



BIOLOGY
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Application of Next Generation Sequencing (NGS) for detection of plant pathogens

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Plant pathogens

- ◎ viruses
- ◎ viroids
- ◎ phytoplasmas
- ◎ bacteria
- ◎ fungi



Why diagnostics is important?

⦿ Practical purposes

- **elucidate** crop losses with unclear etiology/ **new disease**
- **certification** of pathogen-free seed material for growers
- testing **imported/exported plant** material to prevent spread of pathogens between countries and continents

⦿ Research purposes

- **searching** for novel pathogen species
- study of pathogen's population at the level of a single host



Why diagnostics is important?

- ⦿ **Diagnosis** . what caused the **disease** symptoms
- ⦿ **Monitoring** . looking for a specific **pathogen**
- ⦿ **Surveillance** . **prediction** for future risks



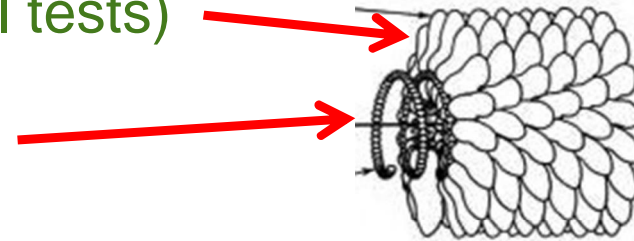
Why diagnostics is important?

- ◎ **Diseases with unknown etiology:**
 - unknown pathogen
 - complicated and inefficient sample preparation
 - difficulties in a transfer to a new host
- ◎ **Pathogens present at low titer**
 - phloem-limited viruses

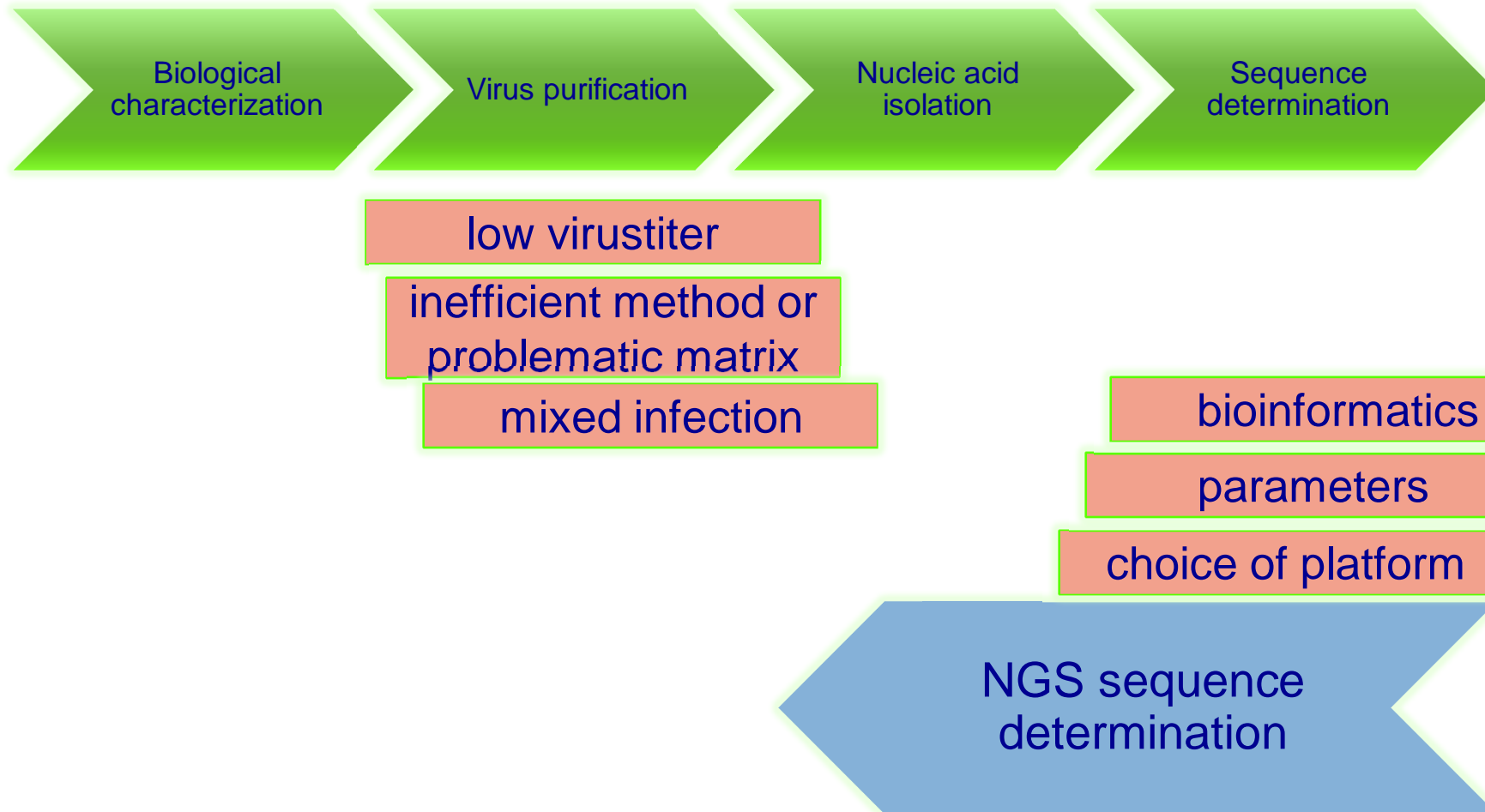


Methods of detection and identification

- Visual inspection, electron microscopy
- Serological detection (ELISA, lateral tests)
- PCR, qPCR, hybridization methods



Traditional diagnostics vs NGS





How?



Sample



Nucleic acid
isolation



Preparation of
sequencing
libraries



Sequencing

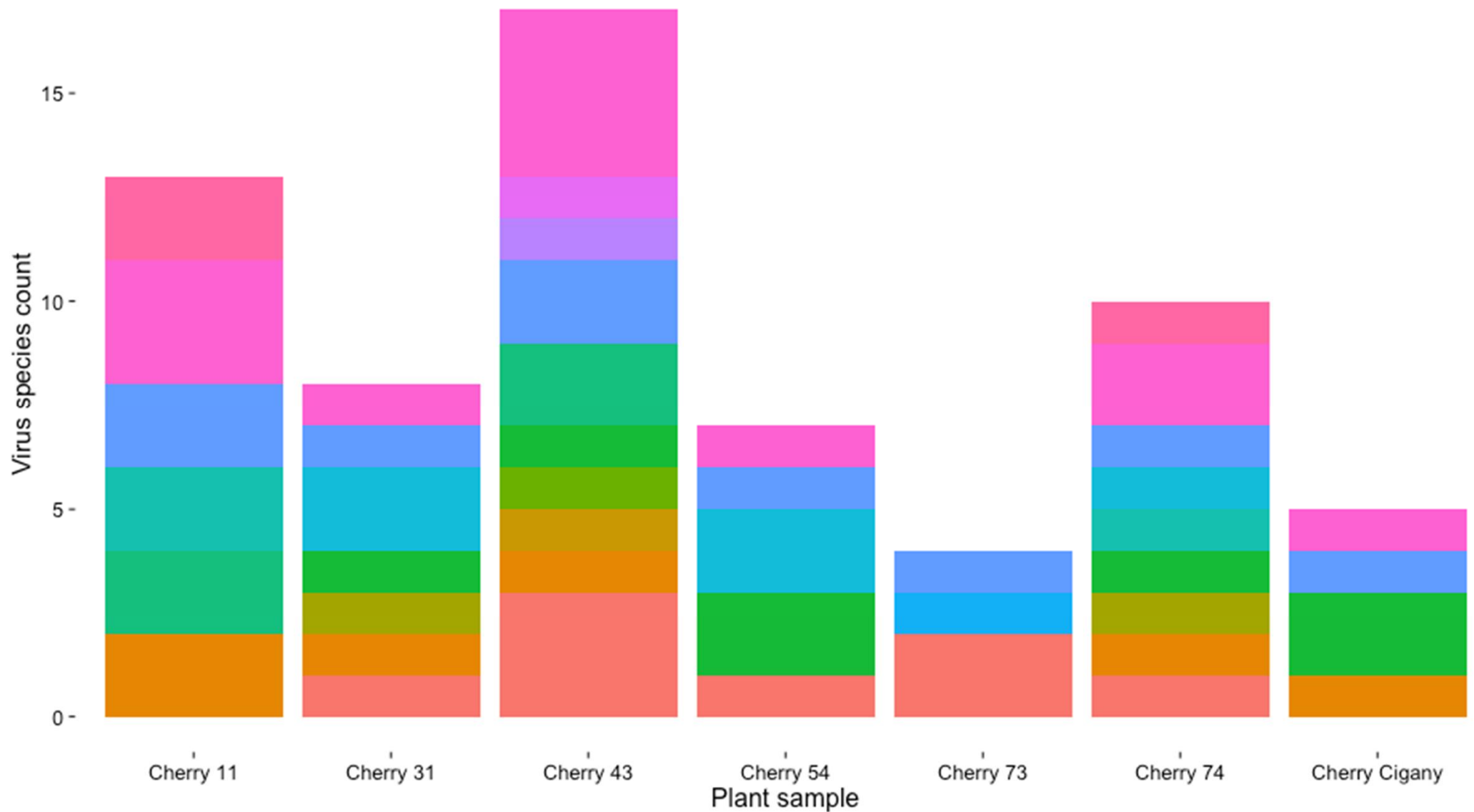


Bioinformatic analysis,
verification of the results

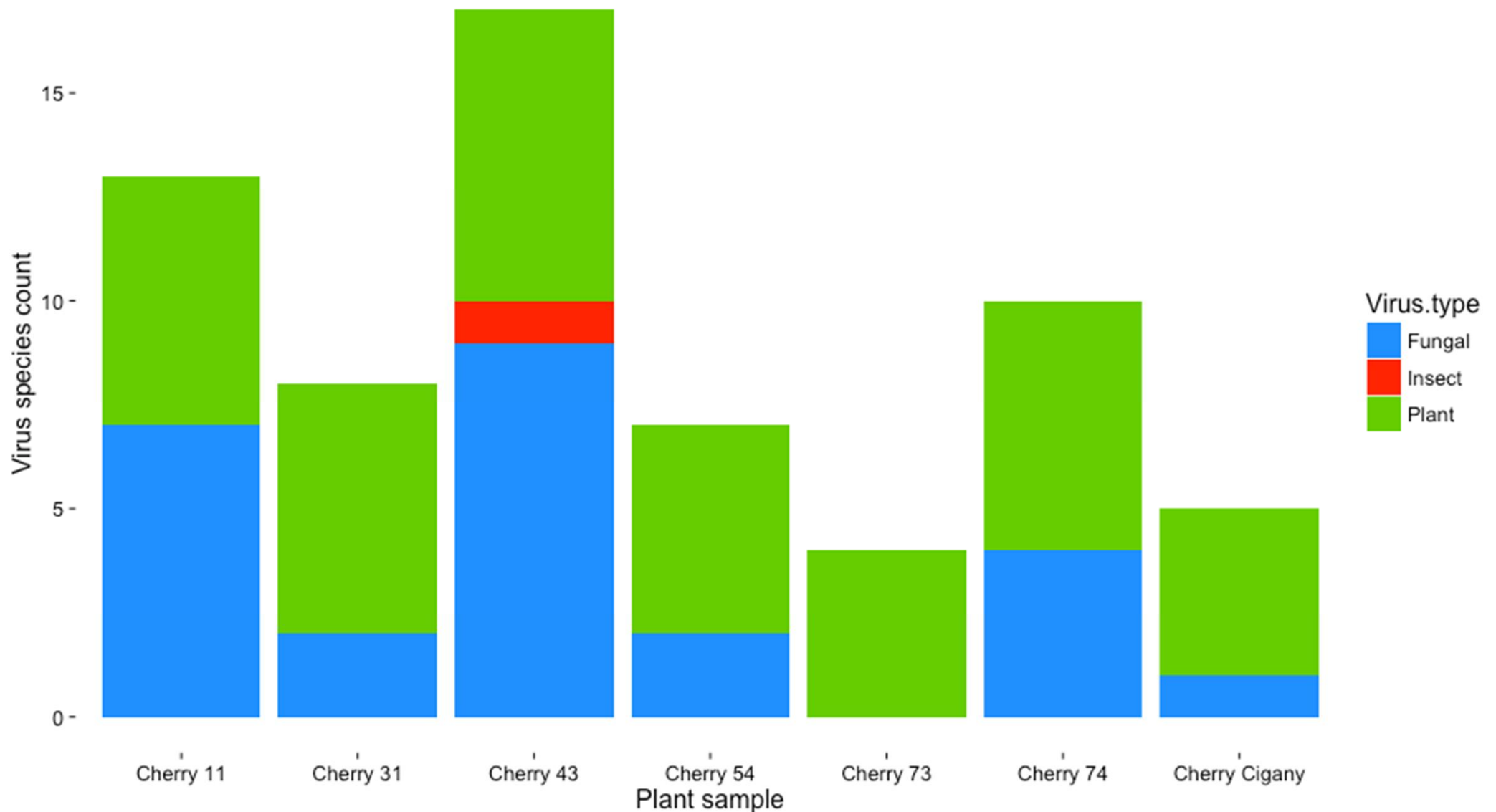




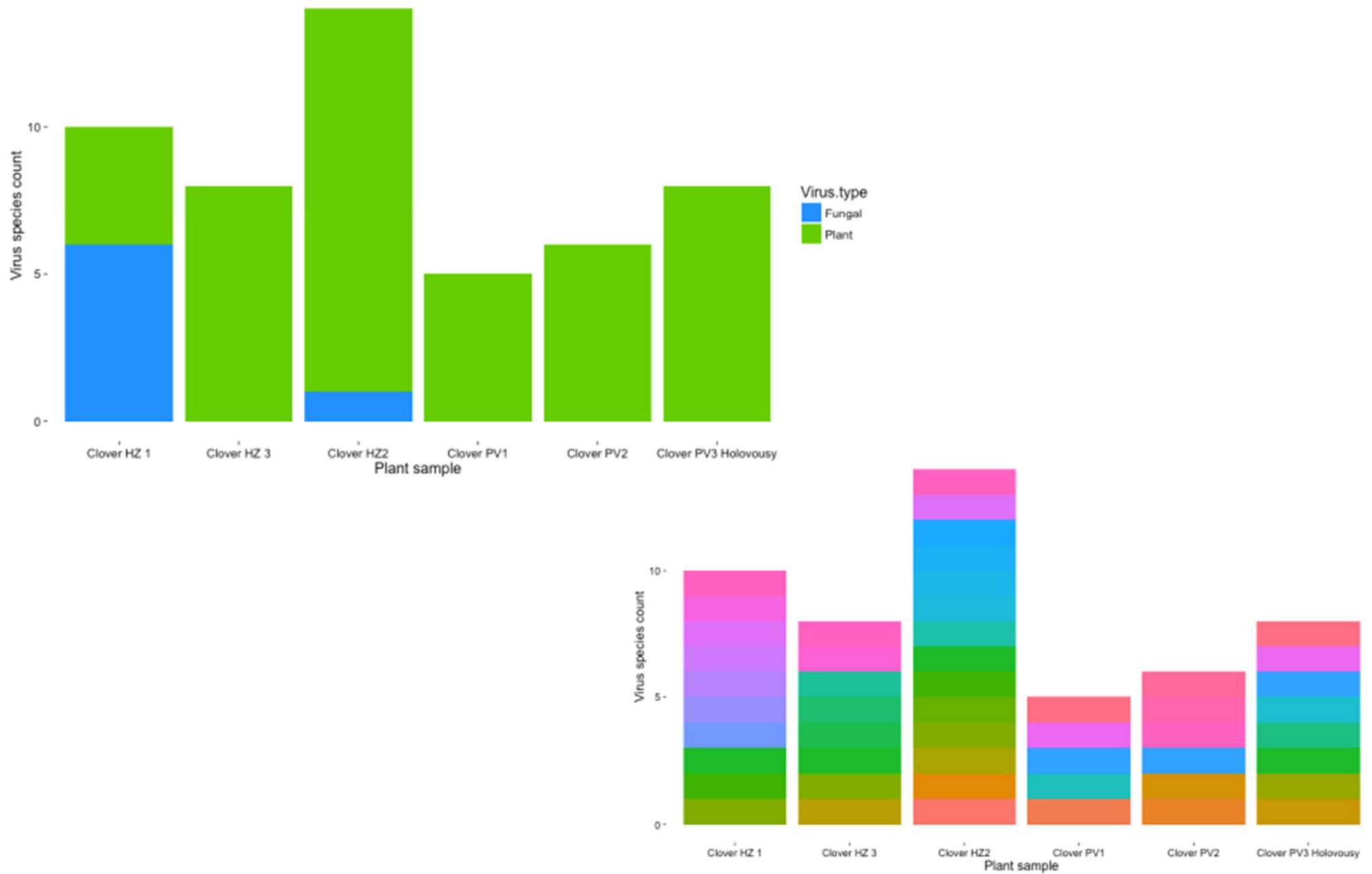
Species diversity of viruses per cherry sample



Species diversity of viruses per cherry sample; sorted by host type



Species diversity of viruses per clover sample





Problematic sample with unclear ethiology

◎ **Strawberry cv a anska rana**

A summer-fruiting cultivar of moderate vigor and marked production of runners.

Early vegetation onset.

Flowers and ripens early.

Very good cropping.

Ripens within 20 days.

Resistant to fungal diseases of strawberry



Problematic sample with unclear ethiology

◎ Strawberry cv. a. anska rana

- known host of:
 - Strawberry crinkle cytorhabdovirus
 - Strawberry mottle sadwavirus





Problematic sample with unclear ethiology

◎ Strawberry cv a anska rana

- known was presence of:
 - Strawberry crinkle cytorhadbovirus
 - Strawberry mottle sadwavirus
- NGS revealed additionally:
 - Cherry leaf roll nepovirus
 - two genotypes of Strawberry mottle sadwavirus



Perspectives/challenges of NGS

- going deeper at the scale of \pm omicsq
- versatility of application
- extra sequence information from a sample

VS

- high analysis cost per sample
- complexity of the data analysis
- biases for low-titer viruses



Transfer of technology

- ⦿ Verified NGS method for the detection of plant pathogens developed at IPMB
- ⦿ License offered to private and government institutions for:
 - Pathogen detection
 - Seed certification (potato, fruit trees)
 - Quarantine purposes

Acknowledgement

Jana Fránová
Jaroslava P ybilová
Josef Źpak
Karel Petrzik
Lenka Hrabáková
Ond ej Lenz

