Biology and control of *Dickeya spp.* affecting potato in the UK

John Elphinstone, Neil Parkinson, Gerry Saddler, Greig Cahill, Rachel Kelly, Leighton Pritchard, Sonia Humphris and Ian Toth

Overview

- History of *Dickeya* in potato
- Diversity and Diagnostics
  - Comparative genomics
  - Development and validation of real-time PCR assays
  - Pathogen diversity
- Extent and risk of spread of *Dickeya* in GB
  - Application of diagnostics
  - Monitoring causes of blackleg in potato crops
  - Surface water contamination
- Control recommendations

Dickeya dianthicola

- Spread since 1950's on ornamentals (C*hrysanthemum & Dianthus*)
- Since found on a number of other ornamental hosts (*Begonia, Cichorium, Dahlia*, *Kalanchoe*, *Sedum*)
- First appeared on European potato in 1970s
- First found on potato in England/Wales in 1990
- Most damaging in warm environments (>25 °C)

Dickeya solani

- Spreading on European potato since at least 2004/5
- Also found on ornamentals such as *Hyacinthia* and *Scilla*
- First detected on potato in GB in 2007 (1-30% wilting plants)
- Becoming the dominant cause of blackleg in some EU countries
- Most damaging in warm environments (>25 °C)



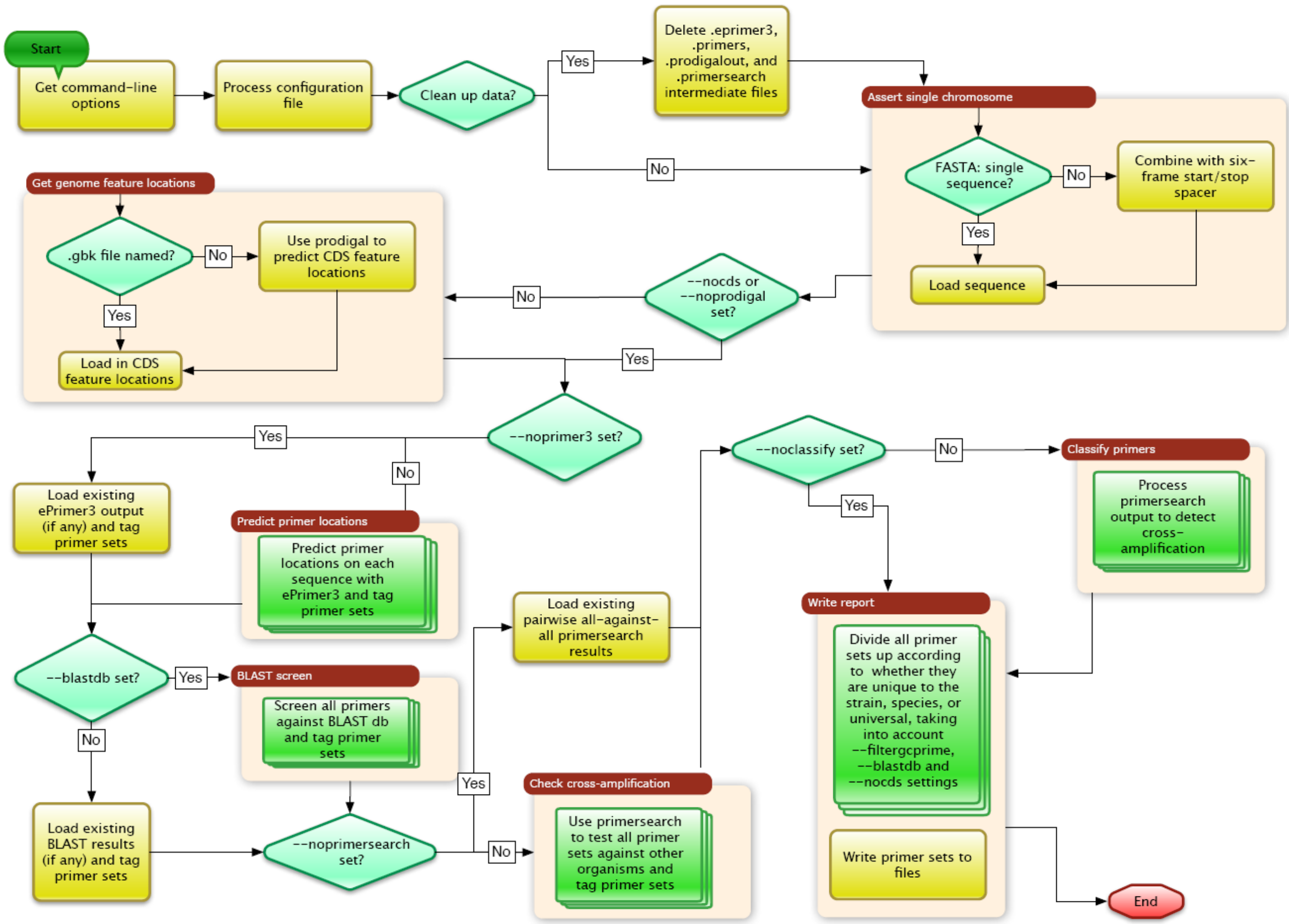
Genome sequencing

Organism	Accession	Source	Approx. size (Mb)
Dickeya solani	IPO2222^T	Potato, Netherlands	4.9
	MK 10	Potato, Israel	4.9
	MK 16	Potato, UK	4.9
	LMG 25865	Potato, Belgium	4.8
Dickeya dianthicola	NCPPB 453^T	*Dianthus*	4.7
	NCPPB 3534	Potato, Netherlands	4.8
	IPO 980	Potato, Netherlands	4.8
	LMG 25864	Potato, Belgium	4.8
Dickeya dadantii subsp. dadantii	NCPPB 898^T	*Pelargonium,* Comoro Is.	4.9
	NCPPB 2976	Potato, Peru	4.8
Dickeya dadantii subsp. dieffenbachiae	NCPPB 3537	*Dieffenbachia*, USA	4.8
Dickeya chrysanthemi	NCPPB 402^T	*Chrysanthemum*, USA	4.8
	NCPPB 3533	Potato, USA	4.7
	NCPPB 516	*Parthenium*, Denmark	4.6
Dickeya paradisiaca	NCPPB 2511^T	Banana*, Colombia*	4.6
Dickeya zeae	NCPPB 2538^T	Maize	4.6
	NCPPB 3531	Potato, Australia	4.6
	Fera RW192	River water, England	4.7
	MK 19	River water, Scotland	4.7
	NCPPB 3532	Potato, Australia	4.6
Dickeya (unclassified clades)	NCPPB 569	Sugar cane, Australia	4.2
	MK 7	River water, Scotland	4.9
	NCPPB 3274	*Aglaonema, St. Lucia*	5.1
'Dickeya aquatica'	Fera 7247^T	River water, England	4.4
	DW0440	River water, Finland	4.3

Pritchard L, *et al*., 2013. Genome Announcements 1:e00087-12. doi:10.1128/genomeA.00087-12.

Pritchard L, *et al*. 2013. Genome Announcements, in press.

Pipeline for primer generation

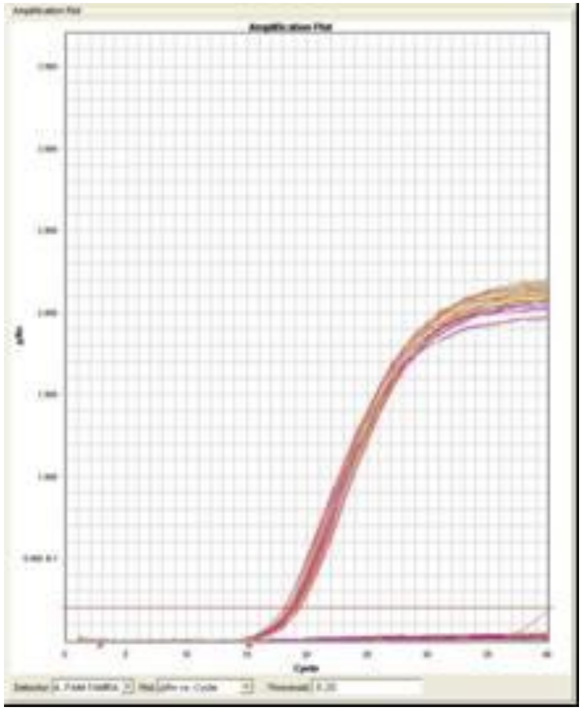


Pritchard L *et al.* 2013. Plant Pathology 62: 587–596.

Selection of real-time PCR primers

Real-time PCR assays

- *D. dianthicola* specific (DIA)
- *D. solani* specific (SOL)
- *D. solani* specific (*fusA*)
- All pectolytic bacteria (PEC)
- *Pectobacterium atrosepticum* (ECA)
- Validated against 70 isolates representing phylogenetic diversity



Pritchard L *et al.* 2013. Plant Pathology 62: 587–596.

Diagnostics validation

Specificity of real time PCR assays

Identification	# isolates tested	# isolates detected				
		DIA	*fusA*	SOL	ECA	ECH
Dickeya dianthicola	7	7	0	0	0	7
Dickeya solani	16	0	16	16	0	16
Dickeya dadantii	16	0	0	0	0	16
Dickeya chrysanthemi	10	0	0	0	0	10
Dickeya paradisiaca	1	0	0	0	0	1
Dickeya zeae	11	0	0	0	0	11
Dickeya unidentified clade DUC-2	5	0	0	0	0	5
Dickeya unidentified clade DUC-3	1	0	0	0	0	1
Dickeya SLCI (‘*D. aquatica*’)	1	0	0	0	0	1
Dickeya SLCII	1	0	0	0	0	1
Pectobacterium atrosepticum	1	0	0	0	1	0
P. carotovorum subsp. *carotovorum*	1	0	0	0	0	0
P. betavasculorum	1	0	0	0	0	0
P. carotovorum subsp. *odoriferum*	1	0	0	0	0	0
P. wasabiei	1	0	0	0	0	0
Pantoea agglomerans	1	0	0	0	0	0
Brenneria quercina	1	0	0	0	0	0
Erwinia amylovora	1	0	0	0	0	0
	70					

Pritchard L *et al.* 2013. Plant Pathology 62: 587–596.

Dickeya MLST Home Page - Mozilla Firefox

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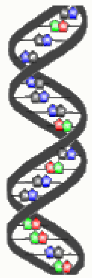
  - V. parahaemolyticus
  - Wolbachia

+ **Mirrors**
+ **Developers**

Dickeya MLST Databases

This site uses two linked databases powered by the BIGSdb genomics platform. The sequence definition database contains allele sequence and MLST profile definitions whereas the isolate database contains provenance and epidemiological information. Further details about BIGSdb can be found in Jolley & Maiden 2010, BMC Bioinformatics **11**:595.



- Information
  - Primers used for amplification and sequencing
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- Policy document
- Submission of data

- BIGSdb software
- Recent publications using MLST in Dickeya research

The use of this database is subject to the terms of the policy document and it should be acknowledged in all publications that make use of it. The preferred format for the acknowledgement can be found in the right-hand sidebar.

This MLST scheme was developed by Rachel Kelly and Gerry Saddler, Diagnostics and Analytical Services Science and Advice for Scottish Agriculture (SASA).

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Status

Sequence database
Sequences: 120
Profiles (MLST): 19
Last updated: 2012-11-02

Isolate database
Isolates: 37
Last updated: 2012-11-02

Start | Dickeya MLST Home ... | Fera - Mozilla Firefox | Document1 - Microsoft ... | Ralstonia | Dickeya solani | EN | 10:01

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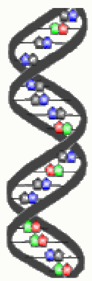

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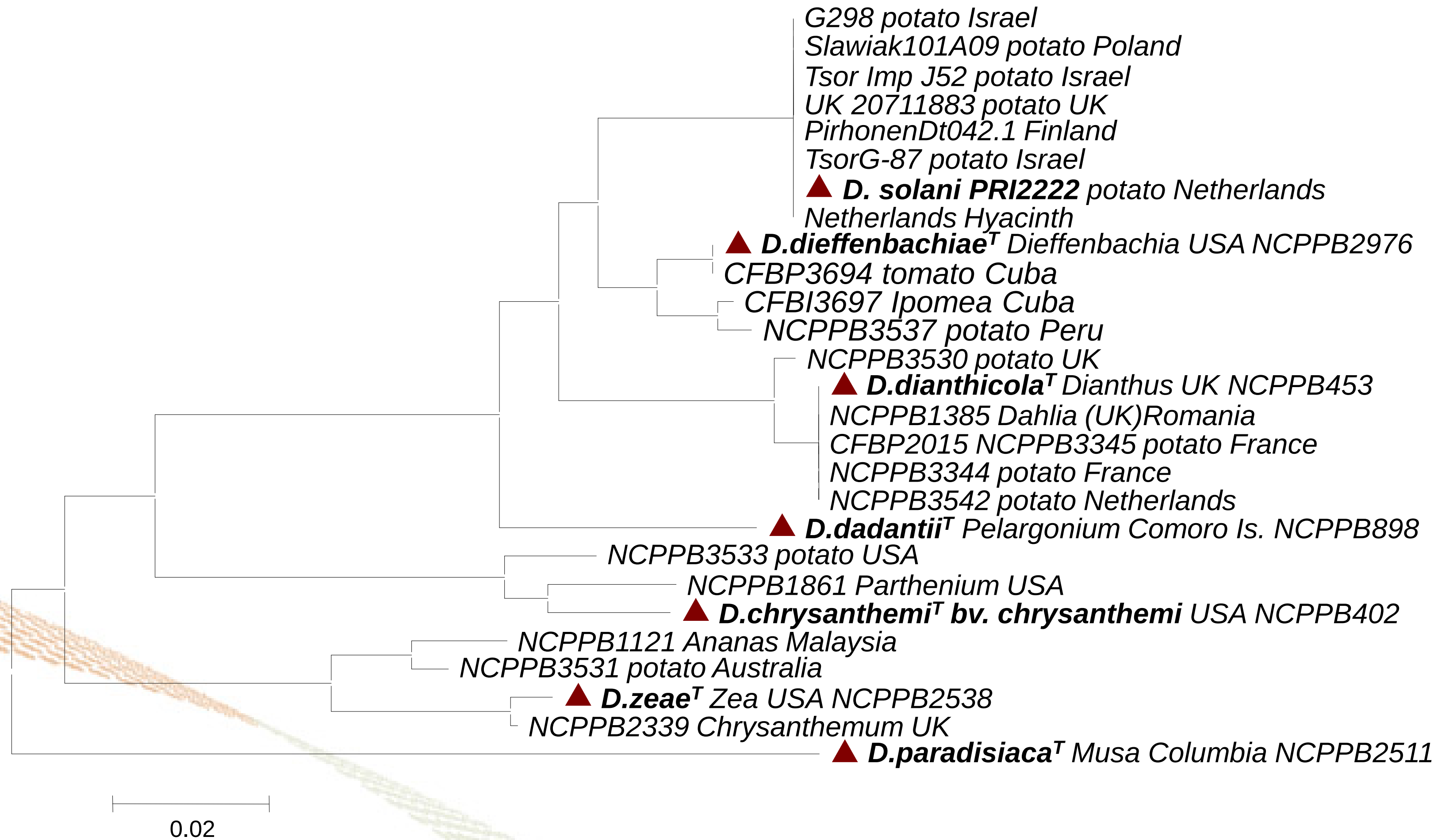
- Web-based multilocus sequence typing (MLST) scheme http://pubmlst.org/dickeya/
- Based on sequence homology between 8 housekeeping genes
- 16S, *recA, dnaN, fusA, gapA, infB, purA, rplB*

Kowalewska MJ *et al*., 2010. Proceedings Crop Protection in Northern Britain pp. 251-256.

Start | Dickeya MLST Home ... | Fera - Mozilla Firefox | Document1 - Microsoft ... | Ralstonia | Dickeya solani | EN | 10:01

Phylogenetic relatedness of EU *Dickeya* panel (*recA* sequence)



Parkinson *et al*., Int. J. Systematic and Evolutionary Microbiology 59, 2388-2393 2009

D. dianthicola VNTR profiles

- 6 VNTR loci identified
- Each locus amplified with fluorescent label
- Amplicon length related to number of repeats
- 19 VNTR profiles amongst 48 reference isolates
- Isolates from ornamentals and potato with linked profiles

Number of consecutive repeats at 6 different loci

Reference	Host	Country	Year	VNTR locus						Profile
				19	19b	29	10	11	28	
IPO 502	Potato	Netherlands	1979	4	5	6	5	5	6	
NCPPB 3534	Potato	Netherlands	1987	4	5	6	5	5	6	
PD 1022	Potato	Netherlands	1987	4	5	6	5	5	6	P1
PD 721	*Kalanchoe*	Netherlands	1986	4	5	6	5	5	6	
NCPPB 3345	Potato	France	1984	4	5	6	5	5	6	
NCPPB 3530	Potato	Jersey	1987	3	4	7	5	3	5	
Fera 2246	*Cichorium*	Netherlands	1987	3	4	7	5	3	5	
Fera 1644	Potato	UK	1996	3	4	7	5	3	5	P2
Fera 1649	Potato	UK	1996	3	4	7	5	3	5	
PD 484	Potato	Netherlands	1984	3	4	7	5	3	5	
Fera 1106	Potato	UK	1992	3	4	7	5	3	5	
PD 554	*Kalanchoe*	Netherlands	1985	3	4	9	9	2	3	
PD 593	*Kalanchoe*	Netherlands	1985	3	4	9	9	2	3	
PD 1325	*Kalanchoe*	Netherlands	1989	3	4	9	9	2	3	P3
PD 1343	*Kalanchoe*	Netherlands	1989	3	4	9	9	2	3	
NCPPB 3730	*Kalanchoe*	Denmark	1990	3	4	9	9	2	3	
NCPPB 3729	*Kalanchoe*	France	1990	3	4	9	9	2	3	
NCPPB 518	*Dianthus*	Denmark	1957	3	4	3	2	3	5	
NCPPB 393	*Dianthus*	UK	1957	3	4	3	2	3	5	P4
NCPPB 429	*Dianthus*	UK	1957	3	4	3	2	3	5	
NCPPB 430	*Dianthus*	UK	1958	3	4	3	2	3	5	
Fera 1104	Potato	UK	1992	1	2	3	2	3	3	
NCPPB 3881	Potato	UK	1993	1	2	3	2	3	3	P5
IPO 771	Potato	Netherlands	1984	1	2	3	2	3	3	
IPO 256	Potato	Netherlands	1975	1	2	3	2	3	3	
NCPPB 394	*Chrysanthemum*	USA	1957	3	3	3	3	3	6	
NCPPB 426	*Dianthus*	UK	1957	3	3	3	3	3	6	P6
NCPPB 2536	*Dianthus*	UK	1973	3	3	3	3	3	6	
NCPPB 1111	*Dianthus*	UK	1961	3	4	3	4	3	6	
NCPPB 453	*Dianthus*	UK	1957	3	4	3	4	3	6	
NCPPB 1609	*Dahlia*	Netherlands	1964	3	4	3	4	3	6	
NCPPB 1956	*Dahlia*	Netherlands	1964	3	4	3	4	3	6	P7
Fera 2272	Potato	Bangladesh	1988	3	4	3	4	3	6	
NCPPB 3529	Potato	UK	1987	3	4	3	4	3	6	
NCPPB 4305	*Sedum*	UK	2002	3	4	3	4	3	6	
PD 1406	Potato	Netherlands	1989	3	2	3	5	3	6	P8
PD 482	Potato	Netherlands	1984	3	2	3	5	3	6	
NCPPB 3138	*Dianthus*	Greece	1980	4	4	3	3	4	4	P9
NCPPB 3139	*Dianthus*	Greece	1980	4	4	3	3	4	4	
NCPPB 452	*Dianthus*	UK	1957	3	5	3	5	3	6	P10
NCPPB 3882	*Dianthus*	UK	1993	3	3	5	2	3	2	P11
CFBP 2598	*Kalanchoe*	Switzerland	1982	3	3	3	2	2	4	P12
Fera 1242	*Dianthus*	UK	1993	3	3	9	8	3	2	P13
NCPPB 2421	*Begonia*	Netherlands	1971	3	4	3	6	3	5	P14
NCPPB 3344	Potato	France	1984	3	2	2	6	3	6	P15
Fera 2279	Potato	Netherlands	1989	4	4	3	5	5	5	P16
NCPPB 3710	Potato	Netherlands	1990	4	5	5	4	5	6	P17
NCPPB 1385	*Dahlia*	Romania	1962	3	3	3	2	3	5	P18
PD 1405	Potato	Netherlands	1989	3	4	3	8	3	6	P19

D. solani VNTR profiles

PRI strain	Origin	Number of tandom repeats at each locus						VNTR Profile
		Host	00048	00099	00082	00094	00102	
3337	France	potato	7	4	5	5	6	1
2276	Poland	potato	7	4	5	5	6	1
3239	UK	potato	7	4	5	5	6	1
2019	Netherlands	hyacinth	7	4	5	5	6	1
2187	Israel	potato	7	4	5	5	6	1
3228	Israel	potato	7	4	5	5	6	1
3296	Israel	potato	8	4	5	5	6	2
3294	Finland	potato	8	4	5	5	6	2
3295	Finland	potato	8	4	5	5	6	2
2222	Netherlands	potato	9	4	5	5	6	3

- 3 VNTR profile types, but all closely related.

D. solani VNTR profiles

VNTR profiles amongst *Dickeya solani* isolates.

Source	Number of isolates	VNTR profile
Potato, England/Wales	27	1
Potato, France	1	
Potato, Israel	2	
Potato, Poland	1	
Hyacinth, Netherlands	1	
Potato, England/Wales	15	2
Potato, Finland	2	
Potato, Israel	1	
River water, England/Wales	2	
Potato, England/Wales	1	3
Potato, Netherlands	1[T]	

[T]*Dickeya solani* type strain (IPO 2222)

D. solani single nucleotide polymorphisms

SNP profiles amongst *Dickeya solani* isolates

Source	Number of Isolates	SNP profile
Potato, Israel	1	1
Potato, Poland	3	
River water, Scotland	3	
Potato, Belgium	1	2
Potato, Netherlands	2	
Potato, Israel	1	3
Potato, Spain	1	4
Potato, Netherlands	1	5
Potato, Netherlands	1[T]	6

[T] *Dickeya solani* type strain (IPO 2222)

Status of *Dickeya* spp. in GB potato

- Annual surveys 2010-2013
- All (~ 800) classified seed stocks per year in England/Wales
- 10% of classified seed stocks per year in Scotland
- Total of 545, 752, 821 and 671 Scottish potato crops surveyed each year.
- Blackleg plants collected at 2 inspections per year.
- Post harvest testing also performed in Scotland
- Isolation on CVP & identification by PCR and MLST

Status of *Dickeya* spp. in GB potato

Blackleg findings in seed potato stocks entered for classification in England and Wales and potato crops surveyed in Scotland

	2010	2011	2012	2013
England and Wales				
% seed stocks surveyed with blackleg	32.1	21.5	33.8	31.0
% with blackleg caused by *D. solani*	2.3	0.5	0.6	0.5
% with blackleg caused by *D. dianthicola*	0.1	0.1	0.6	0
Scotland				
% with blackleg caused by *D. solani*	1.6	0	0	0
% with blackleg caused by *D. dianthicola*	0	0	0	0

- All *Dickeya* findings associated with seed of non-UK origin
- No *Dickeya* found in seed in Scotland
- No evidence of spread to seed stocks of UK origin

Dickeya detected in river water (2009-2012)

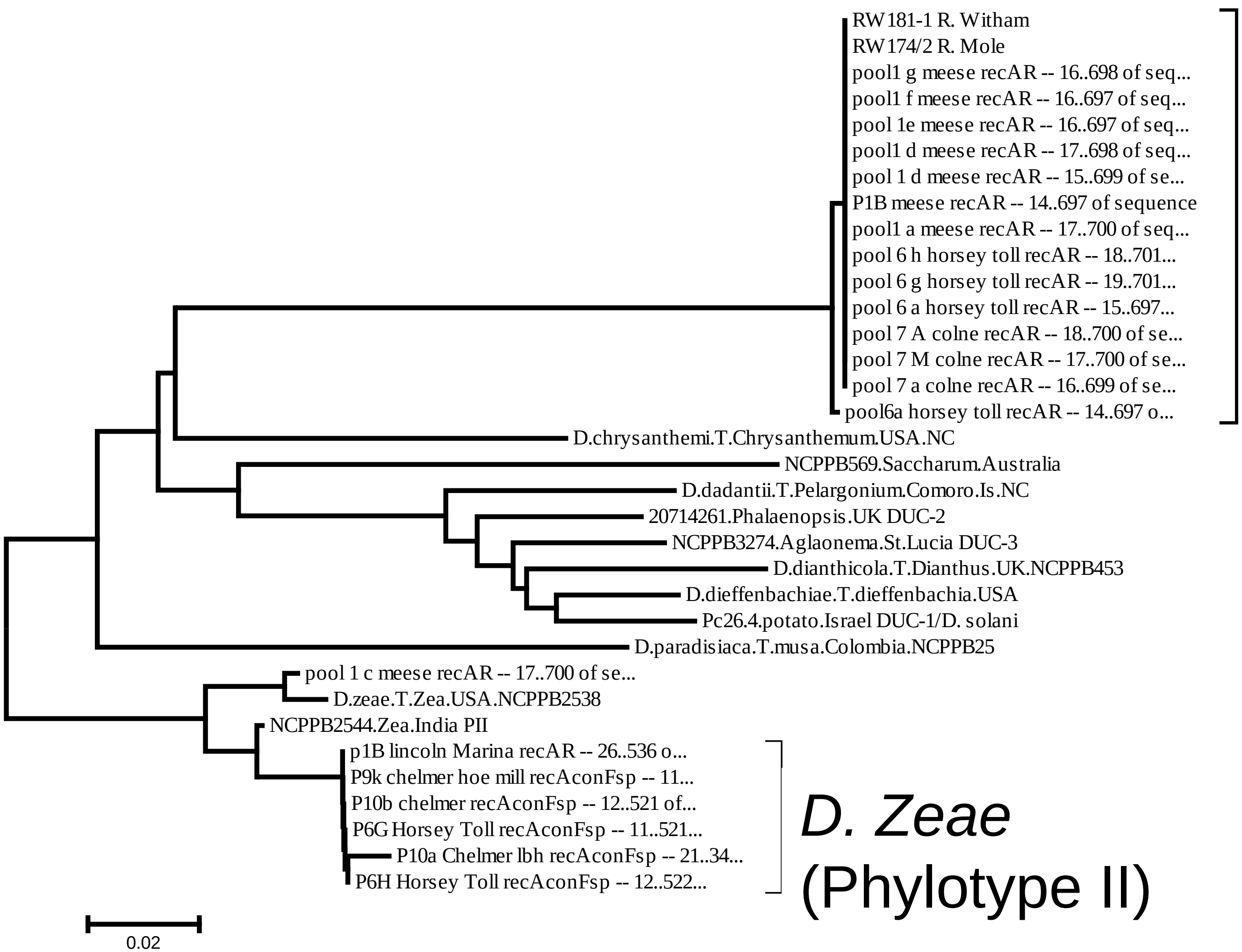
All rivers used for irrigation sampled in Scotland

- *D. solani* (1 river)
- *D. zeae* (1 river)
- Unidentified *Dickeya* sp. (1 river)

60 rivers sampled in England/Wales

- *D. solani* (2 rivers)
- *D. dianthicola* (4 rivers)
- Unidentified *Dickeya* sp. (3 rivers)
- *D. zeae* (13 rivers)
- *'D. aquatica'* (6 rivers)

MLST identification of river water isolates

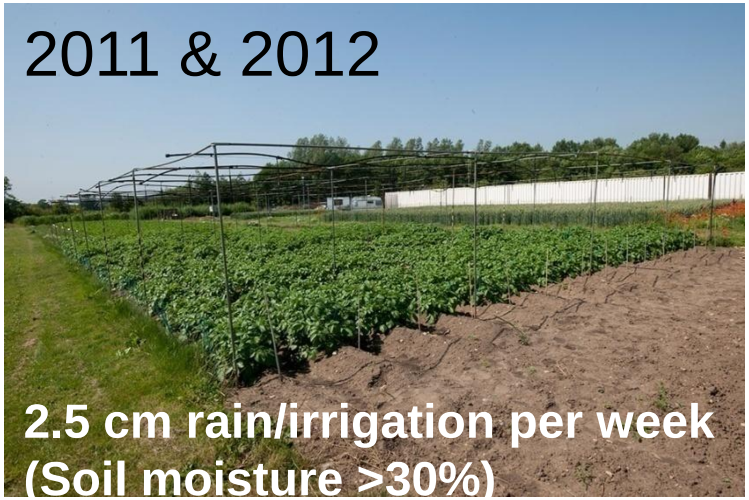


‘*D. aquatica*’

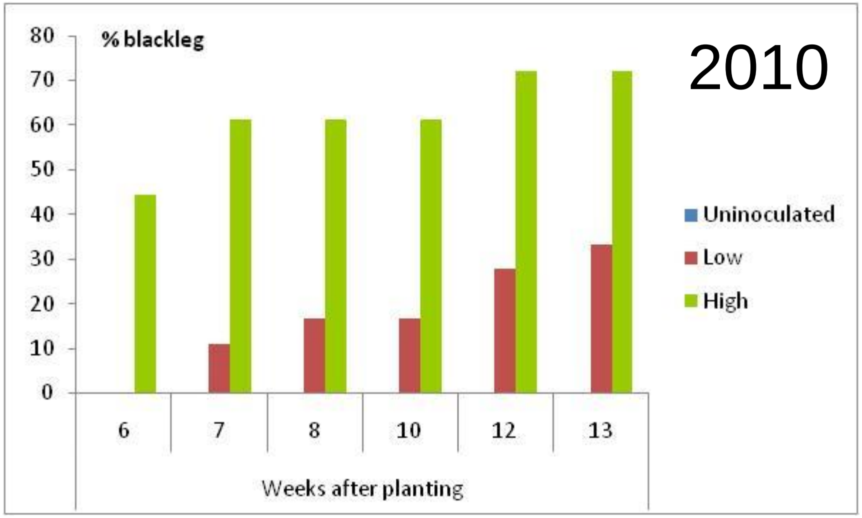
D. Zeae (Phylotype II)

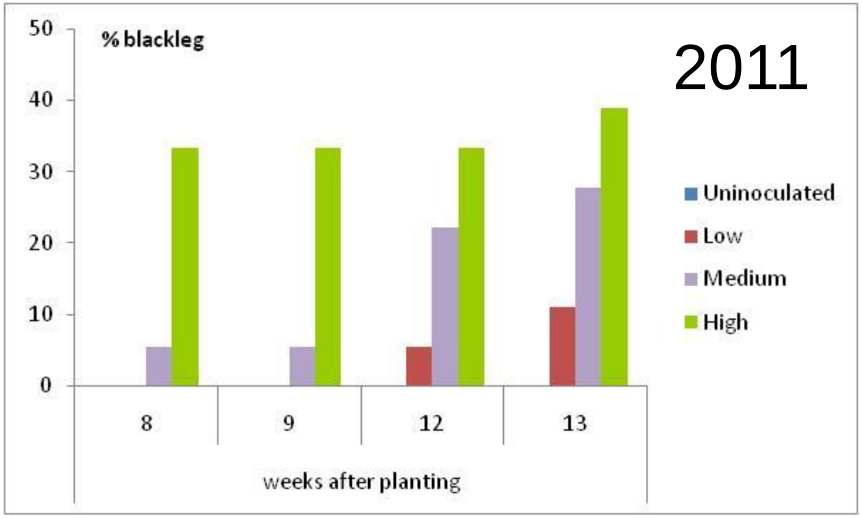
Survival and spread of *Dickeya* in the UK

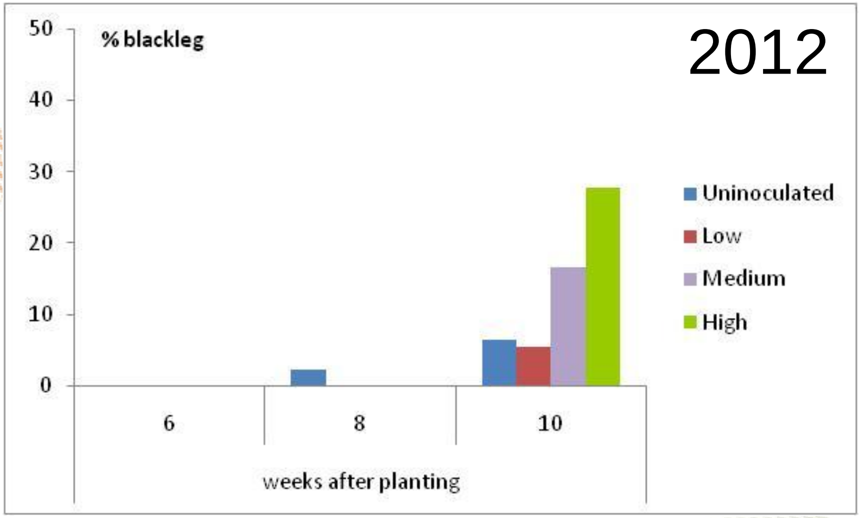
- Field observation plots
- Seed vacuum infiltrated with *D. solani*
  - 10^3 cfu per ml
  - 10^5 cfu per ml
  - 10^7 cfu per ml
- Disease incidence and spread monitored over 3 seasons



Blackleg incidence (cv. Nicola)

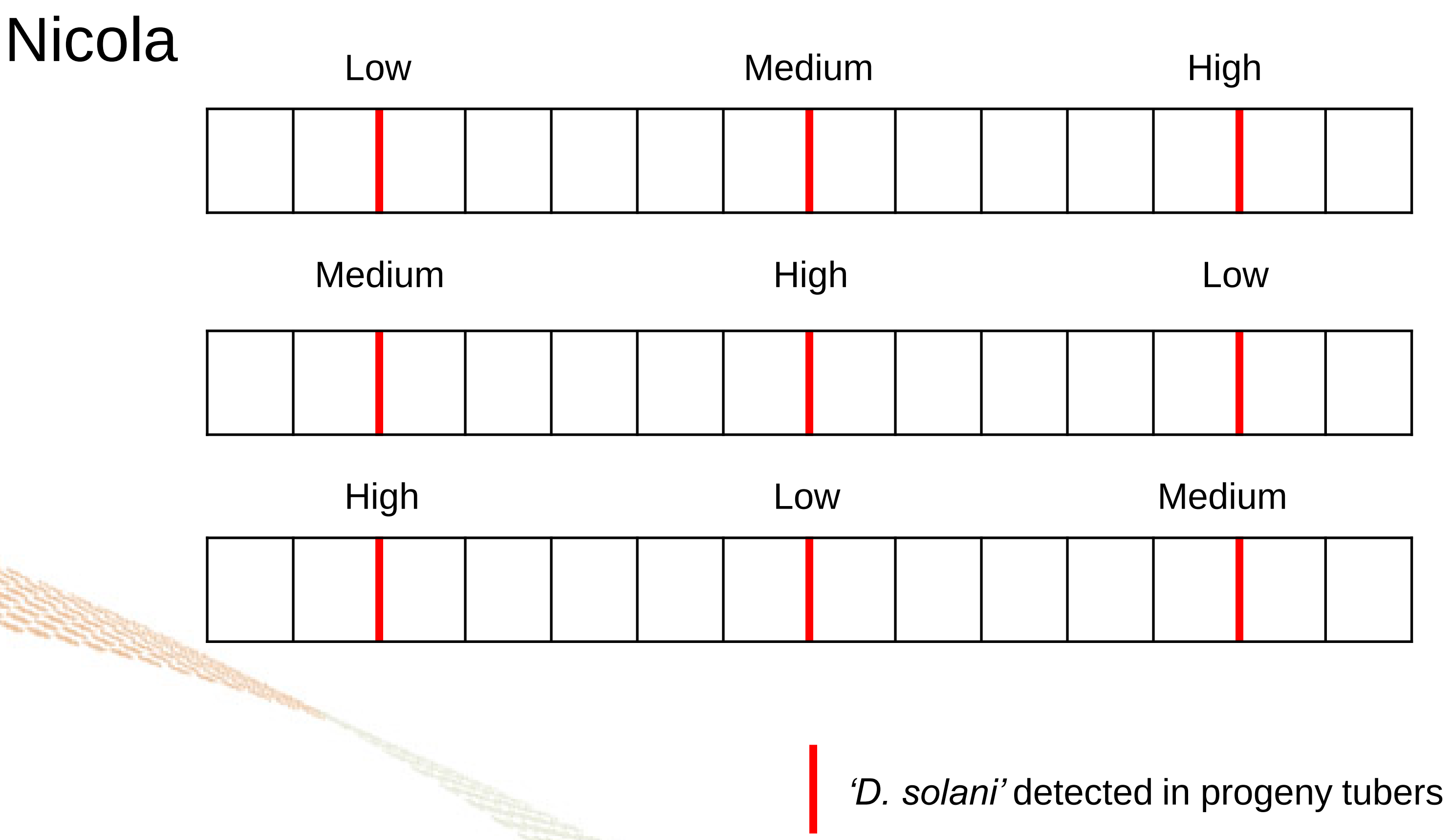




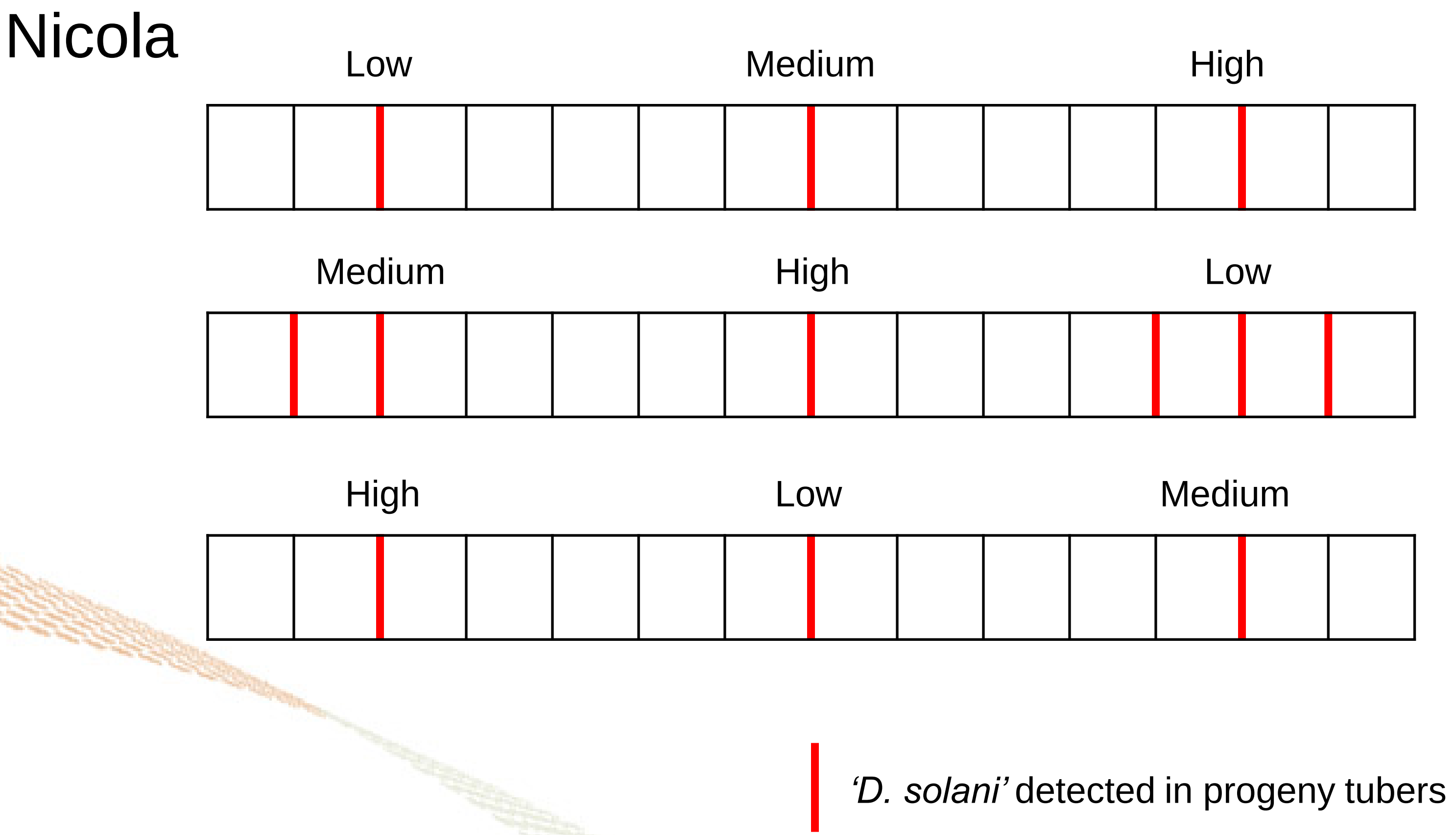


- Blackleg development varied with season
- Always related to seed inoculum level

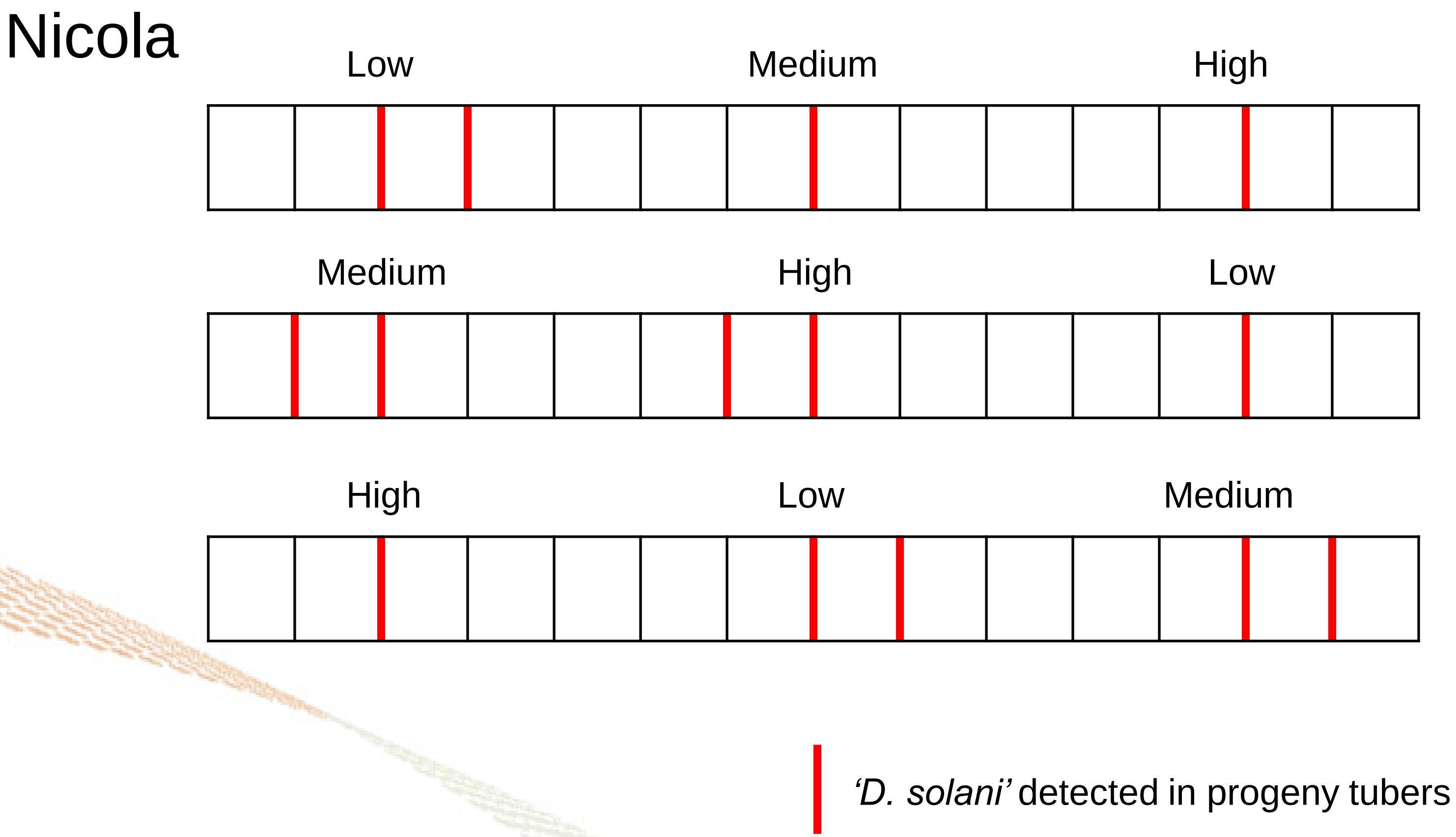
Detection of *D. solani* in 2010 progeny from inoculated & uninoculated rows



Detection of *D. solani* in 2011 progeny from inoculated & uninoculated rows



Detection of *D. solani* in 2012 progeny from inoculated & uninoculated rows



Spread of *D. solani* during handling

Immediate planting in glasshouse (25 °C)

60% non-emergence

14% blackleg

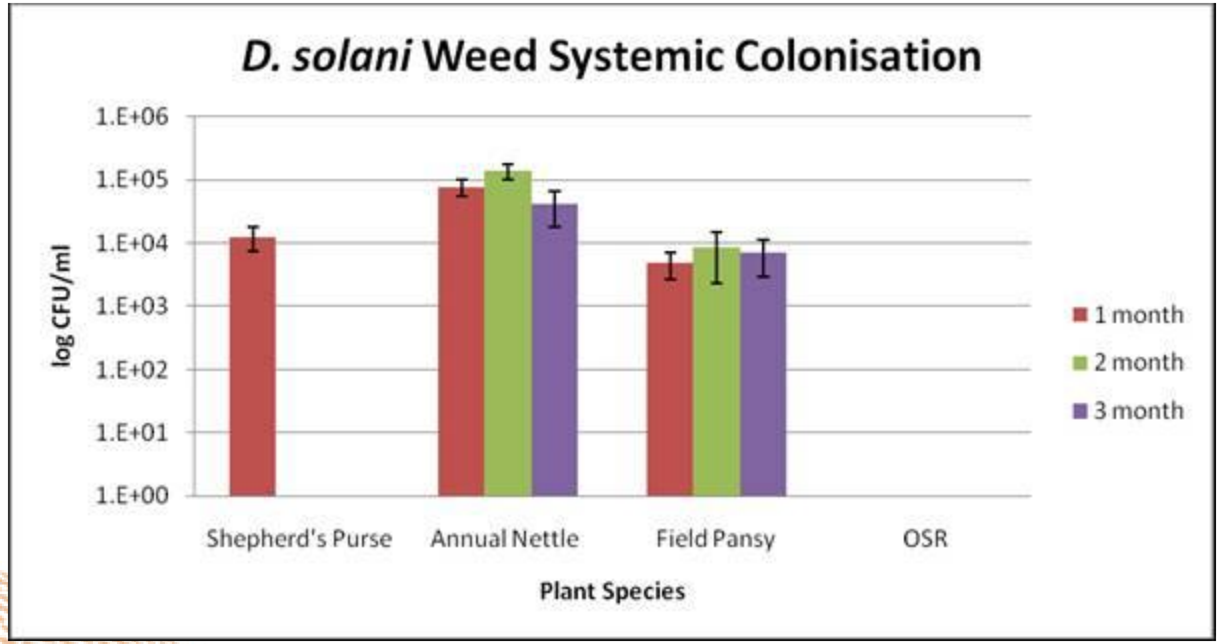
36% ‘healthy’

Disinfectant efficacy

Disinfectant	Active ingedient(s)
Domestos™	sodium hypochlorite
Halamid™	tosylchloramide sodium
V18™	iodophor
Vanoquat™	quaternary ammonium
FAM-30™	acidic-based iodine
GPC8™	glutaraldehyde and quaternary ammonium
Jeyes Fluid™	4-chloro-m-cresol and tar acids
Jet-5™	peracetic acid
Mikrozid AF™	propanol and ethanol
Virkon S™	dipotassium peroxodisulphate, dodecylbenzenesulfonate and organic acids

All 10 common disinfectants killed *D. solani*. *D. dianthicola* and *Pectobacterium atrosepticum* in aqueous suspensions (10^8 cfu per ml) at recommended rates & 5 min. exposure.

Potential survival of *D. solani* on weeds



- Weed species colonised after greenhouse inoculations
- Some (e.g. *Urtica* sp.) developed symptoms

Survival of *D. solani* after harvest of infected ware crops

- 19 crops with 10-30% *D. solani* blackleg
- Detected in soil 2 months after harvest
  - 3-8% *D. solani*; 92-97% *Pcc*
- Not detected in following spring (100% *Pcc*)
- Not detected in nearby watercourses (100% *Pcc*)
- Survived in stored tubers
  - 1-3% *D. solani*; 97-99% *Pcc*
- Not detected in weed rhizospheres
  - *Solanum nigrum*, *Chenopodium album*, *Veronica sp.*, Galium aparine, Senecio vulgaris, *Urtica dioica*

Control recommendations

Control policy based on avoidance of introduction

- Source seed free from infection/contamination
- Scottish legislation (2010)
  - Zero tolerance for *Dickeya* in classification scheme
  - Planting of potatoes with *Dickeya* prohibited
  - Similar precautions in N. Ireland
- England/Wales
  - Tolerance for *Dickeya* same as for *Pectobacterium*
- Avoid irrigation from contaminated watercourses

Questions remaining

- What are principal sources and routes of transmission for contamination of pre-basic seed?
  - Affected varieties more susceptible?
  - Specific pathways of infection?
  - Sources of *Pectobacterium* and *Dickeya*?
  - Importance of waterborne dissemination?
  - Importance of alternative hosts in introduction and long term survival?
- Risk from other *Dickeya* and *Pectobacterium* spp.?
- Storage conditions to limit damage by *D. solani*?

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POTATO COUNCIL