Environmental Sciences Proceedings

MDPI

Proceedings

New Data on Host Range and Geographical Distribution of Dothistroma Needle Blight in Ukraine

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Abstract: Serious pine needle disease, Dothistroma needle blight (DNB), caused by *Dothistroma septosporum* and D. pini was detected in Ukraine in 2004-2005. The aim of this study was to identify the Dothistroma species present on new hosts in Ukraine using different molecular techniques to increase our understanding of the local distribution of these pathogens. The occurrence and distribution of DNB were studied in 2014–2018 and 480 needle samples were collected from 16 different regions in 96 localities, and the presence of DNB was confirmed in 62 of them. The host range of DNB consisted of 8 pine species including 3 subspecies and 2 spruce species, among them *Pinus nigra* subsp. *pallasiana* and *P. sylvestris* were the most frequent hosts. Results showed that both *D. septosporum* and *D. pini* were present on *P. nigra subsp. pallasiana* on the same trees and even in the same needles. Moreover, D. septosporum was found first in Ukraine on *Pinus ponderosa* Douglas, *Pinus banksiana* Lamb and *Pinus contorta* Douglas in the arboretum as well as *Picea pungens* Engelm and *Picea abies* (L.) H. Karst. The suitability for the disease in the different forest types and intensity of the disease are discussed.

Keywords: Dothistroma needle blight; pine species; disease intensity

1. Introduction

Dothistroma needle blight (DNB) has been reported as one of the most serious diseases of conifers with a worldwide distribution and over 100 reported pine host species [1]. DNB is caused by the two morphologically similar ascomycetous fungal species *Dothistroma septosporum* (Dorog.) M. Morelet and *Dothistroma pini* Hulbary [2] and only molecular markers are capable to distinguish these two species [1-2]. *D. septosporum* has a world-wide distribution while *D. pini* has been found in the US and over than 10 European countries such as France, Hungary, Ukraine, Switzerland [1-4].

D. septosporum was first recorded in Ukraine in 1914 in pine plantation and this sample was collected by L. Kaznovsky on Pinus sylvestris L. needles nearby Smela (Kiev province, March 25, 1914: LE 116244, duplicate - CBS 11381) [2]. This species was identified as *Dothistroma septosporum*. Second findings in Ukraine in Mykolaiv and Kherson regions were collected in 2004, DNB was found on *Pinus nigra* subsp. Pallasiana, (Crimean pine) in Ukraine [2] and this species was identified as *D. pini* [3]. Ukrainian isolates from *P. nigra* subsp. pallasiana were identified as D. pini based on both the ITS and the -tubulin sequences [3], presenting the first verified report of this fungus outside north-central USA. Hence, *D. pini* is now known to occur on two continents in 76 countries, and the host range includes 20 newly reported hosts of Dothistroma species [1]. Moreover, two new exotic pine hosts for *D. septosporum*, *Pinus nigra* var. *australica* and *P. nigra* var. *mollet*, were recorded in Western Ukraine [5].

The aim of this study was to identify the Dothistroma species present on new hosts in Ukraine using different molecular techniques to increase our understanding of the local distribution and diseases intensity of these pathogens.

2. Experimental Section

For DNB detection only symptomatic needles were collected in Ukraine in 2014-2018 a from 10-40 years old Scots pine (*Pinus sylvestris*) and from other exotic pine species of different age in 10 different localities (Table 1). The diseases intensity was assessed visually as the rate of the tree crown affected by DNB [6] as follow: up to 20% of crown damage is the low disease severity, 20-40% the middle one, 40-60% high and >60% very high disease severity [6].

The 1-, 2- and 3- year old infected needles were collected from 3 randomly selected trees in every locality (in total, 40-60 needles depending on diseases intensity). Samples were placed in paper bags and kept at -20 °C until processed in laboratory in the same year. Needles were examined with a Nikon C-PS stereo microscope in the laboratory and classified as symptomatic or asymptomatic. Isolations were made from mature conidiomata from symptomatic needles using rolling method and 2 % malt extract agar (MEA) to release conidia [3].

In addition to morphological analysis of fungal sporification structures, different DNA-based techniques were used for species identity. The DNA was extracted from symptomatic and asymptomatic needles from each location using the OMEGA E.Z.N.A. Bio-Tek Fungal DNA Mini Kit D3390-02 (USA), following the manufacturer's instructions. Conventional PCR with specific primers were used to identify the fungus directly in DNA extracted from needles (DStub2-Forward (CGAACATGGACTGAGCAAAAC) and DStub2-Reverse (GCACGGCTCTTTCAAATGAC) for D.septosporum and DPtef-Forward (ATTTTTCGCTGCTCGTCACT) and DPtef-Reverse (CAATGTGAGATGTTCGTCGTG) for identification of *D. pini* [3]. The identity of Dothistroma species from culture was confirmed by sequencing the internal transcribed spacer (ITS) region using the primers ITS5 and ITS 4 [7] and β-tubuline gene region 2 was amplified using the primers using the primers Bt2a and Bt2b [3].

3. Results

The occurrence and distribution of DNB were studied in 2014–2018 and 480 needle samples were collected from 16 different regions in 96 localities, and the presence of DNB was confirmed in 62 of them.

Visual symptoms of DNB observed on needles varied significantly. A clear typical of DNB symptoms were present on individual trees but most of signs were controversial [3] and typical DNB red bands were not generally occurred on infected needles of *Pinus contorta* and *Picea pungens*. Infected needle generally starts to appear on basal part of trees infecting 2- 3- year- old needles of lower branches. DNB in Ukraine is commonly found on ornamental pines in Arboretum, and Botanical gardens although no middle/high damage has been observed. Moreover, damage rate was quite low and pathogen was presented only on lower branches.

The host range of DNB consisted of 8 pine species including 3 subspecies and 2 spruce species among them *Pinus nigra* subsp. *pallasiana* and *P. sylvestris* L. were the most frequent hosts. Diseased pine trees with DNB symptoms were found in various habitats, including planted pine forests and arboretums throughout the country that partially presented in Table 1. Based on a molecular analysis, both *D. septosporum* and *D. pini* were present on *P. nigra* subsp. *pallasiana* on the same trees and even in the same needles.

Table 1. New data on host and locality of DNB in Ukraine (only part of data is shown).

Host	Locality	Coordinates	D. septosporum ITS/ β-tubulin	D.pini ITS/ β-tubulin

Pinus sylvestris	49.622576, 36.390355	Kharkiv, Zmyiv district	+/- (same trees and needles)	-/+ (same trees and needles)
	49.814551, 30.055763	Bila Tserkva, Alexandria	+/+	-/-
	50.047282, 31.396392	Pereyaslavl	+/+	-/-
P. nigra subs.palassiana	50.382611, 30.500463	Kyiv, Botanical Garden Holosiivskyi	+/- (same trees and needles)	+/+ (same trees and needles)
P. ponderosa	50.097505, 36.277293	Kharkiv, URIFFM arboretum	+/+	-/-
P. banksiana	50.027229, 36.229664	Kharkiv, KhNU arboretum	+/+	-/-
P. contorta	50.027229, 36.229664	Kharkiv, KhNU arboretum	+/+	-/-
	48.765469, 30.236940	Uman, arboretum	+/-	-/-
Picea pungens	50.027229, 36.229664	Kharkiv, KhNU arboretum	+/+	-/-
Picea abies	50.097505, 36.277293	Kharkiv, URIFFM arboretum	+/+	-/-
Abies concolor	50.027229, 36.229664	Kharkiv, KhNU arboretum	-/-	-/-

Moreover, the *Dothistroma septosporum* was found and molecularly confirmed first in Ukraine on five non-native conifers *Pinus ponderosa*, *Pinus banksiana* and *Pinus contorta* in the arboretum as well as *Picea pungens* and *P. abies* in Botanical gardens and arboreta (Table 1).

4. Discussion

Having analyzed the distribution of DNB in pine forests, disease severity of infection in Scots pine forest stands and plantations was not very high. The highest disease severity, up to 80%, has been observed in the southern regions on *P. nigra subsp. pallasiana*. On *Pinus banksiana* and *Pinus ponderosa* a DNB was confirmed by only morphological and molecular methods, no typical DNB symptoms and pathogen outbreak have been observed since survey. The analysis of geographical distribution and host range data indicate that DNB is spreading across Ukraine in various habitats, wherever both pine forest and other exotic pines are present. DNB was found in many parts of the country although the disease intensity is low, probably due to the unfavorable climate conditions for DNB development.

Obtained results in this study showed the ability of Dothistroma species to infect the conifers and to cause the damages and different needle losses, but there was no significant influence of diseases intensity with Dothistroma species on the tree decline and dieback. However, further large scale field and pathogenicity studies are required to support this conclusion.

DNB is a serious problem, affecting most of the European countries, and one of the main goals in the possible management strategies is the determination of all the contributing factors of conifer decline in Europe. However, next to infections of DNB as a main cause of the needle dieback, some other pathogens may possibly play role in this phenomenon, including infections with root rot, rust and *Armillaria* species [8]. Therefore, further study of possible interactions of DNB and other pathogens as well as pathogenicity tests are required to understand their common impact on conifers and other possible interactions.

5. Conclusions

The data obtained demonstrated gradual increasing of the geographical range of DNB in Ukraine. In addition to existing 8 pine species, *D. septosporum* was found first in Ukraine on Pinus ponderosa Douglas, Pinus banksiana Lamb and Pinus contorta Douglas in the arboretum as well as *Picea pungens* Engelm and *Picea abies* (L.) H. Karst. The suitability for the disease in the different forest types and intensity of the disease are different throughout country, although generally the disease intensity is low, probably due to the unfavorable climate conditions for DNB development.

Author Contributions:.Conceptualization, K.D. and O.S.; methodology, K.D., D.B. and I.H.; software, D.B., I.H.; formal analysis, K.D., D.B. and O.S.; investigation, K.D., D.B. and O.S.; resources, D.B. and I.H.; writing—original draft preparation, K.D.; writing—review and editing, K.D., D.B. I.H. and O.S.; project administration, I.H. and O.S. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: This work was supported by COST Action FP1102 DIAROD (Determining Invasiveness and Risk of Dothistroma, http://www.cost.eu/COST_Actions/fps/Actions/FP1102?). The research was supported by the Ministry of Education and Science of Ukraine within joint Ukrainian-Lithuanian project No M/93-2018 (Biological control of forest invasive pathogens to preserve biodiversity in European noble forest and woodland ecosystems).

Conflicts of Interest: The authors declare no conflict of interest.

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