About phytopathological and histological aspects of Norway spruce dieback in the Orlické hory Mts.

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ABSTRACT: The condition was evaluated of tissues of declining young spruce stands in selected localities of the Orlické hory Mts. It refers to stands occurring in top parts affected by an air pollution disaster in the last century. Several types of damage of a different symptomatic character were distinguished. A high NO_x input appears to be the risk factor of spruce dieback in the Orlické hory Mts. One of the main causes in the complex of factors is the unbalanced cause of meteorological factors during the year and unbalance in mineral nutrition. The result is decrease in lignification, immaturity of tissues before the winter period and increased susceptibility of damaged tissues to parasites. Several potentially pathogenic species of micromycetes have been identified which can be considered to be significant pathogenic agents in case of declining spruce in the Orlické hory Mts., however, not a main cause.

Keywords: spruce decline; *Gremmeniella abietina*; *Ascocalyx abietina*; *Sirococcus strobilinus*; NO_x

Šach et al. (1999) draw attention to spruce dieback in the Orlické hory Mts. They notice high values of fluorine, chlorine and particularly rapid increase in nitrogen depositions (mainly NO_3) and ozone. These elements work together with a remaining load caused by sulphur oxides. Nárovec (Soukup, Pešková 2000) noticed for the first time the potential occurrence of the fungus *Gremmeniella abietina* (Lagerb.) Morelet (syn. *Ascocalyx abietina* (Lagerb.) Schläpfer – Bernhard) in the Orlické hory Mts. in 1997. Problems of the role of the fungus have been steadily discussed (Klíma 2002; Nárovec 2001, 2002). In abroad, similar symptoms of spruce dieback are connected with activities of the same fungus being termed as Brunchorstia dieback of conifers (Barklund 1989; Solheim 1986; Barklund, Rowe 1981).

At present, Norway spruce decline can be noticed in a number of localities in border mountains such as the Orlické hory Mts., the Kralický Sněžník Mts., the Hrubý Jeseník Mts., the Beskids. Manifestations of spruce decline occur at an age of young-growth stands and small pole-stage stands and sporadically also in mature stands in the Bohemian-Moravian Uplands (Jankovský 2002). In spite of the found spectrum of potential pathogens and pests in dying spruce trees, mechanisms and particularly causes of the decline are not known yet. Even correlations between predisposition stressors (generally abiotic), initiation stressors (where various ecological groups of fungal pathogens appear to be significant) and mortality stressors are not known. Moreover, the role of main stressors is attributed to them without the evaluation of impacts of other factor. In connection with forest decline, impacts of climatic changes on forests are also considered (Janouš 2002; Jankovský 2002; Jankovský et al. 2004).

The top parts of the Orlické hory Mts. rank among the coldest regions of the Czech Republic (CR). Moreover, with respect to the mountain orography (absence of a characteristic climate; the range massif is subject to the most frequently occurring west cir-

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culation nearly at right angle bearing air pollutants through a long-distance transport) they are primarily extreme sites. This unfavourable condition is intensified on semi-disaster clearings not protected by a full-grown forest stand where intoxication of the environment continues even after subsiding the air-pollution ecological disaster being accompanied by changes in geobiocoenoses.

Probably since 1995, such an interaction of negative events occurs that forest stands, predominantly spruce stands decline or even die. Šach (1994) as the first mentions problems concerning shoot maturation in spruce young plantations near Pěticestí (1,004 m altitude) in 1994. In addition to damage to apical shoots by physiological drought, the author describes numerous mechanical disturbances of shoots "in consequence of an extremely high and thus also heavy snow pack".

The aim of the study is to evaluate the condition of tissues of declining young spruce stands in selected localities of the Orlické hory Mts. based on external symptoms, histological analyses of tissues, analyses of the tissue mycoflora etc.

MATERIAL AND METHODS

Assessing the health condition, symptom characteristics

The health condition of spruce young plantations was assessed on the basis of field observations in June to October 2002 and in February 2003 using results from 2000. The study was carried out in top and subtop parts of the Orlické hory Mts. from 800 to 1,100 m altitude. Samples were taken in selected localities of LS (Forest District) Rychnov nad Kněžnou and LS Lanškroun (Table 1).

Histological analysis of tissues

Samples were taken of needles, shoots, macroblasts and branches of various age. The sampled material was documented under fresh conditions and after vital dyeing in the laboratory. Selected samples were preserved in FAA (solution of ethanol and acetic acid with addition of formaldehyde) and manual cuts were made. One part was dyed with phloroglucinol under acid environment (H_2SO_4) for the presence of lignin, the second part of cuts was tested for the presence of starch using a reaction with Lugol solution. The third part was microscoped under native conditions. In needles, attention was paid to the condition of cuticle and stomata, leaf mesophyll, endodermis and central cylinder. In stems, the condition was studied of pith, tracheids of early wood and late wood, width and area of annual rings, the condition of ducts and cambial zone. Special attention was paid to phloem. Selected preparations were evaluated using computer image analysis.

Microbiological analysis of tissues

The presence of a pathogen was identified on the basis of fruit bodies *in situ*. In the laboratory, sampled branches were surface-cleaned and cultivated in moisture chambers. Identification was carried out

Table 1. Characteristics of selected localities in the Orlické hory Mts. (Forests of the Czech Republic)

Plot No.	Forest range	Stand	HS	SLT	Altitude (m)	Age (years)	Species composition by FMP (%)
LS Rychnov nad Kněžnou							
1	Říčky	332 A, non-stocked 101	–	–	ca 800	–	–
2	Říčky	332 A 2a	57(1)	6P1	ca 800	12	spruce 100
3	Orlické Záhoří	425 D 1	73(1)	7K3	ca 950–980	9	spruce 84, blue spruce 10, rowan 3, birch 2, dwarf pine 1
4	Orlické Záhoří	425 D 2	73(1)	7K3	ca 950–980	13	blue spruce 13, spruce 36, birch 5, dwarf pine 5, rowan 2, larch 1, beech 1
5	Říčky	326 A 2	73(1)	7K4	950	11	spruce 65, dwarf pine 20, birch 10
LS Lanškroun							
6	Mladkov	211 D 1	02(1)	7Z1	ca 900–950	9	spruce 100
7	Albrechtice	101 A 1a	02(1)	7Z2	ca 900–950	11	dwarf pine 70, spruce 20, rowan 10
8	Albrechtice	102 C 1a	73(1)	7K3	ca 800	8	spruce 100

LS – forest district, HS – management set of stands, SLT – forest type group, FMP – forest management plan according to macroscopic and microscopic features on fruit bodies. Fungi of an endophytic mycoflora were isolated from tissues of green twigs or from the interface of live and dead tissues. Parts of cleaned samples of about 1–3 mm in size were severed and surface-sterilized using alcohol and sodium hypochlorite and rinsed with alcohol. Cultivation was carried out on Petri dishes with a malt extract agar medium (MEA, 3.5% malt concentration). For the purpose of identification, cultivation was also carried out on other types of media, e.g. on potato agar, flake agar etc. Particular species were identified according to the morphology of cultures and microscopic features of fructification organs if they were created in the culture.

RESULTS

Evaluation of the spectrum of symptoms observed in declining species and their identification with the effect of stressors

Several types of damage with a different symptomatic image were distinguished. It was noted that browning the sapwood part of wood of various intensity was observed in all cases and in some cases even of pith as the manifestation of stress load.

Age class 1 and 2 *Picea abies* (L.) Karst. + *Picea pungens* Engelm.

A. ***Damage to lower parts of the crown roughly to the height of a snow cover.*** Similar damage was also observed in forest nurseries where the snow pack occurred for a long time. In a number of localities, mechanical damage to trees also appeared due to snow. In this type of damage following findings were noted:
   a) damage to needles and annual shoots in the lower part of the crown accompanied with premature abscission,
   b) root system deformations,
   c) irregular occurrence of the fruiting bodies of fungi on dead twigs. *Gremmeniella abietina* or its anamorpha *Brunchorstia pinea* was noted only individually on dead twigs. Mass colonization of tissues was not observed.
B. ***Dieback of spruce trees in the whole crown observed above all at lower locations*** (Hanička, Luisino údolí [Luisa valley] etc.) linked with needle fall; spruce trees show generally bud burst in spring. Similar type of damage was also noticed in several localities in the Bohemian-Moravian Upland (LS Náměšť nad Oslavou, LS Tišnov). The dieback is of focus character being accompanied by needle fall in the whole crown and by the root system flattening and deformations. The occurrence of mites from the group *Oribatei* was also noted, however, their role was unclear. At present, determination is carried out. In Luisino údolí (Luisa valley), spruce spider mite *Oligonychus ununguis* (Jacobi) was also found. The dieback is most evident in stands of the 1st and 2nd age classes; in other localities, damage of the same symptoms was observed even in individual trees of a premature age.
C. ***Damage to the assimilatory apparatus above the snow cover.*** Symptoms are similar as winter transpiration. It is also possible to consider damage due to the coincidence of meteorological effects and short-term air-pollution load including effects of ground ozone. In some localities such as Suchý vrch (Suchý hill), it is possible to note the combination of different types of damage. The most evident is damage to the terminal shoot. It correlates with immaturity of shoots and refers to their incapability to survive the winter period because of the early start of air temperatures below the freezing point, in the course of winter with decreases approaching –20°C. At present, tree species actively separate dead tissues. There is a probable relation to the nitrogen surplus. Individual trees inappropriately respond to the surplus in the course of their elongation of terminal shoots. As for particular symptoms, turning red and needle fall were noticed often connected with leaf spots. Needles were affected roughly above the level of a snow cover and non-lignified shoots died.

Mature stands

Symptoms of chronic damage were found manifesting (in addition to the reduction of the number of needle year-classes) by the formation of secondary branching. Particularly in wind mantles, it is possible to observe marked symptoms of needle frost desiccation (shoot frost desiccation). The phenomenon was also observed in the vicinity of forest nurseries showing damage to plants. In the wind mantles, marked damage was also noted to ground branches roughly at the lower third of the crown similarly as in case of the decline of trees of the 1st and 2nd age classes.

Histological study of shoots of young trees

At higher altitudes (e.g. in the vicinity of Kamenec hill), needles of *Pinus contorta* Dougl. ex Loud. were evidently damaged by ozone. It referred to a needle year-class differentiated in 2000. In a nearby locality situated below the top of Orel hill, needles of blue spruce were found obviously discoloured after bud mutation caused by the combination of low temperatures and high insolation including UV radiation. Functioning the unfavourable factors occurred in 2002, in a period of the mobilization growth stage of buds. Similarly, the combination of low temperatures and high insolation was documented in the youngest leaves of birch discoloured by anthocyanins. In this case, it referred to the Anenský vrch locality.

Young stands of the Orlické hory Mts. looked rather dramatically in consequence of very frequent reddening the needles and death of stems from the youngest needle year-classes to the dieback of whole tree tops after winter in 2001–2002. Based on histological analyses it has been documented that it concerned effects of frost. In needles damaged by frost, it is possible to see cuticle degradation and becoming peripheral cells brown. Moreover, drying out needles due to frost becomes, collapse of mesophyll and finally total collapse of endodermis (Hanisch, Kilz 1990). Changes in the needle volume in various directions also indicate that the stressor influenced from the outside. On the other hand, needles were also documented which showed still live cells with chloroplasts in their mesophyll, however, with destroyed cuticle (detached from walls of epidermal cells), dead outer cells of the mesophyll and, finally, damaged endodermis. It showed evidence of the fact that such needles could not be functional and died even within the growing season (2002). In another case, although the cuticle as well as the regularity of mesophyll cells were generally well preserved, deformations of the endodermal sheath represented serious damage to needles. It can also refer to the result of an insufficient activity of roots documented in another context (chlorosis).

Based on changes in the stem (of various age) xylem structure it was demonstrated that stressors showing marked effects on the differentiation stage of cells occurred repeatedly. Health stems show functional cambium and xylem and phloem regions differentiated in 2002 (from the beginning of the growing season). On the other hand, some stems were damaged to such an extent in the course of growth in 2002 that cambium finished its activity.

In a three-year lateral stem it was evident that already in the first year of growth, reaction wood occurred. During 2001, xylem differentiation of the stem was repeatedly affected by negative influences. In the following year, i.e. in 2002, it referred to two successive waves of the occurrence of reaction. It means that favourable conditions for the cambium activity and differentiation of normal early wood tracheids took such a short time that only their two series originated. In another three-year stem of the first order, reaction wood appeared in 2001 when in the first half of the growing season high temperatures obviously occurred in two stages. These temperatures subsequently affected increased formation of resin ducts. Tracheids are of circular shape being even elliptical in radial direction. In corners, schizogenetic intercellular formations originate. At the beginning of the formation of annual rings in 2002, slight frost damage of cambium occurred which was evident in the first tracheids of early wood.

In a seven-year stem, it is possible to point out the dynamics of the effect of unfavourable mountain conditions which reflect in an irregular secondary growth. In the second year of the stem growth (1997) when a very wide annual ring originated (1 mm in width) numerous tangential fissures occurred. These fissures repeat in a less intensive form in the following year 1998, however, high frequency of resin canals indicates that even then conditions were not optimal. In 1999, a rapid decrease in the annual ring width to less than 50% of the previous year occurred. Then follows the narrowest annual ring created in the growing season of 2000 (annual ring width 0.1 mm). In the course of the following growing season (2001), a somewhat wider annual ring occurred but with very poorly developed late wood. The beginning of wood formation in 2002 was quite promising in July.

Microbiological analysis of spruce tissues – preliminary conclusions concerning the occurrence of pathogens

Identification of fungal pathogens in situ

In 2002, fruit bodies of *Brunchorstia pinea* (Karsten) Höhn. (teleomorph *Gremmeniella abietina* (Lagerb.) Morelet, syn. *Ascocalyx abietina* (Lagerb.) Schläpfer – Bernhard, syn. *Scleroderris lagerbergii* Gremmen) were noted only sporadically on dead twigs. Generally, it referred to fruit bodies from previous years. Even in moisture chambers, mass colonization of spruce tissues by the fungus *Gremmeniella abietina* was not corroborated. Fruit bodies of *B. pinea* var. *pini* (Karsten) Höhn. and *B. pinea* var. *cembrae* Morelet were determined individually. In 2000, *B. pinea* var. *cembrae* Morelet was recorded even on a mass scale; in 2002, this variety was found in Norway spruce only sporadically. In 2002, *B. pinea* var. *cembrae* was found on twigs and needles of *Pinus mugo* L. and *P. contorta*. It is of interest that in

2002, fruit bodies of anamorphous *B. pinea* (Karsten) Hohn. taken in the Orlické hory Mts. in 2000 and deposited in the herbarium of the Department of Forest Protection and Game Management, Mendel University of Agriculture and Forestry Brno developed in a moisture chamber.

In larch, also *Lachnellula occidentalis* (Hahn et Ayers) Dharne and similar *L. suecica* (de Bary: Fuckel) Nannf. with typical orbiculate spores were found. In *Picea pungens* Engelm. with manifestations of dieback, the causal agent Sirococcus shoot dieback of spruce *Sirococcus strobilinus* Preuss. was found in 2002 similarly as in 2000.

Isolation of pathogens

Epicococcum nigrum Link. was the most abundant species. The species is a common component of phyllosphere and dead tissues of plants. It was also frequently found in declining spruce trees from other localities. So far, also other species were determined from samples: *Sordaria* sp., *Fusarium avenaceum* (Fr.) Sacc., *Fusarium cf. oxysporum*, *Cladosporium* sp., *Phomopsis* sp. In all species mentioned above, it is possible to think about their potential pathogenicity on necrosed tissues. Among cultures not determined so far there is none which would be determined from all samples.

Microbiological analysis of pine tissues

Together with spruce dieback, damage to pine plantings was also noticed. Samples were taken from *Pinus mugo* L. and *Pinus contorta* Dougl. ex. Loud. As against spruce, fruit bodies of *Brunchorstia pinea* var. *cembrae* (*Gremmeniella abietina*) were abundantly found in moisture chambers. For the purpose of comparisons, samples were also taken from *Pinus nigra* Arnold at Kunvald in the foothills of the Orlické hory Mts. In addition to needle pathogens *Mycosphaerella pini* E. Rostrup., *Cyclaneusma minus* (Butin) Di Cosmo, Peredo et Minter, *Lophodermium*

Table 2. Survey of examined samples

Habitat	Date	Host	*Brunchorstia pinea* var. *pini*	*Brunchorstia pinea* var. *cembrae*	*Sirococcus strobilinus*	*Epicoccum nigrum*	*Sordaria* sp.	*Fusarium* sp.	*Stemphylium* sp.	*Botrytis cinerea*	*Trichoderma viride*	*Coleosporium*	*Phomopsis* sp. (25–35 × 10–12 µm)	*Cladosporium* sp.
Anenský vrch 425 D 2	4. 6. 2002	*Picea abies*	F	–	–	IS	IS	IS	–	–	–	–	–	–
		Picea pungens	–	–	MC	–	–	–	–	–	–	–	–	–
Anenský vrch 425 D 2	16. 10. 2002	*Picea abies*	–	–	–	–	–	–	–	–	–	–	–	–
Anenský vrch 425 D 1	16. 10. 2002	*Picea abies*	–	–	–	IS	–	–	–	–	–	–	–	–
Anenský vrch 425 C2	16. 10. 2002	*Picea abies*	–	–	–	–	–	–	–	–	–	–	–	IS
Pod Anenským vrchem 327 A 8*	16. 10. 2002	*Picea abies*	–	–	–	IS	–	–	IS	–	IS	–	MC	–
Suchý vrch I	4. 6. 2002	*Picea abies*	F	–	–	–	IS	–	–	–	–	–	–	–
Suchý vrch II	4. 6. 2002	*Picea abies*	–	–	–	–	–	–	–	–	–	–	–	–
Zdobnice školka	16. 7. 2002	*Picea abies*	–	–	–	–	–	–	–	MC	–	–	–	–
Luisino údolí	16. 10. 2002	*Picea abies*	MC	–	–	–	–	–	–	–	–	–	–	–
Nad Vápenkou	16. 10. 2002	*Pinus mugo*	–	MC	–	–	–	–	–	–	–	F (N)	–	–
		Pinus contorta	–	MC	–	–	–	–	–	–	–	–	–	–
Deštné	16. 10. 2002	*Picea abies*	–	–	–	–	–	–	–	–	–	–	–	–

*mature sample tree, F – fructification observed in field, IS – isolation on MEA, MC – fructification in a moisture chamber, (N) on needles *seditiosum* Minter, Staley et Millar also *Brunchorstia pinea* var. *pini* (Karsten) Höhn. was observed on dying twigs and needles.

Survey of examined samples (Table 2)

Except as otherwise provided for, it refers to stands aged 1–20 years. VK – moisture chamber.

Results of evaluating air conditions and the course of weather

Sulphur and nitrogen depositions in 1994–2000

Nitrogen depositions in the open area showed decreasing trends from 1994 to 1997 both in the ammoniac form and the nitrate form (from 25.59 to 16.34 kg/ha/year). In 1998, a rapid increase in nitrogen depositions occurred particularly due to the marked increase of NO_3 to 29.78 kg/ha/year. In following two years, the deposition gradually decreased reaching a comparable value with a minimum in 1997 (16.51 kg/ha/year).

Dynamics of the atmospheric deposition of nitrogen under the spruce stand showed a decrease from 1994 to 1997 (from 57.28 to 49.70 kg/ha/year) which was followed by a marked increase in 1998 particularly due to the increase in NO_3 fallout, viz. 69.97 kg/ha/year. In spite of a negligible decrease in 1999, the fallout increased again to the highest value of 81.66 kg/ha/year in 2000.

As for sulphur in the open area, there was an evident permanent decrease from 32 kg/ha/year in 1994 to 13.2 kg/ha/year in 2000.

Under the stand, the situation was markedly different. A decrease occurred from 82.24 kg/ha/year in 1994 to a value of about 50 kg/ha/year in 1997. Then a rapid increase occurred to 91.30 kg/ha/year in 1998.

Evaluation of the impact of climatic extremes in winter 2001/2002

In the course of November 2001, in the first half decrease in temperatures occurred from +4°C to –10.5°C within two days besides a marked fluctuation during a day under increased radiation. The phenomenon repeated in the last decade when temperatures ranged between –10°C and +1°C accompanied again by increased radiation.

In December, temperatures were constantly below the freezing point but in three terms, the temperature amounted to –17 to –20°C. Solar radiation was of a very low level and soil temperatures gradually decreased. Nearly within two January decades, night temperatures ranged between –5 and –13°C while daily temperatures reached for several times +5°C which represented daily amplitudes as many as 18°C. At the end of the month up to mid-February, increase in temperatures occurred when daily temperatures reached values of about +7°C and night temperatures occasionally decreased below the freezing point. In mid-February, very unfavourable rapid temperature fluctuations between positive and negative values during daily intervals repeated again and positive values were accompanied with high solar radiation. A last temperature reaching –15°C occurred on 22 February.

March was also unbalanced as for temperatures and positive and negative values fluctuated from –7 to +12°C. At the end of March and at the beginning of April, a rapid increase in daily temperatures occurred reaching maximum values of +15 to +18°C whereas night temperatures decreased again below the freezing point. Obviously, it referred to the radiation type of weather because during the day, intensity of solar radiation exceeded 900 W/m^2.

In April, it is possible to find three waves of unfavourable temperature fluctuations about dates 10, 19 and 28 when values of daily temperatures ranged from +5 to +10°C and night temperatures decreased below the freezing point up to –5°C.

DISCUSSION

High NO_x nitrogen inputs (Šach et al. 1999) and unfavourable development of climate particularly the occurrence of extremely dry and rainy periods including temperature extremes appear to be the risk factor of spruce decline in the Orlické hory Mts. It results in decreased lignification, immaturity of tissues before the winter period and increased susceptibility of damaged tissues to parasitic organisms and, together with the potassium deficiency, it results in low resistance during cold periods of the year when with the incidence of first frosts in autumn, a chronic process of the destruction of annual increments begins. In mountain conditions, climatic stressors manifest themselves by high photorespiration and low photosynthetic gain when photosynthates for root systems are missing. High insolation and ozone concentration are manifested by the stress on cell antioxidants, i.e. photooxidation of pigments. In consequence of high photorespiration, there is a low photosynthetic gain when photosynthates for root systems are missing. Temperature fluctuations together with wind and solar radiation under conditions of frozen soil or oxygen deficit (hypoxia) during spring snow thawing result in high transpiration requirements under insufficient activity of roots in combination with the slow water transport and predisposition for damage to membranes in cells of the youngest needle year-classes, particularly in trees occurring in the virginal stage of ontogenesis. It results in the loss of turgor, negative pressure in tissues, air imbibition and death of cells connected with lignin oxidation.

A marked phenomenon of the whole region are deformations of the root system of spruce as a result of impaired parameters of the environment in combination with using unsuitable planting stock and technologies of planting. In this context, it is necessary to mention disturbed mycorrhizal conditions.

Annual shoots are secondarily colonized by fungal pathogens of the Brunchorstia dieback of conifers type as the result of damage to tissues by predisposition stressors. In some localities, activities were also noticed of sucking insect and mites and successive necroses of tissues connected with mycoses (*Fusarium* spp. div., *Cytospora* spp., *Nectria* spp.).

Synergetically unfavourable effects of stressors on spruce young-growth stands is not mitigated or even eliminated by the presence of a parent stand. In some localities, it is even intensified by a top phenomenon and orography of the mountain. Resulting effects consist in acute damage to spruce.

Formation of reaction wood

Virtually in all localities, the creation of reaction wood in an abnormal frequency was observed as one of unspecific responses to an increased stress load. Reaction wood makes possible growth and turgor movements (e.g. of branches, stems etc.) and also changes hydraulic architecture of trees and branches. This type of tracheids can be created under any influence releasing ethylene. For example, it is terrain slope, solifluction, short-term drought, hypoxia, insect attack, parasitic fungi, high and low temperatures, wind etc. Hypoxia occurs in soil under increased groundwater table or long-term snow melting when roots suffer from O_2 deficit; in the above-ground part of plants, conditions of oxygen deficit occur under high, melting and freezing snow cover or glazed frost on branches. The high frequency of the occurrence of reaction wood reflects effects of these natural negative factors. Based on other our studies conducted in numerous localities, the occurrence of reaction wood in spruce increased which could be generally attributed to climatically unbalanced last ten or twenty years.

Role of *Gremmeniella abietina* and other fungi of endophytic mycoflora

The causal agent of Brunchorstia dieback of conifers *Gremmeniella abietina* is considered as a facultative parasite which follows dieback of some conifers, especially pines (Siepmann 1975; Siepmann et al. 1975) and also spruces (Barklund, Rowe 1981). Thus, the infection requires that the trees are susceptible.

Wind-borne ascospores or splash-dispersed conidia infect the bud or shoot scales from May to September. The fungus penetrates the host during autumn and winter above all (Lang, Schütt 1974; Siepmann 1976). Infection is also possible through wounds in the stem and branches (Roll-Hansen 1964) and the infection may happen through needle epidermis (Skilling, Krogh 1970).

Gremmeniella abietina is suggested to have at least three continentally disjuncted physiological races (Dorworth, Krywienczyk 1975; Morelet 1980a). In the Orlické Mts., too varieties were determined of its imperfect stage *Brunchorstia pinea*. While the *B. pinea* var. *cembrae* occurs chiefly in mountains areas, the *B. pinea* var. *pinea* occurs at lower elevations principally on *Pinus nigra, P. mugo* and was described from *Pinus sylvestris* as well (Morelet 1980b).

Gremmeniella abietina is generally considered as an important pathogenic agent in case of spruce dieback in the Orlické hory Mts., however, not a main cause of the dieback (Soukup, Pešková 2000; Národec 2001, 2002). *G. abietina* is not the only factor responsible for the existing condition. It is a case of the secondary colonization of dead tissues under conditions favourable for the fungus development.

According to other observations the function of non biotic predisposition factors were noted. The effect of temperature and humidity is often connected with the winter hardiness and the lignification processes of tissues, which in turn affect the susceptibility to frost damage. Frost damage has been considered to be the primary cause of the differences in resistance to *Gremmeniella*, although after a severe frost damage no fungal infection is necessarily needed to kill the trees (Pomerleau 1971).

Based on analyses carried out in 1970–1986, Uotila (1988) mentions that the greatest damage under participation of *G. abietina* followed after a period when the growing season was characterized by the low sum of temperatures, high precipitation, low insolation and the occurrence of frost days during the growing season, i.e. factors participating in tissue damage.

Edaphic factors often work together with climatic conditions. In several cases, the disease incidence has been greater on fine-textured soils, which formerly may have been spruce stands. On such sites, the roots may suffer from anaerobic conditions.

Disease is sometimes more severe in topographic depressions. In such places, the trees are more often damaged by frosts. Moreover, the spores may recirculate, thus intensifying the amount of inoculum (Dorworth 1972). Sometimes the disease has been found to be more frequent on higher levels above the sea (Roll-Hansen 1972; Roll-Hansen and Roll-Hansen 1973). This might be due to the extreme climatic conditions plus the harmful effects of the snow cover (Senn 1999).

The development of infection is supported by the occurrence of sucking insect on annual shoots of *G. abietina* (Virtanen et al. 1997).

In case of spruce dieback in the Orlické hory Mts., it is possible to note that no biotic agent has been proved which would be present in all damaged spruce trees. From the viewpoint of fungal mycoflora it refers to rather secondary organisms which spread on predisposed tissues. The finding is also based on repeated finds (2000, 2002) of the fungus *Sirococcus strobilinus* in *Picea pungens* where the same symptoms of dieback occur as in *Picea excelsa*.

The occurrence of fungi within tissues can under certain conditions of the host weakening work as a mortality factor. Fungi inside tissues can cause their embolism, perforation of conductive elements, phloem necrosis etc.

Screening for the presence of the fungus in tissues by means of PCR methods would be the definite reply to a question of the importance of *G. abietina* (Hamelin et al. 2000). Application of PCR (polymerase chain reaction) methods would markedly accelerate determination and make possible safe determination of the presence of phytopathogenic fungi in green tissues.

Effects of drought and nitrogen surplus

The significance of dry periods which are an important element affecting forest stands in the Orlické hory Mts. points out Černohous (2000). According to the author, a danger for young stands consists in the decrease of moisture during May and June. Plantings, particularly container-grown plants do not show sufficiently developed root system and, therefore, they cannot reach lower layers of the soil profile for soil moisture. Combinations of water deficit and high nitrogen depositions (smaller extent of root systems) can be another cause of weakening and poor health condition of spruce young plantations.

Atmospheric depositions are commented by Šach et al. (1999) as follows: "High depositions of N in the Orlické hory Mts are above all caused by high depositions of NO_3 which markedly increased in 1998. Nitrogen depositions exceed a critical value for Norway spruce on an open area (according to German sources 11 kg ha/year) on average 2-times (in 1998, however, nearly 3-times), under a mature spruce stand even 5-times (in 1998, however, more than 6-times). " Further, the authors mention: "Particularly in intensively growing young spruce trees, cells enlarged in consequence of luxuriant nitrogen require sufficient amounts of water. However, water is often missing in the Orlické hory Mts. at the present time and the state described results in the spruce tissue damage." The species affected are excited to create disproportionate and low-quality increments characterized by incomplete lignification and decrease allocation of photosynthates to root systems.

CONCLUSION

Histological and meteorological analyses showed that dieback of spruce after winter 2001/2002 was primarily a results of the impact of frost which occurred from autumn to spring in several waves interrupted by warming. In combination with hypoxia (both in soil and under snow cover), with high evaporation requirements of the atmosphere under conditions of reduced transpiration flux and in combination with immaturity of the late summer part of wood within annual rings of 2001, damage to whole shoots occurred. In needles, there is the cuticle degradation and getting brown peripheral cells which indicates that the stressor worked from the outside. Further, local collapse of mesophyll occurs when changes in the needle volume are different in various directions corresponding to the gradual contraction under the loss of water. Drying up due to frost appeared to be the cause of turning red to getting brown of needles. In another case, deformations of an endoderm sheath were found under generally well preserved cuticle and regularity of mesophyll cells. It referred to damage to needles through the effect of an internal factor obviously related to the insufficient activity of roots. Damage to certain needle year-classes by ozone and changes in the structure of the stem xylem (of various age) showed to stress situations which occurred repeatedly in the course of growing seasons. The character of changes corresponded to the increased availability of nitrogen and, on the other hand, to the production of ethylene; these factors affected cells in the period of their volume and differentiation growth stage. According to histological findings, trees were systematically weakened within annual cycles, viz. both during the growing season and the quiescent period. A direct evidence on the SO_2 fallout did not succeed, i.e. gypsum crystals occurred neither on the needle surface nor in the vicinity of epistomatal cavities. Russet colouring of needles was definitely caused by "freezing-out" and obviously corresponded with the unfavourable development of weather in spring 2002 which affected the whole region. Damage to tissues is followed by the secondary colonization of tissues with fungal pathogens which can manifest themselves as mortality factors but not as the only causal agents of dieback. Under certain conditions, fungi of the *Gremmeniella abietina* type can colonize necrotized tissues on a mass scale similarly as after winter 2000. In winter 2001/2002, the concurrence of circumstances occurred which resulted in spruce damage. However, mass colonization caused by the fungus *G. abietina* did not happen. The secondary role of the fungus is also supported by the find of the fungus *Sirococcus strobilinus* on *Picea pungens* with absolutely identical symptoms of damage. Examinations demonstrated, however, the occurrence of the fungus *G. abietina* in affected stands. *G. abietina* was identified in plantings of *Pinus mugo* Turra and *P. contorta* Dougl. ex Loud. Similarly, the occurrence of some other microscopic species such as *Stemphylium* sp., *Epicoccum nigrum, Cladosporium* etc. is a response to damage and subsequent necrosis of tissues.

Possibilities of improving the health condition and general stability of young stands in the Orlické hory Mts. are provided only by the change in the stand environment (even if under a long time horizon). It is possible to suppose that species which will survive unfavourable impacts will easily overcome chronic stress with the increasing age creating a vital capacity to surmount it. The transformation of existing stands to stands differentiated from the aspect of species, age and space has to be carried out cautiously taking into account site conditions using species which proved resistance in mountain conditions, proper genetic origin being suitably grown and planted. The increased proportion of broadleaves will ensure biological soil reclamation and improving the soil environment.

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References

BARKLUND P., 1989. Occurrence of and interaction between *Gremmeniella abietina* and endophytic fungi in two conifers. [Ph.D. Thesis.] Uppsala, Swedish University of Agricultural Sciences: 61–69.

BARKLUND P., ROWE J., 1981. *Gremmeniella abietina* (*Scleroderris lagerbergii*), a primary parasite in a Norway spruce die-back. European Journal of Forest Pathology, *11*: 97–108.

ČERNOHOUS V., 2000. Suché periody v Orlických horách během vegetačního období a jejich potenciální vliv na ujímavost, odrůstání a zdravotní stav smrkových kultur. In: SLODIČÁK M. (ed.), Lesnické hospodaření v imisní oblasti Orlických hor. Opočno, VÚLHM – VS Opočno: 25–29.

DORWORTH C.E., 1972. Epidemiology of *Scleroderris lagerbergii* in central Ontario. Canadian Journal of Botany, *50*: 751–756.

DORWORTH C.E., KRYWIENCZYK J., 1975. Comparisons among isolates of *Gremmeniella abietina* by means of growth rate, conidia measurement, and immunogenic. Canadian Journal of Botany, *53*: 2506–2525.

HAMELIN R.C., BOURASSA.M., RAIL J., DUSABENYAGASANI M., JACOBI V., LAFLAMME G., 2000. PCR detection of *Gremmeniella abietina*, the causal agent of scleroderris canker of pine. Mycological Research, *104*: 527–532.

HANISCH B., KILZ E., 1990. Monitoring of forest damage. Stuttgart, Verlag Eugen Ulmer: 334.

JANKOVSKÝ L., 2002. Nové projevy chřadnutí smrku ve středohorách. Zprávy lesnického výzkumu, *47*: 95–96.

JANKOVSKÝ L., CUDLÍN P., ČERMÁK P., MORAVEC I., 2004. The prediction of development of secondary Norway spruce stands under the impact of climatic change in the Drahany Highlands (the Czech Republic). Ekológia (Bratislava), *23*, Supplement 2/2004: 101–112.

JANOUŠ D., 2002. Změny klimatu a globální oteplování. Lesnická práce, *81*: 12–14.

KLÍMA J., 2002. Orlické hory 2002. Lesnická práce, *81*: 490–492.

LANG K.J., SCHÜTT P., 1974. Anatomische Untersuchungen zur Infektionsbiologie von *Scleroderris lagerbergii* Gr. (*Brunchorstia pinea* (Karst.) von Höhn). European Journal of Forest Pathology, *4*: 166–174.

MORELET M., 1980a. Brunchorstia disease I. Systematic position and nomenclature of the pathogen. European Journal of Forest Pathology, *10*: 268–277.

MORELET M., 1980b. Brunchorstia disease. 2. Distribution in France. European Journal of Forest Pathology, *10*: 354–359.

NÁROVEC V., 2001. K nálezu houby *Ascocalyx abietina* (Lagerb.) Schläpfer-Bernhard v Orlických horách. Lesnická práce, *80*: 268–270.

NÁROVEC V., 2002. Houba *Ascocalyx abietina* (Lagerb.) Schläpfer – Bernhard v dotazech a odpovědích. Lesnická práce, *81*: 324–325.

POMERLEAU R., 1971. Considerations on the cause of conifer damage in plantations attributed to the *Scleroderris* cancer. European Journal of Forest Pathology, *1*: 114–122.

ROLL-HANSEN F., 1964. *Scleroderris lagerbergii* Gremmen (*Crumenula abietina* Lagerb.) and girdling of *Pinus sylvestris* L. Meddelelser Norske Skogsførsaksvesen, *19*: 153–175.

ROLL-HANSEN F., 1972. *Scleroderris lagerbergii*: Resistance and differences in attack between pine species and provenances. European Journal of Forest Pathology, *2*: 26–39.

ROLL-HANSEN F., ROLL-HANSEN H., 1973. *Scleroderris lagerbergii* in Norway hosts, distribution, perfect and imperfect state, and mode of attack. Meddelelser Norske Skogsførsaksvesen, *30*: 443–459.

SENN J., 1999. Tree mortality caused by *Gremmeniella abietina* in a subalpine afforestation in the central Alps and its relationship with duration of snow cover. European Journal of Forest Pathology, *29*: 65–74.

SIEPMANN R., 1975. *Scleroderris lagerbergii* a weak parasite in healthy *Pinus nigra* stands. European Journal of Forest Pathology, *5*: 137–142.

SIEPMANN R., LANG K.J., SCHONHAR S., 1975. Distribution of dieback in *Pinus nigra* caused by *Scleroderris lagerbergii* in the Federal Republic of Germany. European Journal of Forest Pathology, *5*: 185–189.

SIEPMANN R., 1976. Ein Beitrag zur Infektionsbiologie des durch *Scleroderris lagerbergii* verursachten Schwarzkieferntriebsterbens. European Journal of Forest Pathology, *6*: 103–109.

SKILLING D.D., KROGH N.K., 1970. Infection of *Pinus resinosa* tissue fluorescent-labelled spores of *Scleroderris*. Phytopathology, *59*: 1050.

SOLHEIM H., 1986. Dieback of spruce. Norsk Skogbruk, *32*: 12–13.

SOUKUP F., PEŠKOVÁ V., 2000. Napadení poškozených smrků v Orlických horách houbou *Ascocalyx abietina* (Lagerb.) Schläpfer – Bernhard. Lesnická práce, *79*: 472–474.

ŠACH F., 1994. Vyjádření ke stavu smrkových kultur v oblasti Pěticestí na základě pochůzky konané 5. srpna 1994. [Poradenská zpráva pro SKL Rychnov nad Kněžnou.] VÚLHM, VS Opočno: 1.

ŠACH F., FOTTOVÁ D., BALCAR V., ČERNOHOUS V., KACÁLEK D., NÁROVEC V., 1999. Mohou imise škodit v Orlických horách? Lesnická práce, *78*: 452–455.

UOTILA A., 1988. The effect of climatic factors on the occurrence of scleroderris canker. Folia Forestalia, *721*: 1–23.

VIRTANEN T., RANTA H., NEUVONEN S., 1997. Shoot-feeding aphids promote development of *Gremmeniella abietina*, the fungal pathogen causing scleroderris canker disease in conifers. Journal of Phytopathology, *145*: 245–251.

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Fytopatologické a histologické aspekty chřadnutí smrku v Orlických horách

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ABSTRAKT: Hodnotili jsme stav pletiv chřadnoucích mladých smrčin na vybraných lokalitách Orlických hor. Jedná se o porosty ve vrcholových partiích, které byly postiženy imisně ekologickou kalamitou v minulém století. Bylo rozlišeno několik typů poškození s odlišným symptomatickým obrazem. Rizikovým faktorem chřadnutí smrku v Orlických horách je vysoký vstup dusíku NO_x. Za jednu z hlavních příčin v komplexu faktorů je možné označit nevyrovnaný průběh meteorologických prvků během roku a disbalanci minerální výživy. Důsledkem je snížená lignifikace, nevyzrálost pletiv před zimním obdobím, zvýšená vnímavost poškozených pletiv vůči parazitickým organismům. Bylo identifikováno několik potenciálně patogenních druhů mikromycet, které mohou být považovány za významný patogenní agens v případě chřadnutí smrku v Orlických horách, nikoli však za hlavní příčinu.

Klíčová slova: chřadnutí smrku; *Gremmeniella abietina*; *Ascocalyx abietina*; *Sirococcus strobilinus*; NO_x

První reference o chřadnutí smrku v Orlických horách přinášejí Šach et al. (1999). Upozorňují na vysoké hodnoty fluoru, chloru a především prudký vzestup depozic dusíku, především NO_3 a ozonu, které v oblasti působí vedle přetrvávající zátěže oxidy síry. Na možný výskyt houby *Gremmeniella* *abietina* (Lagerb.) Morelet, (syn. *Ascocalyx abietina* (Lagerb.) Schläpfer – Bernhard) upozornil poprvé v roce 1997 Nárovec (Soukup, Pešková 2001). O problematice úlohy této houby se stále diskutuje (Nárovec 2001, 2002). V zahraničí se podobné projevy chřadnutí smrku pozorovaného v Orlických horách spojují právě s aktivitou této houby a označují se jako Brunchorstia Dieback of Conifers (Barklund 1989; Solheim 1986; Barklund, Rowe 1981).

Histologické analýzy prokázaly, že chřadnutí smrku po zimě 2001/2002 bylo v první řadě důsledkem působení mrazu, který se vyskytl od podzimu do jara v několika vlnách přerušovaných oteplením. V kombinaci s hypoxií (v půdě i pod sněhovou pokrývkou), s vysokými evaporačními požadavky atmosféry za podmínek ztlumeného transpiračního roku a v kombinaci s nevyzrálostí pozdně letní části dřeva v rámci letokuhu z r. 2001 došlo k poškození celých prýtů. U jehlic je zřejmá degradace kutikuly a zhnědnutí nejdříve periferních buněk, což poukazuje na to, že stresor působil zvnějšku. Dále dochází k lokálnímu zborcení mezofylu, kdy změny objemu jehlice jsou v různých směrech odlišné a odpovídají postupnému smršťování při ztrátě vody. Příčinou zčervenání až zhnědnutí jehlic bylo tedy jejich mrazové vyschnutí. V jiném případě byla zjištěna deformace endodermální pochvy při celkově dobře zachované kutikule i pravidelnosti mezofylových buněk – jednalo se o poškození jehlice působením vnitřního faktoru, zřejmě souvisejícího s nedostatečnou činností kořenů.

Poškození určitých ročníků jehlic ozonem a změny struktury xylému stonků (různého věku) poukázaly na stresové situace, které se opakovaně dostavovaly i v průběhu vegetačních období. Charakter změn odpovídal na jedné straně zvýšené dostupnosti dusíku a na druhé straně produkci etylenu; tyto faktory měly vliv na buňky v době jejich objemové a diferenciační růstové fáze. Podle histologických nálezů byly stromy soustavně v rámci ročních cyklů oslabovány, a to jak během vegetačního, tak také během klidového období. Přímý důkaz o spadu SO_2 se nalézt nepodařilo, tj. krystaly sádrovce se ani na povrchu jehlic, ani v blízkosti epistomatálních dutin nevyskytovaly. Červenohnědé zbarvení jehlic bylo jednoznačně způsobeno tzv. mrazovým vymrznutím a zřejmě korespondovalo s nepříznivým vývojem počasí na jaře r. 2002, které postihlo celé území. Poškození pletiv je následováno sekundární kolonizací pletiv houbovými patogeny, které se mohou uplatňovat jako faktor mortalitní, nikoli jako jediný původce chřadnutí. Za jistých okolností však mohou houby typu *G. abietina* masově kolonizovat nekrotizovaná pletiva, tak jak k tomu došlo po zimě v roce 2000. V zimě 2001/2002 sice došlo k souběhu okolností, který vedl k poškození smrku, masová kolonizace houbou *G. abietina* však nenastala. Sekundární úlohu této houby podporuje i nález houby *Sirococcus strobilinus* na *Picea pungens* s naprosto shodnými příznaky poškození. Šetření však prokázala přítomnost houby *G. abietina* v postižených porostech. *G. abietina* (Lagerb.) Morelet byla identifikována na výsadbách borovic *Pinus mugo* a *P. contorta*. Stejně tak výskyt některých dalších mikroskopických druhů jako *Stemphylium* sp., *Epicoccum nigrum, Cladosporium* aj. je reakcí na poškození a následnou nekrotizaci pletiv.

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