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Scientific paper - *Artículo científico*

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Abstract

In the present paper the author describes 6 species of quality –non quarantine– *Fusarium* –*Fusarium verticillioides*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium graminearum*, *Fusarium subglutinans*, *Fusarium proliferatum*– identified by him in commercial woody container ornamental production centres of Galicia and Portugal as the result of his work as an independent plant pathologist and researcher. The work was carried out from 2013 to 2020. Some of the references described are the first for Galicia as well as for Spain.

Key words: *Fusarium verticillioides*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium graminearum*, *Fusarium subglutinans*, *Fusarium proliferatum*, Galicia, Portugal, woody ornamentals

Resumen

En el presente trabajo el autor describe un total de 6 especies diferentes de calidad – no de cuarentena– del género Fusarium –Fusarium verticillioides, Fusarium oxysporum, Fusarium solani, Fusarium graminearum, Fusarium subglutinans, Fusarium proliferatum– identificadas por el mismo en los cultivos leñosos ornamentales en contenedor de viveros de Galicia y Portugal como resultado de su labor como patólogo vegetal e investigador independiente. El trabajo fue llevado a cabo del año 2013 al 2020.

Palabras clave: Fusarium verticillioides, Fusarium oxysporum, Fusarium solani, Fusarium graminearum, Fusarium subglutinans, Fusarium proliferatum, Galicia, Portugal, ornamentales leñosas

1. Introduction

Fusarium species have a world-wide distribution and pathogenic species have been recorded from a large number of host plants. In container woody ornamental hosts *Fusarium* species have been traditionally known as responsible of branch cankers, wilts and necroses, cutting root and collar rots, plant wilts, seedlings damping-off as well as root rots. *Fusarium lateritium*, *Fusarium roseum*, *Fusarium oxysporum*, *Fusarium avenaceum* and *Fusarium tricinctum* are the main *Fusarium* species referenced as responsible of these symptoms on ornamental trees and bushes, according to reference literature (Vegh, 1980; Butin, 1995). In conifers *Fusarium* spp. are mainly known as nursery pathogens, causing damping-off, root rots and stunting.

The *Fusarium* species referenced, up to the date of this publication, as pathogens of woody ornamental species in Spain are the following: *Fusarium oxysporum* f. sp. *dianthi* (Melgarejo *et al*., 2010); *Fusarium proliferatum* (Melgarejo *et al*., 2010); *Fusarium verticillioides* (Andrés, 2015; Andrés, 2016 a) and *Fusarium solani* (Andrés, 2015; Andrés, 2016 a).

The *Fusarium* species referenced, up to the date of this publication, as pathogens of conifer species cultivated as ornamentals in Galicia are the following: *Fusarium verticillioides*, *Fusarium subglutinans* and *Fusarium solani* (Andrés, 2016 b).

The detection of *Fusarium circinatum* in conifer plantations in Galicia (NW Spain) (Pintos *et al*., 2006) has led to the intensification of the plant pathology controls of the government Sanitary Services as well as of private Plant Protection Services. The author has carried out independent plant pathology consultancy services to woody ornamental nurseries that produced ornamental conifers –as *Pinus pinaster*, *P. radiata*, *P. sylvestris* and *Cedrus atlantica*– as well as container woody ornamental species, from 2013 to 2020. The results of the pathological surveys are showed in this paper.

2. Material & Method

2.1. Plant production centres included in the study and sampling method

The study has been carried out in eleven woody ornamental production centres, seven of them located in Galicia –five located in the province of Pontevedra, 1 in A Coruña and the other one in Lugo– and four of them in Portugal. The samples taken in this study in field conditions were plant material with symptoms of the disease as well as commercial cuttings, without symptoms of the disease, sampled before being introduced in the nurseries. Each sample contained six plants. The samplings were carried out on each centre, every two or four weeks, intermittently, from 2013 to 2020.

The total number of samples, as well as the origins of them, are specified on tables 1 and 2.

TABLE 1. NUMBER AND ORIGIN OF SAMPLES OF DISEASES PLANTS AND CUTTINGS FROM 2013 TO 2020

NUMBER OF SAMPLES PER YEAR AND LOCATION					
Year	Galicia			Portugal	Total
	Lugo	Coruña	Pontevedra		
2013	9	5	11	0	25
2014	7	9	0	0	16
2015	11	0	1	0	12
2016	29	13	45	16	103
2017	38	11	136	1	186
2018	4	0	137	48	189
2019	0	0	135	48	183
2020	0	0	42	3	45
Total	98	38	507	116	759

TABLE 2. NUMBER AND ORIGIN OF SAMPLES OF CUTTINGS WITHOUT SYMPTOMS FROM 2013 TO 2020

NUMBER OF SAMPLES PER YEAR AND LOCATION					
Year	Galicia			Portugal	Total
	Lugo	Coruña	Pontevedra		
2013	0	0	0	0	0
2014	0	0	0	0	0
2015	0	0	0	0	0
2016	0	0	0	0	0
2017	6	0	11	0	17
2018	0	0	11	13	24
2019	0	0	25	21	46
2020	0	0	17	3	20
Total	6	0	64	37	107

The work was carried out on the base of the results of a total amount of 759 samples 107 of which were of cuttings without symptoms.

2.2. Identification of potential telluric pathogens

2.2.1. Isolation method

The isolation of the pathogen was carried out in the private phytopathological laboratory of the author. Fragments of the stem and root bases of diseased plants were prepared for fungi isolation. The surface of these fragments were disinfected with 0,6% sodium hypochlorite for 4 minutes and plated on PDA (potato dextrose agar) (Rapilly, 1968). Plants with vascular necrosis were disinfected burning their surface after spraying them with alcohol. The fungi were grown under laboratory conditions and microscope observations were carried out every 24 hours during one week.

2.2.2. Isolation method

Fusarium isolates were plated on new PDA petri dishes and were identified following taxonomical criteria described by Nelson *et al* (Nelson *et al*., 1983).

3. Results

The different *Fusarium* species identified from woody ornamental hosts in Galician and Portuguese nurseries were the following:

3.1. *Fusarium verticillioides* (Sacc.) Nirenberg

Isolated hosts: *Pittosporum tobira*, *Pinus pinaster*, *Pinus sylvestris*, *Araucaria araucana*, *Rhododendron*, *Cedrus atlantica*, *Miscanthus sinensis*, *Dianthus caryophillus*, *Pinus radiate*, *Phomrium tenax*, *Thuja occidentalis*, *Pittosporum tobira*

Symptoms observed: damping-off, collar rots, wilts.

Zone: A Coruña, Pontevedra, Lugo, Portugal.

Crops development: cuttings, seedlings and adult plants.

3.2. *Fusarium oxysporum* Schltdl

Isolated hosts: *Dianthus caryophillus*, *Pinus pinaster*, *Pinus sylvestris*, *Cordyline ausytralis*, *Chrysanthemum* × *hortorum*, *Eriostemon myoporoides*, *Gelsenium sempervirens*, *Gardenia japonica*, *Phormium tenax*, *Rosmarinus officinalis*, *Nandina domestica*, *Hebbe speciosa*.

Symptoms observed: yellows, wilts, vascular necrosis, damping-off, root rots.

Zone: A Coruña, Pontevedra, Lugo, Portugal.

Crops development: cuttings, seedlings and adult plants.

3.3. *Fusarium solani* (Mart.) Sacc

Isolated hosts: *Correa reflexa*, *Eriostemon myoporoides*, *Pinus pinaster*, *Pinus sylvestris*, *Dianthus caryophillus*, *Cedrus atlantica*, *Helleborus niger*, *Tibuchina urvilleana*, *Pinus radiate*, *Ligustrum japonicum*, *Camellia japonica*, *magnolia grandiflora*, *Chrysanthemum hortorum*, *Phormium tenax*, *Cleyera japonica*, *Camellia japonica*, *Vinca minor*, *Acacia dealbata*, *Thuja occidentallis*, *Euonymus japonicus*, *Nandina domestica*, *Diosma ericoides*, *Rosmarinus officinalis*, *osmanthus fragans*, *Pittosporum tobira*, *Dracaena marginata*.

Symptoms observed: collar rots, damping-off, wilts, root rots, yellows.

Zone: A Coruña, Pontevedra, Lugo, Portugal.

Crops development: cuttings, seedlings and adult plants.

3.4. *Fusarium graminearum* Schwabe

Isolated hosts: *Cleyera japonica*, *Grevillea lanigera*, *Correa speciosa*, *Viburnum tinus*,, *Chrysanthemum hortorum*, *Phormium tenax*, *Photinia* × *fraserii*, *Thymus vulgaris*.

Symptoms observed: stem rots, collar rots, wilts, foliar necrosis.

Zone: A Coruña, Pontevedra.

Crops development: adult plants.

3.5. *Fusarium subglutinans* (Wollenw & Reinking) P.E. Nelson, Tousson & Marassas

Isolated hosts: *Pinus radiata*, *pinus pinaster*, *Chrysanthemum* × *hortorum*, *Taxus baccatta*, *Grevillea lanigera*, *Phormium tenax*, *Hydrangea macrophylla*.

Symptoms observed: damping-off, wilts, collar rots, root rots.

Zone: Lugo, Pontevedra.

Crops development: seedlings, adult plants.

3.6. *Fusarium proliferatum* (Matshus.) Nirenberg ex Gerlach & Nirenberg

Isolated hosts: *Cotoneaster horizontalis*,

Symptoms observed: collar rots.

Zone: A Coruña.

Crops development: adult plants.

The percentages of incidence of each species on adult plants and cuttings of Galician and Portuguese woody ornamental nurseries are specified on table 3. The highest incidence was obtained by *Fusarium solani* with percentages of isolation of the 7,4% of the total number of samples. Following it we can find *Fusarium verticillioides* –2,8% of the total number of samples–, *Fusarium oxysporum* –2,5 %–, *Fusarium subglutinans* –1,3%– , *Fusarium graminearum* –1,2%– and *Fusarium proliferatum* –0.1%–.

TABLE 3. INCIDENCE OF FUSARIUM SPECIES ISOLATED FROM CUTTINGS AND ADULT PLANTS OF WOODY ORNAMENTAL HOSTS OF GALICIA AND PORTUGAL FROM 2013 TO 2020

Fusarium Species	Number of samples with positive isolation [1]	Percentage of samples with positive isolation [2]	Percentage of incidence of each species [3]
verticillioides	21	2,8	18,1
oxysporum	19	2,5	16,3
solani	56	7,4	48,3
graminearum	9	1,2	7,7
subglutinans	10	1,3	8,7
proliferatum	1	0,1	0,9
Total with positive isolation	116	--	100
Total analized	759	15,8	--

[1] Number of samples with positive isolation of each species

[2] Percentage of samples with positive isolation of each species from a total number of 759 analized samples.

[3] Percentage of incidence of each species from a total number of 116 samples with positive isolation of any *Fusarium* species

The percentages of incidence of each species on cuttings are specified on table four. The highest incidence was obtained by *Fusarium solani* with percentages of isolation of the 5,6% of the total number of samples. Following it we can find *Fusarium verticillioides* –2,8% of the total number of samples– and *Fusarium oxysporum* –0,9%. Either *Fusarium graminearum*, *F. subglutinans* or *F. proliferatum* were not isolated on cuttings

The list of hosts of these species included in this study is specified in the first part of these results. The most polyphagous species is *Fusarium solani* isolated from 26 different hosts, followed by *F. verticillioides* and *F. oxysporum* with 12 different hosts each, *F. gaminearum* and *F. subglutinans* isolated from 8 and 7 different hosts each and *F. proliferatum* with *Cotoneaster horizontalis* as its only woody ornamental host in Galicia.

TABLE 4. INCIDENCE OF *FUSARIUM* SPECIES ISOLATED FROM CUTTINGS OF WOODY ORNAMENTAL HOSTS OF GALICIA AND PORTUGAL FROM 2013 TO 2020

Fusarium Species	Number of samples with positive isolation [1]	Percentage of samples with positive isolation [2]	Percentage of incidence of each species [3]
verticillioides	3	2,8	30,0
oxysporum	1	0,9	10,0
solani	6	5,6	60,0
graminearum	0	0	0
subglutinans	0	0	0
proliferatum	0	0	0
Total with positive isolation	10	--	100
Total analized	107	9,3	--

[1] Number of samples with positive isolation of each species.
[2] Percentage of samples with positive isolation of each species from a total number of 107 analized samples.
[3] Percentage of incidence of each species from a total number of 10 samples with positive isolation of any *Fusarium* species

The symptoms that the diseased plants showed were also diverse being damping-off, collar rots and wilts the most frequently observed, specially detected on *Fusarium verticillioides*, *Fusarium oxysporum* and *F. solani*. Yellows and root rots were also observed. The less frequent symptoms were vascular necrosis, stem rots and foliar necrosis.

4. Discussion

The data just exposed confirm an important way of transmission either of *Fusarium oxysporum*, *Fusarium solani* as well as of *Fusarium verticillioides* on the plant material with foreign origin. This is specially important on economically important diseases as those caused by *Fusarium oxysporum*. This was previously referenced before on the case of *Fusarium oxysporum* f. sp. *dianthi* on soil carnation in Galicia (Andrés, 1995; Andrés et al. 1999).

The taxonomy and determination of the four *Fusarium* species formerly named as *Fusarium moniliforme* sensu Messiaen & Cassini (Messiaen & Cassini, 1968) –*F. verticillioides*, *F. subglutinans*, F. *proliferatum* and *F. circinatum*– is not easy and has an extremely important plant quarantine role as it includes one of the most important conifer quarantine pathogens, *Fusarium circinatum*.

There are no previous references of *Fusarium oxysporum* infecting *Eriostemon myoporoides*, *Gelsenium sempervirens* and *Rosmarinus officinalis* worldwide. (Farr & Rossman, 2020). The reference of this pathogen infecting *Cordyline australis*, *Phormium tenax* and *Nandina domestica* are first references for Spain (Farr & Rossman, 2020; Melgarejo *et al*., 2010).

Although *Fusarium solani* is an important polyphagous pathogen that can infect any ornamental species (Smith *et al*., 1992) it is important to mention the new world references of it infecting *Correa reflexa*, *Eriostemon myoporoides*, *Helleborus niger*, *Tibuchina urvilleana*, *Ligustrum japonicum*, *Magnolia grandiflora*, *Phormium tenax*, *Cleyera japonica*, *Euonymus japonicas*, *Diosma ericoides*, *Rosmarinus officinalis*, *Osmanthus fragans* and *Pittosporum tobira* (Farr & Rossman, 2020; Melgarejo *et al*., 2010). The

references of *Fusarium solani* infecting *Vinca minor*, *Thuja occidentalis*, *Nandina domestica* and *Dracaena marginata* are first references for Spain (Melgarejo *et al*., 2010).

The species determination of *Fusarium* is also important for woody ornamental production centres, in order to design sustainable plant protection programs, due to the increasing problem of resistance to fungicides, specially referenced on species of this genus. Up to the date of publication there are references of resitance of *Fusarium graminearum*, *Fusarium oxysporum* f. sp. *dianthi*, *Fusarium roseum* and *Fusarium solani* to MBC fungicides –Methyl benzimidazole carbamates, with FRAC group code 1– as well as *Fusarium graminearum*, *Fusarium moniliforme*, *Fusarium solani* and *Fusarium verticillioides* to DMI fungicides –Demethylation inhibitors with FRAC code 3–. (FRAC, 2018).

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