primarily, I believe, because of the problems with clearance through E.P.A. It is a beautiful concept but with present regulations there would be considerable trouble getting approval for such a material.

MODERATOR PATERSON: Thank you very much Paul. Next we will hear from Dr. John McGuire concerning the difference in rate of uptake of IAA as influenced by the formulation.

EFFECT OF FORMULATION ON UPTAKE OF 3-INDOLEACETIC ACID IN CUTTINGS[1]

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Abstract. Talc formulations of IAA prepared by dissolving the auxin in alcohol were superior to talc formulations prepared by grinding IAA crystals with talc Similar concentrations of IAA in aqueous solution were taken up faster and produced more roots per cutting Maximum uptake in aqueous solution took place in 24 hours but talc formulations required 72 hours

When talc and ethanol formulations were compared on the basis of adventitious root production in *Ilex* cuttings, it required four and a half times as much IAA in talc to get the same amount of roots as obtained in ethanol formulations

REVIEW OF LITERATURE

Research devoted to methods of application of root promoting chemicals to cuttings has been extensive. As a result, two methods of application have come into widespread use: the concentrated aqueous dip and the talc dust (1, 2, 3, 5, 6, 7, 8, 9, 14, 15). Plant response varies, some responding best to concentrated dips while others give best results with talc dust applications (14, 15). Little information is available on rate of uptake as it is influenced by formulation. It is known that the liquid formulation is taken up in the transpiration stream by diffusion (10, 11, 15, 17, 18). There is less information about talc formulations although there is some evidence that talc alone will stimulate some root initiation (16). It is also known that effectiveness of talc formulation is dependent on the fineness of the talc particles (14).

INTRODUCTION

Further information is needed if a complete understanding of the effect of the carrier or formulation is to be obtained. If a li-

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quid formulation of IAA is more efficient than a talc, perhaps the explanation lies in rate of uptake or in the total efficiency of the uptake process. If the liquid formulation is the most efficient, then the question must be asked, why are talc formulations more effective for cuttings of some species? The following series of experiments were carried out to elucidate some of these questions.

PROCEDURES

Comparison of Two Talc Formulations. Grace (4) found a difference in rooting response in talc formulations when talc was mixed in different ways. These procedures were tested to determine the best method for preparing talc formulations of IAA. Two procedures were used, the grinding method described by Hitchcock, *et al.* (23), which consisted of grinding the auxin with talc in a mortar and pestle. The second method consisted of dissolving the prescribed weight of IAA crystals in sufficient 95% ethanol then pouring the solution over the prescribed weight of talc. After mixing to a slurry, the mixture was dried in a forced draft oven at 25°C. A 0.4% IAA mixture was prepared by each method and compared as rooting stimulants on cuttings of *Coleus blumei* Lour. Each cutting had two leaves and an almost identical leaf surface area. The basal 1/2 inch of the cutting was moistened in water and dipped in one of the talc formulations. Excess talc was removed by tapping the cutting gently. Cuttings were placed in a medium of sphagnum peatmoss and horticultural grade perlite (1:1 V/V) under intermittent mist at a minimum temperature of 20°C. After 10 days, individual roots per cutting were counted. Each treatment included five replications of five samples.

Determination of Quantity of Absorption or Adsorption. It is not possible to compare applications of talc to aqueous dips unless it is known how much of each material is either absorbed in the cutting or adsorbed to the outer surface.

Cuttings of *Ilex crenata* 'Convexa' were dipped in water and allowed to drain for 3 min, then dipped into talc to a depth of 1 inch. Excess talc was tapped off. Cuttings were dried for 3 days at 25°C so the talc could be brushed off. The talc was removed, first by brushing with an artist's brush, followed by washing in 95% ethanol. Ethanol was evaporated and the talc weighed. Five cuttings were treated at one time and the process repeated 65 times.

Mean talc adsorption was 13.62 mg ± 1.82 mg or approximately 2.72 mg/cutting. Uptake of ethanol was measured by dipping the basal inch of each cutting in 50 ml of 40% ethanol. The dilute ethanol was used since ethanolic formulations used to treat cuttings are in that range.

Each cutting was held in the solution exactly 10 sec, simulating the procedure used in applying, concentrated dips. Twenty cuttings were treated at a time and the volume of alcohol lost was measured in a buret. The average volume uptake per cutting was 0.82 ± 0.11 ml for 20 cuttings of approximately 0.04 ml per cutting. The coefficient of variability for replications was 12% for talc and 13% for the ethanol dips.

Determination of Rate of Uptake. IAA was used which contained 1% of the auxin labeled as IAA-2-^{14}C. This was mixed with nonradioactive IAA to make a final formulation of 0.6% IAA in 50% ethanol. This served as a concentrated dip. A talc formulation was prepared by dissolving the IAA in 95% ethanol and mixing with talc as previously described. The mixture contained 1% of the IAA as IAA-2-^{14}C with a total concentration of 0.6% IAA.

A talc treatment containing no IAA served as a check. Materials were applied to the lowest inch of 4-inch cuttings of *Ilex crenata* 'Convexa'. Excess talc was removed by tapping. Cuttings were placed at random in flats containing sphagnum peatmoss and horticultural grade perlite (1:1 V/V), then were placed under intermittent mist at 20°C minimum in normal daylight. Each treatment had three replicates which included nine samples for each harvest date. Three samples were used for ^{14}C analysis, rooting response, and autoradiography. The samples were harvested after 1, 2, 3, 8, 16 and 32 days.

When cuttings were harvested, leaf blades were removed but petioles were retained. The uppermost leaf blade was retained on the ones to be used in autoradiography but all others were cut off.

The upper fourth of the cutting was rinsed in 95% ethanol and the lower three quarters was washed thoroughly in 95% ethanol and scrubbed with a soft bristle toothbrush. The wash process was repeated nine times using a different batch of ethanol each time. These wash solutions were assayed for radioactivity. After cuttings were washed, petioles were removed and the cutting was divided into four 1-inch segments. Segments of all samples in the replicate were pooled for extraction but remained segregated by position on the stem. Stem segments were homogenized in an ice bath in 15 ml of 95% ethanol in a Sorvall Omnimixer. The liquid extract was filtered from the residue and radioactivity of the insoluble portion measured in a Tracerlab planchet counter. Efficiency of the counter was 5%. Data were corrected for self absorption. Soluble extracts were evaporated in counting vials then 6 ml of scintillation solution were added. Samples were counted in a liquid scintillation counter, Nuclear

Chicago, Model 6860. Efficiency ranged from 50-60% depending on the samples.

Samples for auto-radiographic analysis were wrapped in cheesecloth and freeze-dried under a vacuum at -15°C. After all samples from all harvest dates had been obtained, they were re-humidified for 1-1/2 hours to facilitate handling. Cuttings were mounted on 10 x 12 inch sheets on 50% rag content paper with rubber cement. Mounts were pressed in a mechanical screw press to make the surface smooth. They were covered with Saran Wrap and exposed to Kodak Rp X-O-Mat X-Ray film. The film was developed by normal procedures (19).

Comparisons of Formulation at Varying Concentrations. In an attempt to equate the two formulations with actual root initiation of *Ilex* cuttings, comparisons were made of different concentrations of the concentrated dip and the talc formulation. Concentrations were prepared as before except that polyethylene glycol (Carbowax 400) was used as the solvent in the concentrated dips and cuttings were placed in the same environment described previously. Each formulation was made at the following range of concentrations: (given in percent) 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.6, 2.4, 2.8, 3.2. Three replicates of five samples were prepared as before and root counts per cutting were made after 46 days.

RESULTS

Comparison of Two Talc Formulations. The average number of roots per coleus cutting was significantly greater when the talc formulation was prepared by first dissolving the IAA in ethanol. (Table 1) This may be due to more thorough mixing.

Table 1. Number of roots on cuttings of *Coleus blumei* Lour 10 days after treatment

Formulation	Total [y]	$\bar{X}$
Talc IAA 0.4% (grinding)	1287	257.4 b [z]
Talc IAA 0.4% (ethanol)	2564	512,8 a
Talc (no IAA)	437	87.4 c
Control (no talc) (no IAA)	154	30.8 d

[y] Total of five replicates of five samples

[z] Means followed by different letters are significantly different (p 01)

Determination of Amount of IAA Applied to Cuttings. Based on the data obtained from the talc recovery tests and the volume of ethanol lost in the dipping tests it was determined that when a concentrate of 6000 ppm of IAA was used 1.6 x 10^{-2}mg of IAA in talc would adhere to the stem and 2.4 x 10^{-2}mg of IAA in ethanol would be taken up in the cutting in a concentrated dip treatment of the same concentration. The actual amount of IAA recovered as determined by calculating dpm and determining actual molecules of IAA were 3.2 x 10^{-1}mg in ethanol and 2.97 x 10^{-2}mg per cutting in talc.

Determination of Rate of Uptake. A summary of the data obtained by counting ^{14}C in soluble and insoluble fractions is shown in Table 2.

Table 2. Total radioactivity in cuttings of *Ilex crenata* 'Convexa' after application of IAA-2-^{14}C

Formulation	Time (days) 1	2	3	8	16	32
			(dpm)			
Ethanol						
Soluble	1516 5	1126.8	1129.1	1231.6	1238.6	1042 9
Insoluble	9285 1	7526 0	8649 1	9037 3	8581 3	6764 2
Total	10801 6	8652.8	9778.2	10268.9	9819.9	7807.1
Talc						
Soluble	48 9	92 8	78 8	85 8	49 2	43 2
Insoluble	454 0	644 9	921 8	564.1	388.1	473.9
Total	502 9	737 7	1000.6	649 9	437 3	517 1

Adsorption of the ethanol IAA was greatest within 24 hours after application. Uptake in cuttings treated with the talc formulation reached a maximum after 72 hours. Uptake from the talc formulation reached a maximum after 72 hours. Uptake from the talc formulation was always much less than that from the liquid formulation. The ratio of insoluble to soluble forms was greater in the talc formulation. This is not considered to be desirable. Adsorption and transport of ^{14}C can be observed in the autoradiographs as well (Fig. 1 & 2). The greatest amount of uptake is again in the cuttings treated with concentrated dips with the greatest concentration in the basal portion with smaller amounts transported to the apex. In all cases, concentrations were found at leaf scars and near the bark as had been reported previously by McGuire (12).

The variance in total uptake was also reflected in the rooting response Table 3.

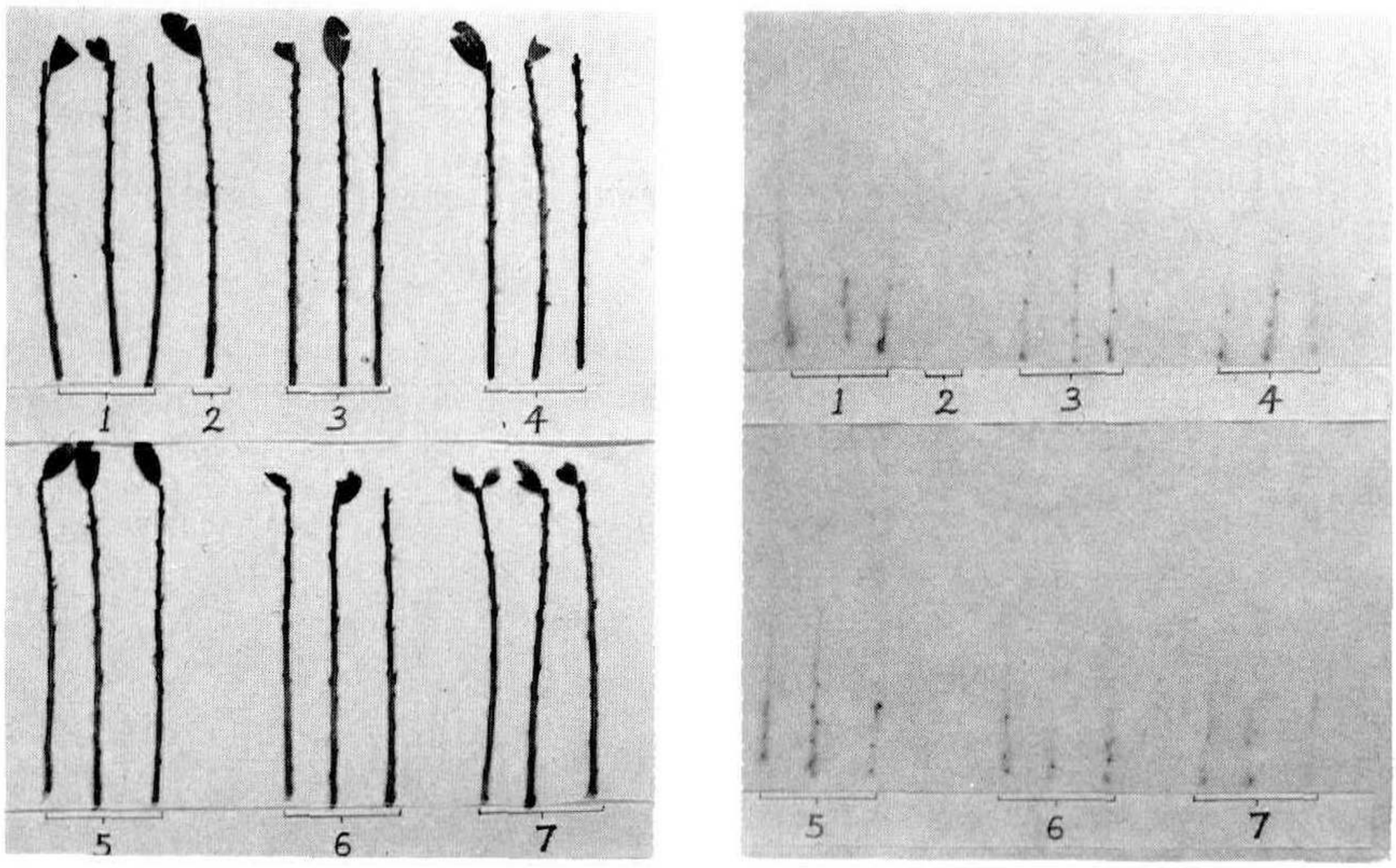

Figure 1. Distribution of radioactivity in cuttings of *Ilex crenata* 'Convexa' treated with 2-^{14}C-IAA in talc formulation. (Left) Original mount. (Right) Radioautograph. Left to right: (1) 24 hours after treatment; (2) untreated cutting; (3) 48 hours; (4) 3 days; (5) 8 days; (6) 16 days; (7) 32 days after treatment.

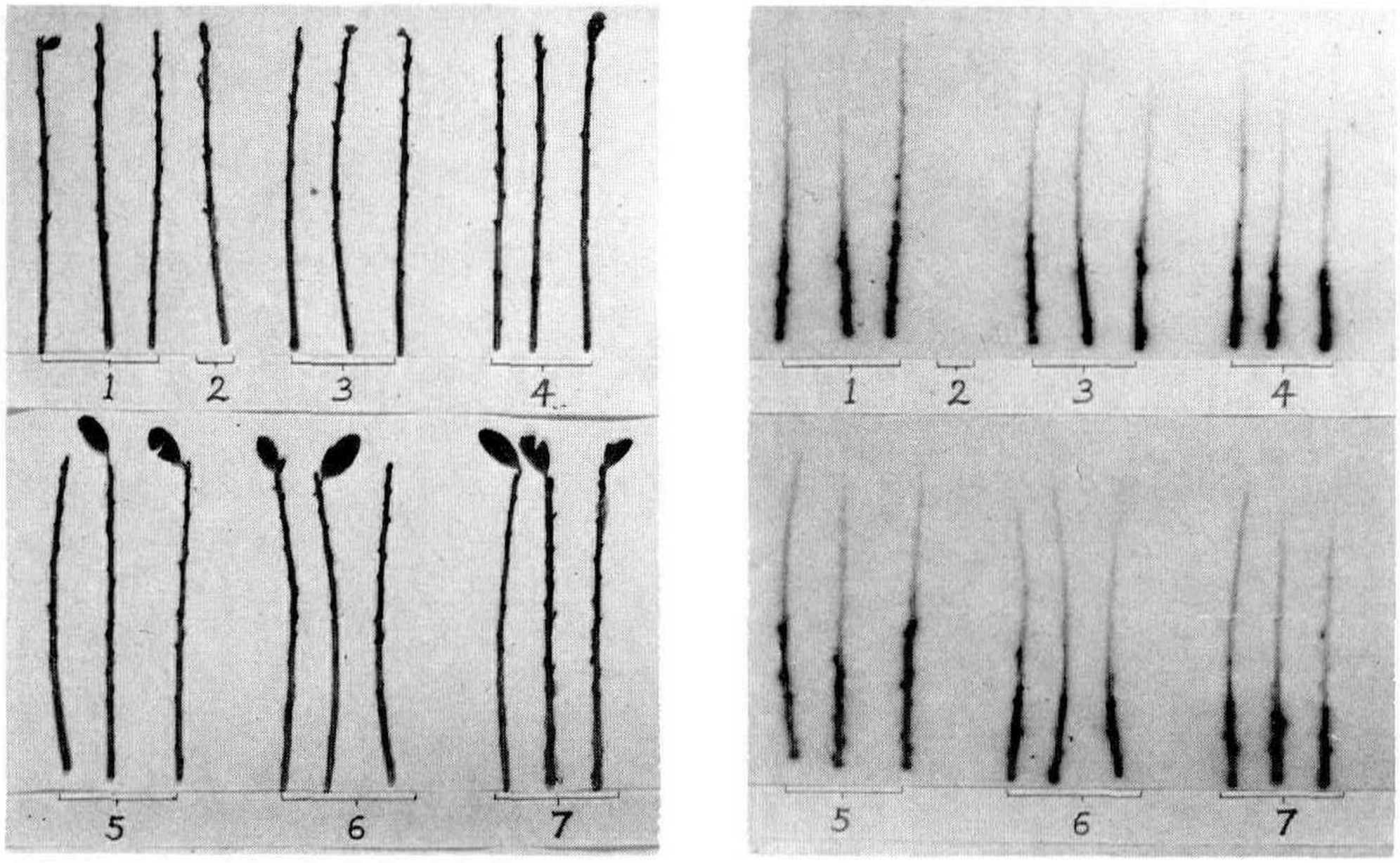

Figure 2. Distribution of radioactivity in cuttings of *Ilex crenata* 'Convexa' treated with 2-^{14}C-IAA in ethanol formulation. (Left) Original mount. (Right) Radioautograph. Left to right: (1) 24 hours after treatment; (2) untreated cutting; (3) 48 hours; (4) 3 days; (5) 8 days; (6) 16 days; (7) 32 days after treatment.

Table 3. Effect of formulation of IAA on rooting of cuttings of *Ilex crenata* 'Convexa' after 32 days

Formulation	Number of roots per replication 1	2	3	$\overline{X}$
IAA Ethanol 6000 ppm	305	277	334	305.3a
IAA Talc 6000 ppm	76	87	89	84.0 b
Talc	53	53	56	54.0 c

Means followed by different letters are significantly different (p .01).

When varying concentrations of IAA in each forumlation were compared it was found again that the liquid formulation was more effective (Fig. 3). When both formulations were used at 0.6% there were four roots per cutting on cuttings treated with the talc formulation and 14 roots per cutting at the same concentration when cuttings were treated with the concentrated dip. It was necessary to use a concentration of 2.8% in talc to get that number of roots.

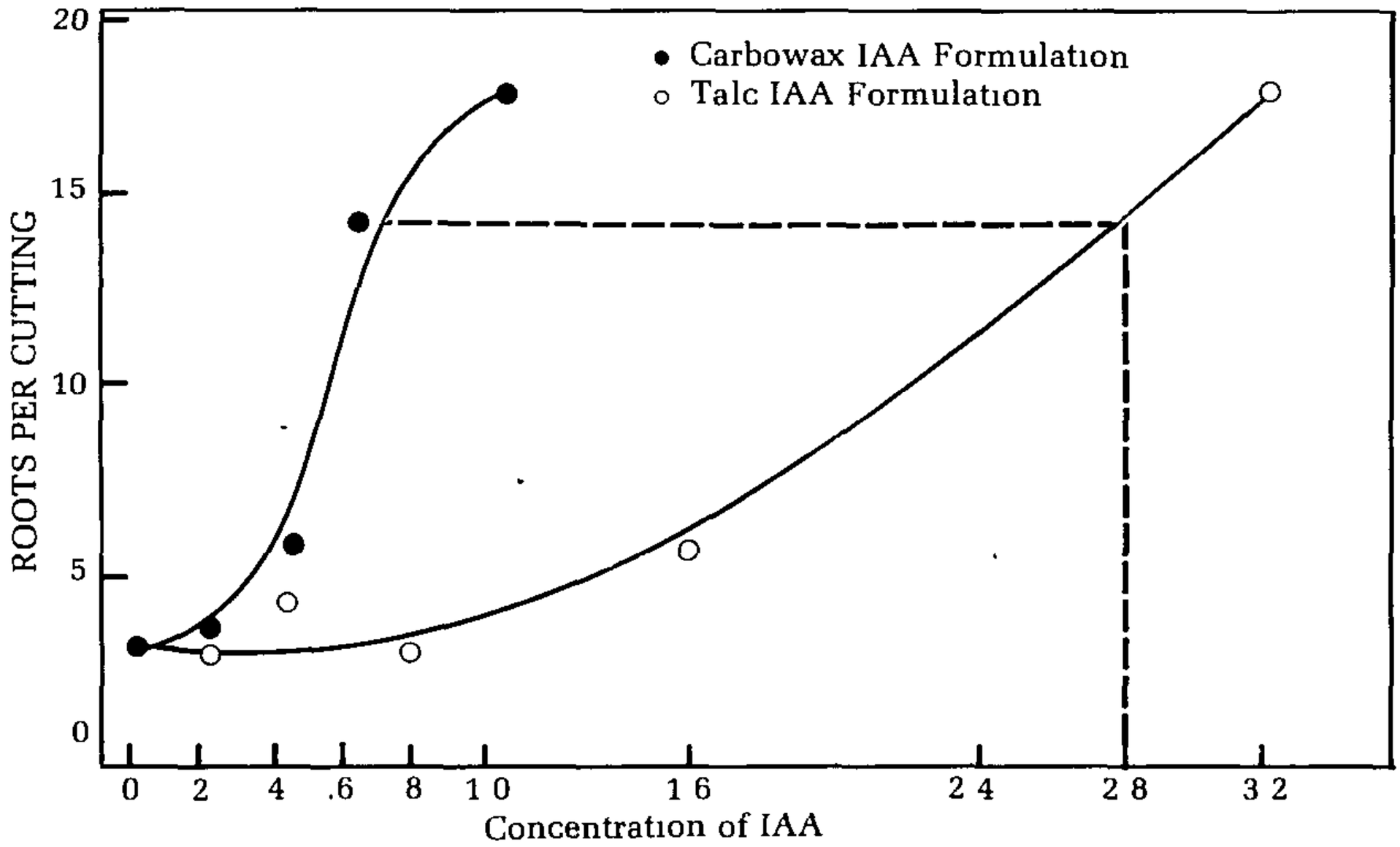

Figure 3. Comparison of relative efficiency of IAA concentrations in liquid (carbowax 400) and talc formulations on rooting cuttings of *Ilex crenata* 'Convexa' (growth period was from February 18 to March 31)

DISCUSSION

It is currently a practice of commercial propagators to use concentrated dips in the range of 10,000 ppm or less but talc formulations in excess of 4% or 40,000 ppm. The difference in relative rate of efficiency of the two formulations found in this

study explains why they can be used at such different concentrations. There can be more to the problem however, since for certain plants a slow rate of uptake at low concentrations may be desirable and, though the talc formulation is less efficient, it may still be preferred for some plants.

Recently Shibaoka (13) found that an application of a dilute aqueous formulation of IAA applied the first day to cuttings of *Azukia angularis* had little effect on rooting, but it enhanced the effect of a similar concentration applied the second day. Treatment only on the second day promoted rooting by 70% and a combination of the two treatments promoted rooting by 200%. A similar phenomenon may occur in temperate woody plants. A slower rate of uptake may provide a suitable level of auxin at a time when endogenous materials are at an optimum level for root initiation.

SUMMARY

1. Dissolving IAA in ethanol prior to mixing with talc produced a superior talc formulation.
2. Concentrated dips were superior to talc formulations.
3. Uptake from the concentrated dip was at a maximum in 24 hours but maximum uptake required 3 days for talc.
4. Root formation was 3.6 to 1 when concentrated dip formulations were compared to talc formulations.
5. Distribution of ^{14}C in the cutting decreased with distance from the base. Much of the ^{14}C was immobile.
6. Though uptake from talc is slow and relatively inefficient, adequate rooting can be obtained if high concentrations are used.

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MODERATOR PATERSON: That was a very interesting and well done piece of research. Thank you for sharing it with us. This concludes this afternoon's program.