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A COMPARATIVE STUDY ON DOTHISTROMA NEEDLE BLIGHT DISEASE ON *PINUS* SPP. IN BULGARIA AND SERBIA

Margarita Georgieva
Forest Research Institute – Sofia
Bulgarian Academy of Sciences

Miroslav Marković
Institute of Lowland Forestry and Environment - Novi Sad

Abstract

Dothistroma needle blight (DNB) diseases caused significant damages on susceptible pine species in some localities in Bulgaria and Serbia where environmental conditions are suitable for its incidence. This study represent the first results of a joint work on the morphological diversity of *Dothistroma pini* isolates from three *Pinus nigra* localities in Serbia and fifteen mountain and urban localities in Bulgaria including a range of five native *Pinus* spp. – *P. nigra*, *P. sylvestris*, *P. peuce*, *P. heldrechii* and *P. mugo*. The potential distribution and abundance of *Dothistroma* spp. were determined by collecting needles with symptoms considered characteristic of the disease. The morphological peculiarities of isolated mycelium cultures were compared and the differences among them were defined. *Dothistroma* fruit structures were most frequently recovered from samples originating from all regions in Serbia, and least frequently from the Bulgarian localities. In Bulgaria, it was established on *P. nigra* plantations in four localities and on *P. heldrechii* trees that is detected for first time in the country as a new host of DNB. Compiling data for the distribution of susceptible hosts in areas predicted to be suitable for range expansion of *Dothistroma* spp. defining the current situation of DNB disease in both countries. Isolations made from symptomatic needles onto general fungal growth media had yielded a range of other fungi long recognised in Bulgaria as causers of *P. nigra* needle and shoot blight disease - *Cyclaneusma niveum* and some saprophytes or secondary pathogens such as *Lophodermium* spp. and *Diplodia sapinea*. The pathogen *Cytospora pinastri* was found for first time on *P. peuce* in Bulgaria.

Key words: Dothistroma needle blight, *Pinus* spp., fungal pathogens, Bulgaria, Serbia

INTRODUCTION

Dothistroma needle blight (DNB) is a needle cast disease of coniferous species, increasing in geographic and host range throughout Europe, attacking trees in both plantation and natural forests, causing significant economic losses and threatening pine ecosystems and biodiversity (Watt et al., 2009). DNB is caused by two morphologically indistinguishable species, *Dothistroma septosporum* (Dorog.) Morelet (the teleomorph *Mycosphaerella pini* Rostrup) and *Dothistroma pini* Hulbary (teleomorph unknown) (Barnes et al., 2004). *D. septosporum* has a worldwide distribution, whereas *D. pini* has only been found in the USA and some areas of Europe, e.g. Bulgaria, Czech Republic, France, Hungary, Russia, Serbia, Slovenia, Switzerland and Ukraine (Petkov, 1993; Barnes et al., 2008; Ioos et al., 2010; Piskur et al., 2013; Queloz et al., 2014). Both pathogens cause necrosis on needles, thus reducing tree growth and vigour, and in some situations results in widespread tree death. In recent years, DNB has grown in parts of Europe from a low priority disease to a serious economic problem (Drenkhan et al., 2016).

In Serbia, *D. pini* was found for first time in 1955 on *Pinus nigra* Arn. plantation in Troglan Bare at Ravna Reka (Krstić, 1958). Subsequently, the fungus was observed on *P. nigra*, *P. contorta*, *P. halepensis*, *P. jeffreyi*, *P. nigra* var. *maritima*, *P. pinea*, *P. ponderosa*, *P. mugo*, *P. sylvestris*, *Picea omorica*, *P. sitchensis* and *Pseudotsuga menziesii* but it has practical significance only for *P. nigra* (Karadžić, 2010). Conidial stage of *D. septosporum* has been determined on the needles of all pine species, and the teleomorph stage (*M. pini*) only on needles of *P. nigra* found for first time in 1979 and detected only in Deliblato sands and Južni Kučaj (Karadžić, 1986).

In Bulgaria, symptoms of DNB infection were observed for first time in 1976 on 3-year-old *P. radiata* seedlings in a nursery near Sozopol, Burgas district (Zlatanov, 1977). In 1992, occurrence of *D. pini* was considered for first time as a causal agent of DNB disease noticed by red bands and fruiting bodies structures on needles of 20-year-old *P. nigra* plantation in the region of Shumen, Varna district (Petkov, 1993; 2000). In the beginning of 2000s, DNB resulted in extensive defoliation and mortality in *P. nigra* plantations along the Black Sea coast area and in stands in the mountain areas where climatic conditions were favourable for *Dothistroma* sp. development (river and lake valleys with frequent fog with high temperature, air humidity and precipitations) (Rossnev et al., 2008). Subsequently, monitoring of health status of *P. nigra* plantations showed a data for increasing of damages caused by *Dothistroma* sp. ((Dobreva et al., 2016), and *P. radiata, P. halepensis* and *P. strobus* trees planted in urban green areas (Georgiev et al., 2017). To reduce losses by DNB, sanitary felling is used to remove severely affected individuals and copper fungicides are applied in nurseries.

The aim of this study was to detect DNB presence in *Pinus* spp. forest stands and urban green areas in different geographical localities in Bulgaria and Serbia, and to compare the intensity of the disease and main morphological and biological characteristics of *Dothistroma* spp. isolates.

MATERIALS AND METHODS

Samples with typically DNB symptomatic needles from 10 trees per site were collected in April 2015 from three *P. nigra* plantations in Serbia: Deliblato sands, Subotica sands and Kovil (Table 1). In Bulgaria, in May 2015, symptomatic needles were investigate for the presence of *Dothistoma* spp. in a range of five native pine species present on different geo-climatic mountain areas and urban localities (Table 1). The needles were gathered from fifteen sites at altitudes between 179 - 2602 m a.s.l.: Sofia city and its outskirts (Vitosha Mt.), Black Sea coast (Burgas and Varna district), Rila, Osogovo and Balkan Range Studied samples were collected from 10 trees per site in forest plantations and stands of *P. nigra*, *P. sylvestris* L., *Pinus mugo* Turra and *P. peuce*

Table 1. Occurrence of fungal pathogens and intensity of disease on studied *Pinus* spp. trees in different localities in Serbia and Bulgaria.

	N	Region	Location	Longitude, N	Latitude, E	Altitude [m a.s.l.]	Host	Pathogens	Severity (%) range	Severity (%) mean
Serbia	1	Subotica	Subotica sand, Hrastovača	46°10´00.50̋	19°39´24.20̋	143	*Pinus nigra*	*Dothistroma pini*	30-85	(44.5)
								Diplodia sapinea	30-50	(35.5)
								Lophodermium pinastri	10-20	(12.5)
								Cyclaneusma niveum	10-20	(15.5)
								Cenangium ferruginosum	10-15	(11.5)
								Gremeniella abietina	5-10	(8.5)
	2	Pančevo	Deliblato sand, Čardak	44°51´19.60̋	21°06´04.90̋	85	*Pinus nigra*	*Dothistroma pini*	10-20	(14.5)
	3	Kovil	St. Mich. Gavr. Monster	45°21´41.70̋	20°03´26.70̋	80	*Pinus nigra*	*Dothistroma pini*	10-20	(15.0)
								Diplodia sapinea	30-50	(37.5)
								Lophodermium pinastri	5-10	(6.5)
								Cyclaneusma niveum	10-20	(15.5)
Bulgaria	1	Burgas	St. Vlas vill.	42°41´28.93̋	23°20´02.36̋	179	*Pinus nigra*	*Dothistroma pini*	30-40	(32.5)
								Lophodermium pinastri	10-20	(11.5)
								Cyclaneusma minus	10-20	(12.5)
	2	Varna	Vehtovo vill.	43°09´25.20̋	27°06´16.06̋	202	*Pinus nigra*	*Dothistroma pini*	30-60	(52.5)
								Cyclaneusma minus	20-30	(27.5)
								Lophodermium pinastri	10-20	(12.0)
	3	V. Tarnovo	Plachkovtsi	43°02´26.20̋	25°22´30.16̋	475	*Pinus nigra*	*Dothistroma pini*	30-40	(35.5)
	4	Sofia	Park City Centre	42°41´28.93̋	23°20´02.36̋	554	*Pinus nigra*	*Diplodia sapinea*	20-30	(25.5)
	5	Sofia	Forest Research Institute	42°37´12.46̋	23°21´14.00̋	655	*Pinus heldreichii*	*Dothistroma pini*	5-25	(14.0)
	6	Sofia	Forestry University	42°39´12.46̋	23°21´27.00̋	588	*Pinus nigra*	*Lophodermium pinastri*	20-30	(22.0)
								Lophodermium seditiosum	10-20	(12.0)
								Diplodia sapinea	20-30	(25.0)
	7	Sofia	Forestry University	42°39´12.46̋	23°21´27.00̋	588	*Pinus peuce*	*Diplodia sapinea*	20-40	(32.0)
	8	Sofia	Technical University	42°39´22.83̋	23°21´11.66̋	596	*Pinus peuce*	*Lophodermium pinastri*	10-20	(17.5)
								Cytospora pinastri	10-15	(12.5)
	9	Kyustendil	Osogovo Mt.	42°27´06.93̋	23°34´15.06̋	723	*Pinus nigra*	*Dothistroma pini*	15-20	(17.0)
	10	Sofia	Vitosha Mt.	42°36´50.23̋	23°14´10.96̋	1400	*Pinus peuce*	*Cytospora pinastri*	10-15	(12.0)
	11	Sofia	Vitosha Mt.	42°36´54.03̋	23°14´15.46̋	1429	*Pinus sylvestris*	*Lophodermella sulcigena*	5-10	(5.5)
	11	Samokov	Rila Mt.	42°15´19.73̋	23°35´45.16̋	1480	*Pinus peuce*	*Lophodermium conigenum*	20-30	(22.0)
	12	Sofia	Vitosha Mt.	42°35´17.93̋	23°17´31.76̋	1787	*Pinus peuce*	*Lophodermium pinastri*	10-25	(17.5)
	13	Sofia	Vitosha Mt.	42°11´12.63̋	23°36´59.36̋	1923	*Pinus peuce*	*Lophodermium pinastri*	20-25	(22.5)
	14	Sofia	Vitosha Mt.	42°34´12.63̋	23°16´59.36̋	2142	*Pinus mugo*	*Sclerophoma pythiophila*	40-50	(45.0)
	15	Samokov	Rila Mt	42°11´18.73̋	23°35´09.15	2602	*Pinus mugo*	*Sclerophoma pythiophila*	40-70	(55.0)

Griseb, and from two trees per urban localities of *P. heldreichii* Christ. planted in the Arboretum of Forest Research Institute in Sofia, *P. nigra* and *P. peuce* growing in the park of University of Forestry and Technical University in Sofia (Table 1). The intensity of DNB and other fungal infection was used to describe the amount of diseases presented in the observed populations. It was expressed by the percentage of the infected host needles (or shoots) covered by symptoms and lesions of the diseases (Kranz, 1988).

The laboratory study was carried out in the period May-July 2015, at the Laboratory of Phytopathology in Forest Research Institute, Sofia. All needles were assessed for the presence or absence of red bands, necrotic lesions characteristic and fruit structures as the early symptoms of the disease.

For culture isolations, DNB symptomatic needles were surface sterilised by washing in a sterilizing solution (96% ethanol for 30 sec and 10% sodium hypochlorite for 30 sec). Samples were then rinsed in sterile deionised water and blotted dry on sterile paper. Methods of *Dothistroma* spp. isolation were applied as described by Mullet, Barens (2012). *Dothistroma* medium (50 g malt-agar, 23 g nutrient agar) and 1 g streptomycin were used for isolation. Under a binocular (stereo) microscope (×30-×60 magnification) the acervuli were rolling on the media. As the acervulus was rolled, the released spores were visible on the surface of the media. The isolates were placed in thermostats at the temperatures of 22°C and their growth was monitored daily. Various cultural and morphological characteristics of isolated fungus were recorded by making visual and microscopic observations. The main characteristics such as mycelial growth, size, colour and separation of mycelia and spores were investigated. The identification of fungal pathogens was carried out by microscopic examination (Zeiss NU2, ×125). The complex of other fungal pathogens was identified after microscopic analyses, based on the appearance of the fruiting bodies, conidia and pure mycelium culture.

RESULTS

In May 2015, well defined symptoms of DNB disease - transverse red bands on green needles with black fruiting bodies (aservuli), erupted through the epidermis (Fig. 1 a,b), were detected on all examined *P. nigra* samples taken from the three selected localities in Serbia (Table 1). The number of ripen aservuli was abundant and conidia were exuded in a mass through a longitudinal rupture of the needles' epidermis where the fruiting bodies appeared (Fig. 1 c). *D. pini* were identified as the causer of DNB disease on all pine needles collected. The assessed intensity of the disease averaged 44.1% for all selected sites. In Subotica sands, severe DNB intensity was detected - infections of two-year-old needles ranged between 30-85% (Table 1). Studies on DNB in *P. nigra* plantations in Deliblato sands and Kovil showed relatively low level of the disease intensity - up to 20%. In the examined plantations, individual trees were severe infected but their participation in the total number of trees was less than 10%). On some pine trees in Hrastovača region (Subotica sands), it was found dead shoots on trees caused by the fungal pathogen *Diplodia sapinea* (Fr.) Dyko et Sutton. Secondary pathogens on needles were also established on the same sample trees but the intensity of diseases they caused were at low rate - up to 20% (Table 2): *Lophodermium pinastri* (Schrad. ex Hook.) Chev., *Cenangium ferruginosum*, *Cyclaneusma niveum* (Pers.) DiCosmo, Peredo et Minter and *Gremeniella abietina* (Lagerb.) Morelet.

Concerning Bulgarian samples, typical DNB symptoms with red bands and black aservuli were detected on needles of four *P. nigra* plantations (St. Vlas, Vehtovo, Plachkovtsi and Kyustendil), and on *P. heldreichii* trees planted in the Arboretum of Forest Research Institute, Sofia (Table 1). This is the first time that *P. heldreichii* is recorded as a host of DNB disease in Bulgaria. On the needles of rest samples, yellow to red bands and parts of dead needle tissue were observed but no fruiting structures of *Dothistroma* spp. appeared.

Amongst all Bulgarian location surveyed, the highest mean DNB intensity was recorded on *P. nigra* trees in Vehtovo (52.5%) followed by Plachkovtsi (35.5%) and St. Vlas (32.5%). Lowest disease intensity was assessed in Sofia region on *P. heldreichii* trees – up to 25% (Table 1). Because of the infection, the oldest needles were shed prematurely and crowns looked whit thin appearance. In contrast to Serbian samples, the formation of aservuli on *P. nigra* needles was starting later – at the end of May, and

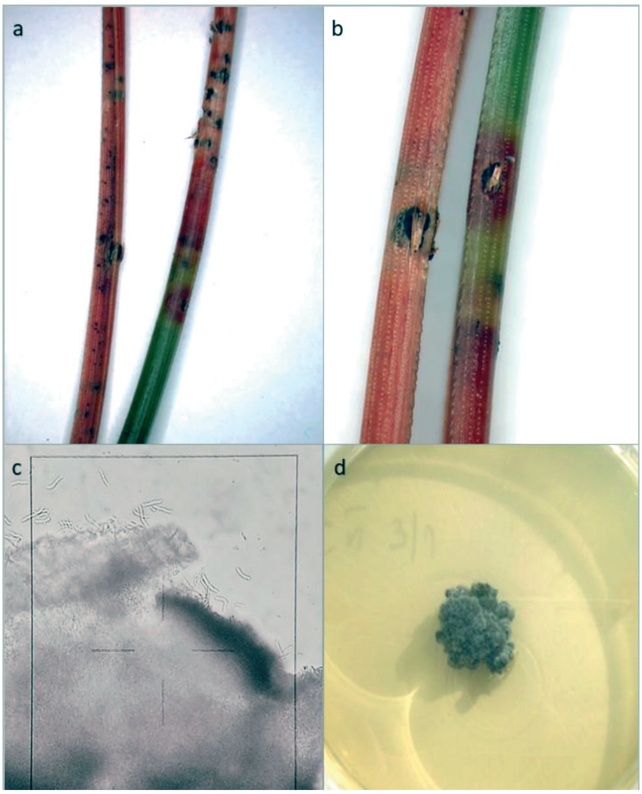

Fig. 1. Symptoms of DNB caused by *Dothistroma pini* on samples collected from Subotica sands, Serbia: a-red bands and fruit bodies; b-aservuli; c-conidia, d-mycelia culture

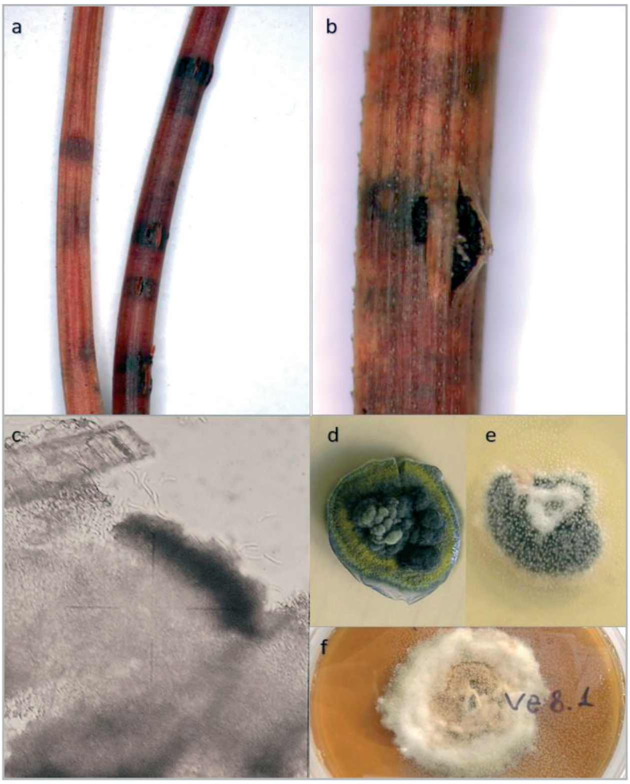

Fig. 2. Symptoms of DNB caused by *Dothistroma pini* on samples collected from Vehtovo vill. Bulgaria: a-red bands and fruit bodies; b-aservuli; c-conidia, d-mycelia culture

the number of ripen fruit bodies on them was lower. These samples were additionally put in wet conditions at 22°C for a week, and afterwards, abundant formation of acervuli appeared and conidia were produced in a slimy, sticky mix on the exposed fruit bodies (Fig. 2a, b).

Identification of *Dothistroma pini* structures was done by microscope observation for both Bulgarian and Serbian samples. Only the anamorphic (asexual) stage features, conidia and conidiophores were noticed (Fig. 1c; 2c). There was no differences between the morphological characteristics of conidia taken from the different localities (Table 2). All investigated conidia were colourless, cylindrical, with two or three septa. Conidial dimensions were between 20-28a2.0-3.0 μm.

Some of the most common *P. nigra* pathogens, previously recognized in sampling areas, were investigated (Table 1): *Lophodermium pinastri, L. seditiosum* Minter, Staley et Millar, *Cyclaneusma niveum* and *D. sapinea*.

On needles of *P. sylvestris, P. mugo* and *P. peuce* white to yellow spots were observed. Signs of the infecting pathogen an symptoms expressed by the host were characteristic and diagnostic for a particular infection disease. *Sclerophoma pythiophila* (Corda) Höhn and *D. sapinea*. This range of parasitic fungi was chosen considering pathogenic incidence on genus *Pinus* and their relative presence frequency on surveyed sites. Most of them are pine needles specific pathogens such as *C. minus* and *D. sapinea*.

The fungal pathogen *Cytospora pinastri* Fr. was detected for first time in Bulgaria on *P. peuce* needles in Rila and Vitosha Mt., causing their premature defoliation. The intensity of the disease was low – up to 10%.

Isolation of *D. pini* from the infected needles on an artificial nutrient medium was extremely difficult. In total of 15 isolations were obtained from Serbian samples. In four weeks, successful pathogen isolation from Serbian samples were noticed, the mycelia grew slowly - about 8-15 mm per one month at 20-22°C. The colour of all isolates were dark grey, and in a month, it turned to black, with stromatic structure (Fig. 1d).

For Bulgarian isolates, 30 mycelium cultures were developed from aservuli on *P. nigra* needles. There was a phenotypic variation in the colour of *D. pini* mycelium culture among the different localities (Fig. 2d). It appeared light grey in isolates from Vehtovo and St. Vlas with a thick consistency to grey-black from Plachkovtsi, Kyustendil and Sofia, producing whitish conidial slime, and agar coloured light reddish-brown. Growth of the culture is characterized by slow mycelial extension and high morphological diversity. Colonies of fungi started to develop in 5-7 days after the inoculation of the medium. It was measured 2-2.5 mm growth per a week at 22°C. Mycelium culture was isolated from 27% of needles that had symptoms considered characteristic of the disease.

DISCUSSION

DNB diseases caused significant damages on susceptible pine species in some localities in Bulgaria and Serbia where environmental conditions are suitable for its incidence. This study represents the first results of a joint work on determine DNB intensity and diversity of *D. pini* isolates from different geo-climatic Serbian and Bulgarian locations where it is known that DNB caused significant damages on *P. nigra* plantations - Deliblato sands, Subotica sands and Kovil in Serbia (Karadžić, 2004; Pap et al., 2016) and from Vehtovo, St. Vlas and Plachkovtsi in Bulgaria (Rossnev et al., 2008). In addition, symptomatic needles samples were collected from natural *Pinus* spp. from high mountain areas (Vitosha and Rila) where the potential DNB hosts as *P. sylvestris, P. peuce* and *P. mugo* were distributed naturally at altitude between 723 and 2602 m a.s.l., and from urban sites in Sofia city where *P. nigra*, *P. heldreichii* and *P. peuce* were planted artificially.

The morphological characteristics of symptoms, reproductive structures and mycelium isolates were used to identify the causative agents of DNB and to infer them to the extent of disease intensity. The potential distribution and abundance of *Dothistroma* spp. were determined by collecting needles that had symptoms considered characteristic of the disease. Infections with *D. pini* was established in all selected Serbian localities and in four *P. nigra* plantations in Bulgaria (Table 1). The disease was observed for first time on the new host *P. heldreichii*, growing in the Arboretum of Forest Research Institute (655 m). The species is consider as a host appearing slight susceptible to DNB (Watt et al.,

Table 2. Diagnostic characteristics of fungal pathogens isolated from ***Pinus*** spp. from Serbia and Bulgaria

Location	Host	Fungal pathogens	Length [µm]	Mean [µm]	Width [µm]	Mean [µm]	Mycelium colour
Serbia							
Subotica sands Deliblato sands	*Pinus nigra*	*Dothistroma pini*	22.0-26.2	24.8	2.0-2.8	2.5	*dark grey to black*
Bulgaria							
Vehtovo	*Pinus nigra*	*Dothistroma pini*	20.0-28.0	25.5	2.0-3.0	2.5	*white, cream, dark grey*
Kyustendil		*Cyclaneusma minus*	80.0-90.0	82.5	2.5-3.0	2.7	*cream*
Vitosha Mountain		*Lophodermium pinastri*	5.0-6.5	5.5	1.0-1.2	1.1	*white*
Vitosha Mountain		*Lophodermium seditiosum*	5.5-8.8	7.2	1.0-1.4	1.2	-
Sofia, Central park		*Diplodia sapinea*	30.0-32.0	31.0	10.0-14.0	12.5	*grey*
Vitosha Mountain	*Pinus sylvestris*	*Lophodermella sulcigena*	-	-	-	-	-
Arboretum, FRI	*Pinus heldrechii*	*Dothistroma pini*	22.0-26.0	25.0	2.0-3.0	2.5	*dark grey*
Rila Mt.	*Pinus peuce*	*Lophodermium conigenum*	4.5-9.5		1.2-2.0	1.6	*white*
Vitosha Mt.		*Lophodermium pinastri*	5.0-6.0	5.4	1.0-1.2	1.2	*white*
Rila Mountain		*Cytospora pinastri*	4.0-8.8	6.2	1.2-2.0	1.5	-
Vitosha Mountain Rila Mountain	*Pinus mugo*	*Sclerophoma pythiophila* *Lophodermium pinastri*	6.0-8.0 8.0-8.8	7.2 8.5	1.0-2.0 1.0-1.4	1.3 1.2	- *white*

2009). Besides, *D. pini* was isolated from *P. heldreichii* symptomatic needles, the damages were with low intensity. In Bulgaria, natural forests of the species are distributed between 1400-2200 m in Pirin Mt. and Slavyanka Mt. (Alexandrov et al., 2004). According to Karadžić (2004) the climatic conditions at altitudes more than 900 m are not favour for *Dothistroma* spp. infections.

The different percent of DNB intensity disease depends on local environmental conditions (Table 1). In Serbia, the most severe intensity was observed on *P. nigra* plantations in Subotica sands (up to 85%), and in Bulgaria - along Black See cost (almost 60%) and in the region of Veliko Tarnovo district (35.5%). The different DNB intensity rate is related to several factors including the aggressiveness of *D. pini*, quantity of inoculum produced and the climatic conditions favour the pathogen's reproductive cycle. Generally, the disease could only cause mortality where the infections levels are high for successive years. There can be a significant economic impact on severely infected forests due to this reduction in timber yield (Drenkhan et al., 2016).

In Bulgaria, DNB was detected on four *Pinus* species: *P. nigra* is the most common host, followed by *P. sylvestris*, *P. heldreichii* and *P. mugo* (Georgieva et al., unpublished). The highest susceptibility to DNB showed *P. nigra* sample trees followed by *P. heldreichii* trees known to have slight susceptibility to the disease in other countries on Balkan Peninsula (Lazarevic et al., 2017). In Serbia *Dothistroma* spp. were isolated from nine *Pinus* spp., two *Picea* spp. and *Pseudotsuga menziesii* (Karadžić, 2010). *D. pini* occurs especially frequently in *P. nigra* plantations on Suvobor, Deliblato Sands, Subotica-Horogoš Sands and Pešter (Karadžić, Milijašević, 2008). Fungicide treatments were applied in Suvobor and Subotica sands. After treatments, health condition and vitality of *P. nigra* plantations were improved and needle infection was reduced to the acceptable level (Pap et al., 2009; Golubovic-Curguz et al., 2013).

For Bulgaria, this is the first attempt for *Dothistroma* sp. mycelium culture isolation from infected symptomatic needles. A variety of mycelium colours from white to dark grey was established among the samples. Such phenotypic variation of *D. pini* mycelium isolates in Slovakia were obtained – white, pale grey to black colour (Ondrušková, 2018). All Serbian mycelium cultures were dark grey to black. According to Kovalski et al. (2016) the colour of the DNB secreted pigment was not a consistent feature of a given fungal isolate, but depended on temperature and pH of the medium, along with a significant interaction among these factors. Lower temperature and lower pH favoured the production of blue pigment.

Isolations made from symptomatic needles onto MEA fungal growth media have yielded a range of fungi long recognised as *P. nigra* associates in Bulgaria. The main pathogens were *Dothistroma pini*, *Cyclaneusma minus, Lophodermium* spp. and *Lophodermella sulcigena*. The pattern of recovery of these fungi has not been consistent with location or tree species and there has been no indication of a consistent association for any of these with the symptoms of red needle cast. Needle and shoot damaging pathogens were also established in other *Pinus* spp. studied. The complex included the pathogen *C. minus, C. niveum* and saprophytes or secondary pathogens such as

Lophodermium spp. and *Diplodia sapinea*. *Cyclaneusma* spp. caused Cyclaneusma needle cast that is a serious disease affects many pine species, present in all continents where they are grown (Watt et al., 2012).

D. sapinea was established on most *Pinus* species from different localities (Table 1). In recent years, it has been one of the most common and widespread pathogens that caused severe damages on young shoots and branches of physiologically weak *Pinus* spp. in a localities with chronic water deficit in both countries (Karadžić, Milijašević, 2008; Dobreva et al., 2016).

For *P. sylvestris*, worse assessed health condition was related to damages caused by *L. pinastri* and *L. seditiosum.* They are the most widespread fungal genera, developing on more than 30 pine species. In particular, *L. seditiosum* is a serious pathogen of pine seedlings in nurseries, causing heavy infections characterized by needle loss in young plantations and nurseries. On the other hand, *L. pinastri* is a common endophyte which can be found also in the same needle occupied by *L. seditiosum*.

The observations of DNB disease in Bulgaria and Serbia revealed the current disease distribution in both counties, causing damages mainly on *P. nigra* plantations. The study was carried out in localities predicted to be suitable for range expansion by *Dothistroma* spp. and included potentially susceptible host species. The mean intensity of disease was assessed as moderate in both countries but expansion of the disease was not observed. The disease could turn in biotic disturbance affecting structure and composition of pine forests in case the climatic conditions become more favourable for the pathogens development. In Britain, DNB intensity has increased to a level where native *P. sylvestris* stands in Scotland - a species generally considered to be of low susceptibility in the past, are under threat. In other parts of Europe (Austria, Czech Republic and Finland), a similar situation is also being observed with native conifer species previously unaffected (Drenkhan et al., 2016). The significance of DNB for pine plantations revealed the need for further investigations detecting DNB distribution and host range susceptible to disease.

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E-mail: margaritageorgiev@gmail.com