Replanting of Vineyards and its Relationship to Vesicular-Arbuscular Mycorrhiza

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ABSTRACT

Grape vesicular-arbuscular mycorrhiza is ubiquitous in New York State vineyards. *Endogone* sp. invades grape roots within 15 days after appearance of the roots in infested soil. The fungus eventually forms numerous arbuscules within the inner cortical cells, and the over-all appearance of the mycorrhiza is that of the Arum type. Presence of old grape roots in the soil, proximity of replant vine to location of a previous vine, and shorter time lapse between old vine removal and replanting increased frequency of occurrence and intensity of mycorrhizal development in replant vines. The addition of fertilizer to replanted soil decreased occurrence and intensity of the mycorrhiza.

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Additional key words: glycol methacrylate embedding, specific replant disease.

Vesicular-arbuscular mycorrhiza, common to many species of flowering plants, has received much study in recent years (4), but there have been no studies on the role of this fungus-root association in replantings of perennial fruit and the possible connection between the fungus, *Endogone*, and specific replant disease.

MATERIALS AND METHODS.—Root material for examination of anatomical detail of the mycorrhiza and for the determination of geographical and cultivar distribution of the mycorrhizal association was collected from vineyards in the Lake Erie and Finger Lakes districts of New York State. These roots were fixed in formalin-propiono-alcohol (FPA), dehydrated in *n*-butanol, embedded in 55-C Tissuemat, sectioned at 10 or 15 μ, and stained with safranin/fast green.

Roots from greenhouse-grown experimental plants were fixed with acrolein and dehydrated with methyl Cellosolve and an alcohol series (1) in preparation for histological examination. At least five root pieces from each plant were selected. After dehydration, two, three, or four root pieces were aligned in 00 gelatin capsules and embedded in glycol methacrylate. After polymerization of the plastic, 3-μ thick sections were cut with glass knives, mounted, and stained with toluidine blue 0 in a sodium benzoate buffer (6), and the percentage of root pieces with mycorrhiza was determined microscopically. Each treatment of two experiments was evaluated for intensity of mycorrhizal development on a 1 = light, 2 = moderate, 3 = heavy scale. The rating of each treatment was calculated by dividing the number of roots with mycorrhiza into the total score for that treatment.

RESULTS.—*Vesicular-arbuscular mycorrhiza of grapes in New York.*—All 20 vineyards sampled contained roots with vesicular-arbuscular mycorrhiza caused by *Endogone* sp. Roots collected in summer or fall always contained mycorrhiza, but those collected in early spring were sometimes without mycorrhiza.

Every cultivar studied had mycorrhiza that was evident in the first sample of roots collected. The cultivars were as follows: *Vitis lubrusca* 'Concord', 'Delaware', and 'Catawba'; French hybrid cultivars, Seibel 1000, Seibel 5279, and Seibel 10898; and rootstock cultivars, Baco, Couderic 3309, and Geneva 1613.

The details of ingress into grape roots by Endogone.—Concord seedlings were potted in vineyard soil and harvested after 6, 10, 15, 21, and 30 days. The root systems were gently washed in water and prepared for microscopic examination as described by Gerdemann (3). Observation of ingress by *Endogone* sp. and other organisms associated with the seedling roots was made with a dissecting microscope. Selected specimens were studied more intensively with a compound microscope.

After 6 days' growth, aseptate Endogone-type hyphae were sparsely coiled around the roots, septate hyphae were numerous, and a few nematodes without stylets and larvae of *Meloidogyne* sp. had penetrated the outer cortical tissue. After 10 days, roots were encoiled by abundant aseptate hyphae, and were appressed with a few hyphae. Synemata of *Stilbum* sp. were attached by short rhizoids to some roots. Rhabditoid nematodes and *Meloidogyne* sp. were more abundant within the roots of this sample. After 15 days, some fully formed appressoria were noted, and in some cases penetration was completed. After 21 days' growth still more appressoria were evident, some newly formed external vesicles were present, ascocarps of *Chaetomium* were associated with sites of phylloxera infestations, and more *Stilbum* synemata were seen. An alga similar in appearance to *Oscillatoria* (Cyanophyta) was locally abundant on the roots harvested after 15, 21, and 30 days' growth.

Internal anatomy of grape mycorrhiza.—The mycorrhiza of New York grapes is the Arum type (2). The fungus gains ingress by rounded, knee-shaped appressoria (Fig. 1-A). Invading hyphae penetrate epidermal cells and ramify, mostly longitudinally, intra-, and intercellularly through the outer cortical cells (Fig. 1-A, C). The hyphae (width, 2.5-7.7 μ) branch toward inner cortical cells where scanty to abundant intracellular arbuscules develop from

TABLE 1. Effect of old grape roots on mycorrhizal development in rootlets of grape seedlings

Grape variety	Vineyard soil	% Root pieces with mycorrhiza		
		Terminal (younger) rootlets	Other (older) rootlets	Roots on or in old grape root pieces
Concord	Without old grape roots	76%	14%	
Concord	With old grape roots	77%	68%	85.2%
Delaware	Without old grape roots	25%	7%	
Delaware	With old grape roots	89%	90%	69%

terminal or lateral hyphal branches (Fig. 1-B). Arbuscules have a main trunk and several primary branches, each bearing numerous smaller ones (Fig. 1-B); this branching may extend beyond the fifth set of branchlets. Typically, a group of arbuscular branches surround the enlarged host nucleus that has a prominent nucleolus. Arbuscular development does not usually involve the innermost layer of cortical cells (Fig. 1-C). The phenomenon referred to as "digestion" (disintegration of arbuscules) was observed frequently. Internal vesicles were present in some greenhouse-grown roots.

Effect of old grape roots on mycorrhiza.—Concord seedlings were potted in screened vineyard soil. Another set of seedlings was planted into soil of the same source, to which was added numerous pieces of old grape roots. The gravel-clay soil was screened and stored in covered metal containers outdoors for 1 year prior to use to reduce phylloxera and fungal levels without our resorting to sterilization or fumigation. Root samples were collected after 55 days and treated as described earlier. Mineral analysis of leaf petioles was made by photoelectric spectrometer (5).

Roots from soil without roots were 9% mycorrhizal as compared with 82% for those from soil plus old roots. Vines from the former soil were smaller, and averaged 5 g (fresh weight). Those from the latter averaged 21 g. Vines from the latter also contained twice as much phosphorus and potassium as did vines with nonmycorrhizal roots, but levels of Ca, Mg, Na, Zn, Fe, Cu, B, and Al were lower, based upon total dry weight.

Inoculum of *Endogone* had probably fallen to low levels in the stored soil, thus addition of old, woody root pieces, carrying a little soil and presumably propagules of *Endogone*, served to increase invasion and mycorrhizal development.

Effect of old roots on age of rootlets invaded by Endogone.—Concord and Delaware seedlings with pruned roots were planted in 5-inch pots filled with vineyard soil, and also in vineyard soil to which had been added old grape roots. After 60 days, each cultivar was sampled by selecting rootlets from three different locations on each root system. One sample consisted of adventitious terminal rootlets that developed from the pruned ends of the original roots. Another sample was taken from near the distal end of the rootlets that had originally been pruned. A third sample consisted of adventitious rootlets that grew on or into old root pieces in those pots with soil amended with such pieces.

There was more mycorrhiza in older roots of both Concord and Delaware from soil plus roots than from those grown in soil alone. Roots from samples in or on old root pieces also had extensive mycorrhizal development (Table 1).

Pruning made positive identification of root age possible, but pruning modifies hormonal balance, and this may have been a factor in the differences in mycorrhizal development.

Microenvironment, nutrient level, and mycorrhiza.—Eighteen two-bud Concord cuttings were planted in "in-row" and "between-row" vineyard soil and "fresh" nonvineyard soil in 5-inch plastic pots. Half the plants of each treatment received 0.04 g of N, P, and K twice during the experiment; the other half received no fertilizer. The plants were allowed to grow for 81 days in a greenhouse, watered daily, weeded frequently, and provided 16 hr light/day. Root samples were handled as described earlier.

Roots from unfertilized in-row, between-row, and fresh soils had about the same amount of tissue with mycorrhiza, but the mycorrhizal development rating was higher for roots from in-row than between-row soil (Table 2). Roots from between-row soil weighed nearly 60% more than those from in-row soil. Vines from fertilized soil had a lower percentage of mycorrhiza, lower rating for mycorrhizal development, and 63± % less phosphorus in leaf petioles than did unfertilized vines.

Mycorrhizal development in grape-free and replanted vineyard soil.—Soil was collected from an old vineyard where grapes had not been grown for 6 years, and from another area of the same vineyard replanted 3 years earlier with Delaware vines. Nine Concord cuttings were potted in each soil and allowed to grow for 78 days in a greenhouse; then the

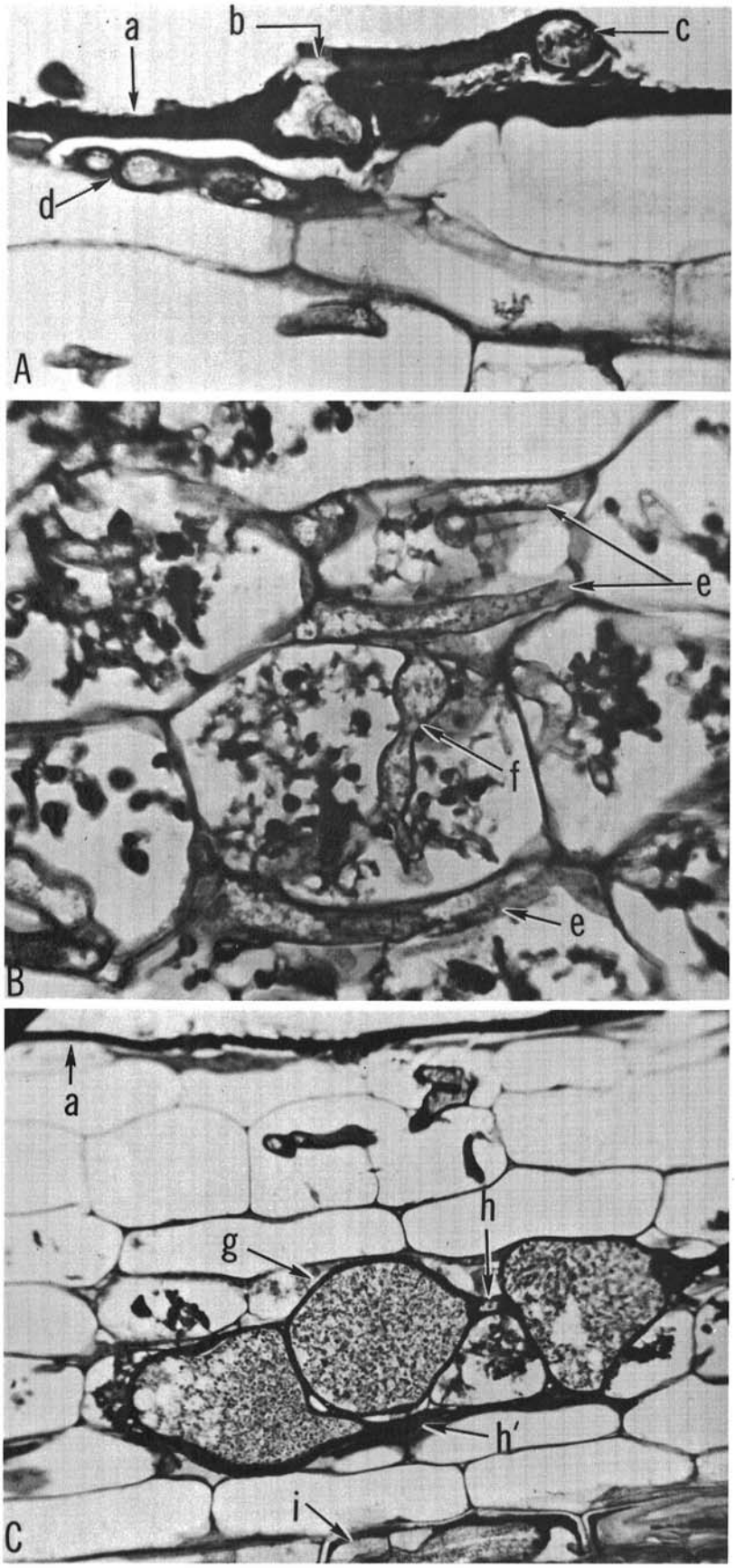
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TABLE 2. Comparison of mycorrhiza in Concord vines grown in "in-row" vineyard soil, "between-row" soil, and "fresh" nonvineyard soil, and the effect of fertilizer on mycorrhizal development

Soil condition	Fertilization[a]	No. root pieces sectioned	Mean plant root weights	% With mycorrhiza	Mycorrhizal development rating[b]
Fresh	–	53	5.53	62%	1.66
	+	65	11.72	55%	1.14
In-row	–	62	3.1	64%	2.10
	+	65	5.9	42%	1.46
Between-row	–	54	5.26	76%	1.29
	+	62	6.96	44%	1.07

[a] At rate determined by soil tests.
[b] Rating scale: 1 = light; 2 = moderate; 3 = heavy.

roots were harvested, sectioned, and examined for mycorrhiza as described earlier.

Fifty-five per cent of root pieces from vines grown in grape-free soil had mycorrhiza, as compared to 87% of those from vines grown in replanted soil. The mycorrhizal development rating for roots from grape-free soil averaged 1.22; for the replanted, 1.68. The vines from replanted soil had root systems averaging 14% heavier than those from grape-free soil, but roots from replant soil were heavy with phylloxera galls. Mycorrhiza developed in close proximity to a phylloxera gall, but not in the gall itself.

DISCUSSION.—Vesicular-arbuscular mycorrhiza is abundant in the roots of grape, and affects the physiology and especially the nutrient balance of grape. The intensity of mycorrhizal development is greater in replant vines exhibiting severe symptoms of grape-specific replant disease than in those not so severely affected. The mycorrhiza is also more abundant and intense in its development in healthy pot-culture vines grown in old vineyeard soil amended with pieces of old grape roots. This apparent contradiction raises the important question of whether vesicular-arbuscular mycorrhiza is a parasite or a beneficial symbiont. Further studies on the relationship between vesicular-arbuscular mycorrhiza and specific replant disease are needed.

The findings that mycorrhiza is regularly abundant in commercial vineyards of satisfactory and exceptional vigor and that vigorous experimental plants have more mycorrhiza than do less vigorous plants suggests that mycorrhiza is beneficial, or perhaps even necessary for normal vine growth. However, caution must be exercised in drawing conclusions from our experimental data because of the presence of amendments of old roots in the soil of the healthy vines. Experiments using known quantities of inoculum of *Endogone* added to rested and replanted vineyard soil could help explain the role of vesicular-arbuscular mycorrhiza in new and replant vineyards.

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Fig. 1. Photomicrographs of mycorrhiza in grape roots. **A)** An appressorium (b) of *Endogone* sp. and attached hypha lying on the root epidermis (a), the hypha in cross section (c), and intraepidermal hyphae (d). **B)** Intercellular hyphae (e) and the main trunk of an arbuscule (f) with intensive branches. **C)** Internal vesicles (g) with attaching hypha longitudinally sectioned (h) and hyphal wall (h'); epidermis (a) and endodermal cell (i). Note *Endogone* hyphae in outer cortical cells above and between (g) and (h).