



Australian Government
Department of Agriculture
and Water Resources

Next Generation Sequencing: A new tool for detecting phytosanitary pests



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1. Testing for viruses/viroids in plants

Current phytosanitary testing methods

- Generic tests:- Visual inspection, bioassays and electron microscope
- Specific tests:- Serological (ELISA) and molecular (PCR)

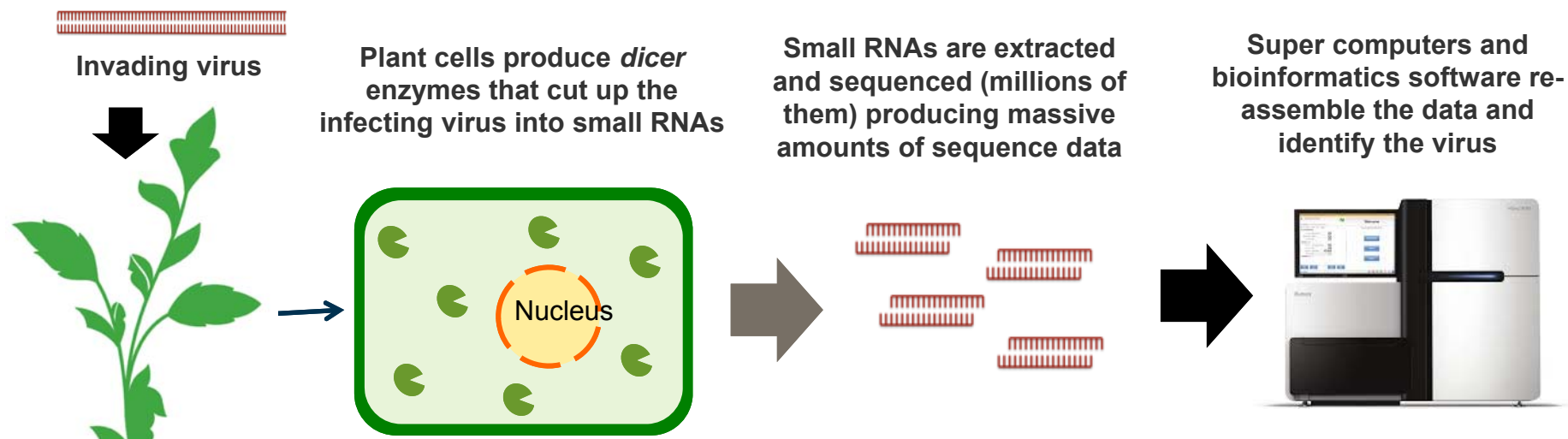


What's wrong with the way we do testing now?

- Many tests, slow and time consuming => (e.g. citrus >20 tests, 3+ years)
- Lots of greenhouse space and bioassays produce ambiguous results
- Requires knowledge of virus to develop and validate tests
- Global expertise in traditional diagnostics is declining

2. Next Generation Sequencing (NGS) - What is it?





- Can sequence complete genomes of pests quickly, cheaply and accurately
- In Australia/NZ, a 4+ year project has used small RNA sequence NGS and the plants '*immune response*'¹ to detect viruses/viroids in plants²



1. Andika *et al.*, Plant J., 2015

2. Roberto *et al.*, BMC 2017

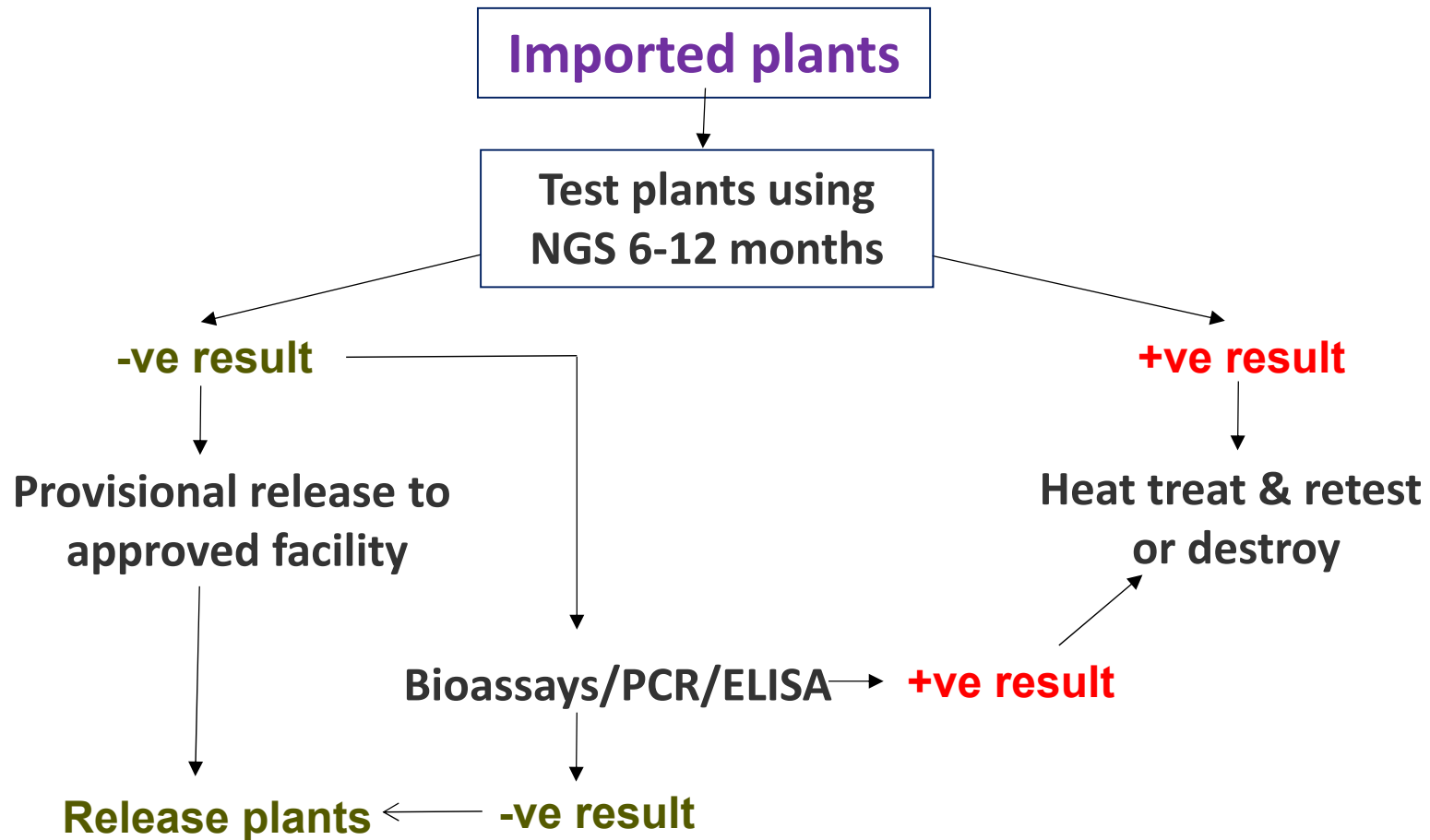
Case study: Current phytosanitary protocols in Australia

Commodity	Phytosanitary period	# of phytosanitary tests (A\$)	Plants tested	Pathogens detected by current methods	Pathogens detected by NGS
Grapevine 	18 months	24 tests (\$4K/cv)	3	4	13
Citrus 	27 months	19 tests (\$8.5K/cv)	5	3	6
Stonefruit 	18 months	23 tests (\$5K/cv)	9	1	1
Strawberry 	18 months	13 tests (\$5K/cv)	6	0	0

NGS

- Reduces phytosanitary period (6-12 months)
- Reduces costs using a single test (<A\$1K)
- Increases accuracy and effectiveness of detecting viruses/viroids

Case study: Using NGS in a phytosanitary context



4. Benefits of using NGS compared to current methods

Compared to current methods, NGS:-

- Improves detection of viruses/viroids and no prior knowledge of virus required
- Is more cost effective (using a single test c.f. many tests)
- Reduces time => faster access to new plants => globally competitive
- Can identify new and emerging threats => surveillance and rapid response to incursions
- Provides opportunity to develop a '*global plant passport*' for movement of plants with no NPPO holdup

5. Challenges using NGS in a phytosanitary setting

- Standard operating procedures and training in use of NGS including:
 - ❖ Sampling issues (when and what tissue to collect)¹
 - ❖ Quality controls for each step are needed
- NGS generates massive amounts of sequence data of genetic variants =>
 - ❖ Interpretation - what does the data mean (no biological context)?
 - ❖ Is the sequence a new pathogen?
 - ❖ Managing plants that '*inhibit*' sRNA response to virus infection²

Note:

1. These challenges exist with current methods so not really new
2. Most viruses of phytosanitary concern do not have capacity to inhibit plant antiviral response system

6. What is happening globally?

Lots happening with NGS - here are just a few examples:

- ❖ Canadian Food Inspection Agency investigating NGS for virus/viroid detection in propagative tree fruit material (2017-19)
- ❖ EPPO workshop on NGS technologies for plant pest diagnostics (Bari, Italy, Nov 2017)
- ❖ Australia/NZ investigating regulatory adoption of NGS (2014-18)
- ❖ EUPRHRESO projects:
 - Application of NGS for detection and diagnosis of viruses and viroids
 - Using NGS as a phytosanitary tool in a regulatory setting
- ❖ USDA Agricultural Research Service investigating NGS on berry crops and grapevine material

7. Where to from here?

IPPC asked to *consider* establishing an international task force including subject matter experts, policy regulators and members of IPPC Technical Panel on Diagnostic Protocols to:-

- ❖ *Identify constraints* in adopting NGS and *how* to address these including developing NGS guidelines and quality controls
- ❖ *Establish* a timeframe to adopt NGS for routine testing of viruses/viroids
- ❖ *Support* international efforts in obtaining scientific evidence on reliability and accuracy of NGS
- ❖ *Support* international proficiency testing to independently assess laboratory capability

7. Take home message

Reminder: Current phytosanitary protocols have got inherent weaknesses

Working together lets *implement* NGS as an Annex to ISPM 27 (Diagnostic protocols for regulated pests)

Arigato

Дякую

Hvala

Todah

Shukran

Dankie

teşekkür ederim

Danke sehr

Děkuji

Blagodaram

THANK

Ngiyabonga

YOU

Khop Khun Mak Kha

Gracias

Grazie

Merci

Köszönöm

Tānan

Obrigado

Kiitos

Do jeh

Спасибо