A Survey of Biotic Relationships in Grape Replant Situations

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ABSTRACT

Bacteria and fungi were more numerous around grape roots growing in replant soil than around those growing in "fresh" nonvineyard soil or rested vineyard soil. Species of *Penicillium, Fusarium, Gliocladium,* and *Roesleria hypogaea* were most commonly isolated. A fluorescent species of *Pseudomonas* was present in the wood of grape roots of high, medium, and low-vigor and dead roots. Amending vineyard soil with pieces of old grape roots suppressed populations of *Penicillium* spp., increased populations of *Tricoderma viride*, and improved growth of grape seedlings; roots of the seedlings had much greater development of endophytic, phycomycete mycorrhizae than those grown in soil minus the old roots. Fertilization did not overcome effects of specific replant disease of grape.

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Grape replant failures often result from the activity of large populations of certain microorganisms; e.g., phylloxera, pathogenic nematodes, Pythium, or Phytophthora (3). In other cases of replant failure, neither obvious biologic or physical factors have been identified; in such instances, the expression "specific replant disease" (5, 11), used to describe similar replant failures of other fruit species, is appropriate. Relatively little is known about the cause of, and microbial associations involved with, grape replant failures. The results of some comparative studies on microorganism populations in replant vineyard, established vineyard, and nonvineyard soils are reported in this paper.

MATERIALS AND METHODS.–*Isolation of fungi and bacteria.*–Fungi were isolated from pieces of grape roots plated on potato-dextrose agar (PDA), oatmeal agar(OA), water agar (WA), and Martin's rose bengal agar (RBA) (6). Roots were cleaned by vigorous washing prior to plating, and surface-sterilized with 1% solution sodium hypochlorite. Identification of fungi was accomplished with Barnett's (1) or Gilman's (4) keys: Species of *Fusarium* were identified according to Messiaen (7). Riddell (10) mounts were used when necessary. Media for bacterial isolations were nutrient agar (NA), PDA (pH-6.8), and soil extract agar (SEA).

Populations of bacteria and fungi in the various soils were compared by plating soil dilutions on plates of PDA, RBA, or SEA. Plates for bacterial counts were incubated in the dark at room temperatures; those for fungal counts were exposed to daylight. Populations were calculated for 1 g oven-dried soil, and compared on the basis of four plate means.

Greenhouse procedures.–Two-bud cuttings of cultivars Delaware, Concord, and Seibel 5279 were callused and rooted prior to planting in plastic pots. In one experiment, Concord seedlings from open-pollinated seed were used. All plants in each experiment were completely randomized on the bench, given 16 hr. fluorescent illumination each day, watered daily, and weeded frequently.

At harvest, the root systems were gently removed from the soil and washed with a water spray. Fresh weights of each root system and its respective shoot (minus original cane) were recorded. In some cases, roots were evaluated for density and general vigor using a scale of 0=dead, 1=poor, 2=fair, 3=good, 4=excellent. All soil of a treatment was thoroughly mixed before sample removal for estimates of microbial populations.

RESULTS.–*Fungi associated with grape roots.*–Root samples consisting of feeder rootlets with lesions, small woody roots, and phylloxera galls were collected throughout two growing seasons from numerous sites along Lake Erie and in the Finger Lakes district (the two major grape-producing areas of New York State). Roots were collected from depths of 2 inches to 2 ft.

Species of *Penicillium, Fusarium*, especially *F. oxysporum* and *F. solani*, and *Gliocladium* were among the most frequently isolated fungi. *Fusarium oxysporum* and several species of *Penicillium* occurred in all types of material studied. Nonsporulating, dark-colored fungi were common in some samples of feeder rootlets with lesions, but absent in others; such fungi were infrequently isolated from phylloxera galls. Similar species of Fungi Imperfecti are associated with roots of grapes in New York and California (3). *Pythium* spp. and *Phytopthora* spp. are commonly associated with irrigated grapes in California (3), but we isolated them only rarely from the nonirrigated vines in New York vineyards.

Fungi from phylloxera galls were frequently the same or similar to those found by Mil'ko (8) in Russia. In New York, *Cylindrocarpon radicicola, F. oxysporum, F. solani, Gliocladium roseum,* and *Penicillium* spp. are commonly associated with phylloxera galls and phylloxera-damaged roots.

The fall-fruiting ascomycete *Roesleria hypogaea* Thum. & Pass is abundant in New York vineyards. The numerous small gray ascocarps are easy to locate on large and small roots of vines low in vigor, and are frequently abundant on rootlets killed by phylloxera.

The fungus was usually isolated only if unrotted, but discolored, wood was used. Bits of bark or rotted wood bearing ascocarps, plated on any of the media, were quickly overgrown with species of *Fusarium, Penicillium, Gliocladium, Tricoderma, Cylindrocarpon*, or *Chaetomium*. The rapid growth of these fungi probably suppressed the slow growing *R. hypogaea* even though it was present in the tissue.

Microflora of living and dead grape roots.—Old grape roots, both living and dead, were collected in a vineyard from which Concord vines had been removed 2 years before. In most roots, the wood was still solid but discolored reddish or bluish-black. Some roots supported sprouts; nearly all bore numerous ascocarps of *R. hypogaea*. Large roots from very productive Concord vines and roots of less productive vines were collected from several vineyards. Roots were scrubbed vigorously with a stiff brush under running water prior to surface sterilization. Separate isolations were made from xylem and phloem.

Three bacterial colonies were randomly selected from each plate for gram staining and physiological tests. Each isolate was rated as oxidative or fermentative in glucose utilization, and on its ability to degrade cellulose (filter paper strips), to produce fluorescent pigment, and to rot white potato discs.

The bacterium most frequently isolated from the wood of grape roots in high, moderate, and low vigor, and from old roots from vineyards several years removed, was a species of *Pseudomonas* that produced a fluorescent pigment, oxidatively utilized glucose; did not degrade cellulose in a basal medium; and produced tan-colored soft rot on potato discs.

A somewhat variable group of gram-negative bacteria that usually utilized glucose fermentatively was isolated from dead bark and partially rotted wood of old roots. The fungi associated with this group of bacteria were mostly species of *Penicillium, Aspergillus*, and *Fusarium*; also found were the other genera previously listed as occurring on bark and rotted wood. The unrotted and sometimes apparently living sections of these same old roots produced isolates of *R. hypogaea, F. oxysporum*, and sometimes a dark-colored, nonsporulating fungus.

Effects of adding old grape roots to vineyard soils.—Concord seedlings were planted in vineyard soil which had been screened and stored in covered garbage cans outdoors for 1 year. Similar seedlings were planted in the soil amended with freshly collected, old grape roots. Plants were harvested after 55 days and weighed. Root samples were sectioned for observation of mycorrhizal development. The microflora of two samples of soil from each treatment were studied, using the procedures described earlier.

Seedlings grown in amended soil averaged 21 g fresh wt whereas those grown in nonamended soil averaged 5 g. Eighty-one per cent of the root pieces from the former were mycorrhizal as compared to 9% from the latter.

Quantitatively, the bacterial populations were nearly the same in the two treatments, but total fungi in the dilution plates were approximately 3 times greater in amended soil. The addition of grape roots to vineyard soil increased threefold the populations of *Penicillium*; this coincides with its abundance in bark and rotted wood of grape roots.

Comparison of soil microflora and vine growth in different soils.—Soil from an old Concord vineyard was collected in April 1969 by scraping away 1-2 inches of soil and removing a sample to a depth of 12 inches. Three types of soil were collected: "in-row" soil from immediately around seven or eight randomly selected vines; "between-row" soil from several places midway between two rows; and "fresh" soil from just outside the vineyard. The plant cover on the fresh soil was predominantly orchard grass, *Dactylis glomerata* L.

All soil of each location was thoroughly mixed, and roots were removed. Subsamples were taken for chemical analysis, and for counts and identification of bacteria, algae, fungi, and nematodes. Eighteen Concord and 18 Seibel 5279 cuttings were planted in each treatment in 5-inch pots. Nine plants of each cultivar in each treatment received 0.04 g each of N, P, and K in solution at 3, and again at 6, weeks after planting; rate was determined on the basis of soil tests.

From the April collection of soil, three separately selected soil samples of each soil type were used to prepare soil dilutions. Total fungal colonies were tabulated, and *Tricoderma viride* and *Penicillium* spp. colonies were counted individually. Modified Bristol's (2) solution was used to determine the presence of algae.

The bacterial population was 28% higher in in-row soil than that of between-row soil as determined by counts of both SEA and PDA plates. The greatest population was in the fresh soil; this was probably due to the orchard grass cover. Fungal populations on PDA were similar quantitatively and qualitatively in all three soils, and consisted primarily of species of *Aspergillus, Fusarium, Penicillium*, and *Tricoderma*. Both *T. viride* and *Penicillium* spp. were slightly more abundant in soil from around vines than from between-row or fresh soil.

The fresh soil had high counts of *Meloidogyne* sp. and *Helicotylenchus* sp. No species of nematode was present in high numbers in either sample from within the vineyard. A unicellular green alga, similar to *Protococcus*, was common to all samples.

The most significant difference in root and shoot weights was in the effect of soil source on each cultivar. As expected, the suppression of root growth was greatest in in-row soil. Also, as expected, the replant effect was most pronounced on unfertilized Concord vines. This was probably the result of Concord being replanted in old Concord soil. Also, Concord may be inherently more susceptible to specific grape replant disease than S. 5279.

A distinct difference in the pattern of growth of Concord, as compared to S.5279, can be seen in the root/shoot ratios of the two cultivars after growth in nonfertilized soils. The ratio for Concord ranged from 1:1.7 in fresh soil to 1:2.5 for in-row soil; but the ratio for S.5279 did not exceed 1:1.2 in any

TABLE 1. The influence of soil source and of fertilization on growth of Concord and Seibel 5279 vines

Cultivar	Soil	Fertilized	Mean root rating[a]	Mean root weight[b] (g)	Mean shoot weight (g)	Root/shoot ratio
Concord	R[c]	–	1.8	3.10[d]	7.84	1:2.5
	B	–	2.2	5.26	9.50	1:1.8
	F	–	2.4	5.53	9.31	1:1.7
	R	+	2.6	5.90	12.48	1:2.1
	B	+	2.7	6.96	13.55	1:2.0
	F	+	3.7	11.72	17.12	1:1.5
Seibel 5279	R	–	2.4	5.54	6.82	1:1.2
	R	+	2.7	6.71	13.46	1:2.0
	B	+	3.4	7.23	13.62	1:1.9
	B	–	3.3	7.33	6.83	1:0.9
	F	–	3.3	8.34	9.57	1:1.2
	F	+	4.0	10.65	16.03	1:1.5

[a] Rating scale: 1 = poor; 2 = fair; 3 = good; 4 = excellent.
[b] Ranked according to mean fresh root weight.
[c] R = "in-row" soil; B = "between-row" soil; F = nonvineyard soil.
[d] Means not connected by lines are significant at .05 level (analysis of variance; Tukey's w test).

TABLE 2. Population dynamics of *Tricoderma viride* and *Penicillium* spp. in "rested"[a] and "replanted"[b] vineyard soils through one growing season

Month of soil collection	Soil type	*Tricoderma viride* 1	*Tricoderma viride* 2	*Penicillium* spp. 1	*Penicillium* spp. 2
		Populations (10^2 colonies/g oven dry soil)			
April	Rested	732	854	366	348
	Replanted to Delaware	88	213	1,176	1,463
June	Rested	462	598	54	245
	Replanted to Delaware	27	54	1,011	1,263
August	Rested	804	595	179	119
	Replanted to Delaware	31	123	1,359	1,234

[a] All vines removed in 1963, 6 years prior to study.
[b] Original vines removed, and vineyard immediately replanted with Delaware in 1966.

treatment except fresh, fertilized soil, where it was 1:1.5. Furthermore, in every treatment, S.5279 produced heavier and more vigorous roots than did Concord (Table 1).

Comparison of microflora and vine growth in rested and replant vineyard soil.—Rested soil was collected from depths of 2 to 12 inches in April 1969 in an old vineyard from which Clinton vines had been removed in 1963. Vines were removed from another part of the same vineyard in late 1965, and the vineyard was replanted in June 1966 with rooted Delaware cuttings. Soil was also taken in April 1969 from around the Delaware replants exhibiting stunted growth and spindly canes, typical symptoms of specific replant disease. The weed cover on the rested soil was primarily sheep sorrel, *Rumex acetosella* L. and pineapple weed, *Matricaria matricarioides* (Less.) Porter. Subsamples of each soil were taken for soil tests and for nematode and soil microflora counts. Additional samples were collected from the same locations for soil microflora counts in mid-June and early August 1969.

After screening, five treatments were established: rested soil, replant soil, rested soil plus old grape roots, replant soil plus old grape roots, and half rested-half replant soil. The old roots, many bearing ascocarps of *R. hypogaea*, were added so that all test vine roots were within less than 1 cm of an old root piece. Nine S.5279 and nine Concord vines were planted in each of the rested and replant soils. During the experiment, all plants were fertilized twice with 0.06 g each of N, P, and K (actuals, liquid solution)

per plant. This was 3 times the recommended amount established by soil tests, and was done to eliminate marco-nutrient deficiency as an experimental factor. After 78 days, the plants were harvested. Two samples of soil per treatment were analyzed for bacterial and fungal populations.

Tricoderma viride was the most common fungus in rested soil, whereas *Penicillium* spp. were most common in replant soil (Table 2). This difference persisted in the field collections of June and August and after growth of Concord vines in the greenhouse. The addition of old grape roots to the soils seemed to have little effect on levels of *T. viride*, but it was correlated with an increase in levels of *Penicillium* in rested soil. Growth of S.5279 brought no noticeable shift in *T. viride* populations in either soil, but the combination S.5279/rested soil was correlated with an increase in populations of *Penicillium* spp. (Table 3).

Higher bacteria counts were recorded for replant soil than for rested soil in samples taken in April, June, and August. After growth of both Concord and S.5279 vines, the bacterial populations in rested and replant soils increased. The increase was least in replant soil planted with S.5279. Addition of old grape roots appeared to cause a slight decrease in the bacterial population of replant soil planted with Concord vines (Table 4).

The preliminary nematode counts of the two soils indicated high populations of *Paratylenchus* (530/250 ml rested soil, and 1,740/250 ml replanted soil). The replant soil also had 350 *Pratylenchus*/250 ml soil. Most root systems grown in replant soil, with and without old roots added, had light to heavy damage by phylloxera.

DISCUSSION.–Evidence tends to support the conclusion that grape specific replant disease is caused by biologic agents rather than physical factors.

TABLE 3. Effect of vine growth and of the addition of old grape roots on populations of *Tricoderma viride* and *Penicillium* spp. in "rested"[a] and "replanted"[b], vineyard soils collected in April 1969

Soil and experimental condition	Populations (10^2 colonies/g oven-dry soil)			
	Tricoderma viride samples		*Penicillium* spp. samples	
	1	2	1	2
After growth of Concord:				
Rested	518	422	549	482
Replanted	94	32	2,625	3,109
Rested with root amend.	833	679	586	1,235
Replanted with root amend.	151	348	1,596	1,456
50% rested/50% replanted	392	609	1,687	2,724
After growth of Seibel 5279:				
Rested	460	658	2,012	1,678
Replanted	278	156	2,500	2,375

[a] All vines removed 6 years prior to collection of soil.
[b] Original vines removed and vineyard replanted with Delaware 4 years prior to collection of soil.

TABLE 4. Bacterial population dynamics in field samples of "rested"[a] and "replanted"[b] vineyard soils through one season and after growth of Concord and Seibel 5279 vines in the greenhouse

Month of soil collection	Soil and experimental condition	Populations (10^2 colonies/g oven dry soil)	
		Sample 1	Sample 2
April	Rested–field	13,079	13,196
	Replant–field	25,735	16,220
June	Rested–field	14,130	20,108
	Replanted–field	49,734	49,731
August	Rested–field	17,262	16,964
	Replanted–field	35,185	33,333
April	Rested–Concord	28,537	20,181
	Replanted–Concord	40,438	30,224
	Rested–S.5279	15,172	21,370
	Replanted–S.5279	20,370	20,656

[a] All vines removed 6 years prior to study.
[b] Original vines removed, and vineyard replanted with Delaware 3 years prior to study.

Observations that grape replant vigor can vary greatly over short distances, depending upon proximity to old vine location, complements this conclusion. We do not assume that any of the microbial species discussed here are necessarily causative agents of grape specific replant disease. The possibility that an as yet unidentified organism causes the disease must be considered.

Understanding the dynamics in replant situations is complicated by the ever-present roots of previous vines. Presence of old roots has been shown to stimulate growth of grape replants here and for other fruit species elsewhere (5, 11). Yet, the worst symptoms of grape specific replant disease are expressed by replants growing closest to an old vine site where old roots are most abundant.

More information on the comparative susceptibilities of *Vitis lubrusca* cultivars (i.e., Concord, etc., and French hybrids, such as Seibel 5279) to specific replant disease would be useful. Knowledge of the growth response of French hybrid cultivars when replanted in old French hybrid sites could be helpful in planning future vineyards, as these cultivars are being successfully planted in old *V. lubrusca* vineyards. Eventual replantings of French hybrids may bring renewed problems with specific replant disease.

The frequent presence of a fluorescent species of *Pseudomonas* in grape roots may be important to grape growth because Mosse (9) has reported that surface-sterilized chlamydospores of *Endogone mossae* did not infect and produce mycorrhiza in sterile apple seedlings until a species of *Pseudomonas* was added. We have found that vesicular-arbuscular mycorrhiza, caused by an *Endogone* species, is regularly associated with grapes in New York.

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