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Fungal Infection of Plants

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INTRODUCTION

Fungi constitute a highly versatile group of eukaryotic carbon-heterotrophic organisms that have successfully occupied most natural habitats. The vast majority of fungi are strict saprophytes; <10% of the ~100,000 known fungal species are able to colonize plants, and an even smaller fraction of these are capable of causing disease. Among the causal agents of infectious diseases of crop plants, however, phytopathogenic fungi play the dominant role not only by causing devastating epidemics, but also through the less spectacular although persistent and significant annual crop yield losses that have made fungal pathogens of plants a serious economic factor, attracting the attention of farmers, plant breeders, and scientists alike.

All of the ~300,000 species of flowering plants are attacked by pathogenic fungi. However, a single plant species can be host to only a few fungal species, and similarly, most fungi usually have a limited host range. The evolution of fungal phytopathogens toward a high degree of specialization for individual plant species may be reflected in the different levels of specialization observed in extant plant–fungal interactions (Scheffer, 1991). The first level may be seen in opportunistic parasites, which enter plants through wounds or require otherwise weakened plants for colonization. These fungal species are usually characterized by a broad host range but a relatively low virulence, that is, they cause only mild disease symptoms. The next level comprises true pathogens that rely on living plants to grow but that under certain circumstances can survive outside of their hosts. Many of the more serious plant pathogens are found at this level; most are highly virulent on only a limited number of host species. Finally, the highest level of complexity is achieved by obligate pathogens, for which the living host plant is an absolute prerequisite to fulfill their complete life cycle.

Therefore, in a simplified view of the evolution toward phytopathogenicity, an ancestral fungus needed first to gain attributes enabling it to live on numerous plant species before refining those traits and/or developing additional devices to increase its virulence on individual plant species, thus gaining an edge on competing pathogens. The strategies pursued by fungal pathogens in this process vary in different types of interactions with their hosts (Keen, 1986). In this review, therefore, I focus on two intriguing questions. First, what are the mechanisms that facilitate the transition of a saprophytic fungus to a plant pathogen? Second, what mechanisms control the degree of virulence on the host once pathogenicity has been established?

ACTIVE PENETRATION OF THE PLANT

To colonize plants, fungal microorganisms have evolved strategies to invade plant tissue, to optimize growth in the plant, and to propagate. Bacteria and viruses, as well as some opportunistic fungal parasites, often depend on natural openings or wounds for invasion. In contrast, many true phytopathogenic fungi have evolved mechanisms to actively traverse the plant's outer structural barriers, the cuticle and the epidermal cell wall. To gain entrance, fungi generally secrete a cocktail of hydrolytic enzymes, including cutinases, cellulases, pectinases, and proteases. Because these enzymes are also required for the saprophytic lifestyle, they are unlikely to represent the tools specifically developed by fungi to implement pathogenesis, and each individual hydrolytic enzyme may not be absolutely necessary for penetration. This does not, however, preclude the adaptation of their structure or their biosynthetic regulation to the specific needs of a pathogen on a particular host plant.

Experiments addressing the role of cutinase illustrate this point. The cuticle covers the aerial parts of living plants and needs to be pierced before other pathogenetic mechanisms can become effective. Therefore, enzymatic degradation of cutin, the structural polymer of the plant cuticle, has been postulated as crucial for fungal pathogenicity, and cutinase is presumed to be a key player in the penetration process. Indeed, several lines of evidence demonstrate the pivotal role of cutinase during the infection process (reviewed in Kolattukudy, 1985). For example, inhibition of the enzyme by using different chemical inhibitors or cutinase-specific antibodies was shown to prevent infection by *Nectria haematococca* (*Fusarium solani* f sp *pisi*) of pea stem segments with intact cuticle but not of those with mechanically breached cuticle. Further support for the role of cutinase came from the observation that a wound pathogen affecting papaya fruits, *Mycosphaerella* sp, that does not produce cutinase, was able to penetrate intact

fruits only when transformed with the cutinase gene from *N. haematococca* (Dickman et al., 1989). However, the importance of cutinase for plant penetration has been questioned (Schäfer, 1994) and may vary between fungi, with other mechanisms compensating for the lack of this enzyme. In addition, cutinase may also be involved in prepenetration processes, for example, by altering the adhesive properties of the cuticle and thus facilitating fungal attachment to plant surfaces (Nicholson and Epstein, 1991) or by releasing signal molecules required for early fungal development on the plant (Kolattukudy et al., 1995).

Alternatively, or in combination with hydrolytic enzymes, some fungi have developed a more complex and sophisticated mechanism to penetrate the cuticle of host plants. In general, phytopathogenic fungi form specialized penetration organs, called appressoria, at the tip of their germ tubes; these organs are firmly attached to the plant surface by extracellular adhesives. As it develops, the porosity of the appressorium wall of mechanically penetrating fungi is markedly reduced by melanin incorporation, allowing high turgor pressure (>8 megapascals; Howard et al., 1991) to build up inside. This pressure is focused effectively on a small area at the base of the appressorium that is kept free of wall material and melanin. From this penetration pore, an infection peg develops and pierces through the cuticle and cell wall, possibly assisted by hydrolytic enzymes (reviewed in Mendgen and Deising, 1993).

Studies of the rice blast fungus *Magnaporthe grisea* have illustrated the importance of melanin for infection peg penetration; melanin-deficient mutants are unable to infect intact plants, but some mutants retain pathogenicity on leaves with abraded (i.e., wounded) epidermis (Chumley and Valent, 1990; Kubo and Furusawa, 1991). Furthermore, melanized appressoria of *M. grisea* were capable of pushing penetration pegs through plastic membranes (Howard et al., 1991). These results suggest that melanin is an essential factor for mechanically penetrating fungi.

Other fungal species, including some rusts, have not evolved a direct penetration mechanism and instead bypass the plant cuticle and outer cell wall by entering through the stomata. These fungi have developed a poorly understood mechanism to locate these openings on the plant surface (Hoch et al., 1987; Correa and Hoch, 1995). Thus, penetration is likely to be controlled by a combination of different factors. In addition to fungal compounds, these factors may include plant surface structures as well as activators or inhibitors of fungal spore germination and germ tube formation.

IMPAIRMENT OF PLANT FUNCTIONS BY FUNGAL TOXINS

After penetration, the next step in a fungal strategy to colonize a plant species is often the secretion of toxins or plant hormonelike compounds that manipulate the plant's physiology to the benefit of the pathogen. This interference can consist simply of killing plant cells for the purpose of nutrient uptake or a more subtle redirecting of the cellular machinery (Keen, 1986); often it is achieved through the production of phytotoxins with varying degrees of specificity toward different plants. Some toxins are host selective (see Walton, 1996, in this issue), whereas others are active in a wide range of plant species.

Phytotoxins have been identified in a broad spectrum of pathogens, but their actual role in pathogenesis remains poorly understood in most cases. However, in some plant–fungus interactions, genetic and biochemical studies revealed that toxins are the determinants of specificity. In these cases, resistance or susceptibility to the fungus always correlates with insensitivity or sensitivity to the toxins. Consequently, these host-selective toxins, which are produced mainly by species of the fungal genera *Alternaria* and *Cochliobolus*, have attracted much attention (see Walton, 1996, in this issue). In contrast, host-nonselective toxins are active on both host and nonhost species. Although this nonselectivity contradicts a role in host-range determination, these toxins may nevertheless have a crucial function during fungal pathogenesis on a particular host. Alternatively, they may be interpreted as remnants of earlier stages of fungal evolution toward phytopathogenicity whose activity may be obstructed in most plants by detoxification or other mechanisms. Two examples illustrate the potential function of host-nonselective toxins.

Which processes are affected by fungal host-nonselective toxins? The mode of action of only a small portion of these toxins has been elucidated. However, several fungal toxins target the plant plasma membrane–localized H^+-ATPase. This enzyme plays a central role in many cellular functions. For example, in mediating ATP-dependent H^+ extrusion, the enzyme helps to establish an inwardly directed proton electrochemical gradient that is required for a number of "uphill" solute transport processes. H^+-ATPase activity is also involved in various turgor-related processes and in the regulation of the intracellular pH (Briskin and Hanson, 1992; Michelet and Boutry, 1995; see also Gianinazzi-Pearson, 1996, in this issue).

This ATPase is activated by the host-nonselective toxin fusicoccin, the major phytotoxic metabolite of the peach and almond pathogen *Fusicoccum amygdali* that appears to be generally active in higher plants (Marrè, 1979; Marrè and Ballarin-Denti, 1985). The ensuing increased uptake of K^+ (and other cations), Cl^-, and water by the stomatal guard cells causes the irreversible opening of the stomata and the disease-typical wilting of leaves (Ballio, 1991). The plasma membrane–localized fusicoccin receptor is a member of the 14-3-3 superfamily of eukaryotic proteins (Korthout and De Boer, 1994; Oecking et al., 1994) that is present in all higher plants (Meyer et al., 1993). Members of the family of 14-3-3 proteins serve a multitude of distinct functions, often through regulating the phosphorylation state of proteins (Aitken et al., 1992). This suggests that fusicoccin may affect a signaling pathway leading to H^+-ATPase stimulation through modulation of protein kinase/phosphatase activity. However, the ubiquitous distribution of the fusicoccin receptor contradicts the limited number of host species of *F. amygdali*. Despite the potential of fusicoc-

cin, an additional as-yet-unknown factor(s) must be responsible for drastically restricting the fungal host range.

A second fundamental plant process, energy transfer during light-driven photophosphorylation in chloroplasts, is inhibited by a different host-nonselective toxin, tentoxin. In sensitive plants, this cyclic tetrapeptide, which is produced by the broad host range pathogen *Alternaria alternata*, causes seedling chlorosis and the arrest of sensitive plant growth. Different from fusicoccin, however, sensitivity to tentoxin varies between plant species (Durbin and Uchytil, 1977). The toxin target appears to be the $\alpha\beta$ subunit complex of the chloroplast coupling factor 1. This is suggested by comparison of the sequences of the β subunit–encoding *atp*B genes from six closely related toxin-sensitive and -insensitive *Nicotiana* species. The presence of a glutamate residue at position 83 correlated with tentoxin insensitivity, whereas an aspartate residue correlated with sensitivity (Avni et al., 1992). In addition, tentoxin binding required the presence of the α subunit (Hu et al., 1993). Despite the identification of a highly specific target for tentoxin action, it has not yet been demonstrated unequivocally that sensitivity of a plant species to the toxin is the only factor responsible for its susceptibility to the pathogen.

In summary, although the mode of action of some host-nonselective fungal toxins has been analyzed in great detail, their contribution to overall pathogenicity of the fungi that produce them remains to be defined. They clearly deserve more attention because their role in pathogenesis may be more significant than generally presumed, but also because they represent valuable tools to study physiological processes in plants (Ballio, 1991).

ELICITORS AND SUPPRESSORS: FUNGAL HOST RANGE DETERMINANTS

A fungus capable of actively penetrating plants and of producing a toxin that affects a fundamental biochemical process has the potential to be a universal phytopathogen. Yet such a pathogen does not exist. Instead, all fungal plant pathogens grow preferentially or exclusively on a limited number of hosts. Therefore, additional factors must exist by which the host range of a pathogen is restricted.

Soon after coming into contact with a plant, fungal pathogens are likely to be detected by the plant and confronted with an active defense system. Clearly then, a successful plant defense response must be based on an effective surveillance system that enables an early recognition of the threat and, as a consequence, the activation of defense-specific processes that act to prevent further fungal development. Successful pathogens, in turn, need to neutralize the plant resistance strategy, and so on. The result of such coevolutionary dynamics is seen in the contemporary highly specialized plant–fungus interactions.

The molecular bases for recognition of potential pathogens by plants outside of gene-for-gene systems (see below) are poorly understood. Plants may recognize an aggressor through non-self factors that are present on the fungal surface (e.g., chitin and glucan fragments) or are secreted by the pathogen (e.g., proteins) and/or through self determinants such as plant cell wall fragments (e.g., oligogalacturonates) that are released by an invading pathogen through the activity of hydrolytic enzymes. After recognition of the pathogen, a multitude of plant resistance–associated reactions is initiated: ion fluxes across the plant plasma membrane, the generation of highly reactive oxygen species (the oxidative burst), the phosphorylation of specific proteins, the activation of enzymes involved in strengthening of the cell wall, the transcriptional activation of numerous defense genes, the induction of phytoalexins, localized cell death at the infection sites (the hypersensitive response [HR]), and the induction of systemic acquired resistance in distal plant organs (Baron and Zambryski, 1995; Kombrink and Somssich, 1995, and references therein; see also Bent, 1996; Crute and Pink, 1996; Dangl et al., 1996; Hammond-Kosack and Jones, 1996; Ryals et al., 1996, in this issue).

Although the actual role of particular defense reactions in restricting further fungal progression in specific interactions has only been incompletely unveiled, plant resistance or susceptibility is presumed to be determined after a sequential exchange of signals between pathogen and host. From a number of fungi, molecules have been isolated that trigger most or at least some of these plant defense reactions. These compounds are called elicitors, and several recent review articles have covered various aspects related to their function (Côté and Hahn, 1994; Ebel and Cosio, 1994; Ebel and Scheel, 1996; Knogge, 1996).

Although some of the more general elicitors such as oligo-*N*-acetylglucosamines and oligogalacturonates are active in several plants, others appear to be species specific. The most extensive data are available for two different elicitors from *Phytophthora sojae.* A glycoprotein derived from culture filtrates of this fungus induces many defense reactions in suspension-cultured cells of the nonhost species parsley but not in the host species soybean. In contrast, a specific heptaglucan elicitor from mycelial walls of the same fungus is active in soybean and other leguminous species but not in parsley (Parker et al., 1988).

An interesting group of small proteinaceous elicitors, termed elicitins, are secreted by species of *Phytophthora* that cause diseases on various plants (Ricci et al., 1989; Yu, 1995). Because elicitins were also found to be produced by another Oomycete, *Pythium vexans*, they may be ubiquitous in this fungal class (Huet et al., 1995). The purified proteins induce necrosis and other defense reactions at the site of application but also distally after their translocation, thus mimicking the effects of fungal infection. In addition, they trigger SAR in tobacco and other solanaceous species. On tobacco, the virulence of *P. parasitica* is inversely correlated with elicitin secretion (Bonnet et al., 1994; Kamoun et al., 1994), implying

that elicitins may be genus-specific determinants of resistance in this (and other solanaceous) species. In contrast, elicitins may be cultivar specific in some *Brassica* species because they have been shown to function as elicitors of necrosis only in a few cultivars of *Raphanus sativus* and *Brassica campestris* (Kamoun et al., 1993b, 1994).

Molecular analyses using a cloned elicitin-encoding gene, *parA1*, from a tomato isolate of *P. parasitica* (Kamoun et al., 1993a) revealed that elicitin genes occur as a multigene family in this fungus. Interestingly, isolates that do not produce elicitins and are virulent on tobacco were found to have retained a set of elicitin genes. This indicates that the virulence of these isolates may be the result of mutations within loci that regulate the transcription of the entire elicitin gene family.

The production of elicitors and the ensuing recognition by the plant are counterproductive for fungal pathogenesis. Therefore, fungi must develop mechanisms to elude recognition by the host or to interfere with plant defense mechanisms. One strategy could include the secretion of suppressors of the defense response (Bushnell and Rowell, 1981). In the most common model, elicitor activity is explained by binding to a specific cell surface–localized plant receptor that initiates a defense-related signal transduction cascade. By comparison, suppressors may interfere directly with elicitor binding, signal transduction, gene activation, or the activity of defense factors from the plant.

Evidence for the actual existence of suppressors came from the observation that successful infection by virulent fungal races frequently renders plant tissues more susceptible to normally avirulent fungi, indicating that plant tissue can be conditioned toward susceptibility (Heath, 1982). To date, suppressors have been described for only a few phytopathogenic fungi (Shiraishi et al., 1991b), those from the pea pathogen *Mycosphaerella pinodes* being the best characterized.

The structurally related glycopeptides suppрescin A and B were purified from germination fluid of *M. pinodes* (Shiraishi et al., 1992). Treatment of pea leaves with a mixture of both suppрescins increased the infection frequency of several otherwise nonpathogenic fungi (Shiraishi et al., 1978, 1991b). Furthermore, suppressor specificity coincided with the host range of *M. pinodes*. The fungus infects different leguminous species to varying degrees. When species that are highly susceptible to *M. pinodes* were treated with suppрescins, they became highly susceptible to the nonpathogen *A. alternata*. Conversely, after suppрescin treatment, *A. alternata* infection levels stayed lower in species that are less susceptible to *M. pinodes*. This observation indicates that the suppрescins may be the determinants of host species specificity for *M. pinodes* (Oku et al., 1980; Shiraishi et al., 1991b).

How do these suppressors allow the fungus to escape the host resistance mechanism? A polysaccharide elicitor also present in fungal germination fluid induces the accumulation of the pea phytoalexin pisatin as well as of two biosynthetic enzymes, phenylalanine ammonia-lyase and chalcone synthase. In the presence of suppрescins, this response is delayed (Yamada et al., 1989). Furthermore, the elicitor-activated transcription of the phenylalanine ammonia-lyase gene was found to be rapidly deactivated upon suppressor treatment of pea tissue (Wada et al., 1995). In vitro studies demonstrated that suppрescin B inhibits the pea plasma membrane H^+-ATPase in a noncompetitive manner (Kato et al., 1993). However, inhibition also occurred in isolated membranes from four nonhost species. By contrast, after treating leaves with the suppressors, cytochemical observations employing the lead precipitation technique in combination with electron microscopy revealed that the ATPase was inhibited only in the host plant, pea (Shiraishi et al., 1991a). Taken together, these data indicate that the suppressors may not function simply by inhibiting elicitor binding to a receptor in pea membranes but rather by affecting the signaling pathway that leads to the activation of the resistance response.

In addition to suppressor formation, several alternative fungal strategies to counter or to avoid the plant defense response can be envisaged. For example, if a recognized elicitor is essential for pathogenesis, the pathogen could develop mechanisms to increase its ability to tolerate the plant defense reactions. If the elicitor component is not crucial, deletion of the respective gene may lead to increased virulence (see below).

TRIGGERING OF PLANT RESISTANCE BY FUNGAL AVIRULENCE GENE PRODUCTS

Genetic analyses of races of fungal pathogens and cultivars of host species demonstrated that pathogen recognition is often determined by the interaction of plant resistance genes with single avirulence genes of the pathogen (Flor, 1955, 1971; also see reviews in this issue by Alfano and Collmer, 1996; Bent, 1996; Crute and Pink, 1996). This gene-for-gene hypothesis may be interpreted in biochemical terms as the interaction of a race-specific pathogen elicitor with either a cultivar-specific plant receptor or alternatively with a cultivar-specific signal transduction compound (Keen, 1982, 1990). In other words, resistant plant cultivars are capable of utilizing specific features of pathogen races to trigger their defense response.

During the past few years, results obtained with a number of pathosystems, mainly involving bacterial pathogens, have corroborated the gene-for-gene complementarity at the molecular level. The small genome size of bacteria and the availability of efficient molecular biology techniques enabled the cloning of many avirulence genes from bacterial pathogens by genetic complementation. A shotgun strategy is, however, not practical for fungi because of their larger genomes and the fact that transformation protocols do not exist for many phytopathogenic fungal species, in particular for most obligate biotrophs. In addition, even if fungi can be transformed, transformation frequencies are usually low. The alternative, a map-based cloning strategy, is not applicable to the Imperfect Fungi, which lack a sexual stage and hence cannot be

Table 1. Cloned Fungal Avirulence Genes

Fungal Species	Avirulence Gene	Gene Product[a]	Activity[b]	Intrinsic Function[b]	Specificity Level	Gain of Virulence[c]
C. fulvum	*Avr4*	135 (105)	Elicitor	?	Cultivar	m
C. fulvum	*Avr9*	63 (28)	Elicitor	?	Cultivar	d
R. secalis	*nip1*	82 (60)	Elicitor	Toxin	Cultivar	d, m
M. grisea	*AVR2-YAMO*	223	Protease?	?	Cultivar	d, i, m
M. grisea	*PWL1*	147	?	?	Species	d?
M. grisea	*PWL2*	145	?	?	Species	d, m

[a] Number of amino acids in the primary translation products and in the processed proteins (in parentheses).
[b] ?, unknown.
[c] m, point mutation; d, deletion; i, insertion.

crossed. Many serious plant pathogens belong to this group of fungi; the identification of their avirulence genes depends on the purification of race-specific elicitors of plant defense responses. To date, avirulence genes have only been cloned from a few fungal species. These include the tomato leaf mold pathogen *Cladosporium fulvum* and the barley leaf scald pathogen *Rhynchosporium secalis*, which are both Imperfect Fungi. In addition, a map-based cloning strategy was recently used successfully to clone avirulence genes from the Ascomycete *M. grisea* (Table 1).

In apoplastic fluids from *C. fulvum*–infected susceptible tomato leaves, two race-specific elicitors, AVR4 and AVR9, were identified (Scholtens-Toma and De Wit, 1988; Joosten et al., 1994) that trigger the HR in tomato cultivars carrying the resistance genes *Cf-4* and *Cf-9* (see Bent, 1996; Hammond-Kosack and Jones, 1996, in this issue). Characterization of the cloned *Avr4* and *Avr9* genes (Van Kan et al., 1991; Van den Ackerveken et al., 1992; Joosten et al., 1994) revealed that the gene products are cysteine-rich preproproteins that are processed by fungal and/or plant proteases to yield active proteins of 105 (AVR4) and 28 (AVR9) amino acids, respectively (Van den Ackerveken et al., 1993; Joosten et al., 1994). Another race-specific elicitor, NIP1, was isolated from culture filtrates of *R. secalis* (Figure 1). The 82–amino acid product of the *nip1* gene is processed to yield a 60–amino acid mature protein that is also cysteine rich (Rohe et al., 1995). In barley plants with the resistance gene *Rrs1*, this elicitor triggers several defense reactions (Hahn et al., 1993). However, the HR is not involved in the resistance of barley to *R. secalis* (Lehnackers and Knogge, 1990).

Genetic complementation and gene disruption were used to analyze the role of these elicitors during pathogenesis. After transfer of the genes into virulent fungal races, transformants were isolated that are avirulent on plants carrying the respective resistance genes (Van den Ackerveken et al., 1992; Joosten et al., 1994; Rohe et al., 1995). In addition, replacement by nonfunctional genes in avirulent races through homologous recombination (Marmeisse et al., 1993; W. Knogge, unpublished data) yielded virulent fungi. Similarly, in a virulent *C. fulvum* isolate, a frame-shift mutation was detected in *Avr4*, leading to a truncated gene product (Joosten et al., 1994). These results demonstrate that the genes *Avr4*, *Avr9*, and *nip1* are sufficient and necessary to condition avirulence in combination with the corresponding plant resistance genes and thus by definition are avirulence genes.

The intriguing questions now are whether the avirulence gene products bind to specific plant receptors and whether these receptors are encoded by the corresponding resistance genes. Several tomato resistance genes including *Cf-9* have been cloned and found to encode proteins with putative secretory signal sequences, single transmembrane domains, and short cytoplasmic tails, indicating their membrane-anchored extracellular localization. In addition, a role of the gene products in recognition is suggested by the occurrence of leucine-rich repeats in the putative extracellular domain (for a more thorough discussion, see Bent, 1996, in this issue). Studies with AVR9 revealed high-affinity binding sites on plasma membranes isolated from Cf-9 plants. However, binding was also detected to membranes from Cf-0 plants lacking resistance to *C. fulvum* and from other solanaceous species, all carrying *Cf-9* homologous genes (Jones et al., 1994; Kooman-Gersmann et al., 1996). Therefore, the question of whether the Cf proteins interact directly with the fungal avirulence gene products is still open for debate (see Hammond-Kosack and Jones, 1996, in this issue).

Which mechanisms allow races of these fungal pathogens to grow on host plants carrying resistance genes? The *Avr9* gene is unique to races of *C. fulvum* that are avirulent on Cf-9 tomato plants but is absent from all virulent races (Van Kan et al., 1991). In contrast, all fungal races contain the *Avr4* gene (Joosten et al., 1994). However, whereas this gene is identical in all races avirulent on Cf-4 tomato plants, the virulent races carry alleles with single nucleotide alterations that frequently affect cysteine residues or, in one case, with a frame-shift mutation. All races transcribe the *Avr4* gene, but the products of the alleles from virulent races were not detectable in the apoplastic compartment of susceptible host cultivars, indicating that the mutated AVR4 proteins are either unstable or not secreted (Joosten et al., 1994; De Wit, 1995; see also Hammond-Kosack and Jones, 1996, in this issue).

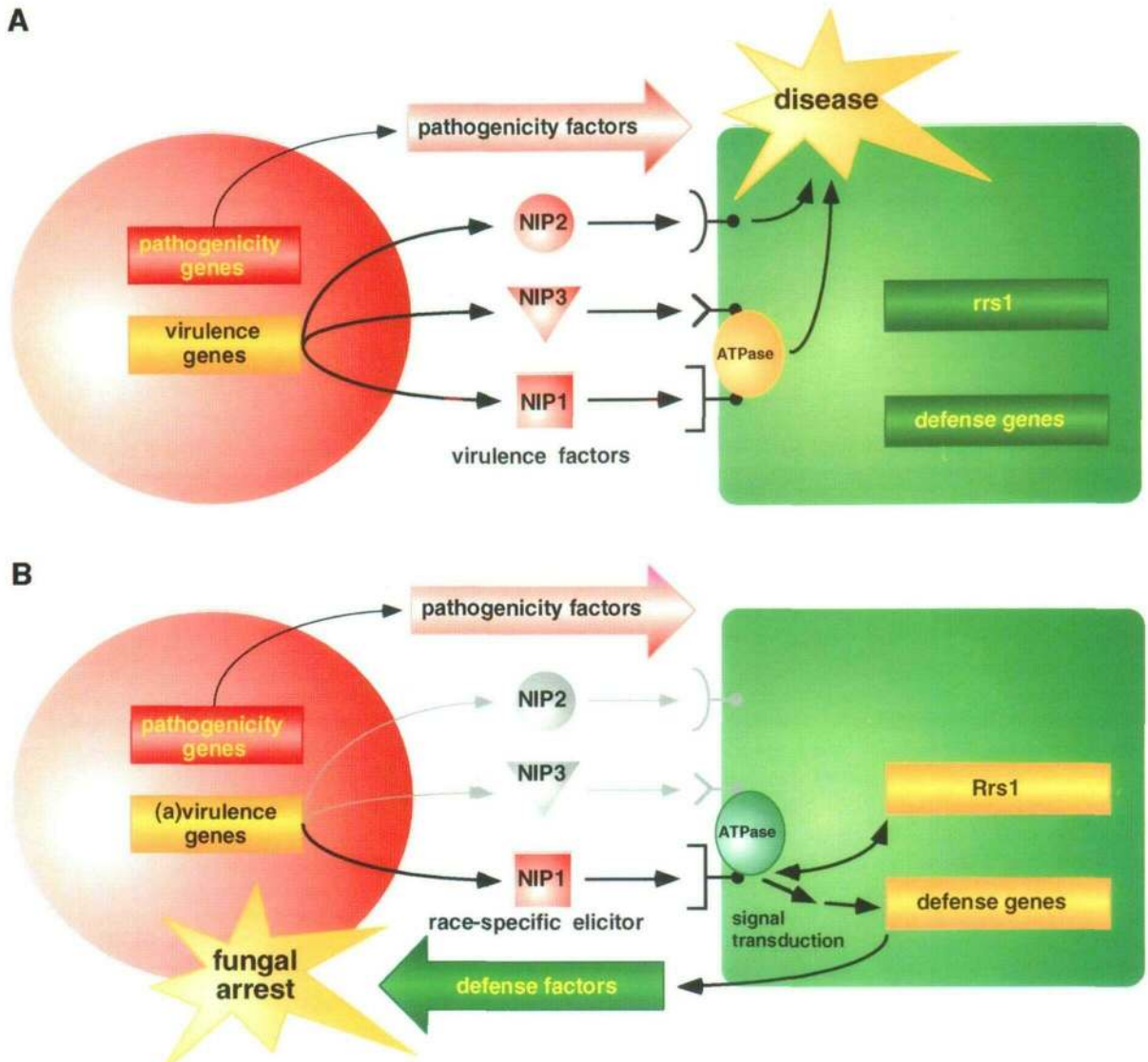

Figure 1. Model of the Interaction of *Rhynchosporium secalis* and Barley.

In addition to factors required for pathogenicity, the fungus secretes a number of virulence factors, including NIP1, but also additional toxic proteins such as NIP2 and NIP3.

(A) Compatible interaction. In susceptible host cultivars lacking the resistance gene *Rrs1*, these factors mediate cell death through stimulation of the plant plasma membrane H^+-ATPase (NIP1 and NIP3) as well as through additional unknown mechanisms (NIP2).

(B) Incompatible interaction. In resistant cultivars, the *Rrs1* gene product is the decisive component in the signal perception and transduction machinery that enables the activation of host defense genes upon specific interaction with one of the virulence factors, NIP1. As a consequence, fungal development is arrested.

R. secalis races that are avirulent on Rrs1 barley carry two classes of *nip1* alleles, both of which encode elicitor-active proteins that differ in three amino acid positions. In contrast, most virulent races lack the *nip1* gene. In addition, not only a highly virulent race but also a race avirulent on an rrs1 cultivar secrete *nip1* gene products that are elicitor inactive. These proteins carry a fourth amino acid alteration at two different positions (Rohe et al., 1995).

The avirulence genes from the imperfect fungi *C. fulvum* and *R. secalis* were isolated after the identification of their products. In contrast, cultivar-specific avirulence genes were isolated from the Ascomycete *M. grisea* by map-based cloning. The *AVR2–YAMO* gene that prevents infection of the rice cultivar Yashiro-mochi resides near the tip of a fungal chromosome. It encodes a 223–amino acid protein that shares a short stretch of amino acid similarity with the active site of neu-

tral Zn^{2+}-proteases (Valent and Chumley, 1994; De Wit, 1995). Mutant analysis revealed that gain of virulence can result from DNA deletion as well as from DNA insertion at the chromosomal tip. In addition, in some virulent isolates, point mutations were identified in the putative protease motif (De Wit, 1995). Although direct evidence for protease activity of AVR2–YAMO is still missing, this avirulence gene product may be functionally different from the *C. fulvum* and *R. secalis* avirulence proteins. The latter are presumably the actual ligands in the resistance-causing signal perception process, whereas the *AVR2–YAMO* gene product may function by releasing an active elicitor from a plant or fungal precursor molecule.

The *PWL* gene family from *M. grisea* exemplifies the concept that resistance at the plant species level can also be controlled by single fungal genes that function in a way very similar to cultivar-specific avirulence genes (Heath, 1991). These genes encode glycine-rich, hydrophilic proteins with putative secretory signal sequences and prohibit pathogenicity on weeping lovegrass (*Eragrostis curvula*). The host range of *M. grisea* includes >50 grass species. The fungus exists in a number of genetically distinct, asexually reproducing populations, only one of which favors rice (Valent and Chumley, 1991). The single species–specific avirulence genes *PWL1* and *PWL2* were isolated from a finger millet (*Eleusine coracana*) and a rice isolate (Kang et al., 1995; Sweigard et al., 1995), respectively.

PWL homologs have been detected in many fungal strains isolated from different host species. However, no correlation has been found between the presence of *PWL* gene sequences in fungal strains and their inability to infect weeping lovegrass, indicating that not all members of this gene family function as avirulence signals. This was substantiated by the finding that two apparently allelic *PWL* homologs, *PWL3* from a finger millet pathogen and *PWL4* from a weeping lovegrass isolate, did not affect the ability of transformed *M. grisea* strains to infect weeping lovegrass. The inactivity of *PWL4* appears to be caused by improper expression of the gene, because it becomes functional when put under the control of the *PWL1* or *PWL2* promoters. By contrast, *PWL3* remained inactive in these experiments, indicating that the gene product is nonfunctional (Kang et al., 1995). In addition, as with the avirulence genes from *C. fulvum* and *R. secalis*, pathogenicity can also be restored by deletion of particular *PWL* genes or be retained in *PWL*-expressing *M. grisea* strains by single base pair changes (Sweigard et al., 1995).

INTRINSIC FUNCTIONS OF FUNGAL AVIRULENCE GENES

If deletion of avirulence genes is advantageous for fungi to overcome recognition by resistant host plants, why is this strategy not always followed by pathogens? Clearly, the role of these genes as avirulence determinants is coincidental and a function of the plant defense mechanism. But what are the genuine gene functions? In the case of the *PWL* genes, sequence comparison and mutation frequencies higher than those in the rest of the *M. grisea* genome strongly indicate that this gene family is highly dynamic and rapidly evolving (Kang et al., 1995). In addition, although spontaneous *PWL2* deletions occurred in fungal strains without affecting the fitness under laboratory conditions, only one field isolate was found to lack *PWL2*-related DNA (Sweigard et al., 1995). Therefore, one can speculate that the *PWL* genes have a function that may be required for fungal fitness in the field.

The *C. fulvum Avr4* gene is induced in plants and expressed during pathogenesis in compatible interactions. In addition, the occurrence of *Avr4* alleles in virulent races suggests a function in virulence. However, a frame-shift mutation detected in the *Avr4* allele from a natural isolate had no visible effect on fungal development in the plant, indicating that the gene is dispensable (Joosten et al., 1994). The *Avr9* gene is activated after fungal hyphae have passed the stomata; it is highly expressed in hyphae growing in the vicinity of vascular tissue. In vitro, *Avr9* expression requires nitrogen-limiting conditions (Van den Ackerveken et al., 1994), possibly reflecting the situation found in the apoplast of tomato leaves. The *Avr9* promoter contains several copies of a sequence motif that was identified as the recognition site of regulatory proteins in *Neurospora crassa* (Caddick, 1992) and *Aspergillus nidulans* (Fu and Marzluf, 1990; Marzluf et al., 1992). Deletion of a number of these elements from the *Avr9* promoter abolishes the inducibility of the gene under low nitrogen conditions, suggesting that they are functional in the transcriptional regulation of the *C. fulvum* gene (De Wit, 1995). Nevertheless, under laboratory conditions, the *Avr9* gene appears to be dispensable for fungal development in the plant, as indicated by the lack of the gene in races virulent on Cf-9 tomato and by disruption mutants. As with the *PWL* genes, a possible role of the gene for fungal development under field conditions has been discussed (De Wit, 1995).

In contrast to these avirulence genes, an actual function is suggested for the *nip1* gene from *R. secalis*. This gene appears to encode a factor that, in addition to its role in determining avirulence, is essential for the expression of virulence (Figure 1). Evidence comes from a fungal *nip1* disruption transformant that displays a level of virulence that is reduced compared with the parental $nip1^+$ race on rrs1 barley. This phenotype is, however, similar to that of wild-type $nip1^-$ races, regardless of the presence or absence of the *Rrs1* gene in the host (W. Knogge, unpublished data). A contribution of NIP1 to fungal virulence is further substantiated by the finding that it is a necrosis-inducing protein in all barley cultivars tested, although at higher concentrations than those required for elicitor activity. Moreover, it is toxic to other mono- and several dicotyledonous plants (Wevelsiep et al., 1991; W. Knogge, unpublished data). At least in part, this host-nonselective toxic activity appears to be based on an indirect stimulation of the plasma membrane H^+-ATPase (Wevelsiep et al., 1993). This observation now raises the question whether the dual functions of NIP1 are mediated through the same plant receptor or whether the elicitor receptor (the one triggering resistance) is distinct from the toxin receptor (the one conditioning disease).

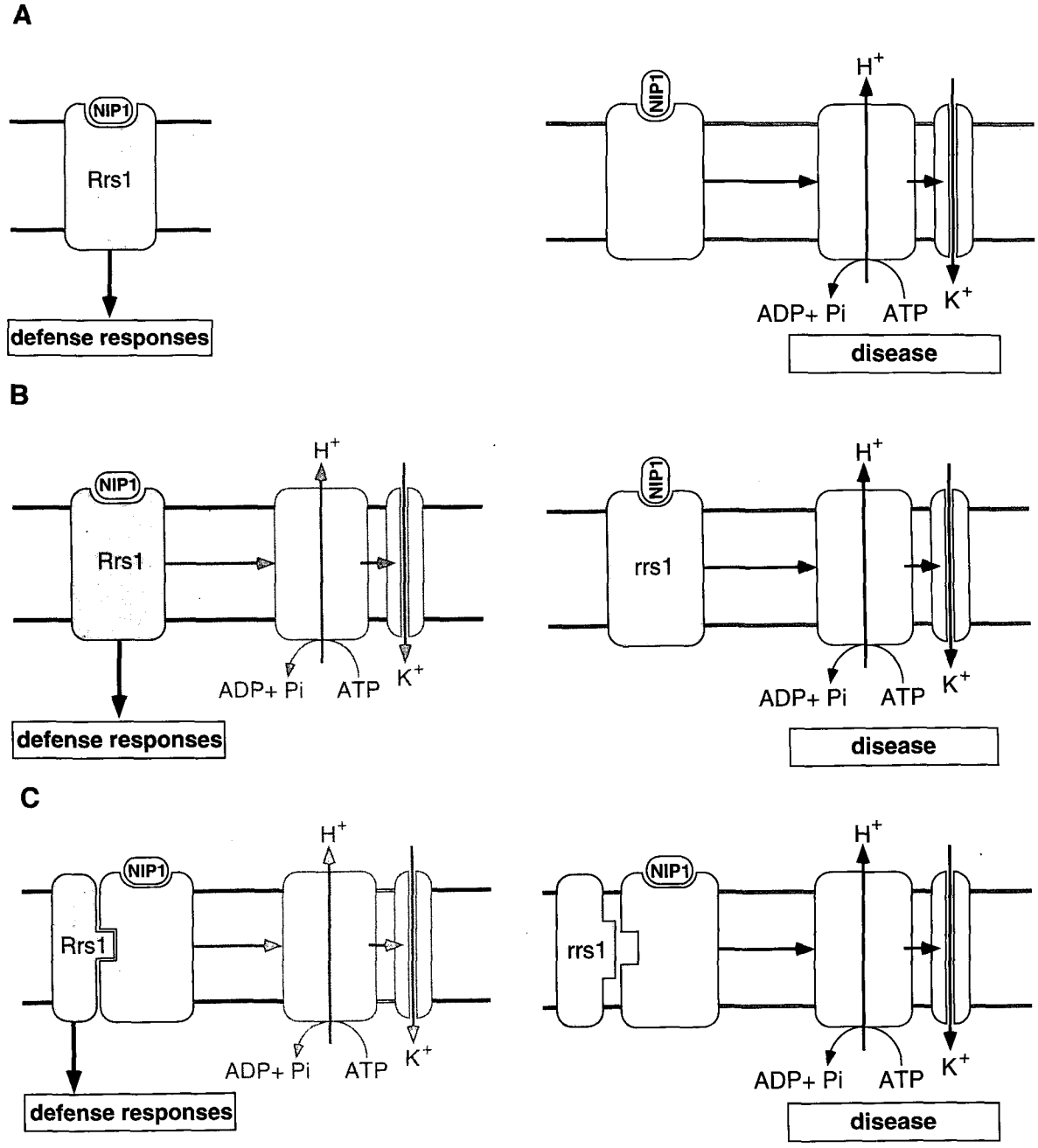

Figure 2. Receptor Models for NIP1 Function.

The avirulence (elicitor) function of NIP1 is depicted on the left, and the virulence (toxin) function of NIP1 is depicted on the right in each panel.

(A) Avirulence activity of NIP1 is mediated through the product of barley resistance gene *Rrs1*, whereas toxic activity results from the interaction with a different plasma membrane receptor. The product of the recessive *rrs1* allele in susceptible plants may not allow efficient binding of NIP1. Alternatively, signal transduction upon binding may be impaired.

(B) Both avirulence function and toxicity of NIP1 are mediated through the same receptor that is encoded by the *Rrs1* locus. Again, differences between the products of the *Rrs1* and *rrs1* alleles in the efficiency of NIP1 binding or in signal transduction may determine whether the plant defense response is triggered.

(C) A single NIP1 receptor mediates the toxic activity. However, it is not encoded by the *Rrs1* locus and requires an interaction with the product of the *Rrs1* gene to initiate the signal transduction pathway leading to the plant defense response. The *rrs1* gene product may interact too poorly with the NIP1 receptor to trigger the defense response. Alternatively, transmission of the signal may be inefficient. In this model, the resistance gene encodes a signal transduction component that may or may not be localized in the plasma membrane.

Further experimentation is also required to determine whether the elicitor receptor is encoded by the resistance gene *Rrs1* (Figure 2).

Current research in several laboratories focuses on the isolation of elicitor receptors from plants. For the heptaglucan elicitor as well as for a 13–amino acid fragment of the glycoprotein elicitor from *P. sojae*, specific binding sites were detected on plasma membranes from soybean and parsley, respectively (*Cosio et al.*, 1992; *Nürnberger et al.*, 1994, 1995). Isolation and cloning of these elicitor receptors should be successful in the near future (Honée and Nürnberger, 1995) and will give further insight into the defense-related signal perception systems of plants. Furthermore, isolation and characterization of the receptor(s) for AVR9, NIP1, and additional avirulence gene products will help to answer the question whether elicitor receptor genes are structurally related to and encoded by plant resistance genes (see Bent, 1996; Hammond-Kosack and Jones, 1996, in this issue).

CONCLUDING REMARKS

Many questions concerning fungal infection of plants remain unanswered. However, research in this field obtains its significance from the fact that these microorganisms are major pathogens of many crop species. An understanding of fungal pathogenicity will not only afford insights into the evolution of fungi but also into the highly dynamic process of their coevolution with plants. In addition, the various factors fungi developed to manipulate the physiology of their hosts to optimize the parasitic lifestyle represent valuable tools to study the affected plant processes. This is clearly demonstrated by the impact of fusicoccin and other phytotoxins on unraveling the roles of the plasma membrane H^+-ATPase in plant cells.

Furthermore, the compounds of fungal origin that are utilized by their hosts to initiate the defense machinery offer the potential for fine-tuned analyses of signaling pathways in plant cells under microbial attack. These analyses, in turn, impinge on cellular functions in healthy plants. Because disease resistance is a response of plant tissues, not only of single cells, one such function is cell-to-cell communication. A major applied goal of this research is to develop strategies to counter the effect of fungal pathogens on crop species. One approach aims to place fungal avirulence genes under the control of defined pathogen-responsive plant promoters (De Wit, 1992). Transformation-mediated combination of such constructs with the complementary plant resistance genes should provide transgenic plants with both components of the switch required to turn on the resistance response. Therefore, experimentation in the coming years is likely not only to deliver answers to the questions raised in the present article but also to utilize the results in molecular breeding approaches to improve resistance of plants to disease.

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REFERENCES

Aitken, A., Collinge, D.B., Van Heusden, B.P.H., Isobe, T., Roseboom, P.H., Rosenfeld, G., and Soll, J. (1992). 14-3-3 proteins: A highly conserved, widespread family of eukaryotic proteins. Trends Biochem. Sci. **17,** 498–501.

Alfano, J.R., and Collmer, A. (1996). Bacterial pathogens in plants: Life up against the wall. Plant Cell **8,** 1683–1698.

Avni, A., Anderson, J.D., Holland, N., Rochaix, J.D., Gromet-Elhanan, Z., and Edelman, M. (1992). Tentoxin sensitivity of chloroplasts determined by codon 83 of β-subunit of proton-ATPase. Science **257,** 1245–1247.

Ballio, A. (1991). Non-host-selective fungal pathogens: Biochemical aspects and their mode of action. Experientia **47,** 783–790.

Baron, C., and Zambryski, P.C. (1995). The plant response in pathogenesis, symbiosis, and wounding: Variations on a common theme? Annu. Rev. Genet. **29,** 107–129.

Bent, A.F. (1996). Plant disease resistance genes: Function meets structure. Plant Cell **8,** 1757–1771.

Bonnet, P., Lacourt, I., Venard, P., and Ricci, P. (1994). Diversity in pathogenicity to tobacco and in elicitin production among isolates of *Phytophthora parasitica*. J. Phytopathol. **141,** 25–37.

Briskin, D.P., and Hanson, J.B. (1992). How does the plant plasma membrane H^+-ATPase pump protons? J. Exp. Bot. **43,** 269–289.

Bushnell, W.R., and Rowell, J.B. (1981). Suppressors of defense reactions: A model for roles in specificity. Phytopathology **71,** 1012–1014.

Caddick, M.X. (1992). Characterization of a major *Aspergillus* regulatory gene, *areA*. In Molecular Biology of Filamentous Fungi, U. Stahl and P. Tudzinski, eds (Weinheim, Germany: VCH Verlagsgesellschaft), pp. 141–152.

Chumley, F.G., and Valent, B. (1990). Genetic analysis of melanin-deficient, nonpathogenic mutants of *Magnaporthe grisea*. Mol. Plant-Microbe Interact. **3,** 135–143.

Correa, A., Jr., and Hoch, H.C. (1995). Identification of thigmoresponsive loci for cell differentiation in *Uromyces* germlings. Protoplasma **186,** 34–40.

Cosio, E.G., Frey, T., and Ebel, J. (1992). Identification of a high-affinity binding protein for a hepta-β-glucoside phytoalexin elicitor in soybean. Eur. J. Biochem. **204,** 1115–1123.

Côté, F., and Hahn, M.G. (1994). Oligosaccharins: Structures and signal transduction. Plant Mol. Biol. **26,** 1379–1411.

Crute, I.R., and Pink, D.A.C. (1996). Genetics and utilization of pathogen resistance in plants. Plant Cell **8,** 1747–1755.

Dangl, J.L., Dietrich, R.A., and Richberg, M.H. (1996). Death don't have no mercy: Cell death programs in plant–microbe interactions. Plant Cell **8,** 1793–1807.

De Wit, P.J.G.M. (1992). Molecular characterization of gene-for-gene systems in plant-fungus interactions and the application of avirulence genes in control of plant pathogens. Annu. Rev. Phytopathol. **30,** 391–418.

De Wit, P.J.G.M. (1995). Fungal avirulence genes and plant resistance genes: Unravelling the molecular basis of gene-for-gene interactions. In Advances in Botanical Research, Vol. 21, J.H. Andrews and I.C. Tommerup, eds (London: Academic Press), pp. 147–185.

Dickman, M.B., Podila, G.K., and Kolattukudy, P.E. (1989). Insertion of cutinase gene into a wound pathogen enables it to infect intact host. Nature **342,** 446–448.

Durbin, R.D., and Uchytil, T.F. (1977). A survey of plant insensitivity to tentoxin. Phytopathology **67,** 602–603.

Ebel, J., and Cosio, E.G. (1994). Elicitors of plant defense responses. Int. Rev. Cytol. **148,** 1–36.

Ebel, J., and Scheel, D. (1996). Signals in host–parasite interactions. In Plant Relationships, G.C. Carroll and P. Tudzinsky, eds (Berlin: Springer-Verlag), in press.

Flor, H.H. (1955). Host–parasite interactions in flax rust—Its genetics and other implications. Phytopathology **45,** 680–685.

Flor, H.H. (1971). Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. **9,** 275–296.

Fu, Y.-H., and Marzluf, G.A. (1990). *Nit-2,* the major positive-acting nitrogen regulatory gene of *Neurospora crassa*, encodes a sequence-specific DNA-binding protein. Proc. Natl. Acad. Sci. USA **87,** 5331–5335.

Gianinazzi-Pearson, V. (1996). Plant cell responses to arbuscular mycorrhizal fungi: Getting to the roots of the symbiosis. Plant Cell **8,** 1871–1883.

Hahn, M., Jüngling, S., and Knogge, W. (1993). Cultivar-specific elicitation of barley defense reactions by the phytotoxic peptide NIP1 from *Rhynchosporium secalis.* Mol. Plant-Microbe Interact. **6,** 745–754.

Hammond-Kosack, K.E., and Jones, J.D.G. (1996). Resistance gene–dependent plant defense responses. Plant Cell **8,** 1773–1791.

Heath, M.C. (1982). The absence of active defense mechanisms in compatible host–pathogen interactions. In Active Defense Mechanisms in Plants, R.K.S. Wood, ed (New York: Plenum Press), pp. 143–156.

Heath, M.C. (1991). The role of gene-for-gene interactions in the determination of host species specificity. Phytopathology **81,** 127–130.

Hoch, H.C., Staples, R.C., Whitehead, B., Comeau, J., and Wolf, E.D. (1987). Signaling for growth orientation and cell differentiation by surface topography in *Uromyces.* Science **235,** 1659–1662.

Honée, G., and Nürnberger, T. (1995). Molecular genetics of fungal plant pathogens and signal perception and transduction in plant–fungus interactions—Workshop of two human capital and mobility networks. Eur. J. Plant Pathol. **101,** 579–583.

Howard, R.J., Ferrari, M.A., Roach, D.H., and Money, D.H. (1991). Penetration of hard substances by a fungus employing enormous turgor pressures. Proc. Natl. Acad. Sci. USA **88,** 11281–11284.

Hu, N., Mills, D.A., Huchzermeyer, B., and Richter, M.L. (1993a). Inhibition by tentoxin of cooperativity among nucleotide binding sites on chloroplast coupling factor 1. J. Biol. Chem. **268,** 8536–8540.

Huet, J.C., Lecaer, J.P., Nespoulous, C., and Pernollet, J.C. (1995). The relationships between the toxicity and the primary and secondary structures of elicitin-like protein elicitors secreted by the phytopathogenic fungus *Pythium vexans.* Mol. Plant-Microbe Interact. **8,** 302–310.

Jones, D.A., Thomas, C.M., Hammond-Kosack, K.E., Balint-Kurti, P.J., and Jones, J.D.G. (1994). Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. Science **266,** 789–793.

Joosten, M.H.A.J., Cozijnsen, A.J., and De Wit, P.J.G.M. (1994). Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. Nature **367,** 384–387.

Kamoun, S., Klucher, K.M., Coffey, M.D., and Tyler, B.M. (1993a). A gene encoding a host-specific elicitor protein of *Phytophthora parasitica.* Mol. Plant-Microbe Interact. **6,** 573–581.

Kamoun, S., Young, M., Glascock, C.B., and Tyler, B.M. (1993b). Extracellular protein elicitors from *Phytophthora*: Host-specificity and induction of resistance to bacterial and fungal pathogens. Mol. Plant-Microbe Interact. **6,** 15–25.

Kamoun, S., Young, M., Förster, H., Coffey, M.D., and Tyler, B.M. (1994). Potential role of elicitins in the interaction between *Phytophthora* species and tobacco. Appl. Environ. Microbiol. **60,** 1593–1598.

Kang, S., Sweigard, J.A., and Valent, B. (1995). The *PWL* host specificity gene family in the blast fungus *Magnaporthe grisea.* Mol. Plant-Microbe Interact. **8,** 939–948.

Kato, T., Shiraishi, T., Toyoda, K., Saitoh, K., Satoh, Y., Tahara, M., Yamada, T., and Oku, H. (1993). Inhibition of ATPase activity in pea plasma membranes by fungal suppressors from *Mycosphaerella pinodes* and their peptide moieties. Plant Cell Physiol. **34,** 439–445.

Keen, N.T. (1982). Specific recognition in gene-for-gene host–parasite systems. Adv. Plant Pathol. **1,** 35–82.

Keen, N.T. (1986). Pathogenic strategies of fungi. In Recognition in Microbe–Plant Symbiotic and Pathogenic Interactions, B. Lugtenberg, ed (Berlin: Springer-Verlag), pp. 171–188.

Keen, N.T. (1990). Gene-for-gene complementarity in plant–pathogen interactions. Annu. Rev. Genet. **24,** 447–463.

Knogge, W. (1996). Elicitors and suppressors of the resistance response. In Resistance of Crop Plants against Fungi, R. Heitefuss, H.H. Hoppe, and H. Hartleb, eds (Jena, Germany: Fischer Verlag), in press.

Kolattukudy, P.E. (1985). Enzymatic penetration of the plant cuticle by fungal pathogens. Annu. Rev. Phytopathol. **23,** 223–250.

Kolattukudy, P.E., Rogers, L.M., Li, D., Hwang, C.S., and Flaishman, M.A. (1995). Surface signaling in pathogenesis. Proc. Natl. Acad. Sci. USA **92,** 4080–4087.

Kombrink, E., and Somssich, I.E. (1995). Defense responses of plants to pathogens. In Advances in Botanical Research, Vol. 21, J.H. Andrews and I.C. Tommerup, eds (London: Academic Press), pp. 1–34.

Kooman-Gersmann, M., Honée, G., Bonnema, G., and De Wit, P.J.G.M. (1996). A high affinity binding site for the AVR9 peptide elicitor of *Cladosporium fulvum* is present on plasma membranes of tomato and other solanaceous plants. Plant Cell **8,** 929–938.

Korthout, H.A.A.J., and de Boer, A.H. (1994). A fusicoccin binding protein belongs to the family of 14-3-3 brain protein homologs. Plant Cell **6,** 1681–1692.

Kubo, Y., and Furusawa, I. (1991). Melanin biosynthesis: Prerequisite for successful invasion of the plant host by appressoria of *Colletotrichum* and *Pyricularia.* In The Fungal Spore and Disease Initiation in Plants and Animals, G.T. Cole and H.C. Hoch, eds (New York: Plenum Press), pp. 205–218.

Lehnackers, H., and Knogge, W. (1990). Cytological studies on the infection of barley cultivars with known resistance genotypes by *Rhynchosporium secalis.* Can. J. Bot. **68,** 1953–1961.

Marmeisse, R., Van den Ackerveken, G.F.J.M., Goosen, T., De Wit, P.J.G.M., and Van den Broek, H.W.J. (1993). Disruption of the avirulence gene *avr9* in two races of the tomato pathogen *Cladosporium fulvum* causes virulence on tomato genotypes with the complementary resistance gene *Cf9.* Mol. Plant-Microbe Interact. **6,** 412–417.

Marrè, E. (1979). Fusicoccin: A tool in plant physiology. Annu. Rev. Plant Physiol. **30,** 273–288.

Marrè, E., and Ballarin-Denti, A. (1985). The proton pump of the plasmalemma and the tonoplast of higher plants. J. Bioenerg. Biomembr. **17,** 1–21.

Marzluf, G.A., Kanaan, M., and Fu, Y.-H. (1992). Molecular analysis of DNA-binding, trans-acting regulatory proteins of *Neurospora crassa.* In Molecular Biology of Filamentous Fungi, U. Stahl and P. Tudzynski, eds (Weinheim, Germany: VCH–Verlagsgesellschaft), pp. 153–166.

Mendgen, K., and Deising, H. (1993). Infection structures of fungal plant pathogens—A cytological and physiological evaluation. New Phytol. **124,** 192–213.

Meyer, C., Waldkotter, K., Sprenger, A., Schlosser, U.G., Luther, M., and Weiler, E.W. (1993). Survey of the taxonomic and tissue distribution of microsomal binding sites for the non-host selective fungal phytotoxin, fusicoccin. Z. Naturforsch. **48,** 595–602.

Michelet, B., and Boutry, M. (1995). The plasma membrane H^+-ATPase. Plant Physiol. **108,** 1–6.

Nicholson, R.L., and Epstein, L. (1991). Adhesion of fungi to the plant surface. In The Fungal Spore and Disease Initiation in Plants and Animals, G.T. Cole and H.C. Hoch, eds (New York: Plenum Press), pp. 3–23.

Nürnberger, T., Nennstiel, D., Jabs, T., Sacks, W.R., Hahlbrock, K., and Scheel, D. (1994). High affinity binding of a fungal oligopeptide elicitor to parsley plasma membranes triggers multiple defense responses. Cell **78,** 449–460.

Nürnberger, T., Nennstiel, D., Hahlbrock, K., and Scheel, D. (1995). Covalent cross-linking of the *Phytophthora megasperma* oligopeptide elicitor to its receptor in parsley membranes. Proc. Natl. Acad. Sci. USA **92,** 2338–2342.

Oecking, C., Eckerskorn, C., and Weiler, E.W. (1994). The fusicoccin receptor of plants is a member of the 14-3-3 superfamily of eukaryotic regulatory proteins. FEBS Lett. **352,** 163–166.

Oku, H., Shiraishi, T., Ouchi, S., and Ishiura, M. (1980). A new determinant of pathogenicity in plant disease. Naturwissenschaften **67,** 310.

Parker, J.E., Hahlbrock, K., and Scheel, D. (1988). Different cell-wall components from *Phytophthora megasperma* f. sp. *glycinea* elicit phytoalexin production in soybean and parsley. Planta **176,** 75–82.

Ricci, P., Bonnet, P., Huet, J.C., Sallantin, M., Beauvais-Cante, F., Bruneteau, M., Billard, V., Michel, G., and Pernollet, J.C. (1989). Structure and activity of proteins from pathogenic fungi *Phytophthora* eliciting necrosis and acquired resistance in tobacco. Eur. J. Biochem. **183,** 555–563.

Rohe, M., Gierlich, A., Hermann, H., Hahn, M., Schmidt, B., Rosahl, S., and Knogge, W. (1995). The race-specific elicitor, NIP1, from the barley pathogen, *Rhynchosporium secalis,* determines avirulence on host plants of the *Rrs1* resistance genotype. EMBO J. **14,** 4168–4177.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.-Y., and Hunt, M.D. (1996). Systemic acquired resistance. Plant Cell **8,** 1809–1819.

Schäfer, W. (1994). Molecular mechanisms of fungal pathogenicity to plants. Annu. Rev. Phytopathol. **32,** 461–477.

Scheffer, R.P. (1991). Role of toxins in evolution and ecology of plant pathogenic fungi. Experientia **47,** 804–811.

Scholtens-Toma, I.M.J., and De Wit, P.J.G.M. (1988). Purification and primary structure of a necrosis-inducing peptide from the apoplastic fluids of tomato infected with *Cladosporium fulvum* (syn. *Fulvia fulva*). Physiol. Mol. Plant Pathol. **33,** 59–67.

Shiraishi, T., Oku, H., Yamashita, M., and Ouchi, S. (1978). Elicitor and suppressor of pisatin induction in spore germination fluid of pea pathogen, *Mycosphaerella pinodes.* Ann. Phytopathol. Soc. Jpn. **44,** 659–665.

Shiraishi, T., Araki, M., Yoshioka, H., Kobayashi, I., Yamada, T., Ichinose, Y., Kunoh, H., and Oku, H. (1991a). Inhibition of ATPase activity in pea plasma membranes *in situ* by a suppressor from a pea pathogen, *Mycosphaerella pinodes.* Plant Cell Physiol. **32,** 1067–1075.

Shiraishi, T., Yamada, T., Oku, H., and Yoshioka, H. (1991b). Suppressor production as a key factor for fungal pathogenesis. In Molecular Strategies of Pathogens and Host Plants, S.S. Patil, S. Ouchi, D. Mills, and C. Vance, eds (New York: Springer-Verlag), pp. 151–162.

Shiraishi, T., Saitoh, K., Kim, H.M., Kato, T., Tahara, M., Oku, H., Yamada, T., and Ichinose, Y. (1992). Two suppressors, supprescins A and B, secreted by a pea pathogen, *Mycosphaerella pinodes.* Plant Cell Physiol. **33,** 663–667.

Sweigard, J.A., Carroll, A.M., Kang, S., Farrall, L., Chumley, F.G., and Valent, B. (1995). Identification, cloning, and characterization of *PWL2,* a gene for host species specificity in the rice blast fungus. Plant Cell **7,** 1221–1233.

Valent, B., and Chumley, F.G. (1991). Molecular genetic analysis of the rice blast fungus, *Magnaporthe grisea.* Annu. Rev. Phytopathol. **29,** 443–467.

Valent, B., and Chumley, F.G. (1994). Avirulence genes and mechanisms of genetic instability in the rice blast fungus. In Rice Blast Diseases, R. Zeigler, P.S. Teng, and S. Leong, eds (Wallingford, UK: Centre for Agriculture and Biosciences International), pp. 111–134.

Van den Ackerveken, G.F.J.M., Van Kan, J.A.L., and De Wit, P.J.G.M. (1992). Molecular analysis of the avirulence gene *avr9* of the fungal tomato pathogen *Cladosporium fulvum* fully supports the gene-for-gene hypothesis. Plant J. **2,** 359–366.

Van den Ackerveken, G.F., Vossen, P., and De Wit, P.J.G.M. (1993). The AVR9 race-specific elicitor of *Cladosporium fulvum* is processed by endogenous and plant proteases. Plant Physiol. **103,** 91–96.

Van den Ackerveken, G.F., Dunn, R.M., Cozijnsen, A.J., Vossen, J.P., Van den Broek, H.W., and De Wit, P.J.G.M. (1994). Nitrogen limitation induces expression of the avirulence gene *avr9* in the tomato pathogen *Cladosporium fulvum.* Mol. Gen. Genet. **243,** 277–285.

Van Kan, J.A.L., Van den Ackerveken, G.F.J.M., and De Wit, P.J.G.M. (1991). Cloning and characterization of cDNA of avirulence gene *avr9* of the fungal pathogen *Cladosporium fulvum,* causal agent of tomato leaf mold. Mol. Plant-Microbe Interact. **4,** 52–59.

Wada, M., Kato, H., Malik, K., Sriprasertsak, P., Ichinose, Y., Shiraishi, T., and Yamada, T. (1995). A supprescin from a

phytopathogenic fungus deactivates transcription of a plant defense gene encoding phenylalanine ammonia-lyase. J. Mol. Biol. **249,** 513–519.

Walton, J.D. (1996). Host-selective toxins: Agents of compatibility. Plant Cell **8,** 1723–1733.

Walton, J.D., Ahn, J.-H., Akimitsu, K., Pitkin, J.W., and Ransom, R. (1994). Leaf-spot disease of maize: Chemistry, biochemistry, and molecular biology of a host-selective cyclic peptide. In Advances in Molecular Genetics of Plant–Microbe Interactions, M.J. Daniels, J.A. Downie, and A.E. Osbourn, eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 231–237.

Wevelsiep, L., Kogel, K.-H., and Knogge, W. (1991). Purification and characterization of peptides from *Rhynchosporium secalis* inducing necrosis in barley. Physiol. Mol. Plant Pathol. **39,** 471–482.

Wevelsiep, L., Rüpping, E., and Knogge, W. (1993). Stimulation of barley plasmalemma H^+-ATPase by phytotoxic peptides from the fungal pathogen *Rhynchosporium secalis.* Plant Physiol. **101,** 297–301.

Yamada, T., Hashimoto, H., Shiraishi, T., and Oku, H. (1989). Suppression of pisatin, phenylalanine ammonia-lyase mRNA, and chalcone synthase mRNA accumulation by a putative pathogenicity factor from the fungus *Mycosphaerella pinodes.* Mol. Plant-Microbe Interact. **2,** 256–261.

Yu, L.M. (1995). Elicitins from *Phytophthora* and basic resistance in tobacco. Proc. Natl. Acad. Sci. USA **92,** 4088–4094.