INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
21–0520/2023/29–4–251–257
DOI: 10.17957/IJAB/15.2027
http://www.fspublishers.org

Full Length Article

First Report of *Fusarium verticillioides* Associated with Resinous Canker in *Pinus greggii* var. *greggii* in Arteaga, Coahuila, Mexico

Ana Karen Guadalupe De León-Torres[1], José Luis Arispe-Vázquez[2*], Abiel Sánchez-Arizpe[1], Ma Elizabeth Galindo-Cepeda[1], Daniel Alejandro Cadena-Zamudio[2], Eladio Cornejo-Oviedo[3], Jorge David Flores-Flores[3], Salvador Valencia-Manzo[3] and Raúl Rodríguez-Guerra[4]

[1]*Departamento de Parasitología, Universidad Autónoma Agraria Antonio Narro. Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México. C.P. 25315*

[2]*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Km 2.5 Carretera Iguala-Tuxpan, Colonia Centro Tuxpan C.P. 40000, Iguala de la Independencia Guerrero, México*

[3]*Departamento de Forestal, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México. C.P. 25315*

[4]*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Km. 31 Carretera Montemorelos-China, C.P. 67400, General Terán, Nuevo León, México*

[*]For correspondence: arispe.jose@inifap.gob.mx

Received 29 April 2021; Accepted 14 February 2023; Published 17 March 2023

Abstract

The objective of this research was to describe the incidence and severity of resinous canker disease on the *Pinus greggii* Engelm. ex Parl. var. *greggii* forest plantation in CAESA at Los Lirios, Arteaga; as well as to morphologically and molecularly identify the pathogen associated with this disease. The causal agent of resinous canker was isolated, purified and identified by morpho-cultural criteria and molecular detection assays from needles, roots, bark, resin and shoots. The percentage of damaged trees (incidence) and the leaf proportion affected by the disease (severity) were evaluated. The inoculation process was performed on 15 specimens of 2-year-old samples. A variance analysis was performed and the comparison between means was made by Duncan test (P = 0.5) using the SAS® 9.1 statistical software. The BLAST nucleotide sequence alignments of the amplicons obtained using the ITS1 and ITS4 primers, identified the isolates as *F. verticillioides* with the GenBank accession number MK790051.1 and identity of 100%. Section 8 presented an incidence of 66.66% and the lowest level of severity was only 24.47%, section 2 presented the highest damage by *F. verticillioides* with 93.75 and 36.56% of incidence and severity, respectively. The first symptoms began between 15 and 30 DAI with chlorotic needles and resin secretion, later these became redaish after 45 DAI. Detection of *F. verticillioides* in the present study could be interpreted a possible association with resinous canker in the studied area, one of the most common fungal pathogens associated with maize.

Keywords: Tree cankers; Forest diseases; Incidence; Severity; Pathogen

Introduction

Forests play important roles in wood production, obtaining fuel, control of soil erosion and maintaining ecosystem functions (Lang *et al*. 2014). The world's total forest area corresponds to 4,060 million ha, which represents almost 1/3 of the earth's surface (FAO 2020a), of which México has 64 million ha (Torres 2020). Forests have long been threatened by a variety of destructive agents, *i.e.*, biotic, as well as abiotic factors (FAO 2021), yet human needs and demands of forest products are an obvious underlying cause of the loss of forest areas (Lovera 2003). The impact of forest pests and diseases has caused a decrease in the global forest area (FAO 2007; Whitehead 2011). In 2015 alone, pathogens and extreme weather conditions damaged 40 million ha (FAO 2020b).

The more important disease-associated pathogens in forest tree nurseries are a soil fungal complex, mainly comprised by *Pythium* spp., *Phytophthora* spp., *Rhizoctonia* spp., *Botrytis* spp. and *Fusarium* spp. (Salas 2002; Benítez *et al.* 2004; Ezziyyani *et al.* 2004). However, the *Fusarium* genus includes important plant-associated pathogens that affect both forest and agricultural species (Summerell and Leslie 2012), also, *Fusarium* species are well recognized as the important causal agent of *Fusarium* root rot (Yang *et al.* 2021). *Fusarium* spp. has been described as the cause of

To cite this paper: León-Torres AKGD, JL Arispe-Vázquez, A Sánchez-Arizpe, ME Galindo-Cepeda, E Cornejo-Oviedo, JD Flores-Flores, S Valencia-Manzo, R Rodríguez-Guerra (2023). First report of *Fusarium verticillioides* associated with resinous canker in *Pinus greggii* var. *greggii* in Arteaga, Coahuila, Mexico. *Intl J Agric Biol* 29:251–257

wilt disease in Mexican pine nurseries in addition to decreasing plant quality, which causes losses of up to 40% in production (Cibrián *et al.* 2008).

Resinous canker disease in pine trees was described for first time in the United States in 1946, reporting the fungal pathogen of the genus *Fusarium* as the causal agent of the disease (Hepting and Roth 1946), specifically *Fusarium circinatum* Nirenberg & O'Donnell (formerly known as *Gibberella circinata*). This disease is widely distributed, affecting up to 60 species of *Pinus* and other conifers, displaying a wide range of adaptability to environmental conditions (Watt *et al.* 2011; Bezos *et al.* 2017*)*, however *F. proliferatum y F. verticillioides*, can cause significant damage to pine seeds and seedlings grown in forest nurseries (Soldevilla 1995; Mansilla-Vázquez *et al.* 1998). In addition to this, it can infect susceptible hosts at any stage of their life cycle in different plant organs, such as: shoots, stems, branches, needles, cones, seeds and roots (Dwinell *et al.* 2001; Wingfield *et al.* 2008). Due to the above, the objective of this research was to describe the incidence and severity of resinous canker disease on the *P. greggii* Engelm. ex Parl. var. *greggii* forest plantation in CAESA at Los Lirios, Arteaga; as well as to morphologically and molecularly identify the pathogen associated with this disease.

Materials and Methods

Study area

The present research was carried out in the forest plantation in the Sierra de Arteaga Experimental Agricultural Field (CAESA) of the Universidad Autonoma Agraria Antonio Narro (UAAAN) at Los Lirios, Arteaga Coahuila, Mexico (Fig. 1). This location is at the 25º24'11.2" and 100º 36'25.46" coordinates.

Incidence and severity of resinous canker

A total of 599 specimens were evaluated in nine sections comprising adult *P. greggii* Engelm. ex Parl. var. *greggii* specimens, corresponding to two lanes with a variable number of trees per section. The trees were observed individually according to symptomatology criteria, that is, presence of resin, descending death and presence of cankers in the cortex (Fig. 2). The percentage of damaged trees (incidence) and the proportion of the leaf area affected by the disease (severity) were evaluated.

Isolation, purification and morpho-cultural identification of the pathogen

Five trees with moderate symptoms (resin exudate, presence of cankers, redaish coloration in the needles and defoliation) were selected from each section. From which resin, roots, needles, branches, bark, cankers and cones (female strobili) were collected. Next, a snippet of 1 cm of the sample was cut and disinfected with 1% sodium hypochlorite for 3 min and subsequently washed three times with sterile distilled water for another 3 min (Martínez-Álvarez *et al.* 2012). Five to eight cuts were placed in Petri dishes with Potato Dextrose Agar (PDA) medium supplemented with oxytetracycline (1 mL/L), in order to be purified by monoconidial cultures in selective culture media, such as Spezieller Nährstoffarmer Agar (SNA) (1 g of KH_2PO_4, 1 g of KNO_3, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KCl, 0.2 g of glucose, 0.2 g of sucrose and 20 g of agar diluted in one L of distilled water) supplemented with gentamicin, (1 mL/L) and carnation leaf agar (CLA) supplemented with gentamicin (1 mL/L) to inhibit bacterial growth. A total of five replicates by plant were kept at 25°C for 168 h. Later, 5 mm. in diameter plugs were taken from the pathogen colonies and added in test tubes with 9 mL of sterile distilled water with constant stirring, from which 60 μL were placed in Petri dishes with SNA and CLA culture media. Twenty-four hours later, one germinated conidia was placed in Petri dishes with SNA and CLA and kept at 25°C for 168 h. Morpho-cultural identification was performed with the aid of a compound microscope, based in color and shape of the colony. Color, length and width of 100 conidia was determined using the DinoCapture 2.0 microscopy imaging software (Dino-lite 2020) using the taxonomic keys of Barnett and Hunter (2006) to determine genus and the Leslie and Summerell (2006) taxonomic keys to determine species.

Molecular identification of the pathogen

Molecular analyses were performed in the Instituto Potosino de Investigación Cientifica y Tecnologica (IPYCIT), using the ITS1 and ITS4 primers to amplify the Internal Transcribed Spacer (ITS) regions of the fungal ribosomal DNA (rDNA) for molecular identification of the isolates. DNA extraction was performed by the Dellaporta method (Dellaporta *et al.* 1983). Visualization of the obtained amplicons was carried out by electrophoresis in a 2% (w/v) agarose gel. DNA yields were quantified on the Thermo Fisher NanoDrop™ 1000 Spectrophotometer and the Applied Biosystems® Veriti™ 96-Well Thermal Cycler to perform the end point PCR. The PCR products obtained were purified following the protocol described using the kit isolate II PCR and Gel kit. Obtained amplicons were sequenced with the dye-labeled dideoxynucleotide method in the Applied Biosystems® 3130 Genetic Analyzer. Obtained nucleotides sequences were analyzed using the BLAST program (Basic Local Aligment Search Tool) for highly similar sequences in the GenBank database of the National Center for Biotechnology Information (NCBI).

Inoculation for pathogenicity tests

The inoculation process was performed on 15 specimens of 2-year-old *P. greggii* samples, with an average height of 86.5 cm and a diameter of 3 cm, which were donated by the

Fig. 1: Area of study in Los Lirios, Arteaga Coahuila, Mexico

Fig. 2: Resinous canker in *P. greggii* Engelm. ex Parl. var. *greggii.* **A**) Healthy trees (healthy foliage, no bark damage); **B**) Slight damage (loss of foliage less than 50 %, slightly chlorotic needles); **C**) Moderate damage (loss of foliage between 55% and 75%, chlorosis, redaish tones, abundant resin secretion and lesions on the bark); **D**) Severe damage (no foliage and presence of cankers)

Society of Commercial Forest Planters of Durango, México. Fifteen days prior to inoculation, the seedlings were taken to CAESA, in order to provide them with a period of adaptation to the climatic conditions of the place. A suspension of 1×10^7 spores/mL was injected with a sterile syringe to the main plant stem and in two upper distal lateral branches of the youngest shoot. Specimens used as controls were injected with sterile distilled water. Daily irrigation was provided for 15 days, subsequently it was performed every third day for 3 months. Evaluations of the percentage of damage were performed every 15 days for three months; the severity was recorded at 45, 60, 75 and 90 days after inoculation (DAI).

Results analysis

Data obtained was adjusted by the arcsine square root transformation. The incidence and severity were determined by a variance analysis (ANOVA) and the means comparison was performed by the Duncan test ($P = 0.5$). Inoculation data was treated by a variance analysis with a factorial arrangement of three factors; factor A = *P. greggii* Engelm. ex Parl. var. *greggii* trees, factor B= the treatments and factor C= the four evaluation dates, with the SAS 9.1 statistical program (SAS 2002; version 9.1, SAS Institute, Cary, North Carolina, USA).

Results

Morpho-cultural and molecular identification

Mycelial growth of isolates presented similar characteristics, such as: abundant, cottony aerial mycelium, white to purple in color (Fig. 3D). Microconidia separated as a chain (Fig. 3A), generally ovoid with one or two septa, of 8.50 and 2.85 μm in length and width, respectively; sparsely branched conidiophores born laterally from the hypha (Fig. 3B). Macroconidia had thin walls and it was canoe-shaped with the basal cell in the shape of a foot (Fig. 3C), generally with three to six septa of 44.5 to 3.15 μm in length and width, respectively. The BLAST nucleotide sequence alignments of the amplicons obtained using the ITS1 and ITS4 primers, identified the isolates as *F. verticillioides* with the GenBank accession number MK790051.1 and identity of 100%.

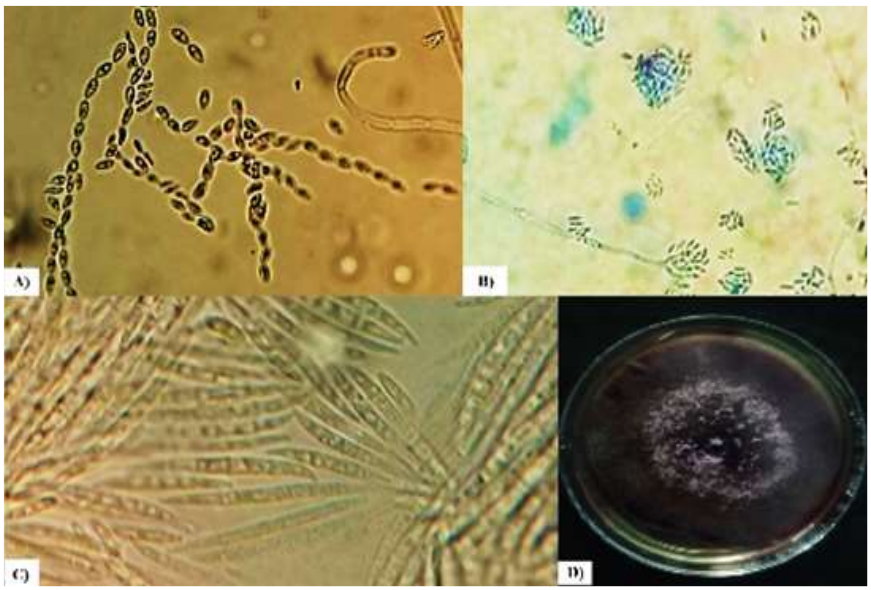

Fig. 3: *F. verticillioides* morphological characters. **A**) Chain microconidia, **B**) Microconidia and conidiophores, **C**) Macroconidia, **D**) Colony

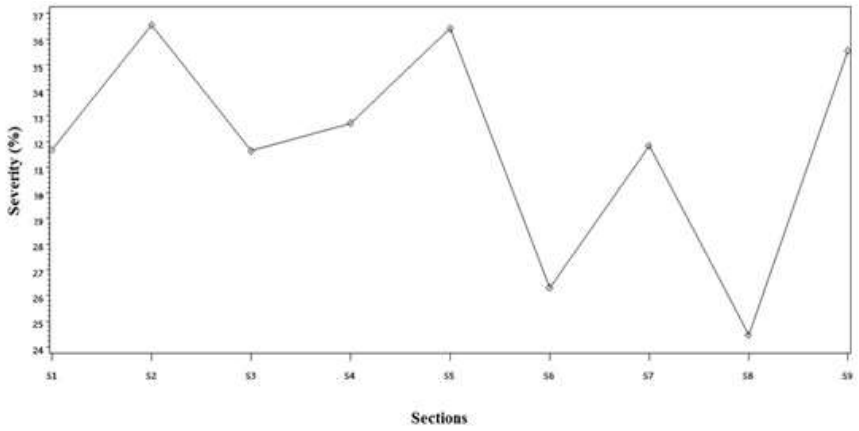

Fig. 4: Severity behavior of the 9 plantation sections of *P. greggii* Engelm. ex Parl. var. *greggii*

Incidence and severity of resinous canker in forest plantations

According to the statistical analysis, there is a significant difference in the severity of the disease between the 9 surveyed sections of the forest plantations (*P = 0.0004*) (Fig. 4 and Table 1), with a coefficient of variation percentage of 63.55%. The presence of the pathogen in the study area was 66.66 to 100%, with section 8 presenting the lowest incidence of 66.66% and a severity of 24.47%, while section 2 presented the highest damage with 93.75 and 36.56% incidence and severity, respectively.

***In vivo* pathogenicity test**

According to the performed analysis, there is a statistical difference between the treatment, as well as in the evaluation dates (*P = 0.0001*) (Table 2). The first symptoms began between 15 and 30 DAI with chlorotic needles and resin secretion, later these became redaish after 45 DAI. At the beginning of the infection, 3% severity was recorded, ending at 90 DAI with the greatest damage severity (22.190%) (Table 2). During the evaluation period, the control group only showed symptoms of chlorosis and scarce resin secretion, that is, the disease control was only 42.41% of the one that was treated with *F. vertcillioides* (Factor B) (Table 2 and Fig. 5).

Discussion

Pine diseases can significantly reduce plant survival in plantations (Chavarriaga *et al.* 2007; Garbelotto and Gonthier 2013; Drenkhan *et al.* 2016). Most of the conifer's diseases are caused by fungal pathogens (Hansen *et al.* 2018). Characteristics of *F. verticillioides* similar to those reported by Booth (1971), Hirata *et al.* (2001), Rahjoo *et al.*

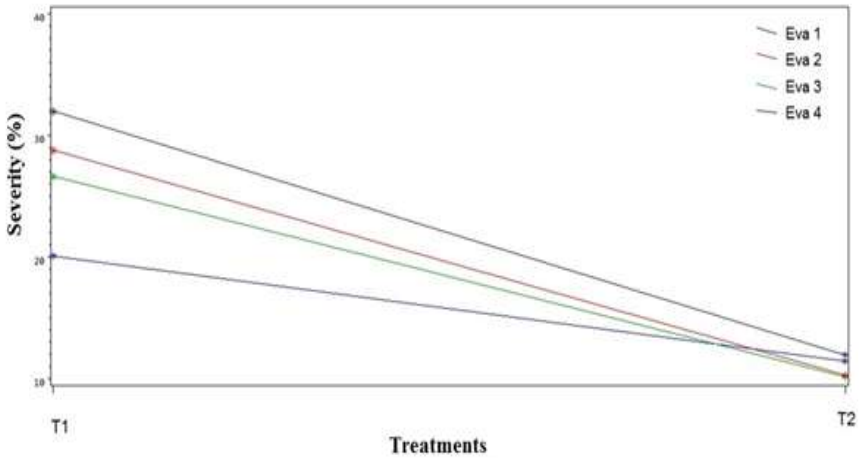

Fig. 5: Severity recorded in pathogenicity tests at 45, 60, 75, 90 days after inoculation. T1 = Seedlings inoculated with *F. verticillioides*, T2 = Control (Non-inoculated plants), Eva 1= 1st evaluation, Eva 2 = 2nd evaluation, Eva 3 = 3rd, evaluation, Eva 4 = 4th evaluation

Table 1: Incidence and severity of the canker disease on the nine plantation sections of *P. greggii* Engelm. ex Parl. var. *greggii*

Section	Incidence (%)	Severity (%)	SD	Ag
1	81.69	31.69	0.84290280	ab
2	93.75	36.56**	1.06443428	a
3	90.62	31.641	0.89501751	ab
4	93.44	32.727	1.01620285	ab
5	87.3	36.429	0.96414598	a
6	76.81	26.304	0.97704482	b
7	84.61	31.846	1.13694536	ab
8	66.66	24.474*	1.13694536	b
9	88.88	35.548	0.99253867	a

SD = Standard deviation, Ag= Statistical aggrupation, equal letters are not statistically different according to the Duncan test ($P = 0.05$), * = Lower disease severity, ** = Higher disease severity

Table 2: Severity development of *F. verticillioides* during the four evaluation dates after inoculation

Factors (B and C)		Means	SD
Treatments (B)	*F. vertcillioides*	26.998 **	7.43311954
	Control	11.452	3.12826189
Evaluation dates (C)	45 DAI	15.159	7.3818026
	60 DAI	19.158	9.4439738
	75 DAI	20.393	9.6443700
	90 DAI	22.190 **	10.8631724

SD = standard deviation; ** = Higher disease severity

(2008), Durán (2013), Pavlovic *et al.* (2016), Chavarri *et al.* (2017), Leyva-Mir *et al.* (2017), Giraldo-Arias *et al.* (2018) and Dharanendra *et al.* (2019). The BLAST nucleotide sequence alignments of the amplicons identified the isolates as *F. verticillioides* with the GenBank accession number MK790051.1. Results exhibited a maximum score of 931 and identity of 100%. *Fusarium* spp. are commonly found to affect the roots of coniferous seedlings, causing damping-off and wilt in pine seedlings (Kwaśna and Bateman 2009; Lazreg *et al*. 2014).

Pitch canker disease of pine trees and Douglas fir is caused by *F. circinatum* (Nirenberg and O'Donnell 1998) which is characterized by resinous stem and branch cankers (McCain *et al.* 1987; Gordon *et al.* 2001) and it can occur at any stage of the life cycle host lifetime, also, disease causes losses in commercial plantations, nurseries and seed orchards, occasionally being a problem in native forests of *Pinus* species (Dwinell *et al*. 2001; Wingfield *et al*. 2008). *F. circinatum* and *Diplodia sapinea* (*Sphaeropsis sapinea*) (Fr.) are two of the more important fungal pathogens that affect *Pinus* species causing canker diseases which can result in significant economic losses in pine tree nurseries, plantations and natural forests worldwide (Swart and Wingfield 1991; Blodgett *et al.* 1997; Burgess *et al.* 2004; Wingfield *et al.* 2008; Iturritxa *et al.* 2013; Bihon *et al.* 2014), for example, pine shoot blight caused by *D. sapinea* is a serious threat to the health of *Pinus densiflora* Siebold & Zucc (Xie *et al.* 2020). However, *F. circinatum* has been reported in most of the world's pine production zones; especially in those where *Pinus radiata* is intensively planted, either on adult forests or nurseries (Hodge and Dvorak 2000; Gordon *et al.* 2001; EPPO 2009; Wingfield *et al.* 2008). Nonetheless, in our investigation the etiological agent of this disease was *F. verticillioides. Fusarium* spp. is well known as a forest nursery pathogen, causing pre-emergence and post-emergence damping off (Dick and Dobbie 2002). The factors that promote the pathogen development are presence of old trees with their roots in process of decomposition, rain frequency, observing an almost insignificant incidence when rain frequency is low (Mendoza *et al.* 2002).

F. verticillioides has been reported as a pathogen in certain pine species, such as *Pinus ponderosa* Dougl. ex Laws. var. *ponderosa* (Ponderosa pine) (Salerno and Lori 2007), *Pinus nigra* (Martin-Pinto *et al.* 2004). Even before the arrival of *F. circinatum* in Europe, *F. verticillioides* was one of the three main fungal pathogens associated with damping-off in European nurseries (Martin-Pinto *et al.* 2008; Romón *et al.* 2008). On the other hand, *F.*

verticillioides is one of the most frequent *Fusarium* spp. associated with conifer seeds and nursery diseases in the USA (James 1986; Fraedrich and Miller 1995). Additionally, *F. verticillioides* isolates have recently been reported from *Pinus strobus* seeds (Ocamb *et al.* 2002) associated to rhizosphere soil and diseased roots of *P. strobus* (Ocamb and Juzwik 1995).

Conclusion

The symptoms reproduced after inoculation of the isolated pathogen to the *P. greggii* Engelm. ex Parl. var. *greggii* trees allowed the association of the phytopathogenic fungi *F. verticillioides* as the etiological agent of the pine resinous canker disease in the CAESA forest plantation.

Acknowledgements

The authors would like to thank the Society of Commercial Forest Planters of Durango, Mexico, for the donation of specimens P. greggii, and to forest plantation of Sierra de Arteaga Experimental Agricultural Field (CAESA).

Author Contributions

AKGDT performed the experiments, JLAV wrote the manuscript and analyzed the experiment data; ASA, Ma.EGC, ECO, JDFF and RRG analyzed the experiment data; SVM revised the manuscript.

Conflict of Interest

Authors declare no conflict of interest.

Data Availability

All datasets presented in this study will be available on a fair request to the corresponding authors.

Ethics Approval

This research does not involve the ethical approval.

Funding Source

To the National Council of Science and Technology (CONACYT) of Mexico how provided a scholarship for graduate student of the first author, to the Universidad Autónoma Agraria Antonio Narro by funding to produce this work.

References

Barnett LH, BB Hunter (2006). *Illustrate Genera of Imperfect Fungi*, 4th Editon. The American Phytopathology Society Press, St. Paul, Minnesota, USA

Benítez T, AM Rincón, AC Limón, C Codón (2004). Biocontrol mechanisms of *Trichoderma* Strains. *Intl Microbiol* 7:249–260

Bezos D, P Martınez-Alvarez, M Fernandez, JJ Diez (2017). Epidemiology and management of pine pitch canker disease in Europe – a review. *Balt For* 23:279–293

Bihon W, MJ Wingfield, B Slippers, TA Duong, BD Wingfield (2014). MAT gene idiomorphs suggest a heterothallic sexual cycle in a predominantly asexual and important pine pathogen. *Fung Genet Biol* 62:55–61

Blodgett JT, EL Kruger, GR Stanosz (1997). *Sphaeropsis sapinea* and water stress in a red pine plantation in Central Wisconsin. *Phytopathology* 87:429–434

Booth C (1971). *The genus Fusarium*, p:213. Commonwealth Micological Institute, England

Burgess TI, MJ Wingfield, BD Wingfield (2004). Global distribution of *Diplodia pinea* genotypes revealed using simple sequence repeat (SSR) markers. *Aust Plant Pathol* 33:513–519

Chavarri M, J Barroyeta, Y Ochoa, N Rumbos, J Alezones (2017). Detección de *Fusarium verticillioides* y fumonisinas en granos de maíz blanco provenientes de los estados Yaracuy y Guárico, Venezuela. *Nova Sci* 9:171–184

Chavarriaga D, WJA Bodles, C Leifert, L Belbahri, S Woodward (2007). *Phytophthora cinnamomi* and other fine root pathogens in north temperate pine forests. *FEMS Microbiol Lett* 276:67–74

Cibrián TD, DS García, MBD Juan (2008). *Manual de Identificación y Manejo de Plagas y Enfermedades en Germoplasma y Planta Producida en Viveros*, p:153. CONAFOR. México

Dellaporta SL, J Wood, JB Hicks (1983). A plant DNA minipreparation version II. *Plant Mol Biol Rep* 18:61–64

Dharanendra S, S Mahadevakumar, HB Hemareddy, KN Amruthesh, S Mamatha, S Kunjeti, KR Sridhar, R Swapnil, KT Vasanthakumar, N Lakshmidevi (2019). Morphological and molecular characterization of *Fusarium verticillioides* (*F. moniliforme*) associated with post-flowering stalk rot (PFSR) of maize in Karnataka. *Kavaka* 53:106–116

Dick MA, K Dobbie (2002). Species of *Fusarium* on *Pinus radiata* in New Zealand. New Zeal. *Plant Prot* 55:58–62

Dino-lite (2020). *DinoCapture 2.0: Software de Imágenes de Microscopio*. Available at: https://www.dinolite.us/en/dinocapture/

Drenkhan R, V Tomešová-Haataja, S Fraser, RE Bradshaw, P Vahalík, MS Mullett, J Martín-García, LS Bulman, MJ Wingfield, T Kirisits, TL Cech, S Schmitz, R Baden, K Tubby, A Brown, M Georgieva, A Woods, R Ahumada, L Jankovský, IM Thomsen, K Adamson, B Marçais, M Vuorinen, P Tsopelas, A Koltay, A Halasz, N La Porta, N Anselmi, R Kiesnere, S Markovskaja, A Kačergius, I Papazova-Anakieva, M Risteski, K Sotirovski, J Lazarević, H Solheim, P Boroń, H Bragança, D Chira, DL Musolin, AV Selikhovkin, TS Bulgakov, N Keča, D Karadžić, V Galovic, P Pap, M Markovic, L Poljakovic Pajnik, V Vasic, E Ondrušková, B Piškur, D Sadiković, JJ Diez, A Solla, H Millberg, J Stenlid, A Angst, V Queloz, A Lehtijärvi, HT Doğmuş-Lehtijärvi, F Oskay, K Davydenko, V Meshkova, D Craig, S Woodward, I Barnes (2016). Global geographic distribution and host range of *Dothistroma* species: A comprehensive review. *For Pathol* 46:408–442

Durán JAB (2013). Primer reporte de *Fusarium thapsinum* Klittick y sus efectos en las inflorescencias del pasto aguja en Yaracuy, Venezuela. *Agron Trop* 63:135–141

Dwinell LD, SW Fraedrich, D Adams (2001). Diseases of Pines Caused by the Pitch Canker Fungus. *In: Fusarium*, pp:225–232. Summerell BA, JF Leslie, D Backhouse, WL Bryden, LW Burgess (Eds). Paul E. Nelson Memorial Symposium. The American Phytopathological Society, St. Paul, Minnesota, USA

EPPO (2009). *European and Mediterranean Plant Protection Organization.* PM 7/91(1): *Gibberella circinata*. *EPPO Bull* 39:298–309

Ezziyyani M, SC Pérez, AA Sid, ME Requena, ME Candela (2004). *Trichoderma harzianum* como biofungicida para el biocontrol de *Phytophthora capsici* en plantas de pimiento (*Capsicum annuum* L.). *Ann Biol* 26:35–45

FAO (2021). Food and Agriculture Organization of the United Nations. *The Threat of the Forests*. Available at: http://www.fao.org/3/y5507s/y5507s02.pdf

FAO (2020a). Food and Agriculture Organization of the United Nations. *Global Forest Resources Assessment 2020*. Available at: http://www.fao.org/forest-resources-assessment/2020/es

FAO (2020b). Food and Agriculture Organization of the United Nations. *Global Forest Resources Assessment 2020*. Main report. Available at: https://doi.org/10.4060/ca9825en

FAO (2007). *Food and Agriculture Organization of the United Nations*, p:26. Forest Department Overview of Forest Pests Argentina Forest Health & Biosecurity Working Papers. Forest Resources Development Service Working Paper FBS/9E

Fraedrich SW, T Miller (1995). Mycoflora associated with slash-pine seeds from cones collected at seed orchards and cone-processing facilities in the south-eastern USA. *Eur J For Pathol* 25:73–82

Garbelotto M, P Gonthier (2013). Biology, epidemiology and control of *Heterobasidion* species worldwide. *Annu Rev Phytopathol* 51:39–59

Giraldo-Arias J, S Celis-Zapata, ND Franco-Sierra, JJ Arroyave-Toro, C Jaramillo-Mazo, JC Correa-Álvarez (2018). Identification of *Fusarium* cf. *verticillioides* as the causal agent of pokka boheng disease in sugarcane in the department of Antioquia, Colombia. *Ingen Cienc* 14:113–134

Gordon TR, AJ Storer, DL Wood (2001). The Pitch Canker Epidemic in California. *Plant Dis* 85:1128–1139

Hansen EM, KJ Lewis, GA Chastagner (2018). *Compendium of Conifer Diseases*. American Phytopathological Society, St. Paul, Minnesota, USA

Hepting GH, ER Roth (1946). Pitch canker, a new disease of southern pines. *J For* 44:742–744

Hirata T, E Kimishima, T Aoki, HI NirenbergI, K O'Donnell (2001). Morphological and molecular characterization of *Fusarium verticillioides* from rotten banana imported into Japan. *Mycoscience* 42:155–166

Hodge GR, WS Dvorak (2000). Differential responses of Central American and Mexican pine species and *Pinus radiata* to infection by the pitch canker fungus. *New For* 19:241–258

Iturritxa E, RJ Ganley, R Raposo, I García-Serna, N Mesanza, SC Kirkpatrick, TR Gordon (2013). Resistance levels of Spanish conifers against *Fusarium circinatum* and *Diplodia pinea*. *For Pathol* 43:488–495

James RL (1986). Occurrence of Fusarium on Douglas-fir Seed and Containerized Seedlings at the Plum Creek Nursery, Pablo, Montana. *In: Forest Pest Management Report*, Vol. 86, pp:1–10. USDA Forest Service, Northern Region, Missoula, Montana, USA

Kwaśna H, GL Bateman (2009). Microbial communities in roots of *Pinus sylvestris* seedlings with damping-off symptoms in two forest nurseries as determined by ITS1/2 rDNA sequencing. *For Pathol* 39:239–248

Lang AC, GV Oheimb, M Scherer-Lorenzen, B Yang, S Trogisch, H Bruelheide, K Ma, W Härdtle (2014). Mixed afforestation of young subtropical trees promotes nitrogen acquisition and retention. *J Appl Ecol* 51:224–233

Lazreg F, L Belabid, J Sanchez, E Gallego, B Bayaa (2014). Pathogenicity of *Fusarium* spp. associated with diseases of aleppo-pine seedlings in algerian forest nurseries. *J For Sci* 60:115–120

Leslie JF, BA Summerell (2006). *The Fusarium Laboratory Manual*, p:1–2. Blackwell Publishing, Hoboken, New Jersey, USA

Leyva-Mir SG, HE Vega-Portillo, HE Villaseñor-Mir, B Tlapal-Bolaños, M Vargas-Hernández, M Camacho-Tapia, JM Tovar-Pedraza (2017). Caracterización de especies de *Fusarium* causantes de pudrición de raíz del trigo en el bajío, México. *Chil J Agric Anim Sci* 33:142–151

Lovera S (2003). *Going to the Roots: Addressing the Underlying Causes of Deforestation and Forest Degradation*. Available at: http://www.fao.org/3/XII/MS12B-E.htm

Mansilla-Vázquez JP, CP Varela, MJ Sáinz-Osés, A Vilariño (1998). *Fusarium* moniliforme Sheldon sobre *Pinus* spp.: Ensayos de patogenicidad. *In: Actas Del IX Congreso de la Sociedad Española de Fitopatología*, p:223. Sociedad Española de Fitopatología. Salamanca, Madrid, Spain

Martínez-Álvarez P, FM Alves-Santos, JJ Diez (2012). *In vitro* and *in vivo* interactions between *Trichoderma viride* and *Fusarium circinatum*. *Silv Fenn* 46:303–316

Martin-Pinto P, J Pajares, J Díez (2008). Pathogenicity of *Fusarium verticillioides* and *Fusarium oxysporum* on *Pinus nigra* seedlings in northwest Spain. *For Pathol* 38:78–82

Martin-Pinto P, JA Pajares, N Nanos, JJ Díez (2004). Site and seasonal influences on the fungal community on leaves and stems of *Pinus* and *Quercus* seedlings in forest nurseries. *Sydowia* 56:243–257

McCain A, C Koehler, S Tjosvold (1987). Pitch canker threatens California pines. *Calif Agric* 41:22–23

Mendoza R, T Hoopen, MJ Kass, D Sánchez, V Krauss (2002) Evaluation of mycoparasites as biocontrol agents of *Rosellinia* root rot in cocoa. *Biol Cont* 27:210–227

Nirenberg HI, K O'Donnell (1998). New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* 90:434–458

Ocamb CM, J Juzwik (1995) *Fusarium* species associated with rhizosphere soil and diseased roots of eastern white pine seedlings and associated nursery soil. *Can J Plant Pathol* 17:325–330

Ocamb CM, J Juzwik, FB Martin (2002). *Fusarium* spp. and *Pinus strobus* seedlings: Root disease pathogens and taxa associated with seed. *New For* 24:67–79

Pavlovic S, D Ristic, I Vucurovic, M Stevanovic, S Stojanovic, S Kuzmanovic, MS Starovic (2016). Morphology, pathogenicity and molecular identification of *Fusarium* spp. associated with anise seeds in Serbia. *Not Bot Hortic Agrobot Cluj-Nap* 44:411–417

Rahjoo V, J Zad, M Javan-Nikkhah, A Gohari, S Okhovvat, M Bihamta, J Razzaghian, S Klemsdal (2008). Morphological and molecular identification of *Fusarium* isolated from maize ears in iran. *J Plant Pathol* 90:463–468

Romón P, M Troya, ME Fernández de Gamarra, A Eguzkitza, JC Iturrondobeitia, A Goldarazena (2008). Fungal communities associated with pitch canker disease of *Pinus radiata* caused by *Fusarium circinatum* in northern Spain: Association with insects and pathogen-saprophyte antagonistic interactions. *Can J Plant Pathol* 30:241–253

Salas B (2002). *Plagas y Enfermedades Forestales en América Central*, pp:212–213. CATIE. Turrialba, Costa Rica

Salerno MI, GA Lori (2007). Association of seed-borne *Fusarium* species on *Pinus ponderosa* with germination and seedling viability in Argentina. *For Pathol* 37:263–271

SAS Institute (2002). *The SAS System for Windows, Release 9.1*. SAS Institute, Cary, North Carolina, USA

Soldevilla C (1995). Marras de origen fúngico (Damping-off) en plantas del género *Pinus* spp. cultivadas en invernadero. *Bol San Veg Plag* 21:87–109

Summerell B, J Leslie (2012). Introducing the genus *Fusarium*. *In*: *Control of Fusarium Species*. Álves-Santos F, JD Casero (Eds). Research Point, Kerala, India

Swart WJ, MJ Wingfield (1991). Biology and control of *Sphaeropsis sapinea* on *Pinus* species in South Africa. *Plant Dis* 75:761–766

Torres RJM (2020). *Estudio de Tendencias y Perspectivas Del Sector Forestal en América Latina al Año 2020*. Informe Nacional México. Available at: http://www.fao.org/3/j2215s/j2215s00.htm#TopOfPage

Watt MS, RJ Ganley, DJ Kriticos, LK Manning (2011). *Dothistroma needle* blight and pitch canker: The current and future potential distribution of two important diseases of *Pinus* species. *Can J For Res* 41:412–424

Whitehead D (2011). Forests as carbon sinks—benefits and consequences *Tree Physiol* 31:893–902

Wingfield MJ, A Hammerbacher, RJ Ganley, ET Steenkamp, TR Gordon, BD Wingfield, TA Coutinho (2008). Pitch canker caused by *Fusarium circinatum* – a growing threat to pine plantations and forests worldwide. *Aust Plant Pathol* 37:319–334

Xie X, J Liang, M Zhang, R Hu, Y Cheng, X Zhang (2020). Endophytic fungal community of *Pinus densiflora* infected by different incidences of *Sphaeropsis sapinea* in a mixed coniferous forest. *Intl J Agric Biol* 23:1044–1050

Yang F, C Liu, L Liu, S Wang, X Jiang, M Li, Y Liu, C Xu, B Su, X Li (2021). Evaluation of antagonistic Bacillus and biochar against soybean root rot caused by *Fusarium oxysporum*. *Intl J Agric Biol* 26:287–293