of affording a sound basis for investing capital in plant breeding and, consequently, stimulating plant development through private enterprise. The more than 4600 Plant Patents issued have been developed by private industry without the help of Government funds. Many of these patents cover food bearing plants, plants that are of better quality, offer higher yields, require less care because of their resistance to insects and disease, and, as a result, make available to the consumer a cheaper, better product. The Plant Patent Act has also led to the development of ornamental plant material which is resistant to disease, drought and cold, all without the aid of Federal funds.

COMMERCIALLY-FEASIBLE MICROPROPAGATION OF MOUNTAIN LAUREL, *KALMIA LATIFOLIA*, BY USE OF SHOOT-TIP CULTURE

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Abstract. The multiplication at rates feasible for commercial production of mountain laurel, *Kalmia latifolia*, by micropropagation using shoot-tip cultures has been demonstrated Shoot-tip explants placed initially in liquid woody plant medium (WPM) supplemented with 4-16 μM N^6-(Δ^2-isopentenyl)-adenine (2iP) produced axillary shoots by 1 to 2 months. These new shoots were excised from the original explant and placed on the same WPM solidified with agar The resultant shoot mass was subcultured monthly Actively multiplying shoot-tip cultures were produced within 6 months A comparison of 7 concentrations of 2iP, varying from 0 to 64 μM, showed that a concentration of 8 μM 2iP produced the greatest number of utilizatble shoots after 8 weeks in culture Stock cultures were maintained or increased monthly by removing and subculturing shoots elongating from the basal mass. Thirty to forty utilizable shoots were harvested from each culture 6 to 8 weeks after the initial subculture Multiplication rates of 8 to 10 times were readily achieved Harvested shoots rooted with 73% success in 4 to 6 weeks when placed in a 100% peat medium in a high humidity chamber. After a period of acclimation, these plants can be treated like young seedlings in commercial production

"We invite you to rediscover the long-neglected laurels, a favorite and familiar American plant. But we warn you that you may experience some frustration, for mountain laurel selections are at present difficult to root and slow growing" (2). However, the situation is improving. A number of programs continue to

find mountain laurel genotypes with superior ornamental value. If named selections are to receive wide distribution, rapid and dependable asexual propagation techniques will be essential. Grafting is feasible but adds considerable expense in production. Success with cuttings varies markedly with the individual genotype and age of the stock plant (2,3). Micropropagation may be a practical solution for the multiplication of unique and desirable mountain laurel selections.

Micropropagation on a potentially commercial scale has been demonstrated for a number of woody species (1,3,5). McCown and Amos (4), working with a number of birch species, have achieved propagation rates that yield 500,000 plantlets per year using only 125 sq. ft. of culture shelf space. Although field growth rates of the micropropagated plants were different than the average seedling, the micropropagated plants did acclimate readily to greenhouse conditions.

Stem-tips were removed from actively growing, 3 year old *Kalmia latifolia* seedlings. After removal of most of the leaves, the explants were dipped in 70% ethanol and then treated for 10 to 15 minutes with 10% household bleach (sodium hypochlorite) with a wetting agent added (0 05% Tween-20). After rinsing 3 times in sterile distilled water and removing any injured tissue, the explants were placed individually in 50 ml Erlenmeyer flasks containing 15 to 20 ml liquid woody plant medium (Table 1) supplemented with 4 to 16 μM 2iP. The liquid medium was changed after 12 and 24 hours and on a daily basis for one week thereafter. After one week the explants were transferred to stationary test tubes and liquid medium was added to a depth of ½ the height of the explant. The medium was changed every 3 weeks. After 1 to 2 months the explants produced axillary shoots (Fig. 1) These shoots were removed when approximately 2 cm long and placed on the same WPM solidified with 0.6% agar. If any exudation from the explants occurred, they were moved to fresh medium. Cultures were transferred to fresh medium once a month and at this time any malformed tissues were discarded. After 6 months in culture, all surviving explants showed active and uniform shoot growth and multiplication (Fig 2).

Cultures were grown in rooms with 24 hour cool white fluorescent lighting (20 $\mu Em^{-2}sec^{-1}$) and temperatures that averaged 28° to 30°C. Culture vessels were either 1 oz or 4 oz glass bottles containing 10 ml and 30 ml of medium respectively, and were capped with Parafilm-M.

The shoot-tip cultures were multiplied by removing several elongating shoots from the basal mass and subculturing the shoots on fresh medium. Dividing and subculturing the basal shoot mass as has been successful with birch stock cultures (4) caused exces-

Table 1. Composition of Woody Plant Medium[1]

Stock		g/l	ml/l	final conc (mg/l)
A	NH_4NO_3	20 0	20	400
	$Ca(NO_3)_2$ 4HOH	27 8		556
B	K_2SO_4	49 5	20	990
C	$CaCl_2$ 2HOH	19 2	5	96
D	KH_2PO_4	34 0	5	170
	H_3BO_3	1 24		6.2
	Na_2MoO_4 2HOH	0 05		0 25
E	$MgSO_4$ 7HOH	74 0	5	370
	$MnSO_4$ HOH	4 46		22 3
	$ZnSO_4$ 7HOH	1 72		8 6
	$CuSO_4$ 5HOH	0 05		0 25
F	$FeSO_4$ 7HOH	5 57	5	27 8
	Na_2 EDTA	7 45		37 3
G	Thiamine HCl	0 2	5	1 0
	Nicotinic acid	0 1		0.5
	Pyridoxine HCl	0 1		0 5
	Glycine	0 4		2 0
H	Myo-inositol	20 0	5	100
I	Sucrose			20 g/l
J	Agar			6 g/l

Stock F Dissolve each component in 200 ml water, heat each, mix while hot, stir as cools to room temp (4-6 hrs) Final vol of 1 liter

[1] Woody Plant Medium (WPM) developed by McCown and Lloyd (University of Wisconsin) for shoot-tip and callus cultures of birch, rose, rhododendron, oak and other dicot ornamental species Hormone supplements usually include benzyladenine (2 to 16 μM) or 2iP (4 to 32 μM) for shoot-tip cultures, and a cytokinin and an auxin (0 4 to 4 μM) for callus stock and callus differentiation Final pH without agar is adjusted to 5 2 using KOH

sive tissue breakdown and exudation which resulted in poor shoot growth.

A comparison of shoot production rates on 0, 2, 4, 8, 16, 32 and 64 μM 2iP was performed to determine the optimum 2iP concentration for shoot multiplication. A 2 cm long shoot from an actively growing shoot-tip culture was subcultured onto 10 ml of medium in a 1 oz bottle supplemented with one of the 7 2iP concentrations. Each concentration was replicated 20 times. After 4 weeks, the 10 best cultures were transferred to fresh medium and then harvested after an additional 4 weeks of growth. Results (Fig. 3) showed an optimal shoot production rate at 8 μM 2iP. Lower levels of 2iP gave fewer but larger shoots and higher 2iP levels caused stunting of shoot growth.

Shoots can be harvested from actively growing cultures 8 weeks after the initial subculture. A 4 oz bottle containing 30 ml of medium supplemeted with 8μM 2iP will produce 30 to 40 utilizable shoots, 1 to 2 cm in length, if the initial subculture contained four 2 cm explants and the tissue was transferred to fresh medium after 4 weeks of growth (Fig. 4). This represents an 8 to 10 time multiplication rate. Harvested shoots were very

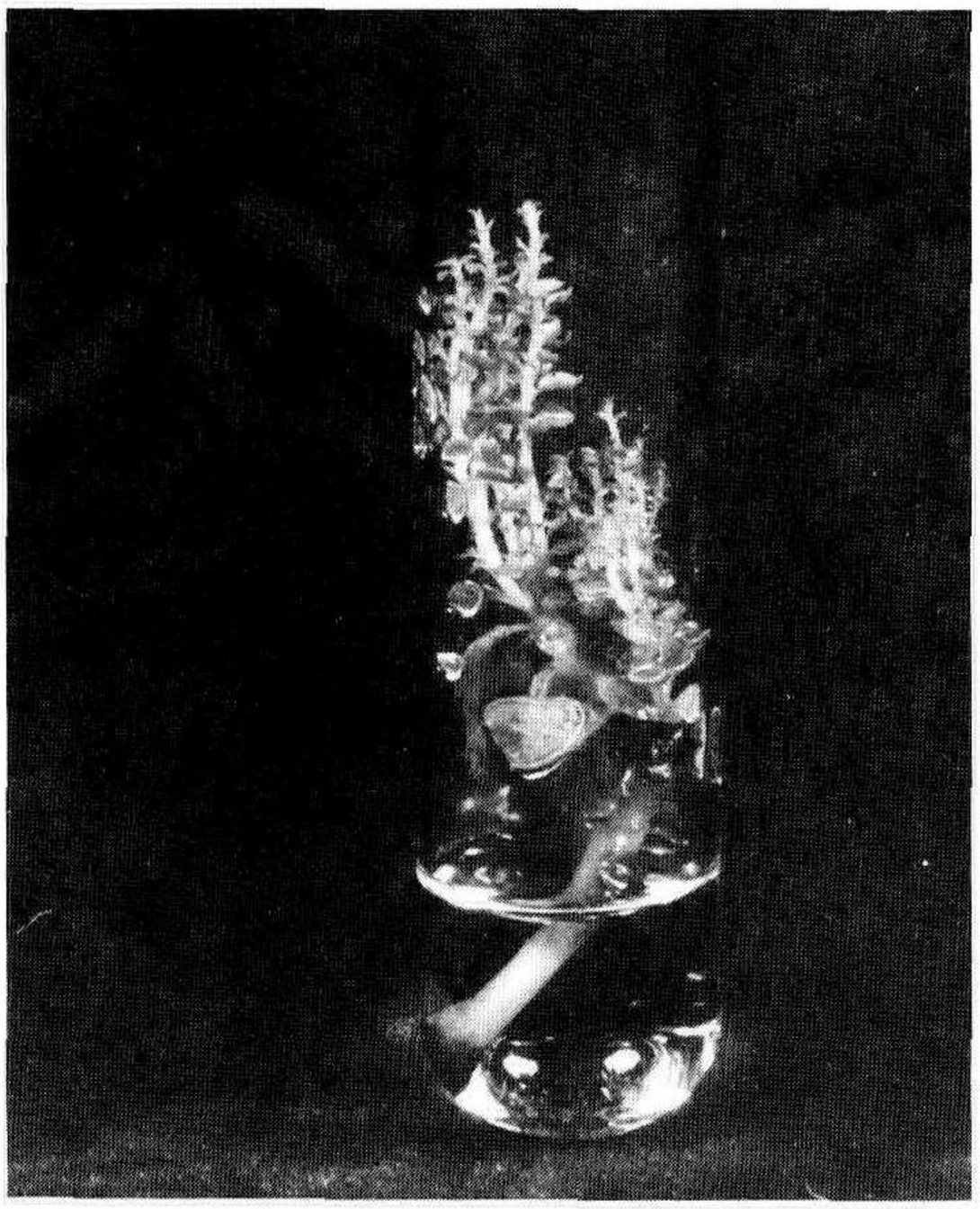

Figure 1. Axillary shoots that have developed from the original explant of mountain laurel, *Kalmia latifolia*, after 1 to 2 months in culture. The medium is woody plant medium (WPM) supplemented with 2iP. The axillary shoots are excised and placed on the same woody plant medium solidified with 0.6% agar for shoot multiplication.

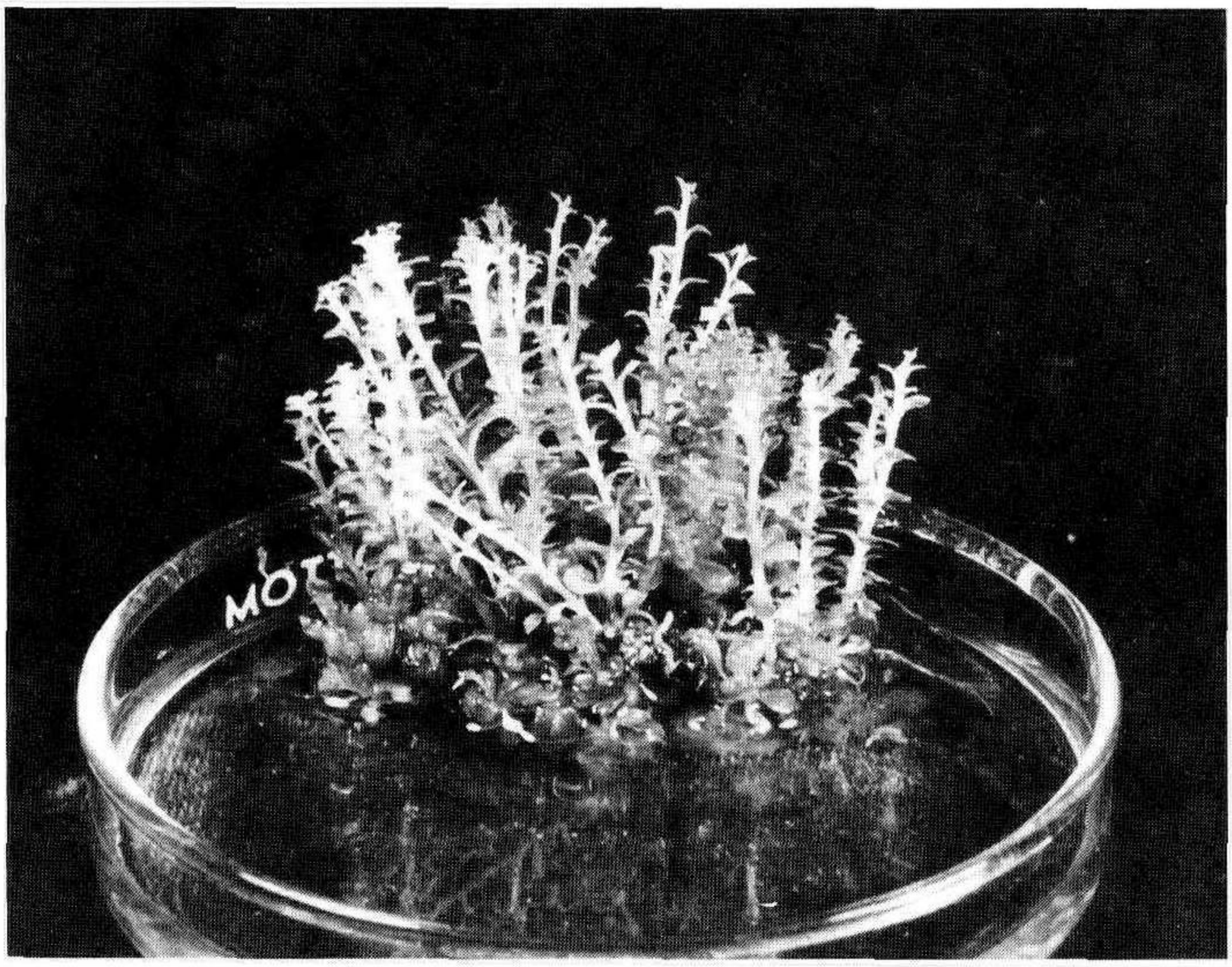

Figure 2. An actively growing shoot-tip culture of mountain laurel, *Kalmia latifolia*, 6 months after isolation of the original explant. Cultures were subcultured at least monthly on woody plant medium (WPM) supplemented with 2iP and solidified with 0.6% agar.

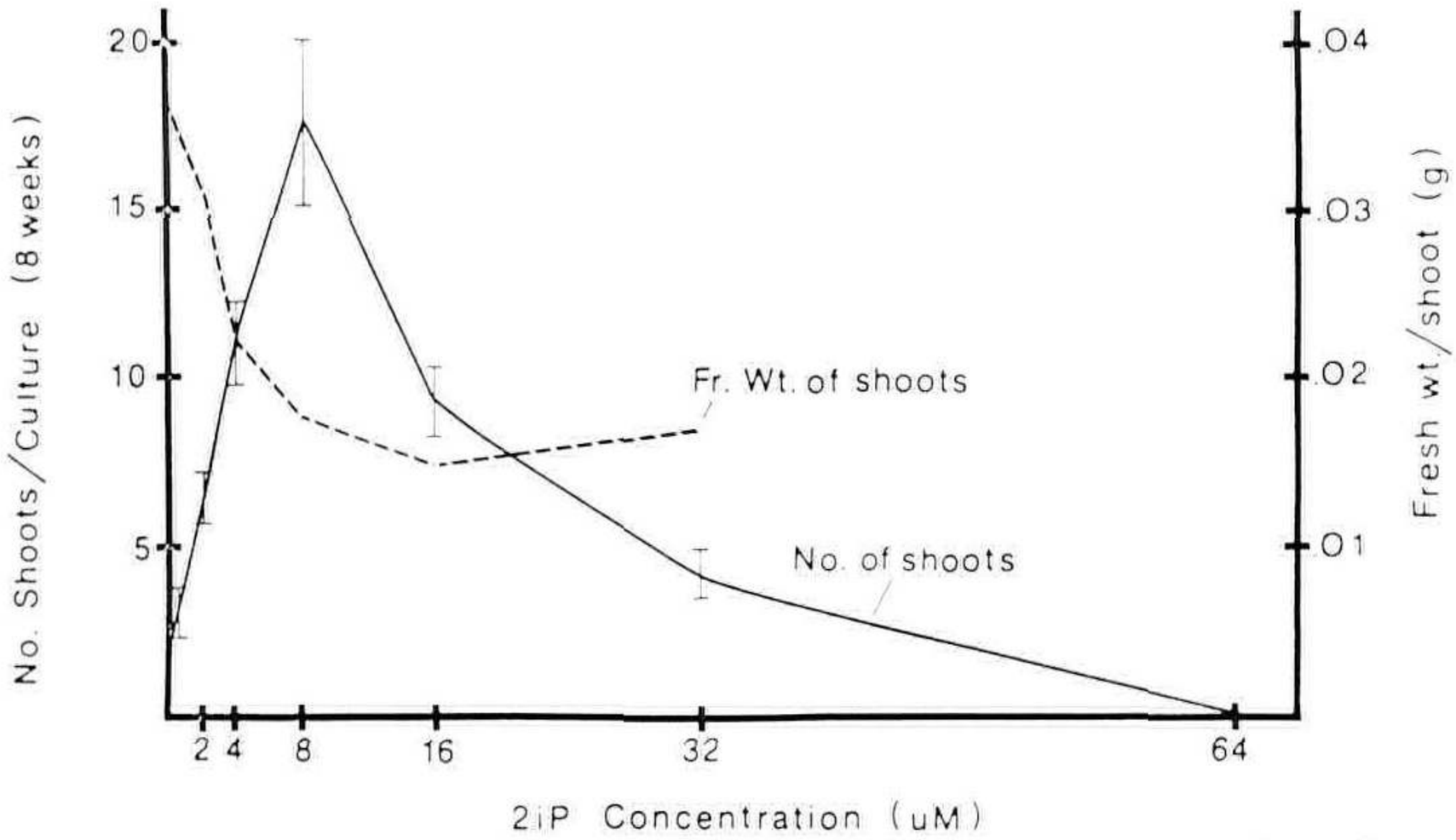

Figure 3. The response of shoot-tip cultures (±SE) of mountain laurel, *Kalmia latifolia*, to the concentration of the cytokinin 2iP (N_6-)Δ_2-isopentenyl)-adenine) in the medium. The medium was woody plant medium (WPM) solidified with 0.6% agar. Cultures were derived from a 2 cm shoot originating from a shoot-tip culture. Cultures were subcultured at 4 weeks onto fresh test medium and shoots harvested after a total of 8 weeks of growth.

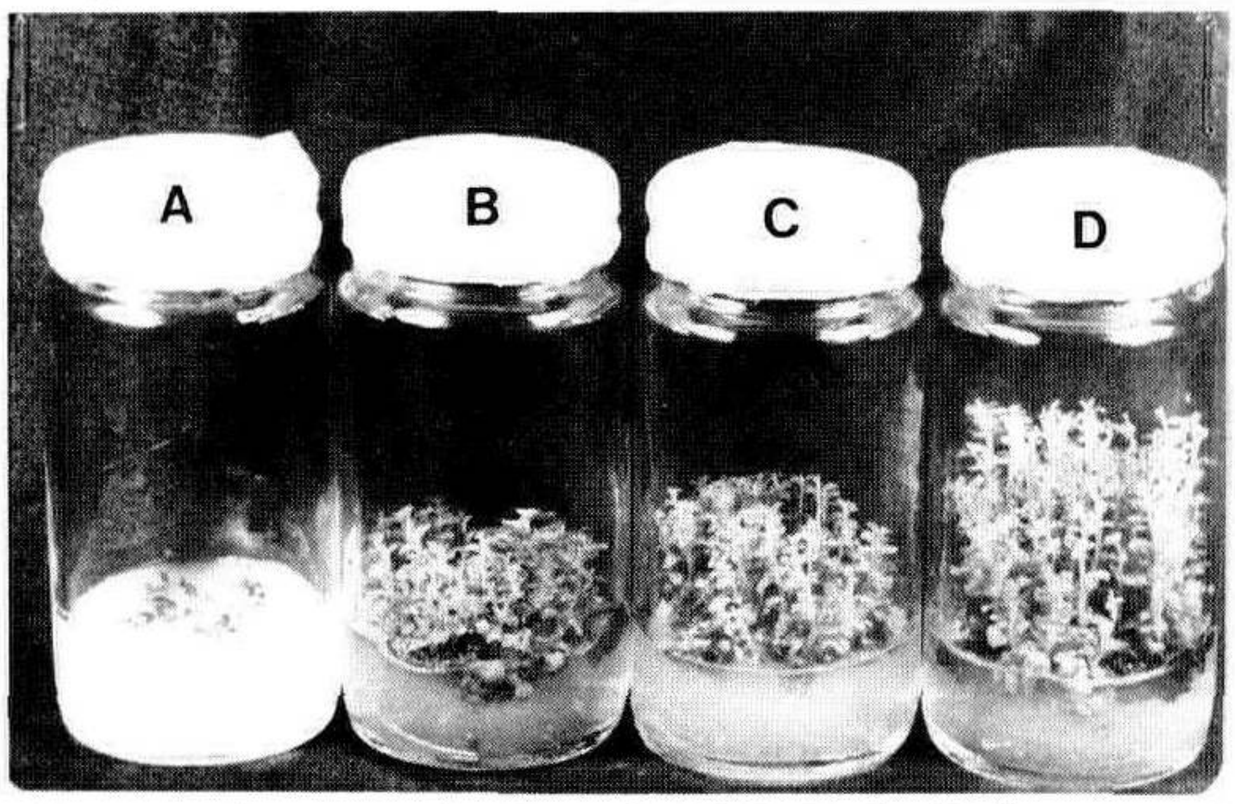

Figure 4. Four cultures of mountain laurel, *Kalmia latifolia*, showing the progressive development of shoot-tip cultures. A, the initial culture consisting of four 2 cm long microshoots; B, the culture after 4 weeks of growth; at this stage the shoot mass will be subcultured to new medium; C, the culture after a total of 6 weeks of growth; D, the final shoot-tip culture ready for shoot harvest and/or further shoot multiplication of the culture.

subject to desiccation and thus were cut into water and remained wet until placed in the rooting environment.

Shoots were rooted in 100% peat medium in a warm (30° to 35°C), high humidity chamber under 24 hour cool white fluorescent light (30 $uEm^{-2}sec^{-1}$). Rooting occurred within 4 to 6 weeks with a 73% success rate. The microcutting showed good root distribution and development (Fig. 5). Hormone treatments did not appear to be necessary. Once rooting occurred, the plants were shifted to a greenhouse and gradually given full sunlight and lower humidity over a 2 week period. At this stage, the plants can be treated as seedlings in normal nursery production programs.

Figure 5. Typical rooted microcuttings of mountain laurel, *Kalmia latifolia*, derived from shoot-tip culture and rooted in a high humidity chamber. A, initial microcutting taken from a shoot-tip culture; B, microcutting after 2 weeks in the rooting chamber; C, microcutting after 6 weeks in the rooting chamber; plant is ready for acclimation to greenhouse environment; D, plant after 2 weeks acclimation in greenhouse; E, plant after 2 weeks further growth in the greenhouse.

The rate of multiplication appears adequate for commercial purposes. Producing an average of 30 shoots per culture in 8 weeks yields at least 7000 shoots per 1 sq ft of culture shelf space per year. With a 73% rooting success, this represents approximately 5000 useable propagules. Although 3-year-old seedlings were used in this study, success has also been achieved with rooted cuttings taken from mature, flowering plants.

Besides rapid multiplication and a minimal space requirement for stock plant maintenance, additional benefits of the use of micropropagation are potentially disease-free propagules, and dependable, easily controlled uniformity of propagules. These factors would be particularly advantageous in accelerated growth programs where a predictable response to cuture conditions is extremely important. Accelerated growth conditions may then be used to overcome the second major problem in commercial production of mountain laurel, the normally slow growth exhibited by most *K. latifolia* plants.

The micropropagation techniques used here employed shoot-tip cultures where shoots evolved from preformed meristems on the original explants and not adventitious shoot regeneration from callus. Thus, given a relatively stable genotype initially, the genetic stability of the culture should remain high. The only abnormality observed is an occasional fastigiation of a shoot in culture. Such abnormalities can easily be selected against in normal stock culture maintenance.

Further research on treatments to increase rooting success and on field growth characteristics of micropropagated plants would be useful.

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Friday Morning, December 12, 1980
NEW PLANT FORUM

Jack Alexander and Gary Koller, Moderators

MODERATOR ALEXANDER: Our first speaker today is Dr. Richard Jaynes.

DICK JAYNES: *Kalmia latifolia* 'Pink Charm' was selected from the progeny of a controlled cross (x1078) made in 1970 between two unnamed pink-flowered selections obtained from Weston Nurseries, Hopkinton, Massachusetts. The plant first flowered in the fourth growing season, 1974, and has flowered every year, except one, since then. The flower buds are red in color (RHS Colour Chart 53C), but less brilliant than the red-buds: 'Nipmuck', 'Ostbo Red', and 'Quinnipiac'. The open flowers are a rich pink being more deeply pigmented than the earlier named 'Pink Surprise'. The inside of the corolla is a relatively uniform pink (RHS 54B but a bit lighter and towards 55B, or 67D). A narrow and deeply red pigmented ring occurs on the inside and near the base of the corolla.

In additlion to floral traits, 'Pink Charm' was selected for the relative ease by which the cuttings root. Small numbers of cuttings have been stuck for each of the past five years in a humidity case, peat:perlite mix (5:2 v/v), bottom heat 70 to 75°F, and no auxin. Cuttings were taken mostly in October, but also December 30 and January 21 (Table 1). Overall success of rooting averaged 76%, or 82% if only the fall stuck cuttings are considered.

Plant habit and foliage of 'Pink Chrm' are characteristic for the species. Limited quantities of cuttings are available from R.A. Jaynes, Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, CT 06504. Also, Briggs Nursery, Olympia, Washington 98501 is propagating this selection by tissue culture, along with three other *Kalmia latifolia* cultivars and is taking orders for small plants.

Kalmia latifolia 'Shooting Star' is a selection from the wild in North Carolina. This cultivar has 5 deeply cut lobes that reflex.