Effects of Variable Diuron and Nitrate on Sucrose Content Plus Enzyme Activity of Sugarcane Grown in Sand Culture

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INTRODUCTION

Although the substituted urea Diuron[2] has found widespread use as a sugarcane herbicide, neither its mode of action nor its influence upon sugar formation is as yet clearly defined. Previous workers reported that urea herbicides affect the Hill reaction (*18,22,25*),[3] sugar metabolism (*21,20*), and ATP synthesis (*22*). *In vitro* studies in Puerto Rico revealed that after 25 months the herbicide still affected ratoon enzymes related to sugar formation and breakdown (*2*). Later experiments showed that traces of Diuron inhibited sucrose synthesis from fructose and glucose-1-phosphate (*3*). Inhibition was competitive, being reversed by high levels of fructose.

Evidence therefore suggests that Diuron might retard sucrose synthesis. Some workers in Puerto Rico suspect that this does in fact happen. Others feel that Diuron increases sucrose yield, possibly by eliminating the competition of weeds for water and nutrients. Yet, field experiments involve such numerous variables that sugar and enzyme variations are not necessarily attributable to Diuron. With this in mind, sand-culture studies were recently conducted in which Diuron and nitrate were given to cane as controlled variables. There were three objectives: 1, To confirm the theory that Diuron will retard sucrose production; 2, to confirm that Diuron, regardless of soil factors, directly influences enzymes related to sugar level; and 3, to test persistence of Diuron-sugar-enzyme relationships under differing conditions of nitrogen status.

MATERIALS AND METHODS

One-eye cuttings of the variety P. R. 980 were planted in HCl-washed "silica shot" contained in glazed, 2-gallon pots with glass wool over the drainage outlets. All seedlings received daily 1 liter of a complete nutrient solution until treatments were begun at 14 weeks of age.[4] Using the most

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[2] 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

[3] Italic numbers in parentheses refer to Literature Cited, pp. 323–4.

[4] Nutrient concentrations, expressed as meq./liter, were supplied as follows: Nitrate, 10; phosphorus, 6; potassium, 5; calcium, 3; magnesium, 2; and sulfur, 2. Micronutrients, expressed as p.p.m., were given as follows: Iron, 1.0; boron, 0.05; copper, 0.02; manganese, 0.50; zinc, 0.05; and molybdenum, 0.01.

uniform plants available a 3 × 3 factorial design was established with three levels each of nitrate (NO_3) and Diuron. There were five replicates. Nitrate concentrations were 1.5, 4.5, and 13.5 meq./liter, and Diuron was provided at rates of 0, 0.05, and 0.50 p.p.m.[5] Each container thereafter received 1 liter of nutrient solution and 1 liter of tapwater daily.

A single harvest was made after 8 weeks of treatment. Leaves +1 to +4 and immature storage tissue (meristem) were frozen in a mixture of Dry Ice and acetone, lyophilized, and ground to a fine powder in accordance with procedures described previously (*1*).

Clarified water extracts of the plant powder were analyzed for total ketose by the method of Roe (23), and for sucrose by the modification of Cardini *et al.* (*17*). Fructose was estimated by subtracting sucrose values from those of total ketose.

TABLE 1.—*Mean values for fresh weight of immature sugarcane supplied with variable nitrate and Diuron in sand culture*[1]

NO_3 (meq./liter)	Grams per plant under indicated treatment—			Mean
	D_0	$D_{0.05}$	$D_{0.50}$	
1.5	182	96	105	101
4.5	185	183	187	185
13.5	196	189	202	196
Mean	161	156	165	

[1] Each figure represents the computed mean of 5 replicates.

Protein was precipitated from water extracts by ammonium sulfate, as described earlier (*1*), and employed for enzyme assay without dialysis. Phosphatase was measured by techniques described previously (*4*), as was β-amylase (*6*), invertase (*5*), polyphenol oxidase (*12*), and peroxidase (*16*). Protein content of the enzyme preparations was determined by the method of Sutherland *et al.* (*24*), and enzyme action was recorded as specific activity (activity units per milligram of protein). Diuron content of leaves was measured by the method of Dalton and Pease (*19*).

RESULTS AND DISCUSSION

FRESH-WEIGHT AND SUGAR RESPONSES

No Diuron toxicity symptoms appeared during the study, nor were any inhibitory growth effects evidenced by fresh-weight data (table 1). As

[5] Thanks are extended to E. I. Dupont de Nemours Co. for a gift of recrystalized diuron.

expected, plants receiving medium and high NO_3 levels revealed marked growth increases. Curiously, the growth increases occurred mainly between the low and medium NO_3 treatments, *i.e.*, between 1.5 and 4.5 meq./liter. Very little additional growth was obtained by increasing NO_3 from 4.5 to 13.5 meq./liter.

Leaf analyses for Diuron clearly reflected the variable herbicide supply via nutrient solutions (table 2). Diuron content ranged about 4 to 6 times higher among high-Diuron treatments than among those receiving the medium level. Traces of Diuron among low-Diuron samples, *i.e.*, from plants given zero Diuron, possibly resulted from contamination of the original seed which was grown upon soil frequently treated with commercial herbicides.

TABLE 2.—*Diuron content of leaves from immature sugarcane supplied with variable nitrate and Diuron in sand culture*[1]

NO_3 (meq./liter)	Diuron content (μg./g. of dry weight)			Mean
	D_0	$D_{0.05}$	$D_{0.50}$	
1.5	0.7	2.6	14.8	6.0
4.5	1.4	2.2	14.8	6.1
13.5	0.7	3.0	11.5	5.1
Mean	0.9	2.6	13.7	

[1] Each figure was derived by analysis of a composite of 5 replicates.

Leaf-sucrose values clearly reflect a depressing effect of Diuron upon sucrose formation (table 3). Interpreting these data from a nitrogen standpoint, it can be said that the low-nitrate treatment, which is known to induce high sucrose (*9,19,20*), was currently able to do so only in the relative absence of Diuron. Conversely, Diuron suppression of sucrose was far more pronounced when NO_3 was low.

It is significant to note that, at the medium level of NO_3, there was an increase of sucrose, in response to 0.05 p.p.m. Diuron (fig. 1). Raising Diuron to 0.50 p.p.m. still suppressed sucrose, yet the implication is clear that at some critical level of nitrogen a limited amount of Diuron can stimulate sucrose formation raher than retard it. This may help account for claims that Diuron has increased sucrose yields in the field. Again, our own data have shown that medium NO_3 was likewise critical with regard to increased growth. Possibly more attention should be given to soil fertility and fertilization programs when developing Diuron recommendations for field usage.

Another important consideration is the mechanism by which Diuron

TABLE 3.—*Mean values for leaf and meristem sugars of immature sugarcane supplied with variable nitrate and Diuron in sand culture*[1]

NO_3 (meq./liter)	Leaf sugars (mg./g. of dry weight)											
	Total ketose				Sucrose				Fructose			
	D_0	$D_{0.05}$	$D_{0.50}$	Mean	D_0	$D_{0.05}$	$D_{0.50}$	Mean	D_0	$D_{0.05}$	$D_{0.50}$	Mean
1.5	155	103	124	127	147	87	81	105	8	16	43	22
4.5	125	138	84	116	118	135	76	109	7	3	8	6
13.5	138	111	156	135	103	91	86	93	35	20	70	42
Mean	139	117	121		123	104	81		17	13	40	

NO_3 (meq./liter)	Meristem sugars (mg./g. of dry weight)											
	D_0	$D_{0.05}$	$D_{0.50}$	Mean	D_0	$D_{0.05}$	$D_{0.50}$	Mean	D_0	$D_{0.05}$	$D_{0.50}$	Mean
1.5	199	242	298	246	41	40	33	38	158	171	167	165
4.5	212	252	242	235	27	25	18	23	214	228	227	223
13.5	200	245	275	240	34	20	20	24	263	222	255	247
Mean	204	246	272		34	28	23		212	207	216	

[1] Each figure represents the computed mean of 5 replicates.

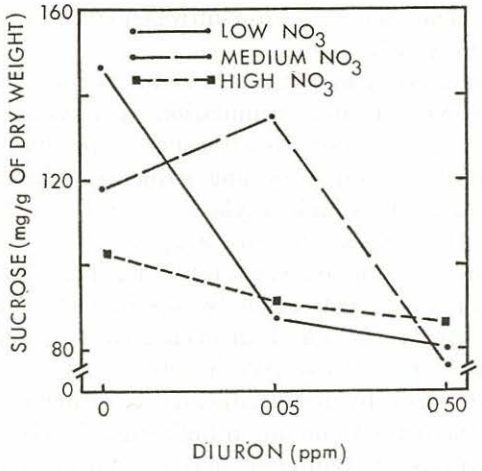

FIG. 1.—Effects of Diuron on leaf sucrose content of sugarcane grown with variable nitrate supply in sand culture.

prevented increased leaf sucrose by low NO_3. The low-NO_3 effect has been vaguely attributed to "physiological stress", and more recently it was found that low NO_3 causes major increases of nucleotides and organic phosphate (*14*). It is also known that Diuron retards ATP synthesis (*22*). Since sucrose precursors are likely involved, it will be interesting to observe in future studies whether Diuron can block the synthesis of cane nucleotides and organic phosphates.

Leaf-fructose values also exemplify strong NO_3 and Diuron effects. In particular, the very low fructose content at the medium NO_3 level reflects again a biochemical status at variance with that of the low- and high-NO_3 plants. Fructose content was consistently high in response to the high-Diuron treatment. Since fructose is able to reverse the Diuron inhibition of sucrose synthesis (*3*), fructose accumulation may indicate an effort on the part of the plants to counteract high Diuron.

Meristem sucrose was generally suppressed both by increasing NO_3 and increasing Diuron (table 3).

DIURON-NITRATE-ENZYME RELATIONSHIPS

Leaf phosphatase activity was stimulated by increasing both NO_3 and Diuron (table 4). This supports earlier observations of both NO_3 effects (*1,15*) and those of Diuron (*2*) upon phosphatase. Phosphatase stimulation is very likely one means of retarding sucrose by limiting the supply of phosphorylated precursors. It should be noted that Diuron failed to increase phosphatase action when the NO_3 supply was low.

Leaf amylase was only moderately stimulated by high NO_3 and high Diuron, and not to the degree we had expected (table 4). Suppressed amylase action is characteristic of low-nitrogen status and of tissues containing high sucrose (*1,15*).

During the present study amylase behaved as if both 1.5 and 4.5 meq./liter were low for NO_3. Diuron stimulation of amylase, if continued or intensified, could possibly interfere with sucrose production by diverting glucose phosphate into a starch-forming sequence. This interpretation is based on our conclusion that high amylase action in cane reflects an active polysaccharide-synthesizing mechanism (*6,7,8,13*).

Diuron stimulated leaf peroxidase (table 4). This agrees with earlier observations of peroxidase activation by residual Diuron in ratoon cane (*2*). Medium and high NO_3 caused an even greater stimulatory effect on peroxidase. Highly active peroxidase has been noted in tissues low in sucrose (*1,9*). Presumably, by increased oxidation of metabolites, it becomes more difficult for the plant to maintain high sugar levels.

With the exceptions of β-amylase and polyphenol oxidase, meristem enzymes did not reveal striking effects of NO_3 or Diuron (table 5). With regard to amylase, the enzyme clearly indicated a distinct nitrogen-Diuron

TABLE 4.—*Mean specific-activity values for leaf enzymes of immature sugarcane supplied with variable nitrate and Diuron in sand culture*[1]

NO_3 (meq./liter)	Phosphatase				Amylase			
	D_0	$D_{0.05}$	$D_{0.50}$	Mean	D_0	$D_{0.05}$	$D_{0.50}$	Mean
1.5	6.4	5.3	6.1	5.9	39	42	45	42
4.5	6.4	7.4	7.7	7.2	39	39	45	41
13.5	8.1	8.3	11.0	9.1	46	51	57	51
Mean	6.5	7.0	8.3		41	44	49	
NO_3 (meq./liter)	Polyphenol oxidase				Peroxidase			
	D_0	$D_{0.05}$	$D_{0.50}$	Mean	D_0	$D_{0.05}$	$D_{0.50}$	Mean
1.5	12	16	21	16	8.0	8.0	14	10
4.5	20	23	20	21	15	19	20	18
13.5	19	18	21	19	21	31	31	28
Mean	17	19	21		15	19	22	

[1] Each figure represents the computed mean of 5 replicates.

TABLE 5.—*Protein content and enzyme specific activity for meristem samples of immature sugarcane supplied with variable nitrate and Diuron in sand culture*[1]

NO_3 (meq./liter)	Protein (mg./g. of dry weight)				Phosphatase				Amylase			
	D_0	$D_{0.05}$	$D_{0.50}$	Mean	D_0	$D_{0.05}$	$D_{0.50}$	Mean	D_0	$D_{0.05}$	$D_{0.50}$	Mean
1.5	17	17	15	16	14	10	19	14	30	34	14	26
4.5	19	23	19	21	15	9	15	13	23	31	38	31
13.5	22	22	23	22	10	10	11	10	36	36	37	36
Mean	19	21	19		13	10	15		29	34	29	
NO_3 (meq./liter)	Invertase				Polyphenol oxidase				Peroxidase			
	D_0	$D_{0.05}$	$D_{0.50}$	Mean	D_0	$D_{0.05}$	$D_{0.50}$	Mean	D_0	$D_{0.05}$	$D_{0.50}$	Mean
1.5	7.6	7.0	8.0	7.5	23	23	26	24	8.2	6.7	9.4	8.1
4.5	7.9	7.2	8.2	7.9	20	19	15	18	7.7	7.0	8.4	7.7
13.5	5.2	7.0	6.6	6.3	12	11	11	11	7.5	6.7	7.1	7.1
Mean	6.9	7.1	7.7		18	18	17		7.8	6.9	8.3	

[1] Each figure represents the computed mean of 5 replicates.

interaction established between 1.5 and 4.5 meq./liter of NO_3. Increasing NO_3 from low to medium suppressed amylase when Diuron was low, but greatly increased amylase action when Diuron was high. Conversely, increasing Diuron from low to high markedly retarded amylase at 1.5 meq./liter NO_3 and activated the enzyme at 4.5 meq./liter NO_3. Again, the interaction itself is not as surprising as is its appearance between two relatively low NO_3 treatments. Thus, the critical nature of NO_3 supply in determining Diuron effects upon a biochemical system is once more demonstrated.

Polyphenol oxidase was generally stimulated by decreasing NO_3 supply. Significantly, variable Diuron scarcely altered the enzyme trends. Abnormally high polyphenol oxidase has been observed previously in plants receiving low nitrogen, and it has been suggested that this enzyme could be used as an indicator of plant nitrogen status (*10,11*).

SUMMARY

Diuron, a substituted urea herbicide, was supplied to sugarcane in factorial combination with variable nitrate. Employing the sand-culture technique, Diuron was given at rates of 0, 0.05, and 0.50 p.p.m., and nitrate at 1.5, 4.5, and 13.5 meq./liter. There were three objectives: 1, To confirm a theory that Diuron is capable of retarding sucrose production; 2, that Diuron rather than soil factors directly influence enzymes related to sugar level; and 3, to study persistence of Diuron-sugar-enzyme relationships under differing conditions of nitrogen status.

Plant growth was not affected by Diuron, but it increased markedly between 1.5 and 4.5 meq./liter of nitrate. Leaf analyses gave an average of about 2.5 and 14 μg. of Diuron per gram of tissue, respectively, for the medium and high Diuron treatments. Variable nitrate did not affect Diuron content.

Diuron suppressed leaf-sucrose content and increased fructose as main effects. However, at the medium nitrate level, sucrose was increased by the 0.05-p.p.m. Diuron treatment. Both Diuron and increasing nitrate suppressed meristem sucrose. Thus, both the ability of Diuron to suppress sucrose and a dependence of Diuron action upon nitrogen status was demonstrated.

Phosphatase and peroxidase were activated by Diuron, confirming earlier observations with ratoon samples from Diuron-treated soil. Amylase was moderately increased by Diuron. Meristem amylase reflected a marked interaction between Diuron and nitrogen. Polyphenol oxidase was greatly stimulated by decreasing nitrate, regardless of Diuron supply.

RESUMEN

A plantas de caña de azúcar cultivadas en arena se les suministró Diuron, que es un yerbicida de un derivado de urea, en tratamientos combinados

con niveles variables de nitrato. Mediante la técnica para cultivos en arena, se aplicó el Diuron a razón de 0, 0.05 y 0.50 partes por millón, y el nitrato a razón de 1.4, 4.5 y 13.5 miliequivalentes por litro. Los objetivos eran tres: 1, Confirmar la teoría de que el Diuron es capaz de retardar la producción de sacarosa; 2, que el Diuron, más bien que los factores del suelo, influye directamente sobre las enzimas relacionadas con el nivel de azúcar; y 3, estudiar la permanencia de las relaciones entre el Diuron, el azúcar y las enzimas bajo los distintos niveles de nitrógeno.

El Diuron no afectó el crecimiento de las plantas, pero éste sí fue afectado marcadamente al aplicárseles entre 1.5 y 4.5 meq/l. de nitrato. Los análisis foliares revelaron, en promedio, un contenido de aproximadamente 2.5 y 14 microgramos de Diuron por gramo de tejido, cuando recibieron los tratamientos mediano y alto de Diuron, respectivamente. Las variaciones en los niveles de nitrato no afectaron el contenido de Diuron.

Los dos efectos principales del Diuron fueron una disminución en el contenido de sacarosa en la hoja y un aumento en el de fructosa. Sin embargo, al nivel intermedio de nitrato, la aplicación de 0.05 partes por millón de Diuron produjo un aumento de la sacarosa. Tanto el Diuron como el nitrato, redujeron la sacarosa en el meristemo. Así quedó demostrado que el Diuron es capaz de reducir la sacarosa y que la acción del Diuron depende del nivel de nitrógeno.

El Diuron activó la fosfatasa y la peroxidasa, confirmando así las observaciones que se hicieron previamente con muestras de plantas de caña de de retoño procedentes de suelos tratados con Diuron. La amilasa aumentó moderadamente con la aplicación de Diuron. En la amilasa del meristemo se observó una marcada interacción entre el Diuron y el nitrógeno. La oxidasa de polifenol se estimuló notablemente al disminuir el nitrato, independientemente de la concentración del Diuron.

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