Silva Balcanica 23(1): 79-88 (2022)
doi: 10.3897/silvabalcanica.23.e90082

RESEARCH ARTICLE

Winter desiccation of dwarf pine (*Pinus mugo* Turra) needles in the area of Belmeken Dam

Sonja Bencheva, Danail Doychev

University of Forestry, Sofia, Faculty of Ecology and Landscape Architecture, University of Forestry, Kliment Ohridski Blvd. 10, 1797 Sofia, Bulgaria, E-mails: sonben@abv.bg, doychev@abv.bg

Corresponding author: Sonja Bencheva (sonben@abv.bg)

Academic editor: Georgi Georgiev | Received 7 July 2022 | Accepted 19 July 2022 | Published 12 August 2022

Citation: Bencheva S., Doychev D. 2022 Winter desiccation of dwarf pine (*Pinus mugo* Turra) needles in the area of Belmeken Dam. Silva Balcanica 23(1): 79-88. https://doi.org/10.3897/silvabalcanica.23.e90082

Abstract

In the summer of 2018, browning and drying of the needles on the dwarf pine (*Pinus mugo* Turra) formations in the vicinity of the dam Belmeken (Rila Mt.) were found. The damages were concentrated mainly in the southern part of the plants and affect the young needles on the tops of the shoots. During the route survey conducted in 2019, almost complete recovery of the affected plants was observed. Symptoms of drying were found only on single bushes. Studies and laboratory analyses gave reason to conclude, that the drying was due to adverse weather conditions in the winter of 2017-2018, when there was significantly less than normal rainfall, and sudden temperature changes and extremes were found. Established in the dry needles, fungi of the genera *Lophodermium, Hendersonia* and *Neofusicoccum* can be characterized as weak parasites, manifested secondarily, or as endophytes, which under physiological stress due to changes in certain environmental factors can become latent pathogens, worsening the condition of dwarf pine.

Keywords

air embolism, *Lophodermium, Hendersonia, Neofusicoccum*

Introduction

Drying, also known as leaf blight, is a physiological problem associated with water supply disorders. The reason for leaf blight may be the lack of water in the soil to supply the roots during periods of drought in summer or in winter when the water in the soil is frozen, but it can also be caused by a pathogen that develops in xylem. Summer leaf blight is a problem mainly for deciduous species. Winter drying (burns) is most often associated with conifers (Bencheva, 2017). Warm, windy and sunny weather during the winter months accelerates transpiration, while the water in the soil may be frozen and indigestible. The damage is due to a sudden change in temperature, which usually occurs at sunset and sunrise. The southern side of the tree, exposed to the sun, is more often damaged. In evergreen conifers, the symptoms of burns appear as necrosis from the tip of the needle down in late winter or early spring, but may not appear until summer. They are easily recognizable by the browning of the needles and drying above the snow level. The part of the tree under the snow is usually not affected. Trees on the periphery of forests and young plants are more susceptible.

During winter drying, the bases of the needles and some whole needles often remain green. In most cases, the damage is insignificant and the trees are restored. Buds at the tops of the branches usually remain viable and the tree can stimulate new growth. However, sometimes the buds, even the affected branches, can be killed, the growth in height and diameter of the damaged trees may lag behind and they are prone to attacks by other organisms. These are *Phacidium infestans* P. Karst., *Lophodermella sulcigena* (Link) Höhn., *Gremmeniella abietina* (Lagerb.) M. Morelet, that cause great damage to pine plantations in mountainous areas of Serbia (Karadžić, Milijašević, 2008), *Mycosphaerella dearnessii* M.E. Barr and *Coleosporium tussilaginis* (Pers.) Lév., found in Poland on dwarf pine (Pusz, Kita, 2014).

The reason for this study is to establish the complex of pathogens that caused mass browning and drying of dwarf pine (*Pinus mugo* Turra) needles in the region of Belmeken in the summer of 2018 (Fig. 1).

Materials and methods

Two route surveys were conducted in the autumn of 2018 (on October 1 and November 2) in the vicinity of the Belmeken dam, located at 1923 m above sea level in the Rila Mountains. The survey site has geographical coordinates 42˚08'55.00"N; 23˚47'03.44"E. It was found that signs and spore-bearing structures specific to some phytopathogens were observed on individual brownish needles of many of the plants in the dwarf pine formations, therefore samples were collected for identification in the Laboratory of Forest Phytopathology of the University of Forestry, Sofia. Twenty plant samples were microscopically examined. A minimum of 50 spores were measured from the different spore formations identified.

Another route survey of the same area was conducted in the summer of 2019 to monitor the development of symptoms.

Results and Discussion

During the route surveys conducted in 2018 an unevenly distributed drying of the needles was found on the dwarf pine formations. The lesions are concentrated

Figure 1. Drying of the needles on the dwarf pine formations near the Belmeken dam

mainly in the southern part of the plants and affect the young needles on the tops of the shoots.

Microscopic analysis of the collected symptomatic plant samples showed the presence of spores of various fungi.

In 3 of the examined 20 plant samples on some of the dried needles were found black pycnidia (Fig. 2), containing pigmented in reddish-brown 3(4)-cell conidia, slightly narrowed at the septa, with dimensions 20(14-24) × 7(5-8) μm, similar to those formed by members of the genus *Hendersonia*.

Conidia of *Hendersonia pinicola* Wehm. Have dimensions close to the ones established by us: 12-20 × 4-7 μm (Funk, 1985). They are dark brown, spindle-ellipsoidal, narrowed at both ends, with 3-4 septa (4-5 cellular), without narrowing at the septa. Spores of the same fungus isolated from needles of *Pinus contorta* Dougl., are sized 6.3 (15.5-22.0) × 19.6 (5.5-6.8) μm (James, 1984), but the author describes the conidia as reddish-brown and slightly narrowed at each septum.

In *Hendersonia acicola* Münch & Tubeuf (Bingzhang et al., 1994) the conidia are initially unicellular, colorless or light brown, and later become yellowish-brown to dark brown, with 2-3 septa, often narrowed at the septa, oval or diamond-shaped, with dimensions 10.2-13.6 × 4.3-6.8 μm.

Based on these data, it can be concluded that the fungus we found is *Hendersonia pinicola* (Phaeosphaeriaceae, Ascomycota).

Hendersonia pinicola has been described mainly as a secondary pest following previous attacks by *Lophodermella sulcigena* (Link) Höhn. – in North America on *Pinus contorta* (James, 1984), *Pinus banksiana* Lamb. and *Pinus strobus* L. (Durand

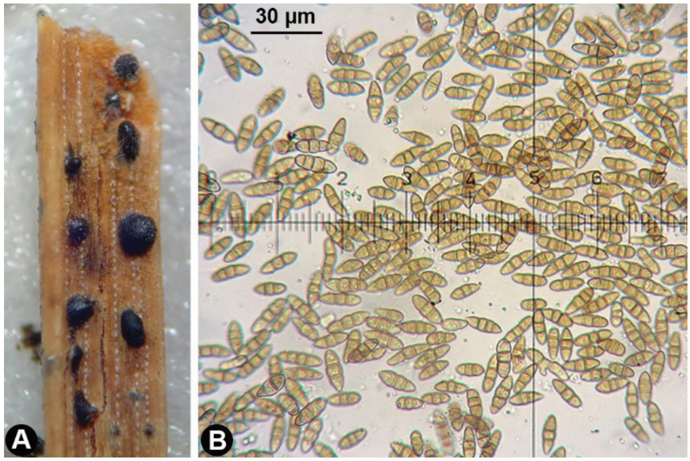

Figure 2. *Hendersonia* sp. – pycnidia (A) and conidia (B)

& Simard, 2012), and in Europe on *Pinus sylvestris* L., *P. mugo*, *P. nigra*, *P. contorta* (Čahtarević, 2013). It is believed that the action of *Hendersonia* spp. can be considered as a natural form of biological control. Studies by Alkanen (1985) in Finland on the relationship between *Lophodermella sulcigena* and *Hendersonia acicola* show that the antagonistic effect of *H. acicola* against *L. sulcigena* occurs only when *H. acicola* infects needles in autumn, but not in spring.

In search of a similar relationship, asci and ascospores from the apothecia identified in 7 of the 20 samples examined on some of the dried needles were measured in the present study (Figs. 3, 4). The dimensions of the ascuses are 135(116-149) × 11(8.1-13.5) μm, of the ascospores – 90(76-113) × 2(1.4-2.7) μm, and of conidia formed in pycnidia (found in only one plant sample) – 4.7(4-5.4) × 1.7(1.4-2.7) μm.

In Europe, *Lophodermella sulcigena* causes *Pinus mugo* disease (Millar, Minter, 1978), which is manifested by redness on the tips of young needles of the current year, which later turn yellow-brown and finally gray. Elongated elliptical black apothecia are located on the killed parts of the needles, below the epidermis, without lips and transverse stromatic lines along the needles. It does not infect all trees in the plantations. *Pinus sylvestris*, *P. nigra* var. *maritima*, *P. contorta* are also hosts. *Lophodermella conjuncta* (Darker) Darker causes a similar change in color, but symptoms develop on biennial needles (Mitchell et al., 1978). The asci of *L. sulcigena* are unitunicate, curved, with 4 or 8 spores, 110-140 × 13-15 μm (100-160 × 15-16 μm at *L. conjuncta*). Its ascospores are 27-40 × 4-5 μm in size, transparent, curved, not septated with a smooth surface, wrapped in a mucous membrane. They are spread by wind in humid weather. Ascospores in *L. conjuncta* are more elongated (75-90 × 3-3.5 μm).

L. sulcigena has been reported in Bulgaria (Dobreva et al., 2016), but there are no data on morphological and DNA analyses. In Serbia, it has been found to attack young, underdeveloped needles from the current growing season (Karadžić, Milijašević, 2008). Infection occurs at the base of the needle, with the part above the

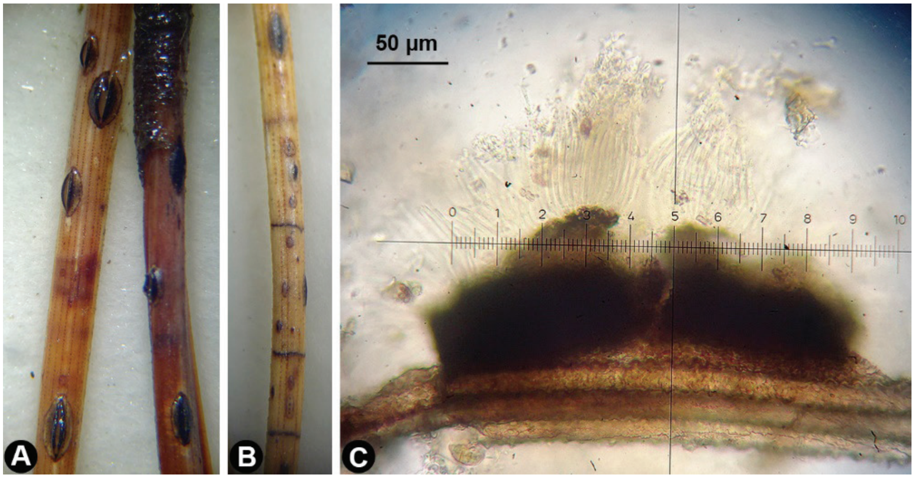

Figure 3. *Lophodermium* sp. – apothecia (A, B, C)

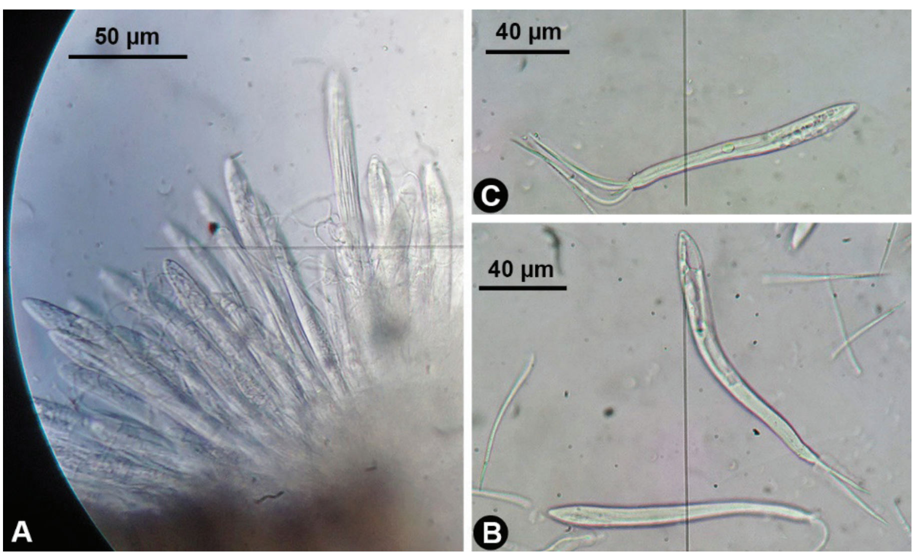

Figure 4. *Lophodermium* sp. –ascuses and ascospores (A, B, C)

site of infection dying and changing color, becoming grayish or light brown, while the part below the site of infection remains green. The gradual killing of the tissues continues until the beginning of November, and only one of a pair of needles may die. The infection rate is highest in years with heavy rainfall in May and June. This fungus is found mainly in mountainous areas, as the ecological conditions at lower altitudes are not favorable for it.

According to Karadžić and Milijašević (2008), the symptoms of *Lophodermella sulcigena* can be confused with those caused by *Lophodermium* fungi. *Lophodermium* *pinastri* (Schrad.) Chevall. is most common on *Pinus mugo* needles in Bulgaria. The fungus develops on 2-year-old needles, but forms sporocarps mainly on dead fallen needles. Apothecia are black, longitudinally opening, with gray, red to yellow-orange lips. Transverse stromatic lines are often formed between the ascocarps. The dimensions of the ascuses are 110-155×9.5-11.5 μm, ascospores 70-110 × 2 μm, and conidia – 4.5-6.25 × 1 μm (Minter, 1981). The apothecia of *Lophodermium seditiosum* (Minter et al., 1978) have colorless or blue-green lips and are often surrounded by a dark line. Stromatic lines between the ascocarps are rarely formed. Ascuses are cylindrical, with 8 ascospores and dimensions 140-170 × 11-13.5 μm, ascospores – filamentous, 90-120 μm long, and conidia – 6-8 μm. They develop on non-fallen needles and cones.

Koukol et al. (2015) report a new species *Lophodermium* found in Poland on *Pinus mugo – L. corconticum* Koukol, Pusz & Minter. The first symptoms of the disease appear after the melting of snow in the spring as yellow spots on 1-2-year-old needles, which later turn completely yellow and fall off prematurely in July. Apothecia of the fungus develop on fallen needles. They are black with pale lips surrounded by a lighter line. In the ascuses, with dimensions 94-142(156) × 8.5-11 μm, filamentous, parallel or spirally wound ascospores with mucous membrane and dimensions (61) 72.5-82.5 (88)×1.5-2 μm are formed. Colorless rod-shaped conidia (3.5-6.5 × 1-1.5 μm) are formed sympodially from the apex of conidiogenic cells.

A new species *Lophodermium pini-mugonis* C.L. Hou & M. Piepenbr., sp. nov. has also been reported on the needles of *Pinus mugo* in Germany (Hou et al., 2009), described only by herbarium materials on twigs with non-fallen needles. This species is characterized by apothecia with wrinkled surface and uneven contour, complex structure of lips cells and elliptical conidia. The dimensions of the cylindrical asci are 90-135 × 9-14 μm, the ascospores 60-90 × 1.5-2 μm, and the conidia 2-3.5 × 0.8-1.2 μm.

Based on the size of ascuses and ascospores, the fungus found by us differs significantly from *Lophodermella sulcigena* and *L. conjuncta*, as well as from *Lophodermium pini-mugonis* and *L. seditiosum*. *Lophodermium corconticum* is close to the size of the ascus, but the ascospores are shorter. To the greatest extent, our sizes are close to those of *L. pinastri*. The latter two species form sporocarps mainly on dead fallen needles as *L. pinastri* develops on 2-year-old needles.

From our known information about the development of the described species of fungi and their morphological data, it can be assumed that the fungus found on dwarf pine in Belmeken is *Lophodermium corconticum* (Rhytismataceae, Ascomycota), but its exact identification is not possible without molecular analysis.

In 2 of the 20 examined plant samples on single needles pycnidia were found, in which unicellular conidia were formed (Fig. 5) with dimensions 13 (8-16) / 5 (4-6.8) μm and length/ width ratio L/W = 2.6 (2-2.7). Their shape is characteristic of the representatives of the family Botryosphaeriaceae, of which the most common of the pines is *Diplodia sapinea* (Fr.) Fuckel (*Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton). However, the size of the conidia in this fungus, although varying quite widely, is significantly larger than that measured by us. According to Phillips et al. (2013) the conidia of *Diplodia sapinea* have dimensions of 25.5-54 × 10-21 μm, and fall within

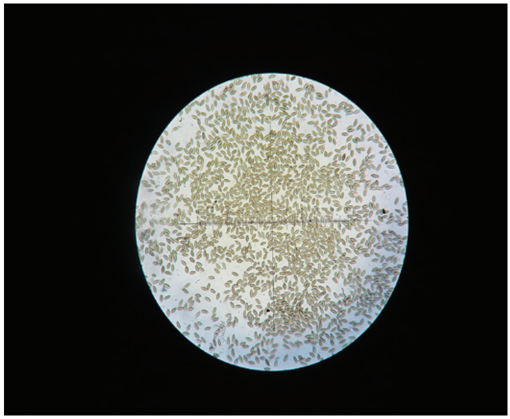

Figure 4. *Neofusicoccum* sp. – conidia

these limits in Hanso and Drenkhan (2009) 30.6-47.1 × 11.8-16.8 μm, but according to Milijašević (2002) they may be smaller 14.8-51.4 × 9.7-20.2 μm.

The L/W ratio of the conidia we measured is less than 3, and the average conidia length does not exceed 18 μm, which according to Phillips et al. (2013) identifies the fungus we found as *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (Botryosphaeriaceae, Ascomycota). According to the same authors, the conidia of this fungus are elliptical, unicellular, colorless, with age they become light brown with 1-2 septa, and their dimensions are (12)13.5-21(24) × 4-6(10) μm, L/W is 3.1-3.2.

Neofusicoccum parvum is distributed worldwide on a large number of hosts (from families Anacardiaceae, Cupressaceae, Ebenaceae, Fagaceae, Juglandaceae, Lauraceae, Moraceae, Myrtaceae, Oleaceae, Pinaceae, Proteaceae, Rosaceae, Rutaceae, Vitaceae). Phylogenetically, this species is in a group of morphologically similar species that can be distinguished only on the basis of data from molecular analyses.

Most members of the Botryosphaeriaceae family have been described as endophytes and latent pathogens (Slippers, Wingfield, 2007), incl. the genera *Neofusicoccum* and *Diplodia*. The development of disease in them is associated with some form of stress (drought, hail, damage from other pathogens, insects, snow, frost) or deteriorating growth conditions for trees.

Zlatkovic et al. (2019) study the genetic diversity of the populations of *N. parvum* and *D. sapinea* from the Western Balkans (Serbia and Montenegro), where damage by members of the Botryosphaeriaceae family is increasingly found. The low genetic diversity and dominance of *N. parvum* and *D. sapinea* on non-native trees leads them to conclude that these species were most likely introduced to the Western Balkans with infected plants (Botryosphaeriaceae endophytic infections are asymptomatic and can easily go unnoticed).

During the 2019 route survey of dwarf pine formations in the area of Belmeken Dam, almost complete recovery of the affected plants was found. Symptoms of drying have been found in single bushes. This fact, as well as the uneven distribution of drying lesions found in the previous year, concentrated mainly on the southern side of the plants and affecting the young needles on the tops of the shoots, both give us reason to look for abiotic causes of the phenomenon.

Similar symptoms have been found in different parts of the world. In some North American states, the needles of many pines, spruces and other conifers turn brown during the winter. This is often observed only in the upper parts of the tree, if the lower branches are under the snow cover, protecting them from wind and sun (Williams, 2018). In Colorado, a sharp drop in temperature in 24 hours by more than 10 °C in November 2014 caused winter drying, especially in the area of dwarf pine and Austrian pine introduction (Ciesla, 2015). The warm weather slows down the hardening of the shoots so far. Damage is most severe in exposed parts of the trees and is observed in late winter and early spring. The side of the tree that faces the prevailing winds, usually from the west, is most susceptible to winter drying.

The reference made for the monthly temperatures and precipitation in the winter of 2017-2018 (Table 1) at the nearest meteorological station to the Belmeken dam on Musala peak (www.stringmeteo.com) shows a tendency of increase in the average monthly temperatures by 0.1-2.8 °C and a reduction in the monthly precipitation amounts to 31-90% of the average monthly norms for a 30-year period (1961-1990). In addition, sharp changes (extremes) in daily temperatures were reported every month, which, for example, in December 2017 for 12 days consistently decreased and increased by more than 15 °C. These conditions are suitable for the occurrence of winter drying or the so-called air embolism.

Table 1. Monthly summaries of rainfall and temperatures for station Musala peak (www.stringmeteo.com)

Year	Month	Temperature ˚C					Precipitations, mm		
		Monthly average		Deviation	Extremes		Monthly averages		% of 1961-1990
		1961-1990	Current		Date	˚C	1961-1990	Current	
2017	November				18.11 21.11 25.11	-3.8 -13.2 -1.8	88	49.5	56
	December	-8.6	- 8.5	+0.1	01.12 05.12 12.12	-1,6 -16.7 -1.5	115	81.3	71
2018	**January**	-10.5	-7.7	+2.8	08.01 18.01 27.01	+0.4 -17.3 -4.0	126	39.5	31
	February	-10.7	-9.2	+1.5	03.02 05.02 18.02 28.02	-4.2 -12.3 -5.1 -17.9	110	39.5	31
	March	-8.5	-6.3	+2.2	01.03 12.03 24.03	-13.3 -0.7 -10.4	130	117	90

At high altitudes, winter weather conditions often cause severe stress, which can cause tree embolism (Maruta et al., 2020). The phenomenon of air embolism in plants occurs in the xylem when the water pressure becomes so high that the air there expands, blocks the vessels with an air balloon and clogging (embolization) of the vessel occurs. This reduces the plant's ability to transfer water from the soil to the leaves. In Japan, Maruta et al. (2020) investigated the onset and recovery from winter embolism in *Abies veitchii* growing near the upper forest boundary. They found a complete (100%) loss of xylem conductivity on the windward side of the trees and 40% on the leeward side. The recovery of conductivity in the xylem on the leeward side begins gradually at the end of winter, while on the windward side it occurs in June. Winter embolism and recovery were also observed in *Pinus mugo* in Austria (Mayr et al., 2019).

Conclusion

The performed researches and laboratory analyses give grounds to conclude that the drying in the dwarf pine formations established in 2018 in the area of Belmeken Dam was caused by unfavorable weather conditions in the winter of 2017-2018, when significantly less than normal rainfall fell and sharp temperature changes and extremes were found.

Future changes in available snow cover may have a strong effect on *Pinus mugo* and other shrubby plants, as reduced snow cover exposes plants to increased temperature stress and impairs recovery processes.

Established in the dry needles fungi of the genera *Lophodermium, Hendersonia* and *Neofusicoccum* can be characterized as weak parasites, manifested secondarily, or as endophytes, which under physiological stress due to changes in certain environmental factors can become latent pathogens, worsening the condition of dwarf pine.

References

Alkanen R. 1985. The occurrence and importance of *Lophodermella sulcigena* and *Hendersonia acicola* on Scots pine in Finland. Karstenia 25, 53–61.

Bencheva S. 2017. Forest phytopathology. Intel Entrans, Sofia, 573 p. (in Bulgarian)

Čahtarević N. 2013. Pine diseases in Western Balkan countries. Master thesis. Mendel University in Brno, 81 p.

Ciesla B. 2015. Winter Drying is a Likely Cause of Brown Pine Needles. Colorado State University Extension (https://extension.colostate.edu/topic-areas/yard-garden/winter-drying-is-a-likely-cause-of-brown-pine-needles/)

Dobreva M., Georgieva M., Dermedzhiev P., Nachev R., Velinov V., Terziev P., Georgiev G., 2016. Fungal pathogens associated with Pinus species in the region of forest protection station Plovdiv in the period 2013-2016. Forest Science 16 (1), 103–116. (in Bulgarian)

Durand J., Simard S. 2012. Les rouges des conifères. Ministère des Ressources naturelles, Québec. 28 p.

Funk A. 1985. Foliar Fungi of Western Trees. Canadian Forestry Service, 160 p.

Hanso M., Drenkhan R. 2009. *Diplodia pinea* is a new pathogen on Austrian pine (*Pinus nigra*) in Estonia. New Disease Reports 19, 14.

He B., Chi Y., Qi G., Yang L., 1994. Study on biological characteristics of *Hendersornia acicola* Munch & Tub. Journal of Northeast Forestry University 5, 37–40.

Hou C.-L., Li L., Piepenbring M. 2009. *Lophodermium pini-mugonis* sp. nov. on needles of *Pinus mugo* from the Alps based on morphological and molecular data. Mycol Progress 8, 29–33.

James R. L. 1984. *Hendersonia* blight of lodgepole pine in Idaho. In Nursey Disease Notes, vol. 9, pp. 2–8.

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.

Koukol O., Pusz W., Minter D., 2015. A new species of *Lophodermium* on needles of mountain pine (*Pinus mugo*) from the Giant Mountains in Poland. Mycol Progress (2015) 14:23.

Maruta E., Kubota M., Ikeda T., 2020. Efects of xylem embolism on the winter survival of *Abies veitchii* shoots in an upper subalpine region of central Japan. Scientific Reports, Nature Research, 10:6594.

Mayr S., Schmid P., Rosner S. 2019. Winter Embolism and Recovery in the Conifer Shrub *Pinus mugo* L. Forests 10, 941.

Milijašević T., 2002. Bioecology of the fungus *Sphaeropsis sapinea* dyko & Sutton – agents of pinus species decline. Glasnik Sumarskog fakulteta, 2002 (86).

Millar, C.S., Minter D.W. 1978. *Lophodermella sulcigena*. CMI Descriptions of Pathogenic Fungi and Bacteria 562, 1–2.

Minter D.W. 1981. *Lophodermium* on Pines. Commonwealth Mycological Institute, Mycological Paper 54 p.

Minter D.W., Staley J.M., Millar C.S. 1978. Four species of *Lophodermium* on *Pinus sylvestris*. Transactions of the British Mycological Society Vol.71 No.2 pp. 295–301.

Mitchell C.P., Millar C.S., Williamson B. 1978. The biology of *Lophodermella conjuncta* Darker on Corsican pine needles. European Journal of Forest Pathology 8, 119–125.

Phillips A.J.L., Alves A., Abdollahzadeh J., Slippers B., Wingfield M.J., Groenewald J.Z., Crous P.W. 2013. The Botryosphaeriaceae: genera and species known from culture. Studies in Mycology 76, 51–167.

Pusz W., Kita W. 2014. Ocena zdrowotności igieł kosodrzewiny (*Pinus mugo* Turra) w Karkonoskim Parku Narodowym The health of mountain dwarf pine needles (*Pinus mugo* Turra) in Karkonosze National Park. Opera Corcontica 51, 41–48.

Slippers B., Wingfield M.J. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants. Fungal biology reviews 21, 90–106.

Williams L. 2018. Browning on spruce and other conifers due to winter drying. Wisconsin DNR Forestry News (https://forestrynews.blogs.govdelivery.com/2018/06/04/browning-on-spruce-and-other-conifers-due-to-winter-drying/).

Zlatković M., Wingfield M.J., Jami F., Slippers B. 2019. Genetic uniformity characterizes the invasive spread of *Neofusicoccum parvum* and *Diplodia sapinea* in the Western Balkans. Forest Pathology, Vol.49 No.1 e12491. https://doi.org/10.1111/efp.12491.