Propagation and inoculation of native nitrogen-fixing shrubs and trees

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Actinorhizal trees and shrubs are nonleguminous species that can form root nodules and fix nitrogen. The microorganism that infects symbiotically and possesses the nitrogenase enzymes is an actinomycete, *Frankia*. Nitrogen gained by the host plant from the symbiosis supports a major fraction of its total growth. Over the years these plants also contribute substantial amounts of nitrogen to the soil.

In nature, the actinorhizal hosts are primarily pioneer species, occupying habitats of recently exposed parent material or of low soil fertility. California has an astonishing diversity and number of actinorhizal species, representing perhaps the richest such assemblage in the world. Several of these natives, notably *Ceanothus* species, have ornamental value. Many are drought-tolerant, including *Ceanothus*, *Cercocarpus*, and *Purshia*. *Casuarina* species, while native to Australia, are also well adapted to California's dry-season conditions.

Actinorhizal plants thus offer potential in landscape situations where soil is poor, water may be a limiting factor, and maintenance is to be minimal. Specifically, these plants may be used effectively for revegetation of disturbed sites, such as mine spoils, construction sites, and hydroelectric projects; as highway plantings or for soil stabilization on road-cuts; in large outdoor public areas; and for soil stabilization in watershed areas. Some species may also have browse value for wildlands conservation.

California has an astonishing variety of native nitrogen-fixing trees and shrubs, such as this *Cercocarpus ledifolius*, that are useful for revegetation of disturbed areas. Successful propagation of cuttings and seedlings is a first step in using them effectively.

The main goal of this project was to collect plant materials from a range of native actinorhizal species and to assess these for successful propagation. For outplanting in poor soils, techniques were explored for supplying *Frankia* inoculant, to permit good nodule establishment.

There are many unanswered questions on the physiological and environmental interactions governing nodule establishment and activity (for example the effects of dry or saline conditions). The potential exists for developing superior or site-adapted plant and nodule material. Propagation and inoculation of clonal material and seedlings was a first step in investigating these questions.

Collection of materials

Plant and inoculant materials were collected to obtain a variety of species at different stages of seasonal growth. Sixteen species from five native actinorhizal genera (*Alnus, Ceanothus*, *Cercocarpus*, *Purshia*, and *Shepherdia*) were tested. Collecting sites ranged from Los Padres National Forest to Sequoia National Forest, and included the UC Davis Arboretum, the Stebbins Natural Reserve, and UC Berkeley Botanical Gardens.

Best survival and rooting of cuttings appeared to result from material taken from vigorous specimen plants, with abundant shoots that clearly achieved considerable current season's growth, with green glossy leaves, and without disease or stress symptoms.

In the field, we excavated a number of *Ceanothus* and *Cercocarpus* plants, in search of root nodules for inoculant. Characteristics of plant vigor correlated with the occurrence of root nodules. Nodules were found in soils that were generally sandy or gravelly, or that contained sandy lenses, often near runoff or dry washes. While ectomycorrhizae were often noted in surface organic soil layers, nodules tended to be located at slightly deeper levels, from 8 inches to 4 feet.

Good specimens for cutting material thus may also serve as good sources for nodules. Time of nodule collection for inoculant is restricted to seasons in which soil moisture is optimal to permit excavation, in our experience late winter and spring. This restriction severely limits the usefulness of nodule inoculation. (In a related project, we have been isolating and testing the *Frankia* organism from root nodules collected in the field, so that we have a controlled source of inoculant for a given host species. Pure isolates will eliminate difficulties inherent in handling soil-based inoculation, and may permit further selection of site-adapted *Frankia* strains.)

Aeroponics tanks

The aeroponics tanks we built were modifications of a design of Zobel *et al.* The tanks operate well with very little maintenance other than a weekly supplement to the nutrient solution.

In an unheated greenhouse, bottom heating with cables in a sand base was necessary during October through April; nutrient solution temperature was thereby maintained between 65° and 75°F. Shade cloth (92%) was used beginning in July, and is now in place year-round.

Cutting propagation trials

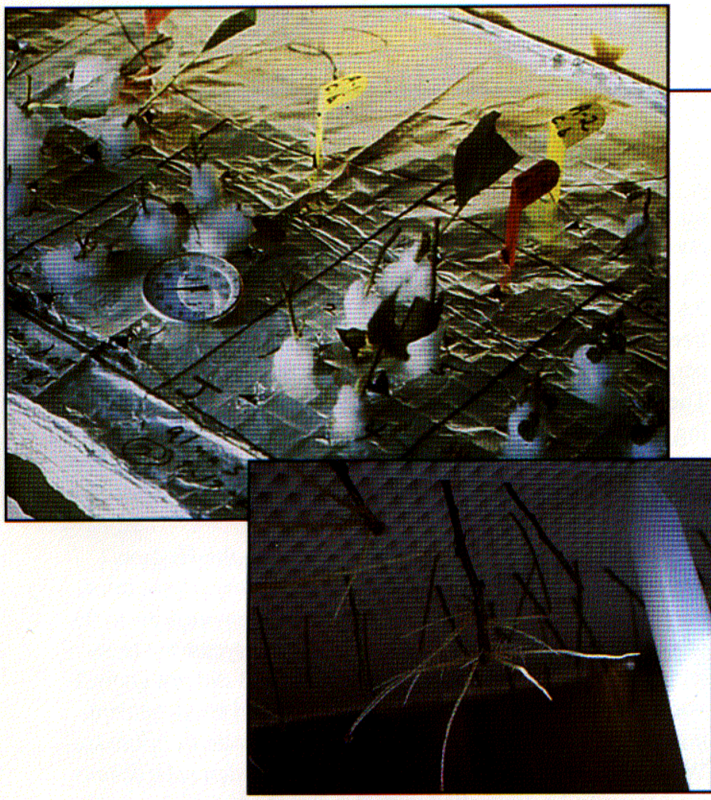
Eight screening experiments were performed during the one-year period of the project, beginning in May 1985. Trials were carried out in either mist bench or aeroponics tanks. Cuttings were prepared from current season's growth of single shrubs as terminal cuttings of slightly flexible woody shoots. Basal ends were dipped in 5,000 ppm IBA and stuck in 1:1 perlite:vermiculite in deionized mist, or suspended in the aeroponics tanks. Four replicates of eight cuttings each per species per treatment were usually prepared. Propagation methods and rootability of species were evaluated using three criteria: percent survival of unrooted cuttings (%SU); percent rooting of surviving cuttings (%R/%SU); and percent survival of rooted transplants (%ST).

Survival of plant materials was a problem repeatedly encountered, both in obtaining rooted cuttings and in ensuring success of transplanting onto the greenhouse bench. Mean survival (%SU) across 13 species was 34 percent in aeroponics and 50 percent in mist. Losses were gradually reduced during the year's experiments. The major difficulty in devising a propagation system seems to be in preventing excessive water loss by transpiration, while at the same time preventing the detrimental effects of water saturation.

In both mist and aeroponics, unrooted cuttings of some species were killed from the base upwards, indicating excess basal moisture. No rot or disease organisms could be associated with this basal dieback. In the mist, some species dropped leaves, indicating intolerance for water-saturated air; in aeroponics, several species dried out from the apex downward, indicating excessive transpirational losses.

For all but one of the species tested in both conditions, rooting was considerably better in mist than in aeroponics. Mean rooting percentage (%R/%SU) across species and treatments was 40 percent in mist and 11 percent in aeroponics. Only *Purshia tridentata* survived well (50%) and rooted better in aeroponics (84%) than in mist (50%).

Conditions contributing to good survival of unrooted cuttings included: 92 percent shade cloth, cool air temperatures,



Aeroponic propagation tank and rooted cutting.

shorter day lengths, still air rather than moving air, and, in the fall, bottom heating of tanks in an unheated greenhouse.

Survival and rooting ability of species tested were divided into four categories:

1. Good survival and relatively easy to root
   Alnus tenuifolia
   Ceanothus griseus
   Ceanothus cv. Julia Phelps
   Ceanothus maritimus
   Ceanothus thyrsiflorus x *leucodermis* (from A.T. Leiser)
   Cercocarpus ledifolius
   Purshia tridentata

2. Good survival, rooting moderate to poor
   Ceanothus hearstiorum
   Ceanothus purpureus
   Cercocarpus betuloides

3. Poor survival, rooting moderate to good
   Ceanothus ramulosus
   Cercocarpus betuloides var. Blancheae
   Purshia glandulosa

4. Poor survival, poor rooting
   Alnus rhombifolius
   Ceanothus foliosus
   Shepherdia canadensis

For the most part, current season's growth that had matured to a woody condition survived better than succulent tips. Suitable cutting material of *Ceanothus* was available beginning in mid-May. For *Cercocarpus* and *Purshia*, the semi-hardwood stage was reached in July to early August, when seed neared maturity; this varied with altitude and latitude. Time to rooting ranged from about six to ten weeks, depending upon species. *Cercocarpus betuloides* cuttings collected in July were successfully rooted in mid-winter after six months' cold storage. Second-year wood of *Cercocarpus ledifolius* also rooted, in midwinter.

Further experiments were carried out for difficult-to- root species, testing effects of mist frequency, rooting compounds, and concentration of rooting compound applied. Improvements in rooting thus obtained were small, and may be related to improved survival.

Transplanting and nodulation

Rooted cuttings were transplanted from mist or aeroponics into pots on the greenhouse bench. Transplant success for the screening trials was quite low; however, in careful studies of selected *Ceanothus* species, virtually 100 percent survival of large numbers of transplants was obtained.

In nodulation trials of three *Ceanothus* species, successful transplants were inoculated with crushed nodule suspensions after establishment in pots. We tested several soil mixes and found that porous media such as perlite favored both plant growth and subsequent nodulation in the *Ceanothus* species. Media amended with peat inhibited nodulation and often produced suboptimally wet soil conditions. Nodules were observed on the plants within six to eight weeks after inoculation.

Nodulation experiments using field-collected rhizosphere soil as inoculant for *Cercocarpus* species have recently had some success. Using crushed-nodule inoculant we obtained nodulation on *Ceanothus betuloides* var. Blancheae. In preliminary trials, the highest percentage of nodulation was obtained in 1:2 perlite:vermiculite and with very low levels of combined nitrogen applied once a week.

Seed germination

Seed germination requirements of four rosaceous actinorhizal species were investigated as an alternative to cutting propagation. Stratification in moist sand for 40 days improved germination of *C. betuloides* var. Blancheae, *C. ledifolius*, and *P. tridentata*. Treatment with hydrogen peroxide improved germination of *P. glandulosa*. Germination of all four species was enhanced in a cool greenhouse as compared with a growth chamber.

Future research directions arising from this study that appear to be most promising are: (1) testing of the correlation between vigor of the source plant and success in rooting cuttings of the difficult-to-root species; (2) continued selection and testing of plant materials and *Frankia* inoculant for optimal or for site-specific performance; (3) continued development of cutting propagation and inoculation procedures.

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