Characterization of *Alternaria* spp. associated to potato crops in Chile

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Potato crop in Chile

Area: 53,485 ha
Yield: 24 t/ha
Production: 1,142,152 tn
60% small scale farmers
Introduction: Early blight
Introduction

- Fungicides commonly used in Chile to control Early blight: difenoconazole, boscalid and strobilurin.

- Difenoconazole: prevents the development of the fungus by inhibiting cell membrane ergosterol biosynthesis.

- Boscalid: inhibiting mitochondrial respiration by binding succinate dehydrogenase (SDH).

- Strobilurin: (QoI, quinone outside inhibitor). They inhibit mitochondrial respiration in fungi by binding to the QoI site of the cytochrome b complex, blocking electron transfer and inhibiting ATP synthesis.

- Reduced early blight control was first observed in 2000 in the USA, where inadequate control by azoxystrobin was caused by a shift in fungicide sensitivity of *A. solani*. A few years later the same situation was observed in Germany.
Azoxistrobin sensitivity in *A. solani* – United States.

Table 3. Mean in vitro concentration that effectively reduces germination by 50% relative to the untreated control (EC$_{50}$ values; µg/ml) of 25 sensitive and 26 reduced-sensitive *Alternaria solani* isolates for four respiratory inhibiting fungicides$^a$

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Sensitive</th>
<th></th>
<th>Reduced-sensitive</th>
<th></th>
<th></th>
<th>LSD (P &lt; 0.0001)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>EC$_{50}$</td>
<td>SD</td>
<td>EC$_{50}$</td>
<td>SD</td>
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<tr>
<td>Azoxistrobin</td>
<td>0.0324</td>
<td>0.0096</td>
<td>0.3788</td>
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<tr>
<td>Famoxadone</td>
<td>0.0168</td>
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<td>Fenamidone</td>
<td>0.3003</td>
<td>0.0856</td>
<td>0.8439</td>
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<tr>
<td>Bосcalid</td>
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<td>0.6330</td>
<td>0.3175</td>
<td>0.1413</td>
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<tr>
<td>LSD (P &lt; 0.0001)</td>
<td>0.1051</td>
<td>...</td>
<td>0.1151</td>
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$^a$ Sensitivity or reduced-sensitivity to azoxistrobin (23); SD = standard deviation; LSD = least significant difference.
Azoxistrobin sensitivity in *A. solani* – Germany

**Figure 2** In vitro azoxistrobin sensitivity assay of *Alternaria solani* wildtype and F129L isolates collected between 2006 and 2011. Columns represent mean EC50 values, i.e. the effective fungicide concentration that inhibited spore germination by 50%. Bars represent standard deviations. Columns with the same letter are not significantly different (Tukey’s b test, \( P = 0.05 \)).

AZ registration: 2007

(Leiminger *et al.*, 2014)
The main mechanism of resistance to QoI has been identified as mutations in the mitochondrial gene, *cytb*.

In *A. solani*, only the F129L amino acid substitution of phenylalanine (F) to leucine (L) at position 129 has been observed (Pasche *et al.*, 2004).

Sequence analysis revealed the occurrence of two structurally different *cytb* genes:
- Genotype I: Intron present
- Genotype II: Intron absence

(Leiminger *et al.*, 2014).
Objetives

- To identify and characterize *Alternaria* spp associated to potato crop in Chile.
- To assess the *in vitro* sensitivity of *A. solani* to QoI fungicides and its relation with F129L substitution.
Methodology

Survey and morphological characterization

Field collection of potato leaves with early blight symptoms from commercial crops in the southern Chile

Single - conidial isolates on PCA

Colony morphology, sporulation patterns and conidial size using taxonomic keys
Methodology

To confirm identity of the isolates molecular tools were used:

1. **Single conidia isolate**
2. **DNA extraction**
3. **PCR with primers ITS5-ITS4 (White *et al.*, 1990). Fragment were excised from the gel**
4. **Alignment**
5. **Sequencing**
In vitro fungicide sensitivity assays of A. solani

a. Sensitivity to azoxystrobin, pyraclostrobin and boscalid: spore germination

Conidia were washed

Conidial suspension was added to water agar amended with each fungicide

0 – 0.01 – 0.1 – 1 – 10 ppm

Conidial germination was determined after 16 hrs of incubation under light.

b. Sensitivity to boscalid and difenoconazole: micelial growth

Culture on PCA

Mycelial growth assessed after 10 days of incubation on 10% PDA amended media with 0 – 0.01 – 0.1 – 1 – 10 ppm of the fungicide
Cultures *A. solani*  

**DNA extraction**  

PCR amplification and sequencing of a cytochrome b fragment for the detection of the F129L mutation


**Sequencing fragment PCR and bioinformatic analysis**
Results

*Alternaria* spp. identification

- *A. solani*
- *A. alternata*
- *A. arborescens*
- *A. tenuissima*
- *A. infectoria*

Images of spores and mycelium for each species.
Results

Phylogenetic tree based on alignment of Alternaria species including ITS sequencing data. The tree was carried out using MEGA software.

A. infectoria

A. solani

Small – spore isolates (A. alternata, A. arborescens and A. tenuissima)

E. pedicillatum
**Results**

*In vitro* fungicide sensitivity assays of *A. solani*

Mean EC$_{50}$ values for *A. solani* isolates obtained from the *in vitro* sensitivity assessment of azoxystrobin, boscalid, difenoconazole and pyraclostrobin.
Results

Ocurrence of genotype I and II of *A. solani* isolates collected in 2013 and 2016 and the presence of F129L mutation

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<th>Genotype II</th>
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F129L TTC → CTC, TTA, TTG

NDSU-F129L: US reference strain (Genotype II F129L)

The F129L mutation was not detected in this population.
Conclusions

• Five Alternaria spp were associated with early blight symptoms in the potato crop in Chile (A. alternata, A. arborescens, A. tenuissima, A. infectoria and A. solani).

• All isolates of A. solani were highly sensitive to azoxystrobin, pyraclostrobin, difenoconazole and boscalid in vitro studies.

• F129L mutation was not detected in this population.

• This information is preliminary and could constitutes the “baseline” for monitoring changes in population sensitivity to QoI fungicides.
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