XYLELLA NDP

Validation and Endorsement process

Rachel Mann

Plant Health AUSTRALIA

NATIONAL DIAGNOSTIC PROTOCOLS (NDPs)

- Essential tools for the accurate and consistent identification of exotic pests and diseases

- Developed for

National Priority Plant Pests (NPPPs)

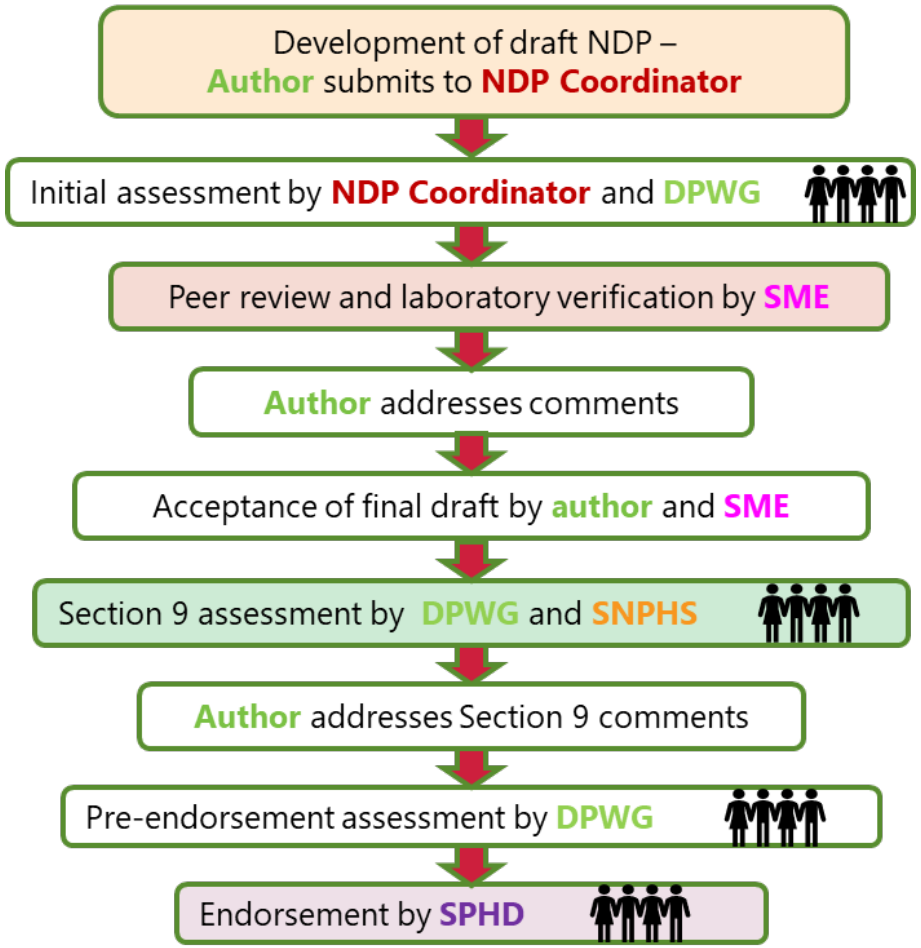
Exotic Environmental Pests (EEPs)

High Priority Plant Pests (HPPs)

NDP 45: Adult *Trogoderma granarium*, dorsal view. Two different coloured setae clearly visible (DPIRD, P Scanlon)

NDP 49: Disease symptoms of *Fusarium oxysporum* f. sp. *cubense* TR4 affecting Cavendish clones at the Coastal Plains Banana Quarantine Station, Northern Territory

NDP DEVELOPMENT

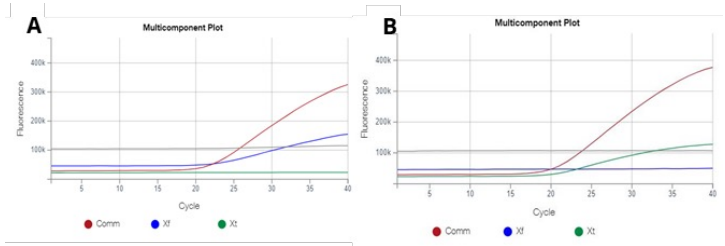


Research projects typically generate draft NDPs

This project

Current progress

NDP DEVELOPMENT



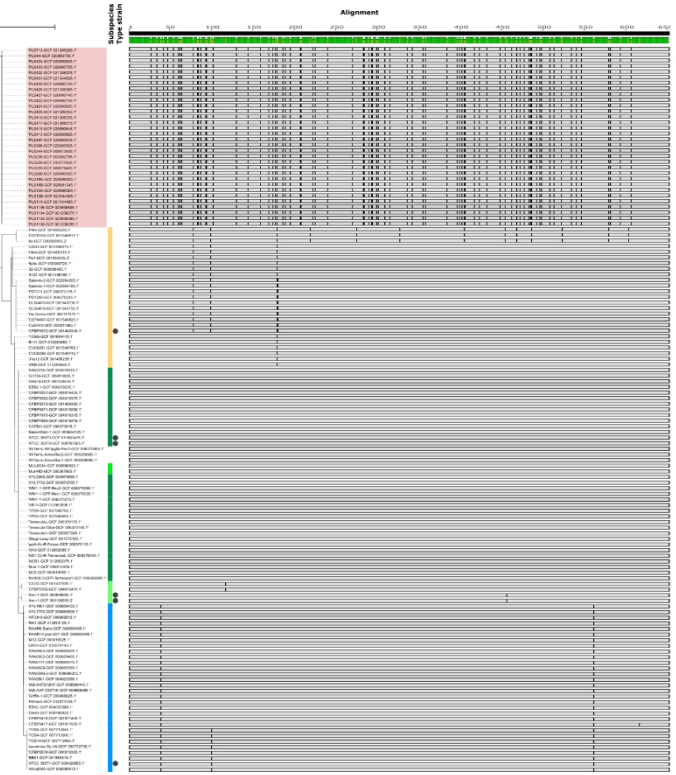
Literature Review

- Existing diagnostics (lab and field)
- Gaps in existing diagnostics

Lack of generic tests for *Xylella* screening

Genome informed assays to detect all known Xylella spp designed in this project:

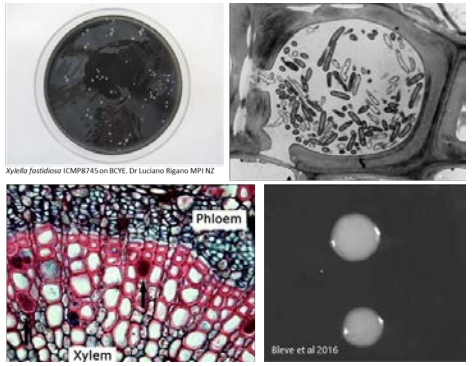
- qPCR assays (DPI NSW)
- endpoint assay (DPI NSW)
  - coupled with MINION for rapid subspecies differentiation
- LAMP assays (AgVIC)



Plant Health AUSTRALIA

NDP DEVELOPMENT

The specificity of **14 PCR assays** (quantitative and endpoint) evaluated on a total of **81 samples:**



Diverse strains of Xf

Closely related bacteria

Xf-infected insect hosts

Xf-infected and uninfected plant host tissue

Collection ID	Sample Type	Host	Exp. Result	Xf-specific Endpoint PCR and LAMP		Xf-specific qPCR						Generic *Xylella* Endpoint PCR			Generic *Xylella* qPCR				
				Minsavage et al 1994 (PCR)	Harper et al 2010 (LAMP)	Harper et al 2010	Agiletti et al 2019	Ouyang et al 2013	Li et al 2013	Francis et al 2006	Dupas et al 2019	Ito & Chiaki 2021	Xylella Generic 1	Xylella Generic 2	Multiplex species differentiation (This project) Xylella spp	Xf	Xt	Ito & Suzaki 2017	Ito & Chiaki 2021
Xylella DNA from culture																			
ICMP 8731	Xff	*Vitis vinifera*	+ve	+ve	46.24	23.9	27.45	26.6	23.63	25.6	23.94	+ve	+ve	+ve	22.14	22.7	UD	19.99	18.56
ICMP 8739	Xfm	*Prunus dulcis*	+ve	+ve	51.86	20.4	30.09	29	23.88	27.41	26.79	+ve	+ve	+ve	24	24.7	UD	20.36	18.87
ICMP 8740	Xfm	*Platanus occidentalis*	+ve	+ve	47.27	23.47	28.86	26.97	24.4	26.9	19.79	+ve	+ve	+ve	30.99	31.7	UD	18.78	16.19
ICMP 8742	Xf	*Ulmus americana*	+ve	+ve	42.35	23.28	26.98	25.29	22.54	24.79	24.21	+ve	+ve	+ve	20.5	20.7	UD	16.46	16.45
ICMP 8745	Xff	*Ambrosia artemisiifolia*	+ve	+ve	49.11	27.28	29.97	28.9	26.47	28.47	26.74	+ve	+ve	+ve	24.88	25.7	UD	22.99	21.87
ICMP 15197	Xff	*Vitis vinifera*	+ve	+ve	38.83	26.64	29.16	29.14	26.65	26.98	20.72	+ve	+ve	+ve	25.05	25.6	UD	24.95	20.43
Closely related bacteria																			
DAR 65801	*Stenotrophomonas*		UD	+ve	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
DAR 72045	*Stenotrophomonas*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	29.44	UD
DAR 75512	*Stenotrophomonas*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
DAR 76132	*Stenotrophomonas*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	18.01	29.41
DAR 77232	*Stenotrophomonas*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	17.36	31.28
DAR 77233	*Stenotrophomonas*		UD	UD	UD	35.69	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	18.47	28.01
DAR 77234	*Stenotrophomonas*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	17.31	29.63
DAR 77236	*Stenotrophomonas*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	18.82	28.87
DAR 77237	*Stenotrophomonas*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
DAR73877	*Xanthomonas vesicatoria*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
DAR35705	*Xanthomonas translucens* pv *translucens*		UD	UD	UD	UD	UD	UD	21.41	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
DAR49849	*Xanthomonas hortorum*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
DAR33337	*Xanthomonas arboricola*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
DAR82645	*Xanthomonas campestris* pv *phaseoli*		UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD

Plant Health AUSTRALIA

NDP DEVELOPMENT

A short list of **five** of the best performing **PCR tests** was further evaluated:

- Endpoint PCR generic *Xylella* spp. assay (This project 2021)
- Generic qPCR *Xylella* spp. and Xt (This project 2021).
- qPCR for Xf by Harper et al (2010, erratum 2013)
- qPCR for Xf by Ouyang et al (2013)
- qPCR for Xf by Dupas et al (2019)

Six different **isothermal assays** were assessed in an Australian setting (including assays designed in this project).

Queensland Government
Department of Agriculture and Fisheries

Ministry for Primary Industries
Manatū Ahu Matua

NSW GOVERNMENT
Department of Primary Industries

GOVERNMENT OF WESTERN AUSTRALIA
Department of Primary Industries and Regional Development

AGRICULTURE VICTORIA

SPECIFICITY OF PCR ASSAYS – AUS NZ

Xylella spp panels

Closely related bacteria panels

A total of 403 individual plant samples

covering 49 host genera

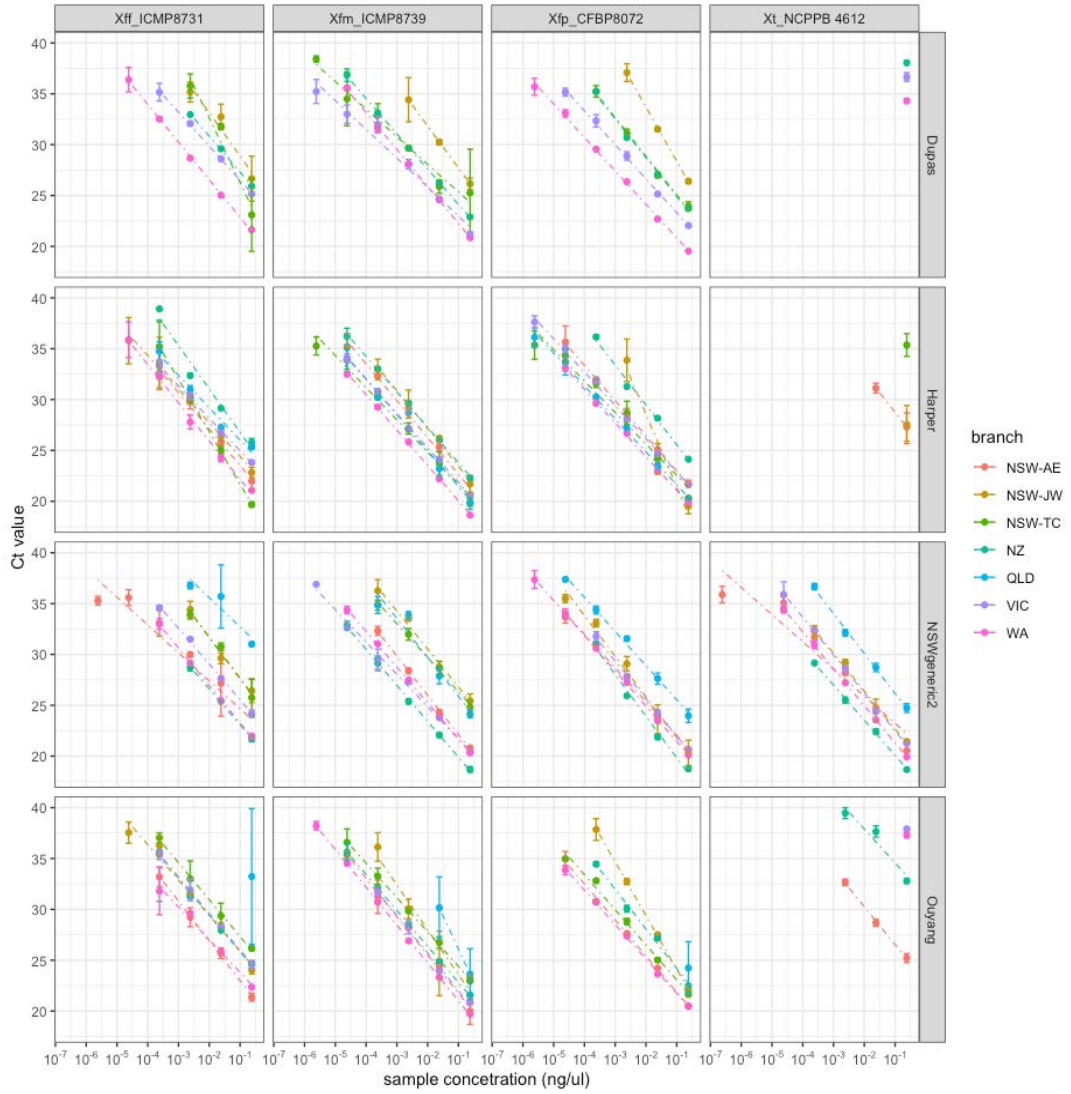
Acer spp.	*Jacaranda mimosifolia*	*Murtus sp (Myrtle)*	*Prunus spp.*
Anacardium occidentale	*Lavandula sp*	*Nandina sp.*	*Psidium guajava*
Bixa orellana	*Liquidambar sp.*	*Nerium oleander*	*Pyrus pyrifolia*
Canna sp.	*Litchi chinensis*	*Nicotiana tabacum*	*Quercus sp.*
Citrus spp.	*Macadamia integrifolia*	*Olea europaea*	*Rosa sp.*
Eugenia uniflora	*Malpighia emarginata*	*Pelargonium sp.*	*Rosmarinus sp.*
Euphorbia sp.	*Malus domestica*	*Persea americana*	*Rubus idaeus*
Fortunella sp	*Mangifera indica*	*Platanus acerifolia*	*Semecarpus australiensis*
Fragaria × ananassa	*Moringa oleifera*	*Plumeria*	*Ulmus*
Ginkgo sp.	*Morus rubra*	*Polygala myrtifolia*	*Vaccinium spp*
Hibiscus sp.	*Murraya koenigii*	*Portulaca sp.*	*Vitis spp*

Plant Health AUSTRALIA

SENSITIVITY

qPCR assays were more sensitive than the endpoint PCR as a screening test

All assays had comparable sensitivity for detection of isolates of different Xf subspecies and no apparent detection bias towards one subspecies was observed.



LABORATORY ASSAYS

The qPCR assay of Harper et al (2010, erratum 2013) provided the most reproducible results when run by different operators using different assay reagents and thermocycling devices.

All five assays are included in the *Xylella* spp. NDP.

The two primary assays recommended are the Harper et al (2010, erratum 2013) qPCR assay and the generic endpoint PCR (this project)

Asian pear samples

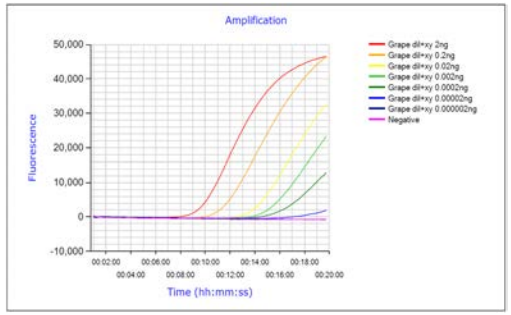
The two primary assays recommended are the Harper et al (2010, erratum 2013) qPCR assay and the generic endpoint PCR (this project)

IN-FIELD DETECTION OF XYLELLA



Goal: In-field detection capability ready for application in the field by field officers included in a revised Australian NDP for *Xylella*

- Isothermal in-field assays (LAMP and RPA) identified as the technology most field ready and transferable to field officers
- Six different isothermal assays were assessed in an Australian setting (including assays designed in this project). Assessed on:
  - Specificity
  - Sensitivity
  - Ease of use by operator
- Recommendation for inclusion in the NDP:
  - LAMP of Harper et al (2010) (Real-time)
    - As published
    - Optigene kit
  - Agdia Amplify RP® XRT kit



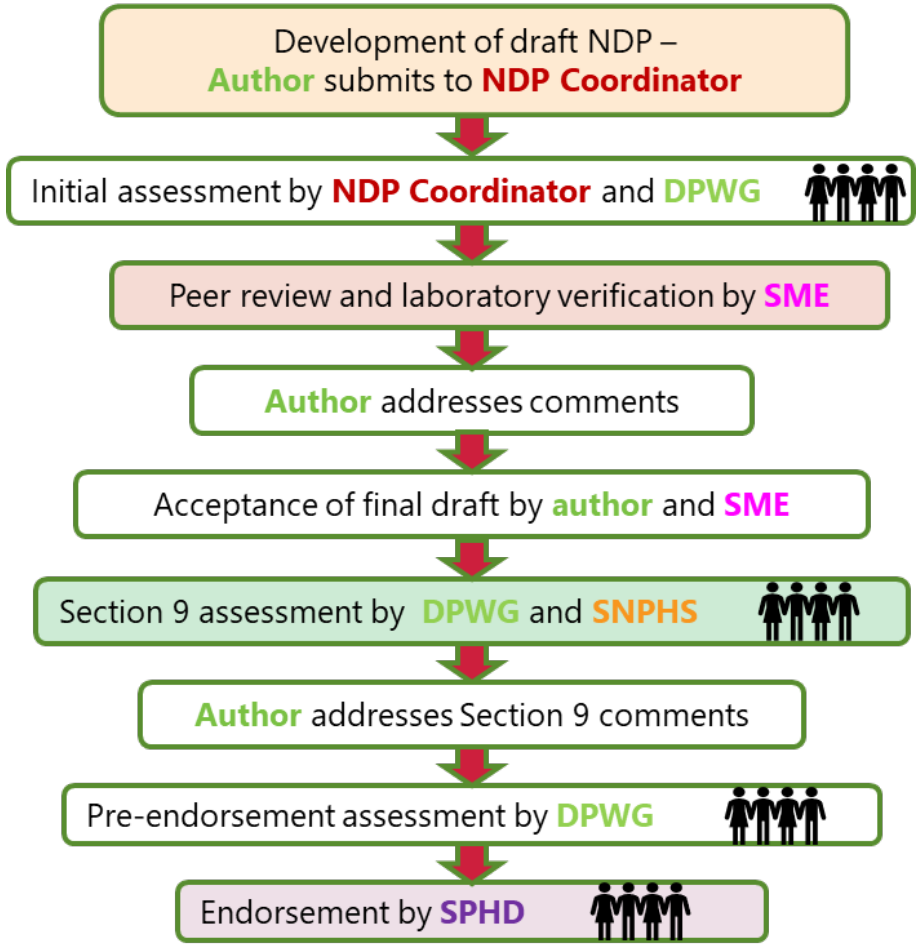
DNA		Harper qPCR	Optigen Xf LAMP kit		Harper LAMP		Agdia	
Conc	Sample Name	Ct Mean	Tp	AD	Tp	AD	RPA Tp	LFD
2.40E-01	XFPD1	21.7	13.9	89.5	7.2	88.7	5:30	Positive
2.40E-02	XFPD2	24.7	16.7	89.4	8.9	88.7	8:00	Positive
2.40E-03	XFPD3	28.1	23.9	88.9	15.4	88.4	13:15	Positive
2.40E-04	XFPD4	31.9	UD	UD	14.9	87.9	UD	UD
2.40E-05	XFPD5	35	UD	UD	22.2	87.9	UD	UD
2.40E-06	XFPD6	37.6	UD	UD	UD	77.5	UD	UD
2.40E-07	XFPD7	UD	UD	UD	UD	77.4	UD	UD

100% specificity against 149 samples
10 -1000 x less sensitive than qPCR

Plant Health AUSTRALIA

BIOSECURITY PREPAREDNESS

- Streamlined NDP endorsement process
- NDP assays are already adopted in 4 major node laboratories in AUS and 1 in NZ
  - Ability to rapidly identify detections to subspecies and ST
- Up to date sampling strategies with images
- Controls available to Australian laboratories



AKNOWLEDGEMENTS

Project Team:

- DPI NSW
  - Toni Chapman
  - Johanna Wong
  - John Webster
- AgVIC
  - Rachel Mann
  - Pragya Kant
  - Fiona Constable
- MPI NZ
  - Robert Taylor
  - Luciano Rigano
- QDAF
  - Rebecca Roach
  - Cherie Gambley
- DPIRD
  - Monica Kehoe
  - Dominie Wright

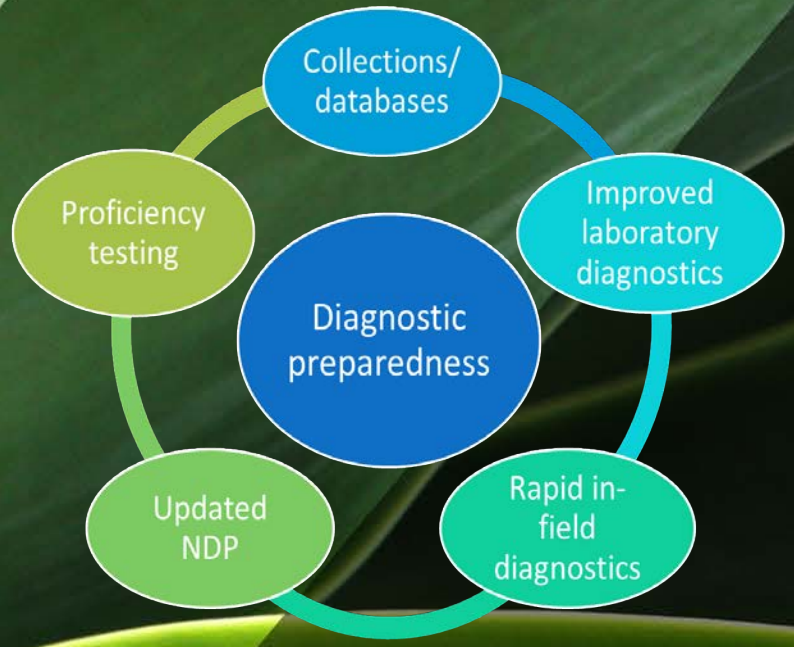
Review:

- Harsh Garg
- Elisse Nogarotto
- David Lovelock
- DPWG
- NSPWG
- SNPHS
- SPHDS

Additional support:

- Craig Elliot
- Mark Whattam
- Greg Chandler
- Penny Measham
- Adrian Dinsdale
- Brendan Rodoni

Project Activities:



Plant Health AUSTRALIA

LET’S GET SOCIAL

Follow us for the latest in plant biosecurity

plant-health-au

planthealthaust

planthealthaustralia

planthealthaust

Plant Health AUSTRALIA