# Occurrence, Aggressiveness and Fungicide Sensitivity of *Alternaria solani* and *Alternaria alternata* Isolates from Potato in the Columbia Basin of Washington

Lydia S. Tymon, Thomas F. Cummings, and Dennis A. Johnson
Department of Plant Pathology, Washington State University, Pullman, WA

Early blight and brown spot on potato are caused by separate species of *Alternaria.* Lesions caused by *Alternaria solani* Sorauer (Ellis)*,* the pathogen responsible for early blight, are distinct in that large concentric rings are formed within a tan colored lesion (Stevenson et al. 2008).  Initially, the lesions are small, dark, and circular, but as they enlarge, they become angular, due to the presence of the leaf veins. Lesions are typically surrounded by a chlorotic halo due to fungal metabolites (Rotem 1994). In comparison, lesions of brown spot, caused by *A. alternata*, tend to be smaller and darker in color, but can be numerous (Nolte 2008).

Both pathogens overwinter as spores or mycelium (Stevenson et al. 2008), but the source of primary inoculum is spores in debris or soil from the previous year. Three-year crop rotations are needed to reduce the initial amount of primary inoculum present. Infection occurs as spores germinate and enter into the tissue through wounds or stomata (Stevenson et al 2008). After lesions form, spores develop within the center of lesions during alternating wet and dry periods. Additional factors determining spore formation are temperature, light, and relative humidity (Stevenson et al. 2008). Multiple cycles of spore production takes place during one season. Spore dissemination is mainly by air currents (Rotem 1994), but secondary mechanisms of dispersal are splashing from rain and irrigation water (Rotem 1994).  Early blight and brown spot are both regarded as diseases of senescing plant tissue, and as plants age, they are more susceptible to infection (Rotem 1994).

**Fungicide resistance-** Traditional management strategies include the use of broad-spectrum fungicides such as mancozeb and chlorothalonil. The benefit to these products is that resistance has not been observed in spite of frequent and prolonged use. Yet, decreased mammal toxicity and a reduction of environmental impact due to residuals on the environment (Rosenzweig et al. 2008) are two reasons for a switch from broad-spectrum to narrow-spectrum fungicides. Fungicide resistance is a regular concern when managing crops, especially when the chemical affects a single site mode of action and the development of fungicide resistance is highly likely with frequent use of these products (Hamm et al. 2008). Quinone outside inhibitor (QoI) fungicides, which include azoxystrobin, pyraclostrobin, and trifloxystrobin, inhibit cellular respiration by interfering with the electron transport chain (Rosenzweig et al. 2008). Strobilurin sensitivity has been conferred by two single point mutations in fungal genomes (Rosenzweig et al. 2008). *A. solani* has recently been reported to be insensitive to strobilurin fungicides in Idaho (Belcher et al. 2010). Currently, there are no records of *A. alternata* populations infecting potato that are resistant to strobilurin fungicides, although *A. alternata* may exhibit a natural insensitivity.

**Frequency of isolates-** Fungal isolations were made from lesions on leaves collected from the Columbia Basin, Bonner's Ferry, ID, and Acequia ID to determine the

prevalence of *Alternaria* species in 2009 and 2010. Of 308 isolates obtained in 2009, 107 (35%) were *A. solani*, 185 (60%) were *A. alternata*, and 16 (5%) isolates were *C. coccodes*. A total of 823 isolations were made in 2010. *A. solani* was isolated 362 (44%) times, *A. alternata* was isolated 354 (43%) times, and *C. coccodes* was isolated 91 (11%) times. Multiple fungi were isolated from the same lesion both years. The frequency of isolation of *A. solani* (19%) from lesions collected in the Columbia Basin in 2009 did not differ from the frequency of isolation of *A. alternata* (17%), even though the frequency of isolations of the two fungi did vary over time (Fig. 1). In 2010, *A. alternata* was typically isolated more frequently than *A. solani* from leaves with lesions collected in the Columbia Basin (Fig. 2). During both years, *A. solani* was isolated less frequently than *A. alternata* at the end of the potato-growing season.

**Pathogenicity and Aggressiveness Assays-** Pathogenicity and aggressiveness assays using 34 isolates collected in 2009 were performed on detached Norkotah Russet leaves. Leaves were collected from potted Russet Norkotah plants that were maintained in the greenhouse. Leaves were then placed into 5.5 cm deep Pyrex baking pans lined with moistened paper towels and fiberglass screen. Detached leaves were sprayed with distilled water until runoff. Ten µL of inoculum was dropped onto filter paper, which was then placed facedown onto the detached leaves. The Pyrex pans were then wrapped into large plastic bags and rubber banded closed. The filter paper was removed after 48 hours, with signs and symptoms of infection typically occurring during this time period. Lesion expansion was determined to assess aggressiveness (Berger 1997). Disease severity was measured as the proportion of leaf area covered by the lesion after 5 days using one *A. solani* isolate and one pathogenic isolate of *A. alternata*.

Pathogenicity was determined by the formation of lesions on detached leaves and aggressiveness was evaluated by lesion expansion (Fig. 3). Initial lesion formation typically occurred within 48 hours of *A. solani* inoculations and within 48 to 72 hours when leaves were inoculated with pathogenic *A. alternata* isolates. All seventeen isolates of *A. solani* (100%) resulted in lesions whereas only nine of seventeen isolates of *A. alternata* (53%) caused lesions. Expansion of lesions caused by *A. solani* occurred more rapidly than those caused by *A. alternata*. The disease severity determined was 12% for *A. solani* vs. 4% for *A. alternata*.

**Frequency of Isolates from Foliage Treated with Fungicides -** Fungal isolations were made from lesions on foliage treated with various fungicides in replicated plots in trials at Quincy, WA (Table 1), and Acequia, ID (Table 2). Sixteen lesions from each fungicide treatment were randomly selected and assayed on modified PDA plates. Plates were scored based upon species of fungi isolated.

In Quincy, the frequency of isolation of *A. solani* and *A. alternata* on lesions assayed from nontreated control plots was 50% and 56%, respectively. The frequency in which *A. solani* was isolated from lesions was less from lesions treated with fungicides than the nontreated control. The frequency of isolation of *A. alternata* was not significantly less than the nontreated control. A fungicide program of Quadris Top rotated with Bravo WS was the most effective at reducing the frequency of isolation of *A. alternata* (Table 1).

Three fungicide trials were done in Acequia, ID. *A. solani* occurred on all lesions assayed from nontreated plots and frequency of isolation was not significantly different among any of the fungicide programs tested. The frequency of isolation of *A. alternata*

on lesions assayed from nontreated control plots was 38%. Although the frequency of isolation of *A. alternata* increased when fungicides were applied in two of the three fungicide treatments, frequency of isolate was not significantly different from nontreated plots. Regardless of the fungicide program, *A. alternata* was isolated less frequently than *A. solani*. These results were consistent in the other two trials (Table 2).

In 2010, the frequency of isolation of *A. solani* exhibited an inverse relationship with the frequency of isolation of *A. alternata* from Quincy, WA, Acequia, ID, and Bonner's Ferry, ID. Multiple regression analysis of the proportion of *A. solani* vs. *A. alternata* from all three locations showed a significant relationship (P<0.001, $R^2$=0.19) (Fig. 4). Separate multiple regression analysis of *Alternaria* spp. from Quincy, WA and Acequia, ID were performed (Fig. 5). No significant relationship between the *Alternaria* spp. was observed in Quincy, WA. However, the multiple regression analysis of Acequia, ID data showed a significant relationship (P= 0.004, $R^2$ = 0.58).

**Discussion-**The strobilurin fungicides are highly desireable for managing early blight in the Columbia Basin due to their reduced impact on human health and the environment (Vincelli 2002). Due to the site specific nature of these chemicals, fungicide resistance is likely and has already been found in populations of *A. solani* in Idaho (Belcher et al. 2010). Applications of only strobilurin fungicides reduced the frequency of isolation of both *A. solani* and *A. alternata* in the fungicide trials, but not significantly when compared to nontreated plots. While these fungicide treatments appeared to be most effective at reducing frequency of isolation of *A. solani* than of *A. alternata* in most locations, Acequia, Idaho was an exception. This indicates that there may be differences in the fungicide resistance of pathogens in populations among locations. Strobilurins that were tank mixed and/or rotated with broad-spectrum fungicides consistently reduced the frequency of isolation of fungi, but as observed in the three locations, frequency of isolation in treated plots was never significantly different from nontreated plots. The unexpected observation of the high frequency of isolation of *A. solani* when treated with Bravo WS at Acequia, ID requires further investigation because fungi typically do not develop resistance to multi-site fungicides.

The fungicide trials were run with multiple tank mixes, different rotations, and at various rates, and were not intended to extract which fungicide is most responsible for controlling infection in plots. Resistance to fungicides can result in significant economic loss and is a major concern when dealing with chemicals that have a specific target. Management programs should include the use of resistant potato varieties, good sanitation practices, as well as fungicide programs with optimized application rates and rotation schedules (Hamm et al. 2008). Fungicide trials allow for the testing of these different programs to determine their effectiveness in pathogen control.

Isolates of *A. solani* were consistently pathogenic on potato and more aggressive than pathogenic *A. alternata* isolates, based on detached leaf tissue assays. Tissue that was inoculated with *A. solani* had a shorter incubation period and more rapid lesion expansion. This may be why the observed disease severity in plots was often highest when frequency of isolation of *A. solani* was approximately 50% or greater. Plots in which A. *alternata* was the primary pathogen isolated from lesions often exhibited lower disease severity. In many of the fungicide trials, treatments that were most effective in reducing *A. solani* in plots often exhibited a higher incidence of *A. alternata*. *A. alternata* has been shown to be a less aggressive pathogen on potato. High populations of *A. solani*

may inhibit *A. alternata* populations from developing, suggesting that competition may be a factor in pathogen population in potato plot.

Figure 1. Frequency of isolation of *Alternaria solani* and *A. alternata* from lesions on potato leaves collected in the Columbia Basin in 2009
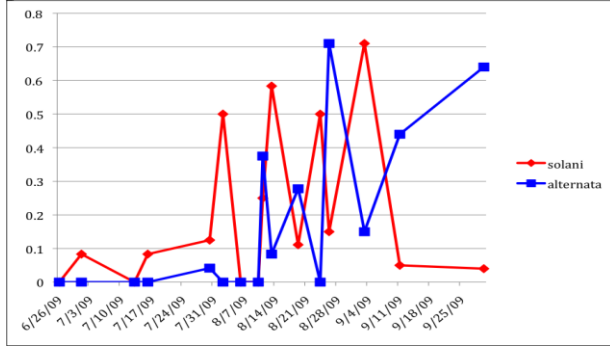


Figure 2. Frequency of isolation of *Alternaria solani* and *A. alternata* from lesions on potato leaves collected in the Columbia Basin in 2010.
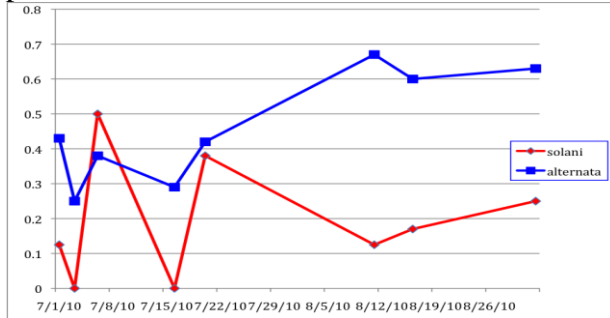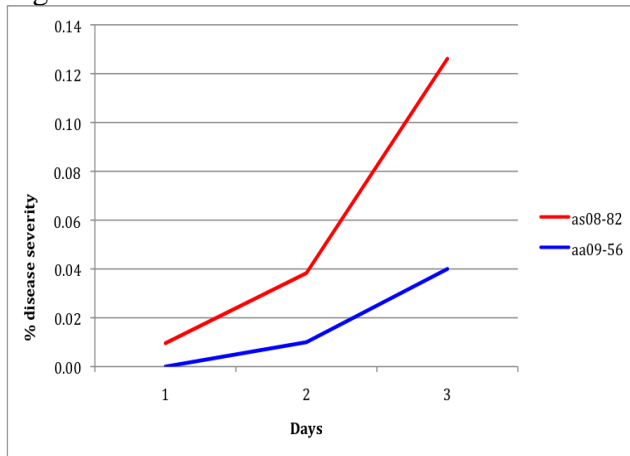


Figure 3. AUDPC of *A. solani* and *A. alternata*

Figure 4. Proportions of assayed potato leaf *Alternaria sp.* lesions from nine fungicide trials at three locations in ID and WA, 2010
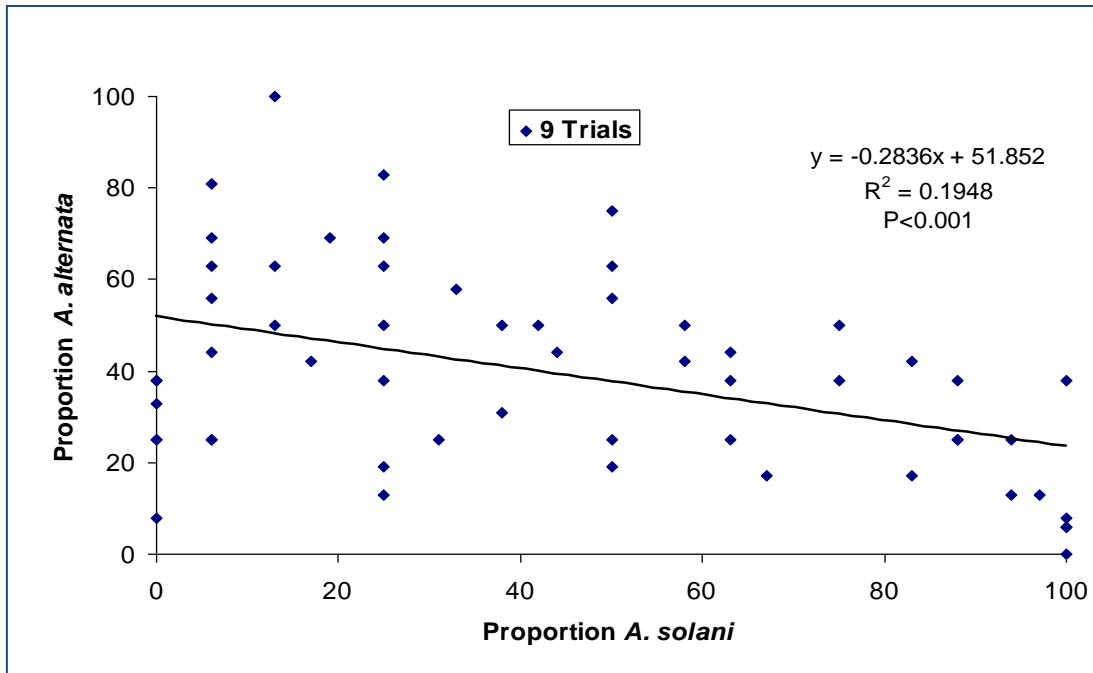


Figure 5. Proportions of assayed potato leaf *Alternaria sp.* lesions from two fungicide trials at Acequia, ID (3) and Quincy, WA (1), 2010.
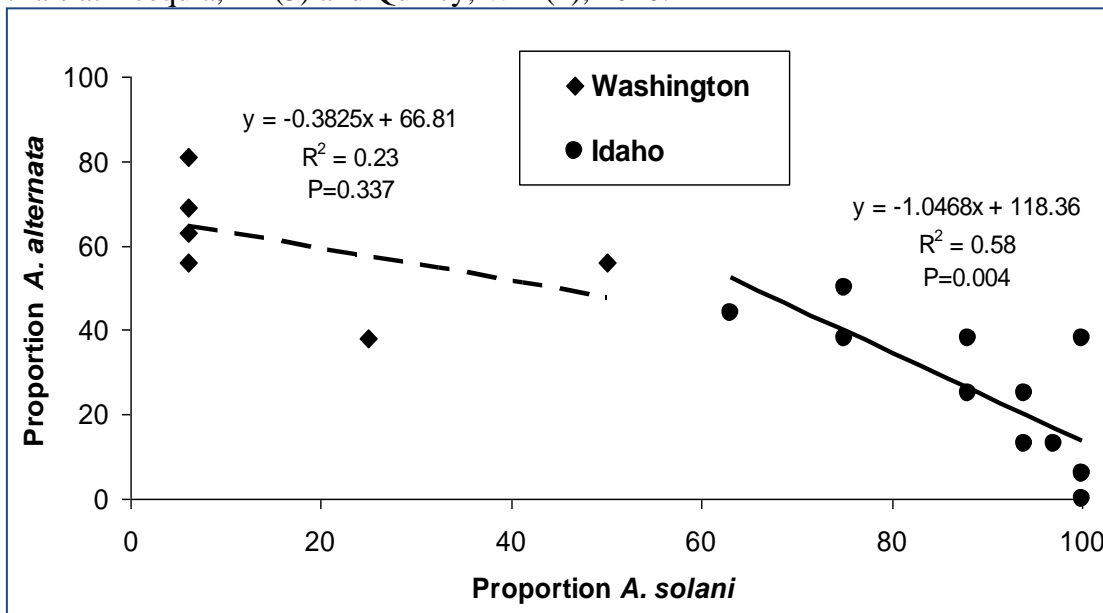
Table 1. Frequency of isolates of *Alternaria solani* and *A. alternata* from lesions on Russet Burbank potato leaves treated or not treated with various fungicides at Quincy, WA

| Treatment | % lesions with *A. solani* | % lesions with *A. alternata* |
|---|---|---|
| Not treated | 50 a | 56 ab |
| Endura, 2.5 oz wt/a (ACE)<br>Bravo WS, 24 fl oz/a (BDF) | 6 b | 56 ab |
| Revus Top, 7 fl oz/a (ACE)<br>Bravo WS, 24 fl oz/a (BDF) | 6 b | 63 ab |
| Quadris Top, 8 fl oz/a (ACE)<br>Bravo WS, 24 fl oz/a (BDF) | 25 ab | 38 b |
| Quadris Top, 8 fl oz/a (AD)<br>Bravo WS, 24 fl oz/a (BEF)<br>Omega, 7 fl oz/a (C)<br>Inspire, 7 fl oz/a (C) | 6 b | 81 a |
| Quadris Top, 8 fl oz/a (AD)<br>Bravo WS, 24 fl oz/a (BEF)<br>Omega, 6.4 fl oz/a (C)<br>Inspire, 6.4 fl oz/a (C) | 6 b | 69 ab |

For most treatments, lesions from 16 leaves were sampled from each treatment. Data includes occurrences of *A. solani* and *A. alternata* on the same lesion.

Table 2. Frequency of isolates of *Alternaria solani* and *A. alternata* from lesions on Western Russet potato leaves treated or not treated with various fungicides at Acequia, ID.

| Treatment | % lesions with *A. solani* | % lesions with *A. alternata* |
|---|---|---|
| **Trial 1** | | |
| Not treated | 75 ab | 38 a |
| Endura, 2.5 oz wt/a<br>Dithane F-45<br>Super Tin | 94 a | 13 a |
| Endura, 2.5 oz wt/a<br>Dithane F-45 1.2 qt/a | 63 b | 44 a |
| Penncozeb, 2 lb/a | 75 ab | 50 a |
| **Trial 2** | | |
| Not treated | 100 a | 6 a |
| Headline, 6 fl oz/a (A-D) | 88 a | 25 a |
| Endura, 2.5 oz wt/a (A-D) | 97 a | 13 a |
| **Trial 3** | | |
| Not treated | 100 a | 6 ab |
| Quadris, 6 fl oz/a | 94 a | 25ab |
| Gem, 3.8 fl oz/a | 100 a | 38 a |
| Tanos, 6 oz/a | 88 a | 38 a |
| Bravo WS, 1.5 pt/a | 100 a | 0 b |

For most treatments, lesions from 16 leaves were sampled from each treatment. Data includes occurrences of *A. solani* and *A. alternata* on the same lesion.

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