Lecanosticta acicola: Biosecurity readiness for New Zealand's plantation forestry

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Basque Country, Spain (Photo: Rebecca McDougal, Scion)

Report information sheet

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Executive summary

The problem

Lecanosticta acicola is a pathogen of pines that is spreading, causing disease and mortality in forests globally. It is major threat to *Pinus radiata* plantation forestry in New Zealand but is not currently present.

Client initiatives

The New Zealand Forest Owners Association (FOA) and the New Zealand Farm Forestry Association (FFA) Forest Biosecurity Committee are aware and concerned about this threat. A biosecurity manager from NZFOA and Forest pathologists from Scion were invited to the Basque Country in Spain in 2019 to investigate diseases in their forests and were surprised by the impact of this pathogen. This pathogen was consequently added to the list of forestry pests and pathogens for the Government-Industry Operational Agreement (GIA-OA) to increase awareness of the pathogen with Biosecurity New Zealand (Ministry for Primary Industries). The NZFOA is interested in investing in the development of readiness plans and activities for this pathogen.

This project

To assist with the development of readiness plans, for example a Threat Specific Readiness Manual and/or Operational Specifications for use during any potential incursion of this pathogen, the NZFOA are interested to understand the literature about this pathogen and knowledge gaps for assisting biosecurity readiness.

Key results

Several comprehensive literature reviews *about Lecanosticta acicola* have been published in the last few years by overseas scientists. Using these reviews and other literature, together with our New Zealand experience with forest diseases and biosecurity knowledge, we have attempted to identify the current knowledge gaps that will inform readiness activities and future research. Several key points were noted during this literature review:

- it was noted that typical silvicultural practices used overseas to keep the disease in check are no longer considered effective.
- there appears to be some conflicting information about the susceptibility of *P. radiata* to *L. acicola*, although we know from contacts in Spain that disease is severe there on radiata pine.
- over the past three years, two *Cedrus* species have been identified as the first non-*Pinus* hosts found to be naturally infected. This, along with warnings from other authors, means we should be aware that other non-*Pinus* hosts may also be susceptible.
- there appears to be variation in the information available describing the known distances that both the asexual and sexual spores of the fungus can travel. Understanding this variation is crucial for planning a response in case this pathogen reaches Australia and/or New Zealand.
- pine species are common in urban and peri-urban locations, which could provide social licence challenges if an incursion response was to occur, and treatments were required. Understanding priority treatment options, such as spraying or burning of infected hosts during an incursion, could inform social licence challenges and how these could be proactively avoided.
- We feel it is important to note that the geographical range of *L. acicola* has increased significantly in the past 10-20 years. There are number of factors that could be contributing to this expansion, including climate change, which may increase the risk of this pathogen to New Zealand.

Implications of results for the client

The knowledge and gaps highlighted in this review demonstrate that *Pinus radiata* in New Zealand plantations is likely a susceptible host, and the whole of New Zealand is likely climatically suitable for the establishment of *L. acicola*. In addition to this, eradication attempts for this pathogen in other parts of the world have not always been successful.

Further work

In view of this, we would support the development of readiness plans and future research directions as described in this review. In particular, the development of a Threat Specific Readiness Manual (or Operational Specifications) for *L. acicola* could provide confidence to the industry in the case of an incursion. We also recommend continuing the work already underway with screening of radiata pine germplasm but suggest this is expanded to look at alternative species that might show greater resistance. Further recommendations are suggested within.

Lecanosticta acicola: Biosecurity readiness for New Zealand's plantation forestry

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1. Introduction

The commercial forestry industry is one of the most important primary industries for New Zealand, second to the dairy and meat industries (StatsNZ) (StatsNZ, 2020). Projections for 2027 indicate that the New Zealand forestry industry is poised to contribute more than $7 billion NZD to the country's gross domestic product (GDP), the highest returns ever recorded for this industry (NZFOA 2023). The success of New Zealand's forest industry is attributed to the extensive planting, constituting approximately 90% of the commercial estate, of the introduced Californian radiata pine, *Pinus radiata* (Ministry for Primary Industries et al. 2023). Strategic selective breeding and customized silvicultural enhancements have resulted in a highly efficient forestry system for *Pinus radiata* in New Zealand, making its pine forests among the most productive in the temperate zone, boasting an average national yield of 27.4 m^3 ha^{-1} $year^{-1}$ (Palmer et al. 2010).

Situated as an island in the Pacific Ocean and fortified by a world-class biosecurity system, New Zealand has successfully averted the establishment of many major forest pests, distinguishing it from more connected countries in the Northern Hemisphere (Ridley et al. 2000, Ridley 2004). However, it is not impenetrable. Damaging pathogens, such as *Dothistroma septosporum*, the causal agent of Dothistroma needle blight (DNB) have established in New Zealand. This pathogen is considered one of the most important pathogens of pine (Barnes et al. 2004) and is responsible for significant impacts on native and introduced pine species around the world (Gibson 1972, Woods et al. 2005, Fraser et al. 2016). *Dothistroma septosporum* entered New Zealand in the 1960s (Gilmour 1967, Barnes et al. 2014) and became widespread across the country causing yield losses that cost the industry millions of dollars annually (Watt et al. 2011). Fortunately, limited genetic diversity of the pathogen due to strict quarantine measures (Barnes et al. 2014), deployment of tolerant planting stock (Carson 1989), and the implementation of management options, such as copper sprays (Gibson et al. 1964, Olsen 1971, Gilmour et al. 1973, Dick 1989) have reduced the impact of this disease on radiata pine in New Zealand.

A similar pathogen to *Dothistroma septosporum* threatens New Zealand. The pathogen, *Lecanosticta acicola* causes brown spot needle blight (BSNB) and is the focus of this review. Like *D. septosporum*, which this fungus is often confused with in terms of symptomology and morphology, *L. acicola* is considered an important pathogen of *Pinus* globally. Briefly, it affects more than 50 different species of pine in the Americas and Europe (van der Nest et al. 2019a) and threatens pines in Africa, Asia and Oceania. The disease causes susceptible pine species to shed their needles prematurely causing stunted or reduced growth, which can result in severe yield loses (Wakeley 1970). To ensure that the New Zealand commercial forestry industry can remain profitable, work is needed to understand the threats that could impact tree health and productivity, including pathogens such as *L. acicola*. This review highlights important facts about the pathogen, including its lifecycle, impact, diagnostics, and management options to help forest owners and managers understand the risks associated with this pathogen and improve the readiness of the industry to manage this pathogen should it arrive in New Zealand.

2. An overview of the genus *Lecanosticta*

Lecanosticta is made up of eleven described species (**www.mycobank.org**) (Table 1). *Lecanosticta acicola* was the earliest described, is the most studied, and the most important in terms of disease impact on various *Pinus* spp. and its distribution across North and South America, Europe, and Asia (**https://gd.eppo.int/taxon/SCIRAC/distribution**). The other *Lecanosticta* species have been described from various *Pinus* spp. in Mesoamerica, namely Mexico, Nicaragua, Guatemala, and Honduras, and include *L. brevispora*, *L. guatemalensis*, *L. gloeopsora*, *L. longispora, L. jani*, *L. pharomachri*, *L. tecunumanii* and *L. variabilis* (Evans 1984, Marmolejo 2000, Quaedvlieg et al. 2012, van der Nest et al. 2019a, Tubby et al. 2023a). *Lecanosticta gaubae* is the only species not found on a *Pinus* species; currently only recorded on *Callistemon viminalis* from Australia (Crous 1999) (Table 1). However, it appears that there may be some taxonomic confusion in the classification of this species (van der Nest et al. 2019a).

Table 1: Summary of *Lecanosticta* species described to-date[1]

***Lecanosticta* species**	**Year first described (as part of *Lecanosticta* genus)**	**Host**	**Locations**	**References**
L. acicola	1922	*Pinus* spp., including *P. radiata, Picea glauca, Cedrus atlantica, C. libani*	North and South America, Europe, Asia	Quaedvlieg et al. (2012) Tubby et al. (2023a)
L. brevispora	2000	*P. oocarpa, P. pseudostrobus*	Mexico, Guatemala, Honduras	Quaedvlieg et al. (2012) van der Nest et al. (2019a)
L. cinerea	1984	*Pinus* spp.	Honduras	Evans (1984) Marmolejo (2000)
L. gaubae[2]	1983	*Callistemon viminalis*	Australia	Von Arx (1983) Crous (1999)
L. gloeospora	1984	*P. pseudostrobus*	Mexico	Evans (1984)
L. guatemalensis	1982	*Pinus oocarpa, P. tecunumanii, P. caribaea,*	Guatemala, Nicaragua, Honduras	Quaedvlieg et al. (2012) van der Nest et al. (2019a)

L. jani	2010	*P. maximinoi,* *P. oocarpa,* *P. tecunumanii*	Guatemala, Nicaragua	van der Nest et al. (2019a)
L. longispora	2000	*P. culminicola*	Mexico	Marmolejo (2000)
L. pharomachri	2010	*P. oocarpa* *P. tecunumanii* *P. patula*	Guatemala, Honduras	van der Nest et al. (2019a), Theron et al. (2022)
L. tecunumanii	2012	*P. tecunumanii*	Guatemala	(van der Nest et al. 2019a)
L. variabilis	1984	*P. arizonica* var. *stormiae,* *P. caribaea,* *P. halepensis,* *P. maximinoi,* *P. oocarpa*	Mexico, Guatemala, Honduras	Evans (1984), van der Nest et al. (2019a)

[1]Note: Two excellent summary tables of the (i) Host and Geographical Range of *Lecanosticta* species and (ii) the taxonomic history of the genus *Lecanosticta* are provided in van der Nest et al. (2019) [doi: 10.1111/mpp.12853] and an interactive online map has been published by Tubby et al. (2023a): portalofforestpathology.com. This table is intended to summarise that data for quick reference.

[2]There is some uncertainty about the taxonomic placement of *L. gaubae*.

Species of *Lecanosticta* are thought to have a similar lifecycle to *L. acicola*, and while these species are thought to have sexual morphs, they have not been discovered and/or described for all species. Asexual spores, or conidia, are airborne (usually in aqueous aerosols like rain splash and fog/mist) and infect attached needles of susceptible pine species. The fungi will invade the needle through natural openings, such as stomata, or through wounds (Kais 1978) using one to four germ tubes where they will colonize the needle tissue and eventually produce conidiomata (Setliff and Patton 1974). Inside the needle, conidiophores will produce conidia toward the exterior of the needle (Evans 1984). Eventually the needle epidermis will erupt, and the conidia are released into the air, and dispersed via rain splash or picked up in fog or mist (see Fig. 1 for *L. acicola* lifecycle). The fungi can overwinter in needles attached to the tree and in cast needles on the forest floor.

For some species, such as *L. acicola* and *L. pharomachri,* sexual reproduction occurs on the necrotic distal parts of living, dead and cast needles (Henry 1954, Jewell 1983). Sexual spores, or ascospores, are produced from the asci and are forcibly ejected from the asci under favourable conditions and dispersed into the environment with rain-splash, wind or fog/mist, when conditions are favourable, including temperature and humidity (Fitt et al, 1989, Kais 1975, Suto, 2002, Tainter and Baker 1996). These spores can infect green needles and continue the cycle. Ascospores are rare when compared to conidia (van der Nest et al. 2019b).

The concentration of species in Mesoamerica and their association with native pines suggests that this is their likely origin (van der Nest et al. 2019b).

3. *Lecanosticta acicola*

3.1 Taxonomy, Biology and Epidemiology

Lecanosticta acicola has gone through several name changes since it was first described as *Cryptosporium acicola* by von Thümen in 1878, including *Mycosphaerella dearnessii* and *Schirria acicola*. Under the 'one fungus one name' rule, *L. acicola* was selected as the valid name and type of the genus (Quaedvlieg et al. 2012), 134 years after it was first described by Thümen.

Warm and wet weather is particularly conducive for the development of BSNB (van der Nest et al 2019a, Tubby et al. 2023a, Wyka et al. 2018). Conidia are released throughout the year at temperatures ranging from 2 to 28°C (Wolf and Barbour 1941, Siggers 1950, Kais 1971, Wyka et al. 2018). Conidia (asexual spores) can germinate between 5 and 35°C, although the upper temperature applies to the southern lineage and northern lineages did not germinate at 32°C (Huang et al. 1995). Suto and Ougi (1998) found that conidia germinated between 10 and 30°C, with an optimum of 25-30°C *in vitro*. High humidity pre- and post-infection is required, and the optimum temperature for infection was 30°C during the day and 21°C at night whereas no symptoms developed with a regimen of 35°C during the day, and 27°C night. Substantial infection occurred when artificially inoculated trees were exposed to three days of high humidity, and this may be due to the conidia requiring 14-52 hours to germinate and the germ tube then having to grow over the surface of the needle prior to entering the stomata (Kais 1975). As noted previously, conidia are dispersed predominantly by rain splash and contribute significantly to rapid disease build-up. There are varying reports about the time of year that conidia are produced and dispersed (Siggers 1950, Skilling and Nicholls 1974, Li et al. 1987, Suto 2002, Wyka et al. 2018) and it appears that conidial dispersal varies depending on rainfall patterns in regions (van der Nest et al. 2019a). It has also been shown that conidia can be naturally dispersed up to 60 m (Wyka et al. 2018); however, longer distance dispersal is possible during strong winds, through insect dispersal, and human assisted mechanisms (Fitt et al. 1989, Suto, 2002).

Ascospores are forcibly expelled into the air and dispersed by wind currents (with or without moisture) and although not reliant, periods of fog, rain, and dew are reported to positively correlate with or increase the rate of ascospore release (Siggers 1939, Kais 1971). Ascospores have been reported at temperatures as low as 10°C but are more commonly found at temperatures above 15°C, and no ascospores were reported at < 4 °C (Kais 1971). There appears to be a bias towards asexual reproduction and in areas with both mating types, the sexual stage is only occasionally found (Janoušek et al. 2016, Wyka et al. 2017, Raitelaitytė et al. 2023).

Lecanosticta acicola overwinters in either asexual or sexual fruiting structures in dead needle tissue on either dead or living needles and mycelium can overwinter in infected needles that remain attached to the host (Siggers 1944).

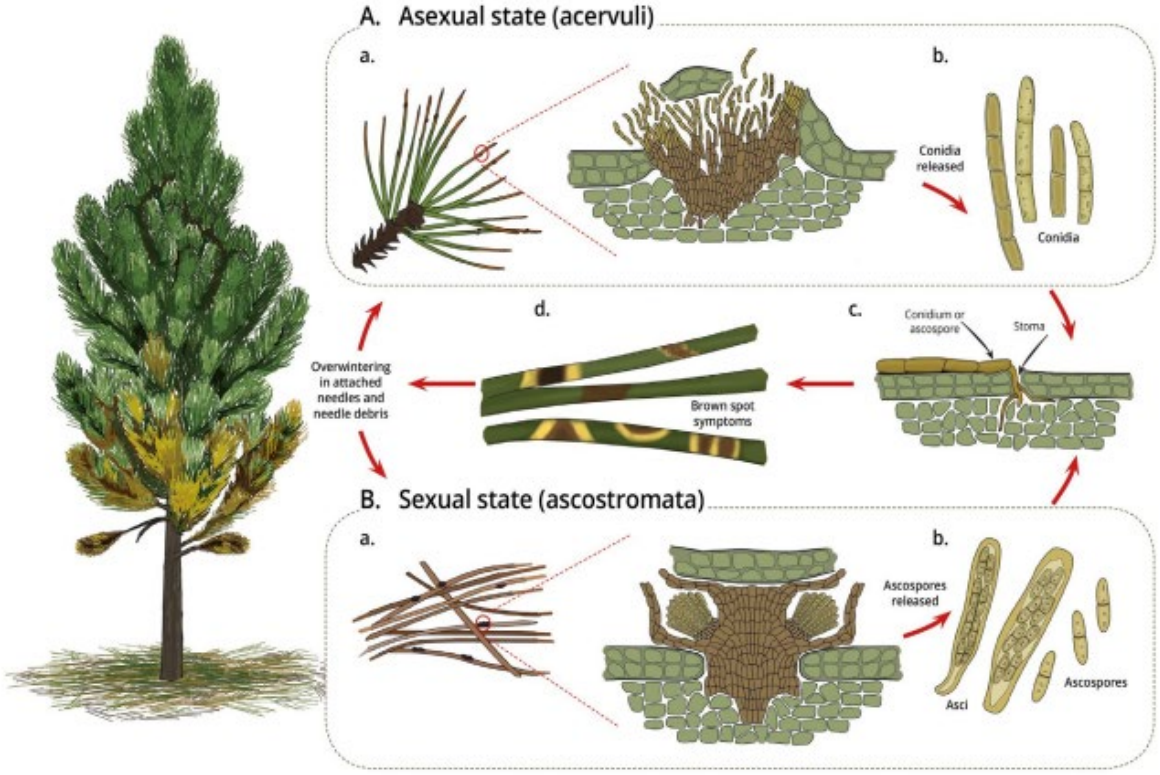

Fig. 1 Life cycle of *Lecanosticta acicola* on *Pinus* spp. (A) Asexual state: acervuli (a) develop on attached needles and needle debris and release conidia (b). Infection occurs through the stomata of new season needles (c), resulting in brown spot symptoms (d). (B) Sexual state: ascostromata develop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

Fig 1. The life cycle of *L. acicola* on *Pinus* spp. From van der Nest et al. (2019a).

Conidia germinate on the needle surfaces by most commonly developing one germ tube from a terminal cell, although some 3-4-celled conidia germ tubes can grow from every cell. The germ tubes are appressed to the needle surface and the growth pattern can be irregular indicating no stomatal attraction. Once the germ tubes penetrate the stomata the germ tube increases in diameter and becomes thick-walled and melanized (Setliff and Patton 1974, Patton and Spear 1978). Light plays an indirect but important role in the infection process since it stimulates the opening of the stomata, allowing the germ tube to enter. Infections can also occur through wounds in the needle (Kais 1978). Once the mesophyll is invaded, acervuli (conidiomata) begin to form, which in turn leads to the formation of conidiophores within the acervuli. Conidia are produced from the conidiophores towards the needle exterior, exerting pressure on the needle epidermis and causing the epidermis to rupture, leaving a flap that partly covers the acervuli (Figure 1), (Wolf and Barbour 1941, Evans 1984). An asymptomatic phase can occur after *L. acicola* establishes within needles, which can last several days (Setliff and Patton, 1974) to 3 months (Skilling and Nicholls, 1974). This appears to be dependent on the strain of the pathogen (Kais 1972) and length of time that needles are wet (van der Nest et al. 2019a).

Lecanosticta acicola is known to produce the toxic compounds LA-I and LA-II, which are heat resistant and non-host specific phytotoxins. These 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, and this has proved to be a reliable indicator of host susceptibility. For instance, *P. thunbergia* cuttings showed little sensitivity to the toxins; however, *P. elliottii* and *P. taeda*, which are both highly susceptible to BSNB, showed high sensitivity to LA-I (Ye and Qi 1999). These toxins seem to be involved in the destruction of mesophyll tissue of the pine needles at the point of infection and the toxins result in dead needle tissues well beyond the tissues where the pathogen originally colonised (Jewell 1983).

Biology and reproduction of *Lecanosticta acicola*

- Conidia (asexual spores) are dispersed by rain splash
- Ascospores (sexual spores) are dispersed by wind currents with or without moisture
- High humidity is required for severe disease expression
- Light plays an indirect but important role in the infection process since it stimulates the opening of the stomata, allowing the germ tube to enter the needles
- The pathogen overwinters in either asexual or sexual fruiting structures in dead needle tissue and mycelium can overwinter in infected needles that remain attached to the host
- Toxins produced by the pathogen seem to kill the mesophyll tissue of the needles resulting in dead tissues well beyond where the pathogen has infected

3.2 *Host range & host susceptibility*

Tubby et al. (2023a) created a collaborative global dataset of *Lecanosticta* presence and absence data using 2,970 independent observations, including absence data from New Zealand based on forest health survey data. They investigated a total of 91 plant taxa during construction of the *Lecanosticta* geo-database of which 70 species are listed as susceptible to *Lecanosticta* species. Sixty-seven of the susceptible species belong to *Pinus*, including *P. radiata*. The other hosts are *Cedrus atlantica, C. libani* and *Picea glauca*; however, the latter was only found to be susceptible in inoculation trials (Skilling and Nicholls, 1974). Pehl et al. (2015) notes that it is likely that most *Pinus* species, and many non-*Pinus* species in the Pinaceae (e.g., *Abies*, *Larix*, *Pseudotsuga*, *Picea* and *Cedrus* species) will prove to be susceptible, particularly under conditions of high inoculum pressure. Interestingly, *Picea abies* did remain uninfected under conditions of heavy inoculum pressure in some European forests (Beenken et al 2018) and following artificial inoculation (Tubby et al. 2023a).

Using data for the susceptible species listed in the database, novel rankings of host susceptibility were described. Of the 70 hosts, eight species were categorised as resistant (no traces of infection have been reported where hosts are located close to infected trees, or in artificial inoculation studies); 31 were categorised as having low susceptibility (trace levels of foliar infection occur); 19 were ranked moderately susceptible (infection clearly visible, but growth not seriously affected) and 23 were apparently highly susceptible (high levels of defoliation and in some cases mortality). Nine species recorded as host taxa have no details of disease impact and were not ranked (Tubby et al. 2023a). The availability of quantitative information describing the impact on forests and trees is limited, although data are reported for some species by Tubby et al (2023a).

Of particular interest to New Zealand are the records on *P. radiata*. According to records, 36% of *P. radiata* had greater than 25% needle infection, with all records originating from Spain (Tubby et al 2023a). In France, *P. radiata* was considered resistant by Chandelier et al. (1994) and Lévy (1996), however *P.* × *attenuradiata* was reported by the former authors to have high susceptibility with high mortality observed after repeated infections of mature trees*. Pinus radiata* was reported as moderately to highly susceptible in Colombia (Gibson 1980) and highly susceptible in Spain (Ortíz de Urbina et al. 2017, Mesanza et al. 2021). There remains uncertainty over impacts of the pathogen on some *Pinus* species, including *P. radiata*, as levels of infection appear to vary across both the European continent and the United States (Tubby et al. 2023a), which maybe a result of climatic differences and unknown differences in the pathogen and host. It should also be noted that the detection of *L. acicola* in Colombia has not been confirmed since and only *L. pharomachri* has been identified in Colombia, using molecular methods (I. Barnes, pers. comm.).

A very recent study examined the physiological, metabolic, and hormonal responses of two pine species, *P. radiata* and *P. pinea,* following infection by *L. acicola* (Monteiro et al. 2024). These pine species have differing levels of susceptibility to *L. acicola*, with *P. radiata* considered more susceptible. The authors show that symptoms were observed in 54% of *P. radiata* and 45% of *P. pinea* seedlings, with those in *P. radiata* being more pronounced. In addition, differences in observed stomatal conductance and transpiration as well as cellular levels of sugars, amino acids, and flavonoids were also observed between the pine species during infection. The authors suggest that identification of specific metabolic pathways can help support the identification of more resistant host genotypes and mechanisms for sustainable disease management.

There are no reports of BSNB on Douglas-fir (*Pseudotsuga menziesii*), New Zealand's second most important plantation species, with a total planted area of approximately 105,000 ha (Kimberley et al. 2017). During a visit to Spain in 2019, it was noted that Douglas-fir was relatively healthy, with some presence of Swiss needle cast observed (McDougal, 2019). However, we should be mindful that new host species, other than pine, have emerged in recent years, namely *C. libani* and *C. atlantica* (Oskay et al. 2020, Schenck et al. 2022). *Dothistroma septosporum* has been found to cause infection on Douglas-fir under favourable climatic conditions and high

inoculum pressure (EFSA 2013). This suggests that there is the potential for host range expansion of *L. acicola* on other coniferous species in the future (Pehl et al. 2015, Ogris et al. 2023). It is difficult to predict the impact of *L. acicola* n New Zealand forests based on our current understanding and the forecasted climate over the next three decades.

3.3 Symptoms on different hosts

It is generally recognised that due to the cryptic nature symptoms of *L. acicola* can be easily confused with those of Dothistroma needle blight (DNB) on many pine hosts (MacLeod et al. 2012, van der Nest et al. 2019a, Laas et al. 2022, Tubby et al. 2023a). The most classic symptoms on most pine hosts are small yellow, light green, or reddish-brown spots at the point of infection, often surrounded by a yellow halo (Fig. 2A-C), which then become brown as the infection progresses (Fig. 2D). These needle spots are often resin soaked (Skilling and Nicholls 1974). Needles with multiple infections can take on a mottled appearance (Phelps et al. 1978) (Fig. 2B). The characteristic brown spots can further develop to form narrow brown bands and tip die-back of the needle occurs to the infection point (van der Nest et al. 2019a, Tubby et al. 2023a) (Fig. 2D). The maturing fruiting structures of the fungus can be seen as small grey spots under the epidermis and develop in the brown spots and bands and other dead tissues. When the fruiting structures mature, using a lens, conidia may be seen oozing as an olivaceous to black tendril (cirrhus) from beneath the flap of host tissue which has been forced upwards by the erumpent fruit body (acervulus), (Fig. 1), (Suto and Ougi 1998). Current season growth normally remains visibly unaffected but older infected needles are prematurely shed, leaving bare branches with tufts of new needles at the branch tips. However, new season growth has been reported to be susceptible under high inoculum loading. Infection is usually most severe in the lower parts of the crown and progresses upwards into the canopy (Suto and Ougi 1998, van der Nest et al. 2019a, Tubby et al. 2023a).

Symptoms can differ on some pine and non-pine species. White pine needle damage (WPND) is a disease that affects the eastern white pine, *P. strobus*, across the northeastern United States. There are four fungal species that are associated with this condition, including *L. acicola*, which is the most prevalent and responsible for symptom development and defoliation (Broders et al. 2015, Wyka et al. 2018). Symptoms of WPND may only be displayed as chlorosis of needles without spotting or banding (Broders et al. 2015). However, Stanosz (1990) reported acervuli developing on light brown bands with borders remaining green on this host. Symptoms on *P. thunbergia* are expressed as small circular greyish green flecks appearing on the distal parts of infected needles, which then develop into brown spots and bands. The brown spots have a slightly darker margin and/or rarely encircled with yellow margins (Fig. 2B). These spots and bands frequently coalesce, and the ends of the needles die while the rest of the needle remains green, as is also seen in other pine species. Infected needles droop and are cast many months later. Severe infection can result in whole needle death before the needles are cast leaving bare branches (Suto and Ougi 1998).

Fig 2: Symptoms of BSNB caused by *Lecanosticta acicola* on pine. A: Symptoms of BSNB on *Pinus mugo* exhibiting only brown spots; Middle: on *P. radiata* showing distinct brown bands; Bottom: on *P. palustris* showing brown spots with yellow halo (Photo from van der Nest *et. al.* 2019a); B: Left BSNB on *Pinus thunbergii.* Right: Symptoms on *P. thunbergii;* C & D: BSNB on pine in Basque Country, Spain 2019 (Photo: R. McDougal, Scion).

On *P. palustris*, the infection begins as brown spots and bands that are surrounded by a yellow halo (Kais 1975). Infected needles usually have three distinct zones; the basal portion, which is green; the middle portion, which is mottled (infected); and the apical portion, which consists of dead tissue. The characteristic spots can remain visible even after the needle dies (Phelps et al. 1978). Adamson et al. (2015) described the first symptoms on *P. ponderosa, P. uncinata, P. mugo*, and *P. mugo* var. *pumilio* as resin-soaked yellowish lesions with a clear orange boarder. They noted that black immature acervuli, in the form of small black spots, occur under the epidermis on dead portions of the needle. Infected needles of *P. sylvestris* start with yellow spots that are often resin soaked; the needles start dying from the tip down until the entire needle turns brown and casts (Skilling and Nicholls 1974, Phelps et al. 1978).

Picea glauca was found to be only mildly susceptible to infection by *L. acicola* after a field inoculation experiment where trees were placed under heavily infected *P. sylvestris* (Skilling and

Nicholls 1974). There is no mention of how symptoms were expressed on *Pi. glauca*. Oskay et al. (2020) made the first report of natural infection of *L. acicola* on a host other than pine, *C. libani*. Their reports note that *C. libani* showed typical BSNB symptoms (Fig. 3A) and that the intensity of infection was less severe compared to native pines. *Lecanosticta acicola* was first reported on *C. atlantica* by Schenck et al. (2022) and trees were exhibiting light to severe defoliation which started in the lower part of the crown. Needles had brown spots and necrosis (Fig. 3B).

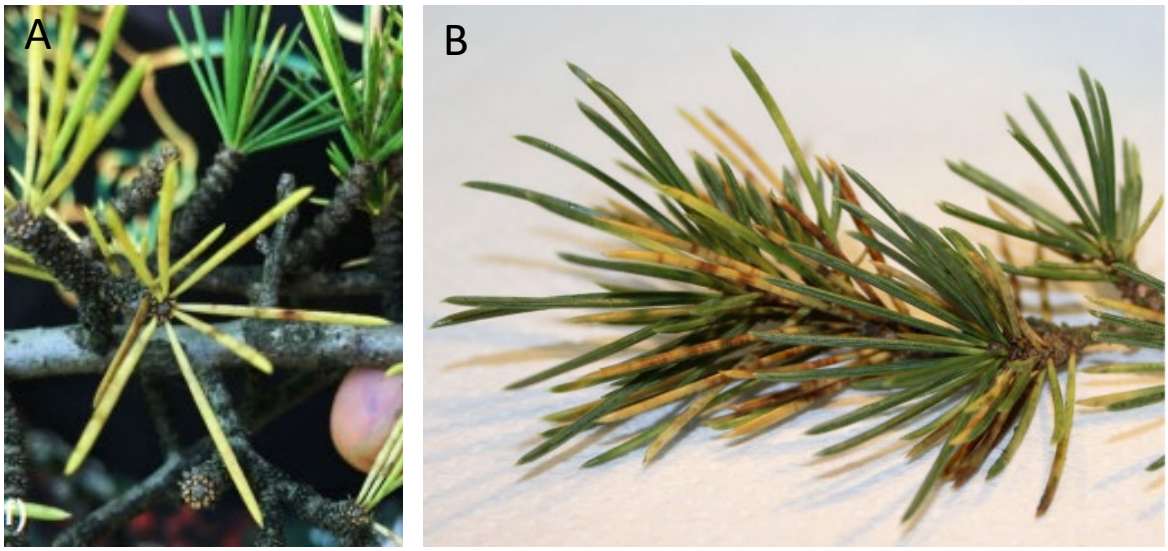

Fig. 3: Symptoms of BSNB caused by *Lecanosticta acicola* on *Cedrus* species**. A:** Symptoms on needles of *Cedrus libani* showing brown spots and necrosis (Photo from Oskay et al 2020); **B:** Symptomatic *Cedrus atlantica* shoot showing needles with brown spots (Photo from Schenck et al. 2022)

4. Surveillance and detection methods - what has been done overseas

Based on the results of climatic modelling, Ogris et al. (2023) noted that New Zealand, while currently free from this disease, has a suitable climate for *L. acicola* to invade and spread across New Zealand forests if it was to arrive here. The authors caution the need to maintain stringent biosecurity practices to limit possible introduction and establishment of this pathogen, particularly with vulnerable plantations of predominantly *P. radiata*.

The endemic nature and long history of disease caused by *L. acicola* in North America means that surveillance for the purposes of eradication is no longer viable. However, in Europe and other parts of the world this is not the case, and considerable effort has been put into surveillance for the purposes of early detection and eradication, as well as limiting the spread of the pathogen once detected. Europe has legislation (Regulation (EU) 2016/2031) to limit the entry and early detection of invasions by non-native pests and pathogens to maximise probability of effective eradication. *Lecanosticta acicola* was listed as an Annex I/A1 quarantine organism under the EU directive 2000/29/ES with requirements for surveillance, containment, and eradication through phytosanitary methods in all European Union countries. Unfortunately, the pathogen still invaded the region.

The main pathway in Europe has been the movement of plants. Traded planting stock is typically subjected to mandatory inspections, including sampling visibly symptomatic tissue in seed beds or in potted plants. Any sample collected is usually subjected to molecular diagnostics using a range of PCR techniques to separate different pathogens. Like *Dothistroma* spp., there is a lag between infection and symptom expression, which likely led to the pathogen's introduction via plant trade and movement. *Lecanosticta acicola* was detected on imported planting stock in Czechia and Belarus with routine inspections (Golovchenko et al. 2020). In other parts of Europe, the pathogen was reported during sampling of exotics in forests, including research projects sampling needle diseases to understand host range and cause, and annual surveys conducted in botanical gardens (Drenkhan and Hanso 2009, EPPO 2012, Mullett et al. 2018). It was also identified during inspections of street trees, parks, and gardens (Cech 1997, Brandstetter and Cech 2003, Jurc and Jurc 2010). Additionally, in some European countries, *L. acicola* was identified as part of their forest surveillance programmes (Markovskaja et al. 2011, Stamenova et al. 2018, Georgieva 2020). The reports were from symptomatic needles, which were sampled and confirmed with laboratory analysis, i.e., with morphology and a species-specific PCR to confirm the identification.

New Zealand also has legislation (Biosecurity Act 1993) that provides the Ministry of Primary Industries (MPI) and others with the authority to keep harmful organisms out of New Zealand. It guides the Ministry on readiness and response, pre-border, and border, as well as long-term management should a pest become established. The Ministry for Primary Industries runs multiple surveillance programs (e.g. High-Risk Site Surveillance [HRSS]) and contributes to a Forest Biosecurity Surveillance system (FBS; owned by the NZFOA) aimed at early detection to enable eradication. This has been internationally reviewed multiple times demonstrating it is fit for purpose (Stevens et al. 2011). The NZFOA also run their own forest health surveillance that is not part of high-risk sites to capture potential risks to plantations (FHS and Non-Model Allocation [NMA] surveillance). The FBS already targets high-risk areas where *L. acicola* could be found should it arrive. It should be noted however, that red needle cast (caused by *Phytophthora pluvialis*) was first recognised in plantation forests (Dick et al. 2014), not in high-risk sites, and so the NMA and FHS parts of the program are still of critical importance to the protection of New Zealand's plantation forests. The import of live plants with *L. acicola* is unlikely given the restrictions on the import of live *P. radiata* and Dougal-fir plants (for a review see (Hood 2018)), and other susceptible species into New Zealand; however, there are other pathways to be cognisant of and are highlighted in Section 7.1.

In Europe, the challenge with plant trade and movement, land borders, and natural dispersion can make this pathogen difficult to manage. Additionally, testing is usually only done if symptoms are identified, and destruction of infected material is sometimes not enough to eradicate the pathogen in time before it spreads to another area (Georgieva, 2020). Part of the problem is that in many newly invaded ranges, we do not always understand pathogen spread within a forest. This requires extensive sampling, frequently and widely, and would be challenging when confused or missed with other infections, such as *Dothistroma* spp., considering the sampling lag between infection and

symptoms. The morphological similarity of *Dothistroma* and *Lecanosticta* has led to taxonomic uncertainty and misdiagnoses in other parts of the world for over 100 years (Tubby et al. 2023a). With *D. septosporum* having been present in New Zealand since the 1960s, this highlights the importance of greater awareness of the differences between these pathogens for New Zealand-based diagnosticians and the need to incorporate molecular (DNA-based) tools for detection and accurate identification.

Lecanosticta acicola is not currently present in Australia; however, *D. septosporum* is. It has been suggested that *D. septosporum* could have travelled from New Zealand to Australia on moist air currents (Edwards and Walker 1978), highlighting the importance of working with our Australian neighbours. Recent actions to re-establish a Trans-Tasman Forest Health and Biosecurity Network will facilitate this type of discussion and information sharing so we can increase our biosecurity awareness through collaboration. This collaboration might include co-investment in projects, shared risk assessments, exchange of technical and operational expertise, visits, testing diagnostic assays, and specific forest-health related workshops and meetings.

5. Diagnostic methods

In New Zealand, work has already been done to test, develop and employ routine assays for the rapid identification of *L. acicola*. Scion had previously tested published molecular assays with *L. acicola* DNA obtained from overseas collaborators (Rebecca McDougal and Josef Janoušek, unpublished data) as a preparedness activity for our own forest diagnostics. The Ministry for Primary Industries – Plant Health and Environment Laboratory (MPI-PHEL) is also testing this species-specific qPCR to identify *L. acicola* from infected material. This has been tested by Scion's Forest Health Reference Laboratory (FHRL) using the MPI-PHEL DNA panel to ensure the assay works consistently between laboratories. Current results indicate that the assay performs in both laboratories and will be included as a tool in our diagnostic toolbox. We currently use a species-specific translation elongation factor 1-α (*TEF1α*) gene region assay for *L. acicola* (+) when collectors and Scion diagnosticians are concerned by the foliar symptoms.

Accurate assays are essential for detecting *L. acicola* considering that the morphology and symptomology can be cryptic. The cryptic nature of this fungus can make sampling and testing difficult, and care needs to be taken to ensure that inspectors can identify the symptoms correctly. Morphologically, *L. acicola* resembles several pine needle infecting fungi. Not only is it confused with other members of the *Lecanosticta* genus but is also confused with important pathogens like *D. pini* and *D. septosporum* (Sydow and Petrak 1924, Barnes et al. 2016). The red banding associated with Dothistroma needle blight (DNB), caused by the toxin dothistromin, is not always evident and can be influenced by the presence of light or shaded conditions. If these bands are not evident or supressed, then initial symptoms may be confused with those caused *L. acicola* (Pehl 2015). The needle spot symptoms of *L. acicola* begin as yellow to grey-green regions on the side of the infection (Fig 2). Overtime these turn brown and are surrounded by a grey halo. Symptoms might differ on different hosts as well. The needles die from the distal part of the needles toward

the base and are eventually cast. The similarities between the symptoms of infection for both *D. septosporum* and *L. acicola* are outlined in Figure 13 of Pehl (2015).

Under the microscope, the conidiomata are pseudopycnidial to acervular and measure 200-800 x 150 – 200 µm. The dark, short-celled hyphae are intracellular and confined to mesophyll cells. The conidiophores are septate, subcylindrical, densely aggregated, dark brown, verruculose and may be branched or unbranched at the base and are 20 – 60 x 4 – 6 µm in size. The conidiogenous cells are holoblastic, terminal, integrated, pale brown and verruculose. The conidia have a truncated base, are straight to curved, with an obtusely rounded apex, are subhyaline to light brown or olive, and have 1 – 5 septae. The base is 2.5 – 3.5 µm diam. and 30-45 x 3-4.5 µm in size. These characters are variable and do overlap with other *Lecanosticta* species; however, are more useful for separating *Lecanosticta* species from *Dothistroma*, i.e., in colour and size of the conidia. To accurately identify this species, molecular techniques are recommended.

Several PCR-based assays have been developed to distinguish between *L. acicola*, *D. pini*, and *D. septosporum* and these have been tested at Scion. These assays use the *TEF1α* (*L. acicola* and *D. pini*) and beta-tubulin 2 (*BT2*) (*D. septosporum*) gene regions to distinguish between the three pathogens (Ioos et al. 2010). Using naturally infected samples, Ioos et al. (2010) developed a conventional PCR method that could differentiate between these three pathogens. The authors also developed a more sensitive and rapid real-time multiplex PCR (qPCR) that could simultaneously target each gene region. Both methods can be used depending on the availability of specialized equipment, such as real-time PCR machines. Studies have used these species-specific primers to confirm the presence of the pathogen in the environment (Adamson et al. 2015, Ortíz de Urbina et al. 2017, Sadiković et al. 2019). Alternative diagnostics assays are also available for differentiation of *D. septosporum* and *D. pini* (McDougal et al. 2011), which can be used for rapid diagnostics.

Comparison of DNA sequences of internal transcribed spacer (*ITS*), *BT 2* and *TEF 1* is another way to accurately distinguish species within the *Mycosphaerellaceae* (Quaedvlieg et al. 2012). A multi-gene phylogenetic analysis of the *ITS, TEF1, BT2,* large subunit of the RNA polymerase II (*RPB2*), and protein-coding *MS204* gene were used by van der Nest et al (2019) to separate cryptic species, which led to the description of four new *Lecanosticta* species. This method is lengthy; requiring a culture of the organism and access to expensive laboratory equipment to amplify and sequence the DNA.

A loop-mediated isothermal amplification (LAMP) tool was recently developed to assist with rapid, in-field diagnostics (Aglietti et al. 2021). This method can distinguish between *D. pini, D. septosporum*, and *L. acicola* using species-specific LAMP primers and fluorescent assimilating probes to target the *BT2* and *TEF1* genes. This method was shown to be specific and sensitive enough to detect and differentiate these pathogens from both fungal and plant material. This assay does not require any specialized equipment and can be used by diagnosticians and surveillance

practitioners in the laboratory or field. This assay has not yet been tested in New Zealand but could be a good tool for inspectors to use as part of their routine sampling.

The bulk of samples processed at Scion's Forest Health Reference Laboratory consists of foliar material, often aimed at confirming the presence of pathogens already established in New Zealand. When symptoms overlap with or resemble those caused by *L. acicola*, it becomes beneficial to collect a subsample for molecular testing. This testing could also encompass various assays targeting high-priority pathogens such as *P. pinifolia*, *P. ramorum*, and *D. pini* simultaneously. Integrating these assays into our diagnostic toolkit involves evaluating existing assays, developing novel ones, exploring nested or combined assay possibilities, and maintaining quality control to ensure sensitivity and specificity, especially with the discovery of new strains or species. This process necessitates obtaining positive controls, acquiring specialized equipment for enhanced capabilities, providing staff training, and revising sampling and processing methods, which will require consultation with collectors and field operations staff. While dedicated funding is essential, incorporating these assays will enhance diagnostic accuracy and efficiency by reducing the time spent on sample diagnosis, i.e., reducing time spent culturing individual isolates and amplifying and sequencing a single gene that often lack sufficient taxonomic resolution necessitating the sequencing of an additional genes for identification. Despite potential development costs, integrating these assays into our routine sample analysis will ultimately save time by streamlining the diagnostic process, enabling simultaneous screening for multiple pathogens rather than one at a time.

Lecanostica acicola is listed as a quarantine pathogen in numerous countries (EPPO, 2022). In the European Union, various measures have been put in place for containment where the pathogen is classified as a regulated non-quarantine pest (European Commission 2019, Laas et al. 2022). In New Zealand, *L. acicola* is classed as an unwanted and regulated organism (Ministry of Primary Industries 2023).

6. Description of the biosecurity threat

6.1 Invasiveness and distribution

Lecanosticta acicola is thought to have originated from Mesoamerica (van der Nest et al. 2019b) and devasted forests in the USA's southern states in the early twentieth century (Tubby et al. 2023a). In 1942 it was found in Spain, and it has now spread to 24 of the 26 European countries where data is available, and parts of Asia (Tubby et al. 2023a). This information can now be accessed via the online Geo-database generated by Tubby et al. (2023a). A screenshot of this is provided in Figure 4.

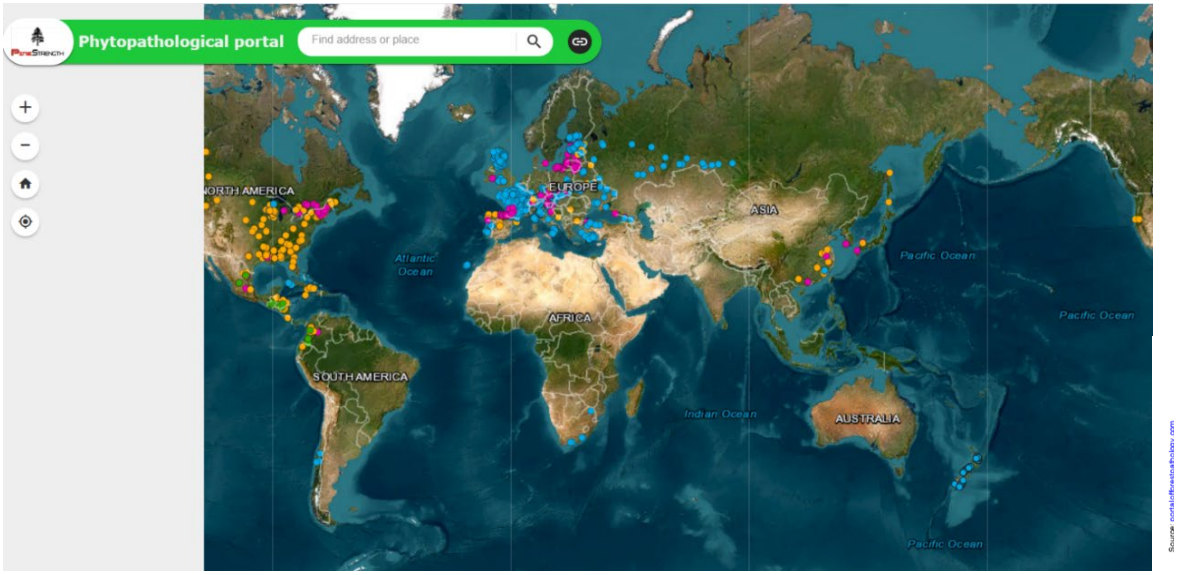

Fig. 4 Open access geo-database for *Lecanosticta acicola***.** Pink: confirmed; Orange: reported but not confirmed; Green: other *Lecanosticta* species confirmed; Blue: not reported. From Tubby et al. (2023): www.portalofforestpathology.com.

Three *L. acicola* linages have been identified based on a *TEF1* phylogeny and other genetic markers (e.g. microsatellite and randomly amplified polymorphic DNA (RAPD) markers), together with observations of cultural morphology. These were classed as the northern linage, currently present in northern USA, Canada, and Europe, and the southern linage, present in southern USA, east Asia, and Europe. The third lineage, which occurs in Mexico, is the most genetically diverse and does not appear to have spread further. It is also potentially made up of one or more cryptic species (a species that cannot be easily distinguished by culture morphology alone and requires molecular markers to differentiate distinct species and their phylogenetic relationships) (Huang et al. 1995, Janoušek et al. 2016, Tubby et al. 2023a).

Lecanosticta acicola is heterothallic, requiring two compatible partners with different mating types, MAT1-1 and MAT1-2, for sexual reproduction to occur (Janoušek et al. 2016). Sexual reproduction of fungi is significant for biosecurity as it is one mechanism by which genetic diversity can increase in a population, potentially leading to adaption of pathogens to new environments and hosts. The presence and ratio of different mating type loci is used to understand the potential for sexual reproduction, i.e., the presence of both mating types in relatively equal proportions can suggest that sexual reproduction is occurring in that population. In a recent genetic analysis of 629 isolates of *L. acicola* from different global locations, both mating types were found in 14 out of 28 populations (Laas et al. 2022). Using statistical analyses, this study showed that equal ratios of the mating types could be found in up to 10 of those 28 populations, including Spain, France, Ireland, Mexico, Slovenia, South-eastern USA, Switzerland, Germany, North-eastern USA and Poland (Laas et al. 2022). Although only half of the populations contain both mating types, even fewer have the mating types in equal proportions, indicating a predominantly asexual mode of reproduction. Given that ascospores (sexual spores) have greater potential for long distance wind-

driven dissemination than the asexual conidia, there is a need to remain vigilant for any successive introductions (van der Nest et al. 2019a, Laas et al. 2022, Tubby et al. 2023a).

Anthropogenic activities, including the movement of plant material, is the primary means of dispersal. This is discussed further in section 7.2.2. The first European report of *L. acicola* in Spain on the non-native *P. radiata* (Martínez 1942) is thought to have originated from plants imported from North America. The known European distribution was limited to Austria, France, Germany, Italy, Spain, Switzerland, and former Yugoslavia (Croatia) until the 1990s (Milatovic 1976, Chandelier et al. 1994, Holdenrieder and Sieber 1995, Pehl 1995, Cech 1997, La Porta and Capretti 2000). However, over the past 10-20 years the pathogen's known geographic range has increased significantly (van der Nest et al. 2019a, Oskay et al. 2020, Laas et al. 2022, Tubby et al. 2023a). Laas et al (2022) used microsatellite and mating type markers to study migration history. They found that there have been several introduction events from North America into Europe and that western Asia populations appear to originate from a genetically similar population in North America. The finding of several shared haplotypes (a genetic type or lineage) across European populations suggests *L. acicola* strains have been moving between countries. More recently, whole genome sequences were generated for 70 isolates of *L. acicola* from the Basque region of Spain. Analysis revealed 39 different haplotypes, and considerable evolution of strains likely from introgression (hybridisation or mating between the two mating-type lineages) between the northern and southern lineages of *L. acicola* (Marcet-Houben et al. 2024). This study also demonstrated that the previously used microsatellites cannot provide the resolution needed to capture the evolution of *L. acicola* and highlight the importance of genomic sequencing technologies for such analyses.

6.2 Hosts and associated impact overseas

As mentioned previously, *L. acicola* has been associated with 70 host taxa (Tubby et al. 2023a), all of which are pine species except for *Pi. glauca* (Skilling and Nicollas 1974), *C. atlantica* (Schenck et al. 2022) and *C. libani* (Oskay et al. 2020) (Table 1). Also, Pehl et al. (2015) noted that most *Pinus* species, and many non-*Pinus* species in the Pinaceae (i.e., *Abies*, *Larix*, *Pseudotsuga* and other *Picea* and *Cedrus* species) will likely prove to be susceptible. *Lecanosticta acicola* is highly damaging to many *Pinus* species and can cause severe defoliation. Repeated infection may result in needle shortening, which further reduces the tree's photosynthetic capacity. In young trees, infection can reduce establishment rates and significant mortality has been observed in some species. Even mature trees can be killed by repeated infections (Tubby et al. 2023). In the southern states of the USA, timber losses caused by *L. acicola* on *P. palustris* and other 'southern' pine species in the 1980s exceeded 453,000 cubic metres per annum (Tubby et al. 2023a). In northern central states of the USA, *L. acicola* rendered millions of Christmas trees unmarketable from the 1960s to the 1980s (Phelps et al. 1978), and the value of amenity or specimen trees has also been significantly affected (Heimann 1997).

When introduced into Bulgaria, the pathogen was deemed highly adaptable to both the novel hosts present in the region at that time and the environmental conditions of the country. It was concluded that it had the potential to inflict severe damage on naturally occurring species of *Pinus* spp. within the country (Georgieva 2020). In Spain, *L. acicola* caused minor damage to native and exotic species occurring in valleys, stands with high tree density, and/or areas with high humidity in the north of the country Mesanza et al. (2023). However, in recent years, more favourable climatic conditions for the pathogen and other potential unknown endogenous factors have caused the disease to spread. The typical silvicultural practices that were keeping the disease in check have become very site-specific with regards to efficicacy (see section 9.2). The severity of disease in some regions is so high that restrictions on the use of highly susceptible pine species are being considered to lower inoculum pressures (Mesanza et al. 2023).

During a visit by FOA and Scion scientists to the Basque country in northern Spain in 2019, the severity of infection on *P. radiata* was noted, as well as infection on other species, such as *P. pinaster*. *Pinus pinaster* is considered to have low susceptibility according to Tubby et al. (2023a) but they do cite infection from some locations. In the Basque Country, *P. pinaster* is a common host of this pathogen (Fig 5A), albeit less susceptible than *P. radiata* (E. Iturritxa, pers. comm). It would be very interesting to know if *P. pinaster* material from New Zealand shows any tolerance to *L. acicola* infection. In the Basque Country some forest sites were being cleared to plant alternative species, such as *Cryptomeria* spp.

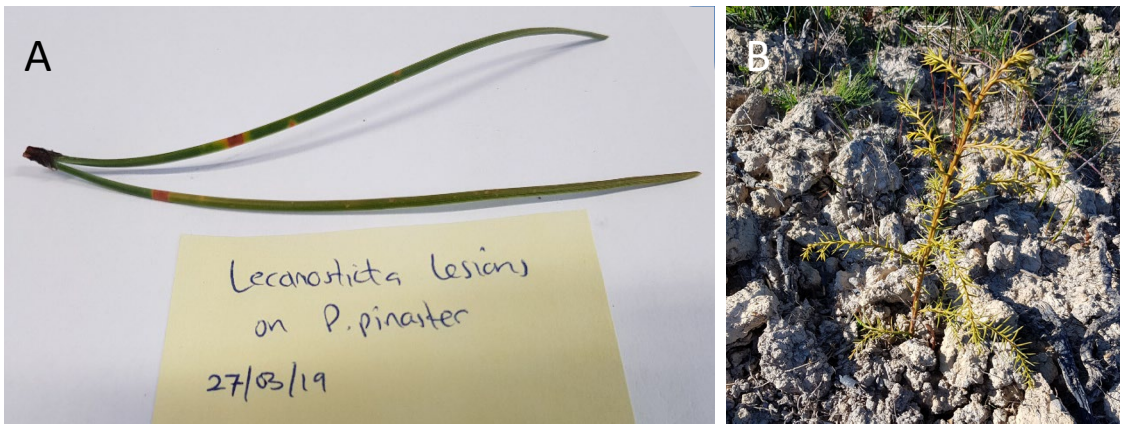

Fig 5. (A) Typical lesions of BSNB on *Pinus pinaster*. (B) Planting of *Cryptomeria* spp. on cleared sites. Both photos taken Basque Country, Spain 2019 (R. McDougal, Scion).

6.3 Climatic conditions for infection and disease

Climate is a critical driver in the lifecycle of this fungal pathogen, influencing its distribution and impact. Warmer regions, such as in the southern states of the USA, allow for multiple disease cycles per annum. Foliar symptoms, conidia and ascospores can be seen throughout the year (Tubby et al. 2023a). In contrast, in the cooler mid-western and northern regions, *L. acicola* tends to have an annual life cycle, which is driven by mist and rain-disseminated conidia, with peaks of infection in late spring and summer (Wyka et al. 2017).

Tubby et al. (2023a) found that the distribution of *L. acicola* geo-database records in relation to maximum monthly temperature to be bimodal, with a peak in numbers of records between 22°C and 23°C, and a second between 32°C and 33˚C, although there are several gaps in these data. The maximum temperature of the warmest month in locations where *L. acicola* was present was 35˚C. The minimum temperature of the coldest month was −24˚C. Release of conidia can occur at temperatures as low as 2˚C in field and ascospores of *L. acicola* have also been trapped in the field at temperatures as low as 5˚C (Kais 1971). Tubby et al. (2023a) also reports that *L. acicola* occurs in regions with wide variation in annual precipitation, from a minimum 407 mm per annum recorded in Alberta, Canada to a maximum annual precipitation of 3157 mm per annum recorded in Colombia. While ascospore release is not reliant on rainfall (Siggers 1939, Kais 1971), conidial discharge is positively correlated with precipitation, and rainfall has been recorded as a significant driver of population expansion. These factors were also considered important in a recent modelling study that showed the wide climatic range renders all of New Zealand as climatically suitable for the establishment and spread of *L. acicola* (Ogris et al. 2023).

In North America, warmer winters, cooler spring temperature, and cumulative precipitation over the spring and summer were important predictors of the presence and severity of WPND on *P. strobus* (see section 2.3) (Wyka et al. 2018). It has also been shown that daily maximum temperature and daily cumulative precipitation were the two best variables explaining *L. acicola* spore abundance in the Basque region of Spain. Low precipitation and average maximum daily temperatures over 25°C resulted in very little spore release (Wyka et al. 2018, Mesanza et al. 2021). Temperature, rainfall, high moisture (leaf wetness) and relative humidity were all significant variables in predicting disease outbreaks, with temperature being the most significant (Ogris et al. 2023).

Climate change is having a significant impact on severity of plant diseases all over the world and was demonstrated for *Dothistroma* as early as 2005 (Woods et al. 2005). A similar situation is being observed with *L. acicola*. Recent changes in climate appear to have caused marked increases in disease intensity in the northern parts of North America (Wyka et al. 2017). *Pinus strobus* is a significant forest species (Wyka et al. 2017, Wyka et al. 2018, Mesanza et al. 2021) in these regions and, until 20 years, ago it was considered moderately to highly resistant to *L. acicola* (Stanosz 1990). However, in the past decade, due to changing climate, i.e., increased summer rainfall (Skilling and Nicholls 1974, Wyka et al. 2017, Wyka et al. 2018) and its impact on inoculum production and dispersion, *L. acicola* has become increasingly damaging to this pine species, causing heavy defoliation, loss of growth, and increased levels of mortality. In Spain, *L. acicola* has been present since 1940s, with only minor impacts on trees in the north of Spain. But recent abnormal climatic conditions have seen an increase in pathogen spread, and disease severity, including mortality, in both native *Pinus* forests and plantations where it was not detected before (Mesanza et al. 2023).

Ogris et al. (2023) tested four modelling approaches to predict the potential future global distribution of *L. acicola* under various climate change scenarios. The outcomes showed the highest relative changes in the pathogen's potential distribution are expected in Africa, Australia, and Oceania, with a 14.1, 26.8, and 175% increase, respectively, compared to the reference period (1971 – 2000). If an incursion was to occur in New Zealand, predictions illustrate that the potential distribution of *L. acicola* will remain relatively stable until 2040 and thereafter suddenly increases to 2060. Ogris et al. (2023) note that 'the very high relative change in *L. acicola* potential distribution in Oceania is largely due to increased potential areas for expansion across New Zealand' and 'the model outputs emphasize the critical importance for these regions of maintaining strict biosecurity measures, as the accidental introduction of *L. acicola* could have hugely significant impacts on forest condition and ecosystem services, given the large areas of vulnerable pine forests.

7. Likelihood of establishment in New Zealand

New Zealand has 1.59 million hectares of *P. radiata* (91 % of the forestry estate as of 2022) (NZFOA, 2023); a host known susceptible to *L. acicola*. While this pathogen is currently absent, modelling has shown that the whole of New Zealand could be climatically suitable for both the establishment and spread of *L. acicola* (Ogris et al. 2023). Climatic data indicate that *L. acicola* could establish in New Zealand irrespective of where the incursion might occur or at what time of year it might occur, although we expect that the risk might be higher in certain regions.

7.1 Potential entry pathways into New Zealand

Human-mediated dissemination of *L. acicola* over long distances primarily occurs through plant movement (van der Nest et al. 2019a, Ogris et al. 2023, Tubby et al. 2023a). New Zealand has strict rules and quarantine measures around the importation of *Pinus, Cedrus*, and *Picea* species, all known hosts of *L. acicola*, which mitigates this pathway. Imports of *Cedrus* and *Picea* are currently allowed from countries known to have *L. acicola* but if an application was received, MPI would be required to reassess potential biosecurity risks, which might lead to changes in import requirements on these species since neither genus has been imported in the past 5 years (Ministry for Primary Industries, Import Health Standard: Importation of Nursery stock: https://www.mpi.govt.nz/dmsdocument/1152/direct) . Despite strict quarantine measures there is always the risk of importation of host genera which have not gone through the proper channels, and this remains a potential pathway. Seed imports are not considered a primary pathway since the pathogen is not seed borne and inoculated seed and needle parts sown in soil did not cause infection of the resulting seedlings. This is maybe due to the low life expectancy (30 to 34 days) of *L. acicola* conidia (Jianren and Chuandao 1988). It should be noted however, that pine seed has been considered as a potential pathway for *D. septosporum* (Tubby et al. 2023b). The current quarantine measures (Ministry for Primary Industries, Import Health Standard: Seeds for sowing; https://www.mpi.govt.nz/dmsdocument/1151/direct) that New Zealand has for seed imports of pine seed lessen any chance of the introduction of this pathogen from seed if individuals are following

correct biosecurity procedures. To the best of our understanding pine seed importation is heavily restricted to avoid the introduction of pathogens, such as pine pitch canker (noting that Import Health Standards have been reviewed by I. Hood (Hood 2018), and these are amended overtime).

The biggest threat might be from contaminants in used cars and machinery, air and sea containers and international travellers intentionally or unintentionally bringing in infected plant material (Brockerhoff and Bulman 2014). Many of the first reports of this disease in European and Asian countries are from amenity tree plantings in parks and botanical gardens (MacLeod et al. 2012) and documented sources of spread include luggage of passengers, forestry tools and equipment, car tyres, clothing and shoes, seeds (if seed lots are contaminated with needles) and insects (EPPO 2023: Lecanosticta acicola (SCIRAC)[Overview]| EPPO Global Database).

Lecanosticta acicola conidia can only be moved over different distances by wind and rain and could be considered an important pathway (Fitt et al. 1989, MacLeod et al. 2012, Suto, 2002). However, ascospores have a greater potential for wind-driven, long-distance dispersal (van der Nest et al. 2019a, Tubby et al. 2023a) much like *Dothistroma septosporum* where long-range dispersal has been considered a major mechanism for international spread of this pathogen (Bulman et al 2016). Conidia of *L. acicola* can also be spread by insects (MacLeod et al 2012), however this is a passive form of movement and since *L. acicola* does not have a specific insect vector (Skilling and Nicholls 1974), insects are not considered a high-risk pathway. If *L. acicola* spores (sexual or asexual) are capable of a trans-Tasman crossing, it could cause considerable concern if an incursion occurred in Australia. We have not been able to source consistent information describing actual distances for 'long-distance dispersal' and believe that this is a major knowledge gap for *L. acicola*.

7.2 Domestic pathways for pathogen spread

7.2.1 Environmental

The spread of *L. acicola* through natural environmental conditions (excluding anthropogenic spread) has been summarised in the table below (adapted from (van der Nest et al. 2019a)) and in previous sections. Briefly, *L. acicola* conidia primarily spread short distance with moisture, such as rain splash (van der Nest et al. 2019a). Although less frequently produced, wind spread ascospores are more likely to contribute to long distance spread (van der Nest et al. 2019a). Long-distance dispersal has been observed for the natural spread of the similar pathogen, *Dothistroma septosporum,* which spread in excess of 1400 metres from its source, suggesting that conidia could travel long-distance by becoming wind-borne through rain-splash droplets (Mullett et al. 2016). This greater dispersal distance of *D. septosporum* could be applied to *L. acicola*, since both produce ascospores and conidia. However, infection severity is expected to decrease with distance from the source inoculum (Mullett, et al. 2016).

The presence of a single mating type of *D. septosporum* in New Zealand was demonstrated (Groenewald et al. 2007), but this analysis has not been repeated, and the presence of *D. pini* is

also not routinely checked. Therefore, the spread of *Dothistroma* within New Zealand is thought to have occurred through movement of conidia and infected material. Dispersal of *L. acicola* may be more efficient than *Dothistroma*, if it is capable of producing both spore types. For *L. acicola* to produce ascospores within New Zealand, both mating types would need to be present. While this does not appear to have happened with *D. septosporum*, it was shown that two different lineages (or strains) of *Phytophthora pluvialis* are present in New Zealand, suggesting that there have been at least two potential introductions of this species (Brar et al. 2018). Measures that reduce the risk of introduction of *L. acicola* will likely reduce the risk of introducing a greater diversity of *D. septosporum*, as well as *D. pini.*

Table 2. Impact of environment on successful spread of *L. acicola*

Environmental spread of *L. acicola*	**Relevant findings**	**Host species**	**Reference**
Spore production	Both relative humidity (>70%) and frequent rainfall are important drivers for production. Occurring from late spring to the end of summer. The frequency of rainfall is more important than duration of rainfall, this could indicate the necessity for leaf wetness.	*Pinus strobus*	(Wyka et al. 2018)
Spore dissemination pathway	Rain-splash spreads conidia between adjacent trees, the mucilage formed in conidial masses prevents dry wind-dispersal. Rainfall patterns are indicated as the main driver for dispersal, due to the variance of peak disease timing found at different geographies. Ascospores produced through sexual reproduction are dispersed long-distances by wind.	NA	(Siggers 1944, Fitt et al. 1989, Tainter and Baker 1996, Janoušek et al. 2016, Wyka et al. 2018, van der Nest et al. 2019a)
Spore travel	Conidia rarely travel more than 3 metres from the canopy but have been observed at over 60 metres. Spore traps have failed to pick-up ascospores in multiple studies. Ascospores have the potential to be dispersed much farther than conidia, since they are carried by wind.	*Pinus strobus*	(Wyka et al. 2018, van der Nest et al. 2019a, Tubby et al. 2023a)
Spore germination	Conidia germinate between 5 and 35 °C. However, the upper limit of germination has some slight variation across studies at different geographies, perhaps implying that pathogen lineages may be adapted to local climate conditions.	*in vitro*	(Siggers 1944, van der Nest et al. 2019a)
Infection	Optimum infection temperature of 30 °C during the day and 21 °C at night. High humidity is a major contributor to disease expression both before and after inoculation.	*Pinus palustris*	(Kais 1975)

Considering the distribution of potential hosts in New Zealand, environmental spread within forests is quite likely. Many (or even the majority) of New Zealand's largest plantation forests, such as Kinleith, Kaingaroa, and Wharerata, are planted in continuous monocultures. Although the effect of tree diversity on the spread of foliar pathogens can be variable, a pan-European study at stand level suggests foliar disease decreases with species richness of conifer species but not broadleaf

species (Nguyen et al. 2016). Although *L. acicola* was not directly studied in this case, high availability of a susceptible host at a landscape level is likely to promote local spread of conidia. Some of these forests, such as Kaingaroa, form a large continuous area (~3000 km^2) which cross different regions. Environmental spread of *L. acicola* through less forested areas may be lower. Use of forestry species in other land uses, should still be considered. For example, *P. radiata* is commonly used as a shelterbelt tree in New Zealand, therefore attention should be given to these shelterbelts as potential sources of inoculum facilitating pathogen spread across non-forested country. Shelter belts or hedgerows have been observed as inoculum reservoirs for other diseases like *Neonectria ditissima* in apple trees (Walter et al. 2015) and dieback in European ash (Chandelier et al. 2017). Some alternative biological dissemination methods have been proposed, such as insects and animals (Skilling and Nicholls 1974, Tainter and Baker 1996); however, little attention has been given to these methods because they are not considered important (van der Nest et al. 2019a).

Pine pitch canker is a destructive disease of pines and the causal agent, *Fusarium circinatum* is one of New Zealand forestry's most unwanted pathogens. This pathogen is also present in Spain, causing huge impacts on *P. radiata* plantations and nurseries (Blank et al. 2019). It has been previously demonstrated that high nitrogen levels can influence the infection of pines by *F. circinatum* (Solel and Bruck 1989, Lopez-Zamora et al. 2007). Considering Spain has elevated levels of nitrogen deposition in the soil (Garcia-Gomez et al. 2014), it would be interesting to understand the influence of nitrogen on *L. acicola* infection of pine. New Zealand's planted forest soils have a wide range of fertility levels (Garrett et al. 2021b). Some locations in New Zealand do have high nitrogen deposition in soils (Parfitt and Baisden 2005, Dyck 2009), especially if the land was previously used for pastoral farming (Garrett et al. 2021a). This could be a potential issue promoting pathogen establishment and infection, but further research is required.

As with the unpredictable nature of climate change, the impact it may have on spread is difficult to quantify. Given the water-borne dispersal of conidia, climate change may promote spread and establishment of *L. acicola* in areas in which rain is predicted to increase, whereas risk will reduce in areas predicted to become drier. Confidence in changes in rainfall is lower than temperature predictions; however, annual rain is predicted to increase in the south and west of New Zealand, driven by spring and winter patterns (Bodeker et al. 2022), which may increase risk for spread and establishment in these regions. It is important to note that rain in the east of New Zealand is decreasing, but this is not reflected by all seasons with a predicted increase in rainfall over summer (Bodeker et al. 2022). As well as general changes in rainfall, there will be changes in extreme weather, such as tropical cyclones (Bodeker et al. 2022) that may contribute to spread through movement of spores, as well as infected needles.

7.2.2 Anthropogenic movement

Movement of *L. acicola* into forests, or between forests/nurseries is likely facilitated by humans. The key route of spread of *L. acicola* in Europe is thought to be through movement of infected plant

material (Tubby, et al., 2003). This is exacerbated by the long asymptomatic phase of *L. acicola* infection which can last up to 3 months (Skilling and Nicholls 1974), allowing plants to be distributed to forests from nurseries with a lack of detection. In addition to movement of plants, other forestry activities, such as a lack of sanitisation of silviculture tools, logging trucks, and other equipment (Tubby, et al., 2023a) may facilitate movement of the pathogen within New Zealand. As *L. acicola* can sporulate on both cast and living needles (Henry 1954, Jewell 1983), diseased material that may be trapped in forestry machinery and moved between forests could contribute to internal spread. An investigation of the risk associated with logging truck movements is currently underway with preliminary results published in an earlier report by Hood et al. (2019), but there is a potential gap in our knowledge around the risk associated with the trade and movement (nationally and internationally) of forestry machinery. It has been speculated that *P. pluvialis* may have arrived in New Zealand on second-hand forestry machinery (Brar et al. 2018).

Tourism has also been implicated as an important factor for the spread of *L. acicola* in Europe, such as the Czech Republic on bog pine (*P. rotundata*) and Slovenia on several pine species (Jankovský et al. 2009, Tubby et al. 2023a). Many of New Zealand's tourist activities include hiking, biking, camping, and other related pursuits that occur within native and exotic forests. Movement of hikers between native forests has already been linked to spread of an important disease of native trees, kauri dieback, and restrictions are in place for access within affected forests (Ministry for Primary Industries 2023). New Zealand's native species have not been tested for susceptibility to BSNB; however, *L. acicola* is typically a pathogen of species in the family Pinaceae (van der Nest et al. 2019a), none of which are indigenous to New Zealand. Between 2022 and 2023, 96 production forests allowed bike access with 335,000 visitors from outside of the host region (NZFOA, 2023). These recreational activities within plantation forests present the risk of internal spread of *L. acicola* if equipment, such as vehicle and bike tyres, and shoes are not cleaned when moving between plantation forests.

8. Incursion response

Case studies indicate that a strong incursion response, while typically aim for eradication, can at least restrict the movement of *L. acicola*. For European locations where efficient pathways for pathogen spread exist, eradication of *L. acicola*, in most cases, have been unsuccessful. van der Nest et al. (2019a) list a table of locations of *L. acicola*, severity of infections, and, where known, eradications methods attempted for those locations. van der Nest et al. (2019a) also note eradication was successful in some areas of Lower Austria (Valley of the river Ybbs) where disease was first observed 1996 to 2000, then no longer detected from 2001/2002. However, the pathogen persists in other areas of Austria, such as Hollenstein/ Ybbs and upper Austria, where eradication has not been successful. Similar stories can be seen across the globe. For example, in Bulgaria, *L. acicola* was first discovered in 2017 on *P. sylvestris* (Georgieva 2020). In 2018, phytosanitary measures, including felling and removal of infected trees, was implemented and the pathogen was not detected from surrounding sites (25km from initial outbreak) in 2019 (Georgieva, 2020). In Lithuania, *L. acicola* was first discovered as a *P. mugo* infection on the Curonian Spit (Markovskaja

et al. 2011). Although initially restricted through removal of infected material (Markovskaja et al. 2011), *L. acicola* is now present throughout the country (Raitelaitytė et al. 2023). Although, the detection of *L. acicola* in new areas may be either new introductions or spread from failed eradications, in each of these case studies, eradication attempts slowed the spread of disease and allowed industry and government time to adjust management practices.

Due to the limited entry pathways (section 4.2) for an island country such as New Zealand, eradication should be attempted as introductions are less likely/frequent. Containing and removing any introductions that do slip through the biosecurity net should limit establishment and internal spread. Similar approaches to those successful in localised eradication around the world could be undertaken in New Zealand. Surveillance and early detection, as outlined in sections 4 and 5, are key to eradication of *L. acicola*. Monitoring should be increased soon after detection to determine the distribution of the pathogen and to understand the success of the containment and eradication attempt (Sosnowski et al. 2009).

Following detection, the area of discovery should be designated a containment zone, and movement of people and host material restricted within the area (Tubby et al. 2023a). Controlled areas require the removal and destruction of all infected material. The destruction method will depend on the location where *L. acicola* was first detected, for example individual infected trees within an urban environment may require a different approach compared to a stand of infected trees within a forest. Techniques for killing plant pathogens are extensively detailed in Sosnowski et al. (2009) and include burning, burying, pruning, composting, soil fumigation, bio-fumigation, soil solarisation, and steam sterilisation. In cases of *L. acicola,* eradication measures tend to involve felling and removal of trees and plant litter with subsequent burning or burying, with varying success (Markovskaja et al. 2011, Georgieva 2020, Tubby et al. 2023a). Attention should be given to the transportation of infected material to burn or burial sites to ensure that there is appropriate containment, this is key for increasing the success of eradication (Sosnowski et al. 2009). As ascospores can persist on dead needles (Fig. 1, van der Nest et al. (2019a)), destruction of the tree alone is likely insufficient to halt disease, and the leaf litter must also be treated. To address lingering inoculum, a soil treatment would be valuable, whether it be fumigation or perhaps soil solarisation using a clear plastic covering over extracted soil (Sosnowski et al. 2009). The area to be managed will likely dictate how feasible it is to treat the topsoil. Monitoring should be continued throughout New Zealand following destruction of infected material to determine how successful eradication is.

9. Treatment and control options

9.1 Chemical

There are several fungicides that are highly effective against *L. acicola*. These have been reviewed by Tubby et al. (2023a) and summarised in Table 3**.** Of these, use of a copper fungicide is the most promising option for immediate use in a New Zealand context. Currently, cuprous oxide is routinely

sprayed at an application rate of 0.855 kg ha^{-1} active ingredient in 2L oil made up to 5L per ha with water for control of DNB (Bulman et al. 2004). Purchase of copper fungicide and application are typically organised by the New Zealand Dothistroma Control Committee (DCC) (Bulman et al. 2004). The knowledge and experience in copper applications as well as environmental effects at a forest scale and therefore this capability will enable the transfer of application for an alternative disease, such as BSNB. The pipeline for developing this process was recently shown by red needle cast of radiata pine caused by *Ph. pluvialis*. Efficacy was first proven in laboratory and pot trials (Rolando et al. 2019), followed by operational trials (Fraser et al. 2022) and is now being incorporated by the DCC for control of red needle cast. In the case of *L. acicola*, copper fungicides are commonly recommended as a Bordeaux mixture (copper (II) sulphate (CuSO4) and quicklime (CaO)) (van der Nest et al. 2019). Tubby et al. (2023a) suggest cuprous oxide as viable control tool also. For control in nurseries, Hedgcock (1929) recommended fortnightly applications in spring of 4-4-50 Bordeaux, with the addition of 0.9 kg oil such as whale-oil or casein soap per 190 litres of solution. Although fixed coppers such as cuprous oxide differ from Bordeaux mixture, the active ingredient in both cases is the copper salt, Cu^{2+}, and have been used interchangeably to treat the same organisms (Lamichhane et al. 2018). Information on the rate of cuprous oxide, or other copper fungicides aerially applied in planted forests for control of *L. acicola* was not readily available. It may be beneficial to determine if application of cuprous oxide at the currently recommended DNB rate is effective against *L. acicola* as it is less difficult to prepare, and more is known on application dynamics and environmental effects. The key time for application of preventative fungicides is prior to infection of new needles, ideally when needles are about half grown (Skilling and Nicholls 1974). This is similar to the timing of DNB sprays (November). In years with high conducive disease conditions, an additional spray one month later may be required (C.A.B.I 1997).

Table 3: Chemical treatments effective against *L. acicola* (adapted from Tubby et al. (2023a)).

Host	Treatment	Reference
P. palustris	Bordeaux mixture (Copper Sulphate & Quicklime), Lime-Sulphur, Calcium Hydroxide, Calcium Caseinate, Chlorothalonil, Mancozeb.	(Hedgcock 1929, Webster 1930, Siggers 1932, Siggers 1944, Kais 1989, Tubby et al. 2023a)
P. palustris and *P. elliottii* seedlings	Benomyl (root-dipping)	(Hedgcock 1929, Webster 1930, Siggers 1932, Siggers 1944, Kais 1989, Tubby et al. 2023a)
Various	Copper containing products (e.g. Bordeaux mixture, Cuprous Oxide)	(Tubby et al. 2023a)
P. taeda and *P. elliottii* seedlings	Carbendazim, Thiophanate-methyl	**(Jianren et al. 1992)**
P. palustris, P. maestrensis seedlings or seedbeds	Mycorrhizal (*Pisolithus tinctorius*) inoculation in combination with fungicide	(Kais et al. 1981, Ferrer et al. 2000)
Generic host species	Felling, burning, burying of infected material	(Tubby et al. 2023a)

9.2 Silviculture

A thorough review of silviculture practices to manage *L. acicola* has been recently accomplished (Tubby, et al. 2023a), these strategies are summarised in Table 4 below. Silvicultural management, such as thinning and pruning that reduce humidity within the canopy, and increase the distance spores must travel to a new host, are typically suggested as a means of disease reduction (Tubby, et al. 2023a). However, the result of pruning and thinning on the levels of foliar disease can be variable. For example, due to role of moisture in spore initiation and spread, if these activities are carried out during wet periods when disease is expected, this may instead contribute to pathogen spread (Tubby et al. 2023a). A key takeaway from the findings presented is the necessity to reduce the potential transfer of spores between trees and to consider the timing of silvicultural activities to occur prior to the expected disease outbreak during dry periods (Kais 1989) and the attention to sterilisation of equipment between sites (Mesanza et al. 2021, Tubby et al. 2023a). Although thinning reduced disease severity in most cases (Tubby et al. 2023a), Ortíz de Urbina et al. (2017) showed that pruning and thinning completed during favourable disease conditions had little effect on disease severity confirming the importance of timing of silvicultural events to occur within certain environmental thresholds where it can be effective. Silvicultural management strategies may not always be effective and are dependent on the conditions, as shown in the example from Spain where silvicultural techniques could not stop the spread of the disease (Mesanza et al. 2023). Further research is required, with a focus on *P. radiata* under climate similar to New Zealand to determine if preventative silvicultural technique, such as alteration of stand density, could reduce disease spread and impact (Tubby, et al. 2023a). Infection risk can be reduced by avoiding the establishment of new stands by those with a history of disease (Tainter & Baker, 1996).

Table 4. Management strategies used to control brown spot needle blight (Summarised from Tubby et al., 2023).

Management strategy	**Reference**
Appropriately storing and caring for seedlings before planting, to ensure plants are not wounded or stressed.	(Tubby, et al., 2023b)
Thoroughly cleaning equipment (like pruning saw blades), machinery and even clothing involved in silvicultural events.	(Nicholls et al. 1973, Mesanza et al. 2021, Tubby et al. 2023a)
Regular controlled burns of infected needle tissue, when infection levels are above 20-30% for fire-resistant species such as *Pinus palustris*	(Demers et al. 2010, Tubby et al. 2023a)
Increasing tree spacing to reduce microclimate humidity and decrease the potential for spore transfer.	(Tubby et al. 2023a)
Regular thinning and/or pruning to reduce humidity and spore spread, while avoiding pruning in wet conditions and when spore production is expected to be active.	(Kais 1989, McIntire et al. 2018, Mesanza et al. 2021, Tubby et al. 2023a)
Employing mixed age silvicultural systems, especially for species where susceptibility varies with tree age.	(Siggers 1932, Tubby et al. 2023a)

9.3 Resistant genotypes

Breeding for resistance against *L. acicola* is a promising option as part of a long-term management plan. Variation in resistance to BSNB has been observed in *P. patulus* (Snyder and Derr 1972), *P*

elliottii (Ye and Li 1988), hybrids of *P. palustris* × *P. elliottii*, and *P. taeda* (Su et al. 2003). Heritability of resistance to BSNB has not yet been recorded for *P. radiata*. However, New Zealand has a very successful breeding programme in place for DNB caused by *D. septosporum*. Although taxonomically, *D. septosporum* and *L. acicola* differ, they share some similarities in symptoms, biology, and behaviour on *P. radiata* (Ogris et al. 2023).

Reviews on host defence against both these pathogens show there are many similarities, including production of resins and pathogenesis-related proteins (Fraser et al. 2016, Tubby et al. 2023a). As tree breeding is a significant investment of time and resources, it would be worth screening current germplasm with high (and low) *Dothistroma* resistance values for *L. acicola* resistance. This would provide an indication of the susceptibility of current stock and provide a baseline for resistance breeding going forward, as well as indication on overlap between resistance breeding between these two needle diseases. As *L. acicola* is currently not present in New Zealand, this screening would need to be carried out by collaborators within countries with established *L. acicola* presence, or within quarantine facilities in New Zealand. There is currently research underway to explore the pathogenicity of *L. acicola* on *P. radiata* germplasm from seven New Zealand seedlots and a Basque control. This research is also exploring endophytes for potential to induce resistance. Field trials are underway in areas that have high loading (incidence) of *L. acicola,* as well as other pathogens and pests. This collaborative project between New Zealand and Spanish researchers is an excellent model of how we can prepare for offshore threats, such as this pathogen (Jenny Aitken and Eugenia Iturritxa, pers. comm.). Consideration of indirect selection with early phenotypes, genomic selections (Alves et al. 2020), marker assisted breeding (Liu et al. 2020), and epigenetics (Amaral et al. 2020) could also enhance the speed of development of resistant germplasm.

Alternatively, New Zealand could look to alternative species that are less susceptible, or hybrids between radiata pine and resistant species (Herron et al. 2020). Known susceptible species are listed in section “Host range & susceptibility”. Tubby et al. (2023a) list conifers *Abies concolor*, *Pi. abies, Pi. koyamae, P. fenzeliana*, *P. heldreichii var. leucodermis, P. massoniana, P. sibirica,* and *P. taiwanensis* as resistant to *L. acicola* based on either artificial inoculations or absence of disease when grown in infected areas. Several of these species are present in New Zealand and have been planted in plantation forestry or forestry trials, providing an opportunity for investigation into the use of alternative species in planting regimes for areas prone to BSNB.

9.4 Emerging technologies

Although this review focuses on traditional methods of disease control that have been used to successfully control *L. acicola*, it is important to also consider alternative control tools, such as emerging technologies or transfers from horticulture.

Understanding the pathogen-host interactions at a molecular level using genomic technologies and gene identification, specifically for effectors, can provide opportunities for accelerated, modern resistance breeding for plants (Vleeshouwers and Oliver 2014). Effectors are small proteins that

interact with host plant receptors and initiate a response, much like an immune system. However, it is becoming increasingly apparent that many effectors do not only induce host immunity but can also interact with susceptibility factors in the host, increasing the chances of infection (He et al. 2020). If susceptibility genes can be identified in the host, these could be targets for biotechnology to help develop resistant lines of the plant host. Recent research aimed at developing durable disease resistance has identified conserved effectors across multiple pathogens of *P. radiata*, including *D. septosporum, Cyclaneusma minus* (Hunziker et al. 2021, Tarallo et al. 2022, Tarallo et al. 2023), and *Ph. pluvialis* (Tarallo, Bradshaw, McDougal, et al. in prep), as well as the development of novel methods for high-throughput screening of plant material (Hunziker et al. 2021). Understanding how the effectors from these pathogens modulate immunity in their pine hosts is becoming clearer. This has also led to a current research program exploring durable resistance to fungal pine pathogens using biotechnology approaches and RNAi (Glenn Thorlby, pers. comm.). The ability to use effectors to identify susceptibility genes in the host will ultimately enable rapid development of resistance and increased opportunities to for preparedness to offshore threats. Understanding how the effector repertoire of *L. acicola* compares to *D. septosporum* could provide information that could lead to protocols for the rapid development of resistant lines of pine and complement screening research already underway.

10. Social licence to operate - lessons from Dutch elm disease

As *L. acicola* affects *Pinus* spp., an exotic species to New Zealand, BSNB will have a greater impact in commercial forests compared to urban forests. In the commercial forestry estate, forest owners should be able to treat BSNB infected trees as they would any other needle disease they currently manage, such as DNB and RNC, with aerial applications of fungicides. There could be some concern from homeowners and communities if the trees were present in gardens or on roadsides and consultations may be needed should aerial spraying be considered an option. It may or may not be of concern for the removal of BSNB affected *Pinus* spp. in urban areas; however, it could come with a cost to homeowners and councils. The Auckland council spends thousands of dollars every year removing elms affected by Dutch elm disease (Simon Cook, Pers. Comm.). Treatments for dutch elm disease are socially accepted as they are targeted, being applied directly through stem injections. This treatment is costly and reserved for special trees.

Eradication efforts in urban areas in Europe that have relied on felling and burning of residues and appear to have been successful in some cases (Tubby et al. 2023a). However, no information is provided by the authors of that review describing the social acceptability of different management techniques in those urban environments. This might be considered a gap in knowledge that could be investigated with overseas practitioners. The authors (Tubby et al. 2023a) do encourage the recording of actions and outcomes in the geo-database, regardless of how successful they are, so that other surveillance teams and forest managers can benefit from that knowledge (Tubby et al. 2023a).

11. Recommended risk management options - surveillance and monitoring

Early detection and surveillance:

- While there are a number of *Lecanosticta* species that infect various *Pinus* species, *L. acicola* should be the focus of readiness activities for biosecurity in New Zealand. However, it might still be beneficial to build collaboration with international researchers (e.g. FABI, Nieker in Spain) to develop diagnostic tools for other *Lecanosticta* species or develop a *Lecanosticta* genus-specific assay.
- Add this pathogen to the list of organisms being surveyed for in high-risk sites* *High-risks sites can be inferred from data generated through modelling of climate and pathway data.*
- Diagnostic tools are ready to be used (tested/validated in-house) and updated as more information becomes available, i.e., new assays, new species, etc.
- Note the potential threat to Douglas-fir and ensure this host is surveyed for any unusual symptoms or typical symptoms of BSNB.

Capacity building:

- Forest Biosecurity surveillance, forest inspectors (e.g., SPS Biosecurity), forest managers as well as scientists who are in the field should be familiar with the disease symptomology on different hosts, but particularly on *P. radiata*. Even if detected and present, on-going surveillance to avoid introduction of additional lineages will remain important.
- Upskilling of diagnosticians to recognise differences in disease symptomology on different hosts, but particularly on *P. radiata*, pathogen morphology and culture morphology, and particularly differentiation from *Dothistroma*. Important to have molecular diagnostics available and most accurate for rapid identification of *L. acicola*, including DNA for positive controls (Scion has obtained this in the past from overseas contacts).
- Further work could be done to streamline needle disease diagnostics using molecular tools, as efficiencies could be achieved, making better use of resources available.
- There is the potential for dispersal via moist air currents from NZ to Australia as postulated for *Dothistroma*, but do currents flow the opposite way? It is assumed that myrtle rust dispersed from Australia to Raoul Island (Toome-Heller et al. 2020). There may be a potential opportunity with the new Aerial Invaders research program at Scion to investigate this.

12. Recommendations on further readiness work (epidemiology, management, new innovative research opportunities)

Readiness activities:

- Work with the Ministry for Primary Industries to develop a Threat Specific Readiness Manual and/or Operational Specifications document, as done previously for *Fusarium circinatum.*
- Development of a Pest Risk analysis might be helpful. This could incorporate climate change scenarios looking forward (as opposed to historical data only).

Education and outreach:

- Use our current channels (forums, Find-a-Pest, workshops, social media etc.) to raise awareness about the symptoms, risks, and prevention methods. *Lecanosticta acicola* does not yet feature on the Find-a-Pest app.
- Develop education factsheets and digital pamphlets to share with those in the field and in diagnostic laboratories.

International Collaboration:

- Continue to support the development of the Trans-Tasman Forest Health and Biosecurity Network with on-going communications and information sharing.
- Working with overseas collaborators on disease management options such as testing the ability of currently operational cuprous oxide formulations (0.855 kg ha^{-1}) to control *Lecanosticta* species.
- Work with overseas researchers to understand the social licence implications of potential control measures for any incursion response, especially in an urban environment, and any cultural considerations that might be necessary.

- Continue working with Neiker scientists (Spain) to explore disease resistance, *P. radiata* genotypes, alternative species, endophyte research for tree resilience and protection, and to understand pathogen populations and evolution. Potentially expanding to screen in other areas e.g. Mesoamerica.
- Continue to develop collaborations with additional overseas forest health researchers, e.g. FABI, to continue knowledge development and increase opportunities to learn about the pathogen and disease management.

Investment in resilient tree varieties:

- International collaboration to screen NZ germplasm in countries affected with *L. acicola* could develop our breeding programmes to deploy varieties that could be rolled out to limit the impact of an incursion should it ever occur.
- Continue the work that has already begun screening of existing *P. radiata* material to understand susceptibility to *Lecanosticta* species (J. Aitken & E. Itturitxa) help inform breeding plans for more resistant stock.
- Screen alternative pine species such as *P. pinaster, P. patula* and *P. coulteri* for susceptibility to *L. acicola* by working with overseas collaborators (as above). There is currently material in production for these species in New Zealand (for example https://www.proseed.co.nz/). Hybrid pine species might also be worth testing.
- Working with overseas collaborators on disease management options.

Research and Monitoring:

- There is a gap in our understanding of the biology of several foliar pathogens, including *L. acicola* and its relatives, as well as the similarly looking *Dothistroma*. This information will be useful to developing management strategies tailored to the pathogens presented here.
- Explore research opportunities for innovative and high-impact, rapid screening of plant material, host-pathogen interactions, and disease treatments e.g., biotechnology, effector biology/genomics, RNAi.
- Understand the disease risk in areas where soil nitrogen levels are high.
- Climate risk modelling to identify high risk areas and provided information on rate of spread. This will inform high risk monitoring sites as part of the biosecurity network. This could also inform pest risk analyses.
- An epidemiological model like that being developed for *Ph. pluvialis* in the Resilient Forests research program could also be informative for *L. acicola* infection of *P. radiata* and could be done collaboratively with overseas researchers.

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