

Title: Quantification of Potato Stem Colonization by *Verticillium dahliae* using real-time PCR

Submitted to NPPGA and the Grower/Processor Consortium

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Research Objectives:

- 1) Develop a real-time duplex PCR assay to enable rapid quantification of *V. dahliae* in potato stem tissue necessary to enhance large scale resistance breeding efforts.
- 2) Assess colonization by *V. dahliae* of russet potato cultivars with purported resistance to Verticillium wilt.

Research Plan:

Eight potato cultivars with russet skin types were selected for evaluation of *V. dahliae* colonization. Among these were known resistant (R), cv. Ranger Russet, and very susceptible (VS), cv. Russet Norkotah, which were included as controls (Hane et al., 2003; Mosley et al., 2001; Jansky, 2009). The remaining six cultivars had been previously reported as moderately resistant (MR) to very resistant (VR), but in most instances stem colonization had not been evaluated; designations were based solely on the presence or absence of wilt symptoms in the field. These cultivars include, Russet Burbank (S/MS/MR/R) (Hane et al., 2003; Lynch et al., 2004; Mosley et al., 1999; Mosley et al., 2001), Umatilla Russet (MS/MR) (Mosley et al., 1999), Dakota Trailblazer (AOND95249-1Russ) (VR) (Jansky 2009), Bannock Russet (VR) (Novy et al., 2002), Alturas (VR) (Novy et al., 2003) and Premier Russet (MR) (Novy, et al., 2008). These cultivars were evaluated in the field and also will be evaluated under greenhouse conditions. Each cultivar was planted into naturally infested ground as well as ground amended with additional inoculum, resulting in low (4 ppg) and high (11 ppg) infestation levels.

Visual assessments for symptoms of Verticillium Wilt (VW) were performed six times in July and August from approximately 45 to 90 days after planting (DAP). General wilting as well as symptoms of unilateral wilting which are characteristic of VW were scored on a percent wilt incidence and severity basis for each treatment/replicate combination.

Sections from the basal region of true stems of five plants in each cultivar/treatment/replicate were collected three times during the growing season. Stems collected at the first two sampling dates were surface sterilized, an approximately one gram disk was excised from each stem, placed into a plastic zip-close bag and sterile distilled water was added at a 1:1 weight to volume ratio. Stem segments then were crushed and 50 µl was spread onto solid NP media. At harvest, when plants were senescent or nearly so, stem samples of the same length again were collected from main stems of an additional five plants. Stem sections were dried, ground and plated onto

solid NP media. Plates from both crushed fresh and dried stem sections were incubated in the dark for 14 to 21 days before examination under 60× magnification using a stereomicroscope.

Real-time PCR primers and probe developed from the trypsin protease gene of *V. dahliae* (Dobinson et al., 2004) and internal control actin gene of *Solanum tuberosum* (Atallah and Stevenson, 2006) were compared to traditional plating methods for pathogen quantification in potato stem tissue. Total genomic DNA was extracted from stem sections as well as dried stem tissue using the FastDNA Spin Kit (MPBio, Inc.) following manufacturer's instructions. The specificity and sensitivity of the assay was determined using fungal cultures and serial dilutions of infected stem tissue, respectively.

Results:

Wilt increased in all cultivars as the growing season progressed and was generally higher in inoculated treatments when compared to non-inoculated, although not always significantly so (Table 2; Figure 1). Wilt severity in susceptible control cv. Russet Norkotah was higher than in any other cultivar. Resistant control cv. Ranger Russet did not display lower wilt severity than all other cultivars. Wilt severity in MS/MR cvs. Russet Burbank and Umatilla Russet was between the two control cultivars, however, wilt in MR cv. Premier Russet was lower than that displayed by R cv. Ranger Russet, sometimes significantly so. Among those cultivars rated VR, Dakota Trailblazer (AOND95249-1Russ), Bannock Russet and Alturas, wilt severity tended to be less than or equal to that observed in R control cv. Ranger Russet.

Stem colonization by *V. dahliae* at the first sampling date was highly correlated with wilt severity observed on August 26 ($r = 0.92$; $P < 0.0001$) (Figure 2). Colonization increased in most cultivars from the first to the second sampling date (Figure 3). S control cv. Russet Norkotah had significantly higher colonization levels at both sampling dates than all other cultivars, while colonization in R cv. Ranger Russet was not significantly different than colonization levels of any of the other cultivars at both sampling dates, excluding cv. Russet Burbank at the first sampling date only. MS/MR cv. Russet Burbank and cv. Umatilla Russet again were colonized at a level between the S and R control cultivars. No *V. dahliae* was detected in MR cv. Premier Russet at the first sampling date, and very low levels were observed at the second sampling date. VR cv. Dakota Trailblazer was colonized at low levels at both sampling dates and cv. Bannock Russet and cv. Alturas were colonized at very low levels at the first sampling date and more moderate levels at the second sampling date.

Real-time PCR efficiencies for amplification of the potato actin gene (97.6%) and the trypsin protease gene of *V. dahliae* (94.9%) were very high using this duplex assay. Real-time PCR successfully amplified the *V. dahliae* in all eight cultivars evaluated, including numerous plants where no *V. dahliae* colonies formed on plates (data not shown). The correlation between real-time PCR Ct values and percent wilt evaluated two weeks after stem sampling ($r = -0.72$; $P = 0.0016$) wilt on August 26 ($r = -0.72$; $P = 0.0016$) were strong.

These results indicate that this PCR assay may be utilized to detect *V. dahliae* in potato stems grown under field conditions, but will also enable quantification of the pathogen in potato plants, providing breeders with the ability to rapidly screen germplasm and distinguish between genetic

resistance and tolerance to the pathogen, thereby decreasing the time needed to make cultivar selections and reducing the labor required to determine the host:pathogen interaction.

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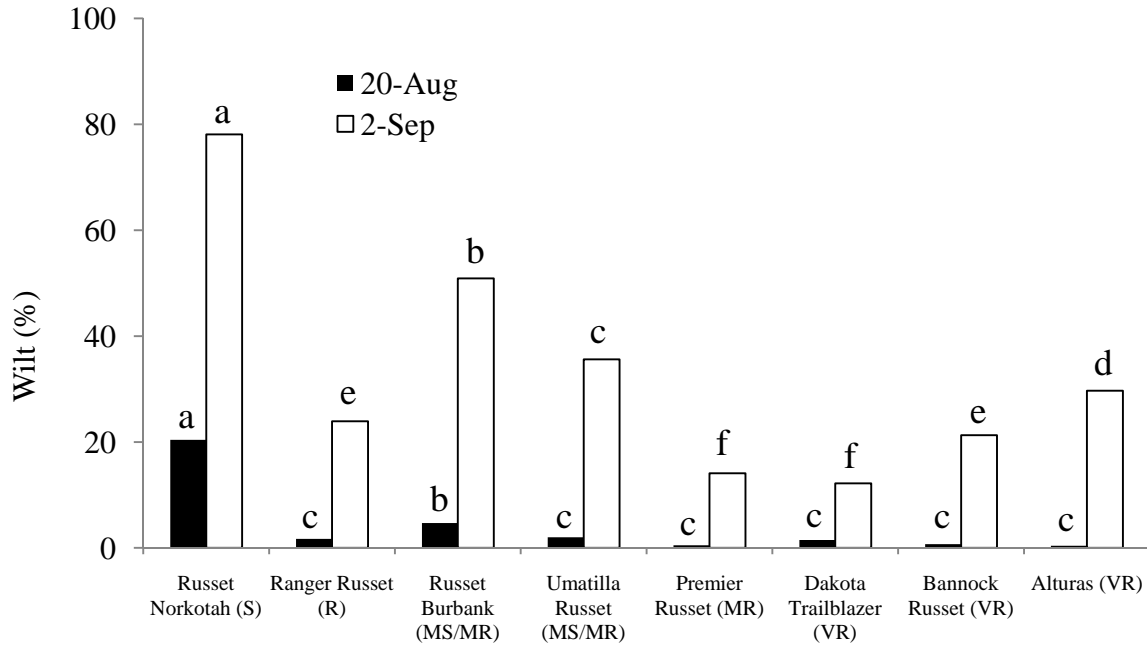


Figure 1. Severity of wilt among eight Russet cultivars with varying reported resistance to *Verticillium* wilt grown in soil infested with *Verticillium dahliae*.

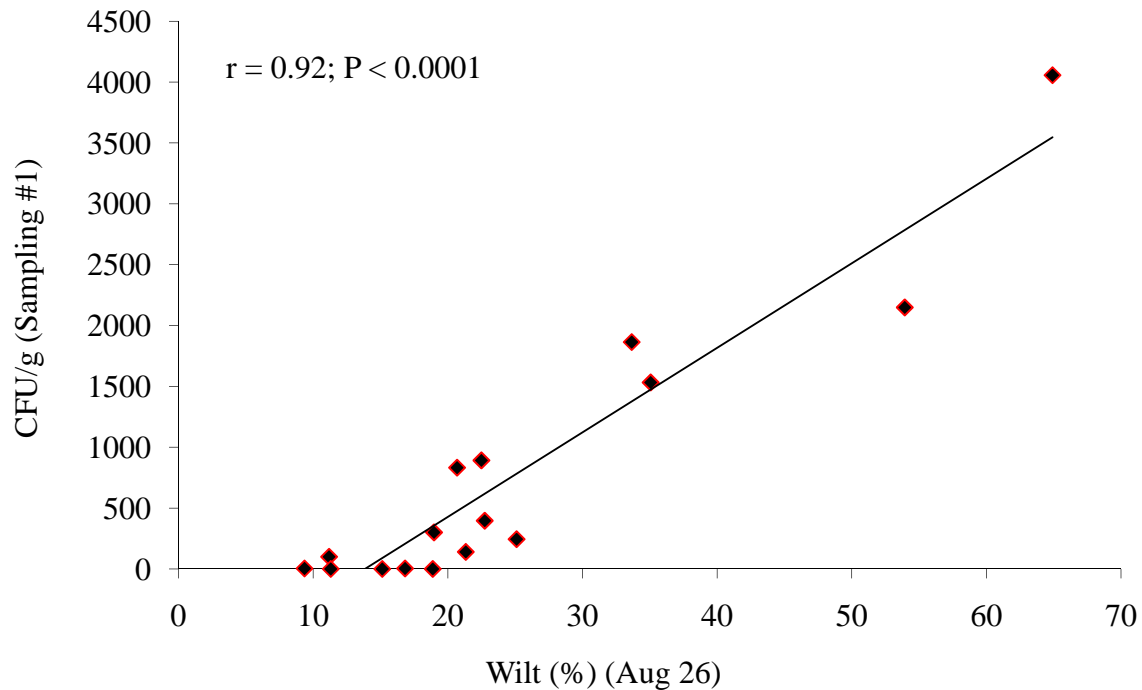


Figure 2. Relationship between colony forming units per gram stem obtained via traditional plating assays on stems sampled at 82 days after planting and wilt severity at 97 days after planting.

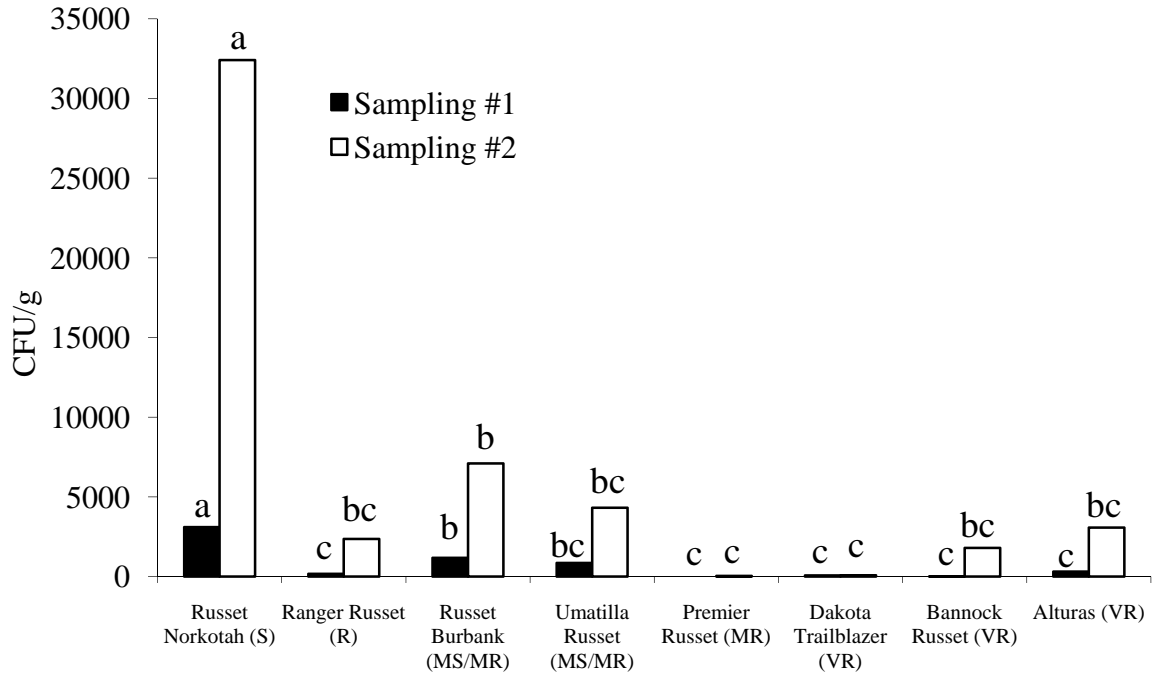


Figure 3. Colony forming units of *Verticillium dahliae* per gram stem obtained from traditional plating assays for eight Russet cultivars with varying reported resistance to *Verticillium* wilt.

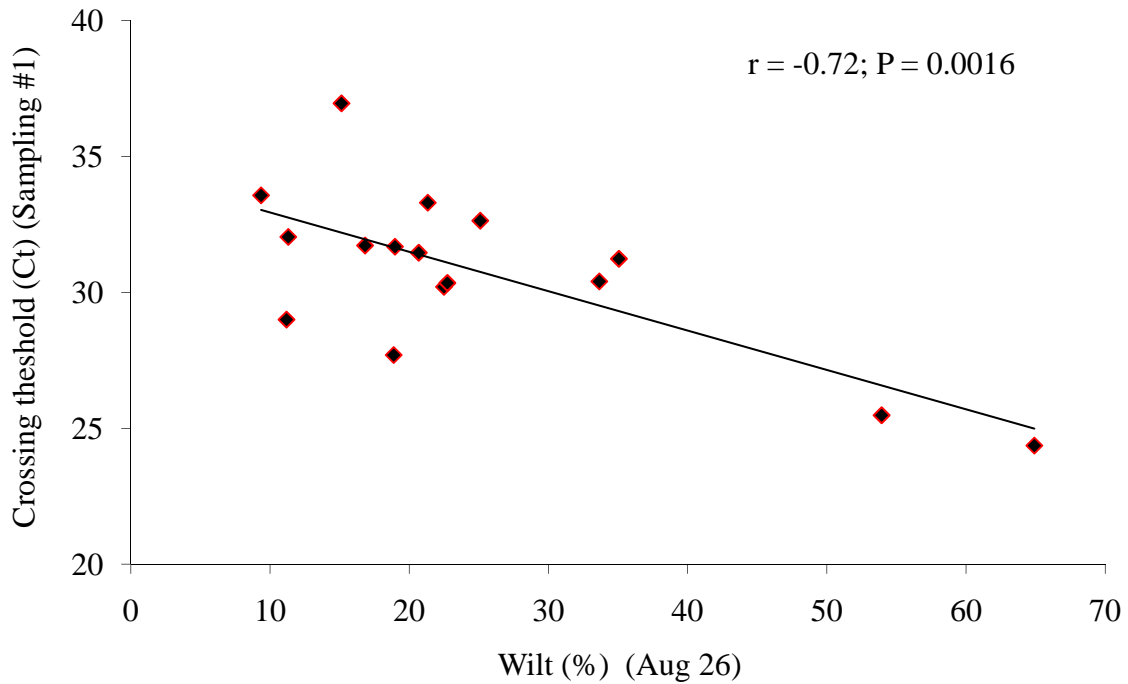


Figure 4. Relationship between crossing threshold values obtained via real-time quantitative PCR assays using primers VTP1-2F/VTP1-2R and Taqman probe VTP1-2P on stems sampled at 82 days after planting and wilt severity at 97 days after plating.

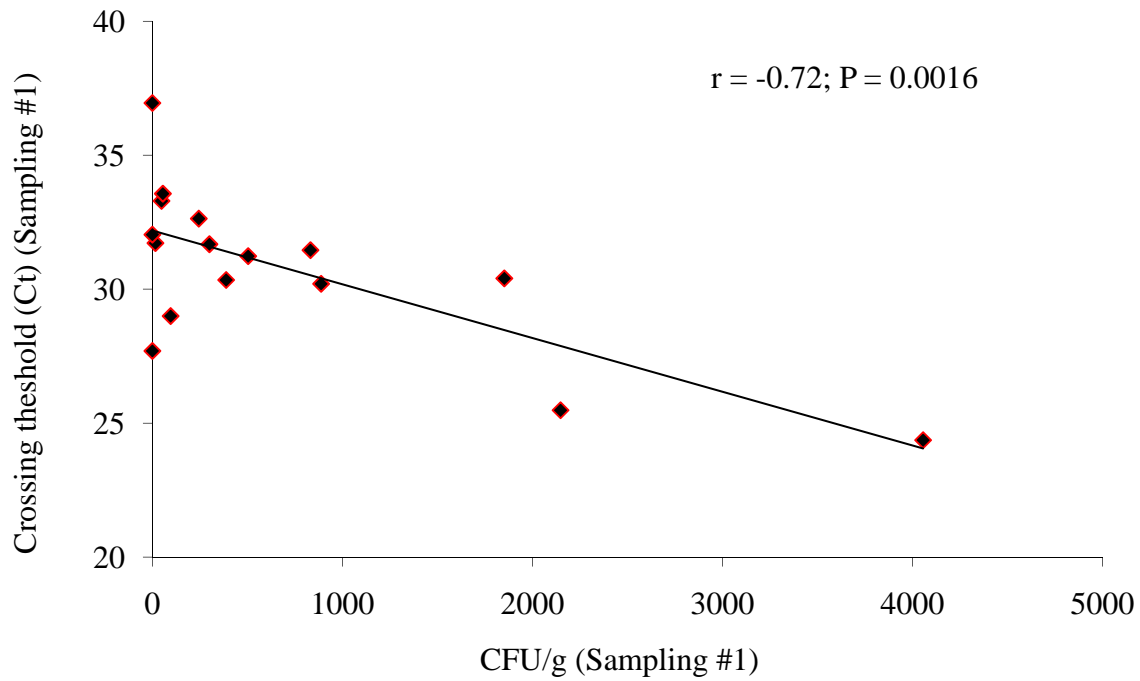


Figure 5. Relationship between crossing threshold values obtained via real-time quantitative PCR assays using primers VTP1-2F/VTP1-2R and Taqman probe VTP1-2P and colony forming units per gram stem obtained via traditional plating assays on stems sampled at 82 days after planting.

Table 1. Percentage of stems wilted among eight Russet cultivars inoculated and not inoculated in-furrow and at side-dress with *Verticillium dahliae*.

Trt	Cultivar	Reported Susceptibility	Infestation Level	Vigor (7-7)	Percent wilted stems							
					7/30	8/4	8/11	8/20	8/26	9/2	9/9	
401	Russet Norkotah	Susceptible check	Low	3.3	3.4	11.8	59.8	62.1	97.6	100.0	100.0	
402	Russet Norkotah	Susceptible check	High	3.0	5.0	23.8	65.8	69.8	99.2	100.0	100.0	
403	Ranger Russet	Resistant Check	Low	3.5	0.0	0.0	3.0	11.3	90.9	98.7	100.0	
404	Ranger Russet	Resistant Check	High	3.3	0.0	0.0	3.4	22.8	94.0	100.0	100.0	
405	Russet Burbank	Moderately Susceptible- Moderately Resistant	Low	3.5	0.0	1.4	2.6	25.4	89.1	100.0	100.0	
406	Russet Burbank	Moderately Susceptible- Moderately Resistant	High	2.8	0.0	1.2	9.1	19.7	97.2	100.0	100.0	
407	Umatilla	Moderately Susceptible- Moderately Resistant	Low	3.0	0.0	0.3	3.1	20.2	89.7	99.3	100.0	
408	Umatilla	Moderately Susceptible- Moderately Resistant	High	3.0	0.0	1.0	6.0	16.0	87.0	100.0	100.0	
409	Dakota Trailblazer	Very Resistant	Low	1.8	0.0	0.0	0.0	10.9	74.8	94.2	100.0	
410	Dakota Trailblazer	Very Resistant	High	1.8	0.0	0.0	0.8	22.2	79.7	100.0	100.0	
411	Bannock	Very Resistant	Low	1.8	0.0	0.0	3.2	7.2	58.7	94.4	99.3	
412	Bannock	Very Resistant	High	1.3	0.0	0.0	7.0	2.6	70.1	97.7	100.0	
413	Alturas	Very Resistant	Low	2.0	0.0	0.0	0.0	2.6	87.7	98.0	100.0	
414	Alturas	Very Resistant	High	2.5	0.0	0.0	0.0	2.3	74.8	99.0	100.0	
415	Premier Russet	Moderately Resistant	Low	2.3	0.0	0.0	3.3	1.4	49.7	90.8	98.8	
416	Premier Russet	Moderately Resistant	High	1.8	0.0	0.0	9.7	5.8	77.2	95.8	100.0	
LSD _{P = 0.05}					0.8	1.3	2.4	7.3	19.4	24.1	NS	NS
	Russet Norkotah	Susceptible check		3.1	4.2	17.8	62.8	66.0	98.4	100.0	100.0	
	Ranger Russet	Resistant Check		3.4	0.0	0.0	3.2	17.1	92.4	99.3	100.0	
	Russet Burbank	Moderately Susceptible- Moderately Resistant		3.1	0.0	1.3	5.8	22.6	93.2	100.0	100.0	
	Umatilla	Moderately Susceptible- Moderately Resistant		3.0	0.0	0.7	4.6	18.1	88.4	99.7	100.0	
	Dakota Trailblazer	Very Resistant		1.8	0.0	0.0	0.4	16.5	77.3	97.1	100.0	
	Bannock	Very Resistant		1.5	0.0	0.0	5.1	4.9	64.4	96.1	99.6	
	Alturas	Very Resistant		2.3	0.0	0.0	0.0	2.5	81.3	98.5	100.0	
	Premier Russet	Moderately Resistant		2.0	0.0	0.0	6.5	3.6	63.5	93.3	99.4	
LSD _{P = 0.05}					0.6	0.9	1.7	5.2	13.7	17.0	NS	NS
			Low	2.6	0.4	1.7	9.4	17.7	79.8	96.9	99.8	
			High	2.4	0.6	3.3	12.7	20.2	84.9	99.1	100.0	
LSD _{P = 0.05}					NS	NS	0.9	2.6	NS	NS	NS	NS

Table 1. (con't).

Trt	Cultivar	Reported Susceptibility	Infestation Level	Vigor (7-7)	Percent wilted stems						
					7/30	8/4	8/11	8/20	8/26	9/2	9/9
401	Russet Norkotah	Susceptible check	Low	3.3	3.4	11.8	59.8	62.1	97.6	100.0	100.0
402	Russet Norkotah	Susceptible check	High	3.0	5.0	23.8	65.8	69.8	99.2	100.0	100.0
LSD _{P = 0.05}				NS	NS	11.7	NS	NS	NS	.	.
403	Ranger Russet	Resistant Check	Low	3.5	0.0	0.0	3.0	11.3	90.9	98.7	100.0
404	Ranger Russet	Resistant Check	High	3.3	0.0	0.0	3.4	22.8	94.0	100.0	100.0
LSD _{P = 0.05}				NS	.	.	NS	NS	NS	NS	.
405	Russet Burbank	Moderately Susceptible-Moderately Resistant	Low	3.5	0.0	1.4	2.6	25.4	89.1	100.0	100.0
406	Russet Burbank	Moderately Susceptible-Moderately Resistant	High	2.8	0.0	1.2	9.1	19.7	97.2	100.0	100.0
LSD _{P = 0.05}				NS	.	NS	NS	NS	NS	.	.
407	Umatilla	Moderately Susceptible-Moderately Resistant	Low	3.0	0.0	0.3	3.1	20.2	89.7	99.3	100.0
408	Umatilla	Moderately Susceptible-Moderately Resistant	High	3.0	0.0	1.0	6.0	16.0	87.0	100.0	100.0
LSD _{P = 0.05}				NS	.	NS	NS	NS	NS	NS	.
409	Dakota Trailblazer	Very Resistant	Low	1.8	0.0	0.0	0.0	10.9	74.8	94.2	100.0
410	Dakota Trailblazer	Very Resistant	High	1.8	0.0	0.0	0.8	22.2	79.7	100.0	100.0
LSD _{P = 0.05}				NS	.	.	NS	NS	NS	NS	.
411	Bannock	Very Resistant	Low	1.8	0.0	0.0	3.2	7.2	58.7	94.4	99.3
412	Bannock	Very Resistant	High	1.3	0.0	0.0	7.0	2.6	70.1	97.7	100.0
LSD _{P = 0.05}				NS	.	.	NS	NS	NS	NS	NS
413	Alturas	Very Resistant	Low	2.0	0.0	0.0	0.0	2.6	87.7	98.0	100.0
414	Alturas	Very Resistant	High	2.5	0.0	0.0	0.0	2.3	74.8	99.0	100.0
LSD _{P = 0.05}				NS	.	.	.	NS	NS	NS	.
415	Premier Russet	Moderately Resistant	Low	2.3	0.0	0.0	3.3	1.4	49.7	90.8	98.8
416	Premier Russet	Moderately Resistant	High	1.8	0.0	0.0	9.7	5.8	77.2	95.8	100.0
LSD _{P = 0.05}				NS	.	.	.	NS	NS	NS	NS

A significant interaction was observed between the main effects of cultivar and inoculation was observed in percent wilted stems on 8/4 ($P = 0.05$).

Table 2. Percentage wilt per stem and total yield among eight Russet cultivars inoculated and not inoculated in-furrow and at side-dress with *Verticillium dahliae*.

Trt	Cultivar	Reported Susceptibility	Infestation Level	Percent wilt/stem							Total Yield (cwt/a)
				7/30	8/4	8/11	8/20	8/26	9/2	9/9	
401	Russet Norkotah	Susceptible check	Low	0.3	1.3	5.5	17.5	53.4	75.0	91.3	523.5
402	Russet Norkotah	Susceptible check	High	1.0	3.0	7.8	22.9	64.2	81.2	96.6	511.5
403	Ranger Russet	Resistant Check	Low	0.0	0.0	0.1	1.5	19.7	26.4	48.8	546.4
404	Ranger Russet	Resistant Check	High	0.0	0.0	0.1	2.0	17.7	21.0	42.4	574.4
405	Russet Burbank	Moderately Susceptible- Moderately Resistant	Low	0.0	0.2	0.1	5.2	32.9	48.7	73.6	506.5
406	Russet Burbank	Moderately Susceptible- Moderately Resistant	High	0.0	0.2	0.3	4.2	34.2	53.3	76.3	485.4
407	Umatilla	Moderately Susceptible- Moderately Resistant	Low	0.0	0.0	0.1	2.2	18.6	36.8	62.8	583.8
408	Umatilla	Moderately Susceptible- Moderately Resistant	High	0.0	0.0	0.4	1.8	20.0	34.2	65.9	562.7
409	Dakota Trailblazer	Very Resistant	Low	0.0	0.0	0.0	1.1	7.3	12.0	21.6	510.5
410	Dakota Trailblazer	Very Resistant	High	0.0	0.0	0.0	2.0	8.9	12.5	21.1	491.2
411	Bannock	Very Resistant	Low	0.0	0.0	0.1	1.2	11.1	18.5	35.0	449.1
412	Bannock	Very Resistant	High	0.0	0.0	0.1	0.2	13.4	24.1	37.8	495.9
413	Alturas	Very Resistant	Low	0.0	0.0	0.0	0.4	19.3	24.2	37.7	578.3
414	Alturas	Very Resistant	High	0.0	0.0	0.0	0.3	19.1	35.0	46.7	501.4
415	Premier Russet	Moderately Resistant	Low	0.0	0.0	0.1	0.1	5.0	10.3	25.7	487.9
416	Premier Russet	Moderately Resistant	High	0.0	0.0	0.2	1.1	11.6	19.1	34.1	424.4
LSD _{P = 0.05}				0.3	0.5	0.7	2.2	3.9	6.0	6.0	78.8
	Russet Norkotah	Susceptible check		0.7	2.2	6.7	20.4	59.2	78.1	94.1	517.5
	Ranger Russet	Resistant Check		0.0	0.0	0.1	1.7	18.8	23.9	45.9	560.4
	Russet Burbank	Moderately Susceptible- Moderately Resistant		0.0	0.2	0.2	4.7	33.4	50.9	74.7	495.9
	Umatilla	Moderately Susceptible- Moderately Resistant		0.0	0.0	0.2	2.0	19.2	35.6	64.2	573.3
	Dakota Trailblazer	Very Resistant		0.0	0.0	0.0	1.5	8.1	12.2	21.4	500.8
	Bannock	Very Resistant		0.0	0.0	0.1	0.7	12.2	21.3	36.2	472.5
	Alturas	Very Resistant		0.0	0.0	0.0	0.4	19.2	29.7	42.6	539.9
	Premier Russet	Moderately Resistant		0.0	0.0	0.1	0.5	7.9	14.1	29.2	456.2
LSD _{P = 0.05}				0.2	0.4	0.5	1.5	2.7	4.2	4.2	55.7
			Low	0.0	0.2	0.8	4.1	22.6	34.7	53.7	523.3
			High	0.2	0.5	13.6	5.4	26.7	39.3	57.6	505.9
LSD _{P = 0.05}				0.1	0.2	0.3	0.7	1.3	2.0	2.0	NS

Table 2. (con't).

Trt	Cultivar	Reported Susceptibility	Infestation Level	Percent wilt/stem							Total Yield (cwt/a)
				7/30	8/4	8/11	8/20	8/26	9/2	9/9	
401	Russet Norkotah	Susceptible check	Low	0.3	1.3	5.5	17.5	53.4	75.0	91.3	523.5
402	Russet Norkotah	Susceptible check	High	1.0	3.0	7.8	22.9	64.2	81.2	96.6	511.5
LSD _{P = 0.05}				0.7	1.1	1.7	4.0	4.9	5.6	3.1	NS
403	Ranger Russet	Resistant Check	Low	0.0	0.0	0.1	1.5	19.7	26.4	48.8	546.4
404	Ranger Russet	Resistant Check	High	0.0	0.0	0.1	2.0	17.7	21.0	42.5	574.4
LSD _{P = 0.05}				.	.	NS	NS	NS	4.5	5.4	NS
405	Russet Burbank	Moderately Susceptible-Moderately Resistant	Low	0.0	0.2	0.1	5.2	32.9	48.7	73.7	506.5
406	Russet Burbank	Moderately Susceptible-Moderately Resistant	High	0.0	0.2	0.3	4.2	34.2	53.3	76.3	485.4
LSD _{P = 0.05}				.	NS	0.2	NS	NS	NS	NS	NS
407	Umatilla	Moderately Susceptible-Moderately Resistant	Low	0.0	0.0	0.1	2.2	18.6	36.8	62.8	583.8
408	Umatilla	Moderately Susceptible-Moderately Resistant	High	0.0	0.0	0.4	1.8	20.0	34.2	65.9	562.7
LSD _{P = 0.05}				.	.	NS	NS	NS	NS	NS	NS
409	Dakota Trailblazer	Very Resistant	Low	0.0	0.0	0.0	1.1	7.3	12.0	21.6	510.5
410	Dakota Trailblazer	Very Resistant	High	0.0	0.0	0.0	2.0	8.9	12.5	21.1	491.2
LSD _{P = 0.05}				.	.	NS	NS	NS	NS	NS	NS
411	Bannock	Very Resistant	Low	0.0	0.0	0.1	1.2	11.1	18.5	35.0	449.1
412	Bannock	Very Resistant	High	0.0	0.0	0.1	0.2	13.4	24.1	37.8	495.9
LSD _{P = 0.05}				.	.	NS	0.8	NS	4.5	NS	NS
413	Alturas	Very Resistant	Low	0.0	0.0	0.0	0.4	19.3	24.2	37.7	578.3
414	Alturas	Very Resistant	High	0.0	0.0	0.0	0.3	19.1	35.0	46.7	501.4
LSD _{P = 0.05}				.	.	.	NS	NS	8.7	NS	NS
415	Premier Russet	Moderately Resistant	Low	0.0	0.0	0.1	0.1	5.0	10.3	25.7	487.9
416	Premier Russet	Moderately Resistant	High	0.0	0.0	0.2	1.1	11.6	19.1	34.1	424.4
LSD _{P = 0.05}				.	.	NS	1.0	2.0	4.9	6.4	NS

A significant interaction was observed between the main effects of cultivar and inoculation in wilt per stems on 8/11, 8-20, 8-26, 9-2 and 9-9 ($P = 0.05$).

A significant interaction was not observed between the main effects of cultivar and inoculation in total yield (cwt/a) ($P = 0.05$).