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

2021

ADAPAR
Agência de Defesa Agropecuária do Paraná

AGRONOMIA
Universidade Estadual de Maringá

Departamento de Agronomia
Universidade Estadual de Maringá

NBA
Núcleo de Pesquisa em Biotecnologia Aplicada
UEM Maringá-PR

William Mário de Carvalho Nunes

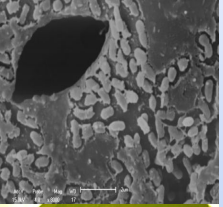
Engenheiro Agrônomo - UEM - 1984
Mestre *em Fitopatologia - ESALQ/USP - 1994*
Doutor *em Proteção de Plantas* - UNESP - 1999
Professor *de Fitopatologia do curso de graduação em Agronomia*
Professor *do Programa de Pós-Graduação em Agronomia (PGA)*
Chefe *do Departamento de Agronomia (DAG)*

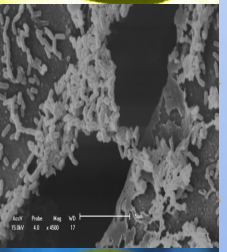
ENADE NOTA 5 AGRONOMIA UEM

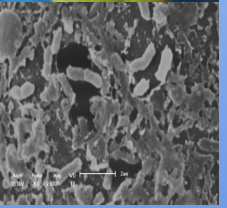
Programa de Pós-graduação em Agronomia
PGA/UEM
Conceito 6 Programa de Excelência CAPES

Parte 2

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**





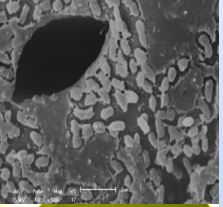


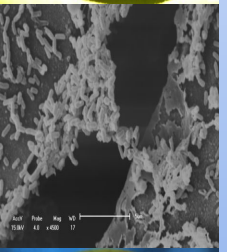
CANCRO CÍTRICO

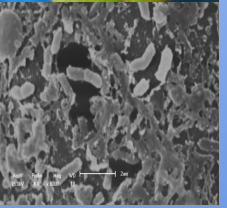
Brasil – 1957 em Presidente Prudente

Paraná – 1957 em Lupionópolis

Hoje no Brasil: São Paulo, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Paraná, Santa Catarina e Rio Grande do Sul

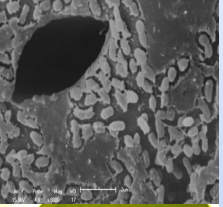


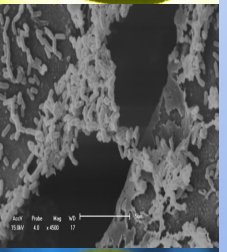


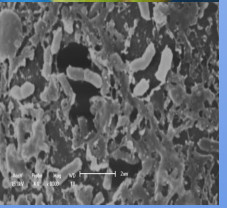


CANCRO CÍTRICO

- doença causada pela bactéria *Xanthomonas citri* subsp. *citri*
- ataca todas as espécies e variedades de citros

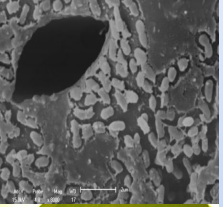


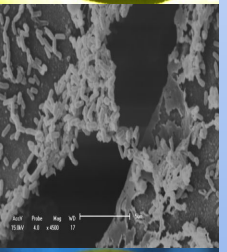


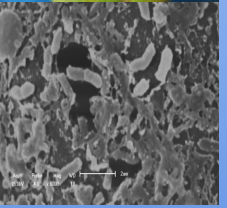


CANCRO CÍTRICO

- provoca lesões nas folhas, nos frutos e nos ramos
- fácil disseminação

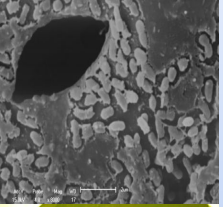


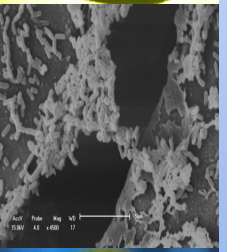


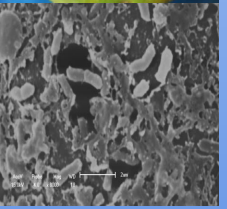


CANCRO CÍTRICO

- cancrose A afeta todas as variedades de citros - disseminada em várias regiões da Ásia, África, Oceania e Américas
- cancrose B é mais agressiva em limões verdadeiros e lima ácida - ocorrência restrita à Argentina, Paraguai e Uruguai
- cancrose C afeta a lima ácida Galego -restrita a algumas regiões do Estado de São Paulo





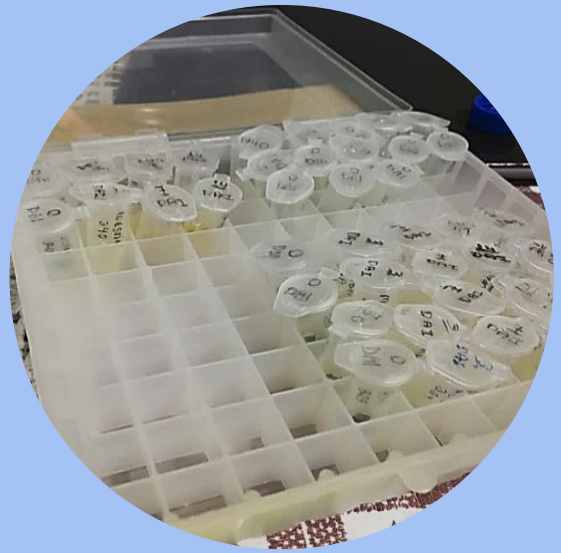


Xanthomonas citri subsp. *citri*

- gram negativa
- bastonete reto
- monotrica
- aeróbia
- colônia amarela
- dimensões: 0,4-0,7 x 0,7-1,6 µm
- temperatura ótima de 28–30ºC para desenvolvimento





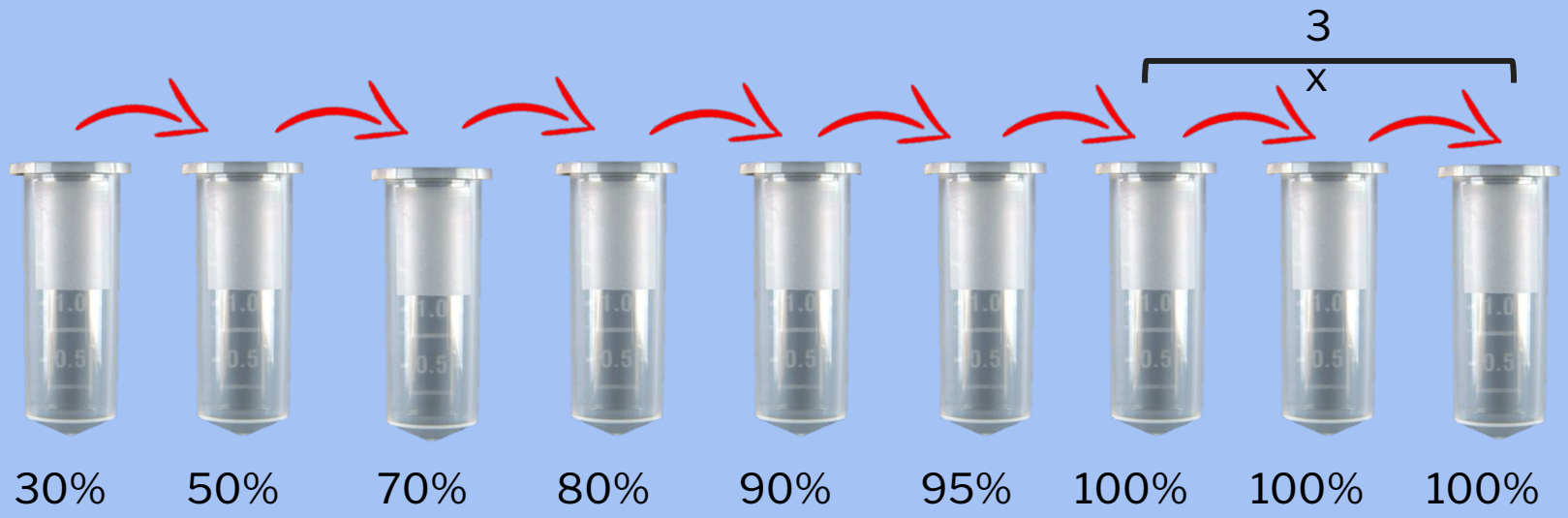


Material vegetal fixado em solução tampão com Cacodilato de sódio 0,1M e Glutaraldeído 2,5%

Desidratação em gradiente crescente de etanol por 10 minutos.

(30, 50, 70, 80, 90, 95 e 100%*)

*No etanol 100%, a desidratação é repetida três vezes.



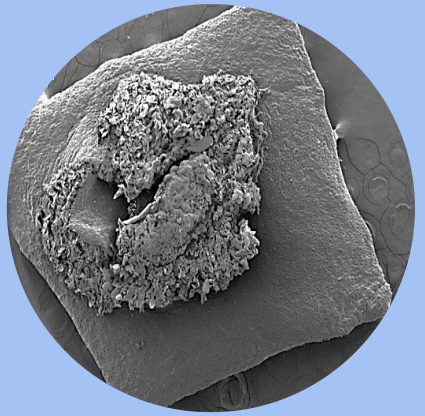

BAL-TEC
CPD



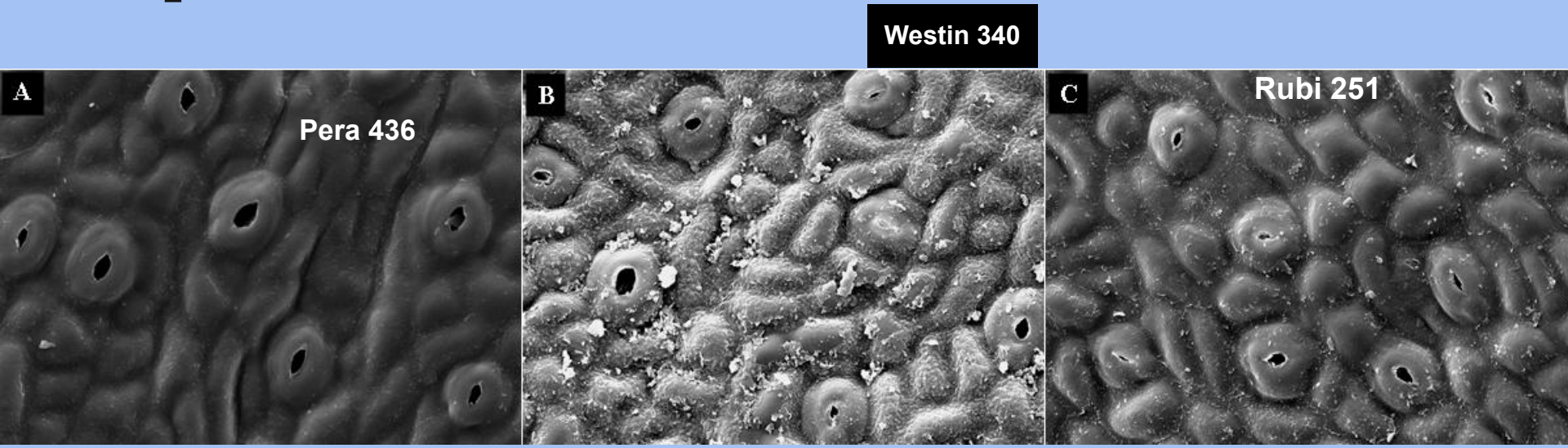






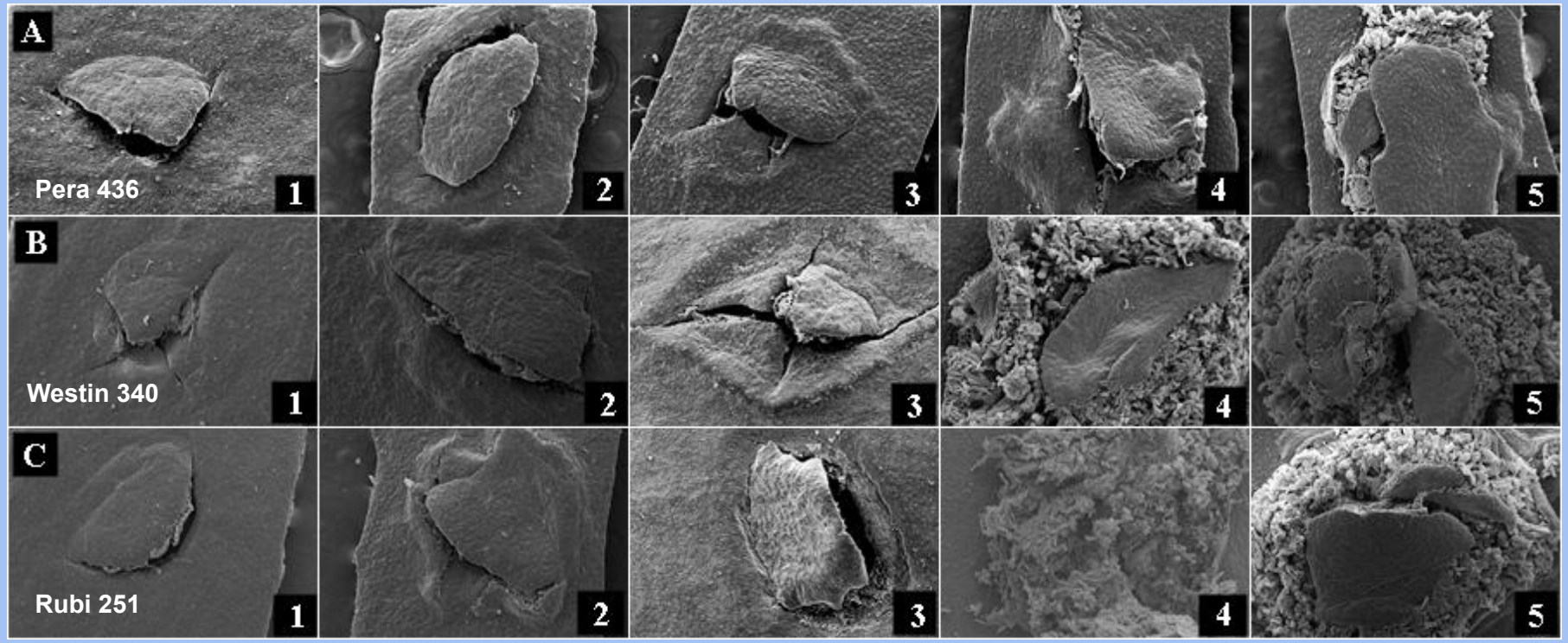


Superfície foliar



Diferenças na estrutura foliar dos genótipos. A: ‘Pera 436’ genótipo considerado mais resistente; B: ‘Westin 340’ e C: ‘Rubi 251’ genótipos considerados mais susceptíveis.

A presença de uma camada de cera mais ou menos resistente e a presença de mais ou menos estruturas na superfície foliar podem facilitar a aderência da bactéria na folha.

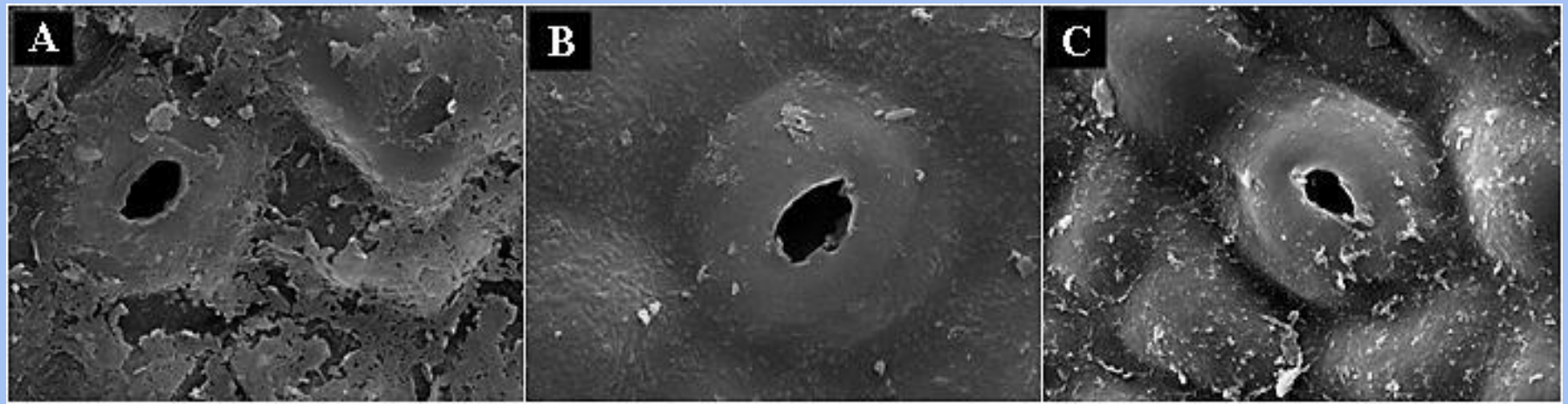


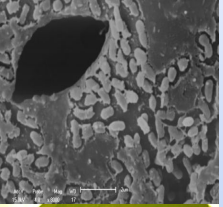
Doença ao longo dos DAI

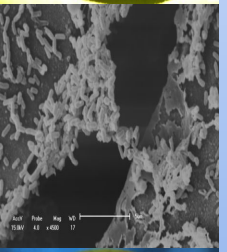
Produção de Exsudatos

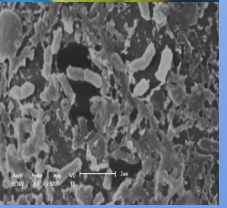
A planta apresenta diversas maneiras para tentar bloquear a entrada do patógeno no seu interior. **Uma delas é a produção de exsudatos.**

A:‘Pera 436’ **B:‘Westin 340’** **C:‘Rubi 251’**



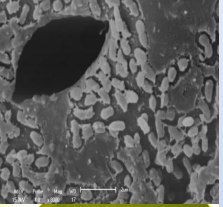


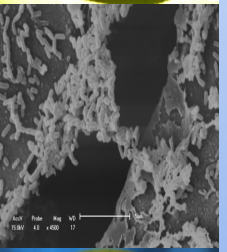


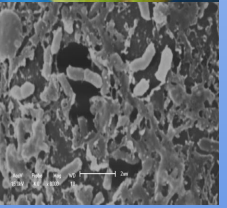


Xanthomonas citri subsp. *citri*

- ausência de plantas cítricas, há declínio na população da bactéria em solos
- sobrevive por vários anos em tecidos desidratados
- lesões nos tecidos que contêm a bactéria representam a principal forma de sobrevivência do patógeno

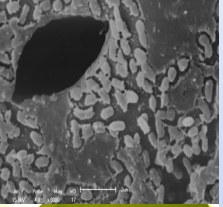


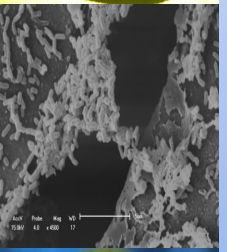


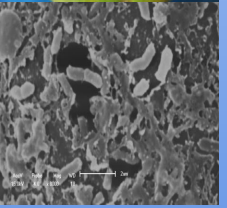


Xanthomonas citri subsp. *citri*

- a bactéria penetra em tecidos novos pelas aberturas naturais ou por ferimentos produzidos por espinhos e insetos

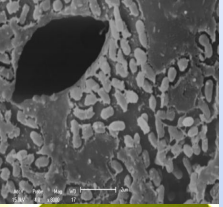


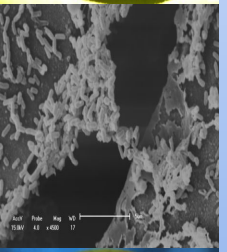


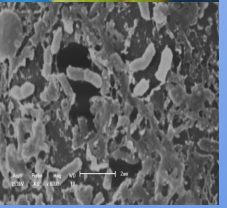


Diagnóstico

- Amostras de parte da planta com sintomas;
- enviar ao Laboratório:
  - isolamento da bactéria;
  - testes com uso serologia;
  - testes com uso de ferramentas moleculares (DNA).
  -

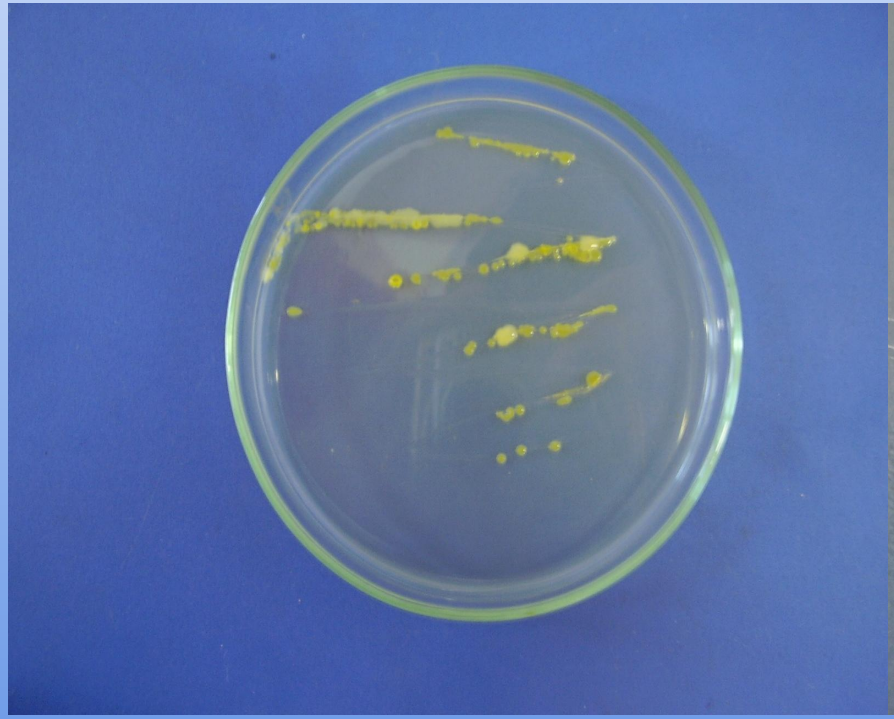
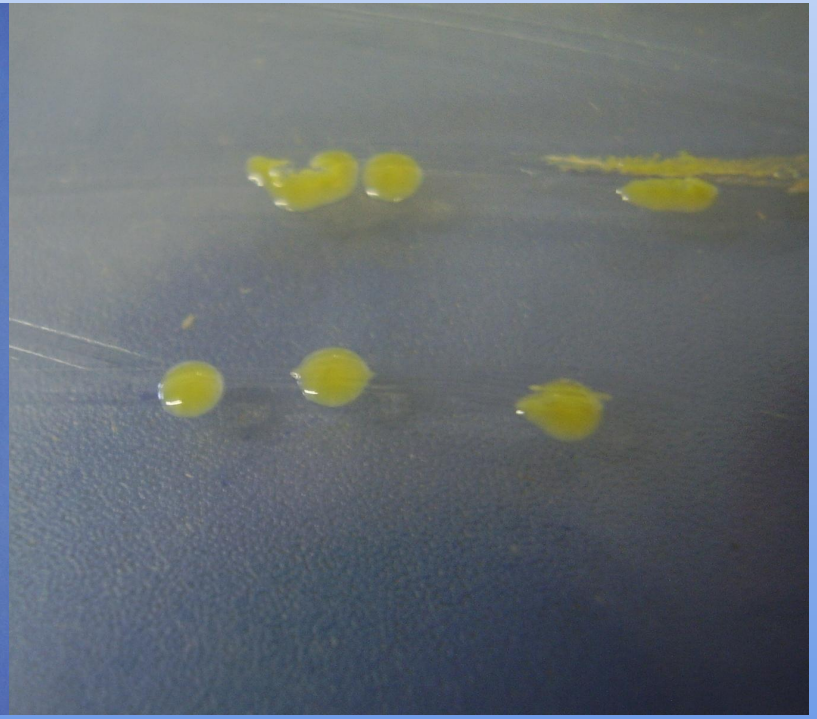






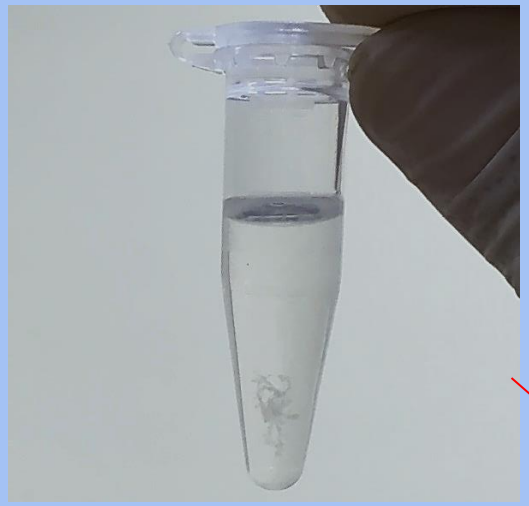
Diagnóstico

- isolamento da bactéria:

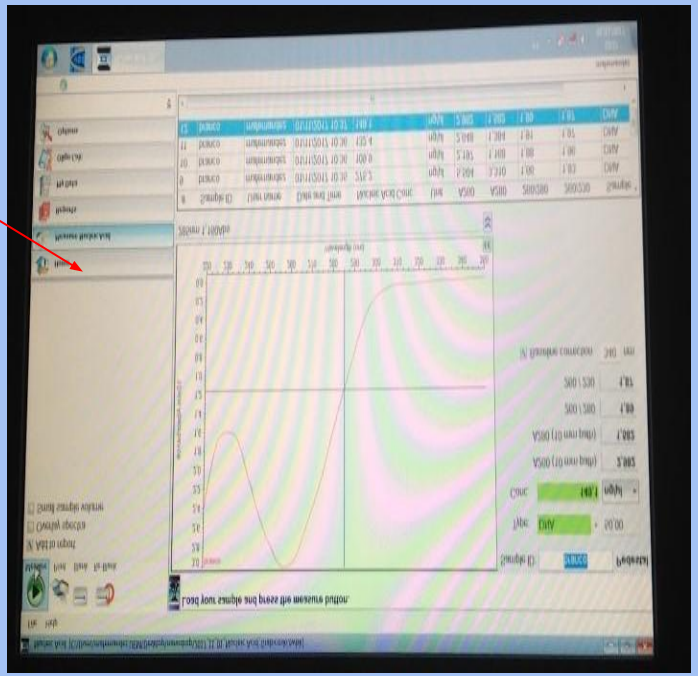



Diagnóstico

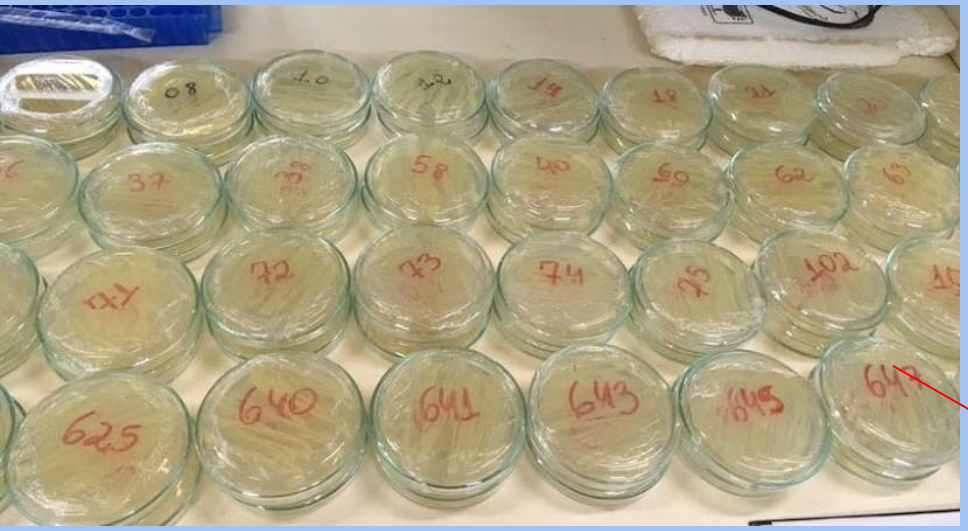
Testes com uso de ferramentas moleculares (DNA):

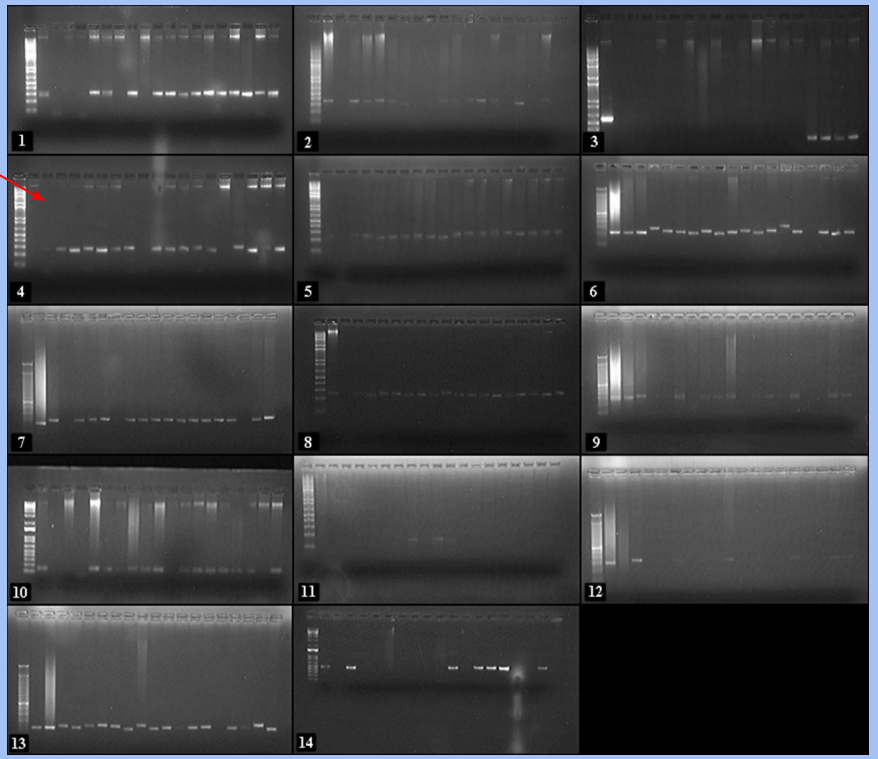




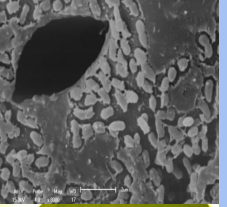


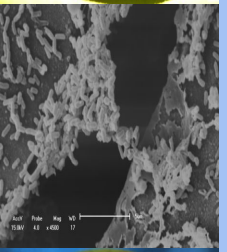
Diagnóstico

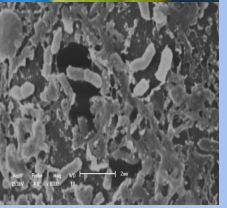




Testes com uso de ferramentas moleculares (DNA):

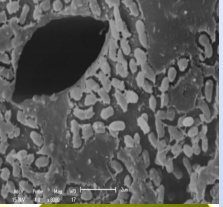


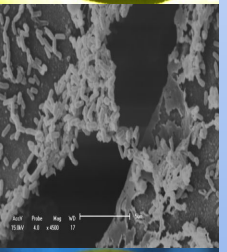


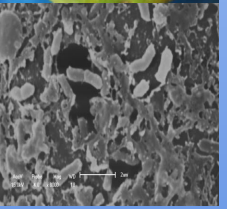


Sintomas

- Lesões salientes, eruptivas e nas folhas aparecem em ambos os lados.
- Aparecem também em ramos e frutos (depreciam comercialmente).
- Na maioria das vezes as lesões apresentam halo amarelado (visível em folhas e frutos verdes).







Sintomas

- erupções salientes, puntiformes e de coloração creme na superfície do tecido afetado

- folhas: as erupções aparecem nos dois lados da folha, e no estágio mais avançado, as lesões velhas ficam corticosas, com centro pardo e um halo amarelo

Sintomas – Folhas





- pequenas lesões salientes, sintomas iniciais do cancro cítrico

Sintomas – Folhas





- lesões circundadas por anel amarelado

Sintomas – Folhas



- lesões corticosas nas duas faces das folhas

Sintomas - Frutos

- pequenas manchas amarelas, com um ponto marrom no centro
- manchas são salientes, mais superficiais, parecidas com verrugas, de cor marrom no centro
- em estágio avançado, as lesões provocam o rompimento da casca

Sintomas - Frutos

- lesões de cancro cítrico não se destacam facilmente da casca de frutos doentes, como as de verrugose, e esta é uma das maneiras de diferenciar as duas doenças quando nos frutos as manchas são salientes, mais superficiais, parecidas com verrugas, de cor marrom no centro.

Sintomas – Frutos



- lesões causadas pelo cancro cítrico em frutos

Sintomas – Frutos



- manchas marrons salientes

Sintomas – Frutos



- Lesões vão se aglutinando e podem causar o rompimento da casca

Sintomas - Ramos



- ramos: Os sintomas nos ramos também são salientes, com lesões corticosas de coloração creme

Sintomas – Ramos





- lesões causadas pelo cancro cítrico em ramos

Sintomas – Ramos



- lesões salientes e de cor parta em ramos

Sintomas – Ramos



- crostas em ramos



Sintomas do cancro cítrico em fruto



Halo amarelo ao redor das lesões



Sintomas de cancro cítrico e verrugose (fungo Elsinöe fawcetti)



Alta severidade da doença em frutos maduros (não é possível observar halos)

Disseminação

- o principal responsável – homem
- curta distância (dentro do próprio pomar): vento associado com chuva,
- média a longa distância (entre pomares e propriedades vizinhas, até entre países): mudas contaminadas e materiais cítricos contaminados

Disseminação

- Larva minadora dos citros (1996)



Disseminação

- larva minadora de citros



Disseminação

- Após ter surgido a primeira lesão, formam-se 1.000.000 de bactérias



Disseminação





Prevenção e Controle

- implantação e renovação do pomar, utilizando mudas sadias
- evitar a instalação de pomares em áreas sujeitas a ventos fortes e constantes
- instalando quebra-ventos e cerca-viva na propriedade

Prevenção e Controle

- pulverizar com bactericidas cúpricos para proteção das novas brotações

- controle da larva minadora com inseticida ou parasitóides

Prevenção e Controle

- plantar cultivares resistentes ou moderadamente resistentes
- inspecionar freqüentemente os pomares
- promover a lavagem e a desinfestação de veículos, máquinas e implementos em pomares (arco rodolúvio)

Prevenção e Controle

- construir silos na entrada das propriedades para o armazenamento dos frutos colhidos
- para as regiões onde a doença não é endêmica, o único método de eliminação da bactéria é a erradicação das árvores contaminadas e todas as demais contidas no raio de 30 metros

Prevenção e Controle



- Erradicação de plantas focos e raio de 30 metros

Arco Rodolúvio



Bins



Mudas Certificadas



Mudas Certificadas



Quebra-Ventos



Quebra-Ventos



Quebra-Ventos



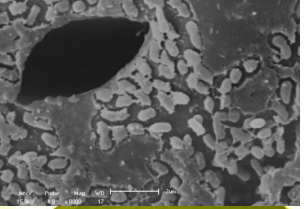

Prof.Dr. Roberto Rezende
Dr. Clive Bock
Prof.Dr.William Nunes
Prof.Dr. Paulo Freitas
Prof.Dr. Lincoln Zotarelli
Prof.Dr. Antonio Costa

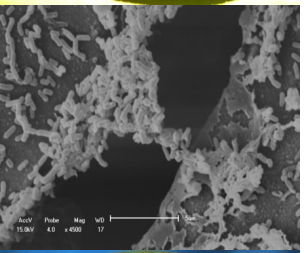
Programa de Pós-graduação em Agronomia
PGA/UEM
Conceito 6
Programa de Excelência
CAPES

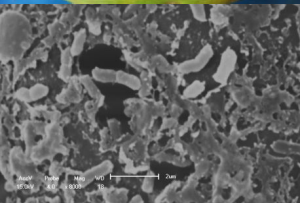
NBA
Núcleo de Pesquisa em Biotecnologia Aplicada
UEM Maringá-PR

Projetos de pesquisa aplicados ao cancro cítrico

Trabalhos desenvolvidos no Núcleo de Pesquisa Aplicada em Biotecnologia







Universidade Estadual de Maringá
Programa de Pós-graduação em Agronomia
Núcleo de Pesquisa em Biotecnologia Aplicada (NBA)

AVALIAÇÃO DA RESISTÊNCIA DE GENÓTIPOS DE LARANJA DOCE (*Citrus sinensis*) AO CANCRO CÍTRICO E ESTUDO DA DIVERSIDADE GENÉTICA DE *Xanthomonas citri* subsp. *citri*

Dra. Aline M. Orbolato Gonçalves-Zuliani
Prof. Dr. William Mário de Carvalho Nunes

MATERIAL E MÉTODOS

MICROSCOPIA ELETRÔNICA DE VARREDURA (MEV)



cortes de 5x5 mm - Long. – estilete Trans. - Criofratura -Antes da inoculação -3°, 7° e 14° DAI

Fixado (Tampão) - Geladeira

Desidratação - Etanol

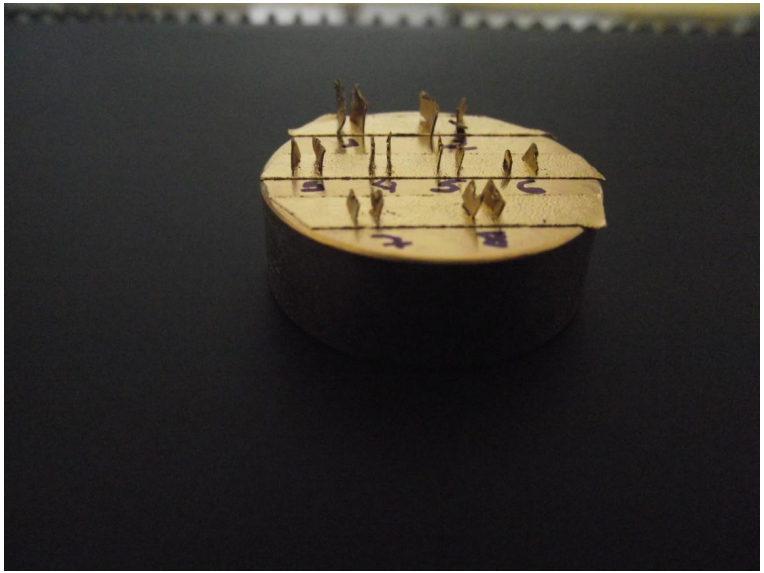
Ponto crítico

Metalização em ouro

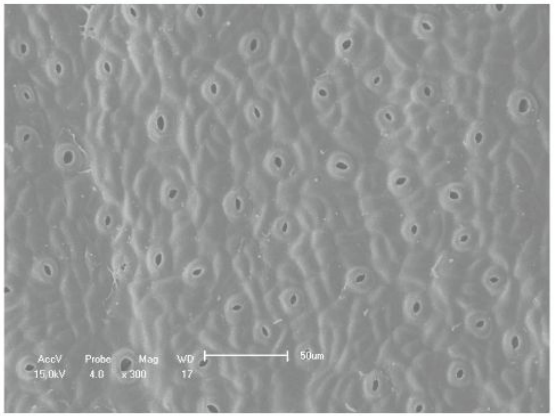


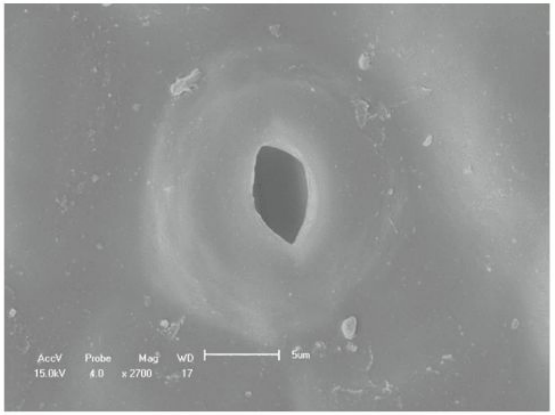
MICROSCOPIA ELETRÔNICA DE VARREDURA (MEV)



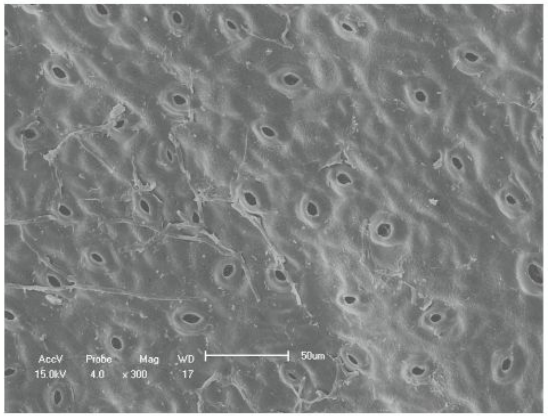


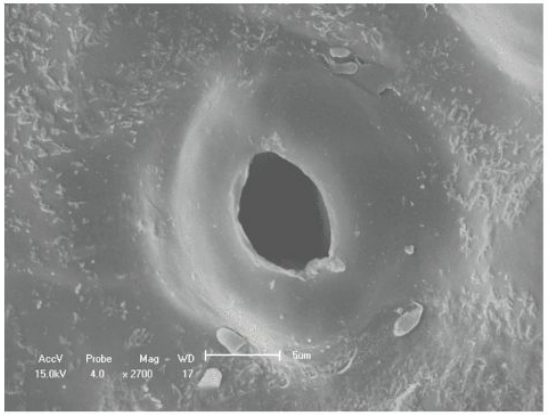
Pêra IAC

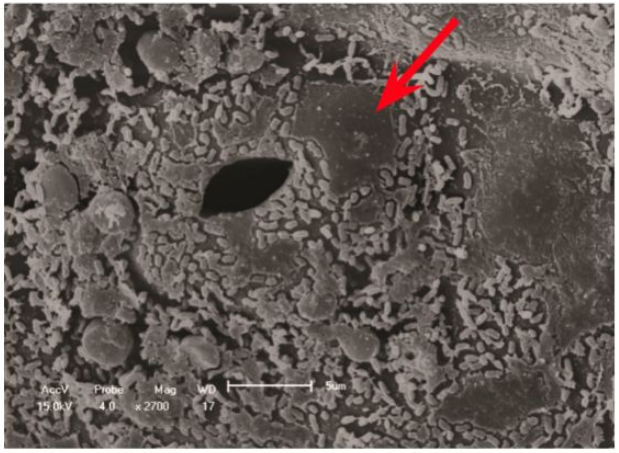
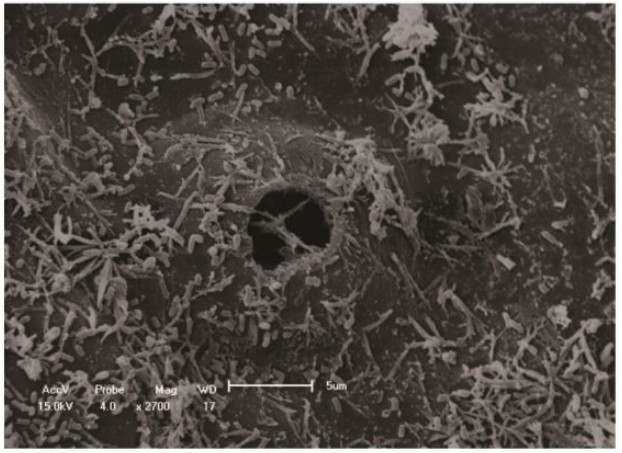
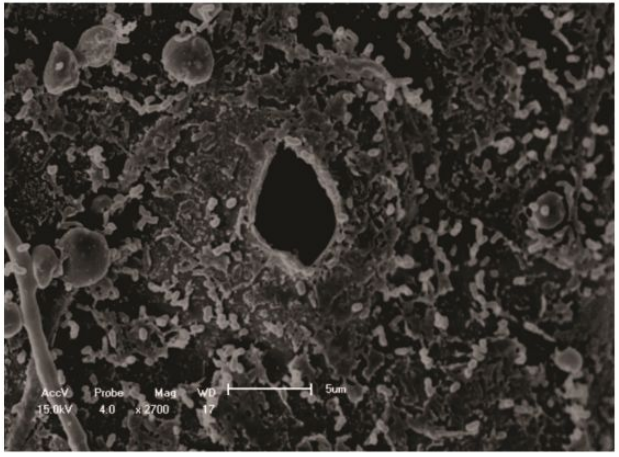
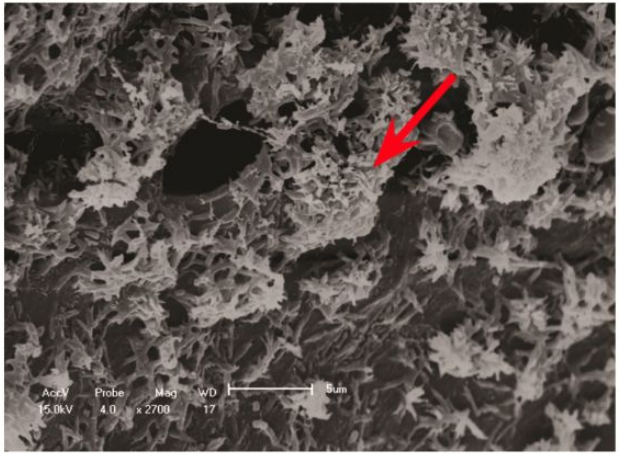


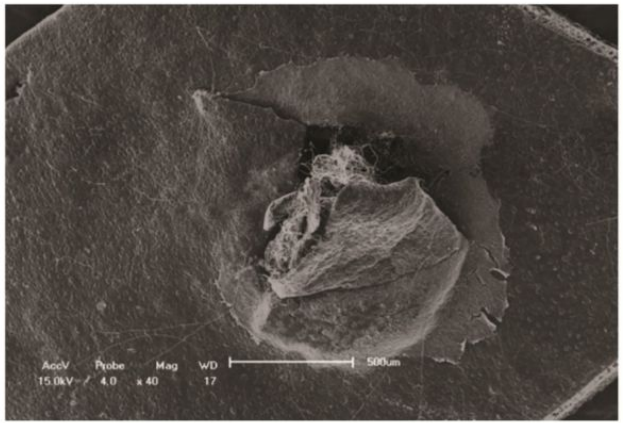
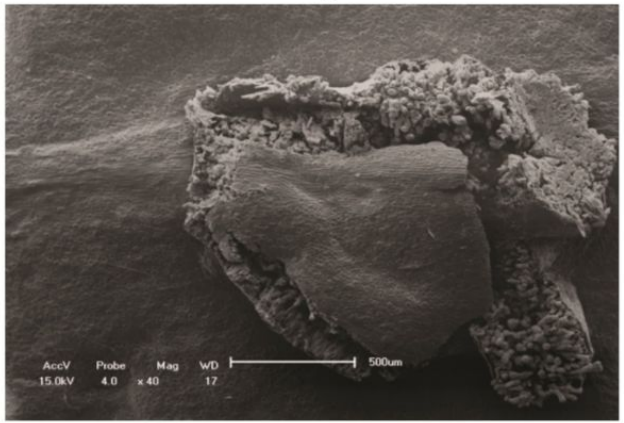
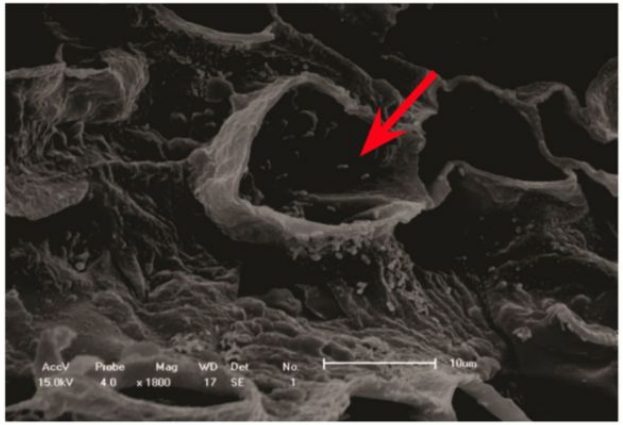
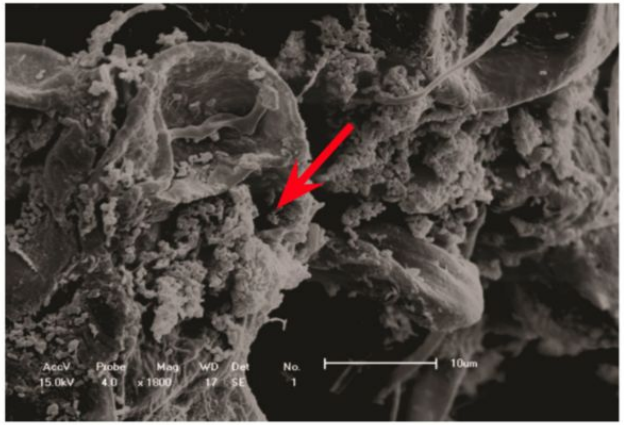


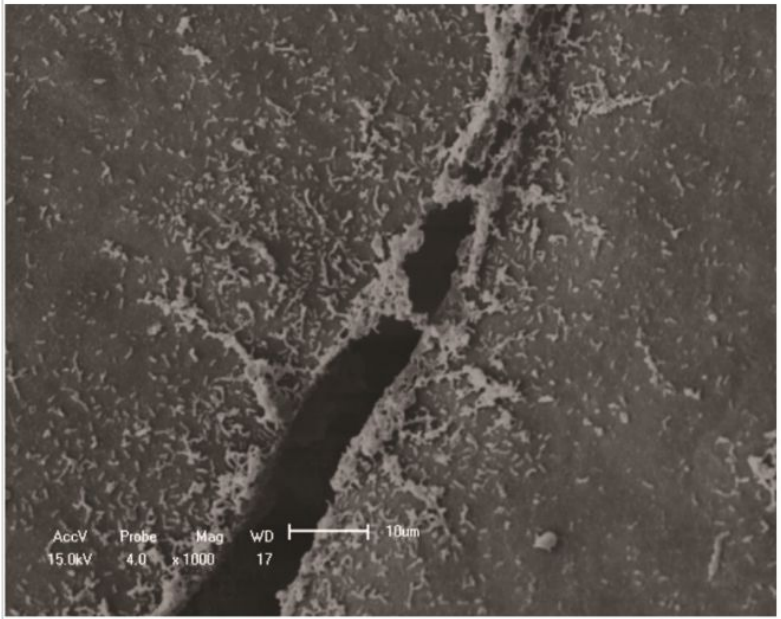
Washington navel





	Pêra IAC	Washington navel
3º DAI		
7º DAI		

	Pêra IAC	Washington navel
14º DAI		
14º DAI		



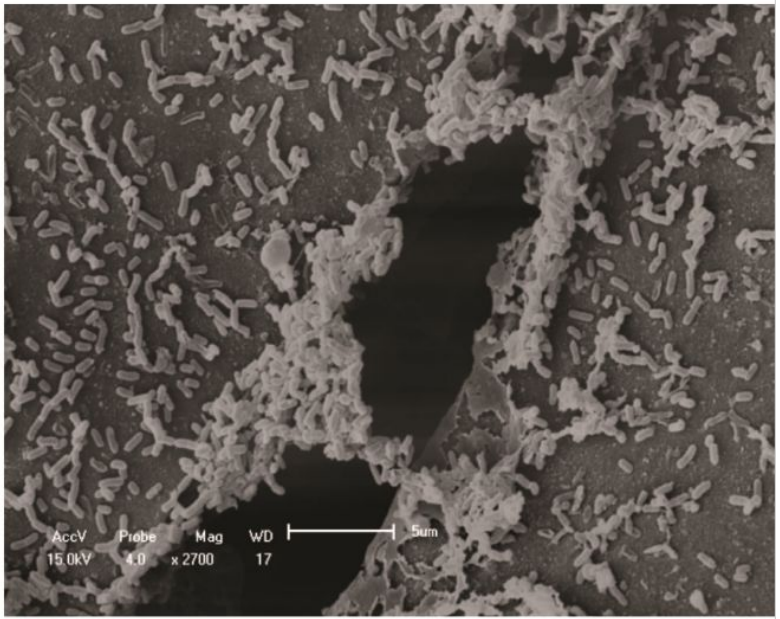


Figura 5. Fotos representativas do ferimento, provocado pela inoculação com perfuração com agulha, na face adaxial de folhas do genótipo Pêra IAC, aos 3°DAI, com aumento de 1000 (A1) e 2700 (A2) vezes.

Equipe de pesquisa XCC

- **Andressa Cazetta**
  - Mestrado
  - Genótipos de citros inoculados com *Xanthomonas citri* subsp. *citri*: um estudo sobre severidade da doença e morfologia da planta.

- **Juliana Glória Franco Roberto**
  - Doutorado
  - Variabilidade Genética e Reação de Espécies Cítricas à Bactéria *Xanthomonas citri* subsp. *citri*

- **Priscila Rossetto**
  - Doutorado
  - Avaliação da resistência de genótipos de laranja doce (*Citrus sinensis*) ao cancro cítrico e expressão gênica da bactéria *Xanthomonas citri* subsp. *citri*

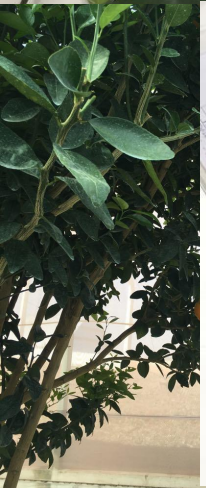
Genótipos de citros inoculados com *Xanthomonas citri* subsp. *citri*: um estudo sobre severidade da doença E morfologia da planta.

Objetivo: avaliar a severidade da bactéria *Xanthomonas citri* subsp. *citri* em 16 genótipos de citros (*Citrus* spp.), analisando se a morfologia da planta pode interferir nos níveis de severidade à bactéria.

Metodologia utilizada

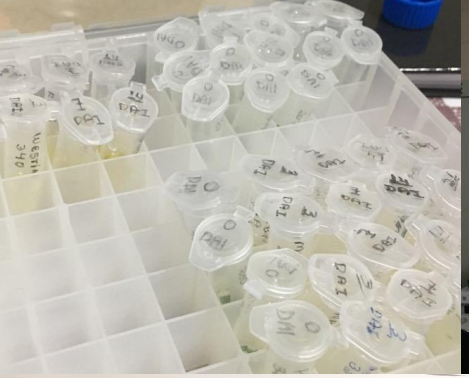
Análise da severidade da doença por folhas destacadas e a análise morfologia da planta através de microscopia eletrônica de varredura



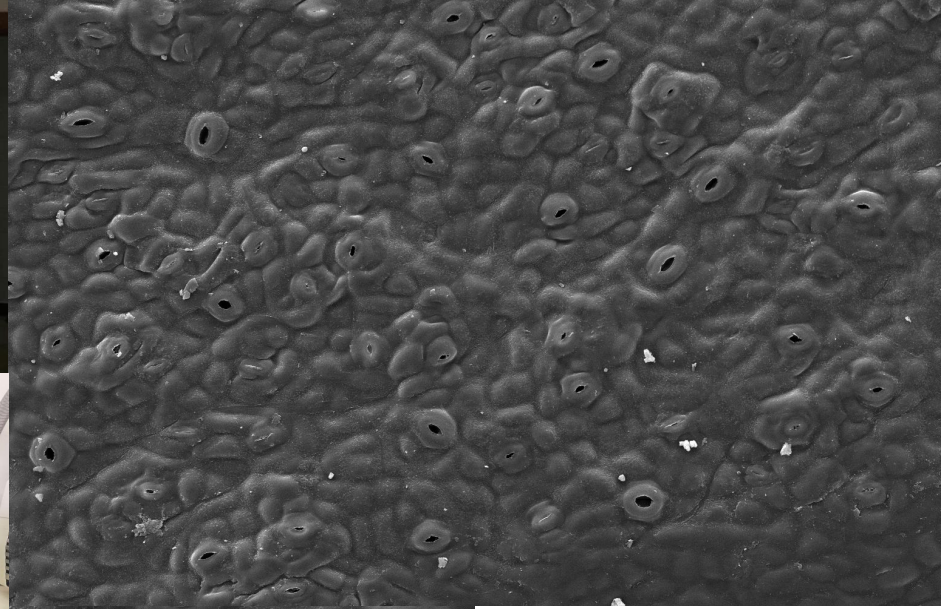




RUBI 25A

mm/inch
1.22 mm
OFF ON ZERO

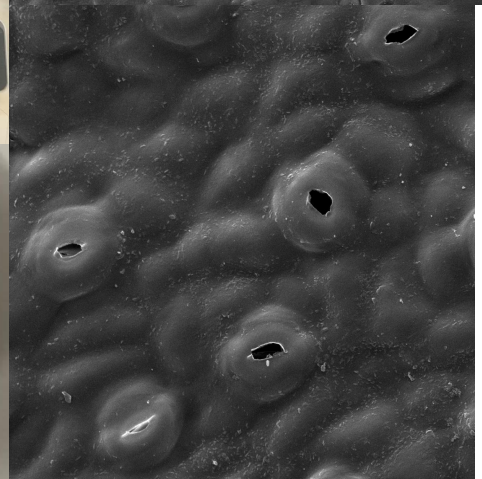



EDAX

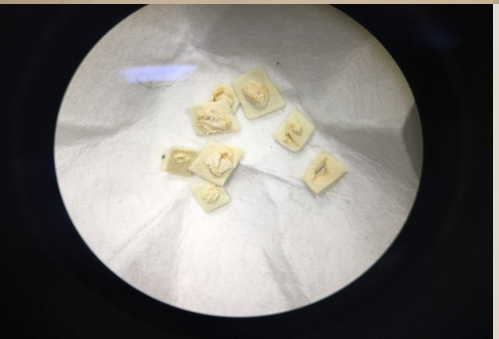



BAL-TEC
CPD 030

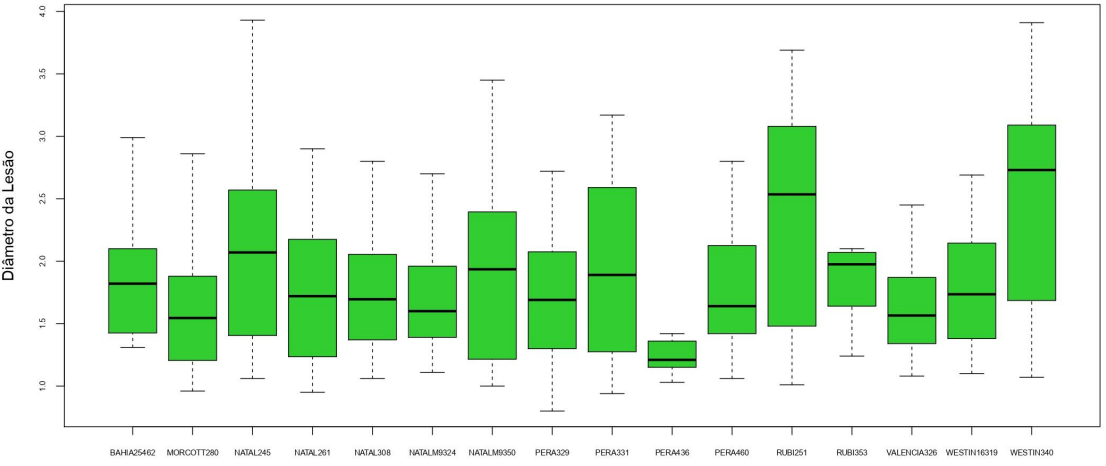

BAL-TEC
SCD 050

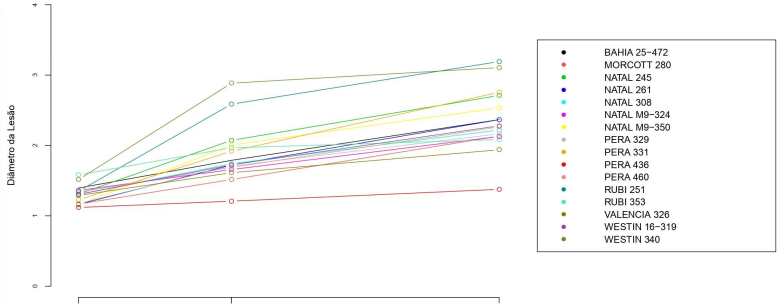


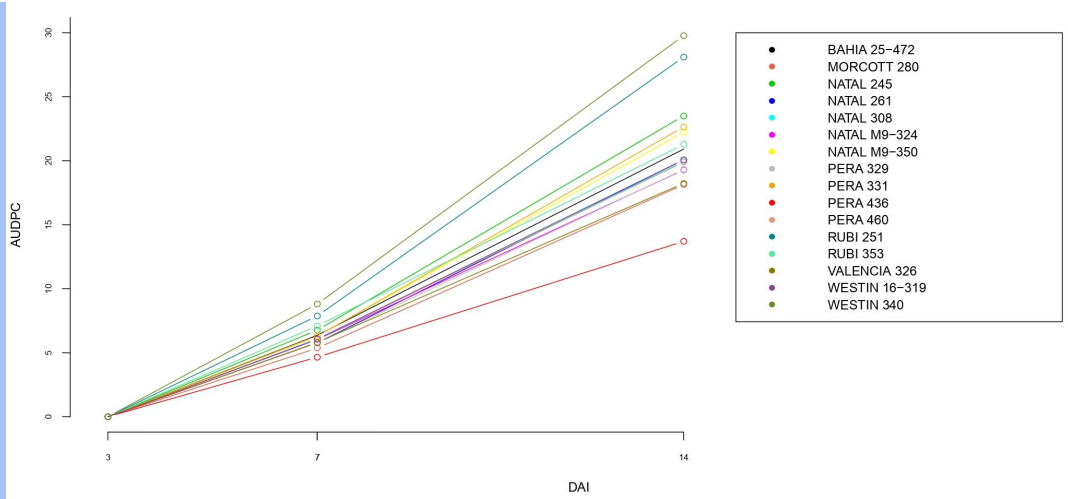


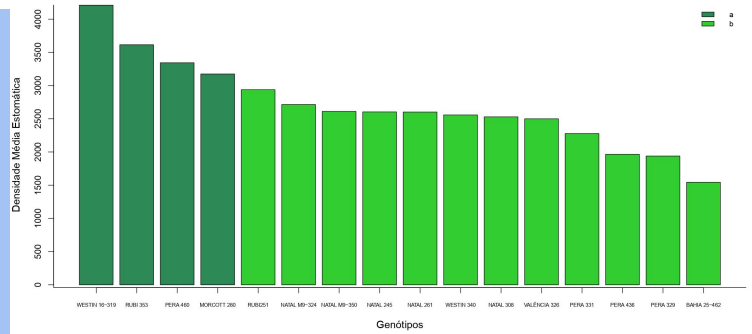


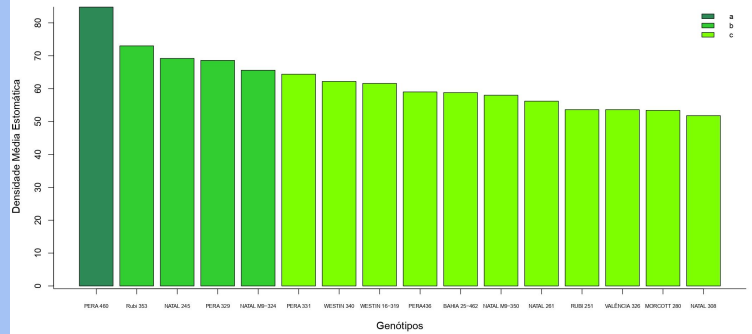



Diâmetro da Lesão
4.0
3.5
3.0
2.5
2.0
1.5
1.0
BAHIA25462
MORCOTT280
NATAL245
NATAL261
NATAL308
NATALM9324
NATALM9350
PERA329
PERA331
PERA436
PERA460
RUBI251
RUBI353
VALENCIA326
WESTIN16319
WESTIN340


Diâmetro da Lesão
BAHIA 25−472
MORCOTT 280
NATAL 245
NATAL 261
NATAL 308
NATAL M9−324
NATAL M9−350
PERA 329
PERA 331
PERA 436
PERA 460
RUBI 251
RUBI 353
VALENCIA 326
WESTIN 16−319
WESTIN 340


AUDPC
30
25
20
15
10
5
0
3
7
14
DAI
BAHIA 25−472
MORCOTT 280
NATAL 245
NATAL 261
NATAL 308
NATAL M9−324
NATAL M9−350
PERA 329
PERA 331
PERA 436
PERA 460
RUBI 251
RUBI 353
VALENCIA 326
WESTIN 16−319
WESTIN 340


Densidade Média Estomática
4000
3500
3000
2500
2000
1500
1000
500
0
a
b
WESTIN 16−319
RUBI 353
PERA 460
MORCOTT 280
RUBI251
NATAL M9−324
NATAL M9−350
NATAL 245
NATAL 261
WESTIN 340
NATAL 308
VALÊNCIA 326
PERA 331
PERA 436
PERA 329
BAHIA 25−462
Genótipos


Densidade Média Estomática
80
70
60
50
40
30
20
10
0
a
b
c
PERA 460
Rubi 353
NATAL 245
PERA 329
NATAL M9−324
PERA 331
WESTIN 340
WESTIN 16−319
PERA436
BAHIA 25−462
NATAL M9−350
NATAL 261
RUBI 251
VALÊNCIA 326
MORCOTT 280
NATAL 308
Genótipos

Projetos de pesquisa aplicados ao cancro cítrico

Trabalhos desenvolvidos no Núcleo de Pesquisa Aplicada em Biotecnologia

Variabilidade Genética e Reação de Espécies Cítricas à Bactéria *Xanthomonas citri* subsp. *citri*

Objetivos:

i) avaliar a severidade do cancro cítrico em tecidos de seis genótipos de citros em condições de casa de vegetação e folha destacada, e análise meticulosa dos estômatos e lesões em Microscópio Eletrônico de Varredura;

ii) comparar diferentes protocolos de extração de DNA de bactérias observando-se a concentração, pureza e integridade do DNA obtido, além da complexidade de execução do protocolo;

iii) avaliar a variabilidade genética de isolados de *Xanthomonas citri* subsp. *citri*, obtidos de diferentes estados do Brasil.

Variabilidade Genética e Reação de Espécies Cítricas à Bactéria *Xanthomonas citri* subsp. *citri*

Metodologia utilizada:

i) inoculação da bactéria *Xanthomonas citri* subsp. *citri* por ferimento através de agulha em 12 genótipos de citros ;

ii) visualização de características fenotípicas de tecidos vegetais de citros em Microscopia Eletrônica de Varredura;

iii) extração do DNA genômico da bactéria *X. citri* subsp. *citri* através de cinco diferentes protocolos e a quantificação do mesmo através de espectrofotômetro;

iiii) amplificação do DNA de 52 isolados de *X. citri* subsp. *citri* utilizando-se a técnica de marcadores moleculares microssatélites.

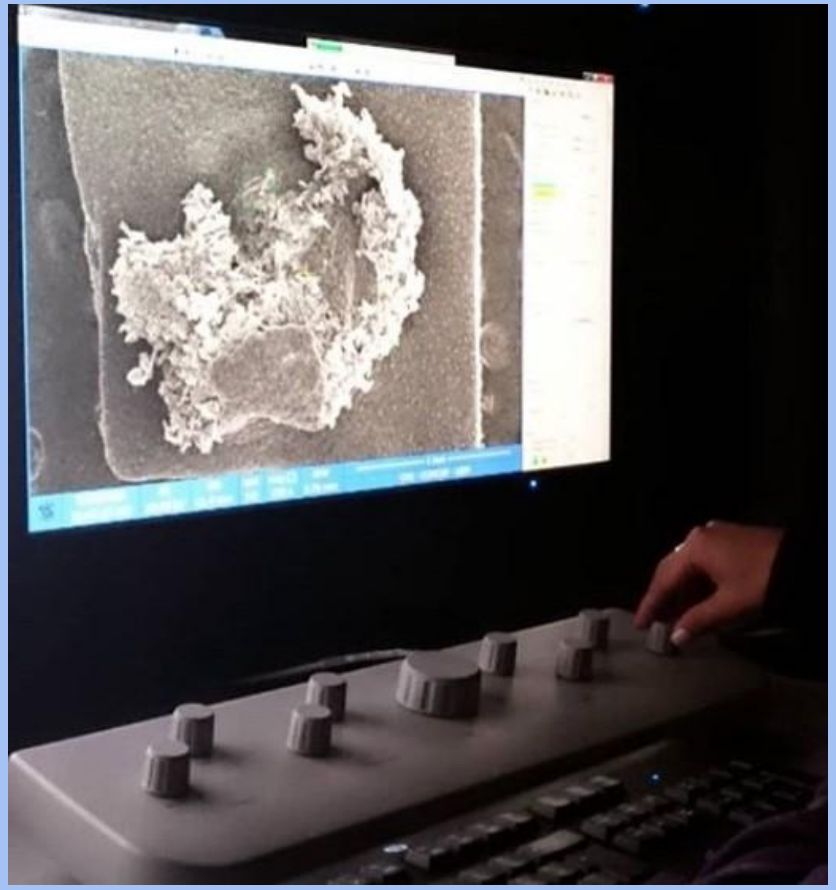


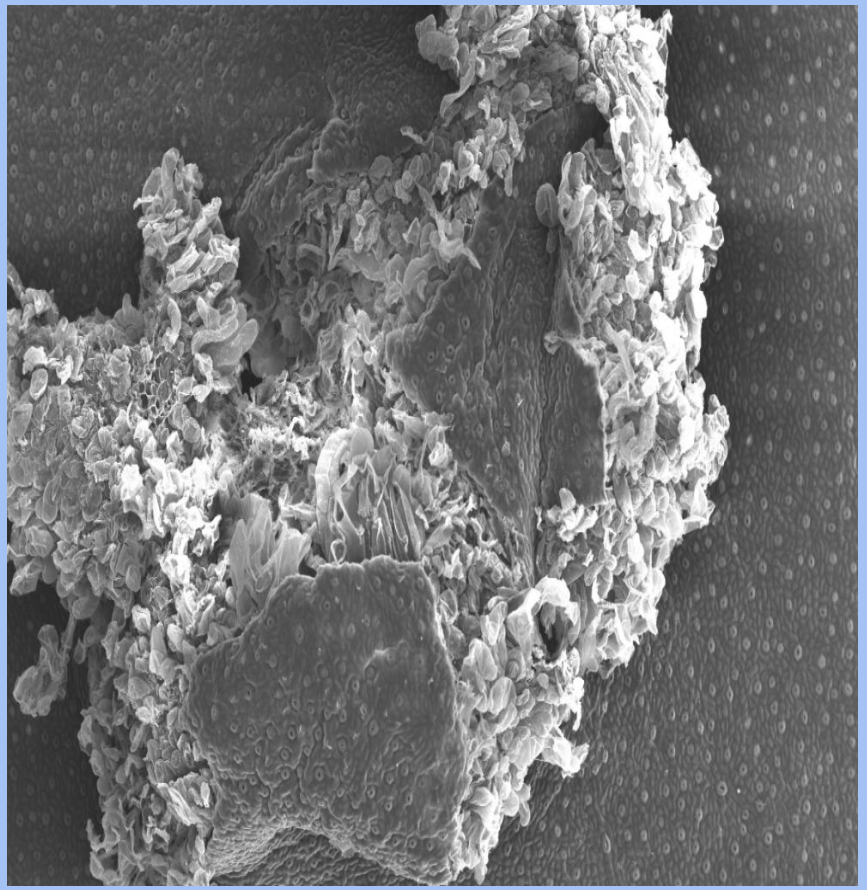

mm/inch
2.71
OFF ON ZERO
mm/inch
3.36
OFF ON ZERO

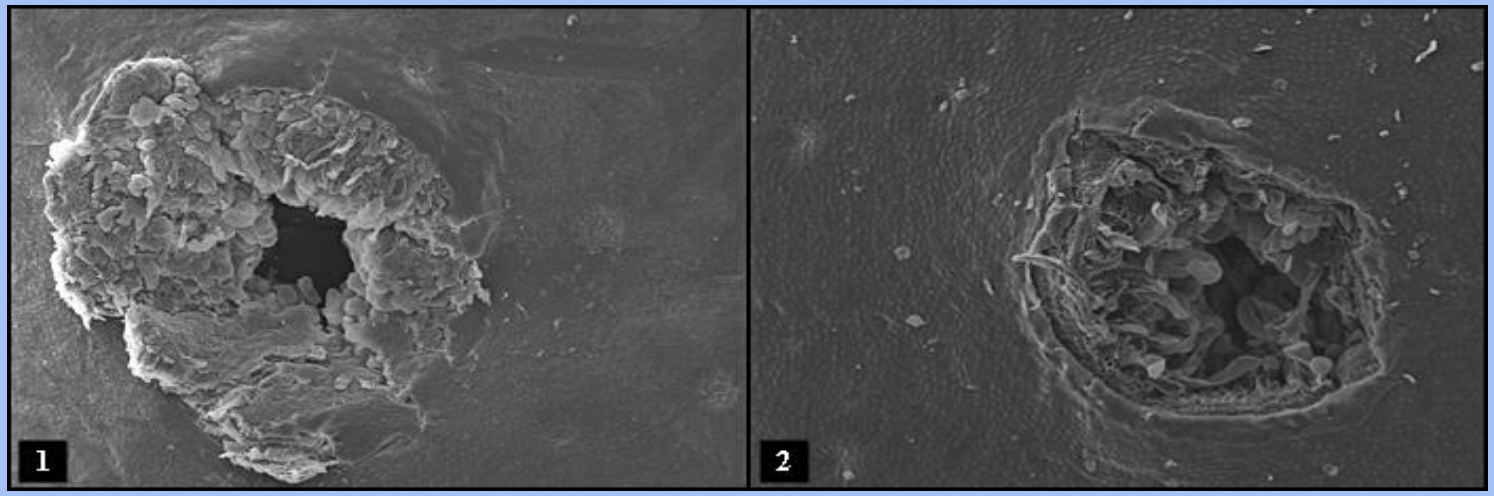

50
ml
45
40
35
30
25
20
15
10

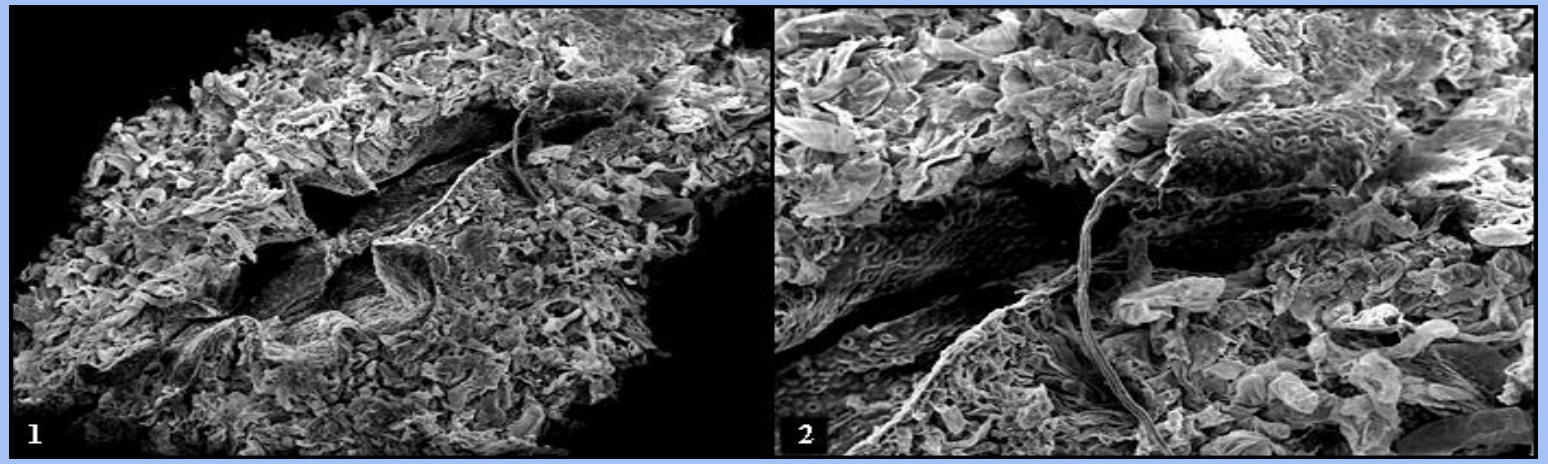


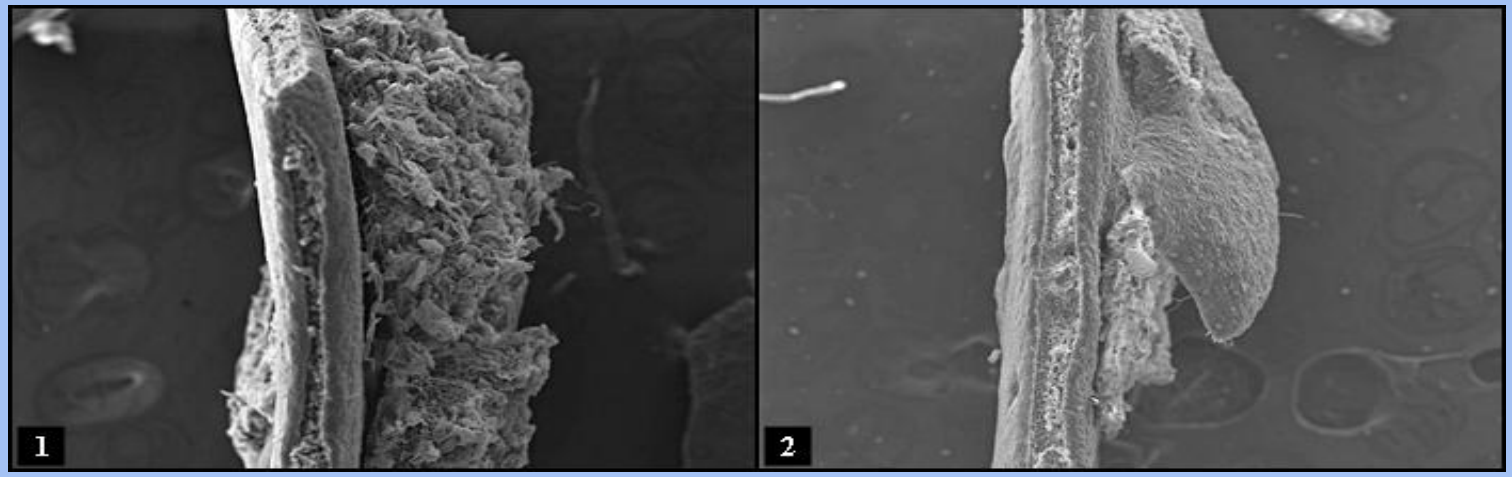

Puccinia Oxyspora

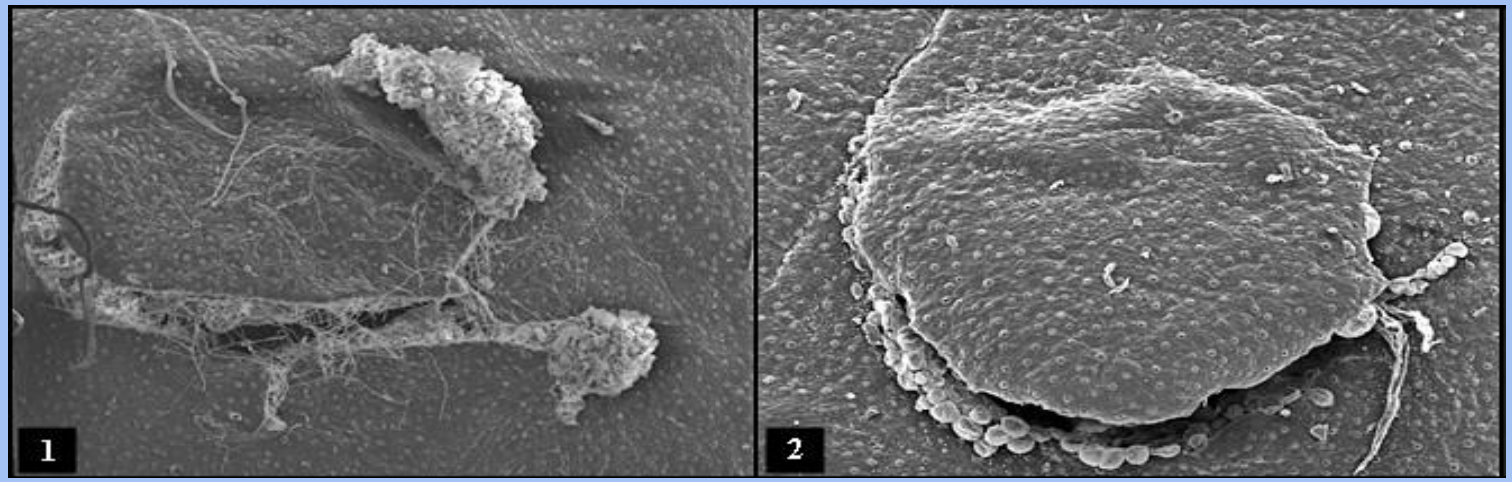


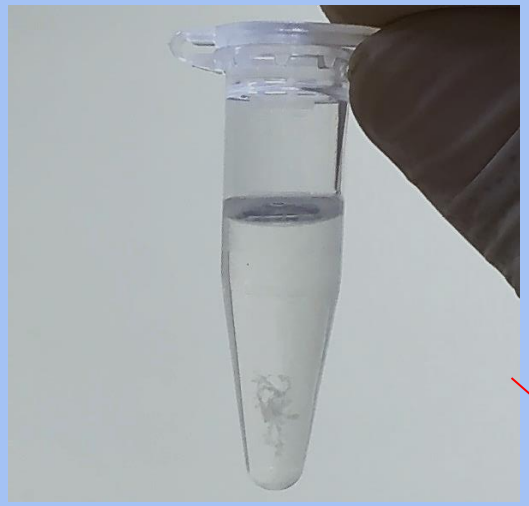



1
2

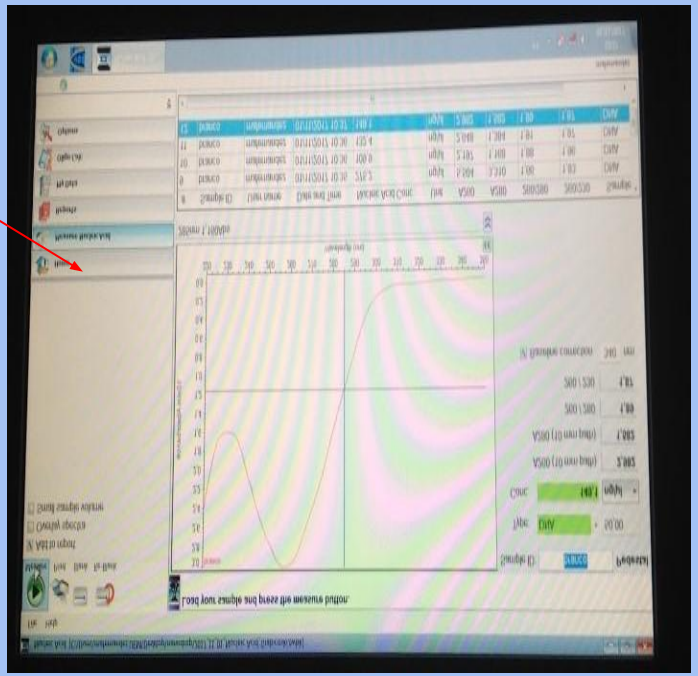

1
2

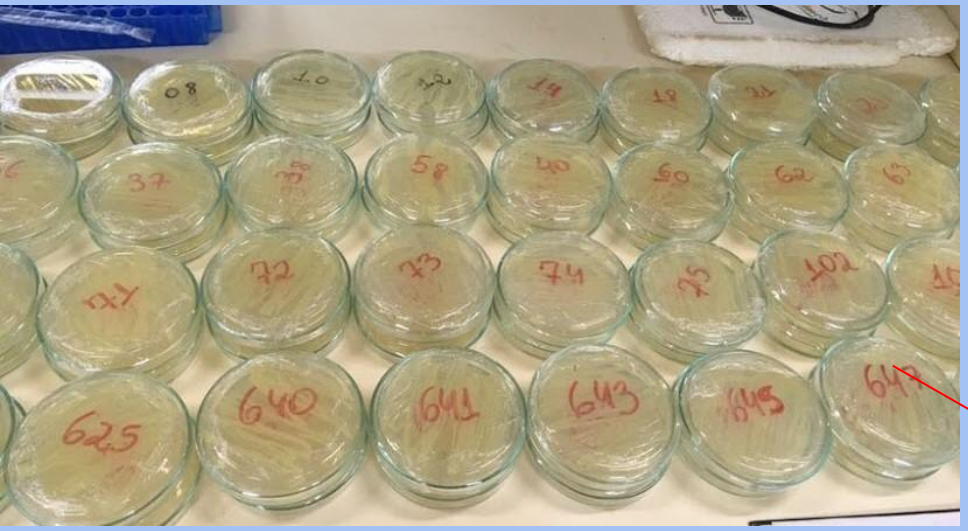

1
2

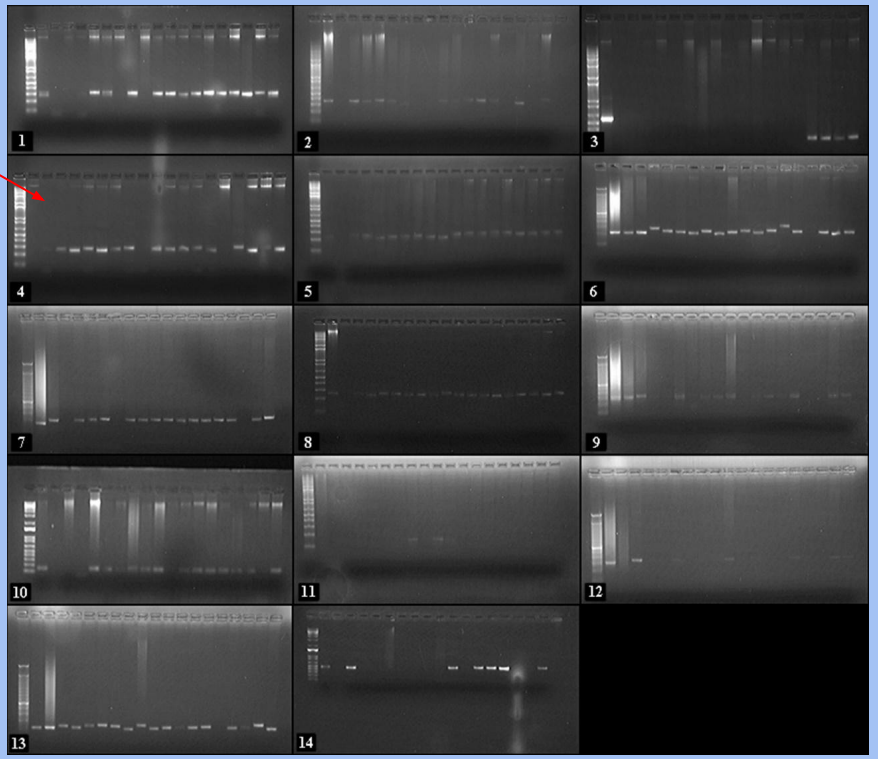

1
2




Thermo
SCIENTIFIC
NanoDrop 2000




625
640
641
643
645

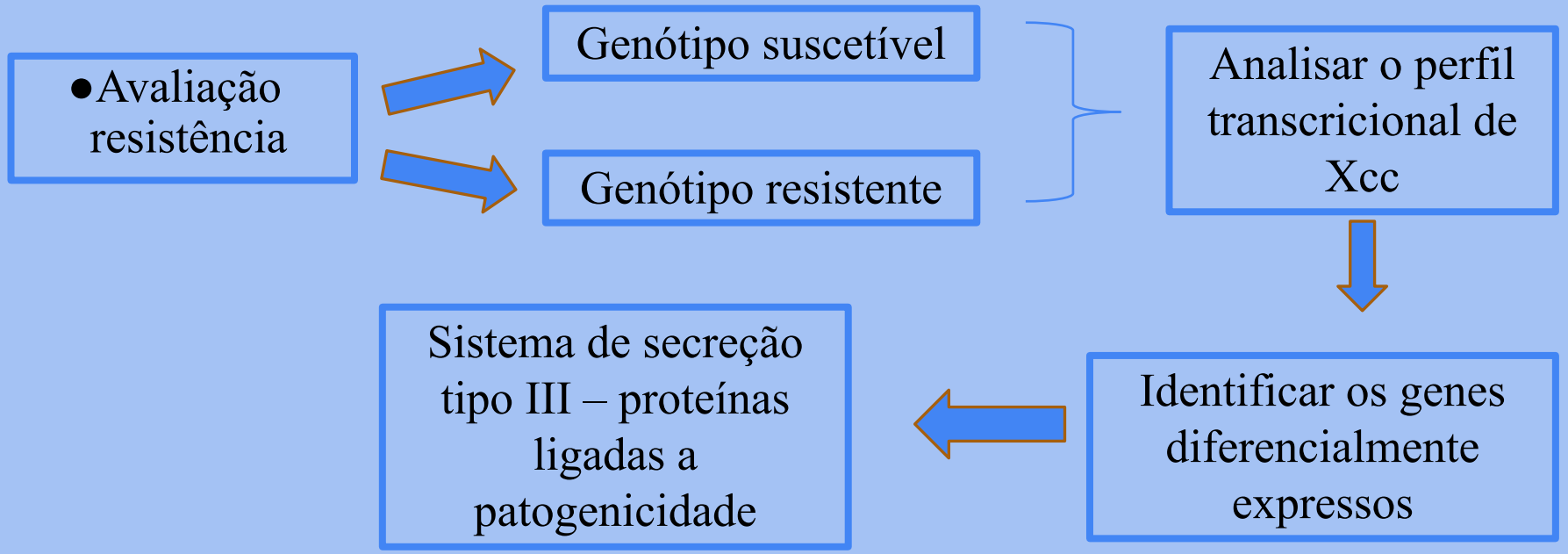

1
2
3
4
5
6
7
8
9
10
11
12
13
14

Avaliação da resistência de genótipos de laranja doce (*Citrus sinensis*) ao cancro cítrico e expressão gênica da bactéria *Xanthomonas citri* subsp. *citri*

Objetivos

- Avaliar a resistência de genótipos de laranja doce (*Citrus sinensis*) ao cancro cítrico;
- Analisar a expressão gênica da bactéria *Xanthomonas citri* subsp. *citri* quando inoculada em dois hospedeiros de citros contrastantes quanto à susceptibilidade;
- Analisar as alterações em relação aos fatores de resistência pós formados (estresse oxidativo) que ocorre nas plantas hospedeiras em resposta à presença do patógeno.

Metodologia



Metodologia

Genótipo suscetível

Genótipo resistente

Analisar as alterações no estresse oxidativo pela determinação da concentração de peróxido de hidrogênio (H_2O_2)

Relação patógeno-hospedeiro

Mecanismos moleculares utilizados pela bactéria para sobrepujar os mecanismos de defesa da planta com diferentes níveis de suscetibilidade ao cancro cítrico.

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

NBA
Núcleo de Pesquisa em Biotecnologia Aplicada
UEM Maringá-PR

CANCRO CÍTRICO

A influência da pesquisa científica no campo

Andressa Cazetta
Juliana Glória Franco Roberto
Priscila Rosseto

A citricultura brasileira

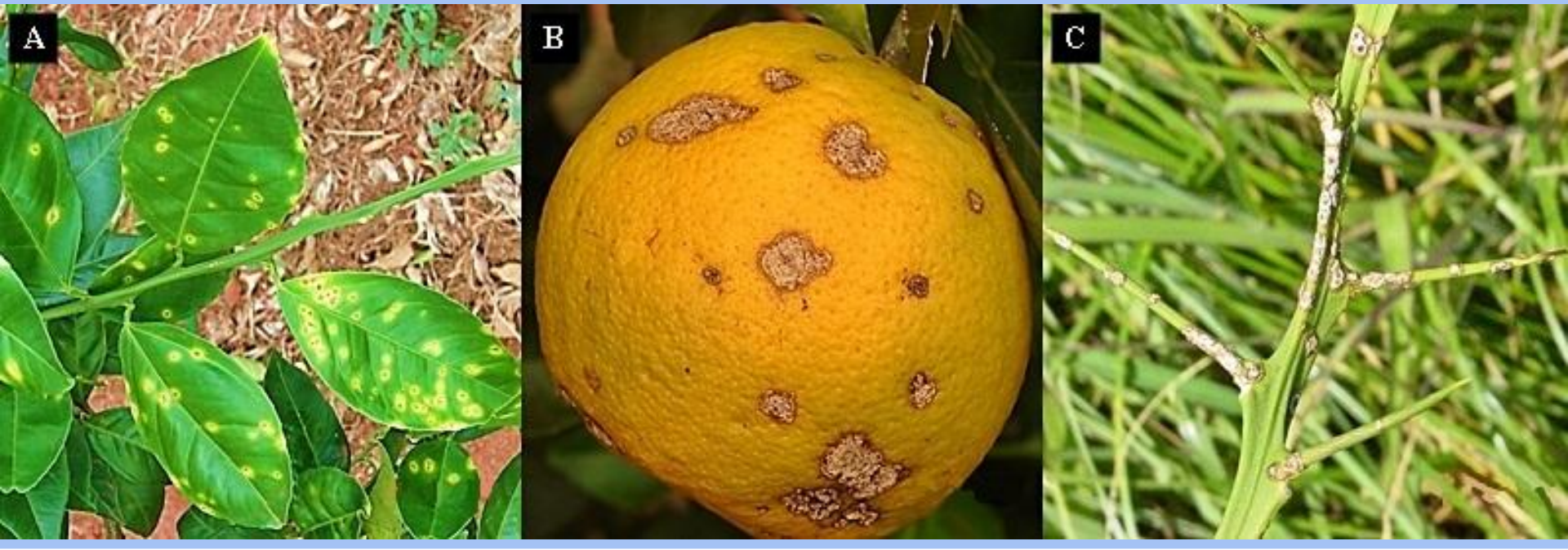
- O Brasil é o maior produtor e exportador mundial de laranja;
- Anualmente movimenta mais de US$ 14,5 bilhões, contribuindo com US$ 6,5 bilhões de PIB (Produto Interno Bruto);
- Principais estados produtores: São Paulo, Minas Gerais e Paraná;
- Produção da última safra: 386,79 milhões de caixas de laranja;
- Exportação: 861.172 mil toneladas do suco de laranja concentrado e congelado e do suco não concentrado para mais de 20 países;
- No ano de 2020 a citricultura gerou 6,33% do total de novas admissões ocorridas na agricultura.

Cancro cítrico

- **Causado pela bactéria *Xanthomonas citri* subsp. *citri***
- **Presente no Brasil desde 1957;**
- **No ano de 2019 foi constatado em 15,01% das árvores do cinturão citrícola brasileiro – 29,3 milhões de plantas;**
- **Índice 28% superior a 2018;**
- **Desfolhação e abscisão prematura de frutos ocorrem em árvores fortemente infectadas;**
- **É uma doença endêmica no Paraná.**

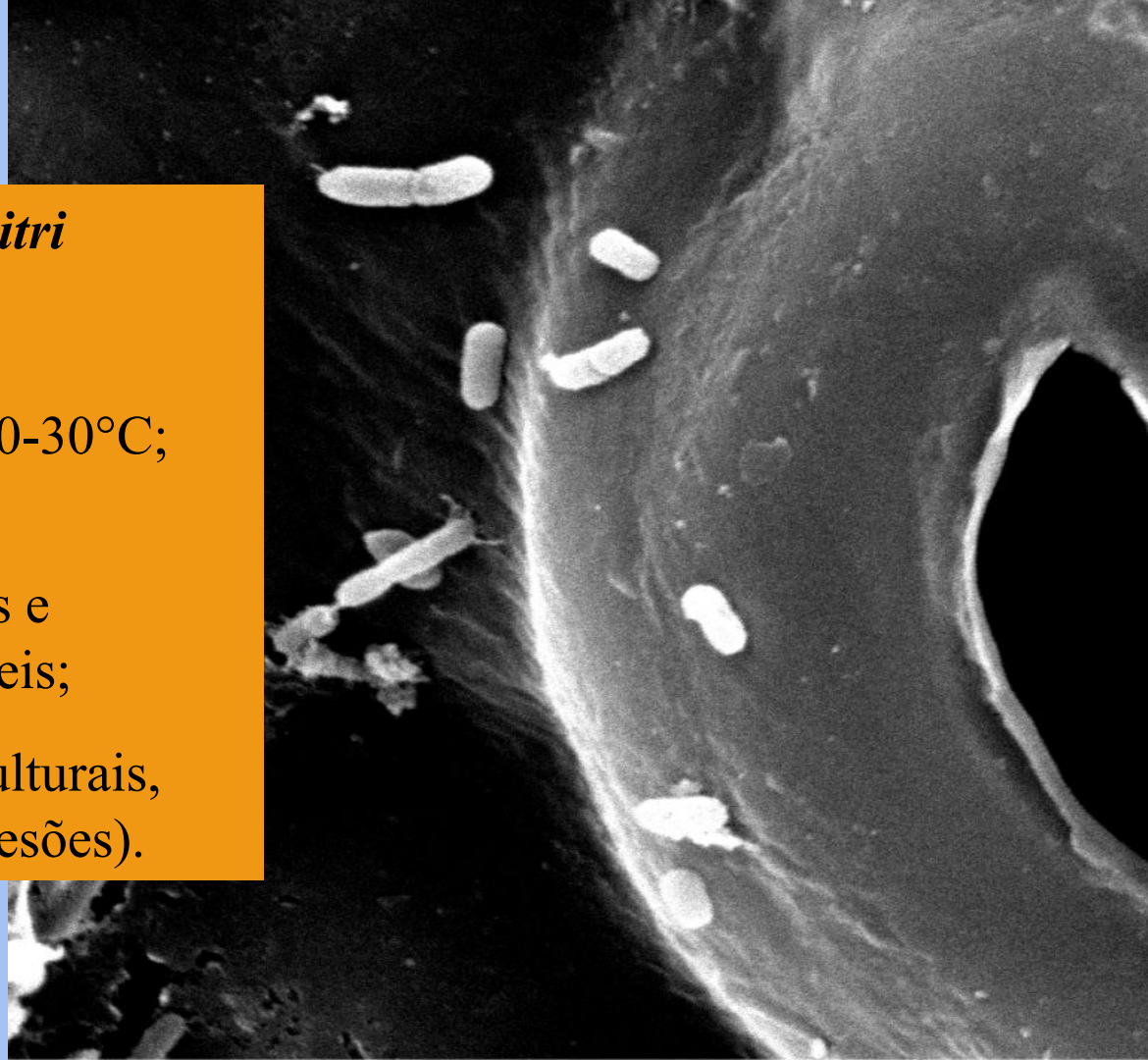
Principais causas, taxa de queda de frutos e caixas perdidas na safra 2019/20:
6º Cancro cítrico: 0,38% (1,80 milhão de caixas)

Fundecitrus (2020).



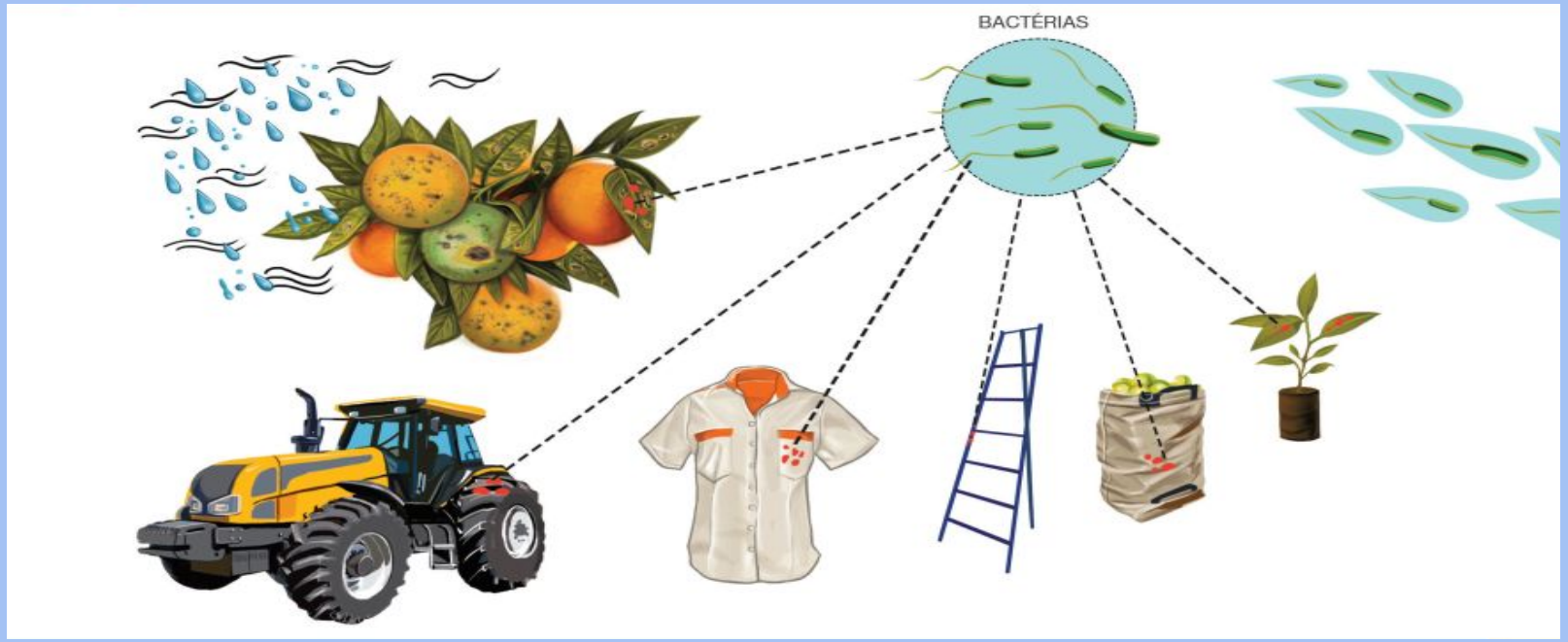
Sintomas de cancro cítrico causado pela bactéria *Xanthomonas citri* subsp. *citri* em laranja (*Citrus* spp.).
A: sintomas em folhas; B: sintomas em fruto; C: sintomas em ramos.

- ***Xanthomonas citri* subsp. *citri***
- **Gram-negativa;**
- **Temperatura ideal:** entre 20-30°C;
- **Alta umidade**
  - Locais de clima tropicais e subtropicais são favoráveis;
- **Sobrevivência:** em restos culturais, vários anos no hospedeiro (lesões).



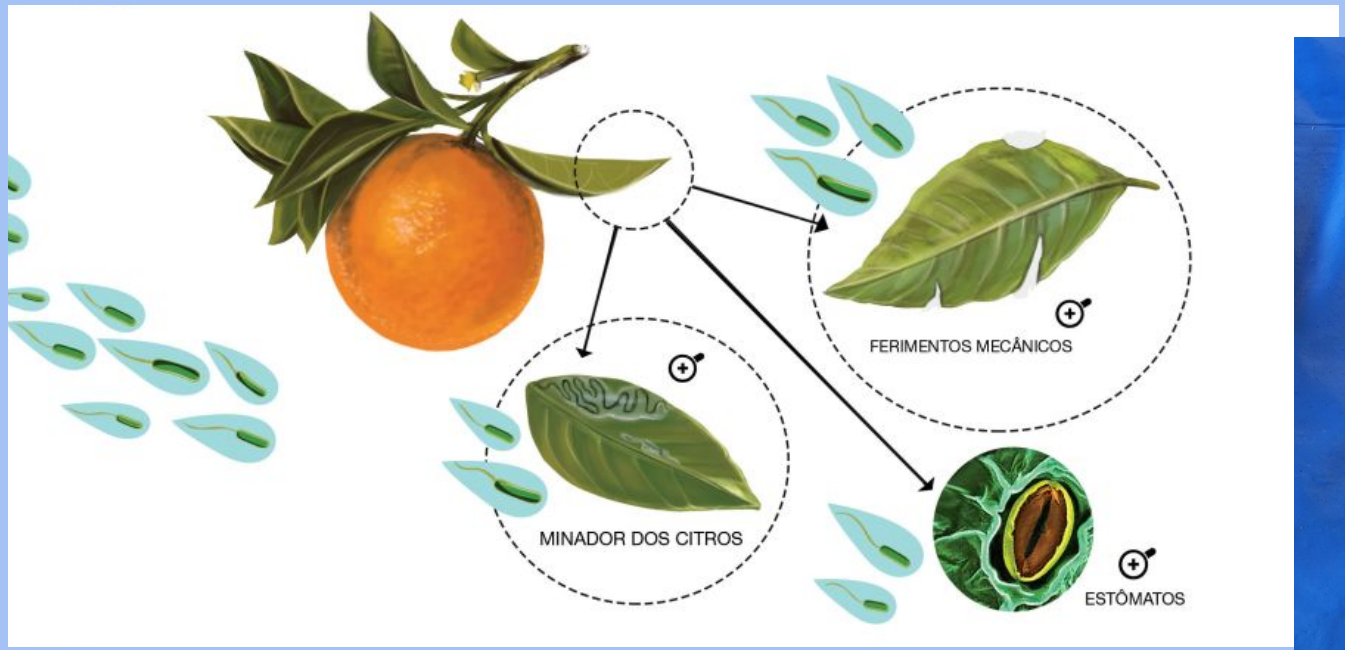
Disseminação

- Curtas distâncias: chuva e vento;
- Longas distâncias: material vegetal, máquinas e ferramentas contaminados.



Penetração

- Aberturas naturais e/ou através de ferimentos;
- Larva minadora dos citros (*Phyllocnistis citrella*) é uma facilitadora.





Medidas de Controle:

- Exclusão e erradicação;
- Evitar ventos fortes e constantes - Quebra-vento;
- Mudas sadias;
- Produtos à base de cobre;
- Controle da larva minadora dos citros;
- Inspeção frequente do pomar;
- Lavagem e desinfestação de veículos, máquinas, implementos e materiais de colheita;



Medidas de controle

- Uso de variedades resistentes ou moderadamente resistentes ao cancro cítrico.

É o método considerado mais econômico e eficiente.

Estudos de Severidade da Doença

- Plantas mais suscetíveis apresentam maior severidade;
- Plantas menos suscetíveis menor severidade.

NBA Núcleo de Pesquisa em Biotecnologia Aplicada
UEM MARINGÁ-PR

na prática...

Pesquisas aplicadas aos estudos de resistência dos diferentes genótipos de citros ao cancro cítrico.

COMO É REALIZADO?

- **Medições da severidade da doença;**
- **Avaliações morfológicas dos genótipos;**
- **Estudos de diversidade genética.**


mm/inch
1.22 mm
OFF ON ZERO





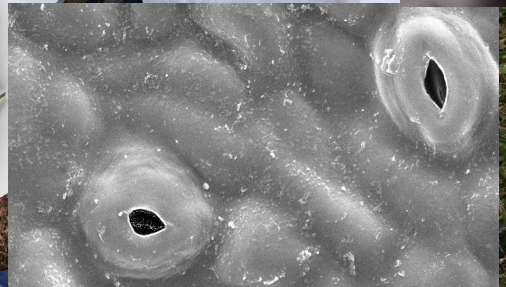









Cancro Cítrico





Dúvidas?



Equipe
NBA/UEM

Literatura



Cartilha_Cancro_Citrico_2013.pdf



http://www.idrparana.pr.gov.br/Noticia/Quebra-vento-e-aplicacoes-de-cobre-reduzem-cancro-citrico-em-mais-de-90

Sistema de Mitigação de Risco (SMR) Cancro Cítrico | Agência de Defesa Agropecuária do Paraná - ADAPAR



Crop Protection 146 (2021) 105679

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Chlorine dioxide, peroxyacetic acid, and calcium oxychloride for post-harvest decontamination of citrus fruit against *Xanthomonas citri* subsp. *citri*, causal agent of citrus canker

Franklin Behlau [a,*], Alexandre Paloschi [b], Tamiris G.S. Marin [a], Talita A. Santos [a], Henrique Ferreira [c], Lenice M. do Nascimento [d]

[a] Fundo de Defesa da Citricultura (Fundecitrus), Araraquara, SP, 14807-040, Brasil
[b] Coordenadoria de Defesa Agropecuária do Estado de São Paulo (CDA), Catanduva, SP, 15803-060, Brasil
[c] Departamento de Biologia Geral e Aplicada, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Rio Claro, SP, 13506-900, Brasil
[d] Associação Brasileira de Citros de Mesa (ABCM), Leme, SP, 13610-070, Brasil

ARTICLE INFO

Keywords:
Sanitization
Sodium hypochlorite
Packing house
Lime
Orange
Lemon

ABSTRACT

After a new regulation on the control of citrus canker (*Xanthomonas citri* subsp. *citri*) came into force in Brazil in 2017, the post-harvest sanitization of citrus fruit became mandatory to prevent dissemination of the causal pathogen and enable the commercial trade of citrus to other states and countries. Although several sanitizers are available worldwide for decontamination of fresh produce, only sodium hypochlorite is legally required by the country for decontamination of citrus fruit traded with other states and countries against *X. citri* in the packing house. Thus, the objectives of this study were to assess the efficiency of chlorine dioxide, peracetic acid, and calcium oxychloride as alternative sanitizers and exposure times of 1 and 2 min for the post-harvest decontamination of citrus fruit against *X. citri*. Sodium hypochlorite, the legally required standard, was used as control. The sanitizers were evaluated regarding the capacity to eliminate live *X. citri* in solution immediately or 1 h after exposure to the products and the efficiency in disinfesting artificially and naturally contaminated lime fruit. An additional assessment was carried out to determine the infective potential of *X. citri* suspensions at different concentrations in order to evaluate the risk of disease dissemination by the remaining bacterial population on the fruit following treatment. All the assessed sanitizers were able to completely eliminate *X. citri* in suspension immediately after treatment. In artificially and naturally contaminated fruit, the sanitizers promoted a significant reduction of 2.4–2.8 and 1.1 to 1.5 $\log_{10}$ cfu/mL, respectively, in the population of live bacteria when compared to the untreated control. In these experiments, the amount of *X. citri* that remained on the fruit ranged from 0.5 to 1.0 and 0.1 to 0.3 $\log_{10}$ cfu/mL, respectively, regardless of the exposure time. The labeled concentrations and treatment periods assessed effectively decontaminated fruit with *X. citri* and demonstrated little or no risk of pathogen spread from treated fruit.

1. Introduction

Brazil is the largest citrus juice producer and exporter in the world (Neves et al., 2020), but accounts for a minor share in the global fresh citrus fruit market, where it ranks well below countries with less expressive citrus growing area, such as Argentina, South Africa and Spain (FAO, 2021; USDA, 2021a). Despite the minor participation in the global market, citrus ranks third in fresh fruit exports from Brazil (Abrafrutas, 2021) with a potential to increase in the near future. The main factors limiting citrus exports from the country relate to phytosanitary restrictions mostly resulting from the occurrence of black spot caused by *Phyllosticta citricarpa*, and citrus canker caused by *Xanthomonas citri* subsp. *citri* (Behlau and Belasque, 2014; Gottwald et al. 2009, 2020; Neves et al., 2010).

Required by the Brazilian Mistry of Agriculture and international trade partners, a new legislation, known as Normative Instruction 21 (IN21), was issued to regulate the control of citrus canker in Brazil after the disease become endemic in some citrus-producing states (Behlau, 2021; Brasil, 2018). For the first time in the country, IN21 allows trees with citrus canker to remain in orchards, but oblige the post-harvest

* Corresponding author.
E-mail address: franklin.behlau@fundecitrus.com.br (F. Behlau).

https://doi.org/10.1016/j.cropro.2021.105679
Received 19 February 2021; Received in revised form 23 April 2021; Accepted 24 April 2021
Available online 29 April 2021
0261-2194/© 2021 Elsevier Ltd. All rights reserved.

behlau_2021_Chlorine_Dioxide_peroxyaceticAcid_calcium_oxychloride_post-harvest_decontamination_citrus_fruit_against_Xanthomonas_citri.pdf

Plant Disease • XXXX • XXX:X-X • https://doi.org/10.1094/PDIS-10-20-2153-RE

e-Xtra*

Relative Contribution of Windbreak, Copper Sprays, and Leafminer Control for Citrus Canker Management and Prevention of Crop Loss in Sweet Orange Trees

Franklin Behlau,[1,†] José Belasque , Jr.,[2] Rui P. Leite , Jr.,[3] Armando Bergamin Filho,[2] Timothy R. Gottwald,[4] James H. Graham,[5] Luis H. M. Scandelai,[1] Isabela V. Primiano,[1] Renato B. Bassanezi,[1] and Antonio J. Ayres[1]

[1] Departamento de Pesquisa e Desenvolvimento, Fundo de Defesa da Citricultura (Fundecitrus), Araraquara, São Paulo 14807-040, Brazil
[2] Departamento de Fitopatologia e Nematologia, Escola Superior de Agricultura "Luiz de Queiroz," Universidade de São Paulo, Piracicaba, São Paulo 13416-382, Brazil
[3] Laboratório de Bacteriologia, Instituto de Desenvolvimento Rural do Paraná, Londrina, Paraná 86047-902, Brazil
[4] United States Department of Agriculture, Agricultural Research Service, Fort Pierce, FL 34945, U.S.A.
[5] Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, U.S.A.

Abstract

The management of citrus canker, caused by *Xanthomonas citri* subsp. *citri*, has been widely studied in endemic areas because of the importance of the disease in several citrus-producing countries. A set of control measures is well established, but no study has investigated the efficiency of each measure individually and their combination for disease suppression. This study comprised a 3-year field study to assess the relative contribution of three measures for the control of citrus canker and reduction of crop losses. Windbreak (Wb), copper sprays (Cu), and leafminer control (Lc) were assessed in eight different combinations in a split−split plot design. The orchard was composed of 'Valencia' sweet orange trees grafted onto 'Rangpur' lime. *Casuarina cunninghamiana* trees were used as Wb. Cu and Lc sprays were performed every 21 days throughout the year. Individually, Cu showed the highest contribution for canker control, followed by Wb. Lc had no effect on reducing citrus canker. Wb+Cu showed the highest efficiency for control of the disease. This combination reduced the incidence of diseased trees by approximately 60%, and the incidence of diseased leaves and fruit by ≥90% and increased the yield in 2.0- to 2.6-fold in comparison with the unmanaged plots. Cu sprays were important for reducing disease incidence and crop losses, whereas Wb had an additional contribution in minimizing the incidence of cankered, non-marketable fruit. The results indicated that the adoption of these measures of control may depend on the characteristics of the orchard and destination of the production.

Keywords: *Casuarina cunninghamiana*, crop loss, integrated management, *Phyllocnistis citrella*, protection, *Xanthomonas citri* subsp. *citri*

Historically, the primary and compulsory strategy for control of citrus canker, caused by *Xanthomonas citri* subsp. *citri* (*X. citri*), in several countries, is the eradication of diseased trees (Behlau 2021; Dopson 1964; Gottwald et al. 2002; Rossetti 1977). The adoption of eradication as the main strategy for control of the disease was encouraged by the characteristics of the pathosystem, such as the restricted host range, the absence of an insect vector, the short interval between pathogen infection and symptom appearance under optimum weather conditions, the low capacity of the pathogen to survive for a long period outside the host, and the predominant dissemination of the bacteria over short and medium distances from the inoculum source (Belasque et al. 2005; Dalla Pria et al. 2006; Danós et al. 1984; Goto et al. 1975; Gottwald et al. 1993; Graham et al. 1987).

†Corresponding author: F. Behlau; franklin.behlau@fundecitrus.com.br

Funding: This work was made possible by the Citrus Research and Development Foundation under grant no. 15-050C, Fundecitrus under grant no. 013757, the Agricultural Research Service under grant no. 58-6618-3-140, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico under grant no. 309261/2016-3.

*The *e*-Xtra logo stands for "electronic extra" and indicates there are two supplementary figures and one supplementary table published online.

The author(s) declare no conflict of interest.

Accepted for publication 24 December 2020.

© 2021 The American Phytopathological Society

Eradication programs have contributed to the elimination or suppression of citrus canker outbreaks worldwide in the 1900s. During most of that time, there were no efficient management measures available, and the disease was considered a threat for the citrus industry (Behlau et al. 2016; Dopson 1964; Hill 1918). However, despite the remarkable success in eliminating or containing the spread of citrus canker, most of the eradication programs were terminated for various reasons over the years in important citrus-growing regions of Argentina, Uruguay, the U.S.A., and in 2017, Brazil (Behlau 2021). Alternatively, the end of these eradication programs forced the development of management approaches to neutralize or minimize the impact of the disease on the fruit quality and crop production (Behlau 2021; Canteros et al. 2017; Gochez et al. 2020; Leite and Mohan 1990).

Since the 1980s, studies conducted in citrus-producing countries where citrus canker became endemic have demonstrated the efficiency of distinct measures to minimize the impact of the disease on yield and fruit quality. The results of these researches associated with field observations over the years resulted in a set of broadly used control measures such as planting resistant or less susceptible citrus cultivars or species, establishment of arboreal windbreaks in the perimeter of the orchards, spray of copper-based bactericides, and control of the citrus leafminer (*Phyllocnistis citrella*; Behlau et al. 2008; Canteros et al. 2017; Gottwald et al. 1993; Gottwald and Timmer 1995; Graham et al. 2011; Leite and Mohan 1990; Stein et al. 2007). Citrus genotypes show a broad spectrum of susceptibility to citrus canker, ranging from highly resistant to highly susceptible (Gottwald et al. 1993, 2002). Planting citrus varieties that are field resistant to citrus canker is the most efficient and inexpensive measure of control. Although it is an important aspect to be

Behlauetal.2021-Relative_contribution_citrus_management.pdf

Pesquisa Agrotóxicos



Marca Comercial	Situação Agrotóxico	Classificação Tox	Empresa Registrante
FEGATEX	Liberado com Restrição de Uso	Cat.5-Impr. Causar Dano Agudo	BR 3 TECNOLOGIA E INDUSTRIA QUÍMICA E FARMACÊUTICA LTDA
QUATERMON	Liberado	Cat.3-Moderadamente Tóxico	Chemitec Agro Veterinária Ltda

fundecitrus.com.br/doencas/cancro

Fotos



Cancro Cítrico

e-Xtra*

Comparison of Resistance to Asiatic Citrus Canker Among Different Genotypes of *Citrus* in a Long-Term Canker-Resistance Field Screening Experiment in Brazil

Sérgio Alves de Carvalho, Centro de Citricultura "Sylvio Moreira", Instituto Agronômico de Campinas (IAC), 13490-970, Cordeirópolis, SP, Brazil; **William Mário de Carvalho Nunes,** Núcleo de Pesquisa em Biotecnologia Aplicada, Universidade Estadual de Maringá, 87020-900, Maringá, Paraná, Brazil; **José Belasque, Jr.,** Departamento de Fitopatologia e Nematologia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, SP, Brazil; **Marcos Antonio Machado,** Centro de Citricultura "Sylvio Moreira", IAC; **José Croce-Filho,** Secretaria de Estado da Agricultura e do Abastecimento do Paraná, Maringá, PR, Brazil; **Clive H. Bock,** United States Department of Agriculture–Agricultural Research Service (USDA-ARS)-SEFTNRL, Byron, GA 31008; and **Zaid Abdo,** USDA-ARS-SAA, Athens, GA 30605

Abstract

de Carvalho, S. A., de Carvalho Nunes, W. M., Belasque, J., Jr., Machado, M. A., Croce-Filho, J., Bock, C. H., and Abdo, Z. 2015. Comparison of resistance to Asiatic citrus canker among different genotypes of *Citrus* in a long-term canker-resistance field screening experiment in Brazil. Plant Dis. 99:207-218.

Asiatic citrus canker (ACC) is caused by *Xanthomonas citri* subsp. *citri*. The disease results in yield loss and renders fruit unfit for the fresh market. A 6-year study in Paraná State, Brazil, was conducted to compare the susceptibility of 186 genotypes of citrus representing sweet orange (*Citrus sinensis*), mandarin (*C. reticulata*), Mediterranean mandarin (*C. deliciosa*), Clementine mandarin (*C. clementina*), Satsuma mandarin (*C. unshiu*), sour orange (*C. aurantium*), lemon (*C. limon*), sweet lime (*C. aurantifolia*), grapefruit (*C. paradisi*), and four hybrids (*C. reticulata* × *Citrus* sp., *C. reticulata* × *C. paradisi*, *C. reticulata* × *C. sinensis*, and *C. unshiu* × *C. sinensis*). Sweet orange (*C. sinensis*) was represented by the most genotypes (n = 141). The number of lesions per leaf was assessed 18 times from 2005 to 2010 (up to 4 times per year). The data were analyzed using mixed-model analysis of fixed and random effects, which showed a total of six resistance-susceptibility groupings of species and hybrids. Based on species, the most resistant genotypes, on average, included Satsuma and lemon (mean lesions per leaf = 4.32 and 4.26, respectively), and the most susceptible genotypes were grapefruit and sweet lime, with 14.84 and 10.96 lesions per leaf, respectively. Genotypes of mandarin, sour orange, Mediterranean mandarin, and sweet orange had intermediate severity (5.48 to 9.56 lesions per leaf). The hybrids also showed a range of ACC severity but all were in the more resistant groupings (5.26 to 7.35 lesions per leaf). No genotype was immune to ACC. The most resistant genotype was 'Muscia' (*C. reticulata*) and the most susceptible was 'Valencia Frost' (*C. sinensis*) (1.86 and 14.78 lesions per leaf, respectively). Approximately one-sixth of the genotypes showed a negative relationship of mean lesions per leaf with time, suggesting increasing resistance as they aged, due to a reduction in either new flush or plant size and structure. These results of the relative susceptibility of different citrus genotypes can be used in future research and to assist in varietal selection or for breeding purposes both within Brazil and other regions where ACC is an issue.

Asiatic citrus canker (ACC; caused by *Xanthomonas citri* subsp. *citri* (ex Hasse)) (50) occurs in several tropical and subtropical citrus growing regions (16,51). It is widespread in southern Brazil, where it has substantial effect on the yield and marketability of fresh citrus and reduced citrus yield (3). Reports from other parts of the world confirmed that it is one of the most important diseases of citrus, as witnessed by the costly and prolonged eradication efforts in Florida (20,31,51) and Brazil (5). Subsequent to cessation of eradication in Florida (21,31), ACC has continued to spread in citrus orchards. *X. citri* subsp. *citri* infects the foliage, fruit, and shoots of the citrus plants, initially causing small water-soaked lesions to develop that enlarge and become erumpent and necrotic. The pathogen is naturally spread in rain splash but wind can dramatically increase the effectiveness of dispersal and infection (8); it can also be spread by farm equipment and by personnel (20). Injury, both that by wind (8) and due to insect feeding, particularly by the Asiatic citrus leafminer (CLM; *Phyllocnistis citrella* Stainton), can increase the incidence and severity of the disease (7,14,29). Severe ACC can result in premature leaf and fruit drop (2,3).

São Paulo State, Brazil, is the largest orange juice producer in the world, and has been fighting an epidemic of ACC throughout the state since 2009. Today, ACC control efforts are almost exclusively the citrus grower's responsibility and, as a consequence, disease foci detection and eradication are less intensive than in previous decades. In 2009, the São Paulo state government altered its eradication policy, making it less effective in suppressing epidemic development (5,6). Furthermore, the newest state law (approved in October 2013) determines the eradication of canker-symptomatic plants only. Thus, for the first time since detection of ACC in Brazil in 1957, neither an eradication zone nor eradication of potentially exposed plants is mandatory. The state survey of ACC conducted in 2012 showed 1.39% of sweet orange orchards had at least one symptomatic tree, which was the highest disease incidence ever observed in São Paulo state. In comparison, from 1999 to 2009, incidence of ACC ranged from 0.08 to 0.70% (6). These data suggest that ACC will probably continue to spread in São Paulo state and, as has occurred in other regions and countries in the Americas, ACC management by growers will be the main disease control strategy.

Some methods are available to manage ACC. These include the use of copper bactericidal sprays (2,3), windbreaks (which might provide some protection; 2,22), systemically acquired resistance (SAR) inducers (25,26), orchard hygiene (8), and, where pathogen arrival is recent or where ACC is not widely distributed, eradication—including in Brazil, where it has been practiced since 1957

Corresponding author: S. A. de Carvalho,
E-mail: sergio@centrodecitricultura.br

*The *e*-Xtra logo stands for "electronic extra" and indicates that a supplementary figure is available online.

Accepted for publication 18 June 2014.

http://dx.doi.org/10.1094/PDIS-04-14-0384-RE
© 2015 The American Phytopathological Society

2015_Comparison of Resistance to Asiatic Citrus Canker Among Different Genotypes of Citrus in a Long-Term Canker-Resistance Field Screening Experiment in Brazil.pdf

e-Xtra*

Comparison of Resistance to Asiatic Citrus Canker Among Different Genotypes of *Citrus* in a Long-Term Canker-Resistance Field Screening Experiment in Brazil

Sérgio Alves de Carvalho, Centro de Citricultura "Sylvio Moreira", Instituto Agronômico de Campinas (IAC), 13490-970, Cordeirópolis, SP, Brazil; **William Mário de Carvalho Nunes,** Núcleo de Pesquisa em Biotecnologia Aplicada, Universidade Estadual de Maringá, 87020-900, Maringá, Paraná, Brazil; **José Belasque, Jr.,** Departamento de Fitopatologia e Nematologia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, SP, Brazil; **Marcos Antonio Machado,** Centro de Citricultura "Sylvio Moreira", IAC; **José Croce-Filho,** Secretaria de Estado da Agricultura e do Abastecimento do Paraná, Maringá, PR, Brazil; **Clive H. Bock,** United States Department of Agriculture–Agricultural Research Service (USDA-ARS)-SEFTNRL, Byron, GA 31008; and **Zaid Abdo,** USDA-ARS-SAA, Athens, GA 30605

Abstract

de Carvalho, S. A., de Carvalho Nunes, W. M., Belasque, J., Jr., Machado, M. A., Croce-Filho, J., Bock, C. H., and Abdo, Z. 2015. Comparison of resistance to Asiatic citrus canker among different genotypes of *Citrus* in a long-term canker-resistance field screening experiment in Brazil. Plant Dis. 99:207-218.

Asiatic citrus canker (ACC) is caused by *Xanthomonas citri* subsp. *citri.* The disease results in yield loss and renders fruit unfit for the fresh market. A 6-year study in Paraná State, Brazil, was conducted to compare the susceptibility of 186 genotypes of citrus representing sweet orange (*Citrus sinensis*), mandarin (*C. reticulata*), Mediterranean mandarin (*C. deliciosa*), Clementine mandarin (*C. clementina*), Satsuma mandarin (*C. unshiu*), sour orange (*C. aurantium*), lemon (*C. limon*), sweet lime (*C. aurantifolia*), grapefruit (*C. paradisi*), and four (mean lesions per leaf = 4.32 and 4.26, respectively), and the most susceptible genotypes were grapefruit and sweet lime, with 14.84 and 10.96 lesions per leaf, respectively. Genotypes of mandarin, sour orange, Mediterranean mandarin, and sweet orange had intermediate severity (5.48 to 9.56 lesions per leaf). The hybrids also showed a range of ACC severity but all were in the more resistant groupings (5.26 to 7.35 lesions per leaf). No genotype was immune to ACC. The most resistant genotype was 'Muscia' (*C. reticulata*) and the most



FEI 2004 nov 11



FEI 2004 nov 12



FEI 2004 nov 12

Experiment location and management. The field experiment was located in Maringá, in Northwestern Paraná state (latitude 23°25′ S and longitude 52°10′ W) at an elevation of 554.9 m. According to Koeppen's climate, it is a tropical climate modified by elevation, with hot summer temperatures and with the warmest month >22°C. The average annual temperature is 16.7°C, the average minimum is 10.3°C, and the average maximum 33.6°C, with an average relative humidity of 66%. The average annual rainfall is 1,500 mm. Frosts are infrequent and concentrated in the winter period. Prevailing winds are from the northeast and the soil of the experimental area classified as typic Haplodox.

The citrus trees were planted in April 2003. Plants were spaced 3.5 m within the row and 5.0 m between rows in a randomized block design with two replications (= blocks), and four plants of each genotype per replicate (plot). As a source of inoculum for natural disease spread to the 186 genotypes tested, every four plants were interspersed by one plant of 'Baia' (*C. sinensis*), which had been inoculated in November 2004 using a bacterial suspension of a highly virulent strain of *X. citri* subsp. *citri* (1) at 10^8 CFU/ml.



FEI 2004 nov 12



FEI 2006 sep 12



FEI 2008 sep 30