Pacific Science (1995), vol. 49, no. 2: 181–191

Vesicular-arbuscular Mycorrhizal Inoculation of Hawaiian Plants: A Conservation Technique for Endangered Tropical Species[1]

R. E. Koske and J. N. Gemma[2]

ABSTRACT: Forty species of plants (including 28 species endemic to the Hawaiian Islands) were evaluated in the greenhouse for their response to inoculation with the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith. Seedlings, cuttings, and established plants were inoculated. Several kinds of growth media were used. Increased growth and survival most frequently occurred when plants were grown in a gravel or fine sand medium that included calcined clay (up to 50% by volume) or sphagnum peat (up to 20%). Significant increases in height, weight, leaf number and size, and survival were noted in 10 of 14 species of seedlings grown in media in which peat content was 20% or less. Mycorrhizae were only rarely present in the non-inoculated plants except for plants grown from cuttings. The latter routinely formed mycorrhizae in the absence of added inoculum. Addition of mycorrhizal fungi to potting mixes appears to have value as a conservation technique for some plants that are difficult to propagate.

Numerous greenhouse studies have shown the benefits of inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi to the growth of a wide variety of plant species (Harley and Smith 1983). Agronomic crops have received the most attention, but relatively few horticultural and wild species have been tested for mycorrhizal responses (e.g., Linderman 1978, 1981, Biermann and Linderman 1983*a*,*b*).

In the Hawaiian Islands, it is estimated that 33% of the flora of ca. 1000 species is endangered or threatened (Vitousek et al. 1987, Wagner et al. 1990). The major causes of this situation are the destruction of habitats (especially by humans, pigs, and goats), damage from introduced pests, and competition from alien plant species (Howarth 1985, Gagné 1988, Smith 1989, Stone 1989). Conservation of many Hawaiian species has been handicapped by our inability to cultivate species in the greenhouse. When plant species can be established and temporarily maintained in a botanical garden, additional time is gained for the protection or acquisition of sites in which the plants can later be outplanted (Theobald 1989).

Hawaiian species are notoriously difficult to propagate in the greenhouse (Wooliams 1976). The frequent observations of the plant propagator, that seedlings "refused to grow larger than 2″, and after 3 months suddenly died" (Wooliams 1976 : 74), could well be a description of the behavior of an obligately mycotrophic species grown in the absence of mycorrhizal fungi (e.g., Janos 1980).

Previous studies have shown the horticultural benefits to be derived from the appropriate use of VAM in the greenhouse (Barrows and Roncadori 1977, Linderman 1978, 1981, Johnson et al. 1980, Johnson 1981, Biermann and Linderman 1983*a*,*b*), but their use in the propagation of rare and endangered species has been overlooked. Indeed, several standard greenhouse practices (e.g.,

[1] This research was funded in part by a grant from the Hawai'i State Division of Forestry and Wildlife administered through the Hawaiian Conservation Biology Initiative program of The Nature Conservancy of Hawai'i and a Faculty Fellowship from the University of Rhode Island. Manuscript accepted 13 April 1994.

[2] Department of Botany, University of Rhode Island, Kingston, RI 02881.

starting seeds and cuttings in "sterile" soil or soilless mixes, extensive use of fungicides, and frequent fertilization) have the effect of suppressing or excluding VAM formation (Biermann and Linderman 1983*a*, Harley and Smith 1983). In a comment that could apply equally to effects of greenhouse practices on VAM fungi, Wooliams (1976: 82) noted that "Hawaiian plants are very susceptible to damage by ... fungicides and will often die more rapidly through their use than without! The same is true to a lesser extent with fertilizers."

The consistent occurrence of mycorrhizae in Hawaiian plants collected from a variety of habitats suggested that ca. 90% of native Hawaiian angiosperms are obligate mycotrophs (i.e., dependent upon the association) (Koske et al. 1992). However, VAM were essentially absent from >100 potted plants grown in the greenhouses of the National Tropical Botanical Garden (NTBG), Kaua'i, Hawai'i (unpubl. obs.), where standard greenhouse methods were in practice (e.g., soilless mixes, frequent fertilization). The discrepancy between the mycorrhizal status of Hawaiian plants thriving in the field and those barely surviving in the greenhouse led us to investigate the effect of VAM on the growth of Hawaiian plants. If plants were found to respond to VAM, an important step toward conservation of Hawaiian and other tropical species would be made.

MATERIALS AND METHODS

A variety of experiments was conducted using seedlings, cuttings, and established plants that had been growing in the greenhouse (Table 1). Of the 40 species of plants tested, 28 were endemic to the Hawaiian Islands. A few species of non-Hawaiian, tropical plants that were in the greenhouse also were included in the study. Where possible, sampling at the end of the experiment was nondestructive, allowing the plants to be outplanted at a later date.

Experiments were performed at NTBG in a screened greenhouse between May 1989 and June 1990 and at the University of

TABLE 1

PLANT SPECIES USED IN INOCULATION EXPERIMENTS

SPECIES	STATUS[a]
Agavaceae	
Pleomele aurea (H. Mann) N. E. Brown	E
Apiaceae	
Peucedanum sandwicensis Hillebr.	E
Araliaceae	
Munroidendron racemosum (C. Forbes) Sherff	E
Tetraplasandra hawaiensis A. Gray	E
Asteraceae	
Dubautia scabra (DC) D. Keck	E
Remya kauaiensis Hillebr.	E
Verbesina encelioides (Cav.) Benth. & Hook.	I
Wilkesia gymnoxiphium A. Gray	E
Convolvulaceae	
Ipomoea pes-caprae (L.) R. Br. subsp. *brasiliensis* (L.) Ooststr.	I
Euphorbiaceae	
Antidesma pulvinatum Hillebr.	E
Chamaescyce remyi var. *remyi* (A. Gray ex Boiss.) Croizat & Degener	E
Fabaceae	
Acacia koa A. Gray	E
Caesalpinia kavaiensis H. Mann	E
Erythrina berteroana Urban	G
Erythrina sandwicensis Degener	E
Sesbania tomentosa Hook. & Arnott	E
Sophora chrysophylla (Salisb.) Seem.	E
Scaevola gaudichaudiana Cham.	E
Scaevola sericea Vahl	I
Lamiaceae	
Plectranthus parviflorus Willd.	I
Lythraceae	
Lythrum maritimum Kunth	I
Malvaceae	
Abutilon eremitopetalum Caum	E
Abutilon menziesii Seem.	E
Gossypium tomentosum Nutt. ex Seem.	E
Hedyotis sp.	E
Hibiscadelphus hualalaiensis Rock	E
Hibiscus clayi Degener & I. Degener	E
Hibiscus waimeae subsp. *waimeae* A. Heller	E
Kokia kauaiensis (Rock) Degener & Duvel	E
Sida fallax Walp.	I
Myrtaceae	
Metrosideros polymorpha Gaud.	E
Pittosporaceae	
Pittosporum kauaiense Hillebr.	E
Primulaceae	
Lysimachia glutinosa Rock	E
Rhamnaceae	
Colubrina oppositifolia Brongn. ex H. Mann	E
Rubiaceae	
Bobea elatior Gaud.	E
Gardenia gordonii Baker	G
Gardenia remyi H. Mann	E

TABLE 1 (continued)

SPECIES	STATUS[a]
Sapindaceae	
Dodonaea viscosa Jacq.	I
Solanaceae	
Lycium sandwicense A. Gray	I
Verbenaceae	
Vitex rotundifolia L. fil.	I

[a]E, endemic in Hawaiian Islands; I, indigenous to Hawaiian Islands; G, greenhouse specimens, not native or naturalized in Hawai'i.

Rhode Island (URI) in glass-enclosed, heated greenhouses between July 1990 and January 1992. Daytime temperatures at URI were 25–35°C (average 27°C) and nighttime temperatures were 16–25°C (average 24°C). Experiments performed before December 1990 at URI were carried out in a greenhouse without supplemental light. After that date, all plants (continuing experiments and new experiments) received 16 hr of light per day of sunlight supplemented with high-pressure sodium vapor lamps giving an intensity at the leaf surface of 350–1375 μEin/m^2/sec.

Several types of growth media were used. In all mixes listed below, ratios of ingredients are given on a volume basis. Based on results from the first part of our studies, the composition of the media and growth conditions were varied during the investigation.

At the completion of each experiment, all plants were measured (typically, the dry weight or height of shoots, although other parameters sometimes were used), and roots of 50–100% of the control and inoculated plants in each experiment usually were collected, cleared, and stained (Koske and Gemma 1989). Stained roots were examined at 40–60 × with a dissecting microscope, and portions of each root system were examined at 400 × with a compound microscope. The presence of vesicles, arbuscules, hyphal coils, and internal hyphae was noted for each specimen. Only those specimens in which arbuscules were found were considered to have formed functional mycorrhizae.

Most experiments were performed with 20 plants (10 inoculated, 10 control). However, because of the scarcity of some species or of viable seed, fewer plants had to be used for some experiments. Data were analyzed using independent t tests (two-tailed), and significance was assigned at $P < 0.05$. Differences in survival of plants were analyzed using the adjusted chi-square test.

An index ("M/NM") to standardize growth response was calculated by dividing the average size of the inoculated (M) plants by the average size of the uninoculated (NM) plants.

Angiosperm systematics and nomenclature are those of Wagner et al. (1990). Endemic species are those that occur naturally only in the Hawaiian Islands, and indigenous species are those that occur naturally in Hawai'i and elsewhere.

Inoculation of Seedlings

All experiments were performed at URI. Most experiments were performed in a gravel-calcined clay mix or a peat-calcined clay mix. Seeds were germinated in the greenhouse in trays of a sphagnum peat : perlite mix (4 : 1). Seedlings (two- to four-leaf stage) were selected for uniform size and vigor, sorted into two groups (inoculated and control), and transplanted to containers filled with the experimental mix. Experiments in 1989 were carried out in plastic pots 10 cm square filled with a 4 : 1 mix of Pro-Mix BX : Oil Dri. Pro-Mix BX (Premier Brands, Stamford, CT 06902) is a peat-based mix (sphagnum peat, vermiculite, and perlite), pH 5.5–6.2, containing sufficient added macro- and micronutrients for initial establishment of seedlings. Oil Dri (Oil Dri Corp., Chicago, IL 60611) is a calcined clay with an average particle size of ca. 3 mm. For the experiments in 1990–1992, cylindrical plastic tubes ("Cone-tainers" [Steuwe and Sons, Corvallis, OR 97333]) were filled with 165 ml of a coarse granitic gravel from a local quarry mixed 1 : 1 with Terra-Green, a calcined clay (Oil Dri Corp., Chicago, IL. 60611) similar to Oil Dri. The pH of the gravel was 5.3. The gravel was sterilized by steam (2 hr) and was

set aside for 2 weeks before being combined with the Terra-Green and used for plants. After mixing, the gravel : Terra-Green mix contained 3.5 ppm NO_3, 55 ppm P, and 53 ppm K. The pH of this mix was adjusted to 6.5 with lime and checked with a pH meter using a 1 : 2 soil : water slurry. Because the clay carrier of the inoculum raises the pH, the pH was measured after the inoculum was added.

A commercially available inoculum of the VAM fungus *Glomus intraradices* Schenck & Smith ("NutriLink," supplied by NPI, Salt Lake City, UT 84108) was used. Three grams of NutriLink (1000 spores per gram) were dispersed throughout the contents of each 10-cm pot in 1989. In the 1990–1992 experiments, 12.5 g was added to each Cone-tainer. Control treatments received an equivalent amount of Oil Dri (in 1989) or the carrier used for NutriLink, an attapulgite clay (supplied by the manufacturer of NutriLink), in 1990–1991. The size of the NutriLink particles ranged from 1.5 to 4.5 mm (average 3 mm).

Plants were watered every other day with tap water. In 1989, pots were fertilized by adding four pellets (ca. 0.5 g) of Sierra Mix (17 : 6 : 12) with micronutrients ("Sierra Mix plus minors") to the surface of the potting medium. Sierra Mix is a slow-release fertilizer with micronutrients (Sierra Chemical Co., Milpitas, CA 95035). Cone-tainers in 1990–1992 were fertilized every 2 weeks with 20 cm^3 of 1/2-strength Hoagland's solution with 1/4-strength P and micronutrients and full-strength Fe (Hoagland and Arnon 1950, Epstein 1972).

Two other mixes were used in 1991 that contained smaller proportions of peat than did the 1989 mix. Plants were grown from January to May, watered every other day, and fertilized with Hoagland's solution as described above. Seedlings of *Acacia koa* were tested in a Pro-Mix BX:fine sand (1 : 4) medium in cylindrical plastic "Deepots" (Steuwe and Sons, Corvallis, OR) measuring ca. 40 by 6 cm and with a capacity of ca. 650 cm^3. The inoculum was 37.5 g of NutriLink. Controls received 37.5 g of attapulgite clay particles. Seedlings of *Dubautia scabra* were grown in Cone-tainers of milled sphagnum peat : gravel : Terra-Green (1 : 2 :2) inoculated with 12.5 g of NutriLink. Controls received attapulgite clay in place of the NutriLink.

At the completion of the experiments, plants were measured and roots were collected, stained, and examined for the presence of VAM.

Inoculation of Cuttings

Cuttings were made from plants growing in the field. In the greenhouse they were stripped of most leaves, cut to ca. 15 cm long, dipped in a rooting hormone mix (Hormex [Brooker Chemical Corp., North Hollywood, California 91609]), and inserted into 10-cm plastic pots containing a 4 : 1 mix of Sunshine Mix no. 1 and Dry Clean. Sunshine Mix no. 1 (Fison's Horticultural Inc., Vancouver, British Columbia, Canada V6H 3V1) is a peat, vermiculite, and perlite medium with added nutrients, similar to Pro-Mix BX, with a pH of 5.6–6.2. Dry Clean (McKay Mfg., Los Angeles, CA 90001) consists of calcined clay pellets, similar in size to the NutriLink pellets. Twelve grams of NutriLink were mixed with the medium in the lower one-third of the pot. Uninoculated controls received an equal amount of Dry Clean in place of NutriLink. Four pellets of Sierra Mix plus minors were added to the surface of each pot. The cuttings were maintained in mist beds in the greenhouse for ca. 1 month and then transferred to greenhouse benches, where they were watered as required. All plants except *Ipomoea pes-caprae* subsp. *brasiliensis* were grown at NTBG in open-air greenhouses at ambient temperature.

Inoculation of Established Plants

Plants that had been growing in the NTBG greenhouse in plastic pots 10 cm square for 1–17 months (after germination) were sorted into two equivalent groups (similar height and general vigor). The height of each plant was measured at the beginning

of the experiment. One group was inoculated with 15 g of NutriLink, and the other received an equivalent amount of Dry Clean. The potting medium that the plants had been growing in before the treatment was Sunshine Mix no. 1. The entire contents of each pot (roots and medium) were removed from the pot, and the lower half of the potting mix was discarded. A few root fragments were collected and later examined to see if VAM were already present. The upper half of the original potting mix remained attached to each seedling. The bottom half of the empty pot was filled with Sunshine Mix : Dry Clean (4 : 1) amended with NutriLink or Dry Clean (the control). Plants were placed into the pots containing the new medium, and four pellets of Sierra Mix plus minors were added to the surface of each pot.

Pots were watered and placed back on the greenhouse benches. During the next 7.5 months, plants were watered as required and fertilized with Sierra Mix pellets at ca. 2-month intervals.

At the conclusion of the experiment, plant height was measured, leaves were counted (some species), and root samples were collected to determined if mycorrhizae had developed in the inoculated and control plants. For some plants, the percentage increase in height was calculated for changes occurring during the duration of the experiment.

Natural Occurrence of Mycorrhizae in Greenhouse Plants

The natural occurrence of mycorrhizae in uninoculated plants from the URI and NTBG greenhouses was assessed by two methods. In the first, pieces of roots collected from plants that had been growing for 1–17 months in the NTBG greenhouse in a peat-based mix (Sunshine Mix) were cleared, stained, and examined. The NTBG plants had been grown with standard practices of greenhouse cleanliness, but without particular attention to preventing introduction of VAM fungi. A second measure of the incidence of VAM was made by keeping records of the number of "control" plants in our experiments that possessed mycorrhizae when the roots were examined at the completion of the experiments.

RESULTS

Inoculation of Hawaiian plants with *G. intraradices* frequently promoted growth and survival. Most consistent increases occurred in the gravel : Terra-Green mix. Responses of plants to inoculation in media containing peat were variable. Some established plants benefited from inoculation. Specific results of each set of experiments follow.

Inoculation of Seedlings

Inoculated plants routinely formed mycorrhizae in the gravel : Terra-Green medium and, in eight of the 12 species tested, were significantly larger or had greater survival than did uninoculated plants (Table 2). Inoculation of plants of *Hibiscus waimeae* subsp. *waimeae* was not effective, and only three of the 10 inoculated plants formed VAM. Survival of seedlings of *Metrosideros polymorpha*, the dominant tree of the Hawaiian forest, was significantly greater in the mycorrhizal plants. After nearly 2 yr, only three of the 10 control plants had survived, but all inoculated plants were alive.

Of 101 root systems of inoculated plants that were examined at the end of the experiment, 87% had formed mycorrhizae. Control plants very seldom formed mycorrhizae (four of 77 plants examined [=4%]), indicating that contamination was not a problem.

In growth mixes containing peat, plants inoculated with *Glomus intraradices* usually were larger than noninoculated plants, and significant responses occurred in five of the nine species tested. Significant increases occurred in *Acacia koa* and *Dubautia scabra*, the two species that were grown in mixes containing 20% peat, and in three of the seven species grown in the Pro-Mix BX : Oil Dri medium. Mycorrhizae were formed by all of the inoculated plants that were examined and by none of the controls. The failure

TABLE 2

RESPONSE OF SEEDLINGS TO INOCULATION WITH *Glomus intraradices*

SPECIES	POTTING MIX[a]	DURATION (days)	M/NM RESPONSE[b]					COMMENT
			DRY WT	HEIGHT	WIDEST LEAF	NO. LEAVES	SURVIVAL[c]	
Abutilon eremitopetalon	Peat-clay	114		1.56	1.51	3.83*	1.33	
Acacia koa	Pro-Mix–sand	94	1.60*				1.00	
Bobea elatior	Gravel-clay	110	1.44**	1.13*			1.00	
Dodonaea viscosa	Gravel-clay	97	1.43*	1.35*			1.00	
Dodonaea viscosa	Peat-clay	45		1.10		1.30	1.00	
Dubautia scabra	Peat-gravel-clay	133	1.26*				3.50*	
Erythrina berteroana	Peat-clay	75		1.02		1.83*	1.00	Stem diameter M/NM = 1.50*
Erythrina sandwicensis	Gravel-clay	61	1.20			1.13	1.00	
Gossypium tomentosum	Gravel-clay	140	0.99				0.60	
Hibiscus clayi	Gravel-clay	140	2.91**				1.00	
Hibiscus waimeae	Gravel-clay	146	0.99				1.00	Only 30% of inoculated plants with VAM
Lycium sandwicense	Peat-clay	114		1.06			1.00	
Metrosideros polymorpha	Gravel-clay	635	1.28				3.33*	
Munroidendron racemosum	Gravel-clay	101	1.09				1.00	
Scaevola gaudichaudiana	Gravel-clay	73	2.62*	1.62*	1.81*		1.00	
Scaevola sericea	Gravel-clay	146	1.20		1.17*		1.00	
Sesbania tomentosa	Peat-clay	77		1.24*		3.55*	1.25	
Sida fallax	Gravel-clay	140	1.40*				1.00	
Tetraplasandra hawaiensis	Gravel-clay	91	1.94*		1.60*		1.11	
Verbesina encelioides	Peat-clay	75		1.19		1.53	1.00	Only 40% of inoculated plants with VAM
Vigna marina	Peat-clay	46				1.31	1.25	

[a] Gravel-clay, gravel : Terra-Green (1 : 1); peat-clay, Pro-Mix BX : Oil Dri (1 : 1); Pro-mix–sand, Pro-Mix BX : fine sand (1 : 4); peat-gravel-clay, Pro-Mix BX : gravel : Terra-Green (1 : 2 : 2).

[b] M/NM = average of mycorrhizal (=inoculated) plants/average of nonmycorrhizal (=control) plants. Significance: *, $P < 0.05$; **, $P < 0.02$.

[c] Survival = percentage of inoculated plants surviving/percentage noninoculated plants surviving.

TABLE 3

RESPONSE OF CUTTINGS TO INOCULATION WITH *Glomus intraradices*

SPECIES	n[a]	DURATION (days)	M/NM RESPONSE[b] HEIGHT	NO. LEAVES	NO. FLOWERS	SURVIVAL[c]	COMMENT
Ipomoea pes-caprae	18	77	1.00	1.18		1.00	
Lycium sandwicense	8	221	0.60	1.20		1.00	All controls with VAM
Lythrum maritimum	10	250	2.25	1.04		1.50	All controls with VAM
Peucedanum sandwicensis	15	236		0.84		1.00	All controls with VAM
Plectranus parviflorus	20	232			1.63	1.00	All controls with VAM
Remya kauaiensis	11	231				100*	All controls died
Vitex rotundifolia	20	258	0.98			100*	All three surviving controls with VAM

[a] n, number of plants in experiment.
[b] See Table 2 for explanation.
[c] When no controls survived and at least 50% of the inoculated plants survived, a survival value of 100 was given.

of *Dodonaea viscosa* to respond to inoculation in this medium was unexpected because the species showed a significant growth increase in the gravel : Terra-Green medium.

Inoculation of Cuttings

It was not possible to assess the effect of inoculation on five of the seven species studied because control plants routinely became mycorrhizal (Table 3). Except for *Ipomoea pes-caprae* subsp. *brasilensis* cuttings, every root system from surviving control plants had formed mycorrhizae at the end of the experiment. Significantly increased survival was noted in inoculated cuttings of *Vitex rotundifolia*. Only three of the 10 control plants survived (and they all had mycorrhizae), whereas all of the inoculated *Vitex rotundifolia* plants survived. All five control plants of *Remya kauaiensis* also died, as did three of the six inoculated plants.

Inoculation of Established Plants

Addition of *Glomus intraradices* to established plants resulted in significantly increased growth or survival in eight of 18 species tested (Table 4). Inoculated plants were an average of 45% taller and had 150% more leaves than control plants. Some plants showed spectacular responses to VAM. Mycorrhizal plants of *Chamaescyce remyi* var. *remyi* were 3.45 times larger than the controls. In eight species (*Caesalpinia kavaiensis, Gardenia gordonii, Hedyotis* sp., *Hibiscadelphus hualalaiensis, Hibiscus waimeae, Pittosporum kauaiense, Pleomele aurea,* and *Sophora chrysophylla*) where significant, positive growth responses to inoculation were not detected, at least some of the control plants also possessed mycorrhizae or some or all of the inoculated plants had failed to form mycorrhizae at the completion of the experiment.

Natural Occurrence of Mycorrhizae in Greenhouse Plants

Mycorrhizal fungi were present infrequently in greenhouse potting mixes. Cuttings were more likely than seedlings to become mycorrhizal in the absence of added inoculum.

Thirty species of plants that had been grown from seed in the NTBG greenhouse in Sunshine Mix for 1 to 17 months before our inoculation experiments were examined. Of 101 root systems examined (each taken from a different pot), only four (one each of *Wilkesia gymnoxiphium, Pittosporum kauaiense, Caesalpinia kavaiensis,* and *Bobea elatior*) were mycorrhizal.

A few seedlings of *Scaevola sericea* and

TABLE 4

EFFECT OF INOCULATION WITH *Glomus intraradices* ON GROWTH OF ESTABLISHED PLANTS IN THE GREENHOUSE

SPECIES	*n*	AGE AT START[a]	DURATION (days)	M/NM RESPONSE[b] HEIGHT	LEAF NO.	% INCREASE	SURVIVAL	COMMENT
Abutilon menziesii	10	390	241	—	2.06*		4.0*	
Antidesma pulvinatum	8	120	237	1.50**	1.67	1.37	1.00	
Bobea elatior	20	225	236	1.30	1.80	1.34	1.13	
Caesalpinia kavaiensis	18	105	241	1.16	1.00	2.75	2.16	VAM in 50% of inoculated plants
Chamaescyce remyi	4	225	237	3.45*	21.7**	9.48*	1.00	
Colubrina oppositifolia	4	210	237				100	Both controls died
Gardenia gordonii	17	510	234	0.93	0.98	0.92	1.00	
Gardenia remyi	8	480	237	1.00	1.90	34.0*	1.00	
Hedyotis sp.	9	240	232	1.2	0.73	1.30	4.00*	
Hibiscadelphus hualalaiensis	7	120	232	2.60	0.59	11.9	2.0	No VAM in inoculated plants
Hibiscus waimeae	20	390	236	1.42*	1.05	1.58*	1.11	
Kokia kauaiensis	22	30	234	1.03	1.39		1.00	
Lysimachia glutinosa	9	180	231	1.32*	1.41*	1.46*	2.40	Root wt. N/NM = 4.31*
Pittosporum kauaiense	11	270	242	1.23	1.18	1.43	1.00	VAM in 50% of inoculated plants
Pleomele aurea	20	180	236	0.96	0.74	6.60	2.50	
Sophora chrysophylla	12	195	236	0.88	0.71	0.89	1.34	No VAM in inoculated plants
Wilkesia gymnoxiphium	20	240	205	1.23	1.08		1.16	

[a] Age (in days) of plants when the experiment was started.
[b] See Table 2 for explanation.

Wilkesia gymnoxiphium in the NTBG greenhouse had field soil added to the Sunshine Mix before our sampling. Of 10 root systems examined in this mix, seven were mycorrhizal. None of these seedlings was used in our growth studies.

In the URI greenhouses, VAM occurred in just three of 198 uninoculated root systems that were examined.

DISCUSSION

Addition of VAM fungi to growth media significantly improved growth and survival of a variety of Hawaiian species in greenhouse cultivation. Best results were achieved by inoculating plants growing in a gravel or fine sand medium amended with clay (e.g., Terra-Green) or peat to aid in water retention. Gravel or sand combined with an enriched peat-based mix (e.g., Pro-Mix BX) was acceptable when the concentration of the enriched mix was 20% (e.g., Pro-Mix BX : fine sand [1 : 4]). When the concentration of peat was 50–80%, significant growth promotion by *Glomus intraradices* often did not occur. The frequent failure of established plants to respond to inoculation appeared to result from the high peat content of the mix.

Biermann and Linderman (1983*a*) tested various combinations and proportions of peat-based mixes and phosphorus fertilization rates for their effects on mycorrhizal benefits in two greenhouse-grown species. Our results confirm many of their findings. In

their study, significant stimulation of growth by VAM fungi in peat-based media occurred only when mixes were amended with soil or sand.

The cause for the frequent failure of mycorrhizae to stimulate growth in mixes with a high proportion of peat in our study is unknown. Mycorrhizae are capable of stimulating plant growth over a limited range of soil P levels and fail to elicit growth responses if P levels are too low or too high (Biermann and Linderman 1983*a*, Harley and Smith 1983). Low P concentration in pots fertilized with Sierra Mix may have resulted in the infrequency of significant growth stimulation in our studies. In our experiments, all inoculated plants had formed VAM in the peat mixes, so it was not a failure to form the association that prevented a positive response. Unidentified inhibitory factors associated with peat were noted by Biermann and Linderman (1983*a*), and further research is needed to resolve the problem. To most consistently obtain benefits from inoculation with VAM fungi, we recommend using mixes with $<20\%$ peat.

Plants may be inoculated effectively soon after seed germination or when older, and cuttings may be inoculated in the rooting medium (e.g., Barrows and Roncadori 1977, Linderman 1978). The high incidence of mycorrhizae in noninoculated rooted cuttings suggested that at least some species carry propagules of the VAM fungi on the surface of their stems. Splashing rain and blowing soil may deposit the fungi on the stems. The two species whose uninoculated cuttings did not form mycorrhizae were *Remya kauaiensis* and *Ipomoea pes-caprae* subsp. *brasiliensis*. The *R. kauaiensis* cuttings were collected at ca. 1.5 m above the ground, and the *I. pes-caprae* subsp. *brasiliensis* cuttings came from a rhizome growing on the nonvegetated area of a beach. All other cuttings were collected within 0.5 m of the ground and could easily have been spattered from raindrops striking the soil surface. Co-dispersal of VAM fungi and vegetative fragments of rhizomatous species has previously been described (Gemma and Koske 1989, Koske and Gemma 1990).

Throughout the study a single species of VAM fungus (*G. intraradices*) was used. The performance of isolates of VAM fungi is variable, influenced by the growth medium, the identity of the host plant, climatic factors, and the genetic makeup of the isolate itself (e.g., Harley and Smith 1983, Wood and Cummings 1992). Other species or isolates may be more effective in greenhouse propagation of Hawaiian plants.

This study was designed to survey the response of a variety of Hawaiian plants to inoculation with VAM fungi under greenhouse conditions. The limited amount of plant materials did not permit factorial experiments to test all combinations of growth media, fertilization, and inoculum. As such, it must be viewed as a preliminary investigation into the use of VAM fungi as a plant conservation technique. The promising results obtained indicate that these VAM fungi have an important role in greenhouse cultivation of threatened and endangered species (Theobald 1989). Routine greenhouse practices (i.e., soilless mixes) appear to effectively exclude VAM fungi. Deliberate inoculation is necessary to ensure their presence.

ACKNOWLEDGMENTS

We greatly appreciate the financial support and interest of the National Tropical Botanical Garden staff and especially acknowledge the contributions of W. L. Theobald, David Lorence, Gregg Koob, and the other members of the NTBG staff. We thank Tim Flynn for collecting and identifying plants, for sending us plant materials, and for leading us in the field. We thank Melany Chapin, Kerin Rosenberger, and Diane Ragone of the Hawaiian Plant Conservation Center for providing plant materials and tending the plants in the NTBG greenhouse, and we are grateful to Bill Garnett of the Waimea Arboretum and Botanical Garden for providing seeds of *Sesbania tomentosa*. Audrey Newman and Colin Bassett of The Nature Conservancy of Hawai'i provided valuable guidance. Laboratory and greenhouse help at URI by Peter Newcomb,

Sardha Suriyapperuma, David Berlinksy, and Eric Roberts is gratefully acknowledged.

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