Rates of S^0 oxidation in nature by *Sulfolobus* were estimated so that a comparison could be made with previous measurements of sulfuric acid production in Yellowstone solfatara areas and in two lakes in a Japanese thermal explosion crater (*10*). Oxidation rates at mean environmental temperatures were estimated by interpolation of 24-hour and 48-hour temperature optimum plots, such as that shown for Moose Pool at 48 hours in Fig. 1. The rates of oxidation of S^0 for the *Sulfolobus* sites were somewhat greater than those calculated for the Japanese thermal lakes and considerably greater than those estimated for the solfataras (Table 1). The amounts oxidized per day represent 1/15 and 1/23 of the total amounts of S^0 assayed in Moose Pool and Sulfur Caldron, respectively. In the previous work, acid production was estimated by measuring sulfate concentrations in runoff waters. The solfataras contain sources of thermal water, but most of their areas consist of soil habitat. A possible explanation for the low rates of oxidation in the solfatara areas may therefore be that *Sulfolobus*, and other S^0-oxidizing bacteria, are more active in aquatic habitats. In addition, it is likely that the estimation of sulfuric acid production based on surface water runoff may give erroneously low results because of loss of acid-rich water by subsurface flow (*10*). Direct measurement of $^{35}S^0$ oxidation probably provides a more reliable means of measuring sulfuric acid production.

No attempt was made to control the amounts of O_2 and CO_2 available to the bacteria during the oxidation assays. Incubation conditions may have deviated in this respect from those in nature, but in view of the linear rates of S^0 oxidation obtained and the relatively short incubation periods employed, our results probably provide a good estimate of oxidation rates in nature. Other pools containing similar numbers of *Sulfolobus* exhibited S^0 oxidation rates comparable to those reported here for Moose Pool and Sulfur Caldron, but sites containing fewer bacteria oxidized S^0 more slowly. The extent to which the observed optimum temperature coincided with the environmental temperature also influenced the rate of S^0 oxidation at a particular site. Temperature optima for S^0 oxidation of 60°, 70°, and 80°C were obtained at other sites, and these optima were frequently higher than the corresponding environmental temperatures. The frequent lack of correspondence between optimum and environmental temperatures suggests that there are different temperature strains of *Sulfolobus* that are incapable of adapting to a wide variety of environmental temperatures.

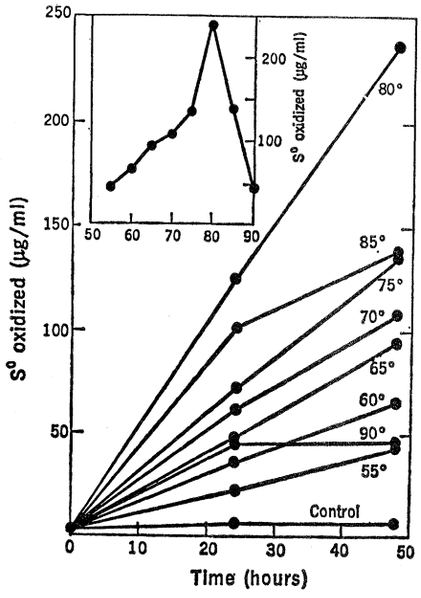

Fig. 1. Oxidation of S^0 by *Sulfolobus* from Moose Pool at different incubation temperatures. Inset shows the amount of S^0 oxidized in 48 hours plotted against incubation temperature.

The data presented here show that *Sulfolobus* is capable of oxidizing large amounts of S^0 to H_2SO_4 at temperatures up to 85°C. Because of the technical problems involved in distributing $^{35}S^0$ throughout soil samples, our study was confined to aquatic samples, but the presence of *Sulfolobus* in soils as hot as 85°C and its ability to fix $^{14}CO_2$ there at this temperature strongly suggest that S^0 oxidation occurs also in soils. *Sulfolobus* is therefore an important geochemical agent in solfatara areas, and its S^0-oxidizing activity at high temperatures obviates the need to postulate movement of sulfuric acid from lower-temperature sites containing *Thiobacillus thiooxidans* to higher temperature sites. Similar radioisotopic approaches could probably be applied to the study of bacterial action in other geochemical processes.

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5. "Moose Pool" is our unofficial designation for an apparently unnamed sulfur-containing pool located about 230 m southwest of Black Dragon's Caldron.
6. D. W. Shivvers and T. D. Brock, *J. Bacteriol.*, in press.
7. Ten-milliliter portions were added to 50-ml bottles containing 2 to 8 mg of $^{35}S^0$ (1 μc/mg). Three bottles were incubated at each of the following temperatures: 55°, 60°, 65°, 70°, 75°, 80°, 85°, and 90°C. Control bottles were incubated at 75°C after the addition of 4 percent (weight to volume) formaldehyde.
8. The ^{35}S-labeled material in the filtrates was insoluble in CS_2 and was precipitable with excess $BaCl_2$, indicating that unoxidized S^0 did not pass through the filters and that oxidized sulfur compounds of oxidation state less than that of SO_4^{2-} were not formed in detectable amounts.
9. Elemental sulfur was measured spectrophotometrically by the method of F. Pachmayr [dissertation, Ludwig-Maximilians University, Munich, Germany (1960), pp. 21–27]; the S^0 was extracted from samples into trichloroethylene and quantitated by determining absorbance at 276 nm, with solutions of known concentration for standardization. Cell counts were made microscopically by using a Petroff-Hausser counting chamber. Temperatures were determined with a telethermometer (model 42SC, Yellow Springs Instrument Co.) equipped with a banjo or Teflon probe, and with maximum-reading mercury thermometers. The surface dimensions of Moose Pool and Sulfur Caldron were measured with a steel tape, and their depths were estimated with a weighted, calibrated rope.
10. R. Schoen, *Geol. Soc. Amer. Bull.* **80**, 643 (1969).

11. Chemical assays were done by K. Boylen. Supported by NSF grant GB-35046 and by the Wisconsin Alumni Research Foundation.

29 November 1972

Toxin from Fusarium moniliforme: Effects on Plants and Animals

Abstract. *A mycotoxin-producing strain of* Fusarium moniliforme *was isolated from southern leaf blight–damaged corn seed. A water-soluble toxin, subsequently purified from the fungus, had an oral median lethal dose of 4.0 milligrams per kilogram in 1-day-old cockerels. The toxin also produced plant growth-regulating and phytotoxic effects on plant systems. Physical and chemical data presented for the toxin suggest a structurally new toxin. The trivial name "moniliformin" has been assigned to the toxin.*

The southern corn leaf blight epidemic in the United States in 1970 seriously reduced yields in the Southeast and Midwest. Additional concern at that time was the possible danger to animals fed blight-damaged corn from toxins produced by either the causal agent, *Helminthosporium maydis* Nisiki

and Miyake (*Cochliobolus heterostrophis* Drechs.), or by other fungi that invaded corn stalks and grain in the field (*1*).

We now report the isolation of a toxigenic isolate of *Fusarium moniliforme* Sheldon, which was found during routine screening (Doupnik and Peckham) for toxigenic isolates of *Helminthosporium maydis* and *Fusarium moniliforme* from naturally infected southern leaf blight–damaged corn seed (*2*).

The toxic principle was extracted with methanol from 2- to 4-week-old fungal cultures grown on cracked corn at room temperature (*2*). It was purified by successive Sephadex LH-20 column chromatography, with the use of methanol as the mobile phase, and by maintaining flow rates at 4 ml/hr. Purification of the toxin (or toxins) was monitored with a cockerel bioassay. Column fractions for assay were dried, redissolved in 5 ml of sterile distilled water, and chicks were dosed orally with 1 ml by crop intubation. A water-soluble compound, which crystallized from methanol solution and produced a single spot when analyzed by thin-layer chromatography (chloroform, acetic acid, methanol; 3 : 3.5 : 0.5, by volume), was found to be the main toxic metabolite of the fungus. The toxin was visualized as a yellow spot at R_F 0.18 with ninhydrin spray after heating at 100°C for 15 to 20 minutes. The trivial name "moniliformin" has been assigned to this toxic metabolite.

At temperatures up to 350°C the compound decomposed without melting. The ultraviolet spectrum of the toxin showed maxima (in methanol) at 229 nm and 260 nm. Major infrared absorptions were 3570, 3370, 3300, and 3130 cm^{-1}, suggesting different hydroxyl groups; and also at 1775, 1705, 1675, 1600, 1100, and 840 cm^{-1}. No shift in the ultraviolet spectrum was observed under acidic or basic conditions, suggesting a lack of phenolic hydroxyl groups. Elemental analysis indicated an empirical formula of $C_6H_{3-6}O_{6-8}$. Elemental analysis also showed that the metabolite contained no nitrogen, phosphorus, sulfur, or chlorine. Low-resolution mass spectral analyses with the use of electron-impact ionization or chemical ionization were unsuccessful because of instability and low volatility of the molecule, suggesting a salt form. Further evidence for a salt form was obtained when analysis of toxin via infrared spectroscopy showed the presence of strong 1600 cm^{-1} absorption (carboxylate function).

The nuclear magnetic resonance (NMR) spectrum of the toxin in dimethylsulfoxide-d_6 solution showed a sharp singlet (nonexchangeable with D_2O) at δ 8.23. No other signals due to toxin were observed in the NMR spectrum and, therefore, integration of the signal at δ 8.23 was not possible. However, a 2,4-dinitrophenylhydrazone derivative of the toxin was prepared, and the sharp signal at δ 8.23 exhibited an upfield shift to δ 6.21 (singlet) and integrated as a single proton when compared to the three aromatic protons on the molecule.

The oral median lethal dose (LD_{50})

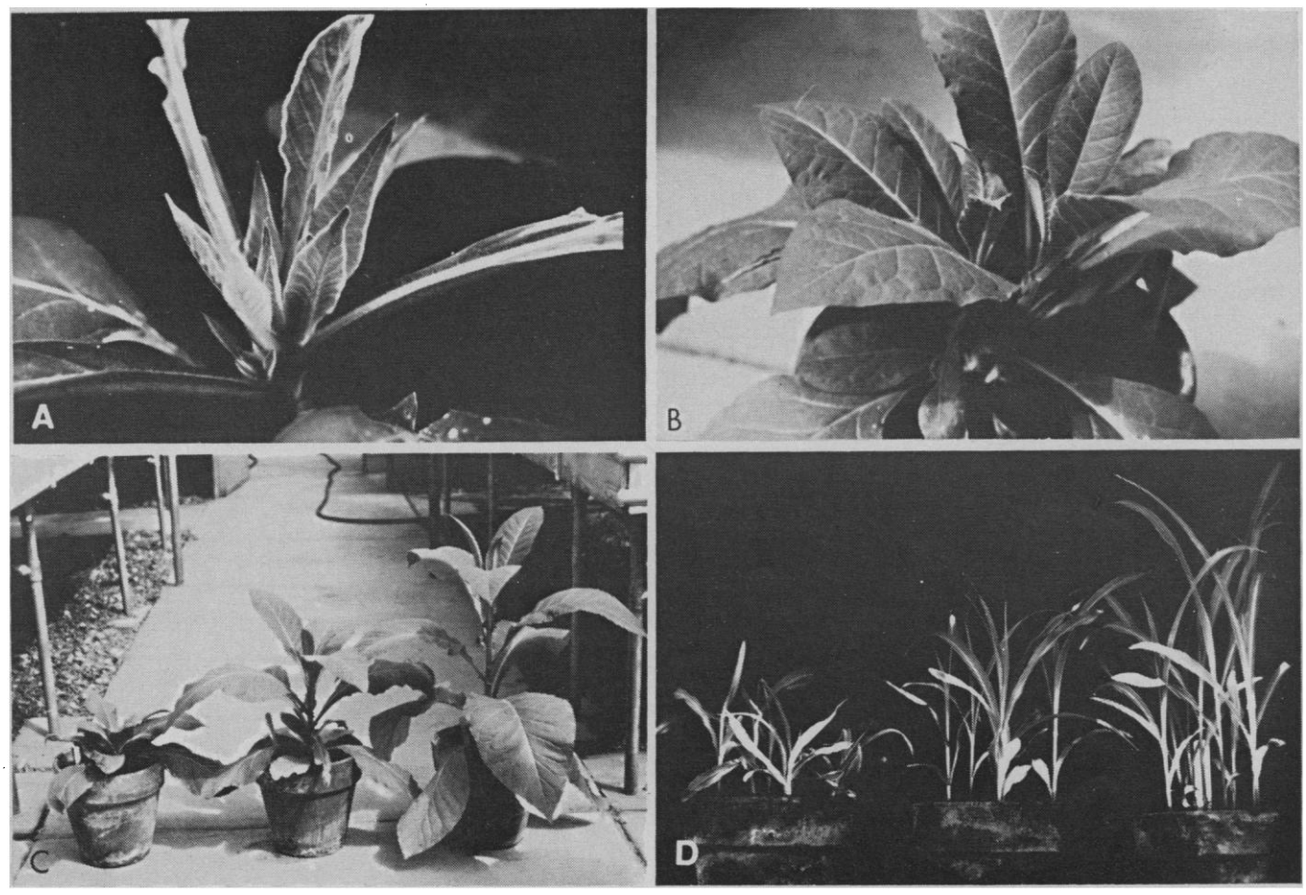

Fig. 1. The effects of *Fusarium moniliforme* toxin (moniliformin) on tobacco plants. (A) Tobacco plant 12 days after treatment with 2000 μg of moniliformin, showing an early stage of the rosetting effect; (B) the same plant 21 days after treatment, showing a later stage of the rosetting effect; (C) long-term effects of moniliformin on tobacco plants 21 days after a single application; left, 2000 μg per plant; middle, 200 μg per plant; right, control; (D) corn plants 14 days after treatment; left, 200 μg per plant; middle, 20 μg per plant; right, control.

of the purified toxin in sterile water solution was 4.0 mg/kg or 160 μg per cockerel. An oral dose lethal to all the animals (LD_{100}) was observed at a dose of 6.25 mg/kg (250 μg per cockerel). Some cockerels receiving a dose of 3.12 mg/kg (125 μg per cockerel) were dead within 24 hours; those surviving for 24 hours recovered with no apparent adverse effects. Increasing the dose beyond 3.12 mg/kg resulted in death of cockerels in a correspondingly shorter time until dosages of 40 mg/kg (1.6 mg per cockerel) resulted in death within 45 minutes.

Gross and histologic lesions in cockerels, dosed at 500 μg and 250 μg, that lived more than 2 hours after dosing, were ascites with edema of the mesenteries and small hemorrhages in the proventriculus, gizzard, small and large intestine, and skin. The cockerels that died within 2 hours (given 1000 and 500 μg per cockerel) had no lesions in organs and tissues of the cardiovascular, digestive, endocrine, hematopoietic, integumentary, lymphoid, musculoskeletal, nervous, respiratory, or urogenital systems. Cockerels dosed with 125 and 62.5 μg of toxin and cockerels that were undosed also showed no lesions.

Studies with plants demonstrated growth-regulating and phytotoxic effects of the toxin. Studies were conducted with 4-mm sections cut from 5-day-old, etiolated wheat coleoptiles (*Triticum aestivum* L. 'Wakeland'). Toxin concentrations of 20 and 200 ppm were assayed in buffered solutions (at *p*H 5.6) containing 2 percent sucrose. Ten sections were placed in test tubes containing 2-ml test solutions and incubated in a roller-tube apparatus for 24 hours at 21°C (*3*). Results demonstrated that wheat coleoptiles were inhibited 24 and 57 percent ($P < .01$) at 20 and 200 ppm of toxin, respectively, relative to controls.

Further tests with aqueous solutions of the toxin containing 0.1 percent Tween 20 were conducted on intact tobacco and corn plants. Solutions were sprayed onto 6-week-old tobacco seedlings (*Nicotiana tabacum* L. 'Hick's') or introduced into the whorls of 1-week-old corn seedlings (*Zea mays* L. 'Norfolk Market White'). Single applications of 1 ml of test solutions containing 20, 200, and 2000 μg of toxin were sprayed onto tobacco seedlings; 0.1 ml of each test solution was placed into corn whorls at 2, 20, and 200 μg of toxin per plant.

Necrosis and interveinal chlorosis were observed at 200 μg per plant on both corn and tobacco plants 1 week after treatment. In addition, distortion of leaf shape and thickening of the midrib caused by hypertrophy or hyperplasia (or both) were quite prominent on tobacco leaves 1 week after treatment with toxin at 2000 μg per plant. A later effect, becoming evident 12 days after treatment, was "rosetting" (Fig. 1A) that presumably was caused by destruction of apical dominance. This effect remained prominent 21 days after treatment (Fig. 1B). Corn plants appeared to be more sensitive to the toxin, since necrosis and chlorosis were more severe than on tobacco plants treated with an equivalent amount of toxin.

The various effects of the toxin on tobacco leaves appeared to be related to the developmental stage of the leaf at the time of treatment. Effects on older leaves were limited to necrotic lesions caused by localized concentration of the toxin resulting from evaporation of spray droplets. Toxic effects on immature leaves were expressed as interveinal chlorosis and leaf-shape distortion. The rosetting or chemical pruning effect probably resulted from the action of the toxin on embryonic leaf tissue, since this effect was not apparent until about 12 days after treatment.

The rosetting or chemical pruning effect observed on tobacco was not apparent on corn, presumably because of the difference in growth patterns (lack of axillary buds in corn). Effects on corn were limited to necrosis, chlorosis, and stunting.

The long-term effect on apical growth of a single application of the toxin on tobacco and corn plants can be seen in Fig. 1, C and D. Tobacco plants treated with 2000 μg per plant showed a marked internodal shortening or chemical pruning effect 21 days after treatment. Tobacco plants treated with 200 μg per plant showed a less severe reduction in height relative to control plants (Fig. 1C). This same trend of height relative to dosage was evident on corn plants (Fig. 1D).

The effect on apical dominance and internodal shortening was evident on tobacco plants for approximately 30 days after treatment. Subsequently these plants gradually overcame the effects of the toxin, and 6 weeks after treatment there was little difference between treated and control plants.

Fusarium spp. are known to produce mycotoxins (that is, F-2 and T-2 toxins) (*4*); however, comparisons of the physical and chemical data from the *F. moniliforme* toxin with data reported for known *Fusarium* toxins suggest a structurally new toxin that produces profound effects in both plants and animals.

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15 January 1973

Electrogenic Sodium Pump in Squid Giant Axon

Abstract. *Squid giant axon possesses a hyperpolarizing electrogenic sodium pump which is stimulated by internal sodium and by external potassium. This conclusion is based on the following observations: strophanthidin depolarizes the membrane and enhances the depolarizing effect of 5 or 10 millimolar external potassium; the magnitude of these effects is directly related to the internal sodium concentration; both effects are abolished by cyanide.*

The giant axon of the squid is one of a few archetypal preparations from which our knowledge of active ion transport has been derived. Ironically, it is one of the last ones to join the growing list of demonstrably electrogenic sodium pumps in animal cells (*1*).