The Growth Regulating Effects of Fungicides on Adventitious Root Formation of Chrysanthemum Cuttings

by

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ABSTRACT

Ten commercial fungicides were applied to cuttings of *Chrysanthemum morifolium* Ramat 'Bright Golden Anne' to determine their effect on adventitious root formation. Thiram, Captan, Botran, and Banrot inhibited root elongation and growth. Root initiation, as measured by root number, was delayed but was not permanently affected. Other treatments had little or no effect on rooting when compared to the control. No noticeable effects on top growth occurred.

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INTRODUCTION

Herbaceous cuttings are rooted under warm, moist conditions, which are highly favorable for disease development. Because of this, the use of fungicides for disease control is a routine practice in commercial plant propagation. Fungicides are applied either to actively control an existing disease problem or to prevent problems from occurring. Basal dips, soil drenches, and sprays are common application methods.

Adventitious roots are defined as "all roots other than those arising from the embryo axis and all their branches formed in normal sequence" (11). They may arise from underground stems, aerial plant parts, or from leaves. Good adventitious root formation is essential for successful propagation of stem cuttings.

Several studies have indicated that in addition to fungicidal properties, some fungicides may have direct growth regulating effects on adventitious root formation and top growth. Effects may be promotive (10) or inhibitive (2,12,17,18,24). This possible growth regulating effect of fungicides is usually overlooked, and researchers and growers alike focus almost exclusively on the pathological action of the fungicide. As a result, beneficial or harmful effects on the plant may go unnoticed.

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LITERATURE REVIEW

Previous studies indicate that the degree of growth regulation caused by fungicides depends greatly on the fungicide, concentration, method of application, and plant species used (12,19,21). Moorman and Woodbridge (22) detected no clear differences in rooting of geranium cuttings drenched with six commercial fungicides. Fungicides tested included formulations of Lesan (fenaminosulf) Truban (ethazol), Benlate (benomyl), Terraclor (quintozene), and Subdue (metalaxyl). However, the same fungicides applied in various combinations and concentrations to polyurethane propagation blocks inhibited rooting of poinsettia cuttings (18). Lesan and Terraclor treatments were the most inhibitory. Previous work done by Lee on poinsettia cuttings indicated that fungicides tested were more inhibitory as drenches than as powders or granular applications (17).

Japanese boxwood cuttings treated with ten commercial fungicides exhibited decreased rooting only with a three-minute Lesan soak prior to planting (23). Lesan applied as a soil drench did not inhibit rooting. Drench applications of Subdue, Banrot, Terraclor, and Lesan resulted in higher root numbers than did preplant soaks at the same concentrations. Benlate and Captan treatments did not affect rooting, although studies on other ornamental species found phytotoxicity with these fungicides (2,12).

In addition to reductions in rooting, regulation of top growth has been reported (15,16,26). Benlate (26) and Truban (16) caused stunting of top growth when applied to poinsettia. Truban-treated rhododendron plants exhibited chlorosis, general stunting, and in extreme cases, death (15). Rooting of rhododendron cuttings was also inhibited by monthly drenches of Truban (6).

Murakishi and Hendrix (24) demonstrated that rooting hormones could be successfully used in combination with some fungicides to overcome phytotoxic effects. Eleven fungicides and six growth substances were applied alone and in combination to carnation cuttings in wilt-infested soil. Rooting inhibition which occurred with several fungicides was overcome by the addition of indoleacetic acid (IAA) or indolebutyric acid (IBA). Hoitink and Schmitthenner (14) found that rooting of rhododendron cuttings was significantly increased by a preplant dip in 5% Benlate or 10% Mertect mixed with IBA . Benlate applied alone as a drench inhibited rooting.

Hare (10) suggested that some fungicides may act as weak auxins to directly promote adventitious root formation. In tests of rooting powder formulations on difficult-to-root cultivars of Chrysanthemum morifolium Ramat, the fungicides Captan and OAC-2582 appeared to possess auxin activity. The concentrations most promotive of rooting fell below the range of known pathological activity, suggesting that the promotion was not a function of pathogen control.

OBJECTIVE

The overall objective of the research was to determine the effect of common commercially used fungicides on adventitious root formation of *Chrysanthemum morifolium* Ramat. The specific objectives were:

1) Identify possible growth regulating effects of fungicides on adventitious root formation.
2) Classify fungicides as promotive, inhibitive, or as having no effect on adventitious rooting.
3) Determine the effect of fungicides on top growth of cuttings during propagation.

MATERIALS AND METHODS

Plant Material Used

Unrooted cuttings of *Chrysanthemum morifolium* Ramat 'Bright Golden Anne' were obtained from Yoder Brothers, Barberton, OH. These were used in all experiments.

The use of *Chrysanthemum morifolium* Ramat in this study was based on several factors. Chrysanthemum is the highest value flowering pot plant and cut flower grown by the floriculture industry. It has a wholesale value of $76 million nationwide (1). Cuttings root quickly and easily, which is a vital consideration. Equally important was the availability of cuttings, which were furnished free of charge by producers (Yoder Brothers, Inc. of Barberton, OH).

Fungicides Tested

Fungicides tested were those determined to be commonly used in chrysanthemum cutting production. Table 1 lists the references used to choose the fungicides tested and several of their important properties. All fungicides were applied at label recommended rates or according to previous research reports.

Intermittent Mist Studies

These treatments were designed to mimic commercial practices by rooting treated cuttings under intermittent mist in the greenhouse.

Table 1 - Fungicides Commonly Used on Chrysanthemums

FUNGICIDE	RATE	PATHOGENS CONTROLLED	REFERENCES
Banrot 40 WP	12 oz/100 gal.	Pythium, Phytophthora, Rhizoctonia, Fusarium, Thielaviopsis	7,8
Benlate 50 WP	16 oz/100 gal.	Botrytis, Rhizoctonia, Thielaviopsis	4,7,8,9, 17,20,25, 26
Botran 50 WP	16 oz/100 gal.	Botrytis	4,7
Captan 50 WP	64 oz/100 gal.	Pythium, Botrytis, Phytophthora	7,8,20, 25, 27
Daconil 75 WP	3/4 Tbsp./gal.	Botrytis	4,7,8,9
Lesan 35 WP	16 oz/100 gal.	Pythium, Phytophthora	7,8,18, 20,22
Maneb 80 WP	8 oz/100 gal.	Botrytis	7,9,25
Terraclor 75 WP	3/4 Tbsp./gal.	Rhizoctonia, Botrytis	7,17,20, 22
Thiram 65 WP	24 oz/100 gal.	Botrytis, Rhizoctonia, Pythium, Phytophthora	7,8,20
Truban 30 WP	10 oz/100 gal.	Pythium, Phytophthora, Rhizoctonia	7,8,22, 25
Daconil 75 WP +Terraclor 75 WP	3/4 Tbsp. each/ 100 gal.	Botrytis, Rhizoctonia	7,8

This approach is especially important since growth regulating effects might be affected by environmental conditions such as temperature.

Experiment 1

Experiment 1 was conducted from November 20 to December 16, 1983. It was a preliminary trial aimed at providing a preview of results for further experiments as well as testing experimental design.

Twenty uniform tip cuttings were used in each of 11 treatments for a total of 220 cuttings. The method of application consisted of a five-minute basal dip immediately prior to sticking. The five treated cuttings were placed in individual plastic flats, and each treatment was replicated in four flats to yield 20 total cuttings. The rooting medium was 1:1 (v/v) perlite:vermiculite. The four replicates of each treatment were spaced in a random manner on the mist bench. A four-hour night interruption (10pm-2am) with artificial tungsten lights was used to keep the cuttings in a vegetative state. The cuttings were kept under mist for 28 days. At the termination of the experiment, fresh and dry weights of both roots and shoots were recorded and a root quality rating determined. The quality rating was on a scale of 1 to 5, (where 1=very poor rooting and 5=good, even rooting), and was judged on the basis of root number, thickness, length, brittleness, and overall rooting uniformity. A fungal infection by Fusarium made it necessary to also rate each cutting for disease incidence, and the percent of each cutting which showed necrosis and disease symptoms was recorded.

Experiment 2

Experiment 2 was conducted from February 14 to March 6, 1984. The experimental design was modified to minimize several problems with the first experiment. To keep the fungicides from settling out of solution during treatment, cuttings were immersed and the solution agitated for five minutes. A soil drench of 1 cup of fungicide solution/flat was applied immediately after planting. A 1:2 (v/v) peat:vermiculite media was used to improve retention of the fungicides in the root zone. The media was also steam-sterilized twice to reduce disease incidence. Fungicide concentrations and method of planting were unchanged from Experiment 1.

Data was collected at 14 and 21 days. At the 14-day harvest, cuttings had less time to grow out of the response, so differences in rooting were much more apparent. The earlier termination also decreased disease problems compared to the 28-day termination in Experiment 1.

At 14 days, data was taken on two of the four flats. Root number and average and maximum root length were recorded and the cuttings were carefully replanted. At 21 days all flats were terminated. Root number, root length (average and maximum), and fresh and dry weights of both roots and tops were measured. A root quality rating was administered on a scale of 1 to 5. No disease rating was necessary, since no disease incidence was observed. All measurements

were made on each cutting so comparisons could be made with the preliminary harvest.

Tissue Culture Studies

In this approach, rooting studies were attempted in vitro on a sterile artificial nutrient medium. This would eliminate all pathological effects of the fungicides, showing only the actual growth regulating effects on the cutting.

Media Preparation

Methods were similar to those used by Earle and Langhans (5). Basal Murashige and Skoog medium was used, with additions of 0.04 mg/l thiamine HCl, 100 mg/l myo-inositol, and 30 g/l sucrose. Kelco polymer (1.5 g/l) was substituted for the Bacto-agar suggested. Three hormones were added:

0.02 mg/l naphthaleneacetic acid (NAA)

2.00 mg/l kinetin

10.0 mg/l gibberellic acid-3 (GA3).

This combination resulted in the best shoot proliferation during the Earle and Langhans (5) study.

The pH of the solution was adjusted to 5.7 before the Kelco polymer was added. The prepared medium was poured into test tube slants (20 ml/tube) and autoclaved at 121°C for 15 minutes.

Explant Preparation

Stock plants of Chrysanthemum morifolium Ramat 'Bright Golden Anne' were grown under greenhouse conditions for approximately four weeks. They were watered at the soil level to reduce contamination. Stem-tip explants were taken to minimize contamination and because apical cells are more likely to be uniform, rapidly-dividing, and free of viruses than those in older tissue (3).

No surface sterilization was used on the shoot tips. All but the youngest leaves were removed by hand. The leaf primordia surrounding the apical dome were removed with sterile tools under a dissecting microscope. The tip was then severed from the rest of the shoot, giving an explant 1.0 mm or slightly smaller in length. Apices were planted singly in the prepared tubes.

Cultural Conditions

Cultures were grown under fluorescent lighting at 64 uEm-2s-1 with a 16:8 (light:dark) photoperiod. The temperature remained constant at 27°C. The developing shoots were subcultured once by transferring multiple shoots to additional tubes for further proliferation.

All cultures were inadvertently disposed of by the lab technicians. Therefore, no fungicide trials were run on these cultures. New cultures were donated by Mr. Steve Lazarz of PanAmerican Plant, Bradenton, FL, but due to time limitations no results were available

at this writing. These materials and methods are included for future reference and studies.

RESULTS AND DISCUSSION

Experiment 1

Results from Experiment 1 are given in Table 2. Overall differences in rooting were erratic and in most instances appeared to result from disease incidence rather than fungicidal effects. No clear trends were noted.

Several problems occurred during Experiment 1 which caused inconclusive results. These include settling of the fungicides during treatment, leaching of fungicides from the media, desiccation of the cuttings as a result of uneven mist, and *Fusarium* infection. In addition, the cuttings were harvested too late and may have grown out of any possible response.

Experiment 2

Clear differences in rooting were observed in Experiment 2 at both 14 and 21 days (Table 3). Severe inhibition of root growth occurred in the Thiram, Captan, Botran, and Banrot treatments, while other treatments appeared to have little effect on rooting. Thiram (12) and Captan (2,12) were among those fungicides previously reported to be phytotoxic to *C. morifolium* cuttings.

At 14 days, root length was severely inhibited and number was slightly inhibited by some of the fungicides such as Thiram, Captan,

Table 2 - Effect of Fungicide Treatments on Adventitious Root Formation and Root Growth in *C. morifolium* at 28 Days[z]

FUNGICIDE	ROOT WT. (g) FRESH	DRY	TOP WT. (g) FRESH	DRY	QUALITY (1-5)	DISEASE (%)
Botran	0.81	0.07	4.69	0.67	2.8	10.0
Benlate	1.10	0.06	4.31	0.53	3.2	35.9
Captan	1.04	0.08	4.40	0.60	2.9	26.5
Dac+Terr	0.80	0.08	3.99	0.58	3.2	12.6
Truban	1.18	0.09	4.55	0.64	3.7	18.5
Maneb	1.05	0.09	4.66	0.69	3.8	24.0
Terraclor	0.98	0.10	4.82	0.79	3.8	12.0
Lesan	1.23	0.10	4.65	0.66	3.9	18.2
Thiram	1.11	0.11	5.21	0.80	4.2	11.4
Daconil	1.15	0.11	4.69	0.69	4.5	14.3
Control	1.09	0.12	4.89	0.76	4.9	7.6

[z]Comparison of the effect of 5-minute pre-plant basal dips with selected fungicides on chrysanthemum cuttings (Experiment 1)

Table 3 - Effect of Fungicide Treatments on Root Initiation and Growth in C. morifolium at 14 and 21 Days[z]

FUNGICIDE	14 DAYS LENGTH (mm)	14 DAYS NO.	21 DAYS LENGTH (mm)	21 DAYS NO.
Thiram	4.6	14.3	8.0	23.6
Captan	5.1	18.2	18.8	22.5
Botran	9.1	12.3	26.0	20.3
Banrot	14.1	16.0	44.5	21.5
Maneb	15.4	25.5	48.3	23.5
Benlate	16.6	20.1	43.8	23.4
Terraclor	19.1	20.5	47.3	23.4
Truban	19.2	23.4	44.3	23.4
Lesan	22.2	22.4	48.8	20.7
Daconil	22.4	27.1	52.8	25.2
Dac+Terr	24.7	26.6	53.0	27.0
Control	28.5	21.7	64.0	20.8

[z]Average root length and root number at 14 and 21 days on chrysanthemum cuttings treated with fungicides. Treatments consisted of a 5-minute soak plus a soil drench at planting. (Experiment 2)

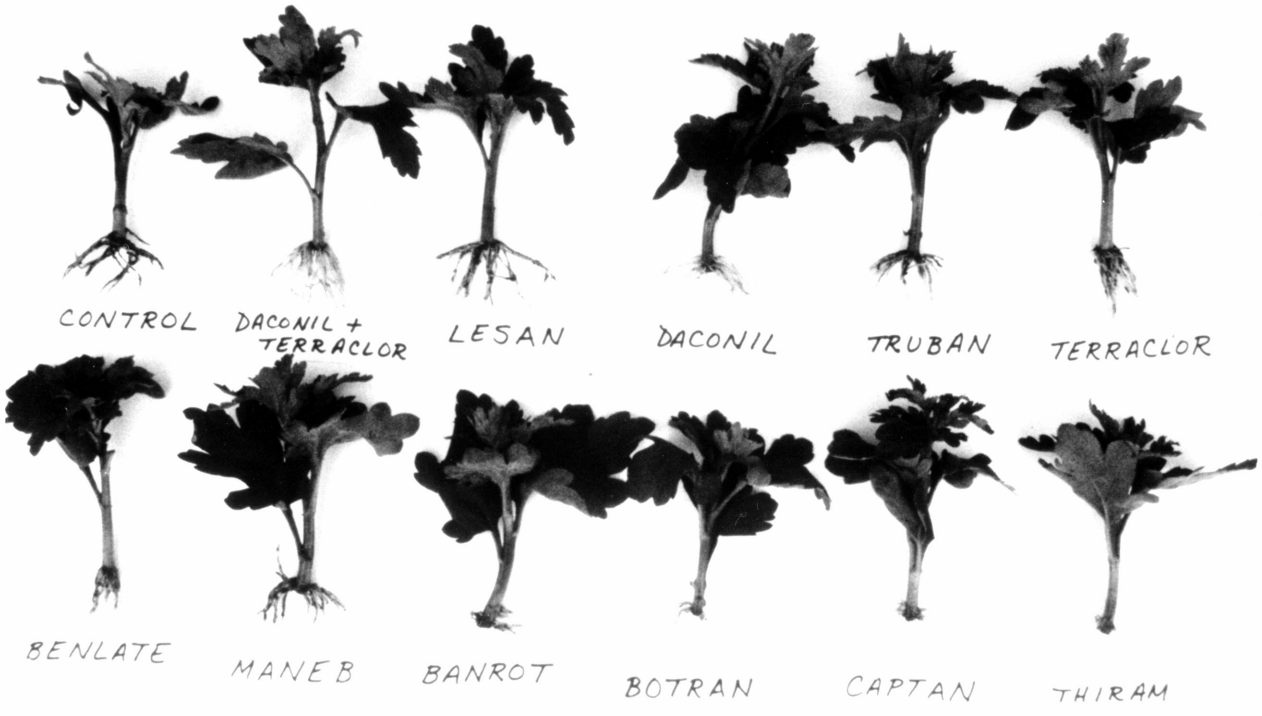

Figure 1. Root development of C. morifolium cuttings 14 days after treatment with various fungicides (Experiment 2).

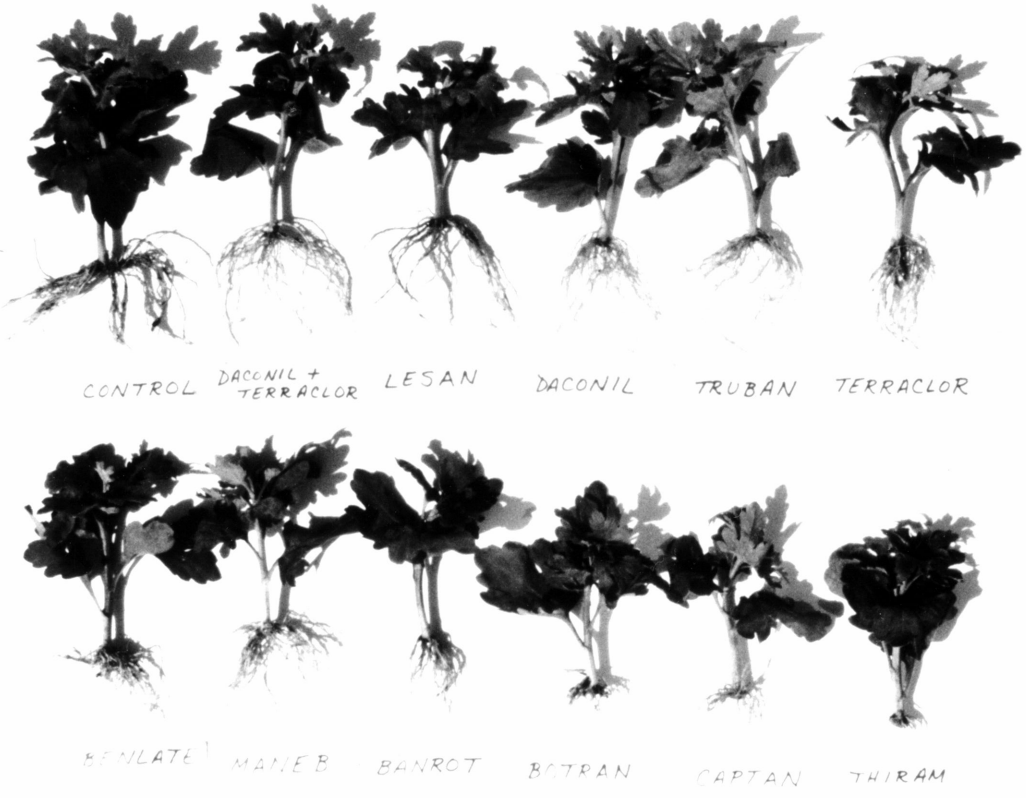

Figure 2. Root development of C. morifolium cuttings 21 days after treatment with various fungicides (Experiment 2).

Botran, and Banrot. At 21 days differences in root length were still apparent but not as dramatic, indicating that the cuttings were growing out of the inhibition. Root number was not significantly different from the controls, indicating complete recovery from the delayed root initiation observed at 14 days.

The primary effect was on root length as opposed to root number. Low root counts obtained at 14 days were no longer apparent at 21 days. However, differences in average root length remained at both 14- and 21-day harvests. Some treatments tended to grow out of the response over time.

This trend supports Arthur's (2) conclusion that some fungicides inhibit root elongation and maturation in chrysanthemum cuttings. Delayed initiation was suggested but not quantified. The root-promoting auxin activity noted by Hare (10) in rooting powders containing Captan is inconsistent with these results. Differences in concentration and method of application may be responsible for this discrepancy. Also, the apparent promotion of rooting in chrysanthemum cuttings demonstrated by Hare (10) occurred at levels below those recommended for fungal control. The enhancement of root initiation by Terraclor reported by Morgan and Colbaugh (23) did not occur in the present study. The inhibition found in this (Table 3) and other studies (2,12) may have resulted from excessive concentrations. Evidence noted by Lee (19) supports this hypothesis.

Root weights and quality ratings were reflective of the trends evidenced by root length (Table 4). Shoot growth was affected little

Table 4 - Effect of Fungicide Treatments on Root and Shoot Growth and Root Quality in C. morifolium at 21 Days[z]

FUNGICIDE	ROOT WT. FRESH	ROOT WT. DRY	ROOT QUALITY	SHOOT WT. FRESH	SHOOT WT. DRY
Thiram	0.17	0.10	1.1	3.51	0.51
Captan	0.27	0.05	1.5	3.58	0.50
Botran	0.39	0.04	2.0	3.49	0.47
Banrot	0.60	0.06	2.5	3.57	0.46
Maneb	0.81	0.08	3.2	3.80	0.50
Benlate	0.83	0.08	3.6	3.45	0.49
Terraclor	0.80	0.07	3.3	3.36	0.41
Truban	0.65	0.07	3.6	3.77	0.52
Lesan	0.84	0.07	3.9	2.86	0.36
Daconil	0.65	0.09	3.8	3.42	0.50
Dac+Terr	0.82	0.09	4.4	3.27	0.44
Control	1.12	0.10	5.0	3.56	0.44

[z]Average root weights (fresh and dry), root quality, and shoot weights (fresh and dry), of fungicide-treated chrysanthemum cuttings 21 days after sticking. Treatments consisted of a 5-minute soak plus a soil drench at planting. (Experiment 2)

by the treatments. This disagrees with results of previous studies (15,16,26) on other species. However, stunting of chrysanthemum may become evident in longer term studies, especially where rooting inhibition is severe and the cuttings do not have sufficient root systems to sustain continued growth.

Auxins may overcome inhibition of rooting when applied with some fungicides (10,14,24). Optimal combinations and concentrations should be determined. Maximum rooting plus effective disease control could thus be achieved and the propagation process improved. Higher quality cuttings and lower production costs would result.

Insufficient work has been done on fungicidal growth regulation. Tissue culture studies would be helpful in determining the physiological effects of fungicides on plants. Other areas, such as media effects on fungicide retention and disease activity (17,23), warrant investigation as well. Further studies will be conducted at a later date.

CONCLUSIONS

Some fungicides inhibited elongation and growth of adventitious roots when applied to Chrysanthemum morifolium cuttings. Thiram, Captan, Botran, and Banrot were found inhibitory in the present study. Root initiation, as measured by root number, was delayed by the above fungicides but was not permanently affected. Cuttings in several treatments tended to grow out of the response over time.

Fungicides should be chosen carefully and screened for possible phytotoxicity before using. Sensitivity to fungicidal growth regulation varies with plant species and other factors (12,19,21). The chemical, concentration, and application method used should be adjusted to minimize harmful effects on the plant.

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