CHAPTER 9

PLANT DEFENCE COMPOUNDS AGAINST *BOTRYTIS* INFECTION

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Abstract. Plants possess a range of tools for combating a *Botrytis* infection. This chapter will describe three types of pre-formed and induced plant defence compounds and discuss their effectiveness in restricting *Botrytis* infection. Case studies are presented on several types of secondary metabolites: stilbenes including resveratrol, saponins including α-tomatin, cucurbitacins, proanthocyanidins and tulipalin A. Evidence is presented suggesting that *Botrytis* species have evolved mechanisms to counteract some of these defence responses. Secondly, we discuss the role of structural barriers and cell wall modification in preventing penetration. Finally the contribution of PR proteins to resistance is discussed.

1. Introduction

In response to pathogens, plants are generally able to mount a spectrum of defence responses, often coinciding with an oxidative burst involving active oxygen species (AOS) that commonly confers resistance to a wide range of (biotrophic) pathogens. The oxidative burst is however not completely effective against *Botrytis* and there is evidence that the pathogen actually benefits from it (Chapter 8). Nevertheless plants possess a range of tools for combating a *Botrytis* infection. This chapter will describe three types of pre-formed and induced plant defence compounds, namely secondary metabolites, structural barriers and antifungal pathogenesis-related (PR) proteins, and discuss their role and effectiveness in restricting *Botrytis* infection. In addition, mechanisms are discussed that suggest that *Botrytis* species have developed strategies to counteract some of these defence responses. Most of the information comes from research on *B. cinerea* but we will also discuss research on other, host-specialised *Botrytis* species where available and appropriate. The terms 'compatible interaction' and 'incompatible interaction' will be used for interactions

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with a host and a non-host plant species, respectively. This is especially relevant for *Botrytis* species with a narrow host range, but in some cases also for *B. cinerea*.

2. Antimicrobial secondary metabolites

There are two types of antimicrobial metabolites: phytoalexins and phytoanticipins (VanEtten et al., 1994). Phytoanticipins are preformed, while phytoalexins are induced by pathogen infection. Excellent reviews on the phytoalexins identified in plants that are hosts for *Botrytis* spp. have been published by Mansfield (1980) and Daniel and Purkayastha (1995). These reviews also discuss the evidence for specific enzymatic degradation of some of these plant defence molecules, for example the metabolism of wyerone derivatives by *B. cinerea* and *B. fabae* in *Vicia faba*. The following section will discuss case studies of secondary metabolites that display inhibitory activity to *Botrytis.* Their chemical structures are very diverse and their modes of action presumably distinct.

2.1. Resveratrol and other stilbenes

Trans-resveratrol (3,5,4'-trihydroxystilbene) is one of the simplest stilbenes. It is a product of the plant secondary phenolic metabolism by the action of resveratrol synthase on p-coumaroyl-CoA and malonyl-CoA. It occurs in unrelated groups of angiosperms (Morales et al., 2000) and has been studied mainly in grapevine where it is the most abundant stilbene (Creasy and Creasy, 1998). In this species, it constitutes one of the major components of wood (Langcake and Pryce, 1976) and acts as a phytoalexin in leaves (Langcake and Pryce, 1977a; Langcake, 1981; Jeandet et al., 1995a). It is also found in the fruit skin (Jeandet et al., 1991).

Besides *trans*-resveratrol, numerous other stilbenes have been characterized in grapevine. These include a 3-O-β-glucoside of resveratrol called piceid that is formed by the action of a glycosyl transferase on resveratrol (Waterhouse and Lamuela Raventos, 1994; Romero-Perez et al., 1999) and a dimethylated derivative of resveratrol (3,5-dimethoxy-4'-hydroxystilbene) named pterostilbene of which the biosynthetic pathway remains to be resolved (Langcake et al., 1979; Pezet and Pont, 1988a). Piceids are a water-soluble form of resveratrol that can be reconverted into resveratrol by plant glycosidases (Ayran et al*.,* 1987). Stressed or *Botrytis*-infected leaves also accumulate oligomers of resveratrol termed viniferins, the most abundant of which being *trans*-ε-viniferin, a resveratrol dehydrodimer (Langcake and Pryce, 1977b; Langcake, 1981) believed to result from the oxidative dimerisation of resveratrol by a plant peroxidase (Langcake and Pryce, 1977c; Langcake, 1981; Morales et al., 1997, 2000) or fungal laccase (Pezet, 1998; Schouten et al., 2002). *Cis* isomers of resveratrol, piceids and viniferins have also been detected in mature fruit and wine (Romero-Perez et al., 1999). They result from the isomerisation of *trans*-resveratrol by UV irradiation (Roggero and Garcia-Parrilla, 1995).

An early study on the antifungal activity of stilbenes revealed that they rapidly inhibit the respiration of fungal cells, probably by acting as uncoupling agents and by forming protein-phenol complexes (Hart, 1981). Based on the structural similarity of hydroxystilbenes and aromatic hydrocarbons, it was inferred that their

mode of action may involve lipid peroxidation by blocking cytochrome c reductase and monooxygenases (Pezet and Pont, 1995). A study on structure-activity relationships of natural stilbenes and synthetic derivatives demonstrated a positive correlation between the biological activity of stilbenes and their hydrophobicity, their ability to form complexes with proteins and the electron-attractivity of their substituents (Pont and Pezet, 1990; Pezet and Pont, 1995). The hydroxystilbenes most active on fungal respiration were pterostilbene and ε-viniferin with respective EC_{50} values of 20 µg/ml (Pezet and Pont, 1988a) and 37 µg/ml (Langcake and Pryce, 1977a).

Resveratrol displayed no immediate toxicity towards *B. cinerea* because of its hydrophilic character and inability to reach its targets in fungal cells (Pezet and Pont, 1995). Long-term incubation of *B. cinerea* with resveratrol, however, led to the inhibition of germination of conidia, as well as the elongation of germ tubes and hyphae (Adrian et al., 1997). Functional analysis of a resveratrol-inducible laccase gene, *Bclcc*2, recently clarified this discrepancy (Schouten et al., 2002). *Bclcc*2 gene replacement mutants had an impaired ability to metabolise resveratrol and displayed improved growth characteristics on resveratrol containing media, suggesting that resveratrol is not toxic in itself, but the BcLCC2 protein is responsible for transforming resveratrol into a fungitoxic substance (member of the viniferins). Such a novel mechanism of activation of a phytoalexin presents obvious advantages for the plant. Plant secondary metabolites may be toxic to the plant itself and often have limited solubility. As resveratrol is one of the most soluble and least toxic stilbenes, it can be stored safely at high concentrations in vacuoles and cell walls (Morales et al., 2000) only to be activated upon contact with a microorganism. The activation of resveratrol into viniferins does not necessarily require the conversion by the pathogen. Increased ε-viniferin production also occurs in grapevine in the absence of a microbe, after UV irradiation (Langcake and Pryce, 1977b). The content ratios of resveratrol and ε-viniferin remained unchanged in several grapevine cultivars after UV irradiation or *B. cinerea* infection, even when their respective concentration increased (Douillet-Breuil et al., 1999; Adrian et al., 2000). Plant enzymes potentially involved in oxidation of resveratrol include peroxidases (Langcake and Pryce, 1977a, b). One constitutive basic grapevine peroxidase exhibits a high affinity for resveratrol (Morales et al., 1997). The peroxidase-mediated oxidation of resveratrol does not yield viniferins *in vitro*. However, the natural association of the benzene rings of resveratrol with cellulose fibres affects the stability of radical reaction intermediates such that peroxidase-mediated oxidation of resveratrol may generate viniferins *in planta* (Morales et al., 2000). Interestingly H_2O_2, a co-substrate of peroxidases, is generated during *B. cinerea* infection (Chapter 8).

Pterostilbene has the highest antifungal activity, but its concentration is less than 5 µg/g in leaves (Douillet-Breuil et al., 1999) and fruit (Adrian et al., 2000) of various grapevine cultivars and is not enhanced by UV irradiation or *B. cinerea* infection. This raised doubts about the capacity of pterostilbene to protect plants against *B. cinerea* (Langcake, 1981; Douillet-Breuil et al., 1999; Adrian et al., 2000). The potency of pterostilbene increases in the presence of glycolic acid, an organic acid that accumulates to high concentrations in immature grape berries.

Pterostilbenes may thus act as a constitutive defence component in berries (Pezet and Pont, 1988b). In contrast, resveratrol acts in an inducible defence reaction against *B. cinerea*. A positive correlation was found between the resveratrol level in grapevine cultivars and their resistance to *B. cinerea* (Langcake and McCarthy, 1979). UV irradiation or *B. cinerea* challenge of leaves and fruit of resistant grapevine cultivars led to resveratrol concentrations up to 750 μg/g, exceeding levels needed for toxicity to *B. cinerea in vitro* (Jeandet et al., 1995a; Douillet-Breuil et al., 1999; Adrian et al., 2000). Local resveratrol concentrations may be more important than those found after grinding whole tissue. The interaction of grapevine leaves with an incompatible *B. cinerea* isolate led to a hypersensitive-like response during which stilbenes preferentially accumulated in the direct vicinity of the fungus (Derckel et al., 1999).

Raising resveratrol levels in crop plants is an attractive option because of the potential of this substance to protect plants from the attack of pathogens and to improve human health (Chiou, 2002) by preventing and curing cancers (Savouret and Quesne, 2002) and guarding against vascular diseases (Hung et al., 2000). In plant species that do not naturally produce stilbenes, the production of resveratrol was achieved by genetic engineering. Introduction of the grapevine stilbene synthase gene *Vst*1 into tobacco (Hain et al., 1993) and barley (Leckband and Lorz, 1998) resulted in resveratrol accumulation and enhanced protection against grey mould. In transgenic kiwifruit, however, the presence of high endogenous levels of glycosyl transferase activity led to the preferential accumulation of piceid over resveratrol and no protection to *B. cinerea* infection was observed (Kobayashi et al., 2000). In plants that naturally have the genetic potential to produce resveratrol, the expression of stilbene synthase is low in unstressed leaves. It can be induced in this organ by a variety of biotic and abiotic elicitors such as $AlCl_3$ (Adrian et al., 1996; Jeandet et al., 1998), UV, paraquat, wounding, H_2O_2, salicylic acid, jasmonic acid, ethylene (Grimmig et al., 1997; Adrian et al., 2000; Lippmann et al., 2000; Chung et al., 2003), methyl jasmonate (Larronde et al., 2003), systemic acquired resistance elicitors (Busam et al., 1997), abscisic acid (Ban et al., 2000) and ozone (Grimmig et al., 1997). The exogenous application of *B. cinerea* cell wall fragments (Liswidowati et al., 1991) or a live soil-borne biocontrol *Bacillus* species (Paul et al., 1998) have also been reported to lead to increased accumulation of resveratrol in grapevine leaves.

Fruit, such as grape berries, behave differently from leaves by expressing significant levels of resveratrol constitutively. One concern is that resveratrol levels decrease during fruit ripening due to lower expression of stilbene synthase (Bais et al., 2000) and increased substrate competition for this enzyme with chalcone synthase, whose activity is stimulated during fruit ripening to provide precursors for anthocyanin accumulation (Jeandet et al., 1995b). Nevertheless, grapes keep their capacity to accumulate higher levels of resveratrol after UV irradiation at any stage of ripening (Adrian et al., 2000) and even after harvest (Cantos et al., 2001). Another concern with resveratrol accumulation in grape is that it only occurs in the skin, implying that resveratrol can only participate in protection of the berry from outside intruders when the fruit skin is intact. Cultivation practices have also been shown to influence resveratrol accumulation in grapevine leaves and fruit. A

negative correlation was found between the resveratrol level and the extent of nitrogen fertilization (Fregoni et al., 2000; Bavaresco et al., 2001) or fungicide spray application (Magee et al., 2002) while increased potassium fertilization correlated with higher levels of resveratrol (Fregoni et al., 2000). The potential of grapevine cultivars to accumulate resveratrol was positively correlated with their tolerance to *Botrytis* (Douillet-Breuil et al., 1999), leading to the suggestion that resveratrol production capacity could be used as a criterion in the selection of resistant varieties (Pool et al., 1981; Creasy and Coffee, 1988; Sbaghi et al., 1995).

The ability of *B. cinerea* to cope with stilbenes should not be underestimated. Several studies did not corroborate a relationship between resveratrol accumulation and disease incidence in grapevine (Magee et al., 2002; Keller et al., 2003). Increasing conidia concentration in synthetic media containing resveratrol led to increased resveratrol catabolism and survival of conidia (Hoos and Blaich, 1990) and a correlation between resveratrol detoxification capacity of *B. cinerea* isolates and their virulence on grapevine leaves has been proposed (Sbaghi et al., 1996). Detoxification of resveratrol and the related pterostilbene has been attributed to their oxidative dimerisation by *B. cinerea* laccases (Hoos and Blaich, 1990; Pezet et al., 1991; Sbaghi et al., 1996; Adrian et al., 1998; Breuil et al., 1999). Application of cucurbitacins led to reduced production of laccases (Sect. 2.3) and correlated with increased resistance of several hosts, even those that do not accumulate stilbenes, such as carrot and cucumber (Bar-Nun and Mayer, 1990; Viterbo et al., 1993a). This suggested that *B. cinerea* laccases are part of a more general 'attack' machinery designed to detoxify phenolic defences from many host plants (Staples and Mayer, 1995). However, the product of one specific laccase gene, BcLCC2, has been implicated in the opposite effect by activating resveratrol into a fungitoxic substance (Schouten et al., 2002). Laccase production by *B. cinerea* increases during late stages of infection or development (Roudet et al., 1992; Manteau et al., 2003) in parallel with a decrease in resveratrol concentration in its host (Adrian et al., 2000; Montero et al., 2003) and can be stimulated *in vitro* by host-derived substances such as phenolics, pectins (Viterbo et al., 1993a) and ambient pH (Manteau et al., 2003). Proanthocyanidins in fruit act as competitive laccase inhibitors (Pezet et al., 1992).

Three laccase genes were described so far (Schouten et al., 2002) and limited information is available on their distribution among *B. cinerea* populations and their physiological role during pathogenesis. This role may prove to be complex since the incubation of *B. cinerea* with resveratrol has been shown to lead to the production of an array of oxidised derivatives, including the fungitoxic ε-viniferin (Langcake and Pryce, 1977a; Keller et al., 2003) and six other dimerisation products: δ-viniferin (Breuil et al., 1998; Pezet et al., 2003a), leachinol F, pallidol and restrytisols A-C (Cichewicz et al., 2000). Some information is available on the toxicity of these individual, pure metabolites towards *B. cinerea*, but one should consider that such *in vitro* tests may not reflect the chemical environment that the pathogen encounters during infection. Laccase activity on resveratrol yields radical quinone intermediates that may react with very distinct (phenolic and other) compounds, giving rise to heterogeneous products. The metabolism of resveratrol *in planta* therefore depends on the temporal and spatial distribution both of the fungal laccase(s), the plant peroxidase(s), their substrate(s), as well as on the enzyme kinetics for each substrate.

2.2. α-Tomatine and saponins

α-Tomatine is a secondary metabolite produced in tomato leaves and unripe fruit (Friedman, 2002). It is a potent antifungal (e.g. Sandrock and VanEtten, 1998) and insecticidal (e.g. Kowalski et al., 2000) compound that interacts with sterols in membranes (Keukens et al., 1995). Already in the 1970s it was reported that α-tomatine inhibits mycelial growth of *B. cinerea* while not affecting germination of conidia (Verhoeff and Liem, 1975). It was proposed that its fungistatic action was responsible for maintaining *B. cinerea* infections in a quiescent state. The reduction in α-tomatine content in ripening fruit (Friedman, 2002) supposedly relieves fungistasis and permits fungal outgrowth. Furthermore, Verhoeff and Liem (1975) reported that *B. cinerea* was able to convert α-tomatine to tomatidine by sugar hydrolysis, thereby actively detoxifying the compound and facilitating fungal outgrowth into tomato tissue prior to the natural drop in α-tomatine levels.

Supporting evidence for the important role of α-tomatine in conferring resistance towards *B. cinerea* came from work of Quidde et al. (1998). Among a set of 13 isolates, one field isolate was identified that lacked α-tomatine degrading ("tomatinase") activity. This isolate, designated M3 and originating from grape, was able to form primary lesions on tomato but these lesions never expanded. Virulence of isolate M3 on *Phaseolus vulgaris* was not affected (Quidde et al., 1998). Contrary to the report of Verhoeff and Liem (1975), the α-tomatine degradation product was identified as β-tomatine (Quidde et al., 1998). On the basis of this conversion it was proposed that the tomatinase should possess xylosidase activity. Quidde et al. (1999) cloned a *B. cinerea* gene homologous to the tomatinase gene *tom*1 from *Septoria lycopersici*. The gene, designated *sap*1, encodes a β-glucosidase active on saponins. Gene replacement mutants deficient in *sap*1 lost the ability to degrade avenacin (a phytoanticipin from roots of oat, a non-host species for *B. cinerea*), yet they remained able to detoxify tomatin, digitonin and avenacoside (a phytoanticipin from leaves of oat). *sap*1-deficient mutants remained able to infect tomato, suggesting that the *sap*1 gene does not encode the true tomatinase. Thus *B. cinerea* can produce at least three distinct saponin-specific glycosidases (Quidde et al., 1999).

2.3. Cucurbitacins

Cucurbitacins are bitter-tasting triterpenoids, produced by the Cucurbitaceae, that are toxic to insects and mammals. There are at least six forms of cucurbitacins, one of which antagonises insect steroid responses (Dinan et al., 1997) while another form disrupts the cytoskeleton in carcinoma cells (Duncan et al., 1996). Application of cucurbitacin I to host tissue prior to inoculation prevented infection by *B. cinerea* (Bar-Nun and Mayer, 1990). When cucurbitacin was added to a *B. cinerea* liquid culture, laccase secretion into the medium was reduced (Bar-Nun and Mayer, 1989) while other enzymes were not affected (Viterbo et al., 1993a). Some cucurbitacin forms were more effective than others (Viterbo et al., 1993b). Radio-labelled cucurbitacin was taken up by *B. cinerea* mycelium suggesting that it acted intracellularly, reducing laccase activity (Viterbo et al., 1994), supposedly as a consequence of repression at the mRNA level (Gonen et al., 1996). These gene

expression studies were however performed with a probe corresponding to the *Bclcc*1 gene, whereas the laccase gene that is induced by gallic acid is in fact *Bclcc*2 (Schouten et al., 2002). The two genes show poor cross-hybridisation (A. Schouten, unpubl.). Neither of these laccase genes is important for virulence on a range of hosts (Schouten et al., 2002). It remains to be resolved whether the reduction of *B. cinerea* infection by application of cucurbitacins (Bar-Nun and Mayer, 1990) is caused indirectly by activation of defence or directly by inhibiting virulence factors.

2.4. Proanthocyanidins

Proanthocyanidins (condensed tannins) are polymeric flavonoids that result from the condensation of two or more derivatives of flavan-3,4-diol. The term was first coined by Weinges et al. (1969) to designate these colourless tannins that form intensely coloured anthocyanidins upon heating with acid. Proanthocyanidins are widely distributed in the plant kingdom and are constitutive components in a number of discrete tissues in most plant organs. In leaves, they are mostly present in vascular tissue and in fruit they preferentially accumulate in the epidermis and seeds (Porter and Schwartz, 1962; Bachmann and Blaich, 1979; Hills et al., 1981; Jersch et al., 1989; Prieur et al., 1994; Prusky, 1996; Souquet et al., 1996). The chemical structure and composition of proanthocyanidins vary among plant species, organs and also with the stage of organ development. In grape berries, the mean degree of polymerisation of the proanthocyanidins, the proportion of epigallocatechin extension subunits and the level of anthocyanin association increase during ripening (Hills et al., 1981; Kennedy et al., 2001).

Because of the potential association of proanthocyanidins with sugars (including pectins), flavonoids and proteins (Kennedy et al., 2001), reports on their toxicity towards *B. cinerea* (conidia germination and germ tube elongation) have yielded opposite results depending on the state of purity of the proanthocyanidins (Hills et al., 1981; Jersch et al., 1989; Hebert et al., 2002). Nevertheless, these studies agree in suggesting that plant proanthocyanidins maintain *B. cinerea* in a quiescent stage, leading to delayed development of symptoms. The transition from quiescence into expansion is triggered during host senescence or ripening and occurs at a less senescent or ripe stage in susceptible varieties (reviewed by Elad, 1997). For example, grapes develop latent infections as early as the blooming stage (McClellan and Hewitt, 1973) while lesions are increasingly likely to start spreading only when the fruit ripens (Hills et al., 1981). The development of quiescent *B. cinerea* infections on grape did not correlate with conidia density (Coertze and Holz, 1997), the stage of ripening (Hills et al., 1981) or the level of resistance of the cultivar to grey mould (Pezet et al., 2003b). Even though the production of phytoalexins after pathogen entry is one way to keep *B. cinerea* quiescent (Sect. 2.1), some fruit, such as strawberries, do not mount a phytoalexin response (Jersch et al., 1989), implying that fungal quiescence can be maintained by additional mechanisms. These may involve the inhibition of fungal pathogenicity factors by proanthocyanidins (Prusky, 1996). *B. cinerea* virulence factors include a range of secreted enzymes (Chapter 7) and proanthocyanidins can complex with proteins (Haslam, 1966, 1974) resulting in non-specific inhibition of the activity of fungal polygalacturonases and

cellulases (Porter and Schwartz, 1962; Hills et al., 1981; Jersch et al., 1989). Proanthocyanidins additionally act as competitive inhibitors of *B. cinerea* laccase, thereby preventing detoxification of the phytoalexin pterostilbene. Pezet et al. (1992) reported an EC_{50} value of 12 µg/ml for the inhibition of *B. cinerea* laccase by grape skin proanthocyanidins. Their levels in grape skin vary from 50 to 250 µg/g dry weight (Hills et al., 1981; Pezet and Pont, 1992) and 14-50 µg/g fresh weight in strawberry (Jersch et al., 1989) depending on the cultivar or the stage of ripening.

Despite the fact that proanthocyanidins do not accumulate in all plant tissues, it is suggested that endogenous proanthocyanidins contribute to maintaining *B. cinerea* in a quiescent state. More tolerant grape and strawberry cultivars accumulate larger quantities of proanthocyanidins (Hebert et al., 2002; Pezet et al., 2003b). Proanthocyanidin content decreases in grape during fruit ripening (Hills et al., 1981) while they remain constant in strawberries (Jersch et al., 1989). However, the ripening-related modifications of proanthocyanidins led to a decrease in their capacity to inhibit *B. cinerea* macerating enzymes (Hills et al., 1981; Jersch et al., 1989; Pezet et al., 1992; Pezet and Pont, 1992). This phenomenon parallels the loss of resistance of these fruit during ripening (Hills et al., 1981; Jersch et al., 1989) and occurs to a larger extent in susceptible cultivars (Pezet et al., 2003b). Finally, the addition of proanthocyanidin at 0.1% at the point of inoculation blocked the development of spreading lesions of *B. cinerea* in susceptible grapevine varieties (Hills et al., 1981).

Prolonging the quiescence of *B. cinerea* infections by increasing the proanthocyanidin content would reduce losses to grey mould, especially after harvest. However, proanthocyanidin levels are constitutive and are not known to be subject to modulation by external elicitors. Moreover, knowledge is lacking on the genes and enzymes involved in the subtle modifications of proanthocyanidin structure that affect their biological activity. The use of proanthocyanidin content as an indicator of grey mould resistance for the selection of cultivars with improved shelf-life has been suggested for grape (Pezet et al., 2003b) and strawberry (Jersch et al., 1989; Hebert et al., 2002). However, proanthocyanidins negatively affect the taste and colour (via anthocyanin binding) of fresh produce and plant-derived food products (Noble, 1990; Gawel, 1998). Attempts to modify their composition may result in a modification of the perception of these products by consumers.

2.5. Non-host resistance

2.5.1. Phytoanticipins of tulip as mediators of *Botrytis* non-host resistance

Tulip bulbs and pistils contain high concentrations of fungitoxic compounds, identified as lactones and termed tulipalins (Bergman et al., 1967; Beijersbergen, 1969). Tulipalin A is found in bulbs, whereas aerial plant parts contain mainly tulipalin B. Tulipalins A and B are stored in a glycosylated form, named tuliposides A and B. Tuliposides are less toxic to fungi than the corresponding aglycosylated lactones and are lactonised into tulipalins when the pH exceeds 5. Plant enzymes may enhance the conversion rate of tuliposides into tulipalin A (Beijersbergen and Lemmers, 1972). The lactone itself is relatively stable *in vitro* at pH values between 5.4 and 6.5 (Beijersbergen, 1969). It was suggested that precursor tuliposides, stored

in acidic vacuoles, are released upon disruption of the vacuolar membranes during cell collapse mediated by pathogens (Schönbeck and Schlösser, 1976). The lactones themselves spontaneously hydrolyse at pH values above 7.5 into the corresponding non-toxic butyric acids (Beijersbergen, 1969).

All *Botrytis* species tested so far are sensitive to pure tulipalin A, except for the tulip-specific pathogen *B. tulipae* (Schönbeck and Schroeder, 1972). Partial or complete fungistasis occurs *in vitro* at 3-5 µM. Higher doses (7-10 µM, more than 30 µM for *B. tulipae*) are lethal to *Botrytis* spp. Mycelium exposed to lethal tulipalin A concentrations does not resume growth when transferred to fresh agar without tulipalin. Microscopic observation of tulipalin A-treated mycelium showed a disappearance of hyphal cell contents and altered autofluorescence of hyphal walls as compared to vital cultures. Conidia are at least three times more sensitive to tulipalin A than mycelium (P. van Baarlen and M. Staats, unpubl.). *B. tulipae* is more tolerant to tulipalin A than other *Botrytis* species. Infection of tulips by *B. tulipae* is associated with a conversion of tuliposides into the corresponding, inactive hydroxylic acids. In contrast, infection by *B. cinerea* leads to a conversion into the active lactones (Schönbeck and Schroeder, 1972). This suggests that *B. tulipae* contains a factor that can hydrolyse the tuliposides or the lactones into the corresponding hydroxylic acids. Indeed, a total protein extract from *B. tulipae* mycelium was able to neutralise tulipalin A; incubation of tulipalin A with this protein extract resulted in loss of toxicity towards sensitive *B. cinerea* isolates (P. van Baarlen and M. Staats, unpubl.). Lactone detoxification was also reported in *Fusarium oxysporum* isolates that produce a lactonohydrolase (Shimizu et al., 1992). It is tempting to speculate that *B. tulipae* possesses an enzyme with similar activity. Such an enzyme might act as a host specificity determinant for *B. tulipae*.

2.5.2. Other monocot secondary metabolites involved in non-host resistance

The genus *Allium* contains various compounds associated with resistance to fungal disease. Some are constitutive inhibitors, such as the phenolic compound catechol that is present in the outer bulb layers of pigmented *Allium cepa* (onion) cultivars where it confers resistance to *Colletotrichum* (Link and Walker, 1933). Onion also produces a class of cyclopentane phytoalexins upon pathogen infection, designated tsibulins (Dmitriev et al., 1990), which accumulate in bulb scales at infection sites during incompatible interactions with *B. cinerea.* Tsibulins inhibited spore germination and germ tube elongation of *B. cinerea in vitro*. The ED_{50} values were lower than the actual phytoalexin content in bulb scale spots, where *B. cinerea* lesion formation was restricted (Dmitriev et al., 1990). Only little accumulation of cyclopentane phytoalexins was observed in onion bulb scales during infection by the specialised pathogen *B. allii* (Dmitriev et al., 1990). *B. allii* seems to either suppress tsibulin accumulation, analogous to the interaction of *B. narcissicola* and its host narcissus (discussed below) or actively degrade tsibulins, as discussed for other antifungal compounds above. It would be of interest to investigate whether *B. allii* is able to break down tsibulin cyclopentanes.

3. Tolerance of *Botrytis* to antifungal metabolites

In many cases *Botrytis* species have adapted to antifungal compounds produced by their host plants by developing mechanisms to counteract their toxicity. In fact the ability to counteract toxicity of phytoalexins and phytoanticipins is often crucial for successful host colonization. As a first line of defence to toxic compounds, fungi possess ATP-binding cassette transporters (ATR) and Major Facilitator (MFS) proteins that are able to excrete a spectrum of chemically unrelated toxic metabolites from the cytoplasm. ATR and MFS proteins serve as membrane pumps with a broad substrate range that expel chemically heterogeneous antifungal compounds at the expense of ATP or proton extrusion (reviewed by de Waard, 1997). These proteins may also be involved in fungicide resistance (Chapter 12). *B. cinerea* possesses a large family of functional transporter genes, some of which confer protection to antibiotics (Schoonbeek et al., 2002) and plant defence metabolites including resveratrol (Schoonbeek et al., 2001). The degree of tolerance that ATR and MFS transporters confer towards these toxicants is in several cases small, only rarely do they provide full resistance. This low degree of tolerance may however be biologically significant as it allows the pathogen time to activate true enzymatic detoxification mechanisms. Such true detoxification mechanisms are specific for each individual antifungal compound, as discussed in Sect. 2.

4. Structural barriers and cell wall modifications

Structural barriers can be mounted during resistance responses and non-specific wound responses (Heath, 2000, 2002) by means of incorporating and cross-linking phenolic compounds at penetration sites. Cell wall modifications may directly pose physical barriers for fungi and they may interfere with degradation of wall components that function as nutrient sources for *Botrytis* species. One form of plant cell wall modification associated with disease resistance is their lignification. The phenylpropanoid pathway generates coumaryl Co-A and cinnamyl Co-A esters that serve as precursors for diverse compounds, including lignin (Dixon and Paiva, 1995). Wall modification occurs in the incompatible interaction of *B. cinerea* with a non-host, wheat. Challenging wheat leaves with *B. cinerea* conidia results in a localised, sharp increase of sinapyl alcohol dehydrogenase, phenylalanine ammonia lyase and peroxidase expression and progressive lignification (Mitchell et al., 1994). The phenylpropanoid pathway also generates phytoalexins (discussed above). The simultaneous formation of structural barriers and phytoalexin production blocks the infection and lesion spread of *B. cinerea* during incompatible interactions with carrot, narcissus and wheat (Garrod et al., 1982; O'Neill and Mansfield, 1982; Mitchell et al., 1994). The relative contribution of wall modifications and phytoalexin production to the effective inhibition of infection is often unclear.

Other wall modifications are established by a class of phenolic amides, mainly hydroxycinnamic acid derivatives and tyramine (McLusky et al., 1999). The synthesis and incorporation in cell walls of amides is associated with the inhibition of outgrowth of *B. allii* germ tubes within modified infection sites (Stewart and Mansfield, 1985). The amides that are produced in onion cells are not fungitoxic to

B. allii and *B. cinerea* conidia and germ tubes up to concentrations of 1 mM (McLusky et al., 1999). The fact that amide synthesis and their incorporation into cell walls were associated with attempted penetrations of *B. allii*, a compatible pathogen of onions, suggests that this defence mechanism provides a general protection against *Botrytis*. The efficiency of the resistance mechanism may depend on spore concentration, H_2O_2 availability and occurrence of other stress factors.

The cell wall modification response (Heath, 2000) has been studied in lily and tulip cultivars in compatible and incompatible interactions, with *B. cinerea*, *B. elliptica* or *B. tulipae.* Cell wall modification was observed during incompatible interactions but not during compatible interactions (P. van Baarlen and J. van Kan, unpubl.). Autofluorescence of cell walls at penetration sites occurred within 18 h upon inoculation of tulip and lily leaves with dry conidia. Fluorescent vital staining of plant cells showed that in an incompatible interaction, the non-host plant cells were not killed upon expression of this resistance response. Germ tubes did not penetrate the modified walls any further. Primary lesion formation was not observed in incompatible interactions, suggesting that wall modifications can effectively restrict *Botrytis* infection. A more extensive study of wall modifications during compatible and incompatible plant-*Botrytis* interactions seems worthwhile, although it will remain difficult to distinguish the contribution to resistance of cell wall modification from that of the concomitant phytoalexin production.

Several methods by which *B. cinerea* could interfere with cell wall resistance responses may be envisaged. The pathogen may actively suppress resistance responses in a compatible interaction. Preliminary microscopy studies have shown that lily cells, at the sites of penetration by *B. elliptica,* did not show the characteristic local yellow cell wall autofluorescence observed in incompatible interactions. Also in the compatible interaction of narcissus and *B. narcissicola*, cell wall modifications were mostly absent (O'Neill and Mansfield, 1982). Both phytoalexin production and wall modification depend upon the phenylpropanoid phenolic acid pathway. It is conceivable that in a compatible plant-*Botrytis* interaction, the cell wall modifications and phytoalexin production are suppressed by (a) secreted fungal factor(s). One study has shown that during a compatible interaction of the cowpea rust fungus (*Uromyces vignae*) with its host, cell wall-associated defence responses are suppressed (Heath, 2002). In this pathosystem, suppression of defence is established through interference with wall-membrane communication via secreted fungal peptides that interfere with integrin proteins that mediate wall-membrane adhesion (Mellersh and Heath, 2001). We are, however, not aware of literature reporting active suppression of defence by *Botrytis*.

5. Pathogenesis-related proteins

Pathogenesis-related proteins (PR proteins) represent a large array of proteins coded by the host plant that are co-ordinately expressed under pathological or related situations. They have been characterised in over 70 plant species and 13 plant families including mono- and di-cotyledonous plants. They are extremely diverse in terms of enzymatic and biological activity and have been grouped into 13 protein families based on primary structure and serological relationships (Van Loon, 1999).

All share several physicochemical properties such as solubility in acidic buffers, resistance to proteolysis, a molecular mass less than 50 kDa and a lack of quaternary structure and glycosylation (Stintzi et al., 1993). They primarily accumulate in plant cell walls and vacuoles. *B. cinerea* infection leads to PR protein induction in many plants (Van Loon, 1985). Fruit tissues differ significantly from other plant organs by accumulating unusually high concentrations of a limited set of PR-like proteins that share sequence similarities with known PR proteins but accumulate with fruit development (most often during fruit ripening) and not after stimulation with pathogenesis-derived signals such as salicylic acid or wounding (Derckel et al., 1998). Grapes, for example, mainly accumulate one thaumatin-like protein and one chitinase which make up 80% of the total soluble protein content of the fruit at harvest (Derckel et al., 1998; Salzman et al., 1998; Waters et al., 1998).

Members of several PR protein families display some toxicity towards *B. cinerea in vitro*. For some of them, this may be caused by their potential to degrade chitin and β-glucan fragments of *B. cinerea* cell walls (Gomez-Miranda et al., 1981; Punja and Zhang, 1993; Simmons, 1994). However, fungitoxicity varies greatly among members of one PR protein family, just like their specific enzyme activity which may differ by up to 250-fold towards a given substrate (Kauffmann et al., 1987; Stintzi et al., 1993). A grape PR-like protein (chitinase) has one of the highest botryticidal activities. It inhibits germination of conidia with an EC_{50} value of 7.5 μg/ml (Derckel et al., 1998) and it restricts the elongation of hyphae (Salzman et al., 1998). The chitinase is present at levels up to 26 μg/g in fruit of resistant cultivars (Derckel et al., 1998; Salzman et al., 1998). The grape thaumatin-like protein, on the other hand, exhibited no toxicity towards *B. cinerea in vitro* (Salzman et al., 1998). In the presence of 1M glucose (a physiological concentration for a ripe grape), however, the toxicity of the grape chitinase was potentiated by as much as 70% and the grape thaumatin-like protein became equally toxic as the chitinase at similar concentrations (Salzman et al., 1998).

Despite their anti-microbial activity *in vitro*, there is little evidence to support a potential role of PR proteins in effective plant disease resistance to *B. cinerea*. The homologous or heterologous expression of PR proteins in transgenic plants, or the infiltration of single PR proteins in leaves, has rarely led to any significant level of protection against *B. cinerea* (Lawton et al., 1993; Datta et al., 1999; Neuhaus, 1999; Van Loon, 1997). Only the coordinated expression of members of different PR protein families yielded some (though limited) degree of tolerance due to synergistic effects (Zhu et al., 1994; Jach et al., 1995; Datta et al., 1999). Additionally, treatments that stimulate the coordinate expression of a large array of PR proteins in tomato leaves such as ethylene treatment (Diaz et al., 2002) or *B. cinerea* primary lesion formation (Benito et al., 1998) did not prevent the development of *B. cinerea* spreading lesions. To the contrary, a higher degree of susceptibility (higher fungal growth as estimated by the level of fungal actin mRNA) correlated with higher levels of PR proteins mRNAs (Diaz et al., 2002). In any case, the commercial potential of plants exhibiting higher levels of PR proteins will be hampered by the fact that PR proteins are associated with several undesirable effects such as the formation of haze in grape juices (Waters et al., 1996) and allergenic reactions (Breiteneder and Ebner, 2000; Hoffmann-Sommergruber, 2000).

6. Conclusions

There are interesting perspectives for altering secondary metabolism in plants to optimise the contents of antifungal compounds, either by transgenes or by classical breeding. The example of enhanced *Botrytis* resistance in transgenic tobacco producing resveratrol (Hain et al., 1993) already provides evidence for its feasibility. Now that the field of plant pathology and breeding has entered the genomics era, it will be increasingly feasible to modulate the levels of secondary metabolites, such as phenylpropanoids, to enhance defence (Dixon et al., 2002). The information on the *Botrytis* genome will be a useful tool. When plants can be equipped with antifungal metabolites for which *Botrytis* has no detoxifying enzymes, it is likely that these metabolites will be more effective. Conversely, when *Botrytis* possesses enzymes that may convert non-toxic metabolites into antifungal compounds (Schouten et al., 2002) one can perhaps make use of these enzymes. When considering the options for enhancing plant resistance to *Botrytis* by modulation of secondary metabolites, it should be taken into serious account that it is often not required to achieve complete resistance as conferred by the classical R-genes. Especially in the case of post-harvest problems, it will be sufficient to delay the disease outbreak and attenuate disease out-growth. We recommend the design of strategies that extend the latent infection phase. Chapter 20 deals in more detail with novel approaches to reduce the damage inflicted by *Botrytis* diseases.

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8. References

Adrian M, Jeandet P, Bessis R and Joubert JM (1996) Induction of phytoalexin (resveratrol) synthesis in grapevine leaves treated with aluminium chloride ($AlCl_3$). Journal of Agricultural and Food Chemistry 44: 1979-1981

Adrian M, Jeandet P, Douillet-Breuil A-C, Tesson L and Bessis R (2000) Stilbene content of mature *Vitis vinifera* berries in response to UV-C elicitation. Journal of Agricultural and Food Chemistry 48: 6103-6105

Adrian M, Jeandet P, Veneau J, Weston LA and Bessis R (1997) Biological activity of resveratrol, a stilbene compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. Journal of Chemical Ecology 23: 1689-1702

Adrian M, Rajaei H, Jeandet P, Veneau J and Bessis R (1998) Resveratrol oxidation in *Botrytis cinerea* conidia. Phytopathology 88: 472-476

Ayran A, Wilson B, Strauss C and Williams P (1987) The properties of glucosides of *Vitis vinifera* and the comparison of their β-D-glucosidase activity with that of exogenous enzymes. An assessment of possible application of enology. American Journal of Enology and Viticulture 38: 182-188

Bachmann O and Blaich R (1979) Vorkommen und Eigenschaften kondensierter Tannine in *Vitaceae*. Vitis 18: 106-116

Bais AJ, Murphy PJ and Dry IB (2000) The molecular regulation of stilbene phytoalexin biosynthesis in *Vitis vinifera* during grape berry development. Australian Journal of Plant Physiology 27: 425-433

Ban T, Shiozaki S, Ogata T and Horiuchi S (2000) Effect of abscisic acid and shading treatment on the levels of anthocyanin and resveratrol in skin of Kyoho grape berry. Acta Horticulturae No. 514: 83-89

Bar-Nun N and Mayer AM (1989) Cucurbitacins - repressors of induction of laccase formation. Phytochemistry 28: 1369-1371

Bar-Nun N and Mayer AM (1990) Cucurbitacins protect cucumber tissue against infection by *Botrytis cinerea*. Phytochemistry 29: 787-791

Bavaresco L, Pezzutto S, Ragga A, Ferrari F and Trevisan M (2001) Effect of nitrogen supply on *trans*-resveratrol concentration in berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. Vitis 40: 229-230

Beijersbergen, JCM (1969) α-methylene-γ-butyrolactone uit tulpen. Thesis, Leiden University (pp. 1-93) Bronder-Offset, Rotterdam, The Netherlands

Beijersbergen, JCM and Lemmers CBG (1972) Enzymic and non-enzymic liberation of tulipalin A (α-methylene-γ-butyrolactone) in extracts of tulip. Physiological Plant Pathology 2: 265-270

Benito EP, ten Have A, van't Klooster JW and Van Kan JAL (1998) Fungal and plant gene expression during synchronized infection of tomato leaves by *Botrytis cinerea*. European Journal of Plant Pathology 104: 207-220

Bergman, BHH, Beijersbergen JCM, Overeem JC and Kaars Sijpesteijn A (1967) Isolation and identification of α-methylene-γ-butyrolactone, a fungitoxic substance from tulips. Recueil des Travaux Chimiques des Pays-Bas 86: 709-714

Breiteneder H and Ebner C (2000) Molecular and biochemical classification of plant-derived food allergens. Journal of Allergy and Clinical Immunology 106: 27-36

Breuil AC, Adrian M, Pirio N, Meunier P, Bessis R and Jeandet P (1998) Metabolism of stilbene phytoalexins by *Botrytis cinerea*: 1. Characterization of a resveratrol dehydrodimer. Tetrahedron Letters 39: 537-540

Breuil AC, Jeandet P, Adrian M, Chopin F, Pirio N, Meunier P and Bessis R (1999) Characterization of a pterostilbene dehydrodimer produced by laccase of *Botrytis cinerea*. Phytopathology 89: 298-302

Busam G, Junghanns KT, Kneusel RE, Kassemyer HH and Matern U (1997) Characterization and expression of caffeoyl-coenzyme A 3-O-methyltransferase proposed for the induced resistance response of *Vitis vinifera* L. Plant Physiology 115: 1039-1048

Cantos E, Espin JC and Tomas-Barberan FGA (2001) Postharvest induction modelling method using UV irradiation pulses for obtaining resveratrol-enriched table grapes: a new "functional" fruit. Journal of Agricultural and Food Chemistry 49: 5052-5058

Chiou RYY (2002) Resveratrol, a promising phytochemical in grape juices, wines and peanuts. Food Science and Agricultural Chemistry 4: 8-14

Chung IM, Park MR, Chun JC and Yun SJ (2003) Resveratrol accumulation and resveratrol synthase gene expression in response to abiotic stresses and hormone in peanut plants. Plant Science 164: 103-109

Cichewicz RH, Kouzi SA and Hamann MT (2000) Dimerisation of resveratrol by grapevine pathogen *Botrytis cinerea*. Journal of Natural Products 63: 29-33

Coertze S and Holz G (1999) Surface colonisation, penetration and lesion formation on grapes inoculated fresh and after cold storage with single airborne conidia of *Botrytis cinerea*. Plant Disease 83: 917-924

Creasy LL and Coffee M (1988) Phytoalexin production potential of grape berries. Journal of the American Society for Horticultural Science 113: 230-234

Creasy LL and Creasy MT (1998) Grape chemistry and the significance of resveratrol: an overview. Pharmaceutical Biology 36 (supplement): 8-13

Daniel M and Purkayastha RP (1995) Handbook of Phytoalexin Metabolism and Action. Marcel Dekker, New York, USA

Datta K, Muthukrishnan S and Datta SK (1999) Expression and function of PR-protein genes in transgenic plants. In: Datta SK and Muthukrishnan S (eds) Pathogenesis-related Proteins in Plants. (pp. 261-278) CRC Press, Boca Raton, Florida, USA

Derckel JP, Audran JC, Haye B, Lambert B and Legendre L (1998) Characterization, induction by wounding and salicylic acid, and activity against *Botrytis cinerea* of chitinases and beta-1,3-glucanases of ripening grape berries. Physiologia Plantarum 104: 56-64

Derckel JP, Baillieul F, Manteau S, Audran JC, Haye B, Lambert B and Legendre L (1999) Differential induction of grapevine defences by two strains of *Botrytis cinerea*. Phytopathology 89: 197-203

De Waard MA (1997) Significance of ABC transporters in fungicide sensitivity and resistance. Pesticide Science 51: 271-275

Diaz J, Ten Have A and Van Kan JAL (2002) The role of ethylene and wound signalling in resistance of tomato to *Botrytis cinerea*. Plant Physiology 129: 1341-1351

Dinan L, Whiting P, Girault JP, Lafont R, Dhadialla TS, Cress DE, Mugat B, Antoniewski C and Lepesant JA (1997) Cucurbitacins are insect steroid hormone antagonists acting at the ecdysteroid receptor. Biochemical Journal 327: 643-650

Dixon RA and Paiva NL (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7: 1085-1097

Dixon RA, Achnine L, Kota P, Liu CJ, Reddy MSS and Wang LJ (2002) The phenylpropanoid pathway and plant defence - a genomics perspective. Molecular Plant Pathology 3: 371-390

Dmitriev AP, Tverskoy LA, Kozlovsky AG and Grodzinsky DM (1990) Phytoalexins from onion and their role in disease resistance. Physiological and Molecular Plant Pathology 37: 235-244

Douillet-Breuil A-C, Jeandet P, Adrian M and Bessis R (1999) Changes in the phytoalexin content of various *Vitis* spp. in response to ultraviolet C elicitation. Journal of Agricultural and Food Chemistry 47: 4456-4461

Duncan KLK, Duncan MD, Alley MC and Sausville E (1996) Cucurbitacin E-induced disruption of the actin and vimentin cytoskeleton in prostate carcinoma cells. Biochemical Pharmacology 52 1553-1560

Elad Y (1997) Responses of plants to infection by *Botrytis cinerea* and novel means involved in reducing their susceptibility to infection. Biological Reviews 72: 381-422

Fregoni C, Bavaresco L, Cantu E, Petegolli D, Vizzon D, Chiusa G and Trevisan M (2000) Advances in understanding stilbene (resveratrol, epsilon-viniferin) – grapevine relationship. Acta Horticulturae No. 526: 467-477

Friedman M (2002) Tomato glycoalkaloids: role in the plant and in the diet. Journal of Agricultural and Food Chemistry 50: 5751-5780

Garrod, B, Lewis BG, Brittain MJ and Davies WP (1982) Studies on the contribution of lignin and suberin to the impedance of wounded carrot root tissue to fungal invasion. New Phytologist 90: 99-108

Gawel R (1998) Red wine astringency: A review. Australian Journal of Grape and Wine Research 4: 74-96

Gomez-Miranda B, Ruperez P and Leal A (1981) Changes in chemical composition during germination of *Botrytis cinerea* sclerotia. Current Microbiology 6: 243-246

Gonen L, Viterbo A, Cantone F, Staples RC and Mayer AM (1996) Effect of cucurbitacins on mRNA coding for laccase in *Botrytis cinerea*. Phytochemistry 42: 321-324

Grimmig B, Schubert R, Fischer R, Hain R, Schreier PH, Betz C, Langebartels C, Ernst D and Sandermann HJr (1997) Ozone- and ethylene-induced regulation of a grapevine resveratrol synthase promoter in transgenic tobacco. Acta Physiologiae Plantarum No. 19: 467-474

Hain R, Reif H-J, Krause E, Langebartels R, Kindl H, Vorman B, Wiese W, Schmeltzer E, Schreider PH, Stocker RH and Stenzel K (1993) Disease resistance results from foreign phytoalexin expression in a novel plant. Nature 361: 153-156

Hart JH (1981) Role of phytostilbenes in decay and disease resistance. Annual Review of Phytopathology 19: 437-458

Haslam E (1966) Chemistry of Vegetable Tannins. Academic Press, London, UK

Haslam E (1974) Polyphenol-protein interactions. Biochemical Journal 139: 285-288

Heath MC (2000) Nonhost resistance and nonspecific plant defenses. Current Opinion in Plant Biology 3: 315-319

Heath MC (2002) Cellular interactions between biotrophic fungal pathogens and host or non-host plants. Canadian Journal of Plant Pathology 24: 259-264

Hebert C, Charles MT, Willemot C, Gauthier L, Khanizadeh S and Cousineau J (2002) Strawberry proanthocyanidins: biochemical markers for *Botrytis cinerea* resistance and shelf-life predictability. Acta Horticulturae No. 567: 659-662

Hills G, Stellwaag-Kittler F, Huth G and Schlösser E (1981) Resistance of grapes in different developmental stages to *Botrytis cinerea*. Journal of Phytopathology 102: 328-338

Hoffmann-Sommergruber K (2000) Plant allergens and pathogenesis-related proteins – what do they have in common? International Archives of Allergy and Immunology 122: 155-166

Hoos G and Blaich R (1990) Influence of resveratrol on germination of conidia and mycelial growth of *Botrytis cinerea* and *Phomopsis viticola*. Journal of Phytopathology 129: 102-110

Hung LM, Chen JK, Huang SS, Lee RS and Su MJ (2000) Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovascular Research 47: 549-555

Jach G, Gornhardt B, Mundy J, Logemann J, Pinsdorf E, Leah R, Schell J and Maas C (1995) Enhanced quantitative resistance against fungal disease by combinatorial expression of different barley antifungal proteins in transgenic tobacco. Plant Journal 8: 97-109

Jeandet P, Bessis R and Gautheron B (1991) The production of resveratrol (3,5,4'-trihydroxystilbene) by grape berries in different developmental stages. American Journal of Enology and Viticulture 42: 41-46

Jeandet P, Bessis R, Sbaghi M and Meunier P (1995a) Production of the phytoalexin resveratrol by grapes as a response to *Botrytis* attack under natural conditions. Journal of Phytopathology 143: 135-139

Jeandet P, Sbaghi M, Bessis R and Meunier P (1995b) The potential relationship of stilbene (resveratrol) synthesis to anthocyanin content in grape berry skins. Vitis 34: 91-94

Jeandet P, Adrian M, Breuil AC, Sbaghi M, Joubert JM, Weston LA, Harmon R and Bessis R (1998) Chemical stimulation of phytoalexin synthesis in plants as an approach to crop protection. Recent Research Developments in Agricultural Food Chemistry 2: 501-511

Jersch S, Scherer C, Huth G and Schlösser E (1989) Proanthocyanidins as basis for quiescence of *Botrytis cinerea* in immature strawberry fruits. Journal of Plant Disease and Protection 96: 365-378

Kauffmann S, Legrand M, Geoffroy P and Fritig B (1987) Biological function of "pathogenesis-related" proteins: four PR proteins of tobacco have 1,3-beta-glucanase activity. EMBO Journal 6: 3209-3216

Keller M, Viret O, and Cole FM (2003) *Botrytis cinerea* infection in grape flowers: defence reaction, latency and disease expression. Phytopathology 93: 316-322

Kennedy JA, Hayasaka Y, Vidal S, Waters EJ and Jones GP (2001) Composition of grape skin proanthocyanidins at different stages of berry development. Journal of Agricultural and Food Chemistry 49: 5348-5355

Keukens EAJ, De Vrije T, Van den Boom C, De Waard P, Plasman HH, Thiel F, Chupin V, Jongen WMF and De Kruijff B (1995) Molecular basis of glycoalkaloid induced membrane disruption. Biochimica et Biophysica Acta 1240: 216-228

Kobayashi S, Ding CK, Nakamura Y, Nakajima I and Matsumoto R (2000) Kiwifruits (*Actinidia deliciosa*) transformed with a *Vitis* stilbene synthase gene produce piceid (resveratrol-glucoside). Plant Cell Reports 19: 904-910

Kowalski SP, Domek JM, Sanford LL and Deahl KL (2000) Effect of alpha-tomatine and tomatidine on the growth and development of the Colorado potato beetle (Coleoptera: Chrysomelidae): studies using synthetic diets. Journal of Entomological Science 35: 290-300

Langcake P (1981) Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, epsilon-viniferin, alpha-viniferin and pterostilbene. Physiological Plant Pathology 18: 213-226

Langcake P and McCarthy WV (1979) The relationship between resveratrol production to infection of grapevine leaves by *Botrytis cinerea*. Vitis 18: 244-253

Langcake P and Pryce RJ (1976) The production of resveratrol by *Vitis vinifera* and other members of the *Vitaceae* as a response to infection or injury. Physiological Plant Pathology 9: 77-86

Langcake P and Pryce RJ (1977a) A new class of phytoalexins from grapevine. Experientia 33: 151-152

Langcake P and Pryce RJ (1977b) The production of resveratrol and the viniferins by grapevines in response to ultraviolet irradiation. Phytochemistry 16: 1193-1196

Langcake P and Pryce RJ (1977c) Oxidative dimerisation of 4-hydroxystilbenes *in vitro*: production of a grapevine phytoalexin mimic. Journal of the Chemical Society, Chemical Communications 7: 208-210

Langcake P, Cornford CA and Pryce RJ (1979) Identification of pterostilbene as a phytoalexin from *Vitis vinifera* leaves. Phytochemistry 18: 1025-1027

Larronde F, Gaudillere JP, Krisa S, Decendit A, Deffieux G, Merillon JM (2003) Air-borne methyl jasmonate induces stilbene accumulation in leaves and berries of grapevine plants. American Journal of Enology and Viticulture 54: 63-66

Lawton K, Uknes S, Friedrich L, Gaffney T, Alexander D, Goodman R, Metraux JP, Kessmann H, Ahl Goy P, Gut Rella M, Ward E and Ryals J (1993) The molecular biology of systemic acquired resistance. In: Fritig B and Legrand M (eds) Mechanisms of Plant defence Responses. (pp. 422-467) Kluwer Academic Publisher, Dordrecht, The Netherlands

Leckband G and Lorz H (1998) Transformation and expression of a stilbene synthase gene of *Vitis vinifera* L. in barley and wheat for increased fungal resistance. Theoretical and Applied Genetics 96: 1004-1012

Link, KP and Walker JC (1933) The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. Journal of Biological Chemistry 100: 379-383

Lippmann B, Mascher R, Balko C and Bergmann H (2000) UV induction of *trans*-resveratrol biosynthesis in the leaves of greenhouse- and *in vitro*-grown potatoes (*Solanum tuberosum* L.). Journal of Applied Botany 74: 160-163

Liswidowati FM, Melchior F, Hohmann F, Schwer B and Kindl H (1991) Induction of stilbene by *Botrytis cinerea* in cultured grapevine cells. Planta 183: 307-314

Magee JB, Smith BJ and Rimando A (2002) Resveratrol content of muscadine berries is affected by disease control spray program. HortScience 37: 358-361

Mansfield JW (1980) Mechanisms of resistance to Botrytis. In: Coley-Smith JR, Verhoeff K and Jarvis WR (eds) The Biology of *Botrytis*. (pp. 181-218) Academic Press, London, UK

Manteau S, Abouna S, Lambert B and Legendre L (2003) Differential regulation by ambient pH of putative virulence factor secretion by the phytopathogenic fungus *Botrytis cinerea*. FEMS Microbiology Ecology 43: 359-366

McClellan WD and Hewitt WB (1973) Early *Botrytis* rot of grapes: time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. Phytopathology 63: 1151-1157

McLusky, SR, Bennett MH, Beale MH, Lewis MJ, Gaskin P and Mansfield JW (1999) Cell wall alterations and localized accumulation of feruloyl-3'-methoxytyramine in onion epidermis at sites of attempted penetration by *Botrytis allii* are associated with actin polarisation, peroxidase activity and suppression of flavonoid biosynthesis. Plant Journal 17: 523-534

Mellersh DG and Heath MC (2001) Plasma membrane-cell wall adhesion is required for expression of plant defense responses during fungal penetration. Plant Cell 13: 413-424

Mitchell HJ, Hall JL and Barber MS (1994) Elicitor-induced cinnamyl alcohol dehydrogenase activity in lignifying wheat (*Triticum aestivum* L.) leaves. Plant Physiology 104: 551-556

Montero C, Cristescu SM, Jimenez JB, Orea JM, Te Lintel Hekkert S, Harren FJM and Gonzalez Urena A (2003) *Trans*-resveratrol and grape disease resistance. A dynamic study by high-resolution laser based techniques. Plant Physiology 131: 129-138

Morales M, Alcantara J and Ros Barcelo A (1997) Oxidation of *trans*-resveratrol by a hypodermal peroxidase isoenzyme from gamay rouge grape (*Vitis vinifera*) berries. American Journal of Enology and Viticulture 48: 33-38

Morales M, Ros Barcelo A and Pedreno MA (2000) Plant stilbenes: recent advances in their chemistry and biology. In: Hemantaranjan A (ed.) Advances in Plant Physiology. Vol. 3 (pp. 39-70) Scientific Publishers, Jodhpur, India

Neuhaus JM (1999) Plant chitinases (PR-3, PR-4, PR-8, PR-11). In: Datta SK and Muthukrishnan S (eds) Pathogenesis-related Proteins in Plants. (pp. 261-278) CRC Press, Boca Raton, Florida, USA

Noble AC (1990) Bitterness and astringency in wine. In: Rouseff RL (ed.) Developments in Food Science 25. Bitterness in Foods and Beverages. (pp. 145-158) Elsevier, New York, NY, USA

O'Neill TM and Mansfield JW (1982) Mechanisms of resistance to *Botrytis* in narcissus bulbs. Physiological Plant Pathology 20: 243-256

Paul B, Chereyathmanjiyil A, Masih I, Chapuis L and Benoit A (1998) Biological control of *Botrytis cinerea* grey mould disease of grapevine and elicitation of stilbene phytoalexin (resveratrol) by a soil bacterium. FEMS Microbiology Letters 165: 65-70

Pezet R (1998) Purification and characterization of a 32-kDa laccase-like stilbene oxidase produced by *Botrytis cinerea* Pers.:Fr. FEMS Microbiology Letters 167: 203-208

Pezet R and Pont V (1988a) Mise en évidence de ptérostilbène dans les grappes de *Vitis vinifera*. Plant Physiology and Biochemistry 26: 603-607

Pezet R and Pont V (1988b) Activité antifongique dans les baies de *Vitis vinifera*: effets d'acides organique et du ptérostilbène. Revue Suisse de Viticulture et d'Arboriculture Horticole 20: 303-309

Pezet R and Pont V (1992) Differing biochemical and histological studies of two grape cultivars in the view of their respective susceptibility and resistance to *Botrytis cinerea*. In: Verhoeff K, Malathrakis NE and Williamson B (eds) Recent Advances in *Botrytis* Research. (pp. 93-98) Pudoc Scientific Publishers, Wageningen, The Netherlands

Pezet R and Pont V (1995) Mode of toxic action of Vitaceae stilbenes on fungal cells. In: Daniel M and Purkayastha RP (eds) Handbook of Phytoalexin Metabolism and Action. (pp. 317-331) Marcel Dekker Inc., New York, USA

Pezet R, Pont V and Hoang-Van K (1991) Evidence for oxidative detoxification of pterostilbene and resveratrol by a laccase-like stilbene oxidase produced by *Botrytis cinerea*. Physiological and Molecular Plant Pathology 39: 441-450

Pezet R, Pont V and Hoang-Van K (1992) Enzymatic detoxification of stilbenes by *Botrytis cinerea* and inhibition by grape berries proanthocyanidins. In: Verhoeff K, Malathrakis NE and Williamson B (eds) Recent Advances in *Botrytis* Research. (pp. 87-92) Pudoc Scientific Publishers, Wageningen, The Netherlands

Pezet R, Perret C, Jean-Denis JB, Tabacchi R, Gindro K and Viret O (2003a) δ-viniferin, a resveratrol dehydrodimer: one of the major stilbenes synthesized by stressed grapevine leaves. Journal of Agricultural and Food Chemistry 51: 5488-5492

Pezet R, Viret O, Perret C and Tabacchi R (2003b) Latency of *Botrytis cinerea* Pers.: Fr. and biochemical studies during growth and ripening of two grape cultivars, respectively susceptible and resistant to grey mould. Journal of Phytopathology 151: 208-214

Pont V and Pezet R (1990) Relationship between the chemical structure and the biological activity of hydroxystilbenes against *Botrytis cinerea*. Journal of Phytopathology 130: 1-8

Pool RM, Creasy LL and Frackelton AS (1981) Resveratrol and the viniferins, their application to screening for disease resistance in grape breeding programs. Vitis 20: 136-145

Porter WL and Schwartz JH (1962) Isolation and description of the pectinase-inhibiting tannins of grape leaves. Journal of Food Science 27: 416-418

Prieur C, Rigaud J, Cheynier V and Moutounet M (1994) Oligomeric and polymeric procyanidins from grape seeds. Phytochemistry 36: 781-784

Prusky D (1996) Pathogen quiescence in postharvest diseases. Annual Review of Phytopathology 34: 413-434

Punja ZK and Zhang YY (1993) Plant chitinases and their roles in resistance to fungal diseases. Journal of Nematology 25: 526-540

Quidde T, Osbourn AE and Tudzynski P (1998) Detoxification of alpha-tomatine by *Botrytis cinerea*. Physiological and Molecular Plant Pathology 52: 151-165

Quidde T, Büttner P and Tudzynski P (1999) Evidence for three different specific saponin-detoxifying activities in *Botrytis cinerea* and cloning and functional analysis of a gene coding for a putative avenacinase. European Journal of Plant Pathology 105: 273-283

Roggero JP and Garcia-Parrilla C (1995) Effect of ultraviolet irradiation on resveratrol and changes in resveratrol and various of its derivatives in the skins of ripening grapes. Sciences des Aliments 15: 411-422

Romero-Perez AI, Ibern-Gomez M, Lamuela-Raventos RM and de la Torre-Boronat MC (1999) Piceid, the major resveratrol derivative in grape juices. Journal of Agricultural and Food Chemistry 47: 1533-1536

Roudet J, Prudet S and Dubos B (1992) Relationship between grey mould of grapes and laccase activity in the must. In: Verhoeff K, Malathrakis NE and Williamson B (eds) Recent Advances in *Botrytis* Research. (pp. 83-86) Pudoc Scientific Publishers, Wageningen, The Netherlands

Salzman RA, Tikhonova I, Bordelon BP, Hasegawa PM and Bressan RA (1998) Coordinate accumulation of antifungal proteins and hexoses constitutes a developmentally controlled defence response during fruit ripening in grapes. Plant Physiology 117: 465-472

Sandrock RW and VanEtten HD (1998) Fungal sensitivity to and enzymatic degradation of the phytoanticipin α-tomatine. Phytopathology 88: 137-143

Savouret JF and Quesne M (2002) Resveratrol and cancer: a review. Biomedicine and Pharmacotherapy 56: 84-87

Sbaghi M, Jeandet P, Faivre B, Bessis R and Fournioux JC (1995) Development of methods using phytoalexin (resveratrol) assessment as a selection criterion to screen grapevine *in vitro* cultures for resistance to grey mould (*Botrytis cinerea*). Euphytica 86: 41-47

Sbaghi M, Jeandet P, Bessis R and Leroux P (1996) Degradation of stilbene-type phytoalexins in relation to the pathogenicity of *Botrytis cinerea* to grapevine. Plant Pathology 45: 139-144

Schönbeck, F and Schroeder C (1972) Role of antimicrobial substances (tuliposides) in tulips attacked by *Botrytis* spp. Physiological Plant Pathology 2: 91-99

Schönbeck F and Schlösser E (1976). Preformed substances as potential protectants. In: Heitefuss R and Williams PH (eds) Physiological Plant Pathology. (pp. 653-678) Springer-Verlag, Berlin, Heidelberg, New York

Schoonbeek H, Del Sorbo G and de Waard MA (2001) The ABC transporter BcatrB affects the sensitivity of *Botrytis cinerea* to the phytoalexin resveratrol and the fungicide fenpiclonil. Molecular Plant-Microbe Interactions 14: 562-571

Schoonbeek H, Raaijmakers JM and de Waard MA (2002) Fungal ABC transporters and microbial interactions in natural environments. Molecular Plant-Microbe Interactions 15: 1165-1172

Schouten A, Wagemakers L, Stefanato FL, Van der Kaaij RM and Van Kan JAL (2002) Resveratrol acts as a natural profungicide and induces self-intoxication by a specific laccase. Molecular Microbiology 43: 883-894

Shimizu S, Kataoka M, Shimizu K, Hirakata H, Sakamoto K and Yamada H (1992) Purification and characterization of a novel lactonohydrolase, catalyzing the hydrolysis of aldonate lactones and aromatic lactones, from *Fusarium oxysporum*. European Journal of Biochemistry 209: 383-390

Simmons CR (1994) The physiology and molecular biology of plant 1,3-beta-D-glucanases and 1,3:1,4-beta-D-glucanases. Critical Reviews in Plant Sciences 13: 325-387

Souquet JM, Cheynier V, Brossaud F and Moutounet M (1996) Polymeric proanthocyanidins from grape skins. Phytochemistry 43: 509-512

Staples RC and Mayer AM (1995) Putative virulence factors of *Botrytis cinerea* acting as a wound pathogen. FEMS Microbiology Letters 134: 1-7

Stewart A and Mansfield JW (1985) The composition of wall alterations and appositions (reaction material) and their role in the resistance of onion bulb scale epidermis to colonisation by *Botrytis allii*. Plant Pathology 34: 25-37

Stintzi A, Heitz T, Prasad V, Wiedemann-Merdinoglu S, Kauffmann S, Geoffroy P, Legrand M and Fritig B (1993) Plant 'pathogenesis-related' proteins and their role in defence against pathogens. Biochimie 75: 687-706

VanEtten HD, Mansfield JW, Bailey JA and Farmer EE (1994) Two classes of plant antibiotics: Phytoalexins versus 'phytoanticipins'. Plant Cell 9: 1191-1192

Van Loon LC (1985) Pathogenesis-related proteins. Plant Molecular Biology 4: 111-116

Van Loon LC (1997) Induced resistance in plants and the role of pathogenesis-related proteins. European Journal of Plant Pathology 103: 753-765

Van Loon LC (1999) Occurrence and properties of plant pathogenesis-related proteins. In: Datta SK and Muthukrishnan S (eds) Pathogenesis-related Proteins in Plants. (pp. 1-20) CRC Press, Boca Raton, Florida, USA

Verhoeff K and Liem JI (1975) Toxicity of tomatine to *Botrytis cinerea*, in relation to latency. Phytopathologische Zeitschrift 82: 333-338

Viterbo A, Yagen B and Mayer AM (1993a) Cucurbitacins, 'attack' enzymes and laccase in *Botrytis cinerea*. Phytochemistry 32: 61-65

Viterbo A, Yagen B, Rosenthal R and Mayer AM (1993b) Dependence of activity of cucurbitacin in repression of *Botrytis* laccase on its structure. Phytochemistry 33: 1313-1315

Viterbo A, Staples RC, Yagen B and Mayer AM (1994) Selective mode of action of cucurbitacin in the inhibition of laccase formation in *Botrytis cinerea*. Phytochemistry 35: 1137-1142

Waterhouse AL and Lamuela Raventos RM (1994) The occurrence of piceid, a stilbene glucoside, in grape berries. Phytochemistry 37: 571-573

Waters EJ, Shirley NJ and Williams PJ (1996) Nuisance proteins of wines are grape pathogenesis-related proteins. Journal of Agricultural and Food Chemistry 44: 3-5

Waters EJ, Hayasaka Y, Tattersall DB, Adams KS and Williams PJ (1998) Sequence analysis of grape (*Vitis vinifera*) berry chitinases that cause haze formation in wines. Journal of Agricultural and Food Chemistry 46: 4950-4957

Weinges K, Bähr W, Ebert W, Göritz G and Marx HD (1969) Konstitution, Entstehung und Bedeutung der flavonoid- Gerbstoffe. Forschung Chemische Organische Naturstoffe 27: 158-259

Zhu Q, Maher EA, Masoud S, Dixon RA and Lamb CJ (1994) Enhanced protection against fungal attack by constitutive co-expression of chitinase and glucanase genes in transgenic tobacco. Bio/Technology 12: 807-812