pISSN 2302-1616, eISSN 2580-2909
Vol 12, No. 1, 2024, pp x
José Luis Andrés Ares
Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v12i1.46769

Quality Phytophthora de Bary and formerly named *Pythium* Pringsh. species isolated from woody ornamental hosts in Galician nurseries, Northwest Spain.

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Scientific paper

Abstract

In the present paper the author describes the quality – not quarantine - *Phytophthora* species isolated from woody ornamental hosts in Galician nurseries, as part of his work as an independent phytopathological consultant. The purpose of the present article is to determine the most important *Phytophthora* and formerly named *Pythium* species that infect woody ornamental plants in nursery conditions in Galicia – Northwest Spain -. The author isolated a total number of 6 species as pathogens to plants with different symtoms: *Phytophthora cinnamomi, P. cactorum, P. palmivora, P. nicotianae, P. cryptogea* and *P. citrophthora*. The author compares the different hosts included in his results with the hosts described by the specialized literature. The author also describes 5 species of the formerly named *Pythium* genus - *Globisporangium ultimum, Globisporangium spinosum, Phytopythium vexans, Pythium aphanidermatum, Pythium periilum* - identified by him in commercial woody container ornamental production centres of Galicia. The work was carried out from 2013 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 of the formerly named *Pythium* species pathogenic to woody ornamental hosts, published on 2016 by the author and his group, employed for the species determinations.

Key words: *Phytophthora, Globisporangium Phytopythium, Pythium.*

INTRODUCTION

During the last 10 years the author has intensified the phytopathological inspections due to the detection of *Phytophthora ramorum* (Werres et al., 2001) in Galicia (Pintos *et al.*, 2004; Pintos *et al.*, 2003), causal agent of the sudden oak death. As a result of these inspections the author detected other species of *Phytophthora* different to *P. ramorum* but without less economical importance for woody ornamental nurseries in Galicia – NW Spain - . In this paper the author carries out a revision of his own phytopathological data from 2013 to 2020 describing the different *Phytophthora* and formerly named *Pythium* species – they have similar root rot symptoms - isolated by him, from woody ornamental hosts, in his own laboratory, as part of his work as an independent phytopathological consultant.

Except for the valuable work carried out by the research group directed by Pérez-Sierra in Valencia, there is a great lack of information about *Phytophthora* species pathogenic to woody ornamental hosts collected in Spanish nurseries. This research group carried out several surveys on woody ornamental nurseries of Eastern

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Spain that pointed out the existence of thirteen different *Phytophthora* species causing disease in the 70 % of the nurseries studied. These were the following: *P. cactorum, P. cinnamomi, P. citrophthora, P. cryptogea, P. drechsleri, P. hibernalis, P. multivora, P. nicotianae, P. palmivora, P. niederhauserii, P. plurivora, P. syringae* and *P. tentaculata*. (Pérez-Sierra et al., 2012).

In Galicia Pintos and his scientific group have recently pointed out the presence of several *Phytophthora* species - *P. ramorum, P. cinnamomi, P. citricola, P. citrophthora, P. cryptogea, P. hibernalis, P. ilicis, P. multivora, P. nicotianae, P. plurivora, P. pseudosyringae and P. syringae* - infecting several woody ornamental hosts in public gardens and parks (Pintos *et al.*, 2023). Considering only nursery stock Andrés references the presence of *Phytophthora nicotianae* infecting several woody ornamental hosts in Galician nurseries– *Ceanothus repens, Dracaena marginatta, Aucuba japonica, Araucaria araucana, Boronia gunni, Hydrangea macrophylla, Leptospermum scoparium, Cotoneaster lacteus, Myscanthus sinensis, Polygala myrtifolia, Correa reflexa, Phormium tenax* and *Rhododendron* -, of *Phytophthora cinnamomi* infecting *Camellia japonica, azaleas, Buxus sempervirens, Arbutus unedo* and *Rhododendron sp.*, of *Phytophthora cryptogea* infecting *Lavandula angustifolia*, and of *Phytophthora cactorum* infecting *Arbutus unedo*, localizing all of these diseases in Galician nurseries. (Andrés, 2016 a, 2016 b, 2017 a, 2017 b, 2017 c, 2018, 2019).

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

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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 coimbatorense, Globisporangium megalacanthum, Globisporangium myriotyllum, Pythium paroecandrum, Pythium periilum, Pythium periplocum, Globosporangium 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 the genus *Phytophthora* de Bary, as well as of the formerly named *Pythium* Pringsh. genus identified by him in commercial woody container ornamental production centres of Galicia as the result of his work as an independent plant pathologist and researcher. The purpose of the present article is to determine the most important *Phytophthora* and formerly named *Pythium* species that infect woody ornamental plants in nursery conditions in Galicia – Northwest Spain -.

MATERIAL AND METHODS

Study area

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 - . The samples taken in this study in field conditions were plant material with symptoms of root, stem or leaf rots. Each sample contained six plants. The samplings were carried out on each centre, every two or four weeks, intermittently, from 2013 to 2020. This technique was carried out in order to isolated the chromistic pathogens in all of the seasons of the year.

Procedures

The isolation of the pathogen was carried out in the phytopathological laboratory of the firm Consultorías Noroeste S.C. Fragments of the stem, root bases and rotted leaves 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). The fungi were grown under laboratory conditions and microscope observations were carried out every 24 hours during one week.

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All of the *Phytophthora* and formerly *Pythium* (*Phytopythium, Elongisporangium, Pythium* and *Globisporangium*) isolates were passed to V8 medium – used to stimulate the production of sporangia, necessary for the morphological taxonomy and determination of species - , grown for one week and then passed to sterile petri dishes with sterile water, where they were grown under white light in order to produce sporangia.

Phytophthora species were identified following taxonomical criteria described by Stamps et al. (Stamps et al., 1990). 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.

RESULTS AND DISCUSSION

The species were all identified following morphological taxonomical criteria. A total number of 11 different species were obtained: 6 belonging to *Phytophthora*, two to *Globisporangium*, two to *Pythium* and one classified as *Phytopythium*.
The different *Phytophthora* species identified from woody ornamental hosts in Galician nurseries were the following:

1.- *Phytophthora cinnamomi* Rands

Isolated hosts: *Pittosporum tobira, Araucaria araucana, Laurus nobilis, Camellia japonica, Rhododendron, Chamaecyparis lawsoniana, Ilexaquifolium, Euonymus japonica.*
Type of crop: soil and container.
Province: A Coruña, Pontevedra, Lugo.

2.- *Phytophthora cactorum* (Lebert & Cohn) J. Schrot (1886)

Isolated hosts: *Ceanothus repens.*
Type of crop: soil and container.
Province: A Coruña.

3.- *Phytophthora palmivora* Butler.

Isolated hosts: *Chamaerops excelsa.*
Type of crop: soil and container.
Province: Pontevedra.

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4.- *Phytophthora nicotianae* Breda de Haan (1896)

Isolated hosts: *Ceanothus repens, Dracaena marginatta, Aucuba japonica, Araucaria araucana, Boronia gunni, Hydrangea macrophylla, Leptospermum scoparium, Cotoneaster lacteus, Myscanthus sinensis, Polygala myrtifolia, Correa reflexa, Phormium tenax, Rhododendron, Thuja plicatta, Eriostemon myoporoides, Lophomyrthusx ralphii, Hydrangea macrophylla, Phormium tenax, Acacia dealbata, Azalea, Grevillea juniperina, Picea abies, Taxus baccata, Eleagnus x ebbingei, Euonymus japonicas, Abelia grandiflora, Diosma ericoides* and *Nandina domestica,*
Type of crop: container.
Province: A Coruña, Pontevedra, Lugo.

5.- *Phytophthora cryptogea* Pethybr & Laff (1919)

Isolated hosts: *Lavandula angustifolia*
Type of crop: container
Province: Pontevedra

6.- *Phytophthora citrophthora* (R.E. Sm & E.H. Sm.) Leonian (1906)

Isolated hosts: *Citrus sinensis*
Type of crop: container
Province: Pontevedra

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

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

Isolated hosts:
Galician nurseries: *Laurus nobilis, Cotoneaster lacteus, Phormium tenax, Pinus pinaster, Diosma ericoides, Dianthus caryophyllus.*
Type of crop: container.
Province - Region: A Coruña, Pontevedra, Lugo – Galicia.

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

Isolated hosts:

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Galician nurseries: *Camellia japonica*.
Type of crop: container
Province – Region: Pontevedra – Galicia.

9.- *Phytopythium vexans* (de Bary) Abad, de Cock, Bala, Robideau, A.M. Lodhi & Lévesque.

Isolated hosts:
Galician nurseries: *Pinus radiata, Photinia x fraserii, Grevillea lanigera, Hydrangea macrophilla, Diosma ericoides, Azalea,* and *Metrosideros robusta*.
Type of crop: container
Province – Region: A Coruña, Lugo, Pontevedra – Galicia.

10.- *Pythium aphanidermatum* (Edson) Fitzp.

Isolated hosts: *Camellia japonica*
Type of crop: container
Province – Region: Pontevedra – Galicia-.

11.- *Pythium periilum* Drechsler

Isolated hosts: *Phyllostachys nigra*.
Type of crop: container
Province – Region: A Coruña – Galicia -.

The data shown in this paper demonstrate the existence of new pathogen-host interactions for Spain. These are the following for *Phytophthora* species: *Pittosporum tobira, Araucaria araucana* and *Euonymus japonica* infected by *Phytophthora cinnamomi*; *Phytophthora cactorum* pathogenic to *Ceanothus repens* and *Thuja plicatta, Eriostemon myoporoides, Lophomyrthus x ralphii, Hydrangea macrophylla, Acacia dealbata, Grevillea juniperina, Picea abies, Eleagnus x ebbingei, Abelia grandiflora, Diosma ericoides* and *Nandina domestica* infected by *Phytophthora nicotianae*. (Pérez-Sierra et al., 2012; Andrés, 2015 a, 2015 b, 2017 a, 2017 b, 2017 c, 2018, 2019; Melgarejo et al., 2010; Mansilla *et al.*, 2005; Farr & Rossman, 2019; Farr & Rossman, 2019 b; Farr & Rossman, 2019 c).

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

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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 (Sanchez 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).

Phytopythium 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 x fraserii, Grevillea lanigera, Hydrangea macrophilla, 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 pathogen-host interactions are first references worldwide, for the formerly named *Pythium* species included in this paper, according to the information obtained by the author of this paper: the isolation of *Globisporangium ultimum* injuring *Laurus nobilis, Cotoneaster lacteus* and *Diosma ericoides;* the isolation of *Globisporangium spinosum* on *Camellia japonica* as well as the isolation of *Phytopythium vexans* on *Photinia x fraserii, Grevillea lanigera, Hydrangea macrophilla, Diosma ericoides as well as on Azalea.* (Farr & Rossman, 2020; Farr & Rossman, 2020 b; Farr & Rossman, 2020 c).

If we compare the optimal temperatures of each species with the Köppen-Geiger temperature classification of the region where they were detected we may conclude thar there is certain influence of this factor on the incidence of the different species: the *Phytophthora* species detected only in Pontevedra – with part of the province with *Csb* climate - Warm-summer Mediterranean climate (Peel at al., 2007) – *Phytophthora palmivora* – 28-30 ºC of optimal cardinal temperatures (Erwin & Ribeiro, 1996) -, *Phytophthora cryptogea* – 25 ºC of optimal cardinal temperatures (Erwin & Ribeiro, 1996) -and *Phytophthora citrophthora* - – 24-28 ºC of optimal cardinal temperatures (Erwin & Ribeiro, 1996) - have optimal cardinal temperatures higher than the species detected in A Coruña or Lugo with all the surface of the province with *Cfb* climate – temperate oceanic climate – as forn example *Phytophthora cactorum* – 22-24 ºC of optimal cardinal temperatures (Erwin & Ribeiro, 1996) -.

The situation is similar if we consider the formerly *Pythium* species: *Pythium aphanidermatum*, only detected in Pontevedra, has a significatively higher cardinal optimal temperatures – 35-40 ºC – than *Pythium periilum* – 25-30 º C -only isolated from plants grown in A Coruña province. (Andrés & Rivera , 2016; Peel at al., 2007).

The information supplied in this article is necessary to design sustainable plant protection programs in woody ornamental nurseries and further research should be done

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in order to determine the influence of climate change in the incidence of the different chromist species.

CONCLUSION

This paper confirms the existence of new pathogen-host interaction for Spain, these are the following: *Pittosporum tobira, Araucaria araucana* and *Euonymus japonica* infected by *Phytophthora cinnamomi*; *Phytophthora cactorum* pathogenic to *Ceanothus repens* and *Thuja plicatta, Eriostemon myoporoides, Lophomyrthus x ralphii, Hydrangea macrophylla, Acacia dealbata, Grevillea juniperina, Picea abies, Eleagnus x ebbingei, Abelia grandiflora, Diosma ericoides* and *Nandina domestica* infected by *Phytophthora nicotianae*; *Laurus nobilis, Cotoneaster lacteus, Phormium tenax, Pinus pinaster, Diosma ericoides* and *Dianthus caryophyllus* infected by *Globisporangium ultimum; Pinus radiata, Photinia x fraserii, Grevillea lanigera, Hydrangea macrophilla, Diosma ericoides* and *Azalea sp.* infected by *Phytopythium vexans.* This paper also confirms the following first references worldwide: *Globisporangium ultimum* injuring *Laurus nobilis, Cotoneaster lacteus* and *Diosma ericoides; Globisporangium spinosum* on *Camellia japonica* as well as the isolation of *Phytopythium vexans* on *Photinia x fraserii, Grevillea lanigera, Hydrangea macrophilla, Diosma ericoides* as well as on *Azalea sp.*.

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Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v12i1.46769

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Annex

Table 1 .- Taxonomycal key to the formerly named *Pythium* genera according to Uzuhashi et al., 2010.

1.- Filamentous sporangia, inflated or not _____ ***Pythium***
1.-Not filamentous sporangia _____ 2
2.-Sporangia ovoid to pyriform, sometimes irregularly shaped _____ ***Ovatisporangium***
2.-Sporangia usually globose _____ 3
3.- Sporangia clavate to elongate _____ ***Elongisporangium***
3.-Sporangia sometimes proliferating _____ ***Globisporangium***
3.- Sporangia not proliferating, secondary hyphae branched complexely _____***Pilasporangium***

Table 2 .- Taxonomycal 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 _____***G. sylvaticum***
2.- Oogonial diameter larger than 40 µm _____ ***G. polymastum***
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 _____ ***G. uncinulatum***
4.- Globose oogonia _____ 5
4.- Globose oogonia not present _____***G. meglacanthum***
5.-Oogonia with 2 oospores _____ ***G. intermedium***
5.- Oogonia with two oospores not present _____ 6
6.- Catenulate oogonia _____ ***G. paroecandrum***
6.- Catenulate oogonia not present _____ 7
7.- Terminal oogonia _____***G. rostratum***
7.- Terminal oogonia not present _____ 8
8.- Catenulate hyphal swellings _____ ***G. carolinianum***
8.- Catenulate hyphal swellings not present _____ 9
9.- Antheridial cells single and oogonia with 38.5 µm in diameter _____ ***G. mastophorum***
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 _____***G. hypoginum***
11.- Monoclinous antheridia _____ 12
11.- Monoclinous antheridia not present _____:_____ ***G. splendens***
12.- Oogonia with 29 µm in diameter _____ ***G. violae***
12.- Oogonia with 29 µm in diameter not present _____ 13
13.- Hyphal swellings larger than 30 µm in diameter _____ ***G. spinosum***
13.- Hyphal swellings larger than 30 µm in diameter not present _____ 14

pISSN 2302-1616, eISSN 2580-2909
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Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v12i1.46769

14.- Hypoginous antheridia _____ 15
14.- Hypoginous antheridia not present _____ ***G. mamillatum***
15.-Diclinous antheridia _____ ***G. irregular***
15.- Diclinous antheridia not present _____ 16
16.-Aerial mycelium in cornmeal agar _____ ***G. ultimum***
16.- Aerial mycelium in cornmeal agar not present _____ ***G. echinulatum***

Table 3 .- Taxonomycal key to the *Pythium* species pathogenic to vegetable and ornamental crops worldwide. (Andrés & Rivera, 2016).

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

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Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v12i1.46769

Table 4 .- Taxonomycal 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 _____ ***O. vexans***
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
_____ ***O. helicoides***
2.- Antheridia wavy or furrowed; oospore wall up to 3.5 µm thick_____ ***O. oedochilum***