The Effects of Wounding and Potato Cultivar on Pathogenicity and Aggressiveness of *Alternaria solani* and *A. alternata*

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The fungi *Alternaria solani* and *A. alternata* cause two infectious diseases of potato: *A. solani* causes early blight and *A. alternata* causes brown spot. Both fungi give rise to concentric rings within the center of the lesions on infected leaves; however, concentric rings are usually more distinct with *A. solani*. Early blight lesions are often surrounded by a chlorotic halo, which is due to the presence of fungal metabolites. Brown spot lesions are generally smaller, darker, and can be numerous. Both fungi form spores on infected and infested plant parts when environmental conditions are optimal and secondary infections can then be repeated. Overwintering occurs as spores or mycelium in soil or in previously infected plant debris. Wind-dispersed spores act as primary inoculum for the following year. Early blight and brown spot are regarded as diseases of senescing tissue, and as plants age they become more susceptible to infection. Tuber yield is impacted when infection of foliage occurs before or when tubers are bulking, resulting in a reduction in tuber size. Infection of tubers occurs only thru wounds, resulting in the formation of dark, sunken lesions, which impact tuber quality.

Early blight and brown spot are difficult diseases to manage. One reason is that the pathogens can produce many spores in a short period. Large numbers of spores are produced in one generation and multiple generations are produced during the season, resulting in high levels of inoculum produced over the growing season. In addition, spores of *A. solani* and *A. alternata* are multicellular and each cell is capable of germinating and initiating hyphal growth, which can rapidly infect and colonize hosts. Traditional management strategies have included the use of broad spectrum fungicides, however, the ability of these pathogens to quickly and repeatedly cause infections has made the timing of fungicide applications critical. Crop rotation helps to reduce initial inoculum levels in a field and can be beneficial in managing both diseases. Potato fields should be rotated out of potatoes for at least two years to augment a disease management program that includes fungicides. In central Washington, potatoes are typically rotated with onion, corn, mint, and small grains.

Alternaria isolates were collected from potato tissue with lesions from the Pacific Northwest over several growing seasons. A third species, *A. triticina,* was also isolated from lesions on potato leaves. *A. triticina* causes leaf blight on wheat, barley, and oats. Morphologically, *A. triticina* is almost identical to *A. alternata*, making it difficult to identify without molecular methods. *A. triticina* has not been previously reported from potato. If *A. triticina* exhibits more or the same aggressiveness as *A. solani* and *A. alternata* on potato, future disease management would be potentially complicated in the Columbia Basin. Another question of concern is whether rotating potato after wheat or barley will increase the risk of infection on potato by *A. triticina*.

Pathogenicity, infection frequency, lesion size, and incubation period contribute to disease development in the field and are considered components of pathogen aggressiveness. Pathogenicity is the ability of a pathogen to cause disease. An isolate of a fungus either causes disease and is pathogenic or it cannot cause disease and is non-pathogenic. Incubation period is the time from inoculation to lesion. Lesion size is a measure of the area of a lesion ($mm^{2)}$. Infection frequency is the proportion of a given number of spores that cause infection. In this study, pathogenicity, incubation period, lesion size, and infection frequency were quantified for

A. solani, *A. alternata*, and *A. triticina* on Russet Norkotah potato leaves in order to determine the impact these *Alternaria* species have on disease development. Resistance was also evaluated for disease development caused by the three *Alternaria* species in several russet potato cultivars.

Materials and Methods

Isolate collection: Potato leaves with lesions were collected in 2008, 2009, 2010, and 2011 from potato fields located in the Pacific Northwest. Potato leaflets with lesions were surface disinfested by dipping leaflets into 70% ethanol for 3-5 seconds. Leaflets were blotted with paper towel and air dried. Tissue was excised from lesion margins and plated onto modified potato dextrose. Cultures were grown from single spores and were maintained on media plates under 24 hour lights at room temperature.

Pathogenicity and aggressiveness assays: Pathogenicity of 195 isolates was assessed on detached leaf tissue. Leaves are collected from mid-canopy of potted Norkotah plants that were maintained in the greenhouse for approximately 2 to 3 months. Standardized spore inoculum was applied to small filter paper squares, which were then placed onto leaves. The filter paper was removed after 48 hours. Incubation period, lesion size, and infection frequency of sixty two isolates of *A. solani* and 136 isolates of *A. alternata* were quantified on non-wounded tissue and one *A. solani* isolate, 3 isolates of *A. alternata*, and 3 isolates of *A. triticina* on non-wounded and wounded detached leaf tissue. Wounding of potato leaves was done using a sterilized scalpel (1 wound/ leaflet). Lesions were photographed at 3, 5, and 7 days post inoculation to record lesion size. Lesion area was determined by using Adobe Photoshop CS4 (San Jose, CA). The increase in lesion area was then used to calculate the area under the lesion expansion curve (AULEC) which integrates lesion areas over time.

Russet potato cultivars were assessed for resistance to infection by *Alternaria* species. Pathogenicity, infection frequency, incubation period, and lesion size of *Alternaria* species were evaluated from inoculated detached leaves. Alturas, Russet Burbank, Ranger Russet, Umatilla, and Russet Norkotah varied in degree of resistance and susceptibility to early blight. Inoculations of the five cultivars were performed on wounded tissue.

Results

Frequency of occurrence – *A. solani* was isolated less frequently than *A. alternata* in 2008, 2009, and 2011 (Fig. 1). *A. solani* and *A. alternata* were isolated at the same frequency in 2010.

Components of aggressiveness- Sixty of 62 (97%) isolates of *A. solani* were pathogenic and 86 of 136 (63%) isolates of *A. alternata* were pathogenic on Norkotah foliage (Table 1). There was no morphological difference observed between pathogenic and saprophytic isolates of *A. alternata*. AULEC, infection frequency, and final size of lesions were greater when leaves were inoculated with *A. solani* than with *A. alternata*. Incubation period was shorter when leaves were inoculated with *A. solani* than with *A. alternata* (Table 1).

Effects of wounding- AULEC was significantly greater on non-wounded leaves inoculated with *A. solani* than when inoculated with *A. alternata* and *A. triticina* (Table 2*)*. AULEC did not differ significantly between *A. alternata* and *A. triticina* isolates on non-wounded tissue. AULEC was greater for *A. solani* than for *A. alternata* and *A. triticina* on wounded leaf tissue. AULEC was significantly greater for *A. alternata* than for *A. triticina* isolates. An interaction was observed

between the species and wounding, which was due to the significant difference in the expansion of lesions caused by isolates of *A. alternata* on non-wounded compared to wounded tissue. This interaction was not observed in the aggressiveness of *A. solani* or *A. triticina* isolates inoculated on non-wounded compared to wounded tissue.

Infection frequency for *A. solani* on non-wounded Norkotah leaves was significantly greater than for *A. alternata* and *A. triticina* (Table 3). There was no significant difference in infection frequency between inoculations of *A. alternata* and *A. triticina* on non-wounded tissue. When leaf tissue was wounded, no significant difference in infection frequency was observed among all three species inoculations.

The final size of lesions was greater on non-wounded tissue when caused by *A. solani* than by either *A. alternata* or *A. triticina*. Final size of lesions was no different when caused by *A. alternata* or *A. triticina* on non-wounded tissue. On wounded tissue, final size of lesions was greater when caused by *A. solani* than those caused by *A. alternata* or *A. triticina* and the final size of lesions caused by *A. alternata* was greater than those caused by *A. triticina.*

The incubation period for *A. solani* was 2 days, regardless of whether or not leaf tissue had been wounded prior to inoculation. The incubation period for *A. alternata* and *A. triticina* on non-wounded tissue was two days longer than on wounded tissue, 4 versus 2 days, respectively.

Potato cultivar- AULEC significantly varied among potato cultivars when inoculated with *Alternaria* species (Table 4). The AULEC of *A. solani* was greater on Umatilla than on Norkotah and on Russet Burbank and was least aggressive on Alturas. The AULEC of *A. alternata* was significantly greater on Ranger and Umatilla than on Alturas, Norkotah, and Russet Burbank. The AULEC for *A. triticina* was significantly greater on Umatilla than on all other cultivars. Within cultivars, the AULEC was significantly greater for *A. solani* than for *A. alternata* and *A. triticina* on Norkotah and Umatilla. There were no significant differences in AULEC among the species observed on Alturas or Russet Burbank.

Discussion-

The frequency of occurrence of *A. solani* observed over the past four years was less than that of *A. alternata*. However, only half of *A. alternata* isolates evaluated exhibited pathogenicity. *A. alternata* was often isolated from lesions also colonized by *A. solani* or *Colletotrichum coccodes*(black dot), suggesting that many of the isolates were involved as secondary invaders, acting as saprophytes or pathogenically only after initial infection by other fungi had occurred. This question will need to be addressed to determine potato foliage requires protection for secondary infection by *A. alternata* after initial infection by *A. solani*.

Infection frequency, AULEC, final size of lesions, and incubation period indicate an isolate's aggressiveness. A comparison of these components of aggressiveness demonstrated that *A. solani* is much more aggressive than *A. alternata* and *A. triticina*. *A. alternata* is not an aggressive pathogen when tissue was non-wounded. When tissue is wounded due to wind and hailstorms, which occur regularly in the Columbia Basin, *A. alternata* may become a more significant pathogen. While wounding of foliage cannot be avoided, efforts can be made to prevent or reduce wounding on tubers since it is required for infection to occur. This can be achieved by delaying harvesting in order to promote skin set and allowing for rapid suberization and wound healing.

A. triticina, which had not been previously known to cause disease in potato, was only mildly aggressive, regardless of whether or not wounding has occurred on leaf tissue. This

suggests that *A. triticina* is a minor pathogen and contributes little to the overall disease severity in a field. *A. triticina* causes leaf blight on cereal grains such as barley, oats, and wheat, with the durum wheat cultivars being very susceptible. Durum wheat is not typically grown in the Columbia Basin, so this pathogen may be of little importance in this region, but may be an issue in areas where durum wheat is grown in rotation with or in proximity to potatoes.

Resistance appears to be an effective means to reduce early blight in the field. Cultivars that were susceptible to infection by *A. solani* were also susceptible to *A. alternata* and *A. triticina*, although to a lesser degree. This suggests that both *A. alternata* and *A. triticina* are less significant pathogens in the field than *A. solani*. Cultivars that mature later in the season generally exhibit moderate to high resistance to infection by all three species of *Alternaria*, which can reduce the number of fungicide applications needed during the season.

Figure 1. Frequency of isolation of *A. solani*, *A. alternata*, and *C. coccodes* from potato leaves and tubers from the Pacific Northwest for 2008, 2009, 2010, and 2011. Multiple fungi were isolated from the same lesion.

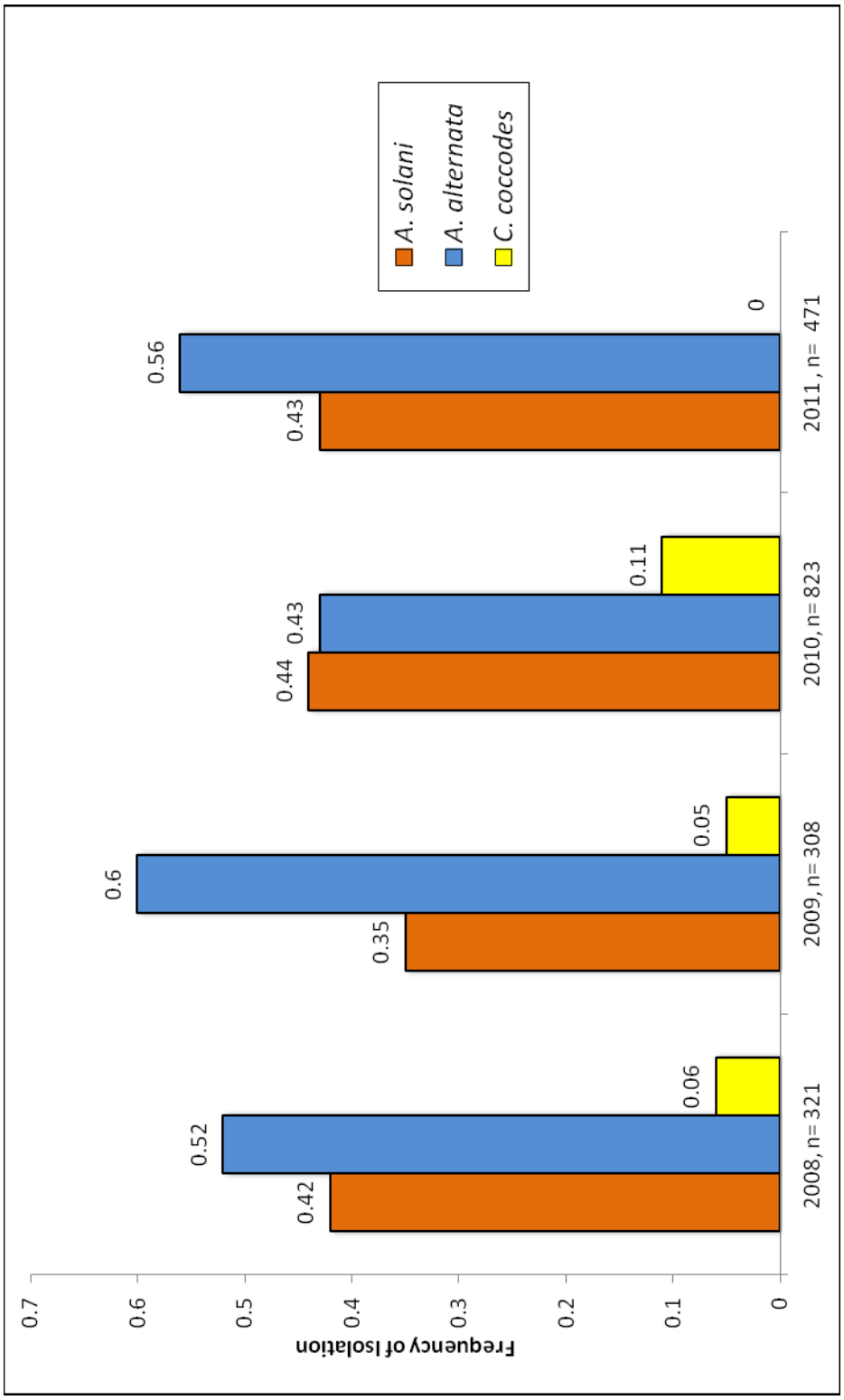

Table 1. Pathogenicity and components of aggressiveness of *Alternaria* species on non-wounded Russet Norkotah leaves.

Component	*A. solani*	*A. alternata*
Pathogenicity (%)	97	63
AULEC	3153	56*
(units over time)		
Infection frequency	1.0	0.3*
(proportion)		
Final size of lesions ($mm^{2)}$	1843	32
Incubation period (days)	2	6

* indicates significance across a row at p=0.05

Table 2. AULEC and frequency of infection of *Alternaria* species on non-wounded and wounded Russet Norkotah leaves.

	AULEC		Frequency of Infection	
Species	Non-wounded	Wounded	Non-wounded	Wounded
A. solani	2001.6a	1714.1a	1.0a	1.0a
A. alternata	36.3b	452.2b*	0.4b	1.0a
A. triticina	16.5b	98.3c	0.3b	0.8a*
UTC	4.0b	24.3c	0.2	0.5b

* indicates significance across a row at p= 0.05
Values with the same letter indicates significance down a column at p=0.05

Table 3. Final lesion size and latent period of *Alternaria* species inoculated onto wounded and non-wounded Russet Norkotah leaves.

	Final size of lesions (mm^2)		Incubation Period (days)	
Species	non-wounded	wounded	non-wounded	wounded
A. solani	1470.4	1091.9	2	2
A. alternata	21.2	322.1	4	2
A. triticina	26.3	233.8	4	2
UTC	4	19.79	6	4

Table 4. AULEC of three *Alternaria* sp. inoculated onto wounded russet cultivars.

Cultivar	*solani*	*alternata*	*triticina*	*control*
Alturas	29c	5b	1b	0a
Norkotah	572b[1]	50b[2]	10b[2]	0a[2]
Ranger Russet	625b[1]	215b[2]	33b[3]	188a[2]
Burbank	103c	0b	0b	0a
Umatilla	1488a[1]	164a[2]	72a[23]	1a[3]

[1,2,3] indicates significance across a row at p=0.05
Values with the same letter indicates significance down a column at p=0.05