TUBER RESISTANCE TO LATE BLIGHT AND AGGRESSIVENESS OF PHYTOPHTHORA INFESTANS ISOLATES IN POTATO TUBERS

by
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INTRODUCTION

Late blight of potato, caused by *Phytophthora infestans*, is a serious problem in the Columbia Basin. Control of late blight on potato vines is essential in order to reduce the incidence and severity of tuber blight. Sporangia can be produced on leaf and stem lesions under cool, humid conditions and get washed down into the soil where tuber infection can occur.

Early researchers found that *P. infestans* sporangia could infect through wounds, eyes, lenticels, and intact skin (Jones *et al.*, 1912). Wounds were most susceptible and intact skin was the least, with eyes and lenticels being about equal. Often tuber resistance to late blight increases with tuber age (Jones *et al.*, 1912; Lacey, 1967; Stewart *et al.*, 1983). Exceptions to this have been observed, however. Spraying tubers with a suspension of sporangia leads to a greater number of lenticels than eyes becoming infected. More lenticels are present on tubers than eyes and as tubers mature lenticels became resistant. Eyes, however, became more susceptible with age. Additionally, when tubers became infected naturally through soil, eyes were the primary infection point four times as often as lenticels (Lacey, 1967). Lacey observed that no infections started through intact periderm.

MATERIALS AND METHODS

Tuber Maturity Trial. Tuber maturity tests were conducted in 1996 and 1997 at the Othello research station in Othello, WA using cultivars Russet Burbank and Ranger Russet. Planting, vine kill, and harvest dates are given in Table 1. The trial was performed in two fields in 1996 and one in 1997 using a randomized complete block design with six replications. Herbicide and fertilizer applications, cultural practices, and overhead irrigation were applied according to standard commercial practices within the Columbia Basin. After harvest, tubers were immediately washed, evaluated for skinning, graded and sorted, then transported to Pullman, WA where they were stored at 48 °F. Tubers weighing 4-12 oz. were selected for inoculation and divided into two lots. One lot, designated as the field lot, was inoculated a few days after harvest and the other, designated as the storage lot, was placed in storage and inoculated six months later.

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In 1996 two isolates of *Phytophthora infestans* (US-1 and US-8) were used to inoculate field lot tubers for field 1. Three isolates (one US-1 isolate, two US-8 isolates) were used to inoculate tubers from field 2. A single US-8 isolate was used to inoculate storage lot tubers for both fields in 1996 and field tubers in 1997. Isolates were obtained from infected foliage in the Columbia Basin during 1994 and 1996. Sporangial suspensions were obtained by washing sporangia from agar plate cultures or sporulating lesions on potato leaves with distilled water. All sporangial suspensions were adjusted to a concentration of 1×10^4 sporangia/ml.

Tubers were dipped in distilled water then inoculated. Inoculations were done by placing a 50 µl drop of sporangial suspension (approximately 500 sporangia) on a 1 cm^2 filter paper. Two inoculated filter papers were placed inoculated side down on tubers, one over an eye and the other over intact periderm tissue. Two tubers (subsamples) were inoculated for each plot and placed in mist chambers where high relative humidity was maintained for 24 hours. All inoculations were performed at 65-70°F. Tubers were then allowed to air dry then stored at 48°F. Infected tubers were evaluated five weeks later for external and internal percent tuber infection. Internal infection was estimated by slicing the tuber into fourths and averaging the percent of discolored tissue at the interface of each slice.

Sequential Sample Trial. The sequential sample trial was conducted at Othello and Pasco, WA in 1997. Russet Burbank and Ranger Russet plots were planted at the Othello research station on April 11 in a randomized complete block design with five replications. Two commercial fields, one of Russet Burbank potatoes planted on April 3 and one of Ranger Russet potatoes planted on March 26, were selected at T and R Farms in Pasco, WA according to a completely randomized design with five replications. Herbicide and fertilizer applications, cultural practices, and overhead irrigation were applied according to standard commercial practices within the Columbia Basin.

All plots were sampled on the same schedule throughout the growing season (Table 2). Sample designation was based on sample time in relation to vine killing with samples 1 through 4 being taken prior to vine kill. The pre vine kill sample was taken the day prior to vine killing (done by vine beating) in Othello, and Post1 and Post2 were taken one and two weeks after the Othello vine kill, respectively. At Pasco, Russet Burbank tubers were not vine killed due to natural senescence and Ranger Russet plants were rolled then desiccated on September 10 using 1 gal/A Des-i-cate and 1 pt/A Diquat. At each sample, one hill of tubers was lifted from each plot and four tubers (subsamples) were selected. Tubers were selected to represent the largest tubers in each hill which were not damaged or misshapen. Tubers were washed gently in running tap water to remove adhering soil, allowed to air dry, and then weighed.

Tubers were inoculated in the same manner as with the maturity trial using a single US-8 isolate of *P. infestans*. After inoculation, tubers were incubated in a mist chamber for 18 hours. After allowing tubers to air dry, tubers were stored at 48°F for four weeks then evaluated as with the maturity trial.

Isolate Aggressiveness Trial. Russet Burbank and Shepody tubers were wound-inoculated with eight isolates representing five genotypes of *P. infestans*. A knife was used to remove a 1 in^2 section of tuber skin.

A sporangial suspension of *P. infestans* was placed into the cut, then the wound was sealed with parafilm. Tubers were incubated at 64°F for two weeks then buried in the field in Othello and Prosser at depths of 4 and 8 inches. Tubers were recovered six weeks later and evaluated as in the previous trials.

Data Analysis. Tuber maturity trial and isolate aggressiveness trial results were analyzed using the PROC GLM procedure of SAS (SAS Institute, Inc., Cary, NC) and sequential sample trial results were analyzed using the PROC MIXED procedure of SAS. When significant differences were found ($P \leq 0.05$) from ANOVA, a Duncan's mean separation was used to determine differences among treatments.

RESULTS AND DISCUSSION

Tuber Maturity Trial. Tubers from the three planting dates of the 1996 maturity trial showed significant differences in skinning (Table 3). The tubers which were planted first had the least amount of skinning. Also, Ranger Russet tubers skinned more easily than Russet Burbank. In 1997 tubers from the first planting date were slightly more skinned than tubers from other planting dates, but the difference does not appear important since the ability to accurately distinguish levels of skinning that low is questionable. In 1997, tubers were left in the ground two weeks after vine kill, which allowed time for skin maturation. In 1996, tubers were harvested one to four days after vine kill.

Contrary to what was expected, tubers showing high levels of skinning in 1996 (late planting) did not develop the highest amount of tuber blight (Table 4). Tubers from both fields planted on the last date had significantly lower levels of external and internal rot compared to tubers from early and mid planting dates. In 1997, however, significant differences were not found. This may be due to the fact that tubers were harvested more than two weeks after vine kill, allowing tuber skin to mature. Any differences in resistance to tuber blight resulting from physiological age were apparently negated by the delay in harvest. In 1996, increased resistance to tuber blight for tubers from the late planting may have been influenced by quicker wound healing time.

In 1996, Ranger Russet tubers were more susceptible than Russet Burbank for internal rot from both fields and for external rot in Field 1. This difference was not observed in 1997, possibly resulting from delayed harvest. External rot was always more severe than internal rot.

Similar trends were seen with tubers in storage (Table 5). For both fields, tubers from the late planting had significantly less tuber blight. Ranger Russet tubers were more susceptible than Russet Burbank tubers.

Sequential Sample Trial. For tubers harvested at Othello, significant differences were not found between Ranger Russet and Russet Burbank tubers for external and internal tuber blight. This was surprising since Russet Burbank vines senesced earlier than Ranger Russet vines. Prior to vine killing, tuber blight susceptibility for tuber eyes remained relatively constant throughout the growing season (Figure 1). After vine kill, susceptibility of tuber eyes increased.

The same trend was observed at Pasco, WA, only significant differences were observed between the two cultivars (Figure 2).

Physical mechanisms of resistance resulting from maturation and suberization of tuber skin increase with tuber age (Lacey, 1967; Stewart *et al.*, 1983). In this study, however, eyes become more susceptible with increasing tuber age. After vine death, physiological mechanisms of disease resistance in tubers may decrease, thus explaining the increased susceptibility. Mode of vine death does not appear to influence this observed increase in susceptibility since this trend was observed when vines were vine beat (both cultivars at Othello), chemically killed (Ranger Russet at Pasco), or allowed to senesce naturally (Russet Burbank at Pasco).

Isolate Aggressiveness Trial. At both Prosser and Othello, tuber decay was similar at both depths. Interactions were observed among cultivars and isolates. Generally, isolates of the US-8 and US-7 genotypes were more aggressive in terms of causing tuber decay than isolates of the US-1 (Table 6). An exception to this was found with the Russet Burbank tubers buried at Othello where a US-1 (Isolate 2) caused a significant amount of decay. High aggressiveness in tubers has been considered a disadvantage to the survival of the late blight fungus from one growing season to the next (Kadish and Cohen, 1992). When the late blight fungus completely decays a tuber, the fungus dies. Therefore highly aggressive isolates of the late blight fungus would be more likely to completely decay tubers, killing themselves in the process and not make it through the winter, compared to less aggressive isolates.

The impact of isolate aggressiveness is only one of many factors which influence pathogen overwintering, however. Even though US-8 isolates are highly aggressive on tubers, thus reducing the likelihood of their survival during the winter, tubers infected with the US-8 strain are more likely to produce infected sprouts (Marshall and Stevenson, 1996). Even though most tubers in this test decayed to a level where the fungus would have died, not all did and tubers in storage or in cull piles may not be decayed to levels lethal to the fungus, allowing the fungus to live through the winter.

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Table 1. Date of planting and harvest for tuber maturity tests conducted in 1996 and 1997

	1996 - Field 1			1996 - Field 2			1997		
	Plant	Vine kill[a]	Harvest[b]	Plant	Vine kill	Harvest	Plant	Vine kill	Harvest
Early	Apr.25	Sept. 6	Sept. 10	Apr.25	Sept. 24	Sept. 25	Apr.11	Sept. 8	Sept. 24
Mid	May16	Sept. 6	Sept. 10	May16	Sept. 24	Sept. 25	May 5	Sept. 8	Sept. 24
Late	June 5	Sept. 6	Sept. 10	June 5	Sept. 24	Sept. 25	May 29	Sept. 8	Sept. 24

[a]All vine kill done using a vine beater.
[b]All tubers harvested using a commercial harvester.

Table 2. Sequential sample information for tubers harvested at Othello and Pasco for the 1997 growing season.

	Date of Sample	Date of Evaluation
Sample 1	July 3	Aug. 1
Sample 2	July 17	Aug. 15
Sample 3	July 31	Aug. 27
Sample 4	Aug. 14	Sept. 11
Pre Vine Kill	Sept. 5	Oct. 3
Post VK 1	Sept. 16	Oct. 7
Post VK 2	Sept. 22	Oct. 15

Table 3. Percent tuber surface skinned during harvest during tuber maturity trial in 1996 and 1997.

	Percent tuber surface skinned[a]	
	1996	1997
Planting Time[b]		
1 (Early)	15 A	8 A
2 (Mid)	23 B	5 B
3 (Late)	36 C	3 B
Cultivar		
Ranger Russet	31 A	6 A
Russet Burbank	19 B	5 A

[a]Percent of tuber skin missing was visually estimated for all tubers harvested from the same plot as tubers rolled over the sorting table. Within a column and group, numbers with the same letter do not differ significantly from each other ($P \leq 0.05$).
[b]Planting time refers to the sequential order of planting, with planting time 1 being the first, or earliest, and planting number 3 being the latest.

Table 4. Effect of planting date and cultivar on percentage of external and internal late blight tuber rot for tubers inoculated at harvest.[a]

	1996 Field 1		1996 Field 2		1997	
	External	Internal	External	Internal	External	Internal
Isolate (Mating type)						
US-8 (A2)	56 A	44 A		45 A	**	**
US-11 (A1)	--	--		17 B	--	--
US-1 (A1)	40 B	21 B		6 C	--	--
Planting Time[b]						
1 (Early)	55 A	37 A	45 A	30 A	44 A	28 A
2 (Mid)	54 A	37 A	35 A	23 A	39 A	18 B
3 (Late)	35 B	23 B	21 B	15 B	42 A	22 AB
Cultivar						
Ranger Russet	58 A	42 A	35 A	27 A	37 A	25 A
Russet Burbank	38 B	23 B	32 A	19 B	46 A	21 A

[a]Tuber rot percentages for planting time and cultivar were calculated without using the US-1 isolate for field 2 due to low infection frequency and severity. Within a column and group, values with the same letter are not significantly different from each other ($P \leq 0.05$).
[b]Planting time refers to the sequential order of planting, with planting time 1 being the first, or earliest, and planting number 3 being the latest.
** The US-8 isolate was the only isolate used in this test.
-- Represents no data.

Table 5. Effect of planting date and cultivar on percentage of external and internal late blight tuber rot for tubers inoculated after a six month storage period.[a]

	1996 Field 1		1996 Field 2	
	External	Internal	External	Internal
Planting Time[b]				
1 (Early)	33 A	17 A	37 A	19 A
2 (Mid)	31 A	13 A	31 A	12 A
3 (Late)	9 B	3 B	30 A	12 A
Cultivar				
Ranger Russet	41 A	18 A	53 A	23 A
Russet Burbank	5 B	3 B	12 B	6 B

[a]Within a column and group, values with the same letter are not significantly different from each other ($P \leq 0.05$).
[b]Planting time refers to the sequential order of planting, with planting time 1 being the first, or earliest, and planting number 3 being the latest.
** The US-8 isolate was the only isolate used in this test.
-- Represents no data.

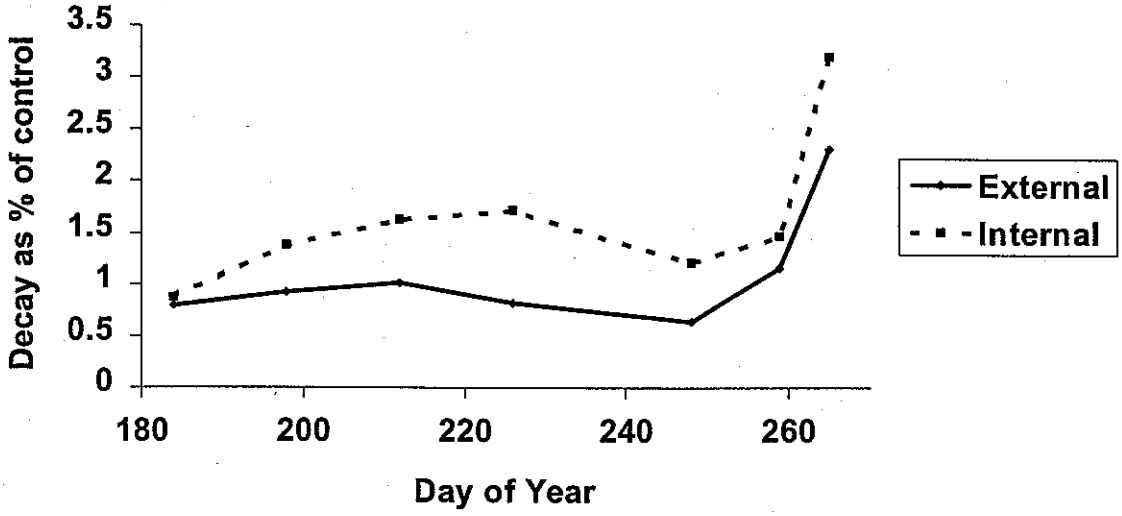

Figure 1. External and internal tuber blight as a percent of blighted control tubers for tubers harvested at Othello, WA. Values were obtained by dividing tuber blight scores for tubers harvested from the field by the corresponding tuber blight scores of tubers inoculated from storage.

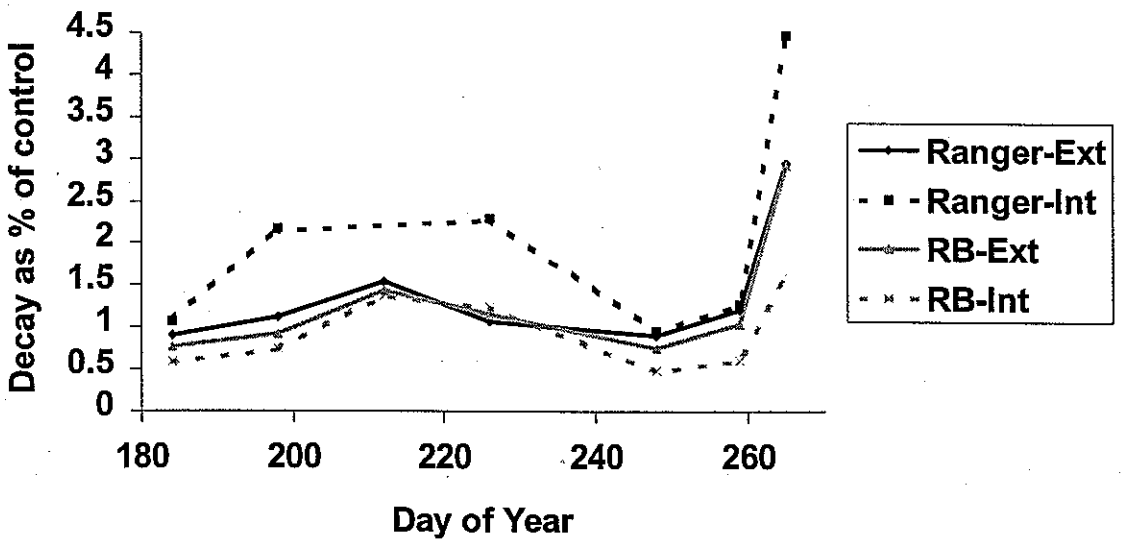

Figure 2. External and internal tuber blight as a percent of blighted control tubers for tubers harvested at Pasco, WA. Values were obtained by dividing tuber blight scores for tubers harvested from the field by the corresponding tuber blight scores of tubers inoculated from storage. RB refers to Russet Burbank and Ranger refers to Ranger Russet tubers. Ext = external tuber blight and Int = internal tuber blight.

Table 6. Percent of external tuber blight caused by isolates of *Phytophthora infestans* on potato tubers buried in soil at two locations in the Columbia Basin for six weeks during the winter.[a]

		Percent external tuber blight		
Isolate	Genotype	Prosser	Othello-Russet Burbank	Othello-Shepody
265	8	55 A	90 A	80 A
584	8	42 B	78 AB	70 AB
444	7	35 BC	79 AB	63 BC
367	7	39 BC	56 C	49 C
94	6	39 BC	66 BC	52 C
416	6	32 CD	32 D	55 C
537	11	26 DE	59 C	27 D
2	1	19 E	77 AB	29 D
382	1	0 F	0 E	0 E

[a]Interactions among isolates and cultivars were present for Othello but not for Prosser data. Values represent surface of tuber discolored due to tuber blight.