Plant Pathology at the University of Wisconsin-Madison

Investigating fungicide resistance in potato early blight complex pathogens of Wisconsin

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Introduction

The potato early blight complex (EBC) refers to the combined disease of early blight caused by Alternaria solani and brown spot caused by A. alternata. Foliar necrotic lesions and chlorosis are common in Wisconsin and are usually first observed in early July and persist to harvest (Fig. 1). Prodominant commercial potato varieties are susceptible, and control is typically achieved with fungicides. Strobilurins or QoI fungicides have been used for about 10 years [1], posing significant single-site-mode of action selective pressure on the pathogens leading to resistance mutations. Amino acid substitutions caused by single nucleotide mutations can lead to partial or complete fungicide resistance. Most commonly detected substitutions are the F129L and the F129A, which may lead to partial QoI resistance, was detected in 96% of the samples, including all isolates from Hancock and Grand Marsh and 90% of isolates from Plover. The spiral plating assay showed that 85% of the isolates were resistant to azoxystrobin (Table 3). There was no significant difference in the resistance detection based on the two methods (p=0.7418).

Mutation in A. solani

Due to the intron between aa129 and aa143 in A. solani, each locus was monitored separately. No G143A substitutions, which may cause complete resistance to QoI, were detected. F129A, which may lead to partial QoI resistance, was detected in 96% of the samples, including all isolates from Hancock

Results

Mutations in A. alternata

For the 30 isolates, no F129L was detected. The G143A mutation was detected in 73% of the samples, including all isolates from Plover and Grand Marsh, and 2 isolates from Hancock. 80% of A. alternata isolates were detected with azoxystrobin resistance on spiral plates (Table 2). There was no significant difference in the resistance detection based on the two methods (p=0.5418).

Mutation in A. solani

Due to the intron between aa129 and aa143 in A. solani, each locus was monitored separately. No G143A substitutions, which may cause complete resistance to QoI, were detected. F129A, which may lead to partial QoI resistance, was detected in 96% of the samples, including all isolates from Hancock

Materials & Methods

Sample collection, isolation, culturing, and identification

Symptomatic potato foliar samples were randomly collected from commercial and research fields in three locations (Hancock, Plover, and Grand Marsh) in mid-August, 2012 (Fig. 1). Single conidium isolations were made on water agar and clarified V8 agar (both adjusted with ampicillin and rifampicin). All cultures were incubated under ambient light at 24°C. Foliar necrotic lesions and chlorosis are common in Wisconsin and are usually first observed in early July

Table 2. Alternaria alternata isolates tested with sampling locations and ratio of QoI resistant isolates.

<table>
<thead>
<tr>
<th>Locations</th>
<th>F129L (partial resistance)</th>
<th>G143A (complete resistance)</th>
<th>Wildtype</th>
<th>Total</th>
<th>Ratio of fungicide resistant isolates</th>
<th>Total resistance on media</th>
<th>Ratio of resistance on media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plover</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>100%</td>
<td>8</td>
<td>80%</td>
</tr>
<tr>
<td>Hancock</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>10</td>
<td>100%</td>
<td>6</td>
<td>60%</td>
</tr>
<tr>
<td>Grand Marsh</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>100%</td>
<td>4</td>
<td>80%</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>28</td>
<td>1</td>
<td>31</td>
<td>100%</td>
<td>18</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 3. Alternaria solani isolates tested with sampling locations and ratio of QoI resistant isolates.

<table>
<thead>
<tr>
<th>Locations</th>
<th>TTG</th>
<th>CTC</th>
<th>TTA</th>
<th>G143A</th>
<th>Wildtype</th>
<th>Total</th>
<th>Ratio of fungicide resistant isolates</th>
<th>Total resistance on media</th>
<th>Ratio of resistance on media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plover</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>90%</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>Hancock</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>100%</td>
<td>7</td>
<td></td>
<td>7</td>
<td>70%</td>
</tr>
<tr>
<td>Grand Marsh</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>50%</td>
<td>3</td>
<td></td>
<td>3</td>
<td>50%</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>27%</td>
<td>23</td>
<td></td>
<td>23</td>
<td>85%</td>
</tr>
</tbody>
</table>

Conclusion

1. The newly designed primers were successful in amplifying the expected genetic regions corresponding to QoI fungicide resistance, which was supported by the spiral plate assay. This PCR-based method provided an efficient way to process a large number of samples to profile QoI resistance of EBC pathogens in a location of interest.
2. The same type of fungicide resistance tended to cluster in one field, suggesting that once a certain mutation type developed and was selected for, large numbers of conidia were produced and may spread throughout the field.
3. For the three locations, most A. alternata isolates showed complete QoI resistance and most A. solani isolates showed partial resistance, which may be due to the structure difference between their genomic DNAs. The relationship between fungicide resistance that may be apparent in the production field and resistance that we may detect in cultures of a relatively small percentage of the pathogen population is not well understood at this time, but the sub-population characterization may be representative of the likely field response to specific fungicide use.

Acknowledgements

We would like to thank the WI Potato & Vegetable Growers Association, the UW-Madison Department of Plant Pathology, and the USDA Hatch for funding. In addition, we thank our grant sponsors for allowing us access to their farms for sample collection, and we acknowledge the laboratory support of Scott Donovan and Alex Phillips in sample collection and pathogen isolation.

Literature Cited


Figure 1. Foliar symptoms and causal agents of the EBC. A) Conidia of A. alternata. B) Foliar symptoms (brown spot) caused by A. alternata. C) Conidium of A. solani. D) Foliar symptoms of early blight; and E) A close up look at the typical “bull’s eye” lesion caused by A. solani.

Figure 2. Locations of pathogen sampling in Wisconsin.

Figure 3. Examples of spiral plate assays showing A. solani isolates with resistance and sensitivity to azoxystrobin.