Fig. 4. Pecan shoots produced from axillary buds of seedlings, using 4 mg/liter BA plus 1 mg/liter IBA for shoot proliferation and 8 mg/liter GA plus 0.1 mg/liter BA to induce shoot elongation.

with 50–75 $\mu Em^{-2}s^{-1}$ of photosynthetically active radiation for 16 hr/day from Gro-Lux fluorescent lights. Temperatures ranged from 22 to 25°C. Explants were incubated from 3 to 6 weeks and evaluated. Periods of incubation on the same medium greater than 4 weeks resulted in a definite decline in explant vigor.

Shoots developing from each node increased as BA levels increased to 8 mg/liter with a maximum of 3 shoots produced by the 8 mg/liter BA treatment. Fewer shoots were produced at higher BA concentrations and the explants died on 32 mg/liter. The 2iP treatments failed to stimulate shoot proliferation and 32 mg/liter was toxic (Fig. 1-A).

Shoot length was unaffected by cytokinin levels up to 2 mg/liter for BA and 1 mg/liter for 2iP, but was reduced at higher concentrations (Fig. 1-B). Concentrations of BA above 1 mg/liter produced longer shoot growth than did 2iP, but growth was less than the controls. As BA increments increased, shoots developed a progressively redder color. Optimum shoot proliferation was at 4 mg/liter BA. BA at 8 mg/liter produced the greatest number of shoots per node, but they were very short and appeared to be injured, however, they would elongate and grow when transferred to the shoot elongation media.

The addition of IBA to the medium, in addition to 4 mg/liter BA, resulted in an increase in both the number of shoots per node and shoot length with concentration up to 1 mg/liter of IBA (Fig. 2). IAA did not affect either shoot number or length.

The synthetic cytokinin (BA) and auxin (IBA) were far more biologically active than were either native compound (2iP and IAA) which either failed to induce a response or the response induced an unfavorable response.

When explants were cultured through the proliferation phase with 4 mg/liter BA for 6 weeks, then transferred and grown 4 weeks in a medium containing 0.1 mg/liter BA with varied GA concentrations, shoot growth increased threefold with 2–8 mg/liter GA (Fig. 3). GA at 2 mg/liter was almost as effective at inducing shoot growth as was 8 mg/liter. GA was toxic at 16 mg/liter and resulted in reduced shoot elongation and leaf browning; levels from 0.5 to 8 mg/liter had no visual effect on morphology or color.

Pecan shoots can be produced from nodal explants of seedlings and can be induced to elongate in preparation for transfer to a rooting medium (Fig. 4). Since shoots appear to originate only from existing nodal buds rather than via adventitious bud formation, the number of shoots that can be produced may be limited. Attempts either to subculture shoots or to induce rooting have been unsuccessful.

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In Vitro Propagation of *Rhododendron catawbiense* from Flower Buds[1]

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Abstract. **Flower pedicels and ovary bases of 4 cultivars of *Rhododendron catawbiense* Michx. were cultured *in vitro* and proliferated granular masses of tissue on Anderson's medium containing indoleacetic acid (IAA) 1.0 or 4.0 mg/liter and 6(γ,γ-dimethylallylamino)-purine (2iP) 5.0 or 15.0 mg/liter. These masses formed numerous shoots when cultured on Anderson's medium with lower levels of growth regulators. The shoots were rooted and grown as plants which appeared normal in vegetative characteristics.**

In vitro propagation of *Rhododendron* has been reported by Anderson (1, 2) and Kyte and Briggs (4) using a low K medium developed by Anderson (3). The shoot tips or small cuttings (2–3 cm) used for explants are very difficult to disinfest because of hairy, sticky surfaces and large size. The *in vitro* propagators of rhododendron obtain only 4–5% success after vigorous disinfestation. Stock plants often have to be raised in a greenhouse to obtain even this yield.

Shoot tip culture was tried on several *R. catawbiense* cultivars, but the tips from outdoor plants proved impossible to sterilize. Since the flowers of various herbaceous perennial plants have proved to be successful sources of explants for *in vitro* propagation (5) this technique was tried with *Rhododendron*. The flowers of *R. catawbiense* proved easy to obtain in a sterile condition because the flowers are borne in a sheath arrangement in a large bud and are covered with outer bud scales which are initiated before the flowers and form an outer covering to protect the flowers.

The following procedures were used for successful *in vitro* propagation of *R. catawbiense album*, 'Nova Zembla', 'Roseum Elegans', and 'Sefton', using flowers as explants. The flower buds were removed from the outdoor stock plants several times during the dormant period from October to April. The complete buds containing 15–20 florets were brought into the laboratory, washed with detergent, and the outer resinous bud scales were removed until the white, papery coverings of the florets were exposed. These buds were then immersed in a solution of 0.5% NaOCl (10% Clorox) and 0.1% po-

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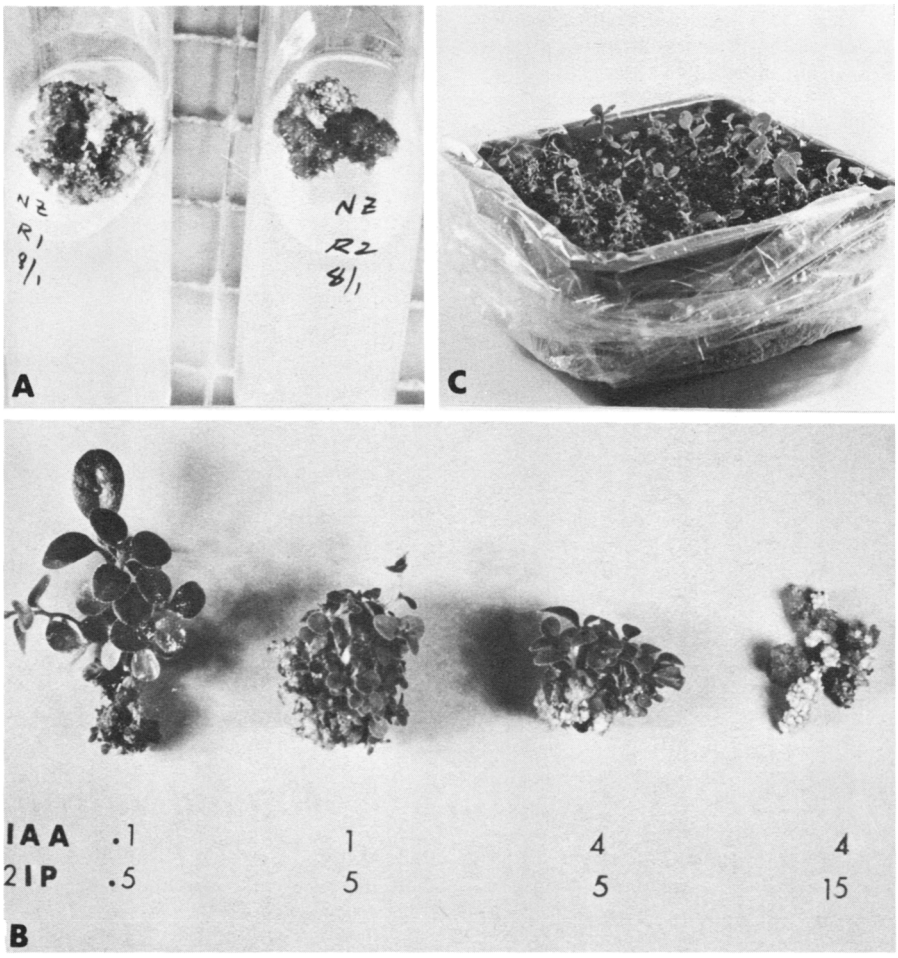

Fig. 1. (A) Proliferation of callus-like material from ovary and pedicel of *R.c.* 'Nova Zembla' flower buds. Left: 1 mg/liter IAA, 5 mg/liter 2iP; right: 4 mg/liter IAA, 15 mg/liter 2iP. (B) Further proliferation of shoots on a new medium with increasing concentrations of IAA and 2iP. (C) Transfer of small shoots and plants to a light-weight growing medium in a plastic bag which will be folded up and tied into a tent.

lyoxyethylene sorbitan (Tween 20) for 20–30 min, rinsed with sterile distilled water, and placed in a plastic petri dish in a sterile hood. The papery covering was then removed and florets were excised from the bud retaining as much pedicel tissue as possible. The florets were then transferred to 25-mm tubes and pressed halfway into an agar culture medium.

A modified Anderson's rhododendron medium (3) was used to stimulate activity in the florets. A factorial arrangement of IAA 0.25, 1.0, and 4.0 mg/liter and 2iP 1.0, 5.0, 15.0 mg/liter were used. Concentrations of IAA/2iP of 1/5 and 4/15 mg/liter stimulated the florets at the pedicel and ovary base to start producing granular masses of callus-like material after 6–8 weeks. The florets seemed to grow better if placed in the dark for 2 weeks before placing under Cool White fluorescent lights ca. 3 klx at 26°C. The granular masses grow slowly and required 3–4 months of growth before transfer (Fig. 1A). The florets from 'Nova Zembla' and 'Sefton' reinitiated growth more easily than *R.c. album* and 'Roseum Elegans'.

These granular masses are obviously a reinitiation of the vegetative growth phase of the plant. A considerable quantity of shoots arose when these masses were transferred to a new medium (Fig. 1B). A high concentration of growth regulator caused the formation of still more granular masses. Groups of shoots were then transferred to the Anderson medium with 4 mg/liter IAA and 1 g/liter activated charcoal for further growth and 10–20% rooted in this medium. The rest of the shoots were easily rooted in a 1 sand:2 sphagnum peatmoss:1 perlite:1 silty loam soil medium in a high humidity environment. A plastic food storage bag 29 × 33 cm in a 10-cm-square container worked well for this purpose. The medium was moistened, sterilized, and placed in the bag with the top folded down (Fig. 1C). The shoots were inserted in the medium and the top of the bag was closed until the shoots are rooted. The bag was then cut away and drainage was established for further growing. This treatment also helped in the establishment of the small rooted shoots.

Although the plants of the above cultivars have not yet flowered, several hundred plants of 'Nova Zembla' and 'Sefton' have been grown and appear quite uniform in leaf characteristics. Several thousand shoots have been cultured from the florets explanted from 2 flower buds of 'Sefton' in 1 year. These could be multiplied by conventional laboratory techniques (4) to give several million at the end of a second year.

These techniques will allow propagation of those *R. catawbiense* cultivars which are difficult to disinfest. In addition, the season for explants is extended and no special care of stock plants is needed to obtain sterile explants. Although these techniques depend on the plant flowering, they do not destroy the shoot growth potential on the mother plant. I have found flowers are more easily cultured into plants than shoot tips.

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