


Diplodia sapinea found on Scots pine in Finland

Michael M. Müller¹  | Jarkko Hantula¹ | Michael Wingfield² | Rein Drenkhan³

¹Natural Resources Institute Finland (Luke), Natural Resources and Bioproduction, Helsinki, Finland

²Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

³Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Tartu, Estonia

Correspondence

Michael M. Müller, Natural Resources Institute Finland (Luke), Natural Resources and Bioproduction, Helsinki, Finland.
Email: micms.muller@gmail.com

Funding information

Eesti Teadusagentuur, Grant/Award Number: PSG136 and IUT21-04; Estonian Science Foundation, Grant/Award Number: PSG136 and IUT21-04

Editor: J. Stewart

Abstract

Diplodia sapinea is an important pathogen of *Pinus* spp. in many parts of the world's temperate and sub-tropical zones. In Central Europe, this fungus has been known since its first description in the 19th century. But it was only in 2007 that the fungus was identified using DNA-markers in northern Europe (in Estonia). Viljo Kujala reported observing *D. sapinea* on logging debris of Norway spruce in Finland in 1945, but we have not been able to verify the identifications from his herbarium samples. In 2004, we collected cones under asymptomatic Scots pine (*Pinus sylvestris*) trees at one location near the southern coast of Finland but failed to isolate *D. sapinea* from the samples. In 2015 and 2016, we collected fallen Scots pine cones in 15 Scots pine stands in various parts of Finland and the fungus was found at five locations in south-western Finland including the location investigated in 2004. Frequency of cones with pycnidia of *D. sapinea* varied from 1% to 12% in the infested stands. No symptoms of *Diplodia* tip blight or resinous cankers were detected in trees in the stands where the cones were collected. The results suggest a recent introduction after 2004 of the *D. sapinea* in Finland.

KEYWORDS

climate, conifer, forest pathology, fungi, latent infection

1 | INTRODUCTION

Diplodia sapinea (Fr.) Fuckel (earlier *D. pinea* and *Sphaeropsis sapinea*) is a pathogen of *Pinus* spp. that causes tip blight, resinous cankers on main stems and branches, dieback, misshapen tops, death of cones, seedling blight, sapwood staining and sometimes root disease in seedlings (Sinclair & Lyon, 2005). Especially in managed *Pinus radiata* and *Pinus patula* plantations of the southern hemisphere, *D. sapinea* has caused large-scale dieback (Chou, 1976; Swart & Wingfield, 1991; Zwolinski, Swart, & Wingfield, 1995). In Europe, the most susceptible native pine species are Austrian pine (*Pinus nigra*), mountain pine (*Pinus mugo*) and Scots pine (*Pinus sylvestris*) (Fabre, Piou, & Desprez-Loustau, 2011; Iturriza et al., 2013; Vujanovic, Arnaud, & Neumann, 2000). In France and Germany, *Diplodia* tip blight and canker damage on Scots pine have increased in incidence and importance in recent decades (Blaschke & Cech, 2007; Fabre et al., 2011). This has also been true on *P. mugo* and *P. nigra* in southern Europe (Georgieva & Hlebarska, 2016; Maresi et al., 2002).

Diplodia sapinea can persist for long periods of time as asymptomatic latent infections in pine hosts (Bihon, Slippers, Burgess, Wingfield, & Wingfield, 2011; Flowers, Nuckles, Hartman, & Vaillancourt, 2001; Stanosz, Smith, Guthmiller, & Stanosz, 1997). Drought and other sources of stress trigger pathogen proliferation and development of disease outbreaks (Blaschke & Cech, 2007; Chhin & O'Brien, 2015; Stanosz, Blodgett, Smith, & Kruger, 2001). Also, hail damage has frequently been mentioned to result in serious disease outbreaks caused by *D. sapinea* on pines and the effect is particularly pronounced in pine stands stressed by drought (Swart & Wingfield, 1991). Infections of pines by *D. sapinea* have also been observed in association with insect damage in the absence of physiological stress (Haddow & Newman, 1942; Swart, Wingfield, & Knox-Davies, 1987a).

Diplodia sapinea is among the most common and widely distributed pathogens of pines worldwide. Consequently, it is interesting that, until recently, it has not caused disease outbreaks in northern Europe where the susceptible Scots pine is one of the dominant tree

species. The pathogen was originally described in the 19th century as *S. sapinea* by Fries in Sweden (Fries, 1823) and as *Sphaeria pinea* by Desmazieres (1842) in France. It has, therefore, been known in Europe for over 170 years. However, DNA-based taxonomy has revealed high diversity within the *Botryosphaeriaceae* with many morphologically similar species (Phillips et al., 2013) and it is questionable whether the initial species descriptions correspond to what we now regard as *D. sapinea*. The first observations of *D. sapinea* in northern Europe (including Fennoscandia, the Baltic countries and northwestern Russia), verified by DNA sequence-based methodology, were from Estonia in 2007 and subsequently from northwestern Russia and Sweden in 2013 (Adamson, Klavina, Drenkhan, Gaitnieks, & Hanso, 2015; Hanso & Drenkhan, 2009; Oliva, Boberg, & Stenlid, 2013). In Estonia, the fungus was initially found only on cones of the non-native Austrian pine but in 2010 it was also collected on needles. The fungus spread throughout Estonia within a few years and was detected in 2012 at one location also on cones, needles and twigs of Scots pine (Adamson et al., 2015). The Russian record on native Scots pine in 2013 was made 110 km from the southeastern border of Finland (Adamson et al., 2015). In Sweden, *D. sapinea* was first detected in 2013 (Oliva et al., 2013), and in 2016, a Diplodia tip blight outbreak occurred in a Scots pine stand in central Sweden (latitude and climate corresponding to that of the south coast of Finland) and in another stand in 2017 in southern Sweden (<https://www.skogsstyrelsen.se/nyhetslista/nytt-fall-av-diplodia-pa-tallbestand/>).

Environmental conditions could previously have prevented *D. sapinea* from becoming established in northern Europe because low winter temperatures are known to correlate negatively with its occurrence in France (Fabre et al., 2011). This view is supported by observations of the temperature response of this fungus in vitro.

Both the maximal and optimal temperature for growth and minimal threshold temperature of *D. sapinea* in artificial growth media are high; 40°C, 28°C and 4°C, respectively (Keen & Smits, 1989; Milijašević, 2006). By comparison, *Gremmeniella abietina*, a pathogen occupying a very similar niche in boreal pine forests (i.e., infecting needles and shoots of Scots pine), occurs in the most northern Scots pine forests of Europe and is able to cause necrosis in Scots pine seedlings at -4°C (Petäistö, 1993). Ettlinger (1945) recorded growth at -3°C, highest growth at 19°C and a thermal death point at 25–28°C for two isolates of this species on agar media. Hence, *D. sapinea* appears to be adapted to considerably higher temperatures than *G. abietina*, which is also consistent with the distribution range of the fungus in warmer regions.

The recent increases in observations of *D. sapinea* in northern Europe coincide with considerable climate warming. In Finland, the warming has been more rapid during the last four decades than ever since temperature recordings were initiated in the country in 1847. During 1980–2010 mean annual air temperature rose by 1°C (Mikkonen et al., 2015). The temperature increase has been highest during November–January, and winters have consequently become milder.

Viljo Kujala (1950) reported observations of *S. sapinea* in Finland. He found the fungus on Norway spruce bark and deposited several samples in between 1945 to 1949 in the herbarium of the Finnish Forest Research Institute. In order to assess the possibility that this fungus was present in Finland in the 1950 s, we sourced and examined the herbarium specimens collected by Kujala. An informal survey of *P. sylvestris* cones in Finland in 2004 failed to result in any isolations of *D. sapinea* at that time. However, in 2015, pycnidia of *S. sapinea* were found on *P. sylvestris* cones in Helsinki. These

TABLE 1 Conidia and pycnidia dimensions of Finnish specimens compared to previously reported

Species or specimen	Geographical origin	Conidia dimensions		Number of septa	Pycnidium width (µm)	References
		length (µm)	width (µm)			
<i>Diplodia pinea</i> A	USA	34–39	12–13	0–1		Palmer, Stewart & Wingfield, 1987
<i>Diplodia pinea</i> B (= <i>D. scrobiculata</i>)	USA	33–34	11–12	0–3		Palmer et al., 1987
<i>Diplodia pinea</i>		30–45	10–16	0–1	≤250	Hansen & Lewis, 1997
<i>Diplodia pinea</i>	Estonia	31–45	14–17	0–1		Hanso & Drenkhan, 2009
<i>Diplodia sapinea</i>	Sweden	30–37	12–15	0 (–3)		Oliva et al., 2013
<i>Diplodia sapinea</i>	The Netherlands and South Africa	30.5–52.5	12.5–20	0 (–3)	300–500	Phillips et al., 2013
Norway spruce bark sampled in 1947–1949 ^a	Finland	38–48	19–26	1		Kujala, 1950
Norway spruce bark sampled in 1947–1949 ^a	Finland	40–53	19–21	0–1	400–800	This study
Pycnidia on Scots pine cones in 2015/16	Finland	33–45	15–18	0	200–250	This study

^aCollected by Viljo Kujala.

TABLE 2 Investigated Scots pine stands and frequency of cones with pycnidia of *D. sapinea*

Site number	Municipality	Locality	Coordinates		Date of sampling	Number of cones collected	Stand age	Symptoms of <i>Diplodia</i> tip blight in pine shoots	Frequency of infected cones (%)
			N	E					
1	Helsinki	Kallahdenniemi	60° 11,4'	25° 08,5'	18.10.2015	100	Mature	No	3
2	Helsinki	Linnanmäki	60° 11,4'	24° 56,3'	11.10.2015	100	Overmature	No	5
2	Helsinki	Linnanmäki	60° 11,4'	24° 56,3'	9.8.2016	100	Overmature	No	12
3	Lohja	Keräkankare	60° 29,0'	23° 55,6'	20.10.2015	100	40–50 years	No	2
4	Kemiönsaari	Helgeboda	60° 11,2'	22° 33,4'	20.9.2016	100	Mature	No	4
5	Eurajoki	Lutta	61° 07,9'	21° 46,1'	14.7.2016	110	Mature	No	0
6	Eurajoki	Lehtimäki	61° 11,5'	21° 43,6'	14.7.2016	110	Mature	No ^a	0
7	Pori	Metsäkulma	61° 31,7'	21° 38,7'	14.7.2016	110	Mature	No	1
8	Nokia	Peltokankaanvuori	61° 30,0'	23° 12,6'	14.7.2016	110	Mature	No	0
9	Joutsa	Koivula	61° 45,1'	26° 08,0'	28.7.2016	110	Mature	No	0
10	Joutsa	Kaurasaari	61° 43,4'	26° 16,6'	19.5.2016	56	Mature	No	0
11	Taipalsaari	Sammaljärvi	61° 14,3'	28° 04,2'	17.8.2016	110	Mature	No	0
12	Savonlinna	Punkaharju	61° 46,5'	29° 20,4'	9.6.2016	100	Overmature	No	0
13	Kauhava	Laakso	63° 13,3'	23° 11,0'	21.6.2016	50	40–50 years	No ^b	0
14	Kontiomäki	Sipola	64° 26,0'	28° 09,4'	19.8.2016	100	Mature	No	0
15	Inari	Inkavaara	68° 59,1'	26° 59,7'	20.8.2016	110	Mature	No	0

^aSome shoot beetle damages. ^bSome Heterobasidion butt rot damages.

contradictory results prompted the present study to determine the current distribution of *S. sapinea* in Finland.

2 | MATERIALS AND METHODS

2.1 | Herbarium specimens

Viljo Kujala noted pycnidia occurring on two samples of Norway spruce bark (no. 406 dated 17.04.1947, from Sippola, Ruotila, Finland and no. 409 dated 4.11.1945 from Tuusula, Ruotsinkylä, Finland) to *Diplodia pinea* (Kujala, 1950) and deposited the samples in the herbarium of the Finnish Forest Research Institute (presently included in the collections of the Finnish Museum of Natural History, LUOMUS). We examined these specimens and found abundant immersed dark brown globose pycnidia similar in appearance. Single pycnidia were aseptically detached with forceps under a microscope and placed into Eppendorf tubes (3–5 pycnidia/tube) for DNA-PCR of the Internal Transcribed Spacer (ITS). Additionally, pycnidia were placed on microscope slides, crushed with a scalpel blade in a droplet of water for microscopic examination (Table 1).

Three methods were used in an attempt to use PCR to amplify DNA of the ITS region using the primer pairs: ITS1/ITS4, ITS5/ITS4 and ITS7A/ITS4 (Bertini et al., 1999; Gardes & Bruns, 1993; White, Bruns, Lee, & Taylor, 1990). These included: (a) disrupting the pycnidia in quartz sand with a glass rod and thereafter extracting DNA with phenol +chloroform (modified from Vainio, Korhonen, & Hantula, 1998, as described by Müller, Valjakka, and Hantula (2007).

Biotoools polymerase (Biotoools B&M Labs, Madrid, Spain) was used following to manufacturer's instructions, (b) DNA extraction by powdering the pycnidia with a glass rod in liquid nitrogen and thereafter dissolving the powder in an extraction buffer containing CTAB followed by heating and extraction in chloroform +isoamyl alcohol as described by Cubero, Crespo, Fatehi, and Bridge (1999). In separate reactions, alternatively Biotoools or Dream Taq polymerase (Thermo Fisher Scientific, Waltham, MA USA) was used following the manufacturer's instructions and (c) rupturing pycnidia at room temperature in Eppendorf tubes containing quartz sand using a glass rod. The sample was then subjected to direct PCR using the Phire Plant Direct PCR kit (Thermo Fisher Scientific, Waltham, MA USA) following the instructions of the manufacturer.

All PCRs were done with undiluted and diluted (1:10 and 1:50) extracts. Two *Diplodia sapinea* strains provided by Dr. Paolo Gonthier (SS 1.2 A.I.A from Torino, Italy and S81 from Trento, Italy) were used as positive controls in all PCR runs. Double distilled water was used as negative template control. The PCR protocol included: 5 min at 95°C and thereafter 25 cycles including a start with 30 s at 95°C, 30 s at 57°C and 1 min at 72°C. The reaction was terminated with 5 min at 72°C and thereafter cooling to +4°C. Amplification success was checked by electrophoresis in gels containing 1.0% agarose in TAE buffer, and the amplification products were visualized by ethidium bromide staining under UV light as described in Müller et al. (2007). Best results were obtained with the primers ITS7A and ITS4, and the PCR products were subjected to sequencing in both directions with this primer pair at Macrogen Inc., Korea (<http://www.macrogen.com>).

2.2 | Field inventory

Scots pine plots were surveyed for the presence of *D. sapinea* by collecting cones on the ground under mature Scots pines and by inspecting these for the presence of *D. sapinea* pycnidia. Cones were collected in an area of ca. 30 ha of the Keräkankare ridge in October 2004 (site 3 in Table 2) and during October 2015–September 2016 from fifteen mature Scots pine stands (Table 2 and Figure 1). In each stand, 50–110 cones were collected from the ground. Only fresh cones that appeared to have fallen during that calendar year were collected. The cones were examined using a dissecting microscope, and the number of cones with pycnidia resembling those of *D. sapinea* was counted. Identification was confirmed by detaching single pycnidia and examining conidial morphology under a microscope. In the case of the 2004 collections, isolations were made from the pith tissue of all the cones on 2% Malt Extract Agar (MEA: 20 g malt extract, 20 g agar 1,000⁻¹ ml water).

2.3 | Fungal isolation and identification

When the conidia matched the shape, colour and dimensions described for *D. sapinea* by Phillips et al. (2013), they were removed from the microscope slide by rinsing them with 1 ml of sterile water and onto water agar plates. The plates were incubated at room temperature for 5 to 20 hr after which several germinating single conidia were lifted using a modified Pasteur pipette and transferred to fresh MEA in a Petri dish. Up to five single conidium isolates per stand (each from a different cone) were transferred to fresh malt extract agar plates covered with a cellophane membrane (Surface Specialities; Wigton, UK). After an incubation period of five days at 20°C, hyphal mass from culture edges was removed and stored at -20°C until DNA extraction. DNA-PCR of these samples was done using the first method described above. The primer pair ITS5/ITS4 was used for PCR. Amplification products were subjected to sequencing in both directions with the primers ITS5/ITS4 at Macrogen Inc., Korea. Global sequence alignments were constructed using Geneious R10 version 10.0.8 (Biomatters Ltd.) and MAFFT alignment algorithms. Sequence comparisons were conducted using NCBI Blast.

3 | RESULTS

The pycnidia abundantly present on Kujala's bark samples were larger (diam. 400–800 µm) than those found on cones freshly collected in pine stands in this study or described earlier for *D. sapinea* (Table 1). Also, conidia from Kujala's bark samples were longer (40–53 µm) and wider (19–21 µm) compared to conidia measured recently from cones in Estonia, Sweden and this study (30–45 and 12–18 µm, respectively). The sequences obtained from pycnidia on Kujala's bark samples also failed to match with sequences of *D. sapinea* in GenBank (National Center for Biotechnology Information, Bethesda, MD). PCR products were obtained from the herbaria

samples only using the Phire Plant Direct PCR kit (Method 3) and using the primer pair ITS7A/ITS4. The best of these sequences, a 244 bp long sequence (from sample 409; GenBank accession number MH260637) was obtained and this sequence was 95% similar to *Aplosporella sumachi*, *Phaeobotryosphaeria porosa*, *Sphaeropsis citrigena* and *S. visci* in GenBank (all equally similar to our sequence) but no match to any species at ≥98%.

Field collected cones in 2004 had no pycnidia on their bracts, and *D. sapinea* was also not isolated from them. In the more recent (2015–2016) field inventory, *D. sapinea* was found on Scots pine cones in five stands. Identification of pycnidia and conidia on the cone scales was based on morphology of pycnidia and conidia as well as on colony appearance of isolates on malt extract agar. The isolates produced on malt extract agar were fluffy, white to grey mycelia and stained the agar dark greenish to grey. The morphological characteristics observed corresponded to those described by Phillips et al. (2013). The pycnidia were immersed, nearly globose, dark brown or black and 200–250 µm in diameter (Table 1). The conidia were thick-walled, ellipsoid, oblong, aseptate, brown in colour, 33–45 µm long and 15–18 µm wide (Table 1). The conidial dimensions fell within the range observed in several previous studies (Table 1).

Identification of *S. sapinea* for the recently collected isolates was verified by ITS sequences of ten isolates originating from stands 1, 2, 3, 4 and 7. All ten sequences (444–515 bp) were identical to each other and identical to numerous sequences in GenBank assigned to *D. sapinea*, *D. pinea* or *S. sapinea*. They were also identical to those obtained recently in Estonia, Latvia and northwestern Russia. The sequences were deposited in GenBank under the accession numbers: Helsinki, Linnanmäki: MH260627, MH260628, MH260629, MH260632, MH260633; Helsinki, Kallahdenniemi: MH260630; Lohja, Keräkankare: MH260631; Kemiönsaari, Helgeboda: MH260634, MH260635; Pori, Metsäkulma: MH260636.

All five Scots pine stands infested by *D. sapinea* were close to the southwestern coast of Finland (Figure 1). The percentage of cones with pycnidia was low: 1%–12% (Table 2). Most of the investigated stands appeared to be healthy, but one suffered from shoot beetle damage, and in one stand, several trees had fallen due to butt rot. None of the 15 investigated stands showed any symptoms typical for diseases caused by *D. sapinea*.

4 | DISCUSSION

The results of this study provide good evidence that the pine pathogen *D. sapinea* is a recent colonist of *P. sylvestris* in Finland. There was no evidence to suggest that it is causing disease problems at present. However, its apparently new area of occurrence and the presence on a *Pinus* spp. known to be susceptible to infection, might suggest that in times of stress, it could become a problem. This would be consistent with similar situations elsewhere in the world.

Both morphological and DNA sequence comparisons have led us to conclude that it is unlikely that the fungus described by Kujala

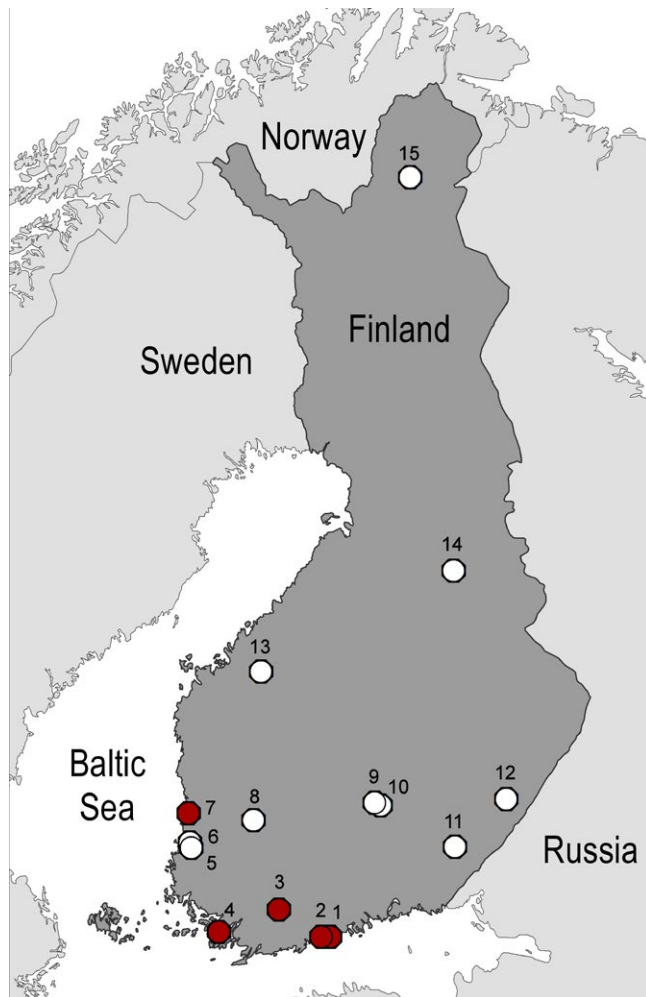


FIGURE 1 Collection sites of fallen Scots pine cones in 2015–2016. *Diplodia sapinea* was found from five sites, indicated by red symbols. White symbols indicate sites where *D. sapinea* was not found. Numbering refers to that used in Table 2

(1950) is *D. sapinea* or the related pine-infecting fungus *D. scrobiculata* (De Wet et al., 2003). Consequently, our observation of *D. sapinea* on Scots pine cones in Helsinki in 2015 appears to be the first true record of this pathogen in Finland. Given that a previous survey in 2004 failed to produce any evidence of the pathogen and the subsequent detection in 2015–2016 of a limited southwestern distribution support the view that the pathogen is a relatively recent invader of Finland.

The frequencies of detection on cones in this study (1%–12%) are relatively low compared to those of 50%–100% frequently recorded in France on *P. nigra* and *P. sylvestris* stands (Fabre et al., 2011). In Latvia and Estonia, detection frequencies of 31% and 12% have been reported on the same pine species (Adamson et al., 2015). Abundance of inoculum on cones is of primary importance for disease development in pines (Fabre et al., 2011; Palmer, McRoberts, & Nicholls, 1988). Though conidial abundance is required for disease progression, conducive environmental conditions and often stress to trees are also required for disease development (Feci, Battisti, Capretti, & Tegli, 2002; Munck & Stanosz, 2010; Palmer et al., 1988).

Winter temperatures correlate positively with the disease severity (Fabre et al., 2011), and especially, winters have become milder but also summers have become warmer during the last decades in Finland (Mikkonen et al., 2015; Ruosteenoja, Räisänen, Venäläinen, Kämäräinen, & Pirinen, 2016). The current distribution of *D. sapinea* only in southwestern Finland may also reflect the importance of mild climate because winters are mildest in that area of this country where the average daily temperature of the coldest month is -9°C and of the warmest month $+16^{\circ}\text{C}$.

The disease history (i.e., disease outbreaks in southern Europe for over 100 years; Luchi, Oliveira Longa, Danti, Capretti, & Maresi, 2014) in Europe suggests that recent climate warming could be an explanation for the current spread of *D. sapinea* to northern Europe. In contrast, *D. sapinea* causes shoot blight and top kill on pines in northcentral United States and even in Ontario, Canada, (Myren & Davis, 1989; Ostry, Moore, Kern, Venette, & Palik, 2012) where winters are colder (but summers are considerably hotter) than in southern Finland. Hence, the explanation for the recent spread of *D. sapinea* to northern Europe remains open to debate. Many studies have hypothesized that the distribution of *Diplodia* shoot blight is climate restricted because of direct effects on the pathogen and/or indirect effects through host susceptibility (Bosso et al., 2017; Fabre et al., 2011; Wyka, Munck, Braze, & Broders, 2018). One justification for this assumption is the fact that *D. sapinea* has been present in Europe for a long time yet has not spread to all areas of this region despite the presence of susceptible pine species.

Diplodia sapinea has been observed on Scots pine at the northern coast of Estonia in 2012 and in northwestern Russia, near St Petersburg (110 km from the Finnish border) in 2013 (Adamson et al., 2015). In Central Sweden, the first observation of the pathogen was in 2013 (Oliva et al., 2013). Consequently, *D. sapinea* could have spread to the southern coast of Finland over the Baltic Sea from Sweden or Estonia or along forested landscapes from northwestern Russia into southeastern Finland.

Diplodia sapinea can survive in a latent state in plant tissues for long periods of time (Bihon et al., 2011). As is true for other members of the *Botryosphaeriaceae* (Marsberg et al., 2017), it is well suited to be accidentally introduced into new environments in infected plant material such as seedlings. Conidia can be disseminated by rain splash and wind-driven rain (Hansen & Lewis, 1997; Swart, Wingfield, & Knox-Davies, 1987b) although their relatively large size might argue against their being blown over long distances such as the Finnish Gulf. It is also possible that the fungus could have been transported to Finland with insects. In this regard, at least one insect, the pine engraver beetle *Ips pini* has been shown to carry *D. sapinea* to logs of *P. nigra* (Whitehill, Lehman, & Bonello, 2007). Several other insect species have also been found to co-occur with *D. sapinea* in pine cones and have been proposed to act as vectors of the fungus (Drenkhan, Voolma, Adamson, Sibul, & Drenkhan, 2017; Feci et al., 2002; Haddow & Newman, 1942; Luchi, Mancini, Feducci, Santini, & Capretti, 2012). Hence, we conclude that the introduction of *D. sapinea* into Finland may have occurred via one or a number of several different modes.

ACKNOWLEDGEMENTS

We are grateful to Juha Puranen for his skilful technical assistance. Prof. Paolo Gonthier is acknowledged for providing us two reference strains of *Diplodia sapinea*. Drs. Kiti Müller and Risto Ojansuu are acknowledged for helping in collection of Scots pine cones. This study was partly supported by the Estonian Science Foundation grants PSG136 and IUT21-04.

ORCID

Michael M. Müller  <https://orcid.org/0000-0003-3699-2139>

REFERENCES

- Adamson, K., Klavina, D., Drenkhan, R., Gaitnieks, T., & Hanso, M. (2015). *Diplodia sapinea* is colonizing the native Scots pine (*Pinus sylvestris*) in the northern Baltics. *European Journal of Plant Pathology*, 143, 343–350. <https://doi.org/10.1007/s10658-015-0686-8>
- Bertini, L., Amicucci, A., Agostini, D., Polidori, E., Potenza, L., Guidi, C., & Stocchi, V. (1999). A new pair of primers designed for amplification of the ITS region in *Tuber* species. *FEMS Microbiology Letters*, 173, 239–245.
- Bihon, W., Slippers, B., Burgess, T., Wingfield, M. J., & Wingfield, B. D. (2011). Sources of *Diplodia pinea* endophytic infections in *Pinus patula* and *P. radiata* seedlings in South Africa. *Forest Pathology*, 41, 370–375.
- Blaschke, M., & Cech, T. L. (2007). Absterbende Weisskiefern – eine langfristige Folge des Trockenjahres 2003? *Forstschutz Aktuell*, 40, 32–34.
- Bosso, L., Luchi, N., Maresi, G., Cristinzio, G., Smeraldo, S., & Russo, D. (2017). Predicting current and future disease outbreaks of *Diplodia sapinea* shoot blight in Italy: Species distribution models as a tool for forest management planning. *Forest Ecology and Management*, 400, 655–664. <https://doi.org/10.1016/j.foreco.2017.06.044>
- Chhin, S., & O'Brien, J. (2015). Dendroclimatic analysis of red pine affected by *Diplodia* shoot blight in different latitudinal regions in Michigan. *Canadian Journal of Forest Research*, 45, 1757–1767.
- Chou, C. K. S. (1976). A shoot dieback in *Pinus radiata* caused by *Diplodia pinea*. I. Symptoms, disease development, and isolation of the pathogen. *New Zealand Journal of Forestry Science*, 6, 72–79.
- Cubero, O. F., Crespo, A., Fatehi, J., & Bridge, P. D. (1999). DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Systematics and Evolution*, 216, 243–249. <https://doi.org/10.1007/BF01084401>
- De Wet, J., Burgess, T., Slippers, B., Preisig, O., Wingfield, B. D., & Wingfield, M. J. (2003). Multiple gene genealogies and microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. *Mycological Research*, 107, 557–566. <https://doi.org/10.1017/S0953756203007706>
- Desmazieres, J. B. H. J. (1842). Neuvième notice sur quelques plantes cryptogames, la plupart inédites, récemment découvertes en France, et que vont paraître en nature dans la collection publiée par l'auteur. *Annales Des Sciences Naturelles Botanique, Sér.*, 17, 91–118.
- Drenkhan, T., Voolma, K., Adamson, K., Sibul, I., & Drenkhan, R. (2017). The large pine weevil *Hylobius abietis* (L.) as a potential vector of the pathogenic fungus *Diplodia sapinea* (Fr.) Fuckel. *Agricultural and Forest Entomology*, 19, 4–9.
- Ettlinger, L. (1945). Über die Gattung *Crumenula* sensu Rehm, mit besonderer Berücksichtigung des *Crumenula*-Triebsterbens der *Pinus* Arten. *Beitr. Kryptogamenflora Schweiz*, 10, 1–73.
- Fabre, B., Piou, D., & Desprez-Loustau, M.-L. (2011). Can the emergence of pine *Diplodia* shoot blight in France be explained by changes in pathogen pressure linked to climate change? *Global Change Biology*, 17, 3218–3227. <https://doi.org/10.1111/j.1365-2486.2011.02428.x>
- Feci, E., Battisti, A., Capretti, P., & Tegli, S. (2002). An association between the fungus *Sphaeropsis sapinea* and the cone bug *Gastrodes grossipes* in cones of *Pinus nigra* in Italy. *Forest Pathology*, 32, 241–247. <https://doi.org/10.1046/j.1439-0329.2002.00286.x>
- Flowers, J., Nuckles, E., Hartman, J., & Vaillancourt, L. (2001). Latent infection of Austrian and Scots pine tissues by *Sphaeropsis sapinea*. *Plant Disease*, 85, 1107–1112.
- Fries, E. M. (1823). *Systema mycologicum*, Vol. 2 (pp. 620). Lund, Sweden: Berlin.
- Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2, 113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Georgieva, M., & Hlebarska, S. (2016). A review of *Sphaeropsis sapinea* occurrence on *Pinus* species in Bulgaria. *Journal of BioScience Biotechnology*, 5, 247–250.
- Haddow, W. R., & Newman, F. S. (1942). A disease of the Scots pine (*Pinus sylvestris* L.) caused by the fungus *Diplodia pinea* Kickx associated with the pine spittlebug (*Aphrophora paralella* Say.). *Transactions of the Royal Canadian Institute*, 24, 1–18.
- Hansen, E. M., & Lewis, K. J. (1997). *Compendium of conifer diseases* (pp. 101). St. Paul, MN: APS Press.
- Hanso, M., & Drenkhan, R. (2009). *Diplodia pinea* is a new pathogen on Austrian pine (*Pinus nigra*) in Estonia. *Plant Pathology*, 58, 797.
- Iturririta, E., Ganley, R. J., Raposo, R., Garcia-Serna, I., Mesanza, N., Kirkpatrick, S. C., & Gordon, T. R. (2013). Resistance levels of Spanish conifers against *Fusarium circinatum* and *Diplodia pinea*. *Forest Pathology*, 43, 488–495.
- Keen, A., & Smits, T. F. C. (1989). Application of a mathematical function for a temperature optimum curve to establish differences in growth between isolates of a fungus. *Netherlands Journal of Plant Pathology*, 95, 37–49. <https://doi.org/10.1007/BF02000880>
- Kujala, V. (1950). Über die Kleinpilze der Koniferen in Finnland. *Communicationes Institute Forestalis Fenniae*, 38, 4.
- Luchi, N., Mancini, V., Feducci, M., Santini, A., & Capretti, P. (2012). *Leptoglossus occidentalis* and *Diplodia pinea*: A new insect-fungus association in Mediterranean forests. *Forest Pathology*, 42, 246–251. <https://doi.org/10.1111/j.1439-0329.2011.00750.x>
- Luchi, N., Oliveira Longa, C. M., Danti, R., Capretti, P., & Maresi, G. (2014). *Diplodia sapinea*: The main fungal species involved in the colonization of pineshoots in Italy. *Forest Pathology*, 44, 372–381.
- Maresi, G., Ambrosi, P., Battisti, A., Capretti, P., Danti, R., Feci, E., ...Tegli, S. (2002). Pine dieback by *Sphaeropsis sapinea* in Northern and Central Italy. In: Forest research Institute Res. Papers. Proceedings of the IUFRO Working Party 7.02.02 Shoot and foliage Diseases, 2001. Hyytiälä Finland pp. 60–67.
- Marsberg, A., Kemler, M., Jami, F., Nagel, J. H., Postma-Smidt, A., Naidoo, S., ... Slippers, B. (2017). *Botryosphaeria dothidea*: A latent pathogen of global importance to woody plant health. *Molecular Plant Pathology*, 18, 477–488.
- Mikkonen, S., Laine, M., Mäkelä, H. M., Gregow, H., Tuomenvirta, H., Lahtinen, M., & Laaksonen, A. (2015). Trends in the average temperature in Finland, 1847–2013. *Stochastic Environmental Research and Risk Assessment*, 29, 1521–1529. <https://doi.org/10.1007/s00477-014-0992-2>
- Milijašević, T. (2006). Effect of temperature on the mycelial growth of the fungus *Sphaeropsis sapinea*. *Glasnik Šumarskog Fakulteta*, 94, 211–222. (DOI <https://doi.org/10.2298/GSF0694211M>)
- Müller, M. M., Valjakka, R., & Hantula, J. (2007). Genetic diversity of *Lophodermium piceae* in South Finland. *Forest Pathology*, 37, 329–337. <https://doi.org/10.1111/j.1439-0329.2007.00504.x>
- Munck, I. A., & Stanosz, G. R. (2010). Longevity of inoculum production by *Diplodia pinea* on red pine cones. *European Journal of Forest Pathology*, 40, 58–63.

- Myren, D. T., & Davis, C. N. (1989). Index of hosts and associated fungi identified by the Forest Insect and Disease Survey in Ontario from 1967 to 1987. I. The pines. Forestry Canada, Great Lakes Forestry Centre (p. O-X-395.). Information Report.
- Oliva, O., Boberg, J., & Stenlid, J. (2013). First report of *Sphaeropsis sapinea* on Scots pine (*Pinus sylvestris*) and Austrian pine (*P. nigra*) in Sweden. *New Disease Reports*, 27, 23.
- Ostry, M. E., Moore, M. J., Kern, C. C., Venette, R. C., & Palik, B. J. (2012). Multiple diseases impact survival of pine species planted in red pine stands harvested in spatially variable retention patterns. *Forest Ecology and Management*, 286, 66–72. <https://doi.org/10.1016/j.foreco.2012.08.017>
- Palmer, M. A., McRoberts, R. E., & Nicholls, T. H. (1988). Sources of inoculum of *Sphaeropsis sapinea* in forest tree nurseries. *Phytopathology*, 78, 831–835.
- Palmer, M. A., Stewart, E. L., & Wingfield, M. J. (1987). Variation among isolates of *Sphaeropsis sapinea* in the north central United States. *Phytopathology*, 77, 944–948.
- Petäistö, R.-L. (1993). Conidial germination and formation of necrosis in pine seedlings by *Gremmeniella abietina* at low temperatures. *European Journal of Forest Pathology*, 23, 290–294. <https://doi.org/10.1111/j.1439-0329.1993.tb00965.x>
- Phillips, A. J. L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfield, M. J., Groenewald, J. Z., & Crous, P. W. (2013). The *Botryosphaeriaceae*: Genera and species known from culture. *Studies in Mycology*, 76, 51–167. <https://doi.org/10.3114/sim0021>
- Ruostenoja, K., Räisänen, J., Venäläinen, A., Kämäräinen, M., & Pirinen, P. (2016). Thermal growing seasons in a warming climate. *Terra*, 128, 3–15.
- Sinclair, W. A., & Lyon, H. H. (2005). *Diseases of trees and shrubs*, 2nd ed. (pp. 130). Ithaca, NY: Cornell University Press.
- Stanosz, G. R., Blodgett, J. T., Smith, D. R., & Kruger, E. L. (2001). Water stress and *Sphaeropsis sapinea* as a latent pathogen of red pine seedlings. *New Phytologist*, 149, 531–538. <https://doi.org/10.1046/j.1469-8137.2001.00052.x>
- Stanosz, G. R., Smith, D. R., Guthmiller, M. A., & Stanosz, J. C. (1997). Persistence of *Sphaeropsis sapinea* on or in asymptomatic shoots of red and jack pines. *Mycologia*, 89, 525–530.
- Swart, W. J., & Wingfield, M. J. (1991). Biology and control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. *Plant Disease*, 75, 761–765.
- Swart, W. J., Wingfield, M. J., & Knox-Davies, P. S. (1987a). Factors associated with *Sphaeropsis sapinea* infection of pine trees in South Africa. *Phytophylactica*, 19, 505–510.
- Swart, W. J., Wingfield, M. J., & Knox-Davies, P. S. (1987b). Conidial dispersal of *Sphaeropsis sapinea* in three climatic regions of South Africa. *Plant Disease*, 71, 1038–1040.
- Vainio, E. J., Korhonen, K., & Hantula, J. (1998). Genetic variation in *Phlebiopsis gigantea* as detected with random amplified microsatellite (RAMS) markers. *Mycological Research*, 102, 187–192. <https://doi.org/10.1017/S0953756297004577>
- Vujanovic, V., Arnaud, M., & Neumann, P. J. (2000). Susceptibility of cones and seeds to fungal infection in a pine (*Pinus* spp.) collection. *Forest Pathology*, 30, 305–320. <https://doi.org/10.1046/j.1439-0329.2000.00211.x>
- White, T. J., Bruns, T., Lee, S. B., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). London, UK: Academic Press.
- Whitehill, J. G. A., Lehman, J. S., & Bonello, P. (2007). *Ips pini* (Curculionidae: Scolytinae) is a vector of the fungal pathogen, *Sphaeropsis sapinea* (Coelomycetes), to Austrian Pines, *Pinus nigra* (Pinaceae). *Environmental Entomology*, 36, 114–120.
- Wyka, S. A., Munck, I. A., Brazee, N. J., & Broders, K. D. (2018). Response of eastern white pine and associated foliar, blister rust, canker and root rot pathogens to climate change. *Forest Ecology and Management*, 423, 18–26. <https://doi.org/10.1016/j.foreco.2018.03.011>
- Zwolinski, J. B., Swart, W. J., & Wingfield, M. J. (1995). Association of *Sphaeropsis sapinea* with insect infestation following hail damage of *Pinus radiata*. *Forest Ecology and Management*, 72, 293–298. [https://doi.org/10.1016/0378-1127\(94\)03459-A](https://doi.org/10.1016/0378-1127(94)03459-A)

How to cite this article: Müller MM, Hantula J, Wingfield M, Drenkhan R. *Diplodia sapinea* found on Scots pine in Finland. *For Path.* 2019;49:e12483. <https://doi.org/10.1111/efp.12483>