however, infrared results suggest the presence of a hydroxyl group and a possible nitrile group in addition to carbon-hydrogen bonds. Ultraviolet results show the lack of a phenolic group previously characterized by Fadl and Hartmann (3) in the extract of the easy-to-root 'Old Home' pear and shown to have a similar Rf value from paper chromatographic studies. The 3 compounds appear to be structurally similar from infrared studies.

In the pure state the 3 compounds are unstable and the instability may result from the molecular arrangement of the hydroxyl and nitrile groups. Two naturally occurring alpha-hydroxy nitrile compounds, mandelonitrile and p-hydroxymandelonitrile, are unstable and undergo appreciable decomposition in solution (2, 8). The unstable nature of the root promoting compounds reported here may explain the lack of rooting response previously reported when juvenile ivy shoot tips were used to assay the root initiating potential of juvenile and mature ivy extracts (4).

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Acidic Root-Promoting Growth Inhibitor(s) Found in *Picea* and *Chamaecyparis*[1]

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Abstract. The acid phase of methanolic extracts of *Picea glauca* var. *albertiana* cv. *Conica* tissue, a difficult-to-root cultivar, had a high content of substances, at Rf 0.9 - 1.0, which inhibited the growth of *Avena* first internode. *Chamaecyparis lawsoniana* var. *fletcheri,* an easy-to-root variety, had a lower content of these substances. The substances stimulated root formation in mung bean cuttings, but no synergistic root-promoting effect was found when they were supplied to mung bean cuttings in the presence of 5 x 10^{-6}M indoleacetic acid (IAA).

The substances at Rf 0.9 - 1.0 showed a dose-response effect over a concn range of 0 to 1.106 g fresh wt. Concn higher than 1.106 g did not further increase the number of roots in either species. An additive effect between extract and IAA was found in *Picea* at concn below 0.276 g and in *Chamaecyparis* at concn lower than 0.110 g and higher than 1.106 g fresh wt. The possibility that root initiation may be determined by hormone balance rather than a single substance is discussed.

Exogenous application of auxin-like substances to the bases of cuttings induces root formation only in tissues which root easily. However the content of endogenous auxin-like substances is not necessarily correlated with rooting capacity in many plant species (15, 10 23). According to Vieitez and Pena (25), the root forming capacity of *Salix atrocinerea* stem cuttings showed a typical yearly periodicity. They concluded that root formation was governed by a complex of factors and not by growth substances alone. Nanda et al. (17) found that low activity of hydrolyzing enzymes was associated with poor rooting in *Populus nigra* cuttings.

Tizio et al. (24) suggested the possibility that rooting depends on hormone balance rather than on a specific auxin. Spiegel (22) found a relationship between rooting capacity and inhibitor content in *Vitis* cuttings. Shibaoka et al. (20) isolated from *Helianthus tuberosum* substances which promoted root formation in *Azukia* and *Phaseolus* cuttings, but inhibited the IAA-induced elongation of *Avena* coleoptile sections. Recently abscisic acid, a substance which induces dormancy in higher plants and abscission in cotton ex-plants, has been found to induce roots in several plant species (2, 5).

We investigated the balance of growth promoting and inhibiting substances in *Picea glauca* var. *albertiana* cv. *Conica*, a difficult-to-root cultivar, and *Chamaecyparis lawsoniana* var. *fletcheri*, an easy-to-root variety.

Materials and Methods

Plant material was collected in the spring of 1970 from field-grown *Chamaecyparis lawsoniana* var. *fletcheri,* Hornibrook and *Picea glauca* (Moench) Voss. var *albertiana* (S. Brown) Sargent cv. *Conica* plants before new growth took place. The material consisted of 5 cm long tip cuttings and, immediately after harvesting, the cuttings were lyophilized and stored at 0°C until used for extraction.

Extraction, purification, and chromatographic separation.

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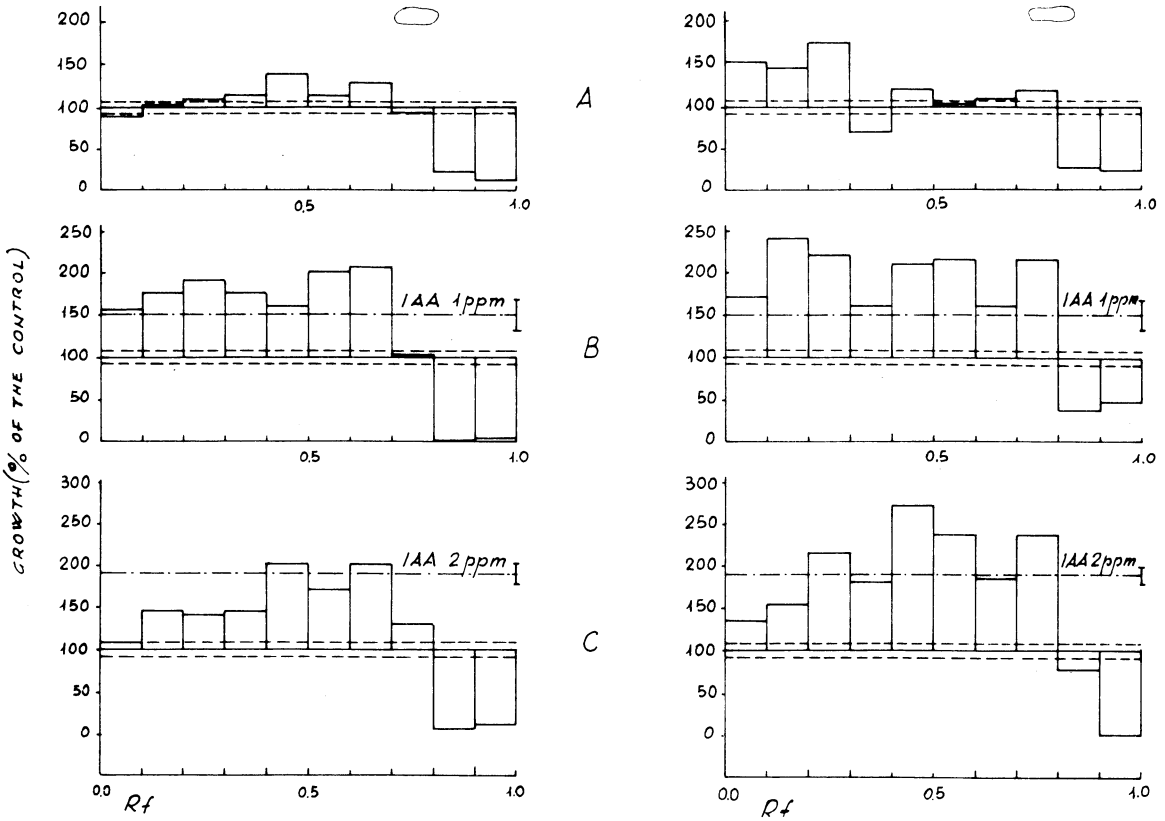

Fig. 1. Histograms of the biological activity of the acid phase of MeOH extracts of *Picea glauca* var. *albertiana* cv. *conica* (left) and *Chamaecyparis lawsoniana* var. *fletcheri* (right). Abscissa: Rf values; ordinate: percent of growth or inhibition of the *Avena* first internode with respect to control.
A – Extract alone. B – Extract plus IAA (1 ppm). C – Extract plus IAA (2 ppm). Distance between the dotted lines is the standard error for control; I indicated the standard error for control with IAA. The spot is the position where synthetic IAA develops in the same solvent systems.

Procedures for extraction and purification of auxins described by Larsen (14) were used. Fresh or lyophilized material was used throughout the experiments.

2.5 g samples of lyophilized material (wood and leaves) were extracted with 50 ml of 80% methanol for 24 hr at 2°C. The extracts were dried to a residue in a vacuum rotavapor at 40°C.

The residue was taken up in 10 ml of redistilled diethyl-ether and a small amount of 3.5% $NaHCO_3$ solution. This was extracted 3 times with pH 8 $NaHCO_3$ solution, 10 ml at a time; the basic ether phase was discarded. The aqueous solution was acidified with 0.1 N HCl to pH 3.5 and extracted 3 times with 10 ml portions of diethyl-ether. The resulting ether fraction was considered as the acid phase. The ether was evaporated and the residue dissolved in 1 ml of 80% methanol. All extracts were stored at -20°C until assayed.

The extract, 0.2 ml in volume representing 500 mg dry wt of tissue, was spotted on Whatman no. 3MM filter paper, 9 cm wide, and partitioned by descending paper chromatography in the dark. Isopropanol:water (8:2 v/v) was used as the solvent and partitioning was continued until the solvent front had advanced 30-32 cm from the starting line (after about 12 hr). At least 2 different extracts with 3 replicates of each sample were chromatographed for each bioassay. Control values are the average of the 10 Rf's of the chromatogram strip run in the same conditions with 0.2 ml of methanol.

Biological test. The acidic phase was bioassayed by either *Avena* first internode or mung bean bioassay immediately after chromatography. The chromatograms were cut into 10 equal parts and each was eluted with redistilled ethyl-ether. The ether was evaporated and residue dissolved in 1 ml of buffer solution and assayed for its auxin activity as described by Nitsch and Nitsch (18).

For the mung bean test, the chromatogram was cut into 10 equal pieces. Mung bean seeds were soaked for 20 min in 20% Clorox, rinsed and sown in a perlite and vermiculite mixture. Low light intensity (90-100 ft-c) was provided for the first 5 days and high light intensity (350 ft-c) for 5 additional days. Six plants were cut 5 cm below the cotyledonary nodes and put in each test tube for 20 hr in the presence of paper chromatogram strips with 6 ml of water or 5 x 10^{-6}M IAA. Plants were then transferred to water, and the roots counted after an additional 5 days.

For the dose-response experiments 50 g fresh wt of *Picea* and *Chamaecyparis* tissues were extracted as described for the lyophilized material and the acidic phase was reduced to 3 ml volume. One ml of the acidic phase was chromatographed and the section between Rf 0.9 - 1.0 was eluted 2 times with 30 ml each of 80% methanol for 20 hr. The eluate was brought to dryness and taken up in 3 ml of methanol (5.53 g of fresh wt of tissue per ml of extract). The eluate was considered a stock solution and used for further dilution in mung bean tests.

Results

Avena first internode test. Without IAA, both species showed a low content of several growth promoting substances (Fig. 1). *Chamaecyparis lawsoniana* var. *fletcheri* extract caused increased internode elongation in the area of Rf 0 - 0.3. *Picea glauca* var. *albertiana* cv. *Conica* extracts showed no growth promotion in this zone but did cause some promotion between Rf 0.4 and 0.6. Strong growth inhibition occurred at Rf 0.8 - 1.0 in both species. Added IAA generally increased growth at all Rfs except the Rf 0.8 - 1.0 region. In *Picea*, the growth inhibiting activity at Rf 0.8 - 1.0 was modified by added IAA. In *Chamaecyparis*, inhibition was decreased with added IAA at Rf 0.8 - 0.9 but increased with 2 ppm added IAA at Rf 0.9 - 1.0.

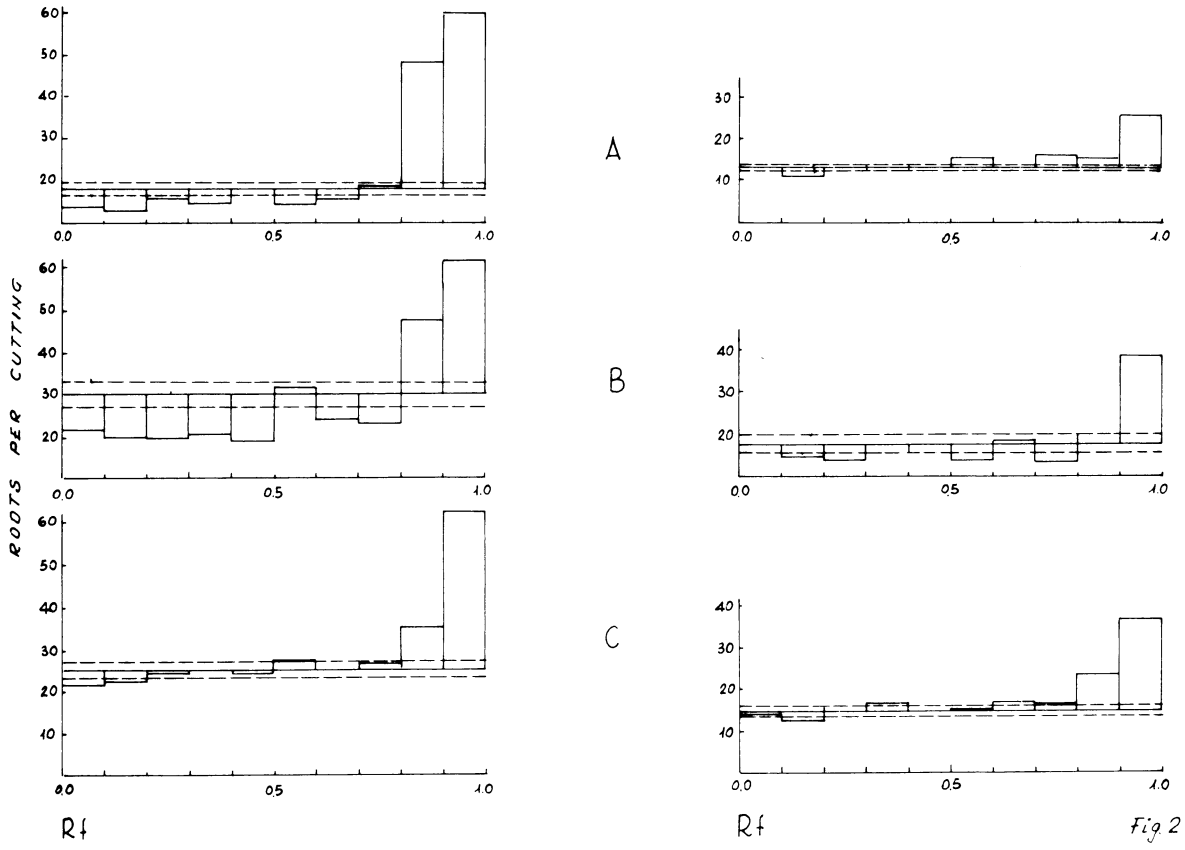

Fig. 2. Histograms of the biological activity of the acid phase of MeOH extracts by the mung bean bioassay. Left *Picea glauca* var. *albertiana* cv. *conica,* right *Chamaecyparis lawsoniana* var. *fletcheri.* Abscissa: Rf values. Ordinate: number roots per cutting.
A – Extract alone. B – Extract plus IAA (5×10^{-6}M). C – Extract alone developed on chromatographic paper prewashed with 2% acetic acid solution. Distance between the dotted lines is the standard error for control.

Mung bean test. Eluates from 0.9 - 1.0, which inhibited *Avena* first internode elongation, had a pronounced root-promoting effect (Fig. 2). This effect, although present in both species, was stronger in *Picea* (difficult-to-root species) than in *Chamaecyparis.* In Abies there was a slight root-inhibiting activity in the area of Rf 0 - 0.8. Added IAA did not stimulate root-promoting activity at Rf 0.8 - 1.0 in *Picea* but it did at Rf 0.9 - 1.0 for *Chamaecyparis.*

To avoid the possibility that this effect was caused by impurities contained in the chromatography paper (13) the papers were washed with a 2% acetic solution or with the chromatographic solvent for 2 days. This reduced the root-promoting activity at Rf 0.8 - 0.9 in *Picea* but increased it in *Chamaecyparis* at Rf 0.8 - 1.0.

Within the extract range between 0 and 1.106 g fresh wt of tissue, the number of roots increased with increasing concn of *Picea* extract tissue (Fig. 3). At the concn of 1.106 g fresh wt of *Picea* and *Chamaecyparis* (Fig. 4) produced 50.0 and 25.8 roots per cutting without added IAA, respectively. The highest concn did not further increase the number of roots in either species. And additive effect for root promotion between IAA and the *Picea* extract was observed at concn below 0.276 g fresh wt of tissue. In *Chamaecyparis* the additive effect was seen at concn lower than 0.110 g and higher than 1.106 g fresh wt of tissue.

Discussion

Chromatographic analysis of growth-promoting and inhibiting-substances showed a similar trend in both difficult-to-root *Picea glauca* var. *albertiana* cv. *Conica* and easy-to-root *Chamaecyparis lawsoniana* var. *fletcheri.* The results observed are considered to reflect the status of growth-promoting substances occurring in the plant tissue since methanolic extraction was used throughout (6). The promotive zone between Rf 0 - 0.3 may be the accellerator α of Kefford (12).

Using the "compensation test" (3), known amounts of synthetic IAA (1 and 2 ppm) were added to each tube containing buffer solution and tissue extract. Although 2 ppm seemed to be high, the concn was selected to observe any possible interaction with inhibitors. By this method inhibitors could be shown which normally would not produce a significant effect in the *Avena* test. This method showed a promotive effect at all Rfs up to 0.8 when 1 ppm of IAA was added (Fig. 1). The promotive effect of IAA was more pronounced in *Chamaecyparis* than in *Picea.* The less promotive effect of 2 ppm IAA may be due to the high concn. This suggests the absence of an inhibitor which counteracts the exogenous applications of IAA.

Growth inhibitory activity occurred at Rfs 0.8 - 1.0 regardless of the addition of IAA in *Picea* but it is partially reduced by added IAA in *Chamaecyparis.* The presence of inhibitors in green plants has been noted by many investigators and a substance associated with dormancy was identified in higher plants (1, 6, 8, 11, 16, 19).

Ferenczy (7) found an inhibitory substances, called inhibitor Omega (Ω), in grape and watermelon. It inhibited *Avena* straight growth but was not tested by the mung bean bioassay. Lane and Bailey (13) found in dormant buds of silver maple a weak inhibiting substance, which had an Rf of 0.9 - 1.0 and was always associated with a faint blue-white fluorescence under UV light. Chromatograms run with no extract showed the same properties at Rf 0.9 - 1.0, unless they were prewashed with a 2% acetic acid solution. In our study, prewashing the paper either with 2% acetic acid or with the chromatographic solvent did not alter the observed effect, suggesting the presence of real substances rather than impurities of the paper.

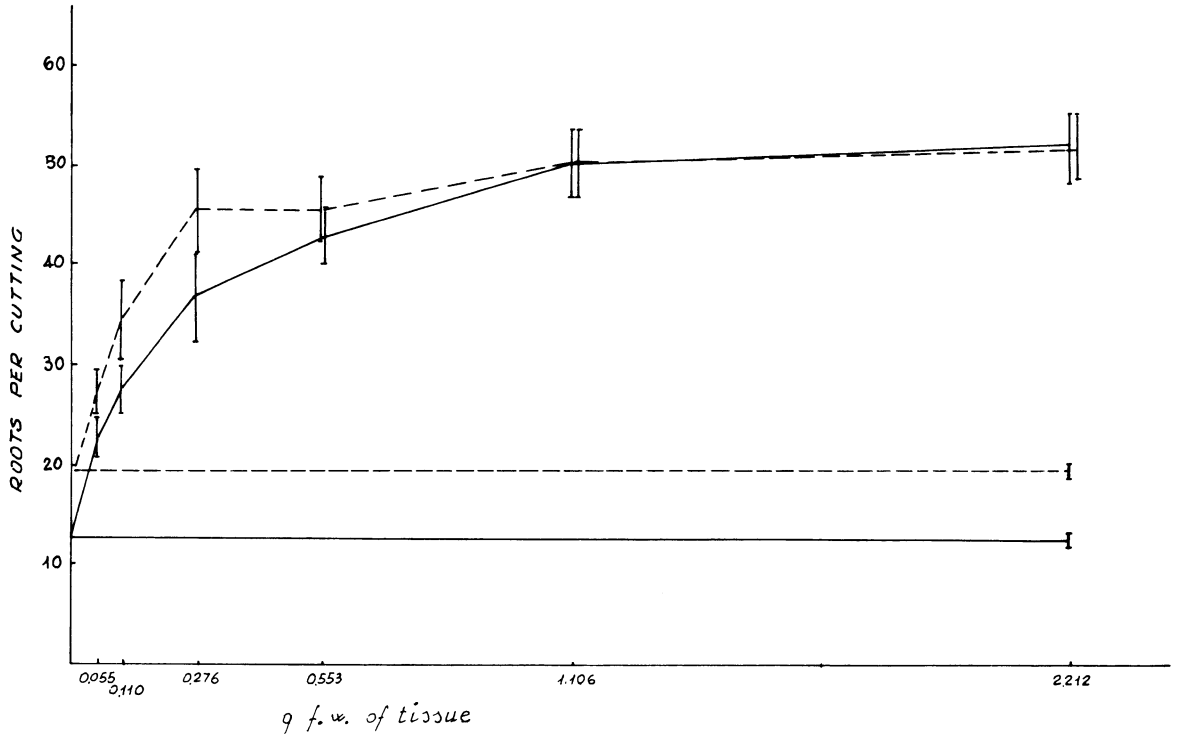

Fig. 3. Dosage response curve of Rf 0.9 - 1.0 fraction extracted from *Picea glauca* var. *albertiana* cv. *Conica* in the presence (dotted line) or absence (solid line) of IAA (5 x 10^{-6}M). Abscissa g fresh wt of tissue in 6 ml of water. Ordinate-numbers of roots per cutting.

The fact that the same substances (Rf 0.9 - 1.0) were detected by two biological tests in different ways (promotion or inhibition) is not contradictory. *Avena* straight-growth is due to cell elongation whereas root formation is primarily due to cell division. Challenger et al. (4) and Shibaoka et al. (20), found a similar situation in apple and *Helianthus tuberosum* tissue extracts, respectively. The first authors suggested that the inhibitory effect observed by them was probably due to the presence of the glycoside phloridizin which is present in apple stem tissue, although the pure glycoside exerted only a small co-factor-like effect. As in Challenger's experiment, we found that inhibition was followed by flaccidity of the *Avena* sections and the lowest part of the mung bean hypocotyls. In the latter, immediately above this zone heavy rooting took place and no visible toxicity occurred in the higher part of the cuttings. Perhaps the promoters induced a concn gradient along the stem, with rooting occurring at the point of optimal concn (21).

If the present inhibitors are compared with those mentioned above, the following conclusions can be made: (a) *Avena* straight growth was inhibited by all inhibitors and a toxic effect was found as with phloridizin, (b) rooting of mung bean cuttings was stimulated by the inhibitors. However the inhibitors of this study had a higher activity in both root stimulation and *Avena* growth inhibition than those reported in the literature (2, 5, 4). Our results indicate that the substances we found are similar in effect to the 3 mentioned inhibitors but compare closer to inhibitor Ω and abscisic acid. Inhibitor Ω has not been tested by the mung bean assay although the results of chromatographic and *Avena* assays were similar to ours.

The high level of root promoting substances in the difficult-to-root cultivar complicated the interpretation. However Tizio (24) suggested that rooting results from a balance of growth substances rather than the presence of a particular one. If this is so, *Chamaecyparis* has a proper balance

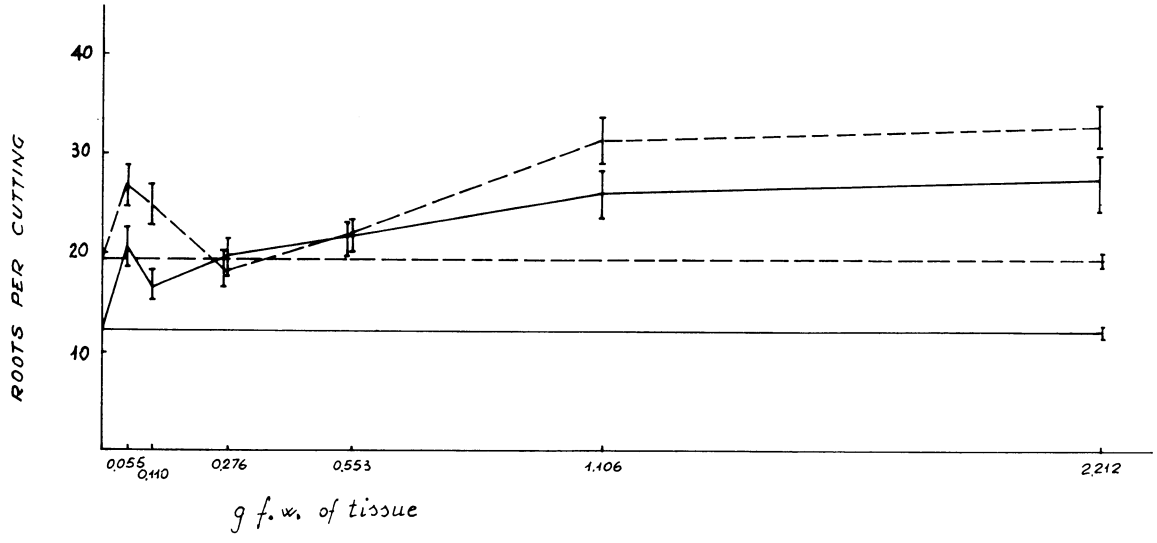

Fig. 4. Dosage response curve of Rf 0.9 - 1.0 fraction extracted from *Chamaecyparis lawsoniana* var. *fletcheri* treated the same as that in Fig. 3.

which stimulates root formation, whereas *Picea* has an unbalanced condition which is not able to stimulate rooting. The inhibitor content of *Picea* may play an important role in this case. The dose-response effect (Fig. 3) of the extracts in the mung bean test may support this idea.

A question which remains unanswered is why, although IAA always influences the rooting process both by cell division and cell extension (9), our extracts of Rf 0.8 - 1.0 had a negative effect in one case (IAA-induced growth of *Avena* sections) and none or a positive effect in the other (mung bean).

The fact that added IAA did not show synergistic effects in inducing rooting in mung bean may be due to an optimal endogenous IAA level in the cutting rather than to an independent mechanism of action.

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Conditions for Opening Cut Chrysanthemum Flower Buds [1]

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Abstract. Cut flower buds of chrysanthemums were opened in sucrose solutions from 0 to 40%. Concentrations of 2% for 'Albatross', 5% for 'Fred Shoesmith', 20% for 'Streamer' and 30% for 'Bright Golden Anne' were optimal under room conditions of 100 ft-c fluorescent light (24 hr); relative humidity, 40 to 70%; temp 19 to 21°C. Sucrose was better than glucose; manitol was toxic. Silver nitrate (25 ppm) and citric acid (75 ppm) resulted in best quality flowers without leaf injury; 200 ppm 8-hydroxyquinoline citrate caused leaf damage. Buds larger than 5.0 cm diam and 10 g fr wt were best for opening in sucrose solutions. Flowers opened in solution in 7-9 days and were better quality and had greater longevity than those opened on the plant in the greenhouse.

Flowers of various species and cultivars are harvested at different stages of development. Usually the flower is cut at the earliest stage that will assure full development in the vase. Many cultivars of roses, gladioli or irises, are normally harvested before they are fully open. Carnations, and most composites (e.g. chrysanthemums, dahlias, gerberas) do not develop well in water and are therefore harvested when fully open.

Recently, practical methods for opening carnation buds have been introduced (2, 3) following the first report by Kohl and Smith (4). A few attempts have been reported on opening composite bud flowers, including chrysanthemums (1, 5, 6, 7).

There are many advantages of being able to harvest chrysanthemums in the bud stage and open them in a chemical solution. The time a crop remains in the greenhouse can be reduced by harvesting the crop early, or by harvesting the late flowers while still in the bud stage, and then opening them in solution. The space saved in shipping buds over shipment of fully opened flowers has been mentioned by Besemer (1) and

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