Cylindrocarpon Species in Pacific Northwest Douglas-fir Nurseries: Diversity and Effects of Temperature and Fungicides on Mycelial Growth

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Abstract

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Douglas-fir nurseries play an important economic role in the Pacific Northwest timber industry, but the early death of seedlings due to disease influences regeneration success. A destructive root disease caused by fungus *Cylindrocarpon* results in the loss of seedlings in early stages of their growth. The objectives of this study were to: (1) identify and determine the diversity of species of *Cylindrocarpon* occurring in three different nurseries in the Pacific Northwest, (2) investigate the effect of temperature on the growth rate of the mycelia of these pathogens *in vitro*, and (3) determine the influence of four common fungicides on the growth of these pathogens *in vitro*. The identity of the species of *Cylindrocarpon* in three different nurseries (two in western Oregon and one in western Washington) was determined using sequences of the ITS region of rDNA. Three species of *Cylindrocarpon* were found; *C. destructans, C. liriodendri* and *C. pauciseptatum*. *Cylindrocarpon destructans* was the dominant species in all three nurseries, making up 61.4% of the isolates, while *C. liriodendri*

was only found in one nursery sample. *Cylindrocarpon liriodendri* grew significantly faster in culture at warmer temperatures than *C. destructans*. The optimum growth temperature for *C. liriodendri* was 25º C, while *C. destructans* grew fastest between 18º and 22º C. There was little growth of *C. destructans* at 30º C. All four fungicides (Cleary 3336F, Dithane 75DF, Heritage and Iprodione E-Pro) tested at different concentrations) (10, 25, 50 and 75% of label active ingredient - i.e., 37, 94, 187, and 281 ppm) reduced the growth of both species of *Cylindrocarpon* mycelia in culture. However, *C. destructans* generally had greater growth reduction than *C. liriodendri*. Cleary and Dithane reduced growth more than Heritage and Iprodione. Dithane at 75% active ingredient concentration had the greatest effect in reducing the growth of both *C. destructans* and *C. liriodendri*. Dithane at 75% concentration was significantly more effective than Cleary in reducing the growth of *C. destructans*, but there was no significant difference between Cleary and Dithane in reducing the growth of *C. liriodendri*. The greater inhibition of *C. destructans* than *C. liriodendri* by the fungicides could have been related to a temperature effect since the study was conducted at 25º C. *Cylindrocarpon liriodendri* grew significantly faster at 25º C than *C. destructans.* Results of this study are useful in identifying and managing *Cylindrocarpon* spp. in Douglas-fir nurseries in the Pacific Northwest.

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DEDICATION

To my parents for encouraging and supporting me to follow my dreams.

INTRODUCTION

Concern over severe destructive root diseases of conifer seedlings in Pacific Northwest USA nurseries has been raised due to the importance of conifers both environmentally and economically. Several fungi (*Fusarium commune*, *Pythium irregular*e, and *Cylindrocarpon* spp.) impact both seedling production and adversely affect regeneration success (Dumroese et al. 2000; Dumroese and James 2005). Douglas-fir (*Pseudotsuga menziesii*), which is one of the economically important conifers in the Pacific Northwest, has been affected by many root rot pathogens (Weiland et al. 2011). One of the common root rot fungi on Douglas-fir seedlings in nurseries is *Cylindrocarpon,* but its identification and role in seedling pathology is poorly understood (James 2012).

This fungus has a wide range of hosts especially in agriculture, including grapevines (Cabral et al. 2012), apples (Tewoldemedhin et al. 2011) and ginseng (Jang et al. 2010). In conifers, *C. destructans* has been implicated in regeneration failures mostly associated with container seedlings. Major losses of Douglas-fir seedlings due to *Cylindrocarpon* root rot in bare-root nurseries have recently attracted the attention of forest pathologists (Weiland et al. 2011).

No previous work has been done to identify *Cylindrocarpon* species on roots of Douglas-fir bare-root seedlings in Pacific Northwest nurseries. New techniques and technology can help us better understand these fungi and the diseases they cause which will be helpful in making better decisions to avoid and control losses. Molecular biology involving DNA analysis has helped us to make a huge step forward in the field of plant pathology with respect to identifying soil pathogens (Ophel-Keller et al. 2008). We can now identify and characterize these difficult to identify species with a lot more reliability so we can understand their relation to root disease and interactions with other pathogens. This knowledge is critical for managing plant disease. Understanding environmental responses of various *Cylindrocarpon* species is essential in understanding their biology and pathology with regards to disease development.

We also need to know how *Cylindrocarpon* species respond to different temperatures since global warming and environmental changes could increase pathogen growth and thus cause even more disease (Sturrock et al. 2011).

Soil fumigation with methyl bromide is the most effective treatment, but Weiland et al. (2011) showed that *Cylindrocarpon* inoculum could survive in residual seedling roots in soils. Methyl bromide, however, is scheduled to be phased out because of its negative environmental effects. Alternatives to fumigation include soil fungicide drenches and biocontrol agents (Agrios 2005). Soil-borne root pathogens like *Cylindrocarpon* can be treated with fungicide chemicals, but they are difficult to control. Also we need to be concerned about the use of chemicals for controlling *Cylindrocarpon* spp. for environmental reasons and the protection of nature. However, few fungicides are registered for use as soil drenches and to date they show little efficacy against *Cylindrocarpon*. Common fungicide drenches include Cleary 3336F, Dithane 75DF, Heritage and Iprodione E-Pro, but their influence has not yet been tested on *Cylindrocarpon*. Fungicides tested to control *Cylindrocarpon* on grapevine plants, which causes black foot disease, include Benomyl, Tebuconazole, Carbendazim, Flusilazole, Cyprodinil, Fludioxonil, Pyraclostrobin, Metiram, Imazalil (Rego et al. 2006, 2009).

The objectives of this study were to: (1) identify and determine the diversity of species of *Cylindrocarpon* on Douglas-fir seedlings occurring in three different nurseries in the Pacific Northwest, (2) investigate the effect of temperature on the growth rate of the mycelia of the fungal pathogen *in vitro*, and (3) determine the influence of the fungicides drenches Cleary 3336F, Dithane 75DF, Heritage and Iprodione E-Pro for controlling this pathogen *in vitro*.

LITERATURE REVIEW

Taxonomy and Diversity of *Cylindrocarpon* (Ascomycota: Hypocreales: Nectriaceae)

Species of *Neonectria sensu lato* (teleomorphs) and their anamorphs in the genus *Cylindrocarpon* are common in tropical and temperate regions. There are many species. Chaverri et al. (2011) report that according to *Index Fungorum* (www.indexfungorum.org) 38 species have been placed in *Neonectria* and 143 in *Cylindrocarpon*; these numbers may be underestimated, however.

The genus *Cylindrocarpon* was first named by H. W. Wollenweber in 1913 with *C. cylindroides* as the type species. Taxonomic revision of *Cylindrocarpon* by Booth (1966) divided the genus into four different groups, based on the presence or absence of microconidia or chlamydospores. In 2001 Mantiri et al. (2001) divided *Cylindrocarpon* into three clades by studying mitochondrial small subunit (mtSSU) rDNA sequences.

Booth's first three *Cylindrocarpon* groups correlate with the three clades of Mantiri et al. (2001). Clade I of Mantiri et al. (2001), the *Neonectria coccinea/galligena*-group, is the teleomorph of *Cylindrocarpon* group I, which has micro- and macroconidia, but no chlamydospores (except *N. ramulariae* which is the anamorph *Cylindrocarpon obtusiusculum* and was originally put in *Cylindrocarpon* group 4 by Booth (1966)). Clade II of Mantiri et al. (200l), the *N. mammoidea/veuillotiana* group, includes *Cylindrocarpon* group II that is characterized by the lack of microconidia and chlamydospores. Anamorphs in the *N.*

radicicola-group (clade III of Mantiri et al. (2001)) belong to *Cylindrocarpon* group III and have both microconidia and chlamydospores.

Phylogenetic studies revealed that *Neonectria* /*Cylindrocarpon* is paraphyletic (Mantiri et al. 2001; Halleen et al. 2004, 2006; Hirooka et al. 2005; Castlebury et al. 2006). Halleen et al. (2004, 2006) tried to resolve this issue by proposing to place the genus *Campylocarpon* in a group with species resembling *Cylindrocarpon.* A multilocus analysis (*act,* ITS, LSU, *rpb1*, *tef1, tub*) by Chaverri et al. (2011) showed five distinct highly supported clades, which are: (1) *N. coccinea*-group and *Cylindrocarpon* groups 1 and 4 (*Neonectria/Cylindrocarpon sensu stricto*); (2) *N. rugulosa*-group (*Rugonectria* gen. Nov.); (3) *N. mammoidea/N. veuillotiana*-groups and *Cylindrocarpon* group 2 (*Thelonectria* gen. Nov.); (4) *N. radicicola*-group and *Cylindrocarpon* group 3 (*Ilyonectria* gen. nov.); and (5) anamorph genus *Campylocarpon.*

Analyses of the sequence data of the mitochondrial small subunit (SSU) ribosomal DNA (rDNA) of some *Cylindrocarpon* species by Mantiri et al. (2001) and Brayford et al. (2004) indicate that *Neonectria/Cylindrocarpon* species which they grouped together were monophyletic. On the other hand both Mantiri et al. (2011) and Brayford et al. (2004) determined that the whole *Neonectria/Cylindrocarpon* clade includes many subclades, which match three of four groups described by Booth (1966). Seifert et al. (2003) analyzed some sequences of *Cylindrocarpon* species from ginseng (*Panax quinquefolius*) and other hosts, based on the partial β-tubulin gene and the nuclear ribosomal internal transcribed spacer (ITS) region. They suggested that the subclades *N. radicicola* and *N. macroconidialis* and a subclade comprising two distinct isolates, one from *Vitis vinifera* in Ontario, Canada, and the other from *Picea* spp. in Quebec, Canada, were monophyletic.

***Cylindrocarpon* Hosts and Pathogenicity**

Cylindrocarpon species are pathogens of both herbaceous and woody plants (Brayford 1993; Domsch et al. 2007) and cause important root diseases of agricultural plants including grapevines, apples, and ginseng as well as conifer seedlings in nurseries. In general they are thought to be weakly pathogenic and are saprobes on dead materials of plants (Brayford 1993) and can be found on the bark of recently killed woody plants (Samuels 1988; Chaverri et al. 2011). Garrett (1956) described *C. destructans* capable of parasitizing only immature, wounded, unthrifty or senescent roots (Garrett, 1956). Ustem et al. (1989) observed that *C. destructans* colonized containerized *Pinus sylvestris* during the dormant winter season in Sweden. This mirrors observations in the Pacific Northwest when seedling held weeks in cold storage can emerge with high levels of *Cylindrocarpon* (Willis Littke, Weyerhaeuser Company, Federal Way, Washington, personal communication).

Some species of *Cylindrocarpon* can cause cankers, root rots, and other diseases on hardwood and coniferous trees as well as nursery seedlings, e.g., *C. destructans* on Douglas-fir (*Pseudotsuga menziesii*) seedlings. Other hosts of *Cylindrocarpon* in nurseries are Engelmann spruce (*Picea engelmannii*), white spruce (*Picea glauca*), lodgepole pine (*Pinus contorta*), western white pine (*Pinus monticola*), western hemlock (*Tsuga heterophylla*), and Sitka spruce (*Picea sitchensis*) (James 2012).

Although *Cylindrocarpon* is one of the most common fungi found on plant roots, the degree of its pathogenicity on conifer seedlings is unclear. Diseased roots appear dark brown and stunted and may be rotted. In nurseries during the growing season shoot stunting may occur later on as well (See photos in Appendix A). Root regeneration capacity may decrease when infected by *Cylindrocarpon.* Survival of nursery-planted seedlings may be seriously affected by *Cylindrocarpon* at levels below minimal detectable infection. To date, conventional isolation and microscopic identification of *Cylindrocarpon* isolates from root tissue is difficult and time consuming. This is complicated by the overlap of diagnostic species characteristics (macroconidia shape, size, etc.) produced in cultures as described by Booth (1966).

Mechanisms of Pathogenicity

Cylindrocarpon is capable of producing phytotoxins, which are responsible for the pathogenicity of the fungi (Andolfi et al. 2011). The phytotoxin nectrolide has been isolated from *C. radicicola* and was identified along with brefeldin A. During a 14 day trial involving growing species of these fungi on liquid culture, about 60% were able to produce 31 to 82 µg per ml of nectrolide. At a concentration of 6 µg per ml the toxin inhibited the growth of germinated blackbutt (*Eucalyptus pilularis*) seedlings. At lower concentrations (2–4 µg per ml) the compound caused severe stunting and blackening of the roots of seedling plants (Evans et al. 1967).

A further investigation of the pathogenicity of *C. didymium* showed that this pathogen produces brefeldin A in vitro causing stunted appearance of tap and lateral roots (Barbetti

2005). If colonization on the rhizoplane is necessary for further root infection, it might be possible that production of this compound would indirectly contribute to the pathogenicity of the fungus. Brefeldin A inhibits mitosis in the root tips of some plants, e.g., in *Vicia faba* L. (Betina and Murin, 1964), and *Allium cepa* L. (Frank 1974). This inhibition results in the stunting of the roots (Sweetingham 1983).

Other studies confirm the capability of *Cylindrocarpon* spp. to cause post-emergence damping-off of seedlings. The main reason for the damping-off of the seedlings was attributed to production of pectic enzymes by these species (Sweetingham 1983).

Cylindrocarpon Diseases on Some Economically Important Hosts

Black foot disease of grapevine

Grapevines (*Vitis* spp.) are important hosts of *Cylindrocarpon* species. Black foot disease has caused significant losses in wine and grape- producing regions especially nurseries (Cabral et al. 2012; Halleen et al. 2006). The infected plant often shows necrotic root lesions as well as loss of root hairs and root biomass. Plants show low vigor with small trunks and short internodes. Total foliage and leaf size decrease and leaves have interveinal necrosis, which mostly leads to plant death (Halleen et al. 2006a; Alaniz et al. 2007, 2009; Abreo et al. 2010). The most common species of *Cylindrocarpon* associated with black foot disease in grapevines are *C. destructans, C. liriodendri, C. macrodidymum* and *C. pauciseptatum.*

Apple replant disease (ARD)

ARD is a soil borne disease impacting apple trees on soils with a history of previous apple cultivation (Tewoldemedhin et al. 2011; Mazzola 1998). Although other pathogens

(*Fusarium*, *Pythium* and *Phytophthora*) can be associated with ARD, *Cylindrocarpon* is also a major component. Some of the common species causing ARD are *C. destructans, C. lucidum,* and *C. heteronema.* *Cylindrocarpon* is both associated with symptomatic apple trees and apple seedlings causing root rot and biomass reduction in plant yield. *Cylindrocarpon lucidum* in particular causes stunting and black lesions on feeder roots (Jaffee et al. 1982; Braun 1991, 1995). *Cylindrocarpon heteronema* is associated with mature apple roots but not the seedlings (Braun 1991) and (its sexual stage) causes *Nectria* canker (Swinburne 1975).

Ginseng root rot disease

Ginseng (*Panax ginseng* and *P. quinquefolius*) are perennial plants in the Family Araliaceae. Ginseng is a valuable medicinal herb in Asian countries and has become an important economic crop in the USA and Canada. Jang et al. (2010) conclude that *C. destructans* is one of the most important pathological species affecting ginseng production. Thick straw mulch and shade cloth provide the best environmental conditions for ginseng cultivation. However, this also provides good conditions for many different pathogens which can affect the plant. The production of ginseng requires 4 to 6 years of cultivation in Korea and about 3 to 4 years of cultivation in North America. Yield loss to disease is proportional to the cultivation period. Seedlings, foliage and roots become infected with different fungal pathogens. Infection of the roots of this crop with *Cylindrocarpon* has a major effect on the production of good quality roots (Rahman and Punja 2005).

Root rot of conifer seedlings

Cylindrocarpon is often isolated from conifer seedling roots (Booth 1966), both in container and bare root nurseries. Like *Fusarium*, *Cylindrocarpon* prefers warm and wet conditions to grow and spread under nursery situations. *Cylindrocarpon* spp. (especially *C. destructans*) are rhizosphere inhabitants (Buscot et al. 1992) and colonize root cortical cells.

Five-needle pine (*Pinus* spp.) seedlings grown in containers (James 1991, 2000, 2004) are the most common hosts of *Cylindrocarpon* spp. Dennis and Trotter (1998) reported low to severe levels of disease of container grown Douglas-fir seedlings in British Columbia. Seedlings with root infections may have severe damage in the root system, but may not show above ground disease symptoms (James 1991, 2000, 2004). This damage might be noticed when pulling seedlings out of containers for planting.

A study by Axelrood et al. (1998) showed that the occurrence of *Cylindrocarpon* on planted seedling was higher than that on those grown naturally. There was a significant linear trend in the *Cylindrocarpon* root colonization in planted seedlings; however, this was not observed for naturally regenerated seedlings. The highest region of colonization of *Cylindrocarpon* species was in roots closest to the root plug remnant. It decreased at distances greater than 10 cm from that region.

The incidence of *Cylindrocarpon* was studied in four different container nurseries in British Columbia growing Douglas-fir and spruce (*Picea* spp.) seedlings KOpe et al. 1996). Percent root colonization of *Cylindrocarpon* root rot on seedling roots was consistently high both in

Douglas fir and spruce. *Cylindrocarpon* was equally prevalent on both Douglas-fir and spruce at the northern interior and south coastal nurseries but there was a significant difference in the occurrence of *Cylindrocarpon* at the southern interior nurseries and the nurseries on Vancouver Island where it was higher in the spruce seedlings compared to Douglas-fir seedlings (Kope et al. 1996).

A number of different of different fungi, including *C. destructans*, were found during an investigation of a root rot dieback of bare-root and container conifer seedling in forest nurseries in Finland where symptoms were found on both Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) (Lilja et al. 1992). Seedling infection symptoms included discoloration of the needles, a partial or total death of the root system and stunted growth. *Cylindrocarpon* spp. was one of the commonly isolated fungi from diseased roots as well as from roots of visually healthy seedlings. The study revealed two new *Cylindrocarpon* species that had not been previously reported from Finland (*C. cylindroides and C. pineum).* None of the collected isolates of *Cylindrocarpon* in this study showed pathogenic properties during *in vitro* tests, although in Swedish laboratory trials unfavorable growing conditions proved to predispose Scots pine seedlings to invasion by the normally saprophytic *C. destructans* (Lilja et al. 1992).

Concern about nursery stock infected with *Cylindrocarpon* spp. and their poor performance after outplanting led to a study by Dumroese et al. (2000). Investigation of *Cylindrocarpon* on western white pine showed that this pathogen persisted longer on out-planted stock than *Fusarium oxysporum* or *F. proliferatum* did on Douglas-fir seedling on a similar site. The results of this experiment show that western white pine seedlings can be infected with *Cylindrocarpon*, but still meet morphological criteria for out planting and yet perform satisfactorily in the field.

Root disease attributed to *Cylindrocarpon* spp. can lead to serious losses both in bareroot and container forest and nurseries in Pacific Northwest. Seedlings with elevated *Cylindrocarpon* levels, but which meet nursery-culling criteria for outplanting, typically perform satisfactorily compared to non-infected seedlings (Weiland et al. 2010). Weiland et al. (2010) suggested that *Cylindrocarpon spp*. had an important influence on the production of bare-root Douglas-fir seedlings Pacific Northwest nurseries. Significant levels of *Cylindrocarpon* spp. were routinely isolated from dead and dying seedlings, buried roots, and asymptomatic seedlings. However, attempts to confirm direct pathogenicity using Koch's postulates have largely failed.

Observations suggest that *Cylindrocarpon* is possibly more adapted to survive soil fumigation because it readily colonizes buried root debris. This species also produces numerous resistant chlamydospores (Booth 1966). Weiland et al. (2010) also implicated transplant seedling contamination with *Cylindrocarpon* as one pathway for movement of this fungus within and between nurseries. Prior to this study no attempt has been made to understand the composition of the *Cylindrocarpon* spp. colonizing Douglas-fir seedlings in bare-root facilities.

Environmental Influences on *Cylindrocarpon* species

Survival of the fungus in soil is dependent on the growth and spread of mycelia and production of conidiospores and chlamydospores. The ability of *Cylindrocarpon* to grow in low concentrations of oxygen makes it capable of colonizing roots to a greater extent than other fungi. Some physiological features of this fungus include rapid spore germination and mycelial growth, great competitive ability, and the ability to use both organic and inorganic nitrogen make it a pioneer in colonizing young root tips. *Cylindrocarpon* is common in alkaline soils (Booth 1966; Kowalski 1982; Kubikova 1965). However, its prevalence in Pacific Northwest soils, which are acidic, suggests it has broader colonization characteristics.

The growth of mycelia, sporulation and chlamydospore production of three different *Cylindrocarpon* spp., including *C. liriodendri* and *C. pauciseptatum*, were investigated at different temperatures, pH and water potentials by Agusti-Brisach and Armengol (2012). These pathogens were studied in association with black foot disease of grapevines. *Cylindrocarpon* isolates were able to grow over a range of temperatures from 5º to 30º C, with an optimum temperature between 20º to 25º C; there was no growth at 35º C. Active mycelia growth was observed over a broad range of soil pH, from 4 to 8. Mycelial growth was greater on amended media (amended with KCl or NaCl) at -0.5, -1.0 or/and -2.0 MPa compared with growth on non-amended PDA (-0.3 MPa), and was reduced at water potential values lower than -2.0 MPa.

Sporulation occurred for most of the species at different temperature, pH and water potentials. *Cylindrocarpon liriodendri* had the highest sporulation capacity compared to other species.

Chlamydospore production was little affected by temperature, pH and water potential. No chlamydospores were produced at 5º and 10º C or -4.0 and/or -5.0 MPa (Agusti-Brisach and Armengol 2012).

Another study on the effect of temperature on the virulence of root fungal pathogens of strawberries in Western Australia by Fang et al. (2011) showed that *C. destructans* infection, particularly on the roots, led to severe levels of disease both on crowns and roots. It also retarded the growth and development of inoculated plants at 22º C. However, in general *C. destructans* was less destructive compared to *Fusarium oxysporum. Fusarium oxysporum* was more virulent at higher temperature, especially at 27º C, but there were no data on the virulence of *C. destructans* at higher temperatures in this study.

Management of *Cylindrocarpon*: Fungicides and Biocontrol Agents

Soil-borne diseases are difficult to manage. Integrated pest management approaches have been successful to some degree in reducing soil disease populations including: fumigation, cover crop manipulation, bare-fallow, tillage, drainage, and increased seedling grading. Soil fumigation with methyl bromide is the most effective treatment, but Weiland et al. (2010) showed that *Cylindrocarpon* inoculum can survive in residual seedling roots in soils treated with operational 350 lb/acre of 67:33 methyl bromide/chloropicrin (MBC) and other fumigants. Methyl bromide, however, is scheduled to be phased out because of its negative environmental effects. The 2009 EPA re-registration fumigant decision mandates smaller fumigant buffer zones around bare-root soil treatments resulting in reduction in fumigant rates.

Compounding the fumigation issue, assays for *Cylindrocarpon* have not been traditionally included as part of pre-and post-fumigation soil pathogen testing. . Kernaghan et al. (2007) showed that there is not a strong relationship between soil chemistry factors and the concentration of pathogen DNA using the qPCR method. Lack of a consistent detection method for quantifying *Cylindrocarpo*n levels compounds the issues of effective control management.

Alternatives to fumigation include soil fungicide drenches and biocontrol agents (Agrios 2005). However, few fungicides are registered for use as soil drenches and to date they show little efficacy against *Cylindrocarpon*. Rego et al. (2006) investigated the effect of some common fungicides on *Cylindrocarpon* spp. of grapevine plants and found that Benomyl, Tebuconazole and the mixtures of Carbendazim with Flusilazole and Cyprodinil with Fludioxonil reduced the incidence of black foot disease *in vitro*. In this study the roots were treated with the fungicides before being planted in the inoculated pots. Rego et al. (2006) also showed that Azoxystrobin was effective in reducing conidia germination, but was ineffective in inhibiting mycelia growth. In a later study Rego et al. (2009) also found out that Fludioxonil and the mixtures of Cyprodinil + Fludioxonil and Pyraclostrobin + Metiram decreased the incidence and severity of these species of pathogens on grapevine plants which were grown in a commercial field with grapevine cultivation history.

Unestam et al. (1989) studied the effect of several different fungicides on the growth rate of mycelia in vitro and showed that most of the fungicides used in their experiment, including Iprodione and Mancozeb, had very limited effect on the radial growth of the mycelia of *Cylindrocarpon*. However, these fungicides suppressed the growth of *Trichoderma*, an antagonist of the *Cylindrocarpon*, resulting in increase in the infection potential of the pathogen. Another study on the effect of fungicides on the root disease severity index showed that most fungicides including Cleary 3336F (Thriophanate-methyl), Iprodione, and Azoxystrobin (the active ingredient in Heritage) significantly decreased the root disease severity index values in *C. liriodendri* and *C. macrodidymum* compared with control treatment (Alaniz et al. 2011).

Commercial biocontrol agents (such as Cease – *Bacillus subtilis*, Actinovate - *Streptomyces lydicus*, Soil Guard – *Gliocladium virens*, and Root Shield – *Trichoderma harzianum*) are registered for controlling seedling root diseases, but they have been only sporadically tested as part of an integrated pest management approach in bare-root and container-grown Pacific Northwest conifers. Initial *in vitro* trials showed some promise for antagonism between these agents and *Cylindrocarpon* (Edmonds et al. 2013). However, more recent analysis has shown that these agents were not successful in controlling *Cylindrocarpon* spp. root infection in Douglas-fir nurseries (Willis Littke, Weyerhaeuser Company, Federal Way, Washington, personal communication).

Various other methods have been tested to control the propagation of *Cylindrocarpon* spp. in crop nurseries, especially in vineyards. Hot-water treatment (Halleen et al., 2007; Bleach et al. 2009; Gramaje et al. 2010), some biological control methods, like chitosan (Nascimento et al. 2007), arbuscular mycorrhizal fungi (Traquair 1994; Petit and Gubler 2005), and ectomycorrhizal fungi (Buscot 1992) as well as fungicides (Halleen et al. 2006, 2007; Rego et al. 2006, 2009) have been used.

Laflamme et al. (1999) studied the effect of chitosan on the morphology, ultrastructure and growth of some *Cylindrocarpon* species including *C. destructans* in vitro. Chitosan caused a reduction in the radial growth of all the fungi involved in the experiment. This substance also caused some alteration in fungal morphological features. Increased vacuolation, retraction and alteration of the plasma membrane, cell wall thickening, hyphal distortion, and cytoplasm aggregation were some of the changes caused by application of chitosan and revealed by light microscope observations.

Hot water treatment can be used for controlling both conidial and mycelia growth of *Cylindrocarpon* spp. (Gramaje et al. 2009). Conidial germination is stopped after 45 minutes at 45º C, while 45 minutes above 48º C are necessary to inhibit mycelial growth. This supports the use of current hot water protocol treatments for 30 minutes at 50º C to control *Cylindrocarpon* spp. (Gramaje et al. 2009). Heat treatment at of 30 minutes at 50º C was found to be effective at preventing germination of macroconidia of *Cylindrocarpon* using root isolates from Douglas-fir (Willis Littke, Weyerhaeuser Company, Federal Way, Washington, personal communication). Some residual spore germination occurred at 30 minutes at 40º C.

MATERIALS AND METHODS

Nursery Locations

Three industrial forest seedling Pacific Northwest bare-root nurseries were selected to obtain *Cylindrocarpon* samples: The Weyerhaeuser Company Mima Nursery located in Thurston County, south of Olympia Washington, the Weyerhaeuser Aurora Nursery in Marion County, Aurora, Oregon, and the IFA Nursery in Clackamas County, near Canby, Oregon. Each nursery annually grows ~10-20 million transplant bare-root Douglas-fir seedlings each year. Douglas-fir seedling culture is similar at each facility, with soil fumigation (MBC) done on 2-4 year cycle. These facilities have been in operation for roughly 30 years.

Mima Nursery

The Mima Nursery is situated on a sandy loamy soil with a 5%slope (NRCS Web Soil Survey 2009). Douglas-fir seedlings (1+1 transplants) used for *Cylindrocarpon* isolation were grown in nursery block 6 during 2010 following a spring fumigation trial of MBC 80:20 (275 lb/ac HDPE (high density polyethylene tarp) or non-fumigated soil. Seedlings were lifted in December 2010.

Aurora Nursery

The Aurora Nursery is on a Canderly sandy loam soil with a 3 to 8% slope (NRCS Web Soil Survey 2009). Douglas-fir 1+1 seedlings previously grown (2009) in Block 17 in non-fumigated soil were used following a greenhouse root growth experiment. Other roots were recovered from previously cropped soils with Douglas-fir during pre-fumigation soil sampling.

IFA Nursery

The IFA Nursery is operated by IFA Nursery, Inc. and is located approximately 8 km northeast of the Aurora nursery on a Canderly sandy loam soil with a 0 to 3% slope (NRCS Web Soil Survey 2009). Root samples were taken from soils previously cropped with Douglas-fir in block 2 during 2009.

Fungal Isolates

Isolates of *Cylindrocarpon* were obtained from the three nurseries. Table 1shows

Table 1. *Cylindrocarpon* culture prefixes and descriptions of isolate origins from Aurora, Canby and Mima nurseries.

Culture Prefix	Origin
AT	Aurora 1+1 DF lifted from the 2009 crop in ARS fumigation trial Block 17, used in a greenhouse survival study. Samples taken post experiment; T = tree number
A-P	Aurora 2010 prefumigation trial Block 14; P = plot number
A-P (*)	*(1) and (2) refers to various root samples plated on Komada's media (cover crop) or (conifer)
AP#1	# refers to one or more isolates taken from a single tree
C-P	Canby nursery samples with the same denoted culture and isolate descriptions above
M-TRT (e.g.,18-Con)	Seedling root isolates taken 1 year after fumigation or control from plots in Block 6 Mima nursery

the culture prefix and origin in each nursery. *Cylindrocarpon* was isolated from Douglas-fir seedling roots or detached buried roots. Roots were first washed and then cut into 1 cm lengths and surface sterilized using a 10% solution of Clorox (5% sodium hypochlorite) for ten minutes. Root tips were washed in deionized water and plated onto Komada's media (Komada 1975). Isolates were then transferred to PDA (Potato Dextrose Agar by Sigma Aldrich) media. Cultures were grown on a new PDA plate at least for two times in (to ensure there were no contaminants) and then single spore isolates were collected for species identification.

***Cylindrocarpon* Species Identification**

DNA extraction and PCR

DNA of fungal cultures was extracted using Qiagen DNeasy Plant Mini Kits. Mycelia were removed from the surface of the PDA plates and placed in Lysing Matrix A tubes provided by MP Bio and DNA was extracted following the Qiagen protocol. The ITS (internal transcribed spacer) region of the rDNA was amplified using universal primers ITS1F, ITS1, ITS5 as forward primers and ITS 2 and ITS4 as reverse primers.

Each PCR assay was performed as follows in a final volume of 25μl:

13.6 μl water, 1.0 μl template DNA, 1.0 μl forward primer (at 4 pmol/μl), 1.0 μl reverse primer (at 4 pmol/μl), 8.4 μl master mix (5 PRIME Hot Master Mix 2.5X). Amplification conditions were as follows: initial denaturation at 94º C for 4 minutes, followed by 35 cycles each consisting of 45 s at 94º C, 45 sec at 54º C (annealing temperature), 1 min at 72º C and a final extension o at 72º C for 10 min.

Obtained PCR products were separated by electrophoresis in 0.8% agarose gel in 1X TBE buffer (Sambrook et al. 1989). Gels were stained with SYBR Green or SYBR Gold and photographed under UV light. PCR amplifications were sent to the sequencing facilities, Department of Biochemistry, University of Washington to be sequenced.

Sequence analysis

Sequences from 21 isolates of *C. destructans* and 9 isolates of *C. liriodendri* from the three nurseries were edited in *Sequencher version 4.9* (Gene codes corporation, Ann Arbor, Michigan, USA) and aligned manually using *Mesquite version 2.75*. Additional sequences were added to the list from GenBank. ACI (Akaike Information Criterion, Akaike 1974) analysis of the aligned sequences with the *J model test 2.1.1* (Posada and Crandall 1998) resulted in the model *SYM+I*. The model *SYM+I* was used for Bayesian analysis in *Mrbayes version 3.2.1*. The number of generations used to make a phylogeny tree was 10000000. *Cylindrocarpon pauciseptatum* sequence from GenBank was used as of the out-group for making the phylogeny tree.

Effect of Temperature on the Growth Rate of Mycelia

Mycelia of 30 different isolates of *Cylindrocarpon* (21 isolates of *C. destructans* and 9 isolates of *C. liriodendri*) (Appendix C) were grown on PDA plates to determine the effects of temperature on growth. Using a loop, a 3 mm plug of active mycelia was placed on PDA agar petri dishes. This step was replicated 3 times for each sample, in order to have multiple observations. Plates were kept for 7 days in incubators, set to one of the following temperatures (5º, 8º, 18º, 22º, 24º, 25º, 28º, and 30º C). The growth of mycelia (distance of colony growing tip to center) was measured twice, once 3 days after inoculation and again 7 days after inoculation. Radial growth rate between the 3rd and 7th day (expressed as mm/day) was used for statistical analysis.

Effect of Fungicides on Mycelial Growth

Four fungicides registered for drench application in conifer nurseries were tested in this study including: Cleary 3336F, Dithane 75DF, Heritage and Iprodione E-Pro. Cleary 3336F is manufactured by Cleary Chemical and is a systemic fungicide used for the prevention and control of turf diseases and the diseases of annual and perennial flowers, bedding plants, foliage plants, ground covers, and deciduous and evergreen trees and shrubs. The active ingredient is:

Thiophanate-methyl (dimethyl 4,4'-o-phenylenebis[3-thioallophanate).

(http://www.clearychemical.com/support/label/4172SL.pdf)

Cleary 3336F is listed as a Group 1 fungicide (Fishel 2006) inhibiting mitosis and cellular division, but having a high risk of developing resistance.

Dithane DF is manufactured by Dow Chemical and is a contact fungicide with registrations on more than 30 vegetable, fruit, field, vine and specialty crops. The active ingredient is Mancozeb, a coordination product of zinc ion and manganese ethylene bisdithiocarbamate (http://www.dowagro.com/usag/prod/060.htm). Mancozeb is listed as a (Fishel 2006) Group M a fungicide with multisite inhibitions, but risk of resistance is not rated.

Heritage 50W is manufactured by Zeneca Inc. and is a broad-spectrum systemic fungicide applied as a foliar spray which controls fungal plant pathogens. The active ingredient Azoxystrobin (a methoxyacrylate compound) is in a new class of fungicides called Strobilurins (http://pmep.cce.cornell.edu/profiles/extoxnet/24d-captan/azoxystrobin-ext.html). Azoxystrobin is listed (Fishel 2006) as Group 11as primarily a respirator inhibitor with high risk of resistance development.

Iprodione E-Pro is manufactured by Nufarm Inc. It is a broad-spectrum fungicide used largely for turf diseases. The active ingredient is Iprodione (3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide) (http://store.parsonspestcontrol.com/iprodione-e-pro.aspx_). Iprodione belongs in Group 2 (Fishel 2006) causing inhibition of lipid and membrane formation, with moderate to high risk of resistance development.

Twelve isolates of *Cylindrocarpon*, identified through PCR analysis were selected for this experiment. Four isolates including three *C. destructans* and one *C. liriodendri* isolate were chosen from the three nurseries (Aurora, Mima and Canby).

Four concentrations of each fungicide were used to measure the effect on *Cylindrocarpon* isolate growth. The fungicide concentrations used were based on: 10, 25, 50 and 75 percent of active ingredient (AI) label rates (i.e., 37, 94, 187, and 281 ppm).

Test fungicide plates contained potato glucose agar (PGA) as the base media with different amounts of each fungicide added to make the specific concentrations. The fungicides shown in Table 2 were added to 1000 mL of sterile PGA agar post-autoclaving in a sterile transfer hood. Each agar + fungicide solution was swirled and 30 ml were poured into petri dishes and allowed to cool. Table 3 shows the fungicide product concentration added to PGA media and Table 4 the final fungicide concentration (AI). The amount of fungicide was varied to allow for differences in the active ingredient concentration so that comparison among fungicides would more clearly reflect concentration rather than label rates. Control plates were made for comparison containing only PGA media.

Table 2. Fungicide trade names, active ingredient and percent active agent ingredient (% A.I).

Fungicide	Agent	% AI
Iprodione E-Pro	Iprodione	23.3
Heritage	Azoxystrobin	50
Cleary's 3336	Thiophanate-methyl	41.3
Dithane 75DF	Ethylene bisdithiocarbamate	75

Table 3. Test fungicide concentrations in mg of fungicide product/l (ppm).

		mg of product/liter		
	High(75%)	Medium (50%)	MidLow (25%)	Low (10%)
Iprodione E-Pro	1206	804	402	161
Heritage	562	375	187	75
Cleary' s 3336	682	455	227	91
Dithane 75DF	375	250	125	50

Table 4. Final concentration (AI) of each fungicide test media in mg/l (ppm)

	mg of fungicide (AI)/liter			
	High(75%)	Medium (50%)	MidLow (25%)	Low (10%)
Iprodione E-Pro	281	187	94	37
Heritage	281	187	94	37
Cleary' s 3336	282	188	94	38
Dithane 75DF	281	188	94	38

Statistical Analysis

Generalized Estimating Equations (GEE) with an exchangeable working covariance matrix were used for analysis of the data from the temperature and fungicide experiments. The GEE method allows for straightforward analysis of correlated outcomes that can be discrete or continuous. It accounts for the correlation caused by repeated sampling of each isolate (Ratclie and Shults, 2008). Indicator variables were included in the model to calculate a mean growth rate for each temperature/species or species/fungicide/concentration combination. In the fungicide experiment, these values were transformed to percentage reduction in growth rate compared to the control and asymptotic variances were calculated using the delta method. A p-value of <0.05 was used to determine significance in these experiments.

RESULTS

Species of *Cylindrocarpon* and Diversity

Amplification products of approximately 750 bases (ITS) obtained from 30 isolates of the fungi from roots of Douglas-fir nursery seedlings and blasting the sequences with FinchTV showed that three species of *Cylindrocarpon* were present in the nurseries (*C. destructans, C. liriodendri* and *C. pauciseptatum. Cylindrocarpon destructans* was the dominant species in all three nurseries and made up 61.4% of the isolates. *Cylindrocarpon liriodendri* was also found in all three nurseries and made up 36.4% of the isolates; *C. pauciseptatum* was found in only one nursery (Aurora, Oregon) and represented 2.2% of the isolates. Appendix B shows all 44 fungal isolates from the Aurora, Canby and Mima nurseries, which were identified to species genetically.

A phylogenetic tree is shown in Figure 1. The phylogeny data show that isolates of *C. destructans* and *C. liriodendri* occurred in all three nurseries and were evenly distributed. Based on this sampling design the nursery location had no effect on the *Cylindrocarpon* species found.

Figure 1 also shows that there were no clusters of any species by hosts (i.e., Douglas-fir or other species). Therefore, the *Cylindrocarpon* isolates identified from Douglas-fir in this study appear to overlap with other hosts.

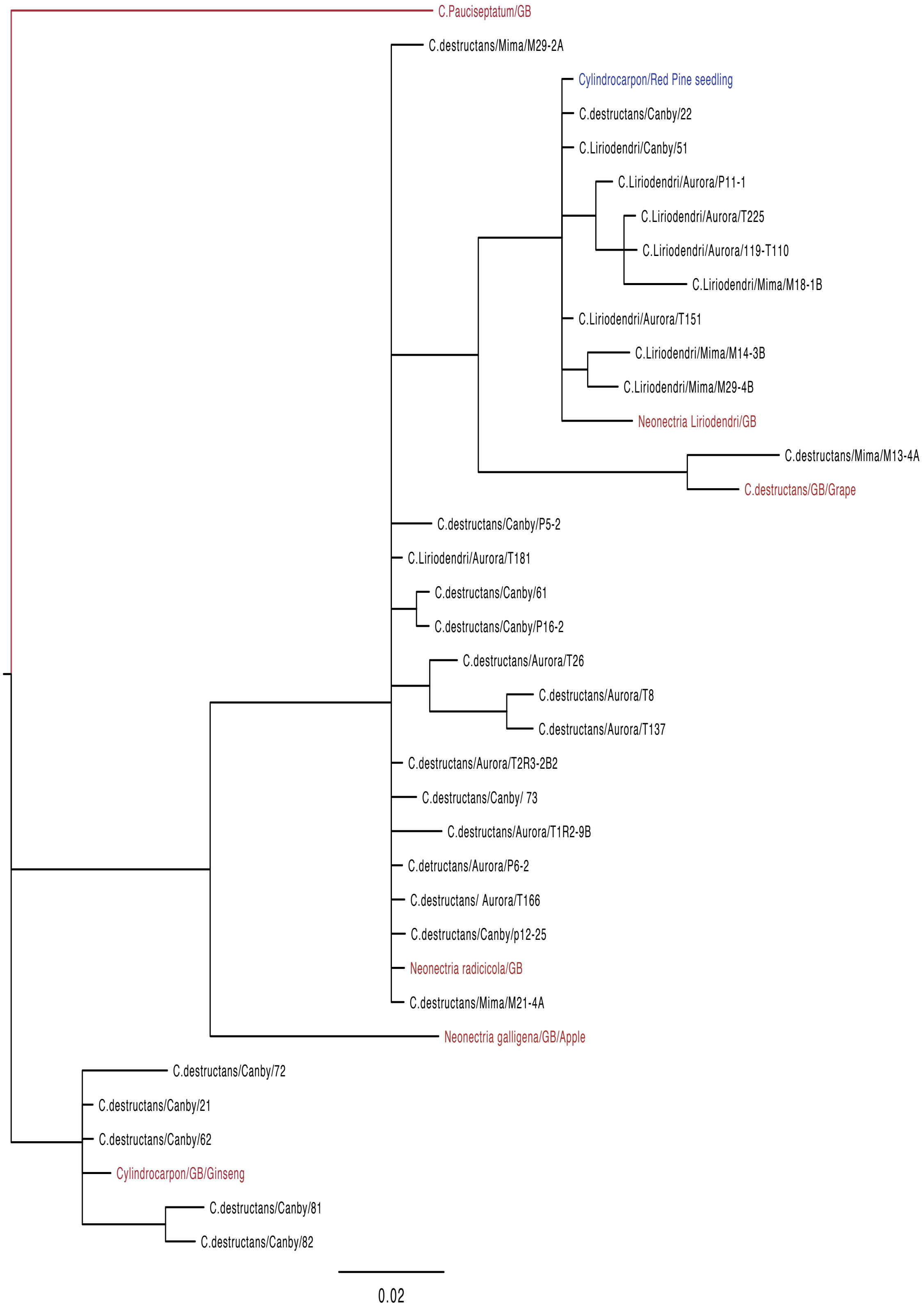

Figure 1. Phylogenetic tree for *Cylindrocarpon* including isolates from the Aurora and Canby nurseries (Oregon) and Mima (Washington) nursery and GenBank (GB - colored entries), C=*Cylindrocarpon*; letters and numbers are isolate names.

Influence of Temperature on *Cylindrocarpon* Growth *in Vitro*

There was a significant difference ($p < 0.001$, Table 5 and Figure 2) between the growth rate in culture on PDA of the major species, *C.destructans* and *C. liriodendri*. *Cylindrocarpon liriodendri* grew significantly faster in culture at warmer temperatures than *C. destructans.* The optimum growth temperature for *C. liriodendri* was 25º C, while *C. destructans* grew fastest between 18º and 22º C. There was little growth of *C. destructans* at 30º C. There was considerable growth of both species at 5º and 8º C. There was no significant effect of nursery location on the growth of the fungal isolates at the different temperatures ($p = 0.61$). The growth data for all the cultures are shown in Appendices C and D.

Influence of Fungicides on Growth of *Cylindrocarpon in Vitro*

All four fungicides (Cleary 3336F, Dithane 75DF, Heritage and Iprodione E-Pro) tested at different concentrations (10, 25, 50 and 75 % of active ingredient, i.e., 37, 94, 187, and 281 ppm) reduced the growth of both species of *Cylindrocarpon* (Figure 3). Raw data on the fungicides in *Cylindrocarpon* isolate growth are shown in Appendices E and F. *Cylindrocarpon destructans* generally had greater fungicide induced growth reduction than *C. liriodendri* (Figures 3 and 4). Cleary and Dithane reduced growth more than Heritage and Iprodione. Dithane at the 75% active ingredient concentration (281 ppm) had the greatest effect in reducing the growth of both *C. destructans* and *C. liriodendri* (Figure 3). Dithane at 75 % concentration was significantly more effective ($p< 0.005$, Table 5) than Cleary 3336 in reducing the growth of *C. destructans* (Figure 3), but there was no significant difference between Cleary and Dithane in reducing the growth of *C. liriodendri* (Figure 3).

Table 5. Statistical differences in growth rates *in vitro* of *Cylindrocarpon* in temperature and fungicide experiments. To determine significance in these experiments, A p-value of <0.05 was used. In this table any value less than 0.05 considered a significant difference between the compared objects.

Comparison	Temperature	Difference in Growth (95% CI)	P-value
C.destructand & C.liriodenrdi	18º C	-0.17 (-0.56, 0.22)	0.398
C.destructand & C.liriodenrdi	24º C	-0.84 (-1.26, -0.43)	<0.001
C.destructand & C.liriodenrdi	25º C	-1.07 (-1.6, -0.54)	<0.001
C.destructand & C.liriodenrdi	28º C	-1.3 (-1.71, -0.89)	<0.001
C.destructand & C.liriodenrdi	30º C	-0.73 (-1.08, -0.39)	<0.001

Comparison	Species	Difference in Growth (95% CI)	P-value
18º C & 22º C	*C.destructans*	-0.09 (-0.27, 0.08)	0.301
18º C & 24º C	*C.destructans*	0.26 (-0.01, 0.54)	0.058
18º C & 25º C	*C.destructans*	0.34 (0.01, 0.66)	0.042
22º C & 24º C	*C.destructans*	0.36 (0.18, 0.54)	<0.001
22º C & 25º C	*C.destructans*	0.43 (0.22, 0.64)	<0.001
24º C & 25º C	*C.destructans*	0.07 (-0.09, 0.24)	0.369

Comparison	Species	Difference in Growth (95% CI)	P-value
18º C &22º C	*C.liriodendri*	-0.3 (-0.52, -0.08)	0.009
18º C & 24º C	*C.liriodendri*	-0.41 (-0.77, -0.04)	0.029
18º C & 25º C	*C.liriodendri*	-0.56 (-1.03, -0.09)	0.02
22º C & 24º C	*C.liriodendri*	-0.11 (-0.39, 0.17)	0.444
22º C & 25º C	*C.liriodendri*	0.43 (0.22, 0.64)	<0.001
24º C & 25º C	*C.liriodendri*	-0.15 (-0.43, 0.13)	0.282

Comparison	Fungicide	Difference in Growth Reduction (%)	P-value
C.destructand & C.liriodenrdi	Heritage at 10 %	0.36 (0.24,0.47)	<0.001
C.destructand & C.liriodenrdi	Heritage at 25 %	0.37 (0.22,0.51)	<0.001
C.destructand & C.liriodenrdi	Heritage at 50 %	0.41 (0.2,0.59)	<0.001
C.destructand & C.liriodenrdi	Heritage at 75 %	0.44 (0.2,0.63)	0.001
C.destructand & C.liriodenrdi	Iprodione at 75%	-0.06 (-0.26,0.15)	0.572
C.destructand & C.liriodenrdi	Cleary at 10%	0.04 (-0.24,0.32)	0.76
C.destructand & C.liriodenrdi	Cleary at 75%	0.11 (-0.18,0.38)	0.45
C.destructand & C.liriodenrdi	Dithane at 10%	0.15 (-0.05,0.34)	0.144
C.destructand & C.liriodenrdi	Dithane at 50%	0.2 (0.07,0.32)	0.003
C.destructand & C.liriodenrdi	Dithane at 75%	0.17 (-0.04,0.36)	0.12
Comparison	Species		P-value
Cleary at 10% & Cleary at 75%	*C.destructans*	0 (-0.02,0.02)	0.918
Dithane at 10% & Dithane at 50%	*C.destructans*	-0.32 (-0.39,-0.24)	<0.001
Dithane at 10% & Dithane at 75%	*C.destructans*	-0.34 (-0.42,-0.25)	<0.001
Heritage at 10% & Heritage at 75%	*C.destructans*	-0.11 (-0.13,-0.08)	<0.001
Iprodione at 10% &Iprodione at 75%	*C.destructans*	0.03 (0,0.05)	0.034
Cleary at 75% & Dithane at 25%	*C.destructans*	0.06 (-0.06,0.19)	0.33
Dithane at 50% & Dithane at 75%	*C.destructans*	-0.02 (-0.06,0.01)	0.204
Comparison	Species		P-value
Cleary at 10% & Cleary at 75%	*C.liriodendri*	0.07 (-0.52,0.61)	0.837
Dithane at 10% & Dithane at 50%	*C.liriodendri*	-0.27 (-0.63,0.19)	0.248
Dithane at 10% & Dithane at 75%	*C.liriodendri*	-0.32 (-0.68,0.16)	0.186
Dithane at 50% & Dithane at 75%	*C.liriodendri*	-0.05 (-0.59,0.52)	0.871
Heritage at 10% & Heritage at 75%	*C.liriodendri*	-0.03 (-0.2,0.14)	0.744
Iprodione at 10% & Iprodione at 75%	*C.liriodendri*	-0.01 (-0.47,0.45)	0.956
Cleary at 75% & Dithane at 50%	*C.liriodendri*	-0.01 (-0.55,0.54)	0.978
Dithane at 75% & Cleary at 50%	*C.liriodendri*	0.03 (-0.56,0.59)	0.937

Dithane at 75% concentration (281 ppm) had the same impact on reduction of the mycelial growth of *C.liriodendri* as Cleary at 10% concentration (37 ppm). There was little effect of concentration for Cleary, Heritage and Iprodione. However, there was a significant effect for Dithane (Figure 4, Table 5). There was significant effect in growth reduction between the species at different concentrations of Cleary, Dithane and Iprodione, except for Dithane at 50 percent concentration (187 ppm) (Table 5). Growth was significantly more reduced in *C. destructans* compared *to C. liriodendri* at all concentrations (Figure 4, Table 5).

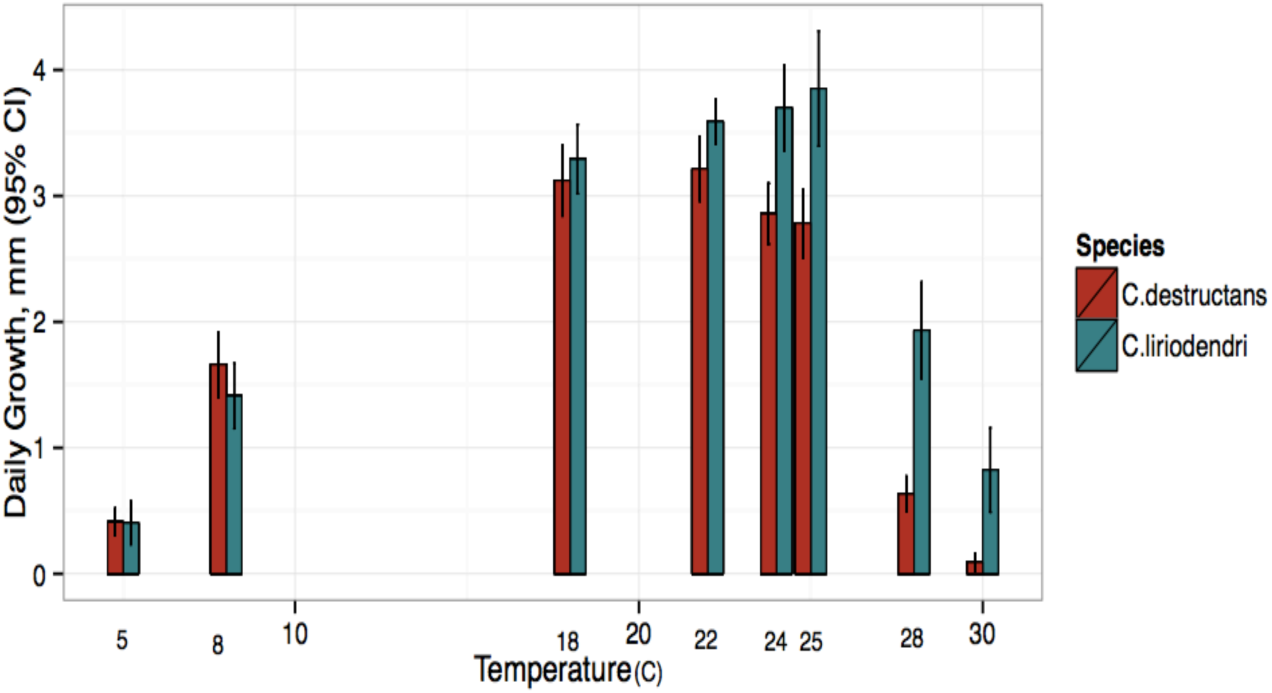

Figure 2. Average radial growth (mm/day) ± 95% confidence interval (CI) of 30 isolates of *Cylindrocarpon destructans* and *Cylindrocarpon liriodendri* on PDA media at 5, 8, 18, 22, 24, 25, 28, and 30º C. See Table 5 for statistical differences and p values.

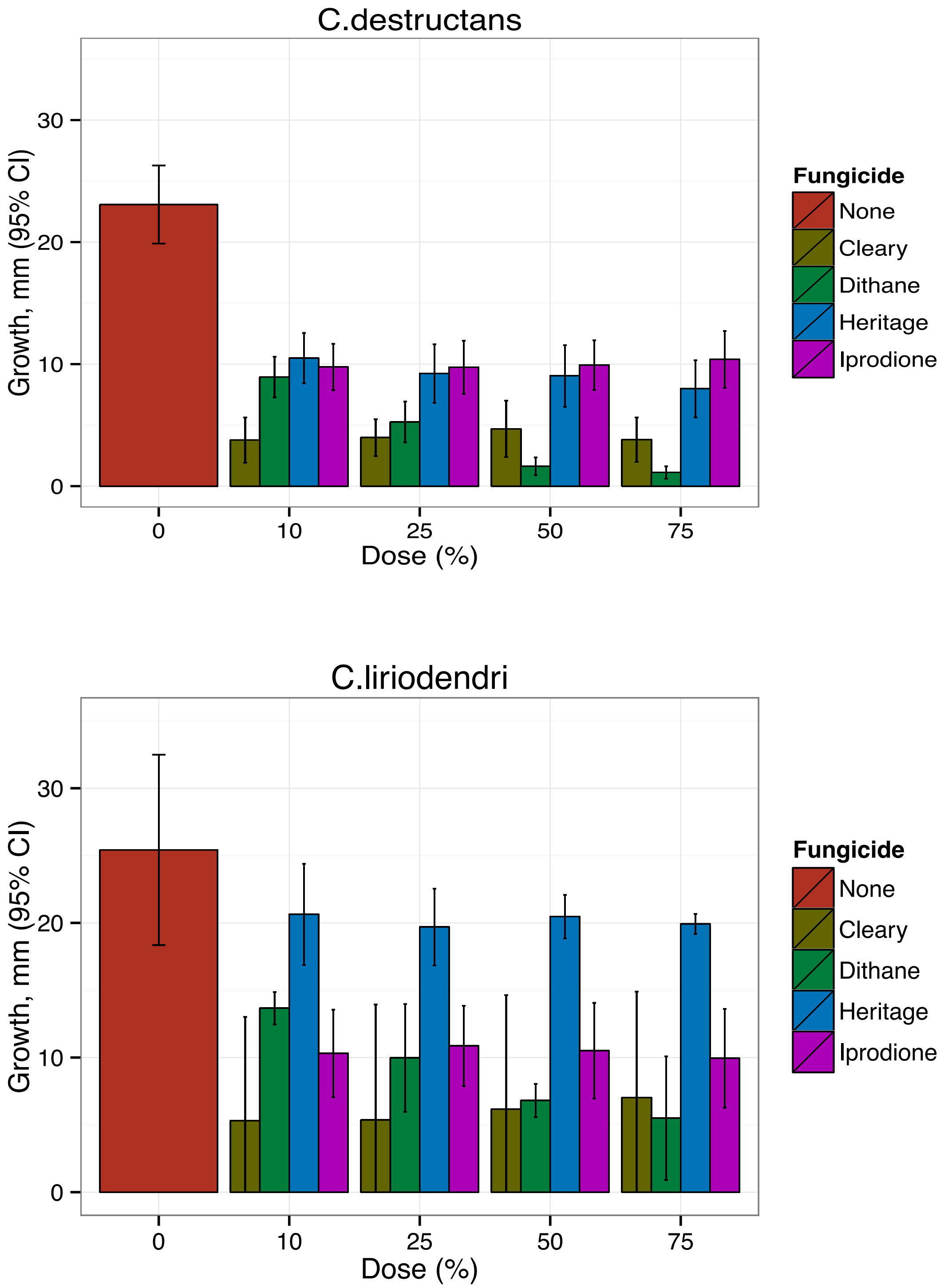

Figure 3. Radial growth (mm/day) ± 95% confidence interval (CI) of *Cylindrocarpon destructans* (upper) and *Cylindrocarpon liriodendri* (lower) on PGA media at 25 C at different doses (0 percent –control, 10, 25, 50 and 75%of label dosage of active ingredient (i.e., 37, 94, 187, and 281 ppm) of four fungicides (Cleary, Dithane, Heritage and Iprodione). See Table 5 for statistical differences and p values.

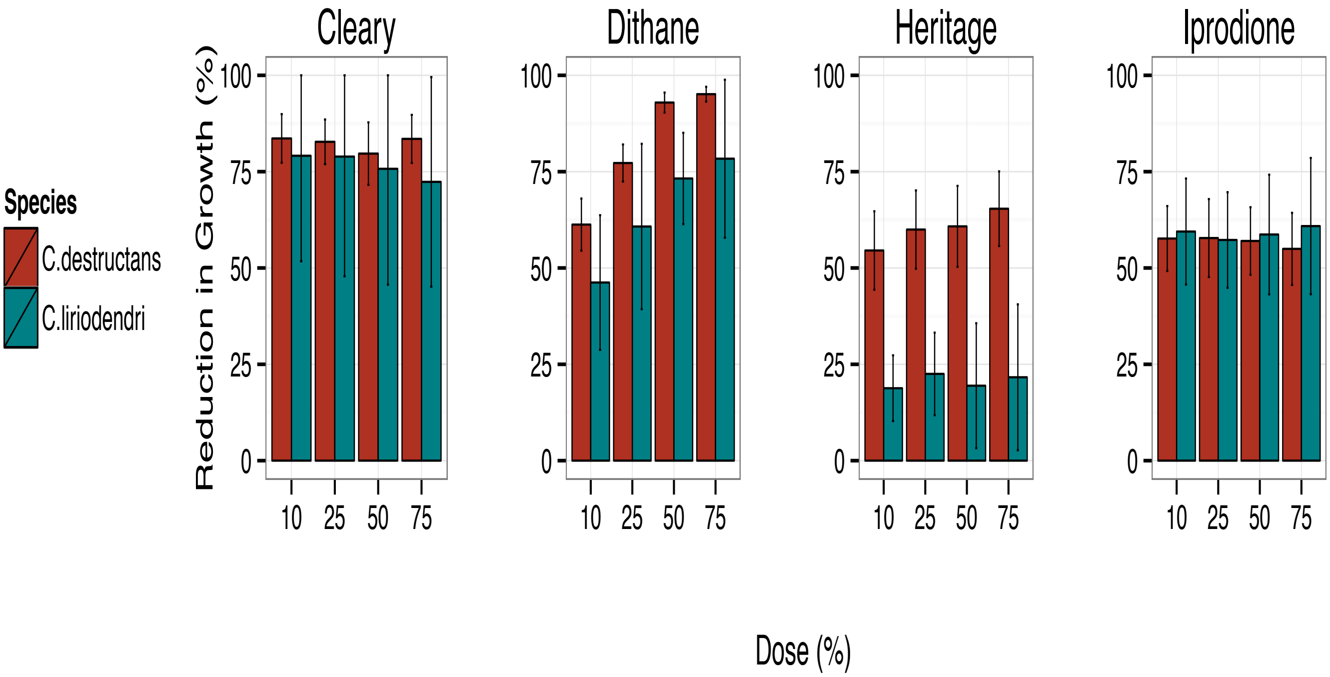

Figure 4: Percent reduction in growth of mycelia ± 95% confidence interval (CI) of *Cylindrocarpon destructans* and *Cylindrocarpon liriodendri* compared to the control (no fungicide) for isolates grown on PGA media with Cleary, Dithane, Heritage, and Iprodione at different concentrations of fungicide active ingredient (10, 25, 50 and 75%) (i.e., 37, 94, 187, and 281 ppm). See Table 5 for statistical differences and p values.

DISCUSSION

Species of *Cylindrocarpon* and Diversity

Species of *Cylindrocarpon* are commonly isolated from roots of both herbaceous and woody plants (Brayford 1993) and cause severe losses in crops and conifer nurseries. Most of the studies of the disease caused by genus *Cylindrocarpon* show *C. destructans* to be the major cause of root disease and losses, especially in the nurseries (Buscot et al. 1992; Rahman and Punja 2005; Halleen et al. 2004). *Cylindrocarpon liriodendri, C. macrodidymum* and *C. pauciseptatum* are other common species (Halleen et al. 2004). Looking at morphological differences alone has not been sufficient to categorize the species of this genus. New molecular methods have aided taxonomic studies of *Cylindrocarpon.*

Molecular identification of the 44 isolates of fungi from roots of Douglas-fir seedlings taken from Pacific Northwest conifer nurseries in this study showed that *C.destructans* was the most common species like the other studies mentioned above. This species comprised 61.4% of the study isolates. The other common species among our isolates was *C. liriodendri* (37.4%). Only one isolate of *C. pauciseptatum* was found and that was from the Aurora nursery. The phylogeny tree produced in this study shows *C. destructans* and *C. liriodendri* are closely related within the genus *Cylindrocarpon*.

To determine differences and show relationships between the sequences of isolates of *Cylindrocarpon* from other hosts sequences of the ITS region of *C. destructans* and *C. liriodendri* were acquired from GenBank and added to the phylogeny tree. ITS sequences of of *Neonectria* (the teleomorph of *Cylindrocarpon*) from different hosts were also selected from GenBank and added to the phylogeny tree. The phylogeny tree did not show any clusters of *Neonectria* and the ITS sequences among anamorphs and teleomorphs showed no significant differences.

The phylogeny tree also indicates that the host type (Douglas-fir, red pine, apple and ginseng) did not have any significant effect on the placement of *Cylindrocarpon* species in the tree. This implies that *Cylindrocarpon* detected in the three Pacific Northwest nurseries has a broad host range. Could it mean that these are agricultural pathogens that have found their way into bare-root conifer nurseries? This is an interesting area for future research.

The data also show that the *Cylindrocarpon* spp. from the different nurseries are evenly distributed across nurseries indicating that the location of the nursery had no effect on the distribution of species. That is not surprising because Mima, Aurora and Canby occasionally share transplant Douglas-fir seedlings between facilities. This could be an intermittent source of cross contamination.

Effect of Temperature on Mycelial Growth

Mycelial growth in culture of *C. destructans* and *C. liriodendri* over a range of temperatures of temperatures from 5º to 30º C was determined. Both species grew at low temperature (5º C) as well as high temperature (30º C). However, there was a difference in optimal growth temperatures for the two species. The highest growth rate for *C. destructans* was between 18º and 22º C, but *C. liriodendri* grew fastest at 25º C. *Cylindrocarpon destructans* also had much less growth at 30º C than C. *liriodendri* and appears to be adapted for growth at cooler temperatures than *C. liriodendri*. These *Cylindrocarpon* species are well suited to grow in the temperature regimes found in Pacific Northwest nursery soils (Temperature Graph). Growth at 5º C also confirms suspicions that *Cylindrocarpon* can also grow on roots at conditions of cooler stored Douglas-fir seedlings (3-5º C). The temperature growth profiles suggest that *C. destructans* may be more active during cool periods such as spring transplanting (April) and during the fall and winter months, while *C. liriodendri* might be active during periods of higher soil temperature (summer).

Agusti-Brisach and Armengol (2012) showed that *C. liriodendri* and *C. pauciseptatum* could grow in a temperature range of 5º to 35º C, but there was almost no growth at 35º C. They found that the optimum growth temperature was 22º C for *C. liriodendri* compared to 25º C in this study. Fang et al. (2011) in a study on the effect of temperature on the virulence of root fungal pathogens of strawberries in Western Australia showed that *C. destructans* grew fastest at 22º C compared to 18º to 22º C in this study. There was no significant difference in the growth of *C. destructans* at 18º and 22º C. Fang et al. (2011) only determined growth rates at 22º and 27º C and not 18º C.

Many forest pathogens, especially foliage diseases and rusts, are expected to increase pathogenicity in response to global warming (Sturrock et al. 2011), but we are not sure how it will influence seedling root pathogens. Temperatures have been rising in coastal, Puget Sound, and east slopes of the Cascade Mountains of Washington since the 1950s. An increase in average Pacific Northwest temperature on the order of 0.2°-1.0° F (0.1°-0.6° C) [(or best estimate average of 0.3° C (0.5° F)] per decade throughout the mid-21st century, has been scaled with global climate models. Temperatures are expected to increase in all seasons with the largest increases in summer.

(http://cses.washington.edu/cig/pnwc/cc.shtml#RecLinksInclude).

Other studies show that over the last 110 years, average annual temperatures have risen by 1.5° F (0.8° C) in the Pacific Northwest (Oregon climate change Research institute, http://occri.net/climate-science/the-climate-of-the-pacific-northwest).

Considering the results of this study global warming might increase root rot disease caused by *Cylindrocarpon* since the pathogen grows well at moderate warmer temperatures. Global warming might also lead to a change in the species dominancy in Pacific Northwest nurseries. *Cylindrocarpon liriodendri* responded better to warmer temperatures than *C. destructans.* As a result it is possible that *C.liriodendri* may become dominant over *C.destructans* with time. However, lack of knowledge on relative pathogenicity on Douglas-fir by these two species makes such future predictions difficult to interpret.

Influence of Fungicides on the Growth of Mycelia

The effect of four drench fungicides, Cleary 3336F (active ingredient: Thriophanate-methyl), Dithane (active ingredient: Mancozeb), Iprodione (active ingredient: Iprodione) and Heritage (active ingredient: Azoxystrobin) on the growth of the mycelia of the *C. destructans* and *C. liriodendri* was investigated. Cleary and Dithane reduced growth more than Heritage and Iprodione. Dithane at 75% concentration was the most effective fungicide among the four used in this study in reducing the mycelia growth of *C. destructans*. It also significantly reduced the growth of *C. liriodendri*. Cleary also significantly reduced growth both for *C.*

destructans and *C. liriodendri*.

Unestam et al. (1989) studied the effect of several different fungicides on the growth rate of mycelia in vitro and showed that most of the fungicides they studied, including Iprodione and Mancozeb (the active ingredient in Dithane), had very limited effect on the radial growth of the mycelia of *Cylindrocarpon*. Unestam et al. (1978) also concluded that fungicides were detrimental to potential biocontrol fungi (e.g., *Trichoderma*) and that this effect and fungicide tolerance could allow *Cylindrocarpon* levels to actually increase. In contrast, Alaniz et al. (2010) showed that Thriophanate-methyl, which is the active ingredient of Cleary 3336F, could significantly reduce the growth of the mycelia of *Cylindrocarpon*, which is similar to my finding. However, Alaniz et al. (2010) also found that Iprodione and Azoxystrobin significantly decreased the root disease severity index values in *C. liriodendri* and *C. macrodidymum* compared with the control treatment.

The greater inhibition of *C. destructans* than *C. liriodendri* by the fungicides could have been related to a temperature effect since the study was conducted at 25º C. *Cylindrocarpon liriodendri* grew significantly faster at 25º C than *C. destructans.*

Cylindrocarpon tolerance to four common drench fungicides labeled for Douglas-fir nursery could present a problem in disease management. First, isolate tolerance may reflect an “adaptation” to these active ingredients as these chemicals have a long history of use in Pacific Northwest nurseries. According to the FRAC fungicide mode of action groupings (Fishel 2006), most of the test fungicides are rated as moderate to highly susceptible to resistance development. There was also some tolerance expressed against Mancozeb a broader spectrum fungicide agent. This suggests that fungicide treatment for *Cylindrocarpon* would likely be more effective if composed of multiple fungicides rather than single fungicide applications. Secondly, these same chemicals have been long used for both foliar and root disease control, so repeated exposure to isolates may have been at low concentrations. Development and testing of new fungicide combinations will be needed to achieve effective control. Lastly, *Cylindrocarpon* is likely being transported between nursery facilities on transplant seedlings. This complicates control measures as resistance once developed and easily spread between facilities. All these factors increase the likelihood that fungicide tolerance will continue to develop within this complex of root pathogens.

CONCLUSIONS AND FUTURE STUDIES

This study represents an initial investigation of the *Cylindrocarpon* species complex present in Pacific Northwest bare-root Douglas-fir nurseries. It also applied recent developments in PCR based technology to help unravel a root-pathogen complex that is difficult to quantify using traditional soil dilution and root isolation assays. Characterization of some fundamental growth relationships against temperature and commonly used fungicides provide some insight to future studies. Effective management of *Cylindrocarpon* caused root disease in bare-root Douglas-fir nurseries will require additional efforts and knowledge on species distribution, pathology and ecological relationships with other root inhabiting microbes.

The major conclusions of this study were:

1. Three closely related species of *Cylindrocarpon* were found in the three study nurseries*;* *C.destructans, C.liriodendri* and *C. pauciseptatum*. This is the first report of *C. liriodendri* and *C. pauciseptatum* occurring as a root colonizer of Douglas-fir in the Pacific Northwest. However, a more intense survey is needed to completely resolve the population structure of *Cylindrocarpon* present in Pacific Northwest conifer nurseries. Verification of the presence of both *C. destructans* and *C. liriodendri* using PCR brings into doubt previous conclusions about *C. destructans* in the earlier literature.

2. *Cylindrocarpon destructans* was the dominant species in all three nurseries (61.4% of the isolates) and was present in all three nurseries, as was *C. liriodendri* (27.4% of the isolates). *Cylindrocarpon pauciseptatum* was present in only one nursery (Aurora, Oregon). Based on the phylogenetic tree there was no clustering of species by host (Douglas-fir, pine, ginseng, or

apple). This implies that this complex may be of agricultural origin adapting to new conifer hosts. If so than, data from other plant species may be applicable to future control efforts in bare-root nurseries.

3. The species response to various temperatures *in vitro* shows that *C. destructans* and *C. liriodendri* have optimum growth rates at different temperatures; *C. destructans* had a lower optimum temperature (18° to 22° C) than *C. liriodendri* (25° C). This indicates different adaptation responses to environmental effects, which are probably caused by basic mutations in coding nucleotides. Predictions of how future climate change will affect *Cylindrocarpon* levels in bare-root facilities will require more information on how temperature affects pathogenicity.

4. Dithane and Cleary 3336F reduced fungal growth in both *C. destructans* and *C. liriodendri.* Dithane at 75% concentration of active ingredient (281 ppm) caused the greatest growth reduction (>80%). *Cylindrocarpon destructans* generally had greater growth reduction than *C. liriodendri*. Thus another feature that is distinguishable between these two species in this study is their different growth response to fungicides. Iprodione and Heritage reduced growth to a lesser extent than Dithane and Cleary. This initial fungicide study indentifies levels of "tolerance" within both species of *Cylindrocarpon*. This suggests that future fungicide control efforts should focus on the testing of multiple fungicide tank mixes versus single fungicide applications.

A number of future studies are suggested below.

Molecular studies help us to the determine which very close related species of fungal pathogens are present in nursery beds and allow us to make better decisions with respect to avoiding and controlling virulent pathogens. They can tell us if the *Cylindrocarpon* spp present in Pacific Northwest nurseries are from agriculture. Mutations in genomic DNA can lead to the evolution of new species which can drastically change the pathogenicity of the fungal isolates leading to greater impacts on hosts. PCR methods to distinguish *Cylindrocarpon* isolates commonly found on Douglas-fir roots in the PNW are the first step in the development of an effective future disease management plan.

In order to understand the differences between *C. destructans* and *C. liriodendri* and their different reactions in various conditions, it would be good to investigate the genes responsible for pathogenicity and growth response. Sequencing the responsible genes for growth response of the two species can give a better picture of understanding the mechanisms which cause differences between these two species.

Looking for genomic differences could help make us aware of any future possible mutations that would cause differences in the distribution and adaptation of the pathogen. This could be helpful for avoiding more damage by fungal pathogens and allow development of a better plan for future nursery cultivation. Fungal genomic DNA can adapt a lot faster than plant genomes.

Are *C. destructans* and *C. liriodendri* equally pathogenic against Douglas-fir and other conifers? High levels of root colonization without seedling death suggest that these species may be root necrotrophs, but this was not investigated in this study. Also important is the genetic relatedness of isolates within and between nurseries. The presence of "unknown" pathogenicity with this genus suggests a need for greater sanitation and inspection of nursery stock grown in one facility and shipped to another facility. It may be possible to identify key gene sequences which could help to resolve individuals who possess pathogenic characteristics from those who have more saprophytic characteristics.

The effect of fungicide chemicals on the environment is of concern. Some of the common fungicides used in this study will not have a significant effect on the control of *Cylindrocarpon*. Nursery management plans should consider that the benefits of using some of these fungicides is less than their harm to the environment. Being more careful about dosages is also important. In this study I showed that in some cases there was no difference between high doses of a fungicide compared to a lower dose. For example, Dithane at 75% concentration (281 ppm) had the same impact on the reduction of the mycelial growth of *C.liriodendri* as Cleary at 10% concentration (37 ppm). Nursery managers need to select the appropriate concentration of effective fungicides for controlling the specific pathogens.

It should be mentioned that results in this study cannot simply be extrapolated to nursery use. There are always differences in the results from lab experiments compared to the field experiments because of the existence of so many variables in the field. Future field studies are needed. However, it is clear that *Cylindrocarpon* spp. pose an integrated pest management challenge to seedling production. A much broader fungicide tolerance screening is needed to develop a more effective preventative control plan. Several effective fungicides will need to be identified to allow for a rotation of use to prevent tolerance buildup. This situation may become direr as the transition away from methyl bromide occurs. Currently effective periodic fumigation with methyl bromide/chloropicrin can reduce soil levels of *Cylindrocarpon* to manageable levels. Because *Cylindrocarpon* can colonize dead and dying roots and possesses resistant chlamydospores it does not appear to amenable to disease control management schemes such as fallow.

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Appendix A. Symptoms of Douglas-fir seedlings with *Cylindrocarpon*

Douglas-fir 1+1 seedlings in spring flush in June at Mima Nursery. This seedlot had a high level (~50%) of *Cylindrocarpon* spp. associated with their roots prior to transplant. The first symptoms of *Cylindrocarpo*n "disease" is failure to elongate the flush resulting in a "bottle brush" appearance. 2006 Nursery Trial Weyerhaeuser Nursery, Little Rock, WA.

Contrast of typical 1+1 DF (left) and those infected with high (~70%) *Cylindrocarpon* levels in the fall (Right).   Infected seedlings are profoundly stunted with small stem caliper and poor root development. 2011 USDA ARS study plots Canby Nursery, Canby, OR.

Appendix B: Fungal isolates identified to species genetically from Aurora, Canby and Mima nurseries.

Isolate	Species name (blasting)	Nursery
C73	*C.destructans*	Canby
C82	*C.destructans*	Canby
C-P12-25	*C.destructans*	Canby
C51	*C.liriodendri*	Canby
C72	*C.destructans*	Canby
C-P16-2	*C.destructans*	Canby
C21	*C.destructans*	Canby
C81	*C.destructans*	Canby
C-62	*C.destructans*	Canby
C61	*C.destructans*	Canby
C22	*C.destructans*	Canby
C-P5-2	*C.destructans*	Canby

Isolate	Species name (blasting)	Nursery
M18-CON-1B	*C.liriodendri*	Mima
M29-CON-4B	*C.liriodendri*	Mima
M29-CON-2A	*C.destructans*	Mima
M14-MBC-3B	*C.liriodendri*	Mima
M13-MBC-4A	*C.destructans*	Mima
M21-CON-4A	*C.destructans*	Mima
M18-CON-2A	*C.destructans*	Mima
M2-MBC-1A	*C.destructans*	Mima
M7-MBC-2A	*C.destructans*	Mima
M9-MBC-2B	*C.destructans*	Mima

Isolate	Species name (blasting)	Nursery
A-P11-1	*C.liriodendri*	Aurora
A-T1R2-9B	*C.destructans*	Aurora
A-P6-2	*C.destructans*	Aurora
A-T2R3-2B2	*C.destructans*	Aurora
A-T151	*C.liriodendri*	Aurora
A-T181	*C.liriodendri*	Aurora
A-T8	*C.destructans*	Aurora
A-T166	*C.destructans*	Aurora
A-T225	*C.liriodendri*	Aurora
A-T137	*C.destructans*	Aurora
A-P12-21	*C.destructans*	Aurora
A-T106	*C.destructans*	Aurora
A-T149	*C.destructans*	Aurora
A-T3R4-5B2	*C.destructans*	Aurora
A-P16-2	*C.destructans*	Aurora
A-P9-1	*C.liriodendri*	Aurora
A-T2R1-4A1	*C.liriodendri*	Aurora
A-P6-1	*C.pauciseptatum*	Aurora
A-T3R1-4A2	*C.liriodendri*	Aurora
A-T2R1-4A2	*C.liriodendri*	Aurora
A-T26	*C.destructans*	Aurora
A-2010-119-T110	*C.liriodendri*	Aurora

Appendix C: Radial growth data (mm/day) of mycelia of isolates of *Cylindrocarpon* from Canby, Aurora and Mima nurseries at 5, 8, 18, 22, 24, 25, 28 and 30 C on PDA media in petri dishes. Three replicate dishes were used for each temperature and culture.

Nursery	Isolate name	Species	5	8	18	22	24	25	28	30
Aurora	A-T110A	*C. liriodendri*	0.25	0.94	3.31	2.94	4.00	4.13	2.38	0.50
Aurora	A-T110B		0.05	0.88	3.19	2.75	3.56	3.44	2.25	0.25
Aurora	A-T110C		0.10	1.06	3.00	3.63	3.56	4.81	2.06	0.25
Aurora	A-P11-1A	*C. liriodendri*	0.75	1.50	4.00	3.81	4.25	4.75	2.56	1.00
Aurora	A-P11-1B		0.75	1.69	4.06	3.75	4.50	4.88	2.69	1.19
Aurora	A-P11-1C		1.25	1.50	3.50	4.00	4.13	5.13	2.69	1.63
Aurora	A-P6-2 A	*C. destructans*	0.25	1.69	3.75	3.56	3.06	2.00	0.25	0.00
Aurora	A-P6-2 B		0.50	1.75	3.63	3.81	2.88	1.25	0.25	0.00
Aurora	A-P6-2 C		0.50	1.69	4.38	3.38	3.13	2.81	0.25	0.00
Aurora	A-T137A	*C. destructans*	0.50	1.13	1.81	3.44	2.44	2.69	0.38	0.00
Aurora	A-T137B		0.50	1.13	3.25	2.94	2.19	2.56	0.38	0.00
Aurora	A-T137C		0.50	1.31	3.50	3.25	2.31	2.88	0.38	0.00
Aurora	*A-T151 A*	*C. liriodendri*	0.25	1.19	3.63	3.75	3.94	4.56	2.00	1.06
Aurora	A-T151 B		0.50	1.63	3.50	3.88	4.19	4.63	2.00	1.63
Aurora	A-T151 C		0.75	1.56	3.63	3.69	4.25	4.75	2.06	1.38
Aurora	A-T166 A	*C. liriodendri*	1.25	1.94	3.50	3.81	3.38	2.50	0.63	0.00
Aurora	A-T166 B		1.13	1.81	3.50	3.75	2.94	2.56	0.63	0.00
Aurora	A-T166 C		0.50	2.50	3.63	3.75	3.00	2.63	0.38	0.00
Aurora	A-T181 A	*C. destructans*	0.25	2.25	3.75	3.69	3.44	2.94	0.63	0.00
Aurora	A-T181 B		0.63	2.50	3.81	3.75	3.38	2.94	0.94	0.00
Aurora	A-T181 C		0.25	2.44	4.00	3.94	3.13	3.19	0.94	0.00
Aurora	A-T1R2-9B A	*C. destructans*	0.75	1.63	3.25	3.75	2.94	3.13	0.13	0.00
Aurora	A-T1R2-9B B		0.75	1.50	3.25	3.75	3.38	3.25	0.13	0.00
Aurora	A-T1R2-9B C		0.75	1.38	3.50	3.75	3.25	3.63	0.13	0.00
Aurora	A-T225A	*C. liriodendra*	0.25	0.88	2.81	3.25	3.25	3.75	2.06	0.94

Aurora	A-T225B		0.13	1.19	3.06	3.69	3.13	3.56	2.19	0.75
Aurora	A-T225C		0.25	1.13	3.25	3.50	3.00	3.69	1.94	0.88
Aurora	A-T26A	*C. destructans*	0.25	1.63	3.13	2.88	2.31	1.75	0.44	0.00
Aurora	A-T26B		0.63	1.94	2.75	3.00	2.25	2.13	0.44	0.00
Aurora	A-T26C		0.63	1.81	3.25	2.69	2.13	2.00	0.69	0.00
Aurora	A-T2R3-2B2 A	*C. destructans*	0.75	2.44	3.81	3.00	3.13	2.56	0.94	0.00
Aurora	A-T2R3-2B2 B		0.75	2.38	3.63	3.31	2.88	2.75	0.81	0.00
Aurora	A-T2R3-2B2 C		0.50	2.19	3.81	3.25	3.00	2.88	0.63	0.00
Aurora	A-T8 A	*C. liriodendri*	0.38	2.06	3.25	3.50	2.75	2.94	1.06	0.00
Aurora	A-T8 B		0.25	2.31	3.19	3.44	2.69	3.19	0.75	0.00
Aurora	A-T8 C		0.25	2.31	3.38	3.38	2.94	3.25	1.00	0.00
Canby	C21 A	*C. destructans*	0.00	0.88	1.56	2.25	2.25	2.25	0.31	0.31
Canby	C21 B		0.00	0.88	1.50	2.56	2.06	2.13	0.88	0.25
Canby	C21 C		0.00	0.88	1.56	2.25	2.44	2.00	0.88	0.31
Canby	C22A	*C. destructans*	0.38	1.69	3.50	3.69	3.06	3.19	1.06	0.00
Canby	C22B		0.38	1.88	3.19	3.63	3.38	3.44	1.06	0.00
Canby	C22C		0.38	1.88	3.56	3.69	3.50	3.38	0.94	0.00
Canby	C51A	*C. liriodendri*	0.50	1.50	3.00	4.00	4.75	4.63	2.88	1.25
Canby	C51B		0.50	1.38	3.06	3.88	4.50	3.94	1.75	2.81
Canby	C51C		0.75	1.63	3.38	4.13	4.69	4.00	1.75	0.94
Canby	C61A	*C. destructans*	0.50	2.13	3.50	3.75	3.38	3.75	0.38	0.00
Canby	C61B		0.50	2.31	3.38	3.56	3.56	3.69	0.38	0.00
Canby	C61C		0.50	2.38	3.50	3.69	3.75	3.25	0.38	0.00
Canby	C-62A	*C. destructans*	0.00	0.75	1.94	2.50	2.38	2.63	1.00	0.44
Canby	C-62B		0.00	0.88	1.75	2.50	2.63	3.44	1.00	0.38
Canby	C-62C		0.00	0.75	1.81	2.63	2.31	3.44	1.00	0.25
Canby	C72A	*C. destructans*	0.00	0.25	1.94	2.88	3.31	3.06		0.56
Canby	C72B		0.00	0.25	2.25	2.63	3.13	2.69	1.38	0.38

Canby	C72C		0.00	0.25	2.25	2.50	2.56	2.75	0.69	0.69
Canby	C73A	*C. destructans*	0.63	2.19	3.69	3.63	2.69	2.56	0.63	0.00
Canby	C73B		0.75	2.19	3.50	3.63	2.75	2.38	0.63	0.00
Canby	C73C		0.50	2.19	3.56	3.63	2.69	2.69	0.63	0.81
Canby	C81A	*C. destructans*	0.50	2.25	3.50	3.50	3.44	3.25		0.00
Canby	C81B		0.25	2.25	3.50	3.75	3.31	3.13	0.69	0.00
Canby	C81C		0.38	2.06	3.63	3.94	3.38	3.50	0.63	0.00
Canby	C82 A	*C. destructans*	0.00	0.63	1.88	2.69	2.38	2.44	0.69	0.56
Canby	C82 B		0.00	0.63	2.00	2.50	2.63	2.56	0.56	0.50
Canby	C82 C		0.00	0.63	2.13	0.00	2.63	0.25	0.38	0.31
Canby	C-P12-25 A	*C. destructans*	0.50	2.19	3.69	3.38	3.06	3.06	0.88	0.00
Canby	C-P12-25 C		0.63	2.19	3.50	3.75	3.19	3.00	1.00	0.00
Canby	C-P12-56 B		0.63	2.25	3.13	4.25	3.13	3.13	0.81	0.00
Canby	C-P16-2A	*C. destructans*	0.75	1.56	2.94	2.69	2.44		0.88	0.00
Canby	C-P16-2B		0.38	1.56	3.00	2.88	2.25	2.38	0.88	0.00
Canby	C-P16-2C		0.50	2.25	2.81	2.88	2.25	1.94	0.88	0.00
Canby	C-P5-2A	*C. destructans*	0.38	1.88	3.13	2.00	1.56	2.00	0.00	0.00
Canby	C-P5-2B		0.31	1.75	3.38	2.81	1.94	2.13	0.00	0.00
Canby	C-P5-2C		0.38	1.88	3.38	2.31	1.38	1.88	0.00	0.00
Mima	M13-MBC-4A A	*C. destructans*	0.13	1.19	3.75	3.94	4.50	4.75	1.31	0.00
Mima	M13-MBC-4A B		0.25	1.38	3.38	3.94	4.19	4.19	1.25	0.00
Mima	M13-MBC-4A C		0.25	1.31	3.63	4.19	4.19	4.31	1.19	0.00
Mima	M14-MBC-3B A	*C. liriodendri*	0.50	1.50	3.31	3.25	3.81	3.75	1.88	0.88
Mima	M14-MBC-3B B		0.25	1.31	3.50	3.69	3.50	3.88	2.13	0.75
Mima	M14-MBC-3B C		0.25	1.25	3.44	3.50	3.50	3.88	1.88	0.94
Mima	M18-CON-1B A	*C. liriodendri*	0.00	1.31	2.25	3.13	3.56	4.13	2.31	0.38
Mima	M18-CON-1B B		0.00	1.44	2.44	3.44	3.63	3.88	2.56	0.38
Mima	M18-CON-1B C		0.00	1.06	2.63	3.25	3.06	3.19	2.69	0.25

Mima	M21-CON-4A A	*C. destructans*	0.50	2.06	3.63	3.75	3.25	3.06		0.00
Mima	M21-CON-4A B		0.13	2.19	3.75	3.25	3.31	2.81	0.25	0.00
Mima	M21-CON-4A C		0.63	2.50	3.69	3.56	2.94	3.13	0.25	0.00
Mima	M29-CON-2A A	*C. destructans*	0.63	2.06	3.50	3.06	2.75	2.88	0.50	0.00
Mima	M29-CON-2A B		0.50	1.38	3.38	3.38	2.56	2.94	0.63	0.00
Mima	M29-CON-2A C		0.50	1.56	3.38	3.25	2.69	2.50	0.75	0.00
Mima	M29-CON-4B A	*C. liriodendri*	0.50	1.13	2.88	3.75	3.44	2.88	0.94	0.75
Mima	M29-CON-4B B		0.50	1.19	3.25	3.38	2.75	2.69	1.06	0.75
Mima	M29-CON-4B C		0.75	1.13	3.25	3.56	3.00	2.06	1.00	0.75

Appendix D: Average radial growth (mm) of mycelia of fungal isolates per day in 8 different temperatures:

NAME/temp	5C	8C	18C	22C	24C	25C
C73	0.62	2.18	3.58	3.62	2.70	2.54
A-P11-1	0.91	1.56	3.85	3.85	4.29	4.91
A-T1R2-9B	0.75	1.5	3.33	3.75	3.18	3.33
C82	0	0.62	2	1.72	2.54	1.75
C-P12-25	0.58	2.20	3.4375	3.79	3.12	3.06
C51	0.58	1.5	3.14	4	4.64	4.18
C72	0	0.25	2.14	2.66	3	2.83
M18-CON-1B	0	1.27	2.43	3.27	3.41	3.72
M29-CON-4B	0.58	1.14	3.12	3.56	3.06	2.54
A-P6-2	0.41	1.70	3.91	3.58	3.02	2.02
A-T2R3-2B2	0.66	2.33	3.75	3.18	3	2.72
A-T151	0.5	1.45	3.58	3.77	4.12	4.64
C-P16-2	0.54	1.79	2.91	2.81	2.31	2.15
C21	0	0.87	1.54	2.35	2.25	2.12
M29-CON-2A	0.54	1.66	3.41	3.22	2.66	2.77
M14-MBC-3B	0.33	1.35	3.41	3.47	3.60	3.83
A-T181	0.37	2.39	3.85	3.79	3.31	3.02
A-T8	0.29	2.22	3.27	3.43	2.79	3.12
A-T166	0.95	2.08	3.54	3.77	3.10	2.56
C81	0.37	2.18	3.54	3.72	3.37	3.29
C-62	0	0.79	1.83	2.54	2.43	3.16
M13-MBC-4A	0.20	1.29	3.58	4.02	4.29	4.41
C61	0.5	2.27	3.45	3.66	3.56	3.56
M21-CON-4A	0.41	2.25	3.68	3.52	3.16	3
A-T225	0.20	1.06	3.04	3.47	3.12	3.66
A-T137	0.5	1.18	2.85	3.20	2.31	2.70
C22	0.37	1.81	3.41	3.66	3.31	3.33
A-T26	0.5	1.79	3.04	2.85	2.22	1.95
A-2010-119T110	0.13	0.95	3.16	3.10	3.70	4.12
C-P5-2	0.35	1.83	3.29	2.37	1.62	2

NAME/temp	28C	30C
C73	0.62	0.27
A-P11-1	2.64	1.27
A-T1R2-9B	0.12	0
C82	0.54	0.45
C-P12-25	0.89	0
C51	2.12	1.66
C72	1.03	0.54
M18-CON-1B	2.52	0.33
M29-CON-4B	1	0.75
A-P6-2	0.25	0
A-T2R3-2B2	0.79	0
A-T151	2.02	1.35
C-P16-2	0.87	0
C21	0.68	0.29
M29-CON-2A	0.62	0
M14-MBC-3B	1.95	0.85
A-T181	0.83	0
A-T8	0.93	0
A-T166	0.54	0
C81	0.65	0
C-62	1	0.35
M13-MBC-4A	1.25	0
C61	0.37	0
M21-CON-4A	0.16	0
A-T225	2.06	0.85
A-T137	0.37	0
C22	1.02	0
A-T26	0.52	0
A-2010-119-T110	2.22	0.33
C-P5-2	0	0

Appendix E: Three replicate measurements of radial growth (mm) of mycelia of single isolates on 4 different concentrations of 4 different fungicides and control:

Fungicide	Concentration (%)	Isolate name	Growth/mm
Cleary	10	A-T26 /1	3.75
Cleary	10	A-T26/2	4.5
Cleary	10	A-T26/3	2
Cleary	10	A-T137/1	3
Cleary	10	A-T137/2	0
Cleary	10	A-T137/3	2
Cleary	10	A-T166/1	2.75
Cleary	10	A-T166/2	3.25
Cleary	10	A-T166/3	2.25
Cleary	10	A-T225/1	1.5
Cleary	10	A-T225/2	0
Cleary	10	A-T225/3	1.5
Cleary	10	C21/1	8.5
Cleary	10	C21/2	7.25
Cleary	10	C21/3	8.5
Cleary	10	C22/1	3.25
Cleary	10	C22/2	2.5
Cleary	10	C22/3	0
Cleary	10	C62/1	9
Cleary	10	C62/2	10.5
Cleary	10	C62/3	9.75
Cleary	10	C51/1	16
Cleary	10	C51/2	14.5
Cleary	10	C51/3	14.25
Cleary	10	M29-C0N2A/1	0
Cleary	10	M29-C0N-2A/2	4
Cleary	10	M29-C0N-2A/3	3
Cleary	10	M13-MBC-4A/1	1
Cleary	10	M13-MBC-4A/2	4
Cleary	10	M13-MBC-4A/3	0
Cleary	10	M21-CON-4A/1	3.5
Cleary	10	M21-CON-4A/2	1
Cleary	10	M21-CON-4A/3	2.75
Cleary	10	M29-C0N-4B/1	0
Cleary	10	M29-C0N-4B/2	0
Cleary	10	M29-C0N-4B/3	0

Fungicide	Concentration	Isolate name	Growth/mm
Cleary	25	A-T26 /1	2.25
Cleary	25	A-T26/2	3.75
Cleary	25	A-T26/3	4
Cleary	25	A-T137/1	1.5
Cleary	25	A-T137/2	2
Cleary	25	A-T137/3	0
Cleary	25	A-T166/1	3.25
Cleary	25	A-T166/2	3.25
Cleary	25	A-T166/3	2.25
Cleary	25	A-T225/1	0
Cleary	25	A-T225/2	0
Cleary	25	A-T225/3	0
Cleary	25	C21/1	9
Cleary	25	C21/2	9
Cleary	25	C21/3	10
Cleary	25	C22/1	3.75
Cleary	25	C22/2	4.25
Cleary	25	C22/3	0
Cleary	25	C62/1	7.5
Cleary	25	C62/2	5.75
Cleary	25	C62/3	5.75
Cleary	25	C51/1	16.25
Cleary	25	C51/2	15.75
Cleary	25	C51/3	16.25
Cleary	25	M29-C0N-2A/1	2.5
Cleary	25	M29-C0N-2A/2	3.75
Cleary	25	M29-C0N-2A/3	5
Cleary	25	M13-MBC-4A/1	5.75
Cleary	25	M13-MBC-4A/2	2.25
Cleary	25	M13-MBC-4A/3	4
Cleary	25	M21-CON-4A/1	4.75
Cleary	25	M21-CON-4A/2	2.25
Cleary	25	M21-CON-4A/3	0
Cleary	25	M29-C0N-4B/1	0
Cleary	25	M29-C0N-4B/2	0
Cleary	25	M29-C0N-4B/3	0

Fungicide	Concentration (%)	Isolate name	Growth/mm
Cleary	50	A-T26 /1	4
Cleary	50	A-T26/2	2.5
Cleary	50	A-T26/3	3.25
Cleary	50	A-T137/1	3
Cleary	50	A-T137/2	2.75
Cleary	50	A-T137/3	3.5
Cleary	50	A-T166/1	3
Cleary	50	A-T166/2	1
Cleary	50	A-T166/3	2.75
Cleary	50	A-T225/1	0
Cleary	50	A-T225/2	1.5
Cleary	50	A-T225/3	2.25
Cleary	50	C21/1	10.75
Cleary	50	C21/2	10.75
Cleary	50	C21/3	9.75
Cleary	50	C22/1	2.5
Cleary	50	C22/2	3.5
Cleary	50	C22/3	4.5
Cleary	50	C62/1	11
Cleary	50	C62/2	11.75
Cleary	50	C62/3	12.75
Cleary	50	C51/1	17
Cleary	50	C51/2	15.75
Cleary	50	C51/3	17.5
Cleary	50	M29-C0N-2A/1	3.25
Cleary	50	M29-C0N-2A/2	1
Cleary	50	M29-C0N-2A/3	1.75
Cleary	50	M13-MBC-4A/1	5.25
Cleary	50	M13-MBC-4A/2	2.5
Cleary	50	M13-MBC-4A/3	4.5
Cleary	50	M21-CON-4A/1	2.75
Cleary	50	M21-CON-4A/2	0
Cleary	50	M21-CON-4A/3	2.5
Cleary	50	M29-C0N-4B/1	1.5
Cleary	50	M29-C0N-4B/2	0
Cleary	50	M29-C0N-4B/3	0

Fungicide	Concentration (%)	Isolate name	Growth/mm
Cleary	75	A-T26 /1	4
Cleary	75	A-T26/2	3.5
Cleary	75	A-T26/3	5
Cleary	75	A-T137/1	1.75
Cleary	75	A-T137/2	2.5
Cleary	75	A-T137/3	1.5
Cleary	75	A-T166/1	2
Cleary	75	A-T166/2	2.75
Cleary	75	A-T166/3	3
Cleary	75	A-T225/1	1
Cleary	75	A-T225/2	3.25
Cleary	75	A-T225/3	3.75
Cleary	75	C21/1	8.75
Cleary	75	C21/2	8.5
Cleary	75	C21/3	7.75
Cleary	75	C22/1	2.75
Cleary	75	C22/2	2.5
Cleary	75	C22/3	2.5
Cleary	75	C62/1	10
Cleary	75	C62/2	8.25
Cleary	75	C62/3	9
Cleary	75	C51/1	17
Cleary	75	C51/2	17.5
Cleary	75	C51/3	16
Cleary	75	M29-C0N-2A/1	3.75
Cleary	75	M29-C0N-2A/2	2
Cleary	75	M29-C0N-2A/3	4.5
Cleary	75	M13-MBC-4A/1	4.5
Cleary	75	M13-MBC-4A/2	0
Cleary	75	M13-MBC-4A/3	0
Cleary	75	M21-CON-4A/1	1
Cleary	75	M21-CON-4A/2	1
Cleary	75	M21-CON-4A/3	0
Cleary	75	M29-C0N-4B/1	2.25
Cleary	75	M29-C0N-4B/2	0
Cleary	75	M29-C0N-4B/3	2.5

Fungicide	Concentration	isolate name	GROWTH/mm
Dithane	10	A-T26 /1	10.5
Dithane	10	A-T26/2	12
Dithane	10	A-T26/3	12
Dithane	10	A-T137/1	7.25
Dithane	10	A-T137/2	7.25
Dithane	10	A-T137/3	7
Dithane	10	A-T166/1	13
Dithane	10	A-T166/2	12.5
Dithane	10	A-T166/3	12.5
Dithane	10	A-T225/1	21
Dithane	10	A-T225/2	15.5
Dithane	10	A-T225/3	0
Dithane	10	C21/1	2.5
Dithane	10	C21/2	5.5
Dithane	10	C21/3	4.25
Dithane	10	C22/1	8.25
Dithane	10	C22/2	8
Dithane	10	C22/3	12.25
Dithane	10	C62/1	6.25
Dithane	10	C62/2	7.5
Dithane	10	C62/3	7.5
Dithane	10	C51/1	13.5
Dithane	10	C51/2	16
Dithane	10	C51/3	14
Dithane	10	M29-C0N-2A/1	11
Dithane	10	M29-C0N-2A/2	11.75
Dithane	10	M29-C0N-2A/3	9.5
Dithane	10	M13-MBC-4A/1	0
Dithane	10	M13-MBC-4A/2	12
Dithane	10	M13-MBC-4A/3	10.5
Dithane	10	M21-CON-4A/1	11.5
Dithane	10	M21-CON-4A/2	10
Dithane	10	M21-CON-4A/3	9
Dithane	10	M29-C0N-4B/1	15
Dithane	10	M29-C0N-4B/2	15
Dithane	10	M29-C0N-4B/3	13

Fungicide	Concentration (%)	Isolate name	Growth/mm
Dithane	25	A-T26 /1	9.25
Dithane	25	A-T26/2	9
Dithane	25	A-T26/3	7.75
Dithane	25	A-T137/1	5.5
Dithane	25	A-T137/2	5
Dithane	25	A-T137/3	5.25
Dithane	25	A-T166/1	7
Dithane	25	A-T166/2	9.25
Dithane	25	A-T166/3	5.5
Dithane	25	A-T225/1	14.25
Dithane	25	A-T225/2	12
Dithane	25	A-T225/3	14.5
Dithane	25	C21/1	1.5
Dithane	25	C21/2	4.25
Dithane	25	C21/3	2.5
Dithane	25	C22/1	3.25
Dithane	25	C22/2	6.75
Dithane	25	C22/3	2.75
Dithane	25	C62/1	2
Dithane	25	C62/2	2
Dithane	25	C62/3	3.5
Dithane	25	C51/1	8
Dithane	25	C51/2	0
Dithane	25	C51/3	7.5
Dithane	25	M29-C0N-2A/1	8
Dithane	25	M29-C0N-2A/2	8
Dithane	25	M29-C0N-2A/3	9.75
Dithane	25	M13-MBC-4A/1	0
Dithane	25	M13-MBC-4A/2	4
Dithane	25	M13-MBC-4A/3	0
Dithane	25	M21-CON-4A/1	1.5
Dithane	25	M21-CON-4A/2	9.75
Dithane	25	M21-CON-4A/3	8.75
Dithane	25	M29-C0N-4B/1	12
Dithane	25	M29-C0N-4B/2	13.5
Dithane	25	M29-C0N-4B/3	8

Fungicide	Concentration (%)	Isolate name	Growth/mm
Dithane	50	A-T26 /1	0
Dithane	50	A-T26/2	2.75
Dithane	50	A-T26/3	5.75
Dithane	50	A-T137/1	4
Dithane	50	A-T137/2	0
Dithane	50	A-T137/3	0
Dithane	50	A-T166/1	5
Dithane	50	A-T166/2	0
Dithane	50	A-T166/3	0
Dithane	50	A-T225/1	10.5
Dithane	50	A-T225/2	10.25
Dithane	50	A-T225/3	0
Dithane	50	C21/1	0.5
Dithane	50	C21/2	1
Dithane	50	C21/3	1
Dithane	50	C22/1	2
Dithane	50	C22/2	0
Dithane	50	C22/3	2.75
Dithane	50	C62/1	0
Dithane	50	C62/2	2
Dithane	50	C62/3	2.25
Dithane	50	C51/1	4.75
Dithane	50	C51/2	5.5
Dithane	50	C51/3	6
Dithane	50	M29-C0N-2A/1	0
Dithane	50	M29-C0N-2A/2	0
Dithane	50	M29-C0N-2A/3	3
Dithane	50	M13-MBC-4A/1	0
Dithane	50	M13-MBC-4A/2	0
Dithane	50	M13-MBC-4A/3	0
Dithane	50	M21-CON-4A/1	0
Dithane	50	M21-CON-4A/2	6.5
Dithane	50	M21-CON-4A/3	5.5
Dithane	50	M29-C0N-4B/1	9
Dithane	50	M29-C0N-4B/2	7.75
Dithane	50	M29-C0N-4B/3	7.5

Fungicide	Concentration (%)	Isolate name	Growth/mm
Dithane	75	A-T26 /1	0
Dithane	75	A-T26/2	0
Dithane	75	A-T26/3	0
Dithane	75	A-T137/1	0
Dithane	75	A-T137/2	0
Dithane	75	A-T137/3	3.25
Dithane	75	A-T166/1	4
Dithane	75	A-T166/2	0
Dithane	75	A-T166/3	0
Dithane	75	A-T225/1	10
Dithane	75	A-T225/2	7.5
Dithane	75	A-T225/3	11.5
Dithane	75	C21/1	0
Dithane	75	C21/2	2.5
Dithane	75	C21/3	0
Dithane	75	C22/1	0
Dithane	75	C22/2	0
Dithane	75	C22/3	2.5
Dithane	75	C62/1	0
Dithane	75	C62/2	2
Dithane	75	C62/3	3
Dithane	75	C51/1	0
Dithane	75	C51/2	0
Dithane	75	C51/3	0
Dithane	75	M29-C0N-2A/1	4
Dithane	75	M29-C0N-2A/2	0
Dithane	75	M29-C0N-2A/3	3
Dithane	75	M13-MBC-4A/1	0
Dithane	75	M13-MBC-4A/2	0
Dithane	75	M13-MBC-4A/3	0
Dithane	75	M21-CON-4A/1	6.25
Dithane	75	M21-CON-4A/2	0
Dithane	75	M21-CON-4A/3	0
Dithane	75	M29-C0N-4B/1	4.25
Dithane	75	M29-C0N-4B/2	7.5
Dithane	75	M29-C0N-4B/3	8.75

Fungicide	Concentration (%)	Isolate name	Growth/mm
Heritage	10	A-T26 /1	13.75
Heritage	10	A-T26/2	12.5
Heritage	10	A-T26/3	13.5
Heritage	10	A-T137/1	8.5
Heritage	10	A-T137/2	9.25
Heritage	10	A-T137/3	9
Heritage	10	A-T166/1	13.25
Heritage	10	A-T166/2	13.25
Heritage	10	A-T166/3	12.5
Heritage	10	A-T225/1	23.5
Heritage	10	A-T225/2	24.5
Heritage	10	A-T225/3	22.75
Heritage	10	C21/1	7.75
Heritage	10	C21/2	6.25
Heritage	10	C21/3	7.35
Heritage	10	C22/1	10.5
Heritage	10	C22/2	11
Heritage	10	C22/3	10
Heritage	10	C62/1	7.25
Heritage	10	C62/2	8
Heritage	10	C62/3	7.75
Heritage	10	C51/1	21.25
Heritage	10	C51/2	23
Heritage	10	C51/3	22.75
Heritage	10	M29-C0N-2A/1	6.25
Heritage	10	M29-C0N-2A/2	8
Heritage	10	M29-C0N-2A/3	6.5
Heritage	10	M13-MBC-4A/1	16.75
Heritage	10	M13-MBC-4A/2	17
Heritage	10	M13-MBC-4A/3	17
Heritage	10	M21-CON-4A/1	10.25
Heritage	10	M21-CON-4A/2	10.5
Heritage	10	M21-CON-4A/3	9.5
Heritage	10	M29-C0N-4B/1	15.5
Heritage	10	M29-C0N-4B/2	15.5
Heritage	10	M29-C0N-4B/3	17

Fungicide	Concentration (%)	Isolate name	Growth/mm
Heritage	25	A-T26 /1	14.5
Heritage	25	A-T26/2	14
Heritage	25	A-T26/3	14.25
Heritage	25	A-T137/1	8.25
Heritage	25	A-T137/2	8.75
Heritage	25	A-T137/3	9.25
Heritage	25	A-T166/1	11.25
Heritage	25	A-T166/2	12.25
Heritage	25	A-T166/3	12
Heritage	25	A-T225/1	21.75
Heritage	25	A-T225/2	20.5
Heritage	25	A-T225/3	23.5
Heritage	25	C21/1	5.5
Heritage	25	C21/2	5.5
Heritage	25	C21/3	5
Heritage	25	C22/1	10.5
Heritage	25	C22/2	9
Heritage	25	C22/3	8.75
Heritage	25	C62/1	5.5
Heritage	25	C62/2	5.5
Heritage	25	C62/3	5
Heritage	25	C51/1	21.25
Heritage	25	C51/2	20.5
Heritage	25	C51/3	21.25
Heritage	25	M29-C0N-2A/1	4.75
Heritage	25	M29-C0N-2A/2	3.75
Heritage	25	M29-C0N-2A/3	4.5
Heritage	25	M13-MBC-4A/1	14.5
Heritage	25	M13-MBC-4A/2	16.75
Heritage	25	M13-MBC-4A/3	14.25
Heritage	25	M21-CON-4A/1	9.5
Heritage	25	M21-CON-4A/2	8.5
Heritage	25	M21-CON-4A/3	8
Heritage	25	M29-C0N-4B/1	14.75
Heritage	25	M29-C0N-4B/2	16
Heritage	25	M29-C0N-4B/3	17.75

Fungicide	Concentration (%)	Isolate name	Growth/mm
Heritage	50	A-T26 /1	13.5
Heritage	50	A-T26/2	13.25
Heritage	50	A-T26/3	14.5
Heritage	50	A-T137/1	6
Heritage	50	A-T137/2	9.25
Heritage	50	A-T137/3	9.25
Heritage	50	A-T166/1	11
Heritage	50	A-T166/2	12
Heritage	50	A-T166/3	12.25
Heritage	50	A-T225/1	21
Heritage	50	A-T225/2	22.25
Heritage	50	A-T225/3	20
Heritage	50	C21/1	6.5
Heritage	50	C21/2	5
Heritage	50	C21/3	6
Heritage	50	C22/1	9.25
Heritage	50	C22/2	8.75
Heritage	50	C22/3	9.5
Heritage	50	C62/1	5
Heritage	50	C62/2	4.5
Heritage	50	C62/3	4.25
Heritage	50	C51/1	22.5
Heritage	50	C51/2	21
Heritage	50	C51/3	22
Heritage	50	M29-C0N-2A/1	3.75
Heritage	50	M29-C0N-2A/2	3
Heritage	50	M29-C0N-2A/3	3.6
Heritage	50	M13-MBC-4A/1	14.5
Heritage	50	M13-MBC-4A/2	15.5
Heritage	50	M13-MBC-4A/3	16.75
Heritage	50	M21-CON-4A/1	8.5
Heritage	50	M21-CON-4A/2	9
Heritage	50	M21-CON-4A/3	9.75
Heritage	50	M29-C0N-4B/1	19.5
Heritage	50	M29-C0N-4B/2	17
Heritage	50	M29-C0N-4B/3	19

Fungicide	Concentration (%)	Isolate name	Growth/mm
Heritage	75	A-T26 /1	12.5
Heritage	75	A-T26/2	12.25
Heritage	75	A-T26/3	12.75
Heritage	75	A-T137/1	7.5
Heritage	75	A-T137/2	6.5
Heritage	75	A-T137/3	7.5
Heritage	75	A-T166/1	11.5
Heritage	75	A-T166/2	11.5
Heritage	75	A-T166/3	11.5
Heritage	75	A-T225/1	21
Heritage	75	A-T225/2	19.5
Heritage	75	A-T225/3	20.75
Heritage	75	C21/1	5.25
Heritage	75	C21/2	5.75
Heritage	75	C21/3	3.75
Heritage	75	C22/1	8
Heritage	75	C22/2	7.5
Heritage	75	C22/3	6.5
Heritage	75	C62/1	3.75
Heritage	75	C62/2	4.25
Heritage	75	C62/3	3.85
Heritage	75	C51/1	20.5
Heritage	75	C51/2	20.5
Heritage	75	C51/3	20
Heritage	75	M29-C0N-2A/1	2.5
Heritage	75	M29-C0N-2A/2	2
Heritage	75	M29-C0N-2A/3	4
Heritage	75	M13-MBC-4A/1	13.5
Heritage	75	M13-MBC-4A/2	13.75
Heritage	75	M13-MBC-4A/3	13
Heritage	75	M21-CON-4A/1	9
Heritage	75	M21-CON-4A/2	7.5
Heritage	75	M21-CON-4A/3	8.25
Heritage	75	M29-C0N-4B/1	18
Heritage	75	M29-C0N-4B/2	19
Heritage	75	M29-C0N-4B/3	20

Fungicide	Concentration (%)	Isolate name	Growth/mm
Iprodione	10	A-T26 /1	10.5
Iprodione	10	A-T26/2	11.25
Iprodione	10	A-T26/3	11.5
Iprodione	10	A-T137/1	8
Iprodione	10	A-T137/2	10.75
Iprodione	10	A-T137/3	10
Iprodione	10	A-T166/1	12
Iprodione	10	A-T166/2	11
Iprodione	10	A-T166/3	13.5
Iprodione	10	A-T225/1	11.75
Iprodione	10	A-T225/2	15.75
Iprodione	10	A-T225/3	15.5
Iprodione	10	C21/1	6.25
Iprodione	10	C21/2	4.5
Iprodione	10	C21/3	4.25
Iprodione	10	C22/1	12.75
Iprodione	10	C22/2	10.5
Iprodione	10	C22/3	13.25
Iprodione	10	C62/1	4.5
Iprodione	10	C62/2	5
Iprodione	10	C62/3	3.5
Iprodione	10	C51/1	8.5
Iprodione	10	C51/2	8
Iprodione	10	C51/3	7
Iprodione	10	M29-C0N-2A/1	12
Iprodione	10	M29-C0N-2A/2	13.5
Iprodione	10	M29-C0N-2A/3	13.5
Iprodione	10	M13-MBC-4A/1	10.5
Iprodione	10	M13-MBC-4A/2	10.25
Iprodione	10	M13-MBC-4A/3	10.25
Iprodione	10	M21-CON-4A/1	11
Iprodione	10	M21-CON-4A/2	9.5
Iprodione	10	M21-CON-4A/3	10.25
Iprodione	10	M29-C0N-4B/1	8.75
Iprodione	10	M29-C0N-4B/2	9.5
Iprodione	10	M29-C0N-4B/3	8

Fungicide	Concentration (%)	Isolate name	Growth/mm
Iprodione	25	A-T26 /1	10
Iprodione	25	A-T26/2	7.5
Iprodione	25	A-T26/3	7.5
Iprodione	25	A-T137/1	10
Iprodione	25	A-T137/2	7.75
Iprodione	25	A-T137/3	9.75
Iprodione	25	A-T166/1	13
Iprodione	25	A-T166/2	14.5
Iprodione	25	A-T166/3	12.5
Iprodione	25	A-T225/1	13.25
Iprodione	25	A-T225/2	16.75
Iprodione	25	A-T225/3	13.75
Iprodione	25	C21/1	4.5
Iprodione	25	C21/2	4.25
Iprodione	25	C21/3	3.25
Iprodione	25	C22/1	11.5
Iprodione	25	C22/2	10.5
Iprodione	25	C22/3	12.5
Iprodione	25	C62/1	3.5
Iprodione	25	C62/2	4
Iprodione	25	C62/3	8.25
Iprodione	25	C51/1	10
Iprodione	25	C51/2	9.5
Iprodione	25	C51/3	7.25
Iprodione	25	M29-C0N-2A/1	12
Iprodione	25	M29-C0N-2A/2	12.75
Iprodione	25	M29-C0N-2A/3	12.75
Iprodione	25	M13-MBC-4A/1	11.5
Iprodione	25	M13-MBC-4A/2	10
Iprodione	25	M13-MBC-4A/3	21.25
Iprodione	25	M21-CON-4A/1	9.25
Iprodione	25	M21-CON-4A/2	9
Iprodione	25	M21-CON-4A/3	9.75
Iprodione	25	M29-C0N-4B/1	10
Iprodione	25	M29-C0N-4B/2	8.75
Iprodione	25	M29-C0N-4B/3	8.5

Fungicide	Concentration (%)	Isolate name	Growth/mm
Iprodione	50	A-T26 /1	10
Iprodione	50	A-T26/2	11
Iprodione	50	A-T26/3	12.75
Iprodione	50	A-T137/1	9.25
Iprodione	50	A-T137/2	9.25
Iprodione	50	A-T137/3	10
Iprodione	50	A-T166/1	12.5
Iprodione	50	A-T166/2	14
Iprodione	50	A-T166/3	9.75
Iprodione	50	A-T225/1	14.75
Iprodione	50	A-T225/2	14.75
Iprodione	50	A-T225/3	15
Iprodione	50	C21/1	5.75
Iprodione	50	C21/2	5.75
Iprodione	50	C21/3	6.25
Iprodione	50	C22/1	12
Iprodione	50	C22/2	14.5
Iprodione	50	C22/3	13.5
Iprodione	50	C62/1	3.75
Iprodione	50	C62/2	3
Iprodione	50	C62/3	4.25
Iprodione	50	C51/1	10
Iprodione	50	C51/2	6.75
Iprodione	50	C51/3	5.75
Iprodione	50	M29-C0N-2A/1	13.25
Iprodione	50	M29-C0N-2A/2	13.25
Iprodione	50	M29-C0N-2A/3	14
Iprodione	50	M13-MBC-4A/1	10.5
Iprodione	50	M13-MBC-4A/2	11
Iprodione	50	M13-MBC-4A/3	9.75
Iprodione	50	M21-CON-4A/1	9.25
Iprodione	50	M21-CON-4A/2	10
Iprodione	50	M21-CON-4A/3	9.5
Iprodione	50	M29-C0N-4B/1	8.75
Iprodione	50	M29-C0N-4B/2	9.25
Iprodione	50	M29-C0N-4B/3	9.5

Fungicide	Concentration (%)	Isolate name	Growth/mm
Iprodione	75	A-T26 /1	11.25
Iprodione	75	A-T26/2	10.25
Iprodione	75	A-T26/3	10.25
Iprodione	75	A-T137/1	10.5
Iprodione	75	A-T137/2	10
Iprodione	75	A-T137/3	10
Iprodione	75	A-T166/1	14.5
Iprodione	75	A-T166/2	15
Iprodione	75	A-T166/3	14.5
Iprodione	75	A-T225/1	16.5
Iprodione	75	A-T225/2	13.25
Iprodione	75	A-T225/3	12.5
Iprodione	75	C21/1	5.5
Iprodione	75	C21/2	5.25
Iprodione	75	C21/3	4.5
Iprodione	75	C22/1	11.75
Iprodione	75	C22/2	14.25
Iprodione	75	C22/3	13
Iprodione	75	C62/1	3.25
Iprodione	75	C62/2	3.75
Iprodione	75	C62/3	4.5
Iprodione	75	C51/1	8.25
Iprodione	75	C51/2	5.25
Iprodione	75	C51/3	5
Iprodione	75	M29-C0N-2A/1	14
Iprodione	75	M29-C0N-2A/2	15.5
Iprodione	75	M29-C0N-2A/3	13.75
Iprodione	75	M13-MBC-4A/1	12.25
Iprodione	75	M13-MBC-4A/2	10.5
Iprodione	75	M13-MBC-4A/3	11
Iprodione	75	M21-CON-4A/1	12.5
Iprodione	75	M21-CON-4A/2	11.5
Iprodione	75	M21-CON-4A/3	7.5
Iprodione	75	M29-C0N-4B/1	10
Iprodione	75	M29-C0N-4B/2	9.5
Iprodione	75	M29-C0N-4B/3	9.25

No fungicide	Isolate name	Growth/mm
Control	M29-C0N-2A/1	21.5
Control	M29-C0N-2A/2	24.25
Control	M29-C0N-2A/3	21.5
Control	M13-MBC-4A/1	36
Control	M13-MBC-4A/2	35
Control	M13-MBC-4A/3	34.5
Control	M21-CON-4A/1	21.25
Control	M21-CON-4A/2	20.5
Control	M21-CON-4A/3	22.75
Control	M29-C0N-4B/1	18.75
Control	M29-C0N-4B/2	16
Control	M29-C0N-4B/3	15
Control	C21/1	20.25
Control	C21/2	19
Control	C21/3	20.75
Control	C22/1	26
Control	C22/2	29.5
Control	C22/3	27
Control	C62/1	21
Control	C62/2	20.5
Control	C62/3	22.5
Control	C51/1	30
Control	C51/2	32.5
Control	C51/3	27.75
Control	A-T26 /1	19
Control	A-T26/2	20
Control	A-T26/3	20.5
Control	A-T137/1	20.5
Control	A-T137/2	17
Control	A-T137/3	17.75
Control	A-T166/1	19.75
Control	A-T166/2	27.25
Control	A-T166/3	17.25
Control	A-T225/1	29.75
Control	A-T225/2	30
Control	A-T225/3	29

Appendix F: Average radial growth data (mm) of mycelia after a week on 4 different concentrations of 4 different fungicides.

Fungal isolate/fungicide	Clear_10%	Clear_25%	Clear_50%	Clear_75%
A-T26/CD1	3.41	3.33	3.25	4.16
A-T137/CD2	1.66	1.16	3.08	1.9
A-T166/CD3	2.75	2.91	2.25	2.58
A-T225/CL1	1	0	1.25	2.66
C21/CD1	8.08	9.33	10.41	8.33
C22/CD2	1.91	2.66	3.5	2.58
C62/CD3	9.75	6.33	11.83	9.08
C51/CL1	14.91	16.08	16.75	16.83
M29-C0N-2A/CD1	2.33	3.75	2	3.41
M13-MBC-4A/CD2	1.66	4	4.08	1.5
M21-CON-4A/CD3	2.41	2.33	1.75	0.66
M29-C0N-4B/CL1	0	0	0.5	1.58

Fungal species/fungicide	Herit_10%	Herit_25%	Herit_50%	Herit_75%
A-T26/CD1	13.25	14.25	13.75	12.5
A-T137/CD2	8.91	8.75	8.16	7.16
A-T166/CD3	13	11.83	11.75	11.5
A-T225/CL1	23.58	21.91	21.08	20.41
C21/CD1	7.11	5.3	5.83	4.916
C22/CD2	10.5	9.41	9.16	7.3
C62/CD3	7.6	5.3	4.58	3.95
C51/CL1	22.3	21	21.83	20.3
M29-C0N-2A/CD1	6.91	4.33	3.45	2.83
M13-MBC-4A/CD2	16.91	15.16	15.58	13.41
M21-CON-4A/CD3	10.08	8.66	9.08	8.25
M29-C0N-4B/CL1	16	16.16	18.5	19

Fungal isolate /fungicide	Ditha_10%	Ditha_25%	Ditha_50%	Ditha_75%
A-T26/CD1	11.5	8.6	2.83	0
A-T137/CD2	7.16	5.25	1.33	1.08
A-T166/CD3	12.6	7.25	1.6	1.33
A-T225/CL1	12.16	13.58	6.91	9.6
C21/CD1	4.08	2.75	0.83	0.83
C22/CD2	9.5	4.25	1.58	0.83
C62/CD3	7.08	2.5	1.41	1.66
C51/CL1	14.5	5.16	5.41	0
M29-C0N-2A/CD1	10.75	8.58	1	2.3
M13-MBC-4A/CD2	7.5	1.33	0	0
M21-CON-4A/CD3	10.16	6.6	4	2.08
M29-C0N-4B/CL1	14.3	11.16	8.08	6.83

Fungal isolate /fungicide	Iprod_10%	Iprod_25%	Iprod_50%	Iprod_75%
A-T26/CD1	11.08	8.33	11.25	10.58
A-T137/CD2	9.58	9.16	9.5	10.166
A-T166/CD3	12.16	13.33	12.08	14.66
A-T225/CL1	14.3	14.58	14.83	14.08
C21/CD1	5	4	5.91	5.08
C22/CD2	12.16	11.5	13.33	13
C62/CD3	4.33	5.25	3.66	3.83
C51/CL1	7.83	8.91	7.5	6.16
M29-C0N-2A/CD1	13	12.5	13.5	14.4
M13-MBC-4A/CD2	10.33	14.25	10.41	11.25
M21-CON-4A/CD3	10.25	9.33	9.58	10.5
M29-C0N-4B/CL1	8.75	9.08	9.16	9.58

Fungal isolate	Control
A-T26/CD1	19.83
A-T137/CD2	18.41
A-T166/CD3	21.41
A-T225/CL1	29.58
C21/CD1	20
C22/CD2	27.5
C62/CD3	21.33
C51/CL1	30.08
M29-C0N-2A/CD1	22.41
M13-MBC-4A/CD2	35.16
M21-CON-4A/CD3	21.5
M29-C0N-4B/CL1	16.58