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The Postharvest Phase: Emerging Technologies for the Control of Fungal Diseases

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Postharvest biological treatment entails a range of different approaches, including strengthening of the commodity's natural defense mechanisms and application of antagonistic microorganisms and natural antimicrobial substances. Postharvest biological treatment has highlighted the potential of antagonistic microorganisms (fungi, bacteria and yeasts) against a limited number of pathogens, and only on specific hosts. Further studies are therefore required to identify antagonists with a broad spectrum of activity. The resistance of fruits and vegetables to postharvest diseases is closely linked to the ripening process, and drops markedly with the onset of tissue senescence: It is now possible to protect the product by inducing disease resistance. Plants produce a large number of secondary metabolites with antimicrobial effect on the main postharvest pathogens. Detailed studies have been conducted on aromatic compounds, essential oils and volatile substances. Combination of the above complementary techniques could well lead to effective control of postharvest fungal diseases.

KEY WORDS: Biological control; postharvest diseases; natural substances; natural defense mechanisms; antagonist microorganisms.

INTRODUCTION

Fruits and vegetables are highly perishable products, especially during the postharvest phase, when considerable losses (due to microbiological diseases, disorders, transpiration and senescence) can occur. Losses which are related to a ready-for-marketing commodity, with a high added value, are of economic importance.

Adequate storage methods, capable of delaying the ripening of fruit and of slowing down the development of pathogens, can prevent or at least reduce losses. Chemical treatment can ensure product protection, but is permitted for only a few species; in addition, public opinion demands a reduction in the use of chemical products (9). This latter issue, along with the appearance of pathogens resistant to fungicides (54) and of iatrogenic diseases (25), have contributed to arousing increased interest in the development of alternative methods for controlling plant pathogens, capable of integrating, if not totally replacing, synthetic fungicides.

The postharvest phase is suited to the application of biological control methods. In a restricted environment parameters such as temperature and relative humidity can be altered, and gas composition of storage room varied; intimate contact between the biological agent and pathogen improves antagonist activity (60), as does the absence of UV radiation (35).

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Along with the use of antagonist microorganisms, emerging postharvest biocontrol technology employs other approaches, such as intensification of the natural defense mechanisms commonly present in the fruit after harvesting and use of natural biocidal substances.

Antagonist microorganisms

A number of microorganisms (bacteria, yeasts and fungi), which effectively control postharvest pathogens, have been identified for postharvest control (61,63); some of these have been patented and registered (42).

Often antagonists are isolated on the surface of plants (51); this natural presence makes them more likely to succeed because of their colonization ability and environmental adaptation (61). Identifying antagonists in the endophyte microbial flora has only recently become a common practice (45), since the presence of bacteria in the tissues of fruits had generally been associated with pathological conditions (50) because such healthy tissues were considered sterile. However, preliminary investigations have revealed the presence of a bacterial population living and reproducing inside healthy fruit. In particular, two strains of *Bacillus pumilus* and *Bacillus amyloliquefaciens* were recognized to be effective against *Botrytis cinerea* in pears (37) and tomatoes (36).

The antagonist activity can be expressed in a number of ways: the most common are production of metabolites (26), competition (59) and direct parasitism, but other mechanisms are involved, for example induced resistance sometimes associated with reduction of pathogen enzyme activity (64).

In recent years the use of antibiotic-producing bacteria has been abandoned (63), in order to prevent the appearance of resistance in pathogen strains for man or animal. In addition, it is extremely difficult precisely to explain the antagonist mechanisms involved, even when they seem to be clear, as for the antibiotic-producing strains. This is the case of pyrrolnitrin produced by *Pseudomonas cepacia*, which can control strains of *Penicillium digitatum* resistant to this antibiotic (32,52).

The use of yeasts as antagonists appears to be quite promising, although the mechanism has not yet been fully elucidated. However, there are indications that competition for nutrients and space is involved, along with direct parasitism as for the US-7 strain of *Pichia guilliermondii*, antagonist of *P. digitatum* in grapefruit, and of *B. cinerea* in apples. In addition to the ability of yeast cells to grow very quickly (and thus to remove nutrients and space from the pathogen) (15), they are able to produce hydrolytic enzymes (β1-3 glucanase) capable of attacking the cell walls of *B. cinerea*, and extracellular polymers that appear to have antifungal activity (14). It is possible to hypothesize the induction of resistance in the host (20) through the accumulation of phytoalexins – like scoparone and scopoletin in citrus fruits (48) – and the production of chitinase.

In the postharvest control of fruits and vegetables it is also possible to use antagonist fungi: dip treatments using strains of *Trichoderma harzianum* (27) and *Trichoderma pseudokoningii* (57) has resulted in the reduction of *Monilinia laxa* in stone fruits and of *B. cinerea* in apples. The *Trichoderma* mechanisms involved differ, depending on antagonist strains; mycoparasitism is the most investigated.

For biological control to be effective, use of antagonists must be compatible with current handling and storage practices – such as low temperature storage – which could otherwise cause a reduction in the effectiveness of bacterial strains (31). Holding the fruits at 20°C for 24 h following treatment with the antagonist was found to enhance pathogen control (37); some strains of yeast have shown a higher level of antifungal activity if maintained at temperatures of 5-6°C (15,47).

Moisture affects the growth and performance of *Candida oleophila*, an antagonistic yeast which controls storage diseases of fruits. Biocontrol of gray mold rot was more effective when *C. oleophila* was applied to fresh wounds rather than to one-day-old wounds; in addition, moisture rapidly becomes a limiting factor for yeast growth (44). Also important is compatibility with certain common treatments, such as wax coating (29,41,46). The use of fungicides should not be excluded for integrated control. In fact, low doses of iprodione (50 ppm) in combination with the antagonist (*B. pumilus* and *B. amyloliquefaciens*) improved control of gray mold in pears, when compared with bacteria or iprodione used alone, while it also resulted in low chemical residue on the fruit (37). Similar results were obtained using yeasts, which were more effective against gray mold in pears when associated with a low dose of TBZ (10). The use of yeasts along with low rates of chemical fungicides against blue mold in citrus fruits has already been stressed by Droby *et al.* (16) as a possible integrated control approach.

The antifungal effectiveness of an antagonist can be increased by addition of substances such as calcium salts or sugar analogs; calcium chloride improves biological control of the yeast *P. guilliermondii* (18), while 2-deoxy-D-glucose, in combination with a mutant strain of *Sporobolomyces roseus*, produces a tenfold reduction in the concentration of the antagonist required for biocontrol of blue mold on apples (30).

It should be stressed that most of the antagonists identified are active against a limited number of pathogens and on specific crops (61) and that effectiveness is often cultivar-specific (Mari, unpublished data). It is also important to avoid strains that may harm plants of economic importance: in fact two strains of *Pseudomonas syringae* (ESC-10 and ESC-11) which partially control postharvest green mold on lemon and orange, were associated with significant virulence in Persian lime (53).

Natural defense mechanism enhancement

Fruits use a wide range of physical and biochemical strategies to defend themselves from attack by pathogenic microorganisms. These include accumulation of phytoalexin, modification of the cell walls and synthesis of antifungal hydrolases (3). However, postharvest resistance of fruit seems to be closely linked to the ripening process: resistance is induced in young tissue by preformed inhibitory substances, which gradually disappear during aging.

Use of low temperatures and atmospheres relatively rich in carbon dioxide and poor in oxygen delays aging of fruit and disappearance of preformed antimicrobial substances, partially – although not always adequately – reducing the losses due to pathogens (21).

Induction of the resistance response in fruit during the postharvest period shows considerable promise, as has been reported in previous reviews (5,13). Interesting results have been obtained using low doses of UV-C irradiation, which stimulate several biological processes such as respiration, biosynthesis of flavonoids and phytoalexin, and elicitation of pathogenesis-related proteins (28). Low-dose UV irradiation protects sweet pepper (1), mango (4), carrot (43) and citrus fruits (49) against postharvest storage rots and extends their storage life, increasing resistance to fungal pathogens. However, it is necessary to study certain aspects of on-line application of UV-C during fruit processing, including factors such as fruit ripening and storage temperature following treatment (17).

Prestorage heating may be useful to inhibit pathogen growth (11) and to stimulate the natural resistance of the fruits (55): in lemons, for example, heat treatment induced the accumulation of scoparone, which increased tissue resistance to infection (33). However, the heat sensitivity of certain fruits and vegetables and the energy required for heat treatment and subsequent refrigeration of the fruit must not be underestimated.

Recently, researchers have shown an interest in induced resistance as a method of controlling postharvest diseases. Induction of resistance involves a whole series of chemical and structural changes, which take place as a result of contact between the potential pathogen and the host (34). The ability to induce resistance has been demonstrated in carrots preserved and inoculated with *B. cinerea* (43); product immunization could be obtained by treatment with hypovirulent strains, elicitors and antagonists (2).

Natural substances

Plants produce a wide range of secondary metabolites; the essential oils produced by different genera are, in many cases, biologically active, endowed with antimicrobic, allelopathic, antioxidant and bioregulatory properties (22,24). The biological activity of the essential oils is due to the action of their components. A study of the fungicidal activity of oils obtained from such genera as *Ocimum*, *Thymus*, *Origanum*, *Anethum*, *Eucalyptus*, *Foeniculum* and *Citrus* against several postharvest pathogens, reveals the marked fungicidal activity of carvacrol (in thyme, origanum oil) and p-anisaldehyde (oxidation product of anethole, found in anise oil) (6). Certain volatile aromatic components produced by fruits during ripening also show antifungal activity. Acetaldehyde has been found effective in postharvest protection of apples (56), sweet cherries (40) and stone fruits (7). Hexanal and benzaldehyde, produced by etheric stone fruit metabolism, also have a fungistatic/fungicidal activity when utilized in postharvest treatment against *Monilinia laxa* and *Rhizopus stolonifer* (8). The use of these substances as antimicrobial agents can be an interesting field of investigation: toxicity to mammals is quite low, and their degree of volatility allows their use for fumigation in cold storage or for active packaging.

Among the numerous natural substances with potential antimicrobic activity are the glucosinolates, a large class of approximately 100 compounds produced by the Cruciferae, the antimicrobic activity of which is widely documented (23). When the cells of the plants which metabolize them are damaged, the glucosinolates come into contact with the enzyme myrosinase. This is also widely present in Cruciferae and catalyzes hydrolysis, producing D-glucose, sulfate ion and a series of compounds such as isothiocyanate, thiocyanate and nitrile, according to the substrate and the reaction conditions, foremost among them being the pH. The antifungal activity of six glucosinolates has been tested on several postharvest pathogens, both *in vitro* (38) and *in vivo* (39), with encouraging results. In particular, glucoraphenine isothiocyanate was seen to be active against *M. laxa* in artificially inoculated ‘Conference’ and ‘Kaiser’ pears, while allyl-isothiocyanate, used as a volatile substance, controls green mold in ‘Conference’ pears inoculated with a thiabendazole-resistant strain (Mari, unpublished data). The potential use of volatile fungicides to control postharvest diseases requires a detailed examination of their biological activity and dispersion in fruit tissues, and the development of a formula which inhibits the growth of pathogens at non-phytotoxic concentrations.

Natural antifungal compounds can be obtained from microbial fermentation; *e.g.* iturin, produced by *Bacillus subtilis* strains, is active against brown rot in peaches (26). An animal-derived polymer, chitosan, has evinced antifungal properties: it stimulates host production of defense enzymes (chitinase) and forms structural barriers in strawberry tissues, but can also delay ripening of strawberry, bell pepper and tomato when applied as a coating (19). Chitosan's compatibility with several antagonistic yeasts also appears to be appreciable, although its feasibility as an antifungal substance has yet to be confirmed in terms of its safety for human consumption and its effect on the organoleptic quality of fruits and vegetables (62).

CONCLUDING REMARKS

These observations make it evident that biological control of the fungal diseases that appear during the storage and sale of fruit is possible. It is clear, however, that none of the alternative methods proposed is capable, for the time being, of giving a level of control that is comparable to that obtained using synthetic fungicides (20). Most of the antagonists identified are active on only a limited number of pathogens, and on specific crops (58). Furthermore, differences in response exist, depending on the cultivar being tested. Therefore, although the postharvest phase appears ideal for the use of biological methods, it still represents a very small market segment for the production industry, and thus development of different antagonists is not justified economically.

Little is known about the fate of natural products in the environment, in foods, or in biological systems, including animals and humans (12). The intense sensory attributes of some of these compounds (*e.g.* isothiocyanate) may be an impediment to their use in fresh commodities. A better understanding of the mode of action and detailed toxicological studies will be needed to enhance their activity and to establish their safety. Counter arguments suggesting the greater safety of natural antimicrobial compounds as compared with synthetic pesticide (20) do exist.

The deliberate induction of defense reactions in advance of infection could inaugurate a biological defense system, but more information is required regarding the physiological changes that occur in harvested tissue. Complementary techniques, capable of improving the effectiveness of alternative methods, are being developed. In any event, the importance of handling practices – to avoid mechanical and physical damage – and of optimal storage conditions, cannot be underestimated.

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