



CENTRO DI SPERIMENTAZIONE E ASSISTENZA AGRICOLA

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CAMERA DI COMMERCIO
RIVIERE DI LIGURIA
IMPERIA LA SPEZIA SAVONA

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**Managing problems caused by
phytopathogenic bacteria during nursery
propagation. Case study: *Acidovorax citrulli***

in una galassia lontana lontana.....



CeRSAA





Cucurbitaceae: A very large botanical family comprising more than 100 predominantly tropical genera, with approximately 650 species.

- Cucurbita L.: squash, zucchini (*Cucurbita pepo* L.).
- Cucumis L.: (*Cucumis sativus*) melon (*Cucumis melo*).
- Citrullus Schrad. ex Eckl. & Zeyh.: water melon (*Citrullus lanatus*).
- Sechium P.Browne: Thorny Chayote (*Sechium edule*).
- Lagenaria Ser.: ornamental squash, rootstocks (*Lagenaria siceraria*).
- Benincasa Savi: wax gourd (*Benincasa hispida*).
- Ecballium A.Rich.: cocomero asinino (*Ecballium elaterium*).
- Bryonia L.: *Bryonia dioica* toxic.
- Dendrosicyos Balf. f.: shrubs cucurbitaceae.
- Luffa Mill.: Luffa gourd (*Luffa cylindrica*).
- Coccinia Wight & Arn.
- Kedrostis Medik.

Cucurbitaceae: Pests and Diseases

Bacteria: 11

Phytoplasmas: 1 group

Viruses: 45

Viroids: 3

Fungi (including Oomycetes): Over 90 pathogenic species

Phytoparasitic Nematodes: 21 species

Insects and Mites: Over 15 genera

Focus on bacterial diseases – key disease

Causal agent	Common name	Main features	Main hosts	Origin (primary)	Infected organs and tissues	Notes
<i>Pseudomonas syringae</i> pv. <i>lacymans</i>	Angular spot	Aerobic gram negative, non forming spore	cucumber, zucchini, squash	Seed (Beneath the seed coat)	Cotyledons, leaves, fruits, stems	Survive on leaf residues for 24 months
<i>Acidovorax citrulli</i>	Bacterial fruit blotch	Aerobic gram negative, non forming spore	Water melon, melon, squash/zucchini	seed	Cotyledons, leaves, fruits, stems	Survive on seed for 30 years
<i>Xanthomonas cucurbitae</i>	Leaf and fruit spot	Aerobic gram negative, non forming spore	squash	seed	Cotyledons, leaves, fruits, stems	
<i>Pseudomonas viridiflava</i>	Leaf spot	Aerobic gram negative, non forming spore	Melon, watermelon, cucumber	Cosmopolitan (weeds, water, ...)		
<i>Pseudomonas syringae</i> pv <i>syringae</i>	Leaf spot	Aerobic gram negative, non forming spore	Watermelon, squash	seed	Cotyledons, leaves	
<i>Erwinia</i> sp, <i>Enterobacter</i> sp, <i>Pseudomonas</i> sp., <i>Bacillus</i> sp.	Seedling bight and necrosis	Aerobic (obligate and non obligate) gram positive (Bacillus) and gram negative	Water melon, melon	Cosmopolitan (weeds, water, ...)	Seedlings	Relevant during grafting phase

Focus on bacterial diseases – secondary disease

Causal agent	Common name	Main features	Main hosts	Origin (primary)	Infected organs and tissues	Notes
<i>Pseudomonas cichorii</i>	Seedling bacterial blight	Aerobic gram negative, non forming spore	Luffa, melon, water melon, cucumber	Seed (?)	seedlings	Relevant during grafting phase
<i>Erwinia</i> sp, <i>Enterobacter</i> sp, <i>Pseudomonas</i> sp., <i>Bacillus</i> sp.	Watermelon rind rot	Aerobic (obligate and non obligate) gram positive (Bacillus) and gram negative	Water melon and melon	Cosmopolitan (weeds, water, ...)	Pericarp	Due to Ca and B deficiency too

Focus on bacterial diseases – post harvest diseases

Causal agent	Common name	Main features	Main hosts	Origin (primary)	Infected organs and tissues	Notes
<i>Pantoea ananatis</i>	Watermelon rind rot	Aerobic gram negative, non forming spore	Melon (post harvest)	Cosmopolitan	Pericarp	

Focus su agenti di batteriosi - esotici

Causal agent	Common name	Main features	Main hosts	Origin (primary)	Infected organs and tissues	Notes
<i>Serratia marcescens</i>	Cucurbits yellowing	Facultative aerobic gram negative	Water melon, melon	Cosmopolitan vector <i>Anasa tristi</i> (nearctic emiptera)	Stems and vascular system	Absent in palearctic area
<i>Erwinia tracheiphyla</i>	Bacterial wilt	Facultative aerobic gram negative	Melon, cucumber	Vector nearctic coleoptera (<i>Acalymna vittatum</i> , <i>Diabrotica undecimpunctata howardi</i>)	Vascular system	Absent in palearctic area

Acidovorax citrulli: A Case Study... But Why?

The bacterium is a key case study due to its combination of efficient seed transmission, high capacity for spread in nurseries, and delayed symptoms in the field, making it difficult to control.

Key Characteristics & Pathogen Profile

- **Bacterial pathogen**
- **Transmitted through infected seed**
- **Pistil inoculation:** Bacterium localized in the **embryo**.
- **Pericarp inoculation:** Bacterial localization outside the **perisperm-endosperm layer**.
- **Easily spread during the multiplication phase** (in the nursery).
- **Capable of becoming endemic in the nursery.**
- **Subtle in field cultivation, potentially manifesting only at harvest.**
- **Able to become endemic in the field** through seeds originating from unharvested fruits.

Acidovorax citrulli

Tassonomia

Kingdom Bacteria (1BACTK)

Phylum Proteobacteria (1PROBP)

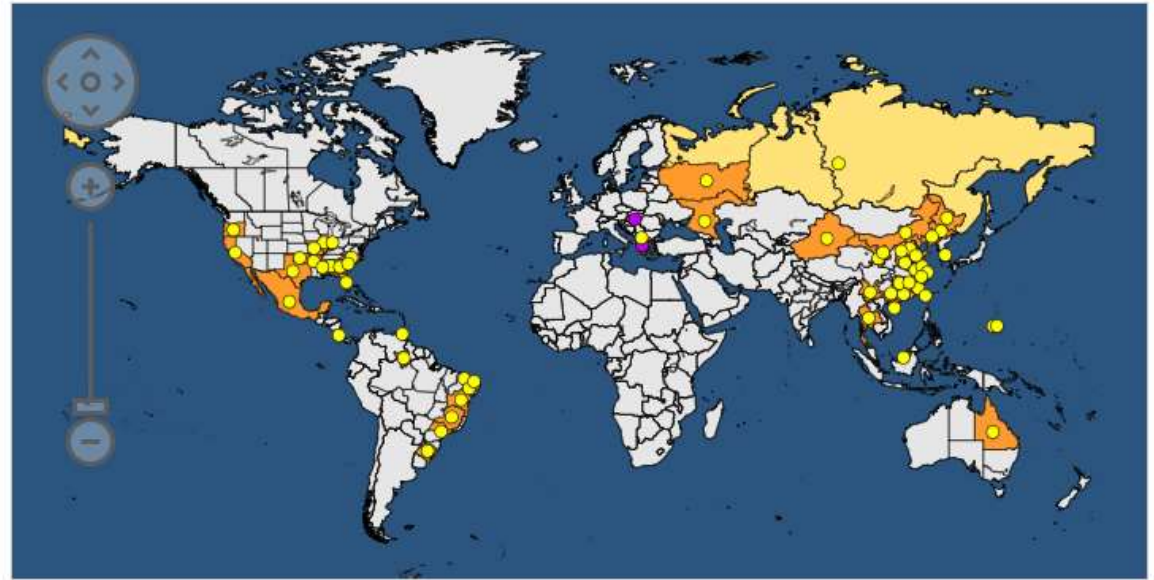
Class Betaproteobacteria (1BETBC)

Order Burkholderiales (1BURKO)

Family Comamonadaceae (1COMAF)

Genus Acidovorax (1ACVRG)

Species Acidovorax citrulli (PSDMAC)



Legend: ● Present ● Transient

Two genetically and pathogenically distinct groups among *A. citrulli* strains.

- Group I strains are primarily isolated from non-watermelon cucurbits
- Group II strains are isolated from watermelon

Hosts

Benincasa hispida (BNCHI)	Experimental
Citrullus lanatus (CITLA)	Major host
Citrullus lanatus var. citroides (CITLC)	Wild/Weed
Cucumis melo (CUMME)	Major host
Cucumis melo var. inodorus (CUMMI)	Major host
Cucumis melo var. reticulatus (CUMMR)	Experimental
<i>Cucumis sativus (CUMSA)</i>	<i>Host</i>
<i>Cucurbita moschata (CUUMO)</i>	<i>Host</i>
<i>Cucurbita pepo (CUUPE)</i>	<i>Host</i>
Lagenaria siceraria (LGNSI)	Experimental
Piper betle (PIPBE)	Host
Solanum lycopersicum (LYPES)	Host
Solanum melongena (SOLME)	Host

A. citrulli has been isolated from tomato plants in Greece causing a leaf spot disease (Malliarakis et al., 2021). In host range studies, symptoms were produced on tomato, aubergine and pepper foliage, but not on fruit.

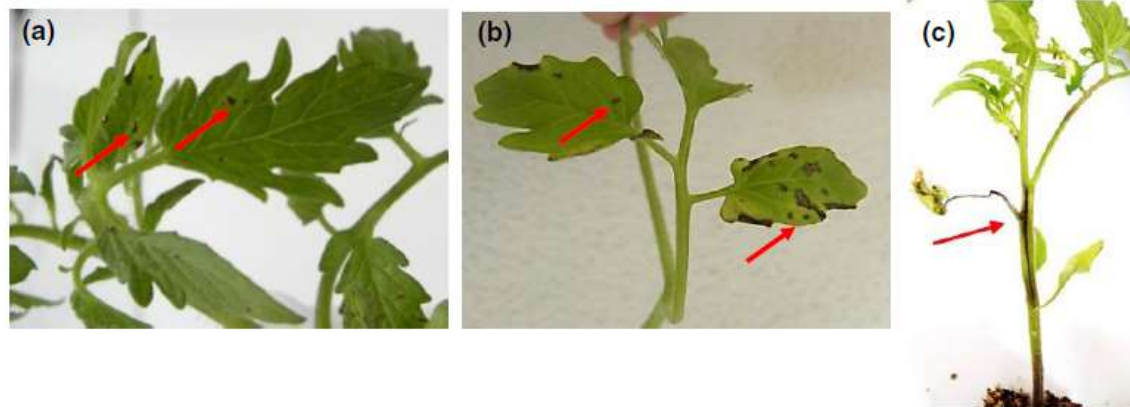


FIGURE 1 Symptoms formed on tomato plants by *Acidovorax citrulli* strains isolated from tomato. (a) A tomato plant with black spots on the leaves from which *A. citrulli* was isolated; (b) symptoms produced on tomato leaves 7 days after spray inoculation; and (c) symptoms produced on the stem 5 days after puncture inoculation [Colour figure can be viewed at wileyonlinelibrary.com]

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ORIGINAL ARTICLE

Plant Pathology | WILEY

Characterization of *Acidovorax citrulli* strains isolated from solanaceous plants

Laura Chalupowicz¹ | Michal Reuven¹ | Orit Dror¹ | Noa Sela¹ | Saul Burdman² | Shulamit Manulis-Sasson¹ 

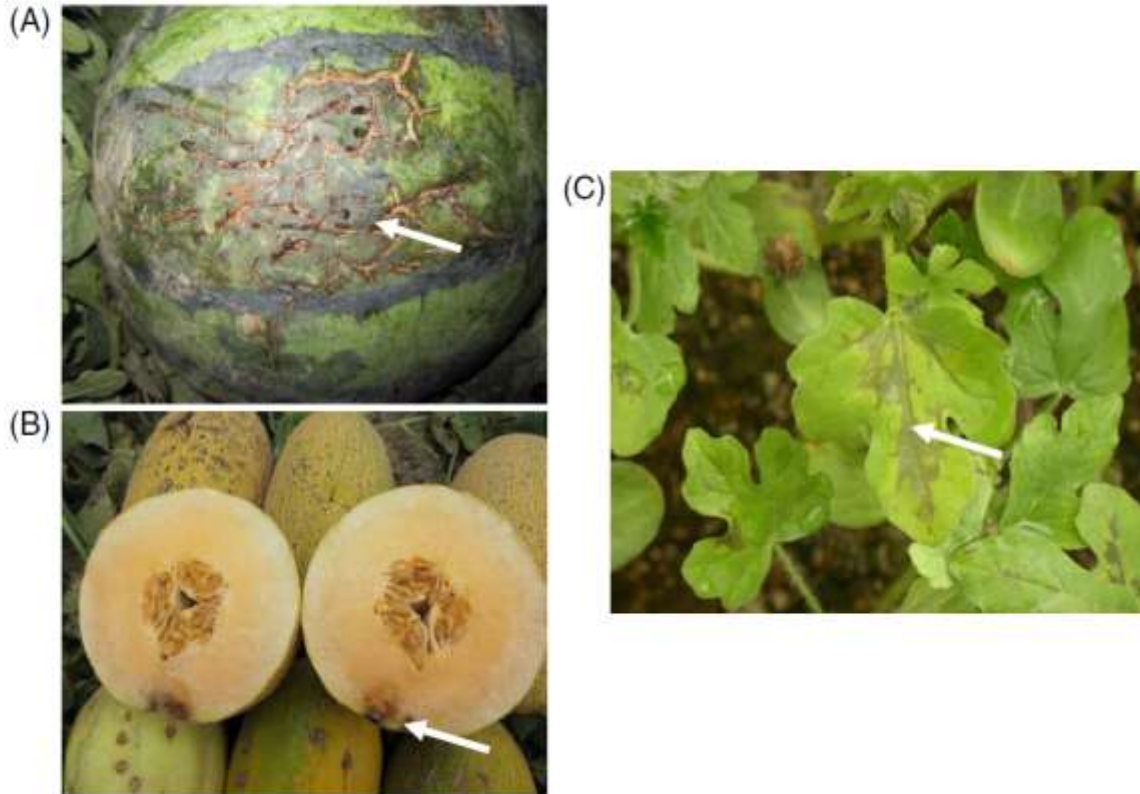


Figure 1. Symptoms of bacterial fruit blotch (BFB) on watermelon (A) and melon (B) under natural field conditions, and on seedlings under greenhouse conditions (C). The arrows indicate the symptoms of BFB.

Symptoms on
watermelon
and melon

Symptoms on melon



Symptoms on zucchini



Symptoms on watermelon



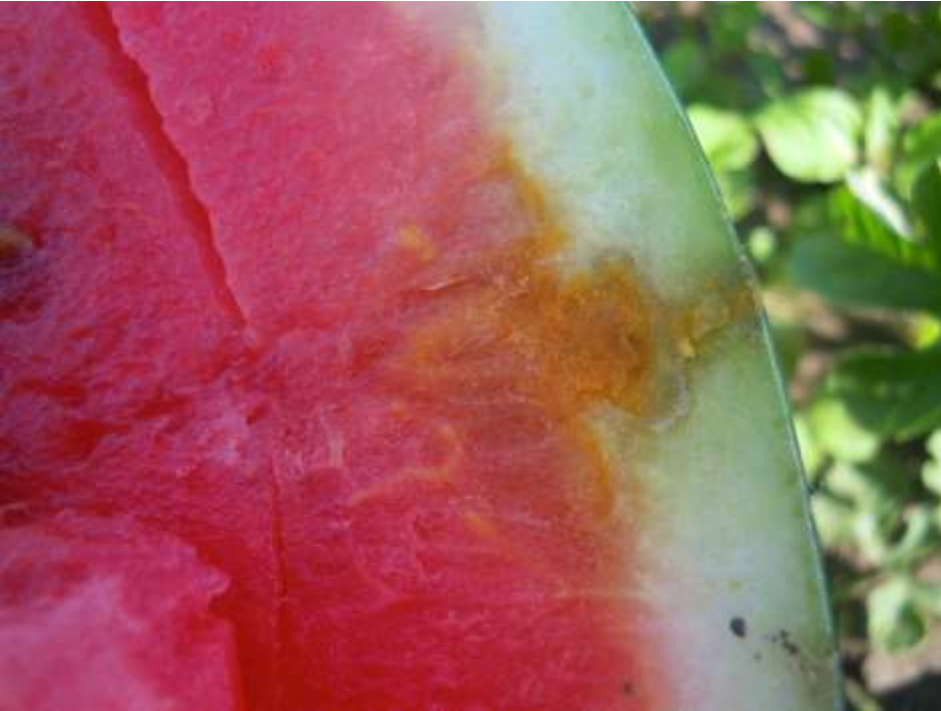
Symptoms on watermelon



Symptoms on watermelon



Symptoms on watermelon



Regulatory aspects – adapted from EPPO Global Database

Continent	country	Note	From
Africa	Morocco	Quarantine pest	2018
America	Chile	A1 list	2019
America	Mexico	Quarantine pest	2018
Asia	China	Quarantine pest	2021
Asia	Iran	A1 list	2018
Asia	Israel	Quarantine pest	2009
Asia	Kazakhstan	A1 list	2017
Europe	Serbia	A1 list	2015
Europe	Türkiye	A2 list	2016
Europe	Ukraine	A1 list	2019
RPPO/EU	EAEU	A1 list	2016
RPPO/EU	EPPO	A1 list	2014

Regulation (EU) 2072 del 2019 – consolidated version July 2025



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Document 02019R2072-20250723

Consolidated text: Commission Implementing Regulation (EU) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2006 and amending Commission Implementing Regulation (EU) 2018/2019

[Access initial legal act](#) In force)

ELI: http://data.europa.eu/eli/reg_impl/2019/2072/2025-07-23

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Table of contents

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II

(Atti non legislativi)

REGOLAMENTI

REGOLAMENTO DI ESECUZIONE (UE) 2019/2072 DELLA COMMISSIONE

del 28 novembre 2019

che stabilisce condizioni uniformi per l'attuazione del regolamento (UE) 2016/2031 del Parlamento europeo e del Consiglio per quanto riguarda le misure di protezione contro gli organismi nocivi per le piante e che abroga il regolamento (CE) n. 690/2008 della Commissione e modifica il regolamento di esecuzione (UE) 2018/2019 della Commissione

No mention of *Acidovorax citrulli*



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Acidovorax citrulli



Nessun risultato per "Acidovorax"!

Forse stavi cercando: [acidovorax citrulli](#) [acidovorax citrulli eppo](#) [acidovorax avenae subsp citrulli eppo](#) [acidovorax citrulli schaad et al](#) [acidovorax citrulli watermelon](#)

HOME

STRUTTURE

DOCUMENTI

ARTICOLI

LINK UTILI

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HOME

STRUTTURE

DOCUMENTI

ARTICOLI

LINK UTILI

No mention of *Acidovorax citrulli*

Why would it matter... to know what steps to take?

PARTE F

ORNQ rilevanti per le sementi di ortaggi

Batteri		
ORNQ o sintomi causati dagli ORNQ	Piante da impianto (genere o specie)	Soglia per le sementi di ortaggi interessate
<i>Clavibacter michiganensis</i> ssp. <i>michiganensis</i> (Smith) Davis <i>et al.</i> [CORBMI]	<i>Solanum lycopersicum</i> L.	0 %
<i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i> (Smith) Vauterin <i>et al.</i> [XANTPH]	<i>Phaseolus vulgaris</i> L.	0 %
<i>Xanthomonas fuscans</i> subsp. <i>fuscans</i> Schaad <i>et al.</i> [XANTFF]	<i>Phaseolus vulgaris</i> L.	0 %
<i>Xanthomonas euvesicatoria</i> Jones <i>et al.</i> [XANTEU]	<i>Capsicum annuum</i> L., <i>Solanum lycopersicum</i> L.	0 %
<i>Xanthomonas gardneri</i> (ex Šutić 1957) Jones <i>et al.</i> [XANTGA]	<i>Capsicum annuum</i> L., <i>Solanum lycopersicum</i> L.	0 %
<i>Xanthomonas perforans</i> Jones <i>et al.</i> [XANTPF]	<i>Capsicum annuum</i> L., <i>Solanum lycopersicum</i> L.	0 %
<i>Xanthomonas vesicatoria</i> (ex Doidge) Vauterin <i>et al.</i> [XANTVE]	<i>Capsicum annuum</i> L., <i>Solanum lycopersicum</i> L.	0 %

PARTE I

ORNQ rilevanti per le piantine di ortaggi e i materiali di moltiplicazione di ortaggi, escluse le sementi

Batteri		
ORNQ o sintomi causati dagli ORNQ	Piante da impianto (genere o specie)	Soglia per le piantine di ortaggi e i materiali di moltiplicazione di ortaggi interessati
<i>Clavibacter michiganensis</i> ssp. <i>michiganensis</i> (Smith) Davis <i>et al.</i> [CORBMI]	<i>Solanum lycopersicum</i> L.	0 %
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First record of *Acidovorax citrulli* in Italy

The NPPO of Italy recently informed the EPPO Secretariat of the first record of *Acidovorax citrulli* (EPPO Alert List) on its territory. In 2009, the bacterium was detected in 2 samples of melon plants (*Cucumis melo*) showing suspect symptoms. These plants had been collected from a farm located in Ferrara province, Emilia-Romagna region. The infected melon crop had been established with plantlets from a nursery in Veneto region themselves issued from seeds supplied by an international seed company. It is noted that Emilia-Romagna is a major production area for melons and watermelons (*Citrullus lanatus*), with approximately 1570 ha for watermelon crops and 1487 ha for melon crops. The other main producing areas are located in Sicilia, Apulia and Lazio for watermelons, and in Sicilia, Lombardia, Apulia, Lazio and Sardegna for melons. Because *A. citrulli* is considered as a serious threat to the Italian production of melons and watermelons, the NPPO of Italy will sample and test imported seed lots for the presence of *A. citrulli*.

The pest status of *Acidovorax citrulli* in Italy is officially declared as follows: **Transient, 1 outbreak under eradication, intensive monitoring to be carried out in the next growing season.**

Sources

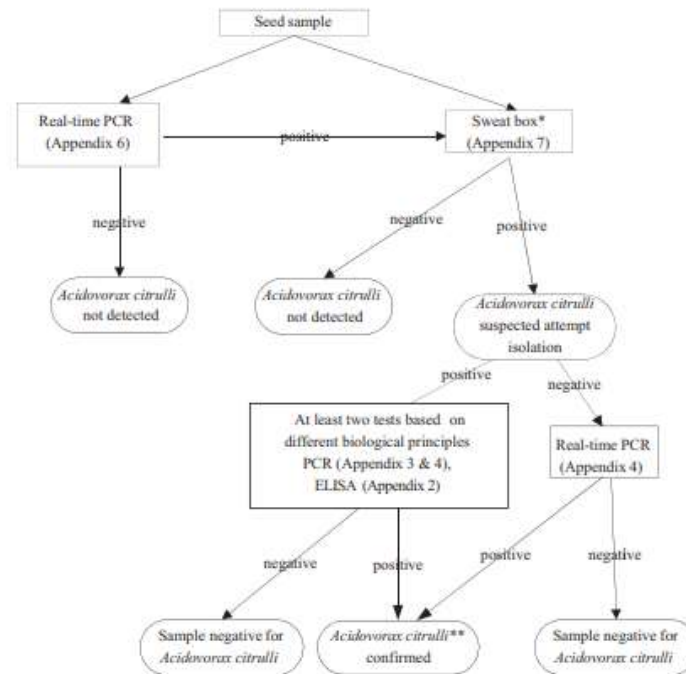
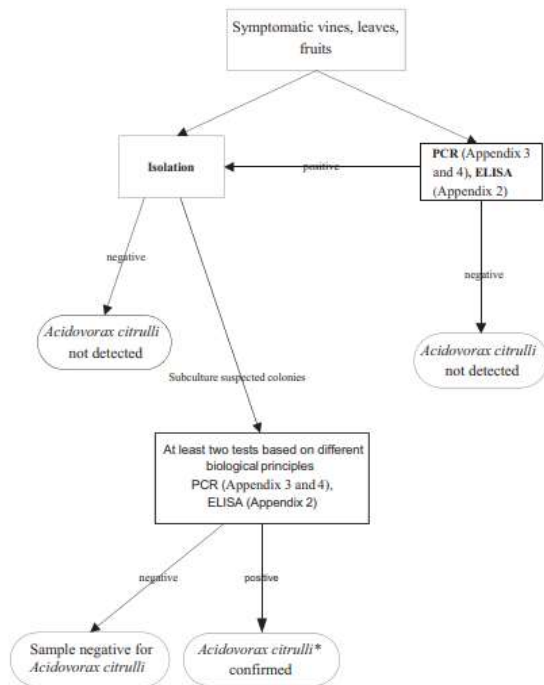
NPPO of Italy, 2010-03.

Associated EPPO Standards

Number	Title	Download
PM3/077(2)	Vegetable plants for planting under protected conditions – inspection of places of production	Download
PM7/127(1)	<i>Acidovorax citrulli</i>	Download

Official diagnostic methods

Minimum seed lot: 10000 seeds



Acidovorax citrulli:

- **24-38°C**, humid environment (**RH > 85%**)
- from infested seed, **infect the seedling** as the cotyledons **emerge from the seed coat**.
- **overhead irrigation** effectively **splash-disperses** bacteria.
- transplants spread the inoculum in field.
- relies on **quorum-sensing** for its pathogenicity: contributes to virulence, twitching motility, seed attachment and biofilm formation
- two-spotted **spider mite** (*Tetranychus urticae*) **might transmits** *A. citrulli*
- **survival** in various types of **soil** only for **3 days**.

Acidovorax citrulli:

High-Risk Production Scenarios

- Nursery: production of grafted plants
- Nursery: continuous coexistence of staggered sowing lots
- Field: cultivation in mini-tunnels (non-walk-in tunnels)
- Field: contemporaneous transplant events coexisting on the same farm

Acidovorax citrulli:

Disease Control Options in the Seed Production Phase

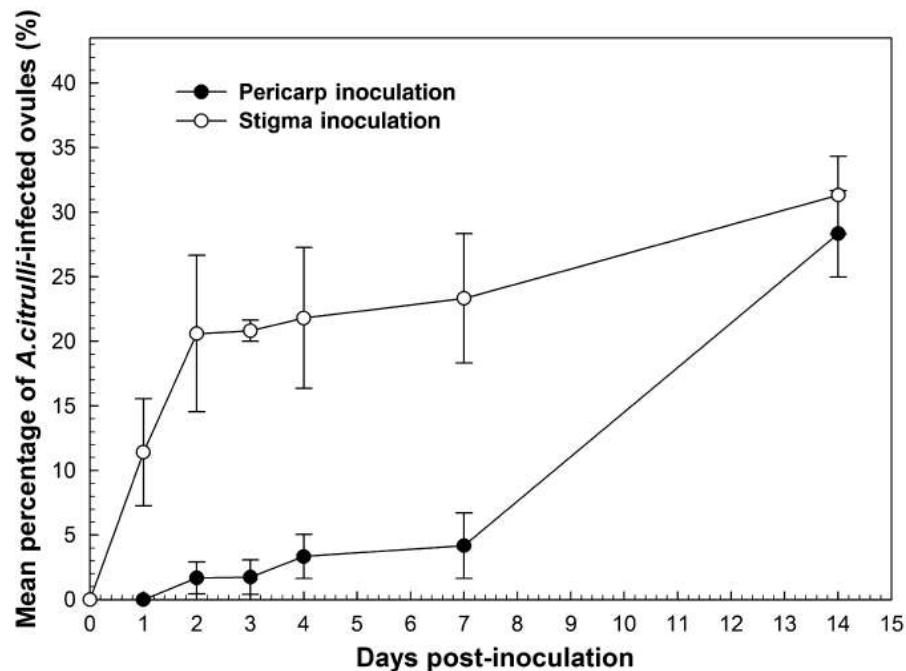
Direct

- Protection during the flowering phase of seed-bearing plants (Fessehaie, A., and Walcott, R. R. 2005. Biological control to protect watermelon blossoms and seed from infection by *Acidovorax citrulli*. Phytopathology 95:413-419)
- Floral infection causes the production of asymptomatic fruits, but with infected seeds.

Indirect

- Preventive actions (including early diagnosis)

Figure 3 Comparison of watermelon seed infection by *Acidovorax citrulli* AAC00-1 after stigma and pericarp inoculation of female watermelon flowers. After inoculation, 20 seeds were sampled from fruits and assayed for *A. citrulli* using semiselective media. Each data point represents the mean percentage of *A. citrulli*-infected seeds (three fruits per sampling time per treatment in two independent experiments) and bars indicate the standard errors of the means.



Pathways of bacterial invasion and watermelon seed infection by *Acidovorax citrulli*

B. Dutta^a, Y. Ha^b, J. T. Lessl^c, U. Avci^d, A. C. Sparks^c, K. L. Johnson^e and R. R. Walcott^{c*}

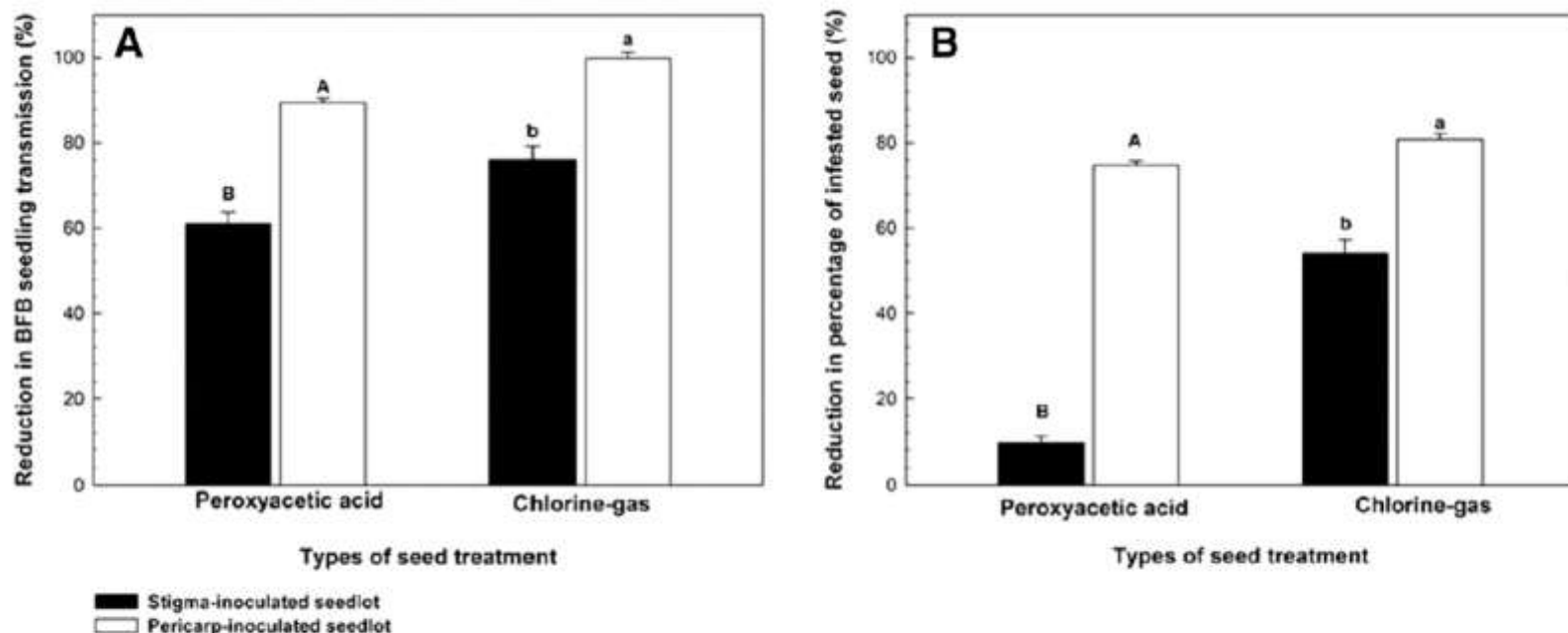


Fig. 3. Reduction in A, bacterial fruit blotch seed-to-seedling transmission and B, percentage of *Acidovorax citrulli*-infested seeds after surface and subsurface antimicrobial seed treatments of watermelon seeds pericarp and stigma inoculated with *A. citrulli*. One gram of seed from two seedlots generated by each inoculation method was subjected to treatment with peroxyacetic acid (1 h followed by overnight drying) and chlorine gas for 9 h. Error bars represent the standard errors of the means with three replicates per treatment in two independent experiments. Means with the same upper or lowercase letters are not significantly different according to Student's *t* test ($P = 0.05$) ($n = 12$).

Dutta, B., Schneider, R. W., Robertson, C. L., and Walcott, R. R. 2016. Embryo localization enhances the survival of *Acidovorax citrulli* in watermelon seeds. *Phytopathology* 106:330-338.

***Acidovorax citrulli*:**

Disease control options during the nursery propagation phase.

Direct

- bacteriophage-based biocontrol of *A. citrulli* was reported in the literature
- Copper based formulations

Indirect

- disease resistance inducers
- without automated overhead irrigation, bacterial fruit blotch develops faster from seedlots with one seed infested with $\geq 1 \times 10^5$ c.f.u than from seedlots with one seed infested with $\leq 1 \times 10^3$ c.f.u.: **keep down population density**
- *Bacillus amyloliquefaciens* strains
- *Bacillus subtilis* strains

Acidovorax citrulli:

Disease control option in field scenarios

Direct

- Copper based fungicides
- Disease resistance inducers

Indirect

- Bacillus based formulations spray
- Hygiene and preventive measures
- Separation of crops
- Avoiding overhead irrigation
- Destruction of infected fruit, avoiding incorporation of discarded seeds into the soil

Final considerations

Effect of copper and dithiocarbamates: NOT AVAILABLE ANYMORE

Dithiocarbamates and copper kill bacteria: dithiocarbamates (DTCs) act as chelators, bringing copper inside the cell. Once inside, copper ions overwhelm the bacteria, causing oxidative stress and disrupting vital enzymes. Dithiocarbamates also directly inhibit enzymes by binding to thiol groups, and can damage the bacterial cell membrane.

Disease resistance inducers

Acibenzolar-s-methyl: induces systemic acquired resistance (SAR) against a wide range of pathogens and pests. It is a synthetic analog of salicylic acid, which triggers a plant's natural defense mechanisms by producing pathogenesis-related (PR) proteins and strengthening cell walls. NOT AVAILABLE ANYMORE

Plant extracts - botanicals

Polyphenolic extracts, terpens, terpenoids

Neem (*Azadirachta indica*) extracts

Pepper (*Piper nigrum*) and geranium (*Pelargonium graveolens*) leaf oils, active against Gram-positive and negative bacteria

Biological Control Agents (BCAs)

Approximately 250 microbial strains, 94 of which are used for disease control, including bacterial ones.

Emerging diseases

Remember that it is mandatory to record/log treatments on the...

REGISTRO DEI TRATTAMENTI (QUADERNO DI CAMPAGNA)

Reporting for each single application

DATA	COLTURA	ESTENSIONE IN ETTARI	FASE DEL CICLO BIOLOGICO (*)				AVVERSITÀ DA COMBATTERE	NOME DEL PRODOTTO	QUANTITÀ (Kg o litri)	Firma dell'utilizzatore se diverso dal titolare dell'Azienda	NOTE
			SEMINA	TRAPIANTO	NUOVO PIANTEGGIO	TRACCOLTA					

in accordance with the current labels of the formulations used

Meeting point: “Prevention avenue”

DISINFESTATION OF GROWING ENVIRONMENTS

SANITATION in absence of the
crop
EPPO PP1/261(1) - Disinfection in
plant production (2008)



Crop protection sector (Reg.
1107/2009) which deserves
greater attention from a regulatory
point of view



Perspectives

- Reconsideration of the relevance of *Acidovorax citrulli* in the seed production phase, regardless of legal regulations (nursery sanitary control).
- Need to reconsider "SANITATION" as a defense technique.
- Introduction of new mechanisms of action.
- RATIONAL adoption of biostimulation systems and techniques

Prodotti biostimolanti ed effetti sulle colture ortofloricole

Roberta Bulgari¹, Giacomo Cocetta¹, Alice Trivellini², Livia Martinetti¹ e Antonio Ferrante¹

¹Dipartimento di Scienze Agrarie e Ambientali, Produzione, Territorio, Agroenergia, Università di Milano

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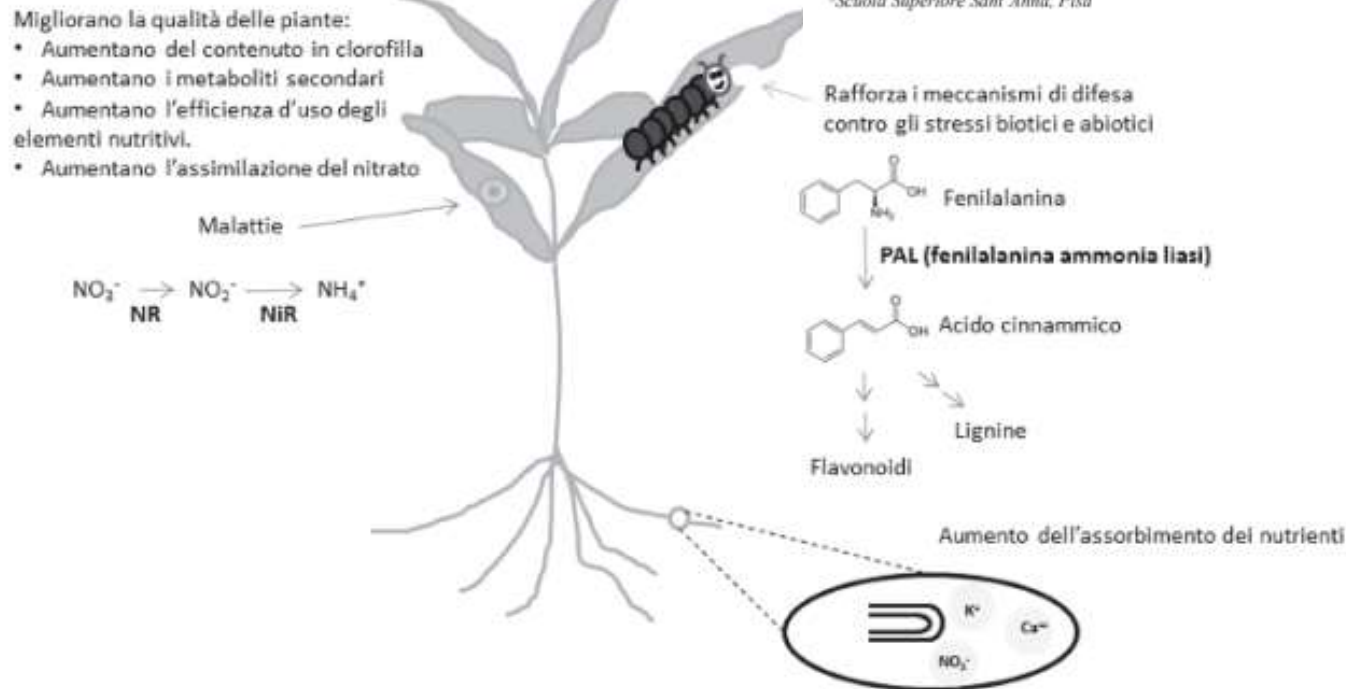
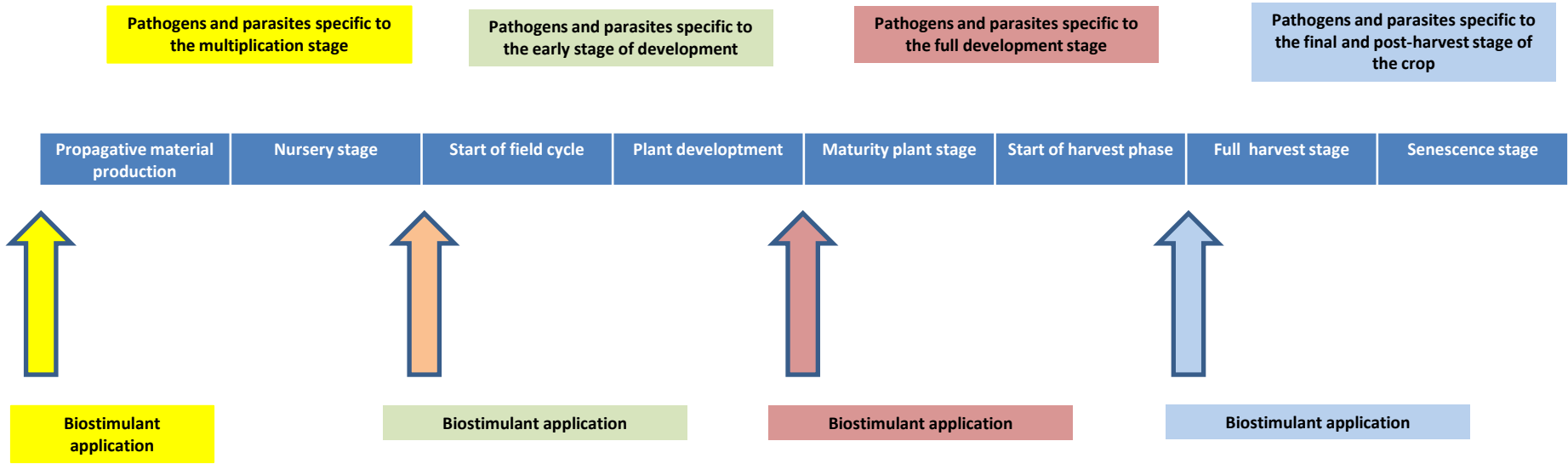


Fig. 1 - Rappresentazione schematica dell'azione dei biostimolanti sulla pianta.

Fig. 1 - Schematic representation of the biostimulants effect on plants.



QUESTION: Do we always have complete knowledge of the interactions between host/environment/parasitic complexes/cultivation technique/FARMER?

The challenges ahead

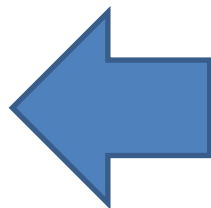
Reconsidering the nursery production scenario within the framework of plant protection product registration regulations

ANNEX III

General principles of integrated pest management

1. The prevention and/or suppression of harmful organisms should be achieved or supported among other options especially by:
 - crop rotation,
 - use of adequate cultivation techniques (e.g. stale seedbed technique, sowing dates and densities, under-sowing, conservation tillage, pruning and direct sowing)
 - use, where appropriate, of resistant/tolerant cultivars and standard/certified seed and planting material,
 - use of balanced fertilisation, liming and irrigation/drainage practices,
 - preventing the spreading of harmful organisms by hygiene measures (e.g. by regular cleansing of machinery and equipment),
 - protection and enhancement of important beneficial organisms, e.g. by adequate plant protection measures or the utilisation of ecological infrastructures inside and outside production sites.

**Healthy propagating material
= pillar of integrated
agriculture (Directive
2009/128/EC)**





IS THE OPERATIONAL SCENARIO THE SAME?



Critical aspects

1. Highly specialized farms
2. Highly technologically advanced protected cultivation systems
3. Non-exhaustive application labels for applications in the horticultural plant propagation sector
4. Maximum number of applications per year (or per crop cycle)
5. Introduction of the concept of application rate per plant for biological pest control
6. Crops "not in contact with the soil" Very high crop density - example tomato

Field 20.000 - 40.000 plants/ha

Nursery standard plants 4.000.000 – 7.500.000 plants/ha

Nursery grafted plants 6.000.000 – 10.000.000 plants/ha

Lack of specific guidelines (evaluation of effectiveness, residues,) dedicated to the scenario "production and multiplication of propagative material" and specifically to pest and disease control

Adoption of the same application GAPs (technical means, application dosages, application intervals, application limitations) of the field production scenario for the "production and multiplication of propagating material"



Objective difficulty – impossibility of producing healthy propagating material (Directive 2009/128/EC)



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Thank You for Your attention

