Lecture Supplements

Plant Bacteriology

Bacterial Disease Management-Part 4

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Part III

Detection and management of major bacterial diseases

Fundamental Principles of Plant Disease Management

- Distribution
- Importance
- Symptoms
- Epidemiology and Biology
- The Pathogen
- Control Strategies

Plant clinic or polyclinic

Mobile clinics

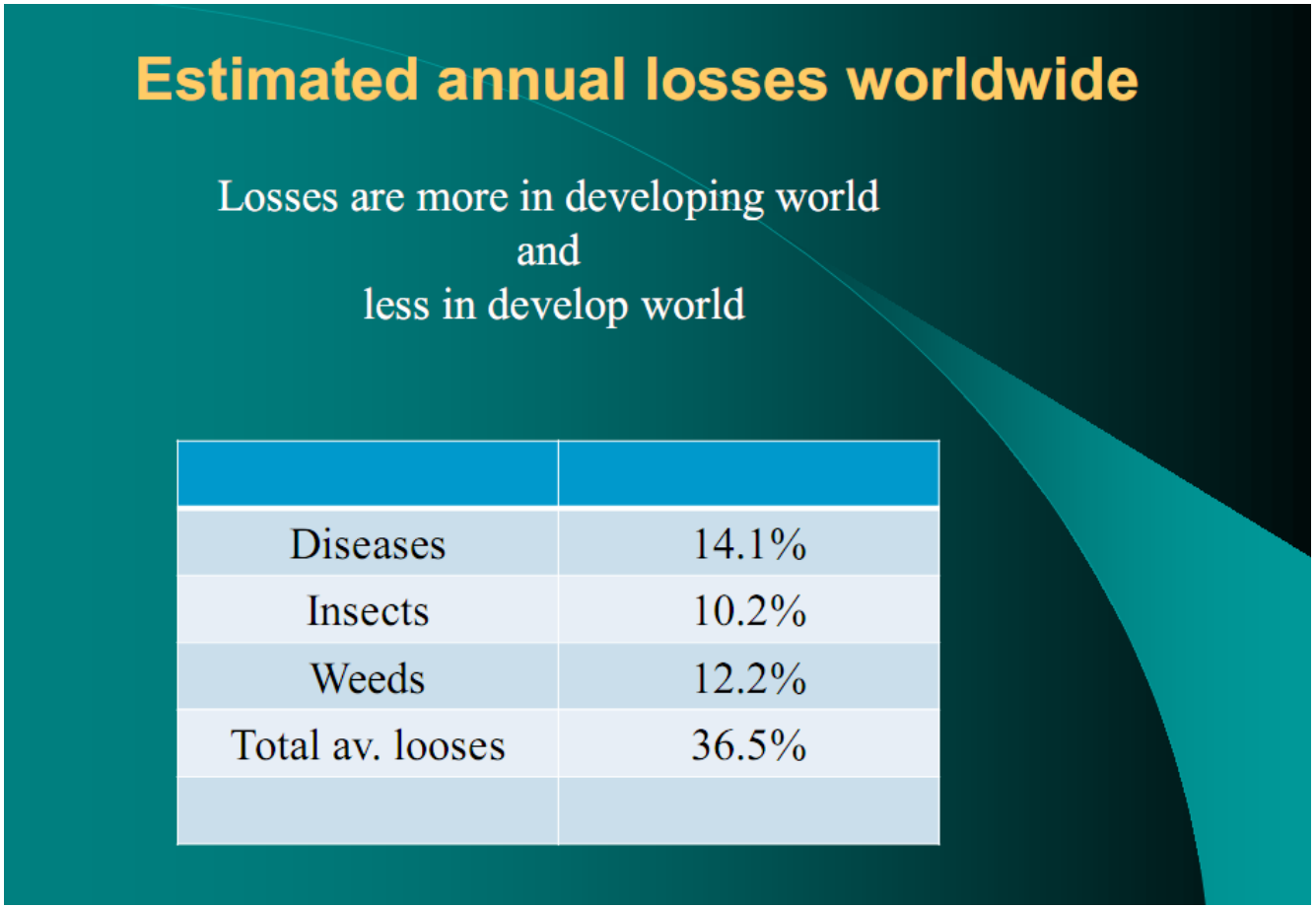
Plant clinic or polyclinic

The concept of dis-ease

- The concept of dis-ease in relation to plant clinic has to be taken in a broader perspective as conceived by grower, i.e. a plant is diseased when it's not at ease.
- An estimated 40% of potential global crop production is lost annually to pests and diseases.
- Reducing this level of crop loss is critical to increasing agricultural productivity, which is essential in achieving the sustainable development goals of zero hunger and no poverty.

Tambo *et al.*,2020;..

Crop losses due to disease and pests
Annual losses worldwide



Diseases	14.1%
Insects	10.2%
Weeds	12.2%
Total av. looses	36.5%

Crop losses due to disease and pests
Worldwide and USA

- All crop pests (pathogens, arthropods, and weeds) combined cause:

1. Preharvest losses of 42%
2. An additional 10% loss after harvest.

- Of these:

- 13% are due to plant pathogens,
- 15% to arthropods, and
- 13% to weeds.

Estimated annual crop losses worldwide Agrois,2005

Attainable crop protection (2002 prices)	$1.5 trillion
Actual crop production (~36.5%)	$950 billion
Production without crop protection	$455 billion
Losses prevented by crop protection	$415 billion
Actual annual losses to world crop production	$550 billion
Losses caused by disease only (14.1%)	$220 billion

Crop losses due to disease and pests
Worldwide and USA

- According to some estimates over 30% of the world's crops are lost in the field, another 15% are destroyed during transit and storage.
- Crop losses in the USA:
  - 9.1 billion lost to disease
  - 7.7 billion to insects
  - 6.2 billion to weeds.

Plant clinic or polyclinic
Results of plant pathology research

- Plant health clinics offer new avenues for applying the results of plant pathology research and for those results to make a positive contribution to farm family livelihoods.

Boa,2007

Plant clinic or polyclinic

The concept of dis-ease

- Plant clinics provide plant health care to farmers in similar way to what human health clinics do for humans.
- Organizational set-up would therefore include:

1. plant pathologist,
2. entomologist,
3. nematologist,
4. Edaphologist, and
5. agronomist.

Edaphology is concerned with the influence of soils on living things, particularly plants.

Plant clinic or polyclinic

Organizational set-up

- Thus, plant clinic is to be broadened to take care of:

1. Plant injury or damage due to insects, weeds, nematodes, mineral deficiency and toxicity, injury due to chemicals and abnormal weather such as frost or sun scorch;
2. Diseases due to plant pathogens.

Plant clinic or polyclinic

Organizational set-up

- Public extension services (government agencies),
- Private sector agrodealer, and
- Cooperative extension centers.

Public health plant clinics

Places for free diagnosis and recommendations

- Plant clinics are meeting places (mostly operating from local markets, community centres and cooperatives), where
- farmers who are struggling with any plant health problem can take samples of their ailing crops to trained plant health extension officers (referred to as plant doctors),
- for free diagnosis and recommendations on how to manage the problem (Boa, 2009, Majuga *et al.*,2018).

Plant clinic or polyclinic
Plant health clinics

- Over 4000 plant clinics have been established in 34 countries worldwide where farmers who are struggling with plant pests and diseases can take samples of their 'sick' crops to trained plant doctors for diagnosis and plant health advice.

Tambo *et al.*,2020

Plant clinic or polyclinic

Accurate diagnosis

Proper sample collection and submission

- Disease diagnosis is a critical initial step for successful disease management.
- Several different diseases and/or plant health problems can cause similar symptoms; therefore, it is important to obtain an accurate diagnosis to choose the best disease-control measures and to know what is affecting your crops or plants.
- Accurate and timely plant disease diagnosis starts with proper sample collection and submission.

Plant clinic or polyclinic

Samples collection and submissions

1. Samples should be collected as soon as disease symptoms develop, and
2. They should be submitted to the Plant Disease Clinic according to guidelines listed in the "How to submit a sample" section.

Plant clinic or polyclinic

Samples collection and submissions

- Collect living plants that exhibit varying stages of decline. Do NOT submit dead plants.
- For herbaceous plants, collect the entire plant, including as much of the root system as possible.
- Dig out (don't pull) several symptomatic plants and shake excess soil from the roots.
- Bundle plants together and wrap roots only in a plastic bag.
- Do not wrap stems in plastic. Wrap the entire bundle of plants in newspaper and place it in a cardboard box.

Plant Disease Clinic,University of Minnesota

Plant clinic or polyclinic

Samples collection and submissions

- For tree wilts, collect branches with a 1/2-inch (25.4 mm) to 1-inch diameter (12.7 mm).
- Collect sample from branches, which are actively wilting, but not totally dead.
- Wrap in plastic to retain moisture. Keep cool until submitted.
- For virus testing, collect symptomatic leaves, stems, or entire plants. Wrap in plastic. It is very important that these samples do not dry out during shipment.
- Do not add extra water to the bags.

Plant clinic or polyclinic

Accurate diagnosis for nematode analysis

- For nematode analysis, ship soil in plastic bags and keep refrigerated until shipped.
- It is important that nematode samples are not exposed to high temperature.

1. Ship samples immediately after collection;
2. Ship samples by overnight delivery or mail early in the week to ensure fast delivery;
3. Plant samples often decompose if left in the post office over the weekend.

Plant Disease Clinic,University of Minnesota

Plant clinic or polyclinic

Private sector

Payment for diagnosis and consultancy

- While diagnosis is extremely important, providing a suitable prescription is still more important.
- It must be clear and easily understandable.
- To make the clinic sustainable, a fee has to be levied for diagnosis and consultancy.

Florida Extension Plant Diagnostic Clinics

- Service provided upon special request (in Quincy clinic):

1. **PCR-based diagnostic services:** Standard rate plus $20.00 (at client request, available for some bacterial diseases such as crown gall, bacterial wilt and Pierce's disease).
2. **MIDI-based diagnostic services:** Standard rate plus $10.00 (at client request for bacterial pathogens).

Quincy: Just a boy's name

Palmateer and co-workers

Distance Diagnostic and Identification System (DDIS)

A new tool for extension diagnostics

Distance Diagnostic and Identification System (DDIS)

A new tool for extension diagnostics

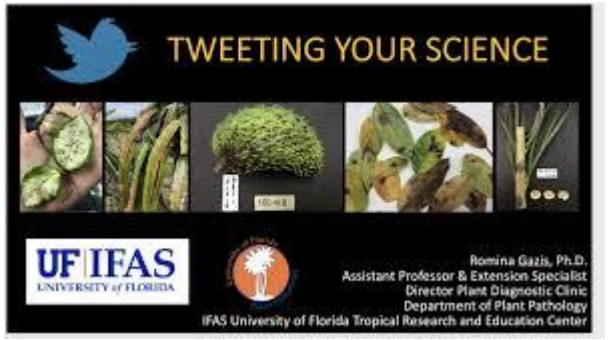
- The Distance Diagnostic and Identification System (DDIS) is used to transmit digital images with related information to enhance plant disease, insect and weed diagnostic capabilities.
- Images, by themselves, are one aspect of a diagnosis.
- They should be used in conjunction with laboratory assays if necessary.

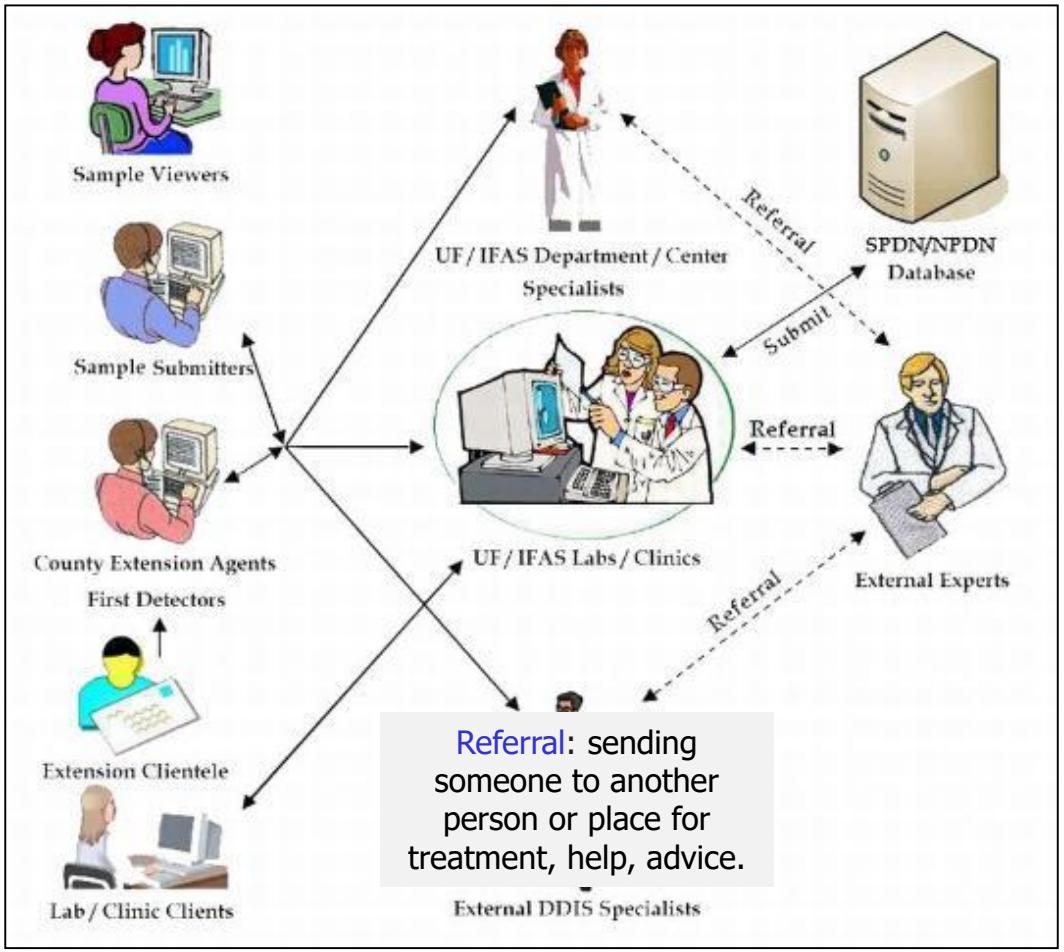
Momol *et al.*,2009

An Updated Web-based Distance Diagnostic and Identification System for Extension

Collaboration among DDIS users and specialists

UF/IFAS: The University of Florida's Institute of Food and Agricultural Sciences



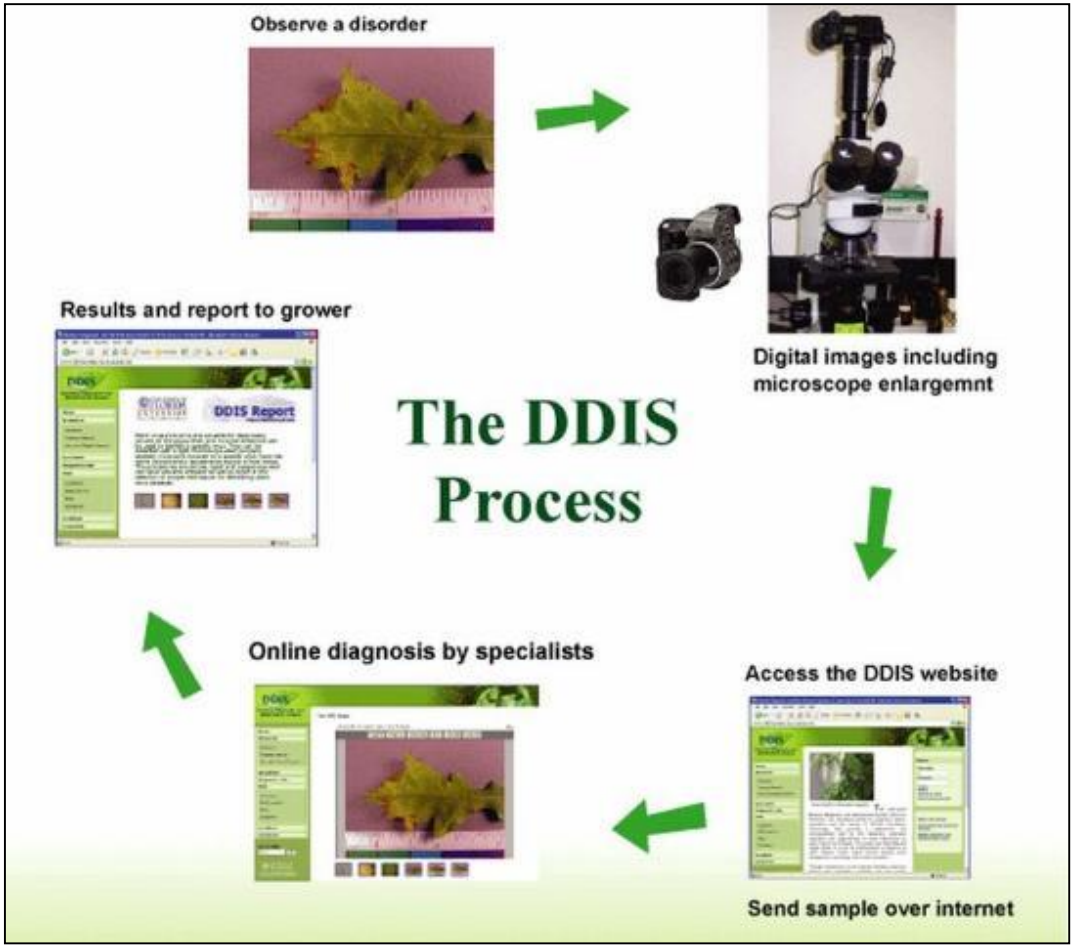


Referral: sending someone to another person or place for treatment, help, advice.

Momol *et al.*,2009;..

An Updated Web-based Distance Diagnostic and Identification System for Extension

DDIS sample submission and diagnosis process



Momol *et al.*,2009

Two smartphones: a Samsung Galaxy J5 (left) and an iPhone 6S (right)

Distance Diagnostic and Identification System (DDIS)

A smartphone is a portable device that combines mobile telephone and computing functions into one unit.

Using Deep Learning for Image-Based Plant Disease Detection

- The combination of increasing global smartphone penetration and recent advances in computer vision made possible by deep learning has paved the way for smartphone-assisted disease diagnosis.
- Using a public dataset of 54,306 images of diseased and healthy plant leaves collected under controlled conditions, we train a deep convolutional neural network (is a class of artificial neural network, most commonly applied to analyze visual imagery) to identify 14 crop species and 26 diseases (or absence thereof).
- The trained model achieves an accuracy of 99.35% on a held-out test set, demonstrating the feasibility of this approach.

Mohanty *et al.*,2016

Using Deep Learning for Image-Based Plant Disease Detection

- To enable a fair comparison between the results of all the experimental configurations, we also tried to standardize the hyper-parameters across all the experiments, and we used the following hyper-parameters in all of the experiments:

1. Solver type: Stochastic Gradient Descent(in contrat),
2. Base learning rate: 0.005,
3. Learning rate policy: Step (decreases by a factor of 10 every 30/3 epochs),
4. Momentum(mass in motion): 0.9,
5. Weight decay: 0.0005,
6. Gamma: 0.1,
7. Batch size: 24 (in case of GoogLeNet), 100 (in case of AlexNet).

Mohanty *et al.*,2016

Using Deep Learning for Image-Based Plant Disease Detection

- Sample images from the three different versions of the PlantVillage dataset used in various experimental configurations.

A. Leaf 1 color,
B. Leaf 1 grayscale,
C. Leaf 1 segmented,
D. Leaf 2 color,
E. Leaf 2 gray-scale,
F. Leaf 2 segmented.

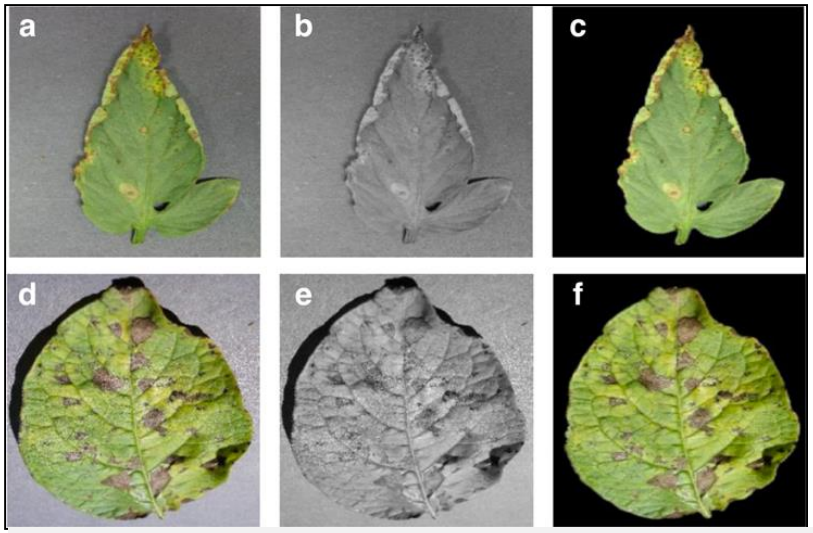


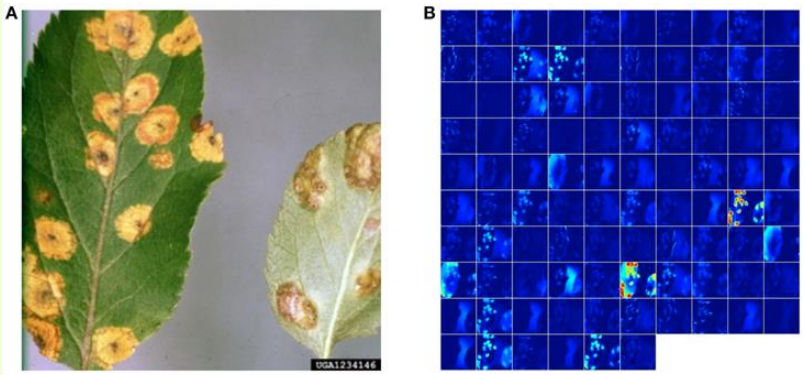
Figure shows the different versions of the same leaf for a randomly selected set of leaves.

Mohanty *et al.*,2016

Using Deep Learning for Image-Based Plant Disease Detection

A. Example image of a leaf suffering from Apple Cedar Rust, selected from the top-20 images returned by Bing Image search for the keywords "Apple Cedar Rust Leaves" on April 4th, 2016. Image Reference: Clemson University - USDA Cooperative Extension Slide Series, Bugwood. org.

B. Visualization of activations in the first convolution layer(conv1) of an AlexNet architecture trained using *AlexNet:Color:TrainFromScratch:80–20* when doing a forward pass on the image in shown in panel b.



Visualization of activations in the initial layers of an AlexNet architecture demonstrating that the model has learnt to efficiently activate against the diseased spots on the example leaf.
Note: AlexNet architecture consists of 5 convolutional layers, 3 max-pooling layers, 2 normalization layers, 2 fully connected layers, and 1 softmax layer.

Using Deep Learning for Image-Based Plant Disease Detection

- **Example of leaf images from the PlantVillage dataset, representing every crop-disease pair used.**
- **(1)** Apple Scab, *Venturia inaequalis* **(2)** Apple Black Rot, *Botryosphaeria obtusa* **(3)** Apple Cedar Rust, *Gymnosporangium juniperi-virginianae* **(4)** Apple healthy **(5)** Blueberry healthy **(6)** Cherry healthy **(7)** Cherry Powdery Mildew, *Podoshaera clandestine* **(8)** Corn Gray Leaf Spot, *Cercospora zeae-maydis* **(9)** Corn Common Rust, *Puccinia sorghi* **(10)** Corn healthy **(11)** Corn Northern Leaf Blight, *Exserohilum turcicum* **(12)** Grape Black Rot, *Guignardia bidwellii*, **(13)** Grape Black Measles (Esca), *Phaeomoniella aleophilum, Phaeomoniella chlamydospora* **(14)** Grape Healthy **(15)** Grape Leaf Blight, *Pseudocercospora vitis* **(16)** Orange Huanglongbing (Citrus Greening), *Candidatus Liberibacter* spp. **(17)** Peach Bacterial Spot, *Xanthomonas campestris* **(18)** Peach healthy **(19)** Bell Pepper Bacterial Spot, *Xanthomonas campestris* **(20)** Bell Pepper healthy **(21)** Potato Early Blight, *Alternaria solani* **(22)** Potato healthy **(23)** Potato Late Blight, *Phytophthora infestans* **(24)** Raspberry healthy **(25)** Soybean healthy **(26)** Squash Powdery Mildew, *Erysiphe cichoracearum* **(27)** Strawberry Healthy **(28)** Strawberry Leaf Scorch, *Diplocarpon earlianum* **(29)** Tomato Bacterial Spot, *Xanthomonas campestris* pv. *vesicatoria* **(30)** Tomato Early Blight, *Alternaria solani* **(31)** Tomato Late Blight, *Phytophthora infestans* **(32)** Tomato Leaf Mold, *Passalora fulva* **(33)** Tomato Septoria Leaf Spot, *Septoria lycopersici* **(34)** Tomato Two Spotted Spider Mite, *Tetranychus urticae* **(35)** Tomato Target Spot, *Corynespora cassiicola* **(36)** Tomato Mosaic Virus **(37)** Tomato Yellow Leaf Curl Virus **(38)** Tomato healthy.

1, Apple Scab, *Venturia inaequalis*; 16, Orange Huanglongbing (Citrus Greening), *Candidatus Liberibacter* spp.; 19, Bell Pepper Bacterial Spot, *Xanthomonas campestris*; 20, Bell Pepper healthy; 29,Tomato Bacterial Spot, *Xanthomonas campestris* pv. *vesicatoria*

Using Deep Learning for Image-Based Plant Disease Detection

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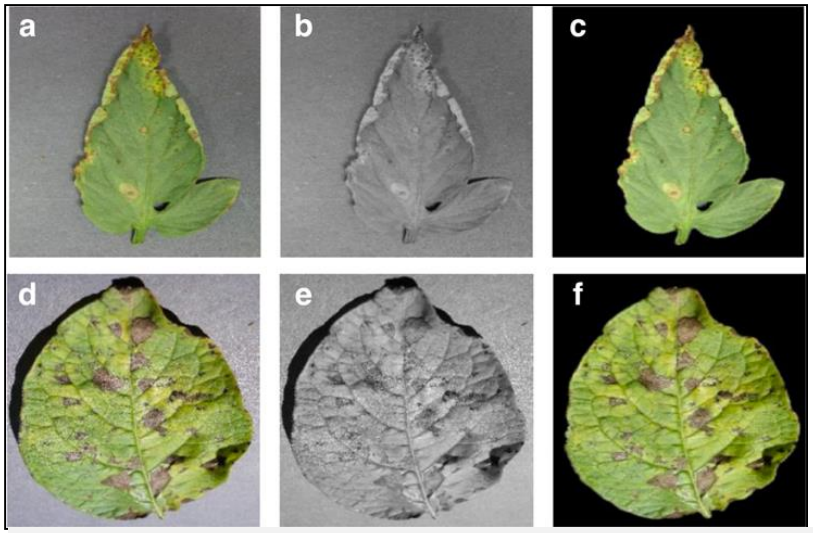

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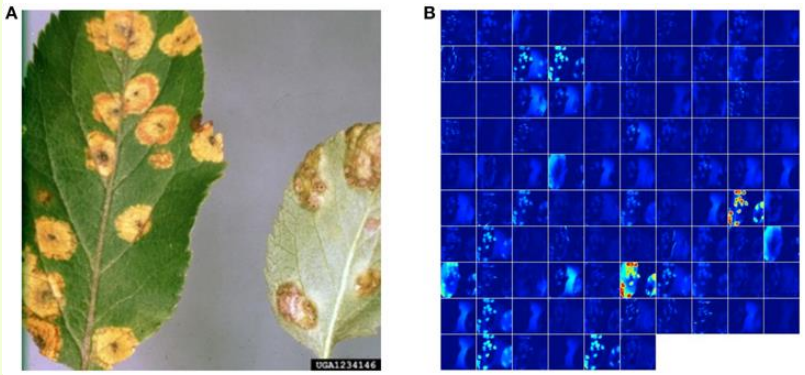

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Rapid diagnosis

Appropriate disease control

- The time taken to get a diagnosis is often critical.
- Rapid diagnosis is needed to allow appropriate control.

Rapid diagnosis
Appropriate disease control

- The development of protocols with higher and well-balanced sensitivity, and specificity for detection of plant pathogens, will have a positive effect on:

1. The sanitary status of the cultivated plants,
2. Reducing the long distance spreading of new, or
3. Emergent pathogens in a globalized world.

López *et al.*,2011

Rapid diagnosis

New detection protocols based on molecular methods

- This should drastically:

1. reduce the need for pesticide treatments,
2. increase the protection of, and
3. enhance the quality of food and the environment, not only in developed countries.

- The accuracy of new detection protocols based on molecular methods will lie behind the availability of plants free of a wide range of pathogens in a near future.

López *et al.*,2011

Diagnosis report
Within 3-14 days

- How long it will take before a diagnosis can be made depends on the bacterial species and the methods available for its identification.
- It may vary from 3-14 days.
- A diagnosis report usually is a letter sent to the original correspondent with the following information:

1. Origin and description of the sample.
2. Name of the bacterium isolated.
3. Name of disease caused by this bacterium and description of its symptoms.
4. Nature (biology, epidemiology) of the pathogen.
5. Nature of the damage.
6. Advice on preventive or control measures.

Janse,2006;..

Rapid diagnosis

Techniques for isolation and identification

- The selected techniques for detection were:

1. Isolation,
2. Immunofluorescence,
3. Enrichment-ELISA,
4. Direct tissue print-ELISA,
5. PCR and enrichment PCR.

- It also included:
- The identification of the bacterium by phenotypic and molecular characteristics and pathogenicity tests.

The purpose of nested PCR is to increase assay sensitivity by re-amplifying the target from a template previously enriched by the first PCR.

Rapid diagnosis

Techniques for isolation and identification

- **Selective media:**
- Usefulness depends on average recovery of target organism at low concentration in mixture with high populations of non target organisms.
- Detection level 10^2 colony-forming units (CFU)/g soil.
- Problems with selective media include:

1. May be strain specific;
2. Only is effective for certain assays (soil, leaves, or seed);
3. Has low recovery efficiency for target organism.

Rapid diagnosis

Techniques for isolation and identification

- **Bioassays:**
- Use host plant to isolate target organism,
- Spraying inocula over leaves or pour inocula over water-soaked leaves (10^5 CFU/ml detection level),
- Carrot slice inoculation for soft rot organisms (10^3 CFU/ml detection level),
- Infiltration (10 to 10^2 CFU/ml detection level).
- Very effective for selecting for target organism.
- Problems with certain soil types and troublesome procedure.

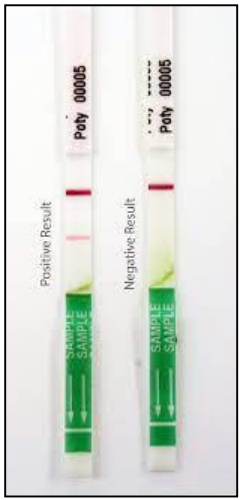
1. Injection;
2. Vacuum infiltration.

Rapid diagnosis

Techniques for isolation and identification

- **Serodiagnostic assays:**
- Cells being detected may be dead.

1. Direct and indirect immunofluorescence: 10^2 CFU/ml detection level.
2. ELISA: 10^4 to 10^5 CFU/ml detection level.
3. Diagnostic test kits(ImmunoStrip): immunostrip assay system is advantageous because of high sensitivity, inexpensive equipment, cost-effectiveness, ease of use.



Rapid diagnosis

Techniques for isolation and identification

- **Genetic assays:**
- PCR (10^2 CFU/ml detection level).
- Cells may be dead.
- Bio PCR-Direct plating followed by PCR. Real-time SYBR Green I assay was developed and evaluated as a biological and enzymatic polymerase chain reaction (Bio-PCR) protocol for the detection of plant bacterial pathogens.

International Diagnostic Protocols

International standards for the diagnosis of regulated pests

International Diagnostic Protocols

Standardization of methods

Phytosanitary regulations of the European Union

- During the last century, movement of goods and persons across the world has increased considerably.
- Natural borders that were once effective barriers to the spread of pests are now under pressure from the increasing volume of international trade.
- As a consequence, the global community has developed cooperative mechanisms to protect plants and the environment from pests.
- The International Plant Protection Convention (IPPC) is an international treaty to protect plant health.

Petter & Smith,2008

International Diagnostic Protocols
Standardization of methods
Normes EPPO Standards

- The first initiatives in developing standards on diagnostic protocols were taken by European and Mediterranean Plant Protection Organization (EPPO).
- Each protocol is intended to contain all the information necessary to detect and positively identify a particular regulated pest.
- Today, more than 300 Standards have been approved by EPPO and are included in this database (paper brochures are no longer available).
- Standards in the series PP1 are divided into:

1. General, and
2. Specific Standards.

EPPO database on PP1 Standards,2021

International Diagnostic Protocols
Standardization of methods
General Standards

- **General Standards** cover all general aspects of efficacy evaluation to help countries in understanding and fulfilling their obligations in the registration of plant protection products.
- e.g. advice on design, conduct, reporting and analysis of trials, phytotoxicity, effects on succeeding crops or adjacent crops, analysis of resistance risk, minor uses, and climatic considerations.
- All General Standards are freely available in the EPPO database on PP1(plant protection products) Standards.

EPPO database on PP1 Standards,2021

International Diagnostic Protocols
Standardization of methods
Specific Standards

- **Specific Standards** cover one type of plant protection product.
- E.g. fungicide/bactericide, insecticide/acaricides, herbicide, plant growth regulator, molluscicides, nematicides, rodenticide, etc.
- Each standard gives details for individual field trials.
- Indications are given on experimental conditions (trial conditions, design and lay-out of the trial etc.), applications of treatments (type, time and frequency of application etc.), mode of assessment, recording and measurement (type, time and frequency of assessment, effects on the crop etc.), reporting of results.

EPPO database on PP1 Standards,2021

Table 1 Published EPPO diagnostic protocols

Diagnostic protocol	
Published Bulletin OEPP/EPPO Bulletin 31(1), 2001	
PM 7/1 (1)	*Ceratocystis fagacearum*
PM 7/2 (1)	*Tobacco ringspot nepovirus*
PM 7/4 (1)	*Bursaphelenchus xylophilus*
PM 7/5 (1)	*Nacobbus aberrans*
Published Bulletin OEPP/EPPO Bulletin 32(2), 2002	
PM 7/6 (1)	*Chrysanthemum stunt pospiviroid*
PM 7/7 (1)	*Aleurocanthus spiniferus*
PM 7/8 (1)	*Aleurocanthus woglumi*
PM 7/9 (1)	*Cacoecimorpha pronubana*
PM 7/10 (1)	*Cacyreus marshalli*
PM 7/11 (1)	*Frankliniella occidentalis*
PM 7/12 (1)	*Parasaissetia nigra*
PM 7/13 (1)	*Trogoderma granarium*
Published Bulletin OEPP/EPPO Bulletin 33(2), 2003	
PM 7/14 (1)	*Ceratocystis fimbriata* f. sp. *platani*
PM 7/15 (1)	*Ciborinia camelliae*
PM 7/16 (1)	*Fusarium oxysporum* f. sp. *albedinis*
PM 7/17 (1)	*Guignardia citricarpa*
PM 7/18 (1)	*Monilinia fructicola*
PM 7/19 (1)	*Helicoverpa armigera*
Published Bulletin OEPP/EPPO Bulletin 34(2), 2004	
PM 7/20 (1)	*Erwinia amylovora*
PM 7/21 (1)	*Ralstonia solanacearum*
PM 7/22 (1)	*Xanthomonas arboricola* pv. *corylina*
PM 7/23 (1)	*Xanthomonas axonopodis* pv. *dieffenbachia*
PM 7/24 (1)	*Xylella fastidiosa*
PM 7/25 (1)	*Glomerella acutata*
PM 7/26 (1)	*Phytophthora cinnamomi*
PM 7/27 (1)	*Puccinia horiana*
PM 7/28 (1)	*Synchytrium endobioticum*
PM 7/29 (1)	*Tilletia indica*
PM 7/31 (1)	*Citrus tristeza closterovirus*
PM 7/32 (1)	*Plum pox potyvirus*
PM 7/33 (1)	*Potato spindle tuber pospiviroid*
PM 7/34 (1)	*Tomato spotted wilt tospovirus*
PM 7/35 (1)	*Bemisia tabaci*
PM 7/36 (1)	*Diabrotica virgifera*
PM 7/37 (1)	*Thaumetopoea pityocamp*
PM 7/38 (1)	*Unaspis citri*
PM 7/39 (1)	*Aphelenchoides besseyi*
PM 7/40 (1)	*Globodera rostochiensis* and *Globodera pallida*
PM 7/41 (1)	*Meloidogyne chitwoodi* and *Meloidogyne fallax*
Published Bulletin OEPP/EPPO Bulletin 35(2), 2005	
PM 7/42 (1)	*Clavibacter michiganensis* subsp. *michiganensis*
PM 7/43 (1)	*Pseudomonas syringae* pv. *persicae*
PM 7/44 (1)	*Xanthomonas axonopodis* pv. *citri*
PM 7/45 (1)	*Cryphonectria parasitica*
PM 7/46 (1)	*Mycosphaerella dearnessii*

Table 1 (continued)

Diagnostic protocol	
PM 7/47 (1)	*Mycosphaerella pini*
PM 7/48 (1)	*Phoma tracheiphila*
PM 7/49 (1)	*Tomato ringspot nepovirus*
PM 7/50 (1)	*Tomato yellow leaf curl* and *Tomato mottle begomoviruses*
PM 7/51 (1)	*Aonidiella citrina*
PM 7/52 (1)	*Diaphorina citri*
PM 7/53 (1)	*Liriomyza* spp.
PM 7/54 (1)	*Lopholeucaspis japonica*
PM 7/55 (1)	*Rhizoecus hibisci*
PM 7/56 (1)	*Scirtothrips aurantii*, *Scirtothrips citri* and *Scirtothrips dorsalis*
PM 7/57 (1)	*Trioza erytreae*
Published Bulletin OEPP/EPPO Bulletin 36(1), 2006	
PM 7/58 (1)	*Burkholderia caryophylli,*
PM 7/58 (1)	*Clavibacter michiganensis* subsp. *sepedonicus*
PM 7/60 (1)	*Pantoea stewartii* subsp. *stewartii,*
PM 7/61 (1)	Candidatus *Phytoplasma aurantifoliae,*
PM 7/62 (1)	Candidatus *Phytoplasma mali*
PM 7/63 (1)	Candidatus *Phytoplasma pyri*
PM 7/64 (1)	*Xanthomonas arboricola* pv. *pruni*
PM 7/65 (1)	*Xanthomonas fragariae*
PM 7/66 (1)	*Phytophthora ramorum*
PM 7/67 (1)	*American plum line pattern ilarvirus*
PM 7/68 (1)	*Eotetranychus lewisi*
PM 7/69 (1)	*Lepidosaphes ussuriensis*
PM 7/70 (1)	*Maconellicoccus hirsutus*
PM 7/71 (1)	*Opogona sacchari*
PM 7/72 (1)	*Tecia solanivora*
PM 7/3 (2)	*Thrips palmi*
Published Bulletin OEPP/EPPO Bulletin 36(3), 2006	
PM 7/30 (2)	*Beet necrotic yellow vein benyvirus*
PM 7/73 (1)	*Gymnosporangium* spp. (non-European)
PM 7/74 (1)	*Popillia japonica*
PM 7/75 (1)	*Toxoptera citricidus*
PM 7/76 (1)	Use of EPPO diagnostic protocols
PM 7/77 (1)	Documentation and reporting on a diagnosis

Published eppo diagnostic protocols

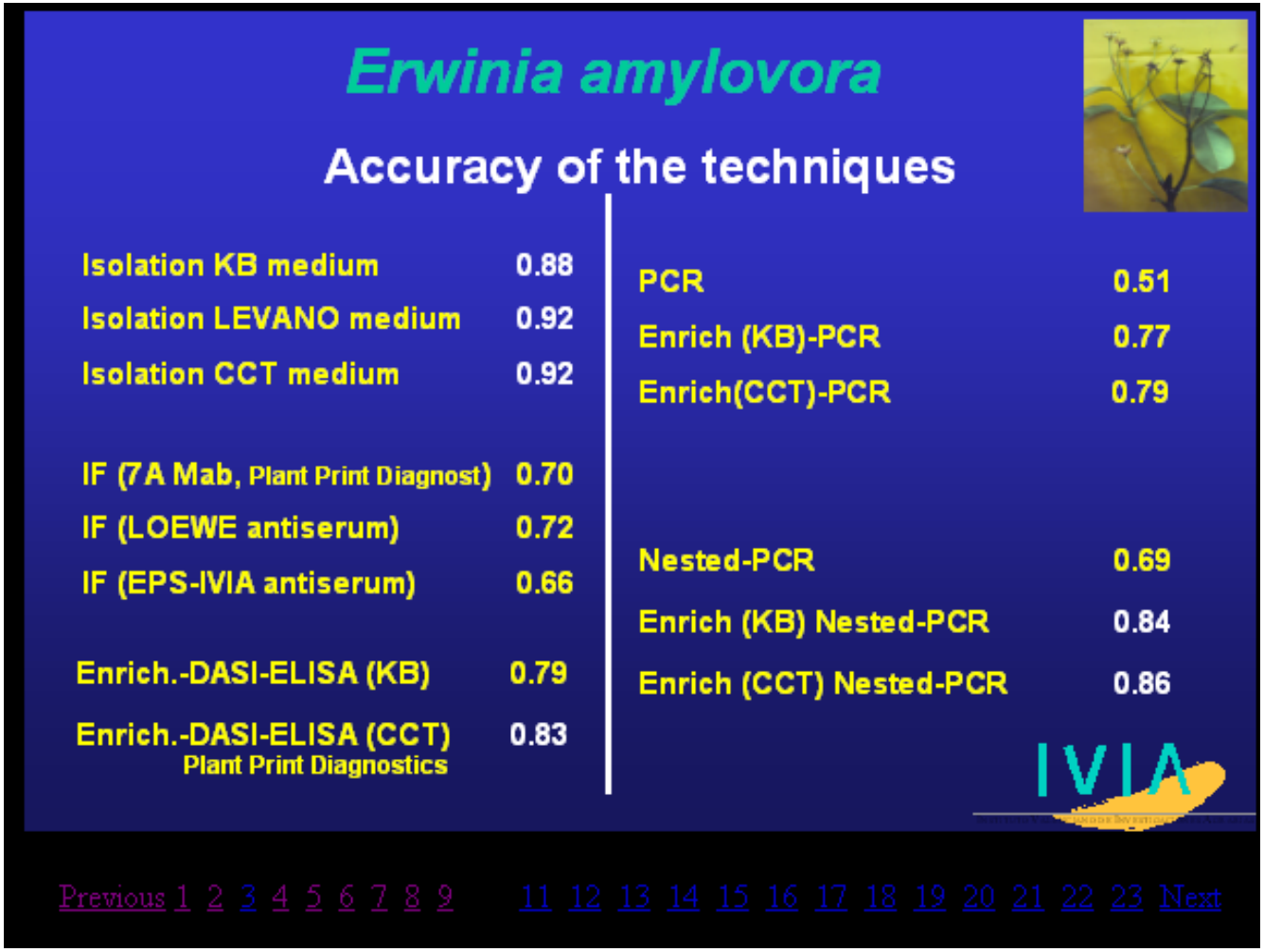
Standardization of methods for diagnosis of *E. amylovora* and *X. fragariae*

EPPO Standards

Lopez *et al.*,2004. Diagnostic protocol for organisms harmful to plants. presented in EPPO Conference on Quality of Diagnosis and New Diagnostic Methods for Plant Pests.

International Diagnostic Protocols
Standardization of methods
Accuracy of the techniques

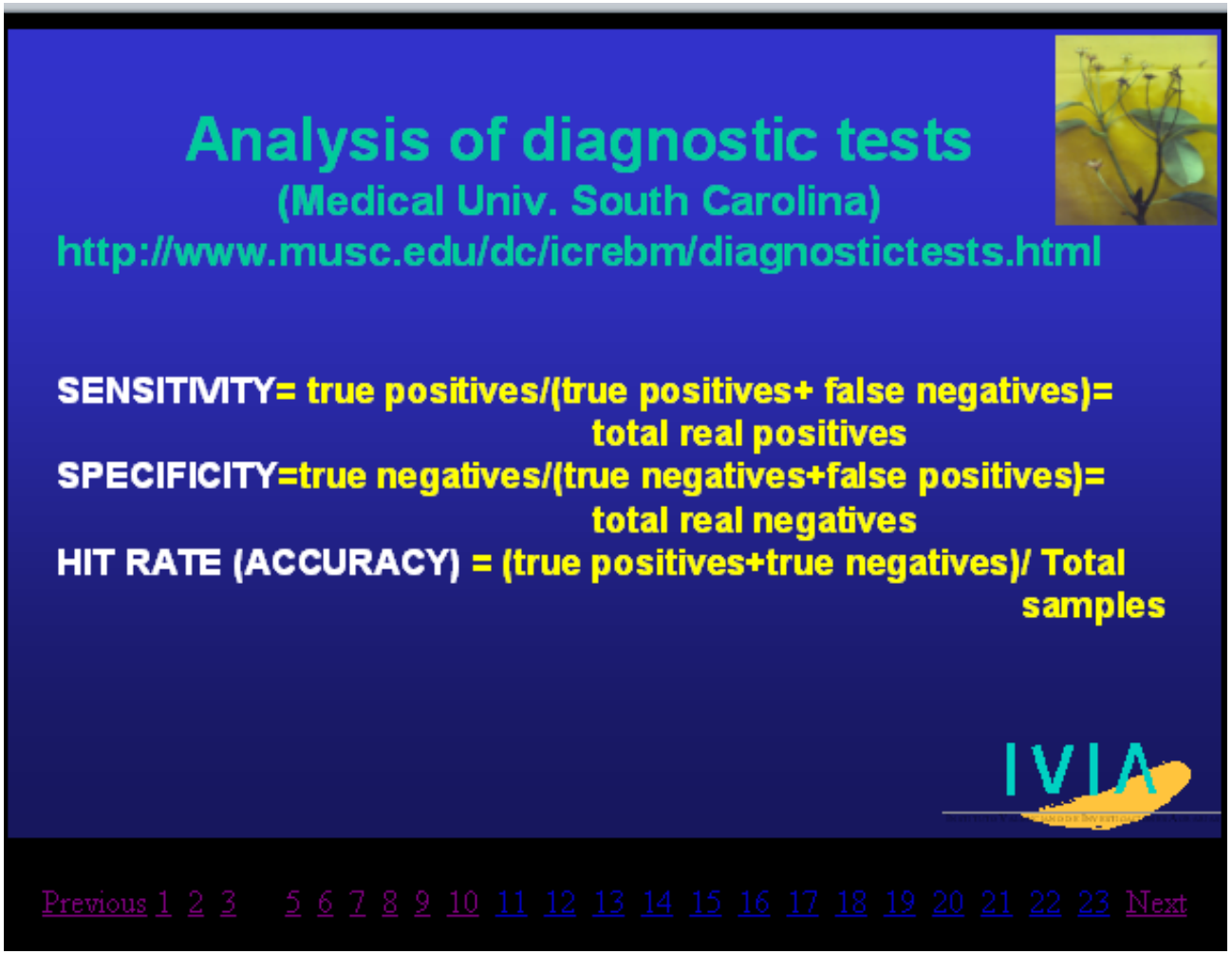
88/100=0.88
0.92/0.88=1.04



International Diagnostic Protocols

Standardization of methods

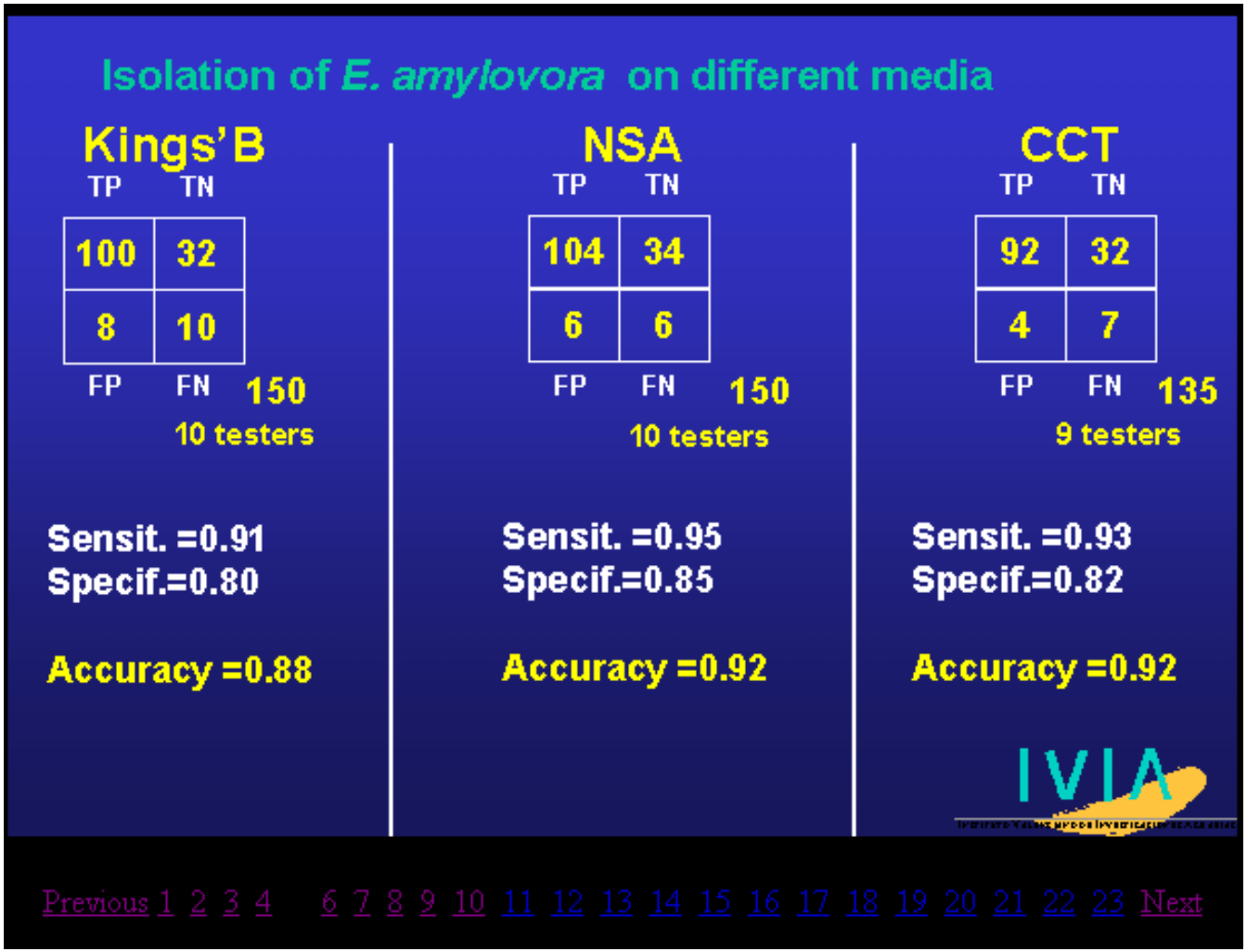
Formula for sensitivity, specificity and accuracy



International Diagnostic Protocols

Standardization of methods

Formula for sensitivity, specificity and accuracy



0.91/0.80=1.03

CABIQ

Windows-based system called CABIQ ('Classification Automatique Bactéries Identification Quarantaine')

Specific computer-assisted identification system of phytopathogenic bacteria

CABIQ:

Specific computer-assisted identification system of phytopathogenic bacteria

- This tool will be released as a Windows-based system called CABIQ ('Classification Automatique Bactéries Identification Quarantaine') incorporating the database.
- About 500 reference strains have been used to initiate the database.
- The CABIQ system, with its database and reference matrices, is a guide on the tests to be done when identifying new isolates.
- This system was designed for phytosanitary regulations of the European Union to **survey for certain quarantine pests and to prohibit their introduction and spread into the EU**.

Poliakoff *et al.*,2005

CABIQ

Windows-based system called CABIQ ('Classification Automatique Bactéries Identification Quarantaine')

- This tool is innovative because it combines:

1. Traditional identification tests(Conventional phenotypic tests and the Biotype 100 (BioMérieux) galleries), and
2. Molecular methods in a single system to provide a more rapid and reliable identification.

Poliakoff *et al.*,2005

CABIQ

Culture collection

List of regulated bacteria studied for the creation of the database

- 14 taxa of regulated bacteria were selected from the EU list, along with representative species of close taxonomic groups, to create a collection of nearly 500 reference strains.
- For each regulated taxon, 15 strains on average were elected including the type strain.
- Strains were selected from ecological and geographical origins which were as different as possible, so that the collection should be representative of potential natural diversity.

Gram group	Genus or family	Regulated taxon
+	*Clavibacter*	*C. michiganensis* subsp. *insidiosus*
		C. michiganensis subsp. *michiganensis*
		C. michiganensis subsp. *sepedonicus*
+	*Curtobacterium*	*C. flaccumfaciens* pv. *flaccumfaciens*
–	*Pseudomonas*	*P. syringae* pv. *persicae*
–	*Xanthomonas*	*X. arboricola* pv. *pruni*
		X. axonopodis pv. *citri*,
		X. axonopodis pv. *aurantifolii*
		X. axonopodis pv. *citrumelo*
		X. fragariae
		X. arboricola pv. *fragariae*
–	*Ralstonia*	*R. solanacearum*
–	*Enterobacteriaceae*	*Pantoea stewartii* subsp. *stewartii*
		Erwinia amylovora

CABIQ

Rules to determine the probable genus of a Gram-positive bacterium

- Rules to determine the probable genus of a Gram-positive bacterium according to the results of orientation tests (O/F, enzymatic activities, production of acid from different carbon sources):

Test results	Probable taxon
Fermentative on Hugh and Leifson (+)	*Enterobacteria* (*Erwinia*/*Pantoea*)
Non-fermentative on Hugh and Leifson (–), fluorescence on King B (+)	*Pseudomonas*
Non-fermentative on Hugh and Leifson (–), fluorescence on King B (–), nitrate reductase (+), oxidase (+)	*Ralstonia*
Non-fermentative on Hugh and Leifson (–), fluorescence on King B (–), nitrate reductase (–), no oxidase (–), cream colonies	*Pseudomonas*
Non-fermentative on Hugh and Leifson (–), fluorescence on King B (–), nitrate reductase (–), no oxidase (–), yellow colonies, mucoid culture	*Xanthomonas*

Poliakoff *et al.*,2005

CABIQ

Measurement of resemblance

- A paired comparison method may be used to check which reference strains are most similar to the unknown isolate.
- After these comparisons, CABIQ calculates the interrelation between the unknown isolate and each taxon through:

1. likelihood coefficient,
2. normalized likelihood coefficient (also called identification score) or Willcox's probability,
3. and relative likelihood.

CABIQ

Measurement of resemblance

- For the example given in Table 7, the unknown isolate is identified as *Clavibacter nebraskensis*, since this taxon has the highest identification score.
- The modal likelihood fraction proves the absolute degree of affinity to the species in the matrix.
- In the case of Table 8, the unknown isolate looks like *Clavibacter sepedonicus* but this identification is not validated.

Subspecies	Oxidase	Levan	Glucose	Erythritol	Esculin
Clavibacter michiganensis subsp. *insidiosus*	0.14	0.10	0.43	0.10	0.90
Clavibacter michiganensis subsp. *michiganensis*	0.10	0.10	0.81	0.10	0.90
Clavibacter michiganensis subsp. *nebraskensis*	0.10	0.90	0.90	0.10	0.90
Clavibacter michiganensis subsp. *sepedonicus*	0.10	0.20	0.67	0.10	0.90
Clavibacter michiganensis subsp. *tessellarius*	0.10	0.80	0.90	0.10	0.90
Unknown isolate	0	1	1	0	1

Table 6 Comparison of an unknown isolate with the positivity frequency matrix of the different subspecies of *Clavibacter michiganensis* given by CABIQ

5 taxa and 19 conventional tests	Score	Modal likelihood fraction
Clavibacter michiganensis subsp. *insidiosus*	5.9%	7.3%
Clavibacter michiganensis subsp. *michiganensis*	9.0%	11.1%
Clavibacter michiganensis subsp. *nebraskensis*	98.7%	100.0%
Clavibacter michiganensis subsp. *sepedonicus*	9.0%	11.1%
Clavibacter michiganensis subsp. *tessellarius*	1.0%	1.2%
Unknown isolate	Successful identification	

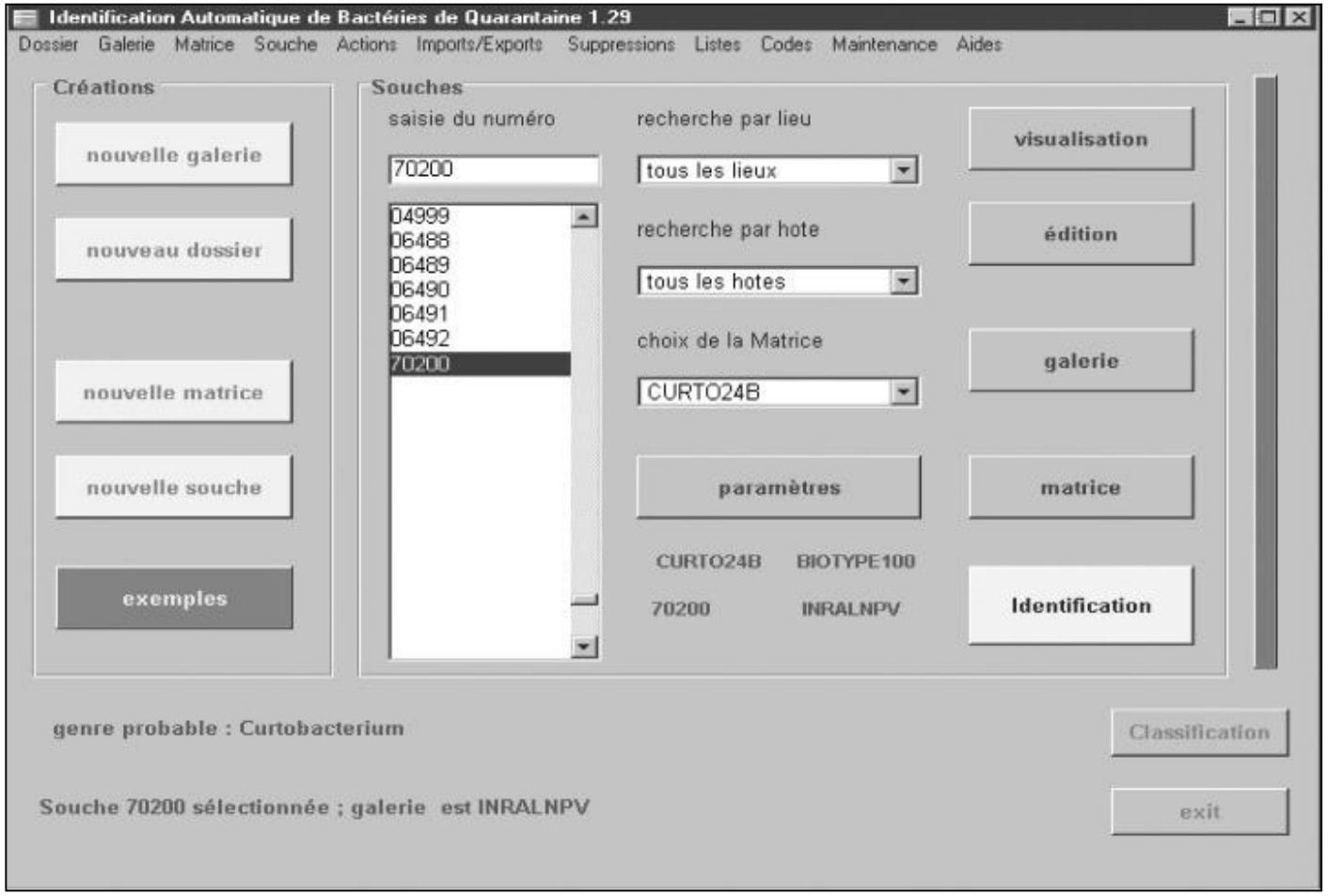
Table 7 Identification score of Lapage and Wilcox and modal likelihood fraction given by CABIQ (example of validated identification)

5 taxa and 19 conventional tests	Score	Modal likelihood fraction
Clavibacter michiganensis subsp. *insidiosus*	0.0%	0.0%
Clavibacter michiganensis subsp. *michiganensis*	0.0%	0.0%
Clavibacter michiganensis subsp. *nebraskensis*	14.7%	4.2%
Clavibacter michiganensis subsp. *sepedonicus*	82.1%	0.3%
Clavibacter michiganensis subsp. *tessellarius*	3.1%	1.5%
Unknown isolate	Non validated identification	

Table 8 Identification score of Lapage and Wilcox and likelihood fraction given by CABIQ (example of non-validated identification)

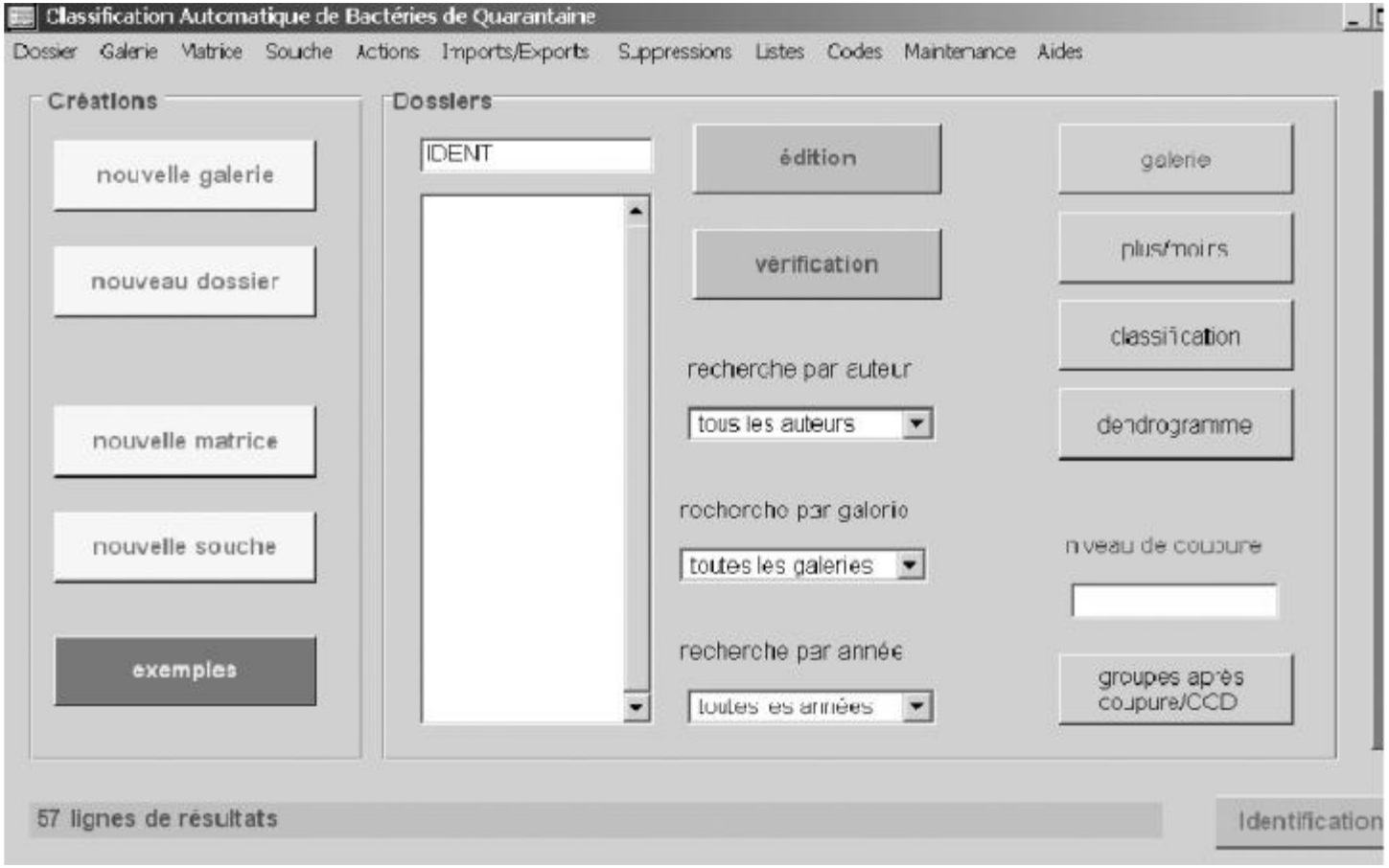
Poliakoff *et al.*,2005

Identification interface of CABIQ



Poliakoff *et al.*,2005

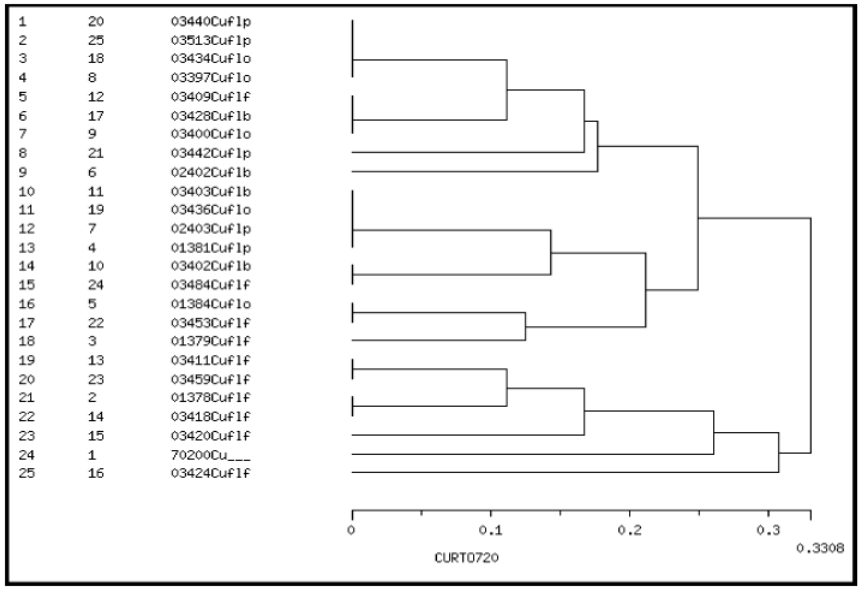
Identification interface of CABIQ



Poliakoff *et al.*,2005

Numerical analysis of phenotypic features with the CABIQ software

- Import and export strains for automatic clustering using the UPGMA (Unweighted Pair Group Method with Average) leading to dendrograms and CDC (coefficients of diagnosis capacity) computations, considering bacterial strains as Operational Taxonomic Units.
- CABIQ software is moreover able to import and export data using classical formats (Dbase, Excel, text, Phylip).
- Clustering of an unknown isolate 70200 in the dendrogram of subspecies of *Curtobacterium flaccumfaciens* given by CABIQ.



Clustering of an unknown isolate

Poliakoff *et al.*,2005

Plant bacterial disease management

Gram-negative bacteria

Acidovorax avenae subsp.*avenae*

Brown stripe disease in rice

Management

- Control of diseases caused by *A. avenae* subsp. *avenae* such as blight/brown stripe of rice, red stripe and top rot of sugarcane, leaf blight of sorghum and leaf blight and stalk rot of maize is most effectively achieved by using pathogen-free seed.
- There is little information on the use of chemicals to control diseases caused by *A. avenae* subsp. *avenae*.
- However,
- The bactericide kasugamycin has been used to control brown stripe disease in rice (Kadota & Ohuchi,1990), and
- Copper sulphate and streptocycline (streptomycin and tetracycline) has been used to control infection in maize (Thind, Randhawa, & Soni,1984).

Fegan,2006

Acidovorax avenae subsp.*citrulli*

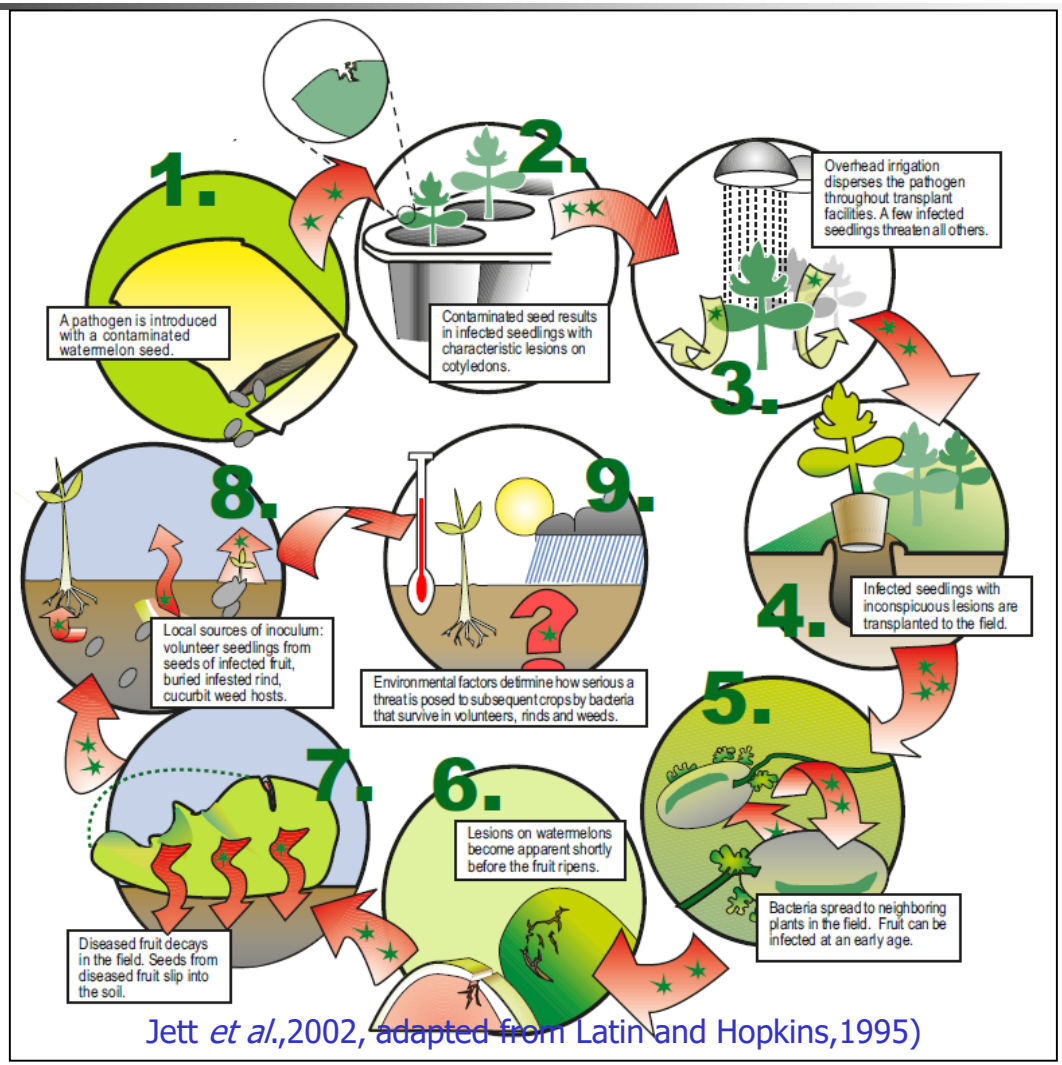
Bacterial Fruit Blotch of Watermelon (BFB)

Economic importance

- BFB is one of the most serious diseases facing U.S. cucurbit seed producers because it can cause up to 00% yield loss (Latin and Hopkins, 1995).

Disease cycle

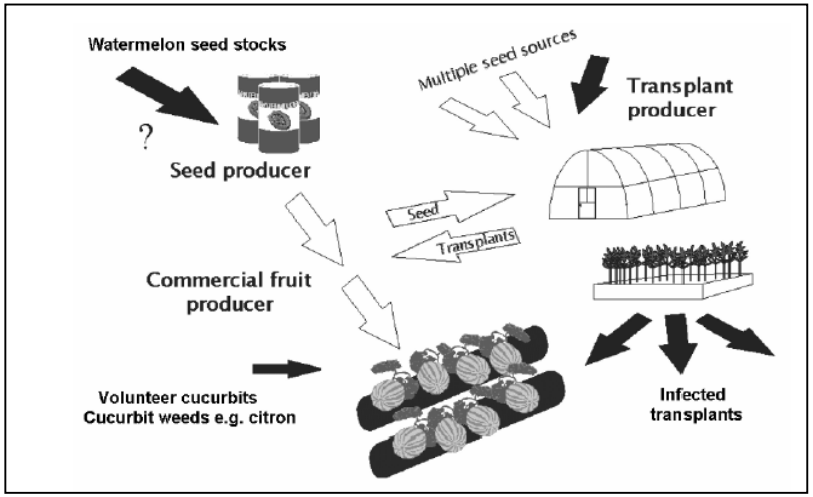
Acidovorax avenae subsp. *citrulli*



Jett *et al.*,2002, adapted from Latin and Hopkins,1995)

Bacterial fruit blotch (BFB) Integrated Management of bacterial fruit blotch of cucurbits

- Relatedness of seed, seedling and fruit production in commercial watermelon production, and the potential for introducing *Acidovorax avenae* subsp. *citrulli* at each stage.
- Black arrows represent potential sources of inoculum.



Ciancio & Mukerji (eds.),2008

Management

- Control of bacterial fruit blotch is best achieved through preventative measures.
- Practice good greenhouse sanitation.
- Purchase watermelon seed that has been tested negative for bacterial fruit blotch in a seedling grow out test.
- Monitor transplants for symptoms.
- If infected plants or potentially infected plants have been transplanted in the field, begin copper spray applications to suppress BFB (Next Table).
- Copper hydroxide fungicides can be tank mixed with most fungicides used to control fungal diseases.
- If symptoms are observed, copper hydroxide should be applied immediately after planting on a 7- to 10- day schedule.
- If BFB has occurred, follow a strict three-year rotation, deep plow after harvest and control all volunteer melons and cucurbit weeds.

Jett *et al.*,2005

Management
Seed Health Testing

- At present, the most widely accepted assay is the greenhouse seedling grow out test, by which samples of seeds (n=10,000–30,000 seeds/lot) are planted under greenhouse conditions favorable for BFB development (>70% relative humidity and 24-35°C). see http://www.seedhealth.org/VEG.Crops.pdf.
- Eighteen days after planting, the seedlings are inspected visually for seedlings expressing BFB symptoms.
- This assay is technically simple and indicates the potential for BFB seedling transmission.
- Walcott *et al*. 2006, reported that the seedling grow out assay could detect only 12.5% (1/8) and 37.5% (3/8) of seedlots (n=10,000 seeds) with 0.01 and 0.1% infested seeds respectively.

Ciancio & Mukerji (eds.),2008

Management
Chemical seed treatments

- Chemical seed treatments with 1% HCl, CaOCl and NaOCl have also been evaluated for seed-borne *A. avenae* subsp. *citrulli*.
- Treatment with 0.5-1% NaOCl or CaOCl for 15-20 min reduced, but did not eliminate BFB seedling transmission.
- On the other hand, while Hopkins *et al*.,1996 reported that seed treatment with 1% HCl for 15 min eliminated BFB infection, Rane and Latin (1992) found that BFB seedling transmission was reduced, but not eliminated, by exposure to 1.8% HCl for 5 min.
- Hopkins recommended that a combination of fermentation and HCl or CaOCl was the most effective seed treatment for BFB.
- However, this treatment might not be applicable for all cucurbit cultivars and species.

Ciancio & Mukerji (eds.),2008

Management
Chemical seed treatments

- Pesticides labeled for suppression of bacterial fruit blotch.

Trade name	Common name	Rate/acre	Comments
Kocide 2000	Copper hydroxide (54%)	1.5 lb	
Kocide 4.5 LF	Copper hydroxide (38%)	1.33 pt	
Kocide DF	Copper hydroxide (61%)	2 lb	5-day postharvest intverval; Apply at 14-day intervals.
ManKocide DF	Copper hydoxide (46%) + Mancozeb	2.5 lb	5-day postharvest interval; Apply at 14-day intervals. pH of spray solution must be above 6.5.
Nu-Cop 50 DF	Copper hydroxide (77%)	2 lb	
Nu-Cop 3L	Copper hydroxide (38%)	1–4 pt	

Jett *et al.*,2005

Management

A. avenae subsp. *citrulli*

Biological control seed treatments

- Fessehaie and Walcott (2005) demonstrated that treatment of *A. avenae* subsp. *citrulli*-infested seeds with *A. avenae* subsp. *avenae* strain 99-2 reduced BFB seedling transmission by 96.5% and 100% under growth chamber and greenhouse conditions, respectively.
- Oliveira *et al.*,2006 have reported several *Bacillus* species with potential to serve as biocontrol seed treatments for *A. avenae* subsp. *citrulli*.

Ciancio & Mukerji (eds.),2008

Burkholderia spp.

- ***B. glumae*** **causes bacterial seedling and grain rot in rice.**
- ***B. plantarii*** **causes bacterial seedling blight of rice.**

Epidemiology
Burkholderis spp.

- It has been established that pathogenic bacteria exist on the phylloplanes of rice plants during the growing season or in rice seeds stored at room temperature during the winter.
- Soil distributions of these pathogens are affected by soil type, pH value, plants cultivated, and weather conditions.
- This disease tends to break out under conditions of unusually high temperatures, especially at night, and frequent rains.
- Yield losses as high as 40% were observed in some fields (Shahjahan,2000b).

Yuan,2004

Management of *Burkholderis* spp.
Chemical control

- **Chemical Control**
- Many bactericides are able to effectively control or suppress the occurrence of seedling rot and panicle rot caused by plant pathogenic *Burkholderia* spp.
- Including antibiotics, copper, and copper-containing compounds.
- Oxolinic acid, a synthetic bactericide developed from quinoline derivatives, inhibits disease development by Gram negative bacteria.
- This compound was highly efficacious for the control of this rice disease, either as seed treatments or foliar sprays.
- It exhibited both preventive and curative effects (Hikichi, 1993).
- A study of the efficacy of oxolinic acid, with FITC-conjugated antibody and fluorescence microscopy, demonstrated that only 3% of seedlings expressed measurable symptoms after treatment, compared with 92% of seedlings from untreated seeds (Hikichi, 1995).

Yuan,2004

Management

Biological control of *Burkholderia* spp.

- Virtually all Bcc species have also been isolated from the natural environment, often from soil samples or from the rhizosphere of various plants.
- The use of *Burkholderia* in agricultural applications is therefore considered a double-edged sword, and a lot of effort has been invested into discriminating between the beneficial environmental (the good) and the clinical (the bad) *Burkholderia* strains.
- The *Burkholderia cepacia* complex (Bcc), are as opportunistic pathogens that can cause severe infections in cystic fibrosis (CF) and immunocompromised patients.
- But many new *Burkholderia* species have been identified in environmental samples that exhibit potentially valuable beneficial traits.
- These species are believed to be safe for applications, as there are very rarely clinical reports that they would pose a risk to human health.

Eberla and Vandamme,2016

Management

Biological control of *Burkholderia* spp.

Avirulent strain of *Burkholderia gladioli*

- **Biological Control**
- The ability of some avirulent strains of *Burkholderia* to restrict the development of rice grain rot (*Burkholderia glumae*) has been confirmed.
- Spraying rice panicles with a mixed suspension of a virulent strain of *B. glumae* and an avirulent strain of *B. gladioli* almost completely controlled the occurrence of the disease in pot and field experiments (Miyagawa and Takaya,2000).
- Similar efficacy was expressed when the seedlings were sprayed with the avirulent *B. gladioli* strain prior to inoculation with the *B. glumae* strain; whereas no suppression was observed when the order of inoculation was reversed (Miyagawa,2000a).
- Similar results were also observed using avirulent strains of *B. glumae* (Furuya *et al.*,1991) and of *B. plantarii* (causes bacterial seedling blight of rice), indicating that pre-treatment of seeds with avirulent strains is an effective biological control method.

Yuan,2004

Dickeya chrysanthemi

Wilt and soft rot diseases

Stem rot of banana

Dickeya chrysanthemi
Wilt and soft rot diseases

- *D. chrysanthemi* pv. *zeae* causes a highly destructive disease of maize in tropical and subtropical countries, particularly under conditions of high temperature and humidity.
- Plants are most susceptible when they are 40-60 days old and symptoms consist of withered leaves and brown, soft and water-soaked stems.
- Infected plants usually emit an unpleasant odour and, when the disease is advanced, collapse.

Bacterial wilt and stem rot of fleshy plants caused by *Dickeya* (*Erwinia*) *chrysanthemi*

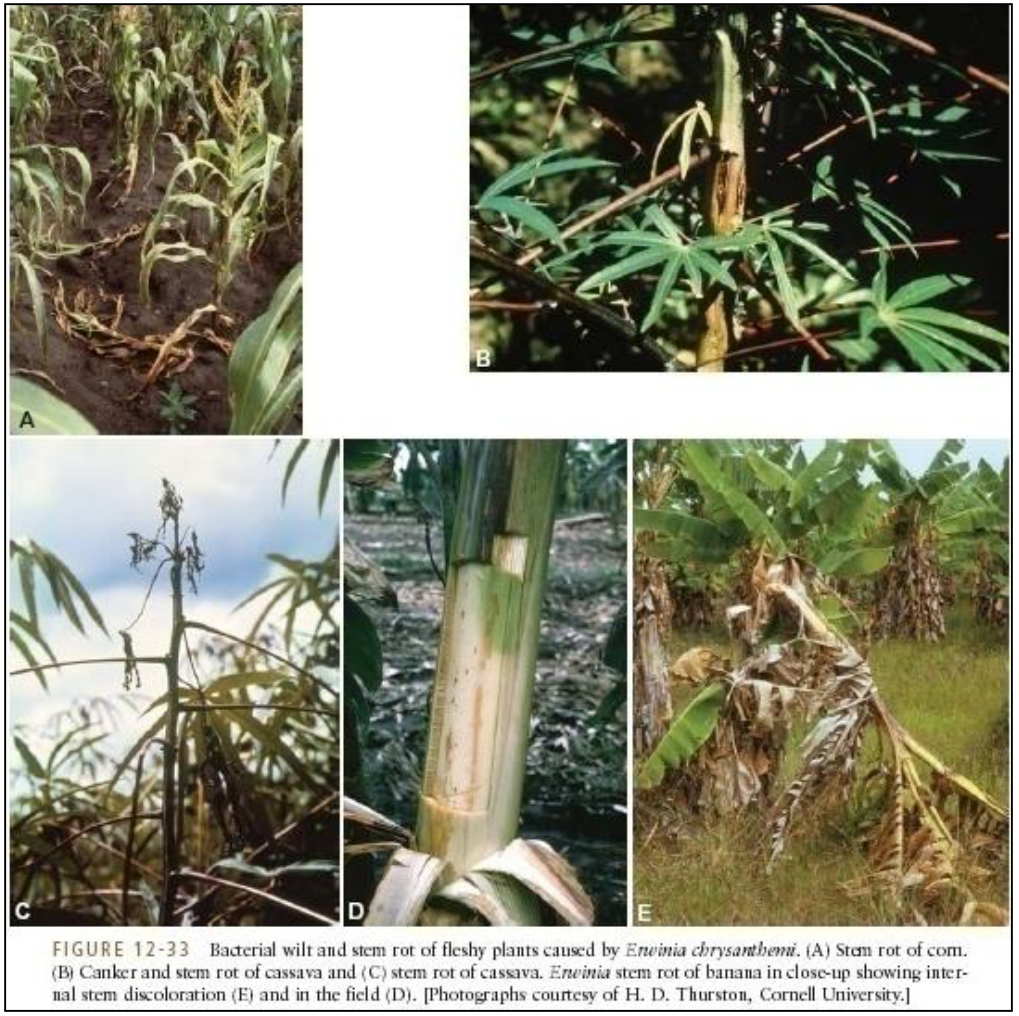


FIGURE 12-33 Bacterial wilt and stem rot of fleshy plants caused by *Erwinia chrysanthemi*. (A) Stem rot of corn. (B) Canker and stem rot of cassava and (C) stem rot of cassava. *Erwinia* stem rot of banana in close-up showing internal stem discoloration (E) and in the field (D). [Photographs courtesy of H. D. Thurston, Cornell University.]

Agrios,2005

Management

- Purchase culture-indexed plants known to be free of the most important bacterial pathogens.
- Discard infected plants.
- Do not use overhead irrigation.
- Pasteurize the propagation bed and medium between crops.
- Do not handle soil or debris on the potting soil surface and then the plant.

Erwinia amylovora

Fire blight

The fire blight of apple and pear

Erwinia amylovora

Hopeful bulletin from the Washington State Agricultural Experiment Station

February,1915

Eternal vigilance is the price of clean orchards.

Washington Agricultural Experiment Station, Pullman, Wash., February, 1915. Popular Bulletin No. 80
Ira D. Cardiff, Director and Botanist.

FIRE BLIGHT

IS THE GREATEST DANGER TO THE FRUIT INDUSTRY

Blight is a PREVENTABLE Disease

Pear Blight, commonly called "Fire Blight," is caused by microscopic, invisible plants (bacteria), growing inside the bark of the tree. No chemical has yet been found which will kill these bacteria without killing the tree.

Blight is the "GREAT WHITE PLAGUE" of the Fruit Industry

It causes losses of $25,000,000 annually to the country. If a foreign foe should invade the country and demand a tenth of this amount, millions would be expended on our army to defeat it. If a fleet of pirates should prey upon our commerce at the rate of $1,000,000 per year, a hundred million would be expended on battleships to combat the foe.

ROUT THE ENEMY—BLIGHT—AT ALL COSTS!

THE DISEASE CAN BE CONTROLLED

Inspection is Necessary

Give the Inspectors Your Support.

Consult County Agriculturist, State Inspector, or Experiment Station for methods.

Write Experiment Station for Bulletin on Blight.

Cleaning Up "HOLD-OVER" BLIGHT is the Best Means of Prevention

THE ONLY KNOWN WAY TO CONTROL BLIGHT IS BY SURGERY

In cutting it out, cut 6 to 24 inches below the canker. DISINFECT TOOLS AND CUTS WITH CORROSIVE SUBLIMATE.

Aphis, flies, ants, and other insects are important carriers of blight. Combat these insects. Birds are the natural enemies of insects. Protect the birds.

Inspect Nursery Stock carefully for blight. Avoid excessive watering of trees.

THERE IS NO PATENT CURE

BEWARE OF THE FAKIR WITH THE "BLIGHT CURES." Do not attempt to cure blight by sprays, tree paints, inoculations, or soil "doctoring."

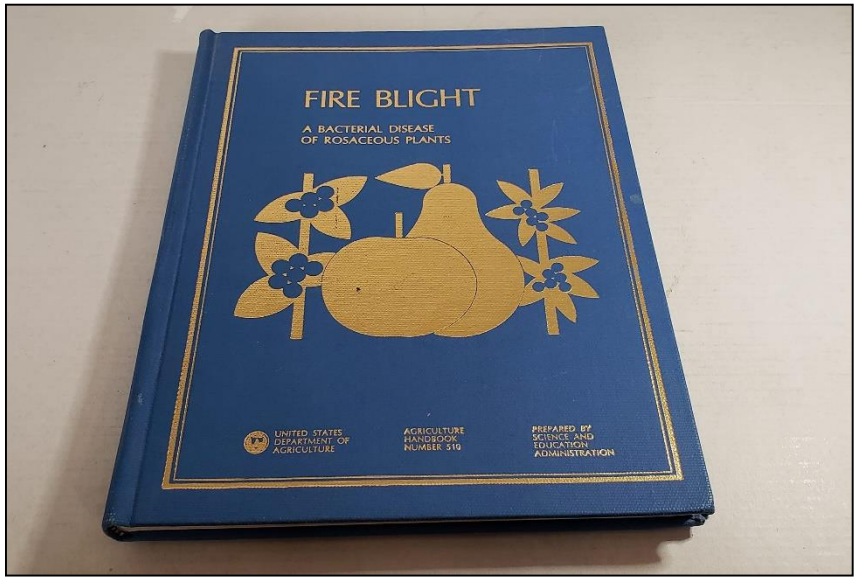
Blight fighting is a community matter. Organize and go after it. Winter is the best time to fight the disease. NOW is the accepted time. Encourage your neighbors to clean up their orchards.

Eternal Vigilance is the Price of Clean Orchards

Books on Fire Blight

Fire Blight: A Bacterial Disease of Rosaceous Plants

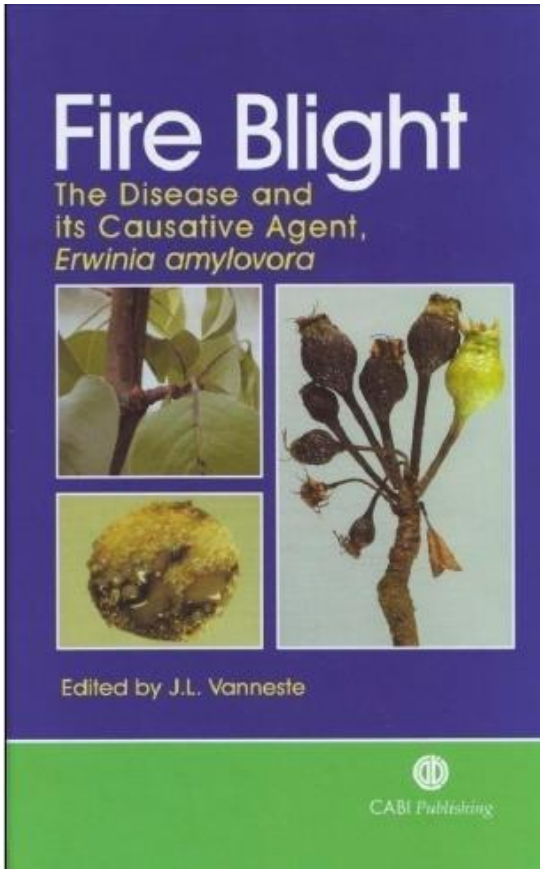
- Tom Van Der Zwet and Harry L. Keil
- 1979
- United States Department of Agriculture.



Books on Fire Blight

Fire Blight: The Disease and its Causative Agent, *Erwinia amylovora*

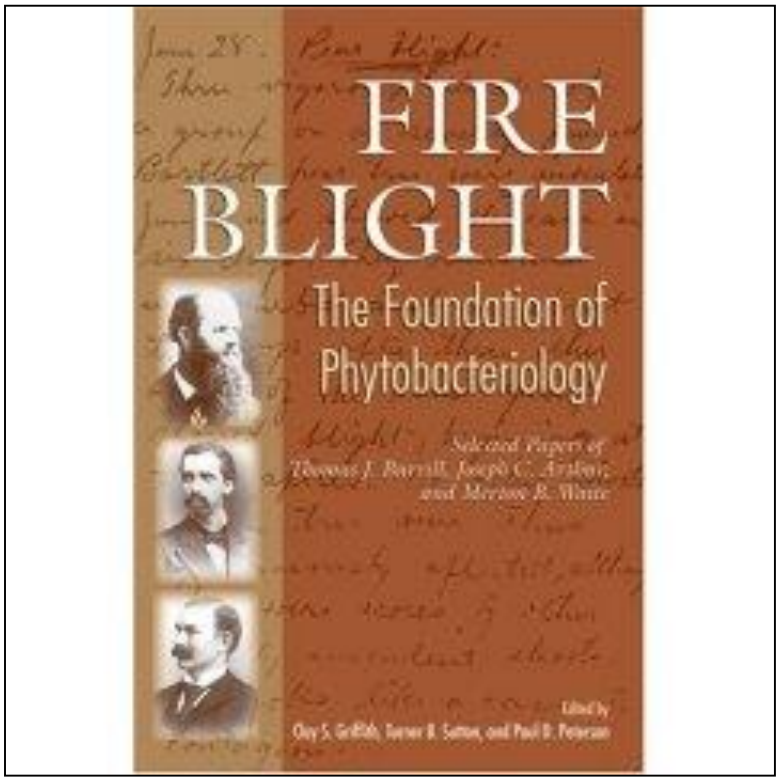
- Edited by Joël L. Vanneste
- 2000
- CABI Publishing
- New Zealand
- 370 pp.



Books on Fire Blight

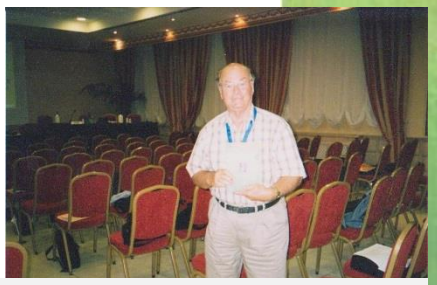
Fire blight: The Foundation of Phytobacteriology

- Clay S. Griffith
- 2003
- American Phytopathological Society
- 144 pages.



Fire blight of Pome Fruits: understanding and managing the disease

Sana Publication- Ordered by Deputy of Horticulture- Ministry of J. Agriculture. 2003, 325 pp. (in Persian with English summary)

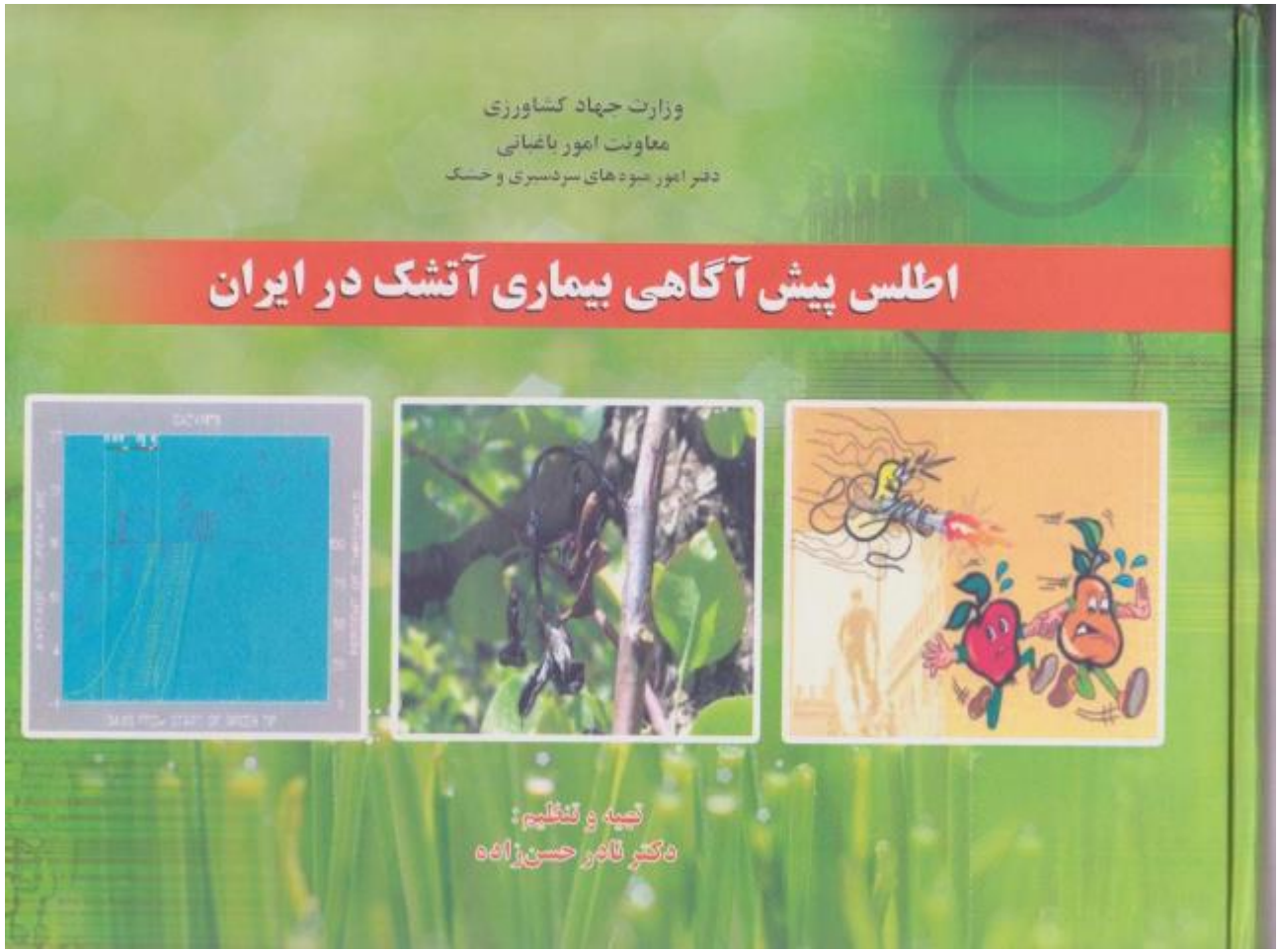


Prof. Tom van der Zwet,2004.Italy

Hassanzadeh,2003

Atlas of fire blight risk assessment in Iran

Sana Publication-Ordered by Deputy of Horticulture- Ministry of J. Agriculture, 2004, 355 pp. (in Persian with English summary)

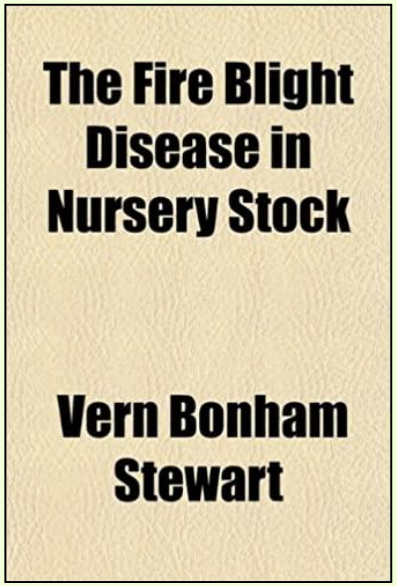


Hassanzadeh,2004

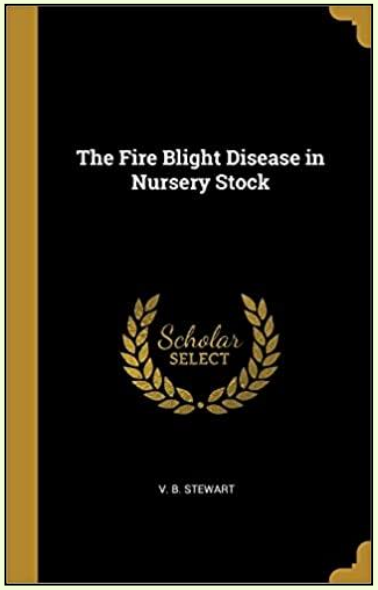
Books on Fire Blight

The Fire Blight Disease in Nursery Stock

- Vern Bonham Stewart
- 2010
- Publisher: General Books
- 50 pages.



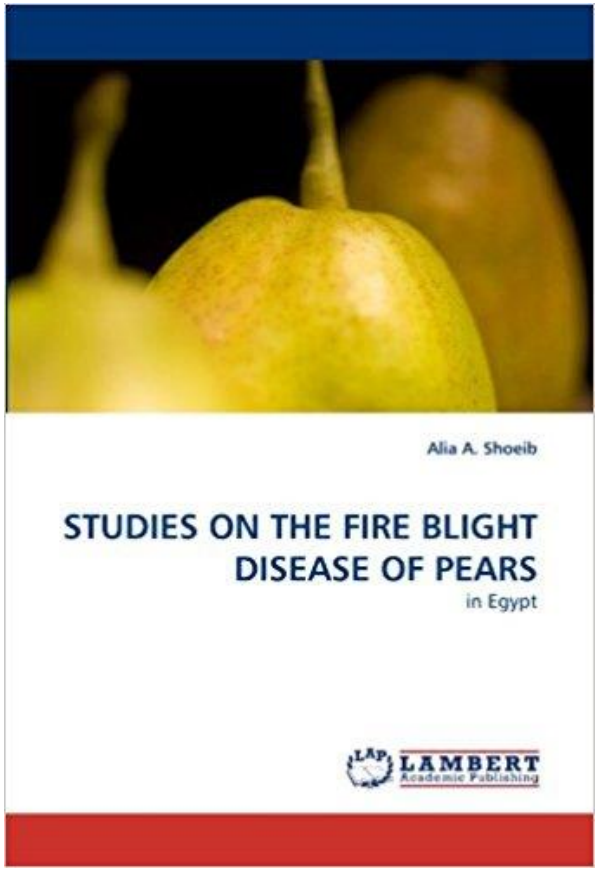
- Vern Bonham Stewart
- 2019
- Wentworth Press
- 58 pages.



Books on Fire Blight

Studies on the fire blight disease of pears: in Egypt

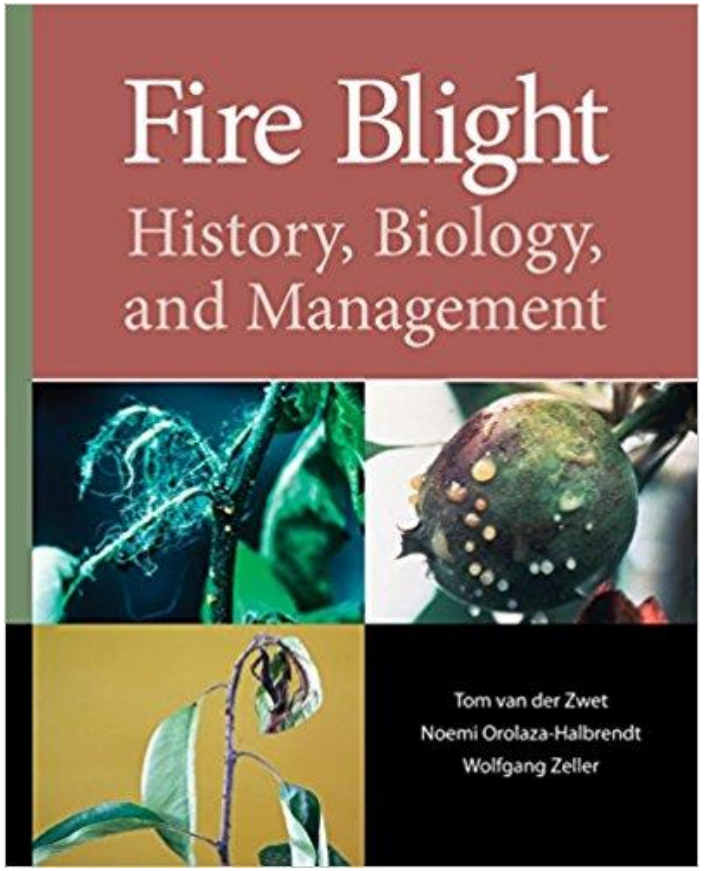
- Alia Abed El-Baky Shoeib
- 2011
- Publisher: LAP LAMBERT Academic Publishing.
- 68 pages.



Books on Fire Blight

Fire Blight: History, Biology, and Management

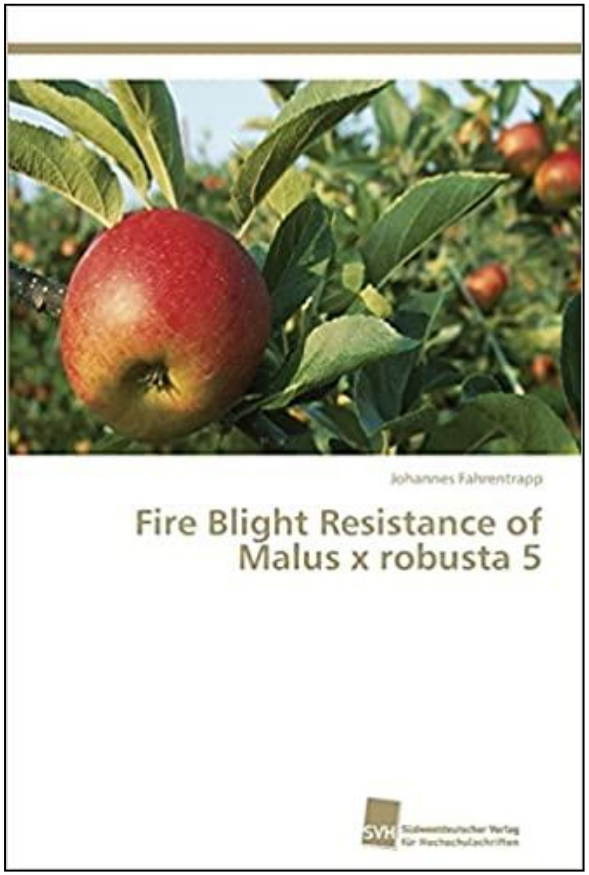
- Tom van der Zwet, Noemi Orolaza-Halbrendt, and Wolfgang Zeller
- 2012
- APS Press
- 460 pages.



Books on Fire Blight

Fire Blight Resistance of Malus x robusta 5

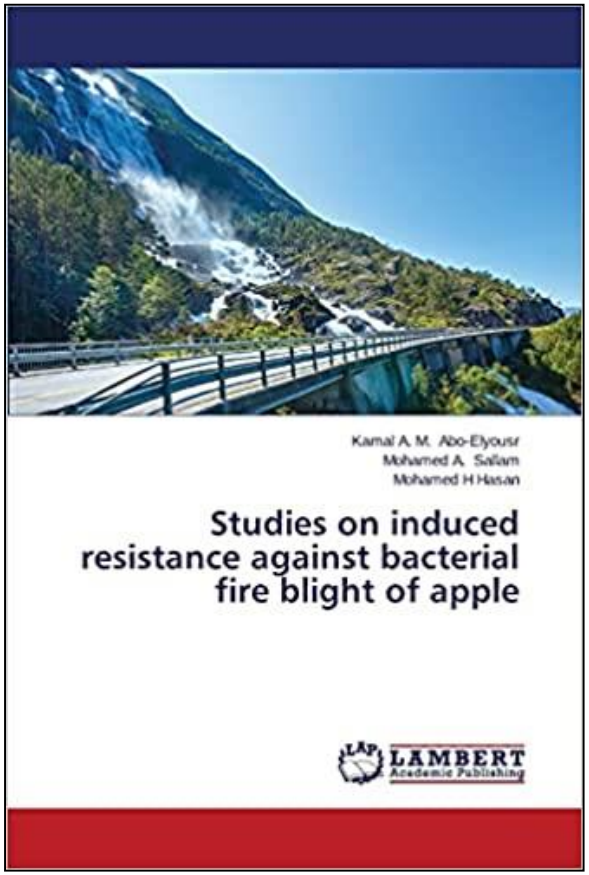
- Johannes Fahrentrapp
- 2013
- Sudwestdeutscher Verlag Fur Hochschulschriften AG
- 168 pages.



Books on Fire Blight

Studies on induced resistance against bacterial fire blight of apple, Egypt

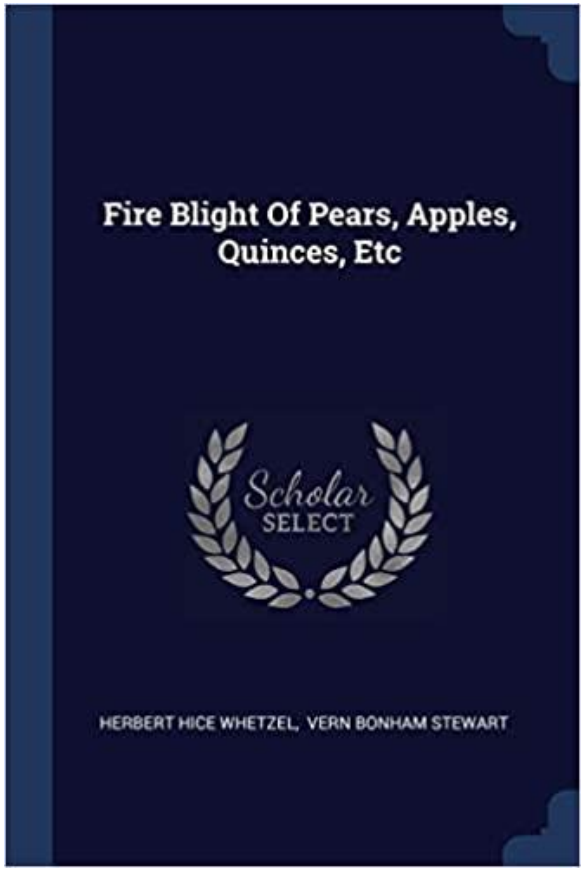
- A. M. Abo-Elyousr Kamal, A. Sallam Mohamed, and Mohamed, H. Hasan
- 2014
- LAP Lambert Academic Publishing, Germany
- 112 pages.



Books on Fire Blight

Fire Blight of Pears, Apples, Quinces, Etc

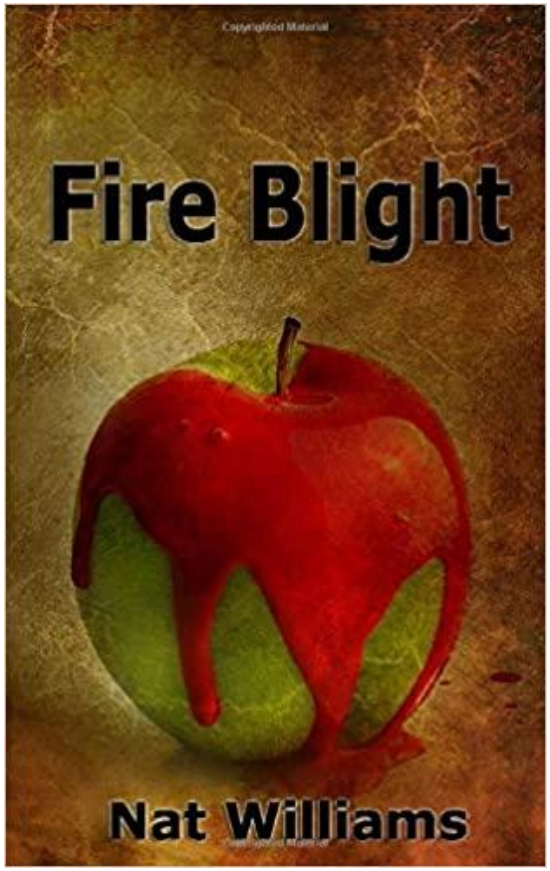
- Herbert Hice Whetzel (Author), Vern Bonham Stewart (Creator)
- 2018
- Sagwan Press
- 30 pages.



Books on Fire Blight

Fire Blight

- Nat Williams
- 2019
- Publisher: Independently published
- 368 pages.



Books on Fire Blight

Some more publications

- van der Zwet, T. and Keil, H.L. 1979. Fire Blight: A Bacterial Disease of Rosaceous Plants. United States Department Agriculture Handbook 510, Washington DC, 200 pp.
- van der Zwet, T. and S.V. Beer, 1992. Fire blight- Its Nature, Prevention and Control: A Practical Guide to Integrated Disease Management. U.S. Department of Agriculture, Agriculture Information Bulletin 631, 83 p.
- Stewart, V.B. 2010. The Fire Blight Disease in Nursery Stock. BiblioBazaar, 60 pages.
- Fahrentrapp, F. 2012. Fire Blight Resistance of Malus x robusta 5. Südwestdeutscher Verlag für Hochschulschriften, 168 pages.
- Brainerd, J. 2016. An Essay on Pear-Blight. WENTWORTH Press, 22 pages.
- Mitrev, S. and E. Arsov. 2018. Presence of fire blight in apple, pear and quince in Macedonia. 60 pp.

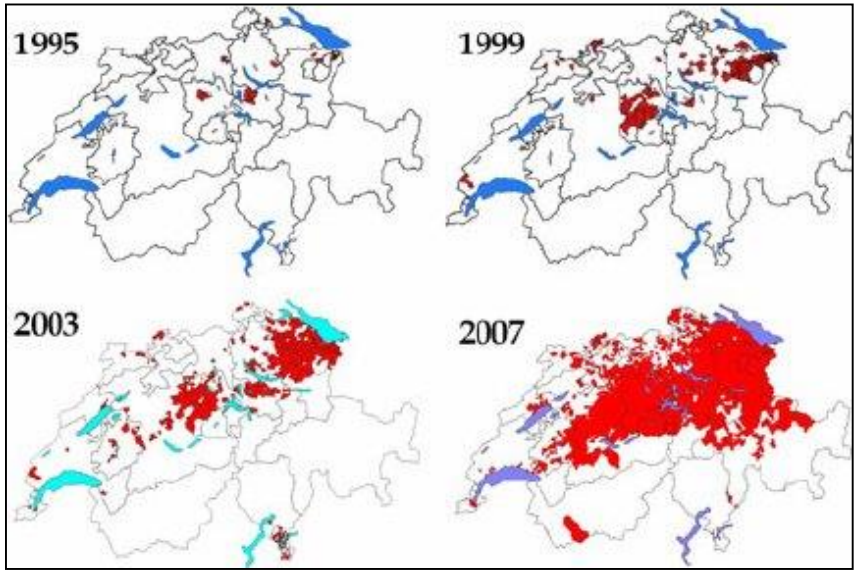
Books on Fire Blight

Some more publications

- Stewart, V. B. 2019. The Fire Blight Disease in Nursery Stock. Wentworth Press. 58 pages.
- Zhao *et al.*, 2019. Fire blight disease, a fast-approaching threat to apple and pear production in China. Journal of Integrative Agriculture. 18(4), 815-820.

Development of the fire blight epidemic in Switzerland, 1995-2007

- The pathogen was introduced into Switzerland from South- West Germany in the 1980s (this explains why the climatically more suitable Ticino has been less affected by fire blight than northern Swiss Cantons).
- It affects tree and shrub species of the family Rosaceae (e.g. Malus, Pyrus, Crataegus) and is favoured by humid and mild springs, as was the case in 2007, when the epidemic reached unprecedented levels (from Holdenrieder *et al.*,2008).



Pautasso *et al.*,2012

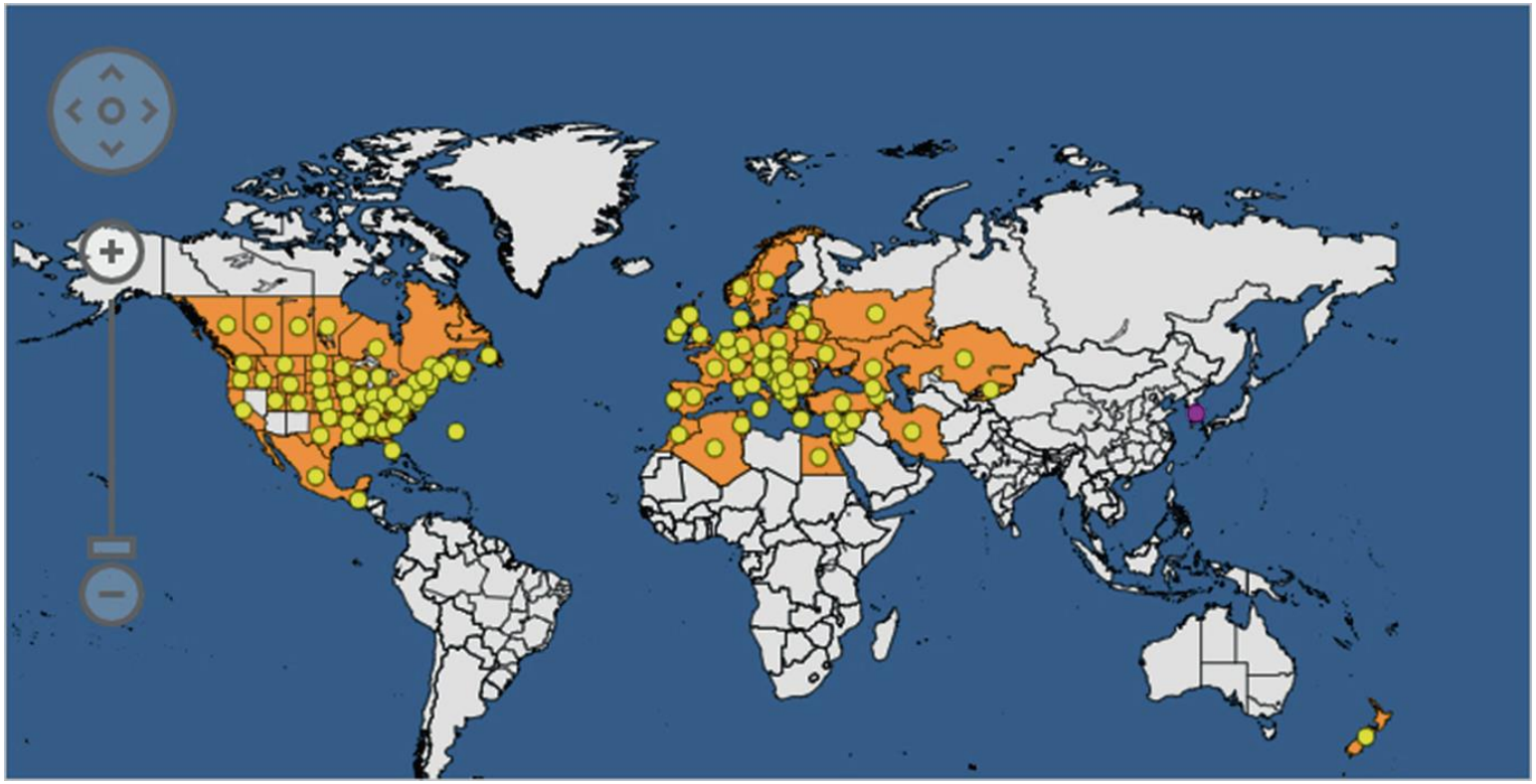
Countries in which fire blight has been recorded, as shown on the distribution map.

Fire blight bacterial disease has been reported from 55 countries around the world (EPPO, 2014).

Europe	Mediterranean Area
Albania	Armenia
Austria	Cyprus
Belgium	Egypt
Bosnia	Iran
Bulgaria	Israel
Croatia	Jordan
Czech Republic	Lebanon
Denmark	Morocco
England	Turkey
France	**Pacific Rim**
Germany	New Zealand
Greece	**North, Central and South America**
Hungary	Bermuda
Ireland	Canada
Italy	Guatemala
Lichtenstein	Mexico
Luxembourg	USA
Macedonia	
Netherlands	
Norway	
Poland	
Romania	
Serbia	
Slovenia	
Spain	
Sweden	
Switzerland	

Vanneste,2000; Peil *et al.*,2009

Microbial battling of fire blight disease on pome fruits



Bastas and Baysal,2022

Panoramic view of apple and pear orchard in which most of the trees were killed by fire blight

Agrios,2005

Young apple orchard destroyed by fire blight

Erwinia amylovora

- Devastation by fire blight (*Erwinia amylovora*) of a 2-year-old high density Gala apple orchard in Michigan after a violent storm.
- Other orchards in the path of the storm had similar losses.

(Courtesy Alan L. Jones)

Agrios,2005

Fire blight disease symptoms

Erwinia amylovora

Wet apple blossoms.
Rain during bloom can be a disaster.

Fire blight leaf symptoms.

Longstroth,2013

Fire blight disease cycle
Erwinia amylovora

- The amount of disease that develops in any given season or orchard of susceptible cultivars will depend on:

1. the number and distribution of sources from which inoculum is available;
2. the inherent genetic susceptibility of scion and rootstock cultivars; and
3. the rate at which new infections occur.

- The availability of primary sources is related to the amount of fire blight that occurred in and around the orchard in the previous year and the thoroughness of grower sanitation practices.

Biggs and Turechek,2018

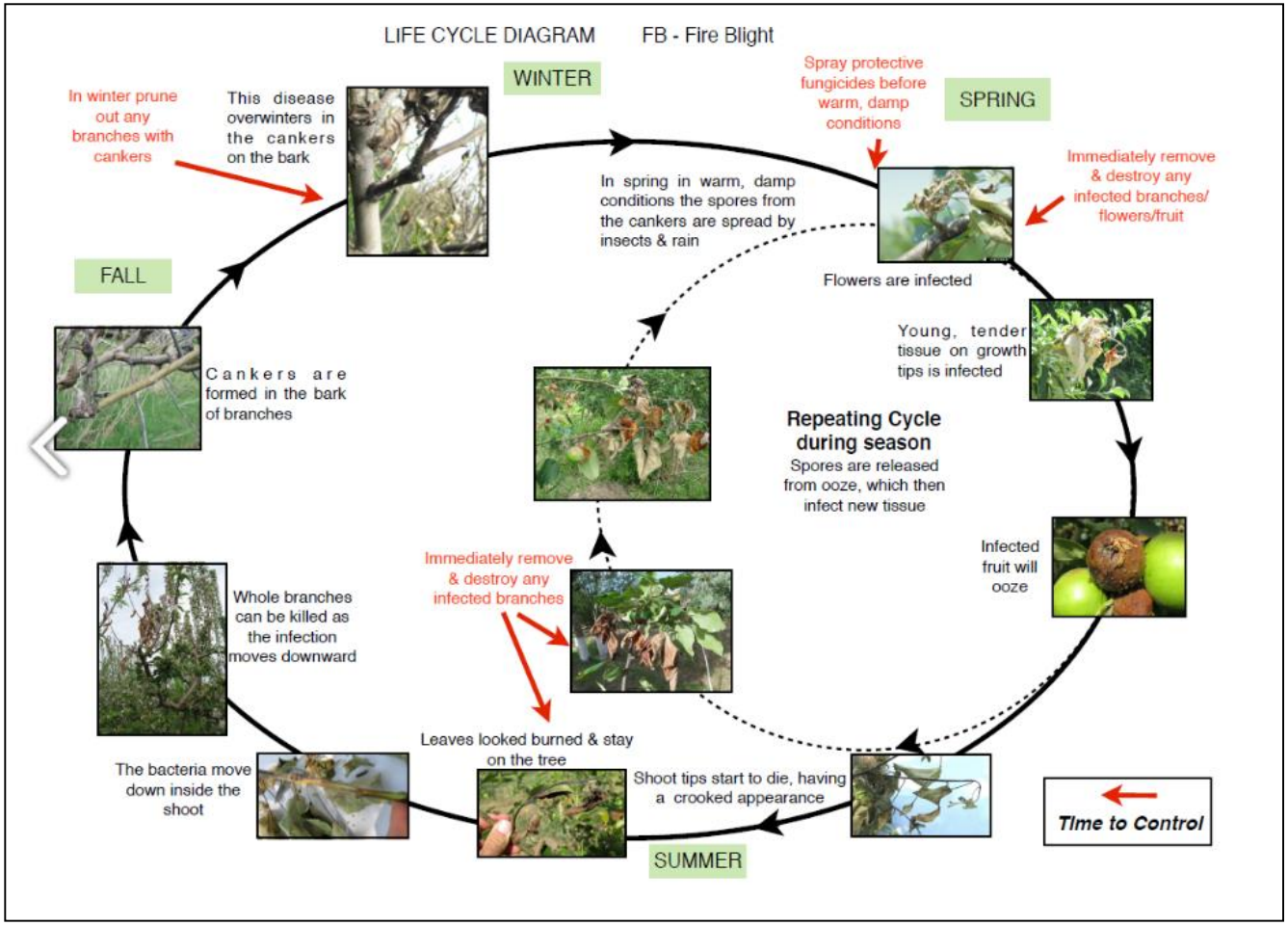
Fire blight disease cycle
Erwinia amylovora

- Fire blight epidemics can begin with a few early infections caused by inoculum that has overwintered in association with the crop.
- As these primary infections develop, they produce abundant inoculum, which is then available for dispersal to other infection sites.
- This process is repeated through many secondary cycles until either the supply of inoculum is exhausted or suitable infection sites.

Biggs and Turechek,2018

Fire blight disease cycle

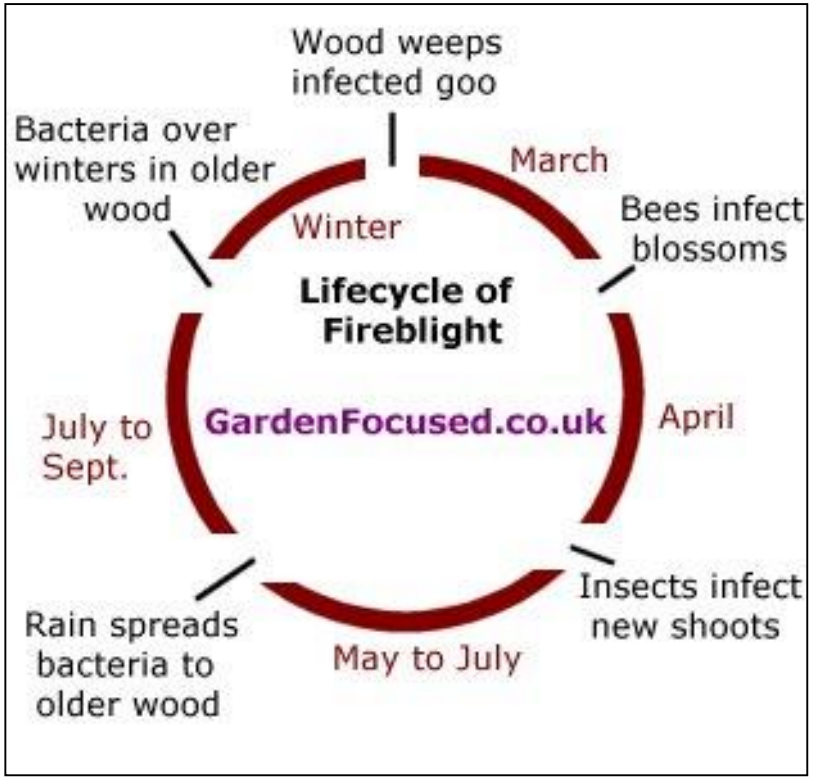
Erwinia amylovora



Fire blight disease cycle

Erwinia amylovora

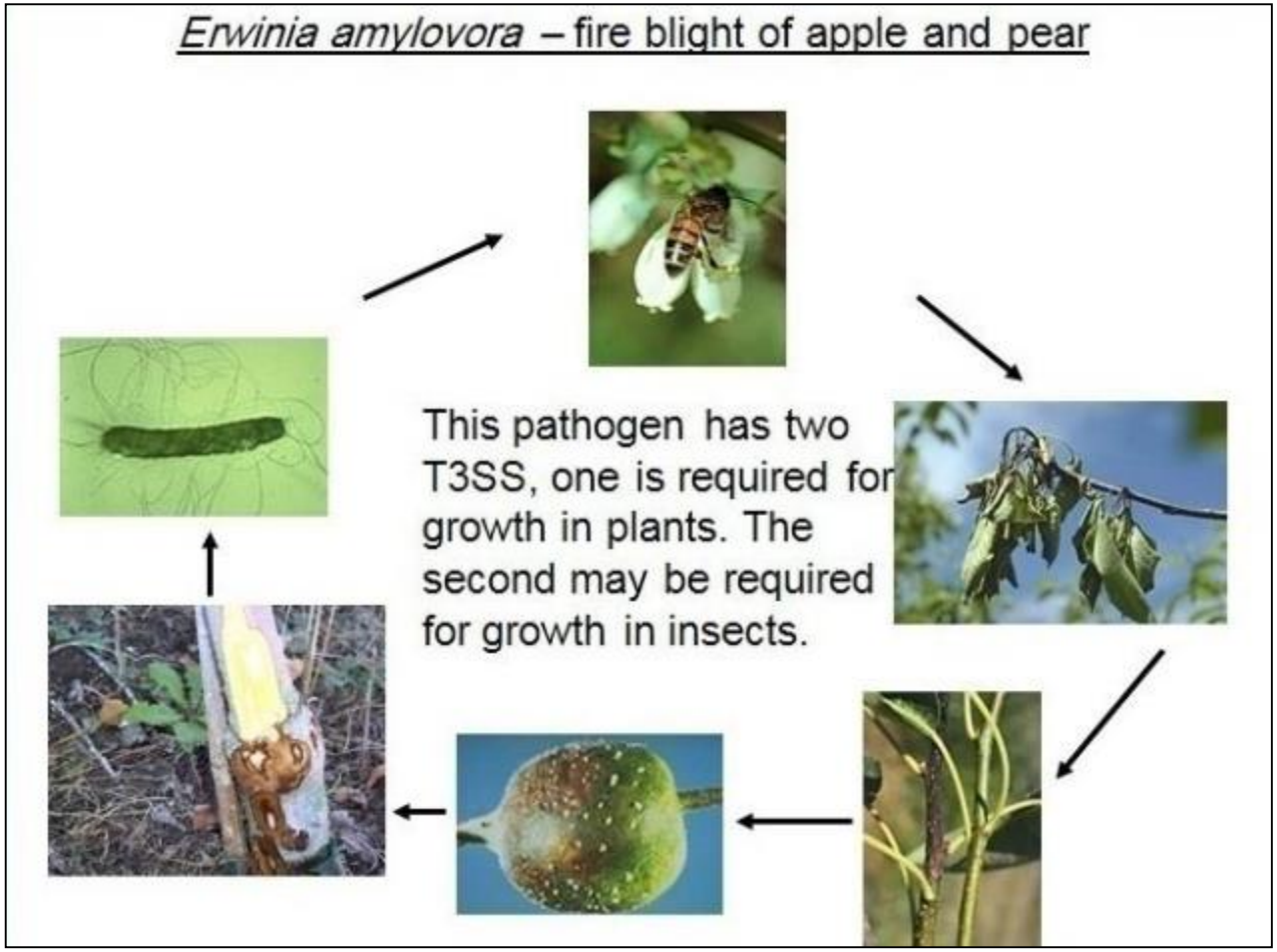
- Infected branches from the previous year is transferred to blossoms and new shoots by bees and other insects.
- Blossom and new shoots very quickly show signs of infection although older wood takes longer to be infected.
- However it is on old wood that the bacteria overwinter ready to restart the infection cycle next year.



Goo: a viscid or sticky substance.

GardenFocused,2020

Disease cycle of the Genus *Erwinia*
Two type three secretion systems(T3SS)
E. amylovora



Etiology

A branch of science concerned with the causes and origins of diseases

- *Erwinia amylovora* causes rapid necrosis of the phloem its hosts.
- It can survive as:

A. An epiphyte (Thomson, 2000), or

B. Endophyte (Vanneste and Eden-Green,2000).

- It also cause latent (symptomless) infections whose detection is not often successful in artificially inoculated hibernating shoots (Crepel *et al*.,1996).

Hibernate: of an animal or plant spend the winter in a dormant state.

Baranauskait,2009

Reaction of *Erwinia amylovora*-like isolates in various diagnostic tests

- *Erwinia amylovora*-like (Ea-like) isolates originating from symptomatic samples of fire blight host plants.
- These isolates identified as *Pantoea dispersa* and *P. agglomerans*.
- These are white variants of mentioned species that occur less frequently than yellow variants.

Ea-like isolate	PTA-ELISA	IF test	HR test on tobacco	PCR	Optimised PCR	BIOLOG
1	+	+	–	+	–	*P. dispersa*
2	+	+	–	+	–	*P. agglomerans*
3	+	+	–	+	–	*P. agglomerans*
4	+	+	–	–	–	nt
5	+	+	–	–	–	nt
6	+	–	–	–	–	nt
RICP Ea 8/95	+	+	+	+	+	*E. amylovora*

Kokošková *et al.*,2005

Identification and diagnostic tests
Erwinia amylovora -like isolates

- Early symptoms of fire blight on blossoms can be mistaken for bacterial blast caused by *Pseudomonas syringae* pv. *syringae*.
- In Korea and Japan a disease of Asian pear similar to fire blight is caused by the closely related bacterium: *Erwinia pyrifoliae*.
- Similarly in Spain, a disease of European pear similar to fire blight is caused by the related bacterium *Erwinia piriflorinigrans*.

Vanneste,2009

Erwinia spp. associated with pear diseases

Erwinia spp.	Disease incited
Erwinia amylovora	The causal agent of the fire blight disease of rosaceous plants in most countries with broader host range.
Erwinia pyrifoliae	Species that are pathogenic to pear trees Nashi pear (*Pyrus pyrifolia*) and European pear (*Pyrus communis*), include in Korea and Japan. symptoms are indistinguishable from those of fire blight in Asian pear trees.
Erwinia piriflorinigrans	Causal agent of pear blossom necrosis.
Erwinia spp.	Found in Japan. Japanese *Erwinia* spp. cause Japanese bacterial black shoot disease of pear (BBSDP) and bacterial shoot blight of pear (BSBP), respectively. Found on several cultivars of pear trees.

Palacio-Bielsa *et al.*,2012

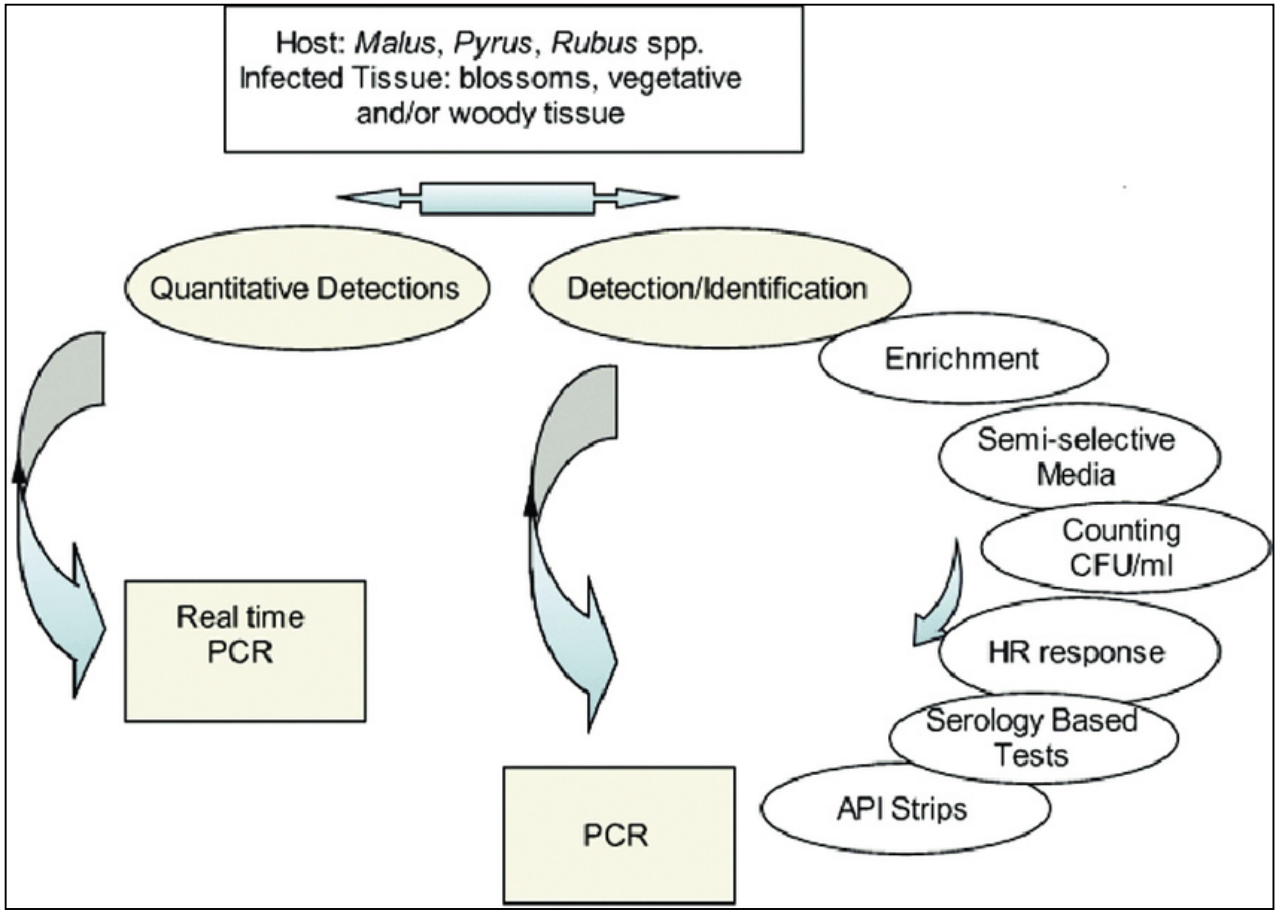
Other necrogenic bacteria

Brenneria rubrifaciens & *Brenneria nigrifluens*

- Cause dry necrotic or wilting symptoms on their host plants.
- Similar life cycle of *E. amylovora*.
- Transmits from diseased walnut trees to healthy trees by mechanical harvester.

Identification and diagnostic tests

Schematic representation of the PCR-based methods for the quantitative and traditional based PCR identification of *E. amylovora*



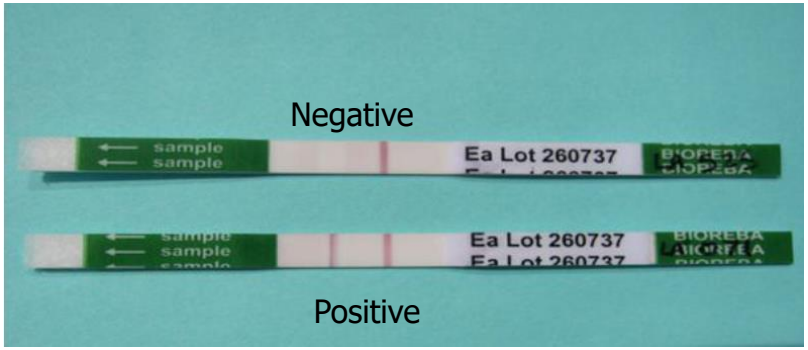
Burns,2009

Immunological test

Ea AgriStrip

Erwinia amylovora

- Ea AgriStrip utilizes antigen-antibody interaction to quickly identify isolates as *E. amylovora* (Bioreba, Reinach, Switzerland).
- Bands appearing on the AgriStrip indicated a positive antigen-antibody reaction targeted specifically to *E. amylovora*.
- The upper Ea AgriStrip is from an assay in which the colony tested was not *E. amylovora*.
- The bottom test is the reaction with a confirmed isolate of the pathogenic bacterium.



Carey,2011

Immunological test
Immunographic lateral flow test
Erwinia amylovora

- Together with industry (BIOREBA AG, CH), we have developed and validated an immunographic lateral flow test similar to a pregnancy test for fire blight, which is simple to use with little training.
- The test has been validated for diagnostic labs to reduce turn-around to 15 minutes for a reliable diagnosis.
- Positive samples can then be confirmed if needed by plating or PCR on test strip extractions without interruption of the daily flow of large numbers of samples.

Duffy *et al.*,2009

Immunological test

The Pocket Diagnostic® Erwinia amylovora rapid test

- The Pocket Diagnostic® Erwinia amylovora rapid test is a lateral flow test designed for the detection of *Erwinia amylovora* using on a wide range of plant material samples, including woody tissues, leaves, roots and soft material.

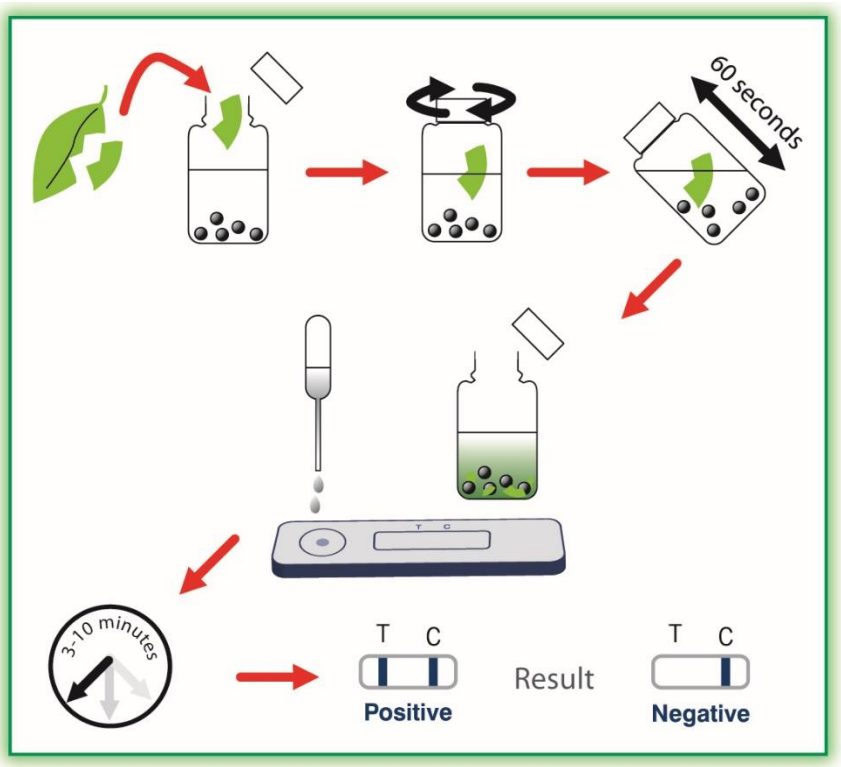
Lateral flow tests are immunoassays for detecting pathogens. It is based on using capillary beds to transport fluid along an antigen-containing matrix. If the antibody is captured by the antigen, a **colorimetric reaction occurs**. This provides a 'yes' (positive) or 'no' (negative) result.

Ephyra Biosciences,2019

Immunological test

The Pocket Diagnostic® Erwinia amylovora rapid test

- Select sample (as detailed below).
- Cut or tear sample into small pieces and put into bottle containing buffer
- Shake firmly for 30-60 seconds to break up the sample and allow the liquid to settle.
- Draw liquid into the pipette – avoid sample debris and air bubbles.
- Keeping the test device level, add 2 drops into the sample well of the device
- Valid results within 10 minutes.



Ephyra Biosciences,2019

Amplification of fire blight bacterium in host flowers

- The rate of colony growth of *Erwinia amylovora* in host flowers is regulated by temperature.
- *E. amylovora* growth is very slow until air temperature rises above 21°C (70°F), with almost no growth below 10°C (50°F).
- Then the rate of division increases rapidly.
- Optimal growth temperature for *E. amylovora* is 27°C (80°F).
- Depending on the strain, growth stops and colony numbers decline above 35 to 39°C (95 to 105°F).

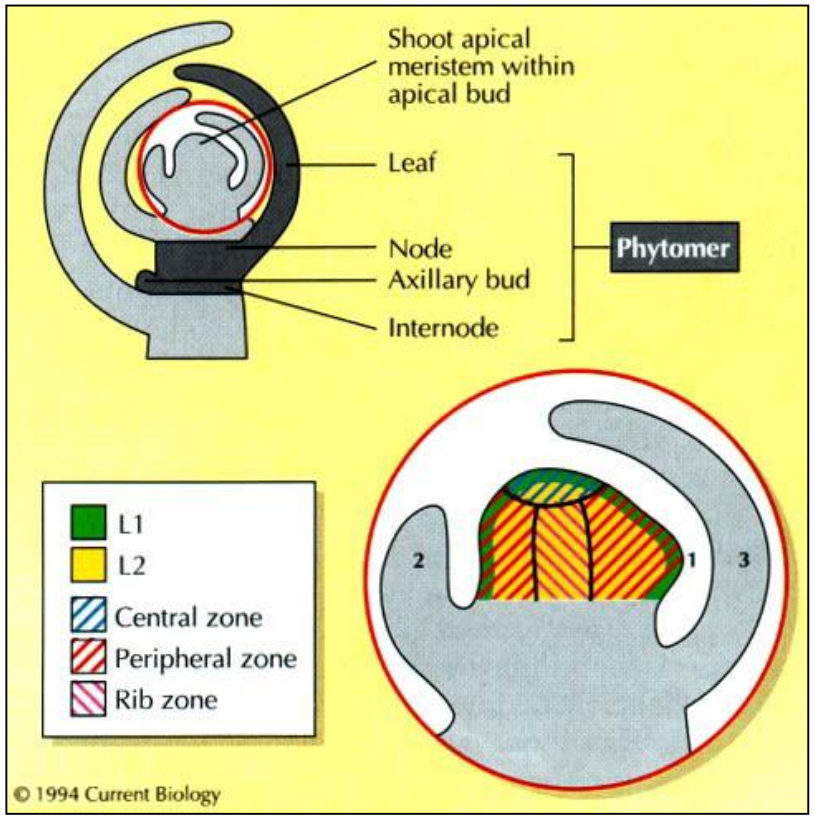
Amplification of fire blight bacterium in orchards

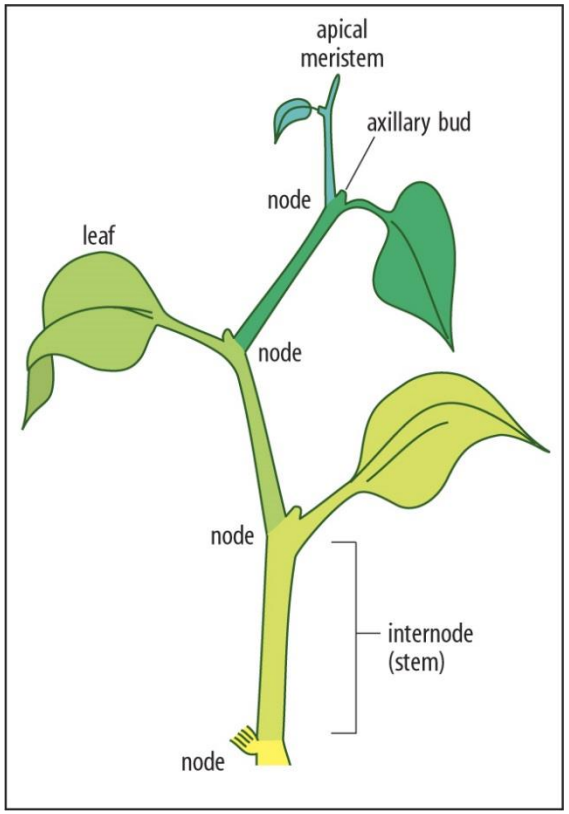
- Where do we see extensive growth -- And spread of the pathogen -- and infection?
- Blossoms
  - Rattail bloom
- Secondary spread from the initial shoot blight strikes
  - Minimum of 50 cells to cause a shoot blight strike.
  - 1,000-10,000 X or more amplification of cells that come out as ooze.
- Targets for management:
  - Initial inoculum, blossoms, prevention of the first shoot blight strikes.

Sundin,2008

Schematic representation of the plant shoot apex

Meristem free from *E. amylovora*





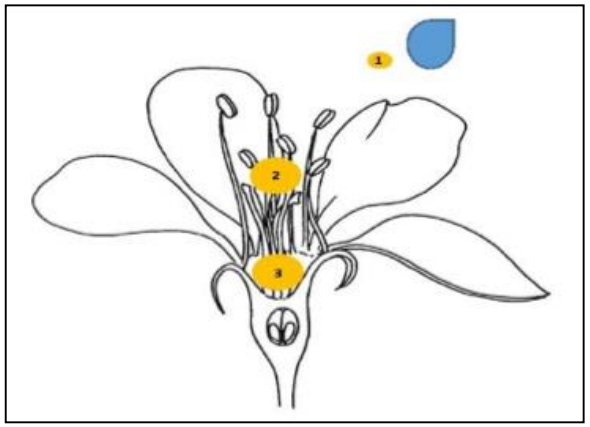
Phytomers are functional units of a plant, continually produced by root and shoot meristems throughout a plant's vegetative life-cycle.

Insects that visit the flowers

Honeybees and flies are the most common and effective vectors

- A syrphid fly feeding on a pear flower with contact of the abdomen on the stigmatic surfaces.
- Some insects often lick the stigmatic surface, while most insects visit the flowers to feed on nectar or pollen.
- Pathogen cells:

1. multiply on the flower stigma, and
2. if rain or dew occur are washed into the floral cup.



Relative fire blight resistance of apple varieties and root stocks

Plant material	Least susceptible	Moderately susceptible	Highly susceptible
Apple cultivar		Ambrosia Cortland Empire Honeycrisp Jersey Mac McIntosh Spartan Sunrise	Gala and Gala-types Ginger Gold Golden Russet Idared Paula Red Wealthy Yellow Transparent
Root stock	M.7 B.9 Cornel-Geneva (CG) series 5	MM.106 MM.111 M.41	M.9 M.26 M.27 Mark Ottawa 3

(Adapted from OMAFRA)

Cultivar susceptibility to fire blight compiled from several sources

very resistant = no control needed; resistant = control needed only under high disease pressure; susceptible = control usually needed where disease is prevalent; very susceptible = control

Apple cultivar	Relative susceptibility	Apple cultivar	Relative susceptibility
Ambrosia	?	Mollies Delicious	Susceptible[1]
Arlet	?	Monroe	Susceptible[2]
Beacon	Susceptible[1]	Mutsu	Very Susceptible[1]
Braeburn	Very Susceptible[2]	Northern Spy	Resistant-Susceptible[1,2]
Cameo	Susceptible[3]	NY674	Susceptible[4]
Cortland	Susceptible[1]	NY75414-1	Susceptible[3]
Creston (BC8m15-10)	Susceptible[3]	Orin	Susceptible[3]
Delicious (Red, all strains)	Resistant[1]	Paula Red	Susceptible-Very Susceptible[1,2]
Elliot	?	Pinova	?
Empire	Resistant[1]	Pioneer Mac	Susceptible[3]
Enterprise	Susceptible[3]	Prima	Resistant[1]
Fortune	Susceptible[4]	Priscilla	Resistant[1]
Fuji	Very Susceptible[2]	Pristine	Susceptible[3]
Fuji 2	Susceptible[3]	R.I. Greening	Very Susceptible[1]
Gala (all strains)	Very Susceptible[2]	Redfree	Resistant-Susceptible[1,2]
GingerGold	Very Susceptible[2]	Rome Beauty	Very Susceptible[1]
Gold Rush	Susceptible[3]	Sansa	Susceptible[3]
Golden Delicious	Resistant-Susceptible[1,2]	Senshu	?
Golden Russet	?	Shizuka	?
Golden Supreme	Susceptible[4]	Smoothee (Golden Del.)	Resistant-Susceptible[1,2]
Granny Smith	Very Susceptible[1]	Spartan	Susceptible[1]
Gravenstein	Susceptible[1]	Spigold	Very Susceptible[1]
Honeycrisp	Susceptible[4]	Stark Bounty	Resistant[1]
Idared	Very Susceptible[1]	Stark Splendor	Resistant-Susceptible[1,2]
Jerseymac	Susceptible[1]	Starkspur (Delicious)	Susceptible[1]
Jonafree	Resistant-Susceptible[1,2]	Stayman	Resistant-Susceptible[1,2]
Jonagold	Very Susceptible[1]	Suncrisp	?
Jonamac	Susceptible[1]	Sunrise	Susceptible[3]
Jonathan	Very Susceptible[1]	Twenty Ounce	Very Susceptible[2]
Liberty	Resistant[1]	Tydeman	Susceptible[1]
Lodi	Very Susceptible[1]	Viking	Resistant[1]
Macfree	Resistant[1]	Wealthy	Susceptible[1]
Macoun	Susceptible[1]	Yataka	Susceptible[3]
McIntosh	Resistant-Susceptible[1,2]	Zestal	?

[1] Ratings from MSU Web site, Nancy J. Butler, "Disease on Apples".
[2] Ratings from WV University, Kearneysville website, K.S. Yoder and A.R. Biggs.
[3] Ratings from Drs. Steven Miller and Alan Biggs in NE183 plot, WV.
[4] Ratings from other field observations.

Breth *et al.*,2001

Disease management
Forecasting systems

Dr. Eve Billing, 2004. Italy. She died peacefully on 18 February 2019, aged 95.

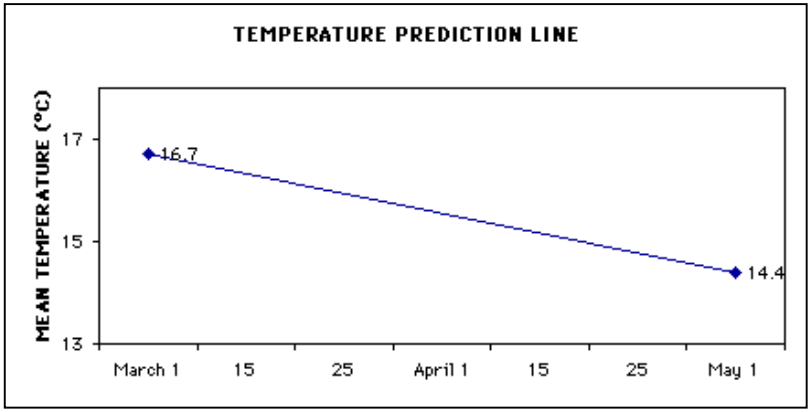
- Implication of forecasting systems such as:

1. Maryblyt,
2. Cougar blight,
3. Billing system,
4. Firescreens, etc.

Plant disease forecasting models are key elements of the decision support systems (DSS) for the management of plant diseases, since they provide guidelines for an efficient and rational use of pesticides in a wide diversity of hazard-disease situations.

Management
Integrated Disease Management
Temperature prediction line

- Prediction of flower colonization by bacteria (*Erwinia amylovora*) is based on the daily mean temperature rising above a line drawn from 16.7 on 1 March to 14.4° on 1 May.
- The blossom blight infection would occur on a day that had a 15.5ºC (60ºF) or higher mean temperature when flowers were wetted.
- Mean temp is High + Low divided by 2.



At moderately warm temperatures (65-75°F/18.3-23.8°C), it has been estimated that the bacterium can double every 20-30 minutes.

Thomson *et al.*,1982

Examples of forecasting models for plant diseases caused by bacteria

Disease (pathogen)	Model	Input variables[y]	Output variables[z]	Reference
Fire blight (*Erwinia amylovora*)	Maryblyt	Daily T_{max} and T_{min} (ºC, ºF or ºK) Rainfall (mm) Phenology Trauma	EIP HWTR BBS CBS SBS TBS	(Lightner and Steiner, 1992; Steiner, 1990)
Fire blight (*Erwinia amylovora*)	CougarBlight	Daily T_{max} (ºC or ºK) Rainfall (mm) Presence of blossoms Blight history in the neighbourhood	Infection risk on flower blossom	(Smith, 1993)
Fire blight (*Erwinia amylovora*)	Billing's Integrated System (BIS95)	Daily T_{max} and T_{min} (ºC, ºF or ºK) Rainfall (mm) Phenology Insect activity Disease incidence	Infection risk on flower Blossom BBS	(Billing, 1996)

BHWTR: B= open flowers, H= EIP>100, W= wetting from rain, dew, T = Average temperature of 15.6°C)and R = Risk prediction. **ºK**, Kelvin temperature scale (300 kelvin = 26.85 degree Celsius).

Morales *et al.*,2017;..

Examples of forecasting models for plant diseases caused by bacteria

Disease (pathogen)	Model	Input variables[Y]	Output variables[Z]	Reference
Bacterial spot on hot pepper (*Xanthomonas campestris* pv. *vesicatoria*)	*Xcv* infection model	Hourly temperature (ºC, ºF or ºK) Rainfall (mm) Wetness duration (h) Wind	Infection risk	(Kim *et al.*, (2014
Bacterial canker of kiwifruit (*Pseudomonas syringae* pv. *actinidiae*)	*Psa* risk model	Hourly Temperature (ºC, ºF or ºK) Wetness duration (h) RH (%)	Infection risk	(Beresford *et al.*, 2017)

Y, T_{max} and T_{min}: maximum and minimum temperatures.
Z, BBS: Blossom blight symptoms; BHWTR: Daily infection risk level; CBS: Canker blight symptoms; EIP: Epiphytic Potential Inoculum; RH: relative humidity; SBS: Shoot blight symptoms; TBS: Trauma blight symptoms. The Kelvin (symbol: **K**) is a unit of measurement for temperature (**300 kelvin = 26.85 degree Celsius**).

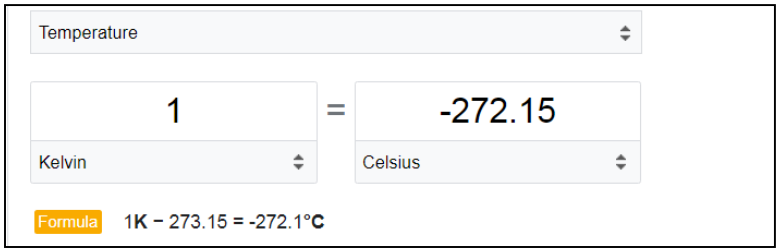
Morales *et al.*,2017

Metric conversion

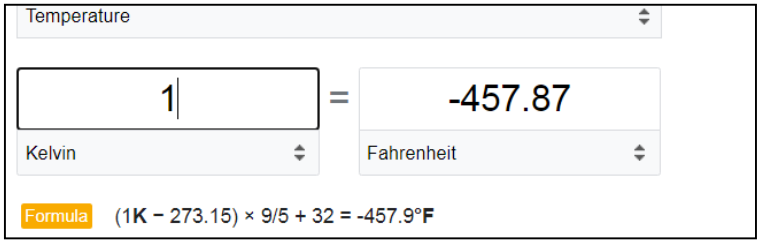
Celsius and Fahrenheit to Kelvin

- Although initially defined by the freezing point of water (and later the melting point of ice), the Celsius scale is now officially a derived scale, defined in relation to the Kelvin temperature scale.
- Zero on the Celsius scale (0°C) is now defined as the equivalent to 273.15K, with a temperature difference of 1 deg C equivalent to a difference of 1K, meaning the unit size in each scale is the same.
- This means that 100°C, previously defined as the boiling point of water, is now defined as the equivalent to 373.15K.

Kelvin (K) to Celsius (°C) degrees conversion.



Kelvin (K) to Fahrenheit (°F) degrees conversion.



Wight Hat Ltd.,2018

Weather stations
Electronic weather station

- A weather station is a facility, either on land or sea, with instruments and equipment for measuring atmospheric conditions to provide information for:

1. weather forecasts, and
2. to study the weather and climate.

Wikipedia,2020;..

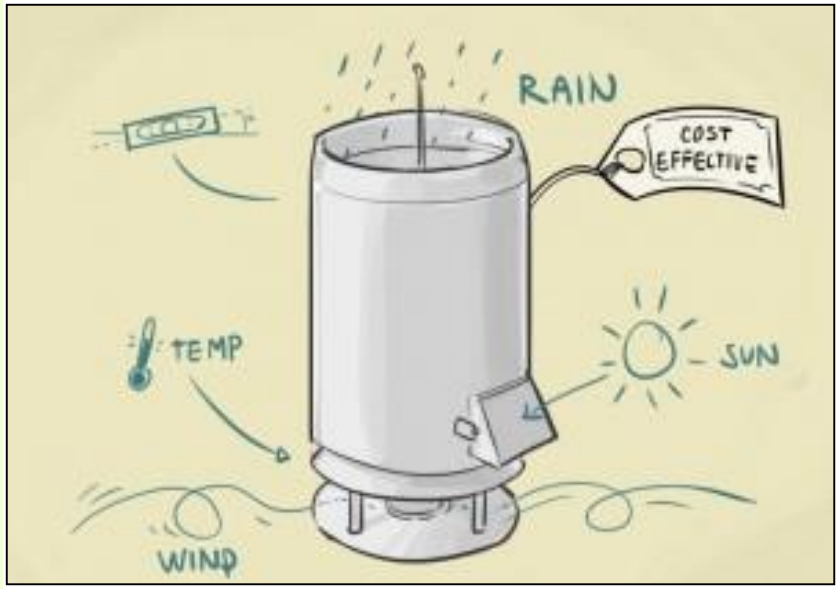
Weather stations
Electronic weather station

- The most efficient weather station equipment is electronic and automated.
- Recording data which is then routed to a computer that runs pest management models, such as a fire blight model.
- Alternatively, data may be downloaded to a computer manually, but it is more convenient to automate that process.

Cooley *et al.*,2015

Weather stations
A Drip-Counting Rain Gauge

- The new rain gauge ten times more sensitive than most tipping bucket gauges.
- The gauge measures precipitation depths as small as 0.02 mm.
- The gauge is also is able to measure events ranging from the formation of dew to heavy storms with intensities of over 200 mm/hr.



Weather stations

AcuRite Iris™ (5-in-1) Weather Station with PC Connect Display (1st Gen). Price:$149.99

- The AcuRite Professional Weather Station with App uses patented Self-Calibrating Technology to provide your personal forecast of 12 to 24-hour weather conditions.
- Self-Calibrating Forecasting is generated from weather data measured by a sensor in your yard - giving you the most accurate forecast available for your exact location.

AcuRite Iris™ (5-in-1) Weather Station with PC Connect Display (1st Gen)

Color Display with PC Connect for 5-in-1 Sensor

- Illuminated color display
- 12 to 24 hour weather forecast
- Patented Self-Calibrating Forecasting pulls data from a sensor in your backyard to give you the most accurate forecast for your exact location
- Weather Ticker™ streams real-time information and alerts
- Programmable weather alarms: temperature, humidity, wind, rain, dew point, heat index and storm alerts
- Measures rain precipitation and rainfall history (inches or millimeters)
- Wind speed: current, peak, and average (MPH or KPH)
- Wind direction with 16 point wind rose
- Indoor and outdoor temperature (degrees Fahrenheit and Celsius) with trend arrow
- Indoor and outdoor humidity (%RH) with trend arrow
- Heat index, wind chill, dew point and "feels like" calculation
- Daily, weekly, monthly and annual high and low records
- Barometric pressure with trend arrow Time and date (month/day)
- Bilingual English or French display
- Tabletop or wall-mountable design
- Indicator for wireless sensor signal strength.

MaryBlyt forecasting system

Forecasting Fire Blight Disease

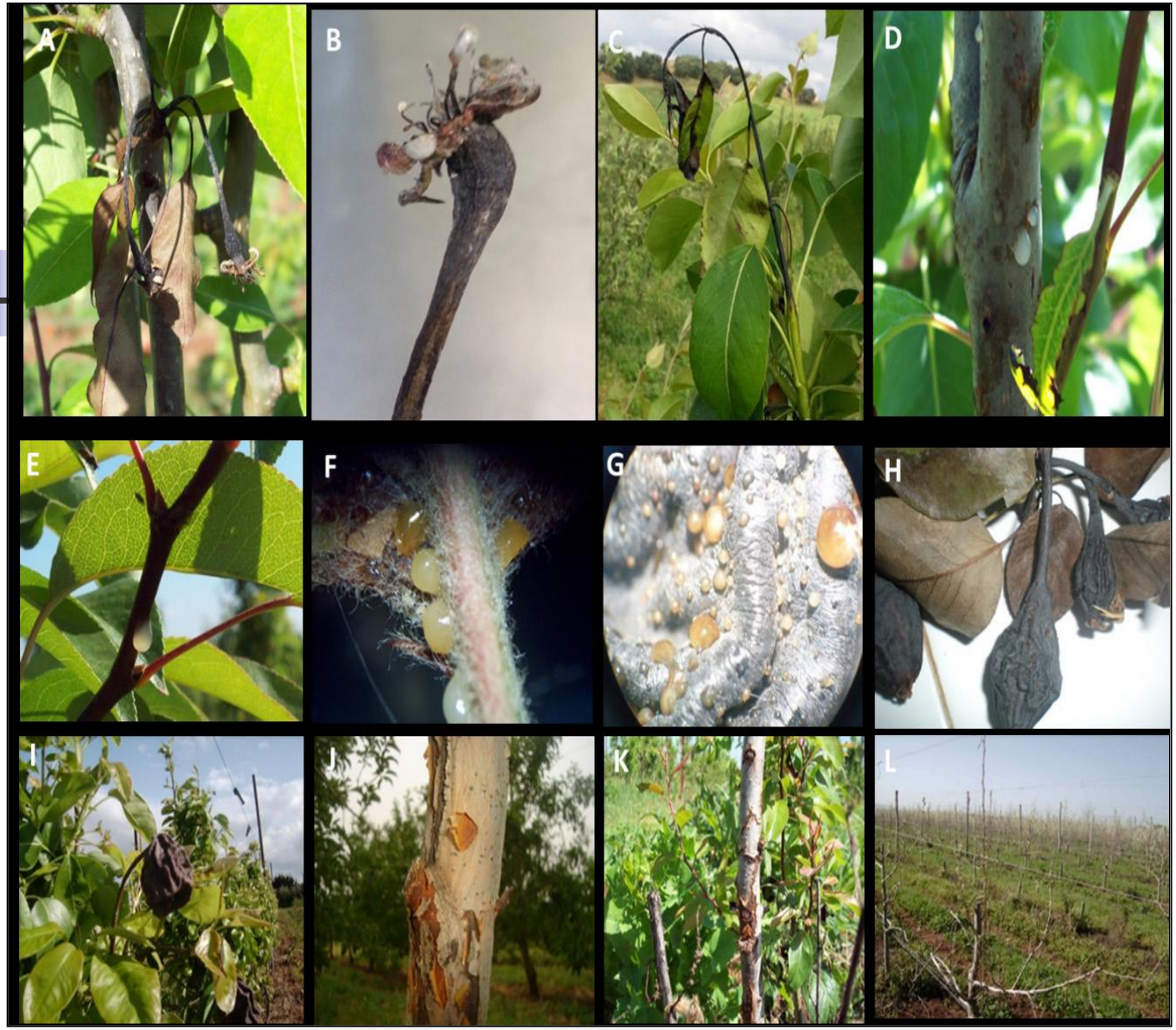
Erwinia amylovora

https://www.youtube.com › watch

Maryblyt tutorial - YouTube

In this tutorial Michelle Cortens, Tree Fruit Specialist, explains how to input data in the **Maryblyt model** for blossom blight…

YouTube · NS Perennia · Khordad 7, 1399 AP



Fire blight Symptoms: A & B - necrotic flowers and leaves; C - necrotic shoot; D, E, F and G - drops of bacterial ooze on pear trunk, leaves and fruits; H and I - mummified immature fruits; J and K - canker of trunk and necrotic tissue; L - field damages.

Bahadou *et al.*,2020

Typical appearance of *E. amylovora* bacterial cultures in the three media

- Typical colony morphology of *E. amylovora* on:

A. King's medium;
B. levan (NSA) medium, and
C. Semi selective CCT medium.

NAS: sucrose nutrient agar medium

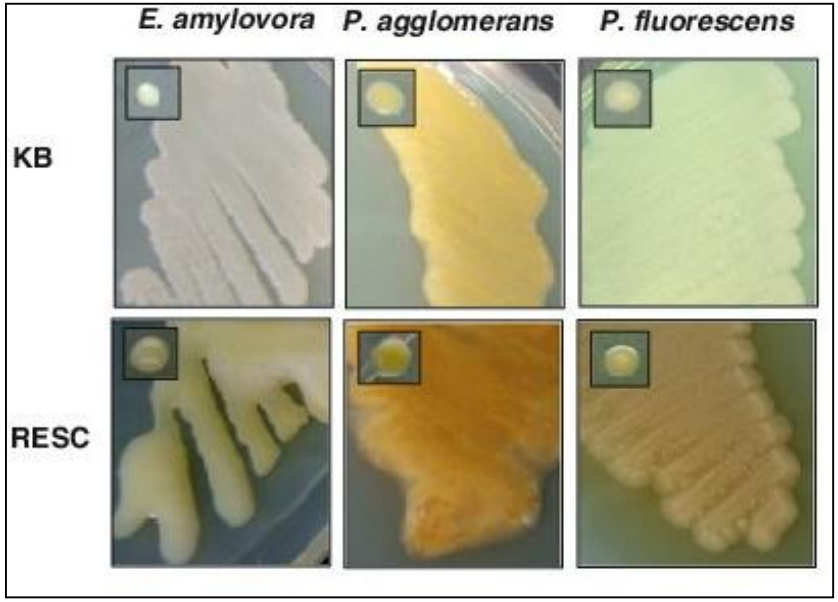
Bulletin OEPP/EPPO Bulletin,2013

Culture media

Two non-selective differential media

KB and RESC

- Growth and colonial morphology of *E. amylovora*, *P. agglomerans* and *P. fluorescens* (strains CFBP1430, EPS411, EPS347, respectively) on differential King's B (KB) medium and non-selective differential RESC medium containing 1.5 mM $CuSO_4$, after 48 h at 26°C.



RESC (Recovery *Erwinia amylovora*- Stressed Cells) medium was used to improve the recovery of *E. amylovora* from plants under unfavorable conditions i.e. unfavorable weather conditions, copper-treated samples, nutrient starvation, etc.. its colonies were easily distinguished by a light yellow color and a high mucus production.

Ordax *et al.*,2011

MaryBlyt forecasting system

Different versions

New 7.1.1 version

1. Maryblyt was originally developed by Dr. Paul W. Steiner, University of Maryland, and Gary Lightner, USDA, AFRS.
2. Maryblyt 7.1 was developed by Dr. Alan R. Biggs, West Virginia University, and Dr. William Turechek, USDA, and **adapted for Windows** from the original source code.

- Maryblyt, the program, the name, and anything to do with it is copyrighted by the University of Maryland and all rights are reserved.

Maryblyt™ ©*2018*

University of Maryland at College Park, All Rights Reserved
West Virginia University, KTFREC, All Rights Reserved

Version 4.0, 4.0a *issued February 1992*
Version 4.1 *issued September 1992*
Version 4.2 *issued March 1994*
Version 4.3 *issued February 1996*
Version 7.0 *issued October 2009*
Version 7.1 *issued May 2014*
Version 7.1.1 *issued August 2018*

The program is available for free by downloading from:

http://grapepathology.org/maryblyt

We request that you do not redistribute it.

Deborah I. Breth; Biggs and Turechek,2018

MaryBlyt: Fire Blight Forecasting Program for Apples and Pears

Maryblyt™ 7.1.1: Main Start Window

- Maryblyt was originally developed by Dr. Paul W. Steiner and Gary Lightner in DOS version.
- Maryblyt 7.1.1 was developed and adapted for Windows by Dr. Alan R. Biggs, and Dr. William.

Maryblyt v.1.1 for Windows: An Improved Fire Blight Forecasting Program for Apples and Pears(2018).

Deborah I. Breth; Biggs and Turechek,2018

MaryBlyt forecasting system Maryblyt™ vers. 4.0-7.1.1

www.maryblyt.com

MaryBlyt v.7.1 and v.7.1.1 for windows
An improved forecasting program for apple and pears

- Earlier versions were able to accept only U.S. units for numbers and dates, and the program would invariably "crash" if the wrong number and/or date formats were used.
- The audible warning beep, which was missing in Version 7.0, was re-established to alert users of an infection event.
- The additional option allowing users to toggle(a key or button on a computer) the beep on and off was also incorporated into Versions 7.1. and 7.1.1.

Turechek,2015; Biggs and Turechek,2018

MaryBlyt forecasting system

A predictive program for forecasting fire blight disease in apples and pears

- Computer based Maryblyt model, began in DOS version.
- Updated to Windows version- Maryblyt 7.1.1

1. Assumes the abundance of inoculum;
2. Predicts potential risk of infection based on occurrence of certain conditions in sequence;
3. Predicts the development of symptoms.

http://www.maryblyt.com

MaryBlyt forecasting system

Four minimum requirements for infection event

Maryblyt™ Output for BB

DH EIP BHWTR

- DH = Degree hours accumulated
- EIP = Epiphytic Inoculum Potential
- B = Blossom present
- H = Degree hour threshold met
- W = Wetting event
- T = Average temperature of 60°F (15.6°C)
- R = Risk prediction

MaryBlyt forecasting system
Multiple phases of fire blight disease

- There are at least five distinct kinds of infections associated with fire blight, not all of which occur every year or with equal intensity.
- The Maryblyt™ program predicts four of these: blossom, canker, shoot and trauma blight.
- A fifth type, rootstock blight, has only recently been characterized and the bases for its prediction are not yet fully understood.

Biggs and Turechek,2018

MaryBlyt forecasting system

Multiple phases of fire blight disease

Rootstock blight

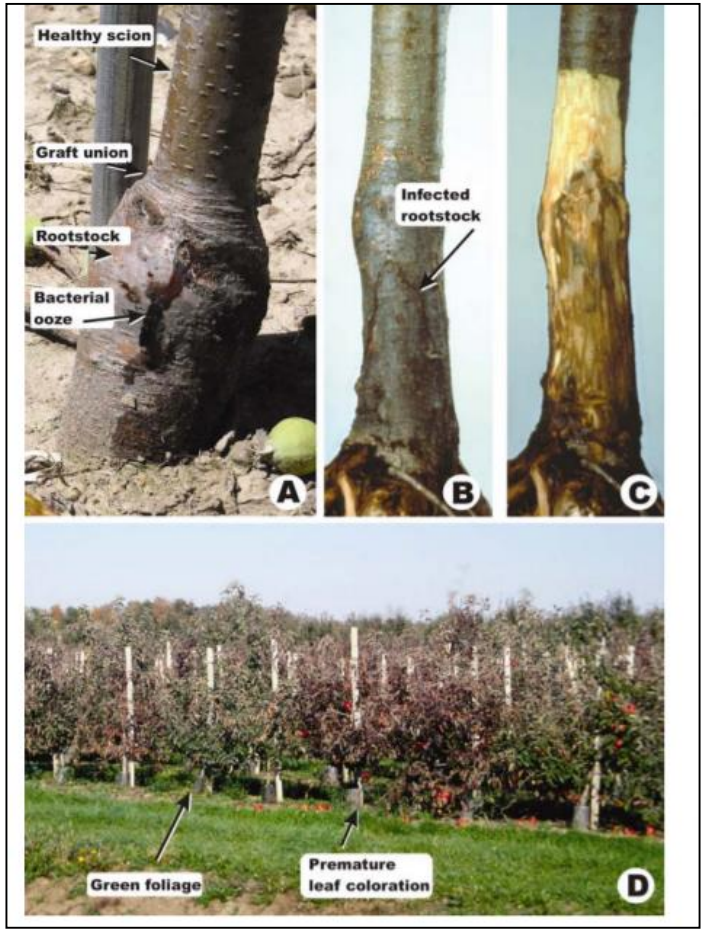
- Rootstock blight is characterized by:

A. the presence of liquid bleeding (bacterial ooze) from the rootstock in early summer,

B. by necrosis;

C. that is more visible when the bark is removed.

D. Trees with rootstock infection often exhibit yellow to burgundy(dark red-purplish colour) foliage about a month before onset of normal autumn coloration.



Norelli *et al.*,2003

MaryBlyt forecasting system
Four phases of fire blight disease

- BBS = blossom blight symptoms
- SBS = shoot blight symptoms
- CBS = canker blight symptoms
- TBS = trauma blight symptom



MaryBlyt forecasting system

The main heat unit "clocks" for predication of infection events

- **Average daily temperature**:
- Average daily temperatures were calculated for keeping track of symptom development.
- **Degree-days** were calculated using the average of the minimum and maximum daily temperatures and began at green tip.
- **Daily degree-hours:**
- Daily degree-hours were calculated to track the bacterial population.
- **Degree-hours** were calculated using six hours at the minimum daily temperature, six hours at the maximum daily temperature, and twelve hours at the average daily temperature and began at first bloom.

MaryBlyt forecasting system
Three cumulative heat unit clocks

- Maryblyt™ integrates the use of three cumulative heat unit "clocks" to indirectly monitor the development of the host, pathogen populations, insect vector availability and symptom development.
- **Cumulative DD > 40°F (4.4°C)** is used in Maryblyt™ to monitor with reasonable accuracy:

1. the age of apple and pear flowers, and
2. the appearance of insect vectors.

- **Cumulative DH > 65°F (18.3°C)** is used in Maryblyt™ to establish the epiphytic infection potential (=EIP) for assessing infection risks.

Biggs and Turechek,2018

MaryBlyt forecasting system

The cumulative heat unit

Epiphytic infection potential (=EIP)

- The EIP is based on data relating cumulative heat units and blossom colonization by the bacteria, but it really surrounded/take place much more (availability of open flowers, bee activity, etc.).
- Thus, an EIP of "zero" does not mean that all bacteria are dead, but only that the risk for infection is low.
- **Once infection occurs, symptom development** (=interaction between a pathogen and a host plant) is predicted using **cumulative DD > 55°F (12.7°C)**.

Biggs and Turechek,2018

Degree day calculation in different plant forecasting models

- There are several related versions of degree-day calculation tools:
- Degree-day is a heat unit calculated on a daily basis, usually using the daily max and min temperature.
- Not all degree days are equivalent or are directly comparable.
- Various formulas are used that produce different results.
- Threshold temperatures and expected degree-days are different in life stages of each causal agents and in turn, predicting risk of disease infection.

IPPC,2018;…

Degree day calculation in different plant forecasting models

- Degree-days: a unit of accumulated heat, used to estimate development of insects, fungi, plants, and other organisms which depend on temperature for growth.
- **Calculation of degree-days**: (one of several methods available)

DDs = avg. temperature minus threshold

- So, if the daily max and min are 80°F(26.6°C) and 60°F(15.6°C), and the threshold is 50(10.0°C), then we accumulate:

(80+60)/2 – 50°F = 20 DDs for the day

IPPC,2018

MaryBlyt forecasting system

Blossom Blight

Blossom blight symptoms(BBS)

- A blossom infection would be triggered if all four of the following events occurred:

1. Blossoms were open with stigmas exposed and petals intact;
2. The daily average temperature was >60°F (15.6°C);
3. Accumulation of at least 198 DH > 65°F (110 DH > 18.3°C) within
   - the last 80 DD > 40°F (44.4 DD > 4.4°C) for apples, or
   - within the last 120 DD > 40°F for pears; and
4. A wetting event occurring as:
   - dew, or
   - Rain >0.01 inch (0.25 mm), or
   - >0.10 inch (2.5 mm) of rain the previous day.

Turechek,2015; Biggs and Turechek,2018

MaryBlyt forecasting system

Blossom Blight

Blossom blight symptoms(BBS)

1. None: If blossoms are not present, the infection risk is NONE.
2. Low: Open blossoms create a LOW risk if no other condition is positive.
3. Moderate: The risk is MODERATE with open blossoms and if one of the other conditions is met,
4. High: with open blossoms and with two of the other conditions, and
5. Infection: an INFECTION occurs if all four conditions are met.

- These values indicate the relative risk of infection, not expected disease severity.

Turechek,2015

MaryBlyt forecasting system

Blossom Blight

Blossom blight symptoms(BBS)

1. Accumulation of a minimum of 198 DH > 65°F (110 DD > 18.3°C) over the last 80 or 120 DD > 40°F (44.4 or 66.7 DD > 4.4°C) after the start of flowering for apples or pears, respectively.
2. First symptoms can be expected with the additional accumulation of 103 DD > 55°F (57 DD > 12.7°C) from the date of infection.

TABLE 1. ***Maryblyt™*** system for reporting the minimum risk for a blossom infection event.

Risk of infection	Requirements for infection			
	Bloom	DH>65°F	Wetting	Ave>60°F
NONE	-	NA	NA	NA
LOW	+	-	-	-
MODERATE	+	+	-	-
or	+	-	+	-
or	+	-	-	+
HIGH	+	+	+	-
or	+	+	-	+
or	+	-	+	+
INFECTION[2]	+	+	+	+

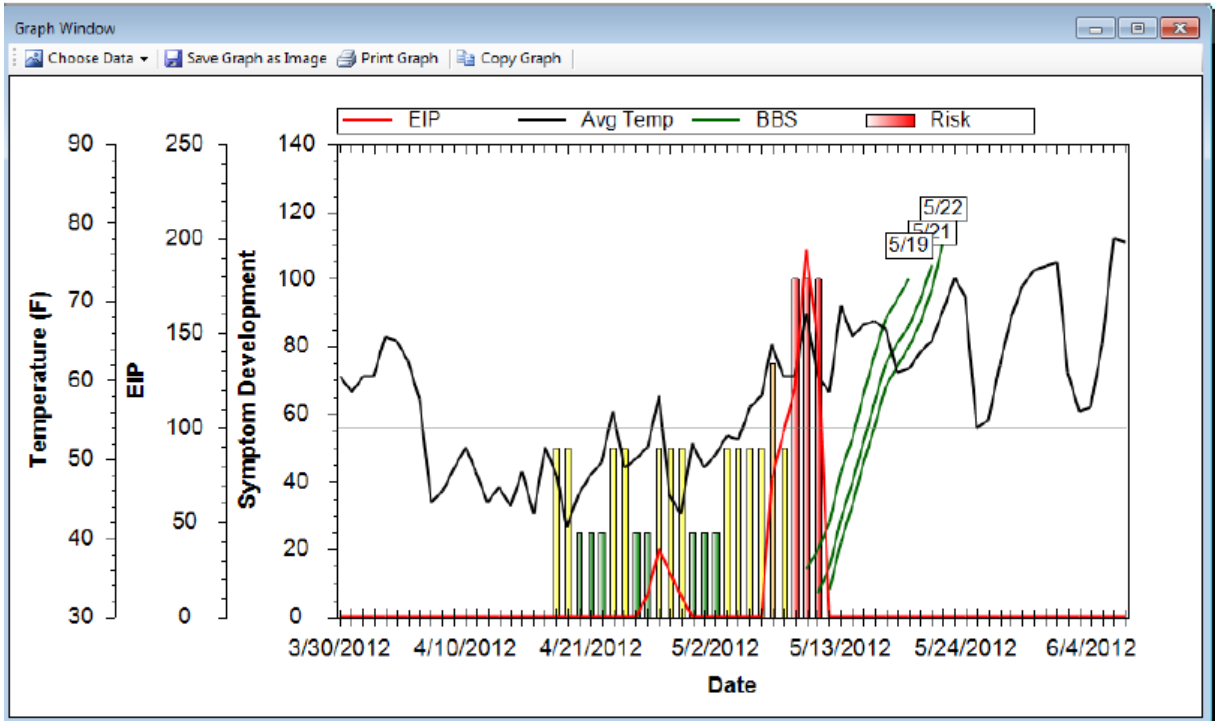
[1] Accumulation of a minimum of 198 DH > 65 °F (110 DD > 18.3 °C) over the last 80 or 120 DD > 40 °F (44.4 or 66.7 DD > 4.4 °C) after the start of flowering for apples or pears, respectively.
[2] First symptoms can be expected with the additional accumulation of 103 DD > 55 °F (57 DD > 12.7 °C) from the date of infection.

Degree hours using a base of 65°F (18.3°C) is used to estimate bacterial population growth, with 198 degree hours> 65° F= 110 DH > 18.3°C) from first bloom needed to build the population to a potentially dangerous level.

Biggs and Turechek,2018;..

MaryBlyt forecasting system

Available variables are: maximum, minimum, and average temperature (shown), EIP (shown) and EIP = 100 reference line (shown), BBS (shown for three infection events), CBS, SBS, TBS, rainfall, and infection risk (shown)



Graphical presentation of Maryblyt™ output example. Variables shown are: average temperature, and EIP = 100 (reference line), blossom blight symptoms (BBS) (shown for three infection events), and infection risk.

Morales *et al.*,2017; Biggs and Turechek,2018

MaryBlyt forecasting system

Blossom Blight

Blossom blight symptoms(BBS)

- If all these four events (open blossom, rain, cumulative degree day and degree-hours) were occurred:
- Blossom blight symptoms(BBS) would become visible with the accumulation of 90 degree days >55°F (50 DD > 12.7°C) from the date of infection.
- At this time the +/– system to identify the four risk categories was implemented.

Turechek,2015

MaryBlyt forecasting system

Shoot Blight

Shoot blight symptoms(SBS)

- Shoot blight as developing following the transmission of the pathogen from systemically invaded tissues to healthy shoots by insects.
- Specifically,

1. winged adults of the white apple leafhopper (*Typhlocyba pomaria* McAtee), and
2. aphids were noted as possibilities.

Turechek,2015

MaryBlyt forecasting system

Canker Blight

Canker Blight symptoms(CBS)

- Overwintering canker activity was defined to indicate when cankers become active.
- This activity was shown to occur after the accumulation of 130 degree-days >55°F (72 DD > 12.7°C).
- Shoot and limb symptoms(CBS) associated with canker blight were expected to become visible with the accumulation of 220 degree-days >55°F (122 DD > 12.7°C) from green tip.

Turechek,2015

MaryBlyt forecasting system

Canker Blight

Canker Blight symptoms(CBS)

- To observe/verify activity, the **bark was removed at the edge of the canker margin(CMS)**.

1. If the margin between the healthy and necrotic tissue is sharp and clear the canker is inactive.
2. If the margin **becomes diffuse or brown streaks** are clearly protruding from the margin, the canker is active.

Turechek,2015

MaryBlyt forecasting system

Trauma Blight

Trauma blight symptom(TBS)

- A trauma infection can be initiated with either:

1. hail damage, or
2. if the temperature dropped below 28°F (-2.2°C) and can expected any time after early bloom with the accumulation of 110 degree-hours >65°F (61 DH > 18.3°C).
3. If a trauma event occurs, symptoms can be expected after the accumulation of 130 degree-days >55°F (72 DD > 12.7°C) after the event.

Turechek,2015

MaryBlyt forecasting system
EIP (Epiphytic Infection Potential)

- EIP (Epiphytic Infection Potential) rises over 100, infection is likely during bloom with rain or heavy dew.
- We are just below the blossom blight (BBS) threshold with an EIP of 99.
- If you still have bloom in the orchard we can still get a fireblight infection with rain because our temperatures are still high enough for good bacterial growth.
- As long as we have average temperatures above 60°F (15.6°C) and bloom present fireblight infections are likely with rain storms.

Michigan State Uni. Extension,2000

MaryBlyt forecasting system

Four minimum requirements for infection event

Maryblyt Risk prediction

Maryblyt Risk prediction

DH	EIP	B	H	W	T	R
144	73	+	-	+	+	H
216	109	+	+	+	+	I
144	73	+	-	+	-	M
408	206	+	+	+	-	H
264	133	+	+	-	-	M
168	85	+	-	-	-	L

L = + M = ++ H = +++ I = ++++

DH = Degree hours accumulated, EIP = Epiphytic Inoculum Potential, B = Blossom present, H = Degree hour threshold met, H = Degree hour threshold met, W = Wetting event, T = Average temperature of 60°F (15.6°C), R = Risk prediction

MaryBlyt forecasting system
Creating a new season file

- To create a new season file, press the "Start New Season" button on the Main Start Window (Figure above). The following dialog will appear (Figure below).

1. Enter the season type (Apples or Pears), and
2. Enter information about the season. The variety, orchard, and description entries are optional.
3. Select either U.S. or metric units of measurement and decimal/date format for US or International.
4. Press OK when finished. This information will be saved with the season file.
5. This information can be edited at a later date by accessing "Season Information…" under the Options menu.

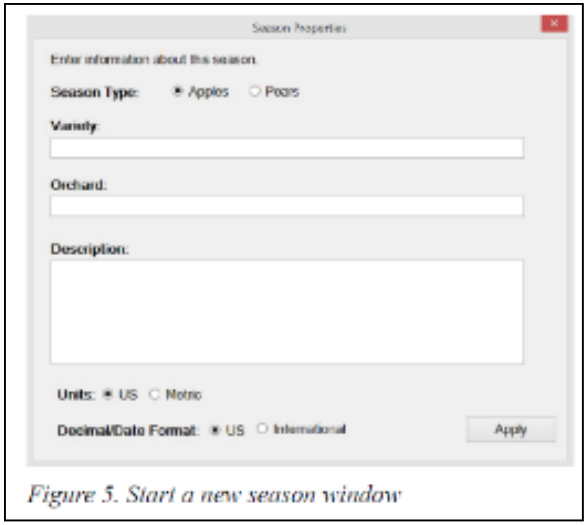
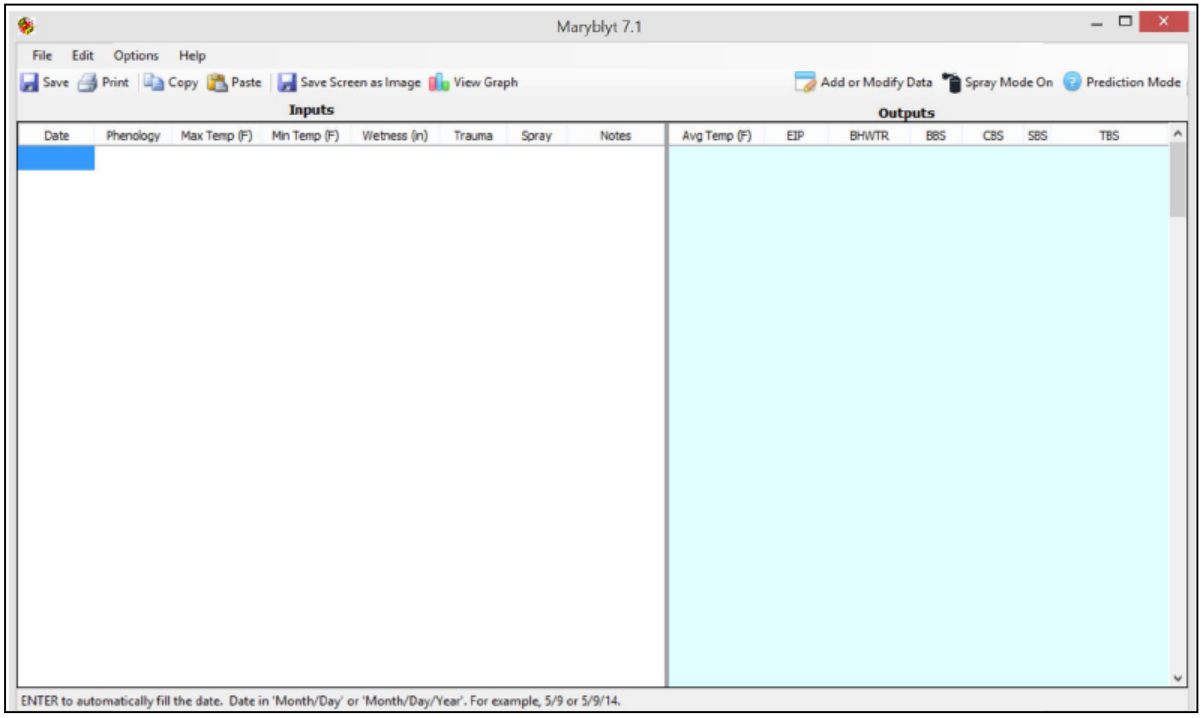


Figure 5. Start a new season window

Biggs and Turechek,2018

MaryBlyt forecasting system
Document Window

- The document window is displayed for a new season, or when an existing season file is opened. The document window is pictured below.



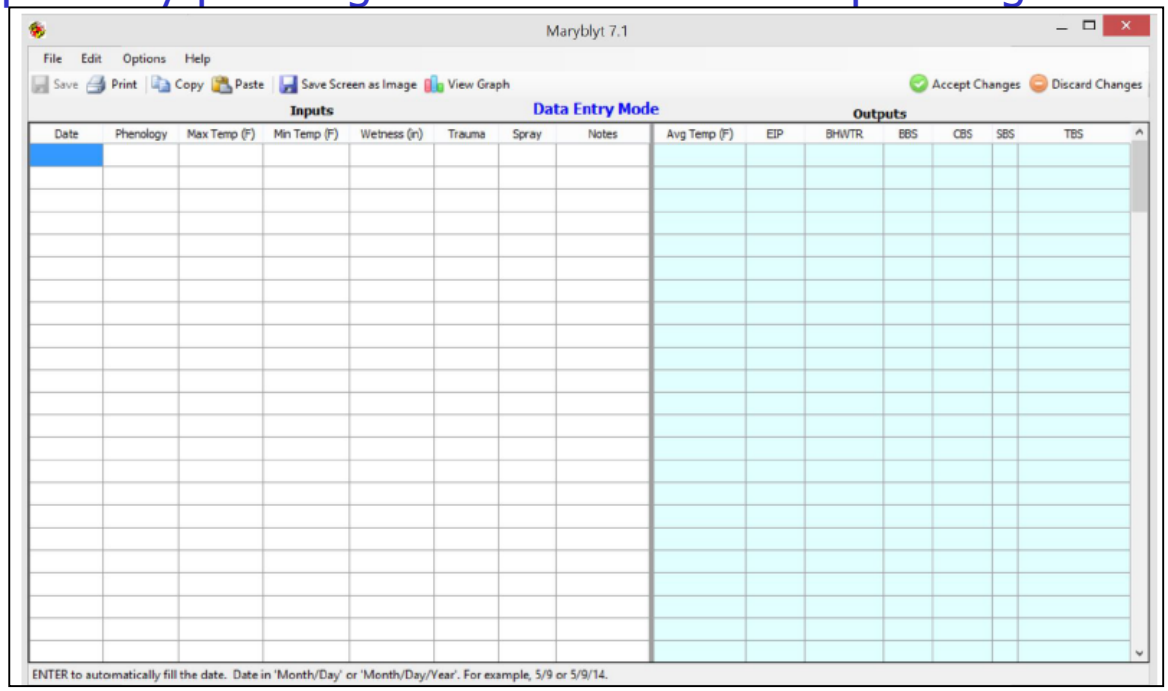
Biggs and Turechek,2018

MaryBlyt forecasting system

Entering Data into MARYBLYT(Data Enry Mode)

Pasting Data from Excel

- Data may also be copied and pasted from Microsoft Excel into Maryblyt™. The spreadsheet data must be ordered exactly as the Maryblyt™ columns are laid out. To paste data, select a cell in the grid and paste by pressing the "Paste" button or pressing CTRL-V.



Biggs and Turechek,2018

MaryBlyt forecasting system

Example of use of the Maryblyt

Prediction Mode

File Edit Options Help

Save Print Copy Paste Save Screen as Image View Graph Exit Prediction Mode

Prediction Mode

Inputs								Outputs						
Date	Phenology	Max Temp	Min Temp	Wetness (in)	Trauma	Spray	Notes	Avg Temp (F)	EIP	BHWTR	BBS	CBS	SBS	TBS
3/14/...	GT	80.0	66.0	0.00				73.0	-	-	-	9	-	-
3/15/...	GT	79.6	43.7	0.00				61.6	-	-	-	14	-	-
3/16/...	TC	68.5	48.3	0.05				58.4	-	-	-	16	-	-
3/17/...	TC	75.1	45.9	0.00				60.5	-	-	-	20	-	-
3/18/...	TC	65.1	52.8	0.00				59.0	-	-	-	22	-	-
3/19/...	TC	73.9	50.5	0.08				62.2	-	-	-	27	-	-
3/20/...	PK	72.2	54.3	0.01				63.2	-	-	-	31	-	-
3/21/...	PK	65.8	49.0	0.01				57.4	-	-	-	33	-	-
3/22/...	PK	76.1	54.5	0.00				65.3	-	-	-	38	-	-
3/23/...	B1	75.0	52.5	0.00				63.8	36	+ - - + M	-	42	-	-
3/24/...	B	69.0	52.0	0.23				60.5	48	+ - + + H	-	45	-	-
3/25/...	B	61.0	48.0	0.00				54.5	32	+ - + - M	-	46	-	-
3/26/...	B	58.0	39.0	0.00				48.5	16	+ - - - L	-	47	-	-
3/27/...	B	55.0	35.0	0.00				45.0	-	+ - - - L	-	47	-	-
3/28/...	B	60.0	42.0	0.00				51.0	-	+ - - - L	-	47	-	-

For phenological bud stage indicators, D (dormant) or ST (silver tip), TC (tight cluster), PK or WB (pink on apples, white bud on pears), BB (full bloom) and B2 (when most primary flowers are gone, but some secondary bloom remains). can be entered early but do not affect the program. Three entries are required for the program to function: (*i*) GT (green tip) is when 50% of the buds show green tissue (this is a biofix to begin predictions); (*ii*) B or B1 (first bloom) is when the first flower opens in the orchard; and (*iii*) petal fall (PF) when the last open flower in the orchard is gone (stops blossom blight predictions).

Degree-days after a starting point (usually called a biofix).

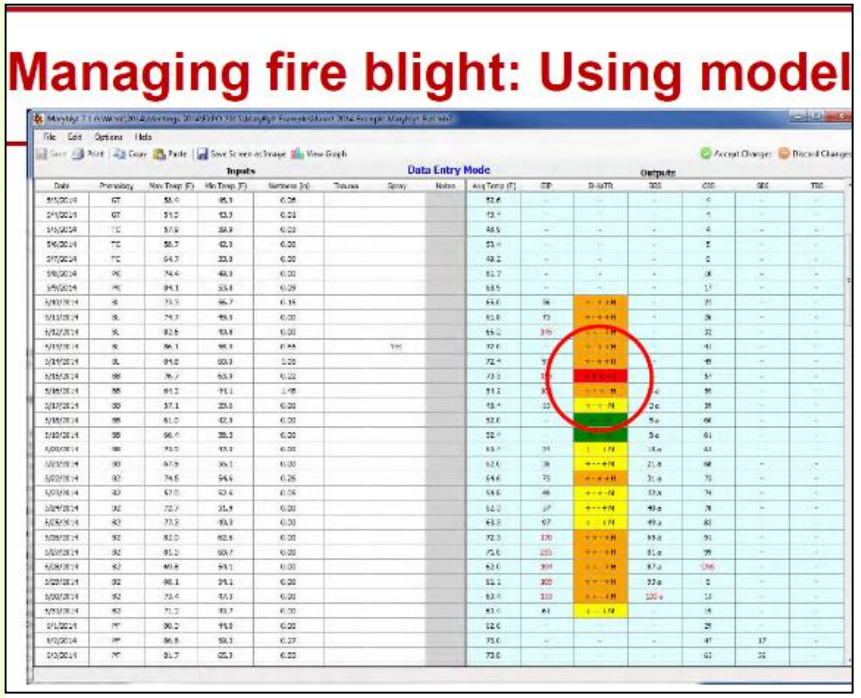
MaryBlyt forecasting system

Click Prediction Mode to demonstrate the simulator in *Maryblyt™*, which uses forecasted weather information to make predictions

Deborah I. Breth

MaryBlyt forecasting system
Prediction Mode

- Do not ignore the warning for canker blight symptoms (=CBS) shown on 4/14.
- In the absence of significant blossom blight, active overwintering cankers provide an inoculum source for the bacteria to be moved by insects, rain, and wind to susceptible shoot tips.
- Locate these first CBS symptoms early and remove all cankers and nearby infected shoots completely.



Biggs and Turechek,2018

Maryblyt v.4.3c, which ran in a DOS window

Very high risk assessment (9 infection days/period) were predicted by MARYBLYT programme based on weather data collected from local synoptic station.

In early versions, some records(limit of 4 characters) were entered/appeared in the Note column.
E.g. trace raining (TRCE), frost (FRST)

File: KHORAM80 - PEAR/HIGH BLOS BLYT/1380

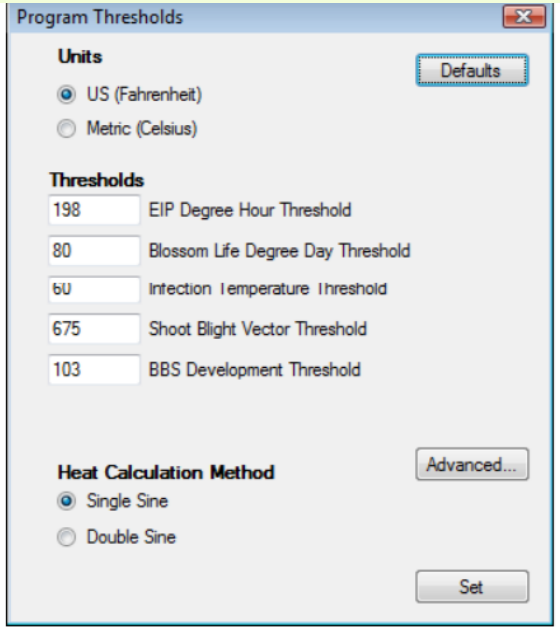
DATE	PH	MAX	MIN	AVG	WET	T	S	DD13	DD04	DH	EIP	BHWTR	BBS	CBS	SBS	TBS	NOTE
1/ 1	PG	21.0	4.0	12.5	0.00			3	8	0	0		0	3	0	0	
1/ 2	PB	21.0	9.0	15.0	0.00			6	19	0	0		0	6	0	0	
1/ 3	PB	21.0	5.0	13.0	0.00			9	27	0	0		0	8	0	0	
1/ 4	PB	19.0	8.0	13.5	13.20			11	36	0	0		0	10	0	0	
1/ 5	PB	11.0	7.0	9.0	14.60			11	41	0	0		0	10	0	0	
1/ 6	PB	13.0	6.0	9.5	0.25			11	46	0	0		0	10	0	0	TRCE
1/ 7	PB	18.0	3.0	10.5	0.00			12	52	0	0		0	11	0	0	
1/ 8	PB	25.0	7.0	16.0	0.00			17	63	0	0		0	15	0	0	
1/ 9	PB	24.0	7.0	15.5	0.00			21	74	0	0		0	19	0	0	
1/10	PB	24.0	9.0	16.5	0.00			26	87	0	0		0	23	0	0	
1/11	PB	25.0	7.0	16.0	0.00			30	98	0	0		0	28	0	0	
1/12	PB	25.0	7.0	16.0	0.00			34	110	0	0		0	32	0	0	
1/13	PB	27.0	8.0	17.5	0.00			40	123	0	0		0	37	0	0	
1/14	PB	26.0	8.0	17.0	0.00			46	136	0	0		0	42	0	0	
1/15	B	26.0	8.0	17.0	0.00			51	148	53	48	+--+M	0	47	0	0	
1/16	B	28.0	10.0	19.0	0.00		?	58	163	133	121	++-+H	0	53	0	0	
1/17	B	24.0	10.0	17.0	0.40		?	63	176	173	158	++++I	0	58	0	0	
1/18	B	20.0	12.0	16.0	7.40		?	66	187	187	170	++++I	6a	61	0	0	
1/19	B	26.0	8.0	17.0	9.70		?	72	200	240	218	++++I	16a	66	0	0	
1/20	B	20.0	9.0	14.5	0.00		?	74	210	253	230	+++-H	20a	68	0	0	
1/21	B	20.0	4.0	12.0	0.00			77	218	200	182	++--M	24a	70	0	0	
1/22	B	23.0	5.0	14.0	0.00			80	227	147	133	++--M	30a	73	0	0	
1/23	B	24.0	7.0	15.5	0.00			84	238	187	170	++--M	38a	78	0	0	
1/24	B	21.0	5.0	13.0	0.00			87	247	160	145	++--M	43a	80	0	0	
1/25	B	22.0	4.0	13.0	0.00			90	255	160	145	++--M	48a	83	0	0	
1/26	B	24.0	4.0	14.0	0.00			94	264	133	121	++--M	54a	86	0	0	
1/27	B	25.0	5.0	15.0	0.00			98	275	173	158	++--M	62a	90	0	0	
1/28	B	22.0	5.0	13.5	0.00			102	284	173	158	++--M	68a	93	0	0	
1/29	B	22.0	5.0	13.5	0.00			105	293	187	170	++--M	74a	96	0	0	
1/30	B	25.0	5.0	15.0	0.00			109	303	200	182	++--M	82a	CMS	0	0	
1/31	B	27.0	8.0	17.5	0.00		?	115	316	227	206	++-+H	91a	8	0	0	
2/ 1	B	27.0	10.0	18.5	0.00		?	121	330	267	242	++-+H	102a	18	0	0	
2/ 2	B	24.0	7.0	15.5	0.00			126	341	280	255	++--M	104b	26	0	0	
2/ 3	B	25.0	5.0	15.0	0.00			130	352	280	255	++--M	102c	34	0	0	
2/ 4	B	27.0	12.0	19.5	0.00		?	137	367	320	291	++-+H	0	46	0	0	
2/ 5	B	30.0	9.0	19.5	0.25		?	144	382	373	339	++++I	0	58	13	0	TRC
2/ 6	B	28.0	15.0	21.5	0.00		?	153	399	400	364	++-+H	16d	74	28	0	
2/ 7	B	27.0	4.0	15.5	0.00			158	410	347	315	++--M	24d	83	37	0	
2/ 8	B	27.0	7.0	17.0	0.00		?	163	423	373	339	++-+H	34d	92	47	0	
2/ 9	B	21.0	9.0	15.0	0.25		?	167	433	320	291	+++-H	40d	98	52	0	TRC
2/10	B	27.0	7.0	17.0	2.90		?	172	446	293	267	++++I	50d	CBS	62	0	
2/11	B	27.0	9.0	18.0	0.00		?	178	459	360	327	++++I	60d		73	0	
2/12	B	29.0	12.0	20.5	2.40		?	186	476	360	327	++++I	74d		86	0	
2/13	B	27.0	15.0	21.0	8.00		?	194	492	387	352	++++I	88d		101	0	
2/14	B	24.0	10.0	17.0	0.00		?	199	505	347	315	++++I	97d		110	0	
2/15	B	19.0	12.0	15.5	5.20		?	202	516	280	255	+++-H	102d		115	0	
2/16	PB	24.0	7.0	15.5	0.00			207	527	0	0		60e		0	0	
2/17	PB	24.0	9.0	16.5	0.00			211	539	0	0		68e		0	0	
2/18	PB	26.0	7.0	16.5	0.00			216	552	0	0		77e		0	0	
2/19	PB	24.0	12.0	18.0	0.00			222	565	0	0		86e		0	0	
2/20	PB	24.0	6.0	15.0	0.00			226	576	0	0		93e		0	0	
2/21	PB	27.0	9.0	18.0	0.00			232	589	0	0		104e		0	0	
2/22	PB	30.0	9.0	19.5	0.00			239	604	0	0		106f		0	0	
2/23	PB	32.0	9.0	20.5	0.00			247	620	0	0		107g		0	0	
2/24	PB	34.0	10.0	22.0	0.00			257	638	0	0		109h		0	0	

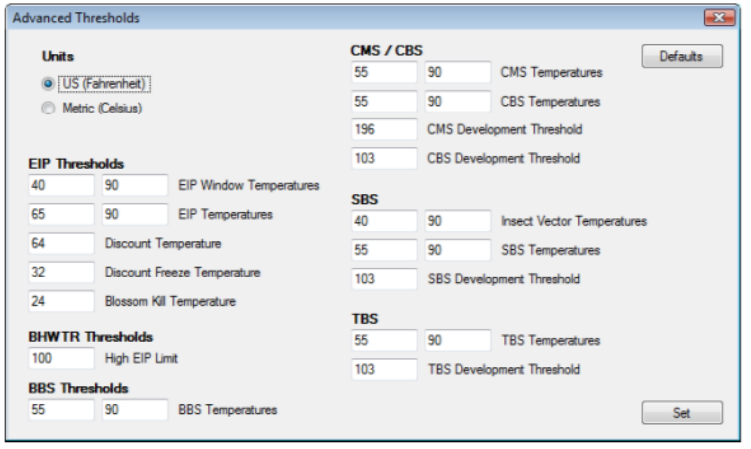
MaryBlyt forecasting system Maryblyt™ 7
The Program Thresholds dialogue box
Modification of program thresholds

- Program Thresholds The MARYBLYT prediction model uses several parameters, or program thresholds, to generate its output.
- These program thresholds are accessible from the Program Thresholds and Advanced Thresholds dialogs.
- Modification of these thresholds is considered advanced usage of MARYBLYT, and detailed descriptions of the thresholds can be found in the accompanying an in MARYBLYT documentation.

MaryBlyt forecasting system
Modification of program thresholds

- The Program Thresholds dialogue box, in addition to accessing all program thresholds, has the added option of setting the spray efficacy; a new feature in version 7.1. This parameter reduces the accumulated number of degree-hours by a factor equal to (100 – % Spray Effectiveness) and subsequent epiphytic inoculum potential calculations are reset to begin from the date of application.





198°F=92.2°C; 80°F=26.6°C
60°F=15.6°C; 675°F=357.2°C;
103°F=39.4°C

Deborah I. Breth;Turechek,2015

CougarBlight Model

Forecasting Fire Blight Disease

Erwinia amylovora

Cougar: large American wild cat

CougarBlight Model

Erwinia amylovora

New and old versions

- The CougarBlight model (Smith,1993;1999 and Smith and Pusey,2011) was developed to help apple and pear managers in the Pacific Northwest USA recognize specific weather conditions that have preceded blight infections.
- The new version of the model will be called Cougarblight 2010, can be downloaded from the following website: http://www.ncw.wsu.edu/treefruit/.
- While old versions of the CougarBlight model are still functional, this new version is recommended for all future risk assessment.

Smith and Pusey,2011

CougarBlight Model

Erwinia amylovora

New and old versions

- The basic concepts of the model have not changed since 1995.
- However, there are significant alterations to the 2010 version of this model relating to the growth rate of *E. amylovora* on stigmas.
- In the past, the CougarBlight model temperature risk numbers were based on the growth rate of *E. amylovora* in broth culture (Schouten, 1987), far different conditions than those found in nature.

Smith and Pusey,2011

CougarBlight Model

Erwinia amylovora

New and old versions

- The basic concepts of the model have not changed since 1995.

1. Orchard fire blight history;
2. Flower life/colony growth;
3. Bacterial growth rate;
4. Wetting as a trigger to infection.

Smith and Pusey,2011

CougarBlight Model

Erwinia amylovora

New version

- There is a basic Excel spreadsheet version of the model in both Celsius and Fahrenheit:

1. The F version: CougarBlight 2010 EZ ver.5.1
2. The C version: CougarBlight 2010 EZ ver.5.1.
3. Very recent version is: CougarBlight2019ver7.

The Cougarblight Model is available https://decisionaid.systems/ for periods when flowers are commonly present on trees (March 11 to Jun 20; Aug 20 to Sept 3).
Only use for periods when you have flowers on your trees.
For a downloadable excel sheet to calculate risk values using farm weather data download CougarBlight2019ver7.
The previous version using daily versus hourly temperatures is CougarBlight2010EZver5_1F.

CougarBlight Model

Erwinia amylovora

New versions

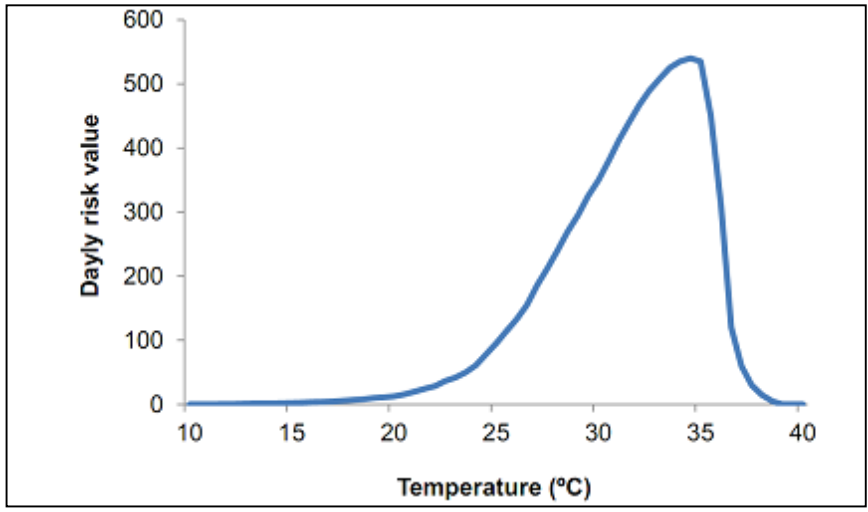
- **Degree hours estimation:**
- A chart was developed to determine the average number of "degree hours" that have occurred on a day with a certain high and low temperature [this chart has been incorporated into the on-line calculator at http://pnwpest.org/cgi-bin/ddmodel.pl?spp=fbl].
- When blossoms are present in the orchard, record this number daily.
- The total number of degree days that have accumulated over the past three days, plus the number predicted for today, equal the "four-day degree hour total."

CougarBlight Model

Erwinia amylovora

New versions

- Average daily risk values related to the daily high temperatures used by CougarBlight, derived from *Erwinia amylovora* population growth on stigmas of detached crab apple flowers held at various temperatures for 24 h.
- Use this version only if you have no access to hourly temperatures.



Daily temperature risk values (TRV) for Cougar Blight were determined by Sum of daily degree hours above 15.5°C (0-600 or more) against degree days in each day.

Smith,1993; Smith and Pusey,2011

CougarBlight Model

Table 1: Daily Degree-Hour Estimate Chart(°F).

Daytime High	Nighttime Low 49.9 F or Less	Nighttime Low 50 F or More
60	0	0
61	1	2
62	2	5
63	5	12
64	10	22
65	14	29
66	20	35
67	26	42
68	33	50
69	42	60
70	52	70
71	62	80
72	74	92
73	87	105
74	100	120
75	115	134
76	130	151
77	146	169
78	162	189
79	178	209
80	195	230
81	212	250
82	228	265
83	243	280
84	257	292
85	266	302
86	274	310
87	280	315
88	285	320
89	288	325
90	290	330
91	288	332
92	287	335
93	284	333
94	280	330
95	274	325
96	267	317
97	260	309
98	254	302
99	246	293
100	238	285
101		275
102		268
103		259
104		250
105		240

Table from: Smith, T. J. Fire Blight Daily Risk Estimation Model Version 98F

CougarBlight Model

Erwinia amylovora

New versions

- Under the Cougar heading, there is a lettered Pathogen Potential (a to e) that is used to estimate the presence of Fire Blight inoculum.
- For each level of inoculum present, a numbered Infection Risk (0 to 4) predicts the severity of an infection.
- **Pathogen Potential:**

a. No Fire Blight in area in past two seasons
b. Fire Blight in local area in past two seasons
c. Fire Blight in local area last year
d. Fire Blight in orchard last year
e. Active cankers present nearby.

Smith,1993; www.specmeters.com

CougarBlight Model

New version

2000C(b)(Celsius)

POTENTIAL FOR PATHOGEN PRESENCE (see below)	LOW	MODERATE	HIGH	EXTREME
No fire blight in your area for the past season	0 - 200	200 - 270	270 - 430	430+
Fire blight in your orchard or your neighbors' orchard last year	0 - 110	110 - 160	160 - 270	270+
Active fire blight cankers are now in or very near your orchard	0 - 30	30 - 110	110 - 200	200+

When blossoms are present in the orchard, record this number daily. The total number of degree days that have accumulated over the past three days, plus the number predicted for today, equal the "four-day degree hour total."

T. J. Smith

CougarBlight Model

Erwinia amylovora

New versions

- This is an update from 2010 where values were calculated from daily vs hourly temperatures.
- Hourly risk values are accumulated for 4 days (96 hrs. i.e. the past three days, plus the present day) the estimated time a flower remains susceptible to infection) to equal a total risk value (TRV).
- The four-day total risk values used as thresholds depend on the fire blight history of the orchard.
- Risk thresholds are reported based on three scenarios which simulate:

1. low,
2. Medium, and
3. high inoculum.

CougarBlight Model

Erwinia amylovora

New versions

1. If growers select 'no fire blight in the orchard last year' in the decision support system (DAS) risk is considered marginal from 0-399 TRV(total risk value), high from 400-799 TRV and extreme above 800 TRV.
2. If growers select 'occurred in the area last year' risk is considered marginal from 0-149 TRV, high from 150-349 TRV and extreme above 350 TRV. This should be the default setting in areas with a history of fire blight problems.
3. If growers select fire blight is 'now active in the area' risk is considered marginal below 79 TRV, high from 80-199 TRV and extreme above 200 TRV.

CougarBlight Model

New version

2000C(b)(Celsius)

.	HIGH TEMP	LOW TEMP	DAILY DEGREE HRS.	.
3 DAYS AGO	21	9	28	.
2 DAYS AGO	28	11	150	.
YESTERDAY	28	8	129	.
TODAY (PREDICTED)	25	10	94	.
.	.	4 DAY TOTAL:	401	WET BLOSSOMS?

EXAMPLE: 3 days ago, 21/9 = 28 degree hours. (See table below for DHr. values) 2 days ago, 28/11 = 150 degree hours. Yesterday, 28/8 = 129 degree hours. Today's predicted temperature, 25/10 = 94 degree hours. The sum of these four days degree hours equals 401 degree hours. If blossoms are wetted, fire blight infection risk is "High."

COUGARBLIGHT: Sums degree hours above 15.5°C for 4 days. EXAMPLE: 3 days ago, 21/9 = 28 degree hours. 2 days ago, 28/11 = 150 degree hours. Yesterday, 28/8 = 129 degree hours. Today's predicted temperature, 25/10 = 94 degree hours. The sum of these four days degree hours equals 401 degree hours.

T. J. Smith

CougarBlight Model

Orchard fire blight history

The three fire blight history scenarios were derived empirically by studying numerous individual fire blight outbreaks

- Thresholds of infection risk relative to different fire blight history scenario are as follows:
- For scenario 1,
- risk categories and temperature risk value ranges are:
- Low 0-150, Caution 150-500, High 500-800, Extreme 800-1000, and Exceptional 1000+.
- For scenario 2,
- risk categories and temperature risk value ranges are:
- Low 0-100, Caution 100-200, High 200-350, Extreme 350-500, and Exceptional 501+.
- For scenario 3,
- Risk categories and temperature risk value ranges are:
- Low 0 (there is no low risk in an infected orchard), Caution 0-100, High 100-200, Extreme 200-300, and Exceptional 301+.

CougarBlight Model
The meaning of risk category terminology
New version

- Low: Wetting of flowers during these temperature conditions has not resulted in new flower blight infections in past years.
- The flowers within a few meters of an active canker may be an exception.
- Caution: Wetting of flowers under these temperature conditions is not likely to lead to infection, but the possibility increases as values approach the upper range.
- Weather forecasts and risk values should be carefully monitored. If antibiotic materials are not being used, blossom protection with other materials should be initiated three or four days prior to entering a high infection risk period. Continue appropriate protective sprays until the infection risk drops below the "high" threshold.

Smith and Pusey,2011

CougarBlight Model
The meaning of risk category terminology
New version

- High: Under these temperature conditions, serious outbreaks of fire blight have occurred. Orchards that recently had blight are especially vulnerable. The risk of severe damage from infection increases during the later days of the primary bloom period, and during petal fall, while blossoms are plentiful. Infection is common, but more scattered when late blossoms are wetted during high risk periods. The potential severity of infection increases if a series of high risk days occur.
- Extreme or Exceptional: Some of the most damaging fire blight epidemics have occurred under these optimum temperature conditions, followed by blossom wetting.
- These infections often lead to severe orchard damage, especially during primary bloom or when numerous secondary blossoms are present. **As the season progresses, secondary blossoms tend to form less frequently, and hot summer**.

Smith and Pusey,2011

CougarBlight Model

Orchard fire blight history

The three fire blight history scenarios were derived empirically by studying numerous individual fire blight outbreaks

- Risk value thresholds of CougarBlight for different orchard blight history scenarios (Smith and Pusey, 2011).

	Low	Caution	High	Extreme	Exceptional
1. No fire blight in the neighborhood last year	0-300	300-500	500-800	>800	-
2. Fire blight occurred in the neighborhood last year	0-100	100-200	200-350	350-500	>500
3. Fire blight is now active in the neighborhood	-	0-100	100-200	200-300	>300

Morales *et al.*,2017

CougarBlight Model

Erwinia amylovora

New version

- It uses temperature data to estimate the growth rate of fire blight bacteria (*Erwinia amylovora*) over the past three days plus the present day, if wetting occurs in the afternoon of evening, or the previous four days if wetting occurs in the morning.
- **Each day blossoms are open, the degree-hours for the noted days are added to obtain a four day degree-hour total.**
- The goal is to determine what sort of growing conditions the bacteria have had while on the stigma during the approximately 96 hours prior to a 3+ hour blossom wetting period.

UC IPM,2019

CougarBlight Model

Erwinia amylovora

New version

- Degree-hours are calculated from average hourly temperatures, or from daily minimum/maximum temperatures based on an estimated degree-hour look up chart (see Table 1).
- Calculation of the "four-day degree-hour total" must be done for each day.
- If blossoms are wetted by rain, four or more hours of dew, or any significant wetting, refer to the degree-hour total and Table 2 below to evaluate the potential risk of infection.

CougarBlight Model

Table 2: Infection risk relative to 4-Day degree-hour total

Potential for Pathogen Presence	Low	Moderate	High	Extreme
No fire blight in area past two seasons	0-350	350-500	500-800	800+
Fire blight in local area past two seasons	0-300	300-500	500-750	750+
Fire blight in local area last year	0-250	250-450	450-700	700+
Fire blight in your orchard or your neighbor's orchard last year	0-200	200-350	350-500	500+
Active cankers present nearby	0-100	100-200	200-350	350+

Table from: Smith, T.J. Fire Blight Daily Risk Estimation Model Version 98F

UC IPM,2019

Billing's Integrated System
Forecasting Fire Blight Disease

Erwinia amylovora

Billing's system for predicting fire blight in a warm dry environment *Erwinia amylovora*

- Billing developed three distinct systems:

1. Billing's original system (BOS) was published in 1980. The top days for infection risk limit was 30°C.
2. A revised system (BRS) in 1990 and 1992. The top days for infection risk limit was 30°C.
3. The third system was called as integrated system (BIS), developed in 1996.

I. The first version of BIS was called BIS95. The top days for infection risk limit was not determined, and

II. The second version, referred as BIS98. BIS98 excludes days with temperatures >32°C from risk day assessments.

Billing,2007

Billing's Integrated System (BIS95)
Erwinia amylovora

- Therefore, integrated system (BIS), was designated as a substitute for:

1. Billing's original (BOS), and
2. Billing's revised system for fire blight (BRS).

- The integrated system (BIS) was based on *E. amylovora* potential doublings derived from *in vitro* growth rates (Billing, 1980, 1992), in order to make it simpler and clearer.

Billing's original system (BOS) (Billing,1984)
Billing's revised system (BRS) (Billing,1992)

Morales *et al.*,2017

Billing's Integrated System (BIS95)
Erwinia amylovora

- Infection risk (IR) depends on:

1. inoculum potential (IP),
2. host susceptibility which is increased by tissue damage, and
3. warmth and wetness at the time of infection.

Morales *et al.*,2017

Billing's Integrated System (BIS95)
Erwinia amylovora
DD18 and DD13

- The model uses two types of degree-day (DD) calculations to help assess the risk of fire blight (Billing, 1996, 1999):

1. DD18 = the sum of daily values above 18°C for the maximum temperature. DD18 calculations begin on the first day of bloom, and continue throughout the bloom period. If the maximum temperature falls to 16-17°C for two days or to 15°C or lower for one day, the DD18 sum is reset to zero.
2. DD13 = the sum of daily values of 0.5°C or more above a 13°C mean. **DD13 calculations begin on the day after each infection risk (IR) day**. DD13 is used to time orchard scouting for signs of new disease.

Morales *et al.*,2017

Billing's Integrated System (BIS95)
Erwinia amylovora

- **A day is classified as infection risk (IR) day if:**

1. The IP is principally coming from ooze and is spread by rain during pre-bloom, blossom, young shoot and fruit growth stages.
2. Rain is ≥ 3 mm and mean temperature on the wet day or the day before is ≥ 13ºC.

- Blossom infection risk (BIR) occurs in situations where IP levels depend on flower colonization by the pathogen and spread by insects.

Morales *et al.*,2017

Billing's Integrated System (BIS95)
Erwinia amylovora

- **A day is classified as a BIR(blossom infection risk) day if:**

1. The DD18 sum is ≥ 17C°, the open flowers are wet by heavy dew, mist, or rain, and the mean temperature is ≥ 15°C on the day of wetting.
2. Rain is ≥ 3 mm and mean temperature on the wet day or the day before is ≥ 13°C.
3. There is no wetting event but the maximum temperature for the day is ≥ 27°C and/or the mean temperature for the day is ≥ 20°C.
4. In addition to degree-day and infection risk calculations based on weather data, the model also involves intensive orchard scouting and systematic recording of host growth stages, insect activity, and disease incidence to determine risk of new disease.

Morales *et al.*,2017

Billing's Integrated System (BIS95)
Erwinia amylovora

- A comparison of the modified form of the fire blight model BIS95 used by Shtienberg *et al.*,2003 with a suggested form for use with incomplete weather records:

BIS95 feature	Shtienberg et al.	Suggested form
Days >32°C	Included	Omitted (6)
Daily rainfall	Wetness >2 h	Rain ≥2.0 mm Wetness >16 h
Potential WIR days[a]	Omitted	Included (5,6)
IR days[b]	Mean temp. ≥15°C Wetness >2 h	Mean temp. ≥15°C Wetness >2 h
	Max. temp. ≥27°C or Mean temp. ≥20°C Wetness 0 h	Max. temp. ≥27°C or Mean temp. ≥20°C Wetness >2h

[a]Wet infection risk (WIR) days are days with ≥2.5 mm rain and mean temperature ≥13°C on the day of rain or the day before. [b]Infection risk (IR) days are days during bloom only, after the sum of degree days above 18°C is ≥17.

Billing,2007

NEWA website

NEWA—Network for Environment and Weather Applications

Forcasting of fire blight caused by *Erwinia amylovora* based on CougarBlight logic

NEWA website

Program's Network for Environment and Weather Awareness (NEWA) on a daily basis

- Growers in the Northeast can use this website to help make:

1. planting,
2. pest management, and
3. a range of other weather- and climate-based agricultural decisions.

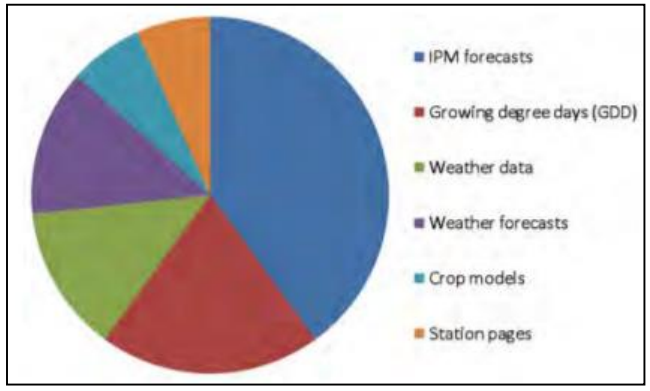
- This website retrieves data from on-farm, grower-owned weather stations throughout the Northeast and in a number of other locations across the U.S.
- Currently, 30 IPM and crop production tools and 13 degree-day tools are freely available from the NEWA website.

NEWA website

Program's Network for Environment and Weather Awareness (NEWA) on a daily basis

- Interactive forecast models automatically compute and display results to inform crop production and precision IPM practices.
- The NEWA website provides:

1. Hourly and daily weather summaries
2. Degree-day tables
3. Plant disease forecasts
4. Insect models
5. Crop production models
6. National Weather Service forecasts.



NEWA website

Program's Network for Environment and Weather Awareness (NEWA) on a daily basis

- NEWA provides automated local weather information and the results of pest forecasts on a daily basis.
- Simple weather recording equipment such as:

1. thermometers,
2. hygrometers, and
3. rain gauges

- placed in orchards will assist the prediction of pest outbreaks.

NEWA website

IPM and crop production forecasts currently available on NEWA

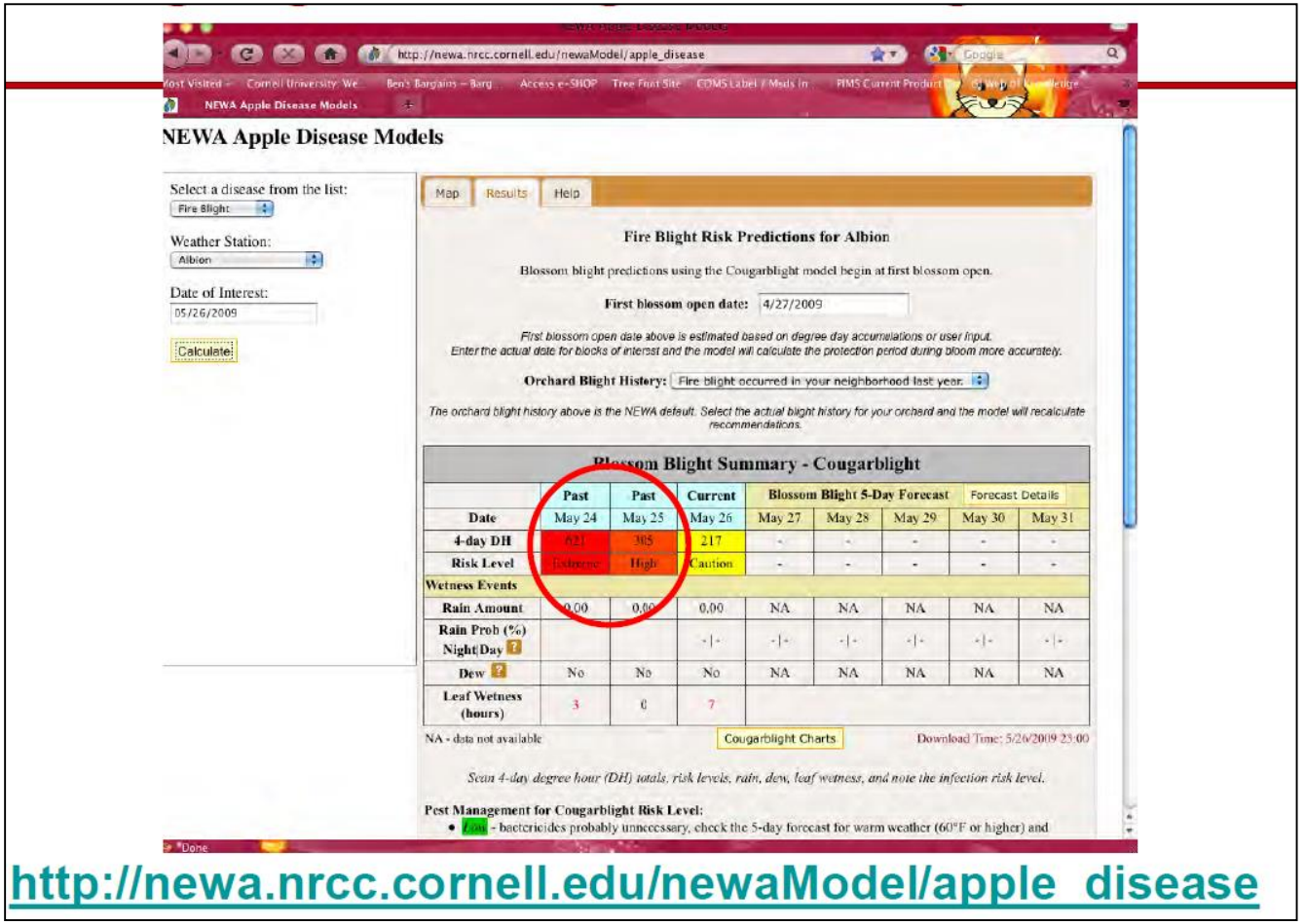
apple scab infection events	black rot of grapes	onion Botrytis blight
apple scab ascospore maturity	grapevine powdery mildew	onion Alternaria blight
fire blight Cougar Blight	Phomopsis cane & leaf spot	onion downy mildew
sooty blotch & flyspeck	grapevine downy mildew DMCast	onion maggot
obliquebanded leafroller	grape berry moth	Stewart's wilt of sweet corn
spotted tentiform leafminer	cabbage maggot	cucurbit downy mildew ipmPIPE
codling moth	tomato early blight TomCast	soybean rust Forecast Center
plum curculio	potato early blight	alfalfa weevil
oriental fruit moth	late blight BLITECAST	turfgrass diseases
apple maggot	late blight decision support system	turfgrass evapotranspiration (ET)

Carroll *et al.*,2011

NEWA website

Program's Network for Environment and Weather Awareness (NEWA) on a daily basis

Based on CougarBlight logic (WSU)



http://newa.nrcc.cornell.edu/newaModel/apple_disease

K.D. Cox

Management
Integrated Disease Management
Erwinia amylovora

- There are several organic remedies you can use for controlling fire blight.

1. First, try to keep your aphid and psylla population under control, since they spread fire blight.
2. Next, remove and destroy all suckers and infected branches.
3. Remove these infected branches as soon as you see them, no matter what the season.
4. Cut as least 12 inches below, if possible, where the fire blight welt is found.
5. Disinfect your tools with a 1:4 bleach solution (one part bleach to 4 parts water) after each cut to help control the fire blight bacteria from spreading from your tools to other areas of the tree or to other trees.

Management
Integrated Disease Management
Cultural practice

- The importance of cultivar susceptibility, can be compounded by grower management practices that either:

1. Reduce, or
2. increase this susceptibility (e.g., prolonged secondary flowering, excessive nitrogen fertilization).

- Modifications to suppress disease management e.g. nitrogen fertilizer in high amounts can stimulate juvenile/succulent growth that is extremely susceptible to fireblight.

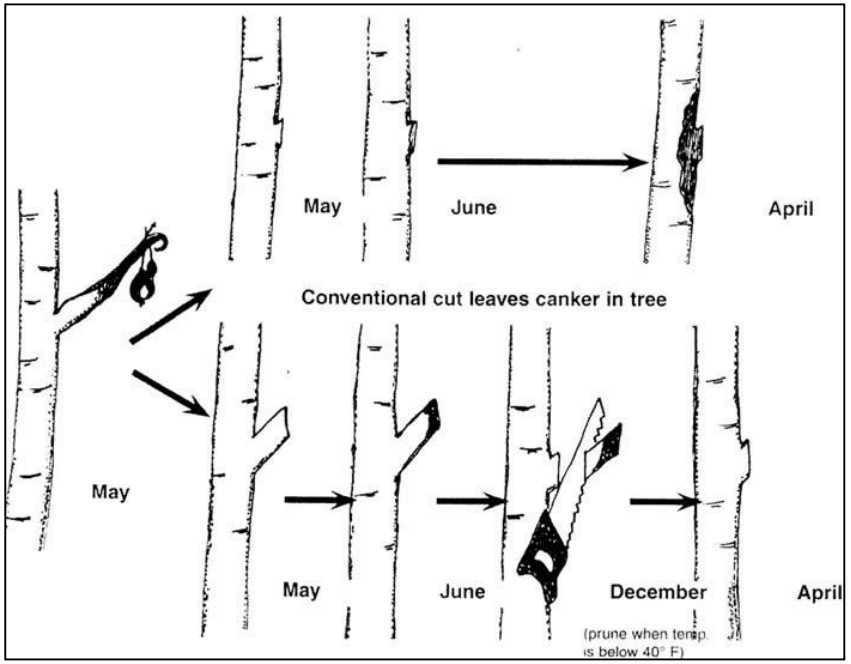
Management

Integrated Disease Management

Ugly stub method

Cultural Practice

- Use the "ugly stub" method for blight removal.
- By making the cut into at least 2-year-old wood and leaving a 3- to 4-inch (1"=2.54 cm), naked, "ugly stub".
- However, the stub and its small tip canker can then be removed safely and completely during the dormant pruning operation so that cankers are not left in the trees.



Ugly stub" cut removes canker in winter.

Steiner,2007

Management
Integrated Disease Management
Chemical control of fire blight

1. To help prevent fire blight, spray the tree regularly while in bloom with a solution of 4 ounces bleach to 3 gallons water.
2. Doing this kills the fire blight bacteria in the blossom so that bees do not spread the blight.
3. You do not want to kill the bees or prevent from visiting the tree during blossom stage because fruit trees are insect pollinated. Doing so would prevent the trees from bearing fruit.
4. High soil acidity will also contribute to fire blight. The more acid the soil the more risk there is to fire blight.
5. If you have a huge breakout of fire blight, you can spray your tree with copper sulfur blend labeled for fire blight.

1 Ounce ≈ 0.03 Liters; 1 Gallon ≈ 3.78 L. or 8 part per 1000 parts (8:1000)

St. Clare Heirloom Seeds

Management

Chemical control of fire blight

- **Copper compounds**
- Copper is an excellent bactericide for fire blight control as the pathogen *E. amylovora* is highly susceptible to copper.
- Copper compounds can be effective in reducing fire blight but their use during spring, when most of the infections occur, is limited because of phytotoxicity.
- This early application should be used yearly for the foreseeable future, as inoculum reduction in orchards will become more and more important.

Possible spray timings and recommended compounds for fire blight management during bloom

Spraying timing	Recommended compound
30-50% Bloom or later	**Antibiotic**
Full bloom	**Antibiotic or Serenade**
Petal fall of the king bloom	**Apogee**
Petal fall - first cover	**Antibiotic**

Serenade (a lyophilized culture filtrate of *Bacillus subtilis* QST 713).

Integrated Disease Management

Chemical control of fire blight

Recommended spray schedule for fire blight control

Starner (Oxolinic acid) is a synthetic antibiotic.

Vanneste,2000

Time of spray application	Chemical	Concentration
Pre-bloom after the swollen bud stage but before bud break	Bordeaux mixture + 1% spray oil or copper oxychloride + oil 1% copper hydroxide + oil 1% or copper oxychloride sulphate + oil 1% (COCS)	250 g Cu hl^{-1} [a]
Blossom period[b]	Copper compounds (Bordeaux mixture, copper oxychloride, etc.) or flumequin (Firestop™, Fructil™) or fosetyl-Al (Aliette™) or oxolinic acid (Starner™) or streptomycin (Plantomycin, Agrept, Agristrep) or oxytetracycline (Mycoshield) or streptomycin + oxytetracycline (Bacterol super)	50–100 g Cu hl^{-1} 300 p.p.m. 3000 p.p.m. (0.3 kg hl^{-1}) 300 p.p.m. 100 p.p.m. 200 p.p.m. 100 p.p.m.
Summer (after storms)[c]	The same as in blossom period	
Autumn[d]	Copper compounds preferably Bordeaux mixture	250 g Cu hl^{-1}

[a] 2500 l ha^{-1} to run off should be applied (van der Zwet and Beer, 1991).
[b] At 3–5-day intervals, or according to the prediction system's recommendation (van der Zwet *et al.*, 1988).
[c] Bactericides should be applied within 24 h after the storm or if possible immediately after the storm (van der Zwet and Beer, 1991).
[d] Two copper sprays during leaf fall in orchards where fire blight has occurred are recommended to reduce the number of active cankers.

Chemical control of fire blight
Antibiotics

- Only a few countries, including New Zealand, allow the use of the antibiotic streptomycin for control of fire blight.
- When used in conjunction with computer-based prediction models, these treatments can be very effective.
- However, strains resistant to streptomycin have been detected in every country where this antibiotic is allowed (Vanneste,2009).
- Oxytetracycline is another antibiotic that differs from streptomycin in that it is bacteriostatic and doesn't kill *E. amylovora* but does act to inhibit its growth.

Chemical control of fire blight
Antibiotics

- Oxytetracycline is equally active on streptomycin-resistant and streptomycin-sensitive strains.
- However, oxytetracycline is not as effective as streptomycin in fire blight control.
- Use the full rate of 1.5 lbs. per acre (0.4047 ha); this antibiotic does not dissolve well, so volumes of at least 100 gallons (≈ 3.78 L) per acre are recommended.
- Also, a surfactant such as Regulaid at one pint per 100 gallons will enhance efficacy.

1 Pound(Ib)=450 gram

Antibiotics

Oxytetracycline is often used with streptomycin in the control of fire blight of pome fruits during blossoming

Agrios,2005

Antibiotics

Antibiotic resistant

Role of mancozeb fungicide or $CaCl_2$ fertilizer

- Plant-grade antibiotics are unlikely to be purer than those used for treating humans.
- Fertilizers and fungicides applied to apple and pear are rich sources of divalent cations.
- The concentration of Mn^{2+} or Ca^{2+} in mancozeb fungicide or $CaCl_2$ fertilizer, respectively, when applied at recommended rates, is similar to the non-physiological concentrations recommended for transformation of bacteria in the laboratory.

	Field application	*In vitro* transformation
$CaCl_2$	~20-50 mM	~50-100 mM
Mg^{2+}	~75 mM	10 mM
Mn^{2+}	~4-8 mM	45 mM

P. S. McManus

Antibiotics

Apple disease control

Trunk injection

- Trunk injection is a target-precise pesticide delivery method that utilizes tree xylem to distribute injected compounds.
- Trunk injection could decrease antibiotic usage in the open environment and increase the effectiveness of compounds in fire blight control.
- In field experiments, after 1-2 apple tree injections of either streptomycin, potassium phosphites (PH), or acibenzolar-S-methyl (ASM), significant reduction of blossom and shoot blight symptoms was observed compared to water injected control trees.

A'cimovi'c *et al.*,2015

Antibiotics

Apple disease control

Trunk injection

- Trunk injection of oxytetracycline resulted in excellent control of shoot blight severity, suggesting that injection is a superior delivery method for this antibiotic.
- Injection of both potassium phosphites (PH), or acibenzolar-S-methyl (ASM), resulted in the significant induction of PR-1, PR-2, and PR-8 protein genes in apple leaves indicating induction of systemic acquired resistance (SAR) under field conditions.

A'cimovi'c *et al.*,2015

Antibiotics
Apple disease control
Trunk injection

- Four cardinally oriented injection ports per tree, positioned approximately 10-15 cm above the ground level, were created by drilling 25.4 mm into the xylem tissue and 9.53 mm in diameter, with a cordless 1500 rpm drill.
- Ports were sealed with Arborplug® no. 4 (Arborjet Inc., Woburn, MA, USA), using screwdriver-like plug tapper and a hammer, with plug positioned just below the bark level to allow port closure with cambium.

A'cimovi'c *et al.*,2015

Antibiotics

Apple disease control

Trunk injection

- Trunk-injected compounds on apple trees for control of blossom and shoot blight in 2012 and 2013.
- Water injected trees served as a control, and non-injected non-inoculated trees were also used as controls.

Treatment	Active ingredient (a.i.)	Dose
Blossom and shoot blight incidence control on 'Gala' apple trees		
ASM 1	Acibenzolar-*S*-methyl 50% (Actigard, Syngenta, AG)	1 × 0.34 g/tree
ASM 2		2 × 0.34 g/tree
PH	Mono- and di-potassium salts of phosphorous acid 45.8% (Phosphojet, Arborjet, Inc.)	2 × 22.5 ml/tree
SS	Streptomycin sulfate 22.4%/17% streptomycin/(Agrimycin, Nufarm, Ltd.)	2 × 1.82 g/tree
Water injected control	–	2 × 520 ml/tree
Shoot blight severity control on 'Jonathan' apple trees		
OTC	Oxytetracycline hydrochloride (ArborBiotic™, MFG Scientific Inc.)	1 × 0.28 g + 2.52 ml of water/each 25.4 mm of DFH*
Water injected control	–	2.52 ml of water/each 25.4 mm of DFH

**DFH, trunk diameter at 30.5 cm height.*

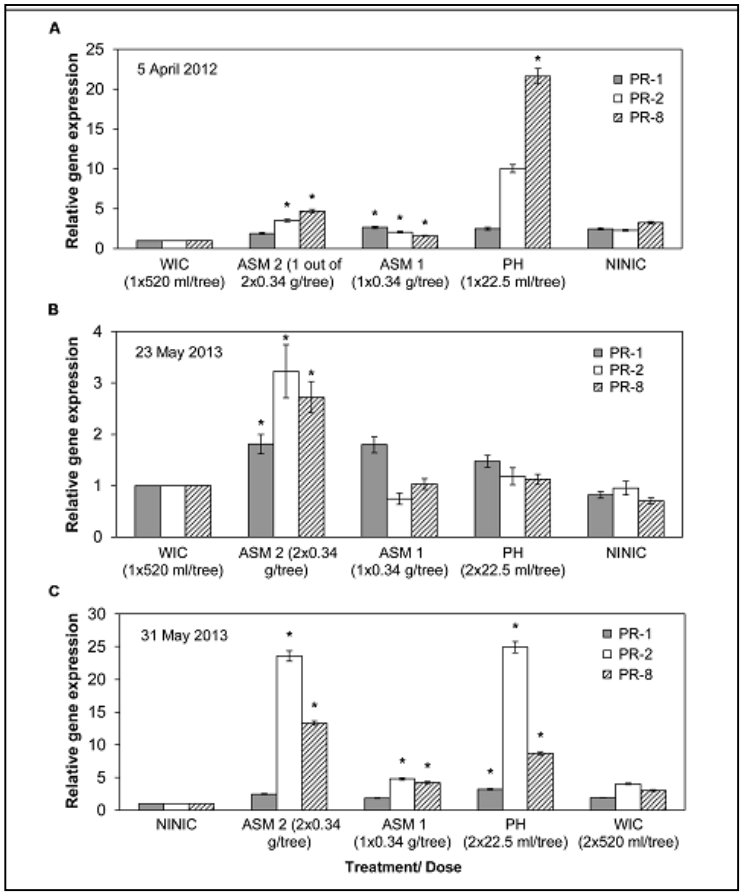
A'cimovi'c *et al.*,2015

Antibiotics

Apple disease control

Trunk injection

- Relative expression of PR-1, PR-2, and PR-8 genes in 'Gala' apple leaves tested by qRT-PCR.
- Samples were collected in 2012 (A) and 2013 (B,C) following trunk injection/s of different compounds.
- From injected 'Gala' apple trees for blossom blight control in 2012 and 2013, 21 leaves and 21 flowers per tree were collected for PR gene expression analysis.



A'cimovi'c *et al.*,2015

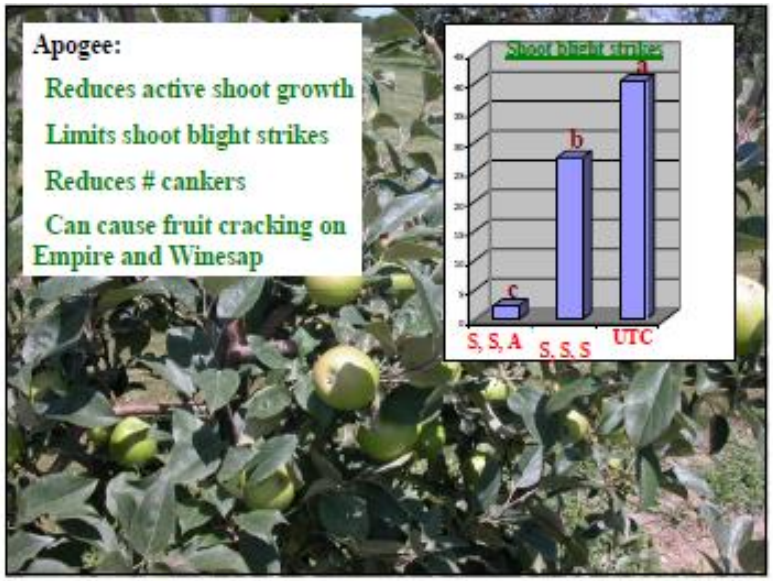
Apogee
The growth regulator(inhibitor)

- Prohexadione calcium(ProCa) is a gibberellin biosynthesis inhibitor which was registered on apples as Apogee® in North America and as Regalis® in Europe.
- As a growth inhibitor inhibits the growth of shoots.
- Shoots exhibiting reduced growth are less susceptible to becoming infected with shoot blight.
- Apogee is the only alternative available for shoot blight control and will become increasingly important in orchards where blossom blight is a problem.
- **Two things are important to remember:**

1. Apogee does not kill the fire blight bacterium;
2. Apogee does not affect the occurrence of blossom blight.

- The spray timing for Apogee for shoot blight control is petal fall of the king bloom.
- The effects of Apogee show up around 10 to 14 days later.
- A single application of Apogee at the high rate is most effective for fire blight control under conditions of high pressure during bloom.

Recommended compounds for fire blight management



Different letters indicate significant differences (Duncan, p≤0.01).

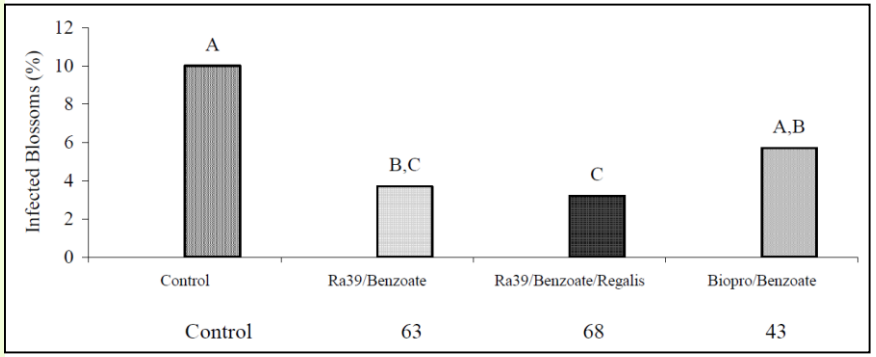
Sundin,2008

Management
Antagonist+aromatic/organicompounds

- Fire blight control with epiphytic bacterium *Rhanella aquatilis*-Ra39 in different combination:

1. Ra39/Benzoate (aromatic compound)
2. Ra39/Benzoate/Regalis (Apogee)
3. Biopro (Ra39+*Bacillus subtilis* BsBD 170)/Benzoate

- on Golden Delicious after natural infection, 2003.
- In field experiments the combination of Ra39 and Na-Benzoate was nearly comparable in the efficacy to streptomycin, 68 to 77%.



Columns with the same letter do not significantly differ following Duncan's multiple range test ($P \leq 0.05$).

Zeller and Laux,2004

Management

Integrated Disease Management

Erwinia amylovora

- **4. Biological control of fire blight**
- In past years, numerous studies approached this problem *via* the application of a range of promising biological control methods.
- These included the use of:

1. Antagonistic bacterial saprophytes;
2. Plant systemic acquired resistance (SAR) inducers, and
3. Construction of transgenic plants resistant to *E. amylovora* by biotechnological methods.

Nagy *et al.*,2012

Management

Integrated Disease Management

Erwinia amylovora

- Further studies on fire blight using biological control measures were directed towards the use of:

1. Yeast;
2. Avirulent strains of *E. amylovora*;
3. Plant extracts and etheric oils, or
4. The use of a new antibiotic produced by symbiotic bacteria of the entomopathogenic nematodes;
5. Another novel and promising method for controlling the fire blight disease could be the use of bacteriophages.

Nagy *et al.*,2012

Management
Integrated Disease Management
Erwinia amylovora

- Several biological materials have EPA labels for use on apple to manage fire blight infection of flowers (blossom blight).
- The commercial products:
- Blightban A506 and C9-1 (containing the bacterial antagonists *Pseudomonas fluorescens* A506 and *Pantoea agglomerans* C9-1, respectively);
- Bloomtime Biological (*Pantoea agglomerans* E325);
- Serenade (a lyophilized culture filtrate of *Bacillus subtilis* QST 713);
- Taegro is a biofungicide that contains *Bacillus amyloliquefaciens* strain FZB24 at 5.0 x 10^{10} cfu/g of the powdered formulation.

Aldwinckle *et al.*,2008

Biological control of fire blight
Blossom Bless

- Two non-pathogenic biocontrol agent bacteria have previously been identified that are useful in spray preparations to reduce fire blight infection through either:
- Direct competition for nutrient resources (*Pseudomonas fluorescens* A506 (Frostban® or Blightban®) or
- Production of antimicrobial compounds (*Pantoea agglomerans* P10c (Blossom Bless®).
- Although these organisms have been shown to be effective, their effectiveness is limited under certain environmental conditions.
- The use of the avirulent genetically engineered *E. amylovora* is designed to more closely match growth and survival niches of virulent *E. amylovora* to better compete and control infection.

USDA/APHIS Environmental Assessment,2004

Management
Biological control of fire blight
Serratia plymithicum J7 culture supernatant

- *Serratia plymithicum* J7 culture supernatant displayed activity against many pathogenic strains of *Erwinia amylovora*, the causal agent of the most serious bacterial disease of apple and pear trees, fire blight, and against *Klebsiella pneumoniae*, *Serratia liquefaciens*, *Serratia marcescens*, and *Pseudomonas fluorescens*.
- Serracin P, a phage-tail-like bacteriocin produced by *Serratia plymithicum* J7, can be a good candidate for the development of a biopesticide against the fire blight pathogen *Erwinia amylovora*.

Jabrane *et al.*,2002

Management

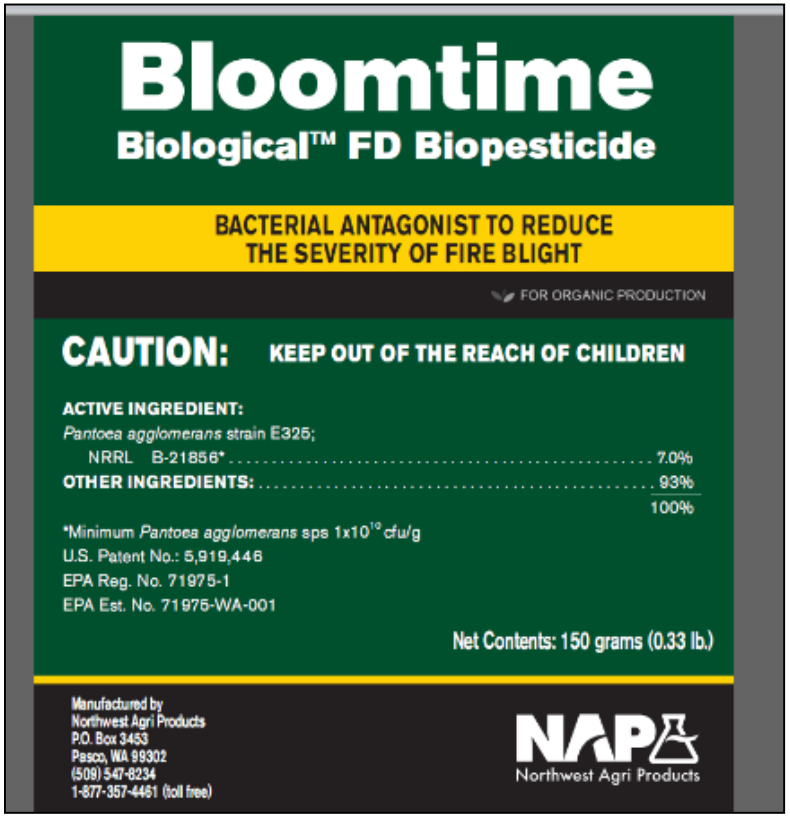
Biological control of fire blight

Blightban

- BlightBan is a *Pseudomonas fluorescens* bacterium that protects blossoms against infection through competitive inhibition of the fire blight pathogen.
- General properties:
- No systemic action (contact activity);
- Various mode of actions and low resistance risk (See bacterial disease management-Part3).
- The BlightBan organism has to arrive at the blossom first to be effective.
- Thus, the spray timings for BlightBan are 20 to 30 percent bloom and 70 to 80 percent bloom.
- BlightBan is compatible with and can be tank-mixed with streptomycin but should not be tank-mixed with Mycoshield (17% oxytetracycline) and only applied at least 48 hours after a Mycoshield application.

Biological control of fire blight
Erwinia amylovora

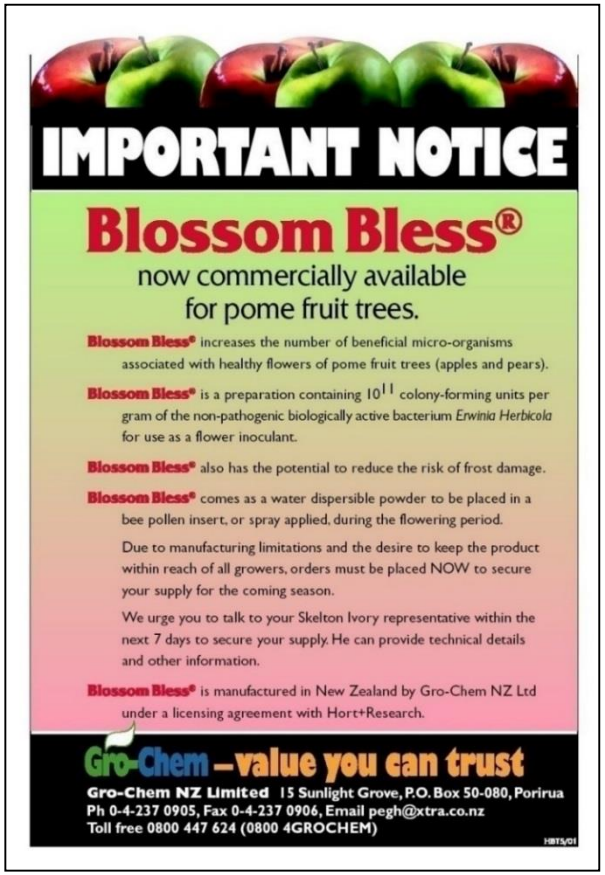
- Bloomtime Biological™ is a naturally occurring *Pantoea agglomerans* E325 that suppresses fireblight.
- For optimal use of this product, apply prior to colonization by the pathogen.



Chris Hale,2009; Northwest Agricultural Products, Inc.

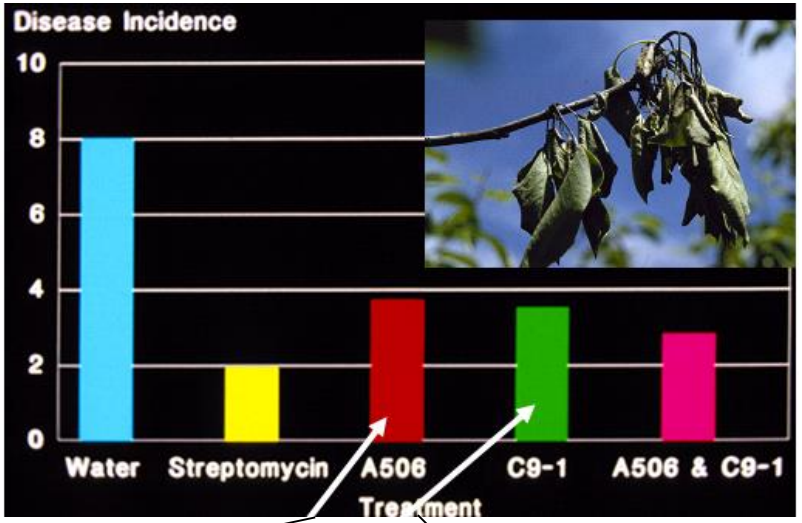
Biological control of fire blight
Erwinia amylovora

- Contains: A minimum of 3×10^{10} CFU/g *Pantoea Agglomerans* P10c in the form of a wettable powder.
- Blossom Bless acts as a protectant, pre-colonising susceptible flower parts and thus preventing the establishment of *E. amylovora*.



Chris Hale,2009

Effectiveness of saprophytic bacteria for fire blight suppression



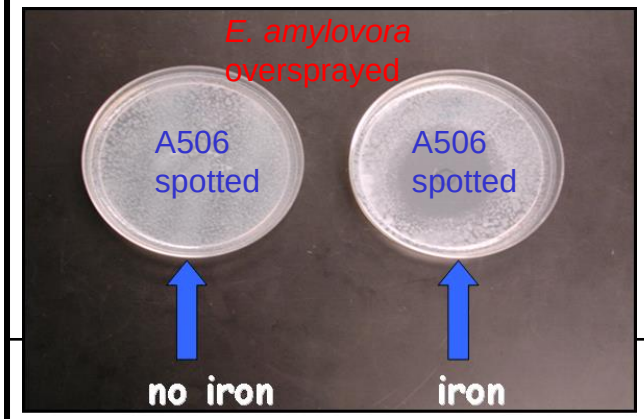
Pseudomonas fluorescens strain A506 (saprophytic epiphyte)

Pantoea agglomerans strain C9-1 (saprophytic epiphyte)

Sonk,2007

Mode of action

P. fluorescens A506 produces an antibiotic toxic to the fire blight pathogen on culture media amended with iron



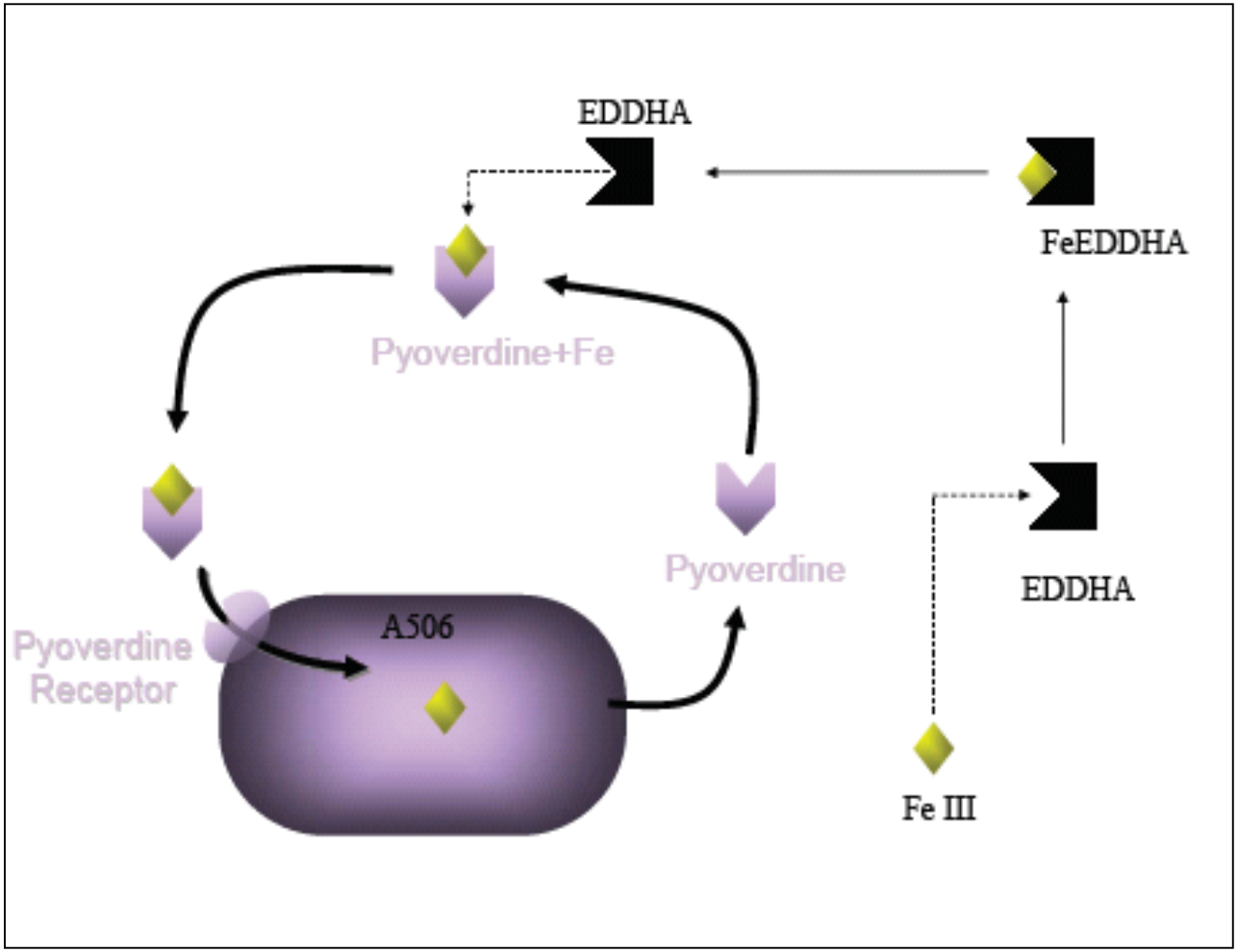
Commercial preparation of saprophytic bacterium that is sprayed onto pear and apple flowers.

Field trials with A506 and iron chelate FeEDDHa (Sequestrene 138)

Sonk,2007

Mode of action

Siderophore-mediated Iron Uptake by strain A506



Management
Other antagonists

Bacterium	Literature
Pantoea agglomerans (syn. *Erwinia herbicola*)	
Strains:	
Eh C9-1 (USA)	Ishimaru et al. (1988)
Eh 252 (USA)	Beer et al. (1984)
Eh 318 (USA)	Wright and Beer (1996)
Eh 1087 (New Zealand)	Kearns and Hale (1996)
Eh 325 (USA)	Pusey (1997)
PA 21889 (Germany)	Laux et al. (1999)
Mode of action: antibiosis (herbicolin, pantocin A and B)	
Pseudomonas fluorescens	
Strain A506: commercialized in 1996 as Blight Ban®A506 (USA)	First isolated in California by Lindow (1984)
Other strains isolated in:	
USA	Thomson et al. (1976)
Germany	Laux et al. (1999)
Turkey	Basim et al. (2002)
Mode of action: competition of sites and nutrients	
Bacillus subtilis	
Strain BsBD 170 commercialized in 2001 as BioPro®(Germany)	Zeller and Wolf (1996)
Strain QST713 commercialized as Serenade by AgraQuest Inc. (USA)	Aldwinckle et al. (2002)
Strain BSF3 isolated in Italy	Bazzi (personal communication 2000)
Other bacteria	
Strain Ra 39 *Rhanella aquatilis*	Laux et al. (1999)

Zeller,2006

Management
Other antagonists

- **Bacteria:**
- *Pseudomonas* spp.
- *Pantoea* spp.
- *Bacillus subtilis*
- **Yeast and yeast like species:**
- *Aureobasidium pullulans*,
- *Candida sake*, and
- *Metschnikowia pullcherrima*.
- Bacteria and yeasts were adjusted to 0.1 optical density at 600 nm using a spectrophotometer, resulting in bacteria at ≈10^8 CFU/ml and ≈3X10^8 CFU/ml.

Pusey *et al.*,2009

Management
Other antagonists

- The use of a new antibiotic produced by symbiotic bacteria of the entomopathogenic nematodes *Xenorhabdus budapestiensis* Lengyel *et al.*, and *X. szentirmaii* Lengyel *et al.*, against the plant pathogens *Erwinia amylovora* (Böszörményi *et al.*,2009).

Nagy *et al.*,2012

Biological control of fire blight

Genetically engineered *E. amylovora* (HrpS- or HrpL-)

- The bacteria have been genetically engineered using the neomycin phosphotransferase (*nptII*) gene from transposon 10 (Tn10) from *Escherichia coli* strain DH5α using molecular biology techniques as detailed in the permit application.
- Insertion of this transposon into the specific *hrp* (**h**ypersensitive **r**eaction on non-host plants and **p**athogenesis on host plants) gene (HrpS- or HrpL-) results in inactivation of the gene and disruption of the disease-causing mechanism within the bacterium, thereby rendering the bacterium avirulent/non-pathogenic.
- The *nptII* gene, which confers resistance to the antibiotic kanamycin (neomycin), has been safely used in many genetically engineered organisms.

USDA/APHIS Environmental Assessment,2004

Biological control of fire blight
Erwinia tasmaniensis and *E. billingiae*

- The genus *Erwinia* comprises phytopathogenic and non-pathogenic species.
- The species *Erwinia tasmaniensis* and *E. billingiae* were isolated from the apple and pear flora.
- *E. tasmaniensis* has a plasmid encoding a bacteriocine.
- A population of *E. tasmaniensis* is apparently more stable in apple flowers of orchards than cells of *E. billingiae* surpassing this species for control of fire blight.
- **The drawbacks with these two antagonists:**
- *E. tasmaniensis* strains are able to cause a hypersensitive response in tobacco leaves in contrast to *E. billingiae*.
- *Erwinia billingiae* often in association with plant pathogens, and are considered as secondary/primary invaders. It causes bacterial canker of mango.

Kube *et al.*,2008; Bergey's Manual of Systematic Bacteriology,2005

Biological control of fire blight
Erwinia tasmaniensis and *E. billingiae*

- It can be assumed that strains of the species *E. billingiae* and *E. tasmaniensis* occur in many places worldwide and may also interfere with colonization of flowers of fire blight host plants by *E. amylovora*.
- Several isolates were screened for their ability to suppress growth of *E. amylovora* on plant tissue such as apple flowers or pear slices.

Geider *et al.*,2008

Biological control of fire blight

Plant extracts

Plant extracts tested against *E. amylovora* *in vitro*/ or *in vivo*.

Vanneste,2000

Plant species	Activity		References
	In vitro	*In vivo*	
Ailianthus altissima (tree of heaven)	+	NT	1
Alchemilla vulgaris	+	+*	3
Allium sativum (garlic)	+	(+)	1
Allium sativum (garlic)	+	NT	2
Berberis lampergiana (barberry)	+	NT	1
Berberis vulgaris (barberry)	+	+	1
Castanea sativa (European chestnut)	+	NT	1
Citrus bergamia (bergamot)	–	NT	2
Cupressus sempervirens (cypress)	–	NT	2
Fallopia convolvulus (climbing buckwheat)	+	NT	1
Hedera helix (ivy)	+	+*	3, 4
Juglans nigra (black walnut)	+	+	1
Juniperus communis (common juniper)	–	NT	2, 5
Mahonia aquifolium (Oregon grape)	+	+	1
Matricaria chamomilla (chamomile)	(+)	NT	2
Mentha × piperita (peppermint)	–	NT	2
Ocimum basilicum (basil)	–	NT	2, 5
Origanum vulgare (origanum)	+	NT	2
Pelargonium odoratissimum	+	NT	1
Pimpinella saxifraga (common burnet saxifrage)	+	NT	1
Pinus sylvestris (Scots pine)	–	NT	2, 5
Polygonum capitatum (polygonum)	+	NT	1
Populus tremula (trembling poplar)	+	NT	1
Potentilla anserina (potentilla)	+	NT	1
Quercus petrea (sessile oak)	+	NT	1
Quercus robur (English oak)	+	NT	1
Reynoutria sachalinensis	+	+*	3
Rheum rabowbarum	+	NT	1
Rhus typhina	+	+	1
Rosa canina (dog rose)	–	NT	2, 5
Rosmarinus officinalis (rosemary)	–	NT	2, 5
Ruta graveolens (garden rue)	+	NT	1
Sabucus nigra (elder)	+	NT	1
Salvia officinalis (sage)	+	NT	1
Salvia sclarea (sclarea sage)	–	NT	1, 2
Satureja hortensis (savory)	+	NT	2
Sedum groenlandiceum	+	NT	1
Senecio spiculosus	+	NT	1
Thymus vulgaris (white thyme)	(+[b])	NT	1, 2
Tilia tomentosa (silver linden)	(+)	NT	2
Viscum album	+	+*	3, 4

*, Induced resistant response; +, Good antibacterial activity; (+), moderate antibacterial activity; (+[b]), slight antibacterial activity (bacteriostatic); –, no antibacterial activity; NT, not tested.

References: 1. Mosch *et al.*, 1989; 2. Scortichini and Rossi, 1991; 3. Mosch *et al.*, 1993; 4. Mosch *et al.*, 1996; 5. Scortichini and Rossi, 1989.

Activity of plant extracts and etheric oils against *E. amylovora*

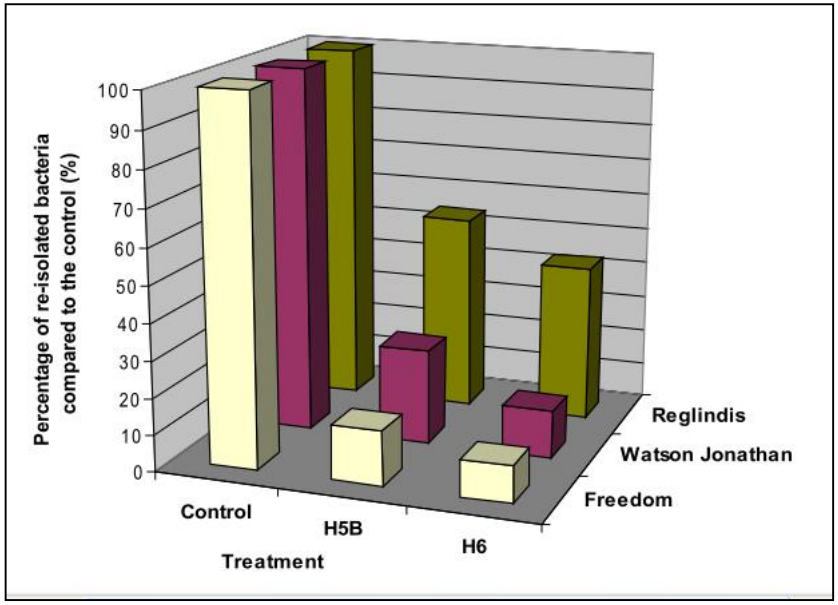
Mode of action	Plant
Protective	*Mahonia aquifolium* *Rhus typhina* *Berberis vulgaris* *Pingiana pinata*
Induction of resistance	*Hedera helix* *Salix alba* *Viscum album* *Alchemilla vulgaris* *Reynoutria*
Antimicrobial	*Origanum vulgare* *Matricaria* *Allium sativum* *Thymus vulgaris*
Plant strengthener	*Tymbra spicata* (BioZell-2000B)

Zeller,2006

Field Phage therapy

Erwinia amylovora

- Effect of phages on *Erwinia amylovora* infection on flowers of different apple cultivars.
- Hungarian phage isolates (H5B, H6) applied in spray inoculation on flowers (10^{10} PFU/ml), significantly reduced the number of re-isolated bacteria on all three apple cultivars tested, compared to the untreated control.
- However, a significant difference was not detectable between the effects of the two phages (data of significant differences not shown).



See also phage section in the file "bacterial diagnostic-Part-1".

Nagy *et al.*,2012

Summarizes the suppressive effects of several biocontrol agents on *E. amylovora* infection

Examples of control of *Erwinia amylovora* in blossoms by biocontrol agents and streptomycin

Biological control agent	Mean % average of disease reduction	
	biological control agent	streptomycin
Pantoea vagans C9-R1[a]	17.0-78.0	65-89
Pantoea agglomerans Eh252	55	75
BlightBan®A506	12,5	61.0
BlightBan®C9-1	33.1	63.3
Bloomtime Biological™ FD Biopesticide	28.5	67.3
Pantoea agglomerans HIP32	46[f]	68
ΦEa1, ΦEa116B, ΦEa116C in mixture	37.0[b] 36.1[c]	NT
Pantoea agglomerans EH 21-5+ ΦEa21-4 Pantoea agglomerans EH 21-5+ ΦEa46-1	50[d]	63[d]
ERWIPHAGE Patent number P0700600	71-75[e]	NT
H5B	85[f]	NT
H6	90[f]	NT

NT not tested, [a] Formerly *Pantoea agglomerans*, [b] Inoculation of *E. amylovora* and phage mixture was carried out on the same day, [c] *E. amylovora* inoculated one day before the application of phage mixture, [d] approximate data, exact data was not available, [e] results were evaluated three and five weeks after treatments, [f] tested only during *in vitro* conditions. Values with different letter indications denote a statistically significant difference.

Nagy *et al.*,2012

Bacteriophage isolation, purification, and titration

Erwinia amylovora

- Bacteriophage Isolation, Purification, and Titration:
- Pond water and wastewater samples were collected in the Apulia region, Italy. The collected samples were filtered through a filter paper of Grade 1, Dia. 75x 100 mm (Whatman, Maidstone, UK) to remove large particles.
- Cellular debris was removed by filtration through 0.22 µm sized pores of a nylon Acrodisc syringe filter.
- The filtrate was centrifuged at 30,000 x *g* for 1 h at 4°C to pellet phage particles. Pellets were resuspended in 2 mL phage buffer (100 mM Tris-HCl(pH 7.6); 10 mM $MgCl_2$; 100 mM NaCl; and 10 mM $MgSO_4$) and mixed with 1 mL of an overnight culture of *E. amylovora* strain PGL Z1 (~10^8CFU/mL) in 20 mL YPG medium.

Sabri *et al.*,2022

Bacteriophage isolation, purification, and titration

Erwinia amylovora

- Bacteriophage Isolation, Purification, and Titration:
- After overnight incubation at 25°C, the mixture was centrifuged at 7000x *g* for 10 min, and the supernatant was filtered through a 0.22 m syringe filter. For initial detection of phages capable of forming plaques on *E. amylovora* in filtrates, spot tests were performed on top of a bacterial layer consisting of 200 L of an overnight culture of *E. amylovora* strain PGL Z1 (~10^8 CFU/mL), mixed with 6 mL of YPG soft agar (i.e., YPG supplemented with 0.7% agar), poured on top of YPGA plates.
- After solidification, 10µL drops of the phage filtrate were spotted on the surface of the soft-agar layer. The drops dried at room temperature, and then the plates were incubated overnight at 25°C. Plates were screened visually for lysis zones, and phages capable of infecting *E. amylovora* were purified from filtrates using the standard double agar overlay method(Kropinski *et al.*,2009).

Sabri *et al.*,2022

Bacteriophage isolation, purification, and titration

Erwinia amylovora

- Bacteriophage Isolation, Purification, and Titration:
- Single clear plaque-forming units were transferred into 1 mL of phage buffer. This process was repeated three times to ensure the isolation of a single phage. In order to obtain high phage titers, isolated phage was amplified on the *E. amylovora* strain PGL Z1 for 24 h, poured through 0.2 µm filters, and precipitated using polyethylene glycol (PEG) 8000.
- Briefly, 200 mL of phage was treated with 15% (w/v) PEG 8000, gently mixed, and incubated on ice for 3 h. Bacteriophages were centrifuged at 13,000 g for 45 min at 4°C, pelleted, resuspended in 5 mL of phage buffer and stored at 4°C. The phage titer was determined through a double-layer assay.

Sabri *et al.*,2022

Bacterial strains used for determining the host range of the bacteriophage IT22 *Erwinia amylovora*

- EP-IT22 specific phage for *E. amylovora*. It was found to be resistant to high temperatures (up to 60°C) and pH values between 4 and 11, and was able to accomplish a complete lytic cycle within one hour.

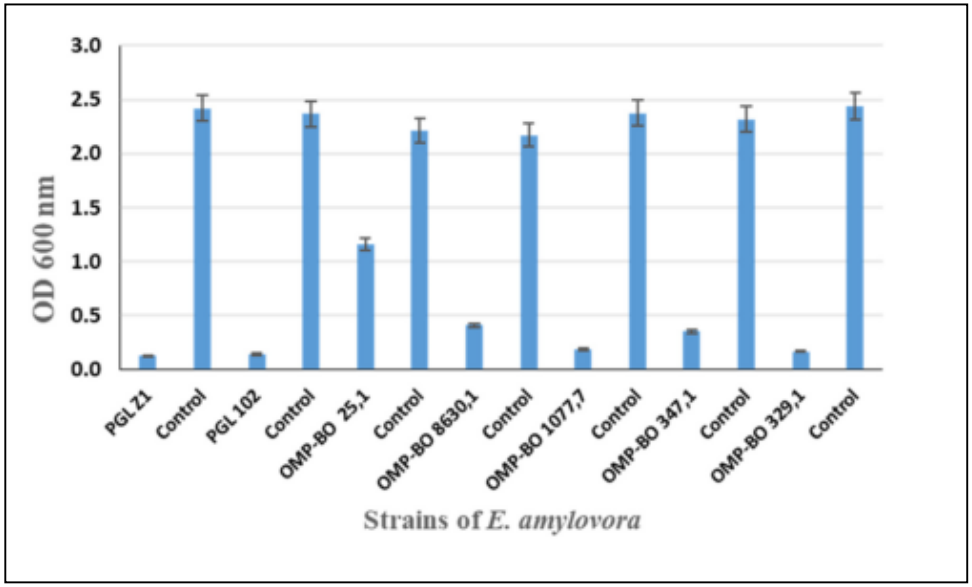
Species	Strains	Hosts	Origins	Isolation	Lytic Activity of IT22
Erwinia amylovora	PGL Z1 *	*Pyrus communis*	Apulia/Italy	2013	++
Erwinia amylovora	PGL 102 *	*Pyrus communis*	Apulia/Italy	2013	++
Erwinia amylovora	OMP-BO 25.1	*Pyrus communis*	Emilia-Romagna/Italy	2000	+
Erwinia amylovora	OMP-BO 8630.1	*Pyrus communis*	Emilia-Romagna/Italy	2010	++
Erwinia amylovora	OMP-BO 1077.7	*Pyrus communis*	Emilia-Romagna/Italy	1994	++
Erwinia amylovora	OMP-BO 347.1	*Crataegus monogyna*	Emilia-Romagna/Italy	2006	++
Erwinia amylovora	OMP-BO 329.1	*Malus domestica*	Emilia-Romagna/Italy	2002	++
Xanthomonas campestris pv. *campestris*	CFBP 1710	*Brassica oleracea* var. *botrytis*	France	1975	–
Pseudomonas syringae pv. *syringae*	CFBP 311	*Pyrus communis*	Indre et Loire-France	1962	–
Dickeya chrysanthemi	CFBP 1346	*Chrysanthemum maximum*	Italy	1969	–

Sabri *et al.*,2022

Bacterial strains used for determining the host range of the bacteriophage IT22 *Erwinia amylovora*

- Host range analysis showing the bacteriolytic effect of phage EP-IT22 against *E. amylovora* strains after 20h of incubation at 25°C. *E. amylovora* strains were infected by phage EPIT22 a MOI of 1.
- *E. amylovora* cultures without phage were used as the controls.
- Error bars represent standard deviations of three replications.

What does a MOI of 1 mean?
The multiplicity of infection or MOI represents the ratio of the numbers of virus particles to the numbers of the host cells in a given infection medium.
0.1 multiplicity of infection (MOI), (i.e., the phage concentration at the beginning of the incubation was 10^7 PFU/mL).



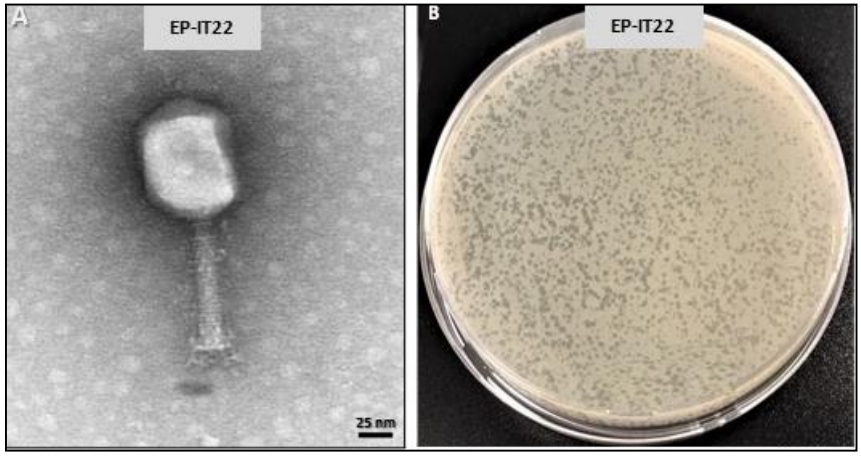
Sabri *et al.*,2022

Bacteriophage isolation, purification, and titration

Erwinia amylovora

A. Transmission electron microscopy (TEM) of EP-IT22 showing a particle with an icosahedral head and a long contractile tail, scale bar= 25 nm.

B. Double-layer assay showing EP-IT22 plaques forming units of ca. 2 mm in diameter.



Sabri *et al.*,2022

In planta biocontrol assay
E. amylovora

- The untreated pear plants infected with *E. amylovora* PGL Z1 showed typical symptoms of fire blight 40 days post inoculation(dpi), as the stems developed necroses and became blackened as if charred; leaves located above the inoculation sites showed wilting, scorching, and dieback symptoms (Figure 8A).
- On the other hand, *E. amylovora*-infected pear plants treated with phage EP-IT22 developed no fire blight symptoms, similarly to those treated with streptomycin sulphate (Figure 8B,C).
- This shows that EP-IT22 is an extremely promising antibacterial agent for the development of an ecofriendly and effective treatment for fire blight disease.

Sabri *et al.*,2022

In planta biocontrol assay
E. amylovora

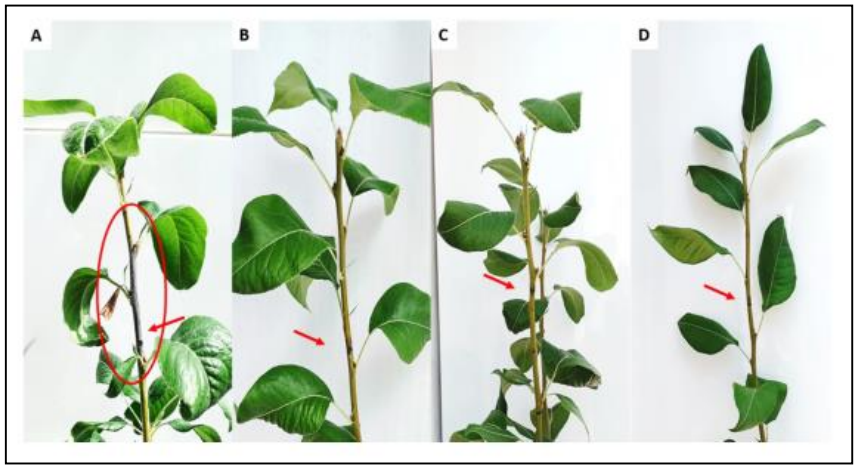
- In planta assay showing the antibacterial effect of EP-IT22 on *E. amylovora* PGL Z1 infection.

A. *E. amylovora*-infected pear plant.

B. *E. amylovora*-infected plant and treated with EP-IT22.

C. *E. amylovora*-infected plant and treated with streptomycin sulphate.

D. A healthy pear plant injected with sterile water.



Arrows indicate inoculation sites. The circle indicates the stem necrosis and leaf scorch symptoms.

Sabri *et al.*,2022

Field Phage therapy

Different plant pathogenic bacteria

AgriPhage™

- AgriPhage utilizes bacteriophages, known as "bacteria eaters" which are naturally occurring organisms that infect and kill only targeted bacteria.
- AgriPhage™ is the first agricultural bactericide based on phage technology, and is currently registered for use on:

1. Tomatoes (*Clavibacter michiganensis* and *Pseudomonas syringae* pv. *tomato*),
2. Peppers (*Xanthomonas campestris* pv. *vesicatoria*),
3. Apples and pears (*Erwinia amylovora*), and
4. Citrus (*Xanthomonas citri*).

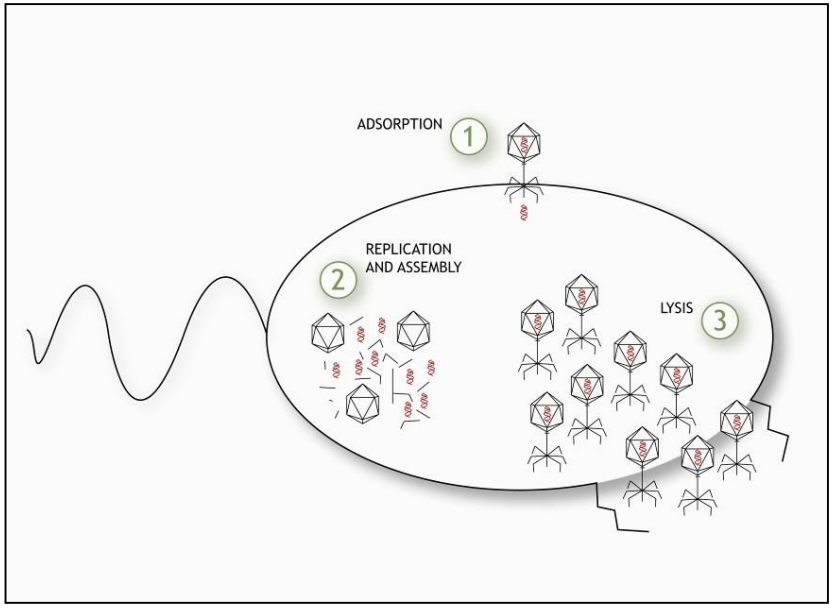
AgriPhage,2022

Field Phage therapy

Different plant pathogenic bacteria

AgriPhage™

- **HOW IT WORKS**
- Bacteriophage destroy bacteria in a process called "lysis".
- Lysing begins the moment a phage comes in contact with a bacterium and results in a release of additional phage within 30 minutes.



AgriPhage,2022

Field Phage therapy
Different plant pathogenic bacteria
AgriPhage™

- **HOW IT WORKS**
- Lysing can be described in three basic phases:

1. Adsorption occurs when a phage encounters a bacterium, attaches its tail fibers, and injects its own DNA into the bacteria. This action can begin as soon as you apply AgriPhage-CMM.
2. New phage can begin replicating and assembling within the bacterial cell, multiplying at a steady rate.
3. Finally, lysis is complete when the cell bursts and releases about 100 new phage into the environment to carry on the process. In this way, AgriPhage-CMM actually increases in efficacy over time.

Field Phage therapy

AgriPhage™

Erwinia amylovora

- OmniLytics, Inc., Certis received EPA approval of AgriPhage-Fire Blight in late 2018, and is today the world's leading provider of agricultural bacteriophage products.
- Certis is proud to make this product available to growers in support of their efforts for successful disease control in this growing season.
- AgriPhage-Fire Blight is a strong fit within an integrated pest management program applied alone or in combination with approved tank-mix partners for maximum fire blight control.

AgriPhage,2022

Field Phage therapy

AgriPhage™

Erwinia amylovora

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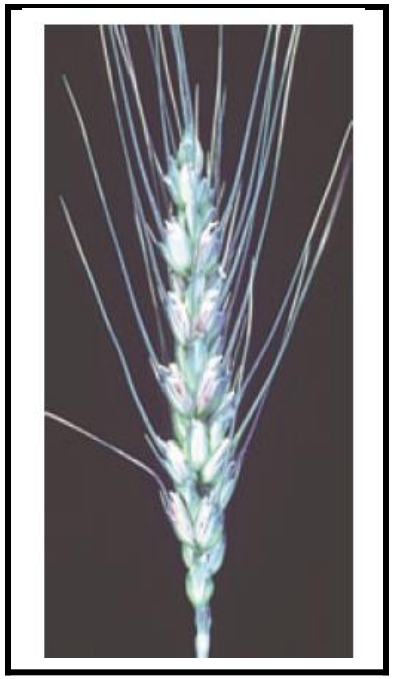
AgriPhage,2022

Erwinia rhapontici

Bacterial pink seed of wheat



Management

- **Disease description and Management:**
- **Bacterial pink seed of wheat caused by *Erwinia rhapontici***
- Symptoms: Kernels appear pink but maintain plumpness and vitreousness.
- More easily seen in durum.
- Survival and Spread: Survives on crop residue.
- Generally invades kernels harvested prematurely or grain in the swath.
- Occurs infrequently.
- No control measures prescribed.

Erwinia tracheiphila

Bacterial wilt of cucurbits

(melon and cucumber)

Disease cycle *Erwinia tracheiphila*

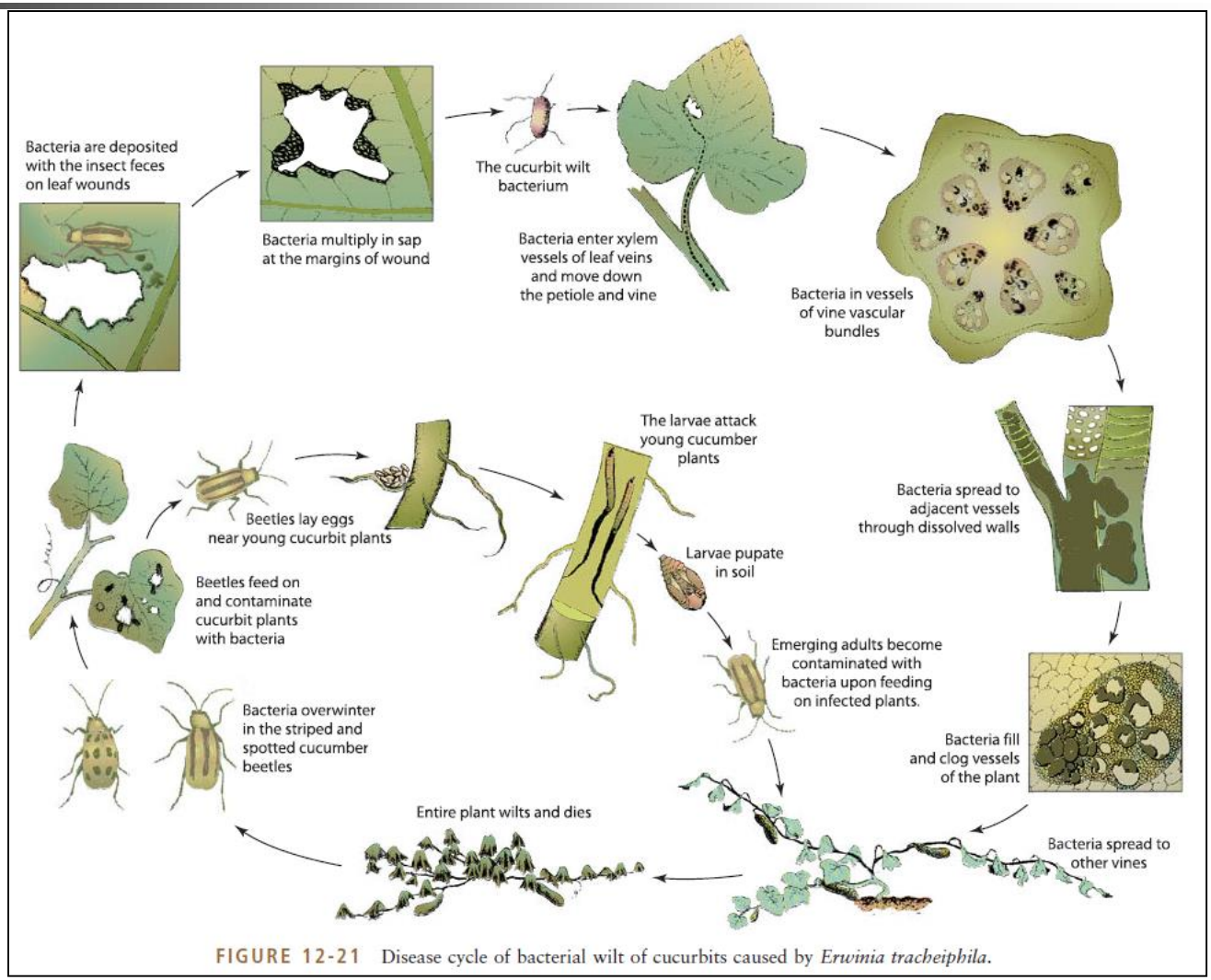


FIGURE 12-21 Disease cycle of bacterial wilt of cucurbits caused by *Erwinia tracheiphila*.

Agrios,2005

Etiology

- The bacteria causing this disease cannot survive in dry, infected plant material for more than a few weeks.
- Instead, the bacteria survive the winter in the gut of adult striped or spotted cucumber beetles.
- When these beetles feed on young cucurbits in the spring, they create small holes in the leaves.
- The bacteria may be deposited on the leaves through beetle feces, and may enter a susceptible host through the beetle feeding wounds.
- Once inside a host plant, the bacteria reproduce and produce gums that block the vascular system.
- Cucumber beetles may also carry the bacteria on their mouthparts and spread the bacteria by feeding on infected, then non-infected plants.

Disease symptoms and vectors

Erwinia tracheiphila

SlideServe,2020

Beetle vectors

	
Striped cucumber beetle	Spotted cucumber beetle

Management
Erwinia tracheiphila

- Once a plant is infected with bacterial wilt, no control of the pathogen is possible.
- Infected plants should be removed and destroyed without injuring nearby plants.
- The best control for this disease is achieved by managing the beetle vector of the pathogen especially the early cucumber beetles.
- Use resistant varieties when available. Watermelon in general is not as susceptible to bacterial wilt as other cucurbits.
- The cucumber varieties County Fair, H 19 Little Leaf, and Saladin tolerate bacterial wilt.
- To avoid squash rot in storage, only fruit from healthy plants should be picked and stored in a clean, fumigated warehouse.

Other necrogenic bacteria

Brenneria rubrifaciens

Deep(shallow) Bark Canker of Walnut

Distribution map

Brenneria rubrifaciens

Management
Brenneria rubrifaciens

- The vast majority of samples were negative for these pathogens.
- Most of the bacteria isolated were most likely random and incidental "opportunistic colonists" utilizing the sap as a food source.
- The cankers aren't evident in young trees, but *B. rubrifaciens* is thought to be present long before there is visual evidence.
- To help control the disease in both nursery and orchard settings, scientists have developed a genetic detection method.
- Understanding the role of QS in *B. rubrifaciens* is providing insights into the long latency period of this disease which has been poorly understood for decades resulting in ineffective disease control strategies.

Field Notes, California University,2015;USDA,2012

Pantoea citrea

Example of an immature pineapple fruit bearing blossoms that are commonly visited by flying insects.

Pink Disease of Pineapple

Disease Cycle

- The disease cycle of pink disease remains unresolved.
- *Pantoea citrea* appears to gain access into the fruit via the numerous florets that grow out from the growing fruit.
- Because there is a relatively consistent correlation between spraying of insecticides during the blooming season and the concomitant reduction of pink disease, insects are thought to play a role in disseminating the pathogen from flower to flower.
- Once entry into the fruit occurs, the pathogen colonizes the intercellular portions of the fruit tissue.
- With time, infected tissues will show a moderate translucent appearance (water soaking) but no soft-rotting symptoms.
- The infected tissues become colored upon canning.

Disease Cycle

- *Pantoea citrea* presumably multiplies in the flowers and becomes the source of inoculum vectored by the insects.
- Because pineapple plants are propagated on a year-round basis, transmission of *P. citrea* from field-to-field sustains pink disease propagation.

Management

- **Chemical control:**
- Current methods of controlling the disease are relatively expensive since multiple applications of insecticides are necessary to maintain low levels of pink disease incidence.
- Insecticide is applied multiple times during the flowering season.

Application of insecticide by means a long arm boom sprayer extending both sides of supplier truck.

Kado,2003

Management

Pantoea citrea

Bacillus gordniae 2061R

- **Biological control:**
- Two applications, two weeks apart, were conducted.
- Each fruit was harvested near maturity and the core was tested for pink disease by heating.
- However, the application of a biocontrol agent (e.g., at the rate of 1 kg of biocontrol inoculum [wet packed weight] per hectare requires 50 liters of culture medium) to such a vast area is perceived as economically unfeasible.

Manual spray application of *Bacillus gordniae* 2061R inoculum covering five 25 acre-replicates during optimum flowering period.

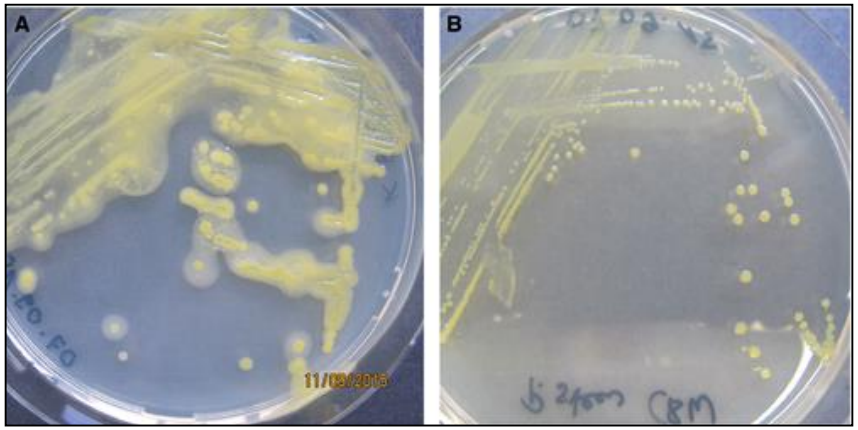
David Stead

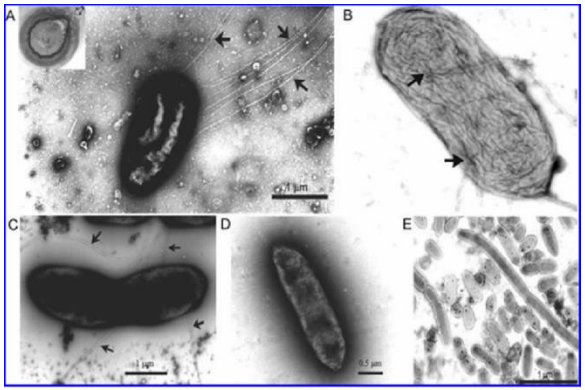
Pantoea stewartii

Stewart's corn wilt

Bacterial wilt (Stewart's disease) of corn
Colony and cells of *Pantoea stewartii*

1. Colonies on nutrient-glucose agar are cream-yellow, lemon-yellow or orange-yellow and flat, raised or convex, respectively.
2. *Pantoea stewartii* subsp. *stewartii* strain on King's B (A) and NBY (B) medium (typical colony morphology).





Herrera, 2008;

Bacterial wilt (Stewart's disease) of corn
Pantoea (*Erwinia*) *stewartii*

	
Early Season Systemic Wilt	Main stalks can be killed if plants are infected by *P. stewartii* at the time of emergence. When main stalks are killed, tillers often begin to grow profusely.

Pataky,2004;DuPont,2012

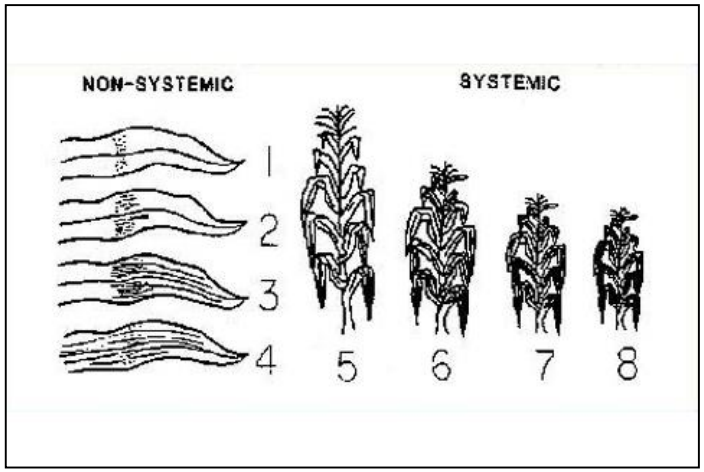
Bacterial wilt (Stewart's disease) of corn
Pantoea (*Erwinia*) *stewartii*

Cavities may form near the soil line in the stalks of plants systemically infected by *P. stewartii*.

Pataky,2004;DuPont,2012

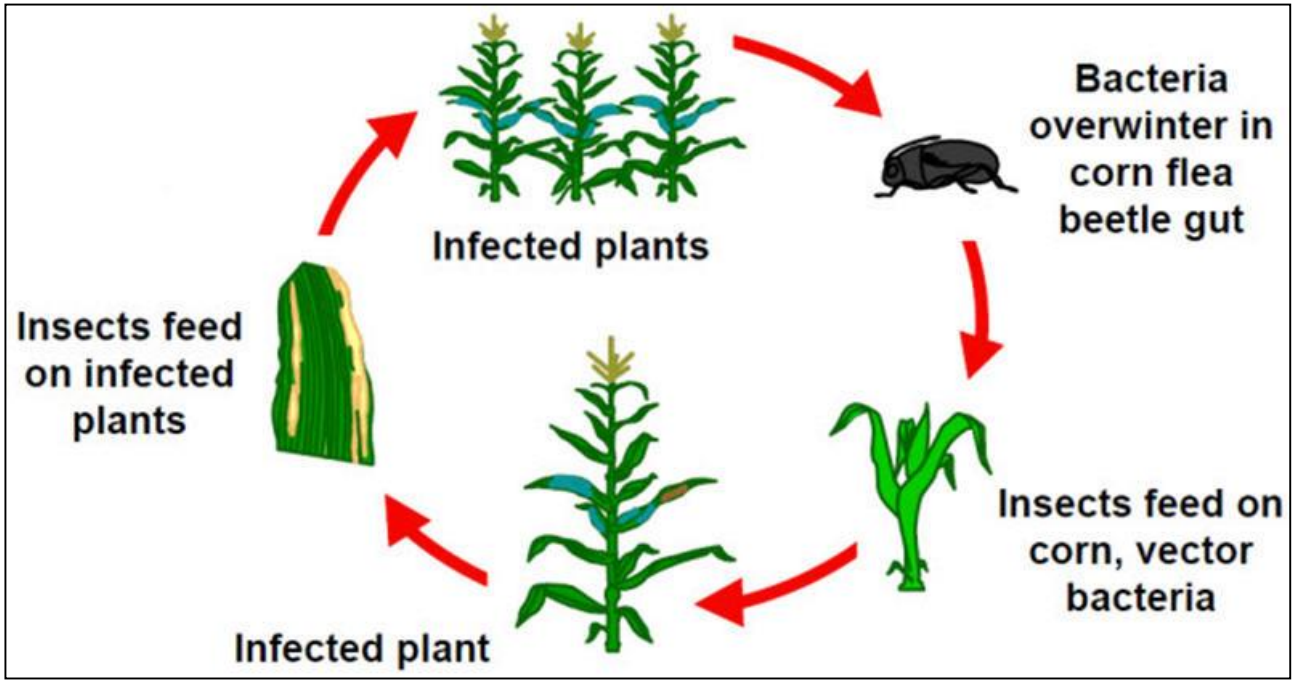
Host reaction to Stewart's wilt

- Reactions of sweet corn hybrids to range from resistant to highly susceptible.



Pataky,2004

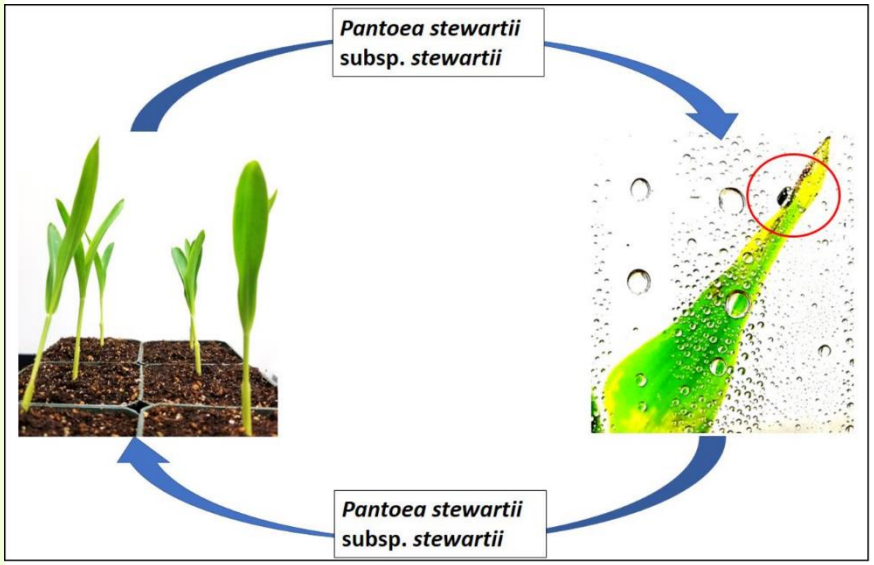
Disease cycle stewart's wilt



DuPont,2012

Bacterial wilt (Stewart's disease) of corn *Pantoea stewartii* subsp. *stewartii*

- This phytopathogen uses quorum sensing (QS) to coordinate cell density-dependent gene expression and successfully colonize corn leading to wilt disease.



Duong,2018

Bacterial wilt (Stewart's disease) of corn *Pantoea stewartii* subsp. *stewartii*

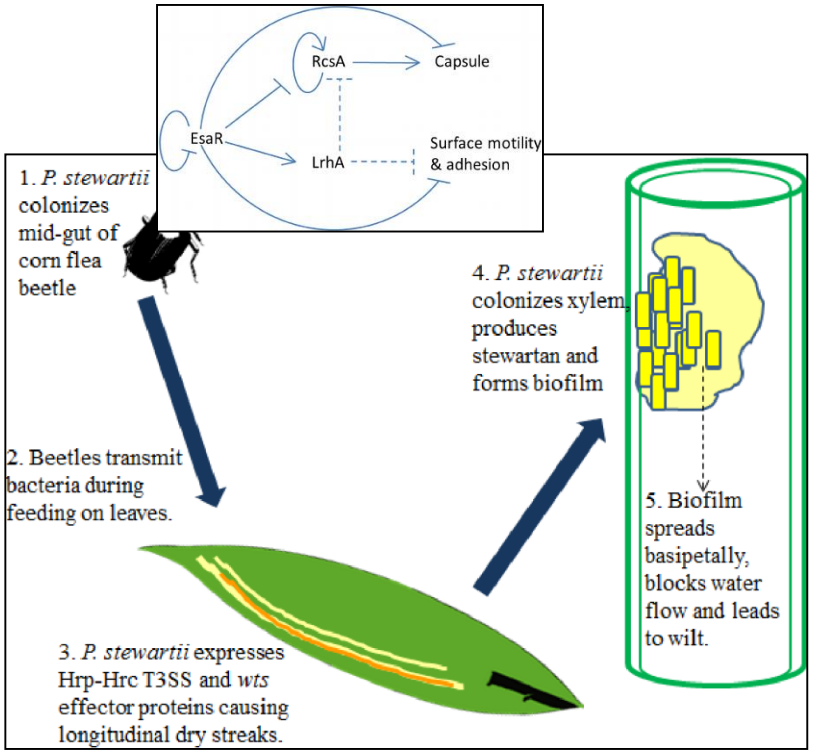
- A complete assembly of the *P. stewartii* genome revealed the existence of a previous unknown 66-kb region in the *P. stewartii* genome believed to contain genes important for motility and virulence.
- In addition, completion of the genome sequence permitted genes for two distinctive Type III secretion systems, used for interactions with corn or the corn flea beetle, to be placed on two mega-plasmids.

Duong,2018

Bacterial wilt (Stewart's disease) of corn *Pantoea stewartii* subsp. *stewartii*

- Regulation is achieved through a quorum-sensing (QS) system consisting of the acyl-homoserine lactone (AHL) synthase, EsaI, and the transcription regulator EsaR.
- The QS master regulator EsaR was shown to regulate two major virulence factors of *P. stewartii*:

1. capsule production, and
2. surface motility.



Ramachandran,2014; Burke,2015; Duong,2018

Stewart's wilt disease

Pantoea stewartii

- Stewart's disease, also known as Stewart's wilt, is caused by the bacterium *Pantoea stewartii*.
- An insect vector, the corn flea beetle plays a critical role in the overwintering survival and plant-to-plant spread of this microorganism.
- The bacterium survives the winter months within the gut of hibernating corn flea beetles.

Pantoea stewartii subspecies *stewartii* (*P. stewartii*), an endosymbiont in the corn flea beetle gut that causes Stewart's wilt disease in corn.

Nutter *et al.*,2011; Duong,2018

The corn flea beetle

Vector

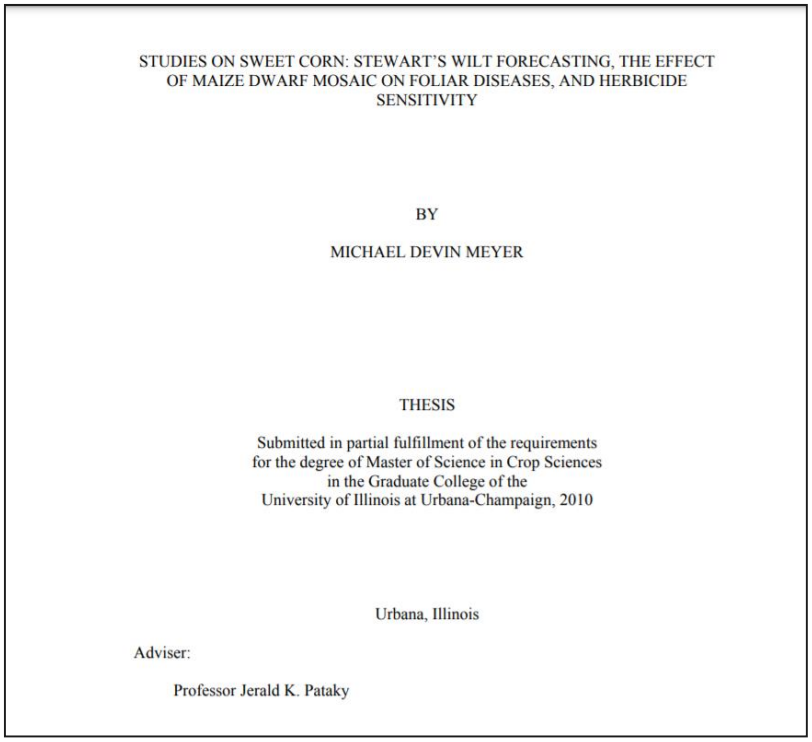
Corn flea beetle on a corn seedling leaf

- The corn flea beetle (*Chatocnema pulicaria*), vector of the Stewart's wilt bacterium (*Pantoea stewartii*) feeds on corn leaves, scraping off the green tissue between veins and causing narrow white strips.

Jackson,2012

Epidemiology and disease management of Stewart's disease

Theses



STUDIES ON SWEET CORN: STEWART'S WILT FORECASTING, THE EFFECT OF MAIZE DWARF MOSAIC ON FOLIAR DISEASES, AND HERBICIDE SENSITIVITY

BY

MICHAEL DEVIN MEYER

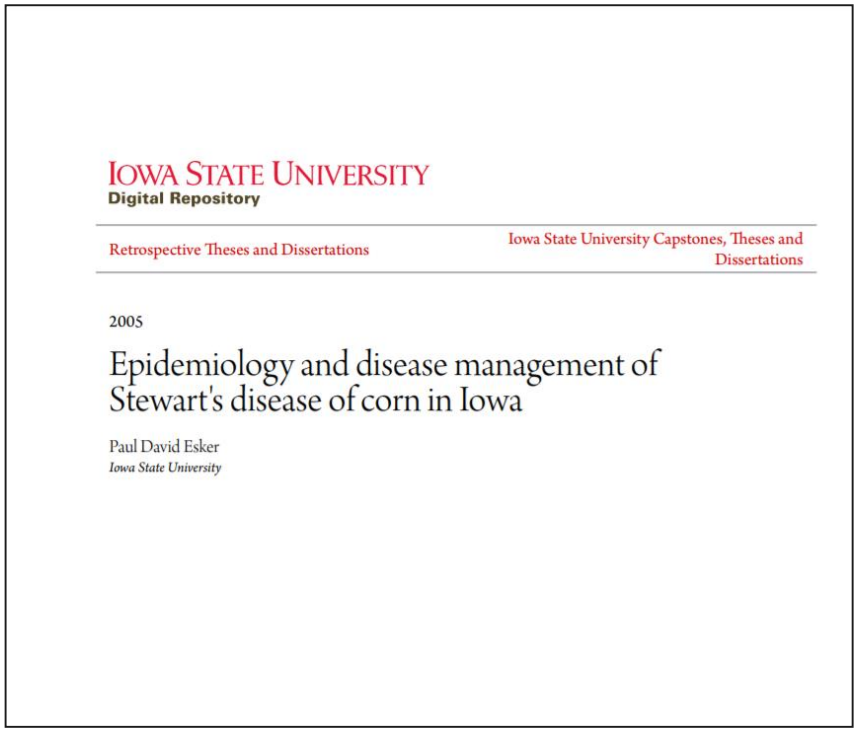
THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Crop Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

Adviser:

Professor Jerald K. Pataky



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Iowa State University Capstones, Theses and Dissertations

2005

Epidemiology and disease management of Stewart's disease of corn in Iowa

Paul David Esker
Iowa State University

Forecasting model for predicting risk of Stewart's wilt disease

- Mild winters during the late 1900s and early 2000s resulted in the occurrence of severe epidemics of Stewart's disease in Iowa.
- However, severe winters and the widespread adoption of planting insecticide-treated seed have greatly reduced corn flea beetle populations throughout Iowa in recent years.

Nutter *et al.*,2011

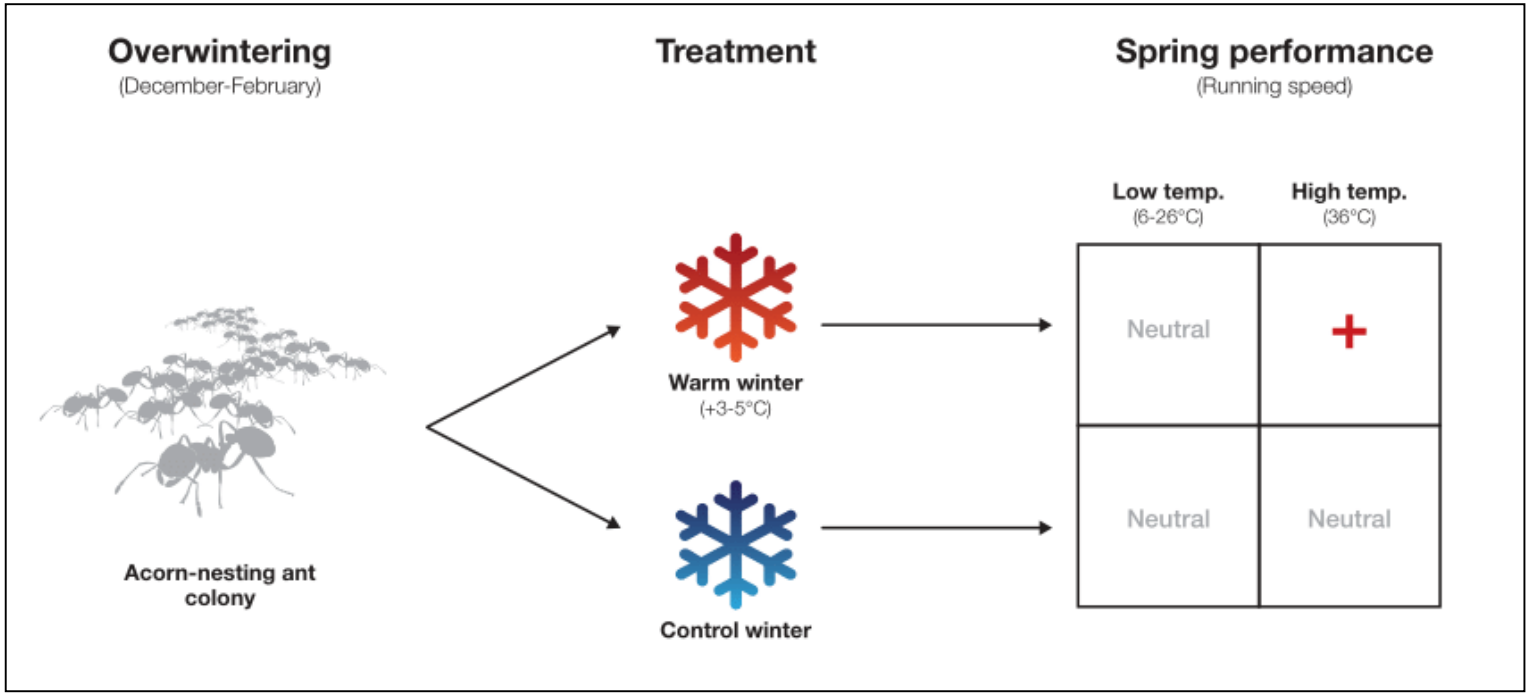
Forecasting model for predicting risk of Stewart's wilt disease

1. If winter temperatures are mild enough for corn flea beetle populations to survive locally, the bacterium will also survive.
2. In the spring, surviving corn flea beetles infested with *P. stewartii* will emerge from grassy areas near corn fields and, as they feed, transmit the pathogen to corn seedlings.

Nutter *et al.*,2011

Forecasting model for predicting risk of Stewart's wilt disease

- A cross-seasonal perspective on local adaptation: Metabolic plasticity mediates responses to winter in a thermal-generalist moth.



acorn-nesting ant 

Williams *et al.*,2005

Forecasting model for predicting risk of Stewart's wilt disease

- Two disease prediction models are available to predict the seasonal and county-level risk of Stewart's disease:

1. the Stevens-Boewe Index Model, and
2. the Iowa State Mean Monthly Temperature Model.

- Both models use the monthly mean winter temperatures for December, January and February to predict the degree to which corn flea beetle populations survived the winter.

Esker *et al.*,2006

Forecasting model for predicting risk of Stewart's wilt disease

- Three forecasting models for Stewart's disease (*Pantoea stewartii* subsp. *stewartii*) of corn (*Zea mays*) were examined for their ability to accurately predict the prevalence of Stewart's disease in Iowa at the county level.

1. The Stevens Model, which is used as a predictor of the early wilt phase of Stewart's disease;
2. The Stevens-Boewe Model, which predicts the late leaf blight phase of Stewart's disease, and
3. The Iowa State Model that is used to predict the prevalence of Stewart's disease.

Esker *et al.*,2006

Forecasting model for predicting risk of Stewart's wilt disease

- High temperatures aggravate disease severity.

1. In general, the disease is not likely to be severe if the sum of the average temperatures for December, January and February is less than 20-24°C.
2. If the sum of the average temperatures for these three months is 32-38°C, the disease is likely to be severe because of increased survival of the vectors.

Forecasting model for predicting risk of Stewart's wilt disease

- All these three forecasting models use mean air temperatures for December, January, and February for a preplant prediction of Stewart's disease risk in a subsequent season.

- **If:** (mean Temp Dec) + (mean Temp Jan) + (mean Temp Feb) 32-38°C

  Then: beetle survival will be high and **Stewart's wilt risk is high.**
- **If:** (mean Temp Dec) + (mean Temp Jan) + (mean Temp Feb) < or equal to 20-24°C
- **Then: beetle survival will be low** and **Stewart's wilt risk is low.**
- In brief, beetle survival used to forecast disease in next season. If sum of DEC, JAN and FEB temperature is less than -1°C, disease will not be severe.

Esker *et al.*,2006;..

Stewart's wilt disease prediction models

The Stevens Model

- In the 1930s, Stevens(2934) associated various levels of the seedling wilt phase of Stewart's disease with a temperature index based on mean daily temperatures in December, January, and February preceding planting (Next Table).
- An additional temperature category was added when the forecast was expanded later to predict the leaf blight phase of Stewart's disease (Boewe,1949).

Stevens, N. E. 1934. Stewart's disease in relation to winter temperatures. Plant Dis. Rep. 18:141-149.
Boewe, G. H. 1949. Late season incidence of Stewart's disease on sweet corn and winter temperatures in Illinois, 1944-1948. Plant Dis. Rep. 33:192-194.

Meyer *et al.*,2010

Stewart's wilt disease prediction models

The Stevens Model

- The Stevens forecast for predicting the seedling wilt phase of Stewart's disease.
- Prediction of the seedling wilt phase is based on
- The abundance of primary inocula as affected by the presence and size of overwintering populations of corn flea beetles.

Index[a]	Mean daily temperature		Prevalence of Stewart's wilt
	Celsius	Fahrenheit	
>100	>0.6°	>33.3°	Destructive
90-100	-1.1° to 0.6°	30° to 33.3°	Intermediate
<90	<-1.1°	<30°	Usually absent
<80[b]	<-2.8°	<27.3°	Absent

[a] Sum of the mean daily air temperature (F) in December, January, and February preceding planting.

[b] An additional temperature index was added when the Stevens forecast was expanded to include the leaf blight phase of Stewart's disease.

>0.6°C = Destructive, -1.1 to 0.6°C = intermediate, < -1.1 = usually absent, < -2.8 = absent.

Meyer *et al.*,2010

Stewart's wilt disease prediction models

The Stevens-Boewe Model

- The Stevens-Boewe forecast can be simplified further by dividing the winter temperature index by 3 to approximate the average winter temperature.

1. If the average daily temperature for December through February is above freezing, >1°C (33°F), flea beetles survive and Stewart's wilt is likely to be severe on susceptible hybrids.
2. If the average daily temperature for December through February is less than -3°C (ca 27°F), flea beetles are not likely to survive, and it is unlikely that Stewart's wilt will be severe.

Pataky,2004

Stewart's wilt disease prediction models

The Stevens-Boewe Model

Stevens-Boewe forecast for Stewart's wilt. Developed by N. E. Stevens in the 1930s and revised in the 1940s by G. H. Boewe at the Illinois Natural History Survey, University of Illinois.

Winter Temp index	Seedling wilt phase	Leaf blight phase
100 or more	destructive	severe
90 to 100	light to severe	severe
85 to 90	nearly absent	moderate
80 to 85	nearly absent	light
below 80	nearly absent	trace

Winter temperature index = sum of average temperature (°F) for December, January, and February.

Pataky,2004

Stewart's wilt disease prediction models

The Stevens-Boewe Model

- The Stevens-Boewe Index predicts the severity (**how much of the corn leaf tissue is infected**) for the mid-to-late season leaf-blight phase of Stewart's disease based on the sum of the mean temperatures for December, January and February.

1. A sum below 80°F/3=26.6°F⟹ -2.9(≈ -3) indicates a negligible risk;
2. 80 to 85 is considered a low risk;
3. 85 to 90 indicates moderate risk; and
4. greater than 90°F/3=(30°F ⟹-1.1°C) is considered a severe risk.

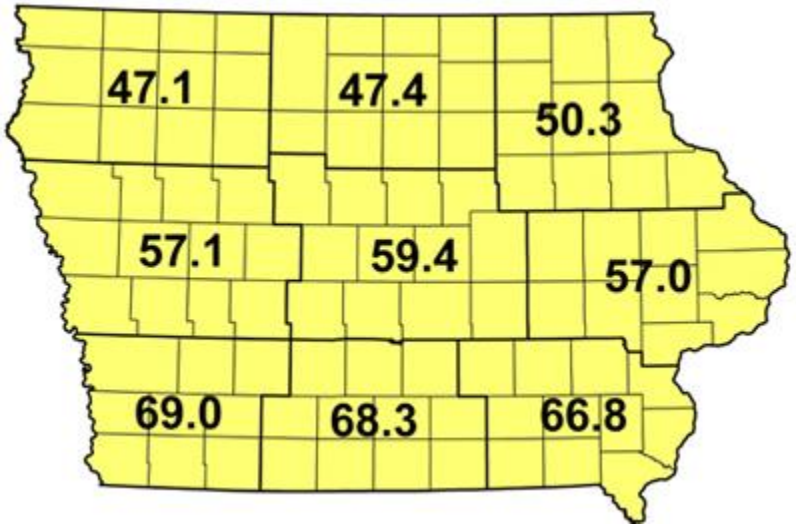
Nutter *et al.*,2011

Stewart's wilt disease prediction models

The Stevens-Boewe Model

- The summed monthly mean temperatures for the nine Iowa agricultural climate districts are presented in the map below:

The Stevens-Boewe risk level for the 2011 growing season is "negligible" for all nine agricultural climate districts in Iowa.
Note: A sum below 80 indicates a negligible risk.



Temperature data (summed mean temperatures for the months of December, January, and February, shown above) was obtained from the Bureau of Climatology, Iowa Department of Agriculture and Land Stewardship.

Nutter *et al.*,2011

Stewart's wilt disease prediction models

Iowa State method

- Recently, a model was developed to predict the occurrence of Stewart's disease at the time of phytosanitary inspections in seed corn fields (Esker *et al.*,2006 and Nutter *et al.*,2002).
- This model, often referred to as the Iowa State method, predicts Stewart's disease from the number of winter months with mean monthly air temperatures above -4.4°C (24°F).

Number of months[a] ≥-4.4°C (24°F)	Predicted risk of occurrence
0	Negligible
1	Low to moderate
2	Moderate to high
3	High

[a] Based on the mean daily air temperature in the December, January, and February preceding planting.

0 = negligible risk, 1 = low risk, 2 = moderate risk, 3 = high risk.

Meyer *et al.*,2010

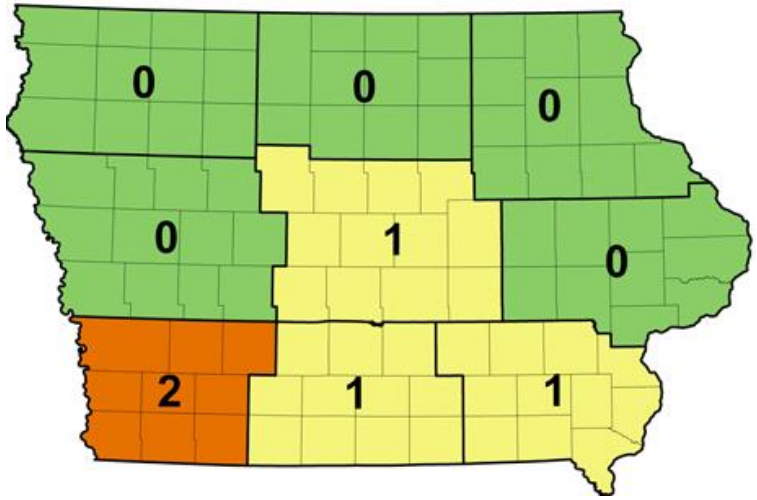
Stewart's wilt disease prediction models

The Iowa State University Stewart's disease model

- The summed monthly mean temperatures for the nine Iowa agricultural climate districts are presented in the map below:

Predicted risk of Stewart's disease in Iowa agricultural climate districts in 2011, calculated using the Iowa State Model.

0 = negligible risk, 1 = low risk, 2 = moderate risk, 3 = high risk.



Continuous snow cover in parts of Iowa from late December through February could have functioned as an insulation blanket to protect corn flea beetles from subfreezing temperatures.

Nutter *et al.*,2011

Forecasting model for predicting risk of Stewart's wilt disease

1. If the average daily temperature for this 3-month period is above freezing, >1°C, corn flea beetle (*Chatocnema pulicaria*), survives and Stewart's may be present.
2. If the average is below -3°C, few flea beetles survive and Stewart's wilt is not likely to be severe.

Continuous snow cover in parts of Iowa from late December through February could have functioned as an insulation blanket to protect corn flea beetles from subfreezing temperatures.

CAB International,2021;..

Management

Stewart's bacterial wilt disease of corn

- Two major control measures are useful in reducing losses to Stewart's disease.
- The first consists of planting tolerant varieties, which will grow and produce well in spite of the presence of the pathogen.
- In general, later maturing varieties are more tolerant of the disease than earlier maturing ones, although several early maturing varieties are available with good tolerance to Stewart's wilt.
- Current sweet corn varieties with tolerance include: Bellringer, Calumet, Capitan, Comet, Defender, Gold Crest, Gold Cup, Gusto, Merit, Midway, NK 199, Pacer, Seneca Chief, Silver Queen, Sprite, Sweet Sue, Titan, Valley Market, Vanguard, Wintergreen, and Yukon.

Management

Stewart's bacterial wilt disease of corn

- The second major control measure is the application of early insecticide sprays for controlling the overwintering flea beetle population.
- In areas where Stewart's wilt is known to be a potentially severe disease, insecticides should be applied when the corn first breaks ground and should be continued for several applications thereafter until the stand is well established.
- Consult your county Extension agent for a list of registered insecticides and rates of application.

Management

Insect Management

Vector of Stewart's bacterial wilt of corn

- Corn flea beetle, the vector of Stewart's disease, can be suppressed with IPM tools such as hybrid selection, scouting and insecticides.
- Areas with potential risk should incorporate resistant hybrids to minimize adult attraction and subsequent egg laying.
- Susceptible hybrids planted in historically infected areas should be planted later to discourage adult colonization.
- Regardless of hybrid selection, all corn fields should be scouted for adult corn flea beetles several times a week during emergence and seedling stages.
- Look for the shiny, black adults feeding on leaves.
- Try to walk quietly, as they are easily disturbed and will jump off the plants. Also look for long, light feeding scars on the leaves.

Nutter *et al.*,2011

Management
Stewart's bacterial wilt disease of maize
Seed Transmission

- **Effect on Seed Quality**
- Seeds harvested from severely infected ears often are deformed, shrunken and discoloured. Germination often is adversely affected.
- For naturally-infected seeds, no pathogen transmission was detected from three seed lots with 3.5, 10 and 35% seed infection, but one infected seedling out of 8127 tested was found in a lot with 9% seed infection.
- Guo *et al.* (1987) have shown that the bacterium disappears from maize seed after 200-250 days at 8-15°C and after 110-120 days at 20-25°C, and recommend storing seed under conditions suitable for eliminating *P. stewartii* subsp. *stewartii*.
- Seed treatment with imidacloprid or thiamethoxam reduced the incidence of Stewart's wilt by 50 to 85% relative to nontreated controls in plots of the susceptible hybrid Jubilee.

Management

Stewart's bacterial wilt disease of maize

Seed treatments

INSECTICIDE	PRODUCT PER ACRE	PHI	Aphids	Armyworms	Corn Rootworm Larvae	Corn Rootworm Adults	Cutworms	European Corn Borer	Grasshoppers	White Grubs	Wireworms	Spider mites
gamma-cyhalothrin Declare *RUP*	Foliar: 0.77 - 1.54 fl oz	21 days	●	●		●	●	●	●			
imidacloprid Attendant 600 Dyna-Shield Imidacloprid 5 Gaucho 600 Senator 600FS	0.72 - 6 fl oz per 80,000 seed unit Use high rate for corn rootworm (protection not adequate in heavy corn rootworm populations)	None	●		●			●		●	●	
indoxacarb Stewart EC	6 - 11.3 fl oz	14 days for grain and stover, 1 day for forage, fodder, silage		●		●		●	●			

North Dakota Field Crop Insect Management Guide,2020

Management

Stewart's bacterial wilt disease of maize

Biological controls

- Biological controls for *P. stewartii* subsp. *stewartii* have not been developed adequately enough to be used.
- A bacteriophage of *P. stewartii* subsp. *stewartii* was isolated from *C. pulicaria* (Woods *et al.*,1981) and partially characterized according to host range, one-step growth experiment behaviour and morphology.
- The host range was limited to 8 of 13 strains of *P. stewartii* subsp. *stewartii* and one strain of *Erwinia herbicola* var. *herbicola* [*Pantoea agglomerans*].
- Woods *et al.*,1981 suggested that under field conditions, *P. stewartii* subsp. *stewartii* might be effectively reduced or eliminated within its beetle vector by virulent bacteriophages.

Pectobacterium and *Dickeya* spp.

Soft rot diseases

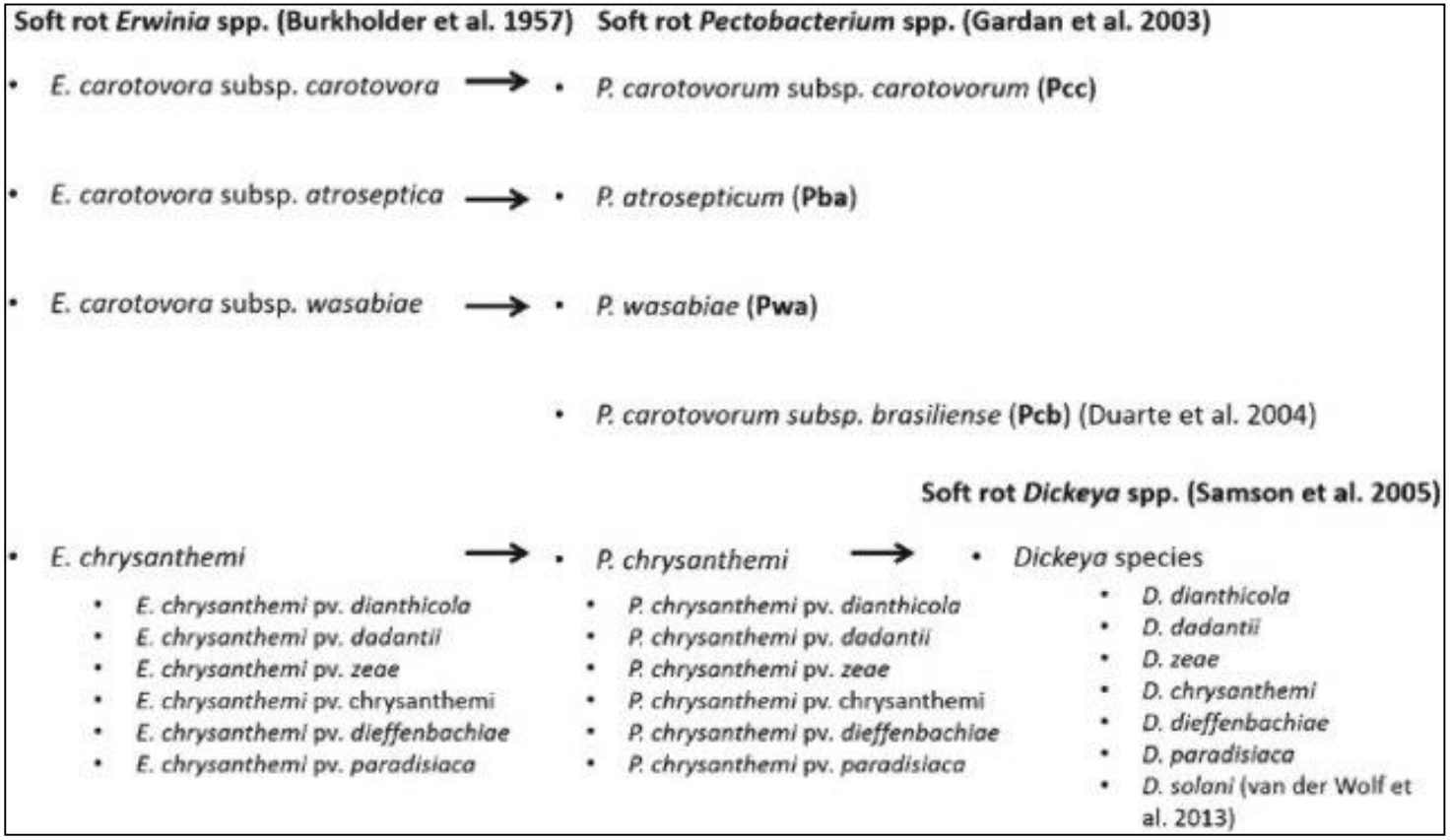
Potato soft rot

Bacterial soft rot diseases

- Soft rots are caused by several bacteria, most commonly:
- *Pectobacterium carotovorum, P. atropsepticum*,
- *Dickeya dadantii* (previously called *Erwinia chrysanthemi*), and
- Certain species of:
- *Pseudomonas*,
- *Bacillus* and
- *Clostridium*.

Bacterial soft rot diseases

Taxonomic classification of *Pectobacterium* spp. and *Dickeya* spp. associated with tuber soft rot and blackleg disease of potato



Czajkowski *et al.*,2014

Bacterial soft rot of vegetables

FIGURE 12-32 Bacterial soft rot of vegetables on (A) cabbage head, (B) carrots, (C) tomato, (D) onion bulb, and (E) celery. [Photographs courtesy of (A, C, and E) Plant Pathology Department, University of Florida, (B) R. J. Howard, W.C.P.D. and (D) G. Q. Pelter, Washington State University.]

Agrios,2005

Etiology
Soft rot protobacteria

- Soft-rot bacteria overwinter in infected fleshy tissues
- in storage,
- in the field,
- garden or greenhouse,
- in the soil (especially in the rhizosphere around the roots of many plants), and
- on contaminated tools, equipment, containers, and in certain insects.

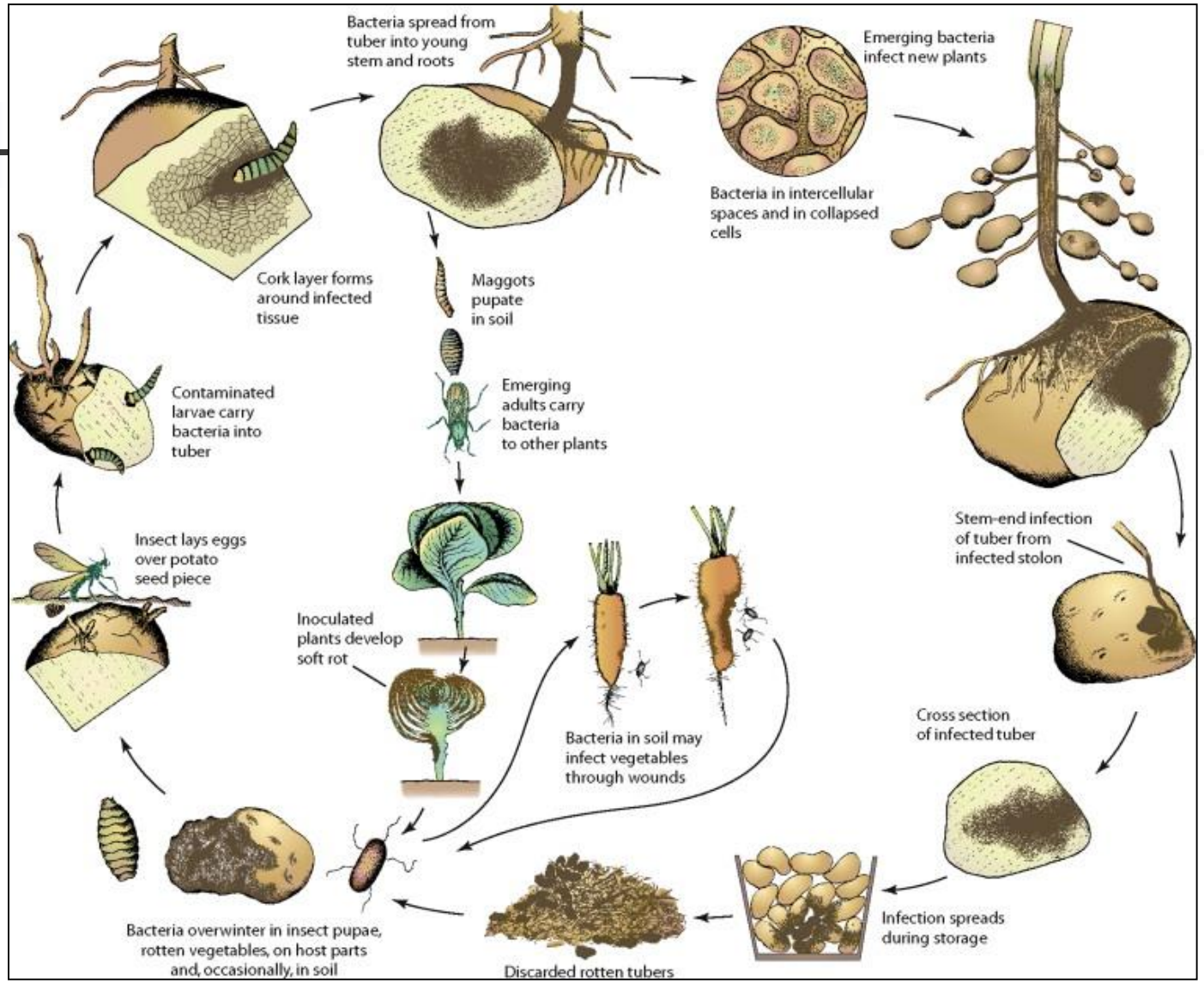
Yield losses

Soft rot caused mainly by different species of the ex. *Erwinia* bacterium(*Pectobacterium* and *Dikeya* spp.)

- Soft rot is the term given to the infection caused mainly by different species of the ex. *Erwinia* bacterium.
- It is an important disease because:
  - It infects a wide range of horticultural crops, and
  - No satisfactory control (chemical or otherwise) currently exists.
- The losses due to soft rot can be huge.
- Yield losses of up to 90% have been recorded in cases with soft rots.

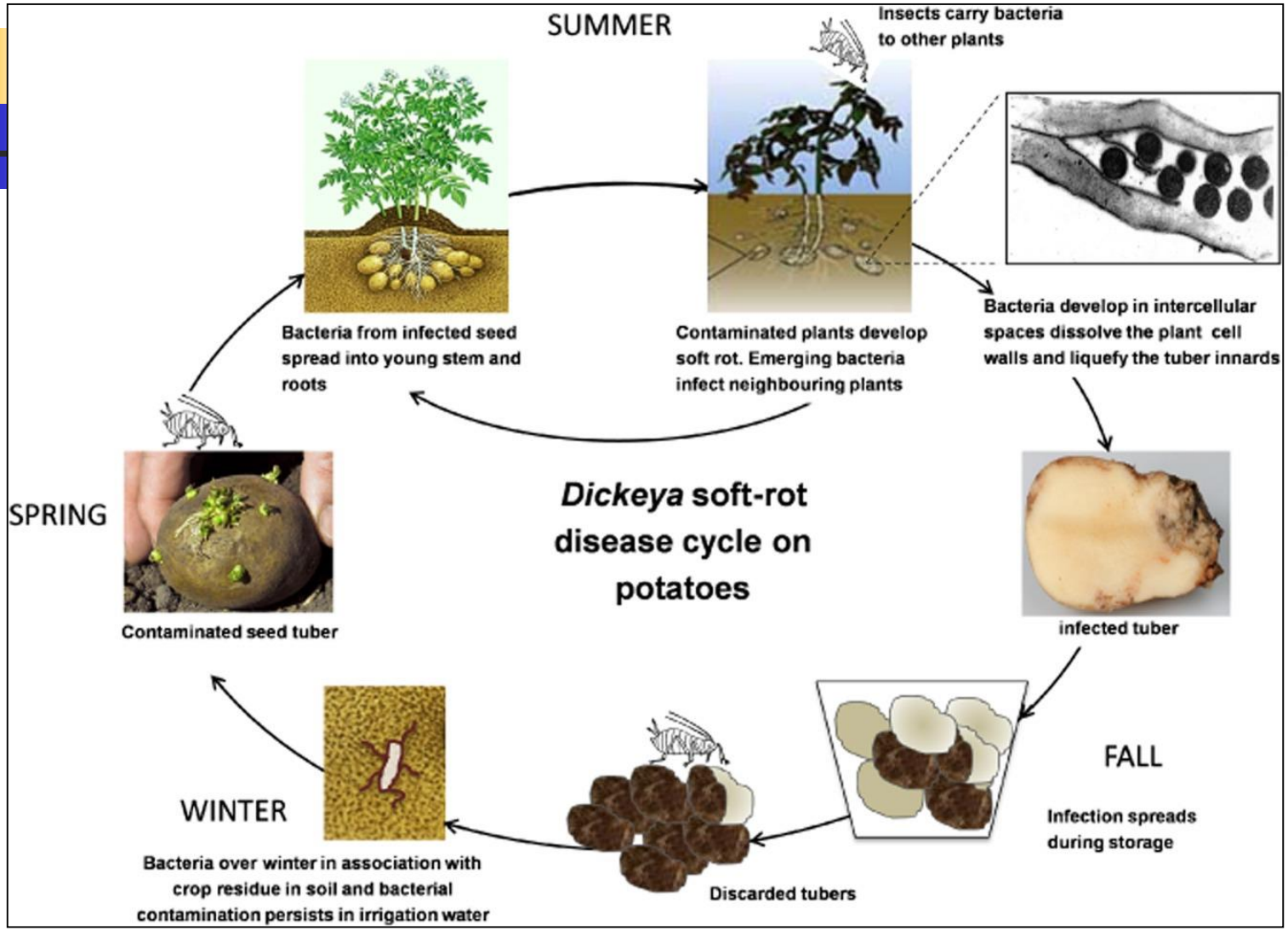
Vanneste;..

Disease cycle of the genus *Pectobacterium*



Agrios,2005

Disease cycle of the genus *Dickeya*



Reverchon and Nasser,2013

Etiology

Soft rot protobacteria

Effect of temperature on soft rot intensity

- Soft-rot bacteria can grow and are active over a range of temperatures from 5 to 35°C, particularly when oxygen is limited.

1. The bacteria multiply rapidly by dividing in half every 20 to 60 minutes under ideal conditions at 18° and 35°C.
2. Minimum temperatures for development is between 2° and 6°C, and
3. Maximum 35° and 41°C, depending on the species of bacterium involved.

- The bacteria are killed at temperatures above 50°C.

Etiology

Soft rot protobacteria

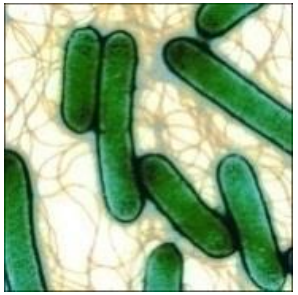
Effect of temperature on soft rot intensity

Temperature ^{0}C	Storage period (Days)	Disease Incidence	Disease Intensity	Weight loss
25	4	100*	19.04 (25.73)	N.D**
	6	100	26.66 (30.71)	21.64
30	4	100	23.80 (29.10)	N.D
	6	100	39.99 (39.17)	32.33
35	4	100	21.90 (27.85)	N.D
	6	100	34.28 (35.77)	25.93
$CD_{0.05}$			8.73 (5.36)	

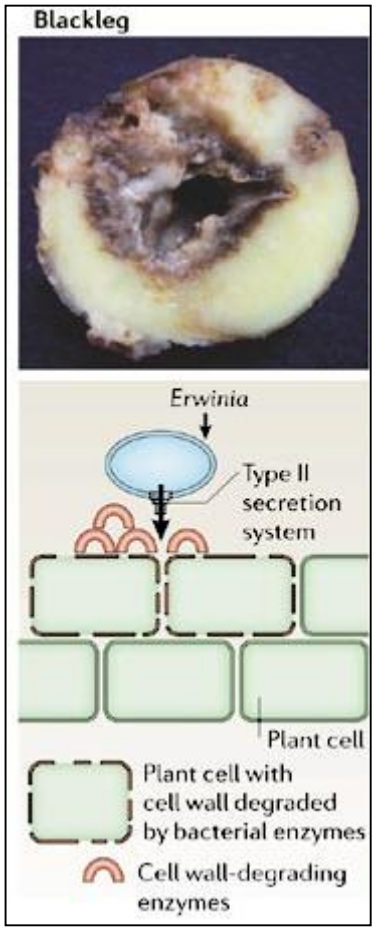
Masoodi *et al.*,2010

Disease symptom & mechanism of action

Pectobacterium atrosepticum



- Disease symptoms caused by *Pectobacterium atrosepticum* and representative virulence mechanism used by this pathogen.
- Soft-rot symptoms are due to the production and secretion of degradative enzymes that destroy the plant cell wall.



Management

Integrated Pest Management (IPM)

Soft-rot *Pectobacterium* spp.

- Bacterial soft rot diseases caused by *Pectobacterium* spp. and *Dickeya* spp. affect a wide range of crops, including potatoes, a major food crop.
- As of today, farmers mostly rely on:

1. sanitary practices,
2. water management, and
3. plant nutrition for control.

Youdkes *et al.*,2010

Management

Integrated Pest Management (IPM)

Soft-rot *Pectobacterium* spp.

- Control practices should include:

1. Early planting, and plant spacings of 15 to 20 cm between plants.
2. Avoiding excessive irrigation.
3. Avoiding close rotations of sugar beets. Good weed control
4. Should be maintained throughout the rotation.
5. Avoiding plant injury.
6. Avoiding excessive nitrogen applications.

Disinfectants for storage rooms, bins, crates or boxes, packing areas, and dump tanks *Pectobacterium* spp.

Compound	Concentration	Comments
Sodium hypochlorite	1,000 to 1,900 ppm	Do not rinse
Peracetic acid (peroxacetic acid)	3,000 ppm	Dip or spray
Formaldehyde	1 pt/15 gal	Wet surfaces thoroughly. Close tightly and ventilate before entering.
Calcium hypochlorite	700 to 5,000 ppm	Rinse all surfaces which will contact plant parts.
Alkyd dimethyl benzyl ammonium chlorite	1,600 ppm	Spray on precleaned, rinsed surfaces. Rinse all food contacting surfaces with clean water before use.

Peracetic acid ($C_2H_4O_3$) is a mixture of acetic acid (CH_3COOH) and hydrogen peroxide (H_2O_2) and is used as a disinfectant.

Babadoost,1990

Management

Calcium treatments may delay decay

Pectobacterium carotovorum

- Calcium is a key component of cell walls, helping to build a strong structure and ensuring cell stability through its interaction with polygalactic acid (Palta, 2010).
- Strengthening of the tuber cell wall with calcium can help reduce the severity of *Pectobacterium carotovora* soft rot in storage.
- Calcium also reduces the level of tuber skin diseases including Black surf and powdery scab leading to better skin finish.

Tuhwe,2015

Management

Calcium treatments may delay decay

Pectobacterium carotovorum

- Adequate calcium has been found to improve cell integrity, thicker skin netting and ensure proper signaling of pathways such as calmodulin (a calcium binding protein that mediates many metabolic activities), thus reducing the incidence and severity of diseases(Rahman and Punja, 2007).
- Previous study by McGuire and Kelman (2010), supported that calcium localized in the cell walls may indeed inhibit tissue maceration, as it produces strong structural rigidity by forming cross-links within the pectin polysaccharides matrix and it does so by combining with pectin in the cell walls forming calcium – pectate which resists maceration.

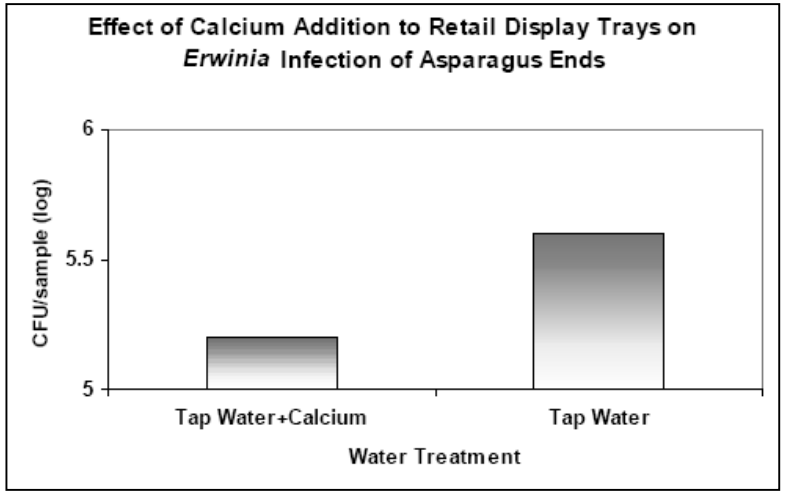
Tuhwe,2015

Management

Calcium treatments may delay decay

Pectobacterium carotovorum

- The addition of 0.5% calcium metalosate to water, in a shipping and retail display tray, reduced the growth of the soft rot pathogen *P. carotovorum* subsp. *carotovorum* in the asparagus spear tissue at the cut end during a three day period at 15°C.
- Typically, decay is not apparent to the unaided eye until the pathogen levels reaches log 5.8 CFU (colony-forming units).



Suslow,2001

Management

Calcium treatments may delay decay

Pectobacterium carotovorum

- Effects of different calcium fertilizers(calcium chloride, calcium sulphate and calcium nitrate) on incidence of soft rot disease.
- There was an interaction ($P<0.001$) between the three calcium fertilizers and variety on soft rot disease incidence.
- All the treatments with calcium had lower disease incidence than treatments without calcium with calcium chloride having the least percentage of soft rot disease incidence followed by calcium sulphate and calcium nitrate respectively.
- From the findings obtained Mondial proved to be more resistant since it had a significantly lower percentage of soft rot disease incidence compared to other Amythest and Mondial, respectively.

Tuhwe,2015

Management

Calcium treatments may delay decay

Pectobacterium carotovorum

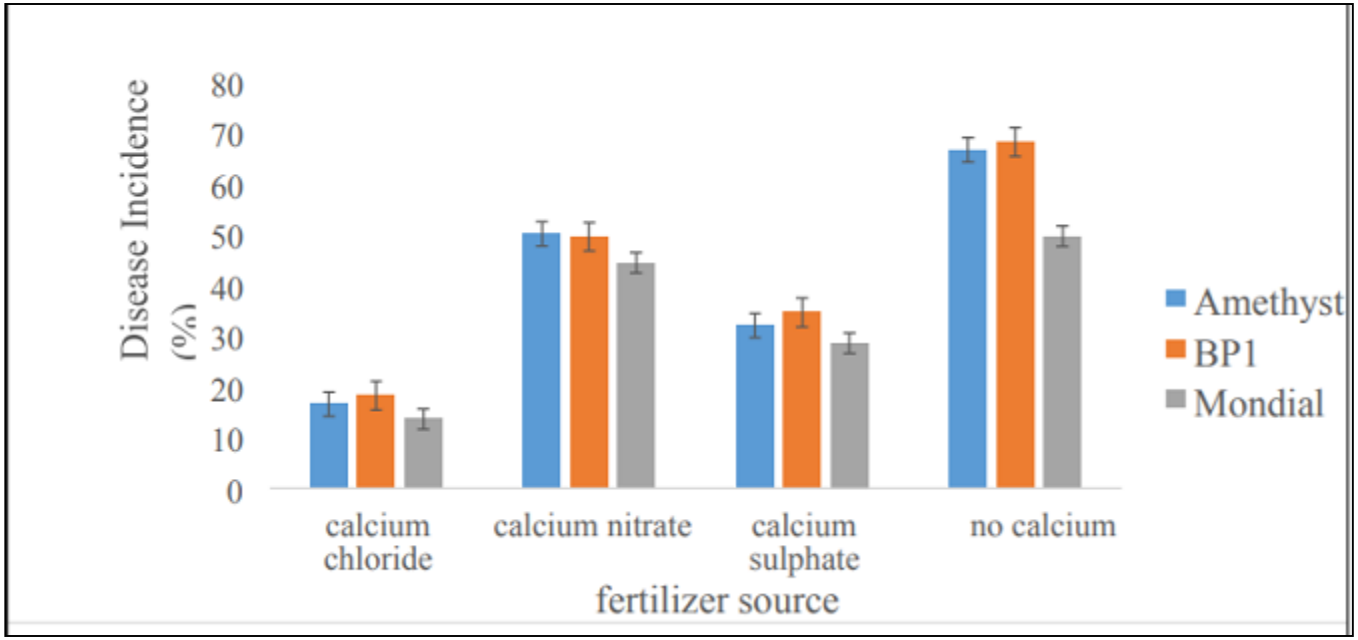
- Application rates of different calcium fertilizer source.

Treatment	Source of Calcium	Application rate/kg source/ha	Ca kg/ha
T1	Calcium Nitrate (19% Ca)	250	35.9
T2	Calcium Chloride (36% Ca)	132.19	47.5
T3	Calcium Sulphate (22% Ca)	215.9	40.1
T4	none	0	0

Tuhwe,2015

Calcium treatments may delay decay
Percent of disease incidence
Pectobacterium carotovorum

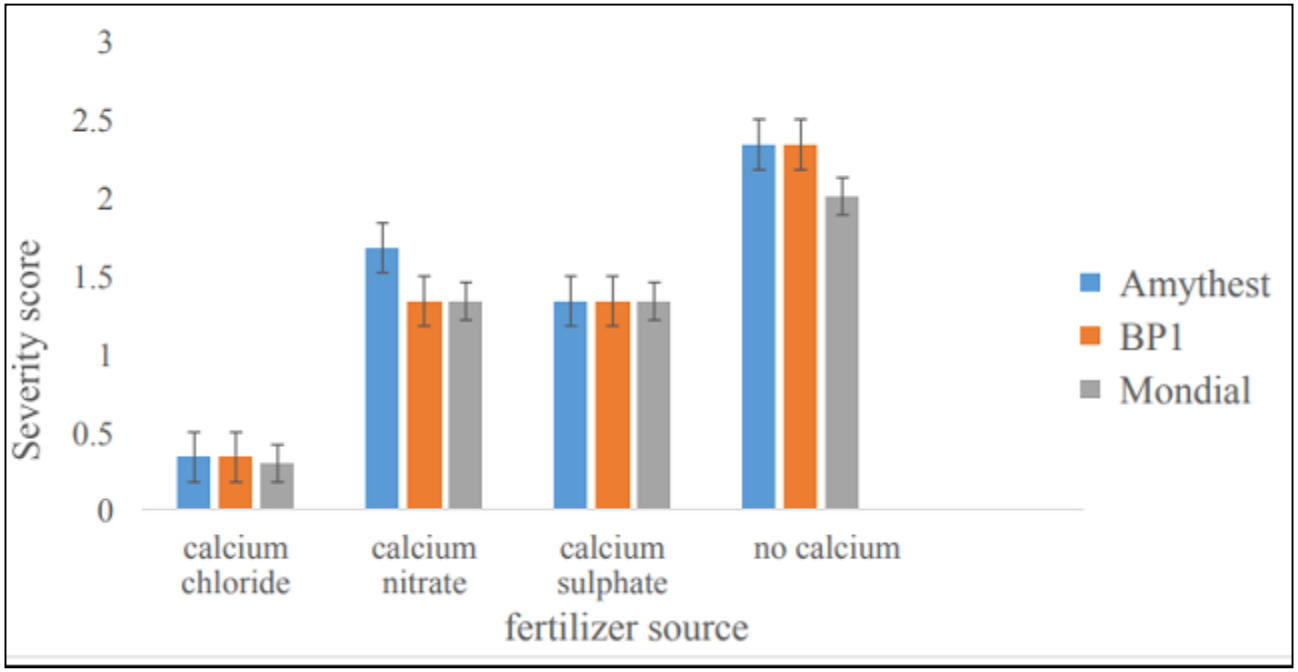
- Effects of different calcium fertilizers(calcium chloride, calcium sulphate and calcium nitrate) on incidence of soft rot disease.



Tuhwe,2015

Calcium treatments may delay decay
Disease severity score
Pectobacterium carotovorum

- Effects of different calcium fertilizers(calcium chloride, calcium sulphate and calcium nitrate) on severity of soft rot disease.



Tuhwe,2015

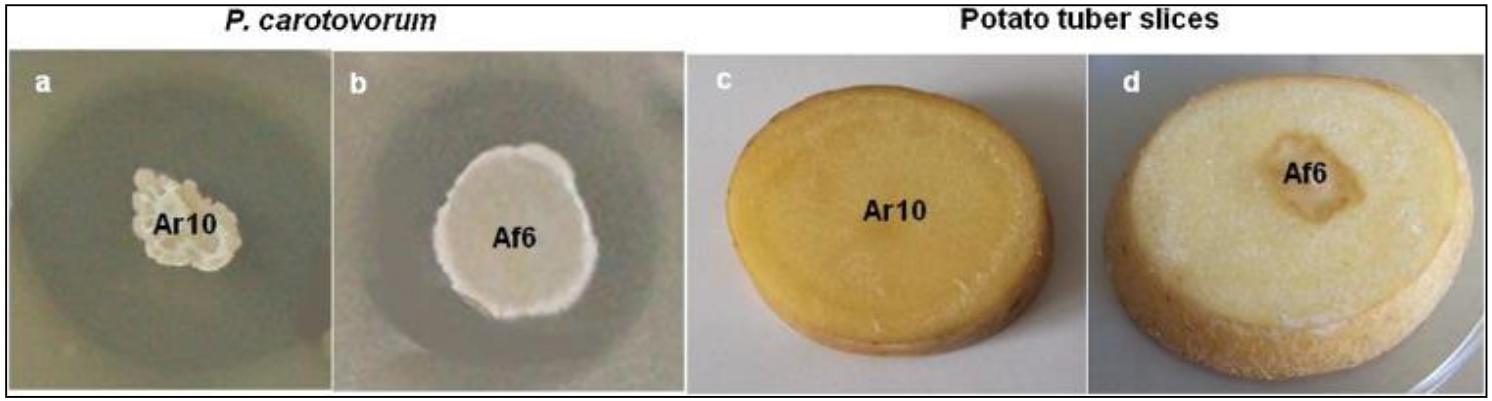
Chemical and biological control of rhizome rot disease of banana *Pectobacterium carotovorum*

- ***In vitro* assays:**
- *In vitro* inhibitory effect of antibiotics, fungicides, botanicals, and bio agents on the growth of the pathogen was tested.
- Among the fungicides, fytolan (a copper oxychloride-based fungicide) at 0.4 per cent concentration gave the maximum inhibition followed by fytolan at 0.3 per cent.
- Among the antibiotics, streptocycline at 300 ppm was found to be the most superior one.
- Garlic extract at 100 per cent concentration was the best botanical for inhibiting the pathogen.

Usha Prasanth Rayirath,2005

Biological control of the soft rot bacterium *Pectobacterium carotovorum* by *Bacillus amyloliquefaciens*

- Treatment of potato tubers with *Bacillus amyloliquefaciens* strain Ar10 for 72 h significantly reduced the severity of disease symptoms (100 and 85.05% reduction of necrosis deep/area and weight loss, respectively).



Strain Ar10 harbored the highest antibacterial activity with an inhibition zone diameter of 18 mm (Fig. 1A). In addition, as strain Ar10 didn't show any visible symptoms of rotting on potato tuber slices (Fig. 1B), this endophytic strain was selected for controlling soft rot of potato tubers.

Azaiez *et al.*,2018

A biological-control agent against Pcc, soft rot disease of Chinese cabbage **Biokeeper**

- A biological-control agent with the trade name "Biokeeper" has been developed for the control of soft rot disease caused by *Pectobacterium carotovorum* subsp. *carotovorum* in Japan.
- This product is formulated from mutants of *P. carotovorum* subsp. *carotovorum* producing a low-molecular-weight bacteriocin, carocin.
- There is strong evidence that avirulent mutant strains of *P. carotovorum* subsp. *carotovorum* effectively control the soft rot disease of Chinese cabbage.

Chuang *et al.*,2007

Biological control of the soft rot bacterium *Pectobacterium carotovorum* by bacterial predators *Bdellovibrio* and like organisms (BALOs)

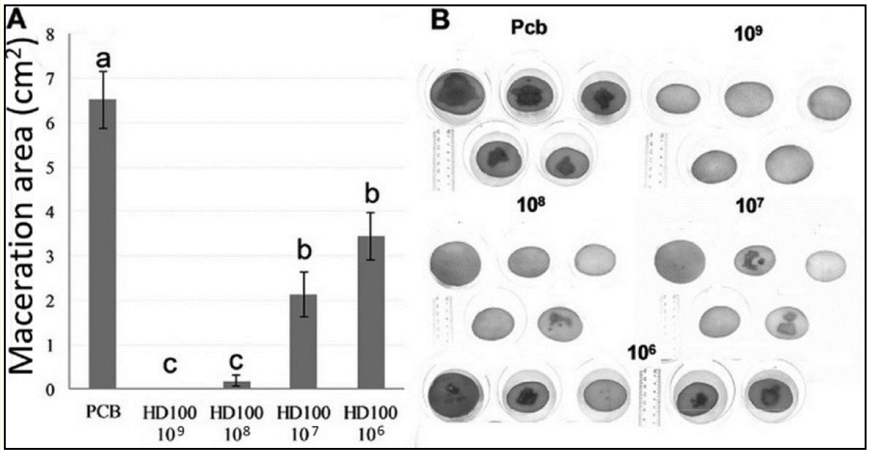
- BALOs are small, motile predatory bacteria found in terrestrial and aquatic environments.
- Bacterial predators *Bdellovibrio* and like organisms (BALOs) to control potato soft rot.
- They prey on a wide range of Gram-negative bacteria, including animal and plant pathogens such as *Pectobacterium* and *Dickeya solani*.
- All BALO strains were highly effective at reducing disease, up to complete prevention.

Youdkes *et al.*,2010

Biological control of the soft rot bacterium *Pectobacterium carotovorum* subsp. *brasiliense* by bacterial predators *Bdellovibrio* and like organisms (BALOs)

A. The effect of *B. bacteriovorus* strain HD100 inoculated at concentrations of 10^6, 10^7, 10^8, and 10^9 PFU. ml^{-1} (10 µl) on tissue maceration in potato slices induced by the soft rot bacterium *P. carotovorum* subsp. *brasiliense* (Pcb) (10 µl, 10^6 CFU. ml^{-1}) inoculated 60 min after the predator.

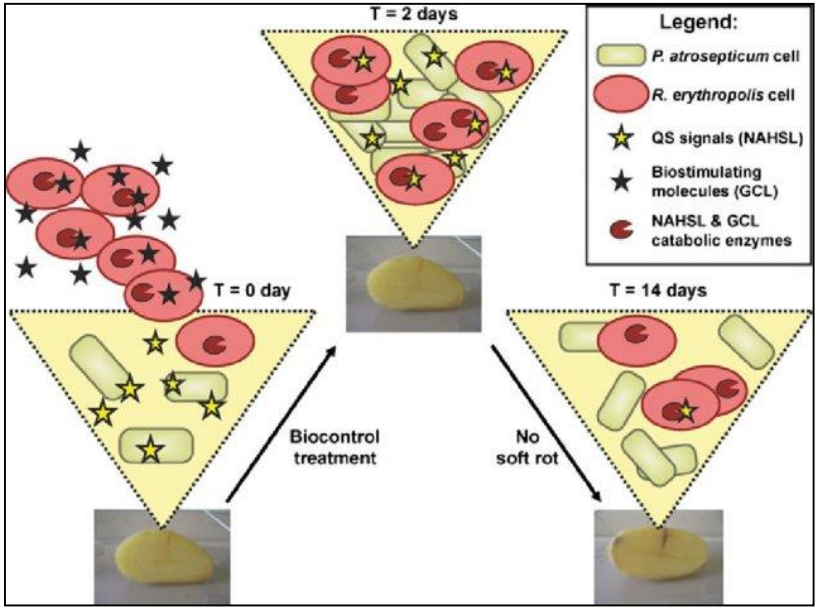
- Macerated tissue appears as dark patches.



Youdkes *et al.*,2010

Key role of pathogen communication in the chronology of events leading to soft rot tuber Quorum sensing

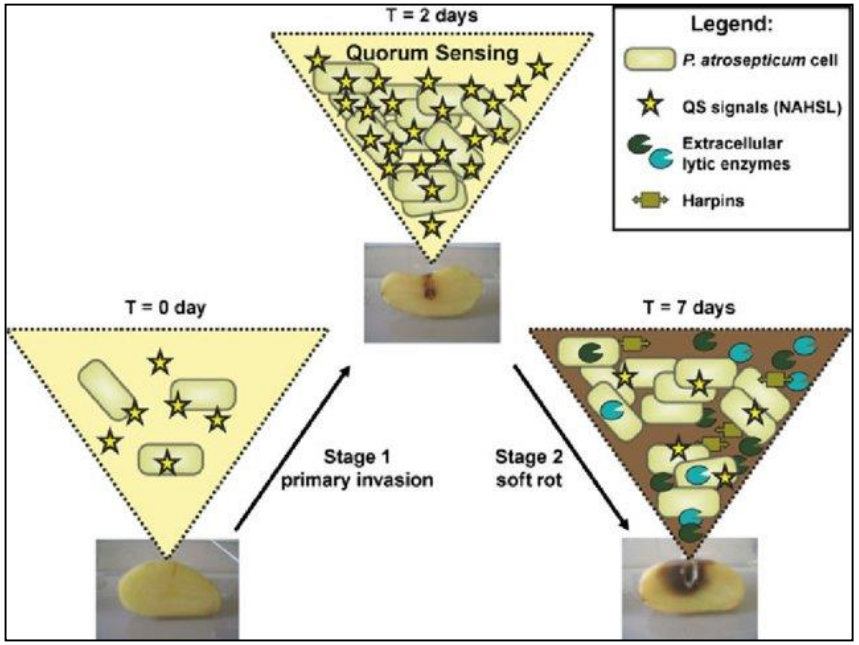
- The addition of gamma-caprolactone (GCL), a structural analog of N-acyl-homoserine lactones (NAHSL), in plant hydroponic systems can promote selective cell proliferation of indigenous or co-inoculated antagonists (i.e. *Rhodococcus erythropolis*) near the plant organs that must be protected.
- *Rhodococcus erythropolis* antagonistic populations in the potato rhizosphere.
- Rhodococcus bacteria have the ability to disrupt the quorum sensing-based communication of *P. atrosepticum* by degrading N-acyl-homoserine lactone signaling molecules and prevent disease.



Crépin *et al.*,2012

Key role of pathogen communication in the chronology of events leading to soft rot tuber
Quorum sensing

- During the primary invasion stage, *Pectobacterium atrosepticum* multiply and produce constitutively small diffusible signaling molecules (N-acyl-homoserine lactones, NAHSL).
- The containment of pathogens in a tuber wound can increase the concentration of signals.
- It is only when the bacteria have the quorum that the effective concentration of NAHSL is obtained and consequently triggers the release of lytic enzymes and harpins responsible for the maceration of the tuber.



Crépin *et al.*,2012

Pectobacterium betavasculorum

Bacterial vascular necrosis and rot (root rot)

Sugar beet soft rot

Etiology
Pectobacterium betavasculorum

- Bacterial vascular necrosis and rot, or *Erwinia* root rot, is caused by *Pectobacterium betavasculorum*.
- Erwinia root rot can occasionally be a highly destructive disease.
- The bacterium is endemic to many native and cultivated soils(disease-endemic areas), and variants of the potato blackleg bacterium (*P. atrosepticum*) can sometimes be pathogenic to sugarbeets.
- Symptoms include, black streaks running up the petioles, froth from crowns, and blackened petiole bases and crown.
- Vascular bundles in the petioles and root will be necrotic, and the root tissue adjacent to the vascular necrosis will turn pink when cut and exposed to the air.
- In late stages, the rot can become an extensive soft or dry rot.

Etiology

Pectobacterium betavasculorum

- Bacterial infection and subsequent disease development is favored by wounds on petioles, crowns, or foliage, excessive nitrogen, excessive moisture, warm temperature (ranging from 20 to 28°C), and increased plant spacing.
- Young plants are more susceptible than old plants.
- The disease can survive in some weeds.
- Splashing water, insects, soil, and contaminated equipment may spread the pathogen.
- *E. betavasculorum* survives between sugarbeet crops in and on unharvested beets, soil, and weeds.
- Several races of the pathogen occur and can also attack tomato, potato, and chrysanthemum (Schwartz and Gent, 2009).

Sugarbeet varieties with known resistance or susceptibility to California isolates of *P. carotovorum* subsp. *betavasculorum* (Ecb)

Cultivar	Resistance to Ecb	Resistance to Other Diseases
USH11	Resistant	None documented
Beta 4776R	Resistant	*Rhizomania*
Beta 4430R	Resistant	*Rhizomania*, Curly Top, *Fusarium* Yellows
Beta 4035R	Resistant	Rhizomania, Curly Top
HH50	Susceptible	None Documented

Zidack and Jacobsen,2001

Management
Pectobacterium betavasculorum

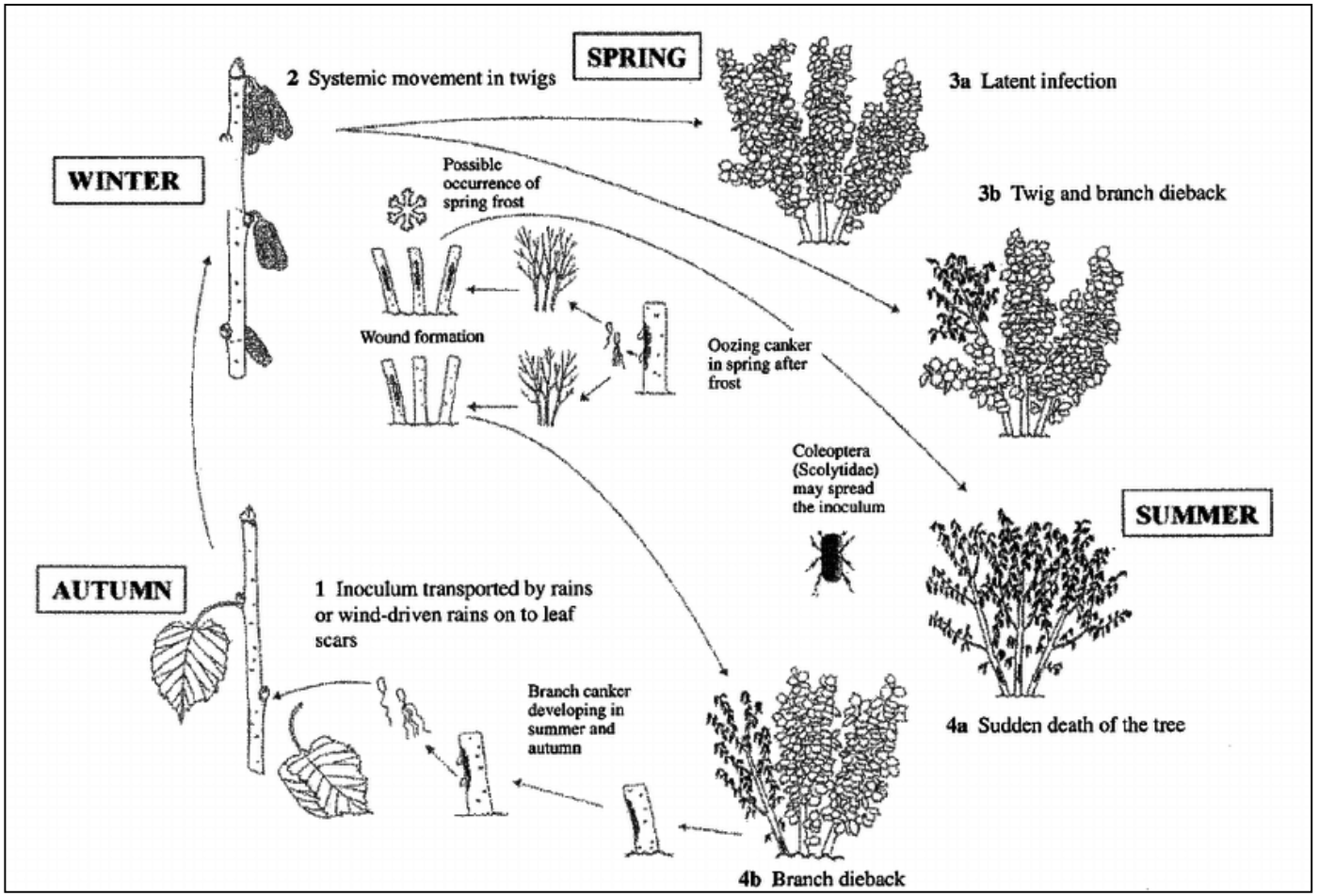
- This is the only important bacterial pathogen of sugar beets.
- Most sugarbeet cultivars have resistance to Pb (ex. *Erwinia* root rot), but losses can still occur.
- Losses are infrequent and sporadic (occurring at irregular intervals or only in a few places; scattered or isolated).
- Yield losses of up to 40% have been reported.
- The disease is controlled solely through use of IPM practices.
- *Erwinia* can be present in areas where excess water and warm temperatures are present. Water accumulates in the hole in the beet crown.
- As this water warms, ideal conditions are established for bacterial activity.
- No chemicals provide effective control of bacterial vascular necrosis.
- No biological control strategies have been developed for bacterial vascular necrosis.

Pseudomonas avellanae

Bacterial canker and decline of hazelnut

Disease cycle

Pseudomonas avellanae



Management

Pseudomonas avellanae

- The production of disease-free nursery plant material is very important.
- In case of completely wilted trees, the roots and suckers also must be removed.
- After a branch is cut, it is advisable to seal the wound with wax or Bordeaux mixture.
- Taking into consideration that *P. avellanae* mainly lives inside the plant, the efficacy of copper-based compounds, the only chemicals allowed in Italy for controlling bacterial disease, is rather low.
- A combination of agronomic techniques and applications of copper-based materials at key times can partially manage the disease.
- Orchards having very acidic soils require lime application to increase the soil pH.

Scortichini,2002

Management
Pseudomonas avellanae

- It is also very important to control the *Scolytidae* by using chromotropic traps.
- Recent progress in the control of bacterial decline has been achieved by artificial induction of systemic acquired resistance (SAR).
- We used acibenzolar-*S*-methyl (CGA 245704, by Syngenta Crop Protection), registered in Europe as Bion (Actigard in the United States).
- The minimum concentration to effect good control without inducing any phytotoxic effect is 5 g a.i. hl^{-1} (25 g a.i ha^{-1}).
- Five applications of Bion applied once a month from late April to July were required to reduce the number of dead trees and branches.
- A final application in September after harvest was also important.

Scortichini,2002

Pseudomonas fuscovginae

Bacterial sheath rot of wheat, rice,..

The disease (sheath brown rot) reduce spike exertion

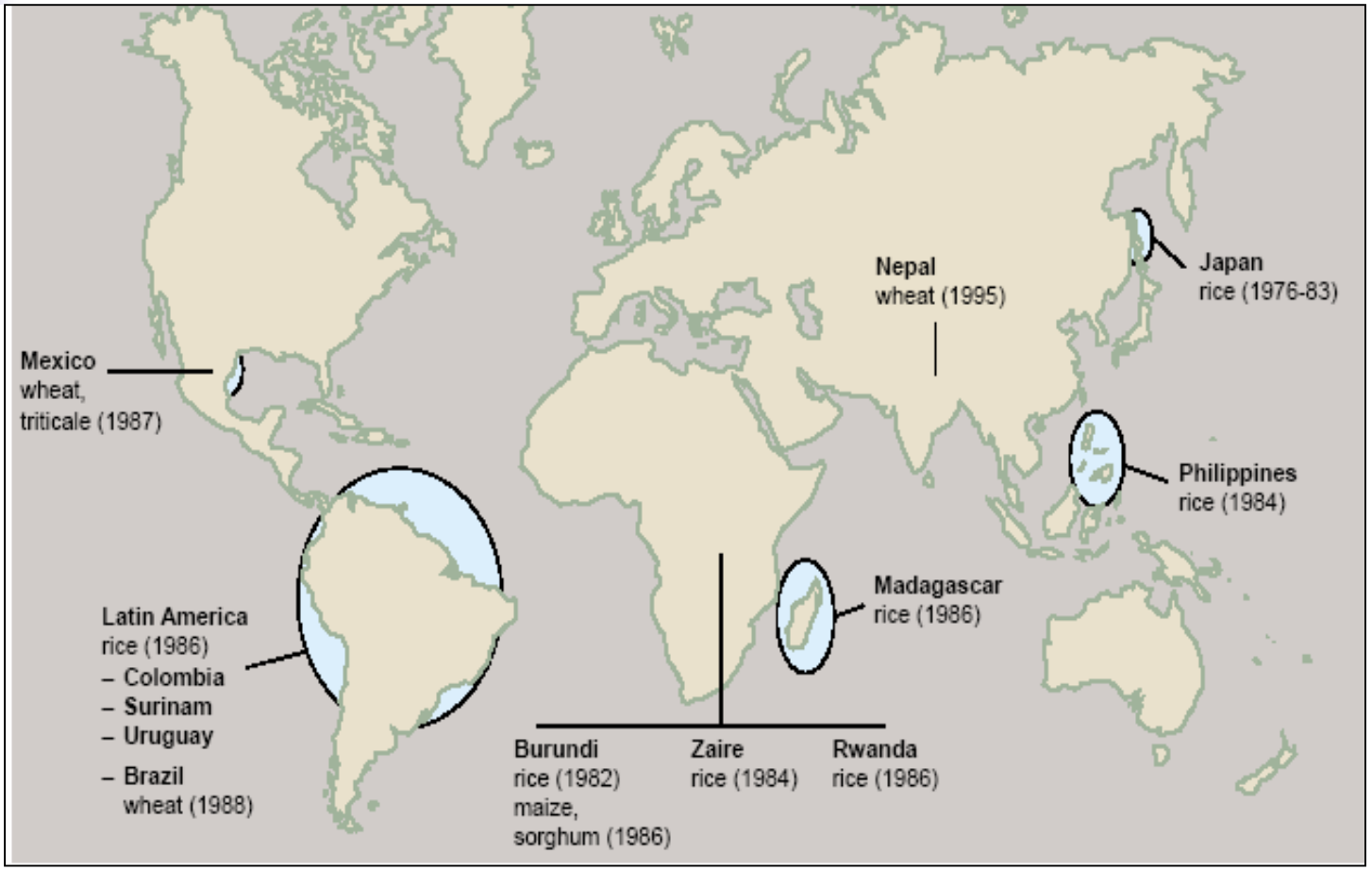
Sheath brown rot, grain discoloration and grain sterility

Pseudomonas fuscovaginae

Pseudomonas fuscovaginae: Its rise not nice for Australian rice.

Dante L. Adorada

Distribution of *Pseudomonas fuscovaginae* and year of identification on different host plants



Host range

- Aside from the rice plant, it is pathogenic to:
- Barley,
- Maize,
- Oats,
- Triticale,
- Wheat,
- *Bromus marginatus* Nees ex Steudel,
- *Lolium perenne* L.,
- *halaris arundinacea* L., and
- *Phleum pratense* L.

Biology and ecology

- Cool daytime temperature, 17-23°C, favors the development of the pathogen.
- It can survive on rice seed at a low level and as an epiphyte on grasses.
- The pathogen can be found on healthy rice leaf blades and sheaths in the rice field.
- Its epiphytic population is observed at the booting stage.
- The seedling rot phase of the disease is noted at temperatures below 20°C.

Management
Pseudomonas fuscovginae

- **Cultural control**
  Eradication by heat treatment (65°C for 6 days or water at 55°C for 20 minutes), and is used for international exchange and for genetic or basic seed.
- **Chemical control**
  - Kasugamycin, only reduced incidence of the pathogen and could be used for commercial certified seed (Zeigler *et al.*,1987, IRRN 12).
  - Streptomycin, alone or in combination with oxytetracycline, controls disease if applied at or few days after panicle emergence (Webster and Gunnell,1992).

Management
Pseudomonas fuscovginae

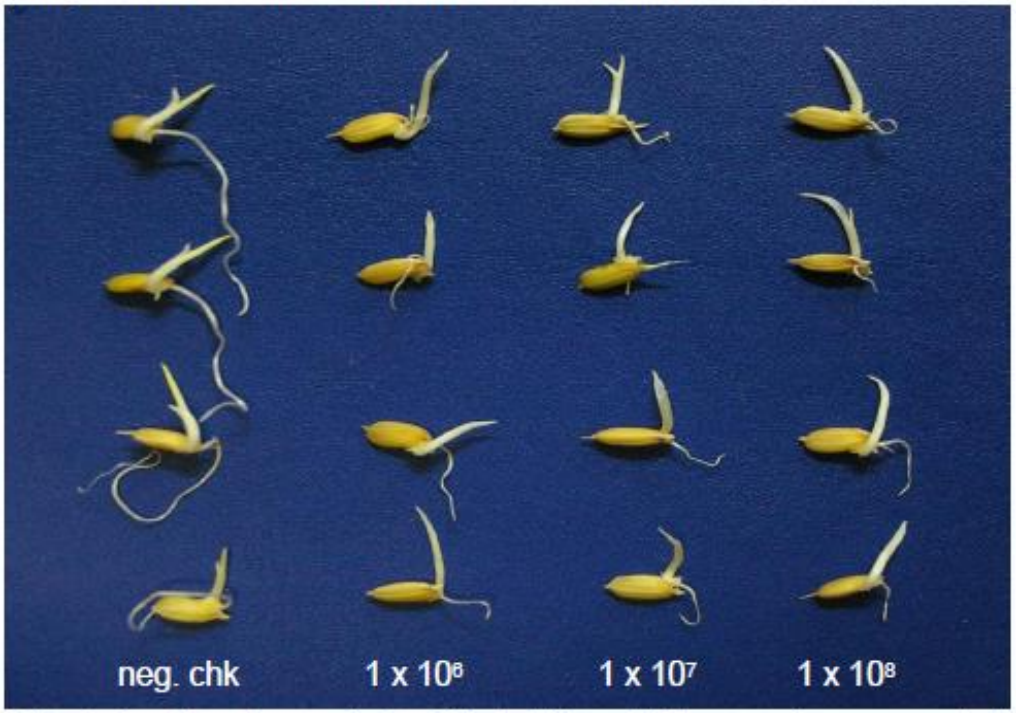
- **Germplasm evaluation for resistance**
- Identify sources of resistance to sheath brown rot in rice for incorporation into breeding program:

1. Effect of soaking rice for 24h on varying concentrations (cfu mL^{-1}) of *P. fuscovaginae*;
2. Inoculation technique comparison experiment.

Management

1. Effect of soaking rice for 24h on varying concentrations (cfu mL^{-1}) of *P. fuscovaginae*

1. Completed test of seed soaking on bacterial suspension for early disease resistance detection

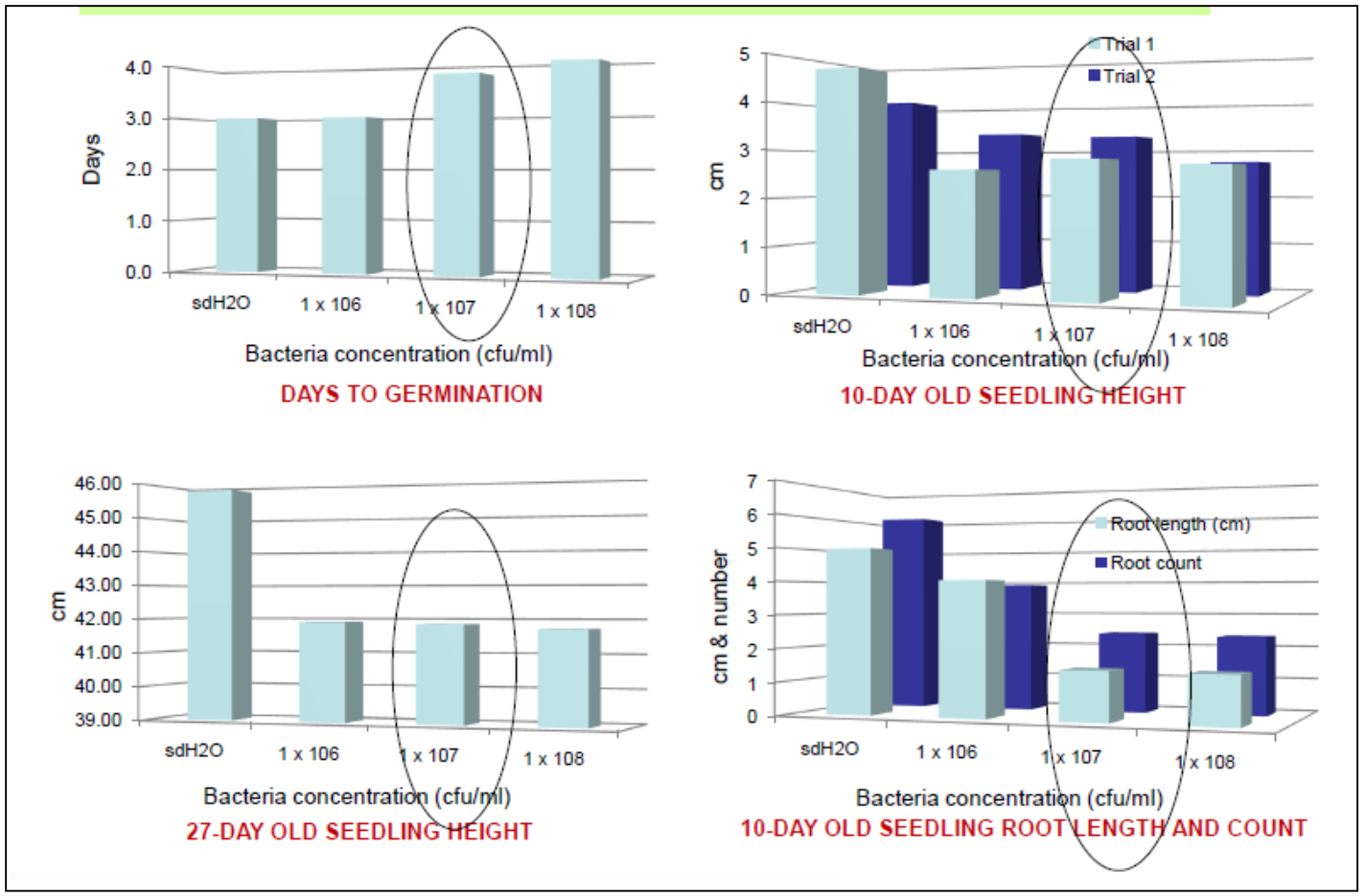


Effect of soaking rice seeds for 24h, in different *P. fuscovaginae* concentrations (cfu mL^{-1}), on seed germination after incubating for 48h at 32°C

Dante L. Adorada

Management

1. Effect of soaking rice for 24h on varying concentrations (cfu mL^{-1}) of *P. fuscovaginae*



Dante L. Adorada

Management

Pseudomonas fuscovginae

2. pin-prick inoculation technique

Pseudomonas savastanoi pv.*savastanoi*

Olive Knot

Disease symptoms on olive
Pseudomonas savastanoi

A. Hyperplasia outgrowths (knots) on olive stems caused by *Pseudomonas savastanoi*, and
B. Extensive knot production on an older olive tree.

Kennelly *et al.*,2007

Disease type

- Olive knot does not kill trees, but it does reduce productivity by destroying twigs and branches, and the flavor of fruit from infected trees may be affected.

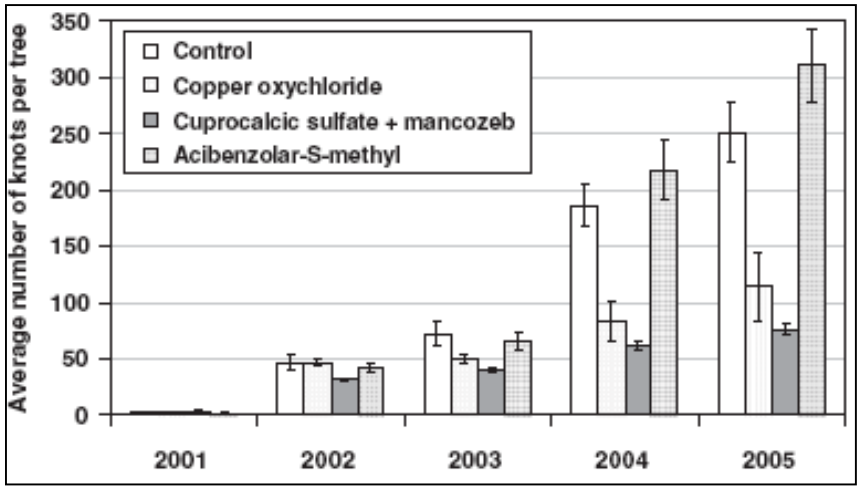
Management

- Bacteria survive in the knots and are readily spread by water at all times of the year.
- Infection occurs at low temperatures, usually in fall or spring. Openings are necessary for penetration of bacteria, and these are provided by leaf scars, pruning wounds, or bark cracks made by freezing.
- Damage can be severe when weather favors disease.
- **Cultural Control**
- Prevent wounding to reduce changes of infection
- Apply copper at post harvest and spring
- Avoid pruning or harvesting in wet weather
- Avoid over fertilizing
- Remove alternate hosts
- Pruning infected trees to remove the disease, burning the prunings.
- Practice good hygiene to minimize spread.

Chemical control

Pseudomonas savastanoi pv. *savastanoi* (Psv)

- Plants of 'Picudo' and 'Arbequina' cultivars were inoculated once with Psv after the plantation and several treatments (copper oxychloride, cuprocalcic sulfate plus mancozeb or acibenzolar-S-methyl, Bion) were applied during fou years.
- The effect of the copper treatments on Psv was more significant in the case of 'Picudo' where the average Psv population densities on untreated control and on treated samples reached 10^4 and 10^2 cfu/g of tissue, respectively.
- Both copper treatments also reduced the proportion of the samples where Psv was isolated.
- No resistance to copper in recovered isolates of Psv was detected.
- Furthermore, the average knot number was significantly lower in the plants treated with copper than in the untreated plants.



Cumulative number of knots per year on cv. 'Picudo' trees after different treatments.
Vertical bars represent the standard deviation of the mean.

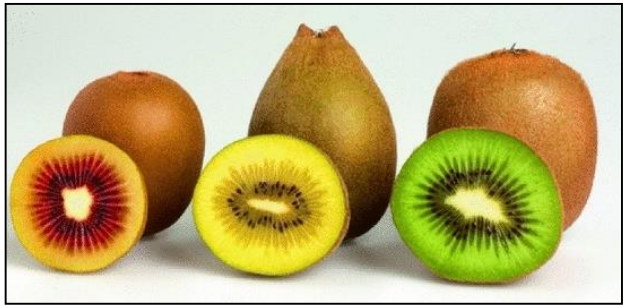
Quesada *et al.*,2008

Pseudomonas syringae pv. *actinidiae*
Highly pathogenic Psa(biovar 3)

Bacterial canker of kiwifruit

Green-fleshed kiwifruit (*Actinidia deliciosa*)

Yellow-fleshed kiwifruit (*A. chinensis*)



Symptoms

Pseudomonas syringae pv. *actinidiae*

- Symptoms of Psa infection are mainly visible in spring and autumn when climatic conditions are favourable.
- The main symptoms include:

1. the production of translucent exudate from tissues,
2. leaf spots in spring that later change to brown, exuding gum from lowers and trunk necrosis.

Balestra *et al.*,2009; EPPO,2020; Narouei Khandan *et al.*,2013

Symptoms

Pseudomonas syringae pv. *actinidiae*

- The symptoms also include:
- Brown-black leaf spots, blossom necrosis, extensive twig dieback;
- Reddening of the lenticels;
- Extensive cankers along the main trunk and leader, and bleeding cankers on the trunk and the leader with a whitish to orange ooze.

Cameron and Sarojini,2013;..

Occurrence

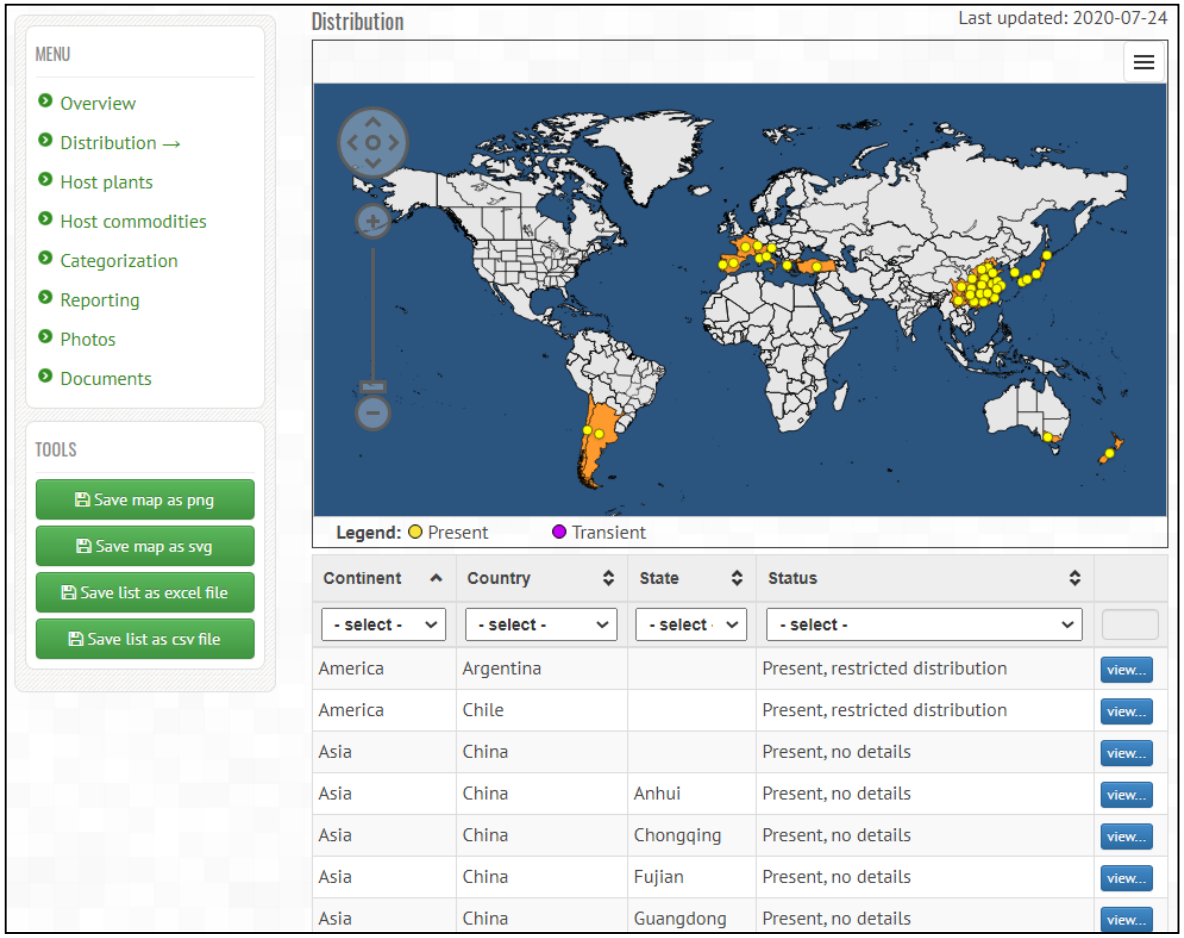
Pseudomonas syringae pv. *actinidiae*

- Many countries in South America, Asia, Europe and Oceania.
- In Asia such as China, Japan, Korea Republic, Turkey.
- Data is lacking on the situation of *P. syringae* pv. *actinidiae* in China.
- In the literature, several papers mention the presence of *P. syringae* pv. *actinidiae* in Iran, but the original publication only refers to *P. syringae* pv. *syringae*.

EPPO,2021

Distribution map

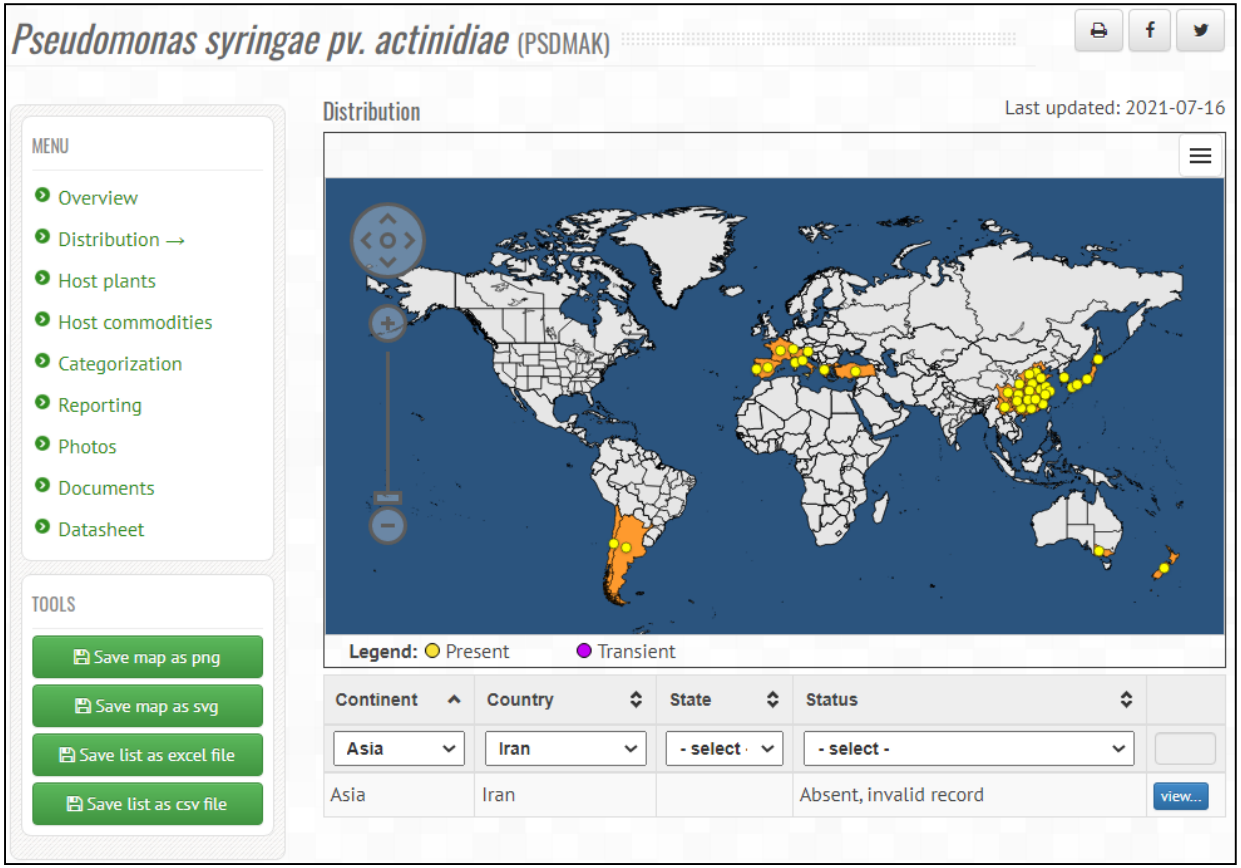
Pseudomonas syringae pv. *actinidiae*



EPPO Website

Distribution map

Pseudomonas syringae pv. *actinidiae*



Mazarei, M. and P. Mostofipour, 1994. First report of bacterial canker of kiwifruit in Iran. *Plant Pathology* **43**(6),1055-1056.

EPPO Website

Bacterial canker of kiwifruit

Pseudomonas syringae pv. *actinidiae*

Economic importance

- The kiwifruit bacterial canker pathogen (Psa), causes enormous economic damages in many kiwifruit producing countries.
- Production of two Kiwifruit varieties i.e. *Actinidia chinensis* var. *chinensis* and *Actinidia chinensis* var. *deliciosa* (cv 'Hayward'), which is mostly concentrated in China, Italy and New Zealand, generates:
- a significant agricultural value of over three billion euros annually,
- with a retail market value worth over ten billion euros (FAO data,2017).

Fujikawa & Sawada,2020

Bacterial canker of kiwifruit

Pseudomonas syringae pv. *actinidiae*

Economic importance

- Formerly, the economic importance of this disease was considered relatively low, until an outbreak in Italy (Latina province) in the spring of 2008 caused economic losses up to 2 million Euros (Balestra *et al.*, 2009).
- Between 2010 and 2012, more than 2000 ha of kiwifruit orchards in Italy have been either destroyed by the disease or cut down, causing severe economic losses for growers, satellite industries, and other stakeholders of the kiwifruit production chain.

Narouei Khandan *et al.*,2013;..

Bacterial canker of kiwifruit

Pseudomonas syringae pv. *actinidiae*

Economic importance

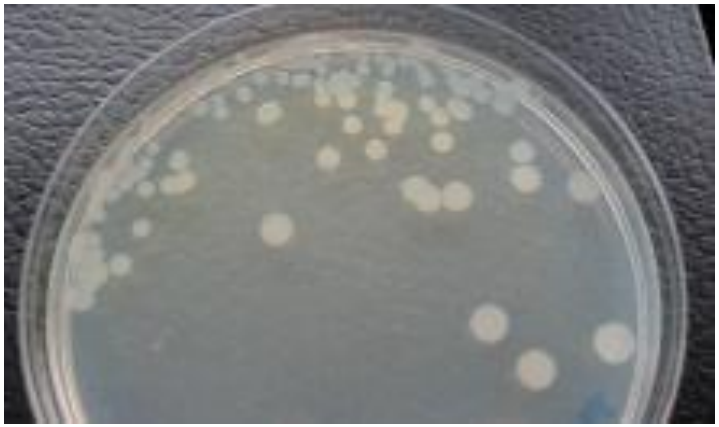
- In New Zealand, it was shown that the virulent strain of biovar 5 (Psa-V) is likely to cost:

1. $310-$410 million over the next 5 years, increasing to approximately
2. $500-$600 million over the next 10 years, and
3. $740-$885 million over the next 15 years (Greer & Saunders, 2012).

Narouei Khandan *et al.*,2013; Vanneste,2017

Diagnostics

Pseudomonas syringae pv. *actinidiae*

	
Morphology of colonies grown for 5 days on King's medium B, supplemented with antibiotics.	Morphology of colonies grown for 5 days on NSA (nutrient-sucrose-agar) medium, supplemented with antibiotics.

EPPO,2020

Diagnostics
Two species and six biovars

- *Pseudomonas syringae* pv. *actinidiae*, the causal agent of bacterial canker of kiwifruit (*Actinidia* spp.), is considered to be a pandemic pathogen and has been isolated around the world over the last 30 years.
- Based on phenotypic, pathogenic, and genomic features, the Pss is grouped into two species and six biovars.
- There are two main species:

1. the highly pathogenic *Pseudomonas syringae* pv. *actinidiae* (Psa/biovar 3), and
2. the less pathogenic *P. syringae* pv. *actinidifoliorum* (Pfm).

County *et al.*,2021

Diagnostics
Two species and six biovars

- *P. syringae* pv. *actinidifoliorum* (Pfm), formerly known as Psa biovar 4 or Psa-LV causes only leaf spots.

1. *P. syringae* pv. *actinidifoliorum* (Pfm), is characterized by its low severity, with the infection not progressing beyond foliar necrotic spots, and not causing important economic and production losses.
2. *P. syringae* pv. *actinidiae* biovar3(Psa3), is able to induce the formation of cankers in stems and conductive branches, compromising the vascular system of the infected plants.

Nunes da Silva *et al.*,2020

Diagnostics
Two species and six biovars

- Strains of *P. syringae* pv. *actinidiae* biovar 1 were isolated in Japan and Italy.
- Strains of *P. syringae* pv. *actinidiae* biovar 2 in South Korea.
- *P. syringae* pv. *actinidiae* biovar 3 was reported first in China and in Italy and some countries.
- *P. syringae* pv. *actinidifoliorum* biovar 4 isolated in New Zealan in 2011.
- *P. syringae* pv. *actinidiae* biovar 5 was recorded in Japan in 2014,
- *P. syringae* pv. *actinidiae* biovar 6.

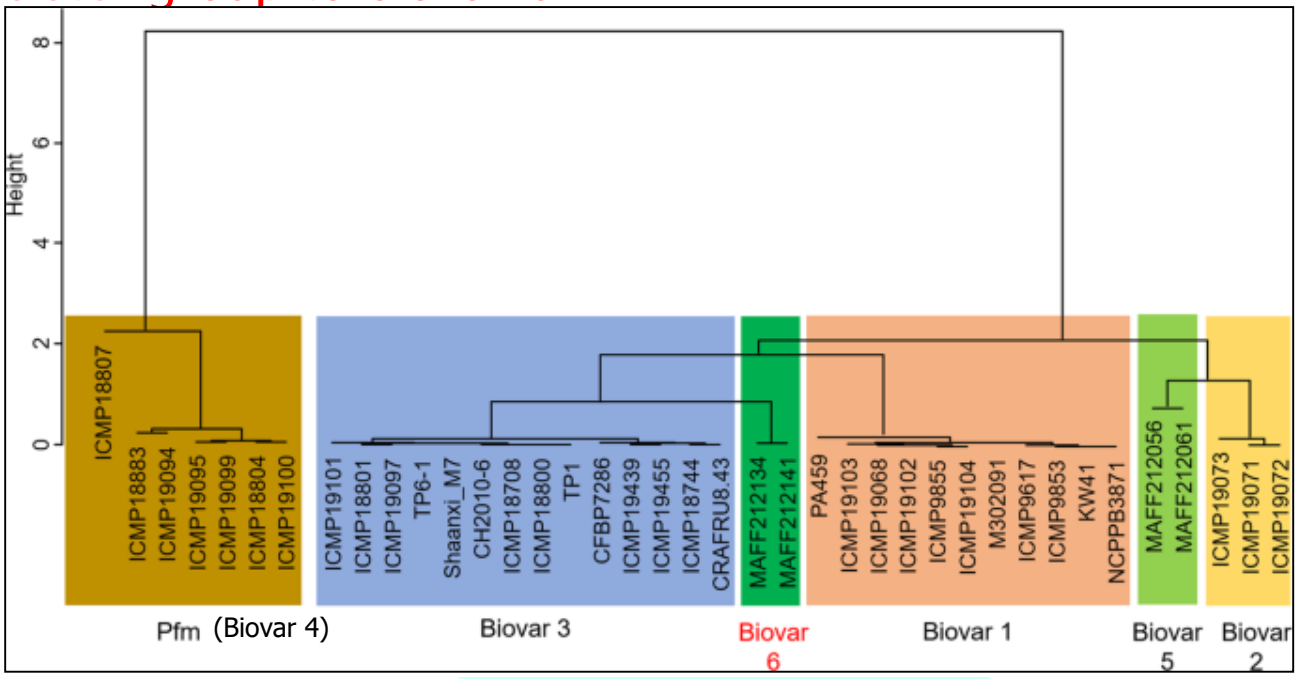
County *et al.*,2021; Fujikawa & Sawada,2019

Bacterial canker of kiwifruit

Pseudomonas syringae pv. *actinidiae*

Biovar characteristics

- The results showed that the two strains of biovar 6 clustered independently of the other Psa biovars and *P. syringae* pv. *actinidifoliorum* (Pfm), and that biovar 3 was the sister group to biovar 6.



Fujikawa & Sawada,2019

Bacterial canker of kiwifruit

Pseudomonas syringae pv. *actinidiae*

Biovar 3 vs. biovar 6

- In 2015, biovar 6, was found in Nagano Prefecture, Japan.
- The genomes of two representative strains of biovar 6 (MAFF 212134 and MAFF 212141) were sequenced and analysed, indicating that their genomes are the somewhat different from biovar 3.

1. Biovar 3 has neither the phaseolotoxin synthesis gene cluster nor the coronatine synthesis gene cluster, whereas
2. Biovar 6 has both clusters and produces both phytotoxins.

Fujikawa & Sawada,2019

Bacterial canker of kiwifruit

Pseudomonas syringae pv. *actinidiae*

Biovar 3 vs. biovar 6

- Another difference:
- Biovar 6 possesses 29 type III secreted effector (T3SE) genes, among which *avrRps4* and *hopBI1* are unique to biovar 6.
- The expression of T3SE genes and two phytotoxin synthesis gene clusters of biovar 6 during the early stages of host infection was investigated.
- These genes could be grouped into three categories:

1. constantly expressed genes,
2. constantly suppressed genes, and
3. temporarily induced genes.

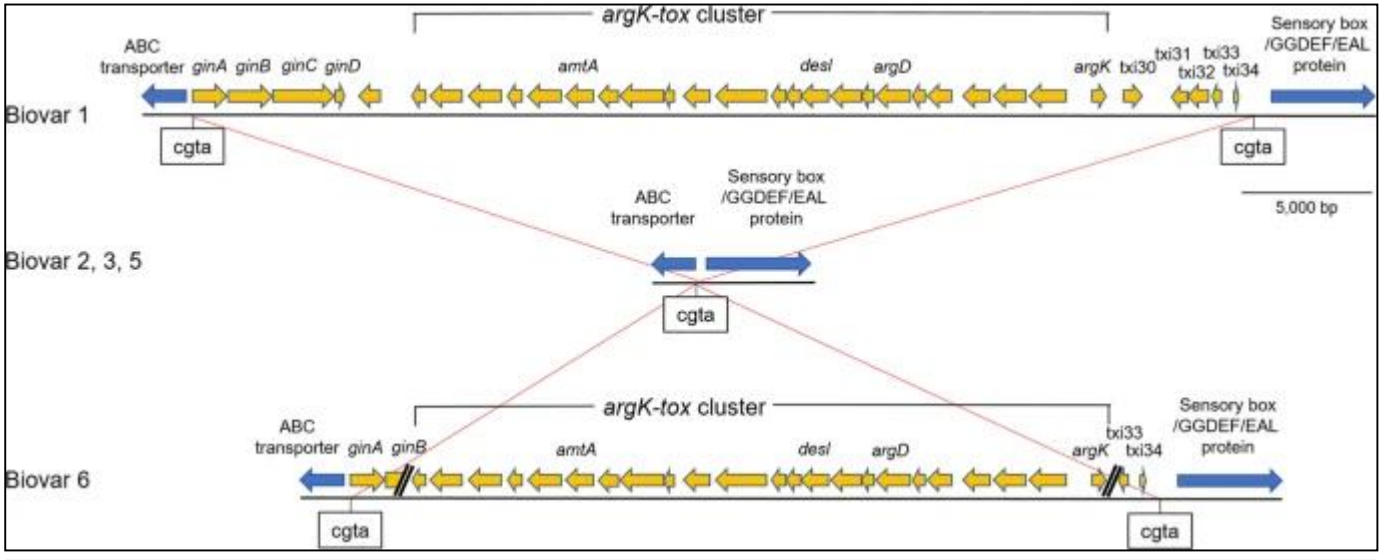
Fujikawa & Sawada,2019

Bacterial canker of kiwifruit

Pseudomonas syringae pv. *actinidiae*

Comparative analysis of the *tox* island region in each biovar

- Locus of the *tox* island, a genomic island which contains *argK-tox* cluster (phaseolotoxin synthesis gene cluster), and its flanking regions in biovar 1 (upper) and biovar 6 (lower) are shown. The corresponding loci in biovars 2, 3, and 5 (middle) are also presented.

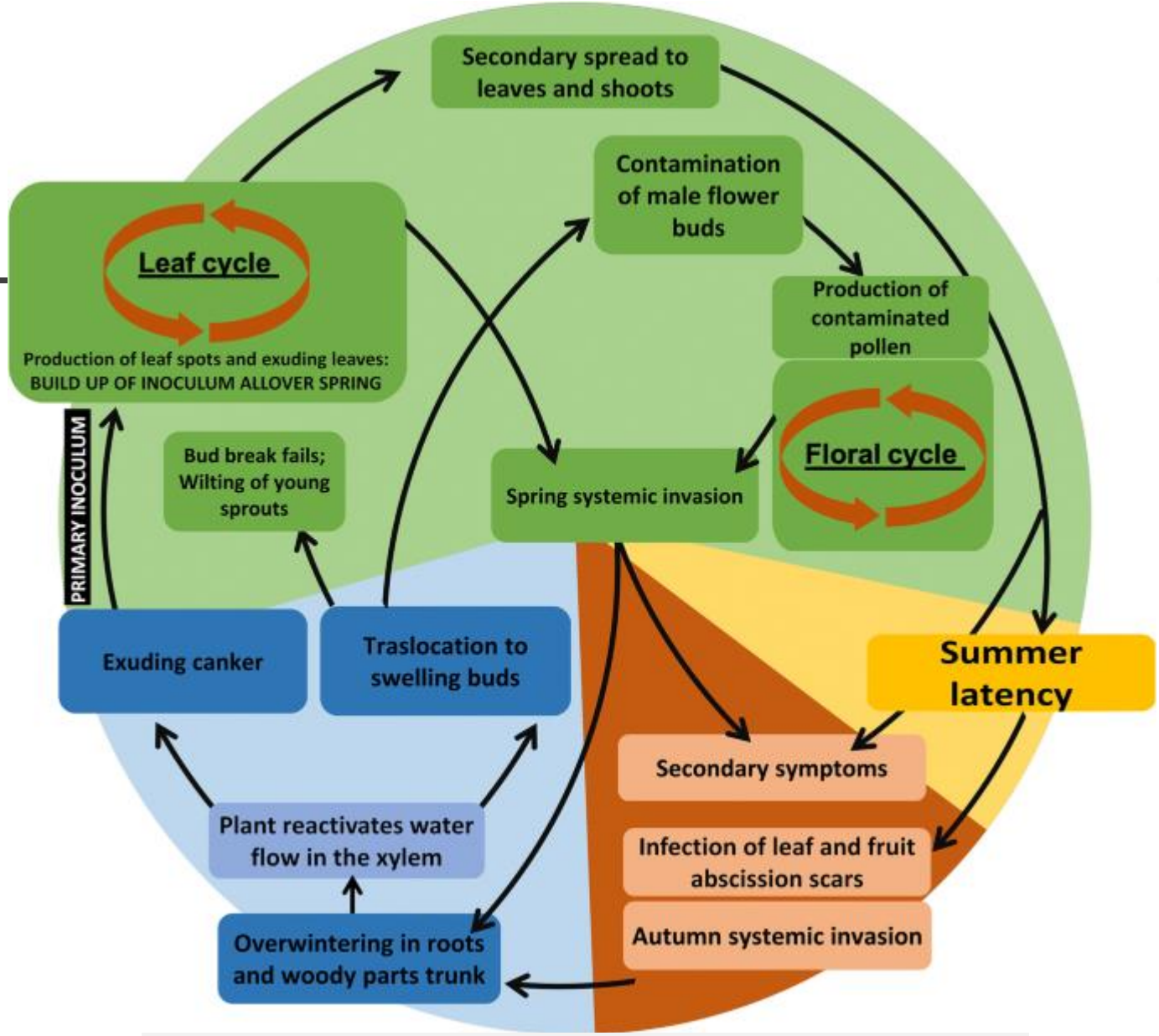


Box arrows indicate predicted genes and their directions. Orange arrows correspond to the *tox* island and blue arrows correspond to its flanking regions.

Disease cycle

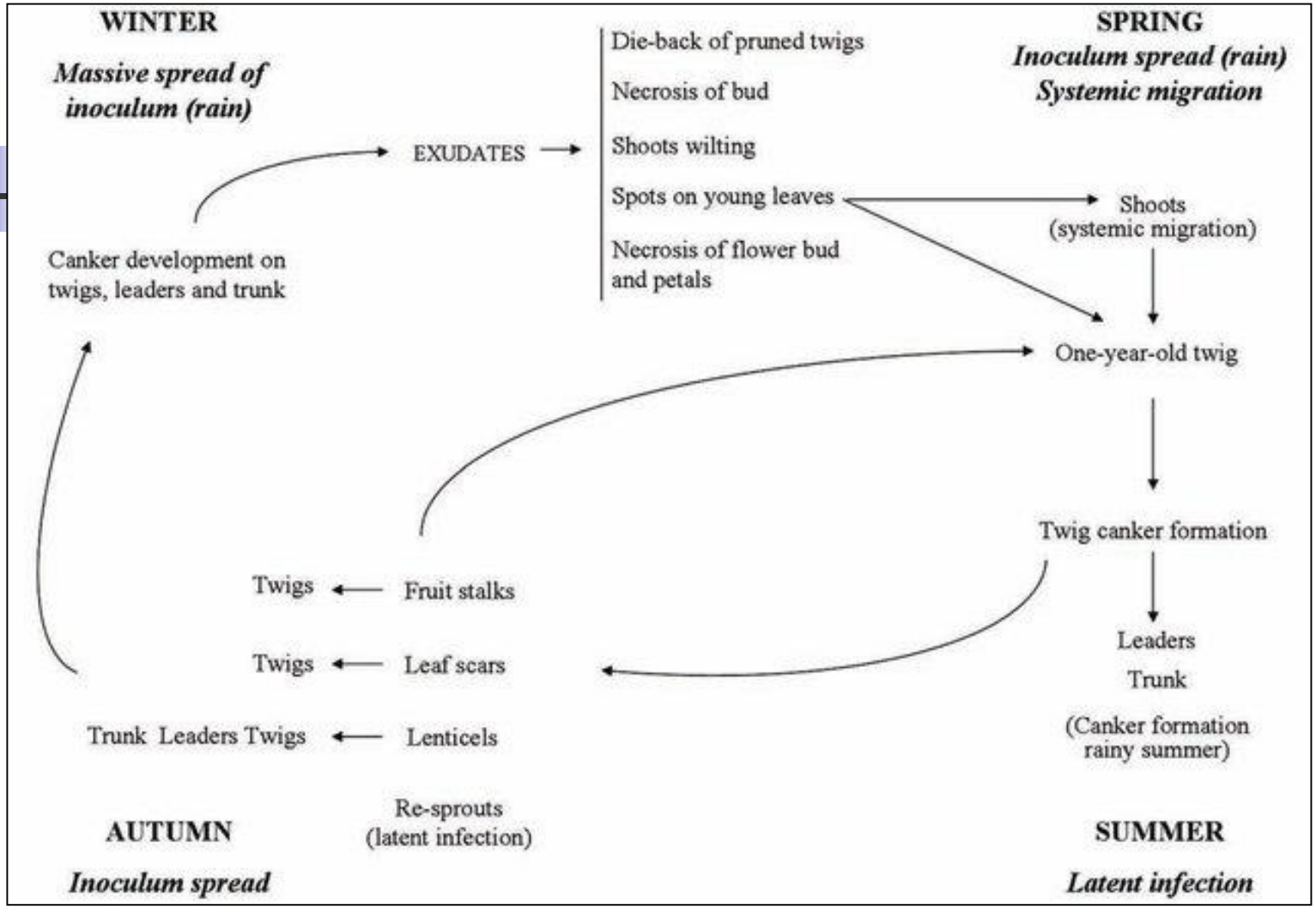
1. Rain and wind can effectively displace bacterial inoculum to the young shoots. Subsequently, flower buds are attacked and show symptoms (i.e. necrosis).
2. Pruning, tying of twigs, irrigation tube scraping the main young trunk which dramatically enhance the possibility of plant colonization of the pathogen through the wounds.
3. Frost is retained as a fundamental predisposing factor.

Scortichini *et al.*,2012



Disease cycle of *Pseudomonas syringae* pv. *actinidiae*

Donati *et al.*,2020

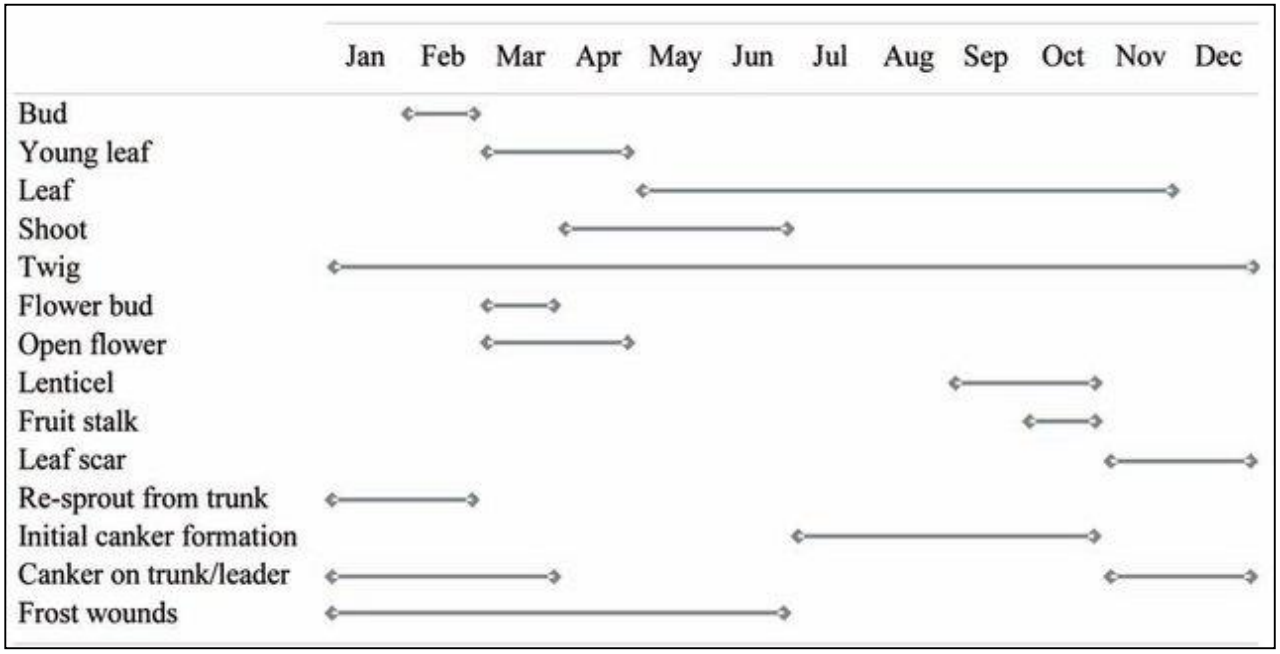


Disease cycle of *Pseudomonas syringae* pv. *actinidiae*

Ferrtante *et al.*,2012

Disease cycle

- Organs and plant parts of *Actinidia chinensis* from which *Pseudomonas syringae* pv. *actinidiae* was consistently isolated and the month when its presence was ascertained.



Scortichini *et al.*,2012; Ferrtante *et al.*,2012

Forecasting Bacterial canker of kiwifruit

Pseudomonas syringae* pv. *actinidiae
(biovar 3)

Bacterial canker of kiwifruit

Influence of temperature and relative humidity on Psa growth in plants

- Experimental inoculations were performed in controlled conditions on 3-year-old potted plants.
- Half of the plants were spray-inoculated with a wild type strain of Psa (biovar 3) and the other half with a green fluorescent protein (GFP) labeling of GFPuv-Psa(GFPuv-Psa) in order to visualize the colonization of the plant tissues by fluorescence stereomicroscope.
- Plants were inoculated with increasing concentrations of the pathogen (approx. 10^2, 10^3, 10^4, 10^5 CFU mL^{-1}) and incubated in different temperature and humidity combinations.

Fujikawa & Sawada,2019

Bacterial canker of kiwifruit

Influence of temperature and relative humidity on Psa growth in plants

- **Combinations** of temperature and relative humidity(%RH) tested (√) for assessing epiphytic and endophytic growth of:
- *Pseudomonas syringae* pv. *actinidiae* in:

1. *A. chinensis* var. *chinensis*,
2. *A. chinensis* var. *deliciosa*

Temperature (°C, ± 2)	Relative Humidity (%, ± 5)			
	20	65	75	90
0			√	
5			√	
7			√	
16			√	
21			√	
24	√	√	√	√
35			√	
40			√	

Pt − 1: epiphytic Psa population at the time point prior T.
AVG: average of all the combinations of temperature and humidity.
Bacterial growth rates were calculated in the logarithmic phase.
For each combination of temperature and RH, the first time point in which an endophytic Psa population occurred (Tn) was considered to estimate the infection threshold. This value was calculated as the average between epiphytic populations at Tn and Tn − 1.

Donati *et al.*,2020

Bacterial canker of kiwifruit

Influence of temperature and relative humidity on Psa growth in plants

- Psa endophytic population was quantified at 3, 6, 24, 48, 72, 96 and 120 h post-inoculation. Symptoms were assessed at 7, 14 and 21 days post-inoculation.
- The infection threshold(IT) was calculated according to the following equation:

$$IT = AVG_{T,RH}\frac{P_t + P_{t-1}}{2}$$

- $_t$: first time point where an endophytic population was found in a specific range of temperature (T) and humidity (RH).
- P_t: epiphytic Psa population at the time point T.

Donati *et al.*,2020

Bacterial canker of kiwifruit

Influence of temperature and relative humidity on Psa growth in plants

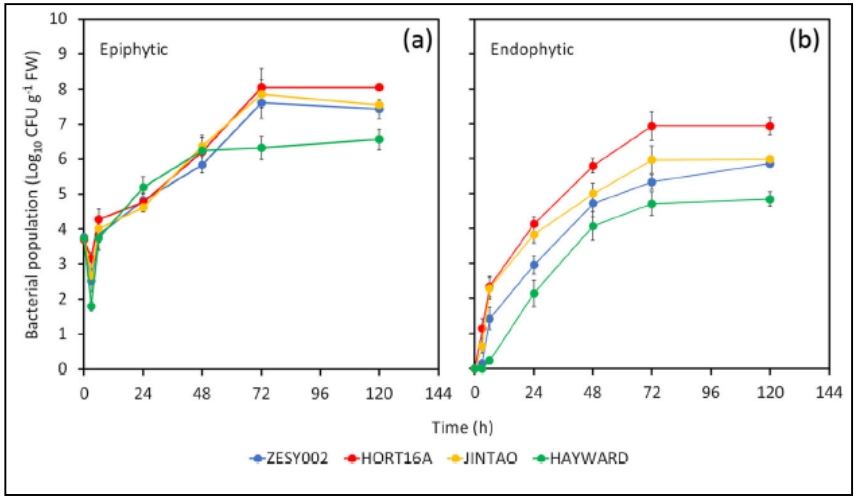
- *P.s yringae* pv. *actinidiae* epiphytic (a) and endophytic (b) growth in four kiwifruit leaves including Hayward.
- The data are the average growth at different combinations of temperature and relative humidity:

1. 16°C, 21°C, 24, 30°C at 65% RH,
2. 24°C at 65%, 75% and 90% RH
3. 24°C at 90% RH.

Maximal population (CFU g^{-1} FW)	Relative Humidity (%, ± 5)		
	65	75	90
Epiphytic	$6.6x10^6$	$1.5x10^6$	$5.0x10^6$
Endophytic	$2.5x10^5$	$1.5x10^8$	$2.5x10^5$

Maximal population (CFU g^{-1} FW)	Temperature (°C, ± 2))			
	16	21	24	35
Epiphytic	$1.9x10^8$	$2.3x10^5$	$1.9x10^6$	$6.8x10^7$
Endophytic	$1.4x10^5$	$1.1x10^6$	$2.0x10^8$	$7.2x10^5$

CFU g^{-1} fresh weight (FW)



The predicted optimal temperature was 21°C for epiphytic growth and 24°C for endophytic growth. Concerning relative humidity, the highest epiphytic growth was observed at 65%, whereas, for endophytic growth the optimal humidity was over 75%.

The investigation of the successful colonization of Psa through various entry points(plant tissues) in the two different species allowed to draw new hypotheses for the different susceptibility between *A. chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa*.

Infection risk (forecasting)model for bacterial canker of kiwifruit

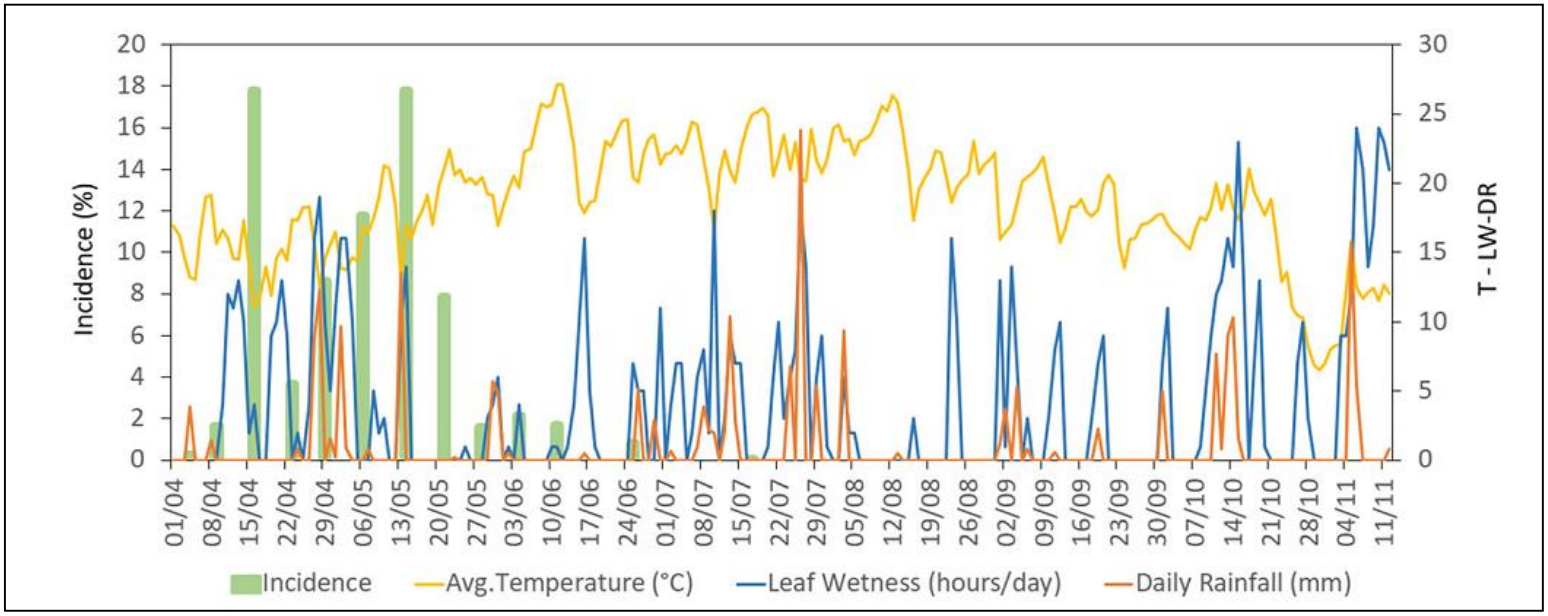
Pseudomonas syringae pv. *actinidiae*

- The environmental conditions also influence the disease incidence and severity in field conditions.
- Experiments conducted in three consecutive years (2013–2015) in three locations suggest that leaf wetness and temperature are the two main drivers of primary symptom occurrence and development (next slide).
- Symptom development concentrates in the spring (April–May), between 7 and 70 days after bud break, as no further increase in leaf spots is observed from late June on.
- In this period, the average daily temperature was about 18.4 ± 3.7°C and symptoms occurred at the same time of periods of leaf wetness.
- Generally, symptoms were observed when leaf wetness in the previous 3 days was lasting more than 4.5 h per day.
- At the increase of temperature around 22.3 ± 2.2°C, symptom occurrence ceased also in presence of leaf wetness periods.
- Finally, the correlation between temperature and relative humidity (%RH) breaks down during temperature falls below a daily average of approximately 15°C.
- During summer, the progress of disease incidence and severity is arrested, most probably as a consequence of temperature, rather than of leaf ontogenetic(origin and development) resistance.

Donati *et al.*,2020

Infection risk (forecasting)model for bacterial canker of kiwifruit

Pseudomonas syringae pv. *actinidiae*



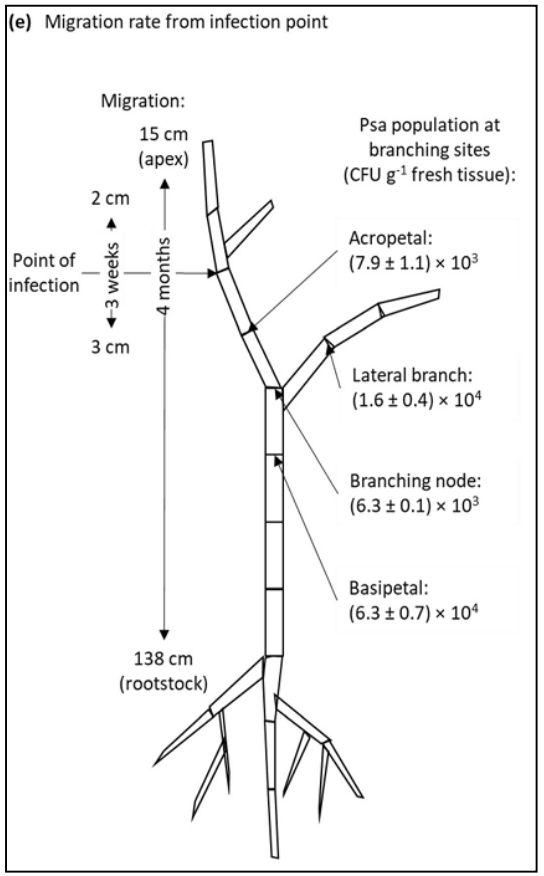
Incidence of *Pseudomonas syringae* pv. *actinidiae* primary symptoms in *A. chinensis* var. *deliciosa* (cv 'Hayward') in relation to daily average temperature, leaf wetness and rain fall. Symptom development had a peak from April(04) to May(05) and almost stopped thereafter. The presented data are calculated as the average of three locations during 2014. Bud break occurred at March 26. Experiments performed in 2013 and 2015 provided comparable results. Temperature low drift (T-LW-DR).

Donati *et al.*,2020

Bacterial canker of kiwifruit

Migration rate of Psa from infection point

- Scheme of the sampling of *A. chinensis* var. *deliciosa* potted plant infected with *Pseudomonas syringae* pv. *actinidiae*, in order to evaluate the rate of migration of bacteria in the plant.
- Psa population was determined in different parts of the plant 3 weeks or 4 months after inoculation in a single point.
- Average endophytic population in the different branching node or section is also reported.



Donati *et al.*,2020

Bacterial canker of kiwifruit

Influence of frost damage on Psa growth in plants

A clear-cut relationship was found between the occurrence of frost events during winter 2007-2008 and the first outbreaks of bacterial canker on *A. chinensis* in the following spring-autumn

The frost events:

Early and late frost events recorded in the areas of *Actinidia chinensis* cultivation of the provinces of Latina (LT) and Rome (RM) from 2007 to 2011 as recorded by thermometers placed in the farms by the Apofruit technical staff.

Apofruit: a fruit and vegetable distributor from the North to the South Italy with its own processing facilities and producers.

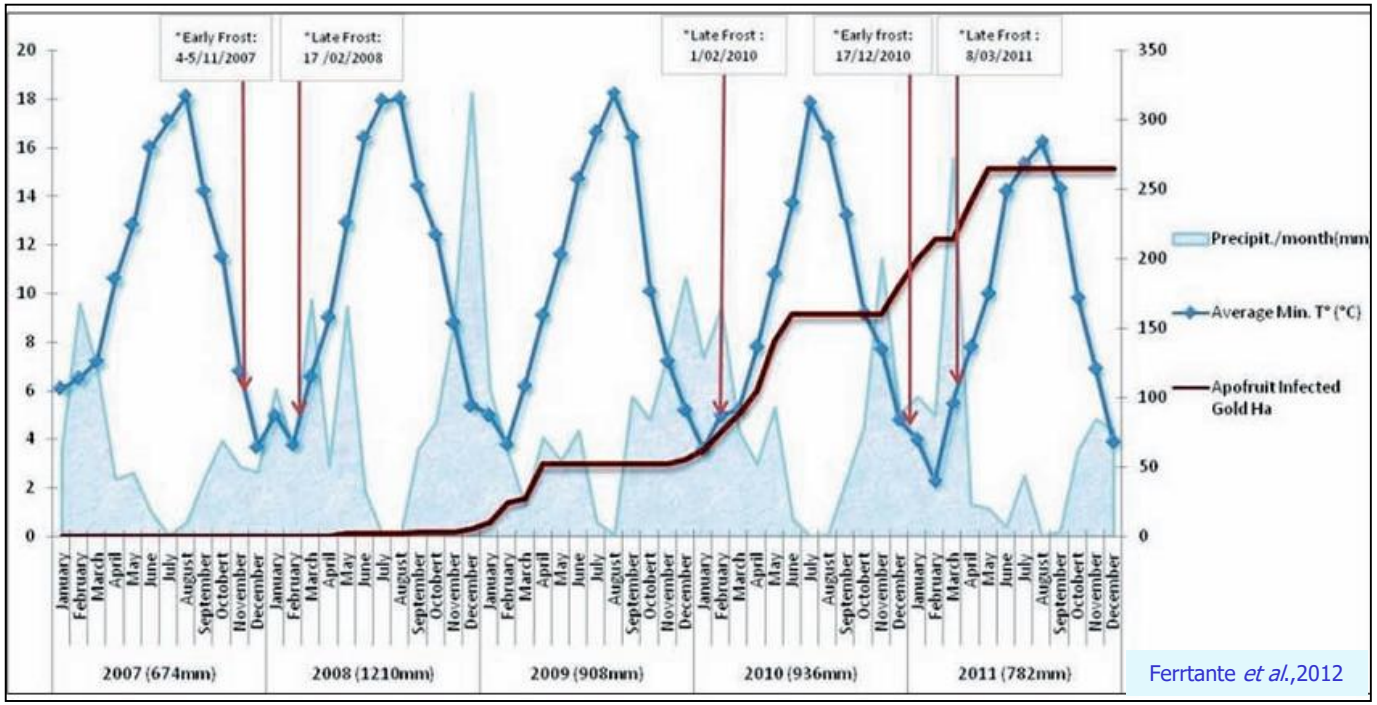
Date of frost event	Municipality/Province	Min T recorded (°C)	Event duration (h)
November 4 2007	Velletri (RM)	- 8	6
	Aprilia (LT)	- 9	6
	Latina (LT)	- 7	4
	Cisterna (LT)	- 5	4
November 5 2007	Velletri (RM)	- 2	4
	Aprilia (LT)	- 2	4
	Latina (LT)	- 1	4
	Cisterna (LT)	- 1	4
February 17 2008	Velletri (RM)	- 8	7
	Aprilia (LT)	- 9	7
	Latina (LT)	- 7	6
	Cisterna (LT)	- 5	6
February 1 2010	Velletri (RM)	- 5	3
	Aprilia (LT)	- 5	3
	Latina (LT)	- 3	3
	Cisterna (LT)	- 2	3
December 17 2010	Velletri (RM)	- 11	10
	Aprilia (LT)	- 12	10
	Latina (LT)	- 8	8
	Cisterna (LT)	- 7	8
March 8 2011	Velletri (RM)	- 8	6
	Aprilia (LT)	- 8	6
	Latina (LT)	- 7	5
	Cisterna (LT)	- 5	4

Ferrtante *et al.*,2012

Influence of temperatures and precipitations in disease incidence

Average temperatures and precipitations recorded from 2007 to 2011 in the kiwifruit production areas of Latina and Rome provinces

- For each year, the total rainfall is reported in brackets. The purple curve shows the increase of bacterial canker incidence in the *Actinidia chinensis* surface area (Ha) tended by Apofruit-Aprilia in the same period. See also Table of the frost events.



Ferrtante *et al.*,2012

Infection risk (forecasting)model for bacterial canker of kiwifruit

Pseudomonas syringae pv. *actinidiae*

- The prediction model for bacterial canker of kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), was developed using the mechanistic scheme composed of the multiplication and the dispersal concepts (Beresford *et al.*, 2017).
- It is based on:

1. the M index(a temperature function),
2. the R index(daily risk indicator), using wetness of 2-day and 6-day rainfall data.
3. R‘ index(modified risk indicator), uses relative humidity (RH) > 81% instead of wetness of 2-day and 6-day rainfall data.

Beresford *et al.*,2017; Morales *et al.*,2017

Infection risk (forecasting)model for bacterial canker of kiwifruit

Pseudomonas syringae pv. *actinidiae*

1. Bacterial multiplication is estimated from a temperature function, the M index, accumulated from hourly air temperature over 3 days for hours when the leaf canopy is wet.
2. The infection risk on the current day is the value of:

- accumulated 3-day M index, called the R index,
- if the rainfall total for the previous 24h is>1mm;

1. Otherwise R (infection risk day) is zero.

Beresford *et al.*,2017; Morales *et al.*,2017

Infection risk model for bacterial canker of kiwifruit

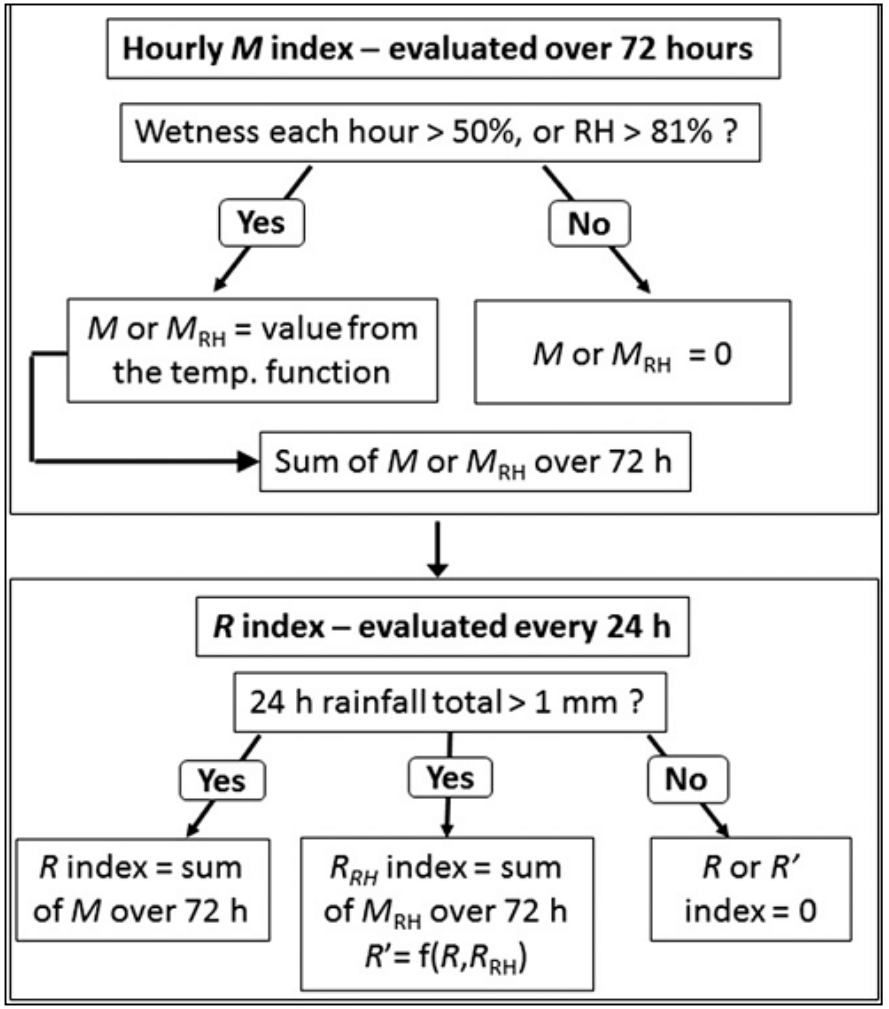
Pseudomonas syringae pv. *actinidiae*

- Scheme for the *Pseudomonas syringae* pv. *actinidiae* **risk model on kiwifruit**.
- **Calculation of the M index:**

1. hourly temperature, and
2. surface wetness data over 3 days and
3. **the R index** from daily rainfall data.

- The **M index** and **R index** (daily risk indicator) or **R' index** (modified risk index) are calculated using hourly relative humidity (RH) and were used in weather forecasting model (Beresford *et al.*,2017).

Morales *et al.*,2017



Two semi-mechanistic model (CLIMEX and MaxEnt) for potential distribution of *P. syringae* pv. *actinidiae*

- The potential distribution of Psa was modelled with two well-used models (CLIMEX and MaxEnt) based on available presence records and environmental data.
- Model projections can provide information to alert decision-makers in kiwifruit-growing regions to prepare for possible incursions of Psa.
- However, in this study because model findings did not agree on the New Zealand validation data, more research is necessary to achieve greater confidence on projections for novel areas

Narouei Khandan *et al.*,2013

CLIMEX

Use of CLIMEX in pest risk analysis for quarantine

- CLIMEX (http://www.ento.csiro.au/climex/climex.htm) is a decision-support system developed by Sutherst and Maywald (1985), in part, to help users to evaluate the risk of establishment of exotic species in relation to climate.
- CLIMEX is now used routinely to define the climatic context for interpretation of local information on species prior to an ecological study.
- Examples of the many applications can be viewed at http://www.ento.csiro.au/climex/bibliography.htm.

To run the specially prepared CLIMEX demo, select CLIMEX demonstration from the Library menu.

CLIMEX

Use of CLIMEX in pest risk analysis for quarantine

- CLIMEX models a species distribution by selecting values for a set of parameters that describe its response to:

1. temperature, moisture and light.
2. Four stress indices (corresponding to cold, hot, wet and dry), and
3. in some cases their interactions, describe the extent to which the population is reduced during the unfavourable season.

Ecoclimatic Index" (EI): The annual potential of a given location or year for population growth is combined with the severity of stresses which depress the size of that population in the unfavourable season, to give an overall measure (the "Ecoclimatic Index" (EI) on a scale of 0-100) of the favourableness of the location or year for the species.

Sutherst *et al.*,2002; Narouei Khandan *et al.*,2013

CLIMEX

Use of CLIMEX in pest risk analysis for quarantine

- The growth and stress indices are combined into an Ecoclimatic Index (EI), to give an overall measure of favourableness of the location or year for permanent occupation by the target species.
- The EI is scaled to an integer between 0 and 100.

1. An EI close to 0 is considered unfavourable for the long-term survival of the species,
2. 1-10 is marginal,
3. 11-25 is favourable, and
4. ≤ 26 is considered very favourable for establishment of that species.

Narouei Khandan *et al.*,2013

CLIMEX

Use of CLIMEX in pest risk analysis for quarantine

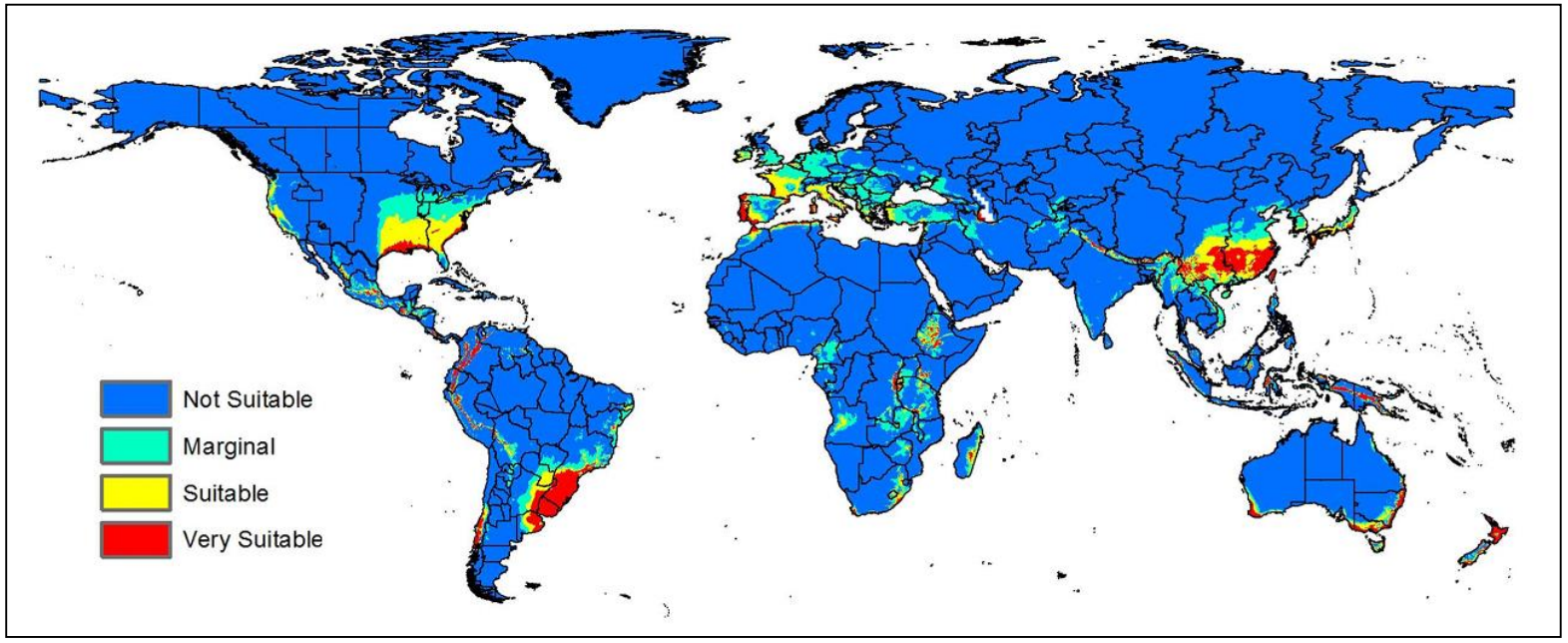
Index	Parameters	Values	Unit
DV0	Lower temperature threshold	5	°C
DV1	lower optimum temperature	12	°C
DV2	upper optimum temperature	20	°C
DV3	Upper temperature threshold	27	°C
SM0	Lower soil moisture threshold	0.5	-
SM1	Lower optimum soil moisture	0.8	-
SM2	upper optimum soil moisture	2	-
SM3	Upper soil moisture threshold	3	-
TTCS	Cold stress temperature threshold	5	°C
THCS	Cold stress temperature rate	-0.00005	$Week^{-1}$
DTCS	Cold stress degree-day threshold	15	°C
DHCS	Cold stress degree-day rate	-0.0001	$Week^{-1}$
TTHS	Heat Stress Temperature threshold	30	°C
THHS	Heat Stress Temperature rate	0.0005	$Week^{-1}$
SDMS	Dry Stress Threshold	0.2	$Week^{-1}$
HDS	Dry Stress Rate	-0.005	Week-1
SMWS	Wet stress threshold (1-10)	2	-
HWS	Wet stress rate	0.001	$Week^{-1}$

The initial temperature index parameters (DV0, DV1, DV2, DV3) were set based on minimum, optimum and maximum temperature requirements of Psa reported by Serizawa *et al.*,1993.

Narouei Khandan *et al.*,2013

CLIMEX

Use of CLIMEX in pest risk analysis for quarantine



Potential distribution of *Pseudomonas syringae* pv. *actinidiae* modelled by CLIMEX. The Ecoclimatic Index (EI) is scaled to an integer between 0 and 100. EI close to 0 indicating that the location is not favourable for the long-term survival of the species. 1-10 is marginal, 11-25 is favourable and ≤26 is considered very favourable for establishment of that species.

Narouei Khandan *et al.*,2013

Management
Pseudomonas syringae pv. *actinidiae*

- Good fertilization, avoidance of overhead irrigation, disinfection of pruning equipment, pruning and destruction of diseased parts, regular inspections of the orchards for disease symptoms, and the use of healthy planting material.
- Chemical control has been implemented in Japan (e.g. with copper compounds and antibiotics), but this has lead to the appearance of resistant strains.
- The development of resistant cultivars and pollinators, effective biocontrol agents, including bacteriophages, and compounds that induce the systemic activation of plant defence mechanisms is in progress.

Scortichini *et al.*,2012;EPPO,2013

Compounds tested against *Pseudomonas syringae* pv. *actinidiae* strains showing inhibition either *in vitro* or *in planta*

Brand name	Active compound	Strain	*In vitro*[a]	*In planta*	References
Antibiotics	Kasugamycin	–	–	50 ppm effective	Serizawa *et al.*, 1989
	Streptomycin	–	–	200 ppm effective	Serizawa *et al.*, 1989
	Ampicillin	All tested	MBC 25 $\mu g\ mL^{-1}$	–	Ferrante & Scortichini, 2010
	Gentamycin	All tested	MBC 10 $\mu g\ mL^{-1}$	–	Ferrante & Scortichini, 2010
	Kanamycin	All tested	MBC 10 $\mu g\ mL^{-1}$	–	Ferrante & Scortichini, 2010
	Streptomycin	All tested	MBC 10 $\mu g\ mL^{-1}$	–	Ferrante & Scortichini, 2010
	Tetracycline	All tested	MBC 10 $\mu g\ mL^{-1}$	–	Ferrante & Scortichini, 2010
	Oxytetracycline	CJW3	MIC 1 $\mu g\ mL^{-1}$	–	Lee *et al.*, 2005
	Oxytetracycline	JJG10	MIC 10 $\mu g\ mL^{-1}$	–	Lee *et al.*, 2005
	Oxytetracycline	JYG10	MIC 25 $\mu g\ mL^{-1}$	–	Lee *et al.*, 2005
	Oxytetracycline	Pal2	MIC 50 $\mu g\ mL^{-1}$	–	Lee *et al.*, 2005
	Streptomycin	CJW3	MIC 10 $\mu g\ mL^{-1}$	–	Lee *et al.*, 2005
	Streptomycin	Pal1	MIC 500 $\mu g\ mL^{-1}$	–	Lee *et al.*, 2005
	Streptomycin	Pal2	MIC 500 $\mu g\ mL^{-1}$	–	Lee *et al.*, 2005
	Streptomycin	PaK2	MIC 500 $\mu g\ mL^{-1}$	–	Lee *et al.*, 2005
	Streptomycin	Pas33	MIC 500 $\mu g\ mL^{-1}$	–	Lee *et al.*, 2005
	Myomycin	Pal1	MIC 50 $\mu g\ mL^{-1}$	–	Han *et al.*, 2003
	Myomycin	Pal2	MIC >500 $\mu g\ mL^{-1}$	–	Han *et al.*, 2003
	Myomycin	PaK2	MIC >500 $\mu g\ mL^{-1}$	–	Han *et al.*, 2003
	Myomycin	PaS33	MIC >500 $\mu g\ mL^{-1}$	–	Han *et al.*, 2003
	Streptomycin	Pal1	MIC >1000 $\mu g\ mL^{-1}$	–	Han *et al.*, 2003
	Streptomycin	Pal2	MIC >1000 $\mu g\ mL^{-1}$	–	Han *et al.*, 2003
	Streptomycin	PaK2	MIC >2000 $\mu g\ mL^{-1}$	–	Han *et al.*, 2003
	Streptomycin	PaS33	MIC >2000 $\mu g\ mL^{-1}$	–	Han *et al.*, 2003
	Streptomycin	Pa1	MIC 3·5 $\mu g\ mL^{-1}$	–	Nakajima *et al.*, 1995
	Streptomycin	Pa11	MIC 3·5 $\mu g\ mL^{-1}$	–	Nakajima *et al.*, 1995
	Streptomycin	Pa423	MIC 400 $\mu g\ mL^{-1}$	–	Nakajima *et al.*, 1995
	Streptomycin	Pa429	MIC 600 $\mu g\ mL^{-1}$	–	Nakajima *et al.*, 1995
	Streptomycin	Pa430R	MIC 500 $\mu g\ mL^{-1}$	–	Nakajima *et al.*, 1995
	Streptomycin	Pa430S	MIC 700 $\mu g\ mL^{-1}$	–	Nakajima *et al.*, 1995
	Streptomycin	Pa431	MIC 400 $\mu g\ mL^{-1}$		Nakajima *et al.*, 1995

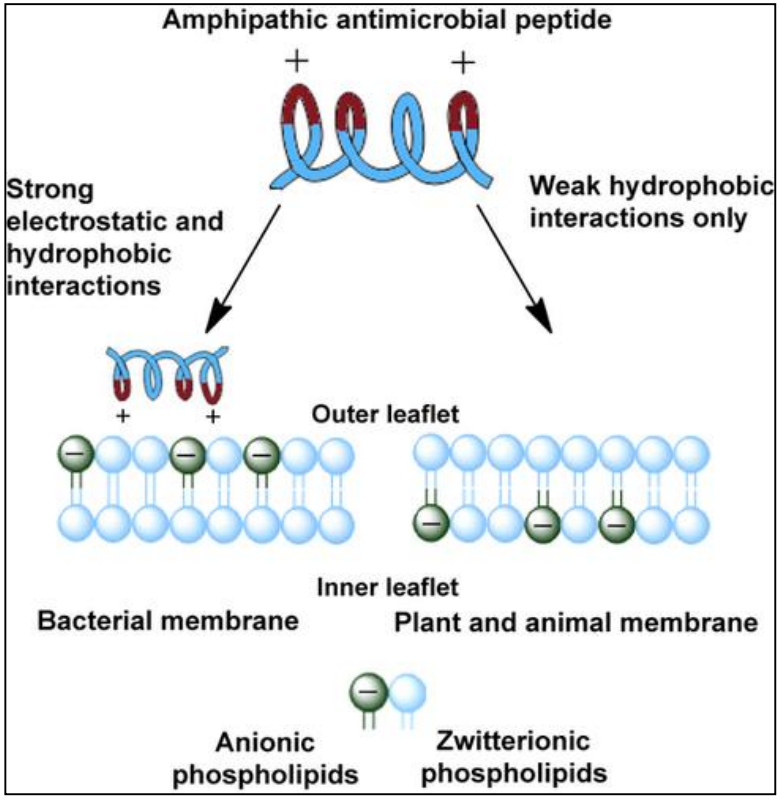
Cameron and Sarojini,2013

Management

Pseudomonas syringae pv. *actinidiae*

Antibacterial peptides

- A theoretical amphipathic α-helical antimicrobial peptide showing selectivity for the bacterial plasma membrane over those of plants and animals.
- Dark grey (red on-line) = cationic (+vely charged) and polar residues,
- light grey (blue on-line) = hydrophobic residues.



Amphipathic molecules are chemical compounds containing both polar and nonpolar (apolar) portions in their structure.

Cameron and Sarojini,2013

Management
Pseudomonas syringae pv. *actinidiae*

- **Useful websites:**

1. Up-to-date information on bacterial canker research progress and on the spread of the disease in New Zealand can be found at: http://www.kvh.org.nz.
2. Daily information on the spread of the disease and on the research being performed worldwide can be found at: http://www.freshplaza.it.

Scortichini *et al.*,2012;EPPO,2013

Pseudomonas syringae pv. *berberidis*

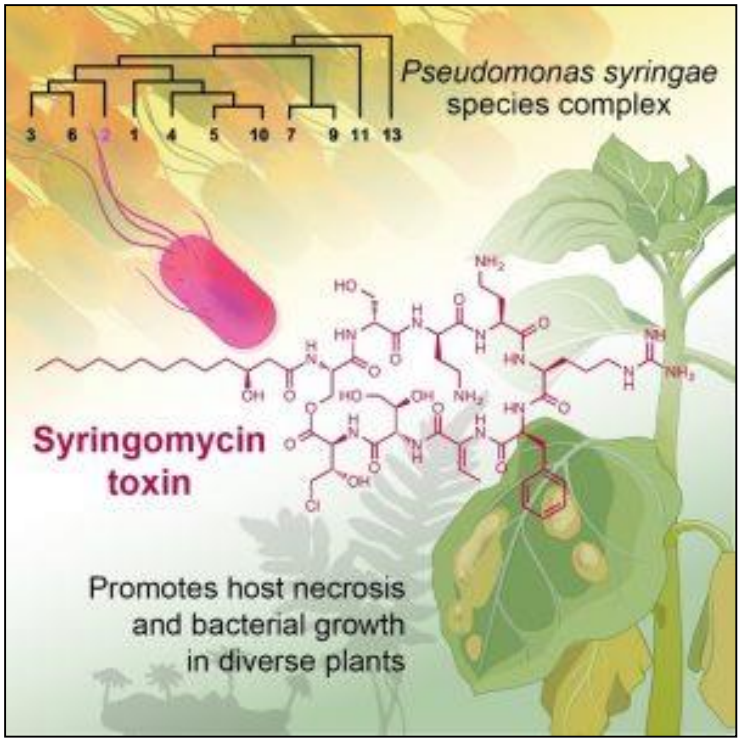
Leaf Spot of Barberry (*Berberis*)

Cause and symptoms

- **Symptoms**
- Lesions first appear water soaked, becoming very dark brown or black. Chlorotic halos usually are present. Leaves may fall prematurely, and lesions may occur on petioles and succulent twigs.
- **Cause**
- *Pseudomonas syringae* pv. *berberidis*, a bacterium.
- The disease is favored by cool, wet weather in spring.
- Two common genetic traits increase the bacteria's ability to cause disease:
- Most produce a powerful plant toxin, syringomycin, that destroys plant tissues as bacteria multiply in a wound.
- Bacteria also produce a protein that acts as an ice nucleus, increasing frost wounds that bacteria easily colonize and expand.

Characteristics of the species
Syringomycin production

- The toxin syringomycin produced by the most widely infectious *P. syringae* strains, and compared its effect on both non-flowering and flowering plants.
- The toxin syringomycin likely interferes with cell membranes across each of the diverse plants.
- A necrotizing toxin enables *Pseudomonas syringae* infection across evolutionarily divergent plants appears in Cell Host and Microbes.

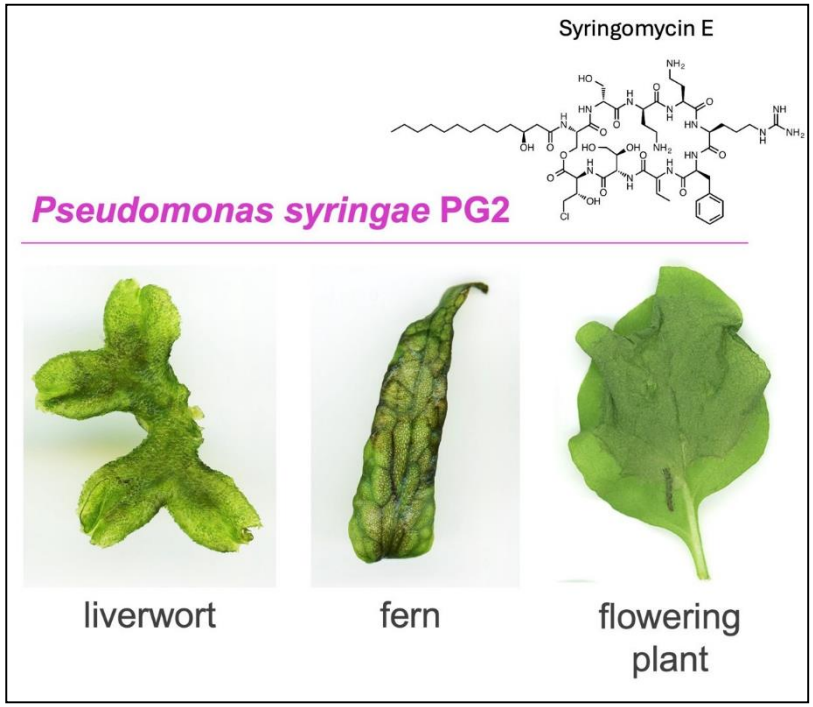


Evolutionary study reveals the toxic reach of disease-causing bacteria across the Plant Kingdom.

Characteristics of the species

Syringomycin production

- Three model plants showing disease symptoms after infection with *Pseudomonas syringae*.
- The toxin Sringomycin shown is critical to establish disease symptoms.



Management

- **Cultural control**
- Keep foliage dry.
- Protect from winter injury.
- **Chemical control**
- Fall and spring applications, used for other crops, may be effective on barberry.
- Fixed copper products may be helpful.
- Junction at 1.3 lb/100 gal water. Useful against copper-resistant bacteria. 24-hr reentry.
- Nu-Cop 50 DF at 1 lb/100 gal water.
- Not specifically registered on barberry so use on a few test plants and observe for phytotoxicity before general use.
- 4-hr reentry.
- Phyton 27 at 1.5 to 3.5 oz/10 gal water.

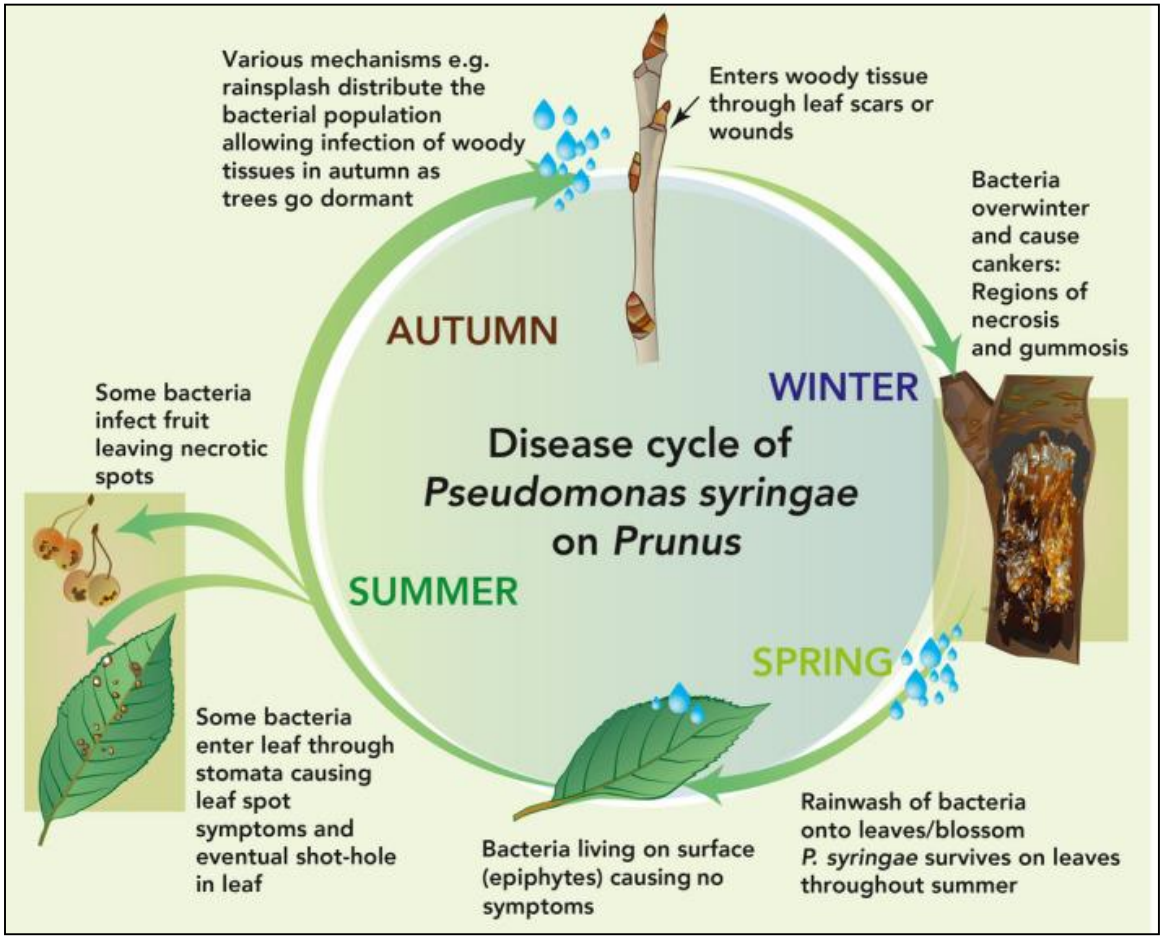
Pscheidt,2007

Pseudomonas syringae pv. *persicae*

Bacterial canker of peach

Disease cycle

Pseudomonas syringae pvs. *syringae, morsprunorum, persicae, avii,..*



Hulin *et al.*,2020

Management

- Caused by *Pseudomonas* spp. currently is almost unattainable, due to the lack of effective chemical or biological control measures, lack (and little available knowledge) of host resistance, and the endophytic nature of the pathogen during some phases of the disease cycle.
- Calcium nutrition- Calcium is important for physiological processes, including resistance to parasites e.g. *P.s.* pv. *persicae* (Vigouroux *et al.*,1989).
- Copper compounds are the standard bactericides for controlling many bacterial diseases.
- Recently, several research groups have screened wild cherry clones for resistance to *P. syringae* pvs. *morsprunorum*, *syringae*, *persicae*, and *avii* in greenhouse- and field- grown plants.

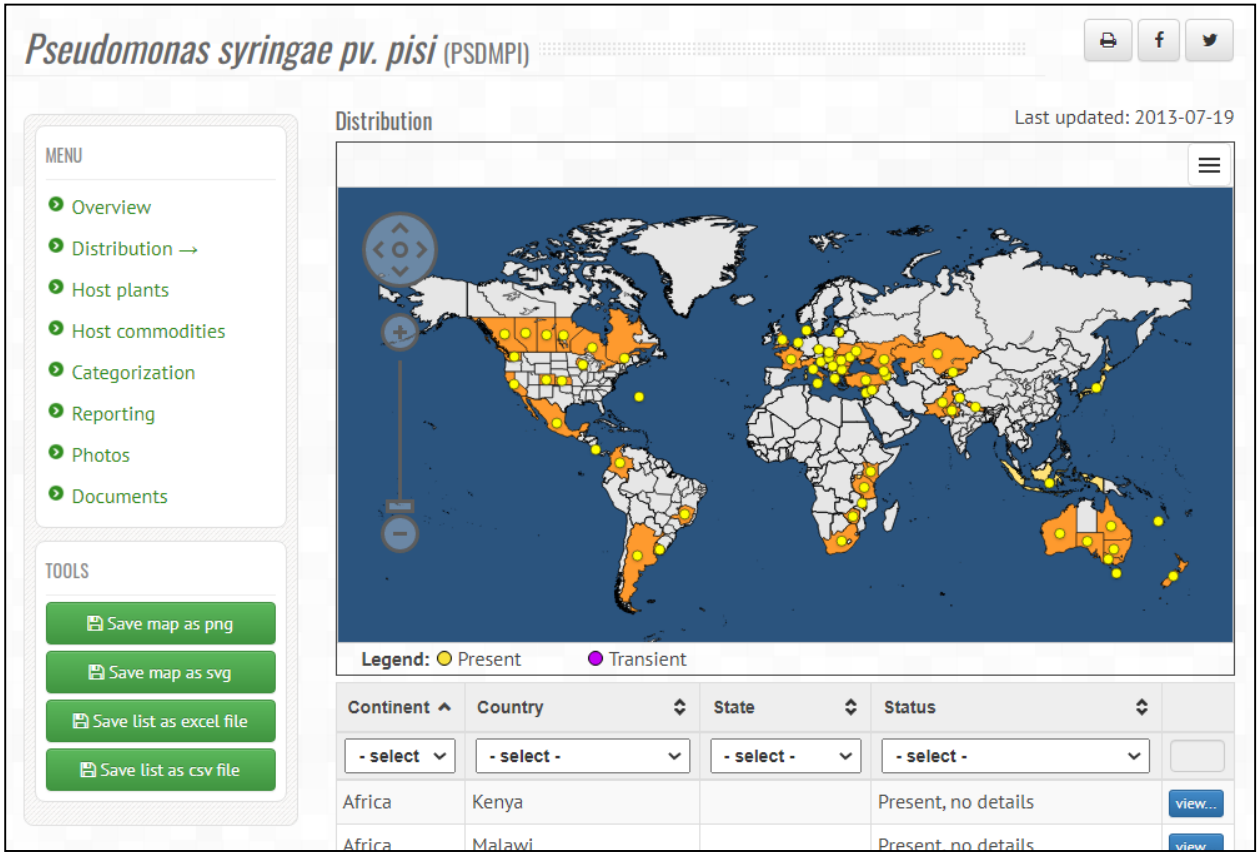
Kennelly *et al.*,2007

Pseudomonas syringae pv. *pisi*

Pea bacterial blight

Distribution

Pseudomonas syringae pv. *pisi*



EPPO Global Database

Management

Pseudomonas syringae pv. *persicae*

- Bacterial blight is a widely distributed and potentially damaging disease of peas.
- The disease is seed-borne i.e. the pathogen can survive on or within seeds between seasons and seed infection remains the most important source of disease outbreaks.
- However, diseased pea debris and infected volunteer plants can also play a role in overwintering and crop rotations are usually considered as part of a strategy in controlling pea blight (Hollaway and Bretag,1997).

Pseudomonas syringae pv. *syringae*

On different hosts

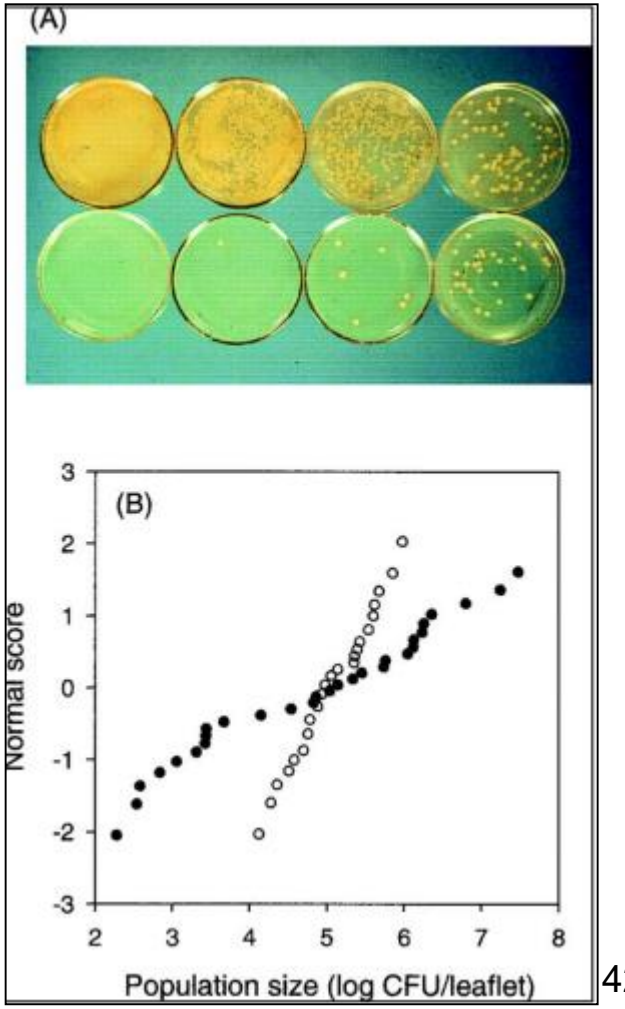
Quantitative variability in population sizes of *P. syringae* on individual leaves

A. Each plate represents an equivalent dilution from washings of different individual rye leaves.

B. Lognormal distribution of population sizes of *P. syringae* on two sets of individual bean leaflets.

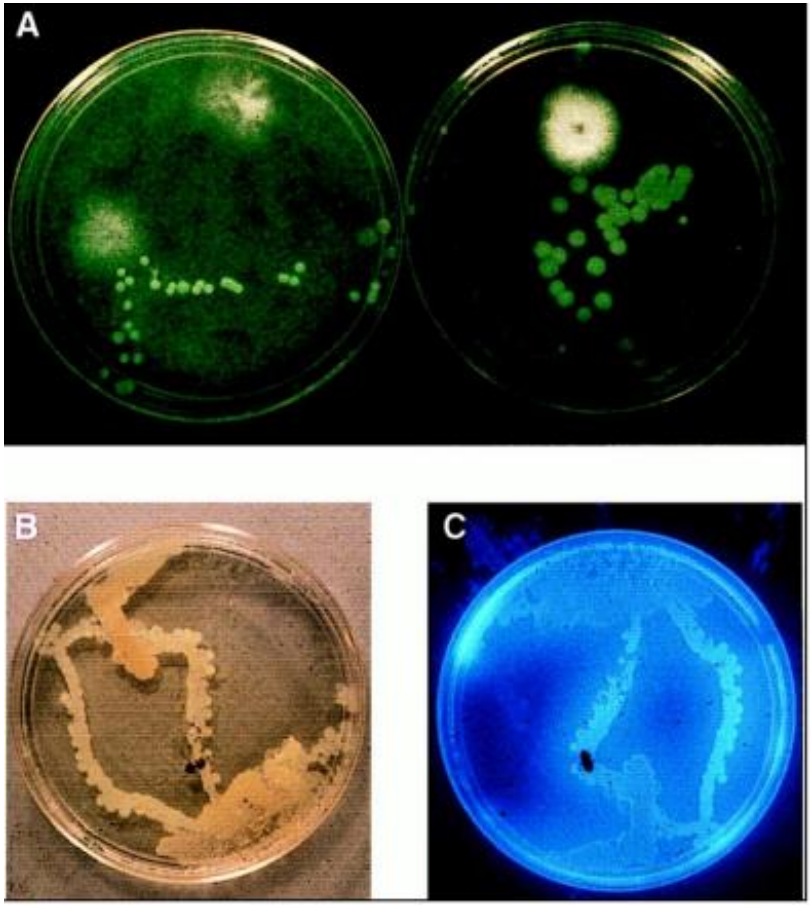
- The mean population size for both sets of leaflets is approximately 5.0 log CFU/leaflet.
- The population variances are 2.5 for set A and 0.23 for set B.

At least 25%, and usually more, of the viable *P. syringae* were recoverable by plating from leaves over a range of conditions in the growth chamber.



Vectors

- (A) Nonrandom distribution of *P. syringae* colonies on a semiselective medium for *P. syringae*. The petri dishes, deployed at canopy height in a bean field, were exposed from 0600 to 0900 h, when leaves were wet with dew.
- (B and C) An insect (*Glischrochilus quadrisignatus*) was trapped in a sterile empty petri dish exposed in a bean canopy in the early morning. The trapped insect was transferred to a petri dish containing King's medium B and allowed to walk over the surface of the medium. The white colonies in panel B are *P. syringae*.
- (C) The colonies fluoresced under UV light. Panel B is reprinted from reference with the permission of the publisher.



Management

Pseudomonas syringae pv. *syringae*

- The pathogen has a wide host range and survives epiphytically. It commonly overwinters in buds and leaf scars of fruit trees.
- In both apple and pear, the disease is favoured by temperatures fluctuating around freezing point, high soil moisture, and high N-fertilization.
- The affected parts turn black in colour and die back.

Basic strategy

- Pruning away diseased plant parts is the only possibility for controlling the disease in apple.
- For controlling blossom blight in pear, a delayed dormant spray with copper-containing chemicals has been recommended, but is frequently not effective.
- Pruning of affected blossoms and shoots may help to reduce spread of the disease. Growing of pear cultivars with a lower susceptibility is recommended.
- The elimination of a gene from a nonpathogenic *Pseudomonas syringae* that codes for ice formation at relatively high temperatures made history (Lindow,1987) in an ice-minus derivative that prevents frost damage when applied to plants.

Pseudomonas syringae pv. *syringae*; *P. syringae* pv. *morsprunorum*

Bacterial canker of stone fruits cherry, apricot, etc.

Canker of apricot

Canker of Cherry

Disease cycle of canker and gummosis of stone fruits caused by *Pseudomonas syringae* pv. *syringae*

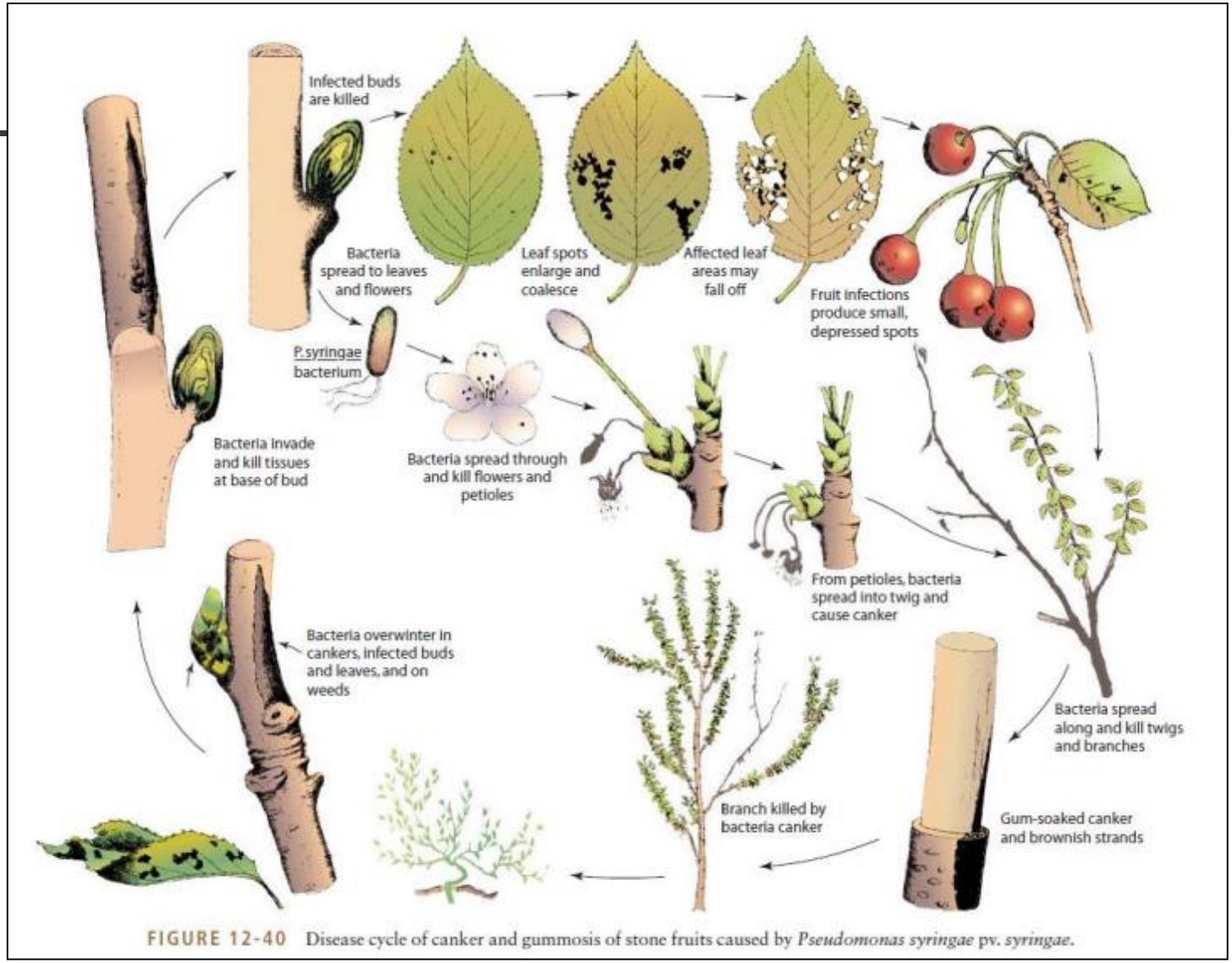
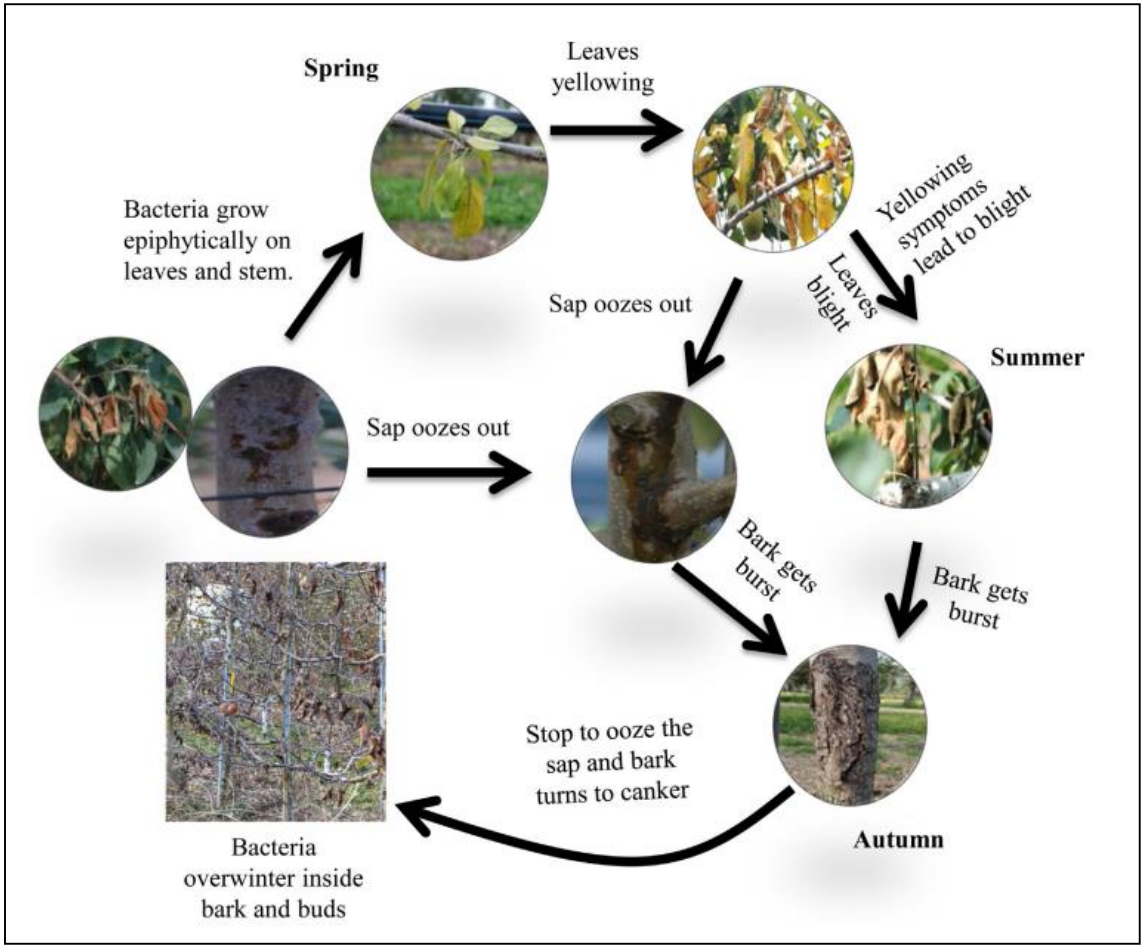


FIGURE 12-40 Disease cycle of canker and gummosis of stone fruits caused by *Pseudomonas syringae* pv. *syringae*.

Agrios,2005

The life cycle of *Pseudomonas syringae* pv. *syringae* (Pss) on the apple tree



Lee *et al.*,2023

Bacterial canker of stone fruits caused by *Pseudomonas syringae* pv. *syringae*; *P. syringae* pv. *morsprunorum*

Management

Pseudomonas syringae pv. *syringae*; *P. syringae* pv. *morsprunorum*

- Delayed pruning may help.
- Of the rootstocks commonly used for cherries in California, Mahaleb is the most tolerant of bacterial canker, Colt is moderately susceptible, and Mazzard is susceptible.
- Copper sprays applied at the beginning and end of leaf fall have given variable control.
- **Treatment Decisions**
- In light, sandy soils and in some heavy soils, control has been achieved with preplant fumigation for nematodes.
- Nematodes stress trees, which predisposes them to bacterial canker.
- The benefits of preplant soil fumigation for control of bacterial canker usually last only a few years; in some areas only limited improvements in disease control occur following soil fumigation.
- Following planting, if bacterial canker occurs in an orchard, treat all trees with fenamiphos in that area of the orchard on a yearly basis until the trees are 8 years old.

Pseudomonas syringae pv. *syringae*

Bacterial canker of citrus

Management

Pseudomonas syringae pv. *syringae*

- **Cultural Control**
- Planting windbreaks and using bushy cultivars with relatively few thorns help prevent wind injury;
- Pruning out dead or diseased twigs in spring after the rainy period reduces the spread of the disease; and
- Scheduling fertilization and pruning during spring or early summer prevents excessive new fall growth, which is particularly susceptible to blast infection.
- **Organically Acceptable Methods**
- Cultural controls and Bordeaux sprays are acceptable for use in organically managed citrus groves.

Pseudomonas syringae pv.*syringae*

Citrus blast

Disease symptoms

- Bacterial blast infections of citrus occur during cool or wet weather during the winter or spring and usually start as black lesions in the leaf petiole and progress into the leaf axil.
- Once the petiole is girdled, leaves wither, curl, and eventually drop.
- Entire twigs may die back.
- Diseased areas are covered with a reddish brown scab.
- Infections result in small black spots on the fruit.

Black lesions in leaf petiole and axil

Withering leaves

UC IPM Online,2009

Management

Pseudomonas syringae pv. *syringae*

- Preventative treatment against bacterial blast alone is generally not economical, but sprays against brown rot or Septoria may provide some protection against bacterial blast.
- Certain cultural practices can reduce the incidence of bacterial blast.
- Pruning out dead or diseased twigs in spring after the rainy period reduces the spread of bacterial blast.
- Scheduling fertilization and pruning during spring or early summer prevents excessive new fall growth, which is particularly susceptible to blast infection.
- Bordeaux sprays applied before the first rain may help prevent bacterial blast.

UC IPM Online,2009

Pseudomonas syringae pv.*tabaci*

Wildfire of tobacco or soybean

Disease cycle of a bacterial leaf blight
Pseudomonas syringae pv. *tabaci*

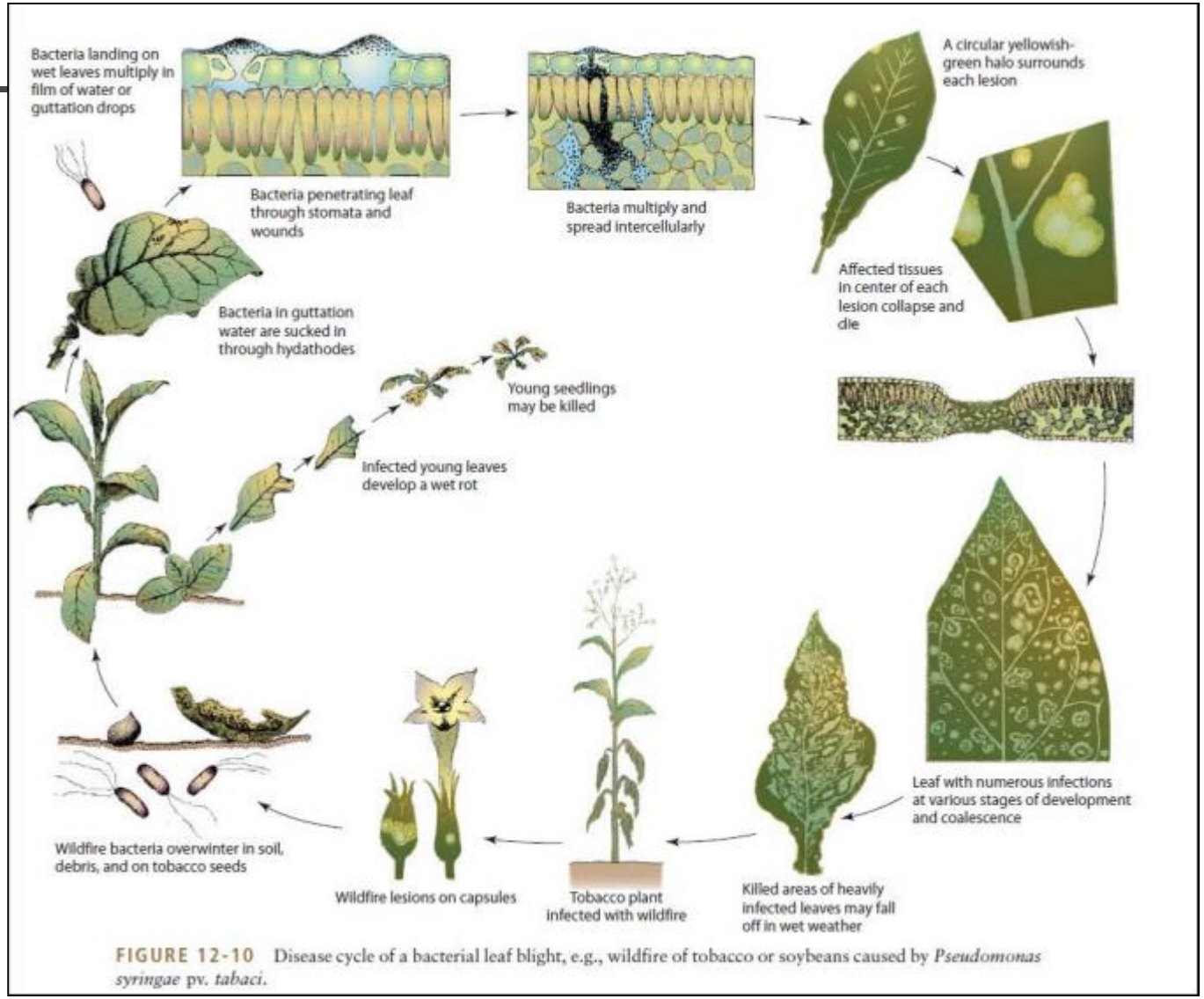


FIGURE 12-10 Disease cycle of a bacterial leaf blight, e.g., wildfire of tobacco or soybeans caused by *Pseudomonas syringae* pv. *tabaci*.

Agrios,2005

Management

Pseudomonas syringae pv.*tabaci*

- Spray streptomycin sulfate 100 ppm solution (½ lb./100 gals. or 1 tsp./gal. water).
- If disease is present, use 200 ppm.
- Use sufficient spray to thoroughly wet plants.
- **For ground beds only**
- Begin application when plants are in two-leaf stage.
- Apply at 5-7 day intervals until disease is under control.
- Remove canvas during warm weather to aid in control.
- Don't water in late afternoon or evening since this will result in plants being wet longer, thus increasing chances of infection.

Pseudomonas syringae pv.*tomato*

Bacterial leaf speck of tomato

Bacterial spot
Xanthomonas perforans

Bacterial speck
Pseudomonas syringae
pv. *tomato*

Management
Pseudomonas syringae pv. *tomato*

- Strategies for bacterial speck(*Pseudomonas syringae* pv. *tomato*) and spot (*Xanthomonas perforans*) are very similar, and require an integrated approach for best results.
- Rotate tomato fields to avoid carryover on crop residue.
- Eliminate any volunteers and weed species (especially solanaceous weeds) that can act as a reservoir.
- Start with clean, healthy transplants.
- Refrain from field activities when foliage is wet to minimize the spread of either bacterium in the canopy and throughout the field.
- Apply bactericidal pesticides as necessary (refer to Table 1).
- When applying copper-based bactericides mix with mancozeb for the control of copper resistant strains, which are prevalent among both pathogens.
-

Management

Pseudomonas syringae pv. *tomato*

Paeinbacillus polymyxa

- Citric acid is effective in controlling food-borne yeasts and bacterial pathogens.
- Growth inhibition of *Pseudomonas syringae* pv.*tomato* ICMP 2844 by citric acid (0.1 mol l^{-1}).
- In addition, a previous study reported that *Paeinbacillus polymyxa* has biocontrol activity against *P. syringae* pv.*tomato*.

Özdemir,2009; Hong,2016

Pseudomonas tolaasii

Bacterial brown blotch disease on mushroom

Bacterial brown blotch diseases

- Caused by many bacteria and a fungus *Verticillium fungicola.*
- The known bacterial diseases of the cultivated mushrooms (*Agaricus bisporus, Pleurotus ostreatus* and *Psalliota edulis*) include:

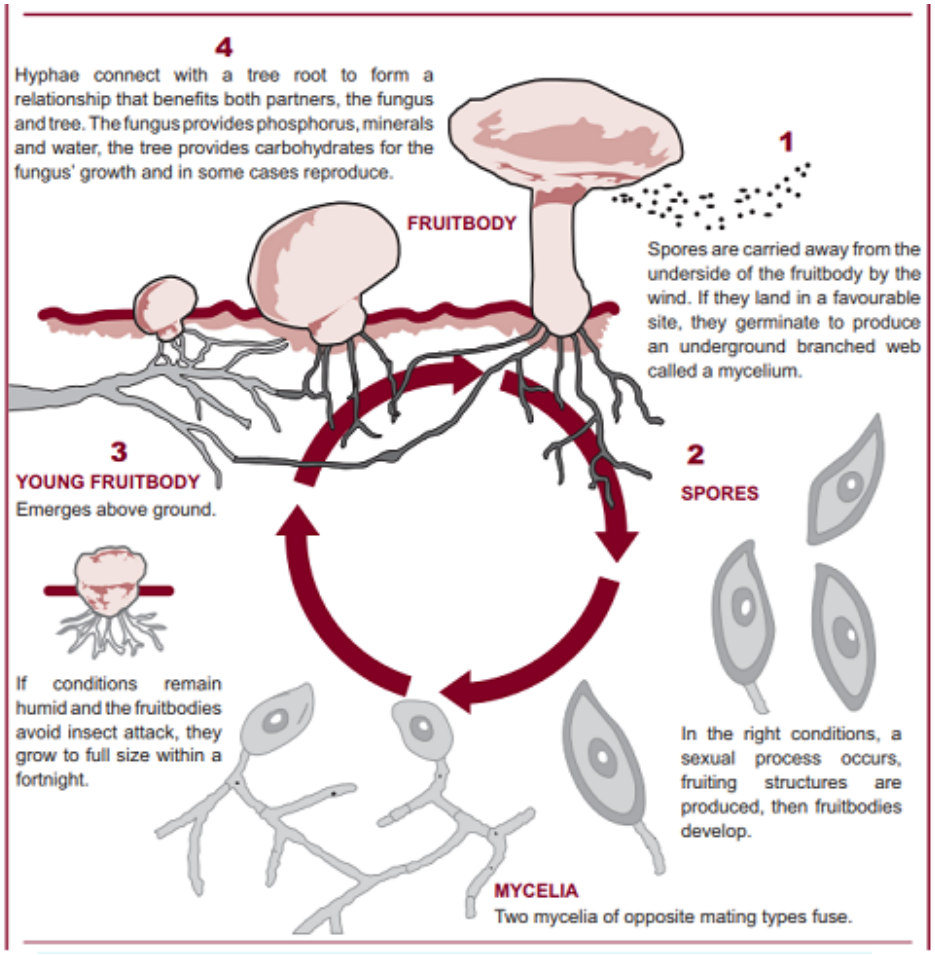
1. Brown blotch (bacterial blotch),
2. Mummy disease,
3. Bacterial pit,
4. Bacterial rot and weeping disease, ginger blotch, and drippy gill.

Bacterial brown blotch diseases
Associated with cultivated mushrooms

- The bacterial strains associated with cultivated mushrooms (*Agaricus bisporus*, *Pleurotus ostreatus* and *Psalliota edulis*) included:

1. *Pseudomonas tolaasii* - The causal agent of brown blotch disease on cultivated mushrooms, is responsible of significant crop losses in mushroom growing houses.
2. *P. agarici* - Affecting mushroom sporophores and causing drippy gill.
3. *Pseudomonas "gingeri"* - Causes a ginger (yellow-brown) blotch symptom.
4. *P. "costantinii"* - Yielded a typical brown blotch symptom.
5. *Pseudomonas* strains responsible for mummy disease.
6. *Pseudomonas "reactans"* (so called white line reacting organisms) - Comprises heterogenic fluorescent pseudomonads belonging to the *P. fluorescens* biovars II, III, or V seem to act as pathogens or as saprophytes in the bacterial community associated to cultivated mushrooms.

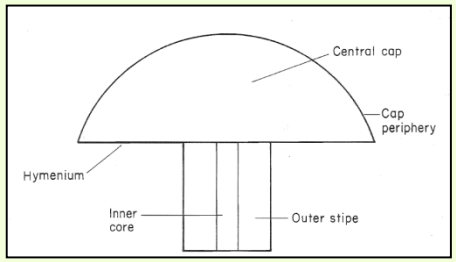
Life cycle of a mushroom

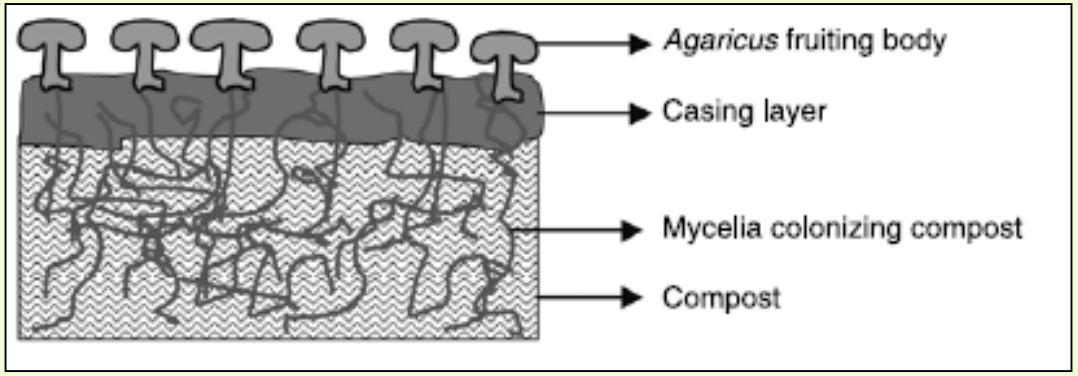


Adapted from geocities.com by Fabio Ricci

Schematic diagram of the compost, casing layer, and fruiting bodies

- Mycelial growth occurs throughout the substrate (compost), and into the peat-based casing layer.
- The casing soil enhances retention of irrigation water on the growing beds, and promotes mushroom fruit body formation.





Sapers *et al.*,2006,Gill,1994

Conditions for disease spread

Pseudomonas tolaasii

- In the production of commercial mushrooms, *P. tolaasii* probably survives between crops on structural surfaces, in debris, and on equipment.
- It can be moved readily from one crop to another on the hands of pickers, on materials or equipment used in harvesting, and by insects, mites, water droplets and mushroom spores.
- Conditions of high relative humidity and surface wetness encourage the expression of symptoms of brown blotch, an important mushroom disease, caused by *P. tolaasii.*
- Dispersal of the microorganisms occurs readily upon watering once the disease is established (Howard *et al.*,1994).

Toxin and volatile compounds
Pseudomonas tolaasii

- Besides tolaasins, other compounds have been reported to be produced by *P. tolaasii* and considered as potential factors responsible for bacterial botch symptoms.
- *P. tolaasii* strains produced the volatile ammonia but not hydrogen cyanide.
- Among the volatiles detected by GC–MS:

1. methanethiol,
2. dimethyl disulfide (DMDS), and
3. 1-undecene.

Lo cantore *et al.*,2015

Management
Pseudomonas tolaasii

- **General control measures**
- Sanitation is the basic control measure for bacterial brown blotch. Follow the **"Basic Practices for Disease and Pest management."**
- Pasteurize substrates thoroughly and use healthy spawn.
- Control mushroom flies. Mushroom flies are well known vectors of the pathogen.
- Try to maintain constant humidity and temperature in growing houses.
- Abrupt temperature and humidity changes increase the incidence of brown blotch.
- Free water on fruiting bodies makes the pathogenic bacteria grow rapidly.
- Try to avoid free water on mushroom surfaces by ventilating after watering.
- Do not water too much.
- Brown blotch is favored by excessive moisture.

Mushroom Growers' Handbook ***1***,2004

Management
Pseudomonas tolaasii

- **General control measures**
- A successful control of brown blotch disease is urgent project that should be developed to promise consistent and stable incomes to the mushroom-cultivating farmers.
- Protection of the mushrooms from the disease is performed by disinfecting under ground water before use, fumigating cultivation bed with hot air, and covering the bed with plastic films.
- Paper bag and bottle cultivations are also good ways of protection.
- Antibiotics and chemical pesticides are not allowed for mushroom cultivation.

rnd.ipet.re.kr/ipetUsr/cm/mdl,2011.In Chinese with English summary

Management
Pseudomonas tolaasii

- **Chemical control**
- Chlorinated water is effective to prevent the brown blotch disease.
- Sodium hypochlorite (NaOCl) and calcium hypochlorite [$Ca(OCl)_2$] are most commonly used.
- Recently Biospot®, sodium dichloroisocyanurate, has also become available.
- Active chlorine content varies among the different formulas and chlorine is well known to be vaporized easily.
- A routine use of 5 ppm chlorinated water (active chlorine concentration) prevents brown blotch incidence.
- If brown blotch is observed in mushroom bags or on mushroom beds, use 20 ppm chlorinated water.

Mushroom Growers' Handbook ***1***,2004

Management
Pseudomonas tolaasii

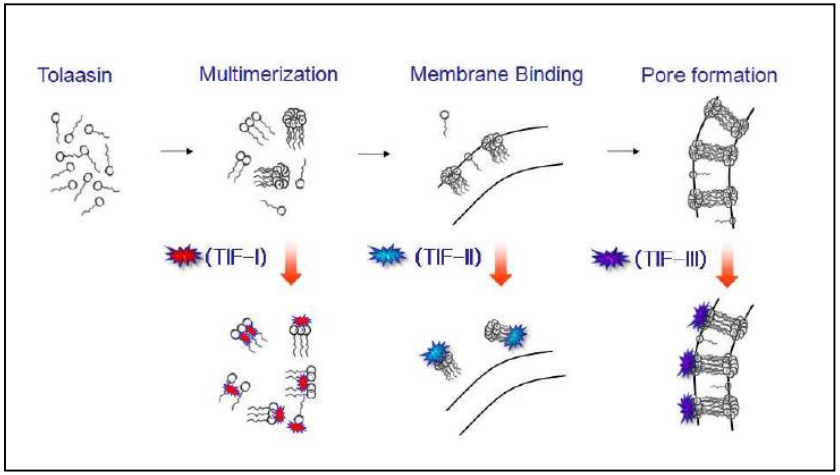
- **Chemical control**
- A 1% aqueous solution of kasugamycin supplied through irrigation water on the second-flush mushrooms drastically reduced bacterial blotch symptoms on these mushrooms at picking stage (Geels,1995).
- In the same study, a sodium hypochlorite-based irrigation treatment showed no beneficial results.
- Pasteurization of the casing layer at 60°C for at least 2 hours resulted in a 2.9 log CFU/g reduction in total bacterial populations.

Sapers *et al.*,2006

Management

Tolaasin ion channel formation can be blocked by chemical compounds named tolaasin-inhibitory factor (TIF)

- Tolaasin, a 1.9 kDa bacterial lipodepsipeptide toxin.
- It forms pores in the cellular membranes of cultivated mushrooms.
- A single tolaasin molecule is not be responsible for the formation of a ion channel across the membrane.
- The tolaasin induced pore formation required the multimerization of tolaasin molecules.

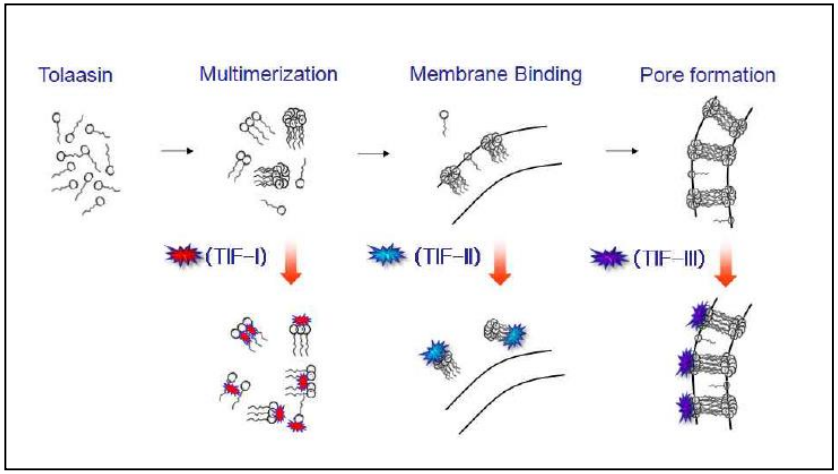


rnd.ipet.re.kr/ipetUsr/cm/mdl,2011.In Chinese with English summary

Management

Tolaasin ion channel formation can be blocked by chemical compounds named tolaasin-inhibitory factor (TIF)

- This multimerization of tolaasin can be blocked by the treatment of various chemical compounds and these compounds named tolaasin-inhibitory factor (TIF).
- This is a practical method to inhibit the cytotoxicity of tolaasin.
- Various chemical compounds, mostly food additives, were investigated to prevent from the brown blotch disease and these compounds named TIFs.



rnd.ipet.re.kr/ipetUsr/cm/mdl,2011.In Chinese with English summary

Management

Tolaasin ion channel formation can be blocked by chemical compounds named tolaasin-inhibitory factor (TIF)

- Among TIF's, glycerin esters of fatty acids, sorbitan esters of fatty acids, sucrose esters of fatty acids, and stearin esters of fatty acids blocked effectively the tolaasin-induced hemolysis.
- These compounds were generally effective at micromolar concentrations.
- The synergic effect of TIFS:
- on the inhibition of tolaasin toxicity was measured When TIF-9, sucrose fatty acid esters, and TIF-16, a polyglyceryl fatty acid ester, were added, tolaasin-induced hemolysis was effectively inhibited.
- In the presence of both TIF's, the inhibition was increased to 3 times.

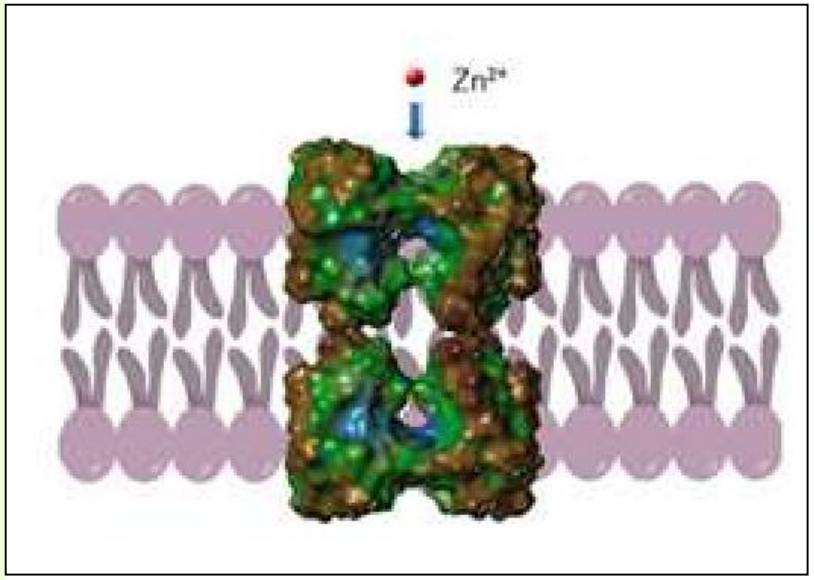
rnd.ipet.re.kr/ipetUsr/cm/mdl,2011.In Chinese with English summary

Management

Tolaasin ion channel formation can be blocked by chemical compounds named tolaasin-inhibitory factor (TIF)

Zinc is a potent tolaasin inhibitor

- Zn^{2+} is a potent tolaasin inhibitor known to bind to tolaasin pore.
- When Zn^{2+} and TIF-9 were added simultaneously, no additive effects were observed at various concentration combinations.
- The inhibition was dominated by Zn^{2+} concentration.



Complete inhibitions of type 1 and type 2 tolaasin (Tol I and Tol II)channels were obtained at 500 μM and 1.2 mM Zn^{2+}, respectively.
Zinc-tolerant bacteria were grown on LB+3 mmol L^{-1}Zn(II).

rnd.ipet.re.kr/ipetUsr/cm/mdl,2011,Cho and Kim,2003; Dell'Amico et al.,2005

Management

Biological control

Antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds

- It is estimated that there are about 140000 species of mushrooms on earth, and of these only 22000 are known and only a small percentage (5%) has been investigated.
- Therefore, there is much to explore about mushroom properties and potential applications.
- Numerous mushroom extracts have been reported as having antimicrobial activity against gram-positive and negative bacteria.
- *Agaricus bisporus*, the most cultivated mushroom in the world, should be highlighted.
- Its methanolic extract revealed MIC=5 µg/mL against *Bacillus subtilis*, even lower than the standard ampicillin (MIC=12.5 µg/mL).

Alves *et al.*,2012

Mushroom extracts with antimicrobial activity against Gram-positive bacteria.

Microorganism	Mushroom[a]	Results
Actinomyces naeslundii	*Lentinus edodes*	CFU = 0–3.30 (± 5.48) × 10^6 MIC = 0.05–20 mg/mL
Actinomyces viscosus	*Lentinus edodes*	MIC = 0.05–20 mg/mL
Bacillus cereus	*Agaricus bisporus, Agaricus bitorquis, Agaricus essettei, Agaricus silvicola, Armillaria mellea, Boletus edulis, Cantharellus cibarius, Clitocybe alexandri, Clitocybe geotropa, Cortinarius sp., Gloeoporus thelephoroides, Hexagonia hydnoides, Hydnum repandum, Hypholoma fasciculare, Irpex lacteus* (M), *Lactarius camphoratus, Lactarius delicious, Lactarius piperatus, Lactarius volemus, Laetiporus sulphureus, Lentinus edodes, Lepista nuda, Leucopaxillus giganteus* (M), *Macrolepiota procera, Meripilus giganteus* (M), *Meripilus giganteus, Phellinus sp., Pleurotus ostreatus* (M), *Pleurotus ostreatus, Ramaria botrytis, Ramaria flava, Rhizopogon roseolus, Sarcodon imbricatus, Sparassis crispa, Tricholoma portentosum*	IZD = 5–21 mm MIC = 5 µg/mL – 100 mg/mL
Bacillus megaterium	*Lentinus edodes*	CFU = 0 (total inhibition)
Bacillus pumilis	*Lentinus edodes*	IZD = 14 mm
Bacillus subtilis	*Agaricus bisporus, Agaricus bitorquis, Agaricus essettei, Agaricus silvicola, Armillaria mellea, Cantharellus cibarius, Clitocybe alexandri, Clitocybe geotropa, Cortinarius sp., Ganoderma lucidum, Hygrophorus agathosmus, Hypholoma fasciculare, Lactarius delicious, Lactarius piperatus, Laetiporus sulphureus, Lentinus edodes, Lepista nuda, Leucopaxillus giganteus* (M), *Meripilus giganteus* (M), *Navesporus floccosa, Paxillus involutus* (M), *Phellinus rimosus, Pleurotus ostreatus* (M), *Pleurotus ostreatus, Ramaria botrytis, Ramaria flava, Rhizopogon roseolus, Sparassis crispa, Suillus collitinus, Tricholoma acerbum, Tricholoma portentosum*	IZD = 5–28 mm MIC = 5 µg/mL – 300 mg/mL
Enterococcus faecalis	*Lentinus edodes*	IZD = 8 mm
Enterococcus faecium	*Lentinus edodes*	MIC > 1.5 – > 5.0 mg/mL
Lactobacillus casei	*Lentinus edodes*	CFU = 5.00 (± 7.07) × 10^{-1} – 9.28 .76) × 10^2 MIC = 0.05–15 mg/mL
Listeria innocua	*Lentinus edodes*	IZD = 8 mm
Listeria monocytogenes	*Lentinus edodes, Pycnoporus sanguineus* (M),	IZD = 11–13 mm
Staphylococcus sp.	*Lentinus edodes*	IZD = 12 mm
Staphylococcus aureus	*Agaricus bisporus, Agaricus bitorquis, Agaricus essettei, Agaricus silvicola, Armillaria mellea, Boletus edulis, Cantharellus cibarius, Clitocybe geotropa, Cortinarius sp., Cortinarius abnormis, Cortinarius ardesiacus, Cortinarius archeri, Cortinarius austroalbidus, Cortinarius austrovenetus, Cortinarius austroviolaceus, Cortinarius coelopus, Cortinarius clelandii, Cortinarius [Dermocybe sp, Dermocybe canaria, Dermocybe kula], Cortinarius fulvoiubatus, Cortinarius ianthinus, Cortinarius memoria-annae, Cortinarius persplendidus, Cortinarius sinapicolor, Cortinarius submagellanicus, Cortinarius tricholomoides, Cortinarius vinosipes, Ganoderma lucidum, Hydnum repandum, Hygrophorus agathosmus, Hypholoma fasciculare, Irpex lacteus* (M), *Lactarius camphoratus, Lactarius delicious, Lactarius piperatus, Lactarius volemus, Laetiporus sulphureus, Lentinus edodes, Lepista nuda, Leucopaxillus giganteus* (M), *Macrolepiota procera, Meripilus giganteus* (M), *Meripilus giganteus, Marchella elata* (M), *Morchella esculenta var. vulgaris* (M), *Navesporus floccosa, Nothopanus hygrophanus* (M), *Paxillus involutus* (M), *Phellinus rimosus, Pleurotus eryngii* (M), *Pleurotus ostreatus* (M), *Pleurotus sajor-caju, Pycnoporus sanguineus* (M), *Ramaria botrytis, Ramaria flava, Sparassis crispa, Suillus collitinus*	CFU = 2.1 × 10^4 IZD = 8–24 mm MIC = 5 µg/mL – 50 mg/mL IC_{50} < 0.01 – ≥ 2.00 mg/mL
MRSA	*Lentinus edodes, Phellinus linteus*	IZD = 12 mm MIC = 500 µg/mL
Staphylococcus epidermidis	*Agaricus bisporus, Hygrophorus agathosmus, Lentinus edodes, Pleurotus sajor-caju, Suillus collitinus*	IZD = 11–27 mm MIC = 7.81–62.5 µg/mL
Streptococcus gordonii	*Lentinus edodes*	MIC = 0.075–50 mg/mL
Streptococcus mitis	*Lentinus edodes*	MIC = 0.075–15 mg/mL
Streptococcus mutans	*Lentinus edodes*	CFU = 2.15 (± 5.58) × 10^5 MIC = 0.1–10 mg/mL
Streptococcus oralis	*Lentinus edodes*	MIC = 0.1 – > 50 mg/mL
Staphylococcus saprophyticus	*Agaricus cf. nigrecentulus* (M), *Tyromyces duracinus* (M)	IZD > 12 mm
Streptococcus pyogenes	*Lentinus edodes*	CFU = 6.0 × 10^4
Streptococcus salivarius	*Lentinus edodes*	MIC = 0.1–10 mg/mL

Alves *et al.*,2012

Mushroom extracts with antimicrobial activity against Gram-negative bacteria.

Microorganism	Mushroom[a]	Results	References
Cupriavidis	*Lentinus edodes*	IZD = 15 mm	[50]
Enterobacter aerogenes	*Agaricus bisporus, Clitocybe alexandri, Hygrophorus agathosmus, Meripilus giganteus* (M), *Paxillus involutus* (M), *Pleurotus ostreatus* (M), *Pleurotus sajor-caju, Rhizopogon roseolus, Suillus collitinus*	IZD = 8–22 mm MIC = 15.62–125 μg/mL	[33, 35, 38, 44]
Enterobacter cloacae	*Armillaria mellea, Clitocybe geotropa, Meripilus giganteus* (M), *Meripilus giganteus, Paxillus involutus* (M), *Pleurotus ostreatus* (M), *Sparassis crispa*	IZD = 10–20 mm	[35, 36]
Enterobacter faecalis	*Armillaria mellea, Clitocybe geotropa, Meripilus giganteus* (M), *Meripilus giganteus, Sparassis crispa*	IZD = 8–14 mm	[35, 36]
Escherichia coli	*Agaricus bisporus, Armillaria mellea* (M), *Armillaria mellea, Boletus edulis, Cantharellus cibarius, Clitocybe alexandri, Clitocybe geotropa, Cortinarius sp., Ganoderma lucidum, Hydnum repandum, Irpex lacteus* (M), *Lactarius camphoratus, Lactarius delicious, Lactarius piperatus, Lactarius volemus, Laetiporus sulphureus, Lentinus edodes, Lepista nuda, Leucoagaricus cf. cinereus* (M), *Macrolepiota procera, Marasmius sp.* (M), *Marasmius cf. bellus* (M), *Meripilus giganteus* (M), *Meripilus giganteus, Morchella costata* (M), *Morchella hortensis* (M), *Navesporus floccosa, Paxillus involutus* (M), *Phellinus rimosus, Pleurotus eryngii* (M), *Pleurotus ostreatus* (M), *Pleurotus sajor-caju, Rhizopogon roseolus, Sparassis crispa, Suillus collitinus*	IZD = 8–27.40 ± 0.19 mm MIC = 250 μg/mL – > 50 mg/mL	[32–36, 38–40, 44, 46, 48–50, 54, 71]
Fusobacterium nucleatum	*Lentinus edodes*	CFU = 2.40 (± 3.11) × 10^2 – 7.56 (± 4.28) × 10^6 MIC = 0.9–20 mg/mL	[53, 54, 67]
Klebsiella aerogenes	*Lentinus edodes*	IZD = 9 mm	[50]
Klebsiella pneumoniae	*Agaricus bisporus, Agaricus bitorquis, Ganoderma lucidum, Lactarius piperatus, Lentinus edodes, Lepista nuda, Pleurotus sajor-caju, Ramaria flava*	IZD = 4–31.60 ± 0.10 mm MIC = 0.5 mg/mL	[11, 15, 33, 39, 46, 47, 49, 50, 55]
Morganella morganii	*Agaricus bisporus, Agaricus bitorquis, Agaricus essettei, Laetiporus sulphurous*	IZD = 4.5 ± 0.5 mm	[15, 48]
Neisseria subflava	*Lentinus edodes*	CFU = 9.49 (± 2.60) × 10^6 – 1.50 (± 0,50)×10^8	[53, 67]
Porphyromonas gingivalis	*Lentinus edodes*	MIC = 0.05–10 mg/mL	[45]
Prevotella intermedia	*Lentinus edodes*	CFU = 2.00 (± 2.83) × 10^1 – 2.60 (± 6.66) × 10^5 MIC = 0.05–15 mg/mL	[53, 54, 67, 68]
Prevotella nigrescens	*Lentinus edodes*	MIC = 0.1–15 mg/mL	[54]
Proteus mirabilis	*Lentinus edodes*	IZD = 4 mm	[11]
Proteus vulgaris	*Agaricus bisporus, Agaricus bitorquis, Armillaria mellea, Clitocybe geotropa, Laetiporus sulphureus, Meripilus giganteus* (M), *Meripilus giganteus, Pleurotus ostreatus* (M), *Pleurotus sajor-caju, Sparassis crispa*	IZD = 5.5 ± 0.5–19 mm	[15, 33, 35, 36, 48]
Pseudomonas aeruginosa	*Agaricus bisporus, Boletus edulis, Cantharellus cibariusCortinarius sp., Cortinarius abnormis, Cortinarius ardesiacus, Cortinarius archeri, Cortinarius austroalbidus, Cortinarius austrovenetus, Cortinarius austroviolaceus, Cortinarius coelopus, Cortinarius clelandii, Cortinarius [Dermocybe sp., Dermocybe canaria, Dermocybe kula], Cortinarius fulvoiubatus, Cortinarius ianthinus, Cortinarius memoria-annae, Cortinarius persplendidus, Cortinarius sinapicolor, Cortinarius submagellanicus, Cortinarius tricholomoides, Cortinarius vinosipes, Ganoderma lucidum, Hydnum repandum, Lactarius camphoratus, Lactarius delicious, Lactarius piperatus, Lactarius volemus, Laetiporus sulphureus, Lentinus edodes, Lepista nuda, Macrolepiota procera, Navesporus floccosa, Phellinus rimosus, Pleurotus sajor-caju, Ramaria flava*	IZD = 6–20 mm MIC = 0.5–100 mg/mL IC_{50} = 0.04 – > 2.00 mg/mL	[10, 32, 33, 39, 40, 45, 46, 48–50]
Pseudomonas maltophila	*Lentinus edodes*	IZD = 6 mm	[11]
Salmonella enteritidis	*Laetiporus sulphureus, Ramaria flava*	IZD = 4–5 ± 1 mm	[37, 40]
Salmonella poona	*Lentinus edodes*	IZD = 9 mm	[50]
Salmonella typhi	*Agaricus bisporus, Ganoderma lucidum, Pleurotus sajor-caju*	IZD = 7.00 ± 0.18–20.60 ± 0.14 mm	[33, 39]
Salmonella typhimurium	*Agaricus bisporus, Armillaria mellea* (M), *Armillaria mellea, Clitocybe geotropa, Ganoderma lucidum, Hygrophorus agathosmus, Irpex lacteus* (M), *Lepista nuda, Meripilus giganteus* (M), *Meripilus giganteus, Morchella costata* (M), *Morchella elata* (M), *Morchella esculenta* var. *vulgaris* (M), *Morchella hortensis* (M), *Navesporus floccosa, Paxillus involutus* (M), *Phellinus rimosus, Pleurotus ostreatus* (M), *Pleurotus sajor-caju, Sparassis crispa, Suillus collitinus,*	IZD = 6–16 mm MIC = 15.62–125 μg/mL	[33–36, 40, 44, 49]
Serratia marcescens	*Lentinus edodes*	IZD = 10 mm	[50]
Veillonella dispar	*Lentinus edodes*	CFU = 1.37 (± 0.31)×10^7 – 2.35 (± 1.09)×10^7	[53, 67]
Veillonella parvula	*Lentinus edodes*	MIC = 0.3–20 mg/mL	[54]
Yersinia enterecolitica	*Agaricus bitorquis, Laetiporus sulphureus, Lentinus edodes, Ramaria flava*	IZD = 5–16 mm	[11, 15, 47]

[a]Acetone, chloroform, ethanol, ethyl acetate, methanol, dichloromethane, ether, xylene, or water extracts. M – mycelium, the other samples refer to the fruiting body. The antimicrobial activity is expressed in CFU (colony forming unities), MIC (minimal inhibitory concentrations), IZD (internal zone diameter), or IC_{50} (concentrations inhibiting 50% of the growth) values

Alves *et al.*,2012

Mushroom extracts with antimicrobial activity against Gram-negative bacteria.

Microorganism	Compound (mushroom)	Results	References
Achromobacter xyloxidans	**6** (*Leucopaxillus albissimus*)	MIC = 32 µg/mL	[69]
Acinetobacter baumannii	**6** (*Leucopaxillus albissimus*)	MIC = 128 µg/mL	[69]
Agrobacterium rhizogenes	Protein (*Clitocybe sinopica*)	MIC = 0.14 µM	[70]
Agrobacterium tumefaciens	Protein (*Clitocybe sinopica*)	MIC = 0.14 µM	[70]
Agrobacterium vitis	Protein (*Clitocybe sinopica*)	MIC = 0.28 µM	[70]
Burkholderia cenocepacia	**6** (*Leucopaxillus albissimus*)	MIC = 16 µg/mL	[69]
Burkholderia cepacia	**6** (*Leucopaxillus albissimus*)	MIC = 32 µg/mL	[69]
Burkholderia multivorans	**6** (*Leucopaxillus albissimus*)	MIC = 16 µg/mL	[69]
Cytophaga johnsonae	**6** (*Leucopaxillus albissimus*)	IZD = 16 mm	[69]
Escherichia coli	Proteins (*Cordyceps sinensis*); **5a, b** (*Ganoderma pfeifferi*); Fraction B (*Pycnoporus sanguineus*); **10** (*Xylaria intracolarata*)	IZD = 4–16 mm MIC = 0.625 mg/mL–100 000 g/L	[42, 57, 60, 61]
Klebsiella pneumoniae	**3** (*Lentinus edodes* M); Fraction B (*Pycnoporus sanguineus*); **10** (*Xylaria intracolarata*)	IZD = 12–22 mm MIC = 0.625 mg/mL	[42, 59, 60]
Proteus mirabilis	**5a, b** (*Ganoderma pfeifferi*)	IZD = 15 mm	[57]
Proteus vulgaris	Protein (*Cordyceps sinensis*); **3** (*Lentinus edodes* M)	IZD = 12 mm MIC = 75 000 g/L	[59, 61]
Pseudomonas aeruginosa	**7, 8a-8 d** (*Cortinarius basirubencens*); **9a–c** (*Cortinarius sp.*); **3** (*Lentinus edodes* M); **6** (*Leucopaxillus albissimus*); Ribonuclease (*Pleurotus sajor-caju*); Fraction B (*Pycnoporus sanguineus*); **10** (*Xylaria intracolarata*)	IZD = 15–16 mm MIC = 128 µg/mL–1.250 mg/mL IC_{50} = 1.5–> 50 µg/mL IC_{50} = 51 ± 6 µM	[10, 42, 59, 62, 64, 68]
Pseudomonas fluorescens	**3** (*Lentinus edodes* M); Ribonuclease (*Pleurotus sajor-caju*)	IZD = 13 mm IC_{50} = 186 ± 12 µM	[59, 62]
Serratia marcescens	**5a, b** (*Ganoderma pfeifferi*)	IZD = 15–16 mm	[57]
Salmonella enteritidis	**10** (*Xylaria intracolarata*)	IZD = 16 mm	[60]
Salmonella typhi	Protein (*Cordyceps sinensis*); Fraction B (*Pycnoporus sanguineus*)	MIC = 0.312 mg/mL – 50 000 g/L	[42, 61]
Stenotrophomonas maltophilia	**6** (*Leucopaxillus albissimus*)	MIC = 32 µg/mL	[69]
Xanthomonas malvacearum	Protein (*Clitocybe sinopica*)	MIC = 0.56 µM	[70]
Xanthomonas oryzae	Protein (*Clitocybe sinopica*)	MIC = 0.56 µM	[70]

M – mycelium, the other samples refer to the fruiting body. The antimicrobial activity is expressed in MIC (minimal inhibitory concentrations), IZD (internal zone diameter), or IC_{50} (concentrations inhibiting 50% of the growth) values

Alves *et al.*,2012

Management

Antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds

- Mushrooms could be a source of natural antibiotics, which can be:

1. Low-molecular weight compounds(LMW), and
2. High-molecular weight compounds(HMW).

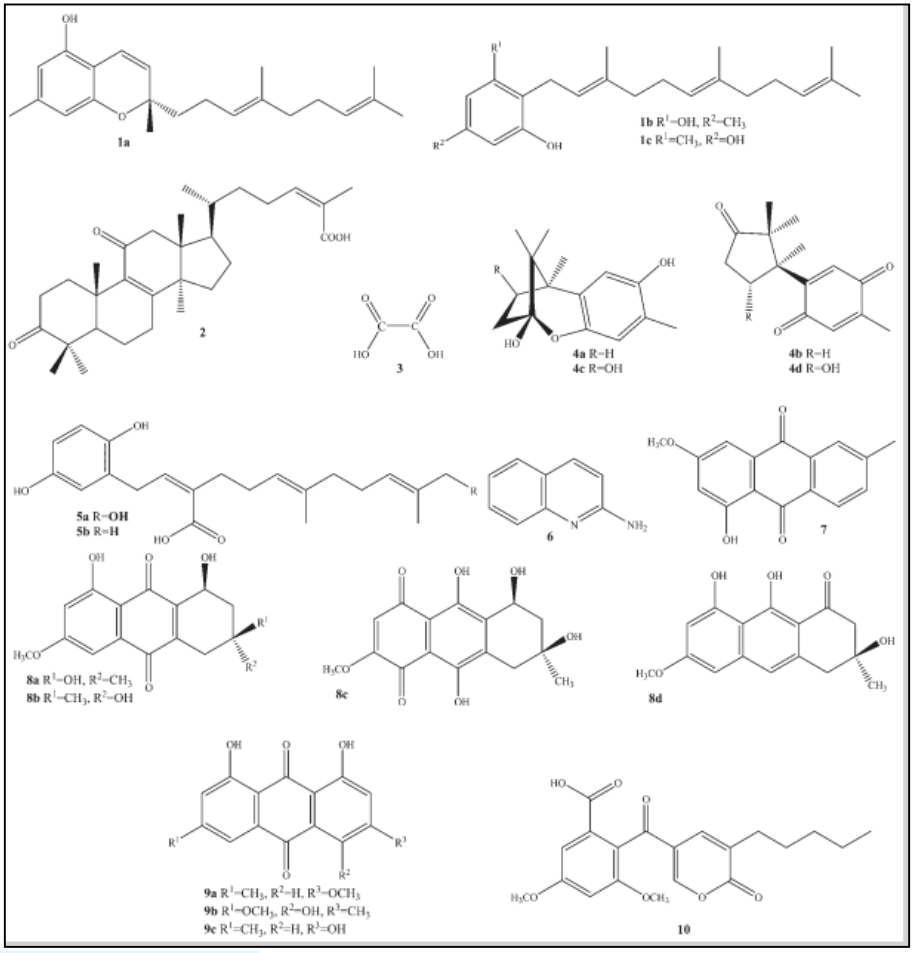
- LMW compounds are mainly secondary metabolites such as sesquiterpenes(a terpene with the formula $C_{15}H_{24}$) and other terpenes, steroids, anthraquinone and benzoic acid derivatives, and quinolines, but also primary metabolites such as oxalic acid.
- HMW compounds mainly include peptides and proteins.

Alves *et al.*,2012

Management

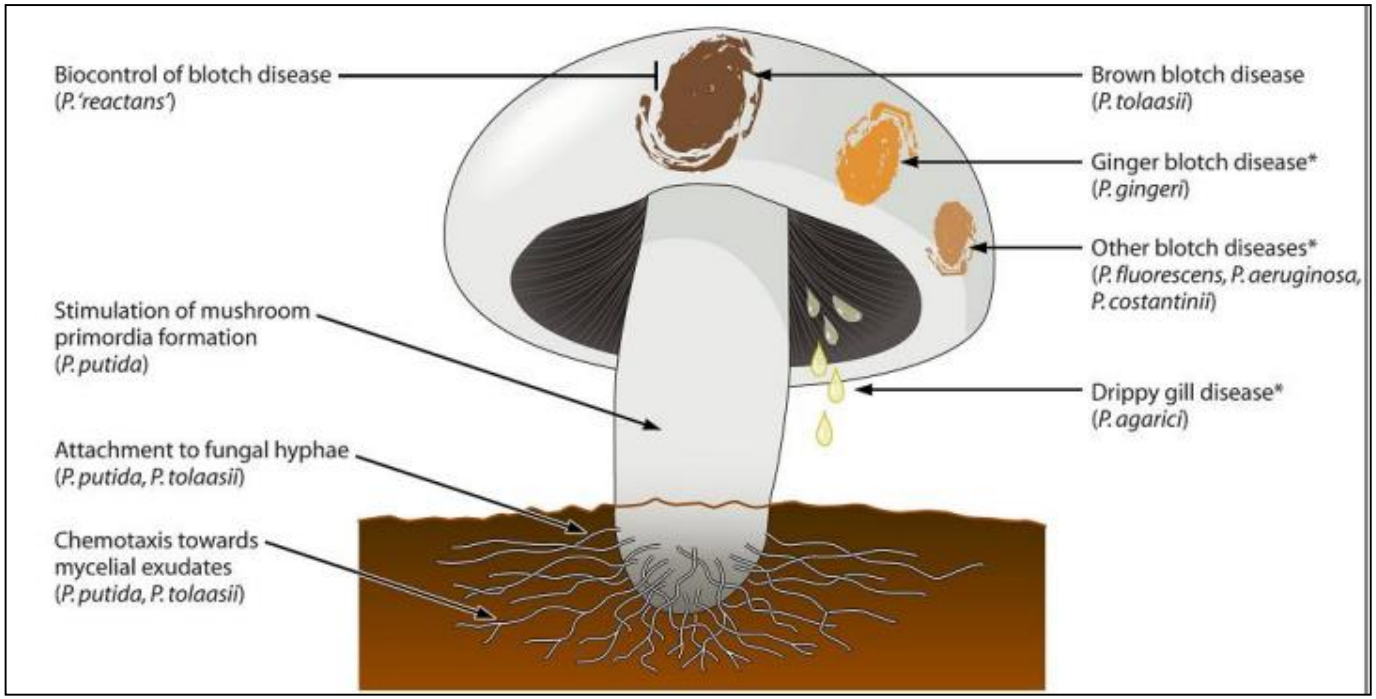
Antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds

Chemical structure of the low-molecular weight (LMW) compounds with antimicrobial potential found in mushrooms.



Alves *et al.*,2012

Management
Biological control
Fluorescent pseudomonad (FP) strains



Interactions of pseudomonads with *Agaricus bisporus* lead to both positive and negative outcomes for the fungus, depending on the bacterial isolate and the developmental stage of the fungus.

Frey-Klett *et al.*,2011

Management
Biological control
Fluorescent pseudomonad (FP) strains

- ***In vivo* test:**
- Efficacy of FP strains against *P. tolaasii* on detached mushroom caps:
- The suspensions of FP strains (10^{11} cfu/ml) were sprayed on freshly harvested detached mushroom caps.
- A suspension of *P. tolaasii* (10^{9} cfu/ml) was sprayed on the caps one hour later.
- Detached caps were kept at high RH (90%) and 24°C for 2 days.
- Disease severity was evaluated by a 0-3 scale:
- (0): healthy caps,
- (1): small spots up to 3,
- (2): numerous small spots,
- (3): extended blackening and rotting (Bora and Özaktan, 2000).

Özaktan *et al.*,2006

Management
Biological control
Fluorescent pseudomonad bacteria

- One of the most recent EPA registrations for a microbial pesticide is the product VICTUS (Sylvan Spawn, PA) which contains the bacterium *Pseudomonas fluorescens*.
- This organism, when applied to commercially grown mushrooms, helps prevent bacterial blotch caused by a closely related but pathogenic species, *Pseudomonas tolaasii*.
- Apparently *P. fluorescens* is a better competitor for nutrients than *P. tolaasii*, and when applied to mushroom caps, excludes the pathogen by utilizing all the available food(J. Parke).
- There is no evidence of antibiosis.
- Two applications with *P. fluorescens* - M 4/2, *P. fluorescens* - M 5/3 and *P. putida* - 39.a strains reduced the severity of disease caused by *P. tolaasii* by 87.34-90.5%, 71.3-71.7% and 67.5-72.6%, respectively (Bora and zaktan,2000).

Management

Biological control

Non-fluorescent and fluorescent Pseudomonad Bacteria

- In our surveys, no *P. tolaasii* was isolated from fresh peat or compost from healthy mushroom beds.
- Therefore, we could isolate three antagonistic bacteria from soil and peat:

1. A2, A nonfluorescent *Pseudomonas* sp. (closest to *Ps. multivoruns*) from soil, and
2. Strains of *Ps. fluorescens* (C12)and *Enterobacter aerogenes* (C10) from peat.

- When the antagonists and the pathogen were added in the ratio of 8 x 10^7: 10^8 cells/ml to unsterilized peat and applied to mushroom trays, infection of mushroom sporophores by the pathogen was effectively controlled.
- *In vitro* studies failed to show lysis or growth inhibition of *Ps. tolaasii* by the antagonists.

Nair and Fahy,1972

Management

Biological control

Non-fluorescent and fluorescent Pseudomonad Bacteria

- **Effects of the bacterial isolates on mushroom in beds**
- Mushrooms were grown in specially designed growth rooms at 16° (1°) and relative humidity 95% (1%).
- Plastic trays were filled with 2 kg of pasteurized compost and inoculated with mushroom spawn.
- After the spawn had colonized the compost, it was covered with a 4 cm layer of unsterile peat, neutralized with calcium carbonate to pH 7.0.
- Turbid suspensions of *Ps. tolaasii* and the antagonists were sprayed on the surface of the peat, singly and in combination.
- Numbers of mushrooms showing blotch symptoms were recorded during a 50 day cropping period.

Nair and Fahy,1972

Management

Biological control

Non-fluorescent and fluorescent pseudomonad bacteria

- Effect of the incidence of brown blotch during a 50-day cropping period of adding antagonists to non-sterile easing peat inoculated with *Pseudomonas tolaasii*.

Ps. tolaasii in casing peat	Mushrooms with brown blotches in presence of			
	Antagonist isolate			No antagonist
	A2	C12	C10	
Added	7·6	9·7	11·1	100
Not added (control)	0	0	0	2·2

Nair and Fahy,1972

Management
Biological control
Non-Pseudomonad bacteria

- The tolaasin-detoxifying bacteria were isolated from wild Agaricales fungi.

1. *Mycetocola tolaasinivorans* and *Mycetocola lacteus* (new species of family Microbacteriaceae) were associated with fruit bodies of wild *Pleurotus ostreatus* and wild *Lepista nuda*, respectively. Both are smooth yellow colonies, variable rod-shaped cells, aerobic growth, Gram-positive, no spore formation, and no motility.
2. An *Acinetobacter* sp. was isolated from fruit bodies of *Tricholoma matsutake*,
3. *Bacillus pumilus* was isolated from *Coprinus disseminatus*, and
4. *Sphingobacterium multivorum* was isolated from *Clitocybe clavipes*.
5. *Pedobacter* sp., a smooth yellow, non-motile, aerobic, Gram negative bacterium was isolated from a *Clitocybe* sp.

- These tolaasin-detoxifying bacteria identified thus far were attached to the surface of mycelia rather than residing within the fungal cells.

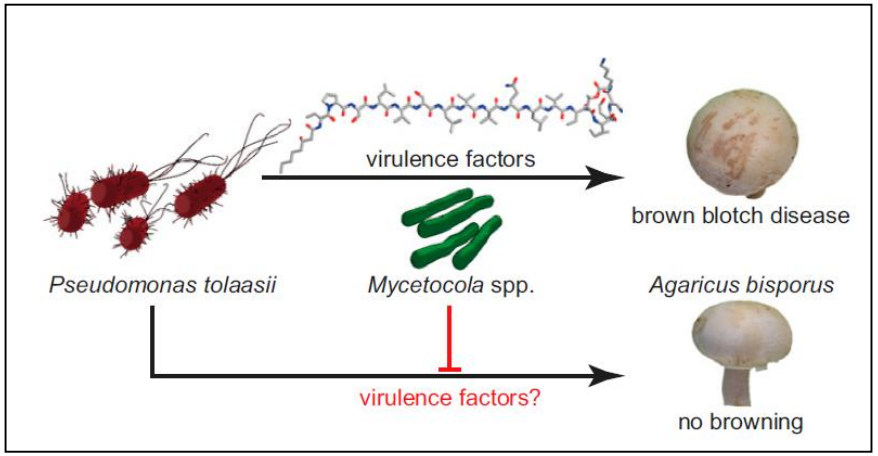
Tsukamoto *et al.*,2002

Plant microbiomes

Helper Bacteria

Mycetocola spp.

- Tripartite interaction of mushroom pathogen, helper bacteria, and fungal host.
- *P. tolaasii* produces virulence factors that cause bacterial brown blotch in *A. bisporus*.
- *Mycetocola* spp. can prevent decay of the mushroom cap.



P. tolaasii relies on two different cyclic lipopeptide toxins during infection: the pore forming toxin tolaasin and the motility conferring biosurfactant pseudodesmin.
On the other hand, *Mycetocola* spp. Produce enzymes provide protection to the mushroom via lipopeptide cleavage.

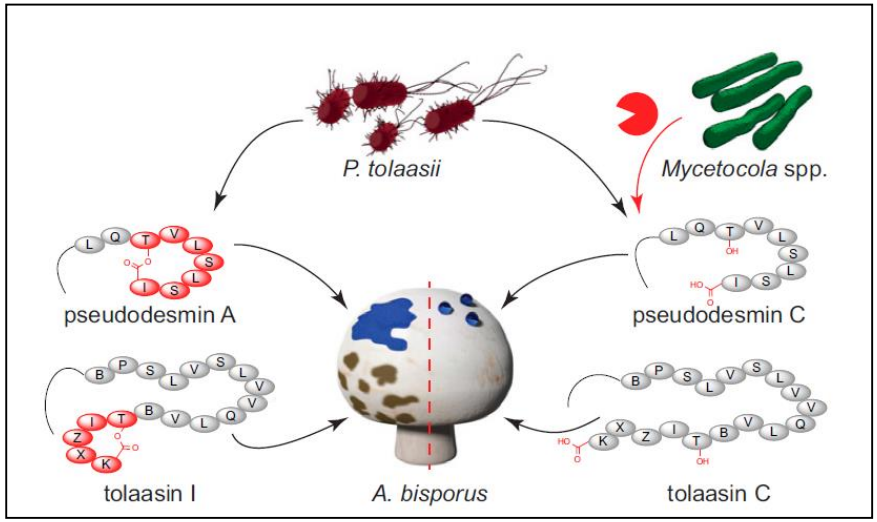
Hermenau *et al.*,2020

Plant microbiomes

Helper Bacteria

Mycetocola spp.

- *P. tolaasii* produces pseudodesmins as swarming agents and tolaasins as toxins against *A. bisporus*.
- In the presence of protective helper bacteria (*Mycetocola* spp.) the virulence factors are cleaved by enzymes, such as TdfL or TdfT.
- One letter code used to represent amino acids in the peptides.



B: dehydrobutyrine;
X: 2,4-diaminobutanoic acid;
Z: homoserine.

Hermenau *et al.*,2020

Management

Isolation of tolaasin-detoxifying bacteria

Effects of talaasin-detoxifying bacteria on potato slices

- Bacteria associated with fungi were collected by soaking fungal fruit bodies in 4 ml of sterile water for 30 min, serially diluted and spread on PSA medium.
- Each of bacterial isolates of 1032 colonies randomly selected was grown in 100 µl of PSB in wells of a 96-well microtiter plate at 25°C for 48 h.
- Then, an equal volume of PSB-Tol was added and further incubated at 25°C for 72 h.
- The 50 µl of samples of the whole cultures were then applied onto potato tuber slices, wherein tuber slices normally become blackened due to the toxicity of tolaasin.
- Bacterial strains that suppressed the occurrence of blackening were scored as tolaasin-detoxifying agents.

Tsukamoto *et al.*,2002

Management

Isolation of tolaasin-detoxifying bacteria

Growth conditions and media

- Bacteria were grown in PSB (potato semi-synthetic broth) or PSA (potato semi-synthetic agar) at 25°C.
- PSA or WPSA composition: $Na_2HPO_4.12H_2O$ 2.0 g, $Ca(NO_3)_2.4H_2O$ 0.5 g, peptone 5.0 g, sucrose 20 g, and 15 g agar added to 1 liter of boiled 300 g potato, pH 7.0.
- PSB-Tol, a medium that contains tolaasin, was prepared by adding the components of PS-broth to the culture supernatants of *P. tolaasii* strains.
- Culture supernatants of *P. tolaasii* strains were obtained by culturing *P. tolaasii* in PSB at 25°C for 48 h, harvested by centrifugation (4 °C, 10,000 x g) and sterilized by placing in boiling water for 10 min.

Tsukamoto *et al.*,1998 and 2002

Management

Isolation of tolaasin-detoxifying bacteria

Growth conditions and media

- **Bacterial growth conditions:**
- Strain type NCPPB2192 of *P. tolaasii* was grown at 25°C under shaking (180 rpm) in 500 mL Erlenmeyer flasks filled with 150 mL of liquid King's B medium inoculated with 1.5 mL of a bacterial suspension containing 10^8 cfu/mL.
- After 48 h incubation cultures were centrifuged (20000*g* for 15 min), and the resulting supernatants were evaluated for the antimicrobial activity against *Bacillus megaterium* following an already established procedure, lyophilized, and stored at -20°C before further processing.

Bassarello *et al.*,2004

Management

Isolation of tolaasin-detoxifying bacteria

Growth material and conditions

- **Growth material and conditions:**
- Commercially prepared pasteurised compost provided by a private company and *A. bisporus* str 512 were used in the study.
- *In vivo* trials were performed in the mushroom growing room of the Department of Plant Pathology of Ege University.
- Hard-plastic trays were 25 x 40 x 16 cm in size and filled with compost (5kg/tray).
- The trays were cased by sterile peat 14 days after the spawn development period.

Özaktan *et al.*,2006

Management
Antimicrobial activity of tolaasins
Indicator organisms

- The antimicrobial activity of HPLC grade tolaasins A-E in comparison with tolaasin I and II against:
- The yeast *Rhodotorula pilimanae*,
- The fungus *Rizoctonia solani*,
- The Gram-positive bacteria *Bacillus megaterium* and *Rodococcus fascians*, respectively, and
- The Gram-negative bacteria *Escherichia coli* and *Erwinia carotovora* subsp. *carotovora*.
- *B. megaterium* and *R. fascians* were the most sensitive test microorganisms.

microorganism	tolaasins minimal inhibitory quantity (μg)						
	I	II	A	B	C	D	E
Rizoctonia solani 1583	0.32	0.64	1.28	2.56	5.12	0.16	5.12
Rhodotorula pilimanae ATCC26423	2.56	5.12	5.12	>5.12	>5.12	2.56	>5.12
Bacillus megaterium ITM100	0.32	0.64	1.28	2.56	>5.12	0.16	2.56
Rodococcus fascians NCPPB3067	0.32	0.64	1.28	1.28	>5.12	0.16	2.56
Escherichia coli K12 ITM103	> 5.12	> 5.12	>5.12	> 5.12	>5.12	>5.12	>5.12
Erwinia carotovora subsp. *carotovora* ICMP5702	> 5.12	>5.12	>5.12	> 5.12	>5.12	>5.12	>5.12

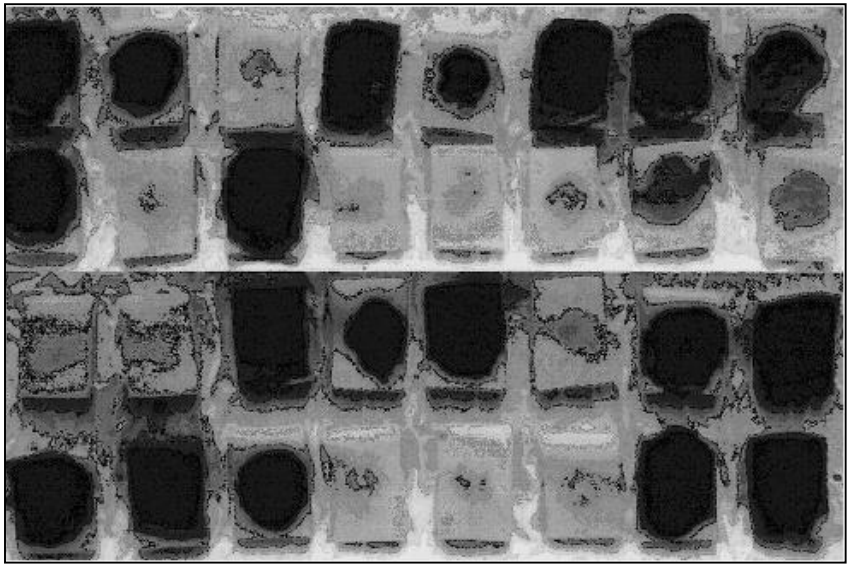
Bassarello *et al.*,2004

Management

Isolation of tolaasin-detoxifying bacteria

Effects of talaasin-detoxifying bacteria on potato slices

- Potato slices treated with PSB-Tol (a medium that contains tolaasin) and tolaasin detoxifying bacteria.
- In the presence of tolaasin detoxifying bacteria remained colorless, but those treated together with non-detoxifying bacteria were blackened.



PSB (potato semi-synthetic broth)

Tsukamoto *et al.*,2002

Management

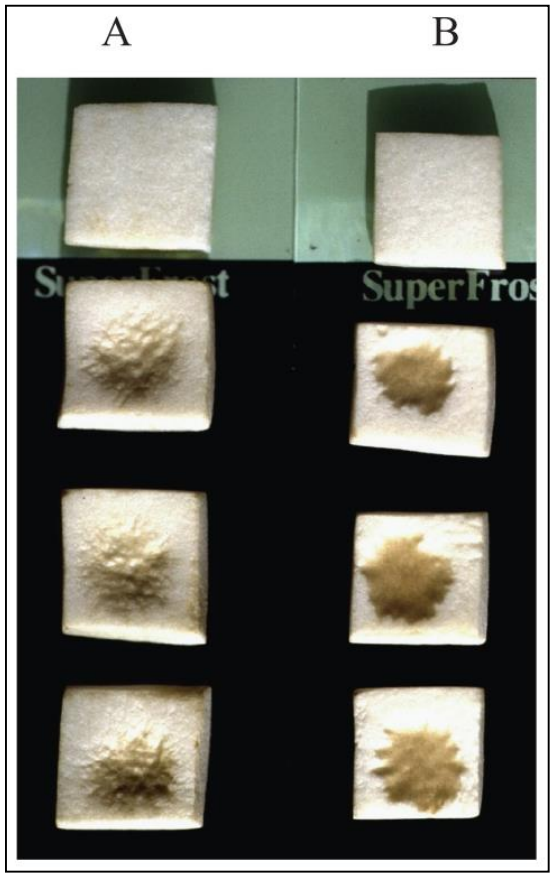
Assay on tissue blocks and whole sporophores of *Agaricus bisporus*

- Brown lesions on tissue blocks of *Agaricus bisporus* (lower three blocks in each treatment), caused by deposition of 5 µl solutions containing:

A. 5.12 µg of *P. reactans* White Line Inducing Principle (WLIP) or lipodepsipeptide, and

B. 0.64 µg of tolaasin I, respectively.

- On upper blocks, 5 µl of sterile water was deposited.
- Brown sunken lesions (pits) were observed with both A and B treatments.
- Sometimes applications of WLIP on mushroom caps before inoculation with *P. tolaasii* protected the mushroom against the bacterium and they did not show discoloration.



Andolfi *et al.*,2009;..

Management

Assay on tissue blocks and whole sporophores of *Agaricus bisporus*

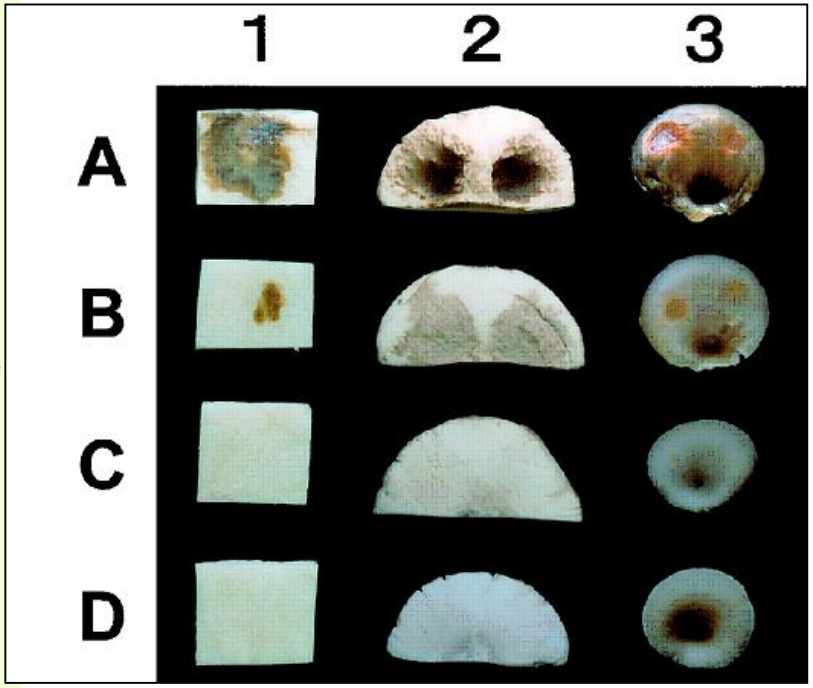
- Recent studies proposed WLIP, the lipodepsipeptide of *P. reactans* as a potential inhibitor of the symptoms of the brown blotch disease of *A. bisporus* caused by *P. tolaasii*.
- Applications of WLIP on mushroom caps before inoculation (with bacterial concentrations higher than the threshold) protected the mushroom against the bacterium and they did not show discolouration.
- The inhibition of the browning was also effective when incubating at low temperatures and during 4 days, but its efficiency at a commercial scale has not been tested yet.

Soler-Rivas *et al.*,2006

Management

Suppression of the brown blotch disease by tolaasin detoxifying bacteria

- Panel A: *P. tolaasii*,
- Panel B: *P. tolaasii* +*Acinetobacter* sp. strain OM-H10,
- Panel C: *P. tolaasii* +*S. multivorum* OM-A8,
- Panel D: A buffer control.
- Lane 1-3: Potato tuber slice, *A. bisporus* fruit body, and *P. ostreatus* fruit body, respectively.
- *Mycetocola tolaasinivorans* OM-F11, *M. lacteus* OM-A1, *Pedobacer* sp. strain OM-E81 and *B. pumilus* OM-F6 also suppressed the disease development to the same extent as *S. multivorum* OM-A8.



Tsukamoto *et al.*,2002

Management

Pseudomonas tolaasii

Populus microbial endophytes

- A direct screening method was developed for isolation of additional anti-fungal endophytes (AFE) from wild poplar extracts.
- By challenging pathogens directly with dilute extracts, eleven isolates were found to be inhibitory to at least two plant pathogen strains and were therefore chosen for further characterization.
- Genomic analysis was conducted to determine if these endophyte strains harbored genes known to be involved in antimicrobial activities.
- The newly isolated *Bacillus* strains had gene clusters

1. for production of bacillomycin, fengicyn, and bacillibactin,
2. while the gene cluster for the synthesis of sessilin, viscosin and tolaasin were found in the *Pseudomonas* strains.

Doty *et al.*,2023

Management

Pseudomonas tolaasii

Known antimicrobial biosynthetic gene clusters

Strain	Total # of BGC	BGC's with Known Antimicrobial Activity [1]	Activity
WPB	12	cepacin A (62%)	antibiotic/antioomycetes
		ornibactin (93%)	antibiotic
		occidiofungin (94%)	antifungal
AFE 1	7	-	
AFE 3	6	-	
AFE 4A	21	difficidin [2] (100%)	antibacterial
		macrolactin (100%)	antibacterial/antifungal
		bacillaene (100%)	antibacterial
		fengycin (86%)	antibacterial
		bacilysin (100%)	antibacterial/antifungal
		bacillibactin (100%)	antibacterial
		lanthipeptide class II	antibacterial
AFE 5	18	sessilin [2] (100%)	antifungal
AFE 8	14	viscosine (100%)	antifungal
		tolaasin (60%)	antibacterial/antifungal
AFE 9	5	-	
AFE 16	5	-	
AFE 21B	21	difficidin (100%)	antibacterial
		macrolactin (100%)	antibacterial/antifungal
		bacillaene (100%)	antibacterial
		fengycin (80%)	antibacterial

AFE, Anti-Fungal Endophytes;
WPB, Wild Populus (poplar) of the species *Burkholderia vietnamiensis*.

WPB and the AFE strains carry a variety of biosynthetic gene clusters with known antifungal and antibacterial activity.

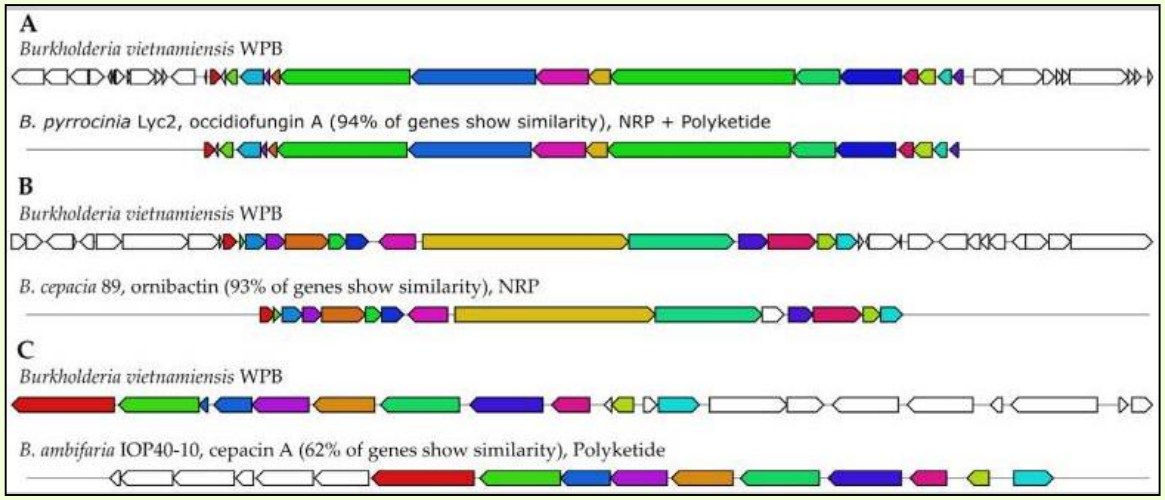
Doty *et al.*,2023

Management

Pseudomonas tolaasii

Organization of the biosynthetic gene clusters identified in *Burkholderia vietnamiensis* WPB

- Orthologous genes shared between the BGCs in WPB (biosynthetic gene clusters, BGCs with antimicrobial activities in WPB) and reference strain are shown with the same color for (**A**) occidiofungin (**A**,**B**) ornibactin, and (**C**) cepacin A.
- The genes not shared between WPB and the reference strain are in white.



Doty *et al.*,2023

Management

Pseudomonas tolaasii

Plant essential oils

- **Disk Diffusion Assay:**
- Ten microliters of a 1:1 serial dilution of each essential oil in 80% (v/v) methanol and 1.6 mg/mL of rifampicin were added to 6 mm diameter sterile blank disks.
- These were placed on the surface of Petri plates containing either 10 mL of KB or WA (0.7% agar) depending on the bacterial species.
- Aliquots of the target bacterial suspensions were added to the media, maintained at 45°C to obtain a final population of about 107 cfu/mL.
- After 48 h of incubation at 25°C, the minimal inhibitory quantity (MIQ), which causes an apparent inhibition zone around the 6 mm diameter disks, was recorded.
- The biological control assays were performed twice with three replicates.

Iacobellis *et al.*,2005

Management

Pseudomonas tolaasii

Plant essential oils

- **Biological control**
- Essential oils of *Matricaria chamommilla*, *Mentha piperita*, *M. spicata*, *Lavandula angusti folia*, *Ocimum basilicum*, *Thymus vulgaris*, *Origanum vulgare*, *Salvia officinalis*, *Citrus limon* and *C. aurantium* and their components; linalyl acetate, linalool, limonene, α-pinene, β-pinene, 1,8-cineole, camphor, carvacrol, thymol and menthol were assayed for inhibitory activity against the three major pathogens of the button mushroom, *Agaricus bisporus* including the bacterium *Pseudomonas tolaasii*.
- The highest and broadest activity was shown by the *Origanum vulgare* (mint family) oil.
- Oregano and Thyme oils and their derivatives may be used as an alternative for the synthetic chemicals.
- Positive although lower effect (55% growth reduction) of olive mill wastewaters(OMW) was reported for *Pseudomonas tolaasii*.

Sokovic´and van Griensven,2006; Soler-Rivas *et al.*,2006

Management

Pseudomonas tolaasii

Olive mill wastes

- **Biological control**
- Cultivation of *Pleurotus* spp. on substrates containing added olive mill waste and wastewaters (OMWW) reduced bacterium-related symptoms caused by *Pseudomonas tolaasii*.
- Two compounds from olive mill waste 4-methylcatechol and catechol were effective as bactericides against *P. tolaasii* (minimum biocidal concentration 5 and 10 mM, respectively).
- Such concentrations were not reached if the wastes were added at 10% v/w; at such levels growth of the bacteria will only be inhibited, not eliminated completely.

Soler-Rivas *et al.*,2006

Management
Biological control
Wild edible mushrooms

- There are roughly 15,000 types of wild fungi in the UK.
- We have provided a simple guide of the best ones to eat and the most important ones not to pick(poisonous).

	Type	Season Start	Season End
 Agaricus Pilatianus	Poisonous	September	November
 Amanita Gemmata	Poisonous	September	November
 Beechwood Sickener	Poisonous	September	November
 Boletus Purpureus	Poisonous	July	November
Boletus Rhodoxanthus	Poisonous	September	November
Boletus Satanoids	Poisonous	June	September
 Brown Roll Rim	Poisonous	August	November
 Clitocybe Dealbata	Poisonous	July	November

	Type	Season Start	Season End
 Agaricus macrosporus	Edible	July	October
 Amethyst Deciever	Edible	June	December
 Bay Boletus	Edible	September	November
 Beefsteak Fungus	Edible	August	November
 Cauliflower Fungus	Edible	September	November
 Cep	Edible	August	November
 Chanterelle	Edible	June	November

Wild Food UK,2013

Management
Biological control
Wild edible mushrooms

Oyster Mushroom(*Pleurotus ostreatus*)

- Various *Pleurotus* (*oyster mushroom* or gilled mushrooms) wild edible mushrooms species have been shown to possess a number of medicinal properties, such as antitumour, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antiplatelet- aggregating, antihyperglycaemic, antimicrobial and antiviral activities.

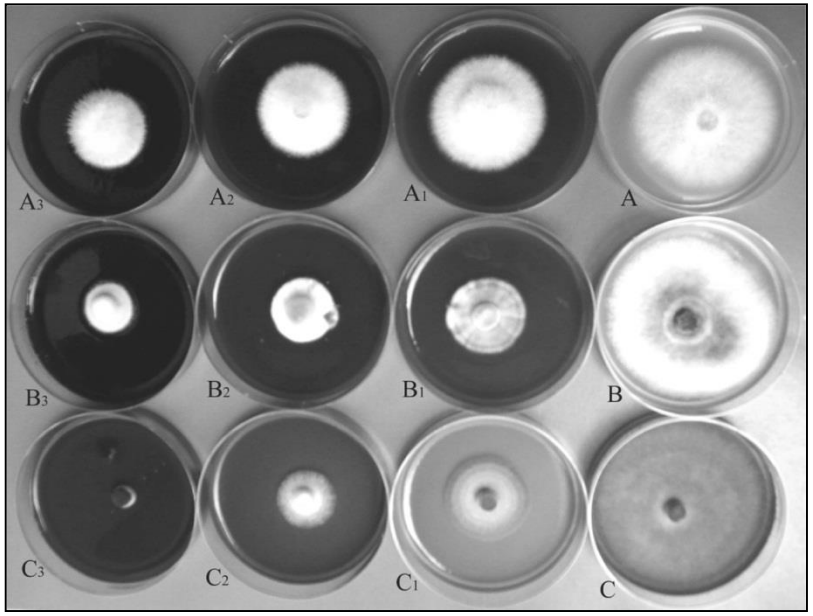
Species		Effective against
P. ostreatus	Crude extracts from fermentation broth	Gram-positive, Gram-negative bacteria and *Aspergillus niger*
	Hexane-dichloromethane extract containing *p*-anisaldehyde	*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Fusarium oxysporum*
	Various extracts; two main unidentified compounds	*Bacillus* spp., *Escherichia coli*, *Vibrio cholerae* and *Salmonella typhi*
P. eryngii	Eryngin – an antifungal peptide	*Fusarium oxysporum* and *Mycosphaerella arachidicola*
	Eryngeolysin – a haemolysin	*Bacillus* spp.
P. sajor-caju	12 kDa ribonuclease	*Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Gregori *et al.*,2007

Management
Biological control
Inedible wild mushrooms

Stereum ostrea

- Inhibitory effects of ethanol extract of *Stereum ostrea* on the mycelial growth of:
  A. *Botrytis cinerea*
  B. *Colletotrichum gloeosporioides*
  C. *Colletotrichum miyabeanus*
- A, B and C indicate control (fresh PDA) of respective fungi. A1/B1/C1, A2/B2/C2. A3/B3/C3 contain 10, 20 and 40 mg/ml crude extract, respectively.



Gregori *et al.*,2007

Management
Biological control
Wild edible mushrooms

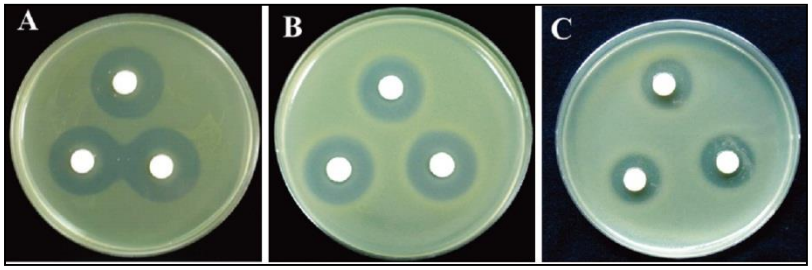
- Clear zones of *Xanthomonas campestris* pv. *campestris* (XCC79) inhibited by culture filtrates of:

A. *Lentinula edodes* (L1);

B. *Clitocybe nuda* (LA82); and

C. *Grifola frondosa* (G1).

- Only the culture filtrate of *Agrocybe cylindracea* showed a clear inhibition zone against *Pectobacterium carotovorum* subsp. *carotovorum*.



- Four culture filtrates strongly inhibited the growth of *Acidovorax avenae* subsp. *citrulli*.
- The culture filtrates of *A. cylindracea*, *Grifola frondosa* and *L. edodes* showed various sizes of growth inhibition zones against *Ralstonia solanacearum*.
- Six culture filtrates inhibited the growth of *Xanthomonas oryzae* pv. *oryzae*,
- Two culture filtrates inhibited the growth of *Erwinia chrysanthemi*, and 13 culture filtrates inhibited *X. campestris* pv. *campestris* and *X. axonopodis* pv. *vesicatoria*.

Chen and Huang,2010

Management
Phage therapy
Terminology

- What are phage lysates?
- A plate lysate is simply a concentrated liquid sample of phage.
- It is obtained by infecting a plate of bacteria with the phage of interest, letting the phage lyse the cells, then adding buffer directly to the plate surface to collect the phages.
- What does a titer of a lysate express?
- Phage titer is expressed in PFU (Plaque Forming Units)/mL.

1. Lysates with a final concentration greater than 10^9 PFU/ml are "High Titer" lysates.
2. Current data suggest that the higher the titer, the more stable the lysate.
3. Full plate titers have higher degree of accuracy.

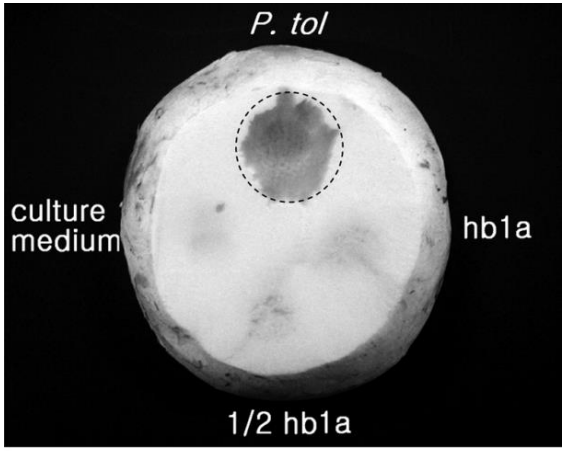
Management
Phage therapy
Terminology

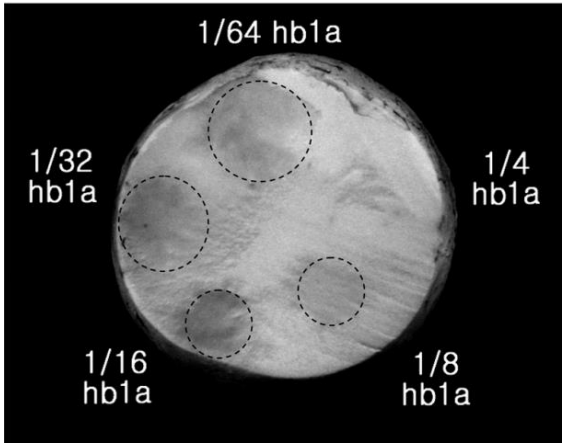
- Phage cocktails: Phages can be combined with non-phage agents such as antibiotics, or phages can instead be combined with other phages possessing different ranges.
- The latter, phage-phage combination therapies, can be described as polyphage treatments. More commonly, these are known as phage cocktails.

Management

hb1a, hb2d, and bp5e bacteriophages of *P. tolaasii*

- Three phages, namely, hb1a, hb2d, and bp5e, were isolated from various sewage samples and chosen from each toxicity group.
- The diluted lysate of phage hb1a by 1,000 times was diluted again as indicated and added to the surface of a mushroom along with *P. tolaasii* (P. tol).
- The mushroom was incubated for 15 h.
- The 1-1/64 hb1a: equal volumes of diluted phage lysate and pathogenic bacteria were added.





Kim *et al.*,2011

A new *Pseudomonas tolaasii* bacteriophages and genomic investigation of the lytic phage BF7

Bf7 bacteriophage of *Pseudomonas tolaasii*

- Several studies have been carried out to isolate bacteriophages against different fluorescent pseudomonads causing diseases (e.g. bacterial blotch), but it proved to be very hard to achieve the desired effect as there are strains of *P. tolaasii*, which are naturally resistant to phage infection (Munsch & Olivier, 1995; Yoon *et al.*,2011).
- Sixteen lytic bacteriophages that infect *Pseudomonas tolaasii* LMG 23423 were isolated from smashed sporocarps of oyster mushroom (*Pleurotus ostreatus*) showing necrotic symptoms.
- The isolation, purification, and host range of these bacteriophages, as well as the morphology and the complete genome sequence analysis of the Bf7 bacteriophage – having one of the widest host ranges of them are described.

Sajben-Nagy *et al.*,2012

Phage isolation, purification, and titration
Bf7 bacteriophage of *Pseudomonas tolaasii*

- Infected mushrooms (5 g of each) were smashed, diluted in 10 mL SM buffer (100 mM NaCl, 8 mM $MgSO_4$, 50 mM Tris–HCl, pH 7.5, and 0.01% gelatin in distilled water), and incubated overnight at 25°C with gentle agitation.
- The mushroom particles and bacterial cells were removed by centrifugation at 4000 g for 20 min at 4°C, then the supernatant was centrifuged at 20,000 g for 60 min at 4°C to collect the phages.
- **Chloroform was added after the centrifugation to eliminate the residual bacterial cells.**
- 150 µL from this mixture was added to 50 µL of *P. tolaasii* LMG 2342 culture(OD= 1) incubated previously at 25°C for 18 h.
- The mixture was diluted in 6 mL of soft Triptic Soy Base(TSB) agar (0.7%), overlaid on 2% agar plates and allowed to solidify.
- The phage plaques were detected after 18 h of incubation at 25°C.

Sajben-Nagy *et al.*,2012

Phage isolation, purification, and titration
Bf7 bacteriophage of *Pseudomonas tolaasii*

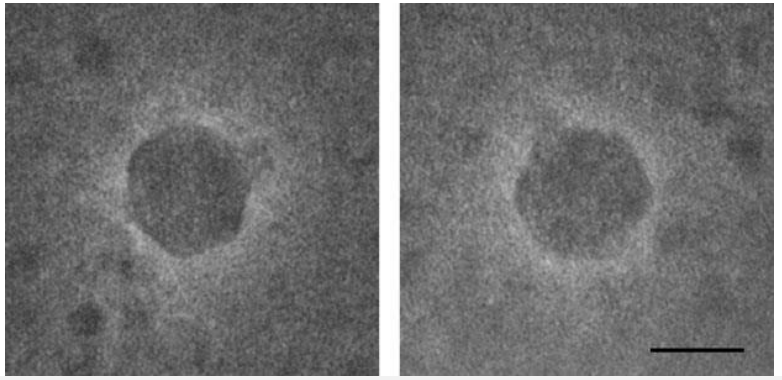
- Phage titers were determined by the double agar layer method (Adams, 1959) with minor modifications.
- Soft TSB with 0.7% agar was used for the top layer.
- Ten-fold serial dilutions were prepared from the phage lysates and added to the host bacteria.
- The mixture was poured onto the bottom agar layer consisting of LB medium.
- Number of plaques was scored after 18-24 h incubation at 25°C.

Sajben-Nagy *et al.*,2012

Morphology of bacteriophage Bf7

Bacteriophage of *Pseudomonas tolaasii*

- On the basis of the electron microscopic studies, the morphology of phage Bf7 seems to be similar to some other bacteriophages infecting the members of the genus *Pseudomonas*.
- We assigned phage Bf7 to the family Podoviridae based on its icosahedral phage head with a diameter of about 60 nm, the short tail

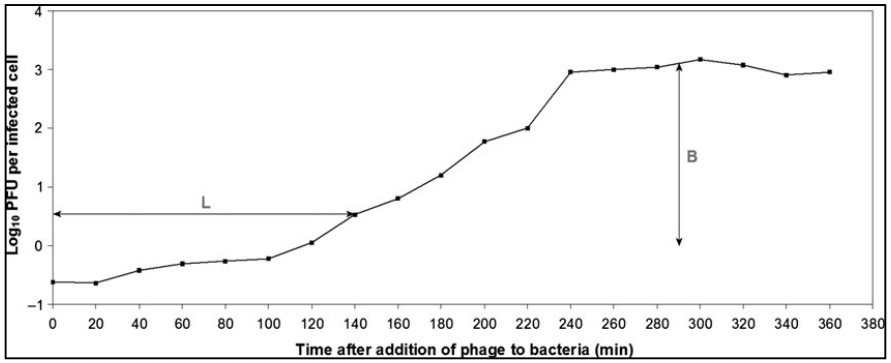


Transmission electron microscopic pictures of bacteriophage Bf7. The scale bar represents 50 nm.

Sajben-Nagy *et al.*,2012

Growth characteristics of bacteriophage Bf7

- Phage Bf7 forms clear plaques (1-3 mm in diameter) after 18 h incubation at 20°C on *P. tolaasii* 2342^{T}.
- This property depends mostly on the temperature.
- At 5, 10, 20, and 25°C clear plaques are formed after 18-48 h incubation, but no plaques are generated at 30 and 35°C.
- This phenomenon is similar to the plaque forming characteristics of phage φGP100 (Keel *et al.*,2002).
- Based on these observations, we performed our further experiments at 20 or 25°C.



One-step growth curve of phage Bf7 on Pseudomonas fluorescens LMG 2342 at 20°C. The growth parameters are indicated: L, latent period; B, burst period.

Phage therapy

23 varieties of *P. tolaasii* classified as three subtypes, Ptα, Ptβ, and Ptγ

- In previous studies, 23 varieties of *P. tolaasii* were isolated from infected mushrooms with disease symptoms and classified into three subtypes, Ptα, Ptβ, and Ptγ, based on their 16S rRNA gene sequences analysis and pathogenic characters.
- In this study, 42 virulent bacteriophages were isolated against these pathogens and tested for their host range.
- Some phages could lyse more than two pathogens only within the corresponding subtype, and no phage exhibited a wide host range across different pathogen subtypes.
- To eliminate all pathogens of the Ptα, Ptβ, and Ptγ subtype, corresponding phages of one, six, and one strains were required, respectively.

Yun *et al.*,2022

Phage therapy

23 varieties of *P. tolaasii* classified as three subtypes, Ptα, Ptβ, and Ptγ

- These 42 virulent bacteriophages were able to suppress the disease completely, as confirmed by the field-scale on-farm cultivation experiments.
- These results suggested that a cocktail (mixture)of these eight phages is sufficient to control the disease induced by all 23 *P. tolaasii* pathogens.
- Additionally, the anti-bacterial effect of this phage cocktail persisted in the second cycle of mushroom growth on the cultivation bed.

Yun *et al.*,2022

Phage isolation, purification, and titration

42 virulent bacteriophages of *Pseudomonas tolaasii*

- Isolation of bacteriophages. Bacteriophages were isolated by using *P. tolaasii* strains as the host strains. Various sewage samples obtained from a rural area of Cheongju, Korea, were mixed with the host bacterial culture for primary isolation of the phages.
- The mixture was added to a 0.75% semisolid agar medium at a 1:2 ratio (v/v) and poured onto a 1.5% solid agar plate. The double-layered plate was incubated for 15 h at 25°C. One of the phage plaques in the incubated plate was chosen and added to the overnight culture of the corresponding *P. tolaasii* host strain.
- Following the method described by Chibani-Chennoufi et al. (2004), phage lysate was a supernatant obtained by centrifuging the culture medium inoculated with the phage and host strain.
- NaCl was added to the phage lysate at a concentration of 10%, incubated for 1 h at 0°C, and centrifuged at 6,000 ×g for 10 min.
- Polyethylene glycol (PEG-6000 as a surfactant/emulsifier was added to the supernatant at a concentration of 10% and incubated for 1 h at 0°C. Phages were collected by centrifugation at 19,000 × *g* for 10 min (XL90; Beckman Coulter, Pasadena, CA, USA).
- The precipitated phages were resuspended in a phage buffer (50 mM tris(hydroxymethyl)aminomethane-HCl, 150 mM NaCl, 20mM NH_4Cl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% gelatin, pH 7.4).
- The resuspended phages were filtered using a 0.2 μm microfilter and stored at -70°C.

Yun *et al.*,2022

Phage titer and bactericidal activity measurement

- Phage titer was determined using the double-layer agar technique (Chibani-Chennoufi *et al.*,2004).
- Phage suspensions were diluted 10^4-10^9-fold and mixed with the culture media of the host bacteria that was previously added to 3 ml of soft agar and poured onto a PAF hard agar plate.
- The plate was incubated for 15 h at 25°C, and plaques formed in the plate were counted.
- To measure the bactericidal activity, the phage lysate at a final concentration of 1% (v/v) was added to the PAF broth containing 5% of host cell culture and incubated at 25°C for 15 h.
- The final concentrations of bacterial strains and phage cocktail were 2×10^6 cfu/ml and 1×10^5 pfu/ml, respectively.
- Bacterial growth was measured by using a UV/Vis spectrophotometer at 600 nm.

Yun *et al.*,2022

Phage typing according to host specificity

Bacteriophages of *Pseudomonas tolaasii*

- Phage typing according to host specificity. Green shades indicate phages selected for phage cocktail preparation.
- [a]O, susceptible to phage. [b] X, resistant to phage.

Pathogen / Phage type		Ptα		
		6264 HK2	HK1 HK3	HK4 HK5
ϕPtα	ϕ6264	O[a]	O	O
	ϕHK1	O	O	X[b]
	ϕHK2	O	X	X

Pathogen / Phage type		Ptβ									
		HK7	HK20 HK21 HK22	HK18 HK19	HK15	HK9	HK8 HK10 HK12 HK17	HK13	HK14	HK16	HK11
ϕPtβ	ϕHK19	O	O	O	X	X	O	X	X	X	X
	ϕHK7	O	O	X	O	X	X	O	X	X	X
	ϕHK22	O	O	X	O	O	X	X	X	X	X
	ϕHK16	O	X	O	X	X	X	X	X	O	X
	ϕHK14	X	X	X	X	X	X	X	O	X	X
	ϕHK11	X	X	X	X	X	X	X	X	X	O

Pathogen / Phage type		Ptγ
		HK23
ϕPtγ	ϕHK23	O

Yun *et al.*,2022

Evaluation of phage therapy on oyster mushroom cultivation

Bacteriophages of *Pseudomonas tolaasii*

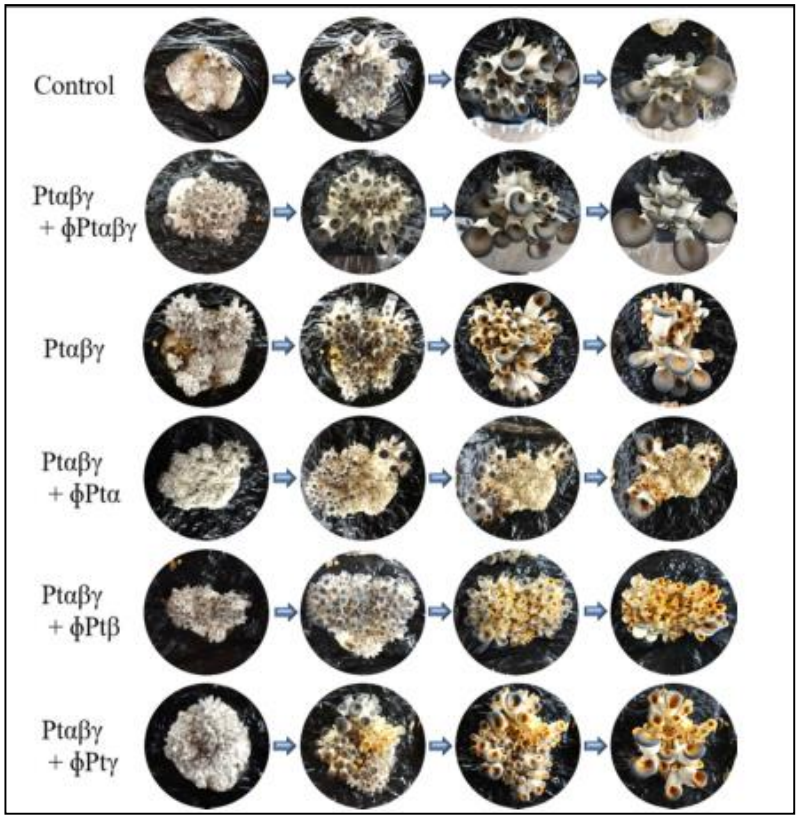
- Seedlings of oyster mushrooms were cultivated in three replicated beds 1 m wide and 2 m long filled with a sterilized sawdust medium. After 3 weeks, when the bed was fully covered with mycelia, the temperature in the cultivation house was lowered to below 18°C to induce the development of the fruiting body.
- The mixture of pathogen culture media (5×10^6 cfu/ml) and the mixture of eight phages (2×10^8 pfu/ml) were sprayed onto the beds.
- Acrylic plate walls 20 cm high were installed to minimize the disturbance among the control and the experimental plots.
- The bacterial mixture (Ptαβγ) and phage mixture (ϕPtα, ϕPtβ, ϕPtγ, and ϕPtαβγ) were made independently and the mixtures applied sequentially.
- There were three replicated mushroom-cultivating beds and each mushroom bed was divided into six sections (Control, Ptαβγ + ϕPtαβγ, Ptαβγ, Ptαβγ + ϕPtα, Ptαβγ + ϕPtβ, and Ptαβγ + ϕPtγ) and each section was 0.3 m wide and 1 m long.
- Five milliliters of each mixture were applied. The ratio of each one of 23 strains in the mixture was about 4% and the 0.5 ml of each bacterial culture was added to the mixture. Each bacterial strain had a level of 5×10^6 cfu/ml in the mixture. Furthermore, the ratio of each phage in the mixture was 12.5% and the 0.5 ml of each phage lysate was added to the mixture and each phage had a level of 2×10^8 pfu/ml.
- Protective effects of the phage cocktail were measured by the simultaneous treatment of phage cocktail right after pathogen treatment and by the treatment of phage cocktail at 12 h after the pathogen treatment, before blotch disease development.

Yun *et al.*,2022

Effects of the phage cocktail on the development of blotch disease

Bacteriophages of *Pseudomonas tolaasii*

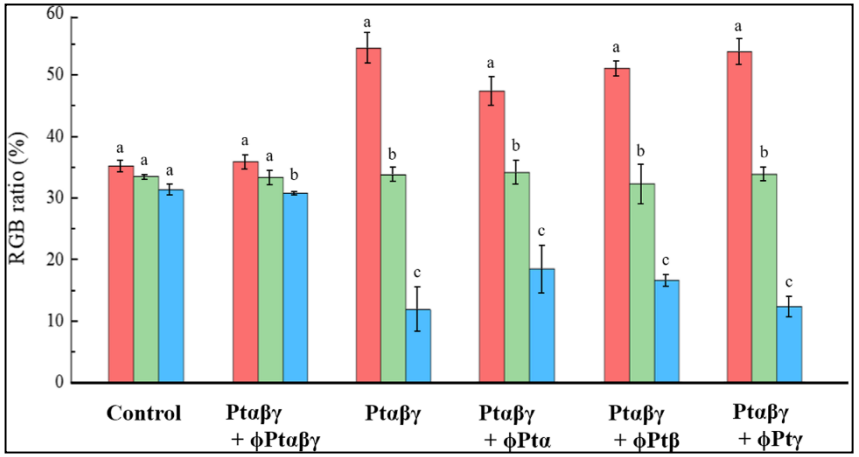
- Effects of the phage cocktail on the development of blotch disease.
- Control: treated with sterilized culture medium.
- Ptαβγ: all 23 strains of *P. tolaasii* pathogen.
- Ptαβγ + ϕPtαβγ: all *P. tolaasii* strains plus eight phages of the cocktail.
- ϕPtα: Ptα phage, ϕ6264.
- ϕPtβ: six Ptβ phages, ϕHK7, ϕHK11, ϕHK14, ϕHK16, ϕHK19, and ϕHK22.
- ϕPtγ: Ptγ phage ϕHK23.
- In each experiment phage mixture was sprayed on the mushroom immediately after pathogen inoculation.



Yun *et al.*,2022

Color analysis of phage cocktail effects
Bacteriophages of *Pseudomonas tolaasii*

- Color component evaluation of the brown blotch area obtained from shelf-cultivated mushrooms.
- Red-green-blue (RGB) ratios were calculated from photos of mushrooms obtained by the control, Ptαβγ + phage cocktail (ϕPtαβγ), and Ptαβγ treatments.
- Those of all pathogens plus one type of phage cocktail treatments were shown in Ptαβγ + ϕPtα, Ptαβγ + ϕPtβ, and Ptαβγ + ϕPtγ.
- Data are mean ± standard error percentages of the results from 7-8 replicates of more than three mushrooms. The same letters are not statistically significances at *P*> 0.05 (Tukey's test).

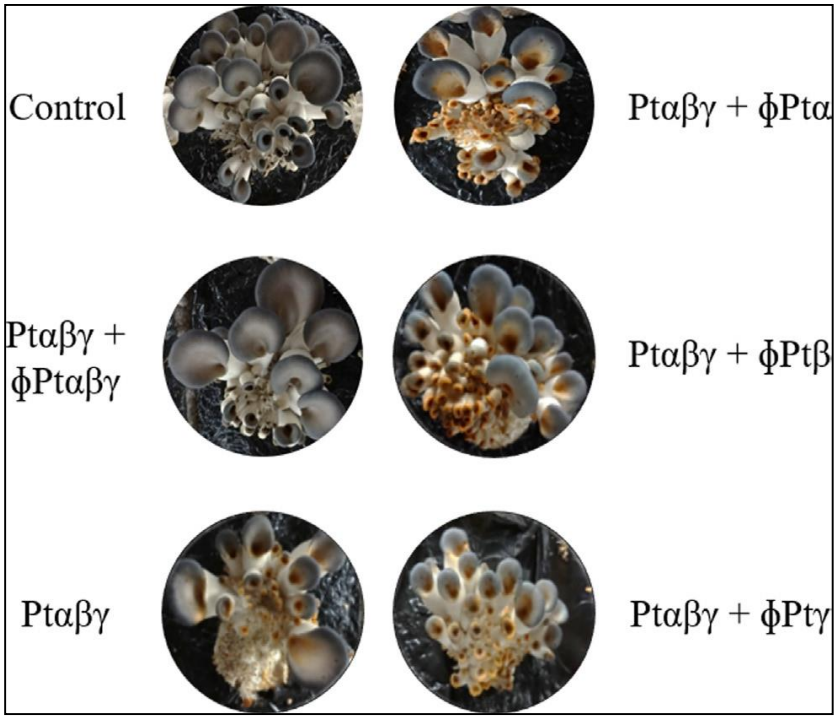


Yun *et al.*,2022

Persistent effect of the phage cocktail during the second cultivation cycle

Bacteriophages of *Pseudomonas tolaasii*

- After the removal of mushrooms from experimental beds, the second growth cycle was monitored without any treatment.
- Disease development was evaluated by brown coloration and shrinkage of the fruiting bodies.
- Ptαβγ: mushrooms grown after 1st generation in the shelf treated with all 23 pathogens,
- Ptαβγ + ϕPtαβγ: mushrooms grown in the shelf treated with Ptαβγ + phage cocktail.



Yun *et al.*,2022

Protective effect of the phage cocktail ensured by the treatment before blotch disease development

Bacteriophages of *Pseudomonas tolaasii*

- Control: treated with sterilized culture medium.
- Ptαβγ: treated with all pathogenic strains of *Pseudomonas tolaasii*.
- Ptαβγ + ϕPtαβγ: the phage cocktail was sprayed on the mushrooms before the development of disease at 12 h after pathogen inoculation.

Yun *et al.*,2022

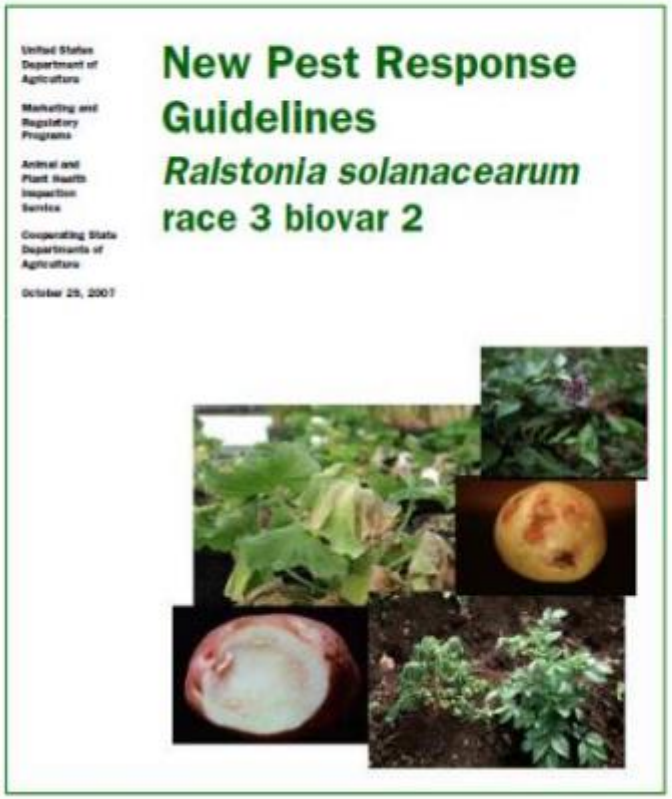
Ralstonia solanacearum

Wilt disease

Potato bacterial wilt

Disease Management Guide

- Disease Management Guide



- Current in formation for detection, control, containment, and eradication of R3b2 according to USDA regulations.

- USDA-APHIS website.
  http://www.aphis.usda.gov

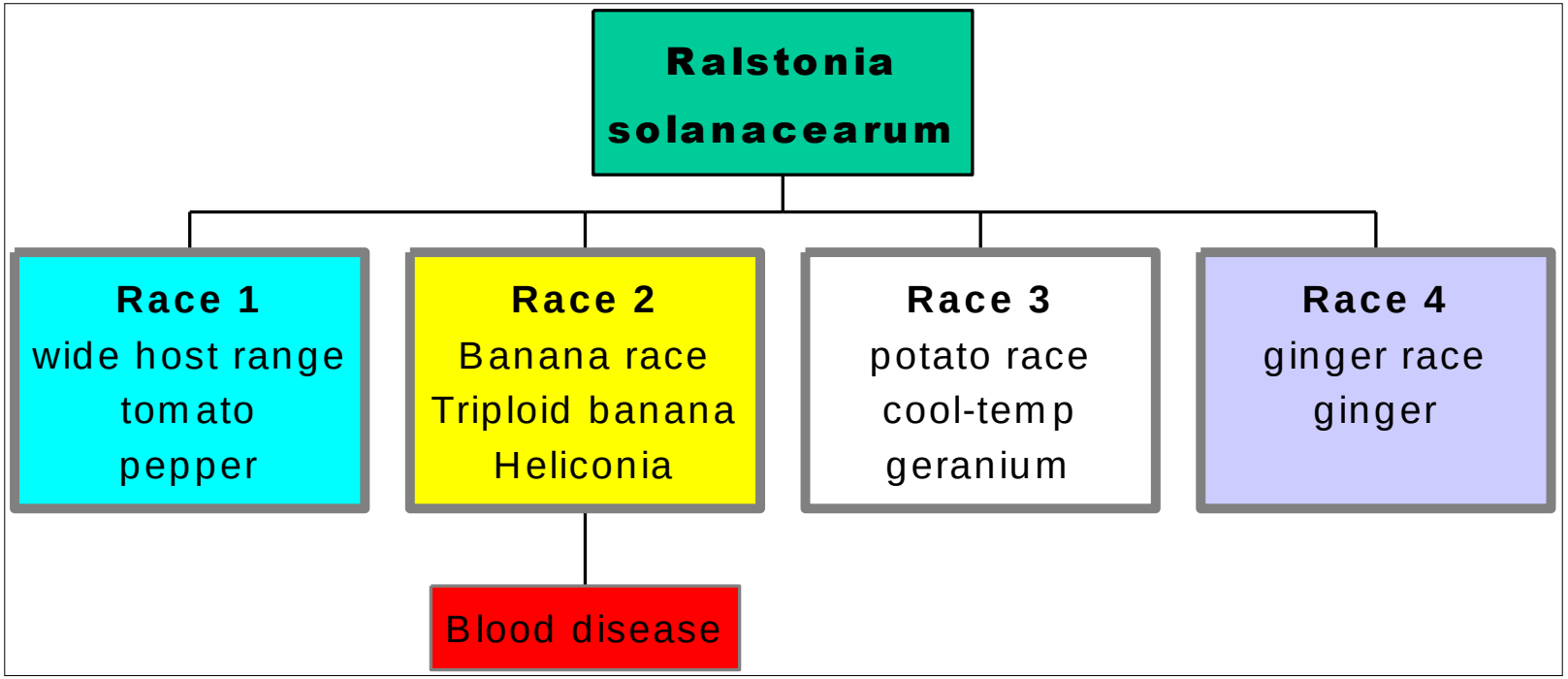
Champoiseau *et al.*,2009

Pathogen diversity
Proposed races

- Based on pathogenicity on different hosts, the strains were separated into five races.

1. Race 1 has a very wide host range and is known as the solanaceous race.
2. Race 2 as the *Musa* race
3. Race 3 as the potato race
4. Race 4 as the ginger race
5. Races 5 as the mulberry race

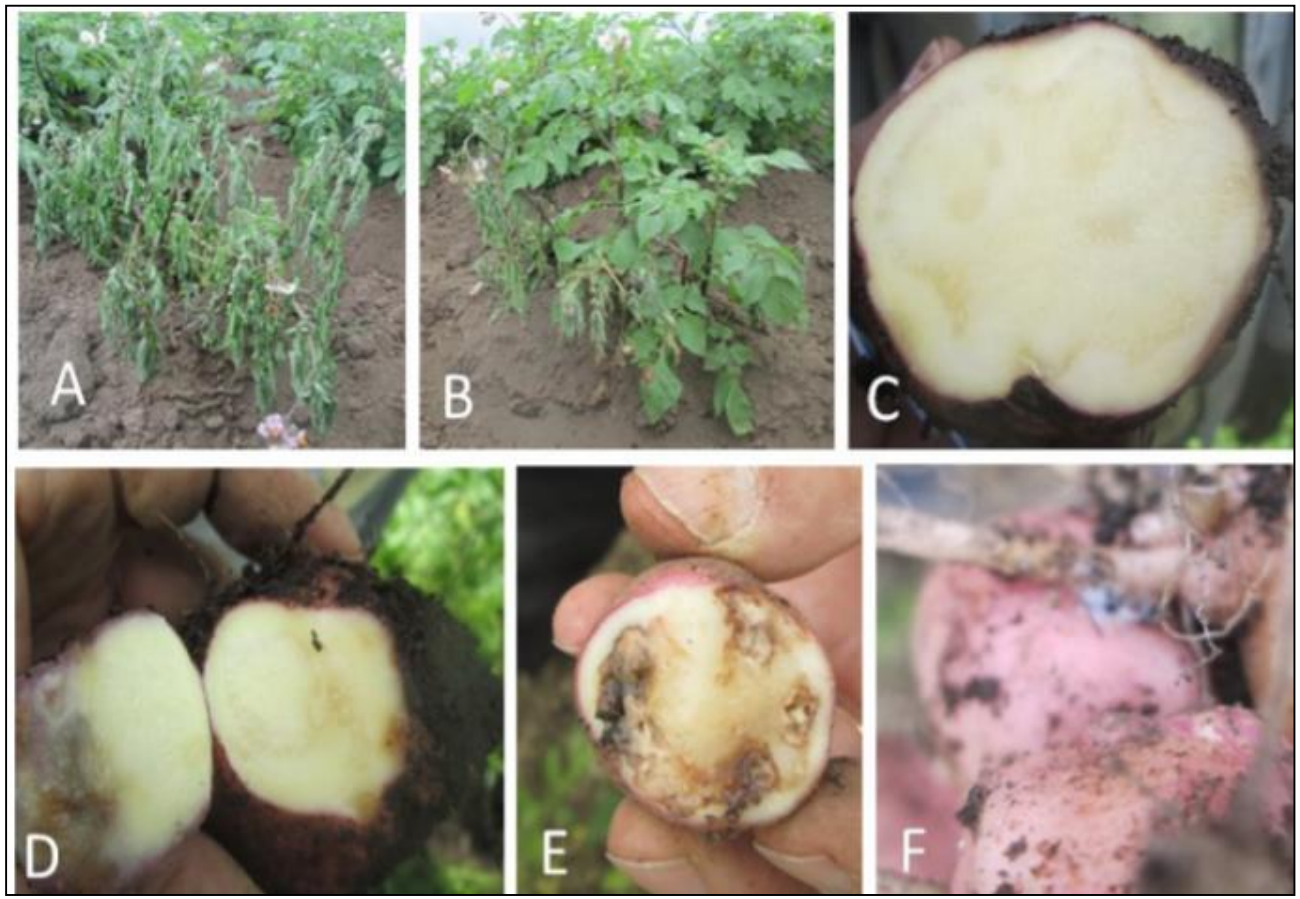
Host ranges



Blood bacterial wilt (BDB) is caused by *R. syzygi* subspecies *celebensis*

Bacterial Wilt (Brown rot)

R. solanacearum (race 3 biovar 2)



Mwamkemwa,2015

Bacterial wilt of tomato

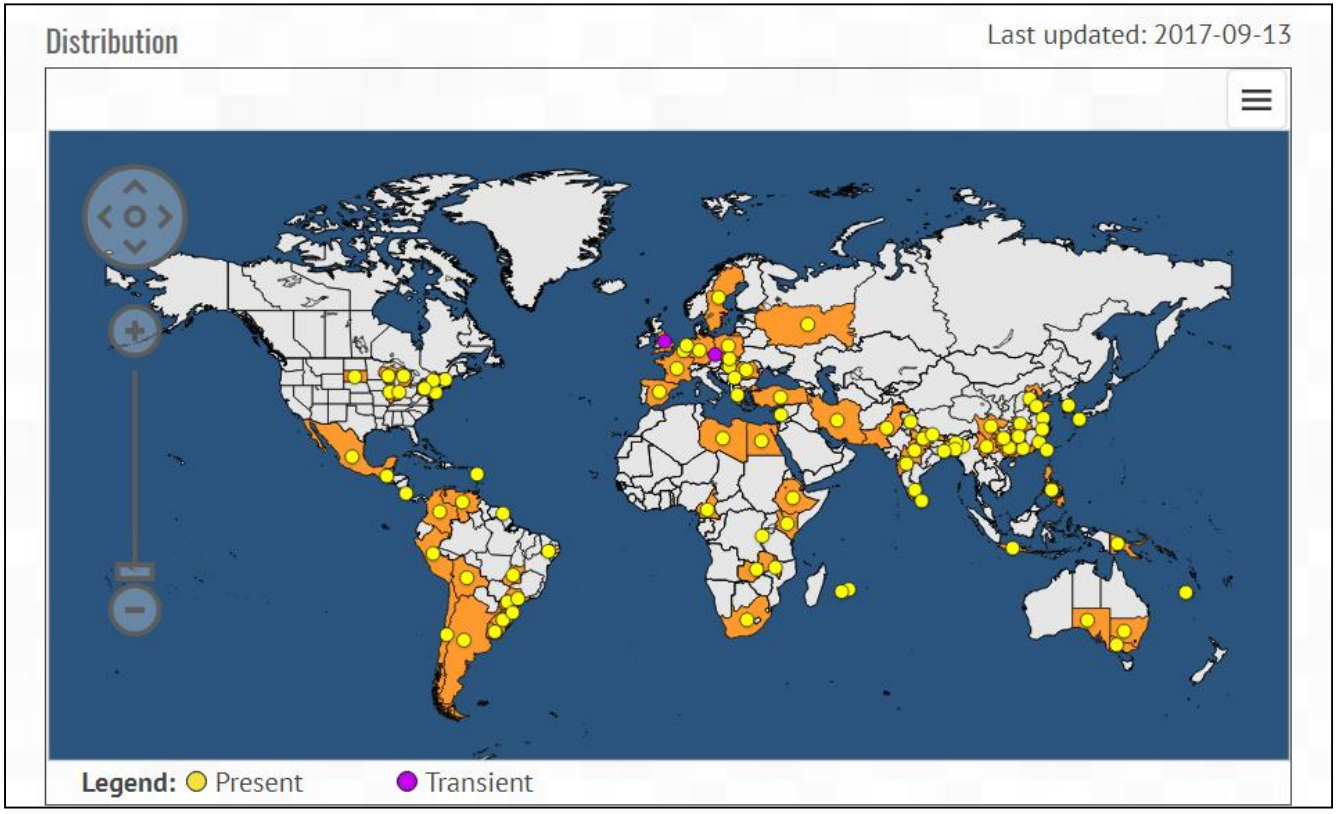
R. solanacearum (race 3 biovar 2)

Southern wilt of geranium
R. solanacearum (race 3 biovar 2)

- Initial symptoms of Southern wilt of geranium caused by *R. solanacearum* showing wilting and curling of leaves .
- Photo courtesy of D. Norman.

Champoiseau *et al.*,2009

Distribution map of *R. solanacearum* race 3: potato, tomato and geranium wilt diseases



The EEPO Global Database maintains an on-line *R. solanacearum* race 3 distribution map which can be found at: https://gd.eppo.int/taxon/PSDMS3/distribution (accessed 02/12/2017). The site also contains incident reports, surveys and other related information.

Host Plants of *Ralstonia solanacearum* Race 3 (Biovar 2)

The following genera contain species that are considered to be potential host plants of *Ralstonia solanacearum* Race 3 (Biovar 2): The names of known natural hosts are in **bold face**.

Ageratum
Amaranthus
Bidens
Brassica (mustards)
Calibrachoa
Capsicum (pepper)
Cerastium
Chenopodium (lambsquarters)
***Cyphomandra* (tree tomato)**
Datura (jimson weed)
***Drymaria* (weed)**
Erigeron
Erodium
Eupatorium
Fagopyrum (wild buckwheat)
***Galinsoga* (weed)**
Gnaphalium
Ipomoea
Leucas
***Lycopersicon* (tomato)**
***Melampodium* (weed)**
Momordica (bitter gourd)
Nicotiana
Oxalis
Pelargonium (geranium)
Petunia
Phaseolus(string bean)
Physalis
Polygonum (weed)
***Portulaca* (purslane)**
Ranunculus
Rumex
Salpiglossis
Salvia
Solanum (egg plant, potato, nightshade, horse nettle, etc.)
Soliva
Spergula
Stellaria
Tagetes
Tropaeolum (nasturtium)
Tussilago
Urtica (perennial stinging nettle)
Verbena
Zea
Zinnia

Epidemiology

- The pathogen infects roots through wounds.
- There is often an association between nematode infection and bacterial wilt, where the nematodes create wounds in the root tissue to allow an entry point for the bacterium to infect the plant.
- The bacterium survives in the soil and can maintain infectious populations over several years.
- Alternative weed hosts may also play a role in survival and overseasoning.

Epidemiology

- Temperature is a major determinant in the distribution of this pathogen, which is widespread in tropical, sub-tropical and warm temperate regions where the mean soil temperature is greater than 15°C.
- Continuous cropping of susceptible plants will also favor infection.
- Bacterial wilt of potatoes is more prevalent in slightly acid to acid soils, as are many soil-borne diseases.

Epidemiology
R. solanacearum (race 3 biovar 2)

- Race 3 biovar 2 (R3bv2) is most severe on plants between 24-35°C (75-95°F) and decreases in aggressiveness when temperatures exceed 35°C (95°F) or fall below 12°C (54°F).
- Active disease at temperatures below 16°C (61°F) is rare.
- R3bv2 can still survive, often in a physiologically latent state known as viable but non-culturable (VBNC).

Champoiseau & Allen,2009

Epidemiology

- The pathogen is also disseminated by:

1. Contaminated farming equipment, in soil on tyres and footwear.
2. Drainage water carrying inoculum through the soil.
3. Infected seed, especially in the case of groundnut.
4. Seedlings raised in infected soil, spreading the pathogen to new areas.
5. Cultural operations such as pruning.

- Moko disease, Banana Blood wilt and fruit rot and Bugtok fruit and peduncle rot are spread mechanically, by insects and on infected plants.

Eradication

Strict regulations (zero tolerance)

R. solanacearum race 3 biovar 2

- Bacterial brown rot is a "zero tolerance" disease, which means that if a single positive plant is found in a field the entire seed lot can be rejected.
- In areas where the bacterium is not known to be established, the first strategy is to prevent introduction and, if inadvertently introduced, subsequent movement of the pathogen.
- Government regulations in the United States include zero tolerance for *R. solanacearum* R3bv2, since it is classified as a Select Agent.
- This zero tolerance includes: reinforcement of quarantine regulations, sanitation protocols, and inspections designed to prevent introduction of infected geranium cuttings produced off-shore.

Champoiseau *et al.*,2009

Eradication

Strict regulations (zero tolerance)

zero-tolerance is implemented by sampling &testing

1 sample = 200 tubers

[1 sample per fraction of 25 tonnes]

preferably from lots in storage

following sound scientific and statistical principles

[based on the biology of the organism]

[considering the particular production systems of the plant material subjected to testing]

infection level p	2,0%	1,0%	0,5%	0,2%	0,1%	0.01%
P	0.98	0.86	0.63	0,33	0.18	0.02

Probability of detection (P) in relation to sampling of 1x200 tubers from a potato lot (considered infinite size) with random distribution of various levels of infected tubers (p)

Vaerenbergh,2006

Management

Chemical control is generally not feasible

- **Cultural practices include:**
  - Rotation with maize or rice for > 2 years.
  - Using healthy seed / certified seed and seedlings, stored under dry cool conditions
  - Planting in cooler conditions, or using resistant rootstock when planting in summer
  - Maintaining clean machinery may aid in the minimizing the spread of the disease.
  - Deep burying of inoculum with tillage, or repeated disk ploughing of dry soil to expose inoculum to the sun can be performed as a control practice.
  - Fallows of 18-months have been shown to control the bacterial wilt of banana, with a shorter period for dry soils.
  - All rhizome tissue and weeds must be removed for an effective fallow. Since major cause of disease spread in bananas is through the replanting of infected suckers, new plantings should be started with tissue cultured plants to restrict the spread of the pathogen.
  - Selecting resistant cultivars of each crop.

Cultural practices

R. solanacearum race 3 biovar 2

- Heat treatment by solarization when used in combination with other control strategies is another method that was shown to reduce *R. solanacearum* populations in soil.
- On geranium, application of phosphorous acid as a drench was recently shown to protect host plants from infection by the bacterium.
- Modification of soil pH by use of acidified nutrient solution or a combination of organic amendment and fertilizers was shown to be very effective in reducing bacterial wilt diseases on diverse hosts.
- Use of suppressive soils was shown to slow infection of tomato seedlings by *R. solanacearum* and reduce bacterial wilt incidence in nurseries.

Patrice,2001

Management

R. solanacearum race 1

Bacteriocins, bacteriophage, non-pathogenic *hrpO* mutants

- Biological control with bacterial antagonists or avirulent strains. However this measure is generally useful only under greenhouse conditions.
- Theoretically, biological control agents (BCAs) may work directly by competing with the pathogen for limited resources in the soil, the rhizosphere, or within the plant, or by producing antibiotics, bacteriocins and bacteriophage.
- BCAs may also work indirectly by stimulating plant defense capabilities.
- BCAs often are nonpathogenic bacteria, but the greatest effort on developing a BCA for *R. solanacearum* has focused on non-pathogenic *hrpO* mutants of the pathogen.
- Unfortunately, although showing promise when tested in controlled conditions, none of the potential BCAs have effectively or consistently reduced BW in field conditions.
- Development of a useful, affordable BCA is unlikely in near term, but progress is being made and there is still hope for success in the future.

Denny,2006

Management
Susceptibility of *Ralstonia pseudosolanacearum* to bacteriophages

- The test was performed using the double layer agar plaque assay method (Bae *et al*.,2012) to determine the susceptibility of *R. pseudosolanacearum* to 12 different bacteriophage isolates.
- Colonies of *R. pseudosolanacearum* suspended in sterile water (OD_{600} value 0.1) were added to 0.6% CPG medium as much as 0.5 mL.
- A total of 1 mL of the bacteriophage suspension was added to the same 0.6% CPG medium.
- Then, 0.6% CPG media which has been added with *R. solanacearum* and bacteriophage was then poured into a Petri dish containing CPG media as the base.
- The media was incubated for 24 to 48 hrs at 30°C. The plaque formed was observed and indicated the ability of bacteriophages to infect *R. solanacearum*.

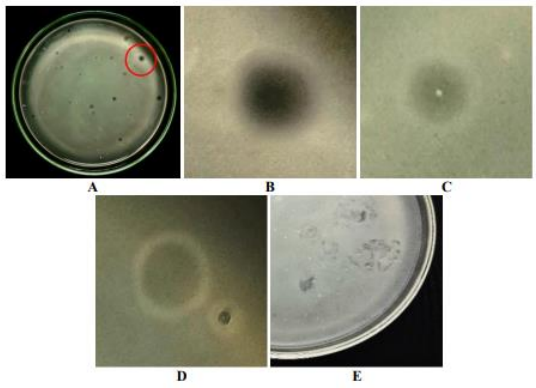
Nurdika *et al*.,2022

Management

Susceptibility of *Ralstonia pseudosolanacearum* to bacteriophages

Bacteria isolate	Bacteriophage isolate											
	NGW	MLG	JTNM	CAB1	CAB2	GRLY	BLKT	MGMK	ASV1	ASV2	HSV2	HSV3
RS19	+	+	+	-	-	-	-	-	+	+	+	+
RS24	+	+	+	-	-	-	-	-	+	+	+	+

Note: (+): Plaque is formed, (-): No plaque formed.



1. Table: Bacteriophage plaque formation on CPG medium with *Ralstonia pseudosolanacearum* causing bacterial wilt disease in tomatoes and eggplants. RS19 and RS24 isolates were susceptible to 7 of the 12 bacteriophage isolates used in this study.
2. Figures: Plaques from bacteriophage that infect *R. pseudosolanacearum*: A. Plaques spread on CPG medium, B. Clear and round plaque morphology, C. Cloudy plaque with a point in the center, D. Cloudy with a halo around it, and E. irregularly cloudy plaque.

Nurdika *et al.*,2022

Management
Biological control
Acinetobacter and *Enterobacter* spp.

- Biocontrol efficacy of strains of *Acinetobacter* and *Enterobacter* as potential biocontrol agents against *Ralstonia* wilt of tomato.
- The disease index was recorded based on a scale of 0-4 as described by Kempe and Sequeira (1983).
- Disease incidence and biocontrol efficiency were calculated as follows:
- Disease incidence = [∑ (The number of diseased plants in this index X Disease index)/(Total number of plants investigated X The highest disease index)] X 100%.
- Biocontrol efficacy = [(Disease incidence of control – Disease incidence of antagonist-treated group)/Disease incidence of control] X 100%.
- Assessment of plant growth promotion by antagonistic strains:
- Biomass increase = [(Average fresh weights of plants treated with antagonist- Average fresh weights of control plants)/Average fresh weights of control plants] X 100%.

Xue *et al.*,2008

Management

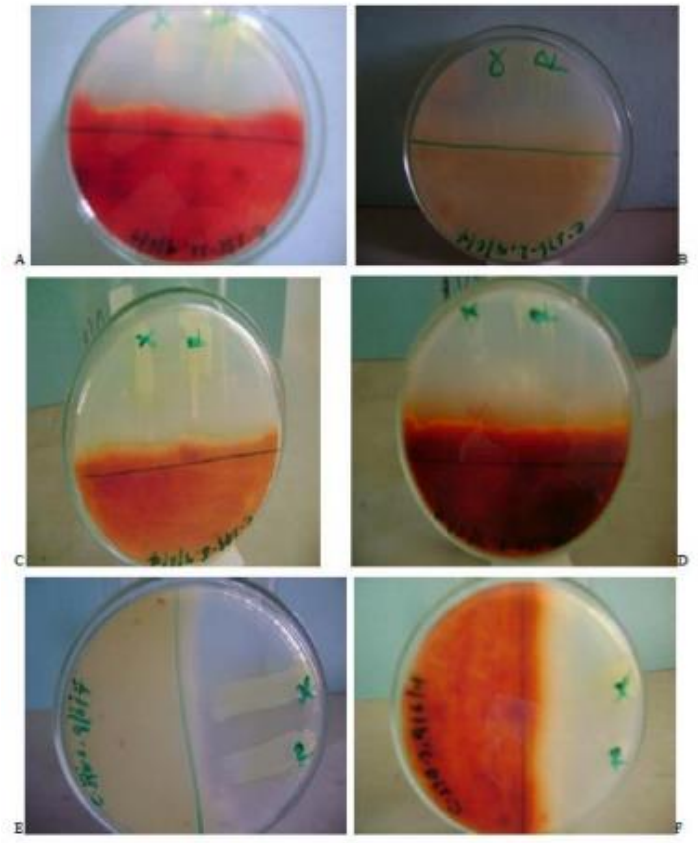
Biological control

Actinobacteria against tomato bacterial wilt (*Ralstonia solanacearum*)

- Some actinobacterial isolates showing significant inhibition ($P \leq 0.05$) against the target pathogen during preliminary screening:

A. IsolateS#132-11
B. IsolateS#176-2
C. IsolateS#149-6
D. IsolateS#130-2
E. IsolateS#196-1
1. Isolate S#174-3

- R=*Ralstonia solanacearum*.



Biratu *et al.*,2008

Plant resistance inducers
R. solanacearum race 3 biovar 2 (R3bv2)

- Application of plant resistance inducers, such as acibenzolar-S-methyl (ASM; Actigard 50 WG), might be used to enhance host resistance against *R. solanacearum* race 3 biovar 2, as it was recently shown to work for broad-host range strains of the pathogen.
- Bacterial wilt incidence was significantly reduced in ASM-treated tomato plants.
- Besides, produced significantly higher tomato yield than the untreated controls.
- This is the first report of ASM-mediated control of bacterial wilt under field conditions.

Patrice,2001; Pradhanang *et al.*,2005

Plant essential oils and systemic acquired

New tactics for bacterial wilt management on tomatoes *R. solanacearum* race 3 biovar 2

- Several different plant essential oils (Palmarosa, Lemongrass, Thymus, S. alpina) were successfully used as soil fumigants to reduce bacterial populations of *R. solanacearum* in tomato.
- Application of stable bleaching powder in conjunction with deep plugging can also be used as it showed significant reduction in bacterial populations of the pathogen in greenhouse and field trials in several geographic areas.
- However, soil disinfection appears to be soil dependent and not universally applicable.
- Drawbacks of this and similar methods(e.g., acibenzolar-*S*-methyl or phosphorous acid) may include environmental damage, cost, and high labor inputs.

Patrice,2001,2009

Plant essential oils and systemic acquired
New tactics for bacterial wilt management on tomatoes
R. solanacearum race 3 biovar 2

- Plant essential oils were used as soil fumigants to manage bacterial wilt in tomato.
- Potting mixture ("soil") infested with *R. solanacearum* was treated with the essential oils at 400 mg and 700 mg per liter of soil in greenhouse experiments.
- *R. solanacearum* population densities were determined just before and 7 days after treatment.
- Populations declined to undetectable levels in thymol, palmarosa oil, and lemongrass oil treatments at both concentrations, whereas tea tree oil had no effect.
- Tomato seedlings transplanted in soil treated with 700 mg/liter of thymol, 700 ml/liter of palmarosa oil, and 700 ml/liter of lemongrass oil were free from bacterial wilt and 100% of plants in thymol treatments were free of *R. solanacearum* (Pradhanang *et al.*,2003).

Patrice,2001

Cultural and chemical practices of banana bacterial wilt (Moko disease)

R. solanacearum (race 2, biovar 1)

- The bacterial wilt diseases of banana are systemic and so once infected a plant must be destroyed in order to prevent further spread of the disease.
- Disinfection of all work tools to being used in the field is of paramount importance.
- Practice of breaking off the male bud from Bluggoe plants was also devised in order to prevent the insect transmission of Moko in Honduras.
- The soil within this buffer zone must then be allowed to fallow.
- There are no useful chemical control measures available for the management of any of these bacterial wilt diseases.
- The only chemical that is effective is methyl bromide used as a soil fumigant.

Plant Heath Australia,2006

Disease management strategies

Additional resources

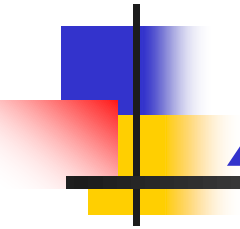
Ralstonia/Bacterial wilt dedicated website

- Visit our *Ralstonia*/Bacterial wilt dedicated website:

  http://plantpath.ifas.ufl.edu/rsol/

- Pest and disease management guides
- Project description, accomplishments
- Real time pest alerts and first reports worldwide
- Protocols, book references and journal articles database
- Web resources
- Photo galleries
- Access to *Ralstonia*-L mailing list

Champoiseau *et al.*,2009



Agrobacterium

Gall diseases

Crown gall

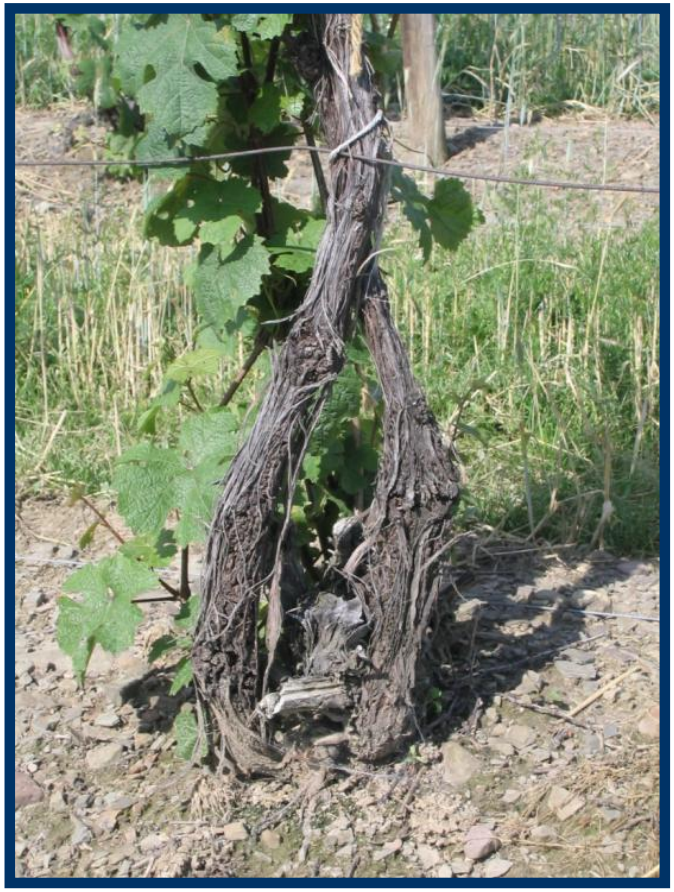
Agrobacterium tumefaciens

Crown gall on the crown and roots of a young apricot tree.
Photo by Joseph M. Ogawa.

Crown gall

Tumors or galls on the lower stem of grape

***Allorhizobium vitis* (= *Agrobacterium vitis*)**



De La Fuente,2009; Hartman,2007

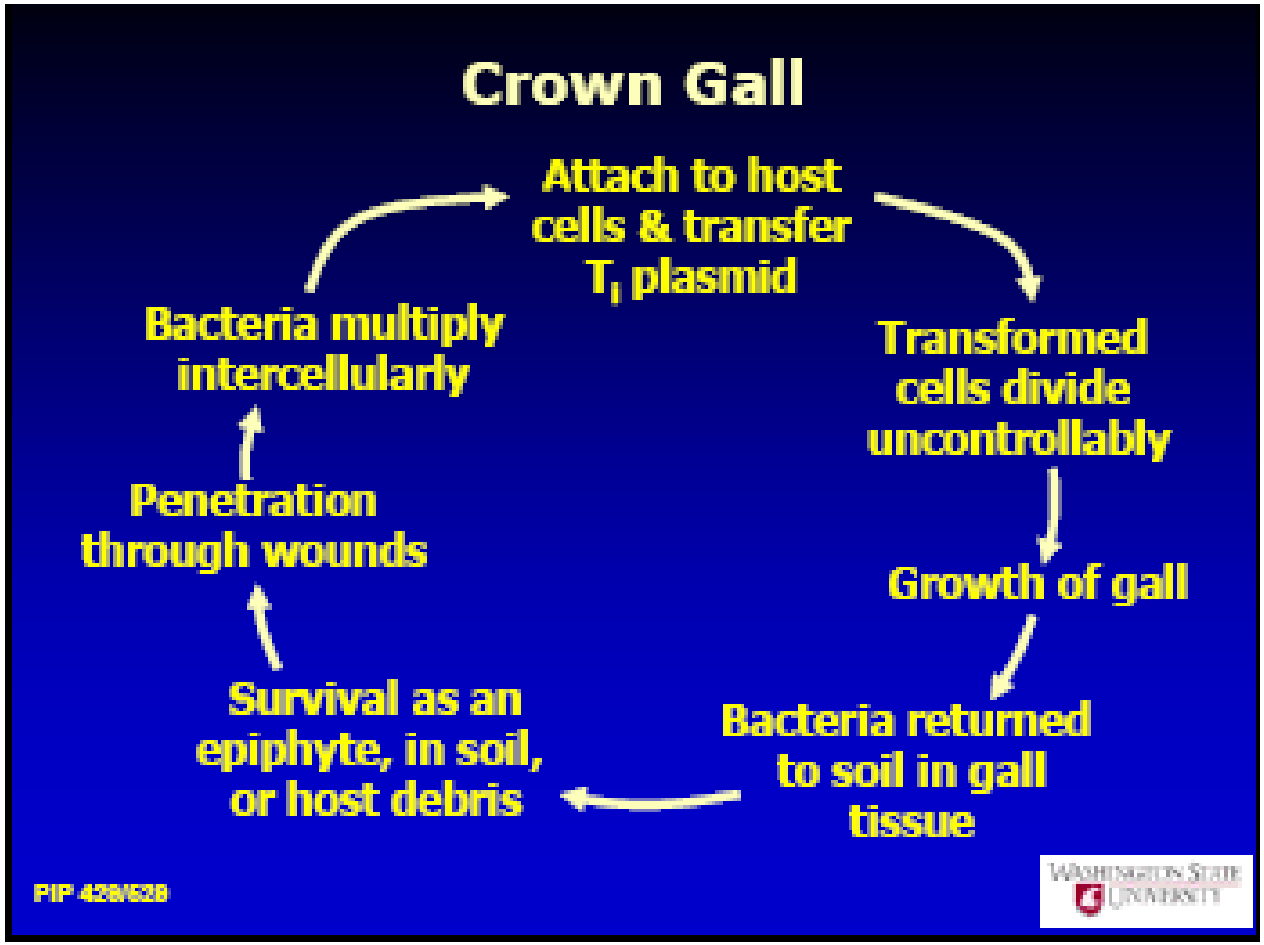
Gall (or burl) on an oak tree trunk, possibly crown gall

Agrobacterium tumefaciens

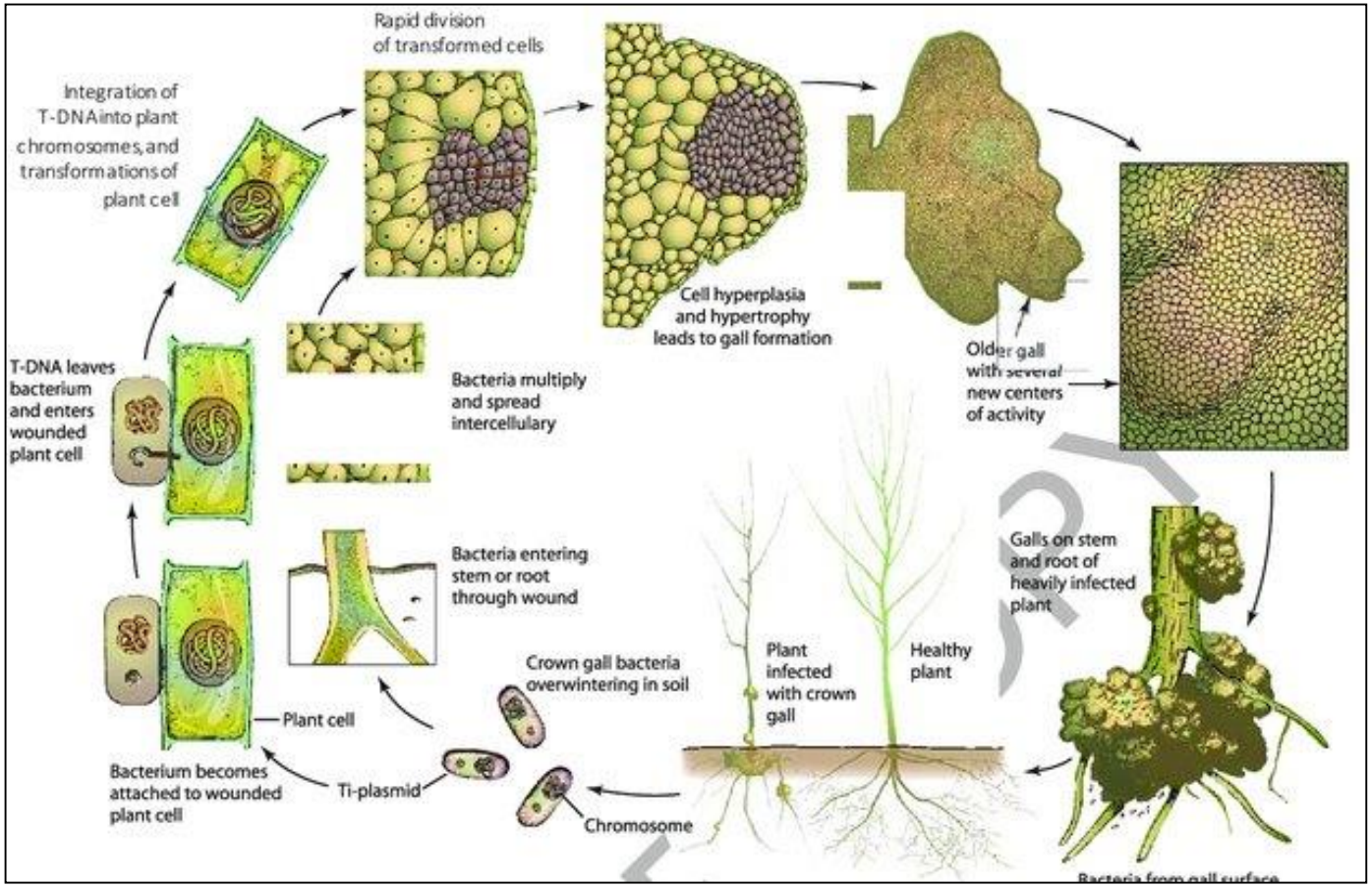
Quercus robur

Alamy Stock Photo

Disease cycle of the Genus *Agrobacterium*



Disease cycle of the Genus *Agrobacterium*



Agrios,2005

Agrobacterium or Rhizobium, which name to use?

Taxonomy and nomenclature within the genus remain controversial

- **Current status**
- Currently there is some confusion over whether *Agrobacterium* or *Rhizobium* is the best name to use for these bacteria.
- Although the taxonomic basis for the reclassification of *Agrobacterium* to *Rhizobium* is supported by some bacterial systematists (e.g., Euzeby,2013),
- Others — particularity molecular biologists — prefer to use the *Agrobacterium* name with which they are familiar.
- ***Allorhizobium vitis* (=*A. vitis*)**

Weir,2013;…

Habitat
Host range

- The genus *Agrobacterium* is represented by species that are indistinguishable from members of *Rhizobium* except that they are pathogenic, producing rhizogenic growths or oncogenic galls rather than symbiotic nodules.
- Both *Agrobacterium* and *Rhizobium* have the unique capacity to induce prolific root formation, nitrogen fixing root nodules and autonomous crown-gall tumors on many higher plants including:

1. Most dicots,
2. Some monocots, and
3. Some gymnosperms.

Vincent,1970; Murugesan *et al.*,2010; Bull *et al.*,2010

Habitat

Host range

Susceptible species

Etiology
Allorhizobium vitis (=*A. vitis*)

- Crown gall of grapevine nursery stock remains a problem because of the systemic nature of the bacterial disease.
- Vineyards in Ontario, Canada are taking a $2-million hit each year from a bacterial disease called crown gall and the situation is getting worse (University of Guelph,1999).

Etiology

Allorhizobium vitis (=*A. vitis*)

Symptoms

- Newly formed galls are often first noticed in June-July and appear as pale-colored, fleshy, convoluted tissue immediately beneath the bark layer.
- Crown gall may develop on any freshly wounded, woody portion of the vine.
- It usually shows up low on the trunk, or at the graft union.
- *A. vitis* also causes areas of root necrosis on infected vines (Burr *et al.*,1987).

Burr,2015

Etiology

New sources of *A. vitis* in environment

- **Shoot tips in vineyards**
- – 2013, 11 of 30 tips from vineyard with crown gall were positive
- – 2014, 16 of 240 tips from two vineyards with crown gall were positive
- **Leaves in vineyard with crown gall**
- – Two leaves from 10 vines
- *Suggestive that *A. vitis* survives on surfaces of grape shoots and leaves.

Burr,2015

Etiology

Sources of *A. vitis* in environment

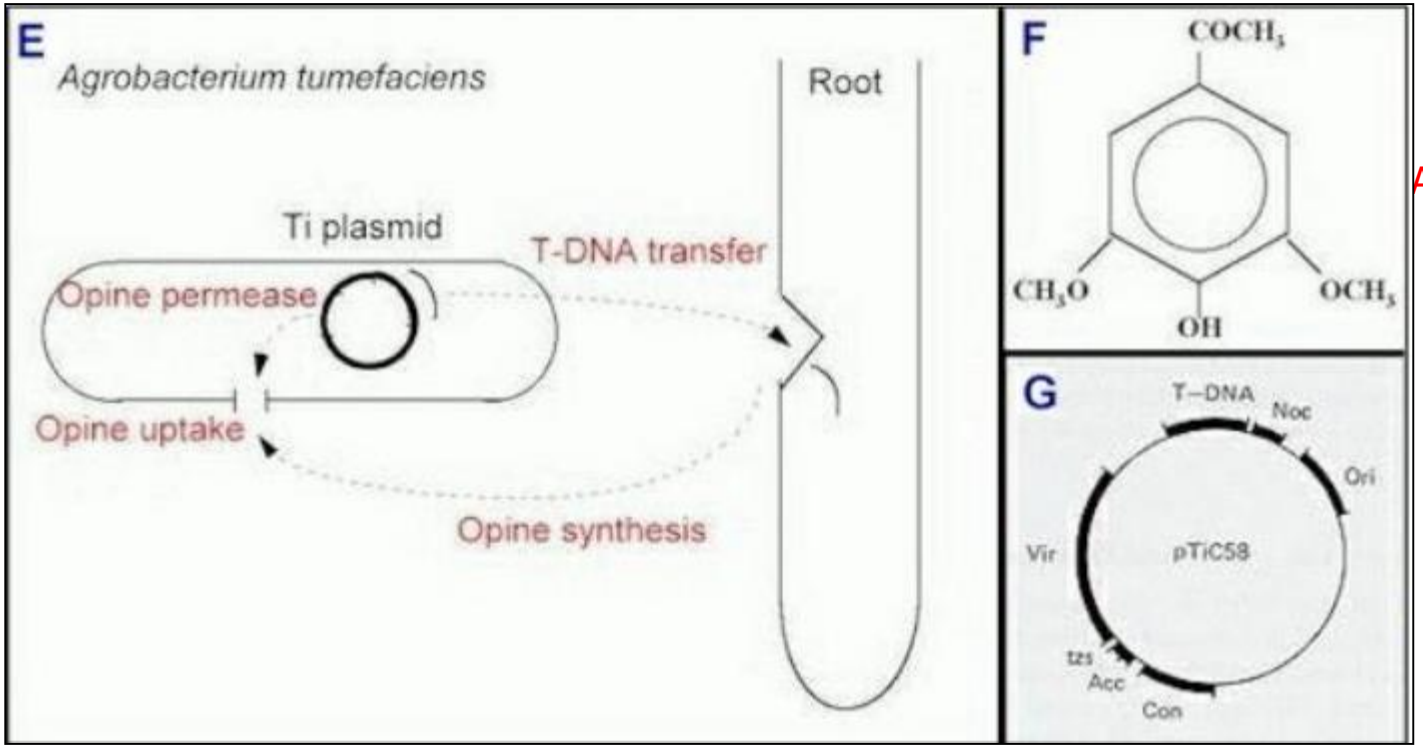
Where Does *A. vitis* Live in the Environment?

- In Grapevines
  - Cultivated and wild grapevines
  - Trunks, canes, roots
  - In dormant buds
- On Grapevines
  - On surfaces of shoots and leaves
- Others to investigate
  - Water, soil, other plants

Burr,2015

Infection process

Chemotaxies toward acetosyringone, a specific phenolic compound



Acetosyringone

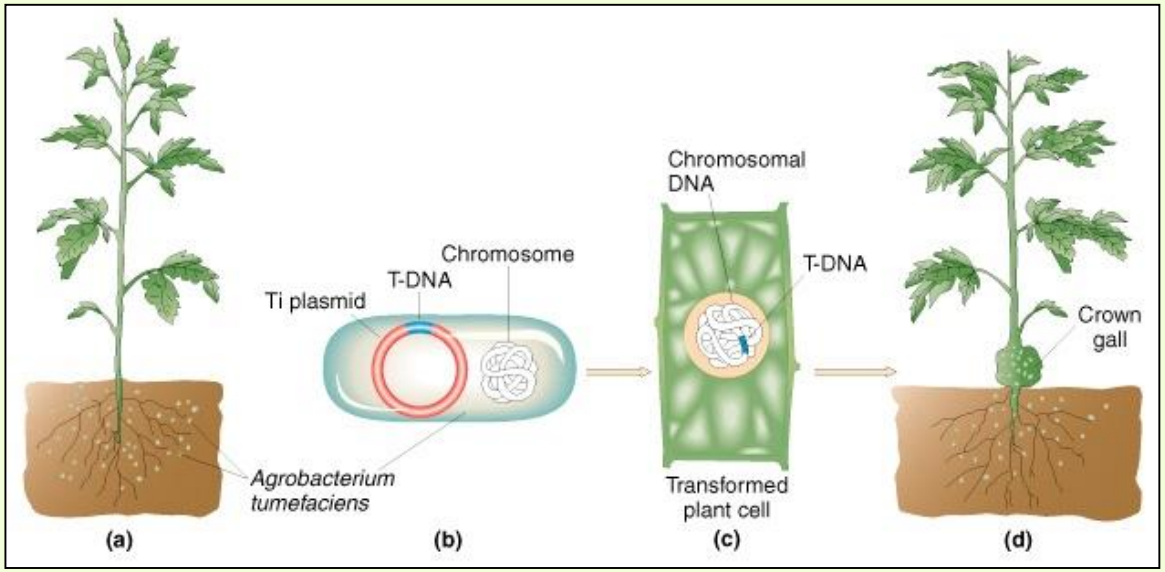
Ti plasmid

Jim Deacon

Infection process

Induction of crown gall disease by the Ti plasmid

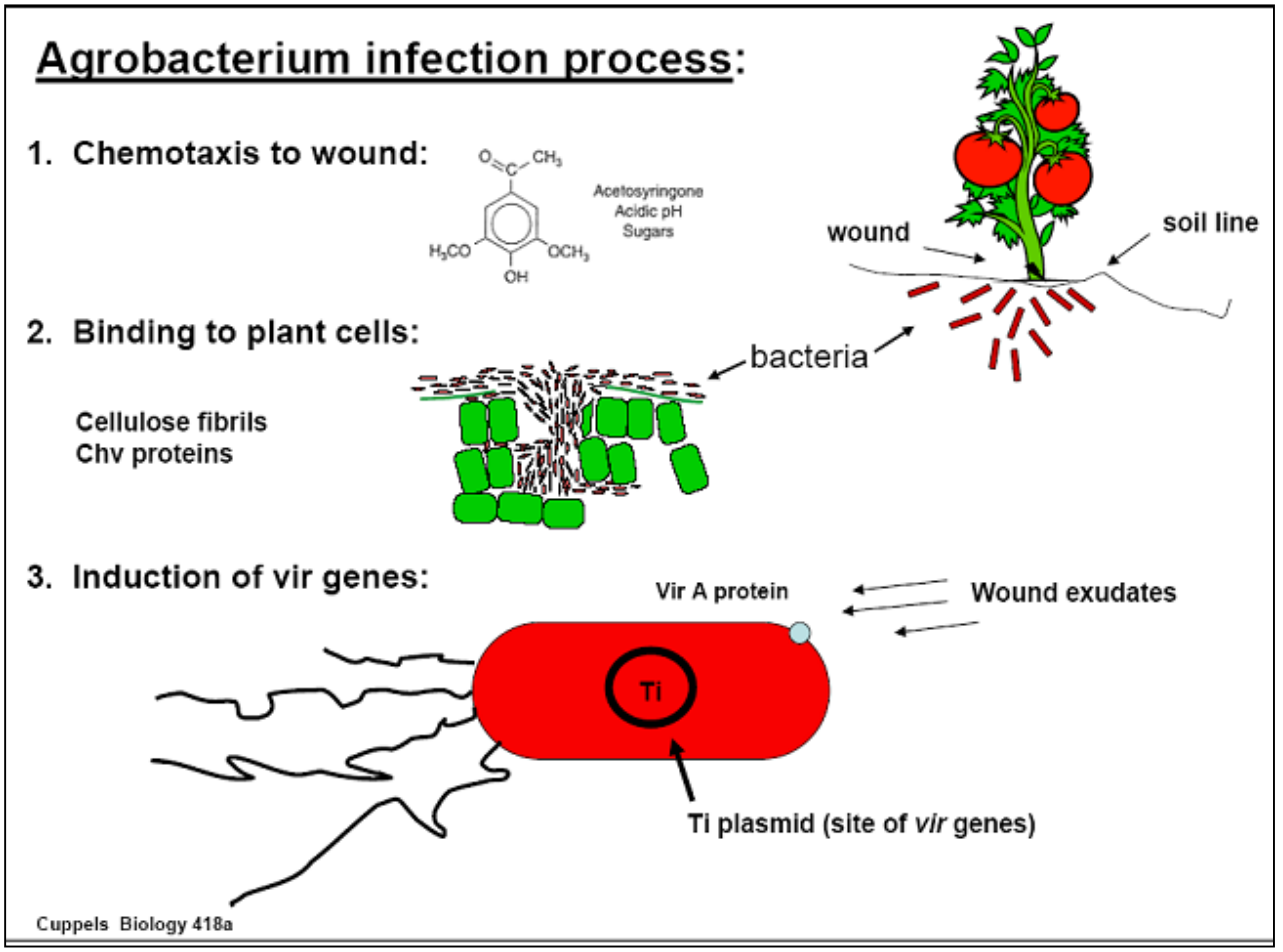
- *Agrobacterium* is a bacterium that grows in association with plant roots. The bacterium carried a naturally-occuring plasmid, Ti, that contains a gene region called T-DNA. In crown gall disease, the T-DNA region is randomly integrated into the host plant chromosomal DNA: this causes the formation of tumor-like growths at the base of the plant. The Ti plasmid thus behaves like a natural vector, and can be used to genetically engineer plants.



Griffiths *et al.*,2000

Infection process

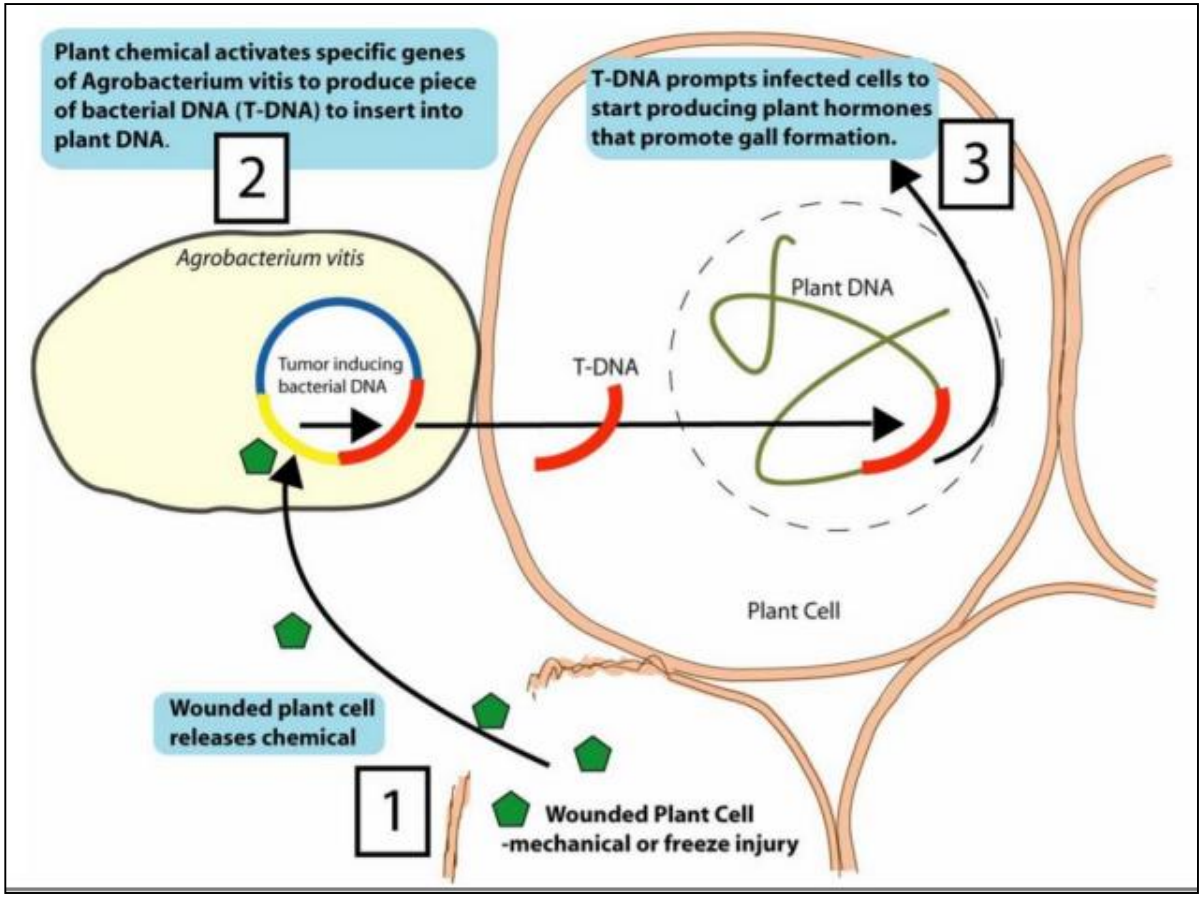
T-DNA transfer into plants



Cuppels,2007

Mode of action

Illustration of *Agrobacterium vitis* transformation of grapevine plant cells leading to the formation of crown gall



Stewart *et al.*,2004

Management

No effective chemicals

Allorhizobium vitis (=*A. vitis*)

- Chemical or agronomical methods are only partially effective (Raio *et al*., 1997).
- Antibiotics can not be used for agricultural purposes, because resistant bacterial strains will develop with widespread use, resulting eventually in loss of effectiveness of the antibiotic against human disease (Olson,2006).
- Since only the *A. tumefaciens* import agrocinopine are sensitive to agrocin 84.
- There are some *Agrobacterium* populations that are insensitive to agrocin 84.

Management

Biological control of nopaline-producing strains of *A. tumefaciens*

- However, for the nopaline-producing strains of *A. tumefaciens* there is a highly effective biocontrol system.
- Crown gall tumors induced by the classic nopaline-type *Agrobacterium tumefaciens* strain C58 synthesize and secrete two families of tumor metabolites:

1. Agrocinopines A and B, and
2. Nopaline.

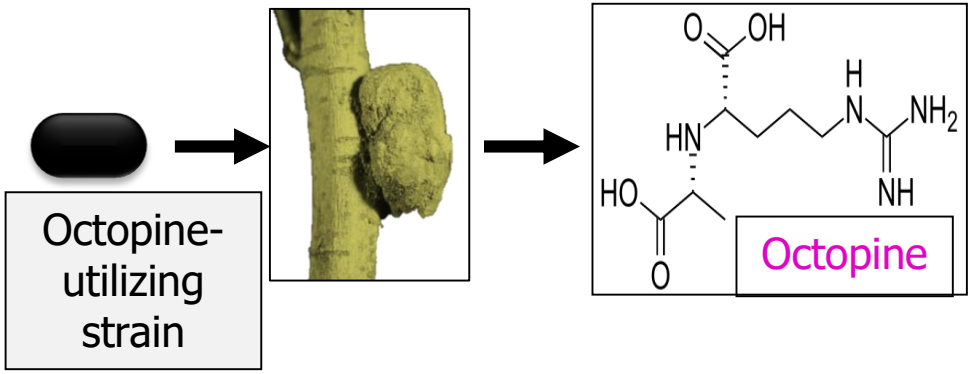
Strain	Characteristics
A. tumefaciens A348	octopine pTi
A. tumefaciens C58	nopaline pTi
Agrobacterium vitis At6	octopine pTi
Agrobacterium vitis S4	vitopine pTi
Agrobacterium vitis F2/5	avirulent strain

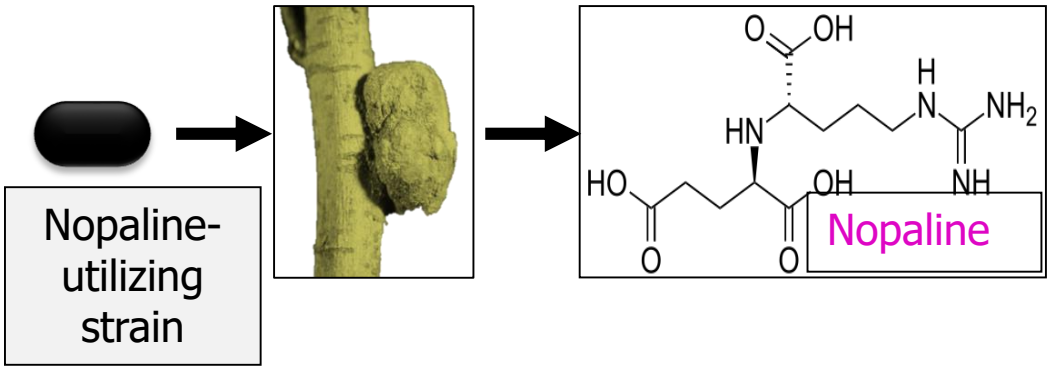
Deacon,?

Opines

The novel plant metabolites

The type of opine is determined by the bacterium, not the plant





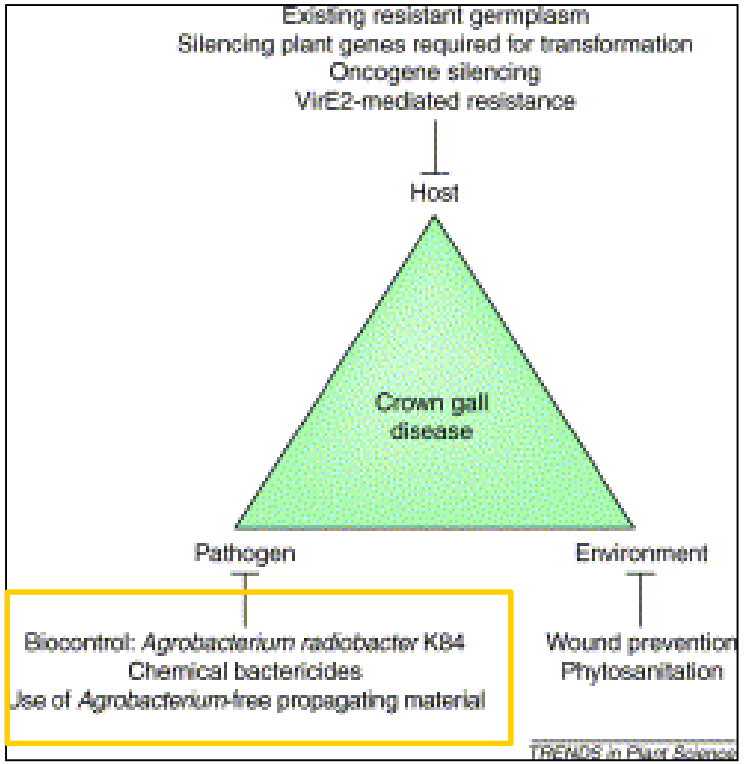
Questions raised:

- What are these compounds?
- Do they cause the tumors?
- How and why do the bacteria cause the plants to make opines?

American Society of Plant Biologists,2014

Management
Before planting
Site selection and soil conditions

- An oncogene is a gene that has the potential to cause cancer.
- In tumor cells, these genes are often mutated, or expressed at high levels.
- Oncogenes are activated and cause cells to grow and divide too quickly because of a mutation.

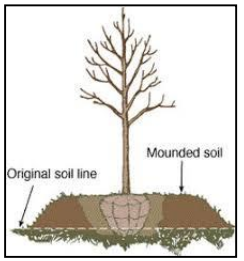


Aescobar and Dandekar,2003;..

Management

Before planting

Current management practices cultivar choice



1. Plant varieties and rootstocks that are tolerant of the disease.

- **Site Selection:**
- Plant vineyards on sites that have good air drainage and well drained soils to minimize freeze injury.
- **Hilling up:**
- Mounding soil over the graft union in the fall protects it from extreme cold events, and ensures survival of scion buds for trunk renewal.

Burr,2015

Management

Before planting

Site selection and soil conditions

- Prepare sites with nutrients and lime before planting if necessary to avoid vine stress due to poor nutrition or low pH.
- Apply lime if the soil is acid.

British Columbia,2015

Management

Before planting

Relative susceptibility of grape rootstocks to crown gall

- Certain rootstocks such as Courderc 3309, 101-14 Mgt, and Riparia Gloire are resistant, whereas Teleki 5C and 110 Richter are susceptible.
- Resistant rootstocks can reduce the amount of crown gall that appears on susceptible scions.
- Select hardy varieties where possible.
- In general, *Vitis vinifera* cultivars are all highly susceptible.

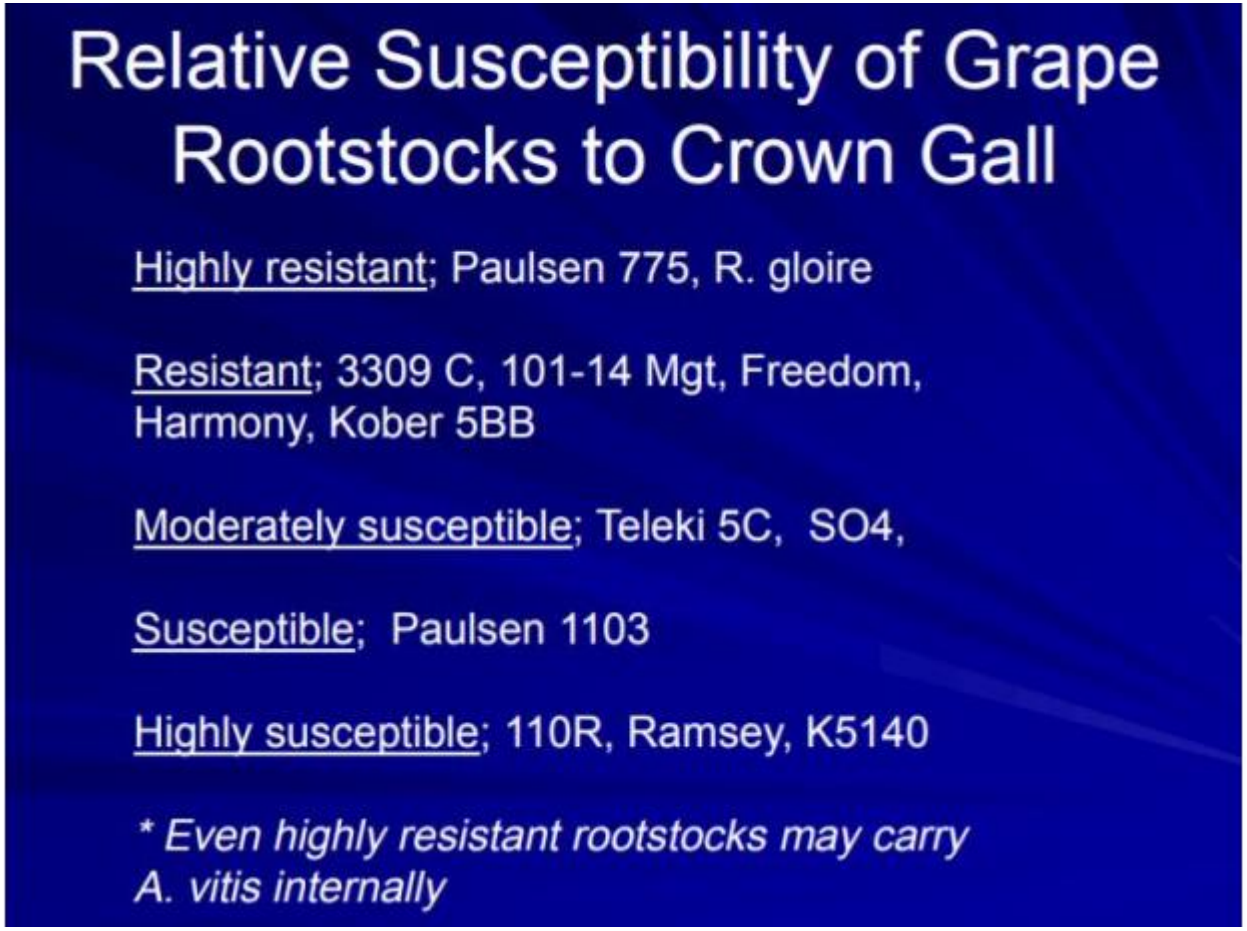
V. vinifera cultivars

British Columbia,2015;..

Management

Before planting

Relative susceptibility of grape rootstocks to crown gall



Burr,2015

Management
Before planting
Pest control programs

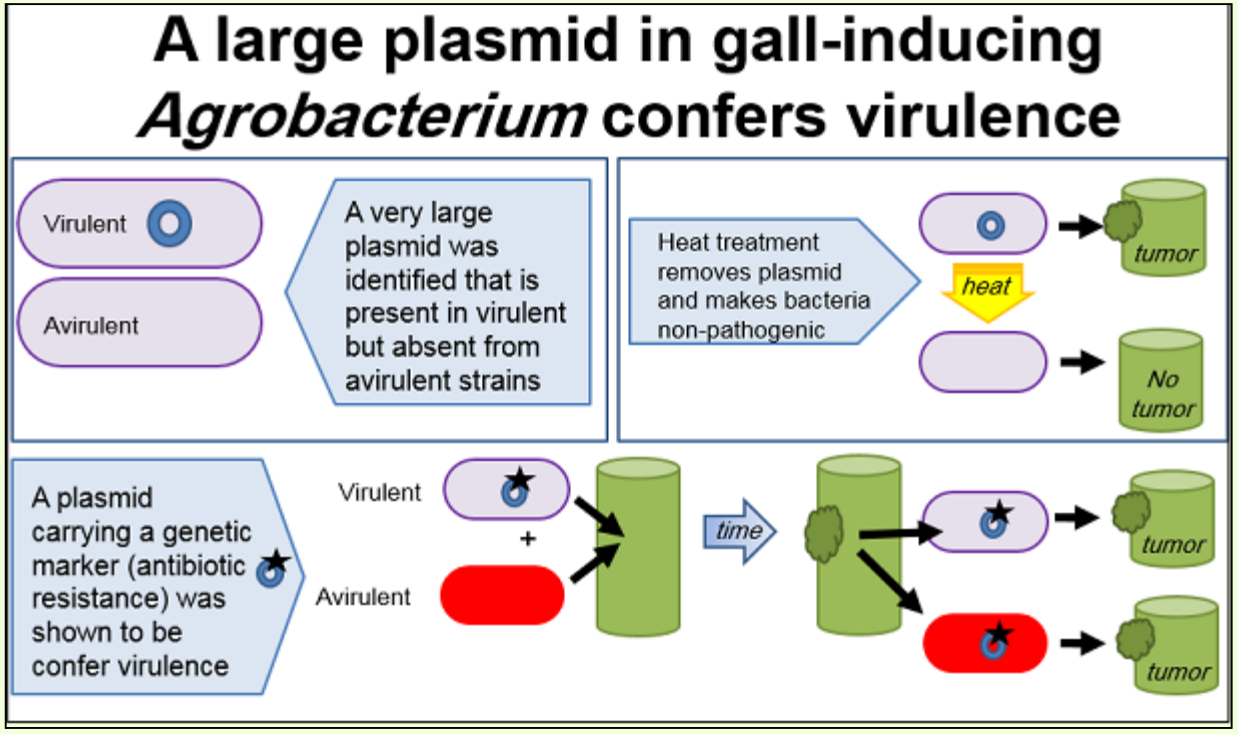
- Consider pest control programs for nematodes and phylloxera aphid before planting through the use of soil fumigation or rootstocks.
- Seeding damage by these pests provides sites for the entry of crown gall bacteria.
- Plant old vineyard areas where crown gall was present only after grapevines have been removed for at least 2 years.
- This is important because crown gall bacteria can survive in the remnants of the old grape plants until the debris decomposes.
- Success in reducing crown gall from the soil by leaving soil fallow or rotating to other crops may vary depending on the amount of vine debris that is left in the soil, and how fast it breaks down.

Management

Before planting

Hot water treatment of vines

- Hot water treatment of vines is effective in reducing crown gall infection levels in planting materials.



American Society of Plant Biologists,2014; British Columbia,2015

Management

Before planting

Hot water treatment of vines

- Hot water treatment of vines is effective in reducing crown gall infection levels in planting materials.

Burr,2015

Management
After planting
Gall of roses

- Disinfect pruning tools between plants.
- Disinfect budding/grafting tools before and after use.
- Bleach (10%; equivalent to 0.6% sodium hypochlorite), or quaternary ammonium-based sanitizer are effective as disinfectants.
- Make sure to prepare fresh stock routinely.

Yiqigao,2017

Management

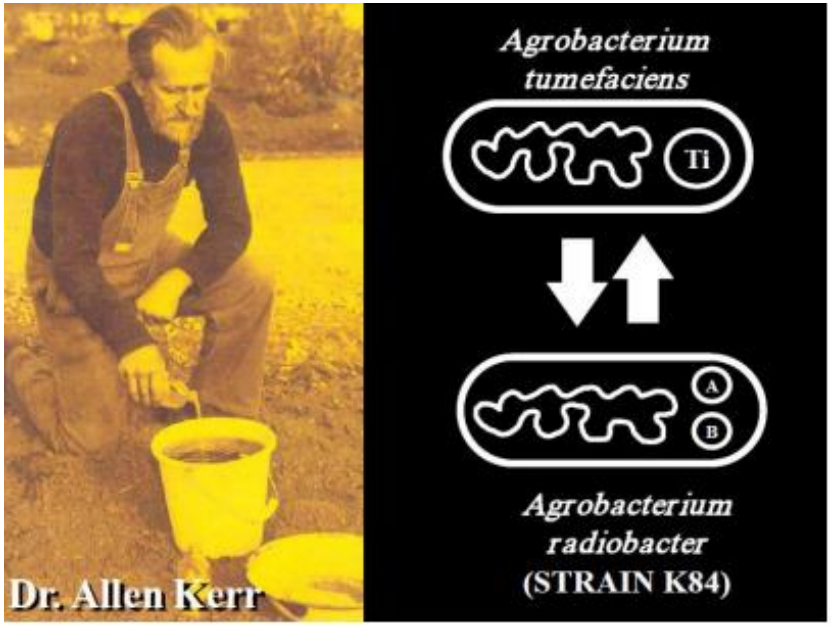
After planting

Flaming the tissue around the margins of a crown gall

Allan Kerr

Pioneer in Biological control of *Agrobacterium*

- Professor Dr. Alan Kerr and his team announced in 1988 that they were able to genetically splice out a small region on the genetic plasmid, which was responsible for the transfer of agrocin 84 from strain K84 to the pathogen bacteria.



Romeiro,2010

Management

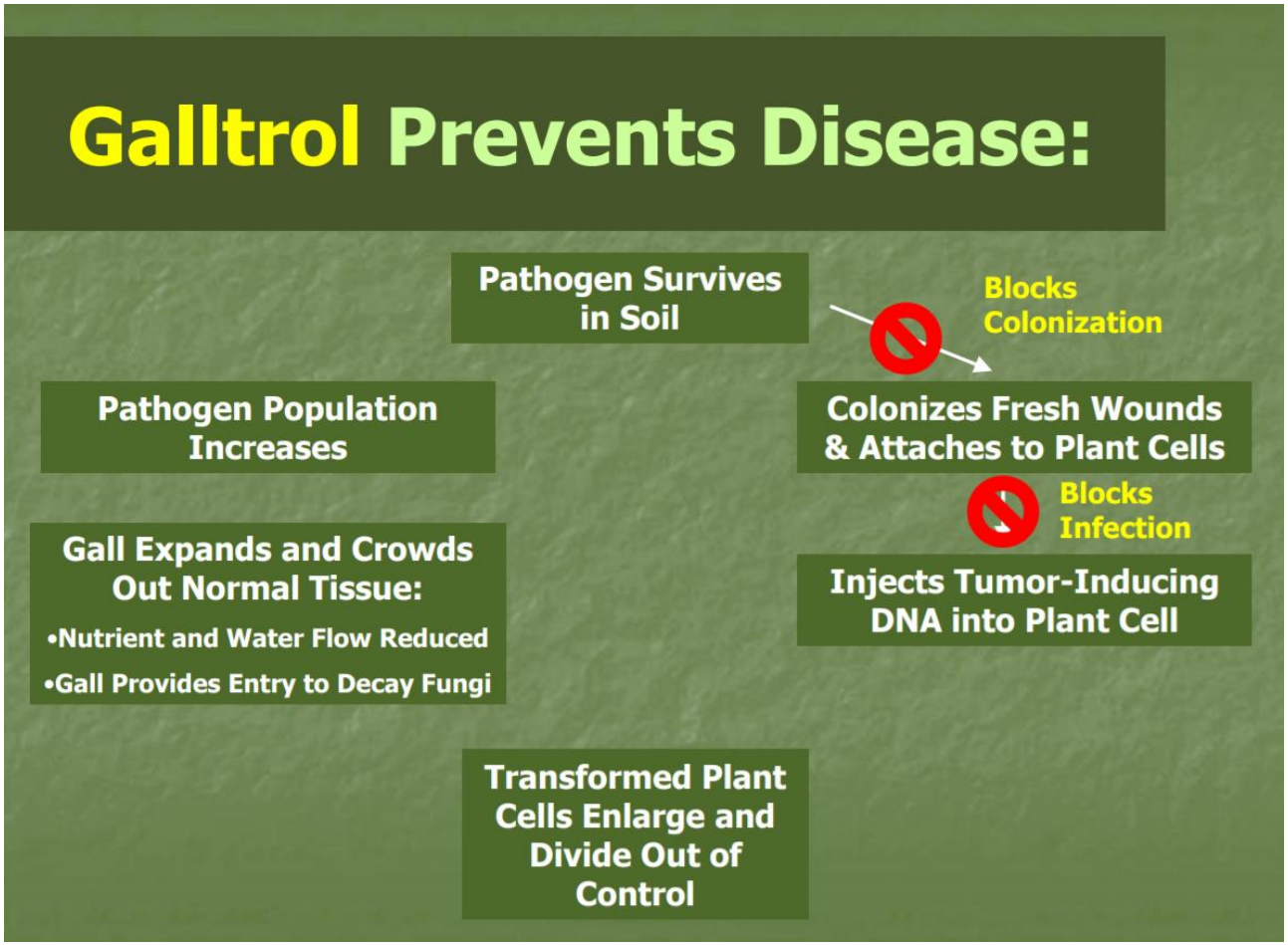
GALLTROL and GALLAX are pure culture of *A. radiobacter* (Strain K84)

AgBioChem, Inc.

Management

Biological control of nopaline-producing strains of *A. tumefaciens*

GALLTROL is a pure culture of *A. radiobacter* (Strain K84)



AgBioChem, Inc.

Management

GALLTROL-A is a pure culture of *A. radiobacter* (Strain K84)

- Timing: as soon after wounding (nursery, delivery to grower, pre-planting) as possible (not more than 12 hours)
- Rate: bacterial growth from 1 plate in 1 gallon water (non-chlorinated)
- Application Method: spray or dip.
- Users: growers and nurseries
- User Cost: $0.10 to $0.15 per tree (much less for nursery seeds, cuttings, liners).

GALLTROL is a pure culture of *Agrobacterium radiobacter* (Strain K84) grown under scientifically controlled conditions to maximize bacterial vigor and numbers. Each plate of GALLTROL contains 120 billion freshly grown, active bacterial cells.

AgBioChem, Inc.

Management

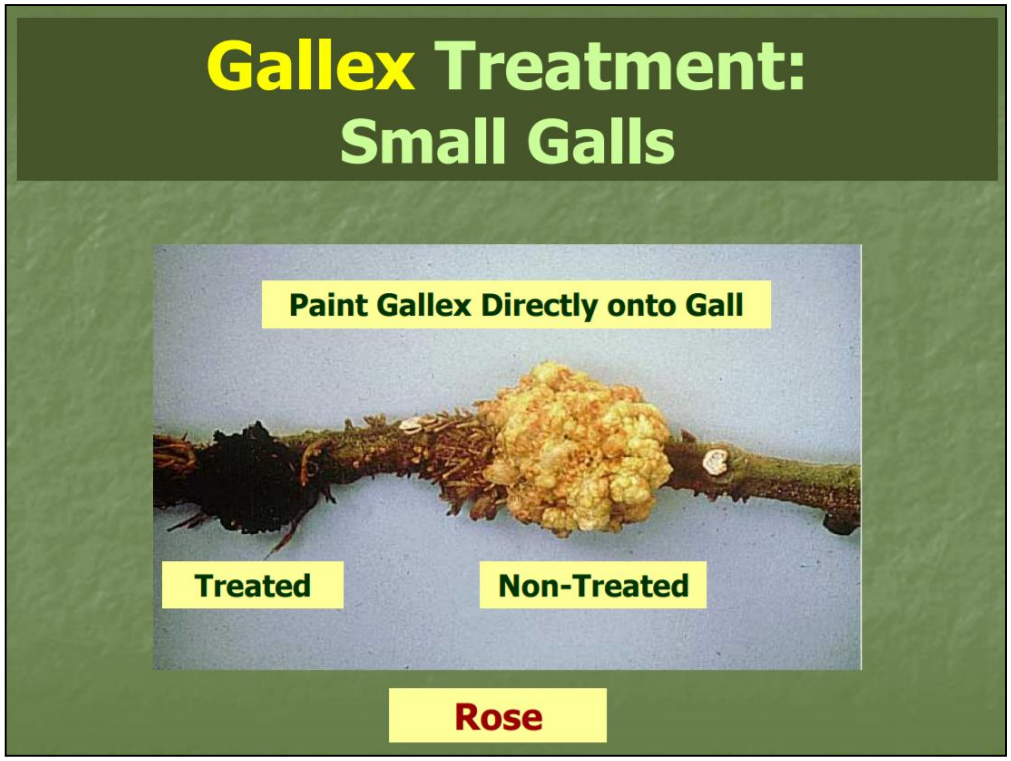
GALLTROL-A is a pure culture of *A. radiobacter* (Strain K84)

Comments: Preventive pre plant treatment only. Acceptable for use on organically grown produce.

AgBioChem, Inc.

Management

GALLEX is a pure culture of *A. radiobacter* (Strain K84)



Comments: For removal of existing galls, apply winter through spring. pre plant treatment only.

AgBioChem, Inc.

Management

GALLEX is a pure culture of *A. radiobacter* (Strain K84)

Gallex: Gall Eradicant

- Kills gall tissue
- Doesn't harm healthy cells
- Controls crown gall and apple burr knot

Gallex: Treatment of Existing Galls

1. Expose gall

2. Remove excess gall tissue (chisel, hatchet, knife)

3. Allow tissue to dry 2-3 days

AgBioChem, Inc.

Management

GALLEX is a pure culture of *A. radiobacter* (Strain K84)

4. Paint Gallex onto cut surface (overlap healthy, intact tissue by 1 inch)

AgBioChem, Inc.

Management

NOGALL is a pure culture of *A. radiobacter* (Strain K1026)

Effective as a preventative treatment

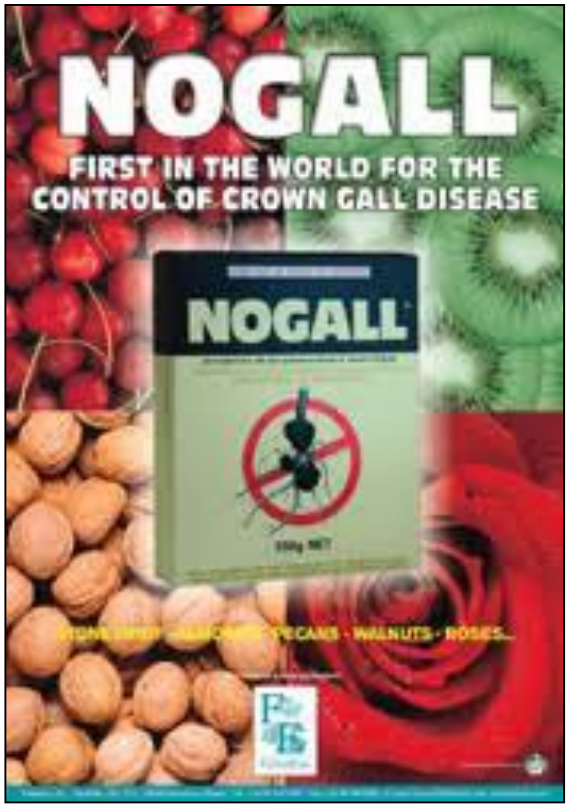
- Use of K84 was stopped in Australia and replaced by K1026 to reduce the breakdown of biological control of crown gall.
- BASF Australia commercially manufactures and markets K1026 under the product name of NOGALL™.
- Strain K1026, an effective agrocin 84-producing derivative of strain K84, which still maintained the ability to control the pathogen, but without the capability of producing an immunity in the disease organism.

Management

NOGALL is a pure culture of *A. radiobacter* (Strain K1026)

Effective as a preventative treatment

- When susceptible plant material is dipped in or sprayed with a suspension of NOGALL™ prior to planting.
- The NOGALL™ K1026 bacteria act by colonizing the wounds and producing antibiotics, which inhibit the pathogen.
- Note: NOGALL™ containing K1026 is ineffective against strains causing crown gall disease in grapes, apples, pears and some ornamentals.



Management

NOGALL is a pure culture of *A. radiobacter* (Strain K1026)

Effective as a preventative treatment

Management

NOGALL is a pure culture of *A. radiobacter* (Strain K1026)

How to use NOGALL

Step 1. Remove leaflet and read instructions and precautions

Step 2. Remove NOGALL peat culture bag and cut top open

Step 3. Empty bag of NOGALL into cool, clean un-chlorinated water

Bio-Care Technology Pty Ltd, Australia

Management

NOGALL is a pure culture of *A. radiobacter* (Strain K1026)

How to use NOGALL

Step 4. Pour water into bag and rinse out residue

Step 5. Mix peat culture and water thoroughly

Step 6. Soak cuttings or root stock well in NOGALL and plant.

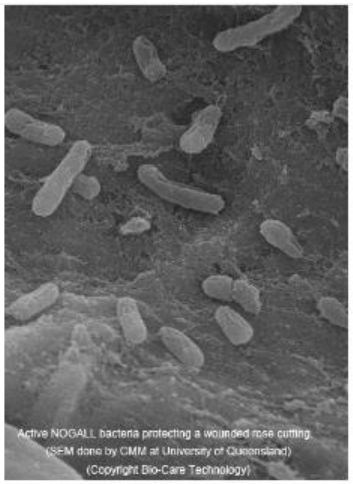
Bio-Care Technology Pty Ltd, Australia

Management

NOGALL is a pure culture of *A. radiobacter* (Strain K1026)

How to use NOGALL

Alternatively, thoroughly spray rootstock with NOGALL dip solution. Loosen bundles and spray till run-off. Increase nozzle orifice size if clogging occurs.



Active NOGALL bacteria protecting a wounded rose cutting

A. radiobacter (Strain K1026) cells

To treat seeds soak in NOGALL dip solution and plant as soon as possible

Bio-Care Technology Pty Ltd, Australia

Management

Biological control of nopaline-producing strains of *A. tumefaciens*

Galltrol-A and Gallax (strain 84), Nogall(strain K-1026)

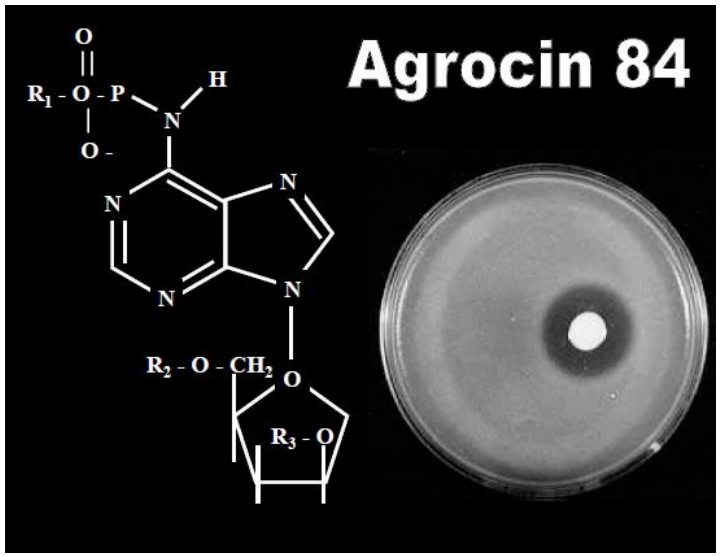
- The nopaline-producing strains of *A. tumefaciens* is a highly effective biocontrol system.
- It has been used in Australia since 1973 - the first commercial biological control agent for any plant disease.
- It is now used world-wide, and marketed by several companies under a range of trade names.

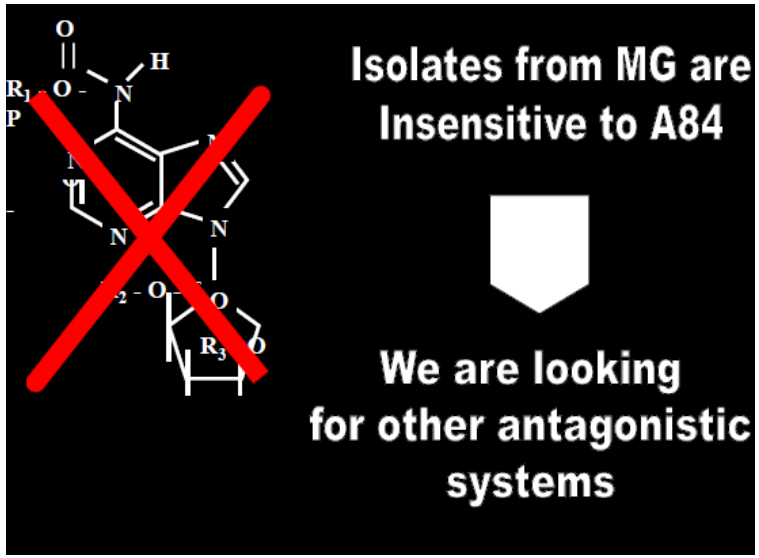
1. GALLTROL and GALLAX are pure culture of *A. radiobacter* (Strain K84).
2. NOGALL is a pure culture of *A. radiobacter* (Strain K1026).

Strain	Characteristics
A. tumefaciens A348	octopine pTi
A. tumefaciens C58	nopaline pTi
Agrobacterium vitis At6	octopine pTi
Agrobacterium vitis S4	vitopine pTi
Agrobacterium vitis F2/5	avirulent strain

Screen for effective antagonists

Senstitive and insenstitive *Agrobacterium* spp. to agrocin 84





Only the *A. tumefaciens* import agrocinopine are sensitive to agrocin 84.

There are some *Agrobacterium* populations that are insensitive to agrocin 84.

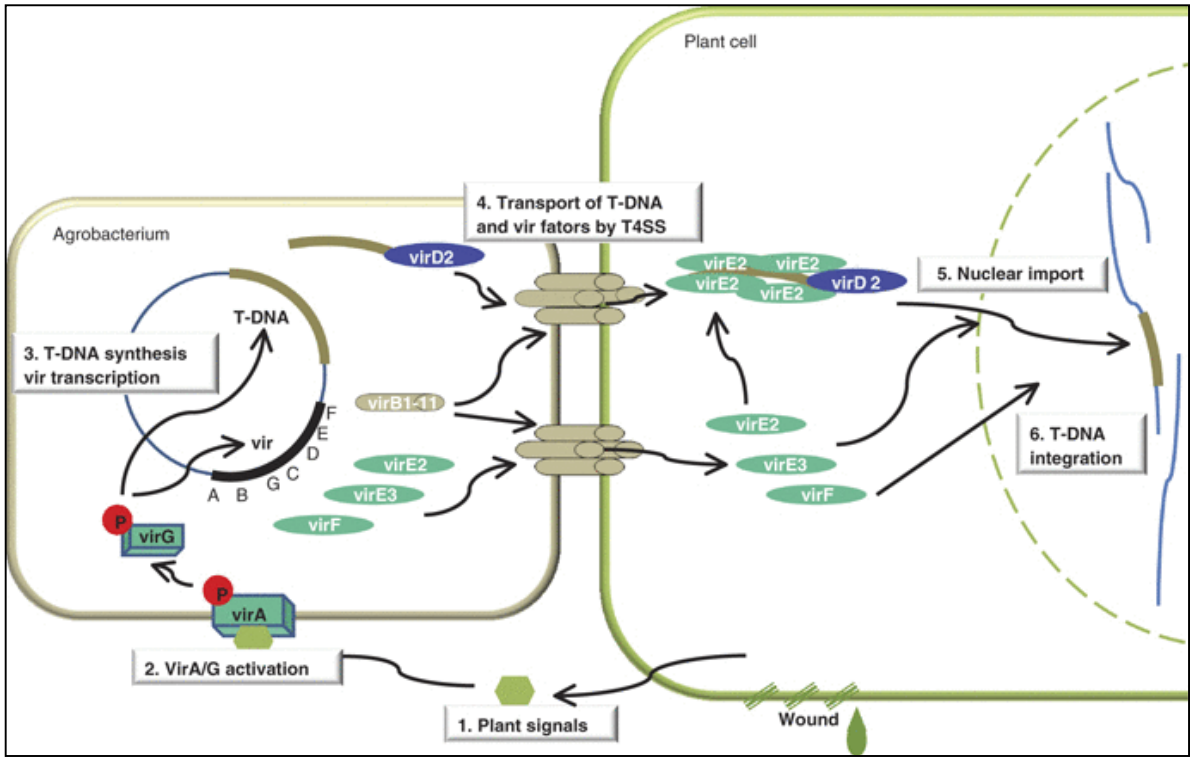
Romeiro,2010; Raio *et al.*,2009

Management

Crown gall of grapevine

Agrobacterium vitis strain E26

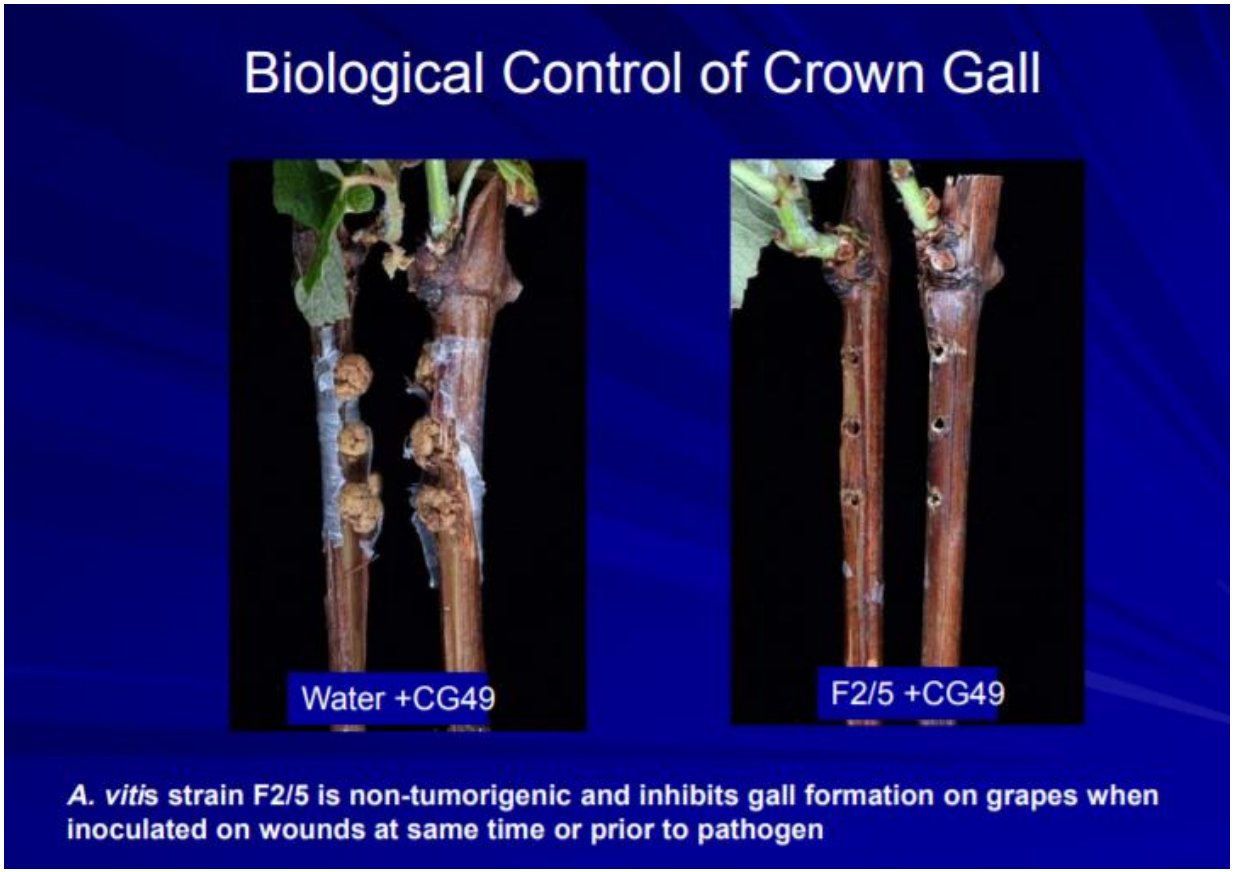
- According to Wei *et al.*,2009, *Agrobacterium vitis* strain E26 without *virA* and *virG* pathogenic determinants is a promising potential biocontrol agent of grapevine crown gall disease.



Management

Crown gall of grapevine

Agrobacterium vitis strain F2/5



Nontumorigenic *Agrobacterium vitis* strain F2/5 and tumorigenic strain CG49.

Burr,2015

Management

Crown gall of grapevine

Agrobacterium vitis VAR03-1

- A nonpathogenic strain of *Agrobacterium vitis* VAR03-1 was tested as a biological control agent for crown gall of grapevine (*Vitis vinifera*).
- When roots of grapevine, rose and tomato were soaked in a cell suspension of antagonists before planting in soil infested with tumorigenic *A. vitis*, *A. rhizogenes*, and *A. tumefaciens*, respectively, treatment with VAR03-1 significantly reduced the number of plants with tumors and disease severity in the three plant species.

Kawaguchi *et al.*,2008

Management

Crown gall of grapevine

Agrobacterium rhizogenes strain K84

- *A. rhizogenes* strain K84 (formerly called *A. radiobacter*) on crown gall of rose and tomato were almost identical, and the inhibitory effect of VAR03-1 on grapevine was superior to that of K84.

Management

Biocontrol control of *Agrobacterium tumefaciens* with 8 bacterial antagonists

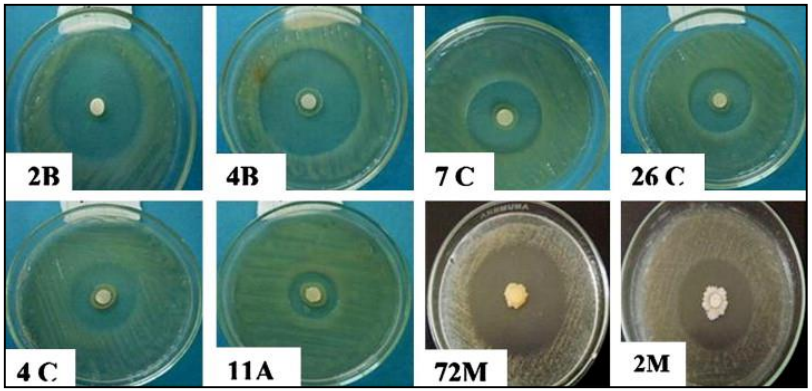
- Eight potential biocontrol agents were screened for their antagonistic effect *in vitro* as well as their efficacy in reducing gall formation *in planta* (rose shoots, kalanchoe leaves and squash fruits).
- They were identified using Biolog microplates system as:
- *Bacillus megaterium*, *Paenibacillus polymyxa*, *Pseudomonas fragi* (two isolates), *Pseudomonas viridiflava*, Pseudomonas asplenii, *Curtobacterium flaccumfaciens* and *Curtobacterum* sp.

Tolba and Soliman,2013

Management

Biocontrol control of *Agrobacterium tumefaciens* with 8 bacterial antagonists

Isolate code	Mean of zone diameter (nearest whole mm)	Isolation origin
2B	30	Rose tumor
4B	29	Rose tumor
7C	28	Rose tumor
26C	28	Galled rose rhizosphere
4C	25	Galled rose rhizosphere
11A	18	Rose tumor
72M	45	Grapevine tumor
2M	40	Galled rose rhizosphere



Tolba and Soliman,2013

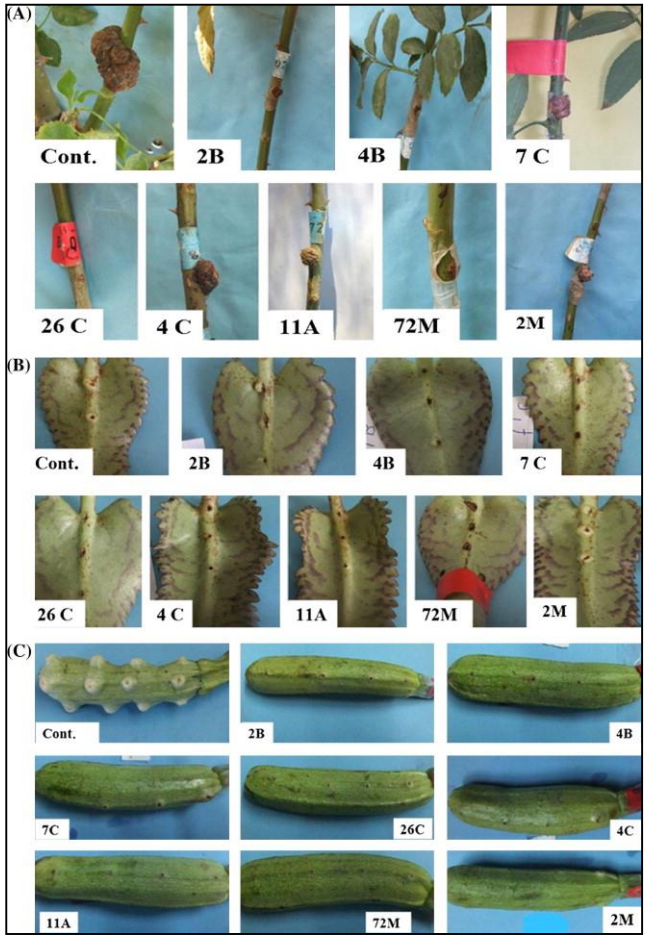
Management

Biocontrol control of *Agrobacterium tumefaciens* with 8 bacterial antagonists

Table 2 Effect of eight antagonistic bacterial isolates on incidence and size of galls induced by *Agrobacterium tumefaciens* on rose shoots, kalanchoe leaves and squash fruits, under artificial conditions.

Isolate	Identified	Gall on rose shoots			Gall on kalanchoe leaves			Gall on squash fruits		
		Size[a] (mm)	Incidence (%)	Reduction (%)	Size[a] (mm)	Incidence (%)	Reduction (%)	Size[a] (mm)	Incidence (%)	Reduction (%)
2B	*Pseudomonas fragi*	2	11.1	88.9	5	11.1	88.9	–	0.0	100
4B	*Pseudomonas fragi*	1	11.1	88.9	3	11.1	88.9	–	0.0	100
7C	*Curtobacterum* sp.	8	66.6	33.3	–	0.0	100	4	75	25
26C	*Curtobacterium flaccumfaciens*	–	0.0	100	–	0.0	100	3	25	75
4C	*Pseudomonas viridilivd*	6	44.4	55.6	–	0.0	100	–	0.0	100
11A	*Paenibacillus polymyxa*	6	55.5	44.5	–	0.0	100	–	0.0	100
72M	*Pseudomonas asplenii*	5	33.3	66.7	–	0.0	100	–	0.0	100
2M	*Bacillus megaterium*	–	0.0	100	6	100	0.0	3	25	75
Control		18	100	0.0	6	100	0.0	10	100	0.0
LSD		2.1	22.3	20.7	2.2	22.9	21.2	2.1	23. 3	20.4

[a] Mean, nearest whole mm.



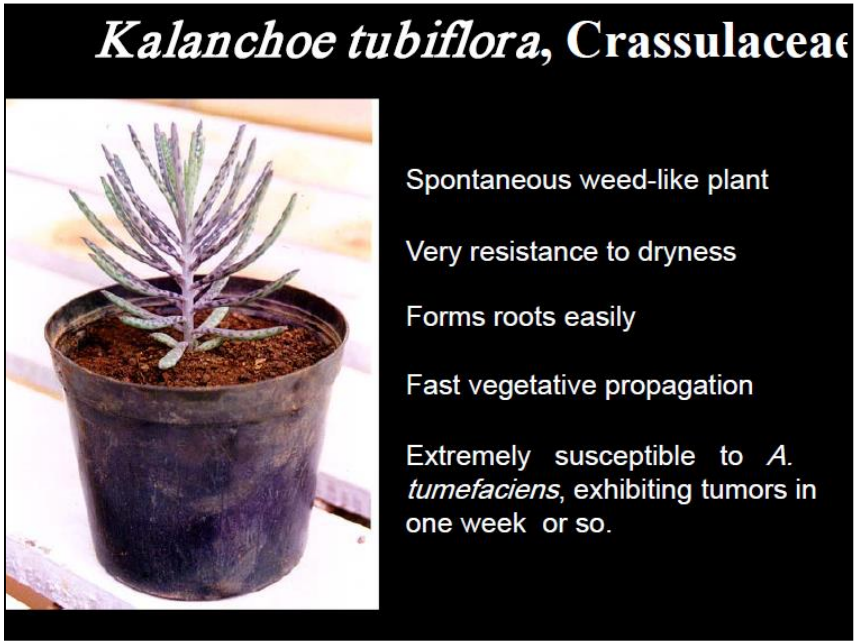
Tolba and Soliman,2013

Kalanchoe tubiflora bioassay

Experimental biocontrol in a greenhouse

Actinomycetes against *A. tumefaciens*

- *Kalanchoe tubiflora* often called "mother of millions"is an erect shrub-like succulent with succulent and tubular leaves.
- Susceptible to *A. tumfaciens*.
- Used as indicator plant in biological control experiments.

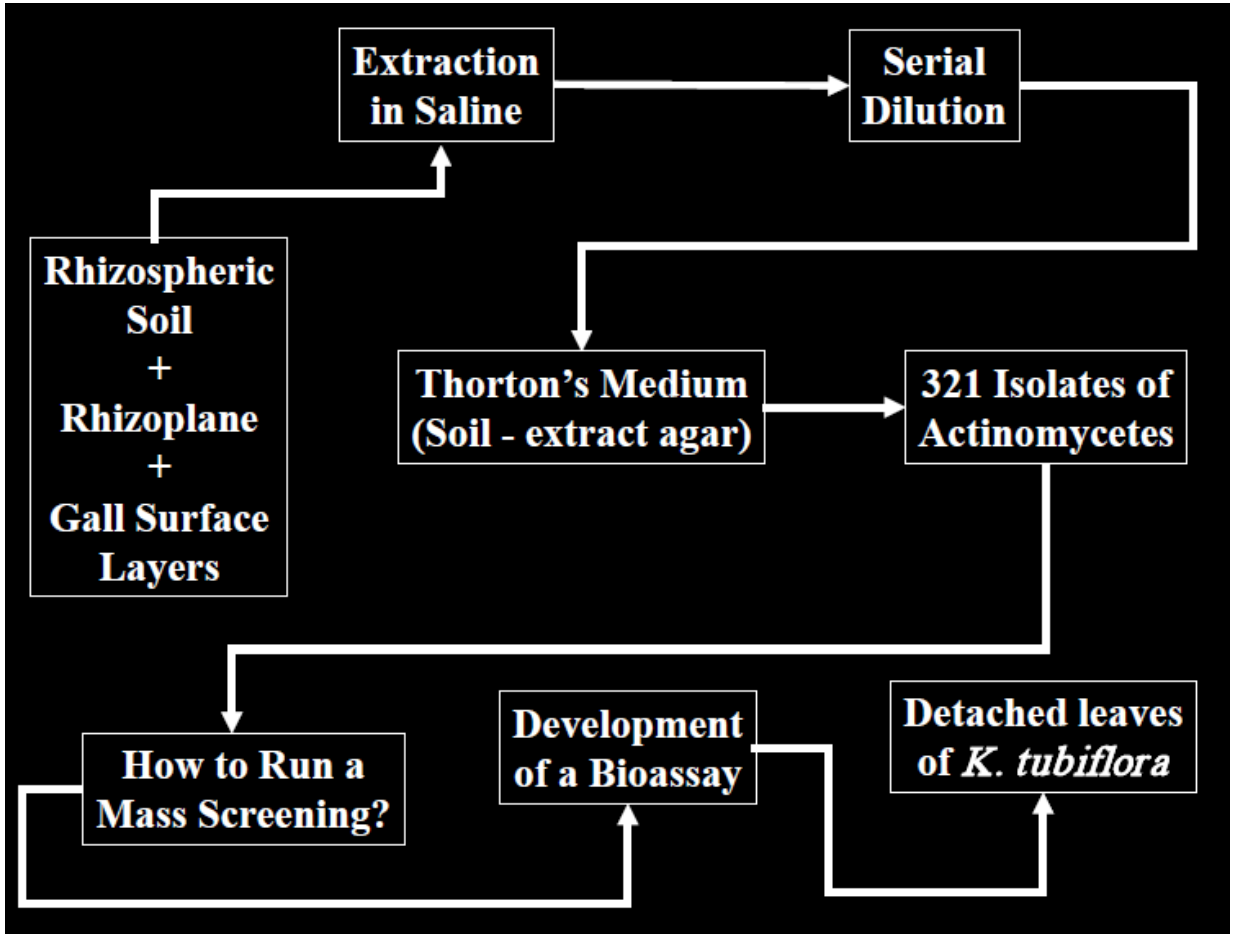


Romeiro,2010;..

Kalanchoe tubiflora bioassay

Experimental biocontrol in a greenhouse

Actinomycetes against *Agrobacterium*

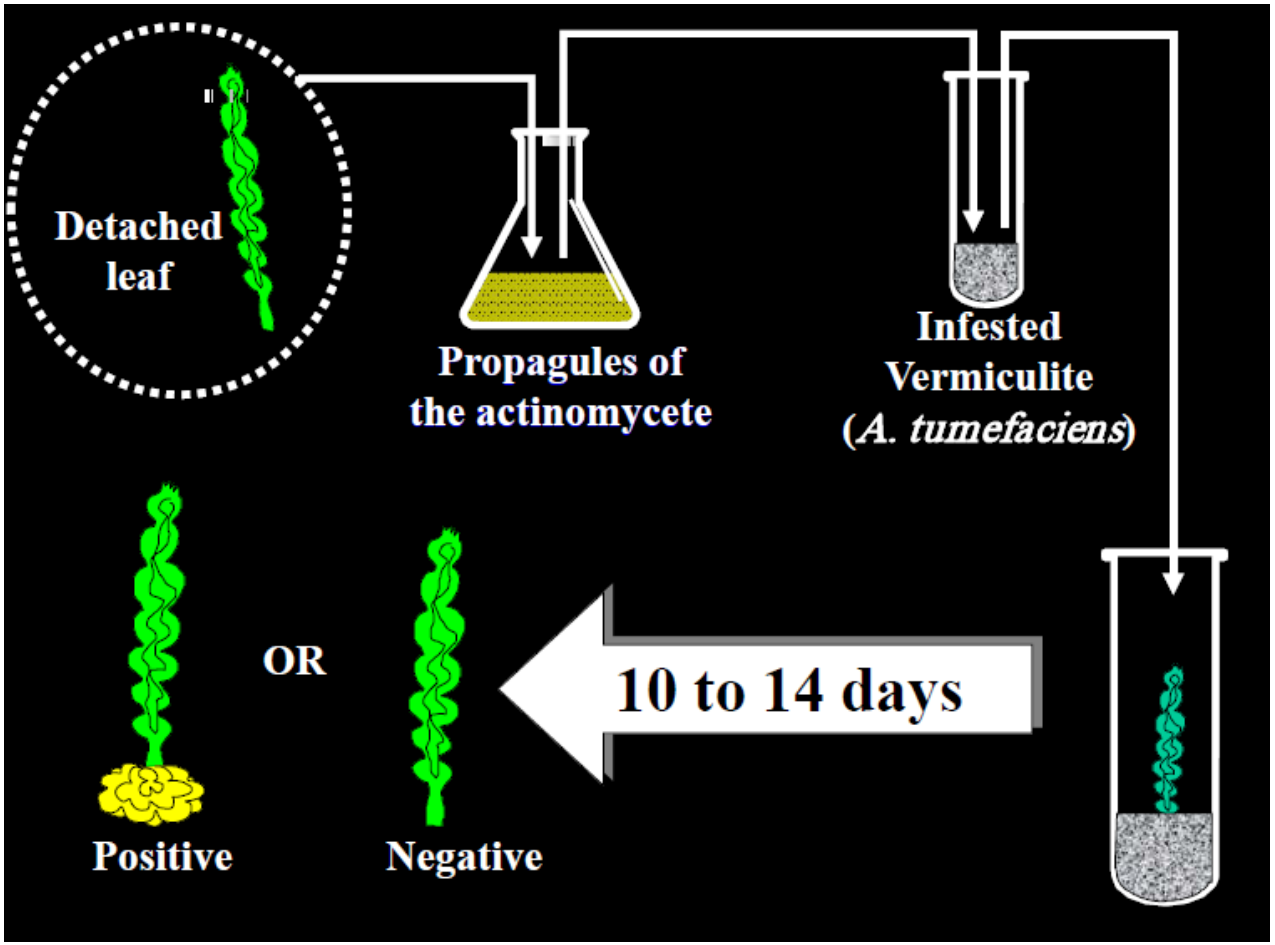


Romeiro,2010

Kalanchoe tubiflora bioassay

Experimental biocontrol in a greenhouse

Actinomycetes against *A. tumefaciens*



Romeiro,2010

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Experimental biocontrol in a greenhouse

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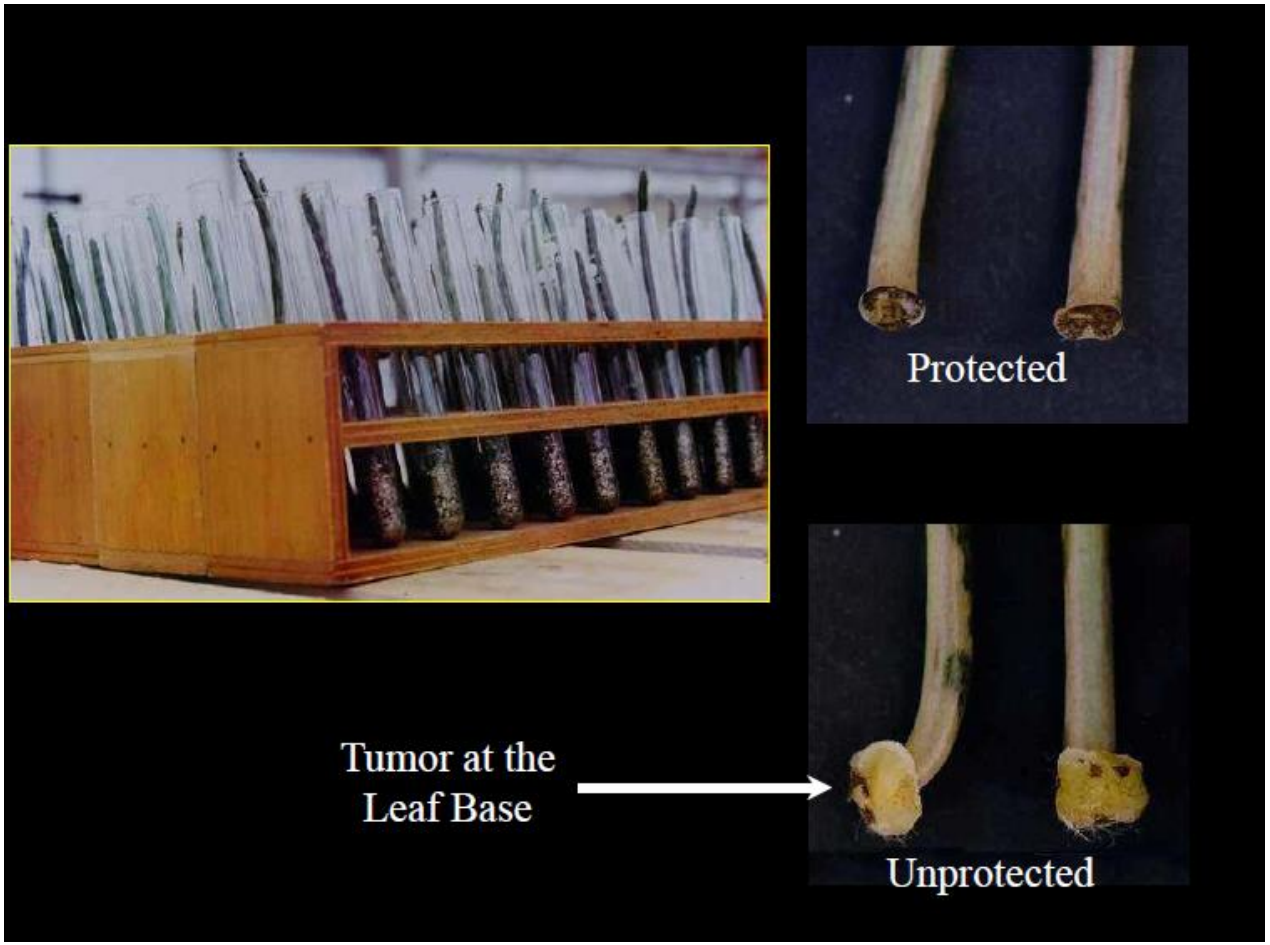

Romeiro,2010

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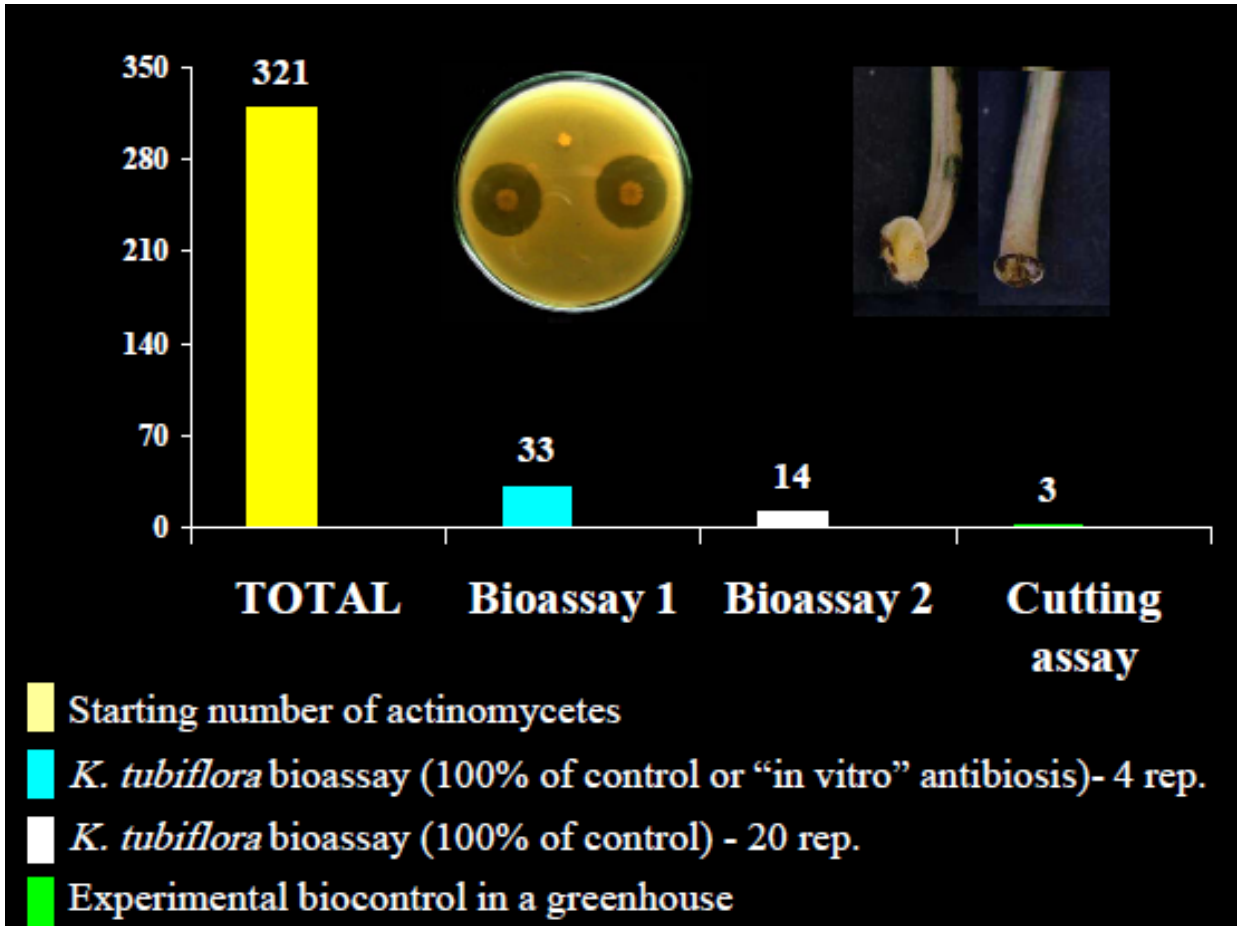

Romeiro,2010

Kalanchoe tubiflora bioassay

Experimental biocontrol in a greenhouse

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Romeiro,2010

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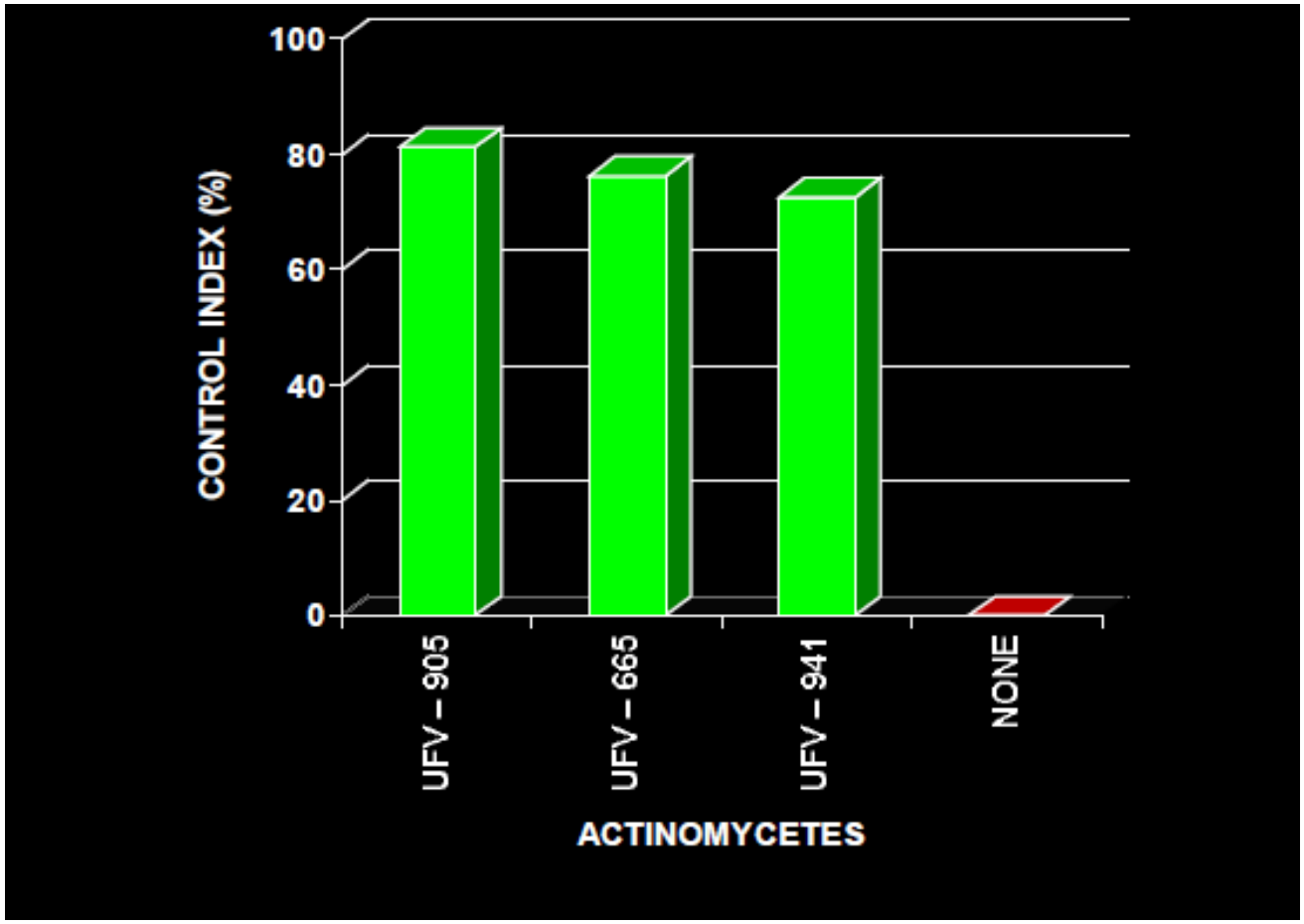
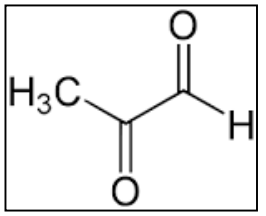


Romeiro,2010

Management
Methylglyoxal (MG)



$C_3H_4O_2$

- Methylglyoxal (MG) is a highly reactive and toxic alpha oxoaldehyde, demonstrating anticancer effect on plant neoplastic tumours.
- Methylglyoxal (MG) is a by-product, a cytotoxic byproduct of glycolysis that induces protein modification.
- It is found in food and living organisms.
- It is formed endogenously in cells due to numerous enzymatic and nonenzymatic reactions.
- It modifies arginine and lysine residues in proteins.
- In *in vivo* studies it was observed that MG destroyed crown gall tumours in *Nicotiana tabacum* produced by *Agrobacterium tumefaciens*, without any adverse effect on the host.

Ray *et al.*,2011;..

Management

Methylglyoxal (MG) vs. cisplatin and ellagic acid

- The efficacy of MG in comparison to other anticancer drugs *viz*. ellagic acid (an antioxidant) and cisplatin (chemotherapy drug) in the treatment of crown gall was investigated.
- A slight degeneration of galls was noted in plants treated with cisplatin and ellagic acid but the plants died subsequently.
- With MG however, crown galls were completely cured and the plants completed their usual life cycle by flowering and producing seeds.
- MG inhibited the respiration of crown gall calluses suggesting that energy depletion resulted in tumour destruction.

Ray *et al.*,2011;..

Management

Garlic powder as an anti-bacterial natural product

- *Rumex* sp. (dock) *Azadirachta indica* (neem oil), jojoba oil, cinnamon oil, soybean oil, compost tea, *Equisetum arvense* (horsetail plant); *Reynoutria sachalinensis* (giant knotweed) and garlic were used against *A. tumefaciens* (Alsup,2004).
- Garlic powder has been shown to have anti-bacterial properties under some conditions (Jonkers *et al*.,1999).

Management

Garlic powder as an anti-bacterial natural product

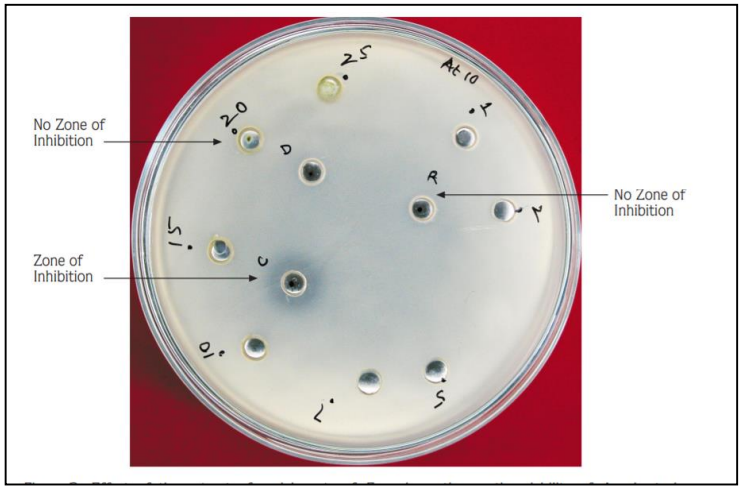
- Solutions containing extracts of the plants will be tested for their ability to inhibit bacterial growth by soaking carrot disks in the solutions and then inoculating the disks with *A. tumefaciens*.

Alsup,2004

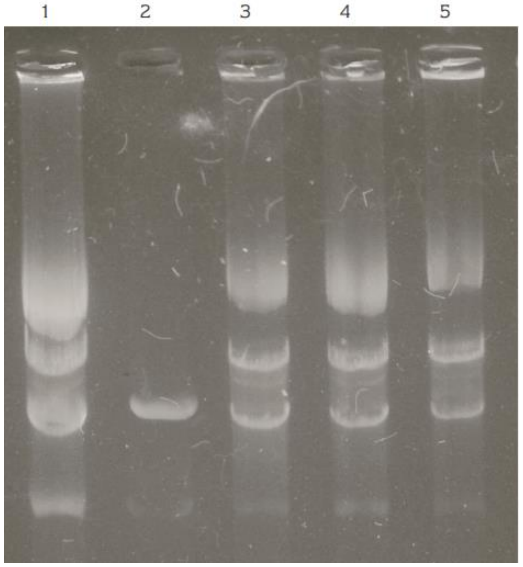
Management

Antibacterial activity of leaf extract of *Fagonia cretica* (Virgin's Mantle) against *A. tumefaciens* on tomato stems

DNA damage analysis



Effect of the extract of aerial parts of *Fagonia cretica* on the viability of *Agrobacterium tumefaciens* (At10) strain. C= Cefixime-USP; R= Roxycithromycin.



Effect of the extract of aerial parts of *Fagonia cretica* on plasmid DNA. The loading sequence is indicated below. 1, Undigested Plasmid DNA (10 µg); 2, Plasmid DNA digested with digested with DNA EcoR1; 3) Plasmid DNA digested with 5 mg/ml of the extract; 4) Plasmid DNA digested with 10 mg/ml of the extract; 5) Plasmid DNA digested with 20 mg/ml of the

Hussain *et al.*,2007

Management

Antibacterial activity of leaf extract of *Fagonia cretica* (Virgin's Mantle) against *A. tumefaciens* on tomato stems

DNA damage analysis

- Single colony of *E. coli* possessing pBluescript was grown for 24 hours at 37°C in Lauria broth containing 0.05 mg/ml ampicillin.
- pBluescript was extracted by using standard protocols.
- The plasmid DNA was treated with 5, 10, 20 and 30 mg/ml concentrations of the plant extract dissolved in DMSO and with EcoR1 as positive control.
- These reaction mixtures were kept at 37°C for 2 hours for the complete digestion of plasmid DNA. After incubation, plasmid DNA was observed on 1% agarose gel.
- The extract at the concentration of 10 mg/ml, 20 mg/ml and 30 mg/ml did not break the DNA backbone, while the plasmid DNA treated with EcoRI produced a single band.

Hussain *et al.*,2007

Management

Antibacterial activity of leaf extract of Brazilian pepper tree against *A. tumefaciens* on tomato stems

Left: plant without application of the leaf extract,
Right: plant treated with the leaf extract of Brazilian pepper tree prepared in hot sterile distilled water.

Ghanney and Rhouma,2015

Management
Future Directions

- Verify that *A. vitis* free vines can be produced via tissue culture.
- Further identify environmental sources of *A. vitis*.

– Epiphytic nature in vineyards

– Survival and spread in wild grapes, soil, water

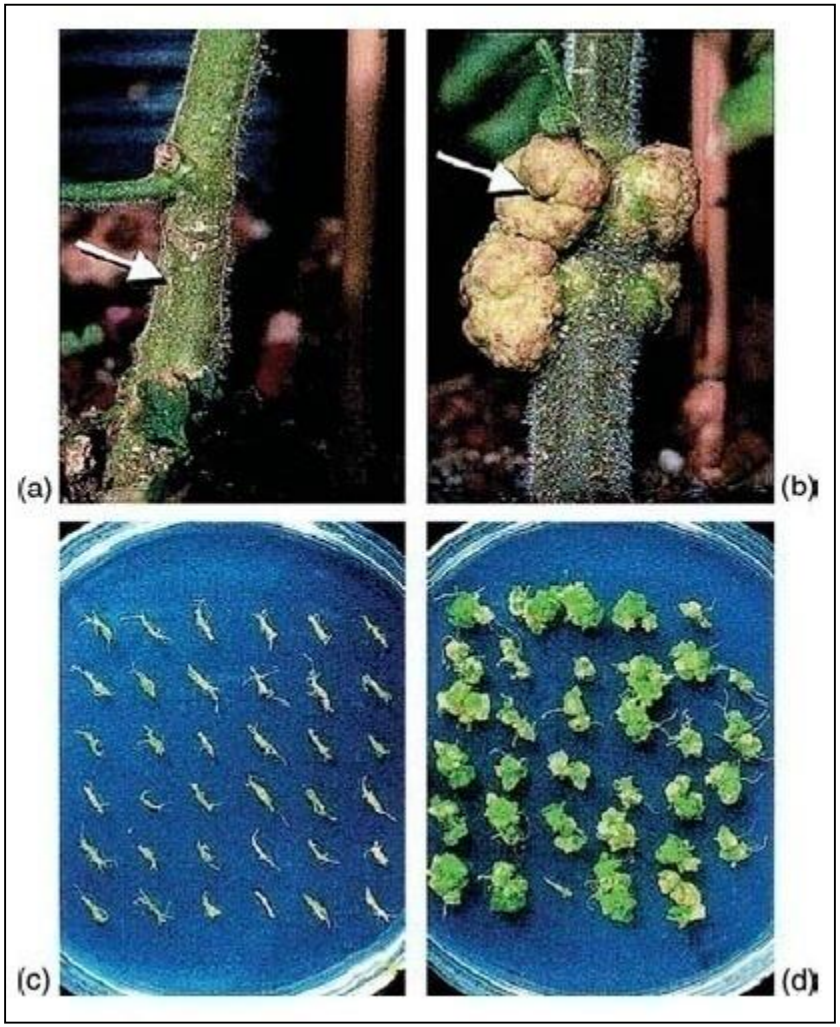
- Further clarify disease triggers
- Develop commercially viable biological control for grape crown gall.

Burr,2015

Control of crown gall
By gene silencing method

- Control of crown gall by silencing of the genes used by the bacterium for the synthesis of IAA and cytokinins:

a) Tomato transgene;
b) Tomato control;
c) Arabidopsis transgene;
d) Arabidopsis control.

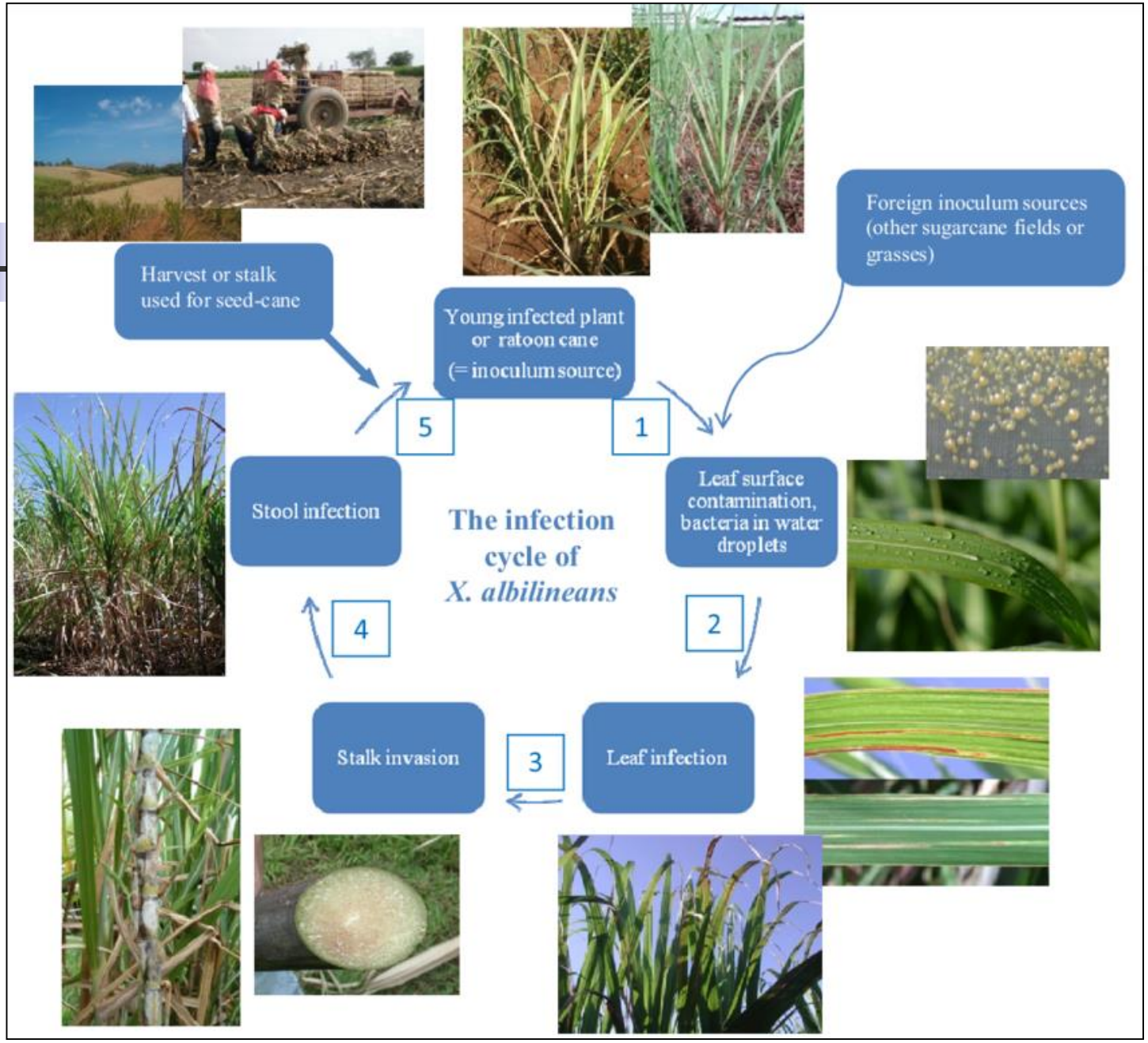


(Courtesy of Escobar *et al.* and Proceedings of the National Academy of Sciences USA).

Strange,2003

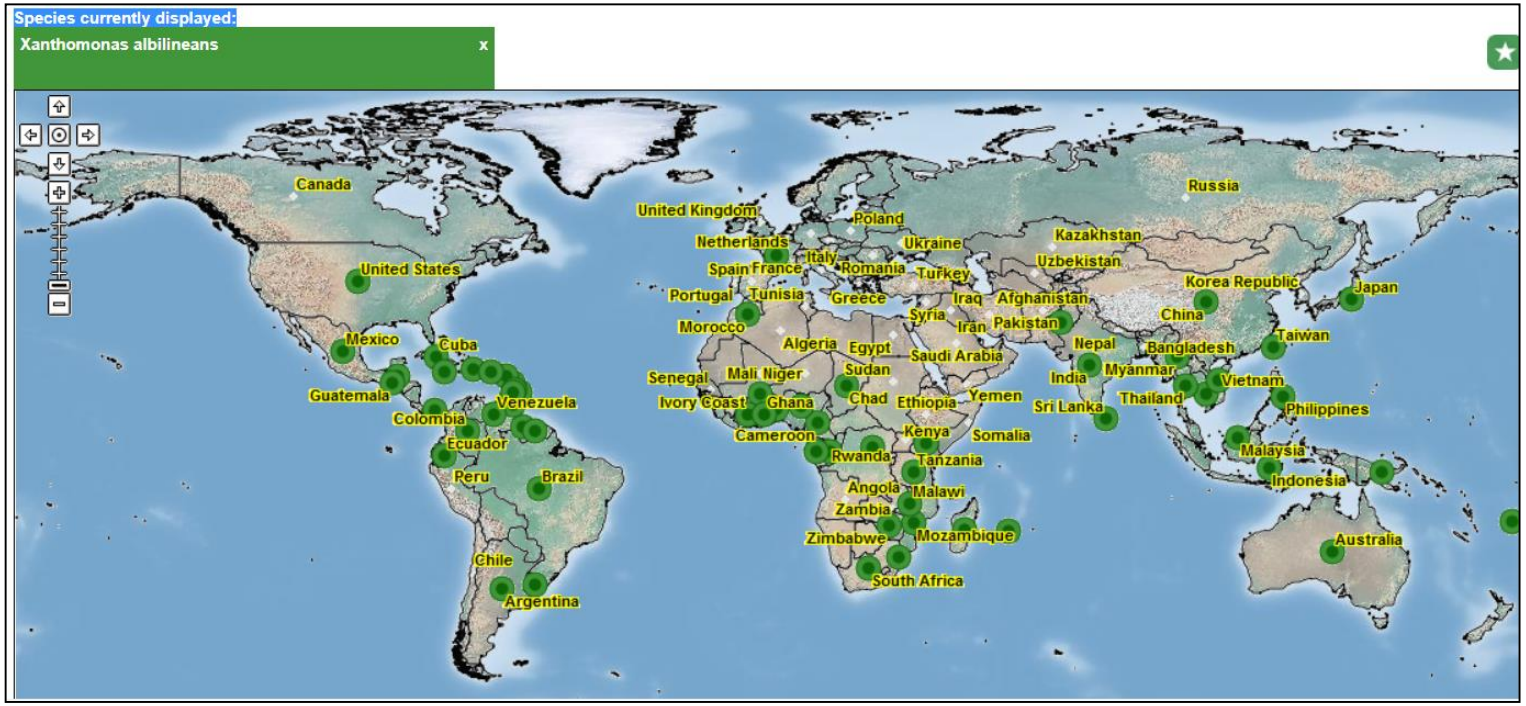
Xanthomonas albilineans

Leaf scald



Daugrois *et al.*,2011

Geographical distribution of sugarcane bacterial leaf scald



Impact

- Leaf scald was responsible for heavy losses in sugarcane at the beginning of the century when noble canes, *Saccharum officinarum*, was cultivated (Ricaud and Ryan,1989). The impact of the disease is reduced by the cultivation of interspecific hybrids but susceptible varieties may be rapidly destroyed.
- Yield losses of 15-20% were recorded in sugarcane variety B 69379 in Guadeloupe (Rott *et al.*,1995).
- Cochereau and Jean-Bart (1989) estimated a loss of 13 tonnes of cane per hectare of infected plants compared with healthy cane.
- In Mexico, leaf scald destroyed 800 hectares under variety Mex 64-1487 (Rott, 1995), while in Mauritius, a sugar yield-depressing effect of up to 31% was observed in variety M 695/69 (Anon,1990).

Management

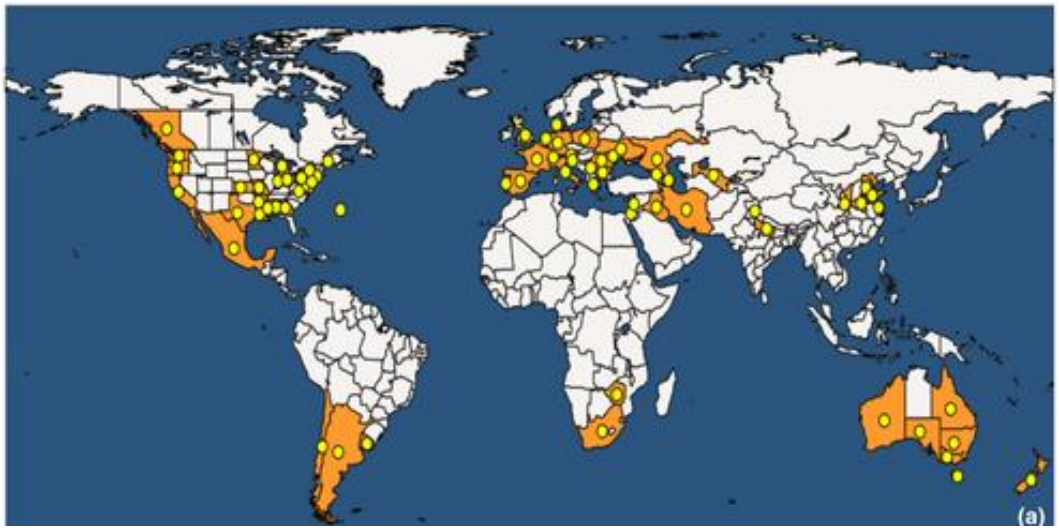
- Leaf scald may be controlled effectively by the simultaneous use of several methods.
- The cultivation of resistant varieties should be supplemented with the use of disease-free cuttings from nurseries.
- These cuttings should be established using sets which have undergone a cold soak/long hot-water treatment in which the cuttings are soaked in cold running water for 48 h followed by hot-water treatment at 50°C for 3 h.
- It is necessary to disinfect cutting instruments regularly when cuttings are prepared and during harvest because *X. albilineans* is transmitted by knives and harvesters.

Xanthomonas arboricola pv. *juglandis*

Walnut bacterial blight(WB)

Worldwide distribution
Xanthomonas arboricola pv. *juglandis*

- Worldwide distribution of *X. arboricola* pv. *juglandis* based on EPPO Global Database EPPO (2021) https://gd.eppo.int.



Yellow: present; purple: transient

Kałużna *et al.*,2021

Walnut tree

The tree produces male flowers on catkins and female flowers on terminal clusters where the fruit develops

Male flowers on catkins.

Female flowers.

Plant Village;..

Pathogenicity test

Inoculation of whole plants

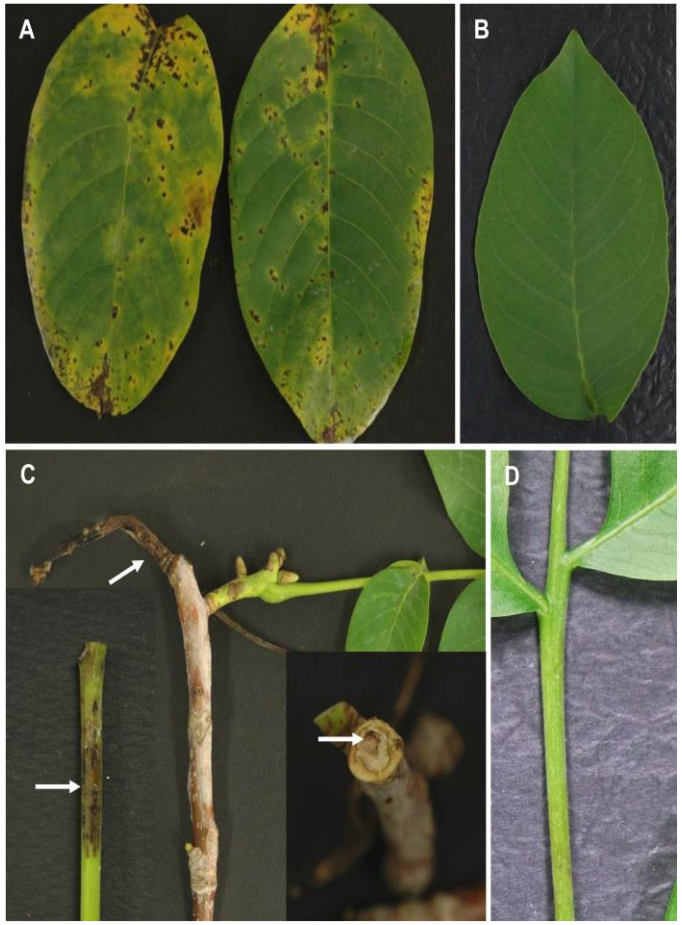
Xanthomonas arboricola pv. *juglandis*

1. Pathogenicity test can be carried out on detached walnut leaves and stem by artificial inoculation:

A. on wounded leaves, 2 weeks after inoculation with yellow halos around the black spots.

C. on inoculated stem 1 week after inoculation (the sites of inoculation noted by arrows).

- A cross-section of the stem shown at the corner represents the disease spread inside the stem tissue.
- B and C, control



Sup Kim *et al.*,2021

Description of the disease

Xanthomonas arboricola pv. *juglandis*

- The optimum temperature at which bacteria can grow is 28-32°C, maximum 37°C, minimum 5-7°C.
- The agent spends the winter in the infected dormant eyes.
- Primary infections occur in the spring when the bacteria multiply in these buds and pass to shoots and fruits.
- The first symptoms of the disease are seen in the leaves.
- One to several black lesions may appear on male catkin flowers in spring.
- Infected young nuts develop black, slightly sunken lesions at the flower end.
- Secondary infections are caused by the spread of the agent with raindrops.
- Once it enters the plant tissue, typical symptoms of the disease occur within 10-15 days.

TaKiMo Agriculture; Ontario,2016

Walnut bacterial blight

Symptoms and signs

Xanthomonas arboricola pv. *juglandis*

- Persian walnuts are more susceptible than black walnuts to this bacterial disease.
- Blight attacks leaves, bark, shoots, and nuts.
- In walnut blight, one to several black lesions may appear on catkins.
- Infected nuts develop black, slightly sunken lesions at the flower end (end blight) when young; more lesions will develop on the sides of the nut as it matures (side blight).
- Shoots develop black lesions, and leaves show irregular lesions on blade.

Description of the disease

Xanthomonas arboricola pv. *juglandis*

- Infections on new shoots do not grow into older wood, so trees are not killed, but
- The nuts can be severely damaged and fail to fill properly.
- Nuts may be infected anytime during the growing season.

Walnut bacterial blight

Symptoms and signs

Xanthomonas arboricola pv. *juglandis*

Male flowers on catkins.

Lamichhane,2014;..

Symptoms and signs

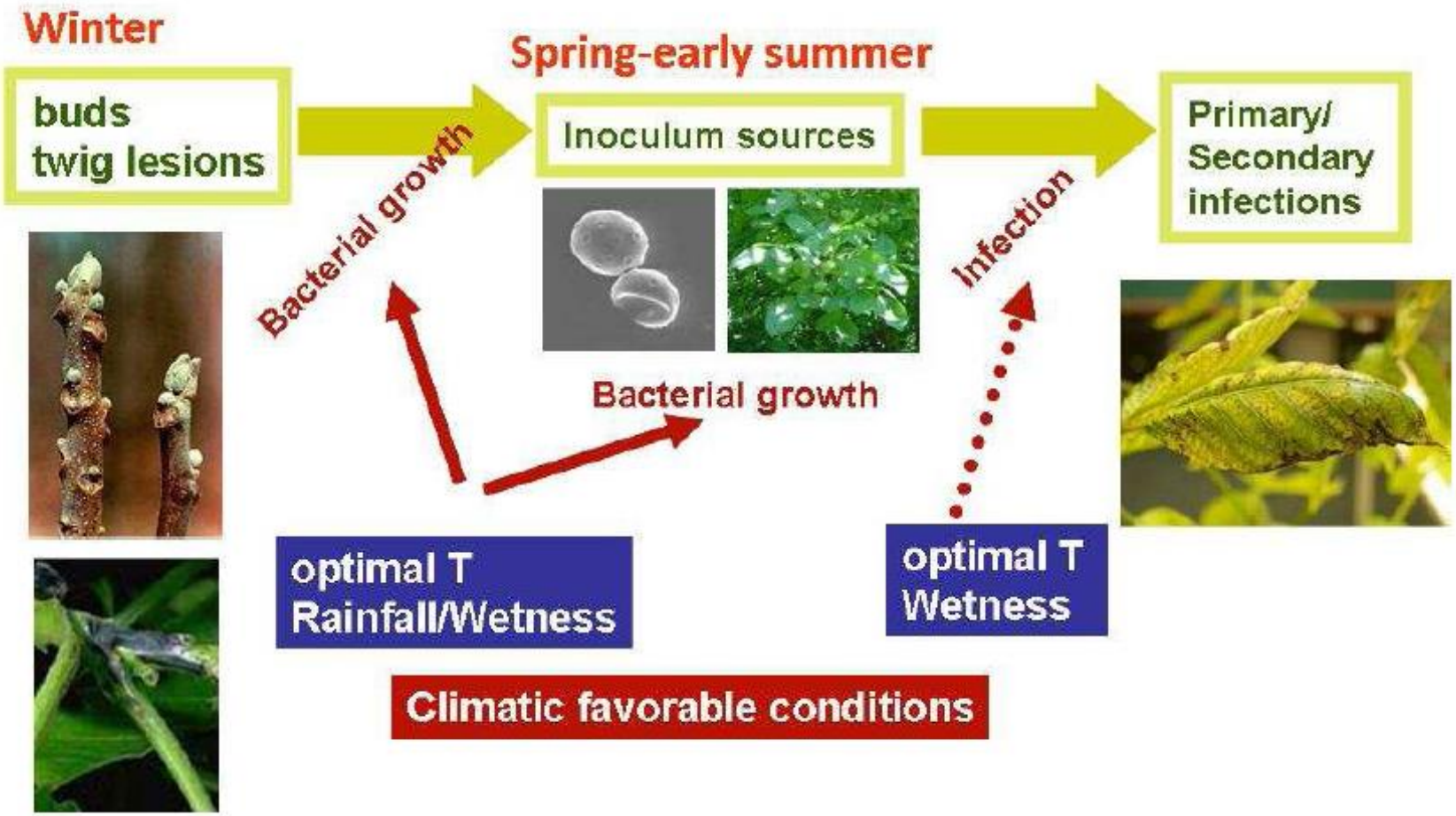
Xanthomonas arboricola pv. *juglandis*

Diseased walnut samples comprising trunks and branches with symptoms of vertical oozing canker (VOC), walnut bacterial blight (WBB) and superficial bark necrosis.

Iličić *et al.*,2021

Epidemiology

Xanthomonas arboricola pv. juglandis



Moragrega co-workers, COST 873,2011

Walnut bacterial blight
Xanthomonas arboricola pv. *juglandis*
Comments

1. All green tissue is susceptible to walnut blight.
2. Economically significant damage occurs when the developing nut is infected.
3. The bacterium that causes walnut blight overwinters primarily in dormant buds.
4. Rain is important for spreading bacteria and aiding infection.
5. Early-leafing varieties are most severely affected, and the disease tends to be more severe in Northern California.

Pathogenicity test

Inoculation of whole plants

Xanthomonas arboricola pv. *juglandis*

1. Pathogenicity test can be carried out on detached walnut leaves and stem by artificial inoculation:

A. on wounded leaves, 2 weeks after inoculation with yellow halos around the black spots.

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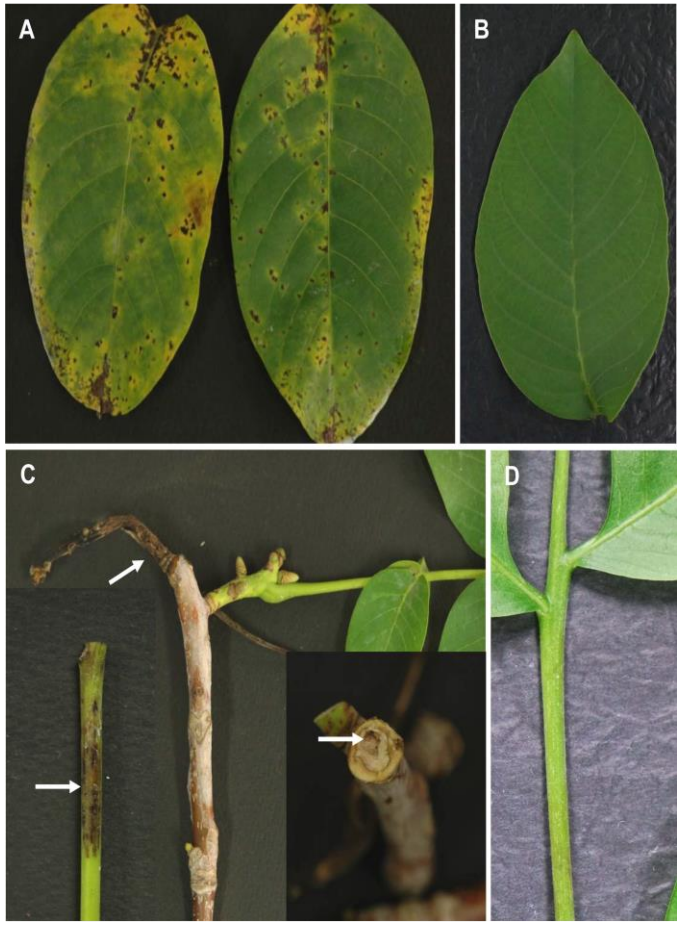


Sup Kim *et al.*,2021

Walnut bacterial blight

Walnut blight resistance in the genus *Juglans*

Artificial inoculation methods

1. Artificial inoculations were performed by spraying the seedlings to run off.

- Among *Juglans* spp. and English walnut varieties sprayed, *J. nigra*, *J. cinerea* and *J. sieboldiana* proved to be highly resistant.

2. Artificial inoculations were performed by injecting a bacterial suspension into the stem. Among English walnut varieties infected by injection:

1. Franquette and Hartley were the most resistant,
2. Feltre and Malizia showed an intermediate susceptibility, while
3. Payne, Serr and Sorrento proved the most susceptible.

Belisario *et al.*,1997

Walnut bacterial blight

Walnut blight resistance in the genus *Juglans*

Artificial inoculation methods

- The host resistance of walnut (*Juglans* spp.) against *Xaj* was evaluated for 18 walnut genotypes using three assays:

1. one under natural conditions (field investigations) and
2. two under controlled conditions (detached leaflet assay and whole plant assay).

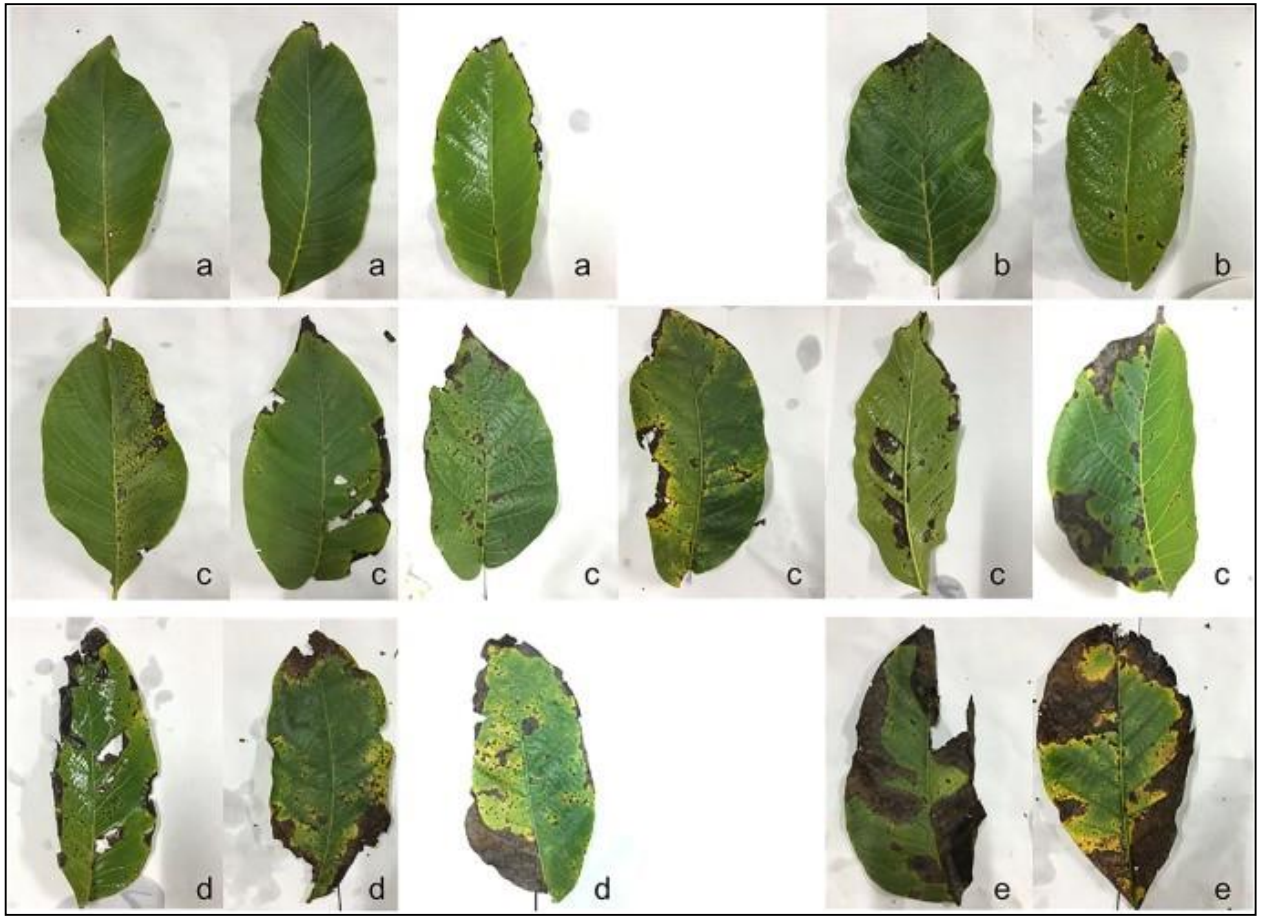
- Five genotypes, 'JS 91', 'JS 92', 'JS 86', 'Qingxiang' and 'JS 71', displayed partial resistance in all assays, suggesting that they may be good candidates for further evaluation.

Jiang *et al.*,2019

Walnut bacterial blight

Walnut blight resistance in the genus *Juglans*

Artificial inoculation methods



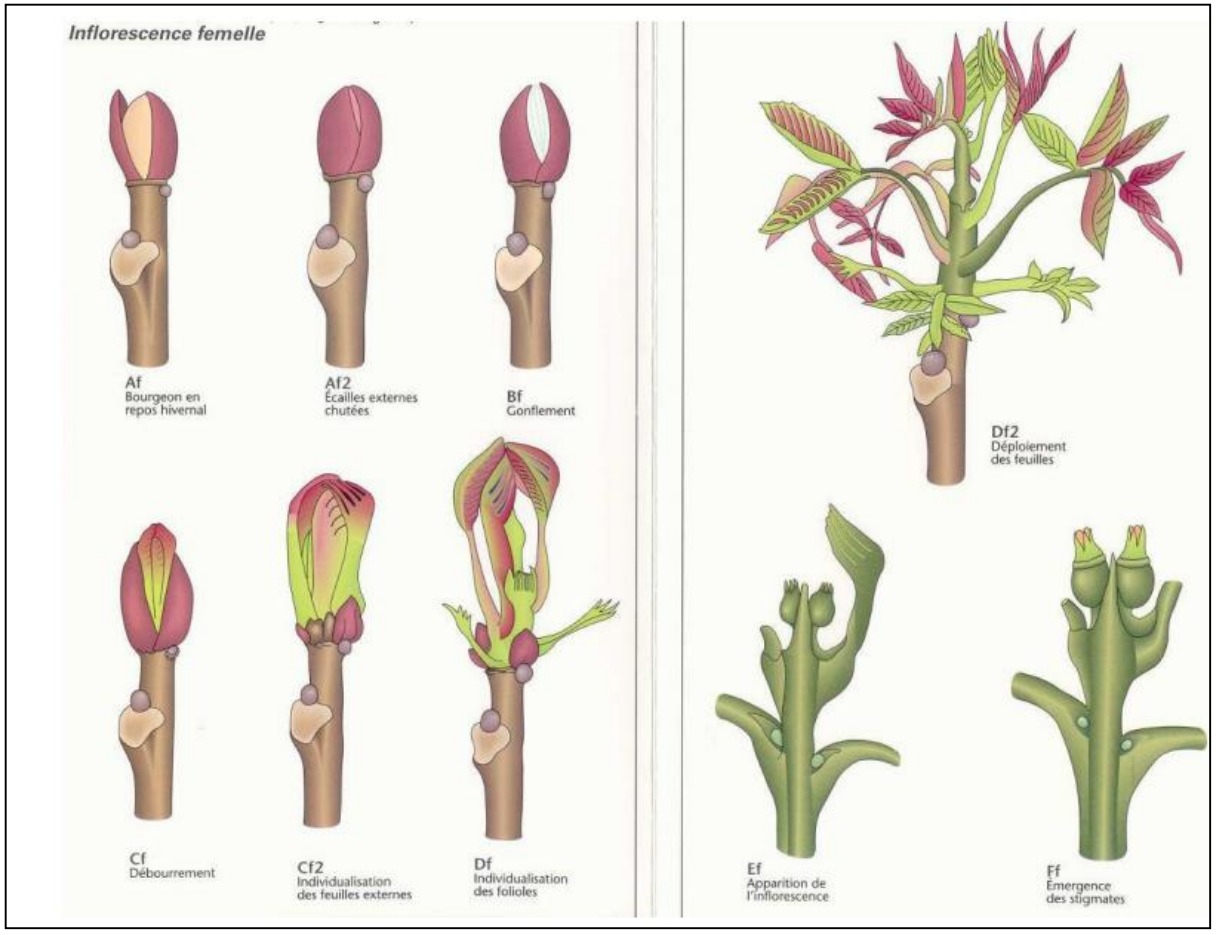
Jiang *et al.*,2019

Phenological stages of walnut buds and femal(pistillate) flowers, from Af to Ff

- Adapted from Germain *et al.*,1999.
- Af – during winter the bud is covered with scales and is dormant;
- Af2 – as early as July the harder outside scales fall and the bud is enveloped by other semi-membranous scales;
- Bf – white bud (the bud inflates, the external scales loosen and a whitish down appears under the ends of the scales);
- Cf – budburst (the bud lengthens and the exterior of the basal leaves are distinguishable);
- Cf2 – the scales and bracts separate and the first leaves begin to separate;
- Df – the first leaves are separate and their leaflets are well individualized;
- Df 2 – the first leaves are completely deployed and the basal leaves are more or less oblique;
- Ef – appearance of the female flowers;
- Ff – appearance of the stigmas.

David Lang,2012

Phenological stages of walnut buds and femal(pistillate) flowers, from Af to Ff

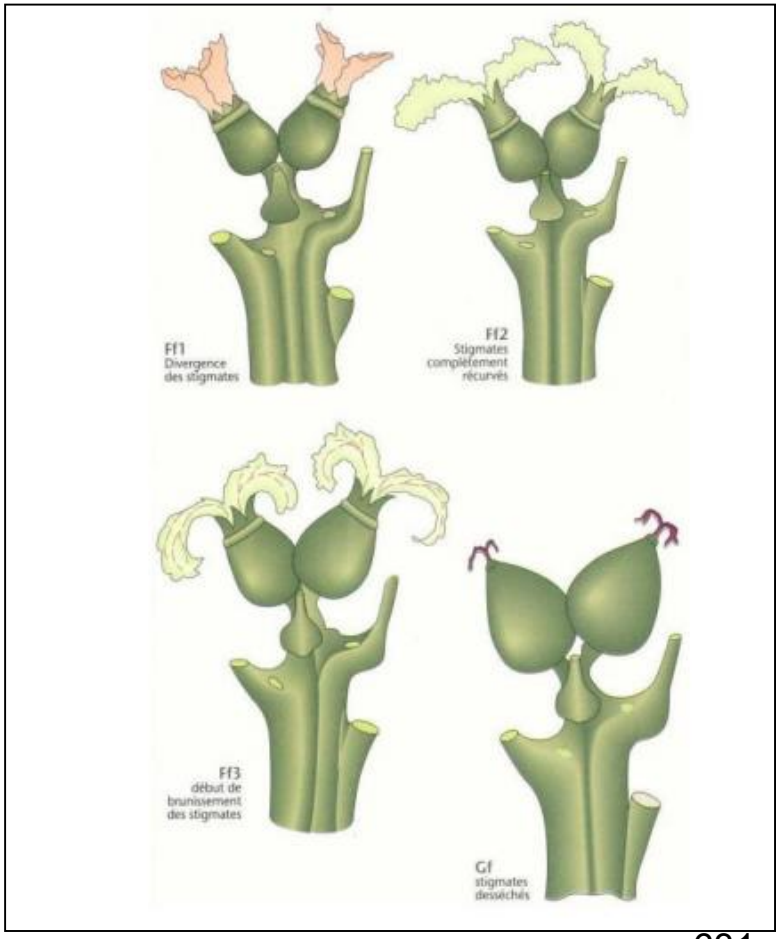


Pistil: the female organs of a flower, comprising the stigma, style, and ovary.

David Lang,2012

Phenological stages of walnut buds and femal(pistillate) flowers, from Af to Ff

- Phenological stages of walnut pistillate flowers, from Ff1 to Gf (adapted from Germain *et al.* 1999);
- Ff1 – the stigmas are yellow to orange colour, with optimum receptivity i.e., full female flowering;
- Ff2 – the stigmas are a green to pale yellow colour and are completely re-curved;
- Ff3 – the stigmas begin to turn a fine brown color;
- Gf – the stigmas dry out and become necrotic.



David Lang,2012

Three walnut blight prediction or forecasting models

- For attempting to predict or forecast walnut blight, utilizing:
  1. orchard history,
  2. bud monitoring, and
  3. Xanthocast.
- All methods have advantages and disadvantages and should be used as information to guide the grower/PCA(Principal component analysis) decision-making process for implementing a preventative management program.

Table 1. Forecasting and Predicting Walnut Blight Risk

Method	How it works	Notes:
Orchard history	Previous June, survey 10 trees for infected nuts: < 50 nuts – low risk 50-150 nuts – high risk >150 nuts – high risk	High disease levels generally mean high bud populations and presence of twig cankers
Bud monitoring	Dormant buds are plated on agar and colony formation counted. See: sacvalleyorchards.com/walnuts/diseases/walnut-blight-bud-sampling/	Need positive identification of bacterial species; twig cankers can also be inoculum source; orchard can quickly go from low-risk to high-risk depending on favorable weather conditions
Xanthocast	Mathematical model using leaf wetness and temperature for forecasting the disease and timing re-treatment intervals	www.agtelemetry.com Values should be combined with information from orchard history and bud monitoring

Adaskaveg *et al.*,2020

Walnut blight forecasting model
Web-based forecasting model
Xanthocast or Walnut Blight Forecast

- This yellow bacterium lives between scales of male and female flower buds, as well as in dead buds and cankers.
- The pathogen can also cause leaf and bud infections.
- Cankers form from fruit infections that progress into the peduncle and stem.
- Rain and temperature are driving environmental conditions.

Agtelemetry *et al.*,2021

Walnut blight forecasting model
Web-based forecasting model
Xanthocast or Walnut Blight Forecast

- The walnut blight forecasting model was developed by Adaskaveg *et al.*,1998-2008.
- XanthoCast is a temperature-mediated surface wetness accumulation model.
- It is a 7-day cumulative index that is based on:

1. temperature,
2. leaf wetness, and
3. walnut phenology.

- The model is currently on two Web sites for different regions of the state.

Walnut blight forecasting model
Web-based forecasting model
Xanthocast or Walnut Blight Forecast

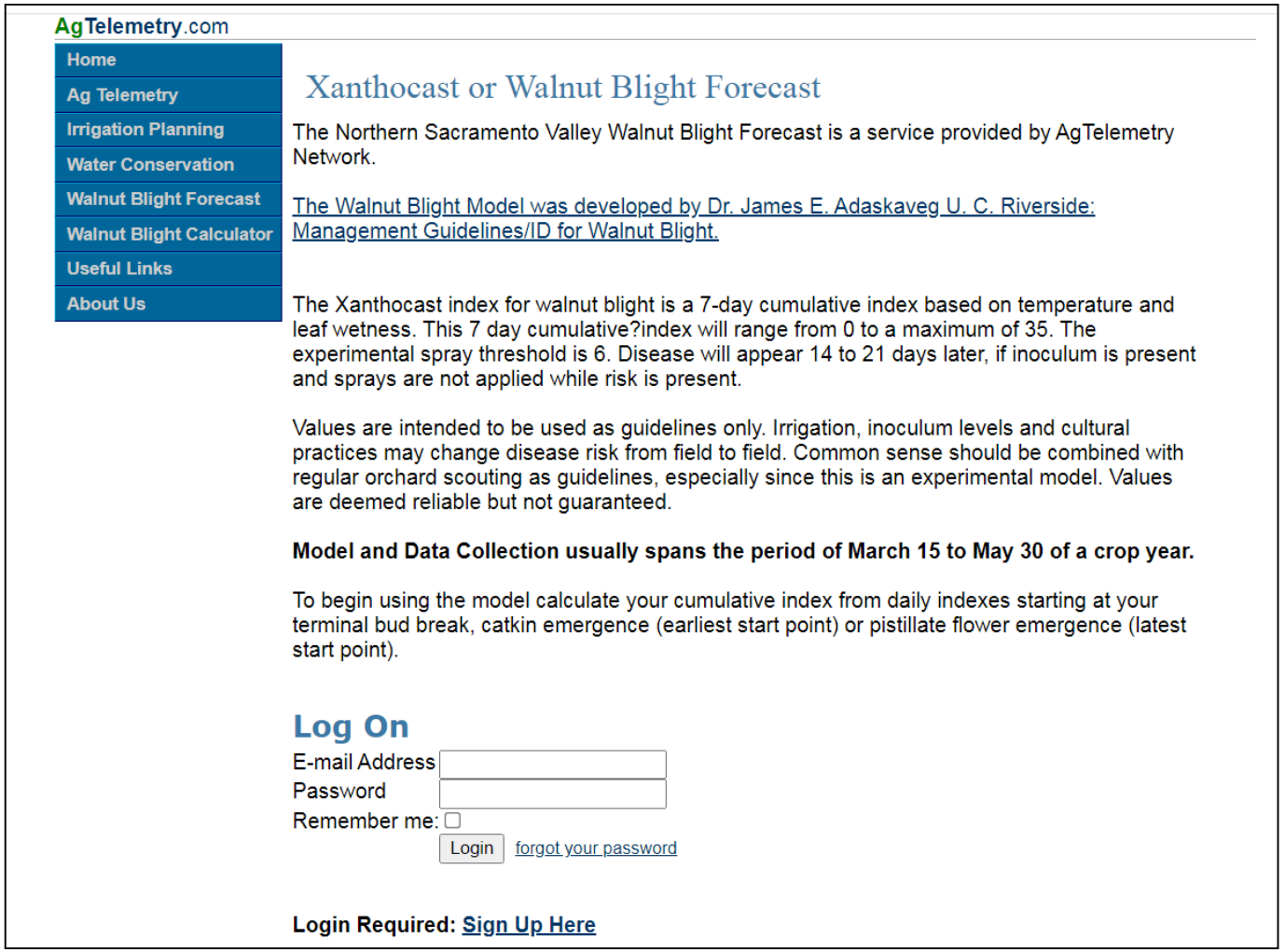
- XanthoCast™, a model that utilizes wetness period duration and temperature for calculating the daily and cumulated daily risk of disease.
- In field trials in California, XanthoCast™ reduced the number of biocide sprays, when compared to a calendar-based spray regime, while providing similar disease control (Adaskaveg *et al.*,2006).

David Lang,2012

Walnut blight forecasting model

A spray forecast software, XanthoCast

website www.agtelemetry.com



Walnut blight forecasting model
Web-based forecasting model
Xanthocast or Walnut Blight Forecast

- The Walnut Blight Model was developed by Dr. James E. Adaskaveg U. C. Riverside: Management Guidelines/ID for Walnut Blight.
- The Xanthocast index for walnut blight is a 7-day cumulative index based on:

1. Temperature, and
2. leaf wetness.

- This 7 day cumulative index will range from 0 to a maximum of 35.
- The experimental spray threshold is 6.
- Disease will appear 14 to 21 days later, if inoculum is present and sprays are not applied while risk is present.

Walnut blight forecasting model
Web-based forecasting model
Xanthocast or Walnut Blight Forecast

- XanthoCast calculates a 7-day cumulative index based on temperature and leaf wetness: in conducive conditions, during prolonged wet springs and rains, sprays should be done at 7- to 10-day intervals to obtain adequate disease control.
- The XanthoCast walnut blight risk assessment tool that tracks:

1. hours of leaf wetness accumulated per day from rainfall, dew, or irrigation water;
2. for three temperature regimes (6-12°C, 12-17°C, and 17-27°C); and
3. calculates the risk or potential for disease in a given location.

Agtelemetry *et al.*,2021; Kałużna *et al.*,2021

Walnut blight forecasting model
Xanthocast or Walnut Blight Forecast
Objectives

1. Evaluate disease development throughout the spring and monitor environmental parameters (e.g., leaf wetness, precipitation, temperature, and relative humidity) that are conducive to bacterial infection of walnut tissues.
2. Evaluate "Pistillate Flower Bloom Cycle" as another phenological parameter for determining start dates in relation to forecasts of favorable environmental conditions for disease development.

Receptivity stages of pistillate flower of 'Serr' walnut: (A) Pre-receptivity stage; (B) Receptivity; (C) Full receptivity and (D) Post-receptivity.

Agtelemetry *et al.*,2008; González *et al.*,2008

Walnut blight forecasting model
Xanthocast or Walnut Blight Forecast
Objectives(continued)

- Continue to determine the reproduction potential of the pathogen on the plant surface using spiral plating technology.
- Evaluate previous year disease levels as a general indicator for "start time" for running the XanthoCast model.
- Continue to evaluate the automated model of XanthoCast with up to a 5-day forecast included in the latest version.

Agtelemetry *et al.*,2008

Walnut blight forecasting model
Xanthocast or Walnut Blight Forecast
Objectives(continued)

- Evaluate early, mid-, and late-spring timings (e.g., male (catkin) vs. female (pistillate) flower emergence or delayed emergence in respect to "Bloom Cycle".
- Evaluate mid- to late spring season timings under natural and simulated rain environments.
- Continue to evaluate walnut genotypes for natural host resistance to walnut blight under simulated rainfall conditions at the Kearney AgCenter (KAC).
- Compare version 481 with a second version, 484 to reduce accumulation of XanthoCast indices at high temperatures.

Agtelemetry *et al.*,2008;..

Walnut blight forecasting model

Xanthomonas arboricola pv. *juglandis*

Xanthocast or Walnut Blight Forecast

1. Each day, determine the number of hours in each of three temperature ranges, from 6 to <12°C, from 12 to 17°C and from >17 to 27°C.
2. Each day, determine the number of hours fruits are wet for each temperature range, with the duration of fruit "wetness" estimated with surface wetness sensor values between 10 and 100%.
3. Each day, add one "point" for every 8, 4 and 1 hrs duration of surface wetness for the 6 to <12°C, 12 to 17°C and >17 to 27°C temperature categories, respectively.
4. Multiply the points for the 12 to 17°C temperature category by 3 then sum the points across each temperature category to get a "daily disease index".

David Lang,2012

Walnut blight forecasting model

Xanthomonas arboricola pv. *juglandis*

Xanthocast or Walnut Blight Forecast

5. Sum each daily disease index for a period of 7 days or until a threshold of 5 is reached. Apply a bactericide as soon as possible after a threshold of 5 is reached.
6. If a threshold of 5 is not reached within 7 days, then sum the daily disease index over a total of 7 days by adding the most recent daily disease index and removing the oldest daily disease index. Apply a bactericide as soon as possible after a threshold of 5 is reached.
7. Seven days after applying the bactericide, begin cumulating the daily disease index again until a threshold of 5 is reached, as previously described.

David Lang,2012

Walnut blight forecasting model
Xanthomonas arboricola pv. *juglandis*
Xanthocast or Walnut Blight Forecast

- Due to the very low disease pressure in 2008, disease incidence was less than 2% at the end of the evaluation period and thus, no disease progress curves could be developed(next slide).
- This is confirmed by XanthoCast since low accumulation of seasonal XanthoCast indices were recorded.
- Average seasonal accumulations of XanthoCast indices among the stations were 69.8 in 2006, 35.3 in 2007, and 8.8 in 2008.

Agtelemetry *et al.*,2008

Walnut blight forecasting model

Xanthocast or Walnut Blight Forecast

XanthoCast indices in forecasting walnut blight

		2006		2007		2008	
No.	Station	XanthoCast Index	Total Precip. (mm)	XanthoCast Index	Total Precip. (mm)	XanthoCast Index	Total Precip. (mm)
1	Butte City	46		43		4	
2	Corning	59		15		5	
3	Durham	63	180.7	36	66.2	7	18.8
4	E Corning	53		ND		6	
5	Gerber	ND	178.4	64	45.2	21	8.6
6	Hamilton City	111		62		10	
7	Jellys Ferry	75		18		12	
8	N Chico	99		ND		4	
9	Nord	81		32		5	
10	Red Bluff	52	166.7	45	55.6	12	11.2
11	Rio Oso	104	139.3	47	58.9	16	2.0
12	S Chico	ND		17		2	
13	SW Gridley	70		41		9	
14	Tehama	98		27		15	
15	W Chico	81	276.1	26	124.1	7	12.2
16	W Corning	ND		15		7	
17	W Cottonwood	50		20		11	
18	W Gerber	75		57		6	
	Yearly Average	69.8	188.2	35.3	70.0	8.8	10.6
Dis.	Chico	60%		27%		<2%	
Incid.**	Durham	64%		45%		<2%	

* - XanthoCast indices were obtained from www.irrigate.net and precipitation data were obtained from CIMIS.
** - Disease incidence of walnut blight in non-treated trees in research trials in W. Chico and Durham, CA (Butte Co.) evaluated in June of each year.

Seasonal accumulation of XanthoCast indices in forecasting walnut blight and total precipitation in N. California from March 15 to May 30 in 2006-2008. It shows high risk of disease incidence in 2006 and 2007 but not in 2008.

Adaskaveg *et al.*,2008

Walnut blight forecasting model

Xanthomonas arboricola pv. *juglandis*

Xanthocast or Walnut Blight Forecast

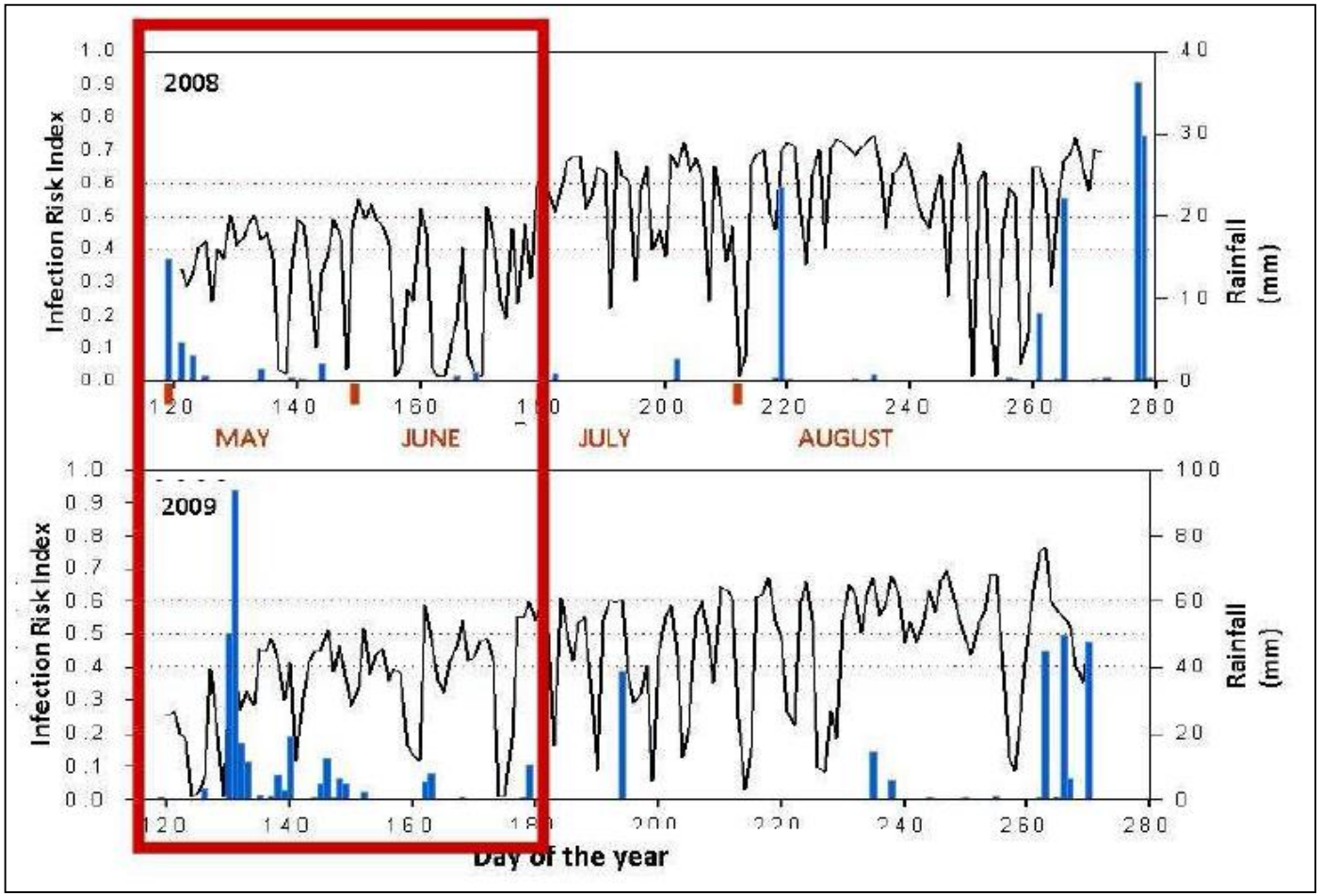
- Average rainfall based on five weather stations for the three seasons was 188.2 mm, 70 mm, and 10.6 mm, respectively.
- This again demonstrates that XanthoCast indices closely reflects the actual weather patterns and predicts the disease potential.
- Differences in XanthoCast indices between sites and years are because the indices are based on three temperature ranges for disease development in addition to leaf wetness and thus, are more accurate for predicting disease than total precipitation alone.

Agtelemetry *et al.*,2008

Walnut blight forecasting model

Xanthocast or Walnut Blight Forecast

Prediction of walnut blight disease risk

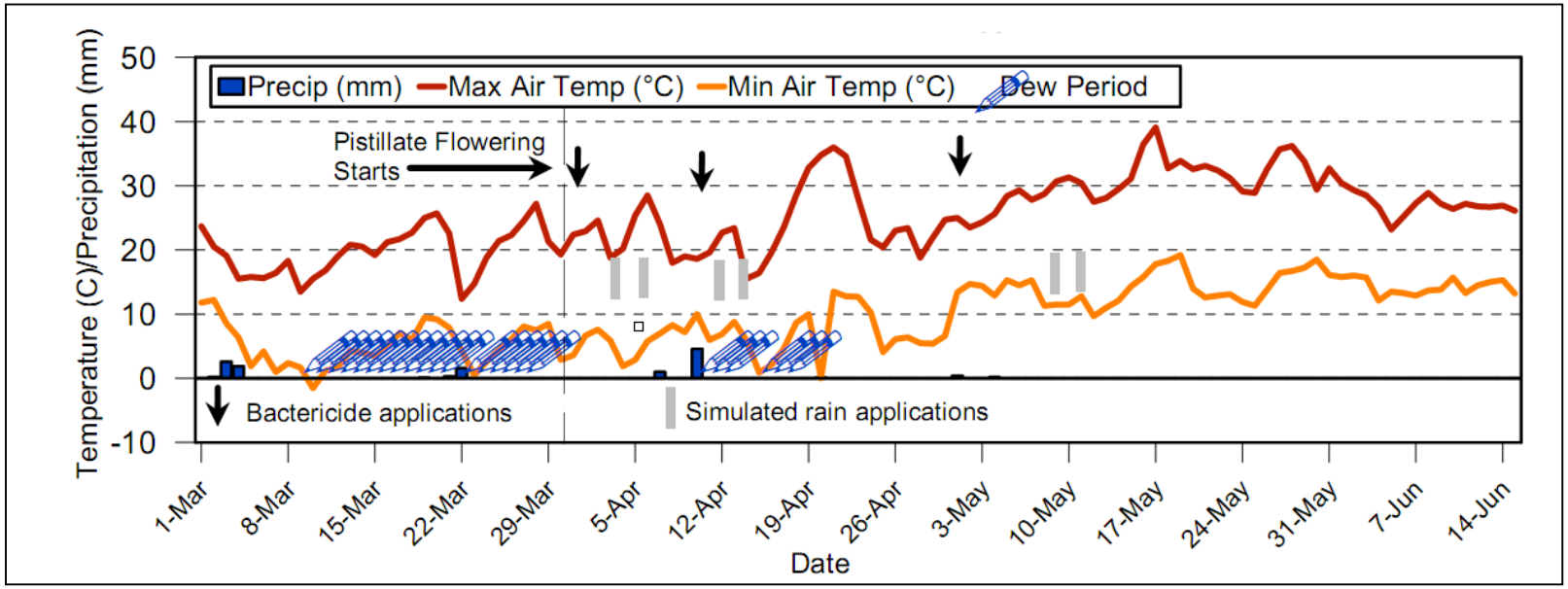


Moragrega co-workers, COST 873,2011

Walnut blight forecasting model

Prediction of walnut blight disease risk for bacteriocide applications

- Timing study with Kocide-Manzate treatments in cv. Vina orchard under simulated rain conditions.



Adaskaveg *et al.*,2009

Walnut blight forecasting model

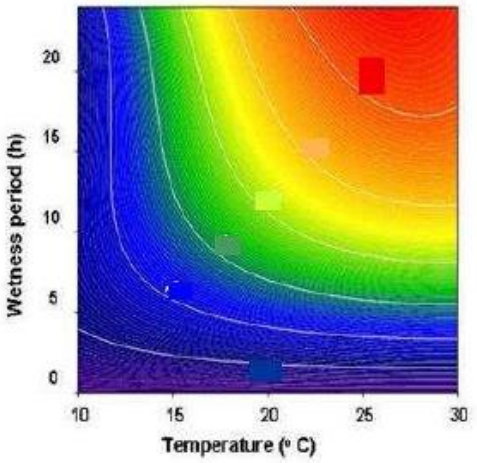
Effect of wetness duration and temperature on disease severity

Xanthocast or Walnut Blight Forecast

Walnut blight forecasting model

Development

Effect of wetness duration and temperature on disease severity



Moragrega co-workers, COST 873,2011

Walnut blight forecasting model

Effect of wetness duration and temperature on disease severity

Xanthocast or Walnut Blight Forecast

Walnut blight forecasting model

Evaluation

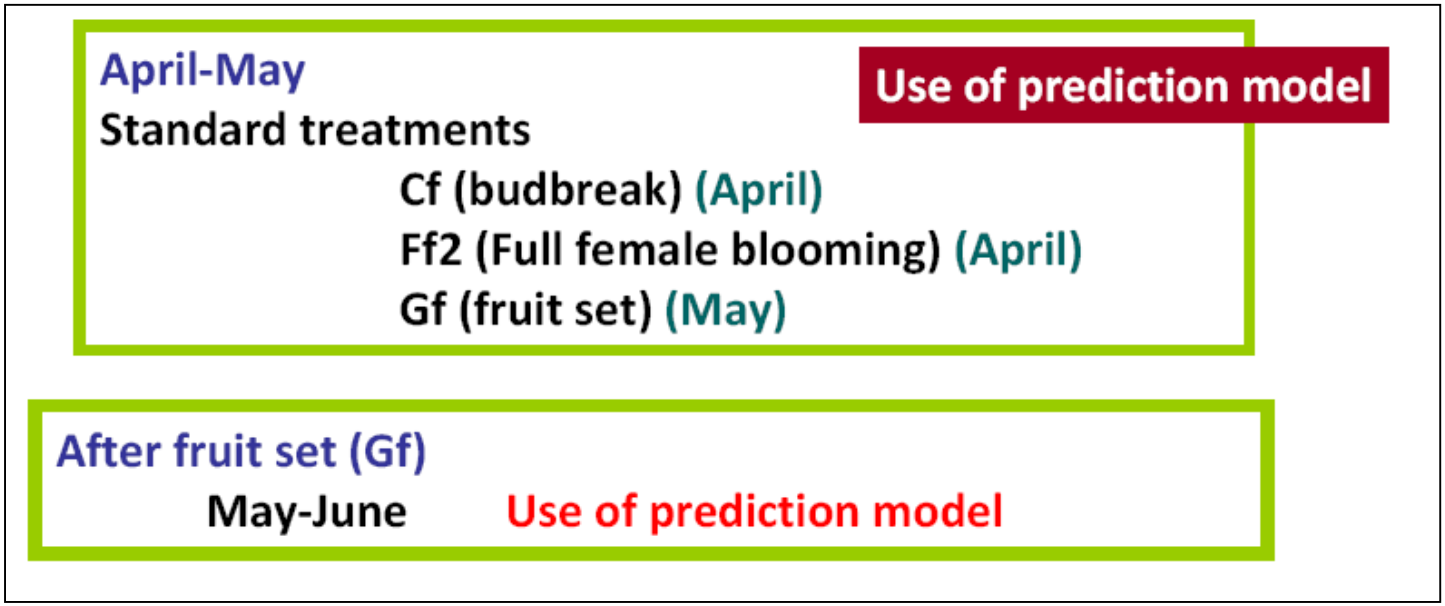
Infection risk predicted by the model ⟷ **Infections in the orchard**

Moragrega co-workers, COST 873,2011

Timing sprays for walnut blight control

Based on phenological and climatic parameters

1. Treatments based on standard methods;
2. Treatments based on predication model.



Moragrega co-workers, COST 873,2011

Management

Chemical control

Copper sprays

- Use fixed copper (50 to 55 percent copper) at the rate of 4 pounds per 100 gallons (3.78 L) of water (3 level tablespoons per gallon).
- Spray 3 times at the beginning and completion of flowering and at nut set.

Walnut blight control

Standard treatments

Copper sprays

Copper sprays

Limitations:

Copper tolerant or resistant strains

Moderate efficacy in disease control

Copper accumulation in soil

Regulation of total amount of copper applied

Increase the efficacy of preventive copper applications

Reduce the number of treatments

Rational control of walnut blight

Moragrega co-workers, COST 873,2011

Management

Bactericide efficacy for different compounds

Material	Resistance risk (FRAC#)[1]	Walnut blight*	Phytotoxicity
Bordeaux	low (M1)	+++	NP
Fixed coppers	medium (M1)	+++	++**
Copper-maneb	low (M1/M3)	++++	NP
Copper-maneb-surfactant	low (M1/M3)	+	NP
Zinc-Copper Bordeaux	low (M1)	+++	NP
Serenade	low	+	NP

***Rating:** ++++ = excellent and consistent, +++ = good and reliable, ++ = moderate and variable, + = limited and erratic, and NP = not phytotoxic. Serenade (a lyophilized culture filtrate of *Bacillus subtilis* QST 713).

Management

Bactericide treatment timing in walnut

Disease	Catkin emergence	Terminal bud break	1 week after bud break	7-10 day intervals[b]	May[c]
Walnut blight (on fruit/nuts)[a]	++	+++	+++	++[b]	+

- Timings used will depend upon orchard history of disease and weather conditions each year.
- Male and female flowers are susceptible beginning with their emergence, depending on wetness and temperatures conducive to disease development.
- A temperature-leaf wetness forecasting model (e.g., XanthoCast) is available for determining optimum timing of bactericide applications.
- Late spring rains are less conducive to disease.

Note: Timings listed are effective but not all may be required for disease control.*

New products with antibacterial activity
Increase copper formulations efficacy

Walnut blight control

Bordeaux mixture

Copper oxychloride

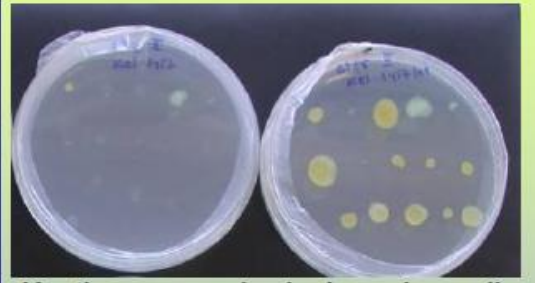
Copper oxychloride + mancozeb

Bordeaux mixture + metallic ions

Fosetyl – Al

Streptomycin

***in vitro* antibacterial activity**



Xanthomonas arboricola pv. *jugandis*

Disease control in walnut plants

Moragrega *et al.*,

New products with antibacterial activity
Increase copper formulations efficacy

Walnut blight control

Disease control in walnut plants

Bordeaux mixture
(2.5 g Cu/l)

Copper oxychloride + mancozeb
(2.5 g Cu/l) *(2.5 g i.a./l)*

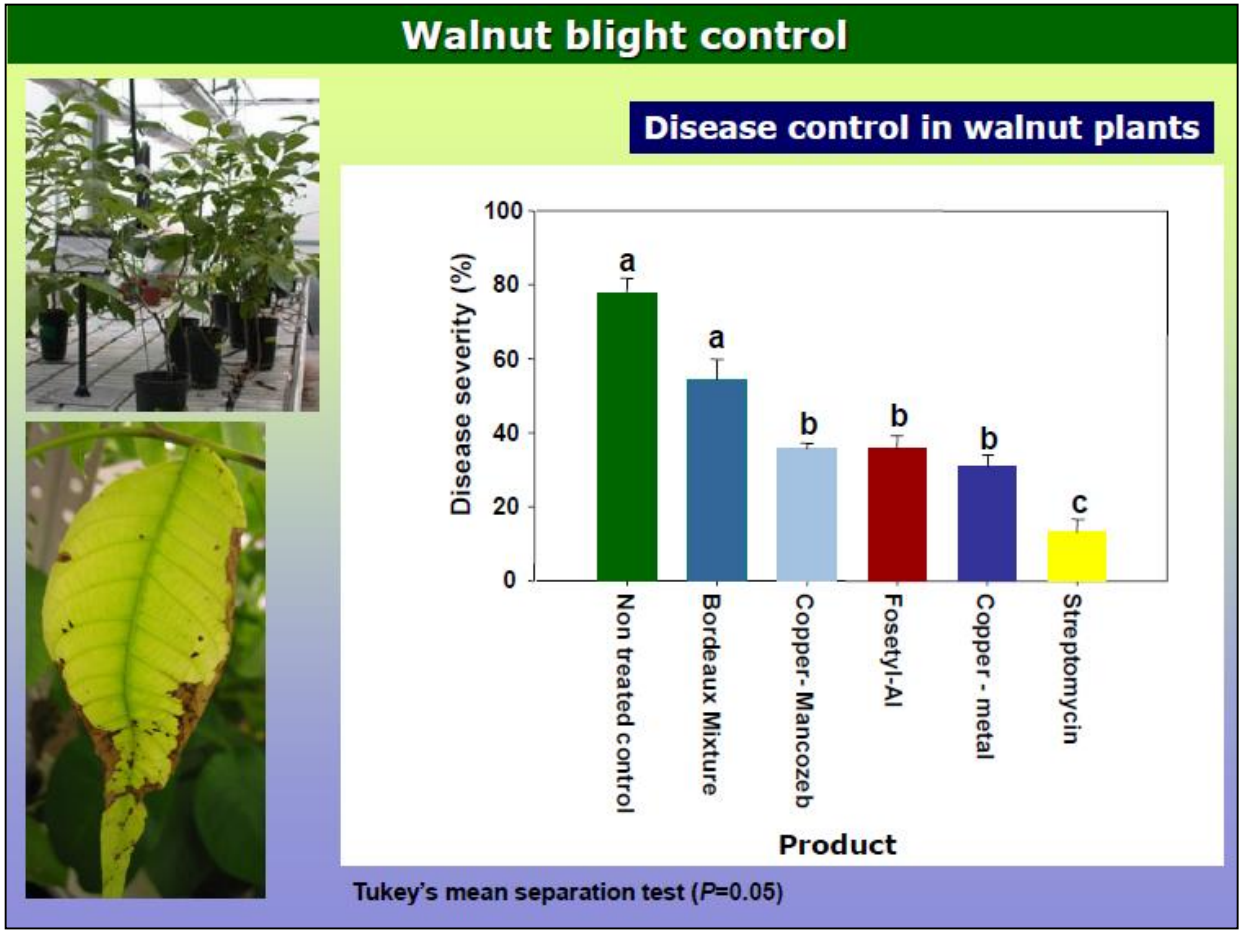
Bordeaux mixture + metallic ions
(2.5 g Cu/l) *(10 µg/ml)*

Fosetyl – Al
(1.86 g $[HPO_3]^{2-}$/l)

Streptomycin
(0.1g/l)

Moragrega *et al.*,

New products with antibacterial activity
Increase copper formulations efficacy



Moragrega *et al.*,

Biological control

Xanthomonas arboricola pv.*juglandis*

Bacteriophage treatments

- Isolates of bacteria were collected from blighted nuts, leaves, buds and petioles of walnut trees from all over New Zealand.
- Bacteriophages which attack *X. campestris* pv *juglandis* were isolated from the soils under the same trees. Phages were readily isolated from a depth of 2.5 cm. In the following spring phages were also isolated from the canopy of trees grown at Lincoln.
- Several phages isolated from the soil were identified under the electron microscope as being from the λ(lamda) phage group, with long unsheathed tails and hexagonal heads.
- Phages isolated from the canopy belonged to several other groups of phages.
- Short term storage had little effect on canopy phage survivability.
- The search to locate effective, hardy phages for use as biobactericides continues.

Mcneil *et al.*,2001

Phage sensitivity test

X. arboricola pv. *junglandis*

- Sensitivity response of three different *Xanthomonas campestris* pv. *Junglandis*.
- Strains to six bacteriophage types isolated from the walnut canopy.

Phage type	Bacterial strain		
	134[2] Lincoln	143 Lincoln	6494 Auckland
$Bp60C_1$	+++	+	+
$Bp60C_2$	+++	+	+++
$Bp60C_3$	+++	+	+++
Bp_{10}	+++	+	-
Bp_{20}	+++	-	-
Bp_{22}	+++	+	-

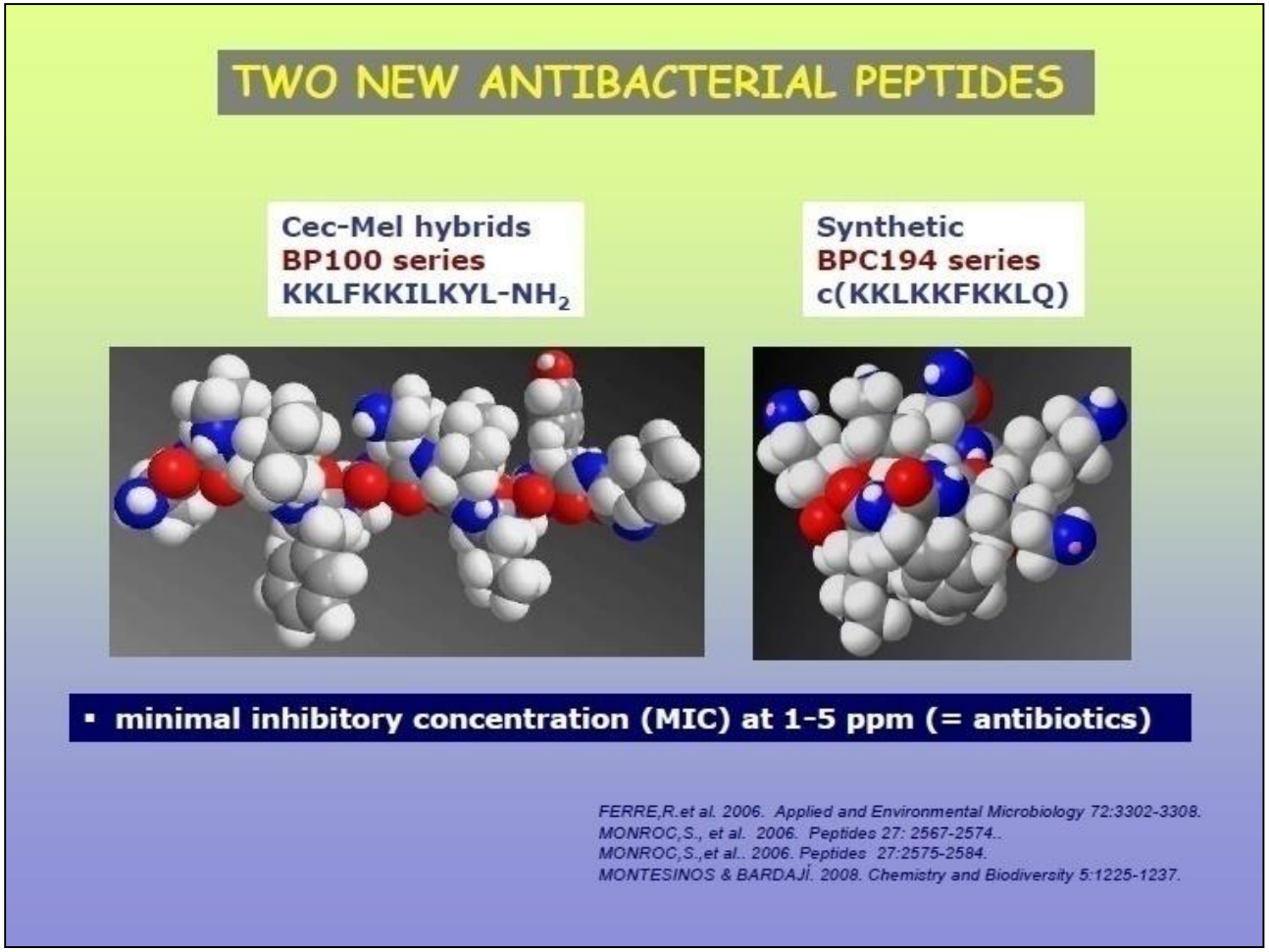
[1]+++ Reactive (total lysis); - Not reactive; + Reactive (lysogenic).
[2]=Strain used for initial isolation.

Mcneil *et al.*,2001

Antimicrobial peptides or proteins

Xanthomonas arboricola pv.*juglandis*

Synthetic AMPs



Moragrega *et al.*,

Antimicrobial peptides or proteins
Synthetic AMPs

Walnut blight control

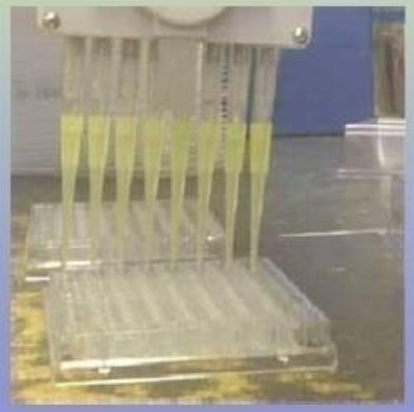
BP100

BPC194
BPC198

New peptides under experimental evaluation
MT07
MT09

***in vitro* antibacterial activity**

Disease control in walnut plants

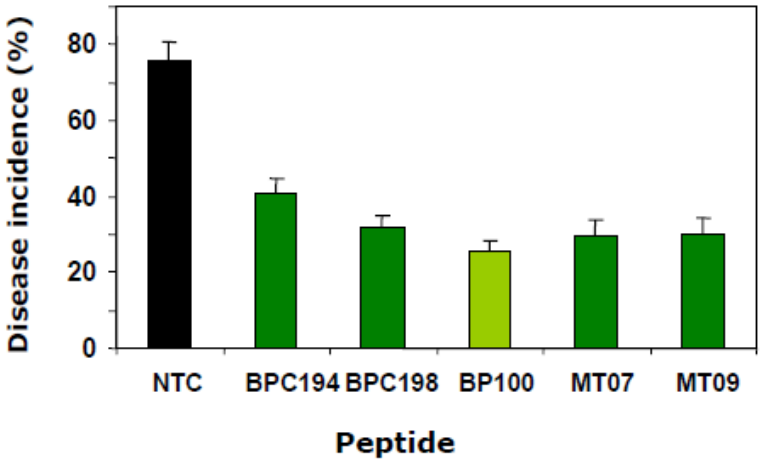


Xanthomonas arboricola* pv *juglandis

Moragrega *et al.*,

Antimicrobial peptides or proteins
Synthetic AMPs

Efficacy of AMPs in walnut blight control



Peptides significantly reduced *Xaj* infection in walnut plants

BP100	**good in walnut blight control**
MT07 and MT09	**reduction of disease incidence similar to BPC series**

Moragrega *et al.*,

Xanthomonas arboricola pv. *pruni*

Bacterial leaf spot of stone fruits

Etiology

Xanthomonas arboricola pv. *pruni*

- Penetration occurs through stomata or lenticels;
- However, surface moisture is needed for bacterial dissemination.

Bacterial spot of stone fruits

Xanthomonas arboricola pv. *pruni*

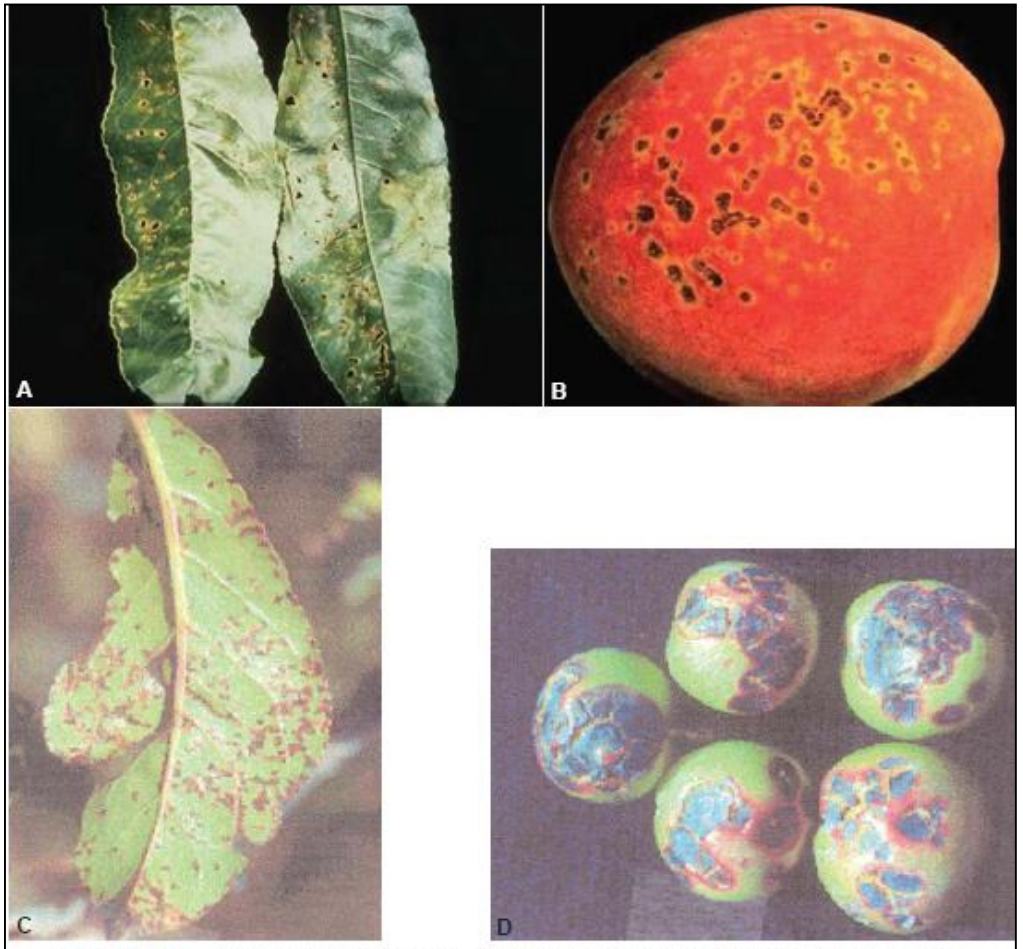


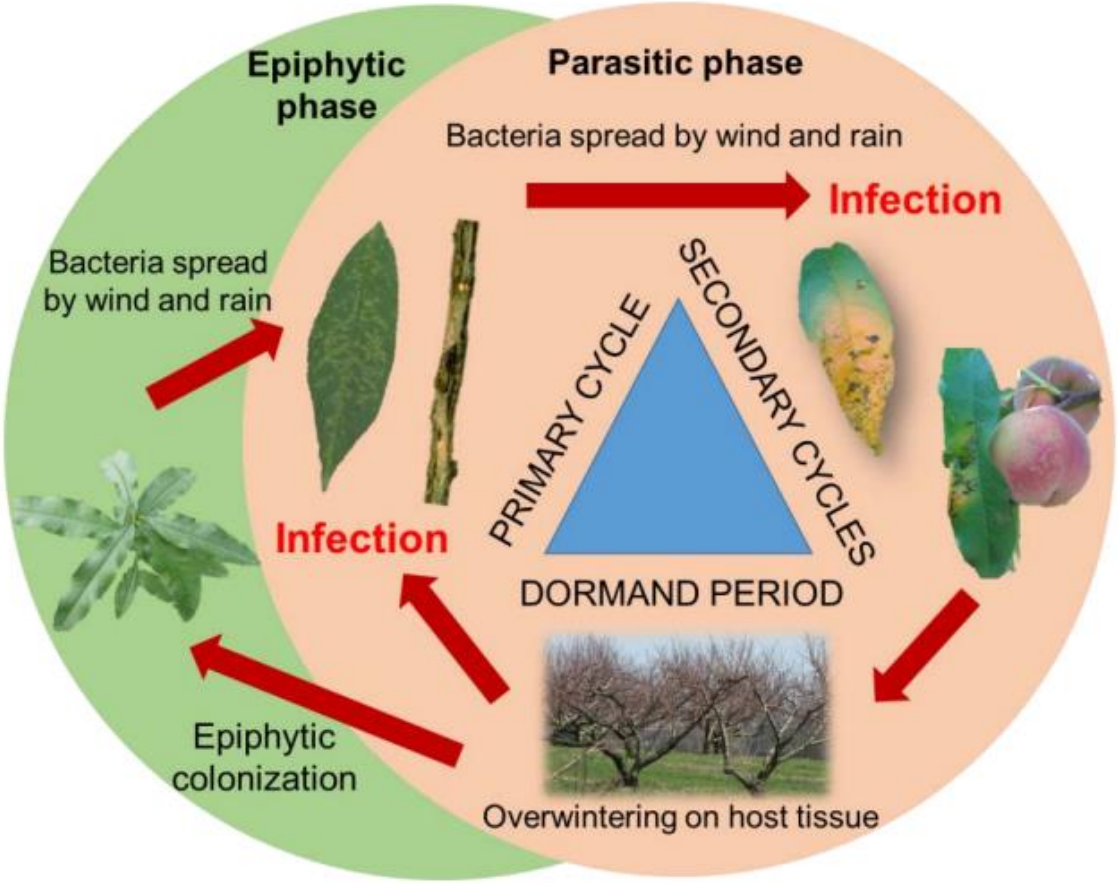
FIGURE 12-18 Symptoms of bacterial spot caused by *Xanthomonas arboricola* pv. *pruni*. (A) Tiny spots and holes on peach leaves. (B) Numerous small, halo-surrounded spots that later turn brown appear on infected fruit. (C) Spots on plum leaf. (D) Large coalescing spots on fruit of susceptible plum variety. [Photographs courtesy of M. Ellis, Ohio State University, (B) K. D. Hickey, Pennsylvania State University, and (C and D) K. Mohan, University of Idaho.]

Agrios,2005

Bacterial spot of stone fruits

Disease cycle

Xanthomonas arboricola pv. *pruni*



Jones and Sutton,1996

Management

Forecasting system for bacterial spot of stone fruits *Xanthomonas arboricola* pv. *pruni*

- The general scheme of the forecasting system comprises three components which operate in sequence, corresponding to these three essential processes:

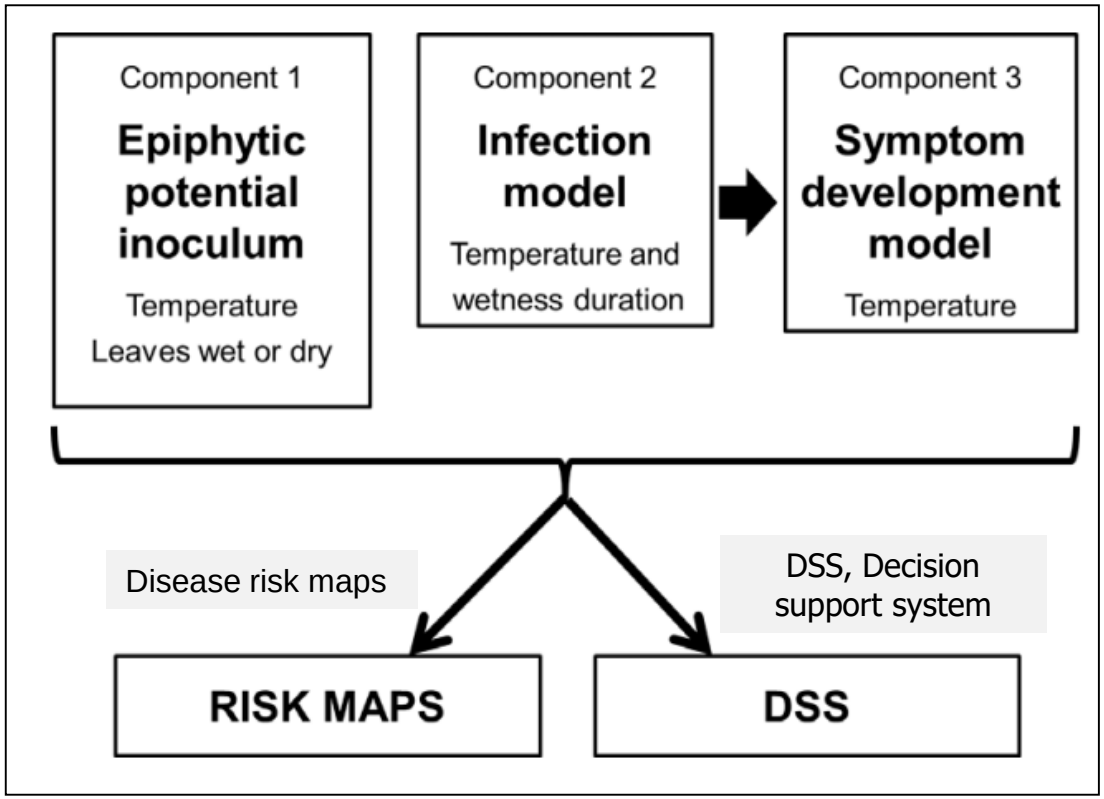
1. the epiphytic inoculum potential,
2. the infection model, and
3. the disease symptom development model.

Morales *et al.*,2017

Management

Forecasting system

Xanthomonas arboricola pv. *pruni*



Conceptualization of the forecasting system for bacterial spot of stone fruit caused by *X. arboricola* pv. *pruni*.

Morales *et al.*,2017

Management

Forecasting system for bacterial spot of stone fruits *Xanthomonas arboricola* pv. *pruni*

- Detached leave assay:
- Disease progress was monitored over time in *Prunus* detached leaves inoculated with suspensions of *X. arboricola* pv. *pruni* at three inoculum densities (10^4, 10^6 and 10^8 CFU/ml), and incubated at constant temperatures from 10 to 35ºC.

1. High final disease severity and short incubation periods were observed at optimal temperatures (20-30ºC) and high bacterial population size (10^6 and 10^8 CFU/ml).

Morales *et al.*,2017

Management

Forecasting system for bacterial spot of stone fruits *Xanthomonas arboricola* pv. *pruni*

- Cumulative degree days (CDD) with a base temperature of 0ºC were calculated, and the CDD-disease progress curves obtained at optimal conditions were used to elaborate the model for predicting disease symptom development.

Morales *et al.*,2017

Management

Forecasting system for bacterial spot of stone fruits *Xanthomonas arboricola* pv. *pruni*

- Greenhouse conditions:
- This model was successfully validated in whole plant assays performed under greenhouse conditions.
- According to the symptom development model 150, 175 and 280 cumulative degree days (CDD) are required for obtaining disease severities of 5, 10 and 50%, respectively.
- This study provides new knowledge on the epidemiology of the bacterial spot disease of stone fruits and offers new possibilities in the management of the disease.

Morales *et al.*,2017

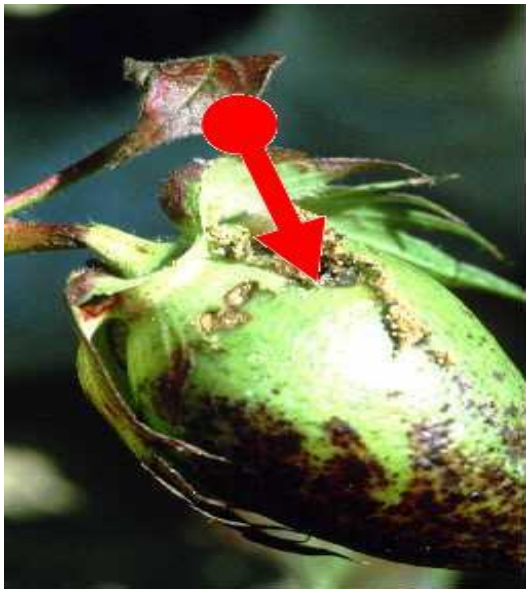
Management

Xanthomonas arboricola pv.*pruni*

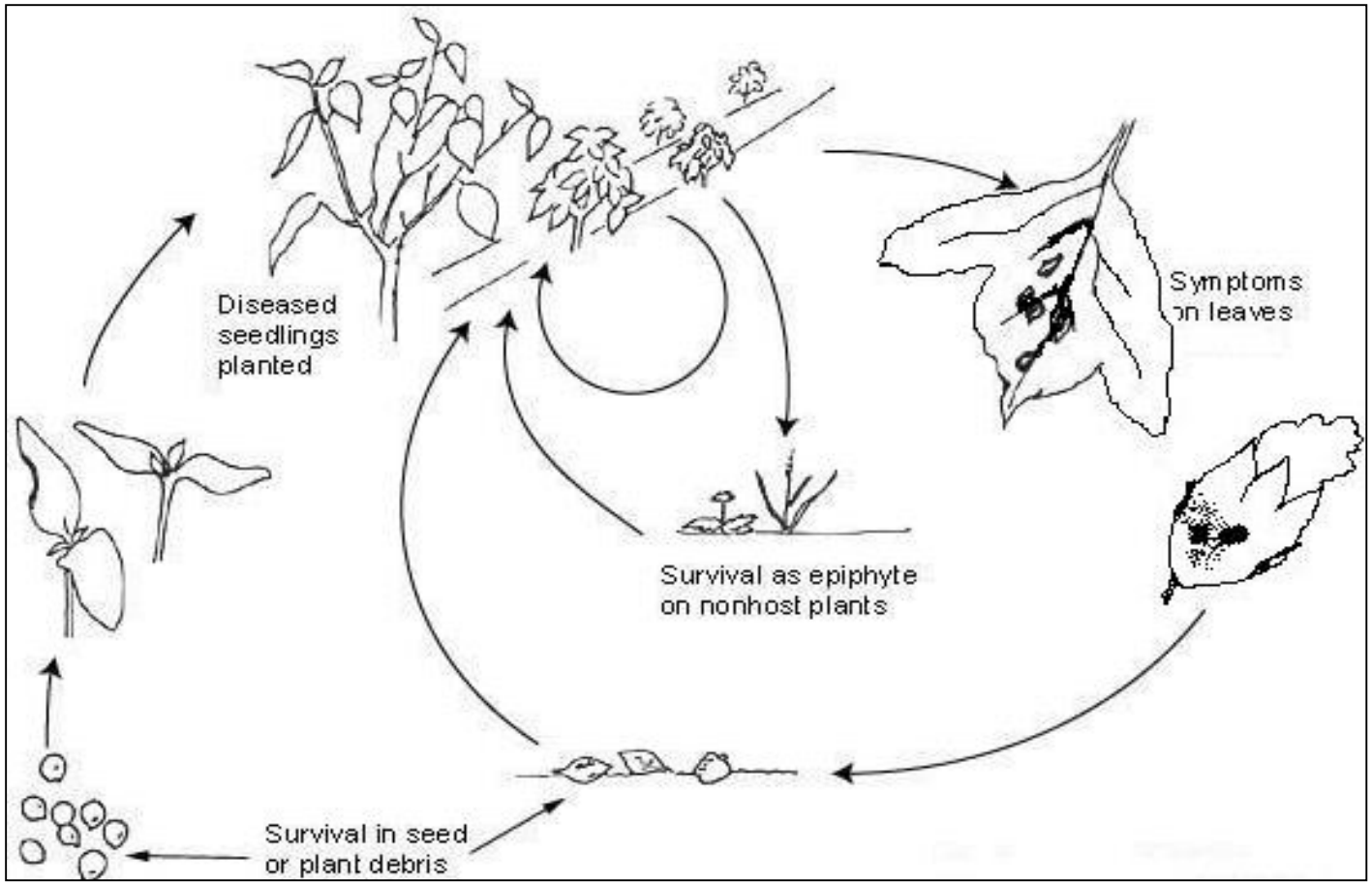
- The best way to prevent bacterial spot is through the use of resistant varieties.
- A good general program of orchard management conducive to production of vigorous trees should be followed to help reduce infection levels.
- During the dormant season, just before bud swell, the trees should be sprayed with a recommended copper fungicide formulation like:
- Kocide 101, Kocide 404, Tri-Basic Copper Sulfate or a Bordeaux preparation.
- At shuck-split, the trees should be sprayed again.
- Also, beginning at shuck-split, an alternative is to spray weekly with a terramycin formulation (Myco Shield).

Xanthomonas axonopodis pv. *malvacearum*

Bacterial blight of cotton



Bacterial life cycle



Innes,1983

Cultivar/race specificity

Xanthomonas axonopodis pv.*malvacearum*

- Strains of *Xanthomonas axonopodis* pv. *malvacearum* that cause bacterial blight of cotton (*Gossypium* spp.) exhibit gene-for-gene (also known as cultivar or race) specificity.
- There are at least 19 well-described races of the pathogen.
- At least 16 different resistance (*R*) genes identified.
- Different *Xanthomonas axonopodis* pv.*malvacearum* races contain different combinations of avirulence (*avr*) genes that define the race and determine cultivar specificity.
- Host cultivars containing *R* genes respond to avirulent races with a hypersensitive response (HR).

Cultivar/race specificity

Xanthomonas axonopodis pv.*malvacearum*

- Bacterial angular leaf spot caused by *Xanthomonas axonopodis pv. malvacearum* (*Xam*) has become an increasing impediment to cotton production worldwide.
- It is generally accepted that resistant cotton varieties are the most effective control approach to this disease.
- Genetic resources of *Gossypium* spp. differ in their resistance to *Xam* ranging from susceptible to highly resistant.
- *Gossypium hirsutum* L. was reported to be one of the best sources of resistance to *Xam*.
- On the other hand, *Xam* was also reported to have a wide range of virulence (Verma, 1986).
- Using ten differential cotton cultivars, Hunter *et al.*,1968 identified 19 physiological races.

Abdo-Hasan *et al.*,2008

Cultivar/race specificity

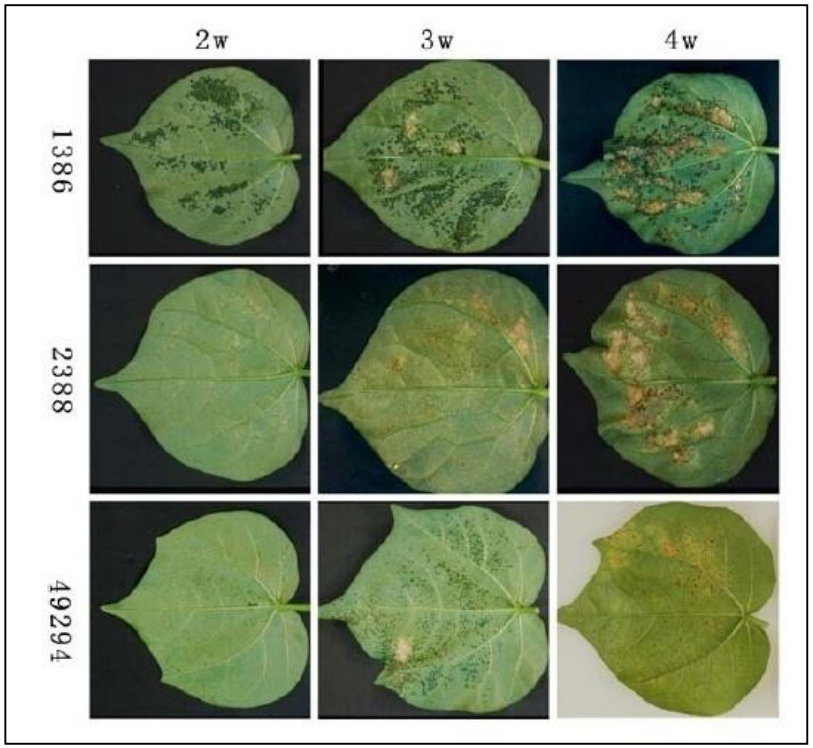
Xanthomonas axonopodis pv.*malvacearum*

- Later studies carried out by Verma and Singh (1974) extended the number to 32 physiological races using only seven cotton cultivars.
- The distribution of these races differs in different countries.
- Race 1 is widespread in Australia, India and USA.
- Whereas races 2 to 5 were recorded in USA and India, race 6 in Nigeria, Zymbawe and India, and race 18 in Australia, USA, Africa, India and Nicaragua.
- Six races of *Xam* (namely 1, 2, 8, 21, 26 and 32) were identified in Syria (Abdo-Hasan, 2002) and four additional races (namely 3, 4, 11, 28) were recorded between 2003 and 2006.

Abdo-Hasan *et al.*,2008;..

Identification of a highly virulent strain of *Xanthomonas malvacearum*

- Comparison of the symptoms caused by race 18 (GSPB 1386),and race 20 (GSPB 2388, ATCC 49294) of *X. axonopodis* pv. *malvacearum* on cotton cv. 'Acala 44'.
- Bacterial strains were suspended in inoculation buffer (0.01 M MgSO4) to a 10^5 cfu ml^{-1} concentration, and then evenly sprayed on the lower side of the first two not fully-grown young leaves (1/3 size of a mature leaf) following the cotyledons as described (Klement *et al.*,1990).



Huang *et al.*,2008

Management

- Infested fields should be harvested as soon as possible.
- Fields that have bacterial blight this year should be planted to a blight-resistant variety next year or rotated to a different crop.
- **Preventative Actions for Bacterial Blight of Cotton:**
  - Plant high-quality, disease free, acid delinted seed.
  - Plant blight-resistant varieties if available.
  - Scout fields and identify infected plants and varieties.
  - Shred stalks and incorporate cotton debris.
  - Do not cultivate or move equipment through fields when foliage is wet.

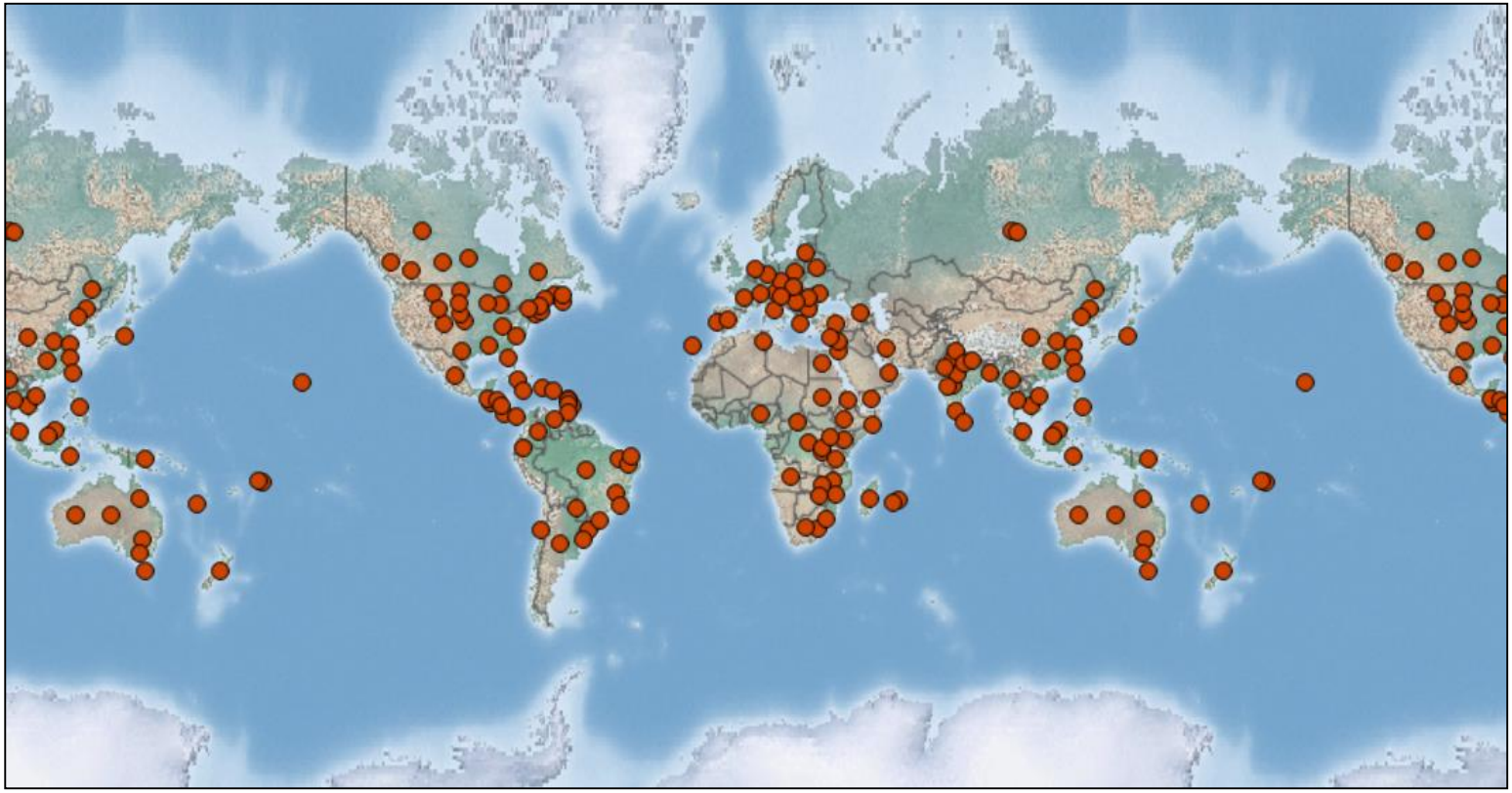
Xanthomonas axonopodis pv. *phaseoli*

Common blight of bean

Common blight of bean

Distribution Maps

Xanthomonas axonopodis pv. *phaseoli*



CABI,2019

Hosts/Species Affected

Xanthomonas axonopodis pv. *phaseoli*

- Many Phaseolus species have been reported as hosts of the seed-borne bacteria *X. axonopodis* pv. *phaseoli*.
- Races of *X. axonopodis* pv. *phaseoli* have been reported on *Phaseolus acutifolius*, *P. calcaratus* and *P. aureus*.
- *P. vulgaris* genotypes have been reported to permit differentiation of races of *X. axonopodis* pv. *Phaseoli* (Zapata,1997).
- *X. axonopodis* pv. *phaseoli*, *X. axonopodis* pv. *alfalfae*, *X. axonopodis pv. glycines* and *X. axonopodis* pv. *vignicola* have all been reported to be pathogenic on common beans (Sabet,1959) and there may be a continuum in host specificity among strains pathogenic to legumes.

CABI,2019

Management

Xanthomonas axonopodis pv. *phaseoli*

Seed treatments

- Seed infection by *X. axonopodis* pv. *phaseoli* can be external or internal.
- Thermotherapy is widely applied for the control of seedborne bacteria (Gondreau and Samson, 1994).
- Both hot water and dry heat have been successful in treating bean seeds for *X. axonopodis* pv. *Phaseoli* (Gondreau and Samson, 1994).
- This involves either incubating for 20 minutes in 52°C water or 23-32 hours in 60°C dry air at 45-55% RH. The latter treatment does not appear to affect seed viability.
- Treatment with an antibiotic such as streptomycin may be used to control external contamination with *X. axonopodis* pv. *phaseoli*, and streptomycin in polyethylene glycol may reduce, but not eliminate, internal populations of *X. axonopodis* pv. *phaseoli*(Liang *et al.*,1992).

CABI,2019

Management

Xanthomonas axonopodis pv. *phaseoli*

Seed treatments

- Chemical control may reduce leaf infection but usually has little improvement on yield.
- Copper compounds may be used (Weller and Saettler, 1976).
- Foliar antibiotic treatment can provide some control but is undesirable because it can result in antibiotic-resistant mutants of *X. axonopodis* pv. *phaseoli*.

Management

Xanthomonas axonopodis pv. *phaseoli*

Biological control by *Rahnella aquatilis* (Ra)

- The effect of the bacterium *Rahnella aquatilis* (Ra) against common blight of bean plant was tested.
- In vitro studies, Ra exhibited inhibitor effect against the pathogen.
- Under greenhouse and field conditions, beanvariety "Giza 6" treated by Ra resulted in marked disease suppression.
- A high decrease of the disease was correlated with a reduction of the bacterial multiplication.
- In physiological studies, bean plants treated by Ra exhibited higher phenolic compounds contents and higher activity of peroxidase (PO) enzyme than untreated plants.
- In conclusion, application of Ra was effective and could be recommended for controlling the bean common blight disease.

Sallam,2011

Management

Xanthomonas axonopodis pv. *phaseoli*

Biological control by *Rahnella aquatilis* (Ra)

- Population of *X. axonopodis* pv. *phaseoli* in shoots of bean plants after treatment with *R. aquatilis* (Ra) (10^6).

Days after application	Treatments		% reduction
	Ra	Control	
7	2 b	3.1 b	35.5
15	3.2 a	5.30 a	39.6

Note: Values in the column followed by different letters indicate significant differences among treatments according to LSD test at 0.05.

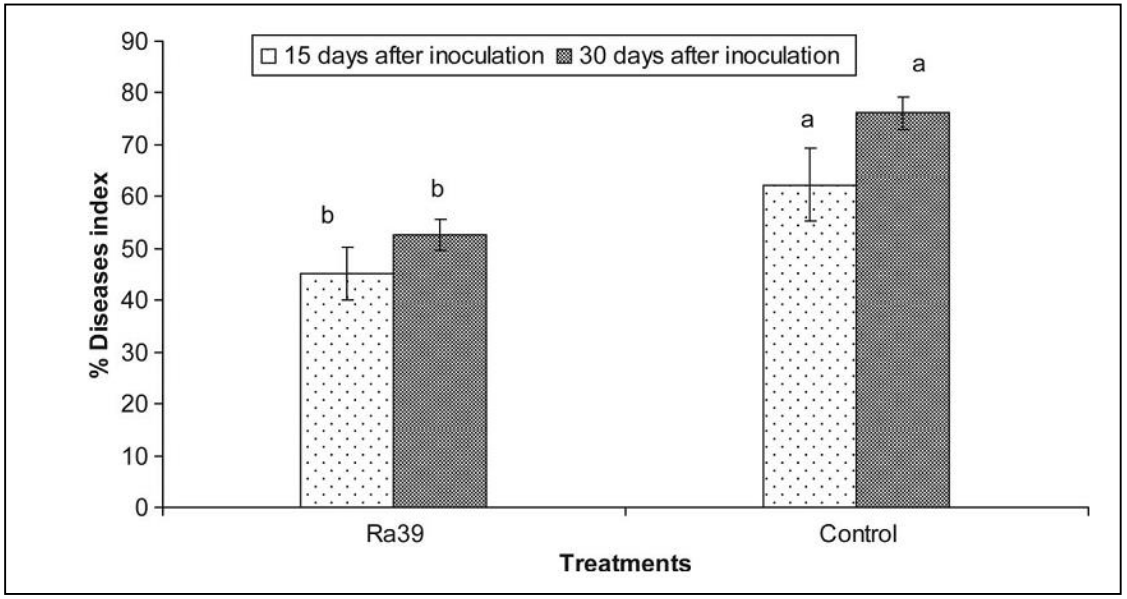
Sallam,2011

Management

Xanthomonas axonopodis pv. *phaseoli*

Biological control by *Rahnella aquatilis* (Ra)

- Disease index of common blight disease on bean plants after treatments with Ra under the greenhouse conditions.



Note: Different letters indicate significant differences among treatments within the same column according to LSD test (P= 0.05).

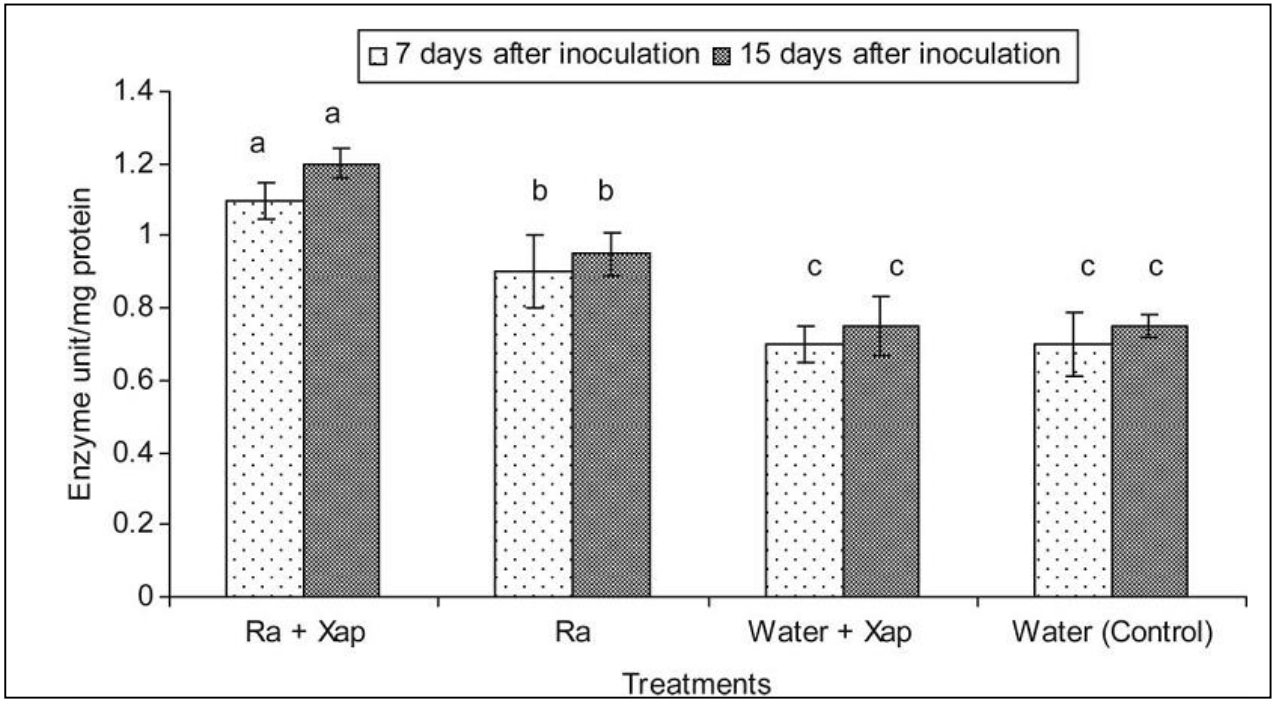
Sallam,2011

Management

Xanthomonas axonopodis pv. *phaseoli*

Biological control by *Rahnella aquatilis* (Ra)

- Effect of Ra on PO activity in bean plants.



Note: Different letters indicate significant differences among treatments within the same column according to LSD test (P=0.05).

Sallam,2011

Management

Xanthomonas axonopodis pv. *phaseoli*

Biological control by bacterial biocontrol agents (BCAs)

- The bacterial biocontrol agents (BCAs) used selected from previous study on the control of *Xanthomonas axonopodis* pv. *phaseoli* Xap) includes: *Bacillus* (DFs093, DFs348 and DFs769), *Pseudomonas* (DFs513, DFs831 and DFs842), *Rhodococcus* (DFs843 and DFs912), and the combinations C01 (DFs093+DFs769+DFs831), C02 (DFs093+DFs769+DFs842) and C03 (DFs093+DFs769+DFs348).
- The symptom development was followed for 10 days.
- The treatments were compared by the area under the disease progress curve for disease incidence, severity, and index.
- The use of combinations of these organisms increased the efficacy of the biocontrol of several strains of the same pathogen.

Obes Corrêa *et al.*,2017

Xanthomonas axonopodis pv. *punicae*

Bacterial Blight of Pomegranate

Bacterial Blight on fruit

Bacterial Blight of Pomegranate

Xanthomonas axonopodis pv. *punicae*

- Symptoms of bacterial blight on young and developing pomegranate fruits.
- Initially, spots are black and round and surrounded by bacterial ooze.
- Under favorable conditions, spots enlarge to become raised, dark brown lesions with indefinite margins that cause the fruit to crack.
- The disease may cause up to 90% yield reduction.

Benagi, APS,2014

Management

- Ravi kumar *et al*.,2006 reported that sprays with streptocycline (500 ppm) + copper oxy chloride (2000 ppm) (33.3%) when compared with control (78.5%) after 8th spray.
- The maximum mean yield of 9.3tons/ha was recorded in streptocycline (500 ppm)+ copper oxy chloride(2000 ppm) followed by 8.50 tons/ha in bromopal (500 ppm)+copper oxy chloride (2000 ppm) the untreated check yielded 2.95 tons/ha.
- Manjula *et al*.,2003 reported that paushamycin (500 ppm), sterptocycline (500 ppm) and K-cycline (500 ppm) were very effective in controlling the bacterial blight of pomegranate.
- Pomegranate crop should be taken only after 3-4 years after planting; pruning should be done in August to September in order to get lesser severity and with less number of sprays.

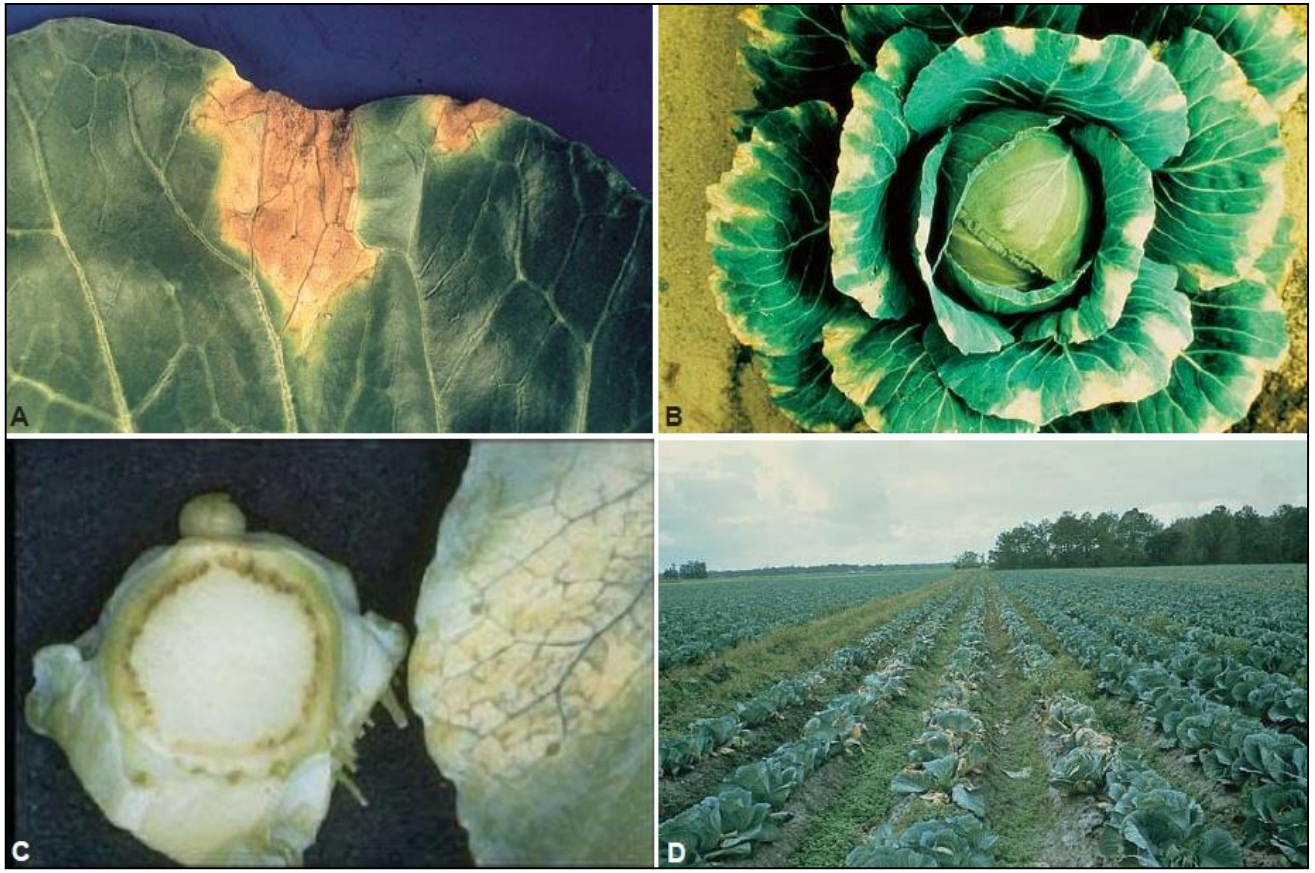
Gamanagatti,2009

Xanthomonas campestris pv.*campestris*

Bacterial wilt or black rot of cabbage

V-shaped lesions

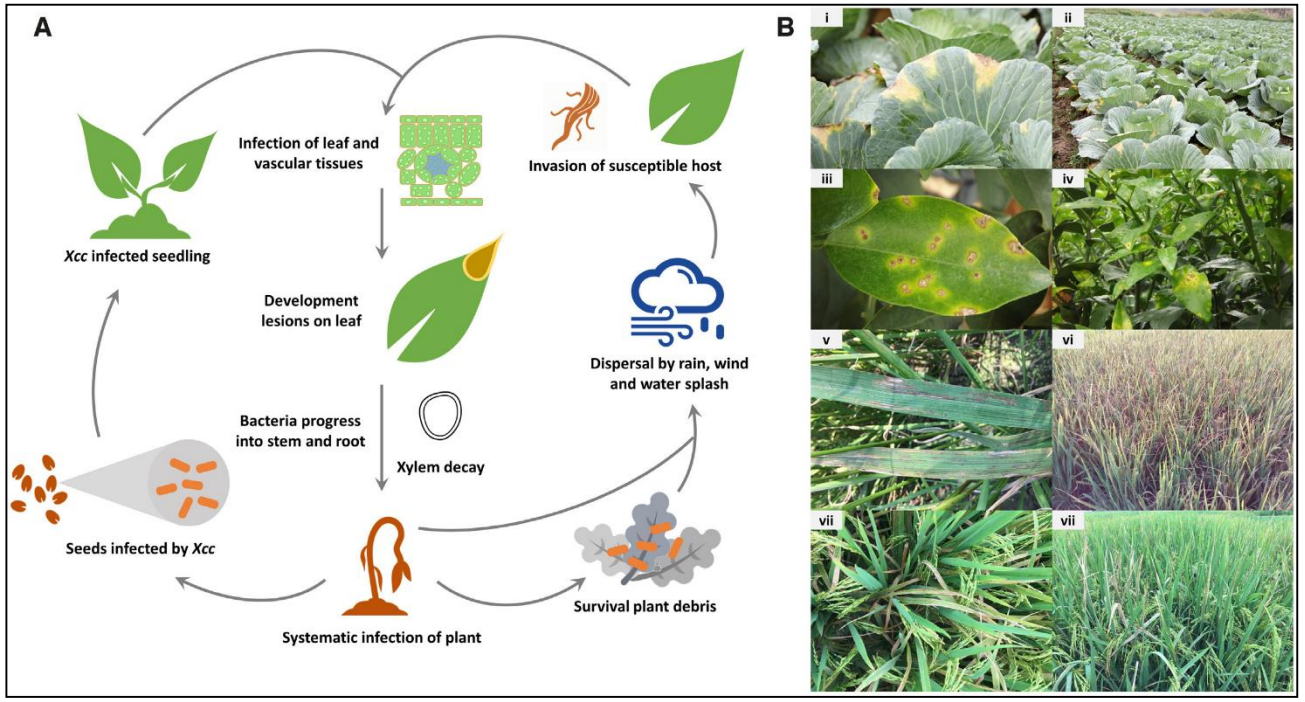
Bacterial wilt or black rot of cabbage caused by *Xanthomonas campestris* pv. *campestris*



Agrios,2005

Bacterial wilt or black rot of cabbage caused by *Xanthomonas campestris* pv. *campestris*

- Life cycle and disease symptoms of *Xanthomonas.* (A), Model illustrating the life cycle of the black rot pathogen *Xanthomonas campestris* pv. *campestris* (Xcc).



An *et al.*,2019

Management
Xanthomonas of Brassicas

- Select seed that has been certified as disease-free.
- Treat seed with hot water to eradicate the bacteria. Treat seed for 15-30 minutes at 50°C, dry, and test for germination. This process must be done carefully and it is recommended that a small sample of seed be tested for the effect on germination first.
- Fumigate or steam sterilize soil in seedbeds and use clean, sterilized seed flats.
- Locate seedbeds where cruciferous crops have not been grown for 4 years and avoid areas that receive run-off from areas previously planted to crucifers.
- Avoid dense seeding rates which can prolong periods of leaf infection and favor pathogen spread.
- Monitor transplants and promptly remove and destroy infected seedlings.
- Do not trim seedlings as the bacteria are easily spread by contaminated tools.
- Practice a three year rotation and control cruciferous weeds.
- Do not work fields when they are wet and avoid overhead irrigation .
- Do not locate cull piles near fields or storage areas.
- Promptly incorporate crop residues after harvest to speed decomposition.

Management
Xanthomonas of Brassicas

- **Chemical recommendations:**
- **Acibenzolar-S-methyl (Actigard 50 WG) :** 1 oz/A (7 dh, REI 12h). Suppression only. Apply preventively in sufficient water to ensure adequate coverage. Do not apply Actigard to plants that are stressed by drought, excessive moisture, herbicide injury, etc.
- **Cupric hydroxide (Kocide 4.5LF) :** 0.6 to 1.3 pt/A (0 dh, REI 24h). Apply as soon as disease appears on a 7-10 day schedule. Tank mixes with Maneb or manex may improve disease control, although not all crucifers are on the Maneb or manex label. Do not apply in a spray solution of less than 6.5 as phytotoxicity may occur.
- **Cuprous oxide (Nordox 75WG):** 3.2 to 2 lb/A (0 dh, REI 24h). Apply as soon as disease appears on a 7-10 day schedule.

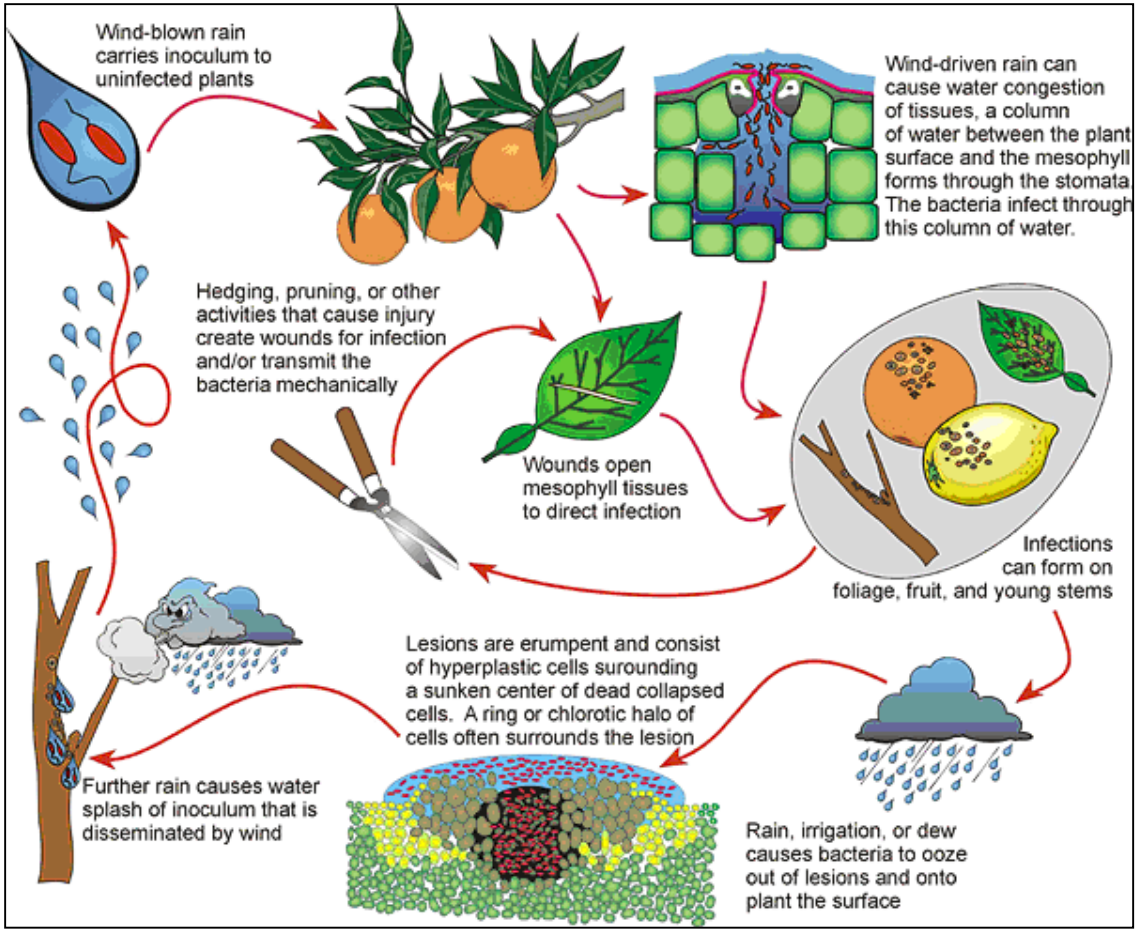
Xanthomonas citri

Bacterial citrus canker

A mesophyll-limited pathogen of citrus

Citrus canker disease cycle

Xanthomonas citri



Gottwald *et al.*,2002

Disease cycle of citrus bacterial canker

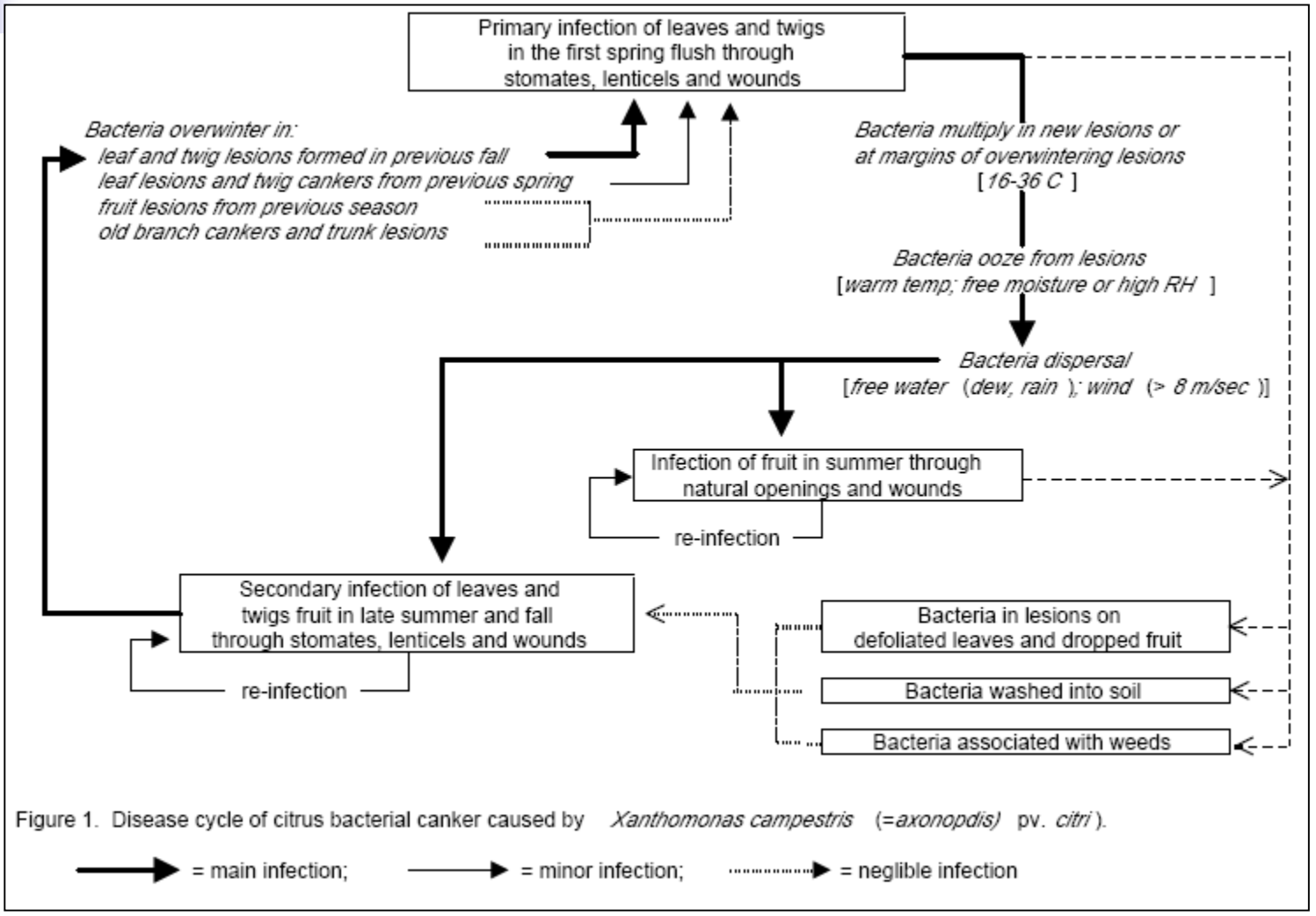
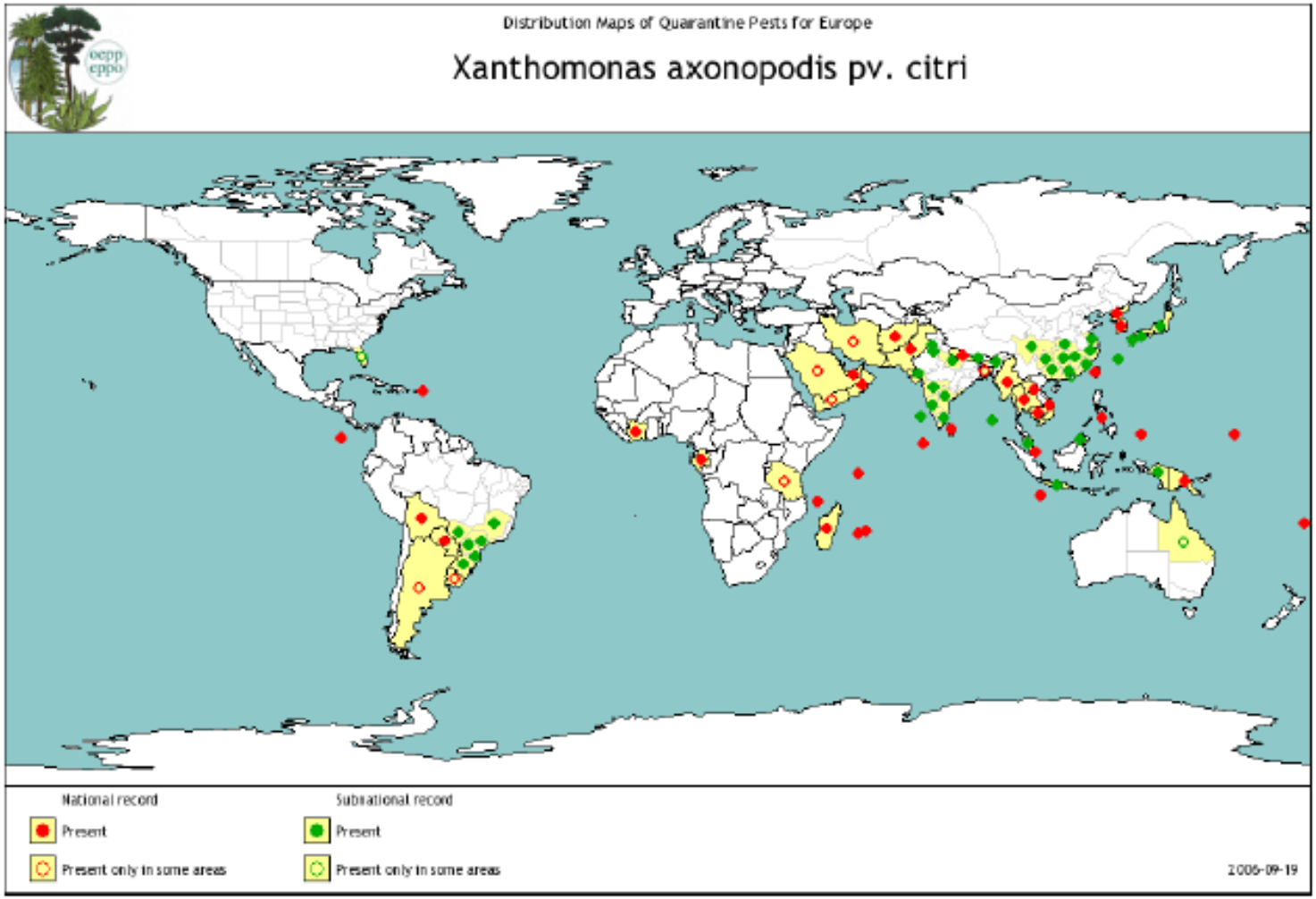


Figure 1. Disease cycle of citrus bacterial canker caused by *Xanthomonas campestris* (=*axonopdis*) pv. *citri*).

= main infection; = minor infection; = neglible infection

Distribution map

Xanthomonas citri



Crop loss

- Severe infections cause defoliation, blemished fruit, premature fruit drop, die-back of twigs and general debilitation of the tree.

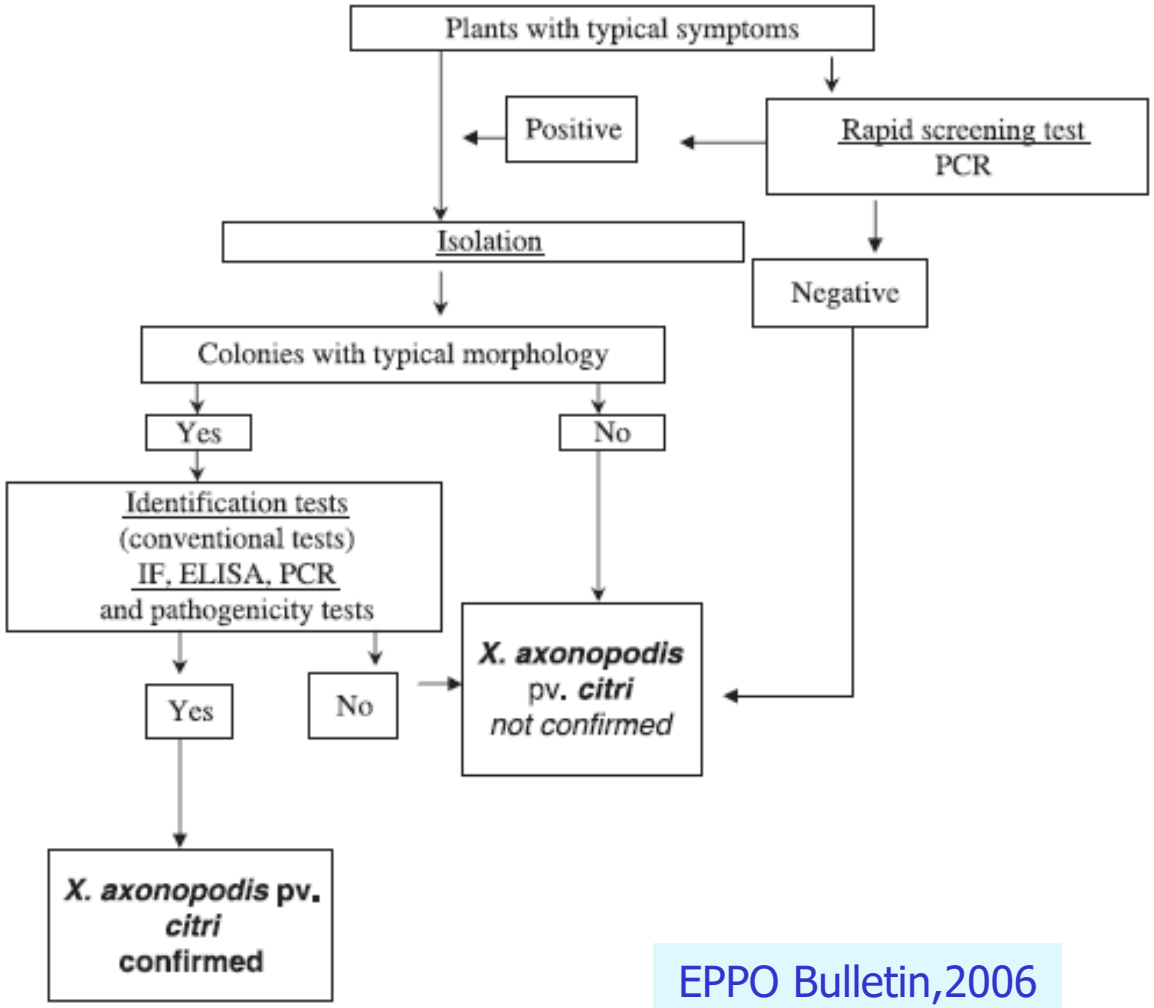
Susceptibility of several citrus varieties and rootstocks to *Xanthomonas axonopodis* pv. *citri*

Highly Susceptible	Moderately Susceptible
1. *Citrus paradisi* Macf., grapefruit 2. *C. aurantifolia* (Christ.) Swingle, acid lime 3. *C. limettioides* Tan., Palestine sweet lime 4. *Poncirus trifoliata* (L.) Raf., trifoliate orange	1. *C. sinensis* (L.) Osbeck, sweet orange 2. *C. aurantium* L., sour orange 3. *C. limon* (L.) Burm., lemon 4. *C. tangelo* J. Ingram & H.E. Moore, tangelo

Moderately Resistant	Highly Resistant
1. *C. reticulata* Blanco, mandarin, tangerine 2. *C. maxima* (Burm.) Merr., pummelo 3. *C. aurantifolia* (Christ.) Swingle, Person or Tahiti lime	1. *C. medica* L., citron 2. *Citrofortunella microcarpa* (Bunge) Wijnands, calamondin 3. *Fortunella* spp., kumquat

Das,2003

Flow diagram for the diagnosis of *Xanthomonas axonopodis* pv. *citri* on symptomatic samples



EPPO Bulletin,2006

Groups of citrus canker-causing bacteria
Strain types

- *Xac*- A, Group "A" strains of *X. axonopodis* pv. *citri* or Canker A (The Asiatic type of canker) originally found in Asia, by far the most widespread and severe form of the disease.
- *Xac*-A* a variant of *X. axonopodis* pv. *citri* pathotype-A with pathogenicity limited to Mexican lime.
- *Xac* -A^W isolated from Key/Mexican lime and more closely related to *Xac*- A & *Xac* -A*.
- Cancrosis B, or false canker (formerly known as B- strain canker) a disease of lemons, Mexican lime, sour orange, and pummelo.
- Key/Mexican lime cancrosis (formerly known as C strain canker).
- Canker D, was reported in 1981 on Mexican lime in Mexico, but its identification remains controversial.
- Citrus bacterial spot (formerly known as Florida nursery strain citrus canker or E strain canker).
- All these five groups can be differentiated from each other by different biochemical, ELISA, PCR-based assays, bacteriophage sensitivity and host specificity.

Sun *et al.*,2004

Groups of citrus canker-causing bacteria
Co-existence of the variant of *X. axonopodis* pv. *citri* pathotypes

- Co-existence of the variant of *X. axonopodis* pv. *citri* pathotypes such as two *Xcc* pathotypes A and A*could further favour the generation of new genetic variants through recombination and horizontal genetic exchange.
- This in turn, led to the generation of new aggressive pathotypes in citrus plantation areas.

Pathotype: An infrasubspecific classification of a pathogen distinguished from others of the species by its pathogenicity on a specific host(s).

Citrus canker/Citrus bacterial spot diseases

- **Citrus canker disease:**
- *X. axonopodis* pv. *citri* (proposed name *X. citri* pv. *citri*) would be considered a host specific disease caused by strains containing pthA pathogenicity genes that are differentially adapted to *Citrus* spp.
- **Citrus bacterial spot disease:**
- *X. axonopodis* pv. *citri* strain type E which later the bacterium reclassified as *X. axonopodis* pv. *citrumelo* (proposed name *X. alfalfae* subsp. *citrumelonis*) is a disease caused by a diversity of strains with differential aggressiveness that represent a heterogeneous group, while the citrus canker group displays a higher homology among its members.

Canker A vs. Citrus bacterial spot lesions

Severe citrus canker infection on Key lime.

Citrus bacterial spot lesions on grapefruit leaves.

Botond Balogh,2006

Management
Xanthomonas citri

- **These programs rely on:**
- Planting resistant citrus cultivars;
- Production of disease-free nursery stock by locating nurseries outside of citrus canker areas and/or indoors;
- Restricting disease spread by:
- establishing windbreaks and fences around groves,
- using preventative copper bactericides and by controlling Asian leafminer.

See also BLO section

Eradication

Strict regulations (zero tolerance)

- Citrus canker, caused by *X. campestris* pv. *citri*, is regarded as a sufficiently serious disease for 20 million trees to have been destroyed in the citrus groves of Florida in an effort to eliminate it (Schoulties *et al.*,1987).
- In March 2006 APHIS (Animal and Plant Health Inspection Service) released a Citrus Health Response Plan, which outlined procedures for managing citrus production in the permanent presence of the disease.

Eradication

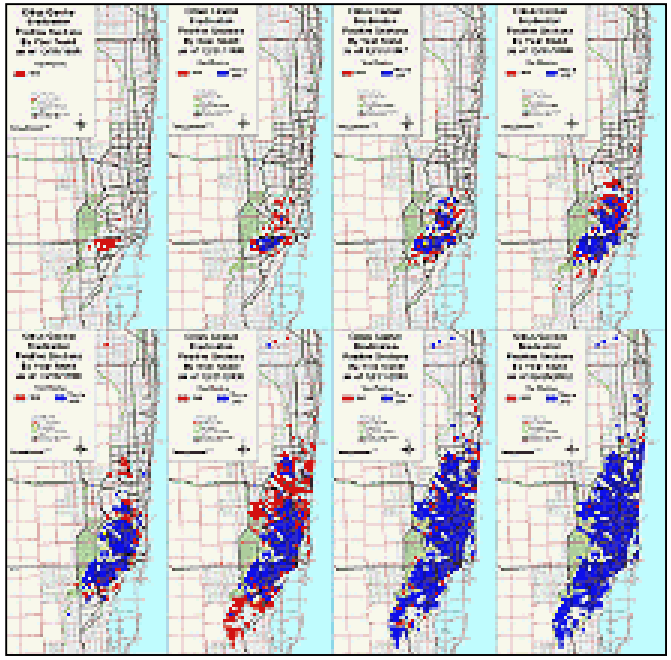
Strict regulations (zero tolerance)

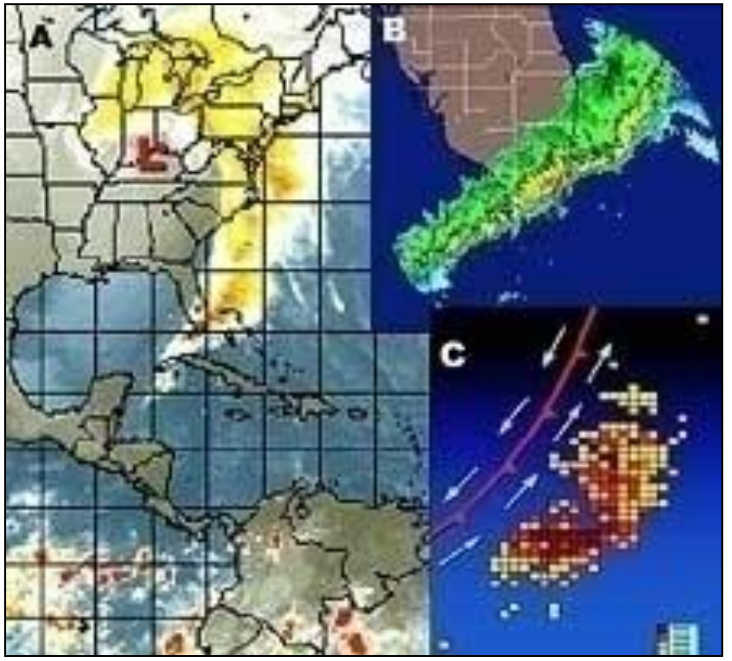
- Most citrus-producing countries free of the disease have strict regulations (zero tolerance) and do not allow importation of fruit or plant materials unless they have passed inspection.
- Considerable effort has been made to eradicate citrus canker from Florida and success has been declared three times, in 1933, 1947, and 1994 (Stackebrandt & Goebel,1994).
- The current eradication effort began in 1998 at a current cost of over 100 million US dollars (T.R. Gottwald *et al.*,2001).

Eradication

Infected area continues to increase

- Extensive eradication efforts in Florida.
- Removal or cutting back of >1.56 million commercial trees and nearly 600,000 yard trees.





Management
Citrus Canker Eradication Program

Typical airblast sprayer in citrus planting used to apply agrochemicals such as copper for control of citrus canker.

Dooryard citrus tree being chipped by mechanical mulcher used to dispose of diseased and exposed citrus trees in residential areas.

Pile of uprooted diseased and exposed citrus trees being burned to eliminate citrus canker from commercial planting.

Front end loader uprooting citrus canker infected trees in commercial grove in Martin County, Florida.

Gottwald *et al.*,2002

Management
Citrus Canker Eradication Program

EQUIPMENT DECONTAMINATION

All equipment used during the control process must be decontaminated:

Dump trucks

Chippers

Stump grinders

Hand Tools

PERSONNEL DECONTAMINATION

Personnel must decontaminate all areas that come in contact with citrus material.

Government outlay costs

The number of trees per square mile was calculated as

$$\frac{trees}{square\ mile} = \frac{trees}{backyard} * \frac{backyards}{square\ mile}$$

Table 8. Government Costs to Eradication Citrus Canker from California

Initial Infestation	Eradication Boundary (feet)	Urban Square miles	Comm. Acreage	Costs Urban	Comm.	Calif. ($000)	U.S.	Total
Small	1900	27	100	3,192	364	1,632	1,924	3,556
	3000	31	100	3,665	364	1,868	2,160	4,028
Large	1900	432	3000	51,577	10,068	26,448	35,196	61,645
	3000	447	3000	53,426	10,068	27,373	36,121	63,494

Table 2. Minimum Eradication Costs for Citrus Canker in an Urban Setting*

Radius (feet)	Square miles	Number of Trees	Costs with no compensation ($)	Costs with Compensation Voucher ($20/tree)	Appraised Value ($400/tree)
125	0.002	1	60	80	460
1,900	0.41	199	11,966	15,955	91,742
3,000	1	497	29,833	39,777	228,720
5,000	3	1,400	83,000	110,000	635,000
15,000	25	12,400	746,000	994,000	5,718,000
20,000	45	22,100	1,326,000	1,768,000	10,165,000

*Monitoring and inspection costs are $90,000 per square mile.

USDA, Washington DC,2000

Controlling Asian leafminer

Asian leafminer (*Phyllocnistis citrella*) tunnels on Swingle citrumelo foliage.

Citrus canker infection in Asian leafminer tunnels.

Botond Balogh,2006

Windbreaks outline an orange grove in Argentina

Management
Contact bactericides

1. **Copper-based bactericides**

- A standard control measure for citrus canker world-wide.
- Copper reduces bacterial populations on leaf surfaces, and multiple applications are needed to achieve adequate control on susceptible hosts.
- Copper-based spray programmes are effective when targeted to the spring leaf flush to protect leaves from the one-half to full expansion stage over a period of 2-4 weeks.
- Fruit are susceptible as they grow from 2.0 to 6.0 mm in diameter for a period of 90-120 days, depending on citrus species (Graham *et al.*,1992b).
- When the incidence of canker infection on spring leaves is reduced, the subsequent infection of fruit is reduced, provided that the treatments are repeated during the summer months as the fruit continue to expand.

Management
Contact bactericides

1. **Copper-based bactericides**

- Since copper diminishes infection by contact effect on bacteria on surfaces, the effectiveness of copper spray programmes is overcome by rains with wind that introduce bacteria directly into stomates.
- In addition to their partial effectiveness under windblown rain conditions, copper bactericides have other possible disadvantages after long-term use, including:

1. Resistance to copper in xanthomonad populations (Rinaldi and Leite, 2000), and
2. The accumulation of copper metal in soils with potential phytotoxic and environmental effects.

Management
Contact bactericides

2. **Other contact bactericides**

- Including antibiotics, are not as effective as copper-based products and the development of antibiotic resistance within xanthomonad populations has occurred.

Phage-based disease control

Xanthomonas citri

Bacteriophage treatment

- Phages varied in their ability to multiply, and the ones that successfully increased in populations on the bacterial host on the leaf surface also reduced disease severity, whereas the ones that were unable to multiply in the target environment did not reduce disease severity.
- Bacteriophages reduced citrus canker disease severity both in greenhouse and field trials.
- The level of control was inferior to chemical control with copper bactericides.
- The combination of bacteriophage and copper treatments did not result in increased control.
- The combination of phage treatment and copper-mancozeb did not prove to be advantageous.
- In summary, bacteriophages show significant promise as part of an integrated management strategy for controlling citrus canker.

Botond Balogh,2006

Field Phage therapy

Different plant pathogenic bacteria

AgriPhage™

- AgriPhage utilizes bacteriophages, known as "bacteria eaters" which are naturally occurring organisms that infect and kill only targeted bacteria.
- AgriPhage™ is the first agricultural bactericide based on phage technology, and is currently registered for use on:

1. Tomatoes (*Clavibacter michiganensis* and *Pseudomonas syringae* pv. *tomato*),
2. Peppers (*Xanthomonas campestris* pv. *vesicatoria*),
3. Apples and pears (*Erwinia amylovora*), and
4. Citrus (*Xanthomonas citri*).

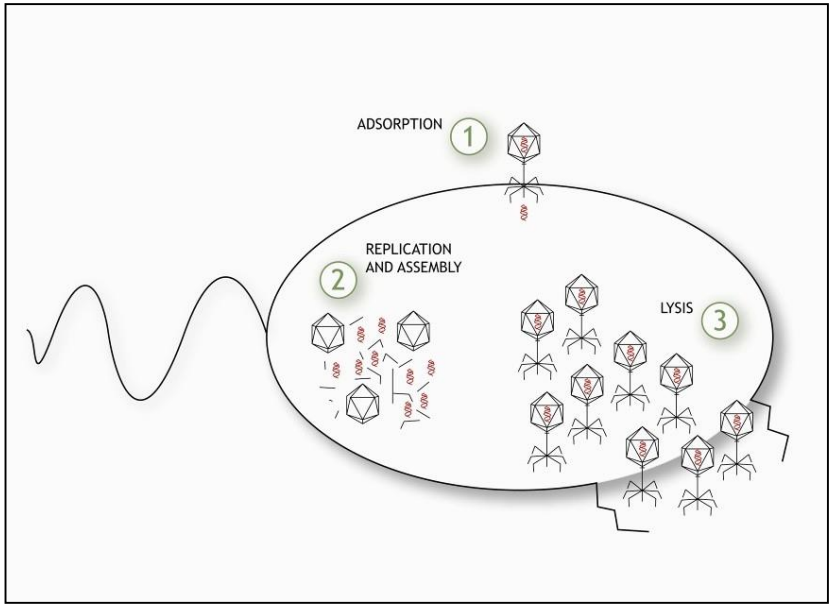
AgriPhage,2022

Field Phage therapy

Different plant pathogenic bacteria

AgriPhage™

- **HOW IT WORKS**
- Bacteriophage destroy bacteria in a process called "lysis".
- Lysing begins the moment a phage comes in contact with a bacterium and results in a release of additional phage within 30 minutes.



AgriPhage,2022

Field Phage therapy
Different plant pathogenic bacteria
AgriPhage™

- **HOW IT WORKS**
- Lysing can be described in three basic phases:

1. Adsorption occurs when a phage encounters a bacterium, attaches its tail fibers, and injects its own DNA into the bacteria. This action can begin as soon as you apply AgriPhage-CMM.
2. New phage can begin replicating and assembling within the bacterial cell, multiplying at a steady rate.
3. Finally, lysis is complete when the cell bursts and releases about 100 new phage into the environment to carry on the process. In this way, AgriPhage-CMM actually increases in efficacy over time.

Field Phage therapy

AgriPhage™

Xanthomonas citri pv. *citri*

- Increase your crop yield and eliminate harmful bacteria using AgriPhage™.
- Citrus Canker is a highly damaging bacterial disease that can cause premature fruit drop along with spotting and damage to mature fruit rendering it unmarketable.
- The causal organism is *Xanthomonas citri* pv. *citri*.
- AgriPhage should be applied at flush to suppress the severity of the disease and to treat active lesions in young leaves.

Disease control plots by of skim milk bacteriophage formulation

Xanthomonas citri

Bacteriophage treatment

Disease control plots in Dilley and Son Nursery, Avon Park, Florida

Management

Xanthomonas citri

Effective inducers of plant resistance (ISRs)

- The compounds acibenzolar-S-methyl, a benzothiadiazole, registered as 'Actigard' in the USA and 'Bion' in Europe and South America (Syngenta Crop Protection), and harpin protein, a *hrp* gene product registered as 'Messenger' (Eden Bioscience) are marketed for the control of certain xanthomond diseases.
- ISR activity slow bacterial growth in rapidly developing leaves to complement the protectant activity of Cu.
- However, early season sprays of ISRs in combination with copper have not proved effective for the control of citrus canker in Southern Brazilian orchards wherein copper is moderately to highly effective (Graham and Leite, unpubl. data).

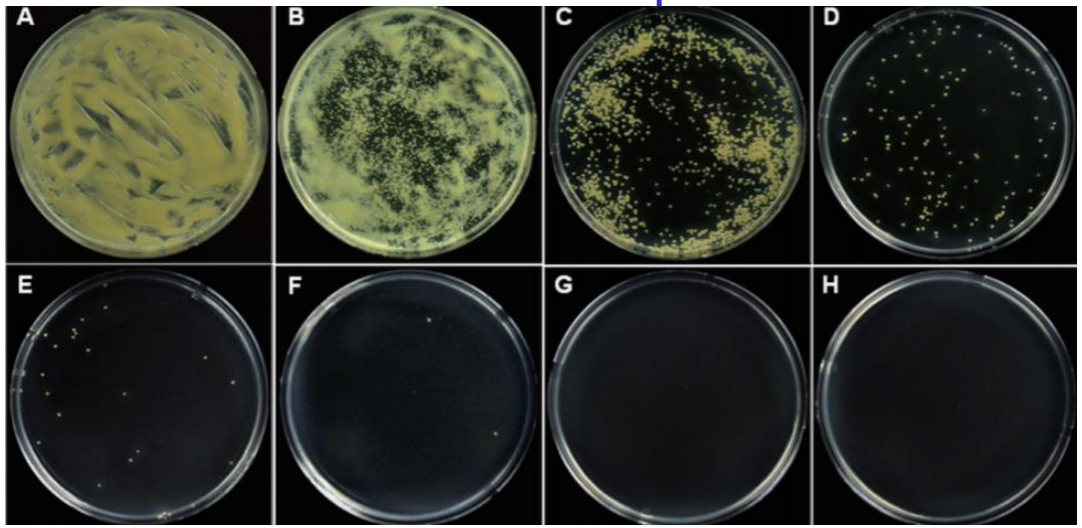
Management
X-irradiation of citrus fruits

- The fruits measuring about 60 mm of diameter were selected for the experiments.
- Fruits were washed under running water, and then dried and cleaned to remove any dirt and microorganisms.
- The fruit samples were sprayed with Xcc suspension at a concentration of 1×10^7 cfu/ml and 0.01% Tween 20 evenly until dew moist.
- After the fruits were completely dried at room temperature, the citrus fruits were exposed to X-irradiation at absorbed doses of 0, 30, 50, 100, 150, 200, 250, and 300 Gy in the same way as the Xcc suspension mentioned above.
- After irradiation, the fruits were placed in a beaker which was filled with sterile water until the fruits were submerged and shaken at 100 rpm at 28°C for 2 h.
- Then 100 µl of the washing solution was prepared by plating on the SSM and incubated at 28°C for 3 days.

Song *et al.*,2015

Management
X-irradiation of citrus fruits

- Suppression of colony formation in the suspension of Xcc on semi-selective medium. The suspension of Xcc was exposed to 0 (A), 100 (B), 150 (C), 200 (D), 250 (E), 300 (F), 350 (G), and 400 (H) Gy of X-irradiation. The plates were coated with 100 μl of the bacterial suspension and incubated at 28°C for 3 days.
- The concentration of the Xcc suspension was 1×10^7 cfu/ml.



Song *et al.*,2015

Xanthomonas euvesicatoria/ Xanthomonas vesicatoria

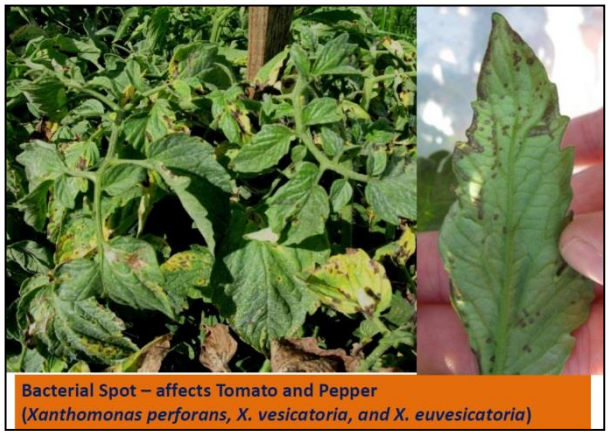
Leaf spot of pepper& tomato

Diseases caused by Xanthomonads

Bacterial spot-causing xanthomonads (BSX)

- In 2004, bacterial spot-causing xanthomonads (BSX) were reclassified into 4 species: *Xanthomonas euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri*.
- The same symptoms can be caused by several pathovars /species.
- e.g.
- Tomato/pepper spot can be caused by:

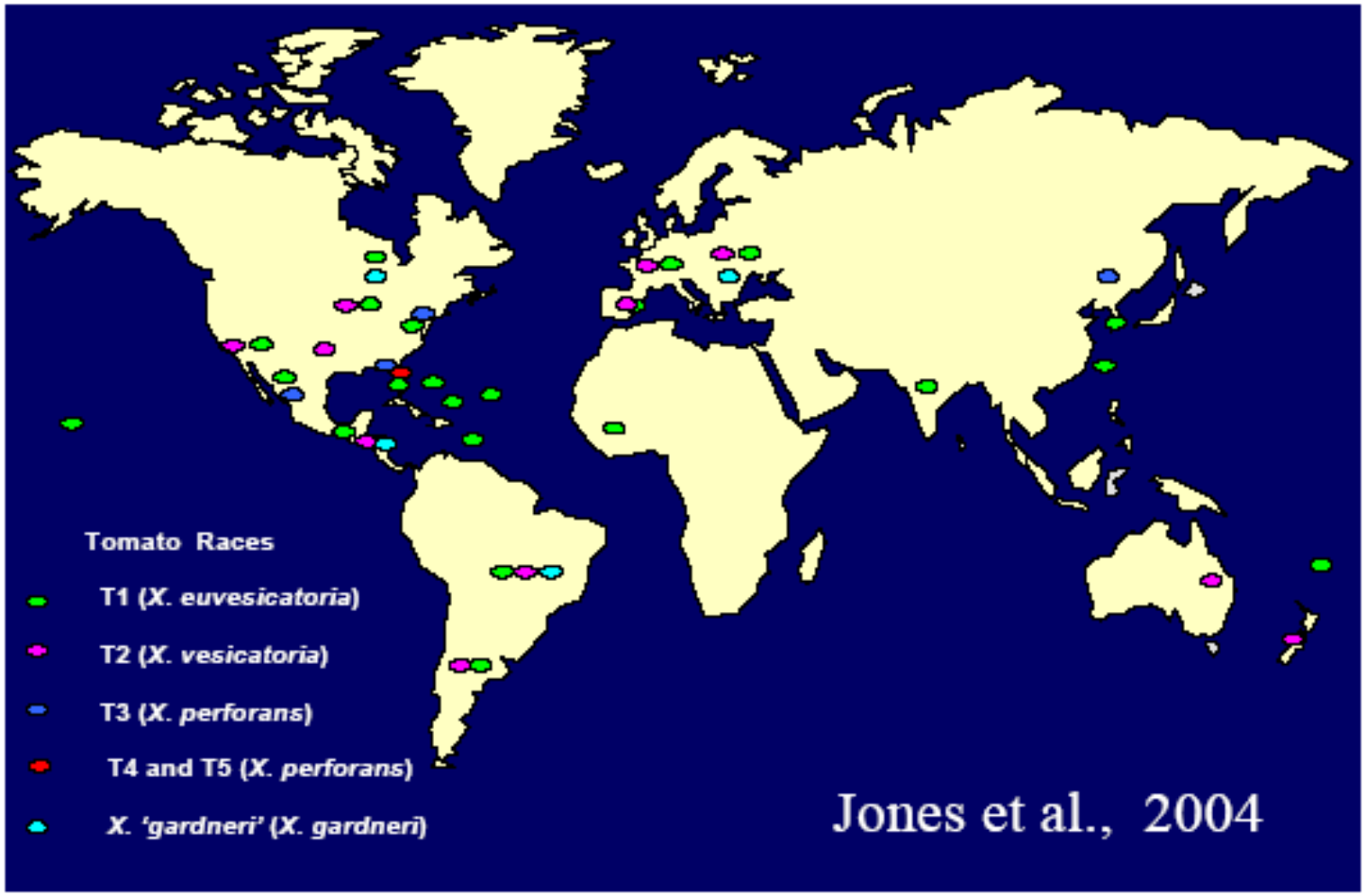
1. *X. euvesicatoria*,
2. *X. vesicatoria*,
3. *X. gardneri*, and
4. *X. perforans*



Bacterial Spot – affects Tomato and Pepper
(*Xanthomonas perforans, X. vesicatoria, and X. euvesicatoria*)

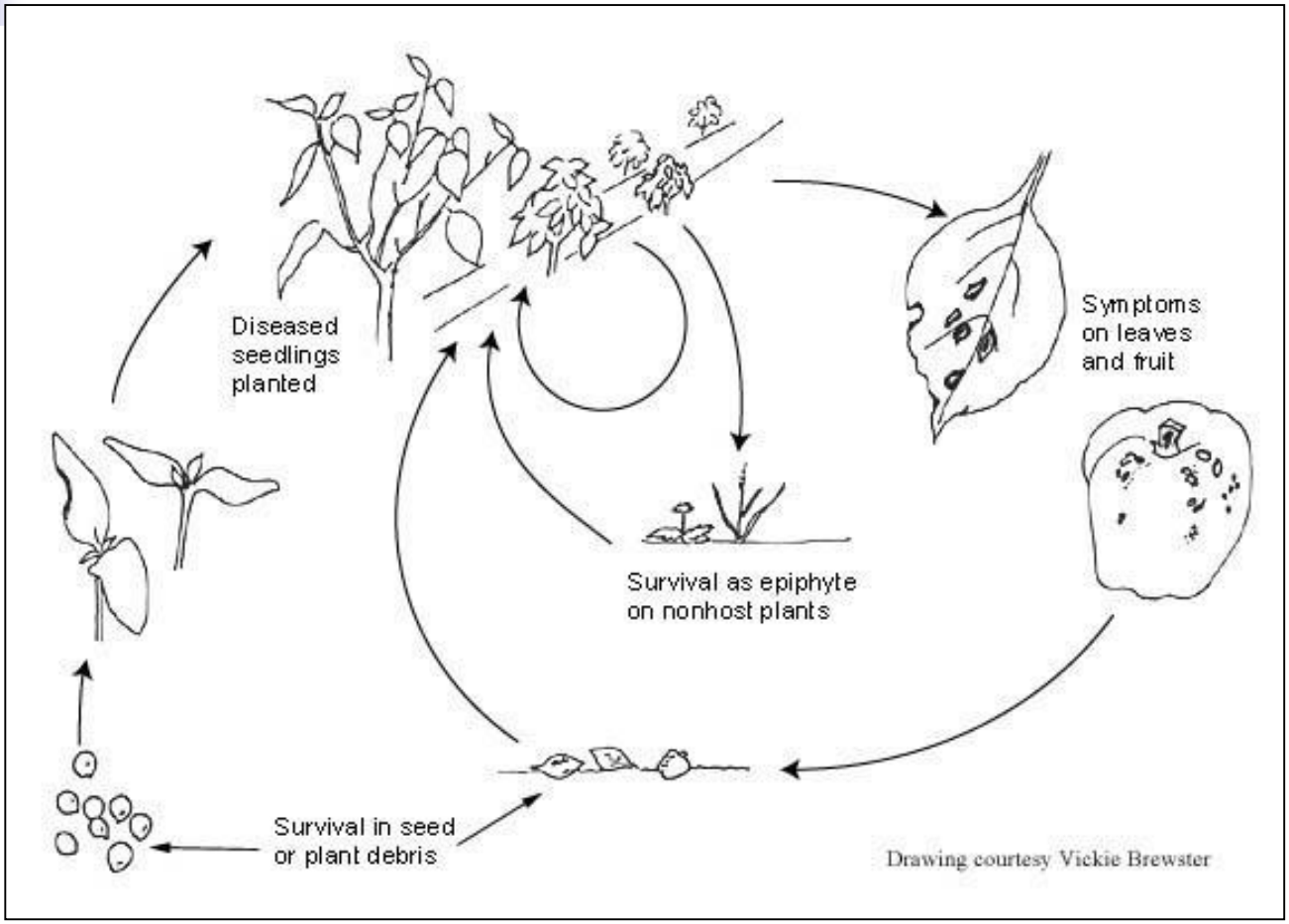
David Stead;..

Tomato leaf spot (*X. vesicatoria*) distribution



Disease cycle

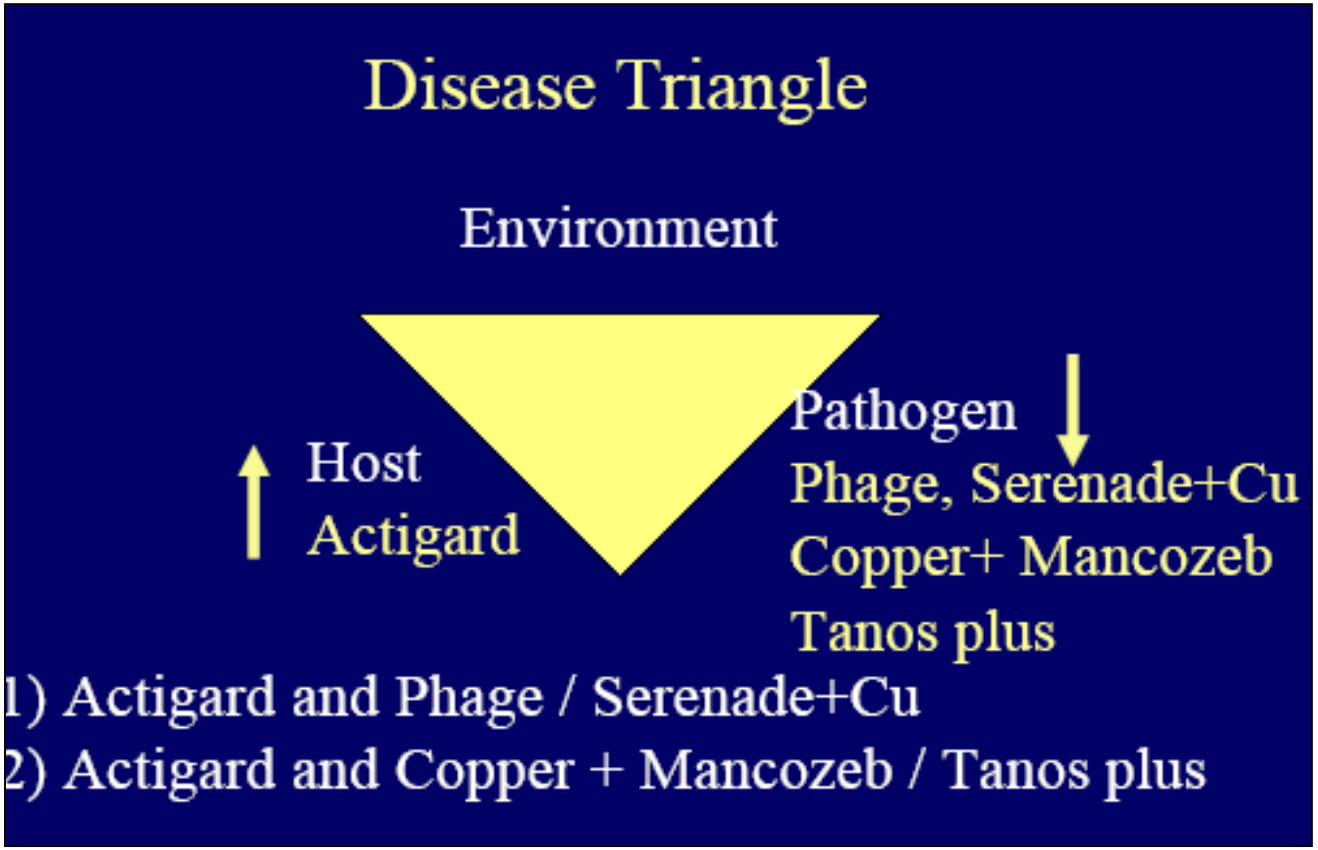
X. euvesicatoria/ X. vesicatoria



Ritchie,2000

Disease triangle

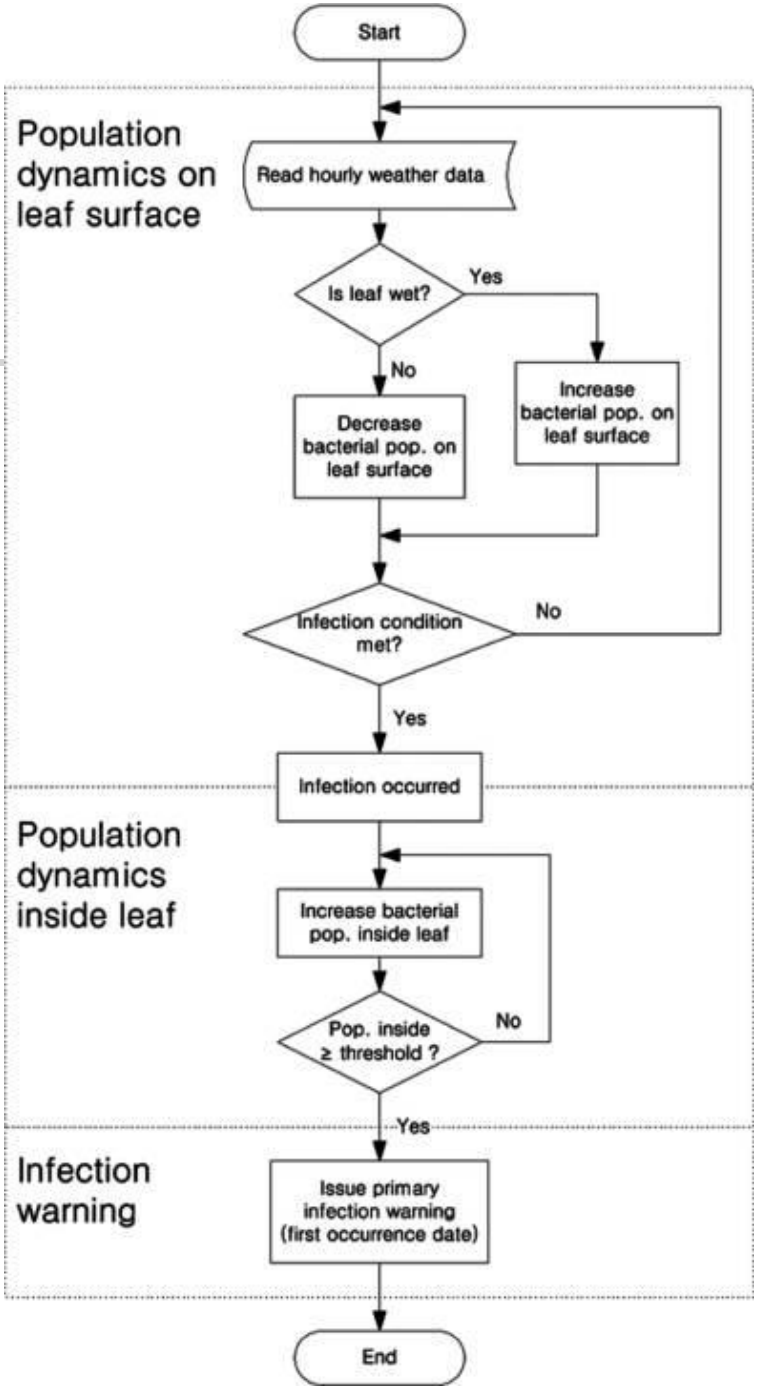
X. euvesicatoria/ X. vesicatoria



Management
Forecasting system
Xanthomonas vesicatoria

- Flowchart of a forecast model for bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* on hot pepper.
- The pathogen population on the surface of leaves increased or decreased depending on wetness.
- If rain levels exceeded 4 mm/h and wind speed was greater than 3 m/s, wounds formed on the leaves.
- Also, if the pathogen population reached 5,000 cells/g, infection was successful.
- Once they could infect inside the leaf, their population decreased by a hundredfold from the infected population and would subsequently grow depending on the temperature.
- Ultimately, the initial infection date was decided when the population was over 10^{15} cells/g.

Kim *et al.*, 2014; Morales *et al.*,2017



Management

Forecasting system

Xanthomonas vesicatoria

- The model for bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) on hot pepper predicts the primary disease infection date (Kim *et al.*,2014).
- **The model is based on:**

1. population densities of the pathogen on a host, which are calculated from one bacterium at the beginning on a leaf.
2. Hourly temperature, relative humidity, and precipitation during the growing season are the input variables for the model simulation.
3. Epiphytic populations of Xcv increase or decrease depending on wetness condition.

Kim *et al.*,2014; Morales *et al.*,2017

Management
Forecasting system
Xanthomonas vesicatoria

- In order to successfully infect, three requirements must be met:

1. pathogens densities have to be above 5×10^3 cells/ml,
2. more than 4 mm/h of rain, and
3. wind speed greater than 3 m/s.

- The pathogen population inside the leaf starts to colonize from 1/100 times of the epiphytic population at the time of entering and its growth inside the leaf depends on temperature.
- When population of *Xcv* inside the host reaches 10^{15} cells/ml, the model warns the timing of the first infection date.

Kim *et al.*,2014; Morales *et al.*,2017

Management
Forecasting system
Xanthomonas vesicatoria

- Evaluation of the spot symptoms followed the 0 to 5 scale:
- 0, no symptoms;
- 1, spots on the first leaf;
- 2, most leaves had spots;
- 3, all leaves had severe spots;
- 4, leaf death.
- These scales were assigned a disease severity value:
- 0 and 1, 0%;
- 2, 50%;
- 3 and 4, 100%.

Kim *et al.*,2014

Management
Forecasting system
Xanthomonas vesicatoria

- **Estimating the doubling time for wet leaves:**
- The increase in pathogen population at a certain time (t) and initial pathogen populations could be determined as follows:

$$N(t) = N(t-1) \times 2^{(W/g(T))}$$

N(t): *Xcv* population at time t

N(t – 1): Xcv population at time t – 1

W: The rate (h/h) of leaf wetness at time t

g(T): doubling time (h) at temperature T

T: temperature at time t

- Doubling time g(T) at a certain temperature T was derived from the following equation:

$$g = t \times \log 2 \times (\log N - \log N_0)$$

t: The hour (h) to take *Xcv* increasing

N: The population size after time t

N0: The initial population size

Kim *et al.*,2014

Management
Forecasting system
Xanthomonas vesicatoria

- **Population decrease: Estimating the half-life when the initial population decreased to half under dry conditions:**
- Based on experiments on dry leaf surfaces, we could estimate the half-life of *Xcv* while the population was decreasing.
- The half-life could be defined as the amount of time before the population reaches half of the initial population density.
- We assumed that the half-life depends on the duration of the dry period, regardless of temperature.
- The equation model for half-life is as follows:

$$N(t) = N(t-1) \times (1/2)^{(1/h)}$$

N(t): The pathogen population at time t

N(t – 1): The pathogen population at time t – 1

h: half-life (h)

Kim *et al.*,2014

Management
X. euvesicatoria/ *X. vesicatoria*

- Use pathogen-free seeds and transplants.
- A variety of treatments can be used to eradicate seed-borne infection as for *C. michiganensis* subsp. *michiganensis*: 0.8% acetic acid for 24 h, 5% HCl for 5-10 h, 1.05% sodium hypochlorite for 30 min, 0.05% $HgCl_2$ for 5 min. Hot water treatment at 56°C for 30 min is another possibility.
- Practice crop rotation with non-host plants such as corn and soybean so that peppers are grown only every 3 to 4 years. However, do not use soybeans in the rotation if white mold (*Sclerotinia sclerotiorum*) has been a problem.
- Deep plow to bury infected crop debris.
- Avoid working in the field when foliage is wet.

Management

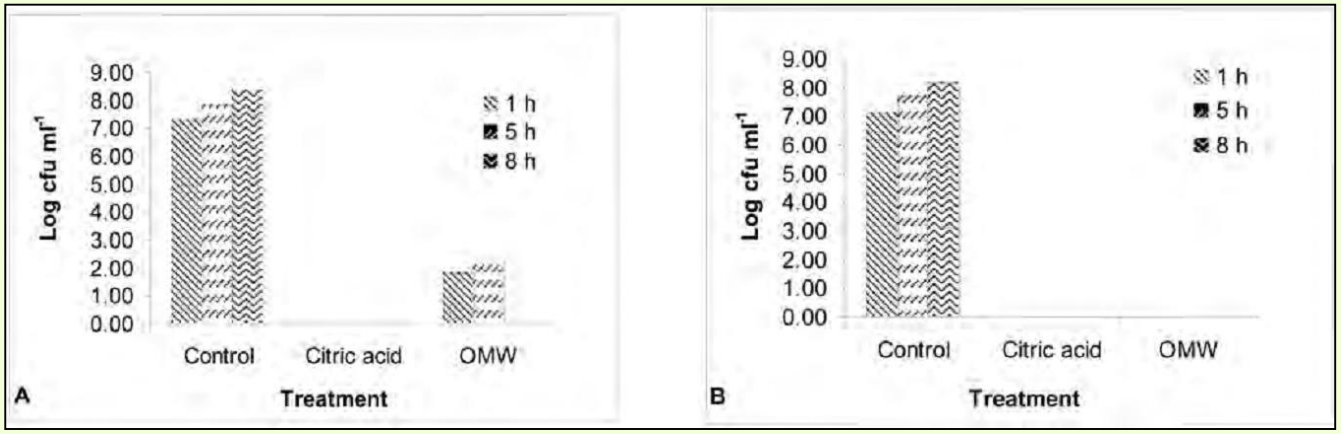
Xanthomomas euvesicatoria/ Xanthomomas vesicatoria

- Eliminate wild host plants such as nightshade and ground cherry in and around field.
- Application of copper-containing pesticides may be helpful for preventing development and spread of bacterial spot.
- None of the currently available pepper varieties are resistant to all known races of the bacterial spot pathogen.
- However, use of varieties resistant to one or more races of the pathogen may provide some control, depending on the races present.

Management

X. euvesicatoria/ X. vesicatoria

- Biological control:
- Growth inhibition of citric acid (0.1 mol l^{-1}) and Olive mill wastewaters, OMW (diluted 1:10) on *Clavibacter michiganesis* ICMP 2550. A. Experiment 1; B. Experiment 2. Results of the third experiment are not shown here since growth was inhibited completely by citric acid and OMW treatments as in Fig. 1B.



Özdemir,2009

Management

Pepper race classification of susceptible and hypersensitive response reactions

- A description of races defined by the hypersensitive response (HR) induced on each pepper host containing the corresponding resistance genes.
- Typical pepper cultivars with no resistance genes, such as Early California Wonder, are used as a positive control to ensure infection occurs with the inoculated bacterial strain.

	Pepper Resistance Gene				
Race	None	*Bsr1*	*Bsr2*	*Bsr3*	*Bsr4*
0	S	HR	HR	HR	HR
1	S	S	HR	HR	HR
2	S	HR	HR	S	S
3	S	S	HR	S	HR
4	S	S	S	HR	HR
5	S	HR	S	S	S
6	S	S	S	S	HR
7	S	S	HR	HR	S
8	S	S	HR	S	S
9	S	S	S	HR	S
10	S	S	S	S	S

S (susceptible)
HR (Hypersensitive Response)

Table taken from Stall *et al.*,2009.

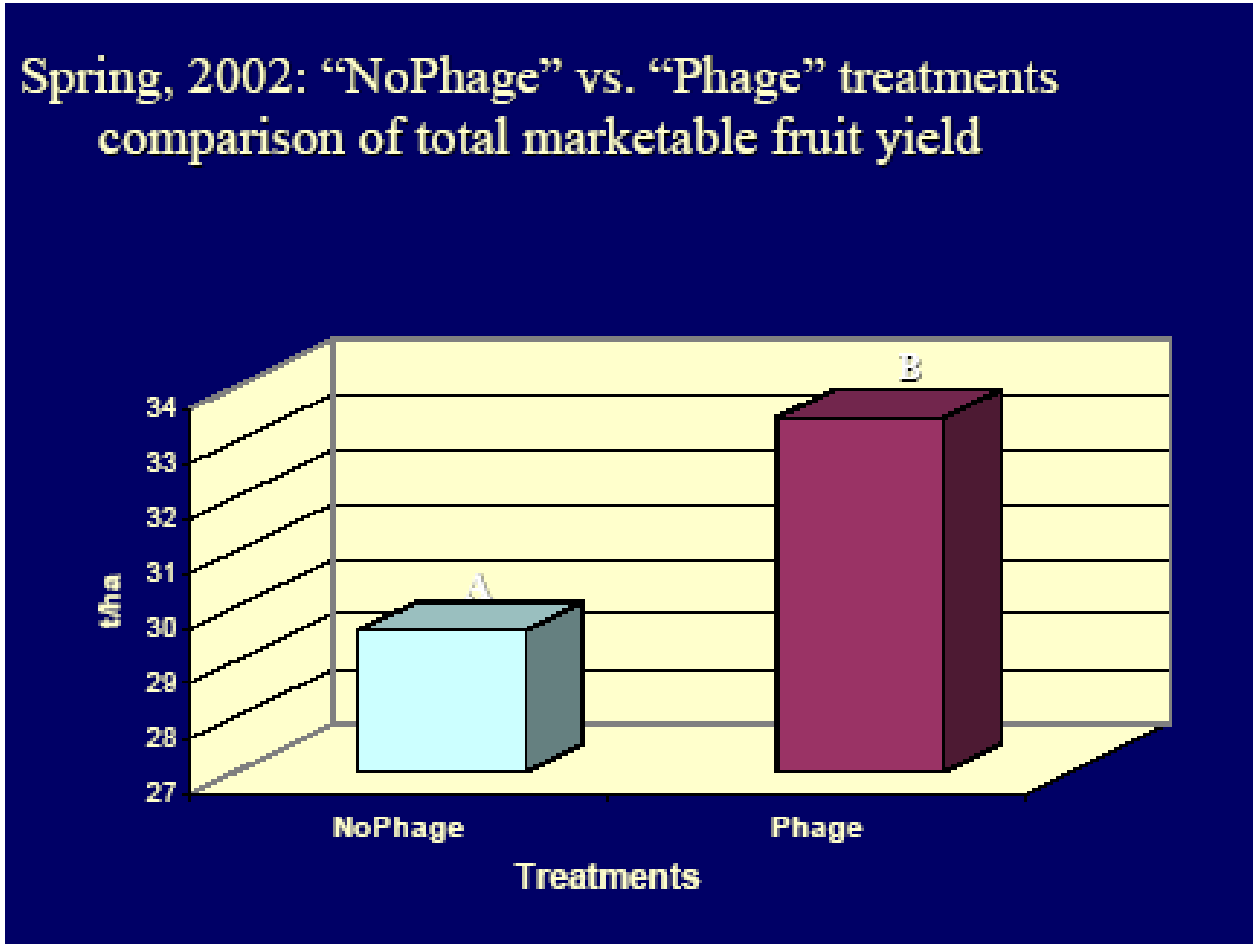
Garton *et al.*,2009

Specific management of *X. euvesicatoria*/ *X. vesicatoria*

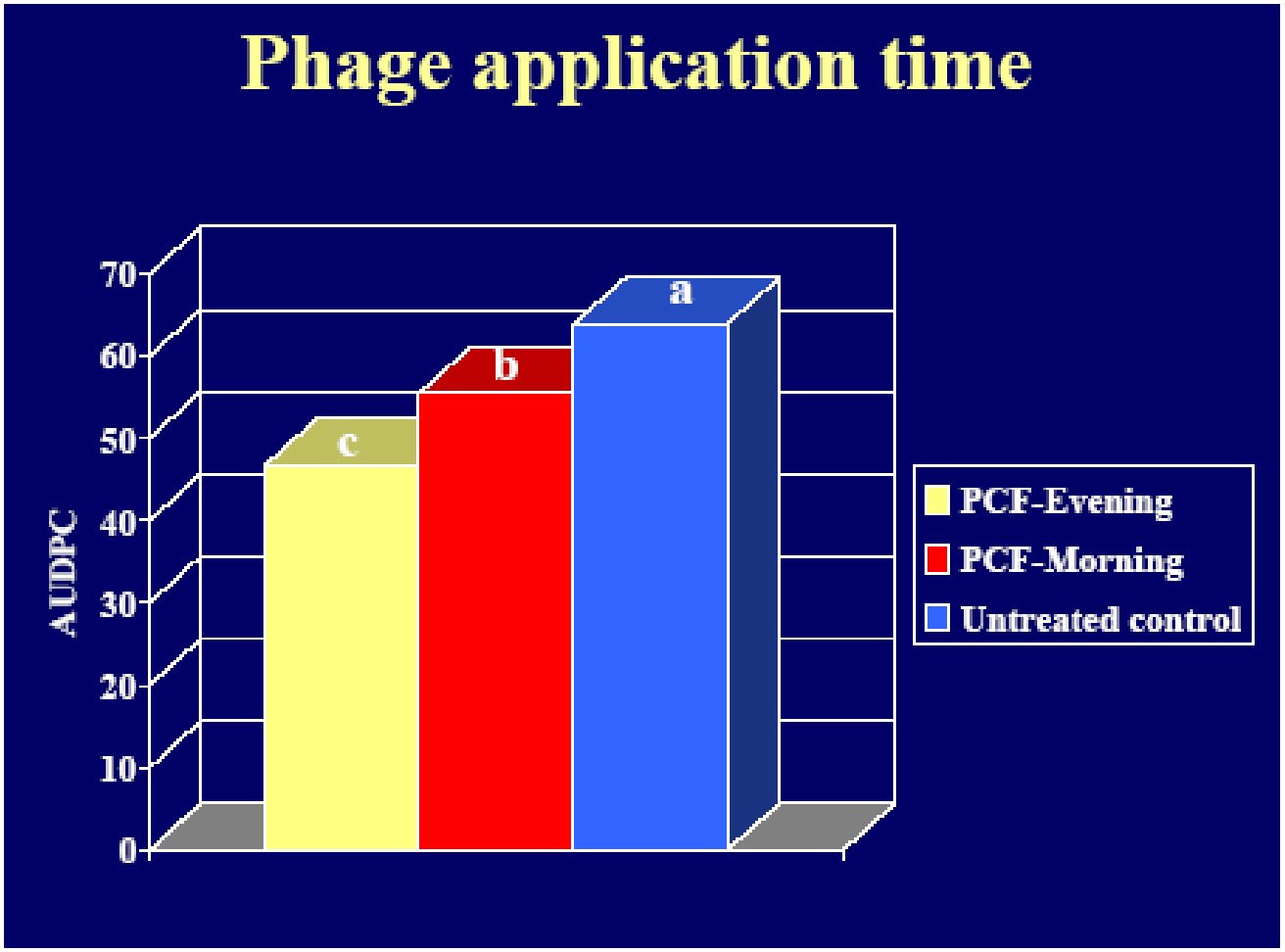
Bacterial Spot Management

- Biological Control
  - Phages (bacteriophages) are viruses that infect bacteria
  - Serenade (*B. subtilis*) plus copper

Biological control of *X. vesicatoria*
Phage treatments



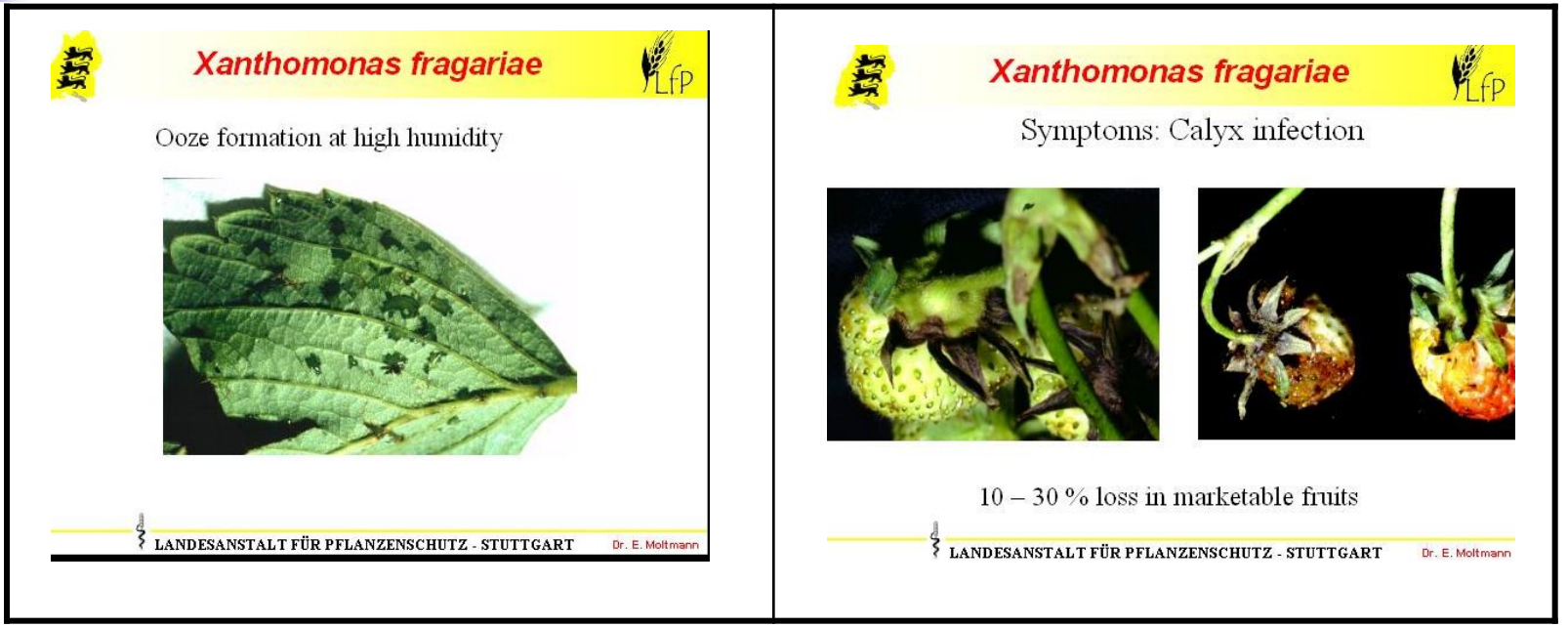
Biological control of *X. vesicatoria*
Phage treatments



Xanthomonas fragraiae

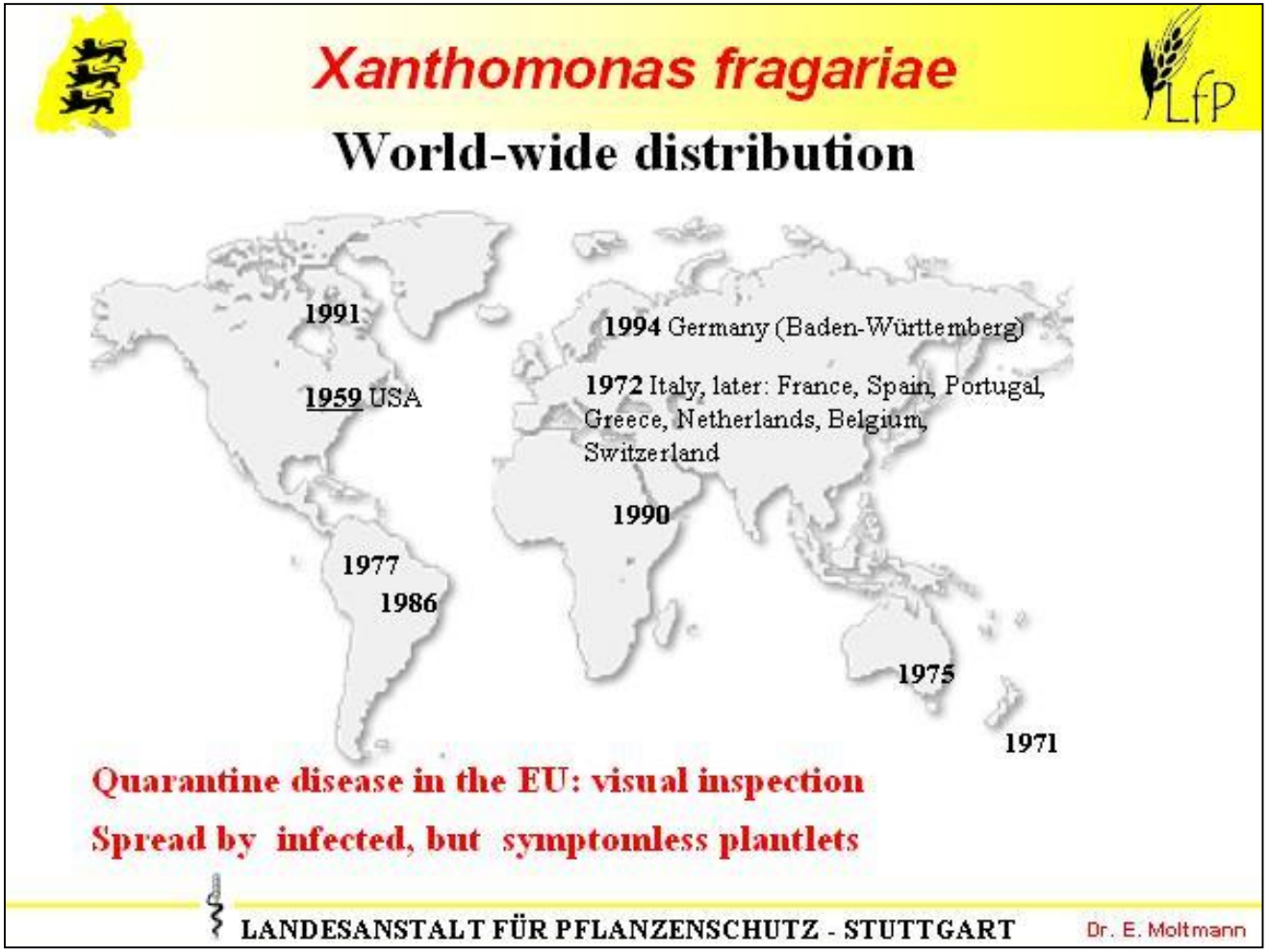
Angular leaf spot of strawberry

Bacterial leaf spot of strawberry
Xanthomonas fragariae



Moltmann & Zimmermann,2004

Geographical distribution of strawberry bacterial leaf blight



Management

- Angular leaf spot is kept to a minimum by using certified planting materials.
- Chemical controls are typically ineffective against this pathogen.
- Copper-containing compounds are registered but have caused phytotoxicity with repeated applications.
- Rotate crops to avoid infesting fields, and avoid overhead irrigation when possible.

Xanthomonas hortorum pv.*carotae*

Bacterial leaf blight of carrot, carrot scab

Bacterial blight symptoms

Xanthomonas hortorum pv.*carotae*

Lesions turn dark brown and shiny, and progress down petiole.

Jodi,2011

Bacterial blight symptoms
Xanthomonas hortorum pv.*carotae*

- Typical pale yellow and mucoid growth of *Xanthomonas hortorum* pv. *carotae* isolates on a sectored plate of YDC after 72 h at 28°C.

International Rules for Seed Testing,2014

Etiology
Xanthomonas hortorum pv.*carotae*

- Bacterial blight of carrot is caused by *Xanthomonas hortorum* pv. *carotae* (old name as *Xanthomonas campestris* pv.*carotae*).
- Moderately virulent, highly seed-borne;
- Primarily causes yield losses due to poor seed germination.
- http://vegetablemdonline.ppath.cornell.edu/factsheets/Carrot_Leaf_Blight.pdf

Jodi,2011

Management
Xanthomonas hortorum pv.*carotae*

- Obtain disease-free seed, heat treat where possible for added security;
- Avoid overhead irrigation;
- Avoid working in wet fields;
- Allow 2-year rotation for debris to break down in field;
- Avoid highly-susceptible varieties which increase inoculum levels;
- Preventive copper sprays.

Jodi,2011

Xanthomonas oryzae pvs. *oryzae* and *oryzicola*

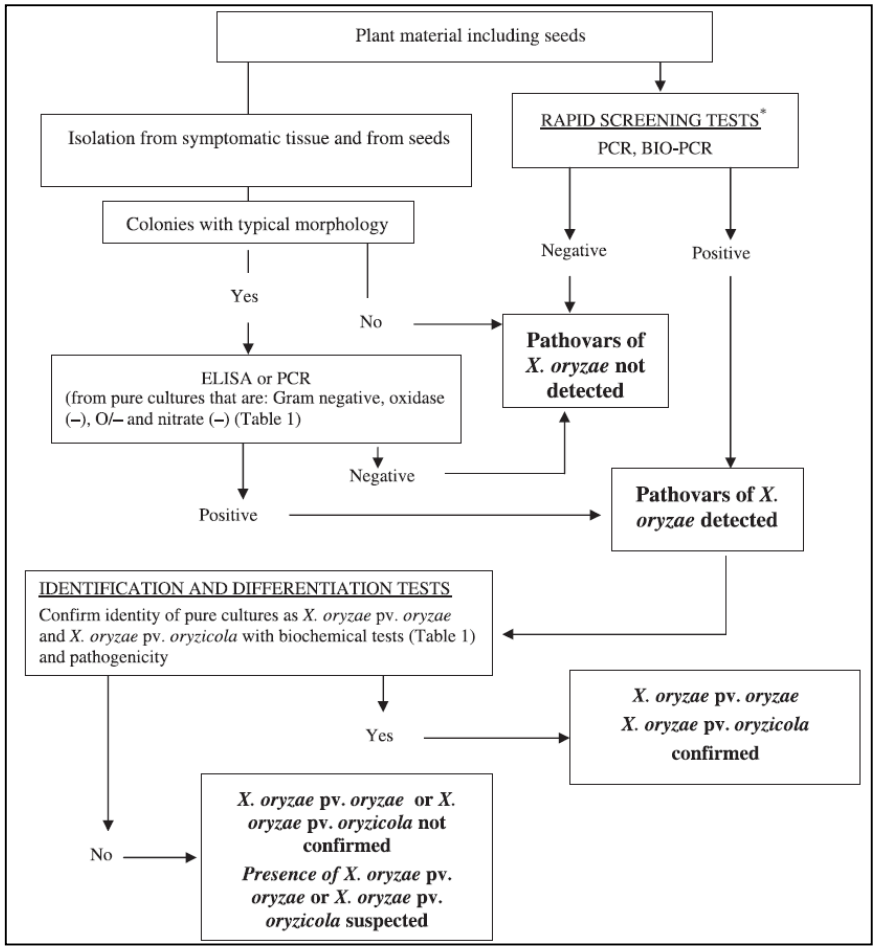
Bacterial leaf blight/streak

Leaf streak
Xanthomonas oryzae
pv. *oryzicola*

Leaf blight
Xanthomonas oryzae
pv. *oryzae*

Flow-diagram for diagnosis of *Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* from leaves and seed of host plants

- For preliminary identification of isolates follow the tests recommended.
- *Rapid screening tests are recommended when no differentiation of the pathovars of *X. oryzae* is needed, but not in the case of new findings.



OEPP/EPPO Bulletin,2007

Management

X. oryzae pvs. *oryzae* and *oryzicola*

1. *Xanthomonas oryzae* pv. *oryzae*

- Use of resistant cultivars and seed treatments help reduce incidence of the disease. Dipping rice seedlings in antibiotic at transplanting has been proposed.
- Systemic bactericides are being developed.
- Bacterization of seeds with fluorescent pseudomonads and *Bacillus* sp. have been tried as a biological control method.
- Since the increase in disease severity in the 1970s and 1980s, varietal resistance has become a very important consideration and there is a large volume of literature on breeding and screening for resistance to leaf blight.
- The existence of different races (see Biology) makes it important to obtain stable (Nayak & Chakrabarti,1986) or adult-plant (Qi & Mew, 1985) resistance.

2. *Xanthomonas oryzae* pv. *oryzicola*

- The bacterial leaf streak pathogen hardly requires any particular control measures except the use of healthy seed.
- Neither treatments nor resistance are mentioned to any significant extent in the literature.

Breeding for resistance

- Comparison of genotypes of rice resistant and susceptible to *Xanthomouas oryzae* pv. *oryzae* and a susceptible genotype transformed with the resistance gene Xa21 (all plants were challenged with the pathogen).
- **From left to right:**
- IRBB21 (resistant),
- IR24 (susceptible),
- TP 309 (susceptible), and
- TP309 (transformed with Xa21).

(courtesy of Pamela Ronald, University of California, Davis, USA)

Strange,2003

Plant growth promotion (*in vitro*) Roll towel method

Plant extracts

- Plant growth-promoting activity of the plant extracts was assessed based on the seedling vigour index by the standard roll paper towel method (ISTA, 1993).

1. Twenty five rice (ADT 43) seeds treated with plant extracts were kept over the pre-soaked germination paper.
2. The seeds were held in position by placing another pre-soaked germination paper strip over it and gently pressed.
3. The sheets along with seeds were then rolled and incubated in growth chamber for 10 days. Three replications were maintained for each treatment.

- The root length and shoot length of individual seedlings were measured and the per cent germination of seeds was also calculated.
- The seedling vigour index was calculated:

Vigor index (VI) = (Mean root length + Mean shoot length) × Germination (%).

Kumar and Balabaskar,2013

Biological control

Xanthomouas oryzae pv. *oryzae*

Bacillus amyloliquefaciens FZB42

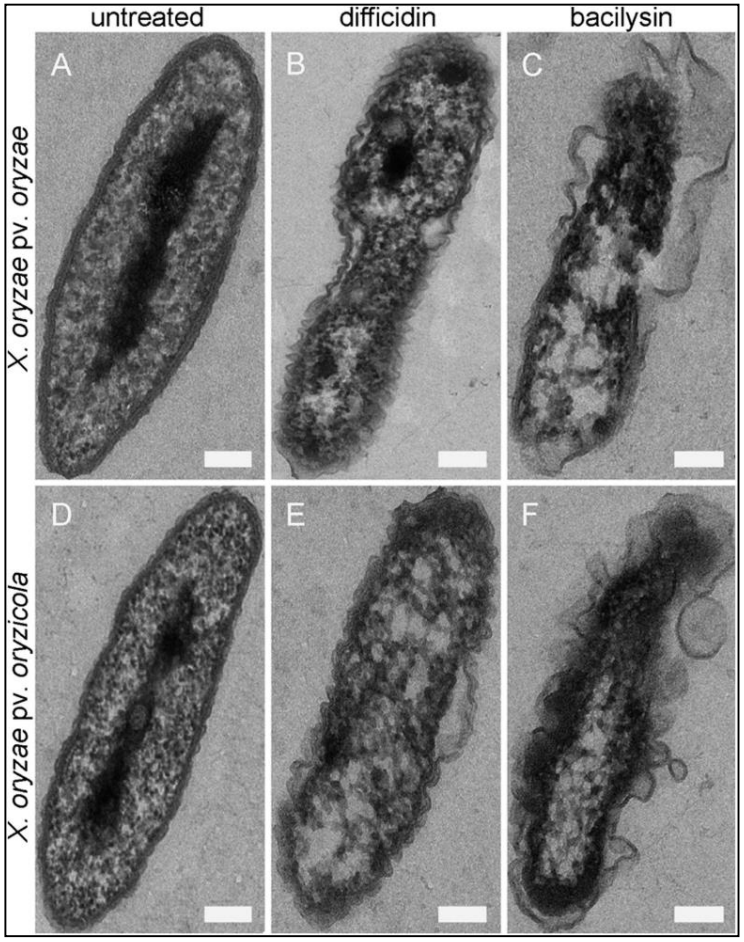
- *Bacillus amyloliquefaciens* FZB42 was shown to possess biocontrol activity against these *Xanthomonas* strains by producing the antibiotic compounds difficidin and bacilysin from *Bacillus amyloliquefaciens*.
- Analyses using fluorescence, scanning electron and transmission electron microscopy revealed difficidin and bacilysin caused changes in the cell wall and structure of *Xanthomonas*.
- Difficidin and bacilysin caused downregulated expression of genes involved in *Xanthomonas* virulence, cell division, and protein and cell wall synthesis.

Wu *et al.*,2015

Ultrastructural effects of 50 μg/ml difficidin or bacilysin on *Xanthomonas* cells after 12 h determined by TEM

A. An untreated *X. oryzae* pv. *oryzae* cell;
B. *X. oryzae* pv. *oryzae* treated with difficidin;
C. *X. oryzae* pv. *oryzae* treated with bacilysin;
D. An untreated *X. oryzae* pv. *oryzicola* cell;
E. *X. oryzae* pv. *oryzicola* treated with difficidin;
F. *X. oryzae* pv. *oryzicola* treated with bacilysin.

- Bars: 0.2 μm.



Wu *et al.*,2015

Biological control

Two antibiotic compounds difficidin and bacilysin from *Bacillus amyloliquefaciens*

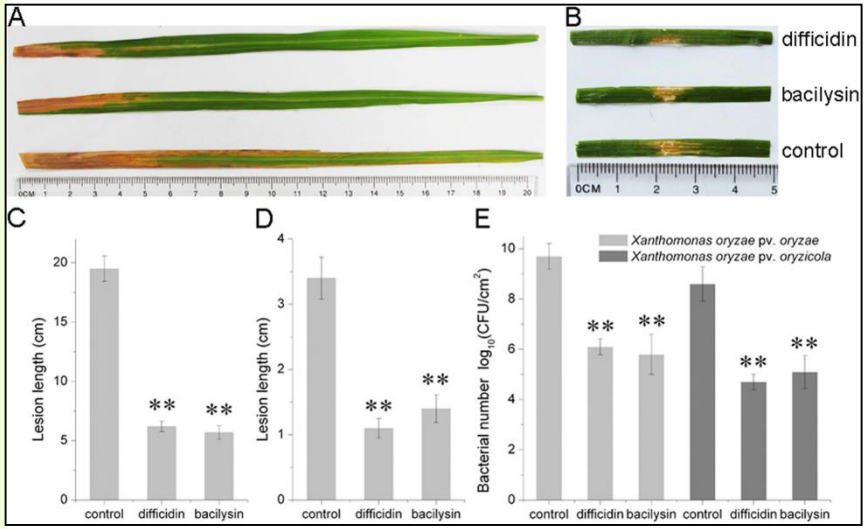
A. Representative result of lesion length symptom tests on the leaves of adult susceptible rice (cultivar 9311, two-month old) after treatment with 50 μg/ml difficidin and bacilysin, respectively.

B. Representative result of water-soaking lesion length tests on rice seedling leaves (cultivar 9311, two-week old) after infiltration with 50 μg/ml difficidin and bacilysin, respectively.

C. Calculated lesion lengths on the leaves of susceptible adult rice.

D. Calculated water-soaking lesion lengths on the leaves of rice seedlings.

E. The number of *Xanthomonas* cells in adult-susceptible rice leaves and rice seedling leaves after difficidin and bacilysin treatments.



➢ The protective rate was calculated by using the following equation: protective rate (%) = (1 – T/C) × 100, where T (treatment) and C (control) are lesion lengths with and without treatment, respectively.

➢ Data are expressed as means ± standard deviation (SD); **indicates an extremely significant difference compared with controls ($P < 0.01$).

Wu *et al.*,2015

Xanthomonas translucens

Bacterial leaf streak(BLS)
of *Triticum* spp.

Host range of *X. translucens* strains

- *Xanthomonas translucens* causes disease in a wide variety of economically-important crops.
- An emerging disease of global importance is bacterial leaf streak (BLS), which is caused by:

1. *X. translucens* pv. *cerealis* (*Xtc*),
2. *X. translucens* pv. *translucens* (*Xtt*), and
3. *X. translucens* pv. *undulosa* (*Xtu*).

- The leaf streak disease of cereals such as wheat, barley,.. caused by Xtc, *Xtt* and *Xtu*, are called black chaff when on the glumes (spikes).

Ali Shah *et al.*, 2019;..

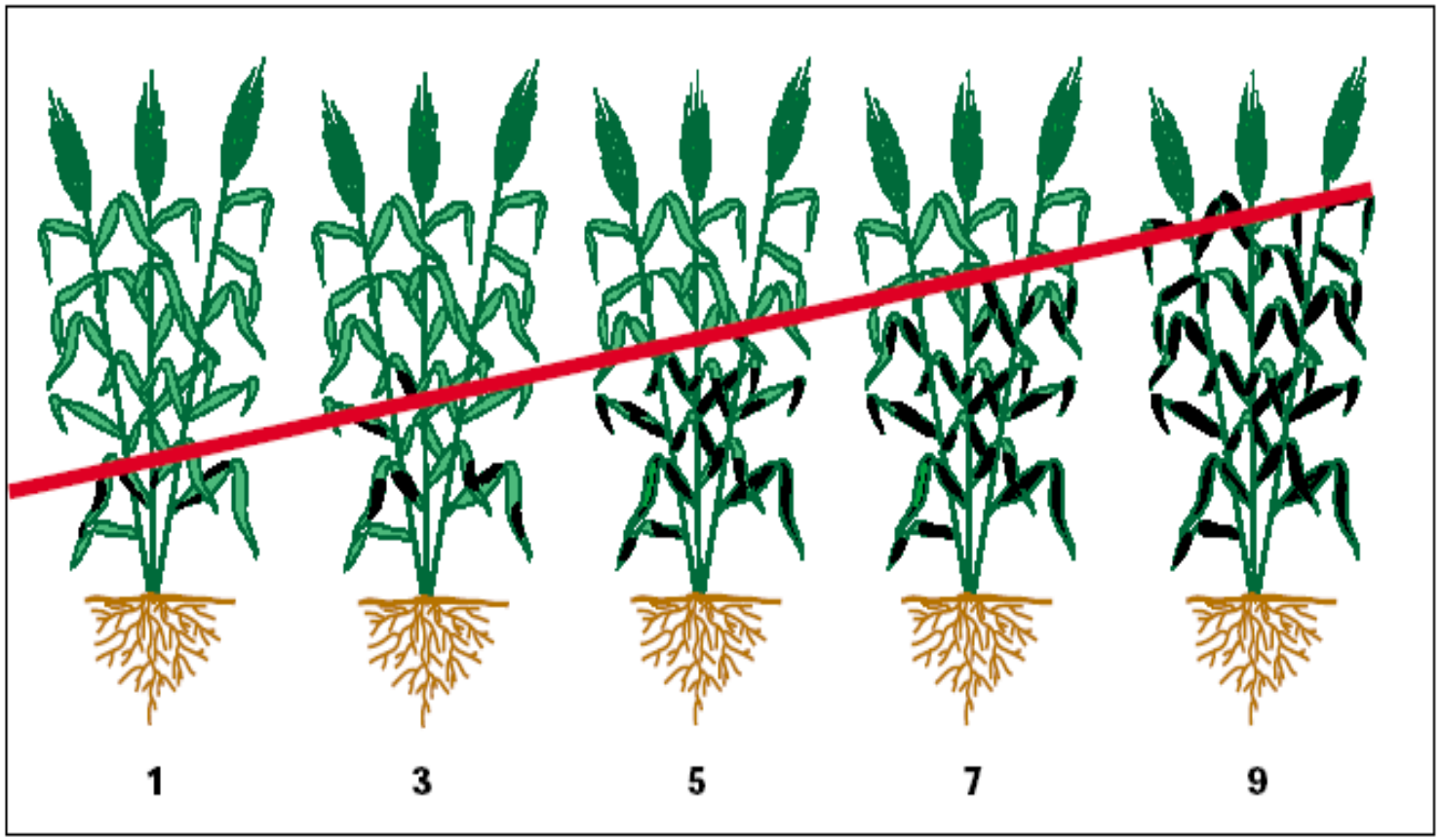
Host range of *X. translucens* strains

Strain[a]	Name	Origin	Species where first isolated	Host range		
				Wheat[b]	Barley	Oat
NCPPB2821	*X.t.* pv. *undulosa*	Canada	*Triticum turgidum* var. *durum* L.	+	+	C
UPB480	*X.t.* pv. *undulosa*	Pakistan	*Triticum turgidum* var. *durum* L.	+	+	C
UPB513	*X.t.* pv. *undulosa*	Mexico	X *Triticosecale* Wittmack	+	+	C
UPB605	*X.t.* pv. *undulosa*	Brazil	*Triticum aestivum* L.	+	+	C
UPB645	*X.t.* pv. *undulosa*	Syria	*Triticum turgidum* var. *durum* L.	+	+	C
NCPPB973	*X.t.* pv. *translucens*	USA	*Hordeum vulgare* L.	(+)	+	–
UPB684	*X.t.* pv. *translucens*	Iran	*Hordeum vulgare* L.	–	+	–
UPB780	*X.t.* pv. *translucens*	Spain	*Hordeum vulgare* L.	–	+	T
NCPPB2820	*X.t.* pv. *translucens*	India	*Hordeum vulgare* L.	–	+	C
NCPPB2822	*X.t.* pv. *secalis*	Canada	*Secale cereale* L.	+	+	C
UPB676[c]	*X.t.* pv. *secalis*	South Africa	*Secale cereale* L.	–	+	T
NCPPB1944	*X.t.* pv. *cerealis*	USA	*Bromus inermis* L.	+	+	C

[a] NCPPB = National Collection of Plant Pathogenic Bacteria, Harpenden, England; UPB = Unité de Phytopathologie Bacterial collection, Louvain-la-Neuve, Belgium.
[b] + = positive reaction, compatibility; (+) = weak positive reaction; - = negative reaction; C = chlorosis; T = translucens spot.
[c] Received as pathovar *translucens* from J. Smith, Small Grains Centre, Bethlehem, South Africa.

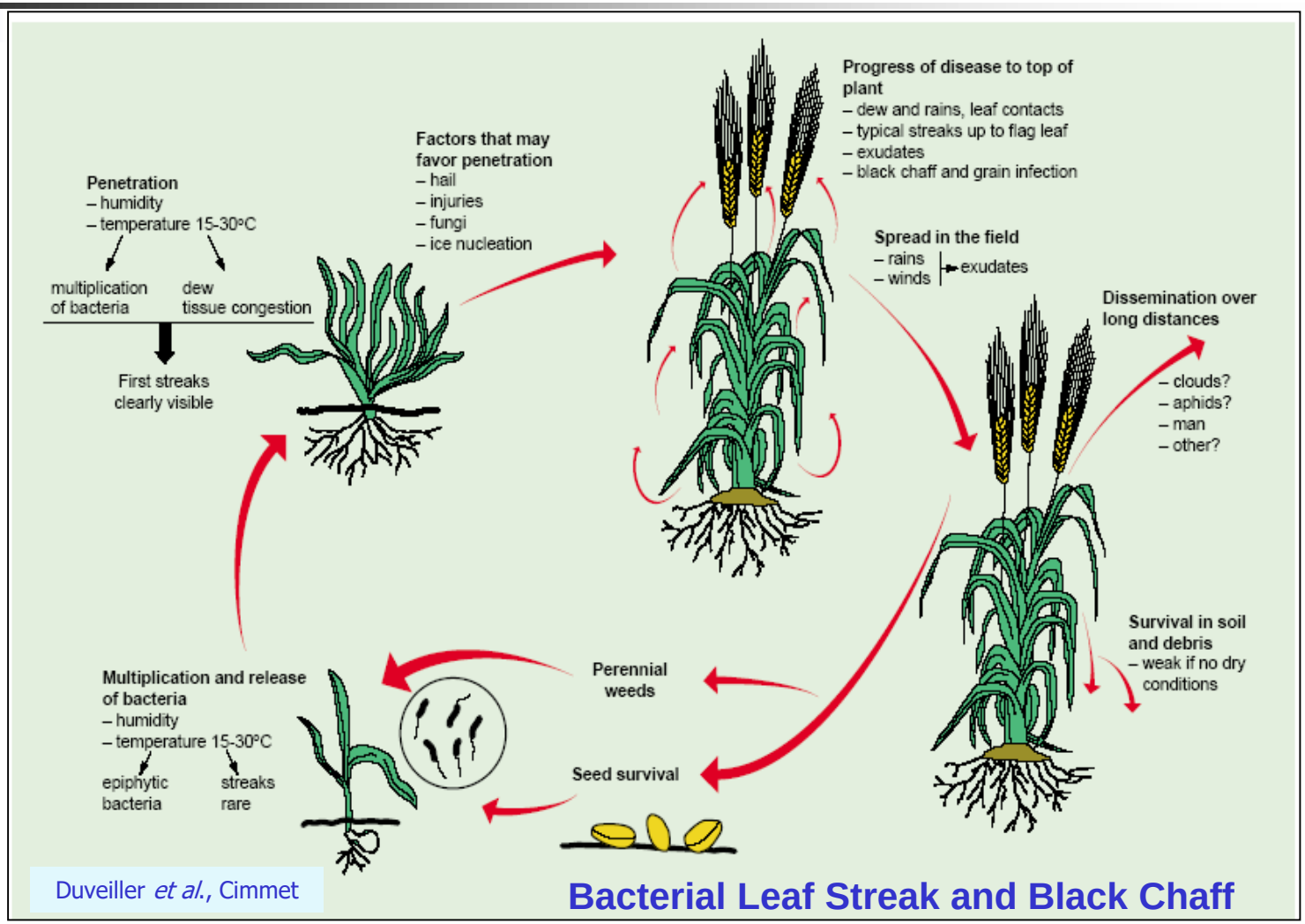
Duveiller *et al.*, Cimmet

The 0-9 scale proposed by Saari and Prescott (1975) for appraising the intensity of all wheat foliar diseases

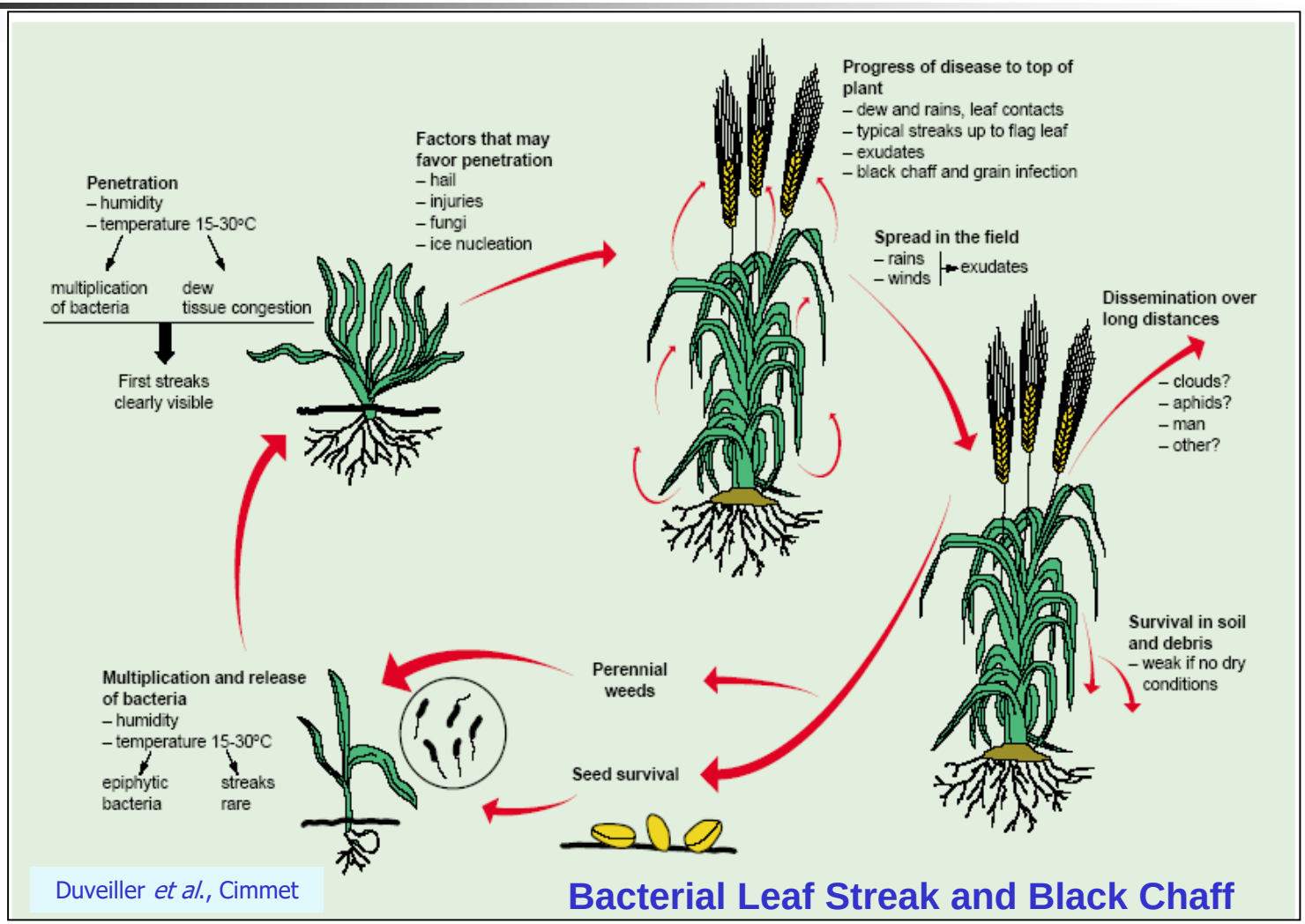


Duveiller *et al.*, Cimmet

Disease cycle of the genus *Xanthomonas*
X. translucens pv. *undulosa*



Disease cycle of the genus *Xanthomonas*
X. translucens pv. *undulosa*



X. translucens pv. *translucens*

Bacterial leaf streak of wheat

Bacterial leaf streak of wheat
Xanthomonas translucens pv. *translucens*

Symptoms on peduncle of soft red winter wheat cv. Exel. Unusually large and dark peduncle lesions develop on this variety in the field even though the leaves are resistant to *X. translucens* pv. *translucens*.
Courtesy: E.A. Milus, University of Arkansas, Fayetteville (US).

Symptoms of bacterial leaf streak caused by *Xanthomonas translucens* in greenhouse conditions
Courtesy: Ebrahim Osdaghi

EPPO Global Database

Black chaff disease of wheat *X. t.* pv. *undulosa* and field screening methods

Disease evaluation scale (Milus and Mirlohi 1994):

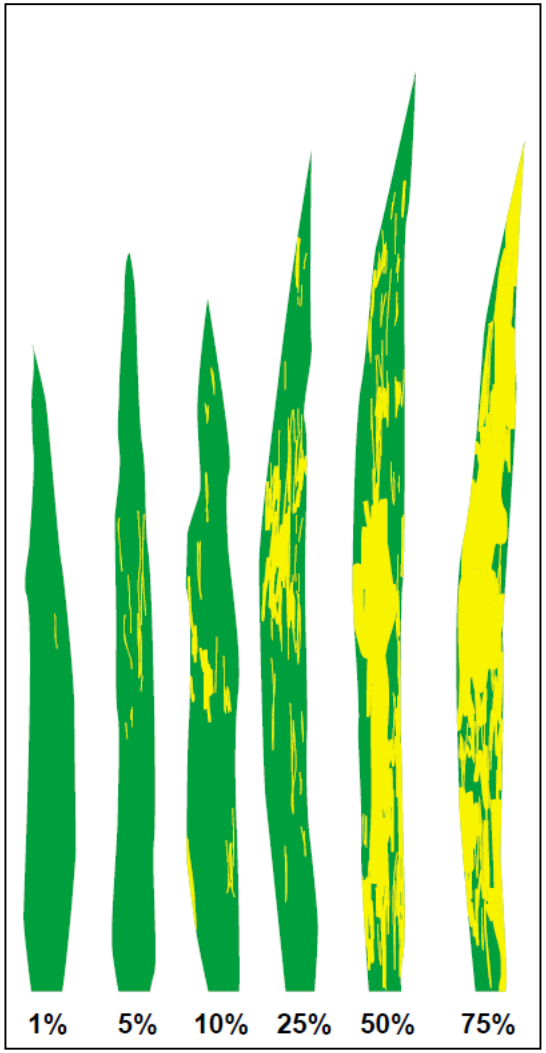
0 = no visible symptoms
1 = chlorosis but no water-soaking
2 = water-soaking less than 10%
3 = water-soaking 10-30%
4 = water-soaking 31-70%
5 = water-soaking 71-100%
6 = water-soaking extended beyond the infiltrated area

Scale for evaluating exudate production (Duveiller 1992):

0 = no symptoms
1 = water-soaking but no exudate
2 = water-soaking with little exudate
3 = water-soaking with readily detectable exudate
4 = water-soaking with abundant exudate

Duveiller,1994a/Cimmet

Standard disease assessment key showing percentages of leaf surface covered by bacterial leaf streak in bread wheat



Duveiller,1994a

Management
X. translucens pv. *translucens*

1. Rotations

- Rotations play in reducing black chaff epidemics.
- Since the major source of inoculum is infected seed, rotations may not play a key role in controlling the disease.
- Straw can harbor viable inoculum from season to season and cause initial infection in the field, but the number of viable bacteria in infested, overwintered straw is reduced when the straw is incorporated into the soil survival of the pathogen seems improbable due to rotation with a non-host crop and its extreme susceptibility to antagonistic bacteria, especially saprophytic *Pseudomonas* fluorescent.

Management
X. translucens pv. *translucens*

2. Seed health

- The best way to limit BLS is to avoid sowing infected seed.
- **Seed Treatments**
  There are no seed treatments that eradicate *X. translucens* without excessive damage to the seed.
- Hot, acidified cupric acetate (Forster and Schaad, 1988; Duveiller, 1989), dry heat (Fourest *et al.*, 1990) and Guzatine Plus (Mehta and Bassoi, 1993) have been shown to greatly reduce seed-borne populations of *X. translucens* and bacterial streak in the field.
- Seed treatment with acidified cupric acetate (0.5 percent) at 45°C for 20 minutes significantly reduced the amount of black chaff in the field.
  However, acidified cupric acetate and dry heat are best suited to small seed lots, and none of the treatments are 100% effective for eradicating the pathogen or preventing transmission to plants (Duveiller, 1994a). Sands et al. (1986) reported a hot-water treatment at 53°C for 10 minutes followed by immediate cooling and drying.

Management
X. translucens pv. *translucens*

3. Biological control

- Thirteen bacterial epiphytes were identified by their ability to reduce *Xanthomonas translucens* pv. *translucens* strain *Xtt*4Rif-2).
- The potential roles of antibiosis and competition for nutrient resources in mediating the observed interactions between the epiphytes and the pathogen were also investigated.
- Only one epiphyte inhibited *Xtt in vitro.* Thus, antibiosis probably was not a major mechanism by which pathogen population sizes and disease severity were reduced.
- Similarity in nutrient utilization between bacterial epiphytes and pathogen strain *Xtt*4Rif-2 was estimated using nutrient-overlap indices.
- Nutrient-overlap indices were not predictive of the ability of epiphytes to reduce pathogen populations or disease severity.
- However, successful antagonists utilized both sucrose and inositol more frequently than poor antagonists.

Stromberg *et al.*,2000

Management
X. translucens pv. *translucens*

- **Biological control**
- Biological control using phyllosphere-inhabiting bacterial epiphytes prior to colonization of *X. translucens* was shown to decrease leaf-associated population sizes of *X. translucens* and subsequent severity of bacterial leaf streak symptoms (Stromberg *et al*.,2000).
- However, because pathogen populations were not completely controlled, these populations can still serve as inoculum sources for dispersal to other plants or hosts.
- Thus, biological control using bacterial epiphytes is not an effective stand alone control measure.

Lindbeck,2011

Management
X. translucens pv. *translucens*

- **Biological control**
- The endofungal bacterium *R. radiobacter* F4 is able to colonize plant roots without specificity and it is able to increase plant resistance against the bacterial leaf pathogens:
- *Xanthomonas translucens* pv. *translucens*, and
- *Pseudomonas syringae* pv. *tomato* DC3000.

Liu et al.,2017

Xanthomonas translucens pv. *pistaciae*

Dieback or decline of pistachio

Dieback or decline of pistachio
Xanthomonas translucens pv. *pistaciae*

- Typical xylem staining and dieback associated with *X. translucens* pv. *pistaciae* (Xtp) infection in pistachio.
- The disease is endemic to Australia and is characterized by trunk and limb lesions, excessive resin exudates, discolouration of mature xylem, stunted growth and shoot dieback.
- Affected trees gradually decline, fail to produce marketable nuts and eventually die.
- Photo credits C. Taylor.

Giblot Ducray and Eileen Scott

Management

Xanthomonas translucens pv. *pistaciae*

- Hygiene practices are of paramount importance and growers are advised to disinfect pruning tools to limit the spread of the disease.
- Drastic pruning, where trees are cut back to secondary or tertiary branches, has shown some benefit in managing severely affected trees and restoring them to productivity.
- Biological control options are being investigated.
- The potential antagonists comprised one isolates of *Bacillus subtilis* and several bacteria isolated from pistachio wood and stored following indications of ability to inhibit *X. translucens* pv. *pistaciae*.

Salowi,2010

Xanthomonas vasicola pv.*musacearum*

Banana *Xanthomonas* wilt(BXW)

Banana bacterial wilt

Xanthomonas vasicola pv. *musacearum*

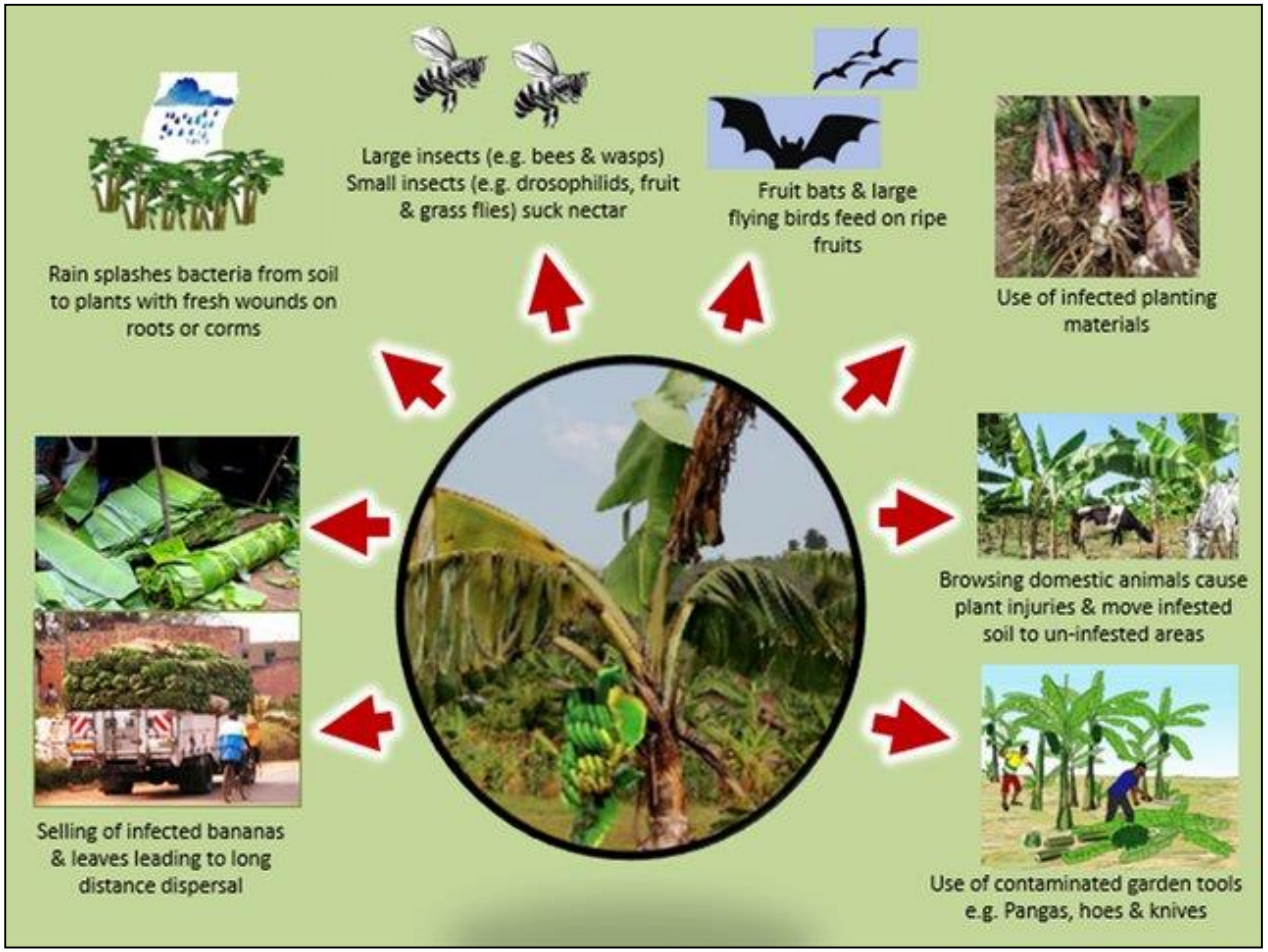
Internal rotting of banana fruit caused by *Xanthomonas vasicola* pv. *musacearum*.

Cross-section of banana pseudostem showing vascular discolouration and oozing of *X. vasicola* pv. *musacearum*.

Reeder *et al.*,2007;..

Banana bacterial wilt

Transmission pathways for the bacterium *Xanthomonas musacearum* causing BXW



Mulugo *et al.*,2022

Management
Xanthomonas vasicola pv. *musacearum*

- The recommended practices to control *Xanthomonas* wilt in Rwanda include:

1. consistent removal of infected mats,
2. burying or burning infected residues,
3. sterilization of farm tools, and
4. timely removal of the male buds (Murekezi 2009; Rutikanga *et al.*,2013).

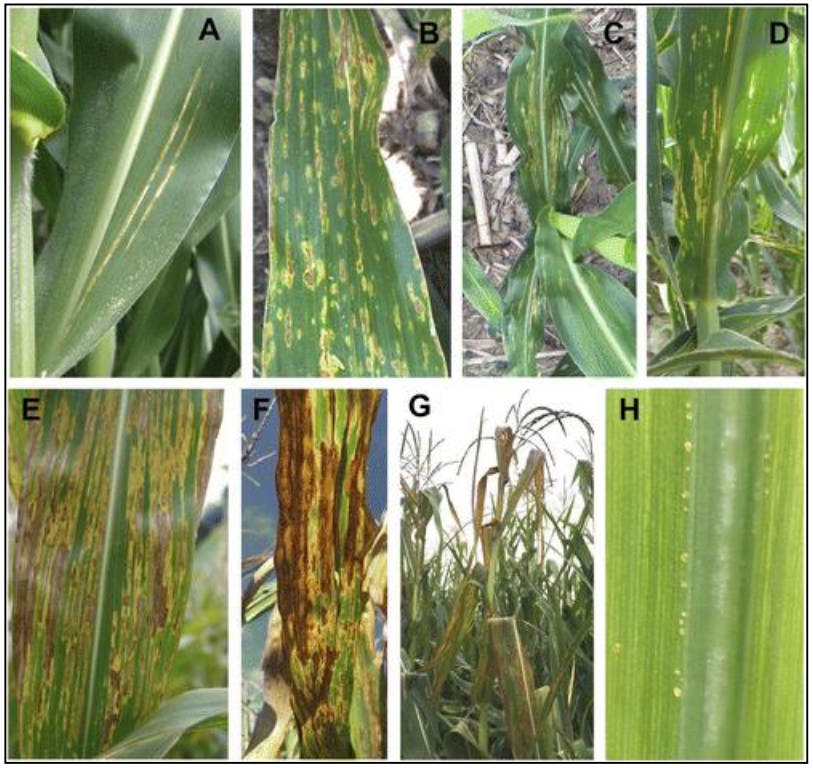
Uwamahoro *et al.*,2019

Xanthomonas vasicola pv.*vasculorum*

Bacterial leaf streak of corn

Symptoms of bacterial leaf streak of corn *Xanthomonas vasicola* pv.*vasculorum*

- Symptoms of bacterial leaf streak of corn including A, early streak lesion development, B, small spot lesion development, C and D, symptom development starting at the base of the plant progressing up resulting in E, F, and G, coalescing severe lesions on upper leaves, and H, bacterial droplet signs of *X. vasicola* pv. *vasculorum* from early lesion development.



Ortiz-Castro *et al.*,2020

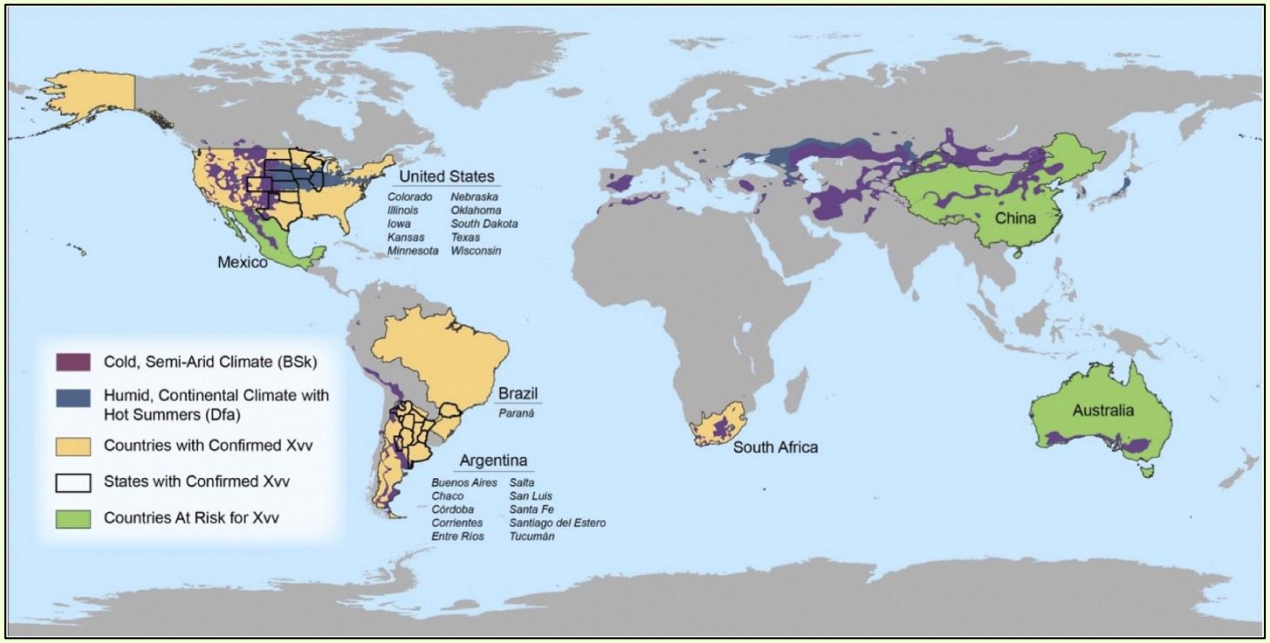
Host range
Xanthomonas vasicola pv.*vasculorum*

- Bacterial leaf streak of corn, caused by *Xanthomonas vasicola* pv.*vasculorum*, has been present in South Africa for over 70 years but is an emerging disease of corn in North and South America.
- Until recently, the only scientific information on this disease primarily focused on South African crops like sugarcane and banana.
- As a result, when the disease was first found affecting corn in Nebraska and Colorado in 2016, there was limited information available.

Ortiz-Castro *et al.*,2020

Distribution of bacterial leaf streak of corn based on climate *Xanthomonas vasicola* pv. *vasculorum*

- Current distribution of bacterial leaf streak of corn. Global map shows countries and states where *Xanthomonas vasicola* pv. *vasculorum* is confirmed (yellow); cold, semiarid regions (purple); humid continental climate with hot summers (blue); and countries at risk for *X. vasicola* pv. *vasculorum* (green) based on climate. Purple and blue regions correspond to the Koppen Climate Classification BSk and Dfa, respectively (EarthData, 2018).

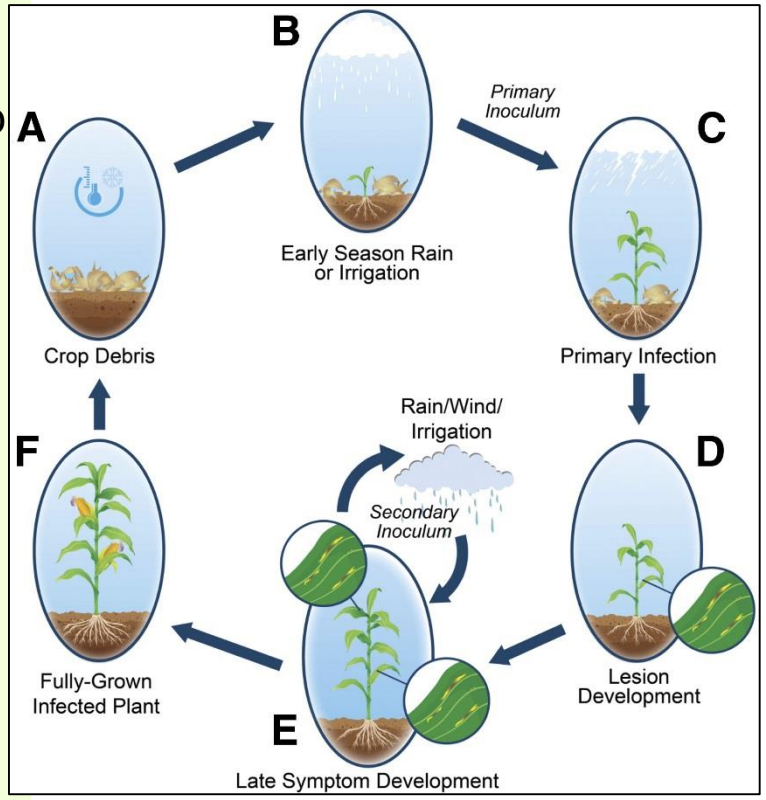


Ortiz-Castro *et al.*,2020

Proposed disease cycle of bacterial leaf streak of corn

Xanthomonas vasicola pv.*vasculorum*

A. survival in crop residue;
B. primary infection from rain or irrigation splash of *X.vasicola* pv. *vasculorum* onto immature lower corn leaves;
C. primary infection through natural opening or wounds;
D. lesion development and production of bacterial ooze droplets on the leaf surface;
E. secondary spread from lower leaves to upper leaves and plant to plant via overhead irrigation and rain splash as well as between field movement through wind-driven rain; and
F. fully grown infected plant ready for harvest.



Ortiz-Castro *et al.*,2020

Management
Xanthomonas vasicola pv.*vasculorum*

- All commercially available corn hybrids are susceptible to BLS, but there is wide variation in disease severity among varieties. This would indicate that resistance is a multigenic trait.

1. tillage to promote the degradation of infested residue, thus reducing inoculum from previous years;
2. Chemical control via bactericides may inhibit disease development;
3. Multiple applications of bactericidal products will likely be impractical in most corn growing situations, as the benefit will not justify the expense.

Ortiz-Castro *et al.*,2020

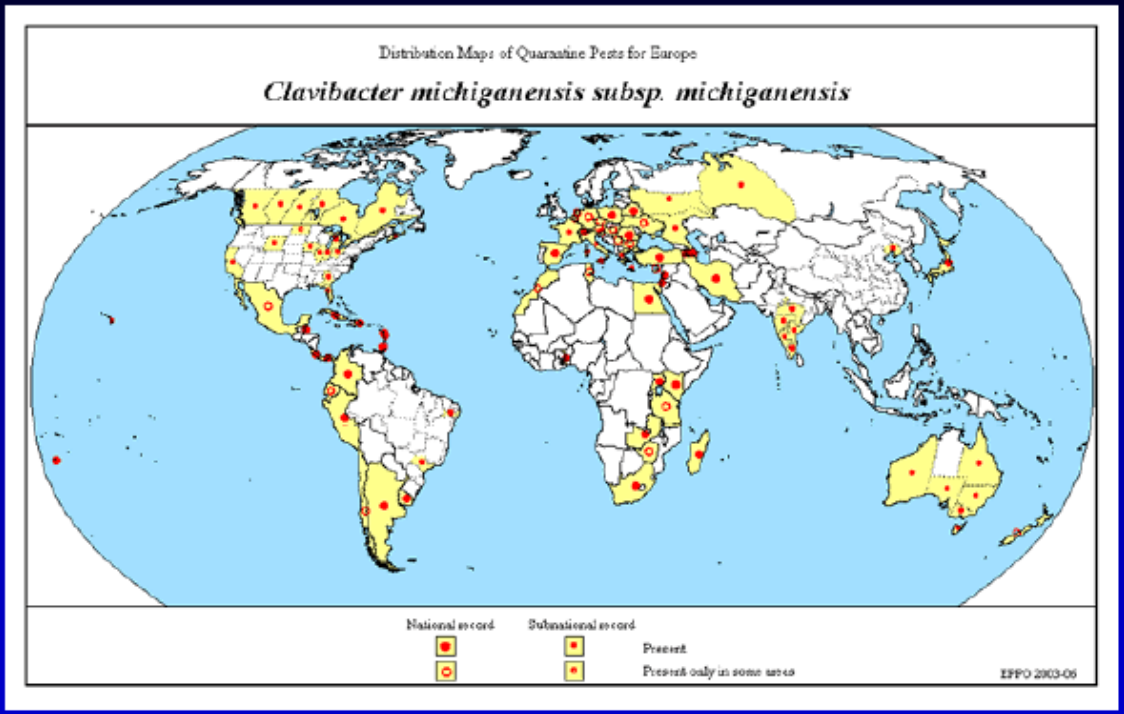
Plant bacterial disease management

Gram-positive bacteria

Clavibacter michiganensis

Bacterial canker of tomato

World Distribution



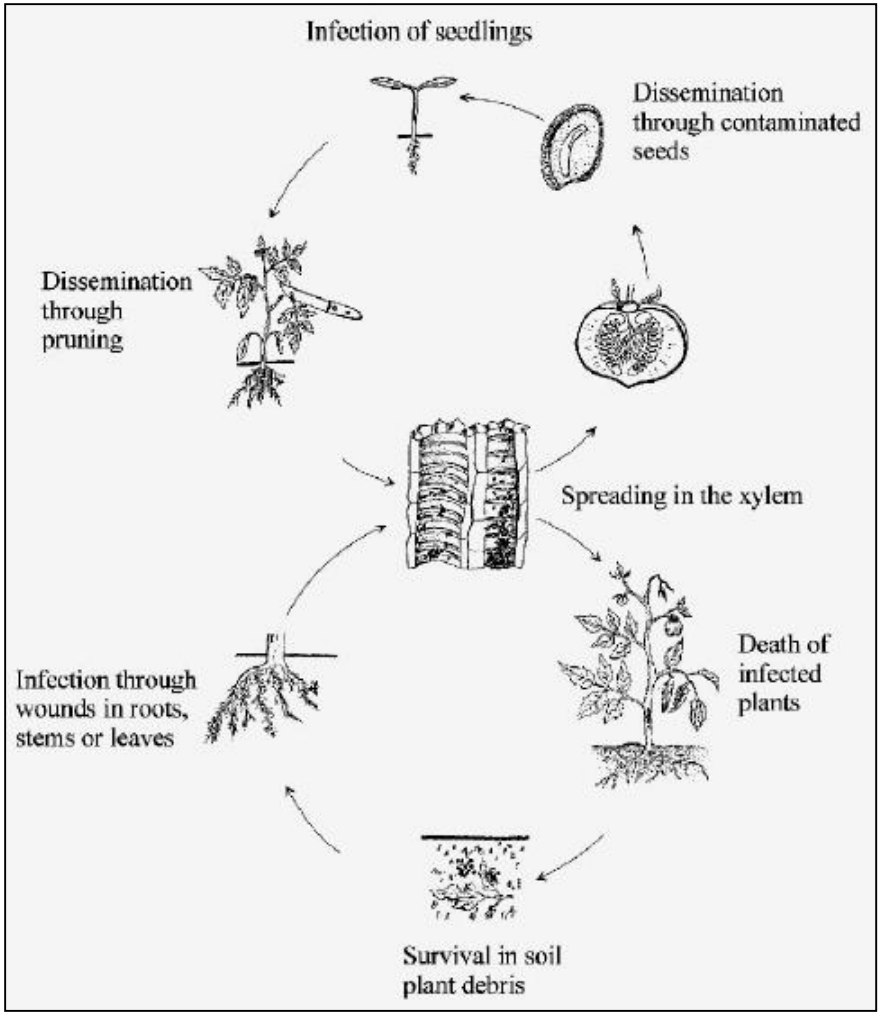
Source: EPPO

Survival and spread of the disease

- The main host of *C. michiganensis* is tomato, but there are also some reports of other *Solanum* subspecies and of the wild plants *Solanum douglasii*, *S. nigrum* and *S. triflorum* as natural hosts.
- Infected tomato seeds grow up and build contaminated seedlings.
- A spread of the disease between single plants occurs via water (rainsplash, irrigation) or by cultural practices as trimming.
- The bacteria infect the host plants via roots or wounds through stomata and other natural openings and invade the xylem vessels, followed by a systemic infection of the host.
- Under natural conditions, it takes a long latent period until the first symptoms appear.

Eichenlaub *et al.*,2006

Infection cycle of *Cmm* in tomato



Eichenlaub *et al.*,2006

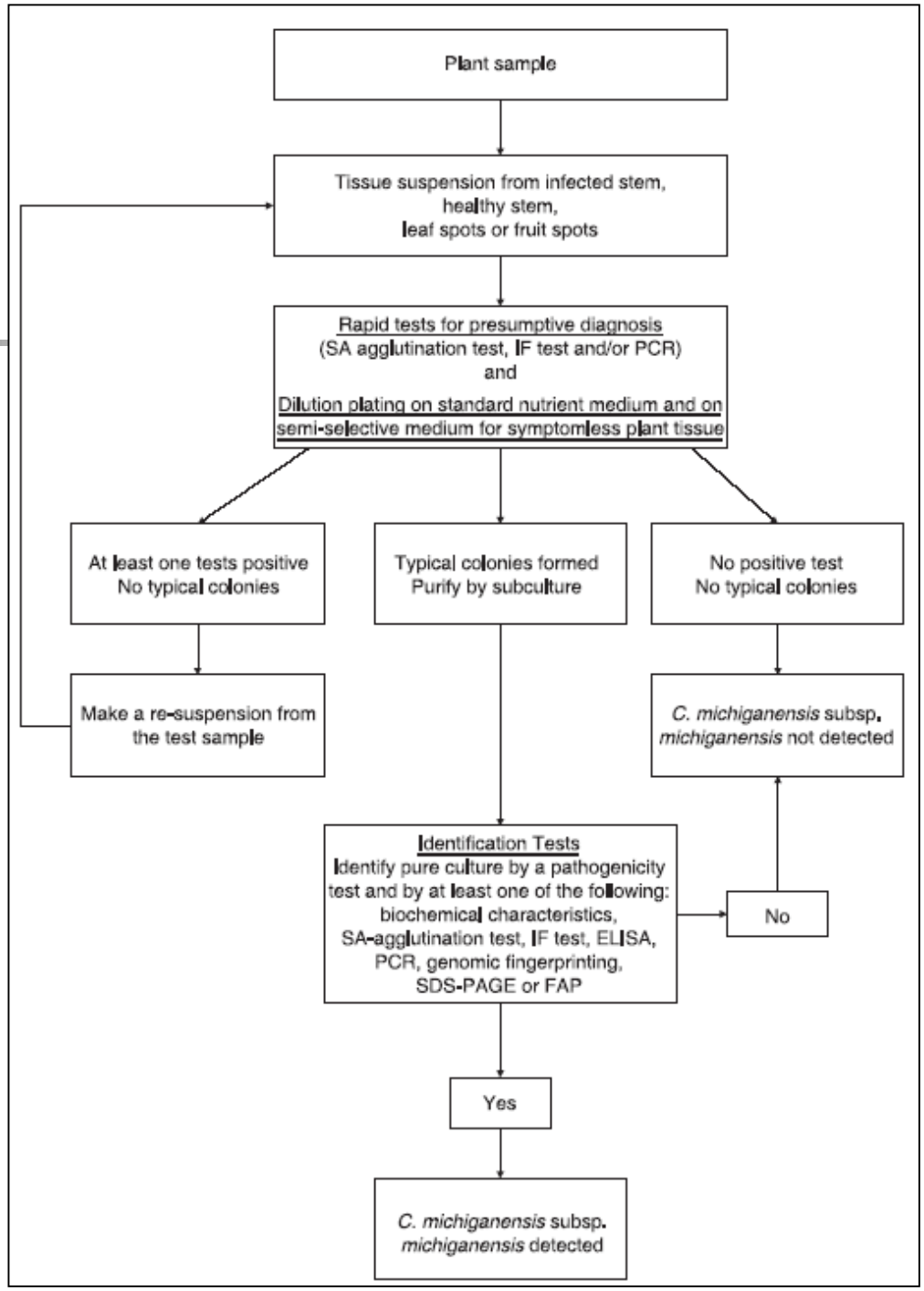
Bacterial wilt and canker of tomato

Clavibacter michiganensis

Agrios,2005

Diagnostic procedure

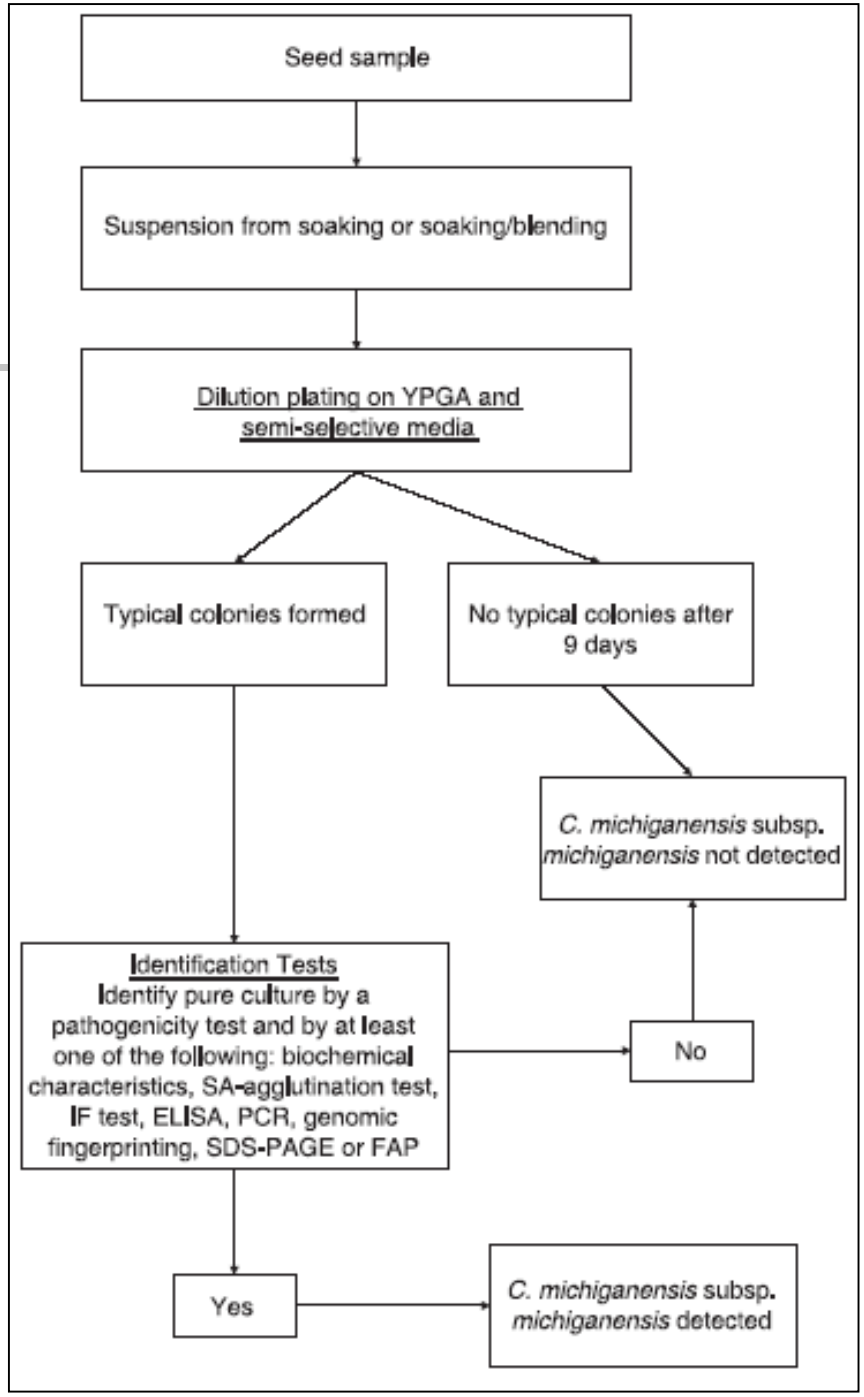
Scheme for detection and identification of *Clavibacter michiganensis* in samples from symptomatic or symptomless tomato plants.



EPPO,2005

The diagnostic procedure

Scheme for detection and identification of *C. michiganensis* in samples of tomato seeds.



Management
C. michiganensis

- At present, neither resistant tomato cultivars nor effective chemical controls of this pathogen are known (Thompson,1986).
- **Phytosanitary Measures**
- A variety of treatments can be used to eradicate seed-borne infection as for *C. michiganensis* subsp. *michiganensis* (EPPO/CABI 1996):
  - 0.8% acetic acid for 24 h, 5% HCl for 5-10 h (also for tobacco mosaic tobamovirus), 1.05% sodium hypochlorite for 30 min, 0.05% $HgCl_2$ for 5 min. Hot water treatment at 56°C for 30 min is another possibility.
  - All imported seed should be treated by such a method, or tested by a suitable procedure (OEPP/EPPO,1992).

Management

C. michiganensis

Fluorescent *Pseudomonas* Pf-17 and Pf-34 and *Paenibacillus alvei* K-165

- Alternatively (or additionally) the seed crop should have been found free from the disease.
- Biological control the soil phase of the disease with with rhizosphere bacteria belonging to *Pseudomonas* sp. (fluorescent *Pseudomonas* isolates Pf-17 and Pf-34) and K-165 strain of *Paenibacillus alvei*.
- These induce systemic resistance in tomatoes against *Clavibacter michiganensis* (*C.m.m.*).
- Fixed copper sprays may help in protecting healthy plants, particularly if only superficial symptoms are present.
- Fixed copper must come in direct contact with the bacteria to be effective.

Management
C. michiganensis

- Hot water treatment is preferred because the bacterial canker pathogen survives inside the seed coat and is not completely eliminated by surface disinfestations using Clorox, acid, or other treatments.
- Soil solarization technique:
- There are now many reports of the successful use of this technique.
- For example, Antoniou, Tjamas and Panagopoulos (1995), working in Greece, found that bacterial canker of tomato, caused by *Clavibacter michiganensis*, was controlled by mulching soil for 6 weeks with transparent polyethylene whereas methyl bromide applied at a rate of 70 g m^{-2} was ineffective.

Field Phage therapy

Different plant pathogenic bacteria

AgriPhage™

- AgriPhage utilizes bacteriophages, known as "bacteria eaters" which are naturally occurring organisms that infect and kill only targeted bacteria.
- AgriPhage™ is the first agricultural bactericide based on phage technology, and is currently registered for use on:

1. Tomatoes (*Clavibacter michiganensis* and *Pseudomonas syringae* pv. *tomato*),
2. Peppers (*Xanthomonas campestris* pv. *vesicatoria*),
3. Apples and pears (*Erwinia amylovora*), and
4. Citrus (*Xanthomonas citri*).

AgriPhage,2022

Field Phage therapy

Different plant pathogenic bacteria

AgriPhage™

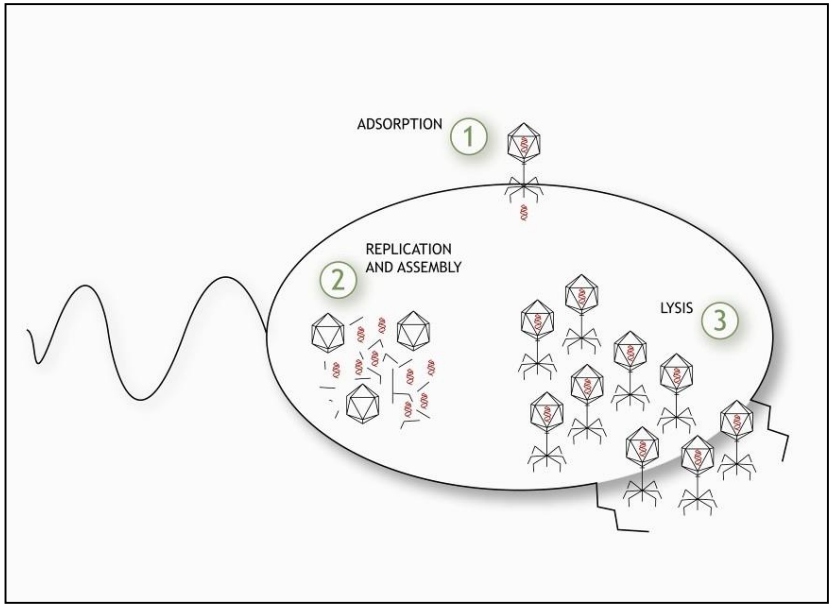
1. AgriPhage Fire blight (*Erwinia amylovora*).
2. AgriPhage XCV (*Xanthomonas campestris* pv. *vesicatoria*)
3. AgriPhage PST (*Pseudomonas syringae* pv. *tomato*)
4. AgriPhage CMM (*Clavibacter michiganensis*).

Field Phage therapy

Different plant pathogenic bacteria

AgriPhage™

- **HOW IT WORKS**
- Bacteriophage destroy bacteria in a process called "lysis".
- Lysing begins the moment a phage comes in contact with a bacterium and results in a release of additional phage within 30 minutes.



AgriPhage,2022

Field Phage therapy
Different plant pathogenic bacteria
AgriPhage™

- **HOW IT WORKS**
- Lysing can be described in three basic phases:

1. Adsorption occurs when a phage encounters a bacterium, attaches its tail fibers, and injects its own DNA into the bacteria. This action can begin as soon as you apply AgriPhage-CMM.
2. New phage can begin replicating and assembling within the bacterial cell, multiplying at a steady rate.
3. Finally, lysis is complete when the cell bursts and releases about 100 new phage into the environment to carry on the process. In this way, AgriPhage-CMM actually increases in efficacy over time.

Field Phage therapy

AgriPhage™

Clavibacter michiganensis

- AgriPhage-CMM is a bactericide used as a preventive and curative product for the suppression of bacterial canker on greenhouse tomato caused by *Clavibacter michiganensis*.
- AgriPhage-CMM may be applied as a foliar spray alone, in alternating spray programs or in tank mixes with other registered crop protect ion products.
- For maximum effectiveness, apply AgriPhage-CMM prior to or at the early onset of disease development or when conditions are conducive to heavy disease pressure.
- Thorough coverage and wetting of all foliage are essential for effective disease control.

AgriPhage,2022

Field Phage therapy

AgriPhage™

Clavibacter michiganensis

- **DIRECTIONS FOR USE:**
- AgriPhage-CMM may be applied as a foliar spray alone, in alternating spray programs or in tank mixes with other registered crop protect ion products.

Seedling Treatment		
RATE	**APPLICATION**	**INSTRUCTIONS**
12 ml of AgriPhage-CMM per 100 square meters of greenhouse space.	Begin applications to seedlings, (at the 4 leaf stage), immediately after planting or grafting, prior to or at early stages of disease development.	Apply treatments in 3-4 day intervals. Use sufficient water to ensure complete coverage.

Hydroponic Greenhouse Treatment		
RATE	**APPLICATION**	**INSTRUCTIONS**
40ml of AgriPhage-CMM per 100 square meters of greenhouse space.	Begin applications prior to or at early stages of disease development and continue throughout the growing season.	Apply treatments in 3-4 day intervals. Use sufficient water to ensure complete coverage.

DO NOT allow effluent or runoff from greenhouses containing this product to enter lakes, streams ponds, or other waters. DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes. The spray solution should be used within 24 hours of being prepared. AgriPhage-CMM may be applied up to and including the day of harvest.

DO NOT allow effluent or runoff from greenhouses containing this product to enter lakes, streams ponds, or other waters. DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes. The spray solution should be used within 24 hours of being prepared. AgriPhage-CMM may be applied up to and including the day of harvest.

AgriPhage,2022

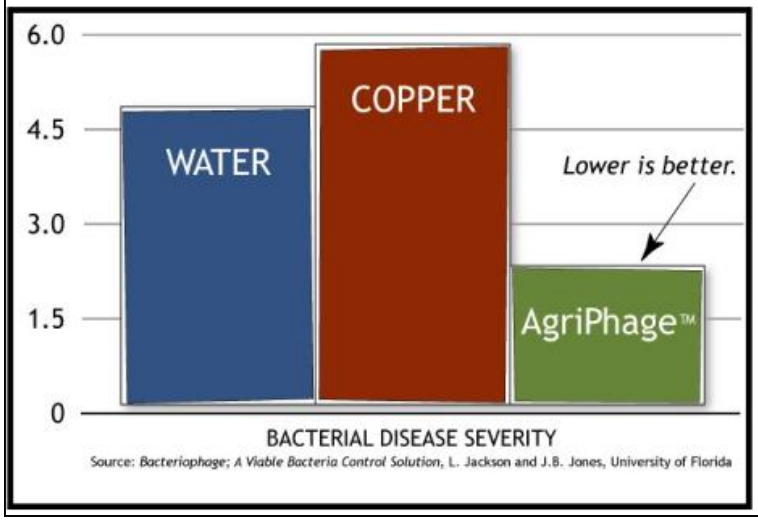
Field Phage therapy

AgriPhage™

Clavibacter michiganensis

- Treating plants preventatively with AgriPhage-CMM can help to prevent the spread of harmful bacteria before damage occurs.
- If bacterial damage has already occurred, bacterial damage has already occurred, AgriPhage-CMM can stop the spread of disease in the infected plant, and can protect surrounding healthy plants from further spread of disease.

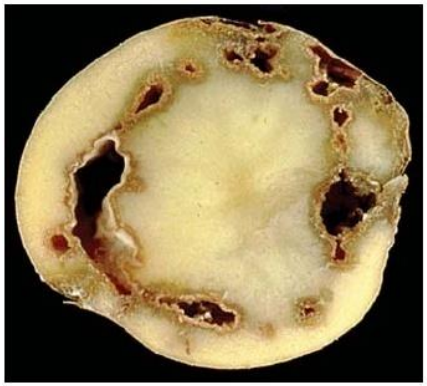
- ☑ Reduce Spread of Disease
- ☑ Increase Fruit Yield
- ☑ Grow Healthier Plants
- ☑ Protect Soil and Beneficial Organisms

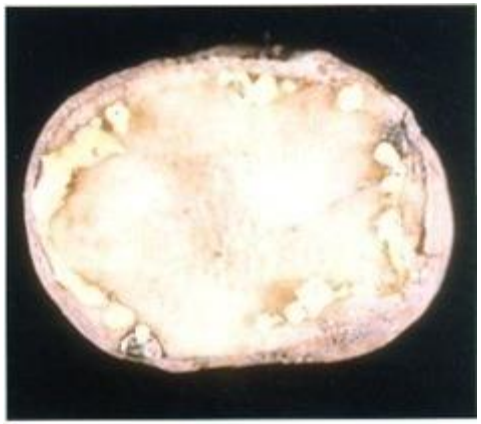


AgriPhage,2022

Clavibacter michiganensis subsp. *sepedonicus*

Ring rot of potato



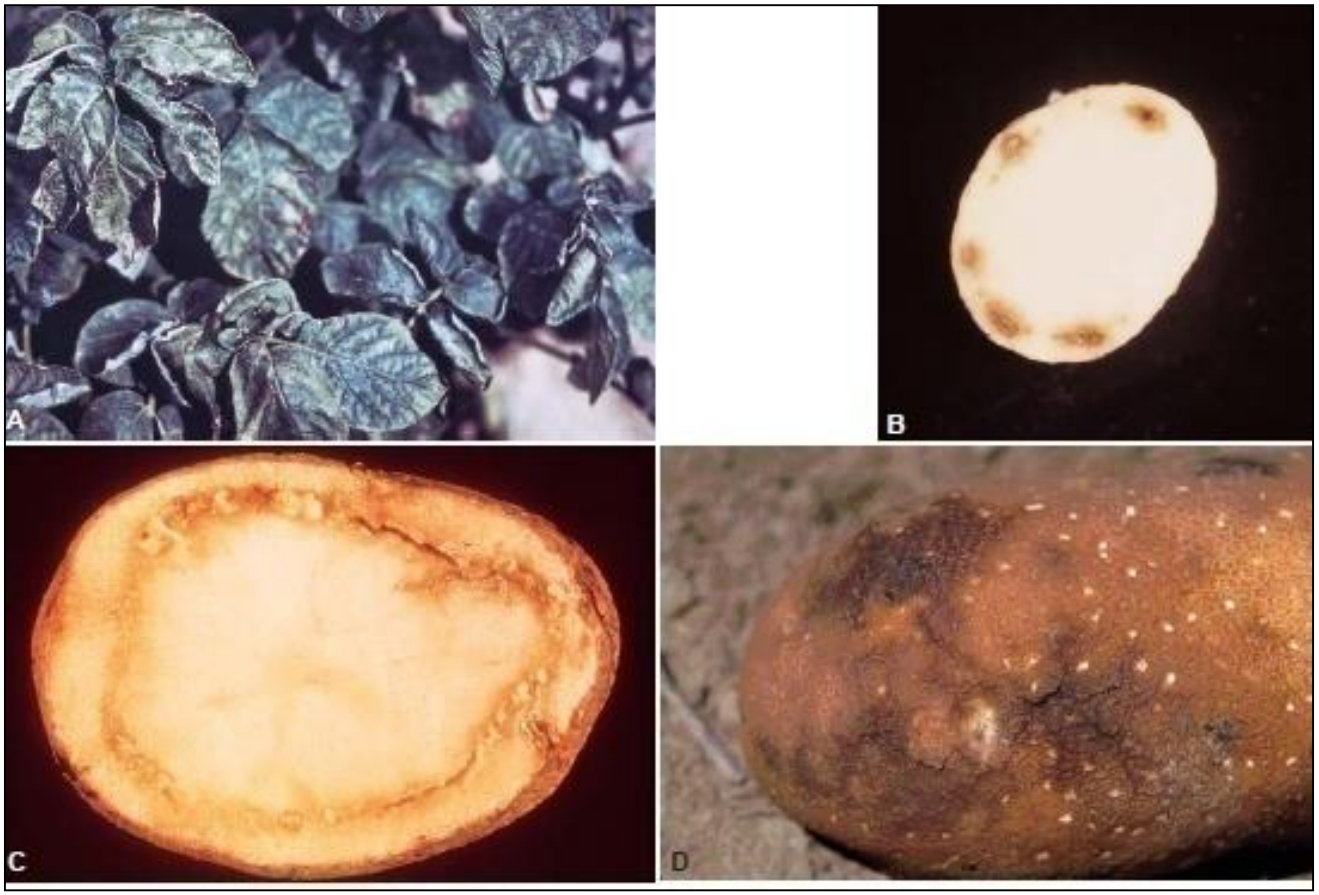


Survival

Clavibacter michiganensis subsp. *sepedonicus*

- The only natural host on which *C. m. sepedonicus* causes disease is potato.
- However, as a natural symptomless host also sugarbeet (Bugbee and Gudmestad, 1988) and in artificial inoculation tests many members of the *Solanaceae* have been described.
- Knowledge of survival is usually essential to intervene in dissemination and for disease management.
- *Clavibacter michiganensis* subsp. *sepedonicus*, causative agent of potato ring rot, is notoriously known for surviving on machinery and packaging material.

Potato ring rot disease caused by *Clavibacter michiganense* subsp. *sepedonicum*



Agrios,2005

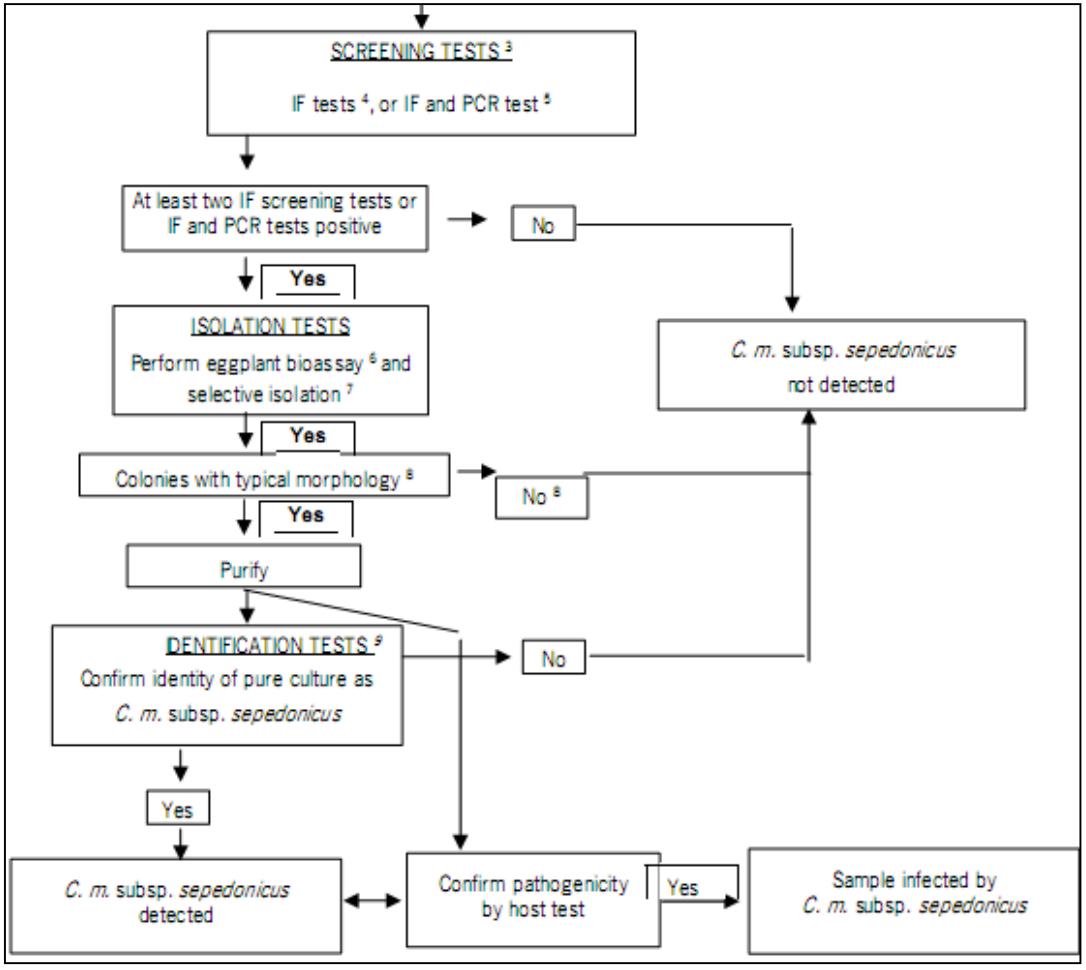
Estimate of value of reduction in bacterial ring rot and spindle tuber

Bacterial ring rot and potato spindle tuber used to be common diseases

	1941-1965 levels	2005 dollars
Bacterial Ring Rot	12%	$2,400,000,000
Spindle Tuber	5%	$2,400,000,000
Total		

- Estimate of acreage fees and inspection fees in US = $5 million/year.
- Can this disease be eradicated?

Scheme for detection and identification of *Cms* in samples of potato tubers



van der Wolf *et al.*,2005

Eradication

Strict regulations (zero tolerance)

Clavibacter michiganensis subsp. *sepedonicus*

- Bacterial ring rot (BRR) is a "zero tolerance" disease, which means that if a single positive plant is found in a field the entire seed lot can be rejected.
- Due to recent changes in Idaho, all seed lots are now lab tested for BRR.
- It's the first state to take such drastic measures, but experts say the move is necessary.

Melanie Epp,2014

Management

Clavibacter michiganensis subsp. *sepedonicus*

- Plant only certified disease-free seed tubers.
- In the U.S. and Canada, certified seed potatoes are produced under regulations mandating zero tolerance for ring rot.
- Although use of certified seed tubers will not guarantee total freedom from ring rot bacteria, it is the best assurance.
- Discontinue use of any lot of seed tubers in which ring rot is found.
- Seed lots known to be contaminated with ring-rot bacteria should never be planted.
- Before handling seed tubers, all containers, tools, knives and mechanical cutters, planters, and other equipment should be thoroughly washed with a detergent solution, rinsed, and then sanitized with a disinfectant.

Management

Clavibacter michiganensis subsp. *sepedonicus*

- When cutting seed tubers, the cutting tool should be periodically washed and sanitized.
- It is essential that this be done before cutting seed tubers from a different source.
- To be effective, disinfectants must be present for a minimum of 10 minutes (preferably 20-30 minutes) on any surface being treated.
- It is much easier to disinfect metal surfaces than wood or burlap.
- Dispose of all infected tubers away from potato production areas.
- Do not plant potatoes for two seasons in any field in hich ring rot has been found.

Clavibacter nebraskensis

Goss' bacterial wilt and blight
(leaf freckles and wilt)

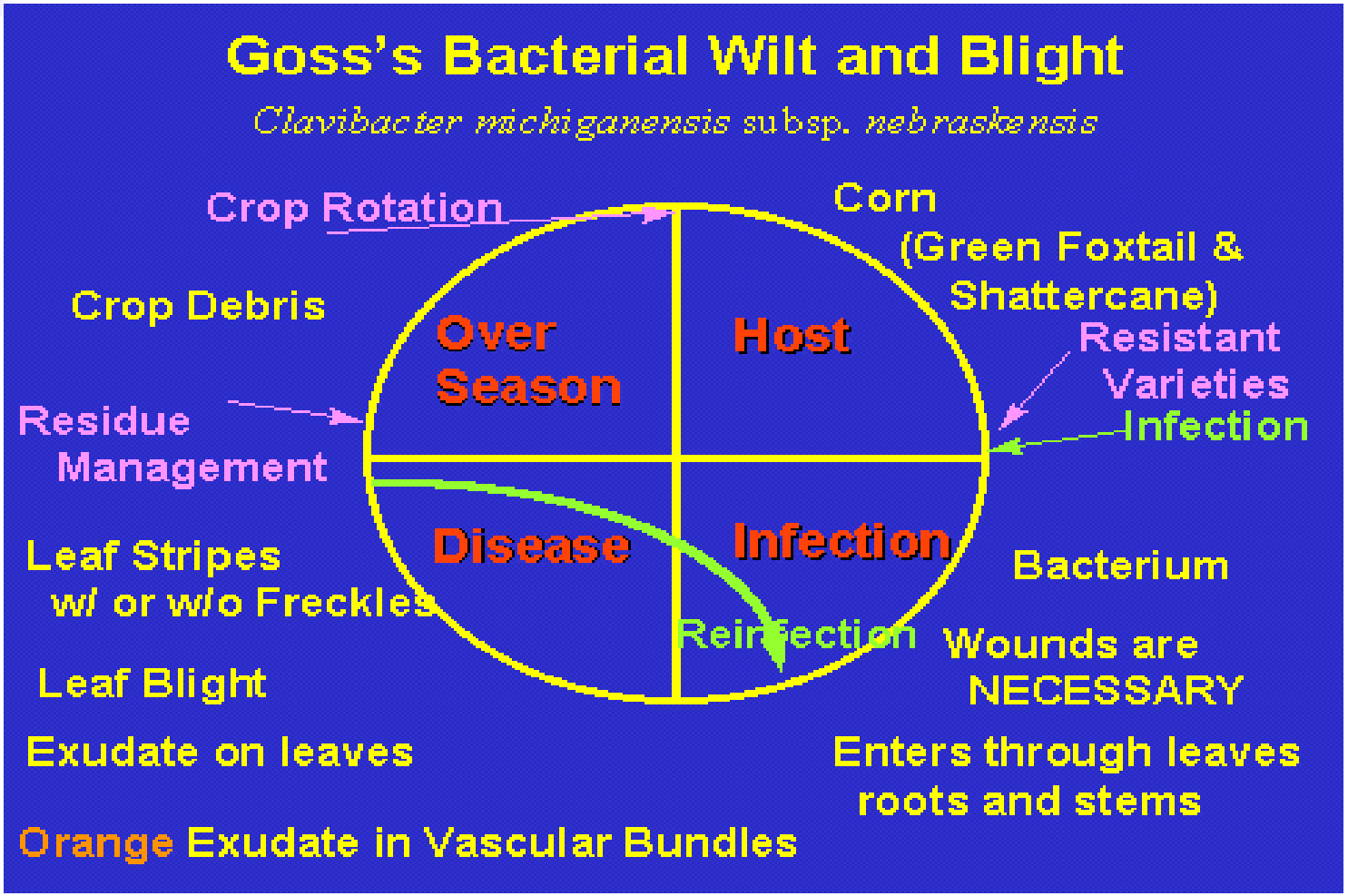
Disease symptoms

Clavibacter michiganensis

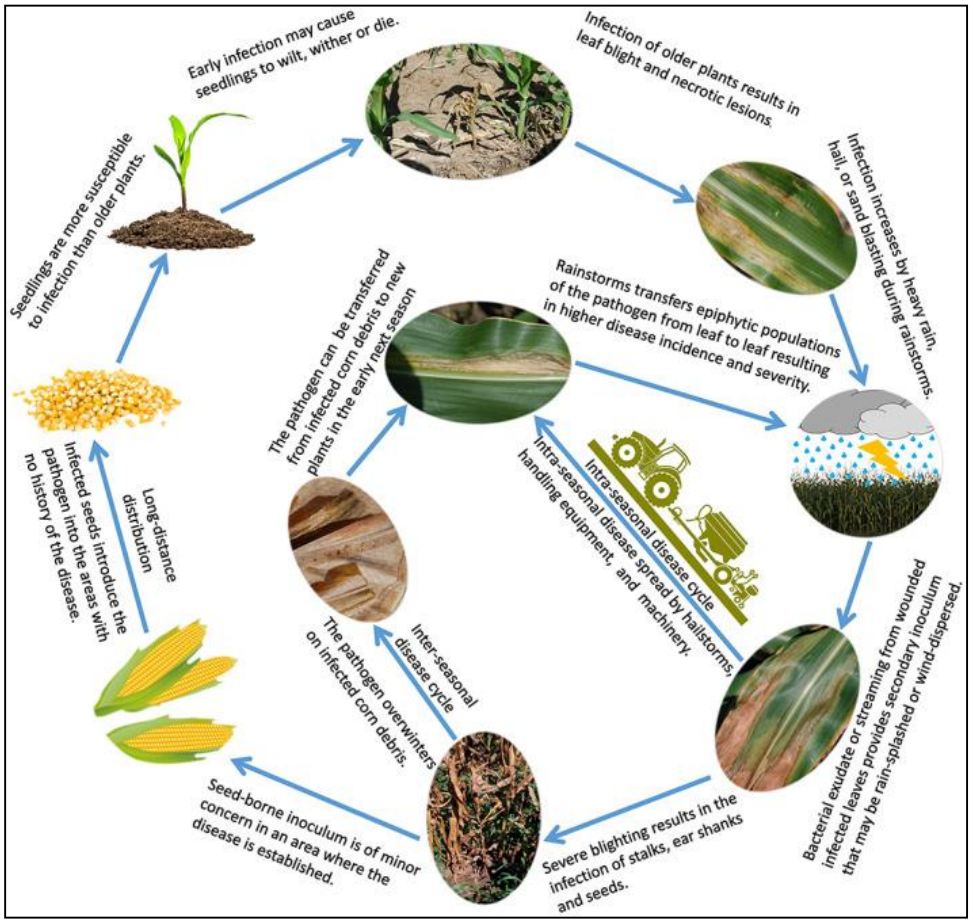
- *C. nebraskensis* is a causative agent of leaf spot ('freckles'), leaf blight and wilt of maize or corn (*Zea mays*).
- This disease is also called Goss's disease.
- Hosts of the bacterium include corn, green foxtail, barnyard grass and shattercane.
- Infection of the plant with *C. nebraskensis* needs wounds and may be direct in leaves or via the roots and stems. The wounding may result from sand, blasting hail, rainstorms, or strong wind.
- The symptoms of *C. nebraskensis* induced maize disease can be easily confused with Stewart's bacterial wilt.
- Characteristic are discrete lesions with water-soaked streaks which are parallel to the leaf veins.
- Drops of bacterial exudate appear on the surface of the leaves when the streaks enlarge.
- After drying these droplets leave a crystalline sheen.

Eichenlaub *et al.*,2006

Disease cycle of
Clavibacter nebraskensis



Disease cycle of
Clavibacter nebraskensis



Osdaghi *et al.*,2023

Management

- **Resistant Hybrids**
- Resistant material is available for field corn and sweet corn but little is available for popcorn.
- **Residue Management**
- Destruction of crop residue will lower the amount of inoculum available.
- However, this practice is not practical in a conservation tillage operation.
- Rotation to a nonhost crop such as soybeans, dry beans or alfalfa also reduces the amount of corn residue and is a more viable option in most situations.

Curtobacterium flaccumfaciens pv. *flaccumfaciens*

Bacterial wilt of dry beans

Disease cycle of
Curtobacterium flaccumfaciens pv. *flaccumfaciens*

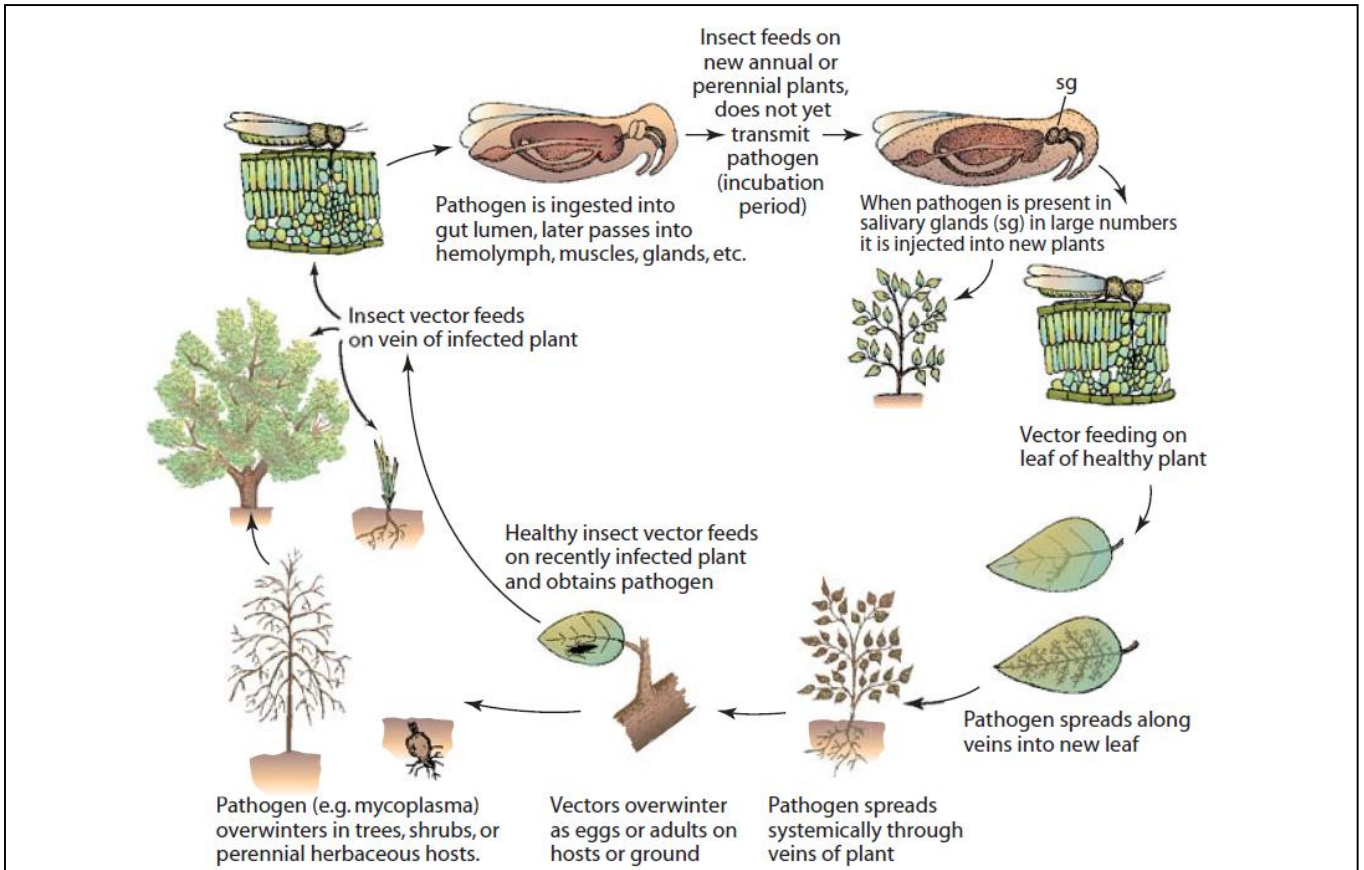


FIGURE 12-55 Sequence of events in the overwintering, acquisition, and transmission of fastidious bacteria, mollicutes, and viruses by leafhoppers and related insect vectors.

See also phytoplasma section

Management

- The resistant cultivar Emerson was developed many years ago by the University of Nebraska specifically for controlling bacterial wilt, but breeding for resistance to this disease was later discontinued.
- Using seed sanitation, producing disease-free seed in dry climates, and using proper crop rotations effectively stopped the introduction of the pathogen while eventually eliminating it from fields where it had been present.
- Similar management measures used for other bacterial diseases, such as foliar applications of copper-based bactericides and seed treatment with antibiotics like streptomycin, also are moderately effective for reducing yield losses and limiting plant-to-plant spread.
- The most important factor, however, is to stop introducing and maintaining the pathogen in dry bean fields.

Leifsonia xyli subsp. *xyli*

Ratoon stunt of sugar cane

Pinkish dicoloration of vascular bundles

Ratoon stunt of sugar cane

- Ratoon stunt disease (RCD) of sugar cane (*Leifsonia* (=*Clavibacter*) *xyli* subsp. *xyli*) caused cane losses of 14 per cent in the first year of cultivation but this increased to 27 per cent in the third year (Grisham,1991).

Ratoon stunting of sugarcane

Leifsonia xyli

- Small with rippled cell wall, Gram (+)
- No flagella
- Grows on complex nutritional media
- Transmitted by seed

www-plb.ucdavis.edu

Cuppels Biology 418a

www.tpp.uq.edu.au

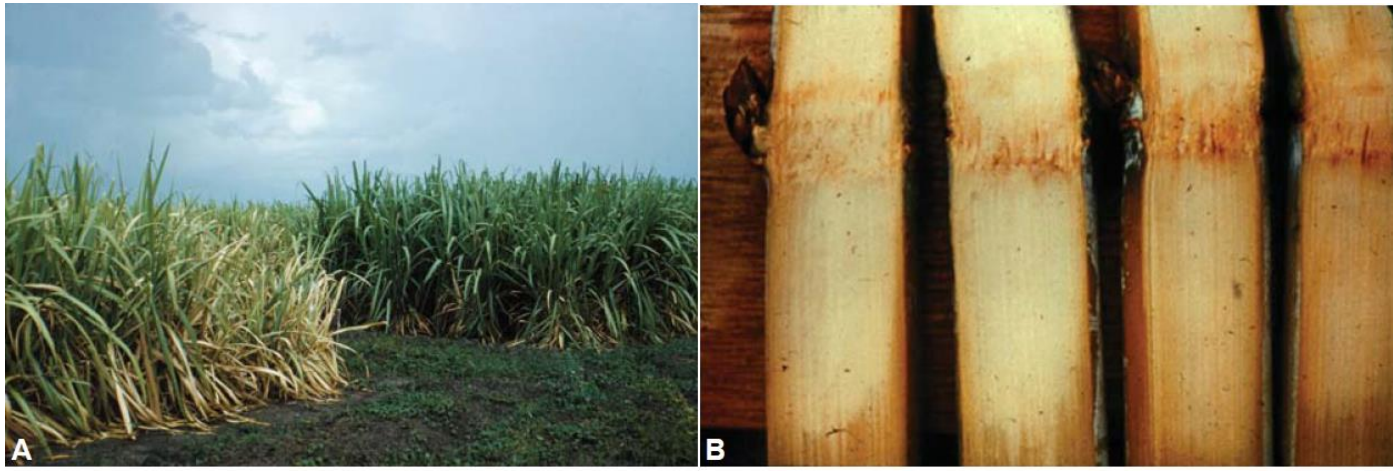


FIGURE 12-49 Sugarcane ratoon stunt disease. (A) Sugarcane planted with infected ratoons (left) and with hot-water treated cane (right). (B) Pinkish discoloration of stem at area of node due to infection by the bacterium. [Photographs courtesy of (A) H. D. Thurston, Cornell University and (B) A. G. Gillespie, USDA.]

Agrios,2005

Management

Ratoon stunt of sugar cane

Leifsonia xyli subsp. *xyli*

- Since RSD bacteria are easily transmitted mechanically, sanitation is important in preventing healthy cane from becoming infected.
- Chemical disinfectants that may be used on cane cutting knives include Lysol, Dettol, ethanol, Mirrol and Roccal.
- At least 5 minutes of contact with the cutting surface is needed to assure disinfection, but a shorter disinfection time will be partially helpful.
- Hot-water treatment (50°C for 2-3 hours) is the method most commonly used to control RSD.
- The use of resistant clones has been shown to control RSD.
- CP 72-2086 has been grown in Florida without either hot-water treatment or sanitation with less than two percent disease incidence in sampled fields.

Rhodococcus fascians

Malformations in a wide variety of host plants

Malformations on tobacco

Rhodococcus fascians
Leafy gall and fasciation diseases

- *Rhodococcus fascians* is the cause of malformations in a wide variety of host plants, the most severe being a leafy gall and shoot proliferations.
- *Agrobacterium* caused crown gall-like tumors and *R. fascians* produced leafy galls and shoot proliferations.
- Outbreaks of the disease on ornamental plants may cause serious financial losses.

Rhodococcus fascians
Leafy gall and fasciation diseases

- Both *R. fascians* and *A. tumefaciens* are known to infect herbaceous and woody plants.
- Both bacteria have a wide host range (over 60 species for *R. fascians*, and hundreds for *A. tumefaciens*).
- In addition, *R. fascians* infects monocots as well as dicots, unlike *A. tumefaciens*, which infects only dicots.

Management
Rhodococcus fascians

- Unfortunately, there is no treatment for either *R. fascians*, therefore steps must be taken to prevent disease.
- NoGall and BlightBan were did not offered any control.
- **Sanitation:**
- Start with clean planting trays, preferably new.
- Potting mix or field soil should be pasteurized (60 minutes at 160 F aerated steam) before use.
- Knives or razor blades should be changed or sterilized between plants during propagation.
- Keep plants off the greenhouse floor and solid surfaces.
- Runoff water can disperse the bacteria.
- Immediately remove and destroy any diseased plants plus any neighboring plants or trays.

Putnam and Miller

Streptomyces scabiei

Common scab

Raised, tan to brown, corky lesions of potato scab.

Disease cycle of common scab of potato caused by *Streptomyces scabies*

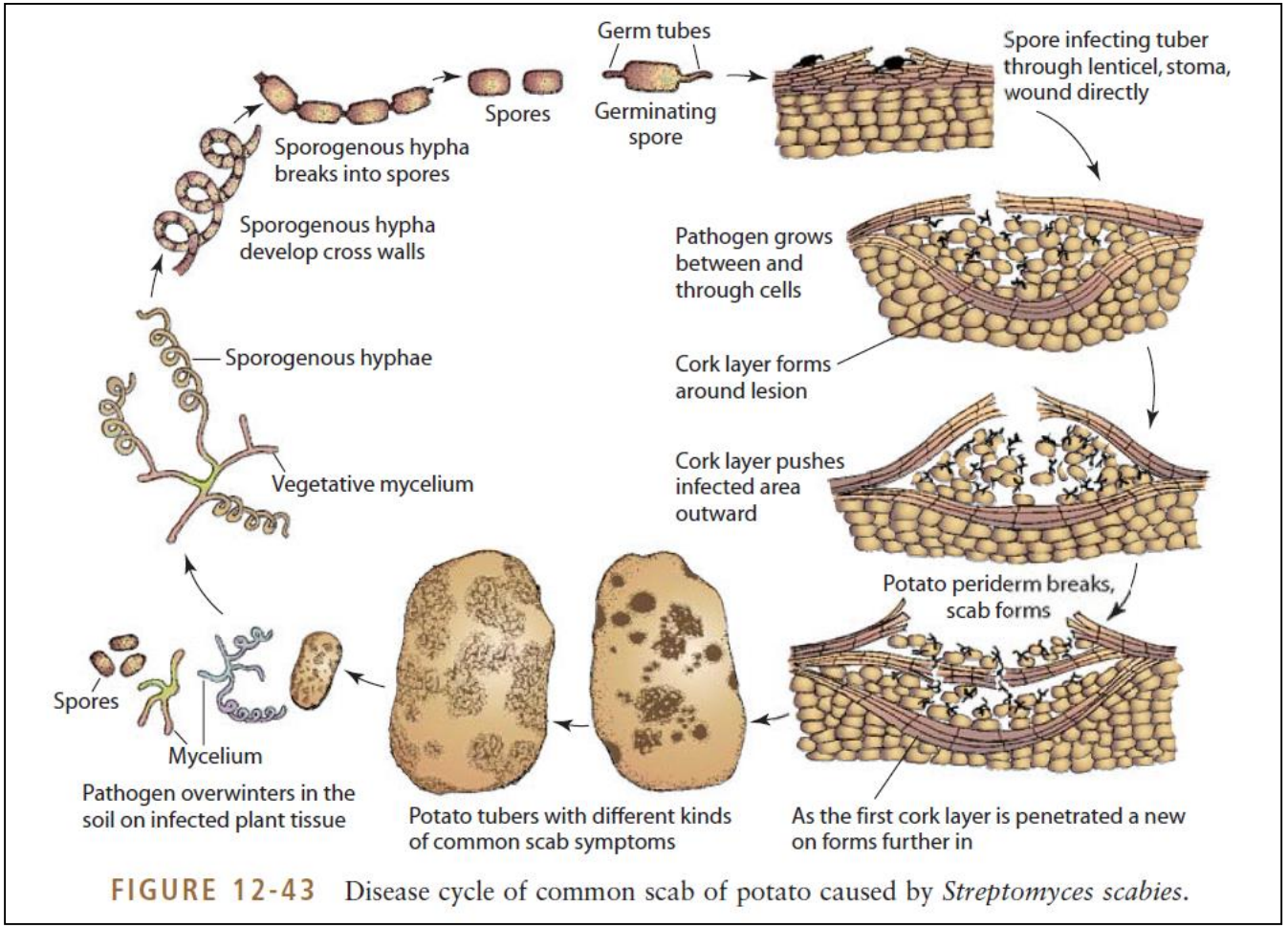


FIGURE 12-43 Disease cycle of common scab of potato caused by *Streptomyces scabies*.

Agrios,2005

Management
Streptomyces scabies

1. Use resistant varieties in fields where scab is a problem.
2. Use scab-free seed and seed treatments to prevent introduction of the pathogen into fields. Seed treatments do not eliminate the pathogen but will provide some suppression of disease. Consult current potato disease-control recommendations for appropriate seed treatments.
3. Rotate heavily infested fields away from potatoes and alternate hosts such as radish, beets, and carrots. Use small grains, corn, or alfalfa in rotations; avoid red clover.
4. Maintain soil pH levels between 5.0 and 5.2 by using acid-producing fertilizers such as ammonium sulphate. Avoid or limit the use of such alkaline-producing amendments as lime and manure.
5. Avoid moisture stress during the 2 to 6 weeks following tuberization.

Loria,1991

Management
Streptomyces scabies

- **Cultural control**

1. Maintaining soil moisture levels near field capacity(-0.4 bars) during the 2 to 6 weeks following tuberization will inhibit infection. However, maintaining high soil moisture may be difficult in some soils, and it is possible that other disease problems may be aggravated by excessive irrigation.

- **Host resistance**

1. Planting resistant cultivars is probably the best and easiest way to combat common scab. However, resistant varieties are not immune and will become infected if soil inoculum densities are high and conditions are favorable.

- **Chemical control**

1. The chemical pentachloronitrobenzene (PCNB), also known as Blocker® (Amvac) has been tested.
2. Pic-plus (chloropicrin) has shown some efficacy in trials.

Management
Streptomyces scabies

- **Fungicides:**
- Mancozeb, coppers, streptomycin, PCNB seed treatment or in furrow application purported to reduce scab
- Generally not effective; not consistent
- May reduce seed-borne inoculum, but no effect on soil-borne inoculum, which is probably the main source.
- **Insecticides:**
- Mocap (etheprop) purported to reduce scab by controlling soil insects (springtails, flea beetle larvae) feeding on tubers that make injuries that can act as entry sites; importance not known.
- **Soil fumigation:**
- Vapam (sodium isothiocyanate) may actually make scab worse by killing suppressive soil micro-organisms
- Continuing work with chloropicrin (tear gas) shows good control of pitted scab (ON, WI, MI, FL).

Secor,2013

Plant bacterial disease management

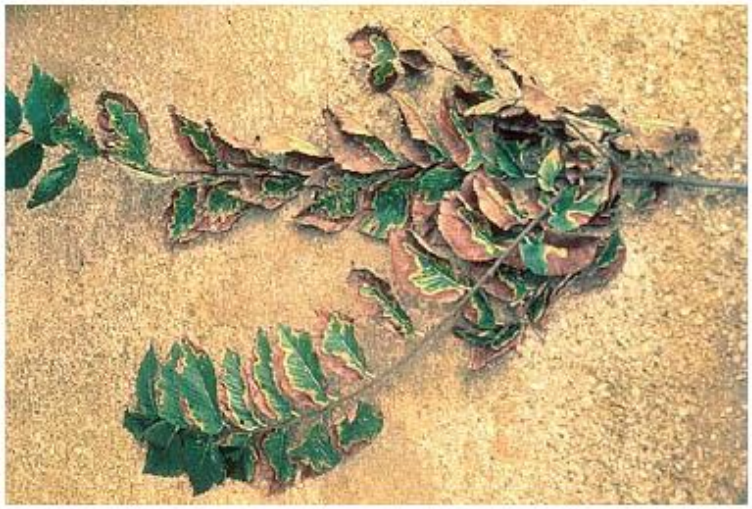
Fastidious bacteria

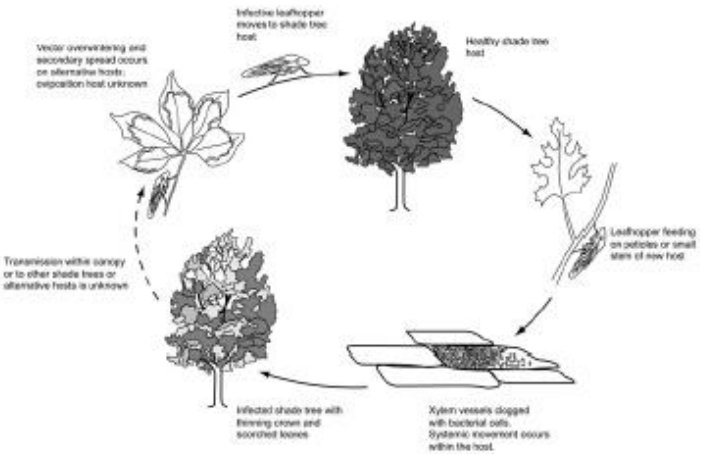
Xylella fastidiosa

Scorch diseases

Pierce's disease

Bacterial Leaf Scorch of Shade Trees





http://www.apsnet.org/education/LessonsPlantPath/BLS/default.htm

BLS Symptoms:

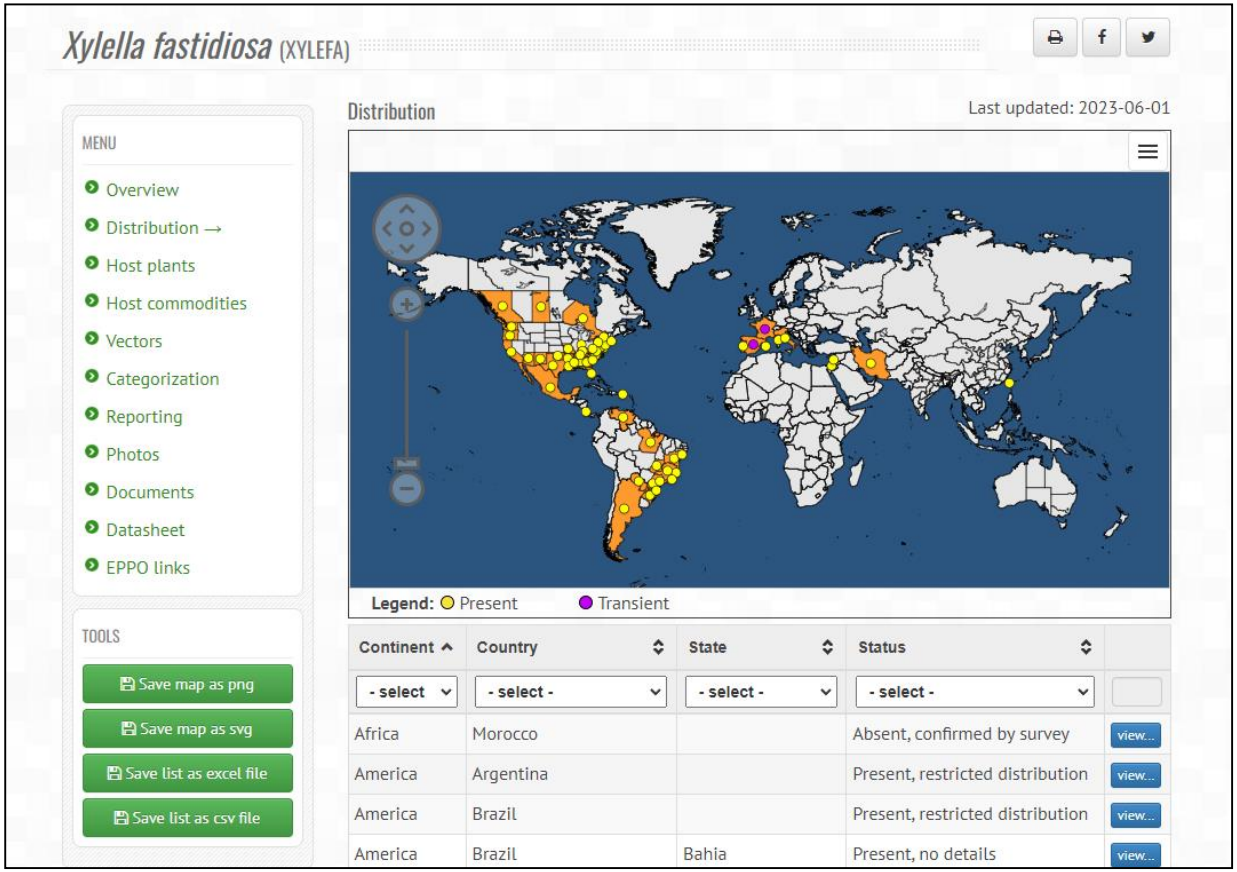
- **Bacterial: irregular, 'tri-color' scorch, older leaves more severely affected**
  - **Abiotic scorch: uniformly affects new and older leaves**

<u>Management for Xylella Diseases</u>

1. **Maintain plant vigor – avoid stresses**
2. **Sanitation – remove dead branches and declining trees**
3. **Plant tolerant plants – Muscadine grapes**
4. **Insecticides to control vectors not effective**

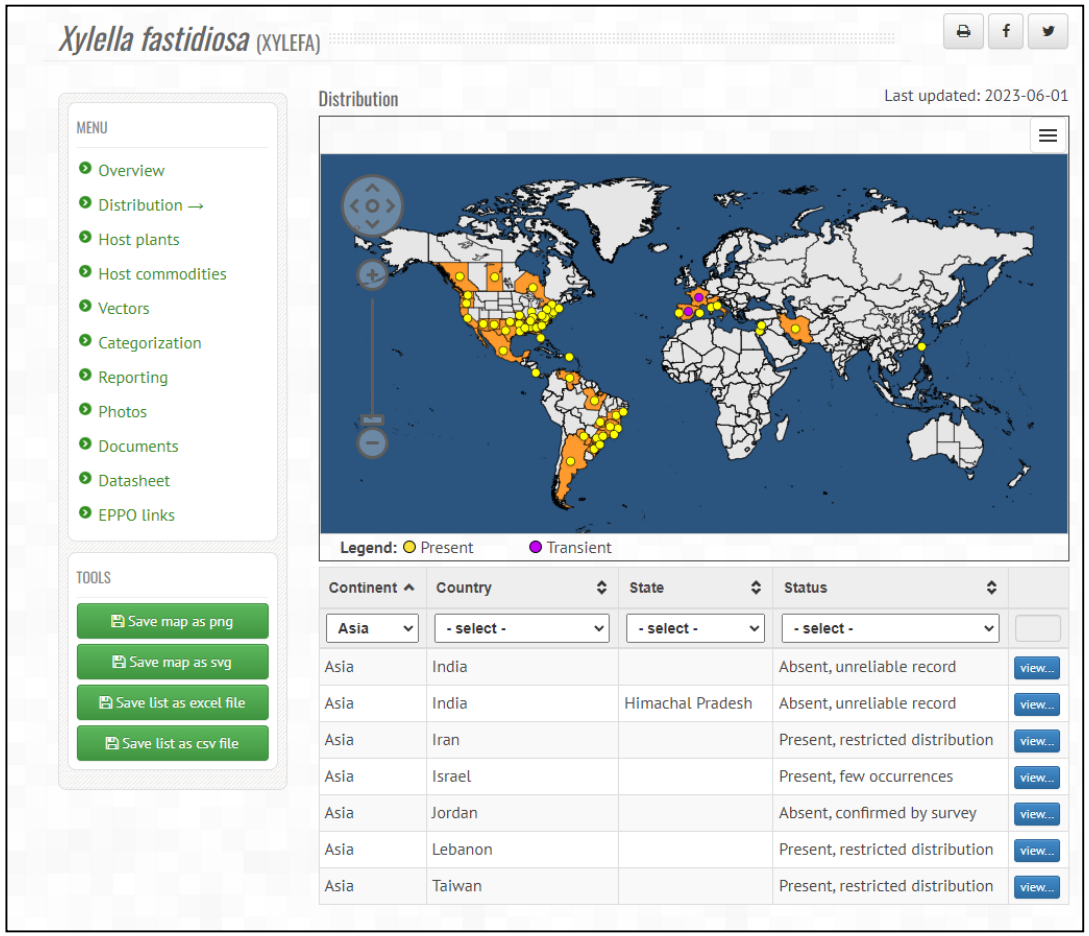
Future: Cross-protection with avirulent *Xf* ?

Geographical distribution of *Xylella* spp. Worldwide



EPPO Global Database,2023

Geographical distribution of *Xylella* spp. In Asia

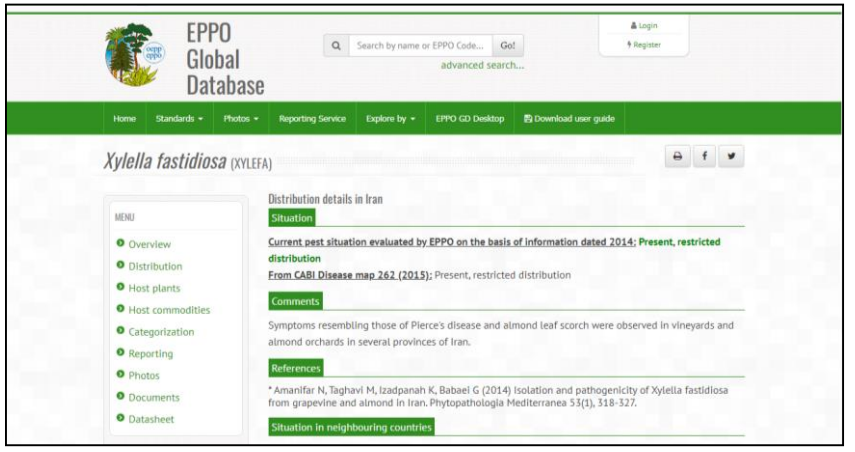


EPPO Global Database,2023

Geographical distribution of *Xylella* spp. In Iran

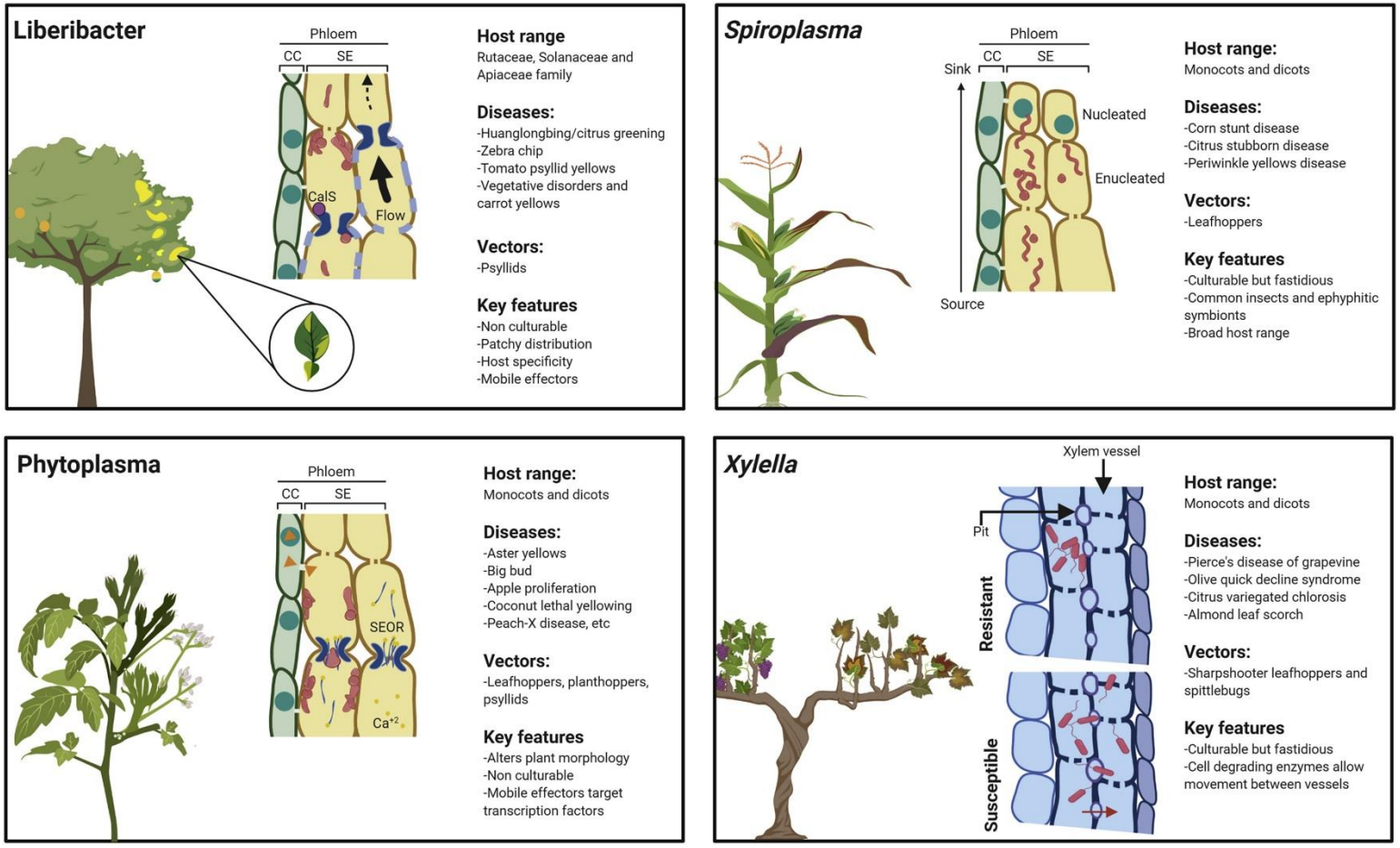
- Symptoms resembling those of Pierce's disease and almond leaf scorch were observed in vineyards and almond orchards in several provinces of Iran.
- Amanifar N, Taghavi M, Izadpanah K, Babaei G (2014). Isolation and pathogenicity of *Xylella fastidiosa* from grapevine and almond in Iran. Phytopathologia Mediterranea 53(1), 318-327.

Asia	India		Absent, unreliable record	view...
Asia	India	Himachal Pradesh	Absent, unreliable record	view...
Asia	Iran		Present, restricted distribution	view...
Asia	Israel		Present, few occurrences	view...
Asia	Lebanon		Absent, invalid record	view...
Asia	Taiwan		Present, restricted distribution	view...



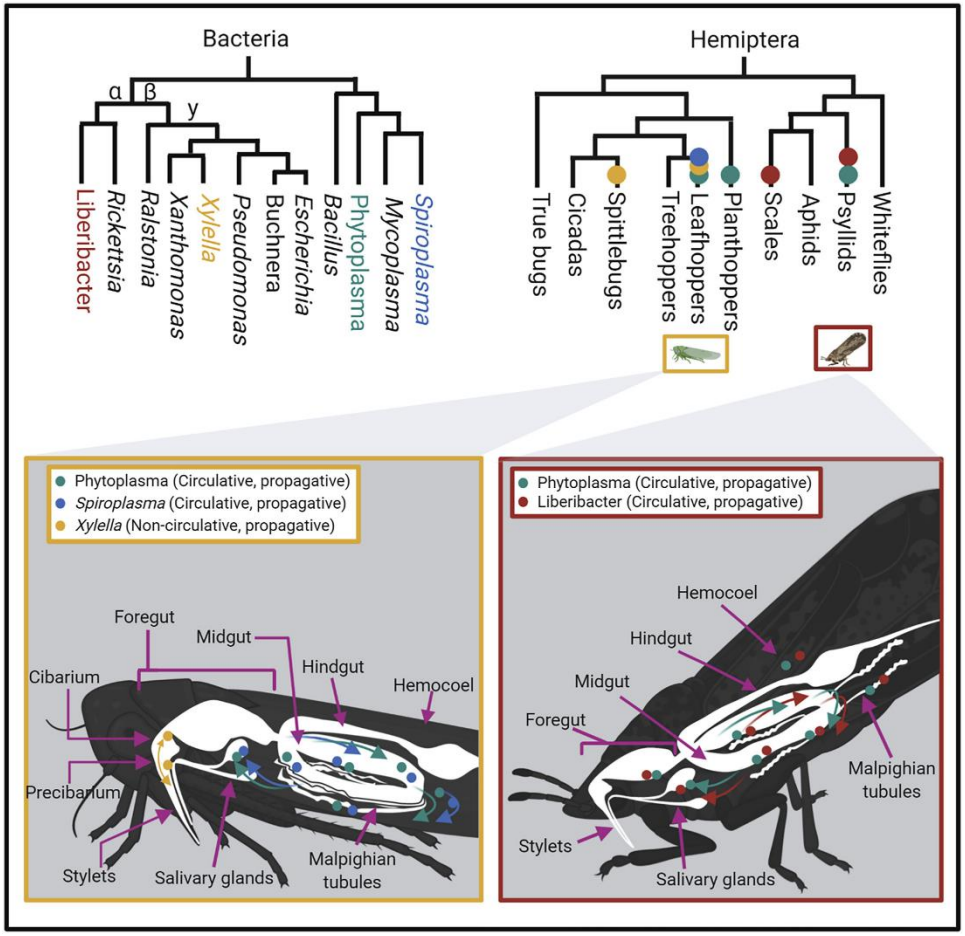
EPPO (European and Mediterranean Plant Protection Organization), online, 2022. EPPO global database. Available online: https://gd.eppo.int [Accessed: 31 August 2022]

Bacterial vector-borne diseases that persist in plant vascular tissues



Huang *et al.*,2020

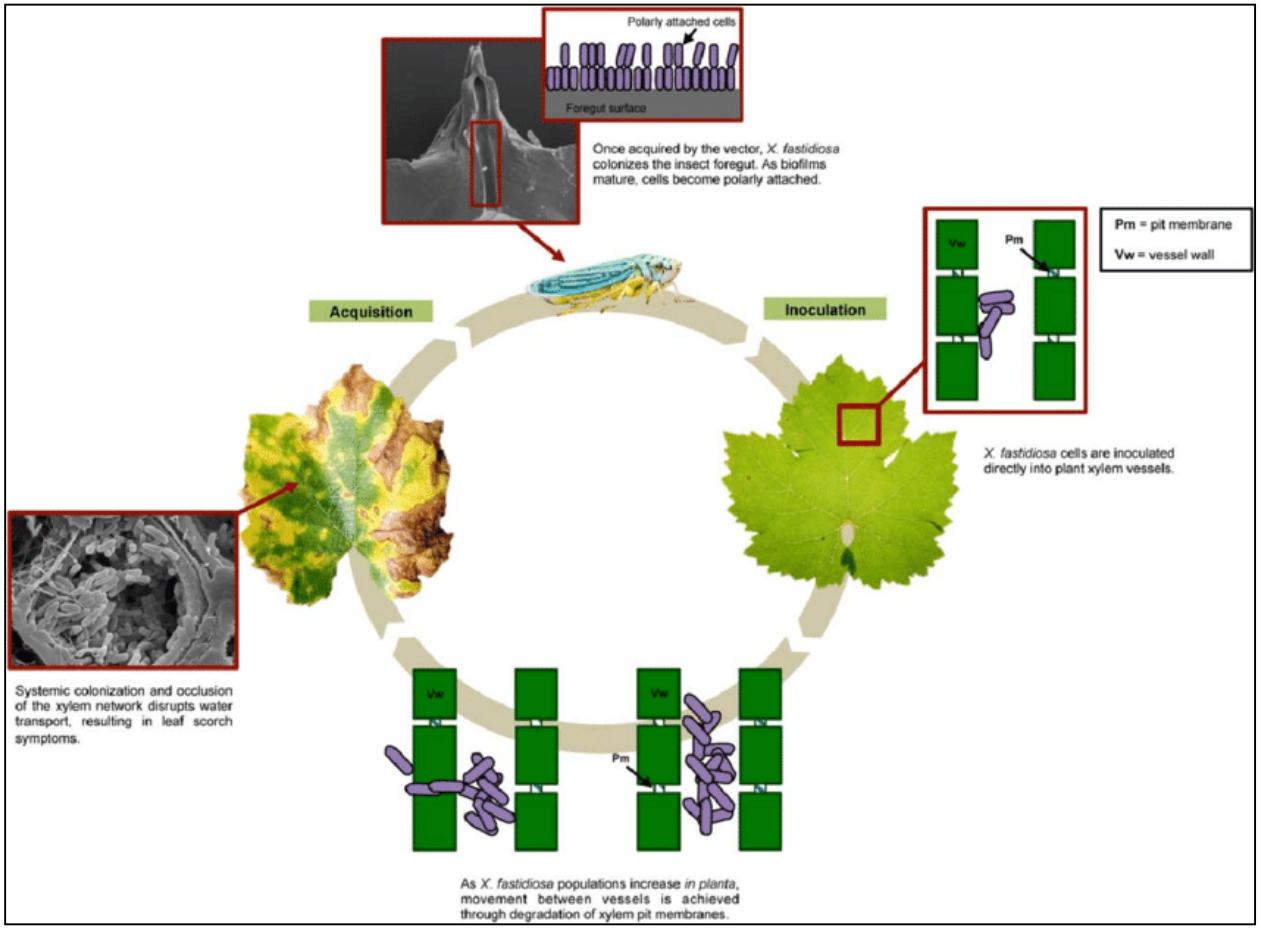
Circulative Propagative and Non-circulative Propagative Bacterial Localization in Insect Vectors through the Perspective of Phylogenetic Relationships



Huang *et al.*,2020

Pierce's disease cycle

Xylella fastidiosa

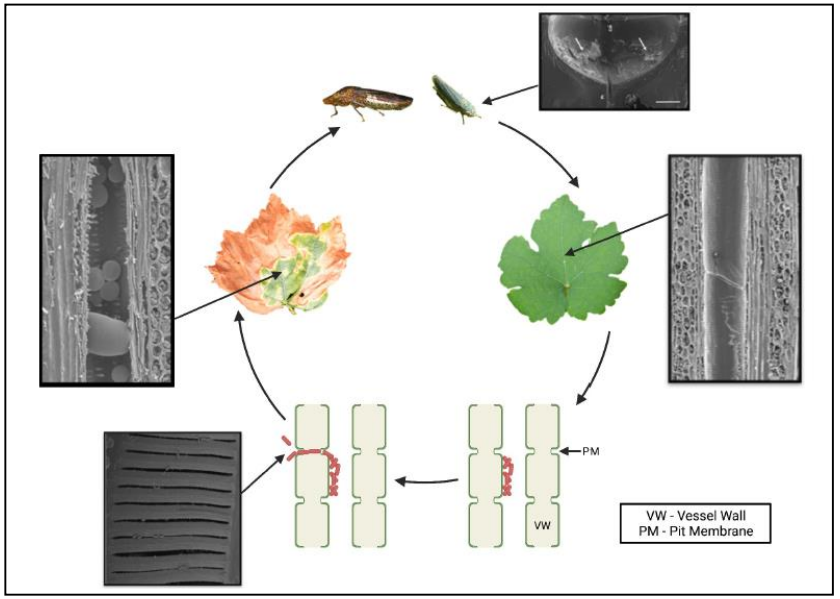


Rapicavoli *et al.*,2017

PD of grapevine cycle

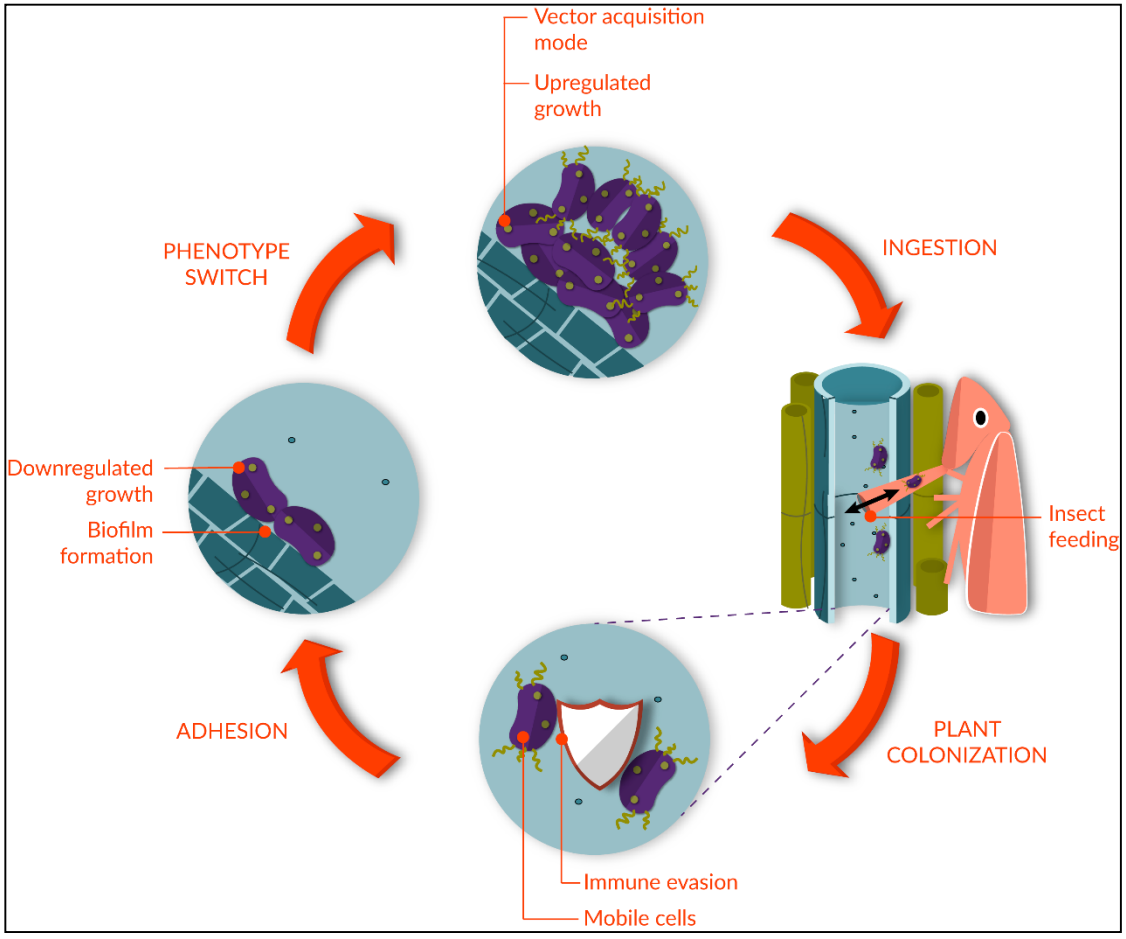
Role of insect vectors, such as the GWSS and the BGSS and excess tylose production in the xylem lead to PD symptom development

- *Xylella fastidiosa* is acquired by its xylem-feeding insect vectors, such as the GWSS and the BGSS, during the feeding process. Once acquired, it colonizes the insect's foregut and forms robust biofilms (**indicated by white arrows**). *Xylella fastidiosa* is transmitted to a new host plant when the insect vector feeds on a new plant and deposits *Xylella fastidiosa* cells directly into the plant xylem. *Xylella fastidiosa* achieves systemic colonization of the xylem by enzymatic degradation of the xylem pit membranes that connect adjacent xylem vessels.
- *Xylella fastidiosa* colonization induces prolific production of balloon-shaped defense related protrusions called tyloses in the xylem. Systemic colonization and vessel occlusion by bacterial biofilms and excess tylose production lead to PD symptom development.
- GSS, blue-green sharpshooter; GWSS, glassy-winged sharpshooter; PD, Pierce disease; PM, pit membrane; VW, vessel wall.



Castro *et al.*,2021

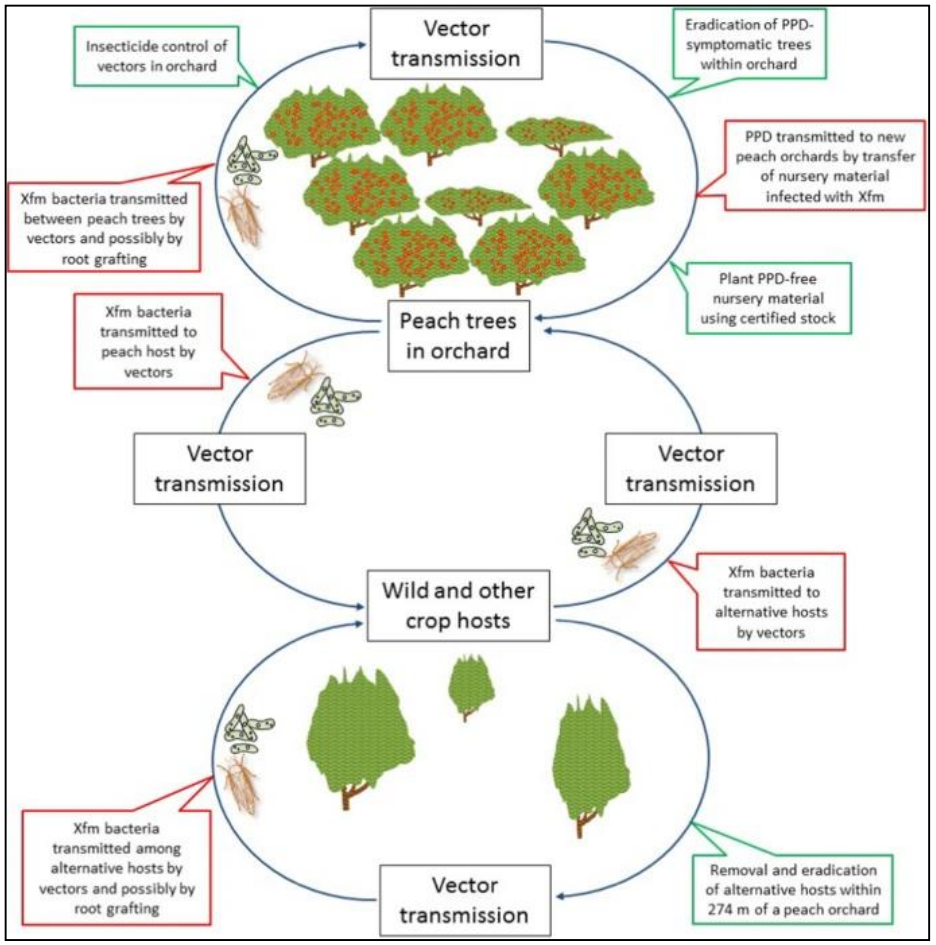
Overview of the different facets of X. fastidiosa's life cycle



Xylencer 2019

The transmission cycle of *Xylella fastidiosa* subsp. *multiplex* (Xfm) between wild hosts and peach by insect vectors

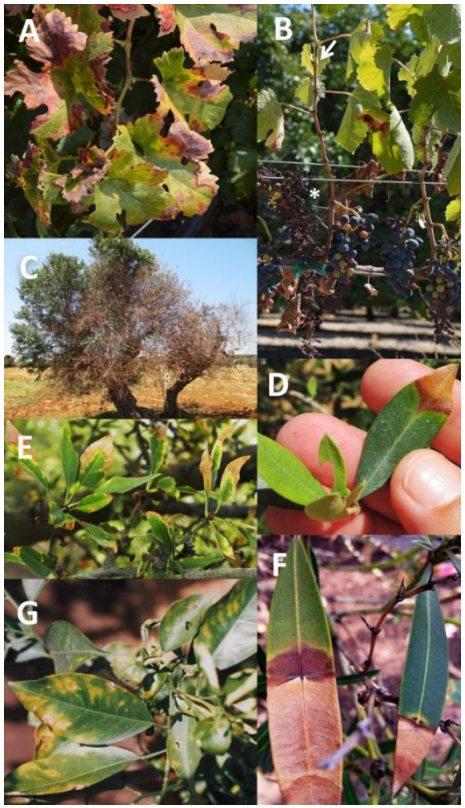
Text boxes outlined in red indicate transmission stages, and text boxes outlined in green indicate points of control for phony peach disease (PPD) caused by *Xylella fastidiosa* subsp. *multiplex* (Xfm).



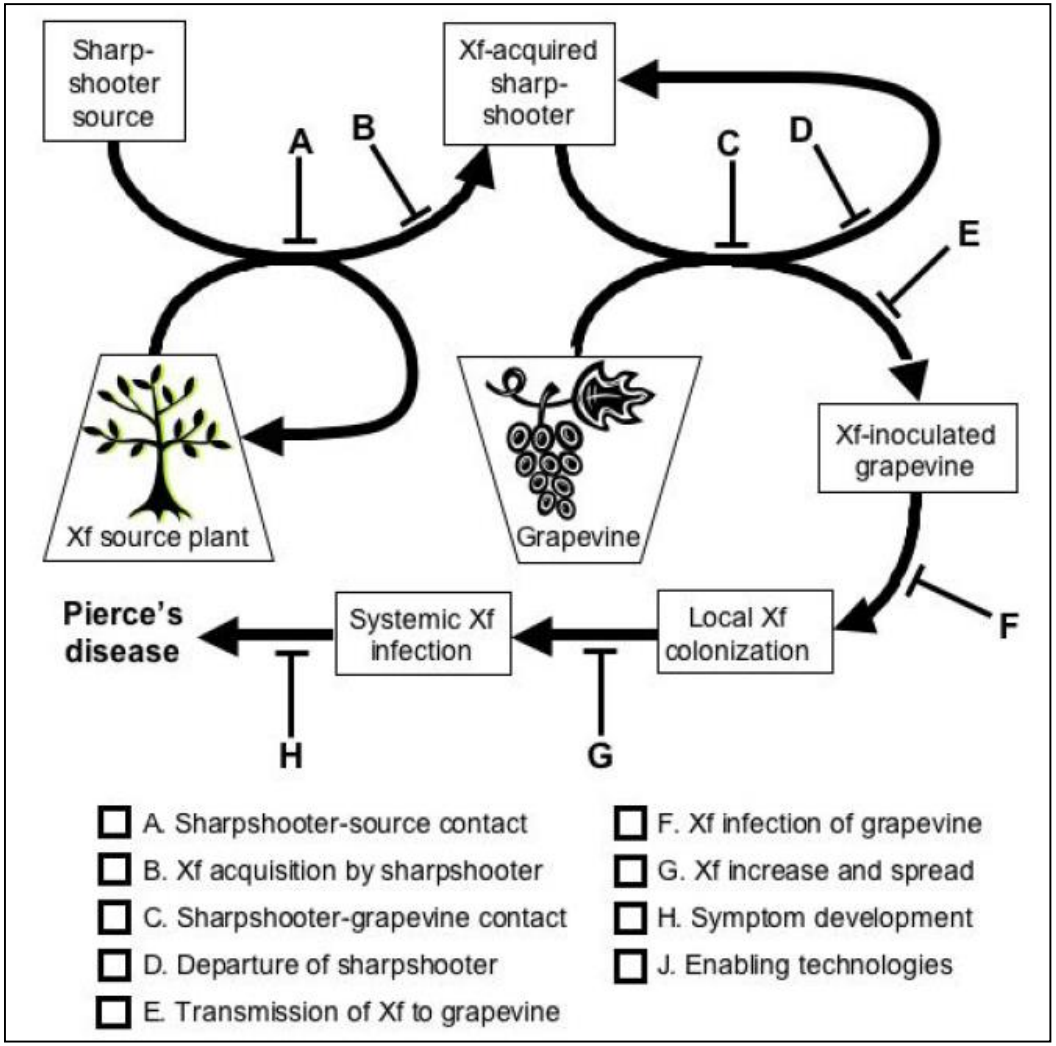
Johnson *et al.*,2021

The glassy-winged sharpshooter, a major vector of *Xylella fastidiosa*

Events leading to the development of Pierce's disease

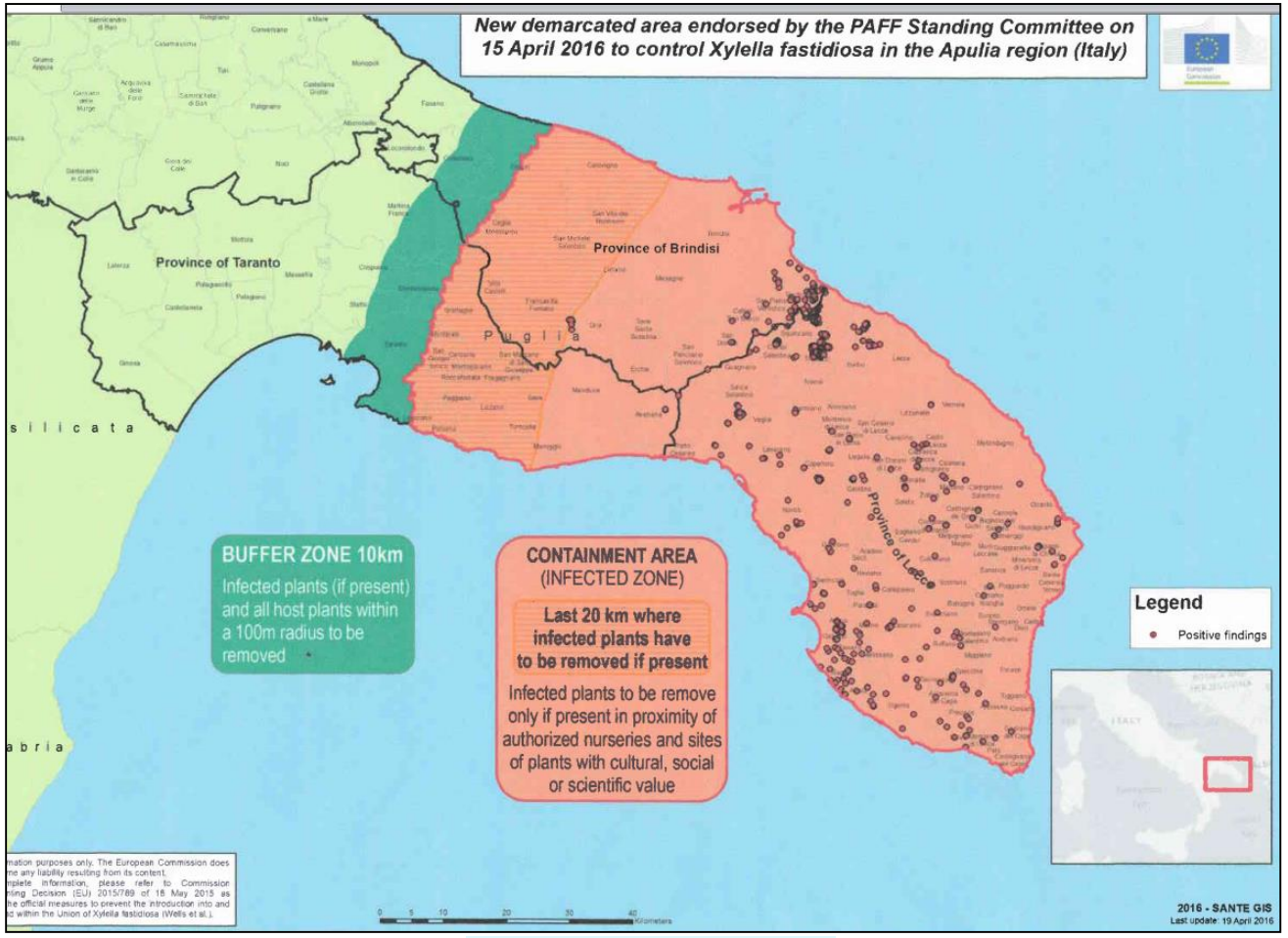


University of California,2007; Rapicavoli *et al.*,2017



New demarcated area to control *X. fastidiosa* in the Auplia region (Italy)

Buffer zone 10 KM



SANTE GIS,2016

Management

Bacterial leaf scorch (*X. fastidiosa*)

Bacterial Scorch

- Improving tree vigor by fertilization and irrigation may extend life of tree
- Injections with commercial brands of oxytetracycline may delay symptoms, but will not cure

Current approaches to control *X. fastidiosa* epidemics

Current approaches to control *Xylella fastidiosa* epidemics

Control of the bacterium
Anti-biofilm compounds
Plant- and microbial-derived compounds
Use of microbial ecology to identify biocontrol strains
Mineral-based compounds

Control of the insect vector
Monitoring of vector populations
Control vector populations by i) weed removal to target juveniles and ii) insecticide applications
Search for natural enemies of the vector
Insect repellent
Pheromones, volatiles and vibrational signals

Plant tolerance/resistance
Germplasm survey for resistance
Search for resistance genes
Breeding for resistance

Integrated pest management (IPM)
Timely removal of Infected plant
Control of vector populations

Morelli *et al.*,2021

Measures of containment/eradication against *X. fastidiosa*

Measures against *Xylella fastidiosa*
(Latest legislative provision: EU Regulation 2020/1201)

Main goal: Prevent the introduction into and the spread within the EU. **How?** (i) Mandatory surveillance programs in all Member States; (ii) Restrictions to the importation of susceptible plant species from Third Countries.

ACTIONS IN CASE OF AN OUTBREAK:

Establishment of the demarcated area:

Infected zone (a radius of at least 50 m around infected plant)
Buffer zone (from 1km to at least 5km according to the situation)
In the demarcated areas: (i) Strict restrictions for the movement of specified plants; (ii) Restrictions for planting

Eradication measures

Removal of all specified host plants
in a radius of at least 50 m around the plant found infected
Intensive surveillance in the buffer zone

Containement measures

Areas in which the bacterium is already widely established
Lighter provisions than those presented in the eradication

Morelli *et al.*,2021

Management

Bacterial leaf scorch of almond (*X. fastidiosa*)

- If discovered early and only in one branch, the infection may be removed by pruning off a primary scaffold 5 to 10 feet below visible symptoms.
- If this is attempted, flag the pruned tree and observe it in subsequent years for indications of the disease.
- If the orchard is young (5 to 10 years old), the best course of action may be to remove infected trees.
- In older orchards (16 to 20 years old), it may be more cost-effective to keep infected trees, because the entire orchard is normally removed between 22 and 25 years of age and infections will probably not significantly impact yields before then.
- The most difficult decision is what to do when infected trees are found in orchards 11 to 15 years old.

Niederholzer 2013; UC Pest Management Guidelines

Management

Bacterial leaf scorch of almond (*X. fastidiosa*)

- The answer may depend on whether there are other young orchards nearby, how long the orchard is expected to last before it is likely to be removed, and once mapped, whether the number of infected trees increases rapidly.
- Sharpshooter populations increase slowly and the insects disperse slowly. Grass-feeding sharpshooters require year-round access to plants on which they can feed and reproduce. Clean cultivation of almond orchards for a 6-week period at any time of the year (like during harvest) should prevent the establishment of in-orchard vector populations.
- Thus, cover crops in almond orchards should not pose a threat. The most common habitats for sharpshooters in the Central Valley are irrigated pastures, alfalfa fields with grass weeds, and permanent cover crops.

Niederholzer 2013; UC Pest Management Guidelines

Management

Bacterial leaf scorch of oak (*Xylella fastidiosa*)

- Laboratory tests are needed for positive diagnosis.
- Submit fresh, symptomatic leaf tissue in late summer or early fall before fall leaf drop to the Extension Diagnostic Clinic.
- Prune out infected branches to prevent further spread in the tree.
- The best management tool for this disease is to maintain tree vigor.
- Fertilizing and irrigating may prolong the life of diseased trees, although the trees may be removed to prevent spread to other nearby healthy trees.
- Control of insect vectors has not been shown to be effective in curbing the spread of this disease.
- In areas where BLS occurs, avoid planting highly susceptible trees.

Management

Bacterial leaf scorch of grape (*Xylella fastidiosa*)

- Insecticide treatments aimed at controlling the vector in areas adjacent to the vineyard have reduced the incidence of Pierce's disease by reducing the numbers of sharpshooters immigrating into the vineyards in early spring.
- Monitor and treat for insect vectors.
- Insecticide treatments of adjacent breeding habitats, such as citrus groves, has been the most effective approach.
- Removing diseased vines as soon as possible when Pierce's disease first appears in a vineyard is also critical to help reduce the infection rate.

Management
Tolerant/resistant host plants

- The degree of control, however, is not effective for very susceptible varieties such as Chardonnay and Pinot Noir or for vines less than 3 years old.
- If a vineyard is near an area with a history of Pierce's disease, plant varieties that are less susceptible to this disease.
- The full list of plant species together with the number of records of tolerant/resistant response for each plant species is listed in Table 12 (see the full text).

EFSA (European Food Safety Authority),2018;..

Management
Tolerant/resistant host plants

Table 12: Number of records in *Xylella* host plant database of tolerant/resistant response for each plant species

Plant species	Number of records
Arabidopsis thaliana	4
Citrus celebica	1
Citrus clementina	2
Citrus jambhiri	2
Citrus junos	1
Citrus latifolia	1
Citrus limettioides	1
Citrus limon	14
Citrus medica	1
Citrus natsudaidai	1
Citrus paradisi	4
Citrus reticulata	9
Citrus reticulata × *C. sinensis* × *C. paradisi*	1
Citrus sinensis	7

Xylella spp. host plant database

Plant species	Number of records
Vitis girdiana	2
Vitis munsoniana	3
Vitis popenoei	1
Vitis rotundifolia	58
Vitis rotundifolia × *V. rupestris*	1
Vitis simpsonii	1
Vitis spp.	76
Vitis tiliaefolia	1
Vitis vinifera	25
Vitis × *champinii*	1
Vitis aestivalis var. *smalliana*	5
Vitis aestivalis var. *smalliana* × *V. simpsonii*	4
Vitis aestivalis var. *smalliana* × *V. vinifera*	1
Vitis nesbittiana	1
Vitis rufotomentosa	1
Vitis shuttleworthii	5
Total	507

EFSA (European Food Safety Authority),2018

Management

Bacterial leaf scorch of sycamore (*Xylella fastidiosa*)

- There is no way to prevent BLS, but if affected limbs are pruned out early in the infection and well below the scorched leaves, a cure may be possible.
- Providing irrigation, mulch and fertilizer to an infected tree can extend the life of the tree.
- Trees with extensive dieback should be removed and replaced with non-susceptible trees.
- Currently, spray treatments are not available.
- However, certified arborists can perform annual root flare injections of antibiotic treatments, using oxytetracycline (such as Bacastat), which can reduce symptoms by suppressing the pathogen.

Clemson University,2015

Impact of moisture stress on disease development

Risk of potential establishment

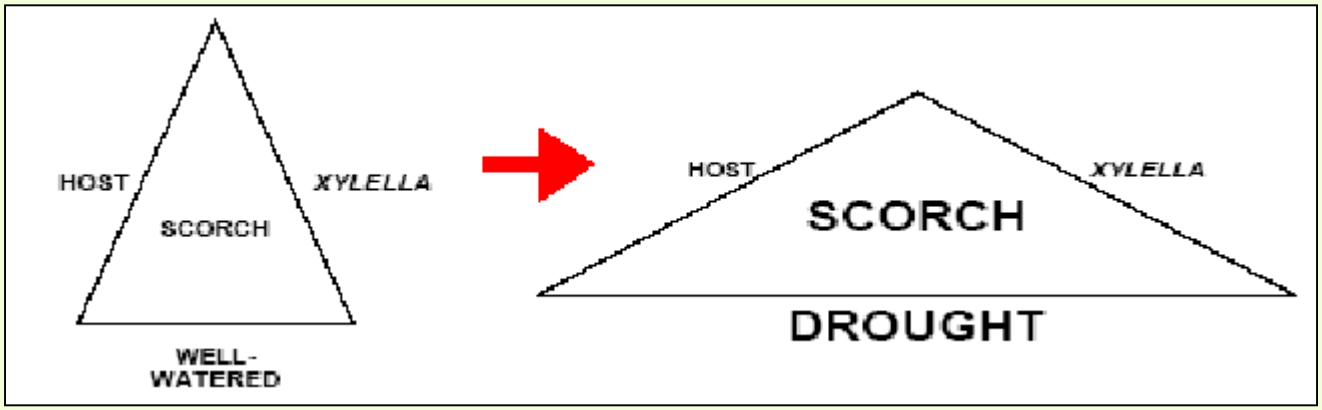
- To assess the risk of potential establishment within the EU, two different approaches were available:

1. firstly, the distribution records of *X. fastidiosa* were intersected with Köppen–Geiger climate zones to identify the climate types present in the EU where *X. fastidiosa* is known to occur (Peel *et al.*,2007; Beck *et al.*,2018). This illustrated that most of the EU territory consists of climate types where the pathogen is known to occur.
2. Next, the potential for establishment was modelled using ensemble predictions encompassing different species distribution model (SDM) techniques that assessed the effects of climate on the distribution of the species (Naimi and Araújo, 2016).

Bragard *et al.*,2019

Impact of moisture stress on disease development

- The component that most often drives the disease process is environmental conditions conductive to disease development.



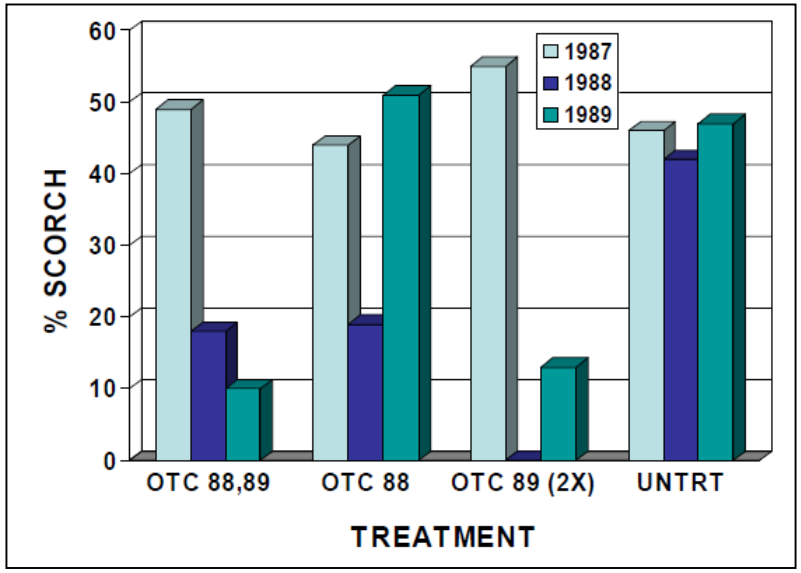
(Figure courtesy of A. McElrone)

Lashomb *et al.*,2001

Impact of oxytetracycline treatment on symptom expression in red oak

Trunk injection

- During the year of treatment (OTC, years 88,89 and OTC 88), symptom expression was reduced approximately 55 to 80%.
- Leaf scorch remained low in trees injected again the following June (OTC 88,89), but returned to 1987 levels if left untreated (OTC 88).
- Injecting twice the label rate was of no added benefit (OTC 89 (2X).
- Currently, a single antibiotic compound (Mycoject®, J. J. Mauget Company) is labeled for leaf scorch of elm and red oak.



(Adapted from B. Fraedrich presentation)

Lashomb *et al.*,2001

Endophytic bacterial populations

Their interaction with *Xylella fastidiosa* in citrus plants

- Bacterial species have been isolated from symptomatic or asymptomatic branches of citrus plants including:
- *Bacillus pumilus*
- *Curtobacterium flaccumfaciens*
- *Enterobacter cloacae*
- *Methylobacterium* spp.
- *Nocardia* sp.
- *Pantoea agglomerans*
- *Xanthomonas campestris.*
- Endophytes are microorganisms that do not visibly harm the host plant but can be isolated from surface-disinfected plant tissue or the inner parts of plants.
- Furthermore, they colonize an ecological niche similar to that of phytopathogens, which might favor them as candidates for biocontrol agents.

Araújo *et al.*,2002

Endophytic bacterial populations

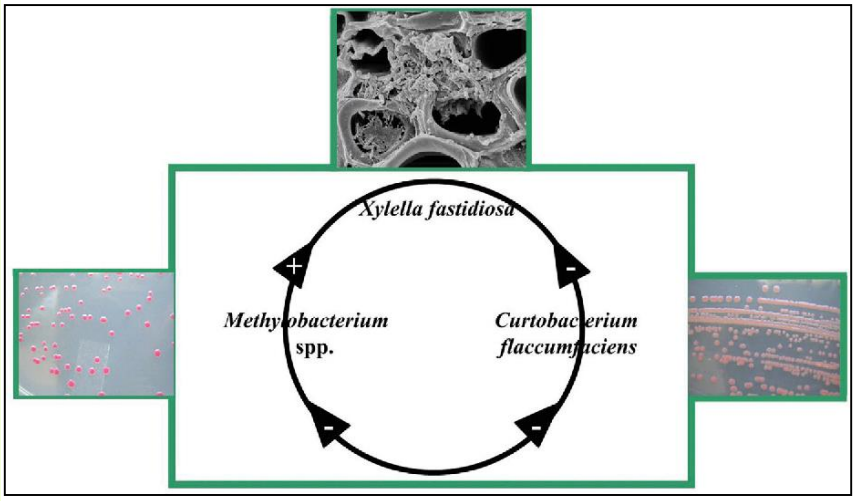
Their interaction with *Xylella fastidiosa* in citrus plants

- Furthermore, several bacterial species have been isolated from the xylem of lemon roots including:
- *Achromobacter* spp.
- *Acinetobacter baumannii*
- *A. lwoffii*
- *Alcaligenes-Moraxella* spp.
- *Alcaligenes* sp.
- *Arthrobacter* spp.
- *Bacillus* spp.
- *Burkholderia cepacia*
- *Citrobacter freundii*
- *Corynebacterium* spp.
- *Curtobacterium flaccumfaciens*
- *Enterobacter cloacae*
- *E. aerogenes*
- *Methylobacterium extorquens*
- *Pantoea agglomerans*
- *Pseudomonas aeruginosa*, and
- *Pseudomonas* spp.

Araújo *et al.*,2002

The diversity of citrus endophytic bacteria and their interactions with *Xylella fastidiosa* and host plants

- Balanced interactions among endophytic bacteria from *Citrus sinensis* and *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis(CVC).
- Photos of endophytic strains of *Methylobacterium* and *Curtobacterium* grown in Petri.



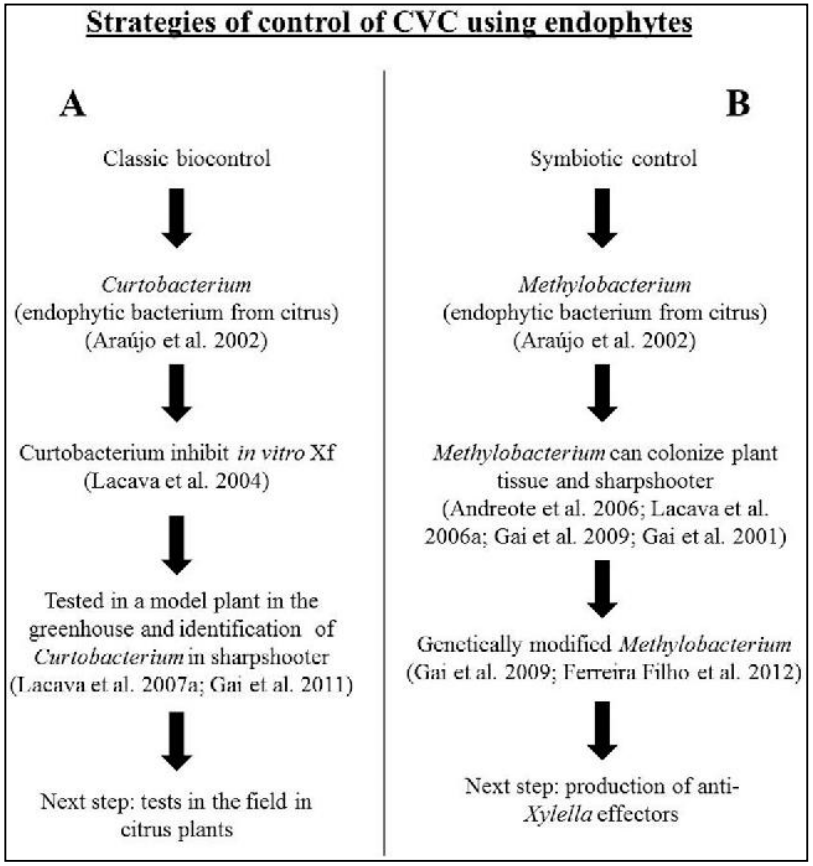
Previous studies have shown that *X. fastidiosa* interacts with the endophytic community in xylem vessels as well as in the insect vector, resulting in a lower bacterial population and reduced CVC symptoms.

Azevedo *et al.*,2016

Hypotheses and strategies to control citrus variegated chlorosis (CVC) using endophytic bacteria from citrus plants

A. We suggest the endophytic bacterium *Curtobacterium flaccumfaciens* as a classical biological control agent. *C. flaccumfaciens* has the ability to colonize plant tissues in the presence or absence of *Xylella fastidiosa* (Xf).

B. Additionally, the endophytic bacterium *Methylobacterium* has been suggested as a qualified candidate for a paratransgenic symbiotic control (SC) strategy.



Azevedo *et al.*,2016

Isolation of endophytic bacteria

- The bark of surface-disinfected branches was removed with a sterilized razor blade, and the branches were cut into pieces 4 to 6 mm long, which were placed on TSA plates amended with 50 µg of benomyl per ml to inhibit fungal growth.
- Incubation was carried out at 28°C for 1 to 12 days to allow growth of endophytic bacteria from the cut pieces and to determine the number of infected fragments.
- In a further experiment, fragments of citrus branches were homogenized in 5 ml of sterile phosphate-buffered saline with a blender and serial dilutions were plated onto TSA.
- The plates were incubated at 28°C for 1 to 20 days or until growth was observed, upon which the CFU were counted and the population density was estimated.

Araújo, *et al.*, 2002

Endophytic bacterial populations

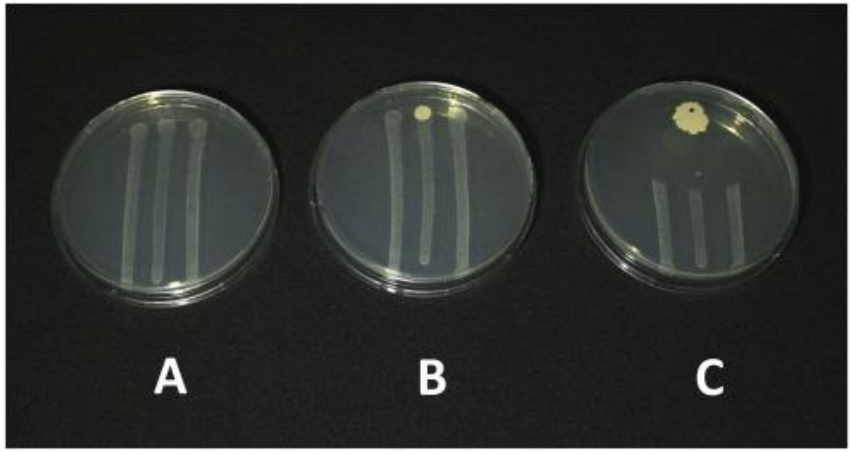
Antagonistic activity of olive endophytic bacteria and of *Bacillus* spp. strains against *Xylella fastidiosa*

- Dual-culture assay of antagonistic activity of *Bacillus* strains against Xylella fastidiosa strain De Donno.

A. Negative control, *X. fastidiosa*;

B. *B. oleronius* strain S95 against *X. fastidiosa*;

C. *B. amyloliquefaciens* strain N3.2 against *X. fastidiosa*.



Zicca *et al.*,2020

Endophytic bacterial populations

Their interaction with *Xylella fastidiosa* in occurrence and intensity of CVC disease symptoms

- **Negative role of *Methylobacterium* spp.**
- is the most frequently found genus associated to *Xylella fastidiosa* subsp. *pauca*, and there is a positive association with the occurrence and intensity of symptoms of citrus variegated chlorosis, CVC.
- This interaction of *Methylobacterium* spp. with *Xfp* may occur by *Methylobacterium* spp. synthesis of pathological factors, such as siderophores and their utilization by *Xfp* (Simionato *et al.*,2006).
- The ability of *X. fastidiosa* to use siderophores produced by endophytic bacteria as source of iron was confirmed.

Lacava *et al.*,2008

Antimicrobial peptides(AMPs)

Against *X. fastidiosa*, the causal agent of pierce's disease

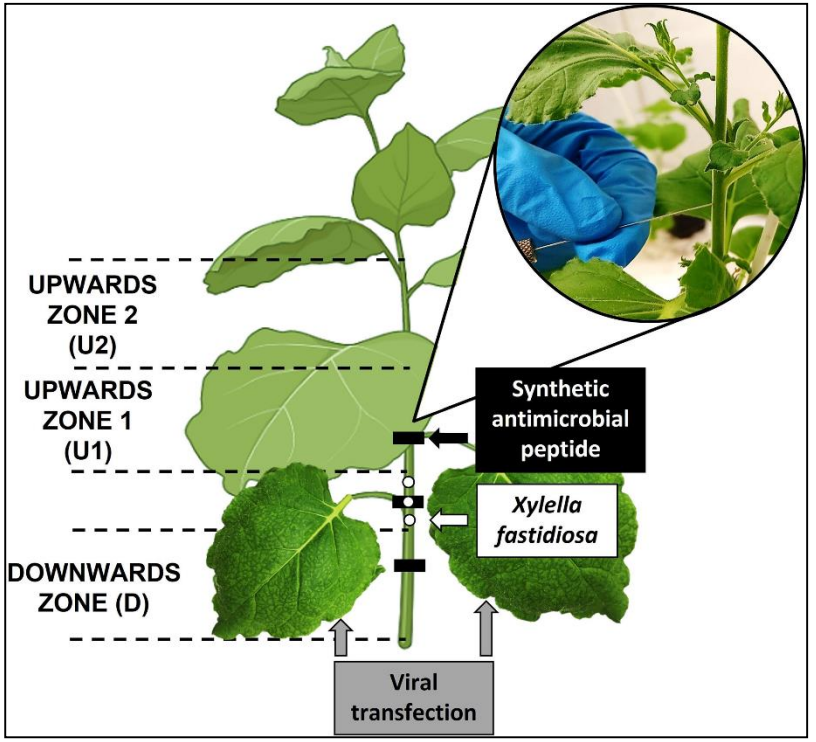
- The minimal inhibitory concentration (MIC) of 12 antibiotics and 18 antimicrobial peptides were determined by agar dilution tests and growth inhibition assays.
- Antibiotics with the lowest MIC for *X. fastidiosa* strains were gentamicin, tetracycline, ampicillin, kanamycin, and novobiocin, chloramphenicol, and rifampin.
- Four of the antimicrobial peptides (Magainin 2, Indolicidin, PGQ, and Dermaseptin) were toxic to all *X. fastidiosa* strains.
- This study shows that antibiotics and antimicrobial peptides have some activity against *X. fastidiosa* and may have application in protecting plants from developing Pierce's disease.

Kuzina *et al.*,2006

Antimicrobial peptides(AMPs)

Against *X. fastidiosa*, the causal agent of pierce's disease

- Briefly, a pathogen suspension, at 10^8 CFU/ml ($OD_{600} \cong 0.3$) was injected with a Hamilton 250 µl syringe including a thin needle with bevel tip.
- The needle end was introduced into approximately one half the plant stem diameter to directly access the vascular system.
- Three inoculations of *X. fastidiosa* suspension of 10 µl each (30 µl of total inoculum/plant, $3x10^6$ CFU/plant) were applied at the same side of the stem in a section of 3 cm at around 10 cm above the soil level.



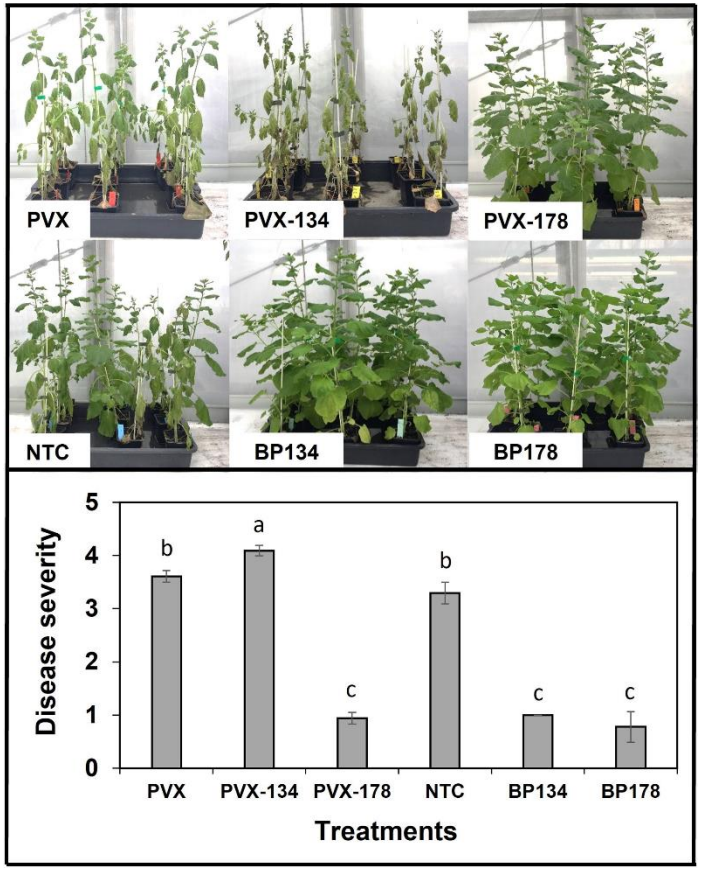
Scheme of pathogen inoculation, peptide application points, and the leaves transfected with PVX constructs, and details of inoculation/delivery process in the stem of the plant.

Baró *et al.*,2022

Antimicrobial peptides(AMPs)

Against *X. fastidiosa*, the causal agent of pierce's disease

- Effect of endotherapy with BP178 or BP134, and by heterologous production of the peptides on *Xylella fastidiosa* infections in *Nicotiana benthamiana* plants.
- The treatments were: PVX, empty vector; PVX-134, vector with BP134 gene; PVX-178, vector with BP178 gene; BP134, synthetic peptide; BP178, synthetic peptide; and NTC, non-treated control.
- Values are the means of nine plants (three replicates of three plants per each treatment), and error bars represent the standard deviation of the mean. Different letters between treatments correspond to statistically significant differences between treatments (Tukey's test, $p \leq 0.05$).



Baró *et al.*,2022

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Kuzina *et al.*,2006

Antimicrobial peptides(AMPs)

Against *X. fastidiosa*, the causal agent of pierce's disease

Strains	MIC* (μgml^{-1})			
	PGQ	Indolicidin	Magainin 2	Dermaseptin
A05	32	32	32	16
B02	32	32	16	16
I03	32	32	16	16
Temecula	32	32	16	16
187	32	16	32	16
237	32	16	16	16
276	16	32	32	32
Dixon	16	32	32	16
Ann1	64	64	64	32
Texas	8	16	8	8

*Mean of the MICs from three independent experiments. AMPs were applied on the top of PD3 agarose plates containing *X. fastidiosa*.

Kuzina *et al.*,2006

Antimicrobial peptides(AMPs)

Against *X. fastidiosa*, the causal agent of pierce's disease

- Recently, in vitro experiments determined antimicrobial effects of peptide conjugates derived from BP100, with exposure resulting in Xf cell death due to the lytic effects of the peptides, and an induction of the viable but nonculturable state (Santiago *et al.*,2018).

Johnson *et al.*,2021

Essential oils

Against *X. fastidiosa* subsp. *multiplex*, the causal agent of phony peach disease(PPD)

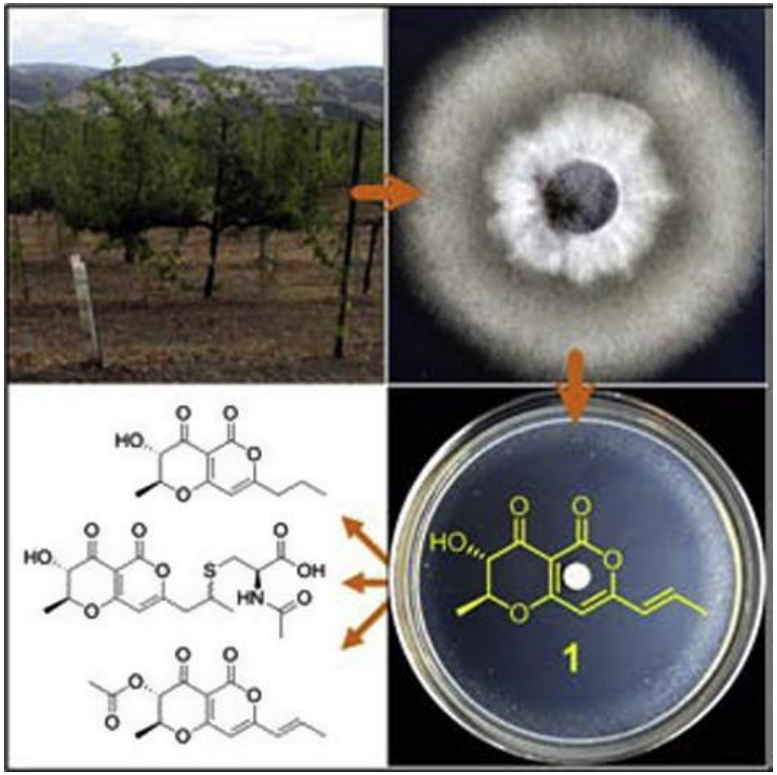
- Various essential oils were screened by Baró et al.,2020 and patchouli essential oils had antibacterial action and thus promise as a natural antimicrobial against Xf.
- Much further research is needed to screen additional antimicrobials, and also once identified, to develop methods of delivery that will control Xf but not harm the host.

Johnson *et al.*,2021

Radicinin as a fungal Phytotoxin

Against *X. fastidiosa*, the causal agent of pierce's disease

- Radicinin (1) as a fungal Phytotoxin produced by a strain of the fungal genus *Cochliobolus* sp. isolated from grapevine inhibits *Xylella fastidiosa in vitro*, suggesting a possible mechanism for tolerance of Pierce's Disease.



Aldrich *et al.*,2015

Radicinin as a fungal Phytotoxin

Xf inhibition assay

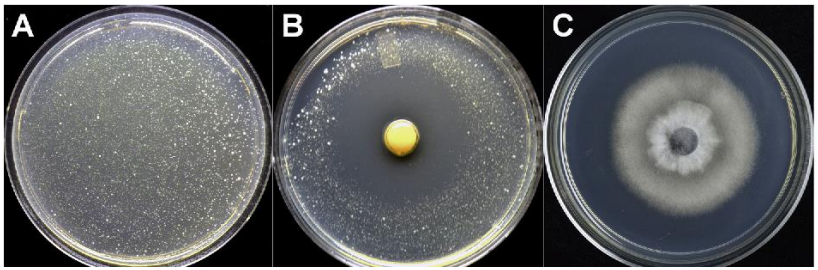
Against *X. fastidiosa*, the causal agent of pierce's disease

- Endophytes may confer grapevine with non-genetic tolerance of Pierce's Disease.

A. uninhibited Xf growth on a control plate,

B. a halo of Xf growth inhibition around an agar plug of an established culture of *Cryptococcus* sp. strain CRY1,

C. complete inhibition of Xf growth around an agar plug of an established culture of *Cochliobolus* sp. strain COC1.



Aldrich *et al.*,2015

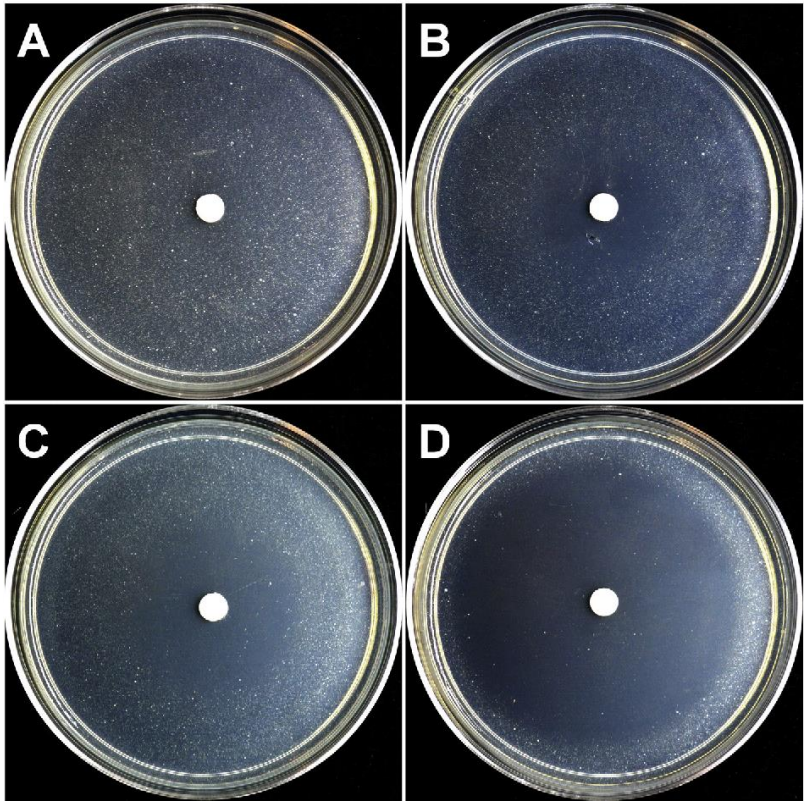
Radicinin as a fungal Phytotoxin

Xf inhibition assay

Against *X. fastidiosa*, the causal agent of pierce's disease

- Dose-dependent inhibition of Xf by radicinin.
- Images of assay plates from the in vitro assay, showing a halo of Xf growth inhibition around filter disks containing radicinin (1) at the following doses:

A. 10 lg,
B. 50 lg,
C. 100 lg and
D. 250 lg.



Aldrich *et al.*,2015

Antimicrobial compounds(antibiotics, phenols, fungal toxins and crude culture extracts) Against *X. fastidiosa* subsp. *pauca*

- Olive quick decline syndrome (OQDS) causes severe damages to the olive trees in Salento (Apulia, Italy) and poses a severe threat for the agriculture of Mediterranean countries.
- A bioassay based on agar disk diffusion method revealed that 17 out of the 32 tested antibiotics did not affect bacterial growth at a dose of 5 μg disk^{-1}.
- When we assayed micro-, ultra- and nano-filtered fractions of olive mill wastewaters, we found that the micro-filtered fraction resulted to be the most effective against the bacterium.
- Moreover, some phenolics (4-methylcathecol, cathecol, veratric acid, caffeic acid, oleuropein) were active in their pure form.
- Noteworthy, also some fungal extracts and fungal toxins showed inhibitory effects on bacterial growth.

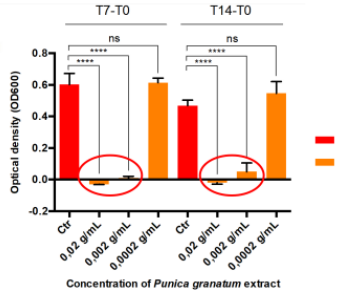
Bleve *et al.*,2017

Antimicrobial compounds

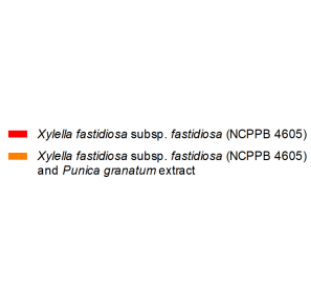
Use of pomegranate-based nano-compounds

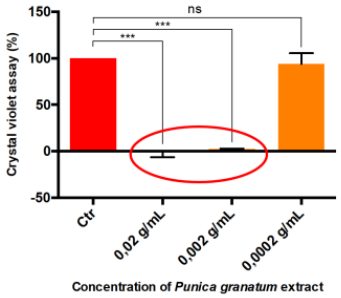
- Antimicrobial activity of Pomegranate peel extract against *Xylella fastidiosa* (Xf) subsp. *pauca*, causal of Olive Quick Decline Syndrome (OQDS).
- Exploring the antioxidant and antimicrobial properties of pomegranate extracts, particularly punicalagin, as a promising and cost-effective solution with low toxicity to higher organisms.

Antimicrobial Activity of Pomegranate Peel Extract against *Xf*

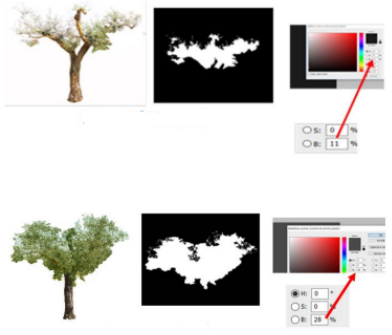


Efficacy of the hydroalcoholic extract of pomegranate peel on the planktonic state of *Xylella fastidiosa* subsp. *fastidiosa* strain Temecula measured as optical density.





Efficacy of the hydroalcoholic extract of pomegranate peel evaluated on the percentage of biofilm formation of *Xylella fastidiosa* subsp. *fastidiosa* strain Temecula

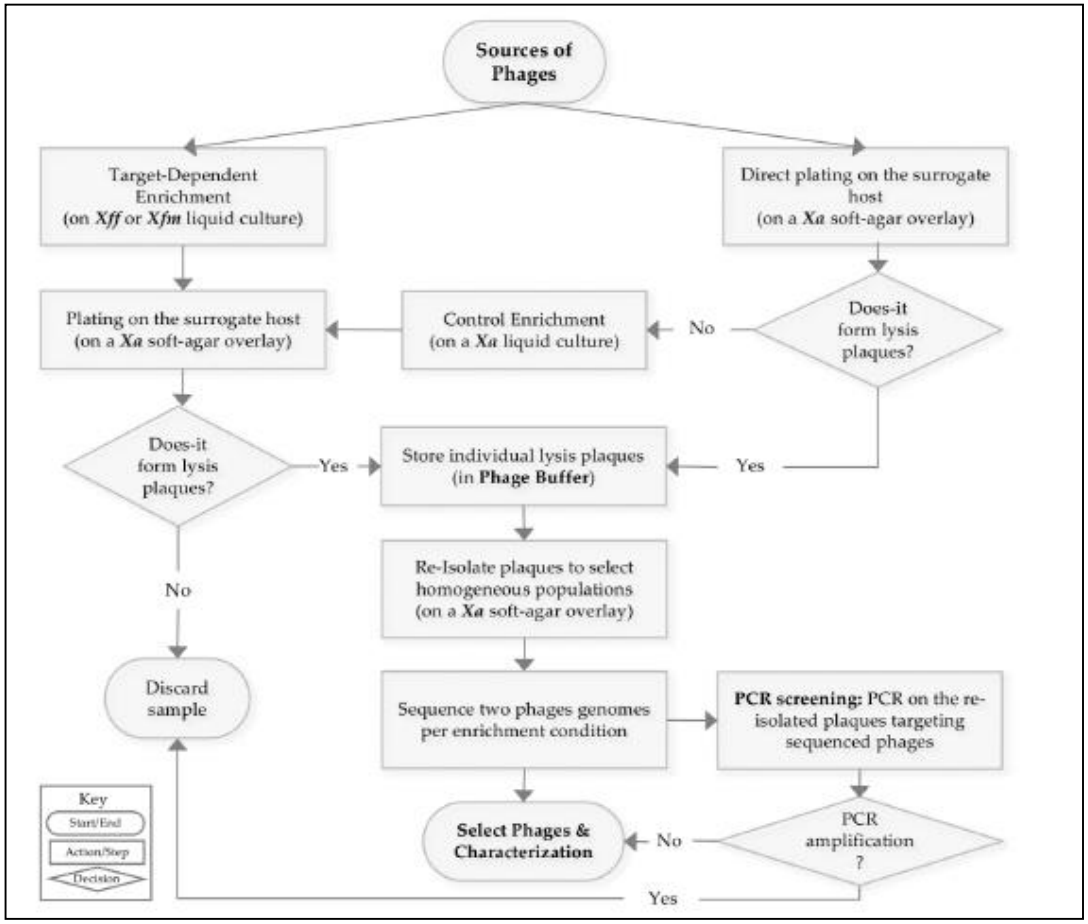


OQDS infected olive tree photographed in May 2018 (above) and two years later (below).
The software "Photoshop CS5" transformed the images into a grayscale with a precise numerical value

Muawiya *et al.*,2023

Novel Virulent Bacteriophages of *Xylella fastidiosa* and *Xanthomonas albilineans*

Workflow for phage isolation



Clavijo-Coppens *et al.*,2021

Novel Virulent Bacteriophages of *Xylella fastidiosa* and *Xanthomonas albilineans*

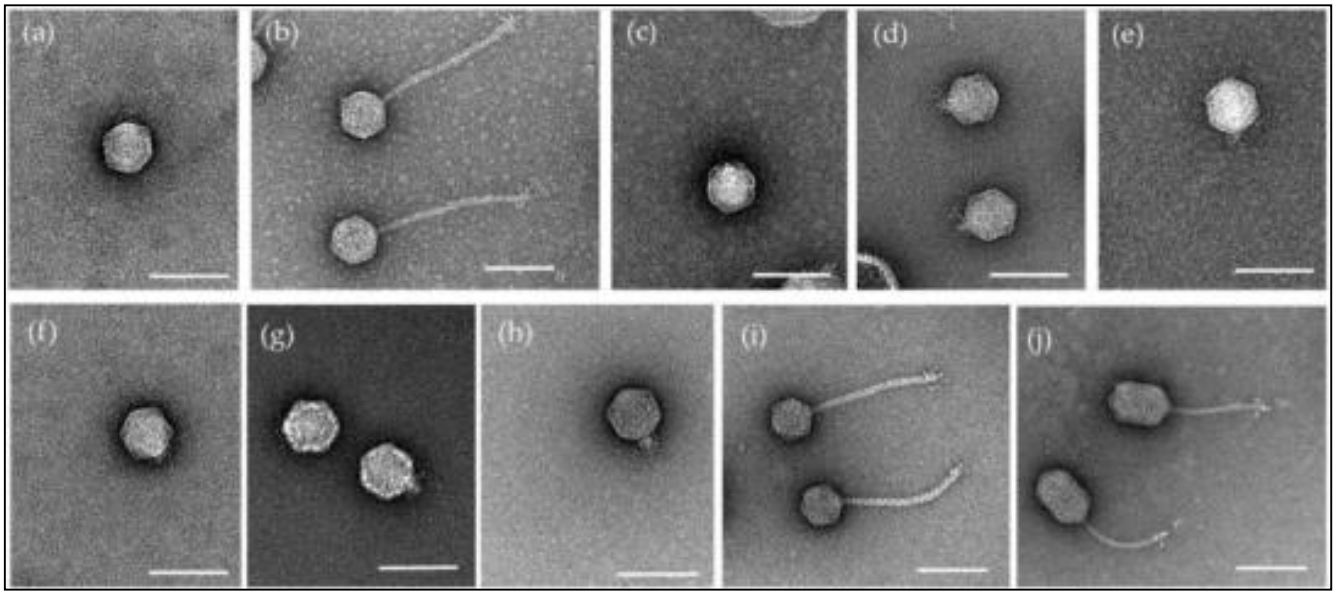
Phages isolated with and without enrichment

Source	Conditions	Isolated Phages	Sequenced Phage Genomes	PCR Groups [a]				Potential New Phages
				FC03	FC44	FC12	FC23	
Plant extracts	Enrichment on *Xff*	NA [b]	-	-	-	-	-	-
	Enrichment on *Xfm*	NA	-	-	-	-	-	-
	Enrichment on *Xa*	NA	-	-	-	-	-	-
Insect extracts	Enrichment on *Xff*	6	LR743523 MW802488	6	-	-	-	0
	Enrichment on *Xfm*	6	MW822538 MW822539	6	-	-	-	0
	Enrichment on *Xa*	12	MW822535 MW822536	11	1	-	-	0
Sewage watersample	Enrichment on *Xff*	6	LR743524 (x2) [c]	-	2	4	-	0
	Enrichment on *Xfm*	6	LR778216 MW822537	1	1	-	3	1
	Direct plaquing on *Xa*	12	LR743531 LR743532	4	4	2	-	1 + LR743530 [d]
Runoff waters sample	Enrichment on *Xff*	NA	-	-	-	-	-	-
	Enrichment on *Xfm*	NA	-	-	-	-	-	-
	Direct plaquing on *Xa*	8	LR743529 LR743528	4	-	-	-	4

Clavijo-Coppens *et al.*,2021

Novel Virulent Bacteriophages of *Xylella fastidiosa* and *Xanthomonas albilineans*

Electron micrographs of isolated phages



Electron micrographs of isolated phages revealed after negative coloration with uranyl acetate 2%. Morphology indicates that the phages belong to two different morphotypes.

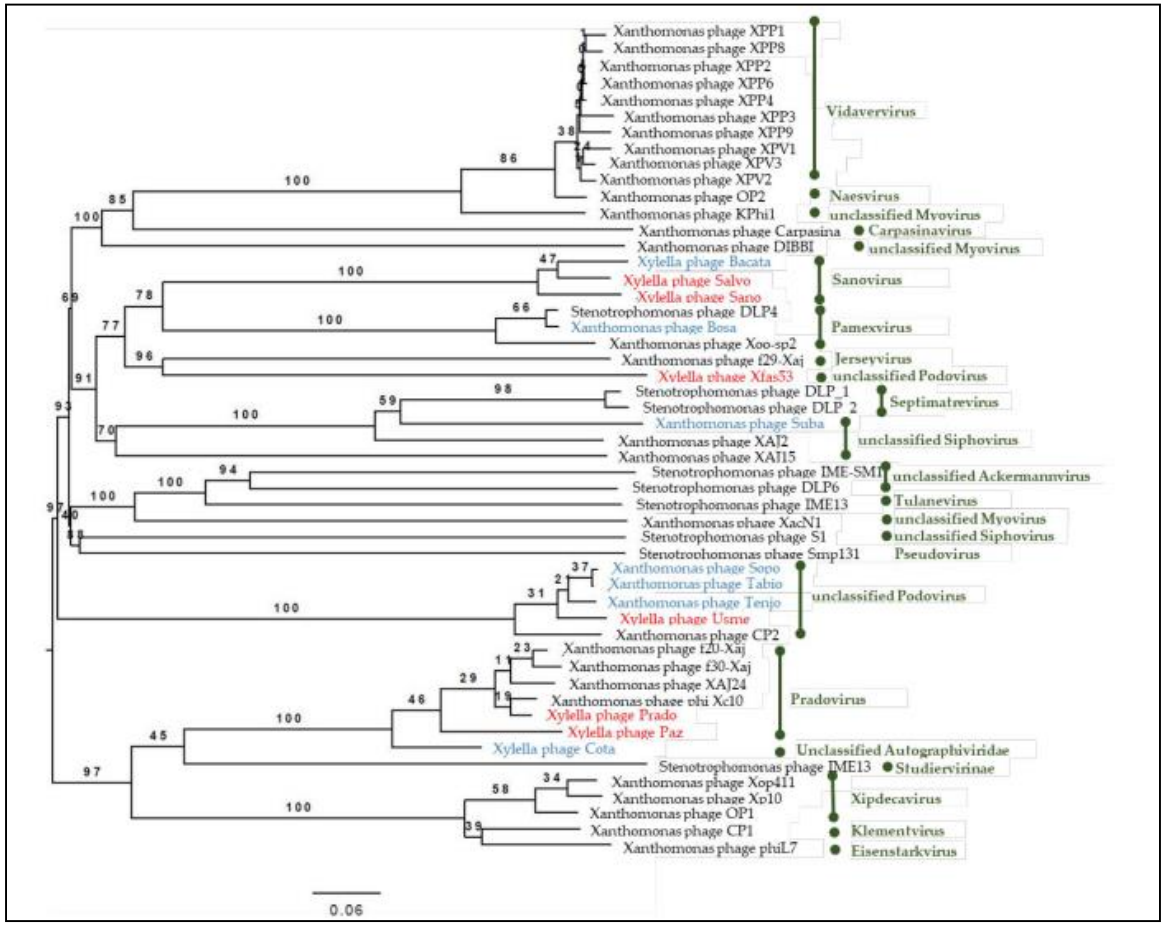
FC03-Usme (a), FC23-Cota (c), FC24-Teja (d), FC28-Sopo (e), FC30-Tabio (f), FC34-Cajica (g) and FC39-Tenjo (h) belong to the podovirus morphotype. FC12-Bacata (b), FC41-Suba (i) and FC44-Bosa (j) belong to the siphovirus morphotype. Scale bar = 100nm.

Clavijo-Coppens *et al.*,2021

Novel Virulent Bacteriophages of *Xylella fastidiosa* and *Xanthomonas albilineans*

Phylogeny of phages infecting Xanthomonads

The phylogenic tree shows the relationships between amino acid sequences for the whole phage genomes obtained in this study and the isolated Caudovirales phages active on various species of the genera *Xanthomonas*, Xylella and *Stenotrophomonas*.



Clavijo-Coppens *et al.*,2021

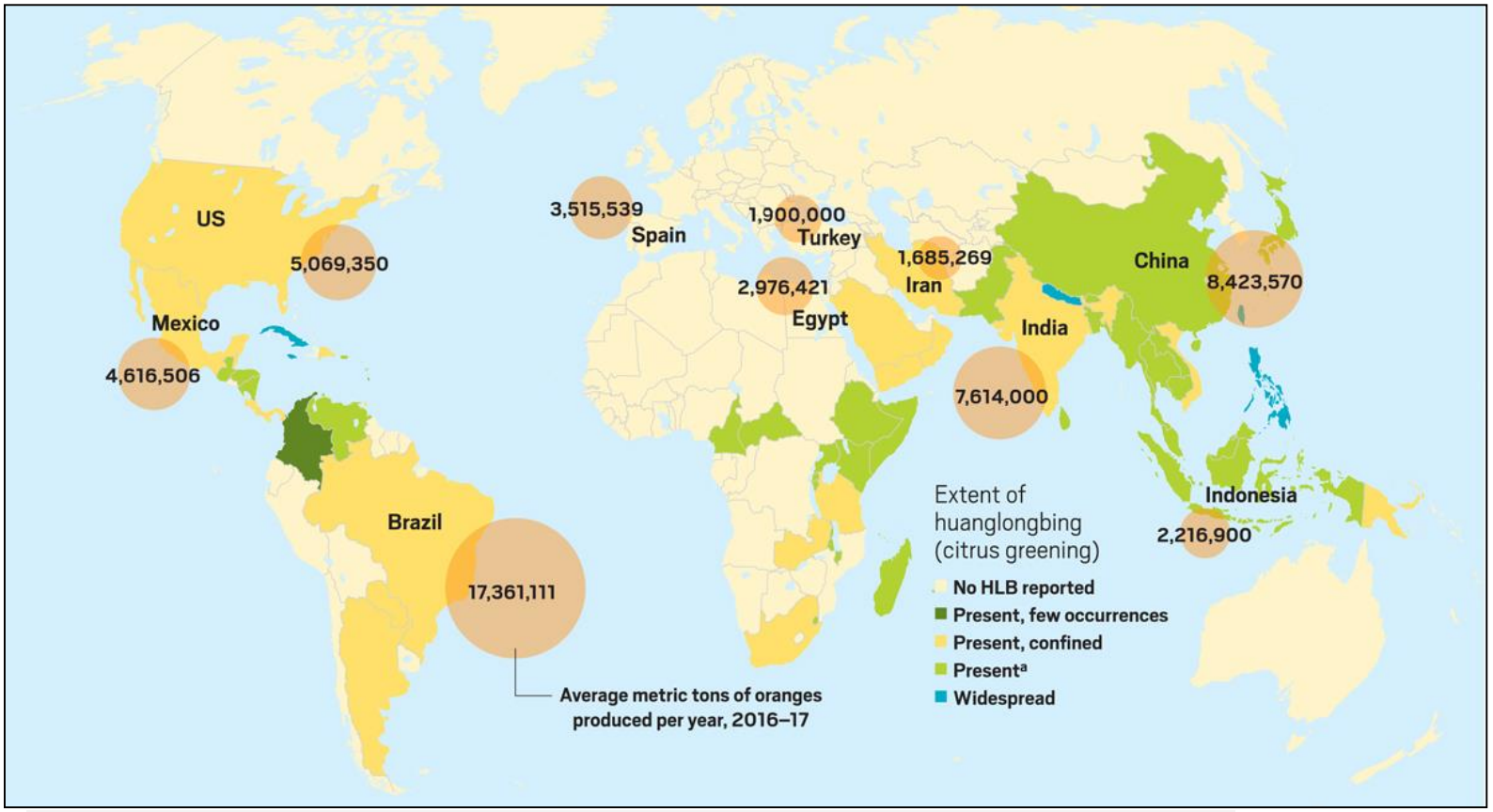
Huanglongbing (HLB) or Citrus greening disease

Hauanglongbing (yellow dragon) disease

HLB on citrus

Geographical distribution of HLB

The bacteria that cause huanglongbing have been detected in 7 of the top 10 orange-producing countries across the globe



[a] Indicates that the disease is present in the area, but to what extent may be unknown.

CAB International's Invasive Species Compendium 2019, Zhang,2019

Citrus greening(Huanglongbing)

Candidatus Liberibacter asiaticus, africanus and americanus

Left, Vein corking and blotchy mottle in HLB-affected leaves. Right, An HLB-affected tree, in which the symptomatic fruit have abscised and fallen to the ground at the base of the tree: note the healthy fruit still hanging on the tree.

Burrow *et al.*,2019

Citrus greening disease

Candidatus Liberibacter asiaticus, africanus and americanus

- Infected trees develop yellow shoots and mottled leaves.
- Trees eventually die; but before that, fruit is distorted and bitter, can't be used for juice.
- Fruit and leaf drop increase as the disease progresses.

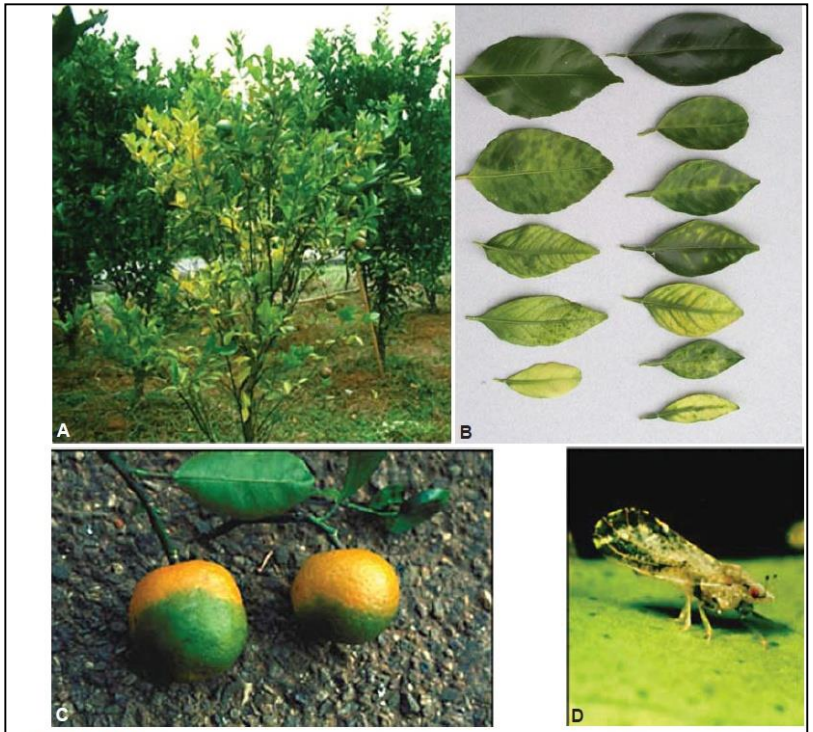


FIGURE 12-51 Citrus greening disease caused by *Candidatus liberobacter asiaticum*. (A) Citrus tree affected by yellow shoot and citrus greening. (B) Leaves of greening-infected orange and lemon trees showing progressive symptoms of the disease. (C) Oranges showing delayed and abnormal coloration due to citrus greening. (C) Citrus psylla, one of the important vectors of citrus greening. [Photographs courtesy of (A, C, and D) T. R. Gottwald and S. M. Garnsey, USDA, Ft. Pierce, FL, and (B) S. P. van Vuuren, ARC-ITSC, Nelspruit, South Africa.]

Agrios,2005; Nesbitt,2014

HLB or Citrus Greening Disease

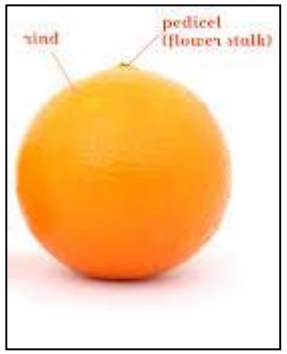
Sectoring of greening in citrus tree canopy

- HLB-affected trees often show only one branch with symptomatic, yellow and mottled leaves.
- Such a yellow branch shows up conspicuously within the green canopy of the tree.

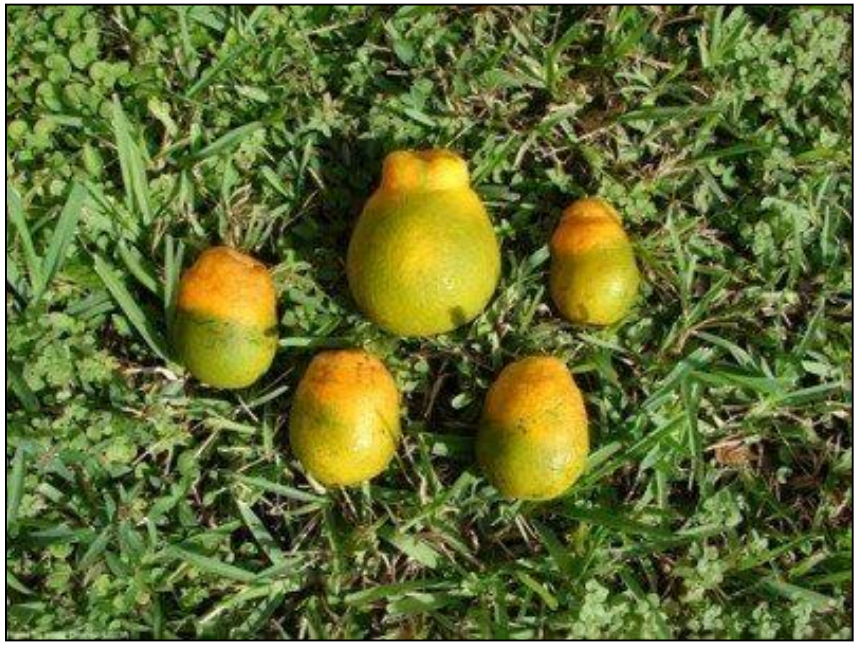
Fruit and leaf drop increase as the disease progresses, usually accompanied by off-season bloom and flushes of growth. Stunting and dieback become more pronounced with time. The tree may survive for several years, but death is inevitable.

State Agricultural Response Team; Nesbitt,2014

HLB or Citrus Greening Disease
Fruit symptoms

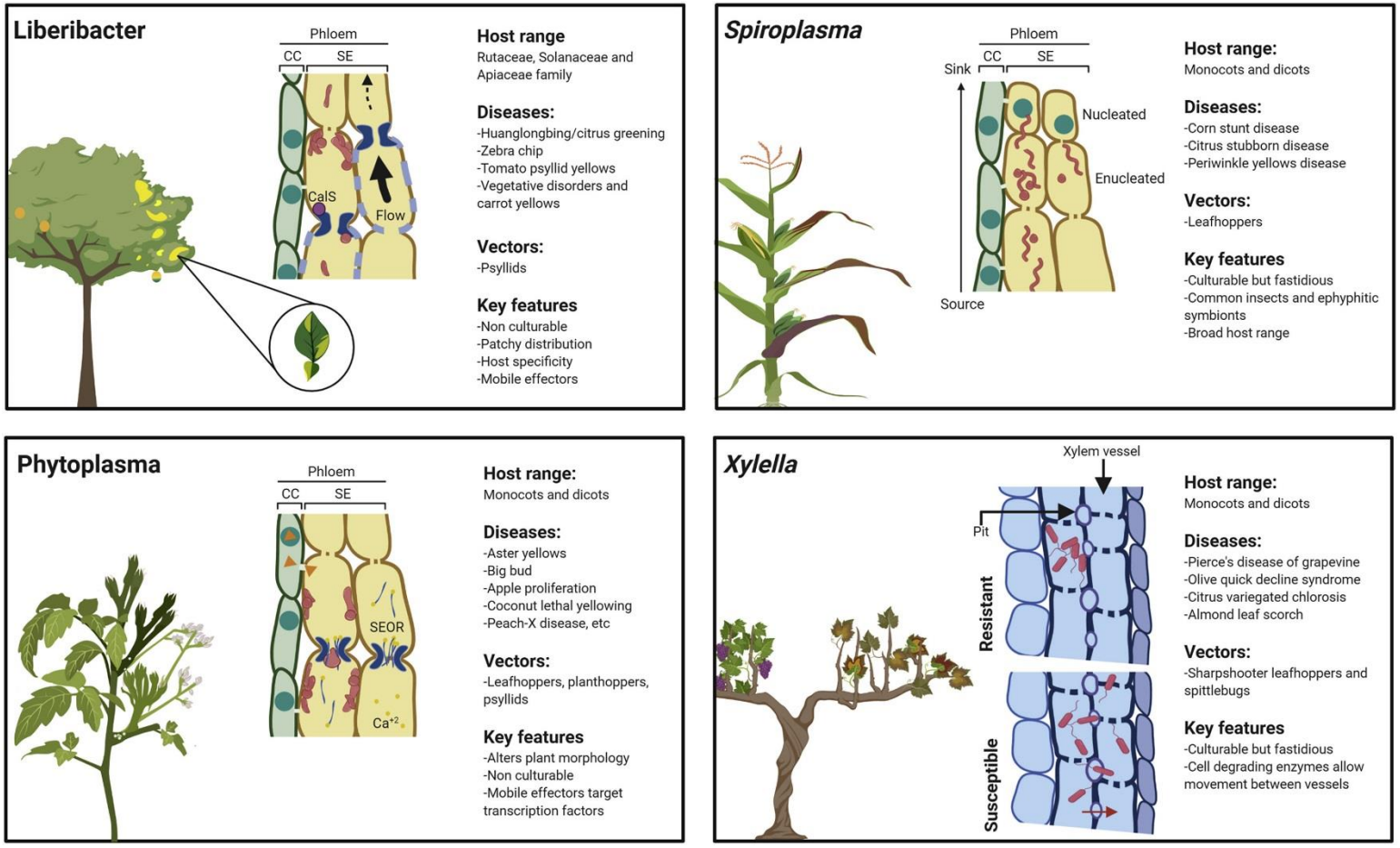


- Fruits on HLB infected trees show color inversion with the presence of brownish, aborted seeds in the fruit.
- Normal fruits break color and turn orange first at the stylar end, the peduncle end being still green.
- HLB affected fruits become orange first at the peduncle end, the stylar end being still green.



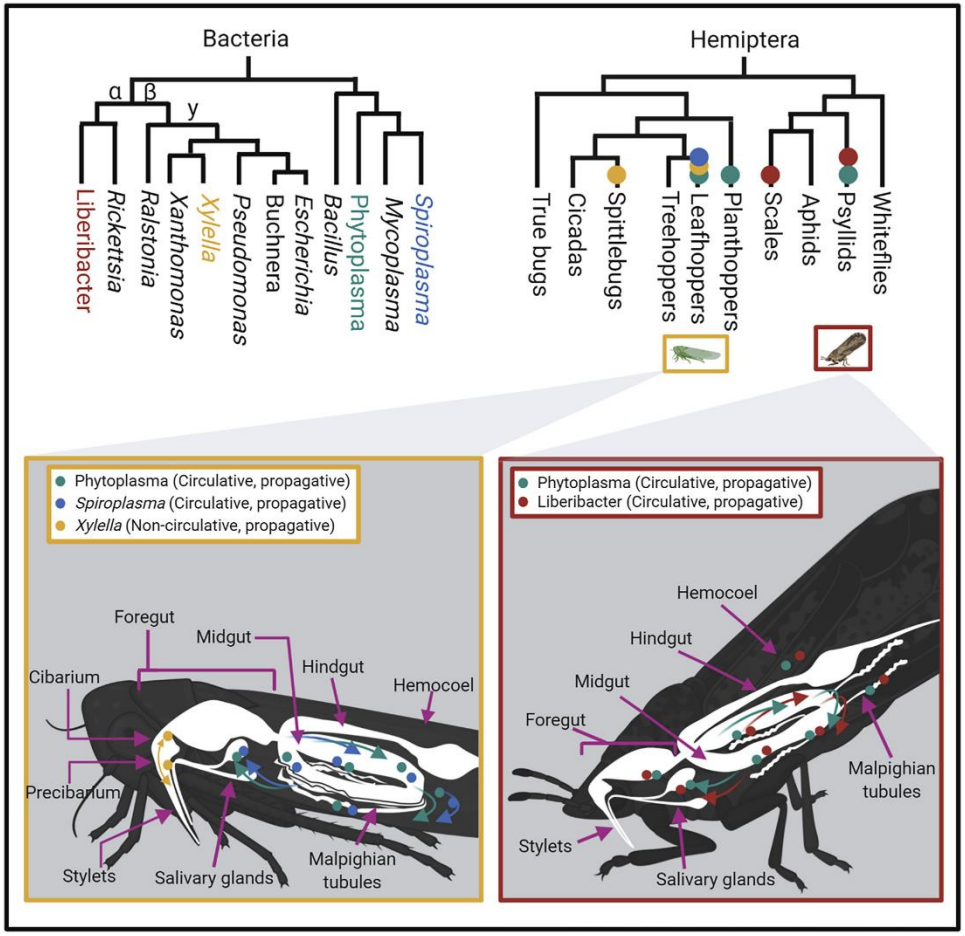
National Plant Protection Centre; Gardening Know How

Bacterial vector-borne diseases that persist in plant vascular tissues



Huang *et al.*,2020

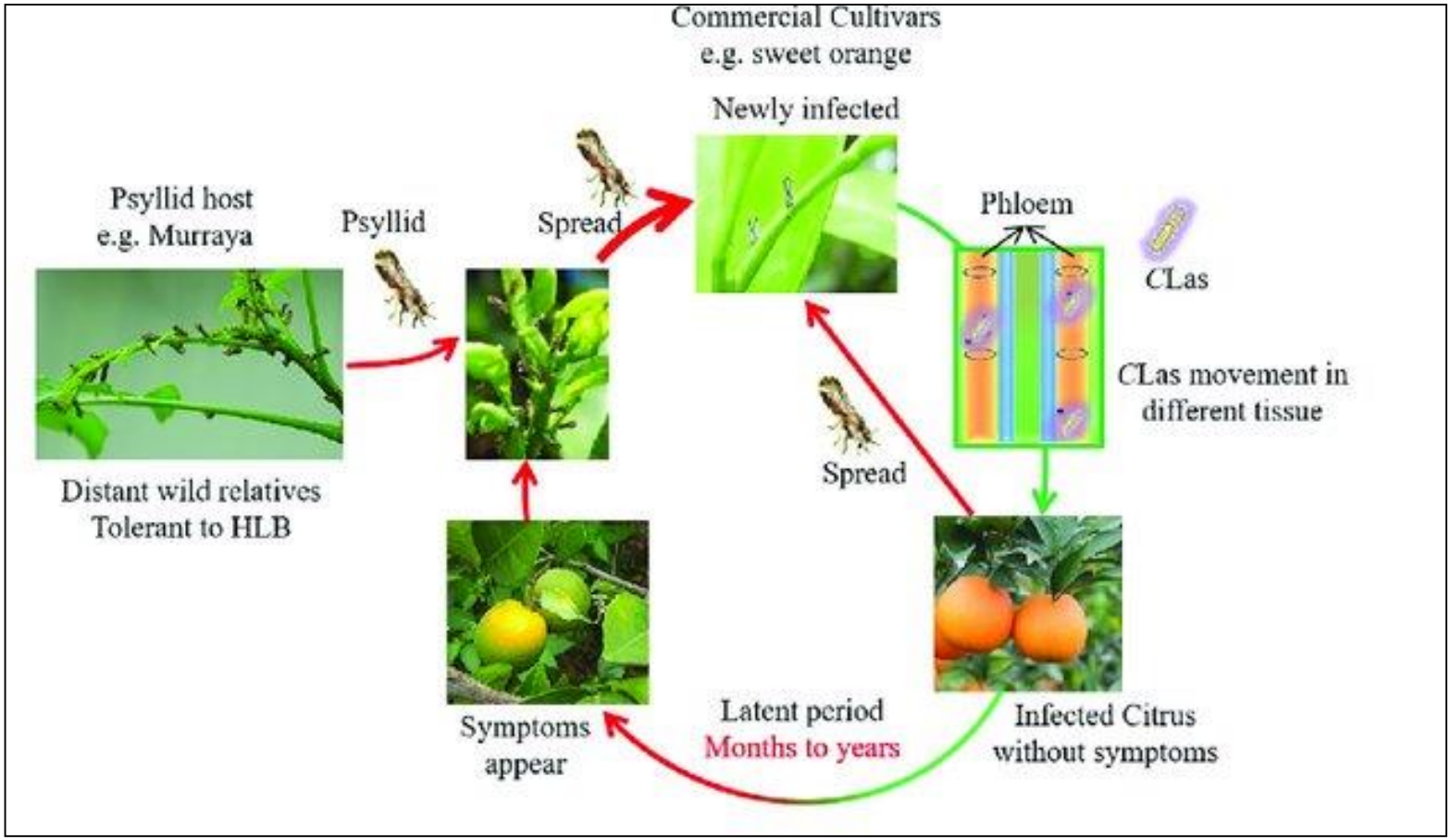
Circulative Propagative and Non-circulative Propagative Bacterial Localization in Insect Vectors through the Perspective of Phylogenetic Relationships



Huang *et al.*,2020

HLB or Citrus Greening Disease

Life cycle

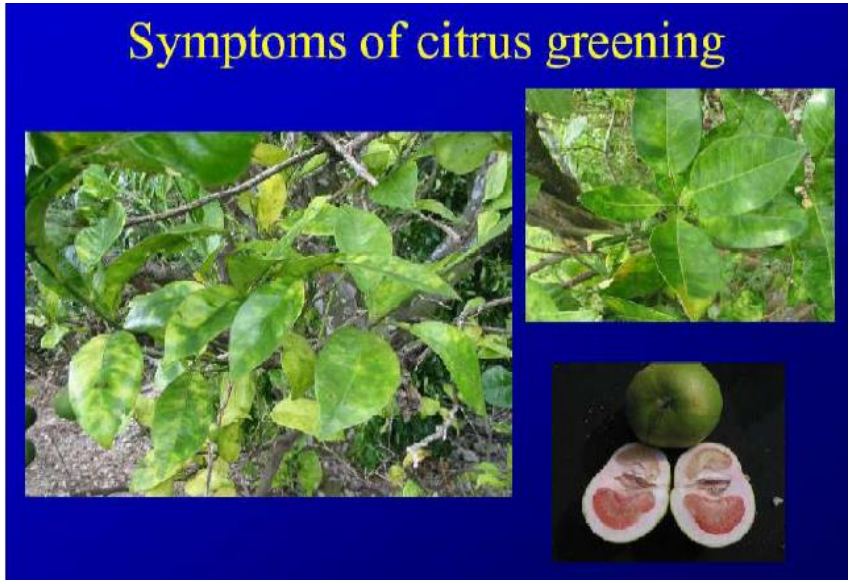


Hu *et al.*,2021

Citrus greening(Huanglongbing)

Association of phytoplasma with *Ca.* Liberibacter asiaticus'(CLas)

- Three species of *Candidatus* Liberibacter cause huanglongbing:
- *Ca. L. asiaticus*,
- *Ca. L. africanus*, and
- *Ca. L. americanus*.
- The Asian form is the most widespread.



Note: Association of phytoplasma with *Ca.* Liberibacter asiaticus'(CLas) in sweet lime was reported for the first from Iran (Saberi *et al.*, 2017).The HLB-associated phytoplasma was a member of peanut witches' broom (16SrII) phytoplasma group. Recently same type of association (phytoplasma with Huanglongbing (HLB) disease) was reported in pomelo (*Citrus grandis*) from India. Here, the pathogen belongs to 16SrXIV Group of phytoplasma, '*Candidatus* Phytoplasma cynodontis'(Ghosh *et al.*,2019).

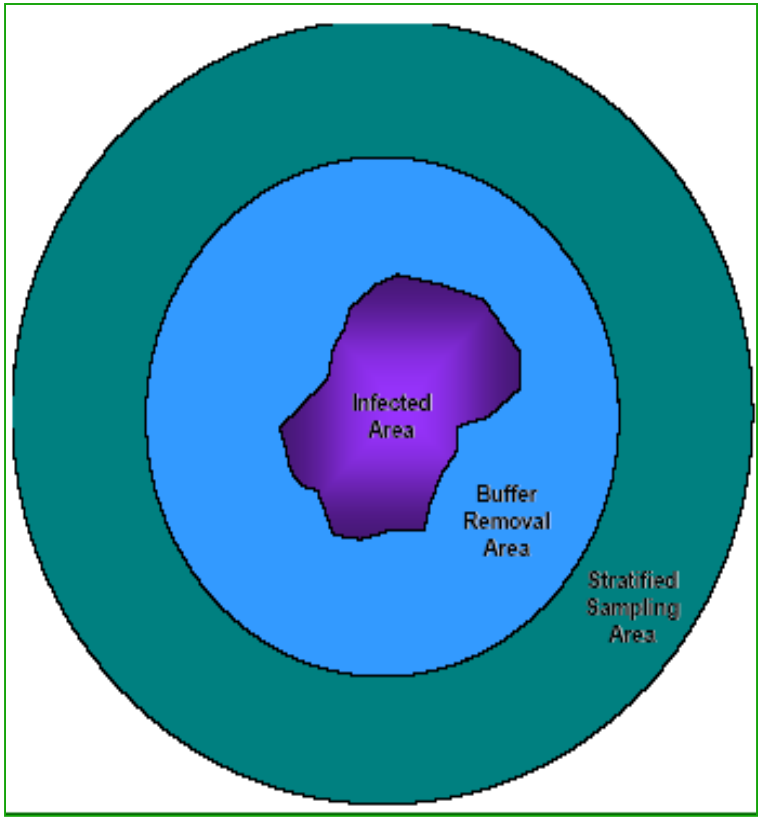
M. J. Davis; Zhang,2019

Survey for Satellite Infestations

HLB or Citrus Greening disease

Candidatus liberobacter spp.

- After one or more infestations are delimited, regulatory and control measures may require the removal of exposed hosts around the known infested areas.
- Further surveys will be necessary to discover satellite infestations or other areas of potential infection.
- Design a sentinel or other stratified survey to accomplish this.
- Figure: Stratified survey to detect satellite infestations After delimiting survey.



Rating of suspect plant tissue samples HLB or citrus greening disease *Candidatus liberobacter* spp.

If symptoms include the following:	Then apply this suspect rating for HLB:
Classic HLB mottle[1] alone or accompanied by one or more of the following symptoms: ◆ Zinc-like deficiency ◆ Yellow veins ◆ Corking veins ◆ Misshapen or oddly colored fruit	High
Non-classic mottle[2] alone or in combination with the following symptoms: ◆ Yellow veins ◆ Vein corking ◆ Chlorotic leaves ◆ Zinc deficiency	Medium
◆ Zinc and other general deficiencies ◆ Mottling resulting from insect injury, fungal diseases, and mechanical damage to leaves ◆ Naturally senescing leaves ◆ Genetic variegation	Low

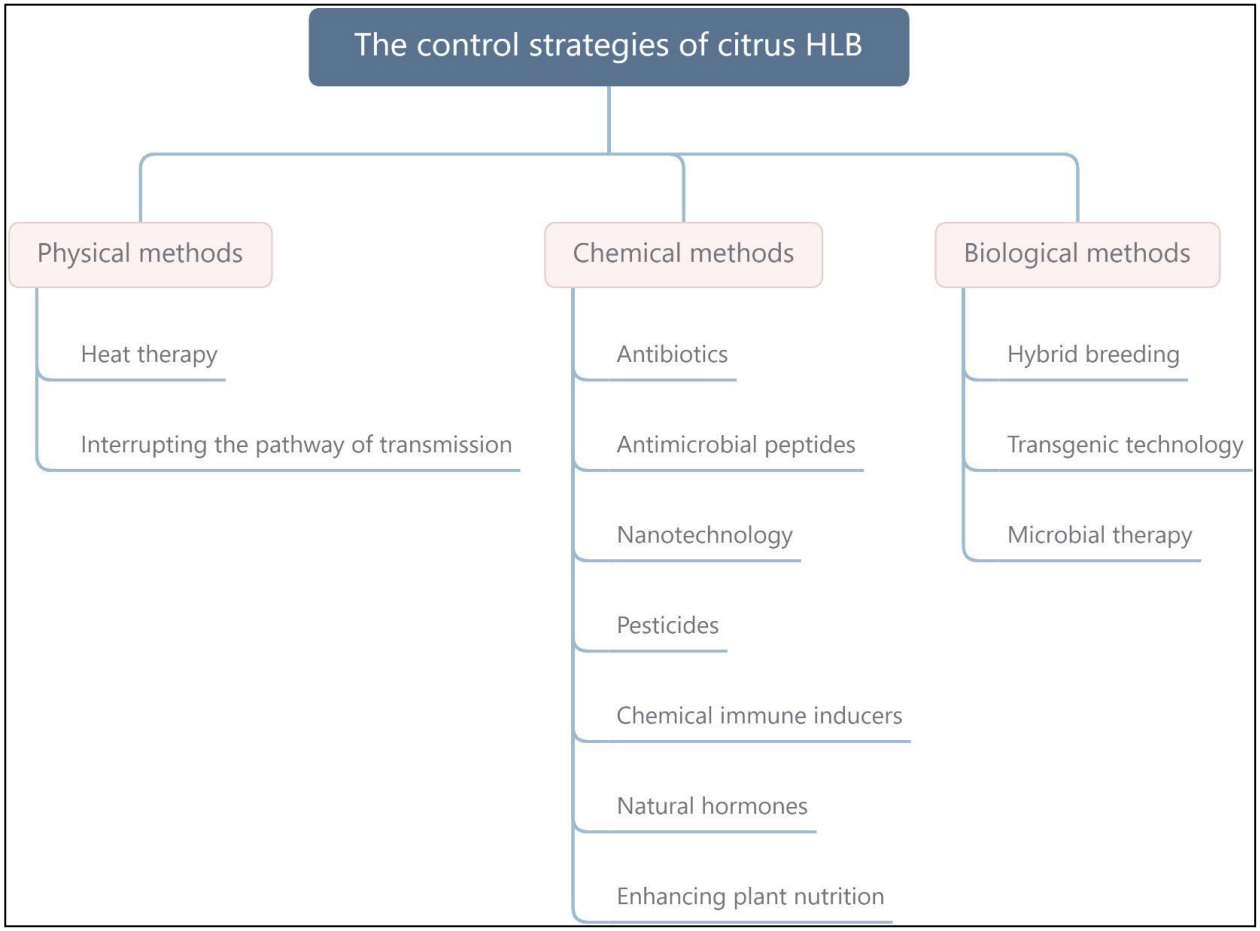
1 Classic HLB mottle is usually visible on both leaf surfaces and mottling/discoloration passes through veins.

2 Non-classic mottle is visible only on adaxial surface and may or may **not** cross veins.

Management

HLB or citrus greening disease

Candidatus liberobacter spp.



Li *et al.*,2012

Management

Antimicrobials that have been tested against HLB infection in studies that incorporated quantification of the phytopathogen

Broad Antimicrobial Class (Target activity)	Antimicrobial Compound(s)	Field/Greenhouse (Applic. method)	Impact on *Ca.* Liberibacter asiaticus (Detection method)	Impact on HLB Symptoms	Potential Side Effects	Reference
Aminoglycosides (inhibit protein synthesis)	Streptomycin	Greenhouse (root drench)	Reduction in population density in leaves by more than 3 log units within 3 months after treatment, yet re-growth to a level close to the starting concentration by the 6-month time point (qPCR)	Not discussed	No phytotoxicity	(Zhang et al. 2011a)
β-lactams (inhibit transpedtidation/ cell wall modification)	Penicillin G	Field (trunk injection)	Concentration-dependent reduction in titer by 6- to 12-fold in leaves of treated trees compared to untreated controls at the 3-month time point after treatment (qPCR)	Slight increases in canopy size	No phytotoxicity; little or no impact on native bacterial populations and penicillin resistance within populations	(Shin et al. 2016)
		Greenhouse (root drench)	Reduction in population density in leaves by more than 3 log units within 3 months after treatment, yet re-growth to a level close to the starting content by the 6-month time point (qPCR)	Not discussed	No phytotoxicity	(Zhang et al. 2011a)
Sulfonamides (inhibit metabolic pathway for folic acid synthesis)	Sulfadimethoxine	Greenhouse (root drench)	Approximately a 9% lower relative abundance (Phylochip), but about twice as high titer (qPCR), in leaves of treated seedlings than controls at the 2-month time point after treatment	Slightly less chlorosis development in canopy of treated seedlings than in that of controls	Partial deleterious effects on relative abundances of native bacteria	(Yang et al. 2016)
	Sulfathiazole	Greenhouse (root drench)	Approximately a 7% lower relative abundance (Phylochip), but about twice as high titer (qPCR), in leaves of treated seedlings than controls at the 2-month time point after treatment	Slightly less chlorosis development in canopy of treated seedlings than in that of controls	Partial deleterious effects on relative abundances of native bacteria	(Yang et al. 2016)
Tetracyclines (inhibit protein synthesis)	Oxytetracycline	Field (trunk injection)	Depending on the amount of injection ports used for application, the population density in leaves decreased 1-3 log units within 1-month after treatment (qPCR). It remained lower in treated trees than controls for 9 months, although population re-growth occurred during this time.	New flushes did not display chlorosis, so the overall canopy appeared healthier	Moderate phytotoxicity – brown discoloration to leaf burning on some young flushes	(Hu and Wang 2016)
Small molecules (inhibit transcription factors produced by *L. asiaticus* that may be essential for pathogenesis and	Tolfenamic Acid	Greenhouse (foliar spray; root drench)	Approximately an 80-95% reduction in the expression of *L. asiaticus* genes *rplJ* and *gyrA* in 75% of the treated seedlings, indicating substantial reduction in viable population (RT-qPCR)	Substantial improvements in fibrous root development and foliage appearance	No phytotoxicity	(Gardner et al. 2016)

Blaustein *et al.*,2017

Management

Control measures other than antimicrobials that have been tested against HLB in studies that incorporated quantification of the phytopathogen

Treatment	Treatment Details	Field/Greenhouse (Applic. method)	Impact on *Ca.* Liberibacter asiaticus (Detection method)	Impact on HLB symptoms	Side Effects	Reference(s)
Thermotherapy	40◦C	Greenhouse	Anywhere from no change to >40-fold reduction in titer (qPCR), depending on the study. About a 9% reduction in relative abundance (PhyloChip)	Some instances of chlorosis-like symptoms being mitigated over time, though some cases of normal symptom progression	Some cases of moderate leaf tissue damage	(Hoffman et al. 2012; Yang et al. 2016; Zhang et al. 2016)
	42◦C	Greenhouse	Anywhere from no reduction to >40-fold reduction in titer (qPCR), depending on the study.	Some instances of chlorosis-like symptoms being mitigated over time, though some cases of normal symptom progression	Some cases of moderate leaf tissue damage	(Hoffman et al. 2012; Zhang et al. 2016)
	45◦C	Greenhouse	Anywhere from 5-fold to 1000-fold reduction in titer in response to treatment (qPCR). Over 80% reduction in relative abundance (PhyloChip)	Chlorosis-like symptoms generally mitigated over time	Some cases of severe leaf tissue damage	(Yang et al. 2016; Zhang et al. 2016; Fan et al. 2016)
	48◦C	Greenhouse	Reductions in titer in leaves of treated trees by about 55%, while that in untreated controls increased by over 300% (qPCR)	Chlorosis-like symptoms mitigated over time	Not discussed	(Fan et al. 2016)
Chemical inducers of plant defenses	AA, BABA, BTH, INA, 2-DDG (used individually or in combination)	Field (spray)	No reduction in titer within leaves; however, the rate of increase was slowed compared to controls (qPCR)	Trees receiving treatment had greater fruit yield and quality than controls	Not discussed	(Li et al. 2016)
Plant-regulating compounds	L-arginine; 6-benzyl-adenine + gibberellins	Greenhouse (spray) and Field (spray)	No reduction in titer within leaves of trees receiving treatments (RT-qPCR)	No phenotypic effects; however, genes involved in plant metabolism and immune response were up-regulated	Not discussed	(Martinelli et al. 2016)
Brassinosteroids	epibrassinolide	Greenhouse (spray) and Field (spray)	Concentration-dependent reduction in titer within leaves by about 160- and 7-fold in greenhouse and field study, respectively (qPCR)	No HLB symptoms on new flushes; genes involved in plant defense response were up-regulated	Not discussed	(Canales et al. 2016)
Micronutrient-based compounds	Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)	Greenhouse (root drench)	Increases in titer (qPCR) and relative abundance (PhlyoChip) in leaves	No effects; symptoms progressed	Not discussed	(Zhang et al. 2016)
	Zineb (i.e., zinc salt of a bis-dithiocarbamate; USA EPA approved agriculture pesticide)	Greenhouse (root drench)	Increases in titer (qPCR) and relative abundance (PhlyoChip) in leaves	No effects; symptoms progressed	Not discussed	(Zhang et al. 2016)
	phosphite combined with Mn-carbonate, Mn-, Cu-, or Zn-metalosate; soluble copper or silver combined with a polymer	Field (spray; trunk injection)	No reduction in titer in leaves (qPCR)	No effects; symptoms progressed	Not discussed	(Gottwald et al. 2012)

Blaustein *et al.*,2017

Management

HLB or citrus greening disease

Candidatus liberobacter spp.

- Green strategies to prevent and control HLB.
- There are mainly three steps:

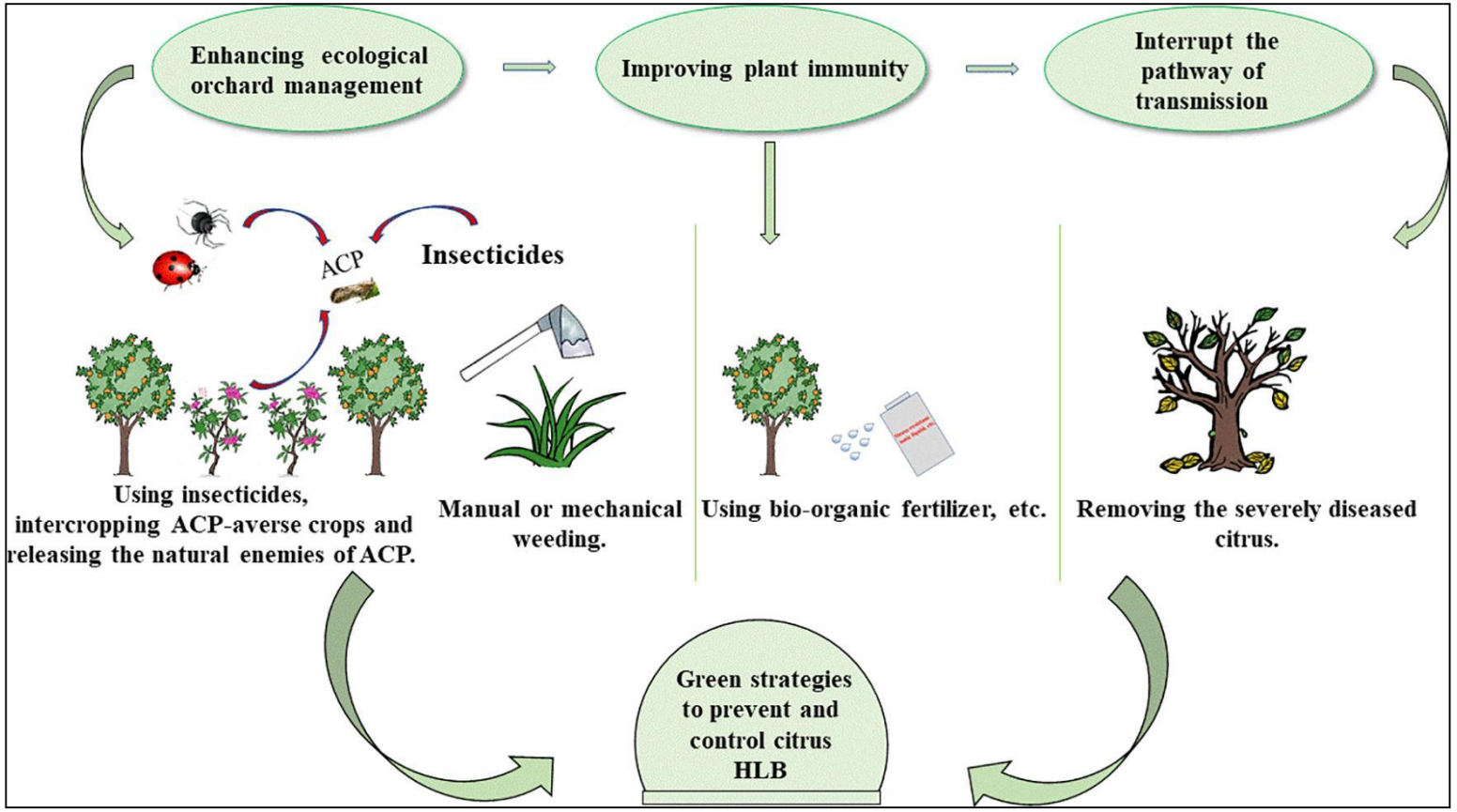
1. first of all, enhancing the management of orchards by manual or mechanical weeding, intercropping ACP-averse crops, and releasing the natural enemies of Asian citrus psyllid(ACP).
2. Secondly, improving plant immunity
3. Using bio-organic fertilizer, spraying metabolic photosynthetic accelerators, and stress-resistant ionic liquids to enhance plant immunity.
4. Finally, removing the severely diseased citrus to cutoff HLB from the source.

Li *et al.*,2012

Management

HLB or citrus greening disease

Candidatus liberobacter spp.



Li *et al.*,2012

Management

HLB or citrus greening disease

Candidatus liberobacter spp.

- **Orchard Sanitation:**
- Periwinkle is a preferred host for the HLB pathogen.
- However, it requires transmission through dodder plants.
- For the Psyllid vector the preferred host for development and multiplication is *Murrya paniculata* (orange jasmin).
- Therefore, these two hosts of the vector and pathogen and the dodder plant must be eradicated from orchards and in the vicinity.

Management

Long term HLB management

Remove alternative hosts

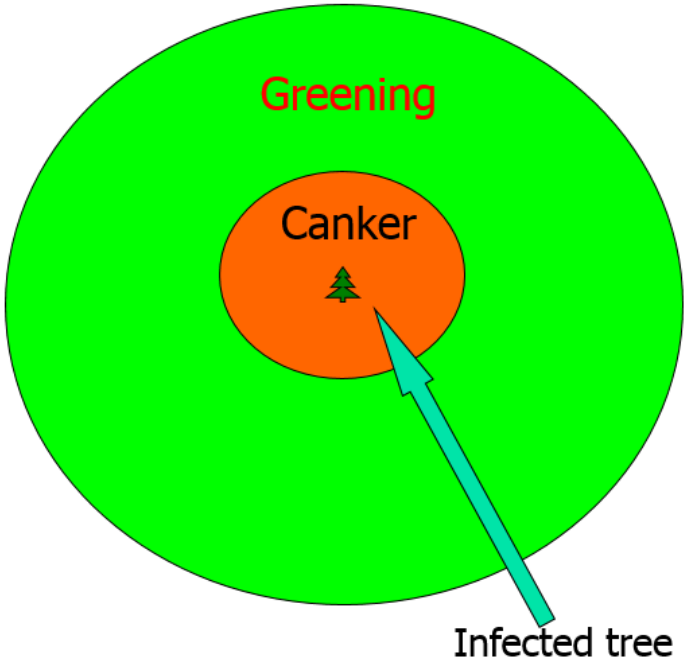
- A compounding difficulty is the spread of infected psyllids from nursery plants.
- Orange jasmine, *Murraya paniculata*, is a preferred host of the Asian citrus psyllid.
- In fact, this psyllid has moved through Florida via sales of untreated orange jasmine in large retail stores.

Management
Cultural control
Exposure radius around an infected tree

- Successful eradication assumes early detection and removal of infected plants as well as all citrus plants potentially infected or exposed within range of the flying psyllids.
- If the disease is detected early enough, prospects for eradication are good, but the difficulty will be the extensive range of psyllid flight.
- For citrus canker, the exposure radius around an infected tree is 1,900 feet.
- For citrus greening, the exposure radius is 9/10 mile! This means we need to destroy infected and potentially infected trees within an area 6.8 times as great as for canker eradication!
- 1 feet=30.45 cm; a mile=1609 m

Management
Cultural control
Exposure radius around an infected tree

- Red: An exposure radius of 1,900 feet is used for citrus canker.
- Green: The estimated exposure radius for citrus greening is 0.9 mile/1448 m (how far Asian citrus psyllids can fly).
- This involves 6.8 times the area for citrus canker.



Schubert and Sapp

Insecticides Registered for Control of Psyllids on Citrus.

Trade Name[1] and Percent AI[2]	Active Ingredients	EPA Reg. No.	Usage
Marathon® II (21.4%)	Imidacloprid [1-[(6-Chloro-3-pyridinyl) methyl]-N-nitro-2-imidaz olidinimine]	3125-549-59807	Ornamentals, fruit and nut trees, and vegetable plants in greenhouses, nurseries, and interior plantscapes
Marathon® 60 WP (60%)	Imidacloprid	3125-492-59807	Ornamentals and vegetable plants in greenhouses, nurseries, and interior plantscapes
Tame 2.4 EC (30.9%)	Fenpropathrin	59639-77	Commercial use on indoor and outdoor ornamental and nursery plants
Dursban® 4E (44.8%)	Chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyri dyl) phosphorothioate]	655-499	Fruit, nut and citrus trees, golf course turf and commerical nursery plants
Discus™ (2.94%)	Cyfluthrin [(RS)-a-cyano-4-fluoro-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate)] (0.70%) and Imidacloprid	432-1392-59807	Ornamentals, non-bearing fruit and nut trees, in field and container nurseries
Chlorpyrifos G-Pro 4 (44.7%)	Chlorpyrifos	79676-9	Commercial nurseries and greenhouses; golf course turf, turf and ornamentals around industrial buildings; turf and ornamentals in road medians

1 Other products might be registered for control of citrus psyllids. Check with APHIS–Environmental Services for more information.

2 AI = Active ingredient

Management
Huanglongbing (HLB) or citrus greening
Biological control

- Two parasites (*Tamarixia radiata* and *Diaphorencyrtus aligarhensis*) have been used with varying admittedly minor degrees of success in Asia and are being introduced in Florida.
- Biological controls have been shown to have some affect on vector populations.
- *Tamarixia radiata* can reduce populations of citrus psyllid.
- In the photo below, *Diaphorencyrtus aligarhensis* inserts an egg into a citrus psyllid nymph.

State Agricultural Response Team

Management
Gene editing

- To save a billion-dollar industry from the infectious disease, also known as huanglongbing, researchers are turning to gene editing, RNA interference, and other advanced techniques.
- But these efforts won't fix the problem overnight.

Zhang,2019

Spiroplasma citri

Stubborn disease of citrus

Uneven fruit size

Spiroplasma plant diseases
Disease symptoms

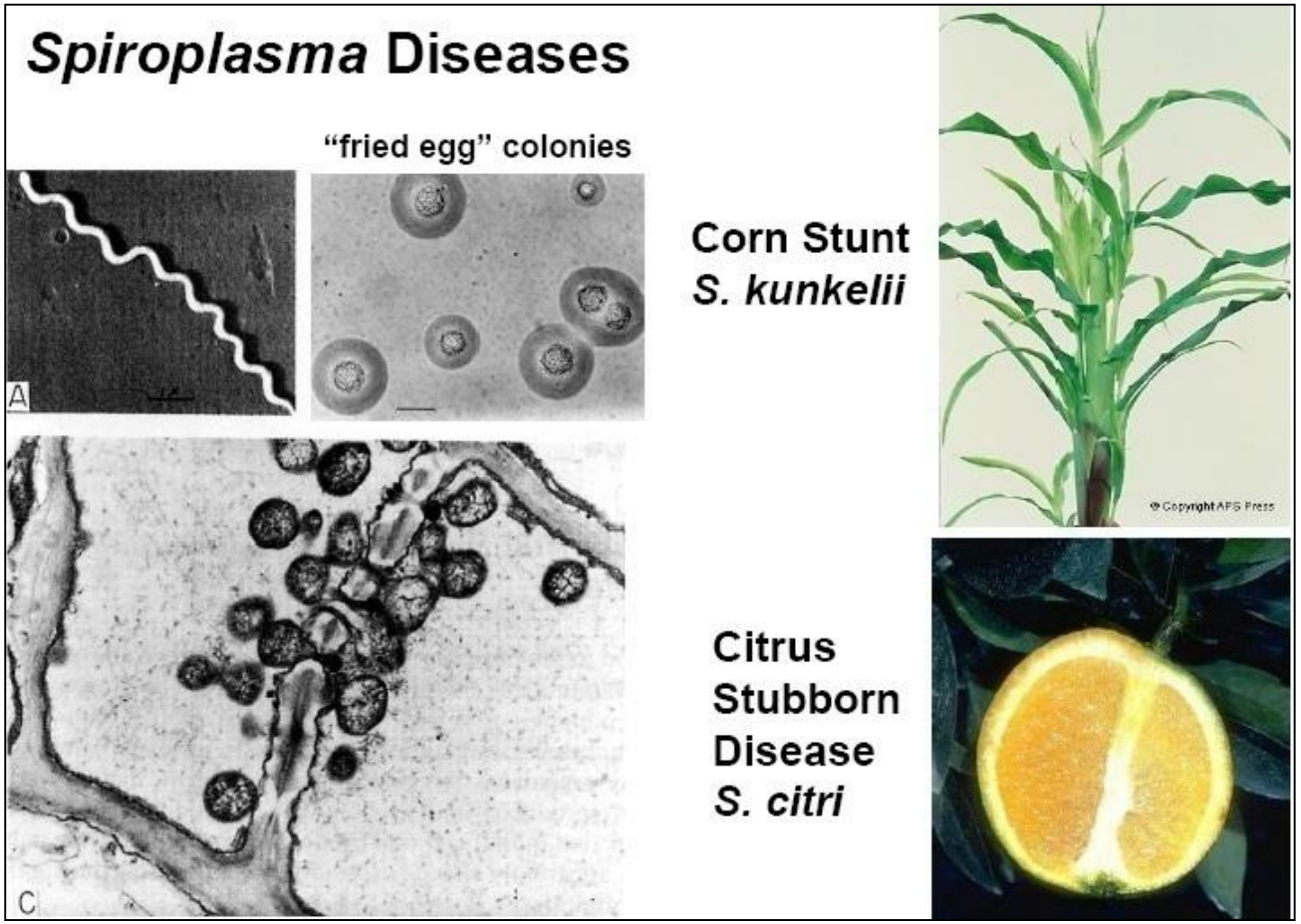
- Out of 38 described Spiroplasma species, only three have been associated with plant diseases and three with arthropod diseases:
- *S. citri*, Citrus stubborn disease (CSD);
- *S. kunkelii*, Corn stunt (CSS), and
- *S. phoeniceum*, causal agent of a periwinkle yellows disease (PYD).

Cacciola *et al.*,2017

Species	Host species	Geographic distribution
Spiroplasma alleghenense	Common scorpion fly *(Panorpa helena)*	USA (WV)
Spiroplasma apis	Honey-bee (*Apis mellifera*)	France
Spiroplasma atrichopogonis	Biting midge (*Atrichopogon* spp.)	USA (MD)
Spiroplasma cantharicola	Soldier beetle (*Cantharis carulinus*)	USA (MD)
Spiroplasma chinense	False bindweed *(Calystegia hederacea)*	China (Jiangsu)
Spiroplasma chrysopicola	Deerfly (*Chrysops* sp.)	USA (MD)
Spiroplasma citri	*Citrus* spp.	USA
Spiroplasma clarkii	Green June beetle (*Cotinus nitida*)	USA (MD)
Spiroplasma corruscae	Lampyrid beetle *(Ellychnia corrusca)*	USA (MD)
Spiroplasma culicicola	Salt marsh mosquito (*Aedes sollicitans*)	Worldwide
Spiroplasma diabroticae	Corn rootworm *(Diabrotica undecimpunctata*)	USA (MD)
Spiroplasma diminutum	Mosquito (*Culex annulus*)	Taiwan
Spiroplasma eriocheiris	Chinese mitten crab (*Eriocheir sinesis*)	China
Spiroplasma floricola	Tulip tree (*Liriodendron tulipifera*)	USA
Spiroplasma gladiatoris	Maryland horsefly *(Tabanus gladiator*)	USA (MD)
Spiroplasma helicoids	Horseflies (*Tabanus abdominalis-limbatinevris*)	USA (MD)
Spiroplasma insolitum	Fall flower (*Bidens* sp.)	USA (MD)
Spiroplasma ixodetis	Black-legged ticks (*Ixodes pacificus*)	USA (OR)
Spiroplasma kunkelii	Corn (*Zea mays*)	America
Spiroplasma lampyridicola	Firefly beetle *(Photuris pennsylvanicus)*	USA (MD)
Spiroplasma leptinotarsae	Colorado potato beetle (*Leptinotarsa decemlineata*)	USA (MD)
Spiroplasma leucomae	Satin moth larvae, (*Leucoma salicis*)	Poland
Spiroplasma lineolae	Striped horsefly (*Tabanus lineola*)	USA (GE)
Spiroplasma litorale	Horsefly (*Tabanus nigrovittatus*)	USA (NC)
Spiroplasma melliferum	Honey bee *(Apis mellifera)*	worldwide
Spiroplasma mirum	Rabbit ticks (*Haemaphysalis leporispalustris)*	USA (GE, MD)
Spiroplasma monobiae	Vespid wasp (*Monobia quadridens*)	USA (MD)
Spiroplasma montanense	Tabanid fly (*Hybomitra opaca*)	USA (MN)
Spiroplasma penaei	Pacific white shrimp (*Penaeus vannamei*)	Colombia
Spiroplasma phoeniceum	Periwinkle *(Catharanthus roseus)*	Syria
Spiroplasma platyhelix	Dragonfly (*Pachydiplax longipennis*)	USA (MD)
Spiroplasma poulsonii	Fruit fly (*Drosophila willistonii*)	South America
Spiroplasma sabaudiense	Mosquitoes (*Aedes stricticus, Aedes vexans*)	France

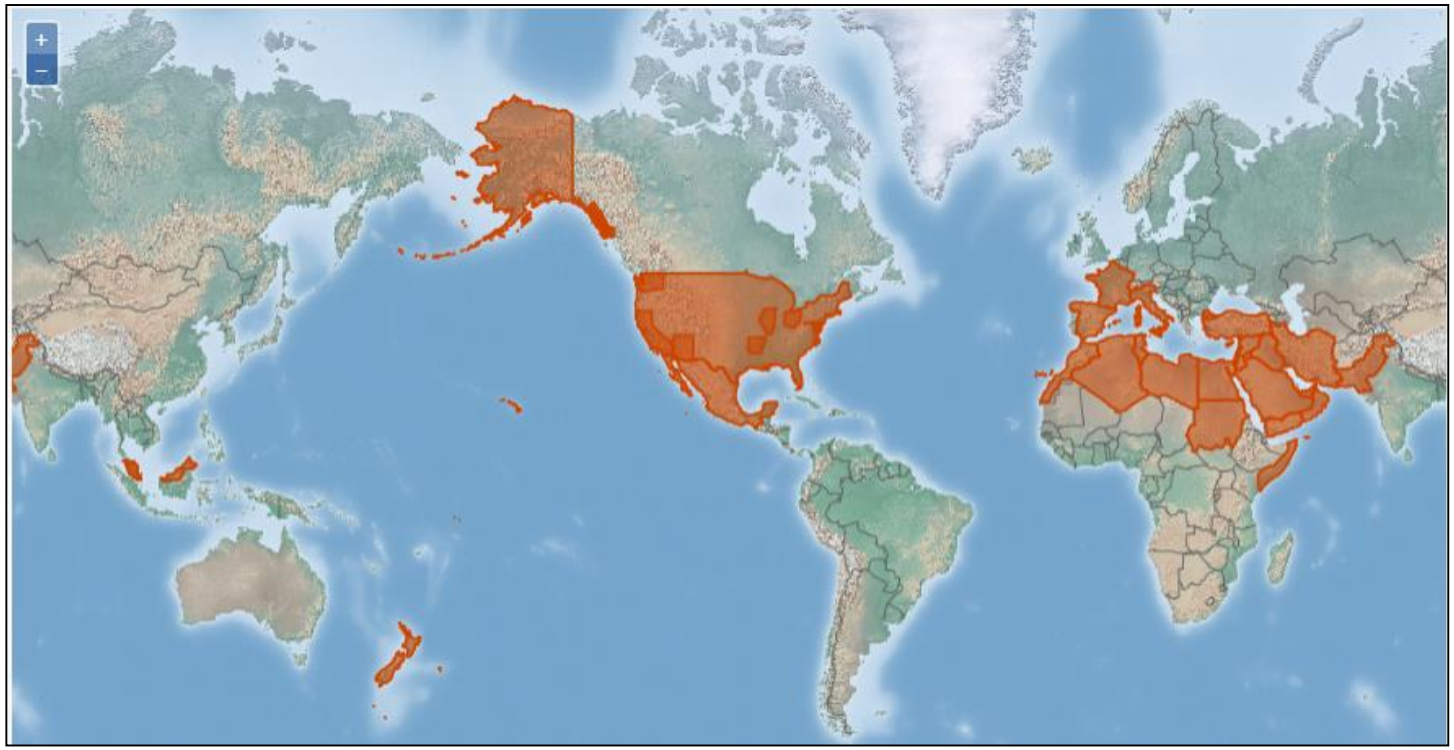
Spiroplasma plant diseases

Disease symptoms



Geographical distribution

Spiroplasma citri



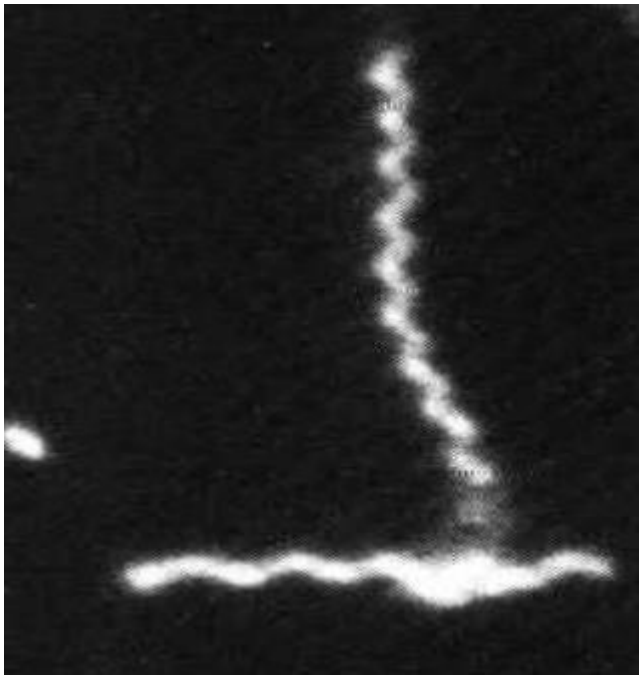
Geographical distribution
Spiroplasma citri

- *S. citri* forms wall-less pleomorphic cells with a characteristic spiral morphology.
- The minimum viable length of a helix is 2.0 x 0.1-0.2 µm.
- The helices are motile by flexing or rotation.
- Some strains are non-motile and non-helical.
- *S. citri* is one of the very few plant pathogenic mollicutes to have been cultured.

Host range and distribution

Spiroplasma citri

- Causes:
1. Citrus stubborn, and
2. Horseradish brittle root.
- Broad Host Range: 79 plant species in 25 plant families.
- Distribution: warm to hot semiarid areas and in deserts mainly in the southwestern U.S. and the Mediterranean countries.



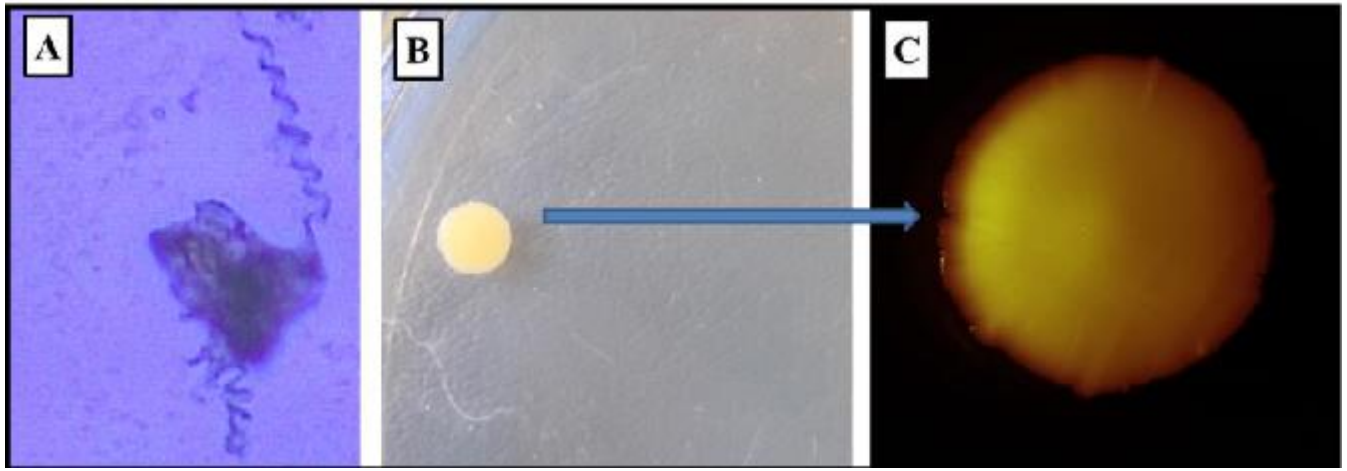
Morphology of *Spiroplasma citri* in C-3G liquid medium

- Morphology of *Spiroplasma citri* in C-3G liquid medium by light microscope.

A. The spiral shape is very obvious in x1000.

B. Fried shape colonies on solid medium 7- 8 days of incubation at 30 ± 2°C.

C. The colony shape by dark field light microscope (400 X).



El-Banna *et al.*,2020

Incidence of citrus stubborn disease (CSD) in citrus samples evaluated by Isolation and by DASELISA

Serial N.	Name	Location	Governorate	Citrus species	Type of sample	Isolation on liquid medium*	Absorbance**
1	EL 1	Damietta	Damietta	Lime	Fruit	+	0.288
2	EL 2	Faiyoum	Faiyoum	Lime	Fruit	+	0.275
3	EL 3	Al Mansouria	Giza	Lime	Fruit	+	0.302
4	EL 4	Al Mansouria	Giza	Lime	Fruit	+	0.298
5	EL K	Kirdasah	Giza	Lime	Fruit	+	0.272
6	EL G	Faculty of Agriculture experimental station	Giza	Volkamer lemon	Leaves	+	0.206
7	EL Be-Fl	Beheria	Beheria	Lemon	Flower	+	0.255
	EL Be-F				Fruit	+	0.288
	EL Be-L				Leaves	+	0.284
8	EL Lux	Al-Odeisat	Luxor	Lime	Leaves	+	0.318
9	EL M	Maadi	Cairo	Lime	Fruit	+	0.254
10	EL I	Ismalia	Ismalia	Lime	Fruit	-	0.138
11	EL B	Benha University	Qalyubia	Lime	Leaves	+	0.206
12	EL T-F	Tersa	Qalyubia	Volkamer lemon	Fruit	+	0.304
	EL T-L				Leaves	+	0.233
	EL T-S				Seeds	+	0.301

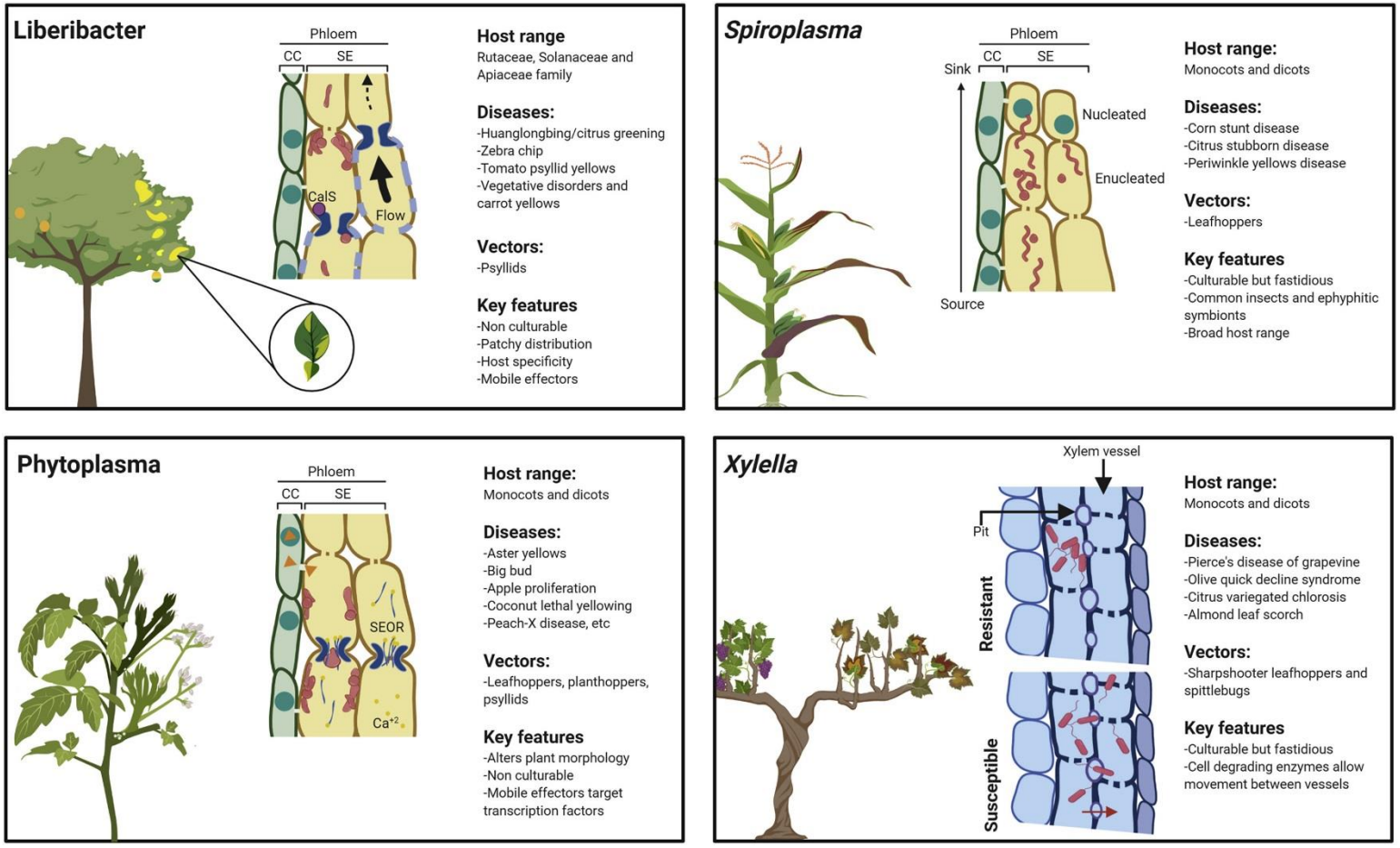
* + = Positive, - = Negative. ** A mean of three reading.
Negative Control: 0.143

El-Banna *et al.*,2020

Vectors

- At least six species of leafhoppers can transmit *Spiroplasma citri*.

Bacterial vector-borne diseases that persist in plant vascular tissues



Huang *et al.*,2020

Circulative Propagative and Non-circulative Propagative Bacterial Localization in Insect Vectors through the Perspective of Phylogenetic Relationships

Huang *et al.*,2020

D-vac

Apparatus for vector collection from fields

Management

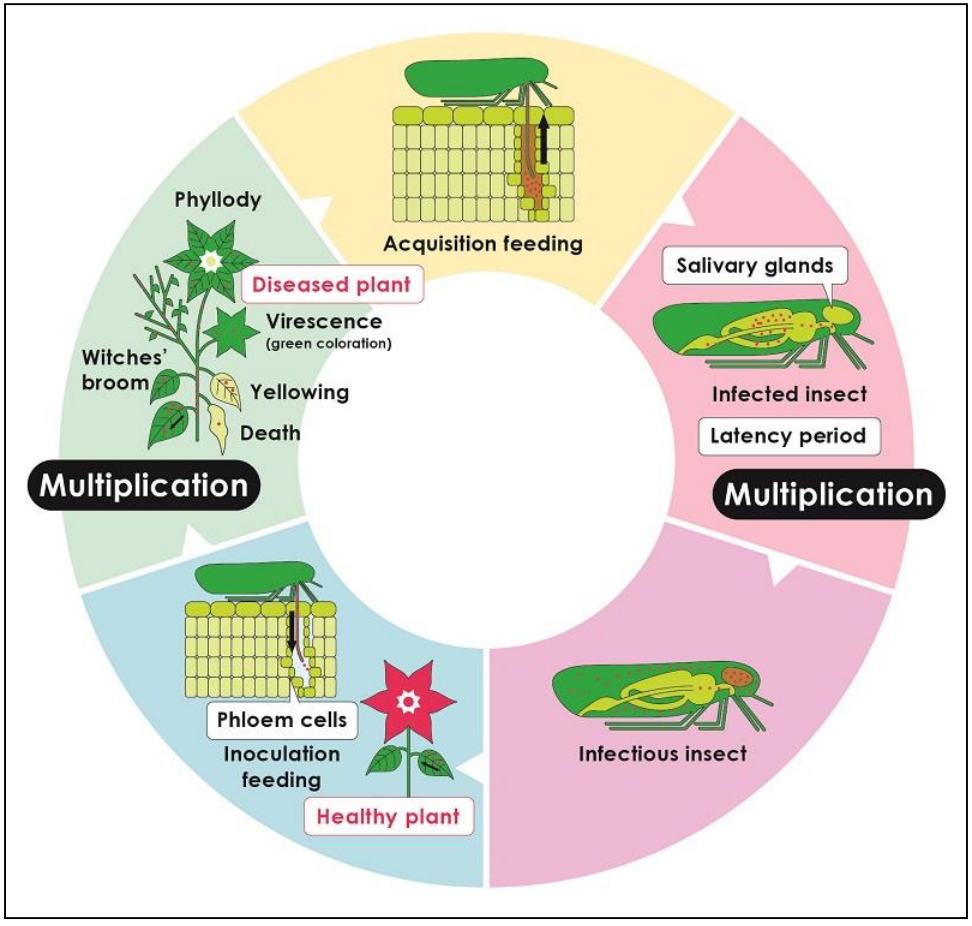
- Management of stubborn disease focuses on preventing the disease and avoiding its spread.
- Preventative measures mainly apply to nursery practices, such as maintaining stubborn-free mother trees for budwood.
- Grafting budwood onto indicator seedlings or culturing leaf and fruit samples in the lab can determine the presence of the stubborn organism.
- No commercial laboratories, however, are currently equipped to carry out these tests. In an established orchard, observe the trees carefully for any signs of stubborn disease in late fall or early winter.
- A sparse crop, a useful diagnostic symptom, becomes apparent as fruit color changes to orange. Map or flag the trees suspected of being infected and recheck the orchard several times during the year to confirm your diagnosis.
- **Cultural Control**
  When planting an orchard, obtain trees from an area that does not have a high incidence of stubborn disease. Replace diseased and unproductive trees. Top working is not advisable because the pathogen moves freely between the scion and rootstock.

Phytoplasmas

Various diseases with yield losses over 300 economically important plant species

Virescence (greening) infection

Phytoplasma infection cycle



Namba and Himeno,2014

Phytoplasma disease symptoms

Aster Yellows

- Yellowing, dwarfing, witches'-broom, abnormal sterile flowers: virescence & phyllody
- Controls:
  - eradicate weedy reservoirs
  - spray leafhoppers
  - partial resistance

Virescence

Floral parts are replaced by vegetative structures

Virescence is closely associated with phyllody (the abnormal development of flower parts into leaves)

Virescence: loss of normal flower color, green flowers.

plp3002.ifas.ufl.edu/pdfs/slides/bacterial_diseases.pdf;..

Phytoplasma disease symptoms

Phyllody: production of leaf like structures in place of flowers

- Development of a flower into a leaf-like structure is called phyllody.
- A healthy hydrangea (left) and a hydrangea showing phyllody due to phytoplasma infection (right).

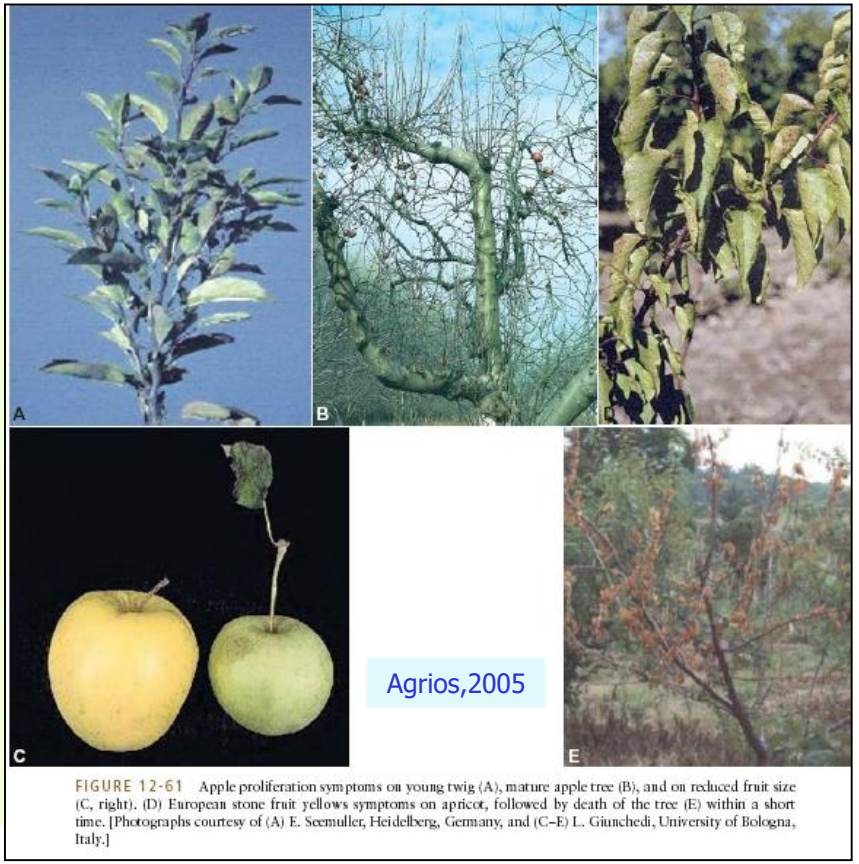
Himeno counters that "If we can utilize the phyllogen protein to induce phyllody without causing disease, we may be able to readily grow ornamental green lilies and other flowers."

Namba and Himeno,2014

Apple proliferation
Phytoplasma

- Apple proliferation symptoms on:

A. Young twig;
B. Mature apple tree and
C. On reduced fruit size (right).
D. European stone fruit yellows symptoms on apricot, followed by death of the tree (E) within a short time.



Agrios,2005

FIGURE 12-61 Apple proliferation symptoms on young twig (A), mature apple tree (B), and on reduced fruit size (C, right). (D) European stone fruit yellows symptoms on apricot, followed by death of the tree (E) within a short time. [Photographs courtesy of (A) E. Seemuller, Heidelberg, Germany, and (C–E) L. Giunchedi, University of Bologna, Italy.]

[Photographs courtesy of (A) E. Seemuller, Heidelberg, Germany, and (C-E) L. Giunchedi, University of Bologna, Italy.]

Pear decline
Phytoplasma

A. Young pear tree showing symptoms of pear decline caused by a phytoplasma.

B. Disruption of phloem at and below the graft union as a result of pear decline infection is responsible for decline symptoms.

FIGURE 12-63 (A) Young pear tree showing symptoms of pear decline caused by a phytoplasma. (B) Disruption of phloem at and below the graft union as a result of pear decline infection is responsible for decline symptoms.

Strange,2003

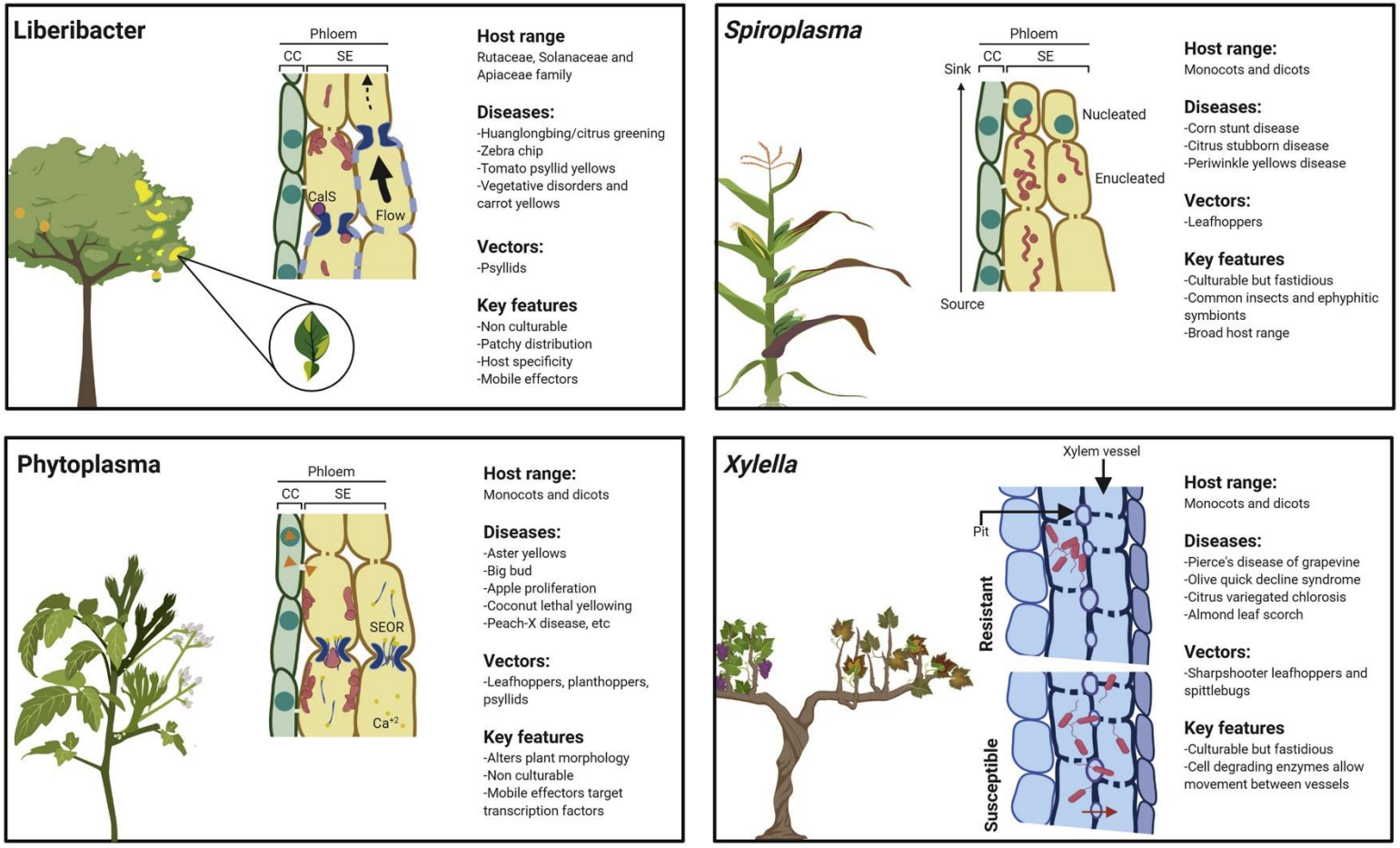
Diagnostic kit

Diagnosing witches' broom disease of cassava

- Namba and his colleagues have developed a highly sensitive diagnostic kit that permits phytoplasma to be detected rapidly and easily that is now used for applications such as diagnosing witches' broom disease of cassava, a major agricultural problem in Southeast Asia.

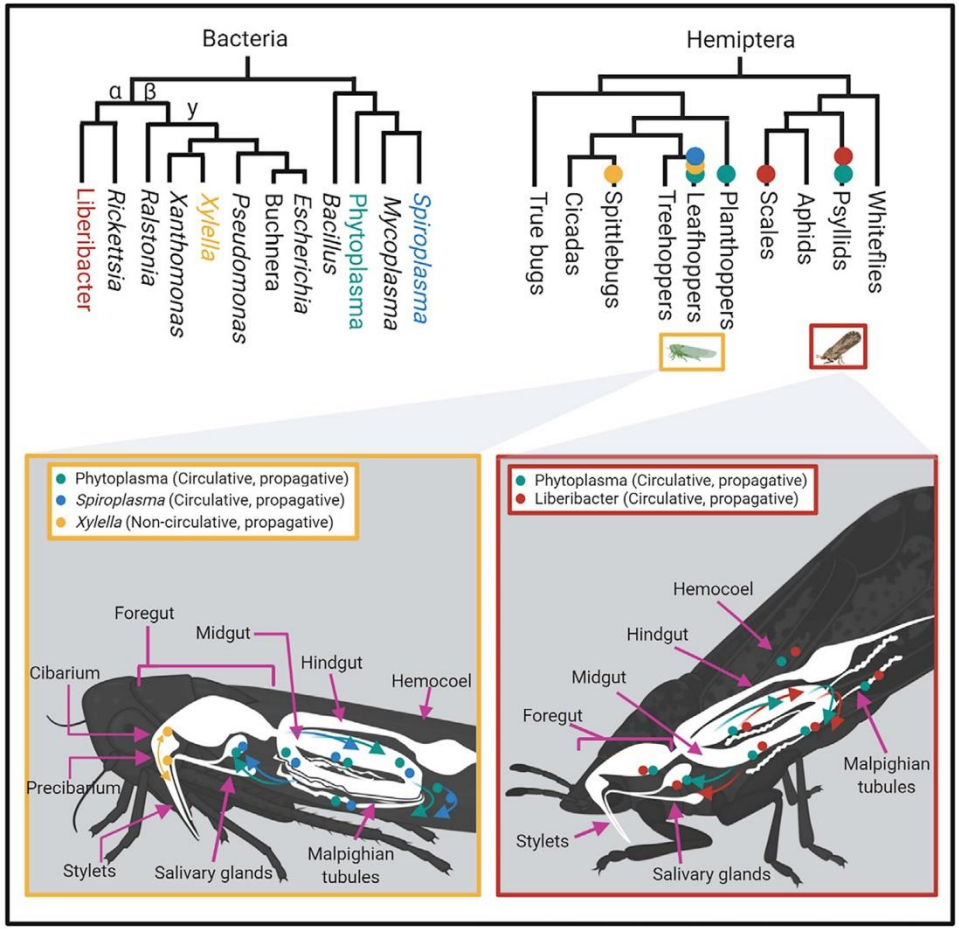
Namba and Himeno,2014

Bacterial vector-borne diseases that persist in plant vascular tissues



Huang *et al.*,2020

Circulative Propagative and Non-circulative Propagative Bacterial Localization in Insect Vectors through the Perspective of Phylogenetic Relationships

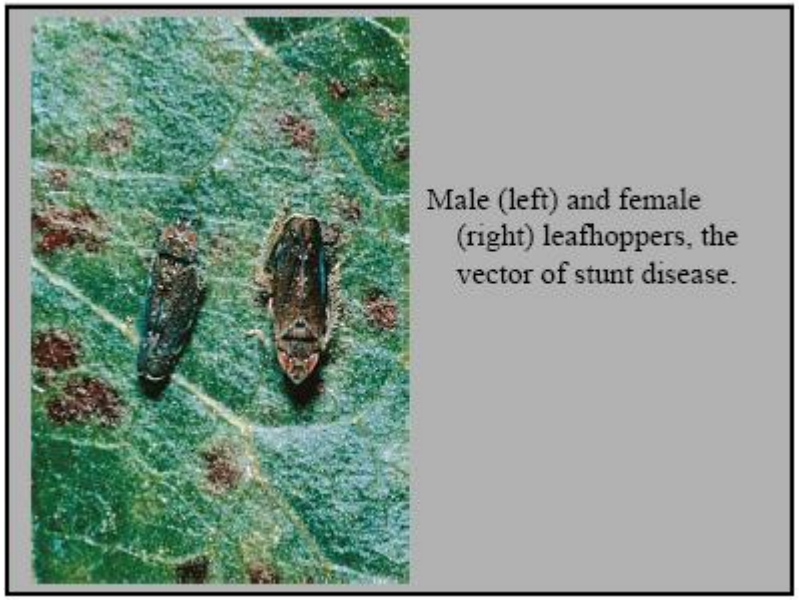


Huang *et al.*,2020

Management

Insecticides to control vector

- Control the leafhopper vector in the crop and nearby weeds early in the season.



Male (left) and female (right) leafhoppers, the vector of stunt disease.

Management

Aster yellows phytoplasma

Insecticides to control vector

- Example of control by application of insecticides to control vector.
- Timing of insecticide is critical.

Management
Pre-treatment

- The control of phytoplasma diseases is difficult and relies mainly on:

1. Insecticide treatments against vector insects and
2. The planting of healthy propagation material.

- Insecticides can be effective in suppressing the vector population but only neonicotinoids actively prevent the transmission from infectious insects visiting the crop (Saracco *et al.*,2008).
- Large scale use of insecticides (in preventing the spread of phytoplasma diseases) has a negative impact on nontarget arthropods, especially mites (Waetermeulen *et al.*,1999) and, possibly, on pollinators (Vorwohl, 1977), and represents a potential threat to human health.
- Planting phytoplasma-free stocks is advisable, but control of vector populations is still required to prevent transmission during the vegetative season (Morone *et al.*,2007).

D'Amelio *et al.*,2010

Management
Elimination methods

- Phytoplasmas can be eliminated from their plant hosts, as they are generally heat labile and are not present in the shoot meristem (Lee and Davis, 1992).
- Furthermore they are sensitive to some antibiotics such as tetracycline (Heintz,1989).
- Several methods have been applied to clean plant material for phytoplasmas, these include:
- *in vitro* tissue culture such as shoot tip (Dale and Cheyne,1993), or
- Micropropagation (Davies and Clark,1994),
- Sometimes in combination with heat or antibiotic treatment.

Nicolaisen,2001

Heat treatment

- Infected plants or dormant propagative tissue can be freed of phytoplasma by heat treatment.
- This technique is used at quarantine facilities when phytoplasma infection is suspected in fruit tree nursery stock being imported into a country.

Warm air	30-37° C	Several days-weeks-month
Hot water	30-50° C	10 min
Hot water	low	72 h

Antibiotic treatments

Antibiotics sensitivity tests

- Curing infected plants with antibiotics or by stimulating the production of specific antibodies is not practicable because:

1. Antibiotics are too costly,
2. Prohibited in several countries, and
3. Do not always provide long-time control.

- Moreover the production of transgenic plants producing antibodies or resistant to these pathogens is still a long way off (Ishiie *et al.*,1967; Chen and Chen,1998; Le Gall *et al.*,1998).

Bertaccini and Duduk,2009

Antibiotic treatments

Antibiotics sensitivity tests

- Phytoplasmas differ from bacteria in that they lack a cell wall or penicillin-binding sites. Therefore, they are resistant to penicillin.
- However, phytoplasmas are sensitive to tetracycline antibiotics.
- It should be also noted that a high concentration of antibiotics is toxic for the plants.
- Hence, three concentrations of antibiotics should be used (100, 500, and 1000 ppm).
- In this experiment, it is important to have many phytoplasma-infected plants and to have enough controls to obtain reliable results.
- One group of plants should be inoculated weekly with tetracycline solution, another with penicillin solution, and the control group with distilled water.
- If, in the experiment, the concentration of antibiotics used is not toxic, then higher concentrations could be substituted.

Antibiotic treatments

Antibiotics sensitivity tests

- The plants that received the penicillin treatment will not show any change in the severity of the symptoms.
- The plants that received the tetracycline treatment will show a reduction in the severity of the symptoms (as long as these symptoms are not evident in the young tissue that will continue to grow).
- If the weekly tetracycline treatment is stopped, the plants will gradually show the symptoms of the disease again.
- Therefore, the sensitivity of the agent to tetracycline is a good indicator of a phytoplasma associated with a disease.

Antibiotic treatment

Antibiotic treatment

Pear decline

Dose: 6-8 g/L tetracycline

Selection of germplasm with host plant resistance to Mollicutes

- Efforts continue to identify germplasm coding for natural resistance to *Mollicutes*, and to incorporate the appropriate genes into various crops and fruit and forest trees *via* selection and breeding programs.
- The resistance thus bred may include resistance to either the pathogens or to the insect vectors.
- Plant defense related proteins, known to be active in responding to invasion by other types of pathogens, may also be effective in responding to mollicute infection.

Bertaccini and Duduk,2009

Systemic acquired resistance
SAR

- The treatment of plants with various agents (e.g., virulent or avirulent pathogens, nonpathogens, cell wall fragments, plant extracts and synthetic chemicals) can lead to the induction of resistance to subsequent pathogen attack.
- SAR by plant activators provided interesting results in the control of a broad spectrum of pathogens, such as bacteria, fungi and viruses.
- Bressan and Purcell,2005 reported a significant effect of plant resistance elicitor benzothiadiazole (BTH) (Bion, Syngenta Crop Protection) in reducing X-disease phytoplasma transmission to *Arabidopsis thaliana* (L.) by the leafhopper.

D'Amelio *et al.*,2010

Systemic acquired resistance (SAR) Bion

- However, little is known about its activity towards phytoplasma diseases and, to our knowledge, only two reports are available on the activity of this chemical on phytoplasmas:
- One towards X-disease phytoplasma transmission to *A. thaliana* studied under controlled conditions (Bressan and Purcell, 2005)and found a significant effect of plant resistance elicitor benzothiadiazole (BTH) (Bion, Syngenta Crop Protection) in reducing X-disease phytoplasma transmission by the leafhopper.
- And the other on grapevine bois noir phytoplasma (Stolbur phytoplasma or '*Candidatus* Phytoplasma solani') under field conditions (Romanazzi *et al.*, 2009).
- In our study we found BTH application against chrysanthemum yellow phytoplasma (CYP) infection in the *Chrysanthemum carinatum* plant. delayed symptom development and phytoplasma multiplication in treated plants compared with the control ones.

D'Amelio *et al.*,2010

Systemic acquired resistance (SAR) Bion

- **Symptom evaluation:**
- The severity of the symptoms of test plants was evaluated three times a week between 11 and 32 dpi (days after the end of the inoculation), and plants were classified into five classes of severity:
- 0= no symptoms,
- 1= yellowing of the apex,
- 2= yellowing and distortion of the apex,
- 3= apex growth stunt,
- 4= severe yellowing and dwarfing of the whole plant and
- 5= plant death.

D'Amelio *et al.*,2010

Terminology

- **Endophytic**: organisms growing inside the host.
- **Epidemiology** is the study of both the distribution of disease (who has it, where, and when), as well as its causes (etiology).
- **Epiphytic**: Organisms growing on the surface of photosynthetic organisms.
- **Etiology** is the (usually unknown) set of causes of the disease. It is a branch of medicine that investigates the causes and origins of disease or the set of factors that contributes to the occurrence of a disease.
- **Phylloplane**: Leaf surface
- **Phyllosphere**: Area surrounding the leaf and impacted by it
- **Rhizoplane**: Root surface
- **Rhizosphere**: A zone which includes the root surface and the soil closely associated with it (generally the soil that adheres to the roots after gentle shaking).

Terminology

- Susceptibility: Inability of the host to resist the attack of the pathogen.
- Tolerance: is a type of defence that minimises crop losses with out restricting the disease development.
- Resistance: Ability of the host to resist the attack of the pathogen
  - Horizontal resistance
  - Vertical resistance
- Disease escape: it is ability of the susceptible host to avoid the damaging disease stress e.g. unfavorable environment/ growth habit

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