Lesnícky časopis - Forestry Journal

LABORATORY EXPERIMENTS WITH GROWTH POTENTIAL OF *CENANGIUM FERRUGINOSUM* TESTED ON NATURAL NUTRITION SOILS

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Kunca, A., Leontovyč, R., 2013: Laboratórne pokusy s potencionálnym rastom *Cenangium ferruginosum* na prírodných živných pôdach. Lesnícky časopis - Forestry Journal, **59**(1): 44–49, 2013, 4 fig., tab. 1, ref. 18, ISSN 0323 – 1046. Original paper.

Začiatkom jari 2012 sa v niektorých oblastiach Slovenska objavilo vážne poškodenie borovíc. Hnednutie ihlíc všetkých ročníkov, nekrózy kôry a rakovinové rany na vetvách boli príznakmi tohto poškodenia. Neboli zistené poškodenia podkôrnym a listožravým hmyzom, koreňové hniloby, sypavky a ani tracheomykózy. Taktiež sme vylúčili pyknidovku beľovú *Sphaeropsis sapinea* ako pôvodcu tohto hynutia borovíc, hoci poškodenie borovíc touto hubou bolo v období rokov 2000 až 2007 najvýznamnejšie a v menšom rozsahu pretrváva stále. Laboratórnymi rozbormi sme zistili prítomnosť huby *Cenangium ferruginosum*, získali sme čisté kultúry a infekčnými testami sme overili patogenitu získaného izolátu. Potenciálnu patogenitu sme testovali ako schopnosť mycélia rásť na prirodzených živných pôdach pripravených z výluhu listov, resp. dreva a kôry vetvičiek vybraných drevín. Poznatky z terénnych šetrení, laboratórnych rozborov a terénnych hodnotení možných predispozičných faktorov sú opísané v tomto článku.
Kľúčové slová: *borovica, hynutie, patogén, Cenangium, sucho, mráz*

Serious pine dieback was reported in early spring from several localities in Slovakia in 2012. Needle necrosis, bark necrosis and twig cankers were the most conspicuous symptoms on diseased trees. There were no or at least not significant damages caused by bark beetles, leaf eating insects, root rots neither tracheomycosis. We also excluded *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton as the main pest agent, which played an important role in *Pinus nigra* Arnold dieback from 2000 to 2007 in Slovakia. Our laboratory inspections revealed *Cenangium ferruginosum* Fr. as the agent responsible for that dieback. We tested its growth capability on different natural nutrition soils in the laboratory to see the potential pathogenecity. This paper describes the pine dieback based on the field inspections and laboratory studies, and we discuss the role of predisposing factors involved in the dieback.
Key words: *pine, dieback, pathogen, Cenangium, frost, drought*

1. Introduction

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

In the past pines overcame 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 climate extremes played an important role as predisposing factors.
2. Invasive needle-cast fungus *Mycosphaerella pini* Rostr. ex Munk ana. *Dothistroma pini* Hulbary was reported for the first time in Slovakia in 1996 (Kunca, Foffová, 2000). At present its occurrence is permanent and it has spread all over Slovakia. It causes the damages mostly in young plantations up to 20 years of their age.

3. *Cenangium 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* has been a serious pathogen on Austrian pines since 2000 through 2007. It still occurs on *Pinus nigra* but after sanitary cuttings many localities have recovered very well (Kunca, 2004).

The aim of the paper is to test the potential pathogenecity of the pathogen *Cenangium ferruginosum* on different natural nutrition soils. We also describe symptoms of *Cenangium ferruginosum* pines dieback observed in the field in 2012, check other pests involved, discuss predisposing factors, map the disease spreading and state prognosis of pines forests health.

2. Material and methods

Foresters with their inquiries were the main source of the information about pines dieback distribution. They invited specialists from the Forest Protection Service settled in Banská Štiavnica (further on mentioned as "FPS") for pest determination and subsequent proper control measures. The FPS specialists visited the localities with the pines dieback, took samples from needles, twigs, bark, roots and soil to look for pest agents in the FPS laboratories. In the field they also evaluated the presence of insects and damages caused by them. Samples were cultivated in wet chambers in the laboratories. Pathogens were cultivated on different natural nutrition soils in the laboratory incubator (Climacell 707) to see its capability to grow on other forest trees and to evaluate the potential pathogenecity for those forest tree species. Carrot agar (later on as "CA"), as a reference nutrition soils, was prepared from 18 g of agar and 40 g of smashed carrot. That was filled up to 1000 ml with distilled water. It was mixed, and filtered after 30 min. Different nutrition soils were prepared alike, but instead of carrot we used the same weight (40 g) of *Quercus petreae* leaves (Ql), *Quercus petreae* twigs (Qs), *Juglans regia* leaves (Jl), *Juglans regia* twigs (Js), *Fraxinus* excelsior leaves (FAl), *Fraxinus* excelsior twigs (FAs), *Fagus sylvatica* leaves (Fgl) and *Fagus sylvatica* twigs (Fgs). The nutrition soils were sterilized under 120 °C for 15 min. in Systec DX-90 autoclave.

Microscopic features of fungal structures were described by stereomicroscope (Leica S8APO) and microscope (Zeiss Axio Scope.A1). Pure cultures were described and speed of growth was measured under 25 °C, 40% of light intensity and 40% of relative humidity. As the FPS has been responsible for the forest health all over Slovakia it has got the information from different parts of Slovakia and all records were marked in the map.

3. Results

From March 2012 to the end of August 2012 there were 6 localities which were visited by FPS specialists and 3 samples were received at the FPS by post. As the FPS specialists on insect damages excluded insect pests as a pine dieback reason, the effort focused on other pests, in particular fungal pathogens. These localities were concentrated in the central southern Slovakia (Fig. 1): Štiavnické vrchy, Javorie, Krupinská planina and Tríbeč. There were also two samples from eastern Slovakia (Slovenský kras and Slánske vrchy) and one from

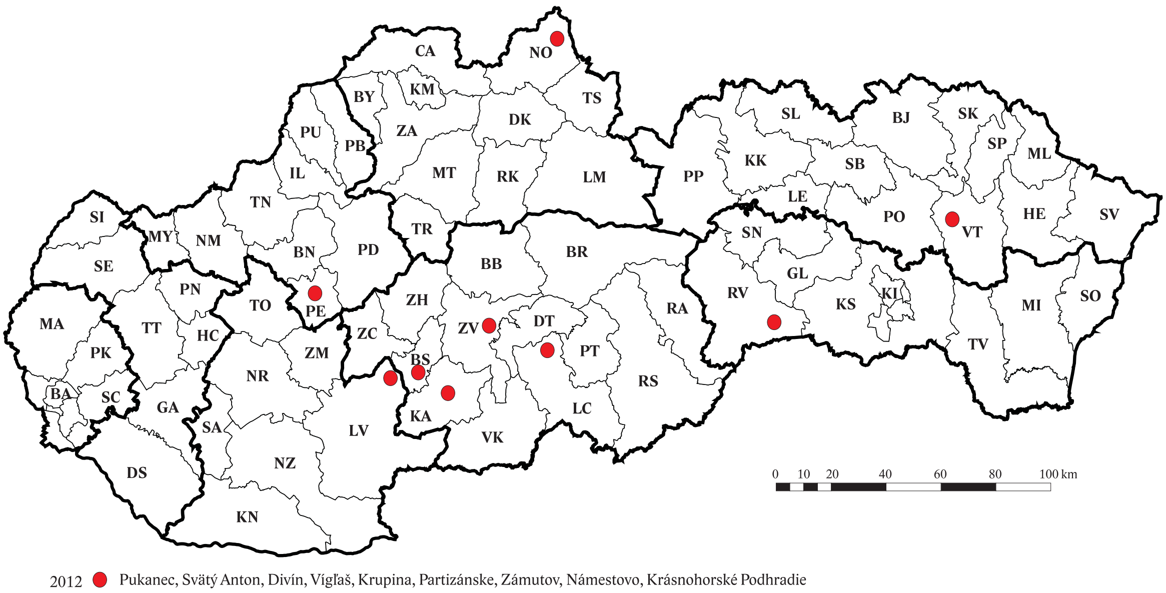

Fig. 1. Pines dieback in Slovakia in 2012

the northern Slovakia (Oravské Beskydy). All together we assumed about 30,000 m^3 of calamity woods of the age from 20 to 100 years.

3.1. Symptoms

The first symptoms on pines were noticed by foresters as soon as in February 2012. The symptoms showed up suddenly, which can be the truth as many of the largely damaged stands are located close to villages and towns and are visible every day by foresters or just by common people. Like that, the colour of needles could not be overlooked by foresters.

Damages in the stands 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 some healthy twigs and moreover, some completely healthy trees were among diseased ones. Symptoms were located in the crown and were limited to the twigs with the diameter not exceeding 10 cm. So, roots, bark and conducted tissues of the trunk were found healthy. Some minor needlecast caused by *Mycosphaerella pini*, *Cyclaneusma* sp., and *Lophodermium* sp., occurred there but just in a small extent. Most needles were free from any signs of pathogens.

The first symptoms were visible on needles of all ages and these needles were first pale green. Later on the base of needles turned brown which is very typical for symptoms caused by *Gremmeniella abietina* (Butin, 1995, Sinclaire *et al*., 1987). However, later on microscopic characteristics of our spores did not fit with size, number of septum and shape of *Gremmeniella abietina* described in common literature (Table 1). Bark tissues under the symptomatic needles were at that time already dry. Under the bark there were black dots which were pycnidia. The pycnidia were also visible on the bark, usually, much easier on Scots pine than on Austrian pine. The bark necrosis with pycnidia often occurred in 10 to 100 cm long sections, ringing the twig, alternated with healthy parts.

Twig samples were cultivated in the wet chambers under laboratory conditions for 3 to 10 days. In spring the spore production was stimulated in grey slimy mass coming out in drops (not tendrils) from the black 0.5 to 1 mm large pycnidia. Conidia were one celled, staminate with 2 to 3 vacuoles at the ends of conidia and sometimes with central vacuole. They measured 6 – 8 × 1.5 – 2.5 µm. The shape and the size of the conidia resembled *Phomopsis pseudotsugae* M. Wilson (Příhoda, 1959).

Fig. 2. Ascospores and ascus of *Cenangium ferruginosum* from the locality Krupina

Sexual stage sometimes occurred in the spring, but much frequently in the summer. The cup like fruiting bodies were first closed like the egg, under wet conditions they spread with the diameter up to 2 mm, standing on a very short stalk. The olive green to grey hymenium was at the upper side of the cup. It contained a lot of paraphysis and there were some asci with 8 ascospores. Clavate asci measured 92.56 ± 13.89 × 14.44 ± 1.87 µm, ellipsoidal ascospores were without septa 10,84 ± 1.04 × 5.88 ± 0.51 µm, but with many small rounded greenish elements (Fig. 2). Ascospores were often concentrated at the apical end of the asci, because that part was a bit dilated.

We did not succeed to get pure culture from the ascospores, but from the conidia. We checked the pathogenecity of the pure culture in the pathogenecity test on twigs of Austrian pine in June 2012 and 4 weeks later we successfully caused the bark necrosis in the infection points. By reisolation we proved the same pathogen which we used in pathogenecity test. Pure culture on carrot agar was brown in the center and white to trans-

Table 1. Microscopic characteristics of two possible fungal pathogens on pines by Butin (1995)

Fungal species	Dimensions		
	Fruiting bodies [mm]	Ascospores [µm]	Conidia [µm]
Gremmeniella abietina	0.5 – 1.2	3 – 4 cells: 14 – 20 × 3.3 – 5.0	3 – 4 cells: 24 – 48 × 2.5 – 3.5
Cenangium ferruginosum	1 – 2	1 cell: 11 – 13 × 5 – 7	1 cell: 5 – 6 × 2 – 3

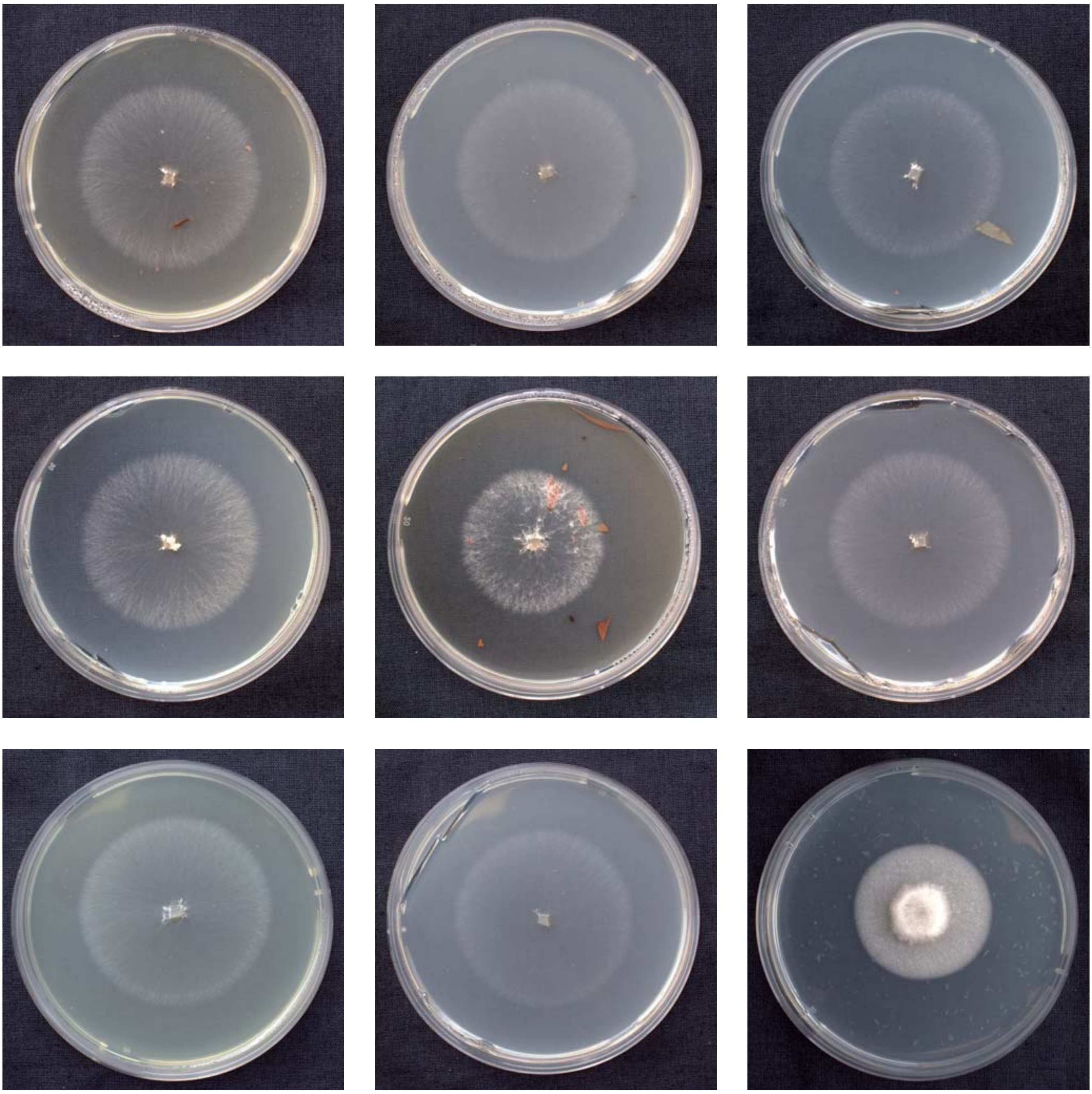

Fig. 3. Culture patterns of *Cenagium ferruginosum* after 9 days of cultivation under 25 °C, 40% of light intensity and 40% relative humidity (1. row: *Fagus sylvatica* leaves, *Fagus sylvatica* twigs, 2. row: *Fraxinus excelsior* leaves, *Fraxinus excelsior* twigs, 3. row *Juglans regia* leaves, *Juglans regia* twigs, 4. row *Quercus petraea* leaves, *Quercus petraea* twigs, 5. row Carrot agar)

parent on the edge, pure cultures on other media looked similarly (Fig. 3). The growth of the culture ranged from 0.19 to 0.29 $cm.day^{-1}$ under 25 °C on different types of nutrition soils (Fig. 4).

Regarding symptoms, signs of pathogens and the culture characteristics we state that the pathogen responsible for the present pines dieback is *Cenangium ferruginosum*.

As the disease is not very common in Slovakia, we consider specific climatic conditions occurring prior to the damage as very important predisposing factors. They correspond to rainy season followed by dry season and strong frost:

– The extremely wet year 2010 through the mid-summer 2011 (continuously at least for 19 months);
– The dry second half of the summer 2011through the end of winter 2011/2012;
– The severe frost in the winter 2011/2012, particularly continuously several weeks from the end of January to the end of February 2012 (Anonymous, 2012).

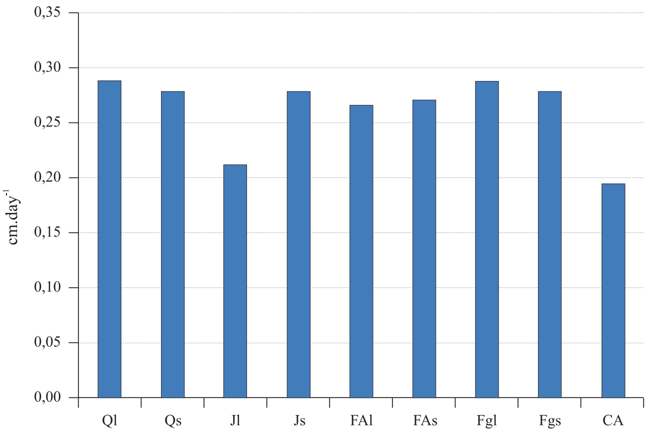

Fig. 4. Averaged daily speed of growth under 25 °C, after 9 days of cultivation on different nutrition soils with 40% of continuus illumination

(Comments: Ql – Quercus petraea leaves, Qs – Quercus petraea stems, Jl – Juglans regia leaves, Js – Juglans regia stems, FAl – Fraxinus excelsior leaves, FAs – Fraxinus excelsior stems, Fgl – Fagus sylvatica leaves, Fgs – Fagus sylvatica stems, CA – Carrot agar).

4. Discussion

C. ferruginosum is often considered as a saprophytic fungus (Butin, 1995; Smith *et al.*, 1988) or a wound pathogen of *Pinus*, *Picea* or *Abies* (Farr *et al.*, 1989). However, some authors (Jurc *et al.*, 2000) see it as a pathogen that overcomes the unsuitable conditions also as an endophytic fungus in pine needles. Fungi of *C. ferruginosum* can cause the dieback only under certain climate conditions, that means high rainfall in the spring followed by a long period of drought (Butin, 1995).

In Slovakia there were supernormal wet 19 months lasting the whole 2010 through July 2011 which could favor pathogen multiplication. In our pathogenecity test we successfully infected pine twigs in June. As ascospores are ripe from the end of spring till the end of summer (Butin, 1995), it is obvious that during that time new pine shoots could be infected. Dry autumn 2011 inhibited proper preparations of pine trees for the winter hibernation. Following winter 2011/2012, trees were again weakened by severe permanent frost lasting from the end of January to the end of February 2012 and thus infected tissues could be successfully colonized by the pathogen. At that time the minimal daily temperature ranged from -15 °C to -20 °C in Banská Štiavnica region and it occurred for about 20 days in January and February 2012 (Anonymous, 2012). At the end of winter, when the sun started to heat the bark, fungal pathogens continued to grow and ringed the twigs by the beginning of spring 2012. When needles needed to transpirate the water in spring, unfortunately they had no supply of it as the twig bark was not functioning at certain damaged parts. This is why symptoms occurred suddenly at the very beginning of spring.

In the Czech republic there were serious *C. ferruginosum* dieback in 2004 and 2010 and they considered the drought as the most important predisposing factor (Pešková, Soukup, 2011). Leontovyč (1962) stated that dry spring, wet summer and dry autumn predisposed pine stands to infection and development of the disease. Moreover, we think that if the winter frost in February 2012 was not so severe, pine trees could overcome the infection. However, we do not know the mechanism how the frost inhibited, postponed or canceled the pine trees defense, but the frost significance in the infectious cycle of *C. ferruginosum* as well as other necrotic fungal pathogens such as *G. abietina*, is discussed by several researchers (Tejerina, Pajares, Diez, 2007; Karadžić, Milijašević, 2008).

As regards fruiting bodies, only flask like black pycnidia occurred on the necrotic bark in groups in spring. If the bark of Scots pine was peeled off, the pycnidia were visible even better. In the lab we found out that conidia were produced and squeezed from pycnidia in grey slimy mass. Apothecia were produced in summer on the same place where pycnidia. It was easier to get pure culture from conidia than from ascospores. In June we infected several twigs of *Pinus nigra* with isolate of C. *ferruginosum* through artificial wounds and even as early as in September the infected twigs died.

In the lab we cultivated *C. ferruginosum* culture on different nutrition soils in order to see the growth rate as well as the mycelium pattern. As it grows on different media prepared from leaves or wood of forest trees, we want to check if it could be able to live on or infect the trees and so if those trees could be hosts for the pathogen. This hypothesis has to be proved and we will work on it in the future.

Once the trees are infected and colonized by pathogens causing bark necrosis in the crown, the trees are strongly stressed to be infested by secondary invaders such as *Ips acuminatus* Gyll., *Ips sexdentatus* Börn, *Tomicus piniperda* L., *Tomicus minor* Htg., Buprestidae, or by other pathogens such as *Armillaria* sp., or *Ophiostoma* sp. (Novotný, Zúbrik, 2004; Kunca, Zúbrik, Novotný, 2007; Zúbrik, Kunca, Novotný, 2008; Zúbrik, Kunca, 2011) and later on some of them might become the primary pest agents.

5. Conclusions

In the time of global climate change and the increasing free global market it is necessary to consider new or rare pathogens as potentially important pests for the future in certain regions. As the *C. ferruginosum* is an important pest agent in some countries in the southern Europe (Tejerina *et al.*, 2007; Jurc *et al.*, 2000) we have to pay an attention to the different aspects of the disease which might play an important role in the future in Central Europe. So far we know that the previous *C. ferruginosum* dieback occurred in Slovakia in 1959 – 1960 (52 years ago). However, we have to know that in the Czech republic 2 diebacks occurred within 7 years (2004 and 2010). It is clear that diebacks like the one in Slovakia in 2012 cannot be overlooked and more research is needed to be able to predict the next occurrence and extent of the damage.

Acknowledgement

This paper was prepared thanks to the financial support of the Slovak Research and Development Agency based on the agreement No. APVV-0045-10 (50%) and the ERDF operational programme Research and Development financing the research project Advanced technologies of trees protection of the juvenile growth stages ITMS 26220220120 (50%).

Literature

ANONYMOUS, 2011: Správa o lesnom hospodárstve v Slovenskej republike za rok 2010 – Zelená správa. [Report on the Status of Forestry in the Slovak republic of 2010 – Green Report]. Bratislava: Ministerstvo pôdohospodárstva a rozvoja vidieka Slovenskej republiky, 84 pp.

ANONYMOUS, 2012: Meteorológia a klimatológia. [Meteorology and climatology]. Bulletin, **18**(2), 45 pp.

BUTIN, H., 1995: Tree Diseases and Disorders. Oxford University Press, New York, 252 pp.

FARR, D.F., BILLS, G.F., CHAMURIS, G.P., ROSSMAN, A.Y., 1989: Fungi on plants and plant products in the United States. APS, St. Paul, 1252 pp.

JURC, D., JURC, M., SIEBER, T.N., BOJOVIC, S., 2000: Endophytic *Cenangium ferruginosum* (Ascomycota) as a reservoir for an epidemic of cenangium dieback in Austrian pine. – Phyton (Horn, Austria), **40**(4): 103-108.

KARADŽIĆ, D., MILIJAŠEVIĆ, T., 2008: The most important parasitic and saprophytic fungi in austrian pine and scots pine plantations in Serbia. Bulletin of the Faculty of Forestry 97: 147-170.

KUNCA, A., 2004: Hynutie porastov borovice čiernej spôsobované hubou *Sphaeropsis sapinea*. [Pine dieback of Austrian pine caused by *Sphaeropsis sapinea*]. In: VARÍNSKY, J. (ed): *Aktuálne problémy v ochrane lesa 2004*, Zvolen: Lesnícky výskumný ústav Zvolen, p. 170-173.

KUNCA, A., FOFFOVÁ, E., 2000: Ohrozenie porastov borovice čiernej fytokaranténnym patogénom *Dothistroma septospora* (Dorog.) Morelet. [Endangered Austrian pine stands by phytoquarantine pathogen *Dothistroma septospora* (Dorog.) Morelet]. In: VARÍNSKY, J. (ed): *Aktuálne problémy v ochrane lesa 2000*, Zvolen: Lesnícky výskumný ústav Zvolen, p. 136-139.

KUNCA, A., ZÚBRIK, M., NOVOTNÝ, J., 2007: Škodlivé činitele lesných drevín a ochrana pred nimi. [Pest agents of forest trees and control methods]. Zvolen: Národné lesnícke centrum, 208 pp.

LEONTOVYČ, R., 1962: Kalamitné odumieranie borovíc na Slovensku v roku 1960 a jeho príčiny. [Pine calamity in Slovakia in 1960 and its reasons]. *Lesnícky časopis*, 6: 429-444.

NOVOTNÝ, J., ZÚBRIK, M. (eds.) 2004: Biotickí škodcovia lesov Slovenska. [Biotic pests of Slovak forests]. Polnochem, a. s., 208 p.

PEŠKOVÁ V., SOUKUP F., 2011: Houbové choroby. [Fungal diseases]. – In: *Výskyt lesních škodlivých činitelů v roce 2010 a jejich očekávaný stav v roce 2011*. Zpravodaj ochrany lesa. Supplementum, (ed. M. Knížek), LOS, VÚLHM, v. v. i., p. 40-45.

PŘÍHODA, A. 1959: Lesnická fytopatologie. [Forestry phytopathology]. Praha: Státní zemědělské nakladatelství, 367 pp.

SINCLAIR, W.A., LYON, H.H., JOHNSON, W.T., 1987: Diseases of Trees and Shrubs. Cornell University, Ithaca, New York, 575 pp.

SMITH, I.M., DUNEZ, J., LELLIOT, R.A., PHILLIPS, D.H., ARCHER, S.A., 1988: European Handbook of Plant Diseases. Blackwell Scientific Publications, 583 pp.

TEJERINA, L., PAJARES, J. A., DIEZ, J. J., 2007: Effects of Assotiated Fungi *Sclerophoma pythiophila* and *Cenangium ferruginosum* on *Gremmeniella abietina* dieback in Spain. *Forest Pathology*, **37**(2): 121-128.

ZÚBRIK, M., KUNCA, A., 2011: Hmyz a huby našich lesov: atlas škôd na drevinách spôsobených hmyzími a hubovými škodlivými činiteľmi. [Insects and fungi of our forests : atlas of damages on trees caused by insect and fungal pest agents]. Zvolen: Národné lesnícke centrum, 200 pp.ZÚBRIK, M., KUNCA, A., NOVOTNÝ, J., 2008: Hmyz a huby : atlas poškodení lesných drevín. [Insects and fungi : atlas of damages of forest trees]. Zvolen: Národné lesnícke centrum – Lesnícky výskumný ústav Zvolen, 178 pp.

Resumé

Borovica patrí k významným hospodárskym a ekologickým drevinám na Slovensku a preto starostlivosť o jej zdravotný stav má veľký význam. V roku 2012 bolo v regióne stredného Slovenska zaznamenané kalamitné poškodenie borovice lesnej a borovice čiernej s objemom do 30 000 m^3. Výskumní pracovníci z Národného lesníckeho centra - Lesníckeho výskumného ústavu Zvolen odobrali vzorky s patogénom, získali čisté kultúry a popísali príznaky poškodenia stromov a porastov a mikroskopické a makroskopické znaky patogéna, ktorého určili ako *Cenangium ferruginosum*. Významná časť článku sa venuje hodnoteniu potenciálnej patogenity huby *C. ferruginosum* pestovaním čistej kultúry huby na prírodných živných pôdach pripravených z výluhov vybraných lesných drevín. Opísané sú vlastnosti čistých kultúr, rýchlosť rastu mycélia a hodnotia sa potenciálni hostitelia patogéna. V závere sú opísané sekundárne škodlivé činitele, najmä podkôrny hmyz, ktoré môžu infikovaný strom napadnúť a spôsobiť jeho odumretie.