Induction of Varying Sugar Levels in Leaves of Immature Sugarcane by Use of Acid Phosphatase Inhibitors

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INTRODUCTION

Previous studies have shown that treatments which generally favor high leaf-sucrose content in cane also bring about a suppression of the acid phosphatases (*2,3,4*).[2] By the reaction

$$R—O—PO_3H_2 + H_2O \rightarrow ROH + H_3PO_4$$

in which R represents a transferable hexose, glyceric acid, or adenylic acid group, it was postulated that the phosphatases were able to deplete the pools of sucrose precursors and energy reserves needed for sugar biosynthesis. The suppression of these enzymes might therefore yield a more favorable supply of phosphorylated metabolites for sucrose production. This paper reviews experiments in which phosphatase inhibitors were applied to sugarcane with the following objectives: 1, To determine whether specific inhibitors could be supplied through the foliage and roots in sufficient quantities to bring about enzyme suppression in living tissues, and 2, to determine whether such suppression would be accompanied by an increase of sucrose.

The fluoride and molybdate[3] anions were initially selected for test inhibitors. Fluoride has long been used *in vitro* as a powerful phosphatase inhibitor (*11,17,18,21*). During our own studies an F concentration of 0.002M in the reaction mixtures removed all traces of phosphatase activity. Molybdenum has more recently been implicated in phosphate metabolism. Possingham (*17*) observed that Mo-deficient tomato plants exhibited a suppressed capacity to convert inorganic phosphorus into organic phosphorus. Spencer (*22*) found that Mo was a strong competitive inhibitor of tomato acid phosphatases. Evans (*10*) suggested that excessive phosphatase activity in Mo-deficient tissues would deplete normal reserves of phosphorylated metabolites and thus indirectly affect the synthesis of ascorbic

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 58–9.

[3] The symbols Mo, F, and Ca refer to molybdenum, fluoride, and calcium, respectively, throughout this paper. The capital letter M represents the word molar. The expression "parts per million" is denoted by p.p.m., and "milliequivalents per liter" by meq./liter.

acid. While studying the Mo nutrition of cauliflower, Alexander (*1*) observed that trace amounts of Mo stimulated acid phosphatase activity, while greater concentrations tended to suppress the enzymes. The latter effect was accentuated by increased Ca or iron supply.

MATERIALS AND METHODS

The initial experiment involved the application of Mo and F solutions as foliar sprays to 14-week-old sugarcane of the variety M.336. The plants had been grown in the greenhouse using No. 2 quartz sand contained in 13-inch clay pots. Twelve one-eye cuttings were planted in each container and the seedlings received 1 liter of nutrient solution[4] plus 2 liters of tap-water daily. At 12 weeks the canes were thinned to the five most uniform plants of each container.

Molybdate was applied in distilled water at concentrations equivalent to 2, 20, and 200 p.p.m. elemental Mo, and F at 1, 10, and 100 p.p.m. Tween 20 was employed as wetting agent at the rate of 0.10 ml. per 100 ml. of solution. Each solution was applied with a Hudson 7.6-liter hand-sprayer at approximately 40 pounds pressure with the nozzle adjusted for a broad, fine-mist pattern. All above-sand areas of the plants were sprayed until thoroughly wet and runoff had begun. To facilitate coverage and to avoid contamination between treatments, each treatment group was, in turn, moved outside the greenhouse to receive its respective application. The containers were arranged in a completely randomized design with four replicates for each of the seven treatments.

Leaf samples were taken for sugar and enzyme assay a few moments prior to treatment, and subsequent samples were taken at 3, 9, 18, and 27 days following inhibitor application. Each sample consisted of the entire blades of leaves +1 to +4[5] from one representative plant. The leaves were inserted in stoppered 38 × 200-mm. pyrex culture tubes and frozen immediately in a mixture of Dry Ice (solid carbon dioxide) and acetone. The frozen tissues were stored in a freezer at −20° C. and over a period of several days were withdrawn to a coldroom where they were dried under vacuum, at 0–2° C., with the aid of a Virtis Roto-Freeze drying assembly. The dried samples were ground in the cold with a Wiley Mill to pass a 60-mesh (0.43 mm.) screen. The powdered leaf material was then sealed in sample jars and stored at −20° C. until extractions could be made at our convenience.

[4] The solution contained the following nutrients expressed as meq./liter: Nitrate, 10; phosphate, 6; potassium, 5; calcium, 3; magnesium, 2; and sulfate, 2. Microelements, expressed as p.p.m., were supplied as follows: Iron, 1.0; boron, 0.05; copper, 0.02; magnanese, 0.50; zinc, 0.05; and molybdenum, 0.01.

[5] The leaf nomenclature employed here is that of Kuijper (*12*), as described by Van Dillewijn (*25*), in which the highest leaf bearing a visible dewlap is designated +1.

A second group of plants was grown in sand culture and treated with Mo and Ca as nutrient variables. The objectives of the second experiment were to test the effects of Mo as an inhibitor of the acid phosphatases when supplied through the roots over a prolonged period, and to determine whether the inhibitory effects could be enhanced by a heavy metal such as Ca. These plants were also of the M.336 variety and were grown under identical conditions to those described above, with the exceptions that a 3 x 3 factorial design with four replicates was employed and only one harvest was taken. The plants were watered daily with 1 liter of solution containing 0, 0.01, or 1.0 p.p.m. Mo, and 1.0, 3.0, or 9.0 meq./liter Ca.

Tissue samples from the second experiment were taken for sugar and enzyme analysis when the plants were 9 weeks of age. Freezing and drying procedures were conducted as described above, with the exception that four representative plants rather than one made up each sample.

Protein and sugar extractions were conducted as follows: Three grams of the freeze-dried tissue from each sample were placed in a stoppered 125-ml. flask containing 40 ml. of distilled water. The mixture was rotated mechanically at slow speed for 1 hour at room temperature (19–20° C.). Most of the tissue debris was removed by expressing the mixture through four layers of Curity absorbent gauze (20 x 12 mesh). The suspension was chilled to 2° C. and centrifuged for 10 minutes at 3,500 r.p.m. A clear, amber supernatant liquid was obtained from which aliquots were withdrawn and clarified for sugar assay by the method of Nelson (*16*).

The remainder of the supernatant liquid was adjusted to pH 7.0 with 2N NaOH and sufficient ammonium sulfate was added to bring the solution to 80-percent saturation. The salt was added slowly with constant stirring and afterward the precipitate-containing samples were allowed to stand at 2° C. for 4 hours in order to increase protein yield. Protein was removed by centrifuge at 3,000 r.p.m. and taken up in 5 ml. of distilled water. The protein solutions were then dialyzed against distilled water for 4 hours. A small quantity of precipitate usually formed during dialysis which was subsequently removed by centrifuge at 3,500 r.p.m. Protein content of the dialyzed preparations was determined colorimetrically by the method of Sutherland *et al.* (*24*). The protein concentrates were refrigerated at 6° C. For a given harvest, all enzyme assays were completed within 48 hours of extraction, utilizing protein dilutions prepared from the concentrates.

Sugar measurements were conducted for both experiments with dilutions of clarified extracts from the freeze-dried tissues. Total ketoses were determined by the resorcinol technique of Roe (*19*), and total reducing sugars were measured by the dinitrosalicylic acid method of Sumner (*23*). Sucrose was distinguished from fructose according to the technique of Cardini *et al.* (*8*), which destroys fructose by heating the samples in the presence of

NaOH. This procedure is specific for reducing sugars and thus enabled us to determine the nonsugar reducing power of the samples. Glucose was calculated by subtracting the fructose values from the corrected total reducing-sugar fraction. Total reducing sugars and glucose were not determined for the second experiment. Details of the sugar-assay procedures have been described previously (*2*).

All phosphatase reactions for the first experiment were run for 10 minutes at 30° C. and pH 4.6. For the second experiment the reaction time was increased to 20 minutes. Each sample was assayed in duplicate, employing a reaction mixture composed of 0.5 ml. of acetate buffer, 0.5 ml. of substrate, and 0.25 ml. of enzyme extract (representing 0.1 to 0.2 mg. of protein). Control vessels received distilled water in place of enzyme and all reactions were terminated with 1 ml. of trichloroacetic acid.

For experiment 1, substrates included β-glycerophosphate (0.01 M), ATP (0.01 M), glucose-1-phosphate (0.025 M), glucose-6-phosphate (0.01 M), fructose-6-phosphate (0.01 M), and 3-phosphoglyceric acid (0.01 M). During the second experiment the concentration of β-glycerophosphate was increased to 0.05 M, ATP to 0.02 M, and glucose-1-phosphate to 0.1 M. All other substrate concentrations were retained. Inorganic phosphorus released by enzymatic action was measured by the phosphomolybdic acid technique as described in a previous article (*2*). For each phosphatase the activity unit was defined as the amount of enzyme catalyzing the hydrolysis of 0.01 mg. of phosphorus under the specified conditions of the assay. In addition to phosphatase, the enzymes peroxidase and amylase were also measured by methods reported earlier (*2*). Peroxidase was not included during the second experiment.

All data collected during these investigations were subjected to statistical analysis of variance and mean values were compared by the Student-Newman-Keuls Q test. With but few exceptions, all variations discussed hereafter were found to be significant at the 5- or 1-percent level.

RESULTS AND DISCUSSION

EXPERIMENT 1: FOLIAR TREATMENTS WITH MO AND F

Results of the Mo and F foliar studies verified the hypothesis that phosphatase inhibitors absorbed through the leaves could suppress phosphatases in the living tissues. Such suppression was usually accompanied by increased sucrose.

A review of table 1 testifies to the unusually large number of significant differences caused by the Mo and F treatments. To a considerable extent, these differences were gained because we were able to select beforehand materials which we knew should alter enzymes intimately associated with sugar formation and metabolism.

More specifically, the application of Mo and F to the cane foliage yielded somewhat sporadic information, indicating that further study is needed as to time, number, and concentration of treatments required to achieve more predictable results. Molybdenum at the medium level (20 p.p.m.) was the only treatment which consistently suppressed phosphatase (fig. 1) and concurrently yielded greater sucrose (fig. 2) at all harvests subsequent to treating the plants. High Mo (200 p.p.m.) affected a similar response with phosphatase (table 2, items 1 to 6) while significant sucrose increases were confined to the 3- and 18-day harvests (table 3; table 1, item 2).

Fluoride treatments did not yield results consistent for all harvests, although at times there was significant suppression of phosphatase and increased sucrose. For example, the treatments of 10 p.p.m. F at 9 days, and 100 p.p.m. F at 18 days, were accompanied by significantly greater sucrose (table 1, item 2; fig. 3) while ATP-ase and glucose-1-phosphatase, respectively, were simultaneously suppressed (table 2, items 2 and 3). An example of the inverse relationship between phosphatase activity and sucrose level is illustrated by the ATP-ase and sucrose curves recorded from the 10-p.p.m. F treatment (fig. 4).

In view of the age of the plants and the fact that only one application of any Mo and F treatment was made, the ability of Mo and F to suppress phosphatase and increase sucrose after 18 days (table 1, item 2, and items 6 to 11) was actually remarkable. For 20 and 200 p.p.m. Mo, this effect was still apparent after 27 days. At the time of treatment the plants were entering a prolonged period of rapid growth and therefore a dilution effect must have been active against treatment materials from the hour they were absorbed. Furthermore, practically all of the foliage which received treatments had passed out of the sampling area (leaves +1 to +4) by the 18- and 27-day harvests. We must also consider that a suppression of phosphatase in the young plants, with the simultaneous increase of ATP and hexose phosphates for sucrose synthesis, does not guarantee that these additional substrates will all be used for sucrose formation. It is probable that a high percentage of these compounds passed on through glycolysis and the Kreb's cycle to take part in the formation of organic acids and energy reserves needed for the synthesis of protein. Perhaps a more logical age at which to apply inhibitors, for a prolonged effect, would be during the 8- to 12-month period when the plants are less concerned with spreading new foliage and are more concerned with the making of sucrose.

We should note the relatively slight suppression of phosphatase needed to bring about changes in sucrose level. Using ATP-ase as an example, the enzyme was not generally suppressed below 70 percent of control values (fig. 1). It should be remembered that this type of enzyme is an essential component of the equilibrium between inorganic and organic phosphate in the plant. In the instance of ATP-ase, the enzyme is believed necessary for

TABLE 1.—*Summary of significant mean differences among sugar and enzyme values recorded for control, variable molybdenum, and variable fluoride treatments applied to immature sugarcane*[1]

Item No.	Data classification	Results for days following treatment indicated				
		0	3	9	18	27
1	Total ketoses	$F_1 > Mo_2^{**}$	$Mo_{200} > Con.^{**}$ F_{100}^{*} $F_1 > Con.^{*}$	$F_{10} > Mo_2^{**}$ F_{100}^{**} Mo_{200}^{**} F_1^{**} $Con.^{**}$ $Mo_{20} > F_1^{**}$ $Con.^{**}$ Mo_{200}^{*}	$Mo_{20} > Mo_2^{**}$ $Con.^{**}$ F_{10}^{**} F_1^{**} $Mo_{200} > F_1^{**}$ F_{10}^{*} $F_{100} > F_1^{*}$	$Mo_{20} > F_{100}^{**}$ Mo_{200}^{**} F_{10}^{**} F_1^{**} $Mo_2 > F_1^{**}$
2	Sucrose	$F_1 > Mo_2^{**}$ Mo_{200}^{*} $F_{100} > Mo_2^{*}$	$Mo_{20} > Con.^{*}$ $Mo_{200} > Con.^{*}$	$F_{10} > Con.^{**}$ F_{100}^{*} Mo_2^{*} Mo_{200}^{*} F_1^{*} $Con.^{*}$ $Mo_{20} > Con.^{*}$	$Mo_{20} > F_{10}^{**}$ Mo_2^{**} $Con.^{**}$ F_1^{**} $Mo_{200} > F_{10}^{**}$ Mo_2^{**} $Con.^{**}$ F_1^{**} $F_{100} > Mo_2^{**}$ $Con.^{**}$ F_1^{**} F_{10}^{*}	$Mo_{20} > F_{100}^{**}$ Mo_{200}^{**} F_{10}^{**} F_1^{**} $Con.^{*}$ $Mo_2 > F_1^{**}$ F_{10}^{*}
3	Fructose		$F_{10} > F_{100}^{**}$ Mo_{20}^{**} $F_1 > F_{100}^{**}$ Mo_{20}^{**}		$Mo_2 > F_{100}^{**}$ F_{10}^{*} Mo_{200}^{*} F_1^{*}	

			$Mo_2 > Mo_{20}^{**}$ $Con. > Mo_{20}^{**}$ F_{100}^{*} $Mo_{200} > Mo_{20}^{**}$ F_{100}^{*} $Mo_2 > F_{100}^{*}$		$Mo_{20} > F_1^{*}$ F_{100}^{*} $Con. > F_1^{*}$ F_{100}^{*}	
4	Total reducing sugars		$Con. > F_{10}^{**}$ F_{100}^{**} F_1^{**} $Mo_{20} > F_{10}^{**}$ F_{100}^{**} F_1^{**}		$Mo_{20} > Mo_{200}^{**}$ $Con.^{**}$ Mo_2^{**} F_{100}^{**} F_1^{**} F_{10}^{**}	
5	Glucose	$Mo_{20} > F_{10}^{*}$ $Con.^{*}$	$Mo_{20} > F_{10}^{**}$ F_1^{**} $Con.^{*}$		$F_{100} > Mo_2^{**}$ F_{10}^{*} $Con.^{*}$	
6	β-glycerophosphate phosphatase		$F_{10} > Mo_{20}^{**}$ Mo_{200}^{**} Mo_2^{**} Mo_{20}^{*} $Con. > Mo_{20}^{**}$ Mo_{200}^{**} Mo_2^{**} $F_1 > Mo_{200}^{**}$ Mo_2^{**} Mo_{20}^{*} $F_{100} > Mo_{200}^{**}$ Mo_2^{**} Mo_{20}^{*}	$F_{100} > Mo_{200}^{**}$	$F_{10} > Mo_{20}^{**}$ Mo_{200}^{**} $Mo_2 > Mo_{20}^{**}$ Mo_{200}^{**} $F_1 > Mo_{20}^{**}$ Mo_{200}^{**} $Con. > Mo_{20}^{**}$ Mo_{200}^{**} $F_{100} > Mo_{20}^{**}$ Mo_{200}^{**}	$F_{10} > F_{100}^{**}$ Mo_{200}^{**} Mo_2^{**} Mo_{20}^{**} F_1^{*} $Con. > F_{100}^{**}$ Mo_{200}^{**} Mo_2^{**} Mo_{20}^{**} F_1^{*}

TABLE 1.—*Continued*

Item No.	Data classification	Results for days following treatment indicated				
		0	3	9	18	27
7	ATP-ase	$F_1 > Mo_{20}^{*}$ Con.* Mo_2^{*} Mo_{200}^{*}	Con. > Mo_2^{**} Mo_{200}^{*} $F_{100} > Mo_{200}^{*}$ Mo_2^{*} $F_1 > Mo_{200}^{*}$ Mo_2^{*} $F_{10} > Mo_{200}^{*}$ Mo_2^{*}	$F_1 > Mo_{200}^{*}$ F_{100}^{*} F_{10}^{*} Con. > F_{10}^{*}	$F_1 > Mo_{200}^{**}$ Mo_{20}^{**} $Mo_2 > Mo_{200}^{**}$ Mo_{20}^{**} $F_{10} > Mo_{200}^{**}$ Mo_{20}^{**} $F_{100} > Mo_{200}^{**}$ Mo_{20}^{**} Con. > Mo_{200}^{**} Mo_{20}^{**}	$F_{10} > Mo_2^{**}$ Mo_{20}^{**} F_{100}^{*}
8	Glucose-1-phosphate phosphatase		$F_{10} > Mo_{20}^{**}$ Mo_2^{**} Mo_{200}^{*} $F_1 > Mo_{20}^{**}$ Mo_2^{**} $F_{100} > Mo_{20}^{*}$ Mo_2^{*}	Con. > Mo_{200}^{**} F_{10}^{**} F_{100}^{*}	Con. > Mo_{200}^{**} Mo_{20}^{*} $F_{10} > Mo_{200}^{**}$	
9	Glucose-6-phosphate phosphatase	$F_1 > F_{10}^{*}$ Mo_{20}^{*} F_{100}^{*} Con.* Mo_2^{*} Mo_{200}^{**}	Con. > $M0_{20}^{**}$ Mo_{200}^{**} Mo_2^{**} $F_{10} > Mo_{20}^{**}$ Mo_{200}^{**} Mo_2^{**}	$F_1 > Mo_{200}^{*}$	$F_{100} > Mo_{20}^{**}$ Mo_{200}^{**} $F_{10} > Mo_{20}^{**}$ Mo_{200}^{**} $F_1 > Mo_{20}^{**}$ Mo_{200}^{**}	$F_{10} > Mo_{200}^{*}$ Mo_2^{*}

			$F_{100} > Mo_{20}^{*}$ Mo_{200}^{*} Mo_{2}^{*} $F_{1} > Mo_{20}^{*}$ Mo_{200}^{*} Mo_{2}^{*}		$Mo_{2} > Mo_{20}^{**}$ Mo_{200}^{**} $Con. > Mo_{20}^{*}$ Mo_{200}^{*}	
10	Fructose-6-phosphate phosphatase		$F_{10} > Mo_{200}^{**}$ Mo_{20}^{**} Mo_{2}^{**} $Con.^{*}$ $F_{1} > Mo_{200}^{**}$ Mo_{20}^{**} Mo_{2}^{**} $F_{100} > Mo_{200}^{**}$ Mo_{20}^{**} Mo_{2}^{**} $Con. > Mo_{200}^{**}$ Mo_{20}^{**} Mo_{2}^{**} $Mo_{200} > Mo_{2}^{*}$	$Con. > F_{1}^{**}$ Mo_{200}^{**} F_{100}^{**} F_{10}^{**} $Mo_{2} > F_{100}^{**}$ F_{10}^{**} F_{1}^{*} Mo_{200}^{*} $Mo_{20} > F_{1}^{*}$ Mo_{200}^{*} F_{100}^{*} F_{10}^{*} $F_{1} > F_{10}^{*}$	$F_{10} > Mo_{200}^{**}$ Mo_{20}^{**} $Con.^{*}$ $F_{1} > Mo_{200}^{**}$ Mo_{20}^{**} $Mo_{2} > Mo_{200}^{**}$ Mo_{20}^{**} $F_{100} > Mo_{200}^{*}$ Mo_{20}^{*} $Con. > Mo_{200}^{*}$ Mo_{20}^{*}	$F_{10} > F_{100}^{**}$ Mo_{200}^{**} Mo_{2}^{**} Mo_{20}^{**} $Con.^{*}$ F_{1}^{*} $Con. > Mo_{20}^{*}$
11	3-Phosphoglyceric phosphatase	$F_{1} > Con.^{**}$ F_{10}^{*} Mo_{20}^{*} Mo_{2}^{*} Mo_{200}^{*} $F_{100} > Con.^{*}$	$Con. > Mo_{2}^{**}$ Mo_{200}^{**} F_{100}^{*} F_{1}^{*} F_{10}^{*} Mo_{20}^{*}		$F_{1} > Mo_{200}^{**}$ Mo_{20}^{**} $F_{10} > Mo_{200}^{**}$ Mo_{20}^{**} $Mo_{2} > Mo_{200}^{**}$ Mo_{20}^{**} $F_{100} > Mo_{200}^{**}$ Mo_{20}^{**} $Con. > Mo_{200}^{**}$ Mo_{20}^{**}	$F_{10} > Mo_{20}^{**}$ Mo_{2}^{**} F_{1}^{*}

TABLE 1.—*Concluded*

Item No.	Data classification	Results for days following treatment indicated				
		0	3	9	18	27
12	Peroxidase	Con. > F_{100}* Mo_{20}* F_1* Mo_2* F_{10}*	Con. > Mo_2** Mo_{20}** F_{100}** F_{10} > Mo_2* Mo_{20}* F_{100}* Mo_{200} > Mo_2* Mo_{20}* F_{100}*	Mo_2 > F_{10}* Con. > F_{10}*		F_{10} > Mo_2** Mo_{20}* F_1*
13	Amylase		Con. > Mo_{20}** Mo_{200}** Mo_2** F_{100}* F_{10} > Mo_2** Mo_{20}* Mo_{200}* F_1 > Mo_2* F_{100} > Mo_2*		F_{10} > Mo_{200}** Mo_{20}** F_1* F_{100} > Mo_{20}** Mo_{200}* Mo_2 > Mo_{20}** Mo_{200}* F_1 > Mo_{20}** Mo_{200}* Con. > Mo_{20}** Mo_{200}* Mo_{200} > Mo_{20}**	F_{10} > F_1** Mo_{200}** Mo_{20}** Mo_2**

[1] The abbreviations and symbols used in this table are defined as follows: The abbreviation Con. represents mean values from the control treatment; the symbols Mo_2, Mo_{20}, and Mo_{200} represent mean values from treatments receiving 2, 20, and 200 p.p.m. Mo, respectively; and the symbols F_1, F_{10}, and F_{100} denote mean values from treatments receiving 1, 10, and 100 p.p.m. F, respectively. The symbol (>) between 2 mean values indicates that the first mean was of greater magnitude than the second. One asterisk denotes significant variance at the 5-percent level, and 2 asterisks at the 1-percent level. For illustration, the notation F_{100} > Mo_2*, appearing under amylase, means that a significantly greater amylase activity was recorded among plants receiving 100 p.p.m. F than among those receiving 2 p.p.m. Mo, and that the mean difference is significant at the 5-percent level.

TABLE 2.—*Mean specific activity values for phosphatases, amylase, and peroxidase, from leaves of immature sugarcane, following foliar application of variable molybdenum and fluoride*[1]

Item No.	Enzyme	Days after treatment	Control	Results under treatment indicated							
				Mo (p.p.m.)				F (p.p.m.)			
				2	20	200	Mo mean	1	10	100	F mean
1	β-Glycerophosphate phosphatase	0	15.5	14.4	16.6	12.4	14.5	19.9	16.5	17.4	17.9
		3	24.1	13.9	17.3	14.7	15.3	22.6	27.4	21.8	23.9
		9	22.3	21.0	22.1	13.7	18.9	22.1	19.9	22.7	21.6
		18	19.5	21.3	12.2	11.2	14.9	20.1	21.7	19.0	20.3
		27	29.8	21.9	21.8	22.0	21.9	24.1	30.7	22.3	25.7
	Mean		22.2	18.5	22.5	18.5	19.8	25.8	23.2	20.6	23.2
2	ATP-ase	0	13.8	13.1	14.3	12.9	13.4	20.1	16.2	17.1	17.8
		3	31.4	17.7	23.1	19.6	20.1	30.4	28.7	30.6	29.9
		9	25.0	21.9	20.4	19.0	20.4	26.0	18.3	18.3	20.9
		18	17.5	22.0	10.4	11.9	14.8	22.4	22.0	19.2	21.2
		27	31.8	24.4	24.0	26.5	24.9	26.3	35.1	27.2	29.5
	Mean		24.0	19.8	18.4	17.9	18.7	25.0	24.1	22.5	23.9
3	Glucose-1-phosphate phosphatase	0	3.2	2.6	2.7	2.6	2.6	3.7	3.1	2.7	3.2
		3	3.1	2.0	2.2	2.8	2.3	4.0	4.2	3.7	3.9
		9	4.8	4.2	3.9	3.5	3.9	4.2	3.3	3.7	3.7
		18	3.5	3.1	2.6	2.1	2.6	2.9	3.2	2.8	2.9
		27	5.1	4.2	3.5	3.9	3.9	4.4	4.3	3.4	4.0
	Mean		3.9	3.2	2.9	2.9	3.0	3.8	3.6	3.3	3.6
4	Glucose-6-phosphate phosphatase	0	4.1	3.9	4.2	2.8	3.6	6.1	4.5	4.1	4.9
		3	7.8	4.2	4.7	4.4	4.4	6.5	7.3	6.7	6.8
		9	5.9	4.8	4.7	4.3	4.6	6.2	5.1	6.0	5.8
		18	4.8	5.5	3.4	3.2	4.0	5.7	5.8	5.9	5.8
		27	8.2	6.1	6.2	6.6	6.3	7.3	8.7	6.9	7.6
	Mean		6.2	4.9	4.6	4.2	4.6	6.4	6.3	5.9	6.2
5	Fructose-6-phosphate phosphatase	0	4.8	4.4	4.4	3.3	4.0	5.6	3.6	3.7	4.3
		3	9.0	3.6	4.8	5.9	4.8	10.4	11.7	10.3	10.8
		9	4.2	3.7	3.7	2.3	3.2	2.6	1.4	1.9	1.9
		18	5.5	6.6	4.0	4.1	4.9	6.7	7.5	6.0	6.7
		27	10.6	8.1	7.8	8.6	8.2	9.6	12.3	8.6	10.2
	Mean		6.8	5.3	4.9	4.8	5.0	6.9	7.3	6.1	6.8

TABLE 2.—*Continued*

Item No.	Enzyme	Days after treatment	Control	Results under treatment indicated							
				Mo (p.p.m.)				F (p.p.m.)			
				2	20	200	Mo mean	1	10	100	F mean
6	3-Phosphoglyceric phosphatase	0	10.8	13.3	13.8	12.7	13.3	19.5	14.6	17.4	17.2
		3	32.0	18.9	23.4	18.8	20.4	24.5	23.6	26.1	24.7
		9	20.2	22.2	19.2	19.4	20.3	27.2	17.6	21.0	21.9
		18	19.2	23.8	11.7	13.1	16.2	24.5	23.9	21.3	23.2
		27	27.5	22.1	22.4	27.8	24.1	24.2	33.4	25.7	27.8
	Mean		21.9	20.1	18.1	18.3	18.8	23.9	22.6	22.3	22.9
7	Amylase	0	35.3	32.1	35.6	34.4	34.0	41.7	42.4	37.2	40.1
		3	32.2	17.3	20.9	20.4	19.5	26.0	29.6	24.6	26.7
		9	39.9	44.5	36.9	37.9	39.8	43.9	52.1	49.2	48.4
		18	38.5	43.2	6.1	27.6	25.6	39.3	54.4	47.9	47.2
		27	26.0	20.1	18.6	23.4	20.7	24.1	35.1	27.2	28.8
	Mean		34.4	31.4	23.6	28.7	27.9	35.0	42.7	37.2	38.3
8	Peroxidase	0	6.2	3.7	3.9	4.7	4.1	3.9	3.6	4.3	3.9
		3	7.0	4.5	4.3	6.1	4.9	5.5	6.3	4.2	5.3
		9	9.5	9.9	7.7	7.7	8.4	7.3	5.8	7.8	6.9
		18	6.2	5.9	5.5	6.5	5.9	5.9	6.2	6.5	6.2
		27	9.3	7.4	7.0	9.3	7.9	8.1	11.1	8.6	9.3
	Mean		7.6	6.3	5.7	6.9	6.3	6.1	6.6	6.3	6.3

[1] Each figure represents the computed mean of 4 replicates.

the provision of inorganic phosphate needed during the phosphorylation of certain intermediates of the glycolytic pathway (*14*). It is therefore essential that the enzymes are not completely inhibited.

It was interesting to observe that Mo and F concentrations which would have been totally lethal to systems in the test tube were accepted by the living tissues without any apparent injury to the plants.

The failure of F to produce a consistent suppression of phosphatase, such as that obtained by Mo at 20 p.p.m., may be due to the relatively foreign nature which F must assume within the plant. Unlike Mo, there is no known role of this element in plant nutrition. The appearance of F in greater than trace amounts could conceivably trigger corrective steps by the plant, such as increased synthesis of enzyme protein to replace that which is inactivated, or synthesis and mobilization of metabolites capable of reacting with F to

TABLE 3.—*Mean values for leaf sugars of immature sugarcane following foliar application of variable molybdenum and fluoride*[1]

Sugar	Days after treatment	Control	Results under treatment indicated							
			Mo (p.p.m.)				F (p.p.m.)			
			2	20	200	Mean of Mo treatments	1	10	100	Mean of F treatments
Total ketoses	0	38.3	30.6	34.4	35.4	33.5	42.0	39.1	40.1	40.4
	3	26.1	29.6	29.8	31.4	30.3	30.7	30.1	27.3	29.4
	9	31.8	35.7	39.6	33.4	36.2	32.3	42.7	35.1	36.7
	18	27.3	28.5	27.1	33.4	29.7	22.8	26.3	29.8	26.3
	27	26.9	29.0	34.2	22.9	28.7	17.9	22.5	26.1	22.2
Mean		30.1	30.7	33.0	31.3	31.7	29.1	32.3	31.7	31.0
Sucrose	0	25.2	20.3	26.3	23.5	23.4	31.1	28.0	28.4	29.2
	3	20.4	23.9	28.3	26.0	26.1	23.1	22.5	25.5	23.7
	9	29.2	32.6	37.1	31.6	33.8	31.5	40.6	33.2	35.1
	18	19.7	19.9	29.4	29.2	26.2	19.5	21.8	27.2	22.8
	27	25.0	27.1	31.4	21.2	26.6	16.8	20.4	23.6	20.3
Mean		23.9	24.8	30.5	26.3	27.2	24.4	26.7	27.6	26.2
Fructose	0	13.2	10.1	8.1	11.7	9.9	10.8	11.1	11.7	11.2
	3	5.6	5.7	1.5	5.3	4.2	7.6	7.6	1.8	5.7
	9	2.5	3.1	2.3	1.8	2.4	.7	2.1	1.9	1.6
	18	7.6	8.5	7.7	4.3	6.8	3.3	4.5	2.7	3.5
	27	1.9	1.9	2.8	1.7	2.1	1.1	2.2	2.6	1.9
Mean		6.2	5.9	4.5	4.9	5.1	4.7	5.5	4.1	4.9
Total reducing	0	14.8	13.0	15.2	16.2	14.8	15.8	13.3	14.9	14.7
	3	15.8	14.7	15.6	13.3	14.5	11.4	11.9	11.5	11.6
	9	16.2	16.2	17.1	14.2	15.8	13.6	14.9	13.4	13.9
	18	12.9	12.6	16.1	13.3	13.9	11.9	10.8	12.2	11.6
	27	9.9	9.8	9.9	10.3	10.0	9.3	7.9	10.1	9.1
Mean		13.9	13.3	14.8	13.4	13.8	12.4	11.8	12.4	12.2
Glucose	0	1.6	2.5	7.2	4.4	4.7	3.6	1.8	3.2	2.9
	3	10.1	9.0	14.1	7.9	10.3	3.9	4.3	9.7	5.9
	9	13.7	13.1	15.2	11.3	13.2	12.9	12.9	11.4	12.4
	18	5.2	4.1	8.4	8.9	7.1	8.6	5.7	10.1	8.1
	27	7.9	7.8	7.1	8.6	7.8	8.2	5.7	7.4	7.1
Mean		7.7	7.3	10.4	8.2	8.6	7.4	6.1	8.3	7.3

[1] Sugar are expressed in milligrams per gram dry weight. Each figure represents the computed mean of 4 replicates.

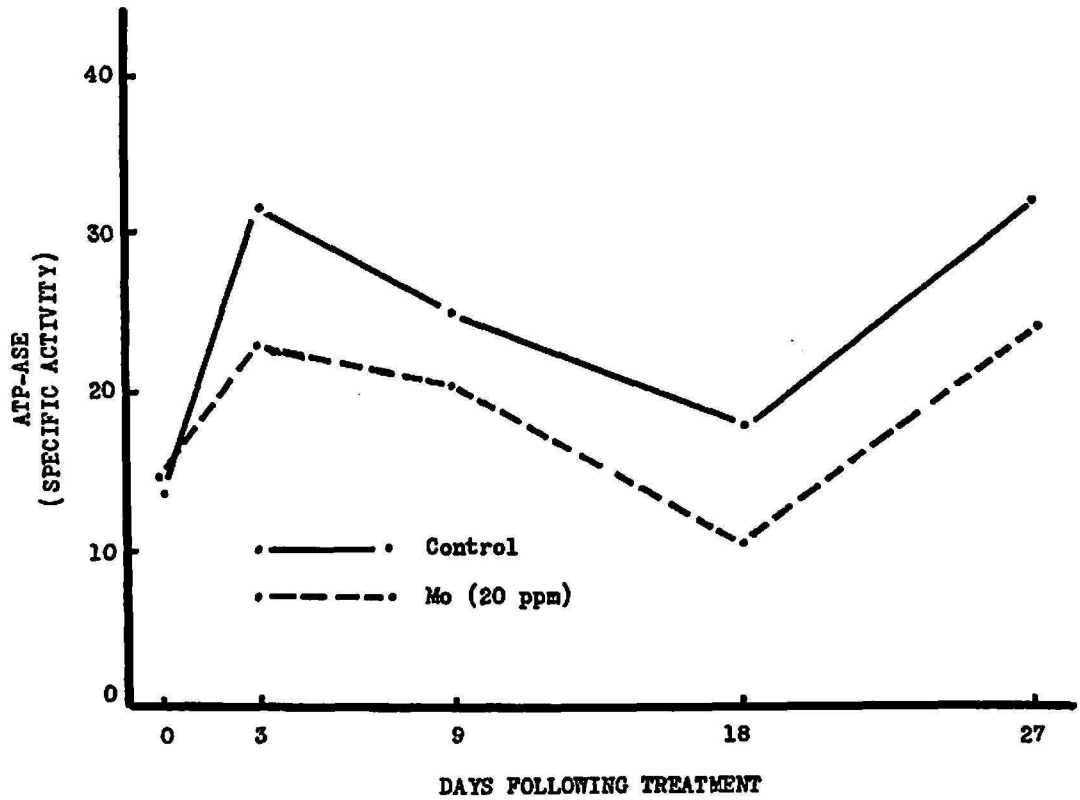

FIG. 1.—Specific activity of ATP-ase from leaves of immature sugarcane following treatment with distilled water (control) and molybdate solution.

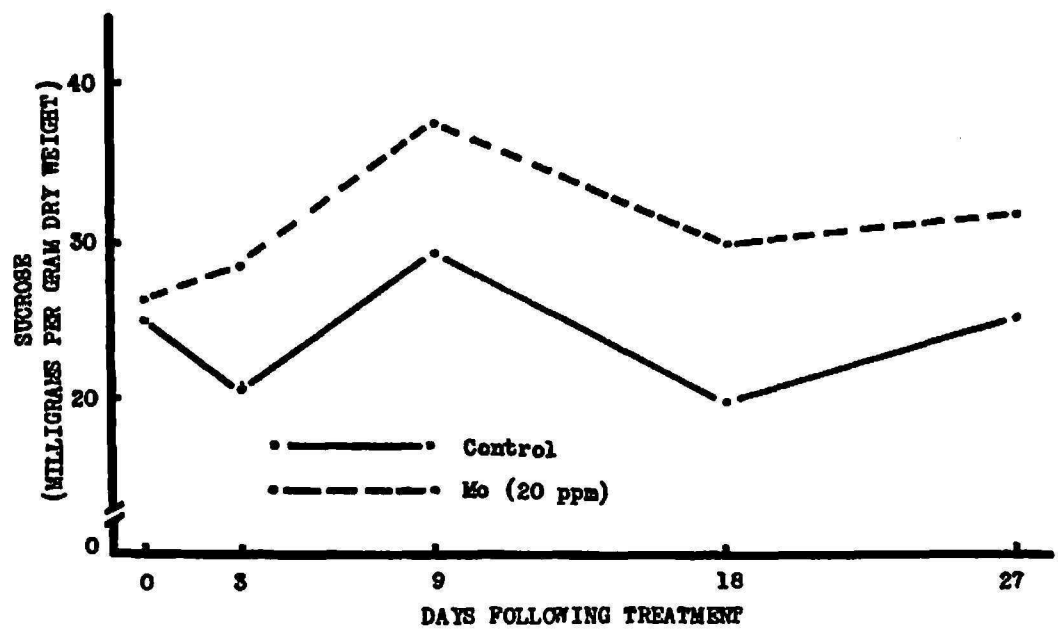

FIG. 2.—Leaf-sucrose content of immature sugarcane following foliar application of distilled water (control) and molybdate solution.

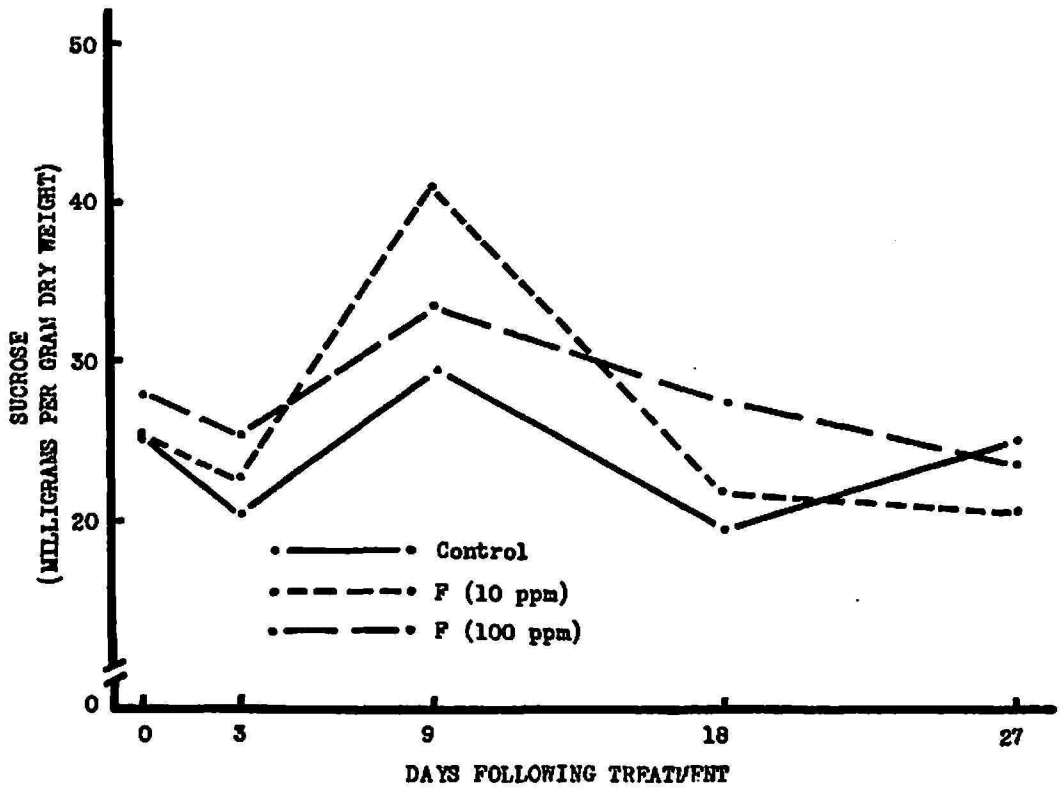

FIG. 3.—Leaf-sucrose content of immature sugarcane following foliar application of distilled water (control) and variable fluoride solutions.

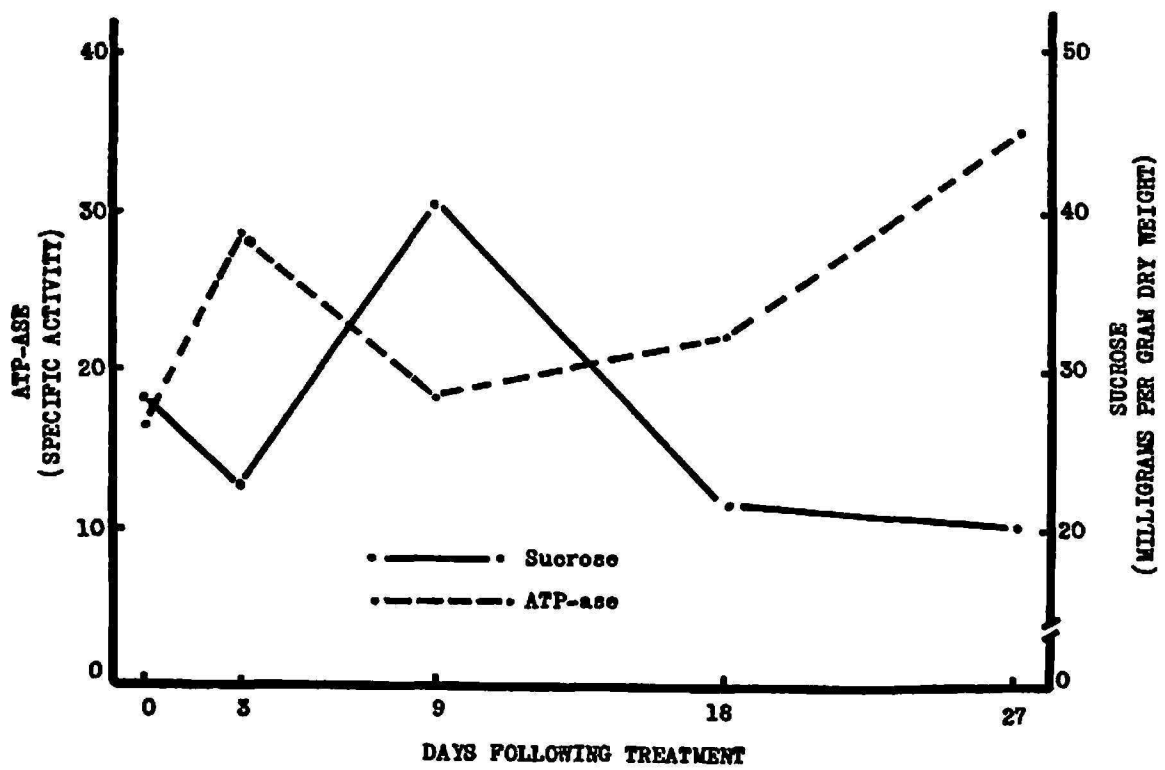

FIG. 4.—Inverse relationship between sucrose content and ATP-ase activity of leaves from immature sugarcane receiving 10 p.p.m. of fluoride.

effectively remove it from critical pathways. As indicated in earlier studies and verified during the second experiment to be discussed later herein, there appears to be a more congenial relationship between the acid phosphatases and Mo. At low concentrations Mo will serve as a phosphatase activator.[6] The inhibitory properties appear only after some critical concentration has been reached. It is therefore conceivable that molybdenum might accumulate among critical enzymes to an inhibitory level without creating undue "alarm" within the plant.

EXPERIMENT 2: CALCIUM-MOLYBDENUM-SUCROSE RELATIONSHIPS

The second experiment, which involved variable Ca and Mo treatments in sand culture, achieved only part of the anticipated results. On the basis of unpublished experiments with cauliflower, we had expected a suppressing effect of high Mo upon the acid phosphatases, and a corresponding increase in sucrose. Increasing concentrations of Ca were expected further to enhance the enzyme inhibition. The results did, in fact, correspond exactly with the data obtained with cauliflower, *i.e.*, increasing Mo first stimulated phosphatases and then retarded the enzymes at the high Mo level (table 4), but this effect of Mo was evident only so long as Ca was supplied at the low concentration (1.0 meq./liter).

Figures 5 and 6 illustrate that the ATP-ase and glucose-1-phosphatase activity curves were inversely related to sucrose level (table 5), or in other words, a stimulation of the enzymes by Mo was accompanied by low sucrose, and enzyme suppression by Mo was accompanied by high sucrose. However, rather than enhance the inhibitory effect, high Ca in some way removed the capability of Mo to inhibit ATP-ase (fig. 7), and the response of glucose-1-phosphatase to Mo was now so transmuted that the enzyme's activity curve lay almost parallel to the curve for sucrose content (fig. 8). The existence of such a Mo $\times$ Ca interaction which is capable of altering enzymes intimately related to sucrose formation could be of considerable importance to cane growers who consider the use of lime.

There is some question as to whether the beneficial effects observed by applying calcium compounds to cane fields are caused by an improved soil pH, or by a more favorable calcium nutritional status. According to Landrau and Samuels (*13*), acid soils in Puerto Rico with a pH below 5.5 have responded to $CaCO_3$ with significant increases of available 96° sugar per acre. The increases were caused primarily by greater tonnage rather than increased sucrose-percent-cane, and trials with soils of pH 6 or more were negative. A combination of lime and superphosphate achieved increased

[6] The term "activator", as used in relation to enzymes, may be defined as any ionic substance which can stimulate enzyme action without actually being required for activity. The term should not be confused with "cofactor".

TABLE 4.—*Mean specific activity values for acid phosphatase and amylase from leaves of immature sugarcane supplied with variable molybdenum (p.p.m.) and calcium (meq./liter) in sand culture*

Ca (meq./liter)	β-glycerophosphatase Mo				ATP-ase Mo				Glucose-1-phosphatase Mo			
	0	0.01	1.0	Mean	0	0.01	1.0	Mean	0	0.01	1.0	Mean
1.0	61.2	73.3	58.9	64.5	70.0	92.8	66.5	76.4	13.5	15.5	11.4	13.5
3.0	57.8	65.8	75.4	66.3	70.3	75.4	94.3	80.0	14.2	13.3	13.1	13.5
9.0	67.1	66.9	67.8	67.3	69.5	83.4	83.9	78.9	14.3	13.8	11.9	
Mean	62.0	68.7	67.4		69.9	83.9	81.6		14.0	14.2	12.1	

	Glucose-6-phosphatase Mo				Fructose-6-phosphatase Mo				3-Phosphoglyceric acid phosphatase Mo			
	0	0.01	1.0	Mean	0	0.01	1.0	Mean	0	0.01	1.0	Mean
1.0	21.7	26.9	19.6	22.7	39.9	52.5	38.2	43.5	88.0	106.8	76.4	90.4
3.0	20.5	23.3	26.4	23.3	37.9	44.4	51.5	44.6	81.5	87.2	102.5	90.4
9.0	23.2	24.2	24.9	24.1	43.4	46.2	46.5	45.4	93.5	95.4	95.1	94.7
Mean	21.8	24.8	23.6		40.3	47.7	45.4		96.5	96.5	91.3	

	Amylase Mo			
	0	0.01	1.0	Mean
1.0	52.3	67.4	59.3	59.0
3.0	59.1	67.2	57.9	61.4
9.0	66.5	54.9	46.8	56.1
Mean	59.3	63.2	54.7	

TABLE 5.—*Mean values for main effects and first-order interactions, in milligrams sugar per gram of dry weight, summarizing the influence of variable molybdenum (p.p.m.) and calcium (meq./liter) supplied to immature sugarcane in sand culture*

Ca(meq/liter)	Total ketoses Mo				Sucrose Mo				Fructose Mo			
	0	0.01	1.0	Mean	0	0.01	1.0	Mean	0	0.01	1.0	Mean
1.0	106.9	73.6	108.9	96.5	81.5	50.6	84.9	72.3	25.4	23.1	24.0	24.2
3.0	91.2	78.4	79.3	82.9	63.8	56.9	56.2	58.9	27.4	21.5	23.1	24.0
9.0	100.3	97.9	74.7	90.9	76.7	76.1	51.7	68.2	23.1	21.8	23.0	22.6
Mean	99.5	83.3	87.6		74.0	61.2	64.2		25.3	22.1	23.4	

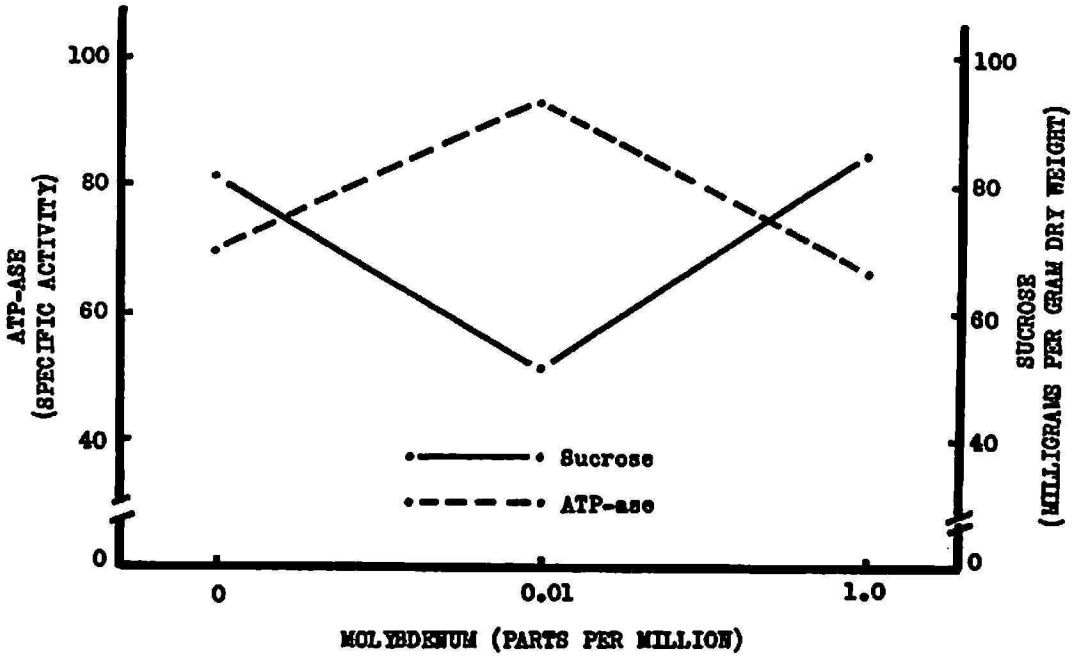

FIG. 5.—Inverse relationship between sucrose content and ATP-ase activity in leaves of immature sugarcane. Plants were supplied with variable molybdenum while calcium was maintained at a low level (1.0 meq./liter).

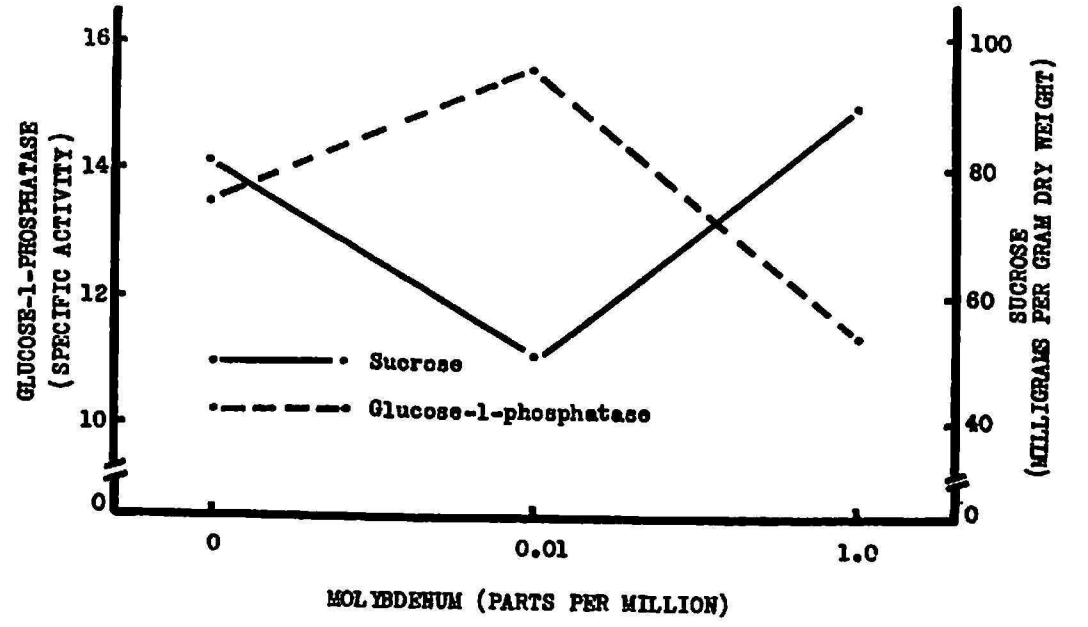

FIG. 6.—Inverse relationship between sucrose content and glucose-1-phosphatase activity of leaves from immature sugarcane supplied with variable molybdenum in sand culture. Plants received a low level of calcium (1.0 meq./liter).

cane tonnage when applied to acid soils in Puerto Rico (*7*). Doty (*9*) reported poor juice purity and quality ratios following lime applications. According to Baver (*6*), Ca-deficient sugarcane grown in water culture contained significantly more sucrose. Similar responses were observed among Ca-deficient cane grown by sand culture in Puerto Rico (*19*).

Results such as those reported by Doty and Baver are in close agreement with our own data, although in this instance the mechanism of Ca action must be a heavy metal antagonism of the normal Mo-phosphatase relationship. On the basis of this and earlier studies (*2*,*3*,*4*), we feel that a close relationship does, in fact, exist between the suppression of the acid phosphatases and increased sucrose content. Molybedenum, so far as we know,

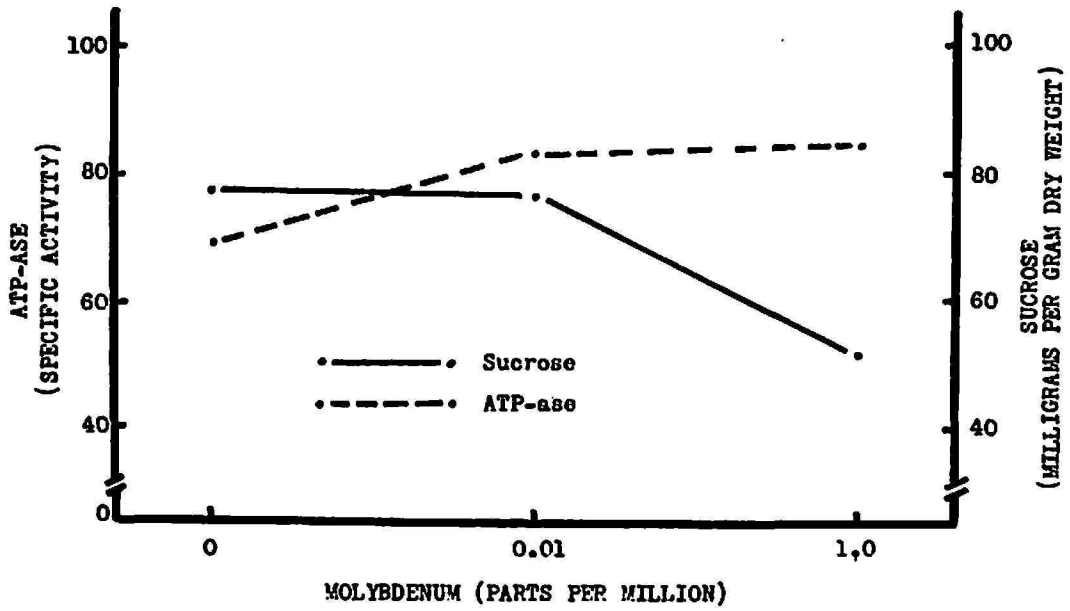

FIG. 7.—Sucrose content and ATP-ase activity of leaves from immature sugarcane supplied with variable molybdenum in sand culture. Plants received a high level of calcium (9.0 meq./liter).

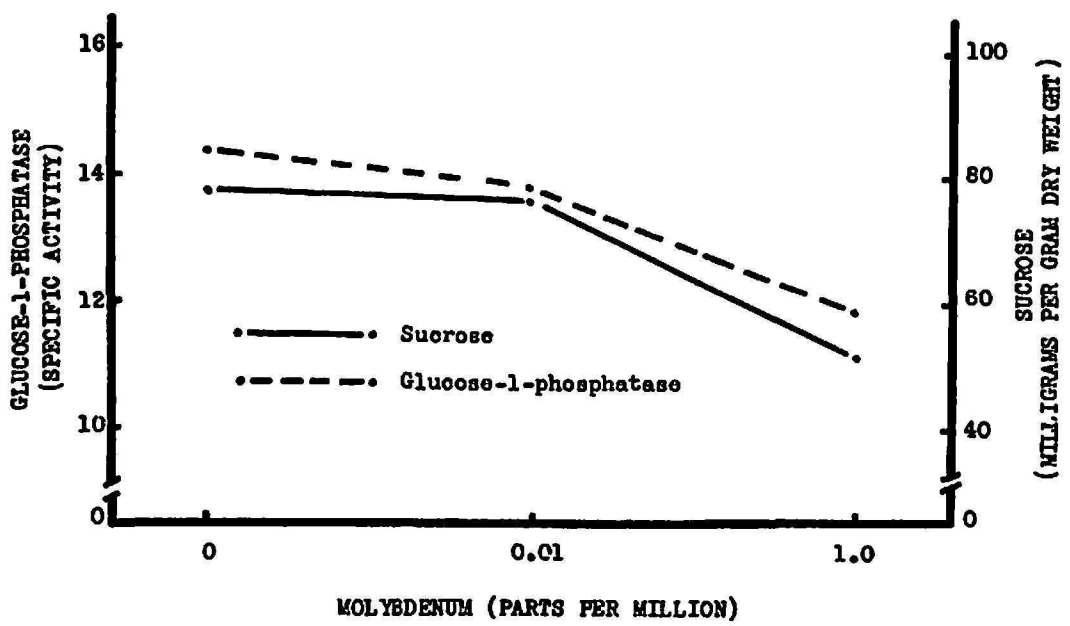

FIG. 8.—Sucrose content and glucose-1-phosphatase activity of leaves from immature sugarcane supplied with variable molybdenum in sand culture. Plants received a high level of calcium (9.0 meq./liter).

is perhaps one of the most effective phosphatase inhibitors normally available to plants. It is possible that by blocking reactive sites on the enzyme or substrate molecule, high concentrations of Ca may "protect" the enzymes from the inhibitory action of Mo, and consequently prevent Mo from inducing a greater sucrose content.

During the present study high Ca did not seem capable of suppressing the acid phosphatases, although the same Ca levels did perform this function with cauliflower. It should also be pointed out that the early stimulating effect of increased Mo, at the low level of Ca, was accomplished using tapwater for both nutrient solutions and supplemental watering. To achieve this effect with cauliflower it was necessary to use water which had been distilled twice under glass. We would thus assume that the local tapwater was extremely lacking in Mo, which is not very likely, or that sugarcane may have a greater requirement for Mo than the *Brassica* representative which was supposed to be among the most voracious Mo users. We might speculate that a plant having an abnormally high consumption of Mo should be in a far more favorable position to produce sucrose, and perhaps it is such a characteristic which could permit sugarcane to produce sucrose at rates unexcelled in the plant kingdom.

Again considering the beneficial effects of lime applied to cane fields with low pH, we should remember that liming is one of the most effective methods of correcting Mo deficiency (*10*). By raising the pH more of the native Mo becomes available to the plant. The same low pH soils which respond to lime are also the soils most likely to be low in available Mo. Thus, to some degree, the beneficial effects of liming sugarcane might be accounted for by a more favorable Mo supply.

Having already pointed out that the medium and high levels of Ca retarded the capability of Mo to inhibit phosphatase, it must also be recognized that the extent of the medium- and high-Ca effects were greatly dependent upon the concentration of Mo being supplied. Figure 9 illustrates that, while variable Ca caused no ATP-ase changes so long as Mo was low, an inverse response to increasing Ca occurred between the medium and high Mo levels. In other words, raising Ca from low to medium at the medium level of Mo caused a slight suppression of ATP-ase, accompanied by a slight increase of sucrose, while the same Ca treatment affected a stimulation of ATP-ase at the high-Mo level, accompanied by a decline of sucrose. We feel that this is just another way of saying that Mo is the key factor in phosphatase suppression. Since a certain number of molybdate ions must accompany the phosphatase for maximum activity, and since some unknown quantity greater than this number will suppress the enzyme, we can regard the Ca effects at the high-Mo level as a kind of mass action, in which enough of the Mo ions are blocked or replaced by Ca to reduce the effective

Mo level to a point below the inhibitory area, *i.e.*, within the range where Mo acts as an activator.

Such reasoning, of course, does not account entirely for the Mo × Ca × sucrose relationships recorded during this study. For example, the lowest sucrose content, aside from that already discussed for the low Ca × medium Mo treatment, was recorded among plants receiving a combination of high Ca and high Mo (table 5). However, phosphatase activity was not so excessively high among these plants as we might have expected on the basis of the original phosphatase-sucrose hypothesis under scrutiny. We are reminded that many enzymatic systems are involved in the ultimate

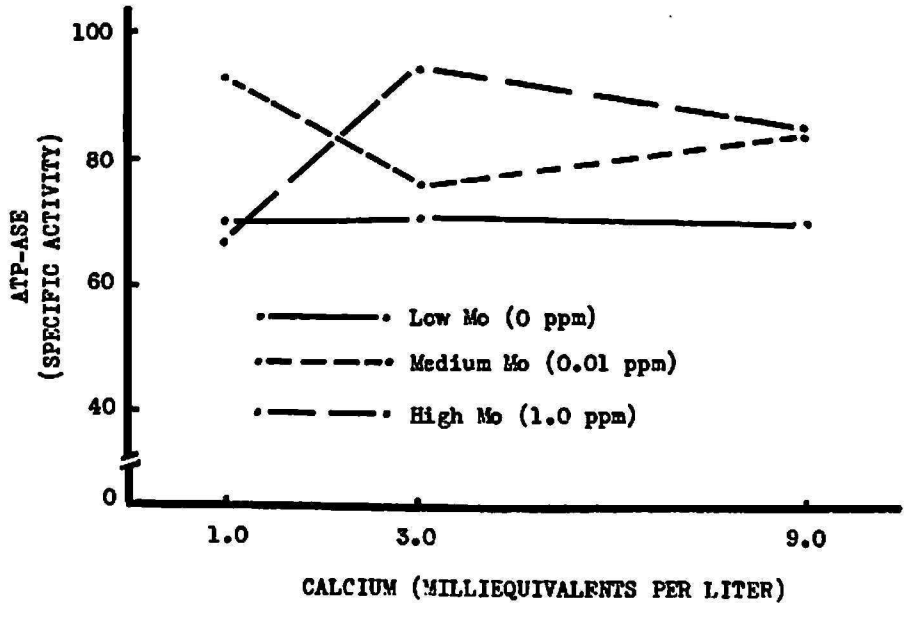

FIG. 9.—Effects of varying molybdenum upon the response of ATP-ase to increasing levels of calcium supplied to immature sugarcane in sand culture.

production of sucrose, and that the ionic properties peculiar to Mo and Ca must permit them to bear upon enzymes other than phosphatases.

A case in point is illustrated by amylase (table 4). This starch-hydrolyzing system was markedly suppressed by the combination of high Mo and high Ca which suppressed sucrose. At the moment, however, it is hazardous to attempt a definition of the sucrose-amylase relationship. Amylase was definitely suppressed by high Ca × high Mo, but it was stimulated by low Ca × medium Mo, and both treatments markedly suppressed sucrose. Amylase-sucrose relationships were observed previously in the M. 336 variety (*2*), and we can be fairly safe in stating that the relationship is somehow associated with the supply of free glucose required for the synthesis of sucrose precursors. The amylase system and its relationships with sucrose are presently being studied in detail at this laboratory.

SUMMARY

Phosphatase inhibitors were applied to immature sugarcane through the foliage and roots to determine whether such materials would suppress phosphatases in living tissues, and whether the induced inhibition would be accompanied by increased sucrose content. Molybdenum, at rates of 2, 20, and 200 p.p.m., and F at rates of 1, 10, 100 p.p.m. were applied as foliar sprays to 14-week-old plants grown by sand culture in the greenhouse. Leaf samples were taken for sugar and enzyme analyses at 0, 3, 9, 18, and 27 days following treatment. A second group of plants, likewise grown in sand culture, was supplied with Mo as a nutrient variable at rates of 0, 0.01, and 1.0 p.p.m. elemental Mo, and with Ca at concentrations of 1.0, 3.0, and 9.0 meq./liter. It was believed that Ca should enhance any inhibitor effects induced by Mo. One harvest was taken for sugar and enzyme analyses when the plants were 9 weeks of age.

A large number of significant sugar and enzyme variations was induced by the variable Mo and F treatments. Phosphatase inhibition was usually accompanied by increased sucrose, although these effects were usually not consistent among all harvests. The most consistent response was obtained with 20 p.p.m. of Mo. This treatment suppressed phosphatases, particularly ATP-ase and glucose-1-phosphatase, and was still accompanied by a higher sucrose content after 27 days. The effects of F were more sporadic. Significant results were recorded from 10 p.p.m. at 18 days and from 100 p.p.m. at 27 days, with phosphatase suppression and increased sucrose appearing concurrently.

It was found that when Mo was supplied as a nutrient variable in factorial combination with Ca, the high level of Mo (1.0 p.p.m.) suppressed phosphatase and was accompanied by an increase of sucrose, but only so long as Ca was supplied at a low level (1.0 meq./liter). Calcium at 3.0 and 9.0 meq./liter retarded or completely eliminated the ability of Mo to suppress phosphatases. The importance that a Ca $\times$ Mo interaction may play in areas where liming is considered beneficial for sugarcane is discussed.

Molybdenum at a medium level (0.01 p.p.m.) was found to act as an activator of the acid phosphatases. This agrees with earlier observations recorded by the author while studying the Mo nutrition of cauliflower.

The results appear to verify the hypothesis that phosphatase inhibitors can be supplied to living tissues without damage to the plants, that phosphatases will be suppressed below normal activity levels, and that the suppression will be accompanied by increased sucrose. The increase of sucrose is apparently a result of a more favorable supply of ATP and phosphorylated hexoses needed for the biosynthesis of sucrose.

RESUMEN

Se aplicaron inhibidores de fosfatasa a caña de azúcar inmadura, a través del follaje y las raíces, para determinar si estos compuestos reprimirían las fosfatasas en tejidos vivos, y si la inhibición inducida iría acompañada de un aumento en el contenido de la sacarosa. Se hicieron aspersiones foliares de molibdeno, a razón de 2, 20, y 200 p.p.m., y de F a razón de 1, 10, 100 p.p.m., a plantas de 14 semanas de edad, sembradas en invernaderos en un suelo arenoso. Se tomaron muestras de hojas a 0, 3, 9, 18 y 27 días después del tratamiento para analizar el contenido de azúcar y de enzimas. A un segundo grupo de plantas, también sembradas en arena, se les suministró Mo como nutrimento variable, a razón de 0, 0.01 y 1.0 p.p.m. de Mo elemental, y Ca a concentraciones de 1.0, 3.0 y 9.0 meq./litro. Creíase que el Ca debe acelerar cualquier efecto inhibidor inducido por Mo. Se hicieron análisis para azúcar y enzimas cuando las plantas tenían 9 semanas de edad.

Con los tratamientos variables de Mo y F, se indujo un gran número de variaciones significativas en el contenido de azúcar y enzimas. La inhibición de fosfatasa iba acompañada usualmente de un aumento en la sacarosa, aunque estos efectos no siempre se repitieron en todas las cosechas. El resultado más consistente se obtuvo con 20 p.p.m. de Mo. Este tratamiento reprimió las fosfatasas, particularmente la ATP-asa y la glucosa-1-fosfatasa, y aún iba acompañado de un alto contenido de sacarosa, después de 27 días. Los efectos de F fueron más esporádicos. Se obtuvieron resultados significativos con la aplicación de 10 p.p.m. a los 18 días y de 100 p.p.m. a los 27 días, produciéndose concurrentemente una retención de fosfatasa y un aumento en el contenido de la sacarosa.

Se encontró que cuando se aplicó Mo como nutrimento variable en combinación factorial con Ca, la alta proporción de Mo (1.0 p.p.m.) retuvo la fosfatasa y estuvo acompañada de un aumento en la sacarosa, pero sólo mientras las aplicaciones de Ca fueron a un bajo nivel (1.0 meq./litro). El calcio aplicado a razón de 3.0 y 9.0 meq./litro. retardó o eliminó completamente la capacidad del Mo para retener las fosfatasas. Aquí se discute la importancia que la interacción entre el Ca y el Mo pueda tener en zonas donde la aplicación de cal se considera beneficiosa para la caña de azúcar.

También se encontró que el molibdeno aplicado a un nivel mediano (0.01 p.p.m.) actúa como activador de las fosfatasas ácidas. Esto concuerda con observaciones previas hechas por el autor mientras estudiaba la nutrición del coliflor con el Mo.

Los resultados parecen corroborar la hipótesis de que los inhibidores de la fosfatasa pueden suministrarse a los tejidos vivos, sin causar daño a las

plantas; que las fosfatasas se reducirán a niveles de actividad bajo lo normal; y que la supresión estará acompañada por un aumento de la sacarosa. El aumento de la sacarosa aparentemente se produce mediante un suministro favorable de ATP y de hexosas fosforiladas, tan esenciales para la biosíntesis de la sacarosa.

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