Involvement of Preformed Antifungal Compounds in the Resistance of Subtropical Fruits to Fungal Decay

Fungal pathogens must perform precise functions and overcome several barriers before they are able to initiate disease in plants. First, the pathogen must locate and adhere to susceptible tissue and then initiate infection (10,12,14). The first plant barriers encountered are generally the cuticle and cell wall, which may be breached by enzymatic (6) or physical (12) assault, or the pathogen may infect through wounds (27). Contact with underlying plant tissues presents the invading pathogen with a different set of barriers, most notably preformed antibiotic compounds and/or morphologic barriers and phytoalexins induced by the plant (13,25,26).

Pathogens that infect fruits are confronted by several problems not normally facing pathogens of vegetative plant tissues. Fruits are generally protected by differentiated integumentary structures, and their physiology changes markedly during development, particularly when ripening occurs. Pathogens frequently infect unripe fruits but cause relatively minor damage until ripening, when they may cause extensive decay. Such quiescent infections have been observed in tropical (15), subtropical (8), and deciduous fruits (9). The resultant decay has great economic importance, since they reduce the shelf life of fruits during storage and transport (27). These disease problems have been further exacerbated by the development of pathogen resistance to fungicides and the withdrawal of pesticides on environmental grounds. Consequently, there is considerable interest in determining mechanisms accounting for the natural resistance of unripe fruits to fungal pathogens and extending its effectiveness to fruits after harvest. One of the possible mechanisms that may account for such differential resistance during ripening is the level of preformed antifungal compounds.

Activity of Preformed Antifungal Compounds

Critical tests of the role of preformed compounds in disease resistance are difficult, primarily because of problems in accurately assessing the quantities of inhibitory compounds that may contact an invading pathogen, uncertainty as to their in vivo biological activity, and difficulty in relating changes in their concentrations to resistance (27). The most compelling evidence for a functional role of preformed antifungal compounds in disease resistance has been produced by using genetically related pathogen strains that differ in their ability to tolerate or degrade a plant antifungal compound or barrier (2,4,5). On the other hand, simple correlations of changes in the level of a preformed compound with resistance may be serendipitous and must therefore be interpreted cautiously. However, if mechanisms accounting for the in vivo concentration of a preformed inhibitory compound are determined, then it may be possible to exogenously modulate these systems and observe whether an altered level of the inhibitory compound and disease resistance are in fact linked. For example, Holowczak et al (11) used exogenously supplied Dl-phenylalanine to alter the levels of phenolic compounds and resistance of apple fruits to the scab disease. In addition to providing a critical test of whether a putative preformed resistance mechanism is physiologically important, this approach may provide treatments that can have practical uses in disease control.

In this paper we discuss the role of preformed inhibitors in fungal decays of avocado (Persea americana Miller) and mango (Mangifera indica L.). We emphasize the interaction of avocado fruit with Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. in Penz. (Fig. 1), in which external modulators have been used to elucidate the nature of a preformed fungal resistance mechanism.

Preformed Antifungal Compounds in Mango Fruits

C. gloeosporioides attacks unripe mango fruits in the orchard. Germinating spores produce appressoria, but the resultant subcuticular hyphae do not develop further until fruit ripen (7). A mixture of antifungal compounds consisting of 5-12-cis-heptadecenyl resorcinol and 5-pentadecenyl resorcinol (Fig. 2) was found at fungitoxic concentrations in the peel but not in the flesh of unripe mango fruits (7,8). The antifungal 5-alkylated resorcinols occurred in unripe fruit of several mango cultivars at concentrations ranging between 154 and 232 µg ml⁻¹ fresh weight but declined to levels between 74 and 125 µg ml⁻¹ in ripe fruits when decay developed (Fig. 3). Inhibition of germ tube elongation indicated an ED₅₀ of approximately 120 µg ml⁻¹, a concentration similar to that present in fruits when symptoms begin to develop.

Several lines of evidence suggested that the preformed antifungal compounds might be involved in the resistance of mango fruits to fungal development: 1) The concentration of 5-substituted resorcinols occurred at fungitoxic levels in the peel of unripe fruits of eight mango cultivars tested and decreased to nontoxic levels in unincubated fruits at the same time that decay appeared in inoculated fruits; 2) the concentration of the 5-substituted resorcinols decreased faster during ripening in disease-susceptible cultivars than in resistant cultivars; 3) delayed reduction in levels of the antifungal compounds was related to delayed decay development; and 4) the flesh of unripe fruits contained subfungitoxic concentrations of the antifungal compounds and was susceptible to fungal attack. These observations suggested, but did not conclusively prove, that the resistance of unripe mango fruits results from fungitoxic concentrations of the antifungal 5-substituted resorcinols present in the peel acting on subcuticular hyphae from germinated appressoria. However, addi-
tional experiments are required to more thoroughly evaluate the role of the preformed resorcinols in resistance.

**Resistance of Avocado Fruits to C. gloeosporioides**

The resistance of unripe avocado fruits to attack by *C. gloeosporioides* was shown several years ago to be correlated with the presence of high concentrations of preformed antifungal chemicals (19). Extracts from the peel of freshly harvested fruits inhibited fungal growth 78% as compared with 7% for extracts from ripe fruits showing disease symptoms. The major antifungal compound was shown to be 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (Fig. 4). This compound inhibited in vitro germ tube elongation with an ED₅₀ of 450 μg·ml⁻¹. Concentrations of the diene in peels of unripe fruits were as high as 1,200 μg·g⁻¹ fresh weight of peel (approximately 1,800 μg·g⁻¹) but decreased 10-fold during ripening (Fig. 5). A second antifungal compound was subsequently purified from unripe avocado fruits and identified as 1-acetoxy-2,4-dihydroxy-n-heptadeca-16-ene (Fig. 4) (20). This monoene was less fungitoxic than the diene, and its concentration in the peel of unripe fruits varied between 500 and 650 μg·g⁻¹ fresh weight (approximately 900 μg·ml⁻¹) and decreased to 40 μg·g⁻¹ fresh weight (approximately 60 μg·ml⁻¹) when decay symptoms were conspicuous. It therefore appeared that the diene accounted for most of the antifungal activity while the monoene had a smaller effect (20). Cultivars that were more susceptible to decay also showed a faster decrease in both compounds as the fruit matured.

The concentration of the antifungal diene in avocado fruits is affected by harvesting as well as by ripening. Freshly harvested fruits showed a rapid, almost 10-fold decrease to subfungitoxic levels 1–2 days after harvest (Fig. 5) (23). Levels subsequently recovered to toxic concentrations. The durations of the decrease and recovery periods were related to fruit maturity, with more mature fruits having a shorter recovery period. The cause of this decrease in diene concentration is not clear but seems to be related to stress occurring during fruit harvest. Nevertheless, fungal development does not occur during this period and is observed only when fruits become ripe (Fig. 6).

Two factors affecting development of *C. gloeosporioides* in the peel of freshly harvested fruits (23) are the time required for germinated appressoria to penetrate the epicuticular wax and the period of time in which the antifungal diene is present at subfungitoxic concentrations. In freshly harvested fruits, the period of decline of the antifungal diene is very short and the pathogen is unable to establish sufficient growth before the subsequent increase in diene levels. Fruits treated with hot water at 55°C, however, showed a delay in recovery of the antifungal diene (23) (Fig. 7). This treatment resulted in a longer period of subfungitoxic diene concentration, and the fungus caused decay in these unripe fruits. All of these observations are consistent with, but do not prove, the view that resistance to fungus development depends on a threshold concentration of the antifungal diene that is present in unripe, but not ripe, fruits.

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**Fig. 1. Symptoms of decay caused by *Colletotrichum gloeosporioides* on a ripe avocado fruit.**

**Fig. 2. Chemical structure of the 5-substituted resorcinols isolated from mango fruits.**

**Fig. 3. Changes in the level of 5-substituted resorcinols during fruit growth and after harvesting in ripening mango cv. Hayden fruits.**

**Fig. 4. Chemical structures of the antifungal diene and monoene compounds isolated from avocado fruits.**
Induction of Avocado Preformed Antifungal Compounds

The possibility that preformed biocides can be induced to higher levels in fruits after fungal infection has not been widely investigated. In avocado fruit, however, concentrations of the antifungal diene increased in response to challenge inoculation with C. gloeosporioides on unharvested or freshly harvested fruits (17). In both cases, the level of the antifungal compound in freshly harvested fruits was enhanced for 2–3 days but fungal resistance was not affected. Certain abiotic treatments also increased the concentration of the antifungal diene (24). For example, freshly harvested fruits exposed to 30% CO₂ showed an initial increase in the diene level immediately upon removal from the high CO₂ atmosphere (Fig. 8). The concentration of the diene then decreased before a second increase that correlated with delayed development of fungal decay. Exposure of avocado fruits to 11 or 16% CO₂ enhanced the initial increase in diene concentration but not the second. The 11 and 16% CO₂ treatments did not affect decay development, suggesting the importance of the second increase in diene level for fruit resistance. These experiments are of interest because they show that the avocado diene compound has some of the characteristics of classical phytoalexins.

Concentration of the Compounds and Decay Resistance

Because disease susceptibility in avocado fruits was closely related to decreased concentrations of the preformed antifungal compounds, the mechanism controlling this reduction may predispose fruits to disease. The antifungal diene (Fig. 4) contains a cis,cis-1,4-penta diene system and serves as a substrate for oxidation by lipoxygenase (18). Several experiments (22) indicated that lipoxygenase activity in avocado fruits was related to degradation of the diene: 1) The apparent specific activity of the enzyme increased by 80% during fruit ripening; 2) partially purified avocado lipoxygenase oxidized the antifungal diene in vitro; 3) treatments with α-tocopherol acetate, an antioxidant that nonspecifically inhibits lipoxygenase, delayed the decrease of the antifungal diene as well as the appearance of disease symptoms; and 4) a specific inhibitor of lipoxygenase, 5,8,11,14-eicosatetraynoic acid, inhibited decay development by C. gloeosporioides.

Lipoxygenase activity in ripening avocado fruit was affected by an endogenous inhibitor, epicatechin, present in avocado peel (21). This flavan-3-ol competitively inhibited lipoxygenase activity. Its concentration decreased from 500 μg g⁻¹ fresh weight of peel in unripe fruits to 8 μg g⁻¹ fresh weight in ripe, symptom-expressing fruits. The concentration of epicatechin in fruits of a highly susceptible cultivar decreased in parallel with decreasing fruit firmness (Fig. 9), and symptom expression occurred when the epicatechin concentration was at its lowest level (21). In a resistant cultivar, however, the initial concentration of epicatechin was much higher and considerable epicatechin was still present in the peel of soft, ripe fruits. Decay resistance was therefore related to the period required for complete decrease of epicatechin levels in softening fruits. These results suggested that lipoxygenase activity and degradation of the antifungal diene might be regulated by epicatechin acting as a trap for free radicals (Fig. 10). This mechanism could, in principle, be exogenously modulated in various ways to test whether corresponding changes occurred in development of anthracnose disease in ripe avocado fruits.

Modulation of Diene Levels and Practical Disease Control

As with the experiments involving mango fruits, the considerable correlative evidence did not conclusively link altered concentrations of the avocado diene with decay resistance. We therefore searched for treatments that would modulate alterations in diene levels in ripening fruits and observed whether they led to concomitant changes in decay resistance. Levels of the antifungal diene in avocado fruit tissue could be increased by exogenously supplying inhibitors of lipoxygenase (16,22). For example, a commercial formulation of butylated hydroxy anisole, Xedaphen-20, sprayed on fruits in a packinghouse during the

Fig. 6. The commercial processes and time frame of harvest, storage, and ripening of avocado fruits.

Fig. 7. Changes in the concentration of the antifungal diene in avocado cv. Fuerte fruits at 20 C after heat treatment at 55 C: (A) Untreated control and (B) 10-min heat treatment.
first day after harvest, provided significant decay control (Fig. 11). Inhibition of lipoxyngeenase activity in these fruits was accompanied by a delayed decrease of the antifungal diene compound and reduced fungal colonization.

Freshly harvested fruits exposed to an atmosphere of 30% CO₂ also contained higher concentrations of the antifungal diene and showed reduced decay (Fig. 12) (24). However, several factors were critical to enhancing the levels of the preformed compound and achieving a significant delay of symptom development: 1) Concentrations of CO₂ below 30% and application for less than 24 hr were less effective, 2) fruits required treatment as soon after harvest as possible, and 3) more mature fruits were less capable of reacting to the CO₂ treatment.

In addition to providing independent evidence suggesting a role for the antifungal diene level in decay resistance, the results with Xedaphen-20 and high CO₂ atmosphere indicate that these treatments may be useful in postharvest decay control. While it is not yet clear from pilot scale experiments whether either treatment will in fact provide economical and effective postharvest disease control, they clearly constitute feasible and environmentally sound approaches.

**Potential Toxicity of Natural Compounds in Food Products**

One of the important considerations involved in attempts to enhance disease resistance by increasing concentrations of natural antifungal compounds is their potential toxicity to animals and humans. No information is available concerning potential toxicity of the avocado antifungal diene and monoene compounds, but several reports exist on toxic preformed compounds in other plants (3). In potatoes, α-solanine and α-chaconine are plasma cholinesterase inhibitors in humans and teratogens in animals. Because of the toxicity of these compounds, the potato cultivar Lenape was withdrawn from the market. Celery, parsley, and parsnip contain the linear furanocoumarin phytalexins psoralen, bergapten, and xanthotoxin, which can cause photosensitization and also are photomutagenic and photocarcinogenic. Sweet potato roots produce phytalexins that are toxic to vertebrates. Thus, preformed or induced plant antifungal compounds can be highly toxic, which may temper enthusiasm for elevating their levels in edible foodstuffs.

An optimal situation would be the induction of high levels of antifungal compounds for a limited period while fruits are at risk of pathogen attack, with decline of these levels before the produce is consumed. There is evidence that this occurs in some cases. In immature peppers inoculated with *Colletotrichum cap-

![Fig. 8. Diene levels (curves at left) and decay development by *Colletotrichum gloeosporioides* (curves at right) in avocado cv. Fuerte fruit peel exposed to ambient air (●) or 30% CO₂ (○) at 20 C. LSD = 0.05.](image)

![Fig. 9. Epicatechin concentration (▲) and firmness (○) of fruits of two avocado cultivars at various days after harvest. Arrows indicate the appearance of decay symptoms caused by *Colletotrichum gloeosporioides*.](image)

![Fig. 10. Apparent relationship between relative diene concentration (as thickness of arrow), lipoxyngeenase activity, and epicatechin levels during avocado fruit ripening and susceptibility to anthracnose decay.](image)
sici, the level of the phytoalexin capsicannol increased, but the compound was absent in ripening fruit at the onset of lesion expansion (1). In avocado, we have shown that the level of preformed compounds can be enhanced during fruit ripening but decreases markedly in ripe fruits. Although additional experimentation is needed, it appears that antifungal diene levels in avocado can be effectively modulated to control disease with minimal risk of toxicity during human consumption.

Conclusions

By means of comparative biochemical approaches, we elucidated putative defense mechanisms controlling postharvest disease in unripe avocado and mango fruits. Levels of the avocado diene compound and the 5-substituted resorcinols were high in unripe, disease-resistant fruit but decreased during fruit ripening, a time when latent fungal infections cause proliferating lesions that make fruit unmarketable. Such correlative evidence, however, does not prove a role for the diene compounds in resistance. To provide more critical tests, we employed various exogenous treatments that modulated the normal disappearance of the diene during the ripening of avocado fruits and observed whether the treatments concomitantly affected the development of fungal decay. The most effective measures were antioxidant and high carbon dioxide treatments, which inhibited decreases of the avocado antifungal diene compound during ripening or reduced the accumulation of higher levels. The fact that modulation of diene levels by these agents led to the predicted changes in fruit resistance constitutes important evidence that the diene is a physiologically important agent effecting resistance to C. gloeosporioides.

Treatments such as the antioxidants that affect levels of preformed antifungal compounds may be useful for practical disease control. For example, we are currently investigating the possibility of applying antioxidants or 30% carbon dioxide treatment of avocado fruits after harvest to control anthracnose disease during fruit storage and shipment. Increasing environmental pressure for reduced pesticide usage encourages experimentation to determine if use of preformed antifungal agents in fruits of other plants can be similarly modulated to provide postharvest disease control.

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Literature Cited

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