Mycorrhizal Status of Two Hawaiian Plant Species (Asteraceae) in a Tropical Alpine Habitat: The Threatened Haleakalā Silversword (*Argyroxiphium sandwicense* subsp. *macrocephalum*) and the Endemic *Dubautia menziesii*[1]

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Abstract: Samples of roots and root-zone soil from the threatened species *Argyroxiphium sandwicense* subsp. *macrocephalum* and the endemic species *Dubautia menziesii*, both members of the Asteraceae, were collected in a tropical alpine area in Haleakalā National Park, Maui, Hawai'i, and examined for arbuscular mycorrhizal fungi (AMF). All root samples exhibited the *Paris*-type of mycorrhizae with arbuscules produced on hyphal coils, and all soil collections included spores of AMF. Spores of *Acaulospora*, *Entrophospora*, *Glomus*, and *Scutellospora* spp. were recovered from this site.

ARBUSCULAR MYCORRHIZAL FUNGI (AMF) form mutualistic associations with the majority of plants and are especially frequent in endemic Hawaiian species (Koske et al. 1992). These fungi are known to play a vital role in scavenging phosphorus and other nutrients from soils and providing them to plants (Read and Smith 1997). The AMF association is especially critical to plants growing in soils with low availability of phosphorus, typical of many of the volcanic soils of the Hawaiian Islands (Gemma et al. 2002). In addition, AMF have been found to confer a significant level of drought tolerance to plants (e.g., Auge 2001).

The rescue of the Haleakalā silversword (*Argyroxiphium sandwicense* DC. subsp. *macrocephalum*) from near extinction in Haleakalā National Park, Maui, Hawai'i, ranks as one of the highlights of Hawaiian plant conservation (Loope and Medeiros 1995). This federally listed threatened species is endemic to a single 1000-ha area in Haleakalā National Park, occurring between 2100 and 3000 m above sea level (Loope and Medeiros 1995). The plants grow in nearly barren areas of loose volcanic cinder in a tropical alpine habitat. Seedling mortality is high in these sites, which are characterized by great diurnal temperature fluctuations (soil temperatures range from −5 to 60°C during the course of a single day), drought stress, and low plant-available phosphorus in the soil (Goldstein et al. 1996, Gemma et al. 2002).

Because of the known benefits of AMF in such stressful environments and the remarkable recovery of the population of silverswords in this nearly barren site, we investigated the mycorrhizal status of plants of *A. sandwicense* subsp. *macrocephalum* growing in Haleakalā National Park. In addition, we examined *Dubautia menziesii* (A. Gray) D. Keck, a codominant at the site.

MATERIALS AND METHODS

Root and soil samples were collected from the Kalahaku area of Mt. Haleakalā (elevation ca. 2825 m; 20° 45′ N, 156° 12′ W) from beneath six specimens of *A. sandwicense* subsp. *macrocephalum* (hereafter referred to as *A. sandwicense*) (Asteraceae) in December 1998. The sampling time in this alpine site (e.g., >1800 m [Carlquist 1980]) was in the wet

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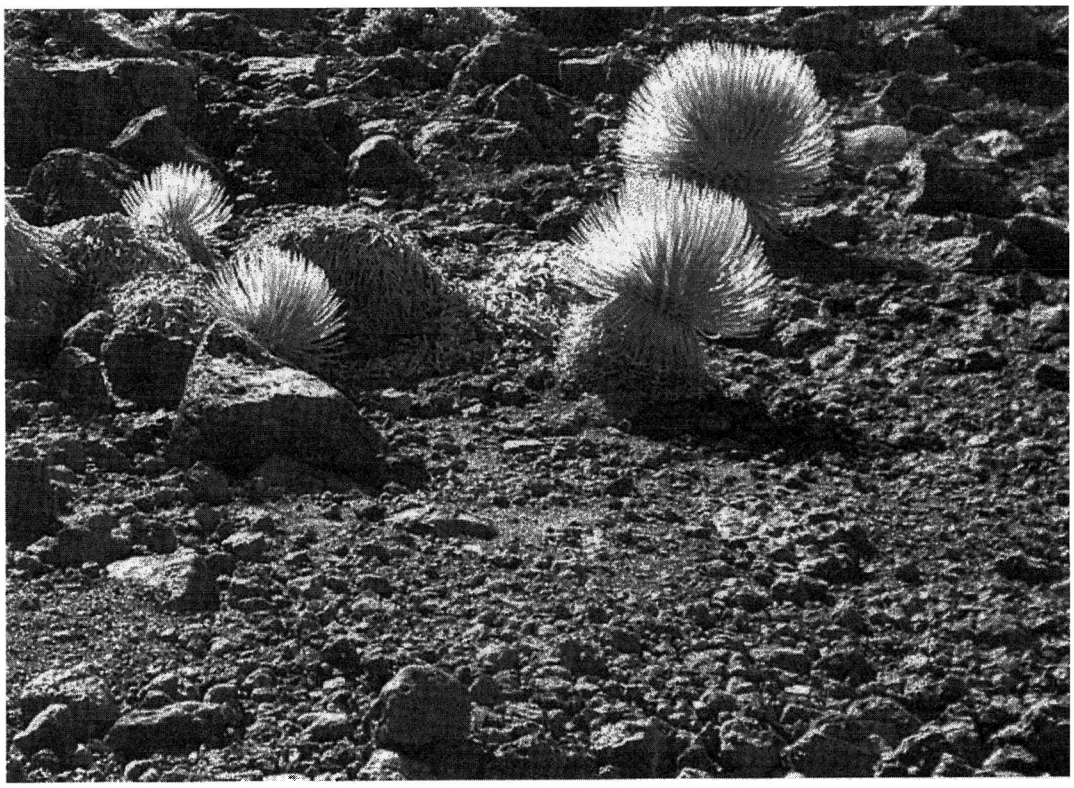


FIGURE 1. Habitat of *Argyroxiphium sandwicense* subsp. *macrocephalum* on Mt. Haleakalā, Maui, Hawai'i. Note low plant cover.

season that lasts from November to April (Price 1983). Care was taken to cause minimal damage to the plants (hence, root samples were small [ca. 6–10 cm long]). The a'ā type of lava dominated the site, and soils were composed primarily of weathered volcanic cinders. Volcanic activity had ceased several hundred years previous to the sampling. The collecting area had very low cover, and plants that were sampled were separated from other plants by >3 m (Figure 1). Other species in the area included *Dubautia menziesii* (Asteraceae), a species endemic to the slopes and crater of Mt. Haleakalā (Wagner et al. 1990), and the indigenous *Styphelia tameiameiae* (Cham. & Schlectend.) F.v. Muell. (Epacridaceae). For comparative purposes, collections also were made from isolated plants of *D. menziesii* (four individuals) from the same area. This species and *A. sandwicense* are closely related and hybridize spontaneously in the field (Wagner et al. 1990). All collections were taken to the laboratory at the University of Hawai'i at Mānoa on O'ahu, and roots were washed, cleared, and stained with trypan blue (Koske and Gemma 1989). Soil samples were refrigerated at 4°C until processed to recover spores.

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 species. Only those species in which arbuscules were found were considered to have functional arbuscular mycorrhizae (AM) and were given an infection rating greater than 0. Extent of

AM infection was quantified by assigning a mycorrhizal index (MI) value of 0 to 3 to stained roots, where 0 indicates no functional AM colonization, 1 = up to 25% of root length colonized (i.e., containing arbuscules, coils, and hyphae), 2 = 25–75% colonized, and 3 = >75% colonized (Koske et al. 1992). The MI of each species was calculated by averaging the MI of each sample of that species that was examined. Although the assessment of AM colonization of roots by the MI method is less precise than the more commonly used methods (e.g., calculation of percentage of length of root system containing AMF structures [Giovannetti and Mosse 1980]), there is little evidence that percentage of root colonization is correlated with mycorrhizal benefit. In several studies, significant increases in growth and drought tolerance resulted from inoculation with AMF when colonization levels were 1.5–7% of root length (e.g., Gemma and Koske 1997, Gemma et al. 1997).

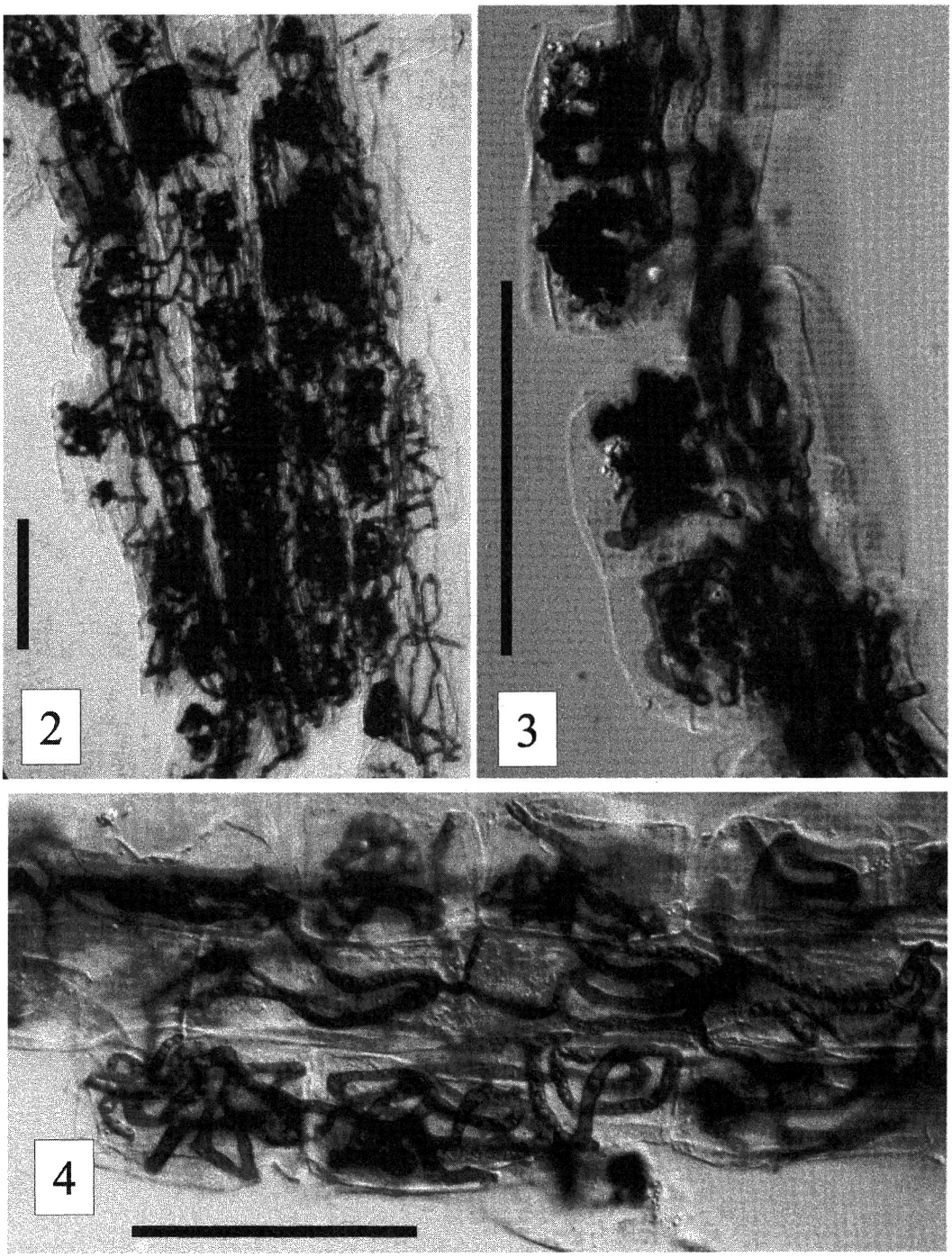
To identify spores of AMF that were associated with the plants whose root systems were sampled, soil samples of ca. 500 cm^3 were collected from each root zone. Spores of AMF were isolated from a 42-cm^3 subsample from each of the 10 collections by wet-sieving and centrifugation in 40% sucrose (Walker et al. 1982). Spores were identified by comparison with type specimens.

Spore abundance data were log-transformed (St. John and Koske 1988), and differences between AMF populations associated with the two host species were investigated by *t* tests. Differences in the frequency of occurrence of individual species were analyzed using a modified χ^2 test incorporating Yate's correction for continuity (Sokal and Rohlf 1981). Soil-solution phosphorus was measured (Fox and Kamprath 1970) in three of the soil samples from root zones of *A. sandwicense*. Three grams of soil was shaken in 30 cm^3 of 0.01 M $CaCl_2$ for 1 hr. The solution was centrifuged, and PO_4 in the supernatant was determined colorimetrically using the molybdenum blue method. Angiosperm systematics and nomenclature are those of Wagner et al. (1990).

RESULTS

All six root systems of *A. sandwicense* were colonized by AMF, and spores of AMF were found in all samples. Internal hyphae were abundant in the roots as were arbuscules and hyphal coils (Figures 2, 3). MI values ranged from 1 to 2, with a mean of 1.4. All root systems of *D. menziesii* were colonized by AMF (Figure 4), with MI values ranging from 1 to 2 (mean = 1.25). All mycorrhizae were of the *Paris*-type with arbuscules arising from hyphal coils in the cortical cells (Figure 3). Vesicles were not observed in any of the 10 samples. Soil-solution phosphorus values were 0.008, 0.009, and 0.009 mg phosphorus per liter.

Spores of AMF were found in all root zone samples. AMF species richness (no. of species recovered/sample) ranged from 2 to 4 (mean = 3.3) for *A. sandwicense* samples and 1 to 4 (mean = 2.5) for *D. menziesii* (Table 1). Spore abundance (all AMF species) did not differ significantly between samples from the two plant species, averaging 47.6 spores per 100 cm^3 soil for *A. sandwicense* and 150.0 for *D. menziesii*. Spores of *Entrophospora infrequens* Hall (Ames & Schneider) were identified. Other AMF species appeared to be undescribed and were identified only to genus and assigned a reference number (e.g., *Glomus* 8156). The AMF species most frequently isolated from root zones of *A. sandwicense* were *Glomus* 8156 (67% of the samples), *Scutellospora* 8156 (67%), *Acaulospora* 8156 (50%), *Acaulospora* 8159 (50%), and *E. infrequens* (50%) (Table 1). Frequency of occurrence of spores in the root zone samples of *D. menziesii* was similar to that of *A. sandwicense*, except that spores of *Scutellospora* 8156 were isolated from only a single sample. In spore abundance, *Scutellospora* 8156 dominated the *A. sandwicense* samples (mean = 19.00 spores per 100 cm^3), and *E. infrequens* was dominant in the *D. menziesii* samples (mean = 108.25 spores per 100 cm^3). Variation between samples was so great that no significant differences between the spore populations of each AMF species beneath different host species were detected.



FIGURES 2–4. Roots of *Argyroxiphium sandwicense* subsp. *macrocephalum* and *Dubautia menziesii* stained for arbuscular mycorrhizal fungi. 2, *A. sandwicense* root with dark-staining internal hyphae and coils in the cortical cells. 3, *A. sandwicense* cortical cells with four prominent arbuscules. 4, *D. menziesii* root with hyphal coils. Scale = 100 μm.

TABLE 1

Frequency and Abundance of Spores of Arbuscular Mycorrhizal Fungi (AMF) in Root Zones of *Argyroxiphium sandwicense* subsp. *macrocephalum* and *Dubautia menziesii*

	Argyroxiphium sandwicense subsp. *macrocephalum* ($n = 6$)		*Dubautia menziesii* ($n = 4$)	
AMF species	Freq.[a]	Abun.[b]	Freq.	Abun.
Acaulospora 8156	50	7.12 ± 4.44	50	17.00 ± 14.37
Acaulospora 8159	50	0.77 ± 0.48	50	17.47 ± 15.13
Entrophospora infrequens	50	3.93 ± 3.05	50	108.25 ± 86.79
Glomus 8155	33	2.78 ± 1.98	25	9.53 ± 9.53
Glomus 8156	67	5.66 ± 3.96	50	7.13 ± 7.13
Scutellospora 8156	67	19.00 ± 14.37	25	1.20 ± 1.20
Scutellospora 8170	17	8.70 ± 8.70	0.0	0.0
All species	—	47.6 ± 15.7	—	150.0 ± 86.2

[a] Percentage of samples from which spores were isolated.
[b] Mean spore abundance per 100 cm^3 of soil ± SE.

DISCUSSION

Examination of root and soil samples from the plants on Mt. Haleakalā revealed that both *A. sandwicense* and *D. menziesii* are AM-forming species. AM have been reported previously from other alpine habitats (e.g., Haselwandter and Read 1980), including from the tropical high mountains of Venezuela (Barnola and Montilla 1997). The latter investigators found AM in 28 of 31 species of plants growing at 3100 m elevation.

One other species of silversword, *Wilkesia gymnoxiphium* A. Gray (a species endemic to the island of Kaua'i), and several species in the closely related genus *Dubautia* in the Hawaiian Islands have been examined for AM (Koske et al. 1992). All three collections of *W. gymnoxiphium* were mycorrhizal (MI = 2.0). *Dubautia ciliolata* (DC.) D. Keck subsp. *ciliolata*, *D. knudsenii* Hillebr. subsp. *knudsenii*, *D. plantaginea* Gaud. subsp. *magnifolia* (Sherff) G. Carr, and *D. scabra* (DC.) D. Keck subsp. *scabra* also were examined, and all but the latter were always mycorrhizal. Three of six specimens of the latter species formed AM. Mean MI values for the *Dubautia* species ranged from 1.0 to 3.0.

Mycorrhizae in the roots of both *A. sandwicense* and *D. menziesii* were similar in appearance and were of the *Paris*-type (Smith and Smith 1997). The presence of this type in the Asteraceae is unusual (Smith and Smith 1997), and the few genera in the family that have been investigated possessed only the *Arum*-type of mycorrhiza. Further studies are needed to evaluate the significance of the Hawaiian results.

Identification of species of AMF is difficult, but several of the species from the Haleakalā National Park collections appeared to be undescribed. Additional material is required to confirm this. None of the species from Haleakalā National Park was isolated during the only other surveys of AMF in Hawai'i, those made from sand dunes on the islands of Kaua'i and Hawai'i (Koske 1988, Koske and Gemma 1996). Spores of *E. infrequens* have been collected from San Miguel Island off the coast of southern California (Koske and Halvorson 1989), in New Zealand (as *Glomus infrequens*) (Hall 1977), and elsewhere in the world (e.g., Koske et al. 1997).

The soil-solution phosphorus values (0.008–0.009 mg/liter) in the collections were very low, but are typical of many natural sites in Hawai'i (Gemma et al. 2002). For comparison, highly fertile agricultural soils have soil-solution levels of ca. 0.20 mg phosphorus per liter (Fox 1981). Of the Hawaiian plant species that have been grown in soils with levels of phosphorus comparable with those in the Haleakalā soil, AM routinely have been

required for significant growth (Miyasaka et al. 1993, Koske and Gemma 1995, Gemma et al. 2002). When seedlings of *D. scabra* were grown in a greenhouse in a soil with low phosphorus, plants that were inoculated with AMF had 100% survival whereas plants without AMF had 72% mortality (Koske and Gemma 1995). Similarly, two Hawaiian species of *Bidens* in the same family as *A. sandwicense* were 3.3 to 5 times larger (shoots) and 6.3 to 16.6 times larger (roots) when grown in low-phosphorus Hawaiian soils in the presence of an AM fungus (Gemma et al. 2002) in comparison with nonmycorrhizal plants.

Seedlings of *A. sandwicense* typically establish from wind-dispersed seeds in the Haleakalā site in protected microsites (e.g., near larger rocks) where extremes of temperature and moisture are less than in exposed locations (Goldstein et al. 1996). The means by which AMF fungi arrive at the same sites in which the seedlings establish is not known. AMF are obligate symbionts, and the fungi do not grow and reproduce in the absence of a live host. However, AMF propagules can persist in the soil in the absence of live plants for 4–5 yr or more (Ferguson and Woodhead 1982, Wagner et al. 2001). Although barren sites typically have no AMF or very low populations of AMF at a depth of several centimeters (e.g., Miller 1987, Koske and Gemma 1997), viable AMF propagules are deposited on the surface (Miller 1987).

Miller's studies in the Red Desert in Wyoming (Miller 1987) of the enhanced deposition of wind-borne spores of AMF in catchments that slowed wind speed may explain how propagules of AMF (e.g., spores, hyphae, mycorrhizal root fragments) and seeds of *A. sandwicense* arrive at the sites of establishment. Wind appears to be an effective dispersal agent, and spores of AMF were found to be carried for up to 2 km by wind in arid sites in Wyoming (Warner et al. 1987). At the Haleakalā site, disturbance of wind flow by rocks probably results in deposition of seeds, propagules of AMF, fine particles, and other debris near the rocks, leading to the establishment of seedlings in these protected sites. A similar pattern of invasion of recent lava flows in Hawai'i by AMF and AM-forming species was reported previously (Gemma and Koske 1990). In those barren sites, plant and AMF propagules accumulated in the cracks in the lava, apparently dispersed by wind and animals.

Such a scenario agrees with the manner in which the Haleakalā silversword population made its recovery over the last 60 yr. The recovery, which saw an increase from ca. 4000 individuals in 1935 to ca. 65,000 in 1993, resulted almost entirely from the use of exclosures to protect the plants from feral goats, domestic cattle, and human vandalism and theft (Loope and Medeiros 1995). Extensive field plantings were not made and no attempt was made to introduce AMF or soil containing AMF to the area (L. Loope, pers. comm.).

It would be of value to know the extent to which *A. sandwicense* is dependent upon AMF. The failure of AMF to be present at a site when seedlings emerge would likely be fatal in the abiotically stressed sites of Haleakalā National Park if *A. sandwicense* is as dependent upon AMF as are many of the Hawaiian species that have been tested (Myasaka et al. 1993, Koske and Gemma 1995, Miyasaka and Habte 2001, Gemma et al. 2002). Measurement of AMF populations in soil (e.g., mycorrhizal inoculum potential) in sites where *A. sandwicense* is established or is likely to become established may be important in understanding the low recruitment and high mortality among naturally occurring seedlings of *A. sandwicense* in Haleakalā National Park (Loope and Crivellone 1986, Loope and Medieros 1994).

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