Increase with Age in Sensitivity of Oat Leaves to Victorin

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ABSTRACT

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The chlorophyll content of first leaves from both 8-day-old and 15-day-old oat seedlings susceptible to *Helminthosporium victoriae* was reduced by treatment with 10 milliunits/ml of victorin. The reduction, however, was greater with the older leaves; when the concentration of victorin was lowered to 1 milliunit/ml, only older leaves showed a significant reduction in chlorophyll content. These results confirmed previous undocumented reports of an increase with age in sensitivity to victorin. A time-course study indicated that the increase in sensitivity to victorin occurred abruptly when the oat seedlings reached 12 days of age. During the preceding two days, the chlorophyll content of both treated and control leaves showed a marked decline. These results suggest that a change in the balance or distribution of endogenous growth regulating substances plays a role in the age-related increase in sensitivity to victorin.

Additional key words: Victoria blight of oats, pathotoxin.

Susceptibility to many plant diseases often increases or decreases as tissues age (5). An increase with age in sensitivity to victorin, the pathotoxic product of *Helminthosporium victoriae* Meehan and Murphy, has been reported for highly sensitive first leaves of oats and also of beans, which are much less sensitive (4). Although the reported increase in sensitivity was quite large (approximately 10-fold), it was not documented with quantitative data. The objective of this paper is to provide such documentation for first leaves of oat seedlings susceptible to *H. victoriae*.

MATERIALS AND METHODS

Oat seedlings were grown in a soil-sand-peat mixture (1:1:1, v/v) in a controlled environment chamber at 23 ± 2 C under a 16-hr photoperiod of 8,800 lux. First leaves, selected for uniformity, were detached at 0900 hours, and their basal ends were recut under water. The cut ends of the leaves then were placed in 10 ml of test solutions in 25-ml Erlenmeyer flasks. The flasks containing the leaves were held for 2 days in a growth chamber under the same conditions of light and temperature used for growing the seedlings. The leaves then were extracted in boiling 95% ethanol and their chlorophyll content was determined by the spectrophotometric method of Arnon (1). Tissue fresh weights were determined at the time leaves were detached.

The source of victorin was the same crude and partly refined material used previously (4). The crude material contained 6.8 mg and the refined 1.0 mg total solids/ml. Each of these preparations inhibited root growth of susceptible oat seedlings 50% when diluted 10^7-fold with distilled water, and hence, each assayed 10,000 units/ml in the standard root growth test (3). Unless otherwise noted, toxin preparations were used at dilutions of 10^6-fold (10 milliunits/ml) and at 10^7-fold (1 milliunit/ml) in all experiments. All tests were run three times, twice with the crude and once with the refined preparation. Since results with the two preparations never differed significantly, the data were pooled. Deactivated victorin solutions diluted 10^6-fold served as controls. Results with these never differed from those obtained with distilled water.

RESULTS AND DISCUSSION

In a preliminary test, first leaves of 9-day-old susceptible oat seedlings (cultivar Victorgrain 48-93) were placed and held for 2 days in victorin solutions which contained 1,000, 100, 10, 1, and 0.1 milliunits/ml. Leaves held in the two highest concentrations of victorin wilted within 24 hr and appeared withered and gray-green in color after 2 days. These leaves retained clearly visible green color even after repeated extraction with boiling ethanol or 80% (v/v) acetone. Similar difficulty has been encountered by others in attempts to extract chlorophyll from leaves infected with powdery mildew (2). Leaves held in the two lowest concentrations of victorin did not differ visibly or in chlorophyll content from controls held in water or deactivated victorin. In contrast, leaves held in 10 milliunits/ml of victorin were lighter green and contained approximately one-third less chlorophyll than control leaves. On the basis of these results, victorin solutions containing 1 and 10 milliunits/ml were used in tests for effects of leaf age on victorin sensitivity.

Results with first leaves of young (8-day-old) and older (15-day-old) seedlings (Table 1) show that the chlorophyll content of leaves exposed to 10 milliunits/ml of victorin was significantly reduced regardless of age. This reduction, however, was much more drastic in older leaves (approximately two-thirds) than in young ones (approximately one-third). This evidence of an increase with age in sensitivity to victorin was reinforced by results with leaves exposed to 1 milliunit/ml (Table 1). At this concentration, victorin caused only a small,

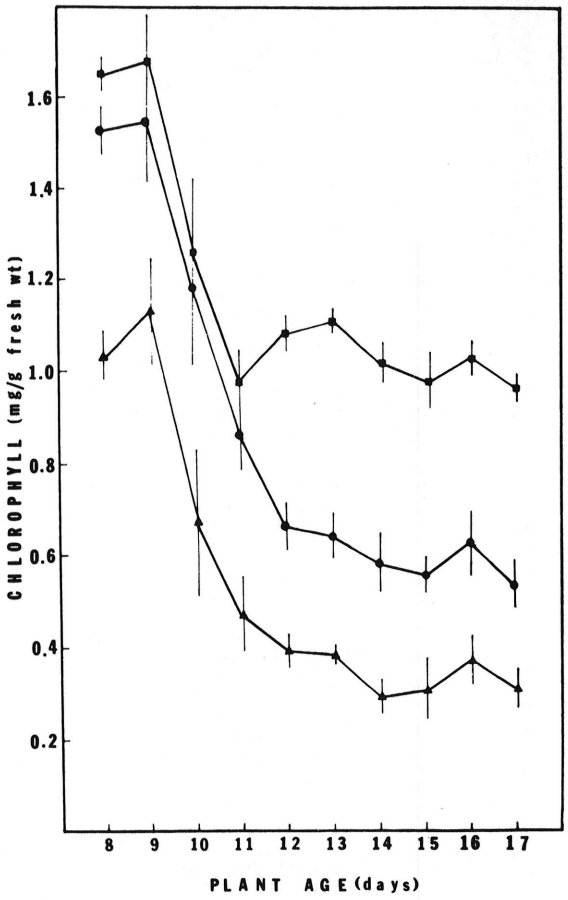

Fig. 1. Pattern of changes with age in the chlorophyll content of first leaves of Victorgrain 48-93 oat plants exposed for two days to solutions of victorin or deactivated victorin. Points are means and vertical bars their standard errors obtained from triplicate samples in three separate tests. Results with leaves exposed to 10 milliunits/ml of victorin (▲) differed significantly ($P = < 0.01$) at all times from control values (■) exposed to deactivated victorin. Results with leaves exposed to 1 milliunit/ml of victorin (●) differed significantly ($P = < 0.01$) from controls only on day 12 and thereafter.

nonsignificant reduction of chlorophyll in young leaves, whereas with older leaves the reduction was large (approximately 50%) and highly significant (Table 1). The fact that the reduction in chlorophyll, expressed as percent of controls, was somewhat larger in old leaves exposed to 1 milliunit/ml of victorin than in young leaves exposed to 10 milliunits/ml confirms previous estimates of an increase in sensitivity of at least 10-fold with age (4). The data in Table 1 also show that the chlorophyll content of old control leaves is about two-thirds that of young ones.

Time-course of increased sensitivity to victorin.—The pattern of changes in chlorophyll content of victorin-treated and control leaves from seedlings 8-17 days old (Fig. 1) shows a sharp decline in all leaves from plants 10 and 11 days of age. The chlorophyll content of control leaves then stabilized at about two-thirds that of 8- and 9-day values, whereas that of leaves exposed to 1 milliunit/ml of victorin, which had previously not differed significantly from controls, continued to decline for another day before stabilizing. In agreement with data in Table 1, leaves exposed to 10 milliunits/ml of victorin were significantly lower in chlorophyll than controls at all ages (Fig. 1). The pattern of changes, however, was similar to that with 1 milliunit except that the decline on day 12 was less pronounced. Taken together, these results indicate that increased sensitivity to victorin occurs abruptly and essentially in one-step when plants reach twelve days of age.

The sharp decline in chlorophyll content of first leaves from 10- and 11-day-old plants suggests that a marked physiological change occurred just prior to the increase in victorin sensitivity. With the oat cultivars and growth conditions used in this study, second leaves exceed first leaves in length for the first time when plants are 10 days old. Thereafter first leaves undergo little if any further elongation whereas second leaves continue to elongate rapidly. Assuming that elongation is under hormonal control, it seems likely that a change in the balance or distribution of endogenous growth-regulating substances plays some role in the increase in sensitivity to victorin.

It has been suggested that increased sensitivity to victorin in older leaves may be the result of a lessened capability of such tissues to repair and recover from the

TABLE 1. Chlorophyll content (mg/g of fresh weight) of first leaves of oat seedlings susceptible to Victoria blight exposed for 2 days to solutions of victorin or deactivated victorin (control)[a]

Oat cultivar	Seedling age (days)	Victorin (milliunits/ml) 10	1	Control
Victorgrain 48-93	8	1.06 ± 0.12[b]	1.63 ± 0.10	1.73 ± 0.07
	15	0.38 ± 0.03[b]	0.65 ± 0.02[b]	0.98 ± 0.04
Fulgrain	8	0.98 ± 0.08[b]	1.48 ± 0.13	1.54 ± 0.14
	15	0.31 ± 0.02[b]	0.57 ± 0.07[b]	1.02 ± 0.09
Park	8	1.12 ± 0.11[b]	1.68 ± 0.18	1.75 ± 0.16
	15	0.44 ± 0.03[b]	0.62 ± 0.04[b]	1.10 ± 0.09

[a]Values are means and their standard errors from results with triplicate samples in three separate tests.
[b]Differs significantly ($P = < 0.01$) from control.

initial effects of this pathotoxin (4). Although the data in this paper do not provide a rigorous test, they are compatible with this hypothesis.

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