Prevalence and Significance of the F129L mutation in *Alternaria solani* from the United States

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ABSTRACT

Alternaria solani isolates collected over a five year period from 2002 to 2006 were evaluated for the presence of the F129L mutation. Overall, 96.5% of these isolates were determined to have reduced sensitivity to QoI fungicides and/or to contain the F129L mutation. The detection of these isolates in areas where conditions are less conducive for the pathogen and where there is less selection pressure by QoI fungicides suggests that the mutation is stable. Field trials were performed in central North Dakota in 2000 when the *A. solani* population was dominated by sensitive/wild type isolates as well as in 2002 and 2003 when reduced sensitive/F129L mutant isolates dominated. These trials support in vitro and greenhouse results indicating that the F129L mutation has affected the field performance of strobilurin-type QoI fungicides. Overall, field trial results suggest that strobilurin-type fungicides no longer provide improved disease control over standard protectant fungicides such as chlorothalonil and mancozeb.

INTRODUCTION

Early blight, caused by *Alternaria solani,* is the most important foliar disease of potato in the Midwestern USA due to heavy inoculum pressure and favorable conditions for the spread and development of the pathogen. Standard protectant fungicides such as chlorothalonil and mancozeb are generally inadequate alone in controlling this disease, therefore, registration of azoxystrobin was important for improved early blight disease control. Reduced sensitivity to azoxystrobin was documented in isolates of *A. solani* collected from Nebraska in 2000, and became widespread in other Midwestern states in subsequent years (Pasche et al., 2004). Little is known about the prevalence of isolates with reduced sensitivity to QoI fungicides outside the central portions of the USA.

Reduced sensitivity observed in *A. solani* has been attributed to the action of the F129L mutation, the substitution of phenylalanine with leucine at position 129 (Pasche et al., 2004, 2005). The G143A mutation, which is responsible for QoI resistance in many other fungi, has been demonstrated to provide cross-resistance among QoI fungicides (Kim et al., 2003), while the F129L mutation has been shown to have a differential effect on fungal sensitivity to QoI fungicides (Pasche et al. 2004, 2005). The objectives of this research were to determine the prevalence of the F129L mutation among *A. solani* isolates collected from commercial potato fields across the USA from 2002 through 2006 and to determine the effect the F129L mutation on disease control of strobilurin-type QoI fungicides via replicated field trials conducted in 2000, 2002 and 2003.

MATERIALS AND METHODS

Fungicide sensitivity evaluations and detection of the F129L mutation. From 2002 to 2006, leaves with early blight lesions were collected randomly from across the USA. This five year survey included samples from 11 potato producing states (Table 1). Isolations to recover *A. solani* and concomitant culture purification and storage were as previously described (Pasche, et al. 2004). The presumptive presence of the F129L mutation was determined via EC_{50} values generated in vitro and/or by real-time PCR. Both methods were performed as previously described (Pasche et al. 2004, 2005).

Field evaluation of early blight fungicides. Field trials evaluating the efficacy of QoI fungicides were conducted in central North Dakota in 2000, 2002 and 2003. All trials were conducted in fields with overhead sprinkler irrigation. In field trials presented here, QoI fungicides were applied five times during the growing season in alternation with five applications of chlorothalonil. The foliar fungicide trial in 2000 was conducted in the presence of a QoI sensitive/wild type *A. solani* population and field trials conducted in 2002 and 2003 were predominated by the presence of the F129L mutation in the early blight fungus.

Chlorothalonil and mancozeb, at a use rate of 1190 g a.i./ha and 1680 g a.i./ha, respectively were used as standard protectant control treatments in the early blight disease control field trials. Azoxystrobin and pyraclostrobin were applied at 113 and 226 g a.i./ha, while trifloxystrobin was applied at 105 and 140 g a.i./ha. These treatments represent the lowest and highest labeled rate for each fungicide. Only the low rate was applied in 2000. Percentage early blight severity was recorded at approximately seven day intervals from the onset of disease development to the end of the season. Foliar disease severity was used to calculate the area under the disease progress curve (AUDPC) (Shaner and Finney, 1977) and then relative area under the disease progress curve (RAUDPC).

Table 1. Number of and percentage F129L mutant isolates of *Alternaria solani* collected from across the United States from 2002 to 2006.

State[a]	2002[b]		2003		2004		2005		2006		2002-2006	
	Total[c]	% Mutant[d]	Total	% Mutant	Total	% Mutant	Total	% Mutant	Total	% Mutant	Total	% Mutant
Nebraska	240	98.8	247	100.0	758	99.0	46	100.0	9	88.9	1300	99.3
Minnesota	-	-	-	-	767	96.7	136	91.9	309	92.2	1212	95.7
North Dakota	181	100.0	225	91.2	230	98.7	66	97.0	58	62.1	760	93.8
Wisconsin	-	-	-	-	-	-	258	96.1	138	89.1	396	93.7
Michgan	-	-	11	100.0	148	94.1	55	98.2	-	-	214	96.7
Texas	-	-	1	100.0	60	100.0	131	98.5	-	-	192	99.0
Colorado	-	-	9	100.0	-	-	20	100.0	78	91.0	107	93.5
Idaho	-	-	-	-	-	-	26	15.4	-	-	26	15.4
Wyoming	-	-	-	-	-	-	13	23.1	-	-	13	23.1
Oregon	-	-	-	-	-	-	-	-	10	60.0	10	60.0
Washington	-	-	-	-	-	-	8	12.5	-	-	8	12.5
Total	421	99.3	493	95.9	1963	98.4	759	91.4	602	87.9	4238	96.5

[a]State from which isolates were originally collected.

[b]Year isolate was collected.

[c]Total number of isolates examined for a given time period.

[d]Percentage of isolates determined to contain the F129L mutation for a given time period.

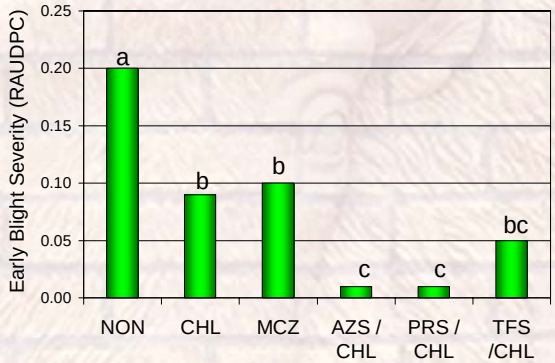

Figure 1. Early blight disease severity, expressed as relative area under the disease progress curve (RAUDPC) from a field trial conducted in central North Dakota in 2000 using cultivar Russet Burbank. A total of ten foliar fungicide applications were performed during the growing season. Treatments included a non-treated control (NON); chlorothalonil (CHL); mancozeb (MCZ); five applications each of azoxystrobin (AZS); trifloxystrobin (TFS); and pyraclostrobin (PRS). All strobilurins were alternated (/) with chlorothalonil. Columns with the same letter are not significantly different according to Fisher's protected least significant difference test ($P < 0.05$).

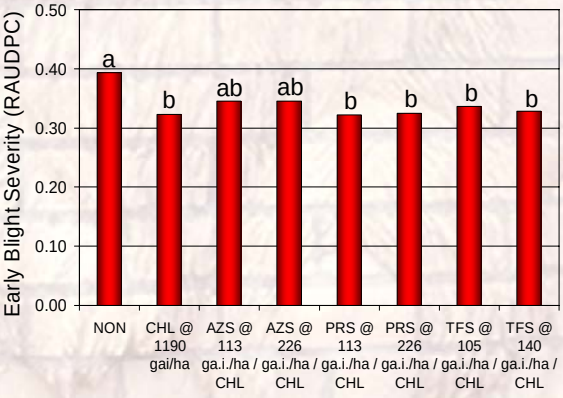

Figure 2. Early blight disease severity, expressed as relative area under the disease progress curve (RAUDPC) from a field trial conducted in central North Dakota in 2002 and 2003 using cultivar Russet Norkotah. Data were combined and represent means of the data set from both years. A total of ten foliar fungicide applications were performed during the growing season. Treatments included a non-treated control (NON); chlorothalonil (CHL); five applications each of azoxystrobin (AZS) at 113 or 226 g a.i./ha; pyraclostrobin (PRS) at 113 or 226 g a.i./ha; and trifloxystrobin (TFS) at 105 or 140 g a.i./ha. All QoI fungicides were alternated (/) with chlorothalonil. Columns with the same letter are not significantly different according to Fisher's protected least significant difference test ($P < 0.05$).

RESULTS AND CONCLUSIONS

Fungicide sensitivity evaluations and detection of the F129L mutation. Between 2002 and 2006, 4238 *A. solani* isolates were collected from 11 major potato producing states across the USA and 96.5% of isolates were determined to have reduced sensitivity to azoxystrobin, dominating the *A. solani* population in potato production areas of the USA (Table 1). In the central portion of the USA the frequency of reduced sensitive/F129L mutant *A. solani* isolates generally ranged from 88-100% in each year of the survey. The exception to this is in North Dakota where only 62% of isolates collected in 2006 were determined to contain the F129L mutation. Among isolates of *A. solani* collected only in 2005 from the Western USA, frequency of the F129L mutant populations were much lower, generally ranging from 12-60%, indicating that the mutation is present in *A. solani* populations with little exposure to QoI fungicides.

Field evaluation of strobilurin-type QoI fungicides. Results from field trials performed in 2000 in central North Dakota, in the presence of a QoI sensitive/wild type *A. solani* population, confirmed that these fungicides represented a class of chemistry that provided control of early blight significantly superior to standard protectant fungicides chlorothalonil and mancozeb ($P < .0001$) (Figure 1). Azoxystrobin and pyraclostrobin provided early blight control superior to that of trifloxystrobin, although not significantly so. Field trials conducted in central North Dakota in 2002 and 2003 demonstrate that early blight disease in plots treated with azoxystrobin, pyraclostrobin and trifloxystrobin were not significantly different from plots treated chlorothalonil and mancozeb alone ($P < .0001$), illustrating that strobilurin-type QoI fungicides no longer provide superior early blight disease control in the field (Figure 2). Furthermore, increasing application rates of the QoI fungicides did not have any affect on the level of disease control provided by these fungicides. In light of these results, new fungicide chemistries are needed to manage *A. solani*.

LITURATURE CITED

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