Genômica de Patógenos: *Xylella fastidiosa*

Alessandra Alves de Souza

Citrus Research Center "Sylvio Moreira"
Agronomic Institute of Campinas - SP

desouza@ccsm.br

Brief history

Strategic vision of FAPESP

- *Development of Molecular Biology*
- *Training of human resources*
- *Multiplier effect*
- *International scientific impact*

✓ Genome sequencing

✓ Microorganism – bacterium

✓ Important for the State of São Paulo

✓ Importance of São Paulo Agribusiness - Brazil

Brief history

Citrus Variegate Chlorosis CVC

-***1992***: *Koch postulates*

- ***1995:*** *vectors*

- ***1997:***

- ***31 %*** *of orchards with incidence*
- ***20 % of progression rate***
- ***3 %*** *in the terminal phase*

1997 – choice of *Xylella fastidiosa* as organism to be sequenced

Citrus Agribusiness

- **COJ: > US$ 1.5 billion a year**
- **Sao Paulo: > 80% production**

Brazilian juice production ≈60% in the world

Fonte: Elaborado por Markestrat a partir de CitrusBR.

Brief history

FAPESP Genoma

Organization for Nucleotide Sequencing and Analysis

The Virtual Genomics Institute

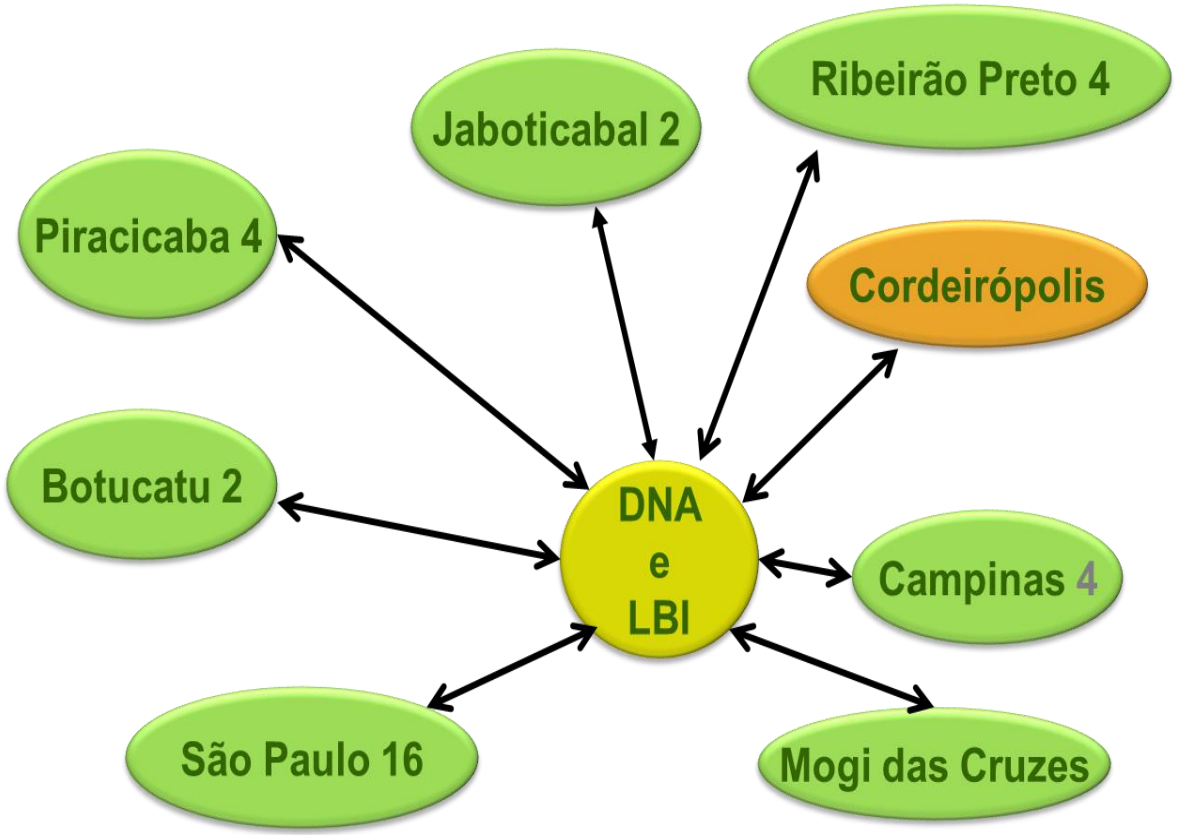
Organization

Steering committee

DNA Laboratories

Sequencing laboratories

Bioinformatics



Cordeirópolis Team

Marcos Machado
Coordinator

Helvecio Coletta Filho
Bacteria growth distribution

Mariangela Cristofani

Eliane Locali

Maria Luiza Targon

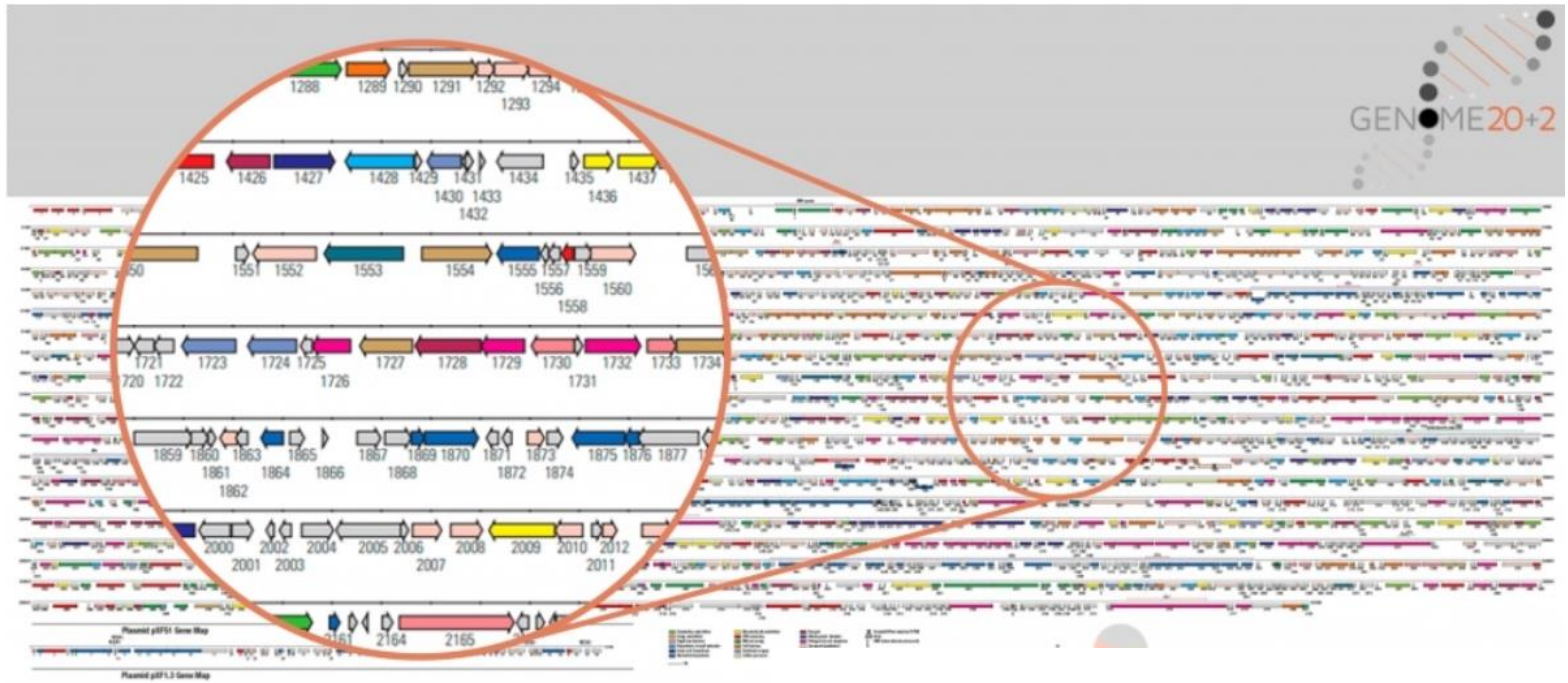
Alessandra de Souza

Marco Takita

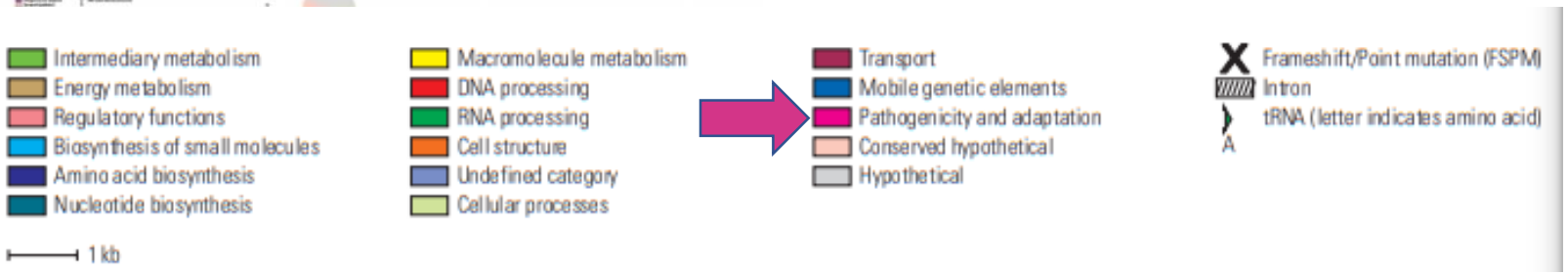
Sequencing team

Genome annotation team

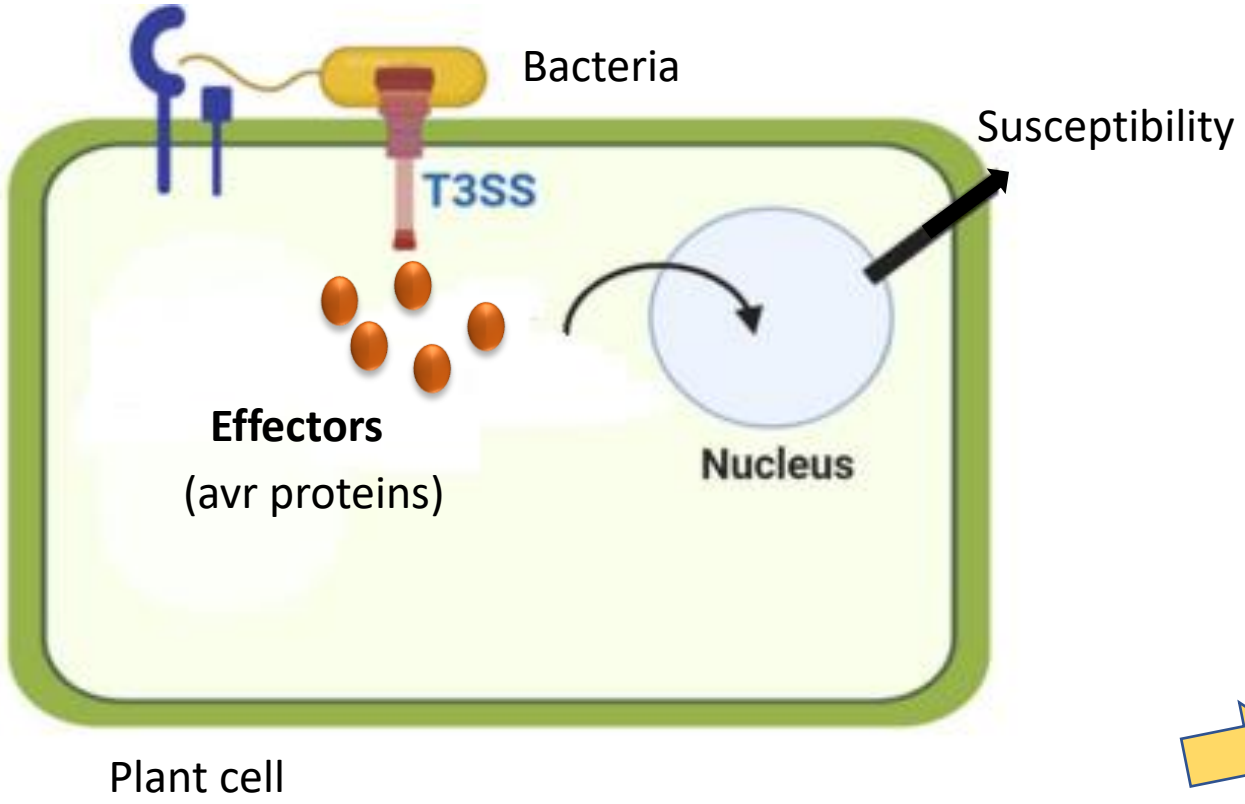
Genome annotation



Category VII - Pathogenicity



Classic model of pathogenicity



Highlights

Absence of avirulence genes

Phytopathogenic bacteria generally have a limited host range, often confined to members of a single species or genus. This specificity is defined by the products of the so-called avirulence (*avr*) genes present in the pathogen, which are injected directly into host cells, on infection, through a type III secretory system[38–40]. BLAST[41] searches with all known *avr* and type III secretory system sequences failed to identify genes encoding proteins with significant similarities in the genome of *X. fastidiosa*. Although the variability of *avr* genes amongst bacteria might account for this apparent lack, the high level of similarity of some components of the type III secretory system argues against this. We suspect that these genes are, in fact, not required because of the insect-mediated transmission and vascular restriction of the bacterium that obviates the necessity of host cell infection. Furthermore, if the differing host ranges of *X. fastidiosa* are molecularly defined, this may be by a quite different mechanism not involving avr proteins.

✓*International scientific impact*

- Publication and cover of *Nature*
- Sequencing of *X. fastidiosa* that causes Pierce's disease in the USA

The Economist, Saturday, 22 July 2000. Page: 109. Issue: 8180.

Brazilian science

Fruits of co-operation

SAO PAULO

SAMBA, football and...genomics. The list of things for which Brazil is renowned has suddenly got longer. Only a few days after publishing, on July 13th, the first-ever sequence of the genome of a plant pathogen, scientists at Sao Paulo's state research

✓ *Training of human resources*
✓ *Multiplier effect*

- 116 researchers' authors – training of several students involved in the project.
- Beginning of the Program of Brazillian Genome network CNPq - *Chromobacterium violaceum*
- Continuing the genome projects by FAPESP, CNPq and FAPs.

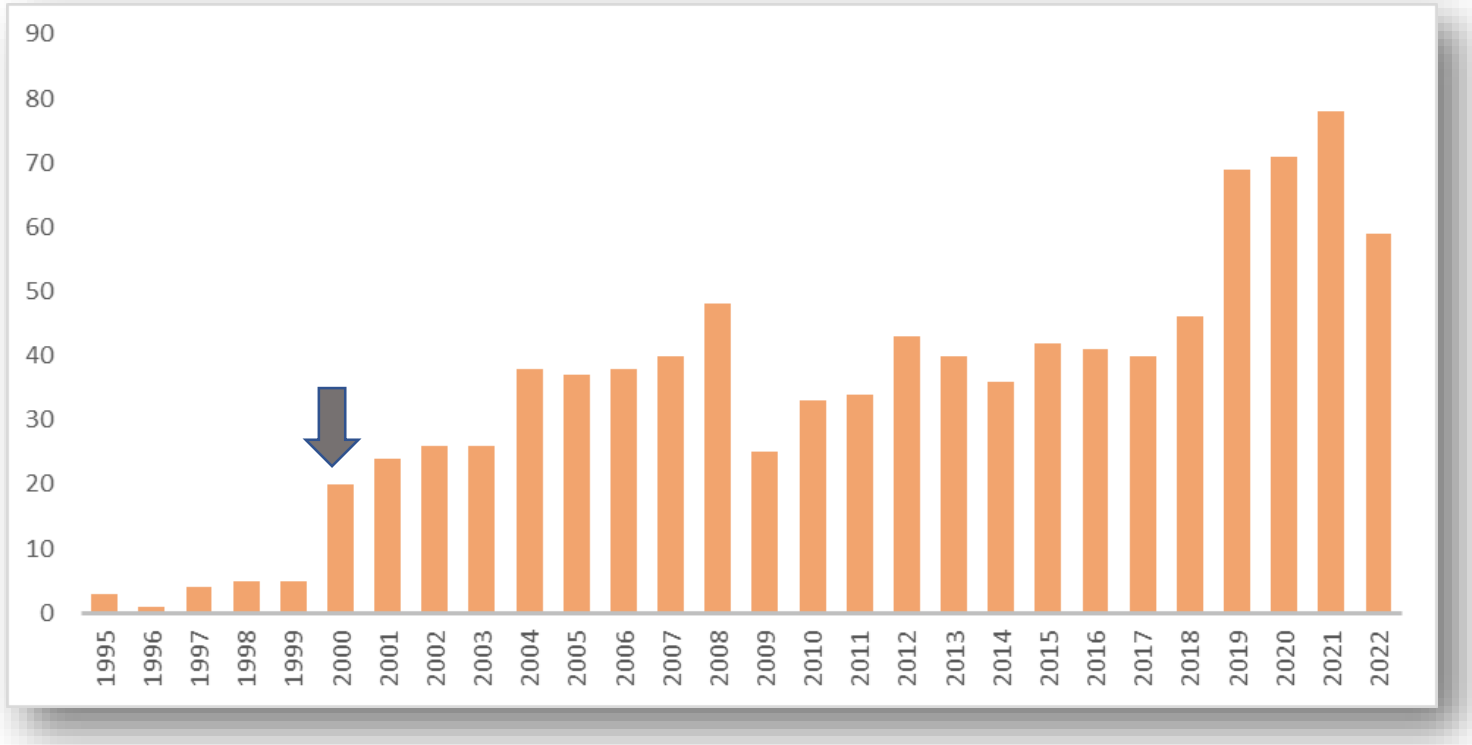
GENOME20+2

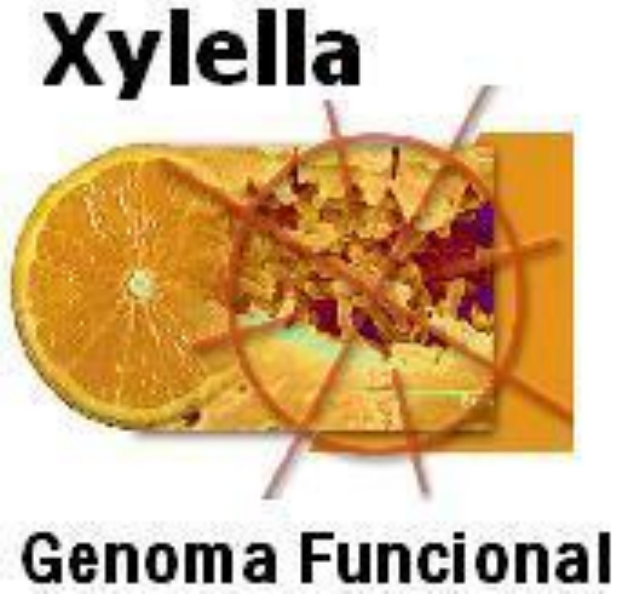
- ✓ *Training of human resources*
- ✓ *Multiplier effect*

BEYOND

- Mechanism of pathogenicity? How to control?

PubMed.gov





19 Projects:
IAC, Unicamp, Esalq, Cena, IB, Fundação Tropical, Mogi das Cruzes, Unaerp, USP, Fundecitrus

GENOME 20+2

Genômica de Patógenos:

UNICAMP

Embrapa Recursos Genéticos e Biotecnologia

IAC INSTITUTO AGRONÔMICO

2001 - 2005

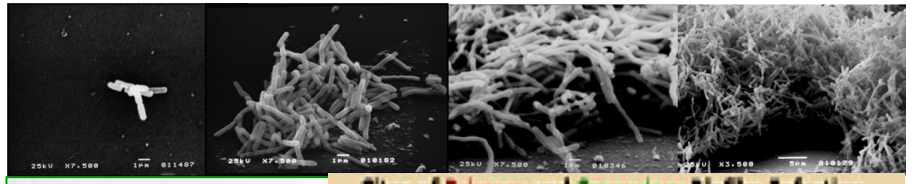
Xylella fastidiosa genome - 2000

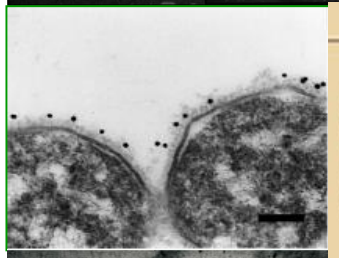
2.800 genes

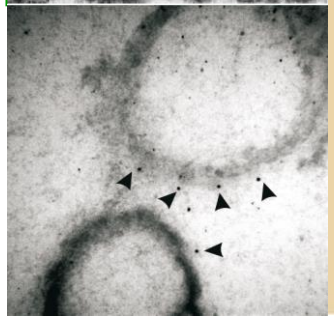
Which involved with pathogenicity?
What are the possible genetic targets for control?
How to achieve these targets?

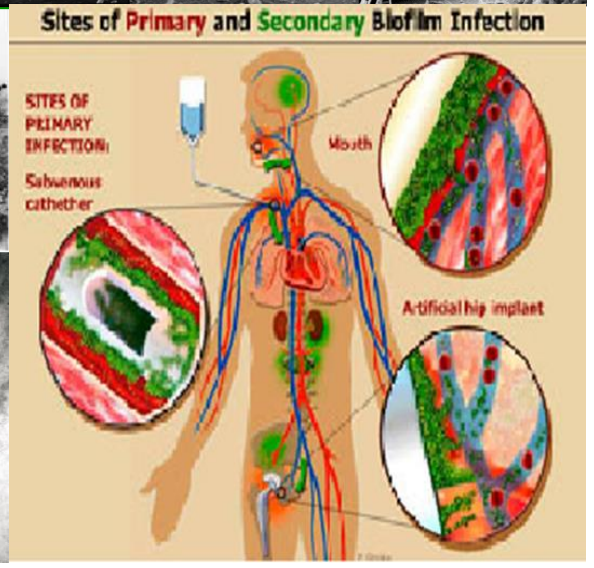
Xylella 2001

Genoma Funcional









Hoiczyk et al., 2000 . EMBO J.

Pathogenicity is associated with the ability of the bacteria to move and colonize (forming biofilm) in the xylem vessels

De Souza et al., Mol. Plant Microbe Interact. 2003.

De Souza et al., Fems Microbiol, 2004

De Souza et al., 2005 Current Microbiol

2005 Bunge Foundation Award - Agribusiness category (youth)

Área: Ciências Agrárias

Ramo: Agronegócio

Premiado (Vida e Obra): ***Ernesto Paterniani*** (São Paulo, 1928). Engenheiro agrônomo, com contribuições na área de seleção e melhoramento genético do milho. Na Escola Superior de Agricultura Luiz de Queiroz (Esalq) da Universidade de São Paulo, foi chefe do Departamento de Genética e coordenador da Pós-Graduação em Genética e Melhoramento de Plantas.

Premiado (Juventude): ***Alessandra Alves de Souza***. Geneticista, com contribuições ao estudo de microorganismos fitopatogênicos e sua interação com a planta hospedeira. Empreendeu pesquisas para encontrar mecanismos de controle para a Clorose variegada dos citros (CVC), ou amarelinho, praga comum nos laranjais paulistas.

PRÊMIO FUNDAÇÃO BUNGE

2005 - Young Geneticist

SBG SOCIEDADE BRASILEIRA DE GENÉTICA

2005 – Young Researcher Project

FAPESP FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO

2006- Entry into the GMB Postgraduate program

UNICAMP

GENOME 20+2

- ✓ *Training of human resources*
- ✓ *Multiplier effect*

BEYOND

- ✓ 10 mestrados
- ✓ 10 doutorados
- ✓ 7 pos-docs
- ✓ 12 TCCs
- ✓ 30 ICs



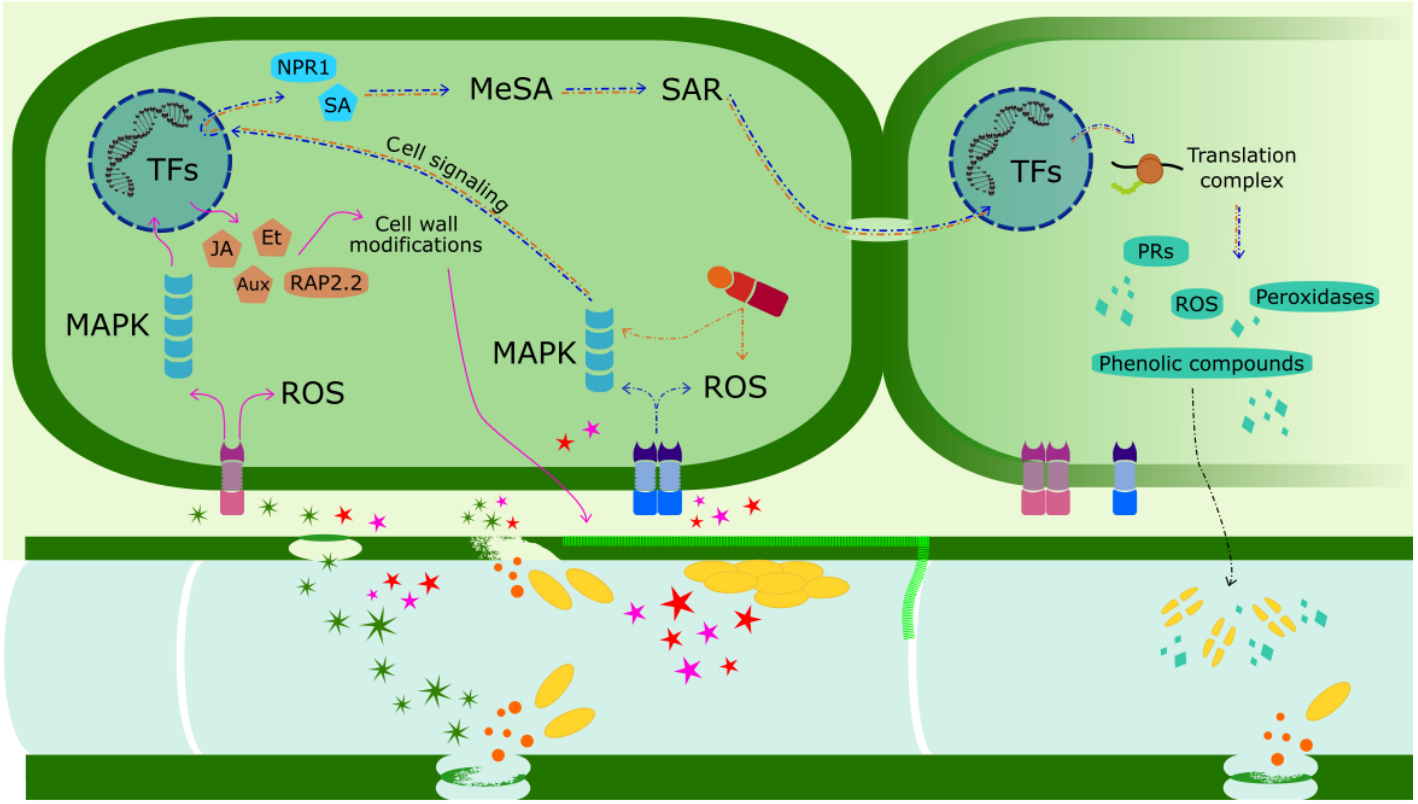







Xylella fastidiosa- plant interaction from basic to applied knowledge

- **Biofilm X planktonic cells**
  De Souza et al., Fems Microbiol, 2004
- **Biofilm development**
  De Souza et al., 2005 Current Microbiol
  De Souza et al., 2006 RAPP, v. 14 p.1-57
  Lorite et al., Plos One 2013
  Granato et al., BMC Genomics 2016
- **Expression of adhesins during biofilm formation**
  Caserta et al., 2010 Appl. Env. Microbiol.
- **Global expression of proteins in mature biofilm**
  Silva et al, 2011 Proteomic science
- **Resistance of biofilm cells to copper and antibiotics**
  Rodrigues et al, 2008 Appl. Microbiol. Biotech
  Muranaka et al., 2012 Journal of Bacteriol.
  Merfa et al., Frontiers in Microbiol., 2016



~ 100 papers

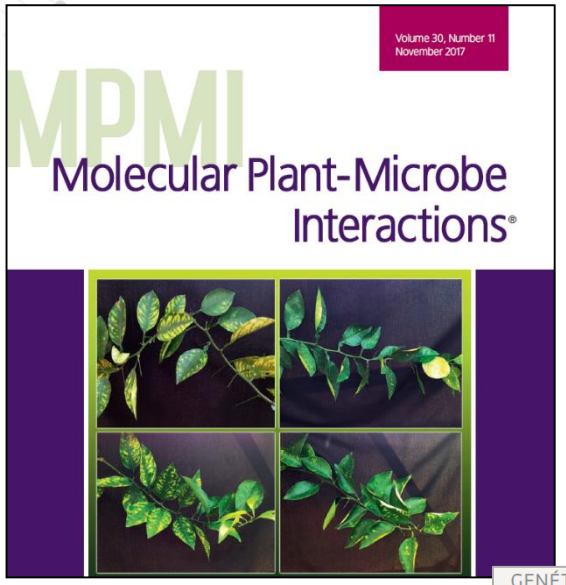
Key genes to be used aiming pathogen control

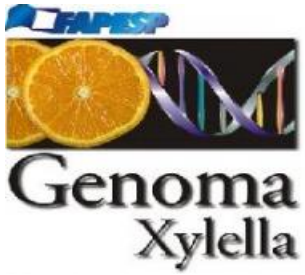
Coletta-Filho, H.Det al. *Trop. plant pathol.* **45,** 175–191 (2020). https://doi.org/10.1007/s40858-020-00358-5

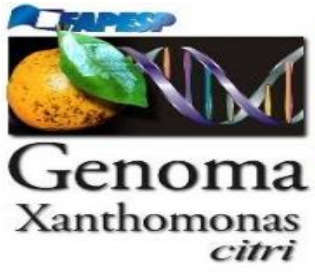
GENOME 20+2

Highlights

Key gene – *rpfF* from Xylella to Sweet orange







Valor ECONÔMICO

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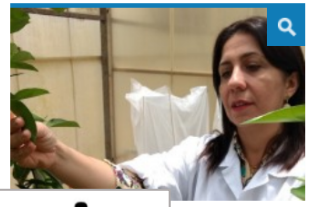
27/11/2017 às 05h00 1

IAC cria laranja transgênica mais resistente ao amarelinho

Por **Bettina Barros | De São Paulo**

Maior produtor e exportador de suco de laranja do mundo, com mais de 80% do mercado global, o Brasil poderá anunciar nos próximos anos um feito marcante dos pontos de vista científico e agronômico, mas que suscita preocupações: o desenvolvimento da primeira laranja transgênica do mundo com



dra Alves de Souza, o IAC

antigas e lmente, à morte

CiB
Conselho de Informações sobre Biotecnologia

NOTÍCIAS E ENTREVISTAS | INFOGRÁFIC E APRESENTAÇÕES

Brasileiras desenvolvem laranja transgênica resistente a doenças

Início / Notícias / Br

A ciência brasileira já tem mais motivo para se orgulhar. O Instituto Agronômico de Campinas (IAC) anunciou o desenvolvimento de uma laranja transgênica resistente a duas doenças: o amarelinho e o cancro cítrico. O amarelinho gera frutos pequenos e de amadurecimento precoce. A doença foi identificada aqui em 1987, no Triângulo Mineiro e em São Paulo. Os impactos do cancro, por sua vez, estão relacionados à desfolha de plantas, à presença de lesões em frutos e à queda prematura de frutos.

Causadas pelas bactérias *Xylella fastidiosa* e *Xanthomonas citri*, respectivamente, essas doenças são antigas e recorrentes nos pomares. Levam à perda de produtividade e, eventualmente, à morte da planta. A pesquisa, que já dura sete anos, foi publicada neste mês pela revista Molecular Plant-Microbe Interactions, da Associação Americana de Fitopatologia.

GENÉTICA

Pesquisa FAPESP

Contra as pragas da citricultura

Pesquisadores obtêm primeira laranjeira transgênica resistente à bactéria *Xylella fastidiosa*

Rodrigo de Oliveira Andrade

A *Xylella fastidiosa*, causadora da clorose variegada dos citros (CVC), é uma bactéria oportunista. Tão logo infecta as laranjeiras, transmitida pela picada

Souza, ambas do Centro de Citricultura Sylvio Moreira do Instituto Agronômico de Campinas (IAC), em Cordeirópolis, interior paulista, conseguiu obter uma variedade de laranjeira transgênica resis-

densidade populacional e do comportamento do microrganismo. Após invadir a planta e aderir à parede do xilema – o conjunto de vasos que leva água e nutrientes do solo para as folhas –, a bac-

SUPERINTERESSANTE.com.br

Cientistas brasileiros inventam laranja transgênica

Desenvolvida em Campinas, nova espécie recebeu DNA de uma bactéria.

UC BERKELEY – Dr. Steve Lindow

Highlights

Key gene – *rap2.2* from tangerine to Sweet orange

Mol Plant Microbe Interact. 2020 Mar;33(3):519-527. doi: 10.1094/MPMI-10-19-0298-R. Epub 2020 Jan 23.

Citrus reticulata CrRAP2.2 Transcriptional Factor Shares Similar Functions to the *Arabidopsis* Homolog and Increases Resistance to *Xylella fastidiosa*

Willian Pereira [1 2 3], Marco Takita [1], Maeli Melotto [3], Alessandra de Souza [1]

Pesquisa FAPESP

Laranjeiras imunes | AGOSTO DE 2020

Variedades desenvolvidas no Instituto Agronômico de Campinas, com acréscimo de gene de tangerina, se mostraram resistentes à clorose variegada dos citros

saopaulo.sp.gov.br

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IAC faz 133 anos com resultado inédito em citros

UC Davis – Dr. Maeli Melotto

Highlights

Key gene – *efr* from Arabidopsis to Sweet orange

Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2021), pp. 1–3 doi: 10.1111/pbi.13629

Brief Communication

The *Arabidopsis* immune receptor EFR increases resistance to the bacterial pathogens *Xanthomonas* and *Xylella* in transgenic sweet orange

Letícia Kuster Mitre[1,2,†], Natália Sousa Teixeira-Silva[1,†], Katarzyna Rybak[3], Diogo Maciel Magalhães[1,2], Reinaldo Rodrigues de Souza-Neto[1,2], Silke Robatzek[3], Cyril Zipfel[4,5] and Alessandra Alves de Souza[1,*]

Centro de Citricultura Sylvio Moreira IAC

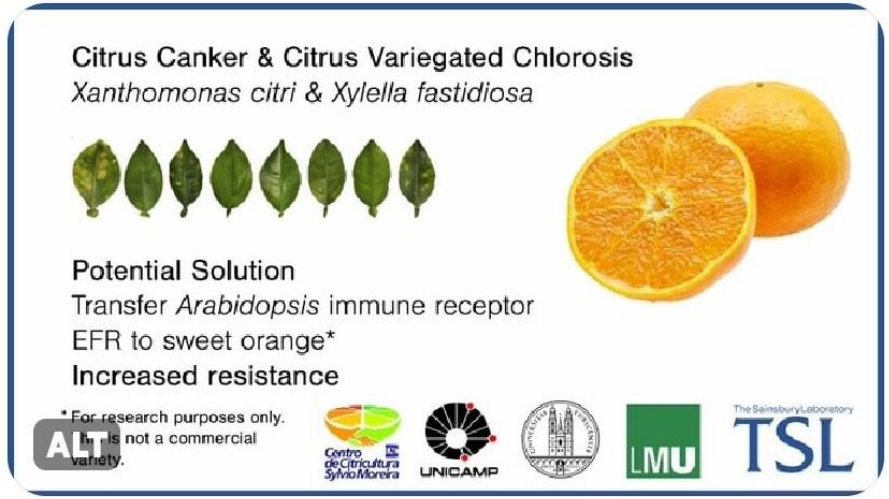
Universität Zürich[UZH] TheSainsburyLaboratory TSL LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Dr. Cyril Zipfel and Dr. Silke Robatzek
Collaborators

TheSainsburyLaboratory TSL

The Sainsbury Laboratory @... · 4d

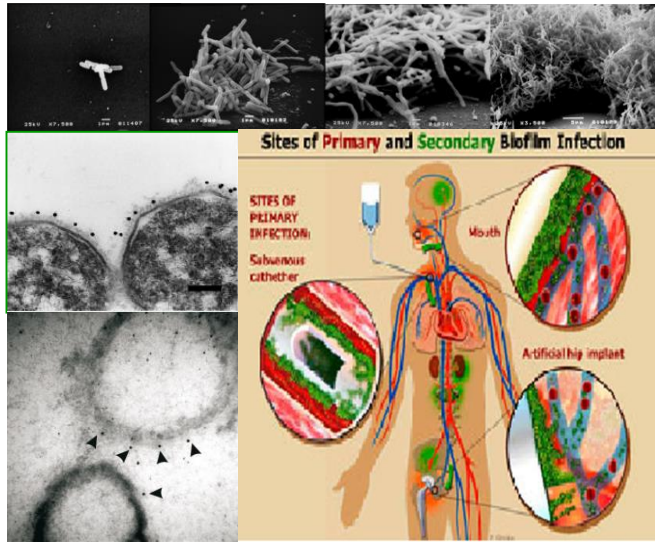
Bacterial diseases can lead to huge economic losses in citrus farming.
For #PlantHealthWeek we celebrate the teams of scientists that are discovering solutions and the international collaborations that bring us together.
#IYPH #IYFV2021
biorxiv.org/content/10.110...



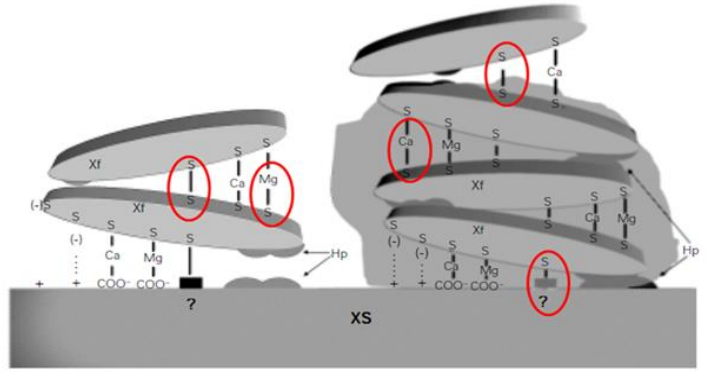
British Society for Plant Pathology

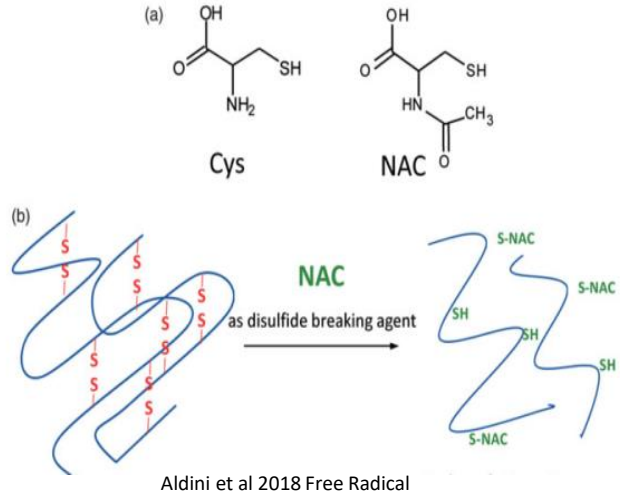

Pineapple- WT
Pineapple- WT
222
Pineapple- mqsR
212
Pineapple- mqsR
211
Pineapple- rpfF
221
Pineapple – rpfF

Xylella fastidiosa- plant interaction from basic to applied knowledge



Proposed model to explain *Xylella fastidiosa* biofilm formation.
Leite et al., 2002 Braz J. Med. Biol. Res 35: 645-650





If *X. fastidiosa* needs disulfide bonds for the function of proteins related with biofilm and movement, then NAC could be used to impair the *X. fastidiosa* colonization.

Aldini et al 2018 Free Radical
DOI: 10.1080/10715762.2018.1468564

Patent INPI 2011

OPEN ACCESS Freely available online 2013 PLOS ONE

N-Acetylcysteine in Agriculture, a Novel Use for an Old Molecule: Focus on Controlling the Plant–Pathogen *Xylella fastidiosa*

Lígia S. Muranaka[1,2], Thais E. Giorgiano[1], Marco A. Takita[1], Moacir R. Forim[3], Luis F. C. Silva[1], Helvécio D. Coletta-Filho[1], Marcos A. Machado[1], Alessandra A. de Souza[1*]

1 Centro de Citricultura Sylvio Moreira, Instituto Agronômico, Cordeirópolis, São Paulo, Brazil, 2 Departamento de Genética e Biologia Molecular, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil, 3 Departamento de Química, Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil

INPI Conceded 2018

USA 2022

How to transform NAC into product to reach the farmer?

Simone Picchi

PROGRAMA FAPESP
PESQUISA INOVATIVA EM PEQUENAS EMPRESAS - PIPE
FAPESP

CiaCamp
da Ciência ao Campo

AMAZON®
AgroSciences

PIPE - Innovative research in small companies

Technology transfer

Pesquisa FAPESP

Suzel Tunes

Edição 276
fev. 2019

Biotecnologia
Genética

IAC INSTITUTO AGRONÔMICO

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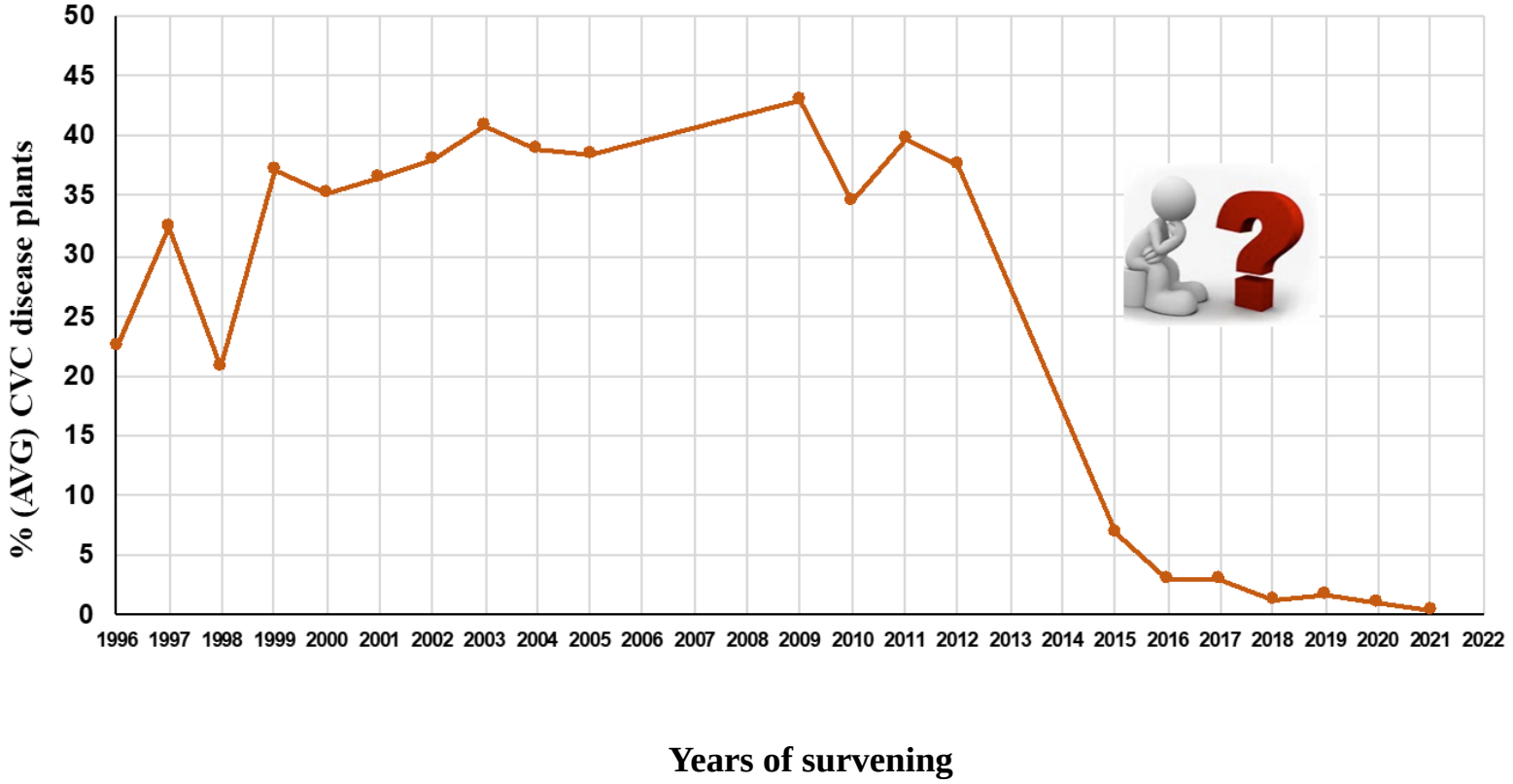
How is the situation of CVC today in São Paulo?

CVC incidence in sweet orange orchards in SP



Source: Fundecitrus

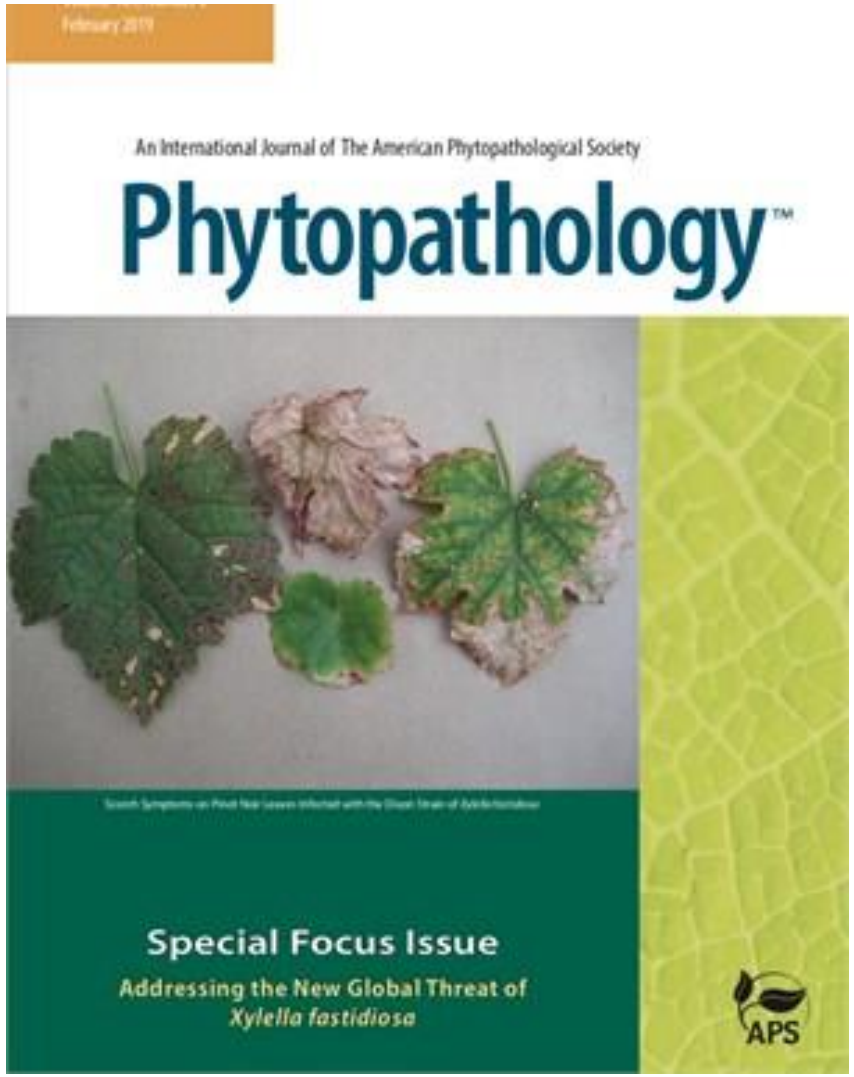
Indexing:

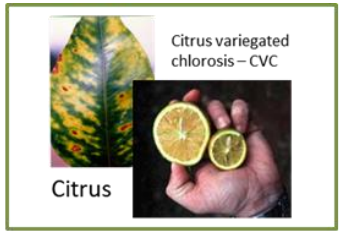
- Budwood mother plants (once a year).
- Budwoods blocks (once a year).
- All nursery plants

Replanting of citrus orchards **(>82%)** by nursery plants produced under the certify program. Indexing against XF and other pathogens.

Pruning of branches with early symptoms of CVC.

Multi-host pathogen – Economic losses in many crops






Grape

Plum

Xylella fastidiosa has wide host range that includes over 350 species of plants, including crops, trees and ornamental plants


Peach


Olive


coffee


Almond

2013

Phytopathologia Mediterranea (2016) 55, 1, 130–135
DOI: 10.14601/Phytopathol_Mediterr-17259

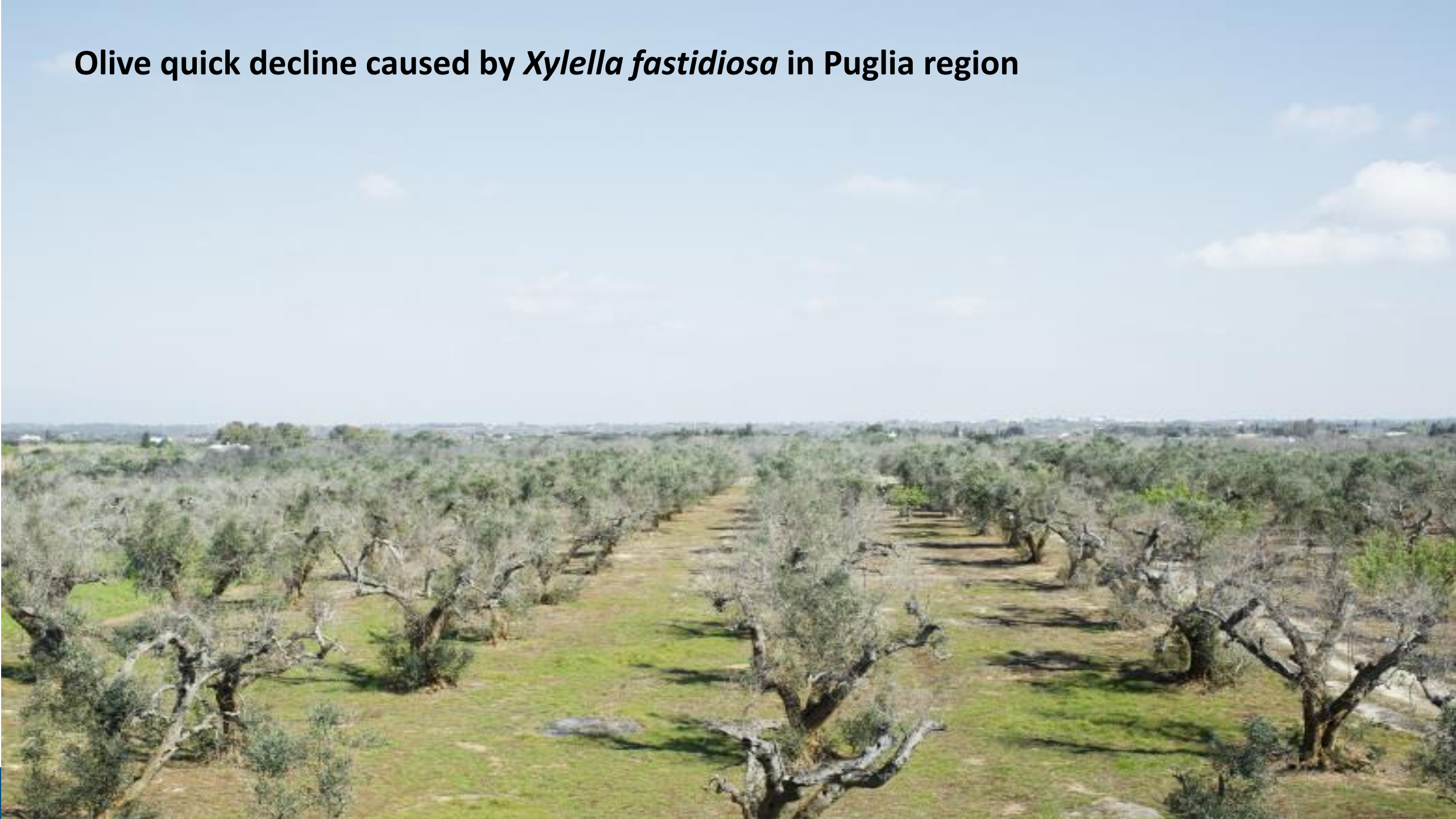
NEW OR UNUSUAL DISEASE REPORTS

First report of olive leaf scorch in Brazil, associated with *Xylella fastidiosa* subsp. *pauca*

Helvécio Della COLETTA-FILHO[1], Carolina Sardinha FRANCISCO[1], João Roberto Spotti LOPES[2], Adelson Francisco DE OLIVEIRA and Luiz Fernando de Oliveira DA SILVA[3]

[1] IAC / Centro de Citricultura Sylvio Moreira, Cordeirópolis, São Paulo, Brazil
[2] ESALQ / USP, Dep. Entomologia e Acarologia, Piracicaba, São Paulo, Brazil
[3] EPAMIG, Maria da Fé, Minas Gerais, Brazil

Olive quick decline caused by *Xylella fastidiosa* in Puglia region



Xylella Detection

2013 – Apulia Italy

CNR - Consiglio Nazionale delle Ricerche -BARI

Xylella Isolation

2014 – Apulia Italy

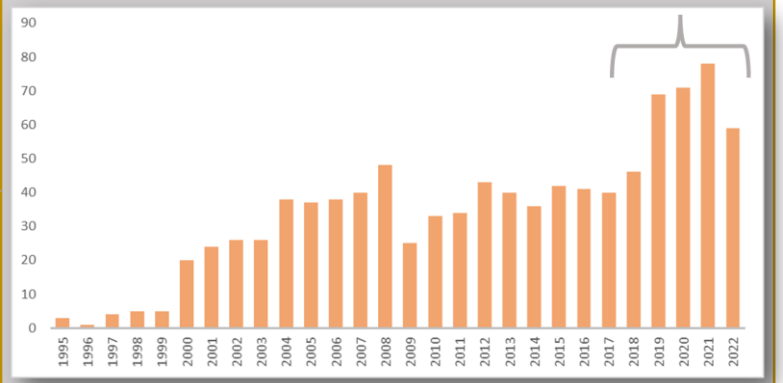
Centro de Citricultura Sylvio Moreira IAC

Dr. Helvécio Della Coletta – Filho

Dra. Maria Saponari from CNR - Bari

Consiglio Nazionale delle Ricerche

Xylella – Olive knowledge



Xylella fastidiosa Network – Brazil as protagonist in basic and applied knowledge

Collaborative Projects- Europe, USA, Brazil

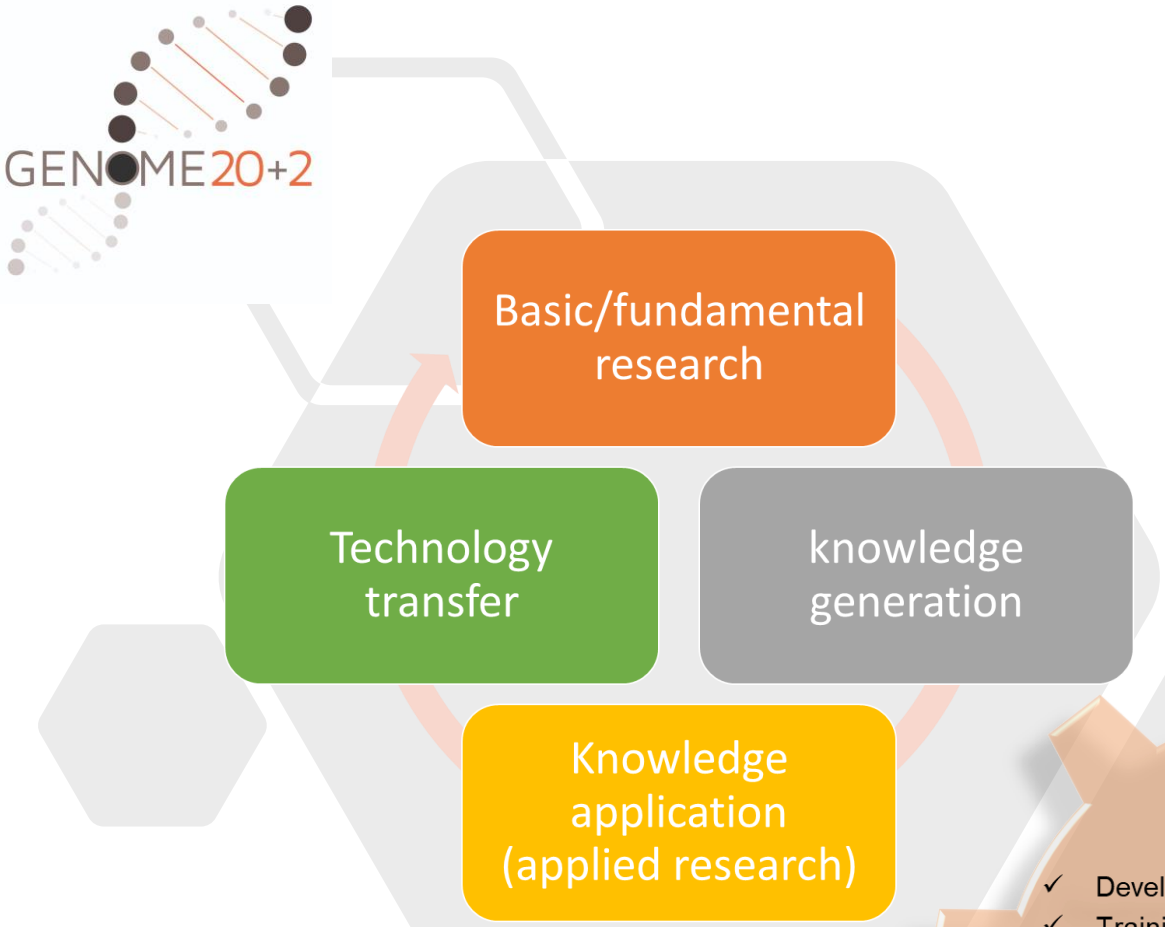
POnTE
PestOrganisms ThreateningEurope

XF ACTORS
Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy

BEXYL
BEYOND XYLELLA

European Commission
Horizon 2020
European Union funding for Research & Innovation

European Commission



Strategic vision - Investment in Basic Research

- ✓ Development of Molecular Biology
- ✓ Training of human resources in strategic areas
- ✓ Multiplier effect
- ✓ International scientific impact
- ✓ Recognition of Brazilian researchers in the international community
- ✓ International interactiona nd research impact
- ✓ increased multiplier effect

- ✓ Applied Research - Problem Solving Technology Transfer
- ✓ Training of students and insertion in the job market (national and international)
- ✓ Entrepreneurship – startups and job creation - (PIPE)

From basic to applied knowledge

Acknowledgment

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