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Effect of Vesicular–Arbuscular Mycorrhizal Fungi on Rooting of *Sciadopitys verticillata* Sieb & Zucc. Cuttings

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Abstract. **A vesicular–arbuscular mycorrhizal fungus in a peat-based medium significantly increased survival, callus development, and rooting percentage of *Sciadopitys verticillata* cuttings over noninoculated cuttings. The presence of a nurse host plant for the mycorrhizal fungi to colonize in the absence of *S. verticillata* roots decreased survival and rooting percentage, but not callus development, relative to the fungus without the nurse host. Among plants that did produce roots, however, there were no significant differences among treatments for root number, weight, or length per cutting.**

When vesicular–arbuscular mycorrhizal (VAM) fungi are used in plant propagation, plants typically are inoculated in containers after micropropagation (Branzanti et al., 1992), after rooting of cuttings, or after seed germination (Yeager et al., 1990). The presence of VAM fungi in the rooting medium increased root development and growth of *Viburnum dentatum* L., but not root initiation (Verkade and Hamilton, 1987). Experiments were conducted with *Sciadopitys verticillata* (umbrella pine) cuttings to test the value of VAM inoculation of cuttings that take up to 6 months to root (Waxman, 1978).

Materials and Methods

Sciadopitys verticillata cuttings were collected on 10 Mar. 1993 from three 15-year-old, 6-m-tall trees in Hatboro, Pa., and treated according to Waxman (1978). The lower 5 cm of the 15- to 20-cm cuttings were immersed in water in a greenhouse for 2 days. Two strips of bark 2 to 3 cm long and 2 to 3 mm wide then were peeled from opposite sides of the bases of the cuttings and they were dipped in plant growth regulator [Hormodin, 3000 ppm 1*H*-indole-3-butanoic acid (IBA) in talc] and inserted into 10-cm square, 500-cm^3 plastic pots containing sphagnum-based potting mix with (Mycorimix; Premier Peat Co., Quebec, Canada) and without (Premier FB mix; Premier Peat Co.) one propagule/gram of the VAM fungus *Glomus intraradix* Schenck & Smith. A third treatment entailed sowing seeds of *Agrostis gigantea* Roth in the mycorrhizal mix with the cutting to act as nurse mycorrhizal host plants for the predicted 6 months until the appearance of *S. verticillata* roots. Cuttings were fertilized monthly with Hoagland's solution with 0.1× phosphorus (Hoagland and Arnon, 1938).

Pots were arranged in a randomized complete-block design on one greenhouse bench with six blocks and 30 plants per treatment in each block and grown under natural photoperiods and at 10 to 30C. After 3 through 6 months of growth under mist controlled by an evaporative sensor, two plants per treatment per block were chosen randomly and harvested. Sampling was limited to living cuttings at the 7-month harvest. Callus development was quantified by counting protrusions on the stem. Colonization of the nurse host plants was measured at 3 and 6 months. Roots were stained with trypan blue (Phillips and Hayman, 1970) and percentage of total root length colonized by mycorrhizal fungi was quantified using the gridline intersect method (Newman, 1966). *Paspalum notatum* Flugge seedlings were planted into pots of mycorrhizal mix without nurse plants, from which cuttings were removed for the 4- through 7-month harvests. These were allowed to grow for 2 months, after which roots were assayed for VAM fungus colonization to quantify the viability of VAM fungi remaining in the pot. Data were analyzed via polynomial regression or analysis of variance and Tukey's method of multiple comparisons ($\alpha = 0.05$).

Results

More plants in the mycorrhizal treatment without *A. gigantea* broke bud (74%) after 3 months than in the other treatments (33% and 45% for control and mycorrhizal mix plus nurse host, respectively). Callus development was greatest in the mycorrhizal mix, followed by the nurse plant and control treatments (Fig. 1). At the end of the experiment, a greater

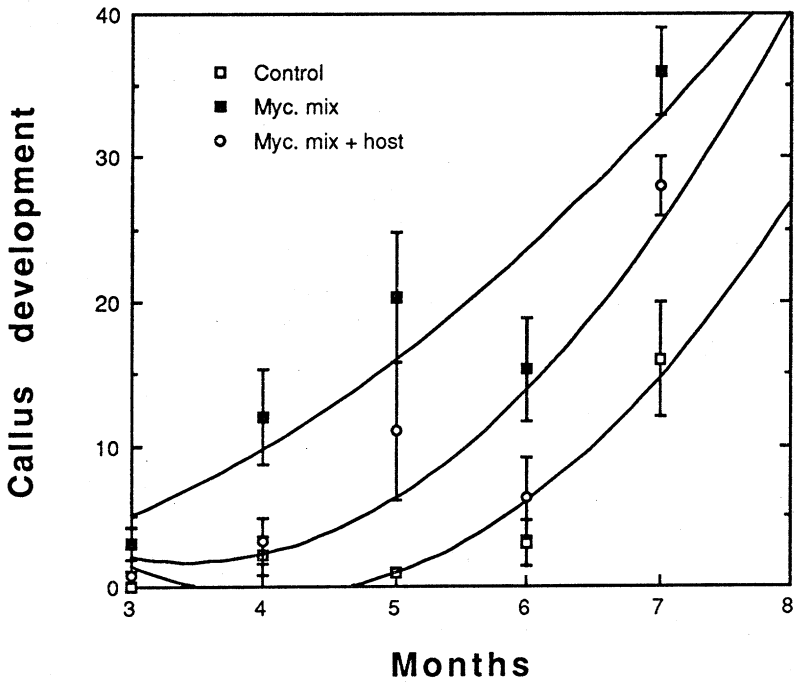

Fig. 1. Callus development on *Sciadopitys verticillata* cuttings in media with and without vesicular–arbuscular mycorrhizal fungi. Data are the means of 12 observations ± SEM and numbers of protrusions of the callus. Equations from polynomial regression: control, $y = 1.7x^2 - 14.6x + 29.1$ ($r^2 = 0.88$); mycorrhizal mix, $y = 0.72x^2 - 0.28x - 0.7$ ($r^2 = 0.82$); mycorrhizal + nurse host, $y = 1.9x^2 - 12.8x + 23.8$ ($r^2 = 0.81$).

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Table 1. Characteristics of *Sciadopitys verticillata* cuttings after 7 months.[z]

Treatment	Survival (%)	Callus development[y] (no.)	Rooting (%)
Control	27 b	16.3 b	17 b
Mycorrhizae	73 a	35.8 a	50 a
Mycorrhizae + nurse host	36 b	28.5 a	25 b

[z]Mean separation within columns by Tukey's method of multiple comparisons, $\alpha = 0.05$.
[y]Numbers of callus protrusions from the stem.

percentage of cuttings in the mycorrhizal mix had survived and produced roots than in the other treatments (Table 1). However, when only those cuttings that produced roots were considered, there were no significant differences in number, length, and fresh weight of roots per cutting because of variability within treatments (data not shown).

The nurse host plants had 9% ± 2% of their root lengths colonized by *G. intraradix* after 3 months and 54% ± 2% at 6 months. Without host roots, the medium in only 25% of pots in the mycorrhizal mix treatment had mycorrhizal fungi that survived 7 months to colonize *P. notatum* test plants.

Discussion

Potting mix containing a VAM fungus increased survival, callus development, and percentage of *S. verticillata* cuttings with roots after 7 months compared to control potting mix. The nurse plant treatment, designed to sustain and increase the mycorrhizal fungi in the medium, depressed the beneficial effects of these fungi. Unlike Waxman (1978), we did not achieve 100% rooting of cuttings, although we followed his method. However, he reported wide clonal variation in rooting percentage. Thus, our low rooting percentage may be a function of the parent trees used in this study.

The apparent influence of VAM fungi on *S. verticillata* is unique. Previously, VAM fungi were thought only to exert an influence on plant growth and development after colonization. Our results clearly show an effect of VAM fungi on plant growth before colonization. To date, VAM fungi have not been shown to synthesize plant growth regulators. This problem may not be resolved satisfactorily until we overcome our inability to grow these fungi in pure culture in quantities sufficient for analysis.

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