slowly and we find that when we transplant, a few have callused but have no roots. These are restuck in flats and caried on under mist, but with no bottom heat. By fall most of these will have rooted.

If the above methods are used, and with patience to wait about 5 months for roots to appear, one should be able to root kalmia cuttings with very acceptable percentages.

ROOTING OF TISSUE CULTURED RHODODENDRONS

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Abstract. Rooting of tissue cultured rhododendrons directly from stage 2 can be successful, however, small changes in the growing environment cause variable survival rates. Uneven crop growth also has been a problem. My research has been redirected towards a stage 3 rooting step in culture in an effort to increase rooting percentages and reduce plant growth variations observed.

INTRODUCTION

The steps in tissue culture propagation involve 3 conceptual stages (3). Stage 1 describes the necessary procedures in establishing plant propagules in the culture environment. The important factors in stage 1 include the selection of the appropriate source of explants; choice of the approprate methods of disinfesting all pathogens from the explants, and determining the appropriate chemical and physical environment for growth and establishment of the culture. Stage 2 is the time when the plant propagules are multiplied. The important considerations of this stage are finding the appropriate growth regulator combinations for propagule multiplication (i.e. shoots for rhododendrons, crowns for strawberries, and bulbs for lilies). The number of times propagules are recycled in stage 2 depends on the genetic stability of the crop and the amount of propagation required. Stage 3 is the term used to describe transition period from the multiplication of propagules and establishing them in the soil environment. After stage 3, the plants can be handled in a similar manner used for growing seedlings.

The stage 1 and stage 2 requirements for propagating rhododendrons have been previously reported (1,2) including revision of the inorganic formula. At the present time about 50 rhododendron cultivars have been established in culture. Other

crops in the heath family that have been successfully established include bearberry, azalea, and kalmia.

During this last year my laboratory has emphasized experimental tests to determine if the in-culture stage 3 could be eliminated in the propagation of a wide variety of crops. The motivation to eliminate the in-culture stage 3 was based on reducing tissue culture costs since each handling after the last multiplication step requires individual handling of each plant. Therefore, using stage 3 prior to establishing tissue cultured plants in soil becomes a labor intensive step. Establishing rhododendron shoots directly into the soil environment was one of several crops intensively studied.

MATERIALS AND METHODS

A cooperative agreement was worked out with Briggs Nursery, Olympia, Washington, and Clay's Nursery, Langley, B.C. Canada, to root tissue-cultured rhododendron shoots directly from stage 2 and then to report back their results.

Research Unit. The planting procedure for the first experiment was to plant the shoots directly into unitized cells that were filled with 50:50 peat/perlite. The planted trays were placed in a closed box made from 1″ × 8″ boards for the box sides and were covered with 7 mil mylar over the top. A wet felt pad was placed under the trays to increase the humidity. The light source was from cool white fluorescent tubes with 16 hrs of ca 400 fc of light. Temperature was maintained at 70-75°F. The plantlets were maintained in the closed box for 5 weeks and then moved to the greenhouse under 40% shade.

Briggs Nursery. The planting mix used was 50:50 sand-peat and several kinds of planting containers were used. The plantlets were placed in a fog chamber with light coming from a combination of natural and supplemental light.

Clay's Nursery. Several different soil mixes were tested including a) peat; b) Jiffy 7's; c) 33% sand, 33% perlite; and d) 67% peat, 67% perlite. The trays of plantlets were placed on a bottom bench with most of the natural daylight shaded. The light source was from cool white fluorescent lights. The trays of plantlets were placed in styrafoam boxes and covered with glass. Temperature was maintained at 70-72°F. The plantlets were kept in this environment for 6 weeks and then placed under a propagation mist system.

RESULTS AND DISCUSSION

Research Unit. Early observation noted that plantlets planted directly into cells take up too much space in a growth room environment because rooting and initial growth of

rhododendrons is slow. There is about a 2-month lag in growth before the rapid plant growth phase begins. The planting mix must be porous to prevent water-logging conditions. Approximately 20% of the plantlets were lost in the laboratory and 20% when moved to the greenhouse, probably indicating that time in the light room should be increased to 6-7 weeks.

Briggs Nursery. A fogging device was used to mist the plantlets. There was a positive correlation of survival with the fog pattern emission.

Another interesting observation made at Briggs Nursery was that a thin layer of sphagnum moss placed on the surface of the planting mix improved survival and rooting.

Clay's Nursery. During the first experiment it was found that the 67% peat, 16% sand and 16% perlite was the best soil mix tested. During the second experiment best result was with 72% peat, 18% perlite and 9% sand and 1 pound of 18-6-12 Osmocote (8-9 mo.) per cubic yard. In all the previous studies no fertilizer had been added to the soil mix. The improved general health and survival of the plantlets indicates that a fertilizer program in the early stages of rooting will be helpful.

The combined results of the experiments show that all the culivars tested can be successfully rooted. There are several cases of variations in cultivar survival ranking between test sites and also between experiments at each site which indicates that both the general condition of shoots from the stage 2 cultures and the propagule rooting environment are important factors in plant survival.

Two major problems became apparent through these rooting experiments: the average survival rates were lower than hoped for, and the uniformity of the crops was variable at all 3 locations. Both of these problems can be aggravated by non-uniform shoots that come directly from stage 2. Some of the shoots are spindly and long and some have a translucent appearance from growing in direct contact with the culture medium.

An alternative method to reduce the problem is to grow the shoots in a stage 3 culture medium for one month. Here the shoots become uniform in appearance, taller, stockier, the base of the shoot becomes callused. Only a small percentage of the shoots produce roots in the stage 3 media. The individual plantlets are then transplanted into individual cells and are established as plants under a normal propagation mist system.

Using the alternative stage 3 in-culture step does not require any more handlings of the plants than planting directly from stage 2 into the soil. It may have advantages of greater

survival and greater crop uniformity. Any method that improves the percentage of usable plants without sigificantly increasing cost will likely be an advantage.

The length of time required in the small cells is about 3 months to reach sufficient size for transplanting into gallon cans.

Table 1. Survival and Growth of Tissue Cultured Rhododendron Shoots Planted Directly From the Shoot Multiplication Medium at 3 Locations.

Experiment 1. (Cuttings planted 4/1/78; survival observations 6/1/78)

	Research Unit		Briggs		Clay's	
Cultivar	Shoots planted	% survived	Shoots planted	% survived	Shoots planted	% survived
Blue Pacific	70	67	120	95	186	96
Pracox	73	89	432	91	241	79
Vulcan	73	88	103	69	199	90
Impeditum	73	64	102	72	210	95
PJM	73	71	120	69	194	88
Cynthia	64	38	204	91	173	90
Rose Elf	73	64	120	86	214	65
Hurricane	73	63	60	90	199	61
Cunningham white	73	64	120	53	183	77
Unique	73	11	120	65	184	58
Total shoots planted	718		1501		1983	
Mean % survival		62		79		80

Experiment 2. (Cuttings planted 6/27/78; survival observations 9/15/78)

	Research Unit		Clay's	
Cultivar	Shoots planted	% survived	Shoots planted	% survived
PJM	150	79	447	90
Hurricane	150	73	301	81
Vulcan	150	73	301	81
Emasculum	150	77	246	76
Jean Marie de Montague	150	60	128	74
Total shoots planted	750		1565	
Mean % survival		72		84

In conclusion, the stages of propagation are schematically shown in Figure 1. Softwood shoot cuttings are surface sterilized and planted on the culture medium in stage 1. Once the new flush of shoots has grown 1-2 cm, these are cut off in stage 2, cycle 1. Small clumps of shoots arise from adventitious buds on the stem and leaves. Stage 2, cycle 2 begins with removing these small clumps and planting them on fresh culture media. Once these are successfully started, normal multiplication rates can be readily achieved of 20-40 shoots/culture in a 10 week culture period. These cultures are continually recycled until the crop requirements are met. The shoots then are transferred to stage 3 either in culture or to a soil environment for about 1 month. The plantlets are then planted into individual

plant cells to grow for approximately 3 months. Then the rhododendrons are ready to be planted into gallon cans.

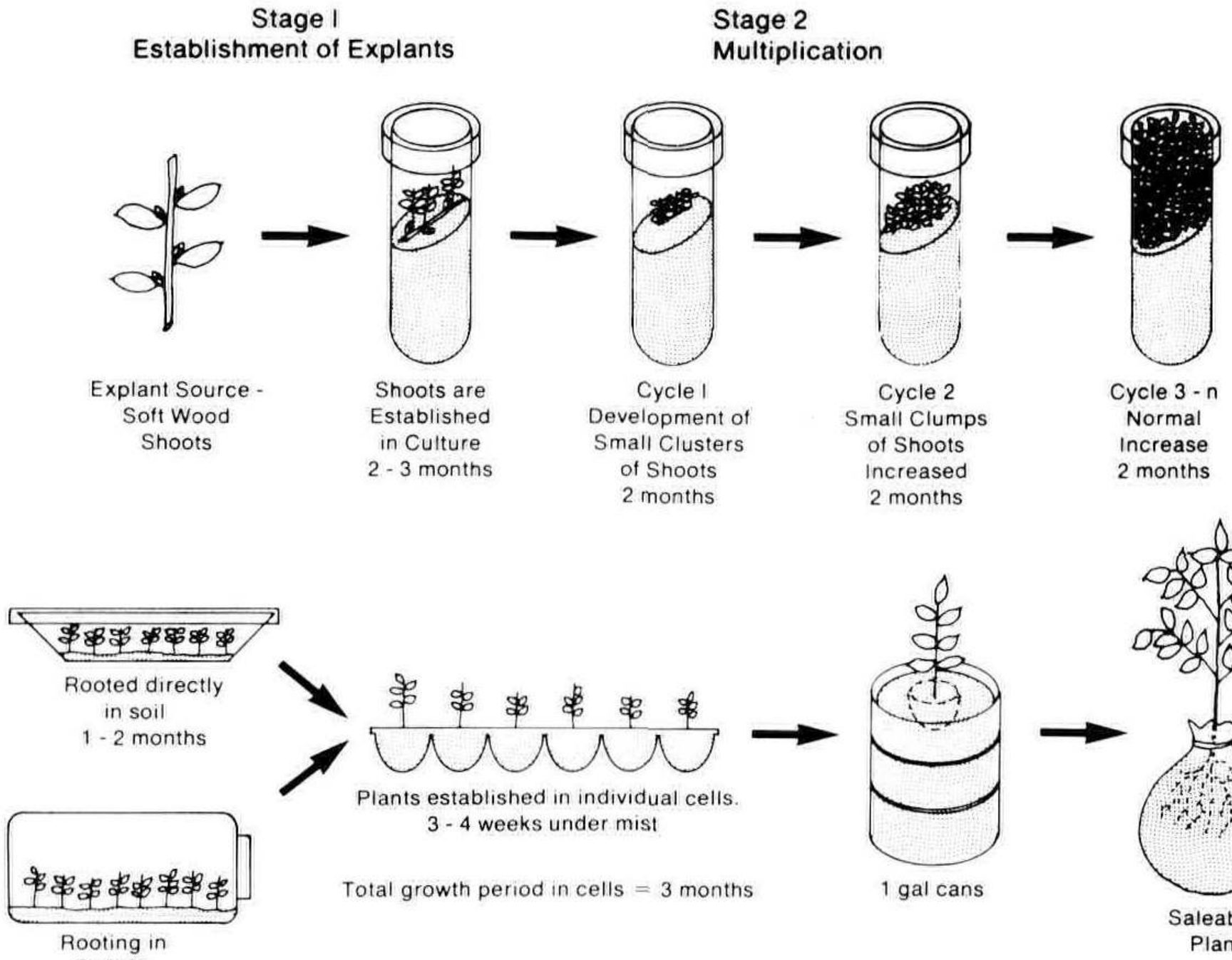

Figure 1. Steps in tissue culture propagation of rhododendron.

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CLONAL PROPAGATION OF WOODY PLANT SPECIES THROUGH TISSUE CULTURE TECHNIQUES

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Abstract. Significant progress has been made in development of tissue culture techniques to meet the requirements for mass clonal propagation of