

Effect of 2-(3,4-Dichlorophenoxy)triethylamine on Tomato *Lycopersicon esculentum* Cv. UCD-82

Wan-Jean Hsu* and Henry Yokoyama

Fruit and Vegetable Chemistry Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Pasadena, California 91106

The application of chemical bioregulator 2-(3,4-dichlorophenoxy)triethylamine (DCPTA) to tomato (*Lycopersicon esculentum* cv. UCD-82) plants resulted in increased fruit yield and plant biomass and better fruit quality. Plants were treated either by seed imbibition or by a foliar application at the three-leaf stage with 5, 10, 20, and 50 ppm of DCPTA solutions in 0.01% Tween 80 only once and allowed to grow to maturity. DCPTA was found to be effective in a concentration level as low as 5 ppm. However, 10 ppm seems to be the optimum effective concentration. At this concentration, DCPTA caused an 86-89% increase in fruit yield, and 16%, 11%, and 30% increases in leaf, stem, and root biomass, respectively. No large changes in pH and total soluble solids were noted in treated fruits. A 0.08% decrease in total titratable acid, an 11% increase in sugar content, a 100% increase in fruit setting, and a 28% increase in main tomato pigment, lycopene, were found in DCPTA (10 ppm) treated fruits.

INTRODUCTION

Chemical bioregulator 2-(3,4-dichlorophenoxy)triethylamine (DCPTA) was first demonstrated to cause stimulation of *cis*-polyisoprene (rubber) synthesis in both greenhouse-grown and field-grown guayule plants (*Parthenium argentatum* gray var. 593) (Yokoyama et al., 1977; Hayman et al., 1983). Later DCPTA was found to increase lipid and protein contents and yield of soybean seed (*Glycine max* L. Merr.) (Yokoyama et al., 1984) and to increase the essential-oil contents in lemon (*Citrus limon* cv. Eureka) (Yokoyama et al., 1986). The enhancement effect of DCPTA upon photosynthesis in detached cotton leaf disks (*Gossypium hirsutum* L.) (Gausman et al., 1984, 1985) and in green algae *Euglena gracilis* Z (Hsu and Yokoyama, 1986) seems to partially explain the effect of DCPTA on the synthesis of unrelated constituents in different plant tissues. To further demonstrate the involvement of DCPTA in photosynthesis by causing increases in the supply and utilization of the carbon atom, DCPTA was applied to other crop plants. In this study we investigate the influence of DCPTA on various aspects of the growth, fruit yield, and fruit quality of tomato plants.

MATERIALS AND METHODS

Plant Materials. Tomato (*Lycopersicon esculentum* cv. UCD-82) plants were individually grown in commercial potting mix (Supersoil) in 2-gal plastic pots and fertilized every 2 weeks with Gro-More brand 20-20-20 soluble fertilizer in a greenhouse. The temperature of the greenhouse was maintained at 28 ± 4 °C day and 18 ± 2 °C night. Plants were grown under 1200-1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ illumination provided by metal halide lamps for a daily photoperiod of 14 h. Each DCPTA treatment group consisted of 10 plants, and all plants were randomly arranged. At the end of the experiment, four randomly selected replicate plants from each group were harvested.

Chemical Treatment. Tomato plants were treated with solutions of DCPTA in 0.01% Tween 80, either by seed imbibition for 16 h before planting or by foliar application at the three-leaf stage.

Chemical. DCPTA was synthesized according to the published method (Schuetz and Baldwin, 1958).

Pigment Analysis. Carotenoid pigments of mature tomato fruits were extracted with acetone and transferred to light

Table I. Effect of 2-(3,4-Dichlorophenoxy)triethylamine on Fruit Yield of Tomato *L. esculentum* Cv. UCD-82^a

treatment	fruit yield, g fresh wt./plant	% increase
Experiment 1 (Plants Treated by Foliar Application at Three-Leaf Stage)		
control	480.32 ^b	-
DCPTA, 5 ppm	735.24 ****	53.07 (31.95-79.25) ^c
DCPTA, 10 ppm	892.00 ***	85.71 (61.48-116.07)
DCPTA, 20 ppm	829.80 ***	72.76 (49.78-101.44)
DCPTA, 50 ppm	540.90 NS	12.61 (-4.98 to 33.93)
ANOVA ^d	***	
linear ^d	NS	
quadratic ^d	***	
Experiment 2 (Plants Treated by Seed Imbibition for 16 h Prior to Planting)		
control	407.40 ^b	-
DCPTA, 5 ppm	545.00 ****	33.78 (11.65-61.86) ^c
DCPTA, 10 ppm	770.10 ***	89.00 (61.04-125.43)
DCPTA, 20 ppm	358.40 NS	-12.00 (-30.04 to 9.91)
DCPTA, 50 ppm	320.60 *	-21.30 (-38.62 to -0.48)
ANOVA ^d	***	
linear ^d	*	
quadratic ^d	*	

^a Each recorded value represents the mean of four samples. ^b Data were subjected to general linear model T test for the null hypothesis against control. ^c Data were treated according to Fieller's theorem at 95% confidence level. Numbers in parentheses are the estimated lower limit and upper limit of percent changes. ^d Data were subjected to ANOVA, linear, and polynomial regression analyses. * NS, *, **, ***, nonsignificant, significant at 5%, 1%, and <1% levels, respectively.

petroleum ether. Lycopene content was quantified by using $E_{1\text{cm}}^{1\%} = 3050$ at $A_{502\text{nm}}$ (Benedict et al., 1985).

Brix Analysis. Total soluble solids (percent), expressed as Brix of tomato fruit, was determined according to the AOAC method for fresh fruit (Horowitz, 1980a).

Sugar Analysis. Sugars in the total soluble solids extracts were separated, determined, and quantified by an HPLC method similar to the AOAC method for sugars in honey (Horowitz, 1980b). A Shimadzu E-6 high-performance liquid chromatograph equipped with a Whatman Partisil 10 analytical carbohydrate column and Micromeritics 771 refractive index detector was used in this study. The mobile phase consisted of aceto-

Table II. Effect of 2-(3,4-Dichlorophenoxy)triethylamine on Growth of Tomato *L. esculentum* Cv. UCD-82^a

treatment	leaf dry wt, g/plant	stem dry wt, g/plant	root dry wt, g/plant	height, cm	no. of flower clusters/plant	no. of fruits/plant
control	67.52 ^b	94.48 ^b	12.20 ^b	40.17 ^b	65 ^b	13 ^b
DCPTA, 5 ppm	70.86 NS ^d	98.63 NS	12.89 NS	41.00 NS	52 ***	24 ***
DCPTA, 10 ppm	78.28 *	105.27 *	15.86 ***	40.67 NS	49 ***	26 ***
DCPTA, 20 ppm	66.53 NS	98.53 NS	12.82 NS	38.00 NS	50 ***	24 ***
DCPTA, 50 ppm	64.70 NS	92.97 NS	11.85 NS	38.33 NS	56 *	13 NS
ANOVA ^c	NS	NS	***	NS	***	***
linear ^c	NS	NS	NS	NS	NS	NS
quadratic ^c	NS	NS	NS	NS	***	***

^a Plant samples were from plants treated by foliar application at the three-leaf stage. Each recorded value represents the mean of four samples. ^b Data were subjected to general linear model T test for the null hypothesis against control. ^c Data were subjected to ANOVA, linear, and polynomial regression analyses. ^d NS, *, **, ***, nonsignificant, significant at the 5%, 1%, and <1% levels, respectively.

Table III. Effect of 2-(3,4-Dichlorophenoxy)triethylamine on Fruit Quality of Tomato *L. esculentum* Cv. UCD-82^a

treatment	Brix (20 °C)	pH	total titratable acid, %	glucose, % fresh wt	fructose, % fresh wt	lycopene, μg/g dry wt
control	5.45 ^b	4.24 ^b	0.36 ^b	1.73 ^b	1.85 ^b	1454 ^b
DCPTA, 5 ppm	5.55 NS ^d	4.09 *	0.36 NS	1.79 *	1.89 NS	1833 ***
DCPTA, 10 ppm	5.60 *	4.09 *	0.28 ***	1.83 **	1.96 **	1857 ***
DCPTA, 20 ppm	5.50 NS	4.16 NS	0.34 *	1.78 *	1.88 NS	1593 *
DCPTA, 50 ppm	5.55 NS	4.11 NS	0.36 NS	1.79 *	1.89 NS	1134 ***
ANOVA ^c	NS	NS	***	***	***	***
linear ^c	NS	NS	NS	NS	NS	***
quadratic ^c	NS	NS	NS	NS	NS	***

^a Fruit samples were from plants treated by foliar application at the three-leaf stage. Each recorded value represents the mean of four determinations. ^b Data were subjected to general linear model T test for the null hypothesis against control. ^c Data were subjected to ANOVA, linear, and polynomial regression analyses. ^d NS, *, **, ***, nonsignificant, significant at 5%, 1%, and <1% levels, respectively.

nitrile/water/1% NH₄OH (80:20:1 v/v) with a flow rate of 2.0 mL/min. Total soluble solids extracts of tomato fruit were first filtered through a MeOH-conditioned Water C₁₈Sep-Pak followed by filtration with a 0.45-μm filter prior to HPLC sugar analysis.

Titrateable Acidity. Total titrateable acidity was determined according to the published method (Dalal et al., 1965).

Biomass Determination. All plants were harvested 95 days after bioregulator treatment. Thus, the plants treated with foliar application were 2 weeks older than the ones treated with seed imbibition. After the tomato fruits were harvested, plants were separated into leaf, stem, and root. Dry weight of plant samples were determined after drying at 50 °C to a constant weight.

Statistical Analysis. Analysis of variance (ANOVA) and Dunnett's test for comparing the mean of control to the mean of each treated group were performed for all the data obtained in this study; the data were also subjected to linear and polynomial regression analyses to find the best fit model for the data (Zar, 1974). Data of fruit yield in Table I were also subjected to analysis based on Fieller's theorem (Zerbe, 1978).

RESULTS AND DISCUSSION

When tomato plants were treated with DCPTA, fruit yield increased (Table I). Increases as high as 86% and 89%, respectively, were obtained in plants treated with 10 ppm DCPTA applied either by foliar application or by seed imbibition. There seems to be a concentration effect in both treatment methods; 10 ppm seems to be the optimum concentration level. The effect of DCPTA increased as the concentration increased from 5 to 10 ppm and then decreased as the concentration increased further. A negative effect was observed in fruit yield of plants whose seeds were imbibed with 20 and 50 ppm of DCPTA. This phenomenon could be due to a prolonged seed imbibition period that resulted in a toxic concentration of DCPTA. It has been shown that DCPTA increased the total activity of key enzymes in isoprenoid biosynthetic pathways such as mevalonic acid kinase and isopentenyl pyrophosphate isomerase (Benedict et al., 1983). It was also observed that at low concentration levels DCPTA stimulated both the β-carotene and lycopene biosynthesis in *Phycomyces*

cultures, while at high concentrations DCPTA stimulated lycopene biosynthesis only (unpublished data). The above observations demonstrated that the mode of action of DCPTA is similar to that of the tetraterpenoid bioregulators (Hsu et al., 1972). DCPTA can exert direct inhibitory effect at higher concentrations in addition to its stimulatory effect. The small negative responses in seed treatment at higher concentrations (20 and 50 ppm) of DCPTA is probably due to the inhibitory effect overwhelming the stimulatory effect.

Biomass increases in leaf, stem, and root were observed in DCPTA-treated plants (Table II). Again, 10 ppm was the optimum effective concentration. Increases of 16%, 11%, and 30% in dry weight were found in leaf, stem, and root, respectively. Plant height was not affected by DCPTA. Total flower cluster initiation decreased in DCPTA-treated plants. However, total fruit numbers were doubled in plants treated with 5, 10, and 20 ppm DCPTA (Table II). This observation showed that low concentrations of DCPTA increased the fruit setting in tomato plants, which accounted for the increase of fruit yield.

During the growth of tomato plants, fruits of the plants treated with low concentrations of DCPTA turned red about 2 weeks earlier than fruits of control plants. The main tomato pigment (lycopene) of mature fruits increased by 26–28% in both 5 and 10 ppm DCPTA treated fruits (Table III). Increases in the total activity of mevalonic acid kinase and isopentenyl pyrophosphate isomerase involved in the tetraterpenoid biosynthetic pathway by DCPTA (Benedict et al., 1983) could account for the increase of lycopene content in treated fruits. During the development of tomato fruit, total titrateable acidity increased as the fruit passed from green-mature stage to pink but declined slightly as the fruit turned red (Dalal et al., 1965). The decrease in total titrateable acidity in fruits treated with 10 ppm DCPTA at harvest indicated that these fruits might be more mature than the control ones. No large changes in pH and total soluble solids were noted in treated fruits. The pH was about 0.1 unit

lower in treated fruits. The highest increase in soluble solids was 0.15 Brix unit found in fruits treated with 10 ppm DCPTA. Tomatoes are classified as acid fruits because their soluble solids are composed chiefly of organic acids and sugar. Total titratable acidity was attributed to citric acid, malic acid, and other organic acids. The simultaneous decrease in total titratable acidity and increase in Brix (Table III) indicated that there might be an increase in sugar content in tomato fruits from plants treated with 10 ppm of DCPTA. In the sugar analysis of the tomato fruit, glucose and fructose were found to be the major sugars in the ripe UCD-82 tomato fruits. Slight increases of glucose and fructose content (5% and 6%, respectively) were observed in fruits treated with 10 ppm of DCPTA as compared to control fruits (Table III).

The increase in biomass of plant, fruit yield, fruit pigment, and total soluble solids in DCPTA-treated tomato plants could be due to the increase of photosynthetic efficiency in treated plants. In this study, DCPTA was applied to the plant at a very early stage of plant development. DCPTA exerted its effect during the whole 95-day growth period and caused the above-mentioned cumulative effects at a much later stage of plant development. This observation clearly indicated that under a short period of influence by DCPTA the plants were stimulated and more efficient photosynthesis was expressed. Further study is needed to determine which photosystem was affected by DCPTA.

ACKNOWLEDGMENT

We thank Ms Linda C. Whitehand for her assistance on statistical analyses of the experimental data.

LITERATURE CITED

- AOAC. *Official Methods of Analysis*, 13th ed.; Horowitz, W., Ed.; Association of Official Analytical Chemists: Washington, DC, 1980a; p 363.
- AOAC. *Official Methods of Analysis*, 13th ed.; Horowitz, W., Ed.; Association of Official Analytical Chemists: Washington, DC, 1980b; p 525.
- Benedict, C. R.; Reibach, P. H.; Madhavan, S.; Stipvanovic, R. V.; Keithly, J. H.; Yokoyama, H. Effect of 2-(3,4-dichlorophenoxy)triethylamine on synthesis of cis-polyisoprene in guayule plants (*Parthenium argentatum* Gray). *Plant Physiol.* 1983, 72, 897-899.
- Benedict, C. R.; Rosenfield, C. L.; Mahan, J. R.; Madhavan, S.; Yokoyama, H. The chemical regulation of carotenoid biosynthesis in citrus. *Plant Sci.* 1985, 41, 169-173.

- Dalal, K. B.; Salunkhe, D. K.; Boe, A. A.; Olson, L. E. Certain physiological and biochemical changes in developing tomato fruit (*Lycopersicon esculentum* Mill). *J. Food Sci.* 1965, 30, 504-508.
- Gausman, H. W.; Yokoyama, H.; Quisenberry, J. E.; Burd, J. D.; Wendt, C. W. Cotton leaf disc photosynthesis increased by DCPTA. *Plant Physiol. Suppl.* 1984, 72, 5.
- Gausman, H. W.; Burd, J. D.; Quisenberry, J.; Yokoyama, H.; Dilbeck, R.; Benedict, C. R. Effect of 2-diethylaminoethyl-3,4-dichlorophenylether (DCPTA) on cotton plant (*Gossypium hirsutum*) growth and phenology. *Bio/Technology* 1985, 3, 255-257.
- Hayman, E.; Yokoyama, H.; Gold, S. Effect of bioregulators on the accumulation of rubber in guayule. *J. Agric. Food Chem.* 1983, 31, 1120-1121.
- Hsu, W.-J.; Yokoyama, H.; Coggin, C. W. Carotenoid biosynthesis in *Blakeslea trispora*. *Phytochemistry* 1972, 11, 2985-2990.
- Hsu, W.-J.; Yokoyama, H. Effect of 2-(3,4-dichlorophenoxy)-triethylamine (DCPTA) on the photosynthetic capacity of *Euglena gracilis* Z. *Abstracts of Papers*, 192nd National Meeting of the American Chemical Society, Anaheim, CA; American Chemical Society: Washington, DC, 1986; Abstract AGFD 45.
- Schuetz, R. D.; Baldwin, R. A. The synthesis and properties of some substituted phenyl- ω -(N,N-dialkylamine)-alkylsulfides. *J. Am. Chem. Soc.* 1958, 80, 162-164.
- Yokoyama, H.; Hayman, E. P.; Hsu, W.-J.; Poling, S. M. Chemical bioinduction of rubber in guayule plant. *Science* 1977, 197, 1076-1077.
- Yokoyama, H.; DeBenedict, C.; Hsu, W.-J.; Hayman, E. Bioregulation of lipid and protein synthesis in soybean by 2-diethylaminoethyl-3,4-dichlorophenylether. *Bio/Technology* 1984, 2, 712-714.
- Yokoyama, H.; Gold, S.; DeBenedict, C.; Carter, B. Bioregulation of essential oils of lemon. *Food Technol.* 1986, 40, 111-113.
- Zar, J. H. *Biostatistical Analysis*; Prentice-Hall: Englewood Cliffs, NJ, 1974.
- Zerbe, G. O. On Fieller's theorem and general linear model. *Am. Stat.* 1978, 32, 103-106.

Received for review May 1, 1990. Accepted July 5, 1990. Reference to a company name or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Registry No. DCPTA, 65202-07-5; lycopene, 502-65-8.