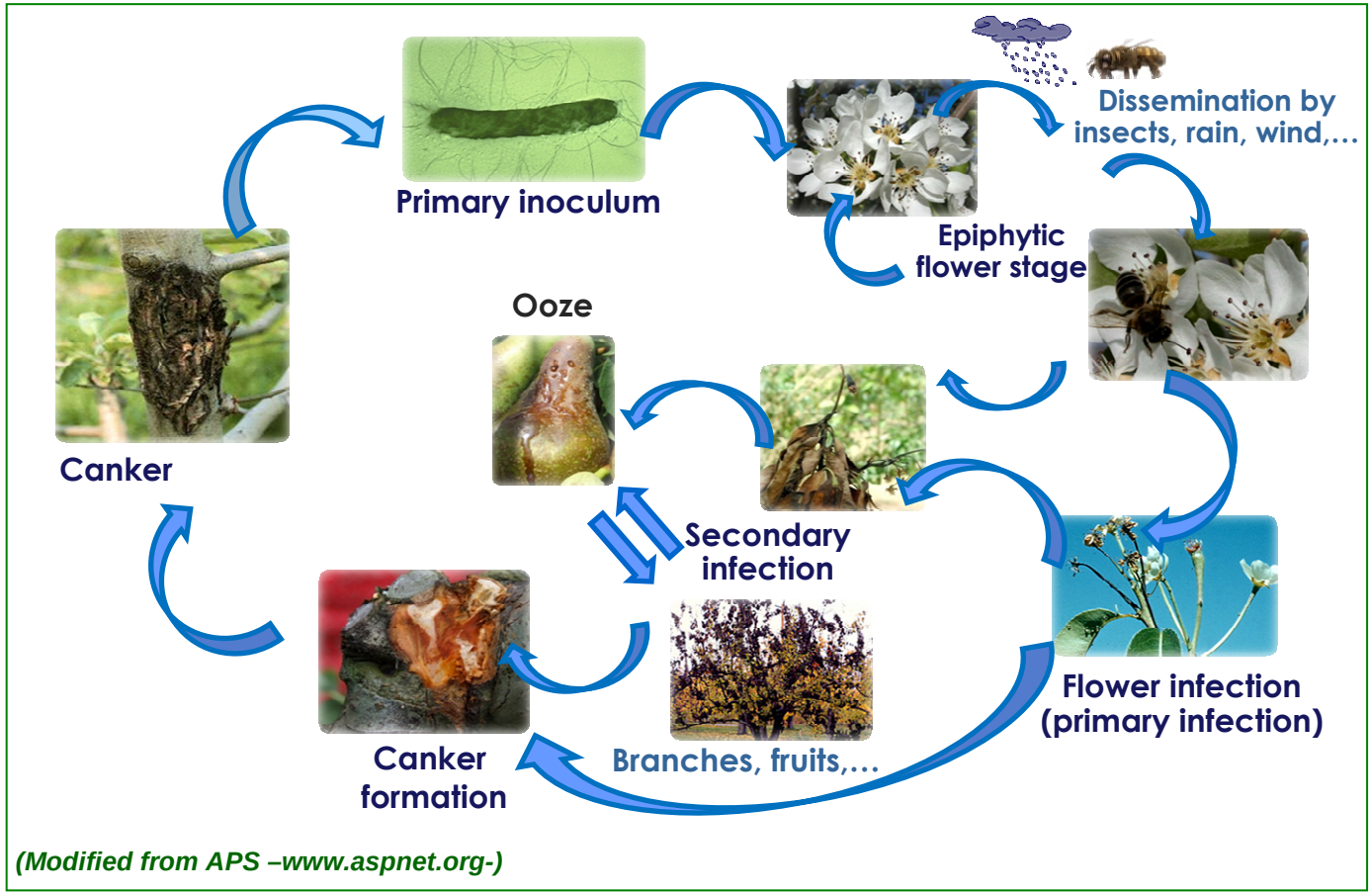
Environmental fitness and survival strategies of *Erwinia amylovora*

María M. López , Elena G. Biosca and Ester Marco-Noales

Instituto Valenciano de Investigaciones Agrarias (IVIA)

ivia
instituto valenciano
de investigaciones agrarias

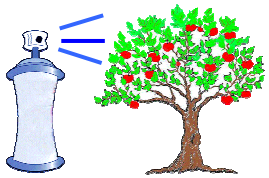
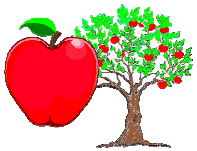
Biological cycle of *E. amylovora*



- Many aspects of this life cycle still remain unclear
- What happens when the pathogen is under stress conditions or outside the host?
- Its ability to survive in asymptomatic plants can lead to undetect some sources of inoculum

❑ **Life of *E. amylovora* outside the host or in the host under non-conducive conditions**

❑ **Determination of survival mechanisms displayed by *E. amylovora* under different situations**

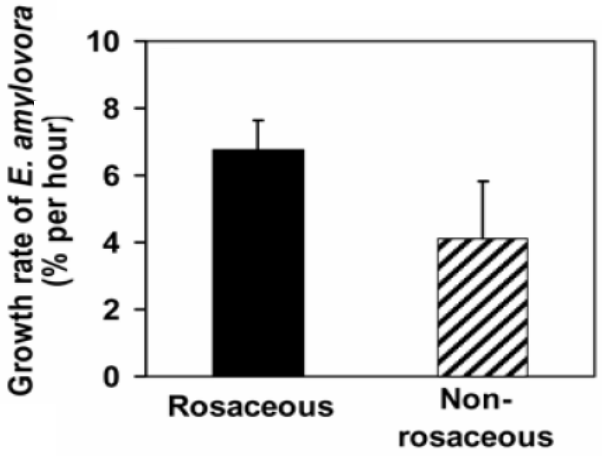
- Under copper stress 
- On apple fruits 
- On fruit flies 
- In water 

ivia
instituto valenciano
de investigaciones agrarias

Epiphytic growth of *Erwinia amylovora*

- In host plants:
  - Kritzmen *et al.* 2003: diagnostic medium
  - Thomson 2006: printing of stigmas

- In non host plants:
  - Floral epiphyte under field conditions
    - *Prunus avium*
    - *Rubus armeniacus*
  - Floral epiphyte in growth chamber



ivia
instituto valenciano de investigaciones agrarias

Latent infections of *Erwinia amylovora*

- Endophytic inoculum and latent infections have been reported in several host plants (Thomson, 2000).
- The sources of primary inoculum have not been demonstrated in many cases.
- Migration of *E. amylovora* followed by GFP and chlorophyll fluorescence imaging shows migration of bacteria in leaves when no symptoms were visible (Heyens and Walcke, 2006).
- There is a need of efficient protocols for bacterial detection in asymptomatic plants (EPPO Bulletin, 2004).

Survival of *Erwinia amylovora* in soil

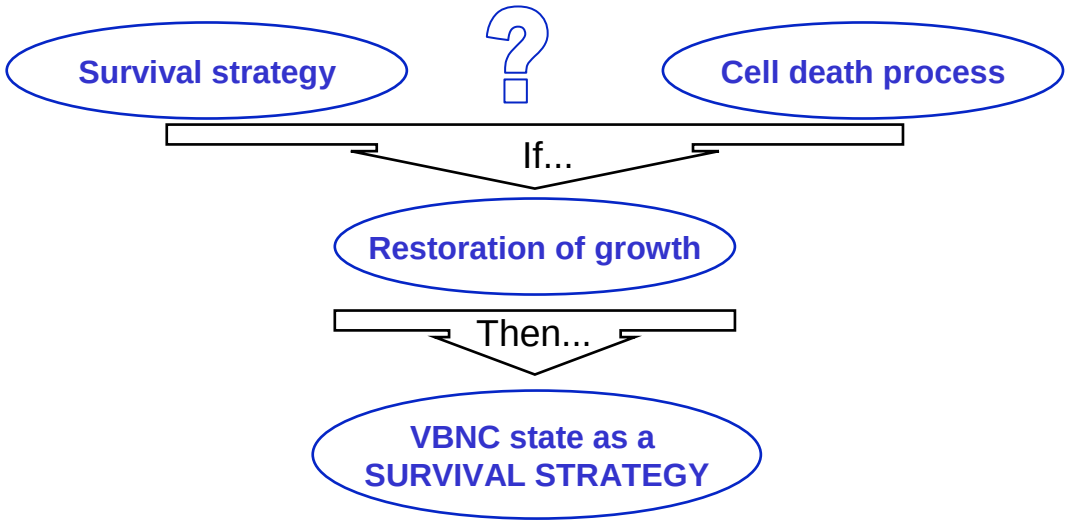
- *E. amylovora* culturability declines faster (4 weeks) in non-sterile than in sterile soil (11 weeks) (Hildebrand et al., 2001).
- Soil-living microarthropods (*Folsomia candida*) can contain large amounts of *E. amylovora* in the gut, but the pathogen is rapidly degradated (Hildebrand et al., 2001).
- Bacteriophages isolated from soil (Schnabel and Jones, 2001; Gill et al., 2003).
- The possibility of VBNC state of *E. amylovora* in soil has not been investigated.

Introduction to some survival strategies

- **Viable but noculturable (VBNC) state:** a physiological state in which bacteria are viable, but unable to divide sufficiently on non-selective solid medium to yield visible growth.

- This state is determined by growth-independent viability assays:
  - Metabolic activitiy
  - Membrane integrity

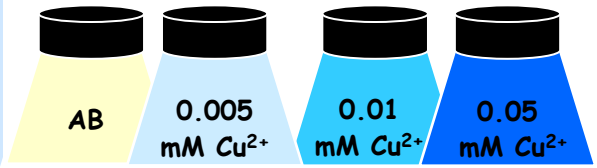
- Nature of VBNC state has been the topic of intense debate:

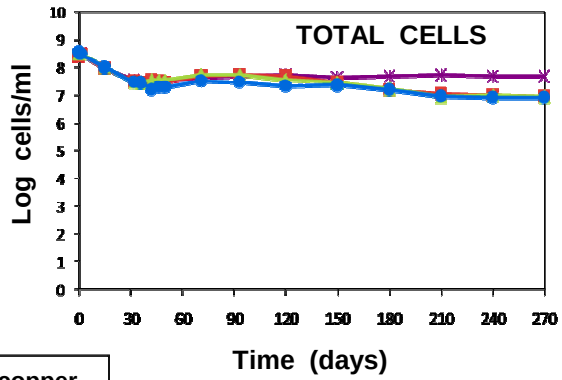


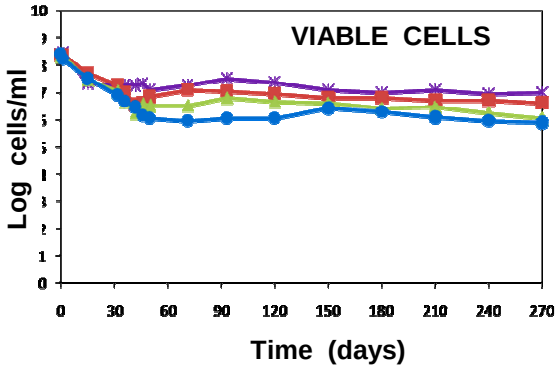
ivia
instituto valenciano
de investigaciones agrarias

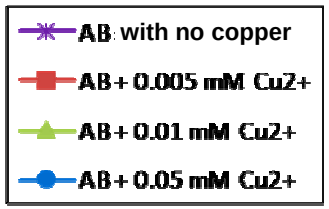
Under copper stress

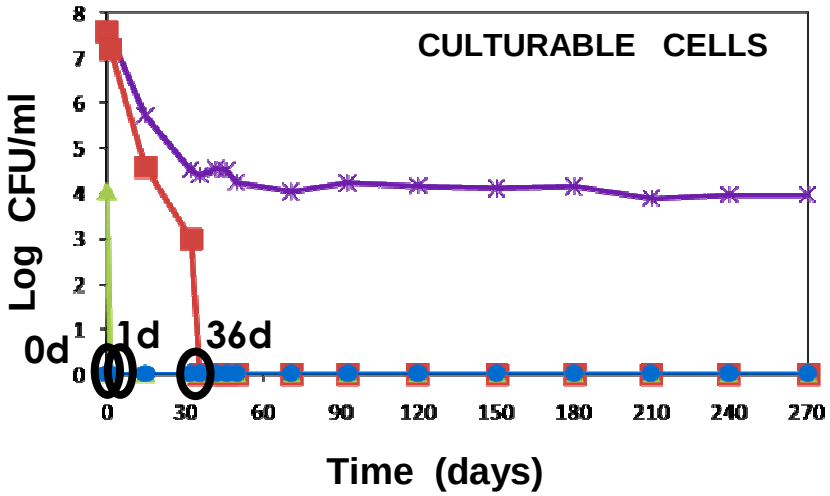
- In the presence of copper, *E. amylovora* adopts the VBNC state.





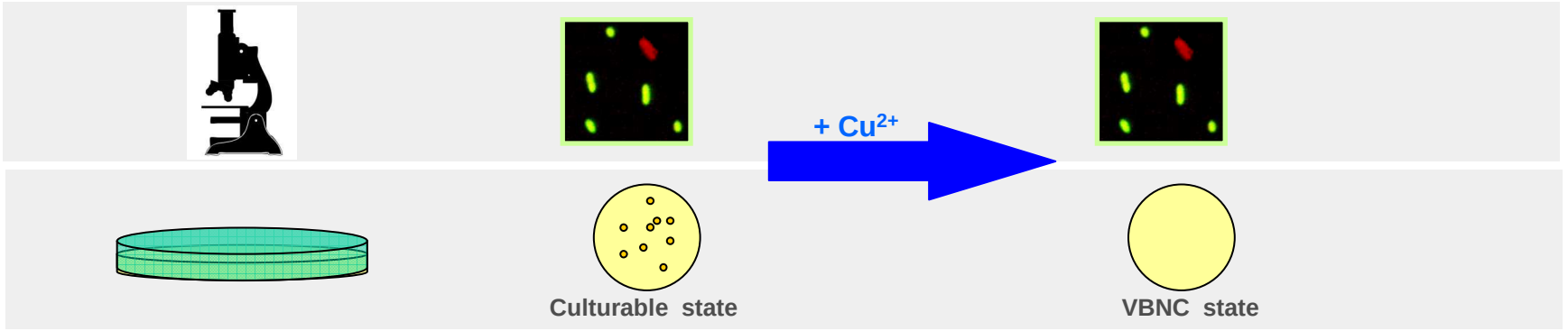






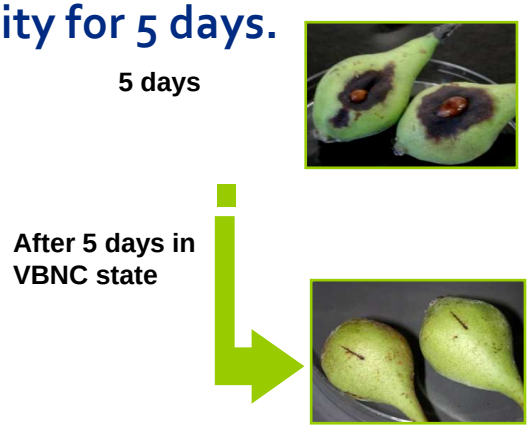
Under copper stress

- Cells become non-culturable but maintain the viability, as determined by culture-independent methods (Live&Dead).



- There is a change in morphology in copper induced-VBNC *E. amylovora* cells.

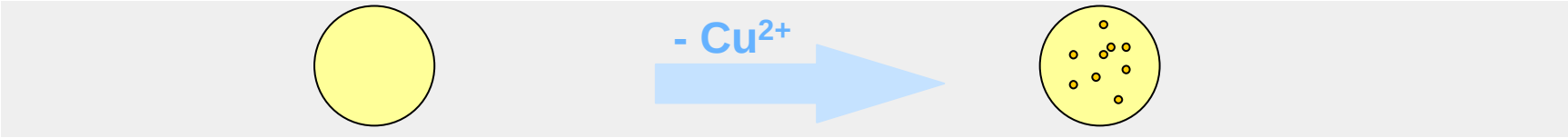
- *E. amylovora* VBNC cells maintained the pathogenicity for 5 days.



Under copper stress

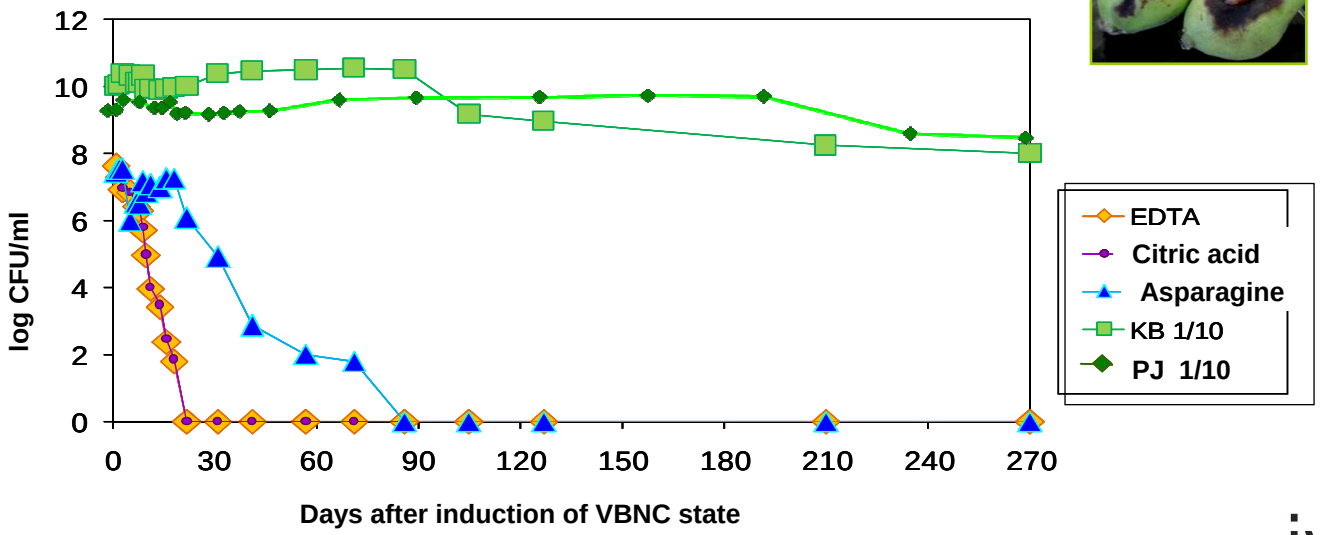
Resuscitation

- The liquid medium KB and pear juice promoted the recovery of culturability of VBNC cells even 9 months after the entry in that cells state.

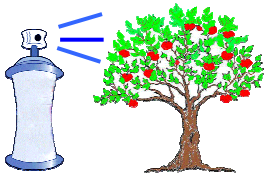


Viable But Non Culturable (VBNC) state

Culturable state



ivia
instituto valenciano
de investigaciones agrarias

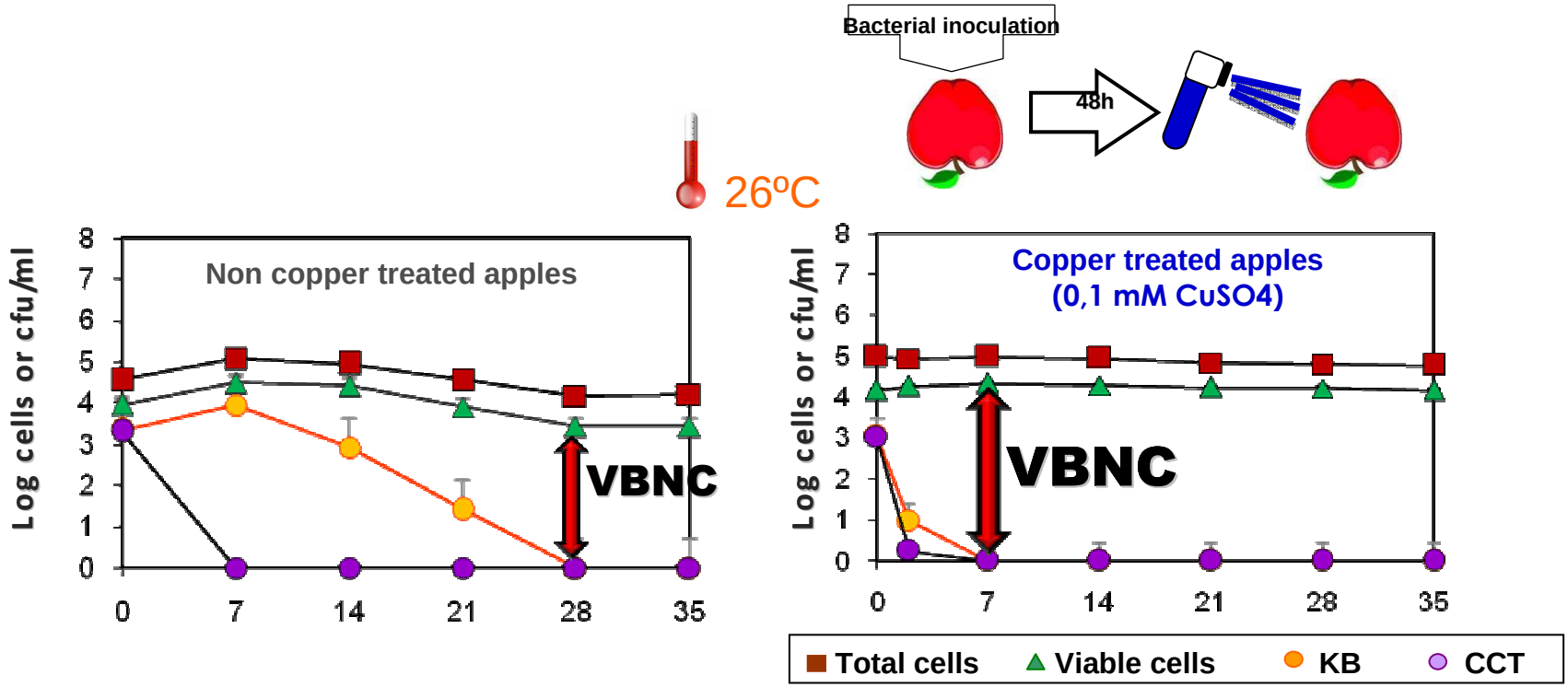


- Copper induces the entry of *E. amylovora* into the VBNC state, much faster as copper concentration increases (from 0.005 to 0.05 mM Cu2+).
- Copper-induced VBNC *E. amylovora* cells retained their pathogenicity only for 5 days.
- In the presence of copper, cells suffer morphological changes.
- Copper-induced VBNC cells are able to resuscitate after, at least, 9 months, regaining their pathogenicity.

Ordax M., Marco-Noales E., López M.M., Biosca E.G. 2006. Appl. Environ. Microbiol. 72: 3482-3488.

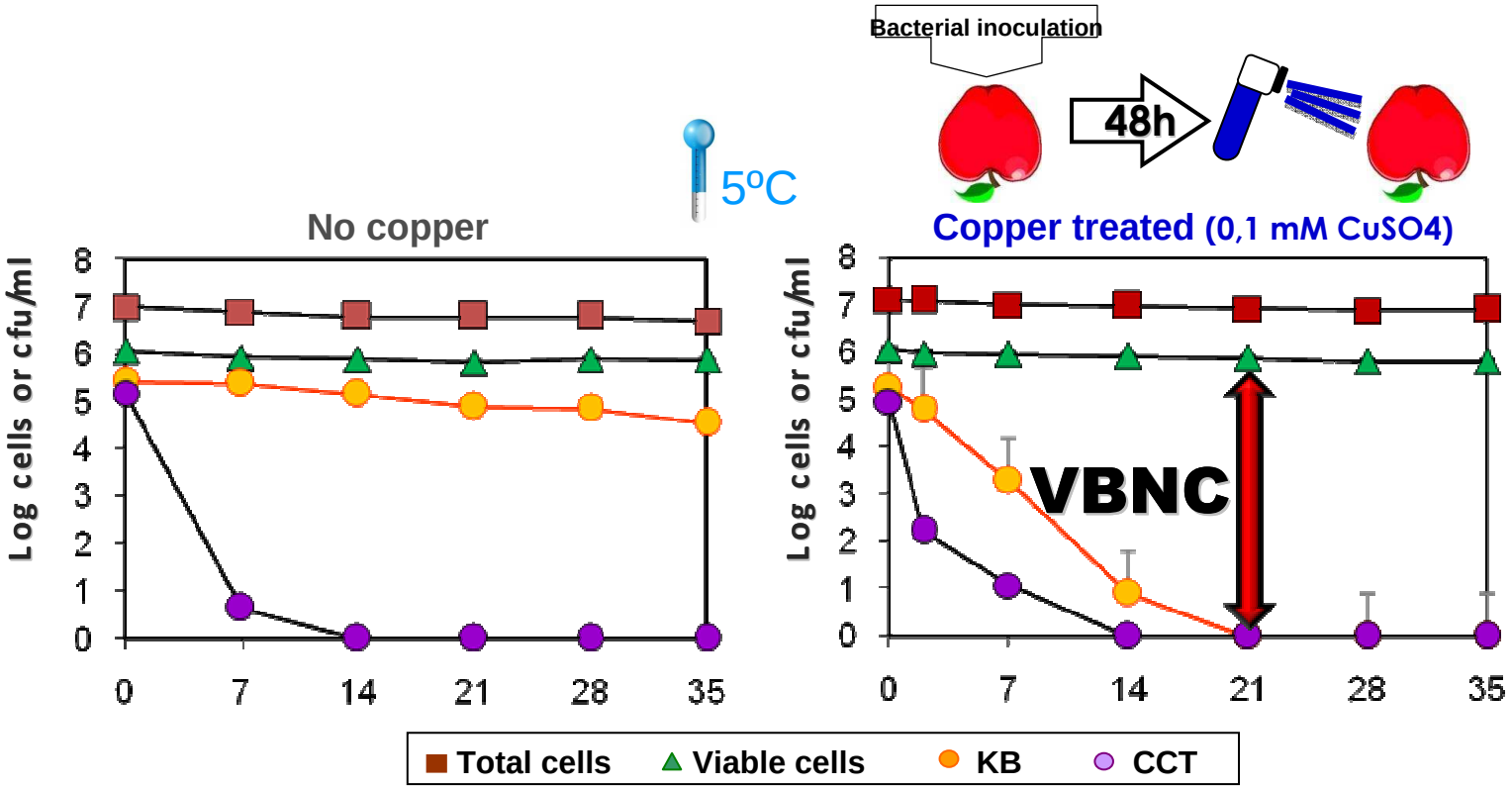
On apple fruits

- At 26ºC, part or whole population of *E. amylovora* adopts the VBNC state, faster in the presence of copper



On apple fruits

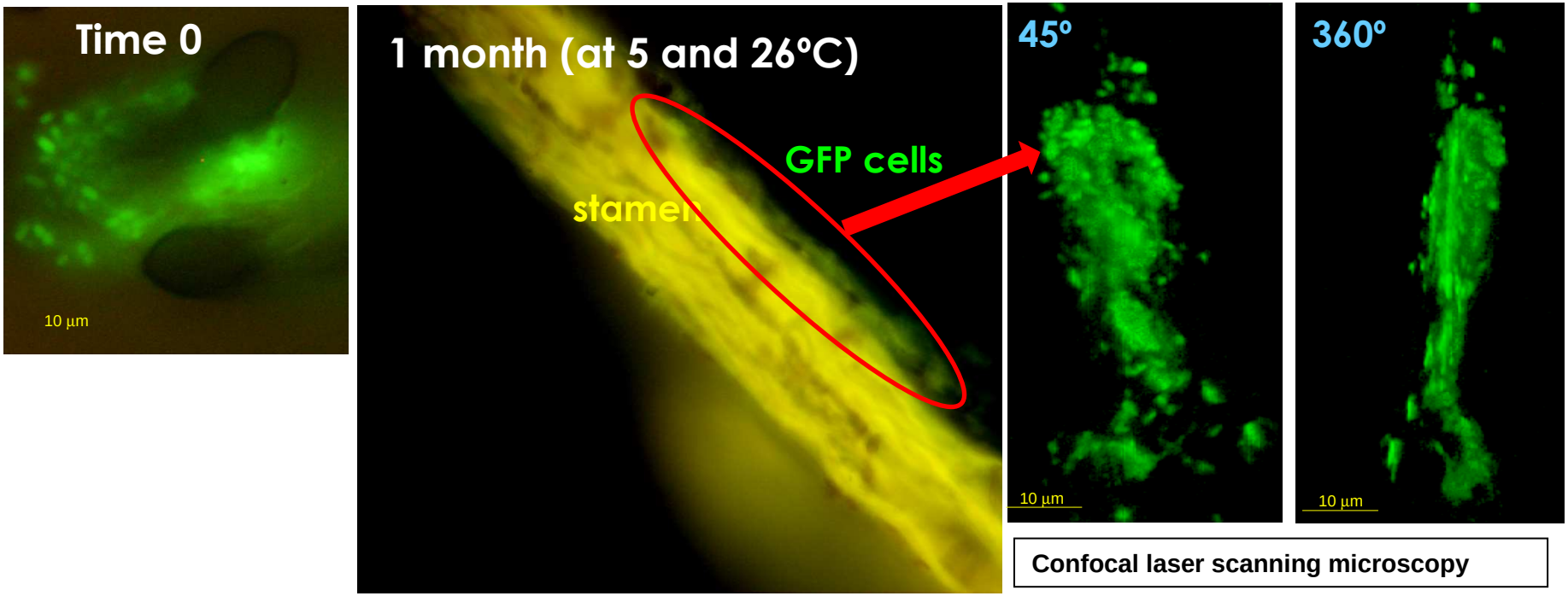
- At 5ºC, *E. amylovora* cells maintain the culturability in the absence of copper, but adopt the VBNC state in the presence of copper, although later than at 26ºC.



- VBNC cells could regain the culturability both *in vitro* (by incubating in KB) and *in vivo* (by pear shoot passage), regaining the pathogenicity.

On apple fruits

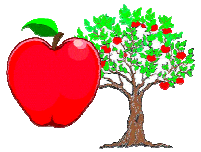
- Multicellular groups of *E. amylovora* along the stamens in mature apple fruit calyces suggests the formation of biofilms as other possible bacterial survival strategy.



Fluorescence microscopy

Confocal laser scanning microscopy

ivia
instituto valenciano
de investigaciones agrarias



- On apple fruits, the culturability of *E. amylovora* cells is dependent on the temperature, being lower at 26ºC than at 5ºC.

- On copper-treated apples, the whole population of *E. amylovora* adopts the VBNC state both at 26 and at 5ºC, although faster at the highest temperature.

- VBNC cells are able to regain the culturability both *in vitro* and *in vivo*, regaining the pathogenicity.

- *E. amylovora* seems to form biofilms as a survival strategy on apple stamens.

Ordax M., Biosca, E.G., Wimalajeewa, S.C., López. M.M., Marco-Noales E. 2009. J. Appl. Microbiol. 107: 110-116.

On fruit flies

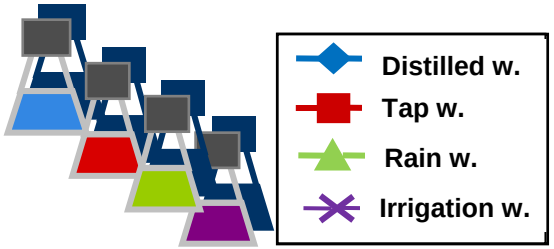
- *E. amylovora* is able to persist in/on *C. capitata* for 14 days in a culturable a pathogenic state. For longer periods, the pathogen losses the culturability but it is able to regain the culturability it through host plant passage.
- *C. capitata* can acquire *E. amylovora* from inoculated mature apples in only 48h, and transmit it in a culturable and pathogenic state to peel and flesh of mature apples.
- *C. capitata* was also able to transmit *E. amylovora* cells to detached pear shoots and whole pear plants.
- These results show that *E. amylovora* manages to survive for a quite prolonged time period in a non-host environment as flies.
- This is the first time that *C. capitata* is reported as a potential vector of a plant pathogenic bacterium.

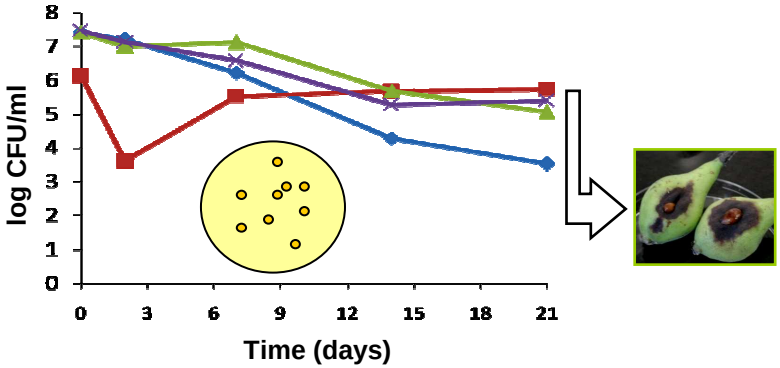
Ordax M., Piquer-Salcedo, J.E., Sabater-Muñoz, B., Biosca, E.G., López M.M., Marco-Noales E. The medfly *Ceratitis capitata* can act as a vector for the fire blight plant pathogen *Erwinia amylovora* (in preparation).

ivia
instituto valenciano
de investigaciones agrarias

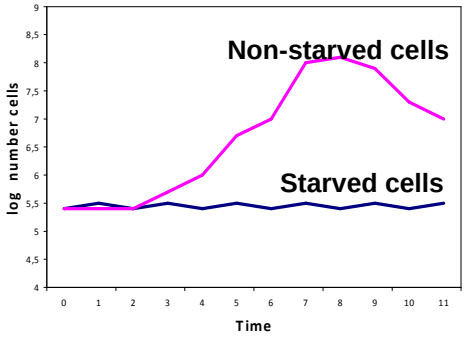
In water

- *E. amylovora* was able to survive in different types of sterilized natural water, maintaining the culturability and pathogenicity.



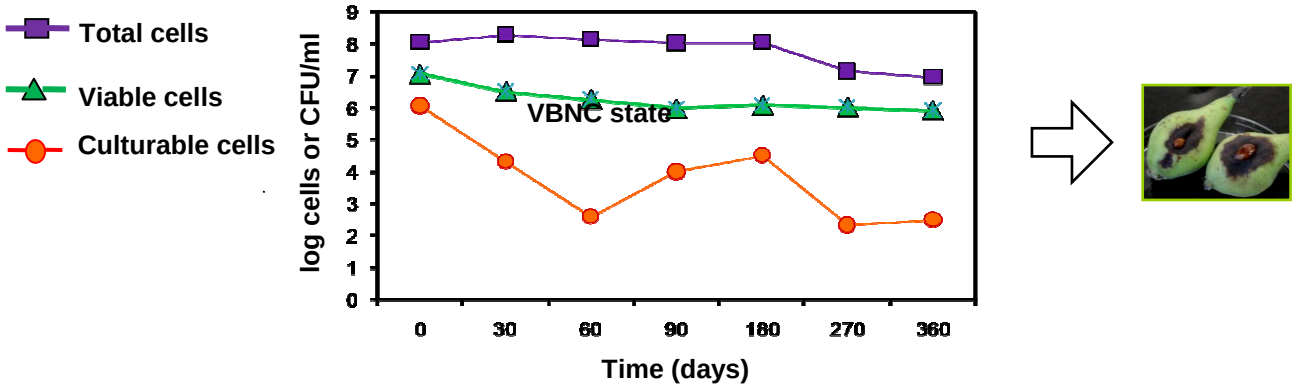


- *E. amylovora* adopted the `starvation survival state´, a physiological state resulting from an insufficient amount of nutrients to permit growth and/or reproduction.

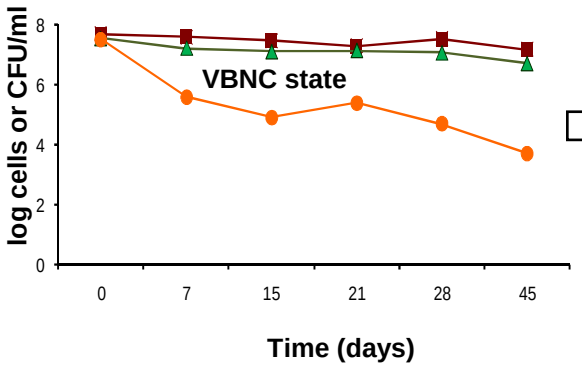


In water

- *E. amylovora* was able to survive in sterilized irrigation water under oligotrophic conditions for 1 year, maintaining the pathogenicity:
  - -some cells in culturable state (`starvation survival state´)
  - -and some cells in the VBNC state

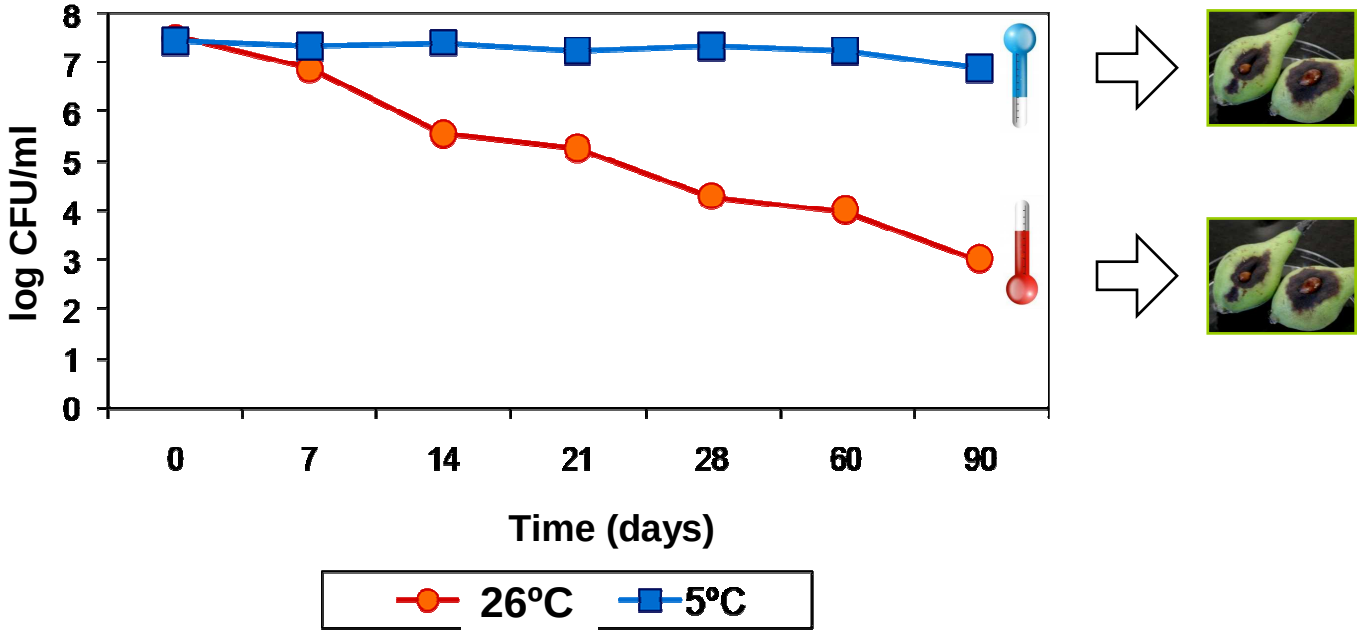


- In non-sterilized rain water, *E. amylovora* was able to survive, at a similar extent than in sterilized water, maintaining its pathogenic potential



In water

- In irrigation water at 5ºC, survival of *E. amylovora* was increased, and cells maintained their pathogenic potential.



ivia
instituto valenciano
de investigaciones agrarias

- *E. amylovora* is able to survive for more than one year in irrigation water.
- The populations of *E. amylovora* in water microcosms are affected by:
  - Chlorine, on short-term survival
  - Nutrient starvation, on long-term survival
- Low temperature does not seem to affect the survival of E. amylovora in water.
- *E. amylovora* maintains its infectivity in water, suggesting that it may be both a reservoir and a dissemination route of fire blight.

Biosca E.G., Marco-Noales E., Ordax M., López M.M. 2006. Acta Hort. 704: 107-112.

Biosca E.G., Santander R.D., Ordax M., Marco-Noales E., López M.M. 2008. Acta Hort. 793: 83-87.

ivia
instituto valenciano
de investigaciones agrarias

CONCLUSIONS

-*Erwinia amylovora* is a very well prepared bacterium to survive in different environments and to support stress conditions in treated trees, fruits, insects and water.

-*Erwinia amylovora* absence of colonies is not an evidence of real absence of viable cells.

- Genomic and transcriptomic analyses of more strains and stress conditions will provide information about the weak points to attack the bacterium for a better disease control

ivia
instituto valenciano
de investigaciones agrarias

Many thanks
for your attention

ivia
instituto valenciano
de investigaciones agrarias