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Prevalence, competitive fitness and impact of the F129L mutation in *Alternaria solani* from the United States

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Abstract

Reduced sensitivity to quinone outside inhibitor (QoI) fungicides conferred by the presence of the F129L mutation was identified in *Alternaria solani* isolates collected in Nebraska in 2000 and in isolates collected from the Midwestern states of Minnesota and North Dakota in 2001. Over 4200 isolates of *A. solani* collected over a 5-year period from 2002 to 2006 from 11 potato-producing states were evaluated for the presence of the F129L mutation utilizing azoxystrobin spore germination assays and real-time PCR. From this population, 96.5% of isolates were determined to have reduced sensitivity to QoI fungicides and/or to contain the F129L mutation. In addition to Midwestern states of Minnesota, Nebraska and North Dakota, *A. solani* isolates containing the F129L mutation were collected from Colorado, Michigan and Texas in 2003, Wisconsin in 2005, as well as from the Western United States including Idaho, Washington and Wyoming in 2005 and Oregon in 2006. The detection of these isolates in areas outside the Midwest suggests that the F129L mutation is stable and present under conditions that are less conducive for the pathogen and where it is under less selection pressure by QoI fungicides. Competitive fitness tests confirm that, while isolates of *A. solani* with the F129L mutation had a decrease in *in vitro* percentage germination compared with wild-type isolates, F129L mutant isolates produced higher disease severity in greenhouse trials than wild-type isolates. Field trials were performed in central Minnesota and central North Dakota in 2000 and 2001 when the *A. solani* population was dominated by wild-type isolates as well as in 2002, 2003, 2005 and 2006 when F129L mutant isolates dominated. These trials support *in vitro* and greenhouse results indicating that the F129L mutation has affected the field performance of all QoI fungicides. Field trial results suggest that these fungicides do not provide improved disease control over standard protectant fungicides such as chlorothalonil and mancozeb.

1. Introduction

Fungicide resistance, as well as reduced sensitivity, has become increasingly important as more fungicides with single-site modes of action are used for disease control. Resistance development has been documented to varying degrees in virtually every chemical mode of action as described by the Fungicide Resistance Action Committee (FRAC). Field resistance to quinone outside inhibitor (QoI) fungicides was first observed in *Blumeria graminis* f. sp. *tritici* isolates collected in 1998 in northern Germany (Sierotzki et al., 2000b; Chin et al., 2001). These isolates

were determined to have EC_{50} values 200 times greater than baseline isolates and were the predominant phenotype found after only 2 years of QoI use, indicating that resistance was stable and not accompanied by a fitness penalty. Subsequent to this, QoI resistance has been documented in several other fungi including *Mycosphaerella fijiensis* (Chin, 2001), *Plasmopara viticola* (Gisi et al., 2000), *Podoshaera fusca* and *Pseudoperonospora cubensis* (Ishii et al., 2001) and *Pyricularia grisea* (Vincelli, 2002).

Prior to the registration of azoxystrobin on potato in 1999 in the United States, protectant foliar fungicides applied to potatoes were only moderately effective in the control of early blight. Because of this, as well as other factors including heavy inoculum pressure and favorable conditions for the spread and development of the pathogen, registration of

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azoxystrobin was important for improved early blight disease control. Immediately after registration, growers in the Midwestern United States frequently performed four to six applications in a single growing season. Reduced sensitivity to azoxystrobin was documented in isolates of *Alternaria solani* collected from Nebraska in 2000, and became widespread in other Midwestern states in subsequent years (Pasche et al., 2004). The term 'reduced sensitivity' was used rather than resistance or insensitivity because resistance factors were determined to be substantially less in *A. solani* than in other previously reported fungi (Pasche et al., 2004).

While QoI resistance in other fungi is primarily due to the action of the G143A mutation, the substitution of glycine by alanine at amino acid position 143 (Sierotzki et al., 2000a, b; Ishii et al., 2001; Kim et al., 2003), 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 has been demonstrated to provide cross-resistance among QoI fungicides (Heaney et al., 2000; Sierotzki et al., 2000a; Ishii et al., 2001; Kim et al., 2003; Köller et al., 2004), while the F129L mutation has been shown to have a differential effect on fungal sensitivity to QoI fungicides (Vincelli and Dixon, 2002; Kim et al., 2003; Pasche et al., 2004, 2005). Resistance factors in *A. solani* isolates with the F129L mutation were $10–15 \times$ for azoxystrobin and pyraclostrobin but only two- to three-fold for trifloxystrobin, famoxadone and fenamidone compared to wild-type isolates (Pasche, et al., 2004, 2005). Concomitantly, early blight control provided by azoxystrobin and pyraclostrobin decreased significantly as a result of the F129L mutation based on growth chamber studies (Pasche et al., 2004). Early blight control provided by trifloxystrobin, famoxadone and fenamidone was not affected by the presence of the F129L mutation in *A. solani* in similar growth chamber studies (Pasche et al., 2004, 2005).

Little is known about the prevalence of isolates with reduced sensitivity to QoI fungicides outside the central portions of the United States. With the detection of reduced sensitivity in *A. solani*, use of QoI fungicides on potatoes has declined dramatically, despite the registrations of several other fungicides in the same class including pyraclostrobin, tryifloxystrobin, fluoxastrobin, famoxadone and fenamidone.

The stability of fungicide resistance ultimately depends on the relative pathogenic fitness of mutated isolates compared with wild-type isolates. Conflicting results have been reported concerning fitness as a result of QoI insensitivity, depending on the pathogen in question (Genet et al., 2006; Heaney et al., 2000), and have not addressed the F129L mutation. 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 United States, to determine competitive fitness of *A. solani* isolates with and without the F129L mutation, as well as to determine the effect the F129L mutation has on disease control of several early blight fungicides via replicated field trials.

2. Materials and methods

2.1. Collection of Alternaria solani isolates

From 2002 to 2005, samples consisting of 30 leaves/field were collected randomly from potato fields which were selected solely on the presence of foliar early blight. Field sampling was done twice during the growing season, once at the first sign of lesion development and again later near the end of the growing season. In addition to this, from 2003 to 2006 samples submitted from growers and colleagues were also evaluated. This 5-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).

2.2. Fungicide sensitivity evaluations and detection of the F129L mutation

In 2002 and 2003, the presumptive presence of the F129L mutation in most isolates was determined via EC_{50} values generated *in vitro* and performed as previously described (Pasche et al., 2004, 2005). In 2004 and 2005, conidial germination was used for approximately 10% of isolates to confirm the presence of the F129L mutation as determined using real-time PCR (Pasche et al., 2005). All real-time PCR reactions were performed as previously described using a 250-bp fragment of the cytochrome *b* gene of *A. solani* that is amplified using sequence-specific primers AS-5F and AS-5R and probe hybridization analysis of the melting point of the DNA fragments (Pasche et al., 2005). All *A. solani* isolates collected in 2006 were tested only using real-time PCR as the method of determining mutation status.

2.3. Competitive fitness evaluations

Competitive fitness comparing QoI wild-type *A. solani* isolates and those containing the F129L mutation was examined using both *in vitro* and *in vivo* methods. Percentage *in vitro* spore germination of 50 spores in each of two replicates, with each experiment performed twice, was used to compare 45 wild-type and 57 F129L mutant isolates utilizing methods previously described (Pasche et al., 2004). *F*-tests were used to compare the two spore germination trials for each isolate. Results from only those isolates in which the two trials were not statistically different were retained for further analysis ($P = 0.05$).

Inoculations of 10 wild-type *A. solani* isolates and eight isolates containing the F129L mutation were performed on greenhouse-reared tomato plants. The percentage infection of two leaves on each of three plants was determined between 4 and 14 d after inoculation using methods

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
Michigan	–	–	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.

previously described (Pasche et al., 2004, 2005). Each experiment was performed at least twice. Variance homogeneity was compared among isolates in the two groups via Levene's test (Milliken and Johnson, 1992). For both *in vitro* and *in vivo* experiments, analysis of variance (ANOVA) was performed on the combined results using PROC GLM in SAS version 9.1 (Cary, NC) and differences between groups were determined using Fisher's protected least significant difference (LSD) test ($P = 0.05$).

2.4. Field evaluation of early blight fungicides

A total of eight field trials evaluating the efficacy of QoI fungicides were conducted in central Minnesota and in central North Dakota from 2000 through 2003, 2005 and 2006. All trials were conducted in fields with overhead sprinkler irrigation and all agronomic practices performed on the field trials were consistent with those employed in the region. QoI fungicides azoxystrobin, pyraclostrobin, trifloxystrobin, fenamidone and famoxadone were evaluated for efficacy against *A. solani*. A typical foliar fungicide program for early blight control in this two-state region includes 10 applications of fungicide. In field trials presented here, QoI fungicides were applied three to six times during the growing season, depending on disease pressure or approach of the foliar disease program. The QoI fungicides were applied in alternation with chlorothalonil and in some years they were also tank-mixed with mancozeb unless otherwise noted. Foliar fungicide trials in 2000 and 2001 were conducted in the presence of a wild-type *A. solani* population and used to illustrate the comparative disease control obtained through the use of strobilurin-type QoI fungicides and a standard protectant fungicide. Field trials conducted in 2002, 2003, 2005 and 2006 were predominated by the presence of the F129L

mutation in the early blight fungus and are used to illustrate the impact of this mutation on early blight disease control. In these years, natural inoculum was supplemented with inoculations using a combination of ten F129L mutant isolates of *A. solani*.

All foliar disease control programs containing QoI fungicides described below were compared with non-treated controls and standard foliar potato fungicides chlorothalonil and/or mancozeb. Chlorothalonil, at a use rate of 1190 g a.i. ha^{-1}, was used as a standard protectant control treatment in every year in which the early blight disease control field trials were conducted (Table 2). Mancozeb was also used as a standard protectant control treatment, at a use rate of 1680 g a.i. ha^{-1}, in 2000, 2003, 2005 and 2006.

In 2000, the strobilurin-type QoI fungicides azoxystrobin (113 g a.i. ha^{-1}), pyraclostrobin (113 g a.i. ha^{-1}) and trifloxystrobin (105 g a.i. ha^{-1}) (Table 2) were applied alone (no tank-mix partner) five times during the growing season. In 2001, azoxystrobin and pyraclostrobin were evaluated comparing three applications of each QoI, versus five applications, in a 10-fungicide application program. Field trials in 2000 and 2001 were conducted in central North Dakota.

Field trials were conducted in central North Dakota in 2002 and 2003 to determine whether the F129L mutation was rate responsive in the field and to evaluate the impact of fungicide rate on early blight disease control. Azoxystrobin and pyraclostrobin were applied alone at 113 and 226 g a.i. ha^{-1}, while trifloxystrobin was applied alone at 105 and 140 g a.i. ha^{-1} (Table 2). The lowest labelled rate is recommended to control early blight and the highest labelled rate of each fungicide is recommended to control late blight. The carboximide fungicide boscalid was also included in the trial and used at a single rate of

Table 2
Fungicide use rates when applied alone, in a tank mix and/or alternated with another chemical for fungicide programs evaluated from 2000 to 2006

Fungicide	Rate (g a.i. ha^{-1})	Application method[a]	Year					
			2000	2001	2002	2003	2005	2006
Chlorothalonil	1190	Alone	X	X	X	X	X	X
	1190	Alt	X	X	X	X	X	X
	785	TM	–	–	–	–	–	X
Mancozeb	1680	Alone	X	–	–	X	X	X
	1680	Alt	–	–	–	–	–	X
	1260	TM	–	–	–	X	X	–
Azoxystrobin	113	Alone	–	–	–	–	–	X
	113	Alt	X	X	X	X	–	–
	226	Alt	–	–	X	X	–	–
	113	TM	–	–	–	X	–	X
Pyraclostrobin	113	Alt	X	X	X	X	–	–
	226	Alt	–	–	X	X	–	–
Trifloxystrobin	105	Alt	X	–	X	X	–	–
	140	Alt	–	–	X	X	–	–
Boscalid	123	Alt	–	–	X	X	–	X
Famoxadone	105	Alt	–	–	–	X	–	–
	105	TM	–	–	–	X	–	–
	140	TM	–	–	–	X	–	–
Fenamidone	200	Alt	–	–	–	X	–	–
Pyrimethanil	300	TM	–	–	–	–	X	X

[a]Fungicide applications made within a fungicide program including full season applications of a single chemical alone, in alternation (Alt) or tank-mixed (TM).

123 g a.i. ha^{-1} (Table 2). All early blight specialty fungicides were applied five times during the growing season.

Famoxadone and fenamidone were also evaluated in Minnesota and North Dakota in 2003 to determine the impact of the F129L mutation on disease control provided by these non-strobilurin QoI fungicides compared with strobilurin-type QoI fungicides. Fenamidone was applied at a rate of 200 g a.i. ha^{-1} (Table 2). Famoxadone was applied at a rate of 105 g a.i. ha^{-1} tank-mixed with mancozeb (1260 g a.i. ha^{-1}) in the North Dakota trial only. Azoxystrobin (113 g a.i. ha^{-1}) was used for comparison in both trials, while pyraclostrobin (113 g a.i. ha^{-1}) and trifloxystrobin (105 g a.i. ha^{-1}) were used only in the Minnesota field trial. All QoI fungicides were applied five or six times during the growing season in a 10 or 12 application program in North Dakota and Minnesota, respectively (Table 2).

Two non-QoI fungicides recently registered for use on potato were also evaluated for early blight efficacy in central Minnesota. In 2005, the anilino-pyrimidine fungicide pyrimethanil was tank-mixed with mancozeb (1260 g a.i. ha^{-1}) and applied four times. In 2006, pyrimethanil was tank-mixed with chlorothalonil and applied twice in alternation with mancozeb. Azoxystrobin alone, no tank mix or alternation, and two applications of azoxystrobin tank-mixed with chlorothalonil alternated with two applications of boscalid and six applications of chlorothalonil were also included in this trial.

All trials were performed under irrigated conditions as randomized complete block designs with four replications. Treatments were planted to cv. Russet Burbank or Russet Norkotah (in central North Dakota in 2002 and 2003 only) in four row blocks 7.5 m in length from 2000 to 2003 and 9.0 m in length in 2005 and 2006. All fungicides were applied with water volumes of 560 l ha^{-1} at 275 kPa to ensure adequate coverage. Percentage early blight severity was recorded in the center two rows at approximately 7-d intervals beginning between June 16 and July 25 (from 52 to 71 d after planting) depending on location, planting date of the trial and disease pressure. Early blight disease evaluations continued for 8–11 weeks, not surpassing 7 d after the final foliar fungicide application. Foliar disease severity was used to calculate the area under the disease progress curve (AUDPC) as follows (Shaner and Finney, 1977):

$$\text{AUDPC} = \sum_{i=1}^{n}[(W_{i+1} + W_i)/2][t_{i+1} - t_i],$$

where W_i is the percentage foliar disease severity at the ith observation, t_i the time in days at the ith observation and n the total number of observations. The relative area under the disease progress curve (RAUDPC) was calculated for each treatment of the replicated trials from each site-year by dividing AUDPC values by the total area of the graph and analyzed using ANOVA (Proc GLM SAS version 9.1, Cary, NC). Mean RAUDPC values were differentiated using

Fisher's protected LSD test ($P = 0.05$). Levene's test of variance homogeneity was performed on data obtained from field trials conducted in North Dakota in 2002 and 2003 (Milliken and Johnson, 1992).

3. Results

3.1. Frequency of the F129L mutation in A. solani

Between 2002 and 2006, 4238 *A. solani* isolates were collected from infected potato foliage and tubers and evaluated from 11 major potato-producing states across the United States (Table 1). Across these 5 years, 96.5% of isolates were determined to have reduced sensitivity to azoxystrobin, dominating *A. solani* populations in nearly all states encompassed by the survey. In the central portion of the United States, states such as Michigan, Minnesota, Nebraska, North Dakota, Texas and Wisconsin, the frequency of reduced sensitive/F129L mutant *A. solani* isolates generally ranged from 88% to 100% in each year of the survey. The exception to this was in North Dakota, where only 62% of isolates collected in 2006 were determined to contain the F129L mutation. Among isolates of *A. solani* collected in 2005 from the western United States, including Idaho, Oregon, Washington and Wyoming, F129L mutant populations were much lower, generally ranging from 12% to 60%. In Colorado where early blight pressure is typically higher than in other western states, 91–100% of isolates tested were determined to have reduced sensitivity to QoI fungicides, and/or contain the F129L mutation.

Among the 4 years in which conidial germination was utilized to determine azoxystrobin sensitivity (EC_{50} values) for *A. solani* isolates, values were within the range of previously published values for azoxystrobin baseline wild-type and reduced sensitive/mutant isolates (Pasche et al., 2004, 2005). Mean azoxystrobin EC_{50} values for wild type ($0.03 \, \mu g \, ml^{-1}$) and F129L mutant ($0.35 \, \mu g \, ml^{-1}$) are based on the mean of 98 and 1157 isolates, respectively.

3.2. Competitive fitness evaluations

Mean percentage *in vitro* spore germination of wild-type *A. solani* isolates (85.7%) was determined to be significantly higher than that of F129L mutant isolates (77.6%) ($P < 0.0001$). Variances were determined to be homogeneous for *in vivo* experiments, and therefore data were combined for further analysis. Mean percentage disease severity on plants inoculated with wild-type *A. solani* isolates was 13.3, significantly lower than the 16.3% infection produced on plants inoculated with isolates containing the F129L mutation ($P = 0.0007$).

3.3. Field evaluation of QoI fungicides

Results from field trials performed in 2000 in central North Dakota, in the presence of a 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 ($P < 0.0001$) (Fig. 1). Azoxystrobin and pyraclostrobin generally provided early blight control superior to that of trifloxystrobin, as has been reported previously (Stevenson and James, 1999), although differences among the QoI fungicides were not significantly different. However, in a year with low early blight disease pressure as in 2001, QoI fungicides were not significantly better than the standard protectant fungicide chlorothalonil but were significantly better than the non-treated control ($P < 0.0001$). No difference in disease control was observed with three versus five applications of azoxystrobin or pyraclostrobin (Fig. 2).

Data from field trials conducted in North Dakota in 2002 and 2003 to determine whether early blight caused by *A. solani* possessing the F129L mutation could be controlled using higher rates of strobilurin-type QoI fungicides were combined after Levene's test demonstrated that variances were homogeneous. The data demonstrate that early blight disease levels in plots treated with azoxystrobin, pyraclostrobin and trifloxystrobin were not significantly different from those in plots treated with chlorothalonil and mancozeb alone, illustrating that strobilurin-type QoI fungicides no longer provide superior early blight disease control in the field (Fig. 3). Furthermore, disease levels in plots treated with azoxystrobin were not significantly different from disease levels in the non-treated control, and increasing application rates of the QoI fungicides did not have any affect on the level of disease control provided by these fungicides. The only foliar fungicide that provided early blight disease control significantly superior to the standard protectant fungicide
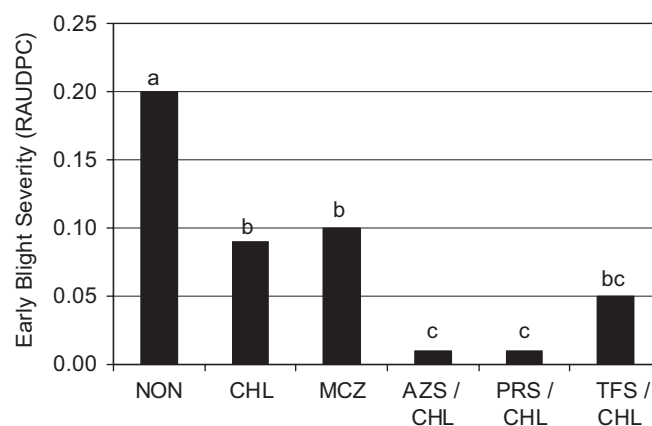

Fig. 1. Early blight severity, expressed as relative area under the disease progress curve (RAUDPC) from a field trial conducted in central North Dakota in 2000 using cultivar Russet Burbank. There was 10 foliar fungicide applications during the growing season. Treatments included a non-treated control (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 ($P < 0.05$).

chlorothalonil was boscalid ($P<0.0001$), which was alternated with chlorothalonil (Fig. 3).

In subsequent trials performed in both North Dakota and Minnesota in 2003, none of the strobilurin-type QoI fungicides registered for use on potato, azoxystrobin, pyraclostrobin and trifloxystrobin provided any substantial increase in disease control over standard protectant fungicides chlorothalonil or mancozeb (Figs. 4 and 5). Non-strobilurin QoI fungicides famoxadone and fenamidone were also examined in these field trials. Disease control provided by the fenamidone, when applied in alternation with chlorothalonil, was comparable to standard fungicides mancozeb or chorothalonil as well as strobilurin-QoI fungicides azoxystrobin, pyraclostrobin
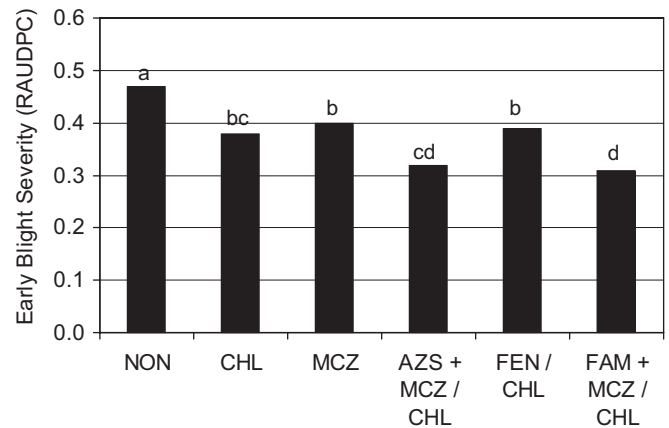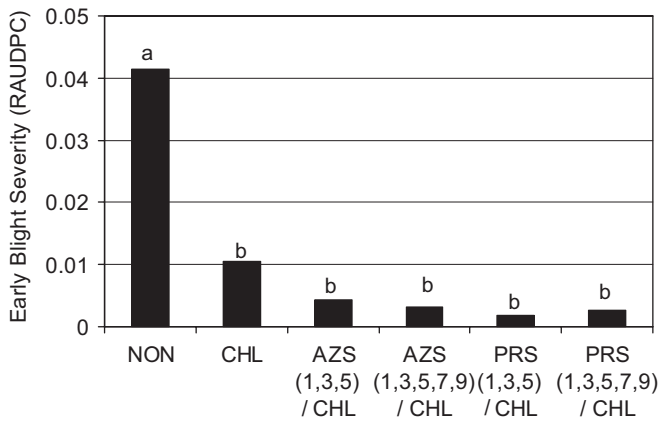

Fig. 2. Early blight severity, expressed as relative area under the disease progress curve (RAUDPC) from a field trial conducted in central North Dakota in 2001 using cultivar Russet Burbank. There were 10 foliar fungicide applications during the growing season. Treatments included a non-treated control (NON); chlorothalonil (CHL); and three (1, 3, 5) or five (1, 3, 5, 7, 9) applications of azoxystrobin (AZS) or pyraclostrobin (PRS) alternated (/) with chlorothalonil. Parenthetic numbers represent the application timing of the QoI fungicides. Columns with the same letter are not significantly different according to Fisher's protected least significant difference test ($P<0.05$).

Fig. 4. Early blight severity, expressed as relative area under the disease progress curve (RAUDPC) from a field trial conducted in central North Dakota in 2003 using cultivar Russet Norkotah. There were 10 foliar fungicide applications during the growing season. Treatments included a non-treated control (NON); chlorothalonil (CHL); mancozeb (MCZ); five applications each of azoxystrobin (AZS) tank-mixed (+) with mancozeb; fenamidone (FEN); and famoxadone (FAM) tank-mixed with mancozeb. 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 ($P<0.05$).
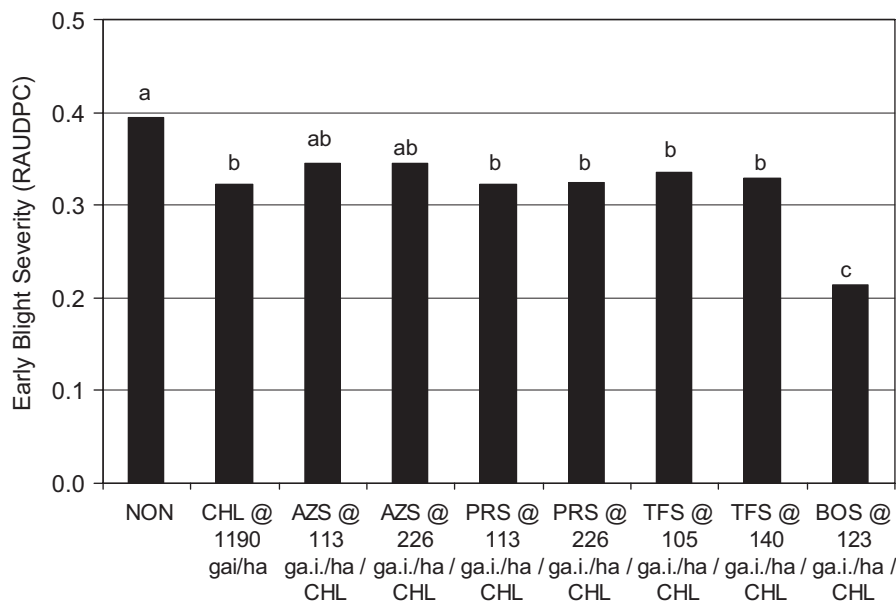

Fig. 3. Early blight severity, expressed as relative area under the disease progress curve (RAUDPC) from field trials conducted in central North Dakota in 2002 and 2003 using cultivar Russet Norkotah. Data were combined and represent means of the data set from both years. There were 10 foliar fungicide applications 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^{-1}; pyraclostrobin (PRS) at 113 or 226 g a.i. ha^{-1}; trifloxystrobin (TFS) at 105 or 140 g a.i. ha^{-1}; and boscalid at 123 g a.i. ha^{-1}. 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 ($P<0.05$).
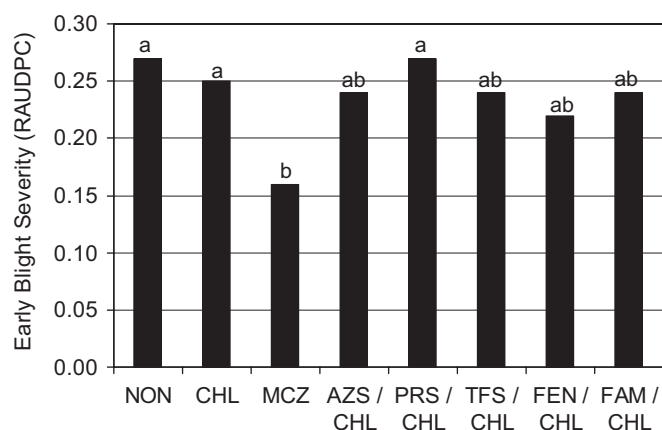
Fig. 5. Early blight severity, expressed as relative area under the disease progress curve (RAUDPC) from a field trial conducted in central Minnesota in 2003 using cultivar Russet Burbank. There were 12 foliar fungicide applications during the growing season. Treatments included a non-treated control (NON); chlorothalonil (CHL); mancozeb (MCZ); six applications each of azoxystrobin (AZS); pyraclostrobin (PRS); trifloxystrobin (TFS); fenamidone (FEN); and famoxadone (FAM). All QoI fungicides were applied in alternation (/) with chlorothalonil. Columns with the same letter are not significantly different according to Fisher's protected least significant difference test ($P < 0.05$).

Fig. 6. Early blight severity, expressed as relative area under the disease progress curve (RAUDPC) from a field trial conducted in central Minnesota in 2005 using cultivar Russet Burbank. There were 11 foliar fungicide applications during the growing season. Treatments included a non-treated control (NON); chlorothalonil (CHL); mancozeb (MCZ); four applications of pyrimethanil (PRM) tank-mixed (+) with mancozeb and alternated (/) with chlorothalonil. Columns with the same letter are not significantly different according to Fisher's protected least significant difference test ($P < 0.05$).

and trifloxystrobin (Figs. 4 and 5). However, famoxadone, when tank-mixed with mancozeb, did significantly improve disease control over chlorothalonil and mancozeb alone in the North Dakota trial ($P < 0.0001$) (Fig. 4) but not in the central Minnesota trial ($P = 0.0065$) (Fig. 5).

Field trials conducted in central Minnesota in 2005 and 2006 demonstrated that the early blight control provided by pyrimethanil, when tank-mixed and alternated with standard protectant fungicides, was not significantly different from the protectants applied alone (Fig. 6 and 7). As in 2003, applications of boscalid significantly improved early blight control when compared with chlorothalonil alone, but were not significantly different from the standard protectant mancozeb (Fig. 7).

4. Discussion

The frequency of the F129L mutation among isolates of *A. solani* collected from across the central portion of the United States appears to have remained stable over the past 6 years since the first detection of reduced sensitivity. There are two likely reasons for this; although QoI fungicides can no longer be considered premium fungicides for the control of early blight in potato, they have been demonstrated to be efficacious against *Colletotrichum coccodes*, the causal agent of black dot (Nitzan et al., 2005). Despite the fact that black dot has traditionally been viewed as a pathogen primarily affecting below-ground potato tissues (Andrivon et al., 1997; Read and Hide, 1995), research indicates that the foliar phase of the disease can contribute to yield reductions (Johnson, 1994) and above-ground stem infection may precede below-ground stem infection (Gudmestad et al., 2007). Foliar applications
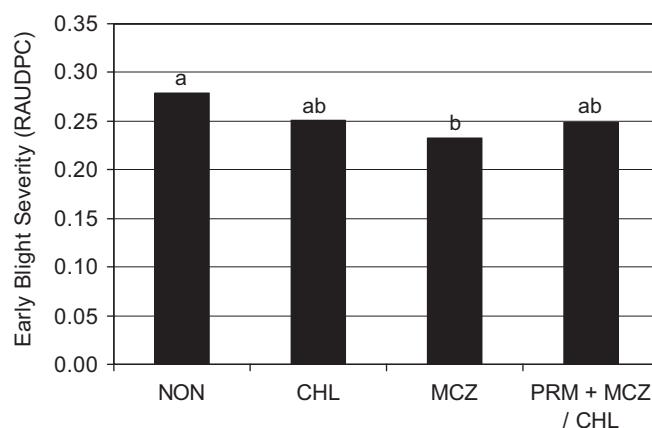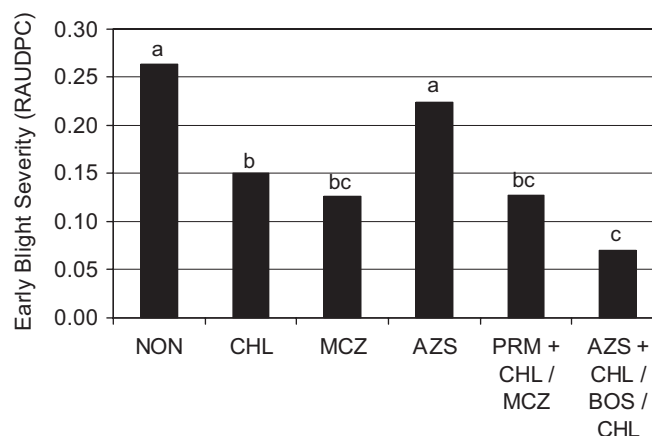

Fig. 7. Early blight severity, expressed as relative area under the disease progress curve (RAUDPC) from a field trial conducted in central Minnesota in 2006 using cultivar Russet Burbank. There were 10 foliar fungicide applications during the growing season. Treatments included a non-treated control (NON); chlorothalonil (CHL); mancozeb (MCZ); azoxystrobin (AZS); two applications of pyrimethanil (PRM) tank-mixed with chlorothalinil and alternated (/) with mancozeb; and two applications of azoxystrobin tank-mixed with chlorothalonil, alternated with two applications of boscalid (BOS) and six applications of chlorothalonil. Columns with the same letter are not significantly different according to Fisher's protected least significant difference test ($P < 0.05$).

of QoI fungicides are used for black dot control in the Midwestern United States (Gudmestad and Pasche, unpublished), and therefore while QoI application frequency has been reduced, selection pressure has not been completely eliminated. The frequency of detection of wild-type isolates did increase slightly in 2005. This can be attributed to the collection of isolates from states in which QoI fungicides have been used sparingly, including

the western states of Idaho, Oregon, Washington, Wyoming and parts of Colorado. It is of interest to note that F129L mutants were detected among the small number of isolates collected from these areas despite very little use of QoI fungicides (J. Miller and D. Johnson, personal communication) compared with Nebraska, North Dakota and Minnesota, where these fungicides have been used near maximum label rates for several years after registration. This suggests that low QoI exposure levels are required to select for F129L mutant isolates and that there is unlikely any significant fitness penalty. Continued research is required to determine the cause of a substantial reduction in frequency of mutant isolates in North Dakota in 2006.

While greenhouse assays are often used to determine competitive fungal fitness, the use of *in vitro* methods including growth rate, sporulation and spore germination to determine fitness has been utilized in several fungi exhibiting fungicide resistance (Malandrakis et al., 2006; Avila-Adame and Köller, 2003; Karaoglanidis et al., 2001). *A. solani* isolates containing the F129L mutation retained similar pathogenicity as measured by greenhouse evaluations, but spore germination was reduced compared with wild-type isolates. These results are similar to those obtained for other fungi resistant to QoI fungicides. In some laboratory-mutated isolates of *Cercospora beticola*, where resistance was conveyed by amino acid substitutions G143S or F129V, a fitness penalty was detected in *in vitro* parameters of growth rate and sporulation capacity (Malandrakis et al., 2006). Additionally, while all mutant isolates could infect sugar beet, they were determined to be less aggressive *in vivo* than wild-type parent isolates. Isolates of *Magnaporthe grisea* containing the G143A or G143S mutation were determined as having no fitness penalty as measured by either *in vitro* colony size or conidia formation (Avila-Adame and Köller, 2003). In contrast, lesion and conidia development on barley leaves of G143A mutants were similar to the wild type, while in G143S mutants studied, these parameters were significantly lower. In G143A mutants of *P. viticola*, *in vivo* experiments revealed that fungicide sensitivity returned when selection pressure was removed (Heaney et al., 2000; Genet et al., 2006) while this was not the case when similar experiments were conducted with G143A mutants of *Erysiphe graminis* (Heaney et al., 2000). This is the first report of a fitness penalty for a fungus exhibiting QoI resistance in field-derived isolates due to the F129L mutation. It is likely, however, that an increase in disease severity caused by F129L mutants of *A. solani* would more than compensate for a reduction in spore germination capacity.

Extensive field studies reported here on early blight control have demonstrated that the level of control provided by strobilurin-type QoI fungicides in the presence of an *A. solani* population dominated by the F129L mutation was not significantly different from control provided by chlorothalonil or mancozeb. Furthermore, it is apparent that *A. solani* isolates with the F129L mutation are not rate responsive to this chemistry. Since QoI

fungicides represent a premium-priced option for disease control relative to mancozeb or chlorothalonil, fungicides with this mode of action should not be recommended for early blight management in areas where the F129L mutation has been detected. The QoI fungicides were developed by agrochemical companies for different markets with a clear dichotomy as to the targeted pathogens. The strobilurin-type QoIs such as azoxystrobin, pyraclostrobin and trifloxystrobin were developed primarily as early blight fungicides. Although each of these fungicides has activity on the late blight pathogen, *Phytophthora infestans*, that activity was not generally regarded as being sufficiently better than mancozeb or chorothalonil alone to justify their expense. In contrast, the non-strobilurin QoI fungicides famoxadone and fenamidone were developed primarily as late blight fungicides but also have some activity against the early blight fungus. Much of the data from early development phases of famoxadone and fenamidone are from trials conducted primarily for late blight control. The evaluation process in field trials changed significantly following the development of reduced sensitivity to QoIs due to the F129L mutation and our discovery of its differential effect on this class of fungicide (Pasche, et al., 2004, 2005). Early blight foliar fungicide trials conducted elsewhere generally agree with those reported here (Stevenson and James, 2003, 2004, 2005, 2006).

In early studies on reduced sensitivity of *A. solani* to QoI fungicides (Pasche et al., 2004, 2005), it was demonstrated that the F129L mutation had a differential effect on this class of chemistry. *In vitro* EC_{50} values and disease control in growth chamber studies demonstrated that azoxystrobin and pyraclostrobin were affected more by the F129L mutation than were trifloxystrobin, famoxadone and fenamidone. However, these data did not reflect the full impact of the mutation on the control of *A. solani*. Although trifloxystrobin, famoxadone and fenamidone were not affected by the F129L mutation to the same degree as azoxystrobin and pyraclostrobin, they were never as efficacious as the strobilurin-type QoIs on a sensitive *A. solani* population, lacking the intrinsic activity of azoxystrobin and pyraclostrobin (Pasche et al., 2004, 2005). The net effect is that the F129L mutation has rendered all QoI fungicides equal in efficacy to that of protectant fungicides such as chlorothalonil and mancozeb.

Contrasting results were observed with the non-QoI fungicides boscalid and pyrimethanil. While pyrimethanil did not improve early blight disease control over standard or QoI fungicides, boscalid proved to be the most efficient fungicide currently available to the potato industry for early blight disease control. Regardless of timing and number of applications, the inclusion of boscalid in an early blight foliar disease management program generally provides the highest level of disease control.

Early blight continues to be an extremely important foliar disease in some potato production areas of the United States; in most years it is more important than late

blight. The direct result is that potato growers in the central portion of the country use a base program of mancozeb or chlorothalonil alternated with fungicides that are effective against *A. solani*. Unfortunately, due to the presence of the F129L mutation two of the most highly efficient fungicides, azoxystrobin and pyraclostrobin, have been rendered less effective in early blight disease control. However, QoI fungicides appear to be an effective early blight management strategy when used in a program approach with other premium early blight fungicides such as boscalid and pyrimethanil and alternated with standard protectants such as mancozeb and chlorothalonil (Pasche and Gudmestad, unpublished). Nonetheless, it is clear from the research reported here that the potato industry in the USA would benefit from additional foliar fungicides, in addition to boscalid, with efficacy against *A. solani*.

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