# Characterization of *Alternaria* spp. associate to potato crops in Chile

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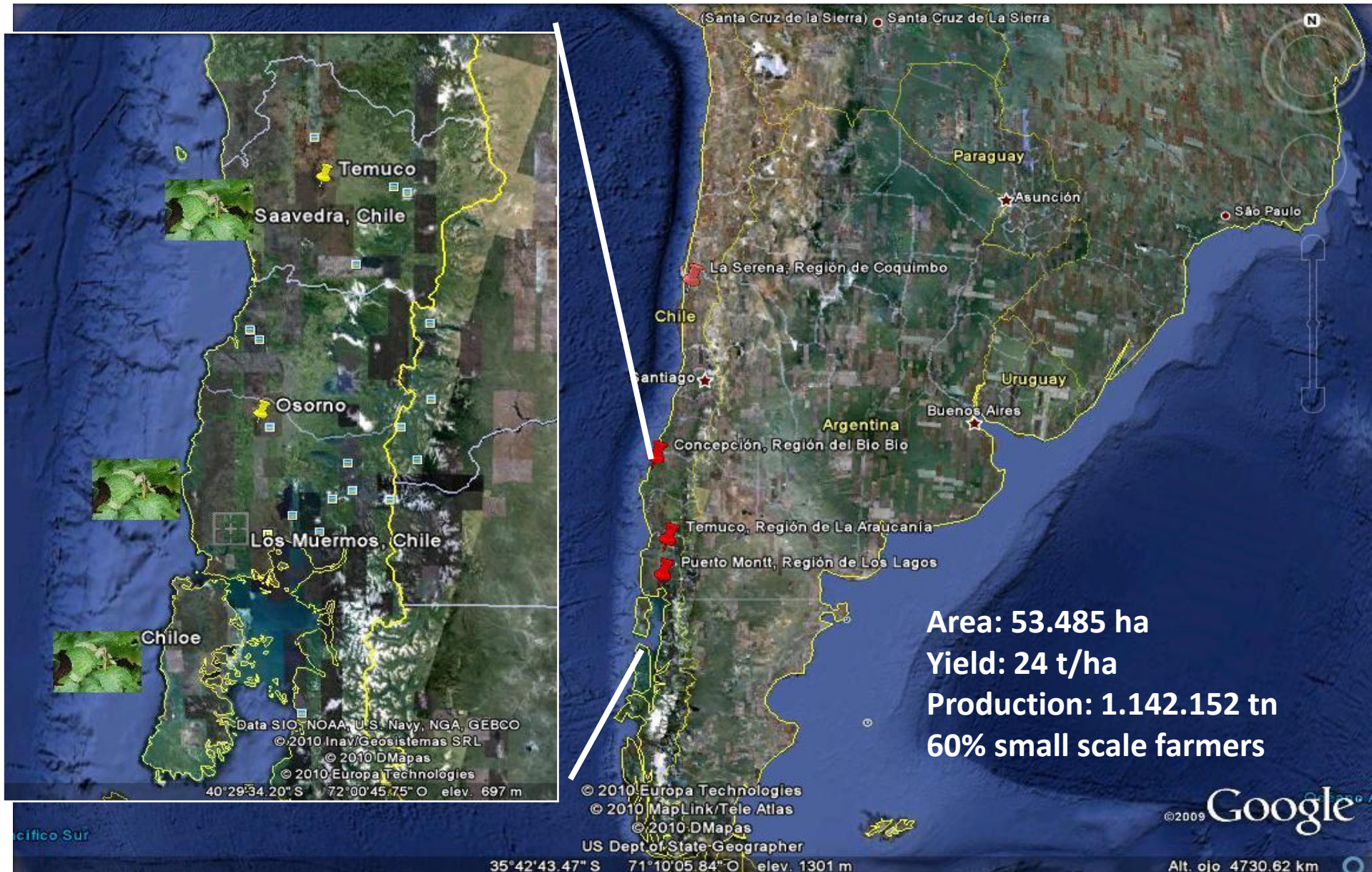
# Potato crop in Chile




**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 mitocondrial 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.

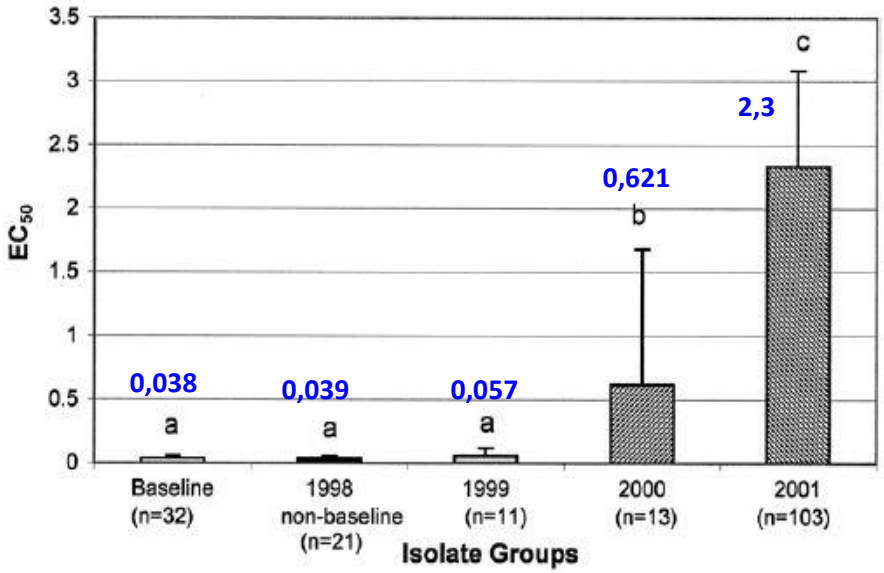



**Fig. 1.** Mean $EC_{50}$ values (effective fungicide concentration that inhibited spore germination by 50%) for *Alternaria solani* isolate groups obtained from the in vitro azoxystrobin assessment with mean separation based upon the least significant difference (LSD). Columns with the same letter are not significantly different ($P = 0.05$). Vertical bars indicate standard deviation for all tests performed on each isolate group.

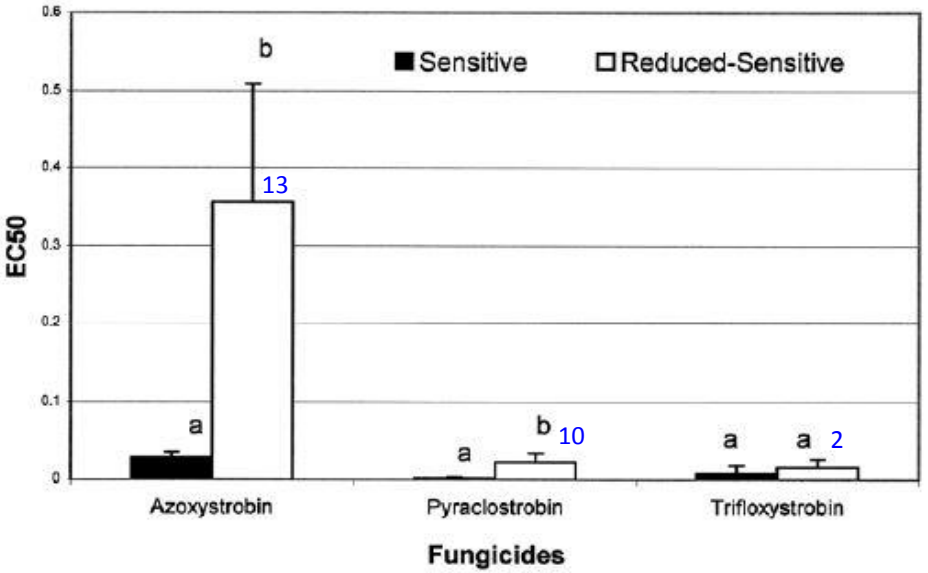



**Fig. 2.** Mean $EC_{50}$ values (effective fungicide concentration that inhibited spore germination by 50%) for sensitive and reduced-sensitive *Alternaria solani* isolates obtained from the in vitro cross-sensitivity assessment of azoxystrobin, pyraclostrobin, and trifloxystrobin. Mean separation provided by Student's *t* tests ($P = 0.05$). Within fungicides, columns with the same letter are not significantly different. Vertical bars indicate standard deviation for all tests performed on each isolate group.

**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]

| | Sensitive | | Reduced-sensitive | | |
|---|---|---|---|---|---|
| **Fungicide** | **$EC_{50}$** | **SD** | **$EC_{50}$** | **SD** | **LSD ($P < 0.0001$)** |
| Azoxystrobin | 0.0324 | 0.0096 | 0.3788 | 0.1458 | 0.0288 |
| Famoxadone | 0.0168 | 0.0113 | 0.0355 | 0.0169 | 0.0057 |
| Fenamidone | 0.3003 | 0.0856 | 0.8439 | 0.6678 | 0.1889 |
| Boscalid | 0.6878 | 0.6330 | 0.3175 | 0.1413 | 0.1786 |
| LSD ($P < 0.0001$) | 0.1051 | … | 0.1151 | … | … |

[a] Sensitivity or reduced-sensitivity to azoxystrobin (23); SD = standard deviation; LSD = least significant difference.

**AZ registration 1998**

(Pasche *et al.*, 2004)
(Pasche et al., 2005)

# Azoxistrobin sensitivity in *A. solani* – Germany

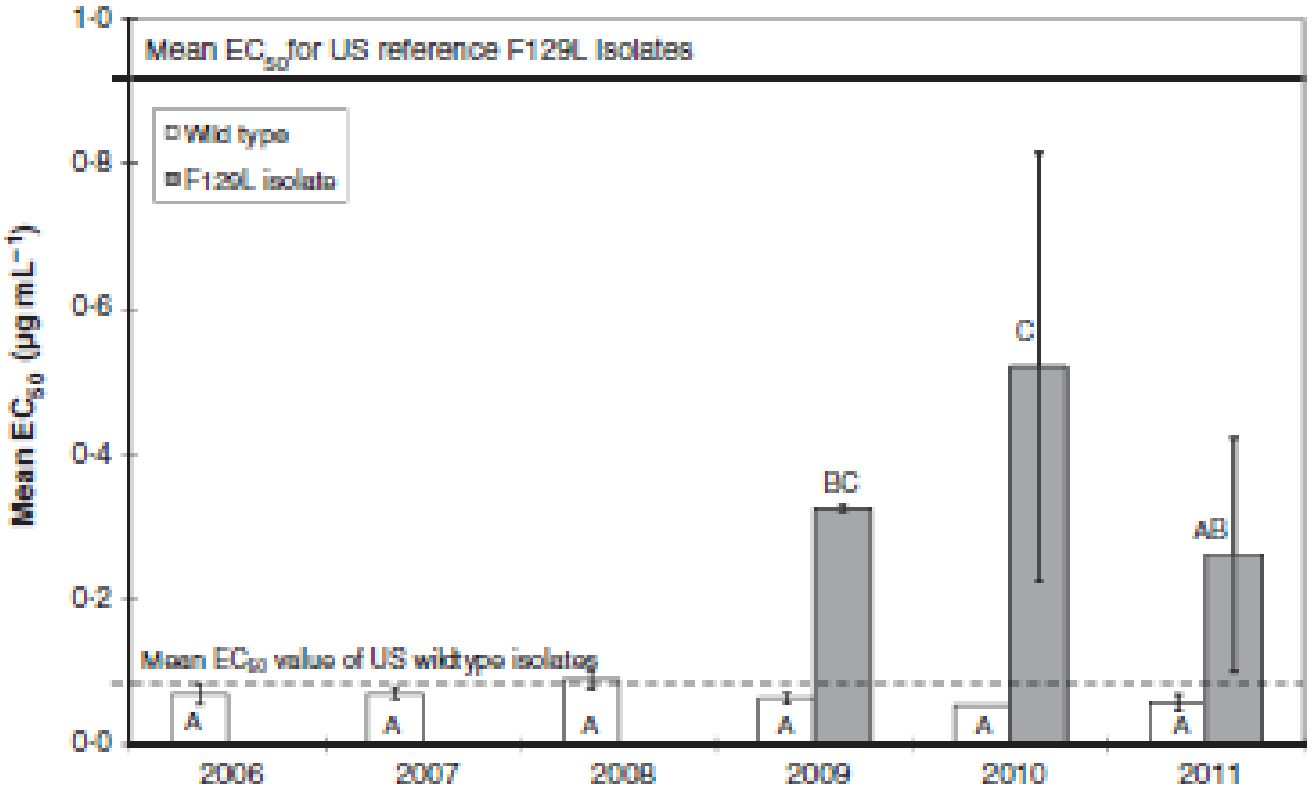



**Figure 2** *In vitro* azoxystrobin sensitivity assay of *Alternaria solani* wildtype and F129L isolates collected between 2006 and 2011. Columns represent mean $EC_{50}$ values, i.e. the effective fungicide concentration that inhibited spore germination by 50%. Bars represent standard deviations. Columns 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 mitocondrial 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).**

**Phenylalanine (TTC)** → **Leucine (TTA, CTC, TTG)**

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

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

Table 3 Occurrence of genotype I and II among German *Alternaria solani* isolates between 2005 and 2011 and their association with F129L mutation

| | | Wildtype | | F129L | |
|---|---|---|---|---|---|
| Year | Total number of isolates | Genotype I | Genotype II | Genotype I | Genotype II |
| 2005 | 6 | 6 | | | |
| 2006 | 24 | 23 | 1 | | |
| 2007 | 20 | 20 | | | |
| 2008 | 5 | 5 | | | |
| 2009 | 39 | 37 | | | 2 |
| 2010 | 10 | 8 | | | 2 |
| 2011 | 99 | 23 | 1 | | 70 |
| Total | 203 | 127 | 2 | | 74 |

# Objetives

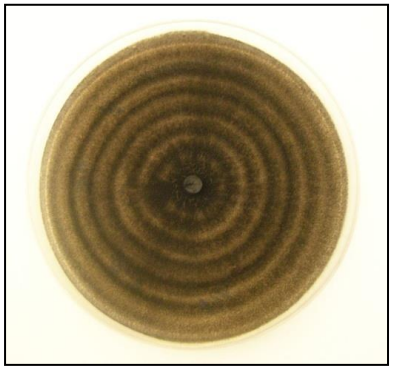
- To identify and characterize *Alternaria* spp associated to potato crop in Chile.
- To assess the *in vitro* sensitivity of *A. solani* to QoI fungicides and its relation with F129L substitution.

# Methodology

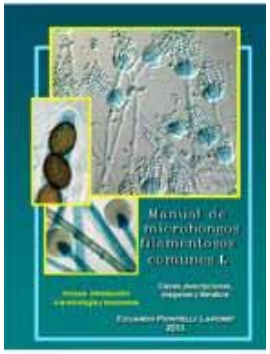
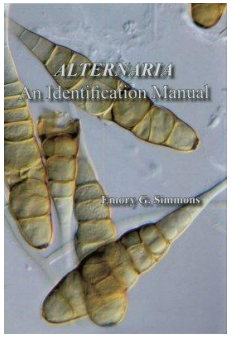
## Survey and morphological characterization

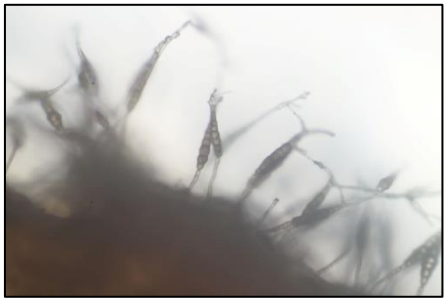


Field collection of potato leaves with early blight symptoms from commercial crops in the southern Chile



Single - conidial isolates on PCA

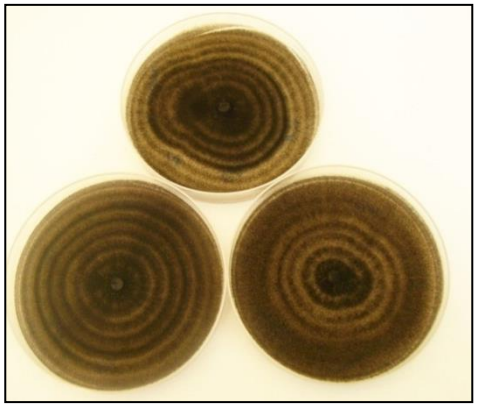





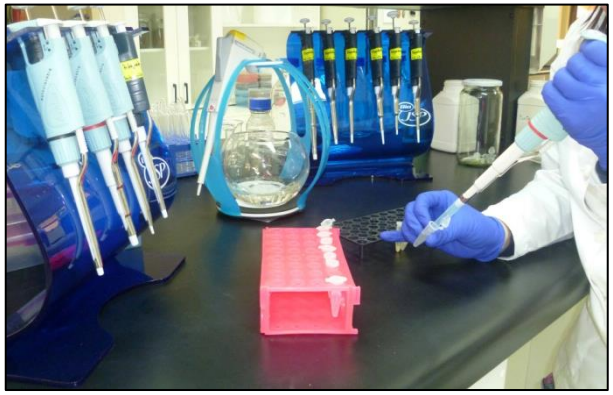
Colony morphology, sporulation patterns and conidial size using taxonomic keys

# Methodology

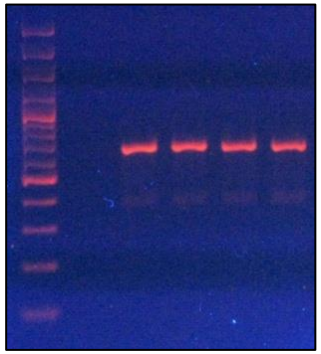
## To confirm identity of the isolates molecular tools were used



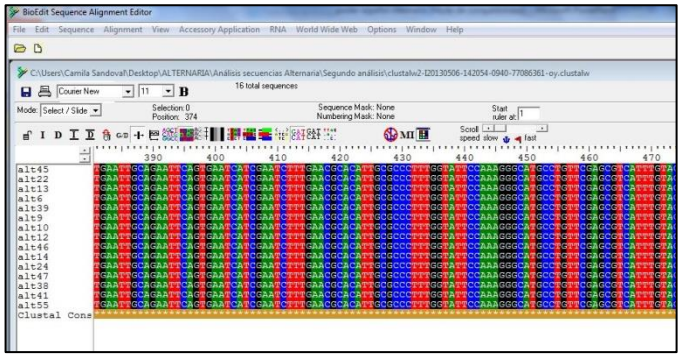
Single conidia isolate



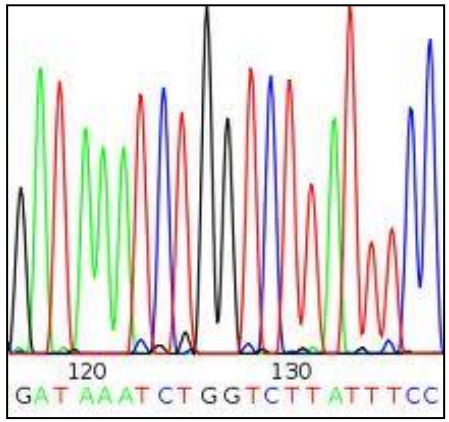
DNA extraction



PCR with primers ITS5-ITS4 (White *et al.*, 1990). Fragment were excised from the gel




Alignment

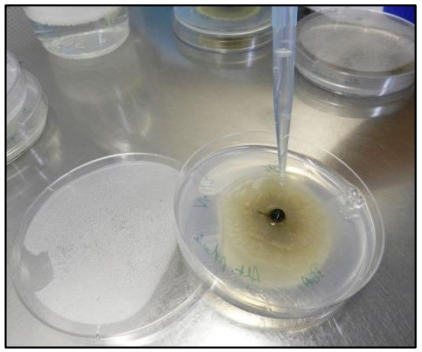



Sequencing

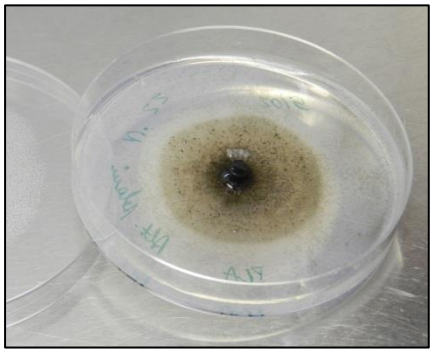
# Methodology

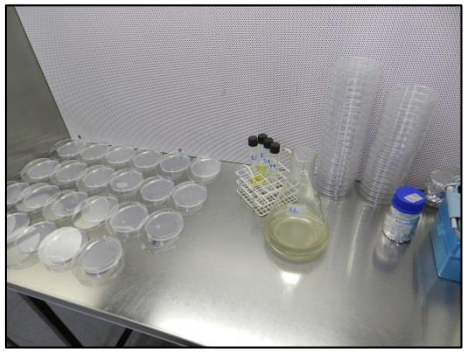
## *In vitro* fungicide sensitivity assays of *A. solani*

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



Conidia were washed



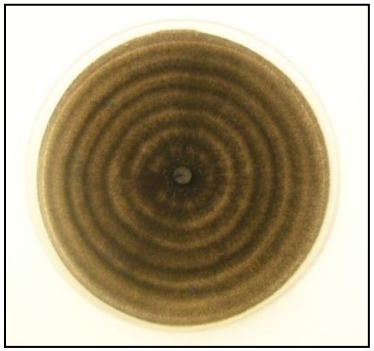


Conidial suspension was added to water agar amended with each fungicide

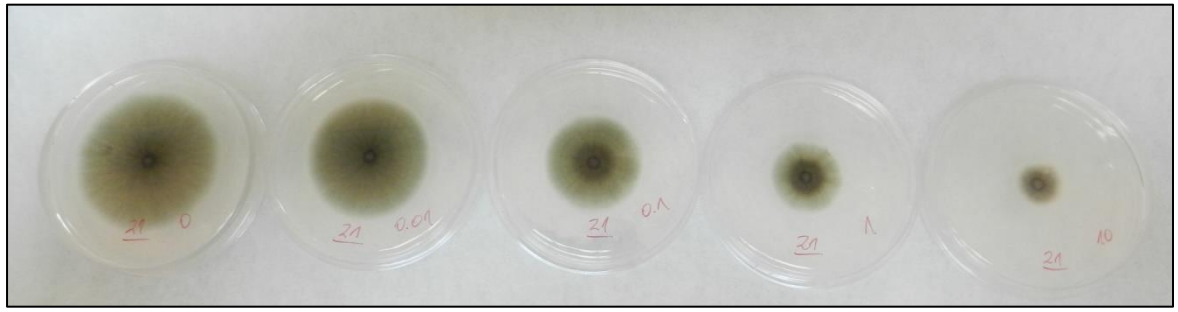
0 – 0.01 – 0.1 – 1 – 10 ppm

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

### b. Sensitivity to boscalid and difenoconazole: micelial growth



Culture on PCA

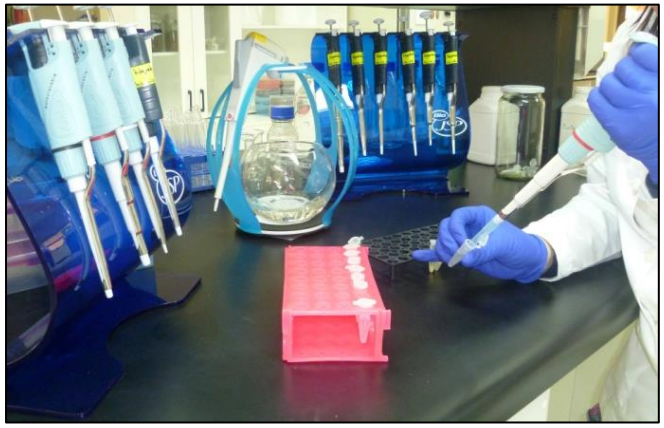


Mycelial growth assessed after 10 days of incubation on 10% PDA amended media with 0 – 0.01 – 0.1 – 1 – 10 ppm of the fungicide

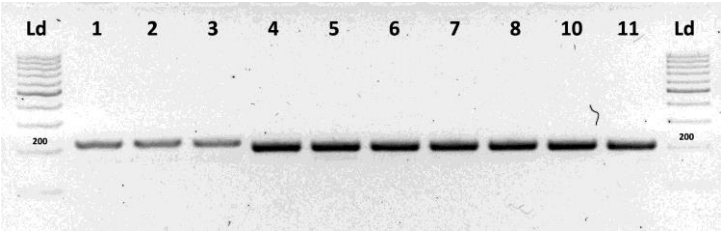
# Methodology

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

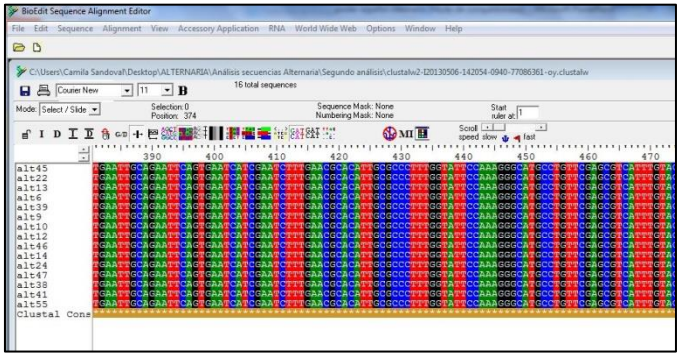
Cultures *A. solani*

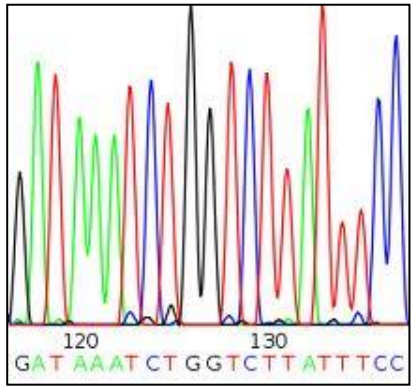


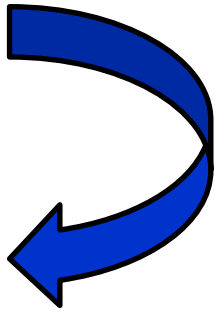
DNA extraction




PCR using specific primers for genotype I and genotype II described by Pasche *et al.*, 2005 and Leiminger *et al.*, 2014.



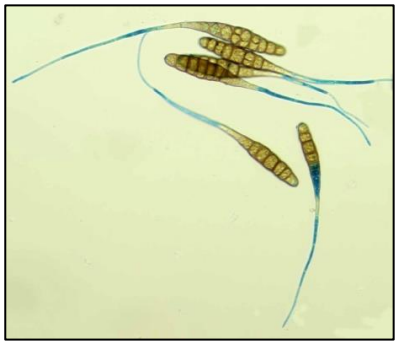



Sequencing fragment PCR and bioinformatic analysis

# Results

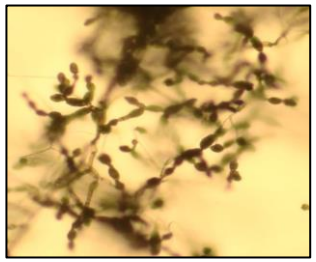
## *Alternaria* spp. identification



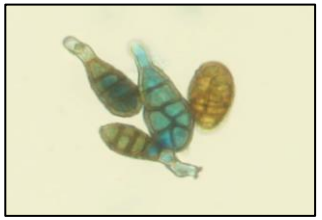
*A. solani*

Large spore

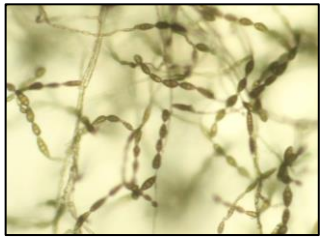
*A. alternata*

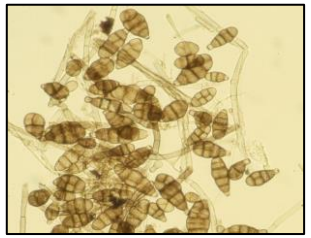


*A. arborescens*



*A. tenuissima*

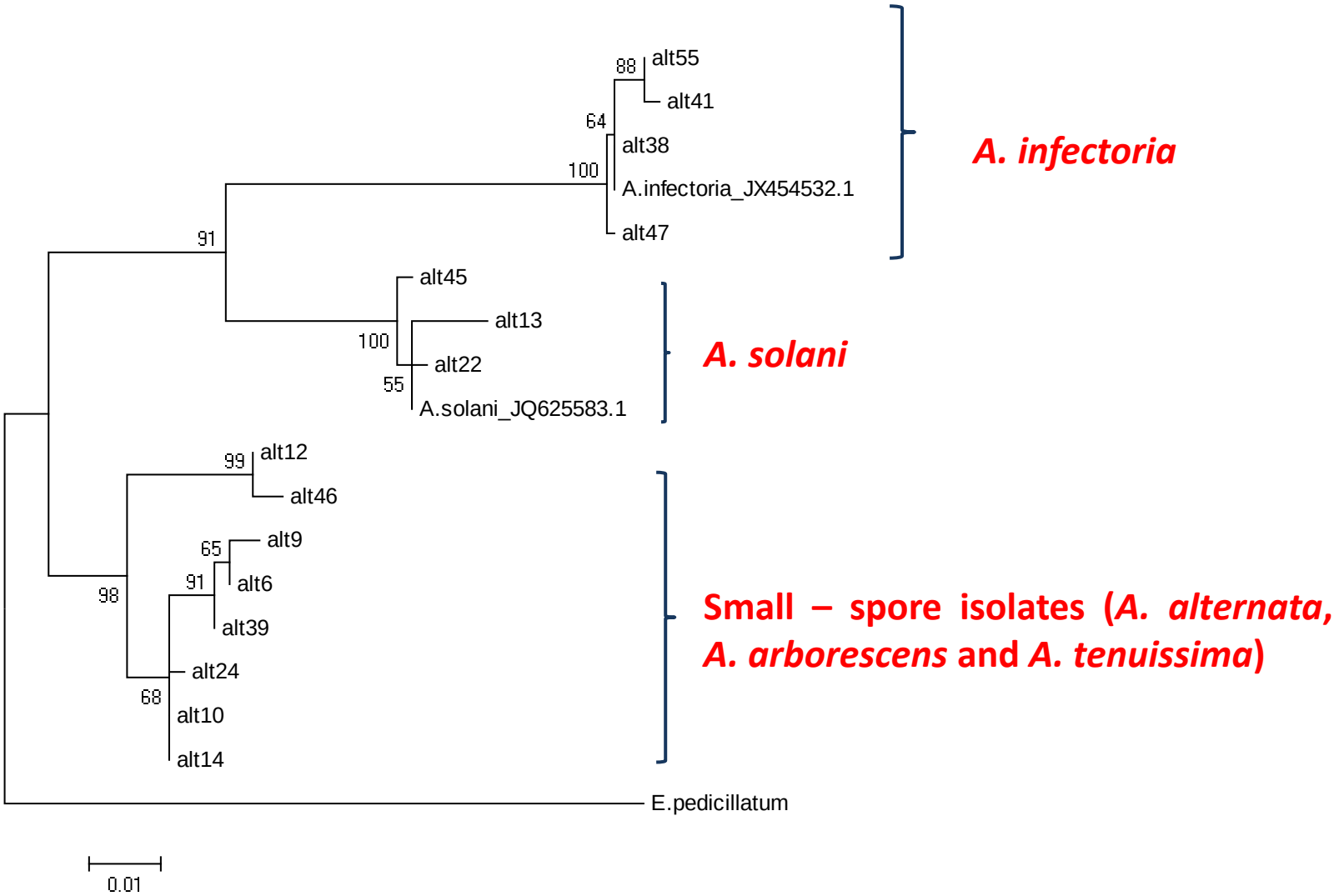




*A. infectoria*

Small spore

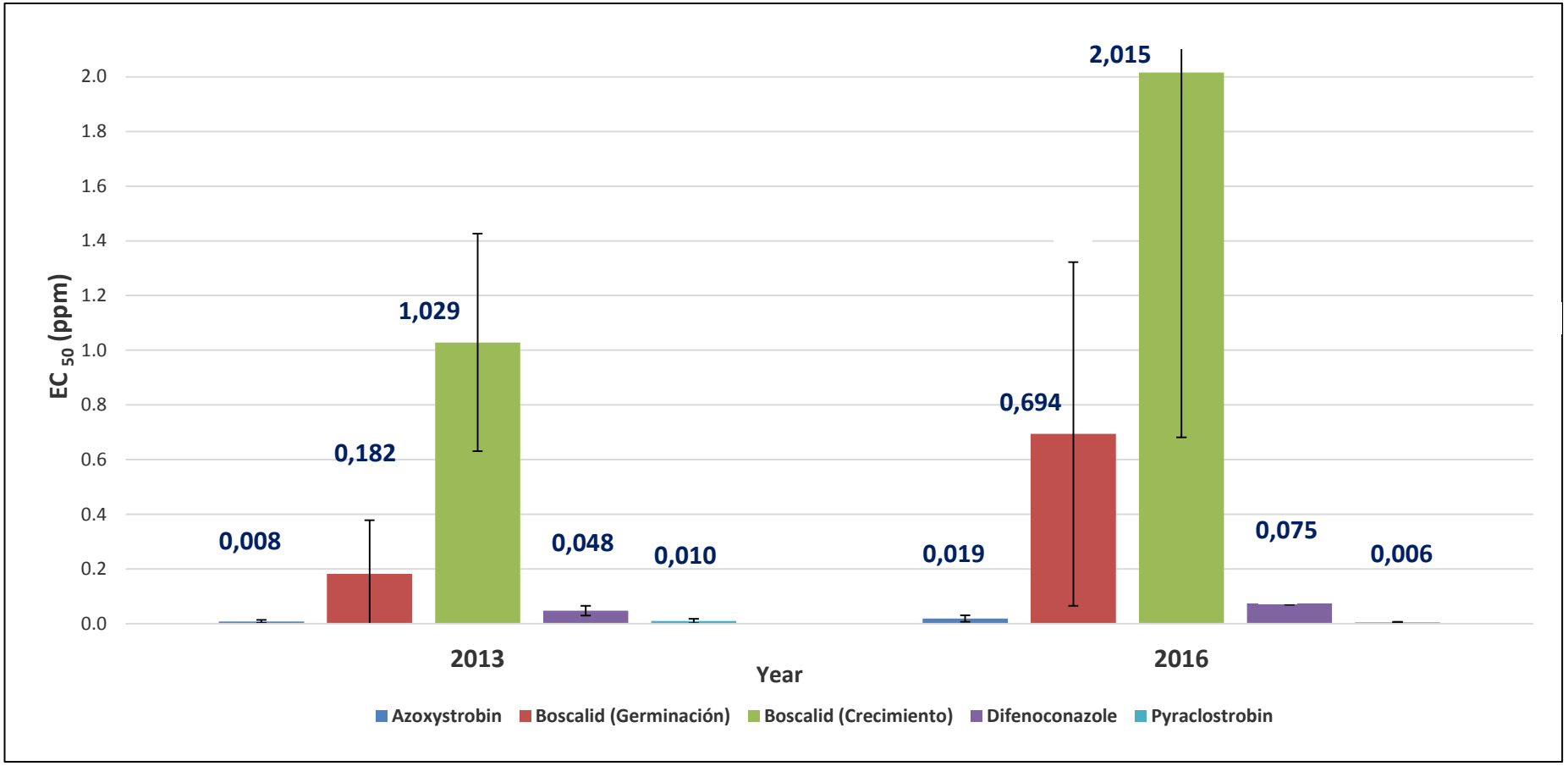
# Results




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

# Results

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



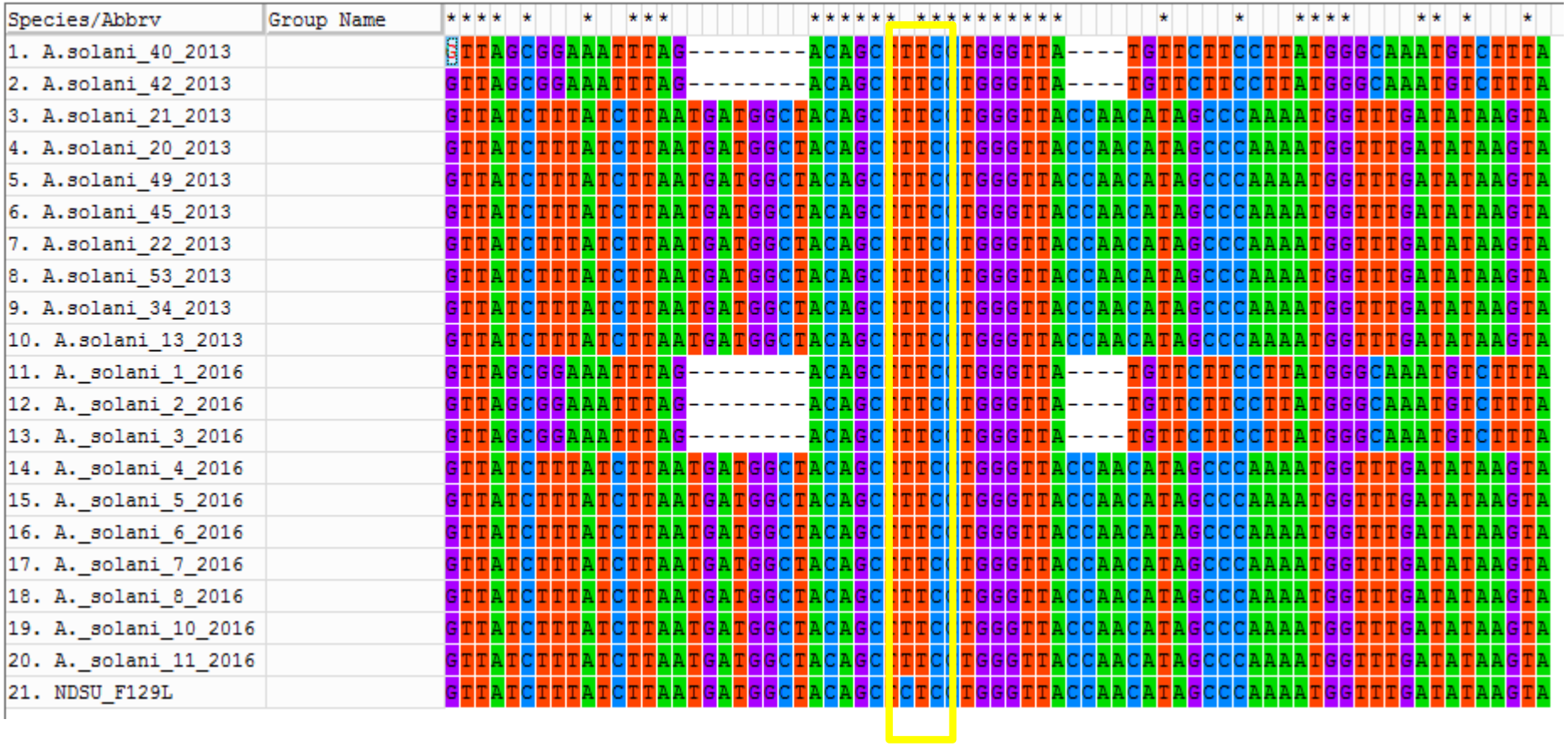

**Mean $EC_{50}$ values for *A. solani* isolates obtained from the *in vitro* sensitivity assessment of azoxystrobin, boscalid, difenoconazole and pyraclostrobin.**

# Results

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

| | | Isolates | | F129L | |
|---|---|---|---|---|---|
| Sample ID | Year | Genotype I | Genotype II | Genotype I | Genotype II |
| 13 | 2013 | | X | | |
| 20 | 2013 | | X | | |
| 21 | 2013 | | X | | |
| 22 | 2013 | | X | | |
| 34 | 2013 | | X | | |
| 40 | 2013 | X | | | |
| 42 | 2013 | X | | | |
| 45 | 2013 | | X | | |
| 49 | 2013 | | X | | |
| 53 | 2013 | | X | | |
| 1 | 2016 | X | | | |
| 2 | 2016 | X | | | |
| 3 | 2016 | X | | | |
| 4 | 2016 | | X | | |
| 5 | 2016 | | X | | |
| 6 | 2016 | | X | | |
| 7 | 2016 | | X | | |
| 8 | 2016 | | X | | |
| 10 | 2016 | | X | | |
| 11 | 2016 | | X | | |

# Results




**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.**

# Acknowledgements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- Chile Papa Consortium


Fundación para la Innovación Agraria

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