



Biologically-based alternatives to synthetic fungicides for the control of postharvest diseases

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Recently, biological control has been advanced as an alternative to synthetic fungicides and considerable success in laboratory and pilot scale tests has been realized utilizing antagonistic microorganisms to control postharvest diseases. Several antagonistic yeasts and bacteria have been isolated and shown to have a broad spectrum of activity against a number of postharvest pathogens on a variety of fruit. However, for biological control methods to emerge as an economically viable option, their consistency and efficacy in controlling postharvest decay needs to be enhanced to a level comparable to that of synthetic fungicides. This could be possible through an integrated strategy that exploits the additive and synergistic effects of different biological approaches.

Keywords: biocontrol agents; postharvest decay; biofungicides; induced resistance

Introduction

Worldwide, postharvest losses have been estimated at 50% and much of this is due to fungal and bacterial infections. Postharvest infection can occur either prior to harvest or during the harvesting and subsequent handling and storage [23]. Disease development during the postharvest phase depends upon the physiological status of the tissue and the constitutive and inducible resistance mechanisms of the harvested produce [21]. In general, most harvested commodities are resistant to fungal infection during their early postharvest phase. However, during ripening and senescence, they become more susceptible to infection [7]. In developing countries, postharvest losses are often severe due to the lack of adequate handling and refrigerated controlled atmosphere storage facilities. While in developed nations losses may be lower, they are often just as serious from the producer's and consumer's standpoint.

Currently, synthetic fungicides are the primary means of controlling postharvest diseases [8,23]. However, growing concerns over the presence of chemical residues in the food chain, the development of fungicide-resistant strains of postharvest pathogens, and the revocation of registration of some of the more effective fungicides, have generated an interest in the development of safer alternatives to synthetic fungicides that are both effective and economically feasible. Several biological control approaches that include the use of antagonistic microorganisms, natural fungicides, and induced resistance have been shown to have a potential as antimicrobial preservative for harvested commodities [11,14,27,29]. The purpose of the present paper is to present an overview of the different biological approaches and the advantages of an integrated strategy.

Biocontrol approaches

Several promising biological control approaches that include use of antagonistic microorganisms, natural fungicides, and induced resistance are available for developing safer technologies for postharvest disease control. Among the proposed alternatives, development of antagonistic microorganisms has been the most studied and substantial progress has been made in this area [31].

Biocontrol microorganisms

In recent years, postharvest pathologists, because of the development of fungicide-resistant strains of postharvest pathogens, and the deregistration of some of the more effective fungicides, have intensified their exploratory and developmental research of biocontrol agents as substitutes for synthetic fungicides. From these efforts, substantial progress has been made and a large body of information regarding postharvest biocontrol antagonists is now available [28]. Several antagonistic microorganisms have been identified and shown to be effective against a number of postharvest pathogens on a variety of harvested commodities [15,17,20,31]. Presently, two antagonistic microorganisms, a yeast *Candida oleophila* Montrocher, and a bacterium *Pseudomonas syringae* are commercially available under the trade names Aspire™ and Biosave™, respectively. Although the mechanisms by which antagonistic yeasts exert their protectant effect have not been clearly elucidated, there are indications that it may involve nutrient competition, site exclusion, direct parasitism, and perhaps induced resistance [6,30].

Natural antifungal compounds

Several antifungal compounds of plant and animal origin are known to reduce the incidence of decay in a variety of harvested commodities. Reduction of postharvest decay was observed with plant extracts [2,22], a variety of essential oils and volatile substances [18,25,26] and natural compounds derived from microbial fermentation [13,16] and animal by-products [9]. Microbial-derived antibiotics

(iturin and pyrrolnitrin) have been shown to reduce postharvest decay of peaches, apples, pears, and strawberries [13,16]. Control of postharvest decay was also reported with chitosan, an animal-derived polymer, and 2-deoxy-D-glucose [9,12]. The control of decay by these agents is believed to originate, in part, from their antifungal property. The potential of naturally occurring biocides as specific target fungicides for the control of postharvest decay will depend on: (1) their safety for human consumption; (2) their effect on the quality attributes of harvested tissue; and (3) whether their use in agriculture could promote the development of antibiotic-resistant strains of animal and plant pathogens.

Induced resistance in postharvest commodities

Recently, considerable attention has been placed on induced resistance in harvested tissue as a potential form of postharvest disease control [11,29]. The reduction of postharvest decay by pre-storage treatment with fungal wall components [1,9,10] and UV light [4,19,24] suggests that induction of defense mechanisms has potential in reducing postharvest decay. Non-ionizing UV-C (from 190–280 nm) radiation has been shown to reduce decay in a variety of commodities and optimum doses of UV-C appear to occur in a rather narrow range depending on the commodity, the type of cultivar, and the physiological status of tissue [5,24]. In several commodities, UV-C treatment triggered a gradual development of tissue resistance that coincided with the induction and accumulation of phytoalexins [3,5,19]. Elicitation of disease-resistant responses was also observed with chitosan treatment. When applied as stem-scar treatment, chitosan caused an induction of several defense enzymes and the formation of physical barriers in strawberry and bell pepper fruit [9,10]. Expression of defense reactions by chitosan treatments seems to be implicated in the restriction of fungal infection. This was supported indirectly by the fact that the pathogen ingress was limited to the epidermal cells ruptured during wounding [13].

Attempts to exploit induced resistance through the application of defense response elicitors are being pursued in many laboratories. Control of postharvest diseases observed so far with various elicitors indicates that induction of defense responses in harvested crops is feasible and may offer a new strategy for disease control. However, since harvested commodities rely on their own reserves to maintain cellular organization, their ability to initiate defense responses is likely to decline with ripening. Any strategy aimed at exploiting the defense potential of fruits and vegetables should take into consideration the physiological status of the tissue and external factors that affect their physiology.

Although the various biocontrol approaches have been shown to reduce postharvest diseases, each alternative comes with limitations that can affect its commercial potential. Most antagonistic microorganisms provide only a protectant effect that diminishes with ripening and has no curative activity. Antagonists are most effective when applied prior to pathogen inoculation, a prerequisite that is difficult to meet under commercial conditions. This may prove to be a liability, especially since commercial packinghouse

fruit often come from different locations with variable inoculum loads, type of infections (latent and quiescent), physiological maturity, and levels of mechanical injury are often processed within 48–96 h of arrival. In most postharvest commodities, the elicitor-mediated resistance often provides non-persistent protection and its expression and magnitude are affected by the physiological state of tissue. The responsiveness of the harvested tissue is likely to decline during the ripening process, a period where the fruit are more susceptible to infection. More importantly, under commercial conditions none of the biological control approaches has been shown to offer consistent disease control comparable to that obtained with synthetic fungicides. For biological control to be accepted as an economically viable option, its consistency and efficacy in controlling postharvest diseases must be enhanced.

Multifaceted approach

The combination of complementary biological approaches for additive and/or synergistic effects could provide greater consistency and efficacy in biological control for postharvest diseases. Such biological strategies should also be expected to have greater stability and effectiveness than the use of a single biocontrol agent. Biocontrol activity of antagonists can be enhanced with several additives. Recently, we have developed a bioactive coating consisting of a unique combination of an animal-derived polymer with antifungal property and an antagonistic yeast. This combination makes it possible to exploit the antifungal and eliciting properties of the polymer, as well as the biological activity of the antagonist.

The results from a series of pilot tests on apple and citrus fruit showed that the bioactive coating was significantly more effective in controlling decay than either the antagonist or polymer alone. The bioactive coating was effective in controlling postharvest decay caused by *Botrytis cinerea*, *Penicillium expansum*, *Penicillium digitatum*, and *Penicillium italicum* on a variety of fruit. In a series of semi-commercial tests conducted in West Virginia, Florida, and California, the bioactive coating was very effective in controlling natural decay of major apple and citrus varieties. The level of disease control obtained with a bioactive coating on apple and citrus fruit was comparable to that obtained with the recommended fungicides Mertect and imazalil. The results obtained from semi-commercial tests demonstrate the potential of bioactive coating as a viable alternative to synthetic fungicides.

Synergistic effects of combined biological agents were also observed with combinations of UV-light treatment and antagonistic yeasts (Chalutz, Volcani Center, Israel and Stevens, Tuskegee University, personal communication) and a biocontrol product 'bioenhancer' which consists of a combination of an antagonistic yeast with a low dose of an antifungal sugar analog. In large-scale pilot tests on apple and citrus fruit the bioenhancer displayed greater stability and effectiveness in controlling natural infection than either the antagonist or sugar analog alone. The level of disease control obtained with the bioenhancer was comparable to that obtained with the recommended fungicides (imazalil and thiabendazole). The activity of bioenhancer appeared to

be due to synergistic interactions between the antagonistic activity of the yeast and the antifungal property of the sugar analog. In laboratory tests the bioenhancer, beside having a protectant effect, displayed a curative activity against major postharvest pathogens in a variety of fruit. The lack of curative activity has been identified as a major limitation of biological approaches. The results obtained from semi-commercial tests demonstrate the great potential of the bioactive coating and bioenhancer as antifungal preservative for harvested commodities. The multiple modes of action of the combined approach should make the development of pathogen resistance more difficult, present a highly effective disease deterrent barrier, provide a greater stability, and greater effectiveness than the approach utilizing single biological agents. However, before bioactive coating and bioenhancer can be used as an alternative to synthetic fungicides for the control of postharvest decay, their safety for human consumption and efficacy under commercial packinghouse conditions needs to be established. Available information suggests that the antagonist and additives in bioactive coating and bioenhancer are safe for human consumption [9,12].

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