

Pines dieback caused by *Cenangium ferruginosum* Fr. in Slovakia in 2012

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Abstract

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Serious pine dieback was reported in early spring 2012 from several localities in Slovakia. Needle and bark necrosis turning to twig cankers were the most conspicuous symptoms. There were no or at least not significant damages caused by bark beetles, leaf eating insects, root rots neither tracheomycosis. *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton was also excluded as the main pest agent, which played an important role in *Pinus nigra* Arnold dieback from 2000 to 2007. Laboratory examination revealed *Cenangium ferruginosum* Fr. as the agent responsible for that dieback. The knowledge on the pine dieback based on the field investigation and laboratory studies are presented and the reasons of the predisposing factors are discussed in the following paper.

Key words

Cenangium, damage, dieback, drought, frost, pine

Introduction

Pines cover 7 % of forest land in Slovakia and belong to the most important forest trees (ANONYMUS, 2011). Scots pine *Pinus sylvestris* L. and Austrian pine *Pinus nigra* Arnold are planted mostly at poor stands such as sandy soils and shallow soils on limestones. However, they also occur on deep nutritional kambisoils and just there they have suffered from a severe damage caused by fungal pathogens in 2012.

In the past pines have overcome several episodes of dieback caused by fungal pathogens:

1. *Cenangium ferruginosum* dieback reported by LEONTOVYČ (1962) occurred in 1959 to 1960 (KUNCA, 2004). It was mentioned that climatic extremes played an important role as predisposing factors.
2. Invasive needle-cast fungus *Mycosphaerella pini* Rostr. ex Munk anamorph *Dothistroma pini* Hulbary was reported for the first time in Slovakia in 1996 (KUNCA and FOFOVÁ, 2000). At present its oc-

currence is permanent and it is distributed all over Slovakia (KUNCA and LEONTOVYČ, 2002).

3. *C. ferruginosum* was found in Nové Mesto nad Váhom in 2001, but without serious dieback (recorded in the Forest Protection Service database in Banská Štiavnica).
4. *Gremmeniella abietina* (Lagerb.) M. Morelet was found in Veľká Fatra Mountains, locality Kráľova studňa, in 2003. Mountain pine (*Pinus mugo* Turra) trees were damaged, but without serious dieback (recorded in the Forest Protection Service database in Banská Štiavnica).
5. *Sphaeropsis sapinea* was a serious pathogen on Austrian pines since 2000 through 2007. It still occurs on *Pinus nigra* but after sanitary cuttings many localities recovered very well (KUNCA, 2004).

The aim of the paper is to describe symptoms of the present pines dieback, check involved pathogens, discuss predisposing factors and map the disease spreading.

Material and methods

The source of the information about pines dieback comes from practical foresters. Specialists from the Forest Protection Service settled in Banská Štiavnica were informed about a pine dieback occurrence and invited in order to determine the pest range, the reason of the dieback as well as to suggest the subsequent proper control measures. Several localities with the pines dieback were visited and marked in the map using Corel Draw 11 (Fig. 1). Samples from needles, twigs, bark, roots and soil were examined for pest agents in the field as well as in the laboratories.

In the field inspections were realized mostly to search for insect agents such as bark beetles (*Ips* spp., *Tomicus* spp.). On the roots *Armillaria* signs (syrro-cium, and rhizomorphs) were looked for. Other signs of biotic agents and dieback symptoms were checked on other parts of trees as well.

In the laboratories samples were cultivated in wet chambers. Prior to isolation of fungal agents, the surface of samples was sterilized by dipping the sample into the 50 % methanol for 30 seconds. Then they were rinsed with distilled water and dried by filter paper. Samples were cultivated on carrot agar that was prepared from 400 g smashed fresh carrot roots, 18 g of agar powder and filled up to 1,000 ml of demineralized water. These ingredients were mixed and sterilized under 121 °C reaching 210 kPa during 15 min. in Systec DX-90 autoclave. Microscopic features of fungal structures were studied by stereomicroscope Leica S8 APO and microscope Zeiss Axio Scope.A1. Pictures of microscopic features were transmitted on the screen using software Micrometrics SE Premium. Pure cultures were cultivated in climatic chambers Climacell 707. Speed of

mycelial growth was measured under 14 °C, 20 °C and 25 °C with 50% of relative humidity and 40% of light intensity. Isolates of pure cultures were re-cultivated in flow box with horizontal air flow AURA HZ 48.

Samples were collected in all the studied localities where pine dieback with similar symptoms appeared (Fig. 1). The age of these stands ranged from 20 to 100 years. All together about 30,000 m³ of infected wood was assumed.

Pathogenicity of obtained isolates was tested on *Pinus nigra* twigs on 10 trees in Banská Štiavnica region in June and July 2012. Bark of twigs was cut in the square shape with 0.5 cm length of its side. A plug 0.5 × 0.5 cm of the pure culture of the pathogen grown on carrot agar for 3–5 weeks was inserted into the wound and that was enwind with a parafilm. Bark necrosis was checked after 3 months.

Results

The first symptoms on pines were noticed at the end of the winter 2011/2012, in February 2012 and they showed up within a very short time. Damages were obvious due to brown needles of the whole crown of all or most trees in the stands. However, some trees among damaged ones had healthy twigs and even there were some completely healthy trees. Symptoms were located in the crown and were bound to the twig's diameter not exceeded 10 cm. So, roots, bark and conducted tissues of the trunk were healthy. Needle cast was found on the needles caused by *Mycosphaerella pini*, *Cyclaneusma* sp., and *Lophodermium* sp., but not in a large extent. Most needles were free from any signs of pathogens.

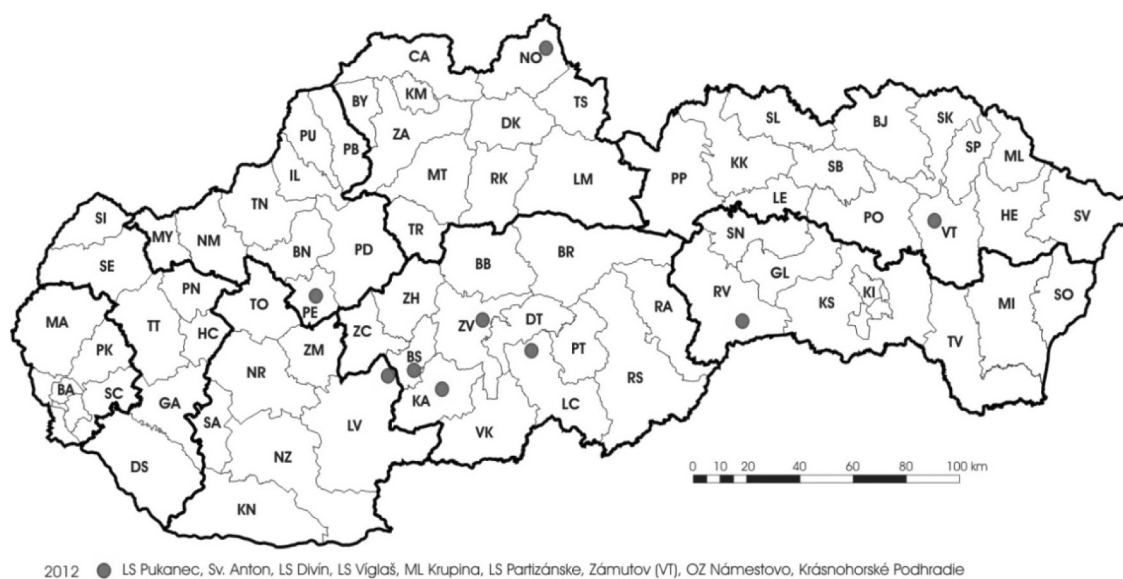


Fig 1. Pine dieback in Slovakia in 2012.

The first symptoms were visible on needles of all ages and these needles were pale green. Later the base of needles turned brown which is very typical for *Gremmeniella abietina* (BUTIN, 1995; SINCLAIRE et al., 1987). However, microscopic characteristics of our spores did not fit the sizes of *G. abietina* described in common literature (Table 1). Tissues under the needles with the changed colour were at that time already dry. When the bark of twigs was cut in strips, black dots with pycnidia were found. The pycnidia were also visible on the bark, however, much easier on Scots pine than on Austrian pine. The bark necrosis often occurred in 10 to 100 cm long sections, ringing the twig alternating with healthy parts.

In twig samples cultivated in the wet chambers under laboratory conditions the spores production was stimulated. The spores came out in drops of grey slimy mass from the black 0.5 to 1.0 mm large pycnidia. Conidia were staminate with 2 to 3 vacuoles at the ends of conidia and sometimes with central vacuole. They measured $7.1 \pm 0.8 \times 1.9 \pm 0.3 \mu\text{m}$ (N = 30). The shape and the size of the conidia resembled *Phacidium coniferarum* (G.G. Hahn) DiCosmo, Nag Raj & W.B. Kendr. (PŘÍHODA, 1959).

Sexual stage, apothecia, seldom occurred among black pycnidia in spring, but they were much more common in the summer. Anyway, the apothecia were first closed like the egg, then spread, with the diameter up to 2 mm, standing on a very short stalk. The hymenium was olive green to grey. It contained a lot of paraphysis and there were some asci with 8 ascospores. Clavate asci measured $92.6 \pm 13.9 \times 14.4 \pm 1.9 \mu\text{m}$ (N = 30), ellipsoidal ascospores were without septa $10.8 \pm 1.1 \times 5.9 \pm 0.5 \mu\text{m}$ (N = 30), but with many small rounded elements. Ascospores were concentrated at the apical end of the asci, because of it that part was a bit dilated so ascospores were grouped in the pile.

Pure cultures were obtained from the slime coming out from pycnidia, not from apothecial tissue. Pure

culture on carrot agar was brown in the center and white to transparent on the edge. The growth of the culture was very slow, up to 2 cm in 4 weeks under 25 °C, and even slower under 20 °C and 14 °C.

Pathogenicity of the obtained isolates was proved by pathogenicity tests. There were necrotic lesions under the bark but not visible on the bark. There was no resin and only dead needles above the infected part showed that infection proceeded. By reisolation it was confirmed that the same pathogen came out as was used in the pathogenicity test.

Regarding symptoms, signs of pathogens and the pure culture characteristics it is evident that *Cenangium ferruginosum* is the pathogen responsible for the pine dieback (Table 1).

Discussion

C. ferruginosum occurs all around the Northern Hemisphere (SINCLAIR et al., 1987). In the Czech Republic there was serious *C. ferruginosum* dieback in 2010 as well as in 2004 and the drought was considered as the most important predisposing factor (PEŠKOVÁ and SOUKUP, 2011). SINCLAIR et al. (1987) believe that severe winter frost is the predisposing factor that determines the following successful infection, especially if winter is preceded by unusually mild autumn weather. Similarly BUTIN (1995) considers drought following several months lasting wet period as the most important predisposing factor.

In Slovakia, the specific climatic conditions in certain regions occurred prior to the damage and could play an important role as the predisposing factors. There are some facts about the climate development:

- There was the extremely wet whole year 2010 through the mid-summer 2011, continuously for at least 19 months.

Table1. Microscopic characteristics of two possible fungal pathogens on pines

Fungal species	Dimensions		
	Diameter of fruiting bodies [μm]	Ascospores [μm]	Conidia [μm]
<i>Gremmeniella abietina</i> by BUTIN (1995)	0.5–1.2	3–4 cells: 14.0–20.0 × 3.3–5.0	3–4 cells: 24.0–48.0 × 2.5–3.5
<i>Gremmeniella abietina</i> by ELLIS and ELLIS (1985)	1.0	4 cells: 15.0–20.0 × 3.0–4.5	3–6 cells: 25.0–40.0 × 3.0–3.5
<i>Cenangium ferruginosum</i> by BUTIN (1995)	1.0–2.0	1 cell: 11.0–13.0 × 5.0–7.0	1 cell: 5.0–6.0 × 2.0–3.0
<i>Cenangium ferruginosum</i> by ELLIS and ELLIS (1985)	up to 3.0	1 cell: 11.0–14.0 × 5.0–6.0	not mentioned

- The second half of the summer 2011 through the end of winter 2011/2012 was dry.
- There was a severe frost ranging from $-15\text{ }^{\circ}\text{C}$ to $-20\text{ }^{\circ}\text{C}$ in the winter 2011/2012, lasting for about 20 days from the end of January to the end of February 2012 (ANONYMUS, 2012).

We believe that *C. ferruginosum* had survived in the pine stands as a saprophyte for decades at those stands. As there were good climatic conditions during supernormal wet 19 months (2010 through July 2011) *C. ferruginosum* population could multiply as a saprophyte on dead pine twigs.

According to KARADŽIĆ and MILIJAŠEVIĆ (2008) the infection of trees is possible throughout the year. However, SINCLAIR et al. (1987) describe that *C. ferruginosum* produces no infectious asexual spores, only ascospores can cause infection. Based on this fact incipient infections begin each year in summer and early autumn and are held in check by host defenses unless these are defeated by environmental damage or by other pests. This pattern would explain the sporadic appearance of symptoms (SINCLAIR et al., 1987). We assume that in Slovakia the infections occurred mostly in summer and early autumn 2011. Then dry autumn 2011 limited proper preparations of pine trees for the following winter hibernation. As later trees were again weakened by the severe permanent winter frost, bark and woody tissues could be colonized by the pathogen that ringed the twigs and top of stems. At the end of winter, the sun heated the bark, needles started to transpire the rest of water supply and symptoms suddenly burst out.

KARADŽIĆ and MILIJAŠEVIĆ (2008) consider *C. ferruginosum* as one of the most dangerous pathogenic fungus on pine trees. By pathogenicity tests realized with mycelial plugs in the summer was confirmed that *C. ferruginosum* is not only a saprophyte but also a pathogen. In 3 months the twigs were ringed by necrosis and the above part was dead. There was no resin on the bark in the infection point (SINCLAIR et al., 1987) and this symptom differs from other bark canker pathogens such as *Sphaeropsis sapinea* (ZÚBRIK et al., 2008; ZÚBRIK et al., 2013).

Pine plantations are situated mostly in south-western Slovakia, in region named Záhorie. They grow on sandy soils and usually suffer from drought. In spite of severe growing conditions the *C. ferruginosum* dieback did not appear in that region. Diseased pine stands were located mostly in volcanic mountains of Štiavnické vrchy and Javorie and in crystalline mountains of Tribeč. These volcanic and crystalline basic rocks offer a good nutrition reserve of elements for soils developed there. The role of nutrition deficiency could be compared in the future analysis.

Once the trees are infected and colonized by pathogens causing bark necrosis in the crown, the trees are strongly stressed and soon can be infested by secondary invaders such as *Ips acuminatus* Gyll., *Ips sexden-*

tates Börn, *Tomicus piniperda* L., *Tomicus minor* Htg., Buprestidae, or by other pathogens such as *Armillaria* sp., or *Ophiostoma* sp. (KUNCA et al., 2007; NOVOTNÝ and ZÚBRIK, 2004; ZÚBRIK and KUNCA, 2011; ZÚBRIK et al., 2008; ZÚBRIK et al., 2013) and later on some of them might become the primary pest agents.

C. ferruginosum is an important pathogen in some countries in southern Europe (KARADŽIĆ and MILIJAŠEVIĆ, 2008; TEJERINA et al., 2007). In Central Europe it used to be considered more as a saprophyte than a pathogen (PŘÍHODA, 1959). Regarding climate change we may get in touch with the pathogenic behavior of *C. ferruginosum* much more frequently than in the past.

Conclusions

Specific climate conditions predisposed pine trees to infection and tissue colonization by *C. ferruginosum*. The disease occurred on deep nutrient soils in mountains in Central Slovakia, but did not occur on sandy soils in pine monocultures of Záhorie region. Pathogen determination was based mostly on microscopic morphological characteristics of ascospores and conidia. Pathogenicity was proven in infectious tests. There were no secondary pests such as *Ips* spp., *Tomicus* spp., *Armillaria* spp., which could accelerate the pine damage.

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