ETHYLENE AND ADVENTITIOUS ROOT FORMATION

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Abstract. Pelargonium peltatum 'Galilee' and a cultivar of Coleus blumei were treated with ethephon at 0, 100, 400, and 800 ppm. Treatment effects on number and length of roots and changes in endogenous levels of root promoting and root inhibiting substances were determined. Rooting was stimulated in both species at most ethephon levels, but 400 ppm seemed to be best. Increased rooting corresponded with increased levels of endogenous rooting promoters and/or decreased levels of inhibitors.

Ethylene, structurally the simplest of plant hormones, is primarily associated with metabolic activity which is inhibitory to growth. These include diminished internode length (15), suppression of bud growth in some cases (4,5), abscision of leaves and fruit (1,5), and flower induction of bromeliads (6).

Ethylene has been tied to endogenous levels of indole-3-acetic acid (IAA) (15). Increased levels of IAA usually lead to increased levels of ethylene which then may diminish the concentration and effectiveness of IAA. The response of a plant to ethephon, (2-chloroethyl) phosphonic acid, is dependent on chemical, physical and environmental conditions. A higher concentration of ethylene was released in plant tissue at higher pH's and higher temperatures (9,10). Duration of the effect of ethylene from an exogenously applied ethylene compound may be as brief as 4 to 24 hours (13) or much longer (5).

The formation of adventitious roots on cuttings is a complex procedure. It involves a synergism and/or direct combination of various root promoting substances (cofactors) with auxin (7,8). This complex then aids in cell division and differentiation into root tissue. Problems which may arise are insufficient quantities of IAA due to lack of synthesis and/or catabolism, lack of one or more of the essential cofactors or other root promoting substances, presence of inhibitors to root initiation, presence of gibberellic acid (GA) which may inhibit cell division (8,15) or a combination of any of the above factors. Some of these problems may be overcome as follows:

- 1. The catabolic effect of IAA oxidase may be overcome by application of IBA, NAA or a phenoxy-compound, which are not rapidly degraded by IAA oxidase.
- 2. The phenolic fraction of the rooting promoters (cofactors) may be added exogenously by compounds such as catechol or rutin.
- 3. The inhibitory effect of GA may be lessened by application of ABA or suitable anti-GA compound such as

Cycocel, B-9 or Phosphon, if GA is a problem. The area which is seemingly uncontrollable is increasing the concentration of the other root initiation promoters (cofactors) and/or decreasing the inhibitors of root initiation.

The role of ethylene in adventitious root formation is little understood and contradictory reports are common. Kawase (8) found ethylene applications would stimulate adventitious root formation in willow and tomato cuttings. Swanson (4) found that adventitious root formation was increased in softwood cuttings of 6 different species of woody plants with treatments of ethephon alone or in combinations with IBA and NAA. Ethephon works independently of IBA or NAA (10). Carpenter (3) succeeded in rooting hardwood cuttings of Juglans nigra after soaking in solutions of ethephon. Ethylene has also shown promise in the rooting of azaleas (11).

METHODS AND MATERIALS

Cuttings. Uniform cuttings of Coleus blumei or Pelargonium peltatum 'Galilee' (4-5 nodes) were rooted under intermittent mist in sand, with 26°C bottom heat. Ethrel¹ (ethephon), 21.3% 2-chloroethyl phosphonic acid, previously adjusted to pH 4.5 with 1 N NaOH was sprayed on the cuttings foliage (both sides) until drip, at the rate of 0, 100, 400 or 800 ppm. A spreader-sticker, Triton B-1956, was added at the rate of 1 drop to 300 ml of solution. Root number and average root length were noted at the end of 7 days for coleus and 12 days for geraniums. There were 3 replications of 5 cuttings for the coleus and 4 replications of 5 cuttings for the geranium. Mean separation was by Duncan's Multiple Range Test after analysis of variance.

Mung Bean Bioassay. Leaf tissue (1.5g) was boiled in 80% ethanol (6 hours after spraying the geranium and 4 hours for the coleus), macerated in a blender, filtered through Whatman No. 1 filter paper and washed with two 30-ml portions of 80% ethanol. The filtrate was concentrated at 40°C to 5 ml under reduced pressure. A 0.25 ml aliquot of this resultant slurry was streaked across the 5 cm width of Whatman No. 3 chromatographic paper which was 45 cm long. After a 6 to 8 hour equilibration in an atmosphere of 100% ethanol, the strips were developed in 80% 2-propanol for 30 cm by descending paper chromatography. The dried strips were then cut widthwise into seventeen 2 cm segments, each of which was equilibrated for 1 hour in a 10 ml vial containing a 4ml solution of $5 \times 10^{-6} \mathrm{M}$ in IAA and $9.4 \times 10^{-7} \mathrm{M}$ in $\mathrm{H_3Bo_3}$. The extra two strips from above the origin and from below the solvent front served as controls.

¹ Amchem Products, Ambler, PA.

Five uniform mung bean (Phaseolus aureus Roxb.) cuttings, sown in vermiculite and grown for 7 days under a 16 hour day at 8.3 klx, 30°C day temperature and 26°C night temperature, were inserted into each vial. There were 3 replications of each treatment. Adventitious roots were counted after 6 days and plotted on histograms according to strip number or Rf value.

RESULTS

Ethephon treatment of geranium at the rate of 400 ppm stimulated the greatest number of roots (Table 1). This was reduced at 100 ppm and further reduced at 0 and 800 ppm. The greatest root length was on the 0 ppm treatment, the least on the 800 ppm, with the 100 and 400 not different than the 0 or 800 ppm treatments. The percent rooting was reduced to 85% at the 0 and 800 ppm levels. The cuttings that did not root would probably have rooted if given sufficient time. There was some leaf abscission at 800 ppm. This data indicates that the ethylene releasing compound, ethephon, will stimulate the formation of a greater number of roots in the 100 to 400 ppm range as compared to the control. The 800 ppm treatment reduced root number, root length and rooting percentage, indicating this concentration was too great for ivy geranium 'Galilee'. The histogram from the mung bean bioassay showed that there was a change in the levels of the various root inducing substances (Figure 1). The highest levels were recorded at the 400 ppm treatment, the lowest levels were recorded at 0 and 800 ppm. These histograms do correspond closely with the rooting study, where the best rooting was at the 400 ppm treatment.

Table 1. The effect of ethephon concentration on the rooting of Pelargonium peltatum 'Galilee' stem cuttings.

Ethephon Concn. (ppm)	Average Root Number	Root Length (mm)	Percent Rooting
0	$6.5 c^z$	11.0 a	85
100	9.0 b	9.9 ab	100
400	12.2 a	10.4 ab	100
800y	5.0 c	7.7 b	85

² Mean separation by Duncan's Multiple Range test at the 5% level.

Ethephon treatment of coleus stimulated the greatest number of roots at 400 ppm when compared to the control (Table 2). This was not different than the 100 and 800 ppm treatments. The shortest roots were found on the 800 ppm treatment. A differential rooting pattern between the ethephon-treated plants and the control was noted. The control cuttings only rooted at the basal node, while the treated plants rooted at the basal node and the internodal region between the bottom nodes. The mung bean bioassay histograms showed an increase in root

y Some leaf abscission of cuttings.

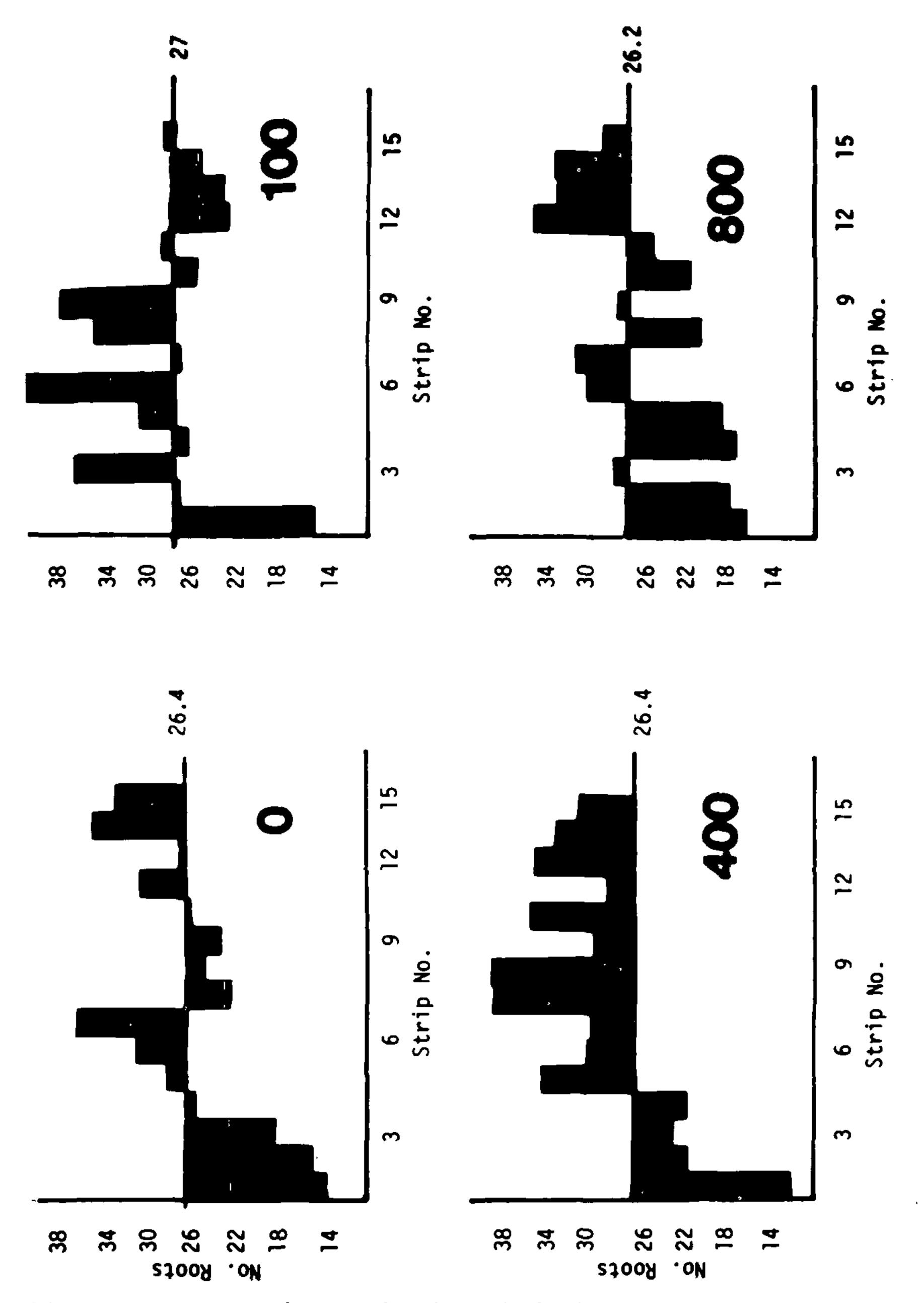


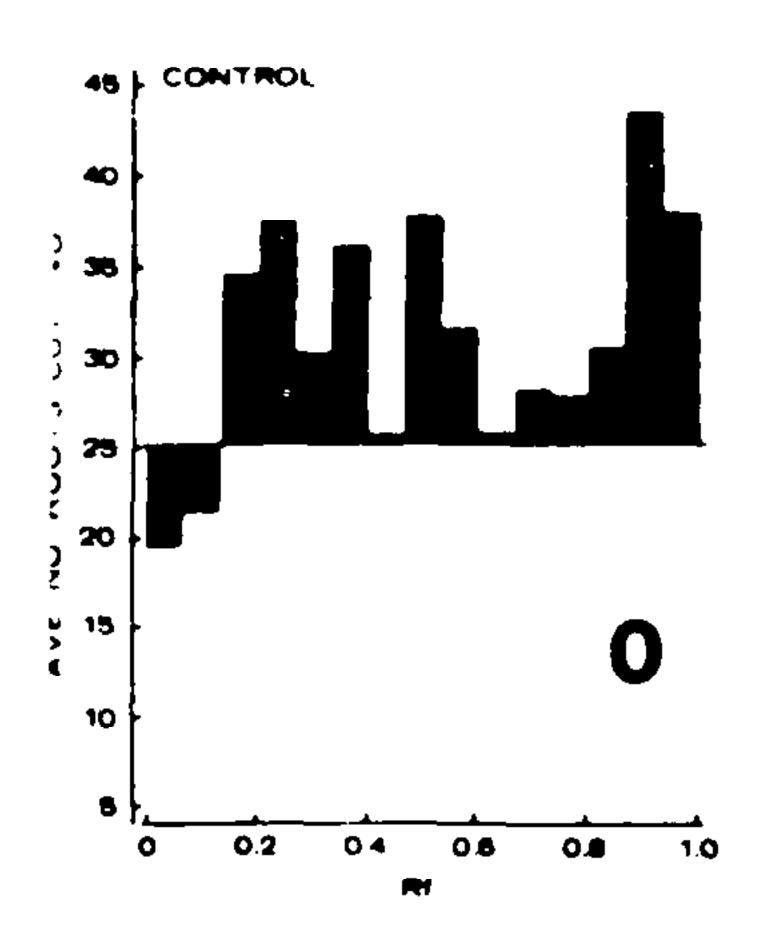
Figure 1. Histograms showing the effect of ethephon at 0, 100, 400 and 800 ppm on the biological activity of *Pelargonium peltatum* 'Galilee', as determined by the mung bean bioassay.

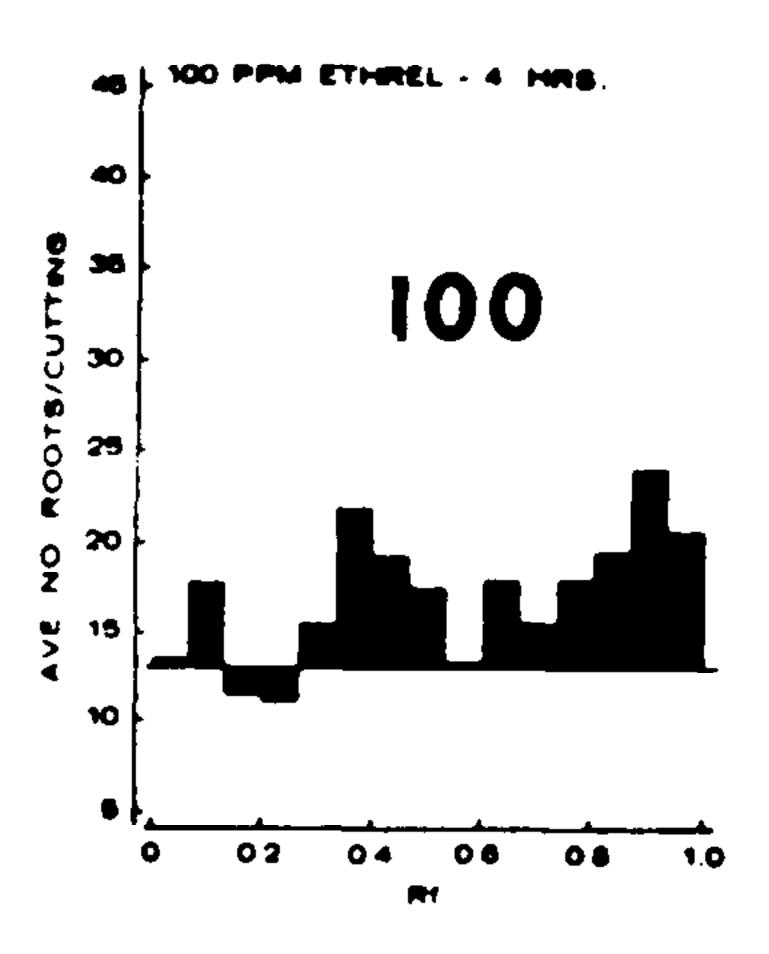
promoting substances, particularly in the 400 and 800 ppm treatments (Figure 2). This also corresponded with the increase in rooting of the cuttings at these levels of ethephon.

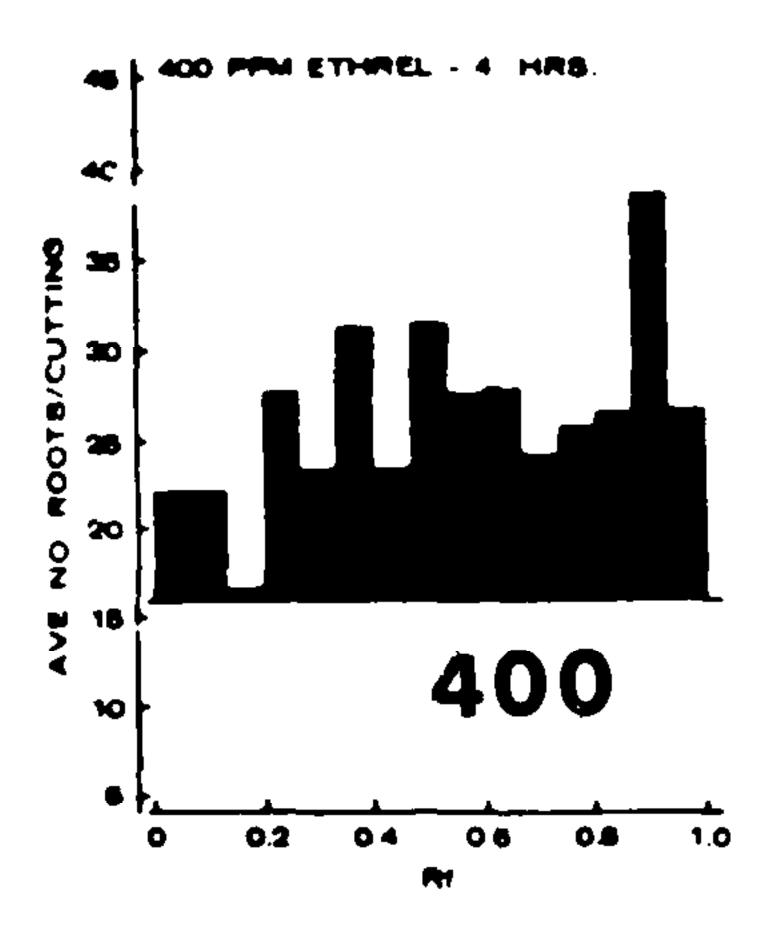
Table 2. Effect of ethephon concentration on the rooting of Coleus blumei cuttings.

Ethephon Treatment (ppm)	Average Number of Roots	Average Length of Roots (mm)
0	16.3 b ^z	15.4 a
100	30.1 ab	12.6 a
400	35.5 a	13.1 a
800	32.4 ab	9.0 b

^z Mean separation by Duncan's Multiple Range Test at the 5% level.







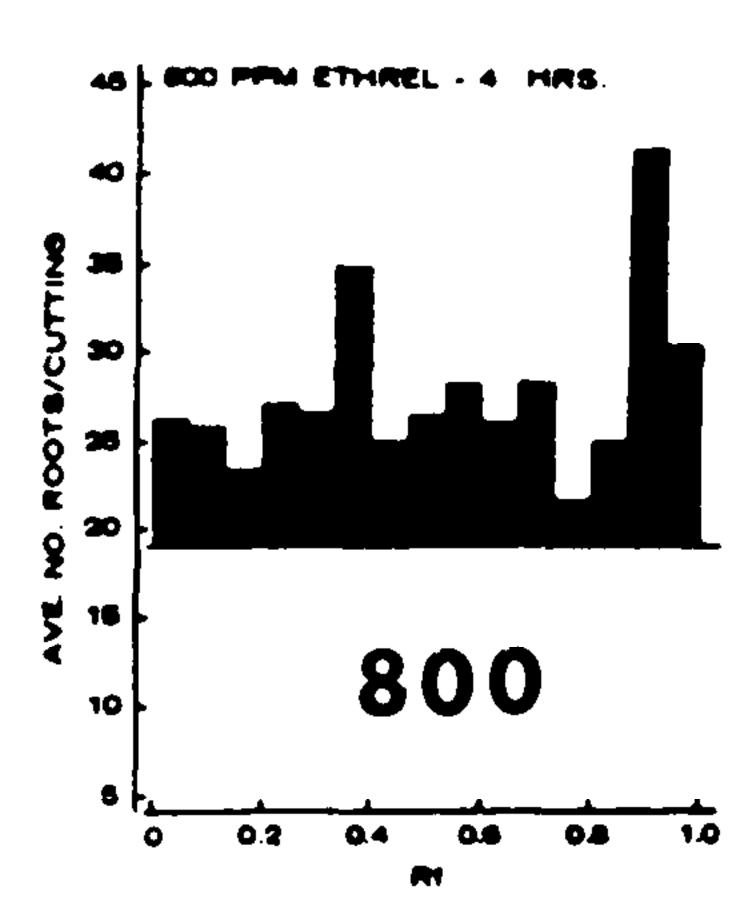


Figure 2. Histograms showing the effect of ethephon at 0, 100, 400 and 800 ppm on the biological activity of Coleus blumei, as determined by the mung bean bioassay.

DISCUSSION

The mode of action of ethylene in the stimulation of adventitious root formation on stem cuttings seems to be a manipulation of various plant metabolites which may induce rooting. This is a concept set forth by Swanson (14) and has been little studied. Ethylene, and ethylene-releasing compounds, may increase the number of endogenous root inducing substances and/or decrease the number of root inhibiting substances. Some of these rooting promoters may be the classical cofactors. The increase in rooting of coleus and ivy geranium 'Galilee' cuttings did correspond very closely with the increase of the various rooting promoting substances as determined by the mung bean bioassay.

The optimum concentration of ethylene for the stimulation of rooting is likely to vary with the species, as well as with other environmental and chemical characteristics. Both temperature and pH play an important role in the release of ethylene from ethephon. More ethylene is released at elevated temperatures and higher pH levels. The ultimate effect on future plant growth, flowering, and fruiting is not known. The increase in the levels of endogenous root promoting substances after treatment with ethylene, may rise and fall within a short period of time. The two species reported on here root very easily and quickly. A more difficult to root species, or one that takes a long time to root, e.g. Taxus sp., may need a delayed application or multiple applications.

In the mass production of easy-to-root plants, ethylene may stimulate quicker rooting, so that plants can be moved faster. Plants which are difficult or impossible to root may be able to be rooted with the proper application of ethylene.

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QUESTION BOX

The question box session was convened at 8:00 pm with Mr. Ralph Shugert and Mr. Ben Minamoto serving as moderators.

MODERATOR SHUGERT: Has anyone propagated Viburnum nudum from seed? If so, what seed treatments were used?

DON SHADOW: The seed, unless picked a little green, will take 2 years to germinate. If picked green and planted in the fall it will often germinate the following spring.

MODERATOR SHUGERT: What is the most successful method of growing Taxus cuspidata (Syn.: T. cuspidata 'Capitata') from seed and how important is the seed source?

ED MEZITT: I collect and clean my own seed, plant them out the same fall, and cover the beds with hay. The seed germinates the second year. We have been using lead arsenate for rodent control.

CASE HOOGENDOORN: Put the seed in sand for one year and then sow it. The seed will germinate the next year. If it is dead it will never come up.

RALPH SHUGERT: Mr. Hoogendoorn hit a very salient point in this matter. It is very important to take a cutting test on any seed. For Taxus, Seeds of Woody Plants in the U.S. states: 90-100 days of warm, 100-120 days of cold; sow it in the spring and maybe the seeds will germinate the next year.