

Development and application of molecular tools to onion neck rot

Martin Chilvers
Dept. Plant Pathology
Washington State University



Botrytis neck rot

- Post-harvest disease
- Seedborne pathogen



Project objectives

1. Determine the identity of *Botrytis* neck rot species isolated from Columbia Basin seed crops
2. Develop a quantitative PCR assay for detection of seedborne *Botrytis* species associated with neck rot of onion

What is PCR ? (polymerase chain reaction)

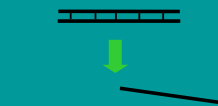
- Amplifies DNA to detectable levels
- Requires primers (short pieces of DNA)
- Primers bind to matching DNA sequences
- DNA is only amplified if primers match
- **SPECIFIC AMPLIFICATION**

Thermal cycler - Cycles temperature



PCR (polymerase chain reaction)

Denature



Anneal



Extend



Identification of *Botrytis* neck rot species in the Columbia Basin

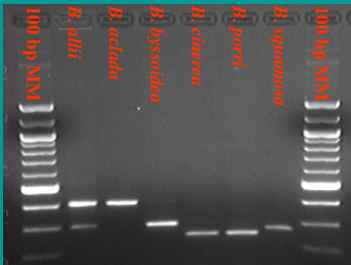
- du Toit demonstrated that *B. aclada/allii* was ubiquitous in the Columbia Basin
- Unable to differentiate *B. aclada* and *B. allii*
 - Due to similar growth patterns in culture
 - And overlapping spore size
- **What species are present?**

du Toit et al. 2004 Plant Disease 88:1061-1068



Identification of *Botrytis* neck rot species in the Columbia Basin

- PCR amplification using *Botrytis*-specific primers
- Cut amplified DNA with enzyme (Apo I)



Nielsen et al. 2002
Plant Disease 86:682-686

Identification of *Botrytis* neck rot species in the Columbia Basin

- Identification of 460 isolates of *B. allii/aclada*
 - *B. aclada* (60%)
 - *B. allii* (40%)
- Implications for future;
 - Epidemiology experiments
 - Fungicide trials
 - Cultivar screening
- **Demonstrates a need for primers that will amplify both species**

WASHINGTON STATE UNIVERSITY
World Class. Since the 1800s.

Project objective

Develop a quantitative PCR assay for detection of seedborne *Botrytis* species associated with neck rot of onion

WASHINGTON STATE UNIVERSITY
World Class. Since the 1800s.

Conventional seed assays

- No industry standard
- Requires knowledge & experience to identify *Botrytis* spp.
- Time-consuming (2+ weeks)
- **Need for a rapid, quantitative, sensitive and reproducible assay**



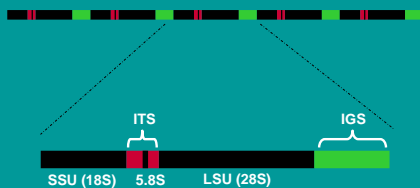
L.J. du Toit



L.J. du Toit

Target locus

Ribosomal DNA

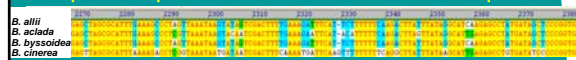


WASHINGTON STATE UNIVERSITY
World Class. Since the 1800s.

Primer design

Forward primer

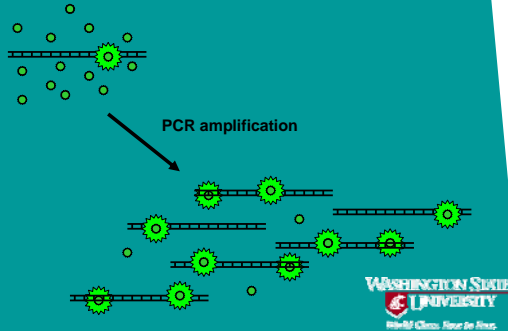
Reverse primer



Primers designed to amplify a 114 bp product

WASHINGTON STATE UNIVERSITY
World Class. Since the 1800s.

SYBR Green I



Primer screening & validation

Fungal isolates (no. of strains)	Strain codes or source
<i>Botrytis aclada</i> (21)	BAS, B328, B330, B346, B351, B352, B363, B364, B368, B369, B390, B393, B403, B436, B463, B511, B514, B519, B523, B529, B514
<i>B. allii</i> (21)	BAS, B331, B332, B338, B365, B374, B377, B381, B382, B414, B423, B442, B454, B516, B520, B529, B627, B636, B678, B679, B683
<i>B. byssoides</i> (1)	ATCC 60837
<i>B. cinerea</i> (4)	BC1, BC2, BC3, BC4
<i>B. porri</i> (3)	BP1, BP2, BP4
<i>Sclerotinia sclerotiorum</i> (1)	Potato plant
<i>Fusarium oxysporum</i> f. sp. <i>cepae</i> (2)	FOC 8, FOC 201A
<i>Acremonium</i> sp. (1)	Onion seed
<i>Alternaria</i> sp. (1)	Onion seed
<i>Aspergillus niger</i> (1)	Onion seed
<i>Aspergillus</i> sp. (1)	Onion seed
<i>Cladosporium</i> sp. (1)	Onion seed
<i>Epicoccum</i> sp. (1)	Onion seed
<i>Fusarium</i> sp. (1)	Onion seed
<i>Penicillium</i> sp. (3)	Onion seed
<i>Rhizopus</i> sp. (1)	Onion seed
<i>Stemphylium</i> sp. (1)	Onion seed
Unidentified hyphomycete (1)	Onion seed
None (1)	Control (onion leaf)

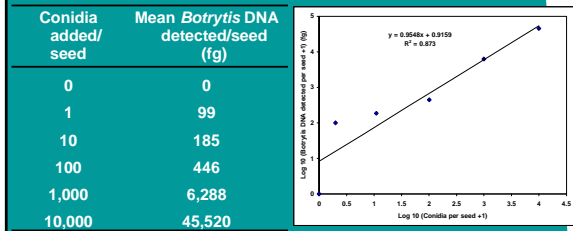
WASHINGTON STATE UNIVERSITY
World Class. Fear No One.

DNA extraction

- 25 onion seed
- Silica - spin column



Sensitivity of assay



Assay validation

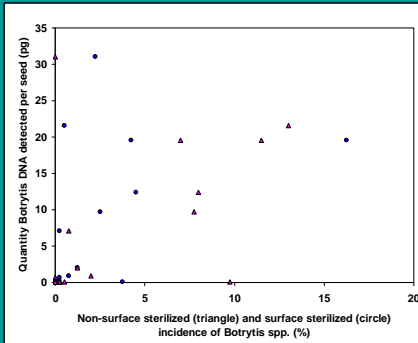
- 23 seed lots (different fields)
- Agar plate assay (4 reps of 100)
 - Surface disinfested
 - Non-surface disinfested
- Q-PCR assay (10 reps of 25)

WASHINGTON STATE UNIVERSITY
World Class. Fear No One.

Results of seed assays

Field	<i>Botrytis</i> DNA (fg) per seed	Surface disinfested (%)					Non-disinfested (%)				
		<i>Botrytis gillus</i>	Asper- porium	Clados- porium	Penic- illium	Rhiz- opus	<i>Botrytis gillus</i>	Asper- porium	Clados- porium	Penic- illium	Rhizo- pus
OR	92.5	3.8	0.8	4.0	3.0	0.3	9.8	17.5	36.5	67.5	0.5
ID	0.0	0.0	58.3	6.8	22.3	0.3	0.0	96.3	53.8	96.0	5.5
ID	31040.0	2.3	12.3	1.3	3.8	0.5	0.0	84.3	65.3	47.0	9.3
ID	19568.0	16.3	19.0	16.0	14.8	0.8	11.5	38.8	98.5	75.3	5.8
WA	12260.8	4.5	9.6	4.3	1.3	3.0	6.0	67.8	78.5	69.0	8.5
WA	2049.2	1.3	36.3	10.5	8.3	1.8	1.3	97.3	89.5	88.8	1.8
AZ	13.3	0.0	53.0	0.3	0.5	0.0	100.0	43.0	9.5	0.3	
AZ	0.2	0.0	80.5	0.5	1.5	0.3	0.0	99.8	22.5	17.0	1.5
CA	0.0	0.0	55.8	6.8	0.3	0.3	0.0	99.8	99.5	36.5	1.5
CA	0.0	0.0	14.3	5.5	0.5	0.8	0.3	90.5	66.3	7.0	1.5
CA	0.0	0.0	5.0	1.0	0.0	0.5	0.0	88.3	59.3	7.8	5.3
CA	38.9	0.0	88.0	23.8	32.3	9.5	0.3	98.0	92.3	80.0	12.5
CA	7112.8	0.3	23.3	1.3	0.0	0.8	0.8	99.5	50.5	3.3	4.0
OR	21581.6	0.5	0.0	1.8	0.5	0.0	13.0	3.0	92.8	33.8	3.8
OR	19524.8	4.3	0.5	8.3	17.3	0.0	7.0	2.0	68.5	91.0	1.3
WA	644.1	0.5	0.8	6.3	82.5	3.8	0.0	2.0	87.3	99.5	10.0
CA	45.5	0.0	37.3	0.0	0.0	0.3	0.5	96.3	2.5	2.8	0.3
CA	8.4	0.0	64.0	38.8	3.0	0.5	0.0	99.8	100.0	27.0	0.8
CA	244.4	0.3	86.3	22.5	21.0	8.3	0.0	98.3	85.5	81.3	8.8
ID	311.4	0.8	0.5	38.8	18.5	0.3	2.0	33.3	99.0	96.3	0.8
-	9691.2	2.5	0.0	3.0	15.0	0.0	9.5	0.8	89.5	99.5	0.0
NZ	37400.0	45.3	0.0	0.5	4.3	2.3	99.3	0.0	46.3	85.5	2.8
Italy	7.5	0.0	56.8	10.5	1.5	0.0	0.0	93.8	64.0	15.0	0.0

Relationship between assays



Pooled comparison of assays

		Quantitative PCR assay	
		Positive	Negative
Conventional agar assays	Positive	25	1
	Negative	13	7

Agreement between assays = 70%



Quantitative PCR assay conclusions

Assay is:

- Specific to *Botrytis* neck rot species
- Sensitive able to detect 10 fg of DNA
- Rapid application

Applications:

- Seed screening/testing
- Quantify *Botrytis* in leaf/bulb tissue
- Tool in epidemiology studies
- Tool in pathogenicity studies



Chilvers and du Toit et al. 2007 Plant Disease 91:599-608

Project outputs

- Plant Disease article: A real-time, quantitative PCR seed assay for *Botrytis* spp. that cause neck rot of onion

© 2006 Plant Management Network.
Accepted for publication 7 August 2006. Published 27 November 2006.

Detection and Identification of *Botrytis* Species Associated with Neck Rot, Scape Blight, and Umbel Blight of Onion

Martin I. Chilvers, Postdoctoral Research Associate, PO Box 646430, Department of Plant Pathology, Washington State University, Pullman 99164-6430; and **Lindsey J. du Toit**, Associate Scientist/Vegetable Seed Pathologist, 16650 State Route 536, Washington State University Mount Vernon NWREC, Mount Vernon 98273-4768

chilvers@wsu.edu or duitoit@wsu.edu

chilvers@wsu.edu or duitoit@wsu.edu

Acknowledgements

- WSU IMPACT Center
- WSU Agricultural Research Center
- CA Onion & Garlic Research Committee
- Columbia Basin Vegetable Seed Association
- NV Onion & Garlic Advisory Board
- Pacific Northwest Vegetable Association (PNVA)
- Onion seed industry
- Dr Tobin Peever (Lab space)