Helminthosporium: Secondary Metabolites, Southern Leaf Blight of Corn, and Biology

Clifford W. Hesseltine,* John J. Ellis, and Odette L. Shotwell

The recent outbreak of the southern corn blight is caused by race T of *Helminthosporium maydis*, a species in the fungus genus *Helminthosporium* which consists of about 175 species, worldwide in distribution, on grasses. Three species of *Helminthosporium*—*H. maydis*, *H. turcicum*, and *H. carbonum*—attack corn and are placed in the genus *Bipolaris*. The southern corn blight fungus has an asexual stage and a sexual stage, *Cochliobolus heterostrophus* or *Ophiobolus heterostrophus*. Names of the sexual and asexual stages and synonyms of the species of *Helminthosporium* were sought in chemical literature to discover the types of compounds produced. Our survey brings together the known secondary metabolites including phytotoxins and pathotoxins which might be suspected mycotoxins. The pathotoxin of *H. maydis*, a polypeptide of unknown structure, induces all of the symptoms of the disease in susceptible corn plants that the pathogen will cause. *Helminthosporium* has been reported once as a human pathogen and is often an allergen.

Great interest has developed concerning the problem of southern leaf blight that caused serious losses to the 1970 corn crop in the United States. Our concern is with the effect of this disease, caused by the field fungus *Helminthosporium maydis* Nisik. and Miyake, on the industrial utilization of corn and its use in foods and feeds. This brief account contains information about the causal agent, its phytotoxins, its life cycle, and the metabolic products formed by it and other species of *Helminthosporium* on cereals. Neither the agronomic aspects nor the extensive literature on the genetics of the fungus is reviewed here.

Helminthosporium is a fungus genus consisting of 175 species that are worldwide in their distribution. Most species parasitize gramineous hosts, including barley, corn, rice, oats, wheat, and sorghum. Typically, its species attack leaves and stems of many grasses. The types of lesions on grasses depend upon the fungus species. Sometimes the lesions are well-defined longitudinal spots with discoloration. Other times they appear as stripes or bands which extend the length of the leaf (Drechsler, 1923). When *Helminthosporium turcicum* infects corn, the affected tissue becomes chlorotic and individual lesions increase in size until they may be several inches long and nearly an inch wide.

The microorganism that produces the southern corn blight disease is one of three species of *Helminthosporium* which attack corn. The other two are *H. turcicum* Pass. and *H. carbonum* Ullstrup. The genus *Helminthosporium* is the generic name given to a great many fungi for the asexual stage of certain *Ascomycetes*. All the species of *Helminthosporium* are characterized by multicelled conidia, with the cells of the conidium arranged in a linear series and never in an irregular fashion as in *Alternaria*, a related form genus. Conidia, besides being multicelled, are pigmented, often in blackish or brown shades. Unlike some parasitic fungi, species of *Helminthosporium* grow readily free of their host and sporulate normally. For example, *H. maydis* will grow luxuriantly on Czapek Dox medium without the addition of any vitamins or growth factors, and uses sucrose as a carbohydrate source. This pattern is in contrast to the parasitic plant rust fungi, which can grow only on the living host tissue. Under proper temperature and moisture conditions, *H. maydis* would be expected to grow on decaying corn parts and plant debris long after the host plant is dead. *Helminthosporium* in pure culture sporulates readily, just as it does on host tissues. Spores on agar germinate in 30 min. In pure culture, *Helminthosporium* colonies are dark to black in rich media, with a cottony gray aerial mycelium. Sporulation is generally better on weak nutrient agars than on media rich in carbohydrates.

When conidia are blown onto the corn plant, they germinate in dew and immediately penetrate the leaf surface. The manner in which the three species of *Helminthosporium* attack corn was studied by artificial inoculation of susceptible and resistant hybrid corns (Jennings and Ullstrup, 1957). *H. maydis* conidia germinated and penetrated the host epidermal cells of both susceptible and resistant hybrids of both young and mature plants. Maximum penetration occurred in 18 hr, with the germ tubes entering the leaf through both the epidermal cells and the stomata. After entering the chlorenchyma tissue of the susceptible plants, the hyphae caused chloroplast destruction and cell wall collapse. The hyphae continued growing through the chlorenchyma tissue, but the vascular tissue was not invaded as it is by hyphae of *H. turcicum*. In *H. turcicum* the mycelium grew into the xylem elements and rapidly grew inside the xylem so extensively that eventually it plugged the water-transporting vessels until there was no water movement, resulting in localized wilting. With resistant corn hybrids, the hyphae of

Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill. 61604

H. maydis, after reaching the chlorenchyma, spread only a short distance and stopped. Evidently the resistant factor(s) in maize to *H. maydis* is present in the chlorenchyma and the resistant factor to *H. turcicum* is present in the xylem elements. Hilu and Hooker (1965) confirmed this work for the northern corn blight pathology.

The imperfect fruiting structures consist of sporophores emerging from the host tissue singly or in clusters. The septate sporophores, when they reach a certain length, produce a single spore (never in chains). After the initial spore has reached maturity, the sporophore grows a short distance beyond the attachment of the first spore and produces another. In this manner a number of spores are produced successively. The spores either fall off the sporophore at maturity or are blown off by the wind.

The release of conidia of *H. turcicum* from corn leaves was studied by Meredith (1965), who demonstrated that conidia are released from corn leaves when they are transferred from moist air to a drier atmosphere, such as occurs during the morning hours. Furthermore, his results indicate that rapidly reducing the vapor pressure induced violent movement of the conidiophores and these movements supplied the energy for conidial release. Increase in light intensity, if air moisture is constant, has no effect on spore release. Although some spores are dislodged by strong air currents, this movement is not the actual cause of conidial release. The asexual spores, or conidia, vary in length and width, but all have a number of cross walls, each at right angles to the elongate axis of the heavy-walled dark spores. In *H. turcicum*, only the sporophores emerge through the stomata of the corn leaves, with all the vegetative hyphae in the host tissue.

The multicelled conidia germinate in two ways. In one group of *Helminthosporium* species, germ tubes emerge from either end of the conidium; in the second group, germ tubes emerge from any cell of the conidium.

In addition to the asexual conidial stage, species in the genus *Helminthosporium* possess a sexual stage, which is always perithecial in nature, bearing asci containing ascospores. Even though the conidial stage of many species of *Helminthosporium* is similar, the sexual stages are quite distinct from species to species and therefore fall in more than two different genera. The perithecial stages will be described below under the appropriate species of *Helminthosporium* attacking corn.

The taxonomy and nomenclature of the genus *Helminthosporium* have been discussed at length by Shoemaker (1959), Sprague (1950), and Hughes (1958). All the species of *Helminthosporium* upon grasses have been segregated into two new genera. Every major cereal crop has one or more species of *Helminthosporium* attacking it. The first genus segregated from *Helminthosporium* is *Bipolaris*, which contains all the species of *Helminthosporium* whose conidia always put out germ tubes only from the ends of the spores. The second genus, *Drechslera*, contains all the species of *Helminthosporium* whose spores germinate from any cell of the conidium. Species left in the genus *Helminthosporium* are forms that grow on woody material rather than on grasses. Also, Shoemaker has pointed out that the correct name is *Helmisporium*, not *Helminthosporium;* but the reasons for this correction cannot be gone into here. The three species of *Helminthosporium* attacking corn are now classified by Shoemaker in the genus *Bipolaris*.

We have summarized the differences in the species of *Helminthosporium* in the key below.

Key to Separation of Species of *Helminthosporium* from Corn (Modified from Luttrell, 1951)

Conidia germinating by germ tubes from ends of conidia only.
- Conidium with protruding conical hilum; conidia with a maximum diameter over 20 μ and a maximum length over 100 μ; causing leaf blight of corn, sorghum, Johnsongrass, and Sudan grass. Sexual stage *Trichometasphaeria.—H. turcicum.*
- Conidium without protruding conical hilum:
  - Conidia dark olivaceous, wall thick; conidia usually straight; maximum diameter of conidia less than 20 μ (25–103 $\times$ 7–18 μ), causing leaf spot of corn. Sexual stage *Cochliobolus.—H. carbonum.*
  - Conidia fuligenous to pale olivaceous, wall relatively thin; conidia curved (25–127 $\times$ 7–21 μ); causing leaf blight of corn. Sexual stage *Cochliobolus.—H. maydis.*

Nomenclature and synonymy of the species of *Helminthosporium* on corn.

Helminthosporium turcicum Passerini
H. inconspicuum Cooke and Ellis
Bipolaris turcicum (Pass.) Shoemaker
Sexual stage:
Trichometasphaeria turcica Luttrell
Common names of disease—"northern corn blight," "leaf blight"
Host species:
Zea mays (maize, corn)—*Sorghum vulgare* (sorghum), *S. halepensis* (Johnsongrass), *S. sudanensis* (Sudan grass), and *Euchlaena mexicana* (teosinte)

Helminthosporium maydis Nisik. and Miyake
Bipolaris maydis (Nisik. and Miyake) Shoemaker
Sexual stage:
Ophiobolus heterostrophus Drechsler
Cochliobolus heterostrophus Drechsler
Common name of disease—"southern corn blight"
Host species:
Zea mays (maize, corn) and *Euchlaena mexicana* (teosinte)

Helminthosporium carbonum Ullstrup
Helminthosporium zeicola Stout
Bipolaris zeicola (Stout) Shoemaker
Charred ear mold and leaf spot
Sexual stage:
Cochliobolus carbonum Nelson
Host species:
Zea mays (maize, corn)

DISTINCTION BETWEEN THE SPECIES ON CORN

H. maydis and *H. carbonum* are very difficult to distinguish since their morphological characteristics are so similar, their measurements overlap to a great extent, and they both have a *Cochliobolus* perithecial stage. *H. carbonum* (*C. carbonum*) has a slightly smaller conidial size, being 25–100 $\times$ 7–18 μ.

In contrast, *H. maydis* can be distinguished from *H. turcicum* by the following characteristics. The conidia of *H. maydis* are strongly curved, moderately tapered toward the ends, and 30–115 $\times$ 10–17 μ in size; they have up to 12 cross walls; and they have a broad, flat scar at the attachment of the conidiophore. The perithecial stage of *H. maydis* is *C. heterostrophus*. The ascocarp is dark and relatively smooth. The asci contain one to eight, typically four, cylindrical ascospores coiled in a close helix. The ascospores have as many as eight cross walls and measure about 125 $\times$ 3 μ.

The conidia of *H. turcicum* are straight to slightly curved, widest at the middle and taper markedly toward the ends, and 45–142 $\times$ 15–25 μ in size; they have up to eight cross walls; and they have a protruding apiculum at the attachment of the conidiophore. The perithecial stage of *H. turcicum* is *T. turcica*. The ascocarp is dark and has short

stiff brown spinelike hairs on its surface. The asci contain one to six, typically two to four, mature ascospores per ascus. The ascospores are fusoid, straight or slightly curved, typically three-septate, and measure 24–48 × 13–17 μ. They are strongly constricted at each septum (cross wall).

SEXUAL STAGES OF *HELMINTHOSPORIUM* SPECIES ON CORN

The sexual stages of the three species of *Helminthosporium* parasitizing corn are known. The first to be reported is Drechsler's (1925) account of *H. maydis*. The sexual or perithecial stage develops when infected corn leaves are incubated in a moist chamber, with the first appearance of the parithecial initials appearing in the corn tissue on the third or fourth day. As the perithecia enlarge, they break through the surface of the corn leaf and in about 2 weeks asci are formed. The mature perithecia are discrete and subglobose with well-defined beaks and ostioles, and with the asci containing typically four multiseptate, fuliginous, filamentous ascospores, each coiled in a helix of about four turns.

Mature ascospores are forced out *en masse* from the ostiole of the perithecium and, if placed in water, germinate very rapidly. Drechsler named the fungus *Ophiobolus heterostrophus*. *Ophiobolus* is a genus containing several species of parasitic fungi on grasses. Later, Drechsler (1934) placed this species in a new genus, *Cochliobolus*. The perfect state of *H. maydis* (*C. heterostrophus*) was found first in pure culture by Nelson (1957), who mated the + and − strains on sterile corn leaves placed in Petri dishes on Sach's nutrient agar. The strains of *H. maydis* are heterothallic, with 511 being in one compatibility group and 484 in the second. The asci produced under these conditions contained one to eight ascospores, although 85% of the asci contained from three to six ascospores. From 0 to 50% of the ascospores germinated.

The sexual stage of *H. turcicum* was discovered by Luttrell (1958) who obtained the ascocarpic stage by mating strains of *H. turcicum* on propylene–oxide sterilized fragments of barley culms partially immersed in Sach's agar. Mature ascocarps were formed in 3 weeks on this medium at 25° C. The perfect stage proved to be *Trichometasphaeria* (*T. turcica* Luttrell). Before this work all the sexual stages of *Helminthosporium* were found to belong in the genera *Pyrenophora*, *Leptosphaeria*, and *Cochliobolus* (a segregate of *Ophiobolus*).

In the genus *Trichometasphaeria* the perithecia are ostiolate, dark brown, and covered with short stiff spinelike hairs on the upper third of the perithecial wall. The asci contain from one to six ascospores which are straight, three to six septate, and typically hyaline, although with aging they may become brown and surrounded by a mucouslike sheath. Because of the shape of its ascospores, the perfect stage of *H. turcicum* is very different from that of *C. heterostrophus*, where the ascospores are twisted and filamentous.

The ascosporic stage of the third species of *Helminthosporium* on corn, *H. carbonum*, was discovered by Nelson in 1959. The mature perithecia were produced on autoclaved pieces of corn leaf inoculated with compatible strains of *H. carbonum* in Sach's medium at pH 4. Like the other two species attacking corn, it is also heterothallic. Ascocarps are produced at 24° C on solid media, such as corn tissue, but not on agar as are the ascocarps of the other two species. Mature ascospores are formed in 22 days. The perfect stage of this fungus belongs in the genus *Cochliobolus*, just as *H. maydis* does, and hence the sexual stage is almost the same. However, the mating types of this species are not cross fertile with those of *H. maydis*.

SPECIES DIFFERENCES IN THE LESIONS ON CORN

Dickson (1956) distinguishes between corn leaf blight and corn leaf spot. The northern leaf blight of corn caused by *H. turcicum* is widely distributed over the world on corn, Sudang rass, Johnsongrass, and other sorghums. Characteristic symptoms are somewhat large irregular lesions on the leaf blades that may join and extend to the leaf sheaths. Under favorable environmental conditions, the entire leaf blade may be killed. Tassel infection is less conspicuous, and ear infection is reportedly rare but darkens kernels when it occurs.

The southern leaf spot is caused by *C. heterostrophus* with the conidial stage as *H. maydis*. The leaf lesions are much smaller and more distinct than those caused by *H. turcicum*. The lesions are buff to reddish brown in color and have a zonate to targetlike color pattern (Drechsler, 1925). Leaf spots caused by *H. carbonum* are similar to those of the southern leaf spot and are often chocolate brown in color. Both parasites causing leaf spots may produce a black moldy growth over the kernels of susceptible varieties, resulting in a charred appearance of the infected ear (Ullstrup, 1941a,b, 1944, 1966).

In the new race T of *H. maydis*, lesions are tan to brown and elliptical, and may reach a size of 2.5 × 1.5 cm. The lesions first appear on the lower leaves and progressively infect the upper leaves. Lesions are also formed on the husks, and from there the mycelium penetrates to the ear through the kernels. The kernels at first are covered with a light-gray mold, which later becomes dark gray to black. Ears are also infected from the silk end. In race O, the lesions are tan to brown and tend to be parallel-sided rather than spindle-shaped. It rarely attacks the ear. In race T, infection to sporulation may occur in as little as 7 days. The reduction in functional leaves predisposes the corn plant to stalk rot by other fungi (Ullstrup, 1970).

The leaf spot and leaf blight diseases are easily confused (Robert, 1953). Although distinct in the early stages of infection, later stages and subsequent reinfections with both leaf blight and leaf spot organisms on the same leaf may coalesce, an effect tending to confuse determination of the major cause. The lesions contain dead leaf tissue. According to Robert (1953) if much of the leaf area is killed, starch formation is lessened and the kernels are chaffy. The blighted leaves are less suitable for fodder because of their lowered nutritive value.

Infections by all three species occur throughout the growing season. Crop residues are the sources of primary inoculum and a single spore is all that is necessary to produce a lesion. Heavy dews, abundant rainfall, and warm summer weather are ideal conditions for infection. The primary lesions become established and produce abundant wind-borne conidia for subsequent secondary infection; thus the older leaves have more extensive as well as more numerous lesions.

Control measures consist mainly of sanitation, proper covering of crop residue, and the use of resistant corn hybrids (Dickson, 1956; Ullstrup, 1966). Seed treatment of Sudan grass with mercury dusts has been effective in reducing its infection (Dickson, 1956).

Fungicides are used in the South to protect the sweet corn crop from *H. maydis* and *H. turcicum*, which are serious limiting factors to the production of high-quality corn. According to Cox (1956) the cost then was about $20–30 an acre to protect the crop. Southern leaf blight is dominant in the fall and late spring crops, while the northern corn blight is found in late winter and spring. This alternation of diseases is believed to be a reflection of the temperature.

The standard fungicide spray was a combination of nabam, zinc sulfate, and zineb. Cox indicates that maneb was also effective.

PHYSIOLOGY OF *HELMINTHOSPORIUM*, ESPECIALLY *H. MAYDIS*

Unlike some plant pathogens, species of *Helminthosporium* are not limited to a single host. Thus, Nelson and Kline (1968) examined the ability of *C. heterostrophus* (*H. maydis*) to induce lesions on 34 gramineous species. Of the 34 species of grasses, 26 hosts were susceptible to one or more of the 150 isolates of *C. heterostrophus* tested. A similar study was made by Kline and Nelson (1968) on *C. carbonum*. They tested 142 isolates of this species on 28 gramineous test species; of this number 19 of the 26 hosts exhibited blight reactions from one or more strains.

Burton (1968) demonstrated that temperatures from 75° to 95° F favored rapid growth of the *H. maydis* in sweet corn ears with 85° F as optimum. Symptoms of the disease developed in 3 days at 85° F after infection.

Braverman and Crosier (1966) stored 32 species of *Helminthosporium* under sterile mineral oil. At the end of 15 years, 16 out of the 32 species remained viable and 12 were pathogenic. On the one hand, both *H. carbonum* and *H. turcicum* were alive and pathogenic after 15 years. On the other hand, *H. maydis* was dead by the end of 10 years. Drechsler (1925) reported that corn leaves infected with *H. maydis* showed no germination of the conidia, but when 3-year-old leaves containing mycelium were placed on agar plates, *H. maydis* grew out from the leaf tissue. Unquestionably the mycelium may remain alive for long periods of time.

Berger (1970) found that *H. turcicum* required 7 hr of relative humidity near 100% at temperatures above 15° C for significant sporulation. Spores were formed during the night hours and released as the humidity decreased during the morning hours. Of all spores released in a day, 50% were released during the 4 hr from 8 a.m. to noon.

Whether conidia of the T strain of *H. maydis* overwinter in the North has not been established, but this question is important because the answer will determine how much initial inoculum will be present for early infection of maize in the spring and early summer. Nelson and Scheifele (1970) studied the northern corn blight organism and concluded from their experiments that two factors are prime determinants of whether the conidia of *H. turcicum* will overwinter or not in Pennsylvania. The first is the genetic nature of the corn hybrid. Some varieties of corn allow more conidia produced from their leaves to overwinter than others. At the same time some races of the northern corn blight fungus overwinter better than others when the corn hybrid is the same. If this situation holds true for the T strain, then overwintering in the North might be possible on certain corn hybrids and not on others.

In considering the growth of *H. maydis* on a corn plant including ears, the question is what type of materials does the fungus use. The only study directed specifically at this problem is a paper by Hale and Roane (1961) who investigated the nutrition not of *H. maydis* but another corn blight organism, *H. carbonum*. The four most common vitamins required for growth by fungi—thiamin, biotin, pyridoxine, and inositol—were tested. Only a slight increase in dry weight of the fungus resulted over a vitamin-free medium. The fungus can readily use galactose, glucose, sucrose, maltose, fructose, and mannose. Furthermore, *H. carbonum* could grow even on a 20% glucose or sucrose medium. An examination of the utilization of amino acids showed the fungus could readily use *l*-glutamic acid, L-aspartic acid, casein hydrolysate, L-asparagine, *l*-glutamine, KNO_3, ammonium tartrate, urea, and glycine. Malca and Ullstrup (1962) investigated the effect of carbon and nitrogen nutrition on growth and sporulation of *H. turcicum* and *H. carbonum*.

A pertinent reference is work by Ullstrup and Miles (1957) who studied the effect of some leaf blights (*H. turcicum* and *H. maydis*) on corn yield. They state that the severity of the disease did not appear to depend so much on the primary infection but rather the development of secondary infections which are influenced by weather conditions. In a severe infection from northern corn blight, the difference in yield between a resistant hybrid and a very susceptible hybrid was 70 bushels per acre. For example, one hybrid which yielded 121 bushels per acre when infected produced only 46.5 bushels. Also, they conclude that a heavy infection of the corn plant 2 or 3 weeks after silking may be expected to cause severe grain losses. If the disease does not become conspicuous until 6–8 weeks after silking, no appreciable reduction in yield occurs. They state the same general rule applied to southern corn blight.

RACE T OF *H. MAYDIS*

In August 1970, Hooker *et al.* published an informative article on their studies with *H. maydis* race T, the fungus race which did so much damage to corn in the United States during the summer of 1970. They point out that 10 years or more ago, workers in the Philippine Islands noticed that corn inbreds and hybrids having the Texas (T) type of cytoplasm for male sterility had increased susceptibility to *H. maydis*. This susceptibility was not commonly observed in the United States until 1969, when seed fields were heavily damaged by *H. maydis*. In their paper Hooker *et al.* (1970) showed that P cytoplasm for male sterility was also susceptible, whereas corn, either with nonsterile cytoplasm or with S and C cytoplasm for male sterility, was resistant.

When corn leaves are inoculated with spores of *H. maydis*, the first symptoms are minute light-green flecks. Within 6 days the susceptible and resistant corns differ in appearance. On the resistant plant the leaf lesions are small and consist of a brown or tan necrotic spot surrounded by a reddish brown border and a chlorotic margin. Lesions on the susceptible plants are larger, consisting of a light-tan necrotic area with a clearly defined pigmented border. As the lesions enlarge they have dark brown borders but little or no chlorosis. Conidia are formed on the individual leaves of susceptible plants within 24 hr, whereas lesions on resistant plants do not enlarge and no conidia are formed. The Hooker group at Illinois also confirmed the production of a pathotoxin from *H. maydis*. This toxin inhibited primary root development of the susceptible plants. The pathotoxin caused a water soaking of susceptible detached leaves in 3 or 4 days, while the resistant corn tissue was not water soaked. The Illinois group suggest, on the basis of their laboratory studies, that a new biotype or race of *H. maydis* with specialized parasitism to plants with T cytoplasm has developed and is now widely distributed in the United States. The obvious solution to the problem is to use lines of corn not containing the T cytoplasm.

Before the work of Hooker *et al.*, Scheifele *et al.* (1970) reported that in field observations inbred lines and hybrids of maize with T male-sterile cytoplasm had increased susceptibility to southern leaf blight.

Corn with T male-sterile type of cytoplasm is susceptible to at least one other fungus disease besides *H. maydis*.

Scheifele and Nelson (1969) and Ayers *et al.* (1970) have shown corn with T cytoplasm is susceptible to *Phyllosticta* leaf spot or yellow leaf blight. All inbred lines of corn with normal cytoplasm are more resistant than corresponding inbred lines with T cytoplasm to *Phyllosticta*.

Smith *et al.* (1970) discuss the physiologic races of *H. maydis*, especially race T. Very recently an entire supplement to the *Plant Disease Reporter* was devoted to this and other aspects of the 1970 outbreak of the southern corn leaf blight (Farrar, 1970).

NATURE OF THE CORN RESISTANT TO *HELMINTHOSPORIUM*

The nature of resistance in nonsusceptible hybrid corns to *H. turcicum* recently was studied by Lim *et al.* (1968). Although all corn plants are infected by both *H. turcicum* and *H. maydis*, in susceptible hybrids the initial infection rapidly spreads, whereas only minute lesions on resistant hybrids develop and the fungus is unable to reproduce itself. These workers conclude that the hyphae of the fungus cannot spread from its initial infection site in resistant corns because it is stopped by a chemical present in the plant. Such chemicals have been given the name phytoalexins. In resistant hybrids, Lim *et al.* (1968) did find that extracts from chlorotic lesions act as inhibitors of spore germination. This inhibition of spore germination was therefore used as an assay to isolate the chemical factor producing resistance to the fungus. In a second paper by Lim *et al.* (1970) the phytoalexin in resistant corn was isolated and some of its chemistry determined.

No phytoalexins could be found in diffusates from either resistant or susceptible corn. However, 3 days after infection diffusates from resistant corn leaves showed phytoalexins to be present. Thus the fungus acted as an inducer of the chemical that affords protection. When the phytoalexins were isolated, two compounds were found with yields of 40 mg/kg of infected leaves. Phytoalexin A has a paper chromatographic R_f value of 0.87 in the upper phase of *n*-butanol, acetic acid, and water (4:1:5), and a uv absorption spectrum with a maximum at 280 mμ. Phytoalexin B has an R_f value of 0.97 and a uv absorption spectrum with a maximum at 270 mμ. Both have a blue fluorescence. Lim and his associates conclude that both A and B are phenolic. The more pathogenic the fungus strain, the higher the concentrations of phytoalexins produced in the resistant hybrids.

PHYTOTOXINS AND PATHOTOXINS

Plant disease fungi are known to produce toxins (phytotoxins) which adversely affect plants. A special group of phytotoxins are ones that reproduce all the symptoms of the disease in the susceptible host just as if the fungus were present (pathotoxins).

An excellent review of phytotoxins and pathotoxins was published by Owens (1969). He points out that pathotoxins may be similar to each other chemically and have the same mode of action towards susceptible host tissue. Therefore, it may be instructive to look at their structures and mode of action. The pathotoxin best known comes from *Helminthosporium victoriae*, the cause of leaf blight of the Victoria variety of oats. This oat variety was imported from South America and was used extensively to breed smut and rust resistance into North American varieties of oats. In 1955 an epidemic of *H. victoriae* leaf blight of oat varieties with Victoria genes occurred in the United States. The result is reminiscent of what happened to the 1970 corn crop after southern leaf blight attacked. The pathotoxin formed by *H. victoriae* was isolated and named victorin and partially characterized. It is a polypeptide with a molecular weight between 800 and 2000 and, therefore, is smaller than polypeptides known to be toxic to animals. Victorin is made up of five or six common amino acids (aspartic, glutamic, valine, glycine, leucine, and/or isoleucine) and a nitrogen-containing sesquiterpene called victoxinine, a compound which is only 1/7500 as toxic and is not host specific. The separate polypeptide moiety of victorin is nontoxic. The primary effect of victorin is believed to be an alteration in cell wall permeability. One needs to remember that the *H. maydis* pathotoxin causes water soaking of the corn tissue. When susceptible oat tissue is treated with victorin, the oat cells begin to lose electrolytes within 5 min. With resistant oat tissue nothing happens. Protoplasts of susceptible oats (cell walls removed) quickly stopped protoplasmic streaming and burst in less than an hour; resistant protoplasts did not.

Pathotoxins are very potent to susceptible host cells. For example, 0.01 μg/ml of victorin is toxic to susceptible oat tissue. The pathotoxin of *H. carbonum* is active at 0.5 μg/ml to corn and the one in *Periconia circinata* is active at 0.1. Each of these toxins are polypeptides.

PHYTOTOXINS OF *H. MAYDIS*

Pringle and Scheffer (1964) reviewed the phytotoxins, pointing out that three fungi, including *H. victoriae*, produced toxins that were host specific and can induce all the symptoms of the plant disease without the fungus being present. The other two plant pathogens, which are relatives of *Helminthosporium*, were *Alternaria kikuchiana* and *P. circinata*. Since this review additional host specific phytotoxins have been found in *H. maydis*, *H. carbonum*, *H. sacchari*, and *Alternaria mali*. The first is of special interest because this is the southern corn blight fungus and was discovered by Smedegard-Peterson and Nelson (1969). Of the seven known examples of host specific toxins, four are from species of *Helminthosporium*.

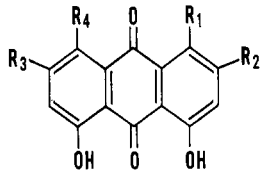
The host specific phytotoxins, now known as pathotoxins, are interesting not only because they affect only one host species, but also because they exhibit the same specificity to susceptible and resistant plants as the pathogen. That is, the susceptible strains of corn are affected by the toxin just as if the fungus were present, while resistant corn strains are not poisoned by the toxin and are not attacked by the fungus. Such pathotoxins should not, therefore, be expected to cause harm to animals. This area of work has not yet been investigated.

The toxin of *H. maydis* is a low-molecular-weight polypeptide that can be isolated by adsorption on charcoal and cation exchange resins, but not on basic ionic exchange resins. Solutions of the toxin give a positive ninhydrin reaction and are stable at pH 3–8 for 16 days, 4° C. The toxin is also stable to autoclaving 30 min, 121° C. Evidence indicates that this toxin is related to the other known five pathotoxins elaborated by *Helminthosporium*, *Alternaria*, and *Periconia*.

SECONDARY METABOLITES OF *HELMINTHOSPORIUM*

One of the first considerations when corn fields were invaded with southern corn blight in the summer of 1970 was whether the mold might form toxic compounds on corn, harmful to man and animals. We made a survey of reports of the secondary metabolic products formed by species of *Helminthosporium*. In so doing, it is necessary to examine

Table I. Polyhydroxyanthraquinones Produced by *Helminthosporium*



Compound	R_1	R_2	R_3	R_4	Color and Crystal Form	Producing Species	Plant Disease Associated with Producing Species	References
Catenarin	OH	CH_3	OH	H	Deep red plates	*H. catenarium* Drechsler		Anslow and Raistrick (1940, 1941)
						H. gramineum Rabenhorst	Leaf stripe of barley	Raistrick *et al.* (1934)
						H. velutinum Link		
						H. tricicivulgaris Nisikado	Yellow spot disease of wheat	
						Penicillium islandicum Sopp		
						Aspergillus amstelodami (Mangin) Thom and Church		
Cynodontin	OH	CH_3	H	OH	Bronze plates	*H. cynodontis* Marignoni	Parasite on Bermuda grass	Raistrick *et al.* (1933)
						H. euchlaenae Zimmerman		Anslow and Raistrick (1940)
						H. avenae Ito Kurib	Leaf spot of oats	Raistrick *et al.* (1934)
						H. victoriae		
Helminthosporin	H	CH_3	H	OH	Dark red needles	*H. gramineum* Rabenhorst	Leaf stripe of barley	Charles *et al.* (1933)
						H. cynodontis Marignoni	Parasite on Bermuda grass	Raistrick *et al.* (1933)
						H. catenarium		
						H. tricicivulgaris Nisikado	Yellow spot disease of wheat	
Tritisporin (ω-hydroxy-catenarin)	OH	CH_2OH	OH	H	Brown needles	*H. tricicivulgaris* Nisikado	Yellow spot disease of wheat	Neelakantan *et al.* (1956)

the chemical literature for compounds not only produced by *Helminthosporium* but also by *Bipolaris*, *Drechslera*, *Ophiobolus*, *Trichometasphaeria*, *Cochliobolus*, *Pyrenophora*, and *Leptosphaeria*. The last five names are the sexual stages of *Helminthosporium*.

An investigation of the literature revealed that Raistrick, in a series of papers beginning in 1933, described a number of polyhydroxyanthraquinone pigments produced by *Helminthosporium*, as well as a polyhydroxyxanthone. Aucamp and Holzapfel (1970) discussed compounds of the polyhydroxyanthraquinone group from both *Aspergilli* and *Bipolaris* whose structure and biosynthetic pathways were related to aflatoxin B_1. Several antibiotics more or less toxic to animals have been reported by Japanese and Italian workers, of which the ophiobolins are actually produced by *H. maydis*, the causative organism of southern corn blight.

The polyhydroxyanthraquinones studied by Raistrick and his associates are pigments closely related in structure (Table I). For example, tritosporin is indeed ω-hydroxycatenarin and can be synthesized from catenarin by brominating the methyl group with a Wohl-Ziegler reaction after the hydroxyl groups are protected by acetylation. Debromination followed by deacetylation led to a compound identical to naturally occurring tritosporin (Neelakantan *et al.*, 1956). Cynodontin has been synthesized from helminthosporin by oxidation with manganese dioxide and concentrated sulfuric acid (Raistrick *et al.*, 1933).

Helminthosporium strains that produce polyhydroxyanthraquinones have been isolated as causative agents of plant diseases such as leaf stripe of barley (Charles *et al.*, 1933), yellow spot of wheat (Neelakantan, *et al.*, 1956), and leaf spot of oats (Raistrick *et al.*, 1934). The compounds are highly colored pigments that fluoresce and are probably responsible for the discolorations noticed on diseased leaves. Other producing species of the mold occur as parasites on grasses—Bermuda grass from Southern United States, "durba" from India, and "teosinte" from states on the Gulf of Mexico (Raistrick *et al.*, 1933). Yields *in vitro* of some of these compounds are high. Helminthosporin, for example, comprises 30% of the weight of dry mycelia (Charles *et al.*, 1933), and catenarin is produced as 15% of dry weight of mycelia (Anslow and Raistrick, 1940).

A polyhydroxyxanthone, ravenelin (I), is produced by *H. turcicum* Passerini, the causative agent of northern corn blight, and *H. ravenelii* Curtis, a parasite growing on grasses in North and South America and China (Raistrick *et al.*, 1936). Raistrick observed that all species in the subgenus *Cylindro-Helminthosporium*, or *Drechslera* (Shoemaker, 1959), produced polyhydroxyanthraquinones, while species in *Eu-Helminthosporium* or *Bipolaris* (Shoemaker, 1959), *H. turcicum*, and *H. ravenelii* formed polyhydroxyxanthones. Mycelia of *H. leersii* Atkinson belonging to *Bipolaris* contain luteoleersin ($C_{26}H_{38}O_7$) and alboleersin ($C_{26}H_{40}O_7$) that are

closely related and readily interconvertible (Ashley and Raistrick, 1938). Luteoleersin is thought to be a substituted quinone or semiquinone and alboleersin the phenol corresponding to luteoleersin.

Sterigmatocystin, a carcinogenic compound structurally related to aflatoxin, was reported to be produced by an unidentified species of *Bipolaris* (*Helminthosporium*) by Holzapfel *et al.* (1966). In 1970, Aucamp and Holzapfel discovered that three polyhydroxyanthraquinones—bipolarin, versicolorin C, and averufanin—were produced by *Bipolaris*. Sterigmatocystin has been known as a metabolite of *Aspergillus versicolor*. Versicolorin C is produced by *A. versicolor*, *A. nidulans*, and *A. flavus*. Averufanin is produced by *A. versicolor* and *A. flavus*. These compounds are significant because their biosynthetic pathways are closely related to those of the aflatoxins, potent carcinogens elaborated by strains of the *A. flavus* series.

Sterigmatocystin was produced by *Bipolaris* on maize meal in very high yields, 1.2 g/kg. This high yield increases the necessity of considering the possibility that sterigmatocystin could be a dangerous mycotoxin. The LD_{50} of the mycotoxin administered intraperitoneally in dimethyl sulfoxide or wheat germ oil was 60–65 mg/kg in albino rats. Degenerative changes were noted in the liver and kidneys of rats that died. Necrosis of kidney and liver cells was revealed by histopathological examinations of tissues (Holzapfel *et al.*, 1966). Another study (Dickens *et al.*, 1966) showed that sterigmatocystin caused not only local sarcomas but also liver tumors and had about 100–250th of the activity of aflatoxin. Subcutaneous injections of sterigmatocystin in rats (0.5 mg twice weekly for 24 weeks) induced local sarcomas, and after 47 weeks liver tumors in two rats. Large doses of aflatoxin B_1 subcutaneously did not produce liver tumors although they caused many more local sarcomas. Oral administration of sterigmatocystin also produces liver tumors in rats (Purchase and van der Watt, 1970).

The structure of sterigmatocystin (II) was finally elucidated by Davies *et al.* (1960) and Bullock *et al.* (1962) on the basis of its conversion to isosterigmatocystin (III) with hot ethanolic potassium hydroxide. Ozonolysis of di-*O*-methyl-isosterigmatocystin resulted in 2 mol of formic acid being formed, establishing the structures of II and III as shown in Figure 1.

Versicolorin C (IV), an orange-red pigment, was first reported by Hamasaki *et al.* (1967) to be produced by *A. versicolor* (Vuillemin) Tiraboschi. It was thought to be a racemate of versicolorin B. Heathcote and Dutton (1969) isolated it as a metabolite of an aflatoxin B_1-producing strain of *A. flavus* with structure IV and believed it could be a common precursor of aflatoxins and sterigmatocystin.

More recently Aucamp and Holzapfel (1970) found that *Bipolaris* sp. and *A. nidulans* formed versicolorin C. In the same study, from corn meal inoculated with *Bipolaris* sp. and *A. versicolor*, they isolated a polyhydroxyanthraquinone identical in structure to the compound averufanin (V). This compound had been produced by a mutant of an *A. versicolor* strain (Holker *et al.*, 1966) and by an aflatoxin-producing strain of *A. flavus* (Heathcote and Dutton, 1969). Aucamp and Holzapfel were the first to report its occurrence as a metabolite of a naturally occurring strain of *A. versicolor* and a *Bipolaris* strain.

They also described the isolation of a new polyhydroxyanthraquinone, bipolarin, VI, from *Bipolaris*. In the proposed scheme by Biollaz *et al.* (1970) for the biogenesis of aflatoxin B_1, acetates are condensed to form a C_{18}-polyketide,

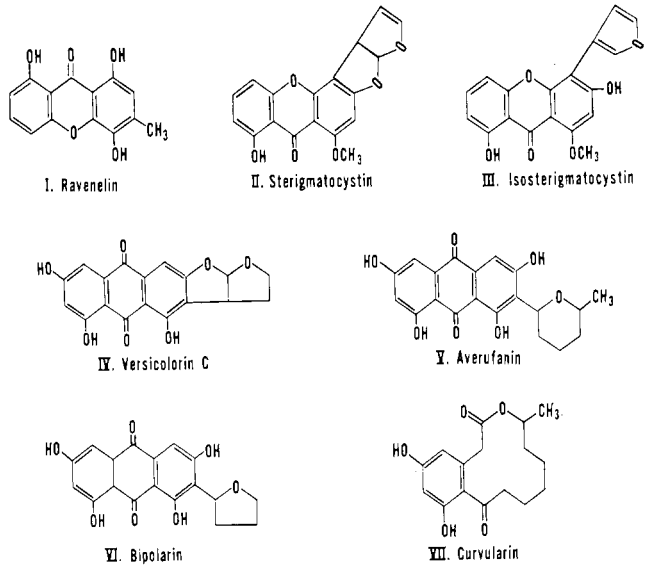


Figure 1. *Helminthosporium* **metabolites with biosynthetic pathways related to aflatoxin B_1 and derivative**

which cyclizes to a polyhydroxynaphthacene. The latter is oxidized to an anthraquinone, which is regarded as a common precursor of versicolorin A, sterigmatocystin, and aflatoxin B_1 (Biollaz *et al.*, 1968). Bipolarin may bridge the gap between the hypothetical C_{18}-polyketide on the one hand and versicolorin C, sterigmatocystin, and aflatoxin B_1 on the other (Aucamp and Holzapfel, 1970). In the same study, curvularin, a macrocyclic lactone, was isolated as a metabolite of *Bipolaris*. Evidence exists for the biosynthesis of curvularin (VII) by the head-to-tail condensation of eight acetate units (Birch *et al.*, 1959). Curvularin readily yielded a naphthol by transannular cyclization leading to the speculation that macrocyclic lactones may be intermediates in the formation of polycyclic aromatic compounds. The finding that curvularin is a metabolite of *Bipolaris* (*Helminthosporium*) supports this hypothesis, particularly when one considers the number of polyhydroxyanthraquinones isolated from *Helminthosporium*.

Members of a family of antibiotics known as ophiobolins have been isolated as metabolites of the fungi that are responsible for southern corn blight and northern corn blight (Figure 2). Nomenclature of the compounds was clarified in a joint paper by a group of Japanese and Italian workers (Tsuda *et al.*, and Canonica *et al.*, 1967) who had been studying them. Ophiobolin A has been reported in the literature as cochliobolin, ophiobolin, ophiobalin, and cochliobolin A. Likewise, ophiobolin B has appeared in papers under the following names: zizanin, ophiobolosin A, zizanin B, and cochliobolin B. Ophiobolin C is also known as zizanin A.

Ophiobolin A is obtained from culture filtrates of *Ophiobolus heterostrophus* (the sexual stage of *H. maydis*), *H. turcicum*, *H. ziziniae*, *H. leersii*, and *H. panici-miliacei* (Scott and Somers, 1969). The structure (VIII) was determined independently by Nozoe *et al.* (1965b) and Canonica *et al.* (1966a). Nozoe and his coworkers based their conclusions on the X-ray crystallographic analysis and on the infrared and nmr spectra of the bromomethoxy derivative. Canonica and his associates studied nmr, infrared, and uv spectra of a number of derivatives and reaction products. Ophiobolin A yields a monoepoxide and an anhydrobis-2,4-dinitrophenylhydrazone. It is dehydrated in acid and

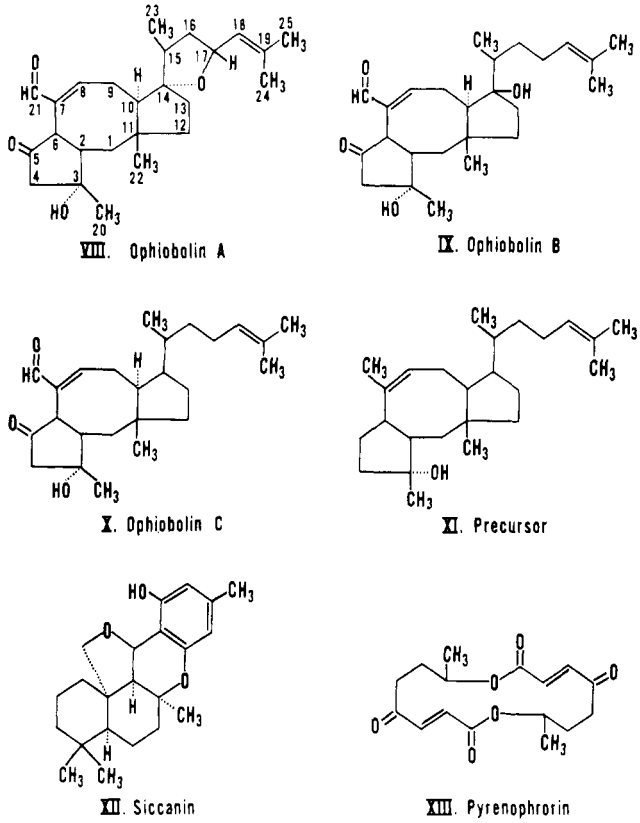


Figure 2. Antibiotics and related compounds produced by *Helminthosporium*

alkaline solutions and reduced with lithium aluminum hydride to two isomeric triols. Oxidations of these, along with other reactions, led to the same structure proposed by Nozoe *et al.* (1965b).

As a metabolite of the southern corn blight organism, the biological activity of ophiobolin A is of particular interest (Nakamura and Ishibashi, 1958). It inhibits the germination and root growth of rice seedlings and causes the same pathogenic symptoms on rice plants as the *O. miyabeanus* infection. Ophiobolin A is toxic to mice. The LD_{50} dose is 238 mg/kg when administered subcutaneously; 21 mg/kg, intraperitoneally; 12 mg/kg, intravenously; and 73 mg/kg, orally. It is specifically active against *Trichomonas vaginalis*, *Trichophyton*, *Glomerella*, *Gleosporium*, and phytotoxic fungi at levels of 1–5 μg/ml.

Three other compounds structurally related to ophiobolin A were isolated from cultures of *O. heterostrophus* (*H. maydis*) and *H. zizaniae* and characterized (Nozoe *et al.*, 1966). These were ophiobolin B and C and anhydroophiobolin. Ophiobolin B is also produced by *Cochliobolus miyabeanus* (*O. miyabeanus*) the perfect form of *H. oryzae*, the organism responsible for leaf spot disease of rice (Ohkawa and Tamura, 1966).

The structure of ophiobolin B (zizanin B) (**IX**) contains a hydroxyl group instead of the oxygen in the tetrahydrofuran ring. In their studies, Canonica *et al.* (1966b) designated the same compound as cochliobolin B. They found ophiobolin B only in young cultures of *H. oryzae*, leading to the conclusion that it is a precursor of A. Ophiobolin B inhibits growth of rice seeds and root formation (Ohkawa and Tamura, 1966). The LD_{50} in mice is 4.4 mg/kg administered intraperitoneally and exhibits activity against many of the same fungi as A (Ishibashi, 1962a).

Ophiobolin C (**X**) produced by *H. zizaniae* along with ophiobolin B (**IX**) is less polar (Nozoe *et al.*, 1966). Ophiobolosin B was isolated from the mycelium of *H. oryzae*, which also formed ophiobolin B. Although the structure of ophiobolosin B has not been elaborated, the compound has the same effect on rice seedlings and fungi as ophiobolin B (Ohkawa and Tamura, 1966).

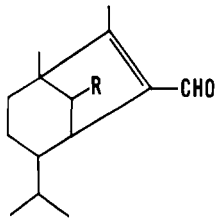
The novel skeletons of the ophiobolins are formed biosynthetically by the head-to-tail condensation of five isoprene units (Nozoe *et al.*, 1967). These are the first examples of C_{25} terpenoids. Evidence for the formation of the skeleton was established by culturing *C. miyabeanus* in a synthetic medium containing [2-^{14}C]mevalonic acid lactone (Canonica *et al.*, 1966c). Labels appeared in carbons 4, 8, 12, 16, and 24 in ophiobolin A. Ophiobolin B (**IX**) was also established as a precursor of A (**VIII**). Both Nozoe *et al.* (1967) and Canonica *et al.* (1967b) established that in the biosynthesis of A and B, molecular oxygen is directly incorporated into the 14-carbon, and the oxygen on the 3-carbon comes from the medium. The pathway for the formation of ophiobolins is **XI** → **X** → **IX** → **VIII** (Canonica *et al.*, 1968; Nozoe *et al.*, 1967). The probable course of the cyclization of the biological equivalent of geranyl farnesyl pyrophosphate was studied by incorporating tritium-labeled and doubly labeled (tritium and ^{14}C) mevalonic acid lactone into fermentations of *C. miyabeanus* and *C. heterostrophus* to produce ophiobolins (Canonica *et al.*, 1967a, 1968; Nozoe *et al.*, 1968c).

Nozoe *et al.* (1968b) isolated from *O. heterostrophus* a new ophiobolin (**XI**) that they had previously described as a possible precursor in the biosynthesis of ophiobolins (Nozoe *et al.*, 1967). At the same time they detected diterpene and sesterterpene hydrocarbons, a sesterterpene alcohol, and squalene by gas–liquid chromatography. They also isolated an acyclic C_{25} isoprenoid alcohol, geranylnerolidol.

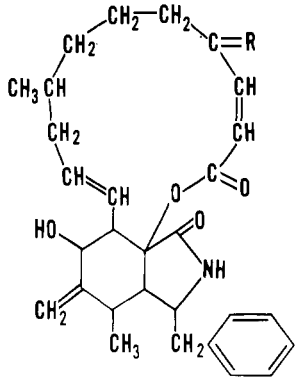
In screening organisms pathogenic to plants for antimicrobial activity, it was found that *H. avenae* (*Pyrenophora avenae*) produced an antibiotic designated as siccanin (Ishibashi, 1962b). The compound is also formed by *H. siccans*. Siccanin was most active against *Trichophyton*, inhibiting the fungi at levels of 0.1 μg/ml. It was potent against at least one species of *Gibberella*, Aspergilli, Penicillia, and *Alternaria* at levels of 5–12.5 μg/ml. Intraperitoneal injection of 500 mg/kg in mice resulted in no adverse affects. Siccanin is a phenolic compound of structure **XII** (Hirai *et al.*, 1967). Orsenellinic acid is probably involved in its biosynthesis. In addition to siccanin, Nozoe *et al.* (1968a) isolated four siccanochromenes—A, B, C, and D—from *H. siccans*.

A second antibiotic, helmintin, is produced by *H. siccans*, the fungus isolated from leaf spots of Italian rye grass. The compound is nonaromatic and shows no absorption in the region 215–340 mμ. Helmintin is most active against *Trichophyton* (Inagaki, 1962). It is active against many of the fungi inhibited by siccanin (Ishibashi, 1962b), but at much higher concentrations. Its reported chemical and physical properties appear to differentiate the two antibiotics. Helmintin, with an empirical formula of $C_{11}H_{18}O_2N_2$, is one of the few secondary metabolites of *Helminthosporium* reported to contain nitrogen. A French patent granted in 1966 reported two fungicidal compounds produced by *H. dematioideum* that also contained nitrogen. They were intraconvertible by oxidation–reduction reactions and had empirical formulae of $C_{29}H_{35}NO_5$ and $C_{29}H_{37}NO_5$. These compounds are probably cytochalasin A and B, discussed later.

Two strains of *Helminthosporium* elaborate compounds



XIV. R = CHO Helminthosporal
XV. R = CH_2OH Helminthosporol

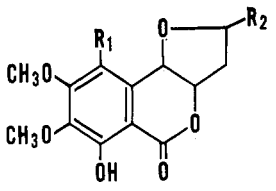


XVI. R = O Cytochalasin A
XVII. R = H, OH Cytochalasin B

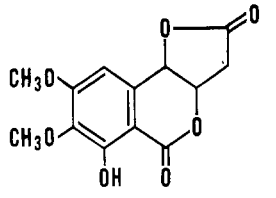
Figure 3. *Helminthosporium* **metabolites with biological activity**

with some antitumor activity. The substance produced by *H. avenae* (*Pyrenophora avenae*), pyrenophorin, is a symmetrical dimer of (−)-7-hydroxy-4-oxooct-2-enoic acid lactone (**XIII**) (Nozoe *et al.*, 1965a). It was first reported by Ishibashi (1961) to be formed by *H. avenae*, a pathogen of oats. Pyrenophorin inhibited pathogenic plant fungi, yeast, and *Trichomonas*. The LD_{50} for mice was 44.1 mg/kg by intraperitoneal injection. Some antitumor effects were observed on Ehrlich ascites of mice. A *Helminthosporium* sp. was discovered to produce a substance with antitumor activity when administered intraperitoneally against four ascitic tumors in mice (Pugh *et al.*, 1962). The compound responsible for the observed activity is 3′-amino-3′-deoxyadenosine (Gerber and Lechevalier, 1962).

Helminthosporal (**XIV**) and helminthosporol (**XV**) are two closely related sequiterpenoid compounds formed by *H. sativum* P.K and B [*B. sorokiniana* (Sace. in Sorok.) Shoemaker]. The toxin, helminthosporal, is responsible for seedling blight, foot and root rot, and leaf spot in cereals causing substantial economic losses in Canada. Structure **XIV** has been determined (deMayo *et al.*, 1962), and the compound, a dialdehyde, has been synthesized (Corey and Nozoe, 1963). Helminthosporol, the compound in which one of the aldehyde groups is reduced to an alcohol, was first isolated by Tamura *et al.* (1963). Helminthosporol stimulates the elongation of leaf sheaths of rice seedlings in much the same manner as gibberellic acid (Kato *et al.*, 1964). Biosynthetic studies suggest these compounds may be formed by nonhead-to-tail linking of three isoprene units (Spencer *et al.*, 1966). It has been shown that helminthosporal and helminthosporol probably do not exist as such in culture liquors, but are formed during isolation procedures from acetals of compounds designated as prehelminthosporal and prehelminthosporol (deMayo *et al.*, 1965). Prehelminthosporal has not been detected in culture liquors, but



XVIII. R_1 = H, R_2 = $CH_2CH_2CH_3$ Monocerin
XIX. R_1 = OH, R_2 = $CH_2CH_2CH_3$ Hydroxymonocerin
XX. R_1 = H, R_2 = CH_2OCOCH_3 Monocerone



XXI. Monocerolide

Figure 4. Benzopyrans

prehelminthosporol, 9-hydroxyprehelminthosporol, and anthraquinone have been isolated as metabolites of *H. sativum* (Aldridge and Turner, 1970a). Helminthosporal stimulates the synthesis of amylase by embryoless barley seeds, but it can also inhibit the gibberellic acid-induced synthesis in barley half seeds (White and Taniguchi, 1969). These studies are being continued in an effort to determine the mode of action by which helminthosporal acts as a phytotoxin.

Cytochalasins A (**XVI**) and B (**XVII**), compounds produced by *H. dematoideum*, have interesting physiological properties (Carter, 1967). They inhibit cytoclasmic cleavage in cultured cells, but at lower doses do not interfere with nuclear division resulting in large multinucleated cells. Higher doses cause nuclear extrusion. Spooner and Wessells (1970) found that cytochalasin B had an effect on the shape of mouse salivary gland epithelium cells interfering with morphogenesis. Cytochalasins A (**XVI**) and B (**XVII**) are macrocyclic lactones containing a benzene ring (Aldridge *et al.*, 1967). The relationship between A and B was established by oxidation of B to A with manganese dioxide.

Recently an antifungal metabolite, monocerin (**XVIII**), has been isolated from culture filtrates of *H. monoceras* (Aldridge and Turner, 1970b). Monocerin inhibits powdery mildew (*Erisyple graminis*) of wheat. Three structurally related benzopyrans, hydroxymonocerin (**XIX**), monocerone (**XX**), and monocerolide (**XXI**), were isolated in small amounts from the monocerin fermentation (Figure 4). Ravenelin (**I**) previously obtained from *H. ravenelii* and *H. turcicum*, the organism responsible for the northern corn blight, was also isolated.

MYCOTOXINS

Besides the occurrence of sterigmatocystin in *Helminthosporium*, Hamilton *et al.* (1968) have reported a second unknown mycotoxin for *C. carbonum* (*H. carbonum*). They showed that certain strains of *H. carbonum* had an acute lethal effect on mice when tested by intraperitoneal injection of culture extracts (lethal within 48 hr). Of the 136 isolates tested for toxicity, 17 were positive (toxic), including 7 isolates from corn. The unknown mycotoxin was produced in a high-protein cereal medium. It was formed in 3 days and levels of toxin were maintained until the 10th day. The unknown toxin was found in the mycelium and once in the culture filtrate. The mycotoxin could be extracted

with ethanol and was stable at 100° C for 10 min. The effect of the toxin was to reduce activity and piloerection, which later led to ataxia, prostration, and respirating difficulty. The animals died without convulsions. None of the animals showed any indication of infection. Evidence of the characteristics of the mycotoxins from several isolates suggested that more than one toxin was present.

Several reviews are available on the secondary metabolites and phytotoxins produced by *Helminthosporium:* Pringle and Scheffer (1964); Scheffer and Pringle (1967); Scheffer and Samaddar (1970); Scott and Somers (1969); Spencer *et al.* (1966); and Wheeler and Luke (1963).

ANIMAL PATHOGENICITY OF *HELMINTHOSPORIUM*

The question has been asked whether danger exists to farmers, elevator operators, and corn millers from toxicity of *H. maydis*. We have checked a number of books on medical and veterinary mycology but were unable to find a single reference to *Helminthosporium* being a pathogen to animals and man. However Dr. K. Kwon, a medical mycologist at the National Institutes of Health, has checked their records for us and kindly furnished us with a reference to a paper published in 1970 in which *Helminthosporium* sp. had caused human infection. Dolan *et al.* (1970) report that *Helminthosporium* sp. were isolated from two human cases in which the fungus had infected the lungs. Both men had recurrent attacks of asthma with cough. Resection of the involved lungs demonstrated bronchiectasis with chronic pneumonia and one case of multiple abscesses. Many colonies of *Helminthosporium* were isolated from pulmonary tissues removed aseptically and mold mycelium was present. The most likely mode of infection was by inhalation of the fungus spores. Dolan and his coauthors state these are the first cases to their knowledge in which *Helminthosporium* has caused infection. Both patients recovered with a clinical course somewhat like that of allergic aspergillosis.

Another aspect of the problem is whether or not *Helminthosporium* can be an allergen. Many cases of humans who are allergic to fungus spores are known. The genus *Alternaria* has been one of the chief culprits, and it is very closely related to *Helminthosporium*. It would therefore not be surprising if *Helminthosporium* would also be an allergen. This proves to be the case, for Gray (1959) cites Feinberg, who made a study of 261 fungus-sensitive patients who were then skin-tested individually as to their sensitivity to a number of fungus genera. At the top of the list was *Alternaria*, which caused 91% of the 261 patients to give a positive skin reaction. The next highest percentage was found with *Helminthosporium* in which 60% of the 261 patients reacted positively. Thus as an allergen it gave a higher percentage of reactions than *Hormodendrum*, and smut spores. Therefore, we can conclude that there is a real possibility that some people inhaling *H. maydis* spores may become ill due to an allergic reaction.

POPULAR ACCOUNTS

Two accounts on the southern corn blight situation were published in 1970: one in *The Wall Street Journal* (Fischer, 1970) and a briefer one in *Science* (Gruchow, 1970). Needless to say, many other items have appeared in farm journals and in newspapers.

LITERATURE CITED

Aldridge, D. C., Turner, W. B., *J. Chem. Soc.* 686 (1970a).
Aldridge, D. C., Turner, W. B., *J. Chem. Soc.* 2598 (1970b).
Aldridge, D. C., Armstrong, J. J., Speake, R. N., Turner, W. B., *Chem. Commun.* 26 (1967).
Anslow, W. K., Raistrick, H., *Biochem. J.* **34,** 1124 (1940).
Anslow, W. K., Raistrick, H., *Biochem. J.* **35,** 1006 (1941).
Ashley, J. N., Raistrick, H., *Biochem. J.* **32,** 449 (1938).
Aucamp, P. J., Holzapfel, C. W., *J. S. Afr. Chem. Inst.* **23,** 40 (1970).
Ayers, J. E., Nelson, R. R., Koons, C., Scheifele, G. L., *Plant Dis. Rep.* **54,** 277 (1970).
Berger, R. D., *Phytopathology* **60,** 1284 (1970).
Biollaz, M., Buchi, G., Milne, G., *J. Amer. Chem. Soc.* **90,** 5019 (1968).
Biollaz, M., Buchi, G., Milne, G., *J. Amer. Chem. Soc.* **92,** 1035 (1970).
Birch, A. J., Musgrave, O. C., Rickards, R. W., Smith, H., *J. Chem. Soc.* 3146 (1959).
Braverman, S. W., Crosier, W. F., *Plant Dis. Rep.* **50,** 321 (1966).
Bullock, E., Roberts, J. C., Underwood, J. G., *J. Chem. Soc.* 4179 (1962).
Burton, C. L., *Plant Dis. Rep.* **52,** 847 (1968).
Canonica, L., Fiecchi, A., Galli Kienle, M., Scala, A., *Tetrahedron Lett.* 1211 (1966a).
Canonica, L., Fiecchi, A., Galli Kienle, M., Scala, A., *Tetrahedron Lett.* 1329 (1966b).
Canonica, L., Fiecchi, A., Galli Kienle, M., Ranzi, B. M., Scala, A., *Tetrahedron Lett.* 3035 (1966c).
Canonica, L., Fiecchi, A., Galli Kienle, M., Ranzi, B. M., Scala, A., *Tetrahedron Lett.* 4657 (1967a).
Canonica, L., Fiecchi, A., Galli Kienle, M., Ranzi, B. M., Scala, A., *Tetrahedron Lett.* 275 (1968).
Canonica, L., Fiecchi, A., Galli Kienle, M., Ranzi, B. M., Scala, A., Salvatori, T., Pella, E., *Tetrahedron Lett.* 3371 (1967b).
Carter, S. B., *Nature (London)* **213,** 261 (1967).
Charles, J. H. V., Raistrick, H., Robinson, R., Todd, A. R., *Biochem. J.* **27,** 499 (1933).
Corey, E. J., Nozoe, S., *J. Amer. Chem. Soc.* **85,** 3527 (1963).
Cox, R. S., *Phytopathology* **46,** 112 (1956).
Davies, J. E., Kirkaldy, D., Roberts, J. C., *J. Chem. Soc.* 2169 (1960).
deMayo, P., Spencer, E. Y., White, R. W., *J. Amer. Chem. Soc.* **84,** 494 (1962).
deMayo, P., Williams, R. E., Spencer, E. Y., *Can. J. Chem.* **43,** 1357 (1965).
Dickens, F., Jones, H. E. H., Waynforth, H. B., *Brit. J. Cancer* **20,** 134 (1966).
Dickson, J. G., "Diseases of Field Crops," ed. 2, 1956, pp 74–114.
Dolan, C. T., Weed, L. A., Dines, D. E., *Amer. J. Clin. Pathol.* **56,** 235 (1970).
Drechsler, C., *J. Agr. Res.* **24,** 641 (1923).
Drechsler, C., *J. Agr. Res.* **31,** 701 (1925.)
Drechsler, C., *Phytopathology* **24,** 953 (1934).
Farrar, L. L., *Plant Dis. Rep.* **54,** 1099 (1970).
Fischer, N. H., "Blight in Corn Fields May Bring Sharp Rises in Prices of Meat, Eggs," *Wall Street Journal,* Aug. 25, 1970.
Fr. Patent 1,437,461 (May 6, 1966); *Chem. Abstr.* **66,** 9972e (1967).
Gerber, N. N., Lechevalier, H. A., *J. Org. Chem.* **27,** 1731 (1962).
Gray, W. D., "The Relation of Fungi to Human Affairs," Henry Holt and Co., New York, N.Y., 1959, pp 480–481.
Gruchow, N., *Science* **169,** 961 (1970).
Hale, M. G., Roane, C. W., *Phytopathology* **51,** 235 (1961).
Hamasaki, T., Hatsuda, Y., Terashima, N., Renbutsu, M., *Agr. Biol. Chem.* **31,** 11 (1967).
Hamilton, P. B., Nelson, R. R., Harris, B. S. H., *Appl. Microbiol.* **16,** 1719 (1968).
Heathcote, J. G., Dutton, M. F., *Tetrahedron* **25,** 1497 (1969).
Hilu, H. M., Hooker, A. L., *Phytopathology* **55,** 189 (1965).
Hirai, K., Nozoe, S., Tsuda, K., Iitaka, Y., Ishibashi, K., Shirasaka, M., *Tetrahedron Lett.* 2177 (1967).
Holker, J. S. E., Kagal, S. A., Mulheirn, L. J., White, P. M., *Chem. Commun.* 911 (1966).
Holzapfel, C. W., Purchase, I. F. H., Steyn, P. S., Gouws, L., *S. Afr. Med. J.* **40,** 1100 (1966).
Hooker, A. L., Smith, D. R., Lim, S. M., Beckett, J. B., *Plant Dis. Rep.* **54,** 708 (1970).
Hughes, S. J., *Can. J. Bot.* **36,** 727 (1958).
Inagaki, N., *Chem. Pharm. Bull.* **10,** 152 (1962).
Ishibashi, K., *J. Agr. Chem. Soc. Jap.* **35,** 257 (1961).
Ishibashi, K., *J. Antibiot. Ser. A* **15,** 88 (1962a).
Ishibashi, K., *J. Antibiot. Ser. A* **15,** 161 (1962b).
Jennings, P. R., Ullstrup, A. J., *Phytopathology* **47,** 707 (1957).
Kato, J., Shiotani, Y., Tamura, S., Sakurai, A., *Naturwissenschaften* **51,** 341 (1964).
Kline, D. M., Nelson, R. R., *Plant Dis. Rep.* **52,** 605 (1968).
Lim, S. M., Hooker, A. L., Paxton, J. D., *Phytopathology* **60,** 1071 (1970).
Lim, S. M., Paxton, J. D., Hooker, A. L., *Phytopathology* **58,** 720 (1968).
Luttrell, E. S., *Phytopathology* **48,** 281 (1958).
Luttrell, E. S., *Plant Dis. Rep. Suppl.* **201,** 59 (1951).
Malca, I., Ullstrup, A. J., *Bull. Torrey Bot. Club* **89,** 240 (1962).
Meredith, D. S., *Phytopathology* **55,** 1099 (1965).
Nakamura, M., Ishibashi, K., *J. Agr. Chem. Soc. Jap.* **32,** 739 (1958).

Neelakantan, S., Pocker, A., Raistrick, H., *Biochem. J.* **64,** 464 (1956).
Nelson, R. R., *Phytopathology* **47,** 742 (1957).
Nelson, R. R., *Phytopathology* **49,** 807 (1959).
Nelson, R. R., Kline, D. M., *Plant Dis. Rep.* **52,** 879 (1968).
Nelson, R. R., Scheifele, G. L., *Phytopathology* **60,** 369 (1970).
Nozoe, S., Hirai, K., Tsuda, K., *Tetrahedron Lett.* 2211 (1966).
Nozoe, S., Suzuki, K. T., Okuda, S., *Tetrahedron Lett.* 3643 (1968a).
Nozoe, S., Morisaki, M., Fukushima, K., Okuda, S., *Tetrahedron Lett.* 4457 (1968b).
Nozoe, S., Morisaki, M., Okuda, S., Tsuda, K., *Tetrahedron Lett.* 2347 (1968c).
Nozoe, S., Morisaki, M., Tsuda, K., Okuda, S., *Tetrahedron Lett.* 3365 (1967).
Nozoe, S., Hirai, K., Tsuda, K., Ishibashi, K., Shirasaka, M., Grove, J. F., *Tetrahedron Lett.* 4675 (1965a).
Nozoe, S., Morisaki, M., Tsuda, K., Iitaka, Y., Takahashi, N., Tamura, S., Ishibashi, K., Shirasaka, M., *J. Amer. Chem. Soc.* **87,** 4968 (1965b).
Ohkawa, H., Tamura, T., *Agr. Biol. Chem.* **30,** 285 (1966).
Owens, L. D., *Science* **165,** 18 (1969).
Pringle, R. B., Scheffer, R. P., *Annu. Rev. Phytopathol.* **2,** 133 (1964).
Pugh, L. H., Lechevalier, H. A., Solotorovsky, M., *Antibiot. Chemother.* **12,** 310 (1962).
Purchase, I. F. H., van der Watt, J. J., *Food Cosmet. Toxicol.* **8,** 289 (1970).
Raistrick, H., Robinson, R., Todd, A. R., *Biochem. J.* **27,** 1170 (1933).
Raistrick, H., Robinson, R., Todd, A. R., *Biochem. J.* **28,** 559 (1934).
Raistrick, H., Robinson, R., White, D. E., *Biochem. J.* **30,** 1303 (1936).
Robert, Alice L., "Plant Diseases, the Yearbook of Agriculture," 1953, pp 380–385.
Scheffer, R. P., Pringle, R. B., "The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction," C. J. Mirocha and I. Uritani, Eds., Bruce Publishing Co., St. Paul, Minn., 1967, pp 217–236.
Scheffer, R. P., Samaddar, K. R., *Recent Advan. Phytochem.* **3,** 123, (1970).
Scheifele, G. L., Nelson, R. R., *Plant Dis. Rep.* **53,** 186 (1969).
Scheifele, G. L., Whitehead, W., Rowe, C., *Plant Dis. Rep.* **54,** 501 (1970).
Scott, P. M., Somers, E., J. AGR. FOOD CHEM. **17,** 430 (1969).
Shoemaker, R. A., *Can. J. Bot.* **37,** 879 (1959).
Smedegard-Peterson, V., Nelson, R. R., *Can. J. Bot.* **47,** 951 (1969).
Smith, D. R., Hooker, A. L., Lim, S. M., *Plant Dis. Rep.* **54,** 819 (1970).
Spencer, E. Y., Ludwig, R. A., Mayo, P. de, White, R. W., Williams, R. E., *Advan. Chem. Ser.* **53,** 106 (1966).
Spooner, B. S., Wessells, N. K., *Proc. Nat. Acad. Sci.* **66,** 360 (1970).
Sprague, R., "Diseases of Cereals and Grasses in North America," Ronald Press, New York, N.Y., 1950, 538 pp.
Tamura, S., Sakurai, A., Kainuma, K., Takai, M., *Agr. Biol. Chem.* **27,** 738 (1963).
Tsuda, K., Nozoe, S., Morisaki, M., Hirai, K., Itai, A., Okuda, S., Canonica, L., Fiecchi, A., Galli Kienle, M., Scala, A., *Tetrahedron Lett.* 3369 (1967).
Ullstrup, A. J., "Southern Corn Leaf Blight in Indiana. A Backgrounder," Purdue Univ., Coop. Ext. Serv., 1970, pp 4–5.
Ullstrup, A. J., *U.S. Dep. Agr., Agr. Handb.* **199,** 1–44, 8 Plates (1966).
Ullstrup, A. J., *J. Agr. Res.* **63,** 331 (1941a).
Ullstrup, A. J., *Phytopathology* **31,** 508 (1941b).
Ullstrup, A. J., *Phytopathology* **34,** 214 (1944).
Ullstrup, A. J., Miles, S. R., *Phytopathology* **47,** 331 (1957).
Wheeler, H., Luke, H. H., *Annu. Rev. Microbiol.* **17,** 223 (1963).
White, G. A., Taniguchi, E., *Can. J. Bot.* **47,** 873 (1969).

Received for review January 29, 1971. Accepted March 29, 1971. Laboratory of the Northern Marketing and Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Ill. 61604. Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.