Role of toxins in evolution and ecology of plant pathogenic fungi

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Abstract. Many fungal pathogens of plants adapt readily to changes in agriculture. Among the most revealing is a fungal group whose species produce host-selective toxins as key determinants of disease. Several lines of evidence support the hypothesis that these fungi evolved from opportunistic, low-grade pathogens by gaining new genetic information leading to toxin production; in some species, toxin production is known to be under single gene control. As a result of this evolution, these fungi became virulent and host-specialized. The best-known model cases belong to the genera *Cochliobolus* and *Alternaria*; there are suggestions of evolutionary lines among these genera, with species that range from saprophytes to opportunists to specialized pathogens. Host specialization can lead to genetic isolation, a first step in speciation. Ability to produce host-selective toxin has allowed these fungi to exploit the monocultures and genetic uniformity of modern agriculture. Destructive epidemics have been the result.
Key words. Fungal evolution; phytotoxins; host-selective toxins; *Cochliobolus*; *Alternaria*; fungal ecology; plant/fungal relationships; genetics of plant/pathogen relations.

Introduction

How do fungal populations become isolated and diverge into separate and distinctive types? The initial step is probably genetic adaptation, or 'microevolution'. I will attempt to show how fungal-produced toxins may be involved in adaptation to selected host plants, and how this could eventually lead to speciation. Where possible, I will base the discussion on observed changes and on data from controlled experiments. Finally, I will discuss how the acquisition by fungi of new genetic potential can help us to understand fungal ecology and plant disease epidemiology.

Morphological simplicity and a paucity of fossil evidence have made all discussions of fungal evolution, especially 'macroevolution', highly speculative; this is certainly true for pathogenicity, or the ability to induce disease. Nevertheless, I believe some useful insights are possible. Plausibility can be strengthened by considering chemical characters and biosynthetic capacities, in addition to morphology and host range. Genetic studies can give a still more plausible basis; after all, such studies on *Neurospora* made fungi respectable organisms for experimentation. Future data on nucleic acid homologies should be useful. Overall, the potential for new knowledge is excellent, in part because observations and preliminary data suggest that genes of filamentous Ascomycetes and related imperfect forms are readily exchanged between species and races[46].

For some time now, an important goal of researchers has been to identify the products of fungal genes for pathogenicity, and the products of plant genes for resistance. Only limited success has been achieved to date. However, the molecular bases of pathogenicity in fungi have been elucidated in several model cases; I refer to the genes for pathogenicity that specify, as final product, toxic compounds with high biological activity and selectivity for specific genotypes of plants: i.e., host-selective (or host-specific) toxins[31, 38]. Plant gene products that determine susceptibility have been identified in at least one of the model cases[3].

Fungal pathogens evolve in response to changes in agriculture

Plant pathologists are well aware of the adaptive nature of fungal pathogens. Genotype changes in crop plants often result in new strains or races of pathogens; the rusts and mildews appear to be especially shifty. In other words, breeding for resistance in crop plants also breeds for selectivity and virulence in pathogenic microorganisms. Our most familiar example is the black stem rust of wheat, the perennial scourge of the grain belt in North America. The fungus is complex, consisting of many races carrying various genes for virulence; the host, too, is complex, and is planted in vast monocultures in mid-North America. Rust-resistant cultivars, developed by plant breeders, can be used to replace susceptible types. Unfortunately, the genetic potential of the fungus is expressed, and new races arise that devastate the new cultivars[42]. The breeder is soon faced with the overwhelming task of incorporating resistance to many virulence genes in rust, plus resistance to unrelated pathogens. To date, there is no practical substitute for this solution to the problem of microbial adaptation.

Ancestral forms of specialized races: Basic steps in microevolution

The presumed ancestral forms of fungal pathogens often have interbreeding populations and wide host ranges; the more evolved forms are adapted to particular hosts and are more virulent on those hosts. These beliefs are supported by studies on *Erysiphe graminis* and *Puccinia graminis*; hybrids between specialized races have wider host ranges among plant cultivars in the Gramineae than

do the parents, but are often less virulent on a given cultivar. Also, isolates of *E. graminis* from wild grasses have wider host ranges than do isolates from crop plants[2]. Generally speaking, the most highly evolved forms in nature are thought to have reached an uneasy equilibrium in which the host and the parasite tend to co-exist.

The genus *Phytophthora* offers an example of evolution in pathogenicity to plants[11]. Most *Phytophthora* species are soil-borne and are not rapidly dispersed over long distances by natural means. However, agricultural practices have moved *Phytophthora* to new environments, resulting in genetic isolation and rapid speciation (evolution). The resulting species show only limited morphological differences, but are diverse in habitat adaptations, especially in selective pathogenicity for host species or genotypes. Morphological differences can develop following genetic isolation on specific hosts. These ideas are supported by chromosome counts, DNA homology, and protein profiles, powerful tools in determining genetic relationships. The conclusion: *Phytophthora* is actively speciating; the prerequisite genetic isolation is provided by host restriction and by changes in ploidy, both of which prevent ready genetic outcrossing[11]. Other fungal groups appear to have similar patterns of change.

These ideas can be carried over to *Cochliobolus* and *Alternaria*, which are of central importance in toxin considerations. For present purposes and as a model, *C. sativus* (anamorph: *Bipolaris*, syn: *Helminthosporium sativum*) may be considered the ancestral type of the genus. This species is a generalized pathogen, with a wide host range in the Gramineae[6,9]. *C. sativus* is variable and diverse; it includes mild, opportunistic forms (possibly even saprophytes), intermediate types, and virulent, relatively specialized forms[13,25,36]. The virulent ones may vary somewhat in morphology and significantly in host range, even when they are genetically compatible with other types. Members of the species are able to produce and excrete a wide variety of secondary metabolites. Most genotypes can colonize intact but senile tissues of many grasses; this attribute is shared with members of many other genera of plant pathogenic fungi that colonize a wide range of host plants.

Differences in pathogenicity and virulence among isolates of *C. sativus* are controlled by relatively few genes. Many characteristics of *C. sativus* are shared with *C. carbonum* and *C. victoriae*; in fact, these so-called species are so closely related that they are sexually compatible[39]. All three 'species' colonize senile and stressed leaves of various grasses. However, *C. victoriae* is virulent and very destructive only to oats (*Avena sativa*) carrying the V_b gene, whereas *C. carbonum* is specialized for growth on *Zea mays* (maize)[36]. In contrast, *C. sativus* attacks the roots, crown, and senile tissues of many grasses, especially wheat and barley. The specificities of *C. victoriae* and *C. carbonum* race 1 are based on excretion of host-selective toxins, each under the control of one gene[39]. Similar patterns of pathogenicity, virulence, and host-selectivity are evident in *Alternaria* and in many other genera of fungi.

Background considerations discussed above are important for understanding the role of toxins in the evolution and ecology of plant pathogenic fungi. As further background, consider the degrees of specialization in the relationships of pathogenic fungi to their hosts; these relationships may be classified roughly into four categories, as follows.

1) *Saprophytes.* There clearly are pure saprophytes that never invade living tissues under any circumstances. There is a common notion that most fungal pathogens evolved from such organisms though there are also conflicting opinions.

2) *Saprophytes that can be opportunistic pathogens.* This is the next step in host-pathogen complexity. The more primitive types in this category have low virulence, require wounds for entry, and need stressed, debilitated, or senile plant tissues for colonization and disease development. Other fungal species or races, slightly more specialized, have evolved structures for penetration of intact tissue, but still fail to colonize and produce disease unless the plant is stressed or senile. Examples are found in the non-specialized species and races of *Cochliobolus*, *Alternaria*, *Botrytis* and many other genera[7,13,36].

3) *Pathogens that are opportunistic saprophytes.* The third category of specialization includes those fungi that are primarily pathogens, but can also exist as saprophytes under special conditions and in culture. This group includes the causal agents of many of our most serious plant diseases. Ordinarily, they are not competitive saprophytes in dead substrates, but survive as dormant structures. Pathogens in this category are usually host selective and virulent to the selected hosts. They appear to have developed mechanisms (by mutation, gene transfer, or genetic recombination) to by-pass, suppress, or ignore the normal tissue responses to disturbance. Resistance in the plant is also more specialized, and selective against a particular pathogen; often, resistance is controlled by a single discrete gene, either dominant or recessive[36,39]. Such resistance may be in addition to, or be superimposed on, the normal plant response to disturbance, which includes phytoalexin production, lignin deposition, and sealing the disturbed area.

Several known mechanisms may account for the ability of a pathogen to suppress or by-pass the usual disturbance response. Such mechanisms include excretion by the pathogen of toxins (both host-selective and non-selective), introduction of pathogen genes into the host cell (example: crown gall), and disruption of normal hormonal controls in the plant tissue (example: *Pseudomonas syringae* pv. *savastanoi* and olive knot)[35,38].

4) *Obligate parasites.* Fungi in this highest category of specialization are represented by the rusts and mildews. These fungi never complete their life cycles without the host plant, although some of them can now be grown in

culture. Their host/pathogen interactions may not differ significantly from those of pathogens in category 3.

Using this classification, *Cochliobolus* and *Alternaria* have species, races, or genotypes in every category except 4 (obligate parasites). The toxin-producing types clearly fit into category 3. In fact, species of a majority of plant pathogenic genera will cover the spectrum, excluding category 4, although toxins are conclusively known for only a few of them. We may think of the *Cochliobolus* and *Alternaria* species as models that show how fungi can become more specialized, moving from category 2 to category 3 by gaining the ability to produce toxic compounds involved in disease development.

Potentials for production of secondary metabolites by fungi

To understand the role of toxins in the ecology and evolution of fungi, we need an appreciation of the abilities of fungi as chemical factories. Species of *Cochliobolus* and *Alternaria*, for example, are capable of producing and excreting a wide variety of compounds. Many are secondary metabolites that are toxic to plants, but are not necessarily known to be involved in disease[43].

Abilities of various species to produce specific metabolites can be used as an indication of relatedness, of possible use in tracing evolutionary lineage. For example, an early suggestion was that *C. victoriae* originated from *C. sativus*; the hypothesis became more plausible when they were found to have certain metabolites in common. All isolates of *C. victoriae* produce victoxinine, and many, but not all isolates of *C. sativus* also produce this compound[36], a sativane closely related to helminthosporal. No other species have been found to produce victoxinine, although several other species of *Cochliobolus* produce helminthosporal. Finally, these and other species of *Cochliobolus* can hybridize, and are similar in morphology[24, 36, 39].

Several of the host-selective toxins are cyclic peptides[21, 43]; thus, the ability of fungi to produce such compounds is pertinent. A number of bacterial and fungal-produced peptide antibiotics and toxins[12, 21] exemplify the widespread abilities of microorganisms to produce and excrete various types of compounds. Such abilities indicate that fungi have the potential to produce metabolites with possible ecological value to the producer. The ability to produce peptides and other compounds is genetically controlled, sometimes by single gene pairs[39]. Mutations may be involved in the ability to produce new compounds, or in changes in the structures. Genetic recombinations may also be important; a fungus may become adapted to a new host, or gain virulence, by acquisition of new genetic information.

Distribution of genes for pathogenicity and virulence in fungal populations

Christensen[5] was the first to recognize the genetic complexity of *Cochliobolus*. His isolates of *Helminthosporium sativum* (anamorph of *C. sativus*) varied greatly in virulence towards wheat and barley, but he did not examine selective pathogenicity for other species. Christensen found cultural mutants for decreased or increased virulence; some mutants attacked previously resistant cultivars of wheat. More recently, long-term use of a field for wheat crops was shown to shift populations of *C. sativus* to more aggressive types than those found in surrounding rotated fields, indicating genetic variability of the original populations[8].

The value of *Cochliobolus* as a model was established by the work of R. R. Nelson. He found that natural populations of *Cochliobolus* are genetically very diverse, containing gene pools for selective pathogenicity for many hosts in the Gramineae. Well-known pathogens in *Cochliobolus* and related genera are highly virulent against specific hosts, but can also contain genes for pathogenicity towards other host species[13–15, 26]; usually, selective pathogenicity is controlled by one or two discrete gene pairs. For example, Kline and Nelson[14] made crosses among 6 isolates of *C. carbonum* that differed in pathogenic capacity. Tests of the F_1 progenies against 6 grass species indicated at least 5 different genes for selective pathogenicity. Some isolates of *C. victoriae* that were virulent on V_b oats were shown to have genes for pathogenicity to several other grasses; one isolate was virulent on 5 of 25 species tested[27]. There are comparable data for *C. sativus*[13], *C. heterostrophus*[28] and *Setosphaeria turcica* (syn.: *Trichometasphaeria turcica*, *Helminthosporium turcicum*)[33].

The flexibility of *Cochliobolus* and related fungi is evident from interspecific and intraspecific mating experiments. *C. victoriae* and *C. carbonum* will mate readily, with a high degree of success[25, 39]. These two species also hybridize with *C. sativus*, but with a low success rate. *C. heterostrophus* was reported to hybridize, but with low success rates, with *C. oryzae*, *C. sorghicola*, *C. carbonum* and *C. sacchari*[24, 25]. Hybridizations between the many fungal species, races, and genotypes allow for many different gene recombinations; selective pressures from new host genes for resistance, or from introduction of new host species, may select from among the recombinants[2, 11, 18].

There are several general conclusions from the work of Nelson and others. It is evident that a given species of *Cochliobolus* can have several specific gene systems for pathogenicity and that some genes are selective for particular host species or genotypes. Another conclusion is that certain interspecific hybrids can have pathogenic potentials greater than the parents. The data are indicative of the potential for evolution of pathogenicity in these fungi.

What does the work on genes for pathogenicity and virulence have to do with the concept of toxins? The connection became evident with the discovery that in some cases the genes for pathogenicity are the same genes that control production of host-selective toxins[38].

Diseases caused by Cochliobolus species: origins, genetics, and toxicology

Cochliobolus victoriae. Victoria blight caused by *C. victoriae* appeared suddenly in Iowa (USA), following widespread planting of oat cultivars carrying the V_b gene for resistance to the prevalent races of crown rust[36]. The V_b gene came from oats imported from Argentina for breeding purposes. Oat is largely self-pollinated, hence the crop maintains genetic uniformity. The new disease became so destructive in 1946–1948 that all cultivars carrying the V_b gene had to be discarded[36]. *C. victoriae* is thought to have originated from *C. sativus*, and is closely related to *C. carbonum*. *C. victoriae* was first described in 1946, but was present in Iowa before V_b oats were introduced in 1942, as shown by isolations from stored grain and herbarium specimens[31, 36]. The newly described fungus was soon shown to produce a potent host-selective toxin, now known as victorin or HV toxin[38]. Toxin-producing ability differentiates *C. victoriae* from *C. carbonum* and *C. sativus*.

The ability of *C. victoriae* to produce toxin is controlled by one gene pair, as shown by fungal mating experiments. *C. victoriae* (tox^+) was crossed with a non-producer (tox^-) and progeny were analyzed for toxin production and pathogenicity. The progeny had a 1:1 ratio of producers and non-producers. All progeny isolates with the tox^+ gene were pathogenic to oats with the V_b gene, but were non-pathogenic to other oat genotypes. When *C. victoriae* was mated with *C. carbonum* race 1 (pathogenic to maize with the *Hm* gene, and producer of another selective toxin), the progeny had a 1:1:1:1 ratio of oat (HV) toxin producers, maize (HC) toxin producers, producers of both toxins, and tox^- isolates[39]. This dihybrid ratio shows that production of each toxin is controlled by a single gene pair. There was a complete correlation of ability to produce each toxin with pathogenicity towards oats and corn.

Victorin is a cyclic peptide, mol. wt 814, containing glyoxylic acid and several unusual amino acids and chlorinated entities, as determined by Macko et al.[22]. There are several chemically related forms. The toxin is extremely active (EC_{50}, 37 nM) against oats with the V_b gene, but does not affect resistant oats or other species at several hundred thousand times this concentration[22, 34].

Cochliobolus carbonum race 1. This fungus was first found by Ullstrup in 1944, in experimental plots of maize that contained the *hm* gene. The maize gene for resistance is dominant (*Hm*), in contrast to the case with *C. victoriae*, where susceptibility is dominant. There is a less specialized form of *C. carbonum* (race 2) that does not produce HC toxin, and that may be the parental type for race 1[36]. *C. carbonum* continues to spawn new races affecting different cultivars of maize[10]; other toxins could be involved, but there are no data.

The special pathogenicity and selectivity of *C. carbonum* depends on excretion of HC toxin. Production is controlled by a single gene pair, as shown by $tox^+ \times tox^-$ matings[39]. The toxin is a small cyclic peptide (mol. wt 436) containing alanine (2 residues), proline, and an unusual amino acid, as determined and confirmed by several research groups[38]. The compound is toxic to *hm* maize at 0.2 μg/ml, and to *Hm* maize (resistant) at 20 μg/ml[32]. The basis of pathogenicity and selectivity of the other *C. carbonum* races is unknown; however, changes in maize cultivars will no doubt spawn still more forms of *C. carbonum*.

Cochliobolus sacchari. The origin of *C. sacchari*, a virulent toxin-producer affecting sugarcane, is not clear. There are some closely-related species of *Cochliobolus* affecting other grasses, and several that are sexually compatible with the sugarcane species[25]. Thus, there are potential sources of origin.

The disease of sugarcane caused by *C. sacchari* appeared suddenly and became severe in Hawaii, Puerto Rico, Java, and other parts of the world when new clones were planted[36]. The crop is vegetatively propagated, and is therefore genetically uniform. Other grasses, including Napier (*Pennisetum purpureum*) in Florida and Hawaii, lemongrass (*Cymbopogon citratus*) in the Florida Everglades, and pearl millet (*P. glaucum*), are affected by the same or very closely related fungi[36]; only the fungus from sugarcane been intensively studied. Resistant cultivars (clones) are used to control the disease, and there are active breeding programs. Plant breeders must always screen new clones for resistance, to avoid introduction of susceptible plants; the toxin is used to screen for resistance to the disease. Sugarcane is genetically complex, and the inheritance of resistance is unknown; however, there are clearly highly resistant, intermediate, and very susceptible clones[37].

Pathogenicity and selectivity of *C. sacchari* depend on production and release of a host-selective toxin[37]. The toxic compound contains four galactose units attached to a sesquiterpene core[21]; isomeric forms differ somewhat in relative toxicity. Analogs with fewer galactose units have been described; at least one of the 3-galactose compounds is highly toxic to some disease-susceptible sugarcane clones, but is harmless to others. In contrast, most of the lower molecular-weight analogs protect susceptible tissue against the toxin[19]. In culture, the galactose analogs are readily converted from one to the other; there are indications that galactose and sesquiterpene are biosynthetic building blocks[23]. Resistant sugarcane and other plant species tolerate all these compounds; however, the other hosts of *C. sacchari* and closely related fungi have never been tested. The significance of the toxicity of one of the 3-galactose analogs[19] for a few sugarcane clones is not clear at this time. It does suggest biosynthetic adaptability that may have ecological and evolutionary consequences.

Cochliobolus heterostrophus. The parental race of *C. heterostrophus* (teleomorph of *Bipolaris* or *Helminthosporium maydis*) was known since 1923 as the cause of a minor

leaf disease of maize in southeastern USA, and of teosinte in Mexico[36]. Race T appeared suddenly in 1968 in Iowa, and caused a devastating epidemic in the maize crop in North America in 1970[47]. The epidemic was possible because the entire maize crop contained Texas male sterile (*Tms*) cytoplasm, for economy in the hybridization procedure; thus, the crop had an unusual degree of genetic uniformity. The new fungal race (T) developed from the old version of the fungus (race O), as indicated by several lines of evidence[36]. Race T soon spread far beyond the geographical range of race O, and caused the most destructive epidemic ever known in crop plants, in terms of financial loss in a single season[47]. Race T soon became rare when *Tms* cytoplasm maize was discarded[18].

The basis of virulence and selectivity by race T is excretion of a host-selective toxin (HMT toxin). Kono and Daly characterized the toxin as a linear polyketol ($C_{41}H_{68}O_{13}$)[17] that uncouples respiration in mitochondria from plants with *Tms* cytoplasm[1], and in mitochondria in intact cells of *Tms* plants[48]. There are no effects on mitochondria in and from resistant maize[38]. HMT toxin production by *C. heterostrophus* is under single gene control[45].

Toxin studies on other genera

Phyllosticta maydis. Another maize disease appeared in north central USA at about the same time as did *Cochliobolus* blight. This second disease was caused by an entirely different fungus, *Phyllosticta maydis*, which may be a new variant of an existing but minor pathogen. *P. maydis* became a problem in certain northern areas for the same reason as did *C. heterostrophus*: the widespread population of genetically-uniform *Tms* cytoplasm maize. A point of exceptional interest is that *P. maydis* produces a selective toxin that is structurally very similar to the toxin produced by *C. heterostrophus* race T[17]; *P. maydis* and HMT toxins also have the same effects on *Tms* mitochondria[36]. This appears to be an example of parallel evolution.

Alternaria species. The history of host-selective toxins from *Alternaria* species is parallel to that of those from *Cochliobolus*. Various chemical structures have been described, and there are parallels in modes of action. At least seven host-selective toxins are known from *Alternaria* species[30], and other toxins have been reported. These toxins affect dicotyledonous plants (strawberry, apple, pear, tangerine, lemon, tobacco, and tomato), in contrast to the *Cochliobolus* toxins, which affect grasses. The one major disadvantage of *Alternaria* studies is that sexual stages for the fungi are unknown, thus less is known of the genetic control of toxin production and its correlative effects on pathogenicity. The *Alternaria* toxins have been characterized[17] and reviewed at length[29,30], so I will discuss only one group as an example.

A. citri (also known as *A. alternata* f. *citri*) is a mild and opportunistic pathogen on fruit and senile leaves of many citrus species[36]. A virulent race appeared suddenly on Emperor mandarin in Australia and later on Dancy tangerine in Florida. The new race differs from the old opportunistic form only in the ability of the new race to produce a potent, host-selective toxin; apparently, the new race is a tox^+ mutant or selection from the old race. Then a third race appeared in Florida, this one pathogenic on rough lemon. The third race produces a toxin that is selective for rough lemon but without noticeable effect on Dancy tangerine[16]. Again, the rough lemon race appears to be a tox^+ mutant from opportunistic *A. citri*. These studies support the hypothesis that simple genetic changes can transform benign, non-specialized fungi into host-selective and virulent forms capable of causing serious epidemics[86].

Periconia circinata. Periconia blight of grain sorghum appeared suddenly in southwest USA in 1924 and soon spread to all sorghum-producing areas of the country. This is another disease of an important crop species with a limited genetic base[31,36]. *Periconia* species are soil-inhabiting fungi, including saprophytes and low grade pathogens on roots[6]. *P. circinata*, which probably arose from such a population, invades roots and crowns of susceptible plants, causing systemic toxemia in the whole plant[40]. The only known hosts of *P. circinata* are cultivars of grain sorghum with the PC gene, one cultivar of sweet sorghum, and shattercane, a weed sorghum[31]. Susceptibility to the fungus and sensitivity to its toxin are controlled by one semi-dominant gene[41]. The fungus has no known sexual stage.

The toxin from *P. circinata* is selective for hosts of the fungus[34,40], and the role of toxin in disease is comparable to that of victorin from *C. victoriae*. The toxin is still not completely characterized, but is known to be a low molecular-weight peptide. Resistant plants tolerate at least 20,000 times higher concentrations than do susceptible plants[34,36].

Members of several other fungal genera are known to produce host-selective toxins[35,38], but these are enough examples to serve as models illustrating the ideas and concerns. Production of non-specific toxins by various fungi no doubt have ecological and evolutionary consequences, but to date there are not sufficient data for a meaningful discussion.

Ecological consequences of toxin production by fungi

We now have convincing evidence that acquisition of a single gene for toxin production can convert a benign, opportunistic pathogen into a virulent, specialized pathogen capable of causing severe epidemics among crop plants. The first striking example of the significance of single gene changes was that of Victoria blight of oats in North America. The causal agent, *C. victoriae*, apparently evolved from *C. sativus*[36]. The single gene in *C.*

victoriae that controls pathogenicity and toxin production made possible a plant disease epidemic of continental proportions. Of course, a prerequisite for this epidemic was extensive planting of a crop with genetic uniformity; most of the oat crop in mid-USA contained a single gene (V_b) making the plant susceptible to *C. victoriae*, sensitive to its toxin[31], and resistant to *Puccinia coronata*. Crops with genetic homogeneity, such as self-fertilized crops (example, oats) or vegetatively propagated ones (examples, sugarcane and citrus) are especially vulnerable.

The V_b locus in oats is composed of two or more alleles that can give the plant intermediate or tolerant levels in the scale from susceptibility to immunity[20]. In nature, without man's interference, it is likely that the most susceptible genotypes would have been eliminated, leaving the tolerant types that allowed survival of both the fungus and the host. In that case, our chances of detecting HV toxin would have been much less, even though it is still the major disease determinant for the intermediate hosts. How many such unknown adaptations have occurred in the past, thereby masking the roles of toxins?

A more recent and even more striking example of the ecological consequences of toxin production by a fungus is the leaf blight disease of maize caused by *C. heterostrophus* race T. The older form of the fungus (race O) was probably the parental type[36]; race T is thought to have resulted from a mutation or some other genetic re-arrangement. The significant fact is that toxin production by *C. heterostrophus* is controlled by one gene[45]. The ecological consequences became apparent when the maize crop in North America became genetically uniform for *Tms* cytoplasm (maternally inherited), and when weather conditions favored disease spread in 1970. *Tms* maize contains a receptor protein in the mitochondrion that binds HMT toxin[3], leading to disruption of the respiratory process[1]; maize lacking *Tms* cytoplasm lacks this protein and is insensitive to the toxin.

Genetic uniformity of the maize crop resulted in selection and population explosion of a fungal strain (race T) that could exploit the situation. Thus a minor, geographically-restricted fungus was transformed into a virulent pathogen that invaded the total range of maize in North America. This is probably the best known example of how a single gene change can create an epidemic of vast proportions, and greatly extend the geographic range of a fungus.

C. sacchari and sugarcane also illustrate two ecological consequences of toxin-producing ability. The genetic control of toxin production by this fungus has not been examined, and the genetic control of resistance/susceptibility in the host is unknown. Nevertheless, the sugarcane disease is another good illustration of how toxin-producing ability can increase a fungal population, and lead to widespread epidemics. The other ecological factor concerns the seasonal occurrence of the disease in sugarcane. Data show that sugarcane becomes insensitive to toxin at temperatures above 32 °C. This explains the seasonal incidence of the disease in Florida and Hawaii, where damage is often severe during the cooler season, but does not develop further during the hot summer. A parallel situation is evident for the black spot disease of Japanese pear, caused by a toxin-producing *Alternaria* species[36].

Blight of grain sorghum, caused by a toxin-producing fungus (*Periconia circinata*) is an even better model for the seasonal incidence of disease. Sorghum is a high-temperature plant, growing well at 35 °C. When plants are held at this temperature for several hours, they become insensitive to the toxin, perhaps because a protein receptor is deranged. When the temperature is lowered to 20 °C, the plants regain sensitivity in 2–3 days, perhaps because new receptors are synthesized[4]. This heat effect on toxin sensitivity fits with disease observations in field and greenhouse. In the southwestern USA, where sorghum is an important crop, *Periconia* blight appeared in the spring, disappeared during the heat of summer, and re-appeared in the fall[36]. Inoculated plants in controlled temperature chambers grew well with no symptoms at 35 °C, but were blighted quickly when the temperature was lowered to 20 °C[4]. There are comparable effects of heat on sensitivity to host-selective toxins from *C. sacchari*, *Alternaria* species, and *C. victoriae*[4,36].

The ecological point of significance is that the seasonal incidence of several plant diseases is determined by heat-induced tolerance of plant tissues to toxins. The parallel between toxin sensitivity and disease incidence is further proof of the key roles of selective toxins in these diseases. Non-selective toxins have been less studied in this regard, but similar patterns would not be surprising. Other plant diseases, not known to involve toxins, also have comparable responses to heat.

Ceratocystis ulmi is a conspicuous example of a toxin-producing fungus that has effects on a host population in natural ecosystems and in plantations. *C. ulmi* has greatly reduced the population of elm trees in North America and Europe. Takai and associates[44] have published extensive data indicating that cerato-ulmin, a low molecular-weight protein, is a key determinant in the development of the disease. The toxin is selective for susceptible elms, but its selectivity is less striking than that of the toxins described above. The American elm, *Ulmus americana*, is becoming more and more rare, although it is not expected to become extinct. There are many examples of virulent pathogens, not known to produce toxins as disease determinants, which have limited or eliminated their hosts in natural ecosystems.

Concluding discussion and summary

Fungal pathogens have clearly adapted or evolved in response to changes in agriculture. This sweeping statement requires background information to be appreciated. In general, plant pathogens may be classified as viru-

lent and specialized, with a limited host range, or as opportunists with wide host ranges but with low virulence except on senile, weakened, or stressed tissues. Many fungal genera contain virulent species, opportunistic species, and pure saprophytes. Some opportunistic and some virulent forms have attributes in common, including genetic compatibility and the ability to produce structures that allow for penetration of living tissues. Such opportunists can become virulent and specialized by acquiring genetic information controlling mechanisms of pathogenicity, such as the ability to excrete toxins.

Fungal species in several genera, including *Cochliobolus* and *Alternaria*, are excellent models for an examination of host adaptation and speciation (evolution). Species and races in these genera range from saprophytes to opportunistic pathogens and virulent, specialized pathogens; the morphological characters and genetic affinities of *Cochliobolus* spp. suggest evolutionary lines. There is good evidence that some species have moved from opportunistic forms to specialized pathogens by gaining (by mutation, gene transfer, or gene recombination) the ability to produce host-selective toxins. These toxins give the producer a striking advantage when growing on certain hosts, and provide the ecological and genetic isolation that are so important as an initial step in evolutionary change. *Cochliobolus* species have the advantage for such studies: the sexual stages are well-known, making them amenable to genetic analysis; and susceptibility/resistance in host plants is known to be genetically controlled. The toxins have now been chemically characterized, and the gene product for at least one plant gene for susceptibility has been identified.

A second objective of this essay was to clarify the role of toxin production in the ecology of plant diseases. We now appreciate the part played by toxins in several explosive epidemics, including Victoria blight of oats, *Cochliobolus* leaf blight of maize, *Periconia* blight of sorghum, leafspot of sugarcane, and *Alternaria* blight of citrus. Also, we can now appreciate the role of toxin production and toxin sensitivity in the seasonal occurrence of some plant diseases. Single gene changes in the control of toxin production, or single gene changes in the control of toxin sensitivity in crops, can have devastating consequences. In general, the resulting epidemics were based on adaptations that allowed the fungi to exploit large monocultures of genetically-uniform host plants. There are potential disasters to major crops involving other fungal genera with adaptive potentials.

The study of the ecological consequences and evolutionary roles of toxin production by plant pathogens is at an early stage; to date, only the host-selective toxins have been examined beyond a superficial level. There are opportunities for a new understanding of many plant diseases, including those involving non-selective toxins, and the discovery of more toxins. We expect that molecular genetics will advance all such studies, and may provide practical uses for our knowledge.

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Phytotoxins as tools in breeding and selection of disease-resistant plants

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Abstract. Conventional plant breeding for resistance to pathogens, although successful, is in many cases still too slow to keep pace with pathogen adaptation, and suffers from the lack of genetic variability in cultivated varieties. Phytotoxins, because of their role in disease development, have been proposed as convenient markers for early screening of resistant genotypes and as selective agents for in vitro selection. The present review summarizes, firstly, the evidence for a genetic correlation between tolerance to toxins and resistance to pathogens, with particular reference to host-selective toxins (HST) and factors affecting early screening. There follows a discussion of results obtained from the use of phytotoxins for in vitro selection of resistant plants. The conclusion is drawn that this practice, while potentially useful in the case of HST, leads to contradictory results when ill-defined toxins or culture filtrates are used. Finally, prospects for future research are adumbrated.
Key words. Resistance of plants to diseases; plant pathogenic fungi; in vitro culture and selection of resistant plants; phytotoxins.

Introduction

Selecting plant genotypes resistant to pathogens has become one of the major tasks of breeders both because of increasing crop losses, and as a result of the general move towards reducing the use of chemicals in agriculture. However, traditional plant breeding for resistance to diseases suffers from several drawbacks, such as the lack of genetic variability, the high costs, the time and space required for screening plant populations under selection,