

A microscopic image of plant tissue, likely a cross-section of a stem or root, showing various cellular structures. A prominent feature is a large, circular, multi-layered structure in the center, possibly a vascular bundle or a specialized cell. The tissue is stained, showing different colors like purple, blue, and green.

Professional Plant Protection

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from woody ornamental hosts in nurseries
sited in Galicia and Northern Portugal

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Scientific paper - *Artículo científico*

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Abstract

In the present paper the author describes 5 species of the formerly named *Pythium* genus –*Globisporangium ultimum*, *Globisporangium spinosum*, *Phytophythium vexans*, *Pythium aphanidermatum*, *Pythium periilum*– identified by him in commercial woody container ornamental production centres of Galicia and Northern Portugal as the result of his work as an independent plant pathologist and researcher. The work was carried out from 1999 to 2020. Some of the references described are the first for Galicia as well as for Spain. The paper includes an annex with the taxonomical clades, published on 2016 by the author and his group, employed for the species determinations

Key words: *Globisporangium ultimum*, *Globisporangium spinosum*, *Phytophythium vexans*, *Pythium aphanidermatum*, *Pythium periilum*.

Resumen

En el presente trabajo el autor describe un total de 5 especies diferentes del anteriormente denominado género Pythium - Globisporangium ultimum, Globisporangium spinosum, Phytophythium vexans, Pythium aphanidermatum, Pythium periilum - identificadas por el mismo en los cultivos leñosos ornamentales en contenedor de viveros de Galicia y del norte de Portugal como resultado de su labor como patólogo vegetal e investigador independiente. El trabajo fue llevado a cabo, de forma intermitente, desde el año 1999 al 2020. El artículo incluye un anexo con las claves dicotómicas, publicadas en el año 2016 por el autor y su grupo, empleadas para la determinación de especies.

Palabras clave: *Globisporangium ultimum*, *Globisporangium spinosum*, *Phytophythium vexans*, *Pythium aphanidermatum*, *Pythium periilum*

1. Introduction

The genus *Pythium* was originally described by Pringsheim in 1858, and *P. monospermum* was selected as the type species. Since then, the classification has been changed by several researchers based on morphological characteristics. All of the members considered by these researchers were englobed in the genus in the current taxonomy defined by Van der Plaats-Niterink (1981).

Recent molecular methods have been used for species identification to supplement the morphological taxonomy by many researchers. These reports have shown that *Pythium* appears to be a nonmonophyletic group that includes several monophyletic groups, and the species are clustered according to sporangial morphology. In this way Japanese researchers emended the genus *Pythium*, describing four new genera segregated from *Pythium s. lato*: *Ovatisporangium*, *Globisporangium*, *Elongisporangium* and *Pilasporangium* (Uzuhashi *et al.*, 2010).

Phytopythium is a new genus of the family *Pythiaceae*, order *Peronosporales* that was described with *Phytopythium sindhum* as the type species by Bala *et al.* (2010). These researchers showed that *Phytopythium sindhum* is a member of the clade K defined by Uzuhashi *et al.* (2010) redefining then the species *Ovatisporangium* as *Phytopythium* and giving priority to this new species name (de Cock *et al.*, 2015).

Members of the formerly named genus *Pythium* are soil inhabiting organisms that are detected all over the world. They are, however, encountered in cultivated soils causing serious problems to various agronomical important crops. They can cause destructive diseases to thousands of plant species. They affect mainly seedbeds of different crops causing seed rot, seedling damping-off, and, occasionally, root rot of different types of adult plants, specially those cultivated in hydroponic systems.

The most important of the formerly named *Pythium* species that are considered pathogens to woody ornamental hosts are the following: *Pythium acanthicum*, *Elongisporangium anandrum*, *Pythium aphanidermatum*, *Globisporangium aquatile*, *Globisporangium deliense*,

Phytopythium helicoides, *Pythium intermedium*, *Pythium irregulare*, *Pythium coimbatorensis*, *Globisporangium megalacanthum*, *Globisporangium myriotyllum*, *Pythium paroecandrum*, *Pythium peritum*, *Pythium periplocum*, *Globisporangium perniciosum*, *Pythium rostratum*, *Globisporangium salpingophorum*, *Globisporangium spinosum*, *Globisporangium splendens*, *Globisporangium ultimum* and *Phytopythium vexans* (Andrés & Rivera, 2016).

In the present paper the author describes the species of the formerly named *Pythium* genus identified by him in commercial woody container ornamental production centres of Galicia and Northern Portugal as the result of his work as an independent plant pathologist and researcher.

2. Material & Methods

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

The study has been carried out in thirteen woody ornamental production centres of Galicia –nine located in the province of Pontevedra, three in A Coruña and the other one in Lugo– and two production centres of Northern Portugal. The samples taken in this study in field conditions were plant material with symptoms of the disease. Each sample contained six plants. The samplings were carried out on each centre, every two or four weeks, intermittently, from 1999 to 2019.

2.2. Identification of potential telluric pathogens

2.2.1. Isolation method

Fragments of the stem and root bases of diseased plants were prepared for fungi isolation. The surface of these fragments was disinfected with 0.6 % sodium hypochlorite for 4 minutes and plated on PDA (potato dextrose agar) (Rapilly, 1968). The fungi and chromists were grown under laboratory conditions and microscope observations were carried out every 24 hours during one week.

All of the formerly *Pythium* (*Phytopythium*, *Elongisporangium*, *Pythium* and *Globisporangium*) isolates were plated on V8 medium, grown for one week and then passed to sterile petri dishes with sterile water, where they were grown under fluorescent light in order to produce sporangia.

2.2.2. Taxonomical criteria for the determination of the formerly named *Pythium* species

The formerly named *Pythium* species were identified following taxonomical criteria described by Uzuhashi *et al.* (2010) as well as by Andrés & Rivera (2016). These taxonomical keys are included on the annex of this paper.

3. Results

The different formerly named *Pythium* species identified from woody ornamental hosts in Galician and Northern Portuguese nurseries were the following:

1. *Globisporangium ultimum* (Trow) Uzuhashi, Tojo & Kakish

Isolated hosts:

Galician nurseries: *Laurus nobilis*, *Cotoneaster lacteus*, *Phormium tenax*, *Pinus pinaster*, *Diosma ericoides*, *Dianthus caryophyllus*.

Northern Portuguese nurseries: *Aloysia citrodora*, *Argyranthemum*, *Ocimum basilicum*, *Ruta graveolens*, *Thymus mastichina*, *Alyssum maritime*, *Passiflora edulis* and *Rosmarinus officinalis*.

Type of crop: container.

Province – Region: A Coruña, Pontevedra, Lugo (Galicia) and Northern Portugal.

2. *Globisporangium spinosum* (Sawada) Uzuhashi, Tojo & Kakish

Isolated hosts:

Galician nurseries: *Camellia japonica*.

Northern Portuguese nurseries: *Origanum vulgare*.

Type of crop: container.

Province – Region: A Coruña, Pontevedra, Lugo (Galicia) and Northern Portugal.

3. *Phytophthium vexans* (de Bary) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque

Isolated hosts:

Galician nurseries: *Pinus radiata*, *Photinia × fraserii*, *Grevillea lanigera*, *Hydrangea macrophylla*, *Diosma ericoides*, *Azalea* and *Metrosideros robusta*.

Northern Portuguese nurseries: *Argyranthemum*, *Passiflora edulis*, *Fuchsia hybrid*.

Type of crop: container.

Province – Region: A Coruña, Lugo, Pontevedra – (Galicia) and Northern Portugal.

4. *Pythium aphanidermatum* (Edson) Fitzp

Isolated hosts: *Camellia japonica*.

Type of crop: container.

Province – Region: Pontevedra (Galicia).

5. *Pythium peritium* Drechsler

Isolated hosts: *Phyllostachys nigra*.

Type of crop: container.

Province – Region: A Coruña (Galicia).

4. Results

Globisporangium ultimum has already been referenced before in Spain (Melgarejo *et al.*, 2010; Andrés & Rivera, 2016 c) as well as in Galicia (Andrés & Rivera, 2016 b) but not on woody ornamental hosts. Therefore, the isolation of such species injuring the hosts sampled on the Galician nurseries – *Laurus nobilis*, *Cotoneaster lacteus*, *Phormium tenax*, *Pinus pinaster*, *Diosma ericoides* and *Dianthus caryophyllus* – are first references of such pathogenic chromist on these hosts in our country (Melgarejo *et al.*, 2010; Andrés & Rivera, 2016 c) as well as in Galicia (Andrés & Rivera, 2016 b).

Globisporangium spinosum has already been referenced before from a greenhouse with a previous *Cucumis sativus* crop, but not on woody ornamental crops (Sánchez *et al.*, 2000). It has not been referenced before in Galicia (Andrés & Rivera, 2016 b). Therefore the references of such pathogen on *Camellia japonica* are also first citations of such pathogen in our country as well as in Galicia (Melgarejo *et al.*, 2010; Andrés & Rivera, 2016 b; Andrés & Rivera, 2016 c).

Phytophthium vexans has been previously referenced in Galicia and therefore in Spain on woody ornamentals (Andrés, 2015) but only on *Metrosideros robusta*, therefore the citations included in

this paper of such pathogen on *Pinus radiata*, *Photinia* × *fraserii*, *Grevillea lanigera*, *Hydrangea macrophylla*, *Diosma ericoides* and *Azalea*, are first references for Spain, and therefore for Galicia (Melgarejo et al., 2010; Andrés, 2015; Andrés & Rivera, 2016 c).

Some of the interactions pathogen-host included in this paper are, according to the information obtained by the author of this paper, first references worldwide: these are isolation of *Globisporangium ultimum* injuring *Laurus nobilis*, *Cotoneaster lacteus*, *Aloysia citrodora*, *Ocimum basilicum*, *Ruta graveolens*, *Thymus mastichina*, *Diosma ericoides* and *Rosmarinus officinallis*; the isolation of *Globisporangium spinosum* on *Origanum vulgare* and on *Cameilia japonica* as well as the isolation of *Phytophthium vexans* on *Photinia* × *fraserii*, *Grevillea lanigera*, *Hydrangea macrophylla*, *Diosma ericoides* as well as on *Azalea* (Farr & Rossman, 2020; Farr & Rossman, 2020 b; Farr & Rossman, 2020 c).

The genus and species determination of the formerly named *Pythium* species 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 these genera. Up to the date of publication there are references of resistance of *Pythium aphanidermatum*, *Pythium irregulare*, *Globisporangium splendens* as well as *Globisporangium ultimum* to Phenylamide fungicides– with FRAC group code 4–; *Pythium aphanidermatum* to QoI fungicides –with FRAC code 3–; *Pythium aphanidermatum*, *Pythium irregulare*, *Globisporangium splendens* as well as *Globisporangium ultimum* to Carbamates –with FRAC code 28– as well as *Pythium aphanidermatum* to phosphonates –with FRAC code 33– (FRAC, 2020).

The species determination, among the formerly named *Pythium* genus, is also important for another reason: plant quarantine. The existence of certain formerly named *Pythium* species considered quarantine pathogens for certain countries, makes the genus determination completely useless for nursery plant producers as well as for plant inspectors. These need quick and sure analysis, that may determine up to species status, in order to avoid introductions and spread of these quarantine pathogens. Among the studied spe-

cies *Phytophthium vexans* is considered a quarantine pathogen in South Korea (List of Regulated Pests of Korea, 2011) as well as in Perú (Lista de plagas cuarentenarias del Perú, 2012). Other formerly named *Pythium* species that are considered quarantine pathogens on certain countries of the world are the following: *Globisporangium splendens*, *Globisporangium paroecandrum*, *Pythium myriotylum*, *Globisporangium echinulatum*, *Globisporangium mamillatum*, *Globisporangium debaryanum*, *Globisporangium sylvaticum*, *Pythium tracheiphilum* as well as *Pythium irregulare* (Andrés, 2015; EPPO, 2020; EPPO, 2020 b).

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TABLE 1. TAXONOMICAL KEY TO THE FORMERLY NAMED *PYTHIUM* GENERA ACCORDING TO UZUHASHI ET AL., (2010)

| | |
|---|-------------------------|
| 1. Filamentous sporangia, inflated or not | <i>Pythium</i> |
| 1. Not filamentous sporangia | 2 |
| 2. Sporangia ovoid to pyriform, sometimes irregularly shaped | <i>Ovatisporangium</i> |
| 2. Sporangia usually globose | 3 |
| 3. Sporangia clavate to elongate | <i>Elongisporangium</i> |
| 3. Sporangia sometimes proliferating | <i>Globisporangium</i> |
| 3. Sporangia not proliferating, secondary hyphae branched complexly | <i>Pilasporangium</i> |

TABLE 2. TAXONOMICAL KEY TO THE *GLOBISPORANGIUM* SPECIES PATHOGENIC TO VEGETABLE AND ORNAMENTAL CROPS WORLDWIDE (ANDRÉS & RIVERA, 2016)

| | |
|--|------------------------|
| 1. Oogonia produced in single cultures | 2 |
| 1. Oogonia not or scarcely produced in single cultures | <i>G. sylvaticum</i> |
| 2. Oogonial diameter larger than 40 µm | <i>G. polymastum</i> |
| 2. Oogonial diameter smaller than 40 µm | 3 |
| 3. Antheridial cells mostly single; oogonia on average 38,5 µm in diameter | 4 |
| 3. Antheridial cells 1- 8; oogonia on average 34 µm in diameter | <i>G. uncinulatum</i> |
| 4. Globose oogonia | 5 |
| 4. Globose oogonia not present | <i>G. meglacanthum</i> |
| 5. Oogonia with 2 oospores | <i>G. intermedium</i> |
| 5. Oogonia with two oospores not present | 6 |
| 6. Catenulate oogonia | <i>G. paroecandrum</i> |
| 6. Catenulate oogonia not present | 7 |
| 7. Terminal oogonia | <i>G. rostratum</i> |
| 7. Terminal oogonia not present | 8 |
| 8. Catenulate hyphal swellings | <i>G. carolinianum</i> |
| 8. Catenulate hyphal swellings not present | 9 |
| 9. Antheridial cells single and oogonia with 38.5 µm in diameter | <i>G. mastophorum</i> |
| 9. Antheridial cells single and oogonia with 38.5 µm in diameter not present | 10 |
| 10. Intercalary oogonia present | 11 |
| 10. Intercalary oogonia not present | <i>G. hypoginum</i> |
| 11. Monoclinous antheridia | 12 |
| 11. Monoclinous antheridia not present | <i>G. splendens</i> |
| 12. Oogonia with 29 µm in diameter | <i>G. violae</i> |
| 12. Oogonia with 29 µm in diameter not present | 13 |
| 13. Hyphal swellings larger than 30 µm in diameter | <i>G. spinosum</i> |
| 13. Hyphal swellings larger than 30 µm in diameter not present | 14 |
| 14. Hypoginous antheridia | 15 |
| 14. Hypoginous antheridia not present | <i>G. mamillatum</i> |
| 15. Diclinous antheridia | <i>G. irregular</i> |
| 15. Diclinous antheridia not present | 16 |
| 16. Aerial mycelium in cornmeal agar | <i>G. ultimum</i> |
| 16. Aerial mycelium in cornmeal agar not present | <i>G. echinulatum</i> |

TABLE 3. TAXONOMICAL KEY TO THE *PYTHIUM* SPECIES PATHOGENIC TO VEGETABLE AND ORNAMENTAL CROPS WORLDWIDE (ANDRÉS & RIVERA, 2016)

| | |
|---|--------------------------|
| 1. Antheridia sessile | <i>P. dissotocum</i> |
| 1. Sessile antheridia not present | 2 |
| 2. Catenulate hyphal swellings present | <i>P. perniciosum</i> |
| 2. Catenulate hyphal swellings not present | 3 |
| 3. Clear yellow oospore present | 4 |
| 3. Clear yellow oospore not present | 5 |
| 4. Diclinous antheridia | <i>P. coloratum</i> |
| 4. Diclinous antheridia not present | <i>P. acanthicum</i> |
| 5. Large antheridial cells with furrows | <i>P. sulcatum</i> |
| 5. Large antheridial cells with furrows not present | 6 |
| 6. Oogonial stalks curved towards the antheridia | <i>P. deliense</i> |
| 6. Oogonial stalks curved towards the antheridia not present | 7 |
| 7. Terminal oogonia present | 8 |
| 7. Terminal oogonia not present | <i>P. salpingophorum</i> |
| 8. Esporangia inflated, oogonial projections blunt | <i>P. periplocum</i> |
| 8. Esporangia inflated, oogonial projections blunt not present | 9 |
| 9. Maximum growth temperatures higher than 40°C | 10 |
| 9. Maximum growth temperatures lower than 40°C | 11 |
| 10. Monoclinous antheridia | <i>P. aphanidermatum</i> |
| 10. Monoclinous antheridia not present | <i>P. myriotylum</i> |
| 11. Fingerlike sporangia present | <i>P. periiulum</i> |
| 11. Fingerlike sporangia not present | 12 |
| 12. Intercalary oogonia not present | <i>P. arrhenomanes</i> |
| 12. Intercalary oogonia present | 13 |
| 13. Antheridia 1-3 present | 14 |
| 13. Antheridia 1-3 not present | 15 |
| 14. Diclinous antheridia present | <i>P. monospermum</i> |
| 14. Diclinous antheridia not present | <i>P. graminicola</i> |
| 15. Antheridia originating 1-5 µm below the oogonia | 16 |
| 15. Antheridia originating 1-5 µm below the oogonia not present | 17 |
| 16. Oospore wall up to 3 µm | <i>P. aquatile</i> |
| 16. Oospore wall up to 3 µm not present | <i>P. torulosum</i> |
| 17. Aplerothic oospore | 18 |
| 17. Aplerothic oospore not present | <i>P. tracheiphilum</i> |
| 18. Monoclinous antheridia | <i>P. coimbatorensis</i> |
| 18. Monoclinous antheridia not present | 19 |
| 19. Diclinous antheridia | 20 |
| 19. Diclinous antheridia not present | <i>P. hydnosporum</i> |
| 20. Oogonial projections acute, 5-7 µm long | <i>P. oligandrum</i> |
| 20. Oogonia projections acute, 5-7 µm long not present | <i>P. volutum</i> |

TABLE 4. TAXONOMICAL KEY TO THE *OVATISPORANGIUM* (ACTUALLY RENAMED AS *PHYTOPYTHIUM*) SPECIES PATHOGENIC TO VEGETABLE AND ORNAMENTAL CROPS WORLDWIDE (ANDRÉS & RIVERA, 2016)

| | |
|--|----------------------|
| 1. Lobed antheridia. Oospores with 17 µm in diameter | <i>O. vexans</i> |
| 1. Lobed antheridia. Oospores with 17 µm in diameter not present | 2 |
| 2. Antheridia long cylindrical, without waves or furrows; oospores wall 4-6 µm thick | <i>O. helicoides</i> |
| 2. Antheridia wavy or furrowed; oospore wall up to 3,5 µm thick | <i>O. oedochilum</i> |