Interactions Between Mycorrhizal Fungi, Soil Fumigation, and Growth of Grapes in California

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Grapevines from all 18 vineyards sampled throughout the grape-growing regions of California were heavily mycorrhizal from natural infestations. *Glomus fasciculatus, Sclerocystis sinuosa, and Glomus macrocarpus* were the mycorrhizal species most frequently associated with grapes in the field. At two sites where methyl bromide (MBr) fumigation was used, some grapevines became severely stunted. This stunting was correlated with a lack of mycorrhizal fungi associated with the roots. When 1,3 dichloropropene (DD) was used as the fumigant, vines were not stunted and were also heavily mycorrhizal. Greenhouse trials indicated that grapevines growing in soil fumigated with methyl bromide became stunted but grew normally when inoculated with mycorrhizal fungi or were grown in non-fumigated grape soil. In one vineyard fumigated with methyl bromide, vines receiving mycorrhizal inoculum grew better and yielded 66% more grapes than vines not receiving mycorrhizal inoculum. In a second field trial, all vines became mycorrhizal within 15 months of planting whether they were inoculated with mycorrhizal fungi or not. However, total growth of all vines inoculated with mycorrhizal fungi were still 13% greater than non-inoculated vines after 19 months. It appears, under certain conditions, that field fumigation with methyl bromide may severely stunt growth of grape vines. These conditions are: 1) effective soil fumigation which destroys the majority of soil-borne mycorrhizal propagules; 2) slow reinvasion of the fumigated soil by mycorrhizal fungi; 3) soil which is deficient in at least one essential nutrient such as phosphorus, zinc, or copper; and 4) planting stock which is non-mycorrhizal.

Deal *et al.* (2), and Possingham and Obbink (8) have shown that grape vines growing in the field are normally mycorrhizal. It has been shown also (3,8) that mycorrhizal fungi can stimulate growth of grapes growing in sterilized soil. It was suggested that mycorrhizal symbiosis is responsible for improved phosphorus nutrition, and perhaps other mineral nutrition as well, in grapes. In the grape growing areas of California, soil fumigation with nematicides or biocides such as methyl bromide (MBr) are common. Fumigation is necessary to alleviate disease problems caused by soil-borne pests. It has been shown by Menge *et al.* (6) that field doses of MBr are capable of destroying mycorrhizal inoculum. Within the past few years, newly planted grapes grown in a small percentage of fumigated vineyards in California have exhibited severe stunting and apparent micronutrient deficiencies. The following study was initiated to determine if the cause of this stunting was related to the destruction of the soil-borne mycorrhizal population by soil fumigants.

Materials and Methods

Survey of mycorrhizal fungi: Soil cores (2.5 cm diam × 22.5 cm long) were taken from the root zone in 18 vineyards located in either Kern, San Joaquin, or Lake County in California. The cores from each location were thoroughly mixed and 500-g soil samples were wet sieved and decanted by the method of Gerdemann and Nicolson (4) to collect chlamydospores and sporocarps of mycorrhizal fungi. Mycorrhizal spores as well as grape roots from each site were used to inoculate rooted Thompson Seedless grape cuttings (*Vitis vinifera* L.) or sudan-grass (*Sorghum vulgare* Pers.) which were grown in an autoclaved sandy soil fertilized with half strength Hoagland's solution (5) without phosphorus. These pot cultures were examined to verify the identification as well as the symbiotic nature of the mycorrhizal fungi.

Observations of grape growth and associated mycorrhizae from fumigated vineyards: Two vineyards were studied; one near Clear Lake, California, and the other near Lodi, California. In both locations, grapevines were severely stunted following tarped, deep (3 M) fumigations with 336-448 kg/ha MBr (98% MBr + 2% chloropicrin). Grape varieties at Clear Lake were Gamay and Cabernet, while at Lodi the variety was Zinfandel. The Clear Lake vineyard was located on land which had been covered with native oak (*Quercus* sp.) and *Ceanothus* sp. and had not been previously planted to grapes. After clearing and preparing the land, the soil was fumigated with MBr to control oak-root fungus, *Armillariella mellea,* found on the oak roots. The Lodi vineyard had produced excellent crops prior to its decline from phylloxera (*Daktulosphaira vitifoliae* Fitch), *Armillariella mellea* and nematodes, *Meloidogyne incognita* and *Xiphinema index.* Soil characteristics for the Lodi and Clear Lake vineyards are given in Table I.

Table 1. Selected soil characteristics from the three grape localities used in this study.

Characteristic or mineral nutrient	Location		
	Clear Lake	Lodi	Davis
Soil type	Manzanita variant loam	Hanford sandy loam	Sandy loam
pH	6.8	7.5	6.7
P (ppm)	5.8	28.0	5.2
K (ppm)	258.0	44.0	114.0
Zn (ppm)	0.5	0.5	0.4
Cu (ppm)	1.0	0.4	2.0
Mn (ppm)	293.0	5.1	12.5

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Fig. 1. Severely stunted Gamay grape vines at Clear Lake. Vines exhibited symptoms of severe phosphorus and zinc deficiencies.

The vineyard in Clear Lake was fumigated with MBr late in 1974. In 1977 and 1978, 1043 Gamay vines and 1299 Cabernet vines were rated as good, intermediate, poor, or dead. Good vines were those which appeared normal and healthy; intermediate vines also appeared healthy but had not yet produced bearing branches; poor vines were stunted and the leaves were usually chlorotic (Fig. 1). Leaf tissues from good and poor vines were analyzed for P, Cu, Zn, and Mn in 1977 using standard methods described elsewhere (1). In 1976 and 1977, two soil cores per vine (2.5 cm diam × 22.5 cm long) were taken from beneath 30-50 good and poor Gamay vines. Roots were screened from each soil sample and cut into 1 cm long sections. Roots were then cleared and stained with lactophenol and Trypan blue (7), and the percentage of 1 mm square root sections containing mycorrhizal hyphae, vesicles, arbuscules or chlamydospores was determined.

The vineyard at Lodi was fumigated in November, 1971, and planted in June, 1972. Four blocks of 520 vines each were treated with: 1) MBr at 336 kg/ha covered; 2) MBr at 448 kg/ha covered; 3) 1,3-dichloropropene (DD) at 2337 L/ha; and 4) DD at 3272 L/ha, respectively. No untreated controls were provided because of long-term nature of the trial and possible source of contamination. Instead, adjacent vines with the same history as the vines used in the trial were examined for nematode and mycorrhizal fungus incidence. In 1975, foliage analysis and percent mycorrhizal infection was determined as indicated above for vines growing in MBr-fumigated, DD-fumigated and adjacent vineyard soils. Mycorrhizal chlamydospores were wet sieved (4) from the soil samples and the number of spores per 200 g of soil was estimated for each treatment. Average grape yields as kg/vine for 520 vines per treatment also were determined from 1975 through 1978.

Greenhouse trials with grape and mycorrhizal fungi: Soil was collected from the root zone of good and poor vines from the Clear Lake vineyard. Thompson seedless grape cuttings rooted in steamed sand were planted in five 15-cm clay pots containing: 1) soil near good vines; 2) soil near poor vines; 3) soil near poor vines plus 10 g of *Glomus fasciculatus* (Thaxt.) Gerd and Trappe (isolate 0-1) inoculum which consisted of roots and soil from a pot containing *Sorghum vulgare* which had been infected with *G. fasciculatus* for 3½ months; 4) soil near poor vines which had been fumigated under a 2-mil polyethylene tarp with MBr (98% MBr and 2% chloropicrin) at the rate of 1.4 kg per m^3 soil for 48 hrs; 5) soil near poor vines which had been fumigated with MBr plus 10 g of *G. fasciculatus* 0-1 inoculum; 6) soil near poor vines which had been fumigated with MBr plus 10 g of *Sclerocystis sinuosa* Gerd. & Bakshi inoculum which consisted of roots and soil from a pot containing a Thompson Seedless grapevine which had been infected with *S. sinuosa* for 16 months. The cuttings were grown for 13 months in a greenhouse at temperatures ranging from 22-27°C. When the vines were harvested, the soil was carefully washed from the roots and dry weights of the vines were obtained.

Field trials: A Zinfandel grape nursery site was established in April, 1975, near Lodi. Virus-free Zinfandel cuttings were planted in the nursery which had been fumigated with 448 kg/ha MBr under a 1-mil polyethylene tarp four months prior to planting. Root samples taken from the nursery in February, 1976, were stained (7), and microscopic examination indicated that the vines were free of mycorrhizal infection. The field site at Lodi was fumigated in March, 1976, with 448 kg/ha MBr under a 1-mil polyethylene tarp.

Soil characteristics of the Lodi field site are shown in Table 1. Vines from the nursery were transplanted into the field in April, 1976. The vines were established within each half of the field in four blocks of 45 vines each. Within two of the blocks in each half of the field, three adjacent 15-vine rows received approximately 216 g/vine of mycorrhizal inoculum placed on the roots at transplant time. Two blocks of three adjacent 15-vine rows received no mycorrhizal inoculum. Mycorrhizal inoculum consisted of soil and roots collected from 4-year-old grape vines grown in field soil fumigated with 3272 L DD/ha. These vines were selected because they were found to be free of nematodes and other root pathogens after a careful examination. All selected vines were found to be heavily mycorrhizal. *Glomus fasciculatus, Glomus constrictus* Trappe, and *Sclerocystis sinuosa* were found associated

with these and neighboring vines and could have accounted for the mycorrhizal infections. The mycorrhizal inoculum was prepared by blending the roots in water and sprinkling on a layer of screened soil, adding another layer of soil and sprinkling again, etc. The soil was then quartered and repiled. The soil was screened, quartered, and repiled a second time and then used as inoculum. Grapes were grown according to standard regional vineyard practices. Yield in kg/ha of grapes and zinc concentrations of foliage were determined in 1977 and 1978. Roots were collected, stained, and assessed for mycorrhizal infections as described above 13 and 16 months after planting.

A second field plot was established at the University of California at Davis field station. Soil characteristics of the Davis site are shown in Table 1. The plot was fumigated with 1120 kg/ha of MBr, 30 cm deep, under 1-mil polyethylene tarp 17 days prior to transplanting grapevines into the field in June, 1975. Fourteen varieties of grape cuttings had been rooted in autoclaved soil (Table 8). One and a half months prior to transplanting to the field, the grapevines were transplanted into milk cartons containing a soil mix of ½ leaf mold and ½ sandy loam soil from Lodi. The soil mix was fumigated with MBr at the approximate rate of 896 kg/ha. One half of the rooted cuttings were inoculated with *Glomus fasciculatus* by dipping the roots in an inoculum slurry at the time of transplanting to the milk cartons. The inoculum slurry was prepared by screening the contents of three pots (1500 g) containing *Sorghum vulgare* which was infected with *G. fasciculatus* for four months, through 1-mm, 350-, 250-, 100- and 40- μm sieves. Roots collected in the 1-mm sieve were blended and added to the mixed contents of the other sieves and a two parts blended inoculum to one part water solution was made. To this mixture 300 mL of a 2% (vol/vol) methyl cellulose (400 cp) solution was added. Examination of roots from several rooted grape cuttings at the time of transplanting to the field indicated that vines inoculated with *G. fasciculatus* were mycorrhizal while those receiving no mycorrhizal inoculum were free of mycorrhizal infection. Vines were transplanted into the field as six split plot blocks. Each block contained four adjacent vines of each variety with the same treatment. Each block contained one row of vines inoculated with *G. fasciculatus* and an adjacent row which did not receive mycorrhizal inoculum. The grapes were grown according to standard regional vineyard practices. The vines were pruned according to prescribed cultural practices three and 15 months after planting. These prunings were dried and weighed and provided a dependable measure of vine growth. Roots were also collected, stained, and assessed for percent mycorrhizal infection, as described above, three and 15 months after planting.

Results

Survey of mycorrhizal fungi: Grapevines from all non-fumigated vineyards sampled were mycorrhizal. Eight mycorrhizal fungi which were found associated with grapevines in Kern, San Joaquin, and Lake counties of California are shown in Table 2. *Glomus fasciculatus, Sclerocystis sinuosa* and *Glomus macrocarpus* Tul. & Tul. were most frequently found associated with grapes in the field. These three fungi plus *Glomus constrictus* were the only ones which were verified as mycorrhizal symbionts of grapes in the greenhouse.

Observations of grape growth and associated mycorrhizae from fumigated vineyards: In the vineyard at Clear Lake three years after fumigation, 53% of the Gamay and 22% of the Cabernet varieties were severely stunted (Fig. 1, Table 3). Although there were no significant differences in the foliar concentrations of phosphorus, zinc, or copper between good and poor vines, poor vines consistently lacked mycorrhizal infections both in 1976 and 1977 while the roots of the good vines were heavily infected with mycorrhizal fungi (Table 4). By 1978, the majority of poor vines had either died or become infected with mycorrhizal fungi and moved into the intermediate or good categories (Table 3).

In the vineyard at Lodi, chlamydospores of mycorrhizal fungi were found associated with adjacent vines but not in the soil fumigated with either MBr or DD (Table 5). Adjacent vines growing in non-fumigated soil were heavily infected with mycorrhizal fungi. Vines grown in the MBr fumigated soil were stunted, yielded an average of only 7.2 kg grapes/vine, and were not infected by mycorrhizal fungi. Vines growing in DD-fumigated soil

Table 3. Growth categories of grape vines following a tarped, deep fumigation with methyl bromide in 1974 at Clear Lake, California.

		% vines in growth category[1]			
Year	Variety	Good	Intermediate	Poor	Dead
1977	Gamay	24	22	53	1
	Cabernet	52	26	22	0
1978	Gamay	36	19	37	8
	Cabernet	70	15	1	14

[1] Good vines were those which appeared normal and healthy; intermediate vines also appeared healthy but had not yet produced bearing branches; poor vines were stunted and the leaves were usually chlorotic.

Table 2. Mycorrhizal fungi associated with grapes in California.

Mycorrhizal symbiont	Location (county)	Number of observations	Formation of mycorrhizae on grape in culture
Gigaspora margarita Becker & Hall	Kern	1	–
Glomus constrictus Trappe	Kern, San Joaquin	2	+
Glomus fasciculatus (Thaxt.) Gerd. & Trappe	Kern, San Joaquin, Lake	12	+
Glomus macrocarpus Tul. & Tul.	Kern, San Joaquin, Lake	4	+
Glomus microcarpus Tul. & Tul.	San Joaquin	2	–
Glomus monosporus Gerd. & Trappe	Kern	1	–
Glomus mosseae (Nicol. & Gerd.) Gerde & Trappe	Kern, San Joaquin	2	–
Sclerocystis sinuosa Gerd. & Bakshi	Kern, San Joaquin, Lake	5	+

Table 4. Foliar analysis and the % of roots of Gamay grape vines which showed structures of mycorrhizal fungi after a methyl bromide fumigation in 1974[1] at Clear Lake, California.

Year	Vine growth category	Foliar analysis			% roots with mycorrhizal structures			
		P (%)	ZN (ppm)	Cu (ppm)	hyphae	vesicles	arbuscules	chlamydospores
1976	Good	—	—	—	100 a	—	—	—
	Poor	—	—	—	0 b	—	—	—
1977	Good	0.16 a	17 a	10 a	100 a	40 a	100 a	100 a
	Poor	0.14 a	19 a	9.8 a	4 b	0 b	0 b	0 b

[1] Values in each column not followed by identical letters are significantly different $P = 0.05$ as defined by Duncan's Multiple Range test.

Table 5. Yield and mycorrhizal structures in roots of grapes 3 years after planting in fumigated[1] or non-fumigated soil at Lodi, California.

Treatment	Yield				Mycorrhizal structures per cm of root[2]			
	1975	1976	1977	1978	hyphae (μm)	vesicles	arbuscles	chlamydospores 200/g soil
Non-treated adjacent vines	—	—	—	—	4,450 a	6.2 a	0.3 b	2 a
Methyl bromide w/cover								
336 kg/ha	6.4	10.5	12.1	8.9	0 b	0 c	0 c	0 b
448 kg/ha	8.0	12.1	12.5	10.4				
1,3-D								
2337 L/ha	12.9	14.6	12.3	14.3	4,440 a	0.9 a	3.7 a	0 b
3272 L/ha	14.7	15.7	13.6	15.4				

[1] Soil was fumigated at a depth of 1 M.
[2] Values in each column not followed by identical letters are significantly different $P = 0.05$ as determined by Duncan's Multiple Range test.

yielded an average of 13.8 kg grapes/vine, and were heavily infected with mycorrhizal fungi (Table 5). The yields in MBr treated vines increased in 1976 and 1977 to about 12 kg/vine, then declined in 1978 to about 9 kg/vine. In DD treated soil, the vines averaged about 14 kg/vine in each of the three years.

Greenhouse trials with grapes and mycorrhizal fungi: Vines grown in refumigated field-fumigated soil associated with poor plants grew better than the vines grown in field-fumigated soil associated with poor plants which was not refumigated, but not as well as vines grown in field-fumigated soil associated with good plants. This indicated that the destruction of some microorganisms associated with poor plants increased growth somewhat, but could not restore normal growth (Table 6). Vines growing in field-fumigated soil taken near poor plants, but inoculated with *G. fasciculatus*, grew better than vines growing in field-fumigated soil taken near good plants. This indicated that mycorrhizal fungi could completely restore normal growth to vines growing in MBr-fumigated soil (Table 6). This result was further reinforced by the fact that vines grown in refumigated field-fumigated soil and inoculated with either *S. sinuosa* or *G. fasciculatus* grew significantly better than non-inoculated vines growing in this soil and equally as well as vines growing in soil taken near good vines in the field.

Table 6. Dry weights of mycorrhizal and non-mycorrhizal Thompson Seedless grapevines grown in the greenhouse in methyl bromide fumigated soils from the Clear Lake vineyard.

Treatment	Mean dry wt of vine[1] (g)
Methyl bromide fumigated field soil:	
associated with good vines	10.1 c
associated with poor vines	1.6 a
associated with poor vines plus *Glomus fasciculatus*	16.0 d
Methyl bromide fumigated field soil refumigated with methyl bromide:	
associated with poor vines	6.0 b
associated with poor vines plus *Sclerocystis sinuosa*	13.3 cd
associated with poor vines plus *Glomus fasciculatus*	11.6 cd

[1] Values not followed by identical letters are significantly different $P = 0.05$ as defined by Duncan's multiple range test.

Field trials: At the Lodi trial during the second growing season after planting (30 months) vines receiving mycorrhizal inoculum grew better and yielded 66% more grapes than vines not receiving mycorrhizal inoculum. Only 11% of the vines inoculated with mycorrhizal fungi were non-bearing or were replants, while 40% of the vines not inoculated with mycorrhizal fungi were non-bearing or were replanted after 18 months of growth (Table 7). Concentrations of zinc and phosphorus in foliage of vines receiving mycorrhizal inoculum were not significantly greater than concentrations in the foliage of vines not receiving mycorrhizal inoculum either six or 18 months after planting. After one year of growth in the field, roots of vines growing in MBr-fumigated soil which did not receive mycorrhizal inoculum were only 20% infected with mycorrhizal fungi. Roots of vines receiving mycorrhizal inoculum were 95% infected with mycorrhizal fungi. Sixteen months after planting, however, most vines not inoculated with mycorrhizal fungi had become naturally infected with mycorrhizal fungi, and 82% of the roots contained mycorrhizal structures. Most of the bearing vines which were not inoculated with mycorrhizal fungi became naturally infected with mycorrhizal fungi soon after planting. However, even 30 months after planting, those bearing vines which received mycorrhizal inoculum yielded 15% more grapes than those which did not receive mycorrhizal inoculum at planting.

In the trial at Davis, all the vines became mycorrhizal within 15 months after planting whether they were inoculated with *G. fasciculatus* or not. Growth of the mycorrhizal vines, as measured by prunings, was not significantly greater than it was for vines not inoculated with *G. fasciculatus* seven or 19 months after planting for

Table 7. Yield and percent of non-bearing vines in mycorrhizal and non-mycorrhizal field-grown 'Zinfandel' grape 30 months[1] after fumigation with methyl bromide at Lodi, California[2].

Treatment	Total Yield kg/grapes/vine	% replanted or non-bearing vines	Yield bearing vine kg/grapes/vine	% plants mycorrhizal 13 months after inoculation	16 months after inoculation
Mycorrhizal	4.8 a	11 a	5.5 a	95 a	86 a
non-mycorrhizal	2.9 b	40 b	4.7 b	20 b	82 a

[1] Vines were planted and inoculated in April, 1976.
[2] Values in each column not followed by identical letters are significantly different $P = 0.05$ as determined by Duncan's Multiple Range test.

any individual varieties. However, total growth of all mycorrhizal inoculated vines was 10% greater than vines not inoculated with the mycorrhizal fungus after seven months of growth and 13% greater after 19 months of growth (Table 8).

Table 8. Dry weights of prunings from grapevines inoculated and non-inoculated with *Glomus fasciculatus* at the Davis field site.

	Dry wts (g)			
	1976		1977	
Grape Variety	−myco	+myco	−myco	+myco
Cabernet	31.9	41.4	378.2	529.8
Chenin Blanc	46.4	55.5	501.7	721.9
Dogridge	36.5	30.6	839.0	544.8
St. George	54.4	59.7	1062.3	1199.9
Petite Sirah	18.8	29.0	202.0	355.5
Thompson Seedless	29.4	35.8	425.4	439.0
Zinfandel	36.6	31.7	295.1	376.9
A × R #1	36.2	49.8	728.6	852.6
Carignane	28.7	50.0	345.9	499.4
Barbera	19.1	20.4	120.3	150.7
Rubired	64.6	55.8	802.2	700.5
Tokay	47.8	50.5	514.4	853.5
Muscat of Alexander	38.3	37.5	378.2	494.9
French Colombard	58.1	61.1	835.4	858.1
Avg.	39.0	43.5	530.6	612.7*

* Indicating significant difference between mycorrhizal and non-mycorrhizal treatments $P = 0.05$ as determined by the LSD test.

Discussion

It has been previously shown that grapevines growing in the field are normally mycorrhizal (2,8) and that mycorrhizal fungi can substantially improve growth of grapes under greenhouse conditions in autoclaved, fumigated or irradiated soil (2,3,8). Results from this study verify these results and extend these principles to field-grown grapes.

Methyl bromide is extremely toxic to mycorrhizal fungi and most field fumigations have the capability to destroy mycorrhizal inoculum in the soil (6). Populations of mycorrhizal fungi were severely reduced by methyl bromide fumigations of grape land at Lodi and Clear Lake, and the effects of the reduced mycorrhizal populations were still evident at these sites five years after planting. Reinvasion of fumigated soil by mycorrhizal fungi occurred both at Clear Lake and Lodi but appeared to be retarded compared to the field trial sites at Davis and Lodi where mycorrhizal fungi colonized non-mycorrhizal plants within 15 months after planting. Typically, vines which are not mycorrhizal become stunted, and if they do not become mycorrhizal within two or three years they die or must be replaced. If the vines can survive the initial period of being non-mycorrhizal and produce some root growth, they generally become mycorrhizal and will begin to grow normally as soon as they are infected.

It appears, under certain conditions, that field fumigation with methyl bromide may severely stunt growth of grape plants. These conditions are: 1) effective soil fumigation which destroys the majority of soil-borne mycorrhizal populations; 2) slow reinvasion of the fumigated soil by mycorrhizal fungi; 3) soil which is deficient in at least one essential nutrient such as phosphorus, zinc, or copper; and 4) planting stock which is non-mycorrhizal.

The soil at Clear Lake was deficient in phosphorus, zinc, and copper while the soil at Lodi appeared deficient in copper, zinc and manganese. Planting stock at both the Clear Lake and Lodi sites were cuttings rooted in the greenhouse and grown in containers with an artificial soil mix. Examination of roots from these plants indicated that they were non-mycorrhizal. Certainly, a minority of all replanted grape vineyards would possess all of the above characteristics which could lead to stunting following fumigation. However, care should be taken to avoid this stunting problem in areas suspected to possess these conditions. Mycorrhizal inoculum is now available commercially from several locations in the United States and could be added to vines when they are transplanted into the field. Mycorrhizal vines may also be purchased. DD which was used at Lodi did not severely reduce mycorrhizal inoculum and did not induce stunting of grape. This fumigant is apparently less toxic to mycorrhizal fungi than methyl bromide and may provide a suitable alternative to methyl bromide when only nematode control is desired.

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