HORTSCIENCE 29(11):1362–1365. 1994.

Vesicular–Arbuscular Mycorrhizae Colonization of Redwood and Incense Cedar Seedlings following Storage

U. Afek[1], L.A. Lippet[2], D. Adams[2], J.A. Menge, and E. Pond
Department of Plant Pathology, University of California, Riverside, CA 92521

Additional index words. Sequoia sempervirens, Sequoiadendrom giganteum, Libocedrous decurrens, Glomus mosseae

Abstract. **Vesicular–arbuscular mycorrhizal inoculum consisting of a mixture of roots of coast redwood [*Sequoia sempervirens* (D. Don)], soil, and spores of *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe was tested for viability and efficacy following storage for 4 or 8 weeks at 4, 9, 15, or 24C and moisture contents of 0%, 6%, 12%, or 17%. Storage regimes did not have any effect on the number of spores of *Glomus mosseae* recovered after storage. However, germinability of the spores decreased from 35% before storage to 10% to 31% during storage, especially under typical ambient room conditions (17% moisture at 24C). Maximum colonization of coast redwood, sierra redwood [*Sequoiadendrom giganteum* (Lindl.) Buchh.], and incense cedar (*Libocedrous decurrens* Torr.) was achieved after inoculation with 1 inoculum : 1 potting mix dilution (w/w). However, plant fresh weight was highest following inoculation with a 1 inoculum : 5 potting mix dilution (w/w). Dried inoculum was effective when stored at 24C, or below 10C when moist.**

Vesicular–arbuscular mycorrhizal (VAM) fungi are important in increasing mineral nutrient uptake and growth of many plants (Harley and Smith, 1983; Menge, 1982b; Mosse, 1973). Mycorrhizal fungi are known to infect roots of coast redwood, and sierra redwood in forests in California (Adams et al., 1990; Mejstrik and Kelly, 1979). The mycorrhizal fungus *Glomus mosseae* occurs naturally in the soil of Ben Lomond State Forest Nursery, Ben Lomond, Calif., and was identified by John A. Menge, Univ. of California, Riverside (unpublished).

Fumigation of soil to control soilborne pathogens and weeds is a common practice in agricultural fields (Haas et al., 1987; Menge, 1982a, 1982b). Fumigation of soil eliminates or greatly reduces VAM populations (Afek et al., 1991; Buttery et al., 1988; Haas et al., 1987; Kleinschmidt and Gerdemann, 1972). Therefore, reintroduction of suitable VAM fungi into fumigated soil before planting is often of primary importance for plant growth (Adams et al., 1990; Afek et al., 1991; Kormanik et al., 1980; South, 1977).

Several species of VAM fungi have been shown to survive in root fragments for up to 6 months at –50 MPa (Tommerup and Abbott, 1981). In an assay of stored soil inoculum with a high moisture content, (i.e., having high water potentials), less VAM colonization occurred than from dried soil inoculum (below –2 MPa) (Tommerup and Abbott, 1981). Plant colonization with VAM fungi also decreased as the storage time of the soil inoculum was increased beyond 2 years (Miller et al., 1985). Germination of spores of VAM fungi is maximal on substrates having water potentials greater than –0.01 MPa (Daniels and Trappe, 1980; Sylvia and Schenck, 1983), although 20% of spores germinated at –3.1 MPa (Daniels and Trappe, 1980).

We, therefore, endeavored to determine the best conditions for storage of VAM inoculum while preserving its potential for colonization of sierra redwood, coast redwood, and incense cedar. We tested the effect of various storage conditions on mycorrhizal spore germination in vitro, and subsequent colonization and growth response of seedlings of sierra and coast redwood and incense cedar following inoculation with the stored inoculum.

Materials and Methods

Storage of VAM inoculum. Mycorrhizal inoculum consisting of a mixture of roots of coast redwood, soil, and spores of *G. mosseae*, taken from the Ben Lomond State Forest Nursery, was used for the experiments. The inoculum was stored in darkness in 30 × 25 × 20-cm plastic boxes, sealed within plastic bags at soil moisture concentrations of 0%, 6%, 12%, or 17% and at 4, 9, 15, or 24C, for a total of 16 combinations, for storage periods of 8 or 16 weeks each.

Each box of each replication contained 10 kg dry weight soil inoculum, and the plastic bags were opened for 5 min every 2 days to permit aeration. The Ben Lomond soil was characterized as follows: water saturation capacity, 38%; pH, 5.6; electroconductivity, 0.87 $dS \cdot m^{-1}$; cation concentrations (milliequivalents per liter), 5.2 Ca, 1.2 Mg, 2.3 Na; exchangeable Na percentage, 1%; nutrient concentrations (parts per million), 1330 N, 37 P, 129 K, 1.34 Zn, 1.04 Mn, 16.64 Fe, 0.15 Cu; and 72% sand, 18% silt, and 10% clay.

Germination of spores. The VAM inoculum was assayed for spores per gram soil and percent germination on 2% water agar (WA) before storage (3 days after collection of the inoculum from the field) and after 8 and 16 weeks of storage under the conditions described above. Inoculum (10 g/sample per treatment) was gently washed with tap water through four stacked sieves, with mesh sizes of 417, 250, 104, and 44 µm. Spores were gently rinsed from the lowest sieve (44 µm), dispensed into 40-ml centrifuge tubes with a 50% aqueous sucrose solution, and centrifuged for 5 min at 1100× *g*. The floating spores were collected and counted. The VAM spores were then surface-sterilized for 3 min with 0.5% NaOCl, washed again with sterilized deionized water, and plated on WA. Percent germination of the spores was determined after 7 days.

Colonization of seedlings. Coast redwood, sierra redwood, and incense cedar seedlings were inoculated with nonstored VAM inoculum (1 week after collection of the inoculum from the field) or with inoculum that had been stored 8 weeks at 0% moisture and 24C or 17% moisture at 4C. These specific conditions were chosen because ≈17% was the average moisture concentration when the inoculum was taken from Ben Lomond soil in early winter. There are two practical options to store VAM inoculum until incorporation into the soil. After its collection, the inoculum can be stored at ≈17% moisture and 4C, or it first can be dried and then stored at 0% moisture and room temperature (≈24C). To save energy, we tested whether reducing moisture from 17% to 0% affected the inoculum quality. At the Ben Lomond State Forest Nursery, soil inoculum is incorporated into fumigated soil before seeding is done with dry inoculum. We did not test inoculum storage at 17% and 24C because this condition reduced VAM spore germination in vitro (Table 1). In this experiment, each replication included 10 kg soil inoculum that was stored in plastic boxes in darkness as described previously. The soil inoculum was diluted with potting mix (Sunshine Mix; Fisons, Vancouver, B.C., Canada) at 1:1, 1:2, 1:5, and 1 inoculum : 10 potting mix (w/w), and placed in 40-cm^3 sterilized plastic pots. Potting mix without inoculum was used as a control. A listing of the exact ingredients in the potting mix is unavailable, but, in general, it consists of vermiculite, sphagnum peat, starter nutrients, trace elements, and a wetting agent.

Seeds of sierra redwood, coast redwood, and incense cedar were soaked in tap water for 24 h, briefly surface-sterilized and dried, and then sealed in Ziplock plastic bags and held for 3 to 4 weeks at 4C before being seeded. This treatment stimulates seed germination (unpublished). After chilling, seeds were sown 5 mm deep in pots (three to four seeds per pot). Pots were watered daily to keep at least the

Received for publication 19 Oct. 1993. Accepted for publication 4 June 1994. Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel. no. 3694-E, 1992 series. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Current address: Dept. of Fruit and Vegetable Storage, ARO, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.
[2]Current address: Dept. of Forestry and Fire Protection, L.A. Moran Reforestation Center, Davis, CA 95617.

surface of the potting mix moist. Seed germination began in ≈2 weeks. After seed germination, watering was done every 3 days. The pots were placed in a completely randomized design on a greenhouse bench under natural photoperiods at 24 ± 2C. Four weeks after planting, seedlings were thinned to one per pot. VAM colonization and plant fresh weight were determined 10 weeks after inoculation. Roots were stained (Phillips and Hayman, 1970), and percent colonization was calculated as the number of colonized sites per 100 sites counted, where one site was considered as a root and grid intersection (Newman, 1966).

Experiments were conducted three times and each treatment included 10 replications. Data were tested by analysis of variance (ANOVA) procedures and regression analysis with the Statistical Analysis System (SAS Institute, 1982).

Results and Discussion

Spore concentration of *G. mosseae* was unaffected by time of storage or moisture or temperature of the soil (Table 1). The mean spore count per gram soil was 98 before storage and 102 after 8 and 16 weeks of storage. The range of spore count per gram soil was 80 to 130. Spore germination before storage was 35% and decreased significantly during the storage periods (10% to 31%), particularly with 17% moisture at 24C (Table 1). The interaction of two storage periods (8 and 16 weeks) × four temperature regimes (4, 9, 15, and 24C) on spore germination was not significant. However, the interactions of two storage periods × four moisture regimes (0%, 6%, 12%, and 17%) and the two storage periods × four temperatures × four moisture regimes on spore germination were significant. In general, germination of stored inoculum was above 29% under low temperature and high moisture conditions and below 13% under high temperature and high moisture conditions. Germination of stored inoculum was maximal after 16 weeks at 9C and 17% moisture and minimal after 8 weeks at 24C and 17% moisture (Table 1).

A significant regression of spore germination on four moisture regimes occurred for 8 and 16 weeks storage at 4, 9, and 24C but not at 15C (Table 1). Thus, at 4 and 9C, spore germination at the high moisture was higher than at the low moisture. However, at 24C, spore germination at the low moisture was higher than at the high moisture. This difference possibly was due to microorganisms that proliferated under these optimal (24C and 17% moisture) conditions and that may affect VAM spores in soils (Afek et al., 1990a; Ames and Linderman, 1978; Menge et al., 1977; Schenck and Kinloch, 1974). Other investigators (Daniels and Trappe, 1980; Sylvia and Schenck, 1983) have reported that maximum germination of VAM spores occurs at water potentials greater than –0.01 MPa; however, 20% of spores germinated at –3.1 MPa (Daniels and Trappe, 1980). We assume, therefore, that VAM inoculum should be stored dry at 24C. However, VAM inoculum with high moisture content can be stored at low temperature where the effect of microorganisms on the inoculum would be diminished.

A significant negative regression of VAM colonization on VAM inoculum, diluted with potting mix, before or after 8 weeks of storage, characterized seedlings of all of the species (Table 2). However, a significant positive regression of fresh weight on the same inoculum dilutions occurred for all of these seedlings (Table 3). For all seedlings, maximum colonization was achieved with a 1 inoculum : 1 potting mix (w/w) dilution both with no stored VAM inoculum or after it had been stored 8 weeks under 0% moisture at 24C or 17% moisture at 4C (Table 2). However, maximum plant fresh weight of seedlings of coast redwood, sierra redwood, and incense cedar was achieved in inoculum : potting mix dilutions of 1:5 and 1:10 (Table 3). We assume that the high inoculum : potting mix ratio promoted early VAM colonization, which has been shown to inhibit growth of plants especially during the initial growth phase (Harley and Smith, 1983). The high ratio of potting mix : inoculum also may have provided a better initial growth medium for the young seedlings.

This study confirms other reports (Daniels and Trappe, 1980; Miller et al., 1985; Sylvia and Schenck, 1983) that it is possible to store VAM inoculum dry without losing colonization potential and without losing its effect on enhancement of plant growth following VAM colonization. VAM inoculum stored in high moisture conditions (such as 17% in Ben Lomond soil) may eventually lose its effectiveness as field inoculum (Menge, 1982b; Menge and Timmer, 1982). Effectiveness of VAM inoculum in the field is highly dependent on inoculum density and proper placement, proper timing, and susceptibility of crops to colonization (Afek et al., 1990b; Ferguson, 1981; Menge and Timmer, 1982). This research shows that storage of dried VAM soil inoculum at 24C for 8 weeks does not adversely affect inoculum germination and seedling root colonization. We thus confirm the practice used in the Ben Lomond State Forest Nursery of incorporating soil inoculum into fumigated soil before seeding as a means of inoculating endomycorrhizal–host (VAM) coniferous crops (Adams et al., 1990).

Literature Cited

Adams, D., T. Tidwell, J. Ritchey, and H. Wells. 1990. Effect of nursery-produced endomycorrhizal inoculum on growth of redwood seedlings in fumigated soil. Tree Planters' Notes 40:7–11.

Table 1. Percent germination[z], on water agar, of vesicular–arbuscular mycorrhizal (VAM) inoculum after 8 or 16 weeks of storage at various temperatures and inoculum moisture concentrations.

	Storage temp (°C)							
	4		9		15		24	
Moisture	Storage duration (weeks)							
(%)	8	16	8	16	8	16	8	16
				Germination (%)				
0	16	22	18	20	18	22	18	24
6	17	15	20	21	17	19	20	21
12	22	29	17	22	20	22	12	16
17	29	30	30	31	17	21	10	12
r^2	0.84**	0.53*	0.57*	0.74**	0.04^{ns}	0.06^{ns}	0.69*	0.86**
Regression model	$15.2 - 0.00008x + 0.005x^2$	$20.5 - 0.6x + 0.07x^2$	$19.1 - 0.9x + 0.09x^2$	$20.4 - 0.6x + 0.07x^2$	$17.5 + 0.2x - 0.01x^2$	$21.6 - 0.3x + 0.02x^2$	$18.7 + 0.1x - 0.04x^2$	$24.1 - 0.5x - 0.01x^2$

Analysis of variance			
Source	df	MS	F value
8 and 16 weeks of storage (A)	1	635.6	138.9**
Four temperature regimes (B)	3	617.1	134.8**
Four moisture regimes (C)	3	211.0	46.1**
A × B	3	7.2	1.6^{ns}
A × C	3	82.5	18.04**
B × C	9	561.5	122.7**
A × B × C	9	21.6	4.7**

[z]Each number is a mean of 10 replications. Regression analysis and analysis of variance were done only for spore germination of 8 and 16 weeks of VAM inoculum storage.

[y]Spore germination before storage was 35% and significantly higher than after 8 or 16 weeks of storage, according to F test significance at $P \leq 0.05$.

^{ns},*,**Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 2. Vesicular–arbuscular mycorrhizal (VAM) colonization (percentage) of coast redwood, sierra redwood, and incense cedar 10 weeks after inoculation with four dilutions of VAM inoculum with potting mix. Inoculum was incorporated before storage or after 8 weeks under two storage conditions: 17% moisture at 4C (17%/4C) and 0% moisture at 24C (0%/24C).

	Colonization (%)[z]		
Inoculum :	Inoculum before	Inoculum after 8 weeks of storage	
potting mix	storage	17%/4C	0%/24C
Coast redwood			
1:1	45	37	35
1:2	41	33	30
1:5	18	14	10
1:10	11	11	9
Control[y]	1	2	1
r^2	0.76**	0.79**	0.76**
Regression model	$48.3 - 4.9x + 0.08x^2$	$37.6 - 3.6x + 0.05x^2$	$35.0 - 3.6x + 0.06x^2$
Sierra redwood			
1:1	39	32	29
1:2	34	28	24
1:5	22	17	13
1:10	12	10	8
Control[y]	1	1	1
r^2	0.86**	0.86**	0.83**
Regression model	$41.1 - 3.6x + 0.06x^2$	$33.6 - 3.0x + 0.05x^2$	$29.6 - 2.8x + 0.04x^2$
Incense cedar			
1:1	36	30	25
1:2	28	21	19
1:5	18	14	13
1:10	11	8	8
Control[y]	1	2	1
r^2	0.82**	0.80**	0.82**
Regression model	$35.9 - 3.2x + 0.05x^2$	$28.9 - 2.7x + 0.04x^2$	$24.7 - 2.1x + 0.03x^2$

[z]Each number is a mean of 10 replications.
[y]Only potting mix.
**Significant at $P \leq 0.01$.

Table 3. Fresh weight (gram per plant) of coast redwood, sierra redwood, and incense cedar 10 weeks after inoculation with four dilutions of vesicular–arbuscular mycorrhizal (VAM) inoculum with potting mix. Inoculum was incorporated before storage or after 8 weeks under two storage conditions: 17% moisture at 4C (17%/4C) and 0% moisture at 24C (0%/24C).

	Fresh wt (g/plant)[z]		
Inoculum :	Inoculum before	Inoculum after 8 weeks of storage	
potting mix	storage	17%/4C	0%/24C
Coast redwood			
Control[y]	0.21	0.19	0.17
1:1	0.25	0.24	0.20
1:2	0.27	0.20	0.22
1:5	0.39	0.35	0.31
1:10	0.49	0.41	0.39
r^2	0.79**	0.71**	0.80**
Regression model	$0.2 + 0.02x - 0.0003x^2$	$0.2 + 0.02x - 0.0003x^2$	$0.2 + 0.02x - 0.0002x^2$
Sierra redwood			
Control[y]	0.22	0.19	0.22
1:1	0.24	0.20	0.21
1:2	0.27	0.24	0.23
1:5	0.33	0.30	0.31
1:10	0.38	0.35	0.33
r^2	0.84**	0.78**	0.68*
Regression model	$0.2 + 0.02x - 0.0002x^2$	$0.1 + 0.01x - 0.0002x^2$	$0.2 + 0.01x - 0.0002x^2$
Incense cedar			
Control[y]	0.20	0.19	0.18
1:1	0.23	0.20	0.21
1:2	0.37	0.34	0.33
1:5	0.68	0.61	0.58
1:10	0.70	0.70	0.64
r^2	0.91**	0.90**	0.92**
Regression model	$0.11 + 0.06x - 0.001x^2$	$0.1 + 0.06x - 0.001x^2$	$0.1 + 0.05x - 0.0009x^2$

[z]Each number is a mean of 10 replications.
[y]Only potting mix.
*, **Significant at $P \leq 0.05$ or 0.01, respectively.

Afek, U., J.A. Menge, and E.L.V. Johnson. 1990a. Effect of *Pythium ultimum* and metalaxyl treatments on root length and mycorrhizal colonization of cotton, onion and pepper. Plant Dis. 74:117–120.

Afek, U., J.A. Menge, and E.L.V. Johnson. 1991. Interaction among mycorrhizae, soil solarization, metalaxyl, and plants in the field. Plant Dis. 75:665–671.

Afek, U., E. Rinaldelli, J.A. Menge, E.L.V. Johnson, and E. Pond. 1990b. Mycorrhizal species, root age, and position of mycorrhizal inoculum influence colonization of cotton, onion and pepper. J. Amer. Soc. Hort. Sci. 115:938–942.

Ames, R.N. and R.G. Linderman. 1978. The growth of Easter lily (*Lilium longiflorum*) as influenced by vesicular–arbuscular mycorrhizal fungi, *Fusarium oxysporm*, and fertility level. Can. J. Bot. 56:2773–2780.

Buttery, B.R., S.J. Park, W.I. Findlay, and B.N. Dhanvantari. 1988. Effects of fumigation and fertilization on growth, yield, chemical composition and mycorrhizae in white bean and soybean. Can. J. Plant Sci. 68:677–686

Daniels, B.A. and J.M. Trappe. 1980. Factors affecting spore germination of the vesicular–arbuscular mycorrhizal fungus, *Glomus epigaeus*. Mycologia 72:457–471.

Ferguson, J.J. 1981. Inoculum production and field application of vescular–arbuscular mycorrhizal fungi. PhD Diss., Univ. of California, Riverside.

Haas, J.H., B. Bar-Yosef, J. Krikun, R. Barak, T. Markovitz, and S. Kramer. 1987. Vesicular–arbuscular mycorrhizal fungus infestation and phosphorus fertigation to overcome pepper stunting after methyl bromide fumigation. Agron. J. 79:905–910.

Harley, J.L. and S.E. Smith. 1983. Mycorrhizal symbiosis. Academic, London.

Kleinschmidt, G.C. and J.W. Gerdemann. 1972. Stunting of citrus seedlings in fumigated nursery soils related to the absence of endomycorrhizae. Phytopathology 62:1447–1453.

Kormanik, P.P., W.C. Bryan, and R.C. Schultz. 1980. Increasing endomycorrhizal fungus inoculum in forest nursery soil with cover crops. Southern J. Applied For. 4:151–153.

Mejstrik, V. and P. Kelly. 1979. Mycorrhizae in *Sequoia gigantea* Lindle. et Grad. and *Sequoia sempervirens* Endl. Ceska Mykologie 33:51–54.

Menge, J.A. 1982a. Effects of soil fumigants and fungicides on vesicular–arbuscular fungi. Phytopathology 72:1125–1132.

Menge, J.A. 1982b. Utilization of vesicular–arbuscular mycorrhizal fungi in agriculture. Can. J. Bot. 61:1015–1024.

Menge, J.A. and L.W. Timmer. 1982. Procedure for inoculation of plants with vesicular–arbuscular mycorrhizae in the laboratory, greenhouse and field, p. 59–67. In: N.C. Schenck (ed.). Methods and principles of mycorrhizal research. Amer. Phytopathol. Soc., St. Paul, Minn.

Menge, J.A., S. Nemec, R.M. Davis, and V. Minassian. 1977. Mycorrhizal fungi associated with citrus and their possible interactions with pathogens. Proc. Intl. Soc. Citriculture 3:872–876.

Miller, R.M., B.A. Carnes, and T.B. Moorman. 1985. Factors influencing survival of vesicular–arbuscular mycorrhiza propagules during topsoil storage. J. Applied Ecol. 22:259–266.

Mosse, B. 1973. Advances in study of vesicular–arbuscular mycorrhizae. Annu. Rev. Phytopathol. 11:171–196.

Newman, E.I. 1966. A method of estimating the total length of root in a sample. J. Applied Ecol. 3:139–145.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55:158–161.

SAS Institute. 1982. SAS user's guide. SAS Inst., Cary, N.C.

Schenck, N.C. and R.A. Kinloch. 1974. Pathogenic fungi, parasitic nematodes, and endomycorrhizal fungi associated with soybean roots in Florida. Plant Dis. Rptr. 58:169–173.

South, D. 1977. Artificial inoculation of fumigated nursery beds with endomycorrhizae. Tree Planters' Notes 28:3–5.

Sylvia, D.M. and N.C. Schenck. 1983. Germination of chlamydospores of three *Glomus* species as affected by soil matric potential and fungal contamination. Mycologia 75:30–35.

Tommerup, I.C. and L.K. Abbott. 1981. Prolonged survival and viability of VA mycorrhizal hyphae after root death. Soil Biol. Biochem. 13:431–433.