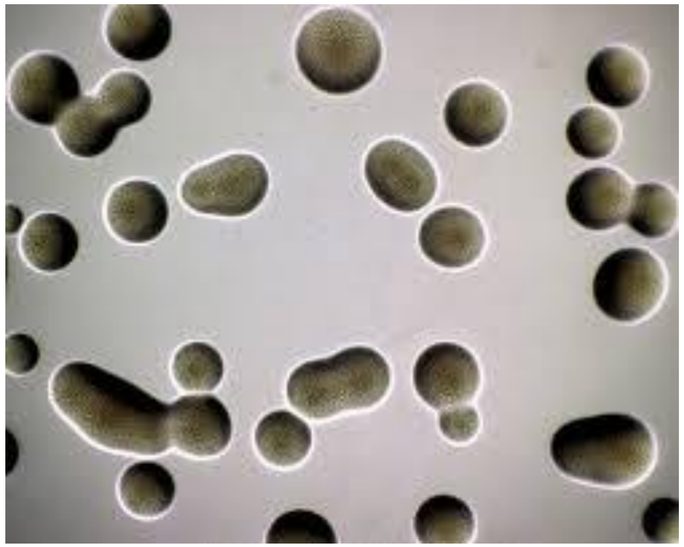
# *Xylella fastidiosa*: state of the art

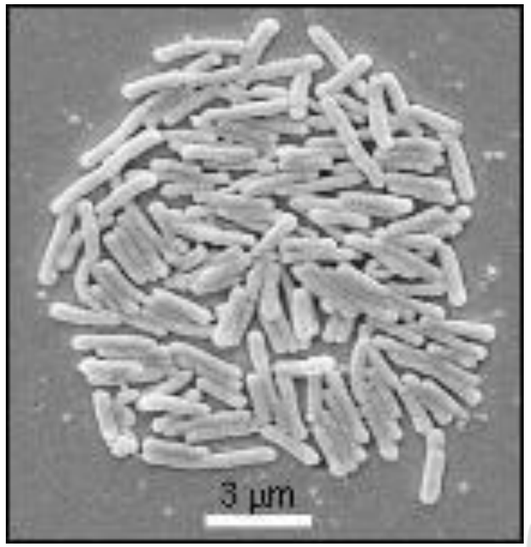
## genetic variability, hosts, symptomatology, distribution

**Donato Boscia**

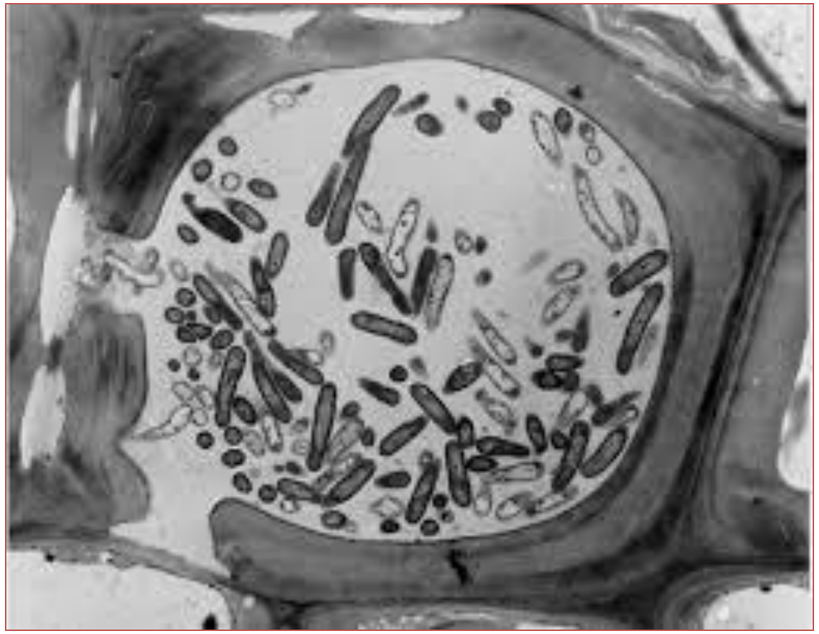
**E-mail: donato.boscia@cnr.it**

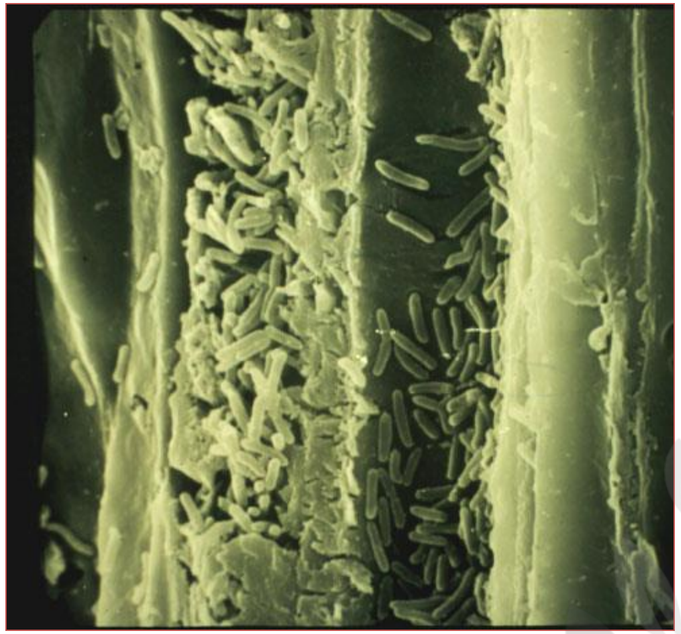
*Xylella fastidiosa* is a Gram-negative bacterium, non sporing, slow-growing  in axenic culture





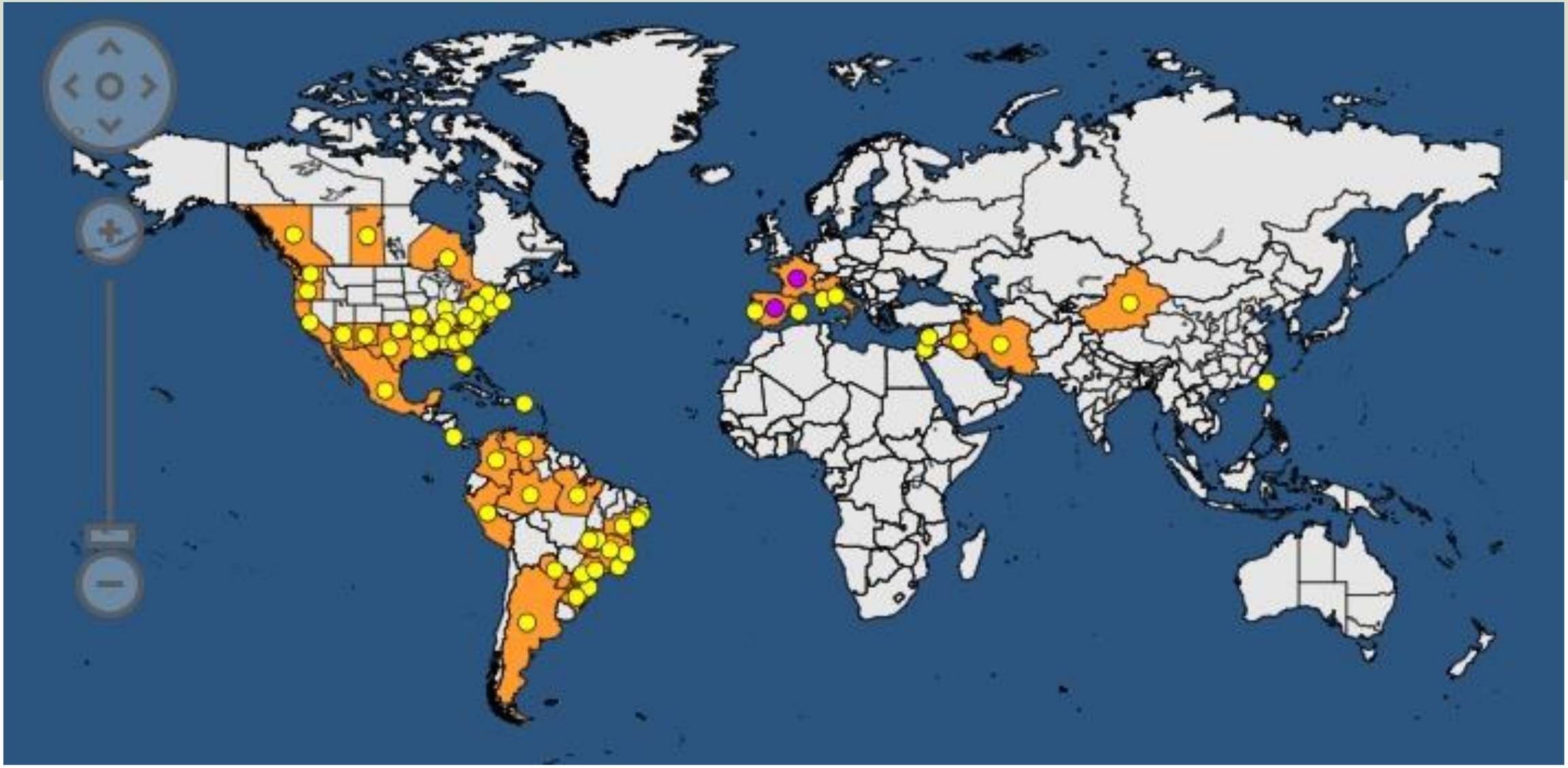
# It localizes  in the xylem vessels





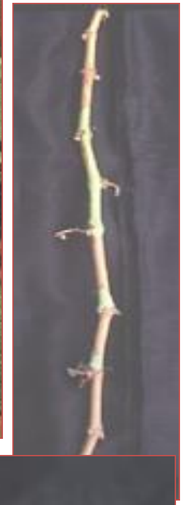
In addition to the movement of infected plants, responsible for the spread over long distances, the natural spread of the bacteria from plant to plant occurs via xylem feeding insects belonging to the Order *Hemiptera*.

# Wide geographical distribution



Its natural host range includes
71 families and 210 genera, for a total of 463 plant species.
It is the recognized agent of infectious diseases
affecting many economically important crops.

# Pierce's disease



Kills grapevines, which cannot be grown in large areas of the USA and the Gulf of Mexico

# Citrus variegated chlorosis

(Central America, Argentina, Brazil)

# Leaf scorch of stone fruits

Almond

Cherry

Plum

# Olive Quick Decline Syndrome

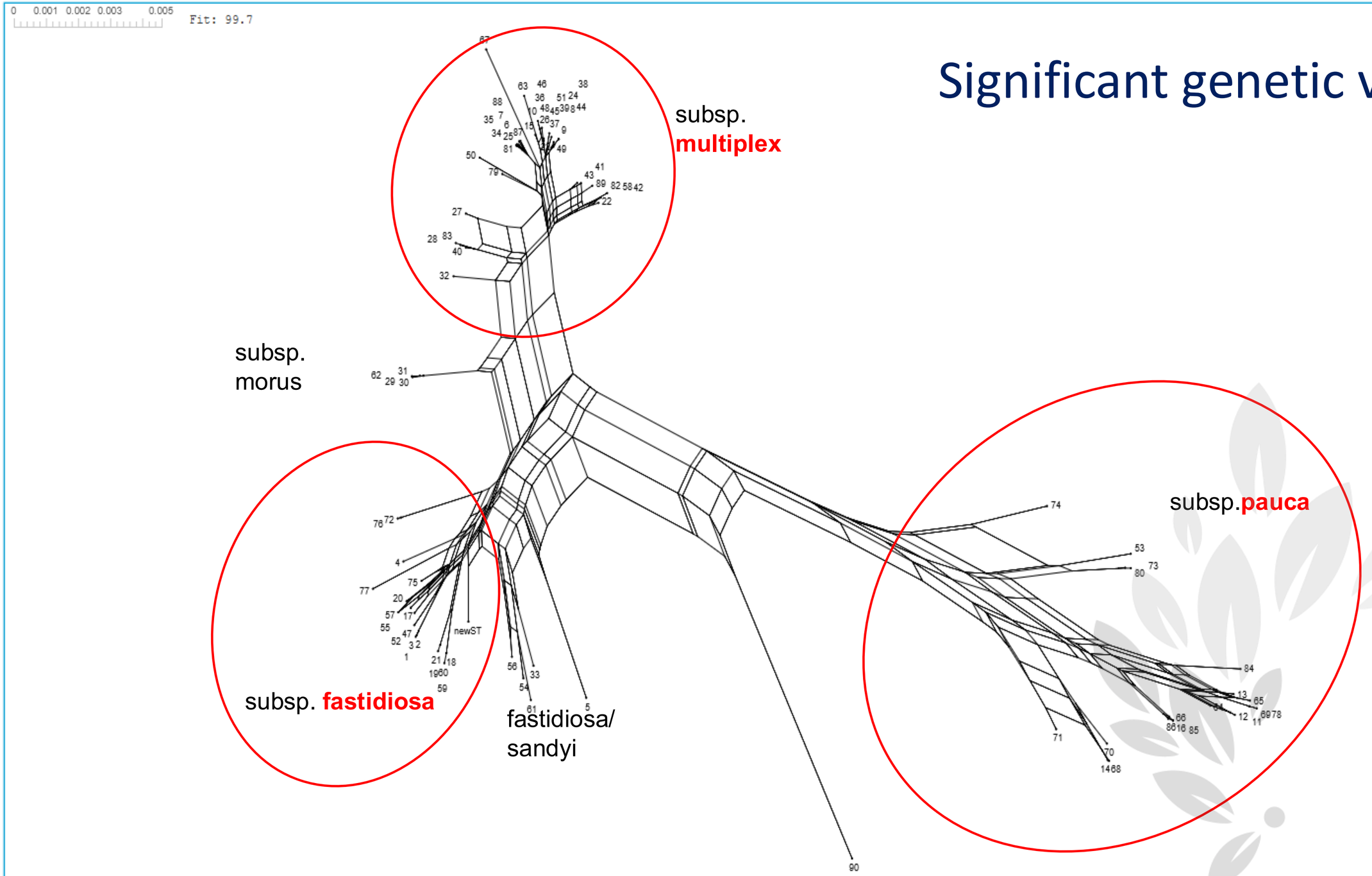
(Apulia, Argentina, Brasil, Balearic islands)

# Leaf scorch of forest and shade trees






# Significant genetic variability



# It is a quarantine organism included in the EPPO A2 list

## EPPO A2 List of pests recommended for regulation as quarantine pests

**- version 2025-09 -**

EPPO recommends its member countries to regulate the pests listed below as quarantine pests (A2 pests are locally present in the EPPO region). The EPPO A2 List is reviewed every year by the Working Party on Phytosanitary Regulations and approved by Council.

# In EU is regulated by



**COMMISSION IMPLEMENTING REGULATION (EU) 2020/1201**

**of 14 August 2020**

**as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells *et al.*)**

## October 2013:
## In Apulia first discovery of established infections of *X. fastidiosa* in EU

Journal of Plant Pathology (2013), 95 (3), 659-668

DISEASE NOTE

# IDENTIFICATION OF DNA SEQUENCES RELATED TO *XYLELLA FASTIDIOSA* IN OLEANDER, ALMOND AND OLIVE TREES EXHIBITING LEAF SCORCH SYMPTOMS IN APULIA (SOUTHERN ITALY)

M. Saponari[1], D. Boscia[1], F. Nigro[2] and G.P. Martelli[1,2]


# 2015: annual monitoring obligation for all member states



DECISIONS

**COMMISSION IMPLEMENTING DECISION (EU) 2015/789**

**of 18 May 2015**

**as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells et al.)**

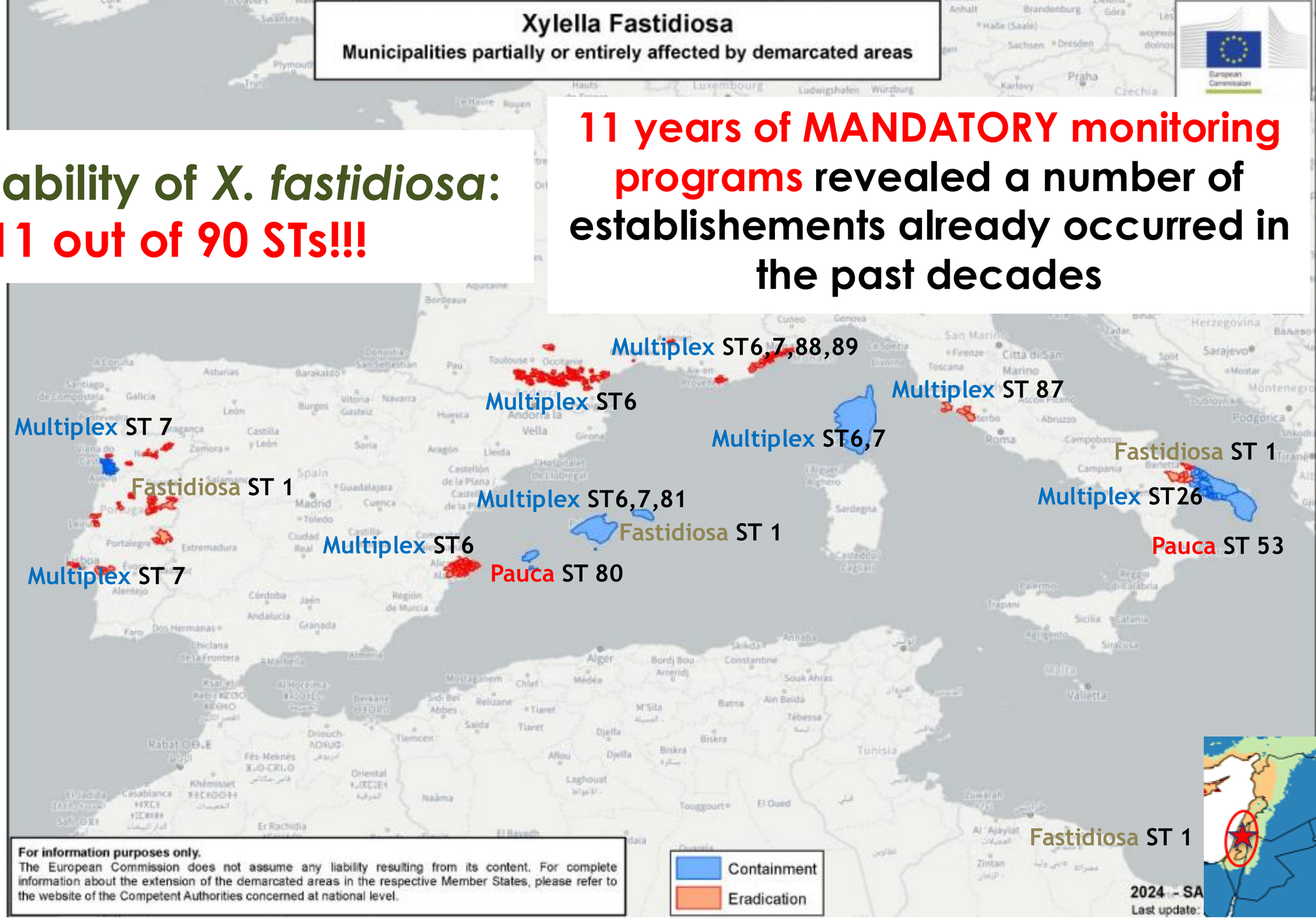
*Article 3*

**Surveys of the specified organism in the territories of the Member States**

Member States shall conduct annual surveys for the presence of the specified organism in their territory on the specified plants.

# Genetic variability of *X. fastidiosa*: in EU 11 out of 90 STs!!!

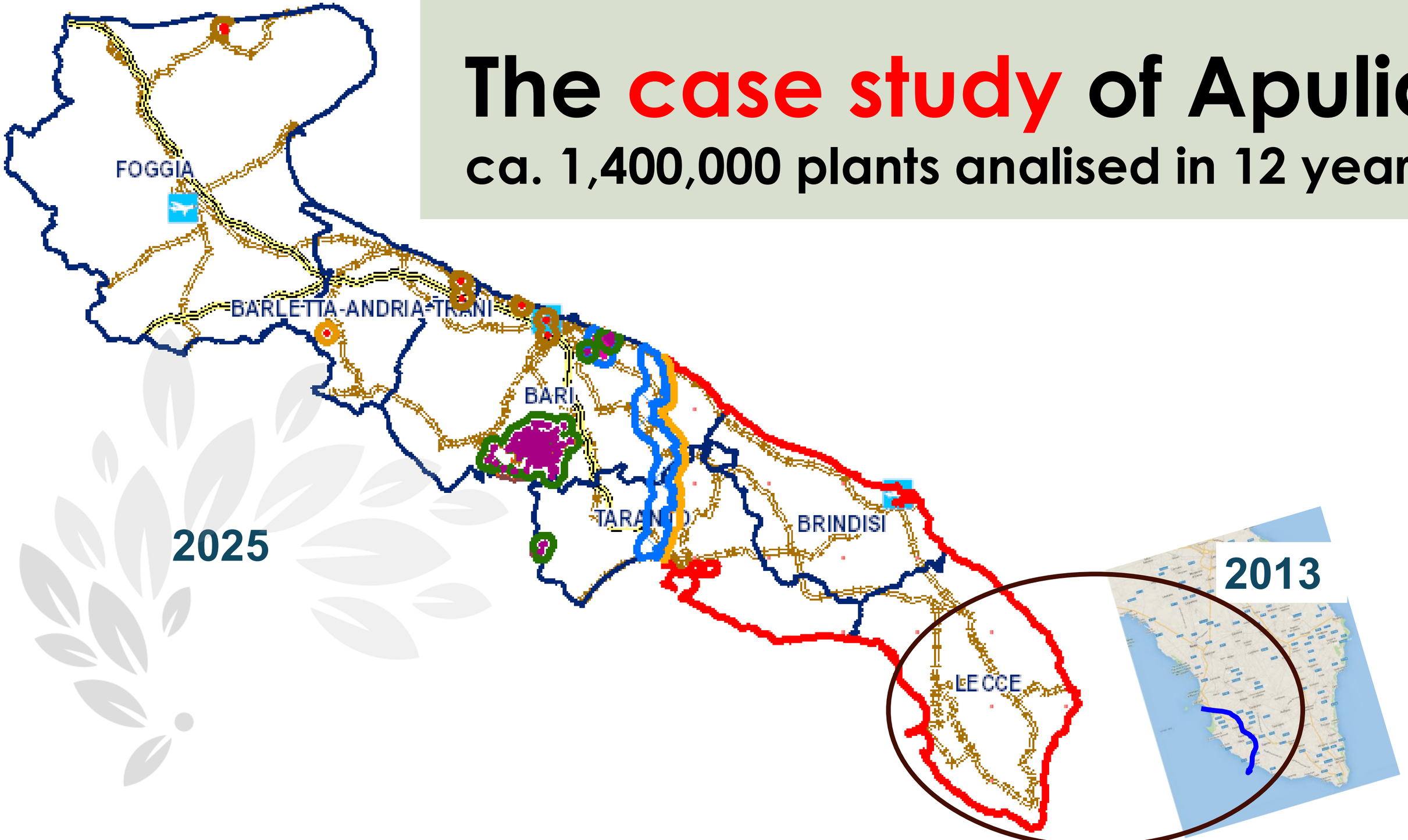
**11 years of MANDATORY monitoring programs revealed a number of establishements already occurred in the past decades**



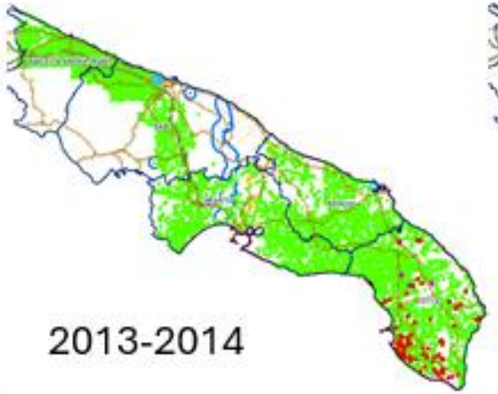
*«pauca» was discovered following the phytosanitary investigations on the devastating OQDS, the other subspecies were found following mandatory monitoring campaigns*

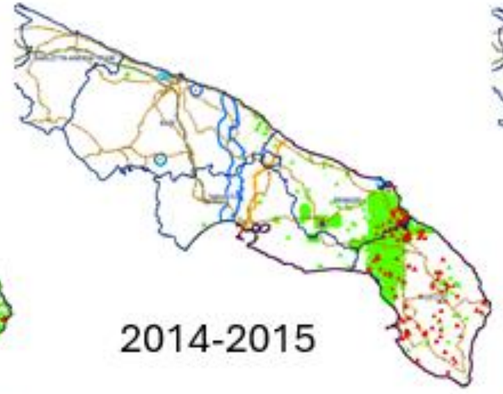
# The case study of Apulia

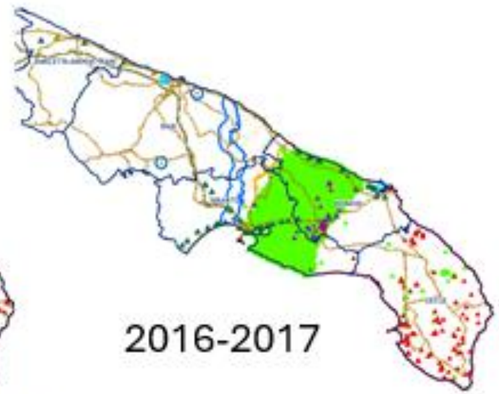
## ca. 1,400,000 plants analised in 12 years!

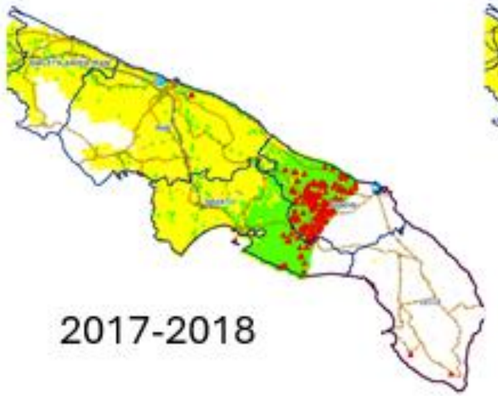


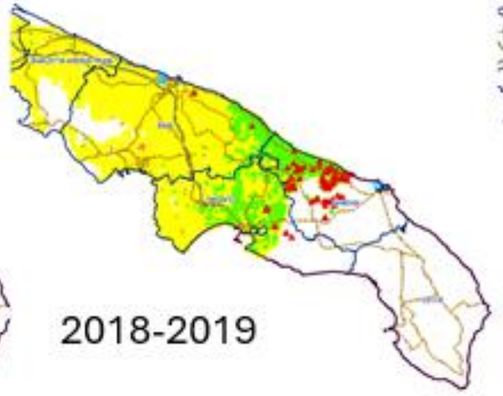
# 2013-2025: annual monitoring campaigns

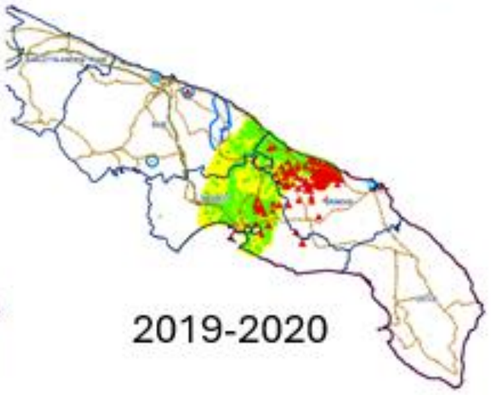

2013-2014


2014-2015


2016-2017


2017-2018

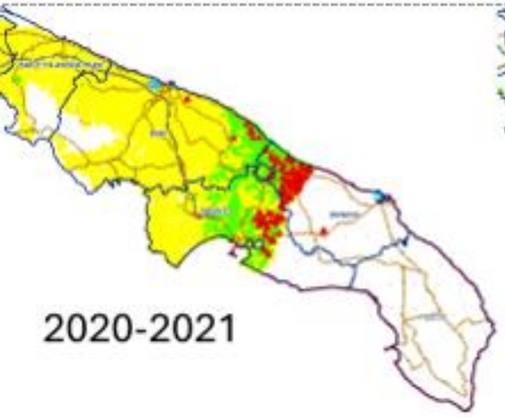

2018-2019

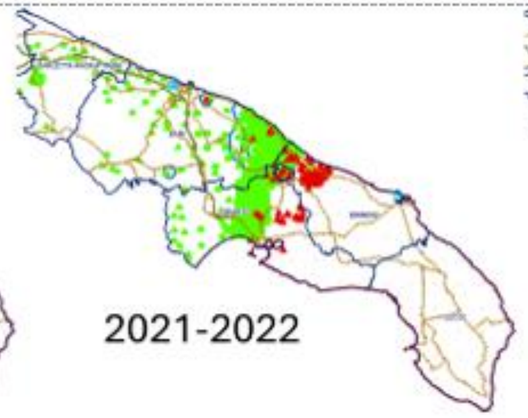

2019-2020

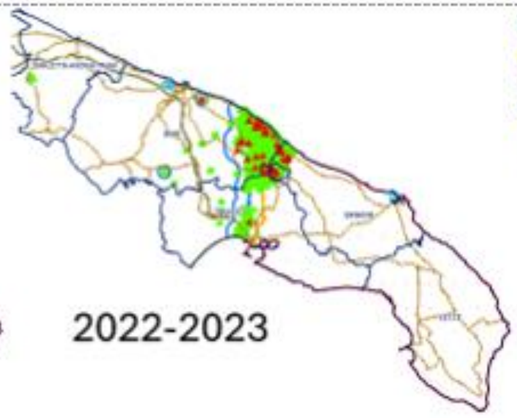
**Green: negatives**

**Red: positives**

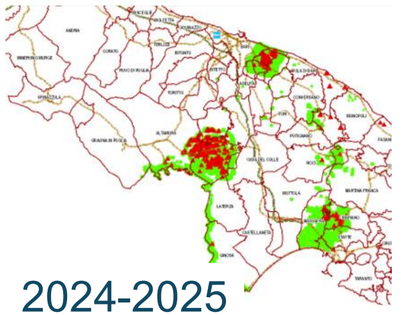
(Yellow: visual inspection)


2020-2021


2021-2022

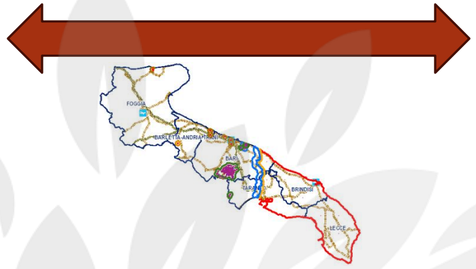

2022-2023

2023-2024

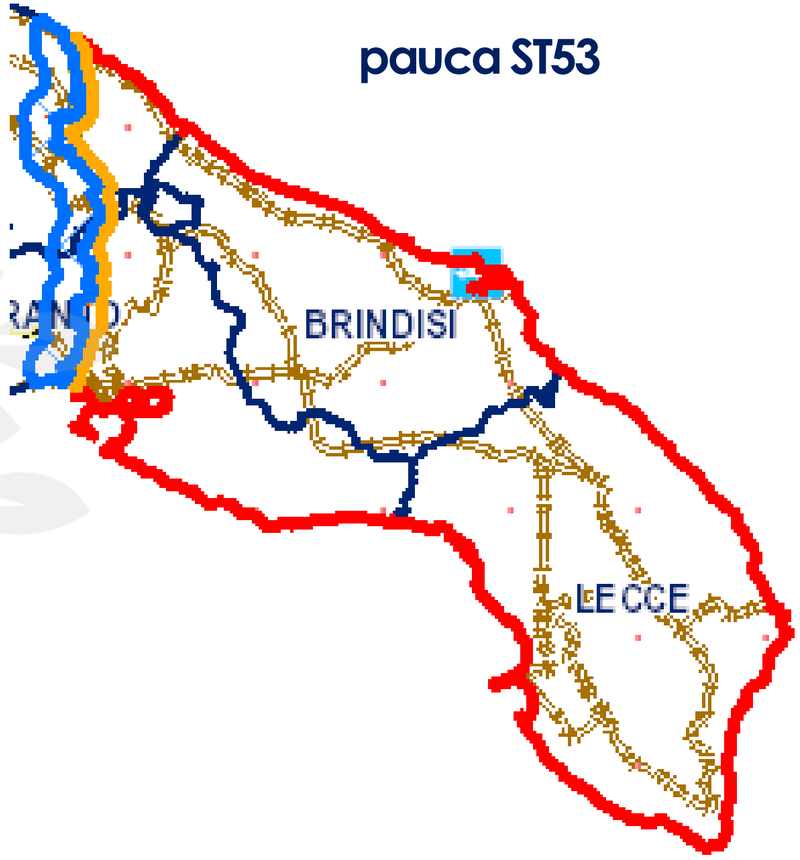

2024-2025

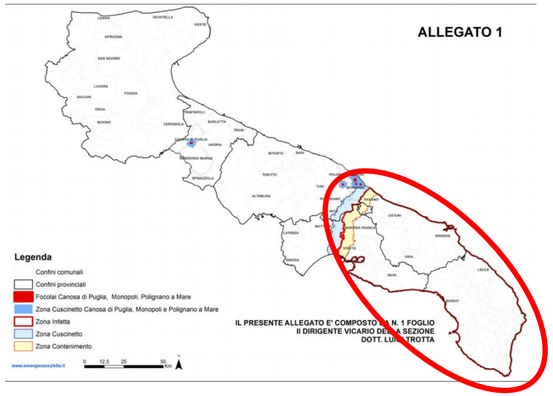
# TWO SCENARIOS


## UNDER ERADICATION



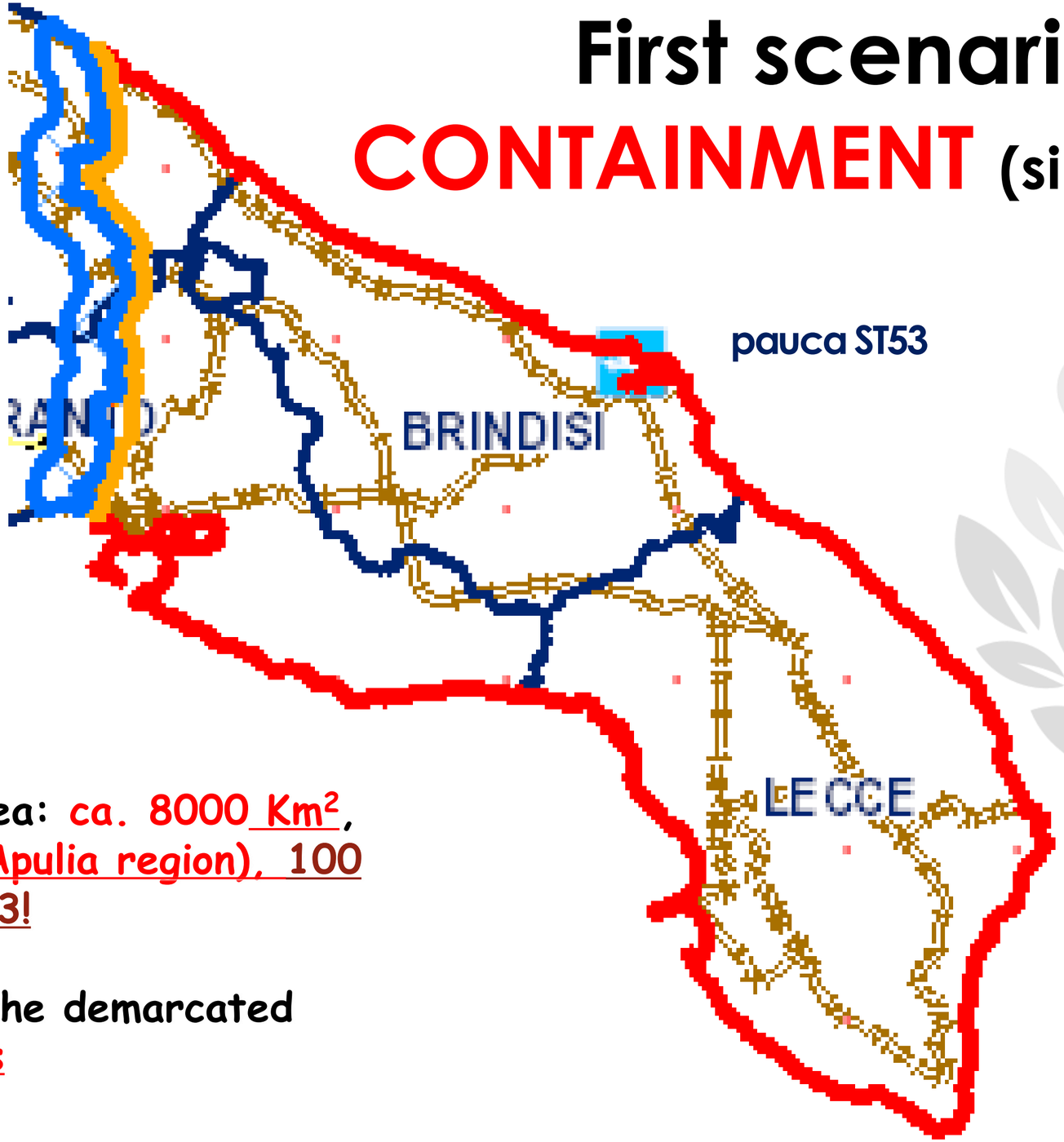
## UNDER CONTAINMENT






# First scenario

## CONTAINMENT (since 2015)




October 2013

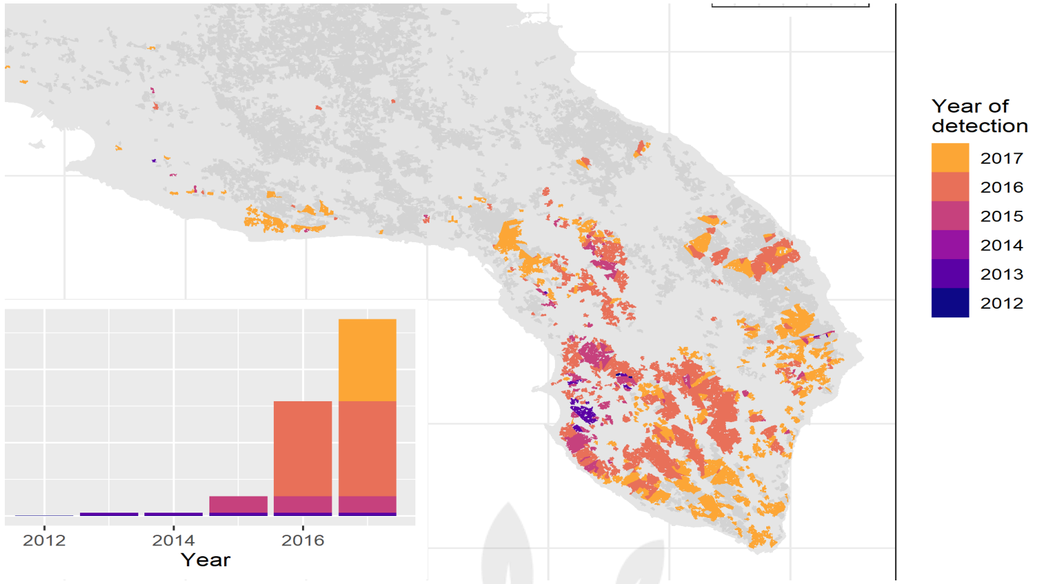
Actual demarcated area: ca. 8000 Km², 160 Km (40% of the Apulia region), 100 times bigger than 2013!

Nr. of olive trees in the demarcated area: over 25 millions

# SUBSP. PAUCA: IMPACT PARTICULARLY SEVERE

**SEVERAL MILLIONS OF TREES**
DEAD OR SEVERELY COMPROMISED

A TOTAL **LOSS OF 132 MILLION EUROS/YEAR** OF PROFITABILITY AND A TOTAL LOSS OF 1,050,000 H OF WORK/YEAR (*CALDERONI ET AL., 2025*)



# Xylella could cost Europe €20 billion, Wageningen University-led study finds

Researchers say the economic costs of Xylella could run to more than €20 billion.

RESEARCH ARTICLE

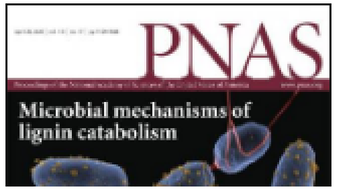
## Impact of *Xylella fastidiosa* subspecies *pauca* in European olives

Kevin Schneider, Wopke van der Werf, Martina Cendoya, Monique Mourits, Juan A. Navas-Cortés, Antonio Vicent, and Alfons Oude Lansink

PNAS April 28, 2020 117 (17) 9250-9259; first published April 13, 2020 https://doi.org/10.1073/pnas.1912206117

Edited by Charles Perrings, Arizona State University, Tempe, AZ, and accepted by Editorial Board Member Simon A. Levin March 3, 2020 (received for review July 16, 2019)



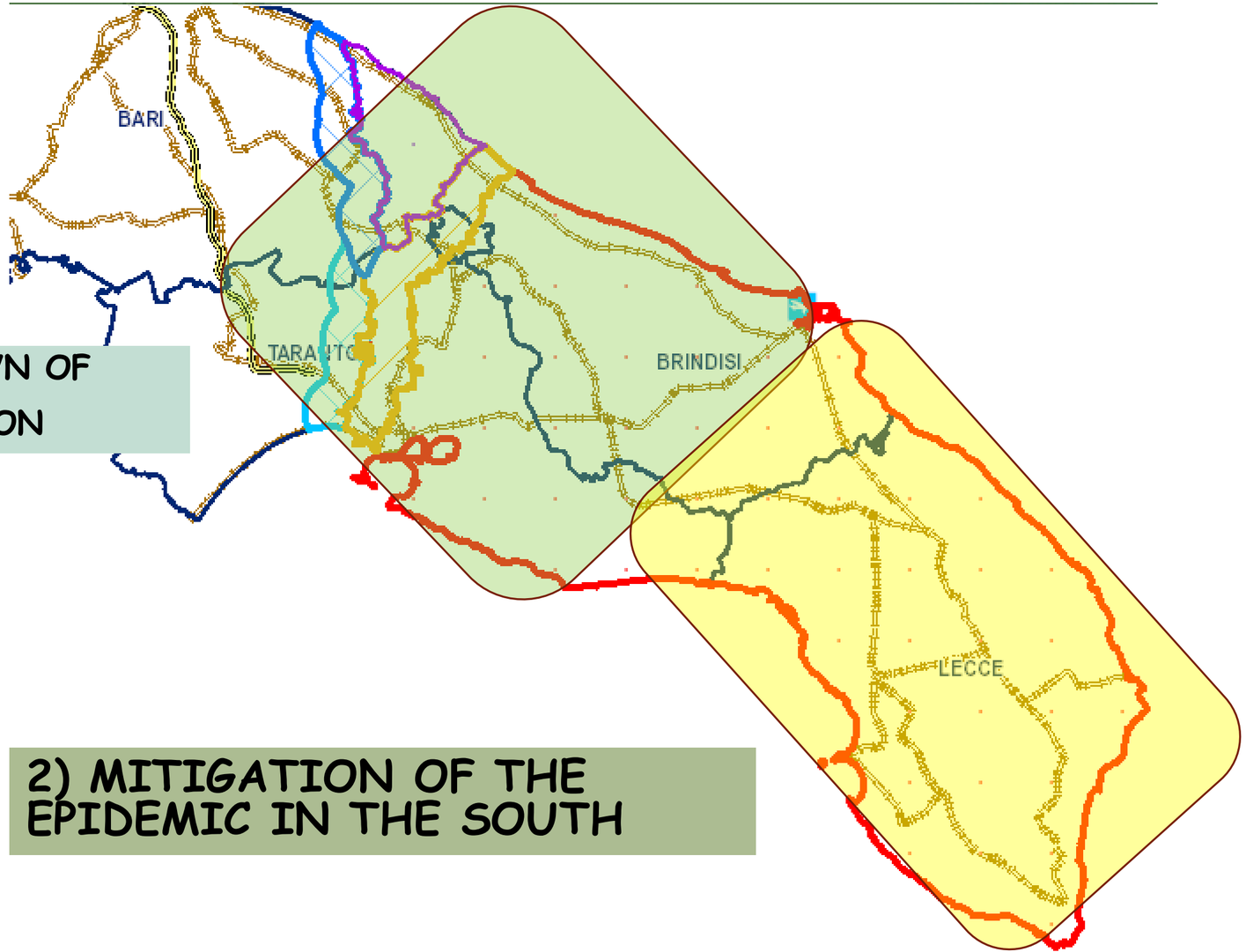


# In addition:

- Loss of land value
- impact on the attractiveness of tourist facilities
- impact on the climate
- growing phenomenon of abandonment
- increase in summer fires
- Etc.

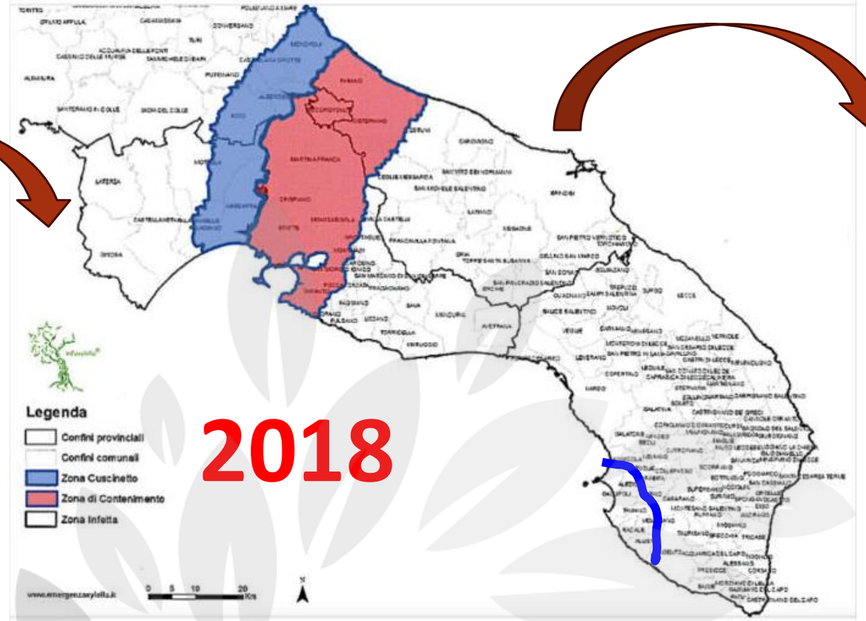
# THE CURRENT STATUS:
# TWO DIFFERENT SCENARIOS

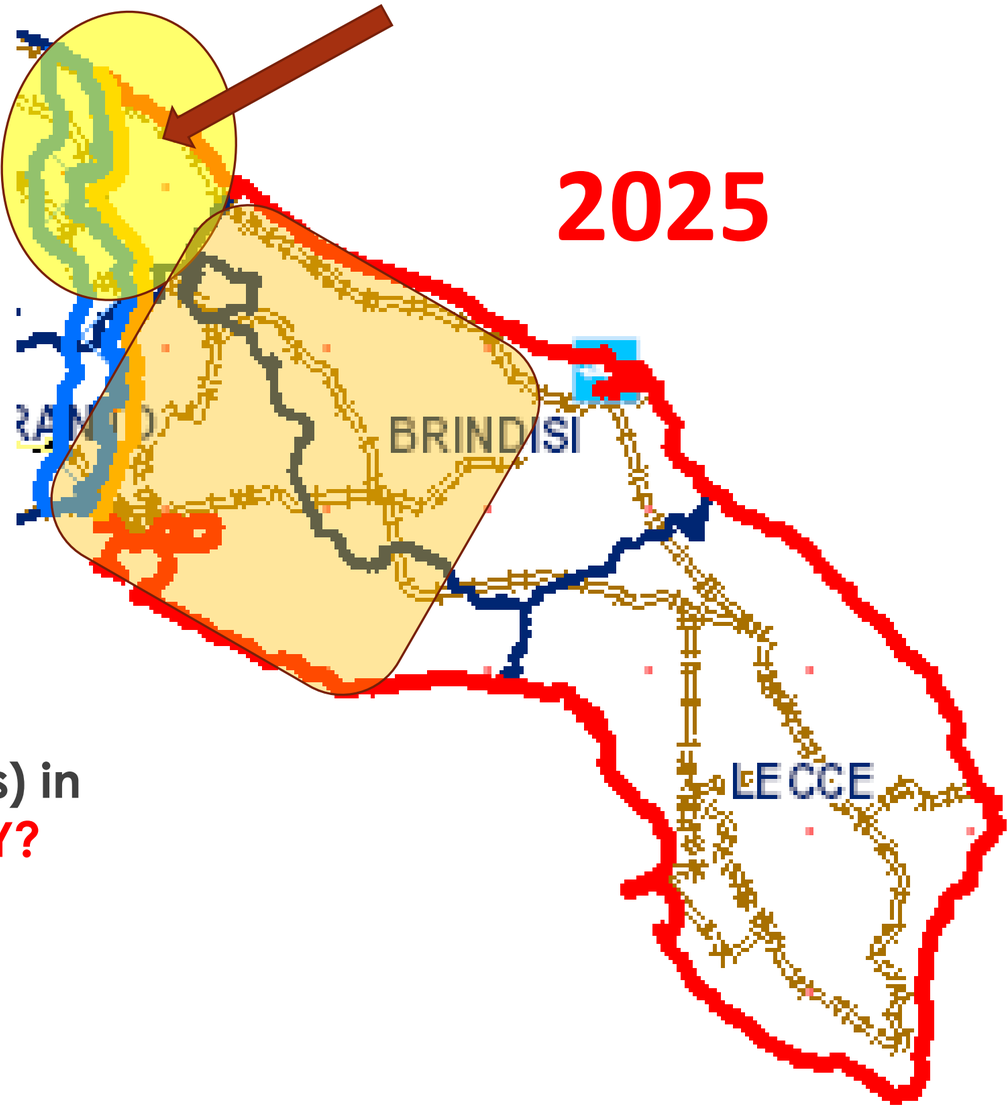
1) SIGNIFICANT SLOW DOWN OF THE NORTHWARD DIFFUSION




2) MITIGATION OF THE EPIDEMIC IN THE SOUTH

# After 2018 significant slowdown in spread


Even the spread over short distances (new outbreaks) in the north is much slower than it was in the south: **WHY?**

# SLOW DOWN OF THE NORTHWARD DIFFUSION: THE REASONS

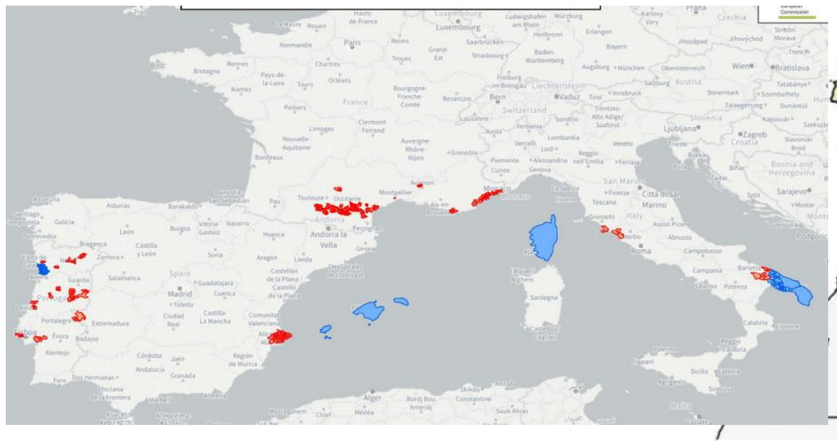
- **Less suitable CLIMATE**
- Different land management (differences in agronomic and phytosanitary practices)
- APPLICATION OF CONTAINMENT MEASURES (monitoring and removal of infected plants, vector control) WHICH HAVE THE OBJECTIVE OF SLOWING DOWN THE SPREAD
- Less abundant VECTOR POPULATION

**Estimated climatic suitability map for X. fastidiosa subsp. pauca** according to a SDM ensemble model (EFSA 2019)

# Estimated climatic suitability map for *X. fastidiosa* according to the SDM ensemble model (PRA EFSA, 2019)

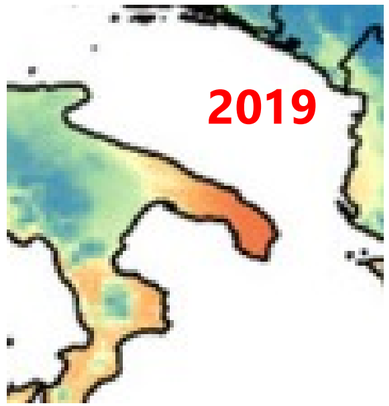
**The climate: a key factor for the epidemioligy of Xf**

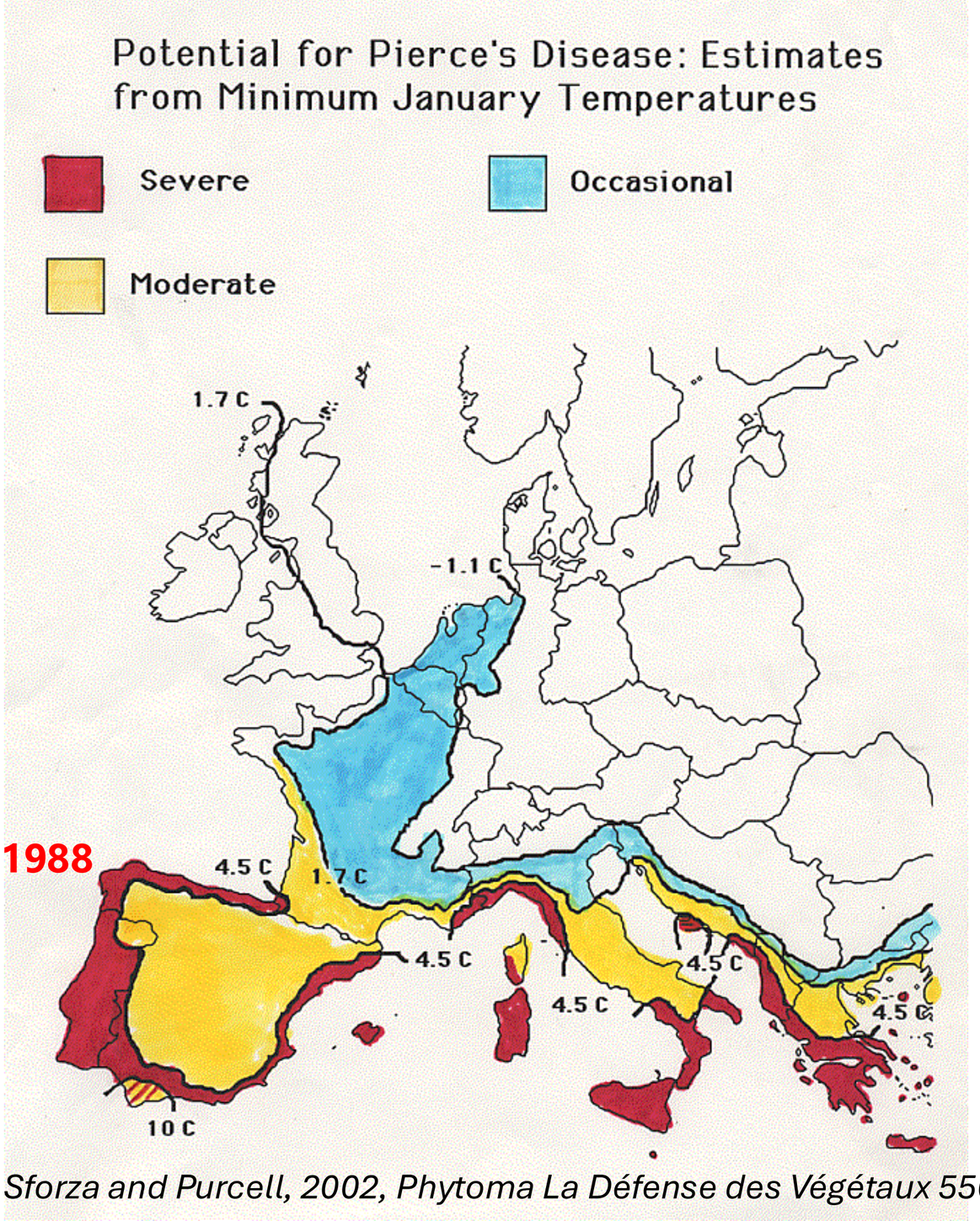
**All the foci discovered so far fall in areas with high/moderate climatic suitability**



Prof. H.
Alexander Purcell

*"**I created this map for a talk that I gave in a symposium in Budapest (in1988?)***
*The article in Phytoma (France) was its first official publication, but it was on the Internet before that."*



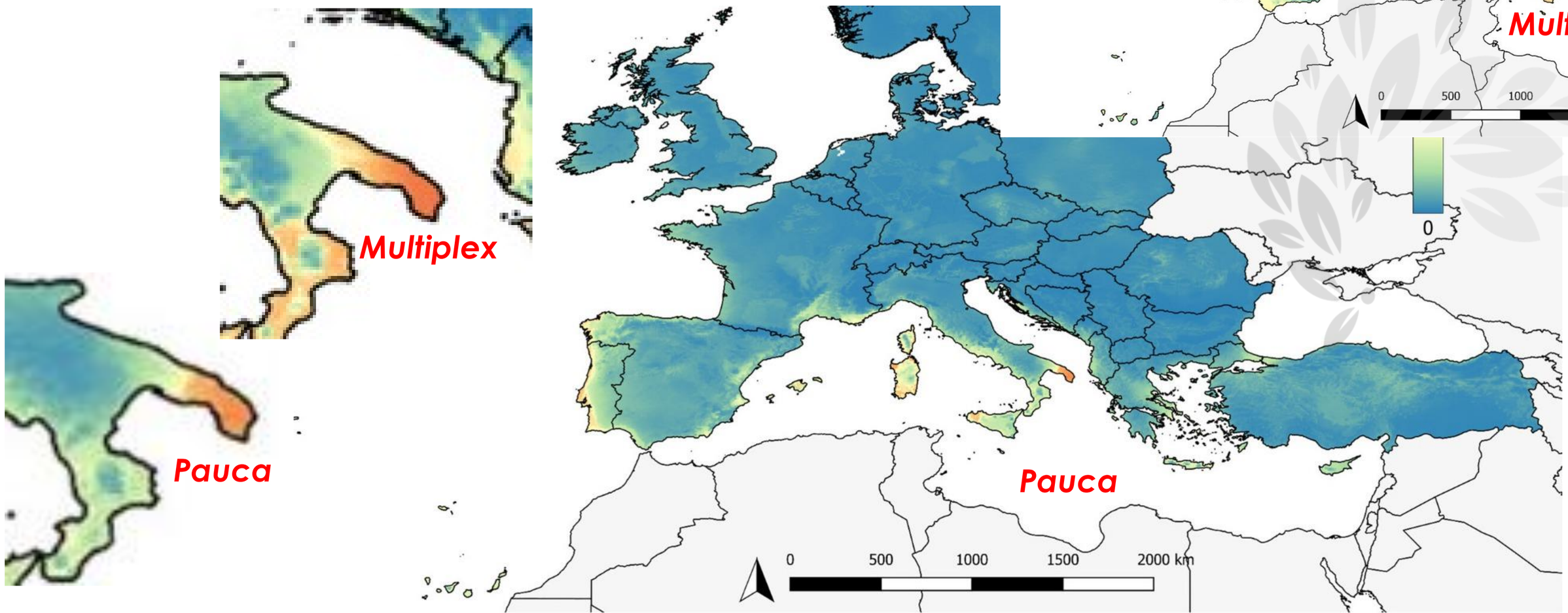




*Sforza and Purcell, 2002, Phytoma La Défense des Végétaux 550:10-14*

# The climatic suitability varies among subspecies

***DIFFERENCES AMONG SUBSPECIES (AND STRAINS) AFFECTS NORTHER BOUNDARIES***

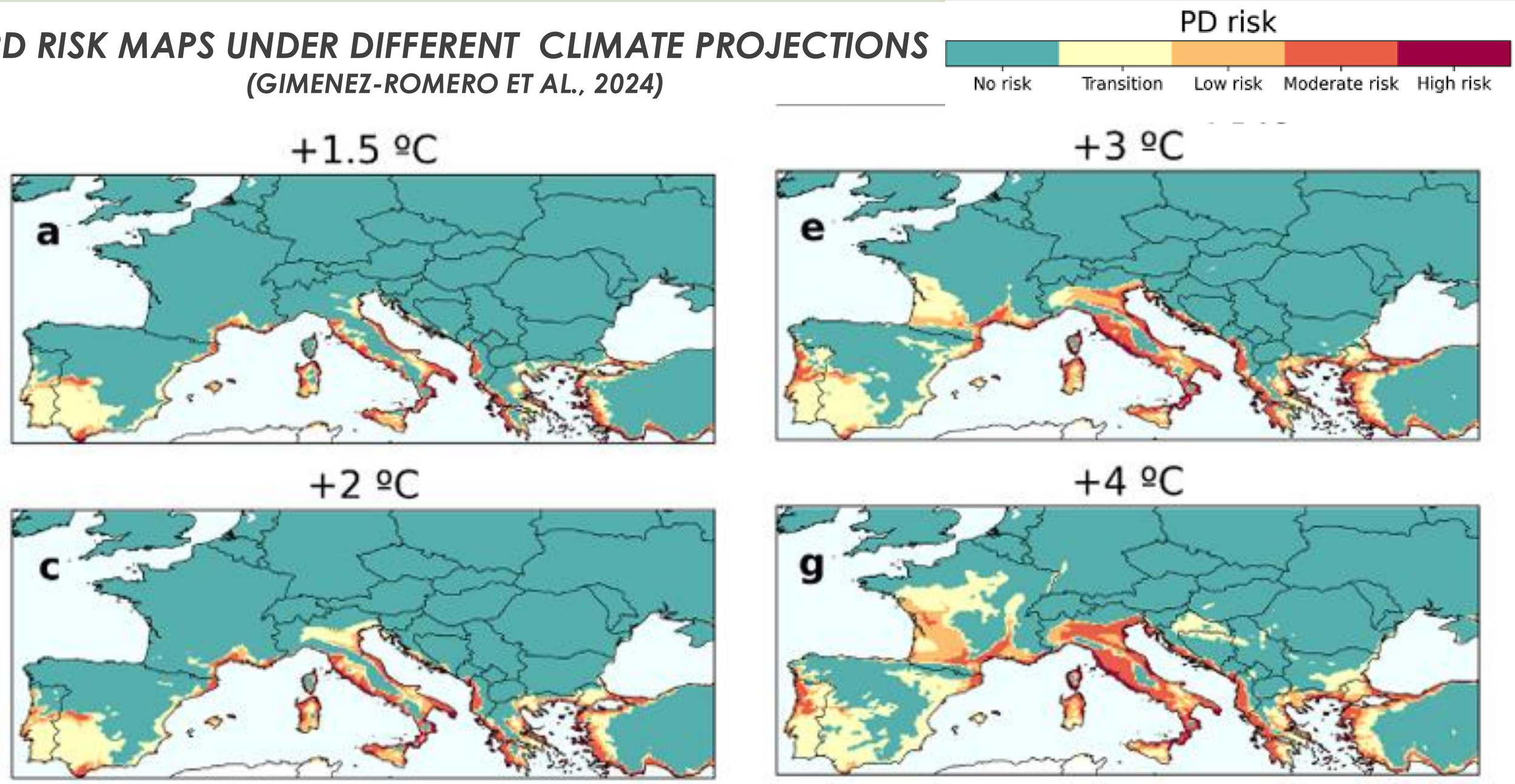
**I.E.: *PAUCA* IS MORE WARM-LOVING THAN *MULTIPLEX* (EFSA, 2019)**



# The effect of the climate change

***PD RISK MAPS UNDER DIFFERENT CLIMATE PROJECTIONS***

***(GIMENEZ-ROMERO ET AL., 2024)***



# SLOW DOWN OF THE NORTHWARD DIFFUSION: THE REASONS

- Less suitable CLIMATE
- **Different land management (differences in agronomic and phytosanitary practices)**
- **APPLICATION OF CONTAINMENT MEASURES (monitoring and removal of infected plants, vector control) WHICH HAVE THE OBJECTIVE OF SLOWING DOWN THE SPREAD**
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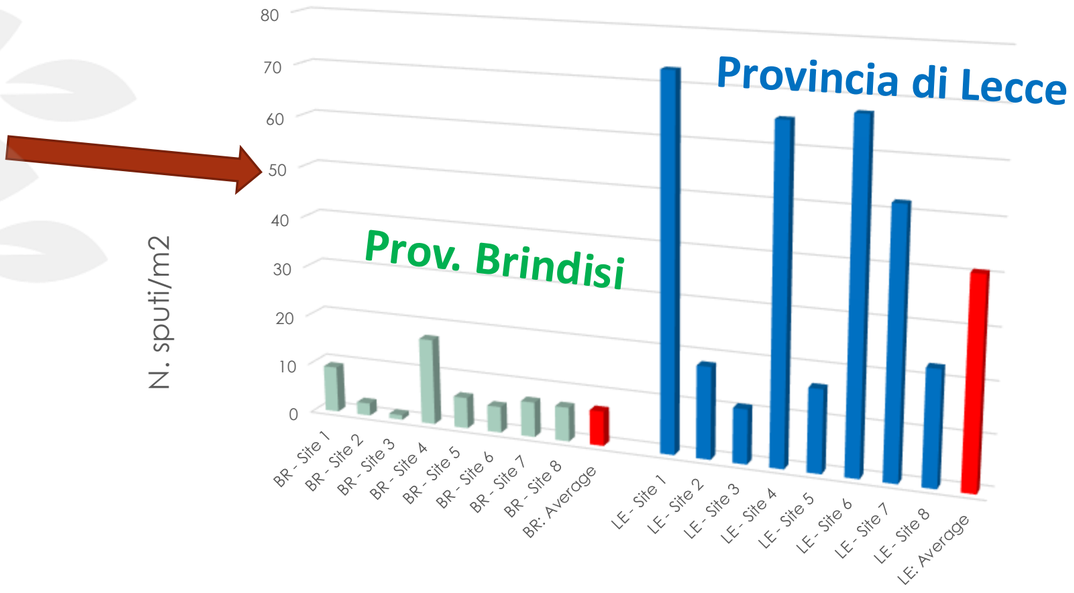
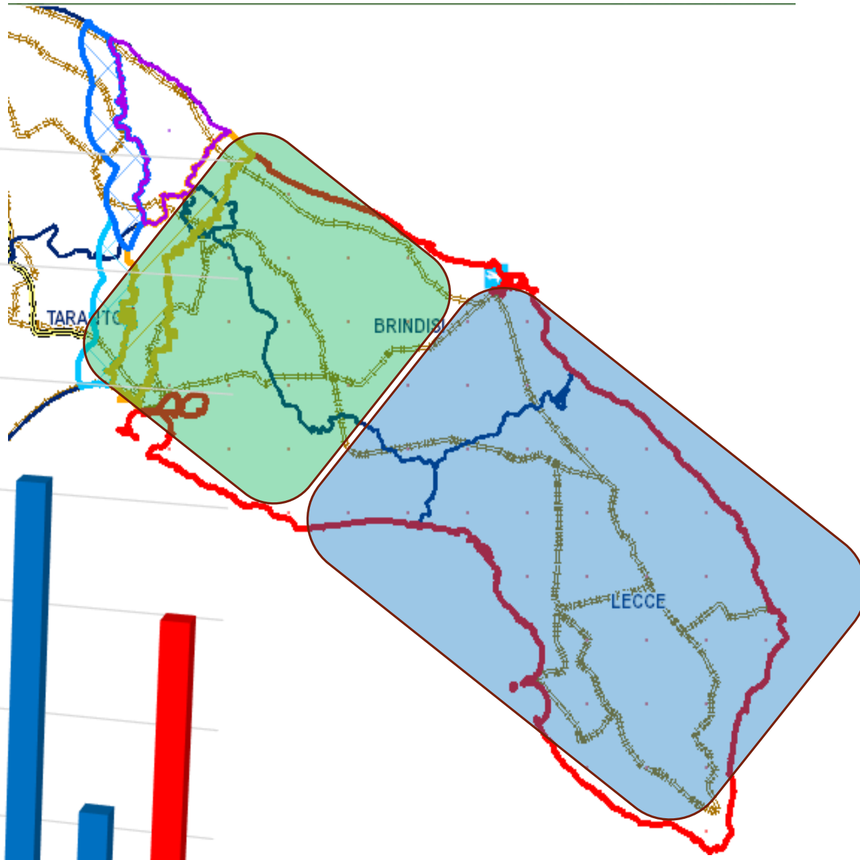


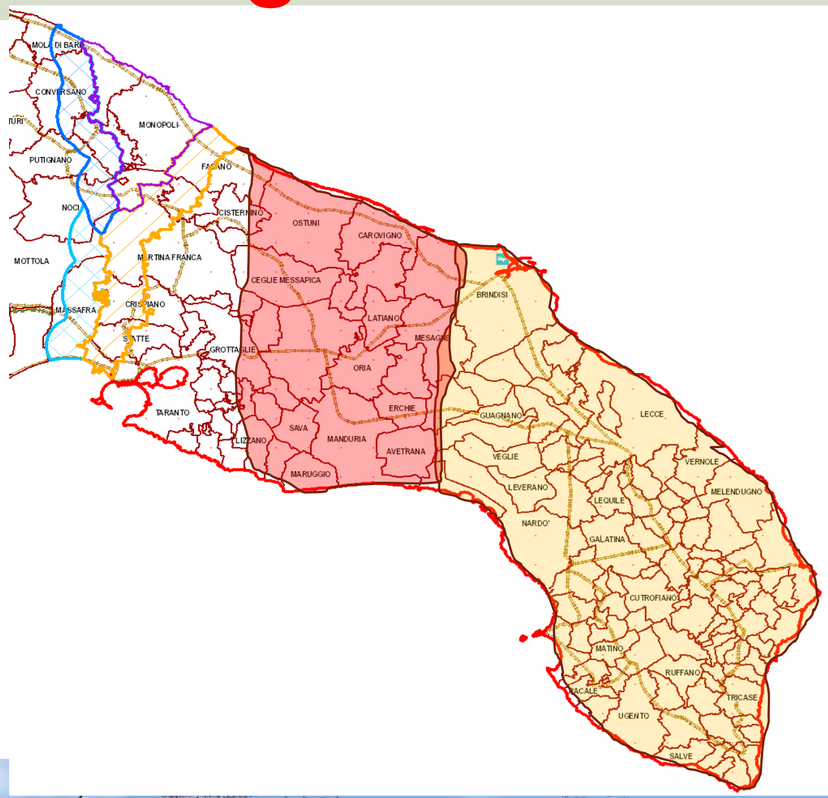
**Population density of juvenile forms of Ps in 8 southern (blue) and 8 northern (green) sites. Data (n. individuals/m2) collected in spring 2023 when the peak of stage 4-5 of juvenile forms was reached. In red the mean**



# CONCLUSIONS

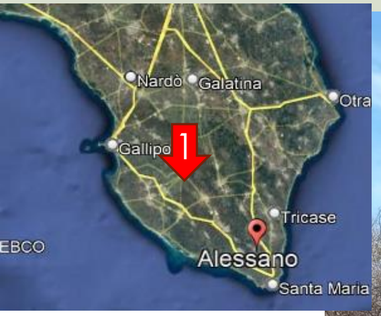
- A notable slow down of the northward diffusion has been observed in the past years
- Several factors seem to contribute to this matter
- A relevant role seems to play the lower vector population abundancy and the different climatic suitability in comparison with the southern part of the Salento

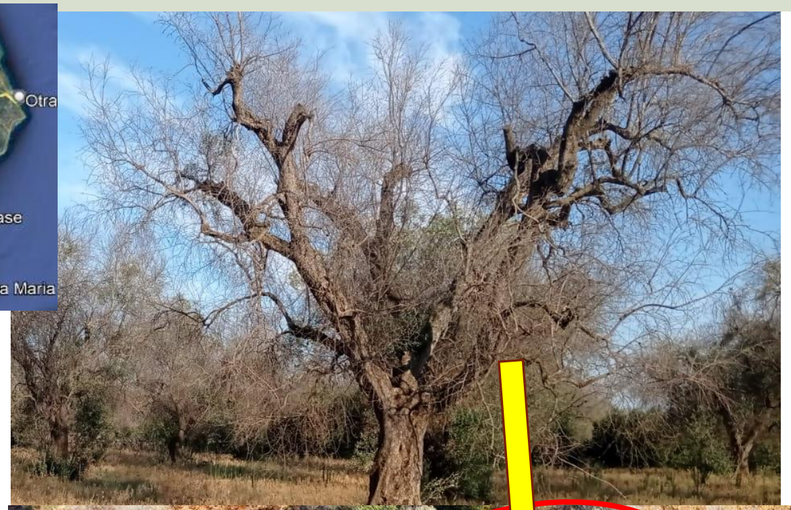
# Mitigation of the epidemic in the south (South of Brindisi)




- **New symptoms of dessication are milder and less frequent (see yellow area)**
- in the last 3-4 years an increasing number of reports of symptoms remittance in survived trees has been recorded in adult trees (age up to 60-70 years old, not century old), more evident in cv. "Cellina" than in "Ogliarola"
- **On the contrary, in the northern territory (red area) the disease is active and turning to the worst**

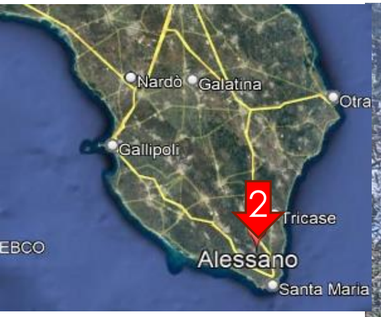
# Few examples



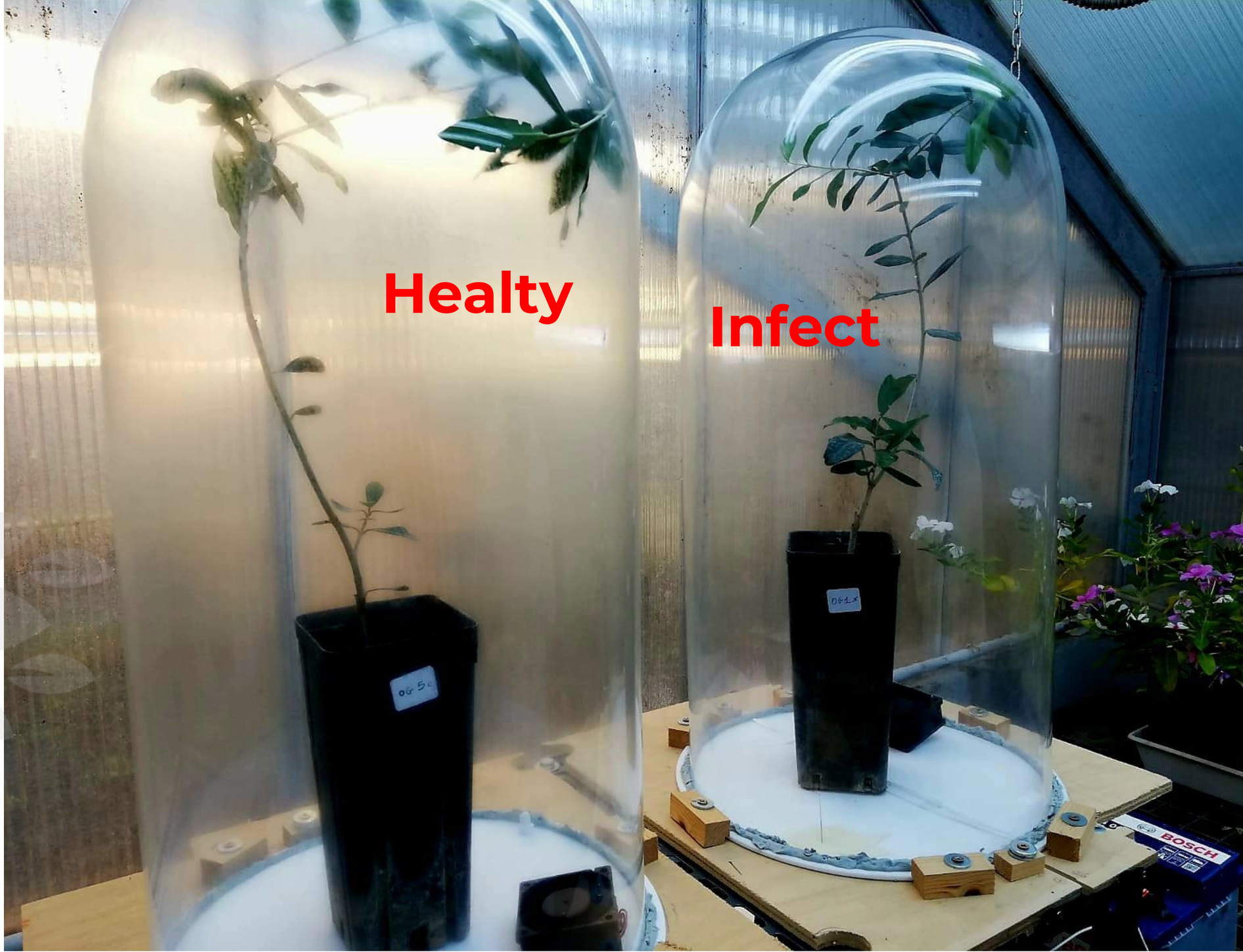
# Cv. Cellina di Nardò


# Cv. Cellina di Nardò




"Partial improvement" is not synonymous with "recovery“

**Reduced transpiration**



# IN RESISTANT GERMPLASM THE BENEFICIAL EFFECTS SEEM TO BE HIGHER

Leccino (resistant), Ugento (le), august 16, 2023

# What has changed?

Occurrence of relevant mutations in the *Xf* population?

As 12 years ago, all new isolated strains harbor ST53:

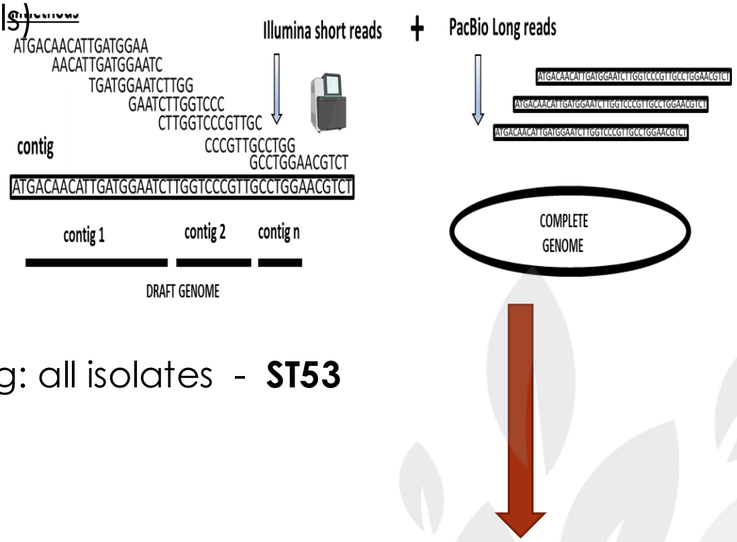
NO EMERGENCE OF NEW/DIFFERENT "ST" REGISTERED SO FAR.

NO EVIDENCES OF RELEVANT GENETIC MUTATIONS

# NO EVIDENCES OF RELEVANT GENETIC MUTATIONS

***Changes in the bacterial genome?***


NGS: on a very limited n. of isolates (1 whole & 3 draft genomes reconstructed using different tools)




MLST typing: all isolates - **ST53**

- ✓ **Mapping the newly recovered whole genome - reads (13,725,672) versus De Donno reference: 99,54% mapped with homogeneous coverage**

So far, the only difference found with the reference strain «De Donno» is a deletion of 38nt repeat sequence

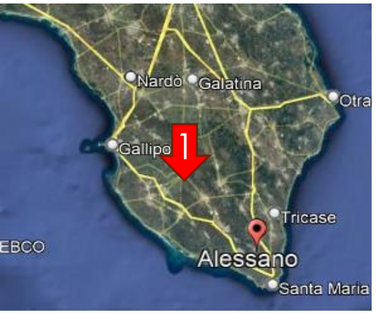
**However it corresponds to an intergenic region >>> no effect on gene function!**

# Wath has changed?

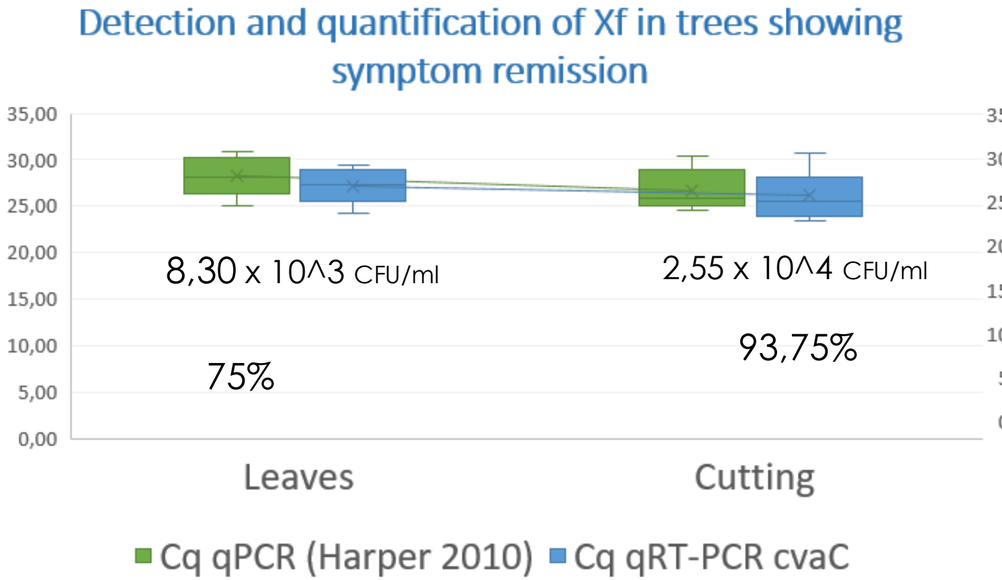
Reduction of bacteral titer in infected plants?

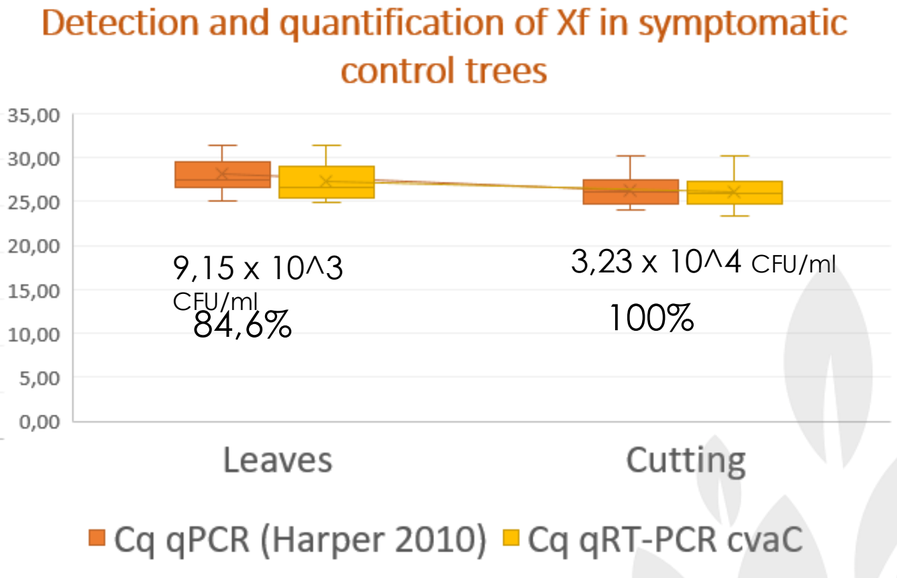
NO EVIDENCES OF SIGNIFICANT REDUCTION

# MONITORING THE XF-STATUS OF THE PLANTS




**Sampling and testing a representative n. of trees: standard detection & estimation of the bacterial multiplication rate by quantification of the bacterial RNA (encoding a microcin-like protein highly expressed in infected trees)**




# Drastic reduction of reservoir of inoculum: the reasons

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**Significant removal of severely damaged olive groves, often replaced (over 3 milions of plants) with resistant cultivars (low bacterium titre when infected)**

# Drastic reduction of reservoir of inoculum: the reasons

## High frequency of fires in olive groves

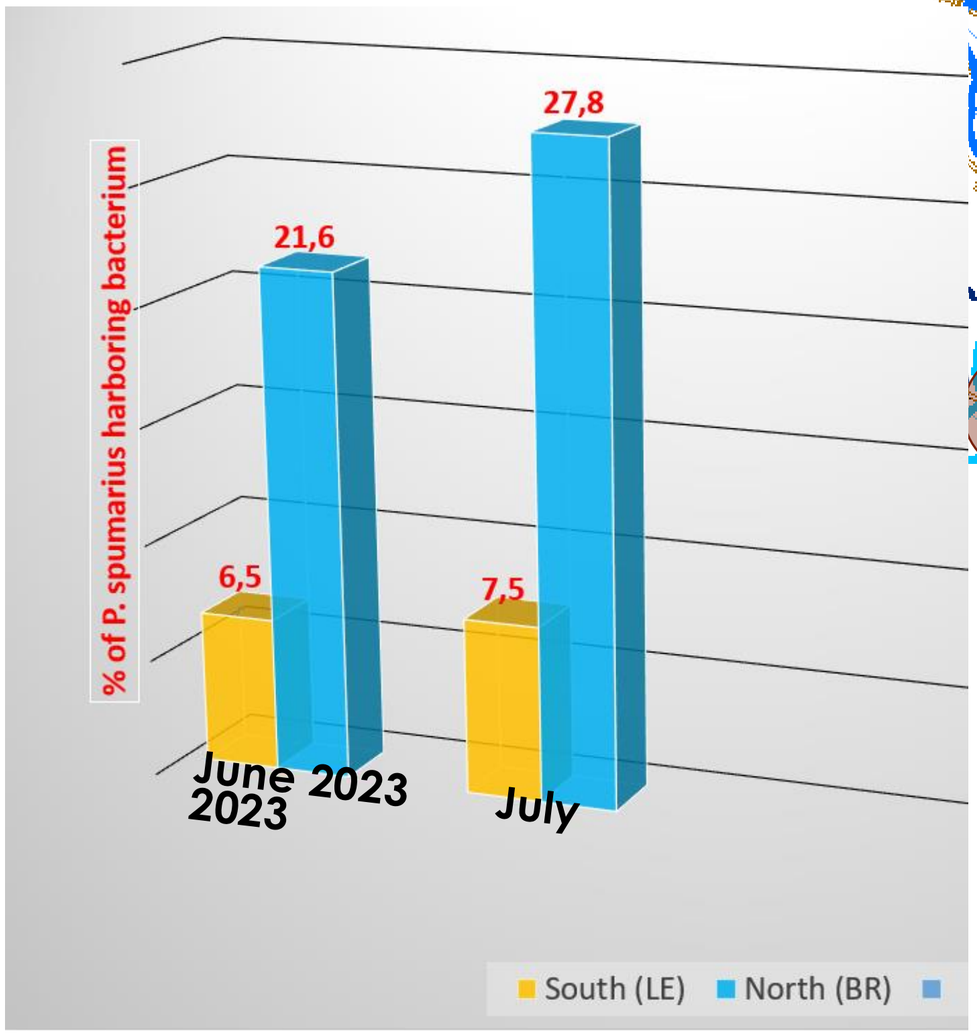
# Drastic reduction of reservoir of inoculum: the reasons

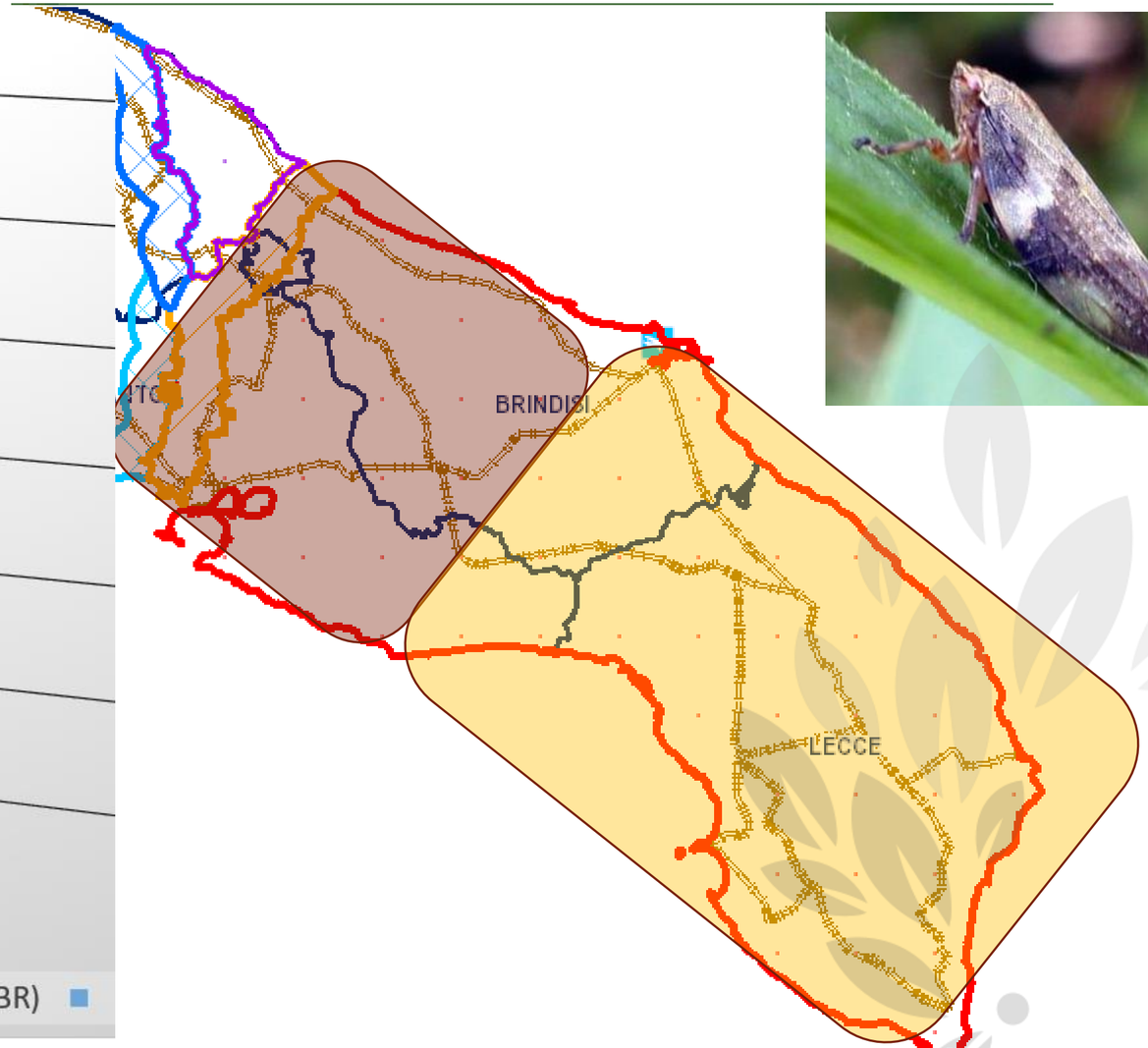
- Massal reduction of vegetation with high concentration of bacterium due to the infection
- Significant removal of severely damaged olive groves, often replaced (over 3 milions of plants) with resistant cultivars (low bacterium titre when infected)
- High frequency of fires in olive groves
- **Proibition of planting higly susceptible cultivars**

**Consequences: Drastic reduction of infected vectors**

# Drastic reduction of infected vectors

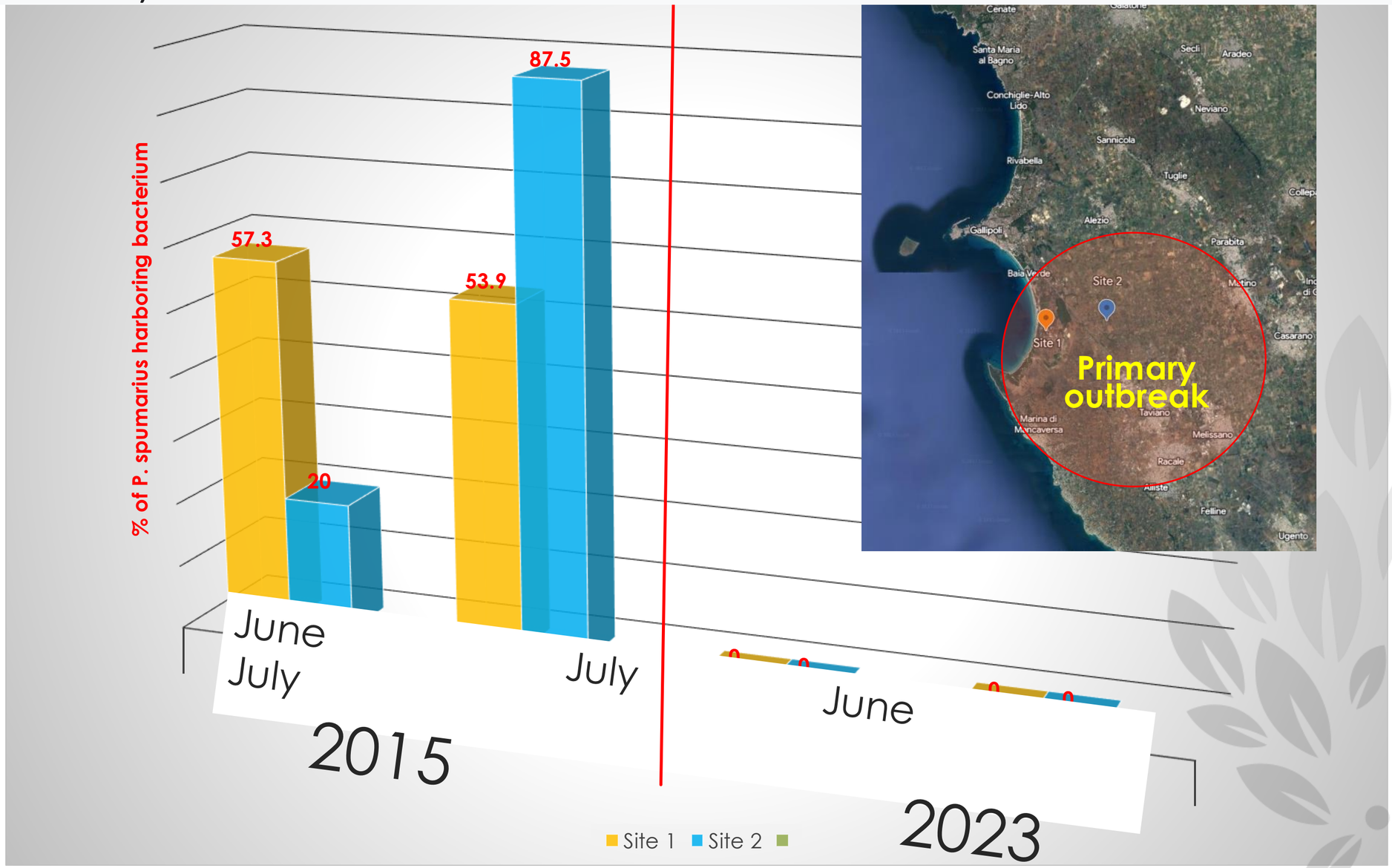
**Comparison of % of infected vectors (qT-PCR) between southern (6 sites, 173 specimens) and northern (5 sites, 141 specimens) areas of the infected zone**



# Drastic reduction of infected vectors

Comparison of the percentage of positive vectors in different years in two sites close to the primary outbreak (80 specimens in 2023)



# Mitigation of the epidemic in the south

## CONCLUSIONS

Reservoir of inoculum drastically reduced!

↓

Vectors efficency dropped

↓

Strong reduction of superinfections

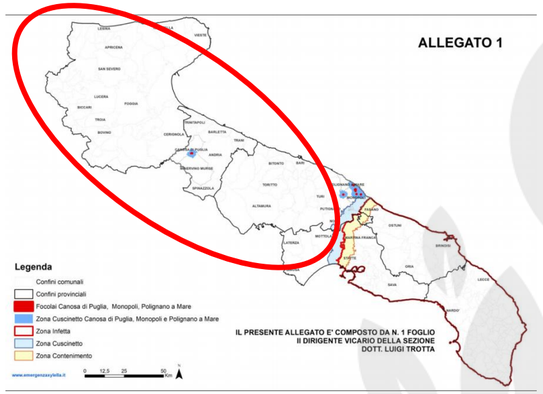
In the mainwhile:

1) no significant mutations are recorded in the bacterial population (still only ST53)
2) the titre of bacterium in infected plants does not show significant reduction in sensitive cultivars

## MAJOR ROLE OF THE SUPERINFECTIONS?

# Second scenario: ERADICATION

## (2024-2025)

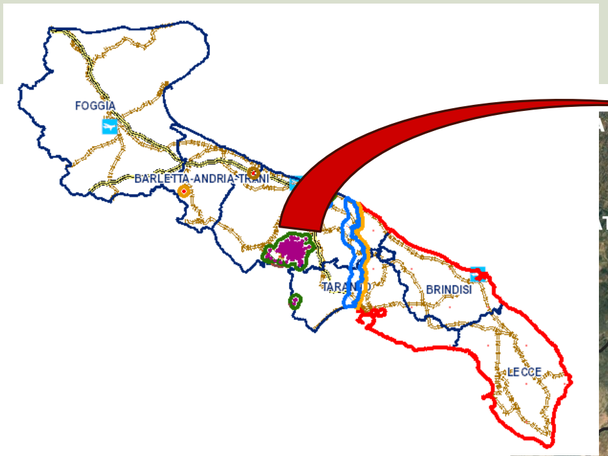




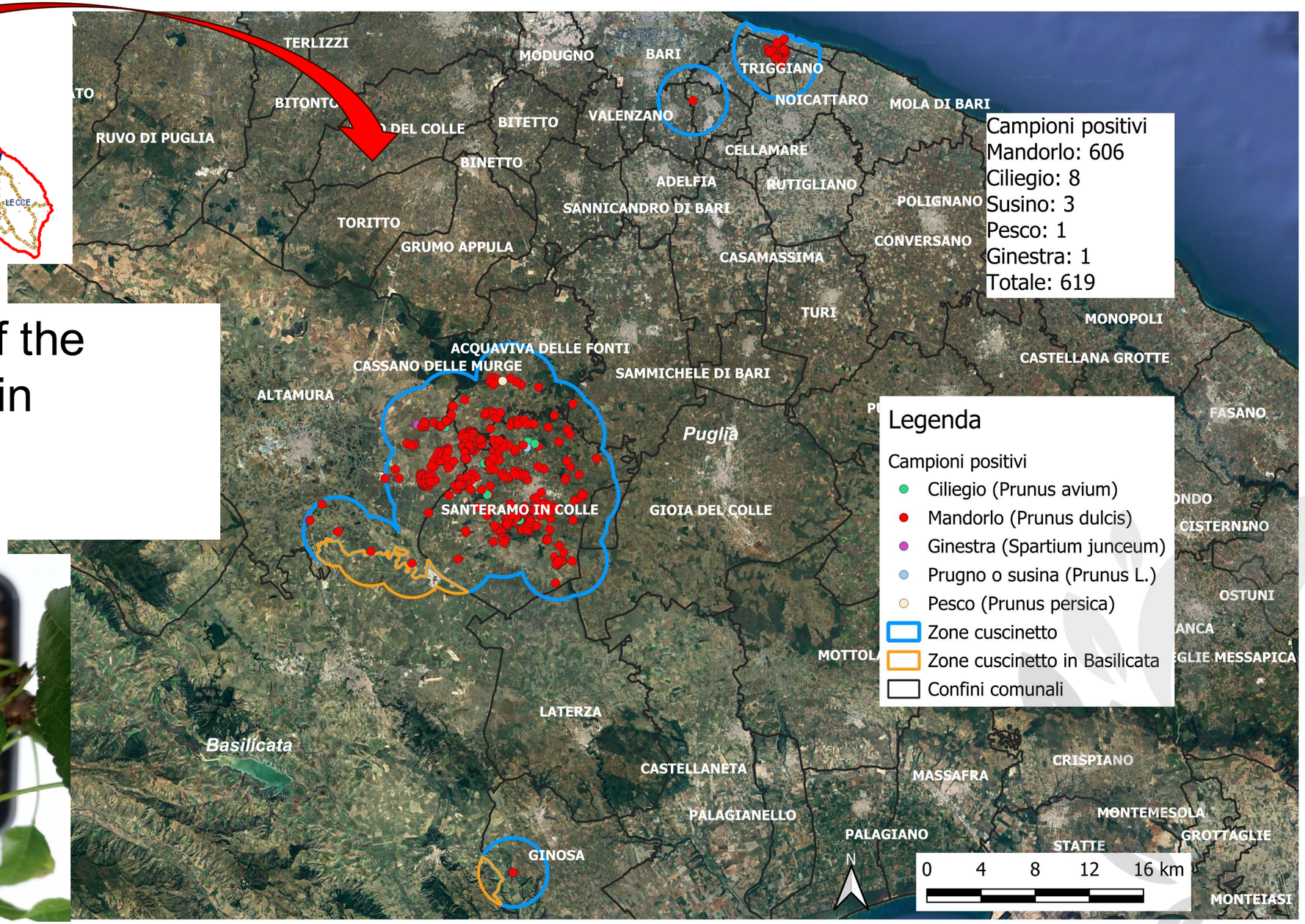
The requirements of the current **EU Regulation** for the control of X. fastidiosa are equally strict **regardless of the subspecies and the impact** of the strain present in a given area.

# multiplex

# First case of ST26 in EU



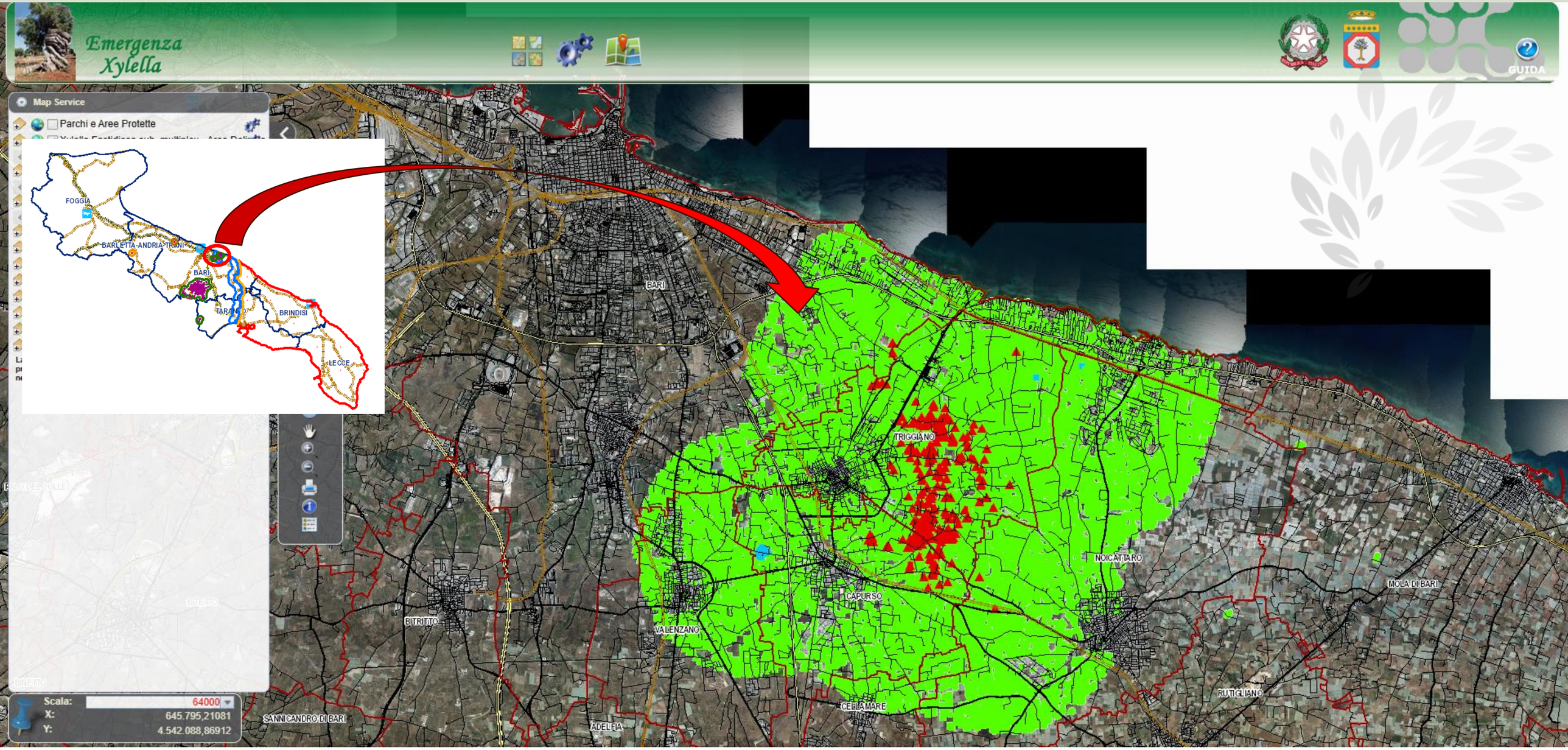

The delimitation of the boundaries is still in progress



# 18 MONTHS OF MONITORING OF MULTIPLEX

- the impact of bacterial infections appears to be negligible; symptoms, if any, are not distinguishable from those of drought, considered physiological in the NON irrigated almonds of this region
- the outbreak was discovered only as a consequence of the surveillance program, but so far in Puglia no one has reported associated diseases
- Beside the almond, the risk of infections of other «specified» plants seems to be very low
- The boundaries of the affected area are not yet identified (suspicion of old introduction and widespread presence)
- Severe impact of the eradication program (regulated as «mandatory»)

# *Xf fastidiosa* ST1 (ca. 8 Km²)



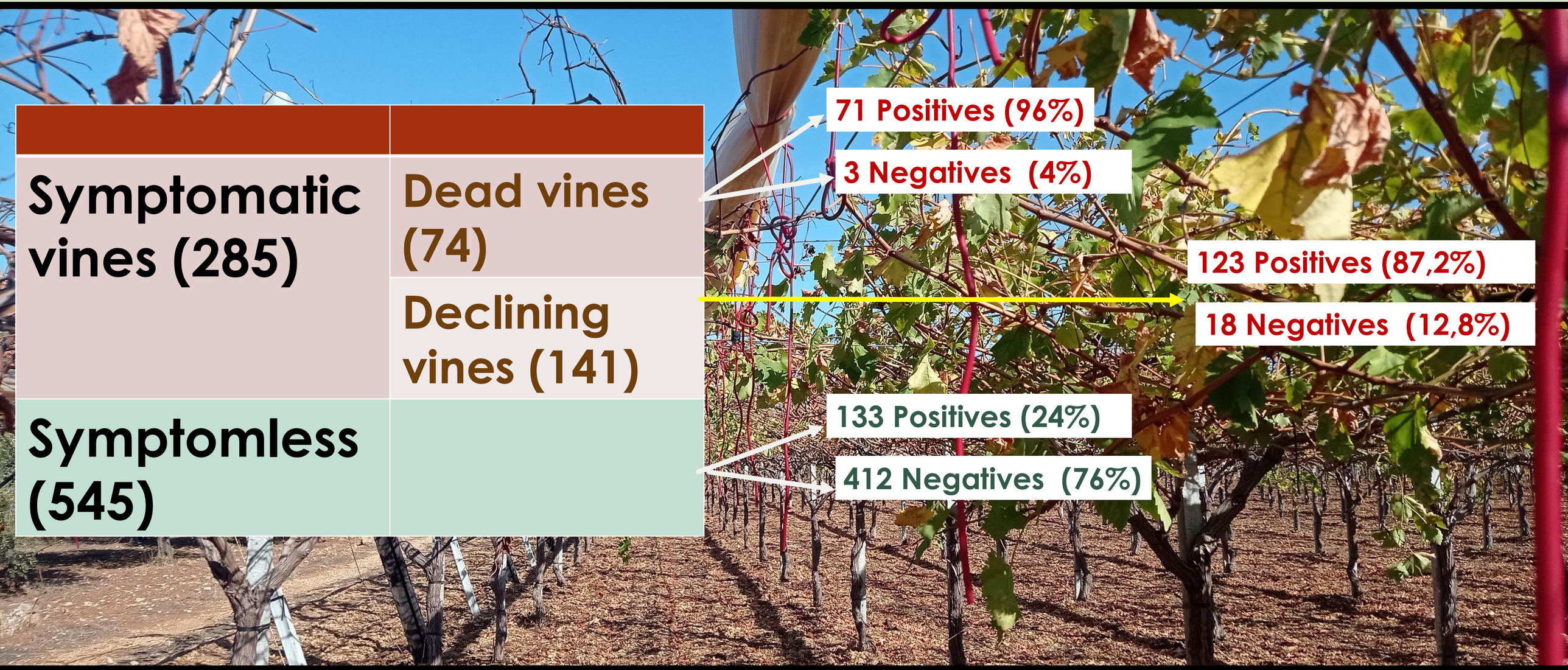
# 20 months of monitoring «fastidiosa»

- **Almond and Grapevine the major hosts**
- **Impact negligible on almond, to be defined in grape (major differences among cvs.)**

## One example of susceptible Cv.: Superior 4 years old

Xf Positive ↓ Negative ↓

# In dept analyses in one vineyard of cv. Superior (4 y old):



| | |
|---|---|
| Symptomatic vines (285) | Dead vines (74) |
| | Declining vines (141) |
| Symptomless (545) | |

71 Positives (96%)

3 Negatives (4%)

123 Positives (87,2%)

18 Negatives (12,8%)

133 Positives (24%)

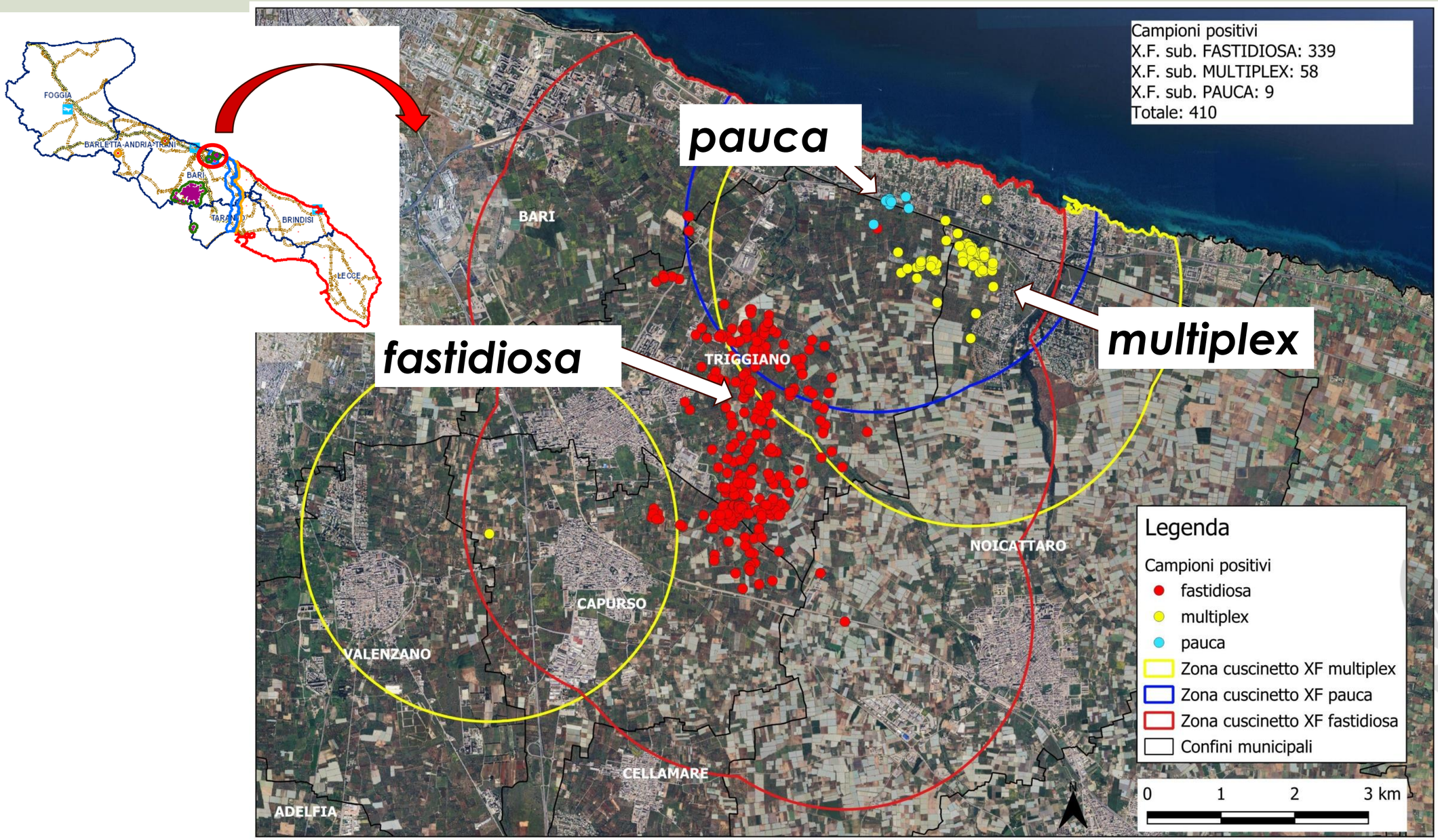
412 Negatives (76%)

# *X. fastidiosa*: The same species includes different pathogens

**different diseases, host plants and impact**

- ***Pauca***
- ***Multiplex***
- ***Fastidiosa***

# A complex picture (few Km from Bari)




REGIONE PUGLIA

# NOT ALL BAD NEWS ARE NECESSARILY BAD (?)

Evidence suggests that **competition between strains may modulate disease prevalence** by influencing strain dominance in both hosts and vectors

i.e.: In the southern part of the island of Mallorca, where **the presence of multiplex (ST81) is higher**, the **incidence of Pierce's disease is lower** than in the rest of the island.

# CONCLUSIONS: major changes since 2013

**The complexity of the situation suggests the adoption of targeted and case-by-case adapted management measures, rather than a one-size-fits-all approach:**

**Is it time to consider revising the EU regulation?**

# THANK YOU!

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RIGENESI