Heat- and Aging-Induced Tolerance of Sorghum and Oat Tissues to Host-Selective Toxins

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ABSTRACT

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Intact sorghum seedlings were held at high temperatures (37/30 C, day/night) and the roots were exposed to host-selective toxin from *Periconia circinata*. After 4 days, the plants had no visible symptoms, whereas toxin-treated plants at 22 C were dead. Assays based on net electrolyte losses from tissues showed that intact plants given brief thermal treatments were highly resistant to toxin, and that full sensitivity was regained within 3 days. Excised sorghum leaves pretreated in water at 35, 40, and 45 C for 15, 4.5, and 0.8 min had 50% as much toxin-induced loss of electrolytes as did controls exposed to water at 22 C. Prior thermal treatments also prevented toxin-induced increases in tissue respiration. Inoculated plants failed to develop symptoms at 37/30 C. Within 3 hr after excision, sorghum leaves at 22 C had 50% as much toxin-induced leakage of electrolytes as did freshly-cut leaves, and by 24 hr excised leaves leaked very little in response to toxin. Toxin sensitivity of excised sorghum leaves was maintained by treatment with kinetin (10^{-4}M). Excision and aging did not reduce sensitivity of oat leaves to toxin from *Helminthosporium victoriae*, although sensitivity was lost after thermal treatments; longer exposure times or higher temperatures were needed to reduce toxin-induced losses in oats than in sorghum. The data are useful for design of toxin assays and may contribute to an understanding of the seasonal development of Periconia blight.

Host-selective toxins are now known to be essential determinants of host specificity, tissue colonization, and pathogenesis by several fungi (13). Such toxins include those produced by *Periconia circinata*, *Helminthosporium victoriae*, *Alternaria kikuchiana*, and *H. sacchari* which affect certain genotypes of sorghum, oats, Japanese pear, and sugarcane, respectively. The environmental factors affecting development of these diseases have had limited attention. Biological effects of the toxins have been the subject of many studies (13, 14); effects of some toxins on tissues can be modified by the presence of cycloheximide and sulfhydryl-binding reagents (6).

Slight changes in temperature are known to have striking effects on development of some plant diseases. For example, normal Kalenchöe cells are altered to tumor cells by *Agrobacterium tumefaciens* at 25-27.4 C (optimum), but the process is completely prevented at 30 C (1). Otani et al.(8) observed that black spot of Japanese pears caused by *A. kikuchiana* does not develop during mid-summer, and that mild thermal treatments (55 C for 2 sec, or 35 C for 16 hr) of leaves eliminates sensitivity to toxin produced by the fungus. Comparable observations were reported later by Byther and Steiner (3) for *H. sacchari* and its host-selective toxin affecting sugarcane. No such data have been reported for other host-selective toxins, although Quinby and Karper (11) observed that development of Periconia blight of sorghum was checked during the hotter months of the growing season.

The first aim of this work was to determine whether or not heat affects the sensitivity of sorghum tissues to toxin from *P. circinata* (PC toxin), and the sensitivity of oat tissues to toxin from *H. victoriae* (HV toxin). Excision and aging of sorghum leaves were found to affect the assays for PC toxin, and the study was extended to include this factor. The results clarify some problems with toxin assays, and should be of help in understanding seasonal development of Periconia blight. An abstract reporting some of the results was published (2).

Some of our data were based on measurements of electrical conductance of ambient solutions containing toxin-treated or control tissues. We did not determine effects of the toxins on ion influx or exchange, or on the kinds of electrolytes involved. For convenience, however, we will refer to an increase in conductance of ambient solutions as an indication of electrolyte leakage. Previous studies have shown that HV toxin prevents uptake of several different ions and other solutes, and promotes loss of these solutes (12, 14).

MATERIALS AND METHODS

Plants.—Two inbred selections of grain sorghum [*Sorghum bicolor* (L.) Moensh, 'Colby'] were used; one is resistant and the other susceptible to *Periconia circinata* and to its host-selective toxin. One gene pair is known to control resistance or susceptibility in these and many other genotypes of sorghum. For most experiments, seedlings were grown for 19 to 23 days in vermiculite watered with White's nutrient solution, containing (in mg/liter):$Ca(NO_3)_2$, 200; Na_2SO_4, 200; KCl, 80;

NaH_2PO_4, 16.5; $MnSO_4$, 4.5; $ZnSO_4$, 1.5; H_3BO_3, 1.5; KI, 0.75; and $MgSO_4$, 36. Plants were grown under Grolux lamps for 15 hr per day, at 22 C. For some experiments which required roots to be in toxin solutions at precise concentrations, seedlings were grown in hydroponic culture. Seeds were germinated for 2 days between moist filter papers, at 22 C. Seedlings then were grown in small vials (five seedlings/vial) with roots in 20 ml of nutrient solution. White's solution was supplemented with $Fe_2(SO_4)_3$ (2.5 μM) plus Na_2EDTA (5.37 μM) to prevent chlorosis. Plants were grown in this solution for 12 days at 22 C, under Grolux lamps for 15 hr/ day, prior to use in each experiment.

Diseased sorghum plants were obtained by planting seeds in soil infested with *P. circinata*. The fungus was first grown in tubes containing an autoclaved mixture of soil, sand, and peat (1:1:1, v/v). After 2-3 wk, the cultures were mixed (1:10, v/v) with steamed potting soil and placed in small pots. Ten seeds were planted in each pot, and at least five pots were used for each treatment. Plants were grown in controlled-environment chambers, with 15 hr light/day.

Oat plants (*Avena sativa* L.) were grown in vermiculite, as described above, for 11 to 17 days. Two cultivars were used; Park is susceptible and Garry is resistant to *H. victoriae* and to its host-selective toxin.

Toxin preparation.—The PC toxin preparation used was an eluate from a charcoal-celite column, prepared as described previously (10). This preparation gave 50% inhibition of root growth by susceptible seedlings at 0.35 μg/ml. The HV toxin preparation was an eluate from an alumina column, prepared as described elsewhere (9). This preparation gave 50% inhibition of growth by susceptible oat roots at .01 μg/ml.

Assays.—The root-growth assay was a modification of an earlier method (13). Sorghum seeds were immersed in water for 2 hr, then germinated between sheets of moist filter paper for 24 hr at 30 C. Germinated seeds were placed in 5-cm diameter petri dishes (five seeds per dish), each containing 5 ml of water or toxin solution. A series of dilutions was used for each assay, which included both resistant and susceptible seedlings. Twenty to 100 seeds were used with each dilution. After 48 hr, the length of the root of each seedling was measured, and the amount of toxin required to give 50% inhibition of root growth was determined.

Electrolyte leakage from leaf tissue (19- to 23-day-old plants) was used as the basis for another assay of PC toxin; this assay was based in part on previous work (5). The second and third leaves above the cotyledons were excised and cut into 0.5-cm pieces. Samples (200 mg each) were enclosed in cheesecloth, submerged in water or toxin solution (10 ml) in small vials, and infiltrated by reducing the air pressure to about 2 cm Hg for 10 min. Vials were held on a reciprocating shaker (100 strokes/minute), and conductances of the ambient solutions were determined at intervals with a conductivity meter equipped with a pipet-type electrode assembly (K = 1.0). The conductance value for leaves in distilled water was used as a correction factor to calculate toxin-induced leakage of electrolytes. A series of toxin dilutions was used, assays were run with triplicate vials for each treatment, and all experiments were repeated.

The HV toxin was assayed by root growth and electrolyte leakage methods. The root-growth assay was slightly modified from the method used in the past (13); the assay end point was the amount of toxin required to give 50% inhibition of growth by susceptible seedling roots. The electrolyte-leakage assay was similar to the PC toxin assay described above, except that 11- to 12-day-old seedlings were used.

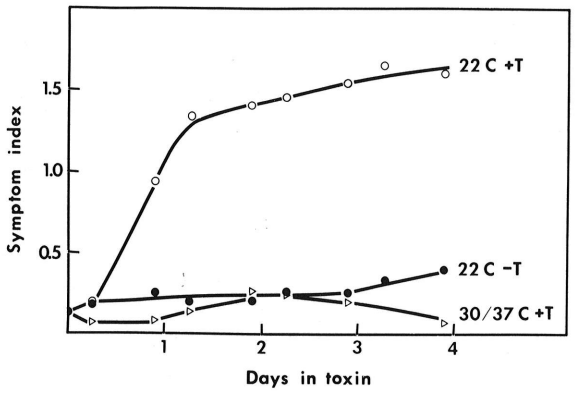

Fig. 1. Effect of temperature on symptoms in sorghum induced by toxin from *Periconia circinata*. Seedlings in nutrient solutions were maintained at 37/30 or at 22/22 C day/night temperatures, beginning 3 days prior to toxin exposure (7 μmg/ml) (+T). Control plants were in nutrient solutions without toxin (−T). The second and third leaves of each seedling were used in the symptom index; each index value was the average number of leaves per plant (15 plants) that had a wilting angle >90 degrees from vertical. The experiment was repeated two times with comparable results.

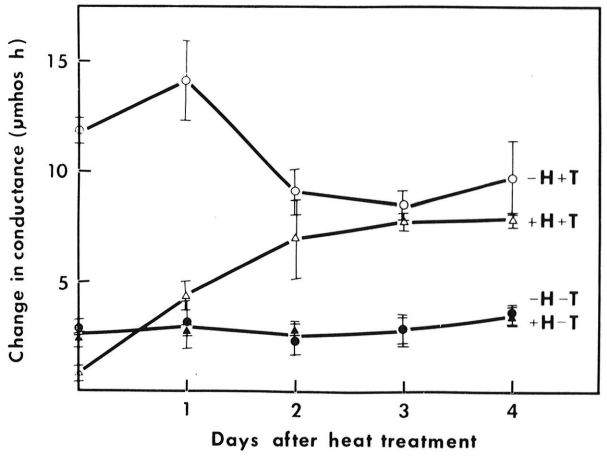

Fig. 2. Time course for recovery of sensitivity of sorghum to toxin from *Periconia circinata*. Intact seedlings were placed at 37 C for 18 hr, cooled to 22 C, and held for various times before exposure to toxin (+T) (70 μg/ml). Controls with (+H) and without (−H) heat, but with no toxin (−T), are shown on lower line; controls with no heat treatment (held constantly at 22 C), but exposed to toxin are shown on upper line. Conductances of ambient solutions were measured during the second and third hours after exposure to toxin. Each value is the mean for three replications; standard deviations are shown. Comparable results were obtained in each of three experiments.

Respiration.—Standard manometric methods (16) were used to determine gas exchange by sorghum leaves. Plants were grown and tissues were manipulated as described for the electrolyte-leakage assay. After toxin or water infiltration, leaf tissues were placed in Warburg flasks on moist filter paper. Oxygen uptake at 30 C was determined, after a 15-min equilibration. Each experiment had duplicate flasks, and all experiments were repeated two or more times.

RESULTS

Effect of temperature on sensitivity of sorghum to PC toxin.—Seedlings were grown in nutrient solutions for 14 days at 22 C. Some then were placed at 37 C during the day (15 hr) and at 30 C during the night, whereas others were held at 22 C during both day and night. After 3 days at these temperatures, an excess of PC toxin (7 μg/ml) was added to the nutrient solution. The second and third leaves of each plant were observed at intervals for wilting, and the average number per plant that had a wilting angle >90 degrees from vertical was recorded as an index value (Fig. 1). Plants held at 22 C were dead within 4 days after exposure to toxin, whereas plants held at 37/30 C had no symptoms of toxicity. Plants held for longer times at high temperatures sometimes developed slight symptoms, indicating that resistance was not complete. Some of the 37/30-C plants were removed from the vials after 4 days and the solutions were tested at 22 C for the presence of PC toxin by placing the roots of susceptible and resistant seedlings in the solutions. The new seedlings of susceptible sorghum wilted and died within 24 hr; resistant seedlings developed no symptoms. Therefore, sufficient toxin to cause symptoms was present throughout the time that susceptible roots were exposed to high temperatures (37/30 C), even though plants were not visibly affected.

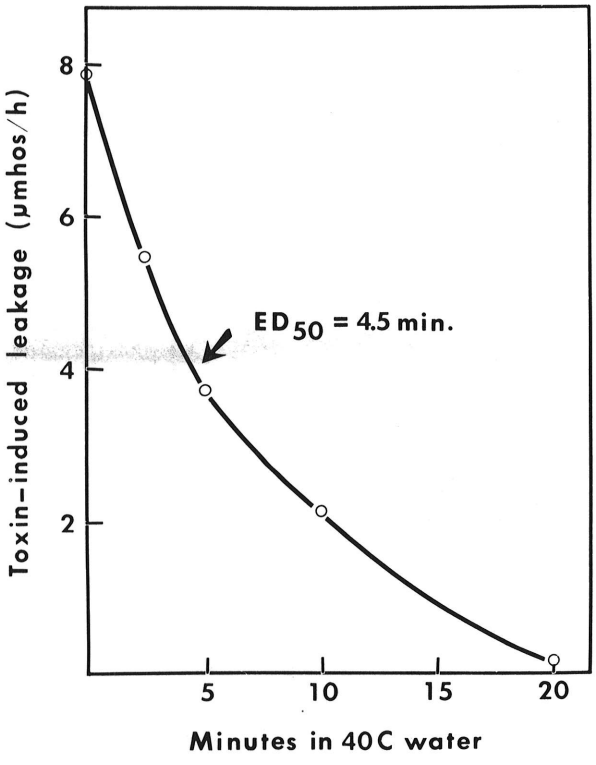

Fig. 3. Thermal exposure times required to decrease sensitivity of sorghum to toxin from *Periconia circinata*. Leaf sections were immersed in water at 40 (± 0.5) C for times indicated, cooled quickly, and exposed to toxin (70 μg/ml). Conductance of ambient solutions was measured during hours 2 and 3 after exposure to toxin. Each value is the mean for three replications; the experiment was repeated with comparable results.

Next, seedlings grown in nutrient solutions or in vermiculite at 22 C were placed at 37 C for 18 hr, followed by return to 22 C for 1 hr. Roots then were exposed to toxin at several different concentrations. Plants held constantly at 22 C also were treated with toxin; control plants without toxin were maintained for each group. The onset of wilt and other toxic symptoms appeared 24 to 48 hr earlier in plants at constant 22 C than in plants with the high-temperature pretreatment. This was true for all concentrations of toxin that caused visible symptoms. The heat treatments caused no visible damage to seedlings without toxin. Water uptake by plants pretreated at 37 C and at 22 C was approximately equal, indicating no significant differences in transpiration during the time of toxin exposure. Resistant seedlings were not visibly affected by toxin.

These results suggested that seedlings pretreated with heat may regain sensitivity to toxin. Intact sorghum seedlings were held at 37 C for 18 hr, then returned to 22 C and tested daily for toxin sensitivity; the first test was made after 1 hr at 22 C. Leaves were cut into pieces, infiltrated with toxin, and assayed by the electrolyte-leakage method. Heat-treated plants were completely insensitive to toxin immediately after exposure to 37 C, but gradually regained normal sensitivity by 2-3 days later (Fig. 2). Transpiration was not a factor in expression of toxicity in this experiment because tissues were submerged in toxin solution or in water during assay.

The temperature and exposure time required to reduce toxin sensitivity was determined more precisely. Sorghum leaf sections were placed in water held at several temperatures from 35 to 50 C for various times, and cooled by immersion in water at 22 C. Leaf sections were then vacuum-infiltrated with toxin solutions or water, and the toxin-induced leakage of electrolytes measured.

TABLE 1. Comparative effects of thermal pretreatments on the sensitivities of oat and sorghum leaves to toxins produced by *Helminthosporium victoriae* (HV toxin) and *Periconia circinata* (PC toxin)[a]

Pretreatment temperature (C)	ED_{50}, minutes[b]	
	Oats/HV toxin	Sorghum/PC toxin
35	>180	15
40	25	4.5
45	3	0.8
50	0.3	0.1

[a]Rates: HV toxin, 2 μg/ml; and PC toxin, 70 μg/ml.
[b]Values are the pretreatment times required to reduce toxin-induced leakage of electrolytes to 50% of the leakage by tissues pretreated at 22 C.

Precautions were taken to insure homogeneity of the samples; also, control tissues were selected from the same batch of leaf pieces and were treated in water at 22 C prior to toxin exposure. The thermal treatments reported here caused little (at 45 and 50 C) or no (at 40 C and below) loss of electrolytes from control tissues in the absence of toxin. An ED_{50} value was estimated for each thermal treatment level; ED_{50} is defined as the thermal-exposure time necessary to reduce the average rate of leakage (during the second and third hours after exposure to toxin) to 50% of the rate for plants exposed to water at 22 C. Sensitivity of sorghum to PC toxin was lost rapidly after relatively mild thermal treatments (Fig. 3, Table 1). For example, the ED_{50} value for a 40-C treatment was 4.5 min. Temperature coefficients for loss of sensitivity were high; the Q_{10} value for 35-45 C was 19. In other experiments, intact plants were exposed briefly to heat by immersing the tops in water. Again, there was a rapid loss of sensitivity to toxin, as measured by the electrolyte assay.

Oxygen uptake is stimulated by PC toxin in susceptible but not in resistant sorghum leaves (14). Therefore, the effect of thermal treatments on toxin-induced increase in respiration was determined. One group of seedlings was held at 37 C for 18 hr in the dark, whereas a control group was held at 22 C in the dark. Leaves then were excised, infiltrated with toxin solutions or water, and respiration measured manometrically. The PC toxin caused a significant increase in oxygen uptake by tissues that had not been heated. There was little or no effect of toxin on respiration by tissues pretreated at 37 C (Fig. 4).

Effect of temperature on susceptibility of sorghum to infection by P. circinata.—The data given above indicate that Periconia blight of sorghum should be less severe at high temperatures (>35 C) than at low temperatures. Susceptible or resistant seeds were planted in pots in infested or noninfested soil and held in a controlled-environment chamber at 20 C. A second set was held in another chamber at 37/30 C (day/night). Seed germination and plant growth were good under all conditions used. Susceptible plants in infested soil at 20 C gradually developed symptoms; by 23 days after planting, 91% were dead, and all others had symptoms of the disease. No plants in infested soil at 37/30 C had symptoms at 23 days, when the experiment was terminated. The experiment was repeated using minor variations in procedure, with comparable results. To determine whether or not the fungus was viable throughout the experiment, some of the high-temperature susceptible plants were moved after 23 days to the 20 C chamber. Plants in infested soil developed symptoms. As a further check, pots that had contained resistant plants at both high and low temperatures were replanted with resistant or susceptible seeds and held at 20 C. Susceptible plants in infested soil developed symptoms.

Results of the inoculation experiments are compatible with the observation that tissues become insensitive to toxin at high temperatures. The experiment does not rule out the possibility that the fungus may make little growth at high temperatures, or that toxin production may be inhibited. Tissue insensitivity, lack of fungal growth, and

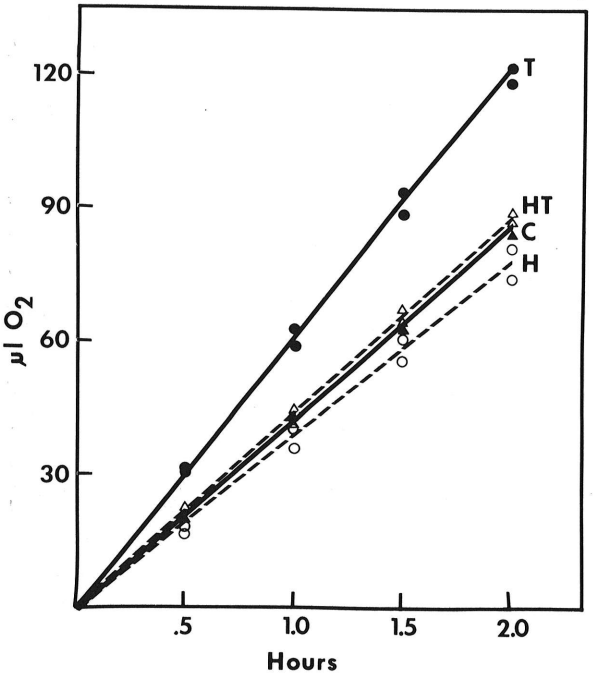

Fig. 4. Effect of toxin from *Periconia circinata* on respiration of heat-treated (HT) and nonheated (T) sorghum tissues. Respiration by heated (H) and nonheated (C) controls without toxin is indicated. Intact seedlings were held at 37 or 22 C for 18 hr in the dark; leaf pieces (200 mg per sample) then were infiltrated with toxin solution (70 µg/ml) or water, and gas exchange at 30 C determined manometrically. Equilibration time was 15 min. Values for two replicates are shown. The experiment was repeated with comparable results.

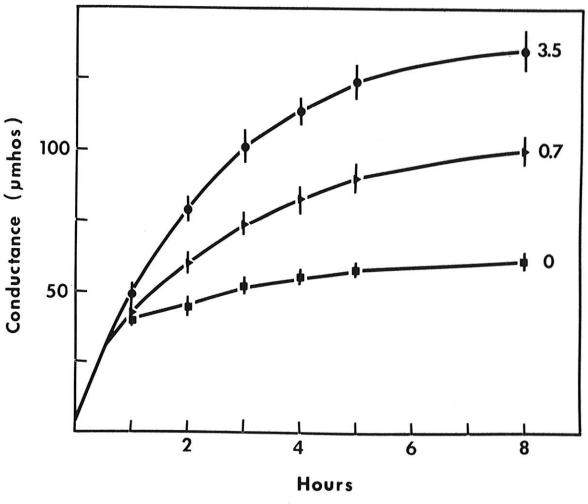

Fig. 5. Effects of two concentrations of toxin on leakage of electrolytes from sorghum. Leaf pieces were infiltrated with toxin from *Periconia circinata* or with water. Conductance of ambient solutions was measured at intervals after exposure to toxin. Each value is the mean for three replications; standard deviations are shown. Comparable results were obtained in each of five experiments. Conductance values at times up to 20 hr after exposure to toxin did not differ appreciably from the values at 8 hr.

lack of toxin each could contribute to lack of symptoms at the higher temperatures.

Effect of temperature on sensitivity of oats to HV toxin.—The electrolyte leakage assay was used to test effects of thermal treatments on sensitivity of oat tissues to HV toxin. Oat-leaf sections were incubated for various times in water at several different temperatures, cooled quickly to room temperature, and placed in toxin solution or water to determine rates of leakage. Results were comparable to those with sorghum and PC toxin, except that higher temperatures or longer exposures were needed to give comparable decreases in sensitivity to toxin (Table 1).

Changes in response of sorghum leaves to PC toxin following excision and aging.—Dosage-response plots showed that the initial rates of toxin-induced loss of electrolytes from sorghum leaves was proportional to toxin concentration, up to a saturating level (5). This was comparable to findings for HV toxin and oats (4). Unlike the response to HV toxin (see below), the rates of change in conductance after exposure of sorghum to PC toxin declined with time (Fig. 5). Declines were apparent for all PC toxin preparations tested, at all concentrations used, and for all exposure times including continuous exposure. The decline was evident with and without vacuum-infiltration of tissues, and was not affected by aeration of the ambient solution. Freezing or boiling of tissues resulted in ambient solutions with conductances of 500 to 600 μmhos, whereas saturating levels of toxin gave ambient solutions with a final conductance of 100 to 150 μmhos. Thus, the decrease in rate of leakage with time was not a result of depletion of total cellular electrolytes in tissues.

One possible explanation was that the decline in rate of toxin-induced leakage resulted from a gradual decrease in sensitivity to toxin. Therefore, sorghum leaves were excised and held in a moist chamber for varying times from 0 to 24 hr. Leaves then were placed in toxin solutions and the rate of loss of electrolytes determined. Freshly excised leaves lost electrolytes rapidly in response to toxin, whereas leaves held for 24 hr prior to exposure had little or no toxin-induced leakage (Fig. 6). Tissues that were excised and aged for 3 hr prior to toxin exposure lost about 50% as much electrolytes as did freshly-cut, toxin-treated tissues. The dramatic loss in response during the first 5 hr after excision indicates that tissues for assays must be cut and used quickly, in a uniform way, or the results will not be reliable.

Other changes in tissues occur quickly after excision, and some are prevented by the addition of kinetin (7). Therefore, leaves from 21-day-old sorghum seedlings were excised, the cut ends placed in vials containing water or kinetin solution (10^{-4}M), and held for 9 hr in light plus 9 hr in darkness. Leaves then were cut into 0.5-cm pieces, infiltrated at reduced pressure with toxin solution or water, and the rate of electrolyte leakage determined. Freshly-cut controls were included. The cut and aged leaves which had taken up kinetin lost electrolytes in response to toxin at a rate comparable to that of freshly-cut tissues (Fig. 7). In response to toxin treatment, excised leaves held for 18 hr without kinetin lost electrolytes at a slower rate than did freshly-cut leaves. In other experiments, leaves were cut into small pieces, infiltrated with kinetin solutions plus toxin, and aged. Under these

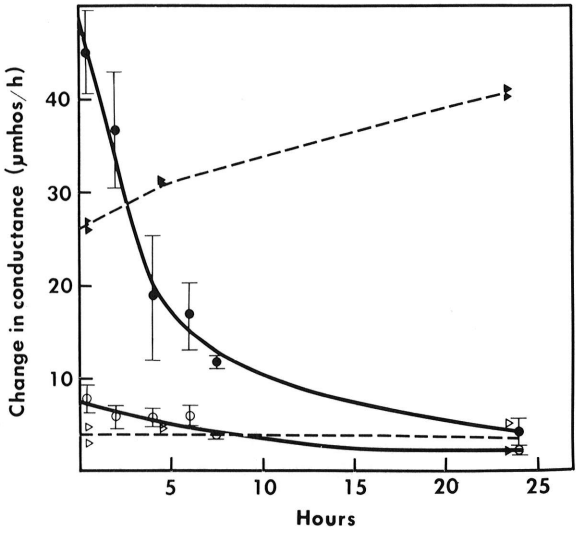

Fig. 6. Effect of excision and aging on toxin-induced loss of electrolytes from oats and sorghum. Leaves were cut and aged for the times indicated, before exposure of oats to toxin from *Helminthosporium victoriae* (2 μg/ml) or of sorghum to toxin from *Periconia circinata* (7 μg/ml). Conductance values were taken during the second hour after exposure. Net electrolyte losses from controls without toxin are shown by the two lower lines. Mean values and standard deviations (three replications) are shown for sorghum (solid lines); values for duplicate samples are shown for oats (dashed lines). The experiment was repeated with comparable results.

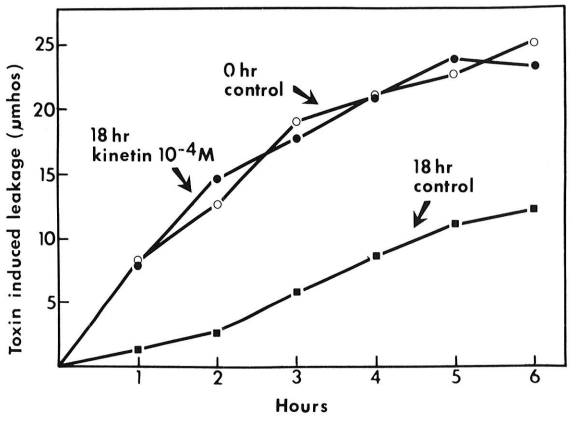

Fig. 7. Maintenance by kinetin of susceptibility to toxin in sorghum tissues. Bases of excised leaves were held in kinetin solution (10^{-4}M) or in water for 18 hr. Leaf sections then were infiltrated with toxin (70 μg/ml) from *Periconia circinata*, or with water, and loss of electrolytes monitored for 6 hr. Values for leaves that were cut and immediately exposed to toxin (0 hr control) are included. Toxin-induced leakage was determined from the conductance reading, by subtracting conductance values for controls (without toxin) and the conductance value for the solution. Each value is the mean for triplicate samples. A Student-Neuman-Keuls multiple range test ($P = 0.05$) indicated that kinetin-treated and 0 hr control values did not differ, and that both differed from the 18-hr control value. The experiment was repeated with comparable results.

conditions, kinetin had little or no effect on loss of electrolytes; leakage declined in the same way whether or not kinetin was present. Other leaves were cut, infiltrated with kinetin, and aged before toxin was added; under these conditions, kinetin gave at least partial maintenance of response to toxin.

Changes in response of oat leaves to HV toxin following excision and aging.—Oat leaves were excised, aged, and treated with HV toxin by vacuum-infiltration, using procedures identical to those described for sorghum and PC toxin. In contrast to the results with sorghum, oat leaves that were excised and aged did not become less sensitive to HV toxin; rather, they appeared to lose even more electrolytes than did toxin-treated, freshly cut leaves (Fig. 8). Oat leaves that were exposed to HV toxin continued to lose electrolytes at a high rate (Fig. 8) until all soluble electrolytes were released. A total electrolyte value for the tissues was determined by thorough homogenization and centrifugation to remove cellular debris. The supernatant liquid and several washings of the pellet were combined and the volume adjusted to that of the ambient solution of toxin-treated tissue of equal weight. The conductance value of the supernatants from homogenized tissue was equivalent to that of the final value for toxin-treated tissue (Fig. 8). Again, this is in contrast to the situation with PC toxin and sorghum.

DISCUSSION

Our results show that sensitivity of sorghum tissues to the host-selective toxin from *P. circinata* is lost after relatively mild thermal treatments and after excision and aging. The criteria used to detect changes in sensitivity were loss of electrolytes, increases in respiration, and development of visible symptoms. Heat-induced losses of sensitivity to other toxins have been reported (3, 8), but there are no previous reports that excision and aging will affect sensitivity to toxins. The findings may contribute to an understanding of disease development in the field, may have application in the design of practical assays, and we hope they will be of use in future studies of the mode of toxin action. If heat directly affects the primary site of toxin action, then resistance in heat-treated tissue should be comparable in some ways to gene-controlled resistance. Thus, we would have the means of studying resistance and susceptibility in a single genotype.

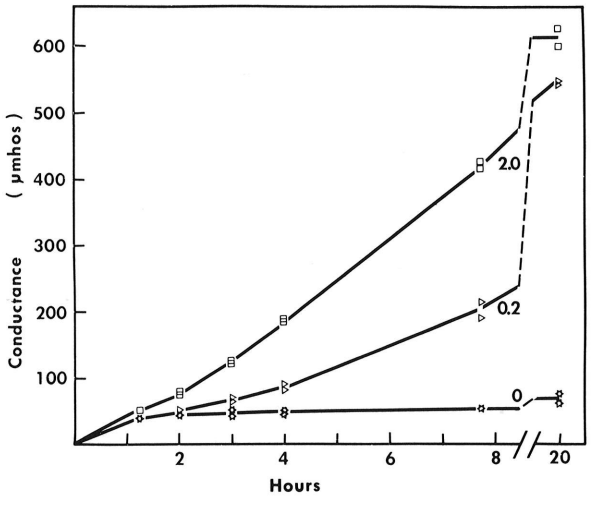

Fig. 8. Loss of electrolytes from oat leaves during a 20 hr exposure to toxin from *Helminthosporium victoriae*. Excised leaves were infiltrated at reduced pressure with toxin solutions (2.0 or 0.2 μg/ml) or water, at 0 time. Values for replicate samples are given.

There are no definitive data on the effects of temperature on development of Periconia blight in the field. However, earlier workers (11) reported that symptoms generally disappeared during mid-summer on the Great Plains. In short-term experiments conducted at the high temperatures which might occur during mid-summer, we found that *P. circinata* did not induce symptoms in sorghum. Comparable observations have been reported for *Alternaria kikuchiana* affecting pears in Japan (8), and for *H. sacchari* affecting sugarcane in Hawaii (3). Disease symptoms on pears and sugarcane develop slowly or are alleviated during the warmer part of the growing season. In both cases, susceptible plants develop resistance to a fungal toxin at temperatures that often prevail during the growing season.

Other researchers have suggested that heat affects sensitivity to toxins by changing labile receptor substances (3). However, other interpretations must be considered. No data to date rule out the possibility that heat affects a secondary process one or more steps removed from the intial site of toxic action; that is, heat may affect a cellular factor necessary for response to toxin, but not the receptor itself. This caution seems especially important for data that are restricted to only one toxic effect, such as loss of elelctrolytes. For this reason, we have used three different responses; each indicates that heat decreases sensitivity to toxin. Thus, we believe that heat affects a site that is at least an early stage in the sequence of toxic action. The gradual recovery of sensitivity to toxin after heating may result from synthesis or reactivation of a receptor or sensitive factor. Loss of sensitivity after excision and aging of sorghum leaves also might result from a change in or loss of toxin receptor sites. However, there are other possible interpretations, because many changes may occur in leaves after they are detached. We have no information on possible changes in ion-transport mechanisms or sizes of electrolyte pools following detachment of leaves.

Studies with protein-synthesis inhibitors and sulfhydryl-binding reagents suggest that cellular factors needed for toxic action are proteins with relatively short half-lives (5, 6). The Q_{10} value for heat-induced resistance is high and within the range said to be characteristic of protein denaturation (15). The half-life for loss of sensitivity by sorghum after excision and aging also is short. The protein-receptor interpretation is compatible with data on kinetin, which showed that the hormone maintained the ability of excised sorghum leaves to leak electrolytes following exposure to PC toxin. Kinetin is known to delay senescence and maintain protein levels in excised leaves (7). Unfortunately, no conclusive data show that these cellular factors are toxin receptors or that all treatments that cause loss of sensitivity are affecting the same cellular factors.

The PC and HV toxins have several similar effects on susceptible tissues, but differences between the two have been observed. For example, sulfhydryl-binding compounds will protect sensitive oat tissues against HV toxin (6), whereas these compounds do not protect sorghum against PC toxin (5). Data presented here demonstrate another difference; PC toxin caused less leakage of electrolytes from excised and aged tissue than from freshly-cut tissue, whereas HV toxin caused at least as much response in aged as in freshly-cut tissues. Also, more heat was required to induce resistance in oats to HV toxin than was needed for sorghum and PC toxin. We have no explanations for these phenomena; differences in mechanisms of action may not be involved. Nevertheless, such variations in response must be considered in development of assays for these and other toxins and in studies of modes of action.

Toxin-induced leakage of electrolytes has been used as the basis of an assay for PC toxin (5). Variability has been a problem at times; therefore the assay has not been as reliable as the electrolyte assay for HV toxin (4). Data on effects of high temperature and excision plus aging have clarified the problems, making possible a much more reliable assay for PC toxin. Tissues must be cut and used promptly to determine initial rates of leakage. Care must be taken to avoid use of plants recently exposed to high temperatures, such as often occurs in the greenhouse.

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