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Influence of Host Plant on the Morphology of the Vesicular-Arbuscular Mycorrhizal Fungus, *Glomus versiforme* (Daniels and Trappe) Berch

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Abstract

The contribution of host genome during the establishment of vesicular-arbuscular mycorrhizal associations was studied by colonizing roots of *Allium porrum*, *Triticum aestivum*, *Helianthus annuus* and *Medicago sativa* with *Glomus versiforme* under identical inoculum and environmental conditions. The morphology of appressoria, internal hyphae and arbuscules differed among the four host species in terms of shape, branching pattern and complexity. Vesicle morphology was similar among the host plants; however, in *Triticum aestivum* roots very few vesicles were formed over a 7 day infection period. The diameter of internal hyphae and arbuscules present in *A. porrum* and *M. sativa* roots were significantly ($P < 0.05$) smaller than those found in *T. aestivum* and *H. annuus* roots. There were no significant ($P < 0.05$) differences in diameter of trunk hyphae, appressoria or vesicles formed by *G. versiforme* among the four host species. Differences in morphology of arbuscles seemed to correlate with root morphology. Coarse, hairless roots contained arbuscules which produced many fine branches. Finer roots with many hairs contained coarse arbuscules which lacked many fine branches. These differences are likely due to the nutritional status of the host.

Keywords: *Allium*, *Glomus versiforme*, *Helianthus*, *Medicago*, *Triticum*, morphology, vesicular-arbuscular mycorrhizae

1. Introduction

Vesicular-arbuscular mycorrhizae (VAM) are among the most prevalent of all symbiotic associations in vascular plants and, because of their potential for crop improvement, are being studied from various perspectives (Powell and Bagyaraj, 1984). One aspect of the association that has received little attention, however, is the role that the symbiont genome plays during the colonization process. One approach to this question is to examine the association of a single VAM species with a range of host plants. Using this approach Bethlenfalvay et al. (1982) determined that the growth enhancement by VAM varied among host plant species: other researchers (Tommerup, 1984; Morton, 1985) have found that the rate and degree of colonization varied among host genomes. Additionally, Daniels-Hetrick and Bloom (1986) found that the host plant affected spore production and subsequent colonization ability of three *Glomus* species.

Several studies have considered the influence of the host genome on the fungal symbiont but the results are conflicting. Abbott and Robson (1979) and Morton (1985) found little effect of the host genome on endophyte morphology while several workers (Gerdemann, 1965; Hall, 1977; Sward, 1978; Jacquelinet-Jeanmougin and Gianinazzi-Pearson, 1983; Daniels-Hetrick et al., 1985) reported some effect of host genome on expression of morphological features in the fungal symbiont. Morphological variation of VAM associations involves the fungal symbiont primarily since few distinctive root (i.e. host) modifications occur, with the exception of some members of the onion family, where a yellow discoloration is apparent (Bonfante-Fasolo, 1984), and in *Allium cepa* in particular, where it has been shown by Fusconi et al. (1986), that root meristematic activity is depressed by mycorrhizal colonization.

Tommerup (1984) found differences in the development of internal hyphae, arbuscules and vesicles of *Glomus caledonium* (Nicol. and Gerd.) Trappe and Gerd. when this species colonized roots of either *Trifolium subterraneum* L. or *Brassica napus* L. This might be expected since *B. napus* is considered to be a non-host for VAM fungi (Gerdemann, 1968). It is interesting that early stages of infection of *B. napus*, including adhesion to the root surface and appressorium formation, although slower to develop than in *T. subterraneum*, appeared to be normal suggesting that there is no incompatibility in *B. napus* at this stage.

In some VAM associations, the fungal symbiont appears to vary little from host to host. *Gigaspora margarita* Becker and Hall showed similar morphological features in colonized roots of *Trachymene* Turcz and *Leptospermum* Sm.

(Sward, 1978). The main difference involved changes in the ultrastructure of hyphae as they penetrated tannin-filled exodermal cells in *Leptospermum*. In a comparison of two isolates of a *Glomus* species with three hosts, *T. subterraneum*, *Erodium botrys* (Cav.) Bertol and *Lolium rigidum* Gaud. under various nutritional conditions, Abbott and Robson (1979) found very little difference in the morphology of the fungal symbionts. These authors suggest that this *Glomus* species may be particularly stable since it has evolved in cultivated, fertilized Australian soils.

The genetic diversity of most VAM fungal species has not been explored and it is possible that species within one genus, e.g. *Glomus*, may show considerable variation.

The objective of this study was to determine the effect of four hosts: leek (*Allium porrum* L.), wheat (*Triticum aestivum* L. Thell), sunflower (*Helianthus annuus* L.) and alfalfa (*Medicago sativa* L.) on the morphology of one VAM species, *Glomus versiforme* (Daniels and Trappe) Berch by examining various stages in the association coupled with morphometric analysis.

2. Materials and Methods

Pot cultures

One year old, 6 litre pot cultures of *G. versiforme* were used. Pot cultures were established by colonizing roots of *Allium porrum* (leek) grown in Turface* using root inoculum of previously colonized leek plants (Brundrett et al., 1985). These pot cultures were maintained in a growth room at a constant temperature of 21°C with 16–8 hr light/dark cycle. Light, 125μE $m^{-2}s^{-1}$ at pot level, was provided by a mixture of cool-white and growlux incandescent tubes. Pot cultures were watered twice weekly with excess deionized water and once per week with 300 ml 1/2 strength Long Ashton nutrient solution. The pots were assessed for inoculum level repeatedly using young leek seedlings prior to the initiation of this experiment.

Host species

Due to seedling size and seedling vigour differences among the four host species, different ages of seedlings were used in order to minimize size differences among the host species.

* Montmorillonite clay, International Minerals and Chemical Corporation, Mundelin, Illinois, 60060

Allium porrum (leek) cv. "Titan" seeds were soaked overnight in sterile distilled water, rinsed several times with sterile distilled water, and placed on paper towels covering fine sand, which had been wetted with distilled water. The seedlings were grown on the paper towels in a sealed container for 5–7 days, after which they were planted in flats containing Turface. At 14 days of age, the seedlings were transplanted to the culture pots.

Triticum aestivum (wheat) cv. "Fredrick" seeds were placed in petri plates on sterile filter paper wetted with 5 ml of sterile distilled water. After 4–5 days the seedlings were planted directly into the *G. versiforme* culture pots.

Helianthus annuus (sunflower) cv. "Russian Mammoth" seeds were husked and surface sterilized in 10% Javex TM (Sodium hypochlorite 5.25%) for 1 min, then rinsed several times in sterile distilled water. The seeds were placed in petri plates on sterile filter paper wetted with 5 ml of sterile distilled water. The seedlings were grown for 2–3 days in the petri plates and were then planted in flats containing Turface. After 7 days, the seedlings were transplanted into the culture pots.

Medicago sativa (alfalfa) cv. "Olinda" seeds were surface sterilized for 8–10 min in 10% Javex. The seeds were then rinsed several times in sterile distilled water and placed in petri plates on sterile filter paper wetted with 5 ml of sterile distilled water. After 4–5 days, the seedlings were planted in flats containing Turface. After 14 days, the seedlings were transplanted into the *G. versiforme* pot cultures.

Morphology comparison

Four culture pots were used and seedlings of each host species were planted randomly in one of the four culture pots. This was repeated 3 times; each time the host species was assigned randomly to a pot. Seedlings of all host species were removed following 7 days of growth in the culture pots.

Roots were excised from shoots at time of harvest and were fixed in formalin-acetic acid-alcohol (FAA) for a minimum of 2 hr before being rinsed in tap water and cleared in 5% potassium hydroxide at 121°for 12 min. The roots were again rinsed several times in tap water and placed in 0.1% Chlorazol Black E stain (made up in a lactic acid-glycerine-water solution). The roots were stained for 1 hr at 63°C (Brundrett et al., 1985), and were then destained in glycerine. At least 20 cleared and stained roots selected at random for each host were mounted on microscope slides using PVLG mounting medium (Omar et al., 1979), and were photographed using Nomarski

interference optics on a Leitz vario-orthomat photomicroscope.

The diameters of internal hyphae, appressoria, arbuscules, trunk hyphae and vesicles were measured using a calibrated eyepiece micrometer on a Leitz vario-orthomat photomicroscope. Appressoria were measured at the point at which the external hyphae began to swell. Arbuscules and vesicles were measured along their longest axis. Several colonized roots from each host species were used to obtain 20 measurements of each fungal structure. Variances were analyzed following a log transformation to normalize the data. The transformed means were compared using a Duncan's Multiple Range Test, $P = 0.05$.

3. Results

Leek

Appressoria were simple, with a lenticular swelling at the site of hyphal attachment (Fig. 1). Penetration occurred through the outer tangential wall of epidermal cells or between epidermal cells. Inter- and intracellular hyphae were straight, with many pegs and hyphal bridges (H-pieces) (Fig. 5). Arbuscules were globose in overall morphology, and several major branches radiated from the central trunk hypha (Fig. 9). Many fine dichotomizing branches developed from the major branches (Fig. 9). Vesicles were elliptical with a large oil drop in the centre, and were formed terminally on inter- and intracellular hyphae.

Wheat

Appressoria were simple and hyphae penetrated the root either through epidermal cells directly or via root hairs (Fig. 2). Inter- and intracellular hyphae were straight with few short pegs (Fig. 6), and several hyphal bridges. Arbuscules were extremely elongate in overall morphology, with one large central trunk hypha giving rise to many very fine branches (Fig. 10). Vesicles were elliptical and contained a large oil drop in the centre. They were formed terminally on inter- and intracellular hyphae. There were few vesicles formed at harvest-time as compared to those formed in the other host genera.

Sunflower

Appressoria were seldom simple, often lobed, and penetration occurred directly through epidermal cell walls, or between adjacent epidermal cells (Fig. 3). Inter- and intracellular hyphae were extremely convoluted and gave rise to many short pegs (Fig. 7). The arbuscules consisted of a main trunk hypha which bifurcated to form two groups of coarse branches (Fig. 11). Elliptical vesicles formed terminally on inter- and intracellular hyphae.

Alfalfa

Appressoria were generally lobed, seldom simple. Hyphae penetrated directly through epidermal cell walls, or between adjacent epidermal cells (Fig. 4). Inter- and intracellular hyphae were straight, with short pegs and some hyphal bridges (Fig. 8). Arbuscules were slightly elongate; one short central trunk hypha gave rise to several main branches which in turn formed many fine branches (Fig. 12). Vesicles were elliptical with a large oil drop in the centre. They were formed inter- and intracellularly as terminal swellings on hyhphae.

Morphometry

G. versiforme formed internal hyphae which were significantly ($P > 0.05$) different in size when in association with *A. porrum* and *M. sativa* than with *T. aestivum* and *H. annuus* (Table 1). Intercellular spaces were not measured, but it is likely that the diameter of intercellular hyphae is partially a function of the space available. Similarly, arbuscules formed by *G. versiforme* in *A. porrum* and *M. sativa* were ($P < 0.05$) significantly different in diameter than those formed in *T. aestivum* and *H. annuus.* Although cortical cells containing arbuscules were not measured, it is believed that arbuscule diameter is not strictly due to cortical cell size, since mature arbuscules usually did not fill the cell in which they were formed. There were no significant differences in the diameters of appressoria, trunk hyphae or vesicles formed by *G. versiforme* with the four host species (Table 1).

Table 1. Mean diameter (μm) of *Glomus versiforme* structures in four host species

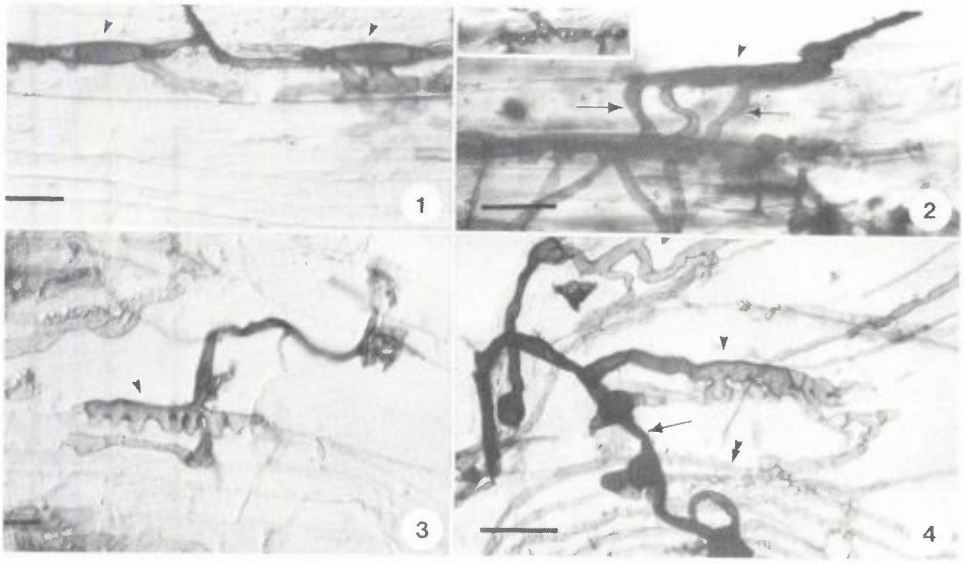
Host Species	Internal Hyphae	Appressoria	Arbuscules	Trunk Hyphae	Vesicles
Leek*	0.57+(3.72) †a**	0.95 (8.91)a	1.87 (74.13)a	0.66 (4.57)a	1.65 (44.67)a
Wheat	0.75 (5.62)b	1.01 (10.23)a	2.09 (123.03)b	0.69 (4.90)a	—
Sunflower	0.78 (6.03)b	1.03 (10.72)a	1.89 (77.62)b	0.69 (4.90)a	1.70 (50.12)a
Alfalfa	0.60 (3.98)a	1.02 (10.47)a	1.74 (54.95)a	0.68 (4.97)a	1.66 (45.71)a
S.E.	0.03	0.04	0.02	0.02	0.02

* Leek (*Allium porrum* L.), Wheat (*Triticum aestivum* L. Thell), Sunflower (*Helianthus annuus* L.), Alfalfa (*Medicago sativa* L.)

\+ Log transformed mean diameter in μm (n=20)

† Numbers in parentheses are de-transformed means

** Means within a column with different letters are significantly different at $P = 0.05$ using a Duncan's Multiple Range Test on the transformed means.



Figures 1–4. Appressoria of *Glomus versiforme* in cleared, stained roots. All bars represent 50μm.

Figure 1. *Allium porrum* root. Two simple appressoria (arrowheads) have formed from one branched hypha.

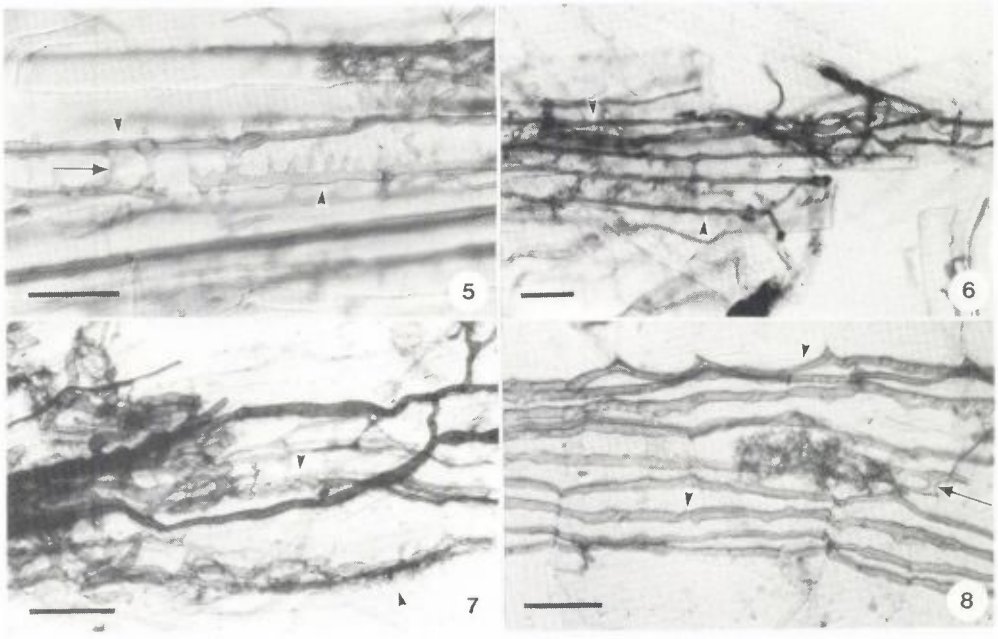
Figure 2. *Triticum aestivum* root with a simple appressorium (arrowhead). Three penetrating hyphae (arrows) have developed from the appressorium. Inset shows a hypha which has entered through a root hair.

Figure 3. *Helianthus annuus* root with a lobed appressorium (arrowhead).

Figure 4. *Medicago sativa* root. A lobed appressorium (arrowhead), external hyphae (arrow) and internal hyphae (double arrowhead) are evident.

4. Discussion

In order to compare the effects of host genome on a VAM symbiont, all host-fungus studies must be done under identical conditions since various factors such as phosphate concentration (Mosse, 1973; Hayman, 1983), light (Furlan and Fortin, 1973) and temperature (Hayman, 1974) can modify the association. The method used to colonize seedlings in this study is very convenient for assessing colonization rates and for comparative studies of host-VAM interactions. Since all culture pots were started from the same in-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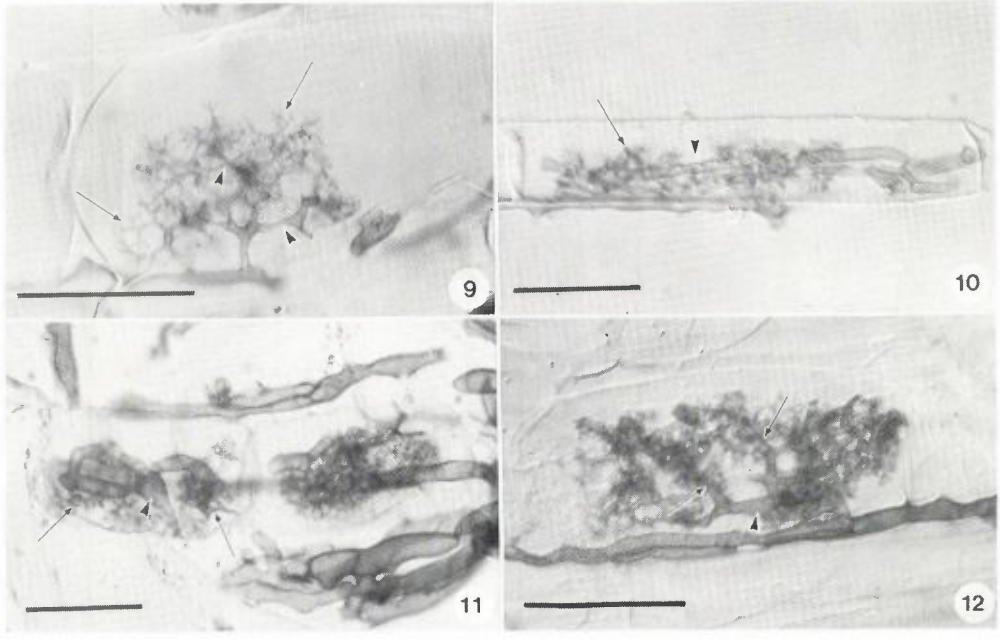
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Figures 5–8. Internal hyphae of *Glomus versiforme* in cleared and stained roots. All bars represent 50μm.

Figure 5. *Allium porrum* root. Straight internal hyphae with numerous pegs (arrowheads) and hyphal bridges (arrows) have formed.

Figure 6. *Triticum aestivum* root with straight internal hyphae (arrowheads) with few, short pegs.

Figure 7. *Helianthus annuus* root. Numerous convoluted internal hyphae with short pegs (arrowheads) are present.

Figure 8. *Medicago sativa* root. Many straight internal hyphae with short pegs (arrowheads) and a few hyphal bridges (arrow) have developed.

Figures 9–12. Arbuscules of *Glomus versiforme* in cleared and stained roots. All bars represent 50μm.

Figure 9. *Allium porrum* root cortical cell. A globose arbuscule consisting of several major branches (arrowheads) which divide to form many fine branches (arrows) is present.

Figure 10. *Triticum aestivum* root cortical cell with an elongate arbuscule with one central trunk hypha (arrowheads) which gives rise to many fine branches (arrow).

Figure 11. *Helianthus annuus* root cortical cells with arbuscules. The trunk hypha (arrowhead) bifurcates to form groups of coarse branches (arrow).

Figure 12. *Medicago sativa* root cortical cell. The arbuscule has a central trunk hypha (arrowhead) which forms several main branches (arrows), which in turn give rise to many fine branches.

oculum source, genetic diversity of the inoculum should be at a minimum. In this study, four host-*G. versiforme* associations were synthesized under identical inoculum and environmental conditions. Differences in the morphology of the fungal symbiont in different hosts were obvious and included shape and complexity of appressoria, straightness of internal hyphae, shape and branching of arbuscules, and amount of vesicle formation over the infection period. The differences among the four host species were distinctive enough such that it was possible to distinguish the host involved in the VAM association by studying colonized roots which were cleared and stained. These observations agree with those of Gerdemann (1965), Hall (1977), Jacquelinet-Jeanmougin and Gianinazzi-Pearson (1983) and Daniels-Hetrick et al. (1985).

Although a time course study was not included, differences in rate of colonization and the development of internal VAM structures were noted. In particular, the difference in vesicle production in wheat was striking. After 7 days in the culture pots, very few vesicles formed in wheat roots compared to numerous vesicles in the other host species.

Plants can be rated as to their mycorrhizal dependency (Gerdemann, 1975); this depends in part on root geometry and root hair production (Mosse, Hayman and Arnold, 1973; Baylis, 1975). In general, plants with coarse, hairless roots are more dependent on mycorrhizae for optimal growth, particularly under low phosphate conditions, than plants with finely branched root systems and numerous root hairs. Since hyphae extend the zone around the root from which ions can be absorbed (Hayman, 1983) it might be expected that hairless roots colonized by VAM fungi would have more external hyphae than roots with abundant root hairs. Differences in arbuscule morphology might also be expected since these are the components of "infection units" (Cox and Sanders, 1974) known to be involved in nutrient exchange (Bonfante-Fasolo, 1984).

We have found that there is a general correlation between root morphology and arbuscule morphology. Leek plants which have coarse, hairless roots possess many small, finely branched arbuscules; wheat and sunflower seedlings form a large number of coarse roots with many root hairs and the fungal symbiont forms fewer large, coarsely branched arbuscules; alfalfa seedlings have a fine root system with a moderate development of root hairs and is intermediate in arbuscule morphology.

Mycorrhizal dependent plants may have evolved VAM associations in which the exchange interface is enhanced by the finely branched nature of the arbuscules. This needs to be tested with several associations using

morphometric analyses. Physical restraints imposed by the host on the fungal structures in terms of size cf cells and intercellular spaces may play a part in determining the ultimate size and shape of the particular structures. However, three of the structures measured in the present study did not indicate significant differences among the four host species. Thus, limitations due to size may not be a significant factor in the association. It is probable that morphological and size differences of the fungus between hosts are due to the interaction of the host's nutritional environment and its genome. Additional host-fungus combinations need to be synthesized to determine the contribution of the host genome to the development of the VAM association.

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