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EFFECT OF ISOTHIOCYANATES ON GERMINATION OF SPORES OF *G. MOSSEAE*

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Although the lack of vesicular–arbuscular mycorrhizal (VAM) colonization in Cruciferae has been attributed to intrinsic characteristics of the cortex, rather than to compounds released in exudates (Ocampo *et al.*, 1980), extracts and volatile compounds from cabbage plants have been shown to reduce spore germination in *Glomus mosseae* (El-Atrach *et al.*, 1989). These compounds also reduced VAM colonization in alfalfa (host) plants to a degree which was influenced by the medium in which the plant was grown and by VAM inoculum potential (El-Atrach *et al.*, 1989).

Glucosinolates are a large group of sulfur-containing glycosides found in the Cruciferae. Although these compounds have no antifungal activity (Mithen *et al.*, 1986) and their amounts in roots are not correlated with the failure to establish VAM symbiosis (Glenn *et al.*, 1988) they can be hydrolyzed, in general after a mechanical injury of the plant cell, by the endogenous enzyme myrosinase (thioglucoside glucohydrolase EC 3:2:3:1) to a variety of products. These include isothiocyanates (Larsen, 1981) which are generally volatile, thermolabile and have marked antifungal activity (Larsen, 1981; Mithen *et al.*, 1986). That the inhibitory effect of root extracts on germination of *G. mosseae* spores maybe due to isothiocyanates is indicated by the fact that the effect disappears after heating to 48°C or when roots are autoclaved prior to extraction and by the inhibition of spore germination by the action of myrosinase on sinigrin (a glucosinolate) or cabbage root extract.

To test the possible role of isothiocyanates in the inhibition of spore germination, roots of cabbabge (*Brassica oleracea*) and tomato (*Lycopersicon esculentum*) (a VAM host-plant used as control) were extracted by grinding root material with 0.1 M Tris–HCl buffer, pH 7. The extracts were sterilized passing them through a millipore filter (0.45 μm). The sterilized tomato root extract was used fresh. The sterilized cabbage root extract was used fresh (i) or heated for 60 min in a 48°C water bath with agitation (ii). Roots of cabbage, autoclaved for 20 min at 120°C, were also used.

In experiment 1 spores of *G. mosseae* were flooded in Tris–HCl buffer (2 ml), as controls, and cabbage or tomato extracts (2 ml) for 60 min, then surface-sterilized (Mosse, 1962) and placed on water–agar in a Petri dish. In experiment 2 (Exp. 2) cabbage extract and solutions of myrosinase and sinigrin were used. The spores were exposed for 60 min in 2 ml of one of the following solutions: Extract (i) (0.5 g ml^{-1}); sinigrin (Sigma) (100 μg ml^{-1}); myrosinase (0.2 U ml^{-1}); 1 ml of sinigrin (200 μg ml^{-1}) plus 1 ml of extract (i) (1 g ml^{-1}); 1 ml of extract (i) (1 g ml^{-1}) plus 1 ml of myrosinase (0.04 U ml^{-1}); 1 ml of extract (ii) (1 g ml^{-1}) plus 1 ml of myrosinase (0.04 U ml^{-1}); 1 ml of sinigrin (200 μg ml^{-1}) plus 1 ml of myrosinase (0.04 U ml^{-1}).

In experiment 3 (Exp. 3) surface-sterilized spores were placed on water–agar as before and the Petri dishes inverted. The lids of each Petri dish were filled with 2 ml of the solutions described in experiment 2, and the dishes were then sealed with necofilm.

In all experiments dishes were kept at 25°C. 25 spores per Petri dish and 10 dishes per treatment were used. After 1 week percentage spore germination was assessed.

In Exp. 1 (Table 1) fresh cabbage extract inhibited spore germination, but inhibition disappeared, when the roots were autoclaved prior to extraction or when the extract was heated to 48°C. When the spores were exposed to tomato root extract, the percentage germination did not differ significantly from controls. These results suggest that the formation of inhibitory substances produced by root extracts is sensitive to high temperatures and that the produced substances are gaseous. This result is in agreement with results found by El-Atrach *et al.* (1989) and suggest that isothiocyanates may act as the inhibitory agents of spore germination. To verify this hypothesis the role of sinigrin (a glucosinolate) and myrosinase on spore germination was tested (Exp. 2 and Exp. 3). As Table 2 shows, the action of myrosinase on sinigrin or on root extracts (i and iii) (cabbage glucosinolates), significantly reduced percentage germination. Furthermore germination was completely inhibited in the

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

	% Spore germination
Control	20 ± 3
Tomato	33 ± 10
Cabbage	7 ± 2
Cabbate incubated for 60 min at 48°C	22 ± 6
Cabbage autoclaved for 60 min at 120°C	18 ± 2

Each number is the mean of ten replicates. SEM are given.

Table 2. Percentage spore germination in the presence of cabbage root extract, sinigrin and myrosinase

		Control	Myrosinase	Sinigrin	Sinigrin plus myrosinase	Extract	Extract plus myrosinase	Sinigrin plus extract	Extract (autoclaved roots) plus myrosinase
% Spore	Exp. 2	51 ± 9	39 ± 6	41 ± 7	23 ± 6	25 ± 5	19 ± 5	23 ± 8	24 ± 4
Germination	Exp. 3	59 ± 11	59 ± 12	62 ± 10	0	0	0	0	0

Exp. 2. Spores exposed to root extract, sinigrin and myrosinase for 60 min. Exp. 3. Spores in the presence of volatile reaction products of glucosinolate hydrolysis. Each number is the mean of ten replicates. SEM are given.

presence of volatile reaction products of glucosinolate hydrolysis. Neither sinigrin nor myrosinase alone had a significant effect on fungal germination. Although myrosinase action is likely to take place only after some kind of mechanical injury to a plant, there is some evidence for low-level production of isothiocyanates in intact plants (Tang, 1973). This together with its possible induction by VAM fungus, may be involved in the absence of VAM colonization in *Cruciferae*.

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