Leaf exudates of barley involved in the defence against *Rhynchosporium secalis*

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Abstract

Rhynchosporium secalis is the casual agent of a foliar disease of barley, which is commonly known as leaf scald. It occurs in every major barley growing area in Australia and the rest of the world, and is especially devastating in cooler, semi-humid growing areas. An initial defence response occurs upon the leaf surface to suppress conidial germination, which is important in reducing the infection efficiency. However, very little is known about the occurrence of this response and the factors involved.

Seedlings of barley cultivars were inoculated with conidia cultured from different isolates of *R. secalis* and examined microscopically at three days post inoculation. There was a substantial reduction in the germination of the isolate H1.1 upon the surface of the barley cultivar Brier. Suppression on other isolate-cultivar combinations was significantly less. Leaf exudates were eluted with water and separated using a Microcon ultrafiltration membrane. The surface structure of the leaves was examined using scanning electron microscopy before and after exudate removal to confirm that only the surface exudates, and not the waxes, were being harvested. An *in vitro* bioassay was used to assess the effect of the exudate on the germination and germ tube growth of *R. secalis* conidia. The low molecular weight fraction showed a strong inhibitory effect on the conidial growth, whereas the high molecular weight fraction stimulated the growth of the conidia.

A separation method was developed for the low molecular weight fraction to resolve the biologically active components. Initial electrophoretic and cation exchange characterisation of the exudate fraction showed that at least two biologically active component(s) had positive charge at pH 1.7 and were UV absorbing. Paper electrophoresis followed by reverse phase HPLC allowed the isolation of three active UV absorbing components. Comparison of the mass spectra, UV spectra, electrophoretic and chromatographic behaviour of various indoles to this component confirmed its identity as gramine (3-(dimethyl aminomethyl) indole). One of the other components was consistent with either an *O*- methyl or hydroxymethyl gramine, based on the mass spectrum, the similarities of the UV spectra and chromatographic behaviour. The third component was proposed to be a flavonoid on the basis of its UV spectra. This work establishes the importance of indoles in the protection of barley seedlings from fungal leaf diseases.

Declaration

This thesis contains no material that has been accepted
for the award of any other degree or diploma in any university and that,
to the best of my knowledge and belief,
contains no material previously published or written by another person,
except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University library,
being available for loan and photocopying.

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