

# *La detecció de Xylella fastidiosa: un repte pels laboratoris.*

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### Reconeixement de símptomes



ucanr.edu



procesoliva.wordpress.com

### Vector identificat



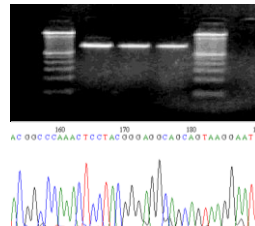
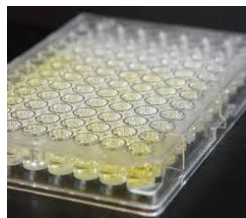
www.britishbugs.org.uk

### Pressa de mostra

aïllament

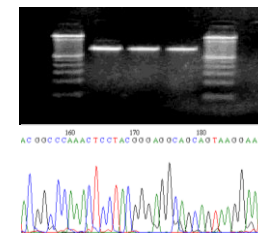
serologia

molecular



### De trampes o lliures

molecular





## Símtomes

- **Cremat de fulles i brots**
- **afebliment generalitzat.**

**Confusió** amb altres causes: agents abiòtics o mediambientals → estrès hídric, vent, salinitat, excés de nutrients...

**Com es diferencia?** Per la distribució  
*Xylella* → en les parts en creixement de la planta  
Altres causes → distribució més generalitzada tant en parts de la planta com en nombre de plantes afectades.

## Època de mostreig:

Primavera

Començament d'estiu

Començament tardor

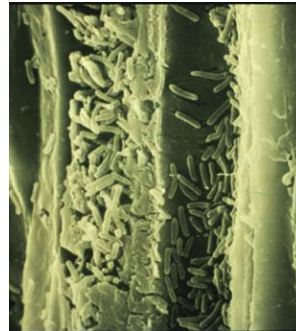




## Pressa de mostra



[www.apsnet.org](http://www.apsnet.org)



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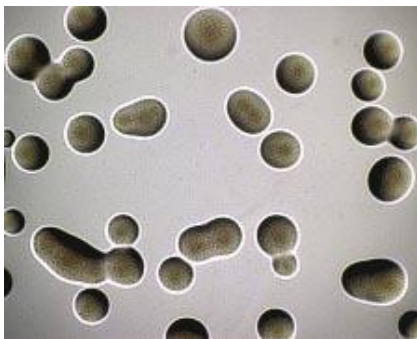


[ftp://ftpfiler.to.cnr.it:21001/Xylella\\_symposium/Workshop%20manuals/WORKSHOP%20MANUAL%20DETECTION%20ENG.pdf](ftp://ftpfiler.to.cnr.it:21001/Xylella_symposium/Workshop%20manuals/WORKSHOP%20MANUAL%20DETECTION%20ENG.pdf)

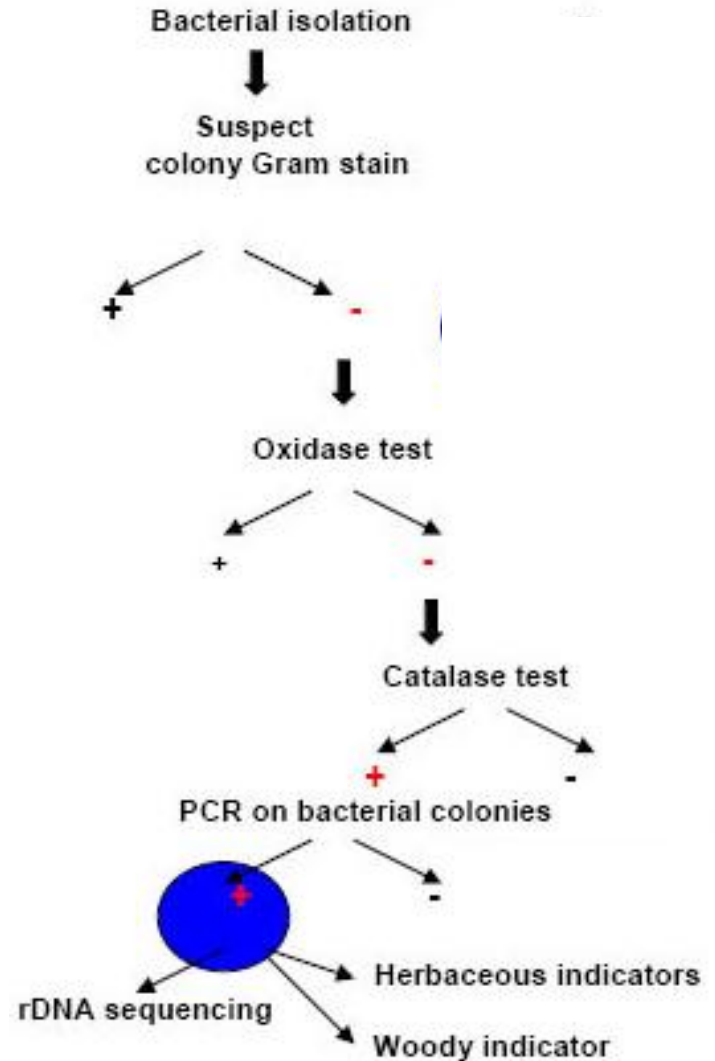
## Aïllament

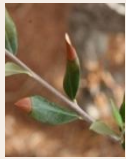
Creixement lent en medi selectiu.  
Colonies petites de color blanc a groc.

- PW (Periwinkle Wilt with Gelrite) Media (Davis et al., 1983)
- PD3 Media (Hopkins and Adlerz, 1988)

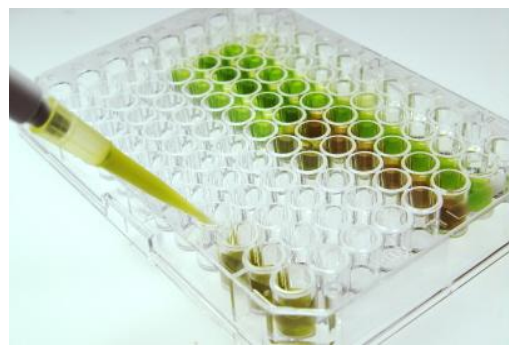
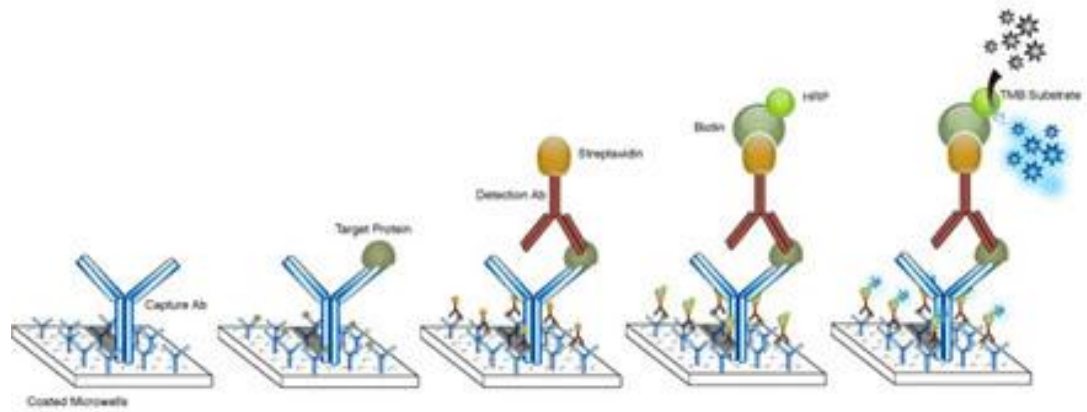


www.apsnet.org  
H. Staniszewska





# Serologia





En el mercat hi ha diferents **Kits ELISA**:

**LOEWE:**

DAS ELISA (Double Antibody Sandwich) using polyclonal antiserum from rabbit  
Hostes: OLIVE, citrus, grapevine, periwinkle, many other economically important trees, shrubs, weed



**AGRITEST:**

DAS ELISA (Double Antibody Sandwich) using polyclonal antiserum from rabbit  
Hostes: OLIVE → *X. fastidiosa* strain CoDiRO

## Molecular

Segons el treball: **DIAGNOSTIC PROTOCOLS. WORKSHOP "CURRENT TOOLS FOR THE DETECTION OF XYLELLA FASTIDIOSA IN HOST PLANTS AND VECTORS"**.  
INTERNATIONAL SYMPOSIUM ON THE EUROPEAN OUTBREAK OF XYLELLA FASTIDIOSA IN OLIVE

[ftp://ftpfiler.to.cnr.it:21001/Xylella\\_symposium/Workshop%20manuals/WORKSHOP%20MANUAL%20DETECTION%20ENG.pdf](ftp://ftpfiler.to.cnr.it:21001/Xylella_symposium/Workshop%20manuals/WORKSHOP%20MANUAL%20DETECTION%20ENG.pdf)

Preparats per:

CNR Istituto per la Protezione Sostenibile delle Piante, Bari, Italy

Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Bari, Italy

### Extracció ADN:

- columnes
- CTAB

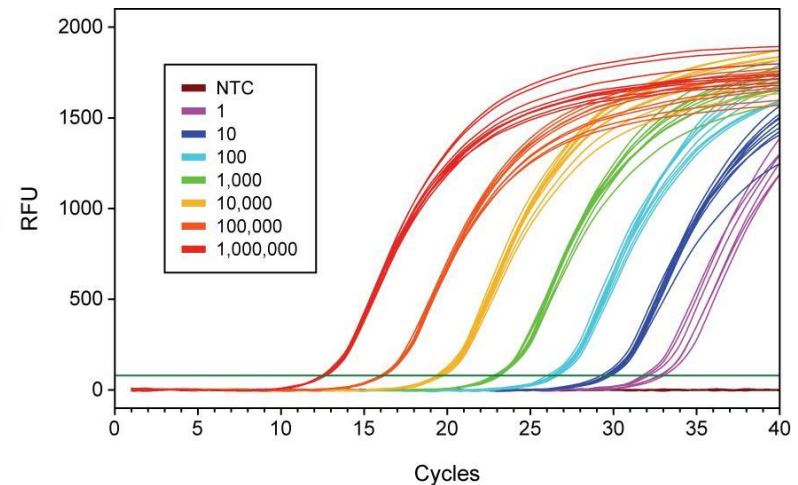
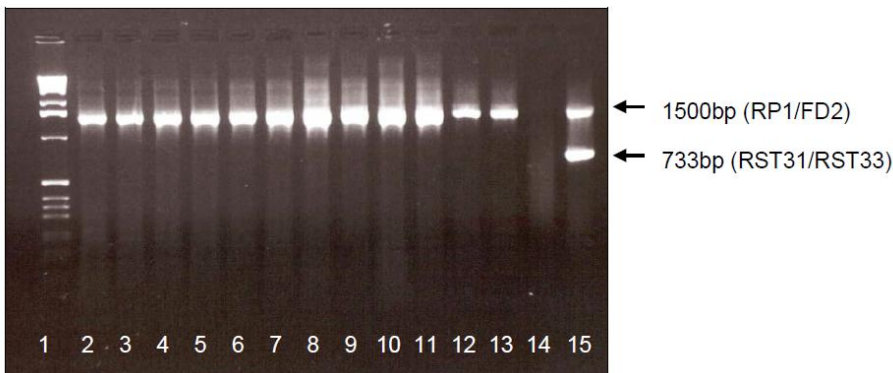




## Molecular

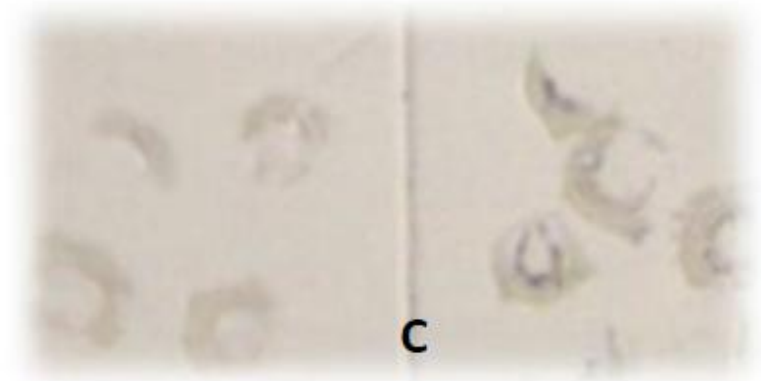
### Amplificació:

- primers RST31/RST33 (Minsavage et al 1994)
- primers FXYgyrR499/RXYgyr907 (Rodrigues et al., 2003)
- primers HL5/HL6, (Francis et al., 2006).
- primers i sonda per PCR a temps real PCR XF-F/XF-R/XF-P (Harper et al., 2010)



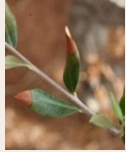
## Mètodes recents:

- Direct tissue blot immunoassay (DTBIA)



Direct tissue blot immunoassay for detection of *Xylella fastidiosa* in olive trees. *Phytopathologia Mediterranea* (2014) 53, 3, 559–564

- Real-time Loop-mediated isothermal amplification (RT-LAMP)



## Comparativa de mètodes per detectar *Xylella fastidiosa*<sup>1</sup>

Technique	Sensitivity <sup>2</sup> (number of bacterial cells)	Cost and labor
Culture from xylem fluid on selective media	1000	Low
ELISA <sup>3</sup>	100,000	Med
PCR	100	High
IC-PCR <sup>4</sup>	<100	High

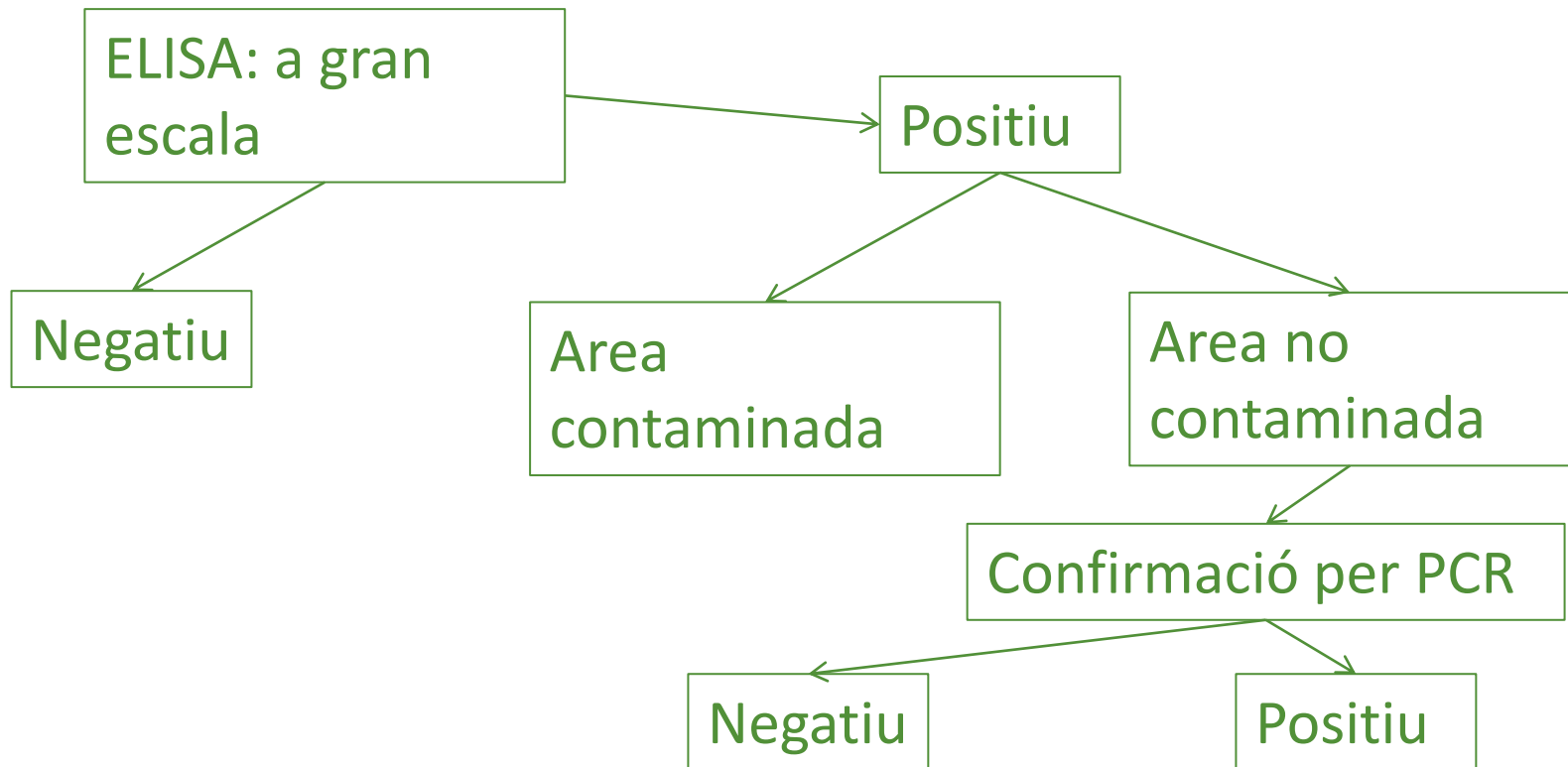
<sup>1</sup> De <http://www.apsnet.org/edcenter/intropp/lessons/prokaryotes/Pages/BacterialLeafScorch.aspx>  
 (Adapted from R. Jordan. 2002. In: Bacterial Leaf Scorch in Amenity trees: A Wide-Spread Problem of Economic Significance to the Urban Forest. Lashomb, J., A. Iskra, A.B. Gould, and G. Hamilton, eds. Vol. NA-TP-01-03: USFS

<sup>2</sup>Lowest approximate number of bacterial cells that the assay can detect.

<sup>3</sup>Enzyme-linked immunosorbent assay

<sup>4</sup>Immunocapture-PCR

## Diagrama de flux del procediment diagnòstic utilitzat a Apulia pel programa de monitoratge.



Gràcies per l'atenció