



The Pine Pathogen *Diplodia sapinea*: Expanding Frontiers

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Abstract

Purpose of Review This review offers a thorough examination of the pine pathogen *Diplodia sapinea*, tracing its historical significance from its initial impact on South African non-native *Pinus* plantations to its recent emergence as a major threat to forests, particularly in Europe. It also highlights recent findings regarding its taxonomic position, genetic studies, and shifts from being perceived as a wound-infecting pathogen to an endophyte in healthy *Pinus* species that causes disease after stress.

Recent Findings Recent years have witnessed a dramatic increase in the damage caused by *D. sapinea* in natural and planted forests. This escalation is apparently strongly linked to climate change. The pathogen's previously confused taxonomic position has been clearly resolved in the *Botryosphaeriaceae*, alongside several sibling species. *Diplodia sapinea* is now well-understood as an endophyte in healthy trees, which has significant implications for studies aimed at understanding its biology. Importantly, robust techniques including those utilizing genome sequences, are now available for rapid identification and population genetic studies of the pathogen and the factors that drive disease outbreaks.

Summary Over the past century, *D. sapinea* has transitioned from a localized problem in South African non-native plantations to a global threat to both natural and planted forests, particularly in Europe. This review underscores the importance of historical context in understanding the evolution of the pathogen's impact. It also lays the foundation for future research endeavours, leveraging modern technologies to address key questions surrounding its biology and ecology.

Keywords Tree health · Climate change · Fungal ecology · Invasion biology

Introduction

Historically the disease caused by *Diplodia sapinea* was viewed as a South African problem and a consequence of planting *Pinus* species outside their native ranges. In fact, globally, the earliest exotic plantations of *Pinus* species were established in South Africa in 1825 [1]. Of particular relevance here is that the country had minimal resources of native trees suitable for the supply of timber products [1]. These first plantations in Genadendal were of *Pinus pinaster*, a species native to the South Atlantic European region and parts of the western Mediterranean. The establishment of the South African Cape Forest Act in 1888, recognized the importance of establishing plantations of non-native species to protect the limited native forest resources from

destruction [2, 3]. This led to numerous *Pinus* species being tested for plantation development and where *Pinus radiata* was identified as most suitable [4]. This species was consequently widely planted in the country from 1910 to 1920. As time passed, it was recognized that *P. radiata* was suitable for establishment only in the winter rainfall region of the county and that *P. patula* and *P. elliotii* were best planted in the summer rainfall areas. The establishment of these plantations was directly linked to *D. sapinea*, which owed its notoriety to the South African forestry situation [2, 4–7].

Early established plantations of pines in the Southern Hemisphere were relatively free of disease or insect pest problems [8]. This follows the “enemy free space” hypothesis proposed by Jeffries and Lawton (1984) [9] and is true for other areas of the world where non-native tree species are established for the first time [10, 11]. By far the most important, and often limiting disease problem in these plantations was die-back due to infections by a fungal pathogen *D. sapinea* [12–16]. While the fungus commonly causes shoot die-back on susceptible *Pinus* spp., much more serious damage to plantations occurs

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Fig. 1 Signs and symptoms associated with *Diplodia sapinea* on *Pinus* spp. (a) Severe die-back of *Pinus patula* after hail damage. (b) Dead and dying needles on a severely affected tree. (c) Infection of the pith moving from tips of branches downwards on a stressed tree. (d) Symptoms of root disease on *P. taeda* showing lesions moving upwards into the root collar region, (e) Sap stain typical on timber after infection. (f) Pycnidial fruiting structures on an infected branch (arrows). (g) Large numbers of pycnida (arrows) on a cone and a common source of inoculum in forests. (h) Typical large brown asexual mitospores (conidia) of *D. sapinea* (scale bar 20 µm)

after hail storms, which are relatively common in South Africa (Fig. 1a, b). Where susceptible *Pinus* spp. such as *P. radiata* and *P. patula* established in plantations are damaged by hail, rapid and dramatic die-back and death of large areas of trees occurs [5, 7, 17–24]. This led to an assumption, one that persisted for many years, that *D. sapinea* was a wound-infecting pathogen. The logic here was that hail resulted in wounds and that these then became infected by the fungus, resulting in tree death. In this respect, it was assumed to be a typical wound related tree pathogen.

While the notoriety of *D. sapinea* as a serious plantation-damaging pathogen was due to Southern Hemisphere plantation situations [8, 13, 25], the fungus was well known in other countries, but mostly treated as of lesser significance. Exceptions were, for example, in the Christmas tree industry of North America where susceptible, and usually non-native species such as *Pinus nigra* and *Pinus sylvestris* are commonly propagated [26, 27]. The fungus was also recorded as problematic in the USA, mostly on non-native *Pinus* spp. in the urban environment, in Christmas tree plantations and in nurseries [26–28] usually associated with stressful conditions. There were also reports for that period of time from Southern Europe (see [29]).

Not a Typical Wound-Associated Pathogen

Prior to the establishment of plantations of non-native *Pinus* species in the Southern Hemisphere, the tree pathogen *D. sapinea* was mostly of mycological interest. The fungus was first described from Sweden as *Sphaeria sapinea* by Fries in 1823 [30] and some years later isolated from *Pinus sylvestris* in France and described as *Sphaeria pinea* by Desmazières in 1842 [31]. It was subsequently treated in various taxonomic studies and given names such as *Macrophomina pinea*, *Sphaeropsis pinea*, *Macrophomina sapinea* and *Sphaeropsis sapinea* [32]. In the later literature, it has mostly been treated as *Diplodia pinea* [33, 34]. Phillips et al. [30] provided a recent taxonomic study and since that time, it has been referred to as *Diplodia sapinea*.

Diplodia sapinea is commonly found on senescent tissues of *Pinus* spp. including shoots, cones and blue stained tissues in many parts of the world. However, its role in causing disease has in many cases not been clear. The first records of

the fungus causing disease were from Kew in 1908 and 1911 [31]. This was linked to disease problems being experienced on *Pinus radiata* in what was then the Cape Colony of the Union of South Africa [2, 35].

For many years, *D. sapinea* continued to be viewed as a wound-infecting pathogen. This was not only in Southern Hemisphere plantation situations, but also in other countries where the fungus was well-known, yet not considered of great importance. A major breakthrough in understanding the biology of the fungus arose at the time when endophytic infections of plants were emerging as important. This was a period when it became clear that healthy trees contain large numbers of fungi in their microbiomes [36–38]. As studies on tree endophytes increased in number, it was recognized that *D. sapinea* was a common inhabitant of healthy pine tissues [19, 39–44]. The first study in which the fungus was isolated from asymptomatic *Pinus* tissue was by Petrini and Fisher [45]. It could be argued that this important discovery effectively changed our understanding of the biology of *D. sapinea*.

Diplodia sapinea is now well-recognised as a common endophyte in the healthy tissues of *Pinus* spp. During this phase of its biology, it exists in an asymptomatic state until the onset of stress [39, 44, 46–48]. This is emerging as true of most fungi in the *Botryosphaeriaceae*; the fungal family in which *D. sapinea* is known to reside [30, 49, 50]. Consequently, the notion that it is primarily a wound-infecting fungus was a veritable “red herring” and is no longer particularly relevant. Thus, for example, the rapid death that occurs in pine trees after hail storms results from *D. sapinea* inoculum, internally in the trees becoming active and rapidly colonizing the tree tissues. This explains the rapid death of trees, which can occur in a matter of weeks, and where the sapwood becomes blue stained (Fig. 1e). Apart from the financial implications of losing a plantation, there’s also the loss of value in the timber affected by the blue stain [22, 51, 52].

Insects are commonly found associated with *D. sapinea* infections [53, 54], but it is most likely that the presence of the fungus arose from endophytic infections rather than having been carried to these damaged tissues by the insects. The fungus is a typical member of the *Botryosphaeriaceae* where spore dispersal is via rain splash and wind. Its large conidia are in no way adapted to being vectored by insects and it would seem most unlikely that insects play a role in the dispersal of *D. sapinea*, even though they are commonly found in the presence of the fungus.

One of the more intriguing and unanswered questions regarding the biology of *D. sapinea* relates to the nature of the stress factors that lead to the onset of active infections (Fig. 1c). For example, it remains unknown what physiological changes occur in susceptible pine trees due to hail

damage, which evidently initiate the infection cycle [9, 21, 23, 44, 55–58]. Several hypotheses have been proposed, including the idea that a physiological shock triggers the quick release of sugars, providing a rapid growth opportunity for the fungus. The occurrence of the pathogen within young pine shoots, as often seen in species like *P. radiata*, may stem from stress placed on rapidly growing shoots during periods when the environment cannot adequately support such growth. This could be due to higher than optimal temperatures or lack of available moisture at the time of shoot extension. The biological nature of stress and how this relates to the onset of tree disease remains very poorly understood [59]. Specifically in the case of *D. sapinea* and other related *Botryosphaeriaceae*, there is a great need to understand the nature of stress and how this intersects with the biology of these fungi [49, 60].

Sources of Inoculum

While *D. sapinea* has long been known to sporulate on the cones of *Pinus* spp [31], Nicholls and Ostry [61] first recognized that this fact is very relevant to our understanding of the biology of *D. sapinea*. This was an understanding that an important source of the inoculum occurs primarily on the cones of *Pinus* spp. (Fig. 1g). Infections are often seen extending from the cones into the branches. The presence of large numbers of *D. sapinea* conidia (mitospores) (Fig. 1h) and pycnidia in which they develop on cones is well-known and has been recorded by many authors [19, 62–64]. There is also evidence that these asexual structures result from the endophytic infections within trees [42]. Other than a predominance on cones, *D. sapinea* inoculum can also be found on dying shoots and needles of pines [6, 20, 21, 61, 65] (Fig. 1f). Nevertheless, according to field observations, the cones seem to be the most abundant source of inoculum in pines.

Swart et al. [6, 65] considered the dispersal biology of *D. sapinea* in South Africa. The motivation for that study was that *P. radiata* developed stem cankers associated with the standard plantation forestry practice of stem pruning at various stages of tree growth. This problem, clearly also associated with stress to trees, was also well-known in New Zealand [66]. It raised the logical question as to when trees might best be pruned to avoid infections from developing. To provide a basis of comparison, the study of Swart et al. [6] compared areas having rainfall in winter and summer. While *D. sapinea* spores were known to be wind-water dispersed, as is typical of pycnidial fungi, the study showed that irrespective of rainfall, temperature dictates the presence of spores in the environment. Consequently, the conclusion of that study was that pruning would best be practiced during winter, despite the fact that

rainfall might also occur at that time. Intriguingly, based on knowledge not available when Swart et al. [6, 65] conducted their studies, infections of pruning wounds might well occur from internal endophytic inoculum and have little to do with spores in the environment. While that question has not been interrogated, currently available tools such as microsatellite markers could easily be applied to provide a reliable answer.

When considering the origin of inoculum that gives rise to *D. sapinea* infections, it is relevant to reflect on the results of pathogenicity tests that have been undertaken with the fungus, including those to satisfy Koch's rules of proof. Most inoculation studies with the fungus have applied mycelial inoculum to artificially wounded tissues [15, 19, 20, 56, 66–69]. The results typically manifest as lesions, the lengths of which can be easily measured and compared. It is obvious that *D. sapinea* can cause lesions on wounded tissues. What is not clear is how accurately wound-induced inoculations apply to the biology of the fungus, which under natural conditions mostly appears to establish disease from previously established internal infections linked to an endophytic life cycle.

Wound-based inoculations with *D. sapinea* mycelium seemed to provide an appropriate proxy [70] for screening trials when the fungus was considered as a wound-infecting pathogen. However, inoculations with mycelium-covered plugs of agar do not reflect a natural infection process; this would require spores as a source of inoculum. Unfortunately, the fungus sporulates notoriously poorly in culture leading to techniques such as adding sterilized pine needles to agar plates to promote sporulation in the *Botryosphaeriaceae* [42]. The situation has recently been resolved by Oostlander et al. [71], who established a protocol to produce large numbers of *D. sapinea* conidia in culture. This makes it possible to easily conduct artificial inoculation tests that emulate a more natural situation. It will surely lead to a greatly enhanced understanding of the biology of the pathogen.

Names and Reproductive Biology

Diplodia sapinea has undergone many name changes since it was first described as *Sphaeria sapinea* in 1823 and later as *Sphaeria pinea* in 1842 [30, 31]. The name *Diplodia pinea*, established by Kickx in 1867, was the most commonly used of these names for many years [34]. As was true in the early days of fungal taxonomy, the applied names were mainly based on opinions regarding various morphological characteristics of the fungus. Arguably the most important discovery relating to the taxonomy of *D. sapinea* emerged when it was recognized as a member of the *Botryosphaeriaceae* [30, 50, 72] (Fig. 2). As is true for most other fungi, this

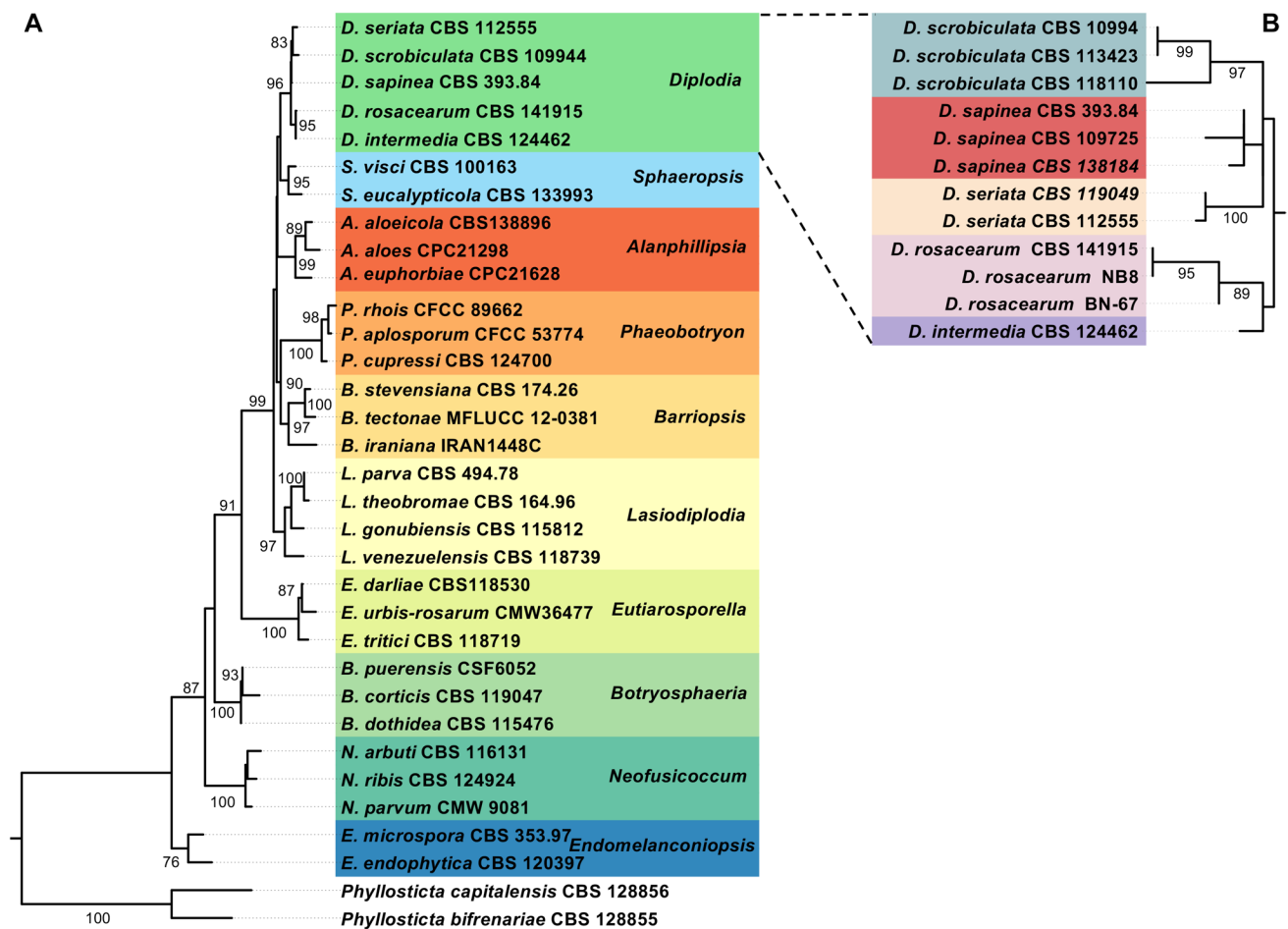


Fig. 2 Phylogenetic placement of *Diplodia* species within the *Botryosphaeriaceae*. **a** Phylogenetic relationships between *D. sapinea* and selected species and genera in the *Botryosphaeriaceae* inferred from ITS and LSU sequence data, with species of the *Phyllostictaceae*

as outgroups. **b** Cryptic species in the *D. sapinea* complex resolved using ITS, LSU, BT and TEF-1a sequence data. Trees were constructed using IQ-TREE with 1000 bootstrap replicates

emerged from the application of phylogenetic inference based on DNA sequence data and the application of the one fungus, one name convention [73, 74] (Fig. 2).

Palmer et al. [75] studied *D. sapinea* infecting *Pinus* spp. in the USA Lake States and recognized obvious differences in the morphology of isolates. This led to the identification of four morphotypes of the fungus, referred to as the A, B, C and I types [75–81]. Later, phylogenetic analyses of DNA sequences and microsatellite markers led to a clarification of these morphotypes as discrete species. Thus, the B morphotype was described as *Diplodia scrobiculata* [82] and isolates of the I morphotype were recognised as *Botryosphaeria obtusa*. Those of the C morphotype were from *Pinus* in Indonesia and indistinguishable from *D. sapinea* morphotype A isolates using the microsatellite markers [82]. Interestingly, isolates of the C morphotype were much more aggressive than other tested isolates [83] and Smith et al. [84] found that the South African population had an unexpectedly high genetic diversity.

Diplodia scrobiculata, which Palmer et al. [75] first recognized as the B morphotype of *D. sapinea*, was particularly interesting as it had comparatively low levels of aggressiveness on the hosts that they tested. It also had conidia that were distinctly pitted and different to the smooth-walled spores of *D. sapinea* [76, 77]. *Diplodia scrobiculata* remained known only from the USA Lake States for many years but was later commonly found in California [85] and in relatively low abundance by Bihon et al. [46] during extensive sampling of *Pinus* spp. in South Africa. It is particularly intriguing that this fungus, unlike *D. sapinea* has hardly spread globally. This might be ascribed to its low levels of aggressiveness or to some other factor such as not being well-adapted to particular environments or hosts, which would have been necessary for it to become established and compete over a diverse range of environments.

Numerous studies have confirmed the differences between isolates of *D. sapinea* and *D. scrobiculata* and their presence

in different parts of the world. These studies have applied various molecular genetic tools including isozyme analyses, RAPD profiles, microsatellite and DNA sequence analyses [80, 82, 86–90]. Likewise, Luchi et al. [91, 92] produced Real Time PCR and High Resolution Melting Curve diagnostic tools specifically for *D. sapinea*, but these do not distinguish it from closely related species. Isolates of *D. scrobiculata* have also been shown to differ in their aggressiveness in inoculation studies [46, 69, 93].

Wingfield and Knox-Davies [94] reported the presence of spermatia in some isolates of *D. sapinea*, but a sexual state has never been found. Given its position in the *Botryosphaeriaceae* [30, 33, 50] sexual structures would resemble those of other taxa in the group having botryose pseudothecia containing bitunicate asci. Having been treated as an exclusively asexual fungus in various studies [81], the results of a population genetics study by Bihon et al. [95] confirmed the earlier study by Smith et al. [84]. These authors showed high levels of genotypic diversity and random association of alleles in various populations of the fungus. This suggested that the fungus has a yet to be discovered cryptic sexual state.

The possibility of cryptic sexual reproduction led Bihon et al. [96] to consider this question more fully. These authors provided the first full genome sequences of *D. sapinea* thus providing the necessary data to consider the reproductive strategy of the fungus. The results showed that *D. sapinea* is heterothallic with individual isolates having apparently functional copies of the *a-1* or HMG domains. In addition to the *MATI-2-1* and *MATI-1-1* genes, they found a *MATI-1-4* gene in the *MATI-1* idiomorph and a novel *MATI-2-5* gene in the *MATI-2* idiomorph. The frequency of occurrence of the two idiomorphs in a population of isolates occurred in a near 1:1 ratio, strongly suggesting that *D. sapinea* has a cryptic sexual cycle.

Hosts, Symptoms and Susceptibility

Although not all areas or species have been extensively surveyed, evidence suggests that *D. sapinea* is primarily a pine-infecting fungus native across the natural range of these trees in the Northern Hemisphere including Europe, Asia, North Africa, North America and South America [97]. In their natural range, *Pinus* spp. differ in their susceptibility to infection. For example, Fabre et al. [29] reported a difference in host susceptibility in France, with *P. sylvestris*, *P. nigra* and *P. pinaster* being infected with decreasing frequency. In natural forests of *Pinus* species in the sub-genus *Pinus* such as *P. banksiana* and *P. resinosa*, together with *P. strobus* (sub-genus *strobus*), Wingfield (unpublished) failed to isolate the fungus from trees of the latter species. This

suggests that the fungus has a preference for species in the sub-genus *Pinus*.

While *D. sapinea* has an apparent preference for certain *Pinus* spp., it is intriguing that it has been found on various other conifers and even hardwoods [98–100]. These records are mostly from studies where DNA sequence data have been applied to the identifications and, although unusual, they are clearly reliable. Intriguingly, Blumenstein et al. [101] found that isolates originating from non-conifer hosts were more aggressive when inoculated on *Pinus* species under water stress conditions, suggesting that host switching under climate change conditions could pose a particular threat. The nature of these infections, and the role that the fungus plays in these apparent non-primary hosts is clearly of interest and deserves further study.

Most research considering the relative susceptibility of *Pinus* species to infection by *D. sapinea* has been conducted in the Southern Hemisphere, where the pathogen has been an impediment to plantation sustainability. This stems back to the earliest report of *D. sapinea* causing a shoot die-back in South Africa [35]. It was clear from some of the earliest plantation programmes that the widely planted *P. radiata* was highly susceptible to infection when trees were subjected to various forms of stress. Damage included shoot die-back, stem cankers and in the most serious cases, rapid death of trees after hail storms [6, 7, 13, 17]. Where trees die, the resulting timber becomes blue-stained leading to a loss of timber value [22, 102]. An intriguing manifestation of infection is a root disease on stressed *P. elliotii* and *P. taeda* in Southern Africa [7]. A similar disease syndrome was later seen on the same *Pinus* species in Hawaii, but caused by *Botryosphaeria dothidea*, a fungus related to *D. sapinea* in the *Botryosphaeriaceae* [103].

In the case of Southern Hemisphere plantation forestry, damage due to *D. sapinea* is clearly associated with particular species of *Pinus*. Although the pathogen has been important in Chile and New Zealand [12, 13, 104, 105] where *P. radiata* is the dominant species planted, hail damage has not been a significant component of the disease. In contrast, where highly susceptible species such as *P. radiata* and *P. patula* are planted in hail-affected areas of South Africa, losses have been very serious [22]. This has resulted in planting less susceptible species such as *P. elliotii* in areas where hail damage occurs. In the case of Christmas tree plantations, mostly non-native species such as *P. sylvestris* and *P. nigra* have been most severely affected, particularly where trees have been subjected to various forms of stress, or insect damage [26, 27].

Most trials aimed at screening trees for tolerance to infection by *D. sapinea* have been those linked to plantation forestry utilizing non-native *Pinus* species [19, 20, 56, 68].

These have generally confirmed field observations where *P. radiata* and *P. patula* are highly susceptible to infection [106]. However, where Smith et al. [19], compared the damage to a number of species due to natural infection after hail damage, they found no significant differences between artificial wound-inoculations and field observations. This suggests that inoculation trials where trees are wounded may provide an inappropriate proxy for disease screening. This is most likely due to the fact that *D. sapinea* symptoms result from prior infections by the fungus, which has become established as part of the endophyte community of the trees [42]. It is consequently necessary to view the results of wound-based inoculation trials with circumspection. Thus, when developing disease screening protocols for resistance or sensitivity of trees to *D. sapinea* it is necessary to consider the biology of the pathogen as an endophyte in healthy *Pinus* spp. Likewise, it is necessary to show that inoculations on juvenile plants accurately reflects the field situation where affected trees are usually established with well-developed woody tissues. In the past, conducting such experiments would have been impossible, but today, genotyping isolates has become an essential tool in the modern plant pathologist's toolkit.

Genetic Diversity

There have been a number of studies considering the global genetic diversity of *D. sapinea*. As a native fungus, and for many years considered to be relatively unimportant in Northern Hemisphere countries, questions relating to its pathways of movement and relevance have mostly focused on plantation forestry in the Southern Hemisphere. The first of these studies was by Burgess et al. [81] that used vegetative compatibility tests and previously developed SSR markers [3, 107] to compare populations of isolates from Australia, New Zealand and South Africa. These populations were chosen specifically to include two countries (New Zealand and Australia) that had long histories of strict quarantine and South Africa where quarantine procedures for the importation of plant material was less rigorous [3]. The results showed that the genotypic diversity of *D. sapinea* was substantially greater in the South African population, thus reflecting multiple introductions into that country and fewer into New Zealand and Australia. That study also showed that the fungus on *P. radiata* in its introduced range was unlikely to have originated on this species and that it had acquired the fungal endophyte from other *Pinus* spp. in the non-native range [107]. Given the common occurrence of *D. scrobiculata* and not *D. sapinea* in California [85], it is conceivable that the former fungus is part of the natural biology of *P. radiata* in the native range of this tree.

The most extensive early study, comparing global populations of *D. sapinea* isolates was conducted by Burgess et al. [85, 87]. That study using microsatellite markers included three populations from the native range of the fungus including USA and Europe and three from plantations of *P. radiata* in the Southern Hemisphere. Unexpectedly, all of these populations had relatively low levels of allelic diversity and high levels of clonality, not only regionally, but across continents. There was no evidence of genetic drift or fixation of alleles in local populations and some genotypes were found across continents. The results supported a long asexual history of the fungus and high levels of anthropogenic movement.

Various studies considered populations of *D. sapinea* globally. Bihon et al. [95] used microsatellite markers to study a relatively large population of the pathogen collected in South African pine plantations. This was shown to be highly diverse and indicative of many discrete introductions, but also providing some evidence of recombination. In a later study, Bihon et al. [96] developed markers for the mating type locus in *D. sapinea* and showed that both mating types are present in South Africa in approximately equal proportions supporting the idea of a cryptic sexual cycle. A subsequent study [108] including isolates from Australia, Argentina, Ethiopia, as well as South Africa suggested shared sources of origin of *D. sapinea* and again evidence that sexual recombination could be occurring.

Recent studies, including comprehensive collections of *D. sapinea* from across Europe, have supported the conclusions of Burgess et al. [85] and Bihon et al. [108] that the fungus has low levels of genetic diversity in Europe. For example, Zlatković et al. [100], using multilocus sequence data and microsatellite markers, found low gene and genotypic diversity in *D. sapinea* from the Western Balkans. This suggested that the fungus is an introduced pathogen that is spreading clonally in that region. Likewise, Mazanos et al. [109] using microsatellite markers and Vegetative Compatibility Group (VGG) analyses found low genetic diversity and high levels of clonality in *D. sapinea* populations in Southern Spain when compared to populations from the Southern USA. The results of that study showed significantly higher levels of diversity than in a previous study in Spain, which the authors ascribed to more intensive sampling from a greater number of tissue types, but the USA collections still showed greater levels of diversity. Brodde et al. [47] also found highly clonal populations of *D. sapinea* across Europe, including pre- and post-outbreak isolates from Sweden. This study included geographically isolated populations, such as from Turkey and other European countries.

Adamson et al. [110] used microsatellites markers to characterize a large collection of isolates from 15 countries of Europe and Western Asia and found that one genotype accounting for 45% of the isolates was dominant.

Furthermore, these authors reported distinct sub-populations of the pathogen in Central/ Northern Europe, Italy and Georgia respectively. This added further support to the numerous studies already mentioned suggesting widely distributed and highly clonal lineages of *D. sapinea* in Europe. In contrast, using a newly developed set of SSR markers, developed from the genomes of *D. sapinea*, Villanoval et al. [111] found higher levels of genetic diversity in Northern Spain and California populations of the pathogen. That study supported evidence, from association of alleles and distribution of mating types, that suggest the existence of a sexual cycle in the fungus. Clearly, despite the numerous population genetic studies on *D. sapinea* including various geographic areas of the world, the question relating to its area of origin has yet to be resolved.

A Rising Challenge in Pine Forests Amidst Climate Change

A number of studies have considered whether variation in the aggressiveness of *D. sapinea* isolates could be related to the presence of mycoviruses. Steenkamp et al. [112] first considered this question but was not able to relate the presence of an unidentified dsRNA element to growth rate in culture or levels of pathogenicity in the fungus. Preisig et al. [113] identified two novel dsRNA Totiviruses in *D. sapinea* and named them *Sphaeropsis sapinea* RNA virus 1 and 2 (SsRV1 and SsRV2). These viruses were different to the one identified in *D. scrobiculata* and named *Diplodia scrobiculata* RNA virus 1 or DSRV1 [114], but neither study considered their roles in pathogenicity. Adams et al. [115] considered the aggressiveness of *Diplodia* isolates including those of morphotype A (*D. sapinea*) and B (*D. scrobiculata*) with and without dsRNA elements and found no consistent patterns relating to the presence of these virus-like elements. These mycoviruses likely represent only a portion of the complex potential mycovirome that is now emerging from studies on the *Botryosphaeriaceae* and other fungi [116, 117].

Although there have been occasional incidences of disease in Europe and North America, particularly since the 1980s, *D. sapinea* has retained a status as being relatively unimportant in the native Northern Hemisphere range of *Pinus*. This situation has changed dramatically in recent years where extensive death of *Pinus* spp. has occurred in various countries of Europe [37]. Where the fungus was virtually unknown and often difficult to find even on pine cones in Scandinavian and Baltic countries [118–120], it now occurs commonly and is considered an important contributor to tree death [47, 48, 101, 121]. This dramatic

change of situation is poorly understood at present, but all indications are that this is due to climate change and conditions that were previously not conducive to disease caused by *D. sapinea*.

As a non-native fungus, *D. sapinea* has had its greatest impact on plantation forestry in the Southern Hemisphere. This can be attributed to the fact that many of the *Pinus* spp. best suited to growing in that environment have high levels of susceptibility to infection. Moreover, plantations are commonly subjected to stressful conditions such as drought, hail and insect damage, and challenging root systems due to various nursery practices, across large areas planted to single species that is not typically associated with trees. Although little is known regarding this topic, successive rotations of trees on the same sites, with a build-up of undesirable microbes and pathogens, could be factors contributing to tree stress, and enhanced susceptibility to infection [122].

The importance of *D. sapinea* is increasing rapidly with substantial damage to *Pinus* spp. being experienced in areas not previously challenged by the pathogen. All available evidence suggests that climate change is the main driver of disease in the newly affected areas [123], particularly those in the northern parts of Europe. This is a complex and relatively poorly understood topic, justifying many newly funded projects aimed at better understanding the biology of *D. sapinea*.

Numerous recent studies seeking to understand the underlying reasons for the many new outbreaks of blight and die-back caused by *D. sapinea* in Europe have considered the population diversity of the pathogen. These studies, that include large numbers of isolates, have found low levels of gene and genotypic diversity and distinctly clonal lineages of the fungus. This supports the early, and at that time intriguing, finding of Burgess et al. [107] that in comparison to isolates from the Southern Hemisphere that had high levels of diversity, those from Europe were distinctly clonal. These studies support the interesting notion that *D. sapinea* could have been introduced into Europe. In contrast, evidence relating to the association of alleles and the distribution of mating types suggests the existence of a sexual cycle in the fungus. It is evident that the question relating to the origin of *D. sapinea* has not been resolved. Future studies including large global populations of the pathogen and the application of genome data will add important new knowledge to this intriguing question.

The *D. sapinea* congener, *D. scrobiculata* was discovered occurring in close association with the former pathogen more than 40 years ago. The fungus has a very similar biology to *D. sapinea* and appears to be native to North America. Although *D. scrobiculata* has been treated as less

aggressive than *D. sapinea*, this may simply be due to the environments and hosts on which it has been found. This is clearly a species that deserves further study, not only relating to its potential threat to *Pinus* species in areas where it is currently not known, but also as a basis for comparative studies such as those relating to infection biology on *D. sapinea*.

One of the often-overlooked impacts of *D. sapinea* is the blue stain that it imparts to the sapwood of infected and recently felled trees. This is a major problem for the commercial industries, particularly in the Southern Hemisphere that market solid wood products. Losses due to *D. sapinea* induced blue stain amount to many millions of US\$ annually and the problem is extremely difficult to manage. Chemical agents are commonly applied to felled trees, but these have negative environmental consequences and overlook the fact that the fungus is already inside the trees. This challenge deserves intensive research to consider new and innovative options such as biological control, which could, for example, include treatments with non-staining endophytic fungi.

Diplodia sapinea has spread to every area of the world where *Pinus* spp. are being cultivated for commercial purposes or as ornamentals. In most of these areas, the fungus was discovered very soon after the pines had become established. And where populations of the pathogen have

been studied, it is evident that it has been introduced into many of these areas a multiplicity of times. The South African example discussed in this paper provides a vivid illustration of this fact. *Diplodia sapinea* is evidently able to spread easily likely via various forms of germplasm including living plant material and seeds.

Concluding Remarks

From being a relatively unimportant in its presumed Northern Hemisphere area of origin, *D. sapinea* is increasingly considered an important factor in the general health of *Pinus* spp. Yet the fungus remains relatively poorly understood in terms of its biology and ecology, at least in comparison to some of the world's better-known tree pathogens (Table 1). Its increasing importance, and the application of modern tools such as those related to genomes and “omics” in general are likely to result in a rapid and valuable increase in our knowledge of every aspect of *D. sapinea* biology. Such studies will also raise our understanding of related fungi as well as tree endophytes and latent pathogens. Importantly, they should also promote a greater understanding of how endophytic fungi in asymptomatic plant tissues are moved globally and how these threats might be better managed.

Table 1 Unanswered questions and research directions for *Diplodia Sapinea*

Gaps in knowledge about <i>D. sapinea</i>	Research that could be undertaken
Where do the isolates of <i>D. sapinea</i> that cause pruning wound infections originate, the environment, or are they endophytic?	Utilize currently available tools such as microsatellite markers to provide a reliable answer.
Do wound-induced inoculations reflect the biology of the fungus? Under natural conditions, do all these arise from previously existing internal infections associated with an endophytic life cycle?	Investigate whether wounding alone results in the same disease as that are induced by inoculations and identify the <i>D. sapinea</i> genotypes involved, using microsatellite markers.
What additional studies are necessary to determine whether meiotic recombination is occurring or if other forms of recombination such as parasexuality, are involved?	Large scale population genomic analyses could provide insights, together with classical molecular genetics studies.
What role is played by genotypes (that are apparently more aggressive) that originate from non-conifer hosts?	Comparing the genomes of a diverse range of isolates, from many hosts including analyses of genomic sweeps, could help to address this question.
Do inoculations on juvenile plants accurately reflect field conditions, where affected trees typically have well-developed woody tissues?	Conduct comparable inoculations on both juvenile and mature trees.
What is the area of origin of <i>D. sapinea</i> ?	Perform a larger sampling of areas where <i>D. sapinea</i> is believed to occur naturally, followed by population genetic/genomic analyses.
What are the best disease management practises for <i>D. sapinea</i> ?	Engage in intensive research to explore new and innovative options, such as biological control, which could include treatments with non-staining endophytic fungi.
What is the exact nature of stress that results in <i>D. sapinea</i> infection and how does this relate to factors such as climate change?	Measure stress through detailed experimentation and couple results to knowledge from genomes and climate change models

Key References

- Swart WJ, Wingfield MJ. Biology and control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. *Plant Dis.* 1991;75:761–6.

A comprehensive review of the topic up to the date of publication.

- Phillips AJL, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Crous PW. The Botryosphaeriaceae: genera and species known from culture. *Stud Mycol.* 2013;76:51–167.

This study provided a fundamentally important taxonomic placement of *D. sapinea*.

- Brodde L, Adamson K, Camarero JL, Castaño C, Drenkhan R, Lehtijärvi A, et al. Diplodia tip blight on its way to the north: drivers of disease emergence in Northern Europe. *Front Plant Sci.* 2019;8:1818. <https://doi.org/10.3389/fpls.2018.01818>.

This paper illustrates how *D. sapinea* is emerging as a serious pathogen in Europe, apparently linked to climate change.

- Slippers B, Wingfield MJ. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biol Rev.* 2007;21:90–106. <https://doi.org/10.1016/j.fbr.2007.06.002>.

A comprehensive review of the *Botryosphaeriaceae*, the family in which *D. sapinea* resides, which considers many factors relating to the biology of these fungi.

- Desprez-Loustau ML, Marçais B, Nageleisen LM, Piou D, Vannini A. Interactive effects of drought and pathogens in forest trees. *Ann For Sci.* 2006;63:597–612. <https://doi.org/10.1051/forest:2006040>.

This paper illustrates the importance of tree stress, which is an important issue relating to the biology of *D. sapinea*.

- Oostlander AG, Brodde L, von Bargen M, Leiterholt M, Trautman D, Enderle R, et al. A reliable and simple method for the production of viable pycnidiospores of the pine pathogen *Diplodia sapinea* and a spore-based infection assay on Scots pine. *Plant Dis.* 2023;107:3370–7. <https://doi.org/10.1094/PDIS-01-23-0107-RE>.

This recent paper has provided a technique that will be important in future studies that seek to understand the biology of *D. sapinea*.

- De Wet J, Burgess T, Slippers B, Preisig O, Wingfield B.D, Wingfield MJ. Multiple gene genealogies and

microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. *Mycol Res.* 2003;107:557–6.

This was the first publication to provide a multi gene genealogy for *D. sapinea*.

- Blumenstein K, Bußkamp J, Langer GJ, Langer EJ, Terhonen E. The Diplodia tip blight pathogen, *Sphaeropsis sapinea* is the most common fungus in Scots pines' microbiome, irrespective of health status—A case study from Germany. *J Fungi.* 2021;7:607. <https://doi.org/10.3390/jof7080607>.

This paper is one of a number that confirms the endophytic nature of *D. sapinea*; a key factor relating to its biology.

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Compliance with Ethical Standards

Conflict of Interest All authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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References

- Legat CE. The cultivation of exotic conifers in South Africa. *Empire Forestry J.* 1930;9:32–63.
- Burgess T, Wingfield MJ. Exotic pine forestry in the Southern Hemisphere: a brief history of establishment and quarantine practices. *South Afr J.* 2001;192:79–83.
- Burgess T, Wingfield MJ. Quarantine is important in restricting the spread of exotic seed-borne tree pathogens in the Southern Hemisphere. *Int Rev.* 2002;4:56–65. <https://www.jstor.org/stable/43740945>.
- Poynton RJ. Tree planting in Southern Africa: the pines (vol. 1), vol. 1. Pretoria: Department of Forestry; 1979. p. 1–576.
- Swart WJ, Knox-Davies PS, Wingfield MJ. *Sphaeropsis sapinea*, with special reference to its occurrence on *Pinus* spp. *South Afr South Afr J for.* 1985;135:1–8. <https://doi.org/10.1080/00382167.1985.9629601>.
- Swart WJ, Wingfield MJ, Knox-Davies PS. Conidial dispersal of *Sphaeropsis sapinea* in three climatic regions of South Africa. *Plant Dis.* 1987;71:1038–40.
- Wingfield MJ, Knox-Davies PS. Observations on diseases in pine and eucalyptus plantations in South Africa. *Phytophylactica.* 1980;12:57–63.
- Wingfield MJ. Pathogens in exotic plantation forestry. *Int Forestry Rev.* 1999;1:163–8. <https://www.jstor.org/stable/42609189>.
- Jeffries MJ, Lawton JH. Enemy free space and the structure of ecological communities. *Biol J Linn Soc.* 1984;23(4):269–86.
- Burgess TI, Wingfield MJ. Pathogens on the move: a 100-year global experiment with planted eucalypts. *Bioscience.* 2017;67:14–25. <https://doi.org/10.1093/biosci/biw146>.
- Wingfield MJ, Slippers B, Roux J, Wingfield BD. Worldwide movement of exotic forest fungi, especially in the tropics and southern hemisphere. *Bioscience.* 2001;51:135–40. [https://doi.org/10.1641/0006-3568\(2001\)051](https://doi.org/10.1641/0006-3568(2001)051).
- Chou CKS. Diplodia leader dieback. Diplodia crown wilt. Diplodia whorl canker. *Forest Pathol New Zealand.* 1984;7:1–7.
- Gibson IAS. Diseases of Forest Trees widely planted as exotics in the tropics and Southern hemispheres: In *The genus Pinus*. Commonwealth Mycological Institute, Kew, and Commonwealth Forestry Institute, University of Oxford. 1979;2:135.
- Gilmour JW. Survey of diplodia whorl canker in *Pinus radiata*. *New Z for Service Res Leaflet.* 1964;5:1–5.
- Marks GC, Minko G. The pathogenicity of *Diplodia pinea* to *Pinus radiata* D. Don. *Aust J Bot.* 1969;17:1–12.
- Millikan CR, Anderson RD. Dead top of *Pinus* spp. *Vic Plantations Austr for.* 1957;21:4–14. <https://doi.org/10.1080/00049158.1957.10675332>.
- Laughton EM. The incidence of fungal disease on timber trees in South Africa. *S Afri J Sci.* 1937;33:377–82.
- Lückhoff HA. Diseases of exotic plantation trees in the Republic of South Africa. In: *Proceedings of the VI FAO/IUFRO Symposium Meeting.* 20–30th July 1964, Oxford 4pp.
- Smith H, Coutinho TA, Wolfaardt FW, Wingfield MJ. Relative susceptibility of northern and southern provenances of *Pinus greggii* to infection by *Sphaeropsis sapinea*. *Ecol Manag.* 2002;166:331–6. [https://doi.org/10.1016/S0378-1127\(01\)00667-3](https://doi.org/10.1016/S0378-1127(01)00667-3).
- Swart WJ, Wingfield MJ. Seasonal response of *Pinus radiata* in South Africa to artificial inoculation with *Sphaeropsis sapinea*. *Plant Dis.* 1991;75:1031–3.
- Swart WJ, Wingfield MJ. Biology and control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. *Plant Dis.* 1991;75:761–6.
- Zwolinski JB, Swart WJ, Wingfield MJ. Economic impact of a post-hail outbreak of dieback induced by *Sphaeropsis sapinea*. *Eur J Pathol.* 1990;20:405–11. <https://doi.org/10.1111/j.1439-0329.1990.tb01155.x>.
- Zwolinski JB, Swart WJ, Wingfield MJ. Intensity of dieback induced by *Sphaeropsis sapinea* in relation to site conditions. *Eur J Pathol.* 1990;20:167–74. <https://doi.org/10.1111/j.1439-0329.1990.tb01127.x>.
- Zwolinski JB, Swart WJ, Wingfield MJ. Association of *Sphaeropsis sapinea* with insect infestation following hail damage of *Pinus radiata*. *Ecol Manag.* 1994;72:293–8. [https://doi.org/10.1016/0378-1127\(94\)03459-A](https://doi.org/10.1016/0378-1127(94)03459-A).
- Eldridge KG. Significance of *Diplodia pinea* in plantations: plantation technical papers. *Vic Forests Comm.* 1957;41:339.
- Peterson GW. Infection, epidemiology and control of Diplodia blight of Austrian ponderosa and scots pines. *Phytopathology.* 1977;67:511–4.
- Peterson GW. Control of *Diplodia* and *Dothiostroma* blight of pines in the urban environment. *J Arboric.* 1981;7:1–5.
- Haddow WR, Newman FS. A disease of the scots pine (*Pinus sylvestris* L.) caused by *Diplodia pinea* Kickx associated with the pine spittle-bug (*Aphrophora Paralella* Say). *Trans Royal Can Inst.* 1942;24:1–18.
- Fabre B, Piou D, Desprez-Loustau ML, Marçais B. Can the emergence of pine Diplodia shoot blight in France be explained by changes in pathogen pressure linked to climate change? *Glob Change Biol.* 2011;17:3218–27. <https://doi.org/10.1111/j.1365-2486.2011.02428.x>.
- Phillips AJL, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Crous PW. The *Botryosphaeriaceae*: genera and species known from culture. *Stud Mycol.* 2013;76:51–167. <https://doi.org/10.3114/sim0021>.
- Waterman AM. *Diplodia pinea*, the cause of a disease of hard pines. *Phytopathology.* 1943;33:1018–31.
- Sutton BC. *Sphaeropsis sapinea*. Coelomycetes. Commonwealth Mycological Institute: Kew, Surrey, UK; 1980. p. 120–1.
- Denman S, Crous PW, Taylor JE, Kang JC, Pascoe I, Wingfield MJ. An overview of taxonomic history of *Botryosphaeria*, and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Stud Mycol.* 2000;45:129–40.
- Punithalingam E, Waterston JM. *Diplodia pinea*. *CMI Descriptions Pathogenic fungi Bacteria.* 1970;273:273–7.
- Fisher J. Two fungus diseases of coniferous trees. *Agricultural J Union South Afr.* 1912;3:389–91.
- Carroll G. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology.* 1988;69:2–9. <https://doi.org/10.2307/1943154>.
- Fisher PJ, Petrini O, Petrini LE, Sutton BC. Fungal endophytes from the leaves and twigs of *Quercus ilex* L. from England, Majorca and Switzerland. *New Phytol.* 1994;127:133–7. <https://doi.org/10.1111/j.1469-8137.1994.tb04267.x>.
- Sieber TN. Endophytic fungi in twigs of healthy and diseased Norway spruce and white fir. *Mycol Res.* 1989;92:322–3.
- Bihon W, Slippers B, Burgess T, Wingfield MJ, Wingfield BD. Sources of *Diplodia pinea* endophytic infections in *Pinus patula* and *P. radiata* seedlings in South Africa. *Pathol.* 2011;41:370–75. <https://doi.org/10.1111/j.1439-0329.2010.00691>.
- Flowers J, Hartman J, Vaillancourt L. Detection of latent *Sphaeropsis sapinea* infections in Austrian pine tissues using nested-polymerase chain reaction. *Phytopathology.* 2003;12:1471–6. <https://doi.org/10.1094/PHYTO.2003.93.12.1471>.
- Smith H, Wingfield MJ, Coutinho TA. The role of latent *Sphaeropsis sapinea* infections in post-hail associated die-back of *Pinus patula*. *Ecol Manag.* 2002;164:177–84.
- Smith H, Wingfield MJ, Crous PW, Coutinho TA. *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *S Afri J Bot.* 1996;62:86–8. [https://doi.org/10.1016/S0254-6299\(15\)30596-2](https://doi.org/10.1016/S0254-6299(15)30596-2).

43. Stanosz GR, Smith DR, Guthmiller MA, Stanosz JC. Persistence of *Sphaeropsis sapinea* on or in asymptomatic stems of red pines. *Mycologia*. 1997;89:525–30. <https://doi.org/10.1080/00275514.1997.12026813>.
44. Stanosz GR, Blodgett JT, Smith DR, Kruger EL. Water stress and *Sphaeropsis sapinea* as a latent pathogen of red pine seedlings. *New Phytol*. 2001;149:531–8. <https://doi.org/10.1046/j.1469-8137.2001.00052.x>.
45. Petrini O, Fisher PJ. A comparative study of fungal endophytes in xylem and whole stem of *Pinus sylvestris* and *Fagus sylvatica*. *Trans Br Mycol Soci*. 1988;9:233–8.
46. Bihon W, Burgess T, Slippers B, Wingfield MJ, Wingfield BD. Distribution of *Diplodia pinea* and its genotypic diversity within asymptomatic *Pinus patula* trees. *Australas Plant Path*. 2011;40:540–8. <https://doi.org/10.1111/j.1439-0329.2010.00649>.
47. Brodde L, Adamson K, Camarero JL, Castaño C, Drenkhan R, Lehtijärvi A, et al. *Diplodia* tip blight on its way to the north: drivers of disease emergence in Northern Europe. *Front Plant Sci*. 2019;8:1818. <https://doi.org/10.3389/fpls.2018.01818>.
48. Brodde L, Åslund ML, Elfstrand M, Oliva J, Wågström K, Stenlid J. *Diplodia sapinea* as a contributing factor in the crown dieback of Scots pine (*Pinus sylvestris*) after a severe drought. *Ecol Manag*. 2023;549:1–9. <https://doi.org/10.1016/j.foreco.2023.121436>.
49. Slippers B, Wingfield MJ. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biol Rev*. 2007;21:90–106. <https://doi.org/10.1016/j.fbr.2007.06.002>.
50. Slippers B, Boissin E, Phillips AJL, Groenewald JZ, Lombard L, Wingfield MJ, et al. Phylogenetic lineages in the Botryosphaeriales: a systematic and evolutionary framework. *Stud Mycol*. 2013;76:31–49. <https://doi.org/10.3114/sim0020>.
51. Da Costa EB. Effect of blue stain on the strength of *Pinus radiata*. CSIRO for Prod Newsl 209 Australia. 1955;209:1–2.
52. Wright JP, Marks GC. Loss of merchantable wood in radiata pine associated with infection by *Diplodia pinea*. *Aust for*. 1970;34:107–19. <https://doi.org/10.1080/00049158.1970.10675516>.
53. Feci E, Smith D, Stanosz GR. Association of *Sphaeropsis sapinea* with insect-damaged red pine shoots and cones. *Pathol*. 2003;33:7–13. <https://doi.org/10.1046/j.1439-0329.2003.00304.x>.
54. Wingfield MJ, Palmer MA. *Diplodia pinea* associated with insect damage on pines in Minnesota and Wisconsin. Melbourne, Australia: Fourth International Plant Pathology Congress; 1983. p. 249.
55. Chou CKS, Zabkiewicz JA. Toxicity of monoterpenes from *P. radiata* cortical oleoresin to *Diplodia pinea* spores. *Eur J Pathol*. 1976;6:354–9. <https://doi.org/10.1111/j.1439-0329.1976.tb00549>.
56. Chou CKS. A shoot dieback in *Pinus radiata* caused by *Diplodia pinea*. II. Inoculation studies. *N Z J Sci*. 1976;6:409–20.
57. Chou CKS. Effect of tree age on *Diplodia pinea* infection of *Pinus radiata*. *Plant Dis Rep*. 1977;61:101–3.
58. Chou CKS. *Diplodia pinea* infection of *Pinus radiata* seedlings: effect of temperature and shoot wetness duration. *N Z J Sci*. 1982;12:425–37.
59. Desprez-Loustau ML, Marçais B, Nageleisen LM, Piou D, Vanini A. Interactive effects of drought and pathogens in forest trees. *Ann Sci*. 2006;63:597–612. <https://doi.org/10.1051/forest:2006040>.
60. Marsberg A, Kemler M, Jami F, Nagel JH, Postma-Smidt A, Naidoo S, et al. *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. *Mol Plant Pathol*. 2017;18:477–88. <https://doi.org/10.1111/mpp.12495>.
61. Nicholls TH, Ostry ME. *Sphaeropsis sapinea* cankers on stressed red and jack pines in Minnesota and Wisconsin. *Plant Dis*. 1990;74:54–6.
62. Fraedrich SW, Miller T. Mycoflora associated with slash-pine seeds from cones collected at seed orchards and cone-processing facilities in the south-eastern USA. *Eur J Pathol*. 1995;25:73–82. <https://doi.org/10.1111/j.1439-0329.1995.tb00321.x>.
63. Munck IA, Smith DR, Sickley T, Stanoz GR. Site related influences on cone-borne inoculum and asymptomatic persistence of *Diplodia* shoot-blight fungi on or in mature red pines. *Ecol Manag*. 2009;257:812–9. <https://doi.org/10.1016/j.foreco.2008.10.023>.
64. Munck IA, Stanoz GR. Longevity of inoculum production by *Diplodia pinea* on red pine cones. *Pathol*. 2010;40:58–63. <https://doi.org/10.1111/j.1439-0329.2009.00607.x>.
65. Swart WJ, Wingfield MJ, Knox-Davies PS. Factors associated with *Sphaeropsis sapinea* infection of pine trees in South Africa. *Phytophylactica*. 1987;19:505–10.
66. Chou CKS, McKenzie M. Effect of pruning intensity and season on *Diplodia pinea* infection of *Pinus radiata* stem through pruning. *Eur J Pathol*. 1988;7:437–44. <https://doi.org/10.1111/j.1439-0329.1976.tb00549>.
67. Burdon RD, Currie D, Chou CKS. Responses to inoculation with *Diplodia pinea* in progenies of apparently resistant trees of *Pinus radiata*. *Australas Plant Path*. 1982;11:37–9.
68. Rees AA, Webber JF. Pathogenicity of *Sphaeropsis sapinea* to seed, seedlings and saplings of some central American pines. *Trans Br Mycol Soc*. 1988;91:273–7.
69. Swart WJ, Wingfield MJ, Palmer MA, Blanchette RA. Variation among South African isolates of *Sphaeropsis sapinea*. *Phytopathology*. 1991;81:489–93.
70. Raffa KF, Brockerhoff EG, Grégoire JC, Hamelin RH, Liebhold AM, Santini A, et al. Approaches to forecasting damage by invasive forest insects and pathogens: a cross-assessment. *Bioscience*. 2022;73:85–111. <https://doi.org/10.1093/biosci/biac108>.
71. Oostlander AG, Brodde L, von Barga M, Leiterholt M, Trautman D, Enderle R, et al. A reliable and simple method for the production of viable pycnidiospores of the pine pathogen *Diplodia sapinea* and a spore-based infection assay on scots pine. *Plant Dis*. 2023;107:3370–7. <https://doi.org/10.1094/PDIS-01-23-0107-RE>.
72. Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WF, Phillips AJ, et al. Phylogenetic lineages in the Botryosphaeriaceae. *Stud Mycol*. 2006;2006(55):235–53. <https://doi.org/10.3114/sim.55.1.235>.
73. Crous PW, Hawksworth DL, Wingfield MJ. Identifying and naming plant pathogenic fungi: past, present and future. *Annu Rev Phytopathol*. 2015;53:247–67. <https://doi.org/10.1146/annurev-phyto-080614-120245>.
74. Wingfield MJ, de Beer ZW, Slippers B, Wingfield BD, Groenewald JZ, Lombard L, et al. One fungus, one name promotes progressive plant pathology. *Mol Plant Pathol*. 2012;13:604–13. <https://doi.org/10.1111/j.1364-3703.2011.00768.x>.
75. Palmer MA, Stewart EL, Wingfield MJ. Variation among isolates of *Sphaeropsis sapinea* in the North Central United States. *Phytopathology*. 1987;77:944–8.
76. Wang CG, Blanchette RA, Jackson WA, Palmer MA. Differences in conidial morphology among isolates of *Sphaeropsis sapinea*. *Plant Dis*. 1985;69:838–41.
77. Wang CG, Blanchette RA, Palmer MA. Ultrastructural aspects of the conidium cell wall of *Sphaeropsis sapinea*. *Mycologia*. 1986;78:960–3. <https://doi.org/10.1080/00275514.1986.12025356>.
78. Palmer MA. Isolate types of *Sphaeropsis sapinea* associated with main stem cankers and top-kill of *Pinus resinosa* in Minnesota and Wisconsin. *Plant Dis*. 1991;75:507–10.
79. Smith DR, Stanosz GR. Confirmation of two distinct populations of *Sphaeropsis sapinea* in the North Central United States using RAPDs. *Phytopathology*. 1995;85:699–704.

80. De Wet J, Wingfield MJ, Coutinho TA, Wingfield BD. Characterization of *Sphaeropsis sapinea* isolates from South Africa, Mexico and Indonesia. *Plant Dis.* 2000;84:151–6. <https://doi.org/10.1094/PDIS.2000.84.2.151>.
81. Burgess T, Wingfield MJ, Wingfield BD. Simple sequence repeat markers distinguish among morphotypes of *Sphaeropsis sapinea*. *Appl Environ Microbiol.* 2001;67:354–62. <https://doi.org/10.1128/AEM.67.1.354-362.2001>.
82. De Wet J, Burgess T, Slippers B, Preisig O, Wingfield BD, Wingfield MJ. Multiple gene genealogies and microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. *Mycol Res.* 2003;107:557–6.
83. De Wet J, Wingfield MJ, Coutinho TA, Wingfield BD. Characterisation of the ‘C’ morphotype of the pine pathogen *Sphaeropsis sapinea*. *Ecol Manag.* 2002;161:181–8. [https://doi.org/10.1016/S0378-1127\(01\)00479-0](https://doi.org/10.1016/S0378-1127(01)00479-0).
84. Smith H, Wingfield MJ, De Wet J, Coutinho TA. Genotypic diversity of *Sphaeropsis sapinea* from South Africa and Northern Sumatra. *Plant Dis.* 2000;84:139–42. <https://doi.org/10.1094/PDIS.2000.84.2.139>.
85. Burgess TJ, Gordon TR, Wingfield MJ, Wingfield BD. Geographic isolations of *Diplodia scrobiculata* and its association with native *Pinus radiata*. *Mycol Res.* 2004;2004(108):1399–406. <https://doi.org/10.1017/S0953756204001443>.
86. Stanosz GR, Smith DR, Guthmiller MA. Characterization of *Sphaeropsis sapinea* from the West Central United States by means of random amplified polymorphic DNA marker analysis. *Plant Dis.* 1996;80:1175–8.
87. Stanosz GR, Swart WJ, Smith DR. RAPD marker and isozyme characterization of *Sphaeropsis sapinea* from diverse coniferous hosts and locations. *Mycol Res.* 1999;103:1193–202. <https://doi.org/10.1017/S0953756299008382>.
88. Zhou S, Stanosz GR. Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analyses of ITS and 5.8S rDNA sequences. *Mycologia.* 2001;93:516–27.
89. Zhou S, Smith DR, Stanosz GR. Differentiation of *Botryosphaeria* species and related anamorphic fungi using Inter simple or short sequence repeat (ISSR) fingerprinting. *Mycol Res.* 2001;105:919–26.
90. Aragonés A, Manzanos T, Stanosz G, Munck IA, Raposo R, Elvira-Recuenco M, et al. Comparison of *Diplodia* tip blight pathogens in Spanish and north American pine ecosystems. *Microorganisms.* 2021;9:2565. <https://doi.org/10.3390/microorganisms9122565>.
91. Luchi N, Capretti P, Surico G, Orlando C, Pazzagli M, Pinzani P. A real-time quantitative PCR assay for the detection of *Sphaeropsis sapinea* from inoculated *Pinus nigra* shoots. *J Phytopathol.* 2005;153:37–42.
92. Luchi N, Pratensi N, Sim L, Pazzagli M, Capretti P, Scala A, et al. High-Resolution melting analysis: a new molecular approach for the early detection of *Diplodia pinea* in Austrian pine. *Fungal Biol.* 2011;2011(115):715–23.
93. Blodgett JT, Stanosz GR. *Sphaeropsis sapinea* morphotypes differ in aggressiveness, but both infect nonwounded red and jack pines. *Plant Dis.* 1997;81:143–7. <https://doi.org/10.1094/PDIS.1997.81.2.143>.
94. Wingfield MJ, Knox-Davies PS. Association of *Diplodia pinea* with a root disease of pines in South Africa. *Plant Dis.* 1980;64:221–3.
95. Bihon W, Burgess T, Slippers B, Wingfield MJ, Wingfield BD. High levels of genetic diversity and cryptic recombination is widespread in introduced *Diplodia pinea* populations. *Australas Plant Path.* 2012;41:41–6.
96. Bihon W, Wingfield MJ, Slippers B, Duong TA, Wingfield BD. MAT gene idiomorphs suggest a heterothallic sexual cycle in a predominantly asexual and important pine pathogen. *Fungal Genet Biol.* 2014;62:55–61. <https://doi.org/10.1016/j.fgb.2013.10.013>.
97. Sinclair WA, Lyon HH. *Diseases of trees and shrubs.* 2nd ed. Comstock Publishing Associates; 2005.
98. Osorio JA, Crous CJ, De Beer ZW, Wingfield MJ, Roux J. Endophytic Botryosphaeriaceae, including five new species, associated with mangrove trees in South Africa. *Fungal Biol.* 2017;121(4):361–93. <https://doi.org/10.1016/j.funbio.2016.09.004>.
99. Zlatković M, Keča N, Wingfield MJ, Jami F, Slippers B. New and unexpected host associations for *Diplodia sapinea* in the Western Balkans. *For Pathol.* 2017;2017(47):e12328. <https://doi.org/10.1111/efp.12328>.
100. Zlatković M, Wingfield MJ, Jami F, Slippers B. Genetic uniformity characterizes the invasive spread of *Neofusicoccum parvum* and *Diplodia sapinea* in the Western Balkans. *Pathol.* 2019;49:e12491. <https://doi.org/10.1111/efp.12491>.
101. Blumenstein K, Bußkamp J, Langer GJ, Terhonen E. *Diplodia* tip blight pathogen’s virulence empowered through host switch. *Front Fungal Biol.* 2022;3:939007. <https://doi.org/10.3389/ffunb.2022.939007>.
102. Schirp A, Farrell RI, Kreber B. Effects of New Zealand sap-staining fungi on structural integrity of unseasoned radiata pine. *Holz als Roh-und Werkst.* 2003;61:369–76.
103. Hodges CS. Pine mortality in Hawaii associated with *Botryosphaeria dothidea*. *Plant Dis.* 1983;67:55–6.
104. Birch TTC. *Diplodia pinea* in New-Zealand. *Rev Appl Mycol.* 1937;16:148.
105. Chou KKS. Crown wilt of *Pinus radiata* associated with *Diplodia pinea* infection of woody stems. *Eur J Pathol.* 1987;17:398–411.
106. Swart WJ, Wingfield MJ, Knox-Davies PS. Relative susceptibilities to *Sphaeropsis sapinea* of six *Pinus* spp. cultivated in South Africa. *Eur J Pathol.* 1988;18:184–9. <https://doi.org/10.1111/j.1439-0329.1988.tb00917.x>.
107. Burgess T, Wingfield BD, Wingfield MJ. Comparison of genotypic diversity in native and introduced populations of *Sphaeropsis sapinea* isolated from *Pinus radiata*. *Mycol Res.* 2001;105:1331–339. <https://doi.org/10.1017/S0953756201005056>.
108. Bihon W, Slippers B, Burgess T, Wingfield MJ, Wingfield BD. Diverse sources of infection and cryptic recombination revealed in South African *Diplodia pinea* populations. *Fungal Biol.* 2012;116:112–20. <https://doi.org/10.1016/j.funbio.2011.10.006>.
109. Manzanos T, Aragonés A, Iturrutxa E. Genotypic diversity and distribution of *Sphaeropsis sapinea* within *Pinus radiata* trees from northern Spain. *Pathol.* 2019;49:1–9. <https://doi.org/10.1111/efp.12550>.
110. Adamson K, Laas M, Blumenstein K, Busskamp J, Langer GJ, Klavina D, et al. Highly clonal structure and abundance of one haplotype characterise the *Diplodia sapinea* populations in Europe and Western Asia. *J Fungi.* 2021;7:634. <https://doi.org/10.3390/jof7080634>.
111. Vilanova L, Caballol M, Zhang K, Olson Å, Barnes I, Wingfield MJ, Oliva J. New simple sequence repeat markers reveal undetected diversity in Spanish and Californian *Diplodia sapinea* populations. *Fungal Genet Biol.* 2024;103937. <https://doi.org/10.1016/j.fgb.2024.103937>.
112. Steenkamp ET, Wingfield BD, Swart WJ, Wingfield MJ. Double-stranded RNA and associated virulence in South African isolates of *Sphaeropsis sapinea*. *Can J Bot.* 1998;76:1412–7. <https://doi.org/10.1139/b98-113>.

113. Preisig O, Wingfield BD, Wingfield MJ. Coinfection of a fungal pathogen by two distinct double-stranded RNA viruses. *Virology*. 1998;252:399–406. <https://doi.org/10.1006/viro.1998.9480>.
114. De Wet J, Bihon W, Preisig O, Wingfield BD, Wingfield MJ. Characterisation of a novel dsRNA element in the pine endophytic fungus, *Diplodia scrobiculata*. *Arch Virol*. 2011;156:1199–208.
115. Adams GC, Wu NT, Eisenberg BE. Virulence and double-stranded RNA in *Sphaeropsis sapinea*. *Pathol*. 2002;32:309–29. <https://doi.org/10.1046/j.1439-0329.2002.00293>.
116. Comont G, Faure C, Candresse T, Laurens M, Valière S, Lluh J, et al. Characterization of the RNA mycovirome associated with grapevine fungal pathogens: analysis of mycovirus distribution and their genetic variability within a collection of Botryosphaeriaceae isolates. *Viruses*. 2024;16:392. <https://doi.org/10.3390/v16030392>.
117. Hough B, Steenkamp ET, Wingfield BD, Read DA. Fungal viruses unveiled: a comprehensive review of Mycoviruses. *Viruses*. 2023;15:1202. <https://doi.org/10.3390/v15051202>.
118. Hanso M, Drenkhan R. *Diplodia pinea* is a new pathogen on Austrian pine (*Pinus nigra*) in Estonia. *Plant Pathol*. 2009;58:797.
119. Oliva J, Boberg J, Stenlid J. First report of *Sphaeropsis sapinea* on scots pine (*Pinus sylvestris*) and Austrian pine (*P. nigra*) in Sweden. *New Dis Rep*. 2013;27:23.
120. Müller MM, Hantula J, Wingfield M, Drenkhan R. *Diplodia sapinea* found on Scots pine in Finland. *Pathol*. 2019;49:1–7. <https://doi.org/10.1111/efp.12483>.
121. Blumenstein K, Bußkamp J, Langer GJ, Langer EJ, Terhonen E. The *Diplodia* tip blight pathogen, *Sphaeropsis sapinea* is the most common fungus in scots pines' microbiome, irrespective of health status—A case study from Germany. *J Fungi*. 2021;7:607. <https://doi.org/10.3390/jof7080607>.
122. Bose T, Hammerbacher A, Slippers B, Roux J, Wingfield MJ. Continuous replanting could degrade soil health in short-rotation plantation forestry. *Curr Rep*. 2023;9:230–20.
123. Caballol M, Serradó F, Barnes I, Camarero JJ, Valeriano C, Colangelo M, Oliva J. (2024) Climate, host ontogeny and pathogen structural specificity determine forest disease distribution at a regional scale. *Ecography* 2024;e06974. <https://doi.org/10.1111/ecog.06974>

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