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# Role of Root Extract and Volatile Substances of non-Host Plants on Vesicular-Arbuscular Mycorrhizal Spore Germination*

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## Abstract

Glucosinolates, substances found in crucifers, are metabolized to antifungal, volatile and nonvolatile isothiocyanates. Vesicular-arbuscular (VA) mycorrhizal symbiosis is not developed in the *Cruciferae*. The effect of exposing root extracts or their gasesous components from host (tomato, alfalfa) and non-host plants (cabbage, rape, radish, spinach) to spores of *Glomus masseae* were studied. The results suggest that the inhibitory effect of root extracts of non-host plants (*Crucifereae*) on spore germination can not be attributed solely to presence of volatile substances and extended to other non-host plants.

## Introduction

Glucosinolates are found in all crucifers. However species differences in concentration and composition exist. There are two major groups, the alkenyl and the indol glucosinolates. The alkenyl glucosinolates are metabolized to antifungal volatile and nonvolatile isothiocyanates (Larsen 1981).

Extracts and volatile compounds of cabbage have recently been shown to reduce mycorrhization and spore germination (El Atrach et al., 1989) in a way similar to that observed with the combination of sinigrin, an alkenyl glucosinolate, and myrosinase (Vierheilig and Ocampo submitted for publication).

The aim of the present work was to study the influence of root extract from VAM host and non-host plants on spore germination of a mycorrhizal fungus.

## Materials and Methods

Plants: Alfalfa (*Medicago sativa*), tomato (*Lycopersicon lycopersicum*), spinach

*Reviewed

(*Spinacea oleracea*), cabbage (*Brassica oleracea*), radish (*Raphanus sativus L.*) and rape (*Brassica napus*). Two varieties of rape were used, rape (0) (Jew Neuf) obtained from "Saaten Union" Hannover F.R.G. with a low erucic acid concentration, and a so-called double zero (00) variety (Arabella) obtained from "Setmundo Saatzucht" Rellingen/F.R.G. In rape (00), the concentration of erucic acid and total glucosinolates is low, although, levels of indol glucosinolates remain approximately constant.

Roots were extracted by grinding 2 g root material with 1 ml 0.1 M Tris Buffer, pH 7. After centrifugation (20 min 8000 g) the extract was passed through a millipore filter. All steps were performed at 4°C.

*Experiment 1*

25 surface-sterilized (Mosse 1962) spores of *G. mosseae* were flooded with the extract for 60 min and then placed in Petri dishes with water agar (1.5%; pH 7).

*Experiment 2*

25 surface-sterilized spores were placed on water agar (1.5%; pH 7) and the Petri dishes inverted. The lid of the Petri dish below the agar was filled with 2 ml of each extract.

Dishes were sealed with nescofilm and stored at 25°C in the dark. Spore germination was examined after 1 week in 10 replications per treatment.

Table 1. Percentage spore germination in the presence of root extracts

| | % spore germination | |
|---|---|---|
| Treatments | Exp. 1 | Exp. 2 |
| Control | 21±4 | 28±5 |
| Cabbage | 0 | 0 |
| Rape (0) | 3±1 | 5±1 |
| Rape (00) | 10+2 | 18+8 |
| Spinach | 12±3 | 31±6 |
| Radish | 2±1 | 10±3 |
| Tomato | 23±4 | 30±6 |
| Alfalfa | 25±7 | 32±7 |

*Exp. 1 Spores incubated with root extract. Exp. 2 Spores in the presence of volatiles compounds. Each number is the mean of ten replications. Standard errors of means are given.

## Results

Extract of all crucifers decreased spore germination. However not all extracts had

the same inhibitory effect. Incubation with cabbage extract resulted in total inhibition, rape (0) and radish showed strong inhibition and rape (00) and spinach inhibited germination to a far lower extent. Tomato and alfalfa seemed to have a slight stimulatory effect, but this was not significant.

Volatile compounds of cabbage extract compltely inhibited germination while rape (0) strongly reduced germination. Spinach, tomato and alfalfa seemed to stimulate germination, but this effect was not significant.

## Discussion

An inhibitory effect of root extract of non-host plants is evident. In rape the differences in inhibition seemed to be related to different alkenyl glucosinolate concentration. A diminution in the resistance of rape (00) to pathogens has already been found (Mithen et al., 1987). Observations of VA-fungal mycelium in the root of rape (00) root but without arbuscules (unpublished observations) confirm that the first steps of colonization might be related with the glucosinolate content of crucifers. Inhibitory components produced by radish seemed to be mainly of nonvolatile nature.

Glenn et al., (1988) related lack of colonization in non-host plants to the lack of attractants rather than to inhibitory factors. Our results suggest that the inhibitory effect of root extract of non-host plants cannot be attributed solely to the presence of isothiocyanates.

Spinach seems to have no inhibitory volatile compounds. However spores incubated in spinach root extract decreased germination, indicating an inhibitory mechanism.

The results obtained in our work suggest that glucosinolates or its hydrolisis products might play a role in non-colonization of crucifers, but this mechanism of inhibition can not be extended to other non-host plants such as Chenopodiacea.

Further studies are needed in order to correlate inhibition of VA-spore germination with inhibition of root colonization.

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## REFERENCES

Allen, M.F. et al., 1989. Responses of the non-mycotrophic plant *Salsola kali* to invasion by vesicular-arbuscular mycorrhizal fungi. *New Phytol.* **111:** 45–49.

El Atrach, F. et al., 1989. Influence of non-host plants on vesicular-arbuscular mycorrhizal colonization of host plants and on spore germination. *Soil Biol. Biochem.* **21:** 161–163.

Glenn, M.G. et al., 1988. Influence of glucosinolate content of *Brassica* (*Cruciferae*) roots on growth of vesicular-arbuscular mycorrhizal fungi. *New Phytol.* **110:** 217–225.

Larsen, P.O. 1981. Glucosinolates. In: The biochemistry of plants. A comprehensive Treatise (P.K. Stumpf and E.E. Conn Ed.), pp. 502–525. Academic Press, New York.

Mithen, R.F. et al., 1987. Resistance of leaves of Brassica species to *Leptosphaeria Maculans. Trans. Br. mycol. Soc.* **88:** 525–531.

Mosse, B. 1962. The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. J. of General Microbiology. **27:** 745–751.