Annu. Rev. Phytopathol. 1999. 37:285–306

PHYTOALEXINS: What Have We Learned After 60 Years?

Ray Hammerschmidt
Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824; e-mail: hammers1@pilot.msu.edu

Key Words phytoalexin, active defense, secondary metabolism, disease resistance

■ **Abstract** One of the best and longest-studied defense response of plants to infection is the induced accumulation of antimicrobial, low-molecular-weight secondary metabolites known as phytoalexins. Since the phytoalexin hypothesis was first proposed in 1940, a role for these compounds in defense has been revealed through several experimental approaches. Support has come, for example, through studies on the rate of phytoalexins in relation to cessation of pathogen development, quantification of phytoalexins at the infection site, and relationship of pathogen virulence to the phytoalexin tolerance. Evidence in support of phytoalexins in resistance as well some recent advances in phytoalexin biosynthesis are reviewed. Criteria for evaluating a role for phytoalexins in disease resistance are also discussed.

INTRODUCTION

Resistance to disease can be described on several levels: These include nonhost resistance, parasite- and race-specific resistance, plant age– and organ-specific resistance, and acquired or induced resistance (38). To fully understand each type of resistance, we need to determine what physical and biochemical factors are needed to stop the pathogen from developing in the tissue after infection.

The physiological/biochemical basis of resistance of plants to fungal, oomycte, and bacterial pathogens has been associated with both preformed and infection-induced antimicrobial compounds (36, 62, 95). For example, preformed antimicrobial compounds are involved in the resistance of oats to *Gaumanomyces graminis* f.sp. *tritici* (73) and onion bulbs to *Colletotrichum circinans* (2). However, the expression of resistance (i.e. defense) in most plant-pathogen interactions cannot be explained by the presence of preformed inhibitors. Most research on resistance mechanisms has shown that the plant uses defenses that are activated after infection to stop pathogen development (22). Many biochemical changes occur in plants after infection, and some of these have been associated with the expression of defense because they have activity against pathogens in vitro.

One type of biochemical response that is strongly associated with defense is the accumulation of phytoalexins, which are defined as low-molecular-weight antimicrobial compounds that are produced after infection (74). The idea that defenses can be activated after infection was crystallized by the phytoalexin hypothesis of Müller & Borger (69), and the study of phytoalexins has been part of the fabric of plant defense research ever since. Phytoalexins have received much attention over the past 60 years, and much of this work has provided new insight into the regulation of gene activation, phytochemical diversity, and the chemistry and biochemistry of secondary metabolites. The topic of phytoalexins and closely related defense responses has been reviewed numerous times (e.g. 5, 21, 22, 32, 36, 62, 82), including several in the *Annual Review of Phytopathology* (17, 25, 51, 52, 70, 94). This review focuses on areas that help illustrate the types of information that can be used to show a role for phytoalexins in defense responses.

PHYTOALEXINS: From Müller to the Present

The year 2000 will be the 60th anniversary of Müller & Borger's paper that first presented the phytoalexin hypothesis (69), and a good summary of Müller's contributions recently was published (40). Müller & Borger demonstrated that prior infection of potato tuber tissue with an incompatible race of *Phytophthora infestans* induced resistance to a subsequent challenge by inoculation with a compatible race of *P. infestans* or a tuber-infecting *Fusarium*. From this work, they hypothesized that the tuber tissue, in response to the incompatible interaction, produced nonspecific substances (phytoalexins) that inhibited further growth of the pathogen and also protected the tissue against later infection by other compatible pathogens. Over 15 years later, Müller demonstrated that bean pod tissues infected with incompatible pathogens produced strongly fungistatic substances (68). These results, along with those being obtained in other systems at that time [e.g. work by Kuć with potato tuber tissue (50)], demonstrated that plant tissues infected with incompatible pathogens could produce antimicrobial compounds. In the early 1960s, the first report on the structure of a phytoalexin, pisatin from *Pisum sativum*, was reported (reviewed in 17). A good summary of the history of phytoalexin research was recently published and shows how phytoalexin research has evolved to encompass many area of plant biology, including biosynthesis, chemosystematics, natural products chemistry, molecular biology, and fungal genetics (77).

The original definition of phytoalexins was very restrictive (68, 69). In 1981, a very general definition of phytoalexins was presented simply stating that phytoalexins are antimicrobial compounds produced after infection or elicitation by abiotic agents (74). Although this definition is easy to apply to induced secondary metabolites, the definition leaves open the question whether phytoalexins are important in defense, and, if so, to what degree.

STRUCTURE AND DISTRIBUTION

Phytoalexins are chemically diverse (5, 21, 32, 36, 52, 62). Examples include simple phenylpropanoid derivatives, flavonoid- and isoflavonoid-derived phytoalexins, sesquiterpenes, and polyketides; representative structures are found in Figure 1. Phytoalexins may be biosynthetically derived from one or several primary biosynthetic pathways. Polyketides such as 6-methoxymellein and sesquiterpenes such as capsidiol are derived from the acetate-malonate and acetate-mevalonate pathways, respectively. Similarly, coniferyl alcohol, a phenylpropanoid phytoalexin from flax, is produced from phenylalanine, a product of the shikimic acid pathway. Other phytoalexins, such as the deoxyanthocyanidin luteolindin and the pterocarpan pisatin, are derived from products of the shikimic acid and acetate-malonate pathways. Finally, phytoalexins like the glyceollins and kievitone are biosynthesized using precursors from three primary metabolic pathways (the shikimic acid, acetate-malonate, and acetate-mevalonate pathways) (52).

Although there is much diversity in chemical structure, many plant families produce phytoalexins that fall into the same chemical class (32, 52). Thus, phytoalexins have been used to examine chemotaxonomic relationships (e.g. 81). The relationships are not always completely clear-cut as some members of a plant family produce phytoalexins that are chemically unrelated to those produced by other members of the same family. This is most clearly demonstrated in the Poaceae, where stilbenes, deoxyanthocyanidins, avenanthramides, and diterpenes have been

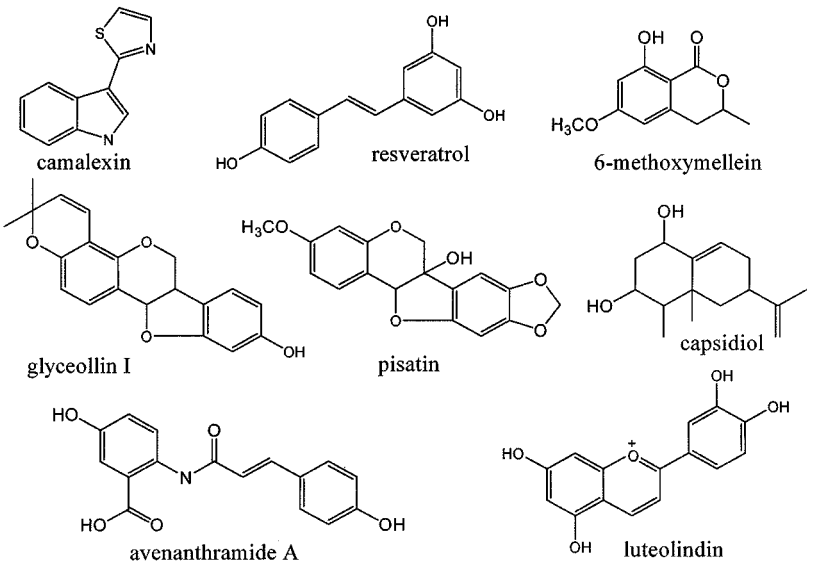

Figure 1 Representative phytoalexin structures.

reported as phytoalexins (reviewed in 32). Although the studies on phytoalexin diversity do not directly shed new light on the role of phytoalexins in defense, the research has provided us with a wealth of information on new chemistry as well as information on the distribution of phytoalexin types and how the spectrum of phytoalexin structures can change as plants are domesticated.

BIOSYNTHESIS AND ELICITATION

Biosynthesis

One approach for evaluating phytoalexins is to clone genes specific for phytoalexin biosynthesis, use these genes to generate transgenic plants that do not produce or overproduce the phytoalexin, and then evaluate the transgenic plant for altered resistance to pathogens. Two problems currently face this approach. First, most of the research on phytoalexin biosynthesis has not focused on enzymes (and the genes that encode these enzymes) that are not specific for phytoalexin biosynthesis. For example, phenylalanine ammonia lyase, chalcone synthase, and hydroxycinnamyl CoA ligases have been extensively studied in relation to flavonoid and isoflavonoid phytoalexin biosyntheses, but these enzymes are also needed for synthesis of other phenolic compounds (21, 70). A similar situation holds true for studies on hydroxymethylglutaryl CoA reductase, a key enzyme in general terpenoid biosynthesis (13). The solution to this problem, as is discussed below, is to focus on phytoalexin-specific genes. The second problem involves the manipulation of plant genes. Specific inhibition of phytoalexin biosynthesis by disruption of a key biosynthetic gene would provide plants that would be ideally suited to evaluate the role of phytoalexins in defense. However, current technology does not allow for specific gene disruption as is possible with bacteria (e.g. 48a) and fungi (e.g. 96).

Thus, the only molecular approach is to transform plants with sense constructs of phytoalexin biosynthetic genes (e.g. 89) or to use antisense constructs as has been done with other defense-associated genes (e.g. 87a).

Several enzymes that appear to be phytoalexin pathway–specific have been identified recently, and the genes encoding some of these have been cloned. For example, in the Solanaceae and Malvaceae, sesquiterpenoid phytoalexins are produced (36, 52). The recent identification and cloning of the gene for the first enzyme specifically required for sesquiterpene production, a sesquiterpene cyclase that converts farnesyl diphosphate into cyclic hydrocarbon, has been reported for several plants (8).

Tobacco and green pepper both produce capsidiol, which is derived from 5-epi-aristolochene (3, 4, 8). 5-Epi-aristolochene synthase, a sesquiterpene cyclase, has been isolated and cloned from both plant species (3, 4, 27). Although 5-epi-aristolochene synthase is not an important regulatory step in capsidiol biosynthesis (49), the expression of this gene appears to be essential to divert farnesyl diphosphate into aristolochene and thus would be a good enzyme for disruption

of the pathway to capsidiol. (+)-delta-Cadinene synthase, the sesquiterpene cyclase that is required for sesquiterpene phytoalexin production in cotton, also has been cloned (11). The cyclase products are hydrocarbons that must be further metabolized to the phytoalexin end product. Capsidiol is hydroxylated in two positions; thus, the recent report of an aristolochene 3-hydroxylase is an important step toward identifying all steps that lead to capsidiol (39).

In pea plants, the terminal step of pisatin synthesis is the O-methylation of (+)6a-hydroxymaackian. A cDNA encoding the enzyme (+)6a-hydroxymaackiain 3-*O*-methyltransferase (76), the last step of pisatin biosynthesis, has been cloned and the authors suggest that this gene will provide another tool to evaluate the role of pisatin (98). In other legumes, the isoflavonoid or pterocarpan phytoalexins need prenylation for full activity. A prenyl transferase that is required for the final steps of glyceollin synthesis in soybean has been identified (25) and, like the methyl transferase needed for pisatin synthesis, would be a key target in understanding the role of these phytoalexins.

The protein responsible for the synthesis of 6-methoxymellein (52), the polyketide-derived phytoalexin of carrot, has been isolated (53, 54). This is a multifunctional enzyme that produces 6-hydroxymellein from malonyl CoA. In addition, the methyl transferase that converts 6-hydroxymellein to 6-methoxymellein has been partially characterized (55).

An enzyme activity that is specific for the synthesis of the avenanthramides (15) (phytoalexins formerly known as the avenalumins) from oats has been detected. The enzyme, hydroxyanthranilate hydroxycinnamoyl transferase, catalyzes the condensation of 5-OH-anthranilic acid and a hydroxycinnamyl CoA ester and is induced in oats by elicitors (41). The avenanthramides are, however, constitutive components of certain parts of oats (15), a fact that would need to be taken into account in assessing results from oat plants that have been transformed to modify phytoalexin production.

Camalexin is the only characterized phytoalexin of Arabidopsis (91). Like all other phytoalexins of the Brassicaceae, camalexin is an indole with the sulfur-containing moiety, in this case a thiazole ring, at the 3-position of the indole (52). Infection or treatment of Arabidopsis with elicitors induces camalexin and the expression of genes of the tryptophan pathway (102, 103). These genes possibly represent a common set of genes needed for the production of indole, a precursor of tryptophan and possibly camalexin (105). Labeling and mutant studies have shown that anthranilic acid, but not tryptophan, is a camalexin precursor (92, 106). Elicited Arabidopsis cell suspensions also accumulate indole and incorporate labeled indole and anthranilic acid into camalexin (105). The thiazole ring of camalexin is derived from the cyclization of cysteine (106). One proposed biosynthetic pathway suggests that indole-3-carboxaldehyde is a precursor that condenses with cysteine to form the immediate precursors to camalexin (10, 106). This is supported by the facts that indole-3-carboxaldehyde is present in Arabidopsis and that ^{14}C anthanilic acid is incorporated into this aldehyde (IA Kagan & R Hammerschmidt, unpublished data). Although no genes that are specific for camalexin have

been identified, the identification of enzymes that catalyze camalexin-specific steps will facilitate the identification of the biosynthetic genes and, eventually, provide a clearer picture of the role of camalexin.

The enzymes or biosynthetic steps described above can potentially be used in assessing the role of phytoalexins via molecular manipulation of the plant from which they were isolated (23, 66). Producing plants that are specifically blocked in phytoalexin synthesis would provide excellent tools to study the relative contribution that the phytoalexins play in defense. However, even though these enzymes appear to be specific for phytoalexin synthesis, experiments that involve the suppression or enhancement of phytoalexin synthesis should also evaluate the effect of the transformation on the expression of other defenses as well as changes in related primary and secondary metabolism that may influence the host-pathogen interaction used to test the plants. Thus, the engineering of new secondary metabolites, like phytoalexins, may not come without risks. Some of the potential problems in engineering new terpenoid biosynthesis have been addressed (66), and at least one example has been reported where engineering a sesquiterpene cyclase into a plant resulted in the production of a totally new and unexpected compound (107).

Elicitation and Elicitors

The production of phytoalexins after infection suggests that a product of the pathogen or the host-pathogen interaction is involved in triggering phytoalexin biosynthesis. A variety of pathogen- and plant-produced molecules, collectively known as elicitors, will induce phytoalexins and other defense responses (33). Some progress has been made in determining if plant cells have receptors for these elicitors (12, 33). Although most elicitors appear to lack any specificity that can be related to the outcome of a host-parasite interaction, some have been shown to have the same specificity as the pathogen has with its host.

The modern synthesis of the gene-for-gene hypothesis states that resistance occurs only when the product of a pathogen avirulence gene interacts with the product of a plant resistance gene (7, 43). Because of the high degree of specificity, gene-for-gene systems provide a good framework to determine if the product of the avirulence gene can also act as a race-/cultivar-specific elicitor of defense responses like phytoalexin accumulation.

Race-specific elicitors, many of which appear to be the products of specific avirulence genes, have been identified and characterized from several pathogens. For example, two specific elicitor proteins produced by the tomato pathogen *Cladosporium fulvum* are the products of two avirulence genes, and when these proteins are infiltrated into leaves of plants with the corresponding resistance gene, the tissue responds with a hypersensitive response (see 19 for review of this system). Although production of phytoalexins by the purified *C. fulvum* elicitors has not been reported, an earlier report indicated that crude preparations containing race-specific elicitors induce phytoalexin in tomato tissue containing the corresponding resistance gene (20).

A race-specific elicitor has been isolated from *Uromyces vigna* (24). This elicitor will likely induce phytoalexin production in cowpea resistant to this race of the pathogen based on the hypersensitive response (HR)-like symptoms induced by treatment of resistant cowpea leaves with the elicitor. The syringolides (67) are another type of specific elicitors produced by *Pseudomonas syringae* pathovars that have the avirulence gene *avrD* (48). These natural products are not the *avrD* gene product, but rather have their synthesis directed by this gene (48). When infiltrated into soybean leaves or added to soybean cell suspensions that have the resistance gene *Rpg4* (46), the syringolides elicit the hypersensitive response and other defenses. Syringolides will also induce glyceollin production in soybean with the *Rpg4* gene (NT Keen, personal communication). *Arabidopsis thaliana* carrying the resistance genes *RPS2* and *RPM1* are resistant to isolates of *P. syringae* that have the avirulence genes *avrRpt2* and *avrB*, respectively. Using a quantative transient expression assay, Leister et al (56a) demonstrated that expression of the *avrRpt2* and *avrB* genes in plant cells induced a resistance reaction in plants that carried *RPS2* and *RPM1*, respectively. This demonstrated that the protein products of these avr genes can function as race-specific elicitors. It would be interesting to see if camalexin, the Arabidopsis phytoalexin, also accumulated in response to the in planta–produced avr gene products.

The nonspecific elicitors (which include proteins, glycoproteins, various types of oligosaccharides, and unsaturated fatty acids) are more difficult to assign a role to in the induction of phytoalexin production by pathogens (33). For example, all races of *Phytophthora infestans* contain the elicitors arachidonic and eicosapentaenoic acids (9). However, the mere presence or absence of these elicitors does not appear to determine how a potato line will react to *P. infestans*. Similary, all oomycetes contain glucans in their cell walls, and true fungi have chitin that can act as a potent elicitor in some plant species, even if the living pathogen does not (33). Characterization of receptors for glucan elicitors suggests that if these elicitors are released from the hyphae, it is possible that a resistance response can be induced (33). An elicitor role for glucans released from hyphae is supported by the observations of Yoshikawa et al (99) who reported that tobacco transformed with a glucanase that will release elicitors from *Phytophthora* cell walls was more resistant to infection. The nonspecific elicitors may play a role in nonhost resistance, and a recent report indicated that the protein elicitors from *Phytophthora* species (the elicitins) that induce phytoalexin accumulation (78) may be involved in the recognition of *P. infestans* as a nonpathogen by *Nicotiana* (44).

EVIDENCE FOR A ROLE IN DEFENSE

Phytoalexins: Defense or Just a Response to Infection?

The current definition of phytoalexins does not include any criteria that would allow discrimination between a role for phytoalexins in defense versus just a response to infection. However, good evidence for a role in defense does exist. In

1981, Keen described several lines of evidence that support a role for phytoalexins in disease resistance (45a). The evidence included data that documented: 1. Localization and timing of phytoalexin accumulation in infected tissue in relation to pathogen development; 2. strong positive correlation of rapid phytoalexin production with incompatible interactions in gene-for-gene plant pathogen systems; 3. association of rapid phytoalexin accumulation with resistance genes that condition rapid restriction of pathogen development; 4. use of metabolic inhibitors that enhance susceptibility and block phytoalexin production; 5. a positive relationship between pathogen virulence and tolerance of phytoalexins; 6. an increase of plant tissue resistance by stimulation of phytoalexin production prior to inoculation. The following sections describe some of the recent and older evidence and approaches that can be used to establish a role for phytoalexins is defense.

Phytoalexins in Race- and Parasite-Specific Resistance

In the past few years, several resistance and avirulence genes have been cloned and sequenced, and evidence is accumulating that the interaction of the resistance gene and avirulence gene products results in the expression of defense genes and, ultimately, cessation of pathogen growth (7, 19, 37, 43, 56, 80, 88). In several demonstrated or putative gene-for-gene systems, resistance has been associated with phytoalexin production (31, 47, 60, 63, 64). Can closer analysis of any of these systems reveal more insight into the role of phytoalexins in defense? Three of these are discussed below.

An examination of Flor's classical gene-for-gene system with flax and the flax rust pathogen *Melampsora lini* reveals that resistance encompasses several levels of host reaction to infection (28). This is also likely to be true for other systems. Can this variation in the resistance phenotype be related to the ability of the plant to express defenses such as phytoalexins?

Flax lines are categorized as resistant to *M. lini* if they receive a rating of 0 (immune) through a rating of 2 (necrosis with some sporulation), and these levels of resistance to race 1 of *M. lini* can be demonstrated by the use of flax isolines that differ by one resistance gene (28). Since resistance (R) genes are thought to act by recognizing the pathogen avirulence gene product (43), could the different resistance genes regulate the expression of defense differently and could this result in differential phytoalexin accumulation that might explain the observed levels of resistance? A cytological study by Littlefield (58) suggested that different resistance genes acted through the expression of different defenses. Could this observation be related to diffences in phytoalexin production?

Keen & Littlefield (47) found that flax produces coniferyl alcohol and coniferyl aldehyde as phytoalexins in resistant flax–flax rust interactions. Using isolines of flax carrying different resistance genes, they showed that lesion size was inversely proportional to the rate of coniferyl alcohol accumulation. In addition, lines with resistance genes that conditioned a 0 infection type produced coniferyl alcohol more quickly than did lines with resistance genes conditioning the 2 infection type. Furthermore, even within the isolines with different genes conditioning a 0

infection type, there was variation in the speed of coniferyl alcohol accumulation that could be correlated with the degree of pathogen restriction.

The resistance of soybean to *Pseudomonas syringae* pv. *glycinea* (Psg) is also a gene-for-gene system, and Keen and co-workers have provided evidence that the resistance conferred by several R genes is associated with glyceollin production (60). A strong correlation between the accumulation of glyceollin and resistance was observed with four races of Psg and nine cultivars of soybean. Using data from three cultivars inoculated with three races of Psg revealed a linear inverse relationship between the amount of glyceollin that accumulated in the tissue and the log of the number of bacterial cells. The fact that there was a linear relationship suggests that there is a quantitative relationship between the amount of phytoalexin produced and the degree of disease resistance.

Mayama et al (63) recently demonstrated a positive relationship between resistance of oats to *Puccinia coronata* and the accumulation of avenanthramides (formerly known as avenalumins). They crossed the oat line Shokan-1 (hypersensitively resistant to *P. coronata* race 226) with oat lines that were very susceptible and analyzed the F_1 and F_2 for resistance, length of infection hyphae, and accumulation of avenanthramide. The F_1 hybrids from both crosses exhibited hypersensitive resistance to race 226. However, cytological observations showed that the pathogen produced longer infection hyphae in both the F_1 hybrids, and chemical analysis revealed that the phytoalexin accumulated to amounts that were intermediate between the Shokan-1 and the two susceptible parents. The F_2 progeny fit an expected 3:1 ratio of resistant to susceptible. However, within the resistant class, about one third of these were highly resistant while the other two thirds were moderately resistant based on cytological evaluation of the pathogen in the host tissues. Thus, one F_2 actually segregated into a 1:2:1 ratio of resistance phenotypes that were correlated with avenanthramide accumulation levels. A similar quantitative relationship between the level of resistance and avenanthramide accumulation was reported for the interaction of other oat varieties with races 202 and 206 (64).

Taken together, the results of the studies described above suggest that R genes can mediate the level of resistance expressed by regulating the amount and/or speed of phytoalexin accumulation. However, these results do not preclude the involvement of other defenses that contribute quantitatively to defense, and do not tell us if the phytoalexins are a direct part of the defense response or are merely a response that is correlated in time and magnitude with the expression of defense. It is important, therefore, to determine if the expression of other defenses followed the same pattern of expression observed for the phytoalexins.

Localization Studies

One approach used to evaluate the role of phytoalexins is to show that they accumulate to inhibitory concentrations at the site of pathogen development (70). Timing and cellular localization studies have also provided evidence that supports a role for phytoalexins in the resistance of cotton to *Xanthomonas campestris* (26, 75) and *Verticillium dahliae* (61), oats to *Puccinia coronata* (65), soybean to

Phytophthora megasperma (34, 100), and carnation to *Fusarium oxysporum* f. sp. *dianthi* (72). Two recent examples are discussed below.

Infection of sorghum seedlings with *Colletotrichum graminicola*, a maize pathogen, results in accumulation of red- to orange-colored 3-deoxyanthocyanidin phytoalexins (71, 70). Snyder & Nicholson (84) showed cytologically that these compounds develop in inclusion bodies that form in the cytoplasm of the plant cell that is being infected. The inclusions are at first colorless and appear in epidermal cells at the time of fungal appressorium maturation. The inclusions migrate in the epidermal cell toward the infection peg and gradually take on an orange-red color as the phytoalexins accumulate in the inclusions. The inclusions eventually coalesce and the phytoalexins are released into the cytoplasm of the infected cell. The amount of phytoalexin in the infected host cells was determined by microspectrophotometry (83). The total phytoalexin content in an inclusion was reported to be 150 M, and it was estimated that each infected cell contained 0.48 to 1.20 ng luteolindin and 0.24 to 0.91 ng apigeninidin per cell. These concentrations are well above what is needed for in vitro toxicity (83).

The resistance of cocoa to *V. albo-atrum* has recently been associated with the localized production of phytoalexins in and around the vessels (16). In addition to two carbon-based phytoalexins, the accumulation of elemental sulfur (as a cyclic octasulfur compound) was detected in tissue extracts. This is the first report of an inorganic phytoalexin. Because of this novel chemistry, the sulfur phytoalexin could be detected by electron microscopy coupled with energy-dispersive X-ray analysis of tissues. The sulfur was localized in the vessels and surrounding parenchyma cells in an orientation that strongly indicated that this phytoalexin could play an important role in defense.

Critical to determining a role for a phytoalexin in defense is a good correlation between the amount of phytoalexin that likely is in contact with the pathogen and in vitro toxicity. However, even though these careful localization studies can provide powerful evidence for a role of the phytoalexin, the results are still only correlations. Unfortunately, no studies have directly shown that the phytoalexin is responsible for stopping the pathogen or that the phytoalexin is actually toxic to the pathogen in the tissue. The apparent in planta insensitivity of *Aphanomyces euteichies* to pisatin and the relationship of the insensitivity to host polar lipids suggest that in some cases localized high phytoalexins levels may not have an effect on the pathogen (87). Use of pathogens produced by mutagenesis that are resistant to the phytoalexin or, as described below, selection of natural variants of the pathogen that are phytoalexin tolerant may provide a means to demonstrate the relationship between in vitro toxicity, in planta levels of phytoalexins, and the contribution of the phytoalexin to defense.

Pathogen Tolerance to Phytoalexins and Virulence

One way to test the role of phytoalexins in defense is to select for pathogenic isolates that are insensitive to phytoalexins. This would allow the evaluation of pathogen

growth into tissues that are producing the phytoalexin and thus provide an assessment of the relative contribution of the phytoalexins to defense. Many pathogens have been shown to detoxify phytoalexins. For two of these, the ability to detoxify the phytoalexin has provided insight into the role of the phytoalexin in resistance.

Early research by VanEtten and co-workers showed that virulence of *Nectria hematococca* to peas appeared to be based, in part, on the ability of the pathogen to detoxify pisatin (reviewed in 94). Biochemical analysis revealed that the detoxification was due to demethylation of pisatin by pisatin demethylase, and genetic analysis showed that pathogenicity segregated with pisatin detoxification (94).

Cloning of *pda*, the gene for pisatin demethylase, provided the tools necessary to evaluate the role of pistatin detoxification in virulence. Disruption of the *pda* gene (96) or transformation of a *pda*$^-$ strain with a pisatin demethylase gene (14) resulted in only a small decrease or increase, respectively, in virulence. This result demonstrated that pisatin detoxification is not the sole factor responsible for virulence. However, the results of this work are important for two reasons. First, they can be used to assess the relative contribution of pisatin to defense because the relatively minor changes in virulence may reflect the actual contribution of pisatin in restricting the pathogen. Second, this work led to the discovery that the *pda* and other virulence/pathogenicity genes are on a dispensable chromosome (93). This explains the very clear genetic results obtained with crosses between virulent and avirulent field isolates.

The virulence of the potato dry rot pathogen, *Gibberella pulicaris*, also appears to be associated with its ability to detoxify the sesquiterpenoid phytoalexins of potato (18, 29). Crosses between pathogenic/phytoalexin-detoxifying strains with nonpathogenic/nondetoxifying strains showed that pathogenicity on potato was inherited with phytoalexin detoxification (18). At least two loci were involved (18). Recently, loss of pathogenicity variability in culture was shown to be correlated with the ability to detoxify rishitin and that high virulence was associated with the greatest detoxification rates in vitro (97). Further studies on the enzymology of rishitin detoxification should eventually lead to the cloning of the gene or genes involved, and this will allow an even more critical evaluation of the role of the terpenoid phytoalexins in potato defense and help determine if the genetics of virulence in *G. pulicaris* is similar to that of *N. haematococca*.

Phytoalexin Mutants

The only plant in which phytoalexin-deficient mutants have been reported is Arabidopsis (30, 31). This plant would seem to be ideal for this work because of the ease of genetic analyses and the fact that Arabidopsis produces only camalexin as its phytoalexin. Five phytoalexin-deficient (*pad*) mutants of Arabidopsis have been isolated. The mutants produce no camalexin to as much 30% of the wild type, depending on the mutant and specific microorganism used in the inoculation.

Studies on disease development in the *pad* mutants have not totally clarified the role of camalexin in disease resistance (30, 31). None of the mutants was

affected in its resistance to incompatible *Pseudomonas syringae* isolates, although the mutant *pad3* did support somewhat more growth of a pathogenic strain of *P. syringae* pv *maculicola*. The mutants also varied in their ability to produce camalexin in response to the maize pathogen *Cochliobolus carbonum* (a very good inducer of camalexin, 106), but the nonhost resistance to this pathogen was not decreased. Some of the more interesting results came from inoculation of the mutants with several avirulent isolates of *Peronospora parasitica* (30). Two of the mutants (*pad1* and *pad5*) responded in a resistant manner to all of these pathogen isolates, whereas *pad2* and *pad3* supported a low amount of growth by two of the isolates. In *pad1*, three of the *Peronospora* isolates grew and sporulated very well and a fourth isolate sporulated to a somewhat lesser extent. The other two isolates did not cause any disease. However, the lack of camalexin data in the *Peronospora* experiments makes interpreting the role of camalexin in this host-pathogen system difficult.

These results, along with the observation that *pad1* reacted to *C. carbonum* by producing wild-type levels of camalexin, suggest that the *pad* mutants may be regulatory rather than biosynthetic. Recent results suggest that this is true for *pad1* (104). Failure to detect accumulation of putative biosynthetic precursors in the mutants or other low-molecular-weight antibiotics (IA Kagan & R Hammerschmidt, unpublished results) further suggests that other *pad* mutants also may be regulatory.

Transgenic Plants

As discussed above, identifying genes that are specific for phytoalexin biosynthesis should allow the role of phytoalexins to be tested by producing transgenic plants that overproduce phytoalexins or do not produce phytoalexins (23). The former approach has been attempted with tobacco (35), tomato (89), and rice (85) plants that were transformed with a grapevine stilbene synthase that is responsible for resveratrol synthesis. The results, especially with tomato and rice, are encouraging as the transformed plants were more resistant to *P. infestans* and *Magnaporthe grisea*, respectively, and suggest that engineering plants with secondary metabolites may provide another tool for plant disease control. Generating phytoalexin-minus plants may be of more use in actually defining the relative contribution of the phytoalexin in defense. However, care must be taken to ensure that blocking the synthesis of the phytoalexin does not result in the accumulation of other compounds in the plant that may interfere with the interpretation of the results.

Analysis of the Evidence

The information presented in the previous sections provides strong evidence for a role of phytoalexins in defense in some plant pathogen systems. However, in many phytoalexin studies, the approaches used to define a role in resistance are not always as detailed as some of the examples described above, and/or the studies do not use more than one approach to evaluate the role of phytoalexins.

Specific criteria can be used to evaluate phytoalexin data, and thus determine if phytoalexins are an important part of plant defense. Three general criteria should be fulfilled to help establish a role for phytoalexins in disease resistance: 1. In race-specific resistance phytoalexin production must be associated with the restriction of pathogen development conditioned by host-resistance genes in race- or parasite-specific resistance or in nonhost resistance; 2. in all types of resistance, phytoalexins must accumulate to antimicrobial levels at the infection site in resistant plants in sufficient concentration to inhibit the pathogen at the time pathogen development is stopped; 3. there must be evidence that the phytoalexins are directly involved in defense, and that this defensive role has a measurable benefit for the plant.

The first two criteria are the easiest to satisfy through direct observation of pathogen development in relation to phytoalexin accumulation. Advances in microscopy, cell sorting, and applications of more sensitive analytical techniques have been used to obtain data that have clarified the role of phytoalexins in some systems. Support can also come from use of metabolic inhibitors that block phytoalexin synthesis, and by evaluating pathogen development into tissues that have accumulated phytoalexins in response to prior treatment with elicitors or avirulent pathogens. These types of results, however, must be carefully evaluated because metabolic inhibitors may suppress other defenses and avirulent pathogens and elicitors induce defenses other than phytoalexins.

The third criterion is more difficult to evaluate as it requires some means of determining the in vivo effect of the phytoalexins, on the pathogen and the relationship between phytoalexin production, defense, and the overall fitness of the plant. The in vivo effect has been addressed to some extent through the use of the cloned *pda* gene, which was used to either transform *pda*$^-$ isolates of *Nectria* or to disrupt the *pda* gene in *pda*$^+$ *Nectria*. These experiments provided information on the contribution of pisatin in defense that was only possible through recent advances in fungal molecular biology. These results also suggest that generating mutants of pathogens that are tolerant of phytoalexins would provide one means of testing the importance of phytoalexins in restricting pathogen development.

Although results to date have not been overly successful, evaluating plant mutants that cannot produce phytoalexins (but are able to express all other defenses) is another approach worth pursuing. Similarly, the use of cloned phytoalexin biosynthesis genes to disrupt phytoalexin biosynthesis would be a very specific approach. Unfortunately the technology to disrupt specific genes in plants (as can be done in bacteria and fungi) has not been developed.

Another approach, as described for plant-herbivore interactions by Karban & Myers (45), is to ask whether the accumulation of phytoalexins is defensive or only a response to attack. It is clear that phytoalexin accumulation is a response, and this is certainly true in cases where hypersensitive response-inducing viruses are shown to elicit phytoalexins that seem to have no obvious effect on virus replication (6) [although there is one example where phytoalexins may effect viruses (86)]. Following the line of thought developed by Karban & Myers (45), if phytoalexins are a defense, there must be some measurable effect on the growth, survival, and/or

reproduction of the plant that can be attributed to the phytoalexin stopping the pathogen. This important question could be addressed easily by using isolines of plants that do or do not produce phytoalexins and then determining if phytoalexin production has any effect on the overall growth and development of the plant when exposed to natural disease pressure. It is to be hoped that molecular technology will become available in the future that will facilitate the development of phytoalexin-producing and -nonproducing isolines that will allow critical evaluation of phytoalexin production on pathogen development as well as provide the tools to assess the contribution of phytoalexin production to plant fitness in field situations.

PHYTOALEXINS: Down on the Farm and Out in the Field

Surprisingly, the study of phytoalexins has relied almost exclusively on well-defined laboratory or greenhouse experiments. These provide a very clean system to study the induction and regulation of these compounds, but plants in natural and agricultural ecosystems are exposed to many stresses other than the attack of one pathogen. Although more complex to set up and to interpret, it would be very useful to determine if a plant growing under field conditions produces phytoalexins as predicted by controlled infection experiments, or if the stresses of day-to-day life in the field result in more or less constant production of phytoalexins.

The accumulation of the stilbene phytoalexin resveratrol was evaluated in vineyard-grown fruit clusters of three cultivars of grapevine that were either healthy or exhibiting 10% natural infection by *Botrytis cinerea* (42). The berries produced resveratrol in concentrations that appeared to be effective in restricting pathogen growth in fruits under environmental conditions that do not favor pathogen development.

In two years of field trials, we have shown that treatment of field-grown soybeans with the herbicide lactofen can reduce the severity of white mold caused by *Sclerotinia sclerotiorum* and that the disease suppression is associated with a significant accumulation of glyceollin in lactofen-treated leaves (EK Dann, B Diers, R Hammerschmidt, unpublished data). Because lactofen also induces production of active oxygen species (79) that have been implicated in plant defense (4a), these data cannot unequivocally support a role for glyceollin in the observed disease suppression. However, a similar problem of interpretation holds true for incompatible interactions between plants and pathogens where multiple defenses are expressed. These field data do, however, demonstrate that phytoalexin accumulation is an inducible phenomenon in plants grown in the field where they are subjected to a variety of stresses.

Even less is known about phytoalexin production and its relation to resistance in natural ecosystems. The induced production of furanocoumarins in certain isolates of wild parsley has been correlated with greater resistance to herbivory (101). Since these compounds have also been described as phytoalexins (36), it would be interesting to determine if resistance to pathogens follows the same pattern. Greater

disease resistance of wild as compared to domesticated *Phaseolus coccineus* was correlated with greater chemical diversity and higher total amounts of isoflavonoids produced after elicitation with $CuCl_2$ (57). The same resistance trend was found with *P. lanutus*, but this could not be explained by phytoalexin diversity or accumulation (the wild *P. lanutus* were, however, more cyanogenic). Although these studies only correlate production of phytoalexins with defense, associating the ability of a plant to produce phytoalexins with its survival and reproduction under natural or agricultural field conditions is an additional type of data that can assess the role of phytoalexins.

PHYTOALEXINS: What Do We Know?

Studies on phytoalexins have generated substantial useful information on secondary metabolism, gene regulation, and novel secondary metabolite chemistries. These results alone have provided valuable contributions to plant biochemistry and molecular biology. However, the real question to be answered is whether phytoalexins are contributors to defense or are just the end product of pathogen-disturbed metabolism that shunts carbon into compounds that are antimicrobial for serendipitous rather than defensive reasons.

When viewed collectively, the data support a role of phytoalexins in plant defense. However, these data are still only correlations with the resistance phenotype. Although studies on the rapidity and localization of phytoalexins provide good evidence for a role, time course and localization studies of other defenses can present exactly the same or very similar conclusions (and correlations) (e.g. 90).

More definitive approaches are needed to determine the role of phytoalexins in plant defense and these approaches can be based on the criteria described above. Transgenic plants or biosynthetic mutants that can no longer produce phytoalexins are perhaps the best way to determine the impact of phytoalexin deficiency on resistance. But to do this correctly, the plants must be evaluated for changes in other defenses that may compensate for the loss of phytoalexin production. These plants should also be tested under field conditions to determine the effect enhanced or suppressed phytoalexin production will have on plant growth and reproduction.

Plants transformed with resveratrol synthase have yielded enhanced resistance to some but not all pathogens (89). These results are similar to those found with transgenic plants expressing certain PR proteins (1, 59). The lack of total resistance in these transgenic plants may be a reflection of the natural contribution of these defenses to resistance to specific pathogens. With this in mind, the small change in virulence of *Nectria* after the *pda* gene was inactivated (96) may be a direct reflection of the actual contribution of pisatin to resistance. Thus it is again important to think about the contribution of phytoalexins to defense in concert with other putative defenses.

As outlined in this review, phytoalexin research has progressed greatly since its inception, and this research has impacted plant pathology, plant biochemistry,

and plant and fungal molecular biology. The phytoalexin concept has provided a framework on which studies of defense have been modeled. As such, phytoalexin research has provided systems to search for specific elicitors of resistance as well as pathogen virulence mechanisms.

Natural products chemistry and our understanding of the enzymology and regulation of secondary metabolites have also benefited directly or indirectly from studies on phytoalexin accumulation and elicitation. As a result of advances in molecular and analytical technology, we also have a much better view of the role of phytoalexins in defense even if the absolute contribution of a phytoalexin to resistance has not been established. The cloning of phytoalexin biosynthetic genes will allow a closer evaluation of these compounds in defense. In addition, use of cloned phytoalexin biosynthetic genes may also provide new strategies for disease control as illustrated with the resveratrol synthase gene.

In conclusion, much has been learned about phytoalexins and from studies of these compounds. Very good evidence now exists that phytoalexins are important in defense, and it is only a matter of time until we can conclusively determine the contribution of these compounds to defense. Clearly, future studies on these fascinating compounds will continue to provide new insights into plant pathogen interactions as well as provide new approaches to disease control.

ACKNOWLEDGMENTS

I thank Ms Brenda Schuster for her assistance in preparing this manuscript. The support of the National Science Foundation (IBN-9220912), the Michigan Soybean Promotion Committee, and the Michigan Agricultural Experiment Station is gratefully acknowledged.

LITERATURE CITED

1. Alexander D, Goodman RM, Gut-Rella M, Glascock C, Weyman K, et al. 1993. Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. *Proc. Natl. Acad. Sci. USA* 90:7327–31
2. Angell HR, Walker JC, Link KP. 1930. The relation of protocatechuic acid to disease resistance in the onion. *Phytopathology* 20:431–38
3. Back KW, He SL, Kim KU, Shin DH. 1998. Cloning and bacterial expression of sesquiterpenoid cyclase, a key enzyme for the synthesis of sesquiterpenoid phytoalexin capsidiol in UV-challenged leaves of *Capsicum annuum*. *Plant Cell Physiol*. 39:899–904
4. Back KW, Yin SH, Chappell J. 1994. Expression of a plant sesquiterpene cyclase gene in *Escherichia coli*. *Arch. Biochem. Biophys*. 315:527–32

4a. Baker CJ, Orlandi EW. 1995. Active oxygen in plant pathogenesis. *Annu. Rev. Phytopathol*. 33:299–321

5. Bailey JA, Mansfield JW, eds. 1982. *Phytoalexins*. Glasgow: Blackie 334 pp.
6. Bailey JA, Vincent GG, Burden RS. 1976. The antifungal activity of glutinosone and

capsidiol and their accumulation in virus-infected tobacco species. *Physiol. Plant Pathol.* 8:35–41
7. Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar SP. 1997. Signalling in plant-microbe interactions. *Science* 276: 726–33
8. Bohlmann J, MeyerGauen G, Croteau R. 1998. Plant terpenoid synthases: molecular biology and phylogenetic analysis. *Proc. Natl. Acad. Sci. USA* 95:4126–33
9. Bostock R, Kuč J, Laine R. 1981. Eicosapentaenoic and arachidonic acids from *Phytophthora infestans* elicit fungitoxic sesquiterpenes in potato. *Science* 212:67–69
10. Browne LM, Conn KL, Ayer WA, Tewari JP. 1991. The camalexins: new phytoalexins produced in the leaves of *Camelina sativa* (Cruciferae). *Tetrahedron* 47:3909–14
11. Chen XY, Wang MS, Chen Y, Davisson VJ, Heinstein P. 1996. Cloning and heterologous expression of a second (+)-delta-cadinene synthase from *Gossypium arboreum*. *J. Nat. Prod.* 59:944–51
12. Cheng J, Boyd C, Slaymaker D, Okinaka Y, Takeuchi Y, et al. 1998. Characterization of a 34-kDa soybean binding protein for the syringolide elicitors. *Proc. Natl. Acad. Sci. USA* 95:3306–11
13. Choi D, Bostock RM, Avdiushko S, Hildebrand DF. 1994. Lipid-derived signals that discriminate wound-response and pathogen-responsive isoprensoid pathways in plants—methyl jasmonate and the fungal elicitor arachidonic acid induce different 3-hydroxy-3-methylglutaryl coenzyme A reductase genes and antimicrobial isoprenoids in *Solanum tuberosum* L. *Proc. Natl. Acad. Sci. USA* 91:2329–33
14. Ciufetti L, VanEtten H. 1996. Virulence of a pisatin-deficient *Nectria haematococca* MPVI isolate is increased by transformation with a pisatin demethylase gene. *Mol. Plant-Microbe Interact.* 9:787–92
15. Collins FW. 1989. Oat phenolics—avenanthramides, novel substituted N-cinnamoylanthranilate alkaloids from oat groats and hulls. *J. Agric. Food Chem.* 37:60–66
16. Cooper RM, Resende MLV, Flood J, Rowen MG, Beale MH, Potter U. 1996. Detection and cellular location of elemental sulfur in disease resistant genotypes of *Theobroma cacao*. *Nature* 379:159–62
17. Cruickshank IAM. 1963. Phytoalexins. *Annu. Rev. Phytopathol.* 1:351–74
18. Desjardins AE, Gardner HW. 1991. Virulence of *Gibberella pulicaris* on potato tubers and its relationship to a gene for rishitin metabolism. *Phytopathology* 81: 429–35
19. DeWit PJGM. 1995. Fungal avirulence genes and plant resistance genes: unraveling the molecular basis of gene-for-gene interactions. *Adv. Bot. Res.* 21:147–85
20. DeWit PJGM, Spikman G. 1982. Evidence for the occurrence of race and cultivar specific elicitors or necrosis in intercellular fluids of compatible interactions of *Cladosporium fulvum* and tomato. *Physiol. Plant Pathol.* 21:1–12
21. Dixon RA. 1986. The phytoalexin response: elicitation, signalling and control of host gene expression. *Biol. Rev.* 61:239–91
22. Dixon RA, Harrison MJ. 1990. Activation, structure and organization of genes involved in microbial defense in plants. *Adv. Genet.* 28:165–34
23. Dixon RA, Lamb CJ, Masoud S, Sewalt VJH, Paiva NL. 1996. Metabolic engineering: prospects for crop improvement through the genetic manipulation of phenylpropanoid biosynthesis and defense responses—a review. *Gene* 179:61–71
24. D'Silva I, Heath MC. 1997. Purification and characterization of two novel hypersensitive response-inducing specific elicitors produced by the cowpea rust fungus. *J. Biol. Chem.* 272:3924–27
25. Ebel J. 1986. Phytoalexin synthesis: the biochemical analysis of the induction

process. *Annu. Rev. Phytopathol.* 24:235–64
26. Essenberg M, Pierce ML, Cover EC, Hamilton B, Richardson PE, Scholes VE. 1992. A method for determining phytoalexin concentrations in fluorescent hypersensitively necrotic cells in cotton leaves. *Physiol. Mol. Plant Pathol.* 41:101–9
27. Facchini PJ, Chappell J. 1992. Gene family for an elicitor-induced sesquiterpene cyclase in tobacco. *Proc. Natl. Acad. Sci. USA* 89:11088–92
28. Flor HH. 1942. Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* 32:653–69
29. Gardner HW, Desjardins AE, McCormick SP, Weisdleder D. 1994. Detoxification of the potato phytoalexin rishitin by *Gibberella pulicaris*. *Phytochemistry* 37: 1001–5
30. Glazebrook J, Ausubel FM. 1994. Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens. *Proc. Natl. Acad. Sci. USA* 91:8955–59
31. Glazebrook J, Zook M, Mert F, Kagan I, Rogers EE, et al. 1997. Phytoalexin-deficient mutants of Arabidopsis reveal that PAD4 encodes a regulatory factor and that four PAD genes contribute to downy mildew resistance. *Genetics* 146:381–92
32. Grayer RJ, Harborne JB. 1994. A survey of antifungal compounds from plants, 1982–1993. *Phytochemistry* 37:19–42
33. Hahn MG. 1996. Elicitors and their receptors in plants. *Annu. Rev. Phytopathol.* 34:387–412
34. Hahn MG, Bonhoff A, Griesbach H. 1985. Quantitative localization of the phytoalexin glyceollin I in relation to fungal hyphae in soybean root infected with *Phytophthora infestans* f. sp. *glycinea*. *Plant Physiol.* 77:591–601
35. Hain R, Reif HJ, Krause E, Langebartels R, Kindl H, et al. 1993. Disease resistance results from foreign phytoalexin expression in a novel plant. *Nature* 361:153–56
36. Hammerschmidt R, Schultz J. 1996. Multiple defenses and signals in plant defense against pathogens and herbivores. *Recent Adv. Phytochem.* 30:121–54
37. Hammond-Kosack KE, Jones JDG. 1997. Plant disease resistance genes. *Annu. Rev. Plant. Physiol. Plant Mol. Biol.* 48:575–77
38. Heath MC. 1995. Thoughts on the role and evolution of induced resistance in natural ecosystems, and its relationship to other types of plant defenses against disease. In *Induced Resistance to Disease in Plants*, ed. R Hammerschmidt, J Kuc, pp. 141–45. Amsterdam: Kluwer
39. Hoshino T, Yamaura T, Imaishi H, Chida M, Yoshizawa Y, et al. 1995. 5-epi-aristolochene 3-hydroxylase from green pepper. *Phytochemistry* 38:609–13
40. Hoxtermann E. 1991. Karl Otto Muller (1897–1978) und die Entdeckungsgeschichte der Phytoalexine. *J. Phytopathol.* 132: 161–67
41. Ishihara A, Miyagawa H, Matsukawa T, Ueno T, Mayama S, Iwamura H. 1998. Induction of hydroxyanthranilate hydroxycinnamyl transferase activity by oligo-N-ascetylchitooligosaccharides in oats. *Phytochemistry* 47:969–74
42. Jeandet P, Bessis R, Sbaghi M, Meunier P. 1995. Production of the phytoalexin resceratrol by grapes as a response to *Botrytis cinerea* under natural conditions. *J. Phytopathol.* 143:135–39
43. Ji C, Smith-Backer J, Keen NT. 1998. Genetics of plant-pathogen interactions. *Curr. Opin. Biotechnol.* 9:202–7
44. Kamoun S, van West P, Vleeshouwers VGAA, de Groot KE, Govers F. 1998. Resistance in *Nicotiana benthamiana* to *Phytophthora infestans* is mediated by the recognition of the elicitor protein INF1. *Plant Cell* 10:1413–25
45. Karban R, Myers JH. 1989. Induced plant responses to herbivory. *Annu. Rev. Ecol. Syst.* 20:331–48
45a. Keen NT. 1981. Evaluation of the role of phytoalexins. In *Plant Disease Control*, ed.

RC Staples, GH Toenniessen, pp. 155–77, New York: Wiley

46. Keen NT, Buzzell RI. 1991. New disease resistance genes in soybean against *Pseudomonas syringae* pv *glycinea*—evidence that one of them interacts with a bacterial elicitor. *Theor. Appl. Genet.* 81:133–38
47. Keen NT, Littlefield LJ. 1979. The possible involvement of phytoalexins with resistance gene expression in flax to *Melampsora lini*. *Physiol. Plant Pathol.* 14:265–80
48. Keen NT, Tamaki S, Kobayashi D, Gerhold D, Stayton M, et al. 1990. Bacteria expressing avirulence gene D produce a specific elicitor of the soybean hypersensitive reaction. *Mol. Plant-Microbe Interact.* 3:112–21

48a. Kelemu S, Collmer A. 1993. *Erwinia chrysanthemi* EC16 produces a second set of plant-inducible pectate lyase isozymes. *Appl. Environ. Microbiol.* 59:1756–61

49. Keller H, Czernic P, Ponchet M, Ducrot PH, Back K, et al. 1998. Sesquiterpene cyclase is not a determining factor for elicitor and pathogen-induced capsidiol accumulation in tobacco. *Planta* 205:467–76
50. Kuć J. 1957. A biochemical study of the resistance of potato tuber tissue to attack by various fungi. *Phytopathology* 47:676–80
51. Kuć J. 1972. Phytoalexins. *Annu. Rev. Phytopathol.* 10:207–32
52. Kuć J. 1995. Phytoalexins, stress metabolism and disease resistance in plants. *Annu. Rev. Phytopathol.* 33:275–97
53. Kurosaki F. 1996. Effect of NADPH-associated keto-reducing domain on substrate entry into 6-hydroxymellein synthase, a multifunctional polyketide synthetic enzyme involved in phytoalexin biosynthesis in carrot. *Arch. Biochem. Biophys.* 328:213–17
54. Kurosaki F. 1996. Transacylase-like structure and its role in substrate channeling of 6-hydroxymellein synthase, a multifunctional polyketide biosynthetic enzyme in carrot cell extracts. *FEBS Lett.* 379:97–102
55. Kurosaki F. 1996. Partial purification and characterization of 6-hydroxymellein-O-methyltransferase from elicitor-treated carrot cells. *Phytochemistry* 41:1023–27
56. Leach JE, White FF. 1996. Bacterial avirulence genes. *Annu. Rev. Phytopathol.* 34:153–79

56a. Leister RT, Ausubel FM, Katagiri F. 1996. Molecular recognition of pathogen attack occurs inside of plant cells in plant disease resistance specified by the Arabidopsis genes *RPS2* and *RPM1*. *Proc. Natl. Acad. Sci. USA* 93:15497–402

57. Lindig-Cisneros R, Benrey B, Espinosa-Garcia FJ. 1997. Phytoalexins, resistance traits, and domestication status in *Phaseolus coccineus* and *Phaseolus lunatus*. *J. Chem. Ecol.* 23:1997–2011
58. Littlefield LJ. 1973. Histological evidence for diverse mechanisms of resistance to flax rust, *Melampsora lini* (Ehrenb.). *Physiol. Plant Pathol.* 3:241–47
59. Liu D, Raghothama KG, Hasegawa PM, Bressan RA. 1994. Osmotin overexpression in potato delays development of disease symptoms. *Proc. Natl. Acad. Sci. USA* 91:1888–92
60. Long M, Barton-Willis P, Staskawicz BJ, Dahlbeck D, Keen NT. 1985. Further studies on the relationship between glyceollin accumulation and the resistance of soybean leaves to *Pseudomonas syringae* pv. *glycinea*. *Phytopathology* 75:235–39
61. Mace ME, Stipanovich RD, Bell AA. 1989. Histochemical localization of desoxyhemigossypol, a phytoalexin in *Verticillium dahliae*-infected cotton stems. *New Phytol.* 111:229–32
62. Mansfield JW. 1999. Antimicobial compounds and resistance: the role of phytoalexins and phytoanticipins. In *Mechanisms of Resistance to Plant Diseases*, ed. AJ Slusarenko, RSS Fraser, LC van Loon. Amsterdam: Kluwer. In press

63. Mayama S, Bordin APA, Morikawa T, Tanpo H, Kato H. 1995. Association between avenalumin accumulation, infection hyphae length and infection type in oat crosses segregating for resistance to *Puccinia coronata* f.sp. *avenae* race 226. *Physiol. Mol. Plant Pathol.* 46:255–62
64. Mayama S, Bordin APA, Morikawa T, Tanpo H, Kato H. 1995. Association between avenalumin accumulation with cosegregation of victorin sensitivity and crown rust resistance carrying the Pc-2 gene. *Physiol. Mol. Plant Pathol.* 46:263–74
65. Mayama S, Tani T. 1982. Microspectrophotometric analysis of the location of avenalumin accumulation in oat leaves in response to fungal infection with *Puccinia coronata*. *Physiol. Plant Pathol.* 21:141–49
66. McCaskill D, Croteau R. 1998. Some caveats for bioengineering terpenoid metabolism in plants. *Trends Biotechnol.* 16:349–55
67. Midland SL, Keen NT, Sims JJ, Midland MM, Stayton MM, et al. 1993. The structures of syringolide-1 and syringolide-2, novel C-glycosidic elicitors from *Pseudomonas syringae* pv *tomato*. *J. Org. Chem.* 58:2940–45
68. Müller KO. 1958. Studies on phytoalexins: I. The formation and the immunological significance of phytoalexin produced by *Phaseolus vulgaris* in response to infections with *Sclerotinia fructicola* and *Phytophthora infestans*. *Aust. J. Biol. Sci.* 11:275–300
69. Müller KO, Borger H. 1940. Experimentelle Untersuchungen über die Phytophthorainfestans-Resistenz der Kartoffel. *Arb. Biol. Reichsanst. Land Forstwirtsch.* 23:189–31
70. Nicholson RL, Hammerschmidt R. 1992. Phenolic compounds and their role in disease resistance. *Annu. Rev. Phytopathol.* 30:369–89
71. Nicholson RL, Kollipara SS, Vincent JR, Lyons PC, Cadena-Gomez G. Phytoalexin synthesis by the sorghum mesocotyl in response to infection by pathogenic and nonpathogenic fungi. *Proc. Natl. Acad. Sci. USA* 84:5520–24
72. Niemann GJ, Baayen RP, Boon JJ. 1990. Localization of phytoalexin accumulation and determination of changes in lignin and carbohydrate composition in carnation (*Dianthus caryophyllus* L.) xylem as a consequence of infection with *Fusarium oxysporum* f.sp. *dianthi* by pyrolysis-mass spectrometry. *Neth. J. Plant Pathol.* 96:133–53
73. Osbourn AE, Clarke BR, Lunnes P, Scott, PR, Daniels MJ. 1994. An oat species lacking avenacin is susceptible to infection by *Gaumanomyces graminis* var. *tritici*. *Physiol. Mol. Plant Pathol.* 45:457–67
74. Paxton JD. 1981. Phytoalexins—a working redefinition. *Phytopathol. Z.* 101:106–209
75. Pierce ML, Cover EC, Richardson PE, Scholes VE, Essenberg M. 1996. Adequacy of cellular phytoalexin concentrations in hypersensitively responding cotton leaves. *Physiol. Mol. Plant Pathol.* 48:305–24
76. Preisig CL, Matthews DE, VanEtten H. 1989. Purification and characterization of S-adenosyl-L-methionine: 6a-hydroxymaackiain 3-O-methyltransferase from *Pisum sativum*. *Plant Physiol.* 91:559–66
77. Purkayastha RP. 1995. Progress in phytoalexin research during the past 50 years. In *Handbook of Phytoalexin Metabolism and Action*, ed. M Daniel, RP Purkayastha, pp. 1–39. New York: Dekkar
78. Rustérucci C, Stallaert V, Milat M, Pugin A, Ricci P, Blein J. 1996. Relationship between active oxygen species, lipid peroxidation, necrosis, and phytoalexin production induced by elicitins in *Nicotiana*. *Plant Physiol.* 111:885–91
79. Scalla R, Matringe M. 1994. Inhibitors of protoporphirynogen oxidase as herbicides:

diphenyl ethers and related photobleaching molecules. *Rev. Weed Sci*. 6:103–32

80. Scofield SR, Tobias CM, Rathjen JP, Chang JH, Lavelle DT, et al. 1996. Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. *Science* 274:2063–65
81. Senevirante GI, Harborne JB. 1992. Constitutive flavonoids and induced isoflavonoids as taxonomic markers in the genus *Vigna*. *Biochem. Syst. Ecol*. 20:459–67
82. Smith CJ. 1996. Accumulation of phytoalexins: defence mechanism and stimulas reponse system. *New Phytol*. 132:1–45
83. Snyder BA, Leite B, Hipskind J, Butler LG, Nicholson RL. 1991. Accumulation of sorghum phytoalexins induced by *Colletotrichum graminicola* at the infection site. *Physiol. Mol. Plant Pathol*. 39:463–70
84. Snyder BA, Nicholson RL. 1990. Synthesis of phytoalexins in sorghum as a site-specific response to fungal ingress. *Science* 248:1637–39
85. Stark-Lorenzen P, Nelke B, Hänssler G, Mühlbach, Thomzik JE. 1997. Transfer of a grapevine stilbene synthase gene to rice (*Oryzae sativa* L). *Plant Cell Rep*. 16:668–73
86. Sun TJ, Melcher U, Essenberg M. 1988. Inactivation of cauliflower mosaic virus by photoactivatable cotton phytoalexin. *Physiol. Mol. Plant Pathol*. 33:115–26
87. Sweigard J, VanEtten HD. 1987. Reduction in pisatin sensitivity of *Aphanomyces euteiches* by polar lipid extracts. *Phytopathology* 77:771–75

87a. Takahashi H, Chen Z, Du H, Liu Y, Klessig DF. 1997. Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels. *Plant J*. 11:993–1005

88. Tang X, Frederick RD, Zhou J, Halterman DA, Jia Y, Martin GB. 1996. Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. *Science* 274:2060–63
89. Thomzik JE, Stenzel K, Stocker R, Schreier PH, Hain R, Stahl DJ. 1997. Synthesis of a grapevine phytoalexin in transgenic tomatoes (*Lycopersicon esculentum* Mill.) conditions resistance against *Phytophthora infestans*. *Physiol. Mol. Plant Pathol*. 51:265–78
90. Thordal-Christensen H, Zhang Z, Wei Y, Collinge DB. 1997. Subcellular localization of H_2O_2 in plants. H_2O_2 accumulation in papilla and hypersensitive response during barley-powdery mildew interaction. *Plant J*. 11:1187–94
91. Tsuji J, Jackson EP, Gage DA, Hammerschmidt R, Somerville SC. 1992. Phytoalexin accumulation in *Arabidopsis thaliana* during the hypersensitive reaction to *Pseudomonas syringae* pv. syringae. *Plant Physiol*. 98:1304–9
92. Tsuji J, Zook M, Hammerschmidt R, Somerville S, Last R. 1993. Evidence that tryptophan is not a direct biosynthetic intermediate of camalexin in *Arabidopsis thaliana*. *Physiol. Mol. Plant Pathol*. 43:221–29
93. VanEtten H, Funnell-Baerg D, Wasmann C, McClusky K. 1994. Location of pathogenicity genes on dispensable chromosomes in *Nectria haematococca* MPVI. *Antonie van Leeuwenhoek* 65: 263–67
94. VanEtten H, Matthews P, Tegtmeier K, Deitert MF, Stein JI. 1989. Phytoalexin detoxification: importance for pathogenicity and practical implications. *Annu. Rev. Phytopathol*. 27:143–64
95. VanEtten HD, Mansfield JW, Bailey JA, Farmer E. 1994. Two classes of plant antibiotics: phytoalexins versus "phytoanticipins". *Plant Cell* 6:1191–92
96. Wasmann CC, VanEtten H. 1996. Transformation-mediated chromosome loss and disruption of a gene for pisatin demethylase decreases the virulence

of *Nectria haematococca* on pea. *Mol. Plant-Microbe Interact.* 9:793–803
97. Weltring KM, Loser K, Weimer J. 1998. Genetic instability of rishitin metabolism and virulence on potato of a strain of *Gibberella pulicaris*. *J. Phytopathol.* 146: 393–98
98. Wu Q, Preisig CA, VanEtten HD. 1997. Isolation of cDNAs encoding (+)6a-hydroxymaackiain 3-O-methyltransferase, the terminal step for the synthesis of the phytoalexin pisatin in *Pisum sativum*. *Plant Mol. Biol.* 35:551–60
99. Yoshikawa M, Tsuda M, Takeuchi Y. 1993. Resistance to fungal disease in transgenic tobacco plants expressing the phytoalexin elicitor-releasing factor, β-1,3-glucanase, from soybean. *Naturwissenschaften* 80:417–20
100. Yoshikawa M, Yamauchi K, Masago H. 1978. Glyceollin: its role in restricting fungal growth in resistant soybean hypocotyls infected with *Phytophthora megasperma* var *sojae*. *Physiol. Plant Pathol.* 12:73–82
101. Zangerl AR, Berenbaum MR. 1990. Furanocoumarin induction in wild parsnip: genetics and populational variation. *Ecology* 71:1933–40
102. Zhao J, Last RL. 1996. Coordinate regulation of the tryptophan biosynthetic pathway and indolic phytoalexin accumulation in *Arabidopsis*. *Plant Cell* 8:2235–44
103. Zhao J, Williams CC, Last RL. 1998. Induction of *Arabidopsis* tryptophan pathway enzymes and camalexin by amino acid starvation, oxidative stress and an abiotic elicitor. *Plant Cell* 10:359–70
104. Zhou N, Tootle TL, Tsui F, Klessig DF, Glazebrook J. 1998. PAD4 functions upstream from salicylic acid to control defense responses in *Arabidopsis*. *Plant Cell* 10:1021–30
105. Zook M. 1998. Biosynthesis of camalexin from tryptophan pathway intermediates in cell-suspension cultures of *Arabidopsis*. *Plant Physiol.* 118:1389–93
106. Zook M, Hammerschmidt R. 1997. Origin of the thiazole ring of camalexin, a phytoalexin from *Arabidopsis thaliana*. *Plant Physiol.* 113:463–68
107. Zook M, Hohn T, Bonnen AM, Tsuji J, Hammerschmidt R. 1996. Characterization of novel sesquiterpene biosynthesis in tobacco expressing a fungal sesquiterpene cyclase. *Plant Physiol.* 112:311–18