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Survival of *Fusarium circinatum* in soil and *Pinus radiata* needle and branch segments

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Survival of *Fusarium circinatum* in colonized pine needles and wood pieces was measured. Naturally colonized branches and their needles were cut into small pieces and placed in mesh bags on the soil surface at two locations in northern Spain. Pieces were recovered periodically, cultured on a selective medium, and microscopically examined to identify the species. After 507 days, *F. circinatum* was recovered from 0 to 27% of the wood pieces and from none of the needles. After 858 days, *F. circinatum* was not recovered from any wood pieces but was found to be present on 1 out of 220 needle pieces analysed. Artificially infested pieces of wood and needles were placed on 5-mm sieved soil either in plastic boxes at controlled temperature or in mesh bags under field conditions. No survival was recorded after 794 days under field conditions and the decline over time occurred more rapidly in inoculated pieces under field conditions. Soil was also infested with conidia of *F. circinatum* and survival was estimated. No conidia were recovered after 224 days at 30 °C, although at 20 and 5 °C the respective populations were 20 and 3700 cfu/g soil. *Fusarium circinatum* was not recovered from 2-mm-sieved soil collected under pitch canker-infected pines. Results indicate that branch segments and needles naturally colonized by *F. circinatum* will not be a potential source of inoculum, and the fungus in soil is not likely to contribute to reinfection of new plantations after 2 years.

Keywords: disease management, Monterey pine, pathogen survival, pitch canker

Introduction

Fusarium circinatum is the causal agent of the disease pitch canker, one of the most important diseases affecting *Pinus* species (Wingfield *et al.*, 2008). In Europe, the disease occurs mainly in northern Spain (EPPO, 2011) where it has been observed in plantations of *Pinus radiata* (Monterey pine) but rarely in *Pinus pinaster* (Iturritxa *et al.*, 2013). *Pinus radiata* is a non-native species of economic importance (Hermoso *et al.*, 2007) that is grown and managed for timber production in even-aged plantations. *Pinus radiata* covers an area of more than 224 000 ha on the Atlantic coast of northern Spain (MAGRAMA, 2016), which represents 93% of the total area occupied by this species in Spain, 47% of which is located in the Basque Country (northern Spain; MAGRAMA, 2016).

Classical symptoms of infection include resinous and sunken cankers with bark retained that progressively girdle the wood causing yellowing of needles and leading to eventual branch death. The disease progresses by infecting multiple branches, which can cause extensive dieback in the upper tree crown and in turn, tree mortality. Unfortunately, *P. radiata* is one of the most susceptible species to *F. circinatum* (Gordon *et al.*, 1998a,b), and evaluation of the Spanish population of *P. radiata* has also shown this population is highly susceptible (Iturritxa *et al.*, 2012). In a recent survey carried out in the Basque Country during the period 2007–9 (Iturritxa *et al.*, 2013), 16.8% of *P. radiata* plots showed symptoms of pitch canker. Medium disease severity (up to 2/3 of the crown with dead branches) was observed in 7.2% of plots and 8.9% of them showed high severity (more than 2/3 of the crown with dead branches). In contrast, none of the other plots with different *Pinus* species evaluated showed symptoms of pitch canker disease, with the exception of one plot of *P. pinaster*.

Management of Monterey pines involves thinning and pruning several times during the growing cycle, and harvesting when trees are 25–35 years old. Debris includes wood that has been removed in thinnings and other management practices as well as logging debris (branches with cones and needles, and small wood debris) generated during harvesting operations. Debris is either left on site or harvested for biomass production. Harvested plantations are replanted, using *P. radiata*, so determining survival times of *F. circinatum* in the pine debris and soil of plantations with pitch canker disease is of particular importance to prevent infection of trees in the new stand.

Existing research on survival of *F. circinatum* is limited. Under natural conditions the pathogen has only been found in its asexual stage (Viljoen *et al.*, 1997;

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Berbegal *et al.*, 2013). While the fungus lacks specialized structures for surviving in soil (Nirenberg & O'Donnell, 1998), conidia are capable of surviving (Wingfield *et al.*, 2008) for an undetermined length of time. The fungus can also survive in or on host debris, where it has been isolated from slash piles (Wingfield *et al.*, 2008) and needle litter (Aegerter & Gordon, 2006) near infested plantations. In pine branches collected in California and maintained under laboratory conditions, the fungus survived for at least 3 years (McNee *et al.*, 2002).

The objective of this study was to determine the survival time of *F. circinatum* in naturally colonized wood debris on soil. Persistence of the pathogen was also measured in artificially inoculated needles and wood pieces under both controlled and field conditions.

Materials and methods

Soil, fungal isolate, and pathogenicity test

Soil used for controlled temperature experiments was collected in the Basque Country in May 2010 from a *P. pinaster* stand where symptoms of pitch canker have never been observed. In the laboratory, the soil was passed through a 5-mm sieve. Moisture content was determined in a subsample of the soil based on the percentage weight lost after oven drying at 100 °C for 48 h. Moisture content was approximately 25% in soil used for measuring survival of conidia, and 7% in soil used for determining survival in needles and wood segments. Hereafter, measurements are given per gram of dry soil to standardize the data. Soil was placed in plastic boxes (measuring 25 × 30 × 13 cm), and stored at 5 °C until used.

The *F. circinatum* isolate used for artificial inoculations in this study (CECT20759) was recovered from a *P. radiata* seedling in Gipuzkoa, Spain, and is associated with the vegetative compatibility group (VCG) A and mating type 2 (Mat-2), which is the only mating type present in the Basque Country (Iturritxa *et al.*, 2011).

For each individual trial, *F. circinatum* isolates recovered from the last sampling point were tested for pathogenicity as follows: small wounds were made on the main stem of *P. radiata* seedlings using a drill bit (2 mm diameter), and a disk of actively growing mycelia on potato dextrose agar (PDA) was placed on the wound. Inoculated seedlings were placed in a plastic bag for 24 h to retain moisture and ensure infection. Ten seedlings were used in each test.

Survival of conidia in infested soil at controlled temperature

A conidial suspension was prepared by adding 10 mL of sterile purified water on the surface of a PDA plate on which *F. circinatum* had been growing for about 10 days at 22 °C. It was rubbed using a spatula and then filtered through washed glass wool. The density of spores was determined using a haemocytometer. Soil (850 g dry weight) was mixed uniformly with 60 000 conidia g^{-1} and placed in each of nine covered boxes. Three boxes of infested soil were randomly arranged and incubated in darkness at each of three temperatures: 5, 20 and 30 °C. The experiment was conducted twice. In order to isolate conidia from the soil, Rose Bengal-amended PPA medium (Leslie & Summerell, 2006) was used. Conidial survival was evaluated at 4, 70, 114 and 224 days after incubation by suspending 10 g of soil in 100 mL sterile distilled water through stirring at 150 rpm for 30 min. Serial soil dilutions (10^{-1}, 10^{-2} and 10^{-3}) were then spread over the surface of Rose Bengal-amended PPA. Ten plates were prepared for each dilution, incubated in darkness at 25 °C for 20 days, and the dilution with colony numbers of less than 100 per plate was chosen to estimate the number of surviving conidia per gram of oven-dried soil. Colonies of *F. circinatum* were identifiable by their pale brown pigmentation and rough margin after 20 days of incubation in darkness at 25 °C. A subsample of colonies was transferred to Spezieller Nährstoffarmer agar (SNA) medium (Leslie & Summerell, 2006) to confirm that these colonies were *F. circinatum* (EPPO, 2009). A subset of colonies from the last sampling time point were tested for pathogenicity as described above.

Survival in infected needles and branch segments at controlled temperature

Segments of *P. radiata* branches (0.5–1.5 cm in diameter and 3 cm in length) and needles obtained from trees where symptoms of pitch canker have never been observed were artificially infected with *F. circinatum*. Branch segments and needles were placed on cultures of *F. circinatum* growing on PDA in Petri dishes that were maintained at 25 °C in darkness for 10 and 6 weeks, respectively. These incubation periods resulted in apparently complete colonization of branches and needles. Nine plastic boxes for needles and nine for branch segments were used. Needles or branch segments were placed on the surface of 1750 g (dry weight) soil and covered with an additional 250 g soil. Three boxes of each were randomly distributed at 5, 20 and 30 °C, and the whole experiment was performed twice. To test for the presence of *F. circinatum*, 15 needles and six branch segments from each box were randomly sampled four times at every 5–6 months. They were washed with tap water, superficially disinfested in 10% commercial bleach (with a concentration <5% sodium hypochlorite) and rinsed with sterile distilled water. They were then dried in the laminar flow hood and placed on selective *Fusarium* agar (SFA) medium, which is recommended for selective isolation from soil debris (Leslie & Summerell, 2006). Survival was calculated as the percentage of needles and branch segments from which the pathogen was recovered after 3 weeks of incubation at 25 °C.

Survival in infected needles and branch segments under field conditions

Survival of *F. circinatum* was measured in both artificially and naturally infected needle and branch segments beginning in February and April 2011, respectively. Needles and branch segments were artificially infected as described above. Infected branches were obtained from diseased *P. radiata* trees in Laukiniz (Basque Country). Branches with cankers were collected, needles were detached and the branches were cut into pieces (1.5–2.5 cm in diameter and 1.5 cm in length). Needles and branch pieces were placed separately into nylon 0.5 cm mesh bags (30 × 45 cm). Two bags of each naturally and artificially infected needles and branch segments (eight bags in total) were placed in two locations 50 km apart: Laukiniz (43°21′15.0″N, 02°55′49.5″W) and Durango (43°09′55.1″N, 02°39′24.5″W). In each location the surface litter was scraped away and the nylon bags were placed directly on the soil surface and then covered with surface litter. Two bags with non-inoculated needles and branch segments were also placed at each of the two locations.

On each sampling date (at 3, 130, 230, 400, 500 and 850 days for naturally infected pieces; and at 6, 160, 340, 440 and 800 days for inoculated pieces), 15 needles and six branch segments were randomly sampled from each bag. The percentage of tissue pieces from which *F. circinatum* could be recovered was determined using the procedure described above.

Statistical analysis

A repeated measures analysis was performed separately for conidia, needles and branch segments under controlled or field conditions, using the GENMOD procedure with SAS software v. 9.3 (SAS Institute Inc.). The experimental design at controlled temperature was a three-factor repeated measures design with the following main sources of variation: trial repetition (conducted twice) and temperature (three levels) repeated over time (four levels) in the plastic boxes (considered as three replicates). A Poisson probability distribution was specified in the model with the log link function. Analysis of pathogen survival data in needles and branch segments under field conditions was performed in the same way, but in this case it was a two-factor repeated measures design (trial location (two levels) repeated over time (six levels) replicated in two net bags) and the link function specified in the regression model was the logit. When interaction terms were not significant, they were excluded from the final model.

Isolation from field-collected soil

Soil was collected from 24 trees from eight plantations of *P. radiata* located in the Basque Country with symptoms of pitch canker in May and June of 2009. Soil was collected from four locations under each tree sampled and a total of about 500 g topsoil (within 15 cm of the soil surface) was collected; any debris in the soil was removed. Soil samples were air dried and passed through a 2-mm sieve. A soil suspension was prepared with 10 g soil in 100 mL sterile distilled water and stirred. One millilitre of this suspension was added to eight plates containing PPA medium (Aegerter & Gordon, 2006) with slight modifications (15 g peptone, 20 g agar, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.2 g PCNB and 30 mg mL^{-1} streptomycin sulphate solution in 1 L of deionized water). The plates were incubated in darkness at 20 °C, and checked for mycelial growth every 2 days. Putative mycelium of *F. circinatum* was successively transferred to SNA medium in order to positively identify it.

Results

Survival at controlled temperature

After 450 days, reisolation of *F. circinatum* on artificially infected branch segments or needles ranged from 85% to more than 90% across all three incubation temperatures: 5, 20 and 30 °C (Fig. 1a,b). There was no significant effect of temperature, days after inoculation, trial, or any interaction term of these factors ($P > 0.05$) on the reisolation of the pathogen. In contrast, the number of colony-forming units (cfu) in infested soil declined over time (Fig. 1c). In the first 70 days, the *F. circinatum* population dropped to a third of the initial level. After 224 days, inoculum densities were about 4000 ± 1800 (standard deviation) cfu/g soil at 5 °C and less than 50 ± 30 cfu/g soil at 30 °C. Recovery of conidia differed significantly between temperatures ($P = 0.064$). The other sources of variation in the experiment, trial and days after inoculation, were also significant ($P = 0.040$ and 0.031, respectively). None of the interaction terms were significant at the level of $P = 0.05$. Contrast analysis revealed that pairwise differences between temperatures were all significant, i.e. conidia survival at 5 °C

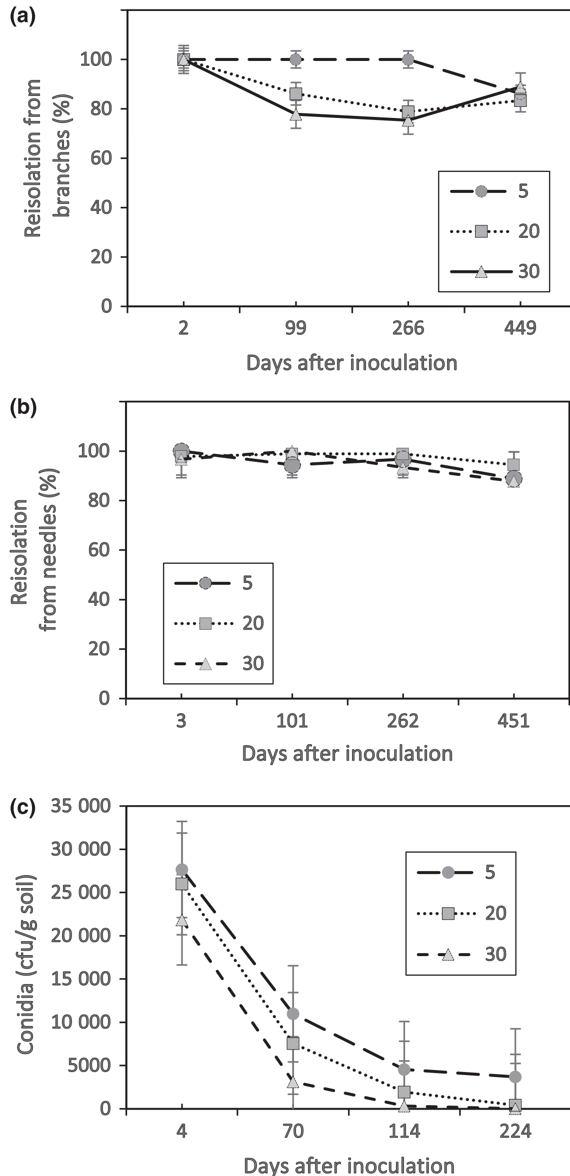

Figure 1 Survival of *Fusarium circinatum* under controlled conditions at 5, 20 and 30 °C in artificially infected branch segments (a) and needles (b) of *Pinus radiata* incubated in soil; and in soil artificially infested with conidia (c). Each point is the mean of two trials with three replicates each. Survival was calculated as the percentage of pieces from which *F. circinatum* was recovered, or the number of colonies per gram of soil.

was significantly greater than at 20 °C, and survival at 20 °C significantly greater than at 30 °C.

Survival under field conditions

Survival in naturally infected branch segments decreased after 403 days on soil (Fig. 2a). Until that time, the pathogen was recovered from more than 80% of branch segments. After 507 days, the pathogen had not survived in any of the wood sampled from the Laukiniz location, whereas it was recovered from 27% of samples from Durango. After 858 days (2 years and 4 months), *F. circinatum* was not recovered from any of the branch segments sampled at either of the two locations (Fig. 2a). The effect of time was significant ($P = 0.0003$) but that of location and the interaction of both factors were not ($P = 0.8516$ and 0.3177, respectively). Survival of the fungus in artificially inoculated branch segments declined after 166 days on soil (Fig. 2b), and the pathogen was not recovered after 443 days. In this case, location and time factors had a significant effect on pathogen survival ($P = 0.0055$ and 0.0024, respectively). The interaction between location and time was not significant ($P = 0.1204$).

For the needles collected from branches with pitch canker symptoms, only 3% of the needles were infected when sampled at day 3. Thereafter, the proportion of needles from which the pathogen was recovered increased up to 403 days in Laukiniz (Fig. 3a). After this time, pathogen survival declined rapidly, and after 507 days no fungi were recovered. After 858 days, needles in bags had deteriorated and were no longer intact. At 858 days *F. circinatum* was detected in one needle piece out of 120 analysed. At the other location, Durango, the proportion of needles from which *F. circinatum* was recovered was also initially low, but to a lesser extent (Fig. 3a). At 230 days onwards, the recovery of *F. circinatum* from the needles decreased, and the pathogen was not recovered from any of the needle pieces after 507 days. Both time and location had significant effects ($P < 0.0001$) on survival, but the time × location interaction was not significant ($P = 0.292$). Survival of *F. circinatum* in artificially infected needles increased up to 166 days, when the

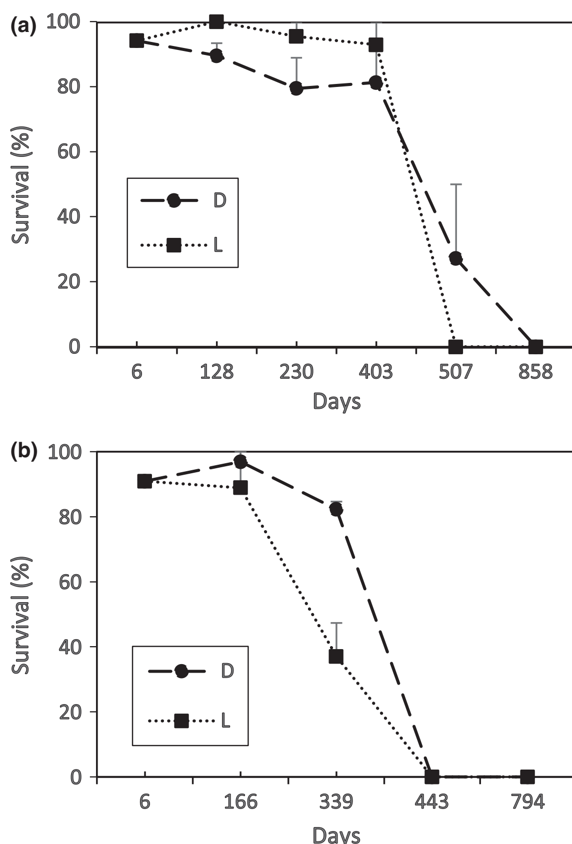

Figure 2 Survival of *Fusarium circinatum* in *Pinus radiata* branch segments (a) naturally colonized, and (b) artificially inoculated, incubated in mesh bags under field conditions at two locations in northern Spain (termed D and L). Each point is the mean of two replicates. Vertical bars are standard errors. Survival was calculated as the percentage of pieces from which *F. circinatum* was recovered.

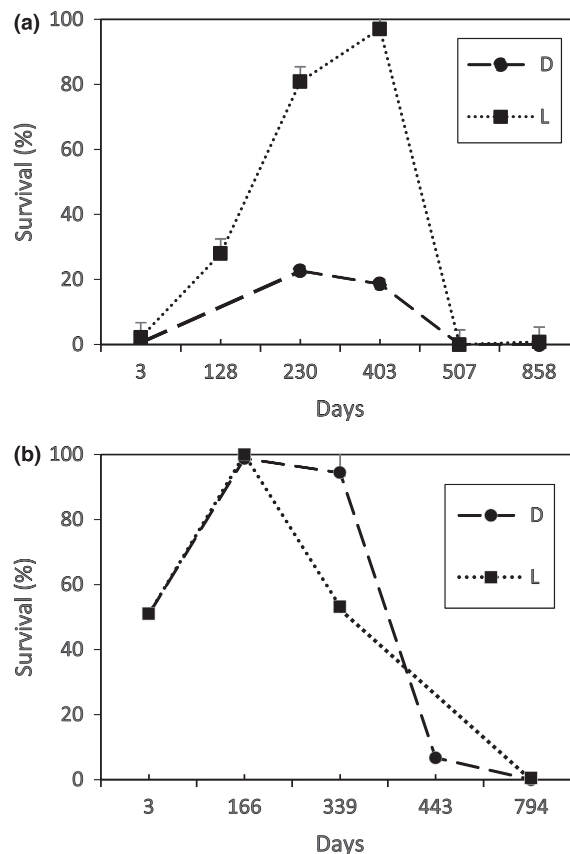

Figure 3 Survival of *Fusarium circinatum* in *Pinus radiata* needles (a) naturally colonized, and (b) artificially inoculated, incubated in mesh bags under field conditions in two locations in northern Spain (termed D and L). Each point is the mean of two replicates. Vertical bars are standard errors. Survival was calculated as the percentage of needles from which *F. circinatum* was recovered. Data is missing for days 128 and 443 for naturally colonized and artificially inoculated, respectively.

pathogen was recovered from 100% of needles at both locations (Fig. 3b). After that day, survival decreased and at day 794, the pathogen was not recovered from any of the samples. Based on ANOVA, the effect of time was found to be a significant factor ($P = 0.0004$), but neither location nor the interaction was significant ($P = 0.2062$ and 0.1432, respectively).

Disease-free needles and branch segments placed in their respective mesh bags were sampled on the same dates at both locations and *F. circinatum* was not recovered (data not shown).

All isolates of *F. circinatum* recovered either from needles or branch segments on the last date sampled were tested for pathogenicity in *P. radiata* seedlings. All isolates resulted in symptoms after 3 weeks of incubation at 25 °C, revealing that they were all pathogenic.

Isolation from soil

Fusarium circinatum was not isolated from soil sampled at any of the eight plantations tested where *P. radiata* with symptoms of pitch canker were present.

Discussion

Branch segments and needles colonized by *F. circinatum*, rather than conidia, play an important role in the long-term survival of this fungus. This study revealed that this pathogen was able to survive for up to 2 years 4 months in naturally infected needles or wood pieces on soil without losing its virulence. The findings of previous studies refer to survival of *F. circinatum* for an undetermined period of time in different substrates such as soil (Gordon *et al.*, 2001), dead host tissue (Wingfield *et al.*, 2008) and specifically, pine litter needles collected near pitch canker-infected pines (Aegerter & Gordon, 2006). Other studies have reported low rates of survival in branches after 3 years of incubation under laboratory conditions (McNee *et al.*, 2002) or in chips and branches for more than 1 year. The survival results presented here were quantified under field conditions on needles and small pieces of wood that had been naturally colonized by *F. circinatum* and were placed on soil under leaf litter to simulate real conditions. Infected branches with needles may fall more frequently from pitch canker infected trees, because the fungus can cause cankers that girdle the branches (EPPO, 2009), rendering them more prone to wind and storm breakage. Woody debris on soil is also generated during thinning and harvesting operations in pine plantations and usually left on site.

The persistence of tree pathogens that do not produce long-resting structures is not uncommon. For example, *Cryphonectria parasitica* survived for more than 1 year in the chestnut blight cankers of stock stems (Prospero *et al.*, 2006); *Diplodia pinea*, *Leptographium serpens* and *Heterobasidium annosum sensu stricto* survived for a period of 3–12 months in dead tissues of *P. pinea* (Santini *et al.*, 2008); and *Gremmeniella abietina* showed conidial germination capacity in 13- to 18-month-old clear cuts of *P. sylvestris* (Witzell *et al.*, 2006). Other *Fusarium* species, such as *F. moniliforme*, *F. proliferatum* and *F. subglutinans*, survive for at least 630 days in maize stalk debris (Cotten & Munkvold, 1998). Similar to *F. circinatum*, these three *Fusarium* species all share a lack of chlamydospores (Nirenberg & O'Donnell, 1998), and one of them, *F. subglutinans*, is closely related to *F. circinatum* (Desjardins *et al.*, 2000).

The survival pattern of *F. circinatum* in this study was similar at both locations for the naturally colonized branch segments. This was not the case for needles, which showed marked differences between the two locations. In the latter case, the incidence of recovery prior to the decline was more notable at Laukiniz. Interestingly, a rapid decline in the recovery of *F. circinatum* in both needles and branch segments was first detected at the 403-day time point, and in both cases there was no recovery beyond 2 years 4 months. During this time, both types of tissue, needles and branches, were exposed to temperature and moisture fluctuations. The highest and lowest mean temperatures occurred in August and January, respectively. These were 18.7 and 2.9 °C at Durango, and 20.6 and 9.3 °C at Laukiniz. Temperature fluctuations may have contributed to reduced viability of *F. circinatum* because there was little variation in recovery of fungus over the first 450 days in artificially infected material incubated at uniform temperature, and *F. circinatum* was absent in almost all of the pieces sampled after 443 days when artificially infected pieces were exposed to field conditions. Humidity may also have played a role in the decline of *F. circinatum* under field conditions. Recovery declined most rapidly between 403 and 507 days in naturally infected needles and branches, and between 339 and 443 days for artificially infected material, which coincided with a decrease in humidity and an increase in temperature for this time period in both locations.

Long-term survival in soil for other species of *Fusarium* is associated with production of chlamydospores, but *F. circinatum* does not form these specialized structures (Nirenberg & O'Donnell, 1998). In the soil viability experiment, where *F. circinatum* conidia had been added to soil and maintained at constant temperatures, no conidia were recovered after 8 months at temperatures of 20 and 30 °C. It is likely that the soil contained scarce organic debris to be colonized saprophytically by the fungus during the experiment, and that cfu estimates corresponded mainly to conidia surviving in the soil. Furthermore, no *F. circinatum* was recovered from soil sampled from plantations below infected trees, suggesting that conidia produced during disease outbreaks did not survive in soil. The fungus may have been present in organic material on the soil surface but this was not included in this study.

In addition to environmental conditions, microbial activity could explain this loss of conidia viability. Information regarding the interaction between *F. circinatum* and other microorganisms in soil is scarce, but some soil microbes have been identified as potential biocontrol

agents (Wingfield *et al.*, 2008), such as *Arthrobacter* spp. (Barrowsbroaddus & Kerr, 1981), common soil bacteria, and *Trichoderma* spp. (Martinez-Alvarez *et al.*, 2012). Previous studies have pointed to the role of soil microorganisms, especially bacteria, in the reduction of *F. oxysporum* f. sp. *lycopersici* populations (Smolinska, 2000) as well as *F. oxysporum* f. sp. *cumini* (Israel & Lodha, 2004). The soil sampling date could also be a factor to consider when evaluating persistence of conidia. Specific periods when maximum sporulation occurs have not been determined for Spanish conditions, but it is known to vary in other geographic locations. For example, it occurs throughout the year in California (Correll *et al.*, 1991) and during the autumn and winter seasons in southeast USA (Schweigkofler *et al.*, 2004). Soil samples were collected in May and June but it is unknown if this time period coincides with maximum sporulation by *F. circinatum* in these regions of Spain.

The results here suggest that *F. circinatum* can survive saprophytically in dead host tissues, which could play an important role in long-term survival. Growth of the pathogen in naturally colonized needles could explain the increased incidence of recovery, although the possibility that part of this increase is due to movement of spores between colonized and non-colonized needles cannot be ruled out.

A single isolate of *F. circinatum* was used for artificial inoculations as well as to study the survival of conidia. Different isolates can vary in their survival ability and response to environmental conditions. However, in the Basque Country (Spain), where this research was carried out, a single common multilocus genotype (Berbegal *et al.*, 2013) defined by eight microsatellite markers is predominant. The isolate used here is representative of this Spanish subpopulation (Iturritxa *et al.*, 2011; Berbegal *et al.*, 2013).

In Spain, regulatory measures are applied to prevent the spread of *F. circinatum*. Specifically, infected forest sites cannot be replanted with *Pinus* species for a minimum of 2 years. The results here indicate that this 2-year wait should be long enough to ensure that naturally colonized needles and branch segments will not be a potential source of inoculum, nor should survival of the fungus in soil contribute to reinfection. However, recent research has reported an endophytic association of *F. circinatum* with grasses found in plantations with infected pines in California and South Africa (Swett & Gordon, 2012; Swett *et al.*, 2014). This suggests that grasses may constitute an important reservoir of inoculum that facilitates persistence of the pathogen. Further studies are required to determine the presence of the fungus in grass in association with *P. radiata* plantations and whether cryptic infection of grass plays a role in the epidemiology of pitch canker in Spain.

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References

Aegerter BJ, Gordon TR, 2006. Rates of pitch canker induced seedling mortality among *Pinus radiata* families varying in levels of genetic resistance to *Gibberella circinata* (anamorph *Fusarium circinatum*). *Forest Ecology and Management* **235**, 14–7.

Barrowsbroaddus J, Kerr TJ, 1981. Inhibition of *Fusarium moniliforme* var. *subglutinans*, the causal agent of pine pitch canker, by the soil bacterium *Arthrobacter* sp. *Canadian Journal of Microbiology* **27**, 20–7.

Berbegal M, Perez-Sierra A, Armengol J, Grünwald NJ, 2013. Evidence for multiple introductions and clonality in Spanish populations of *Fusarium circinatum*. *Phytopathology* **103**, 851–61.

Correll JC, Gordon TR, McCain AH, Fox JW, Wood DL, Schultz ME, 1991. Pitch canker in California: pathogenicity, distribution, and canker development on Monterey pine (*Pinus radiata*). *Plant Disease* **75**, 676–82.

Cotten TK, Munkvold GP, 1998. Survival of *Fusarium moniliforme*, *F. proliferatum*, and *F. subglutinans* in maize stalk residue. *Phytopathology* **88**, 550–5.

Desjardins AE, Plattner RD, Gordon TR, 2000. *Gibberella fujikuroi* mating population A and *Fusarium subglutinans* from teosinte species and maize from Mexico and Central America. *Mycological Research* **104**, 865–72.

EPPO, 2009. *Gibberella circinata*. Diagnostics. *EPPO Bulletin* **39**, 298–309.

EPPO, 2011. Plant quarantine data retrieval system (PQR version 5.3.5). [http://www.eppo.int/DATABASES/pqr/pqr.htm]. Accessed 18 December 2015.

Gordon TR, Okamoto D, Storer AJ, Wood DL, 1998a. Susceptibility of five landscape pines to pitch canker disease, caused by *Fusarium subglutinans* f. sp. *pini*. *HortScience* **33**, 868–71.

Gordon TR, Wikler KR, Clark SL, Okamoto D, Storer AJ, Bonello P, 1998b. Resistance to pitch canker disease, caused by *Fusarium subglutinans* f. sp. *pini*, in Monterey pine (*Pinus radiata*). *Plant Pathology* **47**, 706–11.

Gordon TR, Storer AJ, Wood DL, 2001. The pitch canker epidemic in California. *Plant Disease* **85**, 1128–39.

Hermoso E, Carballo J, Fernandez-Golfin JI, 2007. Structural characterization of *Pinus radiata* D. Don timber from Pais Vasco (Spain) according to standard modifications. *Maderas-Ciencia y Tecnologia* **9**, 223–32.

Israel S, Lodha S, 2004. Factors influencing population dynamics of *Fusarium oxysporum* f. sp. *cumini* in the presence and absence of cumin crop in arid soils. *Phytopathologia Mediterranea* **43**, 3–13.

Iturritxa E, Ganley RJ, Wright J *et al.*, 2011. A genetically homogenous population of *Fusarium circinatum* causes pitch canker of *Pinus radiata* in the Basque Country, Spain. *Fungal Biology* **115**, 288–95.

Iturritxa E, Mesanza N, Elvira-Recuenco M, Serrano Y, Quintana E, Raposo R, 2012. Evaluation of genetic resistance in *Pinus* to pitch canker in Spain. *Australasian Plant Pathology* **41**, 601–7.

Iturritxa E, Ganley RJ, Raposo R *et al.*, 2013. Resistance levels of Spanish conifers against *Fusarium circinatum* and *Diplodia pinea*. *Forest Pathology* **43**, 488–95.

Leslie JF, Summerell BA, 2006. *The Fusarium Laboratory Manual*. Ames, IA, USA: Blackwell.

MAGRAMA, 2016. Anuario Estadística. [http://www.magrama.gob.es/es/estadistica]. Accessed 7 January 2016.

Martinez-Alvarez P, Manuel Alves-Santos F, Diez JJ, 2012. *In vitro* and *in vivo* interactions between *Trichoderma viride* and *Fusarium circinatum*. *Silva Fennica* **46**, 303–16.

McNee WR, Wood DL, Storer AJ, Gordon TR, 2002. Incidence of the pitch canker pathogen and associated insects in intact and chipped Monterey pine branches. *Canadian Entomologist* **134**, 47–58.

Nirenberg HI, O'Donnell K, 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* **90**, 434–58.

Prospero S, Conedera M, Heiniger U, Rigling D, 2006. Saprophytic activity and sporulation of *Cryphonectria parasitica* on dead chestnut wood in forests with naturally established hypovirulence. *Phytopathology* **96**, 1337–44.

Santini A, Pepori A, Ghelardini L, Capretti P, 2008. Persistence of some pine pathogens in coarse woody debris and cones in a *Pinus pinea* forest. *Forest Ecology and Management* **256**, 502–6.

Schweigkofler W, O'Donnell K, Garbelotto M, 2004. Detection and quantification of airborne conidia of *Fusarium circinatum*, the causal agent of pine pitch canker, from two California sites by using a real-time PCR approach combined with a simple spore trapping method. *Applied and Environmental Microbiology* **70**, 3512–20.

Smolinska U, 2000. Survival of *Sclerotium cepivorum* sclerotia and *Fusarium oxysporum* chlamydospores in soil amended with cruciferous residues. *Journal of Phytopathology* **148**, 343–9.

Swett CL, Gordon TR, 2012. First report of grass species (Poaceae) as naturally occurring hosts of the pine pathogen *Gibberella circinata*. *Plant Disease* **96**, 908.

Swett CL, Porter B, Fourie G, Steenkamp ET, Gordon TR, Wingfield MJ, 2014. Association of the pitch canker pathogen *Fusarium circinatum* with grass hosts in commercial pine production areas of South Africa. *Southern Forests* **76**, 161–6.

Viljoen A, Marasas WFO, Wingfield MJ, Viljoen CD, 1997. Characterization of *Fusarium subglutinans* f. sp. *pini* causing root disease of *Pinus patula* seedlings in South Africa. *Mycological Research* **101**, 437–45.

Wingfield MJ, Hammerbacher A, Ganley RJ *et al.*, 2008. Pitch canker caused by *Fusarium circinatum* – a growing threat to pine plantations and forests worldwide. *Australasian Plant Pathology* **37**, 319–34.

Witzell J, Bernhold A, Hansson P, 2006. Survival and vitality of *Gremmeniella abietina* on *Pinus sylvestris* slash in northern Sweden. *Forest Pathology* **36**, 406–12.