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Mechanisms of Resistance of Fruits and Vegetables to Postharvest Diseases

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I. THE BASIS FOR RESISTANCE IN FRUITS AND VEGETABLES

A fungal attack on fruits and vegetables can be separated into stages, including (a) the landing of spores on plant surfaces, (b) attachment of spores to those surfaces, (c) germination of spores, (d) production of penetration structures, (e) penetration into the plant, (f) colonization of plant tissues, and (g) formation of lesions followed by production of new crop of spores. Certain fungi and virtually all bacteria deviate from these generalized stages by not being able to penetrate the unbroken plant surface. These pathogens are restricted to an initial colonization of wounds or other unprotected tissues.

Resistance has been defined as an incompatible interaction between host and pathogen. In contrast, a compatible interaction leads to disease. Unfortunately, these are qualitative terms (the disease does or does not develop) and generally not applicable to the entire postharvest life of the fruit or vegetable. A compatible interaction is the outcome, in most cases, of the ability of the pathogen to overcome various host defenses. Incompatibility, involving processes in the plant that prevent or retard pathogen growth, may be conditioned by a single gene pair: a host resistance (R) gene and a pathogen avirulence gene (Flor, 1971). Although "gene for gene" interactions are extremely important and specific, they are not usually involved in the resistance of fruits and vegetables to postharvest diseases. Instead, resistance to postharvest pathogens is usually the result of several genes interacting in a way that is not well understood. Basic incompatibility between host and postharvest pathogen does occur, however. *Penicillium digitatum*, a pathogen of citrus fruit, does not attack apple or other deciduous fruits. This host specificity defines a relation-

ship between pathogen and plant that results in either the inhibition of pathogen activities responsible for infection and disease or no apparent inhibition of the pathogen at all. The genetic and physiological basis for this kind of specificity is unknown.

Most resistance in fruit and vegetables to postharvest pathogens can be described as a "dynamic" incompatibility. The response of the host's resistance genes to products of a pathogen's avirulent genes prevents or retards pathogen growth under specific host physiological conditions. The physiological state of the host changes, however, as it matures, ripens and senesces. Storage, mechanical injury, temperature extremes, and anoxia also alter host physiology. When physiological changes in the host inhibits defense responses to pathogen activities, the interaction becomes compatible. Pathogens such as *Botrytis* and *Colletotrichum*, which attack a broad range of hosts, usually remain quiescent during fruit development. As fruits ripen, inhibitors responsible for the quiescent state of the pathogen disappear, which allows the pathogen to resume growth. For this type of interaction, quantitative rather than qualitative reactions is the general rule in the postharvest host-pathogen interaction. The following questions apply to quantitative interactions: (a) What is the nature of preformed barriers to pathogen attack? (b) How do R genes trigger defense responses and what are the physiological conditions needed for triggering defense responses? (c) Do antifungal compounds occur in plant tissues, and what is their role in resistance? (d) How are secondary defense responses such as systemic acquired resistance (SAR) elicited and maintained?

II. CONSTITUTIVE RESISTANCE MECHANISMS

A. Inhibition of the Formation of Appressoria

Surface waxes on plants provide a specific signal for the production of appressoria by pathogens such as *C. gloeosporioides* (Kolattukudy et al. (1995). The waxes found on nonhosts do not induce the formation of these structures, which are essential for penetration of the plant surface (see Chap 20). Certain very long chain alcohols found in many plant waxes inhibit the formation of appressoria. For example, the addition of waxes extracted from leaves of broccoli, Jade and *Senecio odoris* to avocado wax inhibited the ability of the latter to induce appressorium formation by a pathogen of avocado. Thus, plant-surface lipids may induce or inhibit spore germination and appressorium formation. The balance between induction and inhibition might be responsible for the selective activation of the pathogen for initiation of parasitism.

B. Regulators of the Germination of Appressoria

Preformed volatile or nonvolatile chemicals in certain plants have been shown to inhibit the germination of appressoria (Muirhead, 1981). The failure of appressoria to germinate may also result from the lack of a triggering factor, i.e., an inducer of germination that appears only during specific periods of fruit life. Flaishman and Kolattukudy (1994) suggested that ethylene produced by the host specifically at ripening could initiate germination of appressoria on the fruit surface. Ethylene at concentrations of less than 1 μL/L, which is much lower than those produced during fruit ripening, induced both spore germination and formation of appressoria by *C. gloeosporioides*. However, Prusky et al. (1996) reported a failure of ethylene treatments to induce decay in unripe avocado fruits that had been inoculated with *C. gloeosporioides*. They concluded that even if ethylene induced

the formation of multiple appressoria, several other changes must occur in the fruit peel before the pathogen could infect.

C. Inhibition of Penetration of Cuticles by Infection Pegs Produced from Appressoria

Several major postharvest pathogens that directly penetrate host surfaces, such as *C. gloeosporioides* and *B. cinerea*, secrete cutinase at the plant/pathogen interface. Dickman et al. (1983) showed that application of cutinase inhibitors or cutinase-specific antibodies to an intact cuticle prevented penetration by *C. gloeosporioides*, whereas no inhibition occurred if the cuticle was breached. Moreover, insertion of genes for the production of cutinase obtained from *Fusarium* into the wound pathogen *Mycosphaerella caricae* allowed the transformants to directly penetrate intact papaya fruit (Dickman et al., 1989). However, Schafer (1994) questioned the importance of cutinase suggesting that certain fungi have mechanisms that compensate for a lack of the enzyme. Van Kan et al. (1997) noted that mutation of the genes for production of cutinase in *Botrytis cinerea* did not affect its ability to penetrate gerbera flowers. They concluded that germination of appressoria and the initiation of fungal attack is dependent on more than one single enzyme.

1. The Cuticle as a Physical Barrier to Fungal Penetration

Resistance in fruit to penetration by postharvest pathogens can be categorized as morphological or chemical. The morphology of the fruit surface may prevent the pathogen from penetrating. The resistance of peaches to infection by *Monilinia fructicola* was correlated with cuticle and cell-wall thickness (Adaskaveg et al., 1989, 1991; Michailides and Johnson, 1992). In peaches, the incubation period (time between inoculation and symptom expression) increased with increasing thickness of the cuticle and cell walls. Peach cultivars that were significantly more resistant had a thicker and denser epidermis than those of susceptible cultivars; resistance was correlated with a delay in penetration of the host by the pathogen and a longer incubation period (Adaskaveg et al., 1989, 1991). Michailides and Johnson (1992) reported a similar phenomenon in nectarines, where the latent period of *M. fructicola* decreased as the thickness of the cuticle decreased.

2. Chemical Inhibitors of the Penetration of Plant Cuticles by Fungi

Bostock et al. (1996) suggested that development of *M. fructicola* in unripe peach fruit is inhibited by phenolic acids in the fruit peel. Chlorigenic acid and caffeic acid are the major phenols in the epidermis and subepidermal cell layers of peach fruit. Although these chemicals might inhibit spore germination or germ tube elongation, the pathogen would appear to be more vulnerable to the effects of phenolics during the penetration stage of pathogenesis. As the penetration peg forces/dissolves its way through the cuticle, the pathogen is intimate contact with epidermal cells. Additionally, penetration is the last stage in pathogenesis before the pathogen colonizes host tissues, which means endogenous food reserves required for respiration and structure development would be at their lowest levels. The concentrations of phenolic acids are especially high in peach genotypes with high resistance to brown rot. With all genotypes, however, concentrations of phenolic acids and resistance to disease decline with fruit maturation. Chlorigenic and caffeic acid appear to contribute to brown rot resistance through an interference with production of cutinase rather than a direct effect on the pathogen.

3. Inhibitors of Pectolytic Enzymes May Also Contribute to Host Resistance to Penetration

Wattad et al. (1997) obtained antisera against purified pectate lyase from *C. gloeosporioides*. The antisera did not affect germination or germ tube growth. However, when applied to germinating conidia on avocado, mango, and banana, disease development was inhibited. Thus, pectate lyase inhibitors might be a constitutive barrier during very early stages of fruit infection.

D. Inhibition of Fungal Colonization by Constitutive Barriers

1. Restriction in Fungal Development Associated with Host Barriers

A highly anionic peroxidase was isolated from the exocarp of unripe tomato fruit (Sherf and Kolattukudy, 1993); it is encoded by a single gene (*tap*). The constitutive expression of *tap* undergoes a twofold increase as immature green fruits develop. As fruits approach their climacteric, however, *tap* transcriptions diminish until they are undetectable in ripe fruits, which are susceptible to decay. When fruit are harvested green, however, *tap* transcripts disappear within 48 h. The rapid decline in peroxidase mRNA levels observed in detached green fruits, may possibly result from loss of a regulatory molecule that originates in the parent plant and functions to sustain the constitutive expression of the gene in maturing green tomatoes. The anionic peroxidase is a key enzyme in polymerization of phenolic polymers, which render the cell walls highly resistant to mechanical and enzymatic disruption (Kolattukudy, 1987; Pearce and Rutherford, 1981). Lignification and suberization of plant cell walls have been suggested as being part of the elaborate defense strategies of the plant. It is, therefore, possible that constitutive expression of an anionic peroxidase in unwounded green tomato fruits represents a barrier to fungal development prior to harvest.

2. The Inhibition of Fungal Development by Preformed Compounds in the Host

Plants produce a diverse array of secondary metabolites that are toxic to fungi (Verhoeff, 1974; Schoenbek and Schlosser, 1976; Swinburne, 1983; Osbourn, 1996b). These compounds might inhibit germinated spores in wounds, germ tubes, or hyphae as they penetrate through wounds or pathogens that have penetrated the host directly and have already overcome the first set of barriers. Certain of these compounds are in a biologically active form, whereas others are inactive precursors that become activated in response to pathogen attack (Osbourn, 1996a). These preformed compounds or "phytoanticipins" differ from the inducible phytoalexins that are synthesized from remote precursors in response to pathogen attack.

Preformed inhibitors of pathogen development tend to be concentrated in the outer layers of plant organs, providing evidence for a chemical barrier against pest attack (Bennet and Wallsgrove, 1994). In onion scales, diffusible preformed compounds like catechol and protocatechuic acid influence fungal growth at the plant surface (Osbourn, 1996b). More often, however, preformed compounds are compartmentalized in vacuoles or organelles in healthy plants, such as gossypol in cotton. Prusky and Keen (1995) found that 85% of an antifungal diene in avocado mesocarp (flesh) was compartmentalized in specific oil cells, whereas the peel contained uniform concentrations. The antifungal activity in the mesocarp appeared to depend on the extent of fungal damage and the amount of

chemical released. The distribution of the dienes is consistent with susceptibility of wounded tissue to fungal attack, whereas the intact peel remains resistant (Kobiler et al., 1994). Specific signals originating from the pathogen could enhance release of preformed compounds from storage to active sites. The amount of chemical available could depend on the plant genotype, plant age, and environmental conditions (Davis, 1991).

There have been numerous attempts to associate natural variation in levels of preformed inhibitors in plants with resistance to particular pathogens, but only a few critical tests have been described (Prusky, 1997). The relationship of preformed compounds to fruit resistance is unclear because of a lack of knowledge about the distribution and concentration of inhibitors in infection courts, the sensitivity of pathogens to inhibitors in the plant, and inhibitor concentrations, and host resistance.

Use of mutant plants that lack preformed inhibitors would allow a direct genetic test of the importance of such compounds in host defense, but this has not been done. Instead, correlative data have been used to evaluate the importance of the concentration of preformed compounds in host resistance. A decrease in concentrations of preformed chemicals in ripening or senescing fruits has been correlated with increased susceptibility to disease in fruit of avocado, mango, and citrus as well as stalks of celery (Prusky et al., 1991, Prusky, 1996). In avocado, the resistance of unripe fruits to infection by *C. gloeosporioides* is correlated with high concentrations of preformed antifungal compounds. The predominant chemical detected was 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene. The concentration of this compound decreased tenfold as the fruit ripened and became susceptible to decay.

A mixture of 5-12-*cis*-heptadecenyl resorcinol and 5-pentadecenyl resorcinol was found at fungitoxic levels in the peel of unripe mango fruits that were resistant to *Alternaria alternata* (Droby et al., 1986, 1987). These chemicals disappeared as the fruit ripened and symptoms of decay appeared in inoculated fruits.

Citrus fruits are resistant to wound pathogens during fruit growth, even though considerable amounts of inoculum maybe present. Ben Yehoshua and coworkers (1992, 1995) suggested that the resistance of young mature green lemons is related to the presence of citral, a preformed monoterpene aldehyde that decreases in older yellow fruits, enabling decay to develop rapidly (Rodov et al. 1995).

Resistance of celery stalks to *Botrytis* could result from the presence of marmesin, the preformed antifungal psoralen precursor. This chemical is almost 10 times more fungicidal than the inducible psoralens that were previously considered to condition celery resistance to insect and microbial pests. A decreased concentration of marmesin during storage, and not the induction of psoralens, is the main factor affecting the resistance of celery to *B. cinerea* (Afek et al., 1995). A differential decrease in preformed compounds during the period of increased susceptibility has been associated with varietal resistance to decay. Fruits of mango cv. Tommy Atkins are more susceptible to decay and experience a more rapid loss of preformed fungicides than do those of "Hayden" (Droby et al., 1986). Also, symptoms of decay appeared earlier in inoculated avocado fruits that lost antifungal compounds more rapidly during ripening (Prusky et al., 1988).

The loss of the natural fungicides in fruits that accompanies ripening may be related to enzymatic changes. The antifungal diene in avocado fruits is as a substrate for oxidation by a lipoxygenase that is activated during fruit ripening (Prusky, 1988; Prusky et al., 1983). Lipoxygenase activity in avocado fruit is affected by epicatechin, an endogenous inhibitor present in the avocado peel (Prusky and Keen, 1993, Prusky et al., 1988). In green fruits this flavan-3-ol competitively inhibits lipoxygenase activity. As the concentration

decreases during fruit ripening, lipoxygenase activity increases until the fruit become completely susceptible.

3. Induction of Preformed Natural Fungicides

Although preformed chemicals have been considered noninducible (Van Etten et al., 1994), significant increases in the level of constitutive fungal inhibitors have been associated with exposure of tissues to different biotic and abiotic elicitors (Prusky and Keen, 1993). Challenge inoculation in unripe fruits can also induce an increase in the concentration of preformed antifungal compounds (Prusky et al., 1990). Inoculation of unripe avocado fruits with spores of a mutant of *Colletotrichum magna*, a nonpathogenic fungus, induced a significant increase in levels of the antifungal diene. The diene remained at antifungal levels for a longer duration (than controls) and symptoms of disease were not observed (Prusky et al., 1994). When fruit were co-inoculated with the mutant and a pathogenic *C. gloeosporioides*, symptom development was delayed as compared with inoculation by *C. gloeosporioides* alone. The effect of the mutant is consistent with the theory that the resumption of fungus development in quiescent infections depends on significant changes that are induced in the host during fruit ripening. These observations suggest but do not always conclusively prove that disease resistance in unripe fruits results from toxic levels of antifungal compounds.

4. Modulation of Preformed Resistance by Pathogens

The possibility that a pathogen's virulence could be determined by its ability to detoxify preformed antifungal compounds in the host was raised by Osbourn (1996b). Preformed saponins exhibit potent antifungal activity and are often present at relatively high concentrations in healthy plants (Osbourn, 1996a). The major saponin in tomato is the steroidal glycoalkaloid α-tomatin, which is particularly concentrated in leaves, flowers, and green fruits (Roddick, 1974). During fruit ripening, however, there is a significant reduction of α-tomatin in the fruit, which suggests a possible function of saponins in the resistance of tomato fruit to certain pathogens. Osbourn et al. (1994a,b, 1995) and Sandrock and Van Etten (1995, 1997) cloned a number of saponin-detoxifying glycosyl hydrolase genes produced by fungal pathogens of tomato fruit. Disruption of the β-tomatinase gene in *Septoria lycopersici* caused the mutants to be sensitive to α-tomatine and non-pathogenic in tomato fruits. A similar approach with similar results was done with *Colletotrichum coccoides*, which causes anthracnose in tomato fruits.

E. Inhibition of Pathogenicity Factors by Constitutive Barriers

1. Preformed Phenols Inhibiting Pathogenicity Factors

Phenols have been reported as possible inhibitors of *B. cinerea* in strawberries. *Botrytis* infects strawberry fruits via the floral parts, but the fungus remains quiescent at the bottom of the receptacle until the fruit matures. Infection by direct penetration through the epidermis of the fruit is relatively rare (Jersch et al., 1989). The inhibition of fungal development in unripe fruit could be entirely attributed to the presence of proanthocyanidins, which are present in a solid layer beneath the epidermis and surrounding the receptacle. These compounds are oligomers, composed of pelargonidin, cyanidin or delphinidin sub-units, with cyanidin being the most common. Since Huth and Schlosser (1982) consider that extracellular hydrolases are essential for pathogenesis of *B. cinerea*, their binding to proanthocyanidins and consequent inactivation would explain the resistance of unripe fruits to this pathogen. When fruits mature, the proanthocyanidins have become highly polymer-

ized and, as such, have lost their inhibitory power (Jersch et al., 1989). Pathogen enzymes produced at this stage produce a rapid development of decay. Similarly, proanthocyanidins have also been found mainly in the skin of the grape. Their concentration in young grapes is sufficient to inhibit the extracellular hydrolases of *B. cinerea* completely, but as the grapes mature and ripen, the inhibitory power declines steadily (Hill et al., 1981).

Inactivation of pectic enzymes by inhibitors has been hypothesized to be a mechanism of host resistance for moderating fungal pathogenicity (Schlosser, 1994). In avocado peel, the epicatechin that inhibits the oxidation of antifungal compounds, as discussed above, also inhibits pectolytic enzymes produced by *C. gloeosporioides*. Purified polygalacturonase and pectate lyase produced by *C. gloeosporioides* were inhibited in vitro by epicatechin (Prusky et al., 1989; Wattad et al., 1994). At 20 μg/mL, epicatechin inhibited the ability of these enzymes to macerate avocado wedges by 64%. Levels of epicatechins in unripe fruit, 350 μg/g fresh weight or about 270 μg/mL, greatly exceed minimum inhibitory concentrations, evidence that this flavan contributes significantly to the resistance of avocado fruits to *C. gloeosoporioides* and *C. musae*.

2. Preformed Proteins Affecting Host Resistance

Plants contain proteinase inhibitors that may inhibit enzymes necessary for pathogen development (Lorito et al., 1994). Plant cell walls contain specific and effective inhibitors of polygalacturonases (PG) of fungal origin. These PG inhibiting proteins (PGIPs) have been found in numerous plant species, including pear fruit (Abu-Goukh et al., 1983a), apple fruit (Yao et al., 1995), pepper fruit (Brown and Adikaram 1983), bean leaves (Cervone et al., 1987), alfalfa leaves (Degra et al. 1988), soybean (Favaron et al., 1994), raspberry fruit (Johnson et al., 1993), and tomato fruit (Stotz et al., 1994). PGIPs are relatively heat stable glycoproteins that inhibit fungal PG by both competitive and noncompetitive mechanisms. Purified pear PGIPs inhibited various fungal PGs, including that of *B. cinerea*, but did not affect endogenous PG activity (Abu-Goukh et al., 1983b). PGIP activity was observed throughout the development of pear fruit (4 to 14 weeks after anthesis). Changes in susceptibility to decay in ripening fruit (cv. Bartlett) were accompanied by a decrease in the concentration of PGIP. PGIPs from different plant species are likely to differ in their inhibition kinetics and target specificity (Abu-Goukh et al., 1983a,b). The PGIP concentration was approximately 100 times more abundant in fruit than in flowers and was not detectable in pear leaves. By contrast, the activity of these proteins in fruit exceeded that in flowers or leaves by 200 or 1400 times, respectively. Stotz et al. (1993) suggested that the pear PGIP promoter might effect a fruit-specific expression of the gene, resulting in the inhibition of *B. cinerea* (Stotz et al., 1993). Powell et al. (1994) reported that transgenic tomato fruits expressing the pear PGIP gene were more resistant to *B. cinerea* than control fruits. However, in field tests, the transgenic tomato line did not have the degree of resistance found in greenhouse tests (Labavitch, personal communication).

Adikaram et al. (1997) described the presence of constitutive chitinase(s) and protease(s) in papaya latex. These enzymes appeared to inhibit infection by *C. gloeosporioides*. Papaya fruit became susceptible to this pathogen only after the latex secretion from the peel or stem slowed.

III. INDUCIBLE MECHANISMS OF RESISTANCE

There is a considerable amount of information on the role of induced antimicrobial chemicals in disease resistance in plants (Kuc, 1987). Most of this information has limited rele-

vance to postharvest pathology, since it has been obtained from plants or organs that are not normally stored or consumed. If a correlation existed in stored fruit or vegetables between accumulation of a chemical and tissue resistance to attack, then induced antifungal compounds could be considered as part of host resistance mechanisms against postharvest pathogens. However, with the exception of highly specific fungal *Avr* gene interactions with host R genes, there is no qualitative resistance in harvested organs. However, an accumulation of toxic levels of phytoalexins in tissues during early stages of infection is a possible indication of their role as a resistance factor. These induced chemicals might restrict pathogen or lesion development but are unlikely to confer absolute resistance (Kuc, 1987). The rate of phytoalexin accumulation is particularly important, as it can determine the outcome of an interaction. Resistant hosts usually accumulate phytoalexins more rapidly than susceptible hosts do (Kuc, 1994). In either case, the amount of phytoalexins produced in response to an elicitor usually diminishes during ripening and/or storage. Such a drop in phytoalexin production was noted after treatment of maturing grapes and stored carrots with abiotic elicitors and was accompanied by a concomitant increase in host susceptibility to *B. cinerea* (Creasy and Coffee, 1988, Mercier et al., 1993a). Other evidence indicating a role of phytoalexins in disease resistance is the reduced pathogen development that accompanies the induction of phytoalexins. Cell-wall hydrolysates of *Glomerella cingulata* enhanced phytoalexin accumulation in pepper (Adikaram et al., 1982). No rot developed on wounded fruits that had been treated with cell-wall hydrolysates 24 or 48 h before inoculation with *B. cinerea*. Carrots, kumquats, and lemons exposed to a low dosage of short-wave ultraviolet (UV) light prior to storage accumulated phytoalexins and became more resistant to storage pathogens (Ben-Yehoshua et al., 1992; Mercier et al., 1993a,b). There was a significant correlation between the levels of 6-methoxymellein in the peel of UV treated carrots and their resistance to *B. cinerea*.

Bramley's seedling apples resist development of *Nectria galligena* prior to harvest apparently because fruit tissues produce fungitoxic levels of benzoic acid in response to fungal attacks. *N. galligena* invades wounds and lenticels of apple fruits before harvest, but fruit rotting does not become severe until after harvest (Swinburne, 1971). Limited colonization takes place following the initial invasion and the synthesis of benzoic acid in the necrotic tissue was readily observed (Swinburne, 1975). A protease produced by the pathogen elicited the formation of benzoic acid (Swinburne, 1975). Two other apple pathogens that infect prior to harvest and then become quiescent, *Diaporthe perniciosa* and *Gloeosporium perennans*, also secreted proteases in vivo. However, five pathogens of apple fruit including—*Penicillium expansum*, *B. cinerea*, *Phytophthora cactorum*, *Sclerotinia fructigena*, and *Aspergillus niger*—do not produce protease in infected tissue, do not induce production of benzoic acid and consequently can successfully infect immature fruit. Benzoic acid is toxic only as the undissociated molecule. When fruits ripen, the tissue pH increases and the acid dissociates, thereby losing its toxicity (Brown and Swinburne, 1973). This, in conjunction with increasing sugar levels, enables the pathogen to degrade benzoic acid and resume active growth.

Several chemicals accumulate in arrested lesions in banana fruit. Resistance of green banana to *C. musae* was associated with a growing necrotic reaction within the peel (Brown and Swinburne, 1980, 1981), and five antifungal compounds not present in healthy tissue were isolated. The concentration of these unidentified compounds diminished as the fruit ripened. Hirai et al. (in Abayasekera et al., 1997) identified one compound as 2-(4′-hydroxyphenyl)-naphthalic anhydride. Luis et al. (in Abayasekera et al., 1997) described six phenalenone-type phytoalexins, called irenolone, emenolone, and musanolones

C to F. However, the antifungal properties of these chemicals have not been clearly determined. Two of the phenalenone group also accumulated when *Phyllosticta musarum* infected fruit. This fungus causes pinhead-sized spots on the peel described as freckles (Abayasekera et al., 1997). The effect of these phytoalexins on development of *P. musarum* is unknown.

Stange et al. (1993) reported that a "wound gum" accumulated in injured lemon exocarp. The gum was composed primarily of 3-[4-hydroxy-3-(methyl-2-butenyl)phenyl]-2-(E)-propenal. This compound increased in concentration as a function of time, resulting in the complete inhibition of the invading pathogen. In several citrus fruits including kumquat accumulation of scoparone (6,7-dimethoxycoumarin) was observed when the fruits were held at 36°C after inoculation with *P. digitatum* (Ben-Yehoshua et al., 1992). However, in lemons stored at 17°C only low concentrations of scoparone were detected in wound-inoculated fruits, perhaps because decay developed rapidly (Kim et al., 1991). Incubation of lemons at 36°C for a period of 3 days, starting 24 h after inoculation, enhanced scoparone accumulation and prevented decay. Inhibitory levels of scoparone were present in the inoculated flavedo 24 h after the beginning of the heat treatment and accumulation continued for 6 days after fruits had been transferred back to 17°C. The heat treatment, which does not induce production of scoparone by itself, may have restricted growth of the pathogen until the phytoalexin had accumulated to toxic levels and/or accelerated the defense response in the presence of the pathogen.

In response to infection by *B. cinerea*, the leaves of *Vitis* spp. produce the stilbene resveratrol, which is converted into the antifungal trimer ε-viniferin (Langcake and McCarthy, 1979; Pryce and Langcake, 1977). The viniferins are constitutive compounds in lignified tissue but induced in leaves. Young leaves are highly susceptible to *B. cinerea* and synthesize only a small amount of resveratrol. As leaves mature, their resistance and their ability to synthesize resveratrol increase concomitantly. With fruits, however, the situation is reversed. Immature grapes synthesize large amounts of resveratrol; but, as they mature, the ability to synthesize it decreases steadily and the fruits become susceptible.

In grapevine leaves, an efficient elicitation factor may alter the activity of the rate-limiting enzyme, leading to stilbene formation. Stilbene synthase activity is the rate-limiting step and various biotic and UV-light treatments activated the stilbene synthase gene to different levels (Schroder et al., 1988. Lanz et al., 1990). In excess of seven genes encode for stilbene synthase, but only two are expressed at higher rates when leaves are exposed to elicitation (Melchior and Kindl, 1991; Weise et al., 1994). A simple way to increase resistance in grapes is to expose the tissues to low levels of ozone (Sarig et al., 1996), which also induces resistance in the fruit to *Rhizopus stolonifer*. Fruit of several *Vitis vinifera* cultivars that had been exposed to ozone for 10 min contained levels of resveratrol, similar to those found in leaves exposed to UV-C (Sarig et al., 1996). Ozone induction of host defenses against disease has been attributed to the induction of the phenylpropanoid pathway and other systems (Eckney-Kaltenbach et al., 1994). In tests with transgenic tobacco (Hahn et al., 1993) and tomato (Kindl, 1994), transfer of the stilbene synthase gene to a plant not capable of forming stilbene led to the production of stilbenes by the transformants, which as a result became resistant to certain diseases.

Phytoalexins are induced at the initial stage of certain fungal infections of pepper fruits. The capsicannol phytoalexins, 1-deoxycapsidion and endesmadienol, were associated with the induction of resistance in *Capsicum annuum* fruits infected with *Glomerella cingulata* (Adikaram et al., 1988). When harvested unripe fruits were inoculated with *G. cingulata*, capsicannol accumulated readily. In ripening fruits, both capsicannol and cap-

sidiol accumulated but, when lesion expansion occurred, both compounds were absent. These compounds may also accumulate in arrested lesions caused by *B. cinerea*.

An isocoumarin, 6-methoxymellein, is the major phytoalexin accumulating in carrot root tissues in response to infection by the storage pathogens *B. cinerea*, *Mycocentrospora acerina*, and *Sclerotinia sclerotiorum* (Coxon et al, 1973, Davis and Lewis, 1981; Garrod et al. 1978; Harding and Heale, 1980, Kurosaki and Nishi, 1983). In carrots held at low temperature and inoculated with *B. cinerea* or *M. acerina*, 6-methoxymellein accumulated to inhibitory concentrations at the site of inoculation. Roots that had lost 10% or 15% of their fresh weight were more susceptible to *B. cinerea* and had a reduced capability of producing 6-methoxymellein (Goodliffe and Heale, 1978).

Lettuce leaves inoculated with *B. cinerea* accumulated the terpenoid lettucenin A (Bennet et al., 1994). By 3 days after inoculation with conidia of this fungus, the phytoalexin concentration in the infection court inhibited spore germination and germ tube growth. Only a few spreading lesions were observed. By contrast, inoculation with mycelia of the pathogen, which apparently overwhelmed the phytoalexin response, led to a large number of spreading lesions.

Stressed or inoculated potato tissues develop numerous phytoalexins and stress metabolites. The chemicals produced and the amounts accumulated in inoculated tissues vary with the pathogen, cultivar, and environment (Ghanekar et al., 1984; Lyon, 1984; Price et al., 1976; Varns et al., 1971). The sesquiterpene risihitin may be associated with the resistance to bacterial soft rot caused by *Erwinia carotovora*, which it inhibits in vitro (Lyon and Bayliss, 1975). Amounts of rishitin detected in tissues with restricted rot were twofold higher than in tissues with extensive rot (Lyon et al., 1975). However, higher incubation temperatures increase tuber susceptibility but do not affect the accumulation of rishitin (Ghanekar et al., 1984; Lyon, 1984; Lyon and Bayliss, 1975). Low oxygen, which suppresses rishitin accumulation, enhanced the development of soft rot (Ghanekar et al., 1984). Potato tubers with resistance to bacterial soft rot accumulate higher levels of phytoalexins than do more susceptible tubers, but whether resistance is dependent on the phytoalexins is not clear.

Inoculation of sweet potato with *Ceratocystis fimbriata* led to accumulation of the furanoterpene, ipomeamarone and the coumarins umbelliferone and scopoletin (Akazawa and Wada, 1961; Minamikawa et al., 1963). The accumulation of these three compounds was rapid in root slices of a resistant cultivar, especially between 24 and 72 h after inoculation. Ipomeamarone levels at 72 h after inoculation were twice as high in resistant as compared with susceptible cultivars, whereas the umbelliferone levels in resistant cultivars were more than three times higher than levels in susceptible cultivars (Minamikawa et al., 1963).

Phytoalexins have also been detected in several other vegetables. Eggplant was found to produce lubumin and other sesquiterpenes in response to inoculation with *B. cinerea*, *Fusarium oxysporum*, and several nonpathogens (Ward et al., 1975). Two phytoalexins have been detected in onions inoculated with *B. cinerea*, 1,3-dion-5octyl-cyclopentane and 1,3-dion-5-hexyl-cyclopentane.

IV. THE INDUCTION OF PATHOGEN DEVELOPMENT BY CHANGES IN THE HOST'S PHYSIOLOGICAL MATURITY

Simmonds (1941) suggested that the resistance of fruits and vegetables to decay could be attributed to failure of the pathogen to produce adequate levels of pathogenicity factors,

such as cutinolytic or pectolytic enzymes. This type of host resistance would disappear as the ripening process produced changes in the cell-wall structure that allowed pathogen attack (Bateman, and Basham, 1976.). However, inadequate pectolytic enzyme activity could result from a lack of sufficient constitutive enzymes and/or inducers for induced enzymes. Alternatively, pathogen enzymes might be produced but activity is blocked by cation cross-linking or pathogen enzymes are inhibited or inactivated by substances present in immature fruit, as described above (Prusky, 1996). *C. gloeosporioides* and *A. alternate* readily macerated (within 1 day) the mesocarp of peeled fruits of unharvested or freshly harvested unripe avocado and mango fruits before the occurrence of any physiological changes associated with ripening. Infection of the peel (pericarp) of the same unripe fruit resulted in quiescent infections that became active 14 to 20 days after inoculation (Kobiler et al., 1994; Prusky, 1996). These observations provide evidence that there is a difference in susceptibility between the peel (pericarp) and the flesh (mesocarp) of unripe avocado and mango fruits and that the biochemical changes in the flesh during fruit ripening are not related directly to activation of quiescent infections in the peel.

Endogenous cell-wall-degrading enzymes in fruit might activate fungal pathogenicity factors, which would make the fruit susceptible. Expression of the tomato PG during the fruit climacteric is temporally correlated with the susceptibility of the fruit to fungal infection. Severity of postharvest diseases in transgenic tomato fruit lines is a function of the level of polygalacturonase expressed by the plant, suggesting that developmentally regulated plant genes can be biochemical determinants of susceptibility. Furthermore, PG-solubilized pectic polysaccharides from tomato may act as signal molecules that specifically induce pathogenicity factors. Thus, a plant and its pathogens might invoke a highly evolved and complementary signaling process that allows the pathogen to cause disease (Labavitch, personal communication).

The presence of blocked cross-linked bonds in the cell-wall pectin may affect enzymatic degradation. The configuration of the polygalacturonic chain allows spaces for the binding of a series of cations between carboxyl groups. The formation of cation bridges between pectic acid molecules may make the cell wall less accessible to enzymes produced by fungal pathogens that cause decay (Tepfer and Taylor, 1981). Increasing the Ca^{2+} content of apples by means of preharvest sprays and postharvest dips reduces postharvest decay (Conway et al., 1988). Such treatments were found to significantly increase the number of salt bridges and consequently the structural integrity of the cell wall, thus reducing the vulnerability of the cell walls to maceration.

V. THE RELEVANCE OF PREFORMED AND INDUCIBLE BARRIERS FOR CONTROLLING POSTHARVEST DISEASES

The manipulation of concentrations of preformed and induced antifungal chemicals in harvested fruits and vegetables is a logical approach to developing new, improved controls for postharvest diseases. This might be accomplished by preventing the loss of inhibitory compounds, enhancing the concentration or activity of inhibitory compounds, or selecting plant lines that are rich in such antifungal compounds. The assumptions underlying this strategy are that such compounds (a) are safe because they are natural; (b) are effective, since they have evolved in nature specifically for protecting the plant against pests; and (c) have enabled existing plants to survive the selection pressure of evolution. However, naturally occurring compounds are not necessarily safe (Osbourn, 1996a); many of the world's most potent poisons are derived from plants, and some plant tissues are extremely

toxic to animals because they contain protective compounds (e.g., potato, tomato, and tobacco foliage). Furthermore, the levels of preformed compounds can change during the hot's lifetime; usually they decrease as the fruit or vegetable becomes ready for market (Prusky and Keen, 1993), thus a specific induction may be required to protect the plant during the marketing period. The exogenous application of naturally occurring defense compounds to protect plants would be uneconomical; their synthesis would be difficult and the cost of their isolation probably high.

In spite of the possible flaws in this scenario, the protection of the host during specific periods of susceptibility by manipulation of the levels of the natural compounds seems to be a safe way to preserve host resistance. For example, high level of the antifungal diene could be maintained in stored avocado fruits, but the inhibitor would be allowed to disappear before consumption of the fruit. An alternative to the external application of defense compounds is their elicitation, specifically or not specifically, within the plant, by regulation of gene expression and hence of the gene product, leading to the synthesis and further accumulation of the desired compounds. Biotic and abiotic factors have been shown to stimulate plant tissues to produce higher levels of performed antifungal compounds (Ingham, 1973; Prusky and Keen, 1993; Van Etten et al., 1994). This type of stimulation may also increase phytoanticipin concentration. The induction of preformed compounds by inoculation of avocado with nonpathogenic strains of *C. magna* suggests that this could be done by biological means. However, the use of biological or physical elicitors (CO_2, UV-C, γ-radiation) has not always proved effective because the host, e.g., avocado, is receptive to the signals only during very specific periods after harvest (Prusky and Keen, 1995).

Triggering early phytoalexin accumulation by treatment of fruits and vegetables prior to or at harvest with elicitors allows treated tissues to develop resistance early in the infection process. However, since many fruits and vegetables are cooled soon after harvest, the process of elicitation may be slowed, thereby affecting the resistance development. But cool temperatures would also slow the degradation of antifungal compounds as well as the pathogen's activities. Phytoalexin levels have been increased experimentally thereby inducing disease resistance in avocado (Adikaram et al., 1992), citrus (Ben Yehoshua et al., 1992), and carrots (Mercier et al., 1993a). To date, however, such treatments have not been adapted to commercial practice.

Suggestions have been made to enhance resistance barriers in plants by incorporation of specific inhibitors of fungal enzymes that detoxify preformed antifungal compounds. If ongoing studies of the preformed α-tomatine suggest that saponin-degrading enzymes may have a more general role in pathogenicity, then inhibitors of these enzymes could become attractive as a basis for disease control strategies (Osbourn et al., 1995; Sandrock and VanEtten, 1997). The extracellular location of the detoxifying enzymes should facilitate approaches involving the use of chemicals or the expression of saponinase inhibitors in genetically engineered plants, since there should be no requirements for inhibitors to penetrate fungal hyphae (Osbourn et al., 1995; Osbourn, 1996a).

The possibility of overexpressing compounds that have preformed activity in a susceptible host that does not express those compounds has also been tested. The transfer of stilbene synthase from grape to a nonproducing plant such as tobacco, leading to the synthesis and accumulation of resveratrol in the transformant, is one exciting example (Hahn et al., 1993); the transgenic tobacco, which synthesized and rapidly accumulated resveratrol, had enhanced resistance to *B. cinerea*. However, most plants have several preformed antifungal barriers and produce secondary metabolites by often complex bio-

synthetic pathways, making the transgenic approach difficult. Another possible way to increase basic constitutive barriers to decay in plants may be to engineer hosts to overexpress constitutive inhibitors such as PGIP or inducible inhibitors. Transgenic tomato plants expressing PGIP were more resistant to fungal attack than the nontransformed parent in the laboratory (Powell et al., 1994), but this resistance was not stable in the field (Labavitch, personal communication). Transgenic tobacco and rape plants containing an inducible bean chitinase gene with a constitutive promoter contain higher basal levels of chitinase and concomitant increased resistance to *Rhizoctonia solani* as compared with control plants (Broglie et al., 1991). In these cases resistance to fungal attack was obtained by "creating a host with a preformed barrier" as a result of engineering the plants to continuously overexpress a chitinase gene. In another study (Neuhaus et al., 1991), tobacco plants that had been transformed with a basic chitinase from tobacco under the regulation of a constitutive promoter accumulated up to 120 times more active chitinase than nontransformed plants. However, the transformed plants were found to be no more resistant to the fungus *Cercospora nicotiana* than were the controls. It is possible that the chitinase produced intracellularly in the transgenic plant was compartmentalized in such a way that it does not come into contact with the penetrating fungus. Alternatively, the activity of chitinase alone may not inhibit invading pathogens; instead, other antifungal chemicals acting in concert with chitinase provide resistance.

The general understanding of the plant biosynthetic pathways that lead to formation of preformed and inducible barriers and of their contribution to disease resistance is of primary importance for the manipulation of these compounds to enhance resistance. Such an understanding offers the possibility that the potential level of preformed barriers might be enhanced by genetic, biological, physical, and chemical means to provide the basis for novel crop protection strategies that could lead to the reduction of pesticide use in the future.

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