Article

Phenotypical and Molecular Characterisation of *Fusarium circinatum*: Correlation with Virulence and Fungicide Sensitivity

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Abstract: *Fusarium circinatum*, causing pine pitch canker, is one of the most damaging pathogens of *Pinus* species. This study investigated the use of phenotypical and molecular characteristics to delineate groups in a worldwide collection of isolates. The groups correlated with virulence and fungicide sensitivity, which were tested in a subset of isolates. Virulence tests of twenty isolates on *P. radiata*, *P. sylvestris* and *P. pinaster* demonstrated differences in host susceptibility, with *P. radiata* most susceptible and *P. sylvestris* least susceptible. Sensitivity to the fungicides fludioxonil and pyraclostrobin varied considerably between isolates from highly effective (half-maximal effective concentration (EC_{50}) < 0.1 ppm) to ineffective (EC_{50} > 100 ppm). This study demonstrates the potential use of simply acquired phenotypical (cultural, morphological) and molecular metrics to gain a preliminary estimate of virulence and sensitivity to certain fungicides. It also highlights the necessity of including a range of isolates in fungicide tests and host susceptibility assays, particularly of relevance to tree breeding programmes.

Keywords: pine pitch canker; mating type; susceptibility; variation; *Gibberella circinata*; pyraclostrobin; fludioxonil

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1. Introduction

Fusarium circinatum Nirenberg & O'Donnell (sexual morph: *Gibberella circinata* Nirenberg & O'Donnell 1998), the causal agent of pitch canker disease, is a highly damaging pathogen of *Pinus* spp. and *Pseudotsuga menziesii* (Mirb.) [1,2]. It affects both mature trees and seedlings, as well as seeds [2]. Symptoms of infection in mature trees include sunken resinous lesions leading to branch and crown dieback, while symptoms in seedlings include damping-off and wilting [3–5]. The pathogen is found in North, Central and South America, South Africa, Asia, and Southern Europe although it has a limited distribution within these regions [2,6]. Presence of pine pitch canker disease is associated with loss of seedlings in nurseries, reduced timber quality and yields along with tree mortality in forest stands, and therefore significant economic losses [3].

The first report of the disease in Europe was from northern Spain in the late 1990s [7,8]. Its incidence has since expanded in Spain and its presence detected in neighbouring European countries [9–13]. A large-scale survey of Spanish *F. circinatum* isolates by Pérez-Sierra et al. [10] revealed the presence of two distinct groups, each group had distinctive morphological features and corresponded to either mating type 1 or 2, as well as having different levels of virulence on certain host species. Although Spanish isolates were well characterized based on cultural and morphological

features and virulence, these characters have never been investigated on an international collection of *F. circinatum*. Berbegal et al. [14] included additional Spanish isolates and a worldwide collection in a population structure analysis using microsatellite (SSR) markers. The study revealed a number of genetic groupings, with the Spanish isolates again being split into two separate groups, each of a single mating type and with a dominant genotype.

Varying levels of susceptibility to *F. circinatum* both between [15–19] and within [20–22] host species is well known. A number of studies have also reported differences in virulence between *F. circinatum* isolates on a single host species [4,10,18,23,24]. Martínez-Álvarez et al. [25] were the first to describe viruses from *F. circinatum* and further work by Muñoz-Adalia et al. [24] revealed that these mitoviruses can significantly increase virulence of *F. circinatum* and decrease survival of infected seedlings. Differences between isolates in sensitivity to certain fungicides and to hot water treatments have also been reported [26,27].

Many phenotypic variables observable in culture, e.g., growth rate, are the result of multiple genes acting together and can therefore be valuable in the identification of different populations or groups with varying levels of fitness [28]. Phenotypic markers are particularly suitable for population genetics of plant pathogens when the genetic basis of the phenotype is known [29]. However, other genetic markers based on more recent molecular approaches (e.g., SSRs, SNPs) are more direct methods of population structure analysis. Where such described groups of a pathogen, using either approach, correspond to varying levels of fitness (e.g., virulence, susceptibility to fungicides) the information is not only of evolutionary significance but may guide and improve disease management. For example, in *Phytophthora lateralis* and *P. ramorum* both morphological and molecular features have been used to describe various lineages and populations that are the result of evolutionary divergence [30–33]. These groups are associated with different virulence levels and geographical distributions and therefore have important ecological and biosecurity implications [30–33].

The aims of the current study were to (i) investigate the population structure of a worldwide collection of *F. circinatum* isolates using phenotypical and molecular characteristics and (ii) determine whether this structure was correlated with virulence and/or fungicide sensitivity of a subset of isolates.

2. Materials and Methods

2.1. Cultural, Morphological, and Molecular Characterization

One hundred and seventy-one *F. circinatum* isolates obtained from diverse worldwide geographical locations and maintained in the culture collection of the Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Spain (Appendix A) were grown on potato dextrose agar (PDA)(Biokar Diagnostics, Allonne, France) and Spezieller Nährstoffarmer agar (SNA) with two 1 cm^2 pieces of sterile filter paper on the agar surface [34]. Plates were incubated in the dark at 25 °C for 10 days. Cultures grown on PDA were used to study culture pigmentation and to classify each isolate as either 'fast' or 'slow' growing based on general growth rate ranges after the incubation period. Cultures grown on SNA were used to determine sterile hyphal characteristics.

Molecular characterization of the isolates, i.e., mating type, multilocus genotype (MLG) and discriminant analysis of principal components (DAPC) cluster membership of each isolate, was obtained from the dataset of Berbegal et al. [14] (Appendix A).

2.2. Temperature-Growth Response

The growth rates of 162 isolates (Appendix A) were investigated at eight temperatures (5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C). A 6 mm diameter plug of mycelium was placed in the centre of a 90 mm diameter PDA plate and two diameter measurements, at right angles to each other, were made of each culture after five to seven days. At each temperature three replicate plates for each isolate were made. The entire experiment (i.e., three replicate plates of each isolate

at each of the eight temperatures) was conducted twice. The mean growth rate at each temperature, in mm per day, was calculated for each isolate.

2.3. Virulence Tests

Twenty isolates from a diverse range of *Pinus* hosts and geographical regions were selected for virulence tests. Two isolates of each of the five most common multilocus genotypes (MLG) of each mating type were selected based on Berbegal et al. [14] (Appendix A).

One-year old seedlings of *P. pinaster*, *P. radiata*, and *P. sylvestris* were inoculated using the method described by Pérez-Sierra et al. [10]. Briefly, a small amount (1–2 mm^2) of mycelium was scraped from the surface of *F. circinatum* cultures grown on PDA and inserted into a wound made on the main stem of the pine seedlings using a sterile scalpel and then wrapped in Parafilm©. Negative controls were wounded and inoculated with a small amount of PDA. Ten seedlings of each pine species were inoculated with each isolate and the entire experiment was conducted twice.

Seedlings were incubated at 20 °C ± 1 on a 12 h light/12 h dark photoperiod and were visually examined every three days for aerial symptoms. A 0–3 rating scale was used to score each seedling (0 = healthy; 1 = yellowing and dieback of basal needles and/or wilting of main apical shoot; 2 = dieback of plant with the majority of needles yellowed or wilted; 3 = dead plant) as described in Pérez-Sierra et al. [10].

The area under the disease progress curve (AUDPC) [35] was calculated using the R package agricolae [36]. At the end of the experiments cultures were obtained from the infected plants to confirm infection by *F. circinatum*.

2.4. Fungicide Sensitivity

The sensitivity to two fungicides, fludioxonil and pyraclostrobin, was determined for selected *F. circinatum* isolates (Appendix A) following the methods described in Berbegal et al. [27]. Briefly, PDA was amended with filtered diluted fungicides after autoclaving to achieve a final concentration of 0.1, 1, 10, and 100 mg a.i. L^{-1} (ppm). Mycelial plugs (6 mm diameter) were placed onto the fungicide amended plates and incubated in the dark at 25 °C. At each fungicide concentration, and no-fungicide control, four replicates of each isolate were prepared and the entire experiment was conducted twice. After five to seven days, when mycelial growth on non-fungicide amended plates covered at least 2/3 of the plate area, two diameter measurements, at right angles, were made of all colonies. Colony diameter on fungicide amended plates was expressed as a percentage of colony diameter on non-fungicide amended plates, converted to probits and plotted against the $\log_{10}$ values of fungicide concentration. The half-maximal effective concentration (EC_{50}) of each fungicide, or the dose needed to reduce mycelial growth by 50%, was then calculated by probit regression analysis.

2.5. Statistical Methods

A preliminary analysis of growth rates indicated that the isolates formed a continuum in temperature-growth response with many overlapping and non-discrete assemblages, therefore the growth rates at all temperatures were used as a single group in a multiple factor analysis (MFA). A MFA allows the combination of both categorical (e.g., culture pigmentation) and continuous (e.g., culture growth rate) variables in the same analysis, giving an equal weighting to each 'group' of variables. Six groups were used: (1) culture pigmentation; (2) visual assessment of culture growth (fast or slow); (3) sterile hyphae morphology; (4) mating type (MAT-1 or MAT-2); (5) MLG or DAPC cluster; and (6) growth rate (at 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C). Hierarchical clustering on principal components (HCPC) was conducted on the MFA results [37]. Analyses were conducted using the R package FactoMineR [38].

A mixed effects model, using the R package lme4 [39], was used to test for differences in susceptibility between host species, differences in virulence between mating types and to explore whether the different MFA groups were related to different levels of isolate virulence.

Experiment, MLG and individual isolate were treated as random effects in the model building. Bonferroni corrections were used to draw out significant differences between hosts, mating types and MFA groups. An analysis of variance (ANOVA), conducted in R [40], was used to investigate whether the different MFA groups were related to different levels of fungicide sensitivity.

3. Results

3.1. Cultural, Morphological and Growth Rate Results

A large variation in culture pigmentation and patterning was observed. The observed pigmentation patterns were divided into five groups: (1) white (2) whitish-purple (3) white with a purple centre (4) whitish-yellow (5) purple. The majority of cultures had a combination of white and purple pigmentation. Striking differences in speed of growth allowed cultures to be categorised as fast or slow growing. Observation of sterile hyphae revealed that each isolate either (a) had coiled sterile hyphae typical of *F. circinatum*; (b) had sterile, but not distinctively coiled hyphae; or (c) sterile hyphae were absent altogether (Figure 1).

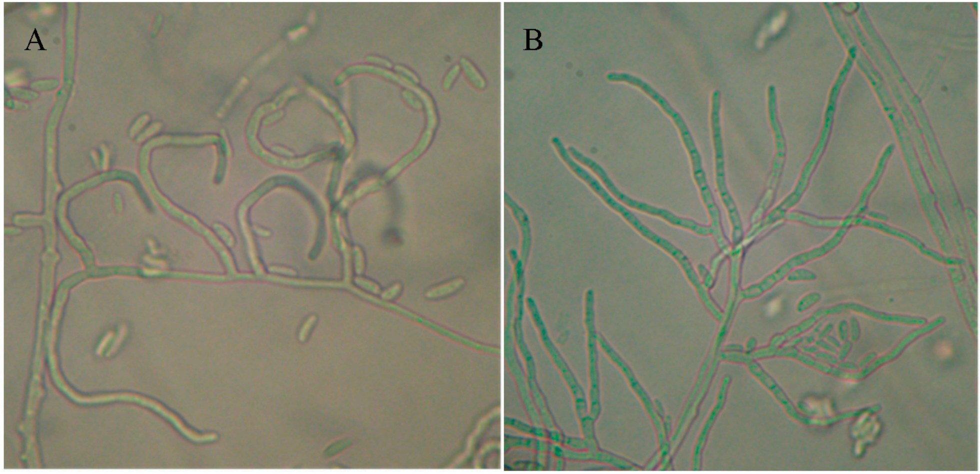

Figure 1. (**A**) An example of *Fusarium circinatum* coiled sterile hyphae (**B**) An example of *F. circinatum* sterile hyphae not distinctively coiled.

Growth rate experiments revealed only minimal growth at 5 °C. The optimum temperature for the fungus was *c*. 25 °C. No growth occurred at 40 °C, however, when these cultures were subsequently incubated at 25 °C growth occasionally occurred, demonstrating that 40 °C was not lethal to the fungus. The minimum, mean and maximum growth rates of all 162 isolates tested at the seven temperatures are given in Table 1.

Table 1. The minimum, mean and maximum daily growth rates (mm per day) of the *F. circinatum* isolates (n = 162) used in this study.

Temperature	Minimum	Mean	Maximum
5 °C	0.060	0.262	0.500
10 °C	0.560	1.042	1.556
15 °C	1.798	3.137	4.181
20 °C	2.492	5.057	6.772
25 °C	3.175	5.856	8.679
30 °C	2.167	4.346	7.573
35 °C	0.127	0.699	2.202

3.2. Virulence and Fungicide Sensitivity Tests

Symptoms developed on all three pine species inoculated with *F. circinatum* isolates. The first symptoms were observed 10 days post-inoculation. The first dead plants occurred 13 days after inoculation (*P. radiata*) and 16 days after inoculation (*P. pinaster* and *P. sylvestris*). All inoculated *P. radiata* plants died by 31 days post-inoculation, all *P. pinaster* plants by 37 days post-inoculation, and the majority of *P. sylvestris* plants by 40 days post-inoculation. Seven, out of the 400 inoculated, *P. sylvestris* seedlings remained alive but symptomatic at the termination of assessments 40 days post-inoculation. No *F. circinatum* cultures were isolated from the negative controls; whereas *F. circinatum* cultures were re-isolated from inoculated seedlings.

The AUDPC values demonstrated that the three host species differed significantly in their susceptibility to *F. circinatum* (χ^2 = 149.73, d.f. = 2, *p*-value < 0.0001) (Figure 2). *Pinus radiata* had the highest AUDPC values (0.01991855 ± 0.0002064786 SE) while *P. sylvestris* had the lowest values (0.01642292 ± 0.0002725392 SE). Host species also differed in terms of rapidity of death, i.e., the days taken for the plant to die (χ^2 = 51.41, d.f. = 2, *p*-value < 0.0001). *Pinus radiata* plants died significantly faster than either *P. sylvestris* or *P. pinaster* however, there was no significant difference between *P. sylvestris* and *P. pinaster* (Figure 3).

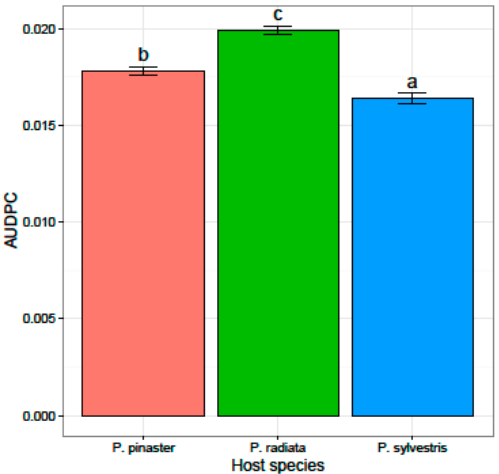

Figure 2. Area under the disease progress curve (AUDPC) values of the three *Pinus* species inoculated. Different letters above bars indicate significant differences in AUDPC values.

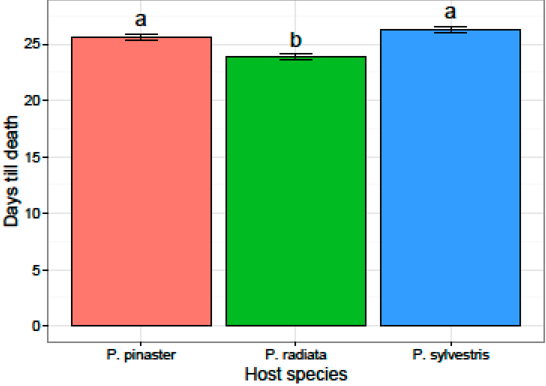

Figure 3. Days till death of the three *Pinus* species inoculated. Different letters above bars indicate significant differences in days till death.

No significant differences in virulence were found between mating types. This was found both when all genotypes (i.e., from all countries) were considered (χ^2 = 2.0, d.f. = 1, *p*-value = 0.1569) and when only the subset of Iberian genotypes was considered (χ^2 = 0.054, d.f. = 1, *p*-value = 0.8155).

The mean EC_{50} values of pyraclostrobin ranged from <0.1 to >100 ppm and those of fludioxonil from 9 to >100 ppm. The mean EC_{50} values of pyraclostrobin and fludioxonil for each isolate are given in Table 2. Pyraclostrobin was more effective at inhibiting mycelial growth than fludioxonil.

Table 2. Mean values (ppm) for half-maximal effective concentration (EC_{50}) for each fungicide on mycelial growth of *F. circinatum* isolates.

Isolate Code	Fludioxonil	Pyraclostrobin
M-4058	38.6	0.1
NRRL26431/MAFF 236397	40	0.1
CBS119864	53	1
CBS119865	9	5
104	69	<0.1
182	50	<0.1
202	>100	0.4
217	47	6.6
122	>100	2
160	30	<0.1
229	>100	18
164	>100	>100
433	65	<0.1
430	>100	<0.1
431	>100	<0.1
389	15	<0.1
453	>100	<0.1
649	61	<0.1
678	54	0.2
623	45	1
625	48	0.6
390	43	<0.1
250	>100	0.1
253	>100	>100
255	>100	>100
822	>100	5
825	22	<0.1
829	40	78
830	9	30
831	>100	75
100	>100	>100
M-8486	60	<0.1
M-8487	>100	<0.1
pv1	>100	<0.1
pv3	>100	0.1
pv8	>100	<0.1
M-3834	>100	2
NRRL25331 M-8386	>100	<0.1
NRLL25332/MAFF240076	60	>100
M-1450	20	<0.1

3.3. Clustering Analysis

Two MFA analyses were conducted. The first, MFA1, used the MLG of each isolate, whereas the second, MFA2, used the DAPC cluster to which the MLG belonged, effectively reducing the 67 MLGs to 5 DAPC groups.

The first two dimensions of MFA1 accounted for 7.67% of the total variation (the first and second dimensions explained 4.32% and 3.35% respectively). MLG, mating type and hyphal morphology group were most strongly correlated with the first dimension (ctr = 29.92, 24.37, and 22.71 respectively) and MLG, the hyphal morphology group and colony pigmentation group were most strongly correlated with the second dimension (ctr = 35.53, 29.13, and 17.24 respectively).

The hierarchical clustering on principal components conducted on the MFA1 results suggested two groups of isolates, and all six data variables were significantly linked with the groups ($p < 0.01$). The main characteristics of each group can be summarised as group A: isolates mainly of MAT-2 with sterile hyphae not distinctively coiled or absent altogether, generally fast growing, growing faster than average at low (10 °C) and optimum (25 °C) temperatures but slower at 35 °C, with a whitish-purple or purple culture pigmentation, MLG 32 (the most common Spanish MLG of MAT-2) and 9 are typical of and found only in this group; group B: isolates mainly of MAT-1 with sterile hyphae coiled, with a white, whitish-yellow, or white with a purple centre culture pigmentation, growing slower than average at low (10 °C) and optimum (25 °C) temperatures but faster at 35 °C, MLG 59 (the most common Spanish MLG of MAT-1) and 62 are typical of and found only in this group.

The first two dimensions of MFA2 accounted for 36.54% of the total variation (the first and second dimensions explained 21.51% and 15.03% respectively). DAPC cluster, hyphal morphology group and mating type were most strongly correlated with the first dimension (ctr = 27.26, 25.20, and 24.42 respectively) and the hyphal morphology group, DAPC cluster and colony pigmentation group were most strongly correlated with the second dimension (ctr = 29.13, 27.81, and 21.19 respectively).

The hierarchical clustering on principal components conducted on the MFA2 results suggested six groups of isolates, and all six data variables were significantly linked with the groups ($p < 0.05$). The main characteristics of each group can be summarised as group A: isolates belong mainly to DAPC cluster 1 with sterile hyphae coiled and of MAT-1, growing slower than average at the optimum (25 °C) but faster at 35 °C; group B: isolates with a whitish-yellow culture pigmentation, belonging to DAPC cluster 1 with sterile hyphae coiled, belonging to MAT-1, growth average at all temperatures; group C: isolates in DAPC cluster 2 with sterile hyphae coiled and of MAT-1, growing slower than average at cool temperatures (10 °C) but faster at high temperatures (35 °C); group D: isolates of DAPC cluster 5 growing slower than average around optimum temperatures (20–25 °C) but average at lower and higher temperatures; group E: isolates of DAPC cluster 4, fast growing and of MAT-2, growing faster than average at all temperatures except extreme high (35 °C); group F: isolates of DAPC cluster 3 with sterile hyphae not distinctively coiled, of MAT-2 and with a whitish-purple culture pigmentation, generally slower growing than average at lower and higher temperatures but average around the optimum (20–25 °C).

All Spanish isolates in MFA1 group A were of MAT-2 with sterile hyphae not distinctively coiled (1 isolate had sterile hyphae absent) and of MLG32. All Spanish isolates in MFA1 group B were of MAT-1 with sterile hyphae coiled. In MFA2 the majority of Spanish isolates were split into groups A and B (corresponding to MFA1 group B) and group F (corresponding to MFA1 group A). Both MFA1 and MFA2 therefore support the grouping of Spanish isolates of Pérez-Sierra et al. [10] and Berbegal et al. [14]. However, isolates from other countries fell into a range of MFA groups not defined solely by mating type or sterile hyphae morphology.

Analysis of virulence and MFA1 groupings revealed a significant interaction between host species and MFA1 grouping ($\chi^2 = 21.9487$, d.f. = 2, p-value < 0.001). Host species had a significant effect independently ($\chi^2 = 127.8558$, d.f. = 2, p-value < 0.001) while MFA1 group had a marginal effect independently ($\chi^2 = 3.2335$, d.f. = 1, p-value = 0.07215) (Figure 4). Across all three host species MFA1 group B isolates produced higher AUDPC values than group A, however this was only significantly different on *P. sylvestris* (Figure 4).

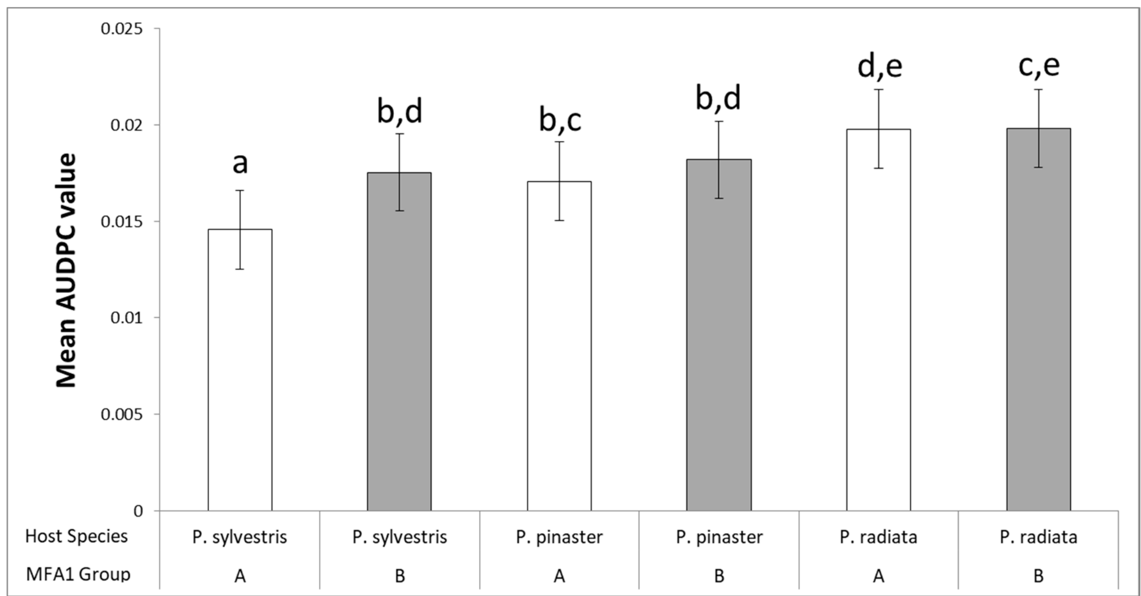

Figure 4. AUDPC values for the three inoculated *Pinus* hosts separated by multiple factor analysis 1 (MFA1) group. Different letters above bars indicate significant differences in AUDPC values.

Analysis of virulence and MFA2 groupings did not reveal an effect of MFA2 grouping independently (χ^2 = 2.2689, d.f. = 4, *p*-value = 0.68642). However host species remained significant independently (χ^2 = 128.0287, d.f. = 2, *p*-value < 0.001), with a significant interaction between host species and MFA2 grouping (χ^2 = 29.2644, d.f. = 8, *p*-value = 0.00028).

However, the MFA2 groups were linked to significant differences in fludioxonil sensitivity between groups (*F* statistic = 3.78, d.f. = 4, *p*-value = 0.016). No difference in pyraclostrobin sensitivity was found between different MFA2 groups (*F* statistic = 0.773, d.f. = 4, *p*-value = 0.553), nor were any differences in either fludioxonil or pyraclostrobin sensitivity found between MFA1 groups (*F* statistic = 0.879, d.f. = 1, *p*-value = 0.357; *F* statistic = 0.565, d.f. = 1, *p*-value = 0.459, respectively).

4. Discussion

This is the first study combining different phenotypical and molecular characteristics to investigate the population structure of a worldwide collection of *F. circinatum* isolates. Two groups defined by sterile hyphae characteristics and molecular markers have been linked to mating type in Spanish isolates in past studies [10,14] and were confirmed here when additional cultural characteristics were included. However, this study revealed that the groups were not as clear when isolates from other countries were included. Nonetheless the groups delineated in this study were related to virulence and fungicide sensitivity.

This study found significant differences in susceptibility to *F. circinatum* between the three host species tested. Such differences in host species susceptibility have been found in other studies (for example [15–19,23,41]). *Pinus radiata* was by far the most susceptible species in this study, which is in agreement with all other studies that have included this host e.g., [18,19,23]. *Pinus pinaster* was more susceptible than *P. sylvestris* according to the AUDPC measure, however there was no significant difference in the number of days it took for the seedling to die, suggesting that *P. pinaster* shows and develops symptoms more rapidly than *P. sylvestris* but mortality occurs within a similar time frame. This is in broad agreement with other studies, for example Martínez-Álvarez et al. [19], working with AUDPC as a measure of susceptibility, found *P. pinaster* to be more susceptible than *P. sylvestris* in two out of three plots yet only significantly different in one plot. Iturritxa et al. [18] found *F. circinatum* lesion length of inoculated seedlings varied between *P. pinaster* provenances with some shorter and

some longer than *P. sylvestris* but none significantly different. In general both *P. pinaster* and *P. sylvestris* are moderately susceptible to *F. circinatum* with *P. pinaster* tending to be slightly more susceptible. However, this is likely to be dependent on provenances of both species and further research is needed to evaluate the relative susceptibility of *P. sylvestris* provenances in order to determine the risk *F. circinatum* poses to the extensive range of *P. sylvestris* in Eurasia.

Fungicide sensitivity was tested on a wide range of *F. circinatum* isolates (n = 40) in this study, whereas most other studies have used a small number of isolates or naturally infested seeds from a single geographical area e.g., [27,41,42]. Both fludioxonil and pyraclostrobin were shown to have a wide range of effects on mycelial growth in the range of isolates tested. Pyraclostrobin had generally lower EC_{50} values, indicating its greater efficacy, yet values ranged from <0.1 ppm (highly effective) to >100 ppm (ineffective). Testing the effect on mycelial growth of four isolates Berbegal et al. [27] also concluded pyraclostrobin had an inconsistent effect, however it was among the most effective fungicides at inhibiting conidial germination of *F. circinatum*. Conversely Berbegal et al. [27] concluded mycelial growth was unaffected by fludioxonil yet the fungicide had inconsistent efficacy for inhibiting conidial germination. In the present study on a wider range of *F. circinatum* isolates fludioxonil had varying efficacy (EC_{50} 9 to >100) on mycelial growth. The results demonstrate that *F. circinatum* isolates vary widely in their sensitivity to these two fungicides, and the same is likely to apply to other fungicides. Such variation is not uncommon in fungal species e.g., [43–45] and highlights the difficulty in recommending a fungicide-based prevention strategy. This variation in fungicide sensitivity may suggest resistance genes or biological mechanisms are already present in natural *F. circinatum* populations, illustrating the adaptive potential of the pathogen. Therefore, if fungicidal control is deployed to combat the pathogen in nurseries, resistance management strategies should be implemented to prevent fungicide resistance quickly rendering individual fungicides ineffective.

A wide range of colony pigmentation and growth rates were observed in the isolates tested. Nirenberg and O'Donnell [46] described colony pigmentation of *F. circinatum* as "greyish white to grey to dark violet at the centre of the colony". This range was grouped into five categories in this study ranging from completely white to fully purple. Mycelial growth rates also encompassed a wide range, with the mean at 20 °C (5.1 mm per day) similar to that reported by Nirenberg and O'Donnell [46] of 4.7 mm per day. Inman et al. [47] also reported a wide range of growth rates from *c.*4.3 to 9.0 mm per day at 20 °C and 6.2 to 10.9 mm per day at 25 °C. These ranges cover a similar spread to those found in the current study yet the values are substantially higher. The most striking morphological feature noted in some of the isolates was the presence of non-coiled sterile hyphae, or in some cases the absence of sterile hyphae altogether. Coiled sterile hyphae are a characteristic feature of *F. circinatum* and gave rise to the species name [46]. Pérez-Sierra et al. [10] were the first to report non-coiled sterile hyphae from Spanish *F. circinatum* isolates, all of which were MAT-2. A wider geographic range of isolates was included in this study and isolates with non-coiled sterile hyphae were found from France, South Africa, Uruguay, and the USA as well as Spain. The majority of these isolates were MAT-2, however some isolates from Uruguay and the USA were of MAT-1. Furthermore, a number of isolates from Canada, Chile, Japan, Spain, and the USA were found with sterile hyphae absent altogether. This variety of sterile hyphae morphology across such a wide geographical range suggests that coiled sterile hyphae may not be as characteristic of *F. circinatum* as previously believed. It is, therefore, recommended to use molecular identification tools to confirm the presence of *F. circinatum*.

This range of phenotypical and molecular characteristics was used to group isolates. The two groups produced by MFA1 split the Spanish isolates by mating type and sterile hyphae morphology exactly as described by Pérez-Sierra et al. [10]. These results support the independent introduction and genetic isolation resulting in population divergence of MAT-2 isolates in Spain as previously reported [14]. However, non-Spanish isolates did not split as clearly, with a small number of isolates present in each of the groups having either different sterile hyphae morphology or the opposite mating type to the majority of isolates in the group. Nonetheless, these two groups did correspond to different virulence levels, with group B (predominantly containing isolates of MAT-1) more

virulent on all host species than group A, although the difference was only significant on *P. sylvestris*. The high susceptibility and rapid death of *P. radiata* likely precluded detection of minor differences in virulence. However for *P. sylvestris*, the least susceptible species tested, the slower development of symptoms allowed small differences in virulence to be discerned. Pérez-Sierra et al. [10] found differences in virulence between the Spanish mating type groups on *P. nigra*, *P. pinaster* and *P. sylvestris* and other studies have also found virulence differences between isolates [4,18,24]. The results exemplify the importance of including a well-characterised set of isolates in virulence tests for breeding programmes where, as a bare minimum, representative isolates of both mating types should be included. The second MFA, which used DAPC instead of MLG as a variable, grouped isolates into six groups rather than the two groups of MFA1. These smaller groups showed differences in fludioxonil sensitivity. In general, the results obtained suggest that a range of easily measured morphological, cultural and molecular characteristics may be of use not only to group isolates into clusters of different virulence but also to predict a new isolate's sensitivity to certain fungicides. This could be useful in disease management and the outlining of biosecurity measures.

Isolates of *F. circinatum* are clearly highly variable in many characteristics, from growth rate to fungicide sensitivity. This variability indicates the pathogen has a high adaptive potential from the genetic diversity already present in the population. For example, the variable and wide ranging growth rates may increase survival by allowing adaptation to various selection pressures or environments. Isolates growing faster than average at higher temperatures would thrive in warmer climates and those growing faster than average at lower temperatures would be more suited to cooler climates. Such differences can be useful in forming groups of isolates which also have other fitness related attributes; in the two groups of MFA1 group A grows faster than group B at 10 °C and 25 °C but slower at 35 °C, with group B more virulent, particularly on *P. sylvestris*. Varying levels of virulence and fungicide sensitivity also indicate adaptive potential, and both of these are likely to be advantageous to the pathogen under various environmental or selection pressures. A greater number of isolates from the delineated groups should be tested for both virulence and fungicide sensitivity to determine the robustness of the groupings.

5. Conclusions

This study demonstrated that *F. circinatum* is a highly variable pathogen, not only in phenotypical and molecular characteristics but also in virulence and fungicide sensitivity. A number of isolates from a wide geographical range (Europe, America, Africa, and Asia) were found with sterile hyphae not distinctively coiled or absent altogether indicating that coiled hyphae are not the diagnostic feature of *F. circinatum* they were once believed to be. Molecular tools are recommended to confirm identity of the pathogen. The use of simply acquired metrics (cultural, morphological and molecular) to group isolates has the potential to be used in the estimation of the virulence or fungicide sensitivity of a new isolate. It is recommended that a well-characterized set of isolates, potentially from each of the major groups found, should be used in virulence and fungicide assays to ensure the full range of the fungus' variability is tested.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Details of the *Fusarium circinatum* isolates [1] used in this study.

Isolate Code	Host Species	Country	Area	Colony Colour [2]	Colony Growth Rate [3]	Sterile Hyphae Morphology [4]	Mating Type	Detailed Growth Rates	MLG [5]	DAPC [6] Cluster	MFA1 [7] Group	MFA2 [7] Group	Fungicide Sensitivity Tests	Virulence Tests
M-4058	*Pinus strobus*	Canada	Ontario	W	F	A	1	-	67	nd	na	na	+	
2010-1038213985	*Pinus radiata*	Chile	Constitución	WP	F	A	2	+	9	4	A	E		+
2010-1249816808	*Pinus radiata*	Chile	Constitución	P	F	A	2	+	9	4	A	E		
3549451339	*Pinus radiata*	Chile	Curicó	WP	F	A	2	+	9	4	A	E		
2010-1454319498	*Pinus radiata*	Chile	Linares	WP	F	A	2	+	9	4	A	E		
2010-1308417545	*Pinus radiata*	Chile	Parral	P	F	A	2	+	9	4	A	E		
441463764	*Pinus radiata*	Chile	Santa Cruz	P	F	A	2	+	9	4	A	E		
4246161911	*Pinus radiata*	Chile	Valdivia	P	F	A	2	+	13	4	A	E		
LNPV217	*Pinus radiata*	France	Cote d'armor	WP	F	NC	2	+	32	3	A	F		
LNPV211	*Pinus* sp.	France	Perpignan	W	F	C	1	+	24	2	B	C		
LNPV216	*Pinus radiata*	France	Vendée	WP	F	NC	2	+	32	3	A	F		
NRRL29945 MAFF237756	*Pinus luchuensis*	Japan	Amami Ohshima	WPC	F	A	1	+	39	5	B	D		
NRRL26431 MAFF 236397	*Pinus luchuensis*	Japan	Kagoshima	W	F	C	1	+	38	nd	B	na	+	
MAFF239859	*Pinus luchuensis*	Japan	Okinawa	WPC	F	A	1	+	38	nd	B	na		
NRRL26432 MAFF236399	*Pinus luchuensis*	Japan	Okinawa	WP	F	A	1	+	39	5	A	D		
E2	*Pinus greggii*	Mexico	Eastern Mexico	WPC	F	C	2	+	9	4	A	E		+
A5	*Pinus patula*	Mexico	Eastern Mexico	WY	F	C	1	+	10	4	B	B		
JAL03	*Pinus douglasiana*	Mexico	Jalisco	WP	F	C	1	+	50	4	B	E		
L-J	*Pinus leiophylla*	Mexico	Michoacan	WY	F	C	1	+	14	4	B	B		
Teo1	*Pinus teocote*	Mexico	Michoacan	WPC	F	C	1	+	7	4	B	E		
Teo3	*Pinus teocote*	Mexico	Michoacan	WP	F	C	2	+	8	4	A	E		
264	*Pinus halepensis*	Portugal	Portugal	WP	F	C	1	+	62	1	B	A		
275	*Pinus halepensis*	Portugal	Portugal	W	F	C	1	+	59	1	B	A		

Table A1. *Cont.*

Isolate Code	Host Species	Country	Area	Colony Colour [2]	Colony Growth Rate [3]	Sterile Hyphae Morphology [4]	Mating Type	Detailed Growth Rates	MLG [5]	DAPC [6] Cluster	MFA1 [7] Group	MFA2 [7] Group	Fungicide Sensitivity Tests	Virulence Tests
276	*Pinus nigra*	Portugal	Portugal	W	F	C	1	+	60	1	B	A		
236	*Pinus pinaster*	Portugal	Portugal	WP	F	C	1	+	60	1	B	A		
240	*Pinus radiata*	Portugal	Portugal	W	F	C	1	+	66	1	B	A		+
252	*Pinus radiata*	Portugal	Portugal	W	F	C	1	+	62	1	B	A		
273	*Pinus radiata*	Portugal	Portugal	WP	F	C	1	+	65	1	B	A		
274	*Pinus radiata*	Portugal	Portugal	W	F	C	1	+	64	1	B	A		
FCC0497 K47 9	*Pinus* sp.	South Africa	Mpumalanga Ngodwana	WPC	F	C	2	+	12	4	B	E		
CBS119864	*Pinus patula*	South Africa	South Africa	W	F	C	1	+	3	4	B	E	+	
CBS119865	*Pinus patula*	South Africa	South Africa	WY	F	NC	2	+	4	4	A	E	+	+
CMWF10 FCC309 K203 5	*Pinus patula*	South Africa	South Africa	WPC	F	C	1	+	16	2	B	C		
NRRL25333 M-8575	*Pinus patula*	South Africa	South Africa	WP	F	NC	2	+	4	4	A	E		+
NRRL25621 CMWF7 FCC140 MAFF240075	*Pinus patula*	South Africa	South Africa	WP	F	C	2	+	26	2	A	C		
CMWF674 KS17 4	*Pinus radiata*	South Africa	South Africa	WP	F	C	1	+	2	2	B	C		
CMWF23 FCC514 K43 8	*Pinus* sp.	South Africa	South Africa	WP	F	C	2	+	28	2	A	C		
CMWF31 FCC133 10	*Pinus* sp.	South Africa	South Africa	WPC	F	C	2	+	1	2	B	C		
CMWF35 FCC124 K42 7	*Pinus* sp.	South Africa	South Africa	WPC	F	C	1	+	6	2	B	C		
CMWF498 FCC116 FGSC9023 2	*Pinus* sp.	South Africa	South Africa	WP	F	C	2	+	4	4	A	E		

Table A1. *Cont.*

Isolate Code	Host Species	Country	Area	Colony Colour [2]	Colony Growth Rate [3]	Sterile Hyphae Morphology [4]	Mating Type	Detailed Growth Rates	MLG [5]	DAPC [6] Cluster	MFA1 [7] Group	MFA2 [7] Group	Fungicide Sensitivity Tests	Virulence Tests
636/06-1	*Pinus nigra*	Spain	Asturias	WY	F	C	1	+	62	1	B	B		
310/06-1	*Pinus palustris*	Spain	Asturias	WPC	F	C	1	+	43	5	B	D		+
104	*Pinus pinaster*	Spain	Asturias	W	S	C	1	+	60	1	B	A	+	
125	*Pinus pinaster*	Spain	Asturias	WP	F	C	1	+	66	1	B	A		
129	*Pinus pinaster*	Spain	Asturias	WP	S	C	1	+	52	1	B	A		
165	*Pinus pinaster*	Spain	Asturias	WP	S	C	1	+	59	1	B	A		
182	*Pinus pinaster*	Spain	Asturias	nd	nd	nd	2	-	nd	nd	na	na	+	
202	*Pinus pinaster*	Spain	Asturias	nd	nd	nd	2	-	nd	nd	na	na	+	
215	*Pinus pinaster*	Spain	Asturias	WP	S	NC	2	+	32	3	A	F		
217	*Pinus pinaster*	Spain	Asturias	WPC	F	C	1	+	59	1	B	A	+	
07/0070-1	*Pinus pinaster*	Spain	Asturias	WP	F	C	1	+	59	1	B	A		
07/0649-1a	*Pinus pinaster*	Spain	Asturias	WPC	S	C	1	+	66	1	B	A		
07/0649-1b	*Pinus pinaster*	Spain	Asturias	WP	F	C	1	-	66	1	na	na		+
488/06	*Pinus pinaster*	Spain	Asturias	WP	S	C	1	+	59	1	B	A		
72	*Pinus radiata*	Spain	Asturias	WPC	F	C	1	+	59	1	B	A		
96	*Pinus radiata*	Spain	Asturias	WPC	F	C	1	+	49	1	B	A		
122	*Pinus radiata*	Spain	Asturias	WP	F	NC	2	-	62	1	na	na	+	
137	*Pinus radiata*	Spain	Asturias	WP	S	C	1	+	59	1	B	A		
160	*Pinus radiata*	Spain	Asturias	WP	F	C	1	+	59	1	B	A	+	
161	*Pinus radiata*	Spain	Asturias	W	F	C	1	+	59	1	B	A		
214	*Pinus radiata*	Spain	Asturias	WPC	S	C	1	+	59	1	B	A		
229	*Pinus radiata*	Spain	Asturias	WP	F	C	1	+	63	1	B	A	+	
244	*Pinus radiata*	Spain	Asturias	W	F	C	1	+	59	1	B	A		
07/0531-1	*Pinus radiata*	Spain	Asturias	WP	S	NC	2	+	32	3	A	F		
07/0650-1	*Pinus radiata*	Spain	Asturias	WPC	F	C	1	+	66	1	B	A		
07/0650-2	*Pinus radiata*	Spain	Asturias	WY	F	C	1	+	59	1	B	B		
487/06 1	*Pinus radiata*	Spain	Asturias	WP	F	C	1	+	59	1	B	A		
499/06-1	*Pinus radiata*	Spain	Asturias	WY	F	C	1	+	64	1	B	B		

Table A1. *Cont.*

Isolate Code	Host Species	Country	Area	Colony Colour [2]	Colony Growth Rate [3]	Sterile Hyphae Morphology [4]	Mating Type	Detailed Growth Rates	MLG [5]	DAPC [6] Cluster	MFA1 [7] Group	MFA2 [7] Group	Fungicide Sensitivity Tests	Virulence Tests
639/06-1	*Pinus radiata*	Spain	Asturias	WPC	S	C	1	+	62	1	B	A		
639/06-2	*Pinus radiata*	Spain	Asturias	WP	F	NC	2	+	32	3	A	F		
639/06-7	*Pinus radiata*	Spain	Asturias	WP	F	NC	2	+	32	3	A	F		
700/05-2	*Pinus radiata*	Spain	Asturias	WP	F	NC	2	+	32	3	A	F		
164	*Pinus sylvestris*	Spain	Asturias	W	F	C	1	+	59	1	B	A	+	+
524/06-2	*Pseudotsuga*	Spain	Asturias	WP	F	C	1	+	59	1	B	A		
433	Pinus nigra subsp. corsicana	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F	+	
76	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
194	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
221	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		+
430	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F	+	
431	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F	+	
434	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
435	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
437	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
438	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
439	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
441	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
442	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
443	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
444	*Pinus radiata*	Spain	Cantabria	WPC	F	A	2	+	32	3	A	E		
445	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
448	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
450	*Pinus radiata*	Spain	Cantabria	WPC	S	NC	2	+	32	3	A	F		
452	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
723	*Pinus radiata*	Spain	Cantabria	WPC	F	C	1	+	58	1	B	A		
1894-08	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		

Table A1. *Cont.*

Isolate Code	Host Species	Country	Area	Colony Colour [2]	Colony Growth Rate [3]	Sterile Hyphae Morphology [4]	Mating Type	Detailed Growth Rates	MLG [5]	DAPC [6] Cluster	MFA1 [7] Group	MFA2 [7] Group	Fungicide Sensitivity Tests	Virulence Tests
1896-08	*Pinus radiata*	Spain	Cantabria	WPC	F	NC	2	+	32	3	A	F		
1900-08	*Pinus radiata*	Spain	Cantabria	WP	F	NC	2	+	32	3	A	F		
389	*Pinus nigra*	Spain	Castilla León	WP	S	C	1	+	53	1	B	A	+	
453	*Pinus nigra*	Spain	Castilla León	WPC	F	NC	2	+	32	3	A	F	+	
649	*Pinus nigra*	Spain	Castilla León	WPC	F	C	1	+	61	1	B	A	+	
678	*Pinus nigra*	Spain	Castilla León	WPC	F	C	1	+	59	1	B	A	+	
821	*Pinus nigra*	Spain	Castilla León	WY	S	C	1	+	59	1	B	B		
729	*Pinus pinea*	Spain	Castilla León	WPC	F	C	1	+	62	1	B	A		
810	*Pinus pinea*	Spain	Castilla León	WY	F	C	1	+	59	1	B	B		
623	*Pinus radiata*	Spain	Castilla León	WP	F	NC	2	+	32	3	A	F	+	
625	*Pinus radiata*	Spain	Castilla León	WP	F	NC	2	-	32	3	na	na	+	
982	*Pinus radiata*	Spain	Castilla León	WP	F	C	1	+	59	1	B	A		
985	*Pinus radiata*	Spain	Castilla León	WPC	F	C	1	+	51	1	B	A		
390	*Pinus sylvestris*	Spain	Castilla León	WPC	F	NC	2	+	32	3	A	F	+	
116	*Pinus nigra*	Spain	Galicia	WP	F	NC	2	+	32	3	A	F		+
250	*Pinus nigra*	Spain	Galicia	WPC	F	NC	2	-	32	3	na	na	+	
253	*Pinus nigra*	Spain	Galicia	WPC	L	C	1	+	62	1	B	A	+	+
255	*Pinus nigra*	Spain	Galicia	W	F	C	1	-	62	1	na	na	+	
822	*Pinus pinaster*	Spain	Galicia	WP	S	C	1	+	62	1	B	A	+	+
823	*Pinus pinaster*	Spain	Galicia	WP	F	C	1	+	41	5	B	D		
825	*Pinus pinaster*	Spain	Galicia	W	F	C	1	+	59	1	B	A	+	+
827	*Pinus pinaster*	Spain	Galicia	WP	F	NC	2	+	32	3	A	F		
828	*Pinus pinaster*	Spain	Galicia	WP	F	NC	2	+	32	3	A	F		
829	*Pinus pinaster*	Spain	Galicia	WY	S	C	1	+	60	1	B	B	+	
830	*Pinus pinaster*	Spain	Galicia	WY	S	C	1	+	59	1	B	B	+	
831	*Pinus pinaster*	Spain	Galicia	WP	F	NC	2	+	32	3	A	F	+	

Table A1. *Cont.*

Isolate Code	Host Species	Country	Area	Colony Colour [2]	Colony Growth Rate [3]	Sterile Hyphae Morphology [4]	Mating Type	Detailed Growth Rates	MLG [5]	DAPC [6] Cluster	MFA1 [7] Group	MFA2 [7] Group	Fungicide Sensitivity Tests	Virulence Tests
100	*Pinus radiata*	Spain	Galicia	nd	nd	nd	1	-	nd	nd	na	na	+	
G1	*Pinus radiata*	Spain	Galicia	WY	F	C	1	+	61	1	B	B		
M-8486	*Pinus radiata*	Spain	País Vasco	WP	F	NC	2	+	32	3	A	F	+	
M-8487	*Pinus radiata*	Spain	País Vasco	WP	F	NC	2	-	32	3	na	na	+	
pv1	*Pinus radiata*	Spain	País Vasco	WP	F	NC	2	+	32	3	A	F	+	
pv14	*Pinus radiata*	Spain	País Vasco	WP	F	NC	2	+	32	3	A	F		
pv15	*Pinus radiata*	Spain	País Vasco	WP	F	NC	2	+	32	3	A	F		
pv2	*Pinus radiata*	Spain	País Vasco	WP	F	NC	2	+	32	3	A	F		
pv3	*Pinus radiata*	Spain	País Vasco	WP	F	NC	2	+	32	3	A	F	+	
pv4	*Pinus radiata*	Spain	País Vasco	WPC	F	NC	2	+	32	3	A	F		
pv8	*Pinus radiata*	Spain	País Vasco	WP	F	NC	2	+	32	3	A	F	+	
pv9	*Pinus radiata*	Spain	País Vasco	P	F	NC	2	+	32	3	A	E		
F1 2053	*Pinus taeda*	Uruguay	Uruguay	WPC	F	C	1	+	48	nd	B	na		
F1 2054	*Pinus taeda*	Uruguay	Uruguay	WPC	F	C	1	+	53	1	B	A		
F1 2186	*Pinus taeda*	Uruguay	Uruguay	WPC	F	C	1	+	47	nd	B	na		
F1 2187	*Pinus taeda*	Uruguay	Uruguay	WPC	F	NC	1	+	31	3	B	F		
D115	*Pinus virginiana*	USA	Alabama	WPC	F	NC	1	+	36	3	B	F		
M-3834	*Pinus radiata*	USA	Berkeley, California	WPC	S	C	1	+	24	2	B	C	+	+
NRRL25331 M-8386	*Pinus radiata*	USA	California	WP	S	C	1	-	24	2	na	na	+	+
CMWF350 FCC986 Fsp34 3	*Pinus* sp.	USA	California	WP	F	C	1	+	23	2	B	C		
FL102	*Pinus elliottii*	USA	Florida	WP	F	C	2	+	45	5	A	D		
FL3	*Pinus elliottii*	USA	Florida	WPC	F	C	1	+	46	5	B	D		

Table A1. *Cont.*

Isolate Code	Host Species	Country	Area	Colony Colour [2]	Colony Growth Rate [3]	Sterile Hyphae Morphology [4]	Mating Type	Detailed Growth Rates	MLG [5]	DAPC [6] Cluster	MFA1 [7] Group	MFA2 [7] Group	Fungicide Sensitivity Tests	Virulence Tests
FL88	*Pinus elliottii*	USA	Florida	WPC	F	C	2	+	44	5	B	D		
M-1001	*Pinus elliottii*	USA	Florida	WPC	S	C	2	+	39	5	B	D		+
M-1025	*Pinus elliottii*	USA	Florida	WP	F	C	2	+	5	5	A	D		
M-1290	*Pinus elliottii*	USA	Florida	WP	F	A	1	+	39	5	A	D		
FK165	*Pinus elliottii*	USA	Georgia	W	F	NC	1	+	18	2	B	C		
M-0956	*Pinus elliottii*	USA	Georgia	WPC	F	NC	1	+	42	5	B	D		
M-0879	*Pinus palustris*	USA	Georgia	WPC	F	C	1	+	43	5	B	D		
FK867	*Pinus taeda*	USA	Georgia	WPC	F	C	2	-	17	3	na	na		+
M-0889	*Pinus taeda*	USA	Georgia	WY	F	A	2	+	34	3	A	E		
NRLL25332 MAFF240076	*Pinus taeda*	USA	Georgia	WP	F	C	2	+	17	3	A	F	+	+
LA4	*Pinus radiata*	USA	Los Angeles county, California	W	F	C	1	+	29	2	B	C		
FSP606	*Pinus radiata*	USA	Marin county, California	WP	F	C	1	+	24	2	B	C		
M-0874	*Pinus taeda*	USA	Mississippi	WPC	F	C	1	+	54	1	B	A		
M-0887	*Pinus taeda*	USA	Mississippi	WPC	F	C	1	+	56	1	B	A		
FSP388	*Pinus radiata*	USA	Monterey county, California	WP	F	NC	1	+	43	5	B	D		+
FSP487	*Pinus radiata*	USA	Monterey county, California	W	F	C	1	+	25	2	B	C		
NRRL25707 MAFF240077	*Pinus caribaea*	USA	North Carolina	WPC	F	NC	1	+	19	2	B	C		
M-0873	*Pinus taeda*	USA	North Carolina	WPC	F	NC	1	+	22	4	B	E		

Table A1. *Cont.*

Isolate Code	Host Species	Country	Area	Colony Colour [2]	Colony Growth Rate [3]	Sterile Hyphae Morphology [4]	Mating Type	Detailed Growth Rates	MLG [5]	DAPC [6] Cluster	MFA1 [7] Group	MFA2 [7] Group	Fungicide Sensitivity Tests	Virulence Tests
M-0912	*Pinus taeda*	USA	North Carolina	WPC	S	NC	1	+	31	3	B	F		
NRRL25708 MAFF 240078	*Pinus taeda*	USA	North Carolina	WY	F	NC	1	+	20	3	B	B		
FSP227	*Pinus radiata*	USA	San Luis Obispo county, California	WP	F	C	1	+	11	4	B	E		
FSP587	*Pinus radiata*	USA	San Luis Obispo county, California	W	F	C	2	+	30	4	B	E		
FSP607	*Pinus radiata*	USA	Santa Cruz county, California	WP	F	C	1	+	27	2	B	C		
FSP255	*Pinus radiata*	USA	Sonoma county, California	W	F	C	2	+	15	4	B	E		
FSP360	*Pinus radiata*	USA	Sonoma county, California	WP	S	C	1	+	39	5	B	D		+
M-1450	*Pinus virginiana*	USA	South Carolina	WPC	F	NC	2	+	21	4	A	E	+	
FK313	*Pinus taeda*	USA	Texas	WPC	F	C	2	+	55	1	B	A		
M-1177	*Pinus taeda*	USA	Texas	WPC	S	C	1	+	35	3	B	A		
M-1061	*Pinus taeda*	USA	USA	WY	F	C	2	+	57	1	B	B		

Table A1. *Cont.*

Isolate Code	Host Species	Country	Area	Colony Colour [2]	Colony Growth Rate [3]	Sterile Hyphae Morphology [4]	Mating Type	Detailed Growth Rates	MLG [5]	DAPC [6] Cluster	MFA1 [7] Group	MFA2 [7] Group	Fungicide Sensitivity Tests	Virulence Tests
M-1057	*Pinus virginiana*	USA	USA	WPC	F	C	1	+	37	3	B	A		
M-0875	*Pinus* sp.	USA	Virginia	WP	F	NC	2	+	33	3	A	F		

[1] Isolates from Chile were provided by E. R. Chávez (Unidad de Fitopatología, Servicio Agrícola y Ganadero, Departamento Laboratorio y Estaciones Cuarentenarias, Santiago, Chile). French isolates were provided by R. Ioos (Station de Mycology, Laboratoire National de la Protection des Végétaux, Malzeville, France). Japanese isolates were provided by NIAS Genebank, National Institute of Agrobiological Sciences, Ibaraki, Japan. Mexican isolates were provided by T. R. Gordon (Department of Plant Pathology, University of California, Davis, CA, USA). Isolates from Portugal were provided by E. Diogo (Laboratório de Micologia, Instituto Nacional de Recursos Biológicos, IP/L-INIA Unidade de Investigação de Protecção de Plantas, Lisbon, Portugal). Isolates from South Africa were provided by B. D. Wingfield (Department of Genetics, FABI, University of Pretoria, Pretoria, South Africa). Isolates from Uruguay were provided by R. Alonso (Laboratorio de Micología, Facultad de Ciencias-Facultad de Ingeniería, Universidad de la República, Montevideo, Uruguay). Isolates from the United States and Canada, were provided by T. R. Gordon (Department of Plant Pathology, University of California, Davis, CA, USA), D. Geiser (Fusarium Research Centre, Pennsylvania State University, University Park, PA, USA) and the NIAS Genebank, National Institute of Agrobiological Sciences, Ibaraki, Japan; [2] Colony colour: W = white; P = purple; WP = whitish-purple; WPC = white with a purple centre only; WY = Whitish-yellow; [3] Colony growth rate: F = fast; S = slow; [4] Sterile hyphae: C = coiled; NC = not distinctively coiled; A = absent; [5] MLG = multilocus genotype; [6] DAPC = discriminant analysis of principal components; [7] MFA = multiple factor analysis.

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