5000 mg·L^{-1}. In August, rooting of >80% and highest root number resulted when cuttings were kept without bottom heat, regardless of auxin level.

After the conclusion of this study, an informal experiment was conducted in a commercial nursery in Tucson, Arizona. Cuttings of *L. candidum* 'Silver Cloud' were taken as described above during the month of October and basal ends were dipped in 2500 mg·L^{-1} K-IBA. Bottom heat of 27 ºC was provided and plants were kept under intermittent mist. After 21 days, rooting percentage of 75% was achieved, while previous propagation of *L. candidum* 'Silver Cloud' in this nursery yielded rooting percentages of less than 20%.

CONCLUSIONS

Leucophyllum candidum 'Silver Cloud', when propagated in June, should be supplied with bottom heat (25–30 ºC) and auxin at 7500 mg·L^{-1}. Later in summer when temperatures are higher, supplemental bottom heat is no longer critical, but auxin at 2500 mg·L^{-1} or higher still enhances rooting percentage and root numbers.

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Actinorhizae and *Ceanothus* Growing®

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INTRODUCTION

Plants in the genus *Ceanothus* grow in rocky and sandy areas. Because of their nitrogen-fixing ability and also because they are pioneer species, *Ceanothus* plants are extremely important in dryland ecological restoration.

Ceanothus plants form symbioses with bacteria in the genus *Frankia*. The actinomycetes nodulate eight plant families representing about 25 genera, collectively called actinorhizal plants (Baker and Schwintzer, 1990). Actinomycetes possess fungal-like structures, including septate filamentous hyphae, vesicles, sporangia, and spores. The bacteria invade root hair cells by the formation of an infection thread (Berg, 1999). Actinorhizae provide plants a ready supply of nitrogen, water, and nutrients, as well as elevated disease resistance.

In nursery conditions, plants in the genus *Alnus* form actinorhizae with air-borne inocula. However, *Ceanothus* plants do not form actinorhizae even though native stands exist in adjacent forests.

The goal of our experiments was to synthesize actinorhizae for *Ceanothus* plants under nursery conditions. We first conducted a literature search, then interviewed researchers, and conducted two greenhouse experiments.

Literature Search and Interviews. We found from the literature search that the genus, *Frankia*, was responsible for the symbiotic relationship. These actinomycetes infect plant roots primarily by root-hair infection. Actinomycetes form vesicles and spores as storage and inoculation organs (Verghese and Misra, 2002; Maunuksela, 2001), and actinomycetes exist in nonhost plant stands or long after host plants have disappeared from a site (Jeong and Myrold, 2001). However, there are few publications focused on how to synthesize actinorhizae in a commercial nursery.

During the interviews with researchers, we learned that one could culture *Frankia* on nutrient media successfully. However, these inocula from artificial cultures did not infect plant roots. Only *Frankia* inocula grown with host plants could infect *Ceanothus* plants.

From the literature search and interviews, we decided that root nodules and adjacent soils were potential inocula for the synthesis of actinorhizae.

EXPERIMENT I

We located one healthy native stand of *Ceanothus velutinus* near our nursery in Corvallis, Montana. We carefully dug into soils under one plant. Root nodules and soil adjacent to nodules were collected and stored in a cooler at 2–5 °C until used.

One-gallon containers were filled with soil mix of 2 peat moss : 1 perlite : 1 vermiculite (by volume). Treated *C. velutinus* seed was sown on top of the soil in each container and covered with perlite 5 mm thick. Seeds were misted with water twice a day until full germination. Seedlings were thinned to one plant per container after full germination. For native soil treatment, screened native soil was banded at 1 inch below the soil surface. For the crushed-nodule treatment, freshly crushed nodules were mixed in distilled water then injected 1 in. below the soil surface after full germination. For the control treatment, soil filling, seed sowing, and thinning were done following the same procedures as the other two treatments, except no inoculation was performed. There were ten replicates for each treatment.

The greenhouse temperatures were set at 25/18 °C, day/night, respectively. High-intensity lights were used as supplemental light. Light intensity for clear days was about 1500 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ and intensity for cloudy days was about 500 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. Photoperiod was set at 16 h. After full germination, plants were fertilized with every third irrigation. Soluble fertilizer solution was injected into the irrigation system to achieve 100 ppm N, 25 ppm P, 100 ppm K, plus all required essential elements. The pH was adjusted to 6.5 with phosphoric acid.

Four months after full germination, all plants were harvested. Half of these plants were subjected to height, caliper, and biomass measurements and the other half was subjected to leaf nitrogen analyses.

Seedlings inoculated with crushed nodules did not form any actinorhizae. Most of these seedlings (75%) inoculated with native soil that was collected adjacent to nodules formed well-developed actinorhizal structures. As expected, control plants did not form any actinorhizae. Actinorhizal plants exhibited superior vigor, produced more biomass, and contained higher nitrogen levels in their leaf tissue than control plants. Although actinorhizal plants tended to be taller with larger caliper than control plants, the results were not statistically significant, perhaps because of the short experimental time.

EXPERIMENT II

A healthy, native, mature plant was dug from a native site and transplanted into a 24-inch wooden box in the nursery. The filling soil media was the same as our experiment soil used in Experiment I. The plant was fertilized with the soluble fertilizer solution as described in Experiment I. This plant, confirmed to be actinorhizal, was allowed to grow in the nursery for a full year before destruction.

Experiment II was conducted the same way as Experiment I, except soil adjacent to nodules and nodules were collected from the cultured plant described above. As

expected, the results were the same as Experiment I. Plants inoculated with soil collected adjacent to the nodules were about 75% actinorhizal. Plants inoculated with crushed nodules did not form actinorhizae, except one plant without well-developed actinorhizal structures. Again, control plants were not actinorhizal.

CONCLUSION

We concluded, from the literature search, interviews, and two experiments, that the vesicles and spores in the soil media collected from native stands or nursery-cultured plants adjacent to root nodules were the most effective inocula for *C. velutinus*. From the two experiments, we learned that actinorhizal plants were much healthier than non-actinorhizal plants, even in nursery conditions. We believe that plants with well-developed actinorhizal structures have a higher chance of survival in native environmental conditions after out-planting on natural sites than non-actinorhizal plants.

RECOMMENDATION AND FUTURE RESEARCH NEEDS

To synthesize actinorhizal *Ceanothus* plants in the nursery, one can collect native soil adjacent to root nodules for the right species and band 1 in. deep from the soil surface in growing containers before sowing. To increase inoculum density in the container, liners should be grown then transplanted into final containers. In this case, inocula can be mixed into liner soil media.

Soil samples collected adjacent to root nodules may contain pathogens. An isolation or purification procedure needs to be developed. Since inoculum density varies from soil sample to soil sample, an inoculum density quantification methodology needs to be developed. Also, factors influencing inoculum density in the soil need to be identified.

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