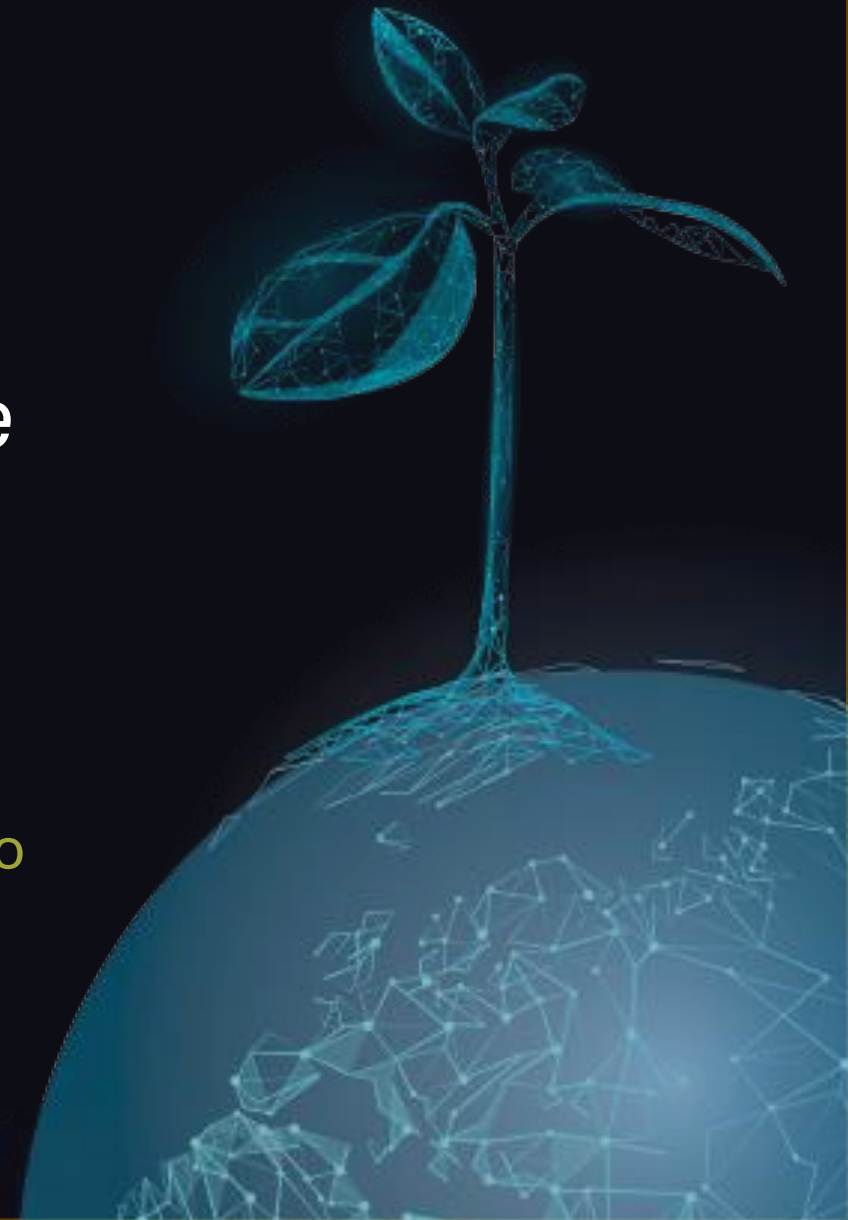




SESSION TWO
*Shifting Landscapes: The
Rise of Novel Pests and
Pathogens*

SPONSORED BY AgroFresh

Chair: Dr Nanike Esterhuizen | Hortgro



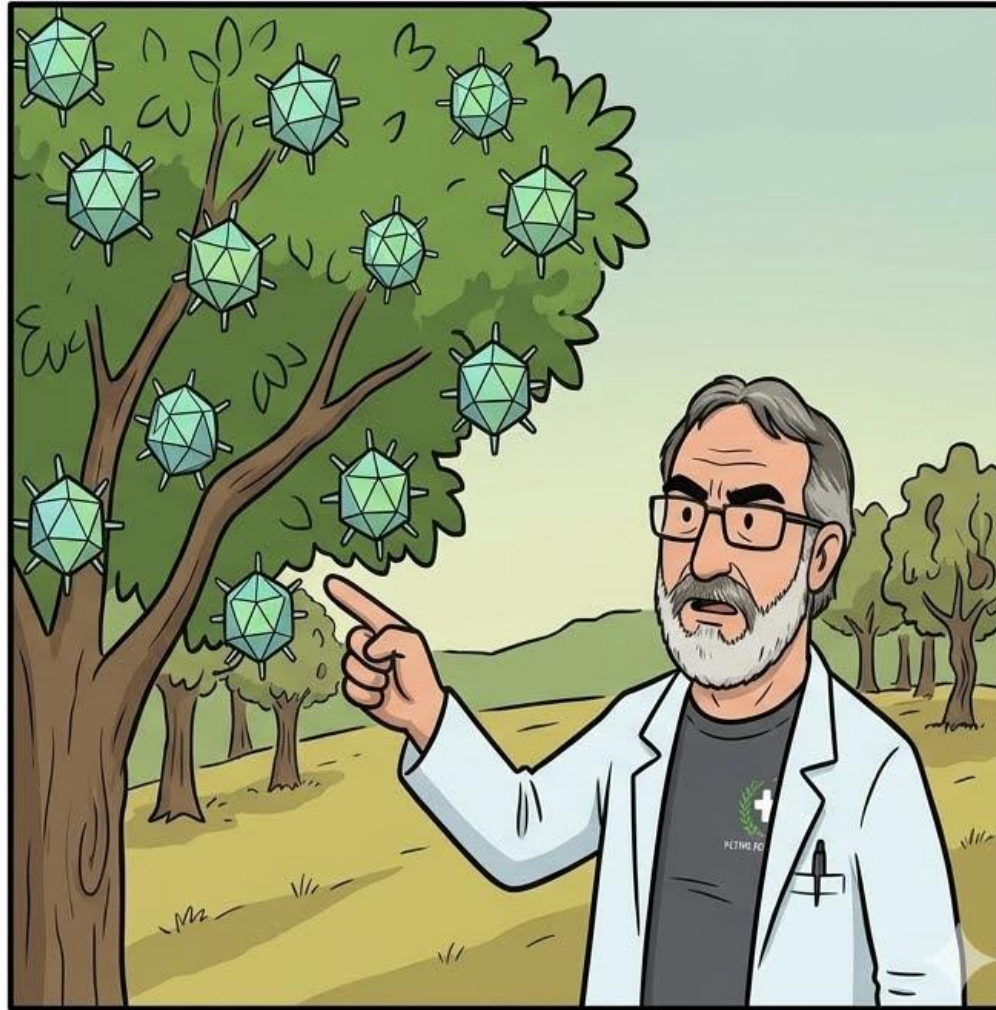
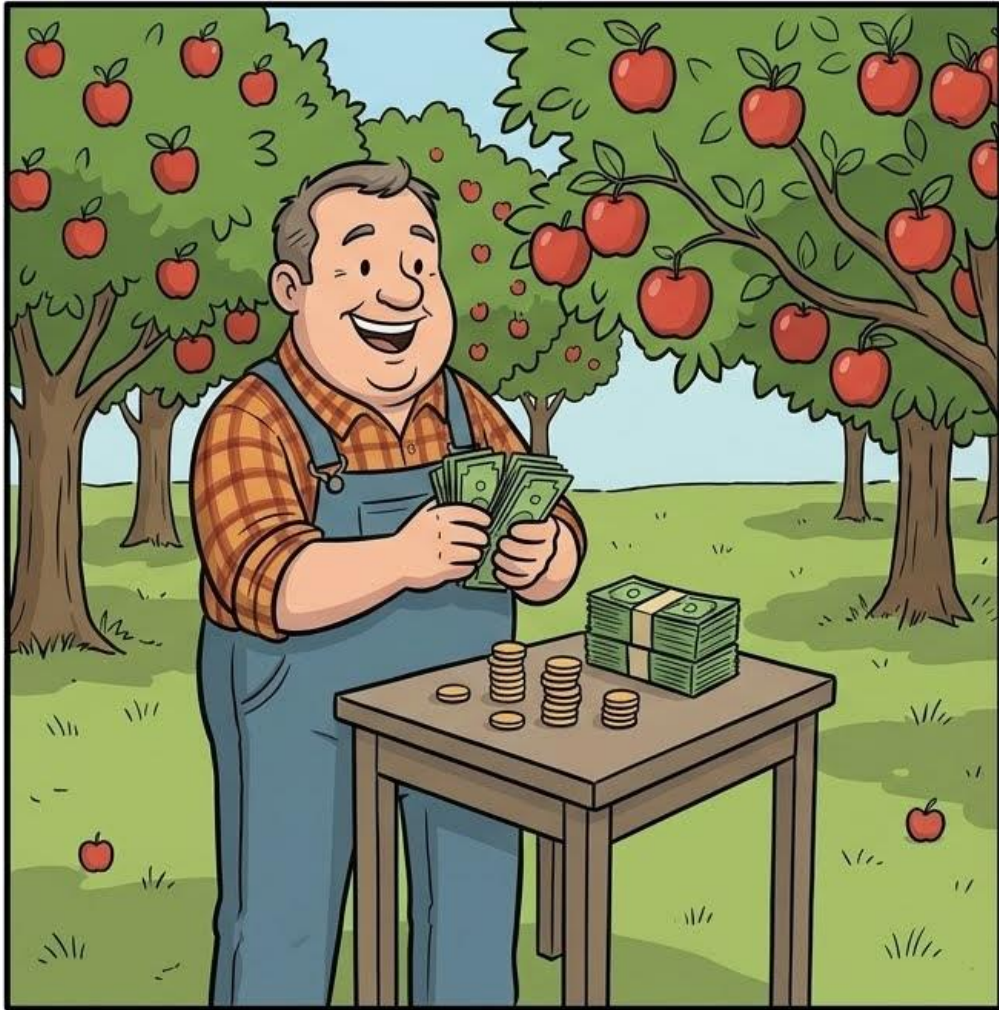


Viruses in your trees?

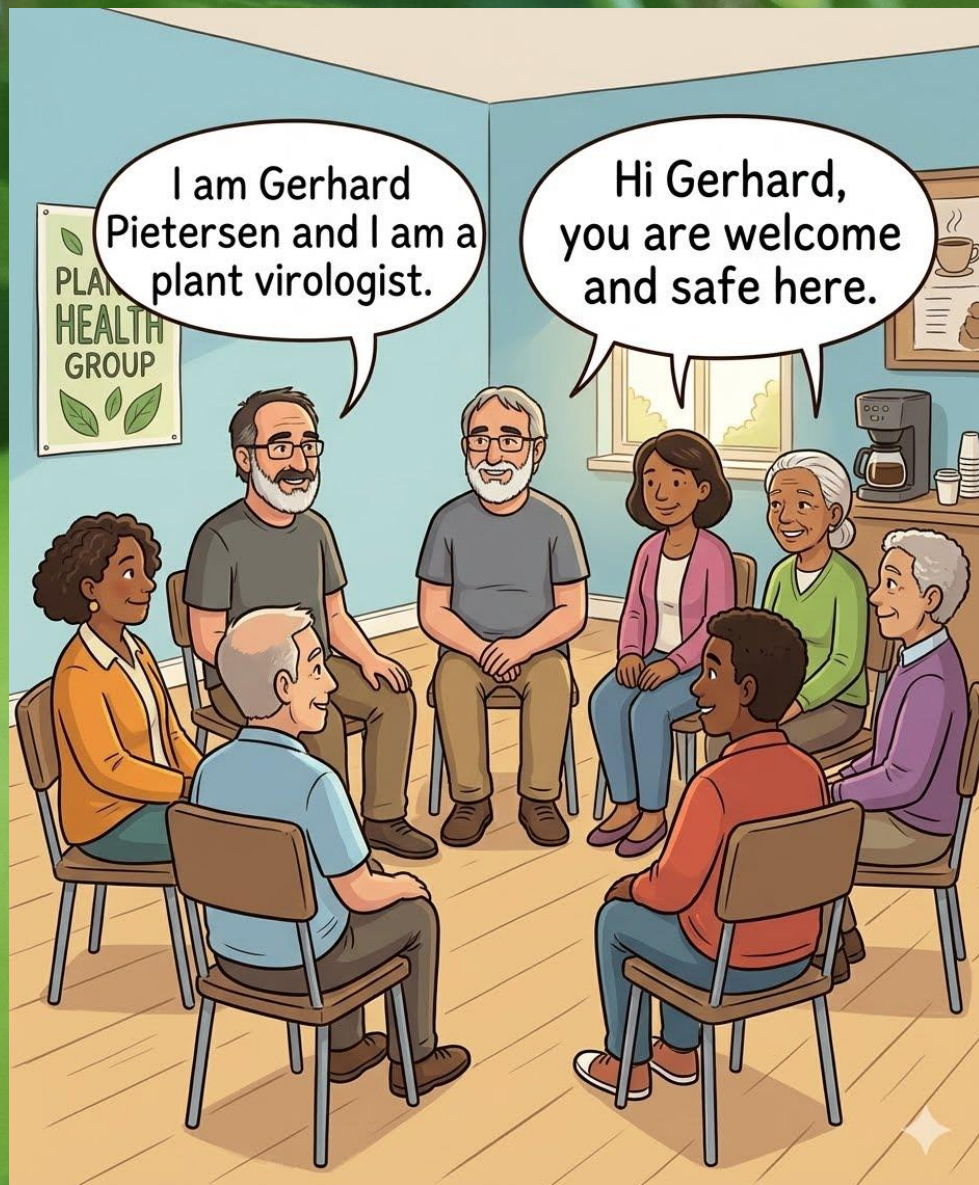
Prof. Gerhard Pietersen | Patho Solutions



Viruses in Your Trees?



PATHO SOLUTIONS



How High throughput sequencing (HTS) has changed plant virology, and the implications for horticultural crops.



The New Era of Plant Virus Detection and Identification

Plant virus detection and identification, which evolved over the decades has recently changed dramatically.

nod.

Identification which used to take months to years 10 years ago...

months to years.

The Old Way (2013)

...can now be done in days and done much better — but interpretation has become much more complex.

Within crops, including the Pome and Stone fruits, tests on plants are revealing large numbers of viruses.

Pome Fruit

Stone Fruit

An awareness as to the proper response to this is required.

Indeed. The field is changing fast.



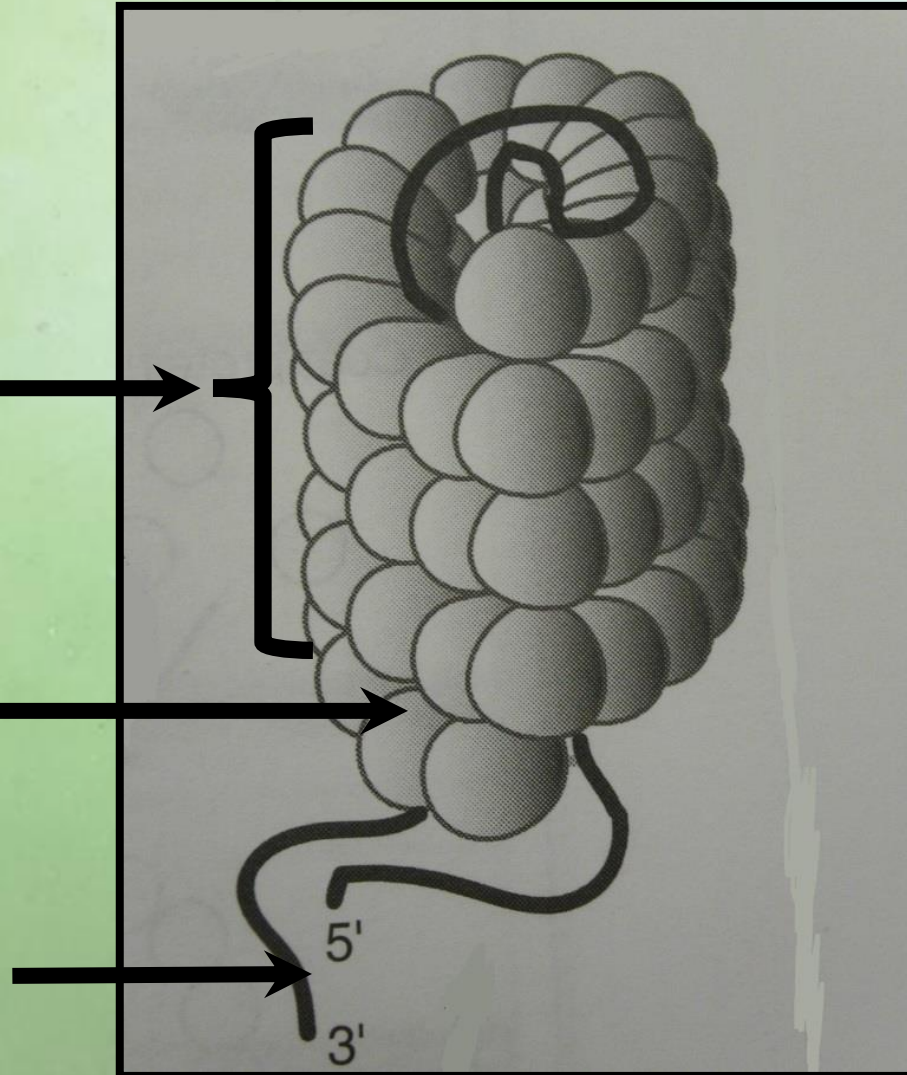
Detection of these viruses reflect changes in virus detection techniques over time.

Virus properties:

Virus particles: Electron microscopy

Virus proteins: Serology

Virus nucleic acids: Molecular Techniques



Virus properties: Transmission, symptoms, host range, vector

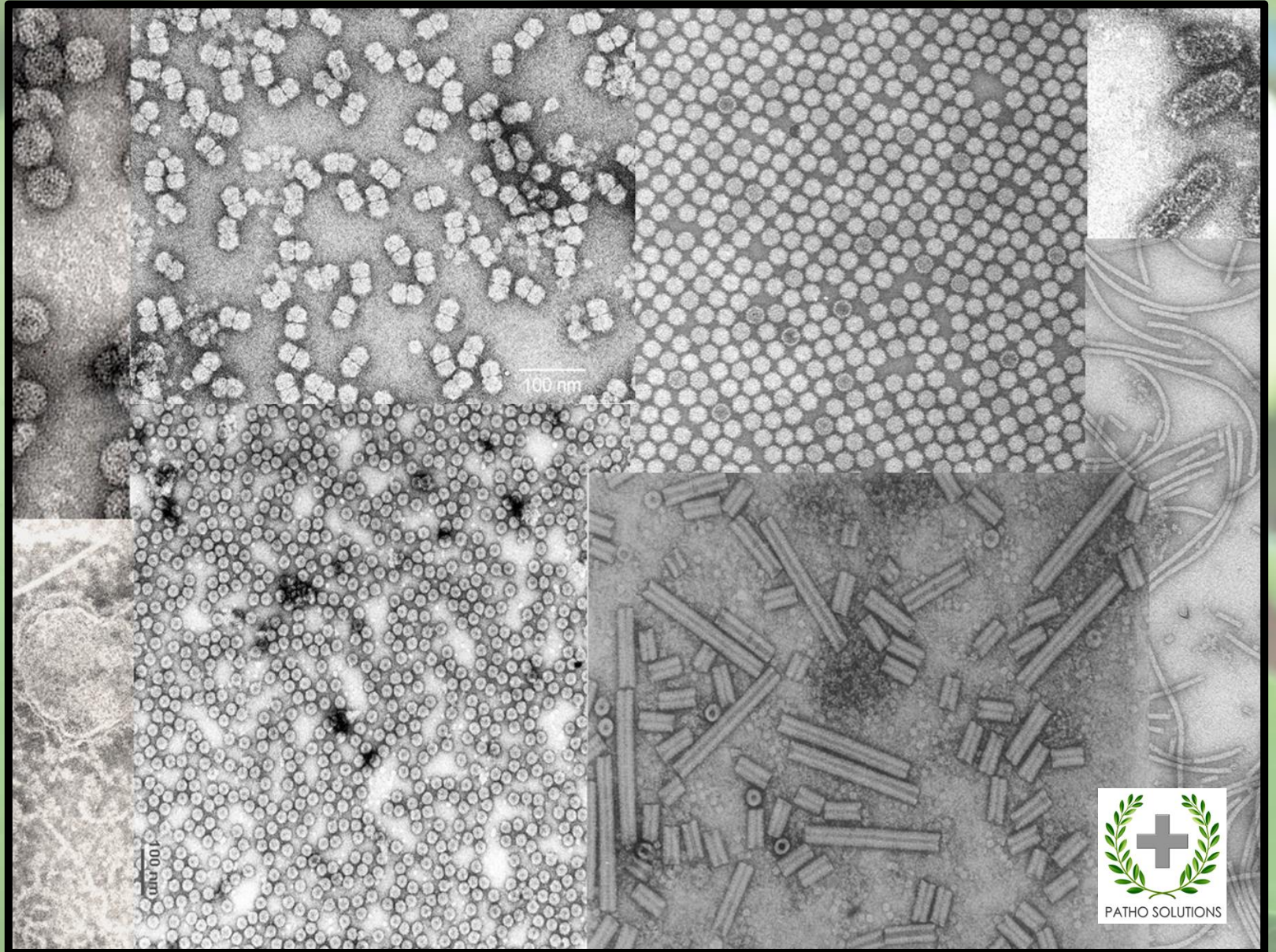
1960s - : Biological indexing.

- **Grafted material onto indicator plants and waited for symptoms — often months.**
- **Could only test few trees.**
- **Didn't detect latent or symptomless plants.**
- **Advantage revealed disease causing viruses**



1970s–90s Virus particles: Electron microscopy.

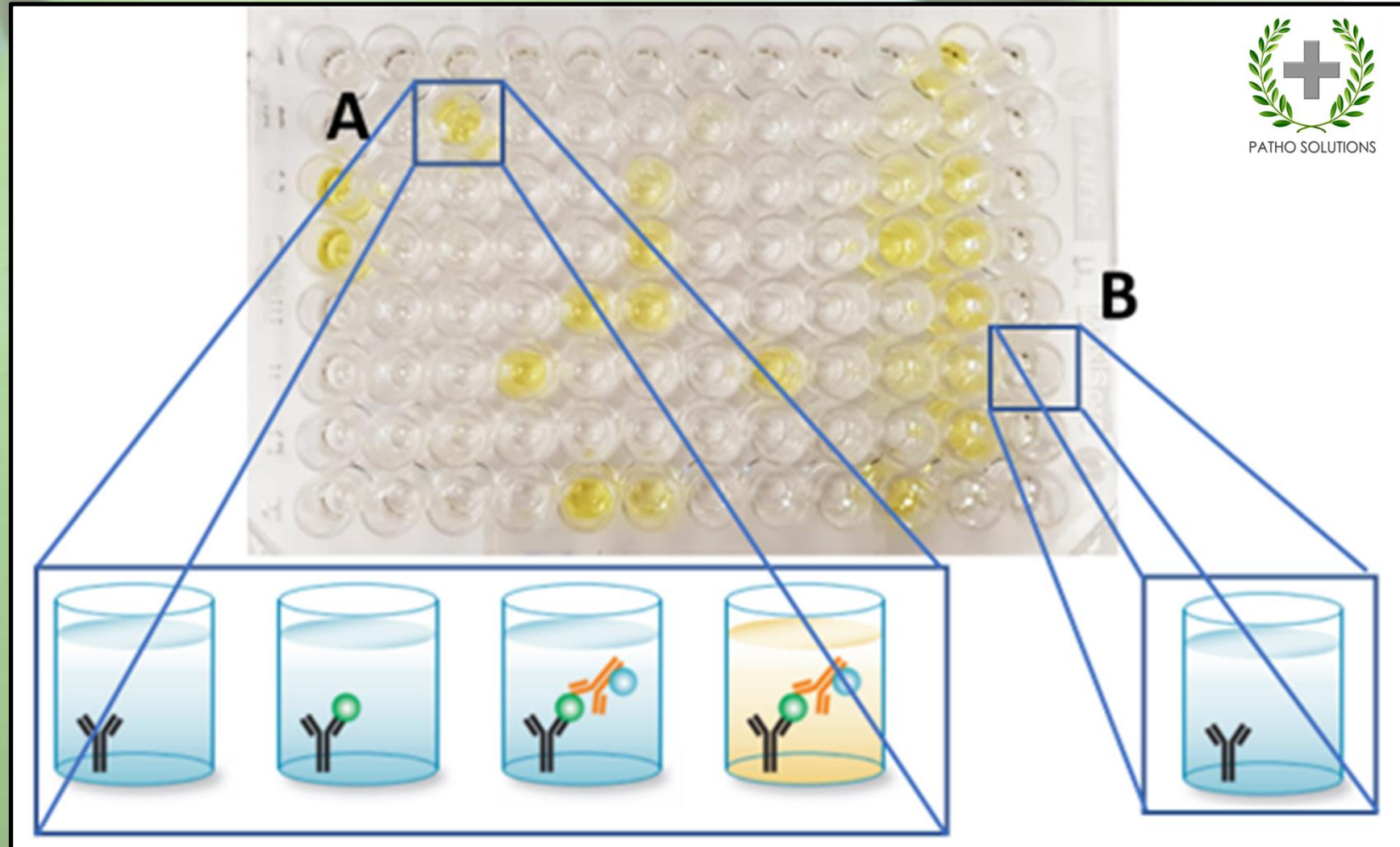
- Could see virus particles but couldn't easily identify which virus.
- Very expensive.
- Could only test small number of samples.



1980s– : Detect virus proteins (used antibodies).

ELISA: Excellent for large scale testing.

Relatively low sensitivity. Could miss virus when titers low (seasonal detection, distribution in plant erratic).



1990s—: Detect viral nucleic acid.

PCR:

Much more sensitive/easier to establish.

Amplified specific viral sequences.

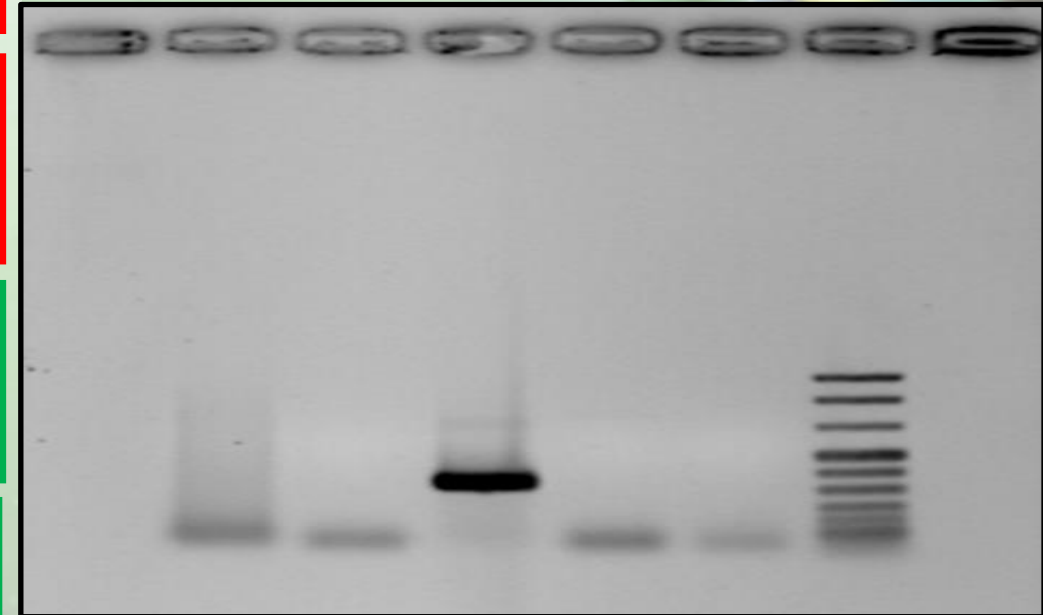
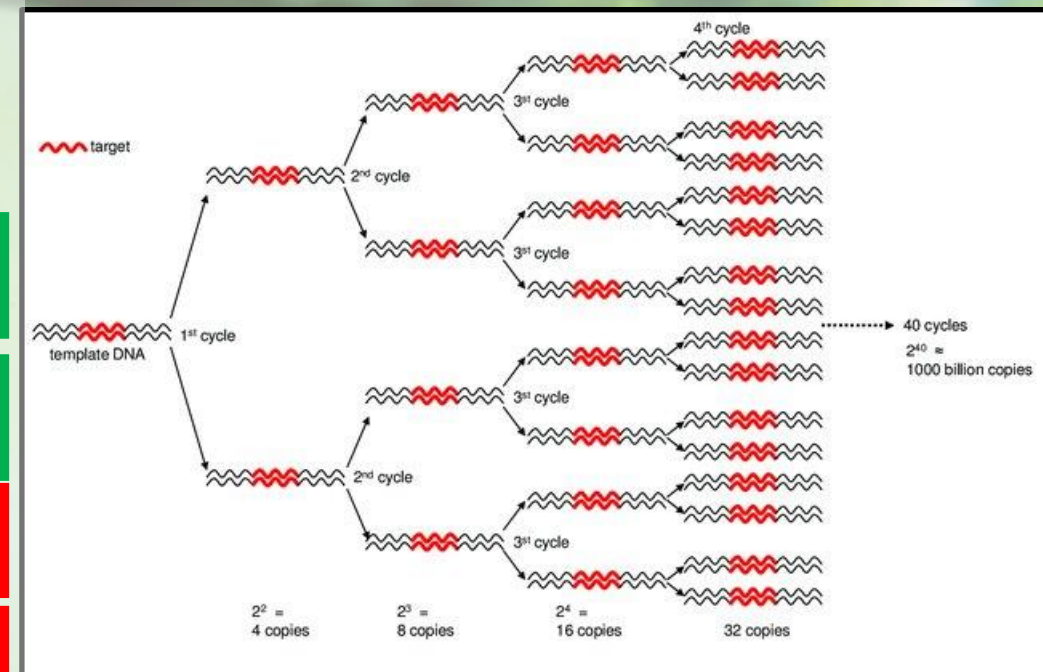
Relatively expensive. Not as robust as ELISA

Technically more difficult than ELISA.

Need to have a sequence available for the virus we were detecting.

Becoming increasingly better as more viral sequences becoming available.

Recently, more feasible on larger scale (real-time PCR, automation)



Traditional Virus Detection

All these methods were **targeted** — you could only find the virus(es) you were looking for.

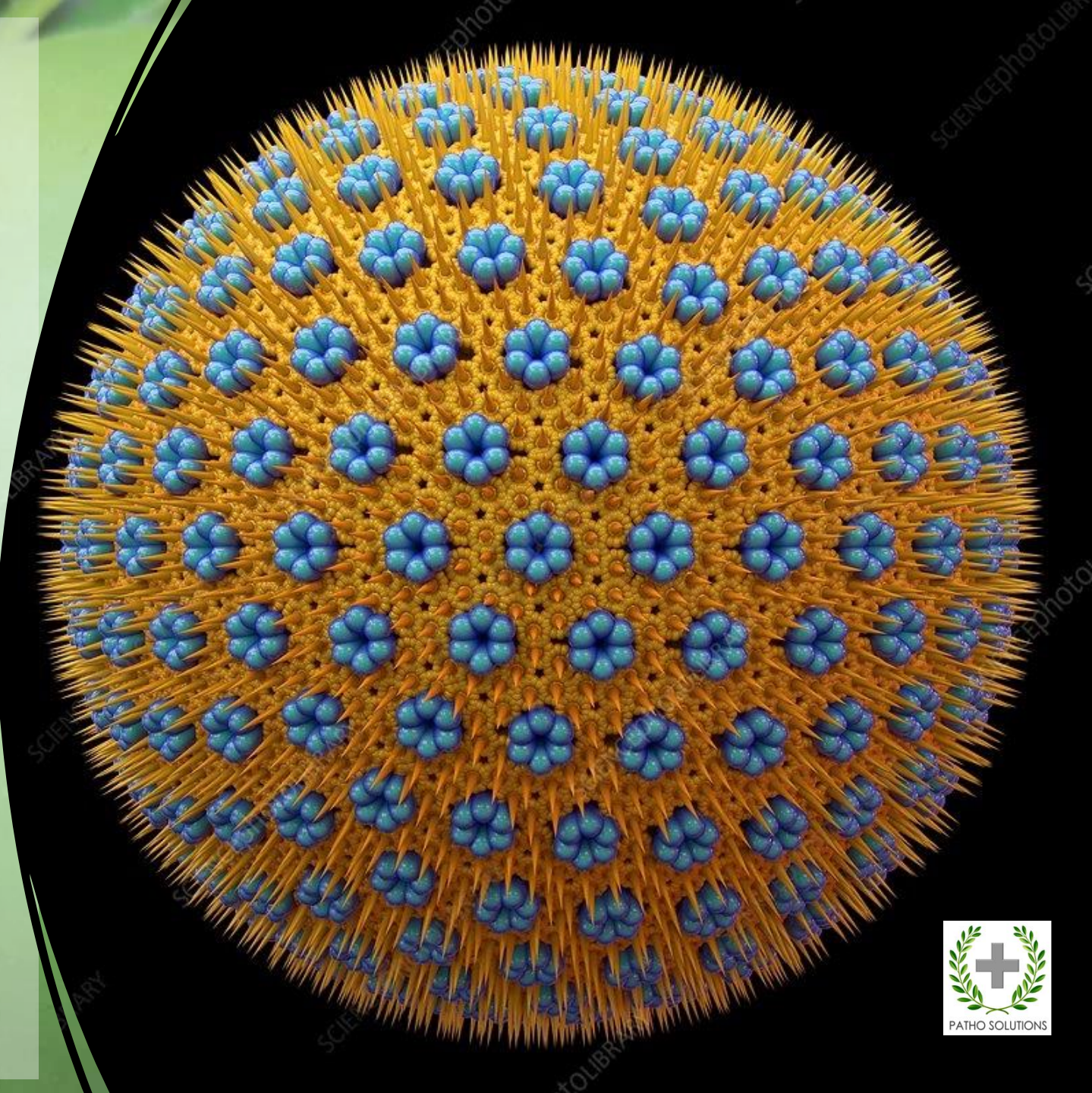


The Game Changer: *High throughput sequencing (HTS), also known as next generation sequencing (NGS).*

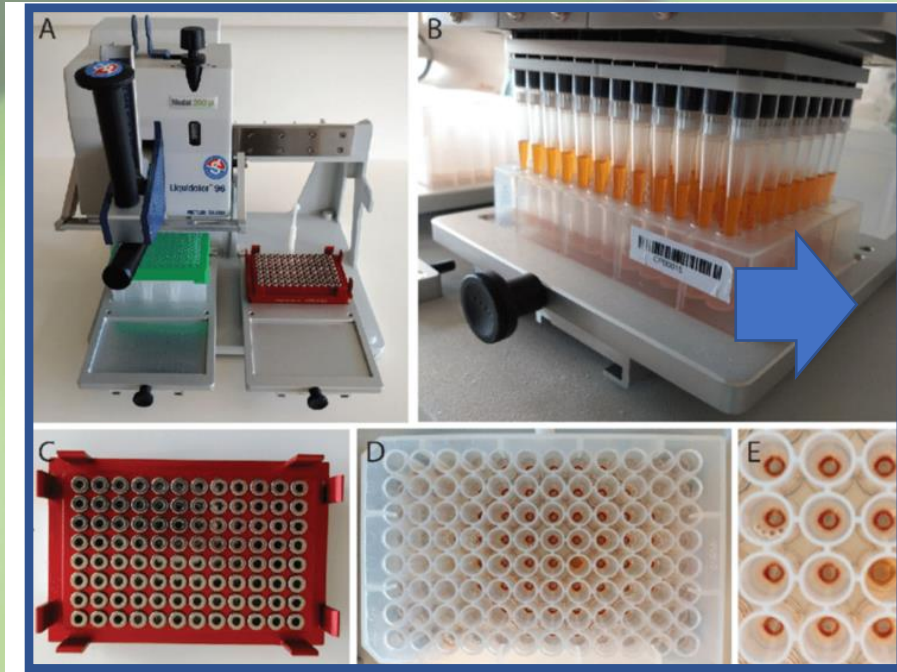
Not just specific virus(es) at a time.

Not limited to known viruses.

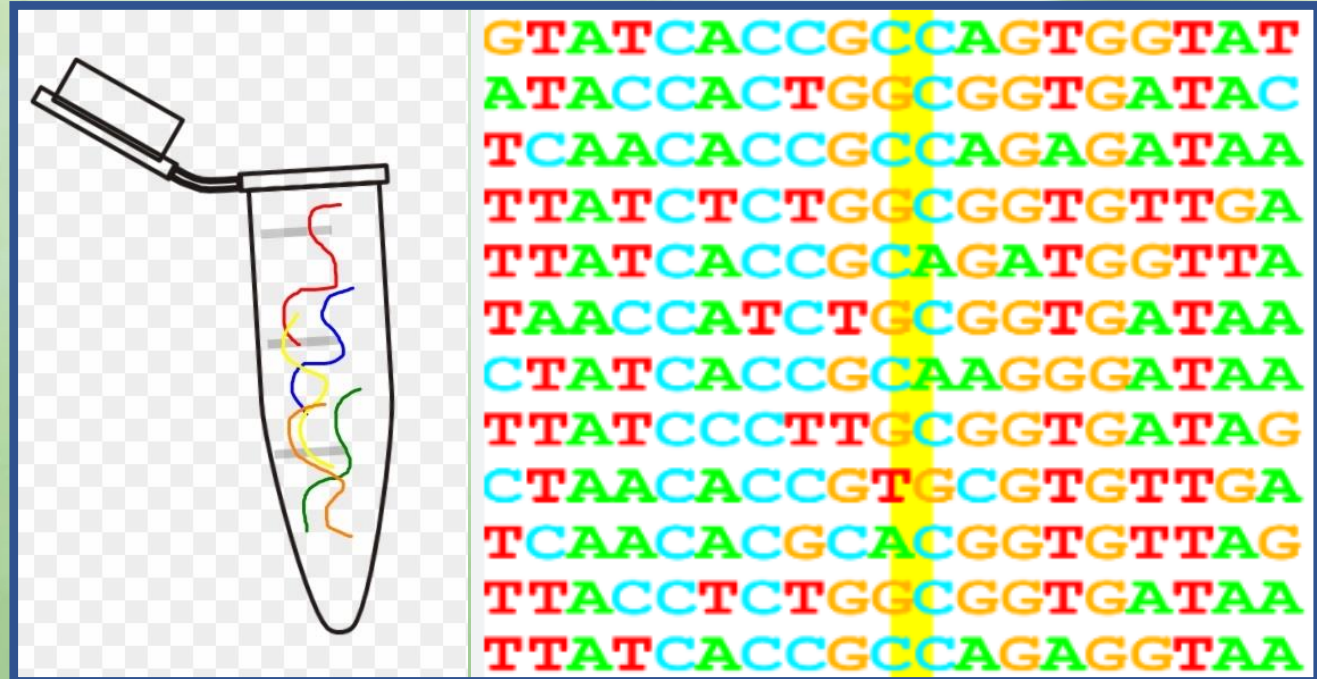
Can detect ALL the viruses in a sample (known viruses, variants of known viruses or viruses distantly related to known viruses).



High throughput sequencing (HTS) for virus detection



Extract nucleic acid



Sequence all the nucleic acid in extract
(plant, viruses, bacteria, fungi).



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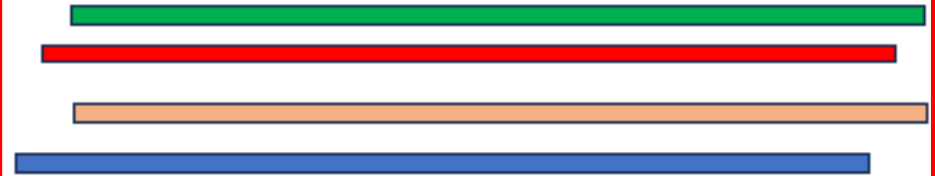
Millions of reads



National Library of Medicine
National Center for Biotechnology Information

GenBank

Virus references



**Assembly of Reads to form
larger contigs**

REFERENCE GENOME



ALIGNMENT/MAPPING



THE REFERENCE GENOME

C C A T G C A T T A C C G C A T G G C A T T A C G T A G C



NEXT-GENERATION SEQUENCING READS (ILLUMINA)

READ DNA FRAGMENT
C C A T G C A T T A C C

READ DNA FRAGMENT
G C A T T A C C G C A T

READ DNA FRAGMENT
C G A T G G C A T T T A

READ DNA FRAGMENT
G G C A T **C** T T A C G T

BWA ALIGNER

BWA ALIGNER

BWA ALIGNER

BWA ALIGNER



THE MAPPING / ALIGNMENT

READ 1 (PERFECT)
C C A T G C A T T A C C

READ 2 (PERFECT)
G C A T T A C C G C A T

READ 3 (PERFECT)
C G A T G G C A T T T A

READ 4 (WITH SNP)
G G C A T **C** T T A C G T

READ 1 (PERFECT)

READ 2 (PERFECT)

READ 3 (PERFECT)

READ 4 (WITH SNP)

100

105

110

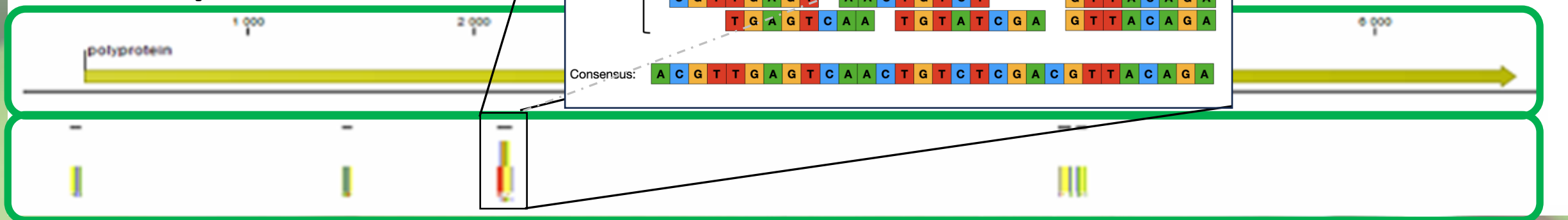
115

120

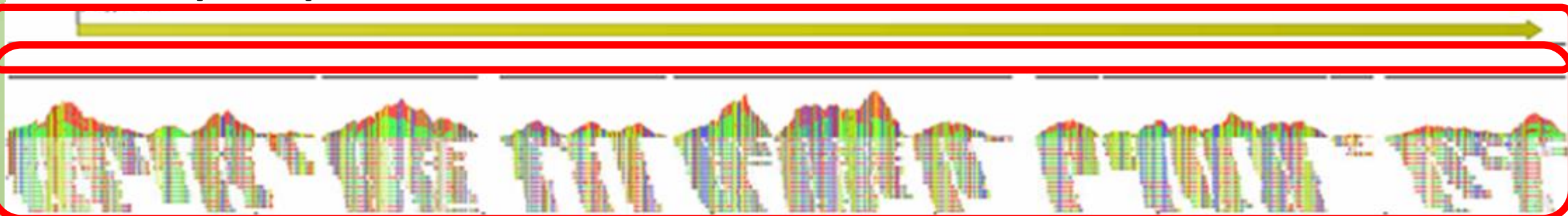
125

Match against thousands reference sequences of plant virus species

An example of a “no match”



An example a positive match: Virus “B”



HTS has revolutionized plant virology — we now detect viruses that were not detectable before.

Consequently, discovering new viruses regularly and discovering viruses in plants previous thought healthy.

Interpretation of findings more complex than with targeted detection methods (variants, strains, related viruses, novel viruses).



Through a combination of PCR and HTS a number of previously unreported viruses and viroids of pome and stone were found in South Africa during the last few years:

Apple rubbery wood virus 1

Apple rubbery wood virus 2

Apple green crinkle- associated virus (ASPV variant)

Citrus concave gum associated virus

Citrus virus A

Citrus jivi-related virus

Hop stunt viroid

Plum viroid -1 (plum marbling)



The logic(?) of plant virus names

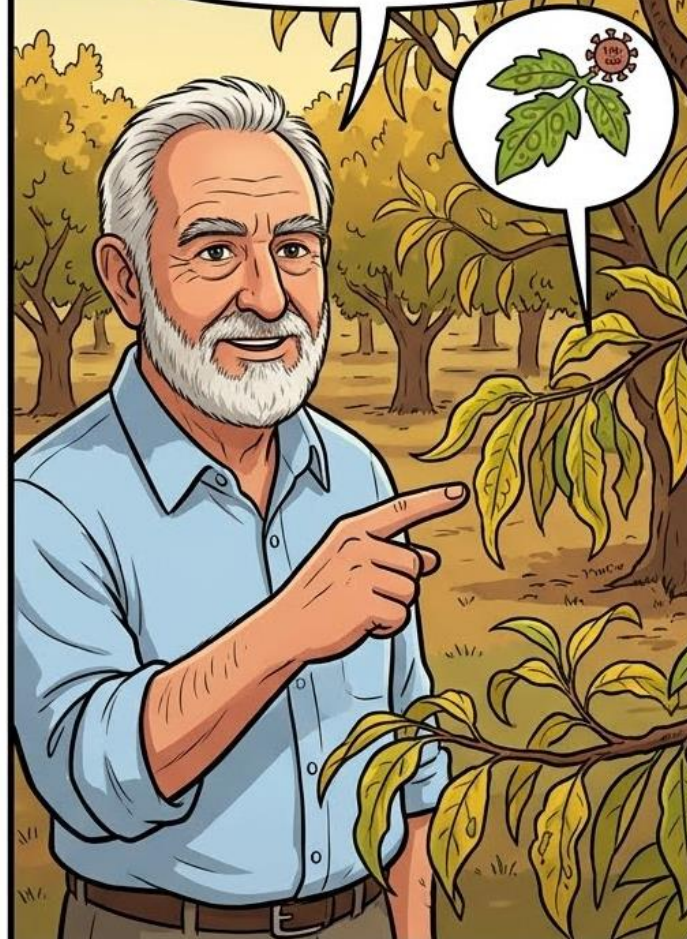
THE DISCOVERY - 1990

I've named a new virus
"Tomato Ringspot Virus"
because it makes ringspots
on a tomato!



THE REVELATION - 2020

The virus causing these
yellow distorted leaves on
peach trees is Tomato
Ringspot Virus!



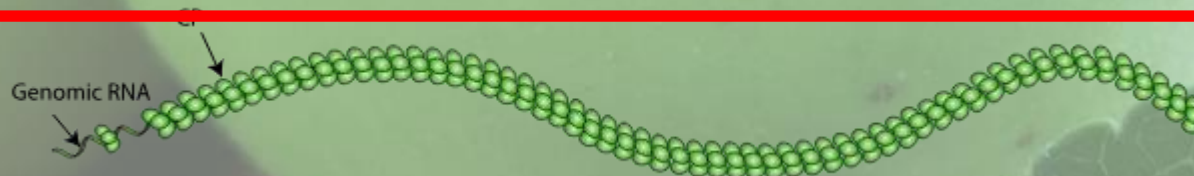
Limitations of HTS:

HTS tells us which viruses are *present*, not which ones are *causing symptoms*.

Trees, especially older ones, often carry several latent or symptomless viruses.

The presence of a virus in a number of trees with a specific symptom but absent in other trees without symptoms is considered an *association*, not proof of *causation*.

Determining causation requires controlled experiments



Don't panic when you see a list of viruses in your trees.

It's tempting to worry, but many of the viruses may have only limited effects.

Many of these viruses may have been present for a long time already, just not detected and identified.

The real risk is overreacting — removing trees or making management decisions based on incomplete information.

Always interpret HTS results with expert input.



Challenges for Plant Virologist today.



HTS has uncovered hundreds of new plant viruses.

For most, we don't know the extent to which they cause disease, reduce yield, or affect quality.

Viruses often occur together in mixed infections. Difficult to find single viruses infecting plants to test for causation.

Testing yield and quality impacts requires replicated field trials over years (also with single viruses). Also, must do trials with virus combinations (some cause diseases only when combined).

Hence, many NGS-detected viruses remain uncharacterized in terms of their biological significance.

Best solution: Certification Schemes.

- . We won't always know which viruses matter yet,**
- . The safest approach is to start with uninfected material.**

Certification programs:

- . Use NGS to detect all known and novel viruses.**
- . Eliminate as many as possible via:**
 - Meristem tip culture,**
 - Heat or cold therapy,**
 - Tissue culture techniques.**
- . Propagate virus-tested, as uninfected planting material as possible.**



spv|dpa

SAGTEVRUGTEPLANTVERBETERINGSVERENIGING
DECIDUOUS FRUIT PLANT IMPROVEMENT ASSOCIATION

HORTGRO

Growing Fruit IQ



Incorporate NGS tests on nuclear material in the certification scheme.

Establish expertise in virus elimination (SAPO, TFC-Bernheim, Patho Solutions).

Implementation on new material entering scheme. Also, retrospective tests on existing material, re-introductions.

IPO Owners of cultivars to subject material for certification.

Encourage growers to only purchase certified planting material.



