First report of *Lecanosticta acicola* on pine and non-pine hosts in Turkey

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Summary

Brown spot needle blight, caused by *Lecanosticta acicola*, is a serious disease of pines worldwide and has become of great concern in Europe over the last decade, with significantly increased outbreaks in pine forests. We examined native and non-native Pinaceae taxa (4 *Cedrus* and 24 *Pinus*) in the Atatürk Arboretum, Istanbul, Turkey for the presence of *L. acicola*.

Needles were sampled from 37 trees at least twice between March 2017 and July 2018. Symptomatic occurrence of the disease was confirmed by isolations, followed by molecular identification via sequencing of the ITS region. *Lecanosticta acicola* was isolated from symptomatic needles of 10 trees from 7 taxa (1 *Cedrus*, 6 *Pinus*). Molecular diagnostics of isolates confirmed the identification of *L. acicola* on *Cedrus libani*, *Pinus sylvestris*, *P. nigra* subsp. *nigra*, *P. nigra* subsp. *laricio*, *P. nigra* subsp. *pallasiana*, *P. nigra* subsp. *pallasiana* var. *fastigiata* and *P. nigra* subsp. *pallasiana* var. *pallasiana* f. *şeneriana* in the arboretum. This paper is the first report of *L. acicola* on *C. libani* and also the first report of the pathogen on a genus other than a *Pinus* spp.; it is also the first report of *L. acicola* occurring in Turkey. The pathogen was clearly able to cause severe damage on native Turkish *Pinus* taxa, including *P. sylvestris* and *P. nigra* subsp. *pallasiana*, and endangered endemic forms of the host in Turkey.

Keywords: Brown spot needle blight, *Mycosphaerella dearnessii*, invasive pathogen, emerging disease, *Cedrus libani*, Anatolian black pine

1. Introduction

Lecanosticta acicola (Thümen) H. Sydow, the causal agent of brown spot needle blight (BSNB) is among the most serious emerging diseases of pines globally and is of great concern in Europe where increased outbreaks have occurred in forests in Austria, Estonia and Spain (Adamson, Laas, Drenkhan, & Hanso, 2018; EPPO, 2016; Laas, Adamson & Drenkhan, 2019; Ortíz de Urbina et al., 2017). *Lecanosticta acicola* was recently redefined as a North American taxon (Van Der Nest, Wingfield, Ortiz & Barnes, 2019b) with a geographical distribution covering Central America, Colombia, USA, Canada, Asia and several European countries (EPPO, 2020;

Van Der Nest, Wingfield, Janoušek & Barnes, 2019a). An almost two-fold increase in reports of the disease have been published in the last ten years and the pathogen is continuing to spread in Europe (Mullett et al., 2018; Van Der Nest et al., 2019a).

Severe blight symptoms resembling those of BSNB caused by *L. acicola* were observed in March 2017 in the Atatürk Arboretum, Istanbul, Turkey, on needles of Anatolian black pine (*Pinus nigra* J.F. Arnold. subsp. *pallasiana* (Lamb.)), along with two endemic forms of this black pine, subspecies (var. *fastigiata* Businský and var. *pallasiana* f. *şeneriana* (Saatçioğlu) Kandemir & Mataracı) and on a Scots pine (*Pinus sylvestris* L.). In addition, needle blight symptoms, with defoliation in the lower crown of a Lebanon cedar (*Cedrus libani* A. Rich) were noticed (Figure 1).

Lecanosticta acicola has been reported from 53 *Pinus* species and hybrids in the wild and from *Picea glauca* (Moench) Voss in a field inoculation experiment (Van Der Nest et al., 2019a). There have been no reports of *L. acicola* on Pinaceae other than *Pinus* spp. under natural conditions. Geographically, Sochi (Russia), on the eastern Black Sea coast, is the closest area to Turkey where *L. acicola* is known to occur (Mullett et al., 2018). The aim of the work reported here was to identify the agent causing needle blight on *Cedrus libani* and various *Pinus* spp. and forms in the Atatürk Arboretum, Istanbul, Turkey.

2. Materials and Methods

2.1 Sampling

The Atatürk Arboretum was established in 1949 in an area of deciduous forest in the northern part of İstanbul, Turkey (41°10'30.53"N, 28°59'5.22"E). The arboretum has a substantial collection of both native and non-native angiosperm and gymnosperm trees. One of the richest collections in this arboretum is that of the Pinaceae, including native and non-native species of *Pinus* L.

Following the initial observations of BSNB-like symptoms on a number of native *Pinus* spp. and on *C. libani* (March 2017), other pines and cedars located close to the symptomatic trees, were examined for BSNB symptoms in March and July 2017 and July 2018. Needle samples were collected, mainly in July 2017 and 2018, from all examined trees. Some trees were sampled on three separate occasions, in March and July 2017, and in July 2018 (Table 1). Needles were sampled from the lower (up to 1.5 meters height) canopies except for the needles of both *P. nigra* subsp. *laricio* and *P. nigra* subsp. *nigra* which were collected from fallen branches with live green shoots since the crowns of these two hosts were out of reach. Needles were placed in sterile plastic bags, labelled, and stored at −20°C.

Severity of BSNB on individual trees was assessed in July 2017 and 2018, following the methods of Bulman, Gadgil, Kershaw and Ray (2004), using 10% increments for scoring the total crown volume with symptoms.

2.2 Morphological diagnosis and fungal isolation

Morphological diagnosis of the causal agent was through examination of symptomatic needles for the presence of conidiomata and conidia of *L. acicola* using dissecting and compound

95 microscopes. Other pathogenic fungi observed during microscopic examinations of needles were
96 also noted.
97 Isolations were made from needles after gently wiping the surface with a tissue soaked in 96%
98 ethanol. Well-developed conidiomata were excised and placed on pine needle agar medium
99 (PNA) (Drenkhan et al., 2013) in 90 mm diameter Petri dishes. Conidiomata were rolled over the
100 surface of the medium to separate individual conidia (Mullett & Barnes, 2012) and incubated at
101 room temperature in the dark for 14 days.
102
103 **2.3 Molecular identification of isolates**
104
105 Pure colonies with morphological features similar to *L. acicola* were subcultured to fresh PNA
106 and incubated in the dark at room temperature for 20 days. Approximately 0.04 g of mycelium
107 from the colony edge was transferred to 2.0 ml micro centrifuge tubes, homogenized using a
108 Retsch MM400 homogenizer (Retsch GmbH, Haan, Germany) with sterile metal beads (Ø 1.6
109 mm) and DNA extracted using the Thermo Scientific GeneJET Genomic DNA Purification Kit
110 (Lithuania) following the manufacturer's instructions.
111
112 Species-specific primer pairs developed for *L. acicola* (LAtef-F/R) targeting EFI-α genes were
113 used to identify the isolates. Conventional PCR was carried out in a TProfessional Thermocycler
114 (Biometra, Gottingen, Germany) as described in Ioos et al. (2010), with modifications
115 (Drenkhan, Adamson, Jurimaa & Hanso, 2014). PCR products were visualized on a 1 % agarose
116 gel (SeaKem® LE Agarose, Lonza) stained with ethidium bromide under UV light using a
117 Quantum ST4-system (Vilber Lourmat SAS, Marne-la-Vallee, France).

For additional confirmation of isolate identity the internal transcribed spacer (ITS) region was amplified by PCR using ITS1-F (Gardes & Bruns, 1993) and ITS4 (White et al., 1990) primers. PCR and ITS region sequencing were carried out as described by Drenkhan et al. (2014). Samples were sent for Sanger sequencing to the Estonian Biocentre in Tartu. Sequences were manually edited and aligned using BioEdit version 7.2.5.

3. Results

A total of 37 trees from 28 taxa (4 *Cedrus* and 24 *Pinus*) were examined for symptoms of BSNB during surveys conducted in March and July 2017 and 2018 in the arboretum (Table 1). Seven of the 28 taxa were native Turkish Pinaceae: *C. libani*, *P. brutia, P. nigra* subsp. *pallasiana* (including two varieties of the subspecies), *P. pinea* and *P. sylvestris* (Table 1).

Black conidiomata and conidia characteristic of *Lecanosticta acicola* were found on needles of ten trees, in microscopic investigations. Ten isolates, one isolate per tree, were obtained from *P. nigra* subsp. *nigra* (1 tree), *P. nigra* subsp. *laricio* (1 tree), *P. nigra* subsp. *pallasiana* (4 trees), *P. nigra* subsp. *pallasiana* var. *fastigiata* (1 tree), *P. nigra* subsp. *pallasiana* var. *pallasiana* f. *şeneriana* (1 tree),*P. sylvestris* (1 tree), and *Cedrus libani* (1 tree) (Table 1, Figure 1).

Lecanosticta acicola species-specific primers gave positive results for all ten isolates, confirming the identification as *L. acicola*. ITS sequences of seven isolates (one isolate per host taxon) showed 100% homology to reference strains of *L. acicola* deposited in GenBank (National

Center for Biotechnology Information, Bethesda, MD). ITS sequences obtained from these seven isolates were deposited in Genbank (Accession numbers: MK797044, MK797045, MK797046, MK797047, MT188701, MT188702, MT188703; Table 1). Fungal cultures were deposited in the Tartu Fungal Culture Collection, Estonian University of Life Sciences (culture collection numbers TFC101133, TFC101134, TFC101135, TFC101136, TFC101139, TFC101140, TFC101141, TFC101142, TFC101143, TFC101144; Table 1).

Lecanosticta acicola was not detected on any of the non-native pine and cedar species (15 *Pinus* spp., *C. atlantica*, *C. deodara*, *C. libani* var. *brevifolia*; Table 1), but was confirmed on two *P. nigra* (subsp. *nigra* and *laricio*), which are not native to Turkey. No *L. acicola* infections were detected on two native pines, *P. brutia* and *P. pinea* (Table 1). The pathogen was therefore confirmed, causing needle disease on 5 native taxa (*C. libani*, *P. nigra* subsp. *pallasiana* including two varieties of the subspecies and*P. sylvestris*), and on 2 non-native host taxa (*P. nigra* subsp. *nigra* and *P. nigra* subsp. *laricio*).

In addition to *L. acicola*, *Dothistroma* sp. and *Diplodia sapinea* were also found on sampled needles in microscopic examinations (Table 1). *Dothistroma* sp. was observed on *P. brutia*, but not on other hosts, including those infected with *L. acicola.* Conversely, *D. sapinea* was observed on most needle samples of hosts infected with *L. acicola* and also with *Dothistroma* sp. (Table 1).

Disease severity, assessed by estimating the percentage of symptomatic crown volume, ranged between 10 and 100 %. Increases of at least 10 % in the second year of assessment were

observed (Table 1). Infection severity was highest on the endemic varieties of *P. nigra* subsp. *pallasiana* (80 to 100 %) and lowest on *C. libani*. The severity of disease on *P. nigra* subsp. *nigra* and *P. nigra* subsp. *laricio* was not assessed in this work, as the trees were over 17 m in height and detailed symptoms in crown (and on needles) were not visible from ground level.

4. Discussion

This report is the first record of *L. acicola* in Turkey. It is also the first record of this pathogen affecting the native Turkish Pinaceae species *Cedrus libani* and *P. nigra* subsp. *pallasiana* (plus two varieties of this pine subspecies). The ability of *L. acicola* to cause disease on *Cedrus libani* is a new global record. Apart from *Picea glauca*, on which a trace of infection (less than 1% of needles) was found after 3 successive years of exposure to natural infection in field inoculations (Skilling and Nicholls, 1974), hitherto there were no reports of *L. acicola* on genera in the Pinaceae other than *Pinus* spp. Moreover, the known hosts for the remaining eight described species in the genus *Lecanosticta* do not include non-*Pinus* hosts (Van Der Nest et al., 2019b).

The closest location to Turkey with a verified record of *L. acicola* was on non-native pines in a Botanical Garden in Sochi (Russia) on the Black Sea coast (Mullett et al., 2018). The pathogen has also been reported from nearby Georgia (EPPO, 2020), Greece (Pantidou, 1973) and Bulgaria (Kovacevski, 1938). However, the EPPO record for Georgia cites Kizikelashvili (1987) who refer to *Dothistroma acicola* (Thüm.) A. Schischk. et N. Tzan. Shishkina & Tsanava produced a series of papers on Dothistroma needle blight disease of *Pinus brutia* subsp. *brutia* var. *pityusa* (as *Pinus pithyusa*) in Georgia in the 1960s in which they clearly describe red

187 banding associated with the disease as well as the hyaline conidia of the fungus, both typical of
188 *Dothistroma* not of *Lecanosticta*. They also directly compared material from Great Britain,
189 supplied by S. Murray who first described *Dothistroma* in Britain (Murray & Batko 1962), with
190 Georgian material and confirmed the fungus to be identical. In the last in their series of papers
191 they renamed *Dothistroma pini* as *Dothistroma acicola* due to finding the sexual stage of the
192 fungus and confusion with the sexual stage of *L. acicola*. Therefore, it is possible that the report
193 from Georgia was of *Dothistroma* sp. not *L. acicola,* a view confirmed by Gibson (1980).
194 Nonetheless, due to Georgia's proximity to both Turkey and Sochi, where *L. acicola* is now
195 known to be present, it would not be surprising if *L. acicola* occurredin Georgia. The reports
196 from Greece from the 1950s (Sarejanni et al., 1954, 1955; Gibson, 1980), however, remain
197 unconfirmed and are therefore not considered reliable (EPPO, 2020), and may well also refer to
198 *Dothistroma* sp. The original report from Bulgaria (Kovacevski, 1938) has subsequently been
199 refuted and almost certainly refers to *Dothistroma* sp. not *Lecanosticta acicola* (Mullett et al.,
200 2018; Van Der Nest et al., 2019a). However, its recent detection in a nursery, which was
201 reported to be eradicated (EPPO, 2020) requires cautious consideration for the presence of *L.*
202 *acicola* in this country.
203 In the Ataturk Arboretum, Istanbul, BSNB caused moderate (i.e. 20 to 40 % of crown infected)
204 to severe (≥ 50 % of crown infected) damage on native pines. Symptom severity on the single
205 individual of *C. libani* was lower than on pines (10 % reaching 20 % in the following year). Not
206 all *P. sylvestris* and *C. libani* individuals examined were affected by the disease.
207
208 Except for subspecies of *P. nigra*, BSNB was not found on non-native pines surveyed in this
209 work in 2017 – 2018, including *P. mugo* or *P. radiata*. In Europe, *P. mugo* appears to be the

most susceptible host of *L. acicola*, followed by *P. sylvestris* in central and northern Europe and the non-native *P. radiata* in southern Europe (Van Der Nest et al., 2019a). Reports from botanical gardens in different climatic regions across Europe also suggest the high susceptibility of *P. mugo. Lecanosticta acicola* was detected only on *P. mugo* with severe symptoms in Italy (La Porta & Capretti, 2000), in Sweden (Cleary, Laas, Oskay & Drenkhan, 2019) and in Germany (Van Der Nest et al., 2019a) and was found on both *P. mugo* and *P. sylvestris* in Ireland (Mullett et al., 2018). The disease was also reported on other non-native pines in Estonia, Latvia and Russia (Adamson, Drenkhan & Hanso, 2015; Adamson et al., 2018; Mullett et al., 2018). In the present work, however, *L. acicola* was not detected on *P. mugo* at the time of sampling, despite the Istanbul Arboretum containing numerous *P. mugo* individuals (probably of various origins and cultivars).

The first report of *L. acicola* in Estonia was on *P. ponderosa* (Drenkhan & Hanso, 2009) in a botanical garden; the pathogen was also later detected on *P. mugo* (Adamson, Drenkhan & Hanso, 2015). In Sweden, the first report of BSNB was from a single dwarf mountain pine cultivar, *P. mugo* "Hesse" (Cleary et al., 2019); the numerous other cultivars of *P. mugo* and *P. sylvestris* present in that arboretum showed no symptoms. It is likely that host species and provenances differ in response to *L. acicola* attack as reported for *Dothistroma septosporum* where both intra- and inter-specific susceptibility varies considerably (Fraser, Brown & Woodward, 2015; Fraser et al., 2016).

Both native Turkish and non-native provenances of *P. sylvestris* were present in the Istanbul arboretum, the local provenances representing the most southern populations of this pine species.

It is possible that this southern provenance of *P. sylvestris* is more susceptible to *L. acicola* than more northern provenances. For example, previous inoculation work demonstrated that Spanish and French provenances of *P. sylvestris* were highly susceptible to *L. acicola*, whereas those from Germany and Austria were less susceptible (Phelps, Kais & Nicholls, 1978; Skilling & Nicholls, 1974).

Although infection severity on the *P. sylvestris* individual was high (reaching up to 80 %), more severe *L. acicola* infections occurred on the two varieties of *P. nigra* subsp. *pallasiana* (100 % for both). Natural occurrence of these varieties of *P. nigra* subsp. *pallasiana* is limited to small populations within the Anatolian black pine forests, where both varieties are in danger of extinction. Hence, the introduction of *L. acicola* may accelerate the disappearance of these trees from their native range.

In comparison to native pines, which were severely damaged by the pathogen, the intensity of *L. acicola* infection on *C. libani* was relatively low, causing defoliation only on lower suppressed branches. The cedar tree infected with *L. acicola* was immediately adjacent to a *Pinus brutia* Ten. var. *brutia* forma *agrophiotii* (Papaj.) Kandemir & Mataraci tree, which was heavily infected with *Dothistroma* sp. *Cedrus libani* is susceptible to infection by *Dothistroma septosporum*, especially under high inoculum pressure (Mullett & Fraser, 2016). Conidia of *L. acicola* can spread only short distances, with a maximum of up to 60 m (Wyka, Munck, Brazee & Broders, 2018); for the infected specimen of *C. libani*, the nearest pines with *L. acicola* infections were approximately 50 m away. It was surprising, therefore, that this cedar tree was infected with *L. acicola* but not with *Dothistroma* sp.

Diplodia sapinea infections were found on all pine host trees infected with *L. acicola*: the co-occurrence of these pathogens will accelerate the rate of damage, possibly leading to mortality. Although not confirmed on all individuals in this work, all *P. nigra* subsp. *pallasiana* var. *fastigiata* individuals were heavily damaged, some even killed, possibly by simultaneous attacks of *L. acicola* and *D. sapinea*. Diplodia shoot blight is common and known to cause severe damage in *P. nigra*, *P. sylvestris* (plantations) and *P. brutia* forests in Turkey (Aday Kaya, Yeltekin, Lehtijarvi, Lehtijarvi & Woodward, 2019; Doğmuș-Lehtıjärvi, Lehtıjärvi, Karaca & Aday, 2007). *Cedrus libani* is also among the known hosts of *D. sapinea* (Zlatkovic et al., 2017), and the pathogen was recently detected on this species in the Atatürk Arboretum (Oskay et al., 2018), however not on the particular *C. libani* tree infected with *L. acicola*.

The work reported in this paper contributes to knowledge on the host range of *L. acicola*, including reporting the presence of the disease for the first time on a non-pine host. Clearly, the potential impact of the pathogen on all Pinaceae requires investigation. The finding that many Turkish pines, as well as Lebanon cedar, are very susceptible to *L. acicola* is of great concern for the future development of forest ecosystems and the forestry industry in Turkey. The additional co-occurrence with *Dothistroma* sp., and *Diplodia sapinea* adds to these concerns.

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References

Adamson, K., Drenkhan, R., & Hanso, M. (2015). Invasive brown spot needle blight caused by Lecanosticta acicola in Estonia. Scandinavian journal of forest research, 30(7), 587-593.

Adamson, K., Laas, M., Drenkhan, R., & Hanso, M. (2018). Quarantine pathogen Lecanosticta acicola, observed at its jump from an exotic host to the native Scots pine in Estonia. Baltic Forestry, 24 (1), 36-41.

Aday Kaya, A. G., Yeltekin, Ş., Lehtijärvi, T. D., Lehtijärvi, A., & Woodward, S. (2019). Severity of Diplodia shoot blight (caused by Diplodia sapinea) was greatest on Pinus sylvestris and Pinus nigra in a plantation containing five pine species. Phytopathologia Mediterranea, 58(2), 249-259.

Bulman, L. S., Gadgil, P. D., Kershaw, D. J., & Ray, P. D. (2004). Assessment and control of Dothistroma needle-blight. Forest Research Bulletin, 229.

Cleary, M., Laas, M., Oskay, F., & Drenkhan, R. (2019). First report of Lecanosticta acicola on non-native Pinus mugo in southern Sweden. Forest Pathology, 49(3), e12507.

Doğmuş-Lehtijärvi, H., Lehtijärvi, A., Karaca, G., & Aday, A. (2007). Sphaeropsis sapinea Dyko & Sutton associated with shoot blight on Pinus brutia Ten. in Southwestern Turkey. Acta Silvatica Lignaria Hungarica, 95-99.

Drenkhan, R., Hantula, J., Vuorinen, M., Jankovský, L., & Müller, M.M. (2013). Genetic diversity of Dothistroma septosporum in Estonia, Finland and Czech Republic. Eur. J. Plant Pathol. 136 (1), 71-85.

Drenkhan, R., Adamson, K., Jurimaa, K., & Hanso, M. (2014). Dothistroma septosporum on firs (Abies spp.) in the northern Baltics. Forest Pathology, 44(3), 250-254. doi:10.1111/efp.12110

Drenkhan, R., & Hanso, M. (2009). Recent invasion of foliage fungi of pines (Pinus spp.) to the Northern Baltics. Forestry Studies/Metsanduslikud Uurimused, 51, 49-64.

EPPO. (2016). Outbreak of Lecanosticta acicola in Salzburg, Austria. EPPO Reporting Service no. 09 - 2016, Num. article: 2016/173. doi:https://gd.eppo.int/reporting/article-5918

EPPO. (2020). EPPO Global Database (available online). https://gd.eppo.int/taxon/SCIRAC/distribution Retrieved 16.02.2020

Fraser, S., Brown, A. V., & Woodward, S. (2015). Intraspecific variation in susceptibility to dothistroma needle blight within native Scottish Pinus sylvestris. Plant Pathology, 64(4), 864-870. doi:10.1111/ppa.12320

Fraser, S., Martín-García, J., Perry, A., Kabir, M.S., Owen, T., Solla, A., Brown, A.V., Bulman, L.S., Barnes, I., Hale, M.D., Vasconcelos, M.W., Lewis, K.J., Doğmuş-Lehtijarvi, H.T., Markovskaja, S., Woodward, S. and Bradshaw, R.E. (2016), A review of Pinaceae resistance mechanisms against needle and shoot pathogens with a focus on the Dothistroma–Pinus interaction. Forest Pathology, 46: 453-471. doi:10.1111/efp.12201

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology, 2(2), 113–118. doi:10.1111/j.1365-294X.1993.tb00005.x

Gibson, I. A. S. (1980). Two pine needle fungi new to Colombia. International Journal of Pest Management, 26(1), 38-40.

Güner, A., Kandemir, A., Menemen, Y., Yıldırım, H., Aslan, S., Ekşi, G., Güner, I. ve Çimen, A.Ö. (edlr.) (2018). Resimli Türkiye Florası, Cilt 2. ANG Vakfı Nezahat Gökyiğit Botanik Bahçesi Yayınları. İstanbul.

Ioos, R., Fabre, B., Saurat, C., Fourrier, C., Frey, P., & Marcais, B. (2010). Development, Comparison, and validation of Real-Time and conventional PCR tools for the detection of the fungal pathogens causing brown spot and red band needle blights of pine. Phytopathology, 100(1), 105-114. doi:10.1094/phyto-100-1-0105

Kizikelashvili, O.G. (1987). The main fungal diseases of Pinus pityusa and measures for their control. Lesnoe Khozyaĭstvo, 12, 55-57.

Kovaćevski, J.C. (1938). Parasitic fungi new for Bulgaria – Fifth contribution. Spisanie na Zemedelskite izpitatelni instituti v Bulgariya, 8, 3–13.

La Porta, N., & Capretti, P. (2000). Mycosphaerella dearnessii, a Needle-cast Pathogen on Mountain Pine (Pinus mugo) in Italy. 84(8), 922-922. doi:10.1094/pdis.2000.84.8.922a

Laas, M., Adamson, K., & Drenkhan, R. (2019). A look into the genetic diversity of Lecanosticta acicola in northern Europe. Fungal biology, 123(10), 773-782.

Mullett, M. S., Adamson, K., Braganca, H., Bulgakov, T. S., Georgieva, M., Henriques, J., Jürisoo, L., Laas, M., Drenkhan, R. (2018). New country and regional records of the pine needle blight pathogens Lecanosticta acicola, Dothistroma septosporum and Dothistroma pini. Forest Pathology, 48(5). doi:10.1111/efp.12440

Mullett, M. S. & Barnes, I. (2012). Dothistroma isolation and molecular identification methods. 22 pp. Available online from:

https://www.forestresearch.gov.uk/documents/305/DIAROD_052012_Isolation_and_identification_97fNCCI.pdf

Mullett, M. S., & Fraser, S. (2016). Infection of Cedrus species by Dothistroma septosporum. Forest Pathology, 46(5), 551-554. doi:10.1111/efp.12214

Murray, J. S., & Batko, S. (1962). Dothistroma pini Hulbary: A new disease on pine in Britain. Forestry: An International Journal of Forest Research, 34(1), 57-65.

Ortíz de Urbina, E., Mesanza, N., Aragones, A., Raposo, R., Elvira-Recuenco, M., Boque, R., Patten, C., Aitken, J., Iturritxa, E.. (2017). Emerging needle blight diseases in Atlantic pinus ecosystems of Spain. Forests, 8(1). doi:10.3390/f8010018

Oskay, F., Lehtijärvi, A., Dogmuş-Lehtijärvi, H.T., & Woodward, S. (2018). First report of Diplodia sapinea on Cedrus libani in Turkey. New Disease Reports, 38, 13-13.

Pantidou, M. E. (1973). Fungus-host index for Greece. Kiphissia, Athens: Benaki Phytopathol. Inst.

Phelps, W. R., Kais, A. G., & Nicholls, T. H. (1978). Brown-Spot Needle Blight of Pines. Forest Insect & Disease Leaflet 44. [Broomall, PA:] U.S. Dept. of Agriculture, Forest Service, Northern Area State & Private Forestry. Retrieved from https://www.fs.usda.gov/treesearch/pubs/10866

Sarejanni, J. A., Demetriades, S.D. and Papiannou, A. J. (1954). Cataloglle commente No. 3 des champignons, bacterieres et virus rencontres sur les plantations cultivees en Grece. Annals of the Benaki Phytopathological Institute, 8, 84-89.

Sarejanni, J. A., Demetriades, S.D. and Papiannou, A. J. (1955). Rapport sommaire sur les principales maladies des plantes cultivees observee en Grece au cours de l'annde 1954. Annals of the Benaki Phytopathological Institute, 9, 3-9.

Skilling, D. D., & Nicholls, T. (1974). Brown Spot Needle Disease: Biology and Control in Scotch Pine Plantations (Vol. NC-109). St. Paul, Minnesota: North Central Forest Experiment Station, Forest Service, US Department of Agriculture.

Van Der Nest, A., Wingfield, M. J., Janoušek, J., & Barnes, I. (2019a). Lecanosticta acicola: A growing threat to expanding global pine forests and plantations. Molecular plant pathology, 20(10), 1327-1364.

Van Der Nest, A., Wingfield, M. J., Ortiz, P. C., & Barnes, I. (2019b). Biodiversity of Lecanosticta pine-needle blight pathogens suggests a Mesoamerican Centre of origin. IMA Fungus, 1(1), 2.

White, T. J., Bruns, T. D., Lee, S., & Taylor, J. (1990). PCR protocols: a guide to methods and applications: part three – genetics and evolution. London: Academic press. Chapter 38, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics; p. 315–322.

Wyka, S. A., Munck, I. A., Brazee, N. J., & Broders, K. D. (2018). Response of eastern white pine and associated foliar, blister rust, canker and root rot pathogens to climate change. Forest Ecology and Management, 423, 18-26. doi:10.1016/j.foreco.2018.03.011

Zlatkovic, M., Keca, N., Wingfield, M. J., Jami, F., & Slippers, B. (2017). New and unexpected host associations for Diplodia sapinea in the Western Balkans. Forest Pathology, 47(3), e12328. doi:ARTN e12328

Table and figure captions

Table 1. Trees monitored for the presence of brown spot needle blight (BSNB) in the Atatürk Arboretum, Turkey between March-2017 and July 2018 (host taxa on which *L. acicola* infection was confirmed are shown in bold, *L. acicola* isolates sequences submitted to GenBank are shown in bold)

Figure 1. Damage caused by *Lecanosticta acicola* on *Pinus nigra* subsp. *pallasiana* var. *fastigiata* (A) and *P. nigra* subsp. *pallasiana* var. *pallasiana* forma *şeneriana* (E), *Pinus sylvestris* (H) and *Cedrus libani* individual (Yellow arrow) infected with *Lecanosticta acicola* located next to *Pinus brutia* var. *brutia* forma *agrophiotii* severely infected with *Dothistroma* sp. (Red arrow) (K). Symptoms and conidiomata of *Lecanosticta acicola* on needles of *Pinus nigra* subsp. *pallasiana* var. *fastigiata* (B, C, D), *P. nigra* subsp. *pallasiana* var. *pallasiana* forma *şeneriana* (F-G), *Pinus sylvestris* (I, J) and *Cedrus libani* (L-M) and conidia of *L. acicola* from mature conidiomata on needles of *C. libani* (N) and *Pinus* nigra subsp. *laricio* (O) at 600x magnification

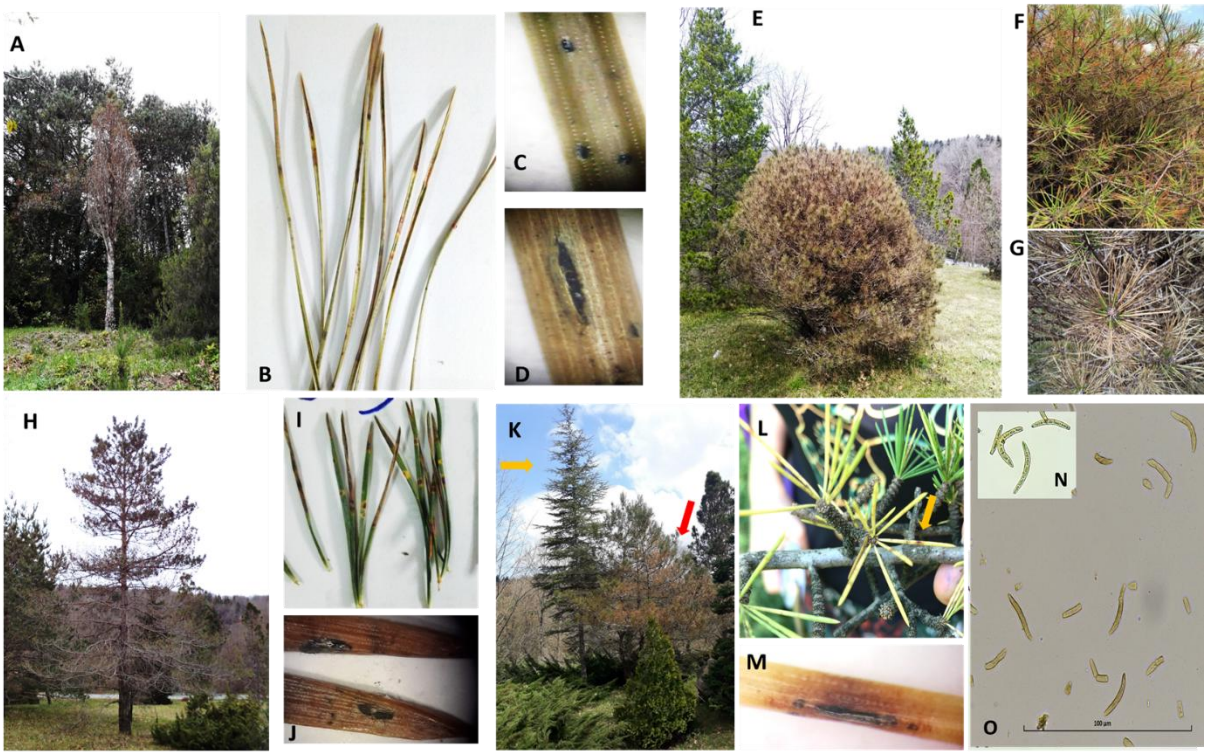

Figure 1. Damage caused by Lecanosticta *acicola* on *Pinus nigra* subsp. *pallasiana* var. *fastigiata* (A) and *P. nigra* subsp. *pallasiana* var. *pallasiana* forma ***şeneriana*** (E), *Pinus sylvestris* (H) and *Cedrus libani* individual (Yellow arrow) infected with *Lecanosticta acicola* located next to *Pinus brutia* var. *brutia* forma *agrophiotii* severely infected with *Dothistroma* sp. (Red arrow) (K). Symptoms and conidiomata of *Lecanosticta acicola* on needles of *Pinus nigra* subsp. *pallasiana* var. *fastigiata* (B, C, D), *P. nigra* subsp. *pallasiana* var. *pallasiana* forma ***şeneriana*** (F-G), *Pinus sylvestris* (I, J) and *Cedrus libani* (L-M) and conidia of *L. acicola* from mature conidiomata on needles of *C. libani* (N) and *Pinus* nigra subsp. *laricio* (O) at 600x magnification .