Alternaria spp. associated to potato crops and its epidemiology in southern Chile

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SUMMARY

Early blight is one of the most important diseases on potato in southern Chile. In this study we could identify five Alternaria groups associated to potato crops (*A. alternata*, *A. tenuissima*, *A. arborescens*, *A. infectoria* and *A. solani*) using morphological and molecular characteristics. At the same time, the Alternaria spore release curve was determined using a spore trap and accumulation of physiological days. This information could be used to predict the appearance of the first symptoms of this disease, which would help the development of an early blight forecast system.

KEYWORDS
Alternaria, early blight, forecast system, spore release.

INTRODUCTION

Early blight, caused by *Alternaria* spp, is the second most important disease in potato in Chile, can infect potato plants developing variable damages depending of the cultivar susceptibility to the disease, agronomic management and favorable environmental conditions. Losses can be attained up to 30% in susceptible cultivars.

This disease affects both foliar and tuber. Initial leaf blight symptoms are observed in the lower part of the plants, on the older leaves. The main symptoms are brown circular spots with concentric rings, surrounded by a bright yellow ring and bounded to the leaflet veins.

However, it has been observed that the symptoms in field are variable, since the incidence and severity of the disease changes according to the season. This situation may be given by the presence of different Alternaria species, the environmental conditions and the amount of pathogen inoculum.

Alternaria species can have their own biological requirements and characteristics such as aggressiveness, fungicide resistance, optimum growth temperature and overwintering, among others. Then, many studies have been done in order to predict the appearance of early blight disease based on favorable environmental conditions and the spore release curve of the pathogen into the air.

Therefore, the aim of this study was to identify *Alternaria* spp. associated to potato plants in southern Chile and to determine the Alternaria spore release curve and their role in the disease development using potato physiological days (P-days).

MATERIALS AND METHODS

Identification of Alternaria spp. associated with potato plants through morphological characteristics and molecular identification.

Potato leaves with early blight symptoms were collected in potato crop from the Araucanía to Los Lagos regions in southern Chile The pathogen was isolated from lesions and single-spore isolates were cultured on potato carrot agar (PCA) with fluorescent light cycles (8h light, 16 h dark) at 25° C for 5 days. Then, each isolate was characterized by colony morphology, sporulation patterns and conidial size. Finally, these results were compared with taxonomic keys following the method of Simmons (2007) and Piontelli (2011).

To perform the molecular analysis, three isolates were cultured of each morphological group on potato dextrose broth (PDB) for 4 days at 25°C with fluorescent light cycles (8h light, 16 h dark). Then, mycelium of each isolates were collected by filtration and were extracted by CTAB method. rDNA from ITS region was amplified with primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Chou et al, 2002). The PCR reaction was performed with an initial denaturation step at 94° C for 5 min, followed by 30 cycles of 94°C for 40s, 58°C for 40s and 72° C for 1 min, and a final extension step at 72° C for 5 min. Amplified fragments were cut from the agarose gel and purified with gel extraction kit. The ITS regions of 15 isolates were sequenced directly in both directions using the primers ITS5 and ITS 4, by Macrogen Korea.

DNA sequences obtained were aligned using the BioEdit Sequence Aligment Editor and were compared with database available in GenBank. Finally, phylogenetic analyses were performed with TreeFinder and the dendrogram was obtained by maximum-likelihood method.

Determination of the Alternaria spore release curve.

During the seasons 2010 to 2013 the Alternaria spore release curve was determined using a spore trap (Sporewatch spore & Pollen sampler, Burkard Scientific, England). This instrument was localized in the field near potato crop. It consist of a rotor carrying an adhesive tape. After 7 days this tape was removed and deposited on a slide for the conidia count under the microscope. P-days were calculated starting at potato plant emergence, using the maximum and minimum day temperatures. This model assumes 7° C, 21° C and 30° C as minimum, optimum and maximum, respectively according to crop development (Gent *et al.*, 2003).

RESULTS AND DISCUSSION

Identification Alternaria spp. associated to potato crops.

According to morphological characteristics that included colony morphology, sporulation patterns and conidial size, four small spore group (*A. alternata*, *A. tenuissima*, *A. arborescens* and *A. infectoria)* and one large group species *(A. solani)* were detected (Figure 1 and Table 1).
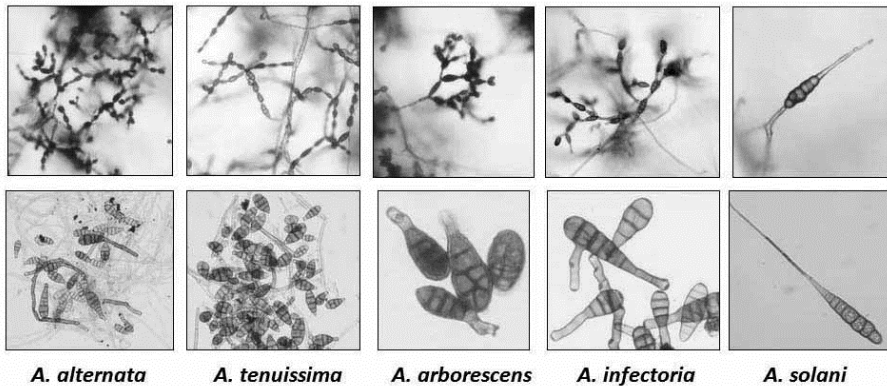
Figure 1. *Morphology of Alternaria species associated to potato crops in southern Chile*

Table 1. *Comparison of morphological characteristics obtained with reference literature*

Species	Conidial size				
	Reference range		Isolate range		
	(length x width)	trans.- long	(length x width)	trans.- long	
	µm	N° septa	µm	N° septa	
A. alternata	20-63 x 9-18	(3-8)-(0-1)	41,78 x 11,92	4-1	
A. tenuissima	32-45 x 11-13	(4-7)-(0-4)	27,66 x 12,15	5-1	
A. arborescens	12-30 x 7-11	(1-4)-(1-2)	29,28 X 11,44	3-2	
A. infectoria	35-40 x 7-9	3-5	35,45 x 8,92	3-3	
A. solani	150-300 x 15-19	(9-11)-(0-4)	200,90 x 19,05	8-2	

Additionally to morphological criteria, three isolates of each morphological group were selected. Then, rDNA region containing the internal transcribed spacer 1, 5.8S rDNA and internal transcribed spacer 2 was amplified with primer pairs ITS5-ITS4 by PCR and then it was sequenced.

After sequencing, we obtained the consensus sequences for each isolate which were compared with database available in Genbank. It was possible to align *A. solani* and *A. infectoria*, corroborating its morphological identification.

As a result of this part of our study, the Alternaria spp. were divided into three groups: the first group included all small-spore isolates, excepting *A. infectoria*. The second group included *A. infectoria*. The last group correspond to *A. solani.* No relation was found between morphological and sequencing data in small-spored isolated.
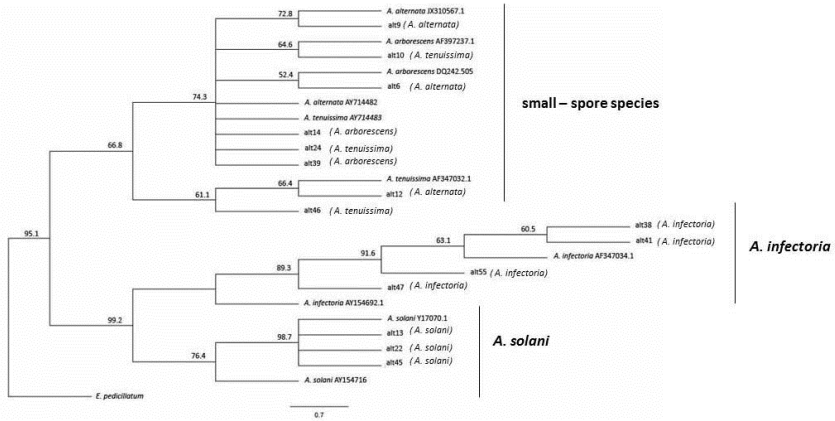
Figure 2. *Dendrogram of Alternaria spp obtained by máximum- likehood method. Sequences from GenBank were incorporated for analysis, () shown identification according to the morphological analysis*

Determination of Alternaria spore release curve.

The Alternaria spore release curve and its correlation with P-days could be used to predict the pathogen infection period. As a result, we observed a similar behavior during the three potato crop season evaluated, where the maximum conidia release were between 200 and 450 P-days. It was possible to differentiate 2 maximum peaks, one between 200 and 300 P-days and another between 350 y 450 P-days (Figure 3). Additionally, the first symptoms of the disease occurred in period of bloom, approximately one week after the first peak of the conidia release curve.
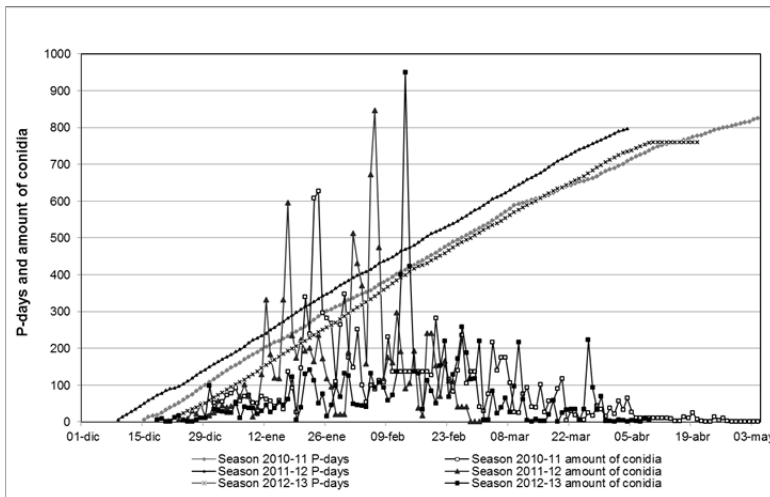

Figure 3. *Potato physiological days value and Alternaria spore release curve, during 2010 to 2013 crop seasons. INIA Remehue, Región de Los Lagos, Chile*

CONCLUSIONS

Five Alternaria groups were identified associated to potato crops in southern Chile: *A. alternata*, *A. arborescens*, *A. tenuissima*, *A. infectoria* and *A. solani* using morphological and molecular characteristics. This information is relevant for future studies of pathogenicity, virulence, fungicide resistance and field trials, because of the possibility of a differential behavior of each species.

The use of P-days and the Alternaria spore release curve can be used to predict the appearance of the first symptoms and support the integrated management of this disease, becoming an alternative for the development of a decision support system for the potato crop in southern Chile.

ACKNOWLEDGEMENTS

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