RESEARCH NOTES

EFFECTS OF MANGANESE DEFICIENCY ON LEAF CHLOROPHYLL AND CERTAIN ENZYMES IN SUGARCANE RAISED IN SAND CULTURE[1]

Manganese (Mn) is an essential micronutrient for plant growth and development. Davis[2] considered Mn as essential for sugarcane from his culture media studies. Manganese deficiency in sugarcane first was reported from Hawaii as 'Pahala blight'. Deficiency of this element also has been reported in the Florida Everglades. Application of Mn with fertilizer ameliorated the deficiency at both locations. Possingham et al.[3] showed that lack of Mn produced marked changes in the structure of spinach chloroplasts. It has been established that manganese is a functional constituent of higher plant chloroplasts,[4] and a close correlation between their manganese content and photochemical activity has been described.[5]

The effect of manganese deficiency on chlorophyll concentration, sugar production and enzyme behavior in the leaves of sugarcane are virtually unknown. Low-manganese effects on leaves of different rank and on sugar-accumulation processes apparently have not been examined. Some evidence suggests that Mn may serve to stabilize acid invertases in immature storage tissue.[6]

Workers in Hawaii[7,8] and Guyana[9] described manganese deficiency in sugarcane grown in solution culture. In Puerto Rico, Samuels and Cibes-Viadé[10] raised four sugarcane varieties in sand culture omitting manganese

[1] Manuscript submitted to Editorial Board March 25, 1971.

[2] Davis, L. E., Manganese as an essential element in the growth of sugarcane, Hawaiian Planters' Record, 35: 393–400, 1931.

[3] Possingham, J. V., Vesk, M., and Mercer, F. V., The fine structure of leaf cells of manganese-deficient spinach, J. Ultrastructure Research 11: 68–83, 1964.

[4] Possingham, J. V., and Spencer, D., Manganese as a functional component of chloroplasts, Australian J. Biol. Sci. 15: 58–68, 1962.

[5] Ibid.

[6] Samuels, G. and Alexander, A. G., Influence of variable manganese and silicon on the nutrition, sugar production, and enzyme activity of immature sugarcane, Proc. Int. Soc. Sugar Cane Technol., 13th Congr., Taiwan, 544–55, 1968.

[7] Martin, J. P., Symptoms of malnutrition manifested by the sugarcane plant when grown in culture solutions from which certain essential elements are omitted, Hawaiian Planters' Record, 38: 3–30, 1934.

[8] Humbert, R. P. and Martin, J. P., Nutritional deficiency symptoms in sugarcane, Hawaiian Planters' Record, 55: 95–102, 1955.

[9] Evans, H., Elements other than nitrogen, potassium, and phosphorus in the mineral nutrition of sugarcane, Proc. 10th Congr. Int. Soc. Sugar Cane Technol., 473–508, 1959.

[10] 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.

FIG. 1.—Manganese deficient sugarcane leaves showing interveinal chlorosis of alternating white and green bands.

from the nutrient solution. They found that Mn-deficiency suppressed cane growth and yield in all except the high-tonnage variety P.R. 980. Samuels and Alexander[11] described a series of main effects and interactions of manganese and silicon on the sugar production and enzyme activity of immature sugarcane.

In the experiments reported herein, Mn-deficiency was induced in immature plants of the P.R. 980 variety to examine the enzyme, chlorophyll, and sugar changes in plants experiencing manganese stress. One-eye cuttings were grown in sand culture following the method described by Samuels and Alexander,[12] except that once-distilled water was used rather than tap water. All plants received a complete nutrient solution except the low-manganese series from which the micronutrient was withheld. Plants were harvested at 5 months of age, by which time the manganese deficiency symptoms were acute. Leaves +1, +2, +3, and +4 were analyzed for chlorophyll content, protein, ATP-ase, acid phosphatase, amylase and sucrose. Analytical procedures were described previously by Alexander,[13]

[11] Samuels, G. and Alexander, A. G., loc. cit.

[12] Samuels, G. and Alexander, A. G., loc. cit.

[13] 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.

TABLE 1.—*Mean values for leaf sucrose, chlorophyll content, ATPase, acid phosphatase, and amylase of sugarcane plants raised with and without manganese in sand culture*[1]

Leaf rank	Chlorophyll μg./ml.		Sucrose mg./g.F.W.		Protein mg./g.D.W.		Specific activity				Amylase	
							ATPase		Acid phosphatase			
	+Mn	−Mn	+Mn	−Mn	+Mn	−Mn	+Mn	−Mn	+Mn	−Mn	+Mn	−Mn
+1	11.2	5.4	25	23	3.9	.075	33.9	17.1	25.4	11.7	40.4	75.9
+2	15.8	5.9	49	26	4.3	.105	20.0	15.8	14.3	10.4	15.2	55.0
+3	12.2	7.8	29	21	4.5	.105	25.4	12.3	18.8	9.5	25.9	38.0
+4	14.5	8.5	32	20	4.3	.106	20.5	11.2	15.3	9.4	22.2	28.2

[1] Each figure represents the mean of two replicates.

and Kumar et al.[14] Chlorophyll values were calculated in accordance with Arnon's equation.[15]

First symptoms of Mn-deficiency were observed as interveinal chlorosis of the middle leaves when the plants were 3 months of age. As the interveinal chlorosis became more pronounced (fig. 1), brown necrotic spots developed in the white stripes, which later coalesced. At about 5 months of age, when the samples were drawn for analyses, stem elongation had ceased and the young emerging leaves were completely necrotic. Plants showing acute deficiency developed lateral shoots. Leaves of these lateral shoots also showed mild interveinal chlorosis.

Results are summarized in table 1. Manganese deficiency caused a considerable depression in the protein content of all leaves irrespective of their rank. Among control plants, sucrose content was highest in leaf +2, verifying earlier observations with field-grown plants;[16] however, there was little difference in sucrose content among leaf ranks of low-Mn plants. Presumably, low manganese had become limiting against photosynthesis potential, whereas in control plants such factors as CO_2 assimilation rate and rate of sucrose transport had come into play.

The two phosphatases studied showed a predominance of activity in leaf +1, and an inverse relationship with sucrose content among leaf ranks. In the Mn-deficient plants a decreasing gradient was observed in the activity of both enzymes from leaf +1 to leaf +4. It is significant that the control leaf having the lowest sucrose content (leaf +1), also showed the highest enzyme activity, a fact which verifies much of the earlier work reported

[14] Kumar, A., Acin, N. M., and Alexander, A. G., Relationships of chlorophyll and enzyme gradients to basipetal sucrose transport in sugarcane leaves. (Unpublished.)

[15] Arnon, D. I., Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*, Plant Physiol. 24: 1–15, 1949.

[16] Kumar, A., Acin, N. M., and Alexander, A. G., loc. cit.

from this laboratory. Alexander[17,18,19,20] observed a similar sucrose-phosphatase relationship in which sucrose increased as the enzyme declined. Alexander and Samuels[21] postulated that the suppression of phosphatase makes possible a more favorable supply of phosphorylated precursors needed for sucrose synthesis.

Low manganese produced a major increase in amylase activity whereas the phosphatases were reduced roughly by half, in all leaf ranks. This offers tacit support for an in vivo regulatory role of Mn which had been implied by earlier in vitro data.[22] Moreover, the low Mn effect on amylase was especially powerful in leaf +2, the rank clearly most active in sucrose production when given adequate Mn. Amylase also showed a negative activity gradient between leaves +1 and +4. The higher amylase activity in the younger leaves was paralleled by low sucrose values in these leaves. An inverse relationship between foliar sucrose content and amylase activity in sugarcane has been observed previously and discussed at length.[23,24,25] The thesis that amylase performs in the conversion of insoluble polysaccharide to sucrose is further substantiated by the sucrose-amylase response to manganese stress. Moreover, the inverse relationship of amylase activity to sucrose content found to exist between young and mature leaves is consistent with the general inability of younger leaves to retain sucrose.

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[17] Alexander, A. G., Sucrose-enzyme relationship in immature sugarcane as affected by variable nitrate and potassium supplied in sand culture, loc. cit.

[18] Alexander, A. G., 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.

[19] Alexander, A. G., Induction of varying sugar levels in leaves of immature sugar cane sugarcane by use of acid phosphatase inhibitors, J. Agr. Univ. P.R. 49 (1): 35–59, 1965.

[20] Alexander, A. G., Effects of tungsten and molybdenum upon sucrose content and hydrolytic enzymes of immature sugarcane, J. Agr. Univ. P.R. 49 (4): 429–42, 1965.

[21] Samuels, G. and Alexander, A. G., loc. cit.

[22] Alexander, A. G., Hydrolytic proteins of sugarcane: Amylase, J. Agr. Univ. P.R. 49 (3): 308–24, 1965.

[23] Alexander, A. G., Sucrose-enzyme relationships in immature sugarcane as affected by variable nitrate and potassium supplied in sand culture, loc. cit.

[24] Alexander, A. G., Behaviour 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.

[25] Alexander, A. G. 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): 204–17, 1968.