Best Management Tactics and Fungicide Resistance in *Alternaria* Populations in the Columbia Basin

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Early blight is caused by *Alternaria solani* and brown spot is caused by *A. alternata*. Both diseases result in lesions on foliage and tubers and yield losses can occur when infection by *A. solani* takes place before or while tubers are bulking. Yield losses due to *A. alternata* have not been documented. Crop rotations of at least three years out of potatoes reduce disease levels. Completely resistant cultivars are not available, but avoiding very susceptible cultivars is important in reducing yield losses.

Fungicides are used to manage both diseases. Approximately two to three fungicide applications are made during the growing season to manage early blight in the Columbia Basin. Number of applications can be as high as ten in other regions. Broad-spectrum fungicides are used for both late blight and early blight. The benefit of these fungicides is that resistance has not been observed in spite of frequent and prolonged use. A switch has occurred over the last two decades from broad spectrum to narrow spectrum (site-specific) fungicides in efforts to reduce potential negative effects on human health and the environment. Fungicide resistance is a concern when managing crops, especially when the fungicide affects a single site and is repeatedly used.

Two narrow spectrum fungicides used in the Columbia Basin are azoxystrobin (Quadris) and boscalid (Endura). Azoxystrobin is a strobilurin, which are quinone outside inhibitors (QoI) that inhibit cellular respiration by interfering with the electron transport chain in the mitochondria. This group of fungicides also includes pyraclostrobin (Headline) and trifloxystrobin (Flint) and has efficacy over a wide range of fungi. Resistance to QoI fungicides has been documented for several fungi infecting various crops.

Boscalid is a carboximide fungicide that also disrupts cellular respiration. The target of boscalid is the succinate ubiquinone oxidoreductase of complex II. Interference with this protein prevents the reduction of quinine. Resistance to boscalid has been observed in multiple fungi and systems, such as *A. alternata* on pistachio and *Botrytis cinerea* on apple. Resistance to boscalid is not conferred by the same point mutation in all organisms, and occurs in all three of the subunit coding regions.

Resistance to azoxystrobin and boscalid has been detected in populations of *A. solani* on potato in the Midwest and in southern Idaho. *Alternaria alternata* resistance to azoxystrobin and boscalid has been reported on pistachio in California and on potato in southern Idaho. Fungicide resistance of *Alternaria* species to azoxystrobin or boscalid on potato has not been reported in the Columbia Basin. Comparison of the two pathogens on potato can help determine if different rates of fungicides are necessary for control. The objective of this research was to assess *A. solani* and *A. alternata* populations in the Columbia Basin for azoxystrobin and boscalid resistance by prescreening isolates using mycelial growth at a threshold concentration. Prescreening was done in an attempt to capture the range of fungicide resistance within the populations of *A. solani* and *A. alternata* in the region. Selected isolates were then assessed for mycelial growth and spore germination at different fungicide concentrations.

MATERIALS AND METHODS

Potato foliage with lesions was collected from commercial potato fields in the Columbia Basin of Washington in 2009 through 2011. Foliage was placed in plastic bags, sealed, and transported to the laboratory in Pullman, WA. Potato leaflets with lesions were surface disinfested and lesion margins were excised and plated on a modified potato dextrose agar. Single spore isolates were obtained from single lesions from single leaflets and grown on mPDA to prepare material for mycelial growth and spore germination assays.

Stock solutions of technical grade azoxystrobin (95.3% active ingredient; Syngenta Crop Protection Inc.) and boscalid (99% active ingredient; BASF Corporation) were prepared in acetone at 10,000 µg/mL and 5000 µg/mL, respectively and azoxystrobin and boscalid were added to 1.5% Difco water agar. Salicylhydroxamic acid (SHAM) was added to media to block the use of an alternate pathway for cellular respiration. Control plates were amended with acetone + SHAM. Media was also amended with 1, 10, and 100 µg/mL of azoxystrobin, and 0.5, 5, and 50 µg/mL of boscalid for final fungicide concentrations.

Assessment of fungicide resistance at a threshold concentration. *A. solani* and *A. alternata* were evaluated for fungicide resistance using mycelial growth and spore germination on water agar amended with azoxystrobin at a fungicide concentration of 100 µg/mL and boscalid at a concentration of 50 µg/mL. Threshold concentrations were based upon concentrations used in previous studies. Fungicide resistance was determined for 50 isolates of *A. solani* and 58 isolates of *A. alternata* that were originally collected from potato in the Pacific Northwest.

For mycelial growth, a 6 mm diameter mycelial plug was plated top down onto fungicide amended water agar and plates were maintained in the lab. Plates were arranged in a completely randomized design with two replicates per trial and the experiment was done twice. Colonies were measured in two perpendicular directions at 3, 5, and 7 days post-plating. Colony area was determined by multiplying the measurements minus the diameter of the agar plug. Area under the growth progress curve (AUGPC) was calculated using the colony area over time for each isolate.

Growth ratio was calculated by dividing AUGPC for each isolate on the fungicide-amended plate by the AUGPC of the control. **Values closer to 1 indicated greater resistance while values closer to 0 indicated greater sensitivity**. A value of 0.5 or greater, or where mycelial growth was reduced by no more than half in comparison to the control, was arbitrarily selected as the boundary to indicate fungicide resistance.

In preparation for spore germination assessment, spores were gently scraped from media using a sterile scalpel and mixed into 1.5mL of sterile distilled water. *A. solani* spore suspensions were adjusted to 10^3 spores/mL and *A. alternata* suspensions were adjusted to 10^4 spores/mL. One hundred fifty µL of the spore suspension was spread plate onto fungicide-amended plates. Plates were placed in the dark and after 24 hours the spore germination of 100 spores was assessed. Germination was defined as the presence of a germ tube at least half the length of the spore, or if multiple germ tubes were present on a single spore. Plates were arranged in a completely randomized block design with two replicates per trial. The experiment was repeated on a second date.

Fungicide concentration analysis. Mycelial growth and spore germination of selected isolates were evaluated at three concentrations of each azoxystrobin and boscalid. Concentrations were 1, 10, and 100 µg/mL for azoxystrobin and 0.5, 5, and 50 µg/mL for boscalid. A subsample of two isolates of *A. solani* and three of *A. alternata* were selected for the assay based upon mycelial growth ratios. Selected isolates for the assay exhibited sensitivity or resistance to either azoxystrobin or boscalid; one isolate of *A. alternata* exhibited resistance to both fungicides.

RESULTS

Assessment of fungicide resistance at a threshold concentration. Growth ratios (indication of sensitivity to fungicide) of *A. solani* (Fig. 1) to azoxystrobin ranged between 0.10 and 0.40 while growth ratios of *A. solani* to boscalid ranged between 0.10 and 0.76 Ten isolates exhibited moderate to high resistance with growth ratios above 0.5. One isolate had a maximum growth ratio of 0.76. Growth ratios of *A. alternata* (Fig. 2) to azoxystrobin ranged between 0.20 and 0.50 while growth ratios of *A. alternata* to boscalid ranged between 0.05 and 0.53.

In 2010, *A. alternata* isolates were less sensitive to azoxystrobin than *A. solani* isolates based upon mean growth ratio and spore germination (Table 1). In 2011, only mean growth ratios suggested that *A. alternata* was less sensitive to azoxystrobin than *A. solani*. Differences in spore germination sensitivity of *A. alternata* and *A. solani* to azoxystrobin were not observed.

Differences were not observed in mean growth ratio and spore germination between *A. alternata* and *A. solani* to boscalid in 2010. However, both mean growth ratios and spore germination were significantly greater for *A. solani* than *A. alternata* in 2011, indicating that *A. alternata* isolates were more sensitive to boscalid than *A. solani*.

The sensitivity of *A. alternata* isolates to azoxystrobin decreased slightly between 2010 and 2011. There was no change in sensitivity of *A. solani* to azoxystrobin between 2010 and 2011. Sensitivity of *A. alternata* to boscalid did not change between 2010 and 2011. However, there was a significant reduction in sensitivity to boscalid observed between 2010 and 2011 isolates of *A. solani*.

Fungicide concentration analysis. Percent spore germination of *A. alternata* (Table 2) was not different than that of *A. solani* at 1µg/mL azoxystrobin, but spore germination of *A. alternata* was greater than that of *A. solani* at 10 and 100 µg/mL azoxystrobin. Percent spore germination of *A. alternata* did not significantly decrease as azoxystrobin concentrations increased, but percent spore germination *A. solani* decreased as azoxystrobin concentration increased.

Percent germination of *A. alternata* on boscalid was less than that of *A. solani* at all concentrations (0.5, 5, and 50 µg/mL). Percent spore germination of *A. alternata* and *A. solani* did not significantly decrease as fungicide concentrations increased.

Growth ratios of *A. alternata* (Table 3) on plates amended with azoxystrobin at 1, 10, and 100 ug/mL were greater than for *A. solani*. For both *A. solani* and *A. alternata*, growth ratios decreased as the concenctration of azoxystrobin increased. On plates amended with 0.5 and 5 µg/mL of boscalid, growth ratio of *A. alternata* was less than *A. solani* whereas no significant difference was observed between growth ratios of *A. alternata* and *A. solani* on

plates amended with 50 µg/mL of boscalid. Growth ratios of both *A. solani* and *A. alternata* decreased as the concentration of boscalid increased.

CONCLUSIONS

Wide ranges of sensitivity to insensitivity (growth ratios) to azoxystrobin and boscalid were observed for *A. solani* and *A. alternata*. Most isolates of *A. solani* and *A. alternata* were sensitive to both fungicides while some isolates exhibited moderate resistance to azoxystrobin and resistance to boscalid. Out of 58 isolates, 3 isolates of *A. alternata* (5% of the isolates) exhibited moderate resistance to azoxystrobin and 2 isolates (3% of the isolates) exhibited resistance to boscalid. Of the 50 isolates of *A. solani* screened, 0 isolates exhibited resistance to azoxystrobin, but 10 isolates (20% of the isolates) exhibited moderate to high resistance to boscalid. The frequency of azoxystrobin and boscalid resistance was low across isolates. However, resistance in both fungal populations could build up rapidly and spread by wind-blown spores if good management practices are not applied.

Fungicide resistance can be minimized by restricting the use of site-specific fungicides (no more than two applications of a site-specific FRAC class per season), tank mixing site-specific fungicides with broad-spectrum fungicides, and alternating mixtures with broad-spectrum fungicides (broad-spectrum fungicides include chlorothalonil (Bravo) and mancozeb). The maximum fungicide rates should be used, fields should be rotated out of potatoes for at least three years, plant stress should be avoided, and adequate plant nutrients should be applied.

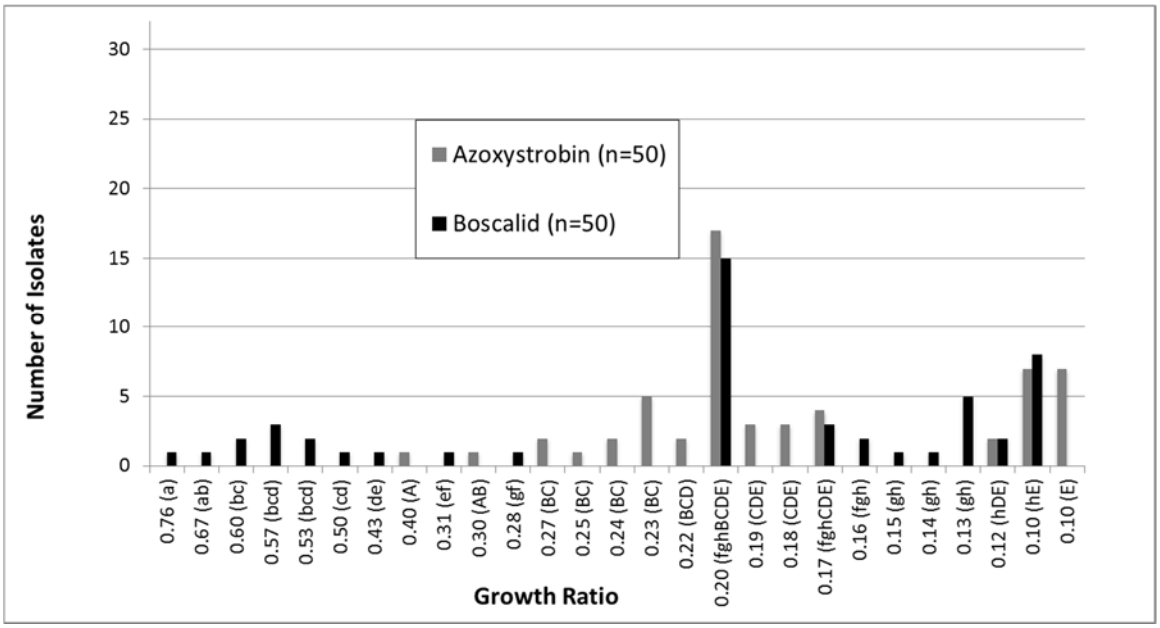

Figure 1. Growth ratios for *A. solani* on azoxystrobin (100 µg/mL) and boscalid (50 µg/mL) amended media. Values closer to 1 indicate greater resistance while values closer to 0 indicate greater sensitivity.

ABC Values indicate significance among isolates grown on azoxystrobin amended plates at P =0.05.

abc Values indicate significance among isolates grown on boscalid amended plates at P =0.05.

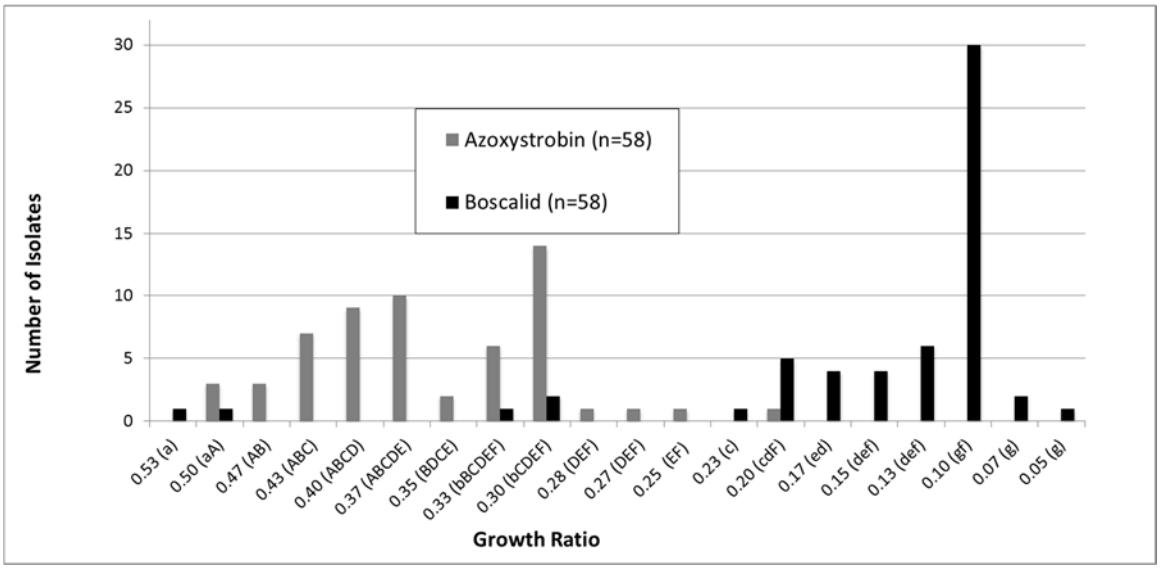

Figure 2. Growth ratios for *A. alternata* azoxystrobin (100 µg/mL) and boscalid (50 µg/mL) amended media. Values closer to 1 indicate greater resistance while values closer to 0 indicate greater sensitivity.

ABC Values indicate significance among isolates grown on azoxystrobin amended plates at P =0.05.

abc Values indicate significance among isolates grown on boscalid amended plates at P =0.05.

Table 1. Mycelial growth ratio and percent spore germination on azoxystrobin and boscalid amended media of 58 isolates of *A. alternata* and 50 isolates of *A. solani* collected in 2010 and 2011.

Mycelial growth ratio	Azoxystrobin + SHAM			Boscalid + SHAM		
	2010	2011	*p*-value	2010	2011	*p*-value
A. alternata	0.36a*	0.33a	0.04	0.20a	0.14a	0.39
A. solani	0.20b	0.21b	0.7	0.20a*	0.40b	<0.0001
Percent spore germination						
A. alternata	0.73a	0.56a	0.12	0.58a	0.53a	0.67
A. solani	0.50b	0.44a	0.52	0.50a*	0.97b	<0.0001

Values with the same letter within a column for each variable are not significantly different at *P* =0.05

* indicates significance across a row at *P* =0.05.

Table 2. Percent spore germination of *A. alternata* and *A. solani* on three concentrations of azoxystrobin and boscalid amended media

	Azoxystrobin concentration µg/mL				Boscalid concentration µg/mL			
Species	1	10	100	*P-value*	0.5	5	50	*P-value*
A. alternata	0.83a	0.87a	0.83a	0.79	0.82a	0.76a	0.69a	0.27
A. solani	0.83a	0.68b	0.50b	0.06	0.96b	0.92b	0.94b	0.05

Values with the same letter within a column for each variable are not significantly different at *P* =0.05.

Table 3. Mycelial growth ratios of *A. alternata* and *A. solani* on three concentrations of azoxystrobin and boscalid amended media.

	azoxystrobin concentration µg/mL				boscalid concentration µg/mL			
	1	10	100	*P*-value	0.5	5	50	*P*-value
Trial 1								
A. alternata	0.8a	0.4a	0.4a	0.01	0.7a	0.3a	0.2a	0.04
A. solani	0.3b	0.2b	0.1b	0.03	0.8a	0.8b	0.2a	0.01

Values with the same letter within a column for each variable are not significantly different at *P* =0.05.