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Growth responses of red pine seedlings to the chemical bioregulator, DCPTA

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Application. Growth of foliage and stems of red pine seedlings was modestly increased by applications of a chemical bioregulator, DCPTA. Further testing is needed with other tree species and treatment methods to realize the bioregulator's potential to increase forest productivity.

Abstract. The chemical bioregulator, 2-(3, 4-dichlorophenoxy)-triethylamine (DCPTA), which has markedly stimulated photosynthesis, carbon allocation, and above- and below-ground growth of several agricultural crops, caused small growth effects on red pine (*Pinus resinosa* L.) germinants and one and two-year-old seedlings, mainly to foliage and stems. At low DCPTA concentrations, i.e. 10 ppm, growth tended to be promoted, whereas at 100 ppm growth appeared to be suppressed. Continued testing of the bioregulator's effect on growth and physiology of other tree species is recommended.

Introduction

Traditional approaches to increase crop yields have sought to improve genetic potential of plant species or to enhance soil and site conditions. A new approach, that of manipulating plant biochemistry with chemical bioregulators, is being intensively studied by agricultural biochemists. Foliar applications of one such chemical bioregulator, DCPTA [2-(3, 4-dichlorophenoxy)-triethylamine], have resulted in substantial increases in productivity of soybean, *Glycine max* L. Merr., and cotton, *Gossypium hirsutum* L. (Gausman et al. 1984; Yokoyama et al. 1984). In field-plot studies with soybeans, foliar applications of 80 ppm DCPTA increased yields of seeds by 35% and plant protein and lipid concentrations by 68 and 20%, respectively (Yokoayama et al. 1984). In greenhouse studies with cotton, treatments of 12.5 to 1250 ppm DCPTA stimulated leaf and stem biomass by about 70% over controls and photosynthesis by 26% on a leaf unit-area basis (Gausman et al. 1984). Plant growth responses to DCPTA are attributed to alterations of plant enzymatic activities, specifically of enzymes that enhance the supply and use of photosynthetically fixed carbon. Root growth has been stimulated by DCPTA treatments, presumably in response to increased photosynthate availability within treated plants.

Despite the marked stimulation of plant productivity that can be induced by chemical bioregulators, notably few plant species have been tested. Effects of DCPTA have been documented with soybean, cotton, and guayule, *Parthenium argentatum* A. Gray (Yokoyama et al. 1977; Benedict et al. 1983), with apparently no tests with tree species. Currently, only soybeans have been tested under field conditions, with the remainder of the studies conducted in the greenhouse (Gausman et al. 1984). Plant age and chemical concentration of DCPTA appear to influence plant responses. Bioregulators such as DCPTA have had less effect on plant processes if applications are too concentrated or are made too late in plant development (Yokoyama et al. 1984).

Provided that DCPTA can also stimulate tree-seedling productivity, chemical bioregulators appear well suited for tree-nursery operations and forest regeneration. Based on agronomic experience, DCPTA treatments could potentially improve early seedling growth, root development, and rate of plantation establishment. In young stands, for example, competitive advantage might be weighted more in favor of desired tree seedlings over other plant species. Herbicide requirements might be reduced, and tree seedlings might be more easily established on more productive sites where competition can limit successful plantation establishment. Significant ecologic and physiologic questions are raised by the use of a chemical bioregulators in forest management. However, chemical bioregulators deserve research attention because they offer a new approach for increasing forest productivity.

The objective of this study was to demonstrate growth responses of red pine (*Pinus resinosa* L.) seedlings to DCPTA, data that are necessary before proceeding with more broad-scale study of the potential role of chemical bioregulators in forestry. Greenhouse experiments were conducted to evaluate effect of DCPTA on height growth and biomass of foliage, stems, and roots. Three experiments evaluated growth effects of DCPTA applied at different concentrations on seedlings of three different ages. A fourth experiment was conducted in a commercial tree nursery (Mead Paper Corporation, Escanaba, Michigan) to evaluate effects of the bioregulator under operational conditions. Germination tests were also conducted to determine effects of DCPTA on germination rate of red pine seed.

Methods

Experiment 1: Newly germinated seedlings

To determine effects of DCPTA treatments on newly germinated seedlings, seeds from an open seed collection made in Michigan (Mich. Dept. of Natural Resources Lot 0006, 1979) were germinated in artificial growth medium (peat, perlite, and vermiculite at 3:2:1 by volume, respectively). Styroblocks with 240 individual planting holes (each 41 cm^3) were used to germinate and grow seedlings. Seedlings were treated with 0, 10, or 100 ppm DCPTA solution with a hand aspirator 4, 5, and 6 weeks after planting. Each treatment supplied about 2 ml of DCPTA solution per seedling. All DCPTA solutions (including controls) contained Ortho X-77 surfactant at 0.01% by volume. There were 96 seedlings per treatment.

Seedlings were irrigated regularly with deionized water to minimize water stress. Fertilization was periodically applied with Peter's 20-20-20, plus micronutrients, in solutions of 150 ppm N. Seedlings were not irrigated for several days following foliar treatments with DCPTA, to minimize leaching of the chemical from foliage. Supplementary light from incandescent lamps increased photoperiod to 15 h per day. Height of all seedlings (288) was measured periodically, and one-half the seedlings treated with each DCPTA solution (48) were harvested 10 weeks after planting. At harvest, tops were divided from roots, and roots were washed free of growth medium with deionized water. Plant parts were oven dried at 65°C for 48 h and weighed. A one-way ANOVA was calculated with DCPTA concentration as the main treatment effect. When an ANOVA's F-statistic was significant, Scheffe's test was used to determine significant differences between means.

Experiment 2: One-year old seedlings

Effects of DCPTA were evaluated with one-year old seedlings that had been grown initially in Styroblock containers at the Mead Paper Nursery in Escanaba, Michigan. The seed source (Mead Lot 10) was Kalkaska County in northern lower Michigan. Prior to potting, initial height above the first lateral root was measured and used to grade seedlings. Extremes in height were discarded. Initial height data were also used as covariates in analysis of covariance (ANACOV) that tested growth responses to DCPTA.

Seedlings were replanted in 0.75-l pots that contained peat, perlite, vermiculite, and calcined clay chips (by volume 6:4:2:1, respectively). Seedlings were treated twice with 0, 10, or 100 ppm DCPTA. DCPTA solutions were sprayed on roots while potting, and a second treatment was sprayed on foliage four weeks after potting, shortly after new shoots and needles had emerged. On both occasions, about 4 ml of DCPTA solution was applied to each plant. There were 27 seedlings per treatment.

Methods for irrigation, fertilization, and lighting were similar to those described in Experiment 1. Seedlings were grown for 12 weeks prior to harvest. At harvest, seedling height was measured, roots were washed free of growth media, and seedlings were subdivided into foliage, aboveground stems, and roots. Plant parts were oven dried at 65°C for 48 h and weighed. A one-way ANACOV with initial height as covariate was calculated with DCPTA concentration as the main treatment effect.

Experiment 3: Two-year old seedlings

Effects of DCPTA were evaluated with two-year old bare-root seedlings that had been grown at the US Forest Service Toumey Tree Nursery in Watersmeet, Michigan. Growing conditions for Experiment 3 were similar to those in Experiment 2 with the following exceptions. The seed source [USFS Lot 0425-2-OTT(5)] for the two-year old, bare root stock was the Ottawa National Forest in western Upper Michigan. Pots contained 1.5 l of growth medium, and seedlings were treated with 5 ml of DCPTA solutions prior to potting and four weeks after potting. In addition to initial seedling height, initial seedling fresh weight was obtained and used to grade seedlings. Both initial height and weight were subsequently used as covariates in ANACOV to test the significance of DCPTA effects.

Experiment 4: Commercially grown seedlings

To evaluate effects of DCPTA on red pine germinants under commercial tree-nursery conditions, red pine germinants were treated with DCPTA and grown under operational conditions at the Mead Paper Inc. Nursery in Escanaba. A total of about 6500 seedlings were grown in Styroblocks such as those used in Experiment 1. Foliage of seedlings was sprayed only once with 0, 10, or 100 ppm DCPTA at 20, 40, or 60 days after planting. Thirty seedlings in each of the 9 treatment combinations (3 DCPTA concentrations X 3 seedling ages at DCPTA application) were randomly selected for height growth measurement 17 and 23 weeks after planting. After 23 weeks of growth, seedlings were harvested. Tops were divided from roots, and plant parts oven dried and weighed.

Experiment 5: Seed germination

To test effects of DCPTA on germination rate of red pine seeds, seeds from the same red pine lot as that used in Experiment 1 were germinated on Whatman 42 filter paper in petri dishes. Filter papers were saturated with 0, 10, or 100 ppm DCPTA solution. For each DCPTA treatment, four replicates of 50 seeds each were germinated in a dark plant-growth chamber held at 25°C. Fresh DCPTA solution was applied every other day, until about 95% of the seeds in the petri dish germinated. Germination, scored when geotropism was expressed by emerging radicles, was observed daily.

Results

Experiment 1: Newly germinated seedlings

Ten weeks after planting, tops of newly germinated seedlings treated with 10 ppm DCPTA averaged about 5.5 mg larger than controls, about a 9% difference (Table 1). Top weight was significantly enhanced by 10 ppm DCPTA as indicated by results of one-way ANOVA and Scheffe's multiple comparison tests. No significant differences were detected in seedling height or root weight that were attributable to DCPTA. Seedling height growth peaked at about 54 mm, irrespective of treatment, at about 13 weeks after planting. Tests of normality and equality of variances indicated that a one-way ANOVA was well suited for analyses of these data.

Table 1. Mean growth response of 10-wk old red pine seedlings to treatments with DCPTA.[1]

DCPTA	Height	Top weight	Root weight	Top/root ratio
(ppm)	(mm)	(mg)	(mg)	
0	50.4 (0.56)	62.6 (1.5)a	19.9 (0.92)	3.2
10	52.0 (0.50)	68.1 (1.6)b	22.0 (0.95)	3.1
100	51.3 (0.60)	65.1 (1.5)ab	21.4 (0.92)	3.0
F-statistic	1.96	3.05	1.28	
Significance	0.14	0.05	0.28	

[1] Standard errors of means are in parentheses. F-statistic of one-way ANOVA results are given along with its significance. Letters following mean and standard errors indicate results of Scheffe's multiple comparison test.

Experiment 2: One-year old seedlings

Results of covariance analysis indicated significant effects from DCPTA treatments on one-year old seedlings (Table 2). Based on mean growth response (adjusted by covariance analysis), seedlings treated with 10 ppm DCPTA averaged about 7% greater stem weight than controls and about 12% greater than those treated with 100 ppm. No differences in seedling height or needle weight were attributable to DCPTA treatments.

Initial height was not correlated with root weight after 12 weeks of growth (Table 2), although initial seedling height was significantly related to initial rootweight. (A sample of 20 seedlings gave a regression of $y = -0.171 + 0.017x$, $r = 0.58$, where y was initial root weight and x was initial height). Instead of ANACOV, a one-way ANOVA was used to test effects of DCPTA on seedling root growth. The ANOVA was significant, and had an F-statistic of 5.76 which is significant at the 0.01 probability level. The conservative Scheffe's test, however, did not indicate that mean root weight was significantly different among the three treatments (Table 2).

The covariate of initial height of seedlings was significantly correlated with subsequent height and stem and needle weight (Table 2). With the exception of root weight, the covariates of initial heights improved substantially the precision of these ANACOV models. Tests of normality and equality of variances indicated that the ANACOV was well suited for these data.

Table 2. Adjusted mean growth responses of one-year old red pine seedlings to treatments with DCPTA[1]. Means adjusted by ANACOV, except for root weight with which the covariate was not significantly correlated.

DCPTA	Height	Stem weight	Needle weight	Root weight	Top/root ratio
(ppm)	(cm)	(mg)	(mg)	(mg)	
0	16.1 (0.20)	587 (19)	1874 (69)	865 (41)	2.8
10	16.3 (0.21)	630 (19)	1949 (70)	969 (47)	2.6
100	15.7 (0.20)	564 (19)	1850 (69)	767 (33)	3.2
Significance of F-statistic	0.09	0.05	0.60	0.005	
Corr.Coef.[2]	0.75	0.49	0.22	0.20	

[1] Standard error of means in parentheses.

[2] Correlation coefficient of initial height and subsequent measures of growth is significant at 0.05 if the coefficient equals or exceeds 0.22.

Experiment 3: Two-year old seedlings

DCPTA caused no detectable differences in growth of two-year old seedlings according to ANACOV. Covariates (pretreatment height or weight) substantially improved the precision of ANACOV (Table 3). Tests of normality and equality of variances indicated that the ANACOV model was appropriate for these data.

Table 3. Adjusted mean growth response of two-year old red pine seedlings to treatments with DCPTA. Means adjusted by ANACOV.[1]

DCPTA	Height	Stem weight	Needle weight	Root weight	Top/root ratio
(ppm)	(cm)	(g)	(g)	(g)	
0	22.7 (0.61)	1.98 (0.06)	6.59 (0.18)	1.83 (0.06)	4.7
10	23.8 (0.63)	1.84 (0.06)	6.49 (0.18)	1.91 (0.06)	4.3
100	24.1 (0.60)	1.90 (0.06)	6.48 (0.17)	1.94 (0.06)	4.3
Initial height					
Corr. Coef.[2]	0.85	0.76	0.61	0.54	
Initial weight					
Corr.Coef.[2]	0.73	0.91	0.93	0.91	

[1] Standard error of mean in parentheses.
[2] Correlation coefficient significant at 0.22 ($p<0.05$).

Experiment 4: Commercially grown seedlings

Height and weight of commercially grown seedlings treated with DCPTA were not altered by DCPTA treatments. Height growth of treated seedlings over controls was modest (about a 5% gain). A subsample of the treated seedlings have been outplanted to monitor field performance in response to DCPTA treatments.

Experiment 5: Seed germination

DCPTA at 10 or 100 ppm did not greatly alter germination rate or total germination of red pine seeds. Seeds of all DCPTA treatments began germinating 5 d following seed set (Table 4). Fifty percent of the seeds in all treatments germinated after 6 or 7 d, and total germination equalled or exceeded 97% by 10 d in all treatments.

Table 4. Percent real germination of red pine seeds as affected by DCPTA solutions.[1]

Days after seed set	DCPTA concentration (ppm)		
	0	10	100
	(% germination)		
4	0	0	0
5	10.0	11.0	14.0
6	41.5	44.5	52.5
7	77.5	79.5	83.0
8	90.5	93.0	93.5
9	95.5	96.5	98.5
10	97.0	97.5	99.0

[1] Sample of 200 seeds per treatment.

Discussion and conclusion

The biological significance of the effects of DCPTA on red pine seedlings is unknown. Growth increases were occasionally detected on germinants and one-year-old seedlings treated with 10 ppm DCPTA, whereas some negative effects appeared to result from 100 ppm DCPTA. Similar positive and negative effects at low and high concentrations, respectively, have been observed with agricultural crops (Yokoyama 1985). The most significant result of these studies was that effects of DCPTA on these pine seedlings were very modest compared with the growth effects found with agricultural crops (Gausman et al. 1984; Yokoyama et al. 1984).

There are serveral possible explanations for the relatively minor effects of DCPTA on pine seedlings. Although the chemical was applied with a surfactant, Ortho X-77, it may not have penetrated through the waxy needles. Red pine is also a determinant growing species. Effects of environmental factors on growth of this species are not always expressed during the current growing season. Bud development and subsequent height growth may have been altered by DCPTA treatments, but this possibility was not tested. In contrast, however, foliage biomass of this species is sensitive to environmental conditions such as drought stress, or N availability during the current growing season. Foliage biomass (i.e. top biomass) was, however, enhanced by the treatments applied in only one of the experiments (Experiment 1, Table 1).

The chemical bioregulator deserves testing with tree species, such as loblolly pine, larch, or cottonwood, that have indeterminant growth characteristics. The effects of DCPTA are currently being tested with larch seedlings by this laboratory.

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References

Benedict, C.R., Reibach, P.H., Madhavan, S., Stipanovic, R.V., Keithly, J.H. & Yokoyama, H. 1983. Effects of 2-3, 4-dichlorophenoxy-triethylamine on the synthesis of cis-polysoprene in guayule plants (*Parthenium argentatum* Gray). Plant. Physiol. 72:897-899.

Gausman, H.W., Burd, J.D., Quisenberry, J.E., Yokoyama, H., Dilbeck, R. & Benedict, C.R. 1984. Effects of 2-diethylaminoethyl-3, 4-dichlorophenylether (DCPTA) on cotton plant (*Gossypium hirsutum* L.) growth and phenology. (Unpublished manuscript, Texas A&M University, College Station).

Kramer, P.J. & Kozlowski, T.T. 1979. Physiology of Woody Plants. Academic Press, New York.

Yokoyama, H., DeBenedict, C., Hsu, W.J. & Hayman. E. 1984. Bioregulation of lipid and protein synthesis in soybean by 2-diethylaminoethyl-3, 4-dichlorophenylether. Biotechnol. 4:712-714.

Yokoyama, H., Hayman, E.P., Hsu, W.J., Poling, S.M. & Bauman, A.J. 1977. Chemical bioinduction of rubber in guayule plant. Science 197: 1076-1078.