Postharvest Pathology

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Postharvest Pathology

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Contents

Recent Developments in Postharvest Pathology

This collection of papers includes some of the presentations given at the International Congress for Plant Pathology held in Turin in 2008 in the session with the above title. Fruit production for human consumption is an important part of the market economy. Any waste due to spoilage and pest infestation, in the field but mostly during the postharvest phase, results in significant economic losses which are more pronounced as the losses occur closer to the time of produce sale. Careful handling of perishable produce is needed for the prevention of postharvest diseases at different stages during harvesting, handling, transport and storage in order to preserve the produce high quality. The extent of postharvest losses varies markedly depending on the commodities and country and are estimated to range between 4% and 8% in countries where postharvest refrigeration facilities are well developed to 50% where these facilities are minimal. Microbial decay is one of the main factors that determine losses compromising the quality of the fresh produce. For the development of an integrated approach for decay management, cultural, preharvest, harvest, and postharvest practices should be regarded as essential components that influence the complex interaction between host, pathogen, and environmental conditions. Orchard practices including preharvest fungicide applications can also directly reduce the development of postharvest fruit decay. Among postharvest practices, postharvest fruit treatments with fungicides are the most effective means to reduce decay. Ideally, these fungicides protect the fruit from infections that occur before treatment, including quiescent infections, as well from infections that are initiated after treatment during postharvest handling, shipment, and marketing. However the wide consumption in human diet of high-quality fresh fruits and vegetables and the increased concerns for the possible toxicity of fungicide residues have lead to the development of new alternative approaches for disease control. One of the alternatives is the use of antagonist applications, either alone or in combination with physical treatments and substances generally regarded as safe. The implementation of these alternatives techniques often requires modifying currently used postharvest practices and development of new formulation for their applications.

Three chapters in this book deal with the mechanisms of host fruit and vegetable resistance. Adikaram and co-workers referred to preformed antifungal substances affecting the resistance of unripe fruits and changes in their level during fruit ripening. Mengiste and co-workers suggested that active processes related to the regulation

of cell death, plant hormone signalling and synthesis are implicated in disease resistance to necrotrophic pathogens during storage. Interestingly Yang and co-workers indicated that a variety of chemical, physical and biological elicitors may modulate inducible mechanisms of resistance.

Two chapters in this book deal with fungal pathogenicity factors and their relationship with the host response. Prusky and co-workers described an interesting mechanism used by postharvest pathogens to modulate host environment (alkalinization and acidification) leading to enhanced pathogenicity, while Gonzales-Candelas and co-workers presented the first wide transcriptome analysis of citrus fruit response to *Penicillium digitatum* infection.

Four chapters in this book deal with subjects related to disease assessments before harvest and their relation to the postharvest treatment of fruits and vegetables. Michailidis and co-workers emphasized the importance of weather and environmental conditions to pathogen infection and suggested different approaches for disease assessment which could be used to predict the incidence of postharvest diseases. Teixido and co-workers suggested the importance of preharvest applications of biocontrol treatment efficacy in combination with nutrients and conclude on the importance of preharvest treatment in postharvest disease control. The other two chapters dealt specifically on the new development of postharvest edible crop in the United States by Adaskaveg and Förster, and in Europe by Mari and co-workers. Both suggested that integrations of combined technologies such as sanitation and use of fungicides, physical and biological agents are of high importance.

Three chapters in this book are dealing with biological control of postharvest diseases and host responses to the biocontrol agents. Janisiewicz, presented a summary of the biocontrol developments in his "Quo Vadis of Biological Control …" chapter and their impact on the industry, while Usall and co-workers described the different technological changes made during the development of new formulations which allow the improvement of biocontrol efficiency. Castoria and Wright referred in their chapter to the different mechanisms of perception and activation of host resistance by the biocontrol agents.

The remaining chapters of the book are focused in specific study cases of crops such as kiwifruit, peaches and grapes, where the integrations of different approaches at the pre and postharvest levels are combined. These represent new types of presentations which were presented in the evening workshops of the ISPP program with excellent attendance. Manning and Beresford described how management and assessment of rot-risk-factors in the vine and storage conditions may allow prevention of botrytis problems. Melgarejo and co-workers described the importance of orchard management in combination with epidemiological assessment to predict risk and optimal handling of fruits.

In summary the Postharvest Pathology sessions included excellent presentations of new and exciting progress at the leading edge of Postharvest Pathology.

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Chapter 1
The Role of Pre-formed Antifungal Substances in the Resistance of Fruits to Postharvest Pathogens

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Abstract Plants contain secondary metabolites with antifungal properties. In fruits they are mostly concentrated in the peel at immature stage and decline during ripening in coincidence with fungal rot development. The information on antifungal systems in immature avocado and mango, reviewed here, suggests that they play a role in natural disease resistance. Immature mangoes have evolved a formidable antifungal system comprising several resorcinols, gallotannins and chitinases. Resorcinols and gallotannins are inhibitory to major postharvest pathogens, *Colletotrichum gloeosporioides* causing anthracnose and *Botryodiplodia theobromae* causing stem-end rot. Their levels are generally higher in resistant cultivars than in susceptible ones. Mango latex, distributed in a fine network of canals in the fruit peel, contains chitinases which have the ability to rapidly digest conidia of *C. gloeosporioides.* Gallotannins and resorcinols decline progressively during ripening and the latex disappears when ripe rot development begins. Retention of latex in the harvested fruit reduces anthracnose and stem-end rot development during ripening. Treatment of harvested fruit with CO_2 or inoculation with certain non-pathogenic fungi increased antifungal resorcinol concentration. Immature avocado fruits possess a pre-formed antifungal system comprising at least five antifungal compounds. The quiescence of *C. gloeosporioides* in the immature fruit has been attributed to the pre-formed antifungal activity of the peel. Lipoxygenase activity increases during fruit ripening, while epicatechin levels decline, suggesting that these events are linked to the decrease in di-ene concentrations. Inhibition of lipoxygenase activity results in retention of antifungal di-ene during ripening increasing fruit resistance. In freshly harvested avocados, the di-ene concentration can be further enhanced by treatment with biotic and abiotic agents.

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1.1 Pre-formed Antifungal Substances

Plants produce a range of chemically diverse secondary metabolites with antifungal activity. Some of these exist in healthy plants in their biologically active forms and others occur as inactive precursors and are activated in response to tissue damage or pathogen attack (Schonbeck and Schlosser 1976; Osbourn 1996). VanEtten et al. (1994) have proposed the term phytoanticipin to distinguish these preformed antimicrobial compounds from phytoalexins, which are synthesized from remote precursors in response to pathogen attack, probably as a result of de novo synthesis of enzymes. Phytoanticipins are low molecular weight, antimicrobial compounds that are present in plants before challenge by microorganisms or are produced after infection solely from preexisting constituents (VanEtten et al. 1994). Certain plants contain high molecular weight constitutive hydrolytic enzymes such as chitinases with considerable antifungal activity and these cannot be accommodated within the umbrella of phytoanticipins, although they seem to play a role in plant defence.

Pathogens infecting fruits have to face challenges that the pathogens infecting vegetative organs do not normally encounter. Fruits are generally protected by mechanical and chemical barriers in the peel and their physiology changes markedly during development, particularly when ripening occurs. Pathogens, when confront unripe fruits, often cause quiescent infections or minor damage. Such quiescent infections have been observed in tropical (Muirhead and Deverall 1981), subtropical (Droby et al. 1987), and deciduous fruits (Hall 1971). The resistance of unripe fruits to fungal decay has been shown to be associated with induced (Adikaram et al. 1982) or preformed (Prusky and Keen 1993) antifungal substances in the peel. The onset of decay coincides with fruit ripening and concurrent decrease in the antifungal compounds to sub-toxic levels. Thus, quiescence may therefore represent a mechanism for avoiding toxic levels of antifungal plant compounds. There is considerable interest in determining mechanisms underlying the natural resistance of unripe fruits to fungal pathogens and extending fruit resistance to postharvest ripening phase.

In certain fruits, the preformed antifungal substances appear to play a supportive role to their arsenal of inducible defences by excluding saprophytic and epiphytic microorganisms. In other fruits such as avocado and mango where the inducible defence system is weaker, the preformed antifungal compounds appear to perform a definitive protective role. This Chapter reviews the preformed antifungal systems in mango and avocado.

1.2 Preformed Antifungal Compounds in Mango (*Mangifera indica*) fruit

Unripe mango fruit peel contains three classes of preformed antifungal substances, gallotannins and resorcinols in the peel tissue, hydrolytic enzyme, chitinase, in the latex.

1.2.1 Resorcinols

Constitutive resorcinol type antifungal compounds were first isolated from the peel of unripe mango fruit cultivars *Tommy Atkins* and *Haden* and identified as a mixture of 5-substituted resorcinols, 5-(12-*cis*-heptadecenyl) resorcinol and 5-pentadecyl resorcinol (Fig. 1.1) (Droby et al. 1986; Cojocaru et al. 1986). Identical resorcinols were also shown in the peel of several other cultivars (Droby et al. 1986). High Pressure Liquid Chromatography of the dichloromethane phase of peel extracts of two Sri Lankan cultivars, *Karutha Colomban* and *Willard*, showed peeks representing 5-(12-*cis*-heptadecenyl) resorcinol, 5-pentadecyl resorcinol and an additional peak due probably to a new resorcinol (Karunanayake 2008). Two other resorcinols, 5(7, 12-heptadecadienyl) resorcinol from the fruit peel (Prusky et al. 1996) and 5-(9, 12-heptadecadienyl) resorcinol from the latex (Oka et al. 2004), have been reported. The concentration of the former did not change significantly during fruit ripening, therefore it did not appear to play a role in fruit resistance. Knodler et al. (2007) in a more recent study identified 3 major and 12 minor C_{15}, C_{17} and C_{19} substituted resorcinols and related analogues, however, their antifungal properties are not yet known.

Resorcinols in mango varieties have been studied in relation to black spot development by *Alternaria alternata* during ripening. In unripe fruit, the fungus

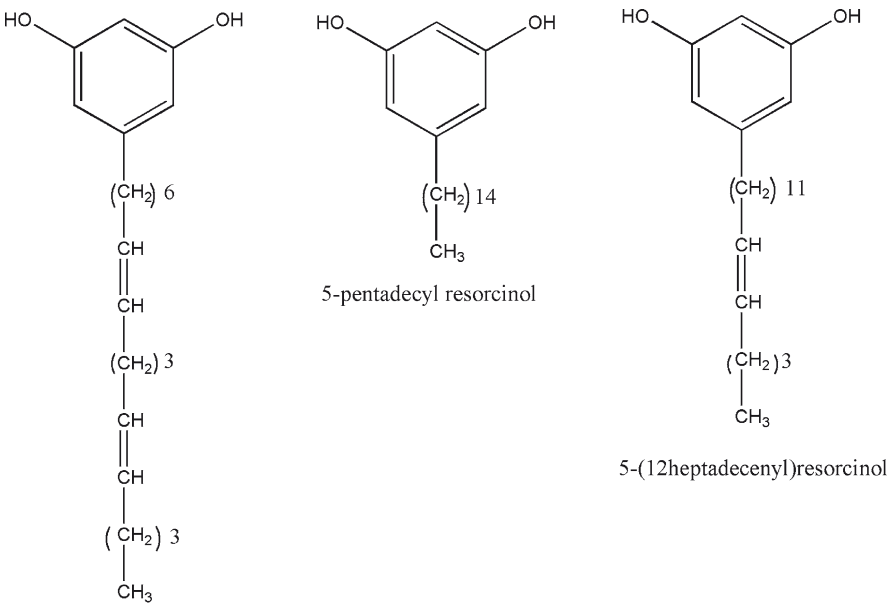

Fig. 1.1 Structures of three resorcinols from mango peel, 5-(7, 12-heptadecadienyl) resorcinol (**a**), 5-pentadecyl resorcinol (**b**), and 5-(12-heptadecenyl) resorcinol (**c**) (Kobiler et al. 1998)

forms quiescent infections. The concentration of resorcinols in the unripe mango fruit peel ranged between 154–232 μg g^{-1} fresh weight and this declined during ripening to about 74–125 μg g^{-1} fresh weight. At the time of *Alternaria* rot development, the concentration also ranged between 74–125 μg g^{-1} FW (Droby et al. 1986). 5-substituted resorcinols were present at fungitoxic levels in the unripe fruit peel and decreased to non-toxic levels, with ripening, in uninoculated fruits at the same time that disease symptoms appeared in inoculated fruits (Prusky and Keen 1993). Eight mango cultivars, *Maya*, *Erwin*, *Palmer*, *Pairi*, *Mabroka*, *Tommy Atkins*, *Haden* and *Keitt* tested gave similar results.

Concentration of resorcinols in the mango fruit was also studied in relation to development of anthracnose disease by *Colletotrichum gloeosporioides* during ripening. In the unripe fruit where the fungus forms quiescent infections, the resorcinols are present at higher concentrations and declined during fruit ripening to very low levels when anthracnose rot development commenced (Fig. 1.2). Recent studies have showed the presence of significantly higher amounts of antifungal resorcinols in mango latex (Bandyopadhyay et al. 1985; Oka et al. 2004) than in the fruit peel (Hassan 2006). The constitutive resorcinols identified in peel extracts could actually be those present in the latex canals of the peel.

The concentration of three resorcinols varied among different cultivars. In cultivars that are resistant to anthracnose disease, there was a higher concentration of 5-(12- *cis*-heptadecenyl), 5-pentadecyl and AR 21 resorcinol (Table 1.1), than in susceptible ones (Karunanayake 2008). A strong positive correlation was present between the level of resorcinols and the degree of resistance to *C. gloeosporioides*. Mango cultivars, *Kensington Pride* and *Keitt* that are more resistant to anthracnose, had the highest concentrations of 5-n- heptadecenyl resorcinol while *Nam Doc Mai* and *Honey gold* susceptible to anthracnose had the lowest (Hassan et al. 2007). As in mango peel, the concentration of 5-substituted resorcinols in mango latex also varied significantly among cultivars (Hassan 2006). A high correlation exists

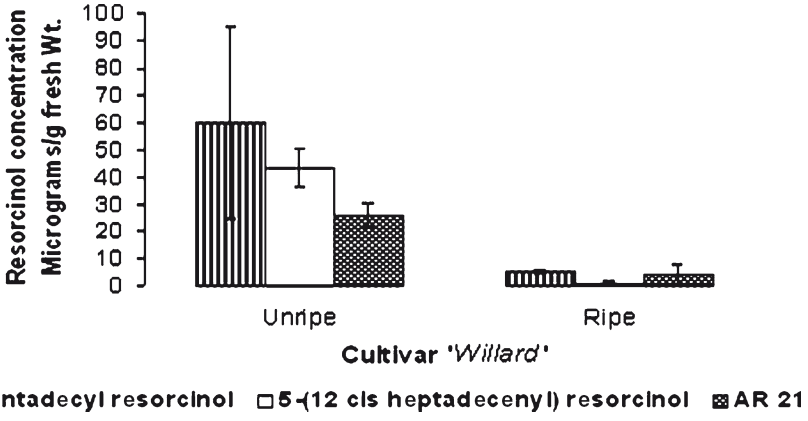

Fig. 1.2 Resorcinol concentration in the unripe and ripe fruit peel of cultivar *Willard*

Table 1.1 The concentration of resorcinols in five Sri Lankan mango cultivars (average of two samples) determined by HPLC

	Concentration of resorcinols μg/g fresh weight			
Cultivar	5 – (12- *cis*-heptadecenyl) resorcinol	5 – pentadecyl resorcinol	Resorcinol derivative	AR 21 Resorcinol
Karutha Colomban (R)	59.9	27.1	532.1	43.2
Rata (R)	91.8	26.8	2,306.3	79.0
Kohu (M)	38.1	8.2	911.4	139.2
Petti (S)	20.7	8.6	47.5	15.3
Willard (S)	34.4	58.3	14.8	25.7

(R) cultivars more resistant, (M) moderately susceptible, and (S) susceptible to anthracnose (Karunanayake 2008)

between the concentration of resorcinols in mango latex and the percentage (w/w) of the non-aqueous phase of mango latex (Hassan 2006).

The concentration of the 5-substituted resorcinols decreased faster during ripening in cultivars like *Tommy Atkins* susceptible to *Alternaria* spot, than in less susceptible cultivars, such as *Haden* (Droby et al. 1986). A similar trend was observed in two Sri Lankan cultivars where the resorcinols in the cultivar *Willard* susceptible to anthracnose declined faster during ripening than in the resistant *Karutha Colomban* (Karunanayake 2008).

1.2.2 Gallotannins

Methanol extract of the mango fruit peel when bioassayed with *Cladosporium cladosporioides* or *C. gloeosporioides* produced a prominent inhibition zone at Rf. 0.00 (Fig. 1.3). The compounds responsible for inhibition were purified and identified as a mixture of three closely related gallotannins (Fig. 1.3). The three compounds vary in the number and the points of attachment of sugar molecules. Earlier 18 different gallotannins have been reported from the mango fruit peel and eight in the pulp (Berardini et al. 2004). The phenolic compounds which were accounted for antibacterial activity, observed in mango seed (Kabuki et al. 2000), showed almost an identical elution profile to gallotannins (Berardini et al. 2004). Young and mature leaves and florets of mango also contain gallotannins (Karunanayake 2008).

The gallotannins are directly inhibitory to the anthracnose pathogen, *C. gloeosporioides* and the stem-end rot pathogen, *Botryodiplodia theobromae.* Antifungal activity due to gallotannins was higher in the peel of unripe fruit at harvesting maturity and declined gradually during ripening. By colour break stage, the antifungal activity had declined by about 20% from what it was at harvest. At the ripe stage, when anthracnose development occurred in cultivar *Karutha Colomban*, the antifungal activity had declined to about 50% of the initial level.

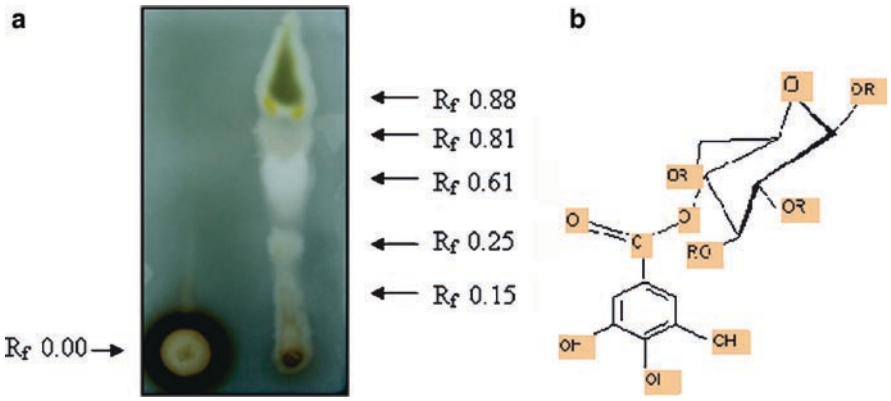

Fig. 1.3 (**a**) Inhibition areas on thin layer chromatography bioassay plates produced by gallotannins in the methanol (*left*) and resorcinols in the dicholomethane extracts (*right*) of mango fruit peel. (**b**) Structure of the antifungal gallotannins

Mango cultivars more resistant to anthracnose such as *Gira* and *Rata* show greater gallotannin activity in their peel than more susceptible cultivars such as *Kohu* and *Willard*. The decline of gallotannin activity during ripening was greater in the more susceptible cultivars.

1.2.3 Chitinase Activity

The peel of unripe mango fruit consists of a network of fine canals with latex which extends to the pedicel. At the abscission point of mango pedicel, the small canals tend to coalesce to form three or four large canals. It is from these that the latex flow spurts out when the fruit is removed from the tree. When the mango latex is removed from the fruit and allowed to settle, it separates into an aqueous and oily phase. The latex is toxic to the conidia of *C. gloeosporioides*, the causal agent of mango anthracnose. When exposed to the undiluted aqueous phase of mango latex, the conidia were gradually digested (Fig. 1.4). During early hours, a slight granulation was visible in the conidia and later the conidial wall was gradually dissolved.

A gel diffusion assay carried out on glycol chitin-enriched agarose confirmed the presence of chitinase enzyme in the aqueous phase of the mango latex. Under UV light (365 nm), the areas hydrolyzed by chitinase appeared dark against blue fluoresced areas containing undigested glycol chitin (Fig. 1.5). Three chitinases with molecular weights 47, 87, and 97 KDa were present in the mango latex (Karunanayake 2008). The level of chitinase activity in the aqueous phase varied with the mango cultivar.

Development of anthracnose (Fig. 1.6) and stem-end rot (Fig. 1.6) during ripening was significantly lesser in fruits from which latex was not drained off after harvest

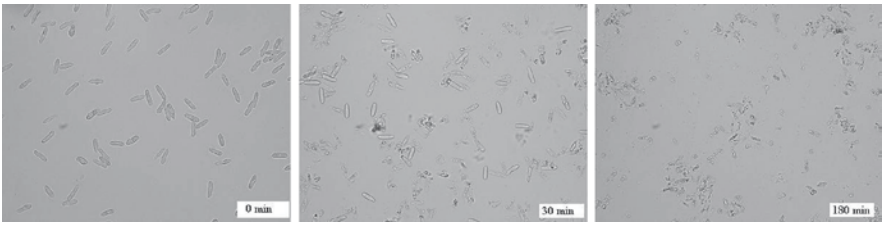

Fig. 1.4 The state of conidia of *C. gloeosporioides* after different periods of exposure to the undiluted aqueous phase of mango latex

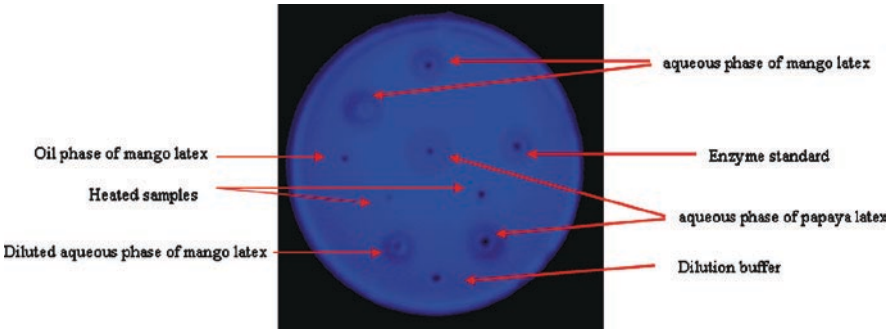

Fig. 1.5 Chitinase activity of the aqueous phase of mango latex, papaya latex (*positive control*), dilution buffer (*negative control*) and a commercial enzyme standard from *S. marcescence*

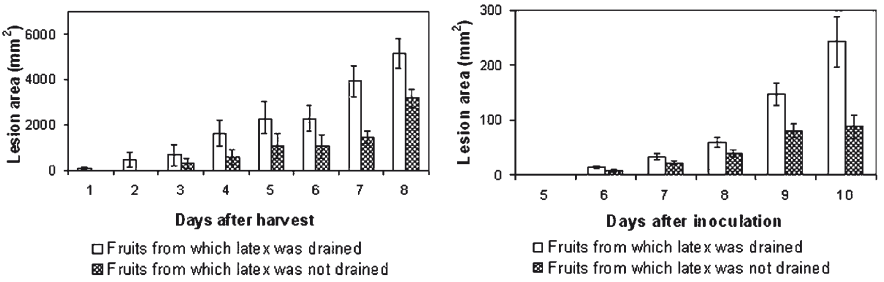

Fig. 1.6 Anthracnose (*left*) and natural stem-end rot (*right*) development in fruits of cultivar '*Willard*' (anthracnose) and '*Karutha Colomban*' (SER) from which latex was drained and not drained

than in fruits from which the latex was drained off. It was subsequently shown that the peel of unripe mango fruits from which latex was not removed after harvest had greater chitinase activity (Table 1.2) which could be the reason for greater fruit resistance in latex-retained fruit.

Table 1.2 Chitinase activity in the fruit peel of cultivar '*Willard*' when the latex was drained after harvest

	Chitinase activity in units/gram fresh weight tissue	
Treatment	Day 1 after harvest	Day 3 after harvest
Latex retained fruits	0.48 ± 0.03	0.29 ± 0.02
Latex drained fruits	0.32 ± 0.08	0.22 ± 0.01

The values given in the table are the average of two independent trials.

1.2.4 Pre- and Postharvest Treatments Enhance Fruit Resistance and Antifungal Activity

Exposure of harvested mangoes to CO_2 at a flow rate of 100 mL of CO_2/min for 24 h significantly reduced anthracnose development, however, the optimum effective dosage of CO_2 varied according to cultivar, 20% CO_2 for *Keitt*, 60% CO_2 for *Tommy Atkins*. A concomitant significant increase of 5-(7,12-heptadecadienyl) resorcinol was also detected in *Keitt* fruits in response to the 30% CO_2 treatment (Kobiler et al. 1998). Dipping fruit in hot water (55° C) for 5 min also resulted in an increase in 5-(7,12-heptadecadienyl) resorcinol.

Dipping the unripe mangoes in a suspension of conidia of *Colletotrichum magna*, not pathogenic on mango, before inoculation with *C. gloeosporioides,* significantly delayed the anthracnose development. A significant increase in 5-(7,12-heptadecadienyl)resorcinol was also detected in mango fruits dipped in a conidia suspension of *C. magna* (Kobiler et al. 1998). Peeling increased five substituted resorcinols in the flesh of peeled mango and the fruits became resistant to *A. alternata* infection (Droby et al. 1987).

Inoculation of unripe fruits with *C. gloeosporioides* resulted in enhanced gallotannins (Sinnaih, Unpublished data) and the total soluble phenol content (Karunanayake 2008). Concurrent histo-chemical tests carried out on inoculated fruit peel at different time intervals supported the findings of chemical tests that tissue phenolics increased following infection. Free phenols can directly act as antimicrobial substances and be oxidized to form quinines which can inhibit extracellular enzymes of the pathogen (Mayer 1987). Chitinase activity increased in the peel following inoculation with *C. gloeosporioides*, however, whether the increased activity is due the same chitinases found in the latex could not be ascertained (Karunanayake 2008).

A field trial was conducted to investigate the influence of soil potassium on postharvest rot development and natural disease resistance. Stem-end rot development was significantly less in mangoes from trees which received three times the annual recommended dose of potassium (2,055 g × 3) compared to those which received the annual recommended level of potassium (2,055 g) or no potassium (Table 1.3).

The fact that the tissue potassium levels were higher in the peel tissues from fruits harvested from potassium-treated trees (16.24 and 12.38 ppm) than the control (11.68 ppm) may indicate that potassium enhances fruit resistance.

Table 1.3 Stem-end rot in fruits harvested from trees which received different levels of potassium

	Lesion area (mm^2) at different days after inoculation				
K level (g)	Day 1	Day 2	Day 3	Day 4	Day 5
0	0	0	558.7[a]	3,873.7[a]	10,572[a]
2,055	0	0	458.8[a]	4,140.1[a]	9,109[a]
2,055 × 3	0	0	248.7[a]	2,394.7[b]	6,834[b]

[a, b]Values followed by the same letter do not differ significantly at the 5% probability level (Duncan's Multiple Range Test)

Gallotannin activity was in fact higher in peel of fruits which received greater potassium, the differences in gallotannin activity were, however, not significant.

1.3 Avocado (*Persea americana*) Fruit

Anthracnose disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and the stem-end rot caused by *Phoma* spp., *Botryodiplodia theobromae* and *Phomopsis* spp. are recognized as major diseases in ripe avocado fruit. Young fruit are usually free from visible symptoms and characteristic decay lesions develop during fruit ripening. The anthracnose disease originates from quiescent infections in the immature fruit long before harvest (Binyamini and Schiffmann-Nadel 1972). In unripe fruit the fungus produces an appressorium, then an infection peg which ceases the growth in the cuticle (Coates et al. 1993) and becomes quiescent.

The quiescence of *C. gloeosporioides* was attributed to the presence of substantial preformed antifungal activity in the immature fruit peel (Prusky et al. 1982; Sivanathan and Adikaram 1989). Avocado peel contains antifungal monoene, 1-acetoxy-2,4-dihydroxy-*n*-heptadeca-16-ene (2) and diene, 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (1) (Prusky et al. 1982; Prusky et al. 1991) and three other compounds 1,2,4-trihydroxyheptadec-16-yne (3), 1,2,4-trihydroxyheptadex-16-ene (4) and 1-acetoxy-2,4-dihydroxyheptadec-16-yne (5) (Adikaram et al. 1992):

$$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COCH_2CHOHCH_2OCOCH_3 \quad (1)$$

$$CH_2{=}CH(CH_2)_{11}CHOHCH_2CHOCH_2OCOCH_3 \quad (2)$$

$$CH_2{=}CH(CH_2)_{11}CHOHCH_2CHOHCH_2OH \quad (3)$$

$$CH{\equiv}C(CH_2)_{11}CHOHCH_2CHOHCH_2OH \quad (4)$$

$$CH{\equiv}C(CH_2)_{11}CHOHCH_2CHOHCH_2OCOMe \quad (5)$$

The most striking structural feature among the five antifungal compounds is the presence of a trihydroxy fragment which could be a precursor to these compounds (Adikaram et al. 1992).

The levels of antifungal di-ene in peel of unripe avocados are subject to complex regulation and may be modulated by lipoxygenase, for which the di-ene is a

substrate (Prusky et al. 1983), and also by the flavan-3-ol epicatechin, an inhibitor of lipoxygenase (Ardi et al. 1998; Prusky et al. 1982). Lipoxygenase activity increases during fruit ripening, while epicatechin levels decline, suggesting that these events are linked to the decrease in di-ene concentrations. In freshly harvested unripe avocado fruits, di-ene 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 (Prusky et al. 1990; Prusky and Keen 1995; Prusky et al. 1985; Prusky et al. 1991; Prusky et al. 1996). Treatment with lipoxygenase inhibitors results in increased disease resistance (Prusky et al. 1985, 1991), offering potential strategies for the manipulation of fruit physiology for control of postharvest diseases. Interestingly, inoculation of freshly harvested avocado fruit with a non-pathogenic mutant strain of *Colletotrichum magna* also confers protection against *C. gloeosporioides*, possibly by the induction of epicatechin and modulation of the level of the antifungal di-ene (Prusky et al. 1994). Taken together, this evidence suggests that for the avocado-*C. gloeosporioides* interaction, preformed antifungal compounds may contribute to the resistance of unripe fruits to decay.

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Chapter 2
Mechanisms of Induced Resistance Against *B. cinerea*

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Abstract *Botrytis cinerea* is a widespread pre-and postharvest pathogen of diverse crops. Current crop protection methods rely on fungicide application and on horticultural practices. Variation for genetic resistance is documented in many crop plant species but has not been utilized. Studies in model and crop plant species are revealing the biological processes that underlie plant responses to infection to *B. cinerea*. The genetic control of pathogen recognition and activation of defense to restrict pathogen ingress and colonization is likely to emerge from such studies. Deeper understanding of resistance mechanisms and their genetic control will aid produce cultivars with genetic resistance to B. cinerea. The genetic components of induced resistance in different plant species and future implications are discussed.

Keywords Botrytis cinerea • genetic resistance • necrotrpohic pathogens • induced resistance

2.1 Introduction

B. cinerea is a ubiquitious fungal pathogen with relative host unspecificity primarily attacking dicot plants but also some monocot species. The fungus causes the gray mold disease resulting in significant crop losses under different production conditions. Gray mold occurs over a wide geographical area, in the open field, in greenhouses and even in storages at 0–10°C. *B. cinerea* is the principal cause of pre- and postharvest disease in grapes, berries, tomatoes and many other crops (Coley-Smith et al. 1980; Williamson et al. 1995; Elad 1997). In grapes, where *B. cinerea* causes "bunch rot", the estimated loss to the vineyard can amount to

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15–40% of the harvest depending upon the season. The losses to strawberries and cut flowers have been estimated at 10–20% (Legard et al. 2000). *B. cinerea* is regarded as an expensive pathogen because of the qualitative and quantitative crop losses it causes and because of its demand for high fungicide treatment. *B. cinerea* also develops fungicide resistance, limiting chemical crop protection options. Chemical protection is also discouraged due to public safety associated with fungicide residues on fresh produce. Consequently, there is an increased effort to identify genetic resistance. Genetic resistance provides cost-effective and sustainable plant protection. However, no robust genetic resistance against *B. cinerea* has been identified in crop plants. There is also a very limited understanding of the biological processes underlying plant responses to necrotrophic pathogens in general and *B. cinerea* in particular. The biology of *B. cinerea* has been studied extensively and the genome of the fungus has been sequenced (Elad et al. 2004; van Kan 2006). The critical factors in *B. cinerea* pathogenesis, pathogen derived effectors, the molecular events associated with infection processes, infection related morphogenesis, and factors that confer the relative host unspecificity are still not fully understood. Equally unknown are the critical factors in plant disease resistance mechanisms in different plant species. In this chapter we will highlight recent progress made towards understanding of plant induced resistance to *B. cinerea*.

2.2 Plant Resistance to *B. cinerea*

Plant resistance to diseases is controlled by a multitude of environmental as well as host and pathogen genetic factors that vary depending on pathogen-host combinations. Figure 2.1 summarizes *B. cinerea* derived disease and/or defense elicitors and the corresponding plant defense mechanisms. Necrotrophic pathogens in general and *B. cinerea* in particular have evolved infection strategies to breach plant defenses (Prins et al. 2000a). These infection strategies involve the secretion of diverse chemical compounds before and during colonization. Some necrotrophic fungi are host specific, producing toxins that promote chlorosis and host cell death only in their hosts (host specific toxins, HSTs) (Wolpert et al. 2002). Many necrotrophic fungi including *B. cinerea* are host unspecific and produce host non-sepecific toxins. There is no HST identified from *B. cinerea* consistent with the host unspecificity of the pathogen. *B. cinerea* produces botrydial, a non-host-specific toxin implicated in the intitation and severity of disease (Colmenares et al. 2002). In addition, *B. cinerea* produces cell wall degrading enzymes, other extracelluar enzymes, oxalic acid, and reactive oxygen intermediates to promote disease and macerate plant tissues (Prins et al. 2000b). These infection strategies differ from obligate pathogens that suppress plant defenses through subtle mechanisms. *B. cinerea* promotes or benefits from host cell death during pathogenesis. Excellent reviews have recently been published on the pathogenesis of *B. cinerea* (van Kan 2006; Williamson et al. 2007).

Plants also have counter defense mechanisms that are built as layers of constitutive and inducible resistance strategies. Broadly, plant defense is composed of primary