Time-Course and Antioxidant Inhibition of Ethylene Production by Victorin-Treated Oat leaves

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ABSTRACT

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Although an increase in ethylene production is one of the most sensitive responses to victorin, no increase in this gas was detected during the first 30 min after susceptible oat leaves were treated with this pathotoxin. These results do not support the concept that ethylene triggers initial changes in permeability and electrochemical potentials that occur within 5 min after exposure to victorin. The results also emphasize the significance of a similar 30-min lag in less sensitive responses to victorin such as increases in respiration, decreases in C_6/C_1 ratios, and other changes clearly linked to metabolism. Antioxidants, of which α-tocopherol was the most effective, inhibited ethylene production by victorin-treated tissues and also suppressed pathologic effects of victorin on losses of electrolytes and chlorophyll. Failure of antioxidants to suppress ethylene production in healthy control leaves lends support to evidence that the pathways leading to ethylene evolution in diseased tissues differ from those in healthy plants.

The ability of victorin, a pathotoxic product of *Helminthosporium victoriae* Meehan and Murphy, to induce in susceptible oat tissues all of the visible and physiologic symptoms of disease found in plants infected by this pathogen is well-established (4,7,13, 15). Initial time-course studies of responses to victorin indicated that changes in permeability, estimated by rates of loss of electrolytes, occur almost instantaneously, but increases in respiration and other changes directly linked to metabolism occur only after a lag of about 30 min (18). These results led to the hypothesis that the initial effect of victorin is on the plasmalemma (4,18,20), which perhaps contains specific receptor sites only in susceptible tissues (7). Despite extensive research, all attempts to provide direct evidence of initial effects of victorin on the plasmalemma or for specific receptors for this toxin have been negative (4,13,16). Thus the site of action of victorin and the significance of the delay in metabolic responses to this toxin remain unknown.

One factor that complicates interpretation of present data on the sequence of changes in victorin-treated tissues is the insensitivity of the metabolic responses that have been studied. Concentrations of victorin 10–100 times higher than those that cause marked effects on permeability and root growth are required to produce detectable effects on respiration (9,18), C_6/C_1 ratios (9), or enzyme activity (6). However, an increase in ethylene production was reported to be at least as sensitive as changes in permeability or root growth (14). The objective of the time-course portion of this study was to determine whether this highly sensitive response occurs with the same delay observed with other metabolic changes or, if not, whether it occurs rapidly enough to be the trigger, postulated by others (2), responsible for changes in permeability and other events in pathogenesis. A second objective was to determine whether lipid antioxidants, known to inhibit ethane evolution in animal systems (10), would inhibit ethylene production and other pathologic changes in victorin-treated oat leaves.

MATERIALS AND METHODS

First leaves of the victorin-sensitive oat cultivar Park, grown for 9 days under conditions previously described (19), were used in all tests. The source of victorin was a partly refined preparation (17) that contained 1.4 mg of total solids/ml, inhibited root growth 50% when diluted 5×10^7-fold, and thus assayed 2,000 units/ml in the standard root growth test (18). A portion of this preparation, diluted to contain 100 units/ml, was detoxified (19), and this solution and distilled water served as controls.

Detached leaves, which had taken up victorin or other test solutions through basal cut ends, were placed in containers sealed with septa, and ethylene production at various intervals was determined by gas chromatography as in a previous study (14). In one series of tests, this procedure was varied by using Beyer and Morgan's vacuum evacuation method (1) in an attempt to measure both evolved ethylene and that contained in the tissue.

The three antioxidants used were sodium ascorbate, propyl gallate, and α-tocopherol, the latter prepared as a mixture in Tween 80 (10); they were serially diluted in 0.005 M KH_2PO_4 to maintain a pH of about 4.5, similar to that of solutions containing victorin. Solutions then were assayed for toxicity by root growth tests and for effects on ethylene production by oat leaves. Thresholds for toxicity and increases in ethylene production for the three compounds fell in the range of 10^{-4} to 5×10^{-5} M. Therefore, these compounds were used at 10^{-5} M in combination with victorin. Methods previously described were used to measure losses of electrolytes (18) and chlorophyll content (17).

RESULTS

Preliminary tests were run to determine which method and time of exposure to victorin, at concentrations (20–200/units/ml) known to cause rapid effects on permeability (5,11,18), would produce the largest increases in ethylene production in short-term experiments. Results with leaves that had taken up toxin solutions for 5 sec to 20 min and with leaf sections that had been vacuum infiltrated with toxin for similar periods indicated that a 1-min uptake resulted in the largest production of ethylene during the first hour after exposure. Similar tests with victorin concentrations lower than 20 units/ml were less effective, hence the 1-min uptake of victorin was adopted as a standard treatment for time-course studies. This standard 1-min treatment with 200 and 20 units/ml caused significant increases in losses of electrolytes from susceptible leaves within 5 and 15 min, respectively.

Results of an initial time-course study, in which only ethylene evolved from tissues was measured, indicated that ethylene production did not significantly increase in response to victorin during the first 30 min after treatment (Table 1). To explore the possibility that ethylene had increased earlier but that the gas remained trapped in the tissues, this test was repeated with a vacuum evacuation technique designed to release such trapped material (1). The results show that the total amount of ethylene recovered was greatly

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TABLE 1. Time course of ethylene production by susceptible oat leaves treated for 1 min with victorin

Treatment	Ethylene (nl/g dry wt/hr) after:[a] 15 min	30 min	45 min	60 min
Not vacuum evacuated				
Victorin (20 units/ml)	15.8 ± 1.4	14.8 ± 0.9	23.9 ± 1.8[b]	34.7 ± 2.3[b]
Victorin (200 units/ml)	14.5 ± 1.6	15.2 ± 1.1	26.4 ± 2.3[b]	37.4 ± 2.8[b]
Victorin (detoxified)	16.7 ± 1.8	17.2 ± 2.1	16.4 ± 1.4	16.8 ± 1.4
H_2O	15.3 ± 1.2	16.7 ± 1.5	16.1 ± 1.2	15.8 ± 1.3
Vacuum evacuated				
Victorin (20 units/ml)	38.8 ± 3.6	54.3 ± 4.8	84.6 ± 5.2[b]	118.2 ± 9.4[b]
Victorin (200 units/ml)	43.6 ± 4.1	57.3 ± 6.1	93.4 ± 6.1[b]	124.6 ± 9.8[b]
Victorin (detoxified)	46.0 ± 3.7	51.4 ± 4.2	48.8 ± 3.5	50.7 ± 5.1
H_2O	52.4 ± 4.1	49.6 ± 3.7	50.7 ± 4.6	53.4 ± 4.8

[a]Values are means; their standard errors were obtained in three separate tests with quadruplicate tissue samples in each test.
[b]Differs significantly ($P = <0.01$) from detoxified victorin and water controls; other differences are not significant at $P = 0.05$.

increased by evacuation in all treatments, but significant increases in response to victorin again occurred only after a 30-min lag period (Table 1). Because this is the same lag found for other metabolic responses to victorin, it seems unlikely that ethylene serves as the trigger for initial rapid changes in permeability (11,18) and electrochemical potentials (5).

Antioxidant effects on victorin-induced ethyelene production.—In these tests, leaves were allowed to take up victorin solutions containing 0.2 units/ml for 1 hr to produce near maximum quantities of ethylene in the range of 400–500 nl/g of dry wt/hr. When these victorin solutions also contained 10^{-5} M α-tocopherol, ethylene production was reduced by more than 50% (Table 2). Propyl gallate was less effective and ascorbate gave only a small, statistically insignificant reduction when combined with victorin. Because toxic effects precluded the use of higher concentrations of antioxidants, victorin concentrations were reduced to 0.02 units/ml in further tests. In these, reductions ranging from 68 to 89% and averaging 78% in victorin-induced ethylene production were obtained with 10^{-5} M α-tocopherol.

Visual observations indicated that antioxidants that inhibited victorin-induced ethylene production also suppressed wilting and leaf discoloration caused by this toxin. To obtain quantitative data, the effects of α-tocopherol on victorin-induced losses of electrolytes and chlorophyll were studied. The results (Table 3) show that both of these pathologic effects of victorin were suppressed by α-tocopherol to about the same extent as ethylene production (Table 2).

DISCUSSION

Failure to detect changes in a highly sensitive response to victorin, ethylene production, during the first 30 min after exposure emphasizes the significance of similar delay in other metabolic responses (9,18) in relation to the site and mode of action of this toxin. It seems unlikely that such a delay would occur if the reported changes in losses of electrolytes (11,20) and electrochemical potentials (5), which occur almost instantaneously, reflect extensive disruption of protoplast permeability. In assessing the role of these very rapid responses to victorin, it should be noted that they have been obtained only with high concentrations (20–200 units/ml) of victorin. Furthermore, analysis of the pattern of these changes—an initial rapid change during the first 5 min of exposure to victorin followed by a period of no change or actual recovery before a second prolonged change occurs after about 30 min—led to the suggestion that the initial change reflects an effect on cell walls (15,16). Evidence that victorin induces a rapid but transient increase in cell elongation (11) and failure to detect selective effects in uptake of labeled compounds by isolated resistant and susceptible protoplasts until after 30 min of exposure to victorin (8) lend support to this suggestion. If this interpretation is correct, the second change in permeability, which occurs after about 30 min and coincides with changes in metabolism, reflects effects of victorin on protoplast permeability. Although present data do not rule out the plasmalemma as the primary site of action of victorin, the 30-min delay in responses that are clearly associated with the protoplast provides ample time for the toxin to act at an interior site.

Inhibitory effects of antioxidants on ethane evolution in animal systems have been linked to the blockage of lipid peroxidation (10) and there is evidence for the participation of oxidized unsaturated lipids in the evolution of ethylene by plants (3). Whether such a mechanism is responsible for the effects of antioxidants found in this study is not known, and in view of uncertainties about the biosynthesis of ethylene (2), it would be premature to speculate about the mechanism responsible for antioxidant inhibition of victorin-induced ethylene production. The fact that antioxidants did not inhibit ethylene production by control tissues (Table 2) is consistent with evidence from sweet potato tissues infected with *Ceratocytis fimbriata* (12) and with victorin-treated oat leaves (9) that the pathway of ethylene production in diseased tissues differs from that in healthy plants.

TABLE 2. Suppression of victorin-induced evolution of ethylene by antioxidants

Treatments	Ethylene (nl/g dry wt/hr)[a]
Victorin (0.2 units/ml)	418 ± 24.6
plus 10^{-5} M α-tocopherol	184 ± 14.8[b]
plus 10^{-5} M n-propyl gallate	274 ± 17.4[b]
plus 10^{-5} M ascorbate	364 ± 21.7
Controls	
10^{-5} M α-tocopherol	17.3 ± 1.2
10^{-5} M n-propyl gallate	18.6 ± 1.6
10^{-5} M ascorbate	21.4 ± 2.2
H_2O	16.2 ± 1.4

[a]Values are means; their standard errors were obtained in three separate tests with triplicate samples in each test.
[b]Differs significantly ($P = <0.01$) from treatment with victorin alone.

TABLE 3. Suppression by α-tocopherol of losses of electrolytes and chlorophyll from victorin-treated oat leaves

Treatments	Conductance change[a] (μmhos/hr)	Chlorophyll[a] (mg/g fresh wt)
Victorin (0.2 units/ml)	21.4 ± 1.8	0.56 ± 0.04
plus α-tocopherol (10^{-5} M)	9.2 ± 0.7[b]	1.16 ± 0.10[b]
Controls		
α-tocopherol (10^{-5} M)	1.7 ± 0.2	1.68 ± 0.14
H_2O	1.9 ± 0.2	1.74 ± 0.19

[a]Values are means, their standard errors were obtained in three separate tests with triplicate samples in each test.
[b]Differs significantly ($P = <0.01$) from treatment with victorin alone.

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