



Biology of the Fire Blight Pathogen *Erwinia amylovora* Under Starvation Conditions: Survival Strategies and Virulence

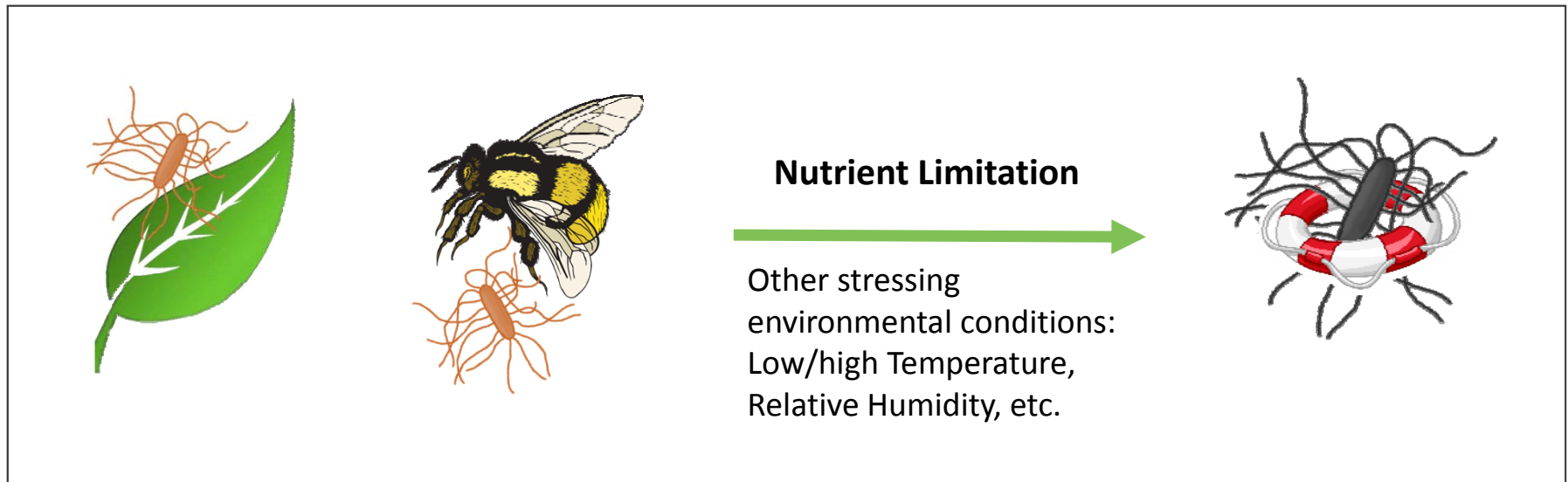
Ricardo Delgado Santander

Unknown Aspects of the *E. amylovora* Life Cycle

- How does *E. amylovora* deal with **nutrient scarcity**?
- Are **roots suitable sites of entry** of *E. amylovora* into the host?

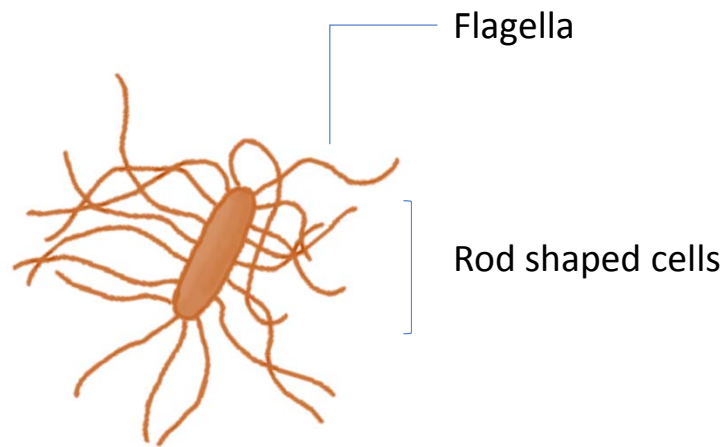
Unknown Aspects of the *E. amylovora* Life Cycle

- How does *E. amylovora* deal with **nutrient scarcity** in cankers, plant surfaces or, in general, outside the host?

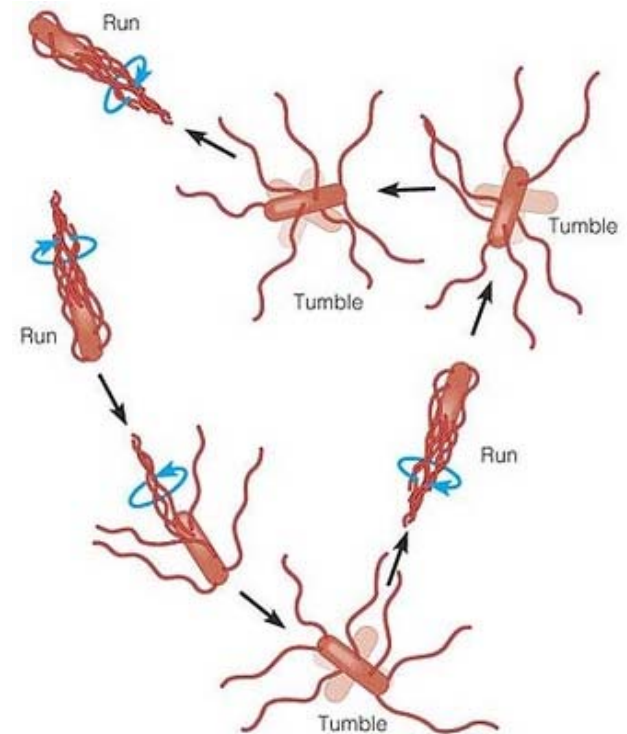


Unknown Aspects of the *E. amylovora* Life Cycle

- How does *E. amylovora* deal with nutrient scarcity in cankers, plant surfaces or, in general, outside the host?
- What happens under **Nutrient Rich** conditions?



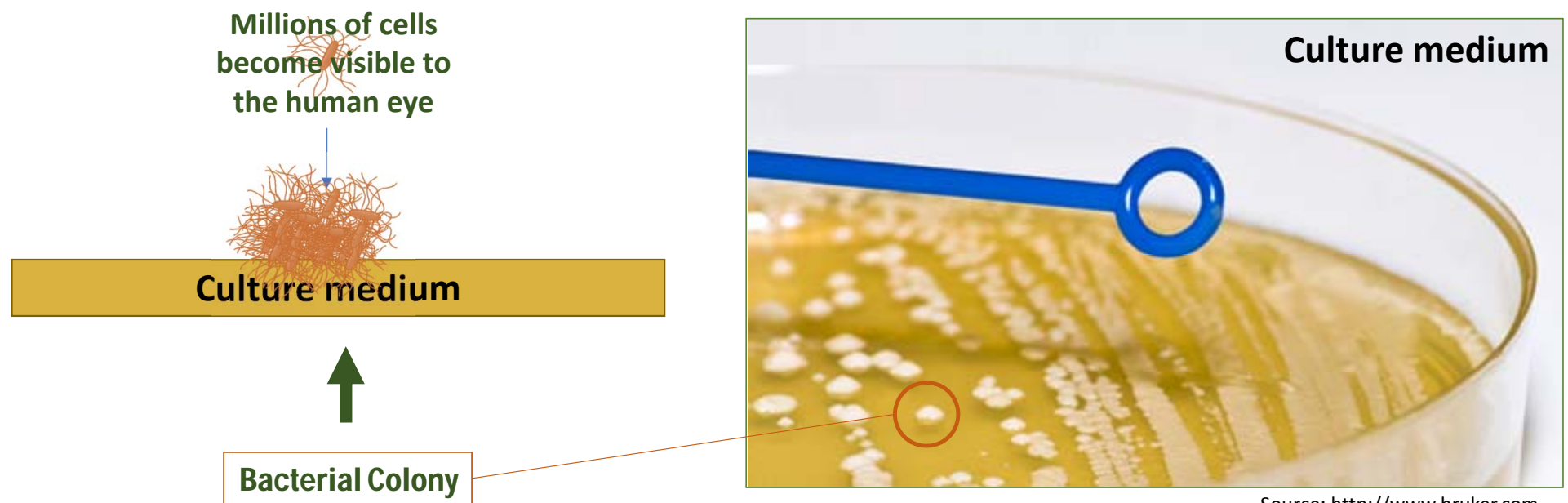
Non stressed cells



Bacterial motility. Source: classes.midlandstech.edu

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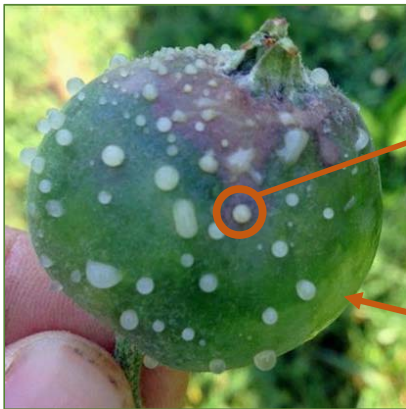
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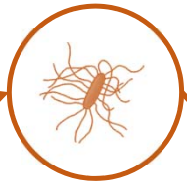
Source: <http://www.bruker.com>

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Fruit blight picture by Scott Nelson



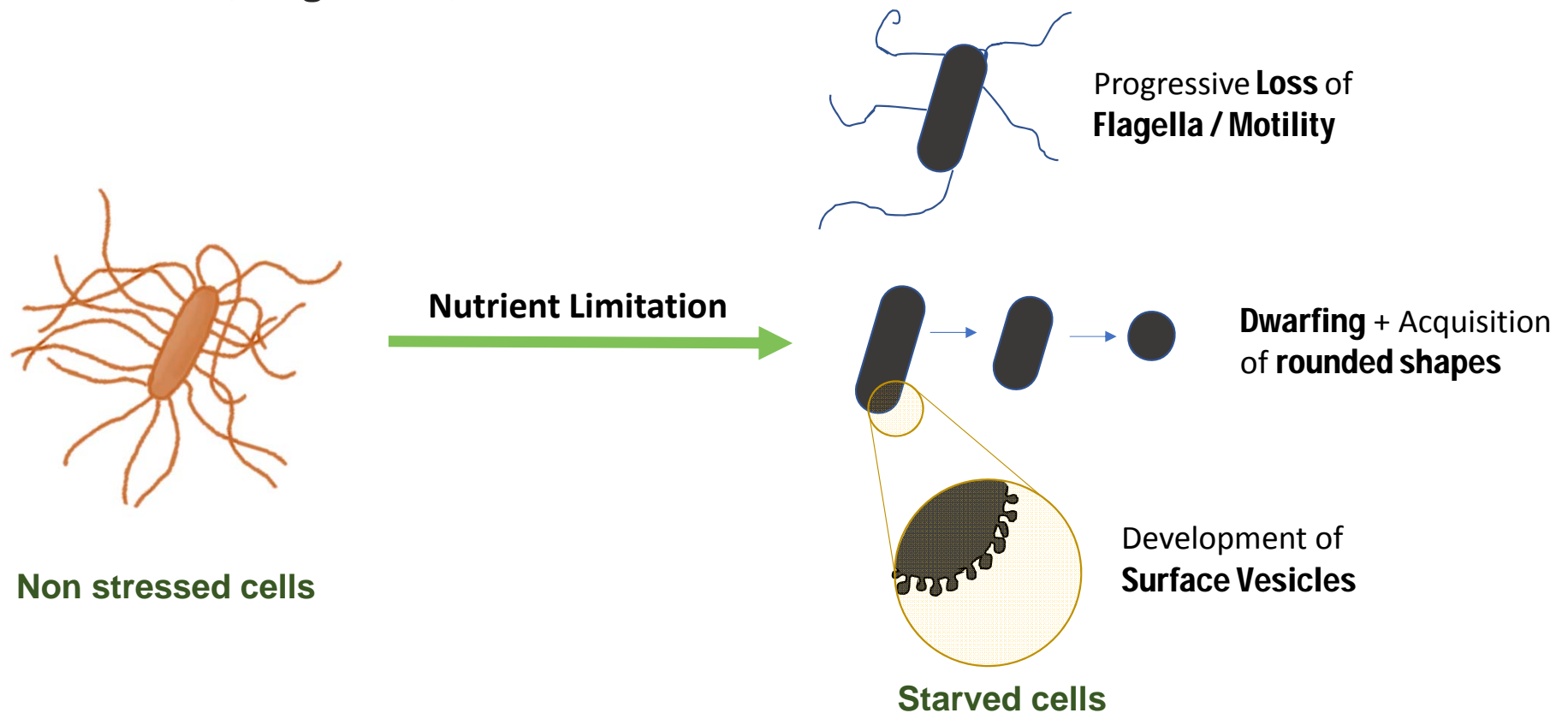
Culture medium

Basis for Plant Disease Diagnosis

Source: <http://www.bruker.com>

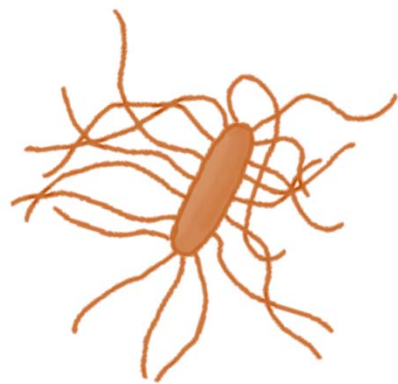
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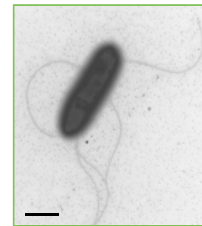
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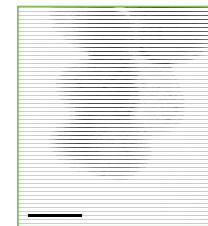


Non stressed cells

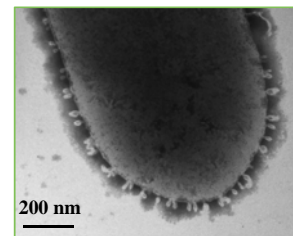
Nutrient Limitation



Progressive Loss of Flagella / Motility



Dwarfing + Acquisition of rounded shapes



Development of Surface Vesicles

TEM images of *E. amylovora* starved cells, by Ricardo D. SaNtander

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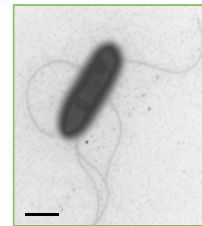
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But what happens with
Bacterial Culturability?

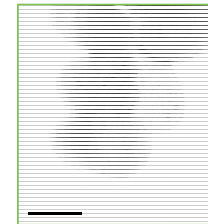


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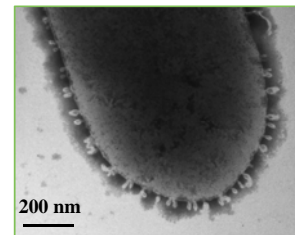
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Progressive **Loss** of
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Development of
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Unknown Aspects of the *E. amylovora* Life Cycle

- Effects of **Starvation** on *E. amylovora* **culturability**

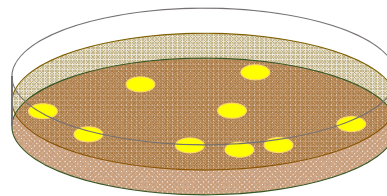
Nutrient rich conditions



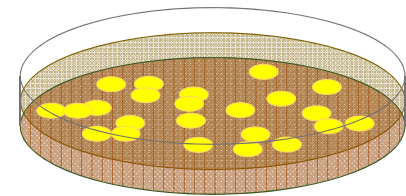
E.g. Within the host
under favorable
growth temperature
conditions

Fruit blight picture by Scott Nelson

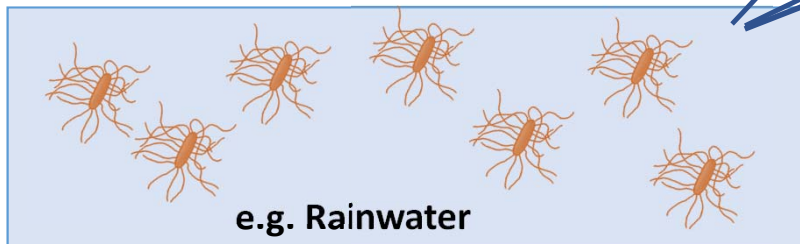
Early infection



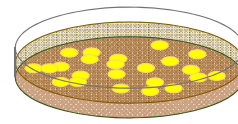
Late Infection



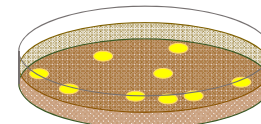
Starvation conditions



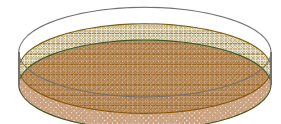
After “days” of
starvation



After “months”
of starvation

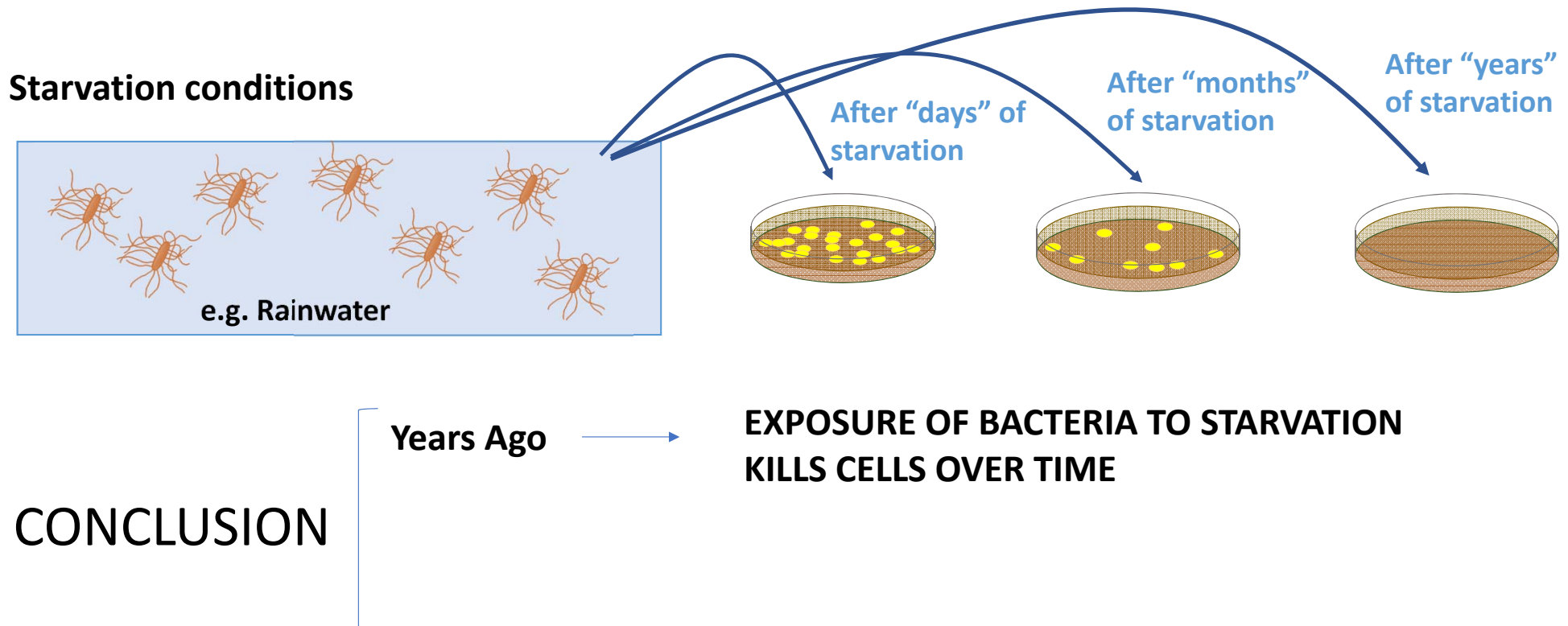


After “years”
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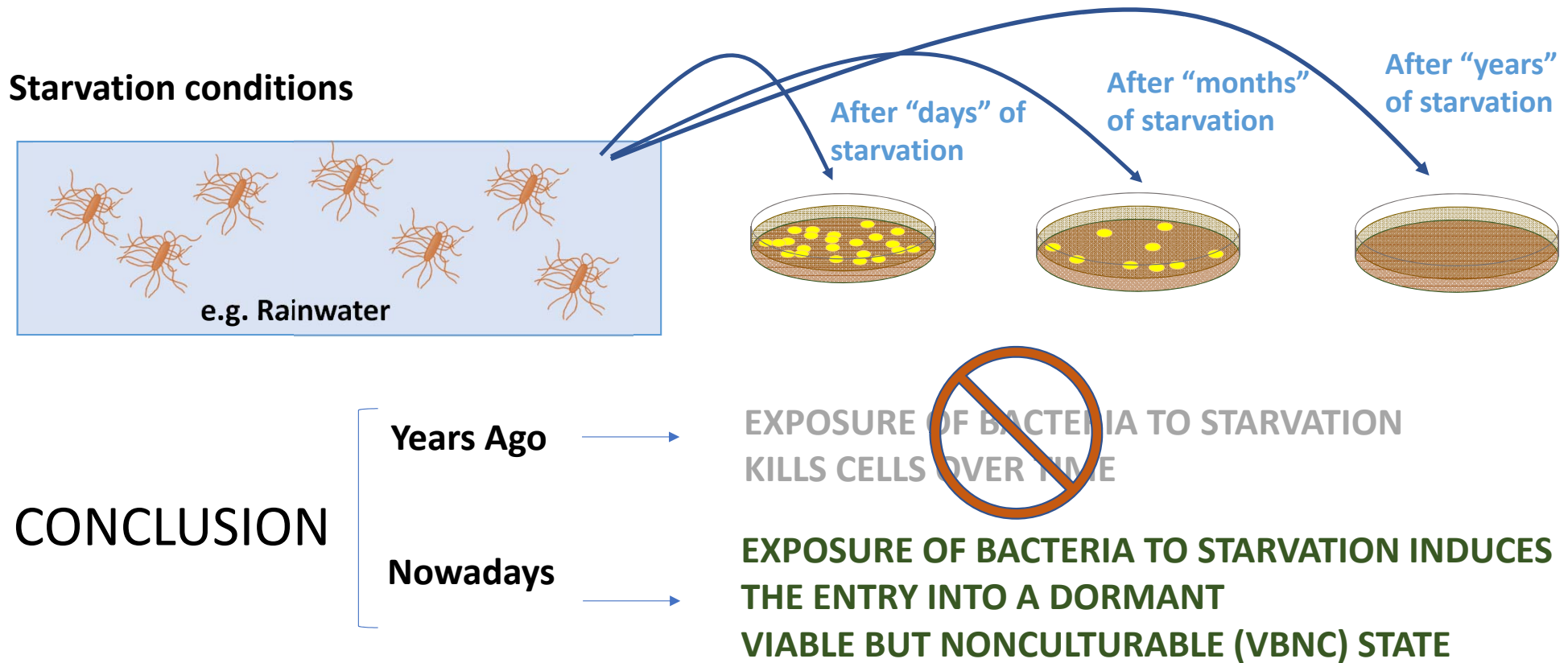
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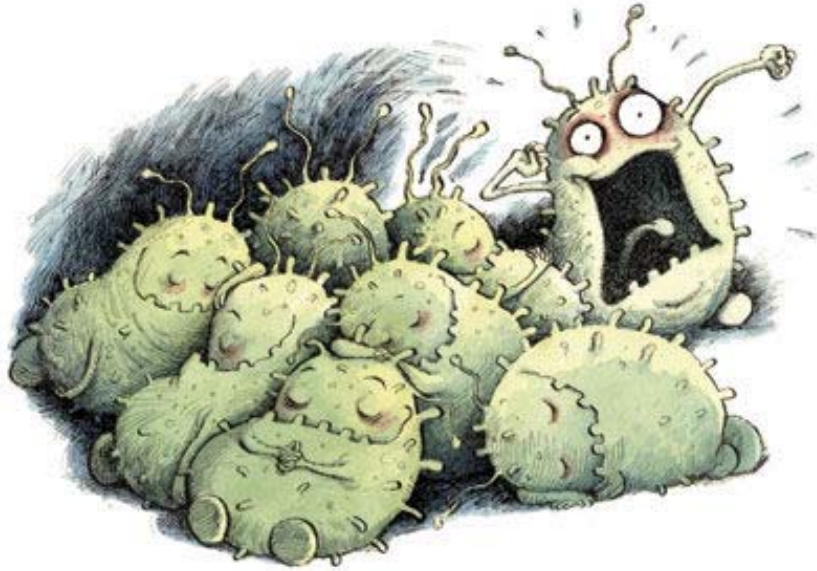
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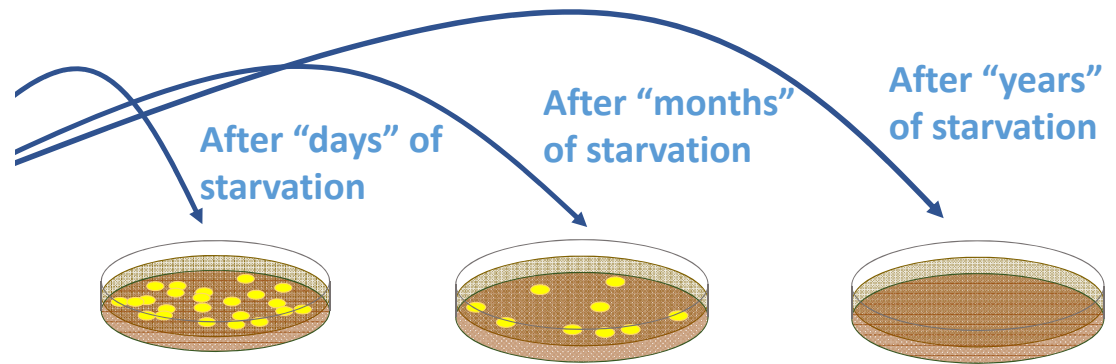


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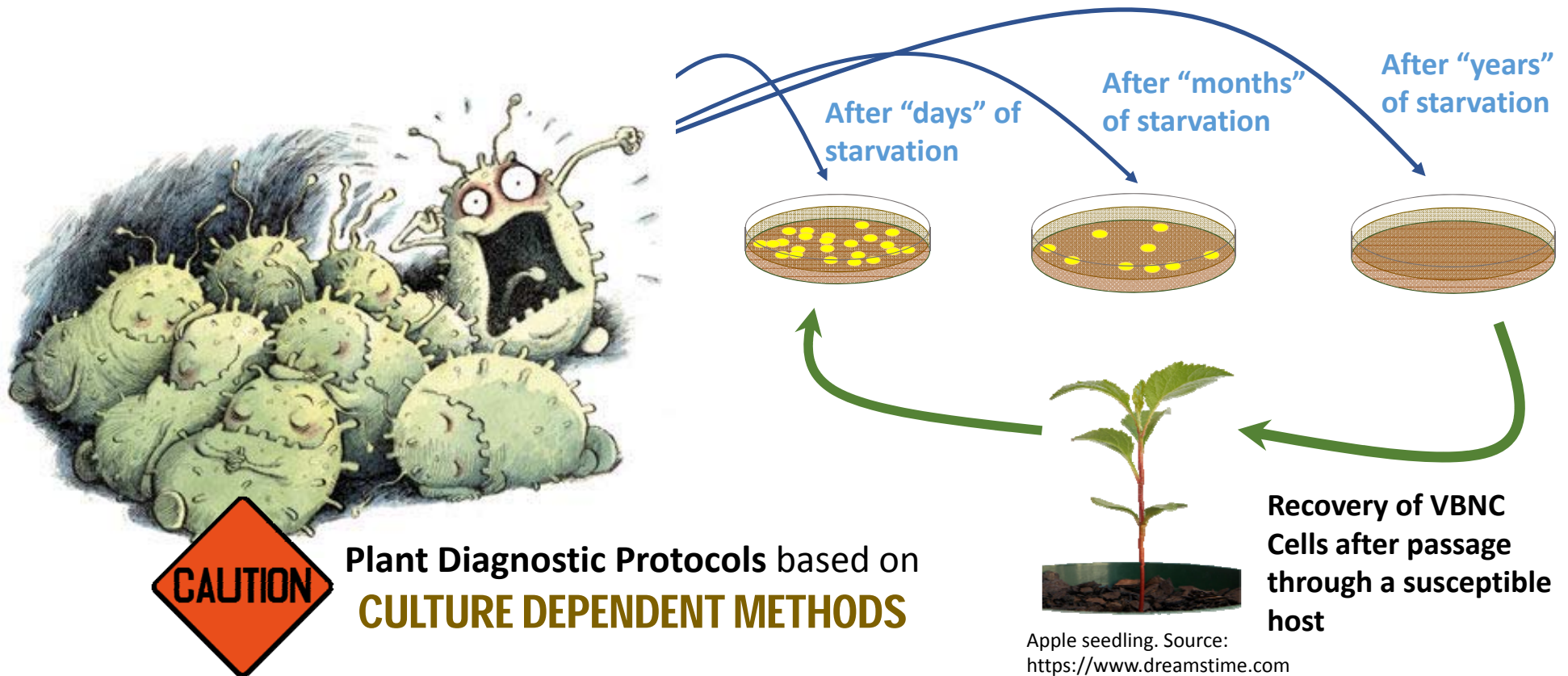


Dormant bacteria by David Parkins



Unknown Aspects of the *E. amylovora* Life Cycle

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Unknown Aspects of the *E. amylovora* Life Cycle

- Effects of **Starvation** on bacterial **culturability**
- **BACTERIAL DORMANCY** → The **Viable But Nonculturable (VBNC) State**



Dormant bacteria by David Parkins

Characteristics of VBNC cells

- Metabolically active
- Increased tolerance to stress (including antibiotics, high temperatures, copper, etc)
- Gene expression and potential virulence.
- Ability to recover culturability after removing the stressing factor, passage through the host, etc

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Dormant bacteria by David Parkins

How can we determine
BACTERIAL VIABILITY in VBNC cells?

VIABILITY STAININGS

MOLECULAR METHODS

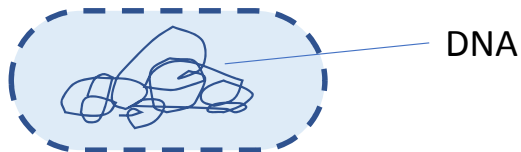
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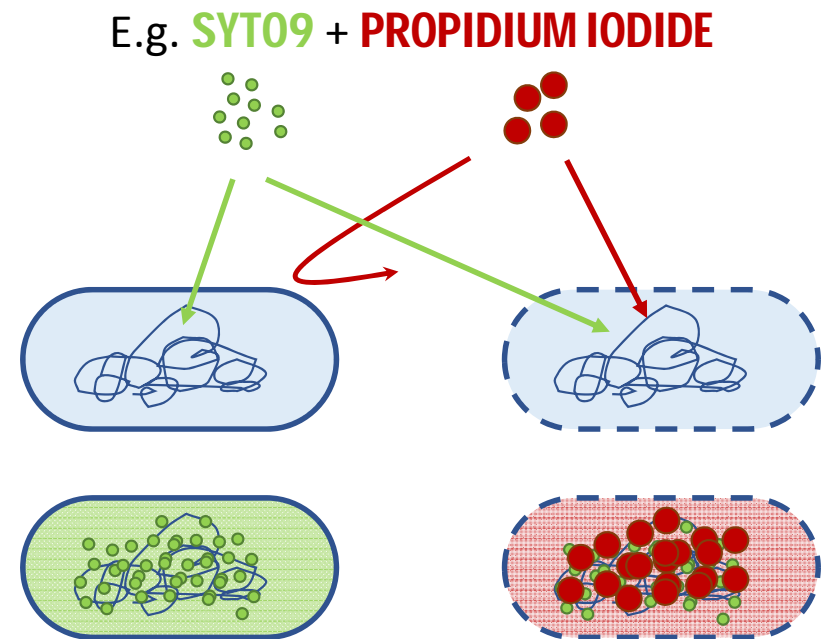
VIABILITY STAININGS



Live Bacteria → Intact membrane



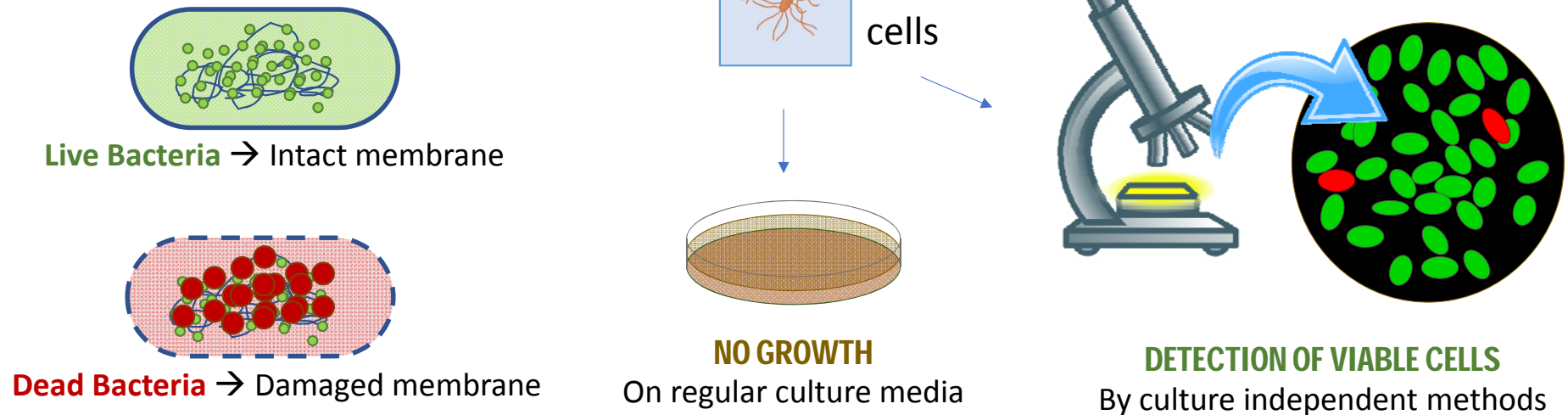
Dead Bacteria → Damaged membrane



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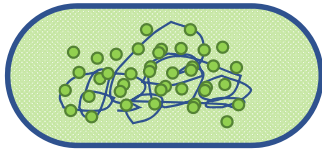
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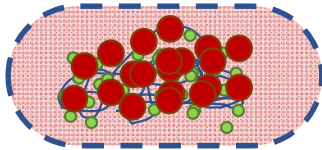
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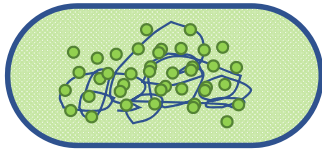
Stresses inducing the VBNC state in *E. amylovora*:

- Copper
- Chlorine (in tap water)
- Starvation in rainwater, distilled water, river water and mineral water
- Starvation in soil
- Starvation in/on insects
- Starvation in the apple calyx
- Starvation in artificial media

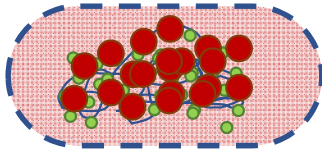
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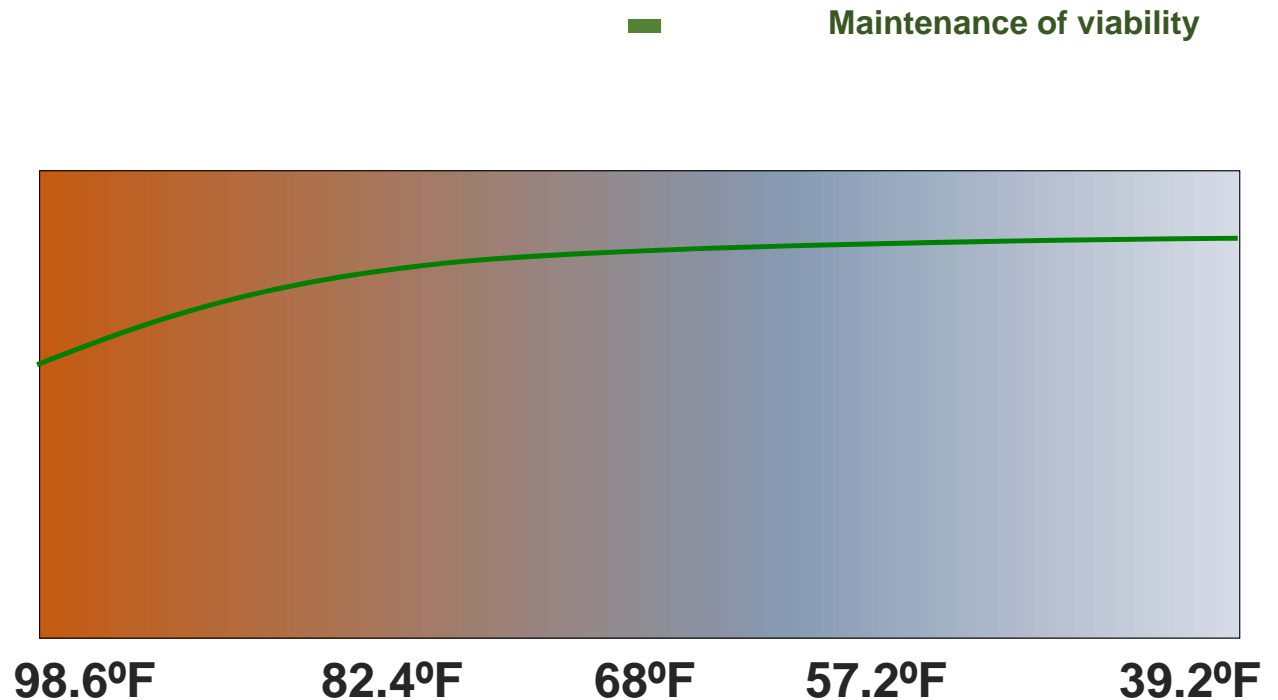
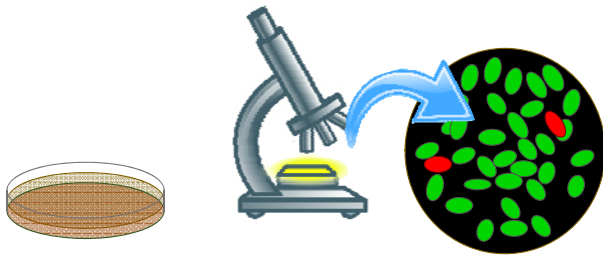
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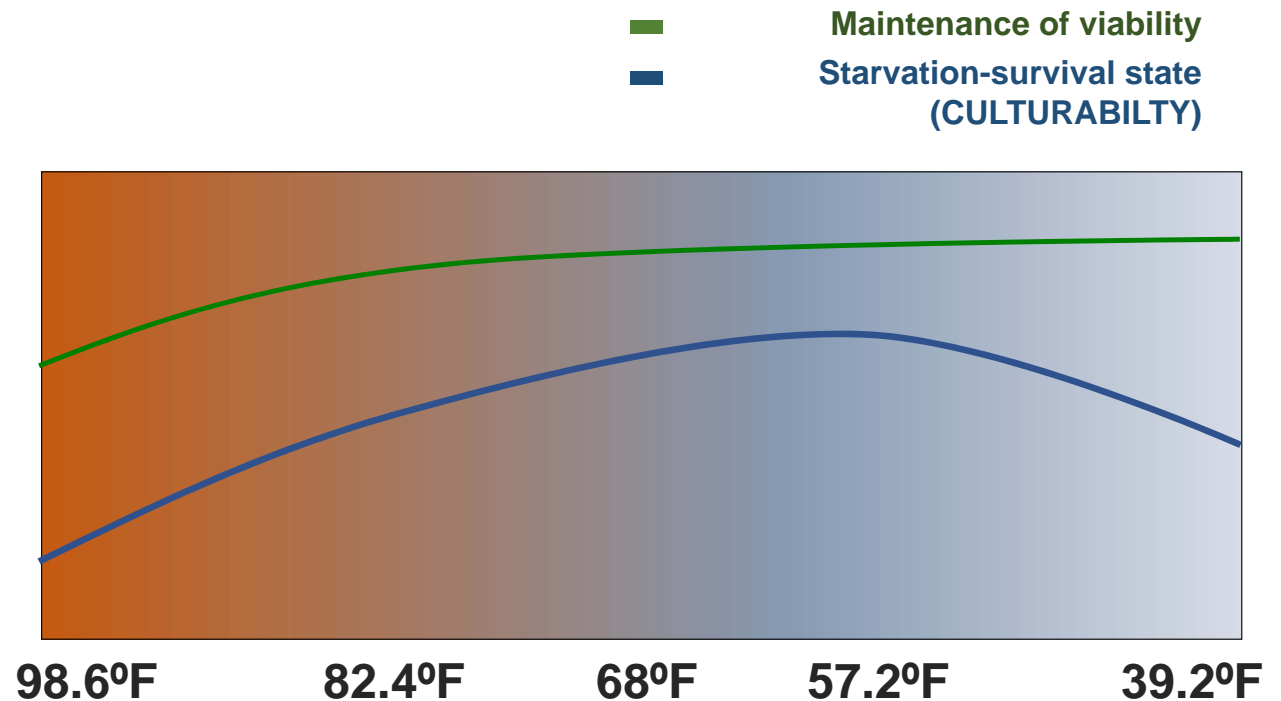
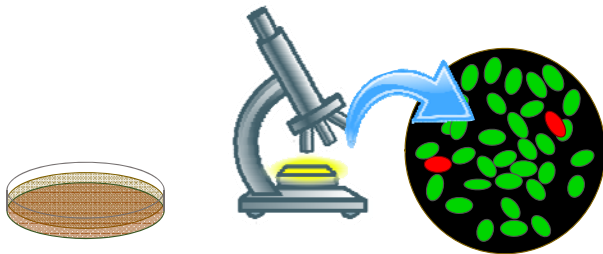
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- Effect of **Temperature** on starvation responses.



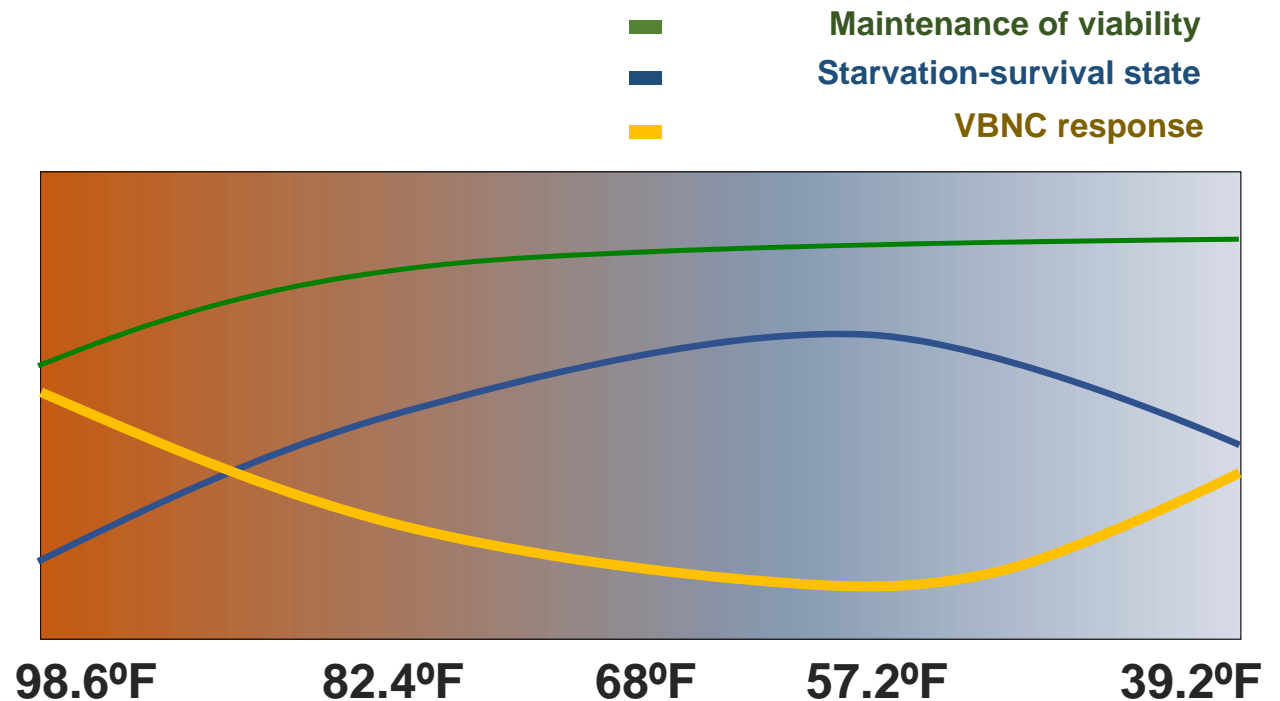
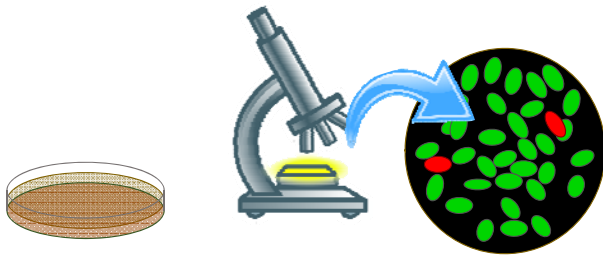
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Is the **VBNC** a stage of the *E. amylovora* life cycle?
Role in Survival / Overwintering / Asymptomatic infections?

CURRENT PROJECT

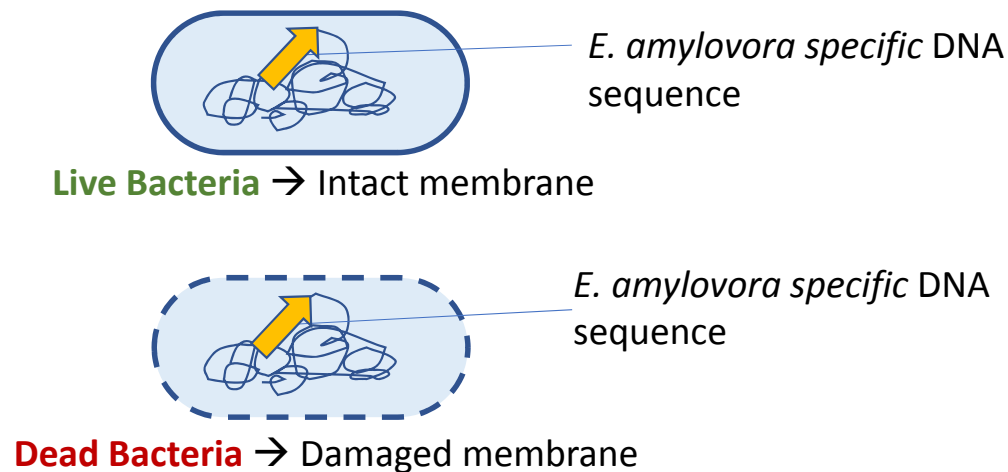
Determine the presence and quantify *E. amylovora* culturable and viable cell populations in cankers of trees of different hosts (apple and pear) and cultivars

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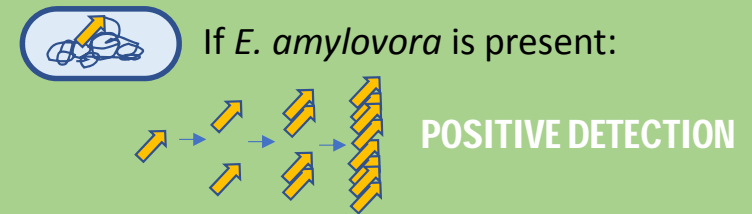
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Molecular Methods

E.g. **digital PCR** + **Propidium Monoazide**



PCR amplification



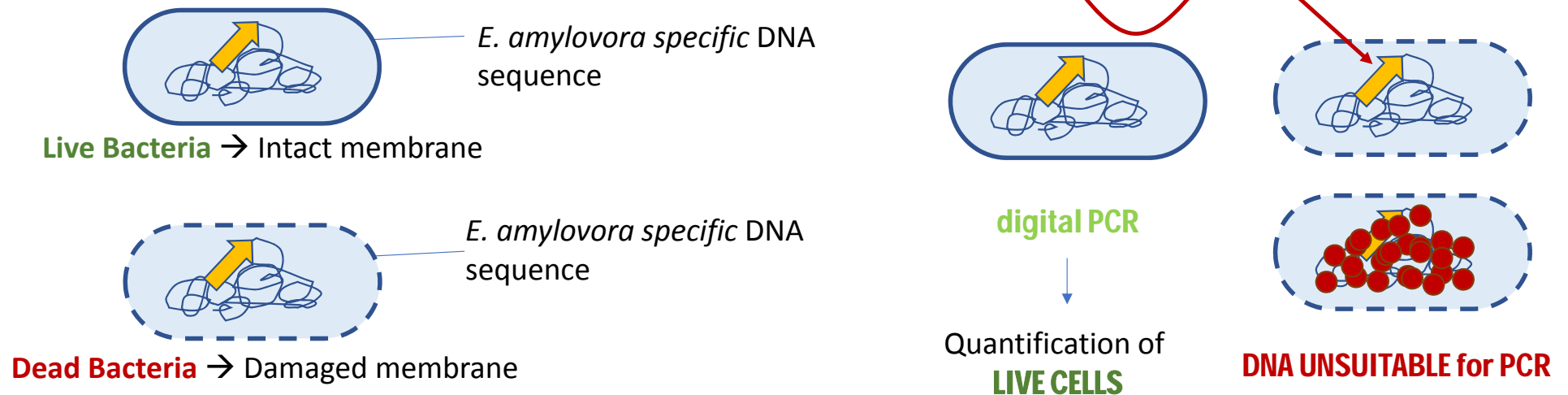
How can we differentiate DNA coming from live or dead cells??

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E. amylovora cells exposed to starvation at 7°C
Plate counts at time 0 → **10^6 CFU/mL**

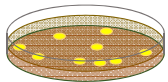
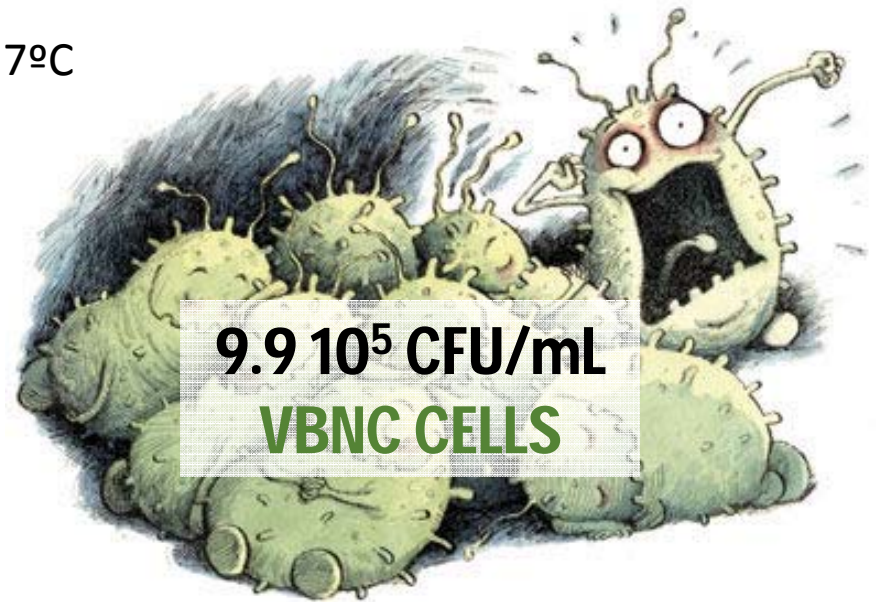


Plate counts after 3 months at 7°C
 10^4 CFU/mL

digital PCR + Propidium Monoazide

after 3 months at 7°C → **10^6 CFU/mL**



Dormant bacteria by David Parkins

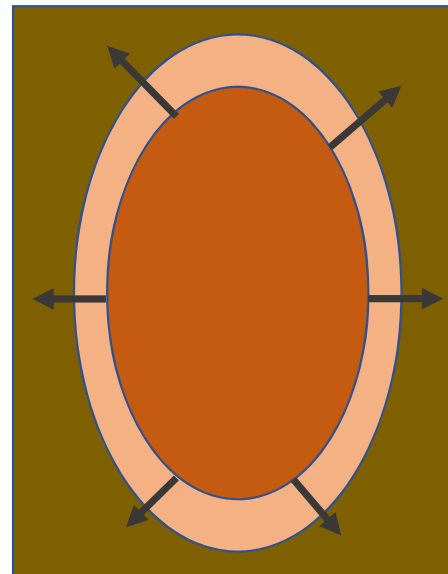
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Canker on apple
(Source: <https://fff.hort.purdue.edu/>)

Average
 2.7×10^4 live cells/canker



Radial decrease of
E. amylovora cell
populations,
outwards from the
canker edge.

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- **Root Blight** → Are roots suitable sites of entry into the host?

360 sampled rootstocks at 7 farms in Champlain Valley (Nov 2016)

	# PCR+ Samples / Total # samples	% Infected Rootstocks
Orchard #1	19/54	35.2
Orchard #2	27/52	51.9
Orchard #3	19/54	35.2
Orchard #4	18/54	33.3
Orchard #5	27/63	42.9
Orchard #6	11/55	20.0
Orchard #7	3/28	10.7

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Previous Data (assays in apple):

- *E. amylovora* cells inoculated into the stem migrate to the roots
- Inoculation of roots does not involve stem invasion
- **In pear?**

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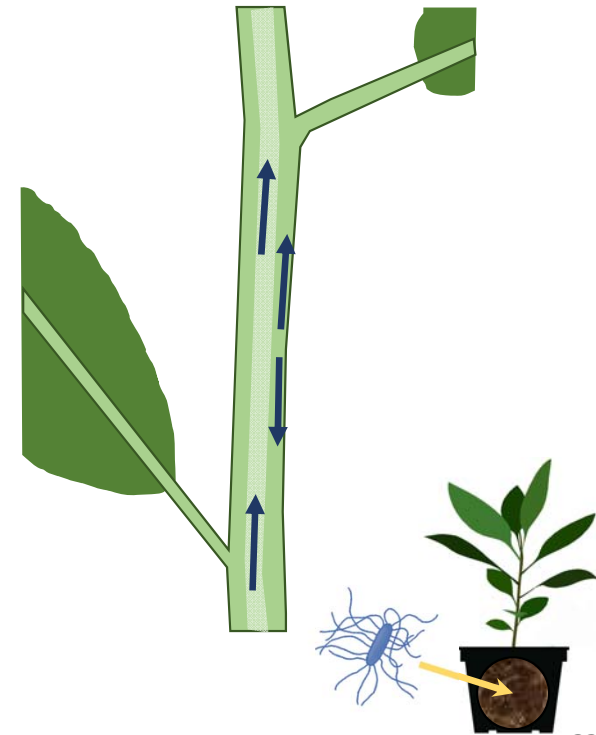
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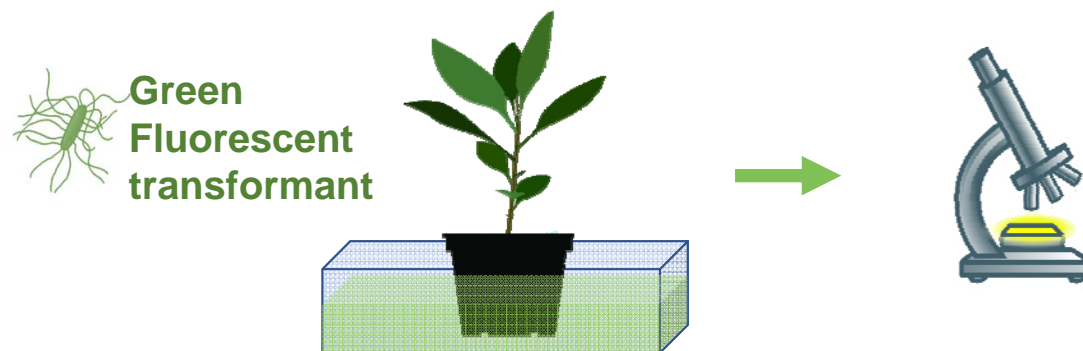


- 100 % of root inoculated plants developed symptoms in the **aerial organs** 2-3 days post-inoculation
- *E. amylovora* migrates from the roots to the stem throughout the vascular vessels
- Cells escape from the vascular cylinder in petioles and the tip of the stem → Fire Blight Symptoms
- Migration of cells through the cortex occurs upwards and downwards

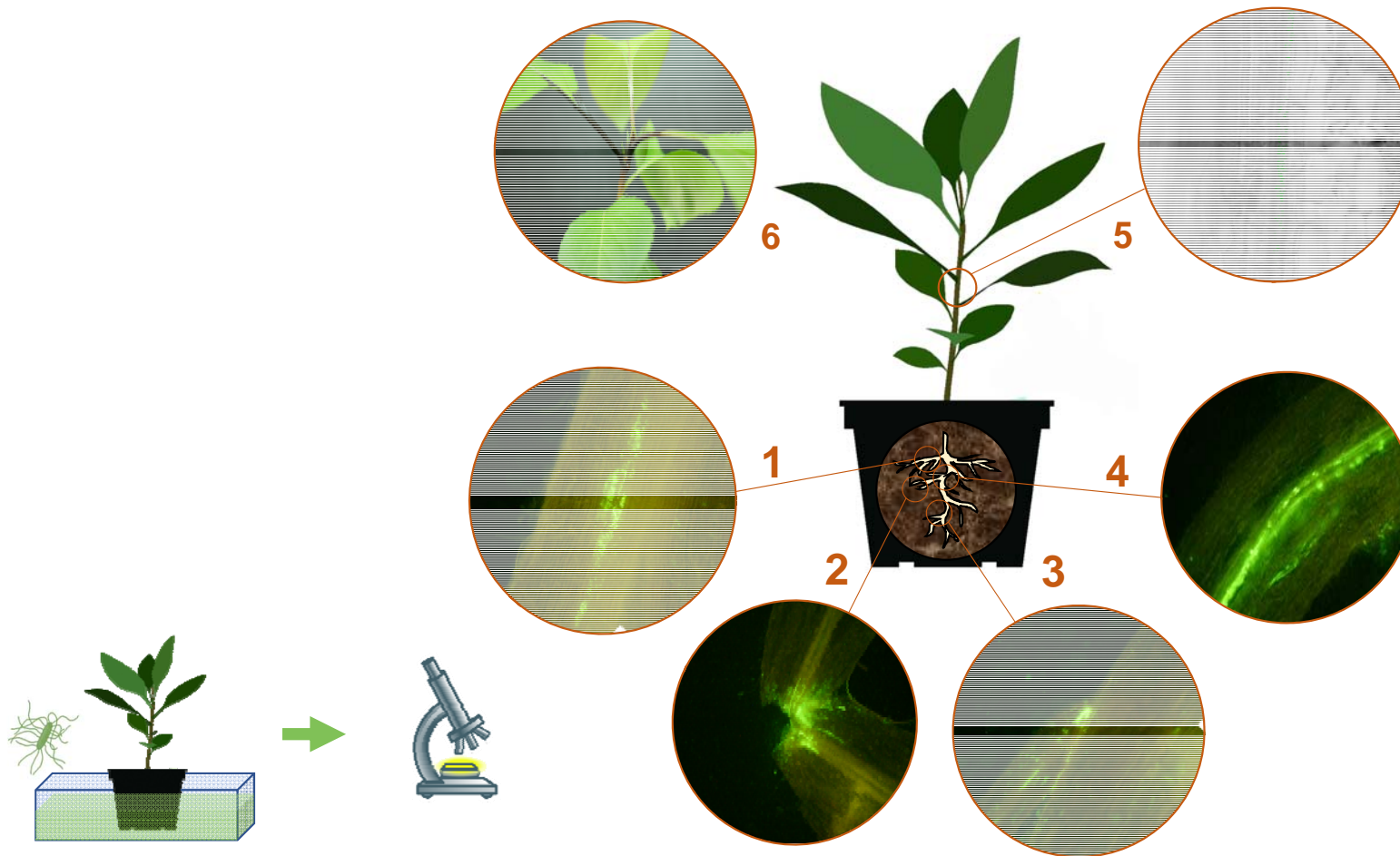


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Acknowledgements

This material is based upon work that is supported by the USDA's National Institute of Food and Agriculture, U.S. Department of Agriculture, Multistate under 2017-18-267: W3185: Biological Control in Pest Management for the project "Population Dynamics of Fire Blight Bacterium *Erwinia amylovora* in Cankers Under Drought Stress and Winter Cold.

