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Effect of Vanadium on Growth, Chemical Composition, and Metabolic Processes of Mature Sugar Beet (Beta vulgaris L.) Plants[1]

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Abstract. As measured 7, 14, and 21 days after the application of 10^{-2} M vanadyl sulfate solution to the foliage of 4.5-month-old sugar beet plants, significantly less growth of the leaves and an increase in the sucrose content of the storage root resulted. Accompanying these alterations were a higher rate of carbon dioxide fixation, a lower rate of respiration, and a decreased rate of nitrate reductase, glutamic-pyruvic transaminase, phosphatase, and invertase activity. The enzymes of sucrose synthesis, sucrose synthetase, sucrose phosphate synthetase and uridine diphosphate glucose-pyrophosphorylase were stimulated. The content of reducing sugar, nitrite N, amino acids and protein was less, and that of nitrate N was greater in the vanadium-treated plants. In the majority of cases the greatest magnitude of change occurred during the first 7 days following treatment. The changes in growth and chemical composition are believed to be closely related to the stimulation or inhibition of the various enzymes by vanadyl sulfate.

Vanadium belongs to the transition group of metals, along with titanium, chromium, manganese and iron. Little is known regarding the effects of V on biological systems. It has been suggested that V can replace Mo in the microbial nitrogen fixation pathway (3). It causes an alteration in mammalian lipid oxidation and synthesis (5); counteracts the action of Mn in flax, soybean, and oat (24); inhibits the growth of *Mycobacterium tuberculosis*, being competitive with Mn and Cr (4); is effective in the inhibition of nitrate reductase in wheat embryo (20) and sugar beet (28); and uncouples oxidative phosphorylation in mitochondria isolated from liver of chicks (9).

Preliminary investigations in our laboratory showed that the foliar application of vanadyl sulfate, ($VOSO_4$), to sugar beet plants at various stages of growth from 1 to 4 months resulted in decreased growth of the leaves. This paper describes the effect of foliar spray on 4.5-month-old sugar beet plants, *Beta vulgaris* L. Along with changes in leaf growth, alterations in chemical composition of the root and of some metabolic processes in both root and leaves were determined.

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Materials and Methods

Sugar beet seed, S.K.E. R-11 (British Columbia Sugar Refining Company, Vancouver, B. C.) was sown in vermiculite and irrigated daily with a modified Hoagland's solution. Nine-day-old seedlings were transplanted singly to 1-gallon glazed ceramic pots containing sandy loam, and grown in a greenhouse for 4 months. At the end of this period 37 pots were transferred to a growth room wherein the growth conditions were a 16-hr photoperiod, 2000 ft-c, with 25° day and 18° night. Another 37 pots were placed in a second growth room which provided a 12-hr photoperiod, 2000 ft-c, and temperatures of 18° during the light and 7.3° during the dark.

When the plants were 4.5 months old their leaves were thoroughly sprayed with an aqueous solution of 10 mM $VOSO_4$, containing 0.2 % (v/v) Tween-20. The characteristics of the plants, described below, were determined 7, 14, and 21 days after the application.

Leaf Blade Area. The expansion of the 7 youngest non-coiled leaves of each of 5 plants was used to indicate the growth of the plant. The leaves were marked and the area determined at the time of application of the $VOSO_4$. The same blades were measured 7, 14, and 21 days after treatment.

Chemical Composition of Root. The beets were trimmed of leaves and small roots, washed, and the crowns removed. The remaining portion was finely chopped, and blended in a Waring Blendor for 3 min. Three 50-gram aliquots of the blended material were used for the determination of reducing sugars, sucrose, and ammonium. Another three 50-gram samples were dried at 85° to a constant weight, then ground, redried and stored over $CaCl_2$ in desiccators.

The methods of Loomis and Shull (12) were used to prepare the extracts of the freshly blended root material for reducing sugar, sucrose, and ammonium determination. Reducing sugar was measured by the arsenomolybdate reagent of Nelson (14). An adaptation of the method of Vickery and Pucher (22) was employed for ammonium measurement.

An aqueous extract of the dry powder was used in the determination of the nitrite, nitrate, and amino acid contents. To 0.5 g of dried powder was added 35 ml of distilled water. After boiling for 5 min the extract was cooled, made to 50 ml volume, centrifuged and finally filtered through glass wool. Nitrite and nitrate contents of the extract were determined by the method of Wooley *et al.* (26). The colorimetric method of Rosen (18) was used for the measurement of amino acid content.

The total nitrogen content of the dried powder was determined by the standard Kjeldahl method.

Samples for the determination of total protein of the root were prepared according to the method of West (25). The measurement of protein content was by the method of Lowry *et al.* (13).

Photosynthesis and Dark Respiration of Leaves. The rate of carbon dioxide exchange of the entire aerial portion of intact potted plants was measured in an open system with a Beckman infra red analyzer IR 215. The plant chamber was a 20-lb capacity polythene bag of 3 mil thickness. The rate of air flow was 1 liter per min, the temperature 29.5 ± 1.5°, and the light intensity for photosynthesis 2000 ft-c. The soil surface in the glazed pots was covered with silicone rubber. The same 3 untreated and 3 vanadium-treated plants, each with 25 leaves, were used for CO_2 exchange measurement immediately prior to and at the 3 intervals following treatment.

Plant Enzymes and Root Respiration. Three untreated plants were harvested at the time of application of the vanadium and 3 treated and 3 untreated plants were used 7, 14, and 21 days after treatment for the determination of enzyme activity in leaves and roots, and of storage root respiration. The roots of each group of 3 plants were combined, as were the leaves. From each aggregate, material was drawn for a minimum of 3 determinations in each case.

The rate of respiration of storage root tissue was determined by the method of Wort and Shrimpton (27) using the Warburg apparatus.

The preparation of all extracts for enzyme activity measurement was carried out at 0 to 4°. The extract used for the assay of glutamic-pyruvic transaminase (transaminase) and nitrate reductase (NRase) was prepared by blending 1 weight of finely chopped leaf blades or root material with 4 weights of cold 0.1 M phosphate buffer, pH 7.8, containing 1 mM GSH, for 2 min. The homogenate was strained through 4 layers of cheesecloth. For transaminase assay the filtrate was diluted 5 times by the addition of cold distilled water. NRase activity was measured in the supernatant prepared by centrifugation of the undiluted filtrate at 20,000*g* for 20 min. Transaminase activity was determined by an adaptation of the method of Reitman and Frankel (16), and that of NRase by a modification of the method of Evans and Nason (6).

The extract for the determination of invertase activity was prepared by blending 100 g finely chopped root or leaf with 100 ml cold distilled water for 2 min. The slurry was squeezed through 4 layers of cheesecloth, and the filtrate was clarified by centrifugation at 10,000*g* for 20 min. Two ml of 10 mM Na_2SO_3, was added to prevent darkening of the supernatant. The solution was then dialyzed with 5 changes of water, for 24 hr. The small amount of precipitate formed during dialysis was removed by centrifugation. The supernatant was used for the determination of invertase activity by the method of Pressey (15).

The extract, prepared according to the method of Hinde and Finch (10) was used for the measurement of the activities of the phosphatases. In the case of phenyl phosphatase and adenosine triphosphatase, 0.6 ml of the enzyme preparation in a total volume of 1.0 ml containing 3 mM phenyl phosphatase, or ATP, and 100 mM acetate buffer, pH 5.1, was incubated for 15 min at 27°. Glucose-6-, and fructose-6-phosphatase activities were determined by incubation of 0.6 ml of enzyme preparation in a total volume of 1 ml containing 100 mM tris-HCl, pH 8.2, and 3 mM glucose-6-, or fructose-6-P. In order to determine the amount of phosphate originating from the enzyme preparation a tube containing appropriate amounts of water, buffer, and 0.6 ml of enzyme preparation in a total volume of 1 ml was incubated at 27° for 15 min. The enzyme action in the mixture was stopped by the addition of 3 ml of 10% trichloroacetic acid. The P_i was determined by the method of Fiske and SubbaRow (7).

The crude extract for the determination of the activity of UDPG-pyrophosphorylase, sucrose synthetase, and sucrose phosphate synthetase was prepared by an adaptation of the method of Rorem *et al.* (17). One hundred grams of fresh and thoroughly washed root or leaf material was finely chopped and blended with 100 ml of 0.05 M phosphate buffer, pH 7.2, for 2 min, at 0 to 4°. The homogenate was then squeezed through 4 layers of cheesecloth. The liquid obtained was centrifuged at

13,000*g* for 15 min. The supernatant was gradually taken to pH 4.9 with acetic acid and immediately centrifuged at 13,000*g* for 15 min. The precipitate was discarded, and the clear yellowish supernatant was left overnight at 4°. The flocculant precipitate which formed was collected by centrifugation and washed several times with 0.02 M, pH 4.9, acetate buffer. The recentrifuged precipitate was then dialyzed against 0.02 M, pH 7.2, phosphate buffer. This enzyme fraction was used to determine the activities of the 3 enzymes. The assay techniques for sucrose synthetase and sucrose phosphate synthetase were essentially the same as those of Rorem *et al.* (17). UDPG-pyrophosphorylase activity was determined according to the method of Gander (8).

In all cases the activities of enzymes were based on the protein content of the enzyme extract, determined by the method of Lowry *et al.* (13).

Statistical Analysis. Analysis of variance and comparison of means were carried out according to Tukey's ω-procedure (21).

Results and Discussion

The effects of vanadium treatment on the growth, composition, and metabolism of the sugar beet plants grown under the 2 sets of conditions were very similar. In this paper the responses of the beets grown at the higher temperatures and longer photoperiod are recorded.

As may be seen in the tables, the difference between the values in the $VOSO_4$-treated plants and those of untreated plants are significant at the 0.01 level in practically every case. In many instances the effect reached its maximum during the first 7-day interval after treatment, and subsequent values tended to remain at this new level.

The application of $VOSO_4$ resulted in a significant inhibition of growth of leaves of the sugar beet plants, measured 7, 14, and 21 days after treatment. The growth increments, in cm^2, of the 7 marked leaves of $VOSO_4$-treated plants during the interval from treatment to the seventh day, to the fourteenth day, and to the twenty-first day were 66, 60, and 58 % of the area increments of the measured leaves of control plants (table I, Fig. 1).

In the roots of treated plants the amount of reducing sugar, on a fresh weight basis, was significantly reduced. The reduction was 9 % when measured on day 7, and it remained at that level during the remaining two 7-day intervals. The content of sucrose rose 28 % above that of the untreated plants during the first 7 days. Thereafter it remained relatively constant (table II).

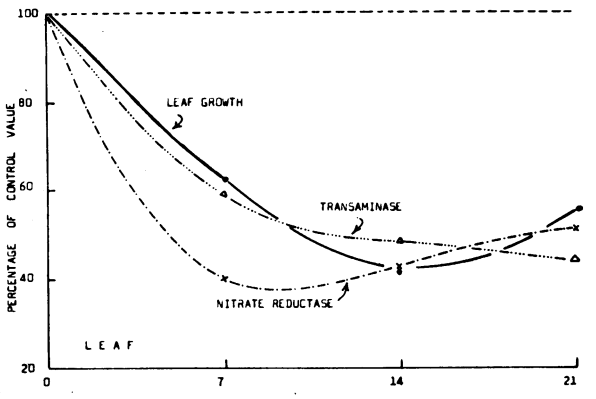
Table I. *Effect of $VOSO_4$ on Leaf Growth, Photosynthesis and Dark Respiration, and on Respiration of Storage Root of Sugar Beet Plants, 7, 14, and 21 Days After Treatment*

Time after treatment	Total area of marked leaves/plant		Increase in area from day 0/plant		Photosynthesis		Dark respiration		Root respiration	
	C[1]	V[1]	C	V	C	V	C	V	C	V
Days	*cm²*		*cm²*		*µl CO_2/dm²·hr*				*µl O_2/g fresh wt·hr*	
0	339	355[2,3]	...	...	1434	1425[3]	443	430[3]	81	. .
7	496	459[5]	157	104	1412	1506[5]	610	493[5]	82	64[5]
14	556	486[5]	217	131	1012	1112[5]	517	376[5]	75	51[5]
21	639	530[5]	300	175	949	1238[5]	351	201[5]	66	55[5]

[1] C = control or untreated plants. V = plants sprayed with $VOSO_4$
[2] Analysis of variance and comparison of means by Tukey's ω-procedure (21).
[3] = not significant at 0.05 level.
[4] = significant at 0.05 level.
[5] = significant at 0.01 level.

Table II. *Effect of $VOSO_4$ on Chemical Composition of the Root of Sugar Beet Plants, 7, 14, and 21 Days After Treatment*

Time after treatment	Reducing sugar		Sucrose		Nitrate N		Nitrite N		Ammonium N		Amino acid		Protein		Total N	
	C	V	C	V	C	V	C	V	C	V	C	V	C	V	C	V
days	*% fresh wt*		*% fresh wt*		*µg/g dry wt*		*µg/g dry wt*		*µg/g dry wt*		*mg/g dry wt*		*mg/g fresh wt*		*mg/g dry wt*	
0	0.17	...	14.3	...	95	...	19	...	143	...	1.9	...	9.2	...	11.4	...
7	0.21	0.19[5]	14.7	18.8[5]	89	125[5]	31	19[5]	163	145[5]	2.3	2.0[5]	9.0	6.3[4]	12.1	12.7[3]
14	0.19	0.17[5]	17.3	19.8[5]	108	149[5]	32	14[5]	124	112[5]	1.8	1.6[5]	8.2	5.5[4]	14.9	12.8[5]
21	0.24	0.22[5]	13.9	17.3[5]	118	119[5]	45	28[5]	98	105[5]	1.7	1.5[5]	8.6	5.9[4]	18.2	13.9[5]



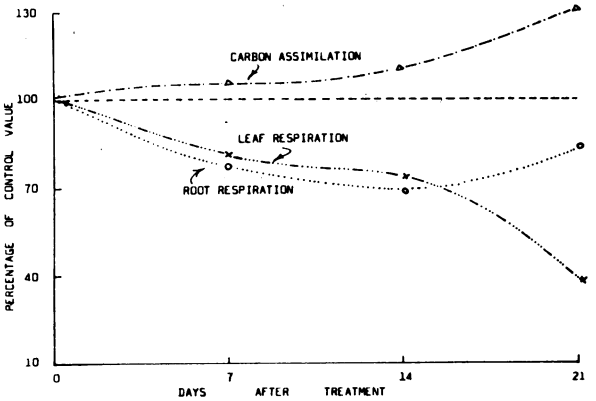


FIG. 1 (Upper). Leaf growth, NRase and transaminase activity 7, 14, and 21 days after treatment of sugar beet plants with $VOSO_4$.

FIG. 1 (Lower). Carbon assimilation, leaf dark respiration, and root respiration 7, 14, and 21 days after treatment of sugar beet plants with $VOSO_4$.

The contents of nitrogenous compounds are given in table II. Ammonium N was lower by approximately 10 % at days 7 and 14. While it decreased in both control and treated plants after day 7 the fall was less rapid in the roots of $VOSO_4$-treated plants. The contents of nitrite N, amino acids, and protein fell steeply during the first 7-day interval to levels 12 to 37 % below controls and remained at the low values until the conclusion of the experiment. The nitrate content of the root rose sharply to a value 39 % greater than in untreated plants, remained at the high level for a week, and then fell to control value by day 21. Total N initially increased slightly, and then steadily declined, showing a reduction of 23 % on the twenty-first day after treatment.

Vanadium treatment resulted in a 20 % decrease in the rate of respiration of the root tissue and also of dark respiration of the foliage during the first 7-day interval. The rate continued to decline in the leaves, but in the root it remained at the low level measured on day 7. On the contrary, net carbon assimilation rose during the period of measurement, becoming 30 % greater than control by the twenty-first day (table II, Fig. 1).

The effect on the activity of enzymes was also greatest during the first week following treatment. The specific activities of the 2 enzymes of nitrogen metabolism, NRase and transaminase, (table III, Fig. 1), were significantly reduced in both leaves and roots. The decreases ranged from 21 to 63 %. Considerable increases in activity of the enzymes of sucrose synthesis, sucrose synthetase, sucrose phosphate synthetase, and UDPG-pyrophosphorylase, occurred in both leaves and roots of treated plants (table IV, Fig. 2). The increases ranged from 6 to 131 %.

Accompanying the increases in the activities of the enzymes of sucrose synthesis there was a diminution of the activities of the hydrolytic enzymes associated with the biosynthesis of sucrose, measured in this experiment (table V, Fig. 3). As indicated, the activities of invertase (Fig. 2), glucose-6-, and fructose-6-phosphatase, ATPase, and also phenyl phosphatase were significantly reduced in the treated plants.

The diminished activity of both NRase and transaminase in the root of vanadium-treated plants is reflected in the reduction of the root's content of

Table III. *Effect of $VOSO_4$ on Activity of NRase, Transaminase and Invertase in Leaves and Roots of Sugar Beet Plants 7, 14, and 21 Days After Treatment*

Plant part	Time after treatment	Nitrate reductase C	Nitrate reductase V	Transaminase C	Transaminase V	Invertase C	Invertase V
	days	*mμ/mole NO_2/mg protein•30 min*		*μ/mole pyruvic acid/ mg protein•2 hr*		*mμmole reducing sugar/mg protein•2 hr*	
Leaf	0	33.4	...	45.5	...	9955	...
	7	20.9	12.1[5]	69.2	28.0[5]	7373	5920[5]
	14	20.8	11.2[5]	63.2	27.5[5]	7150	4695[5]
	21	33.3	15.0[5]	68.3	40.9[5]	9283	4185[5]
Root	0	24.3	...	80 2	...	7843	...
	7	14.5	7.5[5]	94.0	53 4[5]	6089	5559[5]
	14	17.3	6.5[5]	82.2	48.1[5]	3936	3648[5]
	21	19.8	10.2[5]	96.3	76.3[5]	5122	4407[5]

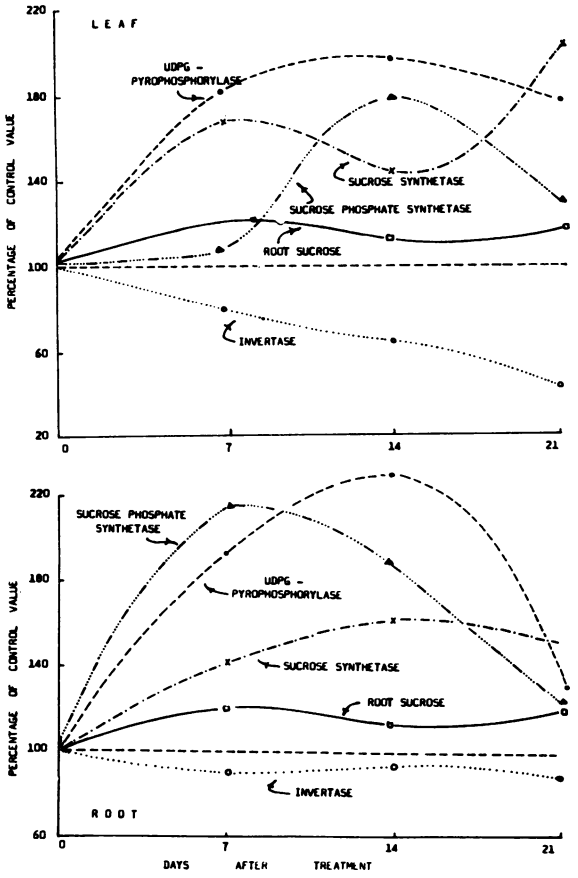


FIG. 2 (Upper). UDPG-pyrophosphorylase, sucrose synthetase, sucrose phosphate synthetase, and invertase activity in the leaf, and content of sucrose in the root 7, 14, and 21 days after treatment of sugar beet plants with $VOSO_4$.

FIG. 2 (Lower). UDPG-pyrophosphorylase, sucrose synthetase, sucrose phosphate synthetase and invertase activity in the root, and content of sucrose in the root 7, 14, and 21 days after treatment of sugar beet plants with $VOSO_4$.

protein. Similarly the significant decrease in the activity of these 2 enzymes in the leaves, coupled with the large reduction in growth of these organs, suggests that the growth reduction may stem, in part.

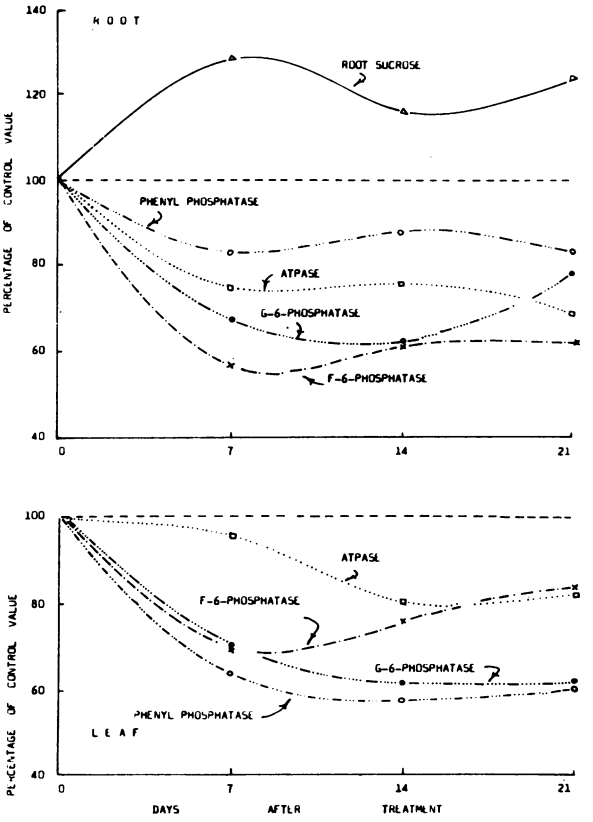


FIG. 3 (Upper). Activity of phosphatases and sucrose content in the root 7, 14, and 21 days after treatment of sugar beet plants with $VOSO_4$.

FIG. 3 (Lower). Activity of phosphatases in the leaf 7, 14, and 21 days after treatment of sugar beet plants with $VOSO_4$.

Table IV. *Effect of $VOSO_4$ on Activity of UDPG-pyrophosphorylase, Sucrose Phosphate Synthetase and Sucrose Synthetase of Leaves and Roots of Sugar Beet Plants*

Plant part	Time after treatment	UDPG-pyrophosphorylase		Sucrose phosphate synthetase		Sucrose synthetase	
		C	V	C	V	C	V
	days	*mμmole glucose-1-P mg protein•15 min*		*mμmoles sucrose/mg protein•2 hr*			
Leaf	0	137.7	...	207.8	...	61.7	...
	7	110.5	199.7[5]	202.4	213.9[5]	61.6	97.9[5]
	14	74.2	145.2[5]	126.2	226.5[5]	72.1	104.5[5]
	21	98.6	177.3[5]	113.9	144.9[5]	57.7	118.0[5]
Root	0	168.9	...	19.6	...	47.6	...
	7	79.3	151.5[5]	11.3	24.2[4]	27.4	38.6[5]
	14	60.7	140.1[5]	13.4	24.4[4]	55.5	89.7[5]
	21	98.2	119.7[5]	18.4	22.0[3]	47.1	70.0[5]

Table V. *Effect of $VOSO_4$ on the Activity of Phosphatases of Leaves and Roots of Sugar Beet Plants, 7, 14, and 21 Days After Treatment*

Plant part	Time after treatment	Glucose-6-phosphatase		Fructose-6-phosphatase		Phenyl phosphatase		ATPase	
		C	V	C	V	C	V	C	V
	days	*mμmoles P_i/mg protein*							
Leaf	0	343.8	...	186.2	...	312.2	...	538.7	...
	7	260.8	186.6[5]	197.6	139.0[5]	195.8	122.5[5]	249.2	237.9[5]
	14	145.3	90.4[5]	155.5	118.4[5]	293.6	151.5[5]	525.2	427.4[5]
	21	119.1	73.7[5]	360.0	300.6[5]	275.8	165.7[5]	570.2	466.0[5]
Root	0	48.0	...	50.1	...	61.5	...	46.7	...
	7	26.5	17.9[5]	12.5	7.0[5]	42.4	35.3[5]	15.7	11.9[5]
	14	29.9	18.7[5]	17.4	10.9[5]	36.8	32.4[5]	11.6	8.8[5]
	21	45.5	36.8[5]	13.5	8.3[5]	40.3	33.3[5]	21.1	14.3[5]

from an amino acid and protein supply stress which was the consequence of vanadium effects on these 2 enzymes, whose activities are involved in the production of amino acids. The increase in nitrate and decrease in nitrite contents also attest to the lower NRase activity in the treated plants. Warrington (24) reported that V supplied through the roots to flax, oats, and soybeans caused a rise in nitrate N. The relationship of growth with activity of NRase in sugar beet plants has been shown by other workers. In vigorously growing peas and cocklebur (23) and in sugar beet leaves (27) NRase activity has been shown to be high, whereas a diminution of activity was measured as the plants matured and growth decreases.

It was shown by Joy (11) that labeled sucrose fed to the root of sugar beet seedlings resulted in no label in the sugars of the young, growing leaves, but amino acids, especially glutamic acid, were the main labeled compounds. The carbon for leaf amino acid skeletons apparently came from the roots. An experiment with sugar beet by Snyder and Tolbert (19) suggests that if the N supply is not limited, plants may preferentially synthesize the citric acid cycle products and their amino acid counterparts, thus less sucrose is stored. It would appear that hexose degradation is required both for the supply of energy needed for nitrate reduction and as a source of material for organic acid synthesis. Thus low NRase activity coupled with weak invertase and phosphatase activities, and a depressed rate of respiration, as found in the vanadium-treated plants, would favor sucrose accumulation.

Other things being equal, a greater sucrose content would be expected to result from the stimulation of carbon dioxide fixation and from the greater activity of the enzymes of sucrose biosynthesis, as well as from the lessened, detrimental, activity of phosphatases involved in several of the reactions leading to sucrose synthesis, as were also measured in the plants which had received $VOSO_4$. Alexander (1) reported that the inhibition of phosphatases in general by Mo resulted in a higher sucrose content in the leaves of sugarcane. He also noted the inverse relationship between ATPase activity and the sucrose content of the leaves (2).

With the phenomenon of "ripening" of the sugar beet are associated decreases in respiratory rate and in N utilization and content, significant reduction in amino acids and protein, and an increase in deposition of sucrose in the storage root. These characteristics were found in the $VOSO_4$-treated sugar beet plants.

The responses of the mature (4.5 months old) sugar beet plant to the application of vanadium in this experiment suggest the possibility that, by the application of $VOSO_4$ to sugar beet plants, "ripening" may be induced, and that the late autumn growth of the beet, which frequently occurs at the expense of stored sucrose, may be considerably reduced by the foliar application of $VOSO_4$ 7 to 21 days before digging the sugar beets.

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