The Italian experience with *Pantoea stewartii* subsp. *stewartii*: Genomics, Diagnostics, and Insect-Mediated Transmission

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Pantoea stewartii subsp. *stewartii* (Pss)

- ✓ Pss causes **stewart's wilt of maize and bronzing disease of jackfruit**
- ✓ In Europe, Stewart's wilt has been reported
- ✓ Pss is a quarantine pest in the **EPPO A2 list**
- ✓ Pss is **Quarantine** pest [(EU) 2019/2072 Annex II A]

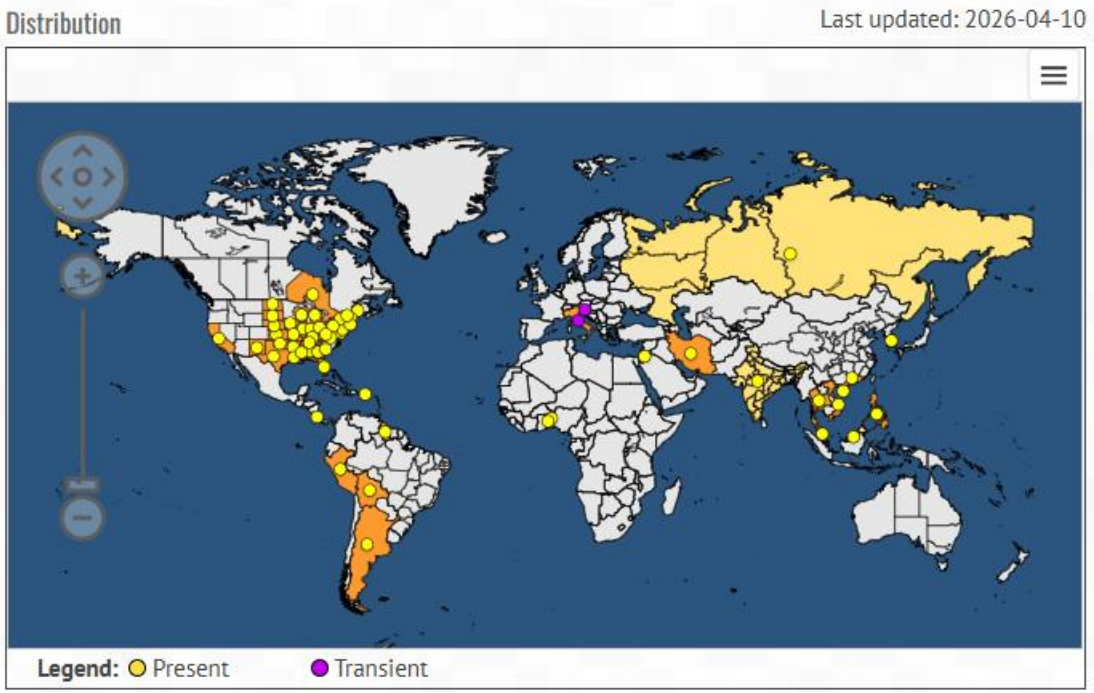
- ✓ Disease symptoms: wilt and leaf blight
- ✓ There are no known asymptomatic infections of corn plants, except for seed infections
- ✓ Two major cycles of infection:
  - **young seedling** stage that leads to rapid **wilting** and death of the plant
  - **mature plants** phase resulting primarily in **vascular chlorosis and necrosis with little wilting**

bacterial leaf blight

bacterial wilt of maize

P. Müller, JKI (DE), PM 7/60(2) Bulletin (2016) 46 (2), 226–236; http://www.apsnet.org/edcenter/intropp/lessons/prokaryotes/Pages/StewartWilt.aspx

Pantoea stewartii subsp. *stewartii* (Pss)



- ✓ Pss is indigenous of **North America**
- ✓ Vectored in the Americas by ***Chaetocnema pulicaria***
- ✓ **Spread** to other parts of the world with maize **seeds trade**
- ✓ In Italy and Slovenia is transient - could this be due to the **absence of a suitable insect vector**, preventing stable establishment? -

Bullet points

- ✓ Phytosanitary restriction and phytosanitary certification for maize seeds Pss free. Stewart's wilt is a **major issue** when **seeds** are destinated for **export**
- ✓ Different tests available for Pss detection: false-positive can occasionally occur with *P. stewartii* subsp. *indologenes,* a non quarantine bacteria pathogen (i.e. Tambog test)

Chaetocnema pulicaria

Pantoea stewartii subsp. *stewartii* (Pss) in Italy

- ✓ The Italian Plant Protection Services carried out the **surveillance of the Italian territory**
- ✓ Pss **presence was notified since 2015**
- ✓ The Italian concerned regions are Emilia-Romagna, Friuli Venezia Giulia, Veneto and Lombardia.

https://lepuntinedelmondo.it/

- ✓ In Italy, investigation on some **insects** in maize fields gave **cPCR positive results** (one insect belonged to the genus *Phyllotreta,* as well as some specimens of *Halyomorpha halys*) (EFSA 2019 doi: 10.2903/j.efsa.2019.5851)

Overall Aim and Integrated Research Strategy

National Reference Framework

Building a **collection representative of Italian Pss** isolates using pathological, biochemical, and molecular methods

Diagnostic Development

Developing and **validating real-time PCR test** to detect Pss and differentiate it from related bacterial plant pathogen.
Interlaboratory comparisons to evaluate the performance criteria of real-time PCR tests for seed

Epidemiological Investigation

Studying **insect vectors** for Pss to understand pathogen **transmission** risks

J.K. Pataky, University of Illinois, Urbana (US).

https://www.ilnuovoagricoltore.it/il-mais-puo-aumentare-la-sua-redditivita-ma-occorre-abbandonare-le-consuetudini/

CHARACTERIZATION OF ITALIAN *Pss* ISOLATES

Characterization of Italian PSS Isolates

Reference Collection Inclusion

Italian representative PSS strains are cataloged in the CREA reference collection with standardized documentation ensuring their value as reference material

Pathological Characterization

Inoculation assays on host plants assess symptom development, aggressiveness, and host response for comparing isolates

Biochemical and Molecular Analysis

Biochemical tests confirm phenotypic traits while molecular sequencing enables high-resolution comparison among isolates

Strain number CREA-DC	Sample name	Year	Source / Location
CREA-DC 1775	Pss_1775	Before 2000	Italy
CREA-DC 1788	Pss_1788	Before 2000	Italy
CREA-DC 1869	Pss_1869	2015	Italy
CREA-DC 1870	Pss_1870	2015	Italy
CREA-DC 1899	Pss_1899	2018	Italy
CREA-DC 1900	Pss_1900	2018	Italy
CREA-DC 1990	Pss_1990	2018	Italy
CREA-DC 2061	Pss_2061	2021	Italy
CREA-DC 2123	Pss_2123	2022	Italy

The Pss strains are tested according to the pathogenicity test, biochemical assay (arbutin, aesculin hydrolysis) and conventional PCR according to the PM 7/60 (2) *Pantoea stewartii* subsp. *stewartii*

Genetic Variability and Phylogeny

Comparative genomic analysis identifies conserved regions, lineage markers, and genetic differences related to pathogenicity

- We generated high-quality **complete genomes of Italian Pss strains** collected between 2015 and 2022 and released them as a community resource
- The analyzed genomes show **high similarity in size and gene content**
- Despite being isolated across **different years** and **geographic** locations, **all Italian strains cluster together**

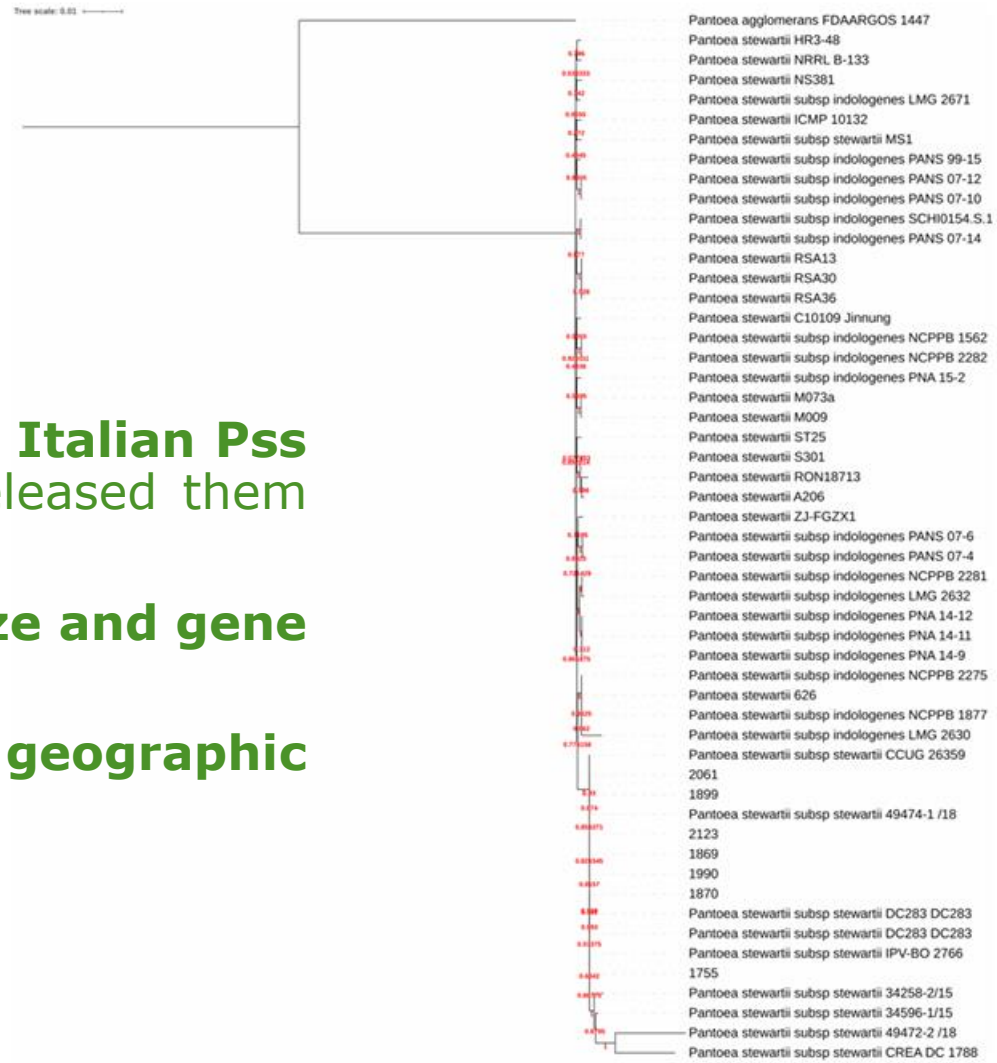
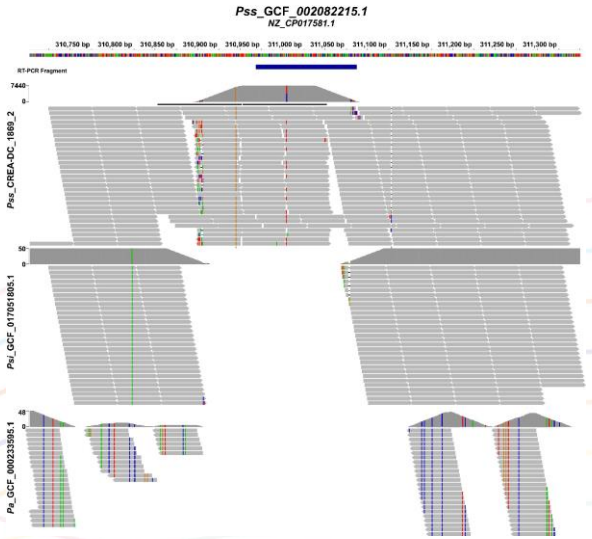


Figure 4. Species tree rooted on *Pantoea agglomerans*. Bootstrap values are reported in red.

REAL-TIME PCR DEVELOPMENT, TPS AND ICL

New molecular tools for the detection of Pss

- ✓ **SYBR green real-time PCR was developed** using genomic data to specifically detect Pss and discriminate closely related bacterial plant pathogen
- ✓ Approximately **30 genomes of Pss and Psi from the NCBI database and from CREA collection** has been compared to identify regions specific to Pss
- ✓ Primers are designed on three different regions and based on primer specificity to Pss, we selected the **ctg3 pair of primers** for further analyzes (named ctg3 and published in **Scala et al., 2023**)



Region amplified by the primer ctg3 selected for real-time PCR

Analytical sensitivity and specificity following EPPO PM7/98 (5) of real-time PCR

- The analytical sensitivity with spiked seed samples was **10^3 CFU/ml**
- The analytical specificity was **100%** (inclusivity and exclusivity)

- **Test Performance Study (TPS):** to validate the Scala et al., (2023) real-time PCR
- **Interlaboratory comparisons (ILC):** to compare of Scala et al., (2023) test with different molecular tests Tambong et al., (2008) and Pal et al., (2019)

- **17 Italian OLs** conducted the TPS and ICL on the same set of samples

ID samples	Type sample
S1	Maize seed extract healty (ES)
S2	ES + 10^4 cfu/ml Pss
S3	Bacterial suspension of 10^6 cfu/ml di Psi
S4	ES
S5	ES +10^5 cfu/ml Pss
S6	ES +10^4 cfu/ml Pss
S7	Bacterial suspension of 10^6 cfu/ml di Pan
S8	ES
S9	ES+10^5 cfu/ml Pss
S10	ES+10^5 cfu/ml Pss
S11	ES
S12	ES+10^6 cfu/ml Pss

Molecular tools for the detection of Pss: TPS of ctg3 and ILC outputs

TPS Scala et al., 2023

- The **diagnostic sensitivity, diagnostic specificity and accuracy** are **100%** with two different Taq polymerases regardless of the DNA extraction methods
- The **reproducibility** was **100%**

Interlaboratory Comparison (ICL)

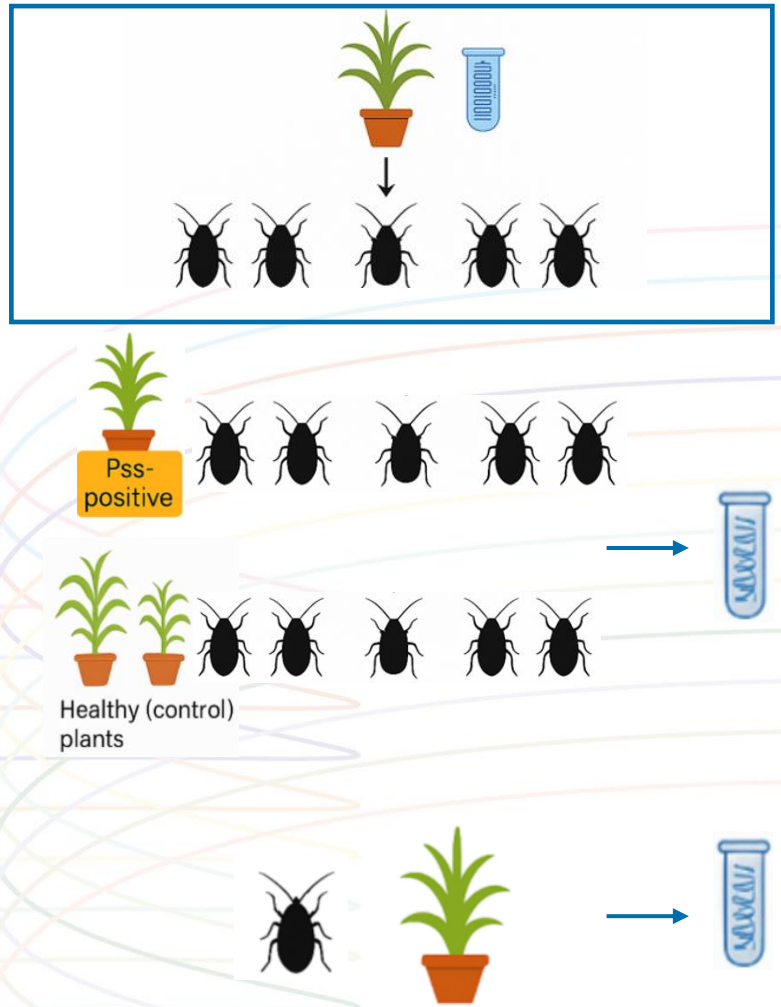
- Tests compared: Pal, Tambong *vs* Scala performance criteria reported in the table

Real-Time PCR	DSE%	DSP%	ACC%
Scala App	100	100	100
Scala Prom	100	100	100
Pal	100	100	100
Tambong	100	83.3	100

VECTOR COMPETENCE EXPERIMENTS

Step I

Experiments to assess if ***Halyomorpha halys*** **can acquire and transmit Pss** to healthy host plants under controlled conditions



Step I – Initial feeding on healthy maize plants -10 days

100 female and 50 male of *Halyomorpha halys,* housed in cages. Five adults were placed with one maize plant for 10 days.

At the end of this period, 6 insects (2 males and 4 females) were randomly selected and analyzed using molecular tests to confirm the absence of Pss

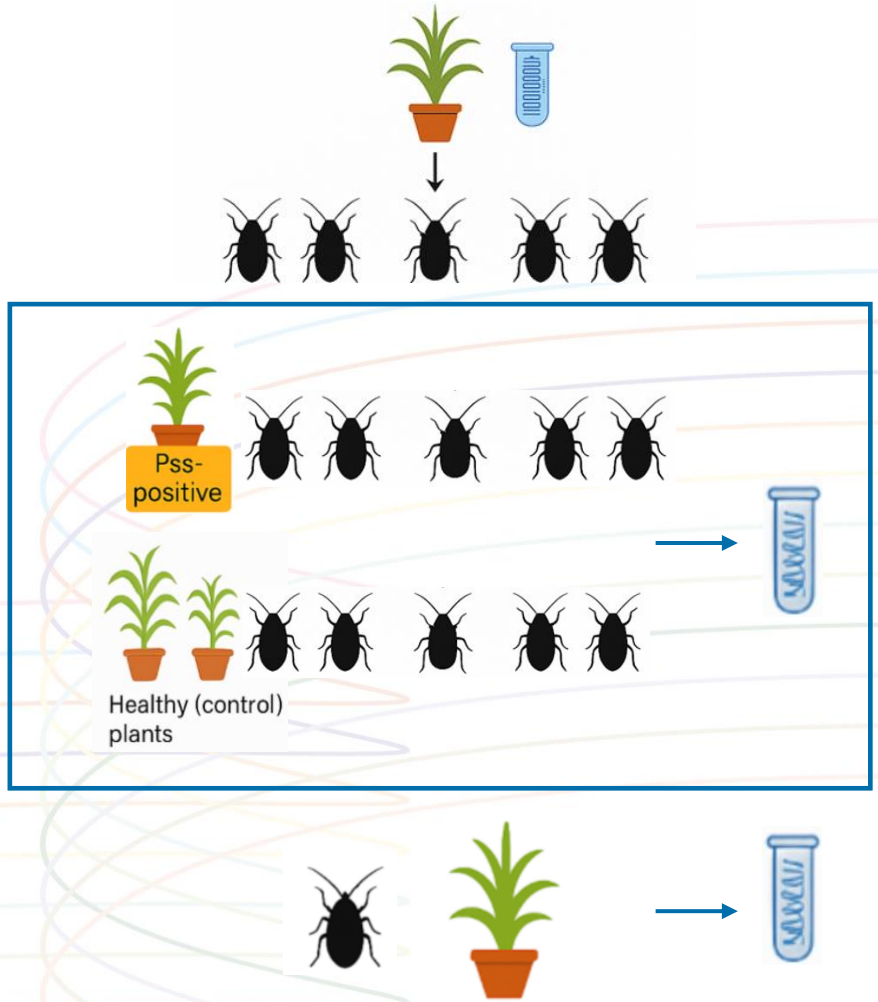
Step II – Exposure to Pss-inoculated *vs* control plants – 7 days

The insects were divided into two groups housed in cages for one week:

- ✓Group 1 (Pss-inoculated plants): 40 males and 80 females housed in cages with maize plants inoculated with Pss
- ✓Group 2 (control plants):5 males and 25 females with healthy maize plants treated with sterile water

In both groups, 5 adults were placed with one maize plant. At the end of this period of feeding, 26 males and 34 females were randomly selected and analyzed by molecular tests to detect Pss in both **head and body**

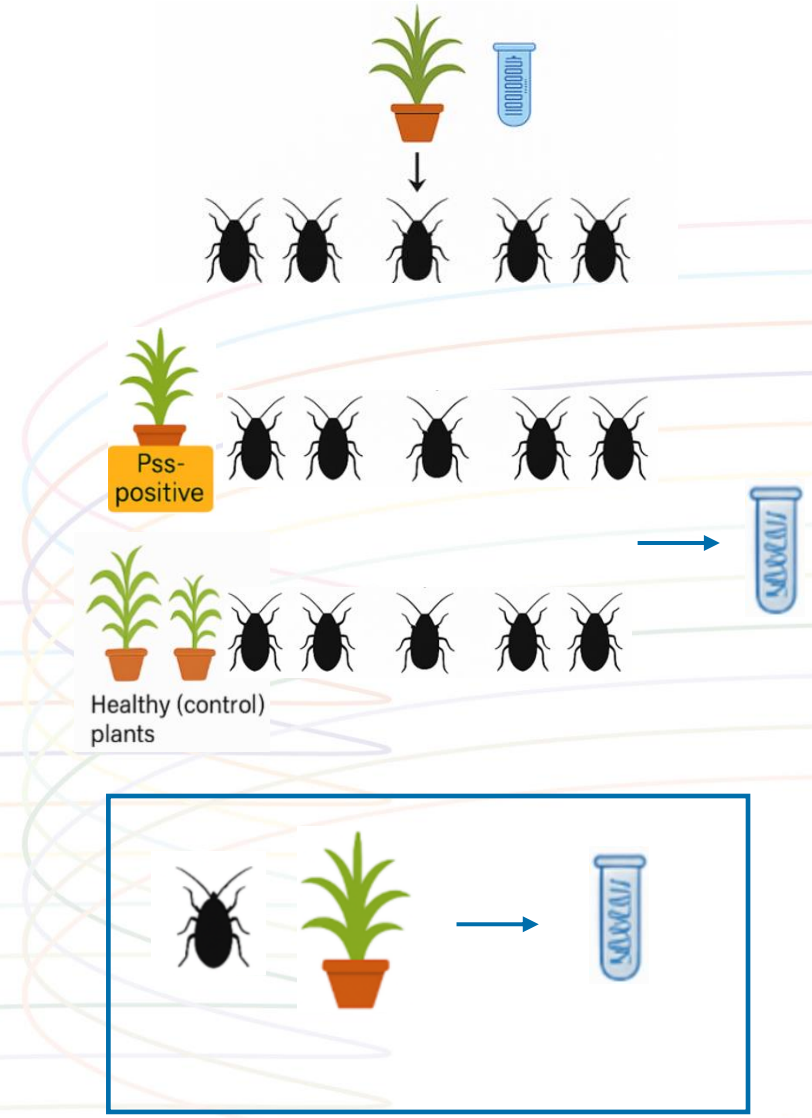
Step II



Step III – 30-day feeding on healthy maize plants

The insects were redistributed into individual cages. Each cage contained one insect and one healthy maize plant.

All the insects (58 previously fed on Pss-inoculated plants and 30 previously fed on healthy control plants) fed on the healthy plants for 30 days, after which all insects and plants were molecularly analyzed to assess the presence of Pss.



Vector Competence Experiments

- Molecular analyses showed that ***H. halys* can acquire and retain the pathogen** after feeding on infected plants
- Pss is randomly **distributed between the heads and the bodies of both** male and female insects
- **All plants from Phase III tested negative** using molecular tests and isolation procedures
- **No Pss transmission** to healthy maize plants was observed

Table 1. Ct and CFU/mL for Pss found in *H. halys*, both males and females, in the different phases of the experiment.

	Insect Part	Step I		Step II		Step III	
		Male	Female	Male	Female	Male	Female
H. halys	Head/Body	7	29	26	34	14	46
H. halys positive to PSS	Head	0	0	24	29	5	37
	Body	0	0	24	32	8	37
H. halys negative to PSS	Head	7	29	2	5	9	12
	Body	7	29	2	2	6	12
Ct range [2]	Head	NA [1]	NA	30–35	25–36	30–35	31–36
	Body	NA	NA	20–35	20–36	29–35	28–36
CFU/mL range [2]	Head	0	0	10^4–10^2	10^5–10^2	10^4–10^2	10^4–10^2
	Body	0	0	10^7–10^2	10^7–10^2	10^4–10^2	10^4–10^2

[1] NA = No amplification signal. [2] Minimum and maximum values obtained in the analyzed samples.

- The insect can ingest the bacterium during feeding, but it **is not able to transmit it into plants**

- *H. halys* is likely a **passive carrier rather than an effective vector** under the tested conditions, highlighting the need for further research to assess potential transmission scenarios and epidemiological risks in Europe

Implications for Surveillance and Plant Health Management

- **Enhanced Pathogen Identification**
  Improved **understanding of pathogen diversity** enables accurate identification and tracing of disease Italian outbreaks

- **Advanced Diagnostic Tools**
  Validated **Scala et al., (2023) real-time PCR to improve detection capabilities**, supporting timely plant health management

- **Informed Risk Assessment**
  Clarification of insect transmission potential guides risk assessments and prioritization of control measures

- Integrated Research Strategy combining pathogen characterization, diagnostics, and epidemiology provide key informations to improve plant health surveillance.

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Thank you for your attention

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