The Propagation of Uluhe Fern (*Dicranopteris linearis*): Vegetative Versus Spores©

Ethan A. Romanchak, Richard A. Criley, and Nellie Sugii
Department of Tropical Plant & Soil Sciences, University of Hawai`i, 3190 Maile Way, Honolulu, Hawaii 96822

Uluhe fern, *Dicranopteris linearis* (Burm.) is an indigenous fern of the Hawaiian Islands. Because there is no established propagation method for this fern, a survey of propagation methods was conducted to determine a protocol suitable for large-scale production. For vegetative propagation, a layering and a division trial were performed in healthy, wild populations. The layering trial tested different concentrations of auxin. Rooted rhizomes and aboveground fern parts were transplanted from the wild into 3-gal pots for the division trial. Preliminary results have yielded no viable asexual method of propagation. For sexual propagation, aseptic cultures were established from spore germination on Steeves Medium (Steeves et al., 1955). Sporophyte genesis in aseptic culture has occurred, though sporophyte growth in vivo has not yet been measured. Preliminary results suggest that the successful propagation and production of uluhe fern will result from propagation by spores.

INTRODUCTION

Uluhe fern (*Dicranopteris linearis*) is an indigenous fern that occurs on all of the main Hawaiian Islands in mesic and wet forests, often covering steep slopes from near sea level to 2,000 m. This species is an early colonizer of landslides and other disturbed sites where it may form dense thickets up to three m deep over large areas of open-canopy, oligotrophic, Hawaiian forests; this is in part due to its indeterminate clonal growth (Palmer, 2003). This fern species is also able to colonize and maintain dominance due to its shallow rhizomes, highly effective leaf area, and phosphorus use efficiency. Uluhe fern is also known to contribute to soil organic matter in the form of leaf litter as soil nitrogen and phosphorus is returned to the poor soils common in tropical ecosystems. And finally, uluhe fern may play an important role in resisting invasions of exotic species into Hawaiian forests due to its role in altering forest floor light regimes (Russell et al., 1998).

Among candidate species for large-scale restoration work in Hawaiian mesic and wet forests, uluhe fern is an obvious choice.

Currently, there is no available procedure to propagate and produce uluhe fern. The objective of this research was to compare vegetative propagation methods with propagation from spores for the production of uluhe fern.

MATERIALS AND METHODS

Vegetative Propagation: Layering Trial. The auxins, indolebutyric acid (IBA 1%) and napthaleneacetic acid (NAA 0.5%) (from Dip 'N Grow®), were sprayed on a scraped nodal section of the rachis of uluhe ferns in the wild. Four auxin treatment doses were used: 0, 500, 1,000, and 2,000 ppm. Twenty uluhe fronds were randomly chosen to receive one of the four-auxin treatments. Five replicates of each treat-

ment were performed. A mixture of moistened peat moss and uluhe leaf litter was wrapped around the treated rachis and covered with plastic wrap then aluminum foil. The 20 experimental units were checked at 4 and 8 weeks and rated for root development.

Division Trial. Terminal rhizomes of uluhe fern containing at least one frond and numerous roots were chosen from a single uluhe fern in the wild. The rhizome was severed from the mother plant then carefully dug and transplanted into the top inch of a 3-gal container filled with native soil. A total of 10 divisions were performed. Containers were kept under 50% shade, watered regularly, and monitored for survival for 8 weeks. The collection site and the division trial site were geographically adjacent; therefore environmental conditions for the division trial site were similar in light quality, day length, and air/soil temperature.

Spore Propagation. Fertile fronds of uluhe were collected from the wild on three of the main Hawaiian Islands (Hawai`i, Maui, and Oahu) for a total of six collections. Individual collections from single genotypes were not pooled. Fronds were left on paper for sporangia to dehisce and spores to disseminate. Approximately 25 µg of uluhe spores from each collection were separated from sporangia using the static electricity created from a small piece of plastic wrap and tweezers; then both the plastic wrap and spores were placed into folded filter paper and secured by a paper clip. The spores were hydrated in water for 10 min and then sterilized in a 10% bleach solution containing 2 mL of Tween® with agitation for 20 min. Using aseptic techniques and working under a hood, spores were rinsed with sterile water and then blotted on sterile tissue to remove excess water from rinsing. Spores were sown on Steeves medium (Steeves et al., 1955) in 90-mm Petri dishes by opening up the folded filter paper containing the plastic wrap and the spores and then blotting the filter paper spore-side down onto the growth medium (plastic wrap was removed). Therefore, the 25 µg of spores were evenly distributed over the surface area of the growth medium. The Petri dishes were sealed with plastic wrap and then placed under 12 h of broad spectrum light from fluorescent tubes at 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in a controlled environment (23 °C). Growth and development were monitored using a dissecting scope weekly for 16 weeks.

RESULTS

Vegetative propagation methods yielded no success**.** The nodal section of the rachis from the uluhe fern layering trial formed no adventitious roots or callus at any concentration of the applied auxin treatments during the 8-week trial. The division trial resulted in the death of all rhizome and frond tissues within 2 weeks of separation from the mother plant in the wild.

Propagation of uluhe fern from spores yielded numerous young sporophytes in vitro. Spores from each of the six collections started to germinate in 2 weeks. Prothalli formation occurred during Week 3 and continued developing until gametangia formation during Week 11. Fertilization and sporophyte genesis occurred over a 2-year period. In vitro 3-year-old sporophytes measure approximately 1.5 cm in height, though little effort had been made to encourage their development after they were formed. The sporophytes have not yet been moved to a Stage 4 medium.

DISCUSSION

Based on results from preliminary trials, the superior propagation method of uluhe fern was propagation from spores. The layering trial was not expected to yield positive results due to the anatomy of the rachis vascular bundles.

The division trial also yielded no positive results. Disturbance to the root system was probably responsible for the immediate desiccation and resulting death of all the divided fern parts. Improved environmental conditions for the divided ferns might have helped survival.

A follow-up study was performed to test whether the division trial failed due to root disturbance or severance from the mother plant. Rhizomes were cut to separate the terminal end (including at least one frond) of the uluhe fern from the mother plant in the wild, but the division was never dug and transplanted leaving the roots undisturbed. The separated terminal end that would normally be dug and transplanted for a division perished within 4 weeks on all 20 of the trials performed. This suggests that division of uluhe fern fails due to disturbance to the roots as well as severance from the mother plant or insufficient root mass at the terminal end.

Efforts continue to increase fertilization and resulting sporophyte genesis for the propagation of uluhe fern from spores. The length of time for fertilization and sporophyte genesis to occur is too long for a commercial production system. Future trials will look at the effect of spore density, media composition, and environmental conditions that will result in increased sporophyte genesis.

Currently, young sporophytes from the wild are successfully being grown in 4-inch containers with native soil. If this species can be successfully grown in containers, then the propagation of uluhe fern from spores may allow for large-scale restoration of open, wet, disturbed sites in the valuable watersheds of the Hawaiian Islands.

LITERATURE CITED

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