Enhanced Germination Rates and Growth of DCPTA-treated Guayule Seed

Ernest P. Hayman and Henry Yokoyama

U.S. Department of Agriculture, Agricultural Research Service, Fruit & Vegetable Chemistry Laboratory, 263 S. Chester Ave., Pasadena, CA 91106

Additional index words. *Parthenium argentatum*, woody plants, growth substances, seed germination

Abstract. A 26- μ M DCPTA seed treatment for guayule (*Parthenium argentatum* Gray) was developed. We obtained a 23% increase in germination rate as measured by the coefficient of velocity (C.V.). Greenhouse seedlings grown from DCPTA-treated seeds exhibited a 22% increase in dry weight and a 95% increase in survival rate under water-limiting conditions. In field-grown guayule, multiple foliar applications of 8.2 mM DCPTA increased the total amount of rubber in roots as much as 58% without affecting the rubber content in the stems. Chemical name used: 2-Diethylaminoethyl-3,4-Dichlorophenylether (DCFTA).

Guayule is a woody perennial shrub that produces *cis*-polyisoprene natural rubber. Natural rubber is required for radial tires and other applications. In those countries where semiarid growing conditions predominate, guayule commercialization could provide a domestic alternative to rubber obtained from tropically grown *Hevea brasiliensis* Muell. Arg. To be commercially practical, guayule cultural practices require either a decrease in production costs, as might be realized through the direct seeding of guayule to the field, or an increase in rubber yield.

The growth regulator DCPTA and its analogs are effective in numerous processes, such as the stimulation of carotenoid biosynthesis in citrus (Coggins et al., 1970), biomass accumulation in soybeans (Yokoyama et al., 1984), and an increase in leaf carbon fixation and biomass in cotton (Gausman et al., 1985). Derepression of DNA-dependent RNA synthesis has been implicated as the mode of action of these bioregulators (Greenblatt et al., 1986).

In guayule, DCPTA has induced an increase in rubber yield of mature field-grown guayule plants (Hayman et al., 1983, 1987). Although numerous demonstrations of the efficacy of DCPTA have been reported for guayule, some studies indicated no significant effects (Bucks et al., 1985). Thus, further study of the optimization of application of DCPTA is needed.

Our objective was to develop and char-

acterize a DCPTA seed treatment for guayule. Proposed benefits of such a treatment include rapid seed germination and enhanced seedling vigor to benefit a direct field seeding program and to provide consistency and efficiency of DCPTA application to effect increased guayule biomass and rubber production.

The protocol for the DCPTA treatment of guayule seed was as follows: Fifty seeds of 'N565' guayule were placed in a polystyrene weighing dish (75 $\times$ 75 $\times$ 25 mm). The seeds were then soaked for 10 hr in 25 ml of 0.05% Tween 80 containing the desired concentration of DCPTA. The DCPTA was synthesized by the procedure of Poling et al. (1975) except for the use of 3,4-dichlorophenol as a starting material. Seeds were harvested by vacuum filtration, rinsed with distilled water, and transferred to a 42.5-mm filter paper circle. Each filter circle was placed on top of a 47-mm diameter cellulose absorbent pad (Gelman Sciences, Ann Arbor, Mich.). Sixteen pads were placed in a covered plastic bio-assay dish (243 $\times$ 243 $\times$ 18 mm; Nunc, Denmark). Distilled water was applied to the filter pads as needed to maintain sufficient moisture for germination. The plastic dishes were kept at laboratory light and temperature conditions.

The effects of DCPTA treatment on seed germination rate were followed by observing radicle emergence at 8-hr intervals. These results were quantified by calculating the coefficient of velocity of germination (C.V.):

$$C.V. = 100(A_1 + A_2 + \dots A_x)/(A_1T_1 + A_2T_2 + \dots A_xT_x)$$

where A = the number of radicles emerging in 8 hr and T = the time corresponding to A (Kotowski, 1926). T₁ began with the first radicle emergence. The C.V. is proportional to the rate of guayule germination and emphasizes the contribution of the earlier germinating seeds.

The data in Table 1 were obtained from a

single experiment employing 50 seeds per replicate and 12 replicates per treatment and are representative of several experiments. Only the 26- μ M DCPTA treatment was effective in increasing the rate of germination when compared with seeds soaked in a Tween 80 solution with no DCPTA (control). The C.V. of guayule seed germination obtained for this treatment was 23% greater than that of the control.

In a similar experiment, eight replicates of 25 guayule seeds each were treated with 26 μ M DCPTX in 0.05% Tween 80. These, along with control seeds (a 2.5-ml 0.05% Tween 80 10-hr soak), were allowed to dry in the laboratory for 1 week before germination in the bio-assay dish. The means of the C.V. for the treated and control samples were 32.8 and 26.7, respectively. The C.V. for the treated seed was 23% greater than that of the control and significantly different from the control at $P = 0.05$, as determined by a two-tailed, unpaired *t* test.

The possible significance of an increased C.V. of germination is 2-fold. Direct seeding of guayule to the field may be of benefit, as faster-germinating seed would be less affected by unfavorable environmental factors. Although the observed differences in the C.V. for DCPTX-treated seeds might be interpreted as small, others (Nichols and Heydecker, 1968) have observed that small differences encountered in germination tests may predict substantial gains in the field. Field trials of DCPTA-treated seed are needed to evaluate this potential benefit.

A second possible benefit of the DCPTA seed treatment is increased biomass accu-

Table 1. Effect of DCPTA concentration on the coefficient of velocity (C.V.) of germinating guayule seed.

DCPTA (μ M)	C.V.*	Increase over control (%)
0	34.5 $\pm$ 3.4	
13	34.8 $\pm$ 4.6	0.9
26	42.3 $\pm$ 6.2	22.6
39	36.7 $\pm$ 4.5	6.4
Significance		
Treatments	***	
Linear	NS	
Quadratic	**	
Cubic	**	

*Mean $\pm$ SD of 12 observations.

NS, **, ***Nonsignificant at $P = 0.05$ or significant at $P = 0.01$ or 0.001, respectively.

Table 2. Effect of various DCPTA treatments on biomass accumulation in greenhouse-grown guayule.

DCPTA		
Mode of application	Concn (μ M)	Dry wt (g/plant)
Foliar	132	3.93 abc ^a
Foliar	66	5.53 d
Foliar	33	4.62 c
Foliar	17	3.61 ab
Seed	26	3.99 bc
Control	0	3.27 a

*Mean separation in column by Duncan's multiple range test. $P = 0.05$.

Received for publication 27 Dec. 1989. WC acknowledge the technical assistance of Charles DeBenedict, Seth Gold, and Bonnie Hayman. WC thank Dale Bucks for providing the guayule seed. Mention of a product or company name does not imply endorsement by the U.S. Dept. of Agriculture to the exclusion of others that may be suitable. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

Table 3. Effect of 8.2-mm foliar applications of DCPTA on rubber and dry weight production of stems + leaves and roots of field-grown guayule.

Plant part	Rubber (%)		Dry wt (g/plant)		Total rubber (g/plant)	
	Treated	Control	Treated	Control	Treated	Control
<i>Harvest 1</i>						
Stems + leaves	7.0	6.8	1354	1346	94.8	91.5
Roots	5.4	4.9	213	171	11.5**	8.4
<i>Harvest 2</i>						
Stems + leaves	9.2	9.8	3274	2765	301.2	271.0
Roots	5.2	5.1	313	203	16.3**	10.3

*,**Significantly different from controls at $P = 0.05$ and 0.01 , respectively.

mulation. We compared seed and foliar application techniques for DCPTA in greenhouse-grown guayule plants to assess this benefit. Seeds of 'N565' guayule were treated with DCPTA and germinated in the laboratory as described above. One week after germination, control and treated seedlings were transplanted to 5-cm-square pots (0.13-liter) containing Supersoil soil mixture (Rod McLellan, S. San Francisco, Calif.) on 1 June 1987. The seedlings were maintained in a greenhouse at $28 \pm 4^\circ\text{C}$ and $18 \pm 2^\circ\text{C}$ day and night cycles, respectively. Plants received a photon flux density of 1200 to 1400 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ photosynthetically active radiation (PAR) as measured by a LI-COR Quantum Sensor (LI-COR, Lincoln, Neb.). After 2 months, seedlings were transplanted to 10-cm-square pots (1.0 liter). Seedlings for foliar treatment were sprayed with 17, 33, 66, or 132 μM DCPTA in Tween 80 (0.1%, w/v) until runoff. Sixteen 6-month-old plants per treatment were harvested, dried at 60°C , and weighed.

We observed a 22% increase in the dry weight of plants obtained from DCPTA-treated seed over that of controls (Table 2). Foliar applications of 33 and 66 μM DCPTA resulted in 41% and 69% increases in dry weight, respectively.

A 95% increase in survival rate was observed for treated seedlings in a preliminary greenhouse study designed to simulate field conditions where inadequate moisture might be encountered. Soil, light, and temperature were as described in the previous experiment. Control and DCPTA-treated seed were sown into flats ($44 \times 44 \times 5$ cm) containing paper planting sleeves (Paperpot: Nippon Beet Sugar, Japan). We used 50 sleeves per treatment, two treatments per flat (a 0.05% Tween 80 + 26 μM DCPTA 10-hr soak and a Tween 80 only control), and replicated the experiment in each of five flats. Water was withheld from 50-day-old seedlings for 4 days. Moisture determinations for soil samples from each of the five flats were obtained. The plants

were then watered and the percentage of plants surviving determined. This process was repeated a second time. For those samples with a soil moisture content of 2% to 6.5%, 42% of the controls and 82% of the seedlings grown from DCPTA-treated seed survived ($P \leq 0.05$ for a two-tailed unpaired t test of the arcsin-transformed data).

If the roots of the seedlings obtained from treated seed were more highly developed or greater in mass than the controls, then the observed advantage in survival rate might be expected. This possibility was examined in DCPTA-treated, field-grown guayule plants. Seedlings of 'N565 II' and '11634' guayule, grown in the greenhouse for 5.5 and 6.5 months, respectively, were transplanted to the field in single treated and control blocks in Riverside, Calif., on 1 Apr. 1985. Intra-row spacings of 0.6 m and interrow spacings of 1 m were used. Plants were treated by a foliar application of 8.2 mm DCPTA in 0.059 Tween 80 (pH adjusted to 9 with 1 N NaOH) first in the greenhouse 2 weeks before transplanting to the field, and five additional times in the field at $\approx 8, 15, 18, 212$, and 26 months. Plants were harvested in February (harvest 1) and August (harvest 2) for 'N565 II' and '11634', respectively. Rubber analyses of seven to nine samples per treatment were performed by ^{13}C -NMR as described by Hayman et al. (1983). Statistical analysis used the single-tailed, unpaired Student's t test.

The roots of guayule plants in both harvests responded to this particular regimen of bioregulator application, but the stems and leaves did not (Table 3). Increases of 37% and 58% in total rubber were obtained for the roots of harvests 1 and 2, respectively. We believe this to be the first report in which guayule roots responded to DCPTA application but the stems and leaves did not. Root : shoot ratio increases of 24% and 32% in dry weight were obtained over that of controls for harvests 1 and 2, respectively. Root : shoot increases of 34% to 48% have been reported with DCPTA-treated cotton seed

(Gausman et al., 1988). The increase in total rubber content of the roots in the two harvests was related to the increase in biomass of the roots as no significant differences in rubber concentration were observed.

Thus, DCPTA seed treatment was effective in increasing the rate of seed germination, as well as the biomass and survival rate of seedlings, all characteristics beneficial to the direct seeding of guayule. The greater survival rate for seedlings derived from DCPTA-treated seed may be the result of an increase in root biomass analogous to that obtained by DCPTA foliar application in the field.

Literature Cited

- Bucks, D.A., R.L. Roth, F.S. Nakayama, and B.R. Gardner. 1985. Irrigation water, nitrogen, and bioregulation for guayule production. *Trans. Amer. Soc. Agr. Eng.* 28:1196-1205.
- Coggins, C.W., G.L. Henning, and H. Yokoyama. 1970. Lycopene accumulation induced by 2-(4-chlorophenylthio)-triethylamine hydrochloride. *Science* 168:1589-1590.
- Gausman, H.W., J.D. Burd, J. Quisenberry, H. Yokoyama, R. Dilbeck, and C.R. Benedict. 1985. Effect of 2-Diethylamino-3,4-Dichlorophenylether (DCPTA) on cotton plant (*Gossypium hirsutum*) growth and phenology. *Biotechnology* 3:255-257.
- Gausman, H.W., H. Yokoyama, J.E. Quisenberry, J.H. Kechthly, and J.D. Burd. 1988. Effect of dimethylamino-3,4-dichlorophenylether (DCPTA) on cotton (*Gossypium hirsutum* L.) root/shoot ratio. *Bul. Plant growth Reg. Soc. Amer.* 16(2):6-7.
- Greenblatt, G.A., C.L. Rosenfield, H. Yokoyama, and C.R. Benedict. 1986. The mode of action of 2-(4-methylphenoxy) triethylamine (MPTA) on carotenoid biosynthesis in cotton cotyledons. *Plant Physiol.* 80:66. (Suppl.)
- Hayman, E., H. Yokoyama, and S. Gold. 1983. Effect of bioregulators on the accumulation of rubber in guayule. *J. Agr. Food Chem.* 31(5):1120-1121.
- Hayman, E., H. Yokoyama, and S. Gold. 1987. Guayule cultivar effect on rubber bioinduction. *J. Agr. Food Chem.* 35(2):186-188.
- Kotowski, F. 1926. Temperature relations to germination of vegetable seed. *Proc. Amer. Hort. Sci.* 23:176-184.
- Nichols, M.A. and W. Heydecker. 1968. Two approaches to the study of germination data. *Proc. Intl. Seed Test. Assn.* 33(3):531-540.
- Poling, S.M., W.J. Hsu, and H. Yokoyama. 1975. Structure-activity relationships of chemical inducers of carotenoid biosynthesis. *Phytochemistry* 14:1933-1938.
- Yokoyama, H., C. DeBenedict, W.J. Hsu, and E. Hayman. 1984. Bioregulation of lipid and protein synthesis in soybean by 2-Diethylamino-ethyl-3,4-Dichlorophenylether. *Biotechnology* 2:712-714.