Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Fisheries, Food Security and Nature

The role of diagnostic tests in outbreak management

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Outbreak management

- Starts with a notification
  - Inspection sample
  - Lab result
  - Other member state or third country
  - Company
  - Private person

Outbreak management

Official sample and official test result?

Context:

Open or closed

Risk of spread (forward tracing)

- Natural
- Through trade

Source of contamination (backward tracing)

How to determine measures for eradication of quarantine organisms

- Task NPPO
  - Legislation (PHR/OCR): prevent introduction and spread of Union quarantine pests

…the competent authority shall immediately take all necessary phytosanitary measures to eradicate the relevant Union quarantine pest from the area concerned…

How to determine measures for eradication of quarantine organisms

- Measures
  - Regulations
  - Contingency plan?
  - First finding or experience?

How to determine measures for eradication of quarantine organisms

- Prescribed measures or Member state can determine measures and treatments
  - Specific for each organism
  - Documents: contingency plan or elimination measures for most relevant organisms for NL
  - Dependent on risks in NL
  - Context dependent, e.g. location and season
- Discussed with specialists
- Report in Scopaff

Role of test results

- Official test result necessary for imposing measures
- Sampling and testing necessary to determine spread
- Lead time test determines speed of investigation

Opportunities and challenges of new techniques

- More sensitive testing -> early detection
  - Early detection -> easier eradication, less measures needed
- Insight in source(s)
  - Phylogenetic analysis (introduction(s) and spread, relatedness)
  - Ancient DNA (history)
- Detection of new species
  - Often more research needed to determine risks
  - Other countries also use techniques such as HTS

  -> Prepare for findings/notifications of new organisms

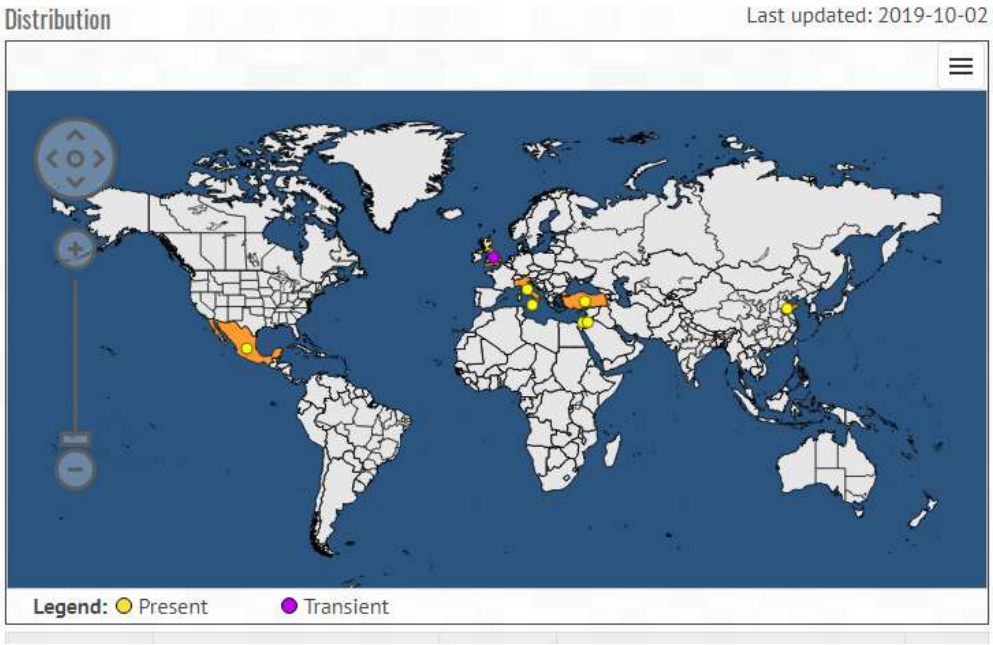
Case study:
Tomato brown rugose fruit virus (ToBRFV)

- Jordan, 2016
- Main hosts: tomato, pepper
- Breaks all known resistance genes/alleles against tobamoviruses in tomato including *Tm-2*^{2}
- Characteristics
  - Highly stable and contagious: easily transmitted mechanically
  - Seed-borne transmission (low efficiency)
  - No vector, but bumble bees can transmit the virus

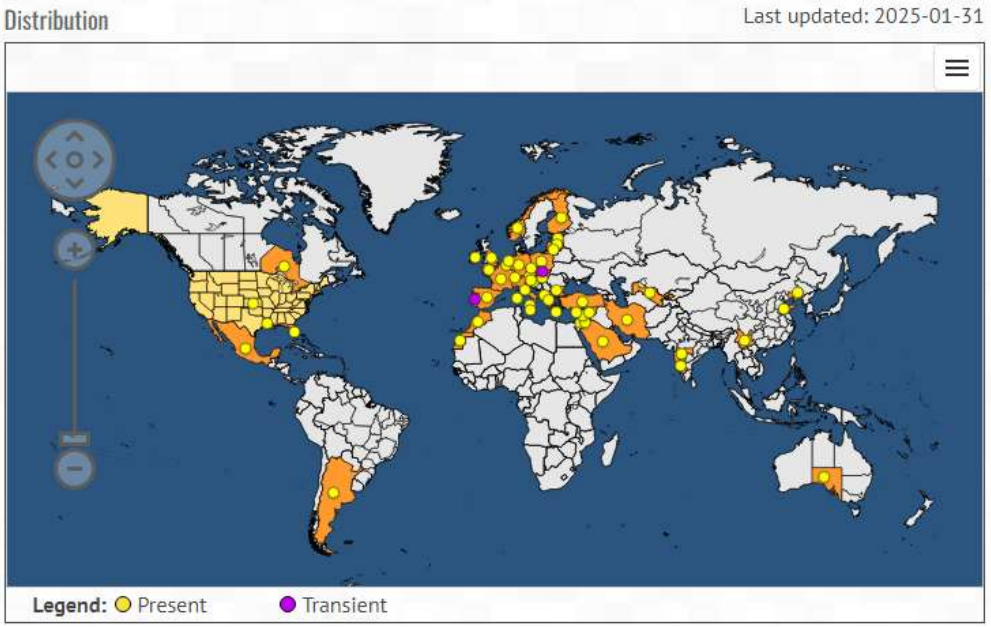
- Strategies: good hygiene and phytosanitary practices
- Diagnostic tests: EPPO PM7/146(2) Tomato brown rugose fruit virus
- Emergency measures (EU) 2023/1032
- RNQP since January 2025

Tomato brown rugose fruit virus (ToBRFV)

2019



2025



ToBRFV outbreaks in The Netherlands

- ToBRFV identified in 2019 at multiple tomato production sites
- NPPO-NL: aim for eradication
- Each finding: try to identify source of infestation (trace back)
- Questions:
  - What are the outbreak dynamics?
  - How many times was the virus was introduced into NL?

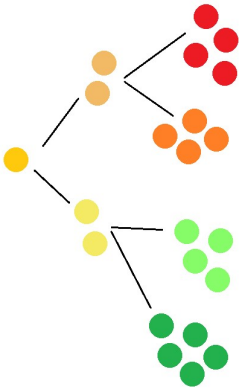
ToBRFV outbreaks in The Netherlands

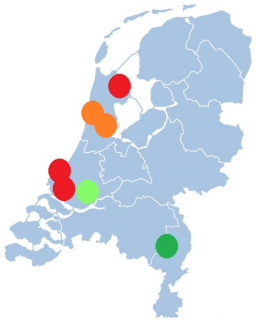
Genome sequences: Input for phylogenetic analyses

- Are clusters associated with certain metadata? (tomato variety, seed batch, geolocation…)

Virus genomes that are most similar, group in a cluster that indicates a shared origin/common ancestor

NIVIP: De novo assembly of complete ToBRFV sequences using validated custom pipeline for virus detection & identification



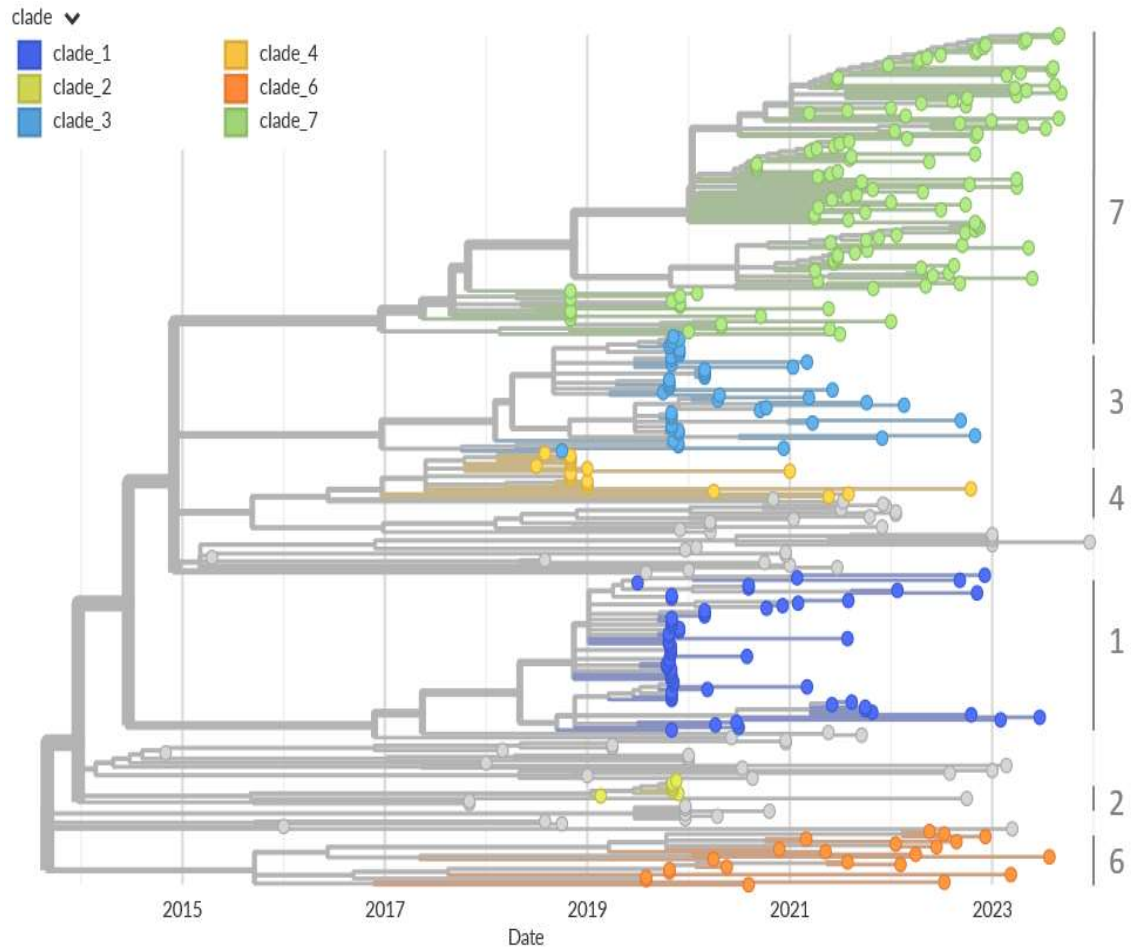


not the actual situation

International cooperation

To integrate and share (anonymized) ToBRFV data:

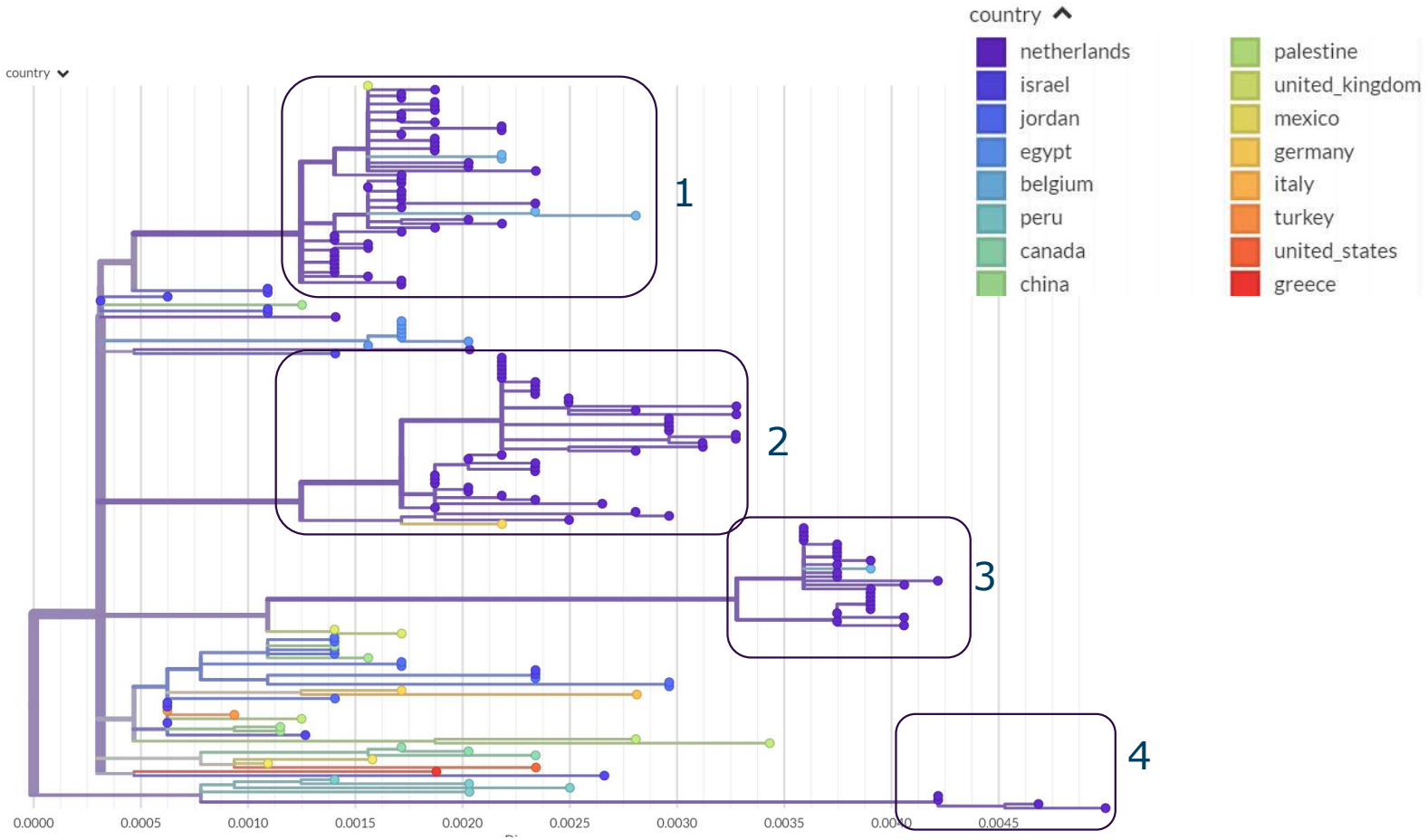
Public tool Nextstrain
https://nextstrain.nrcnvwa.nl/ToBRFV/20220412



A community effort

Sharing data with this interactive online tool will enable the plant virology field to better understand and communicate the diversity and spread of this virus. Organizations are invited to share data or materials for inclusion in the build. Updates will be communicated via Resource Announcements to which contributing organizations are invited as co-author.

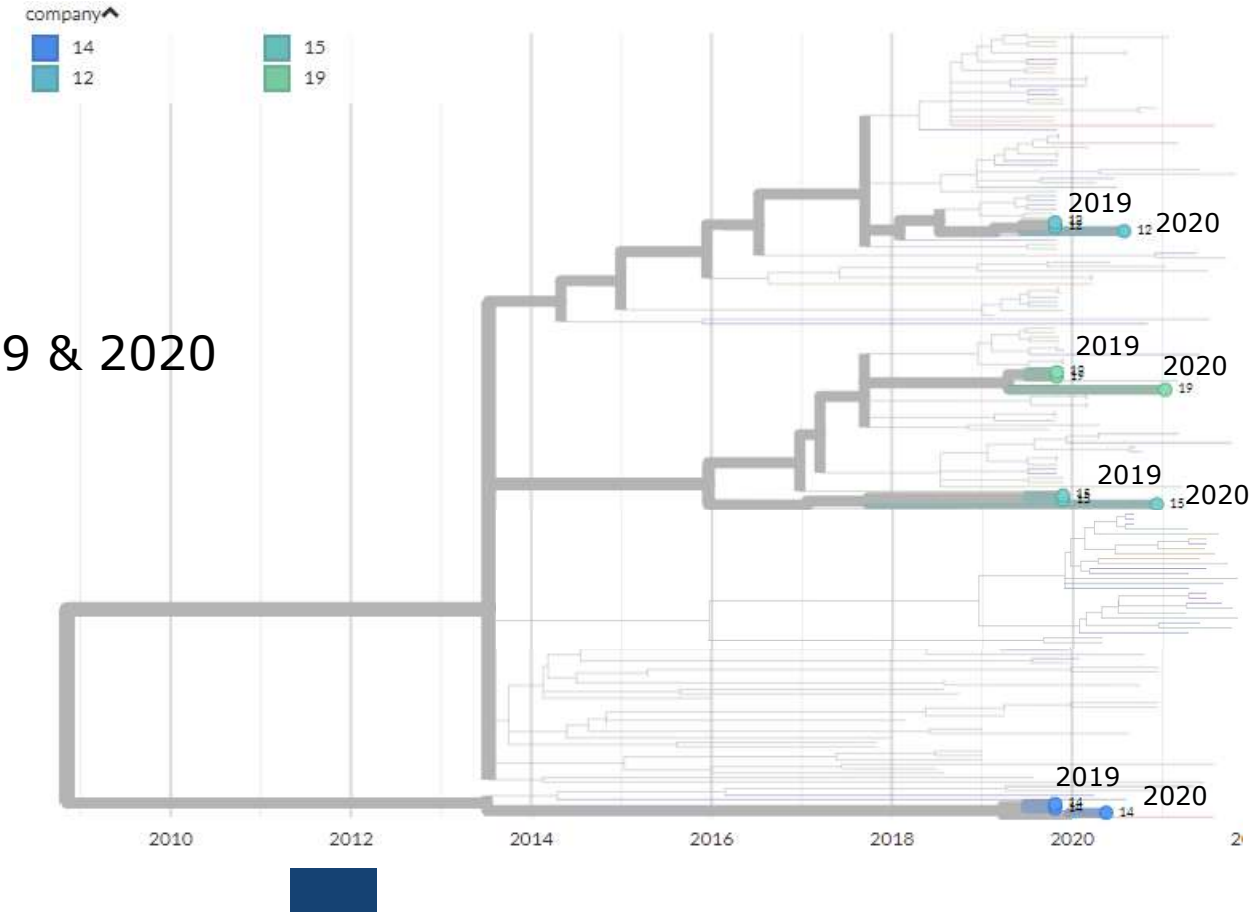
At least 4 independent introductions into NL 2020



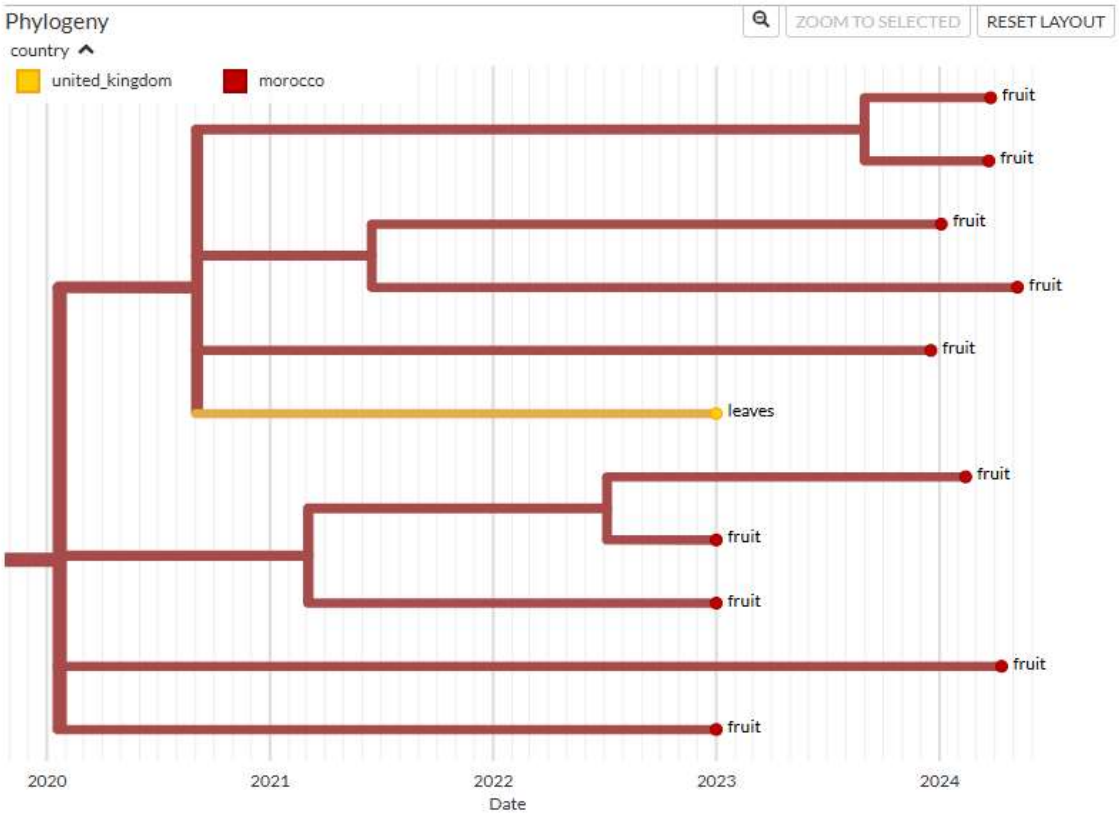
Elimination is extremely difficult

Several growers infested with similar sequence type in subsequent cultivations

Tomato cultivations in 2019 & 2020



Pathways of introduction



Conclusions and summary

- Reliable and fast detection techniques are crucial
- Context is important
- New techniques such as HTS can help investigate sources and spread of quarantine organisms
  - To determine effectiveness of measures
- Communication between laboratory, outbreak management and externally is important!

Questions?