Interrelationships of Gibberellic Acid and Nitrate in Sugar Production and Enzyme Activity of Sugarcane

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INTRODUCTION

Both nitrate stress (*1,2,3*)[2] and foliar treatment with gibberellic acid (*4*) are known to alter the sugar-forming capacity of sugarcane. Work at this Station with nitrate (NO_3) and gibberellic acid (GA) has progressed sufficiently so that each can be used to raise leaf-sucrose production with considerable certainty. However, GA apparently stimulates both growth and sucrose production[3], whereas increasing nitrate seems to promote growth at the expense of sucrose synthesis (*1,2*).

Several questions therefore arise as to field usage of GA in areas of high nitrogen fertilization: 1, To what degree can nitrate be withheld from the plant before growth decline offsets sucrose gains? 2, To what extent will increasing NO_3 supply offset the beneficial effects of GA application? 3, Is there a combination of high NO_3 and GA which will permit both increased tonnage and increased sucrose synthesis as simultaneous effects? This paper summarizes greenhouse and laboratory studies aimed at clarifying nitrogen-GA relationships in immature sugarcane.

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[4] until treatments were begun at 12 weeks of age. A 3 × 3 factorial design was established with three levels each of NO_3 and GA. NO_3 was given at rates of 0, 5, and 30 meq./liter. From past experiments it is felt that "luxury consumption" of NO_3 begins at about 10 meq./liter. Thus, the 5 meq./liter treatment was an attempt to secure near-optimum growth plus the sucrose stimulatory effects of NO_3 deficiency. The 30 meq./liter treatment was purposely excessive, and zero NO_3 was aimed at securing

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 27–8.

[3] Unpublished data.

[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.

nutritional stress with concurrent sucrose increases. GA was applied once as a foliar spray. The 10-percent potassium salt was used in amounts equal to 0, 0.01-, and 0.10-percent solutions of the pure acid. Tween-20 was employed as wetting agent and all above-sand portions of the plants were sprayed until runoff had begun. The high GA level was already known to be in excess of that needed for optimum sucrose synthesis, under conditions of moderate NO_3 status.

A single harvest was made 5-weeks following 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 (*11*), and for sucrose by the modification of Cardini *et al.* (*10*). Fructose was estimated by subtracting sucrose values from those of total ketose. Protein was precipitated from water extracts with solid ammonium sulfate, as described earlier (*1*), and employed for enzyme assay without dialysis. Phosphatase and ATP-ase was measured by techniques described previously (*5*), as was β-amylase (*6*), polyphenol oxidase (*7*), and peroxidase (*8*). Protein content of the enzyme preparations was determined by the method of Sutherland *et al.* (*12*) and enzyme action was recorded as specific activity (activity units per milligram of protein).

RESULTS AND DISCUSSION

FRESH-WEIGHT RESPONSES

Considering that the plants were all healthy at the initial treatment, and that the experiment extended only 5 weeks, the changes in plant weight and appearance were remarkable. Medium GA caused general internode elongation while retaining stockiness of the cane. High GA induced more extensive elongation, so that the canes exhibited a weak and spindly condition plus occasional lodging.

Both NO_3 and GA caused moderate growth increases as evidenced by fresh weights (table 1). Generally, the high treatments were in excess of that needed for maximum growth as main effects, yet a combination of high NO_3 and medium GA gave the greatest yield of the study. Curiously, raising GA to high completely eliminated all NO_3 growth increases. The reader should also note that withholding NO_3 did not severely curtail growth when high GA was applied, and that these plants grew comparably to those given high NO_3 plus zero GA. One might surmise that GA can work well with NO_3 in promoting growth only if GA is used sparingly.

TABLE 1.—*Mean values for fresh weights of sugarcane supplied with variable gibberellic acid and nitrate in sand culture*[1]

NO_3 (meq./liter)	Results when indicated grams per plant were supplied			
	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean
0	80	122	130	111
5	126	132	136	131
30	128	162	122	137
Mean	111	139	129	

[1] Each figure represents the computed mean of 3 replicates; 4 plants were harvested from each replicate.

TABLE 2.—*Leaf and meristem sugar content of sugarcane treated with variable gibberellic acid and nitrate in sand culture*[1]

Leaf sugars (mg./g. of dry weight)—

NO_3 (meq./liter)	Total ketose				Sucrose				Fructose			
	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean
0	120	132	144	132	130	140	139	136	0	0	7	2
5	107	118	121	115	98	118	112	109	9	2	9	7
30	100	109	129	113	87	83	98	89	13	26	31	23
Mean	109	119	131		105	114	116	111	7	9	16	

Meristem sugars (mg./g. of dry weight)—

NO_3 (meq./liter)	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean
0	255	275	315	282	127	172	113	137	127	103	203	144
5	315	284	335	311	91	132	89	104	224	152	246	207
30	368	372	377	372	71	81	77	76	297	290	301	296
Mean	313	310	342		96	128	93	106	216	182	250	

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

SUGAR RESPONSES

Withholding NO_3 succeeded in raising sucrose content in both leaf and meristem tissues (table 2). Sucrose was progressively suppressed by medium and high NO_3. Fructose content was greatly increased by NO_3. The latter suggests that inversion rather than total ketose synthesis was altered by the

nitrogen treatments. GA slightly increased sucrose in leaves, and caused strong sucrose increases in storage tissue when NO_3 was low. However, the GA effect was reduced progressively by increasing NO_3 (fig. 1). As noted earlier (*4*) medium GA was superior to the high GA treatment in stimulating sucrose production. Thus while previous NO_3 and GA effects on sucrose were again verified, in no instance did GA compensate for sucrose lost within increasing NO_3 treatments.

It is concluded from sugar and fresh-weight data that the most favorable treatment was that which withheld NO_3, and stimulated both growth and

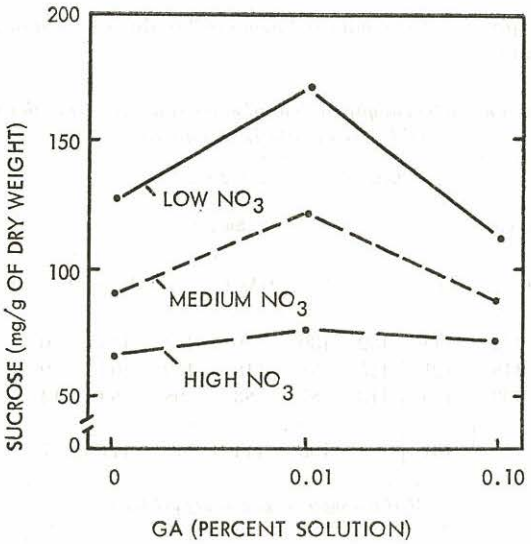

FIG. 1.—Sucrose content of immature storage tissue from sugarcane treated by foliar application of gibberellic acid, and grown with variable nitrate supply in sand culture.

sucrose production with medium GA. Greenhouse data are not directly applicable to field conditions, but the implication remains that GA treatment should be delayed after heavy nitrogen fertilization, and will not prove fully effective if given simultaneously with normal fertilizer programs.

LEAF AND MERISTEM ENZYMES

The most pronounced leaf-enzyme effects were the major stimulation of phosphatase and ATP-ase by NO_3 (table 3). One might also say that these enzymes declined as NO_3 was withheld, and this in itself might help account for much of the GA effects on sucrose. Biochemical consequences of exces-

sive phosphatase and ATP-ase action are thoroughly discussed in an earlier report (*1*). Meristem enzyme data presented in table 4 underscore a striking $NO_3 \times GA$ interaction upon both hydrolytic and oxidative systems. Increasing NO_3 levels severely retarded phosphatase and ATP-ase in the presence of high GA. Conversely, both enzymes were inhibited by increasing GA when NO_3 was high. Peroxidase and polyphenol oxidase were likewise retarded by combining high GA and high NO_3. However, unlike

TABLE 3.—*Mean specific-activity values for leaf enzymes, and leaf-protein content of sugarcane treated with variable gibberellic acid and nitrate in sand culture*[1]

	Leaf enzymes (specific activity)—											
	Phosphatase				ATP-ase				β-amylase			
NO_3 (meq./liter)	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean
0	3.6	4.0	4.6	4.1	4.6	4.9	5.3	4.9	3.7	3.8	3.4	3.6
5	6.4	5.1	5.8	5.8	7.4	5.8	6.9	6.7	3.8	3.0	2.8	3.2
30	7.4	7.2	5.7	6.8	9.6	9.1	7.0	8.6	2.7	2.9	3.0	2.9
Mean	5.8	5.4	5.4		7.2	6.6	6.4		3.4	3.2	3.1	
	Peroxidase				Polyphenol oxidase				Protein (mg./g. of dry weight)			
NO_3 (meq./liter)	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean
0	15.1	20.7	21.0	18.9	15.7	15.4	17.5	16.2	8.0	7.4	7.4	7.6
5	22.6	27.3	25.4	25.1	23.0	21.6	20.9	21.8	5.7	6.8	7.0	6.5
30	23.1	21.8	26.5	23.8	22.0	21.4	18.2	20.5	6.2	6.7	7.7	6.9
Mean	20.3	23.3	24.3		20.2	19.5	18.9		6.6	6.9	7.4	

[1] Each figure represents the computed mean of 3 replicates.

phosphatase and ATP-ase, GA succeeded in activating the oxidases when when NO_3 was withheld.

Evaluation of meristem protein values (table 4) helps clarify the above enzyme responses and gives insight into the action of both NO_3 and GA. NO_3 greatly increased protein and this effect was not reversed by GA. When GA was given, NO_3 caused a threefold increase of protein. Since enzyme specific activity is computed on a protein basis, *i.e.*, activity units per milligram of protein, it follows that specific activity decline from high NO_3 may be offset by the greater amount of protein made available. One might visualize an enzyme dilution effect by high protein, but one which

still permits an equal or greater amount of enzyme action to be accomplished.

Since GA stimulates growth it would not have been surprising to find increased protein content as a result of GA treatment. This did not occur. GA actually suppressed meristem protein when NO_3 was low. The fact that GA does not increase protein synthesis, while NO_3 does, may help explain the ability of GA to promote both growth and sugar formation. Growth stimulation by GA might reflect more efficient utilization of plant

TABLE 4.—*Mean specific-activity values for meristem enzymes, and meristem protein content of sugarcane treated with variable gibberellic acid and nitrate in sand culture*[1]

Meristem enzymes (specific activity)

	Phosphatase				ATP-ase				β-amylase			
NO_3 (meq./liter)	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean
0	9.5	9.7	10.5	9.9	11.0	10.5	10.2	10.6	6.8	5.5	6.9	6.4
5	8.4	8.3	6.4	7.8	8.5	8.0	5.9	7.5	4.5	4.5	5.8	4.9
30	9.0	8.0	5.7	7.6	9.1	7.4	4.9	7.1	5.8	5.2	5.3	5.4
Mean	8.9	8.7	7.5		9.5	8.6	7.0		5.7	5.1	6.0	

	Peroxidase				Polyphenol oxidase				Protein (mg./g. of dry weight)			
NO_3 (meq./liter)	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean	GA_0	$GA_{0.01}$	$GA_{0.10}$	Mean
0	9.2	12.6	16.3	12.7	11.2	16.8	18.0	15.3	15.4	9.5	9.7	11.5
5	7.7	11.4	8.4	9.2	8.9	15.8	10.8	11.8	26.8	24.0	24.4	25.1
30	8.3	8.5	7.5	8.1	10.2	8.9	9.5	9.5	26.3	29.9	29.3	28.5
Mean	8.4	10.8	10.7		10.1	13.8	12.8		22.8	21.1	21.1	

[1] Each figure represents the computed mean of 3 replicates.

constituents already available rather than a mobilization of additional materials. The fact that oxidases were stimulated by the same GA treatments which lowered protein synthesis (*i.e.*, at the low NO_3 level) leads one to suspect a more efficient regulation of catalytic protein.

In conclusion, it must be said that high NO_3 cannot apparently be employed to increase growth simultaneously with GA-increased sugar synthesis. Of course, it is likely that high NO_3 triggers a massive effort by the plant to add new tissues. The withholding of NO_3 may simply restrain the plant and permit more subtle reactions to progress. On the other hand,

recent work has shown that, when NO_3 is in low supply, there are produced additional constituents related to nucleotides (*9*). These apparently contribute to the increased sucrose-forming potential of sugarcane. It might therefore be possible to provide these factors to the plant via secondary treatments, thereby permitting a combination of high NO_3 and GA for greater growth and sugar production than is now possible.

SUMMARY

Variable nitrate (NO_3) and gibberellic acid (GA) were applied to sugarcane in order to clarify NO_3-GA interrelationships which affect growth, sugar production, and enzyme activity. Since both low NO_3 and foliar GA were known to increase sucrose synthesis as separate entities, their combination posed the following questions: 1, To what degree can NO_3 be withheld before growth decline offsets sucrose gains? 2, To what extent will increasing NO_3 supply offset the beneficial effects of GA application? 3, Is there a combination of high NO_3 and GA which will permit both increased tonnage and increased sucrose synthesis as simultaneous effects? Healthy, 12-week-old plants grown in sand culture were treated for 5 weeks. Three levels each of NO_3 (0, 5, and 30 meq./liter) and GA (0, 0.01-, and 0.10-percent solutions of foliar spray) were given in a 3 × 3 factorial combination.

The following results were obtained:

1. Both NO_3 and GA caused moderate growth increases as evidenced by fresh weights. A combination of high NO_3 and medium GA gave the maximum yields recorded. Medium GA stimulated internode elongation while retaining stockiness of the cane. High GA caused excessive elongation plus weakening and occasional lodging of the plants.

2. High GA eliminated all NO_3-induced growth increases. Withholding NO_3 did not seriously curtail growth so long as GA was applied. Plants given GA without NO_3 grew comparably to those receiving high NO_3 without GA.

3. Withholding NO_3 caused major sucrose increases in both leaf and immature storage tissues. Raising NO_3 increased synthesis of total ketoses but caused striking decline of sucrose. GA caused significant sucrose increases in storage tissue when NO_3 supply was low. However, GA-induced sucrose increases could not offset sucrose losses due to high NO_3.

4. The most favorable treatment for growth and sugar production was a combination of low NO_3 and medium GA. This induced moderate growth plus major sucrose increases.

5. Leaf phosphatase and ATP-ase were greatly stimulated by NO_3. High GA alleviated the NO_3 effects.

6. A strong NO_3 × GA interaction affected both hydrolytic and oxidative

enzymes in immature storage tissue. Phosphatase, ATP-ase, peroxidase and polyphenol oxidase were all involved.

7. NO_3 greatly increased protein content of immature storage tissue, but GA had little effect. GA was able to stimulate both growth and sugar formation without the major protein changes characteristic of NO_3 treatments. Practical usage of the NO_3 and GA data are discussed. It is felt that GA treatment should be delayed after heavy nitrogen fertilization, and will not prove fully effective if given simultaneously with normal fertilizer programs.

RESUMEN

Se aplicaron diferentes niveles de nitrato (NO_3) y ácido giberélico (AG) a plantas de caña de azúcar, con el propósito de aclarar las interrelaciones de estos compuestos que afectan el crecimiento, la producción de azúcar y la actividad enzimática. Como ya se sabía que tanto un nivel bajo de NO_3 como de AG en una aplicación foliar aumentan individualmente la síntesis de la sacarosa, la aplicación de estos compuestos en forma combinada sugería las siguientes preguntas: 1, ¿Hasta qué grado puede reducirse el NO_3 sin que la disminución del crecimiento afecte los aumentos de la sacarosa? 2, ¿Hasta qué punto un aumento en la concentración del NO_3 puede afectar la acción favorable de la aplicación del AG? 3, ¿Existe acaso una combinación de AG y NO_3, a una concentración alta, cuyos efectos permitan simultánemente tanto un aumento en el tonelaje como en la síntesis de la sacarosa?

Se trataron plantas saludables de 12 semanas de sembradas y cultivadas en arena, durante un período de 5 semanas. Se aplicaron tres niveles de NO_3 (0, 5 y 30 meq/l) y de AG (en forma de aspersión foliar con soluciones al 0, 0.01 y 0.10 por ciento) en un experimento de diseño factorial de 3×3.

Los resultados fueron los siguientes:

1. Los pesos húmedos indicaron que tanto el NO_3 como el AG causaron un aumento moderado en el crecimiento. Se obtuvo el mayor rendimiento al combinarse el NO_3, a una concentración alta, con el AG a una intermedia. La concentración intermedia de AG estimuló el alargamiento de los entrenudos, sin alterar el grosor de la caña. El AG a un nivel alto causó un alargamiento excesivo, y el debilitamiento y encamado ocasional de las plantas.

2. Los aumentos en el crecimiento inducidos por el NO_3 cesaron en presencia de un nivel alto de AG. La eliminación del NO_3 no impidió seriamente el crecimiento siempre que se aplicó el AG. El crecimiento de las plantas que sólo se trataron con AG fue comparable al de las tratadas con un nivel alto de NO_3, pero sin el AG.

3. La eliminación del NO_3 estimuló considerables aumentos de sacarosa,

tanto en la hoja como en los tejidos tiernos donde se acumula. Al aumentarse el NO_3, aumentó a su vez la síntesis de las cetosas totales pero esto causó una reducción del contenido de sacarosa. El AG produjo aumentos significativos de sacarosa en los tejidos donde se acumula cuando se suministró poca cantidad del NO_3. Sin embargo, los aumentos de sacarosa inducidos por el AG no pudieron compensar las pérdidas de ésta causadas por un nivel alto de NO_3.

4. El tratamiento más favorable para estimular el crecimiento y la producción de azúcar fue la combinación de un nivel bajo de NO_3 y un nivel intermedio de AG. Esto indujo un crecimiento moderado y un aumento mayor de sacarosa.

5. La ATP-asa y la fostatasa foliares fueron muy estimuladas por el NO_3. El AG a una concentración alta aminoró los efectos causados por el NO_3.

6. Una fuerte interacción del NO_3 y el AG afectó tanto las enzimas hidrolíticas como los oxidantes en los tejidos tiernos. También fueron afectadas la fosfatasa, la ATP-asa, la peroxidasa y la oxidasa de polifenol.

7. El NO_3 aumentó en grado sumo el contenido de proteína del tejido tierno, pero el AG apenas tuvo efecto alguno. El AG estimuló el crecimiento y la formación de azúcar sin que se registraran los grandes cambios en las proteínas que son característicos de los tratamientos con NO_3.

Se discute la aplicadión práctica que puede hacerse de los resultados obtenidos en las pruebas con NO_3 y AG. Se cree que la aplicación del AG debe posponerse hasta después que se abone a un nivel alto de nitrógeno, y que su acción no es plenamente efectiva cuando la aplicación se hace simultáneamente con las de los programas corrientes de abonamiento.

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