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Fungal Resistance to Plant Antibiotics as a Mechanism of Pathogenesis

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INTRODUCTION

Most plants produce antimicrobial secondary metabolites, either as part of their normal program of growth and development or in response to pathogen attack or stress. The possibility that these compounds may protect plants against disease has intrigued biologists since the early part of this century (111, 134). Preformed antifungal compounds that occur constitutively in healthy plants are likely to represent inbuilt chemical barriers to infection and may protect plants against attack by a wide range of potential pathogens (87, 120, 140, 186). In contrast, induced antifungal compounds (phytoalexins) are not present in healthy plants but are synthesized in response to pathogen attack or stress as part of the plant defense response and are restricted to the tissue colonized by the fungus and the cells surrounding the infection site (134, 147).

Clearly, if antimicrobial compounds are likely to influence the growth of fungal pathogens during plant infection, the pathogen must be exposed to effective concentrations of antifungal compounds at the time when fungal growth is arrested. Knowledge of the localization and concentration of these compounds within the plant tissue relative to the location of the invading pathogen is therefore valuable. The properties of some antifungal compounds have allowed them to be visualized (and sometimes quantified) in plants by noninvasive techniques. For example, the UV autofluorescence of the triterpenoid saponin avenacin A-1 has enabled this preformed compound to be localized to the epidermal cell layer of oat roots (Fig. 1A), consistent with a role in protecting the roots against invaders (144), and the pigmented 3-deoxyanthocyanidin flavonoid phytoalexins are synthesized in disease-resistant sorghum lines in response to fungal infection, first accumulating in the cell that is undergoing attack (196, 197) (Fig. 1B). Other antifungal compounds that are not readily detectable by simple microscopy have been visualized by a variety of techniques including histochemical procedures and immunolocalization (77, 93, 117). One of the most elegant examples of in situ localization of a phytoalexin involves analysis of the distribution of elemental sulfur (S_8) in the vascular tissue of resistant cocoa (*Theobroma cacao*) genotypes in response to infection by the wilt fungus, *Verticillium dahliae*, by coupled scanning electron microscopy and energy-dispersive X-ray microanalysis (29) (Fig. 1C). Elemental sulfur is one of the oldest known fungicides (212). Recently it has also been shown to accumulate in tomato plants in response to challenge with *V. dahliae* and in tobacco leaves undergoing the hypersensitive response following inoculation with the bacterial pathogen *Pseudomonas syringae* pv. *syringae*, suggesting that elemental sulfur may have widespread significance as an antimicrobial phytoprotectant (30).

Since the growth of a successful pathogen is not arrested by antifungal compounds during infection of its host plant, pathogens must have strategies for circumventing or countering the effects of these compounds. Preformed antifungal compounds are commonly sequestered in vacuoles or organelles of healthy plants, and so necrotrophic pathogens may be expected to encounter fungitoxic levels of these compounds because they cause extensive tissue damage. However, there is also some evidence that preformed antifungal compounds may also affect the growth of biotrophic pathogens during infection of plants (126). Phytoalexin induction may be avoided or delayed by evading recognition by the surveillance system of the host plant. There is also evidence that fungi may produce molecules

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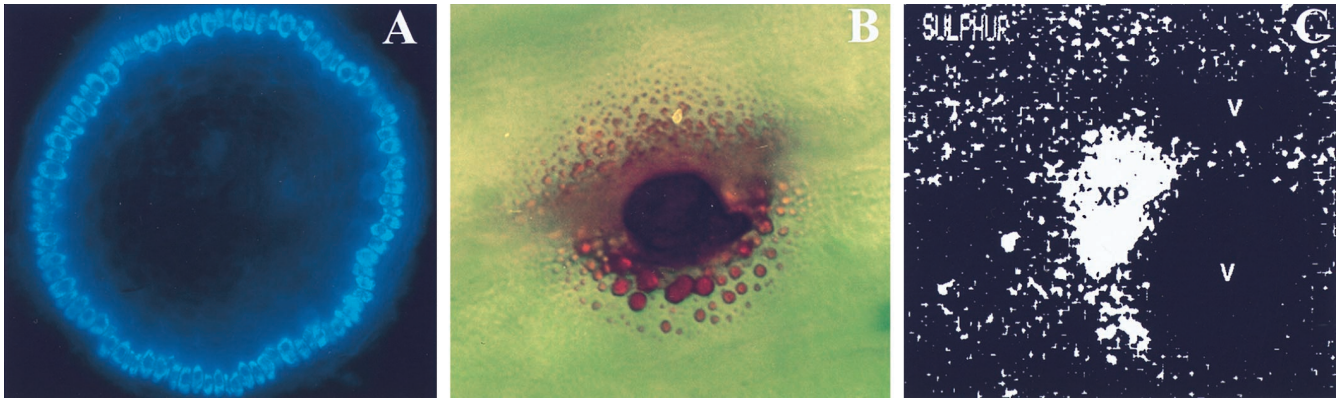

FIG. 1. Localization of antifungal compounds in plants. (A) Fluorescence of the saponin avenacin A-1 under UV light. This compound is localized in the epidermal cell layer of oat roots (144). (B) A resistant sorghum line responding to attack by *Colletotrichum graminicola*, showing the formation and mobilization of vesicles containing pigmented 3-deoxyanthocyanidin phytoalexins toward the site of attempted penetration by an appressorium (visualized by light microscopy) (196, 197). (C) Accumulation of elemental sulfur (S_8) in the vascular tissue of a resistant cocoa line in response to attack by the xylem-invading fungus *Verticillium dahliae* (29). An X-ray map shows sulfur (white) accumulating in a xylem parenchyma cell (XP); xylem vessels are also labelled (V). Panels A and C are reproduced from references 139 and 29, respectively, with permission of the publisher. Panel B was kindly supplied by R. Nicholson.

that suppress plant defense responses including phytoalexin biosynthesis (94, 191, 237). Inevitably, some fungal pathogens will be exposed directly to antifungal compounds during infection of plants and so must be able to tolerate or combat the toxic effects of these compounds. Strategies may include the production of degradative enzymes, innate resistance of the target site, or efflux mechanisms that prevent intracellular antifungal compounds from building up to toxic levels (141, 220). There have been a number of comprehensive reviews on antifungal compounds in plants (11, 17, 73, 87, 101, 102, 120, 139, 140, 186, 192). This review focuses on recent developments in our knowledge of mechanisms of resistance to antimicrobial compounds in phytopathogenic fungi and on the evidence for and against a role for antifungal compounds in plant defense.

PREFORMED ANTIFUNGAL COMPOUNDS

The term "phytoanticipin" has been proposed to distinguish preformed antimicrobial compounds from phytoalexins (216). Some secondary metabolites, such as cyanogenic glycosides, glucosinolates, and some saponins, are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by plant enzymes in response to pathogen attack. These compounds can also be regarded essentially as "preformed," since the plant enzymes that activate them are already present in healthy plant tissue but are separated from their substrates by compartmentalization, enabling rapid activation without a requirement for the transcription of new gene products (216). Although preformed inhibitors are, by definition, present in healthy plants at levels that are anticipated to be antimicrobial, their levels may increase further in response to challenge by pathogens (22, 165). Some antifungal compounds may be present constitutively in one part of a plant but induced as phytoalexins in other organs (24, 82, 216). Numerous constitutive plant compounds have been reported to have antifungal activity in vitro and so have been implicated as antimicrobial phytoprotectants (73, 87, 120, 139, 140, 186, 220). For some of these compounds, their significance in determining the outcome of encounters between potential fungal pathogens and plants has been investigated in detail and is considered below.

Saponins

Structure and biological activity. Saponins are glycosylated triterpenoid, steroid, or steroidal alkaloid molecules which occur constitutively in a great many plant species and which often have antifungal activity (85, 139, 161). These molecules have an oligosaccharide chain, attached at the C-3 position, which may consist of up to five sugar molecules, usually glucose, arabinose, glucuronic acid, xylose, or rhamnose (85). Some saponins also have an additional sugar moiety (normally one glucose) at C-26 or C-28. Examples of saponins that have been studied in relation to their potential role in plant defense against attack by phytopathogenic fungi are shown in Fig. 2. Avenacin A-1 from oat roots (a triterpenoid) and the steroidal glycoalkaloids α-tomatine and α-chaconine (from tomato and potato, respectively) all have a single sugar chain attached to C-3. The steroidal oat leaf saponins avenacosides A and B both have two sugar chains, one at C-3 and one at C-26 (206, 207). Avenacosides A and B are biologically inactive but are converted into antifungal 26-desglucoavenacosides in response to tissue damage or pathogen attack by a plant glucosyl hydrolase that is specific for the C-26 glucose molecule (75, 76).

The major mechanism of antifungal activity of saponins is apparently due to their ability to complex with sterols in fungal membranes and to cause loss of membrane integrity (96, 136, 188), although the precise mechanism is not fully understood (Fig. 3). Electron microscopic analysis and electrical conductivity measurements suggest the formation of transmembrane pores (6, 14, 50, 68, 72, 189), although steroidal glycoalkaloids have been proposed to interfere with membrane integrity by extracting sterols from membranes (95, 96). Aggregation of the saponin-sterol complexes in the membrane may be mediated by interactions between the sugar residues of the saponin molecules (6, 96). The sugar chain attached to C-3 is usually critical for both the membrane-permeabilizing and antifungal properties of saponins, and removal of these sugar residues often results in loss of biological activity (6, 8, 34, 95, 96, 136, 177, 182, 202, 231). Some pairs of steroidal glycoalkaloids that have a common aglycone but differ in the composition of their sugar chains show synergism in their membranolytic and antifungal activity, indicating that some kind of complementation occurs between carbohydrate moieties (57, 96, 177, 178). This

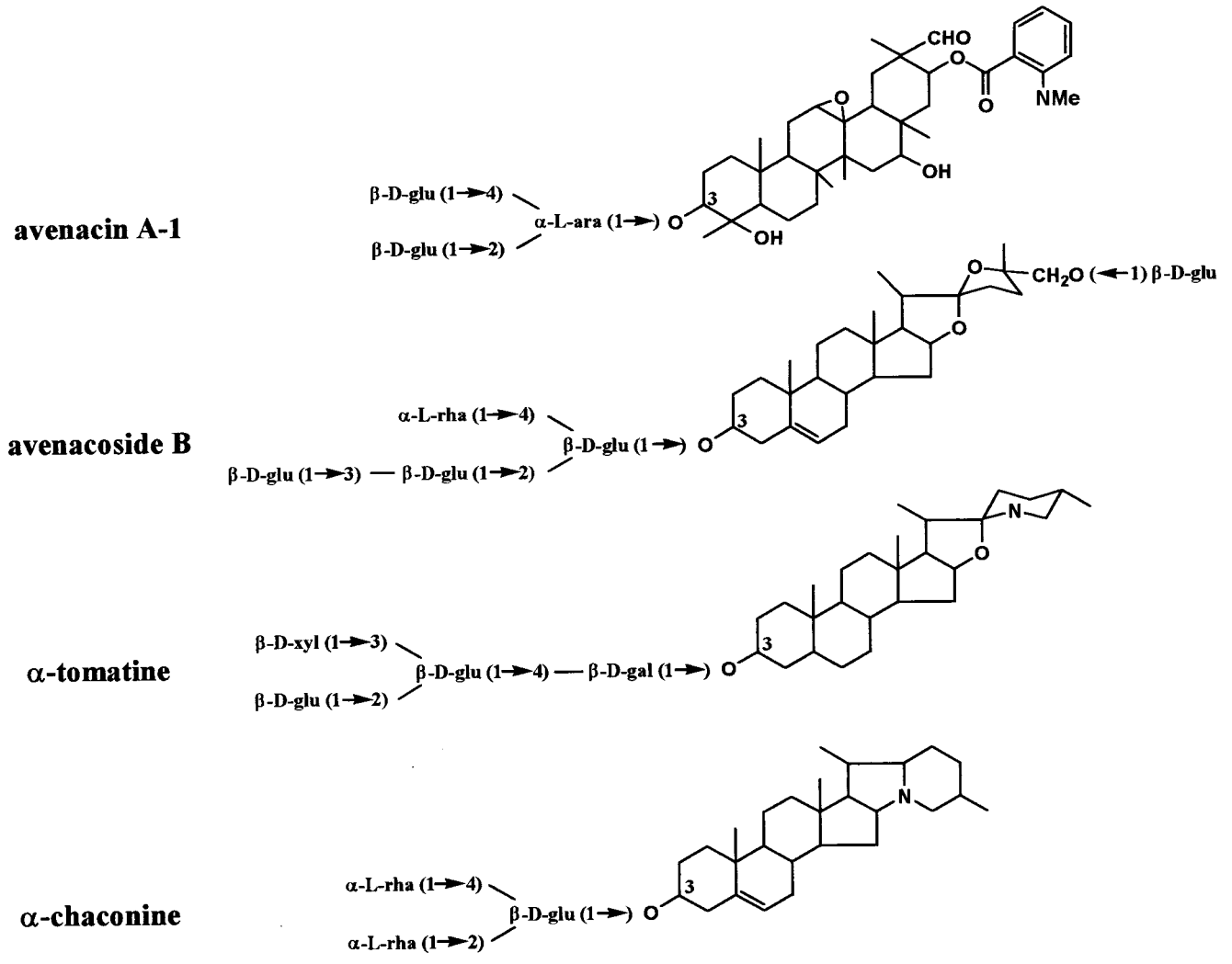

FIG. 2. Examples of antifungal saponins from oats and solanaceous plants. The major oat root saponin avenacin A-1 is one of a family of four related triterpenoid saponins. Oat leaves contain a different family of saponins, the steroidal molecules avenacosides A and B. Avenacoside A is not shown but differs from avenacoside B only in that it lacks the terminal β-1-3-linked D-glucose molecule. The avenacosides are biologically inactive but are converted to the antifungal molecules 26-desglucoavenacosides A and B by a plant enzyme which hydrolyzes the D-glucose molecule attached to C-26. α-Tomatine and α-chaconine are both steroidal glycoalkaloids and are found in tomato and potato, respectively.

synergism is particularly effective at ratios that are similar to those in which the steroidal glycoalkaloids occur naturally (57).

Resistance of fungal pathogens. Fungi that infect saponin-containing plants are often more tolerant of host plant saponins in vitro than are nonpathogens of these plants, suggesting that saponin resistance is a prerequisite for infection (9, 23, 34, 116, 143, 182, 199, 200, 211). Mechanisms of saponin resistance may vary. The resistance of Oomycete pathogens such as *Pythium* and *Phytophthora* to saponins has been associated with the lack of membrane sterols in these fungi (9). The importance of membrane composition is further emphasized by the isolation of sterol-deficient mutants of *Neurospora crassa* and *Fusarium solani* with increased resistance to the steroidal glycoalkaloid α-tomatine (36, 190). Sterol-deficient *F. solani* mutants are able to infect the α-tomatine-rich green fruits of tomato, while the wild type is pathogenic only to the ripe fruits (which contain low levels of the saponin), indicating that resistance to α-tomatine may be important for pathogens of tomato (36, 37). Whereas saponin resistance can be conferred by nondegradative mechanisms, a number of phytopathogenic fungi degrade the saponins of their respective host plants, usually by hydrolysis of sugar molecules from the sugar chain attached to C-3 of the saponin backbone (139, 220). Research on enzymatic degradation of saponins by fungi has focused on pathogens of oat and of solanaceous plants and is considered in greater depth below.

(i) Degradation of oat saponins. The importance of saponin detoxification has been demonstrated for the oat root-infecting pathogen *Gaeumannomyces graminis* var. *avenae*, which encounters the triterpenoid avenacin saponins. This fungus produces the extracellular enzyme avenacinase, which detoxifies avenacins by removal of the terminal D-glucose molecules from the sugar chains (34, 143, 211). Avenacinase is a β-glucosyl hydrolase and is related to fungal cellobiose-degrading enzymes and xylosyl hydrolases (18, 123, 142, 155). Fungal mutants that are specifically defective in the ability to produce avenacinase have been generated by targeted gene disruption and shown to be unable to infect oats, indicating that avenacinase is an essential determinant of host range for *G. graminis* var. *avenae* (18). Avenacin detoxification is not unique to *G.*

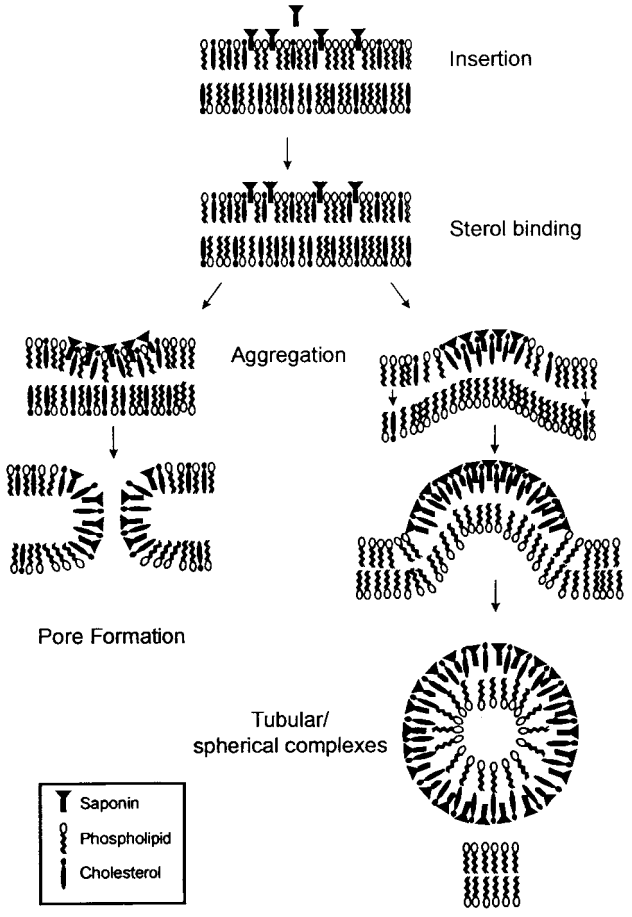

FIG. 3. Proposed models for membrane disruption by saponins. Saponins complex with sterols in membranes, ultimately to form aggregates. Aggregation may then lead to the formation of membrane pores, as shown on the left (6), or to the extraction of sterols from the membrane, with the formation of tubular or spherical complexes outside the membrane (96).

graminis var. *avenae* and has been described for another oat pathogen, *Fusarium avenaceum* (34). A survey of a collection of fungi isolated from the roots of field-grown oat plants has indicated that the ability to hydrolyze sugars from avenacins is widespread among a variety of fungi belonging to different taxonomic groups (23) and may represent a common resistance mechanism for fungi that inhabit oat roots.

Evidence that avenacins are likely to protect oat roots against fungal attack is also emerging from studies of oat variants that lack saponins. Although there is little natural variation in avenacin content in the genus *Avena*, one diploid oat species (*A. longiglumis*) that does not contain avenacins has been identified (144). This species is susceptible to infection by *G. graminis* var. *tritici*, which is avenacin sensitive and is normally unable to infect oats. More recently, the UV fluorescence of the major oat root saponin, avenacin A-1, has been exploited to isolate oat mutants that are defective in the ability to synthesize saponins. These saponin-deficient (*sad*) mutants have increased susceptibility to *G. graminis* var. *tritici* and other fungal pathogens (145a).

Whereas the triterpenoid avenacins are restricted to the roots of oat, foliar pathogens of oat encounter a different family of antifungal saponins, the steroidal 26-desglucoavenacosides, which are generated from the nontoxic avenacosides in response to tissue damage or pathogen attack (115, 116, 137, 206, 207) (Fig. 2). Enzymatic degradation of 26-desglucoavenacosides has been described for two foliar pathogens of oat, *Dreschleria avenacea* and *Stagonospora avenae* (116, 231). Both of these fungi are capable of converting oat leaf saponins to the aglycone nuatigenin by sequential hydrolysis of L-rhamnose and D-glucose. Removal of the terminal L-rhamnose molecule alone is adequate to abolish the antifungal activity of 26-desglucoavenacosides. An avenacosidase enzyme has been purified from culture filtrates of *S. avenae* (231), although it is not yet known whether this enzyme is required for resistance to 26-desglucoavenacosides and pathogenicity to oats.

(ii) Degradation of steroidal glycoalkaloids of solanaceous plants. Steroidal glycoalkaloids are found primarily in members of the family *Solanaceae*, which include the major crops tomato and potato. Tomato plants contain α-tomatine, which has a tetrasaccharide group consisting of two molecules of D-glucose and one each of D-galactose and D-xylose (β-lycotetraose) attached to C-3 (Fig. 2). Assuming a uniform distribution, the concentration of α-tomatine in tomato leaves is estimated to be around 1 mM (8), which is adequate to inhibit the growth of nonpathogens of tomato in vitro (8, 182). Thus, this saponin may be expected to contribute to the protection of tomato leaves against attack by α-tomatine-sensitive fungi. A number of pathogens of tomato produce enzymes that detoxify α-tomatine, generally by the removal of sugars from the C-3 sugar chain. These include *Septoria lycopersici*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *lycopersici*, *Verticillium albo-atrum*, and *Alternaria solani* (reviewed in reference 139). More recently, an extensive survey of a wider collection of fungi has indicated that the ability to hydrolyze sugars from α-tomatine is common among tomato pathogens (182). The degradation of α-tomatine by *S. lycopersici*, *B. cinerea*, and *F. oxysporum* f. sp. *lycopersici* (Fig. 4) has been investigated in detail, although the significance of this process for pathogenicity of these different fungi to tomato has not yet been established.

S. lycopersici, the leaf spot pathogen of tomato, produces an extracellular tomatinase which hydrolyzes the terminal β-1,2-linked D-glucose molecule from the tetrasaccharide moiety of the saponin to give β_2-tomatine (7, 53, 142, 181). Although *G. graminis* var. *avenae* avenacinase and *S. lycopersici* tomatinase enzymes are specific for their respective host saponins, both enzymes hydrolyze the terminal β-1,2-linked D-glucose molecule from their substrates and so have similar mechanisms of action (142) (Fig. 4). Comparison of the properties of the two enzymes and the isolation of the DNA encoding tomatinase has established that tomatinase also belongs to the same family of β-glucosyl hydrolases as the *G. graminis* avenacinase enzyme (142).

The broad-host-range necrotroph *B. cinerea* also infects tomato leaves, causing grey mold disease. There are various reports describing different mechanisms of degradation of α-tomatine by different *B. cinerea* isolates, including the removal of the intact β-lycotetraose group (221) and conversion of α-tomatine to an unidentified, less polar compound which still contains the β-lycotetraose group (214). Quidde et al. (173), however, found that *B. cinerea* isolates from a number of different plants degraded α-tomatine by removal of the terminal D-xylose molecule to give β_1-tomatine (Fig. 4). A *B. cinerea* xylosyl hydrolase that degrades α-tomatine has been purified (173), but its relationship to other fungal glycosyl hydrolases is unclear because the gene encoding the enzyme has not yet been cloned.

In contrast to *S. lycopersici* and *B. cinerea*, the vascular wilt fungus *F. oxysporum* f. sp. *lycopersici* grows mainly in the roots and stems of tomato plants and is likely to encounter lower

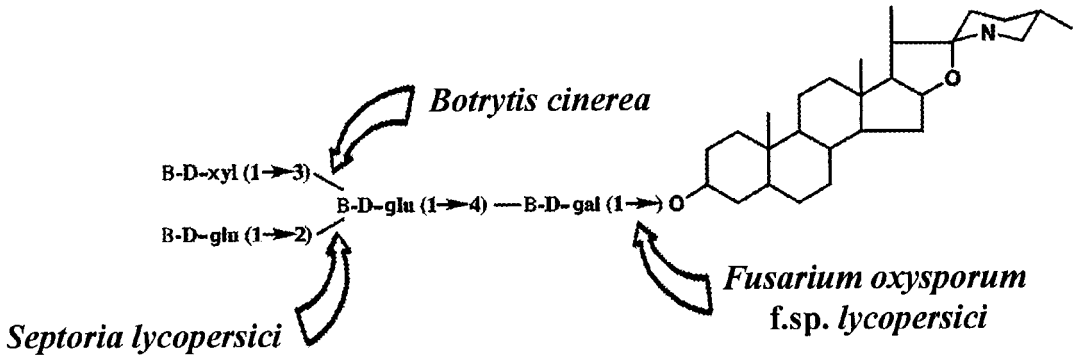

FIG. 4. Detoxification of α-tomatine by some fungal pathogens of tomato. Tomatinases produced by *Botrytis cinerea*, *Septoria lycopersici*, and *Fusarium oxysporum* f. sp. *lycopersici* have different mechanisms of action, as shown by the arrows indicating cleavage sites.

levels of α-tomatine than those experienced by necrotrophic foliar pathogens of tomato (176). Nevertheless, a comparison of the resistance of *F. oxysporum* isolates has indicated that nonpathogens of tomato are generally sensitive to α-tomatine while highly pathogenic *F. oxysporum* f. sp. *lycopersici* isolates show relatively high tolerance, leading to the suggestion that a threshold level of α-tomatine tolerance is required for pathogenicity to tomato (200). *F. oxysporum* f. sp. *lycopersici* produces an inducible extracellular tomatinase activity which detoxifies α-tomatine by cleaving the glycoalkaloid into the tetrasaccharide lycotetraose and the aglycone tomatidine (58, 103) (Fig. 4). The cDNA encoding *F. oxysporum* f. sp. *lycopersici* tomatinase has now been obtained by screening a cDNA expression library with anti-tomatinase antisera, and its identity has been confirmed by expression in *Escherichia coli* (180). The cloned DNA does not have homology to the family of saponin glucosyl hydrolases which contains *G. graminis* var. *avenae* avenacinase and *S. lycopersici* tomatinase, but it does show relatedness to xylanases. Some isolates of *F. solani* also hydrolyze α-tomatine to give the aglycone and β-lycotetraose, but this tomatinase activity differs from the *F. oxysporum* f. sp. *lycopersici* enzyme in molecular mass and immunological properties (104).

Pathogens of potato are also likely to encounter a variety of antifungal compounds, including the steroidal glycoalkaloids α-chaconine (Fig. 2) and α-solanine. *Gibberella pulicaris*, which causes storage rot of potato tubers, can metabolize both of these steroidal glycoalkaloids (227), and an enzyme capable of degrading α-chaconine to β_2-chaconine by removing the terminal α-1,2-linked L-rhamnose molecule has been purified from this fungus (16). Removal of either of the rhamnose molecules from α-chaconine destroys the ability of α-chaconine to disrupt membranes and so is likely to represent a detoxification event (96, 177), although the importance of α-chaconine degradation for pathogenicity of *G. pulicaris* to potato has not yet been assessed by the generation of chaconinase-minus mutants. Although *G. pulicaris* is rarely isolated from tomato plants, it is also able to hydrolyze β-lycotetraose from α-tomatine to give tomatidine, in the same way as *F. oxysporum* f. sp. *lycopersici* (228). The *G. pulicaris* tomatinase gene has been isolated by transforming a cosmid library of *G. pulicaris* DNA into *Aspergillus nidulans* and screening for the ability to metabolize α-tomatine. DNA sequence analysis indicates that the predicted gene product, like *F. oxysporum* f. sp. *lycopersici* tomatinase, is closely related to xylanases (225).

Cyanogenic Glycosides and Glucosinolates

Cyanogenic glycosides are found in over 200 plant species including gymnosperms, ferns, monocots, and dicots (35). They are synthesized by the conversion of amino acid precursors to oximes, which are then glycosylated (86). In healthy plants, cyanogenic glycosides are separated by compartmentalization from enzymes that catalyze their degradation. In response to tissue damage, these glycosides are broken down to release hydrogen cyanide and an aldehyde or ketone (35, 159) (Fig. 5A). The generation of hydrogen cyanide, a potent respiratory poison, may represent a defense mechanism that protects cyanogenic plants against pathogens and herbivores (62, 86). Fungi that infect these plants are generally able to tolerate hydrogen cyanide, and tolerance mechanisms may include cyanide-resistant respiration (110, 156) or enzymatic detoxification (63). The association between the ability to produce the cyanide-detoxifying enzyme cyanide hydratase and the ability to infect cyanogenic plants has been tested for the fungus *Gloeosporioides sorghi*, which is a pathogen of the cyanogenic plant sorghum (220, 223). Cyanide hydratase-deficient mutants of *G. sorghi* generated by targeted gene disruption were more sensitive than wild-type fungi to hydrogen cyanide in vitro, confirming that the enzyme could confer resistance to hydrogen cyanide. Pathogenicity to sorghum, however, was unaffected, indicating either that the ability to tolerate hydrogen cyanide is not required for infection of sorghum by *G. sorghi* or that the fungus has some alternative means of cyanide tolerance which protects it during growth in sorghum tissue.

Glucosinolates are sulfur-containing glucosides that are found in dicots, including members of the *Brassica* genus and the cruciferous weed *Arabidopsis thaliana* (25, 52, 56). These compounds can be subdivided into three major classes determined by the nature of their side chains, which may be derived from aliphatic, indolyl, or aralkyl α-amino acids (Fig. 5B). There are a number of similarities between cyanogenic glycosides and glucosinolates. Both classes of compound are synthesized from amino acids via oxime intermediates, and it has been suggested that some of the biosynthetic steps may be carried out by homologous enzymes (12, 79, 160). Glucosinolates, like cyanogenic glycosides, are activated by plant enzymes in response to tissue damage or pathogen attack (56) and have been implicated in defense against pests and pathogens (25, 66, 132). Activation involves the plant thioglucosidase myrosinase, and degradation gives rise to a variety of products including isothiocyanates, nitriles, and thiocyanates (Fig. 5B). Interestingly, myrosinase belongs to the same family of glu-

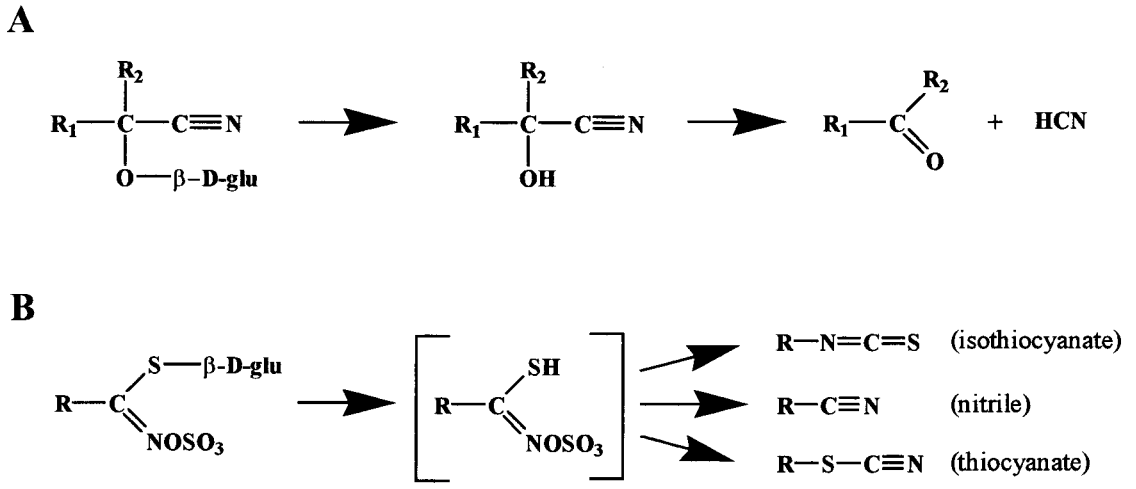

FIG. 5. Examples of cyanogenic glucosides, glucosinolates, and their degradation products. Cyanogenic glycosides and glucosinolates are both activated by plant enzymes in response to tissue damage. Breakdown of cyanogenic glycosides results in the formation of hydrogen cyanide (A), while breakdown of glucosinolate generates products which include isothiocyanates, nitriles, and thiocyanates (B).

cosyl hydrolases as linamarinase, which activates cyanogenic glycosides. This family also includes enzymes that activate saponins with two sugar chains, such as the oat avenacoside saponins (75, 76, 88, 108, 145). Glucosinolates are likely to be localized in the vacuole, while myrosinase is believed to be sequestered in specialized "myrosin" cells, although recent experiments involving the immunological localization of the glucosinolate sinigrin and myrosinase in *Brassica juncea* indicate that myrosinase not only is present in myrosin granules but also may be colocalised with its substrate in protein bodies (93).

The toxicity of isothiocyanates (the major glucosinolate breakdown products generated in *Brassica*), and of other products of glucosinolate hydrolysis to a range of fungi (including some *Brassica* pathogens) has been demonstrated, but the mechanism of toxicity is not known (25, 27, 74, 97, 124, 131, 133, 195). The development of a series of *Brassica napus* lines with contrasting glucosinolate profiles and variable total glucosinolate content has allowed the contribution of glucosinolates to protection against pests and fungal pathogens to be assessed (66, 67). These differences in glucosinolate content had significant effects on *Brassica*-herbivore interactions: increases in the levels of specific glucosinolates increased damage caused by specialist pests and reduced the extent of grazing by generalists (66). However, enhanced levels of glucosinolates were not associated with correspondingly high levels of resistance to *Brassica* pathogens and may even have led to increased susceptibility (67). This is perhaps not surprising, since these pathogens are likely to have become adapted to tolerate glucosinolate breakdown products. Whether the ability of fungal pathogens of *Brassica* to tolerate or detoxify the products of glucosinolate hydrolysis is important for pathogenicity is an area that does not appear to have been investigated in detail.

Cyclic Hydroxamic Acids

1,4-Benzoxazinones are cyclic hydroxamic acids that occur constitutively as glucosides in a number of members of the Gramineae (reviewed in reference 135). They are found in all plant parts but occur at higher levels in the vascular bundles (4, 5). In rye the main hydroxamic acid is a glucoside of DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one), whereas in maize and wheat it is the glucoside of the methoxylated form, DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) (Fig. 6A). These compounds are not found in rice, barley, or oats (135). The glucosides are hydrolyzed in response to infection or physical damage to produce the aglycones DIBOA and DIMBOA, which in turn decompose rapidly to the benzoxazolinones, 2-benzoxazolinone (BOA) and 6-methoxy-2-benzoxazolinone (MBOA), respectively, with liberation of formic acid (84, 230) (Fig. 6). The benzoxazinone aglycones and their degradation products exhibit fungistatic and bacteriostatic activity, as well as antifeeding activity against insects, whereas the parent glucosides are essentially inactive (135). The concentrations of DIMBOA and DIBOA glucosides are highest in young seedlings a few days after germination and then decrease, suggesting that the benzoxazinones act as preformed defense compounds in the juvenile stages of growth (4, 89). They may also act as phytoalexins in certain interactions (22).

Benzoxazinones and benzoxazolinones are inhibitory to a number of fungal pathogens of plants, including *Helminthosporium turcicum* (31), *Stagonospora nodorum* (13), *Microdochium nivale* (222), *Fusarium moniliforme* (175, 229), *Fusarium culmorum* (61), and *Gaeumannomyces graminis* (61). Some isolates of *G. graminis*, *F. culmorum*, *F. moniliforme*, *F. subglutinans*, and of a number of other *Fusarium* species are able to degrade benzoxazolinone compounds to products which are less inhibitory to fungal growth (60, 61, 175, 233). The primary degradation products are *N*-(2-hydroxyphenyl)malonamic acid and *N*-(2-hydroxy-4-methoxyphenyl)malonamic acid, derived from BOA and MBOA, respectively, although 2-amino-3*H*-phenoxazin-3-one has been identified as an additional BOA degradation product generated by *G. graminis* (60, 61, 233) (Fig. 6B). Thus, it appears that a variety of grass pathogens may use common mechanisms for detoxification of benzoxazolinones (60, 61). A detailed investigation of the mechanism of degradation of benzoxazolinones by an *F. subglutinans* isolate has indicated that the first metabolization step of both MBOA and BOA involves the cleavage of the NH—C═O bond, leading to the formation of an *o*-aminophenol intermediate. The aminophenol is then converted to the corresponding malonamic acid by an *N*-malonyltransferase (60) (Fig. 6B).

It is not yet known whether these fungal degradative enzymes are important for infection of plants that produce benzoxazinones. However, *benzoxazineless* (*bx1*) maize mutants that are unable to synthesize DIMBOA are more susceptible

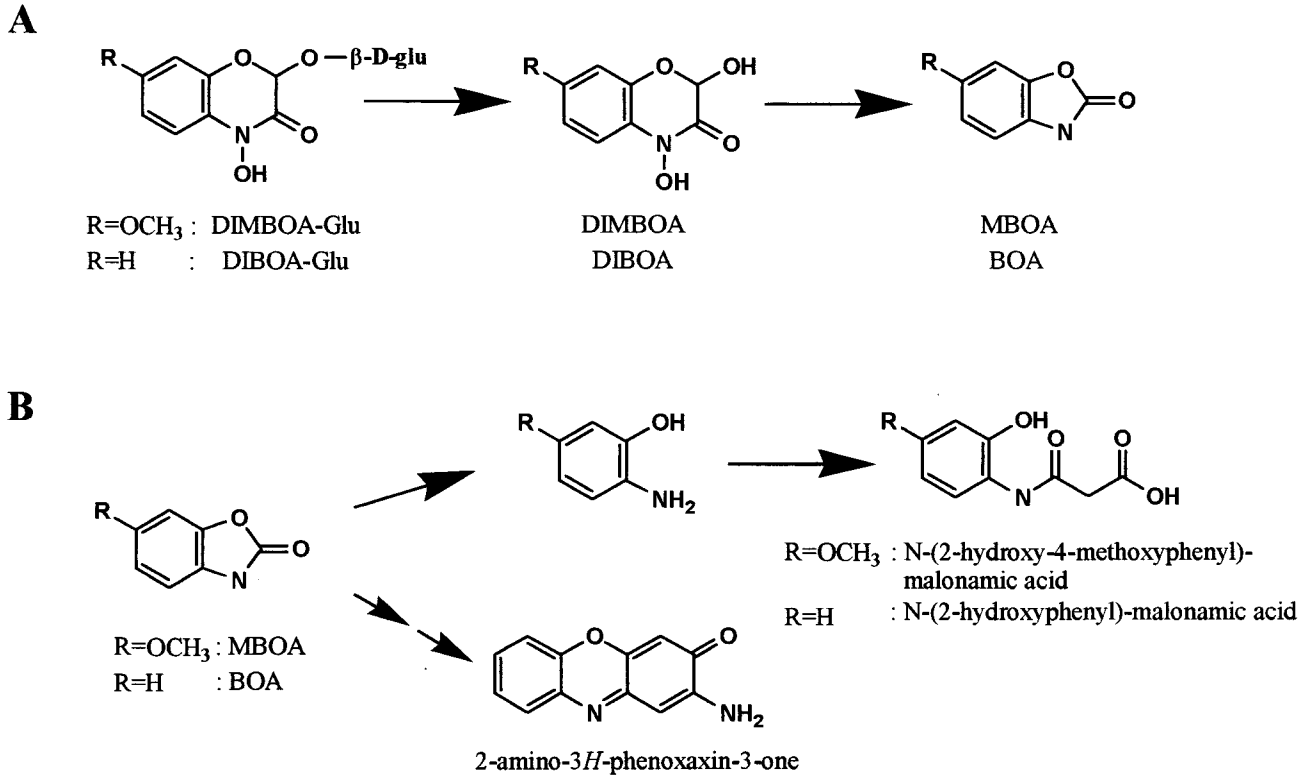

FIG. 6. Cyclic hydroxamic acids and their degradation products. (A) The cyclic hydroxamic acids DIMBOA and DIBOA occur in plants as glucosides (DIMBOA-glu and DIBOA-glu), which are converted to the antifungal aglycones DIMBOA and DIBOA by plant enzymes. These aglycones rapidly decompose to the benzoxazolinones MBOA (6-methoxy-2-benzoxazolinone) and BOA (2-benzoxazolinone), respectively, which are also fungitoxic. (B) Degradation of benzoxazolinones by cereal pathogens. Some isolates of *G. graminis* and of different *Fusarium* species can degrade MBOA and BOA to the less toxic malonamic acids via an *o*-aminophenyl intermediate. Detoxification of BOA to 2-amino-3-*H*-phenaxazin-3-one by *G. graminis* has also been reported.

to insect and pathogen attack (81), indicating that these antimicrobial compounds do play a protective role. The *BX1* gene has been cloned from maize by using the *Mutator* transposon-tagging system and found to encode a tryptophan synthase α homologue which is not required for normal growth of maize (59). This enzyme catalyzes the conversion of indole-3-glycerol phosphate to indole and represents the first committed step toward DIBOA and DIMBOA biosynthesis. Four maize cytochrome P-450-dependent monooxygenase genes *BX2*, *BX3*, *BX4*, and *BX5*, belonging to the *CYP71C* subfamily, have also been isolated, and their products have been shown to catalyze the sequential conversion of indole-3-phosphate to DIBOA (59). Remarkably, *BX1* to *BX5* are all clustered on the same arm of one maize chromosome, representing the first example of clustered genes for secondary metabolism in plants. The transferral of this relatively short pathway into other plants offers exciting challenges for metabolic engineering for improved disease resistance.

Antifungal Compounds and Fruit Ripening

The resistance of unripe fruits to fungal decay has been associated with the presence of preformed antifungal compounds in the peel (164, 165, 201). Pathogens often infect unripe fruits but then remain quiescent, with the onset of decay coinciding with decreases in the concentrations of antifungal compounds to subtoxic levels as the fruit ripens. Thus, quiescence may represent a mechanism for avoiding toxic levels of antifungal plant compounds. This area has been studied in particular detail for interactions involving *Colletotrichum gloeosporioides* and the subtropical fruits mango and avocado. The peel of unripe mango fruit contains a mixture of the antifungal 5-alkylated resorcinols 5-12-*cis*-heptadecenyl resorcinol and 5-pentadecyl resorcinol (51), while avocado peel contains antifungal monoenes and dienes (1-acetoxy-2,4-dihydroxy-*n*-heptadeca-16-ene and 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene) (167, 168) (Fig. 7). The levels of these compounds decrease during fruit ripening, and this decrease occurs more rapidly in disease-susceptible cultivars, suggesting that the presence of inhibitory levels of these antifungal substances in the peel of unripe fruit may arrest fungal growth and cause latency (51, 168, 170).

The levels of antifungal diene in peel of unripe avocados are subject to complex regulation and may be modulated by lipoxygenase, for which the diene is a substrate (166), and also by the flavan-3-ol epicatechin, an inhibitor of lipoxygenase (3, 169). Lipoxygenase activity increases during fruit ripening, while epicatechin levels decline, suggesting that these events are linked to the decrease in diene concentrations. In freshly harvested unripe avocado fruits, diene concentrations can be further enhanced by a variety of biotic and abiotic treatments including challenge with *C. gloeosporioides*, wounding, irradiation, exposure to ethylene (at levels that do not induce ripening) or carbon dioxide, and treatment with lipoxygenase inhibitors (163, 165, 169, 171, 172). Treatment with either carbon dioxide or lipoxygenase inhibitors results in increased disease resistance (169, 171), offering potential strategies for the manipulation of fruit physiology for control of postharvest diseases. Interestingly, inoculation of freshly harvested avocado fruit with a nonpathogenic mutant strain of *Colletotrichum magna*

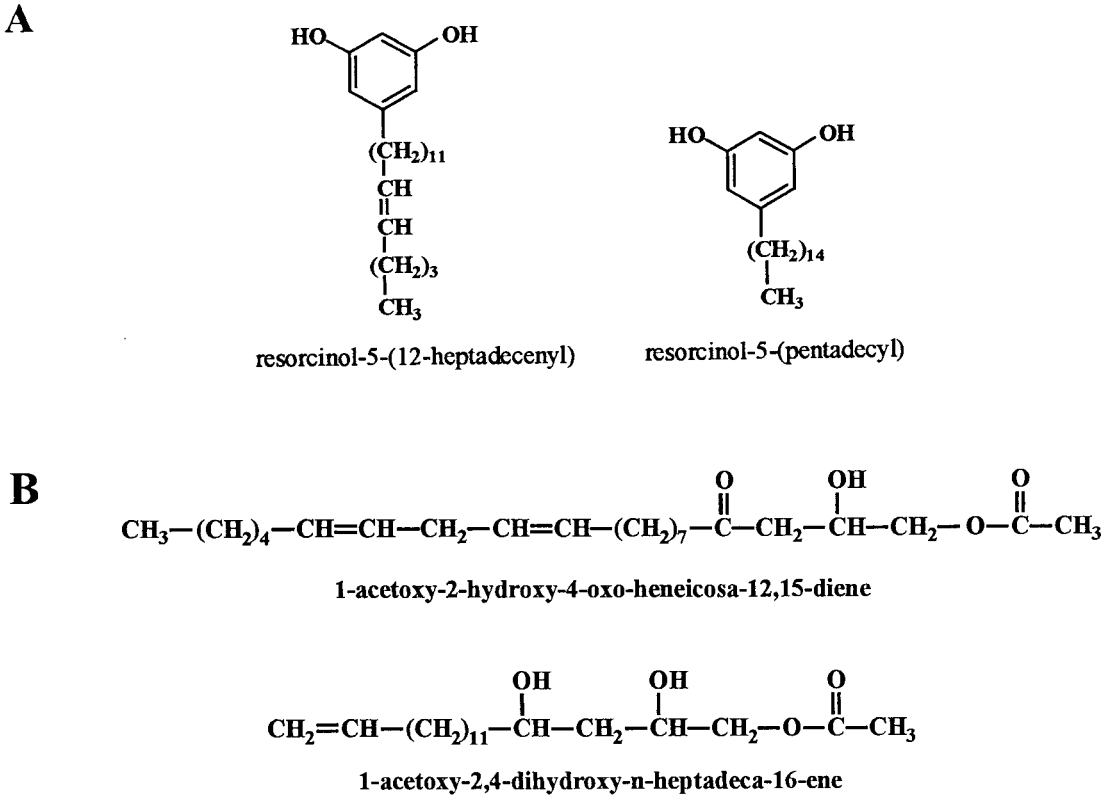

FIG. 7. Preformed antifungal molecules in subtropical fruit. (A) Resorcinols, which occur in the peel of mango fruit; (B) alkenes, which are present in the peel of avocado fruit.

also confers protection against *C. gloeosporioides*, possibly by the induction of epicatechin and modulation of the level of the antifungal diene (162). Taken together, this evidence suggests that at least for the avocado-*C. gloeosporioides* interaction, preformed antifungal compounds may contribute to the resistance of unripe fruits to decay.

PHYTOALEXINS

Phytoalexins are a group of structurally diverse molecules that are generally lipophilic, nonspecific in their antifungal activity, and not particularly potent (73, 192). The mechanisms of antimicrobial action of phytoalexins are not well understood. The basis of their toxicity is likely to involve disruption of membranes, although a variety of physiological and biochemical processes other than changes in membrane permeability may also be affected (179, 192). In contrast to preformed antifungal compounds, phytoalexins are not present in healthy plant tissue and are synthesized from remote precursors in response to pathogen attack or stress, probably as a result of de novo synthesis of enzymes. The accumulation of phytoalexins represents one of an array of induced defense responses associated with plant disease resistance (49, 105). Although both disease-resistant and -susceptible plants may respond to pathogen attack by producing phytoalexins, these compounds generally accumulate more rapidly and to higher levels in resistant plants. Clear correlations between tolerance of host plant phytoalexins in vitro and pathogenicity have been demonstrated for a number of phytopathogenic fungi, and the discovery that many of these fungi are able to metabolize phytoalexins has focused attention on the importance of phytoalexins in restricting the growth of potential pathogens (217, 218). Considerable progress has been made in elucidating the contribution of phytoalexin degradation to fungal pathogenesis, and this part of the review concentrates on plant-pathogen interactions that have been investigated in the most detail in this respect.

Stilbenes

The leaves and fruits of grapevines synthesize a group of related phenolics in response to fungal infection and abiotic treatment. These molecules belong to a group of stilbenes that also occur constitutively in a number of herbaceous and woody plants and which have been postulated to play a role in disease resistance (82, 83). Fungal challenge of vine leaves and berries results in the production of resveratrol (*trans*-3,5,4′-trihydroxystilbene) and related compounds which have antifungal activity toward a number of fungal pathogens, including *Rhizopus stolonifer*, *Plasmopara viticola*, and *B. cinerea* (Fig. 8) (1, 90, 106, 183).

There are a number of indications that stilbenes may contribute to disease resistance in grapevine. There are positive correlations between stilbene production and resistance of vine varieties to fungal attack (42, 106, 183). Furthermore, the ability of *B. cinerea* to infect grapevine has been associated with its capacity to degrade stilbene phytoalexins (184) and laccase-mediated oxidation of resveratrol by a number of enzymes has been described (2, 19, 121, 122, 157, 158). Laccases are polyphenol oxidases that act on a variety of polyphenol substrates and that have been implicated in a wide range of biological processes (205). The contribution of the *B. cinerea* laccases to

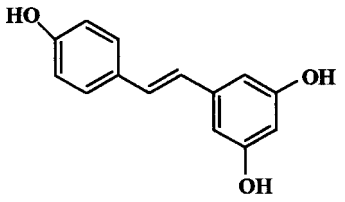

FIG. 8. The stilbene phytoalexin *trans*-resveratrol.

stilbene detoxification and to pathogenicity remains to be determined. However, evidence that stilbenes do protect plants against attack by fungal pathogens comes from experiments in which expression of resveratrol synthase from grapevine in a number of other plant species increased disease resistance (78, 107, 198, 204).

Potato Phytoalexins

During infection of potato tubers, the dry rot fungus *G. pulicaris* is likely to encounter not only the preformed steroidal glycoalkaloids but also phenolics such as chlorogenic acid, caffeic acid, and scopoletin, as well as a number of sesquiterpenes. The levels of many of these compounds increase in response to infection with *G. pulicaris* (47, 100, 174). The sesquiterpenes rishitin, lubimin, and solavetivone are the major phytoalexins that accumulate in potato tubers in response to fungal infection (47, 100). The relationship between resistance to rishitin and lubimin (Fig. 9) and pathogenicity of *G. pulicaris* to potato has been investigated in detail and has provided good evidence to indicate that high levels of pathogenicity are associated with tolerance to both lubimin and rishitin (43, 45, 46, 226) and that degradative mechanisms may be the basis of tolerance (43–46). The ability to tolerate and metabolize rishitin is associated with two independent loci (*RIM1* and *RIM2*), only one of which (*RIM1*) is linked to pathogenicity (44). Genetic analysis of the association between lubimin resistance and pathogenicity has not been carried out because of the lack of fertile lubimin-sensitive isolates.

Analysis of the mechanisms of phytoalexin metabolism by *G. pulicaris* has involved only a few isolates. A rishitin-tolerant isolate degraded rishitin to 11,12-epoxyrishitin (Fig. 9), which was then rapidly broken down to uncharacterized products (64), while a rishitin-sensitive isolate was unable to metabolize rishitin but was tolerant of and could degrade 11,12-epoxyrishitin. Thus, it is not clear whether the initial conversion of rishitin to 11,12-epoxyrishitin represents a detoxification step or whether detoxification occurs as a consequence of the breakdown of 11,12-epoxyrishitin to other products. A lubimin-metabolizing isolate converted lubimin to isolubimin via intermediates (45) (Fig. 9). Isolubimin, however, is still toxic to *G. pulicaris* and was further metabolized to a nontoxic tricyclic compound, cyclodehydroisolubimin. This was subsequently processed to other nontoxic metabolites including 11,12-epoxides (45, 46). It appears that epoxidization, which may be crucial for rishitin detoxification, is of only minor significance for lubimin detoxification. A lubimin-sensitive isolate could also convert lubimin to isolubimin but was unable to process this toxic metabolite further. Since these studies have involved only a very limited number of isolates, it is possible that other pathogenic strains of *G. pulicaris* degrade rishitin and lubimin in different ways. The cloning and subsequent mutation of genes such as *RIM1*, which presumably encode phytoalexin-detoxifying enzymes, will allow the importance of these degradative processes for pathogenicity to be assessed.

Crucifer Phytoalexins

The resistance of the wild crucifers *Camelina sativa* (false flax) and *Capsella bursa-pastoris* (shepherds purse) to different *Brassica* pathogens including *Alternaria brassicae* has been associated with the production of phytoalexins (27, 28, 91, 92). Purification and structure elucidation of phytoalexins following challenge with fungal pathogens revealed that both of these plants produce the thiazoyl-substituted indole camalexin (Fig. 10A) as the major antifungal compound, as well as smaller quantities of a related compound, 6-methoxycamalexin (21, 27, 92). *C. bursa-pastoris* also produces the minor metabolite *N*-methylcamalexin (92). Toxicity tests with *Cladosporium* sp. in-

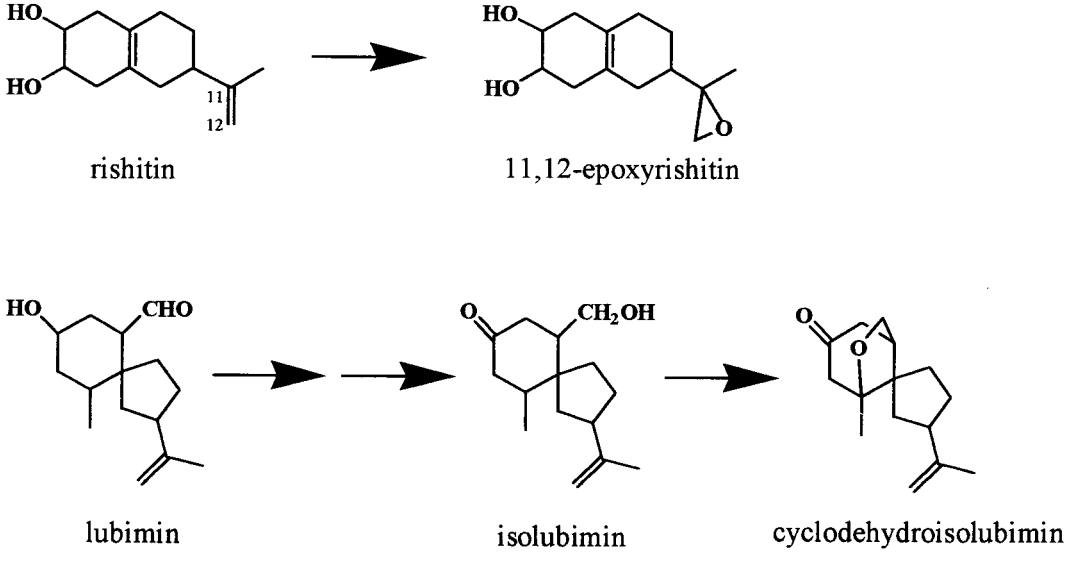

FIG. 9. Detoxification of the potato phytoalexins rishitin and lubimin by *Gibberella pulicaris*.

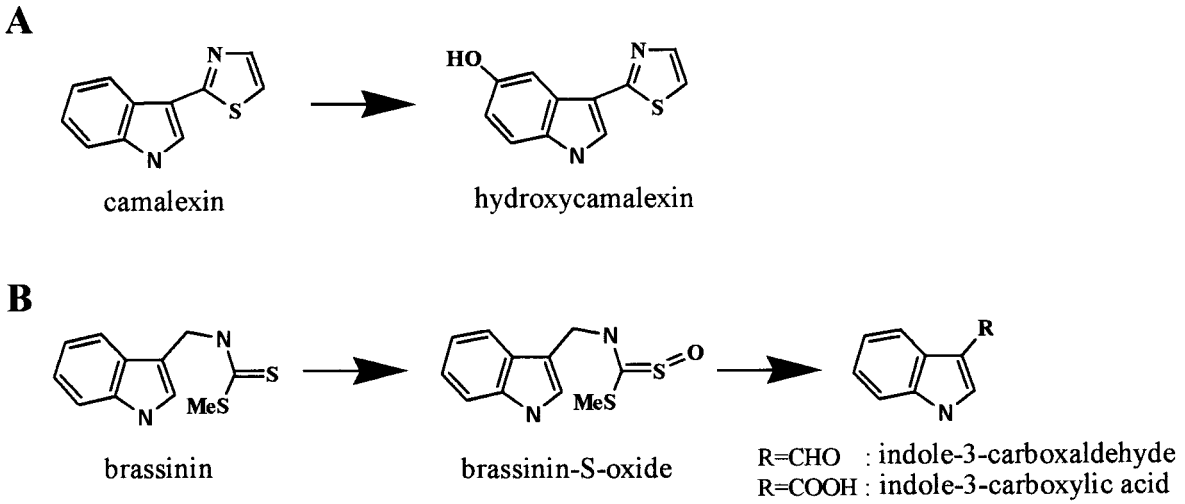

FIG. 10. Detoxification of phytoalexins from crucifers by fungal pathogens. (A) The phytoalexin camalexin is produced by some wild crucifers including *Arabidopsis thaliana*. The root pathogen *Rhizoctonia solani* can metabolize camalexin to 5-hydroxycamalexin, which is less toxic to fungal growth. (B) Brassinin is one of a family of phytoalexins produced by some cultivated brassicas. Brassinin can be oxidized to the less toxic molecule brassinin-*S*-oxide by the blackleg fungus, *Leptosphaeria maculans*, and is then further metabolized to indole-3-carboxaldehyde and indole-3-carboxylic acid.

dicate that camalexin is more toxic to this fungus than is 6-methoxycamalexin (10). Camalexin and related compounds are structurally similar to the synthetic systemic fungicide thiabendazole, which is used extensively for control of plant diseases (21). While camalexin and 6-methoxycamalexin are not present in healthy leaves of *C. sativa*, they do occur in uninoculated roots, and their levels increase in response to inoculation with the root rot fungus *Rhizoctonia solani* (27). *Leptosphaeria maculans* (anamorph *Phoma lingam*) and *A. brassicae* are apparently unable to metabolize camalexin in vitro, but *R. solani* converts this phytoalexin to the less toxic 5-hydroxycamalexin (Fig. 10A) (150–152). Although camalexin detoxification may contribute to the pathogenicity of *R. solani* to *C. sativa*, correlations between detoxification and pathogenicity have not yet been established.

Arabidopsis thaliana also synthesises camalexin on challenge with bacterial and fungal pathogens, and this appears to be the only phytoalexin produced by this plant (208). The amenability of *A. thaliana* to phytopathological studies and to molecular genetics has established it as a model host for the dissection of plant defense mechanisms. A number of mutants of *A. thaliana* that are defective in camalexin biosynthesis have been isolated (69–71). These mutants are known as *pad* (for "phytoalexin-deficient") mutants, and they define five complementation groups representing the genes *PAD1* to *PAD5. PAD1*, *PAD2*, *PAD3*, and *PAD4* are all required for full resistance to avirulent isolates of the downy mildew fungus *Peronospora parasitica*, with some combinations of double mutants showing additive effects on susceptibility (71). The *pad3* mutant is more susceptible to another fungal pathogen, *Alternaria brassicicola* (203), but not to *Botrytis cinerea* (20). This mutant is highly likely to be defective in the production of a camalexin biosynthesis enzyme. The *PAD3* gene has recently been cloned and is predicted to encode a cytochrome P-450-dependent monooxygenase belonging to the same family as the maize enzymes involved in the synthesis of DIBOA, another secondary metabolite derived from indole (235). Some of the other *pad* mutants may have pleiotropic defects in defense responses in addition to phytoalexin deficiency. This is certainly the case for *PAD4*, which plays a central role in defense-related signal transduction (70, 236). Nevertheless, the increased susceptibility of *pad3* to *P. parasitica* and *A. brassicicola* indicates that camalexin probably does contribute to the resistance of *A. thaliana* to at least some pathogens.

Cultivated *Brassica* species such as *Brassica rapa* and *Brassica napus* produce a different group of sulfur-containing phytoalexins belonging to the brassinin family following challenge with pathogens (reviewed in reference 151). Brassinin consists of an indole ring linked to a dithiocarbamate moiety (Fig. 10B). A number of reports describe the metabolism of brassinin and related phytoalexins by *L. maculans*, although the significance of these chemical conversions for pathogenicity has not been established. *L. maculans* can metabolize brassinin (148, 154), brassicanal A (149), brassilexin (151), and cyclobrassinin (153). With the exception of brassinin, however, the rates of metabolism are low, making it unlikely that degradation of these other phytoalexins constitutes a major detoxification mechanism. Brassinin is detoxified in vitro by oxidation of the thiocarbamate group to produce brassinin-*S*-oxide, which is considerably less toxic to *L. maculans* than is brassinin (Fig. 10B) (148). Brassinin-*S*-oxide is subsequently processed to other molecules including indole-3-carboxaldehyde and indole-3-carboxylic acid (148). The rapid conversion of brassinin to less toxic molecules suggests that this detoxification may have relevance for fungal pathogenicity, although further biochemical and genetic evidence is required before any definitive conclusions can be made.

Legume Phytoalexins

Kievitone and phaseollidin. Kievitone and phaseollidin are isoflavonoid phytoalexins that are produced by the legume *Phaseolus vulgaris* (French bean) and can, along with other bean phytoalexins, be detoxified by the pathogen *Fusarium solani* f. sp. *phaseoli* (193, 234). The detoxification of both of these phytoalexins involves hydration of a double bond of the allyl group linked to the isoflavonoid moiety (Fig. 11A). Whereas some isolates of *Fusarium* that are not pathogenic to bean have kievitone hydratase activity, all isolates that are highly pathogenic to bean have this enzyme activity, suggesting a link between kievitone degradation and ability to infect this host (194). The association between phaseollidin degradation

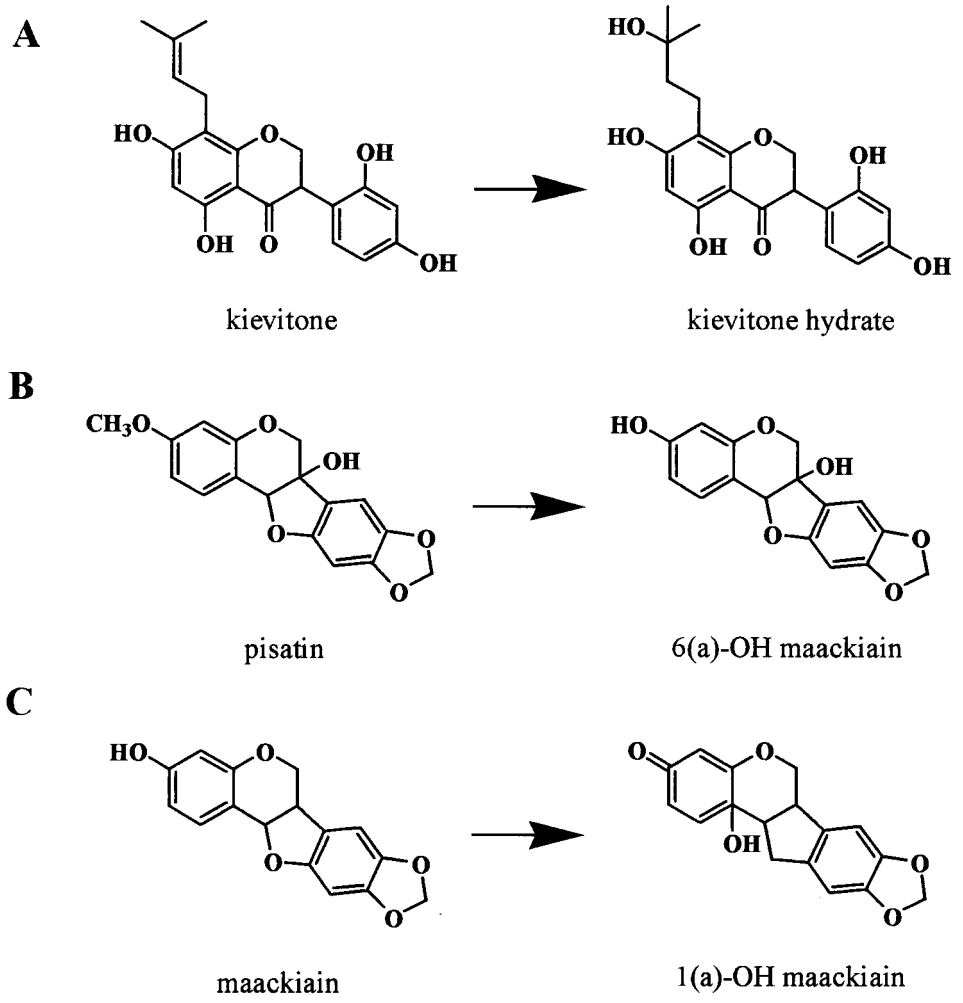

FIG. 11. Isoflavonoid phytoalexins from legumes and some of their fungal degradation products. Kievitone, pisatin, and maackiain are isoflavonoid phytoalexins produced by bean, pea, and chickpea. Kievitone is detoxified by the bean pathogen *Fusarium solani* f. sp. *phaseoli*, while pisatin and maackiain are degraded by isolates of *Nectria haematococca* (*Fusarium solani*), which are pathogenic to pea or chickpea, respectively.

and pathogenicity to bean is less clear. Although a single pathogenic isolate of *F. solani* has both kievitone and phaseollidin hydratase activities, it is not known whether this is true of all isolates that are pathogenic to bean. The phaseollidin hydratase enzyme has been purified, but the gene encoding the enzyme has not yet been cloned (209, 210). The kievitone hydratase gene has been isolated from *F. solani* (109), and its identity has been confirmed by heterologous expression in *Neurospora crassa* and *Aspergillus nidulans*. It should now be possible to directly test the importance of kievitone detoxification in pathogenicity.

Pisatin and maackiain. Isolates of *Nectria haematococca* mating-population VI (MPVI) (anamorph *Fusarium solani*) infect a number of different plants including pea (*Pisum sativum*) and chickpea (*Cicer arietinum*) (215). Although some isolates are pathogenic to both pea and chickpea, the ability to cause disease on these two hosts is not necessarily correlated (215). Both of these plants produce isoflavonoid phytoalexins; pea produces pisatin, while chickpea produces maackiain and medicarpin. Extensive characterization of field isolates of *N. haematococca* MPVI from diverse sources has revealed strong correlations between pathogenicity and the ability to detoxify legume phytoalexins, implying that phytoalexin detoxification contributes to pathogenicity (114, 217, 219).

Isolates of *N. haematococca* MPVI that are pathogenic to pea can detoxify pisatin by cytochrome P-450-mediated demethylation to 6(a)-hydroxymaackiain (125) (Fig. 11B). Natural isolates can be divided into three groups on the basis of pisatin-demethylating ability. Some isolates are unable to detoxify pisatin (Pda^-), some produce low levels of pisatin demethylase activity after prolonged exposure to pisatin (Pda^L), and others are capable of rapid pisatin-induced production of moderate to high levels of pisatin demethylase activity (Pda^H) (118, 224). A total of six pisatin demethylase (*PDA*) genes have been identified by genetic analysis. These include three PDA^L genes and three PDA^H genes (217, 224). The products of all these genes have similar biochemical properties, and the major difference between the two classes seems to be in their expression patterns (65). High levels of pathogenicity to pea are associated with the Pda^H phenotype, suggesting that effective detoxification of pisatin contributes to the ability to cause disease on this plant (217). Further evidence to support this has come from the demonstration that the introduction of a cloned PDA^H gene into Pda^- isolates of *N. haematococca* MPVI or

into other fungal pathogens can enhance the ability to cause lesions on pea (15, 26, 185). Despite this, recent experiments indicate that pisatin detoxification makes only a relatively minor contribution to pathogenicity. Mutants of highly pathogenic isolates that have undergone targeted disruption of the *PDA1* gene (a PDA^{H}-type gene) have an increased level of sensitivity to pisatin that is comparable to that of a naturally occurring Pda^{-} isolate of *N. haematococca* MPVI. However, these mutants show only a moderate reduction in pathogenicity and are still considerably more pathogenic than naturally occurring Pda^{-} isolates (224). *PDA1* is located on a conditionally dispensable 1.6-Mb chromosome (128). In contrast to mutants that are specifically defective in Pda1, transformants that have lost the entire 1.6-Mb dispensable chromosome harboring the *PDA1* gene or that have undergone deletion of a 100-kb chromosomal fragment flanking this gene have clearly reduced pathogenicity. This reduced level of pathogenicity is comparable to that of Pda^{-} isolates, indicating that other genes on the 1.6-Mb chromosome in addition to the *PDA1* gene are required for full pathogenicity to pea (98, 224). These genes do not appear to be required for resistance to pisatin in vitro, and their function is as yet unknown (224).

Very similar experiments have been performed to determine the importance of phytoalexin detoxification for pathogenicity of the same fungus to chickpea. Maackiain and medicarpin are believed to be detoxified by the same enzymes, but only maackian detoxification has been examined in detail (33, 114, 130). Four genes for maackiain detoxification have been identified in *N. haematococca* MPVI (*MAK1* to *MAK4*) (33, 129, 130). Mak1 and Mak2 convert maackiain to 1(a)-hydroxymaackiain (Fig. 11C), Mak3 converts this phytoalexin to 6(a)-hydroxymaackiain, and the products of degradation by Mak4 are unknown. 1(a)-hydroxymaackiain and 6(a)-hydroxymaackiain are both less toxic to fungal growth than is maackiain (40). The *MAK1* and *MAK2* genes are associated with high pathogenicity to chickpea (130). *MAK1* has been cloned and encodes a predicted flavin adenine dinucleotide-binding monoxygenase (33). This gene, like the *PDA1* genes, is located on a conditionally dispensable chromosome (32, 33, 130). Targeted disruption of the *MAK1* gene gave increased maackiain sensitivity (to levels which were comparable to those of naturally occurring Mak^{-} isolates) (55). Mutants generated in this way showed a moderate reduction in pathogenicity to chickpea but were still substantially more pathogenic than naturally occurring Mak^{-} isolates. Unlike the *PDA1* experiments, chemically induced loss of the 1.6-Mb dispensable *MAK1* chromosome did not result in a further reduction in pathogenicity. Transformation of a naturally occurring Mak^{-} isolate with the *MAK1* gene conferred both resistance to maackiain and increased pathogenicity to chickpea, providing further evidence that maackiain detoxification does contribute to pathogenicity. Taken together, these studies of pea- and chickpea-infecting strains of *N. haematococca* indicate that phytoalexin detoxification contributes to but is not the sole determinant of pathogenicity of *N. haematococca* to its legume hosts.

CONCLUSIONS AND FUTURE DIRECTIONS

It is clear that pathogens of plants that produce antimicrobial compounds are often more tolerant of these compounds in vitro than are nonpathogens of these plants, presenting us with the tantalizing hypothesis that resistance to antimicrobial phytoprotectants may be a prerequisite for infection. This is a very simplistic but attractive notion. Obviously, other factors are needed in addition to antibiotic resistance for fungi to be successful pathogens, and the importance of toxins, components of signal transduction pathways, and other pathogenicity determinants is well established (80, 99, 138, 232). However, resistance to antifungal compounds may be a basic requirement for survival in the host plant, and there is increasing evidence to indicate that this is the case, at least for some fungus-plant interactions. This evidence has emerged largely from studies of fungal enzymes that degrade plant antibiotics and also from experiments in which plants with altered levels of antifungal secondary metabolites have been generated.

The demonstration that the root-infecting pathogen *Gaeumannomyces graminis* requires the saponin-detoxifying enzyme avenacinase for infection of oats has set a precedent for the involvement of saponin-detoxifying enzymes in pathogenesis (18), although the general significance of saponin detoxification for other fungal pathogens is not yet known. The increased disease susceptibility of oat variants lacking avenacins adds further support to the importance of saponin resistance for fungal pathogenesis and to the notion that saponins play a role as preformed antifungal phytoprotectants. Similarly, although the significance of degradation of cyclic hydroxamic acids by cereal and grass pathogens has not yet been tested directly, maize mutants defective in DIMBOA biosynthesis have been isolated and are more susceptible to fungal attack than the wild-type maize line (59, 81). This implies that cyclic hydroxamic acids also restrict the growth of potential pathogens and that fungal tolerance of these compounds is likely to be important for pathogenesis. It is also clear that the phytoalexin-degrading enzymes produced by pea- and chickpea-attacking isolates of *N. haematococca* MPVI are required for full pathogenicity, although mutants that are unable to make these enzymes show only a moderate reduction in the ability to cause disease (55, 224). There do not appear to be any reports of the isolation of phytoalexin-deficient mutants of legumes. However, the *pad* mutants of *A. thaliana*, at least one of which is likely to be specifically defective in camalexin biosynthesis, do show increased susceptibility to some fungal pathogens (71, 203), providing complementary evidence from the plant side that phytoalexins contribute to disease resistance. There are also other examples from studies of plants in which the levels of antimicrobial compounds have been altered by transformation-mediated manipulation of gene expression with associated effects on resistance to fungal pathogens (54, 78, 119). These experiments provide further evidence to indicate that antimicrobial compounds protect plants against fungal pathogens.

While there has been considerable emphasis on enzymatic degradation of antifungal compounds, nondegradative tolerance mechanisms are also likely to be important in protecting phytopathogenic fungi against host plant antifungal compounds (36, 110). For example, in addition to the production of pisatin demethylase enzymes, *N. haematococca* MPVI isolates that infect pea have an inducible nondegradative phytoalexin tolerance. This has been attributed to an energy-dependent mechanism that secretes pisatin from mycelium and so may involve membrane transport proteins (39, 41). Membrane transport proteins of the kind associated with multidrug resistance are known to be important for the resistance of bacterial pathogens of animals and plants to host-produced antimicrobial peptides (112, 146). Such proteins may also protect phytopathogenic fungi against plant defense compounds (48). ABC transporter genes whose products have been implicated in phytoalexin resistance have been identified in various filamentous fungi including plant pathogens (38, 113, 187, 213).

While most in vitro studies of the effects of antifungal compounds on phytopathogenic fungi have involved individual purified compounds, many fungi are likely to be exposed to a variety of preformed and/or induced antifungal compounds

during infection of their hosts. Resistance to both α-tomatine and phytoalexins may be required for pathogen ingress and disease development in tomato (200). Similarly, potato pathogens are likely to be subjected to a battery of antifungal compounds including steroidal glycoalkaloids, phenolics, and sesquiterpenes, some of which may have combined or synergistic activity. The relative contributions of these various compounds to restricting fungal growth in plants is therefore difficult to evaluate. It is possible, for example, that the permeabilizing activity of saponins will predispose invading fungi to the toxic effects of other antifungal compounds such as phytoalexins.

Although the significance of some fungal enzymes that degrade host plant antifungal compounds for pathogenicity has been tested by the generation of specific mutants, it is clear from this review that many more have been characterized in some detail but their function is not yet known. For some of these enzymes, the kinetic properties and high substrate specificities for host antifungal compounds are consistent with a primary function as detoxifying enzymes. Others have less favorable properties in relation to antimicrobial substrates and may have activities toward other unidentified substrates in the plant. The contributions of these various degradative enzymes to pathogenicity on antibiotic-producing host plants will become clearer as more fungal mutants are generated by targeted gene disruption. To date, the emphasis on mechanisms of resistance of phytopathogenic fungi to antifungal secondary metabolites has been on degradative enzymes. In the future, we are likely to see a rapid expansion in our knowledge of alternative mechanisms of resistance, such as efflux systems of the kind associated with multidrug resistance, innate resistance due to insensitivity of the target site, and novel mechanisms. The manipulation of plant biosynthetic pathways to alter antibiotic profiles will also tell us more about the significance of antifungal secondary metabolites for plant defense and clearly has great potential for enhancing disease resistance for commercial purposes.

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REFERENCES

1. **Adrian, M., P. Jeandet, J. Veneau, L. A. Weston, and R. Bessis.** 1997. Biological activity of resveratrol, a stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mould. J. Chem. Ecol. **23:**1689–1702.
2. **Adrian, M., H. Rajaei, P. Jeandet, J. Veneau, and R. Bessis.** 1998. Resveratrol oxidation in *Botrytis cinerea* conidia. Phytopathology **88:**472–476.
3. **Ardi, R., I. Kobiler, B. Jacoby, N. T. Keen, and D. Prusky.** 1998. Involvement of epicatechin biosynthesis in the activation of the mechanism of resistance of avocado fruits to *Colletotrichum gloeosporioides*. Physiol. Mol. Plant Pathol. **53:**269–285.
4. **Argandoña, V. H., and L. J. Corcuera.** 1985. Distribution of hydroxamic acids in *Zea mays* tissues. Phytochemistry **24:**177–178.
5. **Argandoña, V. H., G. E. Zúniga, and L. J. Corcuera.** 1987. Distribution of gramine and hydroxamic acids in barley and wheat leaves. Phytochemistry **26:**1917–1918.
6. **Armah, C. N., A. R. Mackie, C. Roy, K. Price, A. E. Osbourn, P. Bowyer, and S. Ladha.** 1999. The membrane permeabilising effect of avenacin A-1 involves the reorganisation of bilayer cholesterol. Biophys. J. **76:**281–290.
7. **Arneson, P. A., and R. D. Durbin.** 1967. Hydrolysis of tomatine by *Septoria lycopersici*: a detoxification mechanism. Phytopathology **57:**1358–1360.
8. **Arneson, P. A., and R. D. Durbin.** 1968. Studies on the mode of action of tomatine as a fungitoxic agent. Plant Physiol. **43:**683–686.
9. **Arneson, P. A., and R. D. Durbin.** 1968. The sensitivity of fungi to α-tomatine. Phytopathology **58:**536–537.
10. **Ayer, W. A., P. A. Craw, Y. Ma, and S. Miao.** 1992. Synthesis of camalexin and related phytoalexins. Tetrahedron **48:**2919–2924.
11. **Bailey, J. A., and J. W. Mansfield.** 1982. Phytoalexins. Blackie, Glasgow, Scotland.
12. **Bak, S., H. L. Nielsen, and B. A. Halkier.** 1998. The presence of CYP76 homologues in glucosinolate-producing plants shows evolutionary conservation of the enzymes in the conversion of amino acid to aldoxime in the biosynthesis of cyanogenic glucosides and glucosinolates. Plant Mol. Biol. **38:**725–734.
13. **Baker, E. A., and I. M. Smith.** 1977. Antifungal compounds in winter wheat resistant and susceptible to *Septoria nodorum*. Ann. Appl. Biol. **87:**67–73.
14. **Bangham, A. D., and R. W. Horne.** 1962. Action of saponin on biological cell membranes. Nature **196:**952–953.
15. **Barz, W., and R. Welle.** 1992. Biosynthesis and metabolism of isoflavones and pterocarpan phytoalexins in chickpea, soybean and phytopathogenic fungi, p. 139–164. *In* H. A. Stafford and R. K. Ibrahim (ed.), Phenolic metabolism in plants. Plenum Press, New York, N.Y.
16. **Becker, P., and K-M. Weltring.** 1998. Purification and characterization of α-chaconinase of *Gibberella pulicaris*. FEMS Microbiol. Lett. **167:**197–202.
17. **Bennett, R. N., and R. M. Wallsgrove.** 1994. Secondary metabolites in plant defence mechanisms. New Phytol. **127:**617–633.
18. **Bowyer, P., B. R. Clarke, P. Lunness, M. J. Daniels, and A. E. Osbourn.** 1995. Host range of a plant pathogenic fungus determined by a saponin detoxifying enzyme. Science **267:**371–374.
19. **Breuil, A. C., M. Adrian, N. Pirio, P. Meunier, R. Bessis, and P. Jeandet.** 1998. Metabolism of stilbene phytoalexins by *Botrytis cinerea*. 1. Characterization of a resveratrol dehydrodimer. Tetrahedron Lett. **39:**537–540.
20. **Broeckhart, W.** Personal communication.
21. **Browne, L. M., K. L. Conn, W. A. Ayer, and J. P. Tewari.** 1991. The camalexins—new phytoalexins produced in the leaves of *Camelina sativa* (cruciferae). Tetrahedron **47:**3909–3914.
22. **Bücker, C., and H. J. Grambow.** 1990. Alterations in 1,4-benzoxazinone levels following inoculation with stem rust in wheat leaves carrying various alleles for resistance and their possible role as phytoalexins in moderately resistant leaves. Z. Naturforsch. Teil C **45:**1151–1155.
23. **Carter, J. P., J. Spink, P. F. Cannon, M. J. Daniels, and A. E. Osbourn.** 1999. Isolation, characterization and avenacin sensitivity of a diverse collection of cereal-root-colonizing fungi. Appl. Environ. Microbiol. **65:**3364–3372.
24. **Cartwright, D. W., P. Langcake, and J. P. Ride.** 1981. Phytoalexin production in rice and its enhancement by a dichlorocyclopropane fungicide. Physiol. Plant Pathol. **17:**259–267.
25. **Chew, F. S.** 1988. Biological effects of glucosinolates. Am. Chem. Soc. Symp. Ser **380:**155–181.
26. **Ciuffetti, L. M., and H. D. VanEtten.** 1996. Virulence of a pisatin demethylase-deficient *Nectria haematococca* MPVI isolate is increased by transformation with a pisatin demethylase gene. Mol. Plant-Microbe Interact. **9:**787–792.
27. **Conn, K. L., L. M. Browne, J. P. Tewari, and W. A. Ayer.** 1994. Resistance to *Rhizoctonia solani* and presence of antimicrobial compounds in *Camelina sativa* roots. J. Plant Biochem. Biotechnol. **3:**125–130.
28. **Conn, K. L., J. P. Tewari, and J. S. Dahiya.** 1988. Resistance to *Alternaria brassicae* and phytoalexin elicitation in rapeseed and other crucifers. Plant Sci. **56:**21–25.
29. **Cooper, R. M., M. L. V. Resende, J. Flood, M. G. Rowan, M. H. Beale, and U. Potter.** 1996. Detection and cellular localization of elemental sulphur in disease-resistant genotypes of *Theobroma cacao.* Nature **379:**159–162.
30. **Cooper, R. M., and J. Williams.** Personal communication.
31. **Couture, R. M., D. G. Routley, and G. M. Dunn.** 1971. Role of cyclic hydroxamic acids in monogenic resistance of maize to *Helminthosporium turcicum*. Physiol. Mol. Plant Pathol. **1:**515–521.
32. **Covert, S. F.** 1998. Supernumerary chromosomes in filamentous fungi. Curr. Genet. **33:**311–319.
33. **Covert, S. F., J. Enkerli, V. P. W. Miao, and H. D. VanEtten.** 1996. A gene for maackiain detoxification from a dispensable chromosome of *Nectria haematococca*. Mol. Gen. Genet. **251:**397–406.
34. **Crombie, W. M. L., L. Crombie, J. B. Green, and J. A. Lucas.** 1986. Pathogenicity of the take-all fungus to oats: its relationship to the concentration and detoxification of the four avenacins. Phytochemistry **25:**2075–2083.
35. **Davis, R. H.** 1991. Glucosinolates, p. 202–225. *In* J. P. D'Mello, C. M. Duffus, and J. H. Duffus, (eds.), Toxic substances in crop plants. The Royal Society of Chemistry, Cambridge, United Kingdom.
36. **Défago, G., and H. Kern.** 1983. Induction of *Fusarium solani* mutants insensitive to tomatine, their pathogenicity and aggressiveness to tomato fruits and pea plants. Physiol. Plant Pathol. **22:**29–37.
37. **Défago, G., H. Kern, and L. Sedlar.** 1983. Genetic analysis of tomatine insensitivity, sterol content and pathogenicity for green tomato fruits in mutants of *Fusarium solani*. Physiol. Mol. Plant Pathol. **22:**39–43.
38. **Del Sorbo, G., A. C. Andrade, J. G. M. Van Nistelrooy, J. A. L. Van Kan, E. Balzi, and M. A. De Waard.** 1997. Multidrug resistance in *Aspergillus nidulans* involves novel ATP-binding cassette transporters. Mol. Gen. Genet. **254:**417–426.

39. **Denny, T. P., P. S. Matthews, and H. D. VanEtten, H. D.** 1987. A possible mechanism of nondegradative tolerance of pisatin in *Nectria haematococca* MP VI. Physiol. Mol. Plant Pathol. **30:**93–107.
40. **Denny, T. P., and H. D. VanEtten.** 1981. Tolerance by *Nectria haematococca* MP VI of the chickpea (*Cicer arietinum*) phytoalexins medicarpin and maackiain. Physiol. Plant Pathol. **19:**419–437.
41. **Denny, T. P., and H. D. VanEtten.** 1983. Characterization of an inducible, nondegradative tolerance of *Nectria haematococca* MPVI to phytoalexins. J. Gen. Microbiol. **129:**2903–2913.
42. **Dercks, W., and L. L. Creasy.** 1989. The significance of stilbene phytoalexins in the *Plasmopara viticola*-grapevine interaction. Physiol. Mol. Plant Pathol. **34:**189–202.
43. **Desjardins, A. E., and H. W. Gardner.** 1989. Genetic analysis in *Gibberella pulicaris*: rishitin tolerance, rishitin metabolism, and virulence on potato tubers. Mol. Plant-Microbe Interact. **2:**26–34.
44. **Desjardins, A. E., and H. W. Gardner.** 1991. Virulence of *Gibberella pulicaris* on potato tubers and its relationship to a gene for rishitin metabolism. Phytopathology **81:**429–435.
45. **Desjardins, A. E., H. W. Gardner, and R. D. Plattner.** 1989. Detoxification of the potato phytoalexin lubimin by *Gibberella pulicaris*. Phytochemistry **28:**431–437.
46. **Desjardins, A. E., H. W. Gardner, and K. M. Weltring.** 1992. Detoxification of sesquiterpenes by *Gibberella pulicaris* (*Fusarium sambucinum*) and its importance for virulence on potato tubers. J. Ind. Microbiol. **9:**201–211.
47. **Desjardins, A. E., S. P. McCormick, and D. L. Corsini.** 1995. Diversity of sesquiterpenes in 46 potato cultivars and breeding selections. J. Agric. Food Chem. **43:**2267–2272.
48. **De Waard, M. A.** 1997. Significance of ABC transporters in fungicide sensitivity and resistance. Pestic. Sci. **51:**271–275.
49. **Dixon, R. A., and C. J. Lamb.** 1990. Molecular communication in interactions between plants and microbial pathogens. Annu. Rev. Plant Physiol. Plant Mol. Biol. **41:**339–367.
50. **Dourmashkin, R. R., R. M. Dougherty, and R. J. C. Harris.** 1962. Electron microscopic observations on Rous sarcoma virus and cell membranes. Nature **194:**1116–1119.
51. **Droby, S., D. Prusky, G. Jacoby, and A. Goldman.** 1986. Presence of antifungal compounds in the peel of mango fruits and their relation to latent infections of *Alternaria alternata*. Physiol. Mol. Plant Pathol. **29:**173–183.
52. **Duncan, A. J.** 1991. Glucosinolates, p. 126–147. *In* J. P. D'Mello, C. M. Duffus, and J. H. Duffus (ed.), Toxic substances in crop plants. The Royal Society of Chemistry, Cambridge, United Kingdom.
53. **Durbin, R. D., and T. F. Uchytil.** 1969. Purification and properties of a fungal β-glucosidase acting on α-tomatine. Biochim. Biophys. Acta **191:** 176–178.
54. **Eckermann, S., G. Schröder, J. Schmidt, D. Strack, R. A. Edrada, Y. Helariutta, P. Elomaa, M. Kotilainen, I. Kilpeläinen, P. Proksch, T. H. Teeri, and J. Schröder.** 1998. New pathway to polyketides in plants. Nature **396:**387–390.
55. **Enkerli, J., G. Bhatt, and S. F. Covert.** 1998. Maackiain detoxification contributes to the virulence of *Nectria haematococca* MP VI on chickpea. Mol. Plant-Microbe Interact. **11:**317–326.
56. **Fenwick, G. R., R. K. Heaney, and W. J. Mullin.** 1983. Glucosinolates and their breakdown products in food and food plants. Crit. Rev. Food Sci. Nutr. **18:**123–301.
57. **Fewell, A. M., and J. G. Roddick.** 1993. Interactive antifungal activity of the glycoalkaloids α-solanine and α-chaconine. Phytochemistry **33:**323–328.
58. **Ford, J. E., D. J. McCance, and R. B. Drysdale.** 1977. The detoxification of α-tomatine by *Fusarium oxysporum* f.sp. *lycopersici*. Phytochemistry **16:**544–546.
59. **Frey, M., P. Chomet, E. Glawischnig, C. Stettnet, S. Grün, A. Winklmair, W. Eisenreich, A. Bacher, R. B. Meeley, S. P. Briggs, K. Simcox, and A. Gierl.** 1997. Analysis of a chemical plant defense mechanism in grasses. Science **277:**696–699.
60. **Friebe, A.** Personal communication.
61. **Friebe, A., V. Vilich, L. Hennig, M. Kluge, and D. Sicker.** 1998. Detoxification of benzoxazolinone allelochemicals from wheat by *Gaeumannomyces graminis* var. *tritici*, *G. graminis* var. *graminis*, *G. graminis* var. *avenae*, and *Fusarium culmorum*. Appl. Environ. Microbiol. **64:**2386–2391.
62. **Fry, W. E., and P. H. Evans.** 1977. Association of formamide hydro-lyase with fungal pathogenicity to cyanogenic plants. Phytopathology **67:**1001–1006.
63. **Fry, W. E., and D. F. Myers.** 1981. Hydrogen cyanide metabolism by fungal pathogens of cyanogenic plants, p. 321–334. *In* B. Vennesland, C. J. Knowles, E. E. Conn, J. Westley, and F. Wissing (ed.), Cyanide in biology. Academic Press, Ltd., London, United Kingdom.
64. **Gardner, H. W., A. E. Desjardins, S. P. McCormick, and D. Weisleder.** 1994. Detoxification of the potato phytoalexin rishitin by *Gibberella pulicaris*. Phytochemistry **37:**1001–1005.
65. **George, H. L., K. D. Hirschi, and H. D. Van Etten.** 1998. Biochemical properties of the products of the cytochrome P450 genes (*PDA*) encoding pisatin demethylase activity in *Nectria haematococca*. Arch. Microbiol. **170:** 147–154.
66. **Giamoustaris, A., and R. Mithen.** 1995. The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. *oleifera*) on its interaction with specialist and generalist pests. Ann. Appl. Biol. **126:** 347–363.
67. **Giamoustaris, A., and R. Mithen.** 1997. Glucosinolates and disease resistance in oilseed rape (*Brassica napus* ssp. *oleifera*). Plant Pathol. **46:**271–275.
68. **Glauert, A. M., J. T. Dingle, and J. A. Lucy.** 1962. Action of saponin on biological cell membranes. Nature **196:**953–955.
69. **Glazebrook, J., and F. M. Ausubel.** 1994. Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens. Proc. Natl. Acad. Sci. USA **91:**8955–8959.
70. **Glazebrook, J., E. E. Rogers, and F. M. Ausubel.** 1996. Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. Genetics **143:**973–982.
71. **Glazebrook, J., M. Zook, F. Mert, I. Kagan, E. E. Rogers, I. R. Crute, E. B. Holub, R. Hammerschmidt, and F. M. Ausubel.** 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:**381–392.
72. **Gogelein, H., and A. Huby.** 1984. Interaction of saponin and digitonin with black lipid membranes and lipid monolayers. Biochim. Biophys. Acta **773:** 32–38.
73. **Grayer, R. J., and J. J. Harborne.** 1994. A survey of antifungal compounds from higher plants 1982–1993. Phytochemistry **37:**19–42.
74. **Greenhalgh, J. G., and N. D. Mitchell.** 1976. The involvement of flavour volatiles in the resistance to downy mildew of wild and cultivated forms of *Brassica oleracea*. New Phytol. **77:**391–398.
75. **Gus-Mayer, S., H. Brunner, H. A. W. Schneider-Poetsch, F. Lottspeich, C. Eckerskorn, R. Grimm, and W. Rüdiger.** 1994. The amino acid sequence previously attributed to a protein kinase or a TCP1-related molecular chaperone and co-purified with phytochrome is a β-glucosidase. FEBS Lett. **347:**51–54.
76. **Gus-Mayer, S., H. Brunner, H. A. W. Schneider-Poetsch, and W. Rüdiger, W.** 1994. Avenacosidase from oat: purification, sequence analysis and biochemical characterization of a new member of the BGA family of β-glucosidases. Plant Mol. Biol. **26:**909–921.
77. **Hahn, M. G., A. Bonhoff, and H. Grisebach.** 1985. Quantitative localisation of the phytoalexin glyceollin I in relation to fungal hyphae in soybean roots infected with *Phytophthora megasperma* f.sp. *glycinea*. Plant Physiol. **77:**591–601.
78. **Hain, R., H. J. Reif, E. Krause, R. Langebartels, H. Kindl, B. Vornam, W. Wiese, E. Schmelzer, P. H. Schreier, R. H. Stocker, and K. Stenzel.** 1993. Disease resistance results from foreign phytoalexin expression in a novel plant. Nature **361:**153–156.
79. **Halkier, B. A., and L. Du.** 1997. The biosynthesis of glucosinolates. Trends Plant Sci. **2:**425–431.
80. **Hamer, J. E., and D. W. Holden.** 1997. Linking approaches in the study of fungal pathogenesis: a commentary. Fungal Genet. Biol. **21:**11–16.
81. **Hamilton, R. H.** 1964. A corn mutant deficient in 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one with an altered tolerance of atrazine. Weeds **12:**27–30.
82. **Hart, J. H.** 1981. Role of phytostilbenes in decay and disease resistance. Annu. Rev. Phytopathol. **19:**437–458.
83. **Hart, J. H., and D. M. Shrimpton.** 1979. Role of stilbenes in resistance of wood decay. Phytopathology **69:**1138–1143.
84. **Hofman, J., and O. Hofnamova.** 1969. 1,4-Benzoxazine derivatives in plants. Sephadex fractionation and identification of a new glucoside. Eur. J. Biochem. **8:**109–112.
85. **Hostettmann, K. A., and A. Marston.** 1995. Saponins. Chemistry and pharmacology of natural products. Cambridge University Press, Cambridge, United Kingdom.
86. **Hughes, M. A.** 1991. The cyanogenic polymorphism in *Trifolium repens* L. (white clover). Heredity **66:**105–115.
87. **Ingham, J. L.** 1973. Disease resistance in higher plants. The concept of pre-infectional and post-infectional resistance. Phytopathol. Z. **78:**314–335.
88. **Inoue, K., M. Shibuya, K. Yamamoto, and Y. Ebizuka.** 1996. Molecular cloning and bacterial expression of a cDNA encoding furostanol glycoside 26-*O*-β-glucosidase of *Costus speciosus*. FEBS Lett. **389:**273–277.
89. **Iwamura, H., E. Nakagawa, and N. Hirai.** 1996. Localization of benzoxazinones that occur constitutively in wheat seedlings. Z. Naturforsch. Teil C **51:**807–812.
90. **Jeandet, P., R. Bessis, M. Sbaghi, and P. Meunier.** 1995. Production of the phytoalexin resveratrol by grapes as a response to Botrytis attack under natural conditions. J. Phytopathol. **143:**135–139.
91. **Jejelowo, O. A., K. L. Conn, and J. P. Tewari.** 1991. Relationship between conidial concentration, germling growth, and phytoalexin production by *Camelina sativa* leaves inoculated with *Alternaria brassicae*. Mycol. Res. **95:**928–934.
92. **Jimenez, L. D., W. A. Ayer, and J. P. Tewari**. 1997. Phytoalexins produced in the leaves of *Capsella bursa-pastoris* (shepherd's purse). Phytoprotection **78:**99–103.

93. **Kelly, P. J., A. Bones, and J. T. Rossiter.** 1998. Sub-cellular immunolocalisation of the glucosinolate sinigrin in seedlings of *Brassica juncea*. Planta **206:**370–377.
94. **Kessman, H., and W. Barz.** 1986. Elicitation and suppression of phytoalexin and isoflavone accumulation in cotyledons of *Cicer arietinum* L. as caused by wounding and polymeric components from the fungus *Ascochyta rabei*. J. Phytopathol. **117:**321–325.
95. **Keukens, E. A. J., T. De Vrije, C. H. J. P. Fabrie, R. A. Demel, W. M. F. Jongen, and B. De Kruijff.** 1992. Dual specificity of sterol-mediated glycoalkaloid induced membrane disruption. Biochim. Biophys. Acta **1110:** 127–136.
96. **Keukens, E. A. J., T. de Vrije, C. van den Boom, P. de Waard, H. H. Plasmna, F. Thiel, V. Chupin, W. M. F. Jongen, and B. de Kruijff.** 1995. Molecular basis of glycoalkaloid induced membrane disruption. Biochim. Biophys. Acta **1240:**216–228.
97. **Kirkegaard, J. A., P. T. W. Wong, and J. M. Desmarchelier.** 1996. *In vitro* suppression of fungal root pathogens of cereals by *Brassica* tissues. Plant Pathol. **45:**593–603.
98. **Kistler, H. C., L. W. Meinhardt, and U. Benny.** 1996. Mutants of *Nectria haematococca* created by a site-directed chromosome breakage are greatly reduced in virulence toward pea. Mol. Plant-Microbe Interact. **9:**804–809.
99. **Knogge, W.** 1996. Fungal infection of plants. Plant Cell **8:**1711–1722.
100. **Kuć, J.** 1982. Phytoalexins from the *Solanaceae*, p. 81–105. *In* J. A. Bailey and J. W. Mansfield (ed.), Phytoalexins. John Wiley & Sons, Inc., New York, N.Y.
101. **Kuć, J.** 1992. Antifungal compounds in plants, p. 159–184. *In* H. N. Nigg and D. Seigler (ed.), Phytochemical resources for medicine and agriculture. Plenum Press, New York, N.Y.
102. **Kuć, J.** 1995. Phytoalexins, stress metabolism and disease resistance in plants. Annu. Rev. Phytopathol. **33:**275–297.
103. **Lairini, K., A. Perez-Espinosa, M. Pineda, and M. Ruiz-Rubio.** 1996. Purification and characterization of tomatinase from *Fusarium oxysporum* f.sp. *lycopersici*. Appl. Environ. Microbiol. **62:**1604–1609.
104. **Lairini, K., and M. Ruiz-Rubio.** 1998. Detoxification of α-tomatine by *Fusarium solani*. Mycol. Res. **102:**1375–1380.
105. **Lamb, C. J., M. A. Lawton, M. Dron, and R. A. Dixon.** 1989. Signals and transduction mechanisms for activation of plant defences against microbial attack. Cell **56:**215–224.
106. **Langcake, P.** 1981. Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ε-viniferin, α-viniferin and pterostilbene. Physiol. Plant Pathol. **18:**213–226.
107. **Leckband, G., and H. Lorz.** 1998. Transformation and expression of a stilbene synthase gene of *Vitis vinifera* L. in barley and wheat for increased fungal resistance. Theor. Appl. Genet. **96:**1004–1012.
108. **Lenman, M., A. Falk, J. Xue, and L. Rask.** 1993. Characterization of a *Brassica napus* myrosinase pseudogene: myrosinases are members of the BGA family of β-glycosidases. Plant Mol. Biol. **21:**463–474.
109. **Li, D. X., K. R. Chung, D. A. Smith, and C. L. Schardl.** 1995. The *Fusarium solani* gene encoding kievitone hydratase, a secreted enzyme that catalyzes detoxification of a bean phytoalexin. Mol. Plant-Microbe Interact. **8:**388–397.
110. **Lieberei, R.** 1988. Relationship of cyanogenic capacity of the rubber tree *Hevea brasiliensis* to susceptibility to *Microcyclus ulei*, the agent causing South American leaf blight. J. Phytopathol. **122:**54–67.
111. **Link, K. P., A. D. Dickson, and J. C. Walker.** 1929. Further observations on the occurrence of protocatechuic acid in pigmented onion scales and its relation to disease resistance in onions. J. Biol. Chem. **84:**719–725.
112. **López-Solanilla, E., F. Garciá-Olmedo, and P. Rodríguez-Palenzuela.** 1998. Inactivation of the *sapA* to *sapF* locus of *Erwinia chrysanthemi* reveals common features in plant and animal pathogenesis. Plant Cell **10:**917–924.
113. **Loser, K., and K-M. Weltring.** 1998. Induction of a polyubiquitin gene (*ubi1*) by potato phytoalexins and heat shock in *Gibberella pulicaris*. Curr. Genet. **34:**404–409.
114. **Lucy, M. C., P. S. Mathews, and H. D. Van Etten.** 1988. Metabolic detoxification of the phytoalexins maackiain and medicarpin by *Nectria haematococca* field isolates: relationship to virulence on chickpea. Physiol. Mol. Plant. Pathol. **33:**187–199.
115. **Lüning, H. U., and E. Schlösser.** 1975. Role of saponins in antifungal resistance. V. Enzymatic activation of avenacosides. Z. Pflanzenkr. Pflanzenschultz **82:**699–703.
116. **Lüning, H. U., and E. Schlösser.** 1976. Role of saponins in antifungal resistance. VI. Interactions *Avena sativa-Drechslera avenacea*. J. Plant Dis. Prot. **83:**317–327.
117. **Mace, M. E., R. D. Stipanovic, and A. A. Bell.** 1989. Histochemical localization of desoxyhemigossypol, a phytoalexin in *Verticillium dahliae*-infected cotton stems. New Phytol. **111:**229–232.
118. **Mackintosh, S. F., D. E. Matthews, and H. D. Van Etten.** 1989. Two additional genes for pisatin demethylation and their relationship to the pathogenicity of *Nectria haematococca* on pea. Mol. Plant-Microbe Interact. **2:**354–362.
119. **Maher, E. A., N. J. Bate, W. Ni, Y. Elkind, R. A. Dixon, R. A., and C. J. Lamb.** 1994. Increased disease susceptibility of transgenic tobacco plants with suppressed levels of preformed phenylpropanoid products. Proc. Natl. Acad. Sci. USA **91:**7802–7806.
120. **Mansfield, J. W.** 1983. Antimicrobial compounds, p. 237–265. *In* J. A. Callow (ed.), Biochemical plant pathology. John Wiley & Sons, Ltd., Chichester, United Kingdom.
121. **Marbach, I., E. Harel, and A. M. Mayer.** 1984. Molecular properties of extracellular *Botrytis cinerea* laccase. Phytochemistry **23:**2713–2717.
122. **Marbach, I., E. Harel, and A. M. Mayer.** 1985. Pectin, a second inducer for laccase production by *Botrytis cinerea*. Phytochemistry **24:**2559–2561.
123. **Margolles-Clark, E., M. Tenkanen, T. Nakari-Setälä, and M. Penttila.** 1996. Cloning of genes encoding β-L-arabinofuranoside and β-xylosidase from *Trichoderma reesei* by expression in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. **62:**3840–3846.
124. **Mari, M., R. Iori, O. Leoni, and A. Marchi.** 1996. Bioassays of glucosinolate-derived isothiocyanates against postharvest pear pathogens. Plant Pathol. **45:**753–760.
125. **Matthews, D. E., and H. D. Van Etten.** 1983. Detoxification of the phytoalexin pisatin by a fungal cytochrome P-450. Arch. Biochem. Biophys. **224:** 494–505.
126. **Melton, R. E., L. M. Flegg, J. K. M. Brown, R. P. Oliver, M. J. Daniels, and A. E. Osbourn.** 1998. Heterologous expression of *Septoria lycopersici* tomatinase in *Cladosporium fulvum*: effects on compatible and incompatible interactions with tomato seedlings. Mol. Plant-Microbe Interact. **11:**228–236.
127. Reference deleted.
128. **Miao, V. P. W., D. E. Matthews, and H. D. VanEtten.** 1991. Identification and chromosomal locations of a family of cytochrome P-450 genes for pisatin detoxification in the fungus *Nectria haematococca*. Mol. Gen. Genet. **226:**214–223.
129. **Miao, V. P. W., and H. D. VanEtten.** 1992. Three genes for metabolism of the phytoalexin maackiain in the plant pathogen *Nectria haematococca*: meiotic instability and relationship to a new gene for pisatin demethylase. Appl. Environ. Microbiol. **58:**801–808.
130. **Miao, V. P. W., and H. D. VanEtten.** 1992. Genetic analysis of the role of phytoalexin detoxification in virulence of the fungus *Nectria haematococca* on chickpea (*Cicer arietinum*). Appl. Environ. Microbiol. **58:**809–814.
131. **Milford, G. F. J., J. K. Fieldsend, A. J. R. Porter, C. J. Rawlinson, E. J. Evans, and P. Bilsborrow.** 1989. Changes in glucosinolate concentrations during the vegetative growth of single- and double-low cultivars of winter oilseed rape, p. 83–90. *In* M. F. B. Dale, A. M. Dewar, R. J. Froud-Williams, T. J. Hocking, D. G. Jones, and B. L. Rea (ed.), Production and protection of oilseed rape and other Brassica crops. Association of Applied Biologists, Warwick, United Kingdom.
132. **Mithen, R.** 1992. Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. Euphytica **63:**71–83.
133. **Mithen, R., B. G. Lewis, G. R. Fenwick, and R. K. Heaney.** 1986. *In vitro* activity of glucosinolates and their products against *Leptosphaeria maculans*. Trans. Br. Mycol. Soc. **87:**433–440.
134. **Müller, K. O., and H. Börger.** 1940. Experimentelle untersuchungen uber die *Phytophthora*-resistenz der kartoffel. Arb. Biol. Reichsasnstalt. Landw. Forstw. Berlin **23:**189–231.
135. **Niemeyer, H. M.** 1988. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. Phytochemistry **27:**3349–3358.
136. **Nishikawa, M., S. Nojima, T. Akiyama, U. Sankawa, and K. Inoue.** 1984. Interaction of digitonin and its analogs with membrane cholesterol. J. Biochem. Tokyo **96:**1231–1239.
137. **Nisius, H.** 1988. The stromacentre in Avena plastids: an aggregation of β-glucosidase responsible for the activation of oat-leaf saponins. Planta **173:**474–481.
138. **Oliver, R., and A. Osbourn.** 1995. Molecular dissection of fungal phytopathogenicity. Microbiology **141:**1–9.
139. **Osbourn, A. E.** 1996. Saponins and plant defence—a soap story. Trends Plant Sci. **1:**4–9.
140. **Osbourn, A. E.** 1996. Preformed antimicrobial compounds and plant defense against fungal attack. Plant Cell **8:**1821–1831.
141. **Osbourn, A. E.** Antimicrobial phytoprotectants and fungal pathogens: a commentary. Fungal Genet. Biol., in press.
142. **Osbourn, A. E., P. Bowyer, P. Lunness, B. Clarke, and M. Daniels.** 1995. Fungal pathogens of oat roots and tomato leaves employ closely related enzymes to detoxify host plant saponins. Mol. Plant-Microbe Interact. **8:**971–978.
143. **Osbourn, A. E., B. R. Clarke, J. M. Dow, and M. J. Daniels.** 1991. Partial characterization of avenacinase from *Gaeumannomyces graminis* var. *avenae*. Physiol. Mol. Plant Pathol. **38:**301–312.
144. **Osbourn, A. E., B. R. Clarke, P. Lunness, P. R. Scott, and M. J. Daniels.** 1994. An oat species lacking avenacin is susceptible to infection by *Gaeumannomyces graminis* var. *tritici*. Physiol. Mol. Plant Pathol. **45:**457–467.
145. **Oxtoby, E., A. Dunn, A. Pancoro, and M. A. Hughes.** 1991. Nucleotide and derived amino acid sequence of the cyanogenic β-glycoside (linamarase) from white clover (*Trifolium repens* L.). Plant Mol. Biol. **17:**209–219.

145a. **Papadopoulou, K., R. E. Melton, M. Leggett, M. J. Daniels, and A. E.**

Osbourn. Compromised disease resistance in saponin-deficient plants. Submitted for publication.
146. **Parra-Lopez, C., M. T. Baer, and E. A. Groisman.** 1993. Molecular genetic analysis of a locus required for resistance to antimicrobial peptides in *Salmonella typhimurium*. EMBO J. **12:**4053–4062.
147. **Paxton, J. D.** 1981. Phytoalexins—a working redefinition. Phytopathol. Z. **101:**106–109.
148. **Pedras, M. S. C., I. Borgmann, and J. Taylor.** 1992. Biotransformation of brassinin is a detoxification mechanism by the "blackleg" fungus. Phytochemistry (Life Sci. Adv.). **11:**1–7.
149. **Pedras, M. S. C., and A. Q. Khan.** 1996. Biotransformation of the *Brassica* phytoalexin brassicanal A by the blackleg fungus. J. Agric. Food Chem. **44:**3403–3407.
150. **Pedras, M. S. C., and A. Q. Khan.** 1997. Unprecedented detoxification of the cruciferous phytoalexin camalexin by a root phytopathogen. Bioorg. Med. Chem. Lett. **7:**2255–2260.
151. **Pedras, M. S. C., A. Q. Khan, and J. L. Taylor.** 1997. Phytoalexins from Brassicas: overcoming plants' defenses. Am. Chem. Soc. Symp. Ser. **658:** 155–166.
152. **Pedras, M. S. C., A. Q. Khan, and J. L. Taylor.** 1998. The phytoalexin camalexin is not metabolized by *Phoma lingam*, *Alternaria brassiceae*, or phytopathogenic bacteria. Plant Sci. **139:**1–8.
153. **Pedras, M. S. C., and F. I. Okanga.** 1998. Strategies of cruciferous fungal pathogens: detoxification of a phytoalexin by mimicry. Chem. Commun. **1998:**67–68.
154. **Pedras, M. S. C., and J. L. Taylor.** 1991. Metabolic transformation of the phytoalexin brassinin by the blackleg fungus. J. Org. Chem. **56:**2619–2621.
155. **Péréz-González, J. A., N. M. E. van Peij, A. Bezoen, A. P. MacCabe, D. Ramón, and L. H. de Graaff.** 1998. Molecular cloning and transcriptional regulation of the *Aspergillus nidulans xlnD* gene encoding a β-xylosidase. Appl. Environ. Microbiol. **64:**1412–1419.
156. **Periera, P. T., M. M. De Carvalho, J. D. Arrabaça, M. T. Amaral-Collaço, and J. C. Roseiro.** 1997. Alternative respiratory system and formamide hydro-lyse activity as the key components of the cyanide-resistance mechanism in *Fusarium oxysporum*. Can. J. Microbiol. **43:**929–936.
157. **Pezet, R.** 1998. Purification and characterisation of a 32-kDa laccase-like stilbene oxidase, produced by *Botrytis cinerea* Pers:Fr. FEMS Microbiol. Lett. **167:**203–208.
158. **Pezet, R., V. Pont, and K. Hoangvan.** 1991. Evidence for oxidative detoxication of pterostilbene and resveratrol by a laccase-like stilbene oxidase produced by *Botrytis cinerea*. Physiol. Mol. Plant Pathol. **39:**441–450.
159. **Poulton, J. E.** 1988. Localisation and catabolism of cyanogenic glycosides. Ciba Found. Symp. **140:**67–91.
160. **Poulton, J. E., and B. L. Moller.** 1993. Glucosinolates. Methods Plant Biochem. **9:**209–237.
161. **Price, K. R., I. T. Johnson, and G. R. Fenwick.** 1987. The chemistry and biological significance of saponins in food and feeding stuffs. Crit. Rev. Food Sci. Nutr. **26:**27–133.
162. **Prusky, D., S. Freeman, R. Rodriguez, and N. T. Keen.** 1994. A nonpathogenic mutant strain of *Colletotrichum magna* induces resistance to *Colletotrichum gloeosporioides* in avocado fruits. Mol. Plant-Microbe Interact. **7:**326–333.
163. **Prusky, D., L. Karni, L. Kobiler, and R. A. Plumbley.** 1990. Induction of the antifungal diene in unripe avocado fruits: effect of inoculation with *Colletotrichum gloeosporioides*. Physiol. Mol. Plant Pathol. **37:**425–435.
164. **Prusky, D., and N. T. Keen.** 1993. Involvement of preformed antifungal compounds in the resistance of subtropical fruits to fungal decay. Plant Dis. **77:**114–119.
165. **Prusky, D., and N. T. Keen.** 1995. Inducible preformed compounds and their involvement in the resistance of plants to pathogens, p. 139–151. *In* R. Reuveni (ed.), Novel approaches to integrated pest management. Lewis Publishers, Boca Raton, Fla.
166. **Prusky, D., N. T. Keen, and I. Eaks.** 1983. Further evidence for the involvement of a pre-formed antifungal compound in the latency of *Colletotrichum gloeosporioides* on unripe avocado fruits. Physiol. Mol. Plant Pathol. **22:** 189–198.
167. **Prusky, D., N. T. Keen, J. J. Sims, and S. L. Midland.** 1982. Possible involvement of an antifungal diene in the latency of *Colletotrichum gloeosporioides* on unripe avocado fruit. Phytopathology **72:**1578–1582.
168. **Prusky, D., I. Kobiler, Y. Fishman, J. J. Sims, S. L. Midland, and N. T. Keen.** 1991. Identification of an antifungal compound in unripe avocado fruits and its possible involvement in the quiescent infections of *Colletotrichum gloeosporioides*. J. Phytopathol. **132:**319–327.
169. **Prusky, D., I. Kobiler, B. Jacoby, J. J. Sims, and S. L. Midland.** 1985. Inhibitors of avocado lipoxygenase; their possible relationship with the latency of *Colletotrichum gloeosporioides*. Physiol. Mol. Plant Pathol. **27:** 269–279.
170. **Prusky, D., R. A. Plumbley, and I. Kobiler.** 1991. The relationship between antifungal diene levels and fungal inhibition during quiescent infection of unripe avocado fruits by *Colletotrichum gloeosporioides*. Plant Pathol. **40:** 45–52.
171. **Prusky, D., R. A. Plumbley, and I. Kobiler.** 1991. Modulation of natural resistance of avocado fruits to *Colletotrichum gloeosporioides* by carbon dioxide treatment. Physiol. Mol. Plant Pathol. **39:**325–334.
172. **Prusky, D., C. Wattad, and I. Kobiler.** 1996. Effect of ethylene on the activation of quiescent infection of *Colletotrichum gloeosporioides* in avocado fruits. Mol. Plant-Microbe Interact. **9:**864–868.
173. **Quidde, T., A. Osbourn, and P. Tudzynski.** 1998. Detoxification of α-tomatine by *Botrytis cinerea*. Physiol. Mol. Plant Pathol. **52:**151–165.
174. **Ray, H., and R. Hammerschmidt.** 1998. Responses of potato tuber to infection by *Fusarium sambucinum*. Physiol. Mol. Plant Pathol. **53:**81–92.
175. **Richardson, M. D., and C. W. Bacon.** 1995. Catabolism of 6-methoxybenzoxazolinone and 2-benzoxazolinone by *Fusarium moniliforme*. Mycologia **87:**510–517.
176. **Roddick, J. G.** 1974. The steroidal glycoalkaloid α-tomatine. Phytochemistry **13:**9–25.
177. **Roddick, J. G., and A. L. Rijnenberg.** 1987. Synergistic interaction between the potato glycoalkaloids α-solanine and α-chaconine in relation to lysis of phospholipid/sterol liposomes. Phytochemistry **26:**1325–1328.
178. **Roddick, J. G., A. L. Rijnenberg, and S. F. Osman.** 1988. Synergistic interaction between potato glycoalkaloids alpha-solanine and alpha-chaconine in relation to destabilisation of cell membranes—ecological implications. J. Chem. Ecol. **14:**889–902.
179. **Rogers, E. E., J. Glazebrook, and F. N. Ausubel.** 1996. Mode of action of the *Arabidopsis thaliana* phytoalexin camalexin and its role in *Arabidopsis*-pathogen interactions. Mol. Plant-Microbe Interact. **9:**748–757.
180. **Roldán-Arjona, T., A. Pérez-Espinosa, and M. Ruiz-Rubio.** Tomatinase from *Fusarium oxysporum* f. sp. *lycopersici* defines a new class of saponinases. Mol. Plant-Microbe Interact., in press.
181. **Sandrock, R. W., D. DellaPenna, and H. D. VanEtten.** 1995. Purification and characterization of β_2-tomatinase, an enzyme involved in the degradation of α-tomatine and isolation of the gene encoding β_2-tomatinase from *Septoria lycopersici*. Mol. Plant-Microbe Interact. **8:**960–970.
182. **Sandrock, R. W., and H. D. VanEtten.** 1998. Fungal sensitivity to and enzymatic degradation of the phytoanticipin α-tomatine. Phytopathology **88:**137–143.
183. **Sarig, P., Y. Zutkhi, A. Monjauze, N. Lisker, and R. BenArie.** 1997. Phytoalexin elicitation in grape berries and their susceptibility to *Rhizopus stolonifer*. Physiol. Mol. Plant Pathol. **50:**337–347.
184. **Sbaghi, M., P. Jeandet, R. Beiss, and P. Leroux.** 1996. Degradation of stilbene-like phytoalexins in relationship to the pathogenicity of *Botrytis cinerea* to grapevine. Plant Pathol. **45:**139–144.
185. **Schäfer, W., D. Straney, L. Ciuffetti, H. D. Van Etten, and O. C. Yoder.** 1989. One enzyme makes a fungal pathogen, but not a saprophyte, virulent on a new host plant. Science **246:**247–249.
186. **Schönbeck, F., and E. Schlösser.** 1976. Preformed substances as potential protectants, p. 653–678. *In* R. Heitefuss and P. H. Williams (ed.), Physiological plant pathology. Springer-Verlag KG, Berlin, Germany.
187. **Schoonbeck, H., G. Del Sorbo, and M. A. De Waard.** The role of ABC transporters in pathogenesis of *Botrytis cinerea*. *In* Modern fungicides and antifungal compounds. Proceedings of the 12th International Reinhardsbrunn Conference, in press. Intercept, Andover, United Kingdom.
188. **Schulz, G., and H. Sander.** 1957. Über cholesterin-Tomatid. Eine neue Molekülverbinding zur analyse und preparativen Gewinnung von Steroiden. Hoppe-Seylers Z. Physiol. Chem. **308:**122.
189. **Seeman, P., D. Cheng, and G. H. Iles.** 1973. Structure of membrane holes in osmotic and saponin hemolysis. J. Cell Biol. **56:**519–527.
190. **Senegupta, S., T. B. Prasanna, and D. P. Kasbekar.** 1995. Sterol 14,15 reductase (*erg-3*) mutations switch the phenotype of *Neurospora crassa* from sensitivity to the tomato saponin α-tomatine to sensitivity to the pea phytoalexin pisatin. Fungal Genet. Newsl. **42:**71–72.
191. **Shiriashi, T., K. Saitoh, H. M. Kim, T. Kato, M. Tahara, H. Oku, T. Yamada, and Y. Ichinose.** 1992. Two suppressors, suppprescins A and B, secreted by a pea pathogen, *Mycosphaerella pinodes*. Plant Cell Physiol. **33:**663–667.
192. **Smith, C. J.** 1996. Accumulation of phytoalexins: defence mechanism and stimulus response system. New Phytol. **132:**1–45.
193. **Smith, D. A., J. M. Harrer, and T. E. Cleveland.** 1981. Simultaneous detoxification of phytoalexins by *Fusarium solani* f.sp. *phaseoli*. Phytopathology **71:**1212–1215.
194. **Smith, D. A., J. M. Harrer, and T. E. Cleveland.** 1982. Relation between extracellular production of kievitone hydratase by isolates of *Fusarium* and their pathogenicity on *Phaseolus vulgaris*. Phytopathology **72:**1319–1323.
195. **Smolinska, U., M. J. Morra, G. R. Knudsen, and P. D. Brown.** 1997. Toxicity of glucosinolate degradation products from *Brassica napus* seed meal toward *Aphanomyces euteiches* f.sp. *pisi*. Phytopathology **87:**77–82.
196. **Snyder, BA, B. Leite, J. Hipskind, L. G. Butler, and R. L. Nicholson.** 1991. Accumulation of sorghum phytoalexins induced by *Colletotrichum graminicola* at the infection site. Physiol. Mol. Plant Pathol. **39:**463–470.
197. **Snyder, B. A., and R. L. Nicholson.** 1990. Synthesis of phytoalexins in sorghum as a site-specific response to fungal ingress. Science **248:**1637–1639.
198. **Stark-Lorenzen, P., B. Nelke, G. Haenssler, H. P. Muehlbach, and J. E.**

Thomzik. 1997. Transfer of a grape stilbene synthase to rice (*Oryza sativa* L.). Plant Cell Rep. **16:**688–673.
199. **Steel, C. S., and R. B. Drysdale.** 1988. Electrolyte leakage from plant and fungal tissues and disruption of liposome membranes by α-tomatine. Phytochemistry **27:**1025–1030.
200. **Suleman, P., A. M. Tohamy, A. A. Saleh, M. A. Madkour, and D. C. Straney.** 1996. Variation in sensitivity to tomatine and rishitin among isolates of *Fusarium oxysporum* f.sp. *lycopersici*, and strains not pathogenic to tomato. Physiol. Mol. Plant Pathol. **48:**131–144.
201. **Swinburne, T. R.** 1983. Quiescent infections in post-harvest diseases, p. 1–21. *In* C. Dennis (ed.), Post-harvest pathology of fruits and vegetables. Academic Press, Ltd., London, United Kingdom.
202. **Takagi, S., H. Otsuka, T. Akiyama, and U. Sankawa.** 1982. Digitonin-cholesterol complex formation—effects of varying the length of the side chain. Chem. Pharm. Bull. **30:**3485–3492.
203. **Thomma, B. P. H. J., K. Eggermont, I. A. M. A. Penninckx, B. Mauch-Mani, R. Vogelsang, B. P. A. Cammue, and W. F. Broekaert.** 1998. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. USA **95:**15107–15111.
204. **Thomzik, J. E., K. Stenzel, R. Stocker, P. H. Schreier, R. Hain, and D. J. Stahl.** 1997. Synthesis of a grapevine phytoalexin in transgenic tomatoes (*Lycopersicon esculentum* Mill.) conditions resistance against *Phytophthora infestans*. Physiol. Mol. Plant Pathol. **51:**265–278.
205. **Thurston, C. F.** 1994. The structure and function of fungal laccases. Microbiology **140:**19–26.
206. **Tschesche, R., and P. Lauven.** 1971. Avenacosid B, ein zweites bisdesmosidisches Steroidsaponin aus *Avena sativa*. Chem. Ber. **104:**3549–3555.
207. **Tschesche, R., M. Tauscher, H. W. Fehlhaber, and G. Wulff.** 1969. Avenacosid A, ein bisdesmosidisches Steroidsaponin aus *Avena sativa*. Chem. Ber. **102:**2072–2082.
208. **Tsuji, J., E. P. Jackson, D. A. Gage, R. Hammerschmidt, and S. C. Somerville.** 1992. Phytoalexin accumulation in *Arabidopsis thaliana* during the hypersensitive reaction to *Pseudomonas syringae* pv *syringae*. Plant Physiol. **98:**1304–1309.
209. **Turbek, C. S., D. X. Li, G. H. Choi, C. L. Schardl, and D. A. Smith.** 1990. Induction and purification of kievitone hydratase from *Fusarium solani* f sp *phaseoli*. Phytochemistry **29:**2841–2846.
210. **Turbek, C. S., D. A. Smith, and C. L. Schardl.** 1992. An extracellular enzyme from *Fusarium solani* f sp. *phaseoli* which catalyzes hydration of the isoflavonoid phytoalexin, phaseollidin. FEMS Microbiol. Lett. **94:**187–190.
211. **Turner, E. M.** 1961. An enzymic basis for pathogen specificity in *Ophiobolus graminis*. J. Exp. Bot. **12:**169–175.
212. **Tweedy, B. G.** 1981. Inorganic sulfur as a fungicide. Residue Rev. **78:**43–68.
213. **Urban, M., T. Bhargava, and J. Hamer.** 1999. An ATP-driven efflux pump is a novel pathogenicity factor in rice blast disease. EMBO J. **18:**512–521.
214. **Urbasch, I.** 1986. Transformation von α-tomatin durch *Botrytis cinerea*. Planta Med. **2:**114–118.
215. **VanEtten, H. D., and H. C. Kistler.** 1988. *Nectria haematococca*, mating populations I and VI. Adv. Plant Pathol. **6:**189–206.
216. **VanEtten, H. D., J. W. Mansfield, J. A. Bailey, and E. E. Farmer.** 1994. Two classes of plant antibiotics: phytoalexins versus "phytoanticipins". Plant Cell **6:**1191–1192.
217. **VanEtten, H. D., D. E. Matthews, and P. S. Matthews.** 1989. Phytoalexin detoxification: importance for pathogenicity and practical implications. Annu. Rev. Phytopathol. **27:**143–164.
218. **VanEtten, H. D., D. E. Matthews, and D. A. Smith.** 1982. Metabolism of phytoalexins, p. 181–217. *In* J. A. Bailey and J. W. Mansfield (ed.), Phytoalexins. Blackie, Glasgow, United Kingdom.
219. **VanEtten, H. D., P. S. Matthews, K. J. Tegtmeier, M. F. Dietert, and J. I. Stein.** 1980. The association of pisatin tolerance and demethylation with virulence on pea in *Nectria haematococca*. Physiol. Plant Pathol. **16:**257–268.
220. **VanEtten, H. D., R. W. Sandrock, C. C. Wasmann, S. D. Soby, K. McCluskey, and P. Wang.** 1995. Detoxification of phytoanticipins and phytoalexins by phytopathogenic fungi. Can. J. Bot. **73**(Suppl. 1)**:**S518–S525.
221. **Verhoeff, K., and J. I. Liem.** 1975. Toxicity of tomatine to *Botrytis cinerea*, in relation to latency. Phytopathol. Z. **82:**333–338.
222. **Wahlroos, Ö., and A. I. Virtanen.** 1958. On the antifungal effect of benzoxazolinone and 6-methoxybenzoxazolinone, respectively on *Fusarium nivale*. Acta Chem. Scand. **12:**124–128.
223. **Wang, P., and H. D. VanEtten.** 1992. Cloning and properties of a cyanide hydratase gene from the phytopathogenic fungus *Gloeocercospora sorghi*. Biochem. Biophys. Res. Commun. **187:**1048–1054.
224. **Wasmann, C. C., and H. D. VanEtten.** 1996. Transformation-mediated chromosome loss and disruption of a gene for pisatin demethylase decrease the virulence of *Nectria haematococca* on pea. Mol. Plant-Microbe Interact. **9:**793–803.
225. **Weltring, K.-M.** Personal communication.
226. **Weltring, K.-M., K. Loser, and J. Weimer.** 1998. Genetic instability of rishitin metabolism and tolerance and virulence on potato tubers of a strain of *Gibberella pulicaris*. J. Phytopathol. **146:**393–398.
227. **Weltring, K.-M., J. Wessels, and R. Geyer.** 1997. Metabolism of the potato saponins α-chaconine and α-solanine by *Gibberella pulicaris*. Phytochemistry **46:**1005–1009.
228. **Weltring, K.-M., J. Wessels, and G. F. Pauli.** 1998. Metabolism of the tomato saponin α-tomatine by *Gibberella pulicaris*. Phytochemistry **48:**1321–1328.
229. **Whitney, N. J., and C. G. Whitmore.** 1959. An antifungal substance in the corn plant and its effect on growth of two stalk-rotting fungi. Nature **183:** 341.
230. **Woodward, M. D., L. J. Corcuera, J. P. Helgeson, and C. D. Upper.** 1978. Decomposition of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one in aqueous solutions. Plant Physiol. **61:**796–802.
231. **Wubben, J. P., K. R. Price, M. J. Daniels, and A. E. Osbourn.** 1996. Detoxification of oat leaf saponins by *Septoria avenae*. Phytopathology **86:** 986–992.
232. **Yoder, O. C., and B. G. Turgeon.** 1996. Molecular-genetic evaluation of fungal molecules for roles in pathogenesis to plants. J. Genet. **75:**425–440.
233. **Yue, Q., C. W. Bacon, and M. D. Richardson**. 1998. Biotransformation of 2-benzoxazolinone and 6-methoxy-benzoxazolinone by *Fusarium moliliforme*. Phytochemistry **48:**451–454.
234. **Zhang, Y., and D. A. Smith.** 1983. Concurrent metabolism of the phytoalexins phaseollin, kievitone and phaseollinisoflavan by *Fusarium solani* f.sp. *phaseoli*. Physiol. Plant Pathol. **23:**89–100.
235. **Zhou, N., and J. Glazebrook.** Personal communication.
236. **Zhou, N., T. L. Tootle, F. Tsui, D. F. Klessig, and J. Glazebrook.** 1998. *PAD4* functions upstream from salicylic acid to control defense responses in *Arabidopsis*. Plant Cell **10:**1021–1030.
237. **Ziegler, E., and R. Pontzen.** 1982. Specific inhibition of glucan-elicited glyceollin accumulation in soybeans by an extracellular mannan-protein of *Phytophthora megasperma* f.sp. *glycinea*. Physiol. Plant Pathol. **20:**321–331.