BACTERIOPHAGE-BASED BIOCONTROL OF ERWINIA AMYLOVORA

Inge Hanssen - R&D Manager

ABIM, October 24, 2023

DCM CORPORATION

- Headquartered in Grobbendonk, **Belgium**. Affiliates in Belgium, the Netherlands, France, Germany, Austria and the USA. Various partnerships with distributors.
- Member of **Group DC**
- DCM **develops**, **registers**, **produces** and **markets**
  - organic substrates,
  - organic fertilisers,
  - biostimulants,
  - and biocontrol products,

  for both consumer and professional use.

DCM CORPORATION

BioSubstrates

Fortified with Microbials

BioFertilisers

MINIGRAN®

Gel Fertilisers

VISCOTEC® - LEAFGEL®

Supported by

BIORATIONALS expert team

BioStimulants

- VITACT®
- IMPULS TD®
- INSTANT TD®
- INTERACT®

Virus-Based Biocontrol

- PMV®-01
- PHACT®

Scientia Terrae RESEARCH INSTITUTE

BACTERIOPHAGES

- **What?**
  - Viruses that infect and replicate within bacteria
  - Recognition via receptor on cell surface

- **Why are they promising for plant protection?**
  1. Narrow host range (strain-level)
  2. No effects on non-target species
  3. Cannot infect human or animal cells, no adverse effects expected
  4. Ubiquitous (in water, soil, plants, animals) but bacteria-specific
  5. Non-persistent (sensitive for environmental factors)
  6. No pathogenic, genotoxic, mutagenic or carcinogenic effects observed in mammals
  7. Do not produce metabolites & not considered toxic

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2022)40

Unclassified

English - Or. English

28 November 2022

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Guidance Document for the Regulatory Framework for the Microorganism Group: Bacteriophages

Series on Pesticides
No. 108

- OECD (2022): Guidance Document for the Regulatory Framework for the Microorganism Group: Bacteriophages. Series on Pesticides No. 108. ENV/CBC/MONO(2022)40.
- https://www.nature.com/news/do-you-speak-virus-phages-caught-sending-chemical-messages-1.21313

PHACT® - NEXT GENERATION BIOCONTROL

- **Exclusive partnership:**
  - between DCM, Scientia Terrae & OmniLytics
  - for the development of bacteriophage-based biocontrol
  - in & around Europe

- **DCM's PHACT® platform refers to:**
  - **PHA**ge-based biocontrol with **ACT**ion against bacteria
  - phage-based biocontrol becoming a **fact**

FIRST TARGET: FIRE BLIGHT IN APPLE AND PEAR (*ERWINIA AMYLOVORA*)

stem canker

blossom blight

shoot blight

IMPACT OF FIRE BLIGHT

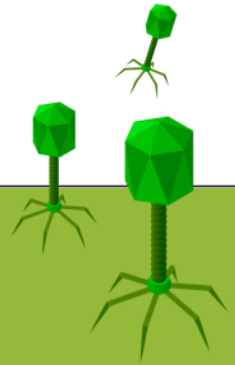
- Found in **> 50 countries**, +/- all EU countries
- Over 200 hosts, main economic issue for **apple and pear trees**
- Important economic impact:
  - **Export** restrictions
  - Cost of **control** (removal of infection, treatments, monitoring, …)
  - **Yield** loss
  - Crop loss: infected trees may need to be **uprooted**
    - E.g. in China: severe outbreak caused yield loss of 30 and 50% and the destruction of more than one million pear trees (EPPO, 2023)

THE OVERALL PATH TO PRODUCT DEVELOPMENT

Library of lytic phages

Library of diverse European *Erwinia amylovora* isolates (>200 isolates)

In vitro screening: bacteriophage candidates vs. bacterial collection



Characterization of selected bacteriophages: molecular (WGS, qPCR), morphological (TEM), genome study

Compilation of bacteriophage mixture to cover the genetic diversity

Optimization of production

Bio-assays & field trials

Scientia Terrae RESEARCH INSTITUTE

OMNILYTICS THE PHAGE COMPANY

DCM

COMPILATION OF AN OPTIMAL BACTERIOPHAGE MIXTURE – A THEORETICAL EXAMPLE

Clear spot = bacterial lysis due to the presence of bacteriophages

HOST RANGE	Phage 1	Phage 2	Phage 3
Bacterium 1	S	S	S
Bacterium 2	R	S	S
Bacterium 3	S	S	R
Bacterium 4	R	R	S
Bacterium 5	S	S	S

Active ingredient = **a phage mixture** that covers the complete range of pathogenic isolates of a specific bacterial disease

COMPILATION OF AN OPTIMAL BACTERIOPHAGE MIXTURE – A THEORETICAL EXAMPLE

Clear spot = bacterial lysis due to the presence of bacteriophages

HOST RANGE	Phage 1	Phage 2	Phage 3	Phage 4
Bacterium 1	S	S	S	S
Bacterium 2	R	S	S	R
Bacterium 3	S	S	R	S
Bacterium 4	R	R	S	S
Bacterium 5	S	S	S	S

Preferentially all bacteria are susceptible to more than 1 phage

PEA-02®

- PEA-02® is a **mixture** of bacteriophages specifically **tailored to cover the EU-wide genetic diversity** of the fireblight pathogen.

- The bacteriophages in PEA-02® infect and replicate within *E. amylovora.*
  - **Curative**: application when the host is present, e.g. based on prediction models such as Maryblyt™
  - **Bactericide**: the bacteriophages kill the bacteria through cell lysis
  - **Self-replicating** and **self-limiting:** can only propagate when *E. amylovora* is present

- **EU registration dossier for PEA-02® is under evaluation**

PEA-02®

- PEA-02® is (at least partially) **systemic** as the bacteriophages are taken up passively by the plant.

  → Movement tracked via bacteriophage-specific Taqman qPCR assay in apple trees

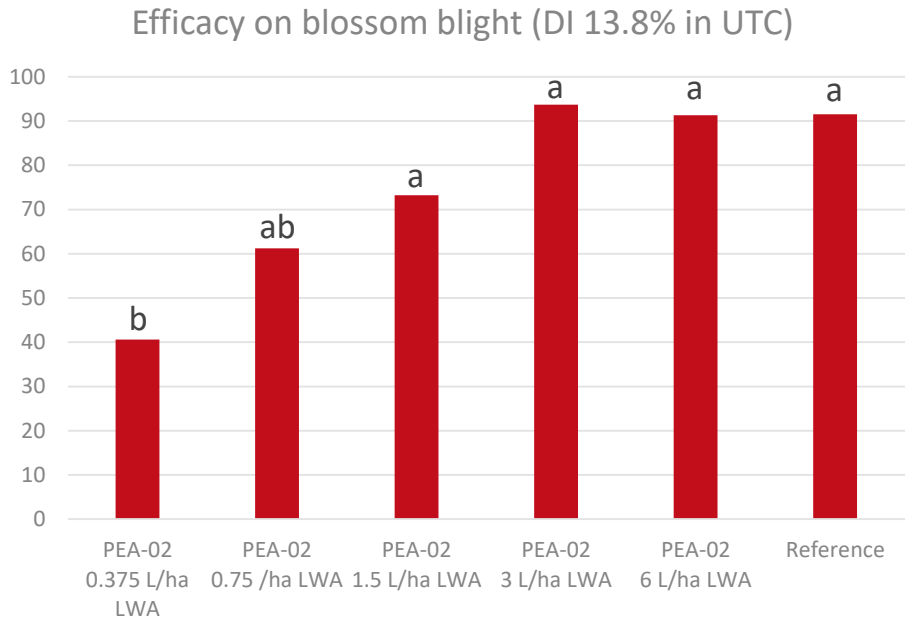
Bacteriophage A	2h	4h	6h
Sprayed leaves	Medium	Medium	Low
Systemic leaves (not-sprayed - covered)	Not detected	Low to medium	Low
Untreated control tree	Not detected	Not detected	Not detected

Its curative, (partially) systemic working mechanism makes PEA-02® a unique biocontrol product

DOSE RANGE FINDING TRIAL – PIEMONT, ITALY, 2022

- Experimental conditions:
  - EPPO Guideline PP 1/166(3) - Efficacy evaluation of bactericides against *Erwinia amylovora*
  - Pear, variety Conference
  - Natural infection, outdoor
- Treatments: 4 x during bloom
  - Untreated control (UTC)
  - PEA-02® at 0.375, 0.75, 1.5, 3 and 6 L/ha LWA
  - Reference
- Assessments:
  - Blossom blight

DOSE RANGE FINDING TRIAL – PIEMONT, ITALY, 2022

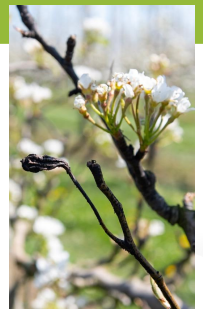


All treatments significantly differ from the untreated control

In total > 40 GEP efficacy trials were performed

TAKE HOME MESSAGES

PEA-02®



- Efficient biocontrol of fire blight in apple and pear orchards
- Tailored to cover the EU-wide diversity of the pathogen *Erwinia amylovora*
- EU registration dossier under evaluation

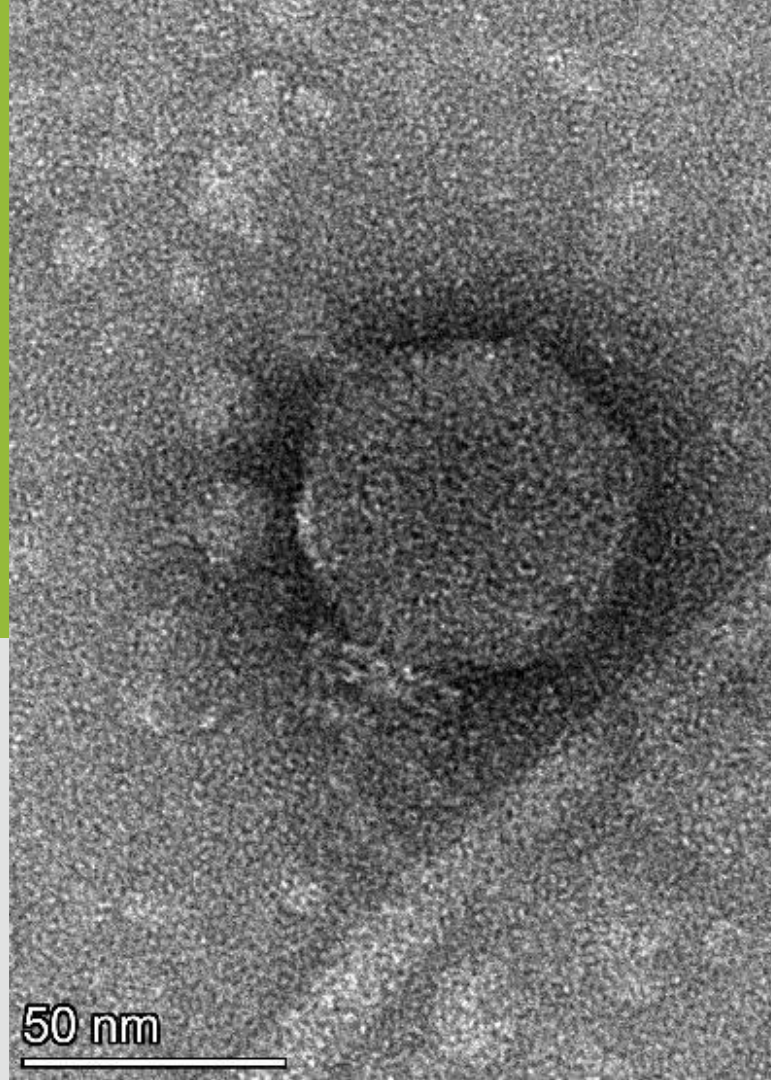
DCM's PHACT® platform: Bacteriophages as Next Generation Biocontrol solutions for bacterial plant diseases

- Control of bacterial diseases without chemicals
- Curative mode of action
- Highly specific without effects on non-target organisms

phact® next generation biocontrol

DCM

THANKS FOR YOUR ATTENTION



Registration process ongoing. Product currently not for sale.