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Occurrence of *Alternaria solani* in Sweden and its sensitivity to strobilurins

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SUMMARY
Potato leaves showing symptoms similar to early blight were collected three times during August - September 2009 in Sweden. Using diagnostic PCR methods *A. solani* was identified in 56% of the 432 samples and *A. alternata* in one single sample. In some of the sampled fields the incidence of early blight was high during September despite one or two applications of strobilurin fungicides. DNA extracted from the samples with confirmed *A. solani* was sequenced in order to determine whether the loss of effect was due to fungicide resistance. None of the *A. solani* sequences showed substitutions at any of the three amino acid positions associated with resistance to strobilurins.

KEYWORDS
Alternaria solani, *A. alternata*, early blight, fungicide sensitivity, strobilurin

INTRODUCTION

The plant pathogenic fungi *Alternaria solani* and *A. alternata* cause early blight and brown spot on potato, respectively. During the last decade the number of reports of early blight has increased in the south-eastern part of Sweden, especially in starch potato crops. Both *A. solani* and *A. alternata* were detected in field trials during 2005 and 2006, but *A. solani* was more frequent (Andersson & Wiik, 2008). A survey of the two *Alternaria*-species performed in Germany showed that both species could be found simultaneously in a field and that they occurred in all areas where potato is grown (Hausladen & Leiminger, 2007). Mancozeb, a common substance in many fungicides used against late blight on potato (*Phytophthora infestans*), has been reported to have an effect on *Alternaria*-species. In Sweden, concerns of the carcinogenic effects and neural tubes defects linked to mancozeb (Belpoggi *et al*., 2002; Nordby *et al*., 2005) has led to far reaching restrictions in the use of this fungicide. As a consequence, the use of mancozeb has drastically declined in Sweden, which may have been one cause of the increase in incidence of early blight. Strobilurins have so far shown efficient control of early blight in Sweden and has helped achieving high potato yields (Andersson & Wiik, 2008). However, in parts of the USA the use of strobilurins no longer gives the desired effect against *Alternaria*-species (Luo *et al*., 2007; Pasche *et al*., 2005; Rosenzweig *et al*. 2008). In the American population of *A. solani,* three different nucleotide substitutions leading to strobilurin resistance have been found. These substitutions are located in the gene encoding cytochrome *b* in

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the amino acid position 129 (F129L). In California, isolates of *A. alternata* infecting pistachio and almonds have a corresponding substitution at position 143, (G143A). This substitution results in a higher degree of resistance compared to F129L.

In Sweden, potato growers are experiencing increasing problems with early blight. There are reports of fields treated with strobilurins where severe attacks of early blight have occurred. The aim of this project was to determine the causal agent of the lesions similar to early blight collected in south-eastern Sweden during 2009 using diagnostic PCR. The occurrence of strobilurin resistance was analysed in the sampled material by sequencing the gene encoding cytochrome *b* in order to identify any relevant amino acid substitutions.

MATERIALS AND METHODS

Identification of *Alternaria solani* and *A. alternata*

Plant material
Sampling was performed at six locations three times during 2009 (early August, late August and in mid September). Twenty-four potato leaves showing early blight symptoms were collected at each location and at each sample time, resulting in 432 samples in total. If no lesions were found at a particular sampling spot, a symptomless leaf was collected instead. The six sampling locations were commercial starch potato fields located in the south-eastern parts of Sweden, five of which were cv. Kuras while one was cv. Kardal. Four of the fields with cv. Kuras were treated twice with strobilurins, either during the second or the fourth week of July.

DNA extraction and identification of causal agent of the lesions
A small leaf piece containing one lesion was cut from each leaflet and was washed twice in sterile distilled water. From each lesion three discs, 2 mm in diameter, were cut from the edge of the lesion containing both healthy and necrotic tissue. The discs were homogenised with five glass beads (3mm ø) in a 2 ml micro centrifuge tube in a Precellys homogeniser. DNA was extracted using a CTAB protocol. The two species were identified using species specific PCR primers developed by Rosenzweig *et al.* (2008) for *A. solani* and Zur *et al*. (2002) for *A. alternata*. Both species and a non template control were included each run. A DNA fragment of the gene encoding cytochrome *b* was amplified and sequenced using a newly developed forward primer (unpublished) and the 143 reverse primer developed by Rosenzweig *et al*. (2008). The PCR-product included the amino acid positions 129, 137 & 143.

RESULTS AND DISCUSSION
The disease incidence increased during the season and in September the majority of the lesions were confirmed to be caused by *A. solani*. The proportion of samples identified as *A. solani* in each field varied between 45% and 81% over the season for cv. Kuras while cv. Kardal had a total incidence of 22%. One sample containing *A. alternata* was identified from the second collection in one of the cv. Kuras fields. All of the 242 samples with confirmed *A. solani* had the wild type version of the gene encoding cytochrome *b* suggesting that the Swedish population of *A. solani* is still sensitive to strobilurins.

CONCLUSIONS

This study showed that early blight in south-eastern Sweden is caused by *A. solani*. *A. alternata* was rarely found on leaves with early blight symptoms. No amino acid substitutions associated with resistance to strobilurins was found in the genome, indicating that these compounds still are effective against the Swedish population of *A. solani*. However, further and continuous investigations must be performed in order to monitor the risk of loss of sensitivity towards strobilurins.

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