Curso para Habilitação de Responsáveis Técnicos para Emissão de CFO/CFOC

2021

ADAPAR
Agência de Defesa Agropecuária do Paraná

AGRONOMIA
Universidade Estadual de Maringá

William Mário de Carvalho Nunes

Engenheiro Agrônomo - UEM - 1984
Mestre *em Fitopatologia - ESALQ/USP - 1994*
Doutor *em Proteção de Plantas* - UNESP - 1999
Professor *de Fitopatologia do curso de graduação em Agronomia*
Professor *do Programa de Pós-Graduação em Agronomia (PGA)*
Chefe *do Departamento de Agronomia (DAG)*

NOTA 5 ENADE AGRONOMIA UEM

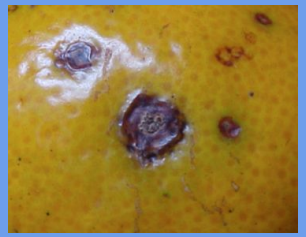
Departamento de Agronomia
Universidade Estadual de Maringá

Programa de Pós-graduação em Agronomia
PGA/UEM
Conceito 6
Programa de Excelência
CAPES

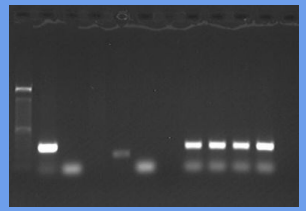
Parte 1

DIA 15/10/21 – SEXTA-FEIRA	
Aspectos sobre classificação taxonômica da praga, monitoramento, tipos de armadilhas, levantamento e mapeamento da praga em condições de campo, identificação, coleta, acondicionamento e transporte da amostra, bioecologia, sintomas, sinais, plantas hospedeiras, ações de prevenção e métodos de controle	
08:00 – 09:00 h	Praga Quarentenária Presente – Pinta Preta dos Citros *(Phyllosticta citricarpa)* **Eng. Agr. Dr. William Mário de Carvalho Nunes - UEM**
09:00 – 10:00 h	Praga Quarentenária Presente – Cancro Cítrico (*Xanthomonas citri* subsp. *citri)* **Eng. Agr. Dr. William Mário de Carvalho Nunes - UEM**
10:00 – 10:15 h	Intervalo
10:15 – 11:00 h	Praga Quarentenária Presente – Greening/HLB (*Candidatus* Liberibacter spp.) **Eng. Agr. Dr. William Mário de Carvalho Nunes - UEM**









Phyllosticta citricarpa, agente causal da Pinta Preta dos citros

Praga Quarentenária A2

ADAPAR
Agência de Defesa Agropecuária do Paraná

PARANÁ
GOVERNO DO ESTADO
Secretaria da Ciência, Tecnologia e Ensino Superior

Departamento de Agronomia
Universidade Estadual de Maringá

NBA
Núcleo de Pesquisa em Biotecnologia Aplicada
UEM Maringá-PR

Impacto da Pinta Preta

Porque está na lista das Pragas Quarentenárias A2?

- Frutos **impróprios** para o mercado *in natura*.

- **Queda prematura dos frutos**, com redução da produtividade.

Embrapa

Embrapa

Embrapa

Impacto da Pinta Preta

Porque está na lista das Pragas Quarentenárias A2?

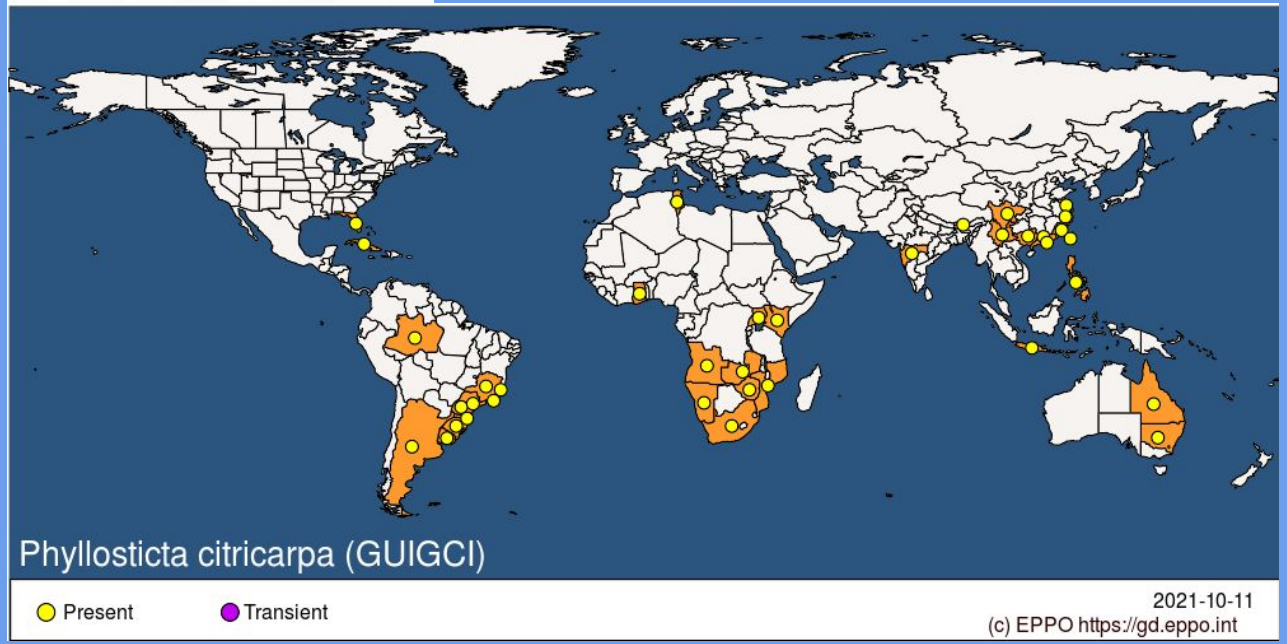
- Afeta **laranjas doces, limões** e **tangerinas**, à exceção da lima ácida ‘Tahiti’.
- A **Erradicação** da doença é considerada **praticamente impossível** - *A incidência pode passar de 10% de plantas com frutos sintomáticos para mais de 80%, em apenas dois anos, se não houver controle* (Fundecitrus).

Ocorrência de Pinta Preta no Mundo

EPPO Global Database

Home | Standards | Photos | Reporting

Phyllosticta citricarpa (GUIGCI)



Continent	Country	State	Status
America	- select -	- select -	- select -
America	Antigua and Barbuda		Absent, confirmed by survey
America	Argentina		Present, restricted distribution
America	Bahamas		Absent, confirmed by survey
America	Belize		Absent, confirmed by survey
America	Bolivia		Absent, confirmed by survey
America	Brazil		Present, restricted distribution

Principais países afetados pela doença:
África do Sul, Austrália, Taiwan, China, Japão , Argentina e Brasil,

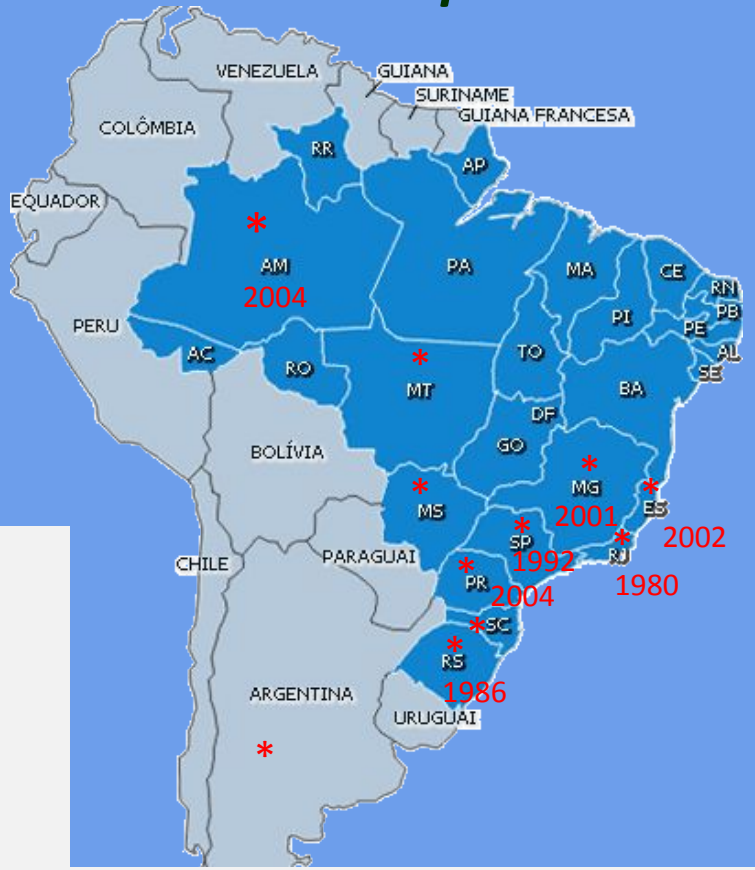
https://gd.eppo.int/taxon/GUIGCI

Ocorrência de *Phyllosticta citricarpa* no Brasil

ANEXO II
Lista de Pragas Quarentenárias Presentes no Brasil - (A2)
https://www.gov.br/agricultura/pt-br/assuntos/sanidade-animal-e-vegetal/sanidade-vegetal/arquivos-quarentena/lista-de-pragas-quarentenarias-ausentes-e-presentes.pdf/view

UNIDADES FEDERATIVAS DE OCORRÊNCIA

Norte: Amazonas,
Sudeste: Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo.
Centro Oeste: Mato Grosso, Mato Grosso do Sul,
Sul: Paraná, Santa Catarina, Rio Grande do Sul



✔ Descrita inicialmente no Estado de São Paulo em 1940 (AVERNA-SACCÁ, 1940)

Etiologia da Pinta Preta

- **Agente causal:** ***Phyllosticta citricarpa***
- ´Current name´ (=nome atual) da espécie, de acordo com www.mycobank.org

- **Classificação**

Reino:	Fungi
Filo:	Ascomycota
Sub-filo:	Pezizomycotina
Classe:	Dothideomycetes
Ordem:	Botryosphaeriales
Família:	Botryosphaeriaceae

mycobank.org/page/Simple%20names%20search

Phyllosticta citricarpa

Taxon name	Phyllosticta citricarpa
Summary	Phyllosticta citricarpa (McAlpine) Aa, Studies in Mycology 5: 40 (1973) [MB#320327]

General information

Classification	Fungi Dikarya Ascomycota Pezizomycotina Dothideomycetes Botryosphaeriales Phyllostictaceae Phyllosticta
Rank	sp.
MycoBank #	320327
Name type	Combination
Gender	Feminine
Authors	(McAlpine) Aa
Year of effective publication	1973
Name status	Legitimate
Sanctioned by	-
Synonymy	

Current name	Phyllosticta citricarpa (McAlpine) Aa, Studies in Mycology 5: 40 (1973) [MB#320327]
Basionym	Phoma citricarpa McAlpine, Fungus diseases of Citrus trees in Australia, and their Treatment: 21 (1899) [MB#226678]
Obligate synonyms	Phyllostictina citricarpa (McAlpine) Petr., Sydowia 7 (1-4): 115 (1953) [MB#303518]
Taxon synonyms	Guignardia citricarpa Kiely, Proceedings of the Linnean Society of New South Wales 73: 259 (1948) [MB#286752] Phyllosticta circumsepta Sacc., Annales Mycologici 12 (3): 305 (1914) [MB#164846] Phyllosticta citricola Hori, Engei no Tome: 627 (1913) [MB#501199] Phyllosticta citricola Hori ex Hara, J. hort. Ass. Japan: 9 (1917) [MB#501198]

Note that the taxonomic opinions listed here are not always up to date and may include errors. Please report them to our curator (Konstanze Bensch)

Associated files



Bibliography

Literature	Aa, H.A. van der. 1973. Studies in Phyllosticta I. Studies in Mycology. 5:1-110
Bibliography links	PubMed Google Scholar PubMed

Mycobank

Studies in Mycology No. 05

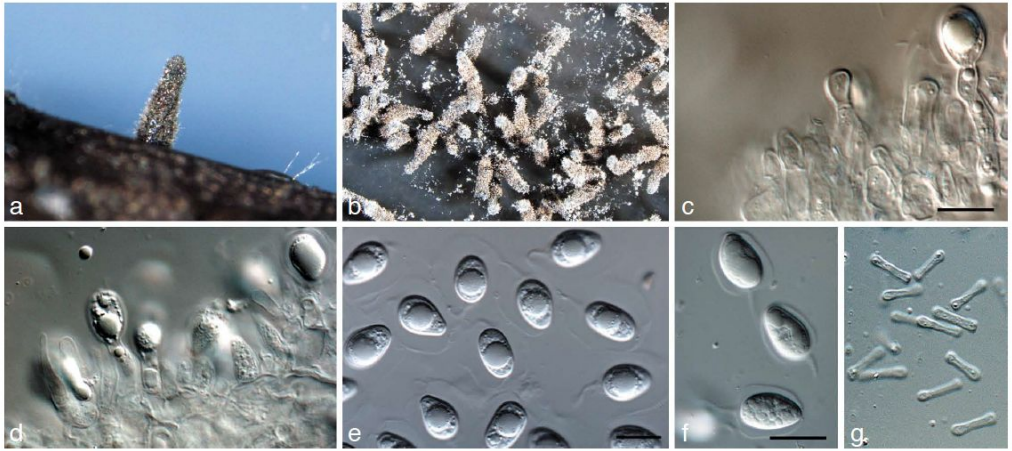


Fig. 2 *Phyllosticta bifrenariae*. a. Pycnidium forming on PNA; b. pycnidia forming on PDA; c, d. conidiophores giving rise to conidia; e, f. conidia; g. spermatia (all: CBS H-20520 holotype). — Scale bars = 10 µm.

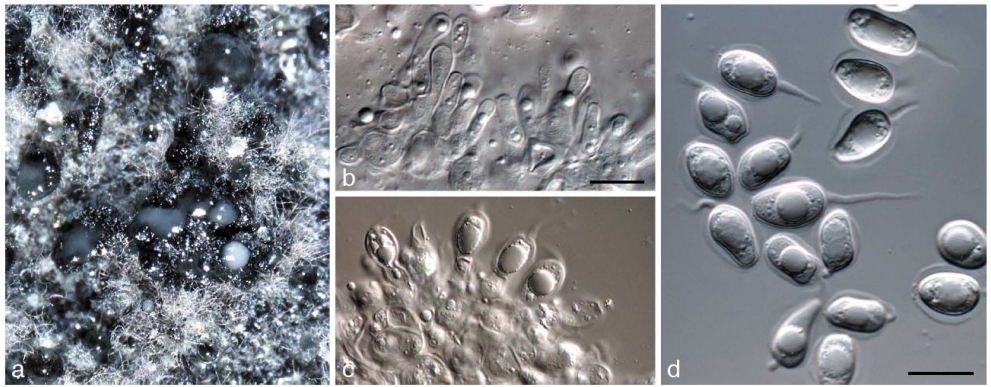


Fig. 3 *Phyllosticta brazilianiae*. a. Pycnidia forming on PDA; b, c. conidiophores giving rise to conidia; d. conidia (all: CBS H-20521 holotype). — Scale bars = 10 µm.

https://gd.eppo.int/taxon/GUIGCI/datasheet https://www.fundecitrus.com.br/doencas/pinta-preta#sintomas

Sintomas

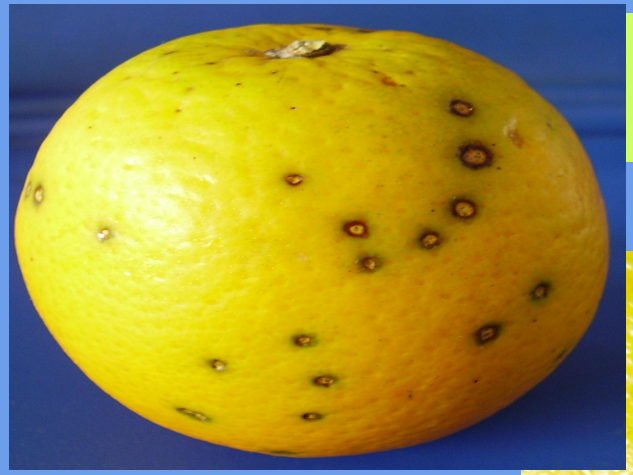




Fotos: Fundecitros

rapa

Sintom











Fotos: NBA

Embrapa

Tipos de Sintomas

(EPPO, 2020)

Mancha dura ou mancha preta

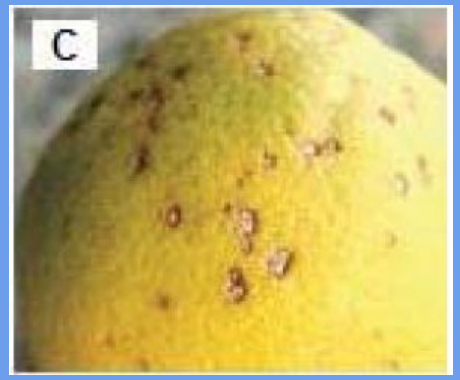


- Sintoma mais comum
- Surgem quando os frutos iniciam a maturação
- Pontuações negras no centro: picnídios

Tipos de Sintomas

(EPPO, 2020)

Manchas sardentas



- Aparecem depos que os frutos atingiram a maturação.
- Lesões levemente deprimidas e avermelhadas.
- As lesões podem coalescer, formando uma grande lesão, ou permanecerem individualizadas.

Tipos de Sintomas

(EPPO, 2020)

Manchas virulentas



- Desenvolvem-se quando os frutos estão maduros .
- Associados a temperaturas elevadas
- Resultam do desenvolvimento e da coalescência dos dois tipos de sintomas anteriores.

Tipos de Sintomas

(EPPO, 2020)

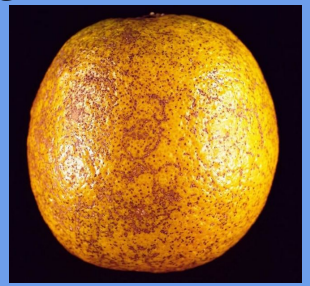
Manchas do tipo falsa melanose



- Manchas irregulares, com textura áspera ao tato, de tamanho variado.
- Manchas predominantemente pequenas (≈2mm)



Melanose



Tipos de Sintomas

(EPPO, 2020)

Mancha trincada



- Manchas superficiais, irregulares, lisas, de tamanho variado, inicialmente de aspecto oleoso ,castanhas, que evoluem para coloração escura.
- Não forma picnidio.
- Normalmente não causa queda dos frutos.
- Aparece em frutos ainda verdes , porém quando os frutos ficam maduros, sua casca apresenta trincas

Tipos de Sintomas

(EPPO, 2020)

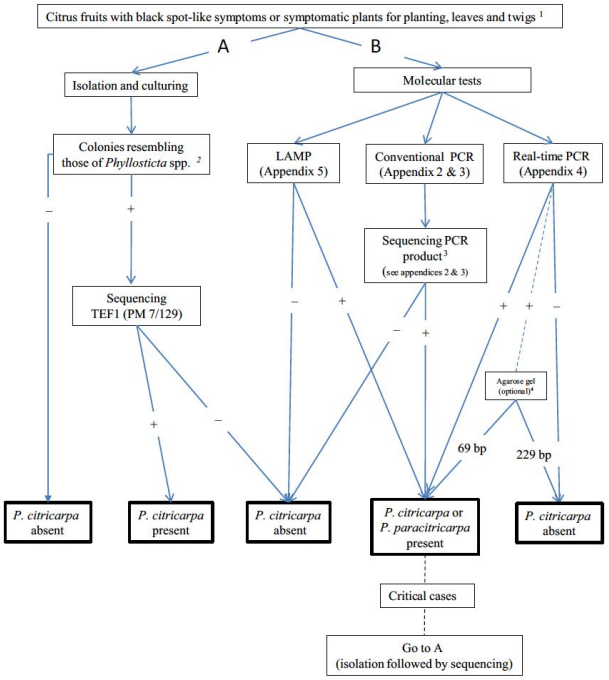
Mancha rendilhada



- Lesões superficiais sem borda definida e textura lisa.
- Aparece quando os frutos estão ainda verdes
- Variante do sintoma de falsa melanose

Diagrama de fluxo dos procedimentos para a identificação de *Phyllosticta citricarpa*

(OEPP/EPPO, 2020; EPP/EPPO Bulletin 50 (3):440-461)



[1] The molecular tests have been validated on fruits not on other plant material
[2] Morphological identification is difficult but may allow the distinction of *P. citricarpa* or *P. paracitricarpa* from other *Phyllosticta* species. However, sequencing is needed to distinguish *P. citricarpa* from *P. paracitricarpa*
[3] To exclude *P. citriasiana*
[4] Depending on internal validation data this step can be omitted

Fig. 1 Flow diagram for the detection and identification of *P. citricarpa*. [Colour figure can be viewed at wileyonlinelibrary.com]

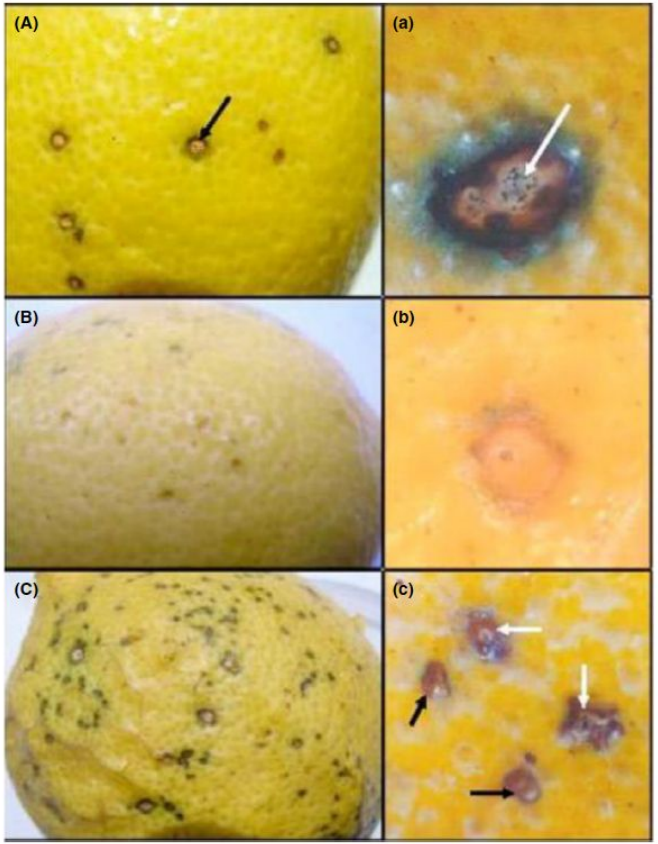


Fig. 2 Hard spot and freckle spot symptoms caused by *P. citricarpa* on sweet orange (*C. sinensis*) and lemon (*C. limon*) fruits: (A) and (a) hard spot lesions on sweet orange (black arrow) with the larger lesions containing pycnidia (white arrow); (B) freckle spot lesions on lemon; (b) freckle spot lesions on sweet orange (the lesions are slightly depressed in the centre and devoid of pycnidia); (C) hard and freckle spot lesions on lemon; (c) freckle spot lesions (black arrows) and intermediate stage between freckle and hard spot lesions with pycnidia (white arrows) on sweet orange. Photographs courtesy of E. Feichtenberger, Instituto Biológico, Sorocaba, Brazil.

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OEPP/EPPO, 2020; EPP/EPPO Bulletin 50 (3):440-461

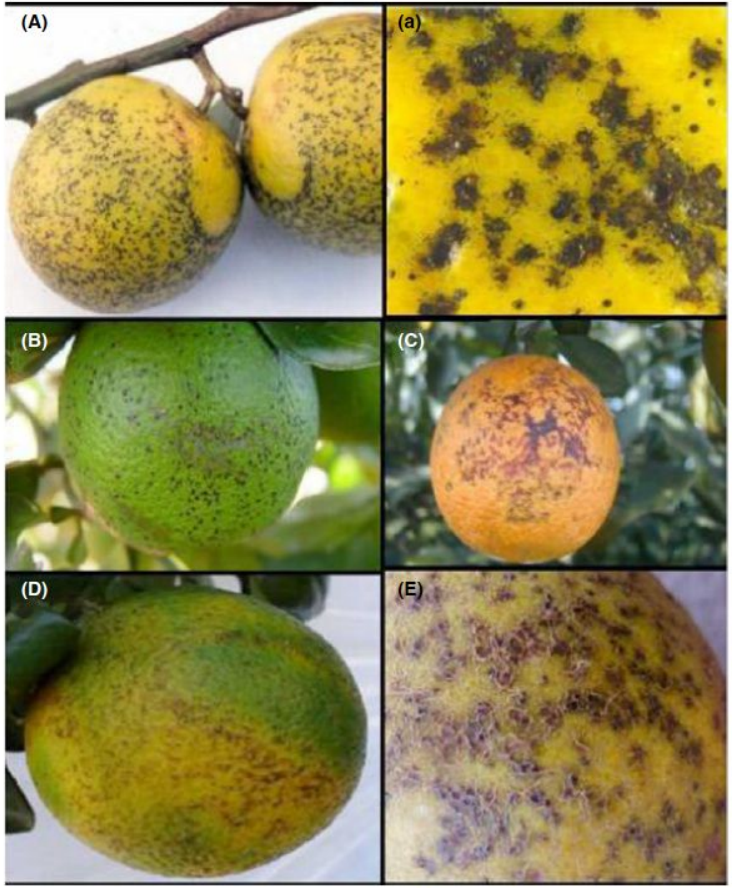


Fig. 3 False melanose, virulent spot, lacy spot and cracked spot symptoms caused by *P. citricarpa* on sweet orange (*C. sinensis*) and lemon (*C. limon*) fruits: (A) false melanose lesions on mature sweet orange; (a) false melanose lesions surrounded by dark specks on mature sweet orange; (B) false melanose lesions on a green sweet orange; (C) virulent spot lesions on sweet orange (the lesions are depressed and extend deeply into the albedo); (D) lacy spot symptoms on a green sweet orange; (E) cracked spot lesions on sweet orange (the lesions are slightly raised, cracked with irregular margins and devoid of pycnidia). Photographs courtesy of FUNDECITRUS (A, B, C, D, E) and E. Feichtenberger, Instituto Biológico, Sorocaba, Brazil (a).

OEPP/EPPO, 2020; EPP/EPPO Bulletin 50 (3):440-461

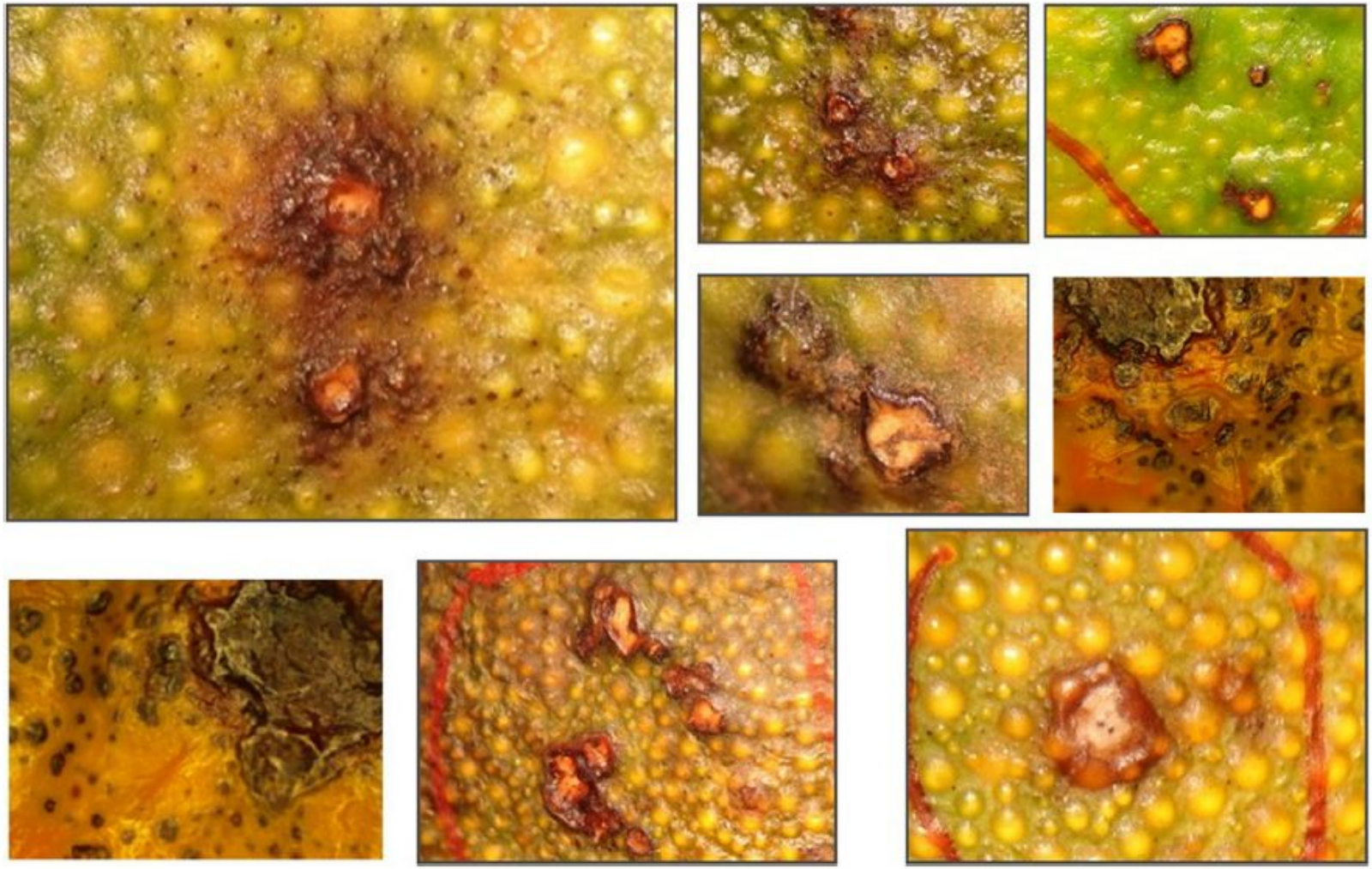


Fig. 4 Constellation symptoms on tangerine hybrid fruits. Photographs courtesy of Instituto Nacional de Tecnología Agropecuaria Argentina.

OEPP/EPPO, 2020; EPP/EPPO Bulletin 50 (3):440-461

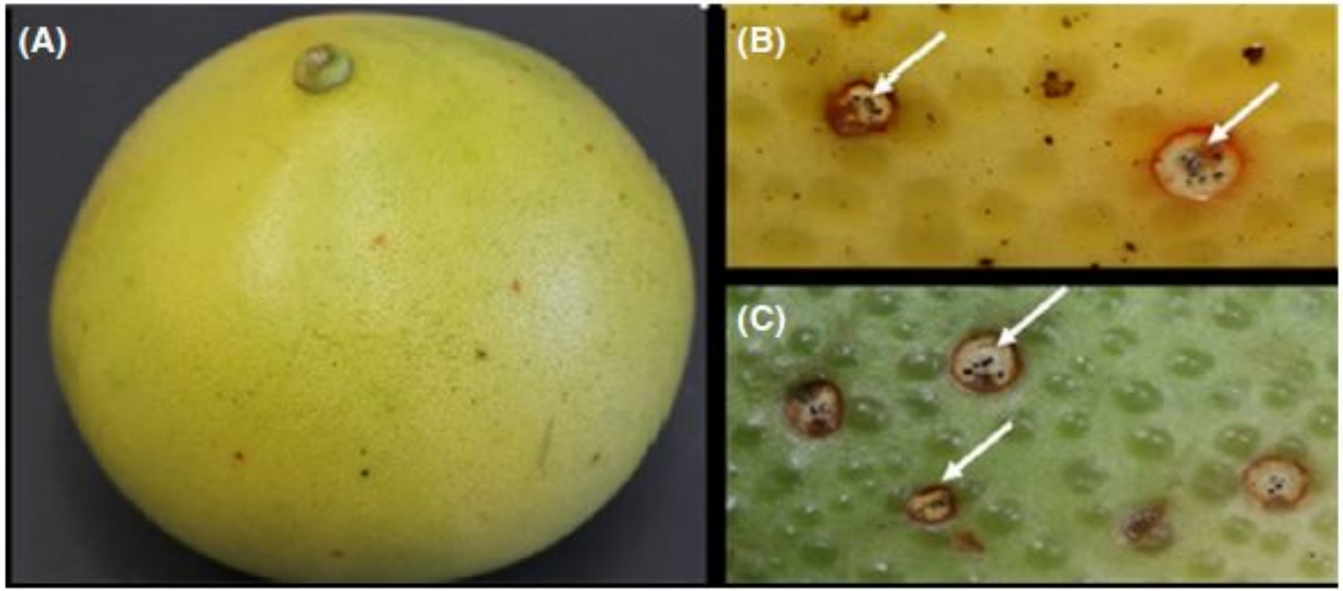


Fig. 5 Tan spot disease caused by *P. citriasiana* on pomelo (*C. maxima*). (A)–(C) Symptoms with lesions containing pycnidia (arrows).

OEPP/EPPO, 2020; EPP/EPPO Bulletin 50 (3):440-461



Fig. 6 Symptoms of citrus black spot caused by *P. citricarpa* on lemon (*C. limon*) leaves. Photographs courtesy of E. Feichtenberger, Instituto Biológico, Sorocaba, Brazil (A) and M. Truter, Plant Protection Research Institute, Agricultural Research Council, Pretoria, South Africa (B).

OEPP/EPPO, 2020; EPP/EPPO Bulletin 50 (3):440-461



Fig. 7 Symptoms of citrus black spot caused by *P. citricarpa* on lemon (*C. limon*) twigs.

OEPP/EPPO, 2020; EPP/EPPO Bulletin 50 (3):440-461

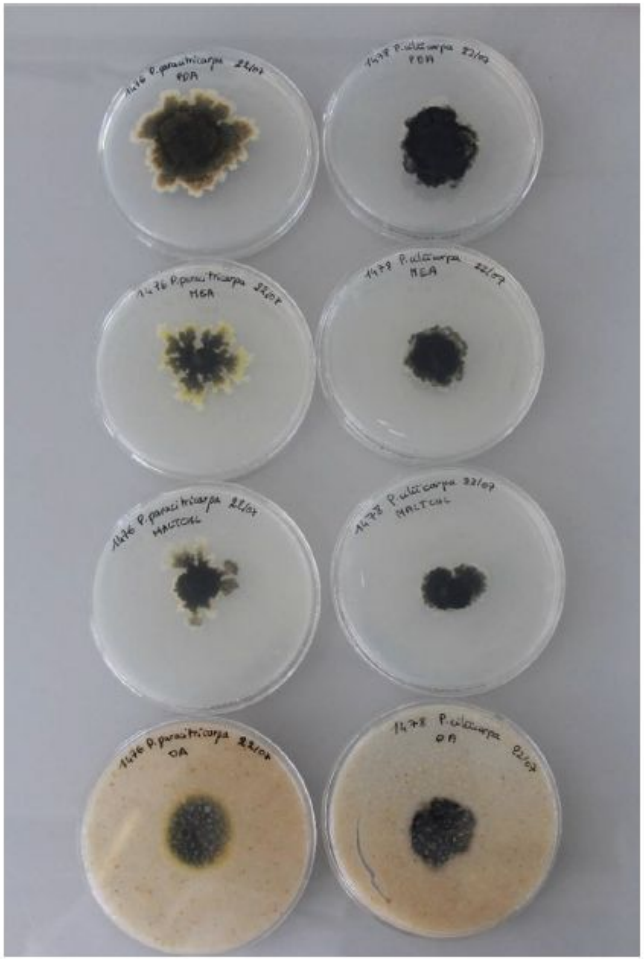


Fig. 8 Cultures of *P. paracitricarpa* (left) and *P. citricarpa* (right) on (from top to bottom) PDA, MEA, MALTCHL and OA (9 days at 26°C 12 h light). Photograph courtesy of J. Hubert, ANSES.

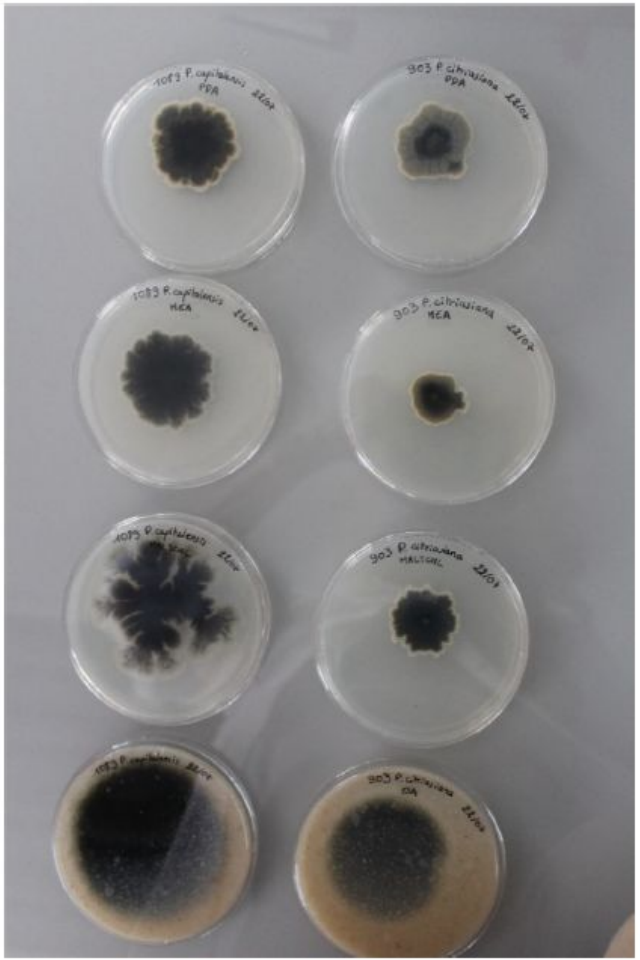


Fig. 9 Cultures of *P. capitalensis* (left) and *P. citriasiana* (right) on (from top to bottom) PDA, MEA, MALTCHL and OA (9 days at 26°C, 12 h light). Photograph courtesy of J. Hubert, ANSES.

OEPP/EPPO, 2020; EPP/EPPO Bulletin 50 (3):440-461

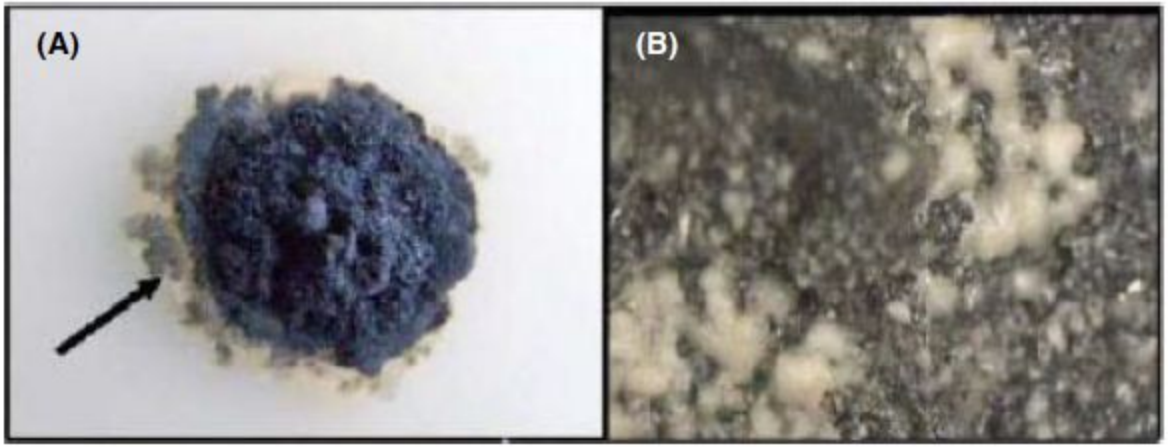


Fig. 10 Colony characteristics of *P. citricarpa*: (A) colony with irregular margin surrounded by a translucent zone of colourless submerged mycelium (arrow) after 30 days of growth on potato dextrose agar at 25°C and a 12 h photoperiod; (B) conidial slime oozing from mature pycnidia. Photographs courtesy of L.E. Diaz, Ministry of Husbandry, Agriculture and Fisheries, Montevideo, Uruguay.

OEPP/EPPO, 2020; EPP/EPPO Bulletin 50 (3):440-461

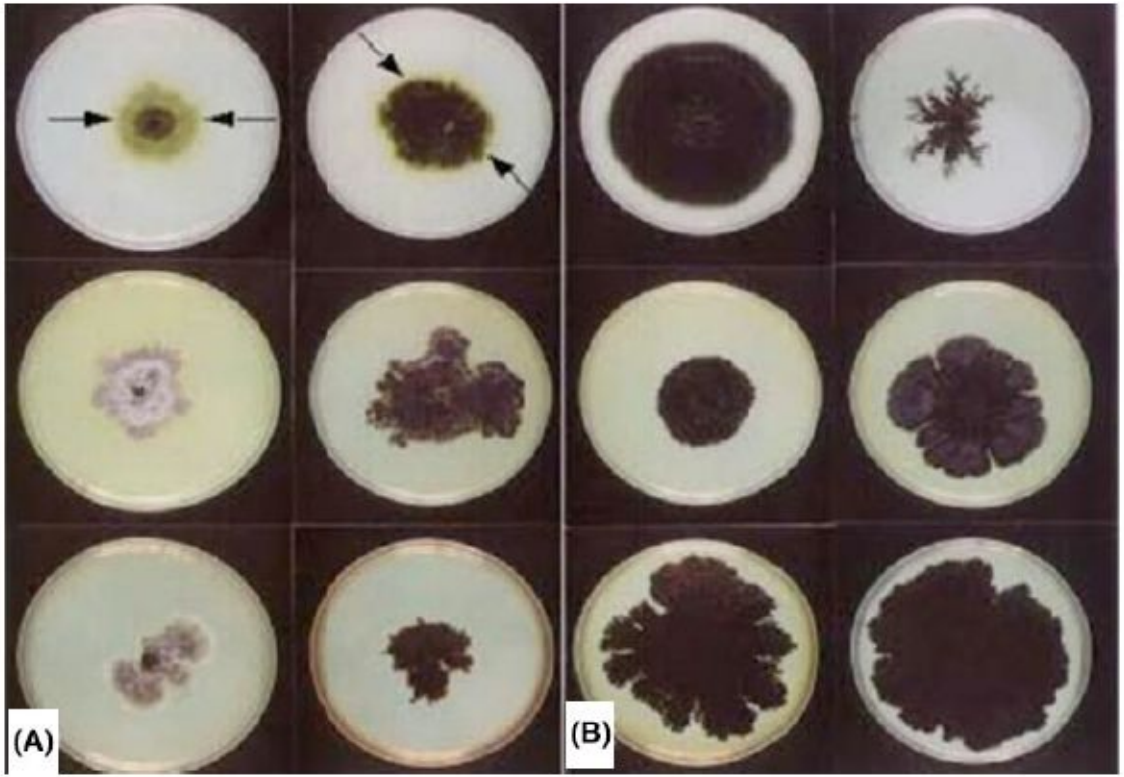


Fig. 11 Cultural characteristics of *P. citricarpa* and *P. capitalensis*: colonies of *P. citricarpa* (A) and *P. capitalensis* (B) after 7 days of growth on oatmeal agar (top row), malt extract agar (middle row) and cherry decoction agar (bottom row) [note that some, but not all strains of *P. citricarpa* form a yellow pigment around the colony on oatmeal agar (A, arrows) and the absence of this pigment in cultures of *P. capitalensis* grown on the same medium (B)]. Photographs courtesy of W. van Lienden, National Plant Protection Service, Wageningen, the Netherlands.

OEPP/EPPO, 2020; EPP/EPPO Bulletin 50 (3):440-461

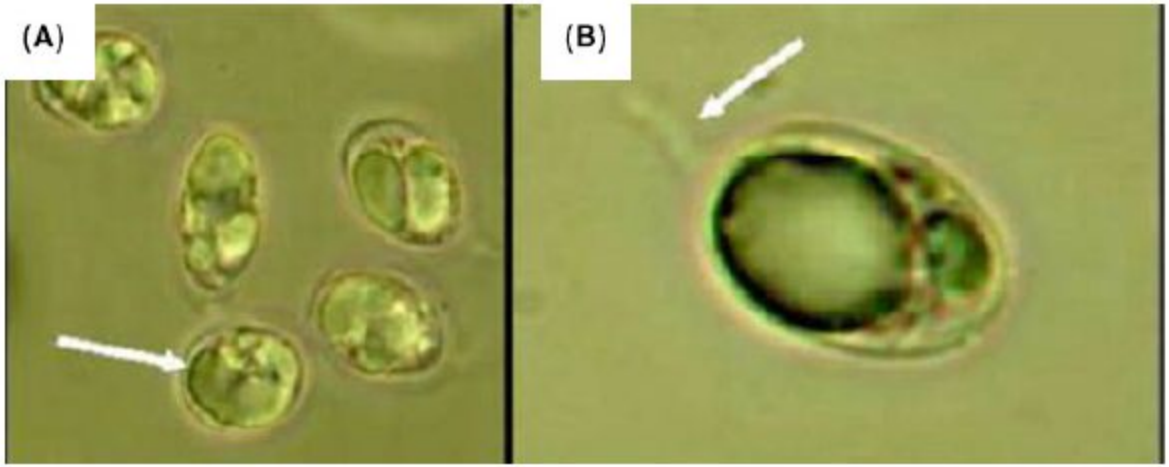


Fig. 12 Conidial morphology of *P. citricarpa*: (A) and (B) conidia with a thin mucoid sheath (A, arrow) and a colourless subulate appendage (B, arrow, magnification 1000 × with immersion oil). Photographs courtesy of L.E. Diaz, Ministry of Husbandry, Agriculture and Fisheries, Montevideo, Uruguay.

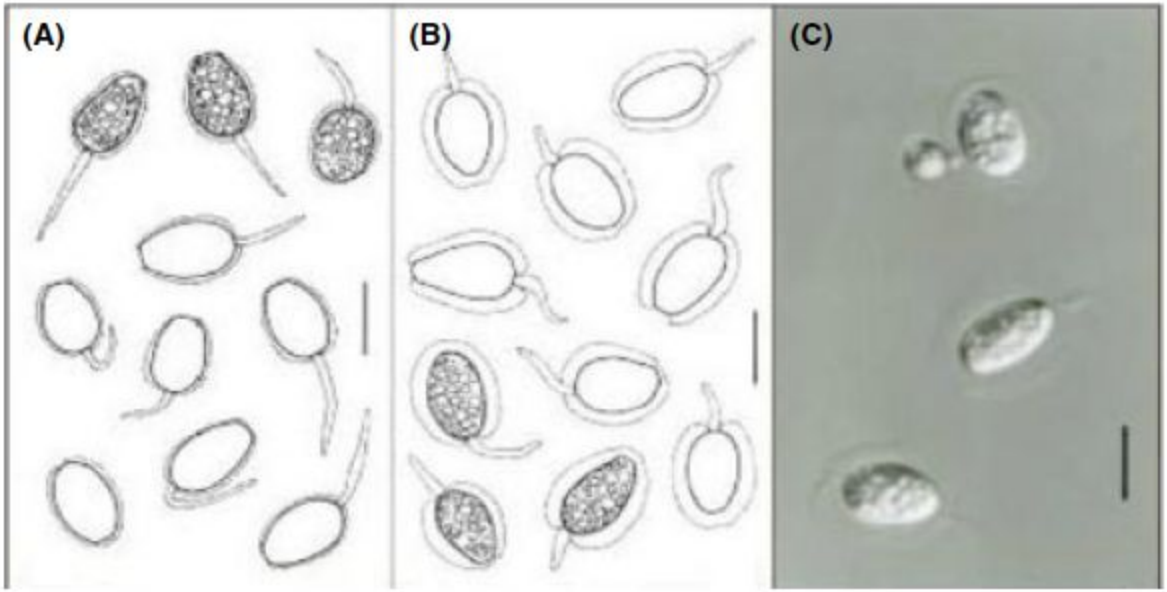
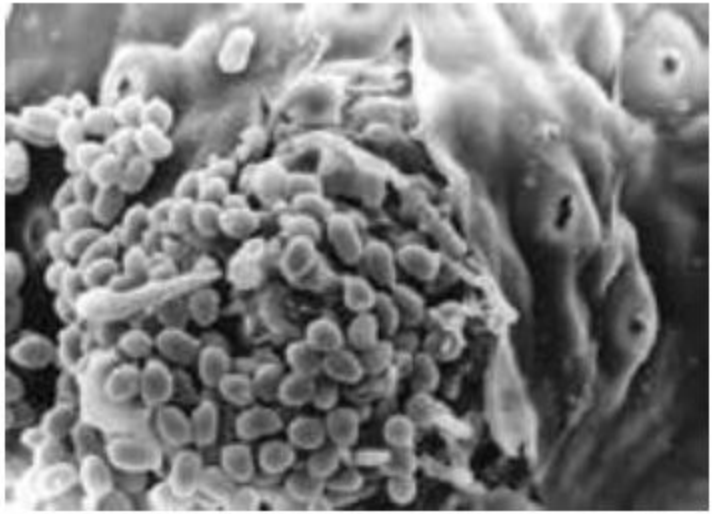


Fig. 13 Conidial morphology of *P. citricarpa* and *P. capitalensis*: (A) conidia of *P. citricarpa* with thin (<1.5 µm) mucoid sheaths; (B) and (C) conidia of *P. capitalensis* with thick (>1.5 µm) mucoid sheaths (scale bar = 10 µm) (photograph C was taken under a light microscope equipped with differential interference contrast). Photographs courtesy of G. Verkley, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands.





Créditos: Eduardo Feichtenberger, Ricardo B. Baldassari e Silvia A. Lourenço

À esquerda, ascas contendo ascósporos de *Phyllosticta citricarpa*. À direita, conídios de *Phyllosticta citricarpa* expostos, após o esmagamento de picnídios.

CARACTERIZAÇÃO MORFOFISIOLÓGICA E MOLECULAR DE ISOLADOS DE *Guignardia citricarpa,* agente patogênico da Mancha Preta dos Citros (MPC).

Marilda Pereira Caixeta

Fevereiro - 2006

Mestrado PGA

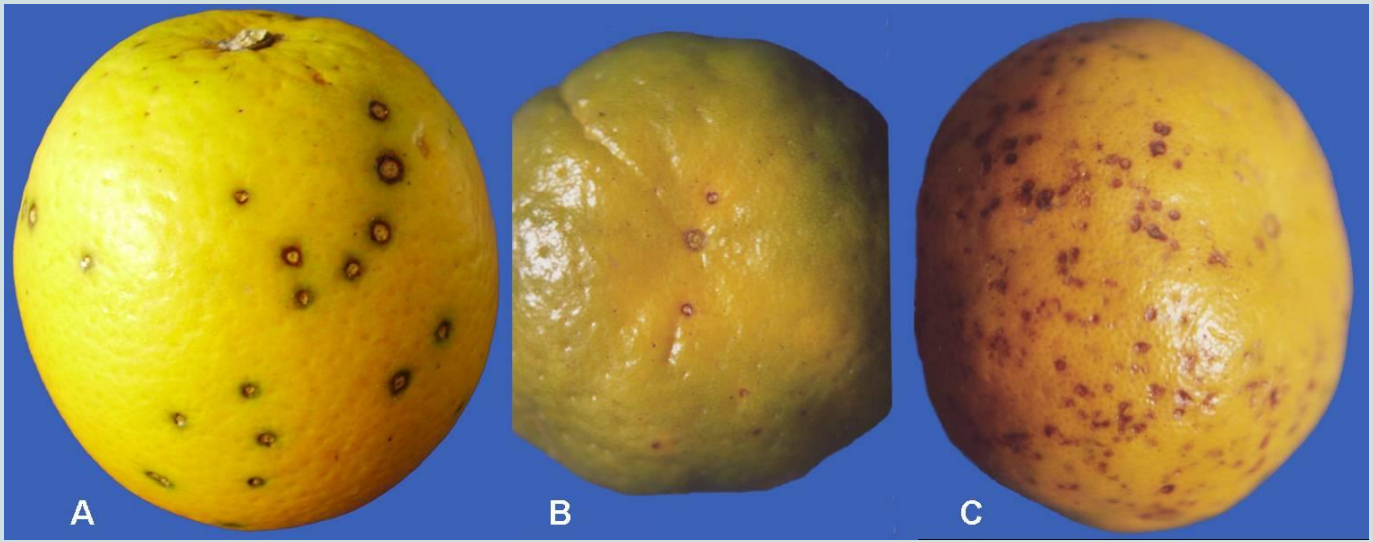
MATERIAL E MÉTODOS

✦ **Local:**

✦**Isolados:**

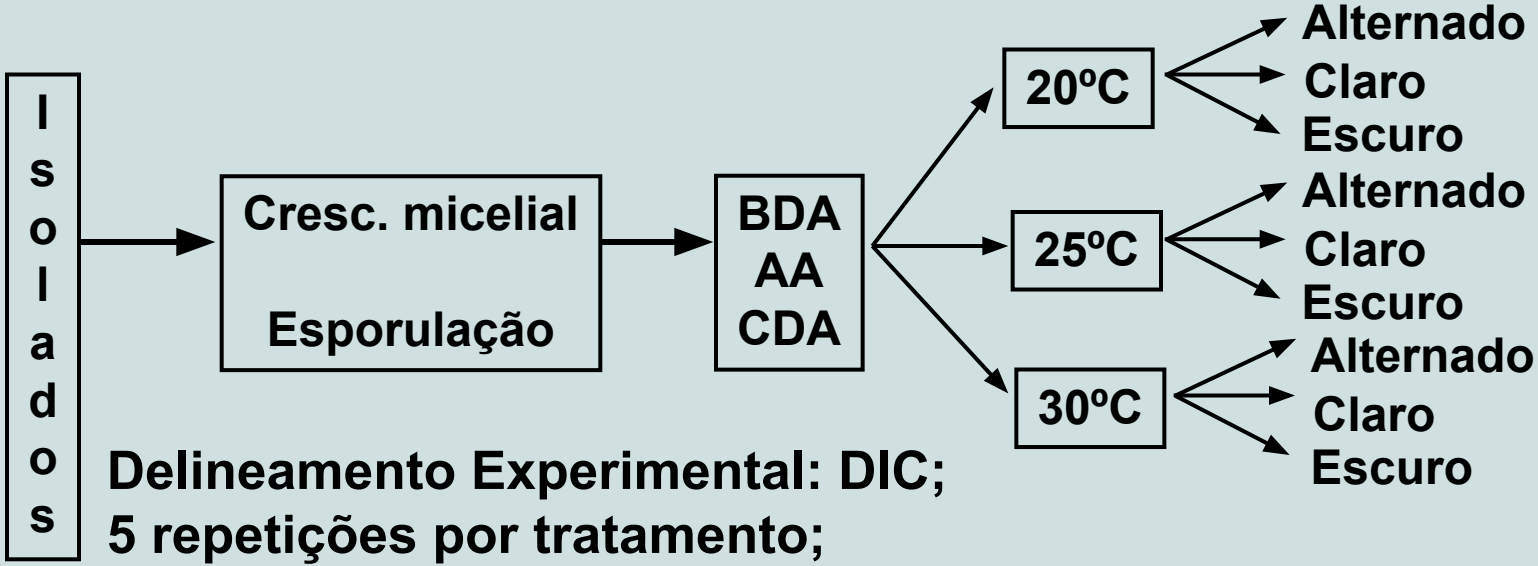
Entidade	Nº de isolados
UFPR	5
ESALQ	3
UNESP	6
UEM	4

ISOLAMENTO



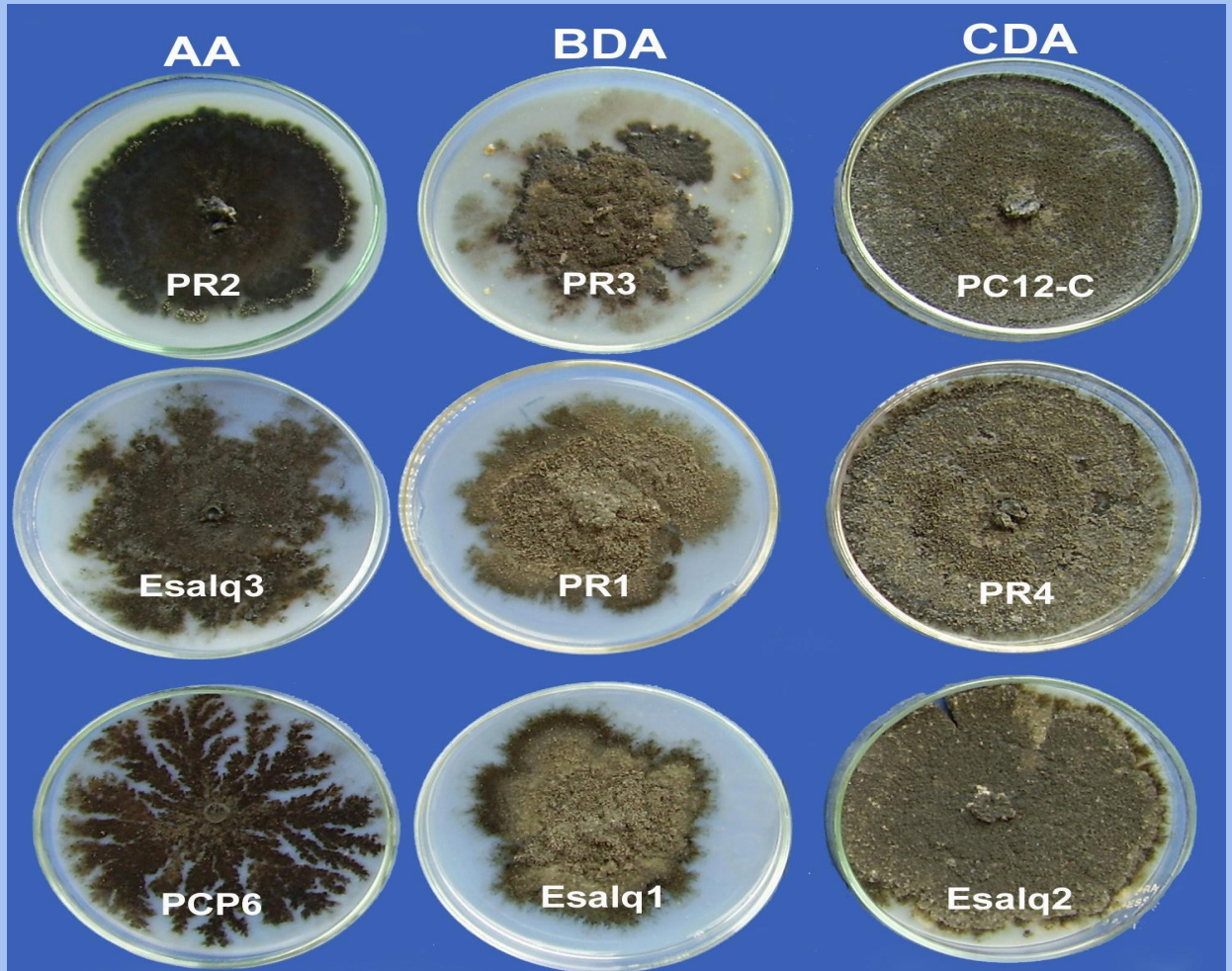
A- Laranja ‘Pêra’;
B- Tangerina ‘Ponkan’;
C- Tangerina ‘Montenegrina’.

CARACTERÍSTICAS ESTUDADAS

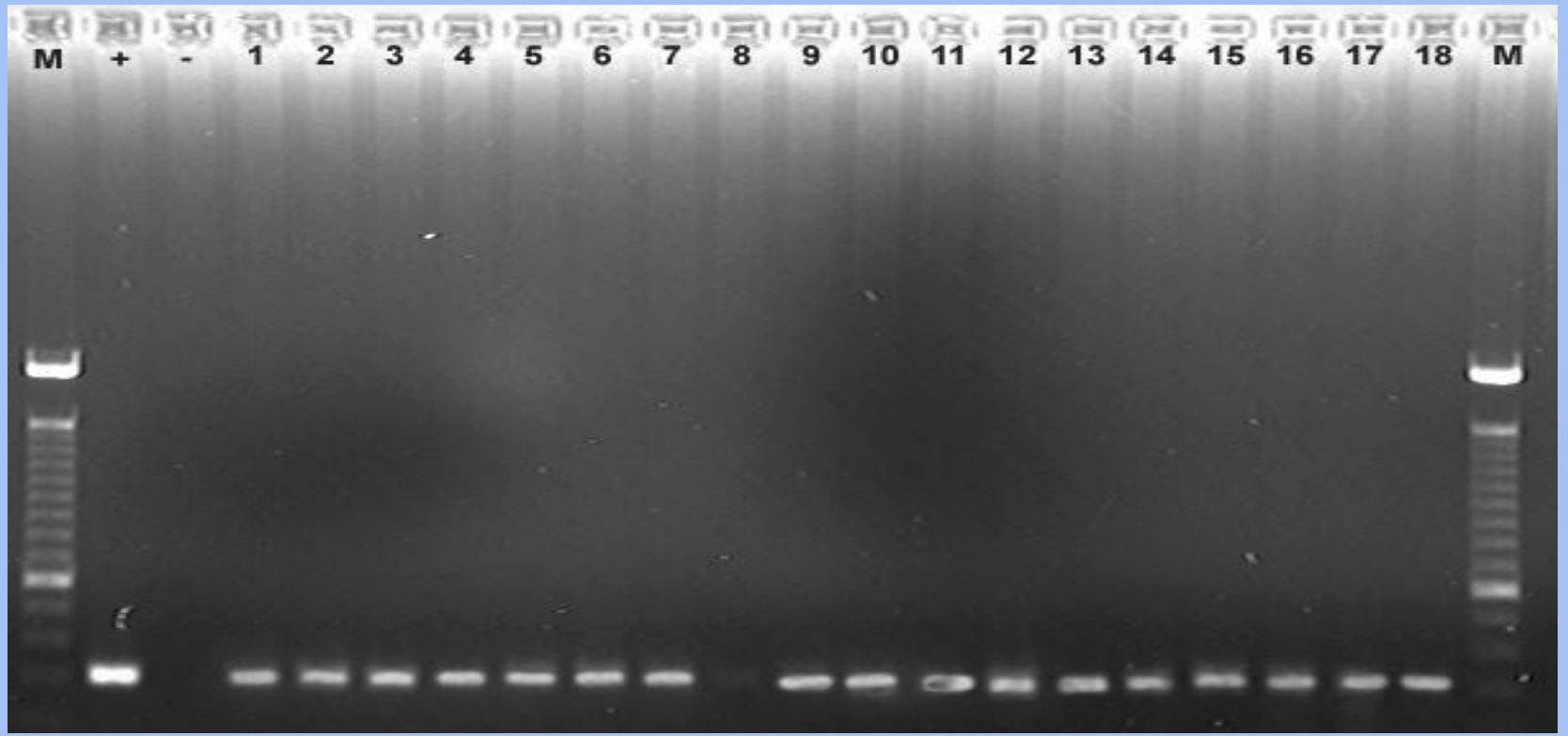


Delineamento Experimental: DIC;
5 repetições por tratamento;
Fatorial 3 x 3 x 3.

Padrões de crescimento micelial apresentado pelos isolados



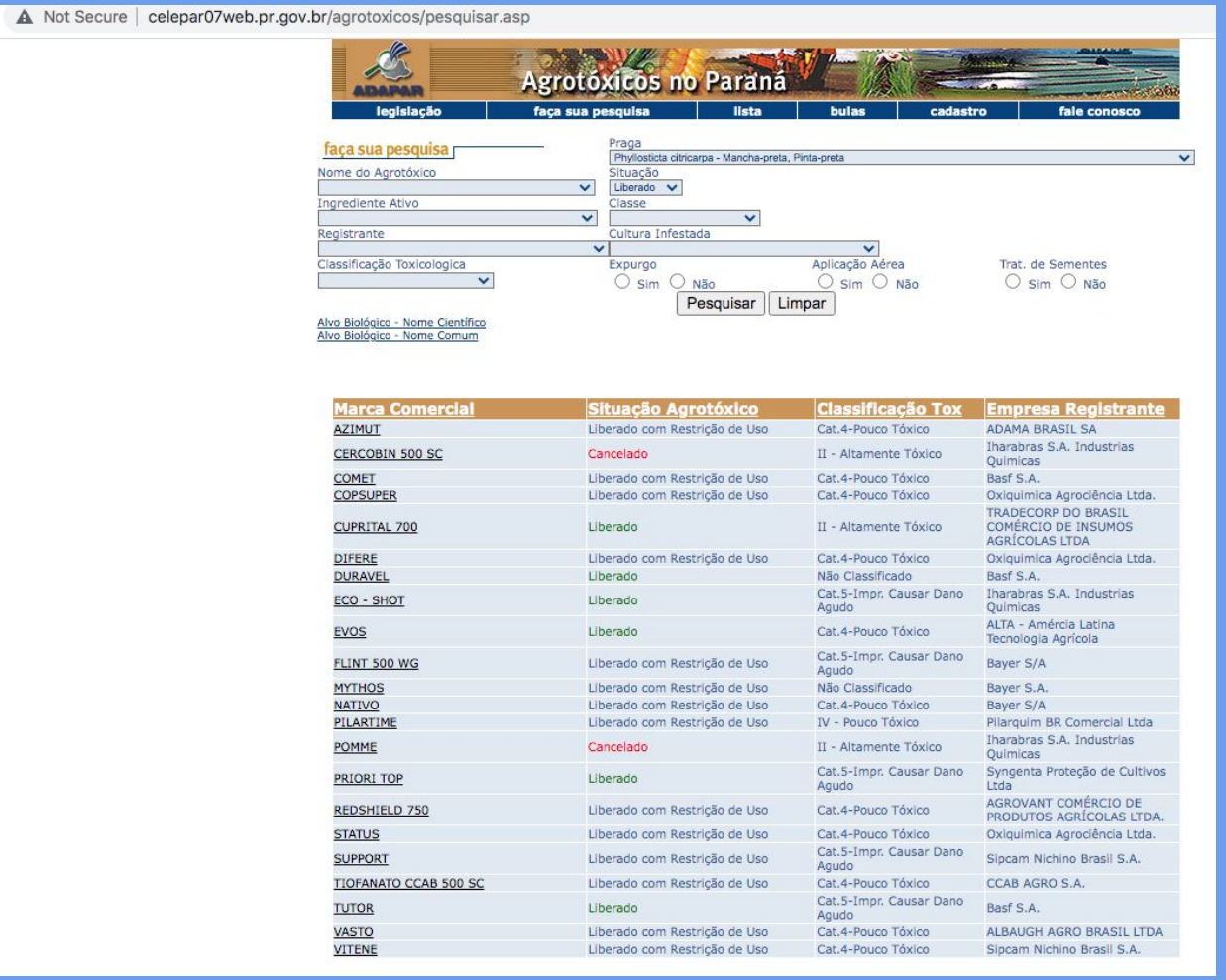
PERFIL ELETROFORÉTICO DOS PRODUTOS AMPLIFICADOS POR PCR DAS AMOSTRAS DOS 18 ISOLADOS











Marca Comercial	Situação Agrotóxico	Classificação Tox	Empresa Registrante
AZIMUT	Liberado com Restrição de Uso	Cat.4-Pouco Tóxico	ADAMA BRASIL SA
CERCOBIN 500 SC	Cancelado	II - Altamente Tóxico	Iharabras S.A. Industrias Quimicas
COMET	Liberado com Restrição de Uso	Cat.4-Pouco Tóxico	Basf S.A.
COPSUPER	Liberado com Restrição de Uso	Cat.4-Pouco Tóxico	Oxiquimica Agrociência Ltda.
CUPRITAL 700	Liberado	II - Altamente Tóxico	TRADECORP DO BRASIL COMÉRCIO DE INSUMOS AGRÍCOLAS LTDA
DIFERE	Liberado com Restrição de Uso	Cat.4-Pouco Tóxico	Oxiquimica Agrociência Ltda.
DURAVEL	Liberado	Não Classificado	Basf S.A.
ECO - SHOT	Liberado	Cat.5-Impr. Causar Dano Agudo	Iharabras S.A. Industrias Quimicas
EVOS	Liberado	Cat.4-Pouco Tóxico	ALTA - Américia Latina Tecnologia Agrícola
FLINT 500 WG	Liberado com Restrição de Uso	Cat.5-Impr. Causar Dano Agudo	Bayer S/A
MYTHOS	Liberado com Restrição de Uso	Não Classificado	Bayer S.A.
NATIVO	Liberado com Restrição de Uso	Cat.4-Pouco Tóxico	Bayer S/A
PILARTIME	Liberado com Restrição de Uso	IV - Pouco Tóxico	Pilarquim BR Comercial Ltda
POMME	Cancelado	II - Altamente Tóxico	Iharabras S.A. Industrias Quimicas
PRIORI TOP	Liberado	Cat.5-Impr. Causar Dano Agudo	Syngenta Proteção de Cultivos Ltda
REDSHIELD 750	Liberado com Restrição de Uso	Cat.4-Pouco Tóxico	AGROVANT COMÉRCIO DE PRODUTOS AGRÍCOLAS LTDA.
STATUS	Liberado com Restrição de Uso	Cat.4-Pouco Tóxico	Oxiquimica Agrociência Ltda.
SUPPORT	Liberado com Restrição de Uso	Cat.5-Impr. Causar Dano Agudo	Sipcam Nichino Brasil S.A.
TIOFANATO CCAB 500 SC	Liberado com Restrição de Uso	Cat.4-Pouco Tóxico	CCAB AGRO S.A.
TUTOR	Liberado	Cat.5-Impr. Causar Dano Agudo	Basf S.A.
VASTO	Liberado com Restrição de Uso	Cat.4-Pouco Tóxico	ALBAUGH AGRO BRASIL LTDA
VITENE	Liberado com Restrição de Uso	Cat.4-Pouco Tóxico	Sipcam Nichino Brasil S.A.

Pesquisa Agrotóxicos

Plantas hospedeiras *Phyllosticta citricarpa*

EPPO Global Database

Search by name or EPPO code. Go!

advanced search...

Login

Register

Home | Standards | Photos | Reporting Service | Explore by

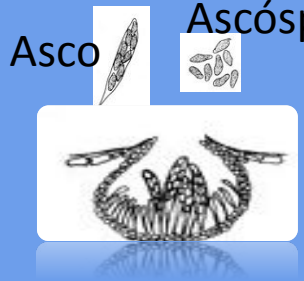
Phyllosticta citricarpa (GUIGCI)

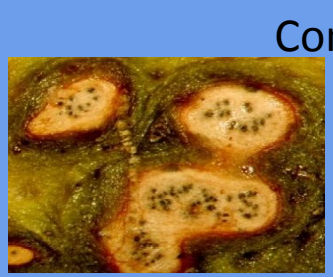
https://gd.eppo.int/taxon/GUIGCI/hosts

Citrofortunella microcarpa (CJFMI)	Minor
Citroncirus (1CJCG)	Minor
Citrus (1CIDG)	Minor
Citrus aurantiifolia (CIDAF)	Minor
Citrus limon (CIDLI)	Major
Citrus paradisi (CIDPA)	Minor
Citrus reticulata (CIDRE)	Minor
Citrus sinensis (CIDSI)	Major
Citrus tankan (CIDTK)	Minor
Citrus x limonia (CIDLO)	Minor
Citrus x nobilis (CIDNO)	Minor
Fortunella (1FOLG)	Minor
Poncirus trifoliata (PMITR)	Minor

	Minor
	Minor
	Minor
	Minor
	Major
	Minor
	Minor
	Major
	Minor
	Minor
	Minor
	Minor
	Minor

Biologia de *Phyllosticta citricarpa*



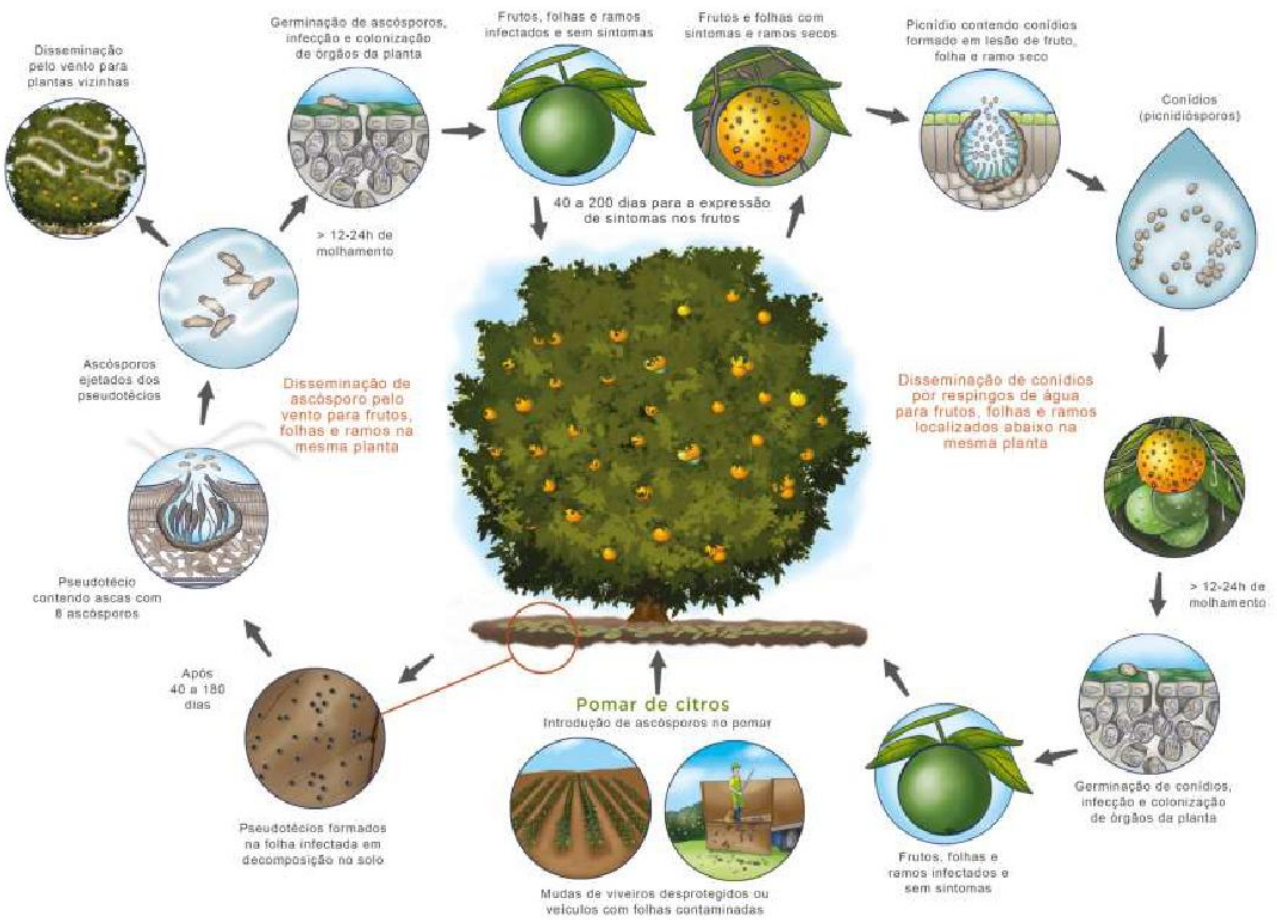




- **Fase sexuada**
- Formada em folhas em decomposição no solo
- Ascósporos = inóculo para o ciclo primário
- Disseminados pelo vento, alcançando longas distâncias
- **Fase assexuada**
- Formada em lesões em ramos, pedúnculo e frutos
- Conídios = inóculo do(s) ciclo(s) secundário(s) da doença.
- Disseminados a curtas distâncias pela ação da água

✔ Infecção com longo período de latência na planta (6 a 8 meses), terminando com a amadurecimento dos frutos (Goes, 1998)

Ciclo da Doença



Condições favoráveis para a ocorrência da Pinta Preta

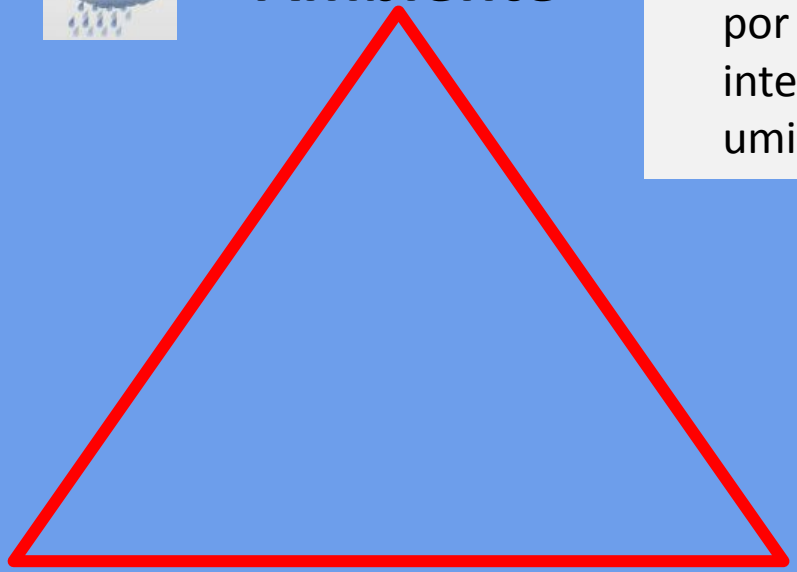


Ambiente

- ✔ A doença é favorecida por altas temperaturas , intensa radiação solar e umidade elevada.







Hospedeiro

Citrus spp.

Patógeno

Conídios



Ascósporos



Disseminação da Pinta Preta

- Mudas infectadas
- Restos de material vegetal
- Conídios e ascósporos

Manejo da Pinta Preta

- **Áreas em que a doença não está presente**

Medidas legislativas de exclusão

Princípio da Exclusão: *não deixar o patógeno entrar em áreas livres da doença*









Phyllosticta citricarpa

✔ Medidas de prevenção: mudas sadias, desinfestação de equipamentos, etc.

Manejo da Pinta Preta

- Áreas de ocorrência da doença
  - Remoção e queima de órgãos infectados das plantas (redução de fontes de inóculo).
  - Manejo da vegetação verde nas ruas de plantio
    -> formação de *mulching* sobre folhas caídas no solo.
  - Uso de decompositores de folhas caídas no solo (uréia)

Manejo da Pinta Preta

- Áreas de ocorrência da doença
  - Eliminação de plantas em estado avançado da doença.
  - Manutenção das plantas em boas condições de nutrição e sanidade.
  - Controle químico

Manejo da Pinta Preta

Controle químico – início após a queda das pétalas

a) **Fungicidas protetores** – cúpricos

b) **Fungicidas sistêmicos** - fungicidas registrados no PR (http://celepar07web.pr.gov.br/agrotoxicos/):

- azoxistrobina (estrobilurina) + difenoconazole (triazol)

✔ Mistura de fungicidas com óleo mineral ou vegetal a 0,25%.

Literatura

apsjournals.apsnet.org/doi/10.1094/PHYTO-05-20-0203-R

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Population Biology

Spatial and Temporal Genetic Analyses of *Phyllosticta citricarpa* in Two Lemon Orchards in South Africa Reveal a Role of Asexual Reproduction Within Sexually Reproducing Populations

Elma Carstens, Celeste C. Linde, Paul H. Fourie, Aletta E. Bester-van der Merwe, Shaun D. Langenhoven, and Adéle McLeod

Affiliations

Published Online: 9 Sep 2021 | https://doi.org/10.1094/PHYTO-05-20-0203-R

FULL TEXT PDF PDF PLUS e-Xtra TOOLS SHARE

Abstract

Citrus black spot (CBS), caused by *Phyllosticta citricarpa*, is a disease that affects citrus worldwide. In different regions of the world where both mating types occur, reports differ as to whether asexually produced pycnidiospores play an important role in the epidemiology of CBS and fruit infections. Therefore, we investigated the potential role of pycnidiospores in two lemon orchards in South Africa by using microsatellite-based analysis of fruit populations over time (two seasons) and space (distance). The two orchards were situated in the semiarid North West province (NW) and subtropical Mpumalanga province (MP). Each population contained both mating types in 1:1 ratios, and linkage disequilibrium analysis indicated a random mating population. A total of 109 and 94 multilocus genotypes (MLGs) were detected across the two seasons in the NW and MP orchards, respectively. P_{sex} analyses indicated that most MLGs probably resulted from sexual reproduction, but there were six predominant MLGs in each orchard that were probably replicated via asexual reproduction. Each of the predominant MLGs was monomorphic for mating type. In the NW, five predominant and widespread MLGs caused 46 and 44% of the fruit infections in the two seasons, whereas in MP, three MLGs caused 34 and 48% of the infections. Asexual reproduction in both orchards was supported by low MLG evenness values in all populations. In both orchards, distance was not a reliable predictor of population genetic substructuring or season. Populations of *P. citricarpa* in the MP and NW orchards were significantly genetically differentiated from each other.

Carstens_phyto-05-20-0203-r.pdf

Summa Phytopathol., Botucatu, v. 32, n. 3, p.295, 2006

2006_Guignardia_Summa.pdf

Ocorrência de pinta preta, causada por *Guignardia citricarpa*, em tangerineiras 'Montenegrina' no sul do Paraná

William Mário Carvalho Nunes, José Croce Filho, José Junior Severino, Carlos Alexandre Zanutto, Dauri José Tessmann, Rudimar Maficioli, Maria Júlia Corazza-Nunes & João Batista Vida.

Núcleo de Pesquisa em Biotecnologia Aplicada, Universidade Estadual de Maringá, Av. Colombo, 5790. CEP 87020-900, Maringá, PR, e-mail: wmcnunes@uem.br

Data de chegada: 08/11/2004. Aceito para publicação em: 16/08/2005.

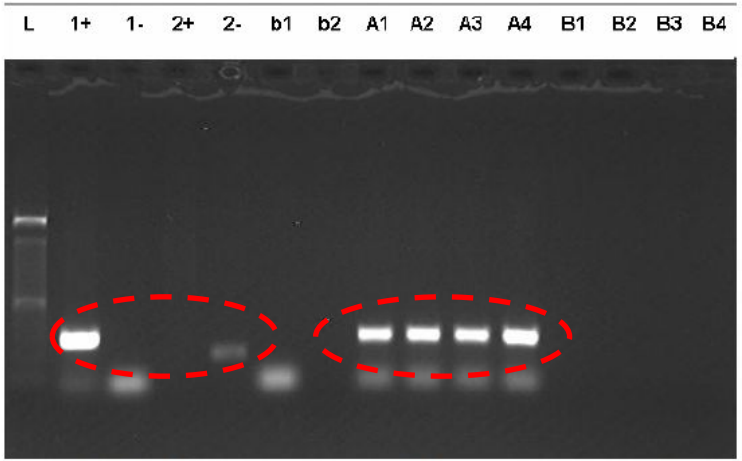


Figura 1. Gel de agarose com o resultado da reação de PCR onde L=marcador molecular 100 pb; 1+= controle positivo (primer op08 e op09); 1-= controle negativo (primer op08 e op09); 2+= controle positivo (primer GCF2 e GCR4); 2-= controle negativo (primer GCF2 e GCR4); b1 e b2= brancos; A1, A2, A3 e A4 = amostras (primer op08 e op09); B1, B2, B3 e B4 = amostras (primer GCF2 e GCR4).



E-book_PintaZPreta_ok.pdf

Bulletin OEPP/EPPO Bulletin (2020) **50** (3), 440–461 ISSN 0250-8052. DOI: 10.1111/epp.12700

Check for updates

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/017(3)

Diagnostics

PM 7/017 (3) *Phyllosticta citricarpa* (formerly *Guignardia citricarpa*)

pm7-017-3-en.pdf

Specific scope

This Standard describes a diagnostic protocol for *Phyllosticta citricarpa*.[1]

This Standard should be used in conjunction with PM 7/76 *Use of EPPO diagnostic protocols*.

Specific approval and amendment

First approved in 2002–09. First revision 2009–09. Second revision 2020–07.

This revision was initially prepared taking into account the IPPC Diagnostic Protocol adopted in 2014 (Appendix 5 to ISPM 27, *Phyllosticta citricarpa* (McAlpine) van der Aa on fruit). However, the EPPO Diagnostic Standard includes other tests developed after the adoption of the IPPC diagnostic protocol and covers additional matrices than fruits. It also includes new species described since the adoption of the IPPC protocol.

DOI: 10.4025/actasciagron.v30i5.5962

Caracterização morfofisiológica e identificação molecular de isolados de *Guignardia citricarpa,* agente patogênico da mancha preta dos citros

Marilda Pereira Caixeta[1], Maria Júlia Corazza[1], Ricardo Ribeiro de Oliveira[2], Carlos Alexandre Zanutto[1], William Mário de Carvalho Nunes[1*] e João Batista Vida[2]

*[1]Núcleo de Pesquisa em Biotecnologia Aplicada, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brasil. [2]Departamento de Agronomia, Universidade Estadual de Maringá, Maringá, Paraná, Brasil. *Autor para correspondência. E-mail: wmcnunes@uem.br*

RESUMO. O presente trabalho teve como objetivo identificar 11 isolados de *Guignardia citricarpa,* agente causal da mancha preta dos citros (MPC), obtidos de frutas cítricas sintomáticas de diferentes regiões geográficas, por meio da PCR e caracterização morfofisiológica das estruturas propagativas, esporulação e crescimento micelial em diferentes meios de cultura, temperaturas e regimes de luz, nas condições de laboratório. Pelo teste de PCR, todos os isolados foram identificados como o patógeno *G. citricarpa.* Os isolados caracterizados foram submetidos às temperaturas de 20, 25 e 30°C, em regime de luz contínua, escuro contínuo e fotoperíodos de 12 horas, durante 24 dias. Utilizaram-se os meios de cultura aveia-ágar (AA), batata-dextrose-ágar (BDA) e cenoura-dextrose-ágar (CDA). Os resultados mostraram que ocorreu interação entre os diferentes meios de cultura, temperaturas e fotoperíodos. O meio de cultura que melhor estimulou o crescimento micelial foi o CDA a 25°C sob o fotoperíodo de 12h. A maior produção de esporos (conídios) foi verificada no meio BDA a 20°C, no fotoperíodo de 12 horas. No meio CDA, não ocorreu esporulação de nenhum isolado. Sob a temperatura de 30°C, foi verificada apenas a produção de hifas e picnídios para a maioria dos isolados, em todos os meios de cultura e fotoperíodo testados.

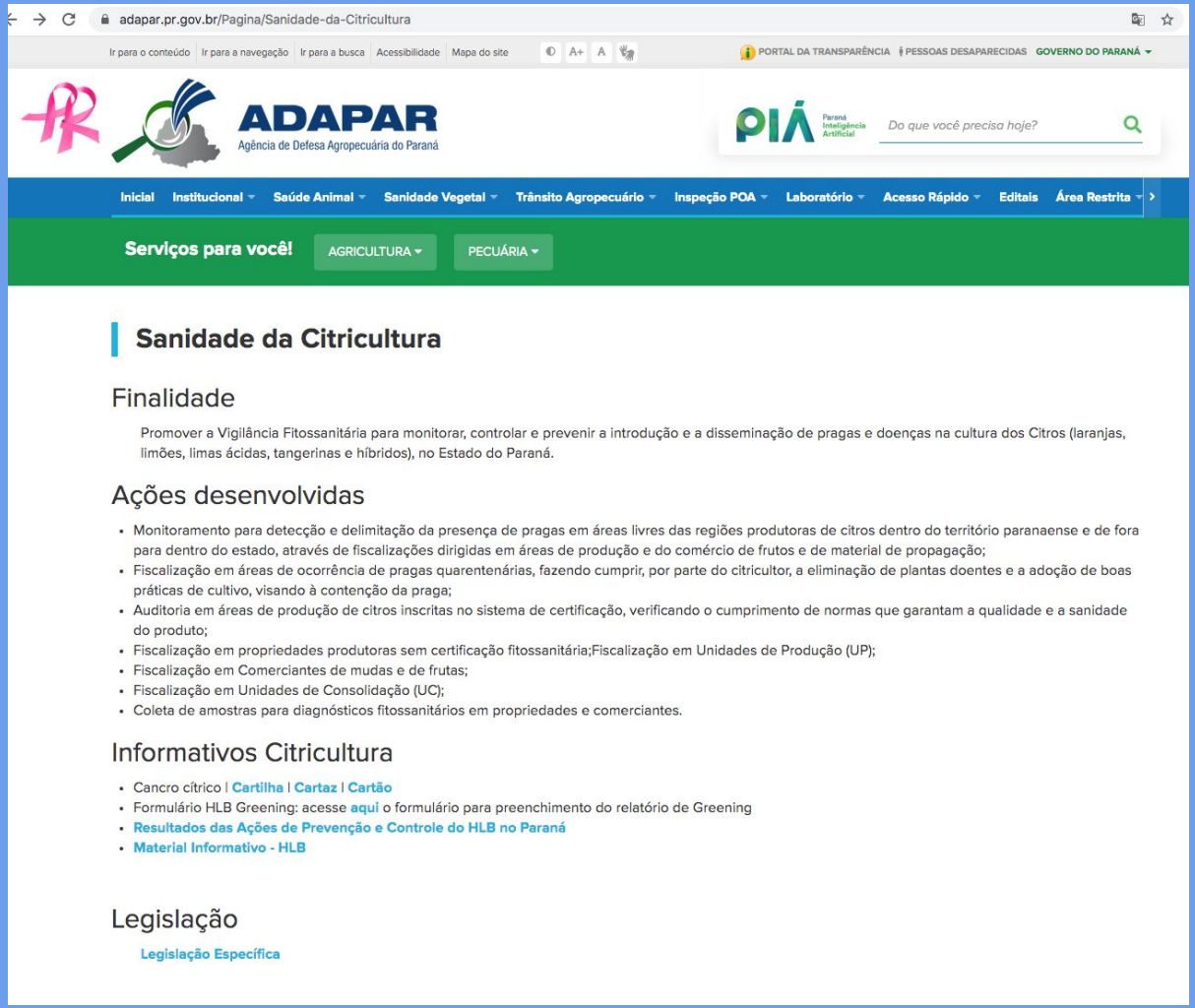
Palavras-chave: *G. citricarpa,* PCR, esporulação, crescimento micelial, temperatura, meio de cultura, fotoperíodo.

2008_Guignardia_Acta.pdf

Legislação Estadual

- **Resolução Estadual Nº 079/90** de 02 de agosto de 1990
  Permite a implantação de citricultura em áreas indenes, desde que saneadas da doença denominada Cancro Cítrico.
- **Resolução Estadual Nº 037/06** de 12 de abril de 2006)
  Proíbe a entrada no Estado do Paraná de plantas e partes de plantas de Murta (*Murraya sp.)*.
- **Resolução Estadual Nº 046/08** de 17 de abril de 2008
  Dispõe sobre o estabelecimento de medidas de defesa sanitária vegetal relativas a produção e a comercialização de mudas cítricas no Estado do Paraná e dá outras providências.
- **Resolução Estadual Nº 059/09** de 22 de abril de 2009
  Dispõe sobre o comércio e o trânsito de frutas cítricas no Estado do Paraná e dá outras providências.
- **Lei Estadual Nº 15.953/08** de 24 de setembro de 2008
  Proíbe o plantio, comércio, transporte e produção da planta Murta (*Murraya paniculata*), por ser vegetal hospedeiro da bactéria *Candidatus liberibacter ssp*., disseminada pelo inseto vetor *Diaphorina citri*, transmissor da praga denominada Huanglongbing (HLB - Greening).
- **resolução estadual nº 047/14** de 17 de junho de 2014
  Revoga a Resolução n° 050, de 30 de Março de 2000 que trata do comércio de frutos cítricos.
- **Portaria Adapar nº 176/15** de 18 de setembro de 2015
  Estabelece as variedades de citros permitidas para produção, comércio e plantio no estado do Paraná, tendo em cista a necessidade de se conter a disseminação da bactéria *Xanthomonas citri* subsp. citri, praga quarentenária A2, agente causal do cancro cítrico.
- **Resolução Estadual Nº 31/18**, de 13 de março de 2018
  Revoga, dentre outras,http:// a Resolução No 059/09, que permitia a comercialização e trânsito de frutas cítricas em território paranaense somente se totalmente desprovidas de ramos e folhas.
- **Portaria Adapar nº 359/19** de 21 de novembro de 2019 **Portaria Nº 139/78** de 31 de agosto de 1978
  Proíbe a venda ambulante de mudas cítricas em todo o território nacional.
- **Portaria Nº 40/09** de 05 de fevereiro de 2009
  Credencia o Laboratório Centro de Diagnóstico "Marcos Enrietti", nome empresarial Secretaria de Estado da Agricultura e do Abastecimento do Paraná.
- **Instrução Normativa Nº 16/03** de 18 de março de 2003
  Proibe a saída de material propagativo de citros (mudas, borbulhas, porta-enxertos), formado ou produzido em viveiros telados e a céu aberto nos Municípios que menciona.
- **Instrução Normativa Nº 20/06** de 31 de julho de 2006
  Adota o Sistema Integrado de Medidas Fitossanitárias para o Manejo de Risco de *Xanthomonas axonopodis* pv.citri em Frutos Cítricos, aprovado pela Resolução GMC nº 48/05, de acordo com o Anexo à presente Instrução Normativa.
- **Instrução Normativa Nº 03/08** de 8 de janeiro de 2008
  Aprova os Critérios e Procedimentos para Aplicação das Medidas Integradas em um Enfoque de Sistemas para o Manejo de Risco - SMR da Praga Mancha Preta ou Pinta Preta dos Citros (MPC) *Guignardia citricarpa* Kiely (*Phyllosticta citricarpa* Van der Aa) em espécies do gênero *Citrus*.
- **Portaria SDA/MAPA n. 317** , de 21 de maio de 2021
  Institui o Programa Nacional de Prevenção e Controle à doença denominada Huanglongbing (HLB) - PNCHLB, cujo agente etiológico é a praga Candidatus Liberibacter spp.
- **Instrução Normativa Nº 01/09** de 05 de janeiro de 2009
  Altera o art. 1º e 4º, da Instrução Normativa nº 03, de 8 de janeiro de 2008.
- **Instrução Normativa Nº 08/12**, de 17 de abril de 2012
  Dispõe dentre outros assuntos sobre a Certificação Fitossanitária de Origem de potenciais hospedeiros do Ácaro Hindu dos Citros *(Schizotetranychus hindustanicus)*.
- **Instrução Normativa Nº 21/18**, de 25 de abril de 2018
  Institui, em todo o território nacional, os critérios e procedimentos para o estabelecimento e manutenção do status fitossanitário relativo à praga denominada Cancro Cítrico *(Xanthomonas citri subsp. citri)*.

https://www.adapar.pr.gov.br/FAQ/Legislacao-da-Sanidade-Vegetal



https://www.adapar.pr.gov.br/Pagina/Sanidade-da-Citricultura

Persoonia 26, 2011: 47–56
www.ingentaconnect.com/content/nhn/pimj

RESEARCH ARTICLE

doi:10.3767/003158511X569169

Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black Spot

C. Glienke[1], O.L. Pereira[2], D. Stringari[1], J. Fabris[1], V. Kava-Cordeiro[1], L. Galli-Terasawa[1], J. Cunnington[3], R.G. Shivas[4], J.Z. Groenewald[5], P.W. Crous[5, 6]

Key words

Guignardia endophyllicola
Guignardia mangiferae
Phyllosticta bifrenariae
Phyllosticta brazilianiae
Phyllosticta capitalensis
Phyllosticta citriasiana
Phyllosticta citribraziliensis
Phyllosticta citricarpa
taxonomy

Abstract We investigated the identity and genetic diversity of more than 100 isolates belonging to *Phyllosticta* (teleomorph *Guignardia*), with particular emphasis on *Phyllosticta citricarpa* and *Guignardia mangiferae* s.l. occurring on *Citrus*. *Phyllosticta citricarpa* is the causal agent of Citrus Black Spot and is subject to phytosanitary legislation in the EU. This species is frequently confused with a taxon generally referred to as *G. mangiferae*, the presumed teleomorph of *P. capitalensis*, which is a non-pathogenic endophyte, commonly isolated from citrus leaves and fruits and a wide range of other hosts. DNA sequence analysis of the nrDNA internal transcribed spacer region (ITS1, 5.8S nrDNA, ITS2) and partial translation elongation factor 1-alpha (TEF1), actin and glyceraldehyde-3-phosphate dehydrogenase (GPDH) genes resolved nine clades correlating to seven known, and two apparently undescribed species. *Phyllosticta citribraziliensis* is newly described as an endophytic species occurring on *Citrus* in Brazil. An epitype is designated for *P. citricarpa* from material newly collected in Australia, which is distinct from *P. citriasiana*, presently only known on *C. maxima* from Asia. *Phyllosticta bifrenariae* is newly described for a species causing leaf and bulb spots on *Bifrenaria harrisoniae* (*Orchidaceae*) in Brazil. It is morphologically distinct from *P. capitalensis*, which was originally described from *Stanhopea* (*Orchidaceae*) in Brazil; an epitype is designated here. *Guignardia mangiferae*, which was originally described from *Mangifera indica* (*Anacardiaceae*) in India, is distinguished from the non-pathogenic endophyte, *P. brazilianiae* sp. nov., which is common on *M. indica* in Brazil. Furthermore, a combined phylogenetic tree revealed the *P. capitalensis* s.l. clade to be genetically distinct from the reference isolate of *G. mangiferae*. Several names are available for this clade, the oldest being *P. capitalensis*. These results suggest that endophytic, non-pathogenic isolates occurring on a wide host range would be more correctly referred to as *P. capitalensis*. However, more genes need to be analysed to fully resolve the morphological variation still observed within this clade.

Article info Received: 31 January 2011; Accepted: 3 March 2011; Published: 22 March 2011.

Chapter 16

Citrus pests in a global world

Alberto Urbaneja[a], Tim G. Grout[b], Santin Gravena[c], Fengnian Wu[d], Yijing Cen[d], Philip A. Stansly[e]
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16.1 Introduction

The agro-ecological citrus system is characterized by richness and abundance of phytophagous species as well as their natural enemies. However, only about 20 of these pests can be considered key, in part due to effective biological control. As early as the fourth century, Chinese citrus growers were known to distribute arboreal nests of the weaver ant, *Oecophylla smaragdina* (F.) (Hymenoptera: Formicidae) to manage pests, such as citrus stink bugs [*Rhynchocoris humeralis* (Thunberg) (Hemiptera: Pentatomidae)], leaf beetles [*Podagricomela nigricollis* Chen (Coleoptera: Chrysomelidae)], weevils [*Hypomeces squamosus* (Fabricius) (Coleoptera: Curculionidae)], and larvae of citrus butterflies and fruit borers (*Anomala cupripes* Hope (Coleoptera: Scarabaeidae)] (Yang, 2002). This practice continued into the 20th century in some parts of Southeast Asia, Burma, and China (Li, 1982). Another early well-known example is the first successful introduction to California of an exotic natural enemy, *Rodolia* (=*Novius*) *cardinalis* (Mulsant) (Coleoptera: Coccinellidae) from Australia to control the cottony cushion scale *Icerya purchasi* (Maskell) (Hemiptera: Monophlebidae) a pest of Asian origin (Fig. 16.1). *R. cardinalis* has now been introduced worldwide with the result that *I. purchasi* is rarely considered more than an occasional pest.

Citrus cultivation has been exported throughout the world's tropics and subtropics from origins in Southeast Asia, unfortunately along with many of its pests but not their natural enemies. This process continues at an accelerated pace in concert with increasing international commerce and travel. Consequently, many citrus pests are now worldwide in distribution and citrus industries have suffered from these and vectored diseases, notably huanglongbing (HLB) and citrus tristeza virus transmitted by psyllids and aphids, respectively. Two other vector and disease combinations: citrus variegated chlorosis (CVC) and citrus leprosis are transmitted by certain Cicadellidae and *Brevipalpus* (Acari: Tenuipalpidae) mites, respectively, and threaten to spread from their origins in South America.

Ever-increasing citrus pest diversity plus evolving pest control methods have resulted in continuously changing management models. The earliest was based on classical biological control, focusing on the introduction and establishment of exotic natural enemies from Southeast Asia to new citrus-growing regions. The next stage following WWII was marked by the growing use of synthetic pesticides capable of controlling principal pests but often without consideration for auxiliary fauna. Secondary pest outbreaks, pesticide resistance, and growing public awareness of health and environmental problems associated with pesticides led to the third stage of integration centered on biological control and selective chemistry. However, the appearance of the insect- and mite-transmitted diseases mentioned above, particularly HLB, has given way to a most recent stage of citrus pest management characterized by a return to intensive pesticide use for vector control.

While globalization and increased international traffic of raw materials have facilitated the distribution of many citrus pests throughout the world, the absence of some of these in specific areas and the influence of different climates and management options vary among zones. In this chapter, citrus pests and their management are detailed for five major citrus-growing areas of the world, Asia, the Mediterranean basin, North America, South America, and Africa. In each of these zones, pests have been classified based on three levels of control: (1) *key pests* for which chemical or biorational intervention is usually necessary, (2) *occasional pests* requiring occasional intervention, and (3) *secondary pests* generally controlled by natural enemies unless upset by the overuse of pesticides.

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Diseases caused by fungi and oomycetes

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17.1 Greasy spot disease of citrus caused by *Zasmidium citri-griseum*

17.1.1 Introduction

Zasmidium citri-griseum (= *Mycosphaerella citri*) belongs to the *Mycosphaerellaceae* family and is the causal agent of the greasy spot (Whiteside, 1970). *Z. citri-griseum* infects citrus inducing leaf spots, premature defoliation, which results in reduced fruit size, fruit drop, and yield loss (Aguilera-Cogley et al., 2017). Rind blotch occurs when the pathogen directly infects the fruit. Greasy spot is widespread in the southeast North America (Florida and Eastern Mexico), Central America, Northern South America, and the islands of the Caribbean (Timmer and Gottwald, 2000). Outside the American continent, Japan, Egypt, and China have also reported greasy spot in citrus to be caused or associated with this same species (Leki, 1986; Haggag, 2012; Huang et al., 2015b). Similar greasy spot-like symptoms have been reported on citrus in other countries, however, this was associated with different *Mycosphaerellaceae* species (Huang et al., 2015b; Aguilera-Cogley et al., 2017).

17.1.2 Disease symptoms

Leaf tissues infected with *Z. citri-griseum* show chlorosis and yellow-brown pustules on the abaxial surface (Fig. 17.1A and B). Fruits infected with *Z. citri-griseum* show a typical rind blemish which significantly reduces the fruit quality for the fresh market (Fig. 17.1C). The pathogen incubation period is long and disease symptoms are only observed after 4–6 months (Mondal and Timmer, 2006). Microscopic observations show that *Z. citri-griseum* exhibits epiphytic growth on the leaf surface (Timmer et al., 2001). Secretion of nutrients or honeydew on the surface of the leaves also maximizes fungus survival (Whiteside, 1974).

17.1.3 Infection process

Z. citri-griseum belong to the *Mycosphaerellaceae* family that are characterized by the formation of pseudothecia that are in contact with the plant tissue. The typically ovoid and bitunicate asci carry ascospores, which also are responsible for the infection (Dewdney and Graham, 2016). Ascospores germinate and form epiphytic mycelia on the leaf surface that penetrate through stomata (natural openings of the abaxial leaf surface). Fungal growth in the leaf tissue is slow and symptoms develop several months later. Decomposing leaf litter in the orchard is the main source of airborne ascospores and primary inoculum (Mondal and Timmer, 2006).

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Chapter 18

Bacterial pathogens of citrus: Citrus canker, citrus variegated chlorosis and Huanglongbing

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18.1 Introduction

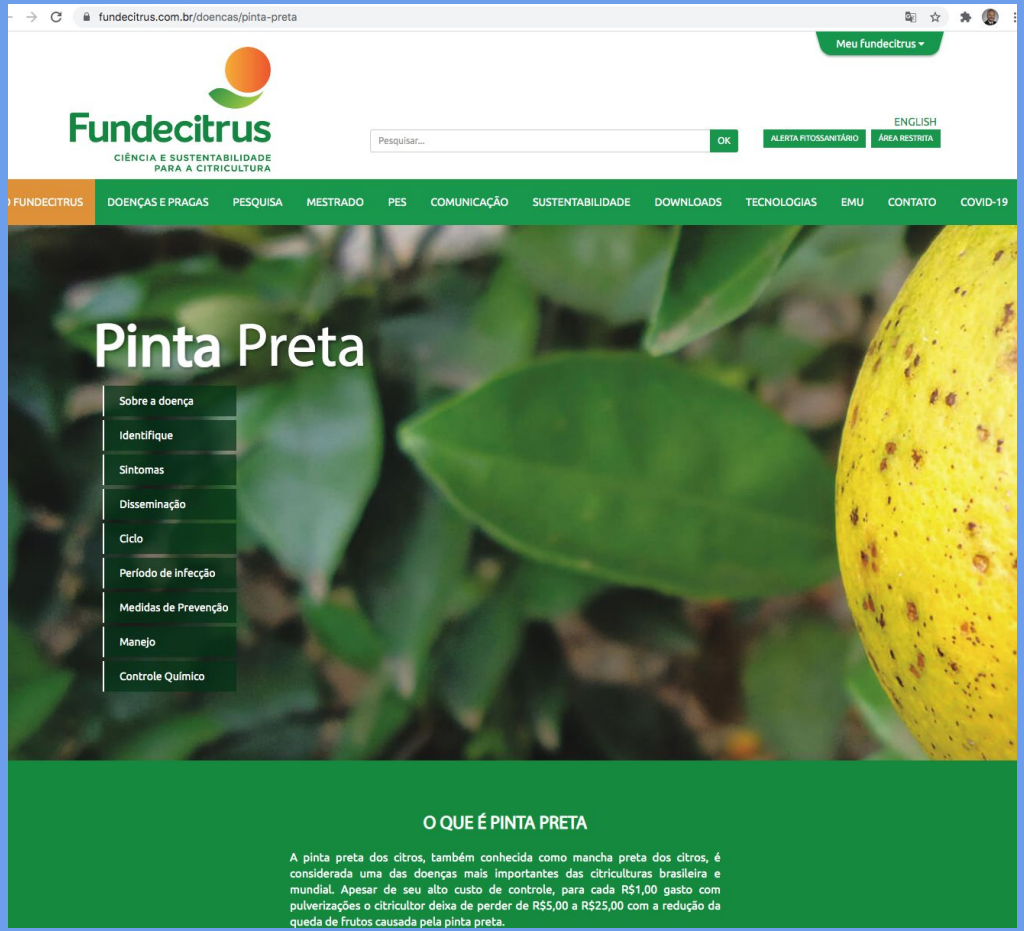
Commercial citrus groves are invariably planted as dense monocultures comprising a few commercial citrus varieties covering large tracts of land, providing little to no natural variation in resistance to any pest/vector, pathogen, or a combination of both with capacity to exploit it. The majority of bacterial pathogens need no insect vector at all, for example, the xanthomonads causing citrus canker, although disease incidence can be dramatically accelerated by leafminers. These xanthomonads enter through stomata or wounds, actively attacking mesophyll cells from the outside and directly affecting citrus cell gene expression by injection of eukaryotic transcription factors. Nevertheless, as discussed in detail below, there is remarkably little variation in resistance among commercially grown citrus genera, species, and varieties to the Asiatic strains of the disease.

Liberibacters are literally injected by their psyllid vectors directly into living phloem cells. Psyllids free of Liberibacters may be introduced into a citrus growing region free of Huanglongbing (HLB), resulting only in the direct damage caused by the psyllid. Independent of vector introduction, Liberibacters may be introduced by planting infected and asymptomatic citrus into a previously uninfected region, but the pathogen cannot spread without an insect vector with host range on citrus. As discussed in some detail below, the excessively long incubation period required before HLB symptoms become recognizable can prevent or frustrate timely detection of such introductions, even with inspections. Epidemics can result from the intersection of these two independent introduction events, as appears to have occurred relatively recently in Florida with HLB. Of course, both events can be concurrent. Again, however, there is remarkably little variation in resistance among commercially grown citrus varieties to Liberibacter infections.

It is useful to distinguish between parasitism and pathogenicity. A parasite is capable of deriving nutrition from, and reproducing on or within, its host(s), and is not always associated with the disease. For example, a Liberibacter-infected pear tree is nearly asymptomatic (Raddadi et al., 2011). A pathogen causes disease, but not always on all hosts. Pathologists typically pay attention only to pathogens on commercially important crops, but pathogens can have a much wider parasitic host range than expected if they cause no disease or cause the disease on weeds. A recurring theme in this chapter involves the worldwide increase in commerce and in transcontinental dispersal of many pathogens and their vectors. The fact that there is often overlap of parasitic, if not pathogenic, host ranges of multiple bacterial species, and the fact that apparent host range limitations of a given pathogen can be disguised by the host range limitations of its insect vector can result in emerging and sometimes surprisingly severe disease threats. In general terms, if a vector is introduced into a geographic region with an unusually wide host range that may include weed or arboreal crops, it is conceivable that the vector may facilitate exposure of previously isolated bacterial populations to one another, thereby facilitating horizontal gene transfer events, either by cell–cell conjugation or by phage transduction within either the insect vector or plant host. Transcontinental dispersal of pathogens and vectors dramatically increases the opportunities for horizontal gene transfer and allows increased recombination frequencies between pathogenic variants of a species and even across genera.

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Site com informações Pinta Preta, Mancha Preta dos Citros, Fungo Guignardia citricarpa



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