American Journal of Potato Research (2019) 96:427–444
https://doi.org/10.1007/s12230-019-09730-x

Symptom Expression of Mainstream and Specialty Potato Cultivars to Bacterial Ring Rot (*Clavibacter sepedonicus*) and Evaluation of in-Field Detection

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Published online: 22 June 2019

Abstract
Bacterial ring rot caused by *Clavibacter sepedonicus* is a zero-tolerance disease in seed potato certification and can cause crop loss and substantial economic damage for affected growers. To address symptom expression and time of expression, 28 cultivars and advanced breeding lines were inoculated with *C. sepedonicus* and grown in North Dakota and Idaho. Cultivars included russet, chip, and specialty types. Foliar ratings were taken, and first symptoms were observed as early as 91 days after planting in Idaho and 55 days in North Dakota. Symptom characteristics were noted for each cultivar. This information is useful to growers and certification officials. Samples of stems, petioles, stolons, and tubers were also collected at different pre-harvest intervals to determine if *C. sepedonicus* can be reliably detected in the lab. The ability to detect *C. sepedonicus* before harvest allows an affected grower to make harvest and management decisions that limit damage caused by bacterial ring rot.

Resumen
La pudrición anular bacteriana (BRR) causada por *Clavibacter sepedonicus* (Cs) es una enfermedad de cero tolerancia en la certificación de papa-semilla, y puede causar pérdida del cultivo y daño económico substancial a los productores afectados. Para ver la expresión de síntomas y tiempo de expresión, se inocularon 28 variedades y líneas avanzadas de mejoramiento con Cm y se cultivaron en Dakota del Norte y Idaho. Las variedades incluyeron tipos russet, de freído, y de especialidad. Se tomaron lecturas foliares y se observaron los primeros síntomas tan temprano como a los 91 días después de la siembra en Idaho, y 55 días en Dakota del norte. Se notaron las características del síntoma para cada variedad. Esta información es útil para productores y oficiales de certificación. También se colectaron muestras de tallos, pecíolos, estolones y tubérculos, a diferentes intervalos de pre-cosecha, para determinar si Cm puede ser detectada confiablemente. La habilidad para detectar Cm antes de la cosecha le permite a un productor afectado hacer decisiones de cosecha y manejo que limiten el daño causado por BRR.

Keywords BRR · CelA real-time PCR

Introduction

Clavibacter sepedonicus (previously re-classified from *Clavibacter michinganensis* subsp. *sepedonicus* [Li et al. 2018]) is the cause of bacterial ring rot in potato and results in tuber decay in the vascular region. This disease is difficult to control and initial infections are soon invaded by secondary organisms such as Pectobacterium soft rotting bacteria. Bacterial ring rot (BRR) is a vascular wilt disease with symptoms that range from a whole stem wilt to stunting of the plant and a myriad of leaf symptoms. Chlorotic regions in leaflets are some of the more common symptoms as well as leaf margin necrosis resulting in a somewhat rolled leaflet with "burned" edges. Survival of the bacteria on equipment and

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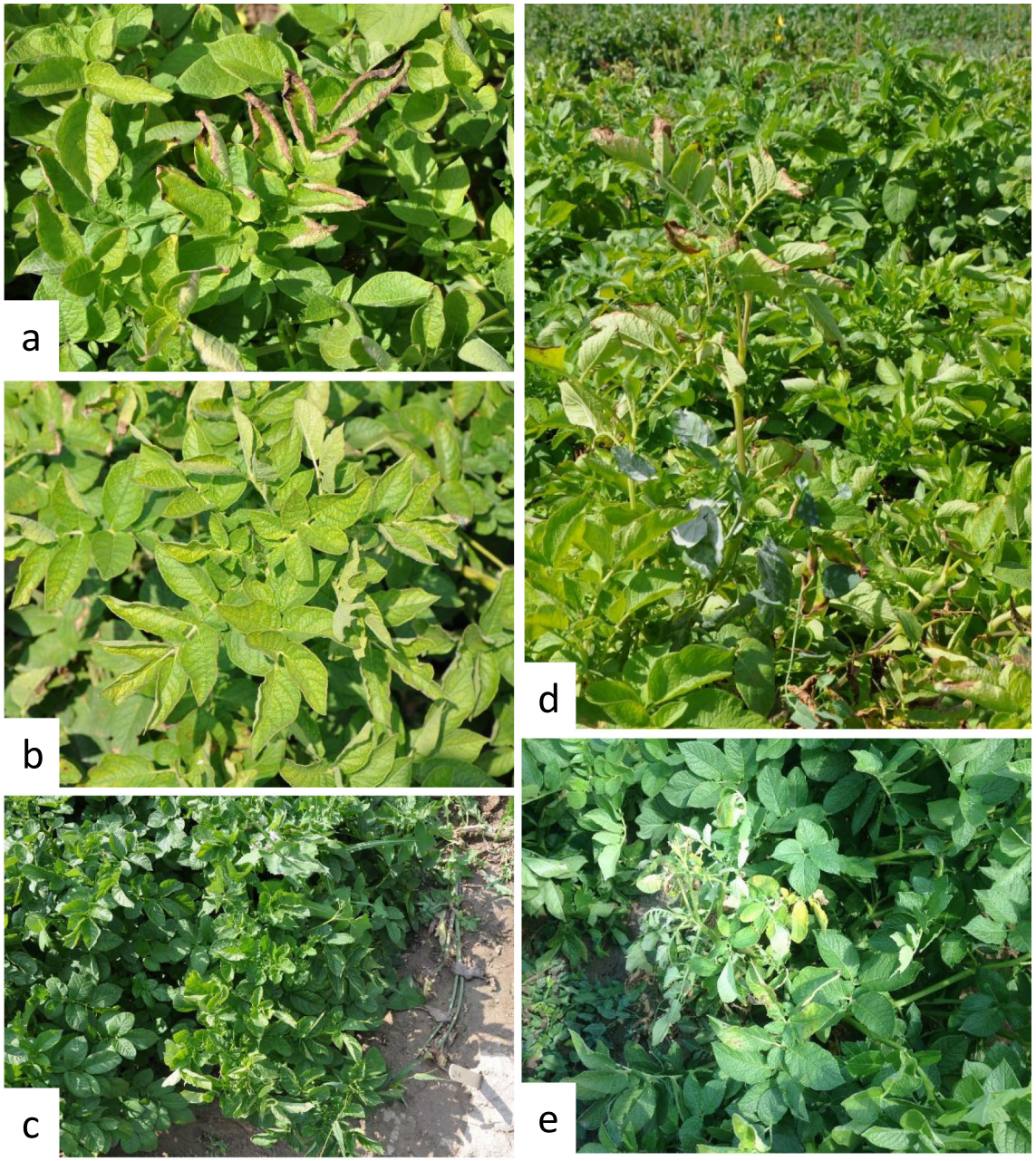

Fig. 1 Foliar symptoms of bacterial ring rot: (**a**) leaflet margin necrosis in A01143–3C, (**b**) interveinal chlorosis in Payette Russet, (**c**) early dwarfing in A03921–2, (**d**) upright stem flagging in A01143–3C, (**e**) whole stem green wilt in Payette Russet. Photos are from plots in Kimberly, Idaho

in storages allows contamination of future crops (Nelson 1980). The ability of Cs to survive cold temperatures on a multitude of surfaces, including belts and rollers of potato handling equipment make the pathogen difficult to eradicate.

BRR is treated as a "zero-tolerance" disease in seed potato certification in the U.S., Canada, and other countries (Gudmestad et al. 1987; Kudela 2007; Logsdon et al. 1957) and detection of even one infected tuber or plant in a seed lot can result in rejection from certification. In addition, many seed certifying states also require a complete "flush-out" of all seed lots on the farm and replacement with new seed as an effective method for eliminating the often-recurring cycle of BRR on a farm. This BRR-management "tool" was adopted as far back as 1931(Logsdon et al. 1957) and again recommended as part of a BRR Task Force in 1987 (Gudmestad et al. 1987) due to widespread problems with BRR.

While some new cultivars include BRR reaction information at the time of their release, not all do. Previous studies also indicate there are groups of cultivars that have asymptomatic tubers produced from infected plants (De Boer and McCann 1990), cultivars that produce asymptomatic plants when infected (Kawchuk et al. 1998), and evidence that infection

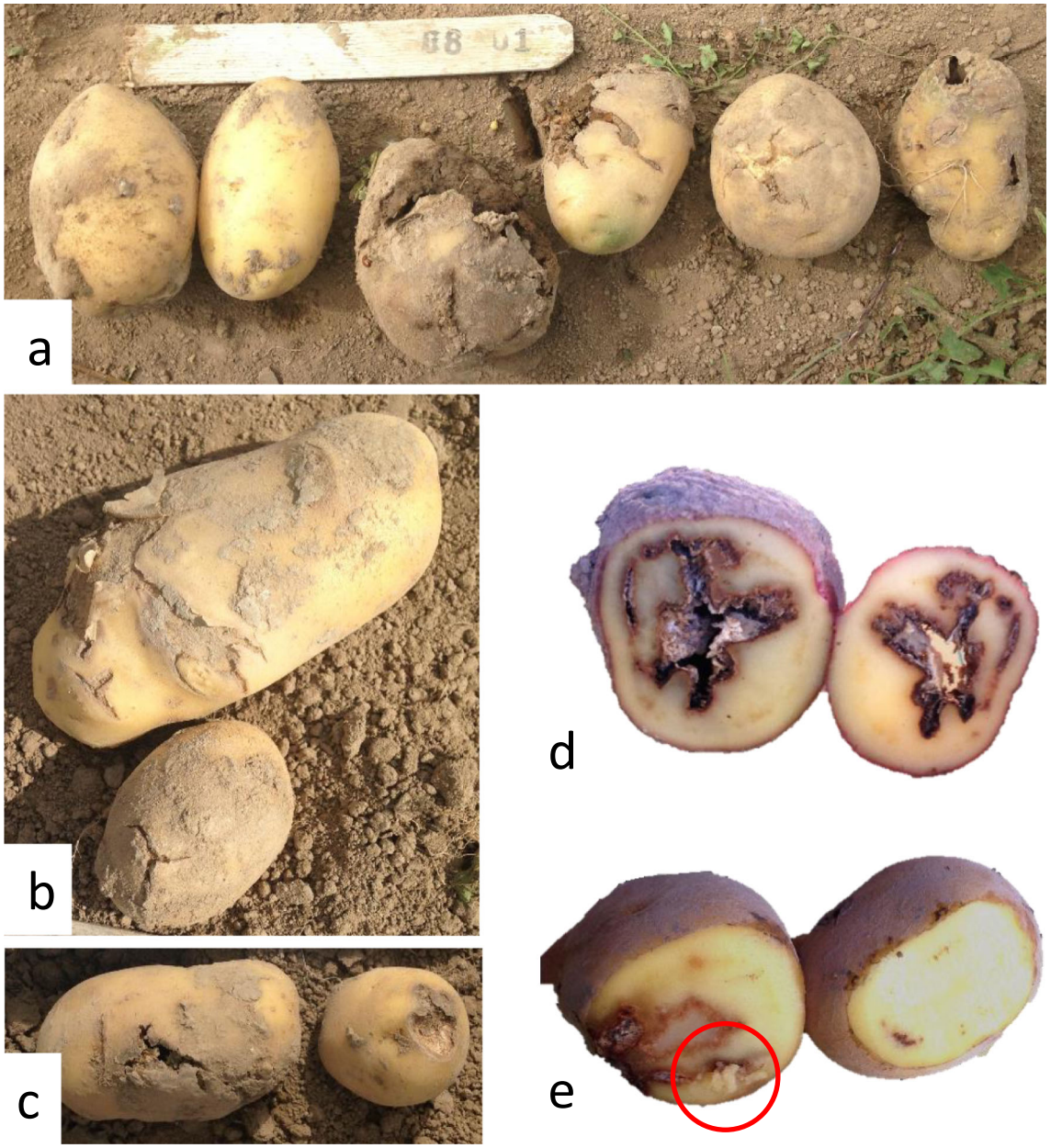

Fig. 2 Bacterial ring rot symptoms in tubers; external tuber cracking in (**a**) Agata, (**b**) Challenger, (**c**) Bintje; internal rot associated with vascular ring in (**d**) Rosara and (**e**) Gala. Gala also showing bacterial ooze (red circle area). Visual evidence of dry rot that is not associated with bacterial ring rot in center of Rosara tuber and edges and center of Gala tuber. Photos are from plots in Kimberly, Idaho

(latency) can remain undetected due to low bacterial cell levels for up to three to four seed generations (Franc 1999). In addition, more and more specialty cultivars are grown in the United States and the number of russeted cultivars for tablestock and processing has also increased. The number of russeted cultivars certified as seed in 2004 was 18 and in 2018 it was 39, while the number of hectares decreased from 26,455 to 21,321 (http://potatoassociation.org/industry/seed).

These cultivars are grown across the U.S. in California, Colorado, Oregon, Idaho, Montana, Washington, North Dakota, Minnesota, Michigan, Wisconsin, New York, and Maine. Knowledge of the type of symptoms characteristic of these new cultivars and the "days to expression" would be beneficial to the industry. In addition, the ability to detect a BRR infection prior to harvest would allow a grower to make management decisions on how to handle the affected crop so that contamination of equipment and storages does not occur. Should an infection occur, proper steps to remove, clean and disinfect exposed equipment and facilities is needed to prevent a reoccurrence of bacterial ring rot (Secor et al. 1997) However, historical records have shown that absence of BRR in a seed program does not mean it is eradicated. In the Czech Republic,

Table 1 Symptoms of bacterial ring rot in cultivars and advanced breeding clones grown in Idaho, 2015. Inoculum dose at 1×10^8 cells/mL

Entry		Number replications ($n = 3$) w/ symptoms @ days after planting				Foliar symptoms[1]	Number replications positive PCR	Number tubers	Number tubers w/ symptoms	
		100	108	115	122				External	Internal
1	A05182–7Y	2	1	1	0	T, F	3	234		
2	Agata	1	2	1	2	MN, IVC, R, ED, F	3	169	9	2
3	Anuschka	0	0	1	0	W	1	104		
4	Bintje	1	0	0	0	MN, IVC	3	249	1	1
5	Blue Belle	1	0	0	0	F	2	361		
6	Cecile	1	1	1	0	IVC, T	2	635		
7	Challenger	0	1	1	2	MN, IVC, W	2	188	2	2
8	Ciklamen	1	0	0	0	W	3	243		
9	Elfe	0	0	2	1	MN, IVC, W	3	363		1
10	Gala (2 reps only)	1	0	0	0	IVC	3	166		2
11	Jelly	1	0	0	0	IVC	3	205	1	1
12	Maris Peer	0	0	0	1	MN, IVC, F	3	379	2	1
13	Melody	0	0	0	0		3	173		
14	Rosara -(2 reps only)	1	0	1	1	MN, IVC	3	143	3	2
15	Victoria-(1 rep only)	0	0	0	0		0	33		
16	Yukon Gem	0	1	1	0	more upright	3	256		
17	Yukon Gold	1	0	3	3	MN, IVC, IVN, F	3	159		
1	A01143–3C	0	2	3	3	MN, IVC, ED	3	232	4	
2	A03921–2	0	0	1	1	MN, IVC, W	3	149		
3	Classic Russet	1	1	1	1	MN, IVC, F	3	142		
4	Clearwater Russet	0	0	0	0		3	134	1	2
5	Mountain Gem Russet	1	0	1	1	MN, IVC, W	3	89	3	
6	Payette Russet	1	1	1	0	MN, IVC, W	3	169	1	1
7	Pomerelle Russet	0	2	3	3	MN, IVC, IVN, F, W	3	153	4	5
8	Targhee Russet	0	1	2	1	MN, IVC, IVN, F, W	3	169		
ck	Red Norland	0	1	2	2	MN, IVC, IVN, W	3	134	10	3
ck	Russet Burbank	0	1	0	0	light green, upright	3	131		
ck	Russet Norkotah (2 reps)	1	2	0	0	MN, IVC, F, W	3	102	3	3

[1] *ED* Early dwarf, *R* Rosette, *IVC* Interveinal chlorosis, *IVN* Interveinal necrosis, *MN* Marginal necrosis, *W* Wilt, *F* Flagging, *T* Twist of leaflets

*a follow up test of all tubers collected from these plots was done with PCR

0% BRR was found in 2005 in certified seed potatoes and only 0.2% in commercial potatoes, then in 2016 0.15% in seed and 0.23% in commercial potatoes was found (Kudela 2007). Infected plants can be found visually during a certification inspection, but if only a few symptomatic plants exist, the chance of detection can be very small. In this case, in an infected seed lot, the next most likely place for detection is in tubers when routine samples are collected by an inspector as shipping trucks are being loaded, but at this point, exposure and possible contamination by Cs has likely occurred. A low level of symptomatic plants makes visual inspection and detection of BRR more difficult. Early studies on the effect of Cs infection caused by low inoculum doses ($< 10^2$ cfu/ml) resulted in low bacterial concentration in the plant resulting in "latent" infections or asymptomatic plants (Nelson 1982). A later study that modeled BRR symptom development (Westra et al. 1994) found that plants given lower doses of inoculum tended to survive the entire season without the development of symptoms. The development of asymptomatic Cs infections has plagued the seed potato industry and illustrates the need for sensitive assays, such as real-time PCR that can detect Cs in infected tissue.

The objectives of these studies were, 1) to characterize the foliar and tuber symptoms on a set of russet cultivars and specialty cultivars grown under Idaho and North Dakota conditions, and 2) to determine if BRR can be reliably detected prior to harvest in stems, petioles, tubers, and stolons using the real-time PCR method described by Gudmestad et al. (2009).

Table 2 Symptoms of bacterial ring rot in cultivars and advanced breeding clones grown in Idaho, 2016. Inoculum dose at 1×10^8 cells/mL

Entry		Number replications ($n = 3$) w/ symptoms @ days after planting							Foliar symptoms[1]	Number replications positive PCR:	Number tubers	Number tubers w/ symptoms	
		85	91	98	105	112	117	125				External	Internal
1	A05182–7Y	0	0	0	0	0	0	0		3	255		
2	Agata	0	0	0	0	0	0	0		2	237	2	
3	Anuschka	0	0	1	0	0	0	0	IVC, MN	1	166		
4	Bintje	0	0	0	0	0	0	0		3	287	1	
5	Blue Belle	0	0	0	0	0	0	0		2	184		
6	Cecile	0	0	0	0	0	0	0		2	407		
7	Challenger	0	0	0	0	0	1	1	W	2	155		
8	Ciklamen	0	0	1	0	1	2	0	MN, W	3	236		
9	Elfe	0	0	0	0	0	0	0		0	209		
10	Gala	0	0	1	1	1	0	1	IVC, MN, F	2	258	1	
11	Jelly	0	0	0	0	1	0	0	W	3	224		
12	Maris Peer	0	0	0	0	0	0	0		2	235		
13	Melody	0	0	0	0	0	0	0		3	301	3	3
14	Rosara	0	2	2	2	2	1	2	IVC, MN, all plants light green	3	146		
15	Victoria	0	1	0	0	2	0	2	IVC, MN	3	227	1	1
16	Yukon Gem	0	0	0	0	0	0	0		2	249	1	
17	Yukon Gold	0	0	0	0	0	0	0		0	198		
1	A01143–3C	0	0	1	0	1	1	3	IVC, MN	3	272	1	1
2	A03921–2	0	0	1	2	1	1	1	IVC, MN, W	3	141	2	
3	Classic Russet	0	1	1	1	0	1	0	IVC, MN, F	3	97		
4	Clearwater Russet	0	0	0	0	0	1	0	IVC, MN, W	2	169		
5	Mountain Gem Russet	0	0	0	0	0	0	1	IVC, MN	3	160		
6	Payette Russet	0	0	1	2	2	2	2	IVC, MN	2	192		
7	Pomerelle Russet	0	1	2	0	3	2	2	IVC, MN	3	115	3	1
8	Targhee Russet	0	0	0	0	0	0	0		2	55		
ck	Red Norland	0	0	0	0	1	1	1	IVC, MN	3	153	1	
ck	Russet Burbank	0	0	0	0	0	0	0		2	162	1	1
ck	Russet Norkotah	0	0	0	0	1	0	0	IVC, MN, W	1	96		

[1] *ED* Early dwarf, *R* Rosette, *IVC* Interveinal chlorosis, *IVN* Interveinal necrosis, *MN* Marginal necrosis, *W* Wilt, *F* Flagging, *T* Twist of leaflets

*a follow up test of all tubers collected from these plots was done with PCR

Materials and Methods

Idaho

Symptomatic Expression of Bacterial Ring Rot

Twenty-eight advanced breeding lines/cultivars were used for these studies. Tubers were inoculated by placing seed pieces (fresh cut lengthwise) into 2 l of Ringer's solution containing Cs cells in a concentration of approximately 10^8 cells/ml for 5 min. A rifampicin mutant of Cs (strain #CIC31) was used for the inoculum. To prepare the inoculum, Cs cells were collected from PDA plates exhibiting good bacterial growth by flooding with Ringer's solution. Cell counts were made using a hemocytometer and inoculum concentration was adjusted to approximately 10^8 cells/m. The Ringer's solution was kept cold prior to adding the Cs and each batch was used for not more than 45 min after which a fresh batch was used for inoculations. Four clones were dipped per batch. Seed pieces were then put into paper sacks and left overnight at room temperature, then planted the next day.

The trial was planted on April 28, 2015 and May 11, 2016 with harvest on Sept. 24, 2015 and Sept 21, 2016.

Table 3 Symptoms of bacterial ring rot inoculated with a low inoculum dose in cultivars and advanced breeding clones grown in North Dakota, 2015. Inoculum dose at 1×10^8 cells/mL

Entry		Number replications ($n = 3$) w/ symptoms @ days after planting								Foliar symptoms[1]	Number replications positive PCR:	Number tubers	Number tubers w/ symptoms	
		62	69	76	83	91	100	107	116				External	Internal
1	A05182–7Y (only 2 reps)	0	0	0	1	0	0	0	0	F		117		
2	Agata	0	0	0	1	1	2	1	1	T, W, IVC, MN, F	3	107	11	9
3	Anuschka	0	0	0	1	0	0	1	0	F		129		
4	Bintje	1	1	1	0	0	0	1	1	ED, IVC, T	3	120	7	7
5	Blue Belle (only 2 reps)	0	0	0	0	0	0	0	0			105		
6	Cecile (only 2 reps)	1	1	2	0	0	0	0	0	ED	2	141		
7	Challenger	0	0	0	0	0	0	0	0		2	167	6	6
8	Ciklamen	0	0	0	1	1	1	2	2	MN, F, IVC	3	201	14	19
9	Elfe	0	0	0	0	0	0	0	0		2	87		
10	Gala	1	1	1	0	0	1	1	1	ED, IVC, T, MN	1	72	1	1
11	Jelly	1	1	1	0	0	0	0	0	ED	2	77		
12	Maris Peer	0	0	0	0	1	1	1	0	MN, W, IVC, F	3	176	14	15
13	Melody	0	0	0	0	1	0	0	1	T, W, F	3	166	9	9
14	Rosara	0	0	0	0	0	0	0	1	MN, T	1	151	7	8
15	Soroya (only 2 reps)	0	0	0	0	0	0	0	0			42	1	
16	Victoria	0	0	0	0	0	1	1	1	IVC, T, MN	2	102	1	3
17	Yukon Gem	0	0	0	1	0	0	2	0	F, IVC	2	103		
18	Yukon Gold	0	0	1	3	3	2	0	0	F, MN, IVC, W	3	56	6	7
1	A01143–3C	0	0	0	0	1	2	2	0	W, IVC, MN, F	3	140	8	9
2	A03921–2	0	0	0	0	0	0	1	0	MN	2	133	6	5
3	Classic Russet	0	0	0	0	1	1	1	1	MN, IVC, F, W	1	84		
4	Clearwater Russet	0	0	0	1	0	0	0	1	IVC, T	3	94	12	14
5	Mountain Gem Russet	0	0	0	0	0	0	1	1	IVC, F, T	3	83	10	10
6	Payette Russet	2	2	2	0	0	0	0	0	ED	1	61		
7	Pomerelle Russet (only 1 rep)	1	1	1	0	0	0	0	0	W, ED	1	3		
8	Targhee Russet	0	0	0	0	0	1	1	1	MN, IVC	2	86		
ck	Red Norland	0	0	0	1	1	2	1	1	MN, F, IVC	3	90	9	10
ck	Russet Burbank	0	0	0	0	1	1	1	1	MN, IVC, F	3	78	4	4
ck	Russet Norkotah	0	0	0	2	1	1	0	0	IVC, F, MN	3	101	20	21

[1] *ED* Early dwarf, *R* Rosette, *IVC* Interveinal chlorosis, *IVN* Interveinal necrosis, *MN* Marginal necrosis, *W* Wilt, *F* Flagging, *T* Twist of leaflets

Plots were set up in a randomized complete block design with three replications. Each plot consisted of a row with seven inoculated seed pieces and an adjacent parallel row with seven un-inoculated seed pieces to serve as a "healthy" control allowing comparisons for visual symptom recording. Plots were located on an ARS research facility in Kimberly, Idaho which is a commercial potato growing region. In 2015, fertilizer applied consisted of 224 N, 166 P, 108 K in Kg/ha and in 2016, it was 308 N, 168 P, 168 K. Regular cultural practices for irrigation were followed and foliar sprays for early blight and late blight were applied as necessary. Evaluations of foliar symptoms consistent with BRR were taken starting at 100 days after planting (DAP) in 2015 and at 63 DAP in 2016 and continued on a weekly basis until harvest. Two to three days prior to harvest, one stem from each plant was removed from a hill and tested for Cs. Subsamples of each stem with tissue taken from a one cm cross section from the crown of each stem (soil level line). Stems were combined into seven plant bulk samples and tested using real-time PCR according to the methods described in Gudmestad et al. (2009) with the following modifications: 1) The fluorogenic probe specific for CelA was labeled with FAM at the 5'end and the 3′ end was modified with BHQ1, 2) the real-time PCR mix consisted of 1X Universal Probes Supermix (BioRad SsoAdvanced™), CelA-F and CelA-R primers at a

Table 4 Symptoms of bacterial ring rot inoculated with a high inoculum dose in cultivars and advanced breeding clones grown in North Dakota, 2015. Inoculum dose at 1×10^{10} cells/mL

Entry		Number replications ($n = 3$) w/ symptoms @ days after planting								Foliar symptoms[1]	Number replications positive PCR:	Number tubers	Number tubers w/ symptoms	
		62	69	76	83	91	100	107	116				External	Internal
1	A05182–7Y	0	0	0	1	0	0	0	0	IVC, F	1	158		
2	Agata	0	0	0	2	1	3	0	0	IVC, F, MN	2	155	2	3
3	Anuschka	2	2	2	1	1	1	0	0	ED, IVC, F, W	1	59	1	1
4	Bintje	1	1	1	0	0	0	0	0	ED	3	76		
5	Blue Belle	0	0	0	1	0	0	0	0	IVC, F	1	106		
6	Cecile (only 2 reps)	0	0	0	0	0	0	0	0		2	239		
7	Challenger	1	1	1	0	0	0	0	1	ED, T, MN	2	157	3	4
8	Ciklamen	2	2	2	0	0	1	1	1	ED, F, MN	1	142		
9	Elfe	0	0	0	0	0	0	0	0		2	120	7	8
10	Gala	0	0	0	1	0	0	0	0	IVC, F	3	155		
11	Jelly	0	0	1	0	0	1	1	0	T, F	3	71	4	3
12	Maris Peer	0	0	0	0	1	1	0	1	F, IVC	3	146		
13	Melody	0	0	0	0	0	0	0	1	IVC	1	108		
14	Rosara	0	0	0	2	2	1	2	1	F, T, MN	2	129	4	4
15	Soroya	0	0	0	0	0	0	0	0		2	93		
16	Victoria	0	0	0	1	1	0	0	0	F, IVC	2	95	2	1
17	Yukon Gem	0	0	1	0	0	0	0	0	IVC	1	80	2	2
18	Yukon Gold	0	0	0	3	2	3	2	0	ED, T, F, IVC, MN	3	52		
1	A01143–3C	0	0	0	0	0	0	0	0		1	105		
2	A03921–2	0	0	0	0	0	1	0	1	IVC, F	2	74	2	2
3	Classic Russet	0	0	0	0	0	1	0	0	W	2	69		
4	Clearwater Russet	0	0	0	1	1	0	0	0	IVC, MN	3	63		
5	Mountain Gem Russet	1	1	1	0	0	0	0	0	ED	2	61		
6	Payette Russet	1	1	1	0	0	0	0	0	ED	1	86	1	1
7	Pomerelle Russet	3	3	3	0	0	2	0	0	ED, W, IVC, F	2	40		
8	Targhee Russet	0	0	0	0	0	1	1	1	IVC, MN, F		79		
ck	Red Norland	0	0	0	0	0	0	0	0		1	77	1	0
ck	Russet Burbank	0	0	0	0	0	0	0	0		2	78	3	3
ck	Russet Norkotah	0	0	0	1	2	1	2	0	F, MN, IVC	2	76	8	9

[1] *ED* Early dwarf, *R* Rosette, *IVC* Interveinal chlorosis, *IVN* Interveinal necrosis, *MN* Marginal necrosis, *W* Wilt, *F* Flagging, *T* Twist of leaflets

concentration of 0.4 μM, Cel-A probe at a concentration of 0.2 μM, 2 μL of sample, and PCR grade water to a final volume of 25 μL. All cycling parameters were the same except for the annealing temperature which was lowered from 60 °C to 59 °C. Samples were considered positive when a crossing threshold (Ct) was less than 35 cycles. These results allowed foliar symptoms to be correlated with an actual positive infected plot. If a plot sample tested negative, then all of the tubers from that plot were bulk tested with the same PCR test to determine if an inoculated plot was actually positive for Cs, again allowing a correlation to be established between the observed symptoms and a positive Cs test.

Pre-Harvest Testing for Cs

A second set of plots were planted for evaluating the feasibility of pre-harvest testing for Cs in plants. A factorial of inoculum dose and cultivar (3 X 2) was planted in a randomized complete block design with four replications. These consisted of 30 hills in each plot, with two cultivars (Red Norland and Russet Burbank) that were inoculated at a high dose (10^8 cells/ml) and a low dose (10^2 cells/ml) of Cs and 30 hills in un-inoculated plots that served as controls.

Beginning at 60 days after planting, individual stems were harvested from each block at

Table 5 Symptoms of bacterial ring rot inoculated with a low inoculum dose in cultivars and advanced breeding clones grown in North Dakota, 2017. Inoculum dose at 1×10^8 cells/mL

Entry		Number replications ($n = 3$) w/symptoms @ days after planting								Foliar symptoms[1]	Number replications positive PCR:	Number tubers	Number tubers w/ symptoms	
		55	61	68	76	83	91	98	105				External	Internal
1	A05182–7Y	0	0	0	0	0	0	0	0		3	197	0	2
2	Agata	2	2	1	0	0	0	1	1	ED, T, MN	3	65	8	10
3	Anuschka	0	0	0	0	0	0	0	0		2	130	1	1
4	Bintje	3	3	2	0	0	0	0	0	ED	3	122	11	13
5	Blue Belle	0	0	0	0	0	0	0	0		3	107	0	4
6	Cecile	1	0	0	0	0	0	0	0	ED	2	221	2	2
7	Challenger	2	1	0	0	0	0	0	0	ED	3	141	35	45
8	Ciklamen	2	3	0	0	0	1	1	1	ED, T, F	3	79	6	16
9	Elfe	1	1	0	0	0	1	1	0	ED, IVC, T, F	1	105	1	2
10	Gala	0	1	0	0	0	0	1	1	ED, T, MN	3	163	11	13
11	Jelly	1	0	0	0	0	0	0	0	ED	3	62		
12	Maris Peer	1	1	0	0	0	0	1	0	ED, IVN	3	132	14	20
13	Melody	1	2	1	0	1	0	0	0	ED, IVC, T	3	143	8	12
14	Rosara	0	0	0	0	0	0	1	1	F, MN, T	3	135	2	10
15	Victoria	0	0	0	0	0	0	0	1	IVC, MN	3	124	16	21
16	Yukon Gem	0	1	0	0	0	0	1	1	ED, T, MN	3	78	6	9
17	Yukon Gold	1	0	0	0	0	0	0	2	ED, MN, F	3	121	11	11
1	A01143–3C	2	0	0	0	0	0	0	2	ED, F, MN, IVC	3	136	0	2
2	A03921–2	1	1	0	0	0	0	1	2	ED, T, MN	3	35	9	10
3	Classic Russet	1	0	1	0	0	0	0	0	ED	3	85	1	3
4	Clearwater Russet	2	2	2	0	0	0	0	0	ED	3	34	14	18
5	Mountain Gem Russet	2	2	0	0	0	0	0	0	ED	3	68		
6	Payette Russet	1	1	0	0	0	0	0	1	ED, MN, IVC	3	91	20	27
7	Pomerelle Russet	0	3	3	1	2	3	2	3	ED, MN, T, IVC, F	3	57	10	11
8	Targhee Russet	2	2	1	1	1	0	0	0	ED, T, IVC	3	61	2	6
ck	Red Norland	0	0	0	2	0	1	0	0	MN, T	3	158	13	17
ck	Russet Burbank	0	1	0	0	1	1	1	1	ED, T, IVC, MN	3	135	20	21
ck	Russet Norkotah	0	1	1	0	0	1	3	3	ED, MN, T, IVC, F	3	89	17	23

[1] *ED* Early dwarf, *R* Rosette, *IVC* Interveinal chlorosis, *IVN* Interveinal necrosis, *MN* Marginal necrosis, *W* Wilt, *F* Flagging, *T* Twist of leaflets

approximately 30-day intervals. In 2016, individual tubers from each harvested plant were also collected. A one-centimeter section from the of crown of each stem (soil level line) or an 11 mm core from the stem end of each tuber was tested using the real-time PCR method described by Gudmestad et al. (2009), with modifications described previously.

Samples were collected at 61, 97, and 125 days post-planting in 2015 and at 61, 90, and 120 in 2016. The cross sections from each plant were placed together in an extraction bag with 6 ml of sterile water and macerated by pounding with a hammer, to obtain a crude extraction. Real-time PCR assays were conducted in duplicate on the original crude extract (1:1) as well as a 1:10 dilution and a 1:100 dilution. A sample was noted as positive if at least two of the three dilutions had a Ct value between 19 and 35. In a few exceptions, if only one of the dilutions was positive, but both duplicates showed similar curves on the Ct graph, that sample was considered positive.

To evaluate the feasibility of testing blind bulk samples in a field scouting situation, composite stem testing was done by combining one-centimeter sections of known positive and negative stems in a 1:199 ratio. Positive stems were selected from the single stem testing to provide a representative sample of the range of Ct values observed for individual stems. Negative stem sections were obtained from the un-inoculated buffer

Table 6 Symptoms of bacterial ring rot inoculated with a high inoculum dose in cultivars and advanced breeding clones grown in North Dakota, 2017. Inoculum dose at 1×10^{10} cells/mL

Entry		Number replications (n = 3) w/symptoms @ days after planting								Foliar symptoms[1]	Number replications positive PCR:	Number tubers	Number tubers w/ symptoms	
		55	61	68	76	83	91	98	105				External	Internal
1	A05182–7Y	1	1	0	0	0	0	0	0	ED	3	126	1	2
2	Agata	2	1	1	1	2	2	1	1	ED, T, MN, F	3	52	4	12
3	Anuschka	0	0	1	0	0	0	0	0	ED	2	124	3	5
4	Bintje	1	0	0	0	0	0	0	0	ED	3	144	22	28
5	Blue Belle	2	1	2	0	0	0	0	0	ED	3	86		
6	Cecile	2	2	0	0	0	0	0	0	ED	1	150	2	3
7	Challenger	2	3	1	0	0	0	0	0	ED	3	124	22	24
8	Ciklamen	1	1	1	0	0	1	1	1	ED, T, F, MN	3	133	18	43
9	Elfe	1	2	1	0	0	0	0	0	ED	1	112	1	1
10	Gala	2	1	0	0	1	0	0	0	ED, T	3	76	1	1
11	Jelly	1	0	0	0	0	0	1	0	ED, T, MN	3	94	10	11
12	Maris Peer	1	0	0	0	0	0	0	0	ED	3	129	9	16
13	Melody	2	1	0	0	0	0	0	0	ED	3	89	19	20
14	Rosara	1	1	1	0	0	0	0	0	ED	3	84	9	14
15	Victoria	2	2	1	0	0	0	0	1	ED, MN	3	60	17	19
16	Yukon Gem	0	0	0	0	0	1	0	0	MN, T	3	95	10	11
17	Yukon Gold	0	0	0	0	0	0	1	1	T, F, MN	3	85	8	15
1	A01143–3C	1	1	0	0	0	0	0	2	ED, IVC, MN, F	3	94	13	17
2	A03921–2	0	0	0	0	1	2	1	1	MN, T, IVC, F	3	58	4	6
3	Classic Russet	0	0	0	0	0	0	0	0		3	38	5	8
4	Clearwater Russet	0	0	0	0	0	0	0	0		3	74	7	12
5	Mountain Gem Russet	3	2	3	1	1	0	0	0	ED	3	40	7	9
6	Payette Russet	2	2	3	0	0	0	0	1	ED, T, MN	3	54	13	18
7	Pomerelle Russet	2	3	3	3	3	3	2	3	ED, T, MN,F	3	32	6	9
8	Targhee Russet	1	2	1	0	0	0	0	0	ED	3	139	5	7
ck	Red Norland	3	1	0	0	0	0	0	0	ED	3	120	13	18
ck	Russet Burbank	2	2	2	1	0	0	1	2	ED, T, MN	3	57	14	17
ck	Russet Norkotah	0	3	3	0	0	1	1	3	ED, MN, T, F	3	42	13	17

[1] *ED* Early dwarf, *R* Rosette, *IVC* Interveinal chlorosis, *IVN* Interveinal necrosis, *MN* Marginal necrosis, *W* Wilt, *F* Flagging, *T* Twist of leaflets

control plots used for the single stem testing or from pre-tested stems obtained from certified seed potato fields. The stem composites were soaked in water with shaking for 24 h and then tested using the real-time PCR method described by Gudmestad et al. (2009) with modifications described previously.

North Dakota

Symptomatic Expression of Bacterial Ring Rot

Trials were conducted in 2015 and 2017 in North Dakota using the same 28 cultivars/breeding lines that were evaluated in in Idaho. In 2015 an additional cultivar, Soroya, was included in the trial but was omitted in the 2017 trial. Cs inoculations were performed with a mix of strains CIC242, 244, 245, and 250. Tubers were inoculated using a vacuum infiltration method by placing 21–25 seed pieces (all with two fresh cut surfaces) into a Nalgene vacuum container with enough Cs NBY broth to cover all the seed pieces. This broth contained Cs cells with 10^{10} cells/mL for high inoculum treatment and 10^{8} cells/mL for low inoculum. To ensure the lids of the bowls remained in place, petroleum gel was applied between the bowls lip and the cover. Once the cover was secure, the bowls valve was opened, and a vacuum pump was attached to remove all air from the Nalgene bowl. The pump remained running with the bowl valve open

Table 7 Number of positive samples for presence of *Clavibacter sepedonicus* in stems collected from field grown plants prior to harvest in Idaho

Year	Cultivar	sample date (days after planting)	10^0 cells/ml (control)	10^2 cells/ml	10^8 cells/ml
2015	Norland	61	0/39[1]	0/40	4/40
2015	Norland	97	0/40	28/40	21/40
2015	Norland	125	Dead[2]	Dead	Dead
2015	RB	61	0/40	2/40	2/40
2015	RB	97	0/38	25/40	26/40
2015	RB	125	0/36	27/34	21/29
2016	Norland	61	0/40	0/40	0/40
2016	Norland	90	0/40	0/40	0/40
2016	Norland	120	Dead	Dead	Dead
2016	RB	61	0/40	0/40	0/40
2016	RB	90	0/40	0/40	0/40
2016	RB	120	0/40	7/46	12/46

[1] number of positive Cs stems/total number stems tested

[2] normal plant senescence at 125 days

for 10 min at 103.4 kPa, after which the valve was closed. The bowl remained under a vacuum for an additional 10 min after which the vacuum was released, and seed pieces were removed from the Cs NBY broth and placed on paper to air dry overnight. The Cs NBY broth was used twice before being replaced with additional

Table 8 Composite testing for presence of *Clavibacter sepedonicus* (Cs) from plants in Idaho. Individual Ct value represents one positive Cs stem sample and Ct composite value represents the one positive stem sample combined with 199 healthy stem samples. Ct values are considered positive when less than 35

Cultivar	Stem sample designation	Dose (cells/ml)	Days after planting	Individual Ct value	Ct value of composite
2015 Stem Composite Testing					
Russet Burbank	B2–1	10^8	125	22.35	28.49
Russet Burbank	A3–6	10^2	97	23.04	33.96
Russet Burbank	C3–9	10^8	97	23.54	32.98
Russet Burbank	C3–1	10^8	125	24.12	29.46
Russet Burbank	C5–1	10^2	125	25.32	29.89
Russet Burbank	D4–5	10^2	97	26.03	34.20
Russet Burbank	C3–3	10^8	125	27.07	32.76
Russet Burbank	D4–3	10^2	125	28.39	32.80
Norland	A4–2	10^2	97	29.53	neg.
Russet Burbank	D4–1	10^2	125	31.82	neg.
Norland	B4–10	10^2	97	31.94	neg.
Norland	C4–1	10^2	97	32.09	neg.
2016 Stem Composite Testing					
Russet Burbank	B2–5	10^8	120	22.33	31.49
Russet Burbank	B2–4	10^8	120	23.52	32.26
Russet Burbank	C3–10	10^8	120	24.21	34.14
Russet Burbank	A1–6	10^8	120	25.10	34.22
Russet Burbank	D3–6	10^8	120	27.63	32.32
Russet Burbank	D4–1	10^2	120	28.21	34.16
Russet Burbank	B2–6	10^8	120	34.25	neg.
Russet Burbank	B2–7	10^8	120	34.77	neg.
Russet Burbank	D4–6	10^8	120	35.41	neg.
Russet Burbank	C5–1	10^2	120	36.05	neg.

Table 9 Percentage of positive bacterial ring rot samples for each plant organ tested at different inoculum levels, North Dakota 2015

Plant Tissue	Week 1		Week 2		Week 3		Week 4		Week 5[3]	
Above Ground Stem										
High Inoculum	33.30%	A[1]	44.40%	A	59.30%	A	55.60%	A	63.00%	A
Low Inoculum	63.00%	B	63.00%	A	88.90%	B	81.50%	B	74.10%	A
Non Inoculum	0.00%	C	0.00%	B	0.00%	C	0.00%	C	0.00%	B
P value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Below Ground Stem										
High Inoculum	37.00%	A	55.60%	A	59.30%	A	59.30%	A	37.00%	A
Low Inoculum	66.70%	A	66.70%	A	74.10%	A	74.10%	A	51.90%	A
Non Inoculum	0.00%	B	0.00%	B	0.00%	B	0.00%	B	0.00%	B
P value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Petioles- Lower Canopy										
High Inoculum	7.40%	AB	18.50%	A	33.30%	A	37.00%	A	14.80%	A
Low Inoculum	25.90%	A	18.50%	A	51.90%	B	37.00%	A	33.30%	A
Non Inoculum	0.00%	B	0.00%	B	0.00%	C	0.00%	B	0.00%	B
P value	0.0146		0.0206		<0.0001		0.0009		0.0013	
Petiole-Mid Canopy										
High Inoculum	22.20%	A	11.10%	A	29.60%	A	37.00%	A	11.10%	AB
Low Inoculum	18.50%	A	11.10%	A	33.30%	A	29.60%	A	29.60%	A
Non Inoculum	0.00%	B	0.00%	A	0.00%	B	0.00%	B	0.00%	B
P value	0.0111		NS		0.0002		0.0003		0.0028	
Petioles- Upper Canopy										
High Inoculum	11.10%	A	11.10%	A	25.90%	A	29.60%	A	0.00%	B
Low Inoculum	14.80%	A	14.80%	A	40.70%	A	33.30%	A	22.20%	A
Non Inoculum	0.00%	A	0.00%	A	0.00%	B	0.00%	B	0.00%	B
P value	NS		NS		0.0002		0.0002		0.0017	
Stolon										
High Inoculum	11.10%	B	14.80%	AB	33.30%	A	44.40%	A	29.60%	A
Low Inoculum	33.30%	A	29.60%	A	59.30%	B	40.70%	A	33.30%	A
Non Inoculum	0.00%	B	0.00%	B	0.00%	C	0.00%	B	0.00%	B
P value	0.0016		0.0071		<0.0001		0.0019		0.0013	
Tuber										
High Inoculum	3.70%	A	7.40%	A	33.30%	A	33.30%	A	63.00%	A
Low Inoculum	14.80%	A	7.40%	A	33.30%	A	37.00%	A	70.40%	A
Non Inoculum	0.00%	A	0.00%	A	0.00%	B	0.00%	B	0.00%	B
P value	NS[2]		NS		0.0001		0.0008		0.0026	

[1] Means separated by the same letter are not significantly different according to Tukey's honest significant difference test ($\alpha = 0.05$)

[2] NS, represent *P* value was not significant at $\alpha = 0.05$

[3] Week 6 and 7 data omitted due to insufficient plants available for collection

broth added as needed to ensure all seed was covered completely within the bowl.

After drying overnight, the inoculated seed pieces were transferred into a paper bag containing enough fir bark to coat each seed piece. The seed pieces were stored at ambient temperature until planting.

The trials were inoculated on May 30–31, 2015 and May 22–23, 2017, planted on June 3, 2015 and June 1, 2017 with harvest October 21–22, 2015 and October 16–17, 2017. Foliar applications of insecticides and fungicides to control Colorado potato beetle, early blight and late blight were applied as necessary.

Table 10 Mean estimate of samples by cultivar for each plant organ tested, North Dakota 2015

Plant Tissue	Week 1		Week 2		Week 3		Week 4		Week 5[3]	
Above Ground Stem										
Ivory Crisp	0.2963	A[1]	0.3704	AB	0.4074	A	0.4444	A	0.3704	A
Russet Burbank	0.2593	A	0.1852	B	0.5185	A	0.3704	A	0.4071	A
Red Norland	0.4074	A	0.5185	A	0.5556	A	0.5556	A	0.5556	A
P value	NS[2]	0.0195	NS	NS	NS					
Below Ground Stem										
Ivory Crisp	0.3333	A	0.3704	A	0.4074	A	0.4074	A	0.4815	A
Russet Burbank	0.2963	A	0.3333	A	0.4074	A	0.4074	A	0.3704	A
Red Norland	0.4074	A	0.5185	A	0.5185	A	0.5185	A	0.5185	A
P value	NS	NS	NS	NS	NS					
Petioles- Lower Canopy										
Ivory Crisp	0.1111	A	0.07407	A	0.1852	B	0.2222	A	0.2222	A
Russet Burbank	0.1111	A	0.1111	A	0.2222	B	0.1852	A	0.1852	A
Red Norland	0.1111	A	0.1852	A	0.4444	A	0.3333	A	0.2222	A
P value	NS	NS	0.0029	NS	NS					
Petiole-Mid Canopy										
Ivory Crisp	0.1111	A	0.07407	A	0.07407	B	0.1111	B	0.1481	A
Russet Burbank	0.1481	A	0.03704	A	0.2593	A	0.2222	AB	0.1111	A
Red Norland	0.1481	A	0.1111	A	0.2963	A	0.3333	A	0.2222	A
P value	NS	NS	0.0101	0.0281	NS					
Petioles- Upper Canopy										
Ivory Crisp	0.07407	A	0.07407	A	0.1111	B	0.1852	AB	0.07407	A
Russet Burbank	0.07407	A	0.07407	A	0.1852	AB	0.1111	B	0.1481	A
Red Norland	0.1111	A	0.1111	A	0.3704	A	0.3333	A	0.1852	A
P value	NS	NS	0.0086	0.0143	NS					
Stolon										
Ivory Crisp	0.1852	A	0.1852	A	0.2593	A	0.2222	A	0.3704	A
Russet Burbank	0.1111	A	0.1111	A	0.2593	A	0.2593	A	0.2222	A
Red Norland	0.1481	A	0.1481	A	0.4074	A	0.3704	A	0.2963	A
P value	NS	NS	NS	NS	NS					
Tuber										
Ivory Crisp	0.03704	A	0.03704	A	0.1481	A	0.1481	B	0.1852	A
Russet Burbank	0.03704	A	0.03704	A	0.2222	A	0.1852	AB	0.1111	A
Red Norland	0.1111	A	0.07407	A	0.2963	A	0.3704	A	0.2222	A
P value	NS	NS	NS	0.0424	NS					

[1] Means separated by the same letter are not significantly different according to Tukey's honest significant difference test ($\alpha = 0.05$)

[2] NS, represent *P* value was not significant at $\alpha = 0.05$

[3] Week 6 and 7 data omitted due to insufficient plants available for collection

Field experiments were located on a NDSU research site near Prosper, ND. The experiment was set up as randomized complete block design with a split-plot arrangement of inoculum treatment and cultivar in three replicates/blocks. Each plot consisted of a row with seven inoculated seed pieces and an adjacent parallel row with seven non-inoculated seed pieces which served as a "healthy" control allowing comparisons during recording of visual symptoms. Evaluations of foliar symptoms began 62 days after planting in 2015 and at 55 days after planting in 2017 and continued on a weekly basis until vines were desiccated on September 30, 2015 and September 19, 2017, respectively. In addition to the typical

Table 11 Mean estimate of bacterial ring rot expression over time, North Dakota 2015

Ivory Crisp	Estimate	*P* value[1]
Above ground stem	0.02222	NS[2]
Below ground stem	0.03333	NS
Petioles- Lower Canopy	0.03704	NS
Petioles- Mid Canopy	0.01111	NS
Petioles- Upper Canopy	0.01111	NS
Stolon	0.04074	NS
Tuber	0.04074	0.0249
Russet Burbank		
Above ground stem	0.04815	NS
Below ground stem	0.02222	NS
Petioles- Lower Canopy	0.02222	NS
Petioles- Mid Canopy	0.01111	NS
Petioles- Upper Canopy	0.01852	NS
Stolon	0.03704	NS
Tuber	0.02963	NS
Red Norland		
Above ground stem	0.03333	NS
Below ground stem	0.02222	NS
Petioles- Lower Canopy	0.03704	NS
Petioles- Mid Canopy	0.03704	NS
Petioles- Upper Canopy	0.03704	NS
Stolon	0.05185	NS
Tuber	0.05185	NS

[1] *P* values were calculated at significance level $\alpha = 0.05$

[2] NS, represent *P* value was not significant at $\alpha = 0.05$

symptoms of BRR which include wilting of leaves and entire stems, interveinal chlorosis, and marginal leaf necrosis, early season symptoms of a dwarf rosette symptom typically associated with the cultivar Russet Burbank were also noted (Westra et al. 1994).

Prior to vine desiccation, two stems from each treatment (cultivar x inoculum dose combination) was randomly sampled for Cs assay. A crude extraction of 1.0 g of stem sample collected from the soil line was crushed in a Ziploc bag with a rubber mallet with 1.0 mL of sterile 1X PBS. The solution collected from this extraction was assayed with PCR using dilutions of 1:10 and 1:100 by real-time PCR (Gudmestad et al. 2009) with the following modifications: 1) The fluorogenic probe specific for CelA was labeled with FAM at the 5'end and the 3′ end was modified with BHQ1, 2) the real-time PCR mix consisted of 1X Universal Probes Supermix (BioRad SsoAdvanced™), CelA-F and CelA-R primers at a concentration of 0.5 μM, Cel-A probe at a concentration of 0.25 μM, 2 μL of sample, and PCR grade water to a final volume of 20 μL.

Pre-Harvest Detection of Cs in Different Plant Tissue

A second field experiment was planted to evaluate the feasibility of pre-harvest testing of above ground plant tissue to detect Cs. A factorial of inoculum dose and cultivar (3 X 3) was planted in a randomized complete block design with three replications. Each treatment consisted of 21 seed pieces in 2015 and 25 seed pieces in 2017 of each cultivar (Ivory Crisp, Red Norland and Russet Burbank) that were inoculated at a high dose (10^{10} cells/mL) and a low dose (10^{8} cells/mL) of Cs and a non-inoculated control.

Beginning 30 days after 50% emergence was reached, three plants from each treatment were collected on a weekly basis for 7 weeks. Weekly sample collections began July 27, 2015 and July 24, 2017 and ended September 10, 2015 and September 6, 2017, respectively. Each plant collected was separated into the type of plant organ for Cs assays: above ground stem, below ground stem, stolon, tuber, low canopy petiole, mid-canopy petiole, and upper canopy petiole. A crude extraction was done by crushing 1.0 g of each sample within a Ziploc® bag with 1.0 ml sterile 1X PBS with a rubber mallet. The solution collected from this process was assayed with PCR using dilutions of 1:10 and 1:100 by real-time PCR (Gudmestad et al. 2009 – modifications as just described).

Statistical Analysis

All statistical analysis was performed using statistical analysis software (SAS) version 9.4. For this study, a mixed model (PROC Glimmix) was used to determine the effects of cultivar and inoculum level on bacterial ring rot expression. Potato cultivars, inoculum treatment, and their interaction were considered fixed effects, while block was treated as random effect. In SAS, BY statement was used to sort the results by week (time) and plant organ tested. In 2015 trial, week 6 and week 7 data were omitted due to insufficient plants available for collection. Furthermore, bacterial ring rot expression in cultivars over time (week) was performed using SAS PROC Glimmix procedure (mixed model).

Results

Bacterial Ring Rot Expression

Examples of foliar symptoms recorded in the tables are shown in Fig. 1 and include, whole stem wilt, early dwarfing, interveinal necrosis, leaf margin necrosis and flagging. Tuber symptoms are shown in Fig. 2 and include tuber cracking, vascular ring degradation, and bacterial ooze emanating from the vascular area. The inclusion of un-inoculated control rows next to each inoculated row allowed the evaluator to distinguish between wilts and other symptoms including natural senescence that is not associated with BRR.

Table 12 Percentage of positive samples for each plant organ tested, North Dakota 2017

Plant Tissue	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7	
Above Ground Stem														
High Inoculum	48.10%	A[1]	85.20%	A	70.40%	A	70.40%	A	92.60%	A	92.60%	A	100.00%	A
Low Inoculum	51.90%	A	77.80%	A	51.90%	B	70.40%	A	81.50%	A	100.00%	A	88.00%	A
Non Inoculum	0.00%	B	0.00%	B	0.00%	C	0.00%	B	0.00%	B	0.00%	B	0.00%	B
P value	0.0001		<0.001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Below Ground Stem														
High Inoculum	85.20%	A	88.90%	A	81.50%	A	81.50%	A	92.60%	A	100.00%	A	92.60%	A
Low Inoculum	64.50%	A	74.50%	A	75.40%	A	63.20%	A	70.50%	A	77.60%	A	67.70%	A
Non Inoculum	0.00%	B	0.00%	B	0.00%	B	0.00%	B	0.00%	B	0.00%	B	0.00%	B
P value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Petioles- Lower Canopy														
High Inoculum	14.80%	A	33.30%	A	22.20%	AB	14.80%	AB	27.00%	AB	37.00%	A	44.40%	A
Low Inoculum	33.30%	B	29.60%	A	25.90%	A	25.90%	A	33.30%	A	30.10%	A	23.60%	AB
Non Inoculum	0.00%	A	0.00%	B	0.00%	B	0.00%	B	0.00%	B	0.00%	B	0.00%	B
P value	0.0006		0.0023		0.032		0.0053		0.0235		0.0002		0.0089	
Petiole-Mid Canopy														
High Inoculum	3.70%	A	0.00%	A	11.10%	A	7.40%	A	12.20%	A	17.20%	A	21.90%	A
Low Inoculum	3.70%	A	3.70%	A	3.70%	A	7.40%	A	0.00%	A	11.10%	A	15.70%	A
Non Inoculum	0.00%	A	0.00%	A	0.00%	A	0.00%	A	0.00%	A	0.00%	A	0.00%	A
P value	NS[2]		NS		NS		NS		NS		NS		NS	
Petioles- Upper Canopy														
High Inoculum	0.00%	A	0.00%	A	0.00%	A	7.40%	A	3.70%	A	7.40%	A	11.10%	A
Low Inoculum	0.00%	A	3.70%	A	3.70%	A	3.70%	A	0.00%	A	7.40%	A	15.70%	A
Non Inoculum	0.00%	A	0.00%	A	0.00%	A	0.00%	A	0.00%	A	0.00%	A	0.00%	A
P value	NS		NS		NS		NS		NS		NS		NS	
Stolon														
High Inoculum	22.20%	A	48.10%	A	40.70%	A	40.70%	A	56.10%	A	72.70%	A	77.80%	A
Low Inoculum	14.80%	A	48.10%	A	88.90%	A	43.50%	A	48.10%	A	61.60%	A	63.90%	A
Non Inoculum	0.00%	A	0.00%	B	0.00%	B	0.00%	B	0.00%	B	0.00%	B	0.00%	B
P value	NS		<0.0001		0.0003		0.0037		<0.0001		<0.0001		<0.0001	
Tuber														
High Inoculum	0.00%	A	4.76%	A	8.30%	A	16.80%	A	30.60%	A	27.80%	A	58.70%	A
Low Inoculum	0.00%	A	3.70%	A	0.00%	A	11.10%	AB	22.20%	A	15.30%	A	16.30%	B
Non Inoculum	0.00%	A	0.00%	A	0.00%	A	0.00%	B	0.00%	B	0.00%	B	0.00%	B
P value	NS		NS		NS		0.0313		0.0233		0.0021		0.0031	

[1] Means separated by the same letter are not significantly different according to Tukey's honest significant difference test ($\alpha = 0.05$)

[2] NS, represent *P* value was not significant at $\alpha = 0.05$

By the time the Idaho foliar symptoms were recorded in 2015 (100 DAP), half of the entries had symptoms. In 2016, visual evaluations were started earlier (63 DAP), but no symptoms were noted until 91 DAP (Tables 1, 2).

In the 2015 trial in Idaho, of the 17 specialty types evaluated only Melody and Victoria failed to show foliar symptoms (Table 1). Melody was positive for Cs in all three replications and Victoria was negative (with only one replication tested – plants in the other two replications did not emerge). However, in 2016, Victoria emerged and was positive in all three replications and showed good foliar symptoms starting at 91 days after planting (DAP). Melody did not show foliar symptoms again in 2016 even though plants in all three replications were positive for Cs.

Table 13 Mean estimate of samples by cultivar for each plant organ tested, North Dakota 2017

Plant Tissue	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7	
Above Ground Stem														
Ivory Crisp	0.4815	A[1]	0.6295	A	0.6296	A	0.5556	A	0.6667	A	0.537	A	0.6111	A
Russet Burbank	0.3333	AB	0.5556	A	0.4444	B	0.6296	A	0.5926	A	0.6296	A	0.6296	A
Red Norland	0.1852	B	0.4444	A	0.1481	C	0.2222	B	0.4815	A	0.5556	A	0.6296	A
P value	0.0327		NS[2]		<0.001		0.0018		NS		NS		NS	
Below Ground Stem														
Ivory Crisp	0.6296	A	0.5926	A	0.6667	A	0.5185	AB	0.6667	A	0.5741	A	0.5556	A
Russet Burbank	0.5556	AB	0.5926	A	0.6296	A	0.6667	A	0.6667	A	0.6667	A	0.6296	A
Red Norland	0.4074	B	0.5926	A	0.4074	B	0.4074	B	0.4815	A	0.6667	A	0.5556	A
P value	0.0447		NS		0.001		0.0145		NS		NS		NS	
Petioles- Lower Canopy														
Ivory Crisp	0.1481	A	0.2963	A	0.1481	A	0.2593	A	0.3704	A	0.3704	A	0.2593	A
Russet Burbank	0.2222	A	0.2222	A	0.2593	A	0.1111	AB	0.1481	AB	0.2593	A	0.1852	A
Red Norland	0.1111	A	0.1111	A	0.07407	A	0.03704	B	0.07407	B	0.03704	B	0.213	A
P value	NS		NS		NS		0.0143		0.0473		0.0009		NS	
Petiole-Mid Canopy														
Ivory Crisp	>0.0001	A	>0.0001	A	0.07407	A	0.03704	A	0.1852	A	0.08148	A	0.2315	A
Russet Burbank	0.07407	A	>0.0001	A	0.03704	A	0.03704	A	>0.0001	A	0.1111	A	0.03704	A
Red Norland	>0.0001	A	0.03704	A	0.03704	A	0.07407	A	>0.0001	A	0.07407	A	0.1111	A
P value	NS		NS		NS		NS		NS		NS		NS	
Petioles- Upper Canopy														
Ivory Crisp	0	A	>0.0001	A	>0.0001	A	0.07407	A	0.03704	A	0.07407	A	0.03704	A
Russet Burbank	0	A	>0.0001	A	>0.0001	A	>0.0001	A	>0.0001	A	0.03704	A	0.07407	A
Red Norland	0	A	0.03704	A	0.03704	A	0.03704	A	>0.0001	A	0.03704	A	0.1389	A
P value	NS		NS		NS		NS		NS		NS		NS	
Stolon														
Ivory Crisp	0.2222	A	0.5185	A	0.3704	A	0.3333	A	0.4444	A	0.5	A	0.4259	A
Russet Burbank	0.1111	A	0.2963	AB	0.2593	AB	0.3333	A	0.4074	A	0.4444	A	0.5556	A
Red Norland	0.03704	A	0.1481	B	0.07407	B	0.1852	A	0.2222	B	0.4074	A	0.4074	A
P value	0.1818		0.0041		0.0065		NS		0.0442		NS		NS	
Tuber														
Ivory Crisp	0	A	0.03704	A	0.07407	A	0.07407	AB	0.1111	AB	0.1481	A	0.1852	A
Russet Burbank	0	A	>0.0001	A	>0.0001	A	0.1852	A	0.2963	A	0.1481	A	0.2593	A
Red Norland	0	A	0.03704	A	>0.0001	A	>0.0001	B	0.03704	B	0.03704	B	0.1111	A
P value	NS		NS		NS		0.0094		0.0182		0.039		NS	

[1] Means separated by the same letter are not significantly different according to Tukey's honest significant difference test ($\alpha = 0.05$)

[2] NS, represent *P* value was not significant at $\alpha = 0.05$

Of the eight cultivars/breeding lines that represent seven russet types and one chipping type only Clearwater Russet and Targhee Russet did not have symptoms in both years. However, all eight entries were positive for Cs in both years for (Tables 1, 2). Three of the eight showed symptoms at 100 DAP in 2015, while two of eight showed symptoms at 91 DAP in 2016.

Foliar symptoms were delayed in 2016 compared to 2015. In 2015, 14 entries had at least one symptomatic plant at 100 DAP. In 2016, 4 entries had symptoms at 91 days and 9 entries had symptoms at 98 DAP. At the end of each year's visual evaluations, foliar symptoms were expressed in 22 total entries in 2015 compared to 16 total entries in 2016. The number of tubers with symptoms were also higher in 2015 compared to 2016.

Table 14 Mean estimate of bacterial ring rot expression over time, North Dakota 2017

Ivory Crisp	Estimate	*P* value
Above ground stem	0.01323	NS
Below ground stem	−0.00265	NS
Petioles- Lower Canopy	0.02513	NS
Petioles- Mid Canopy	0.03869	0.0057
Petioles- Upper Canopy	0.01058	NS
Stolon	0.02778	NS
Tuber	0.0291	0.02
Russet Burbank		
Above ground stem	0.04233	NS
Below ground stem	0.01455	NS
Petioles- Lower Canopy	−0.00529	NS
Petioles- Mid Canopy	0.002646	NS
Petioles- Upper Canopy	0.04058	0.0175
Stolon	0.06349	0.0042
Tuber	0.04894	0.0002
Red Norland		
Above ground stem	0.06746	0.007
Below ground stem	0.02381	NS
Petioles- Lower Canopy	0.005622	NS
Petioles- Mid Canopy	0.01323	NS
Petioles- Upper Canopy	0.01455	0.0379
Stolon	0.06349	0.0009
Tuber	0.01323	0.0302

[x] *P* values were calculated at significance level $\alpha = 0.05$

[y] NS, represent *P* value was not significant at $\alpha = 0.05$

[z] Positive estimate indicate increase over time and negative estimate decrease over time

In the 2015 trial in North Dakota, 23 of 29 potato cultivars inoculated with a high Cs population had foliar symptoms; only cultivars Cecile, Elfe, Soroya, A01143–3C, Red Norland and Russet Burbank did not express BRR (Table 4). Of those inoculated with a low inoculum dose, 25 of 29 cultivars displayed foliar symptoms and only Blue Belle, Challenger, Elfe, and Soroya did not express any symptoms of BRR (Table 3). In 2017, 26 of 28 cultivars inoculated with a high Cs dose exhibited foliar symptoms of BRR and only Classic Russet and Clearwater Russet expressed no foliar symptoms of BRR (Table 6). When these cultivars were inoculated with a low inoculum dose, 25 of 28 expressed foliar symptoms of BRR and only A05182–7Y, Anuschka, and Blue Belle were symptomless (Table 5).

In several cultivars, a symptom of early dwarfing typical of BRR was observed (Fig. 1c). It was more often observed in the North Dakota plots than in the Idaho Plots (Tables 1, 2, 3 and 4).

Pre-Harvest Testing

In the Idaho tests, higher number of positive samples were obtained in the 2015 trial than in 2016. In 2015, detections of Cs in Red Norland occurred in the high and low inoculation plots at 97 days at a rate of 53% and 70%, respectively. At 125 DAP, all the Red Norland plants were dead. In contrast, detections of Cs in Russet Burbank first occurred in a few plants at 61 DAP with higher percentages at 97 DAP and again at 125 DAP with a detection range of 5% to 79%, depending on the sampling date (Table 7).

In 2016, no positive samples were obtained from Red Norland. In Russet Burbank, the date of the first positives was delayed compared to 2016 and the percentage of positives was much lower than in 2015. The range of positives in 2016 was from 15% to 26%.

In the stem testing of one positive/199 negative stem samples, positive PCR results were obtained in 8 of 12 samples in 2015 and 6 of 10 samples in 2016. All negative composite samples in 2015 and 2016 were reached when the individual Ct value of the one positive stem section exceeded 28 PCR cycles (Table 8). Four negative composite samples in 2015 and four in 2016 never reached the crossing threshold and have been recorded as negative in Table 8. In 2015 the individual stem samples corresponding to the negative composites ranged from 29 to 32 Cts, all which would be considered positive. In 2016 the individual samples that corresponded to negative composites ranged from 34 to 36 Cts with one being above the 35 Ct positive threshold.

In the 2015 North Dakota trials, only 5 weeks of collections were recorded due to low emergence and early senescence. The percentage of positive samples for inoculated seed was significantly higher than that of the non-inoculated seed for above and below ground stem samples for all 5 weeks (Table 9). For other plant organs assayed such as petioles in the lower, mid, and upper canopy, stolons, and tubers, the positive detection of Cs was more often significantly higher for inoculated seed than non-inoculated seed in weeks 3–5 compared to weeks 1 & 2 (Table 9).

Comparing just the high and low inoculum results, showed the number of Cs-positive samples assays were not significantly different for below ground stems, mid canopy petioles or tubers. The percentage of Cs positive samples obtained did differ significantly between inoculum doses in above ground stems, lower canopy petioles, upper canopy petioles, and stolons in one or more weeks of collection (Table 9).

There were few differences in the detection of Cs among potato cultivars during the growing season regardless of the plant organ assayed (Table 10). There were no differences in the detection of Cs over the course of the growing season regardless of cultivar of plant organ assayed except for Ivory Crisp tuber samples (Table 11).

In 2017, 7 weeks of plant samples were collected and assayed for Cs. The percentage of positive samples for inoculated seed

was significantly higher than that of the non-inoculated seed for above and below ground stem samples for all 7 weeks. The highest detection levels were correlated with the high inoculum level on below ground stem samples. In these samples >81% of the samples were positive for Cs from the first sampling to the last (Table 12). Regardless of the plant organ sampled, there were few differences in the detection of Cs between inoculum doses (10^8 vs 10^{10}).

There were few differences in the detection of Cs among cultivars regardless of the plant organ samples (Table 13). Across all three cultivars there was a significant increase in the frequency of detection of Cs in tubers over the course of the growing season (Table 14). A similar increase in Cs detection frequency was observed in stolons and upper canopy petioles in Russet Burbank and Red Norland, the mid-canopy petiole of Ivory Crisp, and the above ground stem of Red Norland. In all other instances, Cs detection frequency did not differ among plant organs in all three cultivars.

Discussion

Many seed certification systems in the U.S. include BRR in the criteria for a foliar visual inspection. While more lab testing for Cs is now done on seed lots in the U.S., it is important to know the timing of symptom expression and characteristic symptoms of that cultivar. Typical symptoms observed in the plots included leaf margin necrosis, wilting, rosetting along with tuber symptoms in some cultivars. The tuber symptoms included external cracking observed at harvest. This cracking can be directly caused by secondary breakdown of tissue associated with soft rot and/or dry rot in plants that tested positive for Cs (De Boer 2008; Turkensteen 2005). Tuber symptoms associated with BRR included bacterial ooze from Cs, which can be squeezed out of the vascular area (Fig. 2e). Detection of this disease can also take place when potatoes are moved out of storage for planting. Samples of seed potatoes are then visually inspected for external and internal defects, including those caused by BRR (Whitworth and Davidson 2008). The results here showed that in Idaho in 2016 the earliest detection of symptoms was 91 days after planting. The inoculation technique used here was done to mimic conditions similar to seed cutting practices. A study by Dykstra (1942) compared inoculation techniques and showed that needle injection of Cs into tuber eyes resulted in the quickest expression of symptoms, with 49.7% showing symptoms at 64 DAP and 97% at 105 DAP. In comparison, the technique of dipping cut tubers into an inoculum solution and then immediately planting proceeded at a slower rate, where 64 DAP after planting only 8.3% had symptoms and at 105 DAP, 91.7% had symptoms.

In North Dakota positive samples were detected 54 DAP in 2015 in all seven plant organs and 53 DAP in 2017 in five of the plant organs. Above ground and below ground stems were found to be positive in all weekly collections with only the Ivory Crisp tuber samples in 2015 and the above ground Red Norland stem samples in 2017 showing in increase of positive samples with date progression. In a previous study the onset of ring rot symptom development and maximum disease incidence was influenced most by the location and by location x cultivar interactions (Westra et al. 1994). So, it should be no surprise that there were differences in BRR symptom development between Idaho and North Dakota, regardless of the differences in inoculation method.

This study demonstrates that pre-harvest sampling of stems for Cs is possible using the above ground level stem portion which is consistent with a previous study (Gudmestad et al. 1991). In addition, it shows that a random sampling of stems can be used to run a "blind" test for Cs. However, it appears that the detection limit may need to be one positive stem in less than 200 total stems. Additional studies are needed to determine a reliable detection limit, most likely with a small sample of 100 to 150 stems. Once refined, composite sampling of stems prior to harvest will allow detection of infected seed lots before seed is harvested and placed into storage.

Acknowledgements The authors would like to thank Dr. Rob Davidson and Andrew Houser at Colorado State University for their expertise and for providing Cs inoculum and technical assistance. They also thank Melissa Bertram, Jill Randall, Melinda Lent from University of Idaho, Idaho Falls, Darren Hall from USDA-ARS at Aberdeen, Idaho, Sherry Laug from Idaho Crop Improvement, Idaho Falls, ID, and Dr. Carol Ishimaru from University of Minnesota for providing Cs isolates for inoculum production for the North Dakota trials.

This research was funded by USDA-ARS Federal-State Partnership Potato Program with University of Idaho, North Dakota State University, Idaho Crop Improvement Association and USDA-ARS collaborating.

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