Inhibitory effect of volatiles from rapeseed tissues on *Sclerotinia sclerotiorum,* white mold causal agent

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Abstract

The enzymatic hydrolysis of glucosinolates, typical compounds of Cruciferae, produces molecules with fungitoxic activity known isothiocyanates. We investigated the effects of these volatile compounds released from the leaf, petiole and stem tissues of 5 varieties of rapeseed (*Brassica napus*), black mustard (*B. nigra*) and white mustard (*Sinapis alba*) on mycelial growth of the plant pathogenic fungus *Sclerotinia sclerotiorum.* Tissues were freeze-dried and ground to a powder, then placed in containers with distilled water in sealed, inverted Petri dishes containing potato dextrose agar. Colony diameter of *S. sclerotiorum* was measured after 24 hours. The volatiles released from tissues of various host plants had different inhibitory effects on the pathogen. Growth of exposed fungus colonies was completely inhibited by the volatiles from all tissues of black mustard. Volatiles from rapeseed inhibited growth by up to 40%, but there were significant differences between varieties and between tissue types within a variety. The results indicate that the effectiveness of fungal suppression by *Brassica* crops will depend upon the species and type of plant tissue, which influence the type and concentration of isothiocyanates evolved and the sensitivity of the pathogen.

Introduction

Stem rot or white mold disease, caused by the ascomycete fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is an important threat to oilseed *Brassica* crops worldwide (Lamey 1995). It causes major yield losses in France and Germany and is probably the most important pathogen of oilseeds in central China (Rimmer and Buchwaldt 1995). *Brassica* species and other members of the Brassicaceae contain significant quantities of the thioglucoside compounds known as glucosinolates (GSLs) in their tissues. GSLs are hydrolyzed by the enzyme myrosinase (present endogenously in *Brassica* tissues) to release a range of hydrolysis products including nitriles, thiocyanates and various forms of volatile isothiocyanates (ITCs). These compounds in particular the ITCs, are known to have broad biocidal activity including insecticidal, nematicidal, fungicidal, antibiotic and phytotoxic effects (Brown and Morra 1997; Chew 1988; Rosa *et al.* 1997). Manici *et al*. (1997) exposed plant pathogenic fungi, including *Sclerotinia sclerotiorum*, to eleven glucosinolates and their volatile enzymatic hydrolysis products obtained by myrosinase. They found that the volatiles inhibited fungal growth. *In vitro* inhibition of the pathogen has been proved by Smith and Kirkegaard(2002) using 2-phenylethyl isothiocyanates. Kirkegaard *et al*. (1996) in their investigation on root pathogens of cereals suggested that the degree of fungal suppression by the various *Brassica* tissues is related to the concentration and type of isothiocyanates released, which varied with *Brassica* species, tissue age and tissue type. Having this knowledge, we investigated the effect of different species and tissues of crucifer plants on *Sclerotinia sclerotiorum*.

Materials and methods:

Plant materials: Seeds of the Australian commercial canola (rapeseed, *Brassica napus* L.) varieties AG-Castle, AV-Sapphire, Dunkeld, Oscar, and Rainbow were provided by Dovuro Seeds, Horsham, Australia. The other two species, black mustard (*Brassica nigra* L.) and white mustard (*Sinapis alba* L.) were condiment varieties. Three month old plants, grown in the green house, were dissected into leaves, petioles, and stems. Leaves were selected on the basis of color, shape and size to ensure uniformity between varieties. The leaves, petioles and stems of each variety were placed separately in paper bags and frozen at -20˚C, then freeze dried (Kirkegaard *et al*. 1996). After three days freeze drying, the samples were ground by a soil grinding machine (for tough tissues) or a small laboratory blender (for thin ones), and stored in plastic containers at -20˚C

Effect of Volatiles from Rapeseed Tissues: The bioassay was based on the method of Sexton *et al*. (1999). Four millimeter diameter agar plugs containing hyphae of *S. sclerotiorum* were transferred from the margins of 3-day-old actively growing cultures to 55 mm diameter plates which had a thin layer of ¼ strength potato dextrose agar. 100 mg of ground leaf, petiole or stem tissue of each experimental plant sample was weighed in individual weighing boats (2 ml capacity). The boats containing the ground tissue were placed in the upturned lid of each plate, whilst the inverted bottom containing the fungal plugs was held aside. Distilled water was added to the powder to provide hydrolysis conditions for glucosinolates. Control plates had distilled water only added. The inverted bottoms of the plates were replaced, thereby positioning the fungal plugs over the containers with moistened leaf, petiole or stem tissue. Five mg quantities of mustard powder (Ward McKenzie, Melbourne, Australia) mixed with distilled water was used for comparison with the treatments. The plates were immediately sealed with two layers of Parafilm, and incubated at 25˚C in darkness for 24 hrs. A completely randomized design with three replications was used for the experiment. The diameter of exposed fungus colonies was measured after 24 hour incubation, and growth inhibitory effect of released ITCs was calculated by following formula after the diameter of the inoculum plug was

subtracted:

Growth inhibition = 100 × (Control diameter – Treatment diameter)/Control diameter

Data were analyzed by one-way ANOVA after arcsine transformation.

Results and discussion

Most of the tissues released volatiles that significantly inhibited the growth of *Sclerotinia* (Figure 1). Only the stem of Oscar, petiole and leaves of AG-Castle and leaves of AV-Sapphire did not suppress growth of the fungus relative to the control. All tissues of black mustard had a complete inhibitory effect on the pathogen growth. To determine the fungistatic or fungicidal effect of *B. nigra* volatiles, the weighing boats containing the tissue samples were removed and the fungus was incubated for another two days. No growth was observed from the black mustard leaf and petiole tissue treatments, but the fungus exposed to the black mustard stem treatments resumed growth.

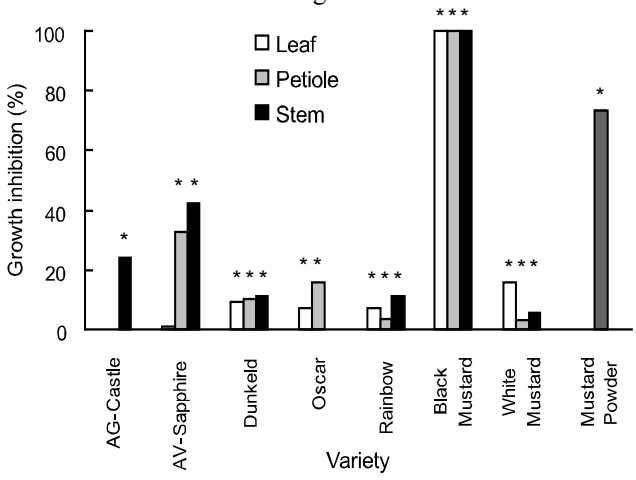

Figure 1. Inhibition of growth of *Sclerotinia sclerotiorum* by volatiles released from ground, freeze-dried tissues of canola and mustard varieties, and mustard powder, in the presence of water. Asterisks indicate growth significantly different ($P < 0.05$) from water controls.

There were significant differences in inhibition of growth of *S. sclerotiorum* between varieties of canola and between tissues within varieties. The greatest inhibition (over 40%) was seen with the stem tissue of AV-Sapphire, while petiole tissue of AV-Sapphire and stem tissue of AG-Castle also gave over 20% inhibition (Figure 1). The leaves of these varieties were not inhibitory, while those of Dunkeld, Oscar and Rainbow were (Figure 1).

The obvious difference between the effect of volatiles of black mustard and the others on *Sclerotinia* shows that Brassicaceae plants could have different glucosinolate components qualitatively and quantitatively. These results support the findings of Kirkegaard *et al*. (1996) in which the degree of fungal suppression by various *Brassica* tissues was related to the concentration and type of isothiocyanates released, which varied with *Brassica* species and tissue type. The relationship between production of growth-inhibitory volatiles and partial resistance to *S. sclerotiorum* in canola is under investigation.

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