Foliar Application of Pyrocatechol to Prevent Late Growth of Sugarbeet and Increase Sucrose Content

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The inverse relationship between the nitrogenous content of the sugarbeet plant and the sucrose concentration of its root has been observed many times (14, 15)[2]. This has led to the suggestion that for a period before harvest, nitrate supply should be greatly reduced to prevent the reinvestment of sucrose in the production of surplus foliage and fibrous roots (15).

The various methods suggested and used to insure the proper level of N at the various ontogenetic stages of the beet include the application of the correct amount of N early in the season to stimulate early growth, but to insure that N be depleted in time for ripening of the plant (15), and the breeding of varieties which will ripen regardless of the level of N in the soil (7). These methods have obvious disadvantages: dependence on average weather conditions, presence or absence of microorganisms, duration of the growing season, and performance and stability of a new variety. An alternative to the above would be the use of selective metabolic inhibitors to induce ripening and reduce the late growth which often occurs at the expense of stored sucrose. Vandyl sulfate (10) and maleic hydrazide (19) have been successfully used for this purpose. This paper describes the use of pyrocatechol in this selective capacity.

Material and Methods

Nine-day old seedlings of sugarbeet, *Beta vulgaris L.*, from the S. K. E. R-11 seed obtained from the British Columbia Sugar Refining Company, were transplanted singly to one-gallon glazed ceramic pots containing sandy loam and grown in a greenhouse for 4 months. When the plants were 4 months old, 31 pots were transferred to a controlled environment room which provided a 16-hour photoperiod, 2000 ft-c, with 25°C light, and 18°C dark temperature.

When 4.5 months old, the leaves of those plants to be treated were thoroughly sprayed with an aqueous solution of 10^{-2}M pyrocatechol (PC) containing 0.2% (v/v) Tween-20. The control plants were sprayed with the surfactants. The PC-treated and control plants were replicated nine times for the determination

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[2] Numbers in parentheses refer to literature cited.

of chemical composition of root and five times for the determination of leaf area. All plants were arranged in a randomized complete block design. The increase in leaf area and changes in the chemical composition of the storage roots were determined 7, 14, and 21 days after application.

Leaf blade area. The expansion of the seven youngest non-coiled leaves of each of five plants was used to indicate the growth of the plant. The leaves were marked with india ink and the area determined at the time of application of PC. The same blades were measured 7, 14, and 21 days after treatment. The formula for blade area was $L \times W \times 0.608$, where L is the blade length and W the maximum blade width. The factor 0.608 was obtained in a previous experiment by the comparison of the product of L and W of 49 representative leaves with their area as determined by a planimeter. The average variation of the calculated area from the actual area was 5.6%.

Chemical composition of root. The beets were trimmed of leaves and small roots, washed, and the crown removed. The remaining portion was finely chopped and blended in a Waring blender for 3 minutes. Three 50-gram aliquots of the blended material were used for the determination of reducing sugar, 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 (4) were used to prepare the extracts of the freshly blended root material for reducing sugar, sucrose, and ammonium determination. The arsenomolybdate reagent of Nelson (6) was used to measure reducing sugar and that of Vickery and Pucher (16) for ammonium.

Nitrite, nitrate, and amino acid contents were determined on an aqueous extract. Thirty-five ml of distilled water were added to 0.5 g of dried plant material, and the mixture was boiled for 5 minutes. The extract was then 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 Wolley et al. (18). The colorimetric method of Rosen (8) was employed for the measurement of the soluble amino acid content.

The standard Kjeldahl method was used to determine the total nitrogen content of the dried powder.

Samples for the determination of total protein of the root were prepared according to the method of West (17). The measurement of protein content was by the method of Lowry *et al.* (5).

Analysis of variance and comparison of means were carried out according to Tukey's w-procedure (13).

Results and Discussion

The area measured 7, 14, and 21 days after the application of PC indicated a considerable reduction in leaf expansion. The maximum reduction in the growth of leaves, 68%, was on the 14th day after treatment (Table 1).

The reducing sugar content of the root of the treated plants was less than the control plants on all three dates of harvest. The reducing sugar contents of the treated plants were 76%, 68%, and 87% of the control values on the 7th, 14th, and 21st day after treatment, respectively (Table 2). The sucrose percentages of the treated beets were higher than the control plants; the actual increases were 11% on the 7th day, 6% on the 14th day, and 9% on the 21st day after treatment (Table 2).

The effect of PC on nitrate N, nitrite N, and ammonium N is given in Table 3 and on amino acids, total N, and protein in Table 4. Nitrate N in the root of treated plants was higher than the control plants on all three dates of measurement. The nitrite N content of the PC-treated plants was lower by 3%

Table 1.—The effect of pyrocatechol (PC) on growth of leaves of 4.5-month-old sugarbeet plants, 7, 14, and 21 days after treatment.

Days after treatment	CK[1]		PC[1]	
	Average leaf area/plant cm²	Increase % from date of treatment	Average leaf area/plant cm²	Increase % from date of treatment
0	338.89	.....	378.41	.....
7	495.86	46.3	452.57	19.6[ns][2]
14	556.28	64.2	465.46	20.3**
21	639.48	88.7	485.50	28.3**

[1]CK = Control or untreated plants
PC = Plants sprayed with pyrocatechol
[2]Analysis of variance and comparison of means by Tukey's w-procedure.
ns = not significant
* = significant at 0.05 level
** = significant at 0.01 level

Table 2.—Effect of pyrocatechol (PC) on reducing sugars and sucrose content of the root of 4.5-month-old sugarbeet plants, 7, 14, and 21 days after treatment.

Days after treatment	Reducing sugars (% of fresh wt.)		Sucrose (% of fresh wt.)	
	CK	PC	CK	PC
0	0.17	.....	14.3	.....
7	0.21	0.16**	14.7	16.2**
14	0.19	0.13**	17.3	18.4*
21	0.24	0.21**	13.9	15.1**

Table 3.—Effect of pyrocatechol (PC) on nitrate, nitrite, and ammonium N content of the root of 4.5-month-old sugarbeet plants, 7, 14, and 21 days after treatment.

Days after treatment	Nitrate N (μg/g dry wt.)		Nitrite N (μg/g dry wt.)		Ammonium N (μg/g dry wt.)	
	CK	PC	CK	PC	CK	PC
0	95	.....	19	.....	143	.....
7	89	100**	31	30*	163	127**
14	108	119**	32	19**	124	117*
21	118	130**	45	25**	98	125**

Table 4.—Effect of pyrocatechol (PC) on the soluble amino acids, total nitrogen, and protein content of the root of 4.5-month-old sugarbeet plants, 7, 14, and 21 days after treatment.

Days after treatment	Amino N (mg/g dry wt.)		Total N (mg/g dry wt.)		Protein (mg/g dry wt.)	
	CK	PC	CK	PC	CK	PC
0	1.9		11.4	.....	9.2	.....
7	2.3	2.1ns	12.0	9.4**	9.0	6.2**
14	1.8	2.4ns	14.9	12.0**	8.2	5.8**
21	1.7	2.1ns	18.2	12.3**	8.6	6.4**

on the 7th day, 41% on the 14th day, and 44% on the 21st day after treatment. Ammonium N of the PC-treated plants was lower than the control plants on the 7th and the 14th day but not on the 21st day. Amino acid content values in the roots of treated plants did not differ significantly from control plant values. Total nitrogen and protein contents were lower than those of the control plants. The percentage decrease in protein content of the treated plants was 31% on the 7th day, 29% on the 14th day, and 26% on the 21st day after application of PC.

The inhibition of growth of the leaves by PC was coupled with reductions in the content of reducing sugars, nitrite, and protein in the root. At the same time, an increase in the content of sucrose and nitrate was observed in the root of sugarbeet treated with PC. High nitrate and low nitrite content of the treated plants may have been the result of diminished activity of the enzyme nitrate reductase. PC is an inhibitor of nitrate reductase activity in sugarbeets. This has been shown both in vitro (20) and in vivo (9).

The increase in sucrose content of the root of PC-treated plants may be attributed to the reduction in growth of the leaves, inhibition of nitrate utilization, and of protein synthesis. Conversions of nitrate to nitrite, hydroxylamine, and ammonia

are energy-requiring processes which must be coupled to carbohydrate breakdown. This was clearly demonstrated by Hamner (1) who found that the application of nitrate to nitrogen-starved tomato plants resulted in the formation of nitrite, the depletion of carbohydrate reserves, and a marked increase in respiration. The data from the experiment with sugarbeet by Snyder and Tolbert (12) suggest that, if the nitrogen supply is not cut down, plants may preferentially synthesize the citric acid cycle products and their amino acid counterparts and thus produce less sucrose. Tracer studies with C^{14} by Joy (3) have suggested that the carbon for leaf amino acid skeleton in sugarbeets comes from the root. In effect, therefore, the PC treatment of sugarbeets has simulated a low N supply by decreasing the plant's ability to utilize N through nitrate reduction.

Higher ammonium on the 21st day and amino acids (soluble) on the 14th and the 21st days may have been the result of PC-promoted protein hydrolysis.

The reduction in the amount of percent-reducing sugars in the root of the PC-treated sugarbeet indicates a possible reduction in the activity of invertase. Other studies have linked growth and invertase activity. For example, Sisakjan et al. (11) found that invertase activity diminished in the root of sugarbeets when the roots assumed storage function. Hatch and Glasziou (2) noted that the rate of elongation of internodes of sugar cane remained correlated with acid invertase irrespective of whether the independent variable was the age of tissue, temperature, or water regime.

The responses of the mature sugarbeet plant to the application of pyrocatechol in this experiment suggest that "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 pyrocatechol 7 to 21 days before digging the sugarbeet. The final result may be a larger content of sucrose in the storage root.

Summary

Pyrocatechol, 10^{-3}M, was applied to the foliage of mature plants of the sugarbeet (*Beta vulgaris L.*). As measured 7, 14, and 21 days after the application, significantly less growth of the leaves and a larger sucrose content of the storage root resulted. Protein, total N, and nitrite N were considerably less, while nitrate N was larger. Ammonium N was lower on days 7 and 14, but it was higher than the control on the 21st day. Changes in the amino acid content of the storage roots of treated beets lacked statistical significance.

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