

Influence of glucosinolate content of *Brassica* (Cruciferae) roots on growth of vesicular–arbuscular mycorrhizal fungi

BY M. G. GLENN^{1,2,*}, F. S. CHEW¹ AND P. H. WILLIAMS²

¹Department of Biology, Tufts University, Medford, Massachusetts 02155, USA

²Department of Plant Pathology, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA

(Received 6 November 1987; accepted 23 June 1988)

SUMMARY

We tested the hypothesis that failure to establish symbiosis with vesicular-arbuscular (VA) mycorrhizal fungi is correlated with glucosinolate concentrations in *Brassica*, a representative genus of the Capparales. *Brassica campestris* and *B. napus* cultivars (brassica) with a range of glucosinolate concentrations (7–524 $\mu\text{mol g}^{-1}$ f. wt in roots) were grown together with the VA mycorrhizal fungi *Glomus mosseae* and *Gigaspora gigantea* in agar. Fungal growth was observed *in situ* but fungi did not penetrate brassica roots. However, normal germ tube growth of the VA mycorrhizal fungi occurred near brassica roots when compatible hosts grew nearby. These compatible hosts developed normal mycorrhizal infections. These results suggest that brassica roots do not produce a diffusible inhibitor of VA mycorrhizal fungi, but lack a diffusible growth stimulus present near roots of compatible hosts.

Key words: Glucosinolates, VA mycorrhizal fungi, Cruciferae, *Brassica*, VA mycorrhiza non-host interaction.

INTRODUCTION

There has been general speculation that the sulphur-containing glucosinolates and/or their labile hydrolysis products, which control many interactions between members of the Capparales, their plant competitors, their pathogenic fungi (reviewed by Glenn, 1983), and their insect herbivores (reviewed by Chew, 1988b), are also responsible for the non-mycorrhizal status of the Capparales (e.g. Bevege & Bowen, 1975; Hayman, Johnson & Ruddledin, 1975; Iqbal & Qureshi, 1976; Malloch, Pirozynski & Raven, 1980; Medve, 1983; St. John & Coleman, 1983).

Field and glasshouse experiments designed to reveal whether cruciferous crops or their residues inhibited growth of VA mycorrhizal fungi in companion plantings of host crops produced negative results (Ocampo, Martin & Hayman, 1980; Ocampo & Hayman, 1981; Powell, 1982). While these studies show that cruciferous crops produce no diffusible inhibitor of growth of VA mycorrhizal fungi, they did not examine the nature of the block to root

colonization, nor did they address the involvement of sulphur compounds.

We tested the hypothesis that glucosinolate content is negatively correlated with establishment of symbiosis between VA mycorrhizal fungi and plants in the Capparales. We examined the response of representatives of two genera of VA mycorrhizal fungi to roots of *Brassica* (a representative genus of the Capparales) cultivars grown in agar. We used a non-destructive method for *in situ* observation of fungal hyphae growing near *Brassica* roots. We focused on the following questions: (1) How do germinating VA mycorrhizal fungi respond to roots of *Brassica* (non-host) and roots of hosts? (2) How do germinating VA mycorrhizal fungi respond to *Brassica* roots containing varying concentrations of glucosinolates? (3) How does the proximity of *Brassica* roots affect the growth of VA mycorrhizal fungi in hosts that normally support symbiosis?

MATERIALS AND METHODS

Fungal and plant cultures

Collection of resting spores and maintenance of fungal cultures of *Gigaspora gigantea* (Nicol. and

* Present address: Department of Biology, Seton Hall University, South Orange, New Jersey 07079, USA.

Gerd.) Gerdemann and Trappe, and of *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe, have been described (Glenn, Chew & Williams, 1985). Because VA mycorrhizal fungi are obligate symbionts, stock cultures were maintained on living host roots. Host seedlings were inoculated with spores of a single fungus species and the two partners were grown in pots of steamed soil. Spores were harvested after four to six months by removing a core of roots from the soil and wet sieving (Gerdemann & Nicolson, 1963) through sieves of mesh sizes 500, 100 and 75 μm . Resting spores were sucked out of the soil debris with a capillary tube attached to an aspirator or by dislodging spores from sporocarps by whirling the debris from the coarsest sieve in a Waring blender for 2–3 s. Spores or sporocarps were surface sterilized in 10% hypochlorite for 2–3 min (spores) or 30–60 min (sporocarps) and rinsed three or four times in sterile distilled water.

Tobacco, *Nicotiana tabacum* L. cv. Burly and tomato, *Lycopersicon esculentum* Mill. cv. Bonnie Best were chosen as model host plants because they produce roots that remain transparent, and shoots that remain compact under the experimental conditions. Five cultivars of *Brassica* were used as non-host models. Three cultivars of canola, Canadian rapeseed (*Brassica campestris* L. cvs. Regent and Candle, and *Brassica napus* L. cv. Altex), had been bred for low glucosinolate concentrations in their seeds and low erucic acid fractions in the seed oil. Two other cultivars of *Brassica* (*B. campestris* cv. Torch, and *B. napus* cv. Midas) were unselected for glucosinolate concentrations. The common term brassica [agricultural crops belonging to the genus *Brassica* (Whitehouse, 1977)] will be used for all these cultivars. Seeds were obtained from A. J. Classen of the Agriculture Canada Research Station, Saskatoon, and from B. R. Stephansson, Department of Plant Science, University of Manitoba, Winnipeg.

Concentrations of glucosinolate in roots were measured for each cultivar, after plants were grown for six weeks in aerated nutrient salts solutions, with and without sulphur (Glenn *et al.*, 1985). Measurement of total glucosinolates, based on glucose release upon glucosinolate hydrolysis, was performed by D. Carlson and H. Tookey at the USDA Northern Regional Research Center, Horticultural and Special Crops Laboratory, Natural Toxicants Research Group, Peoria, Illinois. The values (all $\mu\text{mol g}^{-1}$ fresh weight) for plants grown with sulphur were: *B. napus* cv. Midas, 524; cv. Altex, 186; *B. campestris* cv. Torch, 429; cv. Candle, 186. The values for plants grown without S ranged from 7–73.

Interaction of plant roots with germ tubes

We used two aseptic agar preparations to observe

growth of germ tubes of VA mycorrhizal fungi near roots: (1) using whole plants and (2) using excised cultured root tips. For culture of intact plants, plastic Petri plates (15 cm \times 15 mm or 10 cm \times 20 mm) were filled to a depth of 2–4 mm with 0.8% agar made with a modified Hoagland's nutrient solution containing a low concentration of phosphorus, with or without sulphate (Glenn *et al.*, 1985). Fungal spores set onto the agar surface germinated in 5 d for *Gi. gigantea* and in 10–14 d for *Gl. mosseae*. Aseptically germinated seedlings were placed near spores. Plates were elevated at an angle of approx. 30°, the lower half (where seedling roots grew) shielded from light by covering with black plastic, cardboard or foil, and incubated under continuous cool white fluorescent lights at 27 °C (Glenn, 1983). Some plates were sealed with parafilm, others were moistened by adding 10 ml sterile water after the first week. Both roots and germ tubes (hyphae arising from the spore prior to root penetration) grew through the agar to the surface of the bottom plate, and then along that surface. The plates were inverted for microscopic examination at 30–300 \times . Plates seldom remained aseptic, but most contaminants grew slowly on the mineral salts agar, and it was possible to maintain the plates for 6 weeks. By that time most plants had outgrown the available space.

Interactions between germ tubes and roots were monitored from the time of spore germination until successful mycorrhizas were established (in hosts) or until germ tube growth ceased (in non-hosts). As germ tubes and roots in various stages of development encountered each other, we observed rates and patterns of germ tube growth. All germ tubes of a spore were measured with an eyepiece micrometer every day or two for 2 weeks. The lengths of all germ tubes of a spore were summed and the data were plotted as length against time. Growth rate was estimated as the slope of the best straight line fit by least squares.

To examine the effect of companion host roots on germ-tube growth of VA mycorrhizal fungi near germinating non-host (brassica) roots, six tomato seeds were sown on the agar along the midline (diameter) of a 15 cm \times 15 mm Petri plate; agar contained low-phosphorus nutrient salts with sulphate present. A total of 15–20 *Gl. mosseae* spores were placed in the lower half of the plate. When the spores germinated (10–14 d) and tomato seedling roots were elongating into the agar, six brassica seeds were placed along the midline of the plate. These seeds germinated within 2 d. The density of germ tube branching was measured near the elongating tips of brassica roots. We also set up the reverse companion planting to examine the effects of brassica roots on fungal germ tubes growing near germinating tomato seeds. To ensure we observed effects exerted on VA mycorrhizal fungi by the rhizosphere or root

surface rather than possible effects of root cell contents, only spores whose germ tubes had not yet penetrated any host root were measured. Because VA mycorrhizal fungi have coenocytic cytoplasm, germ tubes which penetrated a root might confound the responses of other germ tubes (from the same spore) which had not yet penetrated roots. The following control plantings were grown with fungal spores: (1) brassica grown alone; (2) brassica grown alone with shoots excised to kill the roots (roots stopped growing and were presumed dead); (3) tomato grown alone.

In addition to using whole plants, we used excised, cultured roots in some experiments, following the methods of Hepper & Mosse (1975). Low phosphate nutrient solution was made with the following modifications: 2% sucrose was added; NaH_2PO_4 was replaced with $9.3 \text{ mg l}^{-1} \text{ KH}_2\text{PO}_4$ (pH was adjusted to 7.0). Excised root tips (10 mm) were incubated in liquid media at 28°C in the dark. To eliminate endogenous sulphur derived from the seed, root tips were grown in the liquid media with and without sulphate through three to six transfers. After approximately 1 week, the cultured root tips had grown and branched. Roots were cut into segments, and each segment with its laterals, was transferred. Sporocarps of *Gl. mosseae* were incubated on water agar at 23°C and germinated in 10–14 d. When spores began to germinate, the excised roots were transferred to solid media (culture media plus 0.8% agar) on Petri plates. The germinating sporocarps were lifted on a small block of agar and set beside the growing root. Plates were sealed with parafilm and returned to a dark, 28°C incubator. Interactions were observed at $30\text{--}300\times$ by inverting the plates under the microscope.

RESULTS

We describe first the growth of fungal germ tubes in agar, then the responses of *Gl. mosseae* and *Gi. gigantea* to host and non-host roots.

Germ-tube growth in agar

Growth of germ tubes of VA mycorrhizal fungi in agar was similar to growth described by others in agar or in soil (e.g. Mosse, 1962; Hepper & Mosse, 1975; Powell, 1976; Hepper, 1981; Koske, 1981). Individual resting spores of *Gl. mosseae* produced germ tubes by regrowth of the old subtending hypha. Lateral branches arose from these germ tubes and over the course of several weeks, formed a coenocytic network of exploratory hyphae covering a circle up to 2 cm diameter. Spores of *Gi. gigantea* produced one or more germ tubes directly through the spore wall. These germ tubes grew in a manner similar to those of *Gl. mosseae*, but the exploratory hyphae often grew 5–10 cm before encountering the

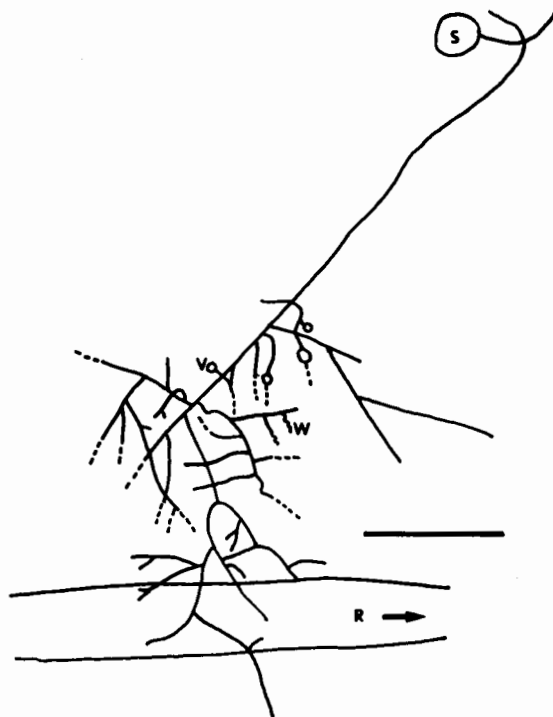


Figure 1. Germ tube branching of *Glomus mosseae* near a tobacco root (host), prior to penetration. A single germ tube is traced from its origin at the spore (S) to its contact with the host root (R). The root is growing towards the right (arrow). Some branches have a wavy appearance (W; see text). The cytoplasm becomes withdrawn in some branches which become septate (dotted lines) but other branches continue to grow. Vesicles (V) formed along the lateral branches of the germ tube are filled with cytoplasm from the branches, which now have septate ends (dotted lines). Tracings of germ tubes which did not intersect the root (upper right) are truncated. Scale line, $500 \mu\text{m}$.

side of the Petri plate (see Koske, 1982). After spore germination, the germ tubes (and plant roots) grew through the agar layer until they reached the bottom surface of the Petri plate. Further germ-tube growth then occurred along the surface of this bottom plate in contact with the agar layer. Occasionally, hyphal (germ tube) branches grew upward from the bottom of the plate into the agar layer. The upward-growing hyphae could be distinguished by their wavy 'corkscrew' appearance (Fig. 1) which may have been a response to increased resistance offered by the agar, compared with the resistance when growing in soil or in contact with agar along the bottom of the plate.

Undisturbed germ tubes grew unbranched for lengths of up to 28 mm. Encounter with a root surface, a root hair, or the Petri plate usually caused branching at the tip. Lateral branching occurred under many circumstances: proximity to another hypha or hyphal damage elicited anastomosis via short bridges; intersection of a root tip with a pre-existing germ tube elicited branches; hyphae branched near host roots. Profuse external branching (outside the root) occurred when a germ tube penetrated a host root, perhaps as a response to nutrient absorption through the arbuscules.

Table 1. Density of branches elicited on *Glomus mosseae* germ tubes by intersection with an elongating root tip

Plant and growing conditions	Inter-sections	Plates	Branches†
Tobacco	34	5	13.1 ± 3.5
Brassica grown with sulphur	40	7	6.6 ± 1.1
Brassica grown without sulphur	46	6	6.2 ± 1.4
Brassica (cv. Torch) grown with sulphur	198	9	3.2*

† The mean number of branches per intersection ± 95 % confidence interval is shown.

* Data from individual intersections in plates were summed so estimate of the 95 % confidence interval is not possible. The range of values per plate (mean density of branches/intersection per plate) was 1.3–5.4.

Growth of germ tubes of *Glomus mosseae* near host and non-host roots

There was a large variance in growth rates of germ tubes in both treatments, which probably reflects individual differences among spores. Mean growth rates for germ tubes near tobacco (host) roots was 5.2 ± 1.0 mm d⁻¹, which was not significantly different from the rate near brassica roots: 6.1 ± 1.2 mm d⁻¹. *B. campestris* cv. Torch was used. These results suggest that growth rates for the complete set of germ tubes of the spore is not influenced by proximity of host or non-host roots.

Table 1 shows the density of branches on germ tubes grown with host and non-host roots (with and without sulphur). In each Petri plate, intersections of elongating roots with pre-existing germ tubes that had not penetrated a root, were located. Within a 2 mm radius of the intersection, the number of branch points on the germ tube was counted. In a preliminary test, the high-glucosinolate cv. Torch appeared to inhibit exploratory germ tube branching as compared with other brassicas, but in subsequent work no differences between cultivars or sulphur treatments were detected. Individual germ tubes branched profusely in the 2 mm area surrounding host roots, and this branching was usually absent near brassica roots. Sample tracings of the germ tube

branching patterns around a host are shown in Figure 1. By comparison, brassica roots elicited fewer and shorter branches. Because this pronounced stimulation of hyphal activity close to host roots was small compared to the overall growth of exploratory germ tubes, it did not significantly alter the mean overall rates (length grown per day) of growth of germ tubes. Our observations confirm those of Powell (1976) who noted no directed growth of hyphae toward the host root in soil culture. Koske (1982) provided evidence for directed growth of aerial hyphae of *Gi. gigaspora*, but we did not observe aerial hyphae.

When a growing host root tip intersected a pre-existing germ tube, hyphal branches arose near the root surface. Table 2 shows the distance from the intersection of root and germ tube to the most distant germ tube branching point that arose after the intersection was formed (see Fig. 1). These distances therefore represent estimates of the radius within which germ tube branches are elicited by the root. Because germ tubes penetrated host roots within a day of intersection, observation of pre-penetration branching patterns involving tobacco were less common than those involving brassica. In tobacco, the branching occurred as much as 2–3 mm from the root surface, in contrast to brassica, where such branches were fewer (Table 1) and closer to the

Table 2. Maximum distance from root surface of hosts and non-hosts to branching points of pre-penetration *Glomus mosseae* germ tubes

Plants	Plates	Inter-sections	Mean max. distance*
Tobacco	4	14	2.7 ± 0.30
Brassica with S (<i>B. campestris</i> cv. Torch)	5	57	0.7 ± 0.15
Brassica without S (<i>B. campestris</i> cv. Torch)	5	60	0.7 ± 0.12

* The mean maximum distance from intersection to branch point, in mm ± 95 % confidence interval, is shown.

root (Table 2). With brassica roots, germ tubes grew up to the root surface and encircled (grew perpendicular to the root axis) or grew parallel to the root axis without contact. Germ tubes formed swellings resembling appressoria but there was no evidence of cell wall penetration. Formation of appressoria was not followed by profuse hyphal branching external to the root, nor by hyphal coils within the cortical cell walls. These events accompany root penetration in hosts and precede internal arbuscule formation.

Rapid formation of papillae on walls of cells resistant to fungal penetration has been suggested as a mechanism of active defence against pathogenic fungi (Zeyen & Bushnell, 1979). Electron microscopic examination of the non-host (brassica) root cells at points of contact with *Gl. mosseae* did not reveal papillae (Glenn *et al.*, 1985).

Growth of Gl. mosseae near excised roots of hosts and non-hosts

Near excised host (tomato) roots, germ tubes branched and penetrated roots. External wavy hyphae and external vesicles were produced near the points of host penetration. In contrast, germ tubes showed little response to brassica roots grown in either nutrient medium. Although germ tubes encircled and occasionally grew along the brassica root surface, there was no evidence of either penetration or extensive pre-penetration branching.

Near excised host roots, the fungus produced numerous fine, highly branched hyphal tufts. Mosse & Hepper (1975) reported observing similar hyphal tufts only near living, excised roots. They commented that these structures resemble internal arbuscules and Hepper (1983) suggested these structures may absorb nutrients from the agar. We also observed these tufts in the vicinity of bacterial contamination away from the roots. The contaminants may release enzymes that make agar carbohydrates available to the fungus, or the contaminants may release some metabolites that are absorbed by the fungal tufts. No contaminants were observed near the excised host roots where hyphal tufts were observed, so the tufts may have been elicited by some effect of the host rhizosphere. Such tufts were

never observed on plates with brassica except near contaminants. This result suggests that brassica roots fail to release some diffusible tuft-eliciting substance.

Hyphal branching of Gl. mosseae near non-host roots growing with host roots

Significantly more hyphal branches occurred near brassica roots when these were growing near tomato roots than occurred near brassica roots growing alone (Table 3 and Figs 2A, B). Data in Table 3 were obtained by counting the number of branch points per germ tube within a 2 mm radius of the intersection of a brassica root and a germ tube. There was no significant difference among the high and low glucosinolate brassica cultivars and the results are combined. In the reverse companion planting, designed to measure the density of germ tube branches near tomato seeds germinating in the presence of brassica roots, emerging tomato roots were so rapidly infected that we were unable to make comparable measurements. When tomato seeds were added to plates containing brassica seedlings, hyphal branching near the tomato radicle occurred even before the true leaves had expanded. Branch proliferation occurred up to 3 mm from the tomato roots, regardless of proximity to brassica roots.

Branch densities for germ tubes near young tomato seedlings (growing alone) were similar to densities near older tomato roots established as companions to germinating brassica. We observed relatively few pre-penetration root-germ tube intersections in tomato growing alone because these roots were so rapidly infected. These results support the idea that exudates of brassica roots lack some diffusible stimulant of growth of VA mycorrhizal fungi, rather than contain a diffusible inhibitor.

Germ-tube growth of Gi. gigantea near host and non-host roots

Interactions of *Gi. gigantea* with host and non-host roots were very similar to results obtained with *Gl. mosseae*. Hyphae of *Gi. gigantea* showed almost no response to brassica roots on media with or without

Table 3. *Effect of presence or absence of host (tomato) companion roots on the density of Glomus mosseae germ tube branches near brassica roots*

Plants	Intersections	Branches*
Brassica + tomato (intact plants)	168	10.8 ± 0.04
Brassica alone (intact plants)	262	3.2 ± 0.08
Brassica alone (shoots excised)	235	2.4 ± 0.04
Tomato alone (intact plants)	40	12.0 ± 1.40

* The mean number of such branch points per intersection ± 95% confidence interval is shown.

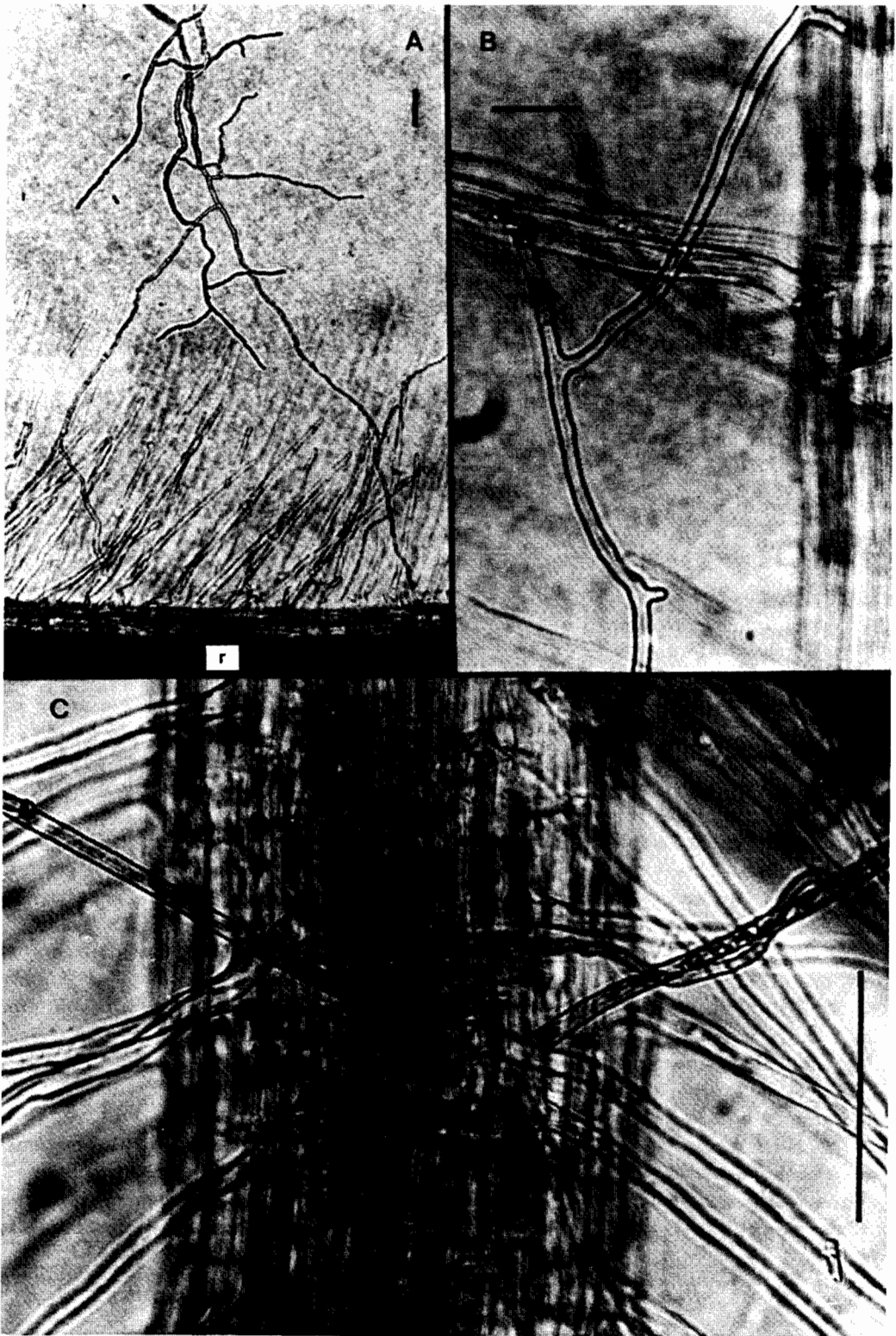


Figure 2. Hyphal growth patterns of VA mycorrhizal fungi near brassica roots growing amidst tomato roots. (A) Frequent, profuse hyphal branching, typical of that around a host root occurs near a healthy brassica root (r). The two longest branches, which grew up to the brassica root surface are empty, as shown by the septa. Due to the tangle of hyphae and roots, it is impossible to determine whether these branches arise from germ tubes or from hyphae attached to a penetrated tomato root. (B) Hyphal branching and appressorium formation on a brassica root hair (a). Branches are short, and the cytoplasm has been retracted, as indicated by septa. Formation of appressoria and branching are growth patterns which typically precede root penetration in hosts. With brassica these initial stages of the penetration process are aborted when the fungal cytoplasm is retracted and roots remain unpenetrated. (C) Hypha of *Gigaspora gigantea*, broken mechanically by an elongating brassica root (shown in the background), carried out normal hyphal repair mechanisms to bridge the break, although the hypha grew amidst root hairs close to the surface of the brassica root. Similar situations were observed in three cases on brassica roots growing without host roots nearby. Size bars, 100 μm.

sulphate, so studies comparing host and non-host interactions were qualitative. Germ tubes branched in response to host roots (tobacco and tomato). Penetration of host roots was followed by proliferation of external hyphae and development of hyphal coils and internal arbuscules. Most penetrations occurred within a few mm of the root tip, but on three occasions, the intersection of an elongating host root tip with a pre-existing hypha was followed by hyphal penetration of an adjacent older root, far behind the root tip. This observation suggests that the passage of the root tip elicited hyphal penetration of a root segment to which the hypha had been previously unresponsive. Such passage of a host root tip never led to penetration of an adjacent brassica root. Observation of *Gi. gigantea* suggests that brassica root exudates do not inhibit hyphal repair in VA mycorrhizal fungi. On three occasions when an elongating root tip severed a stationary hyphal tube, the fungus repaired the break with a hyphal bridge on or near the surface of the brassica roots (Fig. 2C).

DISCUSSION

Three general results emerge from our work. First, glucosinolate concentrations are not correlated with failure of VA mycorrhizal fungi to penetrate brassica roots. Second, brassica roots growing close to compatible host roots are not penetrated by hyphae of VA mycorrhizal fungi. Third, normal symbiosis develops in the compatible hosts grown in proximity to brassica. These results suggest that factors other than glucosinolates are important in determining compatibility of VA mycorrhizal fungi with plants in the Capparales. Our results support the idea that incompatibility between mycorrhizal fungi and brassica is not a by-product of a glucosinolate-related chemical defence as demonstrated against fungal pathogens (e.g. Greenhalgh & Mitchell, 1976; Holley & Jones, 1985). Further, our findings suggest that allelopathic effects of *Brassica* species (e.g. Bell & Muller, 1973; Iqbal & Qureshi, 1976) are not mediated by effects on growth of mycorrhizal fungi in companion (host) plants.

The block to fungal penetration of brassica roots growing in agar appears to involve two steps. (1) Brassica roots lack a diffusible fungal growth stimulant produced by normal hosts, and so fail to elicit hyphal proliferation in their rhizosphere, thus reducing the likelihood that exploratory hyphae contact the root surface, (2) When hyphae contact the root surface, symbiotic interactions fail to develop.

Elliot & Stowe (1971) showed that during 6 weeks of culture, sterile liquid media accumulated glucosinolates produced by *Isatis tinctoria* (woad) at concentrations more than six times that found in roots. These results suggest that substantial amounts of glucosinolates were probably present in the agar

surrounding brassica roots in our experiments, and supports the conclusion that failure to develop mycorrhizas is not due to a diffusible inhibitor.

Anatomically, root penetration by VA mycorrhizal fungi involves entry of the hypha into the root apoplast. The fungus does not penetrate the cell membrane or enter the root cytoplasm (e.g. Bonfante-Fasolo, 1982). Because cell walls and extracellular mucigel offer minimal barriers to the passage of small hydrophilic molecules, substances exuded from the cell membrane diffuse into the agar and may affect fungal growth. Schwab, Menge & Leonard (1983) suggest that this may account for the many observations of profuse hyphal branching near host roots grown in agar compared to soil culture (e.g. Mosse, 1962; Mosse & Hepper, 1975). Schwab *et al.* (1983) estimated the amount of exudate needed to support the symbiosis of the fungus in the apoplast, and suggested that in hosts, the level of exudation from the cell membrane is raised immediately following hyphal penetration into the apoplast. Our results suggest that brassica lacks a sufficient quantity of such an exudate to elicit normal hyphal proliferation in the rhizosphere. If host roots exude more stimulant(s) of fungal growth because their cell membranes become more permeable when plants are phosphate-deficient, brassica may lack such exudates because it is exceptionally tolerant to low phosphate concentrations (Nye, 1981; Grinstead *et al.*, 1982).

Although we observed no root penetration of living brassica cells in agar culture, Tommerup (1984; 1985) observed penetration of one or two cells in 4- to 8-week-old *B. napus* grown in soil and in sand. She suggested that initial stages of fungal colonization of the brassica root closely resembled colonization of the host *Trifolium subterraneum*, but occurred very slowly in non-host brassica. These results are consistent with reports of limited arbuscule formation in cruciferous crops and weeds (e.g. Iqbal & Qureshi, 1976; Medve, 1983; Ross & Harper, 1973; Tommerup, 1985).

Several possibilities might be explored in identifying possible cell-surface barriers to growth of VA mycorrhizal fungi. Firstly, effects of *in situ* isothiocyanate formation (Holley & Jones, 1985) need to be assessed. Isothiocyanates are the major aglycone product of glucosinolate hydrolysis (Ettlinger & Lundeen, 1956), but other products may be involved (reviewed by Fenwick, Heaney & Mullin, 1983; Chew, 1988a).

Secondly, cell surface lectins needed to permit plant-fungal recognition may be absent or deficient in this plant group. Lectins are implicated in specificity of plant infection by pathogenic fungi and bacteria (Sequeira, 1978). Bonfante-Fasolo (1982) has found that wheat germ agglutinin (a lectin that is specific for *N*-acetylglucosamine [chitin]) binds to the cell wall of the VA mycorrhizal fungus *Glomus fasciculatus*.

Thirdly, structural or chemical features of the cell

wall may inhibit fungal growth. For example, Heath (1977) studied growth of the rust fungus *Uromyces phaseoli* on leaf surfaces of eight non-hosts and on membranes mimicking leaf morphology. On most of the non-host leaves and on the membranes, hyphae grew along the surface but failed to penetrate the leaf. This result suggests that the non-hosts did not produce a stimulus necessary to elicit penetration. On cabbage leaves (*Brassica oleracea*) however, even hyphal growth was severely reduced, suggesting that cabbage leaves contain a fungal growth inhibitor not found in the other non-host species.

ACKNOWLEDGEMENTS

We thank C. M. Hepper for spores of *Glomus mosseae*. We thank D. P. Janos, K. A. Pirozynski, P. H. Raven, and I. C. Tommerup for correspondence, but we remain responsible for any errors. We thank J. P. Glyphis, R. K. Robbins and E. Stegmann for suggestions on the manuscript. We thank USDA (CGRO 7900435 to F. S. C.; University of Wisconsin Cooperative Agreement 58-519-b-0-904 to P. H. W.) and Tufts University (Dickens-Olmsted Fund to MGG) for partial support of this work.

REFERENCES

- BELL, D. T. & MULLER, C. H. (1973). Dominance of California annual grasslands by *Brassica nigra*. *American Midland Naturalist* **90**, 277-299.
- BEVEGE, D. I. & BOWEN, G. D. (1975). *Endogone* strains and host plant differences in development of vesicular-arbuscular mycorrhizas. In: *Endomycorrhizas* (Ed. by F. E. Sanders, B. Mosse & P. B. Tinker), pp. 77-86. Academic Press, New York, London.
- BONFANTE-FASOLO, P. (1982). Cell wall architecture in a mycorrhizal association as revealed by cryoultramicrotomy. *Protoplasma* **111**, 113-120.
- CHEW, F. S. (1988a). Biological effects of glucosinolates. In: *Biologically Active Natural Products for Potential Use in Agriculture* (Ed. by H. G. Cutter). American Chemical Society, Washington, D.C. (in the press).
- CHEW, F. S. (1988b). Searching for defensive chemistry in the Cruciferae. In: *Chemical Mediation of Coevolution* (Ed. by K. A. Spencer), pp. 81-112. Academic Press, New York.
- ELLIOT, M. C. & STOWE, B. B. (1971). Distribution and variation of indole glucosinolates in woad (*Isatis tinctoria* L.). *Plant Physiology* **48**, 498-503.
- ETTLINGER, M. G. & LUNDEEN, A. J. (1956). The structure of sinigrin and sinalbin; enzymatic rearrangement. *Journal of the American Chemical Society* **78**, 4172-4173.
- FENWICK, G. R., HEANEY, R. K. & MULLIN, W. J. (1983). Glucosinolates and their breakdown products in food and food plants. *CRC Critical Reviews of Food Science and Nutrition* **18**, 123-201.
- GERDEMANN, J. W. & NICOLSON, T. H. (1963). Spores of mycorrhizal *Endogone* extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46**, 235-244.
- GLENN, M. G. (1983). *Hyphal growth patterns of symbiotic fungi (vesicular-arbuscular mycorrhizae) near host and non-host (Cruciferae: Brassica) roots*. Ph.D. dissertation, Tufts University, Medford, Massachusetts, USA.
- GLENN, M. G., CHEW, F. S. & WILLIAMS, P. H. (1985). Hyphal penetration of *Brassica* (Cruciferae) roots by a vesicular-arbuscular mycorrhizal fungus. *New Phytologist* **99**, 463-472.
- GREENHALGH, J. R. & MITCHELL, N. D. (1976). The involvement of flavour volatiles in the resistance to downy mildew of wild and cultivated forms of *Brassica oleracea* L. *New Phytologist* **77**, 391-398.
- GRINSTEAD, M. J., HEDLEY, M. J., WHITE, R. E. & NYE, P. H. (1982). Plant induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. I. pH change and the increase in P concentration in the soil solution. *New Phytologist* **91**, 199-229.
- HAYMAN, D. S., JOHNSON, A. M. & RUDDLESIDIN, I. (1975). The influence of phosphate and crop species on *Endogone* spores and vesicular-arbuscular mycorrhizas under field conditions. *Plant and Soil* **43**, 489-495.
- HEATH, M. C. (1977). A comparative study of non-host interaction with rust fungi. *Physiological Plant Pathology* **10**, 73-88.
- HEPPER, C. M. (1981). Techniques for studying the infection of plants by vesicular-arbuscular mycorrhizae under axenic conditions. *New Phytologist* **88**, 641-648.
- HEPPER, C. M. (1983). Limited independent growth of a vesicular-arbuscular mycorrhizal fungus *in vitro*. *New Phytologist* **93**, 537-542.
- HEPPER, C. M. & MOSSE, B. (1975). Techniques used to study the interactions between *Endogone* and plant roots. In: *Endomycorrhizas* (Ed. by F. E. Sanders, B. Mosse & P. B. Tinker), pp. 65-75. Academic Press, New York, London.
- HOLLEY, R. A. & JONES, J. D. (1985). The role of myrosinase in the development of toxicity toward *Nematospore* in mustard seed. *Canadian Journal of Botany* **63**, 521-526.
- IQBAL, S. H. & QURESHI, K. S. (1976). The influence of mixed sowing (cereals and crucifers) and crop rotation on the development and subsequent growth of crops under field conditions. *Biologia (Pakistan)* **22**, 287-298.
- KOSKE, R. E. (1981). Multiple germination by spores of *Gigaspora gigantea*. *Transactions of the British Mycological Society* **76**, 328-330.
- KOSKE, R. E. (1982). Evidence for a volatile attractant from plant roots affecting germ tubes of a VA mycorrhizal fungus. *Transactions of the British Mycological Society* **79**, 305-310.
- MALLOCH, D. W., PIROZYNSKI, K. A. & RAVEN, P. H. (1980). Ecological and evolutionary significance of mycorrhizal symbioses in vascular plants (a review). *Proceedings of the National Academy of Sciences (USA)* **77**, 2113-2118.
- MEDVE, R. J. (1983). The mycorrhizal status of the Cruciferae. *American Midland Naturalist* **109**, 406-408.
- MOSSE, B. (1962). The establishment of vesicular-arbuscular mycorrhizae under aseptic conditions. *Journal of General Microbiology* **27**, 509-520.
- MOSSE, B. & HEPPER, C. M. (1975). Vesicular-arbuscular mycorrhizal infections in root organ cultures. *Physiological Plant Pathology* **5**, 215-223.
- NYE, P. H. (1981). Changes in pH across the rhizosphere induced by roots. *Plant and Soil* **61**, 7-26.
- OCAMPO, J. A. & HAYMAN, D. S. (1981). Influence of plant interactions on vesicular-arbuscular mycorrhizal infections. II. Crop rotations and residual effects of non-host plant. *New Phytologist* **87**, 333-343.
- OCAMPO, J. A., MARTIN, J. & HAYMAN, D. S. (1980). Influence of plant interactions on vesicular-arbuscular mycorrhizal infections. I. Host and non-host plants grown together. *New Phytologist* **84**, 27-35.
- POWELL, C. L. (1976). Development of mycorrhizal infection from *Endogone* spores and infected root segments. *Transactions of the British Mycological Society* **66**, 439-445.
- POWELL, C. L. (1982). Effects of kale and mustard crops on response of white clover to VAM inoculation in pot trials. *New Zealand Journal of Agricultural Research* **25**, 461-464.
- ROSS, J. P. & HARPER, J. A. (1973). Hosts of vesicular-arbuscular *Endogone* species. *Journal of the Elisha Mitchell Scientific Society* **89**, 1-3.
- SCHWAB, S. M., MENGE, J. A. & LEONARD, R. T. (1983). Quantitative and qualitative effects of phosphorus on extracts and exudates of sudan grass roots in relation to vesicular-arbuscular mycorrhiza formation. *Plant Physiology* **73**, 761-765.
- SEQUEIRA, L. (1978). Lectins and their role in host-pathogen specificity. *Annual Review of Phytopathology* **16**, 453-481.
- ST. JOHN, T. V. & COLEMAN, D. C. (1983). The role of mycorrhizae in plant ecology. *Canadian Journal of Botany* **61**, 1005-1014.