Implementation of the detection protocol for *Xanthomonas euvesicatoria* in pepper seeds

M. Ferrari[1], B. Xhemali[1-2], D. Giovanardi[1] & E. Stefani[1]

[1]Dept. of Life sciences, University of Modena & Reggio Emilia, Italy

[2]Kosovo Institute of Agriculture (KIA), Peja, Kosovo

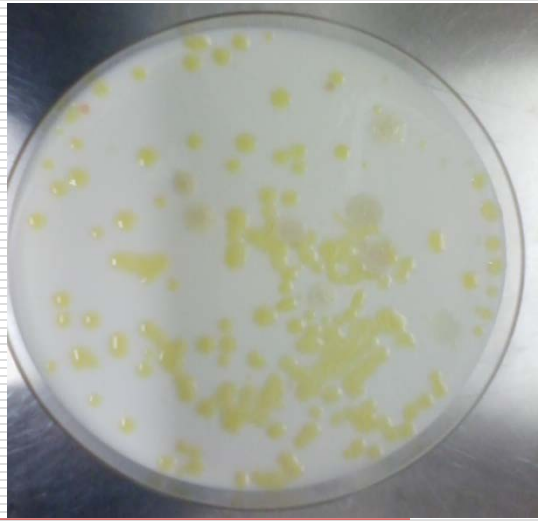
The disease

- Bacterial spot is a worldwide disease, mainly affecting tomato and pepper.
- Symptoms may affect all aerial parts and are particularly severe on pepper.

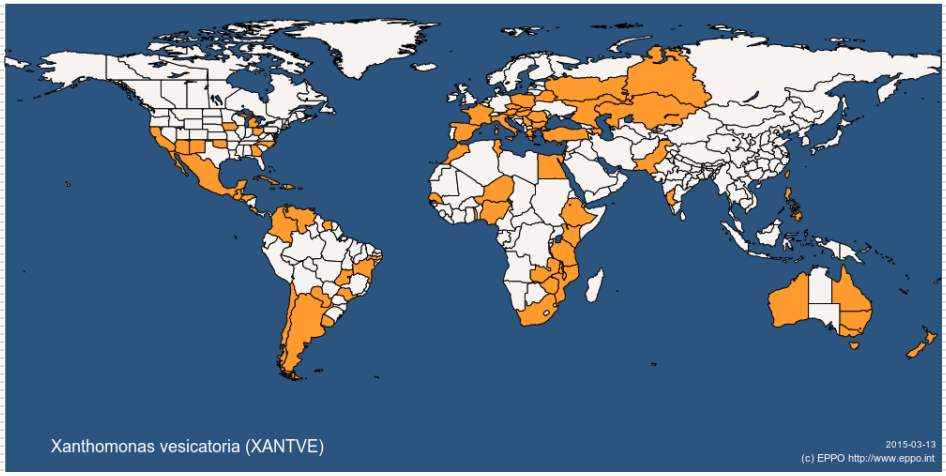
Source: APS

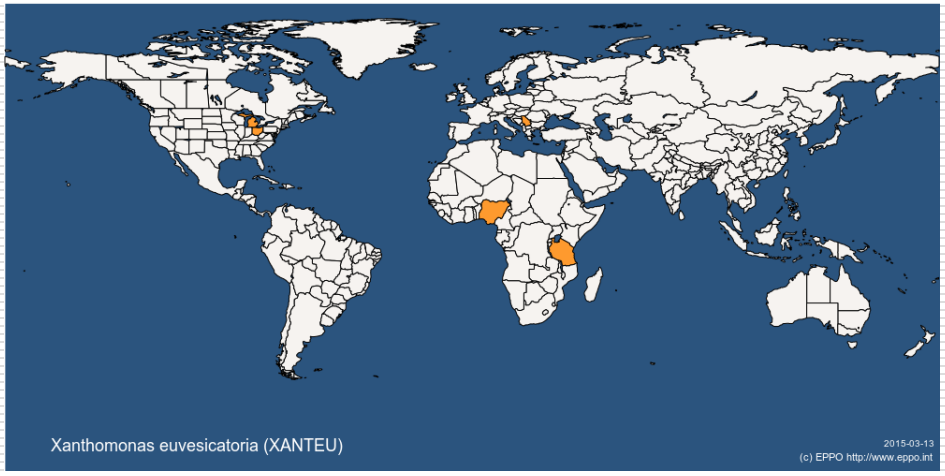
The causal agent

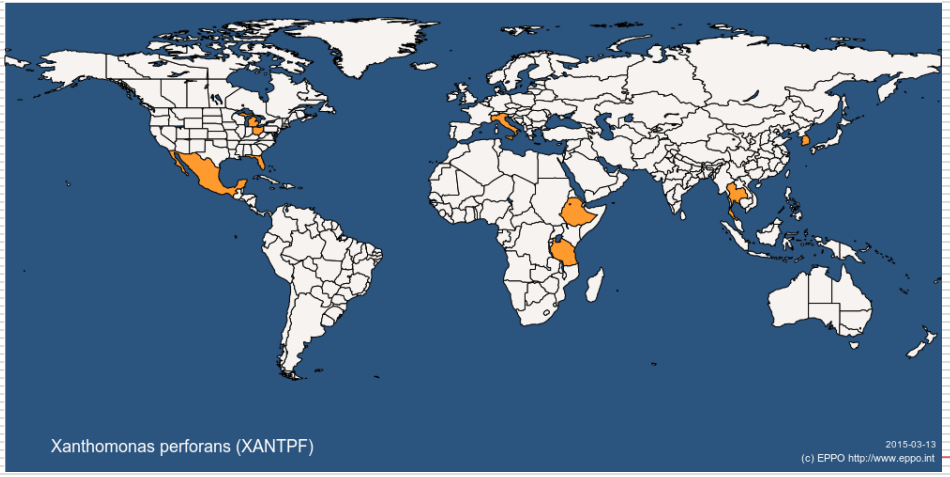
- The causal agent was formerly known as *Xanthomonas campestris* pv. *vesicatoria*.
  - Now reclassified into four species:
    - *Xanthomonas vesicatoria*
    - *Xanthomonas euvesicatoria*
    - *Xanthomonas perforans*
    - *Xanthomonas gardneri*
  - All four species are regulated

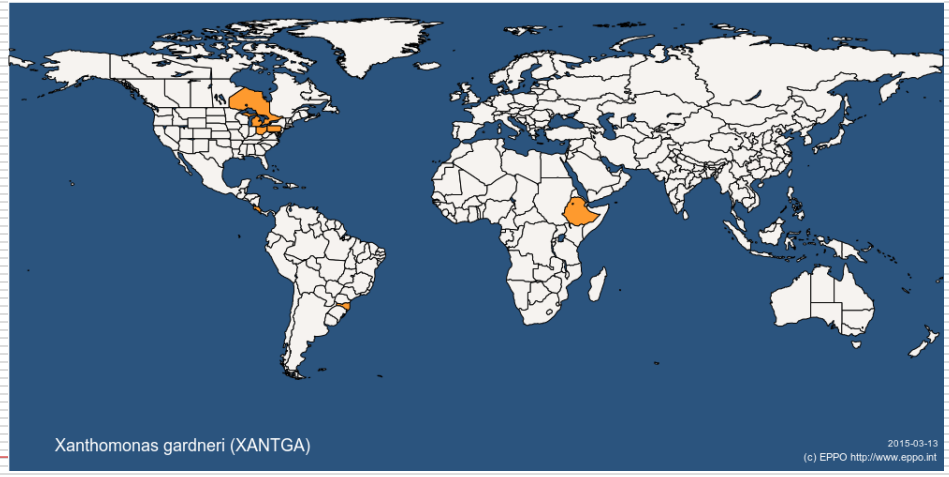


Pathogens distribution

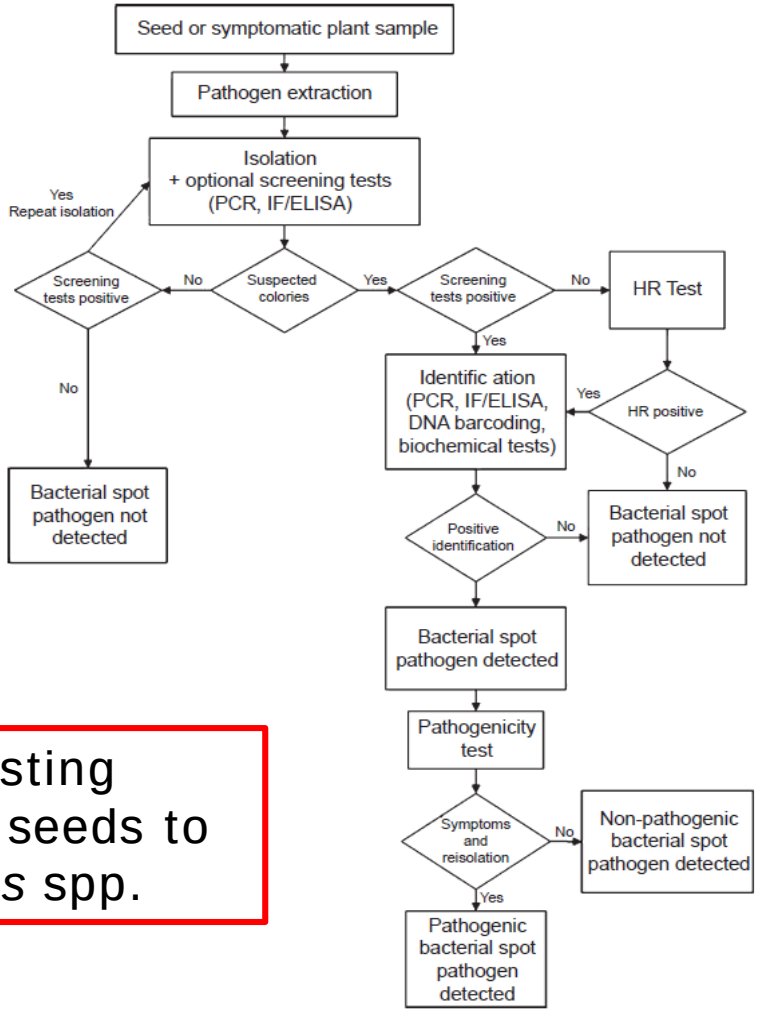








The EPPO diagnostic protocol



Flow diagram for testing tomato and pepper seeds to detect *Xanthomonas* spp.

Objective or our studies

- Implement the diagnostic protocol for pepper seeds.
  - In particular, focusing on *X. euvesicatoria*
- Compare two different DNA extraction methods.
- Compare ELISA diagnostic results with PCR and Direct Isolation on agar media.
- Suggest implementation of the DP

Material and Methods

- During the analytical season 2014 on pepper seeds in Novi Sad (Serbia), 13 seeds lots found *Xeuv* positive
  - Test applied: DAS-ELISA (Loewe)
- The second lab sample was taken for:
  - Direct isolation on YGCA
  - Preparation of seed extracts for PCR
    - Heat shock
    - DNeasy Plant Mini Kit columns (Qiagen)

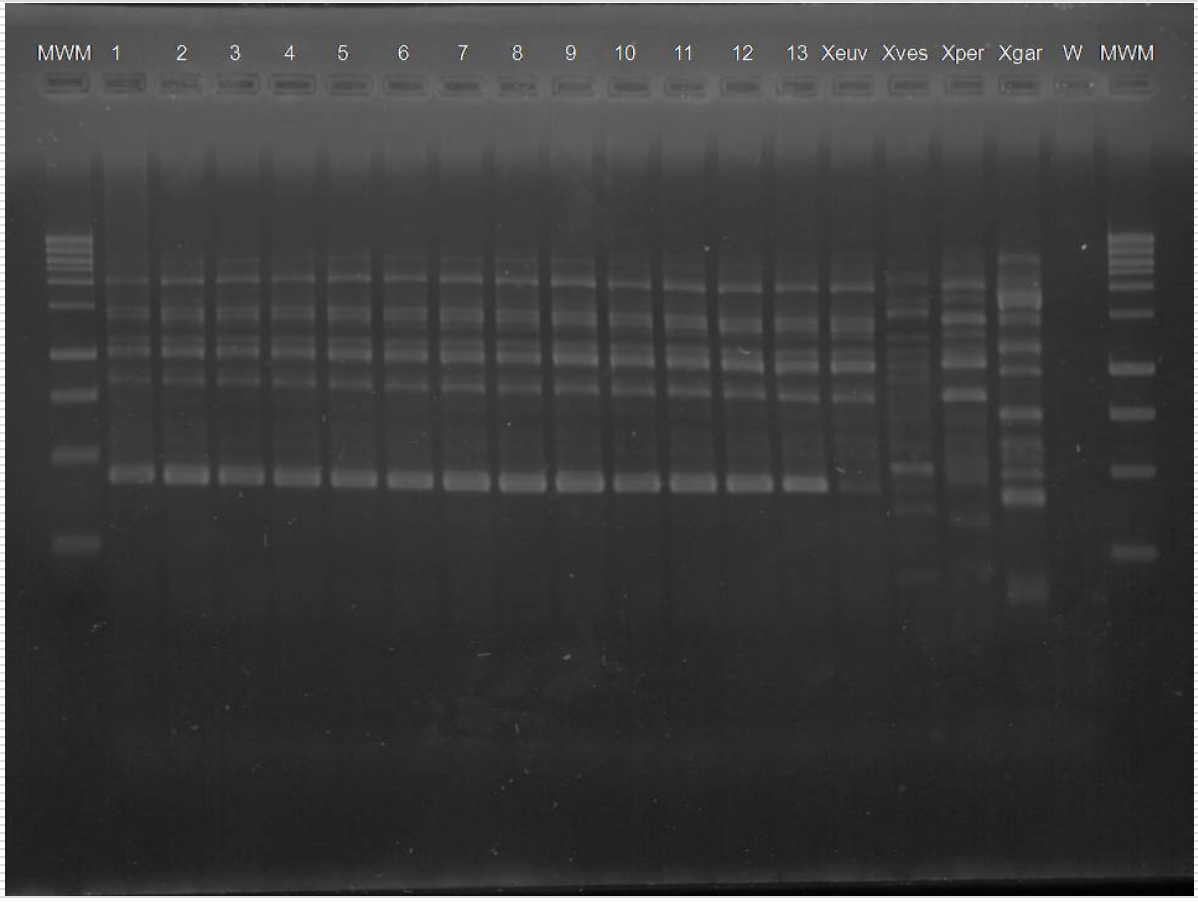
Material and Methods

- PCR protocol applied on seed extracts:
  - EPPO
    - Primer pairs (Koenraadt *et al*., 2007)
- For the identification of putative *Xeuv* colonies obtained on YGCA:
  - BOX, REP, ERIC
- Analysis of genetic profiles:
  - UPMGA Cluster analysis (GelCompar 4.1, Applied Maths, Kortrjik, Belgium)
  - Pearson's correlation coefficient

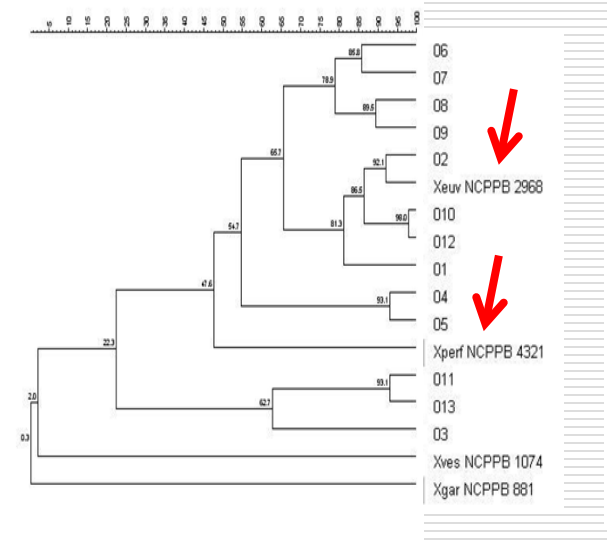
Results

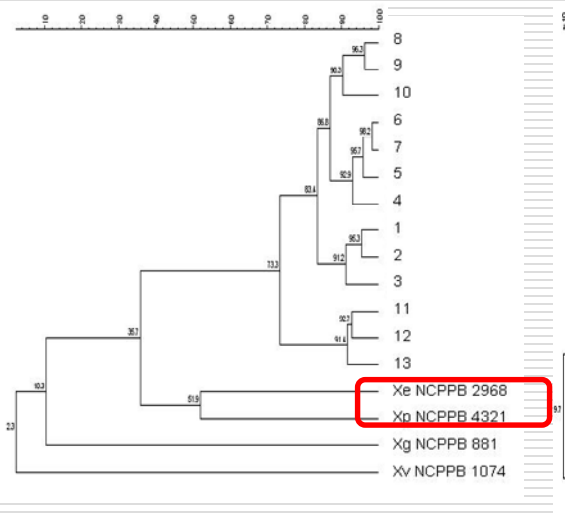
Samples (varieties or breeding lines)	ELISA	Direct isolation, confirmed by PCR on pure cultures and genotyping	**PCR** DNA extraction with heating shock	**PCR** DNA extraction with DNeasy Plant Mini Kit
BL 1 2013/5	+	-	+	+
BL 2 2013/8	+	-	-	-
Novosađanka 1	+	-	-	+
Novosađanka 2	+	-	+	+
Altina	+	-	+	+
Anita	+	-	+	+
Amphora	+	-	+	+
Una	+	-	-	+
Matica	+	+	+	+
Soraksari	+	+	-	+
Vranjeska	+	+	+	+
Plamena	+	+	-	+
Manpryka - ECW	+	-	-	+

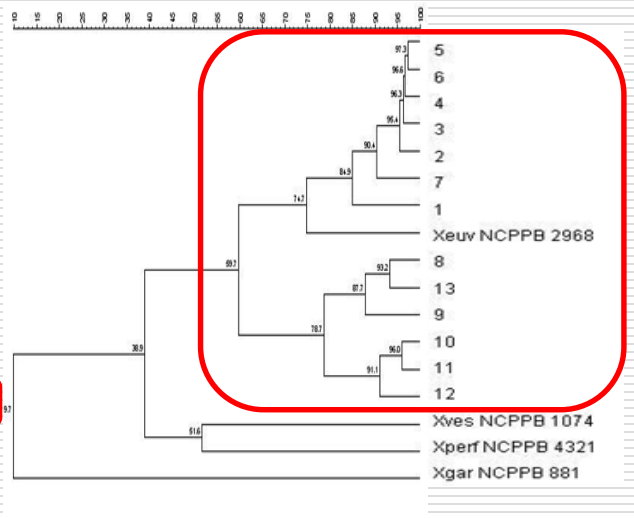
Results – ERIC PCR



Results - Clustering







BOX ERIC REP

Discussion and Comments

- Comparing results:
  - PCR is best done by previous DNA extraction and purification using mini columns
  - PCR confirmed most ELISA results
  - Direct isolation is not so sensitive
- Identifying *Xeuv*
  - Genotyping not always able to discriminate *Xeuv* from *Xper*
  - REP primers are more discriminative than BOX or ERIC

Thank you for your attention!