

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

2023



ADAPAR
Agência de Defesa Agropecuária do Paraná



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)

***Professor do Programa de Pós-Graduação em Genética e
Melhoramento (PGM)***

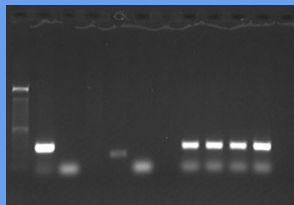
Chefe do Departamento de Agronomia (DAG)



Parte 1

DATA: 25/05/23 (quinta-feira).

08:00 – 09:00 hs - Praga Quarentenária Presente –
Pinta Preta dos Citros (*Phyllosticta citricarpa*)



Phyllosticta citricarpa, agente causal da Pinta Preta dos citros

Praga Quarentenária Presente

INSTRUÇÃO NORMATIVA Nº 38, DE 1º DE OUTUBRO DE 2018

Órgão: Ministério da Agricultura, Pecuária e Abastecimento/Secretaria de Defesa Agropecuária

Pragas Quarentenárias Presentes (PQP)

FUNGOS		
Phyllosticta citricarpa (Guinardia citricarpa)	Citros (Citrus spp.)	Amazonas, Bahia, Espírito Santo, Goiás, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pernambuco, Paraná,
		Rio de Janeiro, Rio Grande do Sul, Santa Catarina e São Paulo

Impacto da Pinta Preta

Porque está na lista das Pragas Quarentenárias Presentes?

- Frutos **impróprios** para o mercado *in natura*.
- **Queda prematura dos frutos**, com redução da produtividade.

Impacto da Pinta Preta

Porque está na lista das Pragas Quarentenárias Presentes?

- 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).*



EPPO
Global
Database

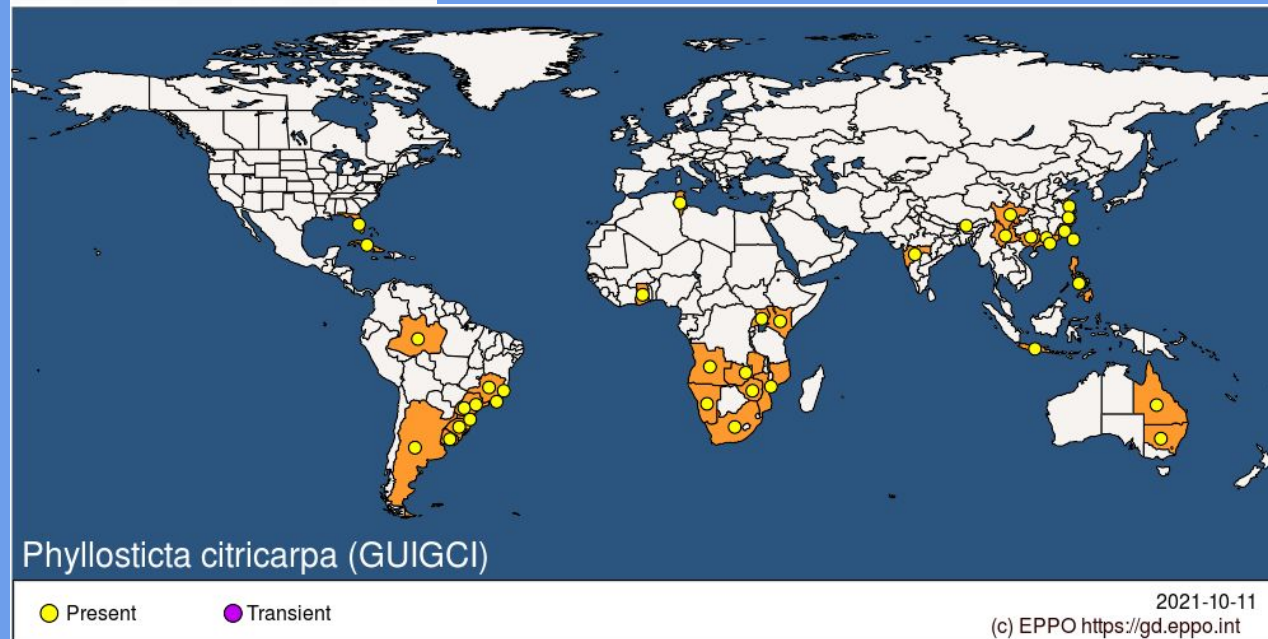
Home

Standards ▾

Photos ▾

Reporting

Phyllosticta citricarpa (GUIGCI)



2021-10-11
(c) EPPO <https://gd.eppo.int>

Ocorrência de Pinta Preta no Mundo

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/as-suntos/sanidade-animal-e-vegetal/sanidade-vegetal/arquivos-quarentena/lista-de-e-pragas-quarentenarias-ausentes-e-presentes.pdf/view>

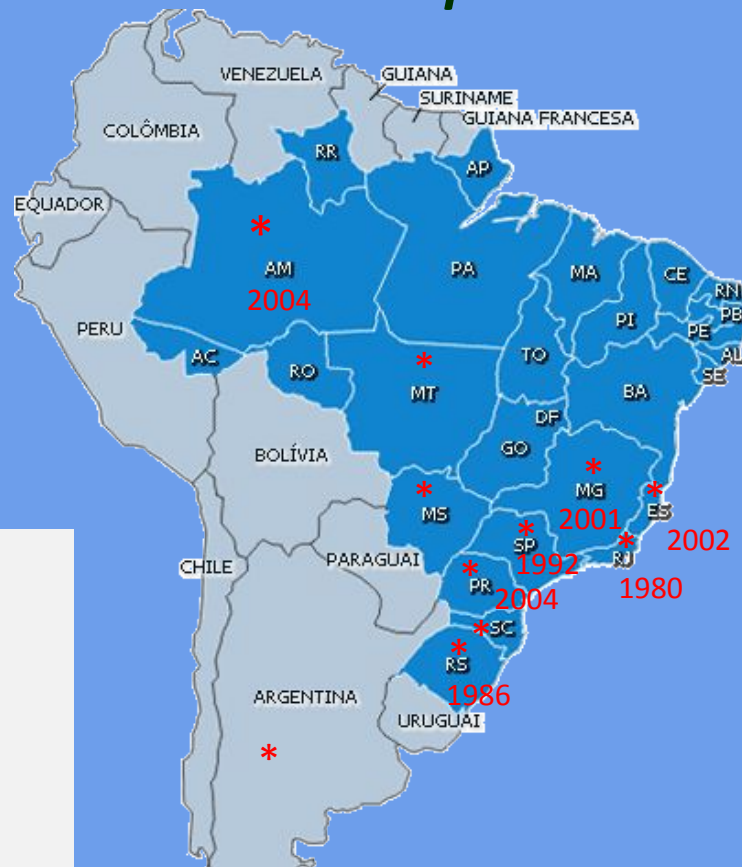
UNIDADES FEDERATIVAS DE OCORRÊNCIA

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)



MAPA, 2023

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

Phyllosticta citricarpa



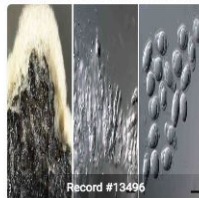
Taxon name Phyllosticta citricarpa
Summary Phyllosticta citricarpa (McAlpine) Aa, Studies in Mycology 5: 40 (1973) [MB#320327]

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, Engeli 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](#)



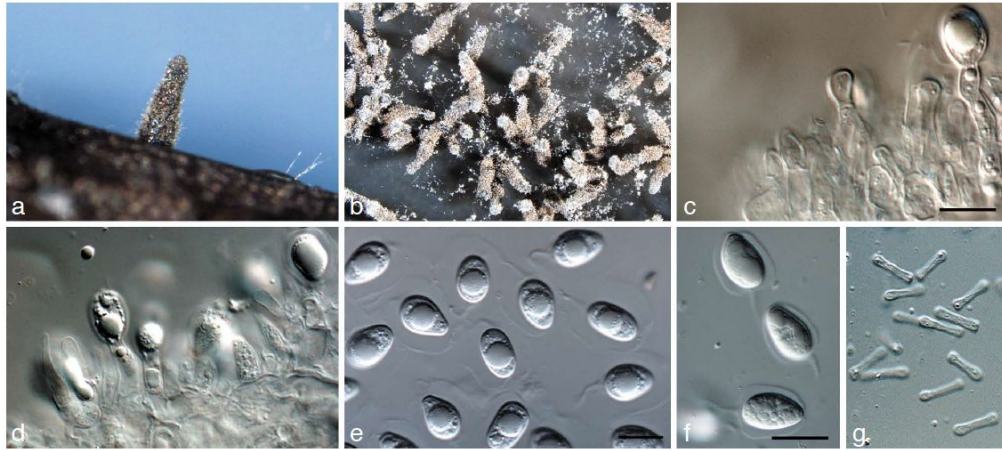


Fig. 2 *Phyllosticta bifrenariae*. a. Pycnidium forming on PNA; b. pycnidia forming on PDA; c, d. conidiophores giving rise to conidia; e, f. conidia; g. spermatia (all: CBS H-20520 holotype). — Scale bars = 10 μ m.

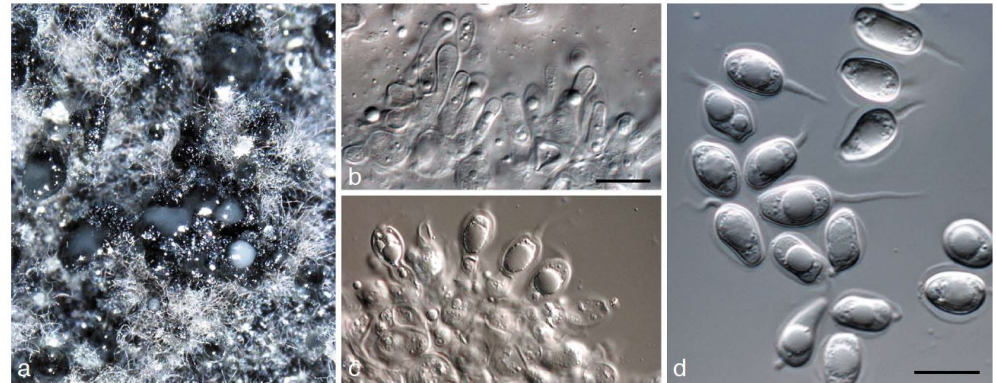


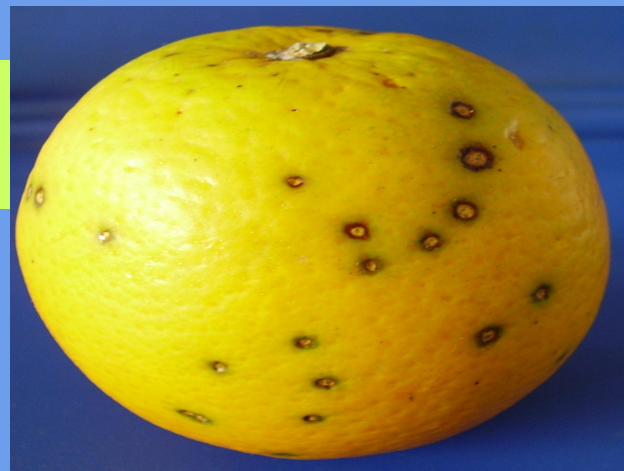
Fig. 3 *Phyllosticta brazilianiae*. a. Pycnidia forming on PDA; b, c. conidiophores giving rise to conidia; d. conidia (all: CBS H-20521 holotype). — Scale bars = 10 μ m.

Sintomas



Fotos: Fundecitros

Sintomas

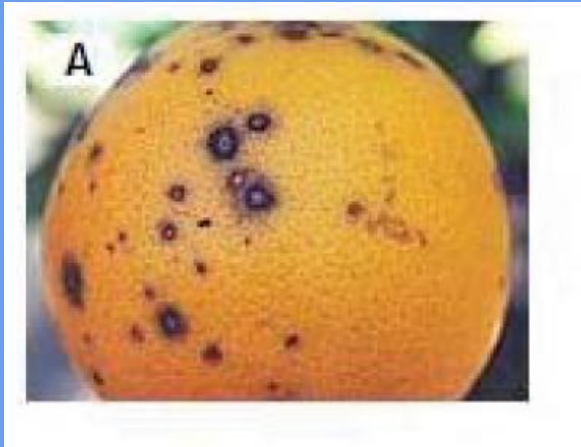


Fotos: NBA

Tipos de Sintomas

(EPPO, 2020)

Mancha dura ou mancha preta

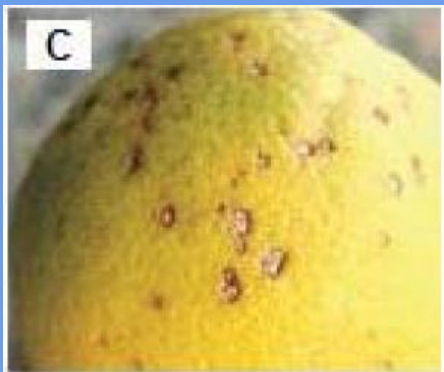


- Sintoma mais comum
- Surgem quando os frutos iniciam a maturação
- Pontuações negras no centro: picnídios

Tipos de Sintomas

(EPPO, 2020)

Manchas sardentas



- Aparecem depois 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

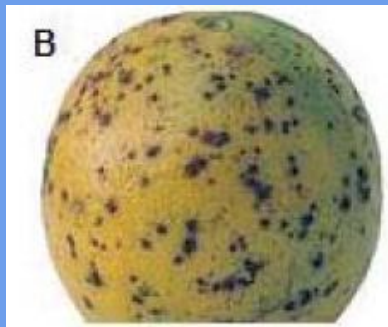


- Desenvolvem-se quando os frutos estão maduros .
- Associados a temperaturas elevadas
- Resultam do desenvolvimento e da coalescência dos dois tipos de sintomas anteriores.

Tipos de Sintomas

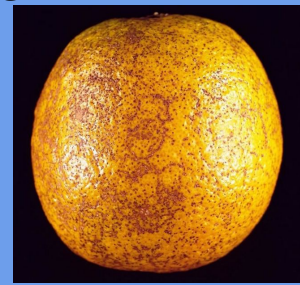
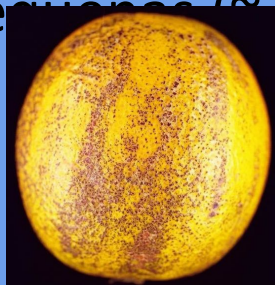
(EPPO, 2020)

Manchas do tipo falsa melanose



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

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 ou fissuras.

Tipos de Sintomas

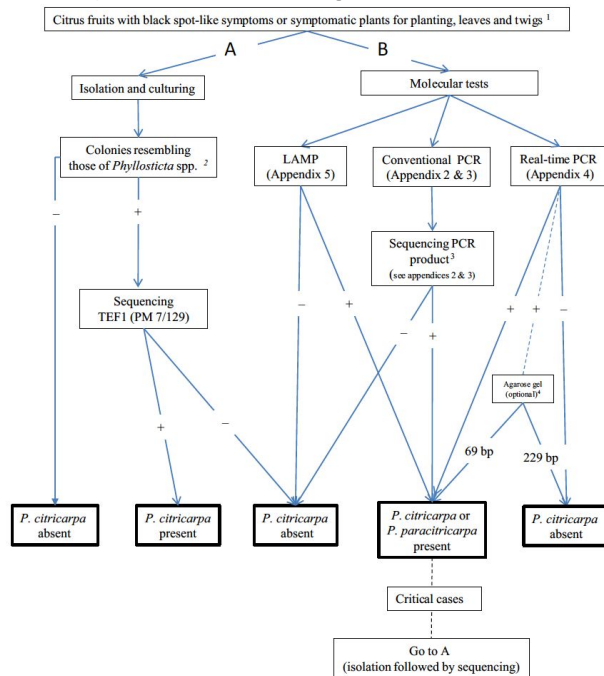
(EPPO, 2020)

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

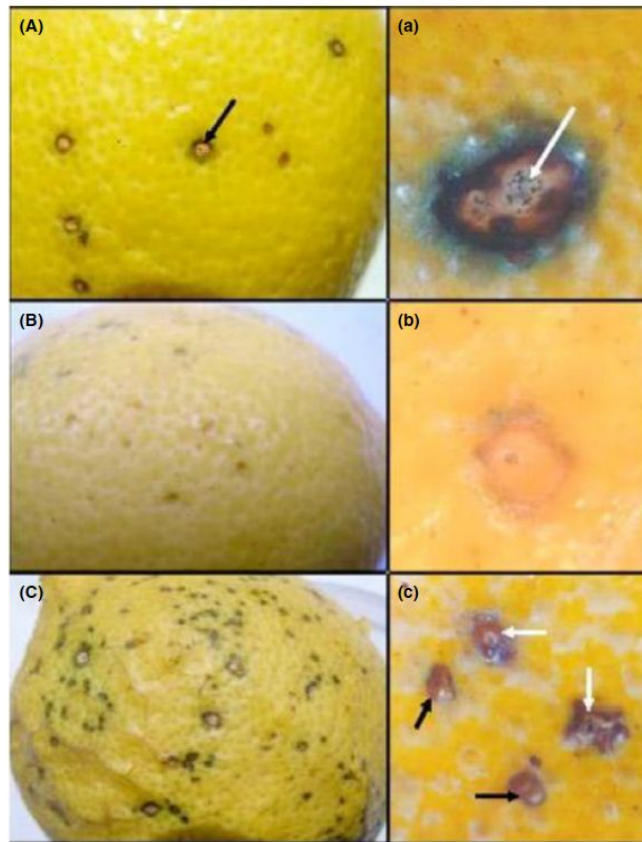


Fig. 2 Hard spot and freckle spot symptoms caused by *P. citricarpa* on sweet orange (*C. sinensis*) and lemon (*C. limon*) fruits: (A) and (a) hard spot lesions on sweet orange (black arrow) with the larger lesions containing pycnidia (white arrow); (B) freckle spot lesions on lemon; (b) freckle spot 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.

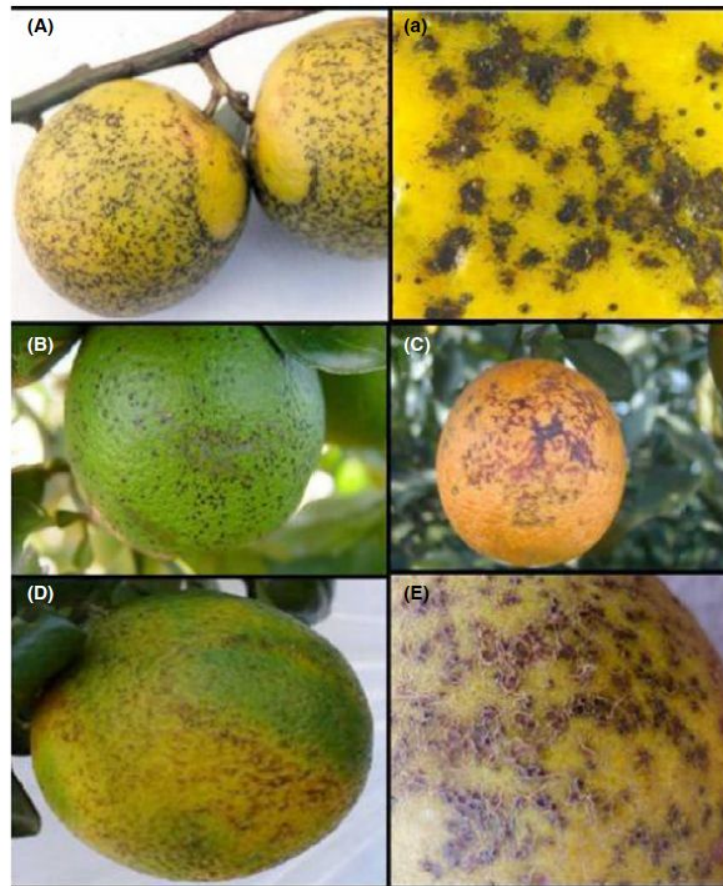


Fig. 3 False melanose, virulent spot, lacy spot and cracked spot symptoms caused by *P. citricarpa* on sweet orange (*C. sinensis*) and lemon (*C. limon*) fruits: (A) false melanose lesions on mature sweet orange; (a) false melanose lesions surrounded by dark specks on mature sweet orange; (B) false 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).

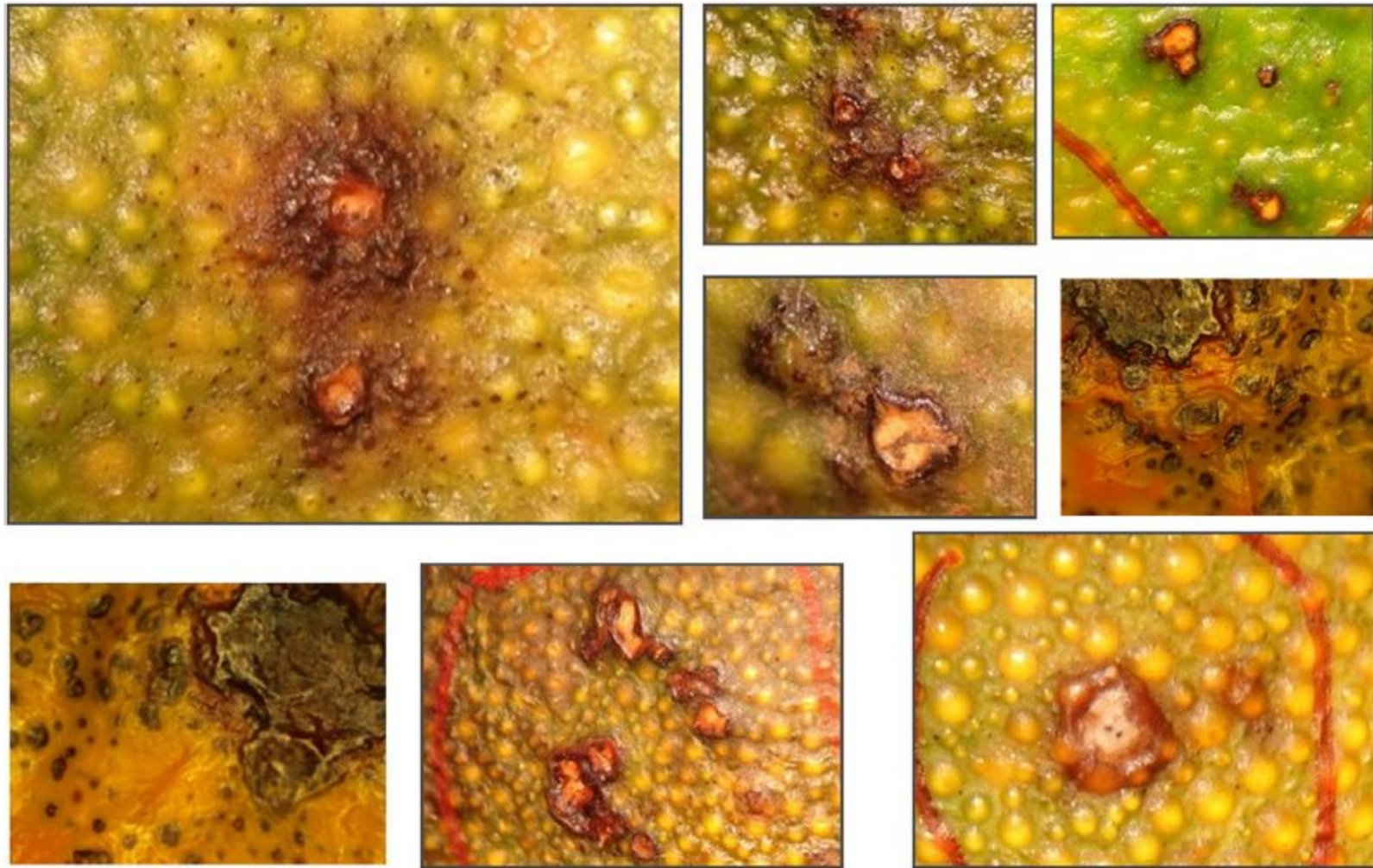


Fig. 4 Constellation symptoms on tangerine hybrid fruits. Photographs courtesy of Instituto Nacional de Tecnología Agropecuaria Argentina.

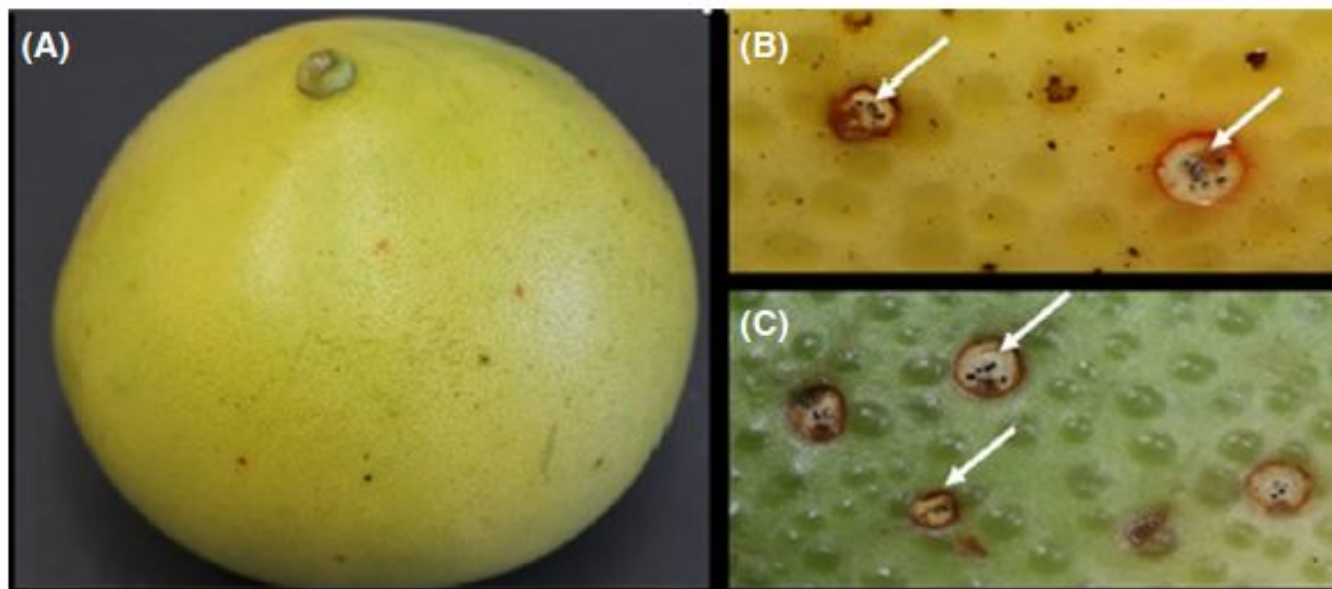


Fig. 5 Tan spot disease caused by *P. citriasiana* on pomelo (*C. maxima*). (A)–(C) Symptoms with lesions containing pycnidia (arrows).



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).



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

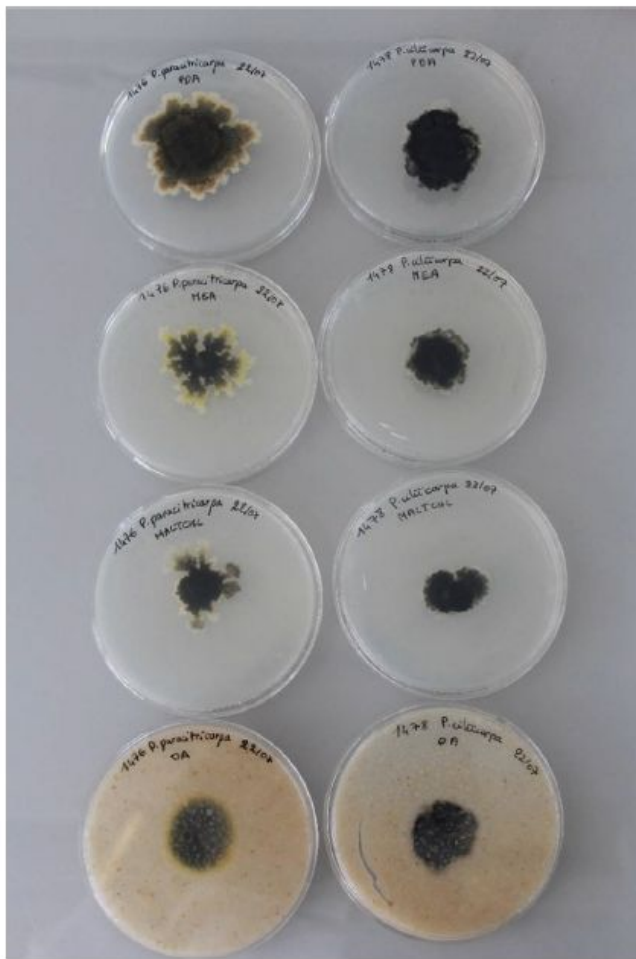


Fig. 8 Cultures of *P. paracitricarpa* (left) and *P. citricarpa* (right) on (from top to bottom) PDA, MEA, MALTCHL and OA (9 days at 26°C 12 h light). Photograph courtesy of J. Hubert, ANSES.

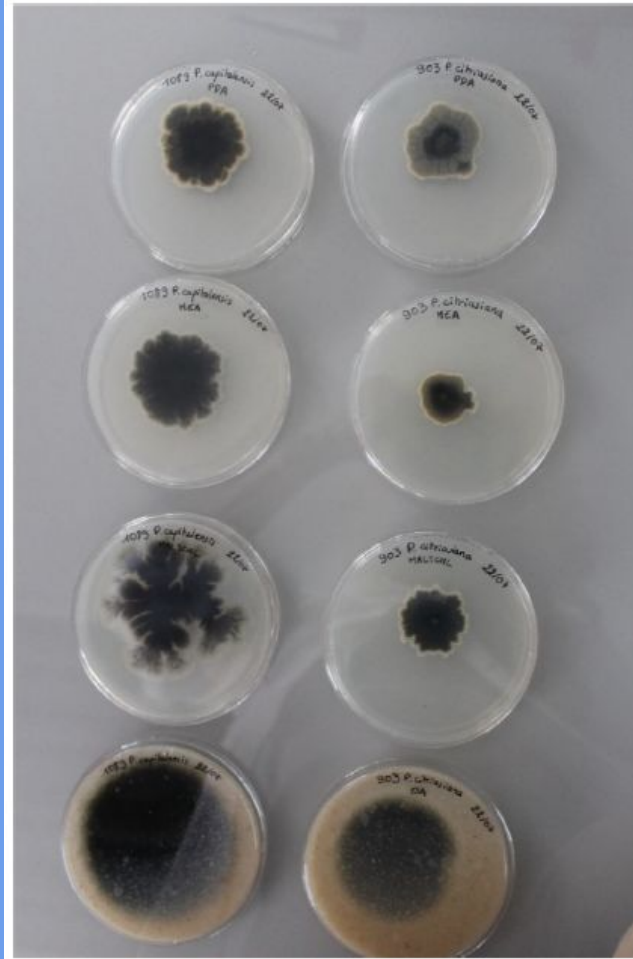


Fig. 9 Cultures of *P. capitalensis* (left) and *P. citriasiana* (right) on (from top to bottom) PDA, MEA, MALTCHL and OA (9 days at 26°C, 12 h light). Photograph courtesy of J. Hubert, ANSES.

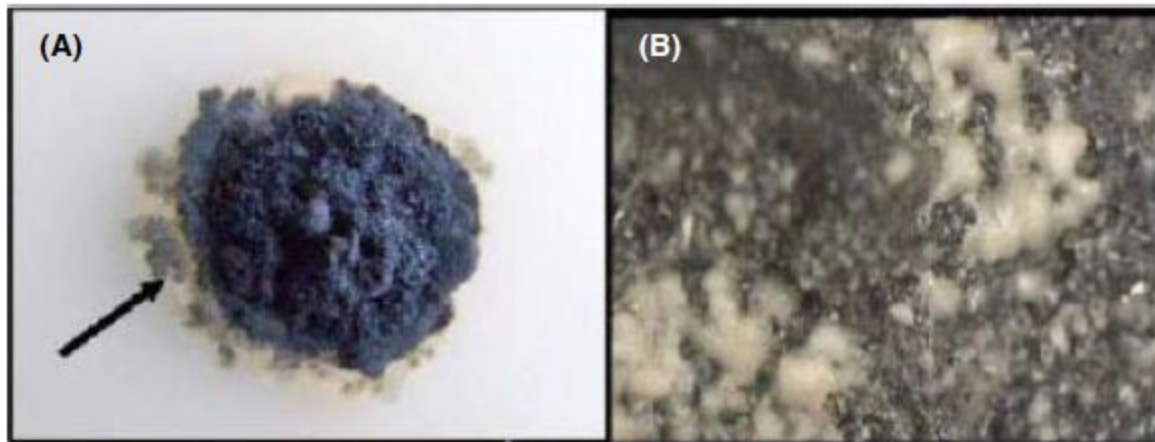


Fig. 10 Colony characteristics of *P. citricarpa*: (A) colony with irregular margin surrounded by a translucent zone of colourless submerged mycelium (arrow) after 30 days of growth on potato dextrose agar at 25°C and a 12 h photoperiod; (B) conidial slime oozing from mature pycnidia. Photographs courtesy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

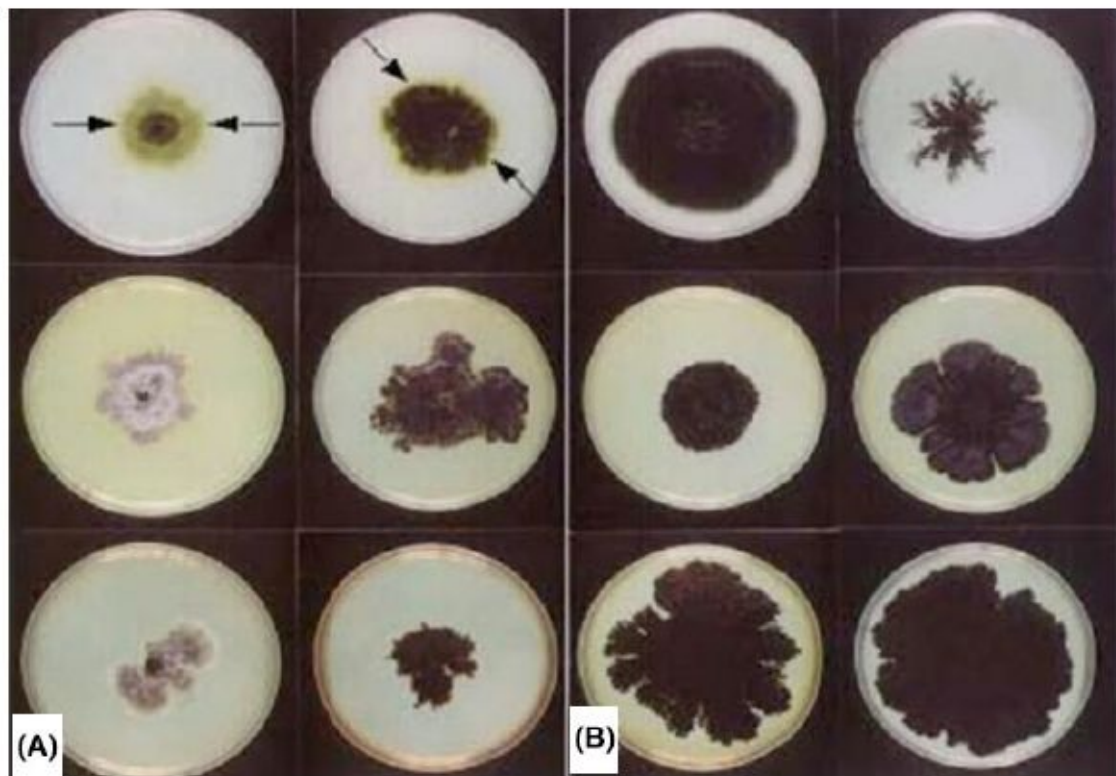


Fig. 11 Cultural characteristics of *P. citricarpa* and *P. capitalensis*: colonies of *P. citricarpa* (A) and *P. capitalensis* (B) after 7 days of growth on oatmeal agar (top row), malt extract agar (middle row) and cherry decoction agar (bottom row) [note that some, but not all strains of *P. citricarpa* 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.

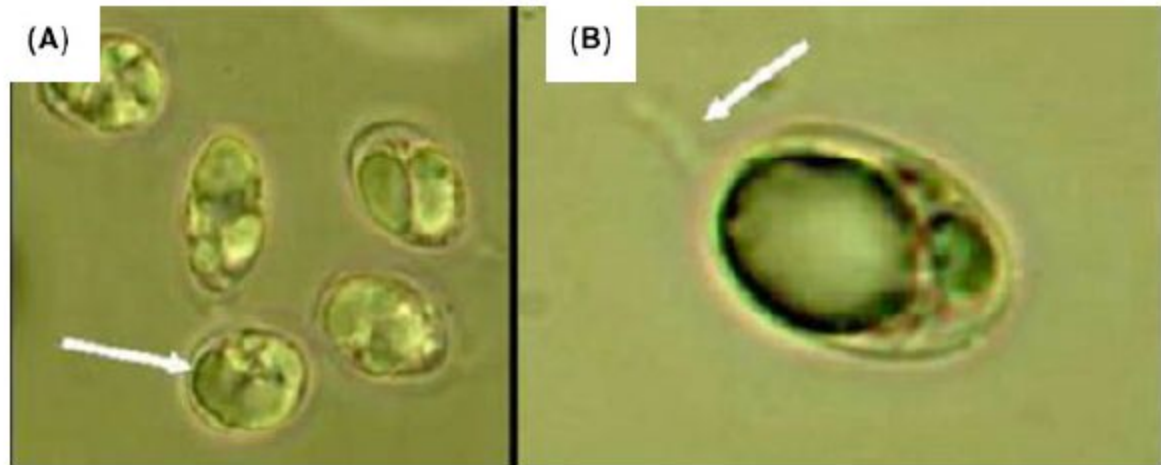


Fig. 12 Conidial morphology of *P. citricarpa*: (A) and (B) conidia with a thin mucoid sheath (A, arrow) and a colourless subulate appendage (B, arrow, magnification 1000 $\times$ with immersion oil). Photographs courtesy of L.E. Diaz, Ministry of Husbandry, Agriculture and Fisheries, Montevideo, Uruguay.

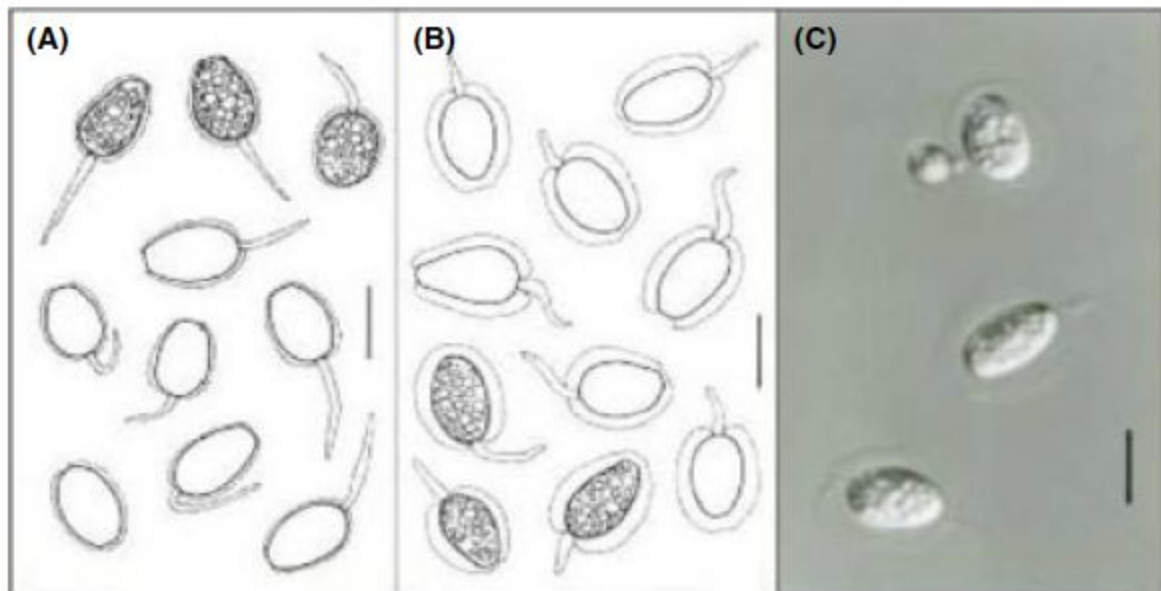
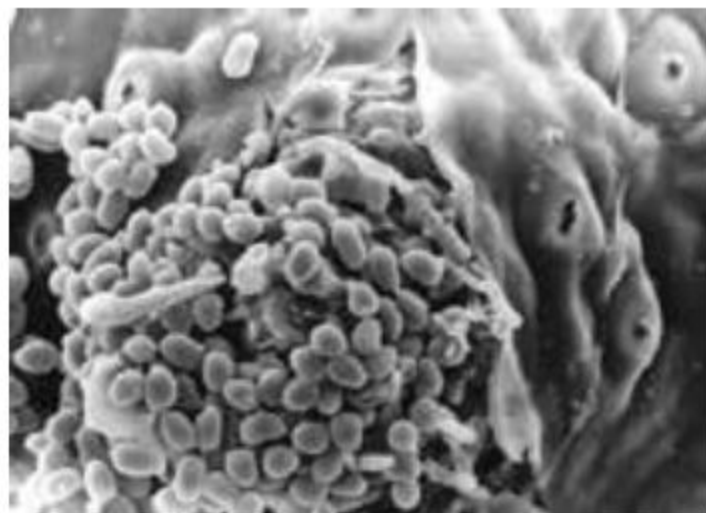
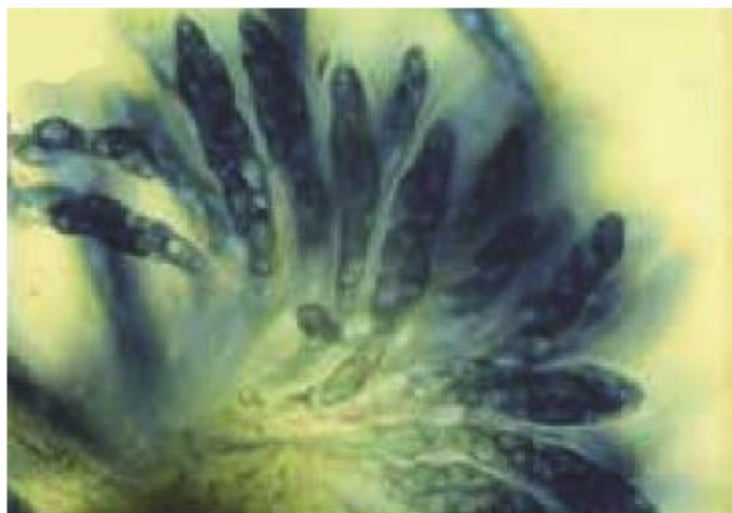


Fig. 13 Conidial morphology of *P. citricarpa* and *P. capitalensis*: (A) conidia of *P. citricarpa* with thin ($<1.5\ \mu\text{m}$) mucoid sheaths; (B) and (C) conidia of *P. capitalensis* with thick ($>1.5\ \mu\text{m}$) mucoid sheaths (scale bar = $10\ \mu\text{m}$) (photograph C was taken under a 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 Sílvia 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

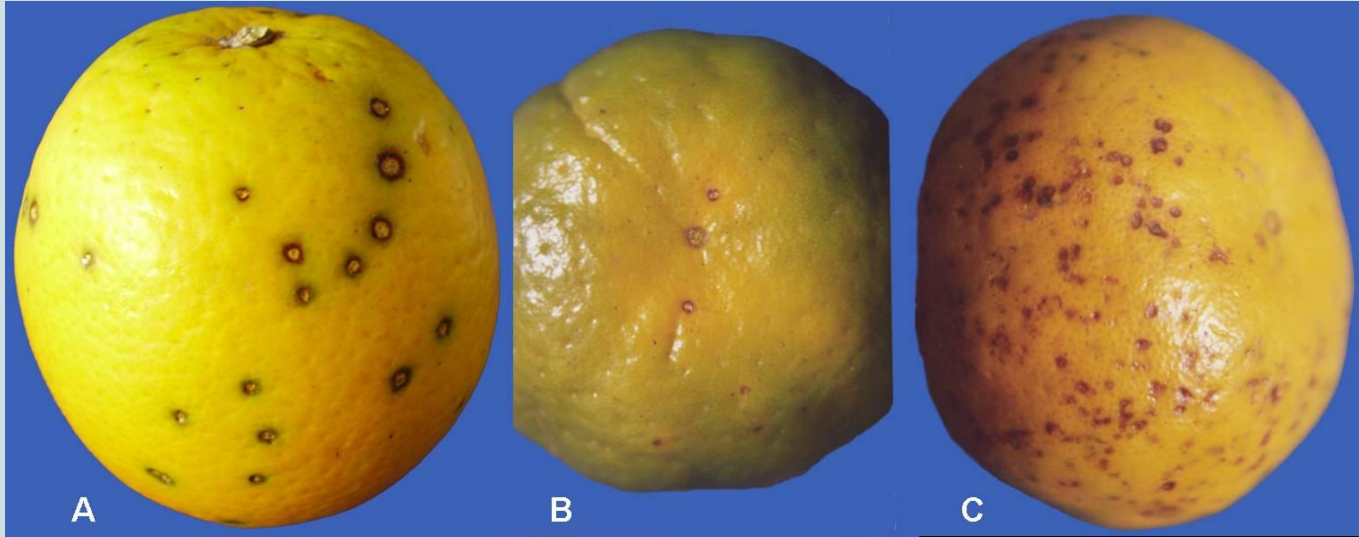
MATERIAL E MÉTODOS

◆ **Local:**

◆ **Isolados:**

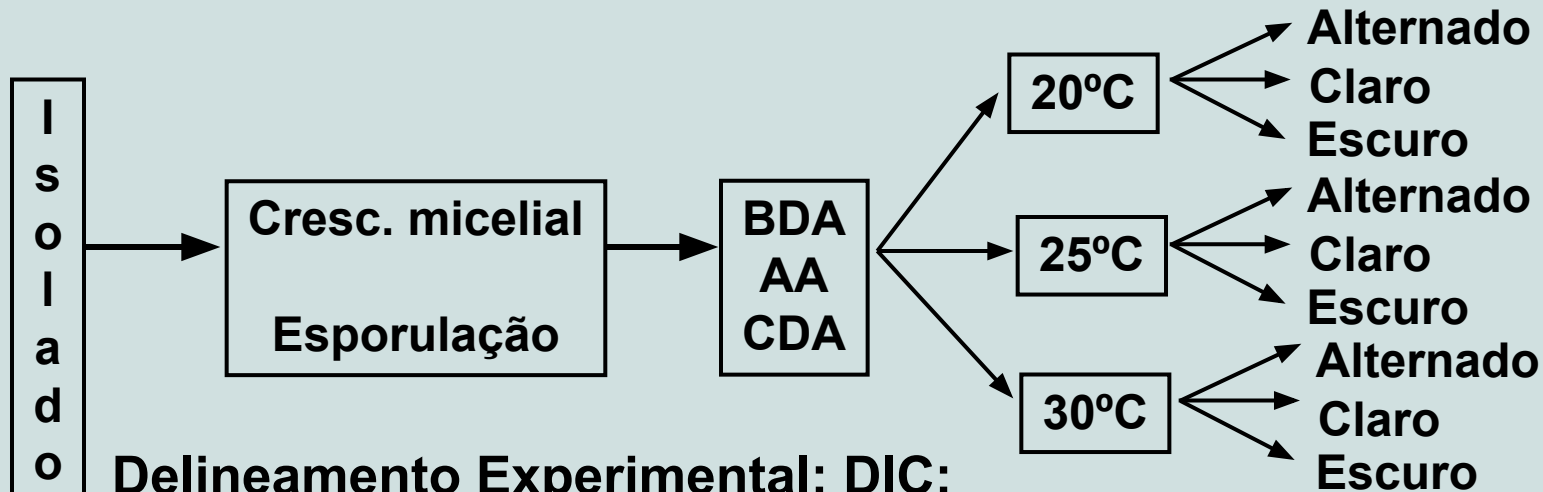
Entidade	Nº de isolados
UFPR	5
ESALQ	3
UNESP	6
UEM	4

ISOLAMENTO



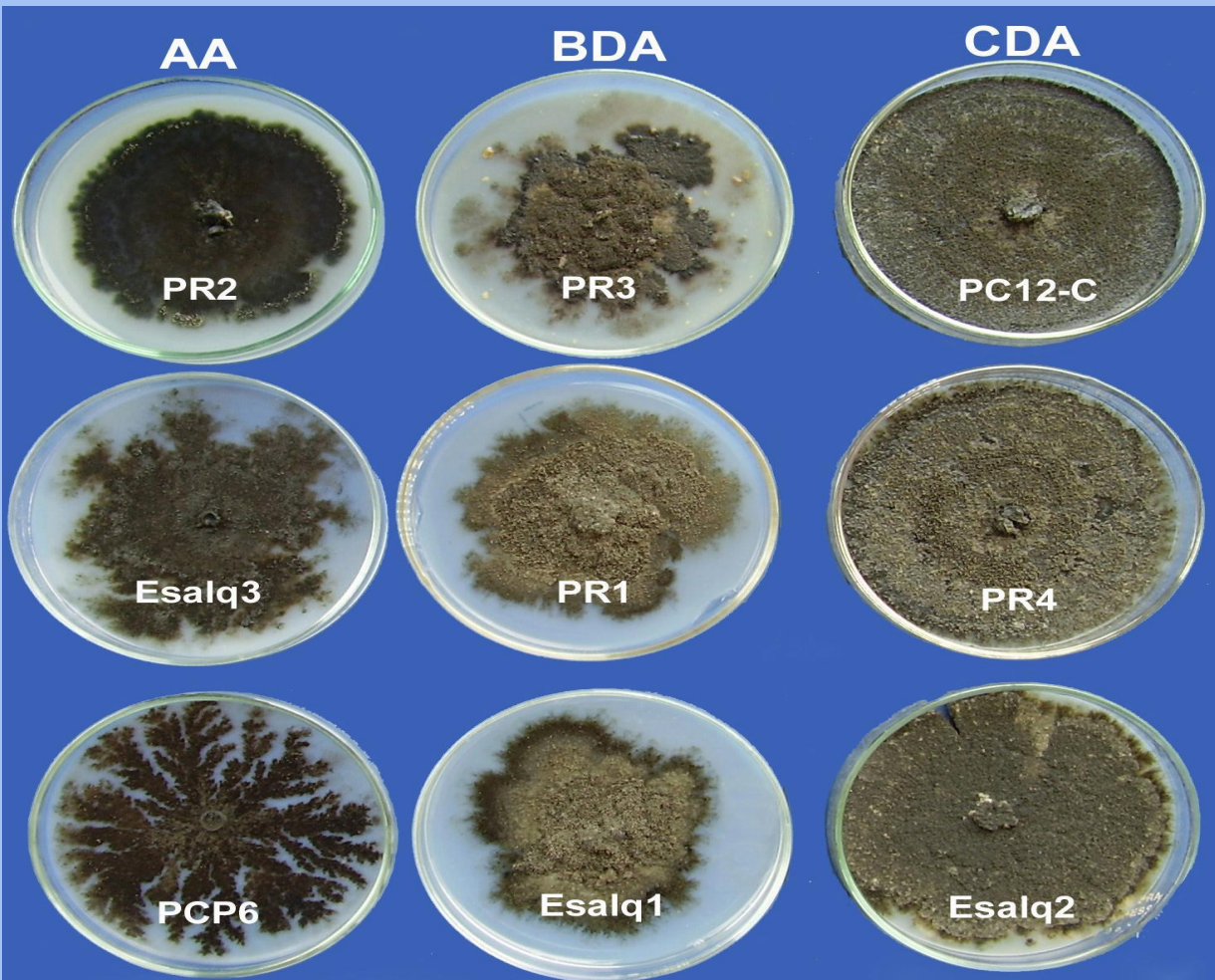
A- Laranja 'Pêra';
B- Tangerina 'Ponkan';
C- Tangerina 'Montenegrina'.

CARACTERÍSTICAS ESTUDADAS

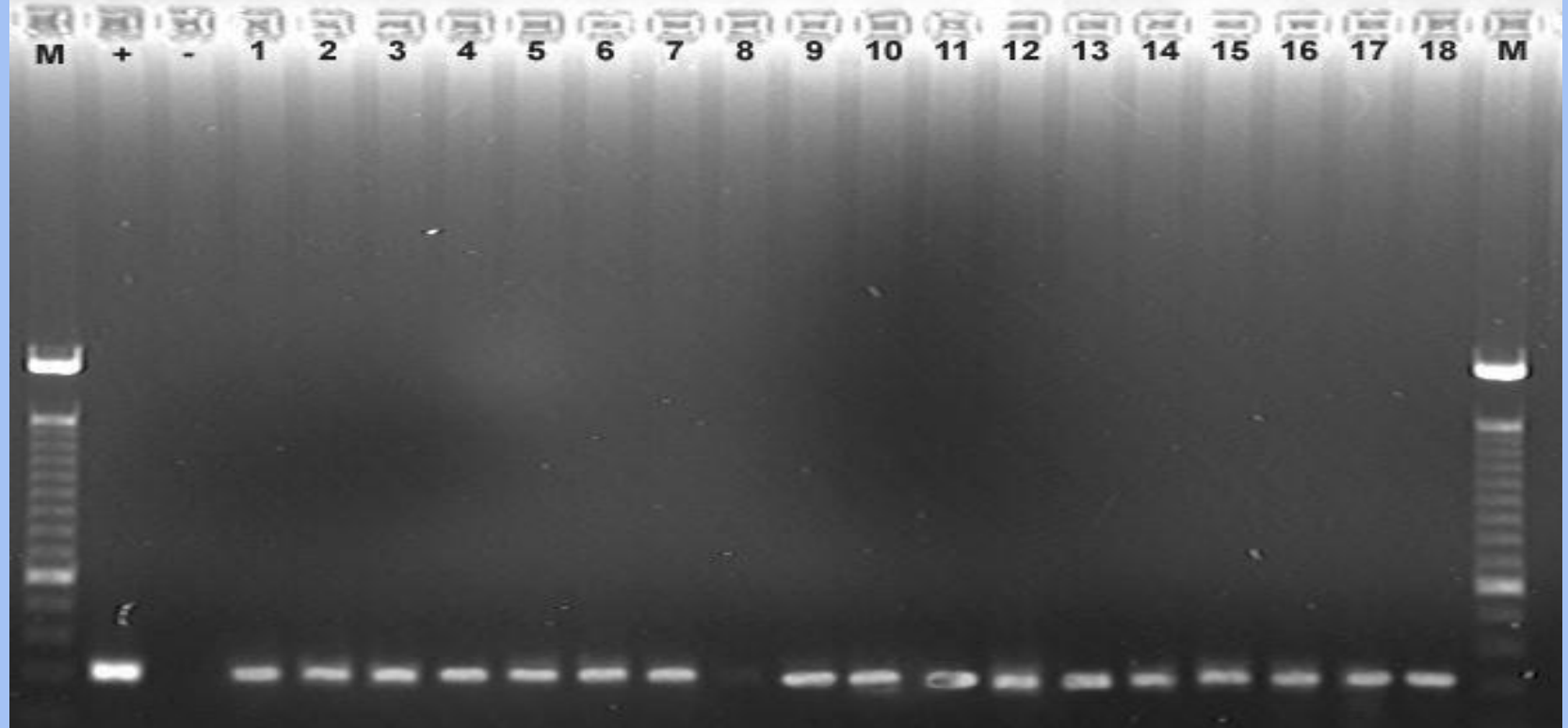


Delineamento Experimental: DIC;
5 repetições por tratamento;
Fatorial 3 x 3 x 3.

Padrões de crescimento micelial apresentado pelos isolados



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











faça sua pesquisa

Nome do Agrotóxico

Ingrediente Ativo

Registrante

Classificação Toxicológica

Praga

Situação

Classe

Cultura Infestada

Expurgo ☐ Sim ☐ Não

Aplicação Aérea ☐ Sim ☐ Não

Trat. de Sementes ☐ Sim ☐ Não

Alvo Biológico - Nome Científico
Alvo Biológico - Nome Comum

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 Agrocência 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 Agrocência 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 - Amércia 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 Agrocência 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*



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Database

advanced search...

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[Explore by](#)

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

Phyllosticta citricarpa (GUIGCI)

Citrofortunella microcarpa (CJFMI)

Minor

Minor

Citroncirus (1CJCG)

Minor

Minor

Citrus (1CIDG)

Minor

Minor

Citrus aurantiifolia (CIDAF)

Minor

Minor

Citrus limon (CIDLI)

Major

Major

Citrus paradisi (CIDPA)

Minor

Minor

Citrus reticulata (CIDRE)

Minor

Minor

Citrus sinensis (CIDS)

Major

Major

Citrus tankan (CIDTK)

Minor

Minor

Citrus x limonia (CIDLO)

Minor

Minor

Citrus x nobilis (CIDNO)

Minor

Minor

Fortunella (1FOLG)

Minor

Minor

Poncirus trifoliata (PMITR)

Minor

Minor



Biologia de *Phyllosticta citricarpa*



□ Fase sexuada

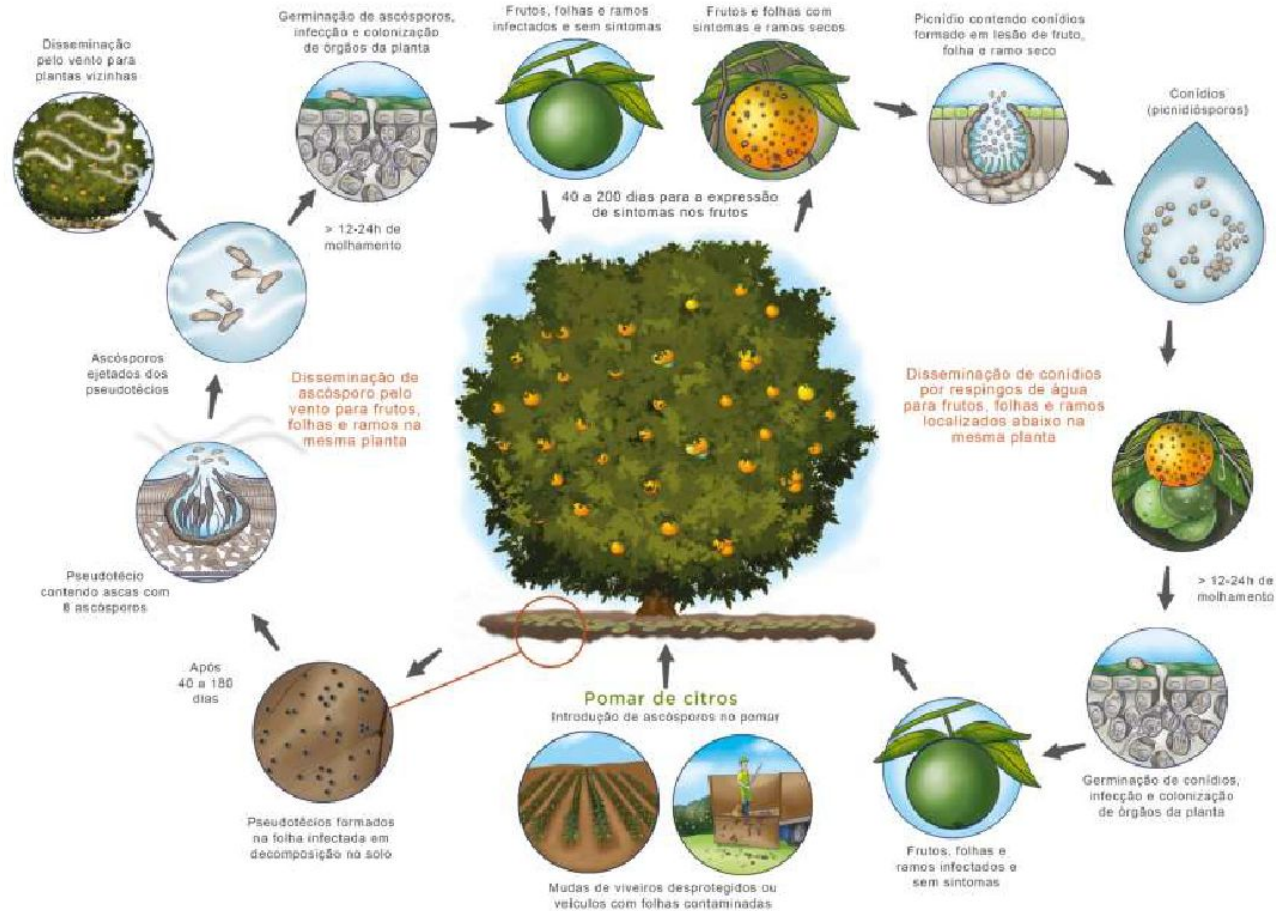
- Formada em folhas em decomposição no solo
- Ascósporos = inóculo para o ciclo primário
- Disseminados pelo vento, alcançando longas distâncias

□ 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



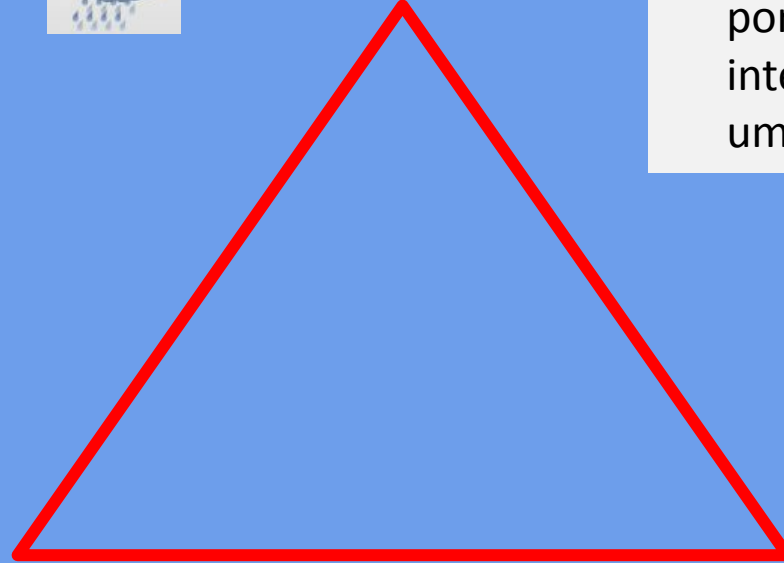
Condições favoráveis para a ocorrência da Pinta Preta



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 saudáveis, 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) +
difenconazole (triazol)

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

Literatura

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

Elma Carstens,^{1,2} Celeste C. Linde,³ Paul H. Fourie,^{1,2} Aletta E. Bester-van der Merwe,⁴ Shaun D. Langenhoven,² and Adèle McLeod^{2,†}

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Accepted for publication 10 November 2020.

ABSTRACT

Citrus black spot (CBS), caused by *Phylosticta 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 semi-arid 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_{max} 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.

Keywords: asexual, *citricarpa*, epidemiology, fruit infections

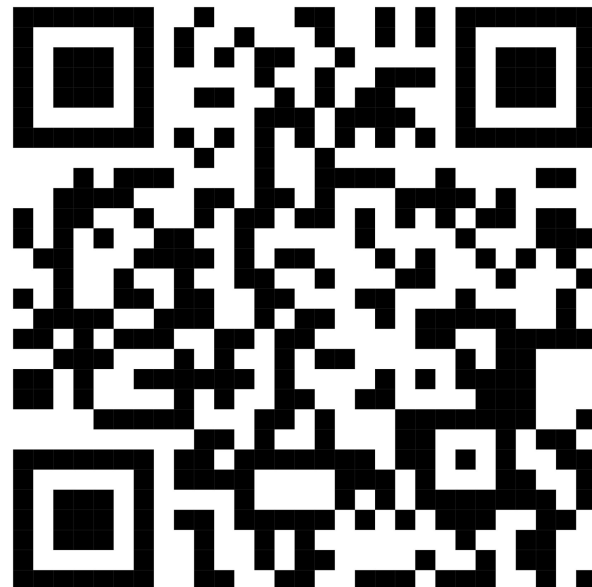
The citrus black spot (CBS) fungal pathogen, *Phylosticta citricarpa* (McAlpine) Aa, which is an economically important pathogen of citrus worldwide, produces two types of spores: waterborne conidia (pycnidiospores) and aerially dispersed ascospores. Different spore types are found on citrus fruit, leaves, branches, and twigs. Fruit lesions are economically the most important, and they often become visible only after fruit color break. The lesions contain pycnidia and pycnidiospores, which are products of asexual reproduction. These asexual structures have also been observed on leaves that are still attached to the trees and abundantly in leaf litter on the orchard floor (Kotzé 1981). Lesions on leaves attached to the tree and in the leaf litter are rare and have been found only on leaves on lemon trees (*Citrus limon*) and infrequently on Valencia orange trees (*Citrus sinensis*) (FAO 2015; Kieley 1948; Kotzé 2000). Dead twigs may also contain lesions producing pycnidia, but these are not common (Kieley 1948; Kotzé 1981, 2000). Living old branches (brown bark without fruits or flower buttons) have been reported as a potential reservoir of the pathogen, although the incidence of the pathogen in these was low (0.8%) (De Oliveira Silva et al. 2017). It has been hypothesized that once infected branches die, pycnidia will be produced on the surface because artificially

inoculated autoclaved branches were a suitable substrate for pycnidial production (De Oliveira Silva et al. 2017). The sexually produced ascospores, formed within pseudothecia, are produced only in fallen leaves (leaf litter). *P. citricarpa* is a heterothallic fungus, and for sexual reproduction to take place two mating types (*MAT 1-1* and *MAT 1-2*) are needed to be in direct physical contact (Kieley 1948; Kotzé 1981; Tran et al. 2017; Wang et al. 2016).

Ascospores and pycnidiospores have been reported to play different roles in the spatial distribution of CBS within orchards and trees (Kieley 1948; Kotzé 1981; Spósito et al. 2007, 2008, 2011). Pycnidiospores, which are exuded in a gelatinous mass, are dispersed over short distances, and their contribution to pathogen spread is generally regarded as within-tree distribution through a water washdown action and to a lesser extent laterally (Hendricks et al. 2017; Kieley 1948; Kotzé 1981, 2000; McOnie 1964a; Spósito et al. 2011). Ascospores are forcibly discharged and dispersed over distances of ≤ 25 m (Spósito et al. 2007) and are considered to be the primary source of between-tree pathogen dispersal within and between orchards (Kieley 1948; Kotzé 1981; McOnie 1964a; Spósito et al. 2007, 2008, 2011).

The importance of pycnidiospores and ascospores as an inoculum source for fruit infections varies in different regions of the world. In South Africa, pycnidiospores have not been considered to play an important role in fruit infections, unless out-of-season, late hanging infected fruit or overlapping fruit crops are present in orchards. Late hanging fruit do not occur in well-managed orchards in South Africa, whereas overlapping fruit crops can sometimes occur in lemon orchards. Ascospores, on the other hand, have been considered by far the most important source of fruit infections (Kotzé 1981; McOnie 1965). A similar situation occurs in Australia (Kieley 1948). However, it should be noted that the older studies (Fourie

Carstens_phyto-05-20-0203-r.pdf



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*The e-Xtra logo stands for "electronic extra" and indicates that five supplementary tables are published online.

The author(s) declare no conflict of interest.

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.

[2006_Guignardia_Summa.pdf](#)

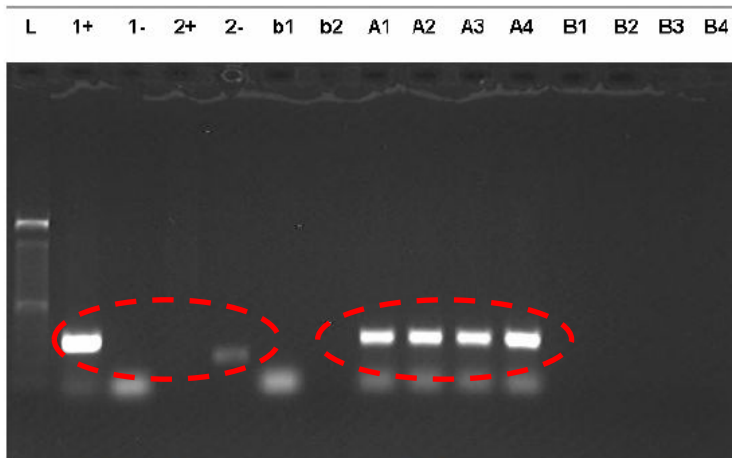
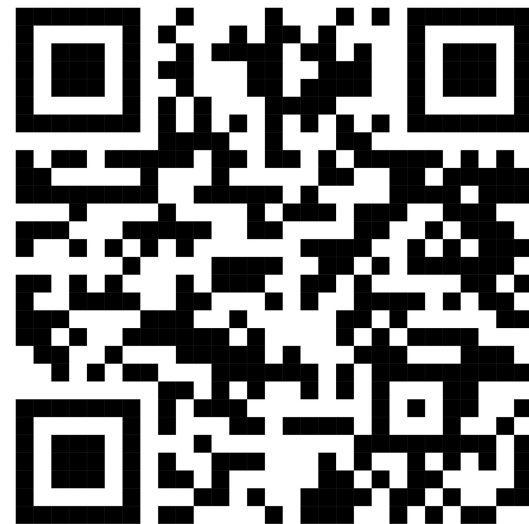


Figura 1. Gel de agarose com o resultado da reação de PCR onde L=marcador molecular 100 pb; 1+= controle positivo (primer op08 e op09); 1-= controle negativo (primer op08 e op09); 2+= controle positivo (primer GCF2 e GCR4); 2-= controle negativo (primer GCF2 e GCR4); b1 e b2= brancos; A1, A2, A3 e A4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



Pinta preta dos citros

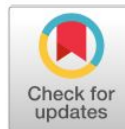


a doença e seu manejo

Geraldo José da Silva Junior | Eduardo Feichtenberger
Marcel Bellato Spósito | Lilian Amorim
Renato Beozzo Bassanezi | Antonio de Goes

[E-book_PintaZPreta_ok.pdf](#)





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*)

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.

[pm7-017-3-en.pdf](#)



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

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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-água (AA), batata-dextrose-água (BDA) e cenoura-dextrose-água (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](#)



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AR **ADAPAR**
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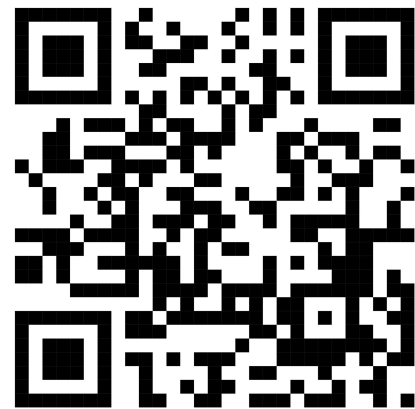
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Legislação sobre Sanidade da Citricultura

Legislação Estadual

- Resolução Estadual Nº 079/90** de 02 de agosto de 1990
Permite a implantação de citricultura em áreas indenens, 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 nº 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 vista 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, 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 Secretária de Estado da Agricultura e do Abastecimento do Paraná.
- Instrução Normativa Nº 16/03** de 18 de março de 2003
Proíbe 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 ssp.*
- 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>



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Sanidade da Citricultura

Finalidade

Promover a Vigilância Fitossanitária para monitorar, controlar e prevenir a introdução e a disseminação de pragas e doenças na cultura dos Citros (laranjas, limões, limas ácidas, tangerinas e híbridos), no Estado do Paraná.

Ações desenvolvidas

- Monitoramento para detecção e delimitação da presença de pragas em áreas livres das regiões produtoras de citros dentro do território paranaense e de fora para dentro do estado, através de fiscalizações dirigidas em áreas de produção e do comércio de frutos e de material de propagação;
- Fiscalização em áreas de ocorrência de pragas quarentenárias, fazendo cumprir, por parte do citricultor, a eliminação de plantas doentes e a adoção de boas práticas de cultivo, visando à contenção da praga;
- Auditoria em áreas de produção de citros inscritas no sistema de certificação, verificando o cumprimento de normas que garantam a qualidade e a sanidade do produto;
- Fiscalização em propriedades produtoras sem certificação fitossanitária; Fiscalização em Unidades de Produção (UP);
- Fiscalização em Comerciantes de mudas e de frutas;
- Fiscalização em Unidades de Consolidação (UC);
- Coleta de amostras para diagnósticos fitossanitários em propriedades e comerciantes.

Informativos Citricultura

- Cancro cítrico | [Cartilha](#) | [Cartaz](#) | [Cartão](#)
- Formulário HLB Greening: acesse [aqui](#) o formulário para preenchimento do relatório de Greening
- [Resultados das Ações de Prevenção e Controle do HLB no Paraná](#)
- [Material Informativo - HLB](#)

Legislação

[Legislação Específica](#)

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

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L. Galli-Terasawa¹, J. Cunnington³, R.G. Shivas⁴, J.Z. Groenewald⁵, 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.



Citrus pests in a global world

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^aCentro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia, Spain, ^bCitrus Research International, Nelspruit, South Africa, ^cPaulista State University (UNESP, Retired) and Member of GCONCI-Consultant Citrus Group, Jaboticabal, Brazil, ^dSouth China Agricultural University, Guangzhou, China, ^eUniversity of Florida—IFAS, Southwest Florida Research & Education Center, Immokalee, FL, United States

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 [*Rhynocoris humeralis* (Thunberg)] (Hemiptera: Pentatomidae)], leaf beetles [*Podagrimela 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.

The Genus Citrus Chapter 16.pdf



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 ring 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).



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

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18.1 Introduction

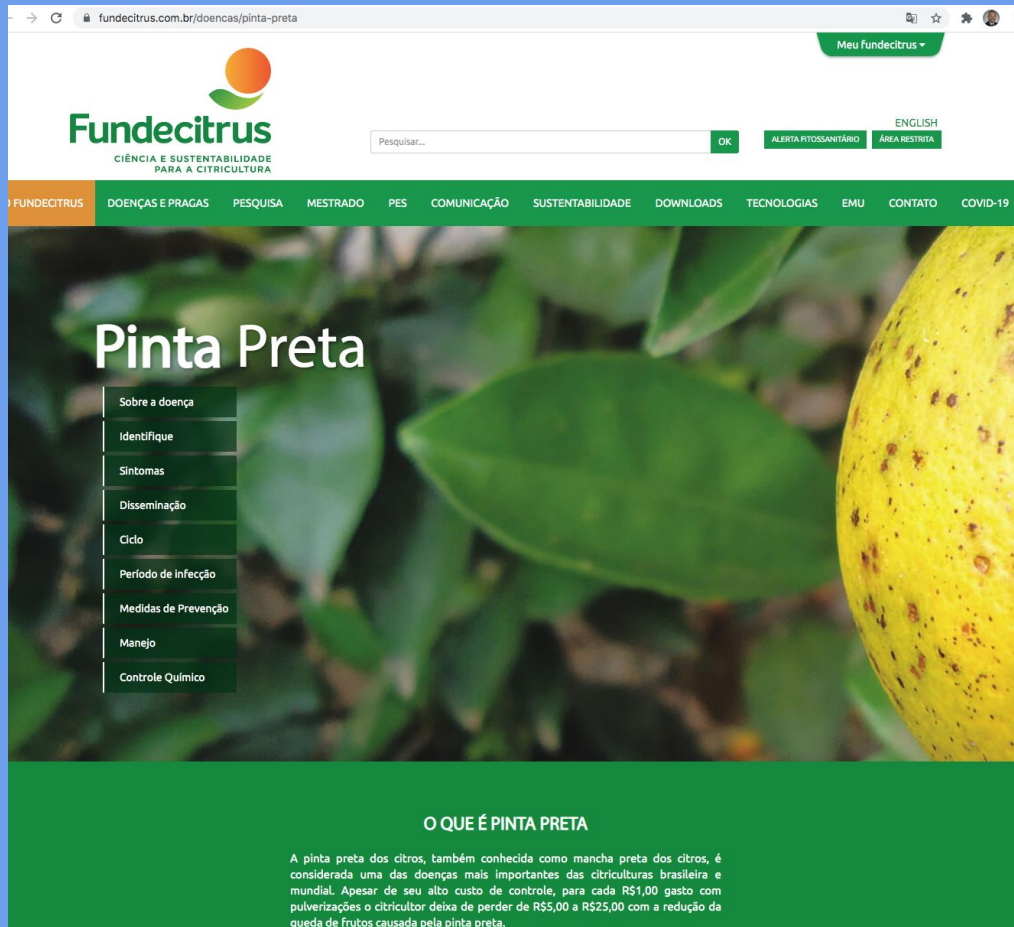
Commercial citrus groves are invariably planted as dense monocultures comprising a few commercial citrus varieties covering large tracts of land, providing little to no natural variation in resistance to any pest/vector, pathogen, or a combination of both with capacity to exploit it. The majority of 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.



Pinta Preta, Mancha Preta dos Citros, Fungo Guignardia citricarpa - Fundecitrus.



The screenshot shows the Fundecitrus website interface. At the top, there is a navigation bar with the Fundecitrus logo (an orange circle with a green leaf) and the text "Fundecitrus CIÊNCIA E SUSTENTABILIDADE PARA A CITRICULTURA". To the right of the logo is a search bar with the placeholder text "Pesquisar..." and a green "OK" button. Further right are links for "Meu fundecitrus", "ALERTA FITOSSANITÁRIO", and "ÁREA RESTRIITA" (with a dropdown arrow). Below the navigation bar is a green horizontal menu with various categories: FUNDECITRUS, DOENÇAS E PRAGAS, PESQUISA, MESTRADO, PES, COMUNICAÇÃO, SUSTENTABILIDADE, DOWNLOADS, TECNOLOGIAS, EMU, CONTATO, and COVID-19.

The main content area features a large background image of a yellow citrus fruit with dark spots (Pinta Preta) and green leaves. The title "Pinta Preta" is displayed in large white text. Below the title is a vertical list of menu items, each in a green box with white text: "Sobre a doença", "Identifique", "Sintomas", "Disseminação", "Ciclo", "Período de infecção", "Medidas de Prevenção", "Manejo", and "Controle Químico".

At the bottom of the page, there is a green section titled "O QUE É PINTA PRETA" in white capital letters. Below the title, there is a paragraph of text in white:

A pinta preta dos citros, também conhecida como mancha preta dos citros, é considerada uma das doenças mais importantes das citriculturas brasileira e mundial. Apesar de seu alto custo de controle, para cada R\$1,00 gasto com pulverizações o citricultor deixa de perder de R\$5,00 a R\$25,00 com a redução da queda de frutos causada pela pinta preta.





Research

Validation of Quantitative and Digital Polymerase Chain Reaction Assays Targeting the Mating Types of *Phyllosticta citricarpa*, the Causal Agent of Citrus Black Spot

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Abstract

Citrus black spot (CBS) is a disease caused by the ascomycetous fungus *Phyllosticta* (formerly *Guignardia*) *citricarpa* (Botryosphaerales, Pezizomycotina) currently present in citrus groves in five counties in southwest Florida. Within Florida, *P. citricarpa* shows limited reproduction via asexual sporulation due to the presence of only one (*MAT1-2-1*) of the two required mating types for sexual reproduction. Here, we present two novel PCR assays standardized in quantitative PCR (qPCR) and digital PCR (dPCR) platforms to distinguish both mating types (*MAT1-1-1* and *MAT1-2-1*) of *P. citricarpa* to monitor for the potential introduction of the *MAT1-1-1* mating type into Florida and a novel protocol for DNA extraction from asymptomatic leaves. During citrus harvesting season 2018–19, fruit lesions as well as asymptomatic leaves adjacent to symptomatic fruit and asymptomatic trees in CBS-infected groves were surveyed for *P. citricarpa* presence and mating types. Results support the presence of only the *MAT1-2-1* mating type in Florida, after surveying more than 1,145 citrus fruit lesions. We also confirmed the limited dispersal ability of the asexual state of *P. citricarpa* in Florida in 10 groves using the enhanced capabilities of the dPCR platform in the detection of *P. citricarpa* directly from asymptomatic leaves with low pathogen inoculum.

Keywords: Ascomycota, asexual state, CBS, Dothideomycetes, dPCR, Florida, *Guignardia*, *Phyllostictaceae*, qPCR, sweet orange

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e-Xtra: Supplementary materials are available online.

The author(s) declare no conflict of interest.



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INSTRUÇÃO NORMATIVA Nº 38, DE 1º DE OUTUBRO DE 2018

O SECRETÁRIO DE DEFESA AGROPECUÁRIA, SUBSTITUTO, DO MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO, no uso das atribuições que lhe conferem os arts. 18 e 53 do Anexo I do Decreto nº 8.852, de 20 de setembro de 2016, tendo em vista o disposto no Decreto nº 24.114, de 12 de abril de 1934, no Decreto nº 1.355, de 30 de dezembro de 1994, no Decreto nº 5.759, de 17 de abril de 2006, na Instrução Normativa nº 23, de 2 de agosto de 2004, na Instrução Normativa nº 6, de 16 de maio de 2005, na Instrução Normativa nº 45, de 29 de agosto de 2018, e o que consta do Processo nº 21000.036807/2018-98, resolve:

Art. 1º Estabelecer, na forma do Anexo desta Instrução Normativa, a lista de Pragas Quarentenárias Presentes (PQP) para o Brasil.

Parágrafo único. A divulgação da lista de que trata *ocapute* de suas atualizações será feita periodicamente, por meio digital, no portal institucional do MAPA - www.agricultura.gov.br.

Art. 2º Esta Instrução Normativa entra em vigor na data de sua publicação.

ANEXO

PRAGAS QUARENTENÁRIAS PRESENTES - PQP

Pragas	Hospedeiros	Unidades da Federação com Ocorrência da Praga
ÁCAROS		
Schizotetranychus hindustanicus	Acácia (Acaciasp.) Cinamomo (Melia azedarach) Citros (Citrus sp.) Coqueiro (Cocos nucifera)	Roraima
	Nim (Azadirachta indica) Sorgo (Sorghum bicolor)	
INSETOS		
	Abiu (Pouteria caimito) Acerola (Malpighia emarginata) Ajuru (Chrysobalanus icaco) Ameixa-roxa (Syzygium cumini)	
	Amendoeira (Terminalia catappa) Araça-Boi (Eugenia stipitata) Biriba (Rollinia omucosa) Caimito (Chrysophyllum cainito)	
	Caju (Anacardium occidentale) Carambola (Averrhoa carambola) Cutite (Pouteria macrophylla) Fruta-pão (Artocarpus altilis)	
	Goiaba (Psidium guajava) Goiaba-araçã (Psidium guineense) Gomuto (Arenga pinnata) Jaca (Artocarpus integrifolia)	





Obrigado

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ANEXO

PRAGAS QUARENTENÁRIAS PRESENTES - PQP

Pragas	Hospedeiros	Unidades da Federação com Ocorrência da Praga
ÂCAROS		
Schizotetranychus hindustanicus	Acácia (Acaciasp.) Cinamomo (Melia azedarach) Citros (Citrus sp.) Coqueiro (Cocos nucifera)	Roraima
	Nim (Azadirachta indica) Sorgo (Sorghum bicolor)	
INSETOS		
	Abiu (Pouteria caimito) Acerola (Malpighia emarginata) Ajuru (Chrysobalanus icaco) Ameixa-roxa (Syzygium cumini)	
	Amendoeira (Terminalia catappa) Araçá-Boi (Eugenia stipitata) Biribá (Rollinia omucosa) Caimito (Chrysophyllum cainito)	
	Caju (Anacardium occidentale) Carambola (Averrhoa carambola) Cutite (Pouteria macrophylla) Fruta-pão (Artocarpus altilis)	
	Goiaba (Psidium guajava) Goiaba-araçá (Psidium guineense) Gomuto (Arenga pinnata) Jaca (Artocarpus integrifolia)	

Bactrocera carambolae	Jambo rosa (Syzygium samarangense) Jambo d'água ou Jambosa (Syzygium aqueum) Jambo amarelo (Syzygium jambos)	Amapá, Pará e Roraima
	Jambo vermelho (Syzygium malaccense) Jujuba ou Maçã-de-pobre (Ziziphus mauritiana) Jujuba chinesa (Ziziphus jujuba)	
	Laranja da terra, Laranja amarga, Laranja caipira Laranja (Citrus aurantium) Laranja doce (Citrus sinensis) Licania (Licania sp.)	
	Limão cayena, Bilimbi, Carambola Amarela (Averrhoa bilimbi) Manga (Mangifera indica) Murici ou Muruci (Bysonima crassifolia)	
	Pimenta-de-Cheiro (Capsicum chinense) Pimenta picante ou Pimenta do Diabo (Capsicum annum) Pitanga vermelha (Eugenia uniflora)	
	Sapotilha ou Sapoti (Manilkara zapota) Tangerina, Mexerica, Mandarina, Bergamota, Poncã (Citrus reticulata Blanco) Tapereba, Cajá-mirim, Cajá (Spondias	
	mombinsinon. Spondias lutea) Tomate (Solanum lycopersicum sinon. Lycopersicum	
	esculentum) Toranja ou Toronja (Citrus paradisi) Bacupari (Garcinia dulcis)	

Anthonomus tomentosus	Acerola (Malpighia spp.)	Roraima
Sternonchetus mangiferae	Manga (Mangifera indica)	Rio de Janeiro
FUNGOS		
Phyllosticta citricarpa (Guinardia citricarpa)	Citros (Citrus spp.)	Amazonas, Bahia, Espirito Santo, Goiás, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pernambuco, Paraná,
		Rio de Janeiro, Rio Grande do Sul, Santa Catarina e São Paulo
	<i>Bananeira (Musasp.)</i> Heliconia spp. Exceto: Heliconia rostrata, H. bihai, H. augusta, H.	Acre, Amazonas, Amapá, Bahia, Espirito Santo, Goiás, Maranhão, Minas Gerais, Mato Grosso do Sul, Mato Grosso,
Pseudocercospora fijiensis (Mycosphaerella fijiensis)	chartaceae, H. spathocircinada, H. librata, H. psittacorum cultivar Red Opal e H. stricta	Pará, Paraná, Rio de Janeiro, Rondônia, Roraima, Rio Grande do Sul, Santa Catarina, São Paulo e Tocantins
Neonectria ditissima (Neonectria galligena)	Maçã (Malus spp.)	Paraná, Rio Grande do Sul e Santa Catarina
PROCARIONTES		
Candidatus liberibacter americanus e Candidatus liberibacter asiaticus	Citros (Citrus spp.) Fortunella spp. Murta (Murraya paniculata) Poncirus spp.	Minas Gerais, Paraná e São Paulo

FUNGOS		
Phyllosticta citricarpa (Guinardia citricarpa)	Citros (Citrus spp.)	Amazonas, Bahia, Espirito Santo, Goiás, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pernambuco, Paraná,
		Rio de Janeiro, Rio Grande do Sul, Santa Catarina e São Paulo
PROCARIONTES		
Candidatus liberibacter americanus e Candidatus liberibacter asiaticus	Citros (Citrus spp.) Fortunella spp. Murta (Murraya paniculata) Poncirus spp.	Minas Gerais, Paraná e São Paulo
PLANTAS INFESTANTES E PARASITAS		
Ralstonia solanacearum raça 2	Bananeiras (Musa spp.) e Heliconiaspp.	Alagoas, Amazonas, Amapá, Pará, Rondônia, Roraima e Sergipe
Xanthomonas citrisubsp. citri	Citros (Citrus spp.), Fortunella spp. e Poncirus spp.	Ceará, Maranhão, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Piauí, Paraná, Roraima, Rio Grande do Sul, Santa Catarina e São Paulo
Xanthomonas campestris pv. viticola	Videira (Vitisspp.) e seus híbridos	Bahia, Ceará, Pernambuco e Roraima
PLANTAS INFESTANTES E PARASITAS		
Amaranthus palmeri	Algodão (Gossypiumsp.), Soja (Glycine max) e Milho (Zea mays)	Mato Grosso

Bactrocera carambolae	Jambo rosa (Syzygium samarangense) Jambo d'água ou Jambosa (Syzygium aqueum) Jambo amarelo (Syzygium jambos)	Amapá, Pará e Roraima
	Jambo vermelho (Syzygium malaccense) Jujuba ou Maçã-de-pobre (Ziziphus mauritiana) Jujuba chinesa (Ziziphus jujuba)	
	Laranja da terra, Laranja amarga, Laranja caipira Laranja (Citrus aurantium) Laranja doce (Citrus sinensis) Licania (Licania sp.)	
	Limão cayena, Bilimbi, Carambola Amarela (Averrhoa bilimbi) Manga (Mangifera indica) Murici ou Muruci (Bysonima crassifolia)	
	Pimenta-de-Cheiro (Capsicum chinense) Pimenta picante ou Pimenta do Diabo (Capsicum annum) Pitanga vermelha (Eugenia uniflora)	
	Sapotilha ou Sapoti (Manilkara zapota) Tangerina, Mexerica, Mandarina, Bergamota, Poncã (Citrus reticulata Blanco) Taperebá, Cajá-mirim, Cajá (Spondias	
	mombinsinon. Spondias lutea) Tomate (Solanum lycopersicum sinon. Lycopersicum	
	esculentum) Toranja ou Toronja (Citrus paradisi) Bacupari (Garcinia dulcis)	
Anthonomus tomentosus	Acerota (Malpighia spp.)	Roraima
Sternochetus mangiferae	Manga (Mangifera indica)	Rio de Janeiro

FUNGOS

Phytllosticta citricarpa (Guinardia citricarpa)	Citros (Citrus spp.)	Amazonas, Bahia, Espírito Santo, Goiás, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pernambuco, Paraná,
		Rio de Janeiro, Rio Grande do Sul, Santa Catarina e São Paulo
	Bananeira (Musaspp.) Heliconia spp. Exceto: Heliconia rostrata, H. bihai, H. augusta, H.	Acre, Amazonas, Amapá,Bahia, Espírito Santo, Goiás, Maranhão, Minas Gerais, Mato Grosso do Sul, Mato Grosso,
Pseudocercospora fijiensis (Mycosphaerella fijiensis)	chartaceae, H. spathocircinada, H. librata, H. psittacorum cultivar Red Opal e H. stricta	Pará, Paraná, Rio de Janeiro, Rondônia, Roraima, Rio Grande do Sul, Santa Catarina, São Paulo e Tocantins
Neonectria ditissima (Neonectria galligena)	Maçã (Malus spp.)	Paraná, Rio Grande do Sul e Santa Catarina

PROCARIONTES

Candidatus liberibacter americanus e Candidatus liberibacter asiaticus	Citros (Citrusspp.) Fortunella spp. Murta (Murraya paniculata) Poncirus spp.	Minas Gerais, Paraná e São Paulo
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Ralstonia solanacearum raça 2	Bananeiras (Musa spp.) e Heliconiaspp.	Alagoas, Amazonas, Amapá, Pará, Rondônia, Roraima e Sergipe
Xanthomonas citrisubsp. citri	Citros (Citrus spp.), Fortunella spp. e Poncirus spp.	Ceará, Maranhão, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pauli, Paraná, Roraima, Rio Grande do Sul, Santa Catarina e São Paulo
Xanthomonas campestris pv. viticola	Videira (Vitispp.) e seus híbridos	Bahia, Ceará, Pernambuco e Roraima
PLANTAS INFESTANTES E PARASITAS		
Amaranthus palmeri	Algodão (Gossypiumsp.), Soja (Glycine max) e Milho (Zea mays)	Mato Grosso

JORGE CAETANO JUNIOR

Este conteúdo não substitui o publicado na versão certificada