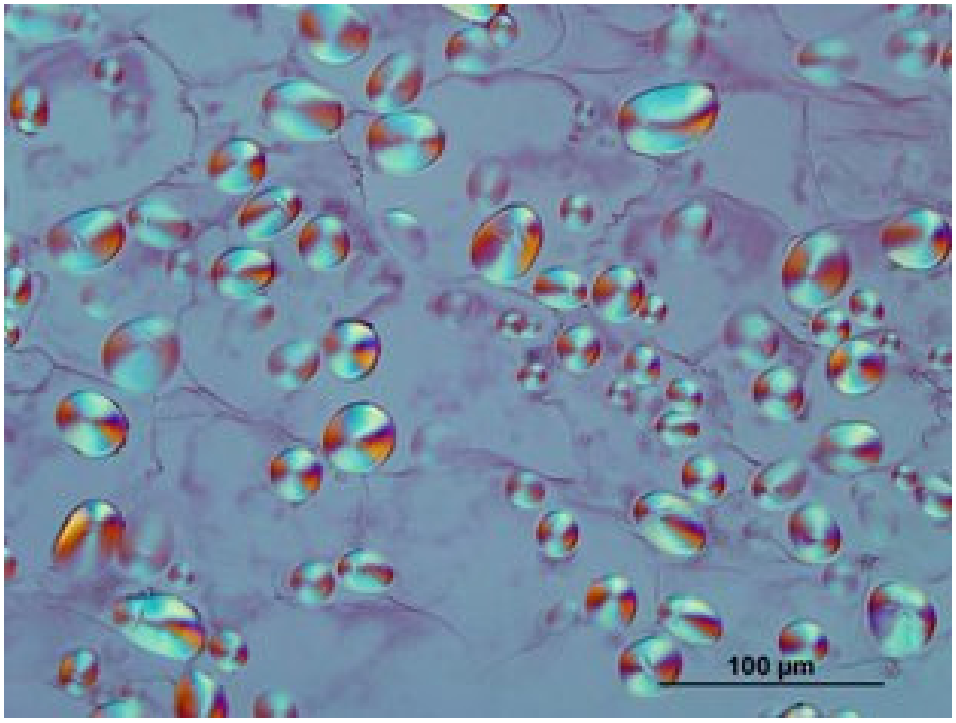
Development of Diagnostic Tools for the Seed Potato Industry



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by

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BSc. Hons. (Biomedical Sciences)

Thesis declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not been submitted for a degree at any tertiary educational institution

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Abstract

The Australian potato industry is threatened by inadequate measures to control the virus health of seed potatoes. Potatoes are vegetatively propagated; therefore infection can result in disease spreading through generations. This has the potential to cause significant economic losses. Virus testing on tuber sprouts is currently conducted by ELISA, however a significant time delay of several weeks can occur while tubers sprout. There is a considerable need for a rapid, quantitative and cost effective virus test directly on bulked samples of dormant tubers to identify infected lots during seed multiplication.

The potato viruses of economic importance in Western Australia are *Potato virus S*, (PVS), *Potato virus X*, (PVX), *Potato leafroll virus*, (PLRV) and *Tomato spotted wilt virus* (TSWV). The main aim of this project was to develop reliable PCR-based methods for multiplex real-time quantitative detection of these viruses in bulked potato tuber samples for seed certification for domestic and export markets.

Knowledge of the distribution of the viruses within tuber tissue was needed to develop more effective methods of RNA extraction. The distribution of the viruses in histological sections of potato tubers was investigated using immunohistochemistry and *in situ* hybridization. All four viruses were found to be distributed at the stolon end of freshly harvested infected potato tubers. Extraction of RNA from tuber tissue is problematic because it contains starches and phenolics which inhibit RT-PCR. Extracting RNA from the tuber peelings would overcome this problem; however one of the viruses, PLRV, is restricted to the

phloem in potato tubers. The distribution of the phloem in the superficial tissue of potato tubers was therefore investigated using histological staining and transmission electron microscopy. The vascular tissue was found to be within 2 mm below the epidermis of the tuber. With this knowledge, total RNA was extracted rapidly and efficiently from bulked potato peelings equivalent to 300 dormant tubers to detect single infections of PLRV, PVX, PVS and TSWV.

For the quantitative detection of these viruses in potato leaves and tuber tissue, specific primers and fluorescent-labeled TaqMan® probes were designed. A real-time multiplex, single tube RT-PCR assay was developed. The main tasks involved primer design, optimization of reagents, standardization of RNA samples from which standard curves for analysis were generated, and identification of a baseline on which to interpret results.

Limits of detection sensitivity were established using a range of virus transcript copy numbers (8×10^1 to 8×10^9 copies of PVX and PVS, 1×10^2 to 1×10^{10} copies of PLRV and 1×10^3 to 1×10^{10} copies of TSWV). The multiplexed assay was validated in blind studies. This high-throughput test is accurate and sensitive, and as a result this test is in the process of being commercialized and used by the seed potato industry of Western Australia as a cost-effective diagnostic tool to detect viruses reliably in bulked samples of dormant potato tubers.

Table of Contents

Publications from this study

Some of the results presented in this thesis have been published and presented at scientific meetings as indicated below.

Mortimer-Jones, Sheila M., Jones, Michael G.K., Jones, Roger A.C., Thomson, Gordon and Geoffrey I. Dwyer (2009). A single tube, quantitative real-time RT-PCR assay that detects four potato viruses simultaneously. *Journal of Virological Methods* 161, 289-296.

Mortimer-Jones, Sheila M., Jones, Michael G.K., Jones, Roger A.C. and Geoffrey I. Dwyer (2008). *Development and validation of a high throughput, one-step, quantitative real-time RT-PCR assay for the simultaneous detection of PLRV, PVX, PVS and TSWV with a rapid RNA extraction method directly from bulked potato tuber samples.* In: Conference Proceedings of the Eighth Australasian Plant Virology Workshop, Rotorua, New Zealand. pp. 9.

Mortimer-Jones, S., Dwyer, G.I., Jones, R.A.C. and M.G.K. Jones (2007). *Localisation of viruses in tuber tissues of potato.* In: Conference Proceedings of the Thirteenth European Association for Potato Research, Aviemore, Scotland. pp. 66.

Mortimer-Jones, S., Dwyer, G.I., Jones, R.A.C. and M.G.K. Jones (2006). *Localisation of viruses in tuber tissues of potato*. In: Conference Proceedings of the Seventh Australasian Plant Virology Workshop, Rottnest Island, Western Australia. pp. 62.

List of Abbreviations

3’	hydroxyl terminus of DNA molecule
35S RNA	transcriptional promoter of CaMV
5’	phosphate terminus of DNA molecule
Bp	base pair
BSA	bovine serum albumin
cDNA	complementary DNA
CP	coat protein
C-terminus	carboxy terminus
cv	cultivated variety (cultivar)
DEPC	Diethyl pyrocarbonate
DIG	digoxigenin
DMPC	di-methyl-propyl carbonate
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
dsRNA	double stranded RNA
E. coli	*Eschericia coli*
EDTA	ethylenediaminetetra-acetate acid disodium salt
ELISA	enzyme-linked immunisorbent assay
GFP	green fluorescent protein
GUS	β-glucuronidase gene
IPTG	isopropyl-B-D-thiogalactoside
Kb	kilobases
KD	kilodalton
LB	Luria-Bertani
M	Molar
Min	minute
MP	movement protein
NBT/BCIP	5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium
NCR	non-coding region
N-terminus	amino terminus
NTP	nucleoside triphosphate
ORF	open reading frame
PCR	polymerase chain reaction
PTGS	Post-transcriptional gene silencing
PVP	Polyvinylpyrrolidone
RdRp	RNA dependent RNA polymerase
RNA	ribonucleic acid
RNAi	RNA interference
RNase	ribonuclease
Rpm	revolutions per minute
RT	reverse transcription
sec	second/s
sgRNA	subgenomic RNA

siRNA	short interfering RNA
TAE	tris-acetate-EDTA
TE	tris-EDTA
U	unit
UTP	uracil tri-phosphate
VIGS	virus-induced gene silencing
Vol	volumes
vRNA	viral RNA
X-Gal	5-bromo-4-chloro-3-indolyl-βgalactopyranoside

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Finally I would like to thank my man, Rhys Jones, who never wavered in his belief in me.

Dedication

I would like to dedicate this thesis to my parents Margaret and Geoffrey Mortimer who strived to give me the best education they could, and to my dear children Gareth, Lewis and Jennifer. Their vacant mother has some sense after all.