# Host-Selective Toxins from *Alternaria citri*

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## ABSTRACT

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Filtrates from cultures of *Alternaria citri* are selectively toxic to rough lemon or tangerine leaves. Two related toxic compounds were isolated from the *A. citri* cultures that are selectively pathogenic to rough lemon. Both compounds affected susceptible rough lemon when 50 μl of solution (0.1 μg toxin per ml) was applied to leaves; tangerine and other nonhost plants were not visibly affected at 1,000 μg/ml. A rough lemon selection that is tolerant to the rough lemon-infecting isolates was affected by the toxins, but symptom development was delayed. Isolates pathogenic to Dancy tangerine yielded a toxin that visibly affected the host species when applied (50 μl, 1.0 μg/ml) to leaves. The tangerine-specific toxin at 1,000 μg/ml did not affect rough lemon or other nonhost species. Several conditions favoring toxin production in culture were determined; especially notable was the stimulatory effect of $ZnSO_4$ in the medium. Procedures are described for purification of toxins, based on solvent extractions and chromatography. Each of the toxins caused leakage of electrolytes from susceptible, but not from resistant, tissue.

A new and highly virulent form of *Alternaria citri* Ellis and Pierce affecting foliage and fruit of Emperor mandarin (*Citrus reticulata* Blanco) was found in Australia in 1962. Pegg (9) reported that filtrates from cultures of the fungus were toxic to susceptible mandarin leaves and fruit, but were not toxic to rough lemon (*C. jambhiri*) and other nonhost plants. In 1974, Whiteside (15) found the same fungal species in Florida, causing a serious problem on Dancy tangerine (*C. reticulata*). Culture filtrates from the Florida isolates had the same specificity as did those from Australia. Whiteside (15) discovered another isolate of the fungus that was highly virulent on rough lemon shoots. Culture filtrates from the rough lemon form of *A. citri* were toxic to rough lemon, but did not affect Dancy tangerine shoots and fruits. Apparently, the two forms of *A. citri* produce different host-selective toxins; our objectives were to isolate, characterize, and determine the biological effects of those toxins. We report herein the methods of isolating homogeneous toxins, along with some preliminary characterization and initial attempts to determine biological effects.

## MATERIALS AND METHODS

Isolates of *A. citri* were obtained from naturally-infected rough lemon and Dancy tangerine trees in Florida. Two rough lemon clones were used; one, from a Florida seed source, is highly susceptible to Alternaria leafspot. The other was a rough lemon selection (Nelspruit 15 from South Africa) that is tolerant or partially resistant to the disease in the field (Whiteside, *unpublished*). Young trees of Dancy tangerine were obtained from the Agricultural Research and Education Center, Lake Alfred, FL, and from the Adams Citrus Nursery, Haines City, FL. All three types of citrus were grown in the greenhouse at temperatures above 18 C, because the trees tend to become dormant at lower temperatures.

*Alternaria citri* was grown in Czapek-Dox solutions (13), without agitation in the dark, for 14–20 days at 20 or 28 C. Cultures were grown in 125-ml Erlenmeyer flasks containing 25 ml of medium, or 1,000 ml Roux bottles, each containing 200 ml of medium. Cultures were harvested by filtration with cheesecloth and Whatman No. 1 filter paper. Cell-free filtrates were prepared by filtration through Millipore membranes (0.22 μm pore size).

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Young shoot-tip cuttings were used for a qualitative test of toxicity; susceptible and resistant cuttings were included in each test. The basal ends were placed in diluted cell-free filtrates or extracts containing toxin, and incubated at 22 C under fluorescent lights to encourage transpiration. Cuttings were observed after 12, 24, and 48 hr for toxic effects.

Unless stated otherwise, a simple, semiquantitative assay that was developed for isolation of toxin was used in all experiments. Young rough lemon or tangerine leaves, not fully expanded, were harvested from greenhouse-grown plants. The leaves were cut into pieces (each 3–4 $cm^2$) and placed on wet foam plastic in trays. A slight wound was made with a glass needle in the center of each leaf piece, and a drop of test solution (50 μl) was placed on the wound; trays were covered tightly with plastic film and placed in an incubator at 28 C in the dark. Serial dilutions of test solutions were used. When toxin was present, a spreading dark lesion developed around the point of application. Data were taken 2 days after treatment. Assay endpoint was the maximum dilution of test solution that caused visible symptoms on the susceptible leaf. The assay was adapted for use with paper or thin-layer chromatography. For example, gel was removed from zones on thin-layer plates, placed on assay leaves, and wet with a drop of water. The gel from $R_f$ zones containing toxin caused a necrotic response in susceptible but not in resistant leaves.

The effect of toxins on leakage of electrolytes from leaves was determined by a method similar to that used for other host-selective toxins (1,2,12). The third and fourth leaves from the tips of growing shoots were selected; these leaves were not expanded fully and lacked the hard cuticle of mature leaves. Leaf disks (1.2 cm in diameter) were cut with a cork borer and infiltrated with toxin solutions or water at reduced air pressure (approximately 2 cm Hg) for 20 min. Disks were then rinsed with water, placed in vials (15 disks per vial; wt approximately 300 mg) each containing 5 ml water, and incubated on a reciprocal shaker (100 strokes/min) at 20–22 C. Conductances of ambient solutions were measured at intervals with a conductivity meter equipped with a pipet-type electrode (K = 1.0). A series of toxin dilutions was used and all experiments were repeated one or more times.

All solvents were of reagent grade and were redistilled immediately before use.

## RESULTS

**Toxicity of culture filtrates.** Cell-free filtrates from cultures of the tangerine- and rough lemon-specific isolates were diluted serially, and drops of each dilution were placed on pieces of tangerine and rough lemon leaves. The leaf pieces were examined for evidence of toxicity at intervals up to 4 days after exposure. Filtrates from the tangerine isolates caused water-soaked spots followed by black necrosis on tangerine leaves, but caused no visible effects on rough lemon leaves. Filtrates from rough lemon isolates caused water-soaking followed by brown lesions on rough lemon leaves, but did not affect leaves of tangerine; assay endpoints were at 1- to 50-fold dilutions. Comparable results were obtained when the experiment was repeated. Thus, the earlier reports of host-selective toxicity from Pegg (9) and Whiteside (15) were confirmed by a different method of testing.

**Effects of culture conditions on toxin yield.** Three pathogenic isolates of *A. citri* from tangerine and three from rough lemon were tested for toxin production. The isolates were grown in three different media: Fries' solution amended with 0.1% yeast extract (11), Czapek-Dox solution, and Richards' solution (13). Incubation temperatures were 21 and 28 C; cultures were harvested after incubation for 10, 15, and 20 days. Toxin yields, as determined by bioassay, were best when cultures were grown in Fries' or Czapek-Dox solutions and harvested after 20 days at 28 C. Toxin titers from the highest yielding isolates of each race varied from one experiment to another, with assay endpoints at 5- to 50-fold dilutions.

Several substances, including $ZnSO_4$, $MnSO_4$, thiamine · HCl, pyridoxine · HCl, folic acid, biotin, and malt extract, were added to Czapek-Dox and Richards' media in attempts to improve yields of rough lemon and tangerine-specific toxins. The experiment differed from the one described above in that cultures were harvested at 14 days. Only $ZnSO_4$ gave significant increases in toxin titers in filtrates of both fungi; $MnSO_4$ increased the titer for rough lemon toxin, but did not affect toxin levels in filtrates of the tangerine isolate. The other additives had little or no effect. Zinc sulfate also had some effect on mycelial growth. The effects of $ZnSO_4$ were more striking for the tangerine than for the rough lemon isolates (Tables 1 and 2). The experiment was repeated twice and the results were confirmed.

**Isolation of toxins.** Many different techniques were tested for isolation of toxins. For the rough lemon-specific toxin, filtrates (40-L batches) were obtained from cultures grown at 28 C for 16–20 days in Roux bottles containing Czapek-Dox solutions enriched with $ZnSO_4$ (2.0 mg $ZnSO_4$/L, or 5 μg $Zn^{++}$/ml). The filtrate was adjusted to pH 2.8 with HCl, and extracted three times with ethyl acetate (filtrate was extracted in batches, and the solvent was redistilled during the process). The ethyl acetate extract was dried with anhydrous $Na_2SO_4$, concentrated under reduced pressure, and extracted four times with an aqueous solution containing 8% (w/v) $NaHCO_3$. The ethyl acetate phase was washed with water, dried, and concentrated again; this fraction contained about 10% of the total (original) activity, as determined by bioassay. The aqueous phase (washings from the acetate extract) was washed with petroleum ether, adjusted to pH 2.8, extracted three times with benzene, followed by three extractions with ethyl acetate. The benzene phase was washed with water, dried with anhydrous $Na_2SO_4$ crystals, and concentrated; this fraction contained approximately 5.0% of the total toxic activity. The ethyl acetate phase was washed with water, dried, and concentrated to about 30 ml. A precipitate was discarded and the supernatant fluid was concentrated to dryness at reduced pressure. This fraction contained about 85% of the original toxic activity.

The fraction containing most of the toxin was chromatographed on a silicic acid column (Mallinckrodt, AR CC-4, 3.8 × 21 cm), which was developed stepwise with varying proportions of benzene, ethyl acetate, and acetone. Toxin was finally eluted from the column with an ethyl acetate-acetone mixture (39:1, v/v). Toxin-containing fractions were re-chromatographed twice on silicic acid columns (2.5 × 20 and 1.8 × 55 cm). In both cases, toxin was eluted with ethyl acetate-acetone mixtures (39:1, 19:1, and 9:1, v/v). The most active preparation, on a dry wt basis, was eluted by the 19:1 mixture; this portion, which contained 6% of the original activity, was toxic in the standard assay with rough lemon leaves at 0.22 μg/ml. This fraction was placed on a silica gel thin-layer plate (Merck 60 F-254) and developed with acetone-benzene-acetic acid (40:10:1, v/v). Most of the host-selective toxic activity was present at $R_f$ 0.45–0.55; this fraction was designated the major toxic fraction. Host-selective toxicity was evident also at $R_f$ 0.6–0.68; this was designated the minor toxic fraction. Thin-layer chromatography of preparations at earlier stages of purification had toxic activity at comparable $R_f$ zones. The toxin-containing zones were extracted five times with acetone, concentrated to dryness, dissolved in chloroform, and filtered.

The major and minor toxins were chromatographed separately on thin-layer plates, with the solvent systems listed in Table 3. Reagents used to detect spots are indicated in Table 4. Only one spot was obtained with each system. Toxin was chromatographed on UV-fluorescing plates; the only UV-quenching area had toxic activity. All attempts to detect more than one substance were negative. The preparation was toxic to susceptible rough lemon leaves at 0.1 μg/ml.

Tangerine-specific toxin was isolated by a similar procedure, modified because of a difference in benzene-solubility. Tangerine toxin was extracted from aqueous solutions with benzene, and was eluted from silicic acid columns with benzene-ethyl acetate mixtures (1:1 and 1:2, v/v). In other respects, the isolation procedures

TABLE 1. Effect of $ZnSO_4$ on toxin production by *Alternaria citri* isolate 325 from rough lemon[a]

| Medium | Concn. of $ZnSO_4 \cdot 7H_2O$ (mg/L) | Mycelial growth[b] (g) | Toxicity: dilution endpoint[c] |
|---|---|---|---|
| Richards' | 0 | 0.72 | 4 |
| | 2.5 | 1.18 | 128 |
| | 5 | 0.96 | 128 |
| | 10 | 0.92 | 128 |
| | 20 | 0.82 | 16 |
| Czapek-Dox | 0 | 0.37 | 0 |
| | 2.5 | 0.79 | 512 |
| | 5 | 0.88 | 256 |
| | 10 | 0.88 | 128 |
| | 20 | 0.96 | 512 |

[a]Duplicate cultures (50 ml medium/200 ml flask) were grown in still culture at 28 C for 14 days.
[b]Average dry wt per flask.
[c]Maximum dilution of culture filtrate that gave definite lesions on test leaves. Tangerine leaves were not affected. Highest dilution in the test was 1,024.

TABLE 2. Effect of $ZnSO_4$ on toxin production by *Alternara citri* isolate 320 from Dancy tangerine[a]

| Medium | Concn. of $ZnSO_4 \cdot 7H_2O$ (mg/L) | Mycelial growth[b] (g) | Toxicity: dilution endpoint[c] |
|---|---|---|---|
| Richards' | 0 | 0.74 | 1 |
| | 2.5 | 2.08 | 1,024 |
| | 5 | 1.80 | 1,024 |
| | 10 | 1.88 | 1,024 |
| | 20 | 1.57 | 1,024 |
| Czapek-Dox | 0 | 0.34 | 1 |
| | 2.5 | 0.94 | 512 |
| | 5 | 1.12 | 256 |
| | 10 | 0.93 | 512 |
| | 20 | 1.13 | 1,024 |

[a]Duplicate flasks (50 ml medium/300-ml flask) were grown in still culture at 28 C for 14 days.
[b]Average dry wt per flask.
[c]Maximum dilution of culture filtrate that gave definite lesions on test leaves. Rough lemon leaves were not affected. Highest dilution in the test was 1,024.

were the same for the two toxins. Tangerine toxin had an $R_f$ value of 0.2–0.3 on thin-layer plates (Merck 60 F-254) developed with benzene-acetone-acetic acid (60:40:1, v/v).

**Specificity of purified toxins.** Toxin from the rough lemon-specific isolate of *A. citri* was highly selective in the standard leaf assay. The homogeneous major and minor toxins caused symptoms on susceptible rough lemon leaves following application of 50 μl of solution containing 0.1 μg toxin per milliliter. When used at 1,000 μg/ml, the major toxin did not affect leaves of the following species: Dancy tangerine, tomato, spinach, soybean, garden bean (*Phaseolus vulgaris*), tobacco (*Nicotiana glutinosa*), datura, maize, and wheat. The minor toxin was tested against Dancy tangerine, grapefruit, tomato, broadbean, garden bean, *N. glutinosa*, *N. tabacum*, wheat, barley, maize, and datura; none was sensitive. Both the major and minor toxins caused visible effects on susceptible rough lemon leaves within 24 hr. The tolerant rough lemon selection had no visible effects at 24 hr; 48–72 hr was required for symptoms to develop.

A partially purified preparation of the rough lemon-specific toxin, containing the major and minor fractions, was tested on rough lemon and tangerine shoot-tip cuttings. The preparation had been chromatographed three times on a Mallinkrodt AR CC-4 column, and was active at 0.22 μg/ml in the assay with cut leaves. Shoot cuttings were allowed to take up solutions containing 4.0 μg of the preparation per milliliter. Within 12 hr, host-specific responses were evident; there were water-soaked spots on susceptible rough lemon leaves, followed by brown necrotic lesions and a little vein necrosis. There were no obvious symptoms on tangerine, even after exposure for 4 days.

The isolates of *A. citri* from Dancy tangerine also produced toxin that was highly selective. This toxin, isolated by the procedures outlined above, caused symptoms in Dancy tangerine leaves when 50 μl of a solution containing toxin at 1.0 μg/ml was applied; rough lemon leaves were not affected by a solution containing 1,000 μg toxin per milliliter (Fig. 1). The tangerine toxin is less stable than the rough lemon toxin; lower stability could be a factor in lower apparent toxicity in the assay, which requires 48–72 hr for completion. Specificity was evident also when shoot tips were allowed to take up solutions (4.0 μg/ml) of this same toxin preparation. Within 12 hr, tangerine leaves had water-soaked spots that developed into black lesions. There was much black necrosis of leaf veins. No symptoms were evident on rough lemon leaves after 4 days, when the experiment was terminated.

In other experiments with shoot cuttings, lower and higher concentrations (1.0 and 10.0 μg/ml) of the toxins gave essentially the same results as described above.

**Characteristics of the toxins.** Partially purified tangerine and rough lemon-specific toxins passed through cellulose dialysis membranes, indicating low molecular weights. Heat-stability of the rough lemon-specific toxins at several pH levels was tested. Toxicity of culture filtrates was not affected significantly at 40 C for 60 min, at pH 2.0, 6.8, and 10.0. Most activity was lost after 10 min at 100 C, at all three pH levels. Purified rough lemon toxins appeared to be somewhat more stable than were the crude preparations. Tangerine-specific toxin from a silica gel column was inactivated approximately 90% after 10 min at 90 C.

The rough lemon-specific toxins and *A. mali* toxin I (14) were chromatographed on thin-layer plates with a number of solvent systems (Table 3). The results suggested that the rough lemon toxins may be less lipophilic than were the host-selective toxins from *A. mali* and *A. kikuchiana* (Table 3). Chromatographic behavior in several solvent systems was used to determine homogeneity of preparations (Table 3).

Several spray reagents were used to detect purified rough lemon-specific toxins on thin-layer plates (Table 4); the only ones of special interest were the negative ninhydrin and the positive terpenoid reactions (vanillin-$H_2SO_4$).

The rough lemon-specific toxins (both major and minor fractions) were soluble in chloroform, methylene dichloride, ethyl acetate, acetone, ethanol, and methanol. They were moderately soluble

TABLE 3. Effects of different solvent systems in thin-layer chromatography (TLC) of toxins produced by two *Alternaria* spp.[a]

| Solvent system[b] | $R_f$ value for toxin | | | |
|---|---|---|---|---|
| | Rough lemon, major | Rough lemon, minor | AM[c] | AK[d] |
| Benzene-*n* hexane-acetone-acetic acid (50:20:20:1,v/v) | 0.04 | 0.06 | 0.10 | 0.20 |
| Chloroform-methanol (9:1) | 0.15 | 0.22 | 0.33 | 0.42 |
| Chloroform - methanol (3:1) | 0.29 | 0.45 | 0.52 | ... |
| Benzene - acetone (2:1) | 0.04 | 0.06 | 0.19 | ... |
| Benzene - acetone (1:4) | 0.31 | 0.48 | 0.53 | ... |
| Ethyl acetate-methanol (3:1) | 0.37 | 0.51 | 0.59 | ... |
| Benzene-acetone-acetic acid (60:40:1) | 0.12 | 0.21 | 0.32 | 0.51 |
| Benzene-acetone-acetic acid (10:40:1) | 0.49 | 0.59 | 0.62 | ... |
| Benzene-ethyl acetate-isopropanol (10:10:1)[e] | 0.25 | ... | ... | ... |

[a]TLC plates: Merck Kieselgel 60 F-254.
[b]All ratios are v/v.
[c]*A. mali* toxin I, prepared by K. Kohmoto in Japan.
[d]*A. kikuchiana* toxin I. Data from MS thesis of Mr. A. Noda, Tottori University, Japan.
[e]Plates were redeveloped and dried five times.

TABLE 4. Color reactions of purified toxins, produced by *Alternaria citri* from rough lemon, with several indicator reagents sprayed on thin-layer chromatograms

| Reagent | Color reaction, rough lemon toxins[a] |
|---|---|
| $I_2$ vapor | Yellow |
| Concentrated $H_2SO_4$ (heated to 105 C) | Black |
| $KMnO_4$ | Yellow |
| $SbCl_3$ in chloroform | Dark brown |
| Vanillin - $H_2SO_4$ | Purple |
| Anisaldehyde in $H_2SO_4$ and acetic acid | Dark brown |
| Diphenylamine-aniline-phosphoric acid | Brown |
| Ninhydrin | None |
| 9. Dragendorff's reagent | None |
| $FeCl_3$ | None |
| 2,4-Dinitrophenylhydrazine | Yellow |
| *o*-Dianisidine | Grey-brown |
| Anthrone | Brown |

[a]Major and minor toxic fractions gave the same reactions.

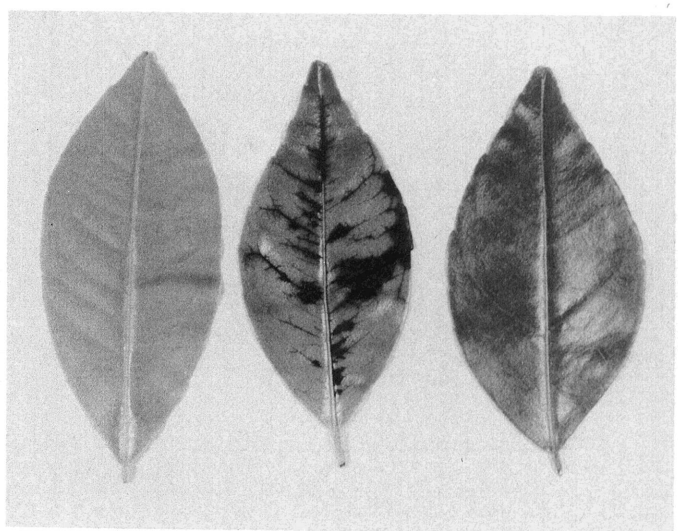

**Fig. 1.** Effect of purified toxin from the tangerine isolates of *Alternaria citri* on leaves of Dancy tangerine (center) and rough lemon (left). An untreated control leaf is on the rght. A drop of solution containing toxin was placed on each side of the midrib of treated leaves, 1 day before the picture was taken.

in water (less than 1 mg/ml) and relatively insoluble in carbon tetrachloride, diethyl ether, benzene, *n*-hexane, and petroleum ether. Rough lemon toxins were less lipophilic than was tangerine toxin, which was soluble in benzene and diethyl ether. Tangerine-specific toxin also was soluble in chloroform, methylene dichloride, ethyl acetate, acetone, and methanol, but was insoluble in petroleum ether.

**Toxin-induced leakage of electrolytes from leaves.** A partially purified preparation from the rough lemon-specific isolate was tested for ability to induce leakage of electrolytes from susceptible and resistant tissues. At 0.67 μg/ml, the preparation caused visible symptoms in susceptible rough lemon leaves. Electrolyte losses were induced by toxin at 20 μg/ml (the lowest concentration used), with increases in losses as toxin concentrations were increased (Fig. 2). There were no toxin-induced losses from tangerine leaves at the highest concentration of toxin (80 μg/ml). In a second experiment, significant toxin-induced losses from rough lemon leaves occurred when toxin was used at 5.0 μg/ml. Comparable results were obtained in a third experiment with toxin at 5.0 μg/ml.

The tangerine-specific toxin also was tested for ability to induce leakage of electrolytes from susceptible and resistant tissue. The toxin preparation caused visible symptoms in Dancy tangerine leaves at 1.25 μg/ml. In the first experiment, toxin was used at 10, 20, and 40 μg/ml; all three concentrations caused significant leakage of electrolytes from tangerine, but not from rough lemon tissues (Fig. 3), with possible saturation of effect at 20 μg/ml. In a separate experiment, toxin was used at 1.25 and 5.0 μg/ml; both concentrations caused leakage (Fig. 4).

## DISCUSSION

Host range and pathogenicity of several plant-infecting fungi is determined by metabolites (host-selective toxins) released from germinating spores and growing mycelium (12). However, relatively few examples of such toxins have been studied in detail. Some of the host-selective toxins have been isolated in crystalline form (10,12), but have not been fully characterized, largely because of lability of the purified preparations. Only one host-selective toxin, that from *A. mali* affecting apple, has been completely characterized and independently confirmed (8,14).

Another difficulty in work on host-selective toxins has been low yields in culture. At first, this was a major problem with *A. citri*; it was solved in part by empirical determination of the best conditions for toxin production. Especially useful was the discovery that toxin yields are greatly increased by adding $ZnSO_4$ to the culture medium. Special requirements for toxin production by plant pathogens are not unusual; for example, *Periconia circinata* produces very little toxin in many media, but gives good yields in Fries' solution amended with yeast extract (12). Attention to special requirements might lead to the discovery of host-selective toxins from other fungi.

Purification of the toxins from two forms of *A. citri* have now been accomplished and characterizations are underway. The procedures described have given high percent recoveries of toxins at each step, up to a point. Low recovery of toxin from silicic acid columns reflects in part the fact that toxin was eluted from the column with three different solutions (ethyl acetate-acetone mixtures; 39:1, 19:1, and 9:1, v/v). The most active preparation (therefore the most purified) was obtained with the 19:1 mixture, and only this one was

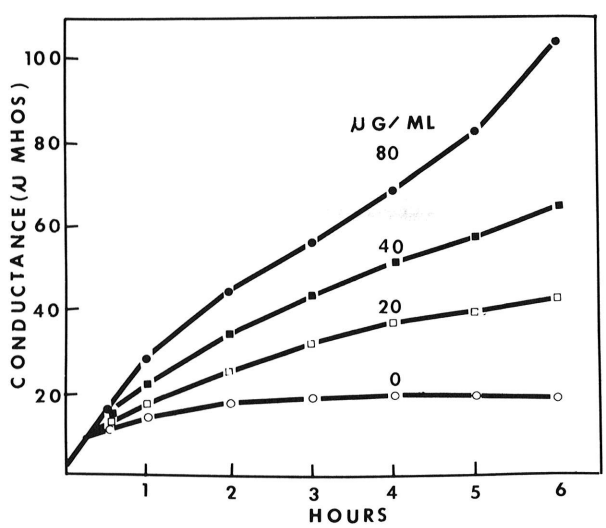


**Fig. 2.** Rates of electrolyte loss from rough lemon leaves induced by toxin from a rough lemon isolate of *Alternaria citri*. Toxin concentrations in μg/ml were 80( ● ), 40( ■ ), 20 ( □ ), and 0 (control) ( ○ ). Losses from toxin-treated and control tangerine leaves did not differ significantly from the 0 control value ( ○ ) shown. Comparable results were obtained in repeated experiments.

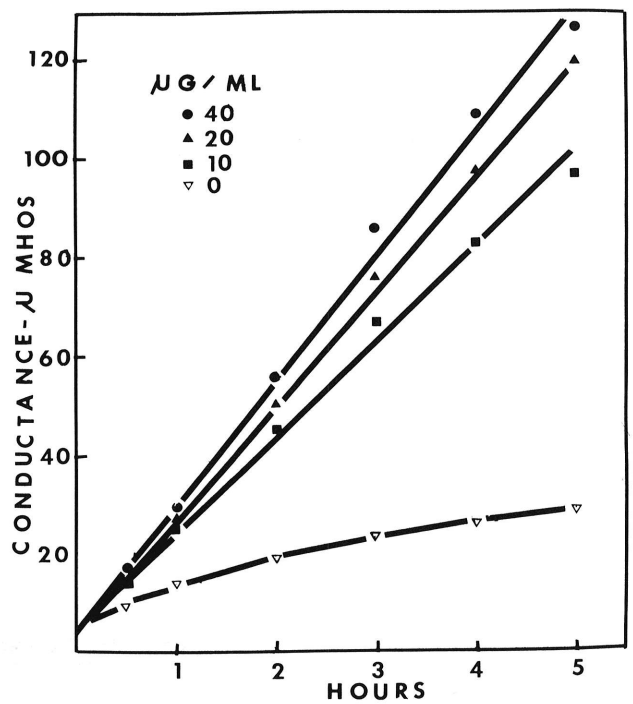


**Fig. 3.** Rates of electrolyte loss from Dancy tangerine leaves treated with relatively high concentrations of toxin from a tangerine isolate of *Alternaria citri*. Toxin concentrations in μg/ml were 40( ● ), 20( ▲ ), 10( ■ ), and 0 (control) ( ▽ ). Losses from toxin treated and control rough lemon leaves did not differ significantly from the 0 control value ( ▽ ) shown.

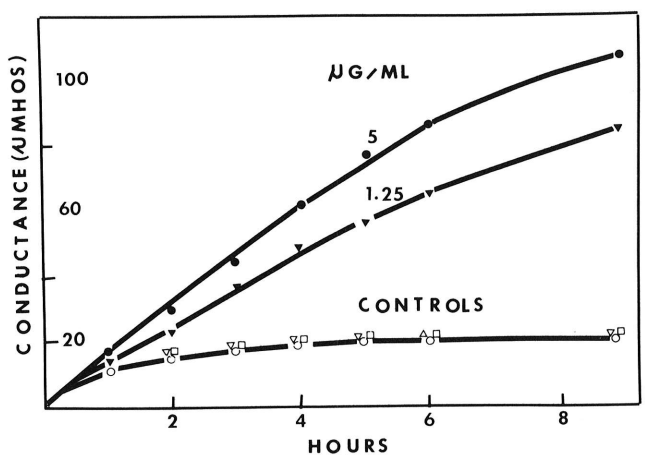


**Fig. 4.** Rates of electrolyte loss from Dancy tangerine leaves treated with low concentrations of toxin from a tangerine isolate of *Alternaria citri*. Toxin concentrations in μg/ml were 5( ● ), 1.25 ( ▲ ), and 0 (control) ( ○ ). Electrolyte losses from toxin treated ( △ ) and control rough lemon ( □ ) leaves are shown.

used in further work.

The limited data now available indicate that the biological effects of the *A. citri* toxins are similar to those of several other host-selective toxins (6,12). A high degree of specificity is evident; tangerine and other nonsensitive species will tolerate at least 10,000-fold higher concentrations of toxin from the rough lemon-infecting fungus than will tissues of susceptible rough lemon. Toxin from the tangerine isolates also has striking specificity, in leaf assays and in tests for induction of electrolyte leakage. Toxin from the tangerine isolates appears to be at least as active as toxin from rough lemon isolates in inducing leakage of electrolytes from susceptible tissue, even though higher concentrations of tangerine toxin were required to induce visible symptoms. The apparent discrepency may be related to the fact that tangerine toxin is less stable than rough lemon toxin. Finally, the data indicate that electrolyte leakage might be used as the basis of a quantitative bioassay (2,12), provided conditions are carefully standardized.

The morphology of *A. citri* resembles that of several other *Alternaria* spp., including *A. alternata* ( = *A. tenuis*). Several fungi in this group are now known to produce host-selective toxins; included are *A. kikuchiana*, *A. mali* (6,7), *A. alternata* f. sp. *lycopersici* (3), an *Alternaria* sp. virulent to certain cultivars of strawberry (5), and possibly *A. eichhorniae* (4). *A. citri* is of special interest because it includes an apparent parental type without pathogenic specialization, and two related forms that are highly specialized pathogens. Each of the specialized pathogens produces different, but perhaps similar, toxic compounds. Thus, a comparison of the chemical characteristics of the toxins has special significance in studies of the basis of pathogenicity and disease development.

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