DEVELOPMENT OF ROOT-PROMOTING SUBSTANCES IN EUONYMUS ALATUS 'COMPACTUS' UNDER INTERMITTENT MIST[1]

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Abstract. In *Euonymus alatus* 'Compactus' plants grown under intermittent water mist during September, leaf senescence and onset of dormancy were delayed and natural root-inducing substances accumulated in the leaves Consequently, stem cuttings from plants grown under intermittent water mist rooted easily, whereas cuttings from non-misted plants rooted with greater difficulty When rutin, a substance similar to those which accumulated in misted leaves, was applied with IBA to stem cuttings from non-misted Euonymus, the cuttings rooted as well as cuttings from misted plants. These results provide an additional explanation for the great success of mist propagation techniques, emphasizing that many substances in addition to auxins and rooting cofactors may play a significant role in rooting

INTRODUCTION

The success of modern mist propagation techniques has been attributed to the cooling effect of the mist on air and leaf temperatures, thus reducing transpiration and respiration rates in cuttings (5, 8). In addition, if nutrients are added to the mist, both water and nutrients can be absorbed by the cuttings with beneficial effects upon rooting (15). However, these effects have not always explained satisfactorily the universal success of intermittent mist propagation.

Stem cuttings of *Euonymus alatus* 'Compactus' are easily rooted, especially in the spring and summer under intermittent mist propagation. However, as plants approach dormancy in the fall, cuttings are more difficult to root, but the difficulty can be partially overcome by use of intermittent mist. The purpose of this study was to investigate the seasonal variation in rooting of cuttings taken from euonymus plants grown under intermittent mist as compared with cuttings from non-misted plants, and to determine what substances were responsible for the effect.

EXPERIMENTAL

In September, two-year-old uniform plants of *Euonymus alatus* 'Compactus' from rooted cuttings were separated into 3 groups of 25

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[1] Contributions CHO-3154-2, U S. Atomic Energy Commission Contract AT (11-1)-3154 Adapted from Lee and Tukey (10).

plants each. From the first group, a sample of leaf tissues was collected for chemical analysis. Simultaneously, 45 stem cuttings from these same plants were wounded slightly, treated with Hormodin (0.8% IBA), and placed in a propagation bench in the greenhouse in a rooting medium of sphagnum peat moss and perlite (1:1 v/v) under intermittent mist with a cycle of 20 sec of mist every 30 min during the day. After 1 month, the cuttings were harvested and the rooting percentage and the diameter of the root ball determined.

The second group of 25 plants was placed in 5-inch plastic pots in the greenhouse under intermittent water mist with the roots and root medium protected from the mist by aluminum foil. For comparison, the third group of 25 plants was grown in the same greenhouse adjacent to the propagation bench with the same environmental conditions, but without mist. One month later in October, 45 stem cuttings were taken from both the misted and non-misted stock plants and were rooted under intermittent mist as described previously. Simultaneously, leaf samples together with the samples collected before propagation were analyzed for a number of plant constituents implicated in root initiation and development (10).

The results of the rooting study showed that cuttings from stock plants misted for 1 month rooted better under intermittent mist (93.3%) than did cuttings from either non-misted stock plants (73.3%), or stock plants before any treatment (70.2%). In addition, root-ball diameters were greater in the cuttings from misted stock plants, indicating not only a greater rooting percentage, but also development of a heavier root system during propagation. These results show an effect of mist on the rate of rooting, for all the treatments would eventually root at or above 95% if left in the bench.

Chemical analyses were made of several constituents in leaf tissues from stock plants before propagation to see if they could be related to the observed differences in rooting. For example, carbohydrates were analyzed as they provide a source of energy and are used in syntheses of other substances important in root initiation. The dry weight of leaves from misted plants was greater (8.4 mg per cm^2) than from non-misted plants (7.5 mg per cm^2) indicating a higher net photosynthetic activity in misted leaf tissues. As a result, leaf tissues of misted euonymus contained more total sugar and starch (Table 1) than did either the non-misted stock plants or the plants prior to treatment.

The concentration of total N in the leaf tissues of non-misted plants was decreased by dilution due to vegetative growth of the plants during the treatment period. The concentration in the misted plants was reduced even more due to leaching by the mist (14).

The carbohydrate-nitrogen relationship (C/N ratio) is used as an indicator of potential rooting. In the experiment, the greater carbohydrate accumulation and decrease of N resulted in a higher C/N

Table 1. Chemical substances in the leaves of *Euonymus alatus* 'Compactus' plants as influenced by 1 month of intermittent water mist.

Substances	Before treatment	After treatment	
		No mist	Mist
	(mg / g dry wt)[d]		
Total sugar	40.0	52.6	57.9
Starch	52.3	48.2	59.5
Total N	31.4	25.6	22.1
C / N ratio[a]	3.0	3.9	5.0
PAL[b]	0.5	0.5	1.4
Chlorogenic acid	0.4	0.9	1.3
Flavonol	2.6	3.9	4.4
Total flavan	4.6	6.7	10.2
Leucoanthocyanin[c]	1.3	2.4	3.6

[a]Carbohydrate-N ratio was calculated by dividing total carbohydrate by total N

[b]Phenylalanine ammonia lyase expressed as μM cinnamic acid / hr / g fresh wt.

[c]Expressed as mg cyanin produced / g dry wt.

[d]Mean amounts of all constituents differ statistically between treatments at the 5% level.

ratio (5.0) in the misted leaf tissues than in non-misted (3.9) , indicating a greater rooting potential in misted stock plants. Whether the C / N ratio had a direct effect in these experiments was not clear, for in spite of a greater C / N ratio in non-misted plants (3.9) than in the plants before treatment (3.0), little difference in rooting was noted.

Phenolic compounds, in addition to auxin, are strong stimulators of adventitious root initiation (2, 3, 6). The biosynthesis of these compounds in plants involves the shikimic acid pathway in which the enzyme phenylalanine ammonia lyase (PAL) is prominent (10). The PAL activity before treatment was 0.5μM cinnamic acid / hr / g fresh wt. There was no apparent increase in the PAL activity in the leaves of non-misted stock plants after 1 month, whereas the leaf tissues of misted stock plants showed a 3-fold increase in PAL activity. Apparently, because of the higher PAL activity, total phenolic content was somewhat higher in the misted leaf tissues than in non-misted tissues. This suggests that the misted plants were still metabolically active (were not dormant) and had an increased potential for production of phenolic substances to promote directly or synergistically root initiation of the cuttings.

Of greater significance than total phenolic content was the qualitative distribution of phenolic compounds in the misted plants (Table 1). Concentrations of chlorogenic acid, flavonols, flavans, and leucoanthocyanins were much greater in the leaves of misted plants than of non-misted plants. All of these substances have been implicated in root initiation (1, 4, 12) and their accumulation may explain in part the propensity for better rooting in the misted plants.

Auxin-like activity in the leaf tissues was determined using the oat coleoptile bioassay (11) in which elongation of the coleoptiles is related to auxin activity. The activity of auxin-like substances in the plants before treatment was considerable (11.6% elongation). However, after one month with no mist, the activity was reduced to a low level (2.6% elongation) suggesting the state of dormancy in these plants in October. In contrast, activity in the misted plants was greatly increased (20% elongation) indicating increased levels of auxin-like substances.

The levels of rooting cofactors, also implicated in root initiation (4, 6, 9), were somewhat greater in misted leaves than in non-misted leaves, and were much greater than the initial levels.

In the previous experiment, one of the dominant effects of the intermittent mist was the apparent enhancement of endogenous root-inducing substances, such as phenolic compounds, rooting cofactors, auxins, and flavonoids. Thus, it was of interest to see if these substances could be added exogenously to non-misted plants to improve rooting. This was tried first with Mung beans, commonly used in the Mung bean bioassay for rooting. Since the effect of auxins has long been known and used in the propagation industry, and since the identity of 2 and perhaps 3 of the rooting cofactors is not firmly established, a flavonoid compound, rutin (quercetin 3-rutinoside), was chosen for this study. Two ml of rutin solutions at concentrations varying between 5×10^{-4} and 10^{-6}M and 2 ml of indoleacetic acid (IAA, 10^{-6}M) were applied to Mung bean cuttings. For comparison, Mung bean cuttings were also rooted with IAA (10^{-6}M) alone, rutin (10^{-6}M) alone, and water alone. Rutin alone and IAA alone did not produce a significant increase in root initiation as compared with the water controls. However, when rutin was added, together with IAA, root initiation in Mung beans was promoted at all concentrations tested, especially at rutin concentrations of 5×10^{-5} and 10^{-5}M. This indicates that rutin, like some other flavonoids, may act synergistically with IAA to produce adventitious roots (13).

A similar experiment was conducted with cuttings of non-misted euonymus, using rutin as an example of a typical flavonoid, and indolebutyric acid (IBA) instead of IAA as the auxin. In August, 450 five-inch stem cuttings (mature) were collected from clonal stock of euonymus growing on the Cornell campus, and were separated into 3 groups of 150 cuttings each. The stem bases of the first group were dipped in a solution containing 2000 ppm IBA and 1500 ppm of rutin.

The second group was treated with 2000 ppm IBA alone, and the third group was treated with water, as the control. All cuttings were then placed in the propagation bench under intermittent mist. Each week 30 cuttings were harvested and rooting percentage and root ball diameters were recorded.

Control cuttings (Fig. 1) treated with water showed no root initiation until the 4th week after start of propagation and, after 5 weeks, only 40% of the cuttings were rooted. Cuttings treated with IBA alone commenced rooting after 3 weeks, but only 65% of the cuttings had rooted at the end of the experiment. In contrast, root initiation was improved in cuttings treated with both IBA and rutin. For example, after 2 weeks, 50% of the cuttings had rooted, and after 5 weeks all were well rooted. In root quality, cuttings treated with both IBA and rutin produced a more compact root system with a root ball diameter more than twice as large as cuttings treated with IBA alone.

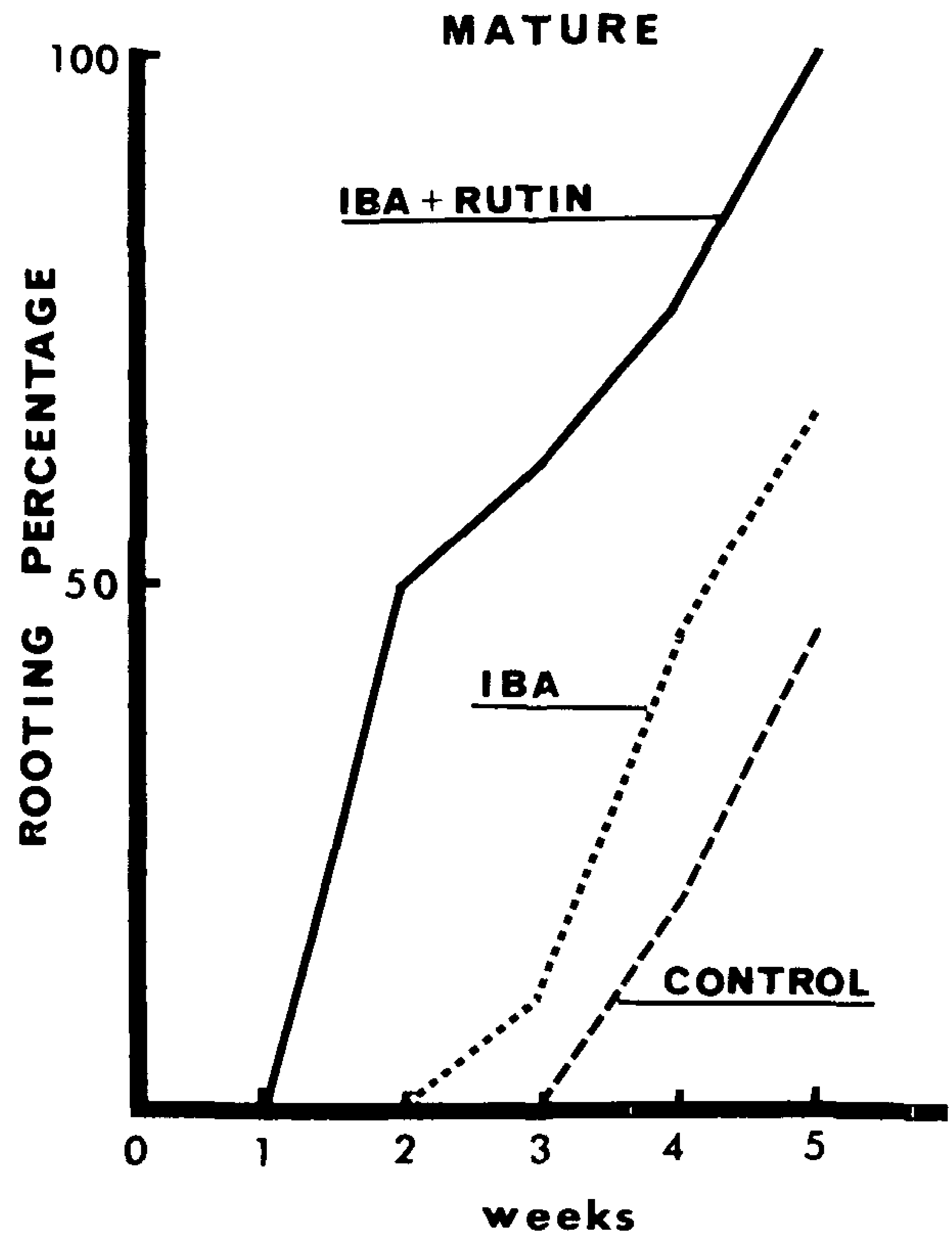

Fig. 1. Rooting of *Euonymus alatus* 'Compactus' cuttings as influenced by applications of IBA (2000 ppm) and rutin (1500 ppm).

In similar experiments conducted with immature cuttings taken in June, there were no significant differences in the rooting percentage and root quality among the 3 treatments. All cuttings were well rooted after 5 weeks, and all had approximately the same root quality rating.

DISCUSSION

When *Euonymus alatus* 'Compactus' plants were grown under intermittent water mist during September, the onset of dormancy was delayed, leaf abscission was delayed, anthocyanin production was inhibited, and there was an increase in the production of natural flavanoid compounds, auxin-like substances, and rooting cofactors. As a result, mature stem cuttings taken from the misted stock plants rooted much earlier and produced a higher quality root system than did cuttings from non-misted plants.

Evidence in the literature supports the suggestion that the accumulation of auxin-like substances, rooting cofactors and flavonoids, may be responsible for improved rootability, including substances such as catechol, pyrogallol, rooting cofactors, chlorogenic acid, quercetin, kaempferol, anthocyanins, leucoanthocyanins, and even "rhizocaline" (1, 2, 3, 4, 6, 9, 10).

When rutin, a flavanol, was applied to mature cuttings from non-misted euonymus, rooting was greatly increased over control plants and similar to rooting of untreated mature cuttings from misted stock plants. However, when immature cuttings were treated with rutin plus an auxin, no significant increase in rootability was noted. This suggests that immature cuttings may have sufficient amounts of natural flavanols or other natural root-inducing substances for good root initiation.

It is not clear how intermittent mist alters the metabolism of the plants. One possible explanation is leaching by the mist of metabolites, including inhibitors, involved in senescence and dormancy (14), allowing synthesis of compounds normally found in vigorously growing tissues. Growth inhibitors have been detected in the leachates of many plants (14). In fact, this was put to practical use in ancient times by plantsmen who placed cuttings in a stream of running water overnight before planting in order to promote root initiation. More recently, immersion of sugar cane cuttings in water prior to planting caused a definite increase in root initiation, due to leaching of inhibitors (7).

The cooling effect of the intermittent mist (5, 8) did not seem to be the primary cause of the observed changes in metabolism, for the temperature changes induced by the mist were a maximum of 3° C, and then only when the mist was on. In addition, accumulation of root-inducing substances noted in these experiments is not usually associated with reduced leaf and air temperatures.

Similarly, the moisture status of the plants did not seem to account for the results, as both the misted and non-misted plants were grown similarly, except for the mist. However, a possible effect cannot be eliminated.

These results offer an additional explanation for the success of modern mist propagation techniques. It has long been known that intermittent mist lowers air and leaf temperatures, thus reducing the rates of transpiration and respiration in cuttings (5, 8). However, the benefits of mist could not always be explained satisfactorily by these effects alone. As these experiments demonstrate, intermittent water mist delays senescence of plant tissues and stimulates an accumulation of natural root-inducing substances in the tissues, thus, in effect, conditioning a plant to better rooting. These results pertain specifically to *Euonymus alatus* 'Compactus' and, although there is evidence that the results are applicable to other species, they have not been tested as yet.

The results reemphasize that there are many endogenous substances in plants which may have a role in root initiation and development, including not only auxins and rooting cofactors, but other phenolic substances such as flavans, flavonols, and anthocyanins. This may be one of the reasons why the results of many rooting studies are difficult to interpret if only one group of root-inducing substances is considered. For the nurseryman, identification and application of these substances to cuttings offers great potential for improved rooting.

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MODERATOR FLEMER: Thank you very much for an interesting and provocative paper; it certainly indicates that mist does something besides just keeping the cuttings turgid.

KEN REISCH: You said that when misting the stock plants they continued to grow, do you mean linear growth regardless of photoperiod in the fall?

HAROLD TUKEY: That is right. There was a period in the fall. when the linear growth slowed down starting at about July when the terminal bud was set. There was a reduction in growth after that but then they began to grow again in October or November and about the end of November the new terminal bud began to grow.

DICK BOSLEY: You mentioned that you had less disease under the mist; would you speculate on this please?

HAROLD TUKEY: The pathologists are quite intrigued as to how this works. It has been suggested that the spores can be washed off by the mist and thus prevent germination. Some pathologists do not believe this but it is true that disease control is better with the mist than without the mist. Whether it is as simple as washing the spores off I do not know.

BRUCE BRIGGS: Can you feed fertilizer through the mist lines to the unrooted cuttings and help rooting?

HAROLD TUKEY: We did not use nutrient mist in these studies but Dr. John Wott, now the Secretary of this Society, did a considerable amount of this work when he was a graduate student at Cornell. The results of his work have been published in previous Proceedings. Specifically, yes you can increase the rootability of cuttings by correct additions of nutrients during the rooting period. Those cuttings which are growing the most, particularly softwood cuttings, respond best whereas the more mature cuttings which are not making a great deal of growth during the rooting period, do not respond particularly well.

MODERATOR FLEMER: Several years ago our Society became interested in the finding and introduction of new plants on a commercial scale A committee under the able chairmanship of Jim Wells was appointed to look into this question and report back to the Society. This report will now be presented to you by Jim Wells.

REPORT OF THE PLANT EVALUATION COMMITTEE

Composed of:

WILLIAM FLEMER III
WILLIAM SNYDER
PETER VERMUELEN
JAMES S. WELLS—CHAIRMAN

Presented by

JAMES S. WELLS

Your Plant Evaluation Committee met four times and I am glad to say that substantial progress was achieved. At our first meeting held November 29, 1970 we first considered whether we needed a scheme for plant evaluation and introduction. It was unanimously agreed that we did. Next followed the consideration of what form it ought to take. Possibilities considered were as follows:

1. A strictly commercial plan for testing, selecting and ultimately introducing first class plants.
2. A non-commercial evaluation system similar to the Royal Horticultural Society in England, the introducing then to be left to the originator.
3. A combination of 1 and 2.
4. A survey type such as the evaluation of hybrid French lilacs carried out by John Wister and published by the Arthur Hoyt Scott Horticultural Foundation.