Using Micropropagation to Conserve Threatened Rare Species in Sustainable Forests

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ABSTRACT. For forests to be sustainable, viable populations of rare plants should be maintained. Where habitat management alone cannot conserve species threatened by human activity, micropropagation may advance species recovery. Micropropagation protocols were developed for Pacific Northwest endemics; *Hackelia venusta*, *Douglasia idahoensis*, *Astragalus* species, and *Cornus nuttallii*. Microshoots and seed were multiplied and rooted on nutrient media containing minimal levels of cytokinin and auxin growth regulators to maintain stable gene expression in plantlets. Acclimatized plantlets were reintroduced to protected habitat or propagated for further environmental experiments. Micropropagation serves a useful offsite role in sustaining Pacific Northwest forests by maintaining viability of certain threatened rare plants. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworth.com]*

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[Haworth co-indexing entry note]: "Using Micropropagation to Conserve Threatened Rare Species in Sustainable Forests." Edson, J. L. et al. Co-published simultaneously in *Journal of Sustainable Forestry* (Food Products Press, an imprint of The Haworth Press, Inc.) Vol. 5, No. 1/2, 1997, pp. 279-291; and: *Sustainable Forests: Global Challenges and Local Solutions* (ed: O. Thomas Bouman, and David G. Brand) Food Products Press, an imprint of The Haworth Press, Inc., 1997, pp. 279-291. Single or multiple copies of this article are available for a fee from The Haworth Document Delivery Service [1-800-342-9678, 9:00 a.m. - 5:00 p.m. (EST). E-mail address: getinfo@haworth.com].

INTRODUCTION

For forests to be sustainable, viable populations of rare plants should be maintained. Many rare woody and herbaceous plants have tangible or potential value to mankind. Increased human activity, however, has altered or eliminated habitat, overharvested plant resources, introduced competitive or pathogenic organisms, or modified the atmosphere to threaten rare endemic forest species and small disjunct populations. Habitat management alone may be unable to prevent extinctions or genetic losses where taxa exist as few meta-populations, occupy narrow ecological niches, or fail to regenerate vigorously (Falk 1992). Off-site conservation can advance species recovery by increasing plant numbers and by reintroducing plant populations into protected habitat (Maunder 1992). As a technology for off-site conservation, micropropagation is fast, uses small amounts of seed or shoots, and may succeed when other methods fail (Fay 1992). Study objectives were to advance, through development and application of micropropagation technique, the recovery of threatened taxa endemic to the forests and rangelands of the Pacific Northwest.

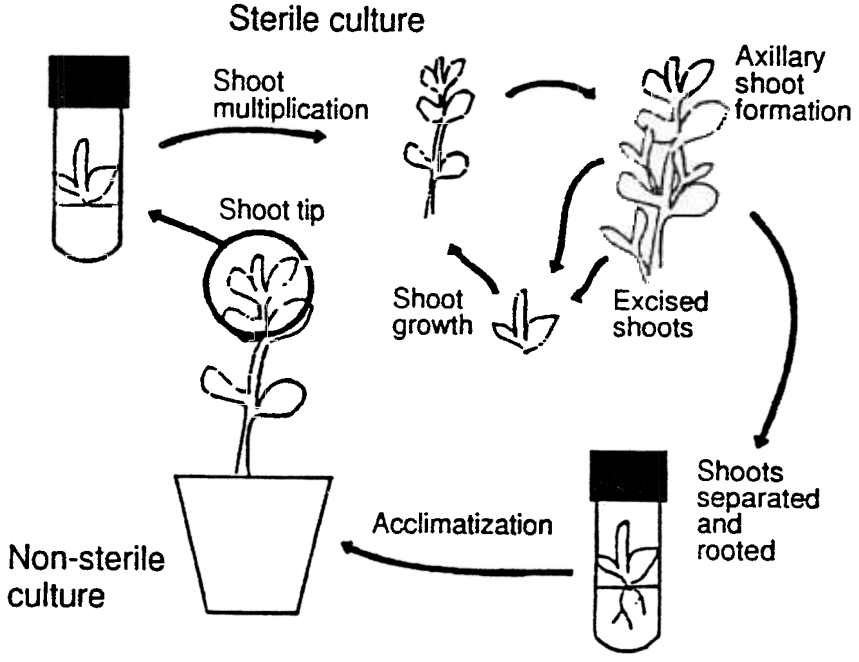
MATERIALS AND METHODS

Hackelia venusta, *Douglasia idahoensis*, *Astragalus* species, and *Cornus nuttallii* were chosen for micropropagation based on perceived threats to their continued existence. Small amounts of seed were collected from abundant sources, but shoot tips were propagated where seed was scarce.

General Micropropagation Technique

Micropropagation involves surface sterilizing seed shoots or buds, inducing shoot growth in sterile culture, multiplying and elongating microshoots, rooting elongated shoots, and acclimatizing plantlets (Figure 1). Seeds or shoots were incubated on a gel containing Murashige and Skoog (MS) nutrient medium (Murashige and Skoog 1962) supplemented with cytokinin or auxin growth regulators to enhance shoot and root formation, respectively.

FIGURE 1. Schematic showing procedure for multiplication of microshoots in sterile culture



Producing True-to-Type Plants

In rare plant recovery, plantlets should be propagated from a comprehensive sampling of the genome and changes in genes or gene expression during culture should be avoided. High concentrations of growth regulators, long culture time, and adventitious shoots produced from callus may result in off-type plants (George 1993), whereas low levels of growth regulators and short culture time promote phenotypically stable axillary growth.

To find low levels of growth regulator adequate for propagation of axillary shoots, microshoots were initially multiplied on MS media containing benzyladenine (BA) from 0 to 10 micromoles. Multiplication rates were selected from plots of BA dose versus microshoot production so that required numbers of plantlets to establish new populations could be produced in minimal time. Minimal BA dosages corresponding to the chosen multiplication rates were predicted by inverse regression (Seber 1977). Microshoots were rooted without auxin treatment where possible or low levels of

indoleacetic acid (IAA) where necessary. Plantlets (rooted microshoots) were transplanted to peat-perlite mixtures in a fog chamber (90% relative humidity) for several weeks and acclimatized to lower humidity and higher light intensity during a season's growth in greenhouse conditions before reintroduction.

APPLICATIONS AND DISCUSSIONS

A Highly Endangered Species

Hackelia venusta or showy stickseed (Boraginaceae), an herbaceous biennial endemic to the Washington Cascades (Hitchcock and Cronquist 1973), comprises a population of fewer than 100 individuals threatened by human activity in its roadside habitat. Because of seed scarcity, 1 shoot from each of 7 plants was multiplied (Figure 2) to develop a BA dose-response curve (Figure 3) for the species. From the plot, we estimated that 3 or fewer axillary shoots could be produced monthly by a low (<0.10 M) level of BA. Because 4 populations were to be reintroduced (1 ramet/genotype/population), 2 shoots/genotype/month needed to be produced in a minimum of 2 months. Inverse regression predicted minimal dosage of 0.02 MBA would produce 2 shoots/month.

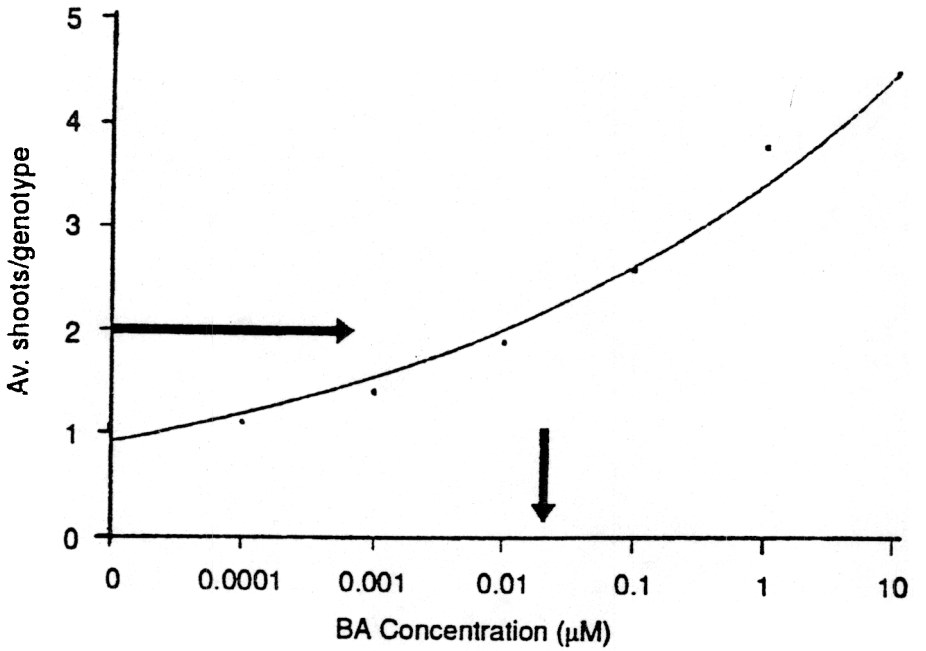
A further on-site sample of 30 genotypes was randomly selected as representative of variation in the population. Microshoots of all 30 genotypes were multiplied (with 0.02 M BA) and rooted at 90-100% on a medium with low dosage of 0.5 M IAA. Plantlets grew vigorously in the greenhouse (Figure 4). First reintroductions resulted in survival of up to 95% of the plantlets (Figure 5) after 9 months on the new forest site. In addition to ongoing plant recovery actions, propagated clones are used to study taxonomy and reproductive biology of the species.

Vulnerability to Climate Change

Douglasia idahoensis (Primulaceae) is a showy herbaceous Idaho endemic (Henderson 1981) found in 24 widely distributed populations (some with fewer than 50 individuals). Climate warming could result in rapid decline of this pre-pleistocene relict confined to subalpine peaks and lacking upslope refugia.

FIGUR Microshoot of *Hackelia venusta* on multiplicatio MS medium containing BA

FIGURE 3. Effect of BA concentration on microshoot multiplication in *H. venusta* after 1 month. Horizontal arrow represents microshoots per genotype to be produced and vertical arrow shows the predicted BA dose used



Microshoots were multiplied on MS medium with 0.04 M BA, and 60% rooting occurred without auxin supplement. Greenhouse and field growth has remained vigorous (Figure 6) for 2 years. We proposed trial plantings be initiated at higher altitude or latitude than current habitat to test the hypothesis that species viability would be enhanced in cooler refugia.

Micropropagating Seed

Micropropagation can increase survival of germinants in often critically small lots of seed collected from rare plants since seed germinated aseptically in culture avoids dampoff and other greenhouse pathogens.

An estimated 156 threatened species of *Astragalus* (Fabaceae) grow in the United States including *A. columbianus*, *A. amblytropis*, and *A. mulfordiae* (Sauer et al. 1979; Falk 1992). A single protocol using MS medium with 0.01 MBA successfully multiplied clones of the 3 species from sterile seedlings (Figure 7). More than 90% of

FIGURE 4. *H. venusta* plantlets (rooted microshoots) transplanted to the greenhouse

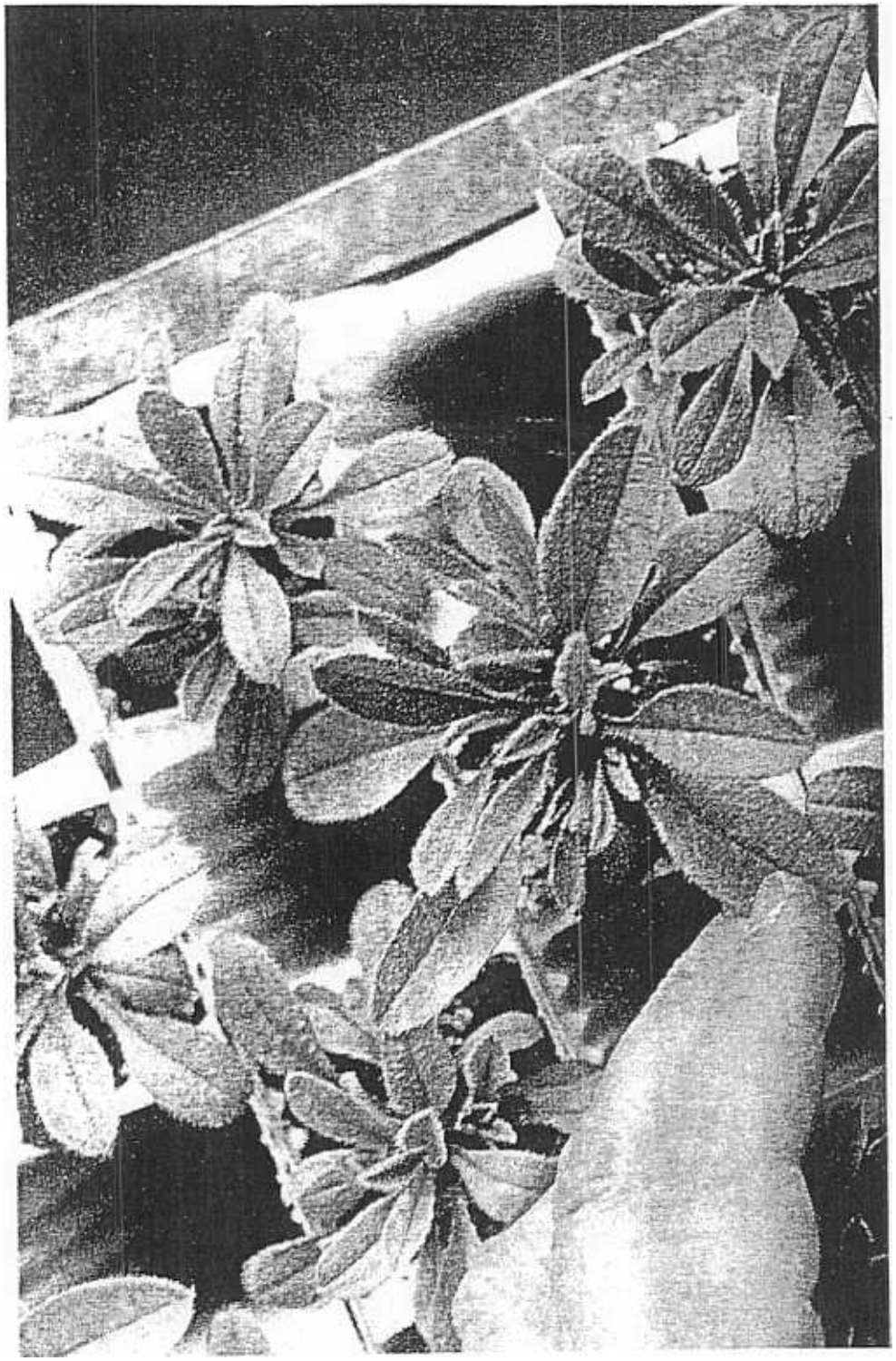
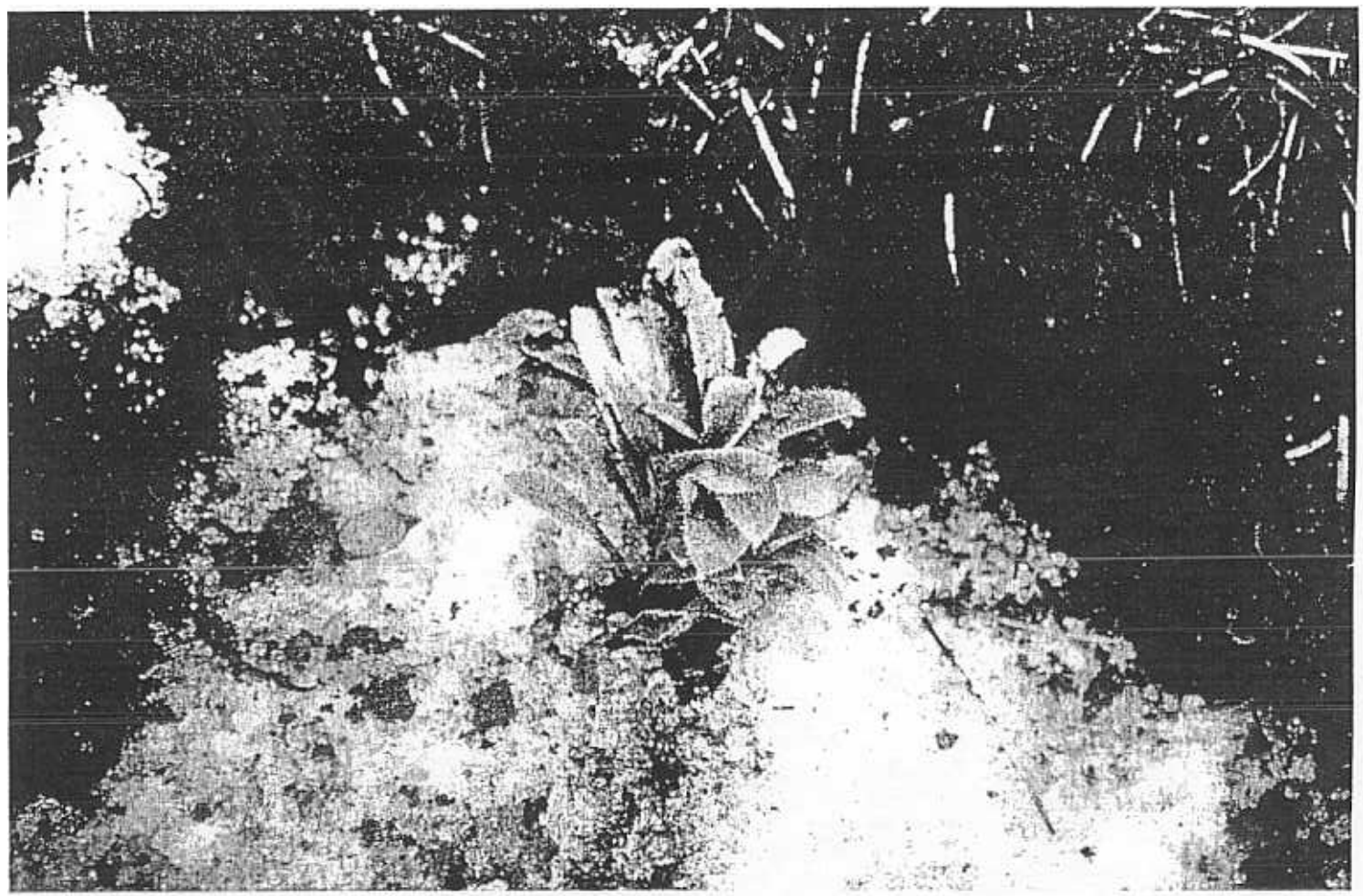
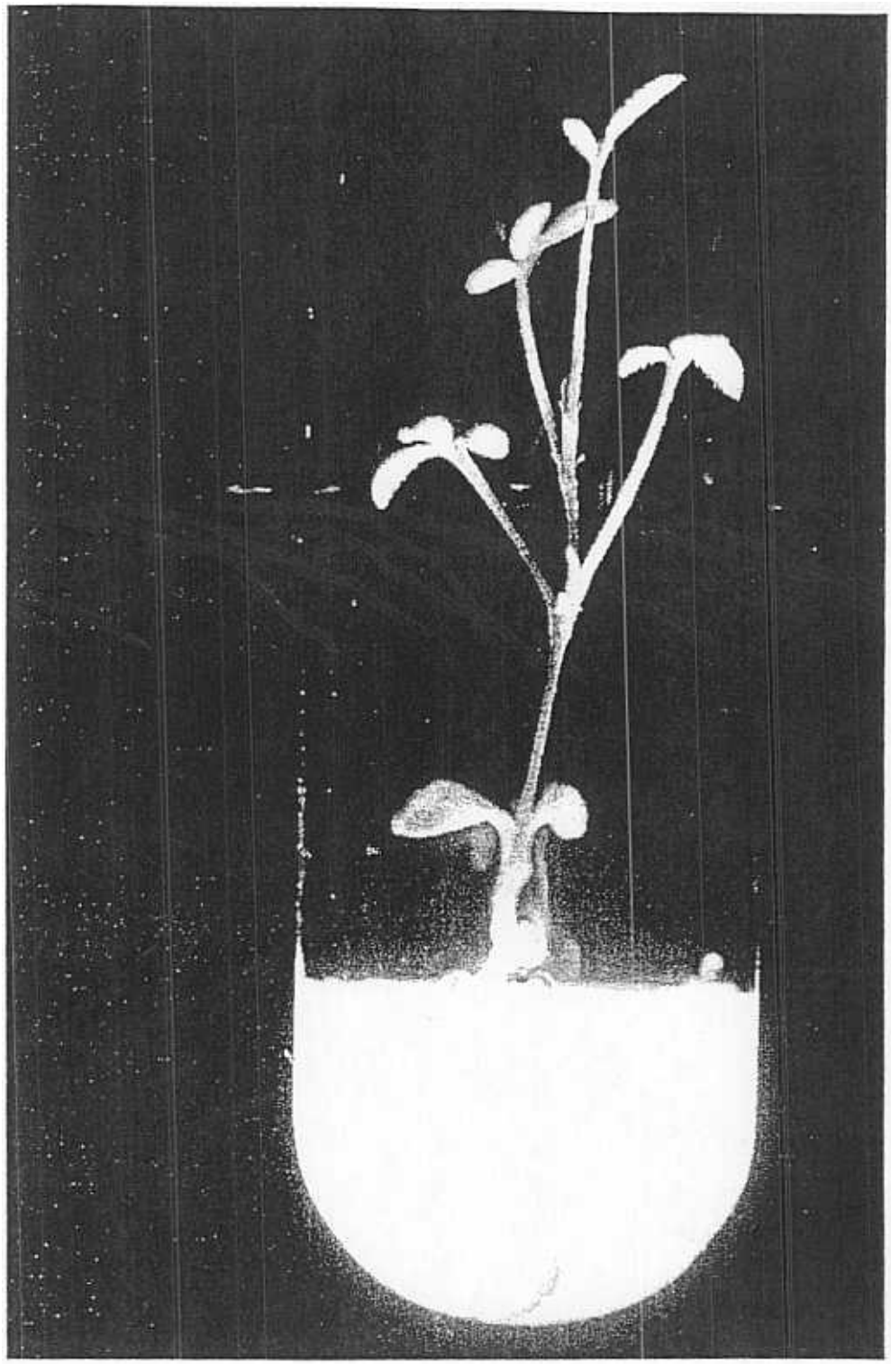


FIGURE 5. *H. venusta* plantlets reintroduced to a forest site



IGURE *Douglasia idahoensis* afte 1 year of greenhouse growth

FIGURE 7. Sterile germinant of *Astragalus columbianus*



A. columbianus and *A. amblytropis* transplants to greenhouse and field survived and produced viable seed. Adaptability of *A. columbianus* is being tested in climate change experiments.

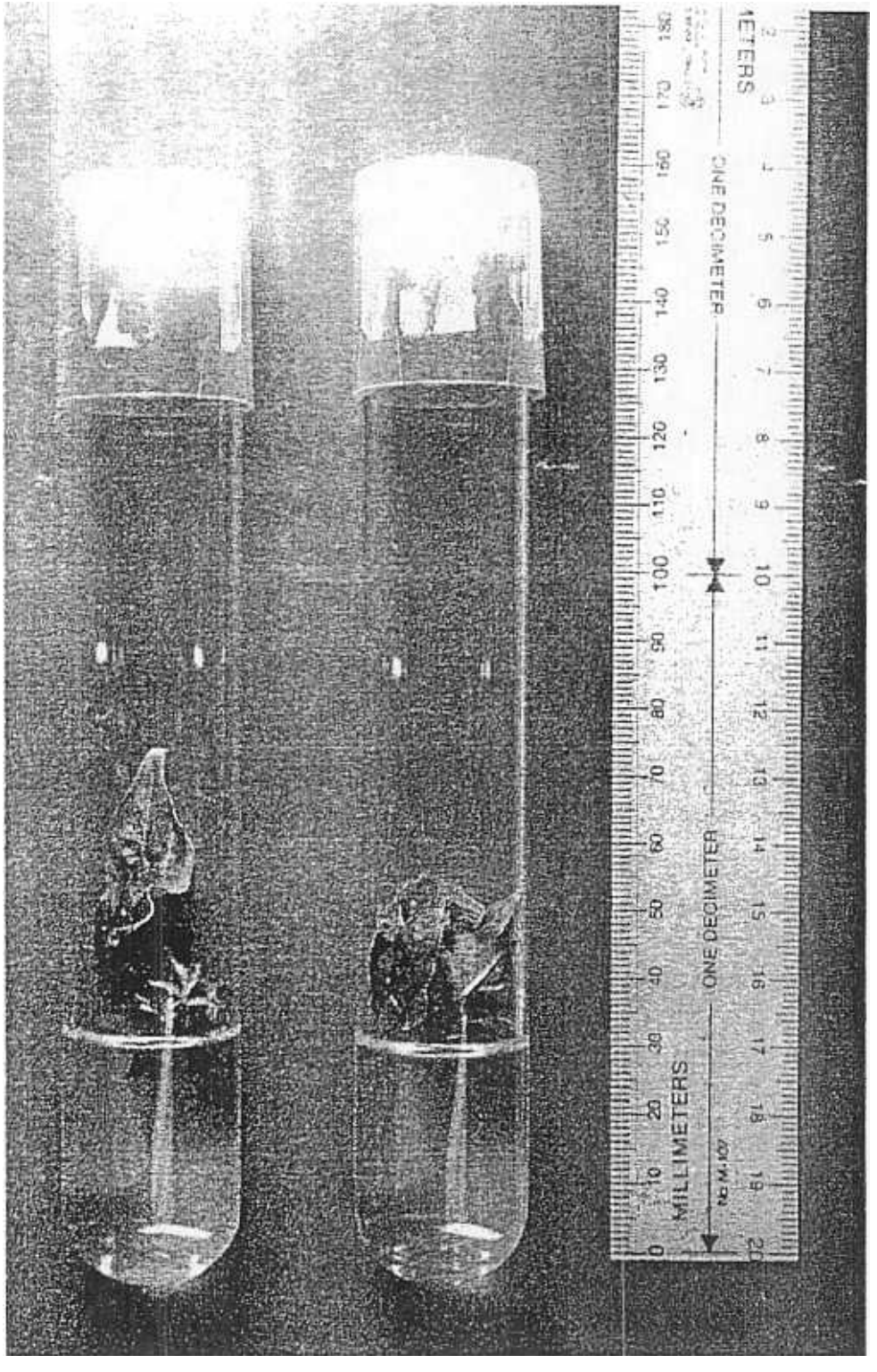
Unique Disjunct Population

Idaho's Pacific dogwood (*Cornus nuttallii*) is a once abundant, but now rare, unique coastal disjunct in the Northern Rockies. The trees, infected with dogwood anthracnose (*Discula destructiva*) produce little seed. Shoots were multiplied and rooted from initially disease-free greenhouse seedlings (Edson et al. 1994). Since transplanted plantlets became infected in the greenhouse after several years, reintroduction is not presently feasible. Present conservation actions are to maintain germplasm as seeds in seed banks at −20°C and as microshoot cultures in gene banks with growth suspended at 1°C (Figure 8) until propagation of resistant genotypes allows reintroduction.

CONCLUSIONS

Micropropagation, as an off-site option, can help maintain biodiversity in sustainably managed forests of the Pacific Northwest where threatened taxa cannot be adequately protected by habitat management alone. Our methodology using low levels of growth regulators and short culture times should be useful to preserve phenotypic stability and advance plant recovery programs when propagation is necessary and feasible.

FIGURE 8. Microshoots of *C nu*



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