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Transmission of [^{14}C]Deoxynivalenol to Eggs following Oral Administration to Laying Hens[1]

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Following a single oral dose of [^{14}C]deoxynivalenol (2.2 mg of DON, 2.4 μCi/bird) low levels of residues were transmitted to eggs. Maximum radioactivity, which occurred in the first eggs laid after dosing (within 24 h), amounted to 1.9 μg DON-equivalents/60-g egg (0.087% of dose); levels dropped rapidly in ensuing eggs. During daily consumption of DON, administered in spiked feed over a 12-day period (2.2 mg of DON/bird per day for 6 days followed by 2.2 mg of [^{14}C]DON, 1.5 μCi/bird per day for 6 days), radioactivity levels increased with each subsequent egg laid up until the last exposure to the toxin; maximum levels accounted for 4.2 μg DON-equivalents/60-g egg. Residues quickly declined once the birds were switched to clean feed. Results indicate that although residues appear to accumulate in eggs, levels do not persist once the contaminated source is withdrawn. Preliminary analysis of egg material showed only about 10% of radioactivity present could be identified as the parent toxin, DON.

Deoxynivalenol (DON, vomitoxin), a *Fusarium*-produced mycotoxin, has been the subject of intensive testing since its identification in eastern Canada and the midwestern United States as an important contaminant of various field crops infected with *Fusarium* fungi (Trenholm et al., 1983; Coté et al., 1984). Since exposure to DON has been associated with a number of toxic effects in farm and laboratory animals (i.e., feed refusal, emesis, anorexia), this has resulted in concern about potentially toxic residues in food products intended for humans, not only contaminated grains but also tainted animal products (meat, eggs, milk) obtained from livestock or poultry previously exposed to DON-contaminated feeds.

Several investigators have studied the transmission of DON from contaminated feed to tissues of livestock and poultry, the toxin being administered either as the pure compound or as naturally or intentionally infected corn or wheat. At detection limits of 10 ng of DON/g of tissue, neither El-Banna et al. (1983) nor Kubena et al. (1985) were able to detect DON in either eggs and/or tissues of poultry fed, respectively, a 4-5 mg of DON/kg diet for periods of between 28 and 190 days or a 9-18 mg of DON/kg diet for up to 35 days. Prelusky et al. (1984) found only trace levels of DON in milk (<4 ng/mL) following a single oral administration of the toxin (2 mg/kg of body weight) to lactating dairy cows. However, in these previous studies the milk and poultry products were analyzed for the presence of DON, but not the presence of possible metabolites. Recent studies though have demonstrated extensive metabolism of DON can indeed occur; between 50 and 75% of the administered dose given to swine (Coppock et al., 1985) or sheep (Prelusky et al., 1986a) appears to be metabolized prior to its elimination. Therefore, it is important from a human health viewpoint to determine whether or not DON, either as the unchanged toxin or as potentially toxic metabolites, can be introduced into the human food chain through edible products obtained from farm animals exposed to DON. A subsequent study (Prelusky et al., 1986b), involving the oral administration of radiolabeled DON to chickens (2.2-mg single dose or 2.2 mg/day for 6 days), demonstrated that measurable levels of residues could be found in tissues: <40 ng DON-equivalents/g for most tissues, except liver, kidney, and gastrointestinal (GI) tract, which were marginally higher. These results suggest that although the parent toxin DON itself may not be transmitted to animal products, metabolites may account for the low levels of residues detected.

The present study to measure the transmission of radioactivity to eggs was part of a larger experiment (Prelusky et al., 1986b) designed to determine the fate of DON in laying hens. DON was administered as the ^{14}C-labeled compound either as a single oral dose or in spiked ration fed to the birds over an extended period.

MATERIALS AND METHODS

Chemicals. Both nonradioactive and ^{14}C-labeled DON were produced biosynthetically and purified as previously described by Miller and Arnison (1986). Purity of each compound (>95%) was established by reversed-phase high-performance liquid chromatographic analysis with a Hewlett-Packard Model 1090 liquid chromatograph (Hewlett-Packard Ltd., Palo Alto, CA) equipped in series with a diode array detector and Berthold Model LB 504 radioactivity monitor. Operating conditions: RP-18 column, 5-μm OD-5A Spheri-5, 25 cm × 4.6 mm (Brownlee Labs Inc., Santa Clara, CA); mobile phase, acetonitrile-water (1:9), flow, 0.8 mL/min; wavelengths monitored, 220, 234, and 254 nm; oven temperature, 40 °C. The original

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Table I. Radioactivity in Eggs from Laying Hens Orally Intubated with a Single Dose of ^{14}C-Labeled Deoxynivalenol (DON)[a]

		dpm		total per egg[e]		
time,[b] h	matl[c]	per g[d]	per egg[e]	dpm	DON-equiv,[f] μg	% dose[a]
24	yolk	24.5 (±11.2)	455			
	ALB	116.7 (±33.0)	4131	4619	1.91	0.087
	MEM	93.1 (±22.1)	33			
48	yolk	56.5 (±39.8)	1051			
	ALB	57.0 (±36.2)	2018	3094	1.28	0.058
	MEM	71.1 (±24.3)	25			
72	yolk	50.9 (±35.1)	947			
	ALB	13.9 (±13.3)	492	1448	0.60	0.027
	MEM	25.0 (±14.1)	9			
96	yolk	10.3 (±8.5)	191			
	ALB	2.0 (±2.3)	71	264	0.11	0.0049
	MEM	5.0 (±3.0)	2			

[a] ^{14}C-labeled DON intubated at 2.4 μCi/bird (5.33 × 10^6 dpm), equivalent to 2.2 mg of DON/bird. [b] Egg collection time, period (h) after dosing. [c] Key: ALB = albumen; MEM = membrane. [d] Mean (±SD). Number of eggs analyzed: 10, 8, 6, and 3 at 24, 48, 72, and 96 h, respectively. [e] Based on a 60-g egg: yolk, 18.6 g; albumen, 35.4 g; shell membrane, 0.36 g. [f] DON-equivalents (DON and/or metabolites) derived from administered [^{14}C]DON; 1000 dpm = 0.412 μg DON-equivalents.

Table II. Radioactivity in Ova Collected from the Ovaries of Laying Hens Orally Intubated with a Single Dose of ^{14}C-Labeled Deoxynivalenol (DON)[a]

	dpm/g of tissue (±SD) at time (h)						
matl[b]	3	6	12	24	48	72	96
yolk	0[c] (±0)	0 (±0)	1.0 (±1.1)	14.3[c] (±10.3)	46.8 (±28.2)	20.8 (±13.9)	13.4[c] (±12.1)
albumen	0[c] (±0)	8.0[c] (±2.8)	20.8 (±14.9)	51.1[d]	39.8 (±47.7)	44.3[c] (±34.6)	10.0[d]
ovum posn in ovary							
1st	0 (±0)	2.0 (±3.2)	28.3 (±12.3)	53.6 (±17.4)	72.3 (±32.1)	35.1 (±15.4)	22.1 (±15.3)
2nd	0 (±0)	2.5 (±2.8)	16.9 (±11.3)	67.8 (±22.4)	83.7 (±27.6)	63.2 (±32.3)	8.7 (±7.2)
3rd	0 (±0)	6.0 (±4.1)	12.8 (±8.2)	85.9 (±28.7)	100.3 (±35.7)	51.6 (±22.7)	12.3 (±8.3)
4th	0 (±0)	3.6 (±3.5)	19.4 (±8.3)	39.3 (±10.4)	34.6 (±18.4)	34.6 (±20.6)	5.1 (±2.1)

[a] Averages (±SD) based on triplicate measurements of three ova/yolk/albumen samples unless otherwise stated; 1000 dpm = 0.412 μg DON-equivalents. [b] Position based on size of individual ovum at time of harvest. Largest ovum considered first for ovulation, second largest in second position, etc. Yolk/albumen data obtained from analysis of incompletely formed egg collected from the isthmus of the oviduct. [c] Calculations based on two samples only. [d] Calculations based on one sample only.

specific radioactivity of the toxin used in this study was 1.3 μCi/mg before dilution.

White Leghorn laying hens (1300–1700 g) were selected at 30 weeks of age, housed in individual laying/metabolism cages, and provided with water and a standard laying ration ad libitum for at least 2 weeks prior to administration of DON.

Single-Dosed Birds. Following a fasting period of 3 h, 35 birds were administered a single dose of 2.2 mg of DON (dissolved in water and absorbed into 5 g of ration) by intubation. Twenty-eight hens received the ^{14}C-labeled DON (2.4 μCi/bird), and seven control birds received only the nonlabeled DON. The standard feed was returned after dosing, and eggs were collected at 24-h intervals until 96 h after dosing. At 3, 6, 12, 24, 48, 72, and 96 h, five birds (four ^{14}C dosed, one control) were sacrificed by CO_2 asphyxiation at each time period, and the four largest ova found in the ovary and any partially formed eggs in the oviduct were collected for radioactive counting.

Continuous-Dosed Birds. Following the 2-week acclimatization period, 24 birds were placed on a restricted amount of feed (110 g/bird per day) consisting of standard laying ration spiked with pure DON to 20 ppm (2.2 mg of DON/bird per day). Hens were maintained on this diet for 6 days, after which time 18 birds were switched to an equivalent DON-spiked ration containing 1.5 μCi of the radioactive toxin. The remaining six birds continued on the nonradioactive diet, and eggs from these birds were used as controls. The laying hens were fed the [^{14}C]DON-spiked diet for a period of 6 days, at which time all birds were returned to normal laying ration ad libitum for 6 days. Eggs were collected daily following commencement of the [^{14}C]DON-spiked ration, and this continued for the remaining 12 days of the experiment. On days 8, 10, 12, 14, 16, and 18 of the experiment (2, 4, and 6 days after beginning [^{14}C]DON and 2, 4, and 6 days after removal of [^{14}C]DON, respectively) four birds (three ^{14}C dosed, one control) were sacrificed by CO_2 asphyxiation, and partially formed eggs and/or ova were collected as above.

Measurement of Radioactivity. At least three laid eggs and three ova of corresponding position in the ovary (largest ovum considered in first position for ovulation, second largest in second position, etc.) were used for daily determination of radioactivity. Background samples were prepared by the same counting procedure with the eggs/ova from control birds that received nonlabeled DON only. The radioactivity in the yolk, albumen, and shell membrane were determined separately.

Samples, in triplicate, weighing 100–200 mg, were burned in oxygen in an auto sample oxidizer (Tri-Carb type 306; Packard Instrument Co., Downers Grove, IL), and the radioactivity of the resulting $^{14}CO_2$ generated by combustion was measured in a Beckman Model LS 3801 liquid scintillation spectrometer (Beckman Instruments, Inc., Irvine, CA) provided with a microprocessor for data reduction and calibration of disintegrations per minute (dpm) values. Quench correction was by automatic external standard, with sufficient counts accumulated to give $<5\%$ error with a 95% confidence interval. Efficiency of combustion and collection of $^{14}CO_2$ was determined to be 98–101%. The radioactivity of yolk and albumen was expressed on a wet-weight basis and that of the shell membrane on a dry-weight basis.

RESULTS AND DISCUSSION

Single-Dosed Birds. Table I shows the level of DON-derived radioactivity found in eggs laid up to 96 h following administration of a single oral dose of ^{14}C-labeled deoxy-

nivalenol (2.2 mg of DON, 2.4 μCi/bird). The transfer of residues into egg tissue occurred rapidly after ingestion of the toxin; radioactivity was not only detected in the first egg laid within 24 h of dosing, but trace levels of radioactivity could be detected in ova harvested from sacrificed hens as early as 6 h after dosing (Table II). Overall though, it would not appear that a single exposure of hens to feed contaminated with low levels of DON would result in significant or persistent residuals in eggs; the health hazard to humans would be minimal. On the basis of a typical egg size of 60 g (31% yolk, 59% albumen, 0.6% shell membrane, 9.4% shell; Romanoff, 1967), in the initial 24 h eggs, which contained the highest total amount of residues, the edible portions accounted for only 0.087% of the dose. This was equal to 1.91 μg DON-equivalents/egg. Subsequent eggs laid contained declining total levels, accounting for 1.28 and 0.60 μg DON-equivalents/egg at respective times of 48 and 72 h posttreatment. By 96 h after dosing, only marginally detectable levels were measured, amounting to 110 ng/egg (0.0049% of dose). This reduction in residual levels in eggs ($t_{1/2} < 24$ h) was similar to the rate of decline previously noted to occur in various tissues (liver, kidney, heart, muscle, etc.) following a single oral dose of [^{14}C]DON to laying hens, where the average elimination $t_{1/2}$ value for ^{14}C was approximately 19 h (range 8.2–33.3 h; Prelusky et al, 1986b).

DON can apparently enter the egg at any stage of its development prior to shell formation. The detection of radioactivity in ova as early as 6 h postdosing indicates that a direct transfer of residues to yolk tissue can occur before ovulation and egg formation in the oviduct. Residual levels measured in individual ova were only slightly higher than that recorded in the yolk of eggs laid the same day, which was probably due to the higher water content in yolk compared to ovum (50% vs. 15%) that diluted the specific radioactivity of yolk material when measured on a per gram (wet-weight) basis. The exception to the above was the lower activity measured in the yolk from the initial 24-h egg, due to the fact that the ovum had probably already been ovulated by the time of dosing and as a result exposed to less toxin than the ova contained in the ovary. Furthermore, it appears DON residues can be incorporated directly into the albumen as well, during the period of egg white formation as the egg passes through the isthmus of the oviduct. This is evident by the increasing radioactivity levels in albumen measured at 6, 12 (incomplete eggs collected from oviduct, Table II), and 24 h (Table I) that cannot be attributed to diffusion of residues from the yolk. Subsequent albumen levels in eggs laid beyond 24 h declined rapidly, presumably due to the declining level of toxin in the body.

Continuous-Dosing Study. Following a more prolonged dosing regimen (6 days cold DON-spiked feed followed by 6 days [^{14}C]DON-spiked feed) with the same daily dose as in the single-dose study, DON residuals in eggs were found to increase with exposure time. Table III shows that peak activity levels occurred in eggs laid on day 13 of the study, which were the first eggs laid after the hens had received their last exposure to the [^{14}C]DON-contaminated diet. As expected, total radioactivity levels in eggs laid subsequent to removal of the adulterated diet were found to decrease with time.

Peak levels (day 13) represented approximately 4.19 μg of DON (and/or metabolites)/average 60-g egg. This accounted for 0.190% of the hen's daily DON consumption. Once the DON was removed from the diet, the decline in DON-derived radioactivity occurred consistently; eggs laid 6 days after removal of [^{14}C]DON from the diet (day 18) contained 20% of peak levels, or about 0.87 μg of DON-equivalents/egg.

Since exposure to [^{14}C]DON was not continued until a plateau level in the eggs was reached, it is unknown to what extent DON would have accumulated in the egg had access to the DON-contaminated feed not been removed. However, the results from the earlier study by El-Banna et al. (1983) suggest that DON does not accumulate in eggs indefinitely. Following the feeding of dietary levels of 4–5 ppm DON to hens for a period of 146 days, they were unable to measure DON in eggs (or tissues) at a detection limit of 10 ng/g of tissue. Similarly, Lun et al. (1984), who fed Leghorn hens approximately 83 ppm DON-contaminated feed for 27 days, were also unable to detect the parent toxin (detection limit 20 ng/g of tissue) in egg material or tissues. However, in both studies, possible metabolites of DON were not included in the analyses. Recently, in the analysis of various tissues obtained from hens exposed to the same continuous [^{14}C]DON dosing regimen as in the current study, Prelusky et al. (1986b) showed that trace levels of radioactive residues could be found in all tissues (<60 ng DON-equivalents/g of tissue), but levels did not increase with continuous exposure to [^{14}C]DON.

Following exposure to [^{14}C]DON, levels of activity rose more rapidly in yolk, increasing 10-fold between days 7 and 13 (22.1 vs. 225.2 dpm/g), compared to levels in albumen, which rose only 3-fold (20.5 vs. 57.6 dpm/g) during the same period (Table III). In partial agreement, Chi et al. (1978) found that following eight consecutive daily administrations of tritium-labeled T-2 toxin to laying hens, radioactivity in the yolk similarly increased with each dose, although the level in the albumen (and shell membrane) increased only until the third day and then remained reasonably constant thereafter. In the current study, at peak levels the yolk and albumen accounted for 66% and 32%, respectively, of the total radioactivity measured in the egg. Residuals in shell membrane, although also increasing significantly (12-fold) between days 7 and 13, contributed only a minor amount (<2%) of the total found in the eggs. It was interesting to note though that the membrane residue levels increased much faster than those observed for albumen, which the membrane surrounds. This possibly indicates a higher affinity of DON and/or metabolites for membrane tissue, compared to albumen.

The evidence that DON and/or metabolites concentrate in yolk is further supported by the data in Table IV, which shows clearly that a high accumulation of [^{14}C]DON-derived radioactivity occurred in ova prior to ovulation. As with yolk, residual levels in ova increased as consumption of [^{14}C]DON-spiked feed continued (up to day 12) and dropped rapidly once DON feeding was discontinued. Analyzed ova from day 12 averaged 0.50 μg (first) to 1.55 μg (fourth) DON-equivalents/g of wet tissue, with maximum levels up to 3.50 μg/g being recorded. Thus, an ovum 10 g in weight could potentially contain up to 35 μg of DON-derived material. The rapid decline in residues after day 12 indicates that DON and/or metabolites are not strongly bound to components in yolk material and appear easily lowered: first, possibly by the blood flow in the ovary prior to ovulation; second, by transmission of toxin from the yolk to the albumen during egg formation.

Although the preferential accumulation of ^{14}C residues in yolk tissue may reflect the lipophilic nature of DON, the solubility characteristics of possible metabolites and how they would partition between yolk/albumen material are unknown. The nature of [^{14}C]DON-derived radioactivity in the eggs has not been accounted for, due in part

Table III. Deoxynivalenol- (DON-) Derived Residues in Eggs Laid by Hens Fed a DON-Spiked Diet over an Extended Period (12 Days)[a]

day of study[b]	matl[c]	dpm		total per egg[e]		
		per g[d]	per egg[e]	dpm	DON-equiv,[f] μg	% daily dose[g]
7 (1)	yolk	22.1 (±10.1)	411			
	ALB	20.5 (±5.9)	726	1147	0.757	0.034
	MEM	26.8 (±9.8)	10			
8 (2)	yolk	32.4 (±11.1)	603			
	ALB	26.8 (±6.8)	949	1582	1.04	0.047
	MEM	84.0 (±15.9)	30			
9 (3)	yolk	39.8 (±16.2)	740			
	ALB	29.2 (±1.9)	1034	1808	1.19	0.054
	MEM	94.0 (±36.9)	34			
10 (4)	yolk	75.4 (±18.6)	1402			
	ALB	28.6 (±5.8)	1012	1456	1.62	0.074
	MEM	117.8 (±43.8)	42			
11 (5)	yolk	118.5 (±18.3)	2204			
	ALB	42.0 (±16.6)	1487	3773	2.49	0.113
	MEM	228.0 (±118.7)	82			
12 (6)	yolk	167.0 (±20.5)	3106			
	ALB	56.3 (±9.6)	1993	5207	3.44	0.156
	MEM	301.0 (±82.7)	108			
13	yolk	225.2 (±19.1)	4189			
	ALB	57.6 (±10.4)	2039	6345	4.19	0.190
	MEM	324.0 (±86.3)	117			
14	yolk	222.4 (±35.9)	4137			
	ALB	50.2 (±10.2)	1777	6016	4.00	0.182
	MEM	284.0 (±35.9)	102			
15	yolk	154.5 (±11.6)	2873			
	ALB	28.5 (±12.1)	1009	3902	2.58	0.117
	MEM	56.3 (±22.7)	20			
16	yolk	122.8 (±14.0)	2284			
	ALB	22.0 (±15.8)	779	3087	2.04	0.093
	MEM	67.0 (±14.0)	24			
17	yolk	87.7 (±26.6)	1631			
	ALB	18.7 (±11.9)	662	2312	1.52	0.069
	MEM	53.7 (±18.0)	19			
18	yolk	41.0 (±25.3)	763			
	ALB	15.3 (±13.8)	542	1316	0.868	0.039
	MEM	30.7 (±34.0)	11			

[a] Unlabeled DON-spiked feed consumed on days 1–6 (2.2 mg/bird per day) followed by [^{14}C]DON-spiked feed consumed on days 7–12 (2.2 mg of DON, 1.5 μCi/bird per day). No DON consumed on days 13–18. [b] Egg collection time, period (days) following first exposure to DON-contaminated feed; time (days) of [^{14}C]DON administration in parentheses. [c] Key: ALB = albumen; MEM = membrane. [d] Mean (±SD) of three to eight eggs. The specific radioactivity of the yolk and albumen calculated on a wet-weight basis and that of the shell membrane on a dry-weight basis. [e] Based on a 60-g egg: yolk, 18.6 g; albumen 35.4 g; shell membrane, 0.36 g. [f] DON-equivalents (DON and/or metabolites) derived from administered [^{14}C]DON; 1000 dpm = 0.660 μg DON-equivalents. [g] Daily dose 1.5 μCi (3.33×10^6 dpm); 2.2 mg of DON/bird per day.

Table IV. Radioactivity in Ova Collected from the Ovaries of Laying Hens Fed a Deoxynivalenol- (DON-) Spiked Diet over an Extended Period (12 Days)[a,b]

matl[c]	dpm/g of tissue (±SD) at time (days)[d]					
	8	10	12	14	16	18
yolk	382.5 (±109.6)	565.3 (±43.3)	780.7 (±474.1)	250.0 (±106.4)	87.5 (±17.7)	62.5 (±88.3)
albumen	214.3[e] (±94.1)	135.3[f]	185.6[e] (±91.9)	97.8 (±45.4)	80.1[f]	42.5[e] (±28.3)
ovum posn in ovary						
1st	1085.0 (±113.1)	779.7 (±239.0)	758.3 (±526.0)	615.0 (±243.8)	193.3 (±34.0)	118.3 (±18.9)
2nd	1182.5 (±60.1)	894.3 (±61.7)	1213.3 (±1188.6)	543.3 (±266.7)	153.3 (±33.3)	98.3 (±7.6)
3rd	1282.5 (±123.7)	958.3 (±111.4)	1798.3 (±1768.7)	428.23 (±149.7)	105.0 (±56.6)	76.7 (±12.6)
4th	1345.0 (±113.1)	1563.3 (±739.1)	2321.7 (±2471.5)	405.0 (±176.8)	57.5 (±3.5)	42.5 (±10.6)

[a] Unlabled DON-spiked feed consumed on days 1–6 (2.2 mg/bird per day) followed by [^{14}C]DON-spiked feed consumed on days 7–12 (2.2 mg of DON, 1.5 μCi/bird per day). No DON consumed on days 13–18. [b] Averages (±SD) based on triplicate measurements of three ova/yolk/albumen unless otherwise stated; 1000 dpm = 0.660 μg of DON and/or metabolites. [c] Position based on size of individual ovum at time of harvest. Largest ovum considered in first position for ovulation, second largest in second position, etc. Yolk/albumen data obtained from analysis of incompletely formed egg collected from oviduct. [d] Egg collection time, days following first exposure to DON-contaminated feed. [e] Calculations based on two samples only. [f] Calculations based on one sample only.

to the low levels of material available for analysis as well as considerable difficulties in isolating the radioactive components. Preliminary gas chromatographic/mass spectrometric analysis of yolk material showed that only about 10% of the radioactivity present could be extracted and identified as the parent toxin, DON. It is possible that the unextracted portion reflects the production of glucuronide conjugates. However, in order to completely establish the potential health risk of these residues to the human population, further studies to elucidate the identities and quantities of any metabolites present are justified.

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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

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