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Mycorrhizal Status of *Gunnera petaloidea* in Hawai'i[1]

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ABSTRACT: Eight collections of the endemic Hawaiian angiosperm *Gunnera petaloidea* ssp. *kauaiensis* were examined for mycorrhizae. Soil-inhabiting roots of all specimens possessed extensive vesicular-arbuscular mycorrhizae (VAM). Aerial roots lacked mycorrhizae. Soil from the root zones of the plants contained propagules of VAM fungi, and spores of two species of VAM fungi were found in the soil. The discovery of mycorrhizae in *Gunnera* adds another symbiont to the *Gunnera-Nostoc* symbiosis.

THE MONOGENERIC ANGIOSPERM family Gunneraceae comprises about 35 species of small to megaphytic perennial herbs. Growing primarily in superhumid tropical, subtropical, or warm temperate environments of the Southern Hemisphere, species of *Gunnera* L. occur naturally in New Zealand, Tasmania, Indonesia, the Philippine and Solomon islands, South and Central America, the Falkland and Juan Fernández islands, southern and central Africa, and Madagascar and extend northward into Mexico and Hawai'i.

Unique among the angiosperms, *Gunnera* forms an intracellular symbiosis with colonies of nitrogen-fixing cyanobacteria that occur in the stems and petioles (Silvester and Smith 1969, Stewart et al. 1983, Bonnet 1990). The nitrogen fixed by the endosymbiont, a *Nostoc* species, is translocated to the host *Gunnera* plant (Silvester and Smith 1969, Bonnet 1990).

A single endemic species, *Gunnera petaloidea* Gaud., occurs in the Hawaiian Islands (Doyle 1990). The species is a conspicuous megaphyte with leaves up to 2.5 m broad and is confined to the wet margins of humid cloud forests of the high islands of Hawai'i (Wagner et al. 1990). Two subspecies are recognized: *G. petaloidea* ssp. *petaloidea*, which occurs on Hawai'i, Maui, Moloka'i, and Kaua'i, and *G. petaloidea* ssp. *kauaiensis* (A. Gray) Doyle, which is a Kaua'i endemic. Although it is very conspicuous where it grows, *G. petaloidea* ssp. *kauaiensis* has been little studied because of its generally inaccessible habitat: the sides of steep cliffs and supersaturated wet banks, along streams, and near the margins of bogs and cliff edges on the Alaka'i and Nāmolokama "plateau" at elevations of 610–1570 m (Doyle 1990).

Like other megaphytic species of *Gunnera*, *G. petaloidea* ssp. *kauaiensis* develops a long, creeping, prostrate to decumbent or ascending stem that is relatively thick (up to 20 cm diam.) and fleshy. Older stems, especially creeping, prostrate, or decumbent portions, produce simple adventitious roots from the internodal regions, some of which may form "prop roots." The numerous roots are relatively stout and reddish when young. Before contacting the soil, these adventitious aerial roots are covered with copious quantities of mucilage, in contrast to the soil-inhabiting roots that appear to lack mucilage.

The phylogenetic affinities of *Gunnera* are poorly known, and controversy has accompanied all attempts to classify both the genus and the family (see Doyle and Scogin 1988*a,b*, Doyle 1990). Until recently the genus was awkwardly included in the Haloragaceae (Cronquist 1981). It now resides in the monogeneric family Gunneraceae, which some workers place within the Haloragales (Dahlgren 1975, 1983, Cronquist 1988). However, inclusion within this order has recently

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been questioned on the basis of phytochemical evidence (Doyle and Scogin 1988*b*).

The purpose of this study was to determine the mycorrhizal status of aerial and soil-inhabiting roots of *G. petaloidea* ssp. *kauaiensis*. Further, since the presence and type of mycorrhizae that a plant or members of a plant family possess have been shown to have value in natural classification schemes (Nakai 1933, Wagner 1974, Trappe 1987), we hoped that this information could be useful for phylogenetic realignment of the genus and family.

MATERIALS AND METHODS

Root samples from eight plants were collected on the island of Kaua'i in July 1987. Collection sites included the Alaka'i Swamp and the mountains above Kilohana and Wailua. Both soil-inhabiting and mucilaginous, aerial roots were collected. In addition, small amounts (ca. 100 cm^3) of soil were collected from around the root systems of the plants for assessing the population of mycorrhizal fungi. In the laboratory, each soil sample was divided into two portions (except for two collections that were divided into three portions, one of which was used for spore extraction, see below) that were then mixed with vermiculite (1:1), and the resulting mixture was placed in 4-in. (10-cm) plastic pots (total number of pots = 16). To determine if propagules of vesicular-arbuscular mycorrhizal (VAM) fungi were present in the soil samples, each pot was planted with two seeds of a "trap plant," cucumber (*Cucumis sativa* L. cv. 'Straight Eight', Burpee Seed Co., Warminster, Pennsylvania 18974). The plants were maintained in the greenhouse at National Tropical Botanical Garden for 8 weeks, receiving water as required.

Roots of field-collected specimens of *Gunnera* and of 8-week-old cucumber plants were washed in water, cleared in 2.5% KOH, and stained with trypan blue in an acidic glycerol solution. Full details of clearing and staining have been published previously (Koske and Gemma 1989). Destained roots were examined at 100–400 × for the presence of structures of mycorrhizal fungi.

Two of the soil samples (one from the Alaka'i Swamp and one from Wailua) were wet-sieved (Gerdemann and Nicolson 1963) to recover spores of VAM fungi. The lower sieve retained spores >45 μm diam.

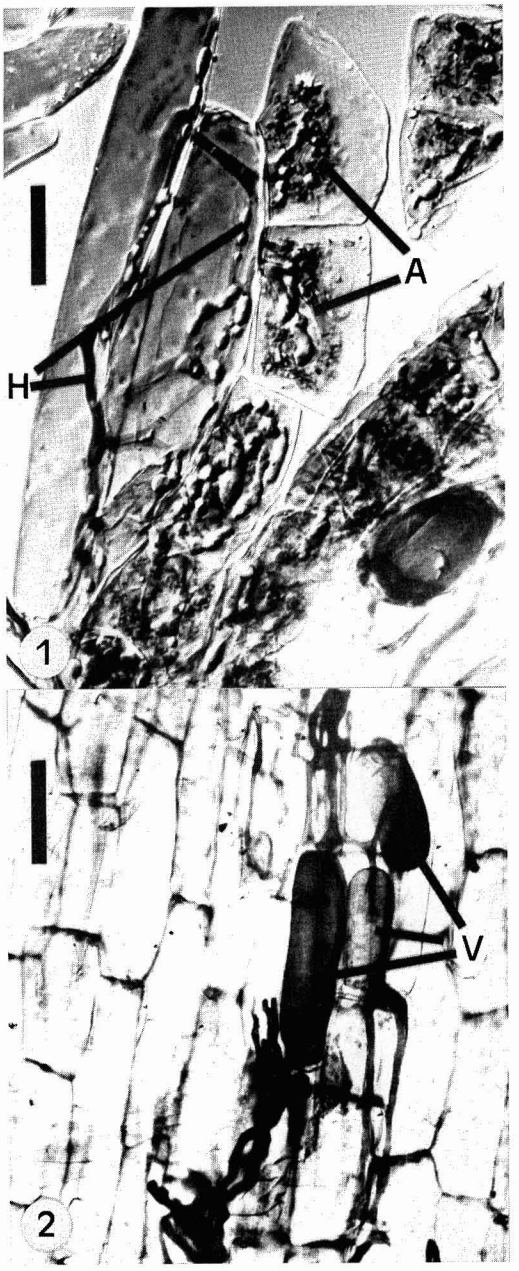
RESULTS AND DISCUSSION

All eight of the terrestrial root samples of *G. petaloidea kauaiensis* were heavily colonized by VAM fungi. Vesicles, arbuscules, hyphal coils, and runner hyphae were present in the roots (Figures 1,2). None of the aerial roots was mycorrhizal. Aerial roots of other plant species seldom have been examined for mycorrhizae. In the Hawaiian endemic tree *Metrosideros polymorpha* Gaud., aerial roots near the soil occasionally contain hyphae and vesicles of VAM fungi (Koske and Gemma, unpubl. obs.), but the lack of arbuscules suggests that the mycorrhizae are nonfunctional.

All 32 of the cucumber plants also had extensive VAM formation, indicative of high populations of propagules of VAM fungi (Moorman and Reeves 1979) in the soils in which *G. petaloidea* ssp. *kauaiensis* grows.

Spores of two undescribed VAM fungi were recovered from the soil samples. Both species have earlier been reported from the island of Hawai'i as *Scutellospora* 816 and *Glomus* 807 (Koske 1988). The species of VAM fungi associated with *Gunnera* on Kaua'i, although apparently endemic to the Hawaiian Islands, are not limited to association with *Gunnera*. The *Scutellospora* species is common in dune soils on the islands of Kaua'i and Hawai'i, and the *Glomus* species occurs in recent lava flows and dunes on Hawai'i Island (Koske 1988, Gemma and Koske 1990; Koske and Gemma, unpubl. data).

The extensive development of VAM in roots of *G. petaloidea* ssp. *kauaiensis* was similar to VAM levels found in other endemic Hawaiian species (Koske et al., in prep.). The major benefit generally attributed to the VAM symbiosis is improved phosphate uptake from the soil, although other benefits are possible, including improved uptake of other soil nutrients, enhanced drought resistance, and increased resistance to pathogenic soil fungi (Harley and Smith 1983). In the nitrogen- and phosphate-deficient volcanic soils where



FIGURES 1,2. Vesicular-arbuscular mycorrhiza of *Gunnera petaloidea* ssp. *kauaiensis*. (1) Arbuscules (A) and internal hyphae (H) in cortical cells. (2) Vesicles (V) and internal hyphae. Bar = 50 μm.

G. petaloidea ssp. *kauaiensis* grows (Foote et al. 1972), the combined contribution of the fungal and cyanobacterial symbionts of this species likely is vital to its survival. Recent work by Osborne (1989) has confirmed the importance of *Nostoc* to plants growing in natural habitats.

The presence of mycorrhizae in all examined specimens suggests that *G. petaloidea* ssp. *kauaiensis* is an obligate mycotroph (Trappe 1987) and has an absolute dependency on the VAM fungi. Such dependence could have important implications when attempts are made to cultivate the species in greenhouse conditions. It may be necessary to ensure that mycorrhizal fungi are present in the potting mix (Gemma and Koske 1988, Theobald 1989).

Discovery of mycorrhizae in its roots establishes *G. petaloidea* ssp. *kauaiensis* as a member of a mutualistic association encompassing four kingdoms—Plantae, Monera, Fungi, and Animalia, as birds apparently are involved in long-distance dispersal of its fleshy fruits (Doyle 1990).

The findings of VAM in *Gunnera* are of uncertain value in judging the appropriateness of the present classification of the Gunneraceae in the Haloragales. Only three other species in the order (all in the family Haloragaceae) have been examined for mycorrhizae. Two lacked mycorrhizae and one was reported to have VAM (Trappe 1987).

On the basis of the degree of mycotrophy of the Hawaiian species and the type of mycorrhiza formed (VAM), the Gunneraceae is not out of place in the highly mycorrhizal subclass Rosidae in which it now is classified (Trappe 1987, Cronquist 1988). Clearly, more data concerning mycorrhizae in other *Gunnera* species and putative relatives are necessary before substantive systematic conclusions can be made.

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