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Serinol as an activator of toxin production in attenuated cultures of *Helminthosporium sacchari*

(helminthosporoside/attenuation/plant disease resistance)

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ABSTRACT **Successive transfer in synthetic medium of spores and mycelial fragments from original toxin-producing cultures of *Helminthosporium sacchari* results in attenuated cultures which do not produce the host-specific toxin helminthosporoside. When attenuated cultures are grown on material obtained from the water wash of sugarcane leaves susceptible to this fungus, the production of heminthosporoside resumes. Cpmpounds that activate toxin production in the fungus are present on the leaf surface and presumably arise via plant metabolism. One activator was identified as a novel free amine, serinol (2-amino-1,3-propanediol). It activates toxin production in attenuated cultures at 1 μM. Several experiments described in this report argue against the selection theory for the attenuation of cultures. The biological significance and some possible mechanisms for the activation of toxin biosynthesis are discussed.**

Variation within a pathogen population may produce highly efficient biotypes that are extremely successful parasites. Conversely, biotypes may arise in which the ability to persist parasitically is greatly reduced or eliminated. This loss of parasitic capabilities has been labeled attenuation (1).

The genus *Helminthosporium* includes pathogens whose conidial stages are responsible for serious diseases of rice, corn, grasses, and cereals (2). Several members of the genus, including *H. victoriae, H. carbonum, H. maydis,* and *H. sacchari,* produce host-specific toxins. These compounds, which are fungal metabolites toxic only to the susceptible host, produce virtually all of the disease symptoms and are critical for pathogenicity of the fungi (3). Since pathogenicity in these fungi is dependent upon their host-specific toxins, the loss of toxin production and attenuation are synonymous. Cessation of toxin production has been observed in cultures of *H. maydis* (4), and *H. carbonum* and *H. victoriae* (5).

Helminthosporium sacchari (Van Breda de Haan) Butler is the causal agent of the eyespot disease of sugarcane. This disease, found in most areas where sugarcane is grown (6), is characterized by the formation of eye-shaped lesions, followed by the development of reddish-brown "runners" which extend from the lesion toward the leaf tip. Runner formation is caused by the host-specific toxin helminthosporoside (7) (2-hydroxycyclopropyl-α-D-galactopyranoside), a fungal metabolite directly involved in the pathogenesis of the eye-spot disease (8).

Fortuitously, we observed that when toxin-producing isolates of *H. sacchari* were grown on a synthetic medium and transferred a number of times through the same medium, they lost their toxin-producing ability: they became attenuated. When an attenuated isolate was transferred to a medium containing an infusion of sugarcane leaves (susceptible clone 51 NG 97), full toxin production was restored. These observations suggested that a compound present in sugarcane is required for toxin biosynthesis.

This report describes the isolation, purification, and chemical characterization of one of several "activators" from susceptible sugarcane that can restore toxin production in these cultures. Some discussion of the importance of this phenomenon to the host–parasite system is also presented.

MATERIALS AND METHODS

Sugarcane. Susceptible (51 NG 97) and resistant (H 50-7209) clones of sugarcane were acquired from R. Coleman, United States Department of Agriculture, Beltsville, Md. Stalks were planted in large plastic buckets and maintained at 22 ± 5° under greenhouse conditions. Mature leaves used in this study were removed from the upper portions of the stalks.

Cultures. Pathogenic strains of *H. sacchari* were maintained on slants of cane leaf agar prepared by extracting leaf sections (1 g of tissue per 5 ml of H_2O) for 15 min at 78° in an isothermal autoclave, filtering the extract through eight layers of cheesecloth, and reducing the volume to ¼ of the original by evaporation, after which agar was added to a final concentration of 2 g/100 ml.

A toxin-producing isolate of *H. sacchari* was successively subcultured on a synthetic medium until it became attenuated. The attenuated culture was sustained on agar slants of the same medium. The medium, a modification of the M-1-D medium described by Filner (9), consisted of $Ca(NO_3)_2$, 1.2 mM; KNO_3, 0.79 mM; KCl, 0.87 mM; $MgSO_4$, 3.0 mM; NaH_2PO_4, 0.14 mM; sucrose, 87.6 mM; ammonium tartrate, 27.1 mM; $FeCl_3$, 7.4 μM; $MnSO_4$, 30 μM; $ZnSO_4$, 8.7 μM; H_3BO_3, 22 μM; and KI, 4.5 μM. The pH was adjusted to 5.5 with 0.1 M HCl. This solution also served as a liquid medium for fungal growth throughout the course of this study. Slants of both strains were stored at room temperature.

Helminthosporoside Production and Assay. Approximately 0.1 g of mycelium from an attenuated culture of *H. sacchari* was transferred to a 50 ml of Erlenmeyer flask containing 10 ml of the liquid medium with or without various test compounds or extracts. The cultures were grown in a Percival incubator at 28 ± 1° under 500 footcandles (5400 lx) of continuous cool white fluorescent light for 5 days unless otherwise indicated.

Helminthosporoside was obtained from fungus cultures by a modification of the procedures of Steiner and Strobel (7). Purification was carried through the 1-butanol extraction step. The 1-butanol phases were combined and the 1-butanol was removed by flash evaporation. The residue was taken up in H_2O for bioassays. The bioassays were conducted according to the methods described by Steiner and Strobel (7).

The quantity of helminthosporoside present in the cultures was estimated with the equation developed by Strobel and

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Steiner (10), which utilizes the length of "runner" symptom as a function of the log of the amount of toxin applied to the leaf. Each assay was repeated four times and the results were averaged. A control attenuated culture was tested in each experiment.

Chromatography. Descending paper chromatography was carried out on Whatman no. 1 filter paper and upon water-washed Whatman no. 541 filter paper in solvent a: 1-butanol: acetic acid:water, 4:1:5, vol/vol.

Eastman Chromagram sheets (6061) coated with silica gel were employed for thin-layer chromatography in the following solvent systems: b, 96% ethanol:water, 7:3, vol/vol; c, 1-propanol:water, 7:3, vol/vol; d, 1-butanol:acetic acid:water, 8:2:2, vol/vol; e, 1-propanol:34% NH_4OH, 7:4, vol/vol; f, 96% ethanol:34% NH_4OH, 7:3, vol/vol; g, 2-butanone:pyridine:water: acetic acid, 70:15:15:2, vol/vol.

Compounds were detected by spraying chromatograms with an alcoholic ninhydrin/3% acetic acid solution and heating at 100° for 10 min.

High-Voltage Paper Electrophoresis. High-voltage paper electrophoresis was performed on a Shandon model L 24 apparatus. Separations were achieved in pyridine:acetic acid: water (1.0:0.5:10, vol/vol), pH 5.02, on water-washed Whatman no. 3 filter paper, 22 × 56 cm, at 12 V/cm for 2.5 hr.

Instrumental Analysis. Melting points were determined on a Fischer Johns melting point apparatus. Infrared spectra were obtained on a Beckman Microspec spectrophotometer using KBr micropellets. Low-resolution mass spectrometry was conducted by Tom Krick, Department of Biochemistry, University of Minnesota, St. Paul, Minn. Mass spectra were obtained on an LKB model 9000 spectrometer with 20 eV on the filament and the probe heated to 80°.

Preparation of Leaf Wash Material. Soluble substances on the leaf were acquired by rinsing both leaf surfaces of sugarcane with deionized, distilled water applied with a squirt bottle. After the debris had been removed by filtration through Whatman no. 1 paper, the solution was taken by dryness by flash evaporation at 45°. The residue was labeled *leaf wash material.*

RESULTS

Acquisition of Attenuated Cultures of *H. sacchari.* A toxin-producing isolate of *H. sacchari* was successively subcultured on the synthetic medium. The fungus was grown for 5 days prior to each transfer, at which time a suspension of mycelium was used to inoculate both the succeeding agar slant and a 500 ml Roux flask containing the synthetic medium. The flasks, after 18 days of incubation, were tested for the presence of helminthosporoside. In two separate experiments, toxin production completely ceased after six successive transfers. Toxin-producing ability was lost abruptly and did not show a gradual decline during successive transfers.

Hyphal Tip and Single Spore Isolates. Classical methods were used to acquire single spore and hyphal tip isolates of attenuated *H. sacchari.* Bioassays revealed that none of the 37 cultures derived from single spore isolates and none of the 17 cultures arising from hyphal tip isolates from the attenuated cultures contained detectable quantities of helminthosporoside.

The Activation Phenomenon and Origin of Activator. When 1 mg of the leaf wash material from clone 51 NG 97 was incubated in 10 ml of the synthetic medium for 5 days with an attenuated culture, there was approximately 2.2 μmol of helminthosporoside produced per mg of dry weight fungal mycelium. This was comparable to the production of helminthosporoside by the original wild-type isolate.

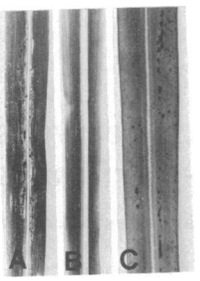

FIG. 1. Symptoms on susceptible sugarcane clone 51 NG 97. (A) After inoculation with a virulent strain of *H. sacchari,* the leaves were placed in an ISCO growth chamber for 24 hr in humid conditions followed by an additional 24 hr incubation period at decreased humidity. (B) After inoculation with an attenuated strain and incubation as with (A) above. (C) After inoculation with an attenuated strain and incubation for 48 hr under humid conditions, followed by an additional 24 hr.

Since the material containing the activator was recovered from the leaf surface, it could conceivably arise from some microfloral component of the surface. To this end, fungal and bacterial isolates from the leaf surface, and within, were cultured on Eckert's medium (11) and medium 523 of Kado *et al.* (12), respectively. Bacteria were incubated at 30° in the dark, with shaking (155 rpm) for 3 days, and fungi were grown in 500 ml Roux flasks at 23° for 18 days. The culture filtrates were tested for their ability to activate a culture of attenuated *H. sacchari.* Of the nine bacterial and 10 fungal cultural extracts tested, none activated the production of helminthosporoside in attenuated *H. sacchari.*

As another test for the origin of the activator, leaves of clone 51 NG 97 were treated with 70% ethanol as a surface sterilant. The leaves were then ground in water in a Sorvall Omnimixer. At a concentration of 1 mg/10 ml of medium, this aqueous extract activated toxin production in assay cultures.

In Vivo* Inoculation with Attenuated *H. sacchari. It seemed reasonable that an attenuated culture of *H. sacchari,* when challenged by a susceptible cane leaf, should be able to recognize the activator, resume toxin biosynthesis, and become pathogenic. Mycelial fragments (*ca* 0.2 g) of the toxin-producing and attenuated cultures were removed from culture plates with a spatula and each sample was mixed thoroughly with 50 ml of sterile water. Leaves of clone 51 NG 97 were inoculated with each culture with the use of an atomizer attached to a compressed air nozzle. The leaves were wrapped in plastic sacks, to maintain high humidity, and incubated in an ISCO growth chamber at 27° day, 16° night, 14 hr/10 hr light/dark cycle. The light intensity was 3,000 footcandles (32,000 lx). After 24 hr of incubation, the sacks were removed and the plants were incubated for an additional 24 hr. The toxin-producing isolate caused the production of typical eye-spot lesions, whereas the attenuated culture caused no symptoms whatever (Fig. 1). However, when the incubation period of the attenuated culture in plastic sacks was increased to 48 hr, followed by the additional 24 hr period, typical eye-spot lesions also developed (Fig. 1).

Source of Activator. Leaf wash material was obtained from the clone 51 NG 97 (susceptible) and H 50-7209 (resistant). The material in each preparation (1 mg/10 ml of culture fluid) was assayed for its ability to activate an attenuated culture of *H. sacchari.* The results showed that the susceptible clone possessed the activator, since 1.6 μmol of toxin per mg of dry weight fungal mycelium were produced. However, no toxin was detectable in the cultures containing the preparation from resistant clone H 50-7209.

Table 1. R_F and $R_{\text{amino acid}}$ values of activator (serinol)

Solvent system	R values	
	$R_{\text{amino acid}}$	R_F
a. 1-Butanol:acetic acid:H_2O, 4:1:5 (vol/vol)		
R_{Tyr}	0.52	
R_{Phe}	0.44	
b. 96% Ethanol:H_2O, 7:3 (vol/vol)		0.00
c. 1-Propanol:H_2O, 7:3 (vol/vol)		0.27
d. 1-Butanol:acetic acid:H_2O, 8:2:2 (vol/vol)		0.40
e. 1-Propanol:34% NH_4OH, 7:3 (vol/vol)		0.35
f. 96% Ethanol:34% NH_4OH, 7:3 (vol/vol)		0.05
g. 2-Butanone:pyridine:H_2O:acetic acid, 70:15:15:2 (vol/vol)		0.000

Solvent a was used for descending paper chromatography, and solvents b–g were employed for thin-layer chromatography. R_{Tyr} is migration relative to tyrosine.

Purification of an Activator. Approximately 1.2 g of solute was obtained from a leaf wash preparation of 8700 cm^2 of leaf surfaces. It was applied in a 5 ml solution to a 1.2 × 5.0 cm column of Dowex 50W-X8 water (200–400 mesh), H^+ form. The column was subsequently washed with 100 ml of water and then eluted with 500 ml of 4 M NH_4OH followed by 100 ml of water. The entire eluate was evaporated to dryness at 45° in a flash evaporator. The residue, which contained the activator, was dissolved in 3 ml of water and subjected to three successive paper chromatographic separations in solvent a for 24, 36, and 24 hr. Following each separation, the strip containing biological activity was eluted with water and the solution was evaporated to dryness. Biological activity was initially found by the trial and error method utilizing the described bioassay test. High-voltage paper electrophoresis was conducted on the residue from the final chromatographic separation in solvent a. Nearly homogeneous activator eluted from the electrophoretogram and was further purified by chromatography on water-washed Whatman no. 541 paper in solvent a for 10 hr. The compound moved as a single band in all of the solvent systems described (Table 1) and as a single band in the electrophoretic system. It reacted with ninhydrin to yield a pink-purple product that turned faintly yellow upon standing.

Identification of the Activator. The infrared spectrum of the activator possessed a broad peak at 2.7–3.0 μm and this is probably a composite of bands attributable to stretching deformations in -OH -NH_2 groups. The intense band found at 6.35 μm is characteristic of the scissoring deformation found in primary amines.

Low-resolution mass spectroscopy did not provide a molecular ion peak (Fig. 2), which is characteristic for aliphatic amines (13). The peak at m/e 75 is attributable to the molecular ion minus 16 (NH_2) and the strong peak at 60 to a $C_2H_6NO^+$ fragment. The base peak at 31 is assigned to $^+CH_2OH$, which is a characteristic fragment of primary alcohols (13).

Collectively, the spectral data suggested that the activator was serinol whose systematic name is 2-amino-1,3-propanediol. 2-Amino-1,3-propanediol was synthesized in a classical manner by refluxing dihydroxyacetone (0.2 g), NH_4Cl (0.4 g), and Raney nickel (0.2 g) in 100 ml of anhydrous methanol for 4 hr at 65°. The reaction mixture was filtered through Whatman no. 1 filter paper and the methanol was removed by flash evaporation. The product was purified in solvent a on water-washed Whatman no. 541 paper with a 3–5% yield. It produced a pink-purple product when sprayed with the ninhydrin solution which, upon standing, faded to yellow.

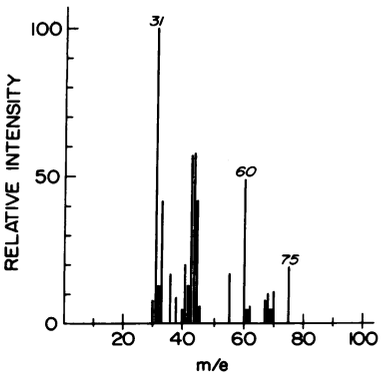

FIG. 2. The mass spectrum of natural serinol. Interpretation of the fragmentation pattern is found in the *text*. m/e is mass/charge.

Both authentic serinol and the natural product were white powders at room temperature. Singly and mixed, both showed initial decomposition at 91° and complete decomposition at 214° (uncorrected). The compounds yielded identical R_F values in solvents a, c, d, and e. Furthermore, as a mixture the compounds moved as a single band in these solvent systems. The same results were obtained when the compounds, singly and mixed, were subjected to high-voltage paper electrophoresis.

The synthetic compound yielded an infrared spectrum identical to the compound isolated from sugarcane, and, considering differences in instrumentation, these spectra compared favorably with the published absorption spectrum of serinol (14). Synthetic serinol yielded virtually the same mass spectrum as the natural product. The unknown activator was presumed to be serinol.

Biological Activity of Serinol. The natural product was assayed at 5-day intervals over a 21-day period at concentrations in the medium varying from 10^{-4} to 10^{-11} molar (Table 2). Serinol was optimally active in the micromolar range with maximum toxin production occurring at day 5. The crude activator at 1 mg/10 ml of medium caused the production of at least three times as much helminthosporoside at day 5 as did serinol at the optimum concentration (Table 2). Serinol, at the

Table 2. Toxin production in attenuated cultures of *H. sacchari* as a function of time and concentration of serinol (natural product)

Serinol concentration (M)	nmol of toxin/mg of dry wt fungus* at days after inoculation			
	5	10	15	21
10^{-11}	0	0	0	0
10^{-10}	0	0	0	0
10^{-9}	0	0	0	0
10^{-8}	0	0	0	0
10^{-7}	7	2.7	0	0
10^{-6}	640	5.2	0	0
10^{-5}	Trace	5.2	0	0
10^{-4}	0	0	0	0
Crude leaf wash, 1 mg/10 ml	2200	0	0	0
Control (no serinol)	0	0	0	0

* At 5 days the dry weight of the fungus averaged 22 mg per flask and at 10 days it was 60 mg.

optimum concentration, activated the production of helminthosporoside in an attenuated culture of *H. sacchari* in a medium containing 1 mg/10 ml of leaf wash material from the resistant clone H 50-7209. Furthermore, serinol (1 μm) caused the production of helminthosporoside in 53% of the single spore isolates and 78% of the hyphal tip isolates of an attenuated culture of *H. sacchari*.

Synthetic serinol also was an activator of helminthosporoside production. At a concentration of 1 μM, it caused the production of 2.9 nmol of helminthosporoside per mg of dry weight of the fungus in 5 days. A dilution experiment similar to the one in Table 2 was also run with synthetic serinol, and the optimum concentration for the activation of toxin production was in the range of 10^{-7} M.

Other Amines as Activators. Putrescine, cadaverine, ethanolamine, or octopine at 1 μM did not activate toxin synthesis in attenuated cultures of *H. sacchari* over either a 5- or 10-day incubation period.

DISCUSSION

Using toxin production in attenuated cultures as a biological assay, at least one activator of helminthosporoside production was isolated. On the basis of comparable spectral, chromatographic, and biological data obtained from its synthetic counterpart, this activator was identified as serinol (2-amino-1,3-propanediol). However, since the crude leaf wash material had greater biological activity than serinol (Table 2), the presence of another activator(s) was suspected. This was substantiated by Pinkerton (15), who showed that a compound, possessing activator properties, in the crude leaf wash material was separated from serinol on a Bio-Gel P-2 column.

Serinol has numerous citations in the chemical literature (16–18). The only reference we found in the biological literature is that of Siddiqueullah *et al.* (19). They describe the biosynthesis of the *p*-nitrophenylserinol moiety of chloramphenicol by a species of *Streptomyces*. To our knowledge, free serinol has not been isolated from biological material (20). Hence, this report appears to be the first for the presence of this biologically active amine.

The common explanation for the occurrence of attenuated cultures in heterokaryotic pathogenic fungi upon culturing on common laboratory media is that nonpathogenic types or mutants outgrow the pathogenic types. We reasoned that if such a selection process were operating, then a few toxin-producing nuclei should be present in the attenuated fungal population, and that one or more cultures derived from the attenuated culture should be toxin producers. However, this did not appear to be the case, since toxin was absent in cultures obtained from single spore or hyphal tip isolates of attenuated cultures. However, 53% of the single spore cultures and 78% of the hyphal tip cultures were activated by micromolar concentrations of serinol. It would seem that if the transfer process had totally selected for nontoxin-producing strains of the fungus, then serinol would have had no effect whatever as an activator.

From this it was evident that two types of cultures were present: (*i*) those upon which serinol had no effect, and (*ii*) those which were activated. The first type of culture may be truly avirulent, lacking the gene for toxin production as postulated. The second type of culture suggests that the multiple transfers of spores and mycelia on the synthetic medium did not select for totally avirulent forms. Rather, it produced forms that did not express toxin production but retained the biosynthetic machinery necessary for toxin production, i.e., the gene for virulence. In these cases, avirulence may be "turned off" virulence rather than an expression of a separate gene for avirulence. The logical conclusion is that serinol somehow plays a role in "turning on" the expression of virulence which is, of course, toxin biosynthesis.

Serinol presumably has its origins in the plant cell rather than in microorganisms located on or within the leaf. For instance, none of the culture fluids from the organisms isolated from leaves contained activating compounds. Further, the aqueous extract of alcohol-treated leaves was capable of activation. Pinkerton (15) has also described a technique for isolating serinol from whole plant parts. Most interesting, however, is the distribution of activators in sugarcane clones *per se*. Clone H 50-7209 (resistant) possessed no activator whatever, but the crude water leaf wash of this clone allowed the fungus to produce the toxin in the presence of 1 μM serinol. Susceptible clone 51 NG 97 contained both serinol and another activator(s). Further studies on the distribution of activators relative to the disease rating of the respective clone should prove interesting, since the mechanism of toxin action in the plant and the activation of toxin production in the fungus are separate biological mechanisms.

The attenuation phenomenon in *H. sacchari* may have some survival value to this organism. In the absence of a suitable host, toxin production would cease. The fungus would revert to a saprophytic mode, existing, perhaps on plant debris or other suitable substrates, until viable host tissue was encountered. Energy otherwise used in toxin production would be conserved. Serinol, encountered on a susceptible host, would activate toxin synthesis and the fungus would assume a pathogenic mode. Advantage would be afforded to a fungal population containing variants capable of this type of existence. The ability to incite disease would be retained and, most importantly, the ability to persist may be markedly increased. Some credence to the notion that the attenuated form can revert back to its pathogenic mode is provided by the inoculation experiments, whereby it took 48 hr at high humidity, rather than 24 hr, for the attenuated form to cause symptom production on a susceptible leaf (Fig. 1). Presumably the additional 24 hr at high humidity was required for activation of helminthosporoside production.

The activation of toxin biosynthesis by serinol may occur by one of several mechanisms: (*i*) serinol may be a direct precursor to helminthosporoside; (*ii*) serinol may serve as inducer (either as a classical or as a gratuitous inducer); and (*iii*) serinol may act as an effector for an enzyme involved in toxin biosynthesis. From the data presented in this report, it does not seem likely that serinol serves as a precursor to the toxin since: (*a*) Toxin synthesis occurs in the fungus, and removal of the fungus from cane agar would result in cessation of the toxin biosynthesis as soon as the available pool of precursor in the fungus was depleted. Helminthosporoside is produced in large quantities in culture (7), hence, the pool should be rapidly used. Yet the toxin is actively synthesized after five or six successive culture transfers. The dilution of an initial pool by five transfers and continual depletion of this diluted pool by toxin biosynthesis seems to preclude serinol as a precursor. (*b*) Single spore and hyphal tip isolates of an attenuated culture exhibited anomalous behavior. Of those cultures containing activator, 47% arising from single spore isolates and 22% arising from hyphal tip isolates were not activated. If activation depended only upon the presence of a precursor, a far larger percentage of the cultures would have produced toxin. Mutants in the biosynthetic pathway of the toxin cannot account for the difference between the observed number of activated cultures and the expected value of 100%. (*c*) Data in Table 2 conflict with the proposed role of a precursor. Serinol has a concentration of optimal activity of

1 μM. Above and below this concentration, activity falls to zero. If the activator were a precursor, biological activity should increase with increasing concentration to a saturation point and then level off. Contrary to expectation, there was no activity at 10^{-4} M. Further, the molar ratio of serinol present to helminthosporoside produced was about 1:1400 at day 5. In view of the objections presented above, the role of activator as a precursor must be viewed with serious skepticism. Insufficient data are currently available for us to intelligently comment on the plausibility of the remaining hypotheses.

Another fact worthy of comment in Table 2 is the reduction of toxin activity to below detectable limits after 10 days. A key feature in the structure of the toxin is an α-galactosidic bond. Therefore, it is likely that reduction in the amount of toxin (Table 2) is caused by α-galactosidase activity in the fungus. Such enzymatic activity, present in cultures of both pathogenic and attenuated strains, appears 6 days after inoculation and increases markedly over a 7-day period (Carrie Ireland, personal communication). Helminthosporoside synthesis probably continues at a normal rate in activated cultures but elevated α-galactosidase activity destroys the toxin as soon as it appears in the culture medium. Perhaps this can also explain the slight differences in the optimal concentrations for activation of toxin production between synthetic and naturally occurring serinol.

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1. Buxton, E. W. (1960) in *Plant Pathology*, eds. Horsfall, J. G. & Diamond, A. E. (Academic Press, New York), pp. 359–401.
2. Dickson, J. G. (1956) *Diseases of Field Crops* (McGraw-Hill Co., New York), p. 517.
3. Strobel, G. A. (1974) *Annu. Rev. Plant Physiol.* **25,** 541–566.
4. Karr, A., Karr, D. B. & Strobel, G. A. (1974) *Plant Physiol.* **53,** 250–257.
5. Scheffer, R. P., Nelson, R. R. & Ullstrup, A. J. (1967) *Phytopathology* **57,** 1288–1292.
6. Martin, J. P. (1961) in *Sugarcane Diseases of the World*, eds. Martin, J. P., Abbott, E. V. & Hughes, C. G. (Elsevier Publ. Co., New York), Vol. I, pp. 167–187.
7. Steiner, G. W. & Strobel, G. A. (1971) *J. Biol. Chem.* **246,** 4350–4357.
8. Strobel, G. A. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1693–1696.
9 Filner, P. (1965) *Exp. Cell Res.* **39,** 33–39.
10. Strobel, G. A. & Steiner, G. W. (1972) *Physiol. Plant Pathol.* **2,** 129–132.
11. Tolmsoff, W. J. (1965) Ph.D. Dissertation, University of California, Davis.
12. Kado, C. I., Heskett, M. G. & Langley, R. A. (1972) *Physiol. Plant Pathol.* **2,** 47–57.
13. Silverstein, R. M. & Bassler, G. C. (1964) *Spectrometric Identification of Organic Compounds* (John Wiley & Sons, Inc., New York), p. 177.
14. Sadtler Res. Lab., Philadelphia, Pa. (1970) *Sadtler Standard Spectra*.
15. Pinkerton, F. D. (1976) Ph. D. Dissertation, Montana State University.
16. Szammer, J. (1969) *Acta Chim. Acad. Sci. Hung.* **61,** 417–421.
17. Schlenk, H. & De Hass, B. W. (1951) *J. Am. Chem. Soc.* **73,** 3921–3922.
18. McKinely-McKee, J. S. (1963) *Biochem. J.* **87,** 43–44.
19. Siddiqueullah, M., McGrath, R., Vining, L. C., Sala, F., & Westlake, D. W. S. (1967) *Can. J. Biochem.* **45,** 1881–1889.
20. Smith, T. A. (1970) *Biol. Rev.* **46,** 201–241.