Stimulation and Retardation of Adventitious Root Formation by Application of B-Nine and Cycocel[1]

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Abstract. Herbaceous cuttings dipped momentarily in solutions of several concentrations of B-Nine produced significantly greater weight and numbers of adventitious roots than did untreated cuttings. Concentrations of 1000 ppm and 5000 ppm were effective, with 2500 ppm optimum. Conversely, similar treatments of Cycocel caused a marked depression of adventitious root production. As rate of Cycocel was increased, production of adventitious roots diminished, suggesting corroboration of research proposing Cycocel's behavior as being that of an "anti-auxin".

Introduction

B-NINE (N-dimethyl amino succinamic acid) has been reported to stimulate root development in dahlia, *Dahlia pinnata* Cav., plants (2). In the same research, root development was inhibited in cuttings taken from plants sprayed with Cycocel, (2-chloroethyl) trimethylammonium chloride.

These preliminary findings indicated a distinct acceleration of rooting by a chemical considered by some authors (2, 3) to be an anti-auxin. Research was therefore initiated to determine individual effects of B-Nine and Cycocel, another growth retardant-type chemical, to ascertain interactions with an established root-promoting chemical, 3-indolebutyric acid (IBA), and to develop a practical application which could be used by commercial propagators.

Materials and Methods

Since excessive time periods were required when cuttings were taken from pre-treated stock plants, attempts were made to achieve similar results through dipping the cuttings in the chemicals to be tested. Uniform 4-inch cuttings of geranium, *Pelargonium hortorum,* Bailey, dahlia, and chrysanthemum, *Chrysanthemum morifolium* Ram., were taken from greenhouse stock plants in the winter and spring. These cuttings were treated with either B-Nine[3] or Cycocel[4] individually or in combination with IBA and rooted in sterile sand under intermittent mist (10 sec per 5 minutes) at a diurnal temperature regime of 60°–70°F. 'Wendy Ann', 'Salmon Irene', 'Carefree Light Salmon' and 'Carefree Picotee' geranium, 'Nita' dahlia, and 'Wanda' and 'Minnwhite' chrysanthemum cuttings were tested. Either the basal inch or the upper ⅔ of the cuttings were dipped for 10 to 15 seconds in distilled water solutions of B-Nine or Cycocel. In some studies, cuttings were allowed to dry and subsequently dipped in talc dilutions of IBA. In one series of experiments, length of treatment was studied utilizing 2500 ppm B-Nine. Experiments were terminated as soon as the control (untreated) cuttings were judged to be at the optimum degree of rooting for potting. After the sand was gently washed from the roots, those over 1 mm were counted, measured, excised and placed in a 30°C oven for 24 hr to obtain dry weight data.

Results and Discussion

In 14 experiments involving geranium, chrysanthemum and dahlia cuttings B-Nine increased adventitious root formation while Cycocel always had a retarding effect. Rooting of cuttings were stimulated most effectively by concentrations of B-Nine at 1000, 2500 and 5000 ppm. Throughout the study condition of the stock plant was observed to affect rates of B-Nine necessary, with 5000 ppm necessary to adequately stimulate rooting of cuttings from slow-growing stock plants.

Tables 1 and 2 illustrate that 'Minnwhite' and 'Wanda' chrysanthemum cuttings produced more root weight and longer roots when treated with B-Nine, but Cycocel treatments caused

Table 1. Effect of B-Nine and CCC stem dips on rooting of 'Wanda' chrysanthemum cuttings. Each value represents the mean of 5 replications.

Treatment	Mean fresh root wt (g)	Mean dry root wt (g)	Mean no. of roots	Mean length of longest root(cm)
Control	2.46	.0401	9.80	2.67
B-9 1000 ppm	3.64	.0596	10.60	2.79
B-9 2500 ppm	4.48	.0868	11.60	3.43
B-9 5000 ppm	4.20	.0790	11.60	4.19
CCC 1000 ppm	1.08	.0220	7.00	1.65
CCC 2500 ppm	0.68	.0103	5.20	1.27
LSD .05	0.66	.0152	2.93	0.86
LSD .01	0.90	.0207	3.99	1.17

Table 2. Effect of stem dips of B-Nine and CCC on rooting of 'Minnwhite' chrysanthemum cuttings. Each value is the mean of 5 replications.

Treatment	Mean fresh root wt (g)	Mean dry root wt (g)	Mean no. of roots	Mean length of longest root(cm)
Control	1.32	.0336	8.0	5.46
B-9 1000 ppm	2.04	.0725	12.6	6.86
B-9 2500 ppm	3.56	.0920	15.2	7.24
B-9 5000 ppm	2.76	.0816	13.2	6.73
CCC 1000 ppm	0.74	.0207	5.0	3.30
CCC 2500 ppm	0.62	.0168	5.4	2.92
LSD .01	0.64	.0116	3.4	1.02

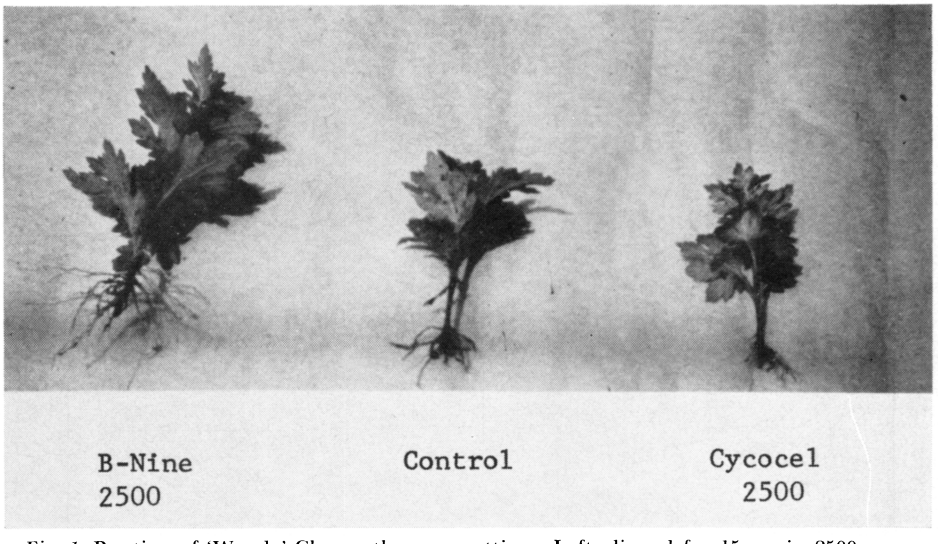

Fig. 1. Rooting of 'Wanda' Chrysanthemum cuttings. Left, dipped for 15 sec in 2500 ppm B-Nine; middle, untreated, and right, dipped for 15 sec in 2500 ppm Cycocel. Note advanced top growth on more heavily rooted B-Nine treated cuttings.

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[3]Prepared as a 5% active formulation with wetting agent by Uniroyal, Naugatuck, Conn., under the trade name "B-Nine".

[4]Prepared as an 11.8% active formulation by American Cyanamid Co., Princeton, N. J., under the trade name "Cycocel".

Table 3. Effect of B-Nine and CCC stem dips on rooting of 'Nita' dahlia cuttings. Data represent mean of 5 replications.

Treatment	Mean dry root wt (g)	Mean no. of roots	Mean length of longest root (cm)
Control	.0155	5.0	5.59
B-9 1000 ppm	.2047	6.8	8.64
B-9 2500 ppm	.1710	6.6	7.37
B-9 5000 ppm	.2008	6.6	8.26
CCC 1000 ppm	.0051	3.2	2.79
CCC 2500 ppm	.0025	3.6	1.52
LSD .05	.0437	NS	2.11
LSD .01	.0596	NS	2.90

Table 4. Effect of duration of 2500 ppm B-Nine stem dips on rooting of 'Wanda' chrysanthemum cuttings. Data are means of 5 replications.

Treatment	Mean dry root wt (g)	Mean no. of roots	Mean length of longest root (cm)
Control	.0372	11.75	2.86
2 sec	.0405	8.40	2.70
15 sec	.0891	13.75	3.33
1 min	.0923	12.25	3.49
5 min	.0275	6.25	2.86
10 min	.0194	5.25	1.75
LSD .05	.0121	2.34	0.86

Table 5. Effect of growth regulator stem dips on rooting of 'Carefree Light Salmon' geranium cuttings. Data are means of 5 replications.

Treatment	Mean fresh root wt (g)	Mean dry root wt (g)	Mean no. of roots	Mean length of longest root(cm)
Control	2.10	.0672	11.00	6.60
B-9 2500 ppm	3.92	.1529	16.00	8.99
B-9 5000 ppm	4.32	.1749	17.80	9.02
CCC 1000 ppm	0.76	.0194	6.60	2.59
IBA 1000 ppm	2.84	.0881	13.60	7.49
B-9 2500 ppm & IBA 500 ppm	4.24	.1684	18.00	8.13
CCC 1000 ppm & IBA 1000 ppm	1.34	.0401	8.80	4.83
LSD .05	0.75	.0247	3.38	1.19
LSD .01	1.00	.0329	4.49	1.57

a severe retardation of adventitious root formation (Fig. 1). 'Minnwhite' cuttings treated with B-Nine also produced significantly greater numbers of roots than did cuttings which were not treated.

All of the B-Nine treatments considerably increased root development with both chrysanthemum cultivars, however B-Nine at 2500 ppm gave the largest dry root weight per cutting. Similar responses were obtained with 'Nita' dahlia cuttings, as all B-Nine levels of treatment caused increases in dry weight of roots per cutting as well as in the length of roots (Table 3).

Dipping the foliage of both geranium and chrysanthemum cuttings produced root formation similar to that of the basal dip treatments. However, foliage dips did not give consistently uniform results, so they were discontinued in favor of stem dips.

In early trials it appeared that 2500 ppm of B-Nine was the most effective rate in promoting adventitious root development. Since a 10 to 15-sec dip had been used, it was deemed of interest to determine if this was the optimum length of time to dip the cuttings. In tests on both geranium and chrysanthemum (Table 4), a one-minute time gave the most rapid root development, but a 15-sec dip was nearly as effective and more conserving of time. Longer dips tended to reduce root development, especially on chrysanthemums. Similar results were achieved using 'Carefree Light Salmon' and 'Carefree Picotee' geranium cuttings.

B-Nine and Cycocel were compared with IBA for root-promoting activity, using IBA at 500, 1000 and 2000 ppm and in combination with several rates of B-Nine and Cycocel. These various combinations were tested on both geranium and dahlia cuttings (Table 5, Fig. 2). IBA at 1000 ppm tended to improve root production, but not as effectively as treatments of B-Nine at 2500 or 5000 ppm. The combination of B-Nine and IBA (e.g. 2500 ppm B-Nine and 500 ppm IBA) did not provide as large an increase in root initiation as did B-Nine alone. However, cuttings treated with both Cycocel and IBA produced roots more nearly like those of untreated cuttings than of cuttings dipped in Cycocel alone.

Fig. 2 depicts 'Nita' dahlia cuttings treated similarly, demonstrating the same type of effects on adventitious root formation. Rooting was more strongly favored by 1000 ppm B-Nine on dahlias than on geraniums and chrysanthemums. B-Nine at all levels was more effective than IBA at the 3 rates tested, while combinations of B-Nine and IBA were essentially no better than B-Nine alone. However, when IBA was added to the Cycocel treated cuttings, adventitious root production again approached that of untreated cuttings, indicating some support for theories of Kuraishi and Muir (1) and others that the effects of Cycocel are opposite those of auxin-like chemicals. Contrary to theories that B-Nine behaves as an anti-auxin (2, 3) B-Nine caused effects which could be construed to be enhancing or replacing auxin behavior.

This research definitely established that cuttings dipped for 15 seconds in 2500 ppm B-Nine promoted root growth on geranium, chrysanthemum and dahlia. This increase was the result of an increase in root length and more root branching. A significantly greater number of roots initiated was frequently observed (e.g. 'Minnwhite' chrysanthemum and 'Carefree Light Salmon' geranium). The dry weight of roots produced by B-Nine treated cuttings was twice that of the untreated. B-Nine at 2500 and 5000 ppm were the most effective rates on geraniums and chrysanthemums while all 3 levels tested seemed to be equally effective on dahlias.

Cycocel, at the rates used, reduced root initiation. No root growth was evident 2 weeks after treatment, compared to fair root initiation by the control cuttings and good root growth on the B-Nine treated cuttings. Higher rates of Cycocel treatment caused greater inhibition of root formation. B-Nine treated plants grew to normal height and bloomed as soon as or earlier than the control plants.

Although data presented are based on single harvest dates, it was observed that cuttings dipped in 2500 ppm B-Nine for 15 sec could be harvested from 5 to 10 days earlier than untreated cuttings if removal from the propagation bench took place at the stage optimum for potting the cuttings. This conceivably could allow a commercial propagator to obtain from 3 to 5 more crops of rooted cuttings

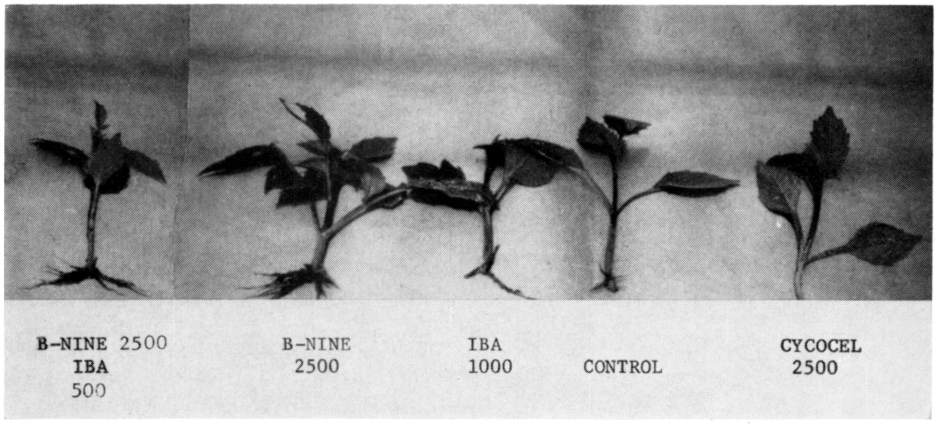

Fig. 2. Effect of growth regulator stem dips on rooting of 'Nita' dahlia cuttings. Figures are ppm.

Morphological and Histological Effects of Ethrel on the Apricot, *Prunus armeniaca* L., as Compared with Those of 2,4,5-Trichlorophenoxyacetic Acid[1]

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Abstract. Apricots on branches sprayed with Ethrel at the beginning of pit hardening abscised, whereas similar treatment with 2,4,5-T stimulated fruit growth and decreased drop. In the stem, gum ducts were formed in the xylem after treatment with Ethrel but not with 2,4,5-T, both at 100 ppm concentrations. Both Ethrel and 2,4,5-T stimulated cambial activity in petioles and midveins, thus increasing phloem and xylem tissues. Both growth regulators induced tylosis formation in petiole xylem. 2,4,5-T treatment caused increase in petiole diameter and leaf blade thickness, through increasing endopolyploidy and thus cell size in ground tissue of the petiole, and in mesophyll, epidermis and vascular bundle sheathes in the leaf blade. Ethrel caused little if any increase in cell size in those tissues, and therefore no obvious increase in petiole diameter and leaf blade thickness.

Introduction

The suggestion has been made that many plant responses thought to be caused directly by auxins may actually be traceable to ethylene, the synthesis of which is enhanced by auxin application (1, 10). Much attention is currently being given to the effects of Ethrel (Amchem 66-329, manufactured by Amchem Products, Inc.), which is a source of gradually released ethylene and is more conveniently applied to plant structures than ethylene itself. The following is an account of some effects of Ethrel when sprayed on branches of the apricot, as contrasted with previously noted effects of the auxin 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) applied to apricot branches in 1965 (3).

Materials and Methods

Ethrel in aqueous solutions at concentrations of 100, 250, and 500 ppm was sprayed on branches of the 'Royal' apricot on April 15, 1968. Each concentration was applied to one branch of each of two 6-year-old trees. Spur leaves were fully expanded at the time of treatment and pit-hardening in the fruits had just begun. Later (May 28), when fruit development was at the beginning of growth period III, the same concentrations were applied to branches of other trees, in order to compare the effects on fruits treated at different phases of fruit development.

Stem and leaf material from control branches and those treated on April 15 was fixed in formalin-propionic acid-alcohol on June 6, and included segments from the bases of current season's and 1-year-old stem growth, ½-cm pieces of petiole bases, and approximately 1-inch long pieces from bases of leaf blades cut at right angles to and including part of the midveins. Some stem pieces were sectioned with the sliding microtome; others, and also petiole and leaf blade segments, were embedded in paraffin and sectioned. Sections were stained with safranin and fast green. Leaves of branches treated on May 28 were collected on August 22 to determine whether Ethrel altered certain leaf blade structures.

'Royal' apricot trees were sprayed on May 22, 1965, with aqueous solutions of 2,4,5-T at concentrations of 50, 100, and 200 ppm (three trees per concentration). Water-sprayed trees were the controls. At that time the spur leaves were fully expanded. Leaves from spurs, and basal pieces of current season's stems were collected on August 3. Segments fixed and the fixative were the same as those for Ethrel-treated material.

Results

Fruits. Application of all concentrations of Ethrel at the beginning of pit hardening caused eventual premature abscission. Ethrel application at the beginning of growth period III did not induce premature abscission, but brought about splitting of the mesocarp in the suture at the stylar end of the fruits.

Stems. Much exudation of gums, which appeared on the surface of the 1- to 5-year-old wood, was caused by the 500 ppm concentration of Ethrel, and progressively less by the 250 and 100 ppm concentrations. Within the xylem of both current season's and 1-year-old regions of the stems treated with 250 and 500, but not 100 ppm, gum pockets and ducts were formed (Fig. 3, 4). In 1-year-old stem regions they developed in current season's but not in previous season's xylem. Apparently the gum pocket initials originated through differentiation of cells of newly formed xylem. Through enlargement and fusion of the gum pockets, the ducts were formed. Exudation of gums following Ethrel treatment of the cherry has been reported by Bukovac et al. (4). Ethrel treatments neither inhibited nor stimulated activity of the cambium, as shown by counts of cells in radial rows of phloem and xylem in treated and control stems. No significant differences occurred between any of the data for stems of the different treatments and controls. No tyloses were induced in the stems.

Leaves. Ethrel at all concentrations stimulated cell division in the cambial zones of the petioles and midribs, thus increasing radial diameters of the phloem and xylem arcs as compared to those of the controls (Fig. 1, 2). Cell proliferation was induced also in some regions of the protoxylem parenchyma and adjacent ground tissue (Fig. 5). In 1 petiole from a branch treated with the 500 ppm concentration, extreme cell proliferation in that region had extended outward to cells of xylem rays and xylem parenchyma in the older parts of the secondary xylem. In consequence of the cell division together with subsequent excessive enlargement of some cells, the adjacent xylem vessels were being or had been obliterated, and the affected regions were highly disorganized in general. No consistent difference in diameters of petioles treated with

per year, depending on crop, cultivar, condition of stock plant and growing conditions. It is also apparent that if a propagator intends to use a growth retardant on stock plants of these species, he should avoid Cycocel.

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