17

Biotechnological Induction of Rooting in *Arbutus menziesii*

Barbara Selemon and Toby Bradshaw

Abstract: This work tests the ability of the bacterium hairy-root (*Agrobacterium rhizogenes)* to induce the formation of adventitious roots on Pacific madrone (*Arbutus menziesii*) stems and cuttings. Madrone stems 6, 9, 12 and 18 months of age are innoculated at different seasons of the year and tested for the production of adventitious roots. Successful rooting of stems with endogenously produced auxin is more effective than externally applied rooting hormone. This rooting allows for more successful commercial propagation and experimental designs with madrone. Vegetative propagation of resistant individual trees and replanting with more vigorous offspring ensures that the Pacific Northwest does not lose this magnificent tree.

Pacific madrone (*Arbutus menziesii*) has proven to be a difficult, if not impossible, species to root with standard methods of stem cutting propagation. Our previous research showed that no roots developed in varying the factors of plant age, season, hormone strength or propagation medium. Other Northwest plant propagators corroborate these negative results with this tree using standard procedures (S. E. Svenson and R. F. Bush personal communication).

Because traditional methods of rooting madrone have been unproductive, we are exploring a biotechnological solution. Specifically, we are testing the ability of the bacterium hairy-root (*Agrobacterium rhizogenes*) to induce the formation of adventitious roots on madrone stems and cuttings (Strobel and Nachmias 1985 and Tripp and Stomp 1997). This bacterium is commonly found in soil. It is a natural genetic engineer. *Agrobacterium rhizogenes* enters a plant stem wound, binds to the surface of the plant cells and then transfers a small segment of bacterial deoxyribonucleic acid (DNA) to the plant cell nucleus. This transferred DNA (T-DNA) contains bacterial genes that direct the synthesis of auxins—the same plant growth regulators commonly used to induce rooting in traditional horticulture; however, unlike this

procedure of dipping a cutting in auxin, the bacterium engineers the plant to make auxin inside the cell. Internally produced auxin is a more effective agent at inducing root production. The plant symptom produced by *A. rhizogenes* is called "hairy root." *Agrobacterium rhizogenes* is used widely to produce roots on various tree species (Han, *et al.* 1996), usually with the intent of producing transgenic plants. The host range of *A. rhizogenes* is very wide, including essentially all dicotyledonous plants such as madrone, monocots and gymnosperms.

METHODS

Seeds from 20 healthy madrone trees are processed (Rodriguez and Shoffner this volume), placed in cold storage (3–5°C) and then placed into cold-moist stratification for 60 days in order to break embryonic dormancy. Seeds are sown into flats and placed in an ideal propagation environment (temperature controlled greenhouse 18–24°C) at the Douglas Research Conservatory at the University of Washington in Seattle. As the seeds germinate, plants are potted into individual 5 inch containers. The seedlings are monitored daily, watered when soil medium has dried out and fertilized once a week with a 200 ppm liquid fertilizer. As adequate growth [the stem growth is at least 5 cm (2 in) and the stem tissue has begun to harden] occurs, 20 plants are sheared to encourage them to branch. Ten of these plants are grown outside to serve as backup stock. Twenty plant stems are inoculated and the inoculation site is wrapped with Parafilm to retain moisture. Roots form within a few weeks of inoculation giving an "air-layered" appearance to the plant. Once roots are initiated, the stem is cut below the roots and the rooted cutting transferred to soil. The bacteria are no longer needed at this point; thus, they are removed with a simple antibiotic soil drench. Stems are inoculated at 6, 9, 12 and 18 months after germination.

DISCUSSION OF PROBABLE RESULTS

We anticipate that the 20 innoculated plants will produce some results in a short period of time (within 3 weeks) following injection with *Agrobacterium rhizogenes*. These "biotechnically layered" plants are detached from the mother plant and encouraged to grow on the newly produced root systems. Observations determine how well the new plantlets survive on the manufactured roots. Results may or may not indicate that one particular genotype responds better than others to this method of rooting. Repeated applications of the bacterium on the

original plant may indicate different responses of the stock plant to this type of treatment. Inoculation at various stages of growth determine whether age is a factor in the successful rooting of plants using *A. rhizogenes*.

DISCUSSION

The results of this experiment may significantly affect how Pacific madrone can be clonally produced in the future. Previous research using conventional means of stem cutting propagation has confirmed that standard propagation techniques are unsuccessful. If proven a successful method, the use of *Agrobacterium rhizogenes* to produce an adventitious root system will be a fairly rapid means of clonally reproducing plants. The costs are low in comparison to tissue culture production (another possible means of vegetative propagation), and the application technique is simple. Both the commercial nursery industry and future researchers working on this species will gain a better tool in reproducing Pacific madrone. The ultimate result could be the development of a practical method of producing disease resistant varieties for replacement of those which have succumbed to the complex of disease and urban stresses that are found in today's environment.

ACKNOWLEDGEMENTS

We wish to acknowledge C.W. Hamilton and D. E. Giblin for their collaborated efforts in an earlier project in which various methods were researched to root *Arbutus menziesii* by stem cuttings. Although the results of this work were that no roots formed during any of the year long trials, this negative response led to the current project of experimenting with bacterium injection. I also wish to acknowledge C. L. Richmond (an undergraduate at the University of Washington) for the many hours of assistance she's given working on this project and B.D. Watson (a research technician working within the Bradshaw lab) for the invaluable knowledge he has shared with me and for his expert technical assistance given in this project.

REFERENCES

Han, K.-H., D.E. Keathley, J.M. Davis and M.P. Gordon. 1993. Regeneration of a transgenic woody legume (*Robinia pseudoacacia* L. black locust) and morphological alterations induced by *Agrobacterium rhizogenes* mediated transformation. Plant Science 88:149–157.

Strobel, G.A., and A. Nachmias. 1985. *Agrobacterium rhizogenes* promotes the initial growth of bare root stock almond. Journal of General Microbiology 131:1245–1249.

Tripp, K.E., and A.M. Stomp. 1997. Horticultural applications of *Agrobacterium rhizogenes* ("hairy-root"): enhanced rooting of difficult-to-root woody plants. Combined Proceedings of the International Plant Propagators' Society 47:527–535.