for their careful performance in the daily care and observation of the birds.

Registry No. DON, 51481-10-8.

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Received for review May 6, 1986. Accepted September 19, 1986.

Guayule Cultivar Effect on Rubber Bioinduction

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The application of DCPTA to guayule plants resulted in increased rubber accumulation. A screening program of an 18-month-old planting of five guayule cultivars, treated with 50 and 100 ppm of three DCPTA analogues, showed that cultivar 11634 responded much more readily to rubber induction by bioregulator than did the other cultivars. At the end of one 120-day period, the rubber content of cultivar 11634 increased from 55.0 to 80.2 g/plant, while cultivar 11619 (chosen as typical of the nonresponsive cultivars) did not have a statistically significant increase. Multiple applications of DCPTA were not necessary to increase rubber production.

The induction of increased rubber accumulation in guayule plants (*Parthenium argentatum* Gray), due to the application of the bioregulator DCPTA [2-(diethylamino)ethyl 3,4-dichlorophenyl ether], has been demonstrated within our laboratory (Yokoyama et al., 1977; Hayman et al., 1983) and by other laboratories (Bauer, 1979; Benedict et al., 1983). The effective use of bioregulators in guayule tissue culture was shown by Pagano and Staba (1983). Evidence has been presented (Greenblatt et al., 1986) that the mode of action of bioregulator compounds such as DCPTA is at the level of DNA-dependent RNA synthesis. Derepression of the controlled DNA sequences leads to increased total activity of key enzymes such as MVA kinase, IPP isomerase, and rubber transferase in the rubber biosynthetic sequence (Benedict et al., 1983).

Certain agronomic questions need to be investigated to optimize the effectiveness of DCPTA-induced rubber accumulation and increased biomass production (Yokoyama et al., 1984). Therfore, we carried out a screening program involving two lesser concentrations of bioregulator than previously tried: three analogues of DCPTA and five guayule cultivars (four of which had not previously been tested with our bioregulator compounds). During the course of this investigation, it was observed that plants of cultivar 11634 responded more readily to bioregulation than did plants of the other cultivars tested. This cultivar effect, as well as the necessary number of spray applications, is the subject of this study.

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MATERIALS AND METHODS

Seeds were germinated in a peat moss and perlite mixture (1:1, v/v) in 1-in. paper cells. Seedlings were greenhouse grown in San Diego County, CA, from January to April, 1982. Seedlings were irrigated as needed. Prior to single-row planting in Brawley, CA, the plants used in the varietal screening experiment were sprayed until runoff occurred with solutions of 50 or 100 ppm DCPTA, DIPTA [2-(diethylamino)ethyl 3,5-diisopropylphenyl ether], or 2,4-DCPTA [2-(diethylamino)ethyl 2,4-dichlorophenyl ether]. These bioregulators were synthesized by methods previously described (Hayman et al., 1983). The bioregulator solutions also contained 0.02% Ortho X-77 spreader (Chevron Chemical Co., San Francisco, CA). All treatments were adjusted to pH 9.0 with 1 N NaOH. The plants used in the experiments as described in Tables II and III were transplanted (nontreated) at 4 months of age. At 20 and 24 months, respectively, 50 plants from a single row were treated with a 2500 ppm solution of DCPTA containing 0.1% Ortho X-77 and 0.1% β-cyclodextrin. Seedlings for all experiments were planted with 14 in. between plants and 3 ft between rows.

Analyses of the rubber content of whole plants, harvested by digging under the roots, were accomplished by use of our ^{13}C NMR method (Hayman et al., 1982) modified as described by Hayman et al. (1983). Plants in Table I were harvested at 18 months. Total rubber content and percent rubber were calculated on an oven-dry basis. Statistical analyses consisted of the analysis of variance

Table I. Effects of Various Bioregulators and Concentrations on Six Guayule Cultivars

cultivar	treatment[a] I	II	III	IV	V	VI	control
11600							
no. of plants	3	4			2	5	3
% rubber	4.3	4.0			3.4	4.3	4.1
total rubber[c]	16.9	19.3			16.1	15.4	17.5
11619							
no. of plants	4	3			3	4	4
% rubber	3.5	5.4[b]			2.9	3.8	3.8
total rubber[c]	13.6	15.8			10.2	9.4	14.4
11634							
no. of plants	3	3	4	3	4		7
% rubber	4.5	4.9	4.5	3.7	6.1[b]		3.0
total rubber[c]	15.3[b]	9.9	20.3[b]	14.4[b]	19.9[b]		7.9
11591							
no. of plants	4	4	3	4			8
% rubber	4.3	4.2	4.0	4.4			4.4
total rubber[c]	10.5	9.4	13.6	10.0			13.1
N576							
no. of plants		4	3	4		4	3
% rubber		4.2	2.3	5.3[b]		3.5	3.3
total rubber[c]		11.4	8.6	12.8		12.5	11.2
11604							
no. of plants	3						4
% rubber	4.5						4.7
total rubber[c]	16.2						19.2

[a] Treatments: I, 50 ppm 2-(diethylamino)ethyl 3,4-dichlorophenyl ether; II, 100 ppm 2-(diethylamino)ethyl 3,4-dichlorophenyl ether; III, 50 ppm 2-(diethylamino)ethyl 2,4-dichlorophenyl ether; IV, 100 ppm 2-(diethylamino)ethyl 2,4-dichlorophenyl ether; V, 50 ppm 2-(diethylamino)ethyl 3,5-diisopropylphenyl ether; VI, 100 ppm 2-(diethylamino)ethyl 3,5-diisopropylphenyl ether. [b] Mean was significantly higher ($P > 0.80$) than the control. [c] Units: gram/plant.

Table II. Comparison of Cultivars 11634 and 11619 for Different Growing Periods

treatment: day	treatment: application	11634: % rubber	11634: n[a]	11634: total rubber, g/plant	11619: % rubber	11619: n	11619: total rubber, g/plant
0	control (April)	7.6	6	40.5	7.9	8	41.4
30	1 spray	7.8	6	56.3	8.2	6	39.1
60	2 sprays	8.8	5	43.0	6.2	5	34.0
90	3 sprays	10.3[b,c]	5	74.0[b,c]	6.6	6	34.4
120	4 sprays	11.0[b,c]	6	80.2[b,c]	8.1	2	48.0
120	control (Aug)	8.1	8	55.0	6.6	6	45.7

[a] n = number of replicates. [b] Mean was significantly higher ($P > 0.80$) than the 0-day control. [c] Mean was significantly higher ($P > 0.80$) than the 120-day control.

and the Neuman–Keuls multiple-range tests.

RESULTS AND DISCUSSION

Four of the five bioregulators applied to plants of cultivar 11634 induced a statistically significant increase in total rubber content (Table I). The total rubber content of cultivars 11600, 11619, 11591, and N576 remained unaffected. Therefore, cultivar 11634 apparently responded better to bioregulator treatment than did the other cultivars. However, one of these cultivars, N576, had been shown previously to respond to chemical induction of rubber (Hayman et al., 1983). Further evidence of a varietal effect, therefore, exists.

To further demonstrate this cultivar effect, an experiment was conducted to monitor rubber accumulation over a 4-month period. Nonsprayed controls were sampled at the beginning and end of this period. Treated samples were obtained at 30-day intervals, and the treated plants remaining were sprayed with DCPTA again. Cultivar 11619, chosen as being typical of the less responsive cultivars, and cultivar 11634 were included. The results from these experiments are shown in Table II. As indicated, both total rubber and percent rubber for the 90- and 120-day treated samples of cultivar 11634 were statistically higher than that of the control. There were no statistically significant differences among the 11619 samples.

Although the results presented in Table II clearly indicate that DCPTA induced rubber accumulation, the relative contribution of multiple applications of DCPTA to rubber induction is difficult to discern. The 90-day sample, for example, apparently benefited from both an additional application of bioregulator and an additional 30-day growth period than did the 60-day sample. Therefore, an experiment was performed to discern the relative contribution of multiple applications of bioregulator to rubber induction. Nonsprayed controls were sampled at the beginning of the experiment and 4 months later at the end of the experiment. All four DCPTA treatments were sprayed at zero time. For each of 3 successive months, one less sample was treated. At the end of the experiment, the plants were harvested, dried,

Table III. Effect of Number of Treatments on Rubber Induction

sample	treatments[a]: day	treatments[a]: application	n[b]	% rubber	total rubber, g/plant
I	0, control (Aug)	0 sprays	8	8.1	55.0
II	0	1 spray	5	10.6	70.7
III	0, 30	2 sprays	7	9.9	57.3
IV	0, 30, 60	3 sprays	8	10.2	59.2
V	0, 30, 60, 90	4 sprays	5	10.0	95.7[c]
VI	120, control (Dec)	0 sprays	6	11.1	68.2

[a] All samples harvested at 120 days except 0-day control. [b] n = number of replicates. [c] Mean was significantly higher ($P > 0.95$) than the 120-day control.

and assayed for rubber content and biomass (Table III). As shown, the total rubber content of the controls increased 24%, while sample V increased from 55 to almost 96 g/plant, which was a 74% increase within a period of 120 days.

There are two possible explanations why only sample V showed an increase in rubber content. Four successive monthly applications of the bioregulator might be necessary to induce rubber accumulation. However, this would seem unlikely from past results. It is also possible that only one application of DCPTA might be necessary but that the time of year of application is critical. This would seem to be the case here. Applying this information to the results in Table II, it appears that the extra applications of DCPTA were not responsible for the increase rubber induction in the 90- and 120-day samples.

Manipulation of the sample V data in Table III showed that the increased total rubber level was the result of a larger than average plant size. The average dry weight was 957 g/plant for the sample V treatment as compared with that of 624 g/plant for the average of all other samples. This represented a 53% increase in biomass. While it seems appropriate to conclude that DCPTA caused a stimulation of biomass accumulation, as reported previously (Yokoyama et al., 1984), there is the possibility that other factors such as nutrient and environmental conditions contributed to the increased plant size. Since the plants in this experiment were planted within a single block, it was not possible to fully evaluate the relative contributions of bioregulation and environmental factors to the biomass increase. However, the conclusions mentioned above regarding single spray applications would seem justified.

Cultivar 11634 responded more readily to rubber induction via DCPTA and its analogues than did other cultivars. Therefore, it would seem likely that there might be other guayule cultivars that are more responsive than those that have been studied. A study of those characteristics of cultivar 11634 that differentiate it from less responsive guayule cultivars could help to lead this selection.

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Received for review June 17, 1986. Accepted November 10, 1986. This work was presented at the 4th International Conference on Guayule Research and Development, Tucson, AZ, Oct 15-19, 1985. Reference to a company 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.

Investigation of Nitrite and Nitrate Levels in Paper Materials Used To Package Fresh Meat

Fiona Scriven, Peter Sporns,* and Fred Wolfe

A method was developed for the analysis of nitrite and nitrate in paper using reduction to nitric oxide and chemiluminescence detection. The potential for fresh meat contamination by nitrite and nitrate from over 50 different samples of paper and paperboard packaging materials was evaluated by this method of analysis. Concentrations of nitrite were determined to be less than 5 μg of NO_2^-/g, with two exceptions containing 14.5 and 19 μg of NO_2^-/g. Nitrate concentrations were generally higher, though 50% of the materials analyzed contained less than 10 μg of NO_3^-/g. The greatest hazard of contamination of fresh meat was determined to be through contact with gummed paper tape, which contains extremely high levels of nitrate (25 800-32 700 μg of NO_3^-/g). Migration studies using giblet bags containing 120 μg of NO_3^-/g in contact with chicken breast meat showed that low but detectable migration of nitrate from the paper packaging material to meat could occur at even these low nitrate levels.

The problems associated with carcinogenic nitrosamines in the food supply has lead to a great deal of scientific investigation in the last two decades (Magee and Barnes, 1967; Archer, 1982; Crosby, 1983). Recently paper packaging materials have been found to contain the nitrosamine *N*-nitrosomorpholine, which could migrate to foods (Hotchkiss and Vecchio, 1983; Hoffman et al., 1982; Sen and Baddoo, 1986). It is probable that paper packaging materials that contain nitrosamines would also contain nitrite and/or nitrate. Also disconcerting is evidence that even highly purified papers such as filter paper can contain nitrite (Fiddler and Gentilcore, 1975) and nitrate (Fawcett et al., 1976).

Although paper packaging materials have been investigated for nitrosamines, there has been no study into levels of nitrite and nitrate that might be present in food contact paper packaging materials. Packaging materials contaminated with nitrite or nitrate would be a major concern when used as wrappings for fresh meat, since moisture from the meat could extract these ions. With the many natural nitrosatable amines and amides present in meat (Singer and Lijinsky, 1976; Spinelli et al., 1974; Velisek et

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0021-8561/87/1435-0188$01.50/0