Effect of the Pathotoxin Victorin on the Pattern of Glucose Catabolism in Susceptible Oats

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ABSTRACT

Decreases in the ratio of $^{14}CO_2$ derived from glucose-6-^{14}C to that from glucose-1-^{14}C (the C_6/C_1 ratio) have been observed in a variety of diseased plant tissues. Victorin, the pathotoxin produced by *Helminthosporium victoriae*, causes a decrease in the C_6/C_1 ratio of susceptible oat leaves. This provides additional evidence that victorin induces metabolic alterations similar to those found in other diseased plants.

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Victorin, the pathotoxin produced by *Helminthosporium victoriae* Meehan and Murphy, causes metabolic alterations in susceptible oat (*Avena sativa* L.) plants similar to those observed in other diseased plants (6). Decreases in the C_6/C_1 ratio (ratio of $^{14}CO_2$ derived from glucose-6-^{14}C to that from glucose-1-^{14}C) have been reported for diseased tissues, and Daly (3) has suggested that this effect in plants infected by obligately parasitic fungi (4, 7) may be caused primarily by the utilization of glucose-^{14}C by the parasite. In bean hypocotyls infected by *Rhizoctonia solani* Kuhn, however, Bateman and Daly (1) attributed the lower C_6/C_1 ratio of tissue apparently uninvaded by the fungus to altered host metabolic activity.

In a single experiment a decrease in the C_6/C_1 ratio was observed in victorin-treated oat leaves (*personal communication* from L. R. Krupka to the junior author regarding work carried out in the laboratory of J. M. Daly in 1961). Since the use of victorin eliminates any possible contribution by the living pathogen, this observation, if verified, would demonstrate that the effect represents a change in host metabolism. We report that victorin causes a striking drop in the C_6/C_1 ratio of susceptible oat leaves.

Oat plants (cv. Victorgrain 48-93, susceptible, and C. I. 7418, a resistant selection obtained from the former) were grown in Jiffy Mix (Jiffy Products of America, West Chicago, Ill.) at 23 C on a 12-h light period (17, 216 lux) for 9-11 days. Leaves were excised 10 min after the onset of the light period and allowed to take up distilled water (controls) or distilled water containing 10 units/ml of crude (5) or refined (ethanol-soluble fraction of freeze-dried crude filtrate) victorin through the cut ends for 4 h under room light (968 lux). After uptake the leaves were rinsed with water, blotted, and cut into 7-mm segments.

Samples of leaf tissue (0.5 g) were placed in 50-ml Erlenmeyer flasks containing 100 μmoles of glucose in 2 ml of 0.02 M KH_2PO_4, pH 4.7. Two ml of glucose-1-^{14}C or glucose-6-^{14}C stock solution (approximately 0.2 μCi/ml), prepared by dissolving crystalline glucose samples (C-1, 0.25 mCi/5.6 mg; C-6, 0.25 mCi/8.9 mg; New England Nuclear, Boston, Mass.) in distilled water, were then added to each flask. The flasks were closed with rubber stoppers, each of which held a phenethylamine-moistened filter paper wick impaled on a pin, and were shaken in a water bath (25 $\pm$ 0.5 C). Victorin-treated tissues were shaken for 2 h; controls were shaken until they had respired an approximately equal amount of CO_2 (4-5 h), as suggested by Daly, et al. (4). Respiratory CO_2 was measured simultaneously, with separate samples in duplicate, by the Warburg direct method (8) with a differential respirometer at 25 C. Values for radioactivity of $^{14}CO_2$, determined by scintillation spectrometry after the paper wicks had been placed in a scintillator solution (2), were converted to disintegrations/min by internal standardization and were corrected for the slightly different activities of the glucose-^{14}C stock solutions and for $^{14}CO_2$ recovered in flasks containing no tissue.

Victorin-treated first leaves of the susceptible oat variety Victorgrain 48-93 exhibited significantly lower C_6/C_1 ratios than those of untreated leaves (Table 1). This indicates that the mechanisms of glucose catabolism differ in treated and healthy leaves. While this effect could result from increased hexose monophosphate pathway activity, it could as well reflect other metabolic fates of glucose. With the toxin treatment conditions used in this work, victorin had no effect on either respiration rate or the C_6/C_1 ratio of resistant leaves. The ratio value of these was within the range of values reported here for untreated susceptible leaves.

TABLE 1. Recovery of $^{14}CO_2$ from glucose-1-^{14}C and glucose-6-^{14}C supplied to Victoria blight-susceptible oat leaves pretreated with victorin (10 units/ml) or distilled water (controls) for 4 h

Experiment[a]	Disintegrations/minute[b]				C_6/C_1	
	Control		Victorin			
	C_1	C_6	C_1	C_6	Control	Victorin
1	6693 ± 453	6355 ± 653	7855 ± 542	4379 ± 210	0.95	0.56
2	7368 ± 461	7346 ± 434	6945 ± 496	4394 ± 19	1.00	0.63
3	5761 ± 399	6754 ± 420	4010 ± 183	3097 ± 113	1.17	0.77

[a]Distilled water dilutions of crude (Exp. 1) or refined (Exp. 2, 3) victorin preparations were used.
[b]Values are means and standard deviations calculated on the basis of triplicate 0.5-g samples of leaf tissue in 50-ml Erlenmeyer flasks containing glucose-1-^{14}C or glucose-6-^{14}C (1.05×10^6 DPM/100 μmoles per flask) in 4 ml 0.01 M KH_2PO_4, pH 4.7.

Interpretation of victorin-induced metabolic abberations must take into account the effects of the toxin on host cell permeability (6). Interference with normal permeability could be reflected in a number of host processes, particularly those of central importance such as respiration. As pointed out by Daly, et al. (4), the interpretation of C_6/C_1 data is influenced by the difference in rates of CO_2 production in healthy and diseased tissues. Allowance for production of equal amounts of CO_2 serves to accommodate cycling of metabolites but does not consider altered host permeability. Loss of control over the entry of exogenous materials (and efflux of endogenous materials) and disruption of cellular compartmentation raise doubts concerning the degree to which the amount of CO_2 produced by diseased tissue reflects uptake and utilization of a particular compound.

Although the data presented herein do not identify the path of carbon from glucose to CO_2, they do demonstrate a marked change in carbohydrate metabolism, a characteristic of other plant diseases. This provides additional evidence of the value of the oat-victorin system as a model for the study of plant disease physiology.

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