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Relationships of Gibberellic Acid to Water and Phosphorus in the Growth, Sugar Production, and Enzyme Behavior of Sugarcane

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INTRODUCTION

It is generally accepted that the maximum sucrose potential of sugarcane requires both a high tonnage of stalks and high degree of sucrose storage. In recent years (*10,13,16,18,26*)[2] a promising stimulant of stalk tissue production has been gibberellic acid (GA).[3] The chemical has also been useful in defining the physiological dimensions for basic studies of chemical growth control (*12*), desiccant action (*15*), and nitrogen stress (*10*).

However, several practical and basic questions persist concerning the physiological limits within which a growth stimulant remains compatible with commercial sugar-growing programs. Can the stimulant be effective in both wet and dry climates, or in cool and warm climates? Can growth stimulation be accountable to a few catalysts, or are all enzymes altered in a common manner? How must the increased growth for this year be reckoned with in the water and nutritional requirements for future ratoons? Such questions will accompany any promising agent for the increase of tonnage.

With this in mind, workers in Puerto Rico are gathering basic enzyme-sugar criteria which will serve as guidelines for managing a balanced growth *vs.* sucrose performance. Reported herein are two GA experiments in which plants were given variable water and P as concurrent treatments. There were three objectives: 1, To evaluate the growth-stimulating capacity of GA within distinct water and P regimes; 2, to discover GA-sugar and GA-enzyme responses which persist regardless of severe physiological stress; and 3, to explore the enzyme basis of water and P performance under extreme conditions of GA-stimulated growth.

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 165–6.

[3] Abbreviations: Gibberellic acid (GA); phosphorus (P); organic phosphorus (PO); adenosine triphosphate (ATP).

MATERIALS AND METHODS

GROWTH AND TREATMENT OF PLANT MATERIALS

Two experiments were conducted in the greenhouse using a sand-culture technique previously described (*1*).[4] All plants were of the variety P.R.980, and GA treatments were initiated at 14 weeks of age. For experiment 1, 3 water levels were established 10 days prior to GA treatment. These consisted of 1, 2, and 4 liters of water daily per 2-gallon container of plants. Regimes of insufficient, ample, and abundant water for growth were thereby created. For experiment 2, variable P treatments of 0, 6, and 30 meq./liter were begun 10 days before GA was applied.

Both experiments employed GA levels equivalent to 0, 0.01, and 0.10 percent of the pure acid. GA source was the 10-percent potassium salt. Tween 20 was used as wetting agent at the rate of 1.0 ml./liter of spray solution. The foliar treatments were applied with a hand sprayer until all above-sand tissues were visibly wet. This required about 25 to 30 ml. for each 14-week-old plant. Within both experiments the treatments were given in 3 × 3 factorial combinations. Randomized block designs were used with three replicates for each of the nine treatments.

For the GA × water experiment a single harvest was taken 34 days after GA treatment. The GA × P study was extended to 52 days before samples were taken. From each replicate four uniform plants were cut at the sand surface and total fresh weights, mature stalk weights, and internode lengths were recorded immediately. The basal 14 inches of leaves +1 to +4 and immature storage tissue were frozen in a mixture of Dry Ice and acetone. They were lyophilized, ground to a fine powder with a Wiley mill, and stored at 0°C until needed for enzyme and sugar analysis.

LABORATORY ANALYSES

Clarified water extracts of leaf and immature storage tissues were analyzed for total ketose by the resorcinol method of Roe (*22*), and for sucrose by the modification of Cardini *et al.* (*17*). Fructose was estimated by subtracting sucrose values from those of total ketose.

Protein was precipitated between 0 and 80-percent saturation with solid ammonium sulfate at room temperature and pH 5.6. The method of Sutherland *et al.* (*25*) was used to measure both the water-soluble protein of tissue samples and the protein content of enzyme preparations. Particular attention was given to hydrolytic and oxidative enzymes which have shown correlations with sugar level in cane. Acid phosphatase and ATP-ase was meas-

[4] Seedlings received only tapwater until 4 weeks of age. Thereafter all plants were given 1 liter of a complete nutrient solution and 1 liter of tapwater daily until water and P variables were established at 88 days.

ured by techniques described earlier (*4*), as were amylase (*6*), invertase (*5*), and peroxidase (*7*).

Total P and organic P (PO) content of leaves were determined as follows: Leaf powder was extracted for 1 hour with 0.1-M acetate buffer, pH 4.63. The buffer contained 0.10 μmole of tungsten per ml. to prevent phosphatase activity (*4*). The extract was centrifuged at 3,000 r.p.m. for 15 minutes. A 1.0-ml. sample of the clarified supernatant was brought to 3.0 ml. with HCl, the final concentration being 6 *N*. Samples were maintained in boiling water for 2 hours. They were cooled, clarified in the centrifuge, and diluted 1:10 with 0.10 M acetate buffer, pH 5.5. Total P was determined by the phosphomolybdic acid technique previously described (*1*). Inorganic P was

TABLE 1.—*Growth values for immature sugarcane supplied with variable water in sand culture and treated with foliar GA*[1]

Water	Mean values for—											
	Total fresh wt. (g./plant)				Millable stalk wt. (g./stalk)				Internode length (inches)			
	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean
	0	0.01	0.10		0	0.01	0.10		0	0.01	0.10	
Liters/day												
1	135	150	184	156	28	51	70	49	1.36	2.96	5.71	3.34
2	273	294	336	301	77	140	163	127	2.95	4.09	5.44	4.16
4	215	383	378	325	70	156	182	136	2.17	4.40	6.26	4.28
Mean	208	276	300		58	116	138		2.16	3.82	5.80	

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

measured in like manner, with the exception that the 6 *N* HCl solution was not heated. Organic P was estimated by subtracting the non-HCl hydrolyzed P from total P. Both organic and total P were recorded as percentage of the dry leaf weight.

RESULTS AND DISCUSSION

EXPERIMENT 1: GA × WATER

Growth Responses to GA and Water

Growth was greatly increased by both GA and water (table 1). As main effects the two factors were about equal in promoting stalk weight, whereas water was more effective than GA in raising total fresh weight. Combining an increase of water supply with foliar GA invariably magnified the growth

response. Considering that zero GA is actually normal for cane, the ability of 0.01-percent GA to double stalk weight was remarkable. The GA effect was more pronounced for stalks than for total fresh weight, indicating a more favorable ratio of stalk to leaf and sheath material in GA-treated plants.

Water supply was an unexpectedly strong factor in controlling the GA growth responses. Plants receiving only 1 liter per day made progressively more stalk tissue as the GA supply was raised. This response was not repeated when 2 or 4 liters of water were given daily. This is of considerable importance in that GA appears to strengthen the plant's efficiency in utilizing a limited water supply. This interpretation is supported by inter-

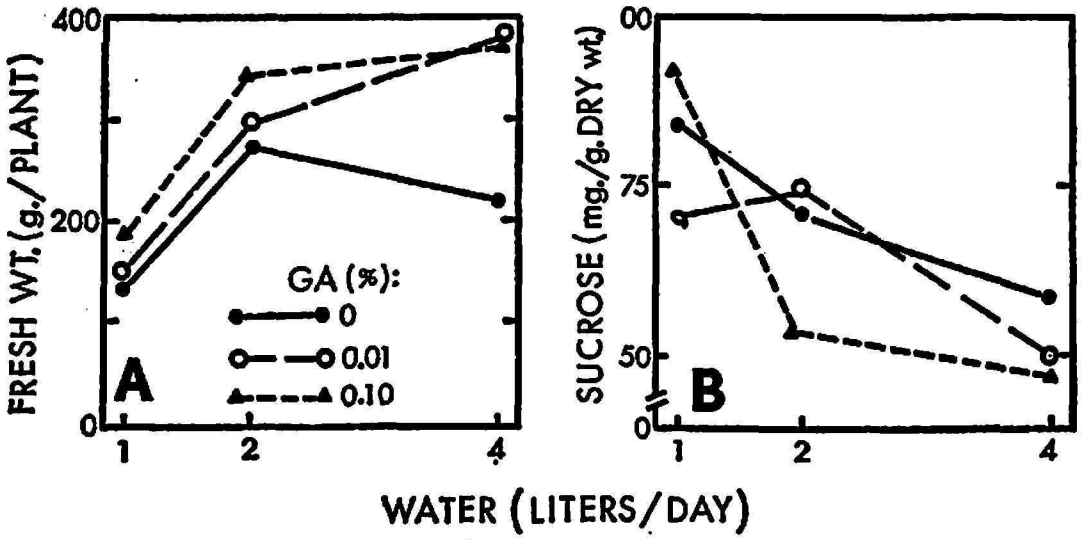


FIG. 1.—Growth and sugar responses of cane to variable water and GA treatment: A, Increased effectiveness of water in promoting growth when plants were treated with GA; B, General decline of sucrose in immature storage tissue as water supply was raised.

node-length data (table 1). It is also evident that GA increased water efficiency, even when water supply was ample (table 1, total fresh weights). When no GA was applied, the use of 2 liters gave excellent growth increases, but 4 liters failed to produce additional growth, and appeared to be in excess of plant needs. However, the plants sprayed with GA gave additional growth responses as water was increased from 2 to 4 liters (fig. 1). Internode lengths again reflect the water-GA relationship. The possibility that water can be utilized more efficiently in the presence of a growth stimulant deserves to be explored in detail. It is an especially pertinent concept for research in light of the increasingly inadequate water supplies for agriculture throughout the world.

With regard to cane production the present experiment has verified that GA can increase stalk weight as well as internode length (*12*). Earlier studies

with GA, under variable nitrate regimes, suggested that for maximum sucrose production GA should be witheld until well after the final nitrogen fertilization (*10*). With regard to water, it appears that GA will not approach maximum growth effectiveness unless an abundant water supply is also provided. This favors the use of GA long before a given crop has begun to ripen, either naturally or by controlled water supply. Cane grown in heavy-rainfall areas would supposedly respond better than the cane of drier regions.

SUGAR RESPONSES

No consistent differences were found in leaf-sugar content among any of the GA- and water-treated plants (table 2). Although growth was restricted

TABLE 2.—*Leaf-sugar content of immature sugarcane supplied with variable water in sand culture and treated with foliar GA*[1]

Water	Mean values (mg./g.) for—											
	Total ketose				Sucrose				Fructose			
	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean
	0	0.01	0.10		0	0.01	0.10		0	0.01	0.10	
Liters/day												
1	84.0	80.6	93.5	86.0	67.2	63.0	80.0	70.2	16.8	17.6	13.0	15.8
2	89.9	95.6	89.9	88.7	71.2	73.0	68.2	70.8	18.7	12.6	23.8	18.4
4	93.5	92.0	90.1	91.9	66.9	71.0	70.6	69.5	21.8	18.9	19.5	20.1
Mean	87.5	88.7	91.9		68.4	69.0	73.1		19.1	16.4	18.8	

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

among the low-water plants, there was no general wilting, and apparently there was sufficient water available to maintain a normal flow of photosynthate from leaf to storage tissues. Conditions of water-impaired transport, as described by Hartt and coworkers (*19,20*), were therefore not achieved with 1 liter of water per day. Sucrose content of immature storage tissue was suppressed both by increasing GA and water as main effects (table 3). However, under conditions of low water supply GA moderately increased sucrose. A similar effect of GA has been observed under conditions of low nitrate supply (*10*). *In vitro* experiments with leaf homogenates have shown that sucrose formation may be stimulated or retarded by GA additives (*14*). In immature storage tissue sucrose gains can often be attributed to invertase decline. GA has been shown to retard invertase in a previous study (*12*), and its enzyme effects during the present experiment warrant a detailed analysis.

ENZYME RESPONSES

Meristem enzymes were generally more sensitive to water and GA variables than was sugar content (table 4). In this instance each treatment was so dependent upon the other for enzyme expression that main-effect values have little meaning. Invertase was severely retarded by high GA, but only when water supply was low. The same was true of ATP-ase, amylase, and polyphenol oxidase (fig. 2). It is clear from table 4 and figure 2 that water supply completely transformed enzyme potential in several critical biochemical areas.[5] Thus some inkling is gained of the mechanisms by which water and GA so clearly affected growth. Undoubtedly a number

TABLE 3.—*Sugar content of immature storage tissue from sugarcane supplied with variable water in sand culture and treated with foliar GA*[1]

Water	Mean values (mg./g.) for—											
	Total ketose				Sucrose				Fructose			
	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean
	0	0.01	0.10		0	0.01	0.10		0	0.01	0.10	
Liters/day												
1	184	207	244	212	71.6	85.2	91.0	82.6	112	122	153	129
2	216	221	214	217	70.4	74.4	53.8	66.2	146	147	160	151
4	210	209	226	215	58.6	50.0	47.8	52.1	151	159	178	163
Mean	203	212	215		66.9	69.9	64.2		139	143	164	

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

of essential enzymes would have shown similar effects of water and GA had they been assayed.

Additional evidence is shown for a GA role in raising water-utilization efficiency. Since invertase, amylase, ATP-ase, and phosphatase are all hydrolytic enzymes, one anticipates their decline when water supply is low. But this occurred only in the presence of high GA. When no GA was given, the enzymes behaved as if 1 liter of water was perfectly adequate and that 2 and 4 liters were excessive. In other words, high GA so increased the sensitivity of the plants to varying water supply that 1 liter now resembled a drought condition, and 2 or 4 liters represented a return to normalcy rather than excessiveness. An easy way to picture the "sensi-

[5] Invertase and amylase are involved in carbohydrate hydrolysis. ATP-ase affects the content of high-energy phosphate, and polyphenol oxidase is considered the principal terminal oxidase in aerial cane tissues (8).

tivity" change is to visualize an increasing percentage of a constant water supply entering into nonenzymatic growth functions when GA is given. The new condition, in which a smaller amount of water is available for hydrolytic reactions, can be likened to a reduction in total supply when no GA is present.

TABLE 4.—*Mean values for immature storage-tissue enzymes from sugarcane given variable water in sand culture and treated with foliar GA*[1]

Water	Mean specific activity for—							
	Phosphatase				ATP-ase			
	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean
	0	0.01	0.10		0	0.01	0.10	
Liters/day								
1	36.6	33.0	8.8	26.1	39.4	34.9	9.2	27.8
2	23.1	26.0	26.1	25.1	32.9	26.9	23.1	27.6
4	29.6	26.1	23.1	26.3	29.6	26.7	23.7	26.7
Mean	29.8	28.4	19.3		34.0	29.5	18.7	
	Invertase				Amylase			
1	10.4	16.9	3.3	10.2	193	152	43	129
2	13.1	12.3	16.2	13.9	80	70	53	68
4	13.8	20.3	14.6	16.2	82	95	109	95
Mean	12.8	16.5	11.4		118	106	68	
	Polyphenol oxidase							
1	18.6	16.7	5.2	13.5				
2	11.0	12.3	10.0	11.1				
4	13.7	13.5	10.8	12.7				
Mean	14.4	14.2	8.7					

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

Under a low-water status the sucrose increases in immature storage tissue (table 3) appear to be a direct function of a strong GA suppression of amylase (table 4). Under a high-water status the GA-sucrose-amylase relationship was reversed. In a strictly academic sense high GA might be considered a ripening agent when water is limiting. Since the plant does not "know" that its water supply is limited, a GA-induced use of water for

nonenzymatic growth functions would serve as an internal withholding of water from amylase or invertase. Again speaking in an academic sense, one can now picture a single treatment forcing the last possible growth and simultaneously retarding enzymes which hydrolyze carbohydrates. This contrasts with the commercial practice of externally withholding water from the entire plant. The latter does bring about ripening, but it is not universally feasible, it is expensive, and it can lower tonnage through restricted growth.

The leaf enzymes phosphatase, ATP-ase, and amylase behaved much like their counterparts in immature storage tissue (table 5). High GA suppressed them only when water was low, and they were stimulated by increasing water primarily when GA was high.

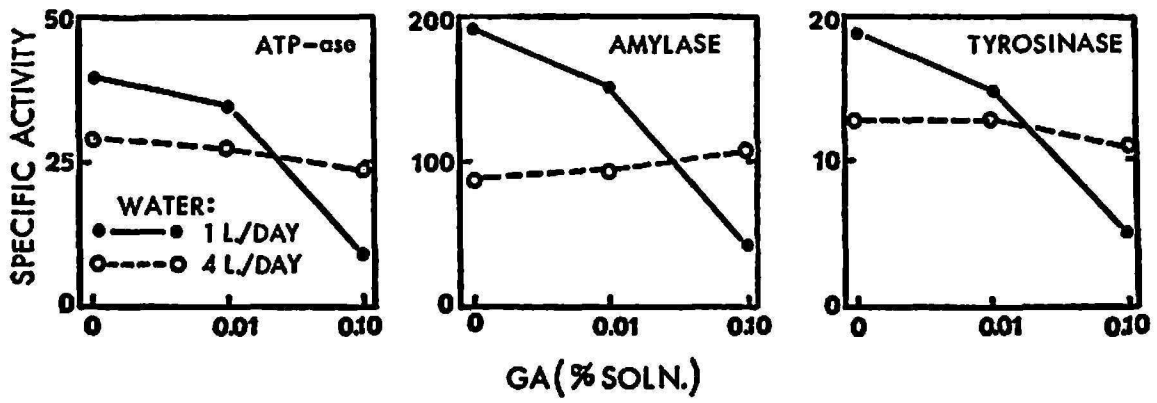


FIG. 2—Alleviating effects of high water supply on the GA suppression of ATP-ase, amylase, and tyrosinase from cane storage tissue.

EXPERIMENT 2. GA × P

Growth Responses to GA and P

The additional 2½ weeks given experiment 2 are reflected in greater fresh weights, particularly those of millable stalks, and a less pronounced GA growth stimulation than was found in experiment 1 (table 6). Small growth increases were still evident for 0.01-percent GA where P was withheld. Mean internode length was greater owing to increased internode number with age, but significant GA elongation was also apparent.

Although no P-deficiency symptoms were recorded, leaf analyses suggest that all low-P plants were experiencing "hidden hunger".[6] Main growth effects for P verify an inadequate P supply for maximum fresh weights (table 6). Internode lengths were also shorter among plants from which P was withheld. Under a limited-P status GA was effective in promoting both

[6] A liberal interpretation of leaf P percentages for 4-month cane is given as follows: 0 to 0.07, visually deficient; 0.07 to 0.15, deficient without symptoms; 0.15 and higher, adequate (*21*).

TABLE 5.—*Leaf-enzyme values for sugarcane supplied with variable water in sand culture and treated with foliar GA*[1]

Water	Mean specific activity for—							
	Phosphatase				ATP-ase			
	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean
	0	0.01	0.10		0	0.01	0.10	
Liters/day								
1	20.7	16.6	13.9	17.1	22.2	22.4	16.8	20.5
2	17.2	22.3	20.3	19.9	24.7	29.6	27.2	27.2
4	15.8	17.9	16.0	16.6	25.0	26.9	25.4	25.8
Mean	17.9	19.0	16.7		24.0	26.3	23.1	
	Amylase							
1	176	201	84	154				
2	186	200	165	184				
4	116	162	130	136				
Mean	159	188	126					

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

TABLE 6.—*Growth values for immature sugarcane supplied with variable P in sand culture and treated with foliar GA*[1]

P	Mean values for—											
	Total fresh wt. (g./plant)				Millable stalk wt. (g./stalk)				Intermode length (inches)			
	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean
	0	0.01	0.10		0	0.01	0.10		0	0.01	0.10	
Meq./liter												
0	359	401	322	361	175	214	170	186	3.72	4.87	6.96	5.18
6	422	387	350	386	191	193	210	198	4.38	5.62	7.56	5.85
30	473	469	431	458	233	247	219	233	4.95	5.70	7.41	6.02
Mean	418	419	368		199	218	199		4.35	5.39	7.31	

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

fresh weight and internode elongation. Under conditions of adequate or abundant P supply, GA also elongated internodes but did not appreciably increase fresh weight. This supports earlier observations that cane responds more readily to GA when under stress (*10,12,13,16*).

Sugar Responses

The GA and P variables had very little effect on sugars of leaf and immature storage tissue. Sugar content was unusually low for both tissues. This may be related to the traditionally heavy growth of greenhouse cane during September and early October in Puerto Rico. Fructose consistently increased in storage tissue as P supply was raised (table 7). High GA moderately raised the leaf sucrose of plants lacking P. This is another example of GA action under stress, because GA treatment of plants given adequate or abundant P led to the lowest sucrose values of the study.

TABLE 7.—*Sugar content of immature storage tissue from sugarcane supplied with variable* P *in sand culture and treated with foliar GA*[1]

P	Mean values (mg./g.) for—											
	Total ketose				Sucrose				Fructose			
	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean	GA (percent soln)—			Mean
	0	0.01	0.10		0	0.01	0.10		0	0.01	0.10	
Meq./liter												
0	132	143	142	139	32.0	35.4	45.6	37.7	100	108	96	101
6	151	139	139	143	25.2	28.0	24.6	25.9	126	111	114	117
30	179	172	164	172	29.8	29.6	23.6	27.7	149	142	140	144
Mean	154	151	148		29.0	31.0	31.3		125	120	117	

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

Enzyme Responses

The GA × P study presented a rare opportunity to evaluate cane phosphatase behavior in relation to P supply. In theory the level of phosphatase present should reflect the PO substrate levels and the current need for the plant to hydrolyze them. Other factors being equal, both P and PO should respond to increasing P supply in the growing medium. Specific PO/P ratios would presumably fluctuate with biochemical needs of growth and maturation, the latter being mediated by phosphorylase and phosphatase action.

Leaf P analyses clearly reflect variations in the nutrient's supply (table 8). Under conditions of zero P supply, leaf P content approached, but did not reach the low levels usually associated with P-deficiency symptoms (0–0.07 percent). A definite luxury consumption is evidenced for leaf samples from the high P treatment. Significantly the PO content also increased with P supply.

Several interesting GA-P relationships were recorded. Total P content was increased to some extent by GA in the zero-P treatment (table 8). The magnitude of the increase, in the area of 0.035 percent, was not great. Yet this amount could mean the difference between "hidden hunger" and severe visible deficiency. Most agricultural plants openly deficient of a major nutrient cannot compare in yield with those having only a hidden need.

It is also significant that about three-fourths of the GA-induced P increase is accountable to the organic form (table 8). The PO fraction should include critical phosphates such as intermediates of sugar synthesis and metabolism, high-energy phosphates, and related P compounds.

TABLE 8.—*Leaf P content of sugarcane supplied with variable P in sand culture and treated with foliar GA*[1]

P	Mean values (percent) for—							
	Total P				Organic P			
	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean
	0	0.01	0.10		0	0.01	0.10	
Meq./liter								
0	.076	.090	.112	.093	0	.014	.027	.014
6	.247	.209	.281	.246	.043	.032	.056	.044
30	.490	.472	.418	.460	.112	.103	.099	.105
Mean	.271	.257	2.70		.052	.050	.061	

[1] Each P value was determined with aliquot samples from 3 combined replicates.

Undoubtedly these give the P increase far more significance than its size would imply. Whereas an improved mobilization of P reserves may account for the total P increase, it is probable that retarded phosphatase or phosphorylase must account for the PO fraction. Phosphatase and ATPase were in fact suppressed by GA (table 9), especially where P supply was low (fig. 3). Since both enzymes are hydrolytic, one explanation for the decline is that water was functionally diverted to nonenzymatic roles under the GA regimes. Water was not originally intended to be limiting in this study and all containers received 2 liters per day.

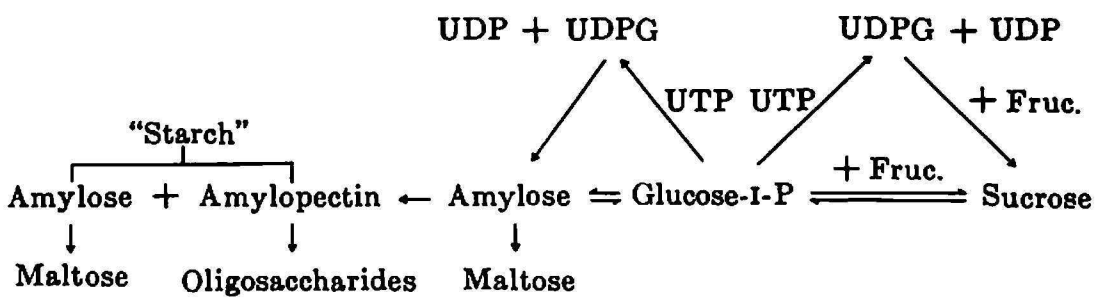
At the medium P level a sharp decline of leaf PO content was noted in response to medium GA (table 8). This is significant in light of increased amylase action in the same samples (table 9), and seems to support the theory that amylase suppression is an essential condition for maximum sugar synthesis (*3*). It has been proposed that amylase suppression permits

TABLE 9.—*Leaf-enzyme values for sugarcane supplied with variable* P *in sand culture and treated with foliar GA*[1]

P	Mean specific activity for—							
	Phosphatase				ATP-ase			
	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean
	0	0.01	0.10		0	0.01	0.10	
Meq./liter								
0	10.5	9.4	8.3	9.4	29.4	22.0	18.8	23.4
6	12.9	13.1	11.4	12.5	27.8	24.5	27.9	26.7
30	11.3	11.8	10.0	11.0	23.8	25.5	21.2	23.5
Mean	11.6	11.4	9.9		27.0	24.0	22.6	
	Amylase				Peroxidase			
0	40.4	39.9	33.4	37.9	93.5	74.1	60.8	76.1
6	38.8	62.4	46.6	49.3	59.0	57.6	62.4	59.1
30	45.6	58.6	47.1	48.9	31.2	45.6	50.7	42.5
Mean	41.6	53.6	42.4		61.2	59.1	57.9	

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

the following reactions to shift to the right (*3,11*):



Excessive amylase action would thus favor starch rather than sucrose synthesis. This activity might then impose a drain upon several sugar and uridine phosphates related to starch formation. The GA-induced loss of PO, coupled with the GA stimulation of amylase (fig. 4) tends to give indirect support to this theory.

Amylase activity in immature storage tissue was increased by P. This was especially true among those plants that received GA (table 10). These values are not especially high, however, and they probably represent a more vigorous growth potential rather than biochemical complications.

Leaf peroxidase was quite sensitive to variable P supply, and to GA in plants from which P was withheld (table 9). The enzyme was about three

times more active in low-P plants when no GA was applied. Cane peroxidase has sometimes been correlated with sucrose level (*1,2*). The enzyme has been studied in some detail but no conclusion has been reached concerning its role in sugarcane (*7*). During the present study its decline generally accompanied a return to adequate or abundant P content (fig. 5). A sug-

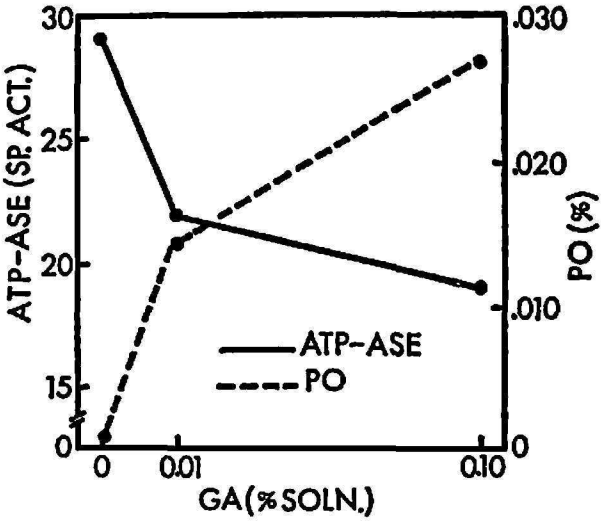


FIG. 3.—Increased PO content and ATP-ase decline in leaves of P-deficient cane treated with GA.

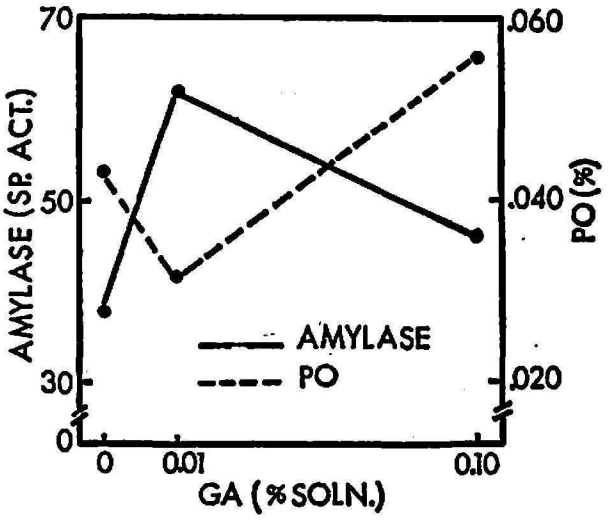


FIG. 4—Inverse relationship between PO content and amylase activity of leaves from sugarcane treated with GA.

gested explanation is that increased peroxidase activity was part of the plant's effort to prevent pathological invasions which often accompany a weakened nutritional regime.

Although P is unquestionably an essential factor in sugarcane biochemistry, fertilizer studies in Puerto Rico have shown little demand by cane for phosphates (*23*). Application of 100 to 150 pounds of P_2O_5 per acre have been adequate for the most P-deficient soils. Up to 400 pounds per acre of

TABLE 10.—*Mean values for immature storage-tissue enzymes from sugarcane supplied with variable* P *in sand culture and treated with foliar GA*[1]

P	Mean specific activity for—							
	Phosphatase				ATP-ase			
	GA (percent soln.)—			Mean	GA (percent soln.)—			Mean
	0	0.01	0.10		0	0.01	0.10	
Meq./liter								
0	9.6	10.5	9.6	9.9	15.7	17.7	16.2	16.5
6	9.2	11.5	10.6	10.4	14.6	13.7	17.5	15.2
30	8.9	9.2	9.8	9.3	11.8	13.6	12.3	12.6
Mean	9.2	10.4	10.0		14.0	15.0	15.3	
	Amylase				Invertase			
0	28.7	24.7	25.7	26.4	7.7	9.0	7.2	8.2
6	29.8	33.9	35.6	33.1	8.9	8.6	10.1	8.8
30	34.1	40.1	41.9	38.7	7.9	8.8	10.1	9.1
Mean	30.9	32.9	34.4		8.2	8.8	8.9	

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

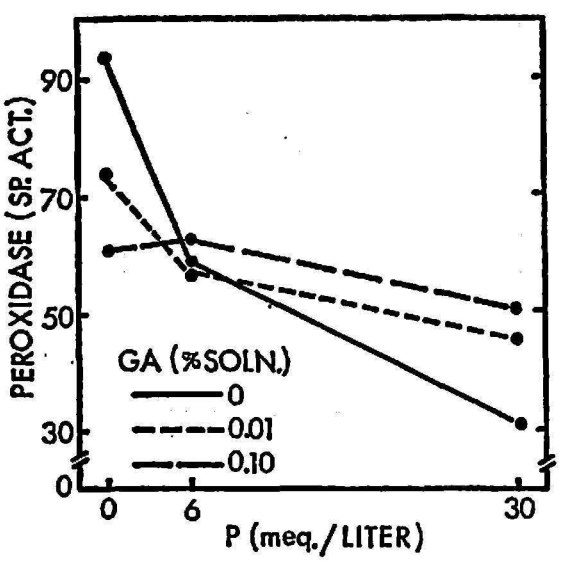


FIG. 5—Leaf peroxidase decline in cane given increasing P in sand culture, and treated with foliar GA.

P_2O_5 have given little to no response. Although field experiments tell us that available soil phosphates are sufficient for plant needs, they are not inherently designed to tell us what these needs are.

Sand-culture studies by Samuels and Cibes (*24*) revealed growth and

yield decline by four varieties after P was withheld for 6½ months. Alexander (*3*) reported sugar and enzyme variations among two varieties when P was withheld between 4 and 12 weeks of age. In a later study it was shown that enzymes respond almost immediately to downward shifts in P supply, whereas several months can pass before symptoms of P deficiency appear (*9*).

It has been suggested that more attention should be given to organic P levels in sugarcane (*14*). The present GA × P study underlines this point. Quite likely the measurement of changing organic P levels would be instructive even if P itself was not a treatment variable.

SUMMARY

Immature sugarcane was subjected to variable water and phosphorus (P) supply and then treated with foliar gibberellic acid (GA). All plants were grown in sand culture and received initial water and P treatments at 88 days of age. Water regimes of inadequate, adequate, and abundant supply were established with 1, 2, and 4 liters of water per day, respectively. Variable P included 0, 6, and 30 meq./liter. Foliar GA was given as 0-, 0.01-, and 0.10-percent solutions. There were three objectives: 1 To determine the effectiveness of GA as a growth stimulant and regulator of sugar-enzyme relationships under conditions of water and P stress; 2, to explore physiological limits within which GA-enzyme relationships persist; and 3, to explore the enzyme basis of water and P performance under extreme conditions of GA-stimulated growth. The following results were recorded:

1. Both water and GA had greatly increased stalk weight and internode length 5 weeks after GA treatment.
2. Water supply strongly affected GA-growth responses. Water-deficient plants were proportionately more stimulated by GA than water-rich plants. However, maximum growth required both GA and abundant water.
3. GA appeared to increase the efficiency of water utilization, regardless of the amount of water supplied.
4. Variable water supply severely transformed the behavior patterns of ATP-ase, amylase, invertase and polyphenol oxidase.
5. GA treatment of low-water plants appeared to increase the severity of water shortage. Hydrolytic enzymes were severely retarded by GA when water supply was low, but not when adequate or abundant water was available. On the basis of growth and enzyme data it was proposed that GA caused an internal redeployment of water so that the net quantity available for enzymatic functions was reduced. It was also proposed that GA might decrease the internal water supply while increasing growth, in contrast to the commercial practice of externally withholding water which decreases growth.
6. Low P was inadequate for maximum growth, but severe P deficiency

was not achieved. GA was proportionally more effective in promoting fresh weights and internode elongation when P supply was low.

7. GA moderately increased leaf P content when P supply was low. The increase was primarily organic P (PO) and this was attributed to GA suppression of phosphatase and ATP-ase. The significance of GA alteration of PO is discussed.

8. Evidence was found of a GA-induced PO decline mediated by increased amylase activity.

9. Leaf peroxidase was extremely sensitive to P supply, and to GA in P-hungry plants. The enzyme was excessively active in low-P × low-GA plants.

10. It is shown that cane growth and enzymology is far more sensitive to P than field experiments have indicated. The importance of PO, phosphatases and phosphorylase, as contrasted to total P content, is stressed.

RESUMEN

A plantas inmaduras de caña de azúcar se les suministró una cantidad variable de agua y fósforo (P), aplicándoseles luego una aspersión foliar de ácido giberélico (AG). Las plantas se cultivaron en arena y recibieron tratamientos iniciales de agua y de P a los 83 días de edad. El suministro de agua se reguló, calificándose de inadecuado, adecuado y abundante, con 1, 2 y 4 litros de agua por día, respectivamente. El P variable consistió de 0, 6 y 30 meq. por litro. El AG foliar se suministró en soluciones al 0, 0.01 y 0.10 por ciento. Fueron tres los objetivos: 1, Determinar la eficacia del AG como estimulante del crecimiento y regulador de las relaciones azúcar-enzima al restringirse el agua y el P; 2, explorar los límites fisiológicos dentro de los cuales las relaciones AG-enzima persisten; y 3, explorar la base enzimática de la acción del agua y el P bajo condiciones extremas de crecimiento estimulado por el AG. Se registraron los siguientes resultados:

1. Cinco semanas después del tratamiento con AG, tanto el agua como el AG habían aumentado grandemente el peso de la caña y la longitud del entrenudo.

2. El suministro de agua afectó marcadamente el efecto del AG sobre el crecimiento. Las plantas carentes de agua fueron proporcionalmente más estimuladas por el AG que las que recibieron agua en abundancia. Sin embargo, el crecimiento máximo requirió tanto AG como agua en abundancia.

3. El AG pareció aumentar la eficiencia de las plantas en utilizar el agua, independientemente de la cantidad suministrada.

4. El suministro variable de agua transformó seriamente los patrones de comportamiento de la ATP-asa, la amilasa, la invertasa y la oxidasa de polifenol.

5. La aplicación de AG a plantas con un suministro restringido de agua

pareció hacer aún más severa la escasez de agua. La acción de las enzimas hidrolíticas fue retardada severamente por el AG cuando el suministro de agua fue bajo, pero no así cuando la cantidad de agua disponible fue adecuada o abundante. A base de los datos que se obtuvieron sobre el crecimiento y la acción de las enzimas, se propone que el AG causó una redistribución interna del agua de tal suerte que la cantidad neta disponible para la función enzimática disminuyó. Se propone, además, que el AG puede disminuir el suministro interno de agua mientras aumenta el crecimiento, en contraste con la práctica comercial de restringir externamente el agua, lo cual reduce el crecimiento.

6. Las cantidades bajas de P fueron inadecuadas para inducir un crecimiento máximo pero no causaron una seria deficiencia de P. El AG fue proporcionalmente más efectivo en aumentar el peso fresco de la caña y en alargar el entrenudo cuando el suministro de P fue bajo.

7. El AG aumentó moderadamente el contenido de P en la hoja cuando el suministro de P fue bajo. El aumento fue primordialmente de P orgánico (PO), lo cual puede atribuírse a la supresión de la fostatasa y la ATP-asa por el AG. Se discute el significado de la alteración orgánica del P por el AG.

8. Se evidenció una disminución del PO inducida por el AG, mediante un aumento en la actividad de la amilasa.

9. La peroxidasa foliar fue extremadamente sensitiva al suministro de P, y al de AG en aquellas plantas deficientes en P. Esta enzima actuó vigorosamente en las plantas que recibieron cantidades bajas tanto de P como de AG.

10. Se demuestra que el crecimiento de la caña y su enzimología son más sensitivos al P que lo que revelan los experimentos de campo. Se señala la importancia del PO, la fosfatasa y la fosforilasa, al contrastarse con el contenido total de P.

LITERATURE CITED

1. Alexander, A. G., Sucrose-enzyme relationships in immature sugarcane as affected by variable nitrate and potassium supplied in sand culture, *J. Agr. Univ. P.R.* *48* (3): 165–231, 1964.
2. ——, Changes in leaf-sugar content and enzyme activity of immature sugarcane following foliar application of indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid, and maleic hydrazide, *J. Agr. Univ. P.R.* *49* (1): 1–34, 1965.
3. ——, Behavior of enzymes governing starch- and sucrose-forming pathways in two sugarcane varieties supplied with variable nitrate and phosphate in sand culture, *J. Agr. Univ. P.R.* *49* (2): 153–75, 1965.
4. ——, Hydrolytic proteins of sugarcane: The acid phosphatases, *J. Agr. Univ. P.R.* *49* (2): 204–28, 1965.
5. ——, Hydrolytic proteins of sugarcane: The acid invertases, *J. Agr. Univ. P.R.* *49* (3): 287–307, 1965.
6. ——, Hydrolytic proteins of sugarcane: Amylase, *J. Agr. Univ. P. R.*, *49* (3): 308–24, 1965.

7. ——, Oxidizing enzymes of sugarcane: Peroxidase, *J. Agr. Univ. P.R. 50* (1): 36–52, 1966.
8. ——, Oxidizing enzymes of sugarcane: Tyrosinase, *J. Agr. Univ. P.R.* 50 (2): 131–45, 1966.
9. ——, High sucrose levels and abnormal enzyme activity as a function of nutritional stress in sugarcane, *J. Agr. Univ. P.R. 51* (4): 325–33, 1967.
10. Alexander, A. G., Interrelationships of gibberellic acid and nitrate in sugar production and enzyme activity of sugarcane, *J. Agr. Univ. P.R. 52* (1): 19–28, 1968.
11. ——, and Samuels, G., Controlled-temperature studies of growth, enzymology, and sucrose production by two sugarcane varieties in Puerto Rico, *J. Agr. Univ. P.R. 52* (3): 179, 204–17, 1968.
12. ——, Growth, enzyme, and sugar responses of immature sugarcane to foliar treatment with 6-azauracil and gibberellic acid, *J. Agr. Univ. P.R. 52* (4): 225–322, 1968.
13. ——, Effects of combined silicon and gibberellic acid on enzyme behavior and sucrose content of immature sugarcane, *Proc. Int. Soc. Sugarcane Technol.*, XIII Cong., 1968.
14. ——, The potential of sugarcane to produce sucrose, *Proc. Int. Soc. Sugarcane Technol.*, XIII Cong., 1968.
15. ——, and Montalvo-Zapata, R., Enzyme studies of sucrose decline in sugar desiccated with Paraquat, *J. Agr. Univ. P.R.* (Submitted for publication May, 1968).
16. Bull, T. A., The effects of temperature, variety, and age on the responses of saccharum spp. to applied gibberellic acid, *Aust. J. Agr. Res. 15:* 77–84, 1964.
17. Cardini, C. E., Leloir, L. F., and Chiriboga, J., The biosynthesis of sucrose, *J. Biol. Chem. 214:* 149–55, 1955.
18. Conference on coordination of basic research programs of sugarcane agriculture, David North Research Center, Queensland, Australia. Personal communication with Dr. A. Vlitos.
19. Hartt, C. E., Kortschak, H. P., Forbes, A. J., and Burr, G. O., Translocation of ^{14}C in sugarcane, *Plant Physiol. 38:* 305–16, 1963.
20. ——, Effect of moisture supply upon translocation and storage of ^{14}C in sugarcane, *Plant Physiol. 42* (3): 338–46, 1967.
21. Personal communication with Dr. George Samuels, Agronomist, Agricultural Experiment Station, Mayagüez Campus, University of Puerto Rico, Río Piedras, P.R.
22. Roe, J. R., A colorimetric method for the determination of fructose in blood and urine, *J. Biol. Chem. 107:* 15–22, 1934.
23. Samuels, G., and Capó, B. G., Research with sugarcane fertilizers in Puerto Rico, 1910–54, Technical Paper No. 16, University of Puerto Rico, Agricultural Experiment Station, Río Piedras, P.R., 1956.
24. Samuels, G., and Cibes-Viadé, H., Influence of mineral deficiencies on the growth and yield of sugarcane, *J. Agr. Univ. P.R. 47* (2): 61–75, 1963.
25. Sutherland, E. W., Cori, C. F., Haynes, R., and Olsen, N. S., Purification of the hyperglycemic-glycogenolytic factor from insulin and from gastric mucosa, *J. Biol. Chem. 180:* 825–37, 1949.
26. Tanimoto, T., and Nickell, L. G., Re-evaluation of gibberellin for field use in Hawaii, *Rept. Haw. Sugar Technol.*, 25th Annual meeting, pp. 184–90, 1966.