Environmentally-friendly management strategies for pitch canker

1. Introduction

Forests are one of the most important ecosystems on Earth. They cover approximately one-third of the world's land mass, are a significant source of commercial products, food, and shelter, and exert a powerful influence on the global carbon cycle. Conifers, mainly composed by *Pinus* spp., account for 112.95 million ha of the European forested area (Rigo et al., 2016). *Pinus* is one of the most ecologically and economically significant tree genus in the world, and, in addition to numerous roles in the ecosystems, pines represent an important source of timber, pulp and paper, seeds, charcoal, resin, and construction materials (Richardson and Rundel, 2000). As a precious natural resource, they benefit nature and society in a multitude of ways and new approaches to pest and disease management are needed that take into account these multiple services and the different stakeholders they benefit, as well as the likelihood of greater threats in the future resulting from globalization and climate change (Boyd et al., 2013; Oliva et al., 2016).

Forest pathogens, especially invasive alien species, introduced into countries as a result of globalization of trade and free market practices, not only result environmental and social impacts, but also important economic losses (Holmes et al., 2009; Vettraino et al., 2015; Wingfield et al., 2015). Together with climatic conditions that can strongly influence the dynamics of forest pathogens and consequent diseases, the role of the increased planting forest monocultures and planting of exotic species as contributing factors to change disease scenarios cannot be ignored (Ramsfield et al., 2016). Phytosanitary regulations often fail to prevent biological invasions (Liebhold et al.,

2012; Eschen et al., 2015; Klapwijk et al., 2016) and, as a result of this, the recorded number of invasive forest pathogens has increased exponentially in the recent past (Stenlid et al., 2011; Santini et al., 2013).

Fusarium circinatum (sexual morph: *Gibberella circinata* Nirenberg & O'Donnell) is a highly virulent invasive pathogen that causes a disease known as Pine Pitch Canker (PPC). The disease is considered as one of the most important of conifers globally (Wingfield et al., 2008); at least 60 species of *Pinus* along with *Pseudotsuga menziesii* (Mirb.) Franco are known to be susceptible to PCC (Dwinell, 1999; Bezos et al., 2017). In forest or plantation trees, the most common symptoms of pitch canker are bleedings, resinous cankers on the main stems, terminal or large lateral branches, wilting of needles and die-back, although roots, shoots, female flowers and mature cones can be also affected. Cankers on the main stems are lethal when the stems are girdled and some trees die as a result of stem breakage due to a loss of structural integrity at the site of canker formation (Hepting and Roth, 1946; Barrows-Broaddus and Dwinell, 1985; Barrows-Broaddus, 1990; Pintos et al., 2008). The fungus also causes mortality in hedge and cutting production, damping-off in seedlings with mortalities of up to 100% (Martínez-Alvarez, Pando, et al., 2014). However, the occurrence of these symptoms also depend on host species, biotic and abiotic conditions (Wingfield et al., 2008). In fact, the presence of asymptomatic yet infested trees and seedlings within infested stands and nurseries is also common for at least 52 weeks (Storer et al., 1998; Mitchell et al., 2004; Kim et al., 2008; Vivas, Zas, et al., 2012; Elvira-Recuenco et al., 2015; Swett et al., 2016).

Fusarium circinatum was first detected in 1945 in the south-eastern United States (Hepting and Roth, 1946) and is now reported from Haiti (Hepting and Roth, 1953),

Japan (Kobayashi and Muramoto, 1989), South Africa (Viljoen et al., 1994), Mexico (Guerra-Santos, 1998), South Korea (Lee et al., 2000), Chile (Wingfield et al., 2002), Uruguay (Alonso and Bettucci, 2009) and Colombia (Steenkamp et al., 2012). Spain was the first European country where the disease was detected, more than a decade ago (Dwinell, 1999; Landeras et al., 2005), and more recently the disease has become established in Portugal (Bragança et al., 2009). *Fusarium circinatum* has also been reported in France (EPPO, 2006) and Italy (Carlucci et al., 2007), although in these countries, PPC has been officially eradicated. *Fusarium circinatum* is included in the A2 list (present in the EPPO region but not widely distributed) of pests recommended for regulation as quarantine pathogens. Consequently, the presence of *F. circinatum* in a European country entails several restrictions. In Spain, for example, this includes the ban of planting susceptible species (*Pinus* spp. and *Pseudotsuga menziesii*) in affected areas or the wood movement from these areas to non infected ones (Spanish Royal Decree 637/2006 and 65/2010), as well as disease monitoring and control in the infested areas.

The European Food Safety Authority has recently established that over 10 million hectares of pine forests in Europe are at risk of *F. circinatum* infection (EFSA, 2010). This estimation was based on current host distribution ranges as well as climatic conditions that are likely to be conducive for disease. Currently, the highest priority in Europe is avoiding the dispersal of *F. circinatum* into disease-free countries. Therefore, efforts have been mainly devoted to controlling of the main pathways of introduction. These include the movement of live plants and plant material, especially seed lots (EFSA, 2010).

Several studies have tested the efficacy of fungicides to control *F. circinatum*, both in *in vitro* (Allen et al., 2004; Mitchell et al., 2004; Carey et al., 2005; Landeras et al., 2006; Ramón-Albalat et al., 2010; Iturritxa et al., 2011; Iturritxa, Mesanza, et al., 2013; Berbegal et al., 2015; Mullett et al., 2017) and *in vivo* for seeds (Muñoz López et al., 2009; Berbegal et al., 2015) or woody material (Serrano et al., 2015). However, although some of them have shown promising results, the utilization of chemicals for this purpose in the European forest environment is highly restricted (Directive 2009/128/EC) and more environmentally friendly management approaches are required. In this regard, biological control and other environmentally friendly methods such as thermotherapy treatments, the use of natural compounds with fungicidal effects, the use of promoters of innate host resistance mechanisms and/or breeding for resistance emerge as attractive alternatives to be implemented in an integrated management approach towards PPC control.

The fate of Pine forests benefits is of increasing concern among scientists and policymakers and the health protection measurements to be implemented depends ultimately on the recognition of and the current state of the problem. In this paper we consider environmentally friendly control strategies that might be applied to manage *F. circinatum*. A general background on biocontrol is provided and this is followed by how it has been applied to control PPC. The review also focuses on the use of hot water treatment to limit the spread of PPC in seed and we discuss genetic resistance against *F. circinatum.* In this way, we attempt to highlight potential areas of research that would ultimately allow for the effective management of this important disease.

2. The use of biological control in combatting *F. circinatum*

Biological control has been applied in agriculture since the beginning of the twentieth century as part of an integrated strategy to decrease the use of fungicide, to increase crop production and to minimize environmental contamination caused by pesticides (Cook and Baker, 1996). However, it has gained relevance in the last decades not just as a result of environmental awareness, but also due to the growing social demand of human friendly management strategies as an alternative to chemical-based products for effective plant disease control. The term biological control has evolved from "any control achieved through a living system with the exception of man" (Cook and Baker, 1996) to "the use of microbial antagonists to suppress diseases, including natural products extracted or fermented from various sources" (Pal and Mc Spadden Gardener, 2006). Here, we refer to biological control as any control measure based on the use of antagonism living organisms, including endophytic fungi, bacteria and viruses.

Several mechanisms of biological control that microorganisms may be employed on plant pathogens weakening or destroying them have been identified. Antibiosis is the inhibition or destruction of one organism by substances (e.g. metabolites, lytic agents, enzymes, etc) produced by an antagonist (Jamalizadeh et al., 2011). Competition occurs when the antagonist competes for limited space or nutrients more efficiently than the pathogen in and/or around the host (Sharma et al., 2009; Francesco et al., 2016). Parasitism or predation occurs when an antagonist is able to attack by either directly killing the pathogen or targeting its propagules (Heydari and Pessarakli, 2010). Induction of the plant defensive machinery, activating pathogenesis-related (PR) proteins (e.g. lytic enzymes, structural proteins, enzymes) involved in hypersensitivity reactions is another mechanism by which antagonists can minimize the disease

incidence (Alves-Santos and Diez, 2011), but this will be treated separately in this review.

As virtually occurs with all the alternative control-measures reviewed in this paper, biological control techniques as the sole control measure is often not effective enough, especially in forests, where complex the biotic and abiotic factors can largely affect the efficiency of the antagonists. One notable exception is the use of the basidiomycete competitor *Phlebiopsis gigantea* (Fr.) Jülich against Heterobasidion root and butt rot caused by *Heterobasidion annosum*. The products Rotstop™ and PG Suspension™ are used commercially in Scandinavia on *Pinus* and *Picea* spp. and *Pinus* spp. in eastern areas of Britain (Pratt et al., 2000). Biological control (and other control approaches) however, is more commonly considered as one component of an integrated management approach (Wingfield et al., 2015; Cazorla and Mercado-Blanco, 2016).

2.1. Endophytic fungi

Microbial diversity, in particular fungi, has been poorly studied, in comparison to macroscopic life forms. Of the currently estimated 5,1 million fungal species on Earth, only 70,000 fungi have been described (Blackwell, 2011). Furthermore, data from of high-throughput sequencing methods have resulted in the estimates the number of fungal taxa on earth from an initial 1.5 million to the ca. 5.1 million (Hawksworth and Rossman, 1997; Blackwell, 2011). And the recognition of many new and cryptic species suggests that the 5.1 million fungi is likely low.

Endophytic fungi are able to live internally within plant tissues, either intercellularly or intracellularly, during at least parts of their life cycle, without causing visible symptoms to their host plants (Petrini, 1991; Wilson, 1995; Saikkonen et al., 1998). Many

141 endophytic fungi can promote growth and provide increased tolerance towards biotic
142 and abiotic stresses (Pirttilä and Frank, 2011; Sanz-Ros et al., 2015). There is increasing
143 evidence that the way by which endophytes induce plant defense reactions, and so
144 conferring a higher tolerance to pathogens, is that interactions between beneficial
145 microorganisms and plants trigger an immune response in plants similar to that against
146 pathogens (discussed below section 3.4) (Zamioudis and Pieterse, 2012) These
147 endophytic fungi have therefore been identified as potentially valuable tools for
148 biological control (Schulz et al., 2002; Strobel, 2003; Backman and Sikora, 2008;
149 Busby et al., 2015).

150 Many endophytic fungi have coevolved with their hosts (Krings et al., 2012) and have
151 been shown to be present in all forest trees in temperate zones (Saikkonen et al., 1998;
152 Sieber, 2007; Partida-Martínez and Heil, 2011). The use of endophytes as biological
153 control agents have mainly been tested in agricultural plant pathosystems, but more
154 recent studies have also demonstrated that endophytes could be used as modifiers of
155 forest diseases (Evans et al., 2003; Martínez-Alvarez et al., 2012; Santamaría et al.,
156 2012; Blumenstein et al., 2015; Romeralo et al., 2015; Martínez-Álvarez et al., 2016;
157 Terhonen et al., 2016).

158 *Trichoderma* species are the best studied fungal biological control agents (BCAs) and
159 are commercially marketed as biopesticides (Vinale et al., 2008; Hermosa et al., 2012).
160 The wide range of specific biocontrol mechanisms for species in the genus is likely
161 linked to the broad spectrum of pathogens on which the *Tricoderma* species has
162 demonstrated its properties as a BCA. This includes species from the genus *Fusarium*,
163 *Rhizoctonia*, *Botrytis*, *Sclerotinia*, *Phytopththora*, and *Phytium*, among many others
164 (Lo, 1998; Howell, 2003; Benítez et al., 2004; Harman et al., 2004; Vinale et al., 2008).

Besides the beneficial role of *Trichoderma* species on plant growth and productivity is universally accepted, a few reports also exist describing a negative effect on plants (Menzies, 1993; Marín-Guirao et al., 2016).

Several *Trichoderma* species, including *T. harzianum* Rifai, *T. viride* Pers., *T. atroviride* P. Karst, *T. asperellum* Samuels, Lieckf. & Nirenberg, *T. virens* von Arx and *T. spirale* Bissett, have been also tested as BCAs against *F. circinatum* (Iturritxa et al., 2011; Moraga-Suazo et al., 2011; Martínez-Alvarez et al., 2012; López-López, Segarra, Vergara, López-fabal, et al., 2016; Martínez-Álvarez et al., 2016). Evidence of the antagonism by *Trichoderma* species towards *F. circinatum* was consistent in *in vitro* experiments. In fact, *Trichoderma* species exerted a significant inhibition of the pathogen growth in *in vitro* assays which ranged from 40-60 % (Moraga-Suazo et al., 2011; Martínez-Alvarez et al., 2012) and up to a 100 % (Iturritxa et al., 2011). However, the antagonism effect of *Trichoderma* species *in planta* was not conclusive. López-López et al., (2016) found that the presence of *T. asperellum* will lead to a reduction in disease incidence of *Pinus radiata* D. Don seedlings both in pre and post-emergence. On the contrary, Martínez-Alvarez et al., (2012) did not find any antagonism exerted by *T. viride* towards *F. circinatum* in *P. radiata* seedlings. This controversy is likely due to the timing of the application of the *Trichoderma* strain, since Martínez-Alvarez et al., (2012) applied the pathogen and the BCA at the same time, and the importance of adding the *Trichoderma* strain at least 7 days prior to *F. circinatum* inoculation has been already reported (Iturritxa et al., 2011; Moraga-Suazo et al., 2011). Accordingly, the application of the same *Trichoderma* strain used by Martínez-Alvarez et al., (2012) one week before *F. circinatum* inoculation has showed promising results on Romanian provenances of *Pinus mugo* Turra and *Picea abies* (L.)

189 Karst. (Martín-García et al., 2017). On the other hand, efficient *Trichoderma* strains in
190 *in vitro* experiments did not exert any antagonism towards *F. circinatum* in the field
191 Martínez-Álvarez et al., (2016), which could be due to the seedling age (two-year-old),
192 environmental conditions or the fact that the endophyte was inoculated in the stem
193 instead of being inoculated in the substrate, taking into account that *Trichoderma* is in
194 general a genus of soil fungi (Samuels, 2006). In this regard, López-López et al., (2016)
195 demonstrated that the microbiota present in the substrate can largely modulate the
196 antagonistic effect exerted by *T. asperellum*. Furthermore, Mitchell et al., (2004)
197 reported that the initial positive response to the *T. harzianum* application in the field
198 diminished rapidly after 180 days. Further studies carried out in the field are therefore
199 needed to unravel the effect of these and other abiotic and biotic factors before
200 implementing the application of *Trichoderma* species as BCAs on *F. circinatum*.

201 Little is known regarding the mechanisms involved in the antagonism by *Trichoderma*
202 spp. on *F. circinatum*. Moraga-Suazo et al., (2011) demonstrated that the capacity of
203 *Trichoderma* spp. to grow rapidly within plant tissues provides these species with a
204 competitive advantage for nutrients and space over *F. circinatum*. This mechanism of
205 "competition" had been already reported for other pathogens, including species in the
206 genus *Fusarium* (Sivan and Chet, 1989; Benítez et al., 2004; Vinale et al., 2008).
207 However, Moraga-Suazo et al., (2011) did not find parasitism in this study, despite the
208 fact that it is well known that *Trichoderma* spp. are able to parasitize other fungi,
209 including hyphae penetration and subsequent dissolution of the host cytoplasm (Howell,
210 2003; Brotman et al., 2010). In fact, Lima et al., (2016, 2017) found several proteins
211 secreted by *T. atroviride* and *T. harzianum*, which have been previously related to
212 mycoparasitism processes. Similarly, several studies have demonstrated that antibiosis

213 is an antagonism mechanism of *Trichoderma* spp. for other *Fusarium* species (Calistru
214 et al., 1997; Ferre and Santamarina, 2010), no information is available for
215 *F. circinatum*. Finally, *Trichoderma* species are known to induce plant defense
216 responses against numerous pathogens (Yedidia et al., 2003; Hoitink et al., 2005;
217 Shoresh et al., 2005; Hibar et al., 2007), but their effect against *F. circinatum* remains to
218 be tested.

219 Several endophytic fungi have also been tested as BCAs on *F. circinatum*. Moraga-
220 Suazo et al., (2011) found that *Clonostachys rosea* (Preuss) Mussat was able to parasite
221 hyphae and reduce the mycelial growth of *F. circinatum* in *in vitro* assays. This study
222 also demonstrated that this endophyte was also able to reduce post-emergence mortality
223 and increase *P. radiata* seedling survival from 5 % to 69 %. More recently, Martínez-
224 Álvarez et al., (2016) found that 138 out of 154 isolates tested from a broad range of
225 genera (*Diaporthe* sp., *Bionectria* sp., *Phomopsis* sp., *Biscogniauxia* sp., *Truncatella*
226 sp., *Macrophomina* sp., *Nectria* sp., *Alternaria* sp., etc) showed an antagonism effect
227 towards *F. circinatum* in *in vitro* experiments. These authors also reported a reduction
228 on the damage caused by *F. circinatum* at field conditions in two-year-old *P. radiata*
229 seedlings previously inoculated with *Chaetomium aureum* Chivers and *Alternaria* sp,
230 which showed a better antagonistic effect in vivo than the four *Trichoderma* species
231 tested. Growth inhibition of *F. circinatum* by *Micromonospora* strains isolated from
232 alfalfa root nodules was also reported by Martínez-Hidalgo, García, & Pozo (2015).
233 These strains were effective to reduce leaf infection caused by *Botrytis cinerea* Pers. in
234 different tomato cultivars as a result of stimulating systemic resistance, but
235 unfortunately they have been not tested on pine seedlings.

Endophytic fungi associated with phloeophagous insects colonizing *Pinus radiata* (*Penicillium chrysogenum* Thom and *Fusarium lateritium* Nees), were both capable of outcompeting *F. circinatum* (Romón et al., 2008). *Penicillium chrysogenum* is well-known for producing large amounts of secondary metabolites with antifungal activity (e.g. penicillins, hypocrellin B, etc) (Meng et al., 2011). Likewise, *F. lateritium* produces several enniatins metabolites, which exhibit strong antifungal activity against *Eutypa armeniacae* Hansf. & Carter (Tsantrizos et al., 1993).

2.2. Endophytic bacteria

Endophytic bacteria, and most likely also endophytic fungi, are characterized by changes in gene expression or regulation of metabolic pathways as compared to their pathogenic equivalents (López-Fernández et al., 2015). This is thought to be due to both, their adaptation to plant tissues and the influence of particular environmental conditions (Schulz and Boyle, 2005). Endophytic bacteria have been traditionally applied in agricultural soils as plant growth-promoters (Rovira, 1965; Souza et al., 2015) and BCAs of plant diseases (Weller, 1988; Alström and Van Vuurde, 2001; Compant et al., 2005). Although the use of endophytic bacteria to control *Fusarium* species is common in agricultural crops (Chen et al., 1995; Singh et al., 1999), it has not been widely applied for the control of PPC.

The available information on endophytic bacteria as BCAs of forest tree diseases is comparatively scarce. Iturritxa et al., (2017) demonstrated that three bacterial species isolated from the endorhizosphere (i.e. *Pseudomonas fluorescens* Migula, *Bacillus simplex* ex Meyer & Gottheil and *Erwinia billingiae* Mergaert) reduced the mycelial growth of *F. circinatum* and the lesion length of two-year-old *P. radiata* seedlings inoculated with *F. circinatum* by 17%–29% and 22%–25%, respectively. Likewise,

Soria, Alonso, & Bettucci (2012) found that *Bacillus subtilis* (Ehrenberg) Cohn and *Burkholderia* sp. inhibited the *F. circinatum* growth *in vitro*. The results from such *in vitro* experiments should be interpreted with caution, but the fact that other studies have also demonstrated the benefit of endophytic bacteria to control pathogens in woody plants (Brooks et al., 1994; Ren et al., 2013; Cazorla and Mercado-Blanco, 2016) emphasizes the potential of these bacteria for practically controlling *F. circinatum*. Future research should thus seek to unravel all aspects related to the use of endophytic bacteria as BCAs, including the safety for human and animal health and for the environment (Vílchez et al., 2016) and the stability of the bacterial genome, since horizontal acquisition of virulence factors may have implications in their use as BCAs (Kelly et al., 2009; Ubhayasekera and Karlsson, 2012).

2.3. Viruses

Mycoviruses are intracellular parasites of fungi, which spread mostly via fungal cell-to-cell contact, i.e. anastomoses. Most of them are composed by dsRNA, while lineal ssRNA and circular DNA genomes are less represented (around 30%) (King et al., 2012). They can be used in virocontrol (biocontrol with viruses) of fungal plant pathogens as was demonstrated by the use of hypoviruses to control the Chestnut blight fungus, *Cryphonectria parasitica* (Murrill) Barr, in Europe (Grente, 1965). The success of chestnut bight virocontrol has inspired researchers throughout the world to search for viral control agents against other fungal diseases, leading to the discovery of many novel virus taxa that mediate hypovirulence in plant pathogenic fungal species (Huang and Ghabrial, 1996; Lakshman et al., 1998; Preisig et al., 2000; Chu et al., 2002; Deng et al., 2003; Kanematsu et al., 2004; Chiba et al., 2009; Yu et al., 2010). In this sense, protoplast fusion is a promising methodology that has achieved the transmission of

hypovirulence caused by viral infection in *Fusarium graminearum*, to other *Fusarium* species (Lee et al., 2011). It should, however, be noted that most of the known mycoviruses have very little, variable or not effects on their hosts (e.g. Hyder et al., 2013; Vainio, Korhonen, Tuomivirta, & Hantula, 2010). Sometimes they may even be considered as mutualists of their hosts (Márquez et al., 2007). Thus, only a tiny fraction of fungal viruses may be considered as potential control agents.

In order to develop an efficient virocontrol application for fungal diseases of plants, the following steps should be taken. Firstly, a virus with the potential to reduce the damage caused by the target pathogen, must be identified. Secondly, a cost efficient method to disseminate the virocontrol agent into the pathogen population should be developed. And thirdly, the level of natural resistance or tolerance against the virocontol agent in nature should be low enough to allow infection of most of the fungal strains.

Fusarium circinatum has been shown to host several species or types of mitoviruses (Martínez-Alvarez, Vainio, et al., 2014; Vainio et al., 2015). However, the role that they potentially play with regards to pathogen virulence is not clear. While they seem to enhance the virulence of *F. circinatum* on one-year-old *P. radiata* seedlings (Muñoz-Adalia et al., 2016), the mycelial growth of *F. circinatum* colonies and spore germination was significantly reduced by the presence of mycoviruses in *in vitro* conditions (Flores-Pacheco et al., 2017). However, much of our understanding of the occurrence of hypovirulence causing viruses on *F. circinatum* remains unknown. This is mostly linked to the fact that the occurrence of these viruses in *F. circinatum* populations in natural distribution areas or introduced areas outside Europe, have not been thoroughly investigated. In addition, viruses other than mitochondrial viruses may have remained undetected thus far.

Development of a cost efficient method for spreading the identified viruses is also complex. The work-intensive manual treatment used in chestnut trees is far too expensive to be used for other forest trees forming continuous and large forests. The nature of *F. circinatum* as a stem canker disease with separate infections on each tree also complicates spreading of intracellular viruses for control purposes. However, the clonal population structure of *F. circinatum* in many areas of introduction (Hammerbacher, 2005; Berbegal et al., 2013) is expected to be a strong advantage for the natural spread of mycoviruses. In fact, active virocontrol methods are no longer required to delimit the spread of Chestnut blight in parts of Italy. This resulted from treatments during several decades, which have increased the frequency of hypoviruses up to a level where each new infection is rapidly attenuated by a natural secondary infection by a hypovirulent *C. parasitica* strain. In other words, the substantial artificial control has turned into a natural disease control with no further treatment costs.

The third step to be taken, the level of natural resistance, has not turned out to be a problem in Chestnut blight virocontrol. Therefore, although resistance and tolerance may be expected to occur against viruses of *F. circinatum*, their effects and evolution should be studied before any conclusions can be made.

3. **Other environmentally-friendly applications for controlling *F. circinatum***

3.1. Thermotherapy treatments

Fusarium circinatum can be transmitted through infested seeds and this is likely to be a major source of nursery infections (Storer et al., 1998; Wingfield et al., 2008). Seeds can carry the pathogen not only externally/superficially, where only the seed surface carries inoculum but also internally/non-superficially where the pathogen is carried

inside the endosperm (Storer et al., 1998; Wingfield et al., 2008). The non-superficial infection can be either active or latent, where seeds germinate and produce asymptomatic seedlings from which the pathogen can be isolated (Storer et al., 1998; Mitchell et al., 2004; Kim et al., 2008; Vivas, Zas, et al., 2012; Elvira-Recuenco et al., 2015). The use of non-infected seeds is obviously the most effective measure to prevent pitch canker in nurseries and to avoid the introduction of *F. circinatum* into areas currently free of the disease (Agusti-Brisach et al., 2012; Berbegal et al., 2015). Numerous treatments have been proposed to eliminate infection from seeds. Chemical treatments, including fungicides, can effectively reduce surface contamination of pine seeds by *Fusarium* spp. (Dumroese et al., 1988; Runion and Bruck, 1988; Allen et al., 2004). However, few can penetrate the seed coat and reduce internal seed contamination without a significant negative effect on seed germination (Agusti-Brisach et al., 2012).

Hot water treatment (HWT) allows heat to penetrate the seeds and control non-superficial infections (Baker, 1962; Gratwich and Southey, 1986; Grondeau et al., 1994). HWTs have been shown to successfully control a number of seed-borne pathogens, including other *Fusarium* species, without a significant negative effect on seed germination (e.g. Bennett & Colyer, 2010; Hermansen, Brodal, & Balvoll, 2000; Toit & Hernandez-Perez, 2005). Jones et al., (2002) reported promising preliminary results of HWT against *F. circinatum* in the southern USA. Immersion of *Pinus palustris* Mill. seeds in 60°C water for two minutes reduced *F. circinatum* to trace levels while still producing high levels of plantable seedlings.

A more extensive study by Agusti-Brisach et al., (2012), using four Spanish isolates of *F. circinatum* and *P. radiata* seeds showed significant survival rate differences between mating types and individual isolates of *F. circinatum* to HWT both in terms of

maximum temperature and duration of the heat treatment. The Spanish MAT-2 isolates were more sensitive to HWT temperatures than the Spanish MAT-1 isolates. Temperatures above 51°C and 50°C were lethal to mycelium of MAT-1 and MAT-2 isolates, respectively, when exposed for 30 minutes. *Pinus radiata* seed germination was reduced with increasing HWT temperatures and duration but this reduction remained below a 30% for temperatures below 53°C. Therefore Agusti-Brisach et al., (2012) concluded that a HWT of 51-52°C for 30 minutes can be used to substantially reduce *F. circinatum* contamination of *P. radiata* seeds. This was also true for the naturally infected *P. radiata* seeds tested in the study, with an incidence of 1 % seeds infected with *F. circinatum* treated at 50°C for 30 minutes in seed lot 1 or 52°C for 45 min in seed lot 2 (Agusti-Brisach et al., 2012).

Iturritxa et al., (2011) demonstrated that *F. circinatum* was totally eliminated from artificially inoculated (and therefore only superficially infected) *P. radiata* seed coat, embryo and gametophyte after a thermotherapy treatment of 55 °C for 8 hours or longer, with no decrease in seed germination. Berbegal et al., (2015) also found *F. circinatum* infection of artificially inoculated *P. radiata* seeds to be negligible after HWT at 52°C for 30 minutes. However, they pointed out that percentage of germination could be reduced as a result of thermotherapy treatment. In the study they found that the HWT combined with subsequent fungicide treatments did not significantly reduce infection compared to the HWT alone. Overall, the results compare well with those of Dumroese et al., (1988) who worked with *P. menziesii* var *glauca* (Douglas fir) seeds infected with unspecified *Fusarium* spp. causing root disease in nurseries in North America. They found that microwave HWT for 90 seconds reaching a maximum of

55.5°C reduced *Fusarium* species in seeds to negligible levels (0.4%) while not significantly reducing seed germination.

The differences observed in seed germination rates obtained in the different studies may be due to initial water soaking for 24 h (prior to hot water treatment) carried out in the studies reporting reduction of germination rates (Agusti-Brisach et al., 2012; Berbegal et al., 2015), while studies not reporting reductions in germination rates (Dumroese et al., 1988; Iturritxa et al., 2011) did not used this initial water soaking but directly applied the hot water treatment, even using higher temperatures and duration of the treatments. This initial 24 hours soaking (priming) stimulates germination process by partial hydration of internal seed tissues, stimulating the activity of starch degrading enzymes and making sugars available for embryo growth (Ashraf and Foolad, 2005; Farooq et al., 2006), and is commonly used as pre-germination treatment for different pine species (Larson and Schubert, 1969) reason why germination could be affected by hot water treatments after soaking seeds 24 hours in water, although further studies are required to confirm it.

These studies show the promise of HWT as a cost-effective, non-toxic approach to reduce *F. circinatum* infection in seeds to a negligible level. However, in many occasions, complete elimination of the pathogen, in the light of the large divergence in outcomes, may be possible only at temperatures detrimental to seed germination. In addition, the temperatures seeds can be exposed to before unacceptable reduction in seed germination occurs will vary between species and provenances, thus complete elimination of *F. circinatum* from seeds of certain species/provenances may be possible using HWT. On the other hand, dielectric heating treatments, microwave and radio frequency technologies, has been used successfully to eradicate other *Fusarium* species

402 in agricultural seeds and, therefore, they could be promising techniques to control *F.*
403 *circinatum* (Leal et al., 2010).

404 Wood material can also be an important source of inoculum of *F. circinatum*, with trade
405 of wood products putatively contributing to long-distance dispersal of the disease
406 (EFSA, 2010). Studies carried out on blocks of wood (30 mm × 10 mm × 5 mm), found
407 that although 56 °C for 30 minutes or longer, reduced the survival of *F. circinatum*,
408 negligible levels (0.1-1 %) would not be obtained until an exposure for 30 minutes to a
409 minimum temperature of 61.7 °C or 68.9 °C (Ramsfield et al., 2010).

410 *3.2. The use of atmospheric pressure non-thermal plasma technology*

411 Atmospheric pressure non-thermal plasma (NTP) is an emerging field that has
412 investigated the application of physical plasma in environmentally way of green
413 biotechnology. In medicine and biology, the term plasma means the liquid component
414 of blood, it is extracellular matrix of blood cells. On the other hand, physical plasma is
415 considered to be the fourth state of matter and it is the most abundant state in the
416 Universe. It is not a human invention, and is present in nature, as fire in the sun, stars, in
417 the tails of comets and as flashes of lightning (Conrads and Schmidt, 2000). The plasma
418 consists of many active particles, radicals, electrons, metastabiles, ions, and radiation.
419 NTP is distinguished from thermal plasma. NTP is not in thermodynamic equilibrium,
420 either because the ion temperature is different from the electron one, or the velocity
421 distribution of one of the species does not follow a Maxwell–Boltzmann distribution
422 (Sankar et al., 2017).

423 The parametric study of plasma for sterilization is of importance in understanding and
424 controlling the deactivation of microbes, because the main sterilizing factors are

strongly dependent on the plasma source type and/or the plasma characteristics (Cheruthazhekatt et al., 2010). Nowadays, atmospheric pressure NTP is more frequently used for the sterilization of both living and non-living materials (Lerouge et al., 2001; Fridman et al., 2008; Moreau et al., 2008; Cheruthazhekatt et al., 2010; Scholtz et al., 2015). It was found that many microbes are inactivated after NTP treatment, bacteria (Baier et al., 2014; Mráz et al., 2014; Ziuzina et al., 2014), fungi (Selcuk et al., 2008; Dasan et al., 2016) and viruses (Wang et al., 2016).

The idea to use plasma to sterilize the surface of seeds dates back to the beginning of the Millennium (e.g. Basaran et al., 2008; Selcuk et al., 2008; Mitra et al., 2014; Zahoranová et al., 2016). NTP (air gas and sulfur hexafluoride) was used for decontamination of haselnuts (*Corylus* sp.), peanuts (*Arachis hypogaea*) and pistacio (*Pistacia vera*) against *Aspergillus parasiticus* and aflatoxins at the nuts surfaces (Basaran et al., 2008). Seeds of wheat (*Triticum durum*), bean (*Phaseolus vulgaris*), chick pea (*Cicer arietinum*), soybean (*Glycine max*), barley (*Hordeum vulgare*), oat (*Avena sativa*), rye (*Secale cereale*), lentil (*Lens culinaris*), and corn (*Zea mays*) were contaminated with spores of *Aspergillus paraciticus* 798 and *Penicillum* MS1982, and then treated by NTP (Selcuk et al., 2008). Depending on the seed surface, plasma gas type, plasma treatment time, and the microbial population density, the percentage of seed infection was reduced to below 1%, without lowering the seed quality below the commercial threshold of 85% seed germination under described plasma conditions (Selcuk et al., 2008).

In the other experiment (Mitra et al., 2014), a significant reduction of the seedborne microbial contamination was observed after 120 s and 300s of NTP treatment in seeds of chick pea (*Cicer arietinum*). Exposure of NTP treatment of 60 s showed an improved

449 seed germination (89.2 %), speed of germination (7.1 ± 0.1 seeds/day), and increased
450 seed vigor, beside a decrease in the mean germination time (2.7 days) compared with
451 controls. In the other experiment (Zahoranová et al., 2016), effect of NTP on wheat
452 seeds artificially infected with pure cultures of filamentous fungi isolated from surfaces
453 of untreated wheat seeds was determined on dead wheat. Result shows the efficiency of
454 the treatment of seeds decreased in the following order: *Fusarium nivale* > *F. culmorum*
455 > *Trichothecium roseum* > *Aspergillus flavus* > *A. clavatus*.

456 The interest of this paper is connected above all on *Fusarium* sp. inactivation, so results
457 of Zahoranová et al., (2016) suggest that NTP technology is good way. NTP causes
458 mortality of *F. culmorum* deposited on polymeric materials surface, the mortality was a
459 result of cell membrane cracking (Stepczyńska, 2016). Above all, it was found that *F.*
460 *oxysporum* f.sp. *lycopersici* (susceptible host plant species *Solanum lycopercicum*) was
461 inactivated after 10 minutes in atmospheric pressure NTP (working gas was Ar)
462 (Panngom et al., 2014).

463 Testing of deactivation of *Pinus radiata* seed surfaces infected by *F. circinatum* was
464 performed by NTP (air gas) for 0 s, 5 s, 10 s, 60 s, 180 s, and 300 s (Sera et al.; unpubl.
465 data). Significant differences were found between the reference sample (0 s) and seed
466 samples without the inoculation and seed samples treated with 60 s of exposure (or
467 more). Infected seeds treated with 60 s (or more seconds) of NTP exposure remained
468 free of mould infection for 12 days of cultivation on an agar surface in Petri dishes.
469 Unfortunately, germination of seeds was observed only in short time exposures. The
470 following research strategy will deal with the methodology elaboration of seed
471 sterilization so that the seeds of pine will retain vitality (germination).

This introduction of alternative environmentally and economically advantageous methods of adjustment of seed would allow to reduce the quantity of fungicides and contribute to the reduction of unwanted residues of xenobiotics in the plants and environment (Zahoranová et al., 2016). This suggests that atmospheric pressure NTP can be efficiently used to control plant fungal diseases by inactivating fungal pathogens and up-regulating mechanisms of host resistance.

3.3. The use of natural compounds

The use of natural compounds for disease mitigation and control of *F. circinatum* is another control measure that is receiving increasing attention. Several compounds derived from plants and other organisms are known to act directly on the pathogen with a fungicidal effect or inhibit the production of mycotoxins by the pathogen.

i. Natural compound with fungicide effect

Isothiocyanates (ITCs) compounds, released during the hydrolysis of glucosinolates (GSL) in cruciferous plants by the endogenous enzyme myrosinase (thioglucoside glucohydrolase, E.C. 3.2.3.1) (Kirkegaard et al., 2000), can control fungi, oomycetes and bacteria. Pure ITC have been shown to be effective in the suppression of mycelial growth and conidia germination of *Fusarium oxysporum* being the conidia the most sensitive to the treatment (Ramos García et al., 2012). Similarly, Smolinska et al. (2003) reported that the treatment with propenyl and ethyl isothiocyanates inhibited mycelial growth, and completely suppressed conidial and chlamydospore germination of all *F. oxysporum* Schltdl. isolates tested. Other isothiocyanates, including ethyl, benzyl, and phenethyl, also displayed a fungitoxic action against *F. oxysporum* conidia and chlamydospores. Based on these results, Smolinska et al. (2003) suggested that

pathogenic *F. oxysporum* isolates infesting nursery soils could be likely suppressed by glucosinates contained by plant species such as *Brassica carinata* A. Braun, *B. nigra* (L.) Koch, and *B. juncea* (L.) Coss.. Reduction in pathogen populations resulting from a green-manure crop are likely achievable since chlamydospores are sensitive to ITCs. In this sense, experiments with commercial *B. carinata* pellets have recently been carried out showing (a reduction of mycelial growth or a fungitoxic action against?) fungicide on mycelial growth of *F. circinatum* (Morales-Rodriguez, C; unpublished data). Application of gorse compost (*Ulex europaeus* L.) obtained from forest cleaning green wastes also decreased the incidence of *F. circinatum* disease when used as a growth medium for *P. radiata* seedlings, but the mechanism involved in this effect stills need to be investigated (López-López, Segarra, Vergara, López-Fabal, et al., 2016).

Plant essential oils may also provide alternatives to the synthetic fungicides currently used for phytopathogenic fungi control. They are a rich source of bioactive molecules (Christaki et al., 2012) and a large number of them have been reported to have antifungal activities (Papaefthimiou et al., 2014). *Leptospermum petersonii* Bailey essential oil, for example, showed antifungal activity against *F. circinatum*, inhibiting mycelial growth (Lee et al., 2008). Essential oils of manuka (*Leptospermum scoparium* J.R. Forst. & G. Forst.) and patchouli (*Pogostemon patchouli* Pellet.) also showed moderate activity against *F. circinatum* (Lee et al., 2009). Iturritxa et al., (2011) screened the effectiveness of several essential oils against *F. circinatum* and they found that the most effective oils were from oregano, Japanese mint and cinnamon, all being 100% inhibitory at their lowest tested dose (10%). Cinnamon, fennel, and clove oils also reduced or inhibited (partially or completely) the *in vitro* growth of *F. circinatum*, showing both fungicidal and fungistatic effects (Iturritxa et al., 2017). This antagonistic

519 effect was demonstrated in infected seeds of *P. radiata* using thymus oil (Iturritxa et al.,
520 2011) and in two-year-old *P. radiata* seedlings using cinnamon, clove or a mixture of
521 both with promising results on the control of *F. circinatum*, while phytotoxicity was
522 caused to one year plant tissues (Iturritxa et al., 2017).

523 Another natural compound that has been widely used for its antiseptic properties for
524 plant protection is propolis (Özcan et al., 2004). In fact, propolis presented a high
525 capacity to inhibit the mycelial growth (68 %) of *F. circinatum* (Silva-Castro et al.,
526 2017). However, the resin obtained from *P. radiata* and five monoterpene components
527 of resin (limonene, α-pinene, β-pinene, camphene, and myrcene) did not inhibit
528 mycelial growth, or affected the germination and/or survival of spores of *F. circinatum*
529 (Slinski et al., 2015).

530 The application of natural compounds, such as essential oils or propolis, seems to be a
531 promising alternative to be implemented in an integrated management approach.
532 However further research is needed to understand the mechanisms involved in this
533 protective effect and know whether there is a phytotoxic effect on the host (Iturritxa et
534 al., 2017), as well as the best method of application of these compounds to plants of
535 different developmental stages.

536 *ii. Inhibition of mycotoxin production*

537 The possible role of natural phenolic compounds in inhibiting fungal growth and
538 mycotoxin production has been of recent interest as an alternative strategy to the use of
539 chemicals. The most important *Fusarium* mycotoxins are trichothecenes, zearalenone,
540 and fumonisins (Ferruz et al., 2016), of which deoxynivalenol, nivalenol, T-2 toxin and
541 fumonisin B_1 are the most produced (Desjardins and Proctor, 2007; Sobrova et al.,

2010), although fusaric acid, beauvericin, enniantin, equisetin, fusarin and many other toxins are produced by *Fusarium* spp. as well (Marasas et al., 1984; Desjardins and Proctor, 2007). Although *Fusarium* species differed in their responses to phenolic acid treatments, significant reductions in toxin concentrations were observed for *Fusarium* trichothecene mycotoxins T-2 and HT-2 (90% reduction) and zearalenone (48 to 77% reduction) (Ferruz et al., 2016). Moreover, the addition of phenolic acids to corn meal agar had a marked dose-dependent inhibitory effect on the radial growth of *Fusarium* species, causing total inhibition in some of the tested species (Ferruz et al., 2016). Boutigny et al., (2010) investigated the *in vitro* effect of natural phenolic acids from wheat bran on type B trichothecene biosynthesis by *Fusarium culmorum* Sacc. . Durum wheat bran contained various monomeric forms of phenolic acids, with ferulic acid being the most abundant. When liquid cultures of *F. culmorum* were supplemented with a natural wheat bran extract, trichothecene production was fully inhibited with a drastic reduction in the expression level of structural trichothecene biosynthetic genes.

3.4. Inducers of resistance

The induction of plant resistance is a potential alternative approach to chemical control that has been widely explored in agriculturally important crop plants to manage plant health. Induced resistance (IR) represents a physiological state of enhanced defensive capacity stimulated by biotic or abiotic elicitors, whereby the plant's innate defenses are enhanced against subsequent challenges. This capacity for augmented defense offers a wide spectrum of protection against biotic and abiotic stresses (Mauch-Mani et al., 2017). Stimuli from pathogens, beneficial microbes, chemicals and abiotic cues can trigger the establishment of induced responses in pines that enhance their protection

against *F. circinatum* (Figure 1). If defense mechanisms are triggered by a stimulus prior to infection by a plant pathogen, disease can be reduced.

Induction of resistance has been observed in young plantations and natural forests of *P. radiata* and is known to be a key process in the interaction between pines and *F. circinatum* (Gordon et al., 2011; Reynolds, Gordon, & McRoberts, 2016). Repeated infection of the same tree resulted in progressively shorter lesions (Table 1), implying an effect of prior infection on susceptibility. This observation explains disease remission in long term monitoring plots (Gordon et al., 2001), in which numerous trees that were initially severely diseased were free of symptoms and infections three years later. Exposure of stems and roots of *P. radiata* seedlings to *F. circinatum* enhanced resistance to shoot infections by the same fungus and promoted survival following subsequent stem inoculations (Bonello et al., 2001; Gordon et al., 2011; Swett and Gordon, 2017). Elevated host resistance occurred both when seedlings were grown in infested sand and when exposure to inoculum resulted from emergence through infested leaf litter (Swett and Gordon, 2017). Being IR operative in nature, susceptibility in populations of *P. radiata* should decline over time following initial exposure to the pathogen.

Could this phenomenon be exploited? From a practical point of view, induction treatments on pines susceptible to the pitch canker disease could be performed in both the nursery and in the field.

In nurseries, resistance against damping off of emerging seedlings caused by *F. circinatum* could be induced by application of several abiotic and biotic stimuli to seeds and plants at the seedling stage (Table 1). Phosphite treatments in *P. radiata* seedlings displayed a significant increase of jasmonic acid (JA), abscisic acid (ABA),

589 and available sugars, and mounted a more robust defense against *F. circinatum* when
590 compared to control plants (Cerqueira et al., 2017). Studies of resistance inducers
591 indicate that some compounds, such as chitosan (Reglinski et al., 2004; Fitza et al.,
592 2013), may be effective in enhancing resistance to *F. circinatum*, whereas others, such
593 as BABA, Bion®, BTH, chitin, Kannar®, Messenger®, methyl jasmonate and salicylic
594 acid (SA) are ineffective (Vivas and Solla, 2012; Vivas, Martín, et al., 2012; Fitza et al.,
595 2013). However, the effectiveness of chitosan against *F. circinatum* seems to be limited.
596 Preventive chitosan application reduced disease incidence in *P. radiata* seedlings
597 inoculated with ca. 100 *F. circinatum* spores/wound, but did not so in seedlings
598 challenged with higher inoculum levels (500 and 8500 spores/wound). Moreover,
599 chitosan systemic induced resistance in seedlings against *F. circinatum* lasted ca 42
600 days in *P. radiata* (Reglinski et al., 2004) and 6 weeks in *Pinus patula* Schiede ex
601 Schltdl. & Cham. (Fitza et al., 2013). The effect of chitosan and phosphite could be due
602 not only to IR, but also to a fungistatic effect. This is consistent with the findings of
603 Silva-Castro et al., (2017) and Cerqueira et al., (2017) who demonstrated that chitosan
604 oligomers and phosphite salts, respectively, were able to inhibit the mycelial growth of
605 *F. circinatum* in *in vitro* conditions. This seems to suggest a direct (pathogen growth
606 inhibition) and indirect (host defense induction) action of chitosan and phosphite,
607 showing that both of them are promising compounds to be used in a potential strategy to
608 control *F. circinatum* infection.

609 As noted above, pine rhizobacterial isolates of *E. billingiae* and *P. fluorescens* protected
610 young *P. radiata* seedlings against *F. circinatum* (Iturritxa et al., 2017), probably as a
611 consequence of a combining effect of mycelial growth inhibition and IR.

612 An important point regarding the potential use of resistance inducers for field protection
613 of forest stands against a quarantine pest such as *F. circinatum* is that resistance
614 inducers just influence plant resistance traits but they do not guaranty inoculum
615 eradication. So, could symptomless infected seedlings with enahanced resistance be
616 used in plantations? And if so, what implications may have on the spread of the disease?
617 In sites where the pathogen is present, the introduction of induced plants could be used
618 to enhance the resistance of a forest. However, according to the current legislation of
619 most countries regulating quarantine pathogens, this strategy would not be allowed.
620 Nevertheless, eradication of *F. circinatum*, as established by current Regulations may
621 be rather complicate in many situations. In Spain, for example, removal and burning of
622 several *P. radiata* plantations was performed following national law RD 637/2006, with
623 costs above 6.000 euro ha^{-1}. Being roots, grasses and soil sources of inoculum, it is
624 mostly unlikely that the fungus would be completely eradicated from a forest. In such
625 cases, improving the resistance of the new plantations by the use of resistance inducers
626 will definitely help to minimize the impact of the disease.

627 Transgenerational induction of defenses is also a putatively way to increase the
628 resistance of seedlings. Recent advances in this field have revealed that the progeny of
629 mother plants subjected to a biotic challenge may be better defended than that of healthy
630 plants (Agrawal, 2002). Although the mechanisms of this non-genetic inheritance still
631 not well known, it is widely accepted that this is a taxonomically widespread
632 phenomenon (Holeski et al., 2012), that may also operates in pine trees (Vivas et al.,
633 2015). Moreover, the abiotic environment in which seeds are produced may also largely
634 affect the progeny performance (Cendán et al., 2013) including its resistance against
635 biotic threats (Vivas et al., 2013). Specifically, favorable abiotic conditions for *Pinus*

636 *pinaster* Ait. mother trees primed their seeds and protected germinating seedlings
637 against *F. circinatum*. Seedlings derived from seeds developed under stressful
638 conditions were more susceptible to *F. circinatum* than seedlings obtained from the
639 same genotypes growing under favorable conditions (Vivas et al., 2013). This result
640 suggests that seedlings would be more resistant towards *F. circinatum* if cones were
641 collected from vigorous trees.

642 Mechanical wounding of mature trees is also a well-known elicitor of induced defensive
643 responses in pines (e.g. Lombardero et al., 2000). Pine seedlings became less vulnerable
644 to subsequent inoculations after being wounded (Kim et al., 2010). In forests infested
645 with *F. circinatum*, however, wounding trees does not seem a good strategy to enhance
646 their resistance as wounds facilite pathogen access (Gordon et al., 2015). Long-term
647 disease remission in forests and plantations by IR seems a better disease management
648 strategy than pathogen eradication.

649 An additional practical consequence of IR in pines, relates to the identification and
650 selection of genotypes able to effectively enhance their resistance after priming (Gordon
651 and Reynolds, 2017). The absence of a negative impact on growth rate associated with
652 IR (Reynolds et al., 2016) suggests that IR may have utility as a tool for management of
653 pitch canker in plantations.

654 Although the occurrence of IR in the pitch canker-*P. radiata* pathosystem has been
655 well-documented, the mechanisms behind IR, the compounds involved in the priming
656 phase, and their efficacy over time are still under investigation. Future of control
657 strategies in forest ecosystems should be directed to an integrated system in which
658 biocontrol agents and natural products play a crucial role.

4. Exploiting genetic resistance against *F. circinatum*

Evidence is accumulating that the exploitation of genetic resistance is one of the most promising alternatives to manage the pitch canker disease (Wingfield et al., 2008; Mitchell, Coutinho, et al., 2012; Gordon et al., 2015). Variation in susceptibility occurs at all possible genetics levels, from among-species (e.g. (Gordon, Okamoto, et al., 1998; Enebak and Stanosz, 2003; Iturritxa et al., 2012; Iturritxa, Ganley, et al., 2013; Martínez-Alvarez, Pando, et al., 2014) to intraspecific genotypic variation (e.g. (Gordon et al., 2006; Matheson, Mark, et al., 2006; Hodge and Dvorak, 2007; Mitchell, Wingfield, Steenkamp, et al., 2012; Elvira-Recuenco et al., 2014), and the range of variation is extremely large. This large variation offers a unique environmentally-friendly and cost-effective opportunity for disease management. With no other absolute means of controlling *F. circinatum*, selection and/or deployment of resistant material for reforesting high risk sites is viewed as one of the few operative ways to reduce the impact of this devastating disease (Mitchell, Coutinho, et al., 2012; Hodge and Dvorak, 2014; Serra-Varela et al., 2017). There are several non-exclusive strategies to exploit the genetic variation in resistance within the genus *Pinus* (described below).

4.1. Selection of resistant species

The first and easiest solution is to select alternative resistant species that may substitute the ones commonly used in large-scale forestry (Gordon, Okamoto, et al., 1998; Hodge and Dvorak, 2000; Iturritxa, Ganley, et al., 2013). This alternative has receive special attention in countries of the Southern Hemisphere (e.g. South Africa, Chile and Colombia and Uruguay), where no native pines occur, and where the forestry industry mainly relies on exotic pine species that are highly-susceptible to *F. circinatum* (e.g. *P. radiata*, *P. patula*, *Pinus elliotii* Engelm.) (Viljoen et al., 1995; Roux et al., 2007; Porter

683 et al., 2009; Mitchell, Wingfield, Hodge, et al., 2012a; b). Independent studies
684 conducted in different countries (South Africa, USA, Spain, South Korea, Central
685 America and Colombia) and laboratories have consistently demonstrated that *P.*
686 *radiata*, *P. patula* and *P. elliotii* are highly susceptible, whereas *Pinus oocarpa* Schiede
687 ex Schltdl., *Pinus canariensis* C. Sm., *Pinus pinea* L. and *Pinus thunbergii* Parl. are
688 highly resistant (Gordon, Okamoto, et al., 1998; Hodge and Dvorak, 2000; Kim et al.,
689 2008; Steenkamp et al., 2012).

690 It is important to note that substituting the main planting species would be complicated
691 when the forestry industry is already well established, centered and fine-tuned around a
692 single or few species. This is for example the case in countries such South Africa, Chile
693 and New Zealand. The search of alternative species would not only imply the selection
694 of those that are resistant to the pathogen, but also those that are well adapted to the
695 local environmental conditions and that are suited to the current industry processes.

696 *4.2. Selection of breeding material from resistant populations*

697 A second strategy to reduce the impact of *F. circinatum* is to select highly resistant
698 populations within species (Gordon et al., 2006; Dvorak et al., 2007; Hodge and
699 Dvorak, 2007). Many pine species, especially those with isolated and fragmented small
700 populations, harbor large intraspecific genetic variation, with population differentiation
701 in many different life-history traits (Grivet et al., 2013), including resistance to biotic
702 threats (e.g. (Zas et al., 2015). Specifically, resistance to *F. circinatum* is known to vary
703 among populations in a wide array of pine species, including *Pinus leiophyla* Schiede
704 ex Schltdl. & Cham. (Dvorak et al., 2007), *P. patula* (Hodge and Dvorak, 2007; Dvorak
705 et al., 2009), *Pinus tecunumanii* Eguiluz & J.P.Perry (Hodge and Dvorak, 2007; Dvorak
706 et al., 2009), *P. oocarpa* (Dvorak et al., 2009), and *P. pinaster* (Iturritxa et al., 2012;

Elvira-Recuenco et al., 2014). In some occasions, among-population variation has been related to geographical and environmental gradients (Hodge and Dvorak, 2007; Dvorak et al., 2009). For example, populations of *P. tecunumanii* obtained from lower altitudes consistently showed increased resistance than those from higher altitudes (Dvorak et al., 2009). These environmental gradients may either be the result of contrasting disease pressures under different environmental conditions in species that naturally coevolved with the fungus (e.g. *P. tecunumanii*) (Dvorak et al., 2009) or the result of other demographic and adaptive processes that have led to correlated responses in *F. circinatum* resistance traits (Elvira-Recuenco et al., 2014).

4.3. Hybridization

Hybridization with resistant species or populations is another alternative that is receiving increasing attention, especially in South Africa, where hybridization between the susceptible *P. patula* and other resistant pine species, particularly *P. tecunumanii*, has been shown to be highly promising for minimizing the current impact of *F. circinatum* in *P. patula* plantations (Kanzler et al., 2014). Hybridization aims at combining the advantages of both parental species. Although interspecific hybrids were originally developed to improve the adaptation of *P. patula* to the particular environmental conditions in South Africa, it soon became evident that the hybrids were much more tolerant to *F. circinatum* than *P. patula*, especially when the highly resistant low elevation populations of *P. tecunumanii* were used for hybridization (Roux et al., 2007; Mitchell, Wingfield, Hodge, et al., 2012b; Kanzler et al., 2014). Other hybrids that have shown good results in terms of tolerance to *F. circinatum* are *P. elliottii* x *P. caribaea* Morelet and *P. patula* x *P. oocarpa* (Roux et al., 2007), although, in general hybridizations between *P. patula* and any other tolerant species improve the resistance

731 to *F. circinatum* (Mitchell, Wingfield, Hodge, et al., 2012b). Family variation within
732 hybrids has also shown to be high, so selection of the parents is a critical process, while
733 mass clonal propagation of selected hybrid offspring may result in substantial genetic
734 gains (Roux et al., 2007; Mitchell, Wingfield, Hodge, et al., 2012b). Vegetative
735 propagation of hybrid material has been shown to be feasible and relatively easy to
736 apply, facilitating the massive deployment of resistant material for reforestation (Ford et
737 al., 2014).

738 Tolerance of family hybrids between *P. patula* and *P. tecunumanii* seems to be more
739 affected by the specific interaction between the parents (Specific Hybrid Ability, SHA)
740 than to general effects of each of the parents (General Hybrid Ability, GHA) (Mitchell,
741 Wingfield, Hodge, et al., 2012b). This indicates that, for operational deployment of
742 hybrid material, selection of the best male and female parents should be accompanied
743 by a deep screening of as many as possible parental combinations. This is especially
744 true when hybridizing with high-elevation *P. tecunumanii* origins, for which large
745 family and within-family variation has been observed (Mitchell, Wingfield, Hodge, et
746 al., 2012b). On the other hand, hybrids with low-elevation origins of *P. tecunumanii*
747 much less variation (Mitchell, Wingfield, Hodge, et al., 2012b; Ford et al., 2014).

748 *4.4. Exploiting within-population variation*

749 Taking advantage of the large within-population variation that commonly occurs in
750 most of the pine species is the last alternative of exploiting genetic resistance as a tool
751 for minimizing the impact of *F. circinatum*. To date, there is ample evidence that
752 resistance to *F. circinatum* widely varied within populations, both in species regarded as
753 susceptible (e.g. *P. radiata*, *P. patula*) and in those considered resistant (e.g. *P.*
754 *tecunumanii*, *Pinus maximinoi* H.E.Moore). Variation in resistance appears to be

quantitative, and has been attributed to polygenic resistance mechanisms (Gordon, Wikler, et al., 1998; Kayihan et al., 2005; Quesada et al., 2010), that is, the result of the integrated action of many genes, each contributing with small effects (Telford et al., 2015).

Heritability estimates of *F. circinatum* resistance are quite variable depending on the species, the population within the species (e.g. (Mitchell, Wingfield, Hodge, et al., 2012a), the material tested (e.g. (Matheson, Devey, et al., 2006), and the screening procedure (e.g. (Aegerter and Gordon, 2006) (Table 2). Nevertheless, despite the relatively high variation in heritability estimates, the level of genetic control is commonly sufficiently high to allow for improving resistance through selection and breeding using classical procedures (Kayihan et al., 2005; Matheson, Devey, et al., 2006; Elvira-Recuenco et al., 2014; Mitchell et al., 2014; Nel et al., 2014). Relatively high coefficients of variation of resistance among genetic entries facilitate large selection differentials that can easily translate into significant genetic gains in resistance.

Gains in resistance can be obtained either by forward recurrent selections, in which the most resistant offspring is selected and breed to obtain new genotypes with increased resistance, or by backward selections, in which the best parents are selected upon the resistance of their progeny, and directly used for producing seeds for reforesting in highly risky sites (Telford et al., 2015). Forward selections are constrained by the long time required for completing the reproductive-selection cycle (and the just moderate narrow-sense individual heritability estimates, see Table 2), but it can lead to notable gains owing to larger differential selections and the opportunity to capture both additive and non-additive genetic variation. On the other hand, backward selections are faster

779 and straightforward to apply but genetic gains are typically not as high due to lower
780 differential selections. Large family heritability estimates (e.g. (Nel et al., 2014)
781 however, favors large genetic gains through family selection. The establishment of
782 depurated seed orchard with resistant selected parents or roughing current seed orchards
783 eliminating those parents that led to highly-susceptible offspring emerges, thus, as a
784 very easy, cheap, quick and operative action that can lead to substantial improvements
785 of *F. circinatum* resistance in new plantations (Vivas, Zas, et al., 2012; Nel et al., 2014).

786 The commercial deployment of resistant pine reproductive material through the
787 selection (and breeding) of species, populations, hybrids, families or genotypes is a real
788 and valuable option to improve *F. circinatum* resistance. The success of the breeding
789 initiatives developed in South Africa since the jump of the disease from the nurseries to
790 the field plantations, has been cataloged as a "story of major success in plantation
791 forestry" (Hodge and Dvorak, 2014), and is the best demonstration that exploiting
792 genetic resistance is a powerful tool against this disease. But this history of potential
793 success is not free of important drawbacks that need to be carefully attended. To
794 properly exploit genetic resistance against *F. circinatum*, special attention should be
795 paid to (i) improve and homogenize the current screening protocols (Oak et al., 1987;
796 Roux et al., 2007), (ii) develope genomic selection protocols (Quesada et al., 2010;
797 Moraga-Suazo et al., 2014; Donoso et al., 2015; Carrasco et al., 2017), (iii) obtain
798 durable and stable resistance across differential environmental conditions, *F. circinatum*
799 strains and tree ontogenetic stages, (iv) quantify the consequences of the putative trade-
800 offs among several life functions (e.g. growth-defense trade-off) (Matheson, Devey, et
801 al., 2006; Vivas, Zas, et al., 2012), and (v) explore other sources of variation with
802 genetic background like inducibility (Agrawal et al., 2002; Moreira et al., 2013),

maternal effects (Vivas et al., 2013) or the genetic variation in the attraction and susceptibility of trees to vectoring insects (Erbilgin et al., 2005).

5. Conclusions, future prospects and challenges: identifying gaps of knowledge

This review suggests that environmentally-friendly control methods could be successful implemented in an integrated management of pine pitch canker. However, plans for this integrated management should differentiate at least three protocols; *i)* avoiding new introductions and spread via plant material, *ii)* control in the nursery and *iii)* control in the field. Thermotherapy and non-thermal plasma technology may be integrated in the management plans developed to avoid the spread of the pathogen. Obviously these techniques should be considered as a valuable complement to rapid detection and efficient monitoring of *F. circinatum*, but not a substitute. In fact, the development of rapid and sensitive diagnostic techniques based on new molecular methods (e.g. High-Throughput Next Generation Sequencing Methods) or DTBIA (direct tissue blot immunoassay), commonly used for detection of viruses and bacteria, is a priority.

On the other hand, endophytic fungi and bacteria as well as the use of natural compounds and inducers of resistance could be implemented in the management plan for the control of the disease in the nursery. However, forest nursery managers should be also aware that their application may be useful to minimize the mortality of seedlings and promote plants more vigorous, but does not entirely remove, the risk of infection. Furthermore, special attention should be paid to the fact that their application might trigger an increase of asymptomatic infected plants. This applies in particular to countries where the pathogen is not widely established (e.g. Europe) and, therefore, avoiding the spread from nurseries to the field via latent, asymptomatic infections is crucial.

827 Exploiting genetic resistance, both selection of alternative species, breeding material
828 from resistant populations, hybridization and exploiting within-population variation,
829 seems to be the only feasible option for the control in the field do date. Further studies
830 are needed to test the susceptibility of alternative species and obtain resistant material,
831 especially in Europe, where the presence of *F. circinatum* is limited to the Iberian
832 Peninsula and a few *Pinus* species are currently affected. The application of viruses has
833 been successful implemented for the control of another forest pathogen in the field
834 (Chestnut Blight Disease) and, therefore, testing the potential hypovirulence of the *F.*
835 *circinatum* mycoviruses should be another priority line of enquiry.

836 Other gaps of knowledge identified in this review are:

837 • To define the temporal and spatial persistence of the different treatments in
838 different scenarios.

839 • To estimate the economic and environmental impact (positive and/or negative)
840 derived of the application of these environmentally-friendly control strategies
841 for pine pitch canker.

842 • To study the genetic background of *F. circinatum* and plant physiological
843 processes to unravel the mechanisms behind plant-fungi interaction, biocontrol
844 and priming strategies that can evidence potential tools.

845

846 **6. References**

847 AEGERTER, B.J., and T.R. GORDON. 2006. Rates of pitch canker induced seedling
848 mortality among Pinus radiata families varying in levels of genetic resistance to
849 Gibberella circinata (anamorph Fusarium circinatum). *Forest Ecology and*
850 *Management* 235: 14–17. Available at:

http://linkinghub.elsevier.com/retrieve/pii/S0378112706004890 [Accessed February 19, 2014].

AGRAWAL, A.A. 2002. Herbivory and Maternal Effects: Mechanisms and Consequences of Transgenerational Induced Plant Resistance. 83: 3408–3415.

AGRAWAL, A.A., J.K. CONNER, M.T. JOHNSON, and R. WALLSGROVE. 2002. Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. *Evolution Int J Org Evolution* 56: 2206–2213. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12487351.

AGUSTI-BRISACH, C., A. PEREZ-SIERRA, J. ARMENGOL, J. GARCIA-JIMENEZ, and M. BERBEGAL. 2012. Efficacy of hot water treatment to reduce the incidence of Fusarium circinatum on Pinus radiata seeds. *Forestry* 85: 629–635. Available at: http://forestry.oxfordjournals.org/cgi/doi/10.1093/forestry/cps074 [Accessed February 19, 2014].

ALLEN, T.., S.. ENEBAK, and W.. CAREY. 2004. Evaluation of fungicides for control of species of Fusarium on longleaf pine seed. *Crop Protection* 23: 979–982. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0261219404000559 [Accessed February 19, 2014].

ALONSO, R., and L. BETTUCCI. 2009. First report of the pitch canker fungus Fusarium circinatum affecting Pinus taeda seedlings in Uruguay. *Australasian Plant Disease Notes* 4: 91–92. Available at: http://www.jstor.org/stable/3761403?origin=crossref.

ALSTRÖM, S., and J.W.L. VAN VUURDE. 2001. Endophytic Bacteria and Biocontrol of Plant Diseases. *In* S. H. De Boer [ed.], Plant Pathogenic Bacteria, 60–67. Springer Netherlands, Dordrecht, Netherland. Available at: http://link.springer.com/chapter/10.1007/978-94-010-0003-1_11.

ALVES-SANTOS, F.M., and J.J. DIEZ. 2011. Biological control of Fusarium. *In* F. M. Alves-Santos, and J. J. Diez [eds.], Control of Fusarium diseases, 250. Research Signpost, Kerala (India).

ASHRAF, M., and M.R. FOOLAD. 2005. Pre-Sowing Seed Treatment—A Shotgun Approach to Improve Germination, Plant Growth, and Crop Yield Under Saline and Non-Saline Conditions. *Advances in Agronomy* 88: 223–271.

BACKMAN, P., and R. SIKORA. 2008. Endophytes: An emerging tool for biological control. *Biological Control* 46: 1–3. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1049964408000832 [Accessed June 15, 2011].

BAIER, M., M. GÖRGEN, J. EHLBECK, D. KNORR, W.B. HERPPICH, and O. SCHLÜTER. 2014. Non-thermal atmospheric pressure plasma: Screening for gentle process conditions and antibacterial efficiency on perishable fresh produce. *Innovative Food Science and Emerging Technologies* 22: 147–157. Available at: http://dx.doi.org/10.1016/j.ifset.2014.01.011.

BAKER, K.. 1962. Thermotherapy of planting material. *Phytopathology* 52: 1244–1255.

BARROWS-BROADDUS, J. 1990. Colonización of cones and seed of Loblolly pine folowing inoculation with Fusarium subglitinans. *Plant di* 74: 1002–1005.

BARROWS-BROADDUS, J., and L.D. DWINELL. 1985. Branch dieback and cone and seed infection caused by Fusarium moniliforme var. subglutinans in a Loblolly pine seed orchard in South Carolina. *Phytopathology* 75: 1104–1108.

BASARAN, P., N. BASARAN-AKGUL, and L. OKSUZ. 2008. Elimination of Aspergillus parasiticus from nut surface with low pressure cold plasma (LPCP) treatment.

900 *Food Microbiology* 25: 626–632.
901 BENÍTEZ, T., A.M. RINCÓN, M.C. LIMÓN, and A.C. CODÓN. 2004. Biocontrol
902 mechanisms of Trichoderma strains. *International microbiology : the official*
903 *journal of the Spanish Society for Microbiology* 7: 249–60. Available at:
904 http://www.ncbi.nlm.nih.gov/pubmed/15666245.
905 BENNETT, R.S., and P.D. COLYER. 2010. Dry Heat and Hot Water Treatments for
906 Disinfesting Cottonseed of *Fusarium oxysporum* f. sp. *vasinfectum*. *Plant Disease*
907 94: 1469–1475. Available at: http://apsjournals.apsnet.org/doi/10.1094/PDIS-01-
908 10-0052.
909 BERBEGAL, M., E. LANDERAS, D. SÁNCHEZ, P. ABAD-CAMPOS, A. PÉREZ-SIERRA, and J.
910 ARMENGOL. 2015. Evaluation of Pinus radiata seed treatments to control Fusarium
911 circinatum : effects on seed emergence and disease incidence. *Forest Pathology*
912 45: 525–533.
913 BERBEGAL, M., A PÉREZ-SIERRA, J. ARMENGOL, and N.J. GRÜNWALD. 2013. Evidence
914 for multiple introductions and clonality in Spanish populations of Fusarium
915 circinatum. *Phytopathology* 103: 851–61. Available at:
916 http://www.ncbi.nlm.nih.gov/pubmed/23678883.
917 BEZOS, D., P. MARTÍNEZ-ÁLVAREZ, M. FERNÁNDEZ, and J.J. DIEZ. 2017. Epidemiology
918 and Management of Pine Pitch Canker Disease in Europe - a Review. *Baltic*
919 *Forestry* 23: 279–293.
920 BLACKWELL, M. 2011. The fungi: 1, 2, 3 … 5.1 million species? *American journal of*
921 *botany* 98: 426–438.
922 BLUMENSTEIN, K., B.R. ALBRECTSEN, J.A. MART??N, M. HULTBERG, T.N. SIEBER, M.
923 HELANDER, and J. WITZELL. 2015. Nutritional niche overlap potentiates the use of
924 endophytes in biocontrol of a tree disease. *BioControl* 60: 655–667.
925 BONELLO, GORDON, and STORER. 2001. Systemic induced resistance in Monterey pine.
926 *Forest Pathology* 31: 99–106.
927 BOUTIGNY, A.L., V. ATANASOVA-PÉNICHON, M. BENET, C. BARREAU, and F. RICHARD-
928 FORGET. 2010. Natural phenolic acids from wheat bran inhibit Fusarium culmorum
929 trichothecene biosynthesis in vitro by repressing Tri gene expression. *European*
930 *Journal of Plant Pathology* 127: 275–286.
931 BOYD, I.L., P.H. FREER-SMITH, C.A. GILLIGAN, and H.C.J. GODFRAY. 2013. The
932 Consequence of Tree Pests and Diseases for Ecosystem Services. *Science* 342:
933 1235773. Available at:
934 http://www.sciencemag.org/cgi/doi/10.1126/science.1235773.
935 BRAGANÇA, H., E. DIOGO, F. MONIZ, and P. AMARO. 2009. First Report of Pitch Canker
936 on Pines Caused by Fusarium circinatum. *Plant Disease* 93: 1079.
937 BROOKS, D.., C.. GONZALEZ, D.. APPEL, and T.. FILER. 1994. Evaluation of endophytic
938 bacteria as potential biological control agents for oak wilt. *Biological Control* 4:
939 373–381.
940 BROTMAN, Y., J.K. GUPTA, and VITER. 2010. Trichoderma. *Current Biology* 20: 390–
941 391.
942 BUSBY, P.E., M. RIDOUT, and G. NEWCOMBE. 2015. Fungal endophytes: modifiers of
943 plant disease. *Plant Molecular Biology* 90: 1–11.
944 CALISTRU, C., M. MCLEAN, and P. BERJAK. 1997. In vitro studies on the potential for
945 biological control of Aspergillus flavus and Fusarium moniliforme by Trichoderma
946 species A study of the production of extracellular metabolites by Trichoderma
947 species. 115–124.
948 CAREY, W. A., S.W. OAK, and S. A. ENEBAK. 2005. Pitch canker ratings of longleaf pine

clones correlate with Fusarium circinatum infestation of seeds and seedling mortality in containers. *Forest Pathology* 35: 205–212. Available at: http://doi.wiley.com/10.1111/j.1439-0329.2005.00404.x.

CARLUCCI, A., L. COLATRUGLIO, and S. FRISULLO. 2007. First Report of Pitch Canker Caused by Fusarium circinatum on Pinus halepensis and P. pinea in Apulia (Southern Italy). *Plant Disease* 91: 1683.

CARRASCO, A., J.L. WEGRZYN, R. DUR??N, M. FERN??NDEZ, A. DONOSO, V. RODRIGUEZ, D. NEALE, and S. VALENZUELA. 2017. Expression profiling in Pinus radiata infected with Fusarium circinatum. *Tree Genetics and Genomes* 13: .

CAZORLA, F.M., and J. MERCADO-BLANCO. 2016. Biological control of tree and woody plant diseases : an impossible task ? *BioControl* 61: 233–242.

CENDÁN, C., L. SAMPEDRO, and R. ZAS. 2013. The maternal environment determines the timing of germination in Pinus pinaster. *Environmental and Experimental Botany* 94: 66–72. Available at: http://dx.doi.org/10.1016/j.envexpbot.2011.11.022.

CERQUEIRA, A., A. ALVES, H. BERENGUER, B. CORREIA, A. GÓMEZ-CADENAS, J.J. DIEZ, P. MONTEIRO, and G. PINTO. 2017. Phosphite shifts physiological and hormonal profile of Monterey pine and delays Fusarium circinatum progression. *Plant Physiology and Biochemistry* 114: 88–99.

CHEN, C., E.. BAUSKE, G. MUSSON, R. RODRIGUEZKABANA, and J.. KLOEPPER. 1995. Biological Control of Fusarium Wilt on Cotton by Use of Endophytic Bacteria. *Biological Control* 5: 83–91.

CHERUTHAZHEKATT, S., M. ČERNÁK, P. SLAVÍČEK, and J. HAVEL. 2010. Gas plasmas and plasma modified materials in medicine. *Journal of Applied Biomedicine* 8: 55–66. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1214021X14600107.

CHIBA, S., L. SALAIPETH, Y.-H. LIN, A. SASAKI, S. KANEMATSU, and N. SUZUKI. 2009. A novel bipartite double-stranded RNA Mycovirus from the white root rot Fungus Rosellinia necatrix: molecular and biological characterization, taxonomic considerations, and potential for biological control. *Journal of virology* 83: 12801–12812.

CHRISTAKI, E., E. BONOS, I. GIANNENAS, and P. FLOROU-PANERI. 2012. Aromatic Plants as a Source of Bioactive Compounds. *Agriculture* 2: 228–243.

CHU, Y., J. JEON, S. YEA, Y. KIM, S. YUN, Y. LEE, and K. KIM. 2002. Double-Stranded RNA Mycovirus from Fusarium graminearum Double-Stranded RNA Mycovirus from Fusarium graminearum. *Applied and environmental microbiology* 68: 2529–2534.

COMPANT, S., B. DUFFY, J. NOWAK, C. CLE, and E. A BARKA. 2005. Use of Plant Growth-Promoting Bacteria for Biocontrol of Plant Diseases : Principles , Mechanisms of Action , and Future Prospects MINIREVIEW Use of Plant Growth-Promoting Bacteria for Biocontrol of Plant Diseases : Principles , Mechanisms of Action , and. *Applied and environmental microbiology* 71: 4951–4959.

CONRADS, H., and M.L.B.-C. SCHMIDT. 2000. Plasma generation and plasma sources. *Plasma Sources Science and Technology* 9: 441. Available at: http://stacks.iop.org/0963-0252/9/i=4/a=301.

COOK, J., and K.. BAKER. 1996. The nature and practice of biological control of plant pathogens. Third Edit. J. Cook, and K. F. Baker [eds.],. American Phytopahological Society, Minnesota.

DASAN, B.G., I.H. BOYACI, and M. MUTLU. 2016. Inactivation of aflatoxigenic fungi

(Aspergillus spp.) on granular food model, maize, in an atmospheric pressure fluidized bed plasma system. *Food Control* 70: 1–8. Available at: http://dx.doi.org/10.1016/j.foodcont.2016.05.015.

DENG, F., R. XU, and G.J. BOLAND. 2003. Hypovirulence-associated double-stranded RNA from Sclerotinia homoeocarpa is conspecific with Ophiostoma novo-ulmi mitovirus 3a-Ld. *Phytopathology* 93: 1407–1414.

DESJARDINS, A.E., and R.H. PROCTOR. 2007. Molecular biology of Fusarium mycotoxins. *International Journal of Food Microbiology* 119: 47–50.

DONOSO, A., V. RODRIGUEZ, A. CARRASCO, R. AHUMADA, E. SANFUENTES, and S. VALENZUELA. 2015. Relative expression of seven candidate genes for pathogen resistance on Pinus radiata infected with Fusarium circinatum. *Physiological and Molecular Plant Pathology* 92: 42–50.

DUMROESE, R.K., R.L. JAMES, D.L. WENNY, and C. GILLIGAN. 1988. Douglas-fir seed treatments: effects on seed germination and seedborne organisms. *In* Proceedings of the combined meeting of the western forest nursery associations, 155–160.

DVORAK, W., G. HODGE, and J. KIETZKA. 2007. Genetic variation in survival, growth, and stem form of <I>Pinus leiophylla</I> in Brazil and South Africa and provenance resistance to pitch canker. *Southern Hemisphere Forestry Journal* 69: 125–135. Available at: http://openurl.ingenta.com/content/xref?genre=article&issn=1991-931X&volume=69&issue=3&spage=125 [Accessed February 19, 2014].

DVORAK, W.S., K.M. POTTER, V.D. HIPKINS, and G.R. HODGE. 2009. Genetic Diversity and Gene Exchange in Pinus oocarpa , a Mesoamerican Pine with Resistance to the Pitch Canker Fungus (Fusarium circinatum). *International Journal of Plant Sciences* 170: 609–626. Available at: http://www.jstor.org/stable/10.1086/597780 [Accessed February 19, 2014].

DWINELL, D. 1999. Global Distribution of the Pitch Canker Fungus. *Current and Potential Impacts of Pitch Canker in Radiata Pine. Proceedings of the IMPACT Monterey Workshop, Monterey, California*54–57.

EFSA. 2010. Risk assessment of Gibberella circinata for the EU territory and identification and evaluation of risk management options. *European Food Safety Authority* 8: 1–93.

ELVIRA-RECUENCO, M., E. ITURRITXA, J. MAJADA, R. ALIA, and R. RAPOSO. 2014. Adaptive potential of maritime pine (Pinus pinaster) populations to the emerging pitch canker pathogen, Fusarium circinatum. *PLoS ONE* 9: 1–21.

ELVIRA-RECUENCO, M., E. ITURRITXA, and R. RAPOSO. 2015. Impact of seed transmission on the infection and development of pitch canker disease in Pinus radiata. *Forests* 6: 3353–3368.

ENEBAK, B.S.A., and G.R. STANOSZ. 2003. Responses of conifer species of the Great Lakes region of North America to inoculation with the pitch canker pathogen Fusarium circinatum. 33: 333–338.

EPPO. 2006. First report of Gibberella circinata in France.

ERBILGIN, N., A.J. STORER, D.L. WOOD, and T.R. GORDON. 2005. Colonization of cut branches of five coniferous hosts of the pitch canker fungus by Pityophthorus spp.(Coleoptera: Scolytidae) in central, coastal California. *The Canadian Entomologist* 137: 337–349.

ESCHEN, R., K. BRITTON, E. BROCKERHOFF, T. BURGESS, V. DALLEY, R.S. EPANCHIN-NIELL, K. GUPTA, ET AL. 2015. International variation in phytosanitary legislation and regulations governing importation of plants for planting. *Environmental*

Science and Policy 51: 228–237.
EVANS, H.C., K.A. HOLMES, and S.E. THOMAS. 2003. Endophytes and mycoparasites associated with an indigenous forest tree, Theobroma gileri, in Ecuador and a preliminary assess- ment of their potential as biocontrol agents of cocoa diseases. *Mycological Progress* 2: 149–160.
FAROOQ, M., S.M.A. BARSA, and A. WAHID. 2006. Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. *Plant Growth Regulation* 49: 285–294.
FERRE, F.S., and M.P. SANTAMARINA. 2010. Efficacy of Trichoderma harzianum in suppression of Fusarium culmorum. 335–340.
FERRUZ, E., S. LORAN, M. HERRERA, I. GIMENEZ, N. BERVIS, C. BARCENA, J.J. CARRAMIÑANA, ET AL. 2016. Inhibition of Fusarium Growth and Mycotoxin Production in Culture Medium and in Maize Kernels by Natural Phenolic Acids. *Journal of Food Protection* 79: 1753–1758. Available at: http://openurl.ingenta.com/content/xref?genre=article&issn=0362-028X&volume=79&issue=10&spage=1753.
FITZA, K.N.E., K.G. PAYN, E.T. STEENKAMP, A. A. MYBURG, and S. NAIDOO. 2013. Chitosan application improves resistance to Fusarium circinatum in Pinus patula. *South African Journal of Botany* 85: 70–78. Available at: http://linkinghub.elsevier.com/retrieve/pii/S025462991300015X [Accessed February 19, 2014].
FLORES-PACHECO, J.A., E.J. MUÑOZ-ADALIA, P. MARTÍNEZ-ÁLVAREZ, V. PANDO, J.J. DIEZ, and J. MARTÍN-GARCÍA. 2017. Effect of mycoviruses on growth, spore germination and pathogenicity of the fungus Fusarium circinatum. *Forest Systems* in press: .
FORD, C., N. JONES, and P. CHIRWA. 2014. Pinus patula and pine hybrid hedge productivity in South Africa: a comparison between two vegetative propagation systems exposed to natural infection by Fusarium circinatum. *Southern Forests: a Journal of Forest Science* 76: 167–175.
FRANCESCO, A. DI, C. MARTINI, and M. MARI. 2016. Biological control of postharvest diseases by microbial antagonists : how many mechanisms of action ? *European Journal of Plant Pathology*711–717. Available at: http://dx.doi.org/10.1007/s10658-016-0867-0.
FRIDMAN, G., G. FRIEDMAN, A. GUTSOL, A.B. SHEKHTER, V.N. VASILETS, and A. FRIDMAN. 2008. Applied plasma medicine. *Plasma Processes and Polymers* 5: 503–533.
GORDON, T.R., S.C. KIRKPATRICK, B.J. AEGERTER, A. J. FISHER, A. J. STORER, and D.L. WOOD. 2011. Evidence for the occurrence of induced resistance to pitch canker, caused by Gibberella circinata (anamorph Fusarium circinatum), in populations of Pinus radiata. *Forest Pathology* 41: 227–232. Available at: http://doi.wiley.com/10.1111/j.1439-0329.2010.00678.x [Accessed February 19, 2014].
GORDON, T.R., S.C. KIRKPATRICK, B.J. AEGERTER, D.L. WOOD, and A. J. STORER. 2006. Susceptibility of Douglas fir (Pseudotsuga menziesii) to pitch canker, caused by Gibberella circinata (anamorph = Fusarium circinatum). *Plant Pathology* 55: 231–237. Available at: http://doi.wiley.com/10.1111/j.1365-3059.2006.01351.x [Accessed February 19, 2014].
GORDON, T.R., D. OKAMOTO, A.J. STORER, and D.L. WOOD. 1998. Susceptibility of five landscape pines to pitch canker disease, caused by Fusarium subglutinans f. sp.

pini. *Hortscience* 33: 868–871.
GORDON, T.R., and G.J. REYNOLDS. 2017. Plasticity in plant-microbe interactions: a perspective based on the pitch canker pathosystem. *Phytoparasitica* 45: 1–8.
GORDON, T.R., A.J. STORER, and WOOD. 2001. The Pitch Canker Epidemic in California. *Plant disease* 85: 1128–1139.
GORDON, T.R., C.L. SWETT, and M.J. WINGFIELD. 2015. Management of Fusarium diseases affecting conifers. *Crop Protection* 73: 28–39.
GORDON, T.R., K. WIKLER, S.L. CLARK, D. OKAMOTO, A.J. STORER, and P. BONELLO. 1998. Resistance to pitch canker disease, caused by Fusarium subglutinans f.sp. pini, in Monterey pine (Pinus radiata). *Plant Pathology* 47: 706–711.
GRATWICH, M., and M.A. SOUTHEY. 1986. Hot-Water Treatment of Plant Material. H.M.S.O., London.
GRENTE, J. 1965. Les formes Hypovirulentes d'Endothia parasitica et les espoirs de lutte contre le chancre du châtaignier. *Académie d'Agriculture de France, Extrait du Proces-verbal de la Séance* 51: 1033–1037.
GRIVET, D., J. CLIMENT, M. ZABAL-AGUIRRE, D.B. NEALE, G.G. VENDRAMIN, and S.C. GONZÁLEZ-MARTÍNEZ. 2013. Adaptive evolution of Mediterranean pines. *Molecular Phylogenetics and Evolution* 68: 555–566.
GRONDEAU, C., R. SAMSON, and D.C. SANDS. 1994. A Review of Thermotherapy to Free Plant Materials from Pathogens , Especially Seeds from Bacteria. *Critical Reviews in Plant Sciences* 13: 57–75.
GUERRA-SANTOS, J.J. 1998. Pitch canker on Monterey pine in Mexico. In T. R. Devey, M.E.; Matheson, A.C.; Gordon (Ed.), Current and potential impacts of pitch canker in radiata pine. Proceedings of the IMPACT Monterey Workshop, Monterey, California. *USA. 30 November to 3 December* 112: 58–61.
HAMMERBACHER, A. 2005. Biology and epidemiology of Fusarium circinatum. University of Pretoria.
HARMAN, G.E., C.R. HOWELL, A. VITERBO, I. CHET, and M. LORITO. 2004. Trichoderma species--opportunistic, avirulent plant symbionts. *Nature reviews. Microbiology* 2: 43–56.
HAWKSWORTH, D.L., and A.Y. ROSSMAN. 1997. Where Are All the Undescribed Fungi ? 623: 8–11.
HEPTING, G.H., and E.R. ROTH. 1953. Host relations and spread of the pine pitch canker disease. *Phytopathology* 43: 475.
HEPTING, G.H., and E.R. ROTH. 1946. Pitch canker, a new disease of some Southern pines. *Journal of Forestry* 44: 742–744.
HERMANSEN, A., G. BRODAL, and G. BALVOLL. 2000. Hot water treatment of carrot seeds: effects on seed-borne fungi, germination, emergence and yield. *Seed Science and Technology* 27: 599–613.
HERMOSA, R., A. VITERBO, I. CHET, and E. MONTE. 2012. Plant-beneficial effects of Trichoderma and of its genes. *Microbiology* 158: 17–25.
HEYDARI, A., and M. PESSARAKLI. 2010. A review of biological control of fungal plant pathogens using microbial antagonists. *Journal of Biological Sciences* 10: .
HIBAR, K., L. DE PHYTOPATHOLOGIE, I.S.A. DE, M. DAAMI-REMADI, M. EL MAHJOUB, D. PHYTOPATHOLOGIE, and I.S.A. DE CHOTT-MARIEM. 2007. Induction of Resistance in Tomato Plants against Fusarium oxysporum f . sp . radicis-lycopersici by Trichoderma spp . 2: .
HODGE, G.R., and W.S. DVORAK. 2014. Breeding southern US and Mexican pines for increased value in a changing world. *New Forests* 45: 295–300.

HODGE, G.R., and W.S. DVORAK. 2000. Differential responses of Central American and Mexican pine species and Pinus radiata to infection by the pitch canker fungus. 241–258.

HODGE, G.R., and W.S. DVORAK. 2007. Variation in pitch canker resistance among provenances of Pinus patula and Pinus tecunumanii from Mexico and Central America. *New Forests* 33: 193–206.

HOITINK, H.A.J., L. V MADDEN, and A.E. DORRANCE. 2005. Systemic Resistance Induced by Trichoderma spp .: Interactions Between the Host , the Pathogen , the Biocontrol Agent , and Soil Organic Matter Quality. 18–21.

HOLESKI, L.M., G. JANDER, and A.A. AGRAWAL. 2012. Transgenerational defense induction and epigenetic inheritance in plants. *Trends in Ecology and Evolution* 27: 618–626. Available at: http://dx.doi.org/10.1016/j.tree.2012.07.011.

HOLMES, T.P., J.E. AUKEMA, B. VON HOLLE, A. LIEBHOLD, E. SILLS, U.S. FOREST, S. NORTHERN, ET AL. 2009. Economic Impacts of Invasive Species in Forests Past , Present , and Future. 38: 18–38.

HOWELL, C.R. 2003. Mechanisms Employed by Trichoderma Species in the Biological Control of Plant Diseases : 87: .

HUANG, S., and S.A. GHABRIAL. 1996. Organization and expression of the double-stranded RNA genome of Helminthosporium victoriae 190S virus, a totivirus infecting a plant pathogenic filamentous fungus. *Proceedings of the National Academy of Sciences* 93: 12541–12546. Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.93.22.12541.

HYDER, R., T. PENNANEN, L. HAMBERG, E.J. VAINIO, T. PIRI, and J. HANTULA. 2013. Two viruses of Heterobasidion confer beneficial, cryptic or detrimental effects to their hosts in different situations. *Fungal Ecology* 6: 387–396. Available at: http://dx.doi.org/10.1016/j.funeco.2013.05.005.

ITURRITXA, E., M.L. DESPREZ-LOUSTAU, I. GARCÍA-SERNA, E. QUINTANA, N. MESANZA, and J. AITKEN. 2011. Effect of alternative disinfection treatments against fungal canker in seeds of Pinus radiata. *Seed Technology* 33: 88–110.

ITURRITXA, E., R.J. GANLEY, R. RAPOSO, I. GARCÍA-SERNA, N. MESANZA, S.C. KIRKPATRICK, and T.R. GORDON. 2013. Resistance levels of Spanish conifers against Fusarium circinatum and Diplodia pinea S. Woodward [ed.],. *Forest Pathology* 43: 488–495. Available at: http://doi.wiley.com/10.1111/efp.12061 [Accessed January 23, 2014].

ITURRITXA, E., N. MESANZA, and J. AITKEN. 2013. Bioensayos de control biológico y químico frente a Fusarium circinatum en Pinus radiata. *In* 6th Spanish National Congress, 1–7.

ITURRITXA, E., N. MESANZA, M. ELVIRA-RECUENCO, Y. SERRANO, E. QUINTANA, and R. RAPOSO. 2012. Evaluation of genetic resistance in Pinus to pitch canker in Spain. *Australasian Plant Pathology* 41: 601–607.

ITURRITXA, E., T. TRASK, N. MESANZA, R. RAPOSO, M. ELVIRA-RECUENCO, and C.L. PATTEN. 2017. Biocontrol of Fusarium circinatum Infection of Young Pinus radiata Trees. *Forests* 8: 32.

JAMALIZADEH, M., H.R. ETEBARIAN, H. AMINIAN, and A. ALIZADEH. 2011. A review of mechanisms of action of biological control organisms against post-harvest fruit spoilage. 65–71.

JONES, J.P., X. SUN, L. ECKHARDT, A. WEBBER, N. HESS, J. BARNETT, and J. MCGILVARY. 2002. Longleaf seedling production: some problems and their solutions. *In* La Agric. Summer, 4–6.

KANEMATSU, S., M. ARAKAWA, Y. OIKAWA, M. ONOUE, H. OSAKI, H. NAKAMURA, K. IKEDA, ET AL. 2004. A Reovirus Causes Hypovirulence of Rosellinia necatrix. *Phytopathology* 94: 561–568.

KANZLER, A., A. NEL, and C. FORD. 2014. Development and commercialisation of the Pinus patula ?? P. tecunumanii hybrid in response to the threat of Fusarium circinatum. *New Forests* 45: 417–437.

KAYIHAN, G.C., D. A HUBER, A.M. MORSE, T.L. WHITE, and J.M. DAVIS. 2005. Genetic dissection of fusiform rust and pitch canker disease traits in loblolly pine. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* 110: 948–58. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15700146 [Accessed February 19, 2014].

KELLY, B.G., A. VESPERMANN, and D.J. BOLTON. 2009. Horizontal gene transfer of virulence determinants in selected bacterial foodborne pathogens. *Food and Chemical Toxicology* 47: 969–977. Available at: http://dx.doi.org/10.1016/j.fct.2008.02.007.

KIM, K., I.J. LEE, C.S. KIM, I.Y. EOM, J.W. CHOI, D.K. LEE, and E.W. PARK. 2010. Resin flow, symptom development, and lignin biosynthesis of two pine species in response to wounding and inoculation with Fusarium circinatum. *Plant Pathology Journal* 26: 394–401.

KIM, Y.-S., K.-S. WOO, Y.-B. KOO, and J.-K. YEO. 2008. Variation in susceptibility of six pine species and hybrids to pitch canker caused by Fusarium circinatum. *Forest Pathology* 38: 419–428. Available at: http://doi.wiley.com/10.1111/j.1439-0329.2008.00558.x [Accessed February 19, 2014].

KING, A.M.Q., M.J. ADAMS, E.B. CARSTENS, and E.J. LEFKOWITZ. 2012. Virus taxonomy: classification and nomenclature of viruses. Ninth Report of the International Committee on Taxonomy of Viruses. A. M. Q. King, M. J. Adams, E. B. Carstens, and E. J. Lefkowitz [eds.],. Elsevier Academic Press.

KIRKEGAARD, J.A., M. SARWAR, P.T.W. WONG, A. MEAD, G. HOWE, and M. NEWELL. 2000. Field studies on the biofumigation of take-all by Brassica break crops. *Australian Journal of Agricultural Research* 51: 445–456.

KLAPWIJK, M.J., A.J.M. HOPKINS, L. ERIKSSON, M. PETTERSSON, M. SCHROEDER, Å. LINDELÖW, J. RÖNNBERG, ET AL. 2016. Reducing the risk of invasive forest pests and pathogens: Combining legislation, targeted management and public awareness. *Ambio* 45: 223–234.

KOBAYASHI, T., and M. MURAMOTO. 1989. Pitch canker of Pinus luchuensis, a new disease of Japanese forests. *Forest Pests* 40: 169–173.

KRINGS, M., T.N. TAYLOR, and N. DOTZLER. 2012. Fungal Endophytes as a Driving Force in Land Plant Evolution: Evidence from the Fossil Record. *In* D. Southworth [ed.], Biocomplexity of Plant-Fungal Interactions, 5–27. Wiley-Blackwell, Oxford, UK. Available at: http://onlinelibrary.wiley.com/doi/10.1002/9781118314364.ch1/summary.

LAKSHMAN, D.K., J. JIAN, and S.M. TAVANTZIS. 1998. A double-stranded RNA element from a hypovirulent strain of Rhizoctonia solani occurs in DNA form and is genetically related to the pentafunctional AROM protein of the shikimate pathway. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 95: 6425–6429. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=27765&tool=pmcentrez&rendertype=abstract.

LANDERAS, E., P. GARCÍA, M. FERNÁNDEZ, M. BRAÑA, A. PÉREZ-SIERRA, M. LEÓN, P.

ABAD-CAMPOS, and J. ARMENGOL. 2005. Outbreak of Pitch Canker Caused by Fusarium circinatum on Pinus spp . in North ... *Plant Disease* 89: 1015.
LANDERAS, L., G. GARCÍA, G. GONZÁLEZ, and B. BRAÑA. 2006. VALORACIÓN DE LA EFICACIA IN VITRO DE DIFERENTES FUNGICIDAS FRENTE A Fusarium circinatum. *In* XIII Congress of the Spanish Society of Phytopathology, H-47.
LARSON, M.., and G.H. SCHUBERT. 1969. Effect of osmotic water stress on germination and initialdevelopment of Ponderosa pine seedlings. *Forest Science* 15: 30–36.
LEAL, I., E. ALLEN, L. HUMBLE, S. SELA, and A. UZUNOVIC. 2010. Phytosanitary risks associated with the global movement of forest products: A commodity-based approach.
LEE, J.K., S.-H. LEE, S.-I. YANG, and Y.-W. LEE. 2000. First report of Pitch Canker disease on Pinus rigida in Korea. *The Plant Pathology Journal* 16: 52–54.
LEE, K.M., J. YU, M. SON, Y.W. LEE, and K.H. KIM. 2011. Transmission of fusarium boothii mycovirus via protoplast fusion causes Hypovirulence in other phytopathogenic fungi. *PLoS ONE* 6: 1–9.
LEE, Y.-S., J. KIM, S.-G. LEE, E. OH, S.-C. SHIN, and I.-K. PARK. 2009. Effects of plant essential oils and components from Oriental sweetgum (Liquidambar orientalis) on growth and morphogenesis of three phytopathogenic fungi. *Pesticide Biochemistry and Physiology* 93: 138–143. Available at: http://linkinghub.elsevier.com/retrieve/pii/S004835750900011X [Accessed February 19, 2014].
LEE, Y., J. KIM, S. SHIN, S. LEE, and I. PARK. 2008. Antifungal activity of Myrtaceae essential oils and their components against three phytopathogenic fungi. 23–28.
LEROUGE, S., M.R. WERTHEIMER, and L. YAHIA. 2001. Plasma sterilization: A review of parameters, mechanisms, and limitations. *Plasmas and Polymers* 6: 175–188.
LIEBHOLD, A.M., E.G. BROCKERHOFF, L.J. GARRETT, J.L. PARKE, and K.O. BRITTON. 2012. Live plant imports: the major pathway for forest insect and pathogen invasions of the US. *Frontiers in Ecology and the Environment* 10: 135–143. Available at: http://www.esajournals.org/doi/abs/10.1890/110198 [Accessed January 22, 2014].
LIMA, F.B. DE, C. FÉLIX, N. OSÓRIO, A. ALVES, P. DOMINGUES, T. RUTE, S. RIBEIRO, and A.C. ESTEVES. 2017. Trichoderma harzianum T1A constitutively secretes proteins involved in the biological control of Guignardia citricarpa. *Biological Control*. Available at: http://dx.doi.org/10.1016/j.biocontrol.2017.01.003.
LIMA, F.B. DE, C. FÉLIX, N. OSÓRIO, A. ALVES, R. VITORINO, P. DOMINGUES, A. CORREIA, ET AL. 2016. Secretome analysis of Trichoderma atroviride T17 biocontrol of Guignardia citricarpa. *Biological Control* 99: 38–46. Available at: http://dx.doi.org/10.1016/j.biocontrol.2016.04.009.
LO, T. 1998. General mechanisms of action of microbial biocontrol agents. 155–166.
LOMBARDERO, M.J., M.P. AYRES, L. LORIO JR, and J.J. RUEL. 2000. Environmental effects on constitutive and inducible resin defences of Pinus taeda. *Ecology Letters* 3: 329–339.
LÓPEZ-FERNÁNDEZ, S., P. SONEGO, M. MORETTO, M. PANCHER, K. ENGELEN, I. PERTOT, and A. CAMPISANO. 2015. Whole-genome comparative analysis of virulence genes unveils similarities and differences between endophytes and other symbiotic bacteria. *Frontiers in Microbiology* 6: 1–15.
LÓPEZ-LÓPEZ, N., G. SEGARRA, O. VERGARA, A. LÓPEZ-FABAL, and M.I. TRILLAS. 2016. Compost from forest cleaning green waste and Trichoderma asperellum

strain T34 reduced incidence of Fusarium circinatum in Pinus radiata seedlings. 95: 31–39.
LÓPEZ-LÓPEZ, N., G. SEGARRA, O. VERGARA, A. LÓPEZ-FABAL, and M.I. TRILLAS. 2016. Compost from forest cleaning green waste and Trichoderma asperellum strain T34 reduced incidence of Fusarium circinatum in Pinus radiata seedlings. *Biological Control* 95: 31–39. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1049964415300724.
MARASAS, W.F.O., P.E. NELSON, and T.. TOUSSOUN. 1984. Toxigenic Fusarium species. Identity and mycotoxicology. W. F. O. Marasas, P. E. Nelson, and T. . Toussoun [eds.],. Pennsylvania State University, Pensylvania.
MARÍN-GUIRAO, J.I., P. RODRÍGUEZ-ROMERA, B. LUPIÓN-RODRÍGUEZ, F. CAMACHO-FERRE, and J.C. TELLO-MARQUINA. 2016. Effect of Trichoderma on horticultural seedlings' growth promotion depending on inoculum and substrate type. *Journal of Applied Microbiology* 121: 1095–1102.
MÁRQUEZ, L.M., R.S. REDMAN, R.J. RODRIGUEZ, and M.J. ROOSSINCK. 2007. A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. *Science* 315: 513–516.
MARTÍN-GARCÍA, J., M. PARASCHIV, J.A. FLORES-PACHECO, D. CHIRA, J.J. DIEZ, and M. FERNÁNDEZ. 2017. Susceptibility of several northeastern conifers to Fusarium circinatum and strategies for biocontrol. *Forests* 8: .
MARTÍNEZ-ALVAREZ, P., F.M. ALVES-SANTOS, and J.J. DIEZ. 2012. In Vitro and In Vivo Interactions between Trichoderma viride and Fusarium circinatum. *Silva Fennica* 46: 303–316.
MARTÍNEZ-ÁLVAREZ, P., R.A. FERNÁNDEZ-GONZÁLEZ, A.V. SANZ-ROS, V. PANDO, and J.J. DIEZ. 2016. Two fungal endophytes reduce the severity of pitch canker disease in Pinus radiata seedlings. *Biological Control* 94: 1–10.
MARTÍNEZ-ALVAREZ, P., V. PANDO, and J.J. DIEZ. 2014. Alternative species to replace Monterey pine plantations affected by pitch canker caused by Fusarium circinatum in northern Spain. *Plant Pathology* 63: 1086–1094.
MARTÍNEZ-ALVAREZ, P., E.J. VAINIO, L. BOTELLA, J. HANTULA, and J.J. DIEZ. 2014. Three mitovirus strains infecting a single isolate of Fusarium circinatum are the first putative members of the family Narnaviridae detected in a fungus of the genus Fusarium. *Archives of Virology* 159: 2153–2155.
MARTÍNEZ-HIDALGO, P., J.M. GARCÍA, and M.J. POZO. 2015. Induced systemic resistance against Botrytis cinerea by Micromonospora strains isolated from root nodules. *Frontier in Microbiology* 6: 922.
MATHESON, A.C., W.R. MARK, G. STOVOLD, C. BALOCCHI, N. SMITH, and C. BRASSEY. 2006. Genetic Variation in Resistance to Pine Pitch Canker and Western Gall Rust in Monterey Pine (Pinus radiata D . Don): Results From a Three- Country Collaborative Field Trial. 253–255.
MATHESON, A. C., M.E. DEVEY, T.L. GORDON, W. WERNER, D.R. VOGLER, C. BALOCCHI, and M.J. CARSON. 2006. Heritability of response to inoculation by pine pitch canker of seedlings of radiata pine. *Australian Forestry* 69: 101–106. Available at: http://www.tandfonline.com/doi/abs/10.1080/00049158.2006.10676235.
MAUCH-MANI, B., I. BACELLI, E. LUNA, and V. FLORS. 2017. Defense Priming: An Adaptive Part of Induced Resistance. *Annual Review of Plant Biology* 68: 485–512.
MENG, L., P. SUN, H. TANG, L. LI, S. DRAEGER, B. SCHULZ, K. KROHN, ET AL. 2011.

Endophytic fungus Penicillium chrysogenum, a new source of hypocrellins. *Biochemical Systematics and Ecology* 39: 163–165. Available at: http://dx.doi.org/10.1016/j.bse.2011.02.003.

MENZIES, J.G. 1993. A strain of Trichoderma viride pathogenic to germinating seedlings of cucumber, pepper and tomato. *Plant Pathology* 42: 784–791.

MITCHELL, R., T. COUTINHO, E. STEENKAMP, M. HERBERT, and M. WINGFIELD. 2012. Future outlook for Pinus patula in South Africa in the presence of the pitch canker fungus (Fusarium circinatum). *Southern Forests: a Journal of Forest Science* 74: 203–210. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-84869988230&partnerID=tZOtx3y1.

MITCHELL, R., M. WINGFIELD, E. STEENKAMP, and T. COUTINHO. 2012. Tolerance of *Pinus patula* full-sib families to *Fusarium circinatum* in a greenhouse study. *Southern Forests: a Journal of Forest Science* 74: 247–252. Available at: http://www.tandfonline.com/doi/abs/10.2989/20702620.2012.741788.

MITCHELL, R.G., M.J. WINGFIELD, G.R. HODGE, E.T. STEENKAMP, and T.A. COUTINHO. 2012a. Selection of Pinus spp. in South Africa for tolerance to infection by the pitch canker fungus. *New Forests* 43: 473–489.

MITCHELL, R.G., M.J. WINGFIELD, G.R. HODGE, E.T. STEENKAMP, and T. A. COUTINHO. 2012b. The tolerance of Pinus patula × Pinus tecunumanii, and other pine hybrids, to Fusarium circinatum in greenhouse trials. *New Forests* 44: 443–456. Available at: http://link.springer.com/10.1007/s11056-012-9355-3 [Accessed January 23, 2014].

MITCHELL, R.G., M.J. WINGFIELD, E.T. STEENKAMP, J. ROUX, S. VERRYN, and T.A. COUTINHO. 2014. Comparison of the tolerance of *Pinus patula* seedlings and established trees to infection by *Fusarium circinatum*. *Southern Forests: a Journal of Forest Science* 76: 151–159. Available at: http://www.tandfonline.com/doi/abs/10.2989/20702620.2014.899061.

MITCHELL, R.G., J. ZWOLINSKI, N. JONES, and T. COUTINHO. 2004. The effect of applying prophylactic measures on the post-planting survival of Pinus patula in South Africa.

MITRA, A., Y.F. LI, T.G. KLÄMPFL, T. SHIMIZU, J. JEON, G.E. MORFILL, and J.L. ZIMMERMANN. 2014. Inactivation of Surface-Borne Microorganisms and Increased Germination of Seed Specimen by Cold Atmospheric Plasma. *Food and Bioprocess Technology* 7: 645–653.

MORAGA-SUAZO, P., A. OPAZO, S. ZALDÚA, G. GONZÁLEZ, and E. SANFUENTES. 2011. Evaluation of Trichoderma spp. and Clonostachys spp. Strains to Control Fusarium circinatum in Pinus radiata Seedlings. *Chilean journal of agricultural research* 71: 412–417.

MORAGA-SUAZO, P., L. ORELLANA, P. QUIROGA, C. BALOCCHI, E. SANFUENTES, R.W. WHETTEN, R. HASBÚN, and S. VALENZUELA. 2014. Development of a genetic linkage map for Pinus radiata and detection of pitch canker disease resistance associated QTLs. *Trees - Structure and Function* 28: 1823–1835.

MOREAU, M., N. ORANGE, and M.G.J. FEUILLOLEY. 2008. Non-thermal plasma technologies: New tools for bio-decontamination. *Biotechnology Advances* 26: 610–617. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0734975008000852.

MOREIRA, X., R. ZAS, and L. SAMPEDRO. 2013. Additive genetic variation in resistance traits of an exotic pine species: little evidence for constraints on evolution of resistance against native herbivores. *Heredity* 110: 449–56. Available at:

http://www.scopus.com/inward/record.url?eid=2-s2.0-84878678000&partnerID=tZOtx3y1.

MRÁZ, I., P. BERAN, B. SERA, B. GAVRIL, and E. HNATIUC. 2014. Effect of low-temperature plasma treatment on the growth and reproduction rate of some plant pathogenic bacteria. *Journal of Plant Pathology* 96: 63–67.

MULLETT, M., A. PÉREZ-SIERRA, J. ARMENGOL, and M. BERBEGAL. 2017. Phenotypical and Molecular Characterisation of Fusarium circinatum: Correlation with Virulence and Fungicide Sensitivity. *Forests* 8: 458. Available at: http://www.mdpi.com/1999-4907/8/11/458.

MUÑOZ-ADALIA, E.J., J.A. FLORES-PACHECO, P. MARTÍNEZ-ÁLVAREZ, J. MARTÍN-GARCÍA, M. FERNÁNDEZ, and J.J. DIEZ. 2016. Effect of mycoviruses on the virulence of Fusarium circinatum and laccase activity. *Physiological and Molecular Plant Pathology* 94: 8–15.

MUÑOZ LÓPEZ, C., E. CUERVO SÁNCHEZ, M. AMPUDIA DÍAZ, A. GASTÓN GONZÁLEZ, J.L. PEÑUELAS RUBIRA, S. IGLESIAS SAUCE, and N. HERRERO SIERRA. 2009. Control químico de Fusarium circinatum Niremberg & O`Donnell en semillas del género Pinus. *In* V Spanish Forestry Congress, 12.

NEL, A., G.R. HODGE, K.E. MONGWAKETSI, and A. KANZLER. 2014. Genetic parameters for Fusarium circinatum tolerance within openpollinated families of Pinus patula tested at screening facilities in South Africa and the USA. *Southern Forests: a Journal of Forest Science* 76: 145–150. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-84899772833&partnerID=tZOtx3y1.

OAK, S.W., G.M. BLAKESLEE, and D.L. ROCKWOOD. 1987. Pitch canker resistant slash pine identified by greenhouse screening. *In* Proc. 19th Southern For. Tree Impr. Conf., 132–139. College Station Texas.

OLIVA, J., C. CASTAÑO, E. BAULENAS, G. DOMÍNGUEZ, J.R. GONZÁLEZ-OLABARRIA, and D. OLIACH. 2016. The impact of the socioeconomic environment on the implementation of control measures against an invasive forest pathogen. *Forest Ecology and Management* 380: 118–127. Available at: http://dx.doi.org/10.1016/j.foreco.2016.08.034.

ÖZCAN, M., A. ÜNVER, D.A. CEYLAN, and R. YETIŞIR. 2004. Inhibitory effect of pollen and propolis extracts. *Nahrung - Food* 48: 188–194.

PAL, K.K., and B. MC SPADDEN GARDENER. 2006. Biological Control of Plant Pathogens. *The Plant Health Instructor*1–25.

PANNGOM, K., S.H. LEE, D.H. PARK, G.B. SIM, Y.H. KIM, H.S. UHM, G. PARK, and E.H. CHOI. 2014. Non-thermal plasma treatment diminishes fungal viability and up-regulates resistance genes in a plant host. *PLoS ONE* 9: .

PAPAEFTHIMIOU, D., A. PAPANIKOLAOU, V. FALARA, S. GIVANOUDI, S. KOSTAS, A.K. KANELLIS, M. SKORIĆ, ET AL. 2014. Genus Cistus: a model for exploring labdane-type diterpenes' biosynthesis and a natural source of high value products with biological, aromatic, and pharmacological properties. *Industrial Crops and Products* 38: 153–159. Available at: http://www.sciencedirect.com/science/article/pii/S0926669012000520%5Cnhttp://linkinghub.elsevier.com/retrieve/pii/S0926669012000520%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4052220&tool=pmcentrez&rendertype=abstract%5Cnhttp://journal.f.

PARTIDA-MARTÍNEZ, L.P., and M. HEIL. 2011. The Microbe-Free Plant: Fact or Artifact? *Frontiers in Plant Science* 2: 1–16. Available at:

http://journal.frontiersin.org/article/10.3389/fpls.2011.00100/abstract.
PETRINI, O. 1991. Fungal Endophytes of Tree Leaves. *In* J. H. Andrews, and S. S. Hirano [eds.], Microbial Ecology of Leaves, 501. Springer New York, New York. Available at: http://link.springer.com/chapter/10.1007/978-1-4612-3168-4_9.
PINTOS, C., R. PÉREZ-OTERO, O. AGUÍN, J.P. MANSILLA, B. GONZÁLEZ-PENALTA, and C. RIAL. 2008. PRESENCIA DE Fusarium circinatum EN ESTRUCTURAS REPRODUCTIVAS DE Pinus pinaster EN GALICIA. *In* XIV Congreso de la Sociedad Española de Fitopatología, 149.
PIRTTILÄ, A.M., and C. FRANK. 2011. Endophytes of Forest Trees: Biology and Applications. A. M. Pirttilä, and C. Frank [eds.],. Springer Netherlands, New York. Available at: http://www.springer.com/gp/book/9789400715981.
PORTER, B., M.J. WINGFIELD, and T. A. COUTINHO. 2009. Susceptibility of South African native conifers to the pitch canker pathogen, Fusarium circinatum. *South African Journal of Botany* 75: 380–382. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0254629908003190 [Accessed February 19, 2014].
PRATT, J. E., M. NIEMI, and Z.H. SIEROTA. 2000. Comparison of Three Products Based on Phlebiopsis gigantea for the Control of Heterobasidion annosum in Europe. *Biocontrol Science and Technology* 10: 467–477.
PREISIG, O., N. MOLELEKI, W.A. SMIT, B.D. WINGFIELD, and M.J. WINGFIELD. 2000. A noval RNA mycovirus in a hypovirulent isolate of the plant pathogen Diaporthe ambigua. *Journal of General Virology* 81: 3107–3114.
QUESADA, T., V. GOPAL, W.P. CUMBIE, A.J. ECKERT, J.L. WEGRZYN, D.B. NEALE, B. GOLDFARB, ET AL. 2010. Association mapping of quantitative disease resistance in a natural population of loblolly pine (Pinus taeda L.). *Genetics* 186: 677–686.
RAMÓN-ALBALAT, A., M. BERBEGAL, A. PÉREZ-SIERRA, J. GARCÍA-JIMÉNEZ, and J. ARMENGOL. 2010. EVALUACIÓN IN VITRO DE FUNGICIDAS PARA EL CONTROL DE Fusarium circinatum. *In* XV Congress of the Spanish Society of Phytopathology, 380.
RAMOS GARCÍA, M., M. HERNÁNDEZ LÓPEZ, L.L. BARRERA NECHA, and S. BAUTISTA BAÑOS. 2012. In vitro Response of Fusarium oxysporum Isolates to Isothiocyanates Application Respuesta in vitro de Aislados de Fusarium oxysporum a la Aplicacion de Isotiocianatos. *Revista Mexicana de Fitopatología* 30: 1–10.
RAMSFIELD, T.D., R.D. BALL, J.F. GARDNER, and M.A. DICK. 2010. Temperature and time combinations required to cause mortality of a range of fungi colonizing wood. *Canadian Journal of Plant Pathology* 32: 368–375.
RAMSFIELD, T.D., B.J. BENTZ, M. FACCOLI, H. JACTEL, and E.G. BROCKERHOFF. 2016. Forest health in a changing world: effects of globalization and climate change on forest insect and pathogen impacts. *Forestry*cpw018. Available at: http://forestry.oxfordjournals.org/lookup/doi/10.1093/forestry/cpw018.
REGLINSKI, T., J.T. TAYLOR, and M.A. DICK. 2004. Chitosan induces resistance to pitch canker in Pinus radiata. *New Zealand Journal of Forestry Science* 34: 49–58.
REN, J., H. LI, Y. WANG, J. YE, A. YAN, and X. WU. 2013. Biocontrol potential of an endophytic Bacillus pumilus JK-SX001 against poplar canker. *Biological Control* 67: 421–430. Available at: http://dx.doi.org/10.1016/j.biocontrol.2013.09.012.
REYNOLDS, G., T. GORDON, and N. MCROBERTS. 2016. Quantifying the Impacts of Systemic Acquired Resistance to Pitch Canker on Monterey Pine Growth Rate and Hyperspectral Reflectance. *Forests* 7: 20. Available at:

http://www.mdpi.com/1999-4907/7/1/20.
RICHARDSON, D.M., and P.W. RUNDEL. 2000. Ecology and biogeography of Pinus: an introduction. *In* D. M. Richardson [ed.], Ecology and Biogeography of Pinus, 3–46. Cambridge University Press, Cambridge.
RIGO, D. DE, C. BOSCO, T.H. DURRANT, J. SAN-MIGUEL-AYANZ, J.I. BARREDO, G. STRONA, G. CAUDULLO, ET AL. 2016. Forest resources in Europe : an integrated perspective on ecosystem services , disturbances and threats. *In* J. San-Miguel-Ayanz, D. de Rigo, G. Caudullo, H. T. Durrant, and A. Mauri [eds.], The European Atlas of Forest Tree Species :, 8–19. Office of the European Union, Luxembourg.
ROMERALO, C., O. SANTAMARÍA, V. PANDO, and J.J. DIEZ. 2015. Fungal endophytes reduce necrosis length produced by Gremmeniella abietina in Pinus halepensis seedlings. *Biological Control* 80: 30–39. Available at: http://dx.doi.org/10.1016/j.biocontrol.2014.09.010.
ROMÓN, P., M. TROYA, M.E.F. DE GAMARRA, A. EGUZKITZA, J.C. ITURRONDOBEITIA, and A. GOLDARAZENA. 2008. Fungal communities associated with pitch canker disease of Pinus radiata caused by Fusarium circinatum in northern Spain : association with insects and pathogen – saprophyte antagonistic interactions. 253: 241–253.
ROUX, J., B. EISENBERG, M.J. WINGFIELD, A. KANZLER, A. NEL, V. COETZEE, and E. KIETZKA. 2007. Testing of selected South African Pinus hybrids and families for tolerance to the pitch canker pathogen, Fusarium circinatum. *New Forests* 33: 109–123.
ROVIRA, A.D. 1965. Interactions between plant roots and soil microorganisms. *Annual Review of Microbiology* 19: 241–266.
RUNION, G.B., and R.I. BRUCK. 1988. ts of Thiabendazole-DMSO Treatment of Longleaf Pine Seed Contaminated with Fusarium subglutinans on Germination and Seedling Survival. *Plant Disease* 72: 872–874.
SAIKKONEN, K., S.H. FAETH, M. HELANDER, and T.J. SULLIVAN. 1998. Fungal Endophytes : A Continuum of Interactions with Host Plants. *Annual Review of Ecology and Systematics* 29: 319–343.
SAMUELS, G.J. 2006. Trichoderma: systematics, the sexual state, and ecology. *Phytopathology* 96: 195–206. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18943925.
SANKAR, P., J.J.J. NIVAS, N. SMIJESH, G.K. TIWARI, and R. PHILIP. 2017. Optical emission and dynamics of aluminum plasmas produced by ultrashort and short laser pulses. *Journal of Analytical Atomic Spectrometry* 32: 1177–1185. Available at: http://dx.doi.org/10.1039/C7JA00133A.
SANTAMARÍA, O., D.R. SMITH, and G.R. STANOSZ. 2012. Interaction between Diplodia pinea or Diplodia scrobiculata and fungal endophytes isolated from pine shoots. 1826: 1819–1826.
SANTINI, A, L. GHELARDINI, C. DE PACE, M.L. DESPREZ-LOUSTAU, P. CAPRETTI, A CHANDELIER, T. CECH, ET AL. 2013. Biogeographical patterns and determinants of invasion by forest pathogens in Europe. *The New phytologist* 197: 238–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23057437.
SANZ-ROS, A. V., M.M. MÜLLER, R. SAN MARTÍN, and J.J. DIEZ. 2015. Fungal endophytic communities on twigs of fast and slow growing Scots pine (Pinus sylvestris L.) in northern Spain. *Fungal Biology* 119: 870–883.
SCHOLTZ, V., J. PAZLAROVA, H. SOUSKOVA, J. KHUN, and J. JULAK. 2015. Nonthermal plasma - A tool for decontamination and disinfection. *Biotechnology Advances* 33:

1108–1119. Available at: http://dx.doi.org/10.1016/j.biotechadv.2015.01.002.
SCHULZ, B., and C. BOYLE. 2005. The endophytic continuum. *Mycological Research* 109: 661–686. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0953756208614690 [Accessed July 19, 2011].
SCHULZ, B., C. BOYLE, S. DRAEGER, A.-K. RÖMMERT, and K. KROHN. 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycological Research* 106: 996–1004.
SELCUK, M., L. OKSUZ, and P. BASARAN. 2008. Decontamination of grains and legumes infected with Aspergillus spp. and Penicillum spp. by cold plasma treatment. *Bioresource Technology* 99: 5104–5109.
SERRA-VARELA, M.J., R. ALÍA, J. PÓRTOLES, J. GONZALO, M. SOLIÑO, D. GRIVET, and R. RAPOSO. 2017. Incorporating exposure to pitch canker disease to support management decisions of Pinus pinaster Ait . in the face of climate change. *PloS one* 12: e0171549.
SERRANO, Y., M. ELVIRA-RECUENCO, M. CONDE, M.T. TROYA, and R. RAPOSO. 2015. In vitro Evaluation of Wood Preservatives to Prevent Dispersal of Pitch Canker Pathogen, Fusarium circinatum. *Journal of Phytopathology* 163: 783–786.
SHARMA, R.R., D. SINGH, and R. SINGH. 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological Control* 50: 205–221. Available at: http://dx.doi.org/10.1016/j.biocontrol.2009.05.001.
SHORESH, M., I. YEDIDIA, and I. CHET. 2005. Involvement of Jasmonic Acid / Ethylene Signaling Pathway in the Systemic Resistance Induced in Cucumber by Trichoderma asperellum T203. *Phytopathology* 95: 76–84.
SIEBER, T.N. 2007. Endophytic fungi in forest trees: are they mutualists? *Fungal Biology Reviews* 21: 75–89. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1749461307000310 [Accessed January 31, 2014].
SILVA-CASTRO, I., J. MARTÍN-GARCÍA, J.J. DIEZ, J.A. FLORES-PACHECO, J. MARTÍN-GIL, and P. MARTÍN-RAMOS. 2017. Potential control of forest diseases by solutions of chitosan oligomers, propolis and nanosilver. *European Journal of Plant Pathology*. Available at: http://link.springer.com/10.1007/s10658-017-1288-4.
SINGH, P.P., Y.C. SHIN, C.S. PARK, and Y.R. CHUNG. 1999. Biological Control of Fusarium Wilt of Cucumber by Chitinolytic Bacteria. *Phytopathology* 89: 92–99.
SIVAN, A., and I. CHET. 1989. The possible role of competition between Trichoderma harzianum and Fusarium oxysporum on rhizosphere colonization. *Disease Control and Pest Management* 79: 198–203.
SLINSKI, S.L., F. ZAKHAROV, and T.R. GORDON. 2015. The Effect of Resin and Monoterpenes on Spore Germination and Growth in Fusarium circinatum. *Phytopathology* 105: 119–125.
SMOLINSKA, U., M.J. MORRA, G.R. KNUDSEN, and R.L. JAMES. 2003. Isothiocyanates produced by Brassicaceae species as inhibitors of Fusarium oxysporum. *Plant Disease* 87: 407–412.
SOBROVA, P., V. ADAM, A. VASATKOVA, M. BEKLOVA, L. ZEMAN, and R. KIZEK. 2010. Deoxynivalenol and its toxicity. *Interdisciplinary Toxicology* 3: 94–99. Available at: http://www.degruyter.com/view/j/intox.2010.3.issue-3/v10102-010-0019-x/v10102-010-0019-x.xml.
SORIA, S., R. ALONSO, and L. BETTUCCI. 2012. ENDOPHYTIC BACTERIA FROM

Pinus taeda L. AS BIOCONTROL AGENTS OF Fusarium circinatum NIRENBERG & O'DONNELL. *Chilean journal of agricultural research* 72: 281–284.

SOUZA, R. DE, A. AMBROSINI, and L.M.P. PASSAGLIA. 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. 419: 401–419.

STEENKAMP, E.T., C.A. RODAS, M. KVAS, and M.J. WINGFIELD. 2012. Fusarium circinatum and pitch canker of Pinus in Colombia. *Australasian Plant Pathology* 41: 483–491.

STENLID, J., J. OLIVA, J.B. BOBERG, and A.J.M. HOPKINS. 2011. Emerging diseases in european forest ecosystems and responses in society. *Forests* 2: 486–504.

STEPCZYŃSKA, M. 2016. Surface Modification by Low Temperature Plasma: Sterilization of Biodegradable Materials. *Plasma Processes and Polymers* 13: 1078–1086.

STORER, A.J., T.R. GORDON, and S.L. CLARK. 1998. Association of the pitch canker fungus , Fusarium subglutinans f . sp . pini , with Monterey pine seeds and seedlings in California. 649–656.

STROBEL, G.A. 2003. Endophytes as sources of bioactive products. *Microbes and Infection* 5: 535–544.

SWETT, C.L., and T.R. GORDON. 2017. Exposure to a pine pathogen enhances growth and disease resistance in Pinus radiata seedlings. *Forest Pathology* 47: 1–10.

SWETT, C.L., P. SCIENCE, L. ARCHITECTURE, C. PARK, and C. SHARON. 2016. Evidence for a Hemibiotrophic Association of the Pitch Canker Pathogen Fusarium circinatum with Pinus radiata. 79–84.

TELFORD, A., S. CAVERS, R.A. ENNOS, and J.E. COTTRELL. 2015. Can we protect forests by harnessing variation in resistance to pests and pathogens? *Forestry* 88: 3–12.

TERHONEN, E., N. SIPARI, and F.O. ASIEGBU. 2016. Inhibition of phytopathogens by fungal root endophytes of Norway spruce. *Biological Control* 99: 53–63. Available at: http://dx.doi.org/10.1016/j.biocontrol.2016.04.006.

TOIT, L. DU, and P. HERNANDEZ-PEREZ. 2005. Efficacy of hot water and chlorine for eradication of *Cladosporium variabile*, *Stemphylium botryosum*, and *Verticillium dahliae* from spinach seed. *Plant disease* 89: 1305–1312. Available at: http://apsjournals.apsnet.org/doi/abs/10.1094/PD-89-1305.

TSANTRIZOS, Y.S., X.-J. XU, F. SAURIOL, and C. HYNES. 1993. Novel quinazolinones and enniatins from Fusarium lateritium Nees. *Canadian Journal of Chemistry* 71: 1362–1367.

UBHAYASEKERA, W., and M. KARLSSON. 2012. Bacterial and fungal chitinase chiJ orthologs evolve under different selective constraints following horizontal gene transfer. *BMC Research Notes* 5: 581. Available at: http://bmcresnotes.biomedcentral.com/articles/10.1186/1756-0500-5-581.

VAINIO, E.J., K. KORHONEN, T.T. TUOMIVIRTA, and J. HANTULA. 2010. A novel putative partitivirus of the saprotrophic fungus Heterobasidion ecrustosum infects pathogenic species of the Heterobasidion annosum complex. *Fungal Biology* 114: 955–965. Available at: http://dx.doi.org/10.1016/j.funbio.2010.09.006.

VAINIO, E.J., P. MARTÍNEZ-ÁLVAREZ, D. BEZOS, J. HANTULA, and J.J. DIEZ. 2015. Fusarium circinatum isolates from northern Spain are commonly infected by three distinct mitoviruses. *Archives of Virology* 160: 2093–2098.

VETTRAINO, A., A. ROQUES, A. YART, J. FAN, J. SUN, and A. VANNINI. 2015. Sentinel Trees as a Tool to Forecast Invasions of Alien Plant Pathogens. 1–15. Available at: http://dx.doi.org/10.1371/journal.pone.0120571.

VÍLCHEZ, J.I., A. NAVAS, J. GONZÁLEZ-LÓPEZ, S.C. ARCOS, and F.J. GUTIERREZ. 2016. Biosafety Test for Plant Growth-Promoting Bacteria : Proposed Environmental and Human Safety Index (EHSI) Protocol. 6: .

VILJOEN, A., M.J. WINGFIELD, G.H.J. KEMP, and W.F.O. MARASAS. 1995. Susceptibility of pines in South Africa to the pitch canker fungus Fusarium subglutinans f . sp . pini. 877–882.

VILJOEN, A., M.J. WINGFIELD, and W.F.. MARASAS. 1994. First report of Fusarium subglutinans f. sp. pini on seedlings in South Africa. *Plant Disease* 78: .

VINALE, F., K. SIVASITHAMPARAM, E.L. GHISALBERTI, R. MARRA, S.L. WOO, and M. LORITO. 2008. Trichoderma – plant – pathogen interactions. 40: 1–10.

VIVAS, M., M. KEMLER, and B. SLIPPERS. 2015. Maternal effects on tree phenotypes: Considering the microbiome. *Trends in Plant Science* 20: 541–544. Available at: http://dx.doi.org/10.1016/j.tplants.2015.06.002.

VIVAS, M., J. A. MARTÍN, L. GIL, and A. SOLLA. 2012. Evaluating methyl jasmonate for induction of resistance to Fusarium oxysporum, F. circinatum and Ophiostoma novo-ulmi. *Forest Systems* 21: 289–299.

VIVAS, M., and A. SOLLA. 2012. APLICACIONES DE BABA Y BTH EN BRINZALES DE PINUS PINASTER PARA LA INDUCIÓN DE RESISTENCIAANTE FUSARIUM CIRCINATUM. *Cuad. Soc. Esp. Cienc. For.* 36: 55–60.

VIVAS, M., R. ZAS, L. SAMPEDRO, and A. SOLLA. 2013. Environmental maternal effects mediate the resistance of maritime pine to biotic stress. *PloS one* 8: e70148. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3724826&tool=pmcentrez&rendertype=abstract [Accessed February 6, 2014].

VIVAS, M., R. ZAS, and A. SOLLA. 2012. Screening of Maritime pine (Pinus pinaster) for resistance to Fusarium circinatum, the causal agent of Pitch Canker disease. *Forestry* 85: 185–192. Available at: http://forestry.oxfordjournals.org/cgi/doi/10.1093/forestry/cpr055 [Accessed December 16, 2013].

WANG, G., R. ZHU, L. YANG, K. WANG, Q. ZHANG, X. SU, B. YANG, ET AL. 2016. Non-thermal plasma for inactivated-vaccine preparation. *Vaccine* 34: 1126–1132. Available at: http://dx.doi.org/10.1016/j.vaccine.2015.10.099.

WELLER, D.M. 1988. Biological Control of Soilborne Plant Pathogens in the Rhizosphere with Bacteria. *Annual Review of Phytopathology* 26: 379–407.

WILSON, D. 1995. Endophyte : The Evolution of a Term , and Clarification of Its Use and Definition. *Oikos* 73: 274–276.

WINGFIELD, M.J., E.G. BROCKERHOFF, B.D. WINGFIELD, and B. SLIPPERS. 2015. Planted forest health: The need for a global strategy. *Science (New York, N.Y.)* 349: 832–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26293956.

WINGFIELD, M.J., A. HAMMERBACHER, R.J. GANLEY, E.T. STEENKAMP, T.R. GORDON, B.D. WINGFIELD, and T.A. COUTINHO. 2008. Pitch canker caused by Fusarium circinatum -- a growing threat to pine plantations and forests worldwide. 319–334.

WINGFIELD, M.J., A. JACOBS, T. A. COUTINHO, R. AHUMADA, and B.D. WINGFIELD. 2002. First report of the pitch canker fungus, Fusarium circinatum, on pines in Chile. *Plant Pathology* 51: 397–397. Available at: http://doi.wiley.com/10.1046/j.1365-3059.2002.00710.x.

YEDIDIA, I., M. SHORESH, Z. KEREM, N. BENHAMOU, Y. KAPULNIK, and I. CHET. 2003. Concomitant Induction of Systemic Resistance to Pseudomonas syringae pv .

lachrymans in Cucumber by Trichoderma asperellum (T-203) and Accumulation of Phytoalexins. 69: 7343–7353.

YU, X., B. LI, Y. FU, D. JIANG, S.A. GHABRIAL, G. LI, Y. PENG, ET AL. 2010. A geminivirus-related DNA mycovirus that confers hypovirulence to a plant pathogenic fungus. *Proceedings of the National Academy of Sciences of the United States of America* 107: 8387–8392.

ZAHORANOVÁ, A., M. HENSELOVÁ, D. HUDECOVÁ, B. KALIŇÁKOVÁ, D. KOVÁČIK, V. MEDVECKÁ, and M. ČERNÁK. 2016. Effect of Cold Atmospheric Pressure Plasma on the Wheat Seedlings Vigor and on the Inactivation of Microorganisms on the Seeds Surface. *Plasma Chemistry and Plasma Processing* 36: 397–414.

ZAMIOUDIS, C., and C.M.J. PIETERSE. 2012. Modulation of Host Immunity by Beneficial Microbes. *Molecular Plant-Microbe Interactions* 25: 139–150. Available at: http://apsjournals.apsnet.org/doi/10.1094/MPMI-06-11-0179.

ZAS, R., X. MOREIRA, M. RAMOS, M.R.M. LIMA, M. NUNES DA SILVA, A. SOLLA, M.W. VASCONCELOS, and L. SAMPEDRO. 2015. Intraspecific variation of anatomical and chemical defensive traits in Maritime pine (Pinus pinaster) as factors in susceptibility to the pinewood nematode (Bursaphelenchus xylophilus). *Trees - Structure and Function* 29: 663–673.

ZIUZINA, D., S. PATIL, P.J. CULLEN, K.M. KEENER, and P. BOURKE. 2014. Atmospheric cold plasma inactivation of Escherichia coli, Salmonella enterica serovar Typhimurium and Listeria monocytogenes inoculated on fresh produce. *Food Microbiology* 42: 109–116. Available at: http://dx.doi.org/10.1016/j.fm.2014.02.007.

Table 1. Priming stimuli and changes induced in seedlings and adult pine trees in nurseries and forests, which successfully enhanced resistance to *Fusarium circinatum*.

Environment	Stimuli	Plant material	Enhanced responses	Long-lasting responses	Reference
Nursery	Phosphite (1%)	0.5-yr-old *P. radiata* seedlings	Increased plant growth and delayed symptom development	ND	Cerqueira et al. 2017
	Chitosan (0.1 mg/ml)	1-yr-old *P. radiata* seedlings	Reduction of disease incidence, lesion length and dead tops	ND	Reglinski et al. 2004
	Chitosan (1 mg/ml)	0.3-yr-old *P. patula* seedlings	Delayed symptom development and reduction of lesion size	ND	Fitza et al. 2013
	Pseudomonas fluorescens and *Erwinia billingiae*	2-yr-old *P. radiata* seedlings	Reduction of lesion size*	ND	Iturritxa et al. 2017
	Clonostachys rosea	1.5-yr-old *P. radiata* genotypes	Reduction of lesion length		Moraga-Suazo et al. 2016
	Substrate infected with high inoculum density of *F. circinatum*	*P. radiata* seeds during germination	Increased survival and reduction of lesion size	ND	Swett and Gordon 2017
Field	Repeated inoculations of *F. circinatum* over time	4-yr-old *P. radiata* trees in the field	-	64% reduction of lesion length of tree over a period of 2 years	Bonello et al. 2001
	Prior exposure to *F. circinatum*	Plantations of 4-yr-old *P. radiata* seedlings	Reduction of lesion length and no reduction of tree growth	ND	Reynolds et al. 2016
	Natural infections of *F. circinatum*	Areas of adult *P. radiata* trees long exposed to the pitch canker	Reduction of lesion length in old *vs* new areas, and 89% of trees in remission	Elevated resistance in trees with a longer period of exposure to *F. circinatum*	Gordon et al. 2011
	Wounding	2-yr-old *P. rigida* and *P. densiflora* seedlings/increased resin flow but similar lignin content	Delay in disease severity	ND	Kim et al 2010

* Probably as a consequence of both antagonism and induced resistance. ND Not determined

1 **Table 2**. Narrow-sense heritability estimates of resistance to *Fusarium circinatum* in different
2 pine species.

Species	Tested material	Age (months)	h_i^2	Reference
P. radiata	More than 500 seedlots, including half-sibs and full sibs	12	0.34-0.49	Matheson et al 2006
P. taeda	Clones of full-sib families from a circular design	12	0.27	Kayihan et al 2005
P. taeda	498 clones from the whole distribution		0.21-0.38*	Quesada et al 2010
P. pinaster	40 half-sib selected half-sib families	6	0.20**	Vivas et al 2007
P. tecunumanii	28 families from 8 high-elevation populations	6	0.59	Mitchell et al 2012
P. tecunumanii	49 half-sib families from 4 low-elevation populations	6	0.01	Mitchell et al 2012
P. maximinoi	105 half-sib families from 13 popupations	7	0.01	Mitchell et al 2012
P. elliottii	49 half-sib selected families	8	0.22	Mitchell et al 2012
P. pseudostrobus	33 half-sib families	7	0.06	Mitchell et al 2012
P. patula	13 half-sib families	7	0.06	Mitchell et al 2012b #43
P. pinaster	165 clones from 47 half-sib families from 10 populations	36	0.45	Elvira-Recuenco et al 2014
P. patula	78 half-sib selected families	10	0.25	Mitchell et al 2014
P. patula	63 half-sib selected families	9	0.52	Mitchell et al 2014
P. patula	65 half-sibs families	5	0.27	Nel et al 2014
P. patula	65 half-sib families	5	0.41	Nel et al 2014
P. patula	140 hald-sib families	6	0.26	Nel et al 2014
P. patula	18 half-sib families	6	0.30	Nel et al 2014
P. patula	73 half-sib families	6	0.32	Nel et al 2014

3 ***** The reported heritability value corresponds to Clonal repeatability

** Heritability for symptom scores. In all the remainder cases the reported heritability corresponds to lesion length after inoculation.

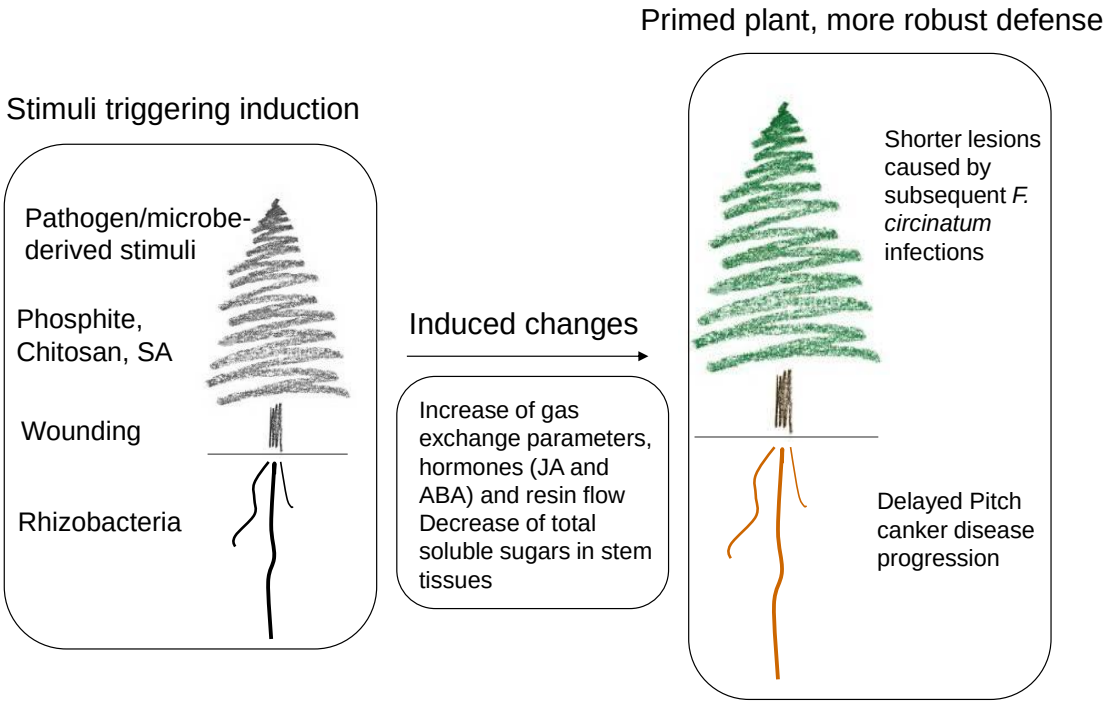

Figure 1. Various biotic and abiotic stressors (left) can trigger an increased defensive capacity of pines to *Fusarium circinatum* (right). Natural infections and artificial inoculations with *F. circinatum*, chemical inducers and mechanical wounding are able to induce local and systemic changes in different parts of the plant.