Characterisation of Mutations in the Succinate Dehydrogenase Gene for Potential Fungicide Resistance in *Ramularia collo-cygni*

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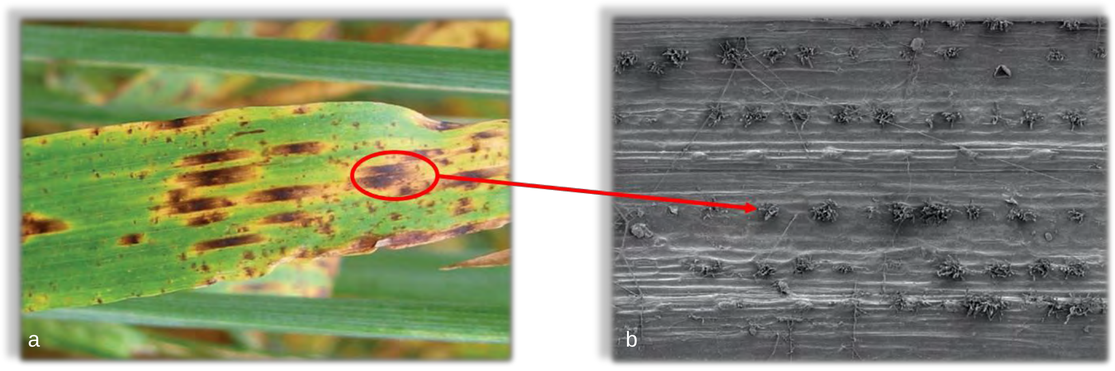
Introduction

The fungus *Ramularia collo-cygni* (*Rcc*) has been present as a major barley pathogen in Scotland since 1998. Since then different fungicides such as strobilurins (QoI), carboxamides (SDHIs), triazoles (DMIs) and chlorothalonil have been used to control the disease. The focus of this study will be on SDHI (Succinate Dehydrogenase Inhibitors) fungicides and potential fungicide resistance that *Rcc* might develop to this chemical group.

The key question of this research is to see what mutations *Rcc* will form if resistance to SDHI's develops and will this resistance cause a fitness penalty?



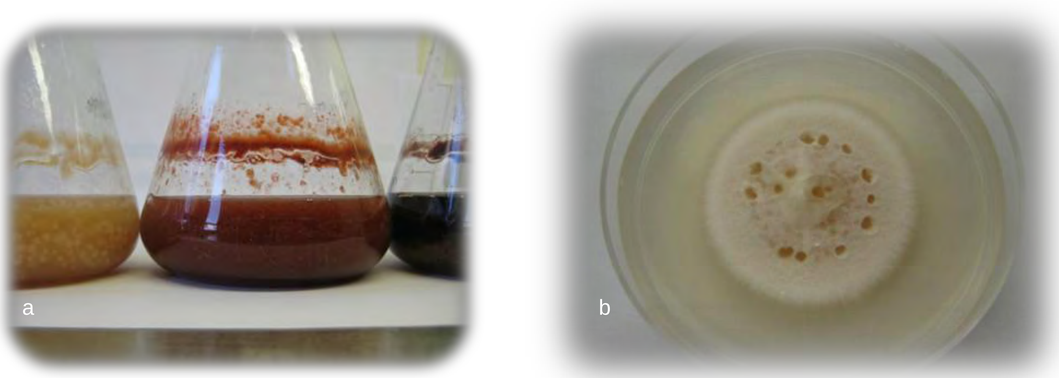
Symptoms of RLS (Ramularia Leaf Spot) (a- black necrotic spots surrounded by a yellow halo) and clinical signs (b- conidiophores growing out of the stomata)

Methods

- *Rcc* has been isolated from the leaves and cultivated on plates and in liquid media to define the optimum conditions for the culture growth.
- Subunit B of the *Sdh* (succinate dehydrogenase) gene was sequenced. Three other subunits A, C and D are being cloned.
- A 96 well plate assay for fungicide sensitivity testing in liquid media and using mycelium suspension was developed and is being optimised.

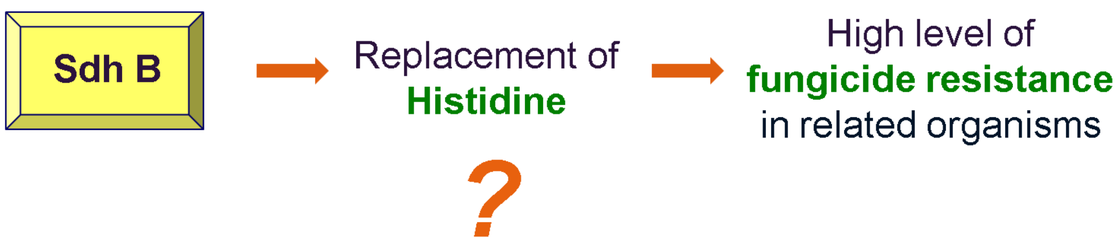
Preliminary Results

- Culture of *Rcc* in the liquid media and on the plate



Rcc in liquid media at different growth stages showing the production of rubelin (a) and a single spore isolate of *Rcc* on agar (b).

- Sequence of subunit B of *Sdh* gene



In *Mycosphaerella graminicola* carboxin resistant isolates had an amino acid (AA) substitution in the *Sdh* B subunit at position **H267Y(L**) changing histidine into tyrosine or leucine, although in other related organisms the position of the mutations may differ slightly.

Preliminary Results (continued)

- 96 well plate assay for fungicide sensitivity testing

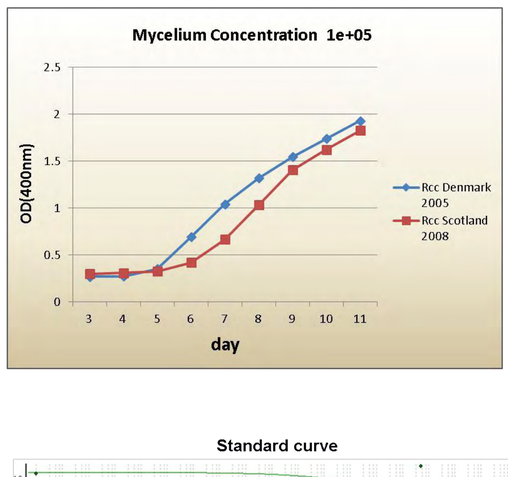


Figure 1. Mycelium growth of *Rcc* over 11 days, at a concentration of 1e+05 mycelium/ml, measured by OD at 400 nm.

Two single spore isolates of *Rcc* were used in this test: one from Denmark, collected in 2005 (blue) and another from Scotland, collected in 2008 (red).

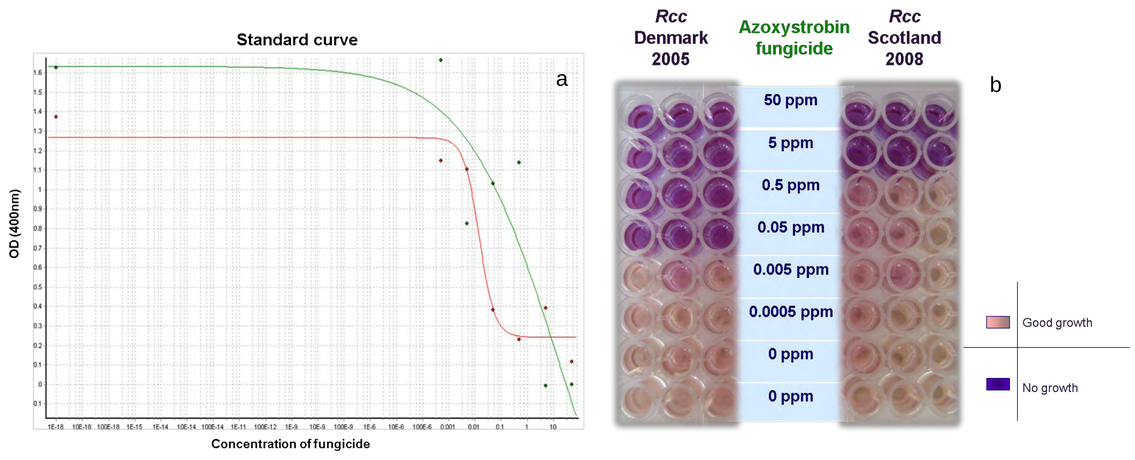
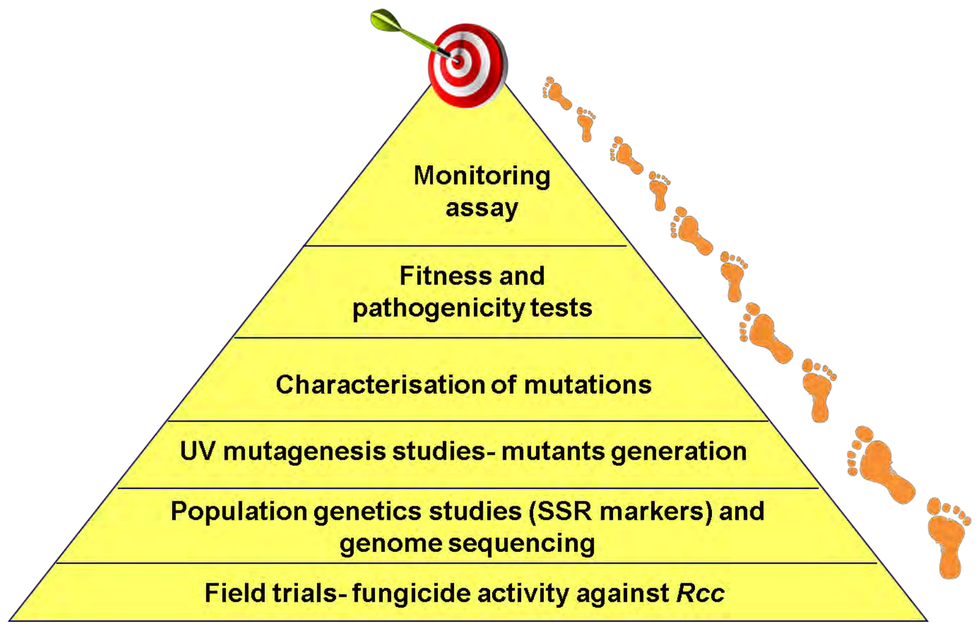


Figure 2. A standard curve (a) and 96 well plate test with Resazurin sodium salt (b) showing the response of two isolates of *Rcc*, from Denmark, 2005 (a-red; b-left) and from Scotland, 2008 (a-green; b-right), to azoxystrobin fungicide at different concentrations.

All the measurements were taken by measuring the OD at 400 nm.

The 96 well plate shows the impact of resistance in *Rcc* to the QoI fungicides. Similar methodology is being established to monitor the sensitivity of *Rcc* to SDHI fungicides.

Further Work



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