

## Pathogen profile

## ***Lecanosticta acicola*: A growing threat to expanding global pine forests and plantations**

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### SUMMARY

*Lecanosticta acicola* causes brown spot needle blight (BSNB) of *Pinus* species. The pathogen occurs mostly in the Northern Hemisphere but has also been reported in Central America and Colombia. BSNB can lead to stunted growth and tree mortality, and has resulted in severe damage to pine plantations in the past. There have been increasingly frequent new reports of this pathogen in Europe and in North America during the course of the past 10 years. This is despite the fact that quarantine practices and eradication protocols are in place to prevent its spread.

**Taxonomy:** Kingdom Fungi; Phylum Ascomycota; Subphylum Pezizomycotina; Class Dothideomycetes; Subclass Dothideomycetidae; Order Capniadales; Family Mycosphaerellaceae; Genus *Lecanosticta*.

**Host range and distribution:** *Lecanosticta* spp. occur on various *Pinus* species and are found in North America, Central America, South America (Colombia), Europe as well as Asia.

**Disease symptoms:** Small yellow irregular spots appear on the infected pine needles that become brown over time. They can be surrounded by a yellow halo. These characteristic brown spots develop to form narrow brown bands that result in needle death from the tips down to the point of infection. Needles are prematurely shed, leaving bare branches with tufts of new needles at the branch tips. Infection is usually most severe in the lower parts of the trees and progresses upwards into the canopies.

**Useful websites:** The EPPO global database providing information on *L. acicola* (<https://gd.eppo.int/taxon/SCIRAC>)  
Reference genome of *L. acicola* available on GenBank (<https://www.ncbi.nlm.nih.gov/genome/?term=Lecanosticta+acicola>)  
JGI Gold Genome database information sheet of *L. acicola* sequenced genome (<https://gold.jgi.doe.gov/organism?xml:id=Go0047147>)

**Keywords:** brown spot needle blight, *Lecanosticta acicola*, *Lecanosticta* species, *Mycosphaerella dearnessii*, pine pathogen, *Pinus* spp.

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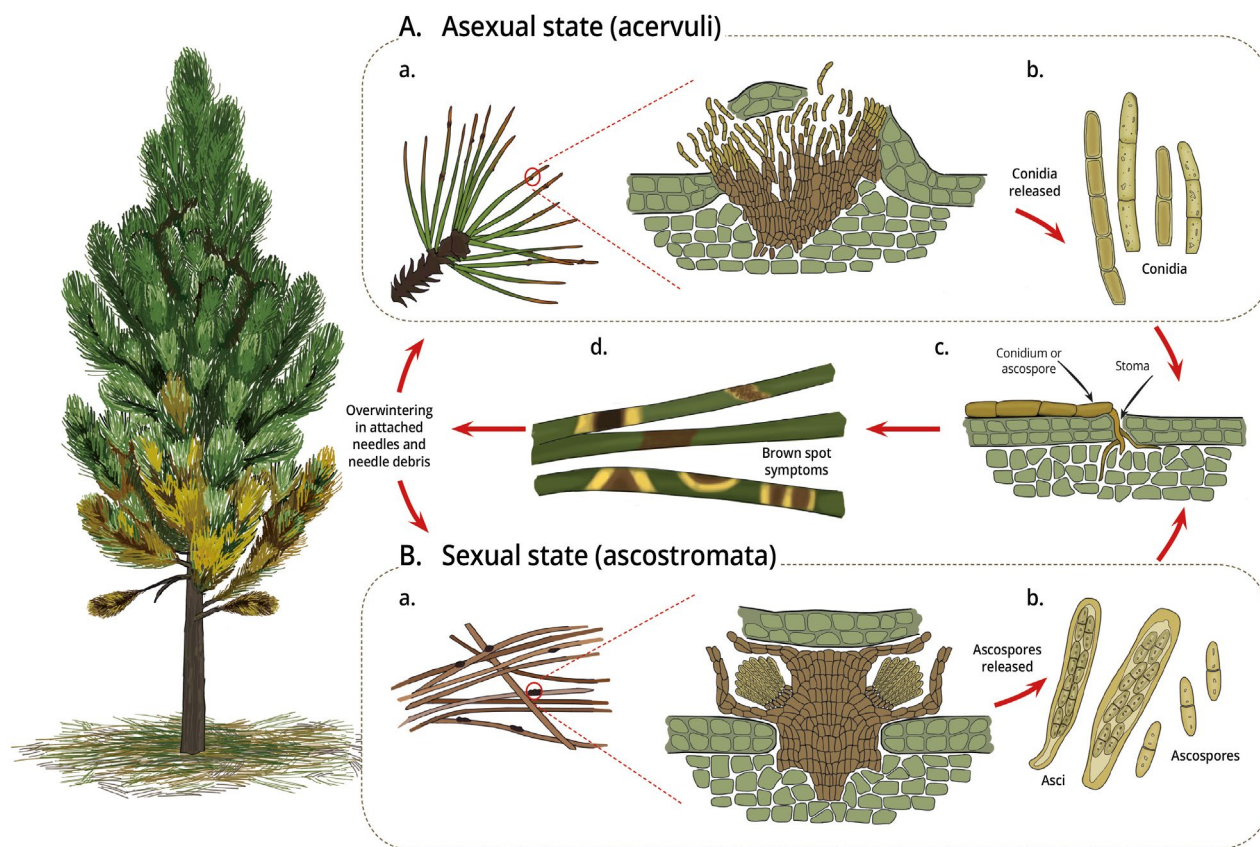
### INTRODUCTION

*Lecanosticta acicola* is an ascomycete fungus that causes a disease of *Pinus* spp. known as brown spot needle blight (BSNB). The pathogen was first described by de Thümen (1878) and it owes its notoriety to a disease problem that arose in the southeastern USA on *Pinus palustris*, better known as long leaf pine in that area (Siggers, 1932). This tree species, which is highly susceptible to infection, is peculiar in having a so-called 'grass' stage during the first five years of its growth. This mass of young needles provides a favourable environment for infection to occur.

The BSNB pathogen completes its life cycle (Fig. 1) on pine needles that are shed prematurely. This leads to reduced or stunted growth that can result in significant yield losses (Wakeley, 1970) or tree death. In some cases, pine plantations have been sufficiently damaged that they have needed to be cleared (Huang *et al.*, 1995; Lévy, 1996; Markovskaja *et al.*, 2011).

*Lecanosticta acicola* has been recorded on 53 different *Pinus* species and hybrids in native and non-native pine stands in the USA, Canada, several European countries and Asia as well as in Central America and Colombia (Table 1). Due to the severity of the disease, the pathogen has been afforded an A1 quarantine status in Africa, Argentina, Chile, Uruguay, Bahrain, Kazakhstan, Ukraine and Russia, and A2 quarantine status in Europe (<https://gd.eppo.int/taxon/SCIRAC/categorization>). However, reports of new outbreaks of the disease in various European countries have increased significantly since 2008 (Adamson *et al.*, 2015, 2018; Anonymous, 2012; Cleary *et al.*, 2019; Hintsteiner *et al.*, 2012; Jankovský *et al.*, 2009a; Markovskaja *et al.*, 2011; Mullett *et al.*, 2018; Ortíz de Urbina *et al.*, 2017).

Quarantine measures rely on accurately identifying the presence of pathogens on symptomatic tissues. This is complicated in the case of *L. acicola* where the symptoms of BSNB closely resemble those of *Dothistroma* needle blight (DNB). DNB is caused by two species: *Dothistroma septosporum* and *D. pini* (Barnes *et al.*, 2016). Due to their similar symptoms,



**Fig. 1** Life cycle of *Lecanosticta acicola* on *Pinus* spp. (A) Asexual state: acervuli (a) develop on attached needles and needle debris and release conidia (b). Infection occurs through the stomata of new season needles (c), resulting in brown spot symptoms (d). (B) Sexual state: ascostromata develop on dead needles associated with previous season infections (a) and release ascospores in spring (b). Infection occurs through the stomata of new season needles (c), resulting in brown spot symptoms (d).

field diagnoses of the causal agent based on symptoms and/or on morphology alone have commonly been incorrect (Shishkina and Tsanova, 1967; Siggers, 1944; Thyr and Shaw, 1964). Consequently, past reports of *L. acicola* based only on morphological descriptions and symptoms must be treated with caution and verified using molecular identification techniques (van der Nest *et al.*, 2019).

*Lecanosticta acicola* has been well-known in the southeastern USA since the early 1900s, but is rapidly spreading in northern parts of the USA, Canada and in some parts of Europe (Broders *et al.*, 2015). Its complete host range is not known but appears to be expanding (Mullett *et al.*, 2018). A recent taxonomic re-evaluation of isolates previously identified as *L. acicola*, applying phylogenetic analyses based on DNA sequences, has led to various isolates being recognized as distinct species (Quaedvlieg *et al.*, 2012; van der Nest *et al.*, 2019). This and a number of recent publications (Adamson *et al.*, 2018; Cleary *et al.*, 2019; Mullett *et al.*, 2018; Ondrušková *et al.*, 2018; Ortíz de Urbina *et al.*, 2017; Sadiković *et al.*, 2019; Schneider *et al.*, 2019; Wyka *et al.*, 2017)

justifies the need for a review of current knowledge regarding BSNB and the *Lecanosticta* species that cause this disease. This is the first review of the topic to be presented in 75 years subsequent to that of Siggers (1944).

## LECANOSTICTA SPECIES

The genus *Lecanosticta*, which includes nine species with the type species being *L. acicola* (previously known as *Mycosphaerella dearnessii*, Table 2), is characterized by stomata and septate, pigmented conidia. The genus was erected by Sydow and Petrak in 1922 (Sydow and Petrak, 1922). The taxonomic history and nomenclature of *Lecanosticta acicola* has been succinctly presented previously (Evans, 1984; Siggers, 1944) and is summarized and updated in Table 2.

*Lecanosticta acicola* is the oldest known species in the genus and owes its notoriety to the disease of long leaf pine, which it was first associated with, in the southeastern USA (Chapman, 1926; Hedgcock, 1929). Although the pathogen was identified in

**Table 1** Host and geographical range of *Lecanosticta* species.

| Country, region, locality                        | Year collected | Host  | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods                            | Report references                      |
|--|----------------|---|---|---|--|--|
| <i>Lecanosticta acicola</i>                      |                |   |   |   |  |  |
| Austria, Lower Austria, Valley of the river Ybbs | 1996–2000      | <i>P. mugo</i> , <i>P. sylvestris</i>                                     | Morphological identifications of the pathogen were performed.                     |   | Infected trees were eradicated after which the disease was no longer detected (2001–2002). | Brandstetter and Cech (2003)           |
| Austria, Lower Austria, Hollenstein/Ybbs         | 2008–2009      | <i>P. sylvestris</i>  | The pathogen was recognized during a forest survey.                               |   |  | Cech and Krehan (2008), Kessler (2009) |
| Austria, Lower Austria, Hollenstein/Ybbs         | 2009–2010      | <i>P. mugo</i> subsp. <i>mugo</i> , <i>P. mugo</i> subsp. <i>uncinata</i> | Symptoms were observed in a survey.   |   |  | Kessler and Krehan (2011)              |
| Austria, Lower Austria                           | 1996           | <i>P. mugo</i>  | Fruiting bodies were observed on pine needles.                                    |   | Isolated occurrence in a garden.   | Cech (1997)                            |
| Austria, Lower Austria, Hollenstein/Ybbs         | 1998           | <i>Pinus</i> sp.  | Symptoms were observed in the field.  |   |  | Brandstetter and Cech (1999)           |
| Austria, Lower Austria                           | 2004           | <i>P. mugo</i> , <i>P. sylvestris</i>                                     | <i>TEF 1</i> sequencing used for identification, both mating types were detected. | *   |  | Janoušek <i>et al.</i> (2016)          |
| Austria, Lower Austria                           | 2010           | <i>P. mugo</i>  | <i>TEF 1</i> sequencing used for identification, both mating types were detected. | *   |  | Janoušek <i>et al.</i> (2016)          |
| Austria, Upper Austria                           | 2010           | <i>P. mugo</i>  | <i>TEF 1</i> sequencing used for identification. Mating type 1 was detected.      | *   |  | Janoušek <i>et al.</i> (2016)          |
| Austria, Upper Austria, Bregenz (Vorarlberg)     | 2011           | <i>P. mugo</i> subsp. <i>mugo</i>   | Symptoms were observed in a survey.   |   |  | Kessler and Krehan (2011)              |
| Austria, Upper Austria, Gmunden                  | 2011           | <i>P. nigra</i> var. <i>nigra</i> , <i>P. mugo</i> subsp. <i>mugo</i>     | ITS sequencing used for identification.   | *   |  | Hintsteiner <i>et al.</i> (2012)       |
| Austria, Upper Austria, Tyrol                    | 2011           | <i>P. mugo</i> subsp. <i>uncinata</i>                                     | Symptoms were observed in a survey.   |   |  | Kessler and Krehan (2011)              |

**Table 1** (Continued)

| Country, region, locality                 | Year collected | Host   | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods   | Report references   |
|---|----------------|--|---|---|---|---|
| Austria, Upper Austria                    | 2012           | <i>P. nigra</i>  | <i>TEF 1</i> sequencing used for identification. Mating type 2 was detected.  | *   |   | Janoušek <i>et al.</i> (2016)                                   |
| Austria, Upper Austria, Tyrol             | 2015           | <i>P. mugo</i> subsp. <i>mugo</i> , <i>P. mugo</i> subsp. <i>uncinata</i> , <i>P. sylvestris</i> | The pathogen was detected during a forest survey and confirmed with laboratory tests (method not specified).  | *   | Detected in area covering more than 60 ha of forest.  | EPPO (2015)   |
| Austria, Graz                             | 2016           | <i>P. mugo</i>   | Infected needles were collected by I. Barnes. Isolations were made by I. Barnes and A. van der Nest, and identified by ITS sequencing. Mating type 2 was detected.  | *   | Trees heavily infected (see Fig. 2A,B).   | I. Barnes, FABI, Pretoria, South Africa, personal communication |
| Austria, Lower Austria                    | 2016           | <i>P. mugo</i>   | Infected needles were collected by T. Cech. Isolations were made by I. Barnes and A. van der Nest, and identified by ITS sequencing. Mating type 2 was detected.  | *   |   | I. Barnes, FABI, Pretoria, South Africa, personal communication |
| Austria, Salzburg                         | 2016           | <i>P. uncinata</i>   | Infected needles were collected by T. Cech. Isolations were made by I. Barnes and A. van der Nest, and identified by ITS sequencing. Mating type 2 was detected.  | *   |   | I. Barnes, FABI, Pretoria, South Africa, personal communication |
| Austria, Upper Austria                    | 2016           | <i>P. mugo</i>   | Infected needles were collected by T. Cech. Isolations were made by I. Barnes and A. van der Nest, and identified by ITS sequencing. Mating type 1 was detected.  | *   |   | I. Barnes, FABI, Pretoria, South Africa, personal communication |
| Belize                                    | 1981           | <i>P. caribaea</i> , <i>P. oocarpa</i>   | Morphological identifications were made. Confirmation is needed as molecular identification did not reveal <i>L. acicola</i> in Central America (van der Nest <i>et al.</i> , 2019).                      |   |   | Evans (1984)  |
| Bulgaria, near Sofia                      | 1938           | <i>P. nigra</i>  | The pathogen was identified based on morphological characteristics. However, the conidial descriptions are not typical of <i>L. acicola</i> and therefore this record is doubtful and should be verified. |   |   | Kovačevski (1938)   |
| Canada, Manitoba                          | 1965           | <i>P. banksiana</i> , <i>P. contorta</i> var. <i>latifolia</i>                                   | Symptoms were observed in the field and the presence of the pathogen was confirmed with morphological identifications.  |   | 50–90% of <i>P. contorta</i> var. <i>latifolia</i> was infected, 20% of <i>P. banksiana</i> was infected. | Laut <i>et al.</i> (1966)                                       |
| Canada, New Brunswick, Quebec and Ontario | 2009           | <i>P. strobus</i>  | <i>L. acicola</i> was reported to occur with <i>Canavergella banfieldii</i> on all trees sampled and confirmed based on morphological characteristics.  |   |   | Laflamme <i>et al.</i> (2010)                                   |

Table 1 (Continued)

| Country, region, locality   | Year collected | Host  | Identification method and additional notes   | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods  | Report references   |
|---|----------------|---|--|---|--|---|
| Canada, Quebec  | 2011           | <i>P. strobus</i> , <i>P. mugo</i>  | <i>TEF 1</i> sequencing used for identification, both mating types detected.   | *   |  | Janoušek <i>et al.</i> (2016)                             |
| China, Jiangsu  | 1958           | <i>P. thunbergii</i>  | Identification method not specified.   |   | Insignificant damage was reported.   | Ye and Wu (2011)  |
| China, Fujian province  | 1982–1985      | <i>P. elliottii</i>   | Morphological identifications of the pathogen.   |   |  | Li <i>et al.</i> (1987)                                   |
| China, Anhui, Fujian, Guangdong, Guangxi, Jiangsu, Jiangxi and Zhejiang provinces | 1986           | <i>P. caribaea</i> , <i>P. clausa</i> , <i>P. echinata</i> , <i>P. elliottii</i> , <i>P. palustris</i> , <i>P. taeda</i> , <i>P. thunbergii</i> | Morphological characteristics were used to identify the pathogen.  |   | <i>P. elliottii</i> , <i>P. taeda</i> and <i>P. thunbergii</i> were severely damaged. <i>P. caribaea</i> , <i>P. clausa</i> , <i>P. echinata</i> and <i>P. palustris</i> were reported as susceptible. | Li <i>et al.</i> (1986), Ye and Wu (2011)                 |
| China, Fujie  | 1988           | <i>P. elliottii</i>   | Morphological characteristics and RAPD analysis were used to identify the pathogen. <i>TEF 1</i> sequencing was further used for identification and mating type 2 was detected by Janoušek <i>et al.</i> (2016). | *   |  | Huang <i>et al.</i> (1995), Janoušek <i>et al.</i> (2016) |
| China, Zhejiang   | 1991           | <i>P. thunbergii</i>  | Morphological characteristics and RAPD analysis were used to identify the pathogen.  | *   |  | Huang <i>et al.</i> (1995)                                |
| China, Jiangxi  | 1992           | <i>P. elliottii</i> , <i>P. thunbergii</i>  | Morphological characteristics and RAPD analysis were used to identify the pathogen.  | *   |  | Huang <i>et al.</i> (1995)                                |
| China, Guanxi   | 1992           | <i>P. caribaea</i> , <i>P. elliottii</i>  | Morphological characteristics and RAPD analysis were used to identify the pathogen.  | *   |  | Huang <i>et al.</i> (1995)                                |
| Colombia, Piedras Blancas and Pereira   | 1978           | <i>P. radiata</i> , <i>P. elliottii</i> , <i>P. patula</i>  | Identification method not specified.   |   | <i>P. radiata</i> severely defoliated but on <i>P. elliottii</i> and <i>P. patula</i> the pathogen was isolated from cast needles found underneath healthy trees.                                      | Gibson (1980)   |
| Colombia, Albán   | 1981           | <i>P. radiata</i>   | Morphological identification, sexual and asexual state were identified.  |   | Plantations were severely defoliated.  | Evans (1984)  |

**Table 1** (Continued)

| Country, region, locality                                   | Year collected | Host  | Identification method and additional notes   | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods  | Report references                   |
|---|----------------|---|--|---|--|-------------------------------------|
| Colombia, Refocosta   | 2011           | <i>P. caribaea</i>  | Infected needles were collected by C.A. Rodas. Isolations were made by I. Barnes. <i>TEF 1</i> sequencing was used for identification and mating type 2 was detected by Janoušek <i>et al.</i> (2016). | *   |  | Janoušek <i>et al.</i> (2016)       |
| Costa Rica, Alajuela  | 1980           | <i>P. oocarpa</i>   | Morphological identification of pathogen.  |   |  | Evans (1984)                        |
| Croatia, Dalmatia   | 1975           | <i>P. halepensis</i>  | Morphological identification of <i>L. acicola</i> .  |   | The pathogen is not as aggressive as in the USA on this host and it seems to only be aggressive where dense canopies are present with high air humidity. Copper fungicides were applied. | Milatović (1976)                    |
| Croatia, Zadar  | Not specified  | <i>P. halepensis</i>  | Forest surveys were conducted. It is not specified in the English abstract whether morphological identifications were performed.   |   | 500 ha of <i>P. halepensis</i> was heavily infected with the pathogen. Highly infected trees and lower infected branches were cut down and it is reported that the trees recovered.      | Glavaš and Margaletić (2001)        |
| Croatia, Zadar  | 2009           | <i>P. halepensis</i>  | <i>TEF 1</i> sequencing was used for identification. Mating type 2 was detected.   | *   |  | Janoušek <i>et al.</i> (2016)       |
| Croatia, Kožino   | 2015           | <i>P. halepensis</i>  | <i>TEF 1</i> sequencing was used for identification. Mating type 2 was detected.   | *   |  | Sadiković <i>et al.</i> (2019)      |
| Cuba, Baracoa, Guantánamo, Plateau of Mayarí and Master Saw | 1980–1998      | <i>P. caribaea</i> , <i>P. cubensis</i> , <i>P. maestrensis</i> | Symptom identification and morphological confirmation of the fungus.   |   | Mostly seedlings in nurseries were infected.   | Lopéz Castilla <i>et al.</i> (2002) |

Table 1 (Continued)

| Country, region, locality  | Year collected | Host   | Identification method and additional notes   | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods   | Report references  |
|--|----------------|--|--|---|---|--|
| Czech Republic, Southern Bohemia, Červené Blato Nature Reserve                       | 2007           | <i>P. uncinata</i> subsp. <i>uliginosa</i>   | Morphological identifications were conducted as well as sequencing of the ITS region. The identity of the pathogen was again confirmed with <i>TEF 1</i> sequencing by Janoušek <i>et al.</i> (2016). Both mating types were detected.   | *   | Heavy defoliation was reported in 2007. No control measures were taken as the incidence was reported in a natural nature reserve. | Jankovský <i>et al.</i> (2009b), Janoušek <i>et al.</i> (2016) |
| Czech Republic, Southern Bohemia, Soběslav, Borkovická Blata National Nature Reserve | 2008           | <i>P. uncinata</i> subsp. <i>uliginosa</i>   | Morphological identifications were conducted as well as sequencing of the ITS region. The identity of the pathogen was again confirmed with <i>TEF 1</i> sequencing by Janoušek <i>et al.</i> (2016). Both mating types were detected.   | *   | No action was taken as the outbreak was in a natural reserve.   | Jankovský <i>et al.</i> (2009a), Janoušek <i>et al.</i> (2016) |
| Estonia, Hiiumaa Island and Käravere   | 2014–2015      | <i>P. mugo</i>   | Symptom identification was confirmed with conventional PCR directly from pine needles. <i>Lecanosticta acicola</i> was isolated from the needles and confirmed with ITS sequencing. Both mating types were detected.   | *   |   | Adamson <i>et al.</i> (2015)                                   |
| Estonia, Tallinn Botanical Garden  | 2006–2008      | <i>P. ponderosa</i>  | Material of <i>Dothistroma</i> was collected and isolated but in culture it was determined to be <i>L. acicola</i> based on culture morphology. The <i>TEF 1</i> sequences were later determined for representative isolates and mating type 2 was detected (Janoušek <i>et al.</i> , 2016). | *   |   | Drenkhan and Hanso (2009), Janoušek <i>et al.</i> (2016)       |
| Estonia, Tallinn Botanical Garden  | 2010–2013      | <i>P. mugo</i> , <i>P. mugo</i> var. <i>pumilio</i> , <i>P. ponderosa</i> , <i>P. uncinata</i> | Symptom identification was confirmed with conventional PCR directly from pine needles. <i>Lecanosticta acicola</i> was isolated from the needles and confirmed with ITS sequencing. Mating type 1 was detected.  | *   |   | Adamson <i>et al.</i> (2015)                                   |
| Estonia, Tartu county  | 2016           | <i>P. sylvestris</i> , <i>P. mugo</i> , <i>Pinus</i> × <i>rhaetica</i>                         | Visual symptom identification was confirmed with conventional PCR and selected isolates were identified using an ITS sequencing PCR. Both mating types were detected.  | *   |   | Adamson <i>et al.</i> (2018)                                   |
| Estonia, Tori and Vasula   | 2012, 2013     | <i>P. mugo</i>   | Symptom identification was confirmed with conventional PCR directly from pine needles. <i>Lecanosticta acicola</i> was isolated from the needles and confirmed with ITS sequencing.  | *   |   | Adamson <i>et al.</i> (2015)                                   |

**Table 1** (Continued)

| Country, region, locality                          | Year collected         | Host                                    | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods  | Report references   |
|--|------------------------|---|---|---|--|---|
| France, South-West, Aquitaine and western Pyrénées | 1993                   | <i>P. attenuata</i> × <i>P. radiata</i> | In field observations were made.  |   | Severe tree mortality was observed. French authorities implemented eradication measures and destroyed 127 ha of trees. | Lévy (1996)   |
| France, Gironde                                    | 1995                   | <i>P. muricata</i>                      | <i>TEF 1</i> and <i>BT 2</i> sequencing used for identification, mating type 1 detected.  | *   |  | Ioos <i>et al.</i> (2010), Janoušek <i>et al.</i> (2016)        |
| France, Landes                                     | 1995                   | <i>P. attenuata</i> × <i>P. radiata</i> | <i>TEF 1</i> and <i>BT 2</i> sequencing used for identification, mating type 2 detected.  | *   |  | Ioos <i>et al.</i> (2010), Janoušek <i>et al.</i> (2016)        |
| France, Pyrénées-Atlantiques                       | 1995                   | <i>P. radiata</i>                       | <i>TEF 1</i> and <i>BT 2</i> sequencing used for identification.  | *   |  | Ioos <i>et al.</i> (2010)                                       |
| France, Ariège                                     | 2009                   | <i>P. sylvestris</i>                    | Forest surveys were conducted.  |   | More than 50% of the trees were affected.  | Alvère <i>et al.</i> (2010)                                     |
| France, Tarn-et-Garonne                            | 2009                   | <i>P. nigra</i> var. <i>laricio</i>     | Forest surveys were conducted.  |   | The trees were moderately affected.  | Alvère <i>et al.</i> (2010)                                     |
| France, Pyrénées-Atlantiques                       | 2012                   | <i>P. radiata</i>                       | <i>TEF 1</i> sequencing used for identification, both mating types were detected.   | *   |  | Janoušek <i>et al.</i> (2016)                                   |
| Germany, Bavaria                                   | 1994                   | <i>P. mugo</i>                          | The pathogen was identified based on morphological characteristics.   |   |  | Pehl (1995)   |
| Germany, Bavaria                                   | 1994, 2000, 2010, 2011 | <i>P. mugo</i>                          | <i>TEF 1</i> sequencing used for identification, both mating types were detected.   | *   |  | Janoušek <i>et al.</i> (2016)                                   |
| Germany, Bavaria, Munich Botanical gardens         | 2018                   | <i>P. mugo</i>                          | Collected by I. Barnes. The identity was confirmed by ITS sequencing. <i>Dothistroma septosporum</i> was also present.  | *   |  | I. Barnes, FABI, Pretoria, South Africa, personal communication |
| Guatemala, El Progreso                             | 1983                   | <i>P. oocarpa</i>                       | Morphological identification methods were used. As <i>L. acicola</i> was not identified in Central America using molecular identification techniques (van der Nest <i>et al.</i> , 2019), this report will need to be verified. |   |  | Evans (1984)  |



Table 1 (Continued)

| Country, region, locality          | Year collected | Host   | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods  | Report references             |
|------------------------------------|----------------|--|---|---|--|-------------------------------|
| Honduras                           | 1980–1983      | <i>P. caribaea</i> , <i>P. maximinoi</i> , <i>P. oocarpa</i> , <i>P. tecunumanii</i> , | Morphological identification methods were used. As <i>L. acicola</i> was not identified in Central America using molecular identification techniques (van der Nest <i>et al.</i> , 2019), this report will need to be verified. |   |  | Evans (1984)                  |
| Ireland, Wexford county            | 2016           | <i>P. mugo</i> , <i>P. sylvestris</i>  | ITS sequencing was used for identification purposes. Mating type 1 was detected.  | *   |  | Mullett <i>et al.</i> (2018)  |
| Italy, Brescia                     | 1997           | <i>P. mugo</i>   | Symptoms were noted in the botanical garden and the presence of the pathogen was confirmed with morphological identifications.  |   | Extensive necrosis and crown defoliation were observed in all 12 of the <i>P. mugo</i> trees present in the botanical garden.  | La Porta and Capretti (2000)  |
| Italy, Brescia                     | 2008           | <i>P. mugo</i>   | <i>TEF 1</i> sequencing used for identification and mating type 1 detected.   | *   |  | Janoušek <i>et al.</i> (2016) |
| Japan, Shimane Prefecture (Honshu) | 1996           | <i>P. thunbergii</i> , <i>P. densiflora</i> (tested in controlled environment)         | The pathogen was morphologically identified.  |   | <i>P. thunbergii</i> was severely infected. Inoculation trials on this host as well as <i>P. densiflora</i> also revealed that <i>P. densiflora</i> is susceptible although it was not reported in the host's natural environment. | Suto and Ougi (1998)          |
| Japan, Shimane                     | 2010           | <i>P. thunbergii</i>   | <i>TEF 1</i> sequencing used for identification, mating type 2 was detected.  | *   |  | Janoušek <i>et al.</i> (2016) |
| Latvia, Salaspils                  | 2012           | <i>P. pumila</i>   | Morphological identification. Later it was confirmed with PCR-based methods.  | *   | Eradication measures were taken.   | EPPO (2012a)                  |
| Latvia, Salaspils                  | 2016           | <i>P. mugo</i>   | Identification was done by ITS sequencing. Mating type 1 was detected.  | *   |  | Mullett <i>et al.</i> (2018)  |

**Table 1** (Continued)

| Country, region, locality   | Year collected | Host   | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods            | Report references   |
|---|----------------|--|---|---|--|---|
| Lithuania, Curonian Spit, Smiltynė Forest District                                | 2009           | <i>P. mugo</i>   | Morphological characteristics as well as ITS sequencing and ITS-RFLP was used to identify the pathogen. This material was again examined by Janoušek <i>et al.</i> (2016) and the identity confirmed with <i>TEF 1</i> . Mating type 1 was detected.                            | *   | A monitoring programme was initiated and infected trees felled and burned. | Markovskaja <i>et al.</i> (2011), Janoušek <i>et al.</i> (2016) |
| Lithuania, Curonian Spit, Smiltynė Forest District and Juodkrantė Forest District | 2010           | <i>P. mugo</i>   | Morphological characteristics as well as ITS sequencing and ITS-RFLP was used to identify the pathogen.   | *   | A monitoring programme was initiated and infected trees felled and burned. | Markovskaja <i>et al.</i> (2011)                                |
| Lithuania, Curonian Spit, near Juodkrante   | 2012           | <i>P. mugo</i> , <i>P. sylvestris</i>  | Morphological identifications and PCR-based methods.  | *   | Phytosanitary methods were implemented.                                    | EPPO (2012b)  |
| Lithuania, Curonian Spit, Smiltynė Forest District and Juodkrantė Forest District | 2014           | <i>P. mugo</i>   | Infected needles were collected by S. Markovskaja. Isolations were made by A. van der Nest. A multigene phylogenetic approach was used to determine the identity of the isolates.   | *   |  | van der Nest <i>et al.</i> (2019)                               |
| Mexico, Puebla  | 1983           | <i>P. patula</i>   | Morphological identification.   |   |  | Evans (1984)  |
| Mexico  | 2000           | <i>P. ayacahuite</i> , <i>P. cembroides</i> , <i>P. halepensis</i>                   | Morphological characteristics were examined.  |   | High disease severity was reported on <i>P. halepensis</i> .               | Marmolejo (2000)  |
| Mexico, Nuevo León  | 2010, 2011     | <i>P. halepensis</i>   | <i>TEF 1</i> sequencing used for identification, both mating types detected. KJ938447–KJ938449 were later identified as <i>L. variabilis</i> (van der Nest <i>et al.</i> , 2019) and the remaining isolates are part of <i>L. acicola</i> lineage 3.                            | *   |  | Janoušek <i>et al.</i> (2016)                                   |
| Nicaragua   | 1981–1983      | <i>P. caribaea</i> , <i>P. maximinoi</i> , <i>P. oocarpa</i> , <i>P. tecunumanii</i> | Morphological identification methods were used. As <i>L. acicola</i> was not identified in Central America using molecular identification techniques (van der Nest <i>et al.</i> , 2019), this report will need to be verified. Both the sexual and asexual state was observed. |   |  | Evans (1984)  |
| Portugal, Minho   | 2016           | <i>P. radiata</i>  | Identification was done by ITS sequencing. Mating type 1 was detected.  | *   |  | Mullett <i>et al.</i> (2018)                                    |

Table 1 (Continued)

| Country, region, locality              | Year collected | Host   | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods                | Report references   |
|--|----------------|--|---|---|--|---|
| Romania, Vrancea                       | 2017           | <i>Pinus</i> sp.   | The pathogen was detected during a forest survey in a 30-year-old plantation.   |   | Eradication reported to be under way in the 19-hectare forest.                 | EPPO (2018)   |
| Russia, Krasnodar region, Sochi        | 2016           | <i>P. mugo</i> subsp. <i>mugo</i> , <i>P. thunbergii</i> | Identification was done by ITS sequencing. Mating type 2 was detected.  | *   |  | Mullett <i>et al.</i> (2018)  |
| Slovenia, Bled                         | 2008–2009      | <i>P. mugo</i> , <i>P. sylvestris</i>                    | Morphological identifications. The identity of isolates on <i>P. mugo</i> were confirmed with <i>TEF 1</i> sequencing and mating type 2 was detected (Janoušek <i>et al.</i> , 2016; Sadiković <i>et al.</i> , 2019). | *   | All affected trees were eradicated.  | Jurc and Jurc (2010), Janoušek <i>et al.</i> (2016), Sadiković <i>et al.</i> (2019) |
| Slovenia, Čatež                        | 2015           | <i>P. mugo</i>   | <i>TEF 1</i> sequencing was used for identification. Mating type 1 was detected.  | *   |  | Sadiković <i>et al.</i> (2019)  |
| Slovenia, Ljubljana                    | 2008–2009      | <i>P. mugo</i> , <i>P. sylvestris</i>                    | Morphological identifications. The identity of isolates from <i>P. mugo</i> were confirmed with <i>TEF 1</i> sequencing by Sadiković <i>et al.</i> (2019).  | *   | All affected trees were eradicated.  | Jurc and Jurc (2010), Sadiković <i>et al.</i> (2019)                                |
| Slovenia, Ljubljana                    | 2013           | <i>P. mugo</i>   | <i>TEF 1</i> sequencing was used for identification. Mating type 1 was detected.  | *   |  | Sadiković <i>et al.</i> (2019)  |
| Slovenia, Tolmin                       | 2016           | <i>P. nigra</i>  | <i>TEF 1</i> sequencing was used for identification. Mating type 1 was detected.  | *   |  | Sadiković <i>et al.</i> (2019)  |
| Slovenia, Trenta                       | 2014–2015      | <i>P. mugo</i>   | <i>TEF 1</i> sequencing was used for identification. Mating type 2 was detected.  | *   |  | Sadiković <i>et al.</i> (2019)  |
| South Korea, Naju                      | 2010–2011      | <i>P. thunbergii</i>                                     | <i>L. acicola</i> symptoms were observed and confirmed with ITS sequencing. <i>TEF 1</i> sequencing was used for identification by Janoušek <i>et al.</i> (2016) and mating type 2 was detected.                      | *   | Low incidence, less than 1%.   | Janoušek <i>et al.</i> (2016), Seo <i>et al.</i> (2012)                             |
| Spain                                  | 1942           | <i>P. radiata</i>  | Probably oldest official report of <i>L. acicola</i> in Europe based on morphological identification.   |   |  | Martínez (1942)   |
| Spain, Cantabria                       | 2012           | <i>P. radiata</i>  | <i>TEF 1</i> sequencing used for identification, mating type 2 was detected.  | *   |  | Janoušek <i>et al.</i> (2016)   |
| Spain, Spanish Atlantic climate region | 2015           | <i>P. nigra</i> , <i>P. radiata</i>                      | Sequenced directly from needles using conventional PCR (loos <i>et al.</i> , 2010). Both mating types were detected.  | *   | <i>Lecanosticta acicola</i> was detected on 44.7% of trees that were surveyed. | Ortiz de Urbina <i>et al.</i> (2017)  |

**Table 1** (Continued)

| Country, region, locality     | Year collected | Host   | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods                          | Report references                           |
|-------------------------------|----------------|--|---|---|--|---|
| Sweden                        | 2017           | <i>P. mugo</i> 'Hesse'                                     | Morphological identification and ITS sequencing.  | *   | Single tree in arboretum that was severely affected.                                     | Cleary <i>et al.</i> (2019)                 |
| Switzerland, Zollikon         | 1995           | <i>P. mugo</i> , <i>P. uncinata</i>                        | Morphological identification of the pathogen.   |   | Control measures were initiated in accordance with the phytosanitary policy of the EPPO. | Holdenrieder and Sieber (1995)              |
| Switzerland, Canton St Gallen | 1999           | <i>P. mugo</i>   | <i>TEF 1</i> sequencing used for identification.  | *   |  | Janoušek <i>et al.</i> (2016)               |
| Switzerland, Canton Zug       | 2009           | <i>P. mugo</i>   | Symptoms were observed in the field. Later, <i>TEF 1</i> sequencing was used to confirm identification (Janoušek <i>et al.</i> , 2016). Mating type 1 was detected.             | *   |  | Angst (2011), Janoušek <i>et al.</i> (2016) |
| Switzerland, Zürich           | 2009           | <i>P. mugo</i>   | Symptoms were observed in the field. Later, <i>TEF 1</i> sequencing was used to confirm identification (Janoušek <i>et al.</i> , 2016). Mating type 1 was detected.             | *   |  | Angst (2011), Janoušek <i>et al.</i> (2016) |
| Switzerland, Bern and Zürich  | 2017           | <i>P. mugo</i>   | Detection with qPCR and a conventional PCR directly from pine needles.  | *   |  | Schneider <i>et al.</i> (2019)              |
| Switzerland, Schwyz           | 2017           | <i>P. sylvestris</i>                                       | Detection with qPCR and a conventional PCR directly from pine needles.  | *   |  | Schneider <i>et al.</i> (2019)              |
| USA, Alabama                  | 1929           | <i>P. palustris</i>  | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.     |   |  | Hedgcock (1929)                             |
| USA, Alabama                  | 1944           | <i>P. echinata</i> , <i>P. palustris</i> , <i>P. taeda</i> | Siggers reported <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified. |   |  | Siggers (1944)                              |
| USA, Alabama                  | 1948–1967      | <i>P. palustris</i>  | Symptoms were observed annually on seedlings and the proportion of seedlings affected were recorded.  |   | In a 4-year study, 78% or more seedlings were infected yearly with <i>L. acicola</i> .   | Boyer (1972)                                |

Table 1 (Continued)

| Country, region, locality | Year collected | Host  | Identification method and additional notes   | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods | Report references            |
|---------------------------|----------------|---|--|---|---|------------------------------|
| USA, Arkansas             | 1929           | <i>P. taeda</i>   | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.        |   |   | Hedgcock (1929)              |
| USA, Arkansas             | 1944           | <i>P. taeda</i>   | Siggers reported <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified.    |   |   | Siggers (1944)               |
| USA, Arkansas             | 1967–1971      | <i>P. sylvestris</i>  | Symptoms were observed in the field and the proportion of needles affected were noted. In some cases, microscopic examinations of conidia were used for identification.            |   |   | Skilling and Nicholls (1974) |
| USA, Florida              | 1929           | <i>P. caribaea</i> ,<br><i>P. glabra</i> , <i>P. palustris</i> , <i>P. taeda</i>  | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.        |   |   | Hedgcock (1929)              |
| USA, Florida              | 1944           | <i>P. attenuata</i> ,<br><i>P. caribaea</i> ,<br><i>P. coulteri</i> ,<br><i>P. jeffreyi</i> , <i>P. glabra</i> , <i>P. halepensis</i> ,<br><i>P. latifolia</i> ,<br><i>P. muricata</i> ,<br><i>P. palustris</i> ,<br><i>P. pinaster</i> ,<br><i>P. pinea</i> , <i>P. ponderosa</i><br>var. <i>scopulorum</i> , <i>P. radiata</i> , <i>P. thunbergii</i> | Siggers reported on <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified. |   |   | Siggers (1944)               |
| USA, Georgia              | 1929           | <i>P. palustris</i> ,<br><i>P. taeda</i> , <i>P. virginiana</i>   | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.        |   |   | Hedgcock (1929)              |
| USA, Georgia              | 1944           | <i>P. caribaea</i> ,<br><i>P. palustris</i> ,<br><i>P. taeda</i> , <i>P. virginiana</i>   | Siggers reported <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified.    |   |   | Siggers (1944)               |

**Table 1** (Continued)

| Country, region, locality | Year collected | Host                                  | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods | Report references            |
|---------------------------|----------------|---------------------------------------|---|---|---|------------------------------|
| USA, Idaho                | 1929           | <i>P. ponderosa</i>                   | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture. According to Siggers (1944) the identification was based on characteristics that do not fit <i>Lecanosticta</i> and therefore this record should be verified. |   |   | Hedgcock (1929)              |
| USA, Iowa                 | 1967–1971      | <i>P. sylvestris</i>                  | Symptoms were observed in the field and the proportion of needles affected were noted. In some cases, microscopic examinations of conidia were used for identification.   |   |   | Skilling and Nicholls (1974) |
| USA, Kansas               | 1929           | <i>P. nigra</i> var. <i>austriaca</i> | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture. According to Siggers (1944) the identification was based on characteristics that do not fit <i>Lecanosticta</i> and therefore this record should be verified. |   |   | Hedgcock (1929)              |
| USA, Kansas               | 1951           | <i>P. nigra</i> , <i>P. ponderosa</i> | Reports in the field and mycological identification.  |   |   | Rogerson (1953)              |
| USA, Kansas               | 1967–1971      | <i>P. sylvestris</i>                  | Symptoms were observed in the field and the proportion of needles affected were noted. In some cases, microscopic examinations of conidia were used for identification.   |   |   | Skilling and Nicholls (1974) |
| USA, Kentucky             | 1929           | <i>P. nigra</i> var. <i>austriaca</i> | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture. According to Siggers (1944) the identification was based on characteristics that do not fit <i>Lecanosticta</i> and therefore this record should be verified. |   |   | Hedgcock (1929)              |
| USA, Kentucky             | 1967–1971      | <i>P. sylvestris</i>                  | Symptoms were observed in the field and the proportion of needles affected were noted. In some cases, microscopic examinations of conidia were used for identification.   |   |   | Skilling and Nicholls (1974) |

Table 1 (Continued)

| Country, region, locality | Year collected  | Host  | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods | Report references   |
|---------------------------|-----------------|---|---|---|---|---|
| USA, Louisiana            | 1929            | <i>P. palustris</i> ,<br><i>Pinus</i> × <i>sondereggeri</i> , <i>P. taeda</i>   | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.   |   |   | Hedgcock (1929)   |
| USA, Louisiana            | 1929–1930, 1960 | <i>P. palustris</i>   | Symptoms were observed and the proportion of seedlings affected were recorded at 4–5 years of age and again at 30 years.  |   | Most of the trees were affected.                                | Wakeley (1970)  |
| USA, Louisiana            | 1944            | <i>P. attenuata</i> ,<br><i>P. caribaea</i> ,<br><i>P. contorta</i> var. <i>latifolia</i> ,<br><i>P. echinata</i> ,<br><i>P. nigra</i> var. <i>laricio</i> , <i>P. palustris</i> , <i>P. pinaster</i> , <i>P. ponderosa</i> var. <i>scopulorum</i> , <i>P. radiata</i> , <i>P. rigida</i> , <i>P. serotina</i> , <i>P. sabiniana</i> , <i>Pinus</i> × <i>sondereggeri</i> , <i>P. taeda</i> | Siggers reported on <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified.  |   |   | Siggers (1944)  |
| USA, Maine                | 2011            | <i>P. strobus</i>   | Isolates were collected and morphologically identified in a survey. These isolates were later identified with <i>TEF 1</i> sequencing and both mating types were detected.  | *   |   | Munck <i>et al.</i> (2012), Janoušek <i>et al.</i> (2016) |
| USA, Maine                | 2011–2012       | <i>P. strobus</i>   | <i>Lecanosticta acicola</i> was identified as part of a complex of pathogens that cause white pine needle damage (WPND). Morphological identifications and selected ITS PCR sequencing was performed to confirm the presence of <i>L. acicola</i> . | *   | It was observed that affected trees were defoliated annually.   | Broders <i>et al.</i> (2015)                              |
| USA, Michigan             | 2016            | <i>P. sylvestris</i>  | <i>TEF 1</i> sequencing used for identification, mating type 2 was detected.  | *   |   | Janoušek <i>et al.</i> (2016)                             |
| USA, Minnesota            | 1967–1971       | <i>P. sylvestris</i>  | Symptoms were observed in the field and the proportion of needles affected were noted. In some cases, microscopic examinations of conidia were used for identification.   |   |   | Skilling and Nicholls (1974)                              |

**Table 1** (Continued)

| Country, region, locality    | Year collected | Host   | Identification method and additional notes   | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods  | Report references             |
|------------------------------|----------------|--|--|---|--|-------------------------------|
| USA, Minnesota and Wisconsin | 1970–1972      | <i>P. banksiana</i> , <i>P. glauca</i> , <i>P. nigra</i> , <i>P. palustris</i> , <i>P. resinosa</i> , <i>P. strobus</i> , <i>P. sylvestris</i> , <i>Picea glauca</i> | Symptoms were observed in the field and the proportion of needles affected were noted.   |   | These species were tested for susceptibility in a field trial by planting the hosts underneath heavily infected <i>P. sylvestris</i> . Four varieties of <i>P. sylvestris</i> , as well as <i>P. nigra</i> and <i>P. resinosa</i> , were the most susceptible. <i>P. strobus</i> was moderately resistant. <i>P. banksiana</i> was the most resistant. Less than 1% of <i>Picea glauca</i> was infected. | Skilling and Nicholls (1974)  |
| USA, Mississippi             | 1929           | <i>P. caribaea</i> , <i>P. palustris</i> , <i>P. taeda</i>   | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.        |   |  | Hedgcock (1929)               |
| USA, Mississippi             | 1944           | <i>P. caribaea</i> , <i>P. palustris</i> , <i>P. pinaster</i> , <i>P. taeda</i> , <i>P. thunbergii</i>   | Siggers reported on <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified. |   |  | Siggers (1944)                |
| USA, Mississippi             | 1952–1953      | <i>P. palustris</i>  | Microscopic identification. Both the sexual and asexual states were observed.  |   |  | Henry (1954)                  |
| USA, Mississippi             | 1966–1967      | <i>P. palustris</i>  | Morphological identifications. Both the sexual state and asexual state were observed throughout the year on infected <i>P. palustris</i> .   |   |  | Kais (1971)                   |
| USA, Mississippi             | 2012           | <i>P. palustris</i> , <i>P. taeda</i>  | <i>TEF 1</i> sequencing used for identification, both mating types detected.   | *   |  | Janoušek <i>et al.</i> (2016) |



Table 1 (Continued)

| Country, region, locality | Year collected | Host                                  | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods                                      | Report references   |
|---------------------------|----------------|---------------------------------------|---|---|--|---|
| USA, Missouri             | 1929           | <i>P. nigra</i> var. <i>austriaca</i> | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture. According to Siggers (1944) the identification was based on characteristics that do not fit <i>Lecanosticta</i> and therefore this record should be verified. |   |  | Hedgcock (1929)   |
| USA, Missouri             | 1947–1949      | <i>P. ponderosa</i>                   | Symptoms were observed in the field and morphological identifications were made. Both the sexual state and asexual state were observed.   |   | All trees were affected. Excessive needle defoliation and in some cases tree mortality was observed. | Luttrell (1949)   |
| USA, Missouri             | 1967–1971      | <i>P. sylvestris</i>                  | Symptoms were observed in the field and the proportion of needles affected were noted. In some cases, microscopic examinations of conidia were used for identification.   |   |  | Skilling and Nicholls (1974)                              |
| USA, New England          | 2016           | <i>P. strobus</i>                     | Severe needle browning was observed and <i>L. acicola</i> was identified as part of a complex of species causing premature defoliation. This is possibly WPND although it was not defined as such.  |   |  | Brazeo (2016)   |
| USA, New Hampshire        | 2011           | <i>P. strobus</i>                     | Isolates were collected and morphologically identified in a survey. These isolates were later identified with <i>TEF 1</i> sequencing and mating type 1 was detected.   | *   |  | Munck <i>et al.</i> (2012), Janoušek <i>et al.</i> (2016) |
| USA, New Hampshire        | 2011–2012      | <i>P. strobus</i>                     | <i>Lecanosticta acicola</i> was identified as part of a complex of pathogens that cause WPND. Morphological identifications and selected ITS PCR sequencing confirmed the presence of <i>L. acicola</i> .   | *   | It was observed that affected trees were defoliated annually.  | Broders <i>et al.</i> (2015)                              |
| USA, New York             | 1976           | <i>P. mugo</i>                        | <i>Lecanosticta acicola</i> was identified with morphological methods and brown spot needle blight symptoms confirmed on trees. Specimens are in the Cornell University Plant Pathology Herbarium.  |   |  | Sinclair and Hudler (1980)                                |

**Table 1** (Continued)

| Country, region, locality | Year collected | Host   | Identification method and additional notes   | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods | Report references |
|---------------------------|----------------|--|--|---|---|-------------------|
| USA, North Carolina       | 1929           | <i>P. echinata</i> ,<br><i>P. palustris</i> ,<br><i>P. rigida</i> , <i>P. taeda</i> , <i>P. virginiana</i> | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.        |   |   | Hedgcock (1929)   |
| USA, North Carolina       | 1944           | <i>P. palustris</i> ,<br><i>P. rigida</i> , <i>P. strobilus</i> , <i>P. taeda</i> , <i>P. virginiana</i>   | Siggers reported on <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified. |   |   | Siggers (1944)    |
| USA, North Carolina       | 1957, 1958     | <i>P. strobilus</i>  | Morphological identifications of <i>L. acicola</i> .   |   |   | Boyce (1959)      |
| USA, Ohio                 | 1944           | <i>P. contorta</i> var. <i>latifolia</i> ,<br><i>P. coulteri</i> , <i>P. jeffreyi</i>                      | Siggers reported on <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified. |   |   | Siggers (1944)    |
| USA, Oregon               | 1929           | <i>P. attenuata</i>  | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.        |   |   | Hedgcock (1929)   |
| USA, Oregon               | 1944           | <i>P. attenuata</i>  | Siggers reported on <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified. |   |   | Siggers (1944)    |
| USA, Pennsylvania         | 1929           | <i>P. rigida</i>   | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.        |   |   | Hedgcock (1929)   |
| USA, Pennsylvania         | 1987–1989      | <i>P. strobilus</i>  | Morphological identifications were done.   |   |   | Stanosz (1990)    |
| USA, South Carolina       | 1876           | <i>P. echinata</i> ( <i>P. variabilis</i> )  | Morphological description of <i>Cryptosporium aciculum</i> .   |   |   | de Thümen (1878)  |
| USA, South Carolina       | 1929           | <i>P. caribaea</i> , <i>P. echinata</i> , <i>P. palustris</i> , <i>P. serotina</i> , <i>P. taeda</i>       | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.        |   |   | Hedgcock (1929)   |
| USA, South Carolina       | 1944           | <i>P. caribaea</i> , <i>P. palustris</i> , <i>P. taeda</i>   | Siggers reported on <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified. |   |   | Siggers (1944)    |

Table 1 (Continued)

| Country, region, locality | Year collected | Host  | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods                          | Report references   |
|---------------------------|----------------|---|---|---|--|---|
| USA, Tennessee            | 1929           | <i>P. rigida</i> , <i>P. taeda</i>  | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.                               |   |  | Hedgcock (1929)   |
| USA, Tennessee            | 1944           | <i>P. palustris</i> , <i>P. ponderosa</i> var. <i>scopulorum</i> , <i>P. rigida</i> , <i>P. taeda</i> | Siggers reported on <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified.                        |   |  | Siggers (1944)  |
| USA, Texas                | 1929           | <i>P. palustris</i> , <i>P. taeda</i>   | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.                               |   |  | Hedgcock (1929)   |
| USA, Texas                | 1929           | <i>P. palustris</i> , <i>P. taeda</i>   | Symptoms were observed on trees inside and surrounding the nurseries.   |   | Low severity recorded. Nursery beds were sprayed with Bordeaux 4-4-50 with good results. | Webster (1930)  |
| USA, Texas                | 1944           | <i>P. caribaea</i> , <i>P. palustris</i> , <i>P. pinaster</i> , <i>P. taeda</i>                       | Siggers reported on <i>Lecanosticta</i> isolates that are in the collections in the Division of Forest Pathology in Louisiana and Maryland, USA. These reports should be verified.                        |   |  | Siggers (1944)  |
| USA, Vermont              | 2008           | <i>P. mugo</i> , <i>P. resinosa</i> , <i>P. sylvestris</i> , <i>P. strobus</i>                        | Forest surveys were conducted and the pathogen identified based on symptomology.  |   |  | Gibbs and Sinclair (2008)                                 |
| USA, Vermont              | 2011           | <i>P. strobus</i>   | Isolates were collected and morphologically identified in a survey. These isolates were later identified with <i>TEF 1</i> sequencing and both mating types were detected.                                | *   |  | Munck <i>et al.</i> (2012), Janoušek <i>et al.</i> (2016) |
| USA, Vermont              | 2011–2012      | <i>P. strobus</i>   | <i>Lecanosticta acicola</i> was identified as part of a complex of pathogens that cause WPND. Morphological identifications and selected ITS PCR sequencing confirmed the presence of <i>L. acicola</i> . | *   | It was observed that affected trees were defoliated annually.                            | Broders <i>et al.</i> (2015)                              |
| USA, Virginia             | 1929           | <i>P. rigida</i>  | Hedgcock reported on collections of the pathogen at the office of Forest Pathology at Washington, D.C. and the Mycological collections of the US Department of Agriculture.                               |   |  | Hedgcock (1929)   |

**Table 1** (Continued)

| Country, region, locality                                | Year collected | Host                    | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods  | Report references                 |
|--|----------------|-------------------------|---|---|--|-----------------------------------|
| USA, Wisconsin   | 1966–1970      | <i>P. sylvestris</i>    | A forest survey was conducted and symptoms of <i>L. acicola</i> was observed.   |   | Approximately 3000 acres in 55 plantations were severely infected. Short leaf French and Spanish <i>P. sylvestris</i> were severely affected. Long leaf <i>P. sylvestris</i> varieties were reported as resistant. | Prey and Morse (1971)             |
| USA, Wisconsin   | 1967–1971      | <i>P. sylvestris</i>    | Symptoms were observed in the field and the proportion of needles affected were noted. In some cases, microscopic examinations of conidia were used for identification. |   |  | Skilling and Nicholls (1974)      |
| USA, Wisconsin   | 1970           | <i>P. resinosa</i>      | Symptoms were observed in the field and morphological identifications were made.  |   |  | Nicholls and Hudler (1972)        |
| USA, Wisconsin   | 2010           | <i>P. sylvestris</i>    | <i>TEF 1</i> sequencing used for identification, mating type 2 was detected.  | *   |  | Janoušek <i>et al.</i> (2016)     |
| <i>Lecanosticta brevispora</i>                           |                |                         |   |   |  |                                   |
| Guatemala, Alta Verapaz, Santa Cruz Verapaz, near Tactic | 2010           | <i>P. oocarpa</i>       | Multigene phylogenetic analysis.  | *   |  | van der Nest <i>et al.</i> (2019) |
| Guatemala, Chimaltenango, Tecpán, Finca La Esperanza     | 2010           | <i>P. pseudostrobus</i> | Multigene phylogenetic analysis.  | *   |  | van der Nest <i>et al.</i> (2019) |
| Guatemala, Lugar, La Soledad, Jalapa site II             | 2012           | <i>P. oocarpa</i>       | Multigene phylogenetic analysis.  | *   |  | van der Nest <i>et al.</i> (2019) |
| Honduras   | 2010           | <i>P. oocarpa</i>       | Multigene phylogenetic analysis.  | *   |  | van der Nest <i>et al.</i> (2019) |
| Mexico   | 2000           | <i>Pinus</i> sp.        | Multigene phylogenetic analysis.  | *   |  | Quaedvlieg <i>et al.</i> (2012)   |

Table 1 (Continued)

| Country, region, locality                                | Year collected | Host                                   | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods | Report references  |
|--|----------------|--|---|---|---|--|
| <i>Lecanosticta gloeospora</i>                           |                |  |   |   |   |  |
| Mexico, Nuevo León, Iturbide-Galeana                     | 1983           | <i>P. pseudostrobus</i>                | Morphological identification. The type was later sequenced using multiple genes (van der Nest <i>et al.</i> , 2019).  | *   |   | Evans (1984), Marmolejo (2000), van der Nest <i>et al.</i> (2019)  |
| <i>Lecanosticta guatemalensis</i>                        |                |  |   |   |   |  |
| Guatemala, Baja Verapaz                                  | 1983           | <i>P. oocarpa</i>                      | The type culture was previously identified as <i>L. acicola</i> based on morphological characteristics (Evans, 1984). Multigene phylogenetic analysis revealed it as a new species, <i>L. guatemalensis</i> . | *   |   | Quaedvlieg <i>et al.</i> (2012), van der Nest <i>et al.</i> (2019) |
| Guatemala, Alta Verapaz, Santa Cruz Verapaz, near Tactic | 2010           | <i>P. oocarpa</i>                      | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)                                  |
| Guatemala, Chiquimula                                    | 2011           | <i>P. oocarpa</i>                      | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)                                  |
| Guatemala, Jalapa, Finca Forestal Soledad                | 2012           | <i>P. oocarpa</i>                      | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)                                  |
| Guatemala, Coban, San Juan Chamelco                      | 2012           | <i>P. oocarpa</i>                      | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)                                  |
| Nicaragua  | 1982           | <i>P. tecunumanii</i>                  | This isolate was previously identified as <i>L. acicola</i> based on morphological characteristics (Evans, 1984). Multigene phylogenetic analysis revealed it to be <i>L. guatemalensis</i> .                 | *   |   | van der Nest <i>et al.</i> (2019)                                  |
| Nicaragua, Matagalpa                                     | 2010           | <i>P. oocarpa</i>                      | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)                                  |
| Honduras, Yoro   | 1981           | <i>P. caribaea</i> , <i>P. oocarpa</i> | These isolates were previously identified as <i>L. acicola</i> based on morphological characteristics (Evans, 1984). Multigene phylogenetic analysis revealed it to be <i>L. guatemalensis</i> .              | *   |   | van der Nest <i>et al.</i> (2019)                                  |
| <i>Lecanosticta jani</i>                                 |                |  |   |   |   |  |
| Guatemala, Alta Verapaz, Santa Cruz Verapaz, near Tactic | 2010           | <i>P. oocarpa</i>                      | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)                                  |
| Guatemala, Chiquimula                                    | 2010           | <i>P. oocarpa</i>                      | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)                                  |
| Guatemala, Jalapa, Finca Forestal Soledad                | 2012           | <i>P. maximinoi</i>                    | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)                                  |
| Guatemala, Jalapa, Finca La Soledad, Mataquesuintla      | 2012           | <i>P. tecunumanii</i>                  | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)                                  |

**Table 1** (Continued)

| Country, region, locality                                | Year collected | Host  | Identification method and additional notes  | Identification verified using molecular methods (*) | Reported severity of infections and applied eradication methods | Report references                               |
|--|----------------|---|---|---|---|---|
| Nicaragua, Matagalpa                                     | 2010           | <i>P. oocarpa</i>   | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)               |
| <i>Lecanosticta pharomachri</i>                          |                |   |   |   |   |   |
| Guatemala, Baja Verapaz, San Jerónimo, Salamá            | 2012           | <i>P. tecunumanii</i>   | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)               |
| Guatemala, Jalapa, Finca La Soledad, Mataquesuintla      | 2010–2012      | <i>P. oocarpa</i>   | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)               |
| Honduras   | 2010           | <i>P. oocarpa</i>   | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)               |
| <i>Lecanosticta tecunumanii</i>                          |                |   |   |   |   |   |
| Guatemala, Baja Verapaz, San Jerónimo, Salamá            | 2012           | <i>P. tecunumanii</i>   | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)               |
| <i>Lecanosticta variabilis</i>                           |                |   |   |   |   |   |
| Guatemala, Alta Verapaz, Santa Cruz Verapaz, near Tactic | 2010           | <i>P. oocarpa</i>   | Multigene phylogenetic analysis. Both mating types were present (Janoušek <i>et al.</i> , 2016).  | *   | Very low.   | van der Nest <i>et al.</i> (2019)               |
| Guatemala, Jalapa, Finca Forestal Soledad                | 2012           | <i>P. maximinoi</i>   | Multigene phylogenetic analysis.  | *   | Very low.   | van der Nest <i>et al.</i> (2019)               |
| Honduras, Santa Barbara, Lago de Yojoa                   | 1984           | <i>P. caribaea</i>  | This isolate was previously identified as <i>L. acicola</i> in a morphological study by Evans (1984). A multigene phylogenetic analysis indicated that this is a new species, <i>L. tecunumanii</i> . | *   | Very low.   | Evans (1984), van der Nest <i>et al.</i> (2019) |
| Mexico   | 2000           | <i>Pinus</i> sp.  | Multigene phylogenetic analysis.  | *   |   | van der Nest <i>et al.</i> (2019)               |
| Mexico   | 2010           | <i>P. arizonica</i> var. <i>stormiae</i> , <i>P. halepensis</i> | Multigene phylogenetic analysis. The isolates were previously identified as <i>L. acicola</i> (Janoušek <i>et al.</i> , 2016) and both mating types were detected.                                    | *   |   | van der Nest <i>et al.</i> (2019)               |

Central America based on morphological characteristics (Evans, 1984), it is now recognized as a Northern Hemisphere pathogen for which phylogenetic analyses of the translation elongation factor 1- $\alpha$  gene (*TEF 1*) sequences have revealed three distinct lineages (van der Nest *et al.*, 2019). One of these lineages includes isolates from Canada, the northern parts of the USA (Maine, Michigan, New Hampshire, Vermont and Wisconsin) and Central and Northern Europe (Austria, Croatia, Czech Republic, Estonia, Germany, Italy, Lithuania, Slovenia, Switzerland) (van

der Nest *et al.*, 2019). A second lineage includes isolates from China, Colombia, France, Japan, Spain, South Korea and the southern part of the USA (Mississippi) (van der Nest *et al.*, 2019). A third lineage includes isolates only from Mexico (van der Nest *et al.*, 2019).

The eight other species described in *Lecanosticta* during the course of the past 35 years are present only in Mesoamerica (Tables 1 and 2) (Evans, 1984; Marmolejo, 2000; Quaedvlieg *et al.*, 2012; van der Nest *et al.*, 2019). Evans (1984) recognized

**Table 2** A summarized history of the taxonomy and nomenclature of the genus *Lecanosticta*.

| Year                        | Species epithet                      | Reference               | Sexual state reported | Country, location   | Host  | Description   | Notes   |
|-----------------------------|--------------------------------------|-------------------------|-----------------------|---|---|---|---|
| <i>Lecanosticta acicola</i> |                                      |                         |                       |   |   |   |   |
| 1878                        | <i>Cryptosporium acicolum</i> Thüim  | de Thüimen (1878)       | Asexual               | USA, South Carolina, Aiken  | <i>Pinus echinata</i> ( <i>P. variabilis</i> )  |   | <i>P. variabilis</i> is a synonym of <i>P. echinata</i> . In Wolf and Barbour (1941), it was mentioned that it was in fact on <i>P. caribaea</i> and that the host was previously incorrectly identified. |
| 1884                        | <i>Septoria acicola</i> (Thüim) Sacc | Saccardo (1884)         | Asexual               | USA, Carolina, Aiken  | <i>P. variabilis</i>  | Saccardo moved <i>C. acicolum</i> to <i>Septoria</i> due to the characteristic septate conidia.   |   |
| 1922                        | <i>Lecanosticta pini</i>             | Sydow and Petrak (1922) | Asexual               | USA, Arkansas and Oregon  | <i>P. taeda</i> and <i>P. palustris</i> in Arkansas and <i>P. attenuata</i> in Oregon | The genus <i>Lecanosticta</i> was erected to accommodate <i>L. pini</i> , a fungus with erumpent stromata and pigmented conidia.  |   |
| 1924                        | <i>Lecanosticta acicola</i>          | Sydow and Petrak (1924) | Asexual               | USA   | –   | The authors recognized that <i>L. pini</i> was <i>C. acicolum</i> . The genus was retained and the name <i>L. acicola</i> was proposed as the valid name.   |   |
| 1926                        | <i>Oligostroma acicola</i>           | Dearness (1926, 1928)   | Sexual                | USA, Florida, Silver Springs                                      | <i>P. palustris</i>   | The sexual state of <i>L. acicola</i> was isolated from old pine needles from which the asexual state was previously isolated. In 1928, it was proposed that the asexual state <i>Septoria acicola</i> fits better in the genus <i>Cryptosporium</i> and that <i>Oligostroma acicola</i> could be <i>Cryptosporium acicolum</i> 's sexual state (Dearness, 1928). |   |
| 1939                        | <i>Schirria acicola</i>              | Siggers (1939)          | Sexual                | USA, Arkansas, Florida, Georgia, Louisiana, North Carolina, Texas | <i>P. palustris</i> , <i>P. taeda</i> , <i>P. thunbergii</i>                          | Ascospores as well as conidia were plated onto media and morphologically examined to come to the conclusion that the sexual and asexual state are connected. <i>Oligostroma acicola</i> was changed to <i>Schirria acicola</i> as erumpent acervuli were observed, characteristic of <i>Schirria</i> .  |   |

Table 2 (Continued)

| Year | Species epithet                  | Reference                       | Sexual state reported | Country, location        | Host              | Description   | Notes  |
|------|----------------------------------|---------------------------------|-----------------------|--------------------------|-------------------|---|--|
| 1941 | <i>Systemma acicola</i>          | Wolf and Barbour (1941)         | Sexual                | USA                      | <i>Pinus</i> spp. | It was recognized that the pathogen was better suited in the Dothideaceae and therefore the fungus was moved to the genus <i>Systemma</i> and all the above names for the sexual state synonymized with <i>S. acicola</i> .   |  |
| 1967 | <i>Dothistroma acicola</i>       | Shishkina and Tsanova (1967)    | Asexual               | –                        | –                 | The name <i>D. acicola</i> was incorrectly assigned to both <i>D. pini</i> and <i>L. acicola</i> . The name was not used in subsequent literature.  | Due to similarities between symptoms caused by <i>Dothistroma</i> and <i>Lecanosticta</i> the asexual states of <i>D. pini</i> (presently <i>D. septosporum</i> ) and <i>L. acicola</i> were synonymized and renamed as <i>D. acicola</i> and furthermore associated with the sexual state <i>Systemma acicola</i> . |
| 1972 | <i>Mycosphaerella dearnessii</i> | Barr (1972)                     | Sexual                | USA                      | –                 | <i>Systemma acicola</i> was synonymized with <i>Mycosphaerella dearnessii</i> . <i>Mycosphaerella dearnessii</i> was assigned as the type for <i>Mycosphaerella</i> subgenus <i>Mycosphaerella</i> section <i>Caterva</i> .   | Mycobank accession number: 318138.   |
| 1996 | <i>Eruptio acicola</i>           | Barr (1996)                     | Sexual                | –                        | –                 | According to Barr (1996), <i>Mycosphaerella dearnessii</i> did not fit the description of <i>Mycosphaerella</i> and the new genus <i>Eruptio</i> M.E. Barr was erected to accommodate <i>Eruptio acicola</i> and <i>Eruptio pini</i> (sexual state of <i>Dothistroma septosporum</i> ).   | The validity of the genus <i>Eruptio</i> was questioned as <i>Lecanosticta acicola</i> phylogenetically clusters with other <i>Mycosphaerella</i> anamorphs (Crous <i>et al.</i> 2001). <i>Eruptio</i> is not widely used in literature.   |
| 2012 | <i>Lecanosticta acicola</i>      | Quaedvlieg <i>et al.</i> (2012) | Both                  | Europe and North America | <i>Pinus</i> spp. | As it was recognized that the genus <i>Mycosphaerella</i> should only be used with the anamorphic genus <i>Ramularia</i> (Crous <i>et al.</i> , 2007; Crous, 2009), <i>Lecanosticta acicola</i> was selected as the valid name and type of the genus under the one fungus = one name rule. An epitype was designated for <i>L. acicola</i> in this study (Quaedvlieg <i>et al.</i> , 2012). | Mycobank accession number: 255702 Epitype CBS H-21113, Ex-epitype CBS 133791.  |



Table 2 (Continued)

| Year                              | Species epithet                   | Reference                         | Sexual state reported | Country, location                    | Host  | Description  | Notes  |
|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------|--------------------------------------|---|--|--|
| Other <i>Lecanosticta</i> species |                                   |                                   |                       |                                      |   |  |  |
| 1984                              | <i>Lecanosticta cinerea</i>       | Evans (1984); Marmolejo (2000)    | Asexual               | Honduras                             | <i>Pinus</i> sp.  | The species name was proposed as the correct name for <i>Gloeocoryneum cinereum</i> .  | This name is not validly published (Marmolejo, 2000) as no basionym was established. The new combination <i>Leptomelanconium pinicola</i> was proposed because it had previously been established that <i>Gloeocoryneum cinereum</i> is a synonym of <i>Strobospora pinicola</i> and that the genera <i>Gloeocoryneum</i> and <i>Leptomelanconium</i> have the same characteristics (Marmolejo, 2000). |
| 1984                              | <i>Lecanosticta gloeospora</i>    | Evans (1984)                      | Asexual               | Mexico, Iturbide-Galeana, Nuevo León | <i>P. pseudoastrobus</i>  | The second valid species in the genus to be described based on morphological characteristics.  | Mycobank accession number: 106975 Holotype IMI 283812 Ex-type IMI 283812.  |
| 2000                              | <i>Lecanosticta longispora</i>    | Marmolejo (2000)                  | Asexual               | Mexico, Nuevo León                   | <i>P. culminicola</i>   | The third species described in the genus based on morphological characteristics.   | No type was assigned but Quaedvlieg <i>et al.</i> (2012) epitypified the species in 2012.  |
| 2012                              | <i>Lecanosticta longispora</i>    | Quaedvlieg <i>et al.</i> (2012)   | Asexual               | Mexico, Nuevo León                   | <i>P. culminicola</i>   | The first phylogenetic study to include this species. An epitype <i>L. longispora</i> was designated here.   | Mycobank accession number: 466255 Epitype CBS H-21111, Ex-type CBS 133602.   |
| 2012                              | <i>Lecanosticta brevispora</i>    | Quaedvlieg <i>et al.</i> (2012)   | Asexual               | Mexico                               | <i>Pinus</i> sp.  | This isolate is the fourth species described in the genus based on phylogenetic inference and morphology.  | Mycobank accession number: 801940 Holotype CBS H-21110, Ex-type CBS 133601.  |
| 2012                              | <i>Lecanosticta guatemalensis</i> | Quaedvlieg <i>et al.</i> (2012)   | Asexual               | Guatemala                            | <i>P. oocarpa</i>   | The ex-type of <i>L. guatemalensis</i> (IMI281598) was initially described as <i>L. acicola</i> based on morphological characteristics (Evans, 1984). The isolate was phylogenetically delineated as a new species in 2012 and subsequently described (Quaedvlieg <i>et al.</i> , 2012). | Mycobank accession number: 801941 Holotype CBS H-21108, Ex-type IMI 281598.  |
| 2018                              | <i>Lecanosticta jani</i>          | van der Nest <i>et al.</i> (2019) | Asexual               | Guatemala, Nicaragua                 | <i>P. maximinoi</i> , <i>P. oocarpa</i> , <i>P. tecunumanii</i> | New species described based on phylogenetic and morphological data.  | Mycobank accession number: 826875 Holotype PREM 62185, Ex-type CBS 144456.   |

Table 2 (Continued)

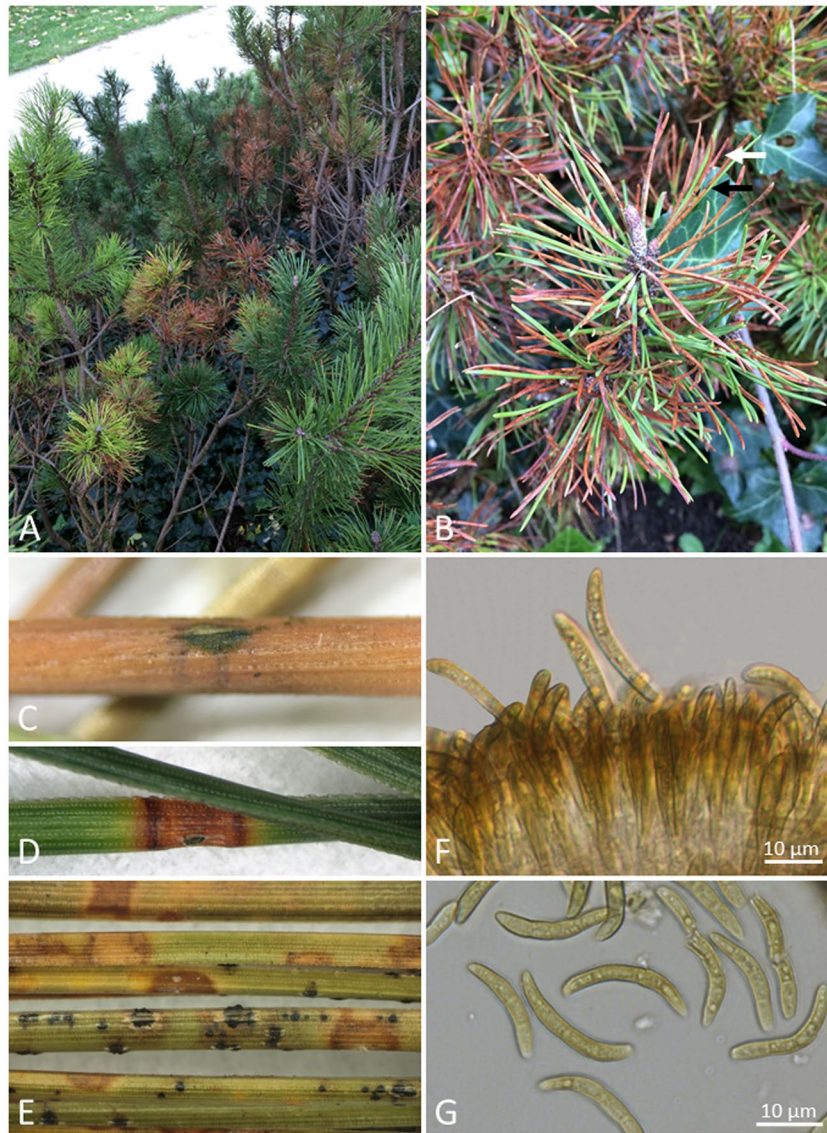
| Year | Species epithet                 | Reference                         | Sexual state reported | Country, location   | Host   | Description   | Notes   |
|------|---------------------------------|-----------------------------------|-----------------------|---------------------|--|---|---|
| 2018 | <i>Lecanosticta pharomachri</i> | van der Nest <i>et al.</i> (2019) | Asexual               | Guatemala, Honduras | <i>P. oocarpa</i> , <i>P. tecunumanii</i>                    | New species described based on phylogenetic and morphological data.   | Mycobank accession number: 826876 Holotype PREM 62188, Ex-type CBS 144448.              |
| 2018 | <i>Lecanosticta tecunumanii</i> | van der Nest <i>et al.</i> (2019) | Asexual               | Guatemala           | <i>P. tecunumanii</i>  | New species described based on phylogenetic and morphological data.   | Mycobank accession number: 826877 Holotype PREM 62191, Ex-type CBS 144450.              |
| 2018 | <i>Lecanosticta variabilis</i>  | van der Nest <i>et al.</i> (2019) | Asexual               | Guatemala, Honduras | <i>P. caribaea</i> , <i>P. maximinoi</i> , <i>P. oocarpa</i> | New species described based on phylogenetic and morphological data. The ex-type was initially described as <i>L. acicola</i> by Evans (1984) based on morphological observations. | Mycobank accession number: 826878 Holotype PREM 62195, Ex-type CBS 144453 = IMI 281561. |

considerable morphological variation amongst his collections of *L. acicola*. In that study, he described a second species, *L. gloeospora* from *Pinus pseudostrabus* in Mexico, and the fungus remains known only from Mexico on this host (Evans, 1984; Marmolejo, 2000). The novelty of this species was recently validated using DNA sequence data (van der Nest *et al.*, 2019).

*Lecanosticta longispora* was first described based on morphological features from *P. culminicola* in Nuevo León, Mexico (Marmolejo, 2000). This species was characterized in a phylogenetic study by Quaedvlieg *et al.* (2012), and was distinguished from *L. acicola* based on differences in the *TEF 1* and  $\beta$ -tubulin 2 (*BT 2*) gene sequences. That study was the first to delineate species of *Lecanosticta* based on phylogenetic inference (Quaedvlieg *et al.*, 2012). These authors included several samples from Central America that had previously been identified as *L. acicola*, as well as the collection used by Marmolejo (2000) to typify *L. longispora*. In their phylogenetic analyses (Quaedvlieg *et al.*, 2012), *L. acicola* was not identified from Central America but two new species, *L. brevispora* and *L. guatemalensis*, were described (Tables 1 and 2).

Evans (1984) observed that ecotypes or morphotypes exist amongst isolates of *L. acicola* in Central America, depending on the altitude and hosts from which the isolations were made. He therefore hypothesized that Central America could be the centre of origin of *Lecanosticta*. This was later supported by analysis of *TEF 1* sequence data that revealed high genetic diversity in this geographical region (Janoušek *et al.*, 2016). An extensive collection of isolates from Central America was recently studied using a phylogenetic approach (van der Nest *et al.*, 2019). Interestingly, *L. acicola* was not identified amongst isolates from Guatemala, Nicaragua or Honduras. Furthermore, the isolates considered to be *L. acicola* by Evans (1984) were sequenced and identified as *L. guatemalensis* and a new species, *L. variabilis* (van der Nest *et al.*, 2019, Table 1). *Lecanosticta brevispora* was identified in Guatemala and Honduras on *Pinus oocarpa* and *P. pseudostrabus* (Table 1), expanding the host range and distribution for that species. Likewise, *L. guatemalensis* was also identified in Guatemala, Honduras and Nicaragua on *P. caribaea*, *P. oocarpa* and *P. tecunumanii* (Table 1). The study of van der Nest *et al.* (2019) introduced four new species, including *Lecanosticta jani* from Guatemala and Nicaragua, *L. pharomachri* from Guatemala and Honduras, *L. tecunumanii* from Guatemala and *L. variabilis* from Mexico, Guatemala and Honduras (van der Nest *et al.*, 2019). Although Central America could not be confirmed as a centre of origin of *L. acicola*, the diversity of species recognized by van der Nest *et al.* (2019) suggests strongly that Mesoamerica is a centre of diversity for *Lecanosticta*.

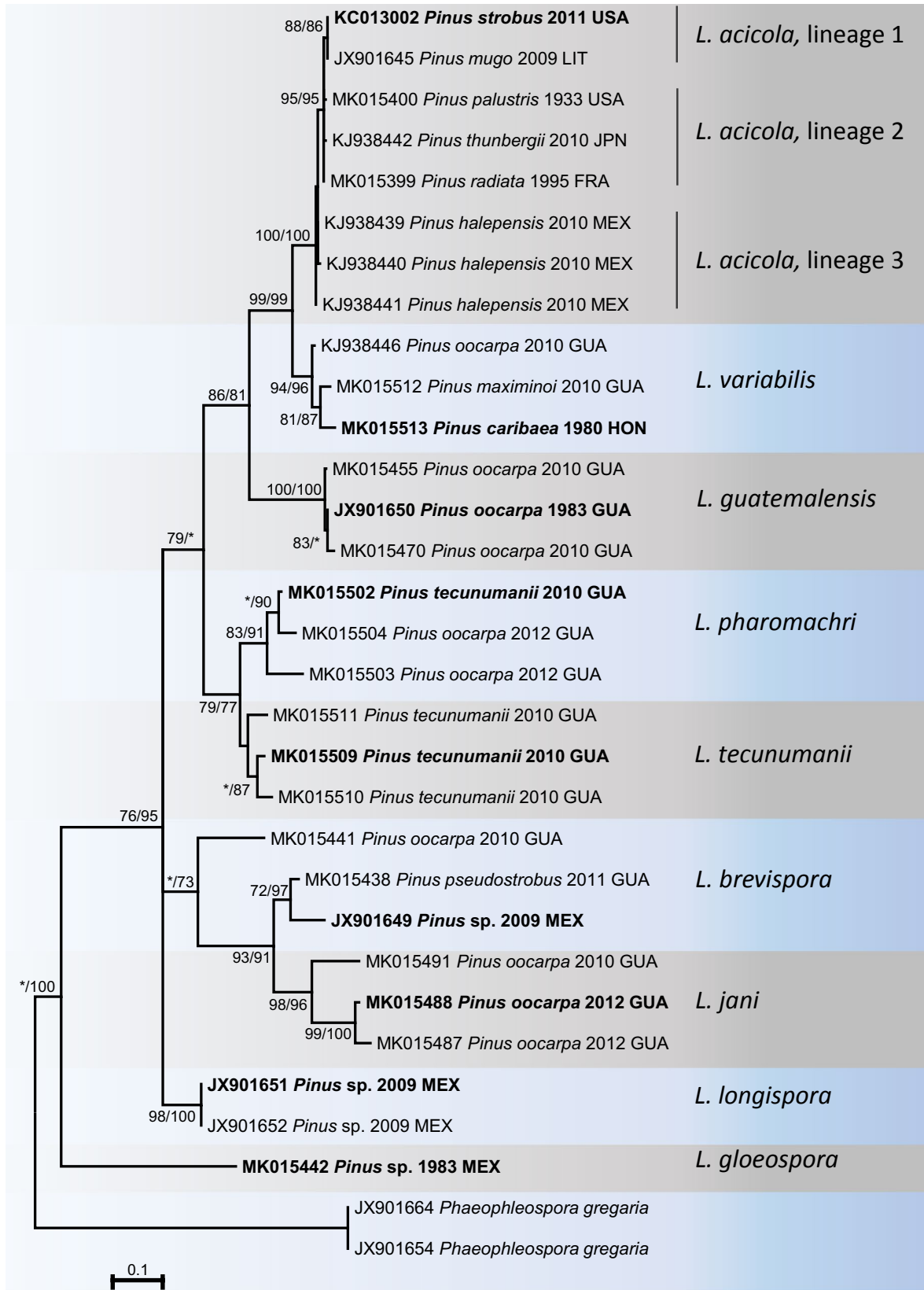
With only one exception, which is probably a taxonomic incongruity, *Lecanosticta* species are all associated with *Pinus* species. Petrak (1954) described *Phragmogloeum gaubae* on *Callistemon sieberi* in Australia (Petrak, 1954). von Arx (1983) attempted to reduce various species with overlapping characteristics to fewer genera and found that *Phragmogloeum* had the same morphological



**Fig. 2** Symptoms of *Lecanosticta acicola*. (A) *Pinus mugo* in Austria displaying symptoms of both brown spot needle blight (BSNB) and Dothistroma needle blight (DNB) on the same branches. (B) Both the characteristic brown spots associated with BSNB (black arrow) and the red banding associated with DNB (white arrow) can be observed. (C)–(E) Symptoms of BSNB vary from only brown spots as observed on *P. mugo* (C) to distinct brown bands as observed on *P. radiata* (D) to irregular mosaic spots as observed on *P. palustris* (E). (F) *Lecanosticta acicola* conidiogenous cells giving rise to conidia on malt extract agar. (G) *Lecanosticta acicola* septate conidia with verruculose surfaces and truncate bases.

characteristics as *Lecanosticta*. He proposed the new combination *Lecanosticta gaubae*. After the genus *Eruptio* was erected to accommodate *Lecanosticta acicola* and *Dothistroma septosporum* (Barr, 1996), *Lecanosticta gaubae* was transferred to that new genus (Crous, 1999). The genus *Eruptio* was further evaluated and it was found that *L. acicola* and *D. septosporum* were not congeneric (Crous, 2009). Consequently, *Lecanosticta* was selected as the correct name for *Eruptio acicola* following the one fungus one name convention (Crous *et al.*, 2009; Hawksworth *et al.*, 2011). Because *Eruptio gaubae* is morphologically similar to *Lecanosticta*, phylogenetic analyses are required to resolve this taxonomic confusion.

*Lecanosticta acicola* is the only species in the genus known to be a significant pathogen. This is particularly important because it is spreading rapidly in Europe and the northeastern parts of North America. Therefore, all data collected over time regarding *Lecanosticta* pertain to the organism that was assigned the name *L. acicola*, and the remainder of the review will focus on this species. However, it is relevant to recognize that other species of *Lecanosticta* cause symptoms similar to those of *L. acicola* and that they have the potential to emerge as pine pathogens if they were accidentally moved to new environments. They would then be recognized as members of a complex of BSNB pathogens.



**Fig. 3** Maximum likelihood (ML) tree representing the nine known species of *Lecanosticta* as well as the three lineages of *L. acicola* generated from the translation elongation 1- $\alpha$  region. ML bootstrap support (>70%) are indicated first, followed by maximum parsimony (MP) bootstrap support values (ML/MP, \* = insignificant value). *Phaeophleospora gregaria* was used as the outgroup taxa. All represented type species are indicated in bold.

## SYMPTOMS OF BROWN SPOT NEEDLE BLIGHT

Symptoms of infection can vary depending on the host species affected. Typically, a small and yellow, sometimes light grey-green or reddish brown, irregular circular spot, with defined margins, appears at the point of infection (Hedgcock, 1929) (Fig. 2C–E). These spots soon become brown as the infections mature and they are often surrounded by a yellow halo (Skilling and Nicholls, 1974). In severe cases, infections can occur on several parts of a needle, leading to more rapid necrosis (Fig. 2E). The characteristic brown spots are the first conspicuous symptoms on the pine needles and this has led to the common name ‘brown spot needle blight’ proposed by Siggers (1932). These brown spots can also appear resin-soaked depending on the host species (Skilling and Nicholls, 1974). In some cases, as has been reported in *P. strobus*, symptoms may only be displayed as chlorosis of the needles without banding (Broders *et al.*, 2015). Infected needles die from the apex to the base (Fig. 2B) and they are eventually shed from the trees (Hedgcock, 1929; Skilling and Nicholls, 1974). Usually only the second- and third-year needles are affected, leaving healthy new growth at the tips of the branches. The new growth tips are then infected in the subsequent season by inoculum on older needles (Skilling and Nicholls, 1974). Generally, infection is more severe in the lower parts of the canopy and then progresses upwards in the trees (Sinclair and Lyon, 2005; Skilling and Nicholls, 1974).

An asymptomatic phase in which *L. acicola* establishes within needles can last several days (Setliff and Patton, 1974) to 3 months (Skilling and Nicholls, 1974). This is dependent on the strain of the pathogen (Kais, 1972) and length of the wet season. This delay in symptom development could lead to the accidental movement of infected plants to new areas.

The symptoms of BSNB (Fig. 2) can easily be confused with those of DNB, which is caused by *Dothistroma septosporum* and *D. pini* (Barnes *et al.*, 2004, 2016). On some host species, symptoms of DNB are similar to those of BSNB (Fig. 2B) but rather than the characteristic brown discoloration and spots, a distinct red band forms around the point of infection in the case of DNB (Pehl and Cech, 2008). However, in some cases the characteristic red banding pattern associated with DNB is not formed or alternatively the red bands are sufficiently dark to give a false impression of brown spots. This can easily lead to incorrect pathogen diagnoses (Barnes *et al.*, 2016; Petrak, 1961).

## LIFE CYCLE

*Lecanosticta acicola* can occur in either its asexual or sexual state (Fig. 1) (Siggers, 1939). The pathogen overwinters in acervuli (asexual) (Fig. 1Aa) or ascostromata (sexual) (Fig. 1Ba) in

the dead tissue of either dead or living pine needles. It can also overwinter as vegetative mycelium in the infected needles that remain attached to the host (Siggers, 1944). Conidia are released in gelatinous masses (Fig. 1Ab) or ascospores are released from asci in ascostromata (Fig. 1Bb) on the needles when the light, temperature and humidity are favourable (Kais, 1975; Tainter and Baker, 1996).

Conidia begin to germinate on the needle surfaces by developing one to four germ tubes, depending on the number of cells in the conidia (Setliff and Patton, 1974). It is uncertain whether the germ tubes are attracted to the stomata, or whether they grow randomly over the needle surface (Patton and Spear, 1978; Setliff and Patton, 1974). Light plays an indirect, but essential role in the infection process as it stimulates the opening of stomata, allowing the germ tube to penetrate the needle (Fig. 1c) (Kais, 1975). Infections can also occur through wounds (Kais, 1978). Once a germ tube enters the stomatal antechamber, it increases in diameter and becomes thick-walled and melanized (Patton and Spear, 1978). Appresoria, such as those found in *Dothistroma* (Gadgil, 1967), have never been seen (Patton and Spear, 1978).

Once the mesophyll tissue has been invaded by *L. acicola* mycelium, conidiomata begin to form. These begin to integrate with the needle tissue and increase in size until they are visible to the naked eye (Wolf and Barbour, 1941). The conidiophores produce conidia towards the leaf exterior (Evans, 1984), which exerts pressure on the needle epidermis. This causes the epidermis to rupture, leaving a flap that partly covers the conidiomata (Wolf and Barbour, 1941). The conidia are released from the conidiomata during wet weather and the disease cycle is repeated.

In the case of the sexual state, asci are formed within the ascostromata on necrotic distal parts of living needles or on dead needles (Henry, 1954; Jewell, 1983). Ascospores are released from asci and dispersed through wind and rain. Asci and ascospores develop more rarely than conidia and have been reported only from Nicaragua, Honduras, Colombia and the southern parts of the USA (Table 1) (Evans, 1984; Henry, 1954; Kais, 1971; Luttrell, 1949; Siggers, 1944). The reports from Nicaragua and Honduras probably represent species other than *L. acicola*.

## TOXIN PRODUCTION

Many plant pathogenic fungi have adapted to produce toxic secondary metabolites in their plant hosts and these could influence colonization and sporulation, as has been seen in *D. septosporum* (Kabir *et al.*, 2015). *Lecanosticta acicola* is known to produce the toxic compounds LA-I and LA-II, which are heat-resistant and non-host specific phytotoxins (Yang *et al.*, 2002, 2005). The two

compounds interact with the host independently and do not promote or inhibit the interaction of one another (Yang *et al.*, 2002). Different *Pinus* species have different reactions to LA-I and LA-II. When rooted cuttings of *P. thunbergii* were exposed to the toxin, they showed little sensitivity to it. In contrast, when *P. elliotii* and *P. taeda*, both highly susceptible to BSNB infection, were exposed to the toxin, the results showed high sensitivity to LA-I (Ye and Qi, 1999). It seems likely that these toxins are involved in the destruction of mesophyll tissue of the pine needles at the point of infection (Jewell, 1983).

## BIOLOGY AND DISSEMINATION

Conidia and ascospores are released throughout the year at temperatures ranging from  $-5.5$  to  $28$  °C (Kais, 1971; Siggers, 1944; Wyka *et al.*, 2018). However, warm and wet weather is particularly conducive for the development of BSNB, irrespective of whether infection takes place by sexual or asexual spores. The conidia do not germinate below  $5$  °C, although most survive this temperature and commence germination once the temperature increases (Siggers, 1944). At the other extreme, tolerance to high temperature was found to vary depending on the strain of *Lecanosticta* involved. It was shown that conidia of isolates from the northern parts of the USA could not germinate at  $32$  °C, whereas cultures isolated from the southern parts of the USA, as well as China, had a germination success of 80% at the same temperature (Huang *et al.*, 1995). This physiological distinction is reflected in population genetic studies which define two lineages of the pathogen in the USA (Janoušek *et al.*, 2016). The success of the pathogen may therefore be a result of isolates in each lineage adapting to local temperature conditions.

The maximum temperature for the germination of *L. acicola* conidia is  $35$  °C (Siggers, 1944). It was also found that high humidity pre- and post-infection is required for high levels of infection (Kais, 1975). The optimal temperature for infection to occur is  $30$  °C during the day and  $21$  °C at night, and Kais (1975) showed that these temperatures gave positive results in inoculation trials.

Conidia are dispersed predominantly by rain splash to adjacent trees, and they contribute significantly to rapid disease build-up in pine stands (Tainter and Baker, 1996). High levels of conidial dispersal were recorded during the rainy season in the USA, especially between late spring and summer, as well as when there were rain spells after a long period of dryness (Kais, 1971). In other reports, conidial production and dispersal were recorded throughout the year (Siggers, 1944). Dispersal was not influenced by the temperature range but conidial release was connected to rainfall patterns. In Wisconsin, two peaks of conidial release were recorded, with the first peak in early summer when young pine needles are present and the second in late summer (Skilling and Nicholls, 1974), which was similar to that found

in the northeastern USA (Wyka *et al.* 2018). In Japan, it was found that conidia were produced by the pathogen from early spring to autumn with peak dispersal in mid-summer. However, for a second year of infection, the dispersal was most abundant from late summer to mid-autumn the following year (Suto, 2002). A study in Fujian province (China) showed that the greatest number of conidia were detected between early spring and mid-summer and again in late summer to late autumn in *Pinus elliotii* plantations (Li *et al.*, 1987). It consequently appears that conidial dispersal varies depending on the rainfall season in any particular geographical region.

Spore traps in several studies failed to capture ascospores (Kais, 1971; Siggers, 1939; Wyka *et al.*, 2018). It was found, however, that conidia could be dispersed to a distance of up to 60 m (Wyka *et al.* 2018). A recent investigation of the dispersal of *Dothistroma*, where the mechanisms of conidial and ascospore dispersal are similar to those in *L. acicola*, showed that conidia could be naturally disseminated over more than 1 km (Mullett *et al.*, 2016). The assumed distance of dispersal in *L. acicola* may, consequently, be similar.

The ascospores of *L. acicola* are forcibly expelled into the air (Wolf and Barbour, 1941) and dispersed by wind currents (Kais, 1971) or rain splash driven by wind (Siggers, 1939). Ascospores can also be released during periods of fog, rain and dew (Tainter and Baker, 1996). Ascospores were recorded in the USA mainly during periods when temperatures were above  $15$  °C and are found in late summer to autumn. Small numbers of ascospores were detected when temperatures were below  $10$  °C (Kais 1971).

The main component that facilitates spread of conidia and ascospores is moisture, but other factors may also aid in their dispersal. Insect dissemination was suggested as a mechanism of conidial spread when two Lepidopteran wing scales were found to have conidia attached to them (Skilling and Nicholls, 1974). Given the biology of *L. acicola*, it seems unlikely that insects are involved in its dissemination. It has also been suggested that animals grazing in forests might aid in dissemination of the conidia when spores stick to their coats or hooves (Skilling and Nicholls, 1974; Tainter and Baker, 1996). Again, this mode of dissemination seems unlikely to be particularly important.

Anthropogenic movement of infected plant material has contributed to the dissemination of many tree pathogens (Wingfield *et al.*, 2015). This has been clearly demonstrated for *Dothistroma septosporum* (Barnes *et al.*, 2014), which has a biology very similar to that of *L. acicola*. A study that used microsatellite markers has demonstrated that two separate lineages of *L. acicola* have most likely been introduced into Europe from North America (Janoušek *et al.*, 2016). Long distance dispersal of *L. acicola* is, therefore, likely to be the result of anthropogenic movement of infected plant material. This would not include seed transmission as *L. acicola* conidia cannot survive on a pine seed's surface

longer than 30 to 34 days and it is thus not considered seed-borne (Jianren and Chuandao, 1988).

## DISEASE MANAGEMENT

Several measures have been suggested to prevent BSNB during plantation establishment. The most effective is to plant disease-free seedlings of superior quality (Cordell *et al.*, 1990; Skilling and Nicholls, 1974). It is also advisable to avoid establishing new plantations alongside old, infected pines that could potentially serve as reservoirs of inoculum (Tainter and Baker, 1996). For natural pine stands, the application of thinning treatments was investigated as a silvicultural practice against pine needle diseases (McIntire *et al.*, 2018). This practice, conducted on native stands of *P. strobus* in the USA, showed promise in reducing the fungal load of *L. acicola*, resulting in reduced severity of the disease over time in stands already infected with the pathogen (McIntire *et al.*, 2018). This practice is recommended as a preventative measure in stands that are at risk of infection by *L. acicola* and other pine needle pathogens (McIntire *et al.*, 2018).

Pruning of infected pines can contribute to the spread of BSNB if it is conducted during rainy or wet periods. This is because conidia are exuded during these conditions and can attach to the pruning shears, providing a means of spread from infected to healthy trees (Skilling and Nicholls, 1974). Cutting blades should be cleaned during pruning and clipped needles and shoots should be removed (Kais, 1978). In the case of infection on *Pinus palustris*, which begins growth as a grass stage, stimulation of growth during the first 3 years of growth reduces the levels of infection (Tainter and Baker, 1996). Because this treatment is economical, effective and environmentally safe, it is widely used in the southeastern USA (Cordell *et al.*, 1990), where BSNB occurs on *P. palustris*.

Breeding for resistance to *L. acicola* has been successfully used to reduce the impact of the disease on *P. palustris* in Alabama. The source population of these trees found in southwestern Alabama was used in breeding programmes (Snyder and Derr, 1972) where seed was made available to the public (Phelps *et al.*, 1978). Since 1982, resistant phenotypes of *P. elliotii* have also been selected for in plantations affected by BSNB in the Fujian province in China. Over time, and using artificial inoculations, resistant clones were selected and resistant seed orchards were established (Ye and Wu, 2011).

Fungicide treatment can protect pine seedlings from infection by *L. acicola*. For example, when *P. palustris* was sprayed with fungicide, the seedlings displayed increased diameter growth in a single growing season, compared to untreated plants (Siggers, 1932). Seedlings, seed orchard trees and Christmas tree plantations have been protected by Bordeaux mixture of copper sulphate and lime, which inhibits conidial germination, by a benomyl root treatment or by ferbam (Fermate®). Chlorothalonil,

a broad-spectrum organochlorine pesticide (products include Bravo®, Daconil® and Maneb®), has also been applied to provide efficient control against BSNB. Chlorothalonil is also very effective against *Lophodermium* needle cast, which could be advantageous when both pathogens are present (Cordell *et al.*, 1990; Kais *et al.*, 1986; Skilling and Nicholls, 1974). Practical details and recommendations concerning fungicide treatment can be found in Skilling and Nicholls (1974). However, the use of chemicals is not considered a desirable solution for disease control due to negative environmental factors and many of these treatments are no longer available.

Controlled burning in pine forests can eliminate competing vegetation and reduce the impact of needle pathogens, especially in *P. palustris* where a grass stage is relevant (Barnett, 1999; Chapman, 1932). This pine species is completely adapted to survive fires as it concentrates all its energy into root development during the first 5 years of growth (Chapman, 1932). Siggers (1934) showed that a single controlled fire can significantly decrease BSNB in *P. palustris* until the next season and that during the initial growth stage, before seedlings begin to increase in height, a winter burn every 3 years is the most beneficial for disease control. The efficacy of controlled burns differs depending on the *Pinus* spp. involved and on the ability to tolerate fire damage.

In countries and regions where *L. acicola* is a quarantine organism, it is suggested that complete eradication of diseased trees or pine stands should be performed once the disease is detected (Pehl and Cech, 2008). This is achieved by felling and burning of infected trees and litter found under infected trees (Sosnowski *et al.*, 2009). In Lithuania, for instance, after positive identification of the pathogen in the Curonian Spit in 2009 and 2010, effective eradication measures were implemented (Markovskaja *et al.*, 2011). Due to this rapid action, the disease has remained under control in that country and is under constant monitoring by the state plant service of the Ministry of Agriculture of Lithuania (<https://gd.eppo.int/taxon/SCIRAC/distribution/LT>). Eradication efforts are, however, not always effective, and the best preventative method is to limit the movement of plant material across borders and between regions. As new knowledge is emerging regarding different genetic entities of the pathogen, including strains of different mating types (Sadiković *et al.*, 2019), the importance of avoiding new introductions is becoming increasingly obvious.

## HOST RANGE, HOST SUSCEPTIBILITY AND GEOGRAPHIC DISTRIBUTION

In an effort to consolidate 140 years of literature with regards to the geographical distribution and host range of *L. acicola*, a detailed list of these data has been compiled (Table 1). This shows that the pathogen has been reported in 31 countries and on 53

pine species and pine hybrids. The majority of the host records on native and non-native trees are from the Americas, followed by Europe. The pathogen has not been found in Africa, Australia or New Zealand and in South America it is known only in Colombia. Of the 69 reports of the pathogen (Table 1), 31 were made in the last decade (2009–2019). This suggests that incidences of the pathogen are most likely increasing.

In North America, the first report of *L. acicola* was in 1876 on native *Pinus echinata* as *Cryptosporium aciculum* (de Thümen, 1878). Since then, the pathogen has been reported in the USA on several susceptible species, including non-native *P. caribaea* and *P. pinea*, and native *P. elliotii*, *P. echinata*, *P. glabra*, *P. ponderosa*, *P. rigida*, *P. taeda* and *P. virginiana* (Hedgcock, 1929; Siggers, 1944; Sinclair and Lyon, 2005; Webster, 1930) as well as on regionally planted exotic species such as *P. attenuata*, *P. coulteri*, *P. muricata* and *P. sabiniana* (Siggers, 1944). *Pinus palustris* seedlings are the most severely affected, largely due to the grass stage associated with early growth and where BSNB can cause complete defoliation (Siggers, 1934). Here it can result in mortality reaching 50% and higher in the southeastern USA (Cordell *et al.*, 1990). New reports of *L. acicola* causing damage on *P. strobus* have emerged since 2005 in the northeastern USA and Canada and these have been attributed to changes in precipitation and climate in the regions (Broders *et al.*, 2015; Wyka *et al.*, 2017, 2018). *Lecanosticta acicola* is also recognized as a component of a complex of pathogens that cause white pine needle damage (WPND) in this region (Broders *et al.*, 2015). Additionally, the pathogen has been reported on *P. banksiana* and *P. contorta* var. *latifolia* in Canada (Laut *et al.*, 1966).

*Lecanosticta acicola* has been reported from 17 European countries (for a complete list of records see Table 1). The pathogen was first recorded in northern Spain in 1942 (Martínez, 1942), where it still occurs on *P. radiata* (Ortiz de Urbina *et al.*, 2017). In southwest Europe, *L. acicola* has caused severe defoliation of *P. radiata* × *P. attenuata*, leading to the felling of 100 ha in the 1990s (Lévy, 1996). *Lecanosticta acicola* is spreading through the valleys in the Alps in Switzerland (Holdenrieder and Sieber, 1995), Austria (Cech, 1997; Hintsteiner *et al.*, 2012), Italy (La Porta and Capretti, 2000) and Slovenia (Jurc and Jurc, 2010; Sadiković *et al.*, 2019), which can be attributed to high humidity in deep valleys or the proximity of lakes. In Europe, *L. acicola* often infects *P. mugo*, a susceptible species on which it has recently caused severe outbreaks in Austria (<https://gd.eppo.int/reporting/article-5139>). It also infects other pine species such as *P. sylvestris* and *P. nigra*. The pathogen has been recorded in several peat bog sites in southern Bavaria (Germany) and southern Bohemia (Czech Republic). These locations are naturally humid throughout the year and the susceptible pine species *P. mugo* and/or *P. uncinata* subsp. *uliginosa* can be heavily infected, leading to considerable mortality. Similarly, *L. acicola* was recorded in the Baltic states (Drenkhan and Hanso, 2009) and, most recently,

also in Sweden (Cleary *et al.*, 2019). These records usually come from stands close to the sea or, very frequently, from botanical gardens or urban areas.

Other pine species such as *Pinus* × *rhaetica* and *P. ponderosa* have also been affected by *L. acicola* (Adamson *et al.*, 2015, 2018). *Lecanosticta acicola* has been present in Croatia on *P. halepensis* for more than 40 years (Milatović, 1976; Sadiković *et al.*, 2019). Interestingly, the pathogen was identified only at a single site in Ireland despite large-scale screening throughout the British Isles (Mullett *et al.*, 2018). From all these records, it is reasonable to conclude that *L. acicola* is spreading in Europe in native and non-native pine species, in plantations and natural forests, and associated with different climatic conditions.

In Asia, BSNB has been reported in China in plantations of non-native *P. thunbergii*, *P. elliotii* and *P. taeda* where the trees were severely damaged by the pathogen (Huang *et al.*, 1995), and on *P. caribaea*, *P. palustris*, *P. clausa* and *P. echinata* that were reported to be susceptible to infection (Li *et al.*, 1986). It was suggested that native pines such as *P. taiwanensis*, *P. fenzeliana* and *P. massoniana* were highly resistant to infection (Huang *et al.*, 1995; Li *et al.*, 1986). BSNB has been reported on native *P. thunbergii* in Japan (Suto and Ougi, 1998) as well as on native *P. thunbergii* in South Korea but the disease was not severe (Seo *et al.*, 2012).

Although some species of *Pinus* seem to not be susceptible to infection by *L. acicola*, the pathogen has the potential to overcome host resistance in a favourable environment and expand its host range, as is suggested for *D. septosporum* and *D. pini* (Drenkhan *et al.*, 2016). For example, *L. acicola* is rarely reported on native *P. sylvestris* in Europe. Considering the importance of *P. sylvestris* in Europe, it will be important to monitor the presence of the pathogen on this host. Only single incidences of *L. acicola* have been reported on *P. sylvestris* in Austria (Cech and Krehan, 2008), Slovenia (Jurc and Jurc, 2010) and most recently in Estonia (Adamson *et al.*, 2018) and Ireland (Mullett *et al.*, 2018). In contrast, *L. acicola* is an important pathogen of *P. sylvestris* grown as part of the Christmas tree industry since the 1960s in the USA (Skilling and Nicholls, 1974). This implies that under favourable conditions this host could be infected by the pathogen. Investigations on the impact of DNB on *P. sylvestris* revealed that there is high intraspecific variability of *P. sylvestris* in Europe and that susceptibility of the host to the pathogen varies between individuals (Perry *et al.*, 2016a,b) and this could also influence the potential importance of *L. acicola*. Unusually high humidity associated with climate change could increase pathogen pressure on *P. sylvestris* (Perry *et al.*, 2016a) and the single incidences in Europe should carefully be monitored. Caution must also be taken when planting susceptible exotic hosts alongside native forests, as this could influence the vulnerability of native forests (Piotrowska *et al.*, 2018).



Of the 69 reports of *L. acicola*, only 22 used DNA sequence comparisons for species verification. This is of concern as there might be an over- or underestimation of hosts affected by BSNB globally. In Central America, for example, *L. acicola* was reported based on identifications using morphological characters. Because the pathogen has not yet been confirmed as occurring in this region using DNA sequences (Quaedvlieg *et al.*, 2012; van der Nest *et al.*, 2019), those reports could be erroneous and may represent different species which could possibly cause new outbreaks if not contained in their native environment.

## MOLECULAR DIAGNOSTICS AND FUTURE PROSPECTS

### Molecular markers used for species identification

Three molecular methods are currently being used to accurately identify *L. acicola*. These include sequencing of various gene regions, an ITS-RFLP method and a conventional PCR that uses species-specific primers. The most common of these approaches is comparison of DNA sequences for the ITS gene region (Adamson *et al.*, 2015, 2018; Cleary *et al.*, 2019; Markovskaja *et al.*, 2011; Mullett *et al.*, 2018). However, the *TEF 1* (Fig. 3) and *BT 2* gene regions have been recommended to distinguish between species of the Mycosphaerellaceae (Quaedvlieg *et al.*, 2012). In order to accurately distinguish between different species of *Lecanosticta*, van der Nest *et al.* (2019) used a multi-gene phylogenetic approach using sequences for the ITS, *TEF 1*, *BT 1*, *MS204* and *RPB 2* gene regions. The outcome was the discovery of four new species, with the ITS and *TEF 1* proving to be the gene regions showing the best amplification success across all species. Pehl *et al.* (2004) developed an ITS-RFLP method to distinguish between *L. acicola*, *D. septosporum* and ten other plant pathogens. However, whether this method remains valid after the recognition of various new species (van der Nest *et al.*, 2019) will need to be established.

Another rapid method allowing for the identification of *L. acicola*, *D. septosporum* and *D. pini* is a conventional PCR that uses species-specific primers (loos *et al.*, 2010). These were developed to partially amplify the *TEF 1* gene for *L. acicola* and *D. pini*, and partially amplify the *BT 2* gene region in *D. septosporum* (loos *et al.*, 2010). Importantly, this method can be used to identify the pathogens directly from infected needles (Adamson *et al.*, 2015; Ortíz de Urbina *et al.*, 2017; Schneider *et al.*, 2019) and is now widely used for preliminary identification of *L. acicola* (Adamson *et al.*, 2018; Sadiković *et al.*, 2019). A multiplex qPCR was also recently developed to detect *L. acicola* as well as *Dothistroma* species from needles simultaneously using probe-labelled primers developed by loos *et al.* (2010) and Schneider *et al.* (2019),

which could become more widely used once that technology is more easily available.

### Population genetic studies

Knowledge regarding the population structure and diversity of pathogens such as *L. acicola* allow for an understanding of migration patterns as well various aspects of their invasion biology. Eleven polymorphic microsatellite markers and mating type primers have been developed for this purpose (Janoušek *et al.*, 2014). The first population genetic study using these markers revealed that two lineages of *L. acicola* were introduced into Europe, possibly on two separate occasions (Janoušek *et al.*, 2016). These results are similar to an earlier study where RAPD analysis of *L. acicola*, collected in the northern and southern parts of the USA and China, showed that the Chinese population originated from the southern USA and that the collection from the northern USA was unique (Huang *et al.*, 1995). A second population genetic study compared populations from Croatia and Slovenia and revealed four distinct populations with possible introductions from other sources within the two countries (Sadiković *et al.*, 2019). Currently available knowledge suggests a Northern American centre of origin for this pathogen (Huang *et al.*, 1995; Janoušek *et al.*, 2016; van der Nest *et al.*, 2019) but further sampling and analyses are required to support this hypothesis. In the population genetic study of Janoušek *et al.* (2016), the microsatellite markers amplified poorly for the *L. acicola* isolates from Mexico and Central America. A later study (van der Nest *et al.*, 2019) showed that these isolates were *L. variabilis*, a new and recently described species.

The study by Janoušek *et al.* (2014) showed that *L. acicola* is heterothallic and that two individuals, one with a *MAT1-1-1* idiomorph and the other with a *MAT1-2* idiomorph, are needed for sexual reproduction to occur. Consequently, to understand whether sexual recombination might occur in a region, it is important to have a knowledge of the mating type idiomorph distribution. Mating type primers that amplify the *MAT1-1-1* and *MAT1-2* idiomorphs and that tested positive for *Dothistroma* species as well as *L. acicola*, *L. guatemalensis* and *L. gloeospora* have been developed (Janoušek *et al.*, 2014). It is, however, not yet known whether these markers will amplify these gene regions for the other, newly described *Lecanosticta* species.

Janoušek *et al.* (2016) considered the global *L. acicola* population and showed that the ratio of mating type idiomorphs in Mississippi, Austria, France and Germany reflected sexual recombination in these regions/countries. In contrast, only asexual reproduction occurs in the Czech Republic and northern parts of America. Using the mating type markers of Janoušek *et al.* (2014), the distribution of *MAT1* and *MAT2* isolates was detected in studies with isolates from Croatia (Sadiković *et al.*, 2019), Estonia (Adamson *et al.*, 2015, 2018), Ireland, Portugal, Russia (Mullett

*et al.*, 2018) as well as Spain (Ortiz de Urbina *et al.*, 2017). In Spain, both mating types were detected whereas only single mating types were detected in all other areas studied. However, in Estonia it was suggested that a second introduction of the pathogen occurred since only MAT1 was initially present but that later both mating types were detected in the same region (Adamson *et al.*, 2015). In populations with equal ratios of mating types or with both mating types present, sexual reproduction could occur, possibly giving rise to more virulent strains. This emphasizes a need to exercise caution and thus to prevent introduction of new strains into regions where the pathogen is already present.

### Future prospects in the age of genomics

Canada's Michael Smith Genome Sciences Centre has recently released a full genome for a *L. acicola* isolate from France ([https://www.ncbi.nlm.nih.gov/assembly/GCA\\_000504345.2#/def](https://www.ncbi.nlm.nih.gov/assembly/GCA_000504345.2#/def)). This genome has not yet been annotated but provides a valuable resource for future studies. Many other genomes of Dothidiomycetes, which have been sequenced and annotated, are available for comparative purposes (de Wit *et al.*, 2012; Ohm *et al.*, 2012). Annotation of putative genes of the *L. acicola* genome, utilizing knowledge of these other genomes, will provide insights into questions regarding many aspects of the biology of *L. acicola*. Opportunities also now arise to sequence the genomes of other *Lecanosticta* spp. and to compare these in order to better understand their relative importance. It will also be possible to follow the *Dothistroma* example where a transcriptomic study considered which genes are expressed during various stages in the infection of *P. radiata* (Bradshaw *et al.*, 2016) and genome sequencing of global representatives of *D. septosporum* revealed that gene copy numbers could play a role in dothistromin production by the pathogen (Bradshaw *et al.*, 2019).

### CONCLUSIONS

*Lecanosticta acicola* has been known in the southern USA for many decades. Consequently, its life cycle, mode of infection, host susceptibility and strategies to prevent infection, particularly on *P. palustris*, have been extensively studied in that region. Yet there is evidence to show that the pathogen, which now has an extensive host range, is spreading rapidly northwards. The reasons for this host range and geographical expansion require further study. Contemporary knowledge has also shown that there have been two introductions of *L. acicola* into Europe. Consequently, BSNB is becoming a disease of great concern in Europe, where it is increasingly being discovered on both non-native and native *Pinus* spp. There are many relevant hypotheses to explain the growing importance of BSNB and these include the effects of climate change, emergence of more aggressive strains of the pathogen and anthropogenic processes leading to new introductions. There is clearly a need for increased attention to and studies of *L. acicola*, particularly in Europe.

Recent studies have shown that there are eight species of *Lecanosticta* in addition to *L. acicola*. All of these other species appear to have a Mesoamerican origin. Much of the literature pertaining to *L. acicola* needs to be reconsidered given the fact that a single name has been widely used to refer to what we now know represents numerous cryptic species. *Lecanosticta acicola* identified based on DNA sequence comparisons has not been found in Central America, suggesting a North American centre of origin. Of the 69 reports of *L. acicola*, only 25 from 12 countries have been confirmed using DNA sequence-based tools (Table 1). Many reports of the pathogen could thus be erroneous and there is an urgent need to resolve this important question.

All the available knowledge regarding BSNB relates to studies on *L. acicola* and these are predominantly from the USA. Nothing is known regarding the relative importance of the remaining eight species of *Lecanosticta*. At least some of these are most likely also important pathogens and their relative threat to global forests and forestry needs to be assessed. A concerted effort must be made to prevent their accidental introduction into new regions of the world and as part of this process DNA sequence-based techniques need to be routinely applied to allow for meaningful identification.

The development of new tools to study *Lecanosticta* spp. and BSNB provides many exciting opportunities to enhance our knowledge of this important group of pathogens. The population structure and diversity of *L. acicola* can now be easily studied in the USA as well as where new invasions occur in Europe, and at levels that were previously not possible. For example, application of the available microsatellite markers will enable a more comprehensive understanding of the pathogen as well as determination of its centre of origin.

Genome sequencing is rapidly becoming cheaper and more readily available, and an isolate of *L. acicola* is already available in the public domain for study. We envisage that all the species of *Lecanosticta* will be sequenced in the relatively near future and many isolates of some species will likely also be studied at this level. These studies, and others relating to the 'omics' level, will surely have a substantial impact on our understanding of a group of pathogens that is growing in importance and relevance. Overall, BSNB (including all species of *Lecanosticta*) has the potential to become a pine needle disease of global importance if proper preventative measures for the spread of the causal pathogens are not implemented.

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## ACCESSION NUMBERS

The aligned dataset used to draw Fig. 3 is deposited in TreeBASE (No. S24301).

## REFERENCES

- Adamson, K., Drenkhan, R. and Hanso, M. (2015) Invasive brown spot needle blight caused by *Lecanosticta acicola* in Estonia. *Scand. J. Forest Res.* **30**, 587–593.
- Adamson, K., Laas, M., Drenkhan, R. and Hanso, M. (2018) Quarantine pathogen *Lecanosticta acicola*, observed at its jump from an exotic host to the native Scots pine in Estonia. *Balt. For.* **24**, 36–41.
- Alvère, M., Aumonier, T. and Kersaudy, E. (2010) Bilan sylvosanitaire 2009 pour Midi-Pyrénées. In: Service régional de l'alimentation. (Département de la Santé des Forêts, ed), pp. 1–7. Direction régionale de l'alimentation, de l'agriculture et de la forêt d'Aquitaine, République Française.
- Angst, A. (2011) Braune Föhren in Gärten und Parks. *Wald Holz*, **92**, 41–42.
- Anonymous (2012) First report of *Mycosphaerella dearnessii* in Latvia. *Eur. Med. Plant Protec. Org. Bull.*, **8**, 5–6.
- von Arx, J.A. (1983) *Mycosphaerella* and its anamorphs. *P. K. Ned. Akad. C Biol.* **86**, 15–54.
- Barnes, I., Crous, P.W., Wingfield, B.D. and Wingfield, M.J. (2004) Multigene phylogenies reveal that red band needle blight of *Pinus* is caused by two distinct species of *Dothistroma*, *D. septosporum* and *D. pini*. *Stud. Mycol.* **50**, 551–565.
- Barnes, I., Wingfield, M.J., Carbone, I., Kirisits, T. and Wingfield, B.D. (2014) Population structure and diversity of an invasive pine needle pathogen reflects anthropogenic activity. *Ecol. Evol.* **4**, 3642–3661.
- Barnes, I., van der Nest, A., Mullett, M.S., Crous, P.W., Drenkhan, R., Musolin, D.L. and Wingfield, M.J. (2016) Neotypification of *Dothistroma septosporum* and epitypification of *D. pini*, causal agents of Dothistroma needle blight of pine. *Forest Pathol.* **46**, 388–407.
- Barnett, J.P. (1999) Longleaf pine ecosystem restoration: the role of fire. *J. Sustain. Forest.* **9**, 89–96.
- Barr, M.E. (1972) Preliminary studies on the Dothideales in temperate North America. *Contributions from the University of Michigan Herbarium*, **9**, 523–638.
- Barr, M.E. (1996) Planistromellaeae, a new family in the Dothideales. *Mycotaxon*, **60**, 433–442.
- Boyce, J.S. (1959) Brown spot needle blight on eastern white pine. *Plant Dis. Rep.* **43**, 420.
- Boyer, W.D. (1972) Brown-spot resistance in natural stands of longleaf pine seedlings. *US Department of Agriculture Forest Service Research Paper*, **SO-142**, 1–4.
- Bradshaw, R.E., Guo, Y., Sim, A.D., Kabir, M.S., Chettri, P., Ozturk, I.K., Hunziker, L., Ganley, R.J. and Cox, M.P. (2016) Genome-wide gene expression dynamics of the fungal pathogen *Dothistroma septosporum* throughout its infection cycle of the gymnosperm host *Pinus radiata*. *Mol. Plant Pathol.* **17**, 210–224.
- Bradshaw, R.E., Sim, A.D., Chettri, P., Dupont, P.-Y., Guo, Y., Hunziker, L., McDougal, R.L., van der Nest, A., Fourie, A., Wheeler, D., Cox, M.P. and Barnes, I. (2019) Global population genomics of the forest pathogen *Dothistroma septosporum* suggest that chromosome duplications influence virulence factor levels. *Mol. Plant Pathol.* **20**, 784–799.
- Brandstetter, M. and Cech, T.L. (1999) Neue Nadelkrankheiten an Kiefer. *Osterreichische Forstzeitung*, **99**, 35–36.
- Brandstetter, M. and Cech, T. (2003) *Lecanosticta* – Kiefernadelbräune (*Mycosphaerella dearnessii* Barr) in Niederösterreich. *Centralblatt für das gesamte forstwesen*, **120**, 163–176.
- Braze, N.J. (2016) Dramatic needle browning and canopy dieback of eastern white pine (*Pinus strobus*) in southern New England. (UMass Extension, Amherst, University of Massachusetts, ed), pp. 1–5. Massachusetts: United States Department of Agriculture.
- Brodgers, K., Munck, I.A., Wyka, S.A., Iriarte, G. and Beaudoin, E. (2015) Characterization of fungal pathogens associated with white pine needle damage (WPND) in northeastern North America. *Forests*, **6**, 4088–4104.
- Cech, T.L. (1997) Brown spot disease in Austria – the beginning of an epidemic. *Forstschutz Aktuell*, **19**, 17.
- Cech, T.L. and Krehan, H. (2008) *Lecanosticta*-Krankheit der Kiefer erstmals im Wald nachgewiesen. *Forstschutz Aktuell*, **45**, 4–5.
- Chapman, H.H. (1926) Factors determining natural reproduction of longleaf pine on cut-over lands in LaSalle Parish, Louisiana. *Yale School of Forestry Bulletin*, **16**, 1–44.
- Chapman, H.H. (1932) Is the longleaf type a climax? *Ecology*, **13**, 328–334.
- Cleary, M., Laas, M., Oskay, F. and Drenkhan, R. (2019) First report of *Lecanosticta acicola* on non-native *Pinus mugo* in southern Sweden. *Forest Pathol.* **49**, e12507.
- Cordell, C.E., Anderson, R.L. and Kais, A.G. (1990) Brown-Spot needle blight. In: Southwide Forest Disease Workshop, (Boone, A.J., Anderson, R.L., Fenn, P., Powers, H.R. and Stambaugh, W.J., eds.) pp. 18–19. Charleston, South Carolina: South Carolina Forestry Commission, Insect and Disease Section, Columbia, South Carolina.
- Crous, P.W. (1999) Species of *Mycosphaerella* and related anamorphs occurring on Myrtaceae (excluding *Eucalyptus*). *Mycol. Res.* **103**, 607–621.
- Crous, P.W. (2009) Taxonomy and phylogeny of the genus *Mycosphaerella* and its anamorphs. *Fungal Divers.* **38**, 1–24.
- Crous, P.W., Braun, U. and Groenewald, J.Z. (2007) *Mycosphaerella* is polyphyletic. *Stud. Mycol.*, **58**, 1–32.
- Crous, P.W., Kang, J. and Braun, U. (2001) A phylogenetic redefinition of anamorph genera in *Mycosphaerella* based on ITS rDNA sequence and morphology. *Mycologia*, **93**, 1081–1101.
- Crous, P.W., Summerell, B.A., Carnegie, A.J., Wingfield, M.J., Hunter, G.C., Burgess, T.I., Andjic, V., Barber, P.A. and Groenewald, J.Z. (2009) Unravelling *Mycosphaerella*: Do you believe in genera? *Persoonia*, **23**, 99–118.
- Dearness, J. (1926) New and noteworthy fungi: IV. *Mycologia*, **18**, 236–255.
- Dearness, J. (1928) New and noteworthy fungi V. *Mycologia*, **20**, 235–246.
- Drenkhan, R. and Hanso, M. (2009) Recent invasion of foliage fungi of pines (*Pinus* spp.) to the Northern Baltics. *Forestry Studies*, **51**, 49–64.
- Drenkhan, R., Tomešová-Haataja, V., Fraser, S., Bradshaw, R.E., Vahalík, P., Mullett, M.S., Martín-García, J., Bulman, L.S., Wingfield, M.J., Kirisits, T., Cech, T.L., Schmitz, S., Baden, R., Tubby, K., Brown, A., Georgieva, M., Woods, A., Ahumada, R., Jankovský, L., Thomsen, I.M., Adamson, K., Marçais, B., Vuorinen, M., Tsoelas, P., Koltay, A., Halasz, A., La Porta, N., Anselmi, N., Kiesnere, R., Markovskaja, S., Kačergius, A., Papazova-Anakieva, I., Risteski, M., Sotirovski, K., Lazarević, J., Solheim, H., Boroš, P., Bragança, H., Chira, D., Musolin, D.L., Selikhovkin, A.V., Bulgakov, T.S., Keča, N., Karadžić, D., Galović, V., Pap, P., Markovic, M., Poljakovic Pajnik, L., Vasic, V., Ondrušková, E., Piškur, B., Sadiković, D., Diez, J.J., Solla, A., Millberg, H., Stenlid, J., Angst, A., Queloz, V., Lehtijärvi, A., Doğmuş-Lehtijärvi, H.T., Oskay, F., Davydenko, K., Meshkova, V., Craig, D., Woodward, S. and Barnes, I. (2016) Global geographic distribution and host range of *Dothistroma* species: a comprehensive review. *Forest Pathol.* **46**, 408–442.

- EPPO (2012a) First report of *Mycosphaerella dearnessii* in Latvia. EPPO Reporting Service no. 8. Num. article: 2012/168. Available at <https://gd.eppo.int/reporting/article-2374> [accessed on April 17, 2019].
- EPPO (2012b) *Mycosphaerella dearnessii* detected again in Lithuania. EPPO Reporting Service no. 11. Num. article: 2012/240. Available at <https://gd.eppo.int/reporting/article-2446> [accessed on April 17, 2019].
- EPPO (2015) Outbreak of *Lecanosticta acicola* in Tyrol, Austria. EPPO Reporting Service no. 10. Num. article: 2015/192. Available at <https://gd.eppo.int/reporting/article-5139> [accessed on April 17, 2019].
- EPPO (2018) New data on quarantine pests and pests of the EPPO Alert List. EPPO Reporting Service no. 11. Num. article: 2018/212. Available at <https://gd.eppo.int/reporting/article-6406> [accessed on April 17, 2019].
- Evans, H.C. (1984) The genus *Mycosphaerella* and its anamorphs *Cercoseptoria*, *Dothistroma* and *Lecanosticta* on pines. *Myc. Papers*, **153**, 1–102.
- Gadgil, P.D. (1967) Infection of *Pinus radiata* needles by *Dothistroma pini*. *New Zeal. J. Bot.* **5**, 498–503.
- Gibbs, J. and Sinclair, S. (2008) Forest insect and disease conditions in Vermont 2008: highlights and management recommendations. In: Vermont Forest Insect and Disease Highlights. pp. 1–3. Waterbury, Vermont: Agency of Natural Resources, Department of Forests, Parks & Recreation.
- Gibson, I.A.S. (1980) Two pine needle fungi new to Colombia. *Trop. Pest Manage.* **26**, 38–40.
- Glavaš, M. and Margaletić, J. (2001) Brown spot needle blight of aleppo pine and protection (English abstract). In: Znanost u potrajnom gospodarenju hrvatskim šumama: znanstvena knjiga, (Matić, S., Krpanj, A., Gračan, J., eds), pp. 277–284. Jastrebarsko, Croatia/Zagreb, Croatia: šumarski fakultet.
- Hawksworth, D.L., Crous, P.W., Redhead, S.A., Reynolds, D.R., Samson, R.A., Seifert, K.A., Taylor, J.W., Wingfield, M.J., Abaci, Ö., Aime, C., Asan, A., Bai, F.-Y., de Beer, Z.W., Begerow, D., Berikten, D., Boekhout, T., Buchanan, P.K., Burgess, T., Buzina, W., Cai, L., Cannon, P.F., Crane, J.L., Damm, U., Daniel, H.-M., van Diepeningen, A.D., Druzhinina, I., Dyer, P.S., Eberhardt, U., Fell, J.W., Frisvad, J.C., Geiser, D.M., Geml, J., Glienke, C., Gräfenhan, T., Groenewald, J.Z., Groenewald, M., de Gruyter, J., Guého-Kellermann, E., Guo, L.-D., Hibbett, D.S., Hong, S.-B., de Hoog, G.S., Houbraken, J., Huhndorf, S.M., Hyde, K.D., Ismail, A., Johnston, P.R., Kadamficer, D.G., Kirk, P.M., Køljalg, U., Kurtzman, C.P., Lagneau, P.-E., Lévesque, C.A., Liu, X., Lombard, L., Meyer, W., Miller, A., Minter, D.W., Najafzadeh, M.J., Norvell, L., Ozerskaya, S.M., Öziç, R., Pennycook, S.R., Peterson, S.W., Pettersson, O.V., Quaedvlieg, W., Robert, V.A., Ruihal, C., Schnürer, J., Schroers, H.-J., Shivas, R., Slippers, B., Spierenburg, H., Takashima, M., Taşkın, E., Thines, M., Thrane, U., Uzman, A.H., van Raak, M., Varga, J., Vasco, A., Verkley, G., Videira, S.I.R., de Vries, R.P., Weir, B.S., Yilmaz, N., Yurkov, A. and Zhang, N. (2011) The Amsterdam declaration on fungal nomenclature. *IMA Fungus*, **2**, 105–112.
- Hedgcock, G.G. (1929) *Septoria acicola* and the brown-spot disease of pine needles. *Phytopathology*, **19**, 993–999.
- Henry, B.W. (1954) Sporulation by the brown spot fungus on longleaf pine needles. *Phytopathology*, **44**, 385–386.
- Hintsteiner, M., Cech, T.L., Halmshlager, E., Stauffer, C. and Kirisits, T. (2012) First report of *Mycosphaerella dearnessii* on *Pinus nigra* var. *nigra* in Austria. *Forest Pathol.* **42**, 437–440.
- Holdenrieder, O. and Sieber, T.N. (1995) First report of *Mycosphaerella dearnessii* in Switzerland. *Eur. J. For. Pathol.* **25**, 293–295.
- Huang, Z.-Y., Smalley, E.B. and Guries, R.P. (1995) Differentiation of *Mycosphaerella dearnessii* by cultural characters and RAPD analysis. *Phytopathology*, **85**, 522–527.
- Ioos, R., Fabre, B., Saurat, C., Fourrier, C., Frey, P. and Marçais, 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 blight of pine. *Am. Phytopathol. Soc.* **100**, 105–114.
- Jankovský, L., Palovčíková, D., Dvořák, M. and Tomšovský, M. (2009a) Records of brown spot needle blight related to *Lecanosticta acicola* in the Czech Republic. *Plant Protect. Sci.* **45**, 16–18.
- Jankovský, L., Palovčíková, D. and Tomšovský, M. (2009b) Brown spot needle blight associated with *Mycosphaerella dearnessii* occurs on *Pinus rotundata* in the Czech Republic. *Plant Pathol.* **58**, 398.
- Janoušek, J., Krumbock, S., Kirisits, T., Bradshaw, R.E., Barnes, I., Jankovský, L. and Stauffer, C. (2014) Development of microsatellite and mating type markers for the pine needle pathogen *Lecanosticta acicola*. *Australas. Plant Path.* **43**, 161–165.
- Janoušek, J., Wingfield, M.J., Monsivais, J.G., Jankovský, L., Stauffer, C., Konečný, A. and Barnes, I. (2016) Genetic analyses suggest separate introductions of the pine pathogen *Lecanosticta acicola* into Europe. *Phytopathology*, **106**, 1413–1425.
- Jewell, F.F. (1983) Histopathology of the brown spot fungus on longleaf pine needles. *Phytopathology*, **73**, 854–858.
- Jianren, Y. and Chuandao, L. (1988) Study on seed transmission of brown spot fungus (*Shcirrhia acicola*). *J. Nanjing Inst. For.* **12**, 21.
- Jurc, D. and Jurc, M. (2010) *Mycosphaerella dearnessii* occurs in Slovenia. *Plant Pathol.* **59**, 808.
- Kabir, M.S., Ganley, R.J. and Bradshaw, R.E. (2015) Dothistromin toxin is a virulence factor in dothistroma needle blight of pines. *Plant Pathol.* **64**, 225–234.
- Kais, A.G. (1971) Dispersal of *Schirria acicola* spores in southern Mississippi. *Plant Dis. Rep.* **55**, 309–311.
- Kais, A.G. (1972) Variation between southern and northern isolates of *Schirrhia acicola*. In: Abstracts of papers for presentation at the sixty-fourth annual meeting of the American Phytopathological Society, 6–10 August, pp. 768. Mexico City, Mexico.
- Kais, A.G. (1975) Environmental factors affecting brown-spot infection on longleaf pine. *Phytopathology*, **65**, 1389–1392.
- Kais, A.G. (1978) Pruning of longleaf pine seedlings in nurseries promotes brown-spot needle blight. *Tree Planter's Notes*, 3–4.
- Kais, A.G., Cordell, C.E. and Affeltranger, C.E. (1986) Benomyl root treatment controls brown-spot disease on longleaf pine in the Southern United States. *Forest Sci.* **32**, 506–511.
- Kessler, M. (2009) Aktuelle Verbreitung der Quarantänekrankheit *Lecanosticta-Nadelbräune* der Kiefer (*Mycosphaerella dearnessii* M. E. Barr) in Hollenstein/Ybbs. *Forstschutz Aktuell*, **48**, 29–30.
- Kessler, M. and Krehan, H. (2011) Neufunde von Quarantäneschadorganismen 2011 in Österreich. *Forstschutz Aktuell*, **53**, 14–16.
- Kovačevski, J.C. (1938) Parasitic fungi new for Bulgaria – Fifth contribution. *Spisanie na Zemedelskite izpitatelni instituti v Bulgariya.*, **8**, 3–13.
- Laflamme, G., Côté, C. and Innes, L. (2010) White pine needle diseases in eastern Canada. In: APS 2010 Northeastern Division Meeting Abstracts. Northampton, Massachusetts: Phytopathology, 101, S260.
- Laut, J.G., Sutton, B. and Lawrence, J. (1966) Brown spot needle blight in Canada. *Plant Dis. Rep.* **50**, 208.
- Lévy, A. (1996) Le point sur le champignon de quarantaine *Scirrhia acicola* dans le sud-ouest de la France. In: La Santé des Forêts, (Département de la Santé des Forêts, ed), pp. 28–30. France: Les Cahiers du DSF.
- Li, C., Zku, X., Han, Z., Zhang, J., Shen, G., Zhang, Z., Zheng, W., Zou, K. and Shi, F. (1986) Investigation on Brown-spot needle blight of pines in China. (English abstract). *J. Nanjing Inst. For.* **10**, 11–18.
- Li, C., Han, Z., Jianren, Y., Zheng, W., Zhang, Z., Zheng, R. and Zhang, H. (1987) Development of brown-spot needle blight in slash pine plantations (English abstract). *J. Nanjing Inst. For.* **1**, 1–7.

- Lopéz Castilla, R.A., Duarte Casanova, A., Guerra Rivero, C., Cruz Escoto, H. and Triguero Issasi, N. (2002) Forest Nursery pest management in Cuba. In: National Proceedings: Forest and Conservation Nursery Associations – 1999, 2000, 2001. (Dumroese, R.K., Riley, L.E., Landis, T.D., technical coordinators, eds), pp. 213–218. Ogden, UT: USDA Forest Service, Rocky Mountain Research Station.
- Luttrell, E.S. (1949) *Schirrhia acicola*, *Phaeocryptopus pinastri*, and *Lophodermium pinastri* associated with the decline of ponderosa pine in Missouri. *Plant Dis. Rep.* **33**, 397–401.
- Markovskaja, S., Kacergius, A. and Treigiene, A. (2011) Occurrence of new alien pathogenic fungus *Mycosphaerella dearnessii* in Lithuania. *Bot. Lith.* **17**, 29–37.
- Marmolejo, J.G. (2000) The genus *Lecanosticta* from Nuevo Leon, Mexico. *Mycotaxon*, **76**, 393–397.
- Martinez, J.B. (1942) The mycoses of *Pinus insignis* in Guipúzcoa. *Publ. Inst. for. Invest. Exp.* **13**, 1–72.
- McIntire, C.D., Munck, I.A., Ducey, M.J. and Asbjornsen, H. (2018) Thinning treatments reduce severity of foliar pathogens in eastern white pine. *Forest Ecol. Manag.* **423**, 106–113.
- Milatović, I. (1976) Needle cast of pines caused by fungi *Schirrhia pini* Funk et Parker and *Schirrhia acicola* (Dearn.) Siggers in Yugoslavia. *Poljoprivredna Znanstvena Smotra – Agriculturae Conspectus Scientificus*, **39**, 511–513.
- Mullett, M., Tubby, K., Webber, J. and Brown, A. (2016) A reconsideration of natural dispersal distances of the pine pathogen *Dothistroma septosporum*. *Plant Pathol.* **65**, 1462–1472.
- Mullett, M., Adamson, K., Bragança, H., Bulgakov, T., Georgieva, M., Henriques, J., Jürisoo, L., Laas, M. and Drenkhan, R. (2018) New country and regional records of the pine needle blight pathogens *Lecanosticta acicola*, *Dothistroma septosporum* and *Dothistroma pini*. *Forest Pathol.* **48**, e12440.
- Munck, I.A., Ostrofsky, W. and Burns, B. (2012) Pest Alert: Eastern white pine needle damage. NA-PR-01-11. In: USDA Forest Service, Northeastern Area State and Private Forestry. Newtown, PA, USA.
- van der Nest, A., Wingfield, M.J., Ortiz, P.C. and Barnes, I. (2019) Biodiversity of *Lecanosticta* pine-needle blight pathogens suggests a Mesoamerican Centre of origin. *IMA Fungus*. **1**, 2. <https://doi.org/10.1186/s43008-019-0004-8>.
- Nicholls, T.H. and Hudler, G.W. (1972) Red pine – a new host for brown spot (*Schirrhia acicola*). *Plant Dis. Rep.* **56**, 712–713.
- Ohm, R.A., Feau, N., Henriessat, B., Schoch, C.L., Horwitz, B.A., Barry, K.W., Condon, B.J., Copeland, A.C., Dhillon, B., Glaser, F., Hesse, C.N., Kosti, I., LaButti, K., Lindquist, E.A., Lucas, S., Salamov, A.A., Bradshaw, R.E., Ciuffetti, L., Hamelin, R.C., Kema, G.H.J., Lawrence, C., Scott, J.A., Spatafora, J.W., Turgeon, B.G., de Wit, P.J.G.M., Zhong, S., Goodwin, S.B. and Grigoriev, I.V. (2012) Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi. *PLoS Pathog.* **8**, e1003037.
- Ondrušková, E., Hečková-Jánošíková, Z., Adamčík, S., Kádasi Horáková, M., Rakúsová-Sládková, D. and Adamčíková, K. (2018) Needle blight caused by *Dothistroma pini* in Slovakia: distribution, host range and mating types. *Scand. J. Forest Res.* **1**–7.
- Ortiz de Urbina, E., Mesanza, N., Aragonés, A., Raposo, R., Elvira-Ruencano, M., Boqué, R., Patten, C., Aitken, J. and Iturrutxa, E. (2017) Emerging needle blight diseases in Atlantic *Pinus* ecosystems of Spain. *Forests*, **8**, 1–18.
- Patton, R.F. and Spear, R.N. (1978) Scanning electron microscopy of infection of Scotch pine needles by *Schirrhia acicola*. *Phytopathology*, **68**, 1700–1704.
- Pehl, V.L. (1995) Lecanosticta-Nadelbräune. Eine neue Kiefernkrankheit in der Bundesrepublik Deutschland. *Nachrichtenbl. Deut. Pflanzenschutzd.* **47**, 305–309.
- Pehl, L. and Cech, T. (2008) *Mycosphaerella dearnessii* and *Mycosphaerella pini*. *Eur. Med. Plant Protec. Org. Bull.*, **38**, 349–362.
- Pehl, L., Burgermeister, W. and Wulf, A. (2004) *Mycosphaerella*-Nadelpilze der Kiefer – Identifikation durch ITS-RFLP-Muster. *Nachrichtenbl. Deut. Pflanzenschutzd.* **56**, 239–244.
- Perry, A., Brown, A.V., Cavers, S., Cottrell, J.E. and Ennos, R.A. (2016a) Has Scots pine (*Pinus sylvestris*) coevolved with *Dothistroma septosporum* in Scotland? Evidence for spatial heterogeneity in the susceptibility of native provenances. *Evol. Appl.*, **9**, 982–993.
- Perry, A., Wachowiak, W., Brown, A.V., Ennos, R.A., Cottrell, J.E. and Cavers, S. (2016b) Substantial heritable variation for susceptibility to *Dothistroma septosporum* within populations of native British Scots pine (*Pinus sylvestris*). *Plant Pathol.*, **65**, 987–996.
- Petrak, V.F. (1954) *Phragmogloeum* n. gen. eine neue Gattung der Sphaeropsideen. *Sydowia*, **8**, 158–161.
- Petrak, F. (1961) Die Lecanosticta Krankheit der Föhren in Österreich. *Sydowia*, **15**, 252–256.
- Phelps, W.R., Kais, A.G. and Nicholls, T.H. (1978) Brown-spot needle blight of pines. US Department of Agriculture Forest Service, pp. 1–6.
- Piotrowska, M.J., Riddell, C., Hoebe, P.N. and Ennos, R.A. (2018) Planting exotic relatives has increased the threat posed by *Dothistroma septosporum* to the Caledonian pine populations of Scotland. *Evol. Appl.*, **11**, 350–363.
- La Porta, N. and Capretti, P. (2000) *Mycosphaerella dearnessii*, a needle-cast pathogen on mountain pine (*Pinus mugo*) in Italy. *Disease Notes*, **84**, 922.
- Prey, A.J. and Morse, F.S. (1971) Brown spot needle blight of scotch pine christmas trees in Wisconsin. *Plant Dis. Rep.* **55**, 648–649.
- Quaedvlieg, W., Groenewald, J.Z., Yáñez-Morales, M.D.J. and Crous, P.W. (2012) DNA barcoding of *Mycosphaerella* species of quarantine importance to Europe. *Persoonia*, **29**, 101–115.
- Rogerson, C.T. (1953) Kansas mycological notes: 1951. *Trans. Kans. Acad. Sci.* **56**, 53–57.
- Saccardo, P.A. (1884) Sylloge Fungorum. *Patavii, Italy*, **3**, 507.
- Sadiković, D., Piškur, B., Barnes, I., Hauptman, T., Diminić, D., Wingfield, M.J. and Jurc, D. (2019) Genetic diversity of the pine pathogen *Lecanosticta acicola* in Slovenia and Croatia. *Plant Pathol.* **68**, 1120–1131.
- Schneider, S., Jung, E., Queloz, V., Meyer, J.B. and Rigling, D. (2019) Detection of pine needle diseases caused by *Dothistroma septosporum*, *Dothistroma pini* and *Lecanosticta acicola* using different methodologies. *Forest Pathol.* **49**, e12495.
- Seo, S.T., Park, M.J., Park, J.H. and Shin, H.D. (2012) First report of brown spot needle blight on *Pinus thunbergii* caused by *Lecanosticta acicola* in Korea. *Disease Notes*, **96**, 914.
- Setliff, E.C. and Patton, R.F. (1974) Germination behaviour of *Schirrhia acicola* conidia on pine needles. *Phytopathology*, **64**, 1462–1464.
- Shishkina, A.K. and Tsanova, N.I. (1967) *Systemma acicola* (Dearn.) Wolf et Barbour: the perfect stage of *Dothistroma acicola* (Thüm.). A. Schischk. et N. Tzan. *Novosti Sist. Nizsh. Rast.* **276**–277.
- Siggers, P.V. (1932) The brown-spot needle blight of longleaf pine seedlings. *J. Forest.* **30**, 579–593.
- Siggers, P.V. (1934) Observations on the influence of fire on the brown-spot needle blight of longleaf pine seedlings. *J. Forest.* **32**, 556–562.
- Siggers, P.V. (1939) Phytopathological note. *Phytopathology*, **29**, 1076–1077.
- Siggers, P.V. (1944) The brown spot needle blight of pine seedlings. *US Department of Agriculture, Washington, D.C. Technical Bulletin*, **870**, 1–36.
- Sinclair, W.A. and Hudler, G.W. (1980) Tree and shrub pathogens new or noteworthy in New York state. *Plant Dis.* **64**, 590–592.
- Sinclair, W.A. and Lyon, H.H. (2005) *Mycosphaerella* diseases. In: *Diseases of Trees and Shrubs*. Ithaca and London: Cornell University Press.

- Skilling, D.D. and Nicholls, T.H.** (1974) Brown spot needle disease – biology and control in Scotch pine plantations. *US Department of Agriculture. Forest Service Research Paper*, NC-109, 1–19.
- Snyder, E.B. and Derr, H.J.** (1972) Breeding Longleaf pines for resistance to brown spot needle blight. *Phytopathology*, **62**, 325–329.
- Sosnowski, M.R., Fletcher, J.D., Daly, A.M., Rodoni, B.C. and Viljanen-Rollinson, S.L.H.** (2009) Techniques for the treatment, removal and disposal of host material during programmes for plant pathogen eradication. *Plant Pathol.* **58**, 621–635.
- Stanosz, G.** (1990) Premature needle drop and symptoms associated with brown spot needle blight on *Pinus strobus* in Northcentral Pennsylvania. In: The American Phytopathological Society, Northeastern Division Annual Meeting, pp. 124. *Phytopathology*.
- Suto, Y.** (2002) Seasonal development of symptoms and conidial production and dispersal of *Lecanosticta acicola* in *Pinus thunbergii*. *Appl. For. Sci.* **11**, 17–22.
- Suto, Y. and Ougi, D.** (1998) *Lecanosticta acicola*, causal fungus of brown spot needle blight in *Pinus thunbergii*, new to Japan. *Mycoscience*, **39**, 319–325.
- Sydow, H. and Petrak, F.** (1922) Ein Beitrag zur Kenntnis der Pilzflora Nordamerikas, insbesondere der nordwestlichen Staaten. *Annales Mycologici*, **20**, 178–218.
- Sydow, H. and Petrak, F.** (1924) Zweiter Beitrag zur Kenntnis der Pilzflora Nordamerikas, insbesondere der nordwestlichen Staaten. *Annales Mycologici*, **22**, 387–409.
- Tainter, F.H. and Baker, F.A.** (1996) Brown spot. In: Principles of Forest Pathology, pp. 467–492. New York, USA: John Wiley.
- de Thümen, F.** (1878) Fungorum americanorum triginta species novae. *Flora*, **61**, 177–184.
- de Wit, P.J., Van Der Burgt, A., Ökmen, B., Stergiopoulos, I., Abd-El Salam, K.A., Aerts, A.L., Bahkali, A.H., Beenen, H.G., Chettri, P. and Cox, M.P.** (2012) The genomes of the fungal plant pathogens *Cladosporium fulvum* and *Dothistroma septosporum* reveal adaptation to different hosts and lifestyles but also signatures of common ancestry. *PLoS Genet.* **8**, e1003088.
- Thyr, B.D. and Shaw, C.G.** (1964) Identity of the fungus causing red band disease on pines. *Mycologia*, **56**, 103–109.
- Wakeley, P.C.** (1970) Thirty-year effects of uncontrolled brown spot on planted longleaf pine. *Forest Sci.* **16**, 197–202.
- Webster, C.B.** (1930) Comments on "Brown-Spot" disease of pine needles in Texas. *J. Forest.* **30**, 763–778.
- Wingfield, M.J., Brouckhoff, E.G., Wingfield, B.D. and Slippers, B.** (2015) Planted forest health: the need for a global strategy. *Science*, **349**, 832–836.
- Wolf, F.A. and Barbour, W.J.** (1941) Brown-spot needle disease of pines. *Phytopathology*, **31**, 61–73.
- Wyka, S.A., Smith, C., Munck, I.A., Rock, B.N., Ziniti, B.L. and Broders, K.** (2017) Emergence of white pine needle damage in the northeastern United States is associated with changes in pathogen pressure in response to climate change. *Glob. Change Biol.* **23**, 394–405.
- Wyka, S.A., McIntire, C.D., Smith, C., Munck, I.A., Rock, B.N., Asbjornsen, H. and Broders, K.D.** (2018) Effect of climatic variables on abundance and dispersal of *Lecanosticta acicola* spores and their impact on defoliation on eastern white pine. *Phytopathology*, **108**, 374–383.
- Yang, B., Ye, J., Bao, H., Liu, J. and Dong, Z.** (2002) Studies on the phytotoxic activities of LA-I and LA-II production by brown spot needle blight fungus (*Lecanosticta acicola*). *Sci. Silvae Sin.* **38**, 84–88.
- Yang, B., Ye, J., Bao, H., Lui, J. and Dong, Z.** (2005) Separation and purification, structure of LA-I toxin produced by brown spot needle blight fungus (*Lecanosticta acicola*). *Sci. Silvae Sin.* **41**, 86–90.
- Ye, J. and Qi, G.** (1999) Studies on the biological specialization of the toxin producing by brown spot needle blight fungus. *J. Nanjing Inst. For.* **23**, 1–4.
- Ye, J. and Wu, X.** (2011) Resistance in nature systems to brown-spot needle blight of pine in China. In: Fourth international workshop on the genetics of host–parasite interactions in forestry, pp. 107. Eugene, Oregon, USA: US Department of Agriculture.