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Review

Pine Pitch Canker (PPC): Pathways of Pathogen Spread and Preventive Measures

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Abstract: *Fusarium circinatum* (Nirenberg and O' Donnell) is the causal agent of pine pitch canker (PPC) disease, one of the most devastating forest diseases worldwide. Long-distance spread occurs mainly through the movement of infected seeds whereas at regional level, the movement of seedlings, substrates, or containers may play an important role in fungal dispersal. Invasion of nurseries takes place via infected seeds and further spread can occur by planting contaminated seedlings, especially due to the possibility of infected plants remaining symptomless. Once established, *F. circinatum* spreads by rain, wind, and insects. The natural spread of the pathogen is limited due to the short dispersal distances of the spores and the fairly short flight distances of disseminating insects. In this review, we summarize the currently known dispersal pathways of the pathogen, discussing both natural and human-assisted processes. With the purpose of understanding how to best intervene in the disease's development in nurseries and forests, we outline the epidemiology of the pathogen describing the key factors influencing its spread. Preventive measures to control the spread of *F. circinatum* locally and globally are described with special emphasis on the challenges in implementing them.

Keywords: pine pitch canker; *Fusarium circinatum*; dispersion; invasive species; environmentally friendly management

1. Introduction

The geographic barriers that have stabilized the distribution of the world's biota for millions of years have been eroded by human activity in recent centuries, and many plant and pathogen species have consequently moved beyond their native range [1,2]. Invasive alien species are organisms introduced accidentally or intentionally into a natural environment where they are not normally found, and where they can cause serious negative consequences for the native biodiversity. In the forest, invasion by fungal and fungal-like pathogens are closely linked to emerging infectious diseases [3,4]. There are a number of examples of intercontinental invasions of fungal pathogens including the arrival of *Ophiostoma novo-ulmi* Brasier from North America to Europe with rock elm logs [5], which led to the pandemic of Dutch elm disease, as well as the introduction of the chestnut blight agent, *Cryphonectria parasitica* (Murr.) Barr. from Asia to North America through plant propagation material [6]. The pine pathogen *Fusarium circinatum* (Nirenberg and O' Donnell) causing pine pitch canker (PPC) disease was introduced from its native region in Mexico [7] to different continents probably by contaminated seeds, and is also currently present throughout Southern USA, Africa, Asia, and Europe [8].

Fusarium circinatum has been found to be pathogenic to over 60 pine species and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), thus posing a significant risk to natural pine forests, nurseries, and commercial pine plantations [8]. The pathogen can infect different host tissues such as branches, stems, shoots, cones, seeds, and roots of all ages. Infections can occur at any time of the year but PPC symptoms vary depending on host susceptibility and environmental conditions [9–11]. The pathogen infection sometimes affects large surfaces of cortical and subcortical tissue of the trunk [12]. This results in reduced tree growth and timber loss associated with conspicuous resin exudates (pitch) bleeding in response to the fungal infection [13]. Resin bleeding coats the trunk and lower branches below the point of the infection. At this point, the breakage of these branches and even the stem can often occur due to loss of structural integrity or windstorms and the tree eventually dies [14,15]. Root infection promotes brown discoloration and disintegration of the root cortex [16].

Besides cankers, the disease causes mortality of cones and female flowers, reduction in seed quality [17,18], as well as wilting and crown dieback caused by water flow obstruction [14]. On infected branches, cones affected by the pathogen may also abort before reaching full size, but may sometimes remain symptomless depending on the timing and severity of infection [18]. Seeds can be colonized by *F. circinatum* internally and externally, but with no visible impact until seed germination [12,19]. Seeds with embryo and coat infection are 2.4 times more likely to express symptoms than seeds with only

external infection of the seed coat. The pathogen is located mainly on the seed coat [20]. Establishment of *F. circinatum* in nurseries usually is associated with extensive seedling mortality leading to significant economic losses [14]. Infected seedlings show damping off symptoms: the needles turn red, brown, or chlorotic and die from the base up [21], but also a symptomless phase has been documented [22,23].

Since the first record of PPC in 1945 in the South-Eastern United States [24], the disease has spread to other parts of the USA causing substantial economic losses in plantations and the native pine stands [25–30]. After that, PPC was reported from different regions of North, Central and South America, South Africa, Asia [14] and more recently from Spain [31,32] and Portugal [33]. In Italy [34] and France [35] the pathogen was reported in gardens and nurseries, but eradication efforts were successful, and the countries are currently declared disease free.

The spread of *F. circinatum* to countries currently free of the disease, such as New Zealand and Australia, is anticipated to cause significant economic losses [36]. In Australia, keeping *Pinus radiata* (D. Don.) forests free of *F. circinatum* in the next 30 years would produce a benefit of at least 3 million dollars per year [36]. In Chile, for example, between 2006 and 2012, the elimination of 4.3 million plants (0.65% of total production) due to the presence of *F. circinatum*, caused economic losses of around 332,000€ [37]. In addition, the full economic costs of the introduction of invasive pests and pathogens include more than the direct damage or costs associated with monitoring and combating them. They also include the ecological impacts on ecosystem services upon which humans rely [38]. Socioeconomic benefits derived from European forests and, in particular, pine forests are unquestionable. Diseases such as PPC could significantly reduce forestry activities and associated employment contributing to the depopulation and population ageing in rural areas of Europe. In Spain, PPC has resulted in severe crop and yield losses in forest plantations and nurseries. The negative impact has been a consequence of reducing revenues due to the ban on planting susceptible species (*Pinus* spp. and *P. menziesii*) in infected areas [39,40], the high costs invested in monitoring and control, and bans on the export of timber and other products. In addition, *Eucalyptus* spp. plantations that have become popular after PPC have a shorter rotation period, lower social acceptance, and a very narrow genetic pool. This, together with the fact that these types of plantations have not coevolved with the native insects and fungi increase the probability of appearance of pests and diseases [41,42].

Phylogenetic analyses have shown that *F. circinatum* belongs to the "American" clade of the *Fusarium fujikuroi* Nirenberg complex suggesting its origin to be in Mexico [7,43–45]. This is also supported by population studies placing Mexico as a point of origin for *F. circinatum*. Wikler and Gordon [7] showed that *F. circinatum* had the highest genetic diversity in Mexico compared to other populations worldwide. In addition, the very low level of genetic differentiation among *F. circinatum* populations between Mexico and South Africa [7,46] confirmed previous hypotheses that the fungus was introduced into South Africa from Mexico with contaminated seeds [47]. Population differentiation analysis performed by Berbegal et al. [46] revealed similarities among *F. circinatum* populations from Spain, France, Portugal, Uruguay, and the USA and clearly separated Spanish and Portuguese populations from those identified in South Africa, Mexico, and Chile. This suggests that the USA would have been the source of the *F. circinatum* introduction to Spain, which in turn is the probable source of Portuguese and French populations. This hypothesis is supported by the geographic proximity among these countries but also by very high frequencies of many shared genotypes in Spain [46,48], where the disease was well established before it was officially detected in France and Portugal.

This review provides a comprehensive description of the pathways of *F. circinatum* spread and clarifies its epidemiology by outlining the process of infection and describing environmental factors influencing disease development. In order to enhance our readiness to limit future damage by this pathogen, this information is combined with our current understanding of its human-assisted pathways of spread between and within the geographical areas and an updated list is provided of available preventive measures, in order to minimize the risk of spreading, is given. Moreover, the review identifies weak points in current regulations and provides suggestions for implementation.

2. Biology and Ecology of the Pathogen

Fusarium circinatum can reproduce sexually and asexually. The mating is controlled by two alleles at a single mating type locus MAT1 and MAT2 [49]. Strains of opposite mating type can cross, but in nature both mating types must be present at the same host for sexual reproduction to occur [50,51]. The sexual stage, involving perithecia, has never been observed in the field [14], and, therefore, the pathogen spreads mainly asexually through the production of micro- and macro-conidia on infected host tissues [46,51–53]. Notably, the presence of both sexual and asexual modes of reproduction places it in the group of most difficult-to-control fungal diseases, since sexual reproduction may imply genetic recombination, which can lead to the emergence of strains with enhanced virulence [54].

Fusarium circinatum can infect vegetative and reproductive parts at all ages of the host [14,55], although it appears to grow more rapidly in succulent than in older, more lignified tissue [56]. Natural infections occur presumably through wounds of the potential host, regardless of their origin as natural or artificial [56]. However, this theory has been called into question by a recent study, which has demonstrated that *F. circinatum* is also able to colonize seedling stem and mature tree branches that were artificially inoculated without wounds [57]. The disease progress depends on environmental conditions that affect the establishment of the infection (outlined below), the presence of a wound [57], and its depth [28]. Under laboratory conditions, once the conidia germinate on host tissue, the fungus moves first radially via medullary rays, phloem, and cortex, advancing towards the pith of the stem [58]. Subsequently, tangential invasion takes place through the outmost phloem and cortex, parenchyma rays, resin ducts, and axial tracheids in xylem. The pathogen rapidly reaches the nutrient-rich phloem, releasing laccase enzymes that degrade lignified tissue [59], and produce conidiophores and conidia that spread to different parts of the same plant. In addition, *F. circinatum* seems to induce formation of traumatic resin ducts (TRDs), a conifer defense mechanism. The pathogen uses resin ducts for vertical colonization of new zones and high amounts of resin accumulation are thought to restrict the supply of water [60] contributing to plant death.

With root infection, different types of hyphae (e.g., bulbous or narrow) are able to penetrate and colonize the root without causing apparent damage and switch to an active pathogenic phase when the pathogen colonizes the stem [22,23]. Thus, *F. circinatum* displays hemibiotrophic behavior, being capable of living inside the host remaining symptomless even for over a year [19,20,22,23,61].

Across its area of occurrence, PPC is principally or exclusively a disease of planted pine trees [62], but the latent endophytic phase of *F. circinatum* may occur in other species as well. Surveys in California (EEUU), South Africa, and Spain revealed natural colonization by the PPC pathogen of grass species [63–65] including two species of *Poaceae* family as well as five species of herbaceous dicot families (*Asteraceae*, *Lamiaceae*, *Rosaceae*). The grasses tested grew in the understory of a PPC-affected *P. radiata* and *P. muricata* D. Don plantations [64,65] and in a nursery where *Pinus* (primarily *P. patula* Schl. et Cham.) and *Eucalyptus* species grow [63]. All *F. circinatum* isolates found as endophytes in grasses were pathogenic to artificially inoculated pine seedlings, and produced similar lesions to known virulent isolates obtained from symptomatic pine trees [63,64]. In Spain, the fungus was only found in the aerial part of non-symptomatic hosts, not in roots, and it was the same haplotype (as revealed from analysis of microsatellite markers) as the one causing symptoms on pines [61]. Non-symptomatic grasses can therefore constitute a source reservoir of inoculum in a plantation, and the potential risk of spread of the fungus across non-symptomatic hosts constitutes a new scenario that needs to be considered. In fact, there are doubts if pines are the primary resource utilized by this fungus in nature. Instead, *F. circinatum* might be primarily a commensal associate of grasses and only incidentally infect pines [62].

Disease severity is greater in coastal than inland locations in the US, and has been ascribed, among other factors, to longer periods of free moisture on host surfaces owing to frequent intervals of fog in the coastal zone [66]. A similar coastal effect was described also in Northern Spain [67,68] and confirmed by a species distribution model developed for PPC in Spain [69–71]. According to this model, only distance to the coast, precipitation, and temperature were significant variables explaining

the PPC dispersal. However, it seems that the promotive effect of temperature on spore germination is opposite to the effect on spore production since Dvořák et al. [72] found a negative effect of high air temperature and free superficial moisture on the fructification of the pathogen in Galicia (NW Spain). Although in this zone located close to the coast the inoculum was detected through the whole season, the highest amounts were detected after 2–4 weeks of relatively low temperature (around 10 °C) and leaf wetness. This finding shows certain limitations of the thermo- and hydrophilic character of *F. circinatum*, partly supporting the results of Garbelotto et al. [73] who found the highest density of the aerial inoculum during the cool rainy months of the year (November to March) in Northern California.

Variation in susceptibility of pines to *F. circinatum* occurs at various genetic levels from among-species differences (e.g., [15,74]) to intraspecific variation (e.g., [75–77]). In greenhouse studies, it has been shown that lesion size is primarily influenced by the interaction between the host and the pathogen, and less by environmental conditions [27]. The most susceptible species is *P. radiata*, one of the most planted conifers of the southern hemisphere [14,28]. In Europe, the pathogen has been found in forest stands of *P. radiata* in Spain [32] and *P. pinaster* (Ait.) in Spain [48,78] and Portugal [79]. It was previously reported in association with different *Pinus* species in nurseries of both countries [33,48]. In Italy, *F. circinatum* was found to infect numerous trees of *P. halepensis* Mill. and *P. pinea* L. in urban parks and gardens before eradication [34]. Cones and seedlings of *P. pinaster* are highly contaminated in some areas of Northern Spain, and almost half of the seed lots surveyed provided positive isolations of *F. circinatum* [80]. It is also known that artificially infected *P. pinaster* seedlings develop cankers, and may even die [15].

3. Pathways of Spread of the PPC Pathogen

3.1. Natural Spread

Environmental factors may help to explain the particular epidemiology of PPC in some countries. In South Africa, 15 years after being regularly recorded in pine nurseries across the country, *F. circinatum* was identified for the first time in plantation trees [81]. However, the slow establishment of pitch canker in the plantations and the actual timing of these introductions remain unclear. In Chile, *F. circinatum* is known to be present only in nurseries [37], but the disease has never established itself in plantations, probably due to suboptimal climatic conditions or a lack of suitable insect wounding and vectoring agents [82]. The same situation might occur also in Northern Europe where the disease could find suitable conditions in nurseries and glasshouses, but has less chance to thrive in the forest. The equation of environmental variables, the vectors, and the suitability of the plant material that lead to the establishment of the disease in field, are particularly complex.

3.1.1. Weather Conditions

Wind favors the dissemination of *F. circinatum* conidia from infected plants [66]. High concentrations of spores have been detected at distances of at least 1000 m following the wind direction from infected *P. radiata* plants, which indicates wind dispersal over midrange distances [66,72]. The dryness of the atmosphere and the exposure to UV have been suggested as factors reducing the viability of the thin-walled airborne spores of the pathogen [11,66], possibly explaining its limited natural dispersal range. Wind dispersal of spores explains why it is very likely that pine seeds in an infested stand get superficially covered and are able to carry inoculum of *F. circinatum* regardless of the health status of the tree [19]. Dispersal of airborne spores of *F. circinatum* and other *Fusarium* spp. depends also on the rain, since macroconidia need to be in touch with raindrops for flying [83].

The seasonality of the inoculum dispersal was investigated in Northern California (USA) and Spain. The occurrence of spores was detected throughout the year [72,73] although as noted before, their delicateness and quick decrease of viability limit their dispersion by wind, especially in a dry atmosphere. The conidia of *F. circinatum* germinate over a wide range of temperatures, slowly at 10 °C and progressively faster as the temperature rises, up to an optimum around 20–25 °C [84]. For

this reason, infection rates tend to be lower in winter than during warmer periods [84], and it is unlikely to become established in natural forests of northern latitudes. High temperatures (20–26 °C) could favor infections only if relative humidity (RH) is high and/or free moisture is present on plant tissues [11,66,73].

Other meteorological events such as hail or windstorms increase the number of infection courts for the pathogen. Infection increases with fresh wounds compared with old wounds, with the presence of water droplets and with deeper wounds [11,84]. Snow could also affect the incidence of PPC: when snow falls in areas where it is normally infrequent, branch breakage could happen due to accumulating snow, and may open the way to *F. circinatum* [85]. The large wounds formed after branch breakage are perfect entrance courts for the *F. circinatum* spore infection after wind dispersal.

3.1.2. Disease Vectoring

Fusarium circinatum has been reported to be associated with several bark beetles species in *P. radiata* plantations in the Basque Country (Northern Spain) by Romon et al. [86] e.g., *Pityophthorus pubescens* (Marsham, 1802) (25%), *Hylurgops palliates* (Gyllenhal, 1813) (11.96%), *Ips sexdentatus* (Börner, 1776) (8.57%), *Hypothenemus eruditus* Westwood, 1836 (7.89%), *Hylastes attenuates* Erichson, 1836 (7.40%), and *Orthotomicus erosus* (Wollaston, 1857) (2.73%). However, in *P. radiata* plantations in Cantabria (Northern Spain), lower rates of phoresy were found for *P. pubescens* (1.05%), *I. sexdentatus* (0.9%), *H. attenuatus* (1.6%), and *Tomicus piniperda* (around 4%) [87,88]. In *T. piniperda*, *F. circinatum* was isolated with 15%, 13%, 15%, and 33% success from larvae, pupae, F1 young adults, and parents, respectively, of the 118 specimens collected from *P. radiata* trees showing symptoms of PPC [87]. Furthermore, laboratory experiments demonstrated the ability of *T. piniperda* to transfer *F. circinatum* to healthy shoots. Thus, *T. piniperda* is considered as a plausible vector of this pathogen [87]. However, when *F. circinatum* is introduced into pine trees by this bark beetle species, it is less damaging than when entering via mechanical wounds [89]. Bark beetles seem not only to act as vectors but also as wounding agents by boring their breeding and feeding galleries and promoting infection. Thus, the presence of bark beetles in PPC affected stands could increase the incidence of the disease even if insects are not carrying the pathogen [90].

An additional factor in the success of the disease transmission is the amount of inoculum carried by insect vectors. The amount of inoculum acquired by each beetle depends on the extent to which the pathogen colonizes and sporulates on the walls of each pupal chamber [91]. It is also likely to be influenced by the length of time the beetle remains within the chamber. An individual will carry more inoculum of the pathogen if environmental conditions are likely to favor the development of *F. circinatum* in pupal chambers and breeding galleries [92]. In vitro, *F. circinatum* grows optimally at 25 °C [84] while it does not survive exposure to temperatures above 50 °C [28].

Most species of *Pityophthorus* have two to four generations per year in California [93], and the summer emerging adults could be expected to be less infective than spring emerging adults as the time spent in pupal chambers is short, reducing the probability of the developing adults coming into contact with spores. In Northern Spain the phoretical association between *P. pubescens* and *F. circinatum* ranged between 0% and 2.04% [90]. In North America phoresy rates vary depending on the insect species. For example, *F. circinatum* was found on the 0–13.69% of *Pityophthorus carmeli* (Swaine, 1918) while it appeared to be 0%–2% on the *Pityophthorus setosus* (Blackman, 1928) in Monterey Peninsula in California [94]. In addition, relative humidity (RH) may influence the phoresy rate. More PPC lesions per tree appeared when plants were in touch with *P. setosus* at a 100% of RH than when kept at ambient RH of 49–82% [95].

Following the emergence of the insect vectors, the time taken for insect dispersal will depend on the availability of suitable pines for breeding or maturation feeding. Arriving at a suitable pine may be haphazard, but there is evidence that beetles are more likely to land and feed on certain types of pines [96]. Bonello et al. [97] reported the ability of *Pityophthorus* spp. to discriminate between healthy and PPC diseased branches, preferring symptomatic branches due to the increasing ethylene

emission. Species like *P. carmeli* and *P. setosus* in California showed an exploratory behavior, i.e., insects tasted several twigs before making a definitive choice for feeding [94]. This behavior increases the chances of these species acting as wounding agents or vector insects of the PPC pathogen. However, highly diseased trees become less suitable for the sporulation of the pathogen [73], which may have a balancing effect on the further spread of the disease.

Many other insects (e.g., shoot and foliage feeders, bark beetles, wood and root borers, suckers and predators) have also been recognized as potential carriers or vectors of PPC in European and non-European countries [98–100].

3.2. Human-Assisted Spread

Globalization plays a key role in the current geographic redistribution of pathogens, host plants, and vectors that are moved through international transports and travel. International trade in living plant materials and plant products is amplifying the already high risk of spreading of *F. circinatum* to disease-free regions. Species belonging to the genus *Pinus* are among the most widely traded trees worldwide and in Europe [101]. The EU regulation of international trade of plants is based on interception of symptomatic plants but latent infection can easily escape inspections [102]. Since *F. circinatum* can be endophytic in its host and other plant species, the control of these pathways is particularly challenging.

3.2.1. Seeds and Plants for Planting

The seed movement has been one of the most important pathways of introduction of *F. circinatum* into new countries, and has been documented in Chile [103], South Africa [47,81], and Spain [46]. Pine seeds are also potential inoculum sources of the pathogen in nurseries [14,104]. They can be easily acquired on the most popular multinational e-commerce platforms, where phytosanitary certification is not always applied and therefore represent a risk of introduction and spread on a global scale [105].

Seeds from infected pine forests can carry PPC inoculum both on the surface and in the inner tissues, either causing damage to embryo and gametophyte tissues, or as an asymptomatic endophyte [19,20,26]. The mechanisms of seed infection by *F. circinatum* are unknown, but external infestation may in part be due to air-borne spores entering cones at the time of pollination or through small cracks or wounds when cones mature [14]. The vertical transmission of the pathogen (from parents to progeny) must not be discarded since *F. circinatum* has been detected both in open and shut pine cones [80].

The extent of seed contamination depends on the pine species and on environmental conditions [106]. Infection rates of seed lots may largely vary. For instance, *F. circinatum* was isolated from up to 83% of seeds from cones of infected branches of *P. radiata* in California [19], while in North West Spain it was isolated from 45% of seed lots from the field in *P. pinaster* and *P. radiata* [80]. Elvira-Recuenco et al. [20] found that less than 1% of seeds collected from infected *P. radiata* trees in Northern Spain harbored *F. circinatum*. In the latter case, the pathogen was present on the coat of all infected seeds, in some of them it was also infecting the gametophyte, and in a small proportion, the embryo. In North America, the pathogen was also isolated from *P. taeda* seeds, with external presence varying between 7% and 61%, and internal infection between 1% and 34% [18]; also in *P. elliottii* seeds, where up to 30% had internal infection [107]. However, due to the fact that *F. circinatum* is a quarantine pathogen in the EU (included in EPPO/OEPP's A2 list), the infection rate of seed lots is not as important as the percentage of lots with any infected seed, since a single infected seed is enough to introduce the pathogen in a disease-free location.

Recently, this pathogen known to have colonized as an endophyte several herbaceous plants worldwide [63,64,108], was also detected in asymptomatic *Hypochaeris radicata* L. seeds in Europe [64], suggesting that seeds of taxonomically unrelated species could also act as an inoculum reservoir.

In forest nurseries, *F. circinatum* causes pre- and post-emergence damping off and mortality of established seedlings [19,20]. If PPC is established in seedlings it may easily spread further [109,110] since seedlings can be asymptomatic during the trade and develop the disease only after they are

transplanted [14,19,20,111]. In Chile, where *F. circinatum* is a quarantine fungus, the Agricultural and Livestock Service suggests plant analysis in nurseries prior to planting. The official authorization considers the plants to be free for planting if fewer than 10% of the total number of analyzed plots test positive. Additionally, artificial inoculation of *F. circinatum* under laboratory conditions on *Picea abies* (L.) Karst. and *Larix decidua* Mill. seedlings showed that symptomless plants hosted viable pathogen inoculum for at least 8.5 months after inoculation [111]. This finding warns of the risk of dispersion in non-regulated species (i.e., genus *Pinus* and the species *Pseudotsuga menziesii*; [112]).

Elvira-Recuenco et al. [20] studied the transmission rate of *F. circinatum* from *P. radiata* seeds to seedlings, using two different inoculum doses (10^4 or 10^6 spores·mL^{-1}). The percentage of asymptomatic seedlings from the inoculated seeds was 1% (two out of 203) 15 months after inoculation. Fungal populations were analyzed in dead seedlings from 77 to 281 days post-inoculation during the experiment. In roots, *F. circinatum* was estimated between 10^5 and 10^7 cfu·g^{-1} fresh weight in symptomatic plants for both inoculum doses and up to 10^4 cfu·g^{-1} in asymptomatic plants. When the roots are inoculated, it has been suggested that shoot symptoms in seedlings only develop when fungal colonization reaches the root collar, while the plants remain symptomless if the fungus stays only below ground [22,23].

3.2.2. Wood and Bark

Due to the presence of susceptible hosts throughout Europe, care should be taken in handling industrial wood and fuel wood from diseased areas. The risk of dispersing the fungus in infected wood, branch segments, naturally colonized needles, and chips is relatively low [113,114], although this pathway is theoretically possible. Insects that are carried within the wood, and later emerge from it, especially if it still has bark attached, might disseminate the pathogen. While insects are effectively eliminated by chipping, pathogen survival is not affected [115]. The viability of the pathogen declines with time, but it may survive in untreated wood for at least 18 months [116].

Packaging material made of coniferous wood represents a high risk in the dissemination of *F. circinatum*. The wood used for packaging is usually inferior-quality timber in terms of physical imperfections and presence of bark. For instance, wood from trees damaged by *F. circinatum* could also be used in wood packaging entering global trade routes [117]. Conifer bark carrying the pathogen may constitute an additional dispersal pathway. Bark is increasingly traded as natural mulch for ornamentals and the pathogen may be present in it [112].

3.2.3. Dispersion Via Soil

Although *F. circinatum* is not a common resident in soil (since it does not develop chlamydospores), its conidia may persist in soil and plant debris for a variable time depending on soil humidity and temperature [28,118,119]. In nurseries, reused containers that have carried soil represents a reservoir of inoculum resulting in asymptomatic infections associated with *F. circinatum* [120]. Conidia inoculated to soil were not recovered after 224 days when kept at 30 °C, whereas at 20 and 5 °C, the populations were 20 and 3700 cfu/g soil, respectively [114]. Under field conditions, the pathogen was not recoverable from naturally infected wood chips and needles after two years, and nor from soil collected under pitch canker-infected pines [114].

3.2.4. Other Pathways of Spread

The PPC fungus is efficiently dispersed by human actions, such as transfer of seeds, plants and other commodities, tourism, or movement of forestry workers and other forest visitors. Non-evident pathways such as movement of wood packing material with contaminated insects, or residues of contaminated plant material in vehicles or in camping equipment [121,122] must be considered. A serious concern is the intercontinental trade of plants through postal services that are in many cases non-compliant with the phytosanitary requirements defined in the Directive 2016/2031 [123]. Therefore,

specific rules for internet trade should be agreed on, while information campaigns are needed to raise awareness among target groups.

Geographic factors and management intensity of planting sites influence the severity of the PPC as revealed by a survey carried out in California [124,125]. A greater percentage of *P. radiata* trees in the smaller size class (<15 cm of diameter at breast height -DBH-) were free of PPC (69.8%) compared with trees in the larger (>15 cm DBH) size class (45.6%) [124]. Moreover, trees with smaller crown heights (the distance from ground level to the base of the live crown) had higher ratings of disease severity than trees with taller crown heights. Disease was significantly more severe, and progressed more rapidly in managed stands than in wildland areas [124]. It was suggested that higher levels of disease in managed landscape types were due to human activities that intensify the movement of inoculum into and within the managed forest. Ferchaw et al. [125] pointed out that site preparation also affected seedling survival. In particular, pile and burn sites were estimated to have higher survival rates than lop and scatter sites.

Runion and Bruck [126] found that the incidence of PPC disease was negatively correlated with tree spacing. Similarly, Blakeslee et al. [127] found that thinning of *Pinus taeda* (L.) could significantly reduce the incidence and severity of *F. circinatum* infections. The effect is probably more attributable to the alleviation of drought stress than to a reduction in the local spore load of inoculum by removing infected material [128,129]. Indeed, increase of susceptibility to PPC during water stress is higher when trees are planted at high-stand densities [14]. Thinning in combination with fertilization resulted in similarly high PPC levels as observed with fertilization alone [127]. However, fertilization alone caused longer cankers and higher percentages of infections [130]. Heavy fertilization may make pine seedlings tissues more succulent, facilitating fungal entry, influencing the physiological capacity of pines to resist pathogen attacks, and potentially also increasing the attractiveness of pines to insects that may act as vectors causing suitable infection courts for the pathogens [131]. Specifically, applications of high levels of nutrients such as nitrogen, potassium, and phosphorus, both in soil and foliage, have been found to increase disease severity [132,133]. Nitrogen was the principal nutrient that increased the PPC severity, while potassium and phosphorus had an additive effect when both were applied with nitrogen [130]. Outbreaks of PPC have been associated with poultry manure [134], applications of chemical fertilizers [127,130,132,135], and with high levels of nitrogen emissions from air-conditioned chicken houses [133].

Several studies have indicated that pruning wounds are potential infection sites, whereas infections frequencies declined as wounds aged [11,67]. Gordon et al. [129] recommended carrying out pruning operations during cool and dry periods to minimize the risk of infection. Bezos et al. [67] found more dead trees in unpruned plots than in pruned ones, which was related to the decrease of the quantity of inoculum in the air and surrounding trees due to pruning and wood removal.

4. Pathway-Specific Preventive Measures

Once the pathways of dispersal have been described and understood, key stakeholders to engage and implement management actions to prevent spread of the pathogen can be identified [136]. This review illustrates that *F. circinatum* is spread by multiple pathways (Figure 1), and therefore can be controlled only by way of a multitude of complementary actions.

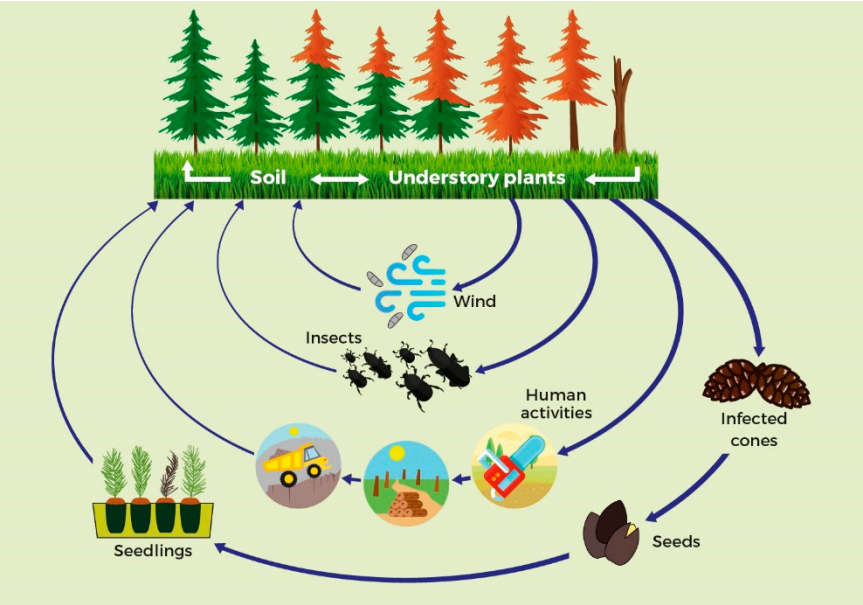

Figure 1. Spreading pathways in the pine pitch canker disease [137].

Different measures to prevent the spread of PPC can be applied depending on whether an area is disease free or if the pathogen is already established. The best way to protect native plants against a new pathogen is the prevention of entry by controlling the known pathways of introduction. Restricting the transport of any infected materials are recommended guidelines for prevention of entry and, just in case, containment of the disease [102].

4.1. Measures to Suppress Natural Spread

4.1.1. Species Selection and Diversity

Natural spread of *F. circinatum* is fairly limited [71], and therefore fostering tolerant host conifers in between plantations might slow down the spread of the disease. However, as *F. circinatum* can infect non-coniferous species endophytically [63,64], it has become evident that it may not be possible to stop the spread of the pathogen by enlarging the physical gaps and discontinuities in the occurrence of host conifer species.

In areas where *F. circinatum* occurs, it is highly recommended to avoid planting susceptible clones or species that are already forbidden under Spanish regulations [39]. The risk of seed contamination should be considered if broadscale seed sourcing, beyond the current seed transfer guidelines, is planned as a part of restoration and adaptive regeneration actions. For instance, northward movement of tree seeds from southern, drought-tolerant tree populations to northern areas has been suggested as a potential means to improve the genetic potential of these forests to adapt to the drier, warmer conditions of the future [138,139]. If such practices are adopted, they should include measures to ensure that the seed lots are free of *F. circinatum*.

Although most of the pine species along with Douglas fir are susceptible to PPC, some alternative trees for planting appear to be more resistant, such as *Chamaecyparis lawsoniana* (A. Murray) Parl. that has showed resistance to *F. circinatum* both in laboratory and in field conditions [15]. The use of resistant-evaluated provenances may help to avoid or reduce disease outbreaks in pine plantations and seed orchards [14,126]. Strategic development of tree resistance requires substantial investment in dedicated programs when eradication of lethal non-native phytopathogens invading defense-free space (i.e., attacking evolutionarily naive host plants) is determined to be impossible [140]. Recently, multiple test of susceptibility of different provenances were carried out under the framework of the European COST action FP1406 "Pine Pitch Canker Strategies for Management of *Gibberella circinata*

in Green Houses and Forests" (PINESTRENGTH), that brought together 36 countries focused on the problem of pine pitch canker disease [111,141,142]. Genetic control, by means of hybridization, breeding, or genetic selection for tolerance, were shown as one of the best long-term methods for the management of PPC [143]. Breeding programs are mainly recommended in populations that are highly susceptible and productive [69]. In addition, a mix of multiple provenances of seeds can be used to increase diversity in order to facilitate adaptation of planted forests to new phytosanitary risks as a result of climate change [138].

4.1.2. Biocontrol Strategies

Interest in using microbiome, i.e., the collective assembly of microorganisms in the rhizosphere, plant external surfaces, and internal tissues to predict the spread and impact of pathogens [144], as a tool in biocontrol has increased during recent decades [145]. The potential of microbes (fungi, bacteria) as biological control agents (BCAs) is based on an array of mechanisms, including the ability of microbes to produce toxins or parasitize the pathogen, or compete with it for the nutritional niche inside plants [146,147]. Moreover, microbiome can influence the pathogen indirectly if the host's defensive metabolism is stimulated by the associated microbes [148]. In order to suppress *F. circinatum* through manipulation of pine microbiome, or to predict disease spread as a function of microbiome composition, more information would be needed about the microbiome associated with healthy and diseased pine phenotypes. For example, it remains to be proven whether taxonomic signatures that are specific to the health status of pines [149] can actually be distinguished in the pine- *F. circinatum* pathosystem, or if high variation among habitats and individuals obscures such comparisons.

Symbiotic mycorrhizal fungi are also an important component of pine microbiome. Their relevance for the general vitality and defensive capacity of pines is linked to the crucial role of mycorrhiza in the uptake of nutrients and water [150]. Whether and how the mycorrhizal colonization is linked to the degree of susceptibility of pines to *F. circinatum* has not been studied yet. In other pathosystems, however, colonization by mycorrhizal fungi has been shown to suppress certain diseases of pines [151] or some *Fusarium* pathogens [152,153]. Therefore, further information about possible direct and indirect effects of mycorrhiza on *F. circinatum* would be needed to assess the ecological importance of these root symbionts for the disease development, and to evaluate possibilities to support pine resistance by promoting mycorrhizal colonization, e.g., in nurseries.

4.1.3. Management of Insect Vectors

The insect-mediated spread of *F. circinatum* varies by region and insect species [14]. In addition, it involves complex interactions that must be elucidated in order to design successful control methods for potential vector insects of PPC. The short-distance flight of insect vectors limits the dispersal of the pathogen to local scale. However, insects can act as wounding agents providing an entry point for the disease [98] that favors PPC establishment. In forest or plantations, a reliable method of monitoring the population of insect vectors is the use of volatile compounds such as pheromones [154]. Interventions for the control should be adapted to the different Forest Management Approaches, considering the gradient of management intensity allowed in each silvicultural system [98]. Due to the increasingly restricted use of pesticides in EU forests [155], mechanical controls are gaining more interest. Strategies such as the elimination of coarse woody debris colonized by insects before their emergence or the avoidance of storage of freshly cut logs in forests have shown effective results [156–158]. Recently, literature-based recommendations in the management of insect vectors of PPC have been compiled by Fernández-Fernández et al. [98,99]. In international trade, the suppression of the long-distance spread of potential insect vectors implicates the debarking of conifer wood destined for packaging material [159]. The desired management of insect vectors in nurseries involves the integration of various strategies relating to food lures, semichemical, and biological controls. In addition, the implementation of proper nursery hygiene practices, such as avoiding water accumulation, which reduces the population of insects is crucial from a phytosanitary point of view.

4.2. Measures to Prevent Human-Assisted Spread

4.2.1. Good Nursery Practices

In forest nurseries, vehicles, tools, equipment, footwear, gloves, and hands should be routinely sanitized by using a wheel bath or footbath in chlorine solutions, and spraying or washing with a 70% alcohol solution (Figure 2). Prevention of seed-borne infections of nursery seedlings should be considered when designing regulations counteracting PPC [129]. Thermotherapy has been identified as a potential tool against *F. circinatum* in seeds [143]. In particular, hot water treatment following the internationally standardized protocol ISPM No. 15 (52 °C for 30 min) seems to be a better treatment of pine seeds than hydrogen peroxide and fungicides against *F. circinatum* [160]. However, implementing this regulatory standard could lead to an almost 28% of *F. circinatum* survival according to Ramsfield et al. [161]. In this study, it was pointed out that temperatures of 61.7 and 68.9 °C for 30 min result in 99% and 99.99% mortality of the pathogen, respectively. Therefore, an urgent revision of the lethal temperature of the PPC pathogen is needed since hot water treatment of seeds could easily be implemented as an environmentally sound and affordable standard in commercial nurseries in order to minimize the risk of the spread of the PPC disease [160,162]. There is, however, no way to completely remove internal infections [19]. Thus, restrictions on seed imports should be considered, while it is advisable to use local seeds that have been collected from trees growing in a favorable maternal environment with the temperature, moisture, and soil characteristics suitable to the ecology of the tree species [163].

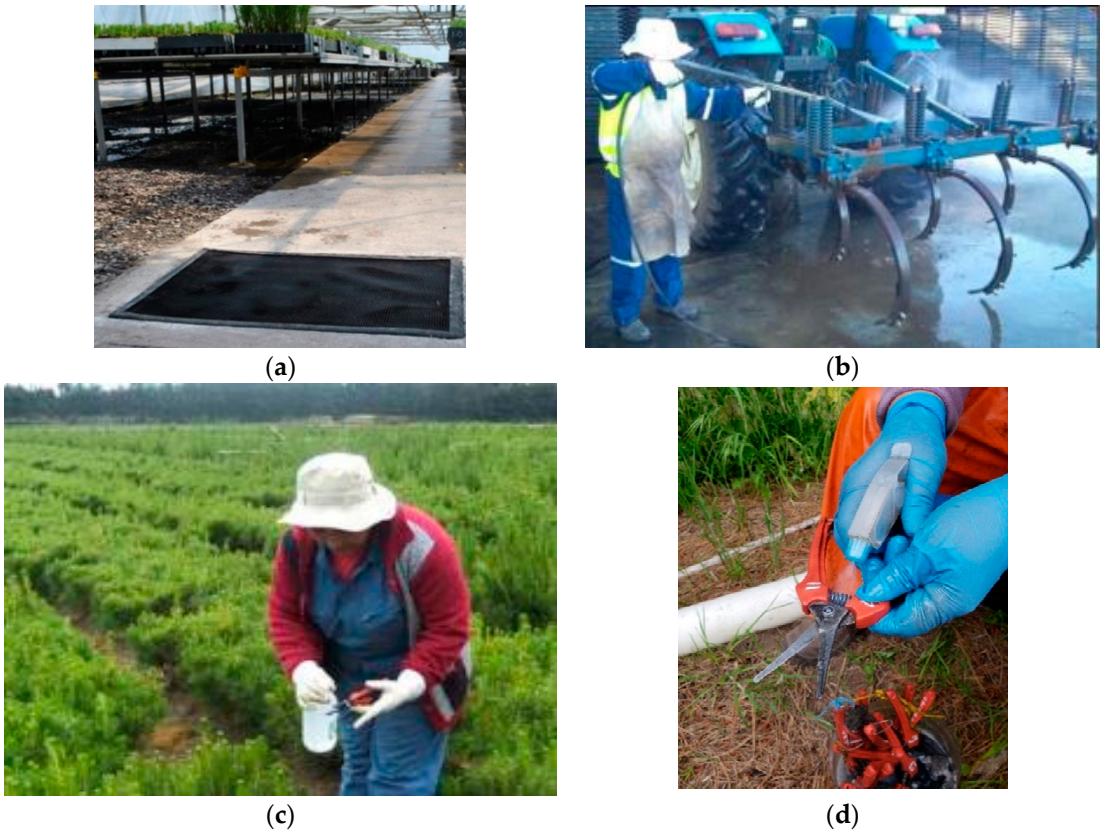

Figure 2. Management actions against *Fusarium circinatum* in Chile: (**a**) footbath; (**b**) disinfection of machinery with 70% ethanol; (**c**,**d**) glove and tool disinfection with ethanol. Photos by Mr A. Rotella-Bioforest SA.

Seedling nurseries may disseminate *F. circinatum* locally and regionally [66]. Sanitation practices are critical to avoid new infestations and spreading of the disease to the field. Removal or burning

of diseased seedlings including their root systems is beneficial. In addition, the use of pathogen-free irrigation by using chlorination or ozone treatment of water, and the use of sterile substrate helps to minimize the incidence of *F. circinatum* associated with asymptomatic seedlings [14,120]. Containerized plants, established from disease-free seeds and grown in carefully sterilized containers, could be favored over bare-root seedlings when possible [129]. The production of seedlings should be in areas where the absence of the organism has been verified (non-demarcated areas) in order to reduce the risk of new long-distance introductions. In Spain, demarcated areas include a part in which the presence of the organism has been verified and another part that acts as a security zone, both with prohibition of the exit of susceptible species [39].

In nurseries already infested by the pathogen, proper hygiene and sterilization practices are important to prevent disease outbreaks. Immersion in hot water (90 °C for 10 s, or 80 °C for 30 s) may be used to clean trays and containers of soil residues (Figure 3). Water with hydrogen peroxide at an oxidation-reduction potential value of 360 mV for 6 h, or autoclaving have also been found to be effective. Using steam at 87 °C for 40 min to treat the substrate for plant production has been found efficient against *F. circinatum* if applied before filling the cavities of trays (Figure 3). Due to the possibility of infected grasses serving as a cryptic reservoir, careful weeding of nurseries and planting areas is needed to reduce inoculum [64,65]. Movement of infested material should be avoided even into areas where pitch canker is already present since the introduction of *F. circinatum* strains that differ in virulence could lead to changes in local populations [129]. In Spain, the declaration of a positive of *F. circinatum* in a nursery forces disinfection of the entire nursery and a ban on the production of susceptible plants (officially *Pinus* and *Pseudotsuga*) [39].

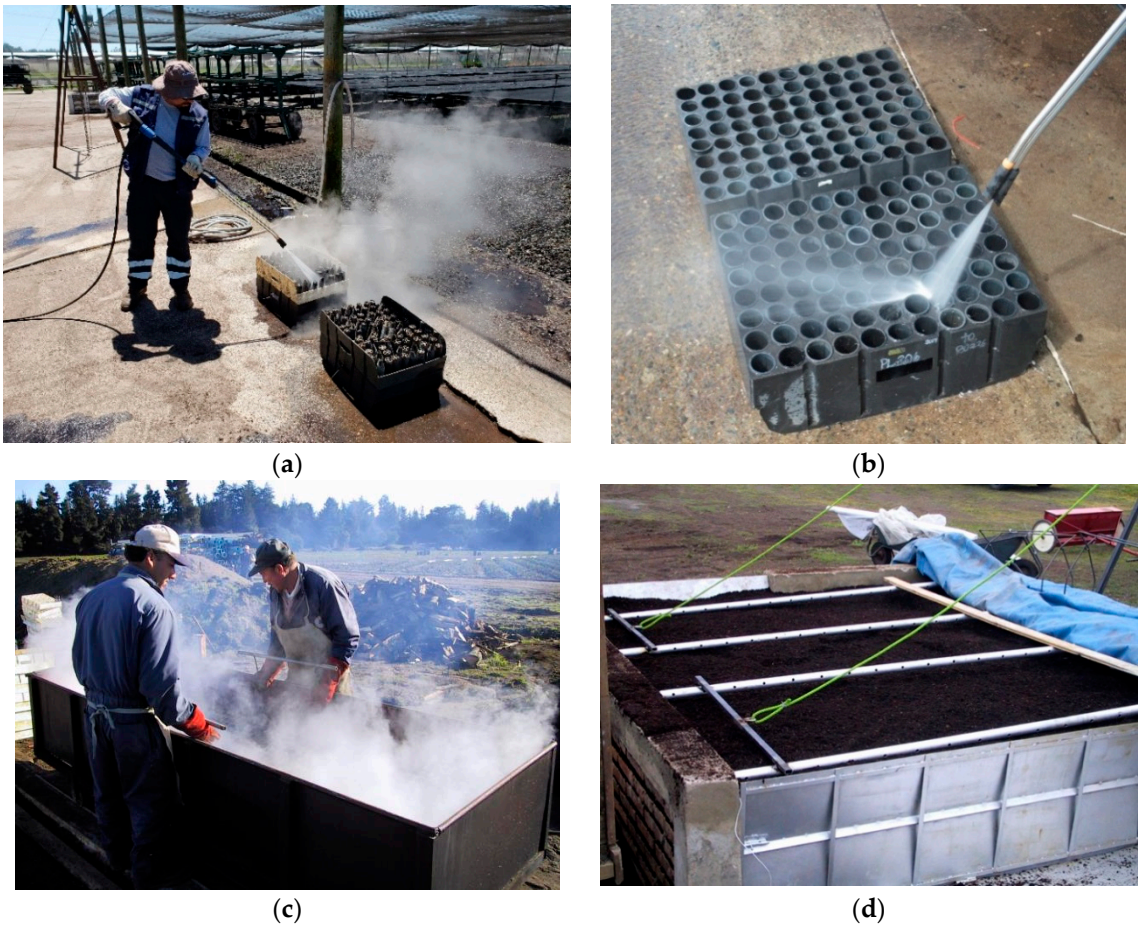

Figure 3. Management actions against *Fusarium circinatum* in Chile: (**a**,**b**) tray washing; (**c**) manual disinfection of trays with hot water; (**d**) substrate sanitation with steam. Photos by Mr A. Rotella-Bioforest SA.

As alternatives to chemical pesticides, BCAs have been used in agricultural soils and proven to be effective in protecting crops against *Fusarium* spp. Antagonistic microbes in soil may suppress the development of *F. circinatum* [164,165] and could thus be used as a part of Integrated Pest Management (IPM) strategies. BCAs that target *F. circinatum* have gained considerable support in recent research and were recently reviewed in Martín-García et al. [143]. In general, the control of woody plant diseases using BCAs is highly context dependent [165,166] and pathogen-specific but it seems to be more effective when applied as a preventive treatment [142,167–169].

Other control methods around nurseries include nursery inspections, maintaining a robust microbial community that will inhibit root-infecting pathogens, and avoiding practices that predispose trees to disease [129]. Culling symptomatic seedlings during lifting of nursery beds can reduce the dispersal of the fungus to other outplantings and minimize exposure of inoculum to healthy seedlings [74]. Attention should be paid to avoid the movement of soils associated with diseased seedlings into non-infested areas of the nursery [112]. The problem of PPC persistence in soil and plant debris is of primary concern in Europe, where the import of soil and growing media containing soil or organic matter from the majority of non-European countries is forbidden, while the importation of pot plants from outside EU is allowed if a phytosanitary certificate is provided [170]. The new rule "Regulation EU 2016/2031" [123], which applies from 14 December 2019 does not mention this contradiction.

4.2.2. Early and Accurate Detection of the Pathogen

Currently, one of the biggest challenges in PPC management is the potential for seedlings to sustain latent infections of *F. circinatum*, having been evident in the last update of the situation of the PPC pathogen in Spain and Portugal [79]. The official surveys carried out between 2017 and 2018 show that the new nursery detections were in asymptomatic plants in both countries. Since visual inspections are not sufficient for a preliminary diagnosis, regular monitoring in seedling nurseries should not only cover symptomatic plants, but also asymptomatic ones. For instance, on site detection of specific signatures of volatile organic compounds or spectral changes could provide means to detect early infections [171]. The development and implementation of reliable diagnostic protocols is fundamental for the early and accurate detection of *F. circinatum* [172].

Molecular identification of *F. circinatum* can be carried out by conventional or real-time Polymerase Chain Reaction (PCR), directly in planta, in soil, or after trapping airborne propagules. Over years, several tests have been published, with different objectives. Ramsfield et al. [161] developed a duplex conventional PCR to detect *F. circinatum* in soil and in host tissue. Schweigkofler et al. [173] and Fourie et al. [174] developed a real-time PCR using SYBR Green dye to detect airborne conidia of *F. circinatum* trapped in the vicinity of infected stands or in nurseries, whereas Dreaden et al. [175] used this technology to detect the fungus in seeds. Real-time PCR using primers and hydrolysis probe targeting different loci in the genome of *F. circinatum* were applied to the detection of the pathogen in seeds [176,177], woody tissue [177,178], and insects [177,179]. Given the increasing number of cryptic taxa described within some species of the *Fusarium* genus, including *F. circinatum* [45], one might expect that the specificity of these tests can be challenged with sibling but non-target species. In this respect, a recent international collaborative study assessed the transferability and performance of these tests using a wide panel of *F. circinatum* and other *Fusarium* spp. DNA, in a standardized manner [172].

4.2.3. Preventive Silvicultural Treatments

In field, specific measures and silvicultural methods can reduce the probability of the pathogen entering new areas. Primarily, the characteristics of the planting site can have a pronounced effect on PPC epidemiology and should be selected with special emphasis on the physical and chemical properties of the soil. Susceptibility to PPC pathogen increases in shallow soils, where trees can suffer from water stress during severe droughts or roots face depletion of oxygen during waterlogging [14,106,126,180].

An association between drought and rapid spread of the disease has been observed in Florida [181] and California, with high mortality of stands located on soils with poor water holding capacity [182].

Removal of branch tip cankers caused by *F. circinatum* has not been shown to be effective at eliminating or slowing the progress of the disease [129,183]. In addition, as noted above wounds favor infections the pathogen [67], making this cultural practice together with pruning not recommended. If pruning is necessary, sterilization of tools by disinfectants with 70% ethanol should be carried out before and after the activity to avoid contamination, even within the same tree. Early harvest of pine stands under influence of *F. circinatum* may be required if the disease incidence is high, and a shortened rotation time should be considered in forest management plans. Harvest operations should start from healthy areas towards infected areas in order to reduce risks of spreading. Vehicles operated in infected areas transport notable amounts of plant material and soil being able to act as spore carriers to disease-free areas [112]. To prevent these pathways of spreading, high sanitary standards for vehicles such as "clean at entry and exit" should be imposed. Besides these recommendations, the risk of disease can be reduced by limiting the silvicultural operations to cool, dry periods, which are less favorable for infection and insect vectors activity [129]. An effective science-based forest management program is important for prevention and control of PPC.

Ferchaw et al. [125] evaluated how silvicultural methods affect survival, growth, and PPC infection in *P. radiata* seedlings. The proposed strategy consists of the use of resistant genotypes and avoiding lop and scatter treatment after tree cutting, increasing seedling survival. In addition, treated openings over 0.20 ha can improve the growth and PPC resistance of the seedlings. Heavy fertilization is directly related to the increase in the severity of PPC, as previously described; therefore, it is advisable to control the application of these products.

4.2.4. Legislation Based on the Current Specific Knowledge

The fact that *F. circinatum* can occur asymptomatically in pines and other plants suggests that the current European legislation [112] will not meet the necessary requirements to stop new introductions of this pathogen, since it is only considered for diseased plants of the genus *Pinus* and of *Pseudotsuga menziesii* [109]. Therefore, revision of current regulations regarding export and import of conifer seeds and other reproductive material (e.g., seedlings, scions) is necessary. Avoidance of any movement of logs, soil, litter, and wood chips from infested areas would minimize the risk of introducing *F. circinatum* propagules to areas free of disease [112]. The Council Directive 2000/29/EC specifies that isolated bark must undergo a heat treatment until the core temperature within each piece reaches at least 56 °C for at least 30 min, according to EPPO Standard PM 10/6 [184]. As noted above, the lethal temperature of *F. circinatum* should be revised in order to guarantee its complete elimination. The efficacy of other methods such as composting [116] or fumigation against the PPC pathogen, is not known. Conifer wood, whether traded in the form of packaging, chips, or wood waste should be stripped of its bark [159] and the most reliable measure would be the prohibition of bark export from areas where the pathogen is known to occur. In addition, economic incentives for actors to keep trade materials clean should be developed [185]. Information campaigns targeting crucial actors such as nurseries, plant retailers, forest managers, and owners should be organized to increase awareness of the risks with different pathways.

5. Concluding Remarks

The multiple pathways of spread make *F. circinatum* challenging to prevent, especially when basic knowledge is still lacking such as the role of insect vectors or microbiome in the spreading processes. The recent discovery of the endophytic colonization of non-coniferous species by *F. circinatum* illustrates the importance of the biological and ecological knowledge for the design of effective intervention strategies. Eradication of the disease may be feasible only if its entry is detected at a very early stage, which is why new methods for early detection and diagnosis of *F. circinatum* in seeds, plants, and vector insects are urgently needed. To ensure that the new, science-based strategies to suppress PPC comply

with existing practices, regulations, and policies [109], it is important that these strategies are developed through collaborations between phytosanitary authorities and researchers. Opinion-building actions, such as The Montesclaros Declaration [186], advocating the crucial target groups about the risks and measures to mitigate them are also needed to suppress the further spread of *F. circinatum* in nurseries and forests.

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