## RUSSET POTATO VARIETIES WITH RESISTANCE TO POTATO MOP-TOP VIRUS AND COORDINATED VARIETY AND VIRUS RESISTANCE RESEARCH EFFORTS.

- Jonathan Whitworth and Rich Novy. USDA-Agricultural Research Service, Aberdeen, Idaho, USA
- Charles Brown. USDA-Agricultural Research Service, Prosser, WA, USA (retired)
- \*jonathan.whitworth@usda.gov

EAPR Pathology & Pests Section Meeting, Neuchatel, Switzerland, 2019



#### Mop top virus symptoms – Scotland 2007





#### Mop top virus symptoms – California, USA 2015







#### PMTV

Russet Burbank

Modoc





Shepody A02267-5PY



## Samples taken 2001 and 2002, surveillance study Xu et al. 2004, Plant Dis. 88:363-367

**Table 3.** Incidence of *Potato mop-top virus* (PMTV) detection in the western, central, and eastern zones of the United States and Canada

	Number of states and	Number of potato lots							
Zone <sup>a</sup>	provinces tested	Tested	Positive for PMTV						
Western	10	1,133	29						
Central	8	163	34						
Eastern	12	1,859	76						
Unknown	Unknown	66	0						
Total	30	3,221	139						

<sup>a</sup> Zones correspond approximately, but not entirely, to Pacific plus Mountain time zone, Central time zone, and Eastern plus Atlantic time zone for the western, central, and eastern zones, respectively.

### U.S./Canada Virus Management Plan

(at shipping point)

 Table 1: Eligibility of Seed potatoes related to the Percentage of Internal Tuber

 Necrosis, apparently caused by PVY complex, PMTV or TRV

Percentage Internal Tuber Necrosis Scored*	Seed intended for Recertification	Seed intended for Commercial Production
Less than or equal to 0.5%	Eligible	Eligible
Between 0.5 and 2.0%	Eligible if negative lab test for PVY <sup>N/NTN</sup> , PMTV, TRV	Eligible
More than 2.0%	Not eligible	Not eligible

Notes:

\* Internal tuber necrosis will be calculated as percent incidence {(number of tubers with internal necrosis/number of tubers sampled) x100}.



#### Potato Mop Top Virus

- Vector Powdery scab (Spongospora subterranea)
- Infected spore balls of *S.* subterranean survive up to 18 even in the absence of potatoes
- Reduced quality (necrotic arcs and rings)
- Primary infected tubers can be symptomless, but sensitive cultivars can form ringspots or arcs
- Foliar symptoms rare (in USA)
- Emerging disease

# Routine screening for PMTV and TRV resistance, Pacific Northwest

#### **Materials and Methods**

- PMTV
  - Done in infested research field in Prosser, WA area
  - Field fumigated to remove any stubby root nematode vectors
  - Measurements are done by slicing tuber into 4 long sections
  - Counts of necrosis on 8 sides of long sections = severity index
  - Any sign of internal necrosis used to calculate variety percentage
- TRV
  - Done in infested research field in Prosser area
  - Necrosis measurements done as above

#### Materials and Methods (cont.)

- Random tuber samples were tested for PMTV using RT-PCR (n=30) and real-time PCR (n=20).
- Results showed that only PMTV was detected in tubers
- PMTV tuber necrosis increases during storage (4% 1<sup>st</sup> yr, 10% 2<sup>nd</sup> yr)
- PMTV may be latent (low titer) resulting in no symptoms, so classification should be insensitive vs. sensitive
- A lot of russet types are insensitive to PMTV necrosis
  - Russet types are also more resistant to powdery scab

	2015 Average Disease Severity Index of Potato Mop Top Virus																																
25		Breeding line/variety					PMTV %				PMTV serious defects %						-																
		Pomere	elle	Ru	sse	t							3	<mark>8.4</mark>											0	).7							
20	-	Mounta	ain	Ge	m F	ในร	sse	t					7	'.5											1	2						-	-
		Classic	Rus	sset	t								5	5.8											1	4							
15		Russet	Bui	rba	nk								5	5.8											1	7							-
		Targhee Russet				9.1						1.7																					
10		A06021-1T			9.1					3.8						-	_																
		A03921-2			27.6					9.8																							
5	Ranger Russet				20.0					6.3							_																
	Clearwater Russet					35.8					11.9																						
			ĪI	I		II	I		Ţ		Ī			± 1																			
	POR06V12-3	Red La Soda CO03202-1RU AO6191-1 W6234-4Russ Ivory	Pomerelle (A02062-1TE)	AF4281-3	Norkotah	0KU5U39-4	Classic Russet	(112-201200) 100 100 100 100 100 100 100 100 100	CO97087-2RU	AC03452-2W RB	Targhee (A01010-1)	Premier Russet	AF4296-3	A05180-7Y	A02138-2	Kennebec	A06021-1T	A025U/-2IB A003123-2	Shepody	OR08014-1	Victoria	Ranger	A08014-11TE Teton Russet	Challenger	Snowden	AC96052-1RU	AU6914-3CK	ATX05202S-3W/Y	A0012-5	A03921-2	Clearwater Russet		7





#### The insensitive story of PMTV

			JUST	VISUAL	<mark>&amp; VIS</mark>	<mark>TESTED PC</mark> SUAL SYMP	R TOMS	TESTED PCR & NO SYMPTOMS					
Year	# clones/cvs represented	# Tubers Evaluated	# Tuber/w int	<mark>% w/int</mark>	# Tubers Tested w/int	# Pos	<mark>% Pos w/int</mark>	# Tubers Tested w/o int	# Pos	<mark>% Pos w/o int</mark> <mark>"INSENSITIVE"</mark>			
2012	63	8686	1155	<mark>13.30%</mark>	168	115	<mark>68.45</mark>	410	90	<mark>21.95</mark>			
2014	51	6835	435	<mark>6.36%</mark>	120	35	<mark>40.97</mark>	390	77	<mark>19.74</mark>			

From work of Prosser group: Crosslin, Brown, Quick, Hamlin



- 1. Purple Marker Asymptomatic 1
- 2. Purple Marker Asymptomatic 2
- 3. Purple Marker Asymptomatic 3
- 4. Purple Marker Asymptomatic 4
- 5. Purple Marker Asymptomatic 5
- 6. Purple Marker Asymptomatic 6
- 7. Purple Marker Asymptomatic 7
- 8. Purple Marker Asymptomatic 8
- 9. Purple Marker Asymptomatic 9
- 10. Purple Marker Asymptomatic 10
- 11. Purple Marker Asymptomatic 11
- 12. Purple Marker Asymptomatic 12
- 13. Purple Marker Asymptomatic 13
- 14. Purple Marker Asymptomatic 14
- 15. Purple Marker Asymptomatic 15
- 16. Purple Marker Symptomatic
- 17. Purple Marker Asymptomatic Bulk #1-7
- 18. Purple Marker Asymptomatic Bulk #8-15
- 19. + Control (tuber)
- 20. + Control (tuber)
- 21. Control (tuber)
- 22. Control (tuber)
- 23. Control (tuber)
- 25. Control (tube
- 24. H<sub>2</sub>O









Kylie Swisher USDA-ARS

#### Varieties at Washington Screening Trials

Variety	Year	Incidence	Severity	Use			
Pomerelle Russet	2014	0.8	0.2	Early maturity, process/fresh			
	2015	3.4	0.7				
La Belle Russet	2014	2014 0.0		Early maturity, process/fresh			
	2015	9.1	3.8				
Castle Russet	2014	0.5	0.1	Medium maturity,			
	2015	0.0	0.0	process/fresh			
(@ North Dakota)	2015	0.0	0.0				
Clearwater Russet	2014	22.5	10.2	Medium/late maturity,			
	2015	35.8	11.9	Process/fresh			

### Different trial - <u>Seed Lot testing</u>- PMTV and TRV

-essentially a survey for virus amounts in the industry

Washington state

- Chuck Brown/Mark Pavek
  - 2016: 0.29% TRV-positive tubers 1.76% PMTV-positive tubers
  - 2017: 0% TRV-positive tubers 4.07% PMTV-positive tubers
  - Varieties included: Umatilla, Ranger, Shepody, Nordonna, Norkotah, Agata, and Jelly
  - All + tubers were symptomless carriers in yr. 1, and all but one tuber were symptomless carriers in yr. 2

Oregon state

• Frost

- 88 seed lots assayed for TRV and PMTV
- No TRV or PMTV was detected in OR seed lot trials

## PCR test for detection of PMTV from soil sample (last week! First Look Phytopathology)

Development and application of a reverse transcription real-time PCR (RTqPCR) and droplet digital PCR (RT-ddPCR) assays for the <u>direct detection of</u> <u>Potato mop top virus in soil</u>. B. Pandey, I. Mallik, N.C. Gudmestad, Phytopathology 2019

- RT-qPCR and droplet digital (RT-ddPCR) assays of the total RNA extracted directly from soil to detect PMTV
- Both assays detected PMTV from all soil types used in samples with less than 10 PMTV copies per  $\mu l$
- First report of real-time PCR and droplet digital PCR for detection of PMTV directly from soil.

**RNA** extracted from Primer/probe Sequence (5'-3') **RNA** extracted from RNA extracted from Amplicon (Reference) (Fluorophore) size (nt)<sup>a</sup> Sss sporosori b manually spiked soile naturally infested Ct<sup>c</sup> value ± SD<sup>d</sup> soil samples<sup>f</sup> Ct value ± SD (Detection (Detection Ct values frequency) frequency) (Detection frequency) PMTV 601 F ACCGAACGCTTTGGTGGAAGT 157  $25.87 \pm 0.59$  $34.19 \pm 0.89$ >35 CGGAAGCTTCTCTCGGACCT PMTV 601 R (100%)(30%)(25%)PMTV 603 P CGTCTTGCCCGCCCTTCGCCGT (This study) (FAM)<sup>g</sup> PMTV 501 F TGACGCTTGGGACCATGAGC 144  $24.80 \pm 0.56$  $34.36 \pm 1.16$ >40PMTV 501 R GACACCTGGCTCAACACGCT (0%) (100%)(20%)PMTV 503 P ACTTCCTCACGGCAGCCTTCATGGCC (This study) T (FAM) **PMTV 401 F** GGAAGTTCACTTGTTGGTGATAAA 140  $22.58 \pm 0.38$  $27.47 \pm 0.91$  $\sim 25$  to  $\sim 31$ PMTV 402 R GAAGCTTCTCTCGGACCTAATC (100%)(100%)(90%) PMTV 403 P TGTAGCGTTGCAGACAGTGAACGG (This study) (FAM) PMTV-1948F GTGATCAGATCCGCGTCCTT 70  $25.48 \pm 0.19$  $35.23 \pm 0.68$ > 35 PMTV-2017R CCACTGCAAAAGAACCGATTTC (100%)(40%)(10%)PMTV-1970 ACC AGA ACT ACG GTG CCG CGT (Mumford et CG (FAM) al. 2000)

Table 1. Primers and probes for PMTV detection and their preliminary test results.

<sup>a</sup> nt – nucleotide

<sup>b</sup> RNA with approximately 1×10<sup>3</sup> µl<sup>-1</sup> copies of viral target.

<sup>c</sup>Ct - Mean cycle threshold and only positive reactions were used in the calculation of the mean Ct value

#### Story of how to use new tools

- Soil type loamy fine sand
- By 1906 sub-irrigation was used in the area
- By 1970s sprinkler irrigation was replacing sub-irrigation
- One of the areas implicated in Xu et al. 2004 surveillance paper

USDA ARS Idaho Soil - S. subterranea detection and quantification, 10-13-17, NDSU lab Mallik and Pandey.

Submitted by: Jonat Whitworth							
Sample	Fluor	Target	Cq	SQ	Sporosori/g soil	PS	ΡΜΤν
IDS-2	FAM	Sporosori	24.12	1057.02	4.2E+03	М	Positive
IDS-7	FAM	Sporosori	24.45	1064.84	4.3E+03	Μ	Positive
Positive Control	FAM	Sporosori	18.42	354853.1	1.4E+06	Н	
Negative Control	FAM	Sporosori	-	-	-	Ν	
Water Blank	FAM	Sporosori	-	-	-	Ν	

- S. Subterranea detection by van de Graaf et al.
   2003 primers
- PMTV detected in soil samples using real-time RT-PCR, NDSU lab protocol



#### Pomerelle Russet: Early maturity, process/fresh



Castle Russet: Medium maturity, process/fresh



Solutions to grower problems with PMTV

- Ability to survey fields for presence of PMTV
- Varieties that have little to no symptoms in infested fields.

#### • CURRENT STUDY

- Pomerelle and Castle Russet resistant
- Russet Burbank and Clearwater Russet susceptible
- Planted in infested field
- <u>At harvest:</u>
  - tuber symptoms will be rated
  - Samples for PCR confirmation of PMTV will be taken from symptomatic and asymptomatic tubers





Thanks to:

Whitworth & Novy crew

#### Technicians

- Darren Hall
- Brian Schneider
- Mark Fristad
- Ian Fullmer
- Benjamin Hudson

#### Post-docs

- Greg Elison
- Jae Park