Simultaneous Changes in the Rate and Pathways of Glucose Oxidation in Victorin-Treated Oat Leaves

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ABSTRACT

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In time-course tests, victorin (the selectively toxic product of *Helminthosporium victoriae*) induced in susceptible oat (*Avena sativa*) leaves simultaneous changes in oxygen uptake rate and the C6/C1 ratio (ratio of $^{14}CO_2$ derived from glucose-6-^{14}C to that from glucose-1-^{14}C). The former increased gradually; the latter dropped abruptly. Although decarboxylations of both C1 and C6 increased, C1 more so than C6, the ratio did not change significantly after the initial drop. In dosage-response tests based on a fixed treatment period and varying toxin concentration, victorin stimulated oxygen uptake only if it reduced the C6/C1 ratio. Victorin treatment increased anaerobic CO_2 production but decreased the anaerobic CO_2/aerobic CO_2 ratio. The respiratory uncoupler 2, 4-dinitrophenol increased both oxygen consumption and the C6/C1 ratio of healthy leaves. The results indicate that victorin simultaneously increases respiration rate and the activities of both the pentose phosphate pathway and the glycolysis-Krebs cycle pathway and that the pentose phosphate pathway is not primarily responsible for increased respiration.

Additional key words: resistant oats, radiolysis.

Increased pentose phosphate pathway (PPP) activity is among the explanations that have been offered to account for the commonly observed increase in oxygen consumption by diseased plant tissues (17). In keeping with this, the C6/C1 ratio (ratio of $^{14}CO_2$ derived from glucose-6-^{14}C to that from glucose-1-^{14}C) decreases in many diseased tissues (1, 2, 4, 5, 7, 16). In virus-infected bean (2), rusted wheat (16), and tobacco leaves inoculated with necrosis-inducing viruses (6, 7) the C6/C1 decrease and the respiratory increase apparently occur at about the same time during pathogenesis. Taking the C6/C1 drop to mark the onset of increased PPP activity, this pattern of change is consistent with a PPP-mediated increase in oxygen uptake. However, in rusted bean and wheat (4) and in tobacco leaves systemically infected by viruses (6, 7) the onset of increased respiration precedes the C6/C1 drop. Daly (3) has argued that the C6/C1 decrease in rust diseases is largely due to metabolic activity of the pathogen. Thus, one problem in determining the role of the PPP in pathological respiration is the assessment of the contribution of a living pathogen to the observed changes. A second problem is the likelihood that the 24-hr sampling interval used in time-course studies of pathogenesis (2, 4, 6, 7) was not sufficient to pinpoint the onset of respiratory changes.

Within the group of diseases characterized by C6/C1 reductions and respiratory increases, other lines of evidence concerning PPP involvement in increased respiration have shown inconsistencies. Rusted safflower showed reduced anaerobic CO_2 production rates and fluoride insensitivity of added respiration (5), both of which are consistent with a PPP role in respiration. In the bean rust and wheat rust diseases, increased anaerobic CO_2 output was observed, and fluoride sensitivity was found to be an unreliable indicator of respiratory pathway changes (4). In addition, virus-infected bean showed fluoride insensitivity of added respiration but greatly increased anaerobic CO_2 production (2).

The use of pathogen-produced toxins to induce disease symptoms eliminates some major methodological problems inherent in other experimental systems. Victorin, the selectively toxic product of *Helminthosporium victoriae* Meehan and Murphy, accurately reproduces the physiological symptoms of Victoria blight in oats (*Avena sativa* L.) and induces physiological changes similar to those observed in other diseased plants (14). Any contribution by a living pathogen to observed changes in oats is eliminated through the use of victorin to induce the disease. In addition, victorin treatment compresses the events of pathogenesis to a few hours (8, 14). This makes possible continuous observation of change in a single tissue sample and eliminates the variability that accompanies

the sampling of populations of healthy and diseased plants over a period of several days.

Among the victorin-induced changes in susceptible oat leaves are increased respiration rates (8) and decreased C6/C1 ratios (13). The latter is consistent with the interpretation that victorin increases oat leaf PPP activity. Therefore, the oat-victorin model seemed an ideal system with which to examine the question of whether the PPP mediates increased oxygen uptake in diseased plants. The purposes of this investigation were to determine whether: (i) the onset of increased respiration precedes the C6/C1 drop in victorin-treated oat leaves, (ii) victorin treatment affects anaerobic CO_2 production, and (iii) victorin and 2, 4-dinitrophenol affect glucose oxidation similarly. Part of this work has been reported (12).

MATERIALS AND METHODS

First leaves of 9- to 11-day-old plants (oat cultivar Victorgrain 48-93, susceptible, and C. I. 7418, resistant) were used throughout. Growth conditions, lots of glucose-1-^{14}C and glucose-6-^{14}C, and victorin preparations were those used previously (13). Tissues were treated with distilled water dilutions of crude or refined toxin preparations which, undiluted, assayed approximately 10,000 units/ml (11) and 2,000 units/ml, respectively. Tissues treated with deactivated victorin served as controls (18).

In dosage-response measurements of C6/C1 ratios excised leaves were allowed to take up solutions of victorin at various concentrations for 4 hr (refined toxin, susceptible leaves) or 24 hr (crude toxin, resistant leaves) through the cut ends. Leaves were then rinsed, blotted, cut into 7-mm segments, and 0.5-g samples were placed in 50-ml Erlenmeyer flasks containing 4 ml of 0.01 M KH_2PO_4, pH 4.7, and 250 units of penicillin-G per ml, 100 μmoles of carrier glucose, and about 0.4 μCi [9.0×10^5 disintegrations per min (DPM)] of either glucose-1-^{14}C or glucose-6-^{14}C. Flasks were closed with CO_2 traps (13) and were shaken in a water bath (25 C). In each test the toxin-treated tissues which respired at the highest rate were shaken for 2 hr, whereas controls and treated tissues which respired at lower rates were shaken until they had evolved about the same amount of CO_2 (4-5 hr for controls). These time adjustments were based on simultaneous measurement of gas exchange rates with separate samples in each test.

In time-course measurements of C6/C1 ratios 0.75-g samples of untreated susceptible tissue were placed in flasks containing the above isotope solution, with the exception that 50 μmoles of carrier glucose per 4 ml were used. Flasks were shaken for 1.5 hr (25 C), at which time the CO_2 traps were discarded and replaced with fresh ones. At the end of the next 30-min period the CO_2 traps were removed for ^{14}C measurement to give time zero readings and were replaced with fresh ones. At this point 1 ml of deactivated or refined victorin was added (final concentration 15 units/ml), and at 30-min intervals thereafter the CO_2 traps were removed for $^{14}CO_2$ measurement and replaced with fresh ones. Alternatively, 1 ml of buffer or 2, 4-dinitrophenol was added (final concentration 10^{-4} M) in some tests.

Radioactivity of trapped CO_2 was measured as previously described (13). Data were converted to DPM by internal standardization (counting efficiency about 85%) and were corrected for background and radiolysis of the labeled glucoses. In contrast to background radioactivity, radiolysis contributed significantly to $^{14}CO_2$ recovery in tissue flasks. The extent of decomposition of glucose-1-^{14}C was three to five times that of glucose-6-^{14}C, with three separate lots of glucoses purchased from two suppliers. Three replicate samples were used in each test.

Gas exchange rates were measured with or without a filter paper wick and 0.2 ml of 20% KOH in the center well of Warburg flasks containing 0.25 g tissue (two or three samples per treatment) and 2 ml of the buffer-glucose solutions used in the $^{14}CO_2$ recovery tests. Anaerobic CO_2 output was measured after flasks were flushed with N_2 for 15 min. Flasks were shaken in a differential respirometer (25 C), and readings were begun after a 15-min temperature equilibration. In time-course tests, 0.5 ml of victorin or 2, 4-dinitrophenol was added from the flask side arm at time zero.

RESULTS

Dosage response tests.—With a 4-hr treatment, victorin concentrations which stimulated oxygen

TABLE 1. Oxygen consumption and C6/C1 ratios of oat leaves treated 4 hr (susceptible) or 24 hr (resistant) with various concentrations of victorin or deactivated victorin (control)[a]

Concentration (units/ml)	Number of tests	Control		Victorin	
		O_2	C6/C1	O_2	C6/C1
		Susceptible tissue			
0.002	2	8.2	1.00	8.0	1.00
0.02	5	9.4	0.98	10.9	0.92
0.2	5	9.4	0.98	15.7[b]	0.68[b]
2.0	3	10.3	0.98	25.1[b]	0.67[b]
10	3	9.8	. . .	25.0[b]	. . .
		Resistant tissue			
10	2	9.1	1.03	9.5	1.02
100	2	9.1	1.03	10.7	0.95
200	3	9.6	1.03	13.2[b]	0.76[b]

[a]Data are means obtained with two (O_2) or three (C6/C1) samples in each test. Oxygen data are μmoles per gram fresh weight per hour.

[b]Differ significantly from controls ($P<0.01$).

TABLE 2. Recovery of $^{14}CO_2$ from glucose-1^{14}C and glucose-6-^{14}C supplied to susceptible oat leaves after a 4-hr treatment with deactivated victorin (control) or victorin (0.2 or 2.0 units ml)[a]

Treatment	Glucose uptake period (hr)	DPM $^{14}CO_2$	
		C1	C6
Control	4-5	4636	4562
Victorin	2	4997	3407
Difference		361 ± 176	1155± 216

[a]Values are means and mean differences (with standard errors) obtained with triplicate 0.5-g samples in each of five tests. Difference in C6 is significant ($P<0.01$).

consumption reduced the C6/C1 ratio of susceptible oat leaves (Table 1). In no test was one change observed in the absence of the other. These ratio decreases are similar in magnitude to those reported for 10 units/ml (13). The maximum C6/C1 drop occurred at a 10-fold lower dosage than that required for a maximum change in oxygen uptake rate.

With treatments that ranged from 4 hr to 24 hr, oxygen uptake rates and C6/C1 ratios of resistant leaves were unaltered by dosages which affected susceptible leaves in 4 hr. However, a 24-hr treatment with 200 units/ml decreased the C6/C1 ratio and increased oxygen uptake (Table 1). The latter confirms an earlier report (18). A 24-hr treatment with refined toxin (200 units/ml) also produced these changes. The C6/C1 ratio of healthy resistant leaves and the magnitude of the toxin-induced drop were similar to those found with susceptible leaves, but the maximum respiratory increase was only about 40%, as compared with increases of more than 100% in susceptible leaves. Thus, results with both cultivars suggested that the ratio drop was accompanied by a relatively small increase in respiration rate. With both tissues toxin dosages which stimulated oxygen uptake decreased the respiratory quotient (0.95-1.00 for controls) by only about 5%. Krupka (8) also found essentially no change in the susceptible oat leaf respiratory quotient with victorin treatment. The resistant tissue C6/C1 reduction is an additional indication that resistant and susceptible oats differ quantitatively, rather than qualitatively, in response to victorin (18).

Victorin dosages which stimulated gas exchange by susceptible leaves increased the rates of decarboxylation

TABLE 3. Aerobic and anaerobic CO_2 production by susceptible oat leaves treated 4 hr with deactivated victorin (control) or victorin (2 units/ml)[a]

Treatment	μmoles/g fresh weight/hour		Ratio
	Aerobic	Anaerobic	(Anaer./Aer.)
Control	9.2	3.6	0.39
Victorin	21.0	5.4	0.26
Difference	11.8 ± 0.54[b]	1.8 ± 0.52	0.13 ± 0.05

[a]Values are means and mean differences (with standard errors) obtained with duplicate samples in each of three tests during the lst hr after flasks were flushed with nitrogen and allowed to temperature equilibrate.

[b]$P<0.01$. For other differences $P<0.05$.

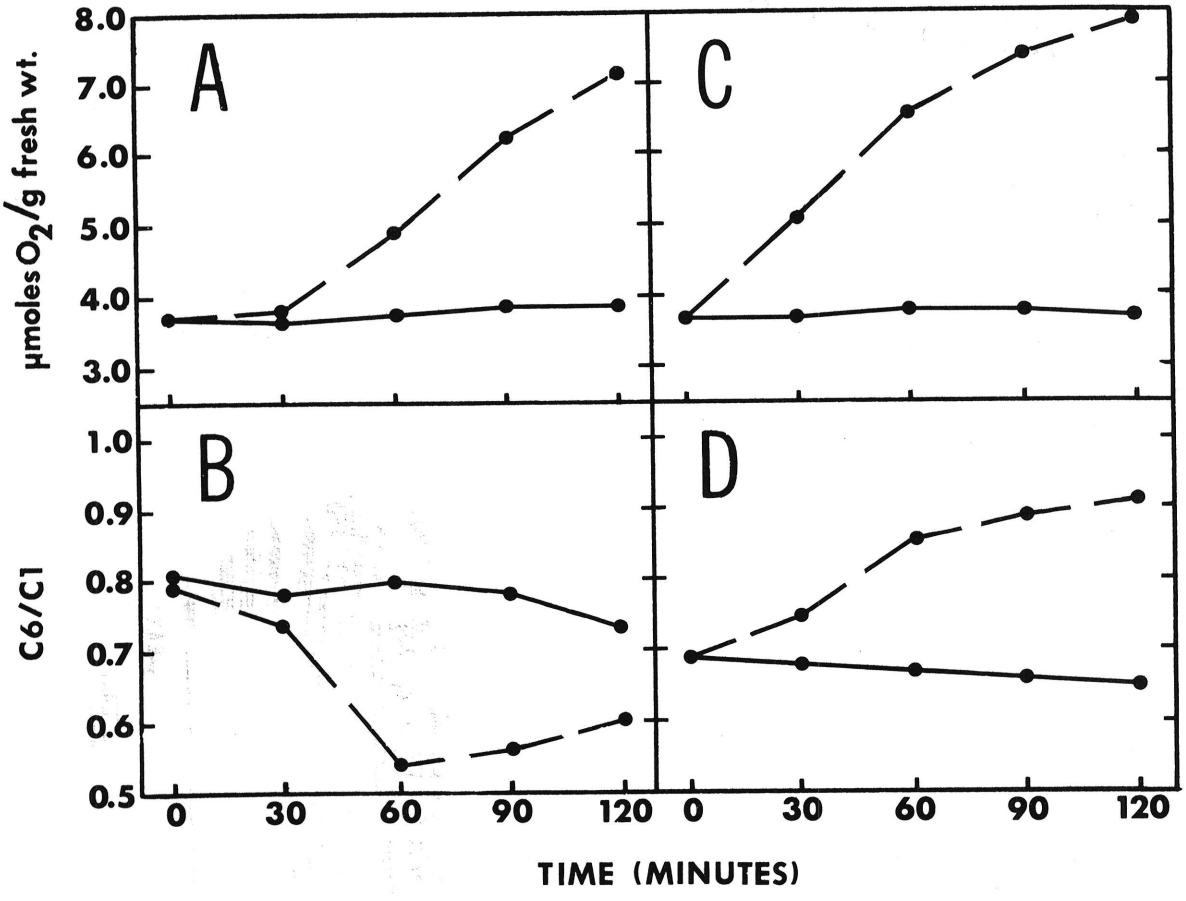

Fig. 1-(A to D). Time-course changes in O_2 consumption and C6/C1 ratios of susceptible oat leaves after application of victorin (**A, B**): final concentration 15 units/ml) or 2,4-dinitrophenol (**C, D**): final concentration 10^{-4}M) at time zero. Solid lines: controls; broken lines: victorin or 2,4-DNP. Each point represents the average of three determinations in each of three tests. Treated vs. control comparisons: (A, B) $P < 0.01$ at 60-120 min, (C) $P < 0.01$ at 30-120 min, (D) $P < 0.01$ at 60-120 min.

of both C1 and C6 of glucose (Table 2). The data show that treated leaves decarboxylated as much C1 in 2 hr as did controls in 4-5 hr. Based on this time difference C6 output also increased, but to a smaller extent. The fact that total C6 decarboxylation in diseased leaves was less than in controls shows that C6 output did not increase to the degree that the respiration rate did. In contrast, the output of C1 increased to about the same extent as the respiration rate, hence the statistical equality of the C1 data. Similar $^{14}CO_2$ data were obtained with resistant leaves treated 24 hr with 200 units/ml, but the increases were smaller.

In a separate series of tests, the C6/C1 ratio of susceptible leaves was determined for five consecutive 1-hr intervals after a 4-hr victorin treatment (2 units/ml). Representative values were 0.61, 0.63, 0.61, 0.61, and 0.59, respectively. Corresponding control values were 1.19, 0.96, 0.93, 0.86, and 0.83, respectively. The diseased tissue ratio ramined significantly below that of controls and did not increase with time. The decline in the control ratio cannot be explained.

Victorin treatment of susceptible leaves also increased CO_2 production under nitrogen, but to a smaller extent than in air (Table 3). This resulted in a decreased anaerobic CO_2/aerobic CO_2 ratio. Similar ratio decreases have been found in other diseased plants (17). Although the absolute value of this ratio is of little interpretational use, a decrease in the ratio does suggest inhibition of an apparent Pasteur effect.

Time-course tests.—In time-course tests samples of untreated susceptbile leaf tissue were placed in flasks containing the glucose-^{14}C solutions, and victorin then was added. Netiher the C6/C1 ratio nor the rate of oxygen uptake changed significantly for 30 min after application of toxin. During the second 30-min period the ratio decreased and oxygen uptake rose (Fig. 1-A, B). The ratio did not fall further thereafter, but the oxygen uptake rate continued to increase. These results are consistent with those of the dosage-response tests. Thus, the two changes appeared simultaneously, but the C6/C1 ratio drop was more abrupt than the respiration rate rise. The toxin concentration used in these tests (15 units/ml) was selected because it consistently induced the respiratory increase after a 30-min lag period. Higher concentrations, up to 100 units/ml, did not induce the change sooner, and with lower concentrations the lag period increased.

In extended tests the C6/C1 ratio of victorin-treated tissue remained significantly below that of controls and did not approach unity with increasing time after the initial drop. For the six consecutive 1-hr periods after the decrease, representative values for diseased tissue were 0.61, 0.68, 0.65, 0.62, and 0.61, respectively. Corresponding control values were 0.84, 0.89, 0.85, 0.85, 0.80, and 0.81, respectively.

The respiratory uncoupler 2, 4-dinitrophenol (2, 4-DNP) increased oxygen consumption of susceptible oat leaves, as previously reported (8), but it also increased the C6/C1 ratio (Fig. 1-C, D). Both victorin and 2, 4-DNP caused simultaneous increases in C1 and C6 decarboxylations during the period in which the respiratory increase appeared (Fig. 2). With victorin, the ratio drop was due to a more rapid appearance of C1 than C6 as $^{14}CO_2$. This is consistent with the dosage-response test results. With 2, 4-DNP, the increase in C6 decarboxylation exceeded that of C1 on a percentage basis. Neither agent significantly altered the tissue respiratory quotient. Since 2, 4-DNP- induced changes were apparent within 30 minutes, their absence with

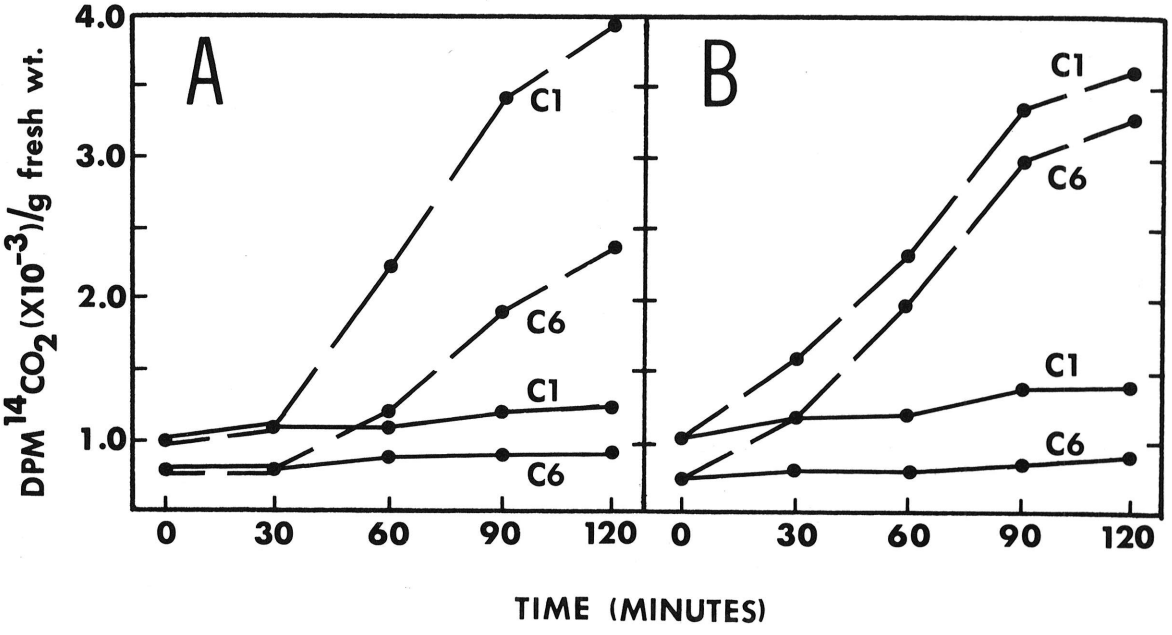

Fig. 2-(A, B). Time-course changes in decarboxylation of glucose-1-^{14}C and glucose-6-^{14}C by susceptible oat leaves after application of victorin **A)** final concentration 15 units/ml) or 2, 4-dinitrophenol **B)** final concentration 10^{-4}M) at time zero. Solid lines: controls; broken lines: victorin or 2, 4-DNP. Each point represents the average of three determinations in each of three tests. Treated vs. control comparisons: (A) $P<0.01$ for C1 and C6 at 60-120 min, (B) $P<0.01$ for C1 and C6 at 30-120 min.

victorin before the second 30-min period cannot be attributed to limitations of the methods used.

The reason that control C6/C1 values differed in dosage-response and time-course tests is not known. Substances present in the deactivated victorin preparation, the concentrations of which differed in the two types of tests, were not responsible for this ratio difference in that (i) leaves allowed to take up distilled water for 4 hr also showed ratios near unity (13) and (ii) tissues floated on buffer alone (Fig. 1-D) showed ratios similar to those of tissues floated on buffer containing deactivated victorin (Fig. 1-B) in time-course tests. Further, replacement of the buffer with distilled water in time-course tests did not produce ratios near unity (Rawn, *unpublished*). Despite differences in control ratios, victorin clearly induced similar changes in both cases.

DISCUSSION

Decreases in the C6/C1 ratio of diseased plants typically have been taken as an indication of increased pentose phosphate pathway (PPP) activity. Three lines of evidence indicate that this interpretation holds for the victorin-induced C6/C1 drop in oats. First, victorin decreased the C6/C1 ratio by stimulating decarboxylation of C1 to a greater extent than that of C6. Second, as expected on the basis of its uncoupling action, 2, 4-dinitrophenol increased the C6/C1 ratio by increasing rates of decarboxylation of both C1 and C6 such that the rate of C6 decarboxylation gradually approached that of C1. Third, victorin decreased the anaerobic CO_2/aerobic CO_2 ratio. Since the PPP is aerobic, its increased contribution to aerobic CO_2 output would not have an anaerobic complement. These changes have been cited by others as evidence of increased PPP activity in various diseases (2, 5, 7, 16), and they provide additional evidence of the similarity between victorin-treated oats and other diseased plants (14).

Although the increase in PPP activity, indicated by the C6/C1 drop, coincided with the respiration rate increase, the time-course data are not consistent with a major PPP contribution to victorin-induced respiration. Victorin caused simultaneous increases in C1 and C6 decarboxylations, but the ratio did not increase after the initial drop. This rules out any significant contribution of PPP cycling to the added C6. Therefore, the C6/C1 ratio should have continued to decline as the respiration rate and C1 decarboxylation increased, if the PPP were mediating the added respiration. The maximum ratio drop was confined to a single 30-min period and was accompanied by only a small respiratory increase.

The observation that victorin-induced oxygen uptake is mediated largely by a fluoride-sensitive mechanism (8) indicates that the glycolysis-Krebs cycle pathway (GKP), rather than the fluoride-insensitive PPP, largely is responsible for increased respiration. A GKP activity increase also is indicated by increased anaerobic CO_2 output (Table 3) and increased concentrations of Krebs cycle acids (10) and related amino acids (9) in victorin-treated leaves. An increase in GKP activity would account for the simultaneous increases in C6 decarboxylation and respiration rate that accompany the PPP activity increase. It also would reduce considerably the apparent PPP contribution to glucose oxidation, since the GKP decarboxylates C1 and C6 equally. The magnitude of the 2, 4-DNP-induced respiratory increase indicates that the GKP can accommodate the respiration levels found with victorin treatment. However, uncoupling apparently is not the stimulus for the victorin-induced changes in view of the opposite effects of victorin and 2, 4-DNP on the C6/C1 ratio.

The foregoing is not inconsistent with the interpretation that the PPP contributes to increased respiration in diseased plants; it is the magnitude and timing of the contribution that have been in doubt. None of the reports of increased PPP activity (2, 5, 7, 15) quantitates the PPP respiratory contribution. In diseased tissues that show C6/C1 reductions, tests involving anaerobic CO_2 production rates and effects of inhibitors on respiration rates have given mixed results (2, 4, 5, 6, 16). Increases in PPP activity and respiration rate apparently coincide in some diseased tissues (2, 6, 7, 16) but not in others (4, 6, 7). In diseases incited by obligately parasitic fungi the pathogen's contribution to observed changes further complicates interpretation (3). Since fungi show low C6/C1 ratios (1, 16), this contribution may account for the fact that ratios associated with sporulation in rust diseases (4, 5, 16) are considerably lower than those in victorin-treated oats, where changes are clearly attributable to altered suscept metabolism. As yet, it is not clear that the PPP is responsible for either the onset or the magnitude of increased respiration in any diseased tissue.

The view suggested here for victorin-treated oats is that increased PPP activity makes only a small contribution, if any, to the development of induced respiration and its maintenance at a maximal level well into pathogenesis. This may be the case in other diseases as well, in view of the many similarities between victorin-treated oats and other diseased plants.

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