Host-Selective Toxins and Their Role in Plant Diseases

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Summary. Toxins with unusual characteristics are involved in some destructive diseases of plants. Certain parasitic fungi produce toxins of low molecular weight that selectively affect the host plant; nonhosts are tolerant. These toxins have diverse structures, including cyclic peptides and linear polyketols. Genetic and other data show that resistance to each fungus is based on tolerance to its toxin. The same fungal genes control toxin production and ability to cause disease. Little is known about toxic action, although one toxin selectively affects mitochondria. Plant cell membranes are affected; this may allow the fungus to colonize tissues. Resistant cells may lack toxin receptor sites.

Toxins from disease-inducing microorganisms are major factors in the development of a number of destructive diseases of plants. Two classic cases are often cited: the vast losses of oat production in North America in 1946 to 1948 and of maize production in 1970 to 1971 caused by fungi of the genus *Helminthosporium*. In both cases, toxins were the major factors in the destructive process (*1, 2*).

A number of other plant-infecting fungi and bacteria are now well known as toxin producers with economic impact. However, our concern in this article is with the contributions of toxin studies to an understanding of plant diseases at the molecular and ecological levels.

Toxins involved in the development of plant disease often are classified as host-selective (specific) or nonspecific; we are concerned here primarily with those in the selective category, for reasons that will be apparent. Selective toxins are produced by fungi that are specialized or restricted to certain plant cultivars and are toxic only to hosts of the fungus that produced the toxin. Nonspecific toxins, in contrast, are toxic to many plants, regardless of whether or not the plants are hosts of the producing microorganism (*1, 2*). As factors in disease development, nonspecific toxins may be comparable to toxins involved in certain animal diseases; nothing comparable to host-selective toxins is known for animal diseases.

The word toxin has various meanings, so we must define our terms. The plant-affecting toxins known to date have low molecular weights and are not antigenic, in contrast to toxins affecting animals. Plant pathologists reserve the word toxin for products of microbial pathogens that cause obvious damage to plant tissues and that are known to be involved in disease development. The latter restriction is necessary because of the many simplistic claims for toxic substances found in microbial cultures, for which there is no evidence of a role in disease development. (*2*). We must also keep in mind that toxic compounds involved in disease may affect the outcome of infection at concentrations well below the level required for obvious damage to tissues and that chemical determinants of disease are not necessarily toxic. These problems in usage do not negate the usefulness of the word toxin.

Toxins, as defined here, are probably not the only disease determinants at the disposal of plant pathogens. Other determinants may include release of hormone-like compounds and extracellular enzymes (*2*). The role of such factors is clearly defined in very few cases; further discussion is outside the scope of this article. The biochemical bases of disease development, disease resistance, and host specificity for plant diseases other than the ones involving host-selective toxins are largely unknown; there appear to be unidentified toxins or other factors that determine hosts.

Host-selective toxins are now known from 14 fungal species in six genera. Several are listed in Table 1 along with their shorthand designations. An example of the effect of selective toxicity is shown in Fig. 1. Toxins most studied by U.S. and Canadian workers include those from *Helminthosporium* species: *H. victoriae*, affecting oats; *H. maydis* race T, affecting maize with Texas male sterile (Tms) cytoplasm; *H. sacchari*, affecting sugarcane; and *H. carbonum* race 1, affecting maize. The other center of research on selective toxins is Japan, where investigators have concentrated on selective toxins from *Alternaria* species, especially *A. mali*, affecting apples, and *A. kikuchiana*, affecting the Japanese pear. Other well-known selective toxins are produced by *A. alternata* f. *lycopersici*, affecting tomatoes; *A. citri*, affecting citrus; *Periconia circinata*, affecting grain sorghum; *Phyllosticta maydis*, affecting Tms maize; and *Corynespora cassiicola*, affecting the tomato. In all cases there are susceptible and resistant genotypes of each host species. Other plant species are highly tolerant of the toxins (*2, 3*).

Selective Toxins as Key Determinants of Disease

There were many early reports of culture filtrates with selective toxicity to plants, but most of the claims were inconclusive and were soon disputed. A host-selective preparation from *A. kikuchiana*, cause of the black leaf-spot disease of Japanese pears, was reported in 1933 and was the first case to be confirmed. The work was overlooked until about 1950, when research was resumed (*4*). A much greater stimulus to research was provided by the report that cell-free culture filtrates of *H. victoriae* are very toxic to and very selective for a certain genotype of oats (*5*). The report was soon confirmed and extended (*4*). Over the ensuing years, the list of fungi that produce host-selective toxins gradually grew: *P. circinata* in 1961 (*6*); *H. carbonum* race 1 in 1965 (*7*); *A. mali* in 1966 (*8*); *H. maydis* race T in 1970 (*1, 2*); *H. sacchari* in 1971 (*9*); *A. alternata* f. *lycopersici* in 1976 (*10*); and strains of *A. citri* affecting either Dancy tangerine or rough lemon in 1979 (*11*). There are others (*2*).

Several pioneering studies established the significance of host-selective toxins in plant disease development. The most conclusive data came from genetic studies of the host plants and the fungi; the

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Fig. 1. Selective effect of *Periconia circinata* toxin on sorghum seedlings. Equal amounts of toxin were added to the nutrient solution of susceptible and resistant seedlings.

conclusions were supported by histological data. HV toxin from *H. victoriae*, which is released by germinating spores (*1, 4*), was shown to be a factor in initial colonization of oat tissues; the toxin also causes the early physiological changes characteristic of infections (*12*). Toxin concentrations used in many experiments were high enough to disrupt plant cells, which was often interpreted to mean that the fungus kills cells and colonizes dead tissue. Work with HC toxin from *H. carbonum* indicated that this interpretation is unlikely and that HC toxin has a more subtle role. HC toxin is slower to act and is less disruptive than HV toxin; initial effects of HC toxin are stimulatory to several metabolic processes (*13*). The histological data showed that sensitive cells are not killed or obviously damaged during the early stages of colonization by *H. carbonum* and that inhibitory substances from host cells do not account for resistance to this and related fungi. Toxinless mutants of *H. carbonum* and wild-type *H. victoriae* penetrate susceptible maize tissues and induce a necrotic fleck characteristic of a resistance reaction; HC toxin appears to inhibit this response (*14*). Cells in oat tissues also live compatibly with *H. victoriae* during early stages of infection, indicating that no more than minute levels of toxin are present at that time (*12*). Release of tracer amounts of toxins apparently allows the producing fungus to grow at the expense of host tissues. Toxin accumulates later, resulting in host cell death and systemic toxemia.

Genetic Studies of Toxin Production and Toxin Sensitivity

The genetic controls of resistance and susceptibility to *H. victoriae* and *H. carbonum* are well known. In each case, the reaction is controlled by a single pair of alleles; resistance to *H. victoriae* is recessive and to *H. carbonum* race 1 is dominant. Tolerance and sensitivity to each toxin is controlled by the same pair of alleles that controls resistance and susceptibility to the fungus. All oat and maize genotypes that are susceptible to these fungi are sensitive to their respective toxins. All plant species and genotypes that are resistant to the fungi are tolerant of their toxins. Genotypes that are intermediate in resistance are intermediate in sensitivity to the toxins. In oats the range of reaction to the fungus and its toxin is controlled by a gene locus (V_b) that has two or more alleles with semidominance; some alleles give high resistance, others are intermediate (*1*). In maize the reaction to *H. carbonum* and its toxin is controlled by a major gene locus with at least two alleles. There is also a minor gene for resistance (it gives a low level of resistance) on another chromosome (*1, 15*), but the effects of this gene on reaction to toxin are unknown. There is little information on genetic controls of resistance and susceptibility to toxins from other fungi, but there appear to be comparable patterns. Resistance and susceptibility to *H. maydis* T and to *Phyllosticta maydis* and its toxin are maternally inherited (*1*).

Genetic control of toxin production by several fungi has been examined. All isolates of *H. carbonum* and *H. victoriae* that produce HC and HV toxins also induce disease in their respective hosts; all isolates that fail to produce the toxins also fail to induce disease in oats and corn. These relations hold with wild-type isolates and with toxinless mutants (*1*). Some isolates of the two fungi are sexually compatible; the sexual stage is known as *Cochliobolus*. Matings of *C. victoriae* with *C. carbonum* race 1 gave progeny that produced HV or HC toxin, both toxins, or neither toxin in a 1:1:1:1 ratio. The progeny that produced only HV toxin caused disease only in oats with the V_b gene; those with only HC toxin caused disease in maize with the *hm* gene; those that produced both toxins caused disease in oats and maize of the susceptible genotypes; and those that produced neither toxin did not affect either plant species. Without exception, disease-inducing ability was correlated with toxin-producing ability (*16*).

There are also data on the genetic control of toxin production by *H. maydis* T. A single gene was shown to control virulence to corn with Tms cytoplasm; the same gene controls production of the chemically defined toxin that is essential for high virulence (*17*). The patterns of genetic control of toxin production by other fungi are not known, but the ability of isolates to produce host-selective toxins is known to be correlated with the ability to induce disease (*1*).

There are some limited uses for the host-selective toxins, and there is a potential for further applications. Plant breeders have used toxins to screen oat populations for resistance to *H. victoriae*, grain sorghum for resistance to *P. circinata*, and sugarcane for resistance to *H. sacchari* (*1*). Furthermore, there is widespread interest in using toxins to screen for resistant lines in cell culture research. Perhaps the greatest use will be in genetic engineering, once the techniques for gene transfer in higher plants are perfected. The best available models for gene manipulation to achieve disease resistance appear to be those involving the selective toxins. These models provide chemically characterized molecules, the final end products of genes for disease-inducing ability. The models also have single genes in the host plant that control resistance and susceptibility to these fungi; the same genes control sensitivity and tolerance to their respective toxins. An entirely different possibility is that we may find or make molecules that recognize many other plant species, leading to the development of highly selective herbicides.

Host-Selective Toxins as Factors in the Incidence of Plant Disease

Plant disease epidemics sometimes follow the introduction and widespread use of superior but genetically uniform crop cultivars. Such cultivars frequently are vulnerable to new or highly adaptable pathogens; thus, plant breeders must guard against "breeding" new pests along with new crop genotypes. A well-known example is the oat blight caused by *H. victoriae* that followed widespread planting of oats with a gene for resistance to *Puccinia coronata*. The southern leaf blight of maize, caused by *H. maydis* T, became devastating when most of the U.S. crop was planted in hybrids with Tms cytoplasm, used for economy in seed production. Epidemics caused by *Periconia circinata* in grain sorghum and *H. sacchari* in sugarcane had agronomic backgrounds comparable in some ways to that of the oat blight epidemic (*1*).

The role of a selective toxin in the

incidence of a plant disease is well illustrated by *H. maydis* and maize. Before 1968 *H. maydis* in the United States was, for some reason, confined largely to the Southeast and to the southern fringes of the Corn Belt. A new race of *H. maydis*, now known as race T, appeared in 1968; race T is indistinguishable from the old race (race O), except for the ability to produce HmT toxin that is specific for maize with Tms cytoplasm. Race T quickly spread throughout the Corn Belt, presumably as a result of its ability to produce HmT toxin. Race T overwintered throughout the Corn Belt, and was destructive again in 1971. After 1971 Tms maize was replaced by normal (N) cytoplasm maize, and *H. maydis* virtually disappeared from most of the Corn Belt. Race T may be a race O mutant that arose in the Corn Belt or was introduced from abroad (*2*).

There are examples of new plant diseases that are caused by new, toxin-producing races of *Alternaria* species. *Alternaria citri* was known in most citrus-producing areas as a weak or benign pathogen on senile tissues of various citrus crops. Virulent races of the fungus became locally destructive in Australia in 1964 and in Florida in 1974. The Australian race specifically affected the Emperor mandarin, whereas the Florida race affected the closely related Dancy tangerine (*2*). The new races have the same morphology as the previously known, nonspecialized form of *A. citri*, but differ in that the new races release metabolites with selective toxicity to the susceptible cultivars (*11*). The obvious hypothesis is that the new diseases resulted from the ability, acquired by mutation or otherwise, to produce toxins. Another race of *A. citri* occurs on the rough lemon in Florida; this race produces still another toxin that is host-specific (*11*). Similar or even better examples of toxin-producing forms of *Alternaria* have appeared in Japan (*8*).

Toxic effects also help to explain the seasonal occurrence of disease outbreaks. Temperatures > 34°C cause sorghum and sugarcane to lose sensitivity to toxins from *P. circinata* and *H. sacchari*, respectively; sensitivity is restored after several days at lower temperatures (*2, 18*). The sorghum disease was a problem early in the growing season, often disappeared in midsummer, and was again prevalent late in the season (*2*). Inoculated sorghum plants grow well with no symptoms at 35°C but quickly succumb at 22°C (*42*). A similar seasonal situation with the sugarcane disease is evident in Hawaii and south Florida (*2*). Sensitivity to toxin thus appears to be the major factor in the seasonal incidence of both diseases.

Chemical Structures of the Host-Selective Toxins

Characterizations of the host-selective toxins were delayed for many years, but good progress is now evident. Pringle and Braun (*19*) made the first attempts, but their data were not sufficient to suggest a structure for HV toxin. The molecule was thought to contain several amino acids and a base, later characterized as a sesquiterpene (*20*). The first selective toxin to be adequately characterized, a cyclic depsipeptide, is the product of *A. mali* (Fig. 2A); the structure was confirmed and the molecule was synthesized by Japanese investigators (*21*). Next, it was found that HmT toxin from *H. maydis* is not a single compound, but is a series of related linear polyketols with 35 to 45 carbon atoms (Fig. 2B) (*22*). Related but smaller compounds with selective toxicity to Tms maize were synthesized (*22*). More recently, the toxin from *A. alternata* f. *lycopersici* was shown to be a 1-aminodimethyl-heptadecapentol (*23*). *Helminthosporium sacchari* produces toxins containing β-1,4-galactofuranoside plus a sesquiterpene (three isomers); there are

Table 1. Frequently used shorthand designations for host-selective toxins. There are resistant and susceptible host genotypes for each.

Producing fungus	Species affected	Toxin designation
Helminthosporium carbonum race 1	Maize	HC
Helminthosporium maydis race T	Maize	HmT
Helminthosporium sacchari	Sugarcane	HS*
Helminthosporium victoriae	Oats	HV†
Perciconia circinata	Sorghum	PC
Phyllosticta maydis	Maize	PM
Alternaria kikuchiana	Japanese pear	AK
Alternaria citri (lemon race)	Rough lemon	ACL
Alternaria citri (tangerine race)	Dancy tangerine	ACT
Alternaria mali	Apple	AM
Alternaria alternata f. *lycopersici*	Tomato	AL

*Also known as helminthosporoside. †Also known as victorin.

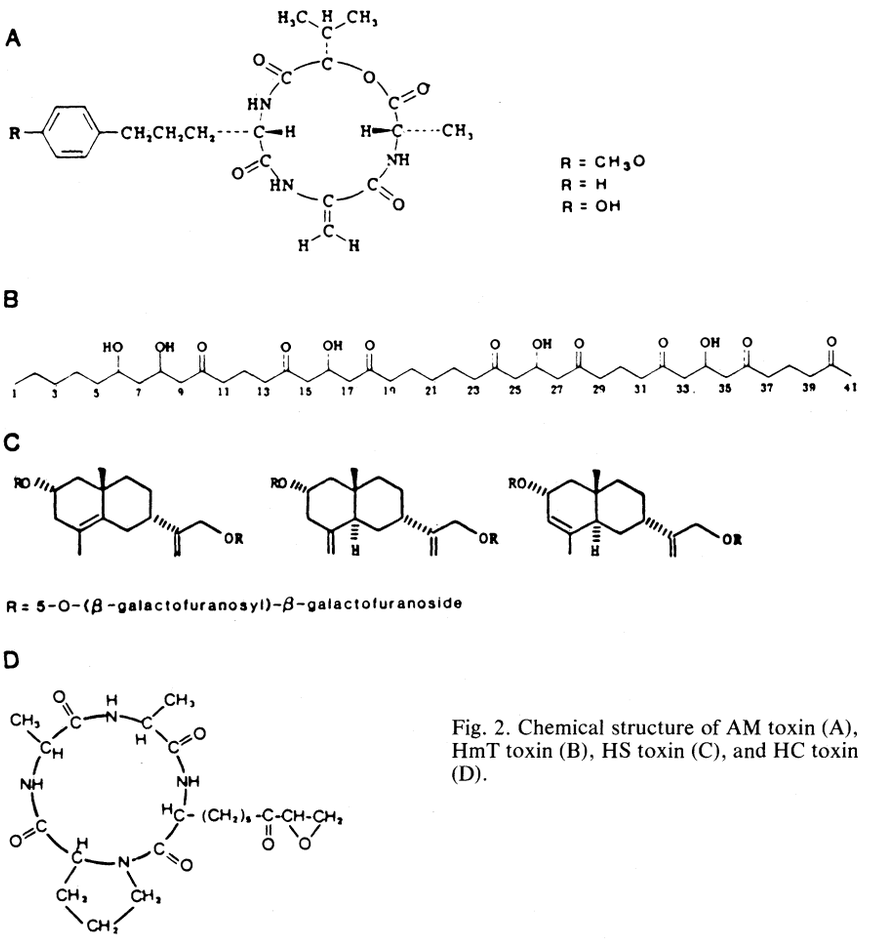

Fig. 2. Chemical structure of AM toxin (A), HmT toxin (B), HS toxin (C), and HC toxin (D).

two galactose units on either side of the sesquiterpene core (Fig. 2C) (*24*). HC toxin from the maize leaf-spot pathogen (*H. carbonum*) is a cyclic tetrapeptide with an epoxide group (Fig. 2D) (*25*). The selective toxin from *A. kikuchiana* was characterized as an ester of *N*-acetyl-β-methylphenylanine and a decatrienolic acid with an epoxide group; a second known form contains a β-demethylated derivative (*26*).

In contrast to early expectations, host-selective toxicity is not characteristic of a group of related compounds; it is obvious that diverse structures are involved. The toxins also appear to act at diverse sites in plant cells.

Toward an Understanding of Toxic Action

Host-selective toxins cause the visible and physiological changes that are characteristic of infected plants. Gross physiological effects include changes in respiration, cell permeability, protein synthesis, and CO_2 fixation. Most of the changes appear to be secondary to prime or initial biochemical lesions, as indicated by the single-gene control of sensitivity and by experiments with isolated organelles (*1*). Single-gene control is compatible with a hypothesis of long standing: susceptible cells have receptor or sensitive sites for toxins; such sites are lacking in resistant cells, or the sites have less affinity for toxin (*4*). The hypothesis is still viable, but to date conclusive proof is lacking.

To our knowledge, the first report of a cellular site of action for host-selective toxin was that of Miller and Koeppe (*27*), who found a striking difference in mitochondria isolated from Tms and N cytoplasm maize. HmT toxin inhibited malate oxidation and stimulated oxidation of reduced nicotinamide adenine dinucleotide (NADH) by Tms mitochondria, but did not affect N mitochondria even when used at 1000-fold higher concentrations. The effects involved uncoupling of oxidation and phosphorylation, as shown by inhibition of adenosine triphosphate (ATP) synthesis (*28*). However, binding of toxin to a mitochondrial site may not be firm; when mitochondria were washed within 5 minutes of toxin application, the toxic effect was reversed (*28*).

The mitochondrial site for HmT toxin soon became controversial, because the early reports gave no evidence for an effect on mitochondria in intact cells. This question has since been pursued at length. Electron micrographs of toxin-treated roots and protoplasts showed an early and selective loss of matrix density in Tms but not N mitochondria (*29*). Also, the mitochondria in intact, toxin-treated tissues of Tms maize were nonfunctional, as indicated by their failure to contract in the presence of 2-deoxyglucose; mitochondria in N tissues were functional under the same conditions (*30*). Finally, ATP levels declined in protoplasts of Tms maize within 3 minutes after exposure to toxin (*29*). These lines of evidence show conclusively that mitochondria in intact cells are affected by HmT toxin.

An exact site for a toxic lesion in the mitochondrion has been suggested but is not known with certainty. Various observations appear to identify the site of action as the inner membrane of the mitochondrion, perhaps a small section of the electron transport chain. This was inferred from differences in rates of oxidation and phosphorylation and by differences in the degree of uncoupling, depending on the substrate used (*28, 31*). Also, these and other findings indicate that HmT toxin has more subtle effects than does a classic uncoupler such as 2,4-dinitrophenol. Several reports indicate that toxin causes Tms mitochondria to leak NAD^+ but not NADH or malate dehydrogenase; depletion of NAD^+ might be the basis of some of the toxic effects. Detailed discussions of the mitochondrial effects are available (*31, 32*).

The maternal inheritance of sensitivity to HmT toxin is compatible with a toxic effect on the mitochondrion. Most investigators favor a single site for the toxic lesion in the mitochondrion. However, other sites of action have not been eliminated, and more than one site in the mitochondrion is conceivable (*33*). So far, the main indications of an extramitochondrial site are reports that toxin inhibits K^+-stimulated adenosine triphosphatase (*34*), an activity associated with the plasma membrane. The reports are controversial (*35*).

It is rational that sensitivity to HmT toxin is associated with organization or expression of the mitochondrial genome. Restriction endonuclease analyses of mitochondrial DNA confirm a difference between the Tms and N types (*36*). Also, Tms mitochondria contain a 13,000-dalton polypeptide not found in N mitochondria, but lack a 21,000-dalton polypeptide present in N mitochondria (*36*). However, no cause and effect relations with toxin sensitivity have been established.

Toxin HS from *H. sacchari* has been the focus of much controversy. Specific binding of HS toxin to a protein in the plasma membrane of susceptible sugarcane cells was reported (*37*). A similar protein in resistant cells failed to bind HS toxin, and toxin sensitivity was transferred to resistant cells with the toxin-binding protein (*38*). All this work relied heavily on the use of ^{14}C-labeled toxin with relatively low radioactivity and on crude protein preparations in equilibrium dialysis and gel filtration experiments. Careful examination of the data indicates that the results are not conclusive. Indeed, severe criticisms have been published (*39*), and an attempt to confirm the findings was not successful (*40*). More recent data suggest that purified proteins from resistant and susceptible sugarcane will bind HS toxin, but only the protein of the susceptible plant will bind toxin in the glycolipid environment found in the cell (*41*).

The action of HS toxin is still an open question. There must be a specific receptor or sensitive site, as indicated by the use of nontoxic analogs that competitively inhibit damaging effects of the toxin on sugarcane tissues (*42*). Nevertheless, we are not likely to obtain conclusive proof of receptor sites by the methods used to date. More sensitive techniques or much higher radioactivity in the toxin preparation will be required. The site is not necessarily in the plasma membrane, nor is the toxin necessarily bound firmly.

Toxin HV, affecting oats, was used in the first studies of host-selective toxicity. Highly purified preparations of the toxin were not available in most of these studies, but there is reason to believe that the data are reliable. The preparations were active on susceptible tissues at < 0.1 ng/ml, whereas resistant tissues tolerated a millionfold higher concentration. Resistant tissues were used as controls in all experiments, with no apparent damage. Also, inactivated toxin was used as a control in representative experiments, with no apparent effects on susceptible or resistant tissues. The underlying hypothesis of these experiments was that the toxin has a receptor or sensitive site in the susceptible cell. Other hypotheses concerning the action of HV toxin were found to be inadequate; among them, the possibility that resistant tissues inactivate the toxin more rapidly than do susceptible tissues (*1*).

No effects of HV toxin on cell-free preparations have been reported. In intact tissues, a drastic effect on the plasma membrane is evident (*1*), but this could be secondary to an initial toxic lesion. Treatment of susceptible tissues with sulfhydryl- and carbonyl-binding reagents gave protection against the effects of HV toxin. Sensitivity to the toxin was lost after 12 hours of exposure to cycloheximide; sensitivity was regained 48 hours after removal from cycloheximide.

These and other results are compatible with the hypothesis of a protein receptor with a short half-life (*1*). To our knowledge there have been no successful attempts to prove binding of HV toxin to tissues, protoplasts, or protein preparations. However, the methods used to date may not be adequate to detect the minute amounts of toxin sufficient for damaging effects.

Responses of susceptible tissues to HC toxin differ markedly from those to any other known host-selective toxin. HC toxin caused rapid but transitory increases in negative electropotential across the plasma membrane (*43*). The toxin also stimulated the uptake of certain solutes, presumably by affecting the plasma membrane. Nitrate uptake was doubled, and there were increases in the uptake of Na^+, Cl^-, methylglucose, and leucine. The effects were selective, because uptake of NO_2^-, K^+, Ca^{2+}, phosphate ions, SO_4^{2-}, and glutamic acid was unchanged (*13*). The accumulated ions were held against a gradient for 6 hours or more; eventually, the toxin-treated tissues became leaky and necrotic. In contrast, HV, HS, and PC toxins cause rapid loss of electrolytes, especially K^+ (*1*).

Effects of HC toxin on cell permeability indicate a complex role of the host-selective toxins in disease development. The toxins appear to do more than simply kill cells and provide a nutrient soup for growth of the fungus. Histological observations of fungal hyphae growing in host tissues that are not obviously damaged (*14*) are consistent with this view. The concentration of toxin at the site of a single fungal cell–plant cell confrontation has not been determined but probably is very low. Effects of concentrations below those required for visible toxicity are also unknown. The toxins probably provide an environment at the infection site that is conducive to growth and sporulation of the fungus. This might occur through several mechanisms: inhibition of disturbance responses, such as lignin deposition in the host; prevention of the usual export of nutrients from disturbed cells; and stimulation of imports that favor fungal growth. These are areas for future research.

Concluding Remarks

By restricting this discussion to the so-called host-selective toxins, we do not mean to imply that nonspecific toxins are of minor significance in plant disease development. Clearly, nonspecific toxins are involved in certain plant diseases. For this reason, several toxins from bacteria of the genus *Pseudomonas* have been characterized; their modes of action are understood better than those of the host-selective toxins. Fusicoccin, tentoxin, and rhizobitoxine are other well-known nonspecific toxins (*2, 44*). However, the unique features of the host-selective toxins make them of special interest for studies of the molecular basis of disease development and disease resistance in plants. Most of the selective toxins are required by the producing microorganism to colonize tissue and induce disease (*3*). Some nonspecific toxins may be required for pathogenicity; however, the ones known to date are thought to contribute to the severity of disease without being required for tissue colonization and disease induction. Many other toxic compounds are produced by microorganisms in culture, but few of these have been established as determinants of plant diseases (*2*).

Several categories of research will be important in the near future for progress in understanding toxins that affect plants. First, there must be increased emphasis on evaluating the role of each potential toxin in disease. Genetic analysis is our best available tool for this purpose. Next, we must be concerned with enlarging the concept with more types of plant-affecting toxins. This will require detailed knowledge of the chemical characteristics and structures of as many toxins as possible. An understanding of the mechanisms of action of each toxin is essential, along with an understanding of why certain genotypes are not affected. Several notable successes with some nonspecific toxins (*2*) indicate that problems with modes of action are becoming more tractable. Finally, we need to elucidate the roles of toxins in epidemics and in the seasonal incidence of plant diseases. So far, consideration of the ecological and epidemiological aspects of toxins has been very limited.

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