Unravelling the little cherry disease complex at European scale to improve transnational diagnostics and management of the disease (EURAVELCH)

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Aim of the project

Getting a better insight in the pathogenesis of the host plants with little cherry disease symptoms by means of high throughput sequencing (NGS) (unravelling the little cherry disease complex)

Project partners

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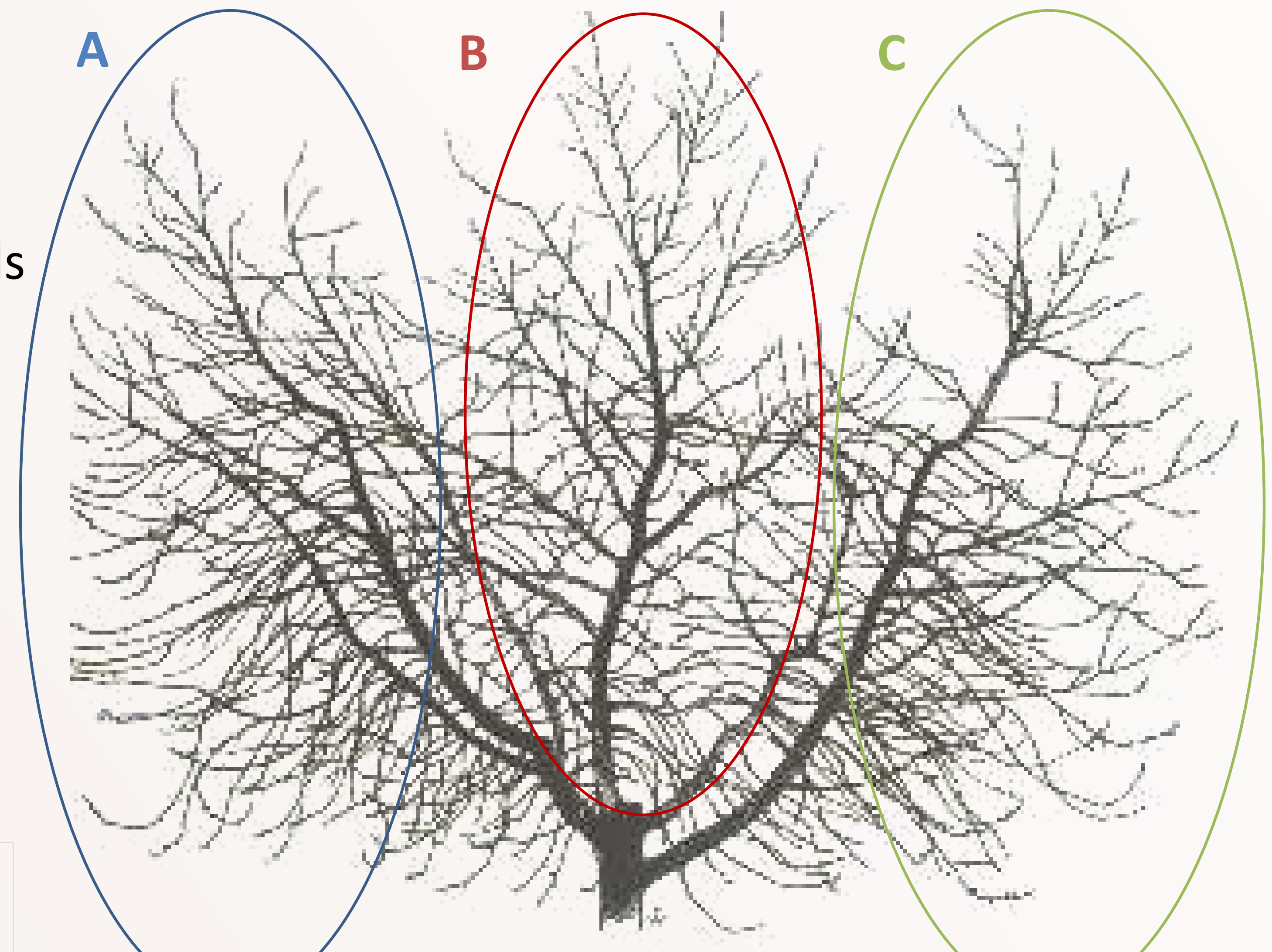
Main project tasks

1. **Obtaining more information about the EU-wide spread** of little cherry viruses (LChV), and **raising awareness**.
2. **Optimization and standardization of diagnostics**, taking into account the population diversity at national, European and worldwide levels (Networking & NGS - Method performance study).
3. **Enhancing knowledge on the little cherry disease epidemiology through NGS**.
4. Reflecting on consequences for future EU quarantine **legislation**.

Case study: Epidemiology/diagnostics on a Prunus avium *rootstock carrying 3 ≠ cv. grafts*

Link disease symptoms and targeted diagnostics:

- ✓ Several different specific PCR methods
- ✓ PCR result interpretation depends on
  - Choice of tests
  - Seasonal variation
  - Reliability of the individual tests
- ✓ Sequencing results:
  - Fragmented
  - Low resolution



A : cv. Hedelfinger
B : cv. Bigarreau-Burlat
C : cv. Bigarreau-Reverchon
Grafted 5-6y ago on same rootstock

D rootstock *(wild P. avium)*
CVA, PDV, LChV1, ?
(not sequenced by NGS yet)

Link disease symptoms and untargeted diagnostics (NGS):

- ✓ Unbiased and broader view
- ✓ NGS result interpretation depends on
  - Sequencing strategy
    - Virus enrichment
    - dsRNA, smallRNA, totRNA
  - Depth of sequencing
  - Bioinformatics pipeline
- ✓ Sequencing results:
  - Across genome variation study possible

Case study strategy:
SmallRNA sequencing
NEBNext Ultra RNA Lib kit *
Illumina NextSeq v2 *
3M reads; 1x 50bp
VirusDetect automated pipeline
SearchSmallRNA ref. genome mapping

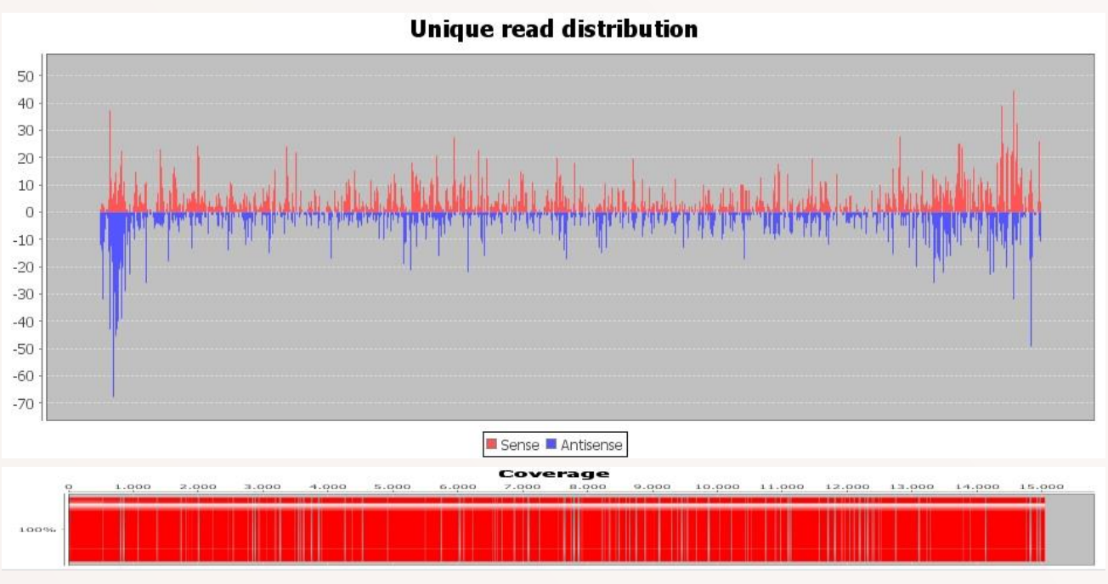
* Admera Health, USA

A : CVA, PDV + novel virus ?
B : CVA, PDV, SLRSV, LChV1, LChV2
C : CVA, PDV, SLRSV, LChV1

? *≠ virus/host interaction ? (cv ? rootstock ? …)*

Direct advantages of the NGS strategy:

- ➢ *Strawberry latent ringspot virus* was not picked up by traditional strategy !
- ➢ Detection of novel viruses.
- ➢ Endpoint PCR detection of LChV-1&2 often erratic.
- ➢ Full genomes of CVA, PDV, LChV1, LChV2 obtained.



Better epidemiological insight
Allows standardisation of diagnostic methods

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