

# PEPEIRA

## PepMV: epidemiology, economic impact and pest risk analysis

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# WP 3 objectives

## ■ PepMV characterization and detection

- Determine occurrence and spread of PepMV isolates and strains over Europe
- Most important biological and genetic characteristics of the different strains and isolates of PepMV across (and outside) Europe
- Determine possible risks of PepMV strains and variants on tomato and other Solaneceous crops
- Develop and evaluate accurate detection and diagnostic methods
- Determine risk of seed transmission



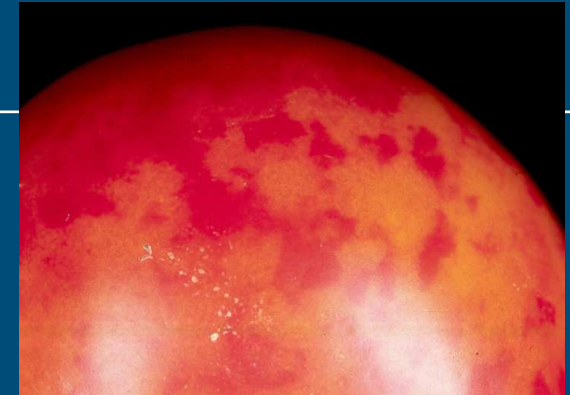
# Overview of presentation

- Update on the virus
  - Strains and isolates
- Biological and genetic characteristics
- Development of diagnostics
- Occurrence and incidence
- Seed transmission



# Pepino mosaic virus

- Virus first found in Peru (1974) on pepino (*Solanum muricatum*)
- Potexvirus (*Flexiviridae*)
  - $\pm$  500 nm flexuous particle
  - Mechanical transmission
  - Very persistent and infectious
- Present in Spain on tomato in 1998, Netherlands 1999 and spreading since



# PepMV strains

- 4 PepMV strains recognized
  - Pepino (or Peruvian) = type strain (Jones et al 1980)
  - EU-tomato strain (Netherlands 1999)
  - US1 strain = 2005 USA (2007 Can. Islands)
  - Chile-2 strain = 2007 USA
  - Recombination also occurs (US2)
- No clear distinction between strains on plants
- Differentiation on basis of sequence homology



# PepMV strains

- Strong biological differences between isolates of one strain
  - Mild and 'aggressive' isolates
  - Leaf and fruit symptoms
  - Yield and fruit quality
- Unknown factors influence symptoms on leaves and fruits
  - Climate
  - Cultivar



# Biological characterization of PepMV strains

- Impact on other Solaneceous crops
  - Standardized isolates
  - EU-tomato, Chile-2 and US1
  - Symptoms and effects on
    - *Solaneceous* crops: tomato, potato, pepper, aubergine and tobacco
    - Local crop cultivars



# Risks for other Solaneceous crops

- Mechanical inoculations of 5 -10 plants with PepMV isolate
  - Score local and systemic symptoms
  - Test by ELISA
  - Confirm by back-inoculation





# Risks for other Solaneceous crops

## ■ Tomato

- Nearly always systemic symptoms and always ELISA positive in both leaves and roots

## ■ Pepper

- In general no symptoms and rather seldom leaves ELISA positive

## ■ Potato

- In general no symptoms and about 1/3 leaves ELISA positive. No roots or tuber infection

## ■ Aubergine

- Often systemic symptoms and in general ELISA positive in leaves and roots



# Genetic characteristics



- Typical potyvirus
  - 6412 nts
- Many sequences available
  - > 10 full-length sequences
- Distinction between strains by sequence differences
  - Virus-wide and strain specific primer sets (qRT-PCR)
- No correlation between sequences and biology



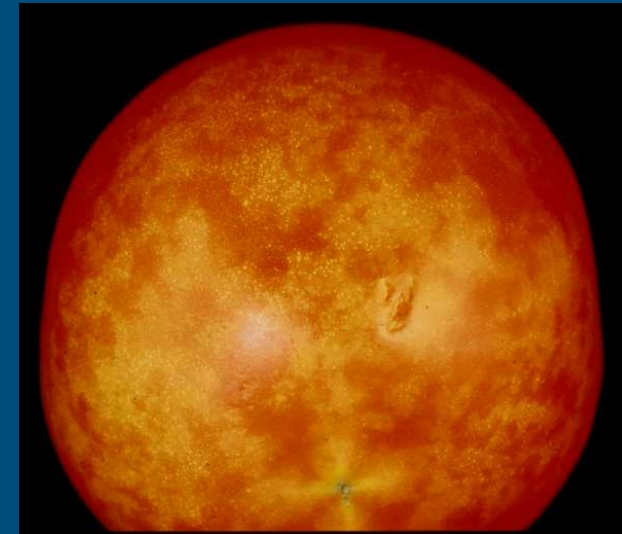
# Development of diagnostics

- Serology
  - DAS-ELISA antisera available (several suppliers)
  - Different strains but CPs highly homologous
  - Antisera compared: minor differences in reactivity but no differences in sensitivity
- PCR
  - Conventional RT-PCR and q-RT-PCR primer sets developed and tested
- Selection of diagnostic methods made for ring test



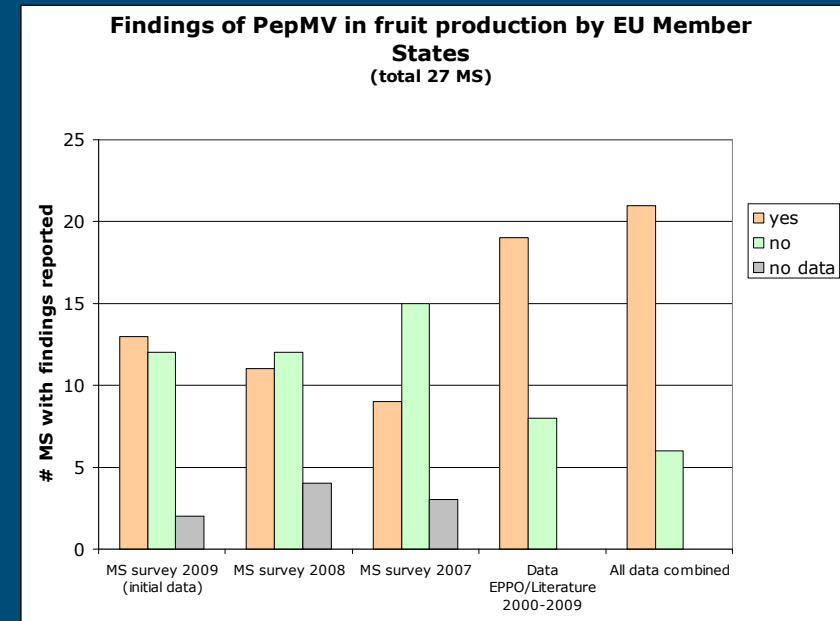
# Occurrence and incidence

- First report in Europe in 1999
  - Netherlands and UK
  - Present in Spain in 1998
- Reports worldwide
  - USA and Chile: new strains
- Presence in Europe
  - National surveys mandatory
    - Seeds of EU origin
    - Imported seeds third countries
    - Tomato plants for planting
    - Tomato fruit production
    - Fruits on the market



# Occurrence and incidence

- Present in Europe
  - Established in a few countries
  - 'Present' in some countries
  - Under various levels of control
- Main transmission route still under debate



# Seed transmission

## ■ Set-up of test

- Seeds collected from crop grown by Belgian partner
- Infected with mixture of EU-tomato and Chile-2 strain
- > 100.000 seeds harvested, 3 batches, 6-12 weeks after infection

## ■ Seeds only marginally cleaned

- Pectinase treatment only to remove fruit flesh
- No additional disinfection
- Distributed to partners and sown within 3 weeks after harvest



# Seed transmission

## ■ Seed testing

- Seeds distributed to 10 partners (official permits!)
- Seedlings tested 6 weeks after germination
- Batches of 10 seedlings, standardized ELISA protocol

## ■ Test results

- 87.780 seedlings tested
- 23 pooled batches found positive
  - 1/1887, 3/3538, 19/3353
- 0.026% seed transmission



# Seed transmission

- Seed transmission confirmation
  - In line with earlier results from the Netherlands, Spain
  - Seed transmission re-confirmed
  - 'Worst-case scenario'
- Practical implications
  - EU-directive: no seed harvest from infected crops
  - Rigorous testing of crops, thorough disinfection (acid-treatment) plus seed testing by seed companies
  - Transmission risk small





On behalf of the consortium:

Thank you for your attention!

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