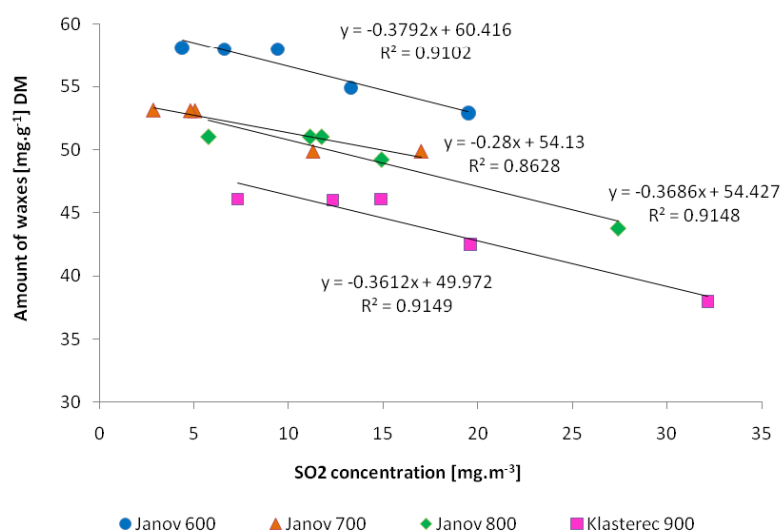
Within the period of monitoring, the decreasing amount of epicuticular waxes was demonstrated on leaves with the increasing altitude of monitored stands, which is proved by a determined dependence $R^2 = 0.8969$ ($y = -0.0473x + 86.24$). Above-limit concentrations of sulphur oxides resulted in the degradation of epicuticular waxes and decreasing



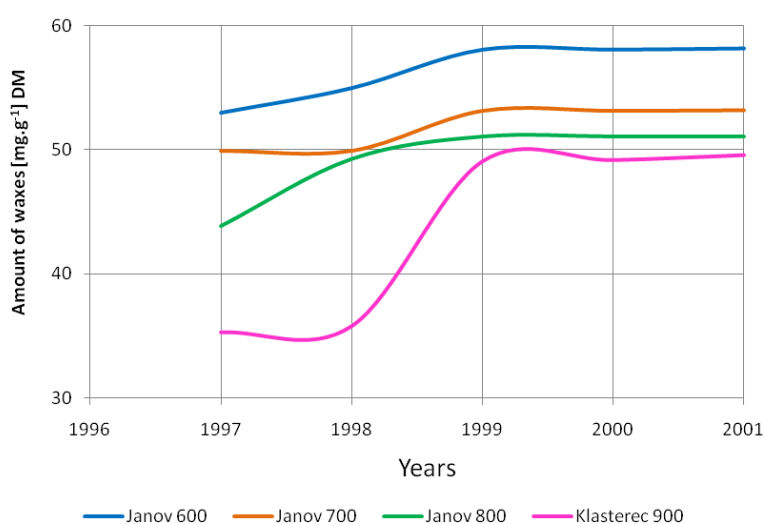
2: Amount of epicuticular waxes in birch (*Betula pendula*) in lower altitudes



3: Amount of epicuticular waxes in birch (*Betula pendula*) in higher altitudes



4: Regression between concentration of sulphur dioxide and amount of epicuticular waxes at different altitudes



5: Trend in amount of epicuticular waxes after the main SO_2 attack

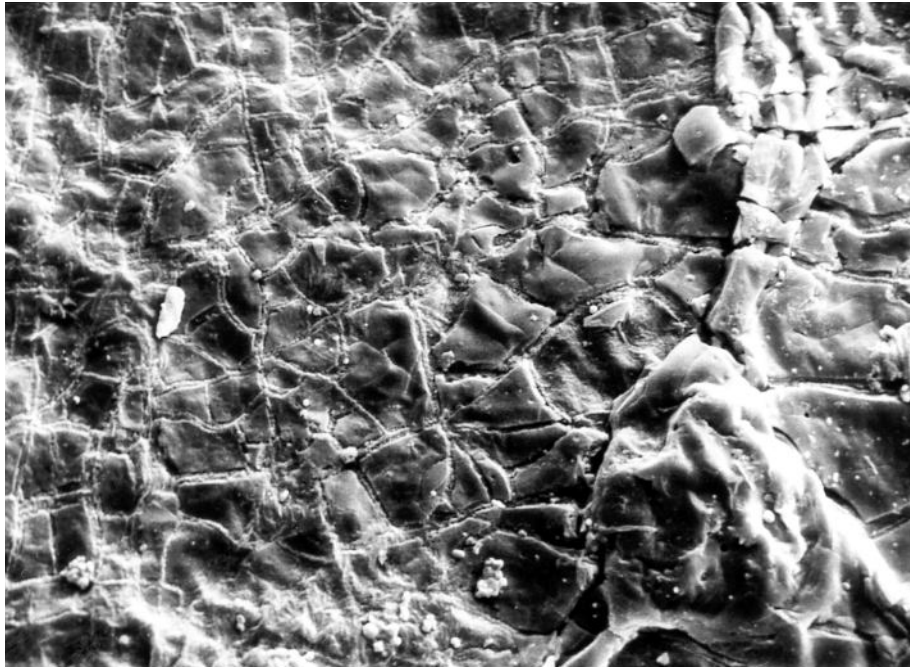
their total amount on the leaf surface, which was expressed by high dependences evident from Fig. 4.

Fig. 5 characterizes the level of the measured amount of epicuticular waxes at silver birch growing in the immediate vicinity of stations measuring the sulphur oxide concentrations in the period 1997 to 2001.

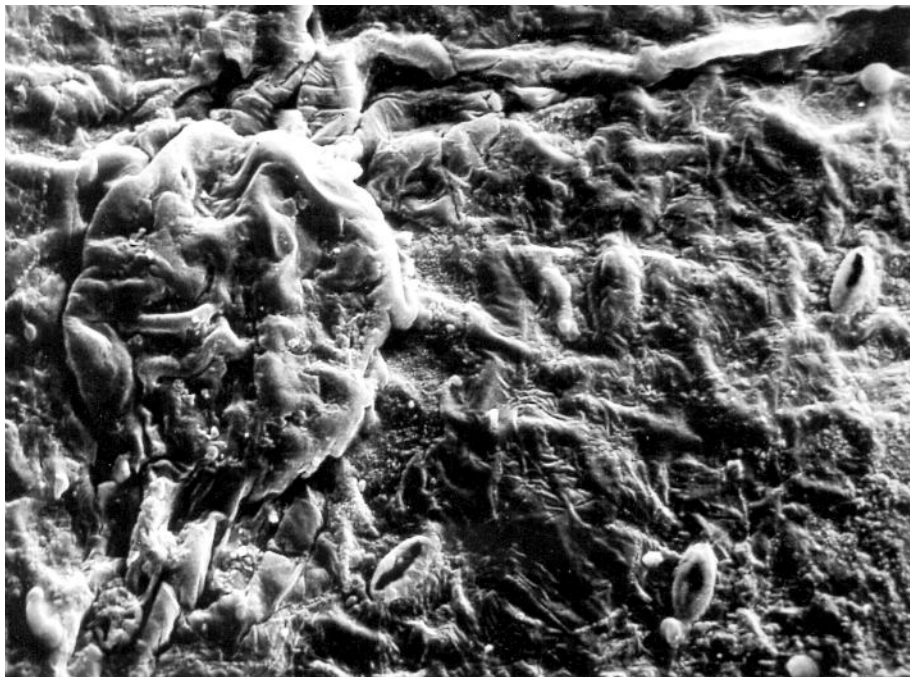
In 2000, measured values of sulphur oxides decreased to insignificant values throughout the altitudinal gradient, however, since 2000, gradual increasing the ground ozone concentrations occurred in forest stands. In 2001, the average concentration of ground ozone was measured (in the Ore Mountains at an alt. 800 m at CHMI stations) for the period May to August, namely $91.00 \mu\text{g} \cdot \text{m}^{-3}$, which exceeds a limit for forest trees amounting to $80 \mu\text{g} \cdot \text{m}^{-3}$. In 2002, the average value of ozone

concentrations $92 \mu\text{g} \cdot \text{m}^{-3}$ was determined for the same period. Since 2003, above-limit concentrations of ground ozone were measured at six long-term monitored plots occurring at altitudes 600 to 1 000 m (Fig 8).

At all higher-situated plots, lower amounts of epicuticular waxes were measured on birch leaves as against the previous year. Increased concentrations of ground ozone resulted in the higher degradation of epicuticular waxes, which became evident in the destruction of waxes (Fig. 6) and their total loss. Fig. 6 characterizes the damaged structure of epicuticular waxes of birch occurring on a considerably exposed plot Janov (alt. 900 m). On the lowermost monitored plot (alt. 600 m), the epicuticular waxes loss on birch leaves or damage to wax structures did not occur (Fig. 7).



6: Damaged epicuticular waxes structure on birch leaves



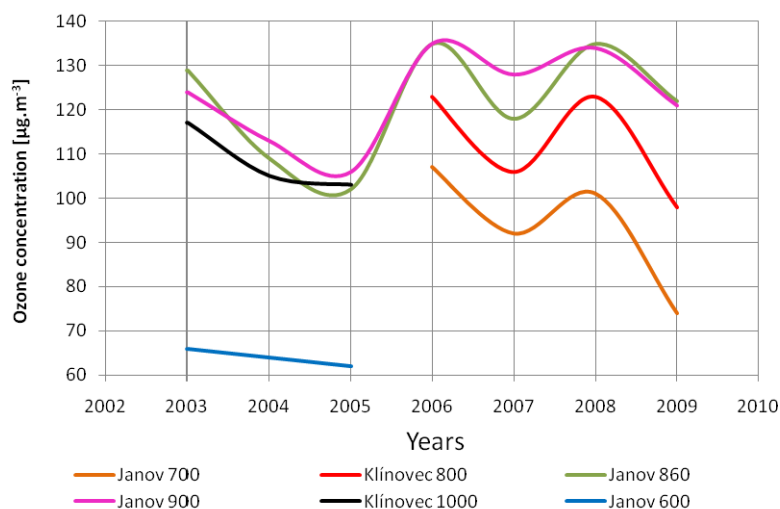
7: Intact epicuticular waxes structure on birch leaves

In next years (2003–2009), the above-limit amount of ground ozone was measured at all localities occurring at altitudes exceeding 800m in the period May to August (Fig. 8).

In 2004 and 2005, concentrations of ground ozone at localities over 800m were lower than in 2003, which affected positively the amount of waxes on leaves. Another increase of the ground ozone concentration occurred in 2006, which resulted in the loss of epicuticular waxes in stands at higher

altitudes. In 2007, a slight increase of epicuticular waxes and decline of ozone concentrations occur. In 2008, when ground ozone concentrations increased again, the total decrease of epicuticular waxes occurred at all stands at altitudes over 800m alt.

From 2003 to 2009, it was possible to note a visible damage to the assimilatory apparatus of silver birch (in addition to the loss of epicuticular waxes) at localities occurring at an altitude over 800m. Statistical evaluations demonstrated high



8: Measured values of ozone concentration in the altitudinal gradient from 600 to 1 000 m of the Ore Mts

dependence of the amount of epicuticular waxes on birch leaves depending on measured concentrations of ground ozone in stands. This dependence increased with the increasing altitude of monitored silver birch stands. At stands occurring at an altitude of 900m, the coefficient of determination $R^2 = 0.7704$ ($y = -0.2661x + 77.585$) was found. At stands occurring at an altitude of 1000m, the dependence was even higher, namely $R^2 = 0.9666$ ($y = -0.1344x + 55.937$).

In a period with above-limit concentrations of ground ozone, chlorotic spots were created on leaves (without damaged venation) in stands at higher locations, which were evident also on the abaxial face of leaves at the end of August. Visible symptoms of negative effects of ozone became evident several weeks after the development of leaves (leaf unfolding), particularly at localities situated towards south or south-west. This finding corresponds with data of Günthardt-Georg, Vollenweider (2003) and with the description of symptoms of visible damage caused by effects of ground ozone (Novotný *et al.*, 2009).

Epicuticular waxes play an important role as a barrier between the leaf surface and the ambient environment. Their insufficient degraded layer cannot already protect the assimilatory apparatus from external effects, pathogens, UV radiation, fungal attack, viruses and insect, which is proved by papers of a number of authors, such as Karnosky *et al.*, (2002), Mengden (1996), Percy *et al.*, (2002). Thus, knowledge concerning the development of the condition of epicuticular waxes under the effect of changing air pollution can contribute to elucidate damage to trees in areas, which have occurred under the impact of anthropogenic factors.

CONCLUSIONS

The 13-year study of damage to the assimilatory apparatus by anthropogenic effects using the

method of the evaluation of changes in epicuticular waxes on the surface of silver birch proved considerable effects of sulphur oxides and above-limit concentrations of ground ozone. At the beginning of monitoring (1997 to 2000) epicuticular waxes showing the protection role for the assimilatory apparatus were considerably damaged by high concentrations of SO_2 in the period May to September. Degradation of waxes manifested itself by the total loss of epicuticular waxes on the surface of leaves and their destruction. Statistical evaluation demonstrated the high dependence of the epicuticular wax decline on above-limit concentrations of sulphur oxides. The loss of epicuticular waxes on silver birch leaves increased with the increasing altitude of birch stands. Since 2001, the marked decrease of air pollution load in the form of sulphur oxides occurred, however, at localities situated at higher altitudes (over 800 m), a new phenomenon appeared, namely high concentrations of ground ozone. From 2003 to 2009, ground ozone concentrations largely exceeding a limit for forest trees were measured in all monitored stands (above 800m alt.). The highest concentrations of ground ozone were measured in birch stands in 2006 when a mean value reached $135 \mu\text{g} \cdot \text{m}^{-3}$ in the period May to August. Above-limit concentrations of ground ozone resulted in the loss epicuticular waxes at silver birch stands occurring at higher altitudes, which proved high statistical dependence. On leaves of birch growing at altitudes over 800m, visually noticeable disturbances of the assimilatory apparatus typical of damage caused by ground ozone were also noted. The leaf cuticle, particularly its external wax layer, creates an important barrier from toxic substances. However, if this protective layer is insufficient, negative effects can become evident also in other physiological processes and in reduced resistance to pathogenic viruses and fungal diseases.

SUMMARY

Monitoring the damage to epicuticular waxes to silver birch (*Betula pendula* Roth.) in the changing air pollution spectrum of the Ore Mountains.

A long-term study of the assimilatory apparatus damage in silver birch using the evaluation of changes of epicuticular waxes on the leaf surface revealed a highly negative influence of above-limit concentrations of sulphur dioxide and ground ozone on the condition of epicuticular waxes. Epicuticular waxes have a unique role in the protection of assimilatory apparatus against unfavourable environmental conditions. Their decrease and destruction influence other physiological procedures. Knowledge of epicuticular waxes condition under the influence of changing immissions may help to explain the damage of tree species in the areas that were and still are affected by anthropogenic factors. The amount and structure of epicuticular waxes were studied according to long-term proved methods. Concentrations of sulphur dioxide and ground ozone were measured directly in the observed stands. During the period 1997 to 2009 the immission spectrum in the Ore Mountains changed. At the beginning of the study high concentrations of sulphur oxides were the main anthropogenic factor even in the summer period. Degradation of epicuticular waxes that have a protective role on the assimilatory apparatus increased according to increasing altitude of the stands. Since 2001 the measured concentrations of sulphur dioxide fell down to minimal values, however an above-limit concentration of ground ozone appears as a new phenomenon that causes damage of the assimilatory apparatus in the forest tree species. High concentrations of ground ozone damage the assimilatory apparatus including the destruction of epicuticular waxes. This procedure is accompanied by visible leaf damage. Above-limit concentrations of ground ozone in the period April to September negatively influenced the assimilatory apparatus in all silver birch stands (*Betula pendula* Roth.) growing in the altitudes above 800m. Obtained results are confirmed by high determinance coefficients in the statistical analysis.

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