# Toxins in Plant Disease: Structure and Mode of Action

Toxins may cause disease symptoms by inhibiting enzymes or changing the permeability of membranes.

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The concept that plant pathogens cause disease by producing toxic substances dates back about a century. Evidence for its general validity, however, has accumulated only recently. The difficulty has been that most plant disease symptoms are the result of a complex interplay of several factors. Some pathogens, in addition to producing one or more toxins, may also excrete enzymes which degrade cell walls, causing tissue disorganization; they may destroy plant hormones, causing abnormal growth of the host; or they may physically block the water-conducting vessels of the host by their prolific growth or by production of viscous polysaccharides. Because of this complexity, proof of the role of a toxin as one of several interacting factors causing a disease symptom is often difficult to obtain, even though the presence of toxic substances in cultures of most plant pathogens is easily demonstrated. Despite these problems, our growing knowledge of the biochemistry of symptom causation clearly establishes the role of toxins as a dominant one in most plant diseases.

In plant pathology a toxin is generally defined as a nonenzymic substance which injures plant cells or disrupts their metabolism. Plant toxins are of lower molecular weight than are animal toxins, and most do not induce antibody formation when injected into animals. Although the structure and mode of action of relatively few toxins have been thoroughly investigated, sufficient information is now available to permit an integrated presentation of the subject. I will discuss here only those toxins produced by pathogens for which data on their chemical nature and mode of action are available. This includes two antimetabolites, several host-specific polypepetides, and a number of miscellaneous compounds of low molecular weight. Pathogen-produced enzymes and plant hormones will not be considered.

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## Antimetabolite Action of Wildfire Toxin

Wildfire disease in tobacco is a much cited example in which one symptom is caused by an antimetabolite synthesized by the pathogen. The phytopathogenic bacterium *Pseudomonas tabaci* produces a toxin which causes chlorosis in the form of a halo around the necrotic locus of infection (*1*). The toxin, isolated from cultures of the bacterium, is capable of inducing chlorosis in many plant species other than tobacco (*2*).

Woolley and associates (*3*) first isolated wildfire toxin and proposed a tentative structure, $\alpha$-lactylamino-$\beta$-hydroxy-$\varepsilon$-aminopimelic acid lactone (*4*), a modified dipeptide lactone. Later, an attempted synthesis led them to postulate that the correct structure was an unspecified structural isomer of the one first proposed (*5*).

The mode of action of wildfire toxin was investigated concurrently by Braun (*6*) using sterile culture filtrates of *P. tabaci* which contained the toxin and the unicellular green alga *Chlorella vulgaris* as a test organism. Growth of this alga was inhibited by the toxin, but the inhibition could be prevented by adding extracts of liver or yeast. L-Methionine was found to be the factor capable of preventing toxicity to chlorella, thus suggesting that the toxin was an antimetabolite of L-methionine. In support of this reasoning, Braun further demonstrated that a competitive relationship existed between the amount of toxin in the medium and the amount of L-methionine required to prevent growth inhibition. However, attempts to prevent chlorosis in tobacco leaves by adding L-methionine failed.

Additional support for the methionine antimetabolite theory was sought with a newly discovered structural analog of methionine, methionine sulfoximine (MSO) (Fig. 1), an animal convulsant isolated from bleached wheat flour. Methionine sulfoximine caused chlorotic halos when injected into tobacco leaves, and it inhibited growth of chlorella (*1*). As in the case of wildfire toxin, MSO toxicity to chlorella, but not to tobacco, was prevented by simultaneous addition of L- methionine. The parallel toxicities exhibited by the toxin and MSO were ascribed to the hypothesis that both were structural analogs and antimetabolites of L-methionine. Further evidence to support this hypothesis was that mutants of chlorella selected for resistance to MSO were equally resistant to the toxin (*1*).

The pioneering work of Braun and Woolley made a large contribution to the understanding of certain plant diseases. They first alerted plant pathologists to the possibility that pathogens may disrupt the metabolism of their hosts by producing antimetabolites. This important concept has stood the test of time. Nevertheless, their hypothesis that wildfire toxin was specifically an antimetabolite of methionine discounted several incongruities. (i) The tentative structure proposed for wildfire toxin bore little structural resemblance to methionine. (ii) Methionine failed to prevent toxicity to tobacco leaves by either wildfire toxin or MSO. (iii) The parallel toxicities of toxin and MSO and resistance of chlorella mutants to both compounds, while suggesting similar mode of action, did not necessarily implicate methionine antagonism. As Braun (*1*) pointed out, the mechanism of MSO toxicity was not fully understood, since MSO inhibition of bacterial growth could be reversed equally well by either methionine (*7*) or glutamine (*8*). Further, MSO had been reported to inhibit a glutamine-synthesizing enzyme from *Staphylococcus aureus* and sheep brain (*9*). In recognizing this ambiguity Braun (*1*) tested L-glutamine

for its ability to prevent MSO toxicity to chlorella; none was observed. Finally, the possibility existed that methionine prevented MSO and toxin inhibition in chlorella by preventing uptake of these two compounds, as was later found to be the case for MSO entry into animal cells (*10*), and not by competing for a metabolic site within the cell.

Following this initial work, which demonstrated the similarities in biological activities of MSO and wildfire toxin, evidence continued to accumulate that MSO was an inhibitor of glutamine synthesis and that amelioration of MSO toxicity in animals by methionine was due to indirect effects (*11*). Also, rather high concentrations of glutamine were shown to prevent MSO toxicity to wheat embryos (*12*). Thus, MSO became recognized as an antimetabolite of glutamic acid and not of methionine in some organisms.

The question of the mode of action of wildfire toxin was reopened by Sinden and Durbin and associates when they found that injection of toxin into the brain of rats caused convulsions similar to those caused by MSO (*13*). They proceeded to demonstrate that both wildfire toxin and MSO inhibited glutamine synthetase prepared from rat brain (*13*) and from pea (*14*). This enzyme catalyzes the reaction

$$\text{glutamic acid} + NH_3 + ATP \overset{Mg^{2+}}{\rightleftharpoons} \text{glutamine} + ADP + P_i$$

in which $\gamma$-glutamyl phosphate is postulated to be an enzyme-bound intermediate (*15*).

Toxin inhibition of glutamine synthetase from pea was a mixed-type, that is, a combination of partially competitive and noncompetitive inhibition with respect to glutamic acid (*13*). Ronzio and Meister (*15*) have attempted to explain a similar mixed-type inhibition of glutamine synthetase by MSO by postulating a two-step inhibition process. The rate of the first step, presumably binding of MSO to the catalytic site, is competitive with glutamate. The second step, formation of MSO phosphate which remains tightly bound to the enzyme, is not reversible by glutamate, and hence, is noncompetitive. The parallel inhibition kinetics of toxin and MSO toward glutamine synthetase would suggest a similar mechanism of inhibition by the two compounds.

The structural analogy of MSO and glutamate (*15*) is shown in Fig. 1. Apparently, the oxo and imino functions of MSO are necessary for attachment to the enzyme binding sites normally occupied by the $\gamma$-carboxyl group of glutamate and ammonia. Lack of these functions in methionine explains why it neither inhibits glutamine synthetase nor protects the enzyme against MSO inhibition (*11*).

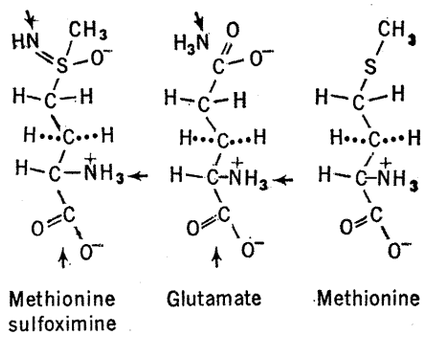



Fig. 1. Possible orientations of methionine sulfoximine, glutamate, and ammonia on glutamine synthetase. Arrows indicate possible sites of attachment. [Taken from Ronzio and Meister (*15*).] The structure of methionine is shown to illustrate the lack of structural analogy to the other two compounds regarding the $\gamma$-carbon functional groups.

Sinden and Durbin further related this in vitro enzyme inhibition to cause of symptoms in tobacco. They found that concentrations of toxin or MSO inhibitory to glutamine synthetase in vitro caused chlorosis in tobacco leaves (*13*). Treated leaves also suffered a buildup of ammonia to concentrations about seven times that of untreated leaves. Both chlorosis and ammonia buildup were prevented by simultaneous injection of high concentrations of L-glutamine into the leaves. This probably means that either glutamine prevented cellular uptake of the toxic compounds or that it protected glutamine synthetase from being inhibited. Either action would account for the lower ammonia levels.

On the basis of their results, Sinden and Durbin propose that inhibition of glutamine synthetase may be the primary action of tobacco wildfire toxin (*13*). They further suggested that the chlorosis symptom is more likely caused by toxic amounts of ammonia or other intermediates of nitrate metabolism rather than by induced deficiency of glutamine. The ammonia presumably arises from continued nitrate reduction by the plant while one of its main pathways for ammonia incorporation into organic compounds is blocked.

One remaining question is the structure of wildfire toxin. Sinden and Durbin (*16*) recently reported that the toxin contains a threonine moiety, which was not originally found by Woolley *et al.* (*3*).

*Pseudomonas tabaci* is only one of several species of this genus that cause diseases in various plants. Do all phytotoxic pseudomonads produce the same toxin? Sinden and Durbin (*16*) have isolated a toxin from *P. coronafaciens* (causing halo blight of cereals) cultures which is identical to wildfire toxin. On the other hand, they have obtained considerable evidence to suggest that the halo-inducing toxins from *P. phaseolicola* and *P. tomato* (bean and tomato pathogens) are different from wildfire toxin (*17*). Also, DeVay, Sinden, and co-workers (*18*) have isolated a polypeptide toxin from cultures of the peach tree pathogen, *P. syringae* [= *P. morsprunorum* (*19*)].

## Antimetabolite Action of Rhizobium Toxin

Rhizobial-induced chlorosis in soybeans is the second example of a disease thought to be caused by an antimetabolite produced by the pathogen. This disease arises from a rather unique association between two organisms, an association that is both symbiotic and pathogenic. Certain strains of the legume root-nodule bacterium *Rhizobium japonicum* fix nitrogen in an apparently normal fashion and simultaneously synthesize a toxin which induces chlorosis in the new leaf growth of the soybean host plant (*20*). This toxin has been isolated from the nodules and chlorotic leaves of diseased plants (*20*) and from the culture medium of the bacterium (*21*).

The toxin from *R. japonicum*, "rhizobitoxine" (*22*), is not host-specific. At low concentrations it causes chlorosis in seedlings of many plants (*20, 23*). Because the toxin apparently causes all of the disease symptoms and because toxin production in the nodule is essential to disease expression (*20*), rhizobitoxine may be classified as a primary disease determinant. This disease is defined as those aspects of the association that are not part of the normal symbiotic relationship and that are clearly deleterious to the host.

The precise structure of the toxin has yet to be determined. It is known to be a basic sulfur-containing amino acid. Upon desulfurization, it yields a nontoxic amino acid (termed unknown *Y*)

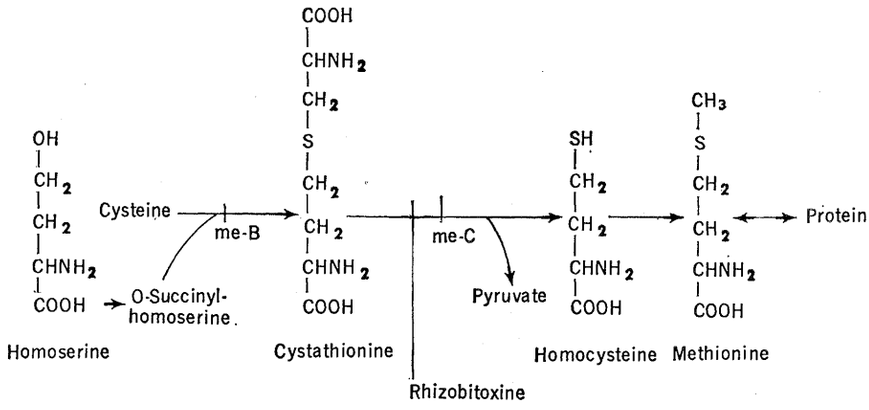



Fig. 2. Pathway of methionine biosynthesis in *Salmonella typhimurium*. β-Cystathionase catalyzes the cleavage of cystathionine to form homocysteine and is inhibited by rhizobitoxine. Metabolic blocks of methionine-requiring mutants, me-B and me-C, of *Salmonella* are indicated.

that also occurs naturally in soybean nodules and in *R. japonicum* cultures (*24*). Unknown *Y* has been tentatively identified as an ether derivative of homoserine (*24*), R-O-$CH_2$-$CH_2$-CH ($NH_2$)-COOH, where R is a three-carbon moiety containing an amino and a hydroxyl group (*25*).

Several lines of evidence suggest that the toxin is composed of two molecules of unknown *Y* joined through a thioether linkage (*24*). This interpretation is attractive considering mode of action hypotheses, as will be discussed later, but other interpretations have not been ruled out.

A logical approach to determining the mode of action became possible once the toxin was known to be a sulfur-containing amino acid. One would obviously suspect toxin interference with the metabolism of one of the naturally occurring sulfur-containing amino acids. Initially, attempts to prevent chlorosis in sorghum seedlings by adding methionine to the root solution along with the toxin gave ambiguous results. Chlorosis was apparently reduced but at methionine concentrations that bordered on being toxic (*26*).

*Salmonella typhimurium* proved to be a much more suitable organism for investigating the mechanism of action. Growth of this bacterium was inhibited by low concentrations of rhizobitoxine (0.25 mmole/liter), and the inhibition could be completely prevented by simultaneously adding small amounts of L-methionine or its immediate precursor, homocysteine (*22*). The toxin inhibition was not ameliorated, however, by adding earlier precursors of methionine, namely, cystathionine and homoserine (Fig. 2).

The possibility that cystathionine was simply not entering the cells was eliminated by performing the same experiment with a methionine-requiring mutant (me-B in Fig. 2) that can utilize cystathionine as a source of methionine. The same results were obtained as with the wild type.

These experiments suggested that the toxin was inhibiting the enzymatic cleavage of cystathionine to form homocysteine, thereby inducing a deficiency of methionine. We found this to be true in experiments with the isolated cleavage enzyme (*22*). The apparent $K_m$ of β-cystathionase for its substrate cystathionine was 0.36 mmole/liter while the apparent $K_i$ for the toxin was 0.02 μmole/liter. Thus, the enzyme affinity for the toxin was over 10,000 times that for its substrate. The toxin inhibition was mixed type.

It was concluded that the primary site of action of rhizobitoxine in *Salmonella* was the inhibition of β-cystathionase. The deficiency of methionine induced thereby resulted in decreased rates of protein synthesis and, consequently, decreased growth.

The question as to whether this same mechanism was operative in plants was investigated with a strain of the unicellular green alga *Chlorella pyrenoidosa* that is extremely sensitive to the toxin (*26*). Addition of L-methionine to the culture medium along with the toxin provided partial relief of growth inhibition.

The effect of rhizobitoxine on β-cystathionase isolated from spinach leaves has been determined (*27*). As in the case of *Salmonella*, the toxin proved to be a potent inhibitor of this enzyme. Thus, rhizobitoxine is an antimetabolite of cystathionine in both *Salmonella* and spinach. A spinach seedling develops chlorosis from 1 μg of toxin added to its root solution.

Thus, there is considerable evidence that the mechanism of rhizobitoxine action in higher plants is the same as in *Salmonella* (*27*). Although the pathway of methionine biosynthesis in plants has not been fully elucidated, there is evidence to suggest it is similar to that in *Salmonella* (*28*). β-Cystathionase activity has been observed in cell-free extracts of a number of higher plants, thus suggesting its involvement in methionine biosynthesis (*29*). It seems likely, therefore, that β-cystathionase inhibition is a major, if not the sole, site of rhizobitoxine action in higher plants and hence the basis of this disease.

What structural features of rhizobitoxine account for its antimetabolite action against cystathionine? To answer this question we need to know both what part of the cystathionine molecule (Fig. 3) is involved in binding to the β-cleavage enzyme and where the sulfur substituent is located in the toxin molecule. Neither are known. However, a thioether substituent on C-4 of unknown *Y* would provide analogy to the four-carbon moiety of cystathionine, while the same substituent on C-3 would resemble the three-carbon moiety (Fig. 3).

## Host-Specific Polypeptide Toxins

Several phytopathogenic fungi are known to produce polypeptide toxins. These are highly specific compounds that only affect the host of the pathogen, and they are primary determinants of the diseases in which they are involved (*30*). They are smaller than animal polypeptide toxins and resemble certain polypeptide antibiotics and animal hormones, both in size and structure (*31*). The apparent similarities among these various classes of biologically active polypeptides may even extend to their modes of action. Certain members of each class are postulated to exert their biological influence primarily by altering the permeability of cell membranes (*32*).

*Helminthosporium victoriae* causes blight only in Victoria variety of oats or in its derivative cultivars (*30*). Victoria was imported from South America and used extensively to breed smut and crown rust resistance into North American oat varieties. Shortly after the release of these cultivars, in about 1955, "Victoria blight" became epidemic throughout the United States.

The primary determinant of this disease, a potent toxin called victorin, was discovered by Meehan and Murphy in 1947 (*33*). Pringle and Braun isolated and partially characterized the toxin as a polypeptide derivative with a molecular weight between 800 and 2000 (Table 1) (*34*). Mild alkali treatment cleaved the toxin into a biologically inactive polypeptide moiety composed of five or six common amino acids and a nitrogen-containing sesquiterpine moiety called victoxinine (*30, 35*). Victoxinine is 1/7500 as toxic as the intact toxin, is not host-specific, and is produced in a free form by both pathogenic and nonpathogenic isolates of *H. victoriae*. Its role as a disease determinant is therefore doubtful, except as an integral part of the toxin.

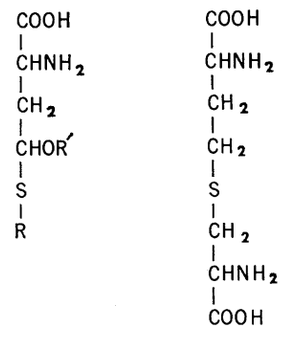
The cleaved peptide moiety of victorin is nontoxic and, when added to solutions of the intact toxin, reduces its toxicity (*36*). This suggests that the peptide is competing for toxin receptor sites and, therefore, that toxin specificity is conferred by its peptide moiety. The finding that sulfite also reduces toxicity without affecting the toxin is some evidence for the involvement of carbonyl groups in the receptor site (*30*). According to a preliminary report, bisulfite and semicarbazide also protect tissue against toxin-induced electrolyte loss into a bathing solution (*37*).

Although many secondary effects of the toxin from *H. victoriae* have been reported, the primary effect is now postulated to be an alteration in cell membrane permeability. Wheeler and Black (*38*) showed that toxin-treated or infected tissue from a susceptible oat variety rapidly begins to lose electrolytes into a bathing solution. This electrolyte loss commences within 5 minutes after toxin treatment, has a low temperature coefficient characteristic of a physical process, and is induced by 1/50 the concentration required to increase respiration. Tissue from resistant oat varieties was not affected, which shows that these are host-specific and not general effects.

Further evidence for the membrane-alteration theory was obtained by Samaddar and Scheffer (*39*) with plant cell protoplasts, that is, cells stripped of their cell walls by enzymic digestion but retaining an intact cell membrane. Protoplasts from susceptible oat tissue quickly stopped protoplasmic streaming and burst within 1 hour after toxin treatment. Protoplasts from resistant oats were little affected.

Luke and co-workers (*40*) have obtained electron-microscopic evidence for selective toxicity toward cell membranes. The endoplasmic reticulum, nuclear envelope, and mitochondrial membranes were more resistant to toxin damage. These findings have been corroborated by studies showing that neither succinoxidase activity (*41*), respiratory control (*42*), nor permeability (*38*) of mitochondria isolated from susceptible oat tissue was affected by toxin treatment.

*Helminthosporium carbonum* is closely related to *H. victoriae* but pathogenic to certain corn hybrids. The fungus produces a host-specific toxin in pure culture, which has been isolated and crystallized by Pringle and Scheffer (*43*) (Table 1). Acid hydrolysis yielded amino acids or amino-containing degradation products. This fungus also produces a second toxic polypeptide, carbtoxinine. It is much less toxic (Table 1) and lacks host specificity. Unlike victoxinine, there is no indication that carbtoxinine is a part of the toxin from *H. carbonum*. Its role as a disease de-




Rhizobitoxine Cystathionine

Fig. 3. A possible structure of rhizobitoxine illustrating analogy to the four-carbon moiety of cystathionine.

Table 1. Properties of polypeptide and related plant toxins.

| Toxin | Host-specific | Host | Lowest toxic concentration ($\mu$g/ml) | Chemical nature | Elemental proportions, or known products of hydrolysis |
|---|---|---|---|---|---|
| *Helminthosporium victoriae* toxin (victorin) | Yes | Oats | 0.01 | Polypeptide derivative, ninhydrin negative | Aspartate, glutamate, valine, glycine, leucine and/or isoleucine victoxinine |
| Victoxinine* (*H. victoriae*) | No | Oats | 75 | *N*-containing sesquiterpene | $C_{17}H_{29}NO$ |
| *Helminthosporium carbonum* toxin | Yes | Corn | 0.5† | Polypeptide, ninhydrin negative | Amino compounds |
| Carbtoxinine (*H. carbonum*) | No | Corn | 25† | Polypeptide, ninhydrin positive | Amino compounds |
| *Periconia circinata* Toxin A | Yes | Grain sorghums | 0.1 | Polypeptide | Six alanine, four aspartate two glutamate, two serine |
| Toxin B | Yes | | ? | Polypeptide | ? |
| *Corynebacterium sepidonicum* toxin | No | Potato | 500 | Glycopeptide | $C_{48}H_{96}O_{48}N$; MW21,450; threonine, serine, glycine, alanine, lysine, two methionine, glucose, mannose, and two unidentified sugars |
| *Corynebacterium michiganense* toxins | No | Tomato | 400 | Glycopeptides | I: $C_{42}H_{82}O_{40}N$; MW129,700<br>II: $C_{478}H_{940}O_{449}N$; MW>200,000<br>III: $C_{27}H_{52}O_{25}N$; MW35,280<br>All contain six amino acids and four sugars |
| *Colletotrichum fuscum* toxin (colletotin) | No | Digitalis | 60 | Glycopeptide ? | Glucose, galactose, mannose; peptide moiety ? |

* Occurs naturally in free form and also as a constituent of *H. victoriae* toxin. † Concentration causing one-third to one-half inhibition.

terminant is questionable. Corn tissue treated with the toxin from *H. carbonum* shows none of the secondary metabolic disturbances observed with the toxin from *H. victoriae* (*44*). Dark $CO_2$ fixation appears to be stimulated (*45*).

*Periconia circinata* is the cause of "milo disease" in certain grain sorghums. In 1948 Leukel (*46*) discovered that a host-specific toxin was involved in milo disease. Two host-specific polypeptide toxins, termed A and B, were subsequently isolated from culture filtrates by Pringle and Scheffer (*47*) (Table 1). Toxin A, the major toxin, (molecular weight <2000) may possess a nonamino acid moiety. It induces metabolic changes in susceptible corn tissue in a fashion resembling that of the toxin from *H. victoriae* (*35*).

## Glycopeptide Toxins

Most polysaccharides excreted by certain phytopathogenic bacteria cause wilting of the host plant by physical obstruction of water-conducting vessels (*48*). Several polysaccharide toxins possess a polypeptide moiety which increases the possibility that their mechanism of action is not merely physical obstruction.

Four glycopeptide wilt toxins have been isolated from two species of *Corynebacterium* and partially characterized (Table 1) by Rai and Strobel (*49*). They proposed that the toxins play a major role in production of disease symptoms (*49*). Several lines of evidence, including autoradiography of cells treated with labeled toxin and electron-microscopic observations, suggest that the mode of action involves membrane changes and not plugging of vascular elements.

Colletotin is a nondialyzable toxin produced by the phytopathogenic fungus *Colletotrichum fuscum*. Goodman (*50*) partially purified the toxin and obtained good evidence for a polysaccharide moiety. Proof of the existence of a suspected peptide moiety is still lacking. The toxin appears to act by causing disintegration of leaf mesophyll and palisade tissue (*51*).

## Other Toxins

The structures of several plant toxins produced by fungi are shown in Fig. 4. Most are secondary disease determinants, that is, they contribute to a disease but are not absolutely essential for its development. As will be noted, the role of several in symptom causation is unclear.

*Fusarial Wilt Toxins.* Because fusarial wilt is an economically important disease of many crops, it has received much attention (*52–59*). The fusarial wilt syndrome appears to be the result of complex interactions of a pathogen-produced plant hormone, several toxins, and toxic enzymes. The syndrome usually includes epinasty, plugging and browning of xylem vessels, necrosis, wilt, and ultimately death. Various toxins have been implicated, including fusaric acid, dehydrofusaric acid, $\alpha$-picolinic acid, phytonivein, novarubin and several related compounds, and lycomarasmin—but not all in the same diseased plant.

Fusaric acid (Fig. 4) is produced by cultures of many species of *Fusarium* (*53*), and this acid has been detected in tomato (*54, 55*) and wilted cotton (*56*). It forms a weak chelate with metal ions (*60*) and inhibits rice catalase by forming a complex with its heme-Fe prosthetic group (*61*). The inhibition depends upon the presence of the carboxyl group in the fusaric acid molecule and can be reversed by addition of $Fe^{3+}$. The toxin does not inhibit enzymes lacking bound metals. The actual concentrations of fusaric acid in the host tissue need to be known before metal-enzyme complexing can be established as a mechanism of action.

Fusaric acid also caused electrolyte loss, wilting of tomato cuttings (*57, 53*), and changes in cell permeability of rice tissue (*54*) and of *Rhoeo discolor* epidermis (*62*). Some cells are affected by as little as $10^{-9}M$ fusaric acid (*55*). Electrolyte leakage also occurred from fusaria-infected tomato leaves (*63*). $\alpha$-Picolinic acid (Fig. 4) and dehydrofusaric acid, the *n*-butylene analog of fusaric acid, apparently have toxic properties and mechanisms similar to those of fusaric acid (*62, 64*).

Phytonivein was isolated from cultures of the watermelon wilt organism, *F. oxysporum* f. *niveum* (*58, 65*). It has an empirical formula $C_{29}H_{46}O_2$ and many properties of steroids. At low concentrations it permanently wilts watermelon seedlings.

Kern and Naef-Roth have isolated four phytotoxic pigments (naphthazarin derivatives) from various cultures of *Fusarium solani* and from diseased pea (*66*). The structure of one, novarubin, is shown in Fig. 4 (*67*). The severity of disease expression in pea from infection or from toxin treatment was diminished by adding certain metal ions, providing indirect but not conclusive evidence for the involvement of these pigments in symptom causation (*66*). The four toxins presumably cause wilting in seedlings of various plants by affecting the water permeability of the cell membranes.

Lycomarasmin is a toxin produced by *Fusarium oxysporum* f. *lycopersici*. The suggested role of lycomarasmin as a secondary determinant of tomato wilt disease is a matter of controversy (*52, 53, 57*). Woolley (*68*) postulated lycomarasmin to be the modified tripeptide shown in Fig. 4. The synthesized compound was as toxic as the natural product (*69*). Lycomarasmin causes wilting of tomato cuttings (*53*). Its toxicity is increased tenfold by the presence of iron, with which it forms a weak chelate (*53, 70*), but it is detoxified by other metal ions (*58*). In the presence of iron, lycomarasmin causes increased transpiration of tomato cuttings and increased water permeability of epidermal strippings from *Rhoeo discolor* leaves (*58*). When *Tradescantia* leaves were treated with lycomarasmin, protoplasmic streaming ceased within 30 minutes (*52*). Damage to cell membranes appears to be an early effect.

Blast disease is one of the two principal diseases of rice. Certain symptoms—leaf desiccation and yellowing and stunting of seedlings—are attributed to two toxins produced in cultures of the fungal pathogen *Piricularia oryzae* and in diseased plants (*71*). One toxin was identified as $\alpha$-picolinic acid (Fig. 4). The other, piricularin, was obtained crystalline by Tamari and Kaji (*71*), who proposed a tentative empirical formula of $C_{18}H_{14}N_2O_3$. It exhibited some specificity for susceptible varieties, and 1 $\mu$g/ml enhanced respiration in rice seedlings.

Helminthosporium leaf spot, the other principal disease of rice, is caused by the fungus *Cochliobolus miyabeanus* (*Helminthosporium oryzae*). Nakamura and co-workers (*72*) have isolated the toxin ophiobolin (Fig. 4) from cultures of the fungus and have obtained evidence for its production in diseased plants (*73*). Treatment of rice leaves with 10 $\mu$g of ophiobolin per milliliter causes necrotic spots resembling those of the disease and inhibition of rice seedling root elongation.

Ophiobolin induces production of

phenolic compounds in rice leaves (*72*), a phenomenon also observed with some other plant toxins. Subsequent oxidation and polymerization of these phenolics produces the brown pigments apparent in necrotic spots.

A related fungus, *Cochliobolus sativus*, causes common root rot of cereals, particularly wheat and barley. Fungal attack of barley seedlings causes necrosis and stunting, effects that can be reproduced by treatment with helminthosporal. This toxin, isolated from culture filtrates of *C. sativus*, is a sesquiterpenoid dialdehyde (Fig. 4) (*74*). It is probably produced by the fungus during invasion.

Helminthosporal inhibits both electron transfer and oxidative phosphorylation in isolated mitochondria (*75*). The site of electron transfer inhibition appears to be between flavoprotein dehydrogenase(s) and cytochrome c. Inhibition of these respiratory processes may constitute the mechanism of phytotoxicity, since seedlings treated with helminthosporal suffer respiration inhibition. It is interesting that a reduced analog of helminthosporal, namely helminthosporol, has gibberellin-like growth-promoting properties.

*Alternaria solani* causes early blight disease of various Solanaceae such as potatoes and tobacco. Alternaric acid, a hemiquinone derivative (Fig. 4) (*76*), has been isolated from culture filtrates of the fungus (*77*) and is thought to be a secondary determinant of this disease (*57, 58*). Alternaric acid causes severe wilt of seedlings of many plants at 5 $\mu$g/ml (*53, 77, 78*).

Leaf and stem blight in zinnia, sunflower, and marigold has been attributed to the fungus *Alternaria zinniae*. Several symptoms in zinnia, including stem withering, chlorosis, and leaf-tip curling, appear to be caused by zinniol, a toxin isolated from culture filtrates of the fungus (*79*). The structure of zinniol is shown in Fig. 4 (*80*). At a concentration of 500 $\mu$g/ml it causes rapid loss of pigment from cut red beet stems and drastic shriveling of the stems, which suggests that cell membrane damage is an early effect (*79*).

Ascochitine is another example of a phytotoxic hemiquinone derivative. It was isolated from culture filtrates of *Ascochyta fabae*, the causal fungus of brown spot disease of broad bean, and characterized by Oku and Nakanishi (Fig. 4) (*81*). Application of 10 $\mu$g of ascochitine per milliliter to broad bean coleoptiles causes the brown necrotic spots characteristic of this disease. Concentrations of 100 $\mu$g/ml cause epidermal cells of *Rhoeo* discolor to leak pigment and to lose plasmolytic ability, which indicate alteration of the cell membrane (*81*). Ascochitine was detoxified by equimolar concentrations of $Fe^{3+}$, yielding a red complex (*82*).

Chestnut blight, caused by the accidentally imported fungus *Endothia parasitica*, virtually eliminated the American chestnut tree about 40 years ago. Gäuman and co-workers have isolated two toxins from cultures of the pathogen which are highly active in causing changes in water permeability of cells of *Rhoeo discolor* (*83*). Diaporthin, $C_{30}H_{18}O_{10}$, is active at $10^{-11}M$, while skyrin, a dianthraquinone (Fig. 4), is active at $10^{-6}M$ (*84*). Both are about equally toxic to tomato shoots. Although diaporthin induces symptoms of chestnut blight, apparently neither toxin has been isolated from diseased tissue.

## Problems and Prospects

We are now aware of the wide variety of compounds that cause disease symptoms; we know the chemical structure of a number of them, and we understand the mode of action of several. Still, we are unable to describe, in molecular detail, the mechanisms by which most toxins damage plant cells. With the few exceptions noted, the

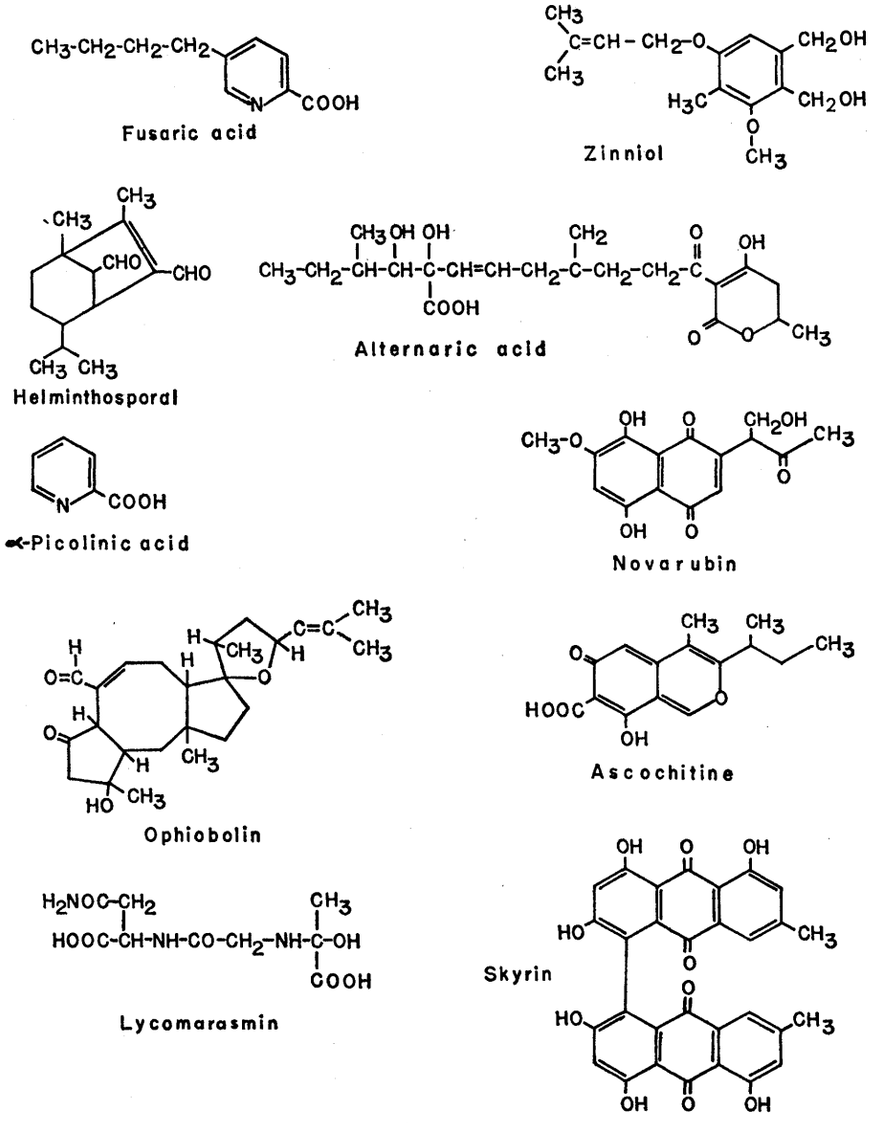



Fig. 4. Structures of pathogen-produced plant toxins: fusaric acid (*53*), zinniol (*80*), helminthosporal (*74*), alternaric acid (*76*), $\alpha$-picolinic acid (*71*), novarubin (*67*), ascochitine (*81*), ophiobolin (*72*), skyrin (*83*), and lycomarasmin (*68*).

most common earliest sign of toxin damage to cells is an alteration in water or ion permeability of the cytoplasmic membrane. Does this mean that each of these chemically diverse compounds reacts with a specific membrane component to alter its permeability characteristics? Is there any physical relationship between the water permeability and selective metabolite permeability characteristics of membranes? Is the membrane, in fact, the primary site of action?

The most interesting of toxins in regard to these questions are the host-specific polypeptides. The one most studied, the toxin from *Helminthosporium victoriae*, causes membrane damage within minutes at very low concentrations and exhibits specificity for the cytoplasmic membrane. One would like to know the amino acid sequence of its polypeptide moiety and whether it is in fact cyclic, as lack of ninhydrin reactivity would suggest. With this knowledge, it would be possible to investigate the relation between structural alterations—amino acid sequence, deletion, addition, and so forth—and biological activity. This approach has been widely used in studies of polypeptide antibiotics and hormones that affect membrane permeability (*31, 32, 85*).

One class of modified cyclic peptide antibiotics, represented by valinomycin, facilitates movement of alkali ions across membranes and in so doing alters their semipermeability (*31, 86*). The enniatins are members of this class synthesized by various species of *Fusarium*. Although enniatins injure tomato cuttings at low concentrations, their role in fusarial wilt has not been established (*55*). Studies relating possible capacities of polypeptide plant toxins to form ion complexes and their solubilities in lipids might be fruitful.

The mechanisms by which other toxins affecting membrane permeability operate may be more amenable to study than the polypeptide toxins. They are usually easier to isolate, more stable, and easier to label with a radioisotope. Use of labeled toxins should indicate whether or not the cell membrane is the primary site of action. Analogs of toxins of low molecular weight would be easier to prepare for determining the structural requirements for toxicity and the nature of the receptor site. Some of this work has been done with fusaric acid (*61*) and the toxin from *H. victoriae* (*30*).

Investigations of the way in which toxins affect membrane transport need not be limited by the complexities of intact cells. Membrane sacs (or vesicles), reconstituted from disrupted cytoplasmic membranes of bacteria, have been used to study selective transport of amino acids (*87*). They offer a simplified system which retains the selective transport properties of the intact cell membrane. Artificial membranes have also been proved useful for studying antibiotic-facilitated transport of ions (*86*), for determining membrane constituents necessary for antibiotic action of certain polyenes (*88*), and for predicting the effect of unsaturated hydrocarbon chains on water permeability of bean root membranes (*89*).

In the investigation of any subject one attempts to identify model systems from which he may generalize. The models of toxin-induced diseases of plants are not yet clear. The two antimetabolite toxins discussed are both produced by pathogenic bacteria. Are they typical of bacterial diseases? The host-specific polypeptide toxins are produced by apparently typical fungal pathogens, but are their toxins typical of pathogenic fungi in general? Are there yet-to-be discovered classes of plant toxins that will typify a large group of diseases?

These are questions of a relatively new interdisciplinary phase of plant pathology. With the techniques now available for isolating and identifying plant toxins and for studying their reactions with living cells, we can expect many exciting developments as we come to further understand plant disease.

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# Mitochondrial DNA: Advances, Problems, and Goals

Studies of size and structure of mitochondrial DNA relate to biogenesis and function of this organelle.

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The concerted efforts of biologists and biochemists have led to the discovery that deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are distinct components of cytoplasmic organelles, in particular of mitochondria and chloroplasts. Various problems pertaining to the heredity and biogenesis of mitochondria and chloroplasts, and to the structure, function, and synthesis of nucleic acids and proteins in these organelles, have been reviewed (*1–8*). It is characteristic of our present age of rapid scientific developments, involving exponentially growing numbers of individuals and teams, that the pertinent literature on mitochondrial DNA, which was in its infancy in the early 1960's (*8*), far exceeds the scope of an article in these pages today. I restrict this discussion, therefore, to the more recent advances in the structure and biochemistry of mitochondrial DNA and present some of the problems we confront at the present level of knowledge and insight (*9*).

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## Structure and Size of DNA Molecules

The most common conformation of mitochondrial DNA of multicellular animal cells is a double-stranded circle with a perimeter of 4.7 to 5.5 microns (*10, 11*) corresponding to a molecular weight of $9 \times 10^6$ to $10 \times 10^6$ daltons. Circular mitochondrial DNA has been described in most classes of vertebrates, including man (*12–15*), birds (*16*), and amphibians (*17*); it was found in sea urchin (*18*) and as a minor DNA component of mitochondria in yeast (*19*). The circular DNA molecules observed in the electron microscope consist of a mixture of highly twisted forms and loosely twisted, or open, types (Fig. 1). The twisted structure is typical of covalently closed DNA, and the loosely twisted forms may represent DNA with one or more single-strand scissions (nicked DNA). Other factors, however, are known that determine the degree of coiling. The DNA molecules can be separated on gradients consisting of cesium chloride and ethidium bromide by use of the principle that less dye (at high concentrations) binds to covalently closed circles than to nicked or linear DNA, and different buoyant densities are imparted to the various molecules regardless of base composition (*12*).

The very small differences in length that have been observed for mitochondrial DNA's from various cell types are frequently due to technical factors in the hands of different investigators. Generally, the ionic strength of the medium (hypophase) upon which the DNA molecules are spread as a DNA-protein monolayer affects the molecular lengths significantly (*20*). The molecules may shorten by about 10 percent at ionic strengths above 0.1 mole per liter, compared with distilled water as a hypophase. The existence of true size differences was shown by spreading different mitochondrial DNA's together as mixtures. The measurements of size obtained from a mixture of mitochondrial DNA from mouse fibroblasts (L cells) and from chicken liver followed a bimodal distribution with peak categories (4.7 to 4.8 $\mu$ and 5.1 to 5.2 $\mu$, respectively) corresponding to the size observed when each DNA was spread individually. The existence of small size