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Combining biological control with physical and chemical treatments to control fruit decay after harvest

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Abstract
Purpose of review: This article reviews research on combining biological control with alternatives to conventional fungicide treatment in order to reduce postharvest decay on fruits. The basis for selection of the alternative treatments, the effectiveness of the combined treatments, and the feasibility and readiness of their implementation are discussed.
Findings: Biological control can be easily combined with a variety of other alternative treatments, some of which result in an additive or even synergistic effect in improving control of fruit decay. The effectiveness of the combined treatments was often comparable with conventional fungicide treatments. The combinations were often complementary; the alternative treatments provided an eradicative effect that was short lived while biocontrol had a long lasting effect on protecting the wounds.
Directions for future research: More large scale trials are needed to prove the feasibility of combining these treatments. The cost analysis of these treatments is also needed in order to determine the practicality of their implementation. To increase the spectrum of pathogens which can be successfully controlled, research is needed to address other host/pathogen interactions, especially latent infections of fruits.

Keywords: postharvest biocontrol; alternative treatments; integrated control; fruit rots

Abbreviations

2-DOG	2-Deoxy-D-glucose
BCPD	Biological Control of Postharvest Disease
BRR	Black Root Rot
CA	Controlled Atmosphere
HWRB	Hot Water Brushing
IAA	Indole-3-acetic Acid
JA	Jasmonic Acid
MA	Modified Atmosphere
MAP	Modified Atmosphere Packaging
MCP	1-Methylcyclopropene
MeJa	Methyl Jasmonate
SA	Salicylic Acid
SBC	Sodium Bicarbonate
SC	Sodium Carbonate
TBZ	Thiabendazole

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Introduction

It has been 25 years since research first showed that biological control could be successfully used on harvested fruits when brown rot of peach was effectively controlled using the soil isolated bacterium *Bacillus subtilis* B3 [1*]. Since then, the development of biological control of postharvest disease (BCPD) has gone through various phases, including the search for bacterial and yeast antagonists naturally occurring on fruit, upscale tests under semi-commercial conditions, pilot tests, toxicology tests, and registration of the first commercial products [2**]. Currently there are more than two dozen programs world-wide working on various aspects of BCPD, and new products are being registered for postharvest application in various countries [3**]. As the commercial use of BCPD increased, originally perceived limitations of this approach become a reality. This spurred significant interest into research on addressing the limitations of the registered products, as well as antagonists in the developmental stage. One of the main approaches has been combining biological control with alternatives to conventional fungicide control, which by themselves may not provide commercially acceptable control but may complement the limitations of the biological control treatment [4*]. In general, these alternatives could be divided into physical and chemical methods. Some of them have direct effects on the pathogens but others may act indirectly by increasing fruit resistance to the pathogens or retarding senescence. In this review we discuss various combinations of these treatments with biological control on fruits after harvest.

Physical treatments

Heat treatments

Hot air

Prestorage heat treatment has been shown to beneficially affect fruit quality during storage. Heat may be applied to fruit and vegetables by hot water dips, vapour heat (steam), or hot dry air [5*] or by hot water rinsing and brushing [6]. Early work with strawberries indicated that exposure to hot air at 43°C for 30 min at a relative humidity of 98% or 60 min at 90% relative humidity effectively reduced postharvest decay during 4 subsequent days at 16°C [7]. In 'Spartan' and 'Golden Delicious' apples exposed to 38°C for 4–6 days and then stored at –1°C for 4–7 months, fruit softening was suppressed and naturally occurring decay, largely due to *Corticium* and *Penicillium* sp., was reduced [8]. Similarly, exposure at 40°C for 2–4 days maintained firmness of stored 'Golden Delicious' fruits [9]. In addition, Klein *et al.* [10] found that the best heat treatment for 'Anna' and 'Granny Smith' apples was exposure to 38°C for 4 days. Later work indicated that heating 'Golden Delicious' apples inoculated with *Penicillium expansum* for 4 days at 38°C completely inhibited decay development [11]. These workers concluded that the effect of heating on fruit decay caused by *P. expansum* is not only a result of direct inhibition of fungal germination and growth by high temperature, but the formation of an antifungal substance in the heated peel may also play a role.

Combining heat treatment of 'Gala' apples after harvest (38°C, 4 days) with pressure infiltration of a 2% $CaCl_2$ solution, and antagonist treatment (*Pseudomonas syringae*) reduced blue mould decay caused by *P. expansum* more than the treatments applied alone [12]. After 6 months in storage at 1°C, no decay lesions developed on fruit that were heated after inoculation with *P. expansum* or any combination of *P. expansum*, the antagonist or $CaCl_2$. Heat treatment had little effect on lesion size when the fruit were inoculated with *P. expansum* after heat treatment in the parallel lots. $CaCl_2$ alone, the antagonist alone and heat plus $CaCl_2$ all reduced the incidence of decay by approximately 25%, whereas heat plus the antagonist reduced it by 70%. $CaCl_2$ plus the antagonist or $CaCl_2$ plus the antagonist and heat reduced decay incidence by 89% and 91%, respectively. Similar decay reduction occurred when $CaCl_2$-treated 'Golden Delicious' fruits were inoculated with *Botrytis cinerea* prior to heat treatment [13]. It is important to treat fruit with calcium prior to rather than following heat treatment. An electron microscope study revealed that epicuticular wax on the surface of non-heated fruits exhibited numerous deep surface cracks that formed an interconnected network on the fruit surface. The epicuticular wax of heat-treated fruits did not exhibit a similar network of deep cracks. This apparent obstruction or elimination of deep cracks may limit penetration of the $CaCl_2$ into fruits [14]. On 'Gala' apples, less decay developed on fruits treated with heat (38°C, 4 days) or cold storage (1°C, 4 days) before wound inoculation with *P. expansum* and the antagonist *P. syringae* or one of the two yeast antagonists than on fruits not treated with these temperatures, after storage for 7 days at 20°C or 3 months at 1°C. The addition of any of the antagonists before heat treatment also reduced lesion size and incidence of decay [15]. Heat treatment was shown to be effective in sanitising the fruit and enhancing the wound healing process [16, 17]. Factors other than wound healing may be involved in this process. Enhanced production of phenolic substances near wounded tissue is known to be a common response to wounding. These phenolics may be incorporated into lignified cell walls during wound healing or may be directly toxic to the pathogen [18]. On 'd'Anjou' pears stored for 4 days at –1°C after wounding, compounds such as callose, gums, pectic substances, starch, suberin and phenolics increased in wound tissue [19]. The accumulation of these compounds appears to coincide or precede the increase in resistance to decay. The same wound healing response was accelerated and occurred after 1 day when fruits were heated at 28°C [17].

Prestorage heat treatment at 26.5°C for 2 days is recommended for 'Anjou' and 'Bosc' pears prior to rapid cooling to –1 to 0°C in cold storage [20]. On apples, heat treatment has the added benefit of improving fruit colour, especially in 'Golden Delicious' fruits, but does not lead to softening, since it inhibits the synthesis of cell wall hydrolytic enzymes

[16]. In another study, heat and 1-methylcyclopropene (MCP) treatment were combined with biocontrol to reduce postharvest decay of 'Golden Delicious' apples [21]. Fruits were treated with MCP, wounded and inoculated with *P. expansum* alone or with a heat tolerant yeast antagonist, the same one as used in the previous study [15]. After incubation at room temperature for up to 48 h, the apples were heated at 38°C for 4 days or moved to cold storage for 5 months. The combination of heat treatment with the yeast antagonist were more effective than either treatment alone, with a resulting reduction in decay incidence of 96–100%. The addition of MCP had no beneficial effect on decay reduction and even slightly increased lesion diameter. On heat-treated apples the antagonist population increase was greater than on non-heated fruits. Because the heat treatment reduces the population pressure and competition of other microorganisms in the apple wounds, it is likely that the heat tolerant yeast had an advantage in colonising the surface and competing with the pathogen. Similarly, in other work, biocontrol of green mould of citrus (caused by *P. digitatum*) by a bacterial strain *Pseudomonas glathei* was significantly improved when the inoculated fruits were incubated at 30°C for 24 h before being moved to 25°C [22]. The incubation conditions prevented the fungal spores from germinating, but the bacterial populations increased greatly in the wounds. In another study, an antagonist, *Metchnikowia pulcherrima* [23], was integrated with MCP, heat treatment and stored in controlled atmosphere (CA; 1.1% O_2, 1.8% CO_2) to control bitter rot and blue mould decay on apples caused by *Colletotrichum acutatum* and *P. expansum,* respectively [24]. MCP treatment increased decay caused by *C. acutatum* and *P. expansum* but these decays were controlled on MCP-treated apples by a combination of the antagonist and heat treatments. The antagonist controlled *C. acutatum* more effectively than *P. expansum*, while *P. expansum* was more effectively controlled by heat treatment.

In a subsequent study, the addition of sodium bicarbonate (SBC) to heat treatment and two yeast strains further reduced decay caused by these pathogens on 'Golden Delicious' apples [25]. Both yeast antagonists reduced decay caused by *P. expansum*, whereas heat or heat in combination with either antagonist eliminated decay. Either heat or the antagonists alone reduced decay caused by *C. acutatum*, but a combination of the two was required to eliminate decay caused by this pathogen. Adding SBC to heated or antagonist-treated fruits had little effect on decay caused by either pathogen but when used on non-heated fruits it significantly reduced decay caused by *P. expansum* after 4 months at 0°C. A similar study with 'Golden Delicious' apples that used heat treatment (38°C, 4 days) and one strain of *M. pulcherrima* from the previous study [25], alone or in combination with a different antagonist (*Cryptococcus laurentii*), had similar results [26**]. Research combining biocontrol with heat treatment shows promise as being as effective as chemical control in controlling decay of apple fruits caused by postharvest pathogens. A successful postharvest decay control strategy should have both eradicant and protectant properties. While heat treatment acts as an eradicant in that it significantly reduces the pathogen population on the fruit surface, it provides little residual protection. The residual (protectant) protection from the antagonist adds to the control provided by the heat treatment to provide a complete decay control strategy.

Hot water dipping

The use of hot water treatments was one of the earliest nonchemical methods of control investigated to reduce postharvest decay. Research done in 1922 indicated that brown rot caused by *Phytophtora* spp. on lemons was prevented by dipping the fruit for 2 min in water at 46–49°C [27]. Later work was done with peaches inoculated with *Metschnikowia fructicola* to control *Rhizopus stolonifer* [28]. Peaches that were dipped for 7 min at 49°C, 3 min at 54°C or 2 min at 60°C, either 1 or 24 h after inoculation, had approximately 70% less decay than non-treated peaches after 2–6 days . Hot water treatment of fruits and vegetables to control postharvest pathogens is usually applied at temperatures above 40°C. Many fruits and vegetables can tolerate temperatures of 50–60°C for 5–10 min without a loss in quality. In addition, genetic differences among fungi result in a great deal of variation in their sensitivity to high temperature. *M. fructicola*, for instance, is more heat sensitive than *P. expansum* [29*]. Hot water treatments have several advantages over the use of chemicals to reduce postharvest decay. They are of short duration, easily monitored, leave no chemical residue on the fruit surface, and pathogens may be eradicated even after they have entered the fruit. Since biocontrol of postharvest diseases has little eradicative activity, a combination of hot water treatment followed by biocontrol would be effective in providing both eradicant and protectant activities. Research was conducted to determine the effects of hot water treatment (55°C for 30 s) and an antagonist, *C. laurentii*, alone and in combination, in reducing black rot of strawberries caused by *R. stolonifer* [30]. As stand-alone treatments, hot water dips and the antagonistic yeast reduced the percentage of infected wounds from 96.7% to 65% and 63.3%, respectively. However, in fruits treated with the combination of hot water dips followed by *C. laurentii*, the percentage of infected wounds was only 43%.

To develop an integrated method to control postharvest diseases of banana, an antagonist (a member of the *Burkholderia cepacia* complex) and hot water dips, alone and in combination, were tested for their efficacy in reducing anthracnose, crown rot and blossom end rot of bananas caused by *Colletotrichum musae* [31]. The optimum temperature and exposure time were determined to be 50°C for 3 min. The most effective concentration of *B. cepacia* was 10^{10} CFU/mL and the addition of 2% Tween 20 to the bacterial suspension increased its efficacy. While either treatment alone reduced decay severity by approximately 50%, the combination of a hot water dip with the antagonist resulted in almost complete control of the fungus.

Hot water rinsing and brushing

In addition to hot water dipping, a more recent technology, hot water brushing (HWRB) has shown promise [6, 32**, 33]. This technique involves hot water rinsing and brushing to clean and disinfect freshly harvested produce. Treatment time is no longer than 30 s at temperatures between 48 and 53°C [32**]. Fresh produce is rinsed with pressurised hot water by nozzles from above while rolling on brushes made from soft synthetic bristles, which tends to clean and disinfest the produce. Treating stone fruits with HWRB at 60°C for 20 s and then dipping them into a cell suspension of the antagonistic yeast *Candida* spp. (10^8 CFU/mL) 24 h after inoculation with *P. expansum* reduced decay development on stone fruits by 60% compared with the controls [34]. The combination of HWRB and *Candida* spp. had no significant additional effect against *M. fructicola* compared with HWRB alone.

Vapour (steam)

A strategy for postharvest control of black root rot (BRR) of carrot, caused by *Theilaviopsis basicola*, combined steam heat using a steam pressure of 4 bar (maximal produce surface temperature was measured at 85°C) and Shemer, a biocontrol product containing the yeast *M. fructicola* (Agrogreen, Minrab group, Ashdod, Israel) [35*]. After treatment, the carrots were stored for 1 month at 0.5°C and then transferred to a shelf-life simulation room at 20°C for 8 days. Treatment of carrots with Shemer before storage did not affect BRR disease incidence but steam reduced the incidence of decay by approximately 47% during the shelf-life period after cold storage. Applying *M. fructicola* after a 3 s exposure of the carrots to steam resulted in a synergistic effect that reduced BRR decay by 86%. It was concluded that the improved efficacy of *M. fructicola* might be the result of either pathogen weakening or induced resistance in heat-treated carrot tissue [34]. The antagonistic yeast might be able to colonise the carrot wounds more effectively due to reduced competition from other microflora that are killed by the heat treatment.

Controlled or modified atmosphere

CA or modified atmosphere (MA) has been found to be effective in delaying the onset of ripening and senescence in stored fruits and vegetables, with much of the initial research done on apples [36, 37]. Generally, harvested produce has more resistance to infection by potential pathogens earlier in their postharvest life, and as fruits and vegetables ripen and senesce, they become more susceptible. Therefore, delaying ripening also delays the time at which produce becomes more susceptible to decay, thus prolonging their storage life [38*]. The effect of CA on growth and development of various decay-causing fungi is variable and temperature related [37]. The development of apple decay caused by *P. expansum*, for example, was inhibited more effectively in CA than in normal air cold storage [39]. The incidence of blue mould decay caused by *P. expansum* was reduced on apples stored at 4°C under CA conditions of 3 kPa O_2 and 5 kPa CO_2 but only slightly reduced when held in an atmosphere of 3 kPa O_2 and 0 kPa CO_2 [40]. In a later study, apples inoculated with *P. expansum* and stored at 0°C under CA conditions of 3% O_2 and 2% CO_2 or 1% O_2 and 0% CO_2 had significantly less blue mould, produced less ethylene and were firmer than fruits stored in air [41]. Therefore, CA storage affects both the pathogen and the host. Decay development was retarded because growth, sporulation, or enzyme activity of the pathogen was reduced, and the improved physiological condition of the host enabled it to resist the pathogen more effectively. 'Golden Delicious' apples wound-inoculated with *P. expansum* and treated with various combinations of SBC and two antagonists (*M. pulcherrima, C. laurenti*) had 30% smaller lesions when stored in CA (1.4% O_2 and 3% CO_2) than in the air after 2 months storage at 1°C [42]. Combining SBC with either or both of the antagonists in air storage reduced lesion diameters by 95 to 98%, but the best control was achieved with the combinations of *C. laurentii* plus SBC and the two antagonists plus SBC stored in CA, where no decay developed. After 4 months in storage, all of the control fruits stored in air or CA were totally decayed, and while either antagonist alone in both air and CA storage significantly reduced decay, the only combination that completely controlled blue mould was the two antagonists plus SBC stored in CA. The CA conditions had no adverse affect on antagonist populations. Similar results were obtained in a subsequent pilot trial under commercial storage conditions, using the same treatments except the CA regime, which was 1.5% O_2 and 2.0% CO_2 , showing that the combination of antagonists and SBC under CA conditions would be a viable alternative to chemical control [43**].

The biocontrol potential of the yeast *Candida sake* (CPA-1) against *P. expansum* decay of 'Golden Delicious' was maximised on apples stored in CA (3% O_2 and 3% CO_2) at 1°C where reduction in decay incidence was 97% compared with only about 40% in ambient or air storage [44]. The antagonist growth was compatible with all of the atmospheres tested. The biocontrol capability of the yeasts *Trichosporon* sp. and *Cryptococcus albidus* against *B. cinerea* and *P. expansum* was evaluated in 'Golden Delicious' apples and 'Jingbai' pears at 1°C in air and under CA (3% O_2 and 3% CO_2 or 3% O_2 and 8% CO_2) conditions [45]. The application of the antagonists controlled decay caused by both pathogens better on apples than on pears, and more effectively in CA than in air. *Trichosporon* sp. was a more effective antagonist than *C. albidus*.

In another study, the combined effect of modified atmosphere packaging (MAP) (polyethylene bags; O_2 levels = 1 to 15%; CO_2 levels = 0 to 15% and ambient conditions as well) and the application of a bacterial antagonist (*Erwinia* sp.) to control grey mould caused by *B. cinerea* on 'Golden Delicious' apples was investigated [46]. Grey mould on fruits stored under these MA conditions was not affected by O2 levels but it was controlled when CO_2 was increased from 0–15%, and when the bacterial antagonist (*Erwinia* sp) was added. How-

ever, the antagonist was most effective under ambient conditions. In another study, the control of brown rot caused by *M. fructicola* on sweet cherry treated with the antagonist *Cryptococcus infirmo-miniatus* was improved from an incidence of 47% to 2% by MAP (5.1% O_2 and 11.4% CO_2) [47]. In summary, CA or MAP can affect both the pathogen and the host. Decay development is retarded because growth, sporulation, or enzyme activity of the pathogen is reduced, and the improved physiological condition of the host enables it to resist decay more effectively. Since there is a direct relationship between antagonist population and biocontrol efficacy [48], it is important that the antagonist populations are not negatively affected by the MAP or CA conditions.

Ultraviolet light

Ultraviolet light below 280 nm (UV-C) can be used to reduce fungal decay in harvested fruits and vegetables. This is achieved by direct germicidal action and induction of resistance [49**, 50]. The induced resistance appears to be stronger and longer lasting in plant storage organs such as sweet potatoes, carrots or potatoes [51–53] than in fruits such as apple, peach, citrus fruits, grapes or tomato, although significant reductions in postharvest decay have been observed among these fruits after UV treatment [49**, 50, 54–56]. The efficacy of the UV-C treatment is affected by a variety of factors including, positioning of the fruit in relation to the irradiation source, type of fruit, cultivar or fruit maturity, which can cause significant variations in efficacy. The induction of resistance in table grapes was greatly enhanced when UV-C irradiation was combined with a preharvest chitosan treatment [57]. Using UV-C lamps above and below a conveyer improved the distribution of the irradiation and reduced the variation in fruit response to the treatment [58]. Combining UV-C treatment with the biocontrol agent *Debaromyces hansenii* improved control of brown rot (caused by *M. fructicola*) of peach, green mould (caused by *P. digitatum*) on tangerines, and Rhizopus rot (caused by *R. stolonifer*) on tomato and sweet potato [59**]. The efficacy of the combined treatments, which also included $CaCl_2$, was equivalent to benomyl treatment of peaches, and even superior to dichloran treatment of sweet potatoes. Implementation of the UV-C irradiation treatment to complement biocontrol should not be difficult. In fact, some apple packinghouses already use UV irradiation boxes with the intention of reducing fungal inoculum on fruits. However, to achieve more consistent control, the UV-C treatment procedure will have to be developed for each commodity, and even then significant variation in efficacy of the UV-C treatment can be expected.

Microwaves

The feasibility of using microwaves to kill different pests on harvested commodities has been explored for almost two decades [60, 61]. Two microwave frequencies have been approved by the United States Federal Communication Commission for heating application, 915 and 2,450 MHz. The 915 MHz microwaves have higher energy and penetrate more deeply in fruits and vegetables than 2,450 MHz [62]. It was shown to be promising for the eradication of a quarantine pest, codling moth, on sweet cherry [63]. The larvae of this pest reside and feed in the centre of the fruit and microwaves can raise the temperature to the desired level in the centre of the fruit quickly. The quality of fruits treated with the microwaves was comparable to untreated controls or fruits treated with methyl bromide. The 2,450 MHz microwave (used in kitchen type microwave ovens) has been successfully used to control postharvest grey mould and blue mould of peaches caused by *B. cinerea* and *P. expansum*, respectively, under laboratory conditions [64]. Treatment of peaches with 2,450 MHz microwave for 2 min followed by the application of an antagonist, *C. laurentii,* reduced decay incidence caused by *R. stolonifer* to 23.7% compared with 95% decay in the control, to 75% when using the antagonist alone, and to 42.1% after microwave treatments alone [65*]. Microwave treatments did not harm the fruits. Similar treatments on pears reduced the incidence of blue mould, caused by *P. expansum*, from 100% in the control to 72.6% after microwave treatment, to 65.5% using the antagonist only, and 20.2% after the combination of the antagonist and microwave treatments. The treatments did not impair major fruit quality indices [66]. Microwave treatments have eradicative activity but impart no residual protection. Again, the alternative treatment complements biological control, which generally cannot eradicate the pathogens but has persistent protective activity.

Ozone

Ozone has traditionally been used as a water disinfectant throughout the world [67]. It can also be used to reduce fungal spore contamination in water used for handling fruits and vegetables in packinghouses, and therefore reduce new infections [68**]. Ozonated water, however, is not effective in controlling infections from wounds on pears or citrus fruits inoculated before ozone treatment [69, 70]. An ozone atmosphere causes abnormal development of fungal colonies and greatly reduces germination of the detached spores depending on the species [71]. Ozone at 0.3–1.0 ppm in air retarded production of fungal spores and slightly reduced the rate of decay by major fruit pathogens such as *B. cinerea*, *Mucor piriformis, P. expansum, Penicillium digitatum and Penicillium italicum* on strawberries, grapes, peaches, and citrus fruits [72–76]. By inhibiting spore production ozone can reduce the number of spores in the packinghouse atmosphere and thereby effectively reduces the number of infection cycles, especially on citrus fruits [69].

Ozone treatment at 0.1 mg/g significantly reduced populations of filamentous fungi, yeasts and bacteria on the berry surface of table grapes after exposure for 1 min, and by more than 90% after 10 min in most cases [77]. This could negatively affect biocontrol agents applied to the surface of the fruit. However, the application of the biocontrol agent *Muscodor albus*, which produces inhibitory volatiles, in tea bags placed in grape cluster bags, reduced decay incidence on berries artificially infected with *B. cinerea* from 91.7% (control) to 21.2%. The ozone treatment alone or in combination with

M. albus reduced incidence of decay to 19.3% or 10.1%, respectively [78**]. A rye culture of *M. albus* in the tea bags survived the ozone treatment, indicating that this treatment can be applied before or after ozone treatment. This makes integration of ozone and *M. albus* easily achievable. On organically grown grapes, where the natural incidence of grey mould was 31%, treatment with a combination of *M. albus* and ozone reduced decay incidence to 3.4%, which was a greater reduction than either treatment alone, after 1 month of storage at 0.5°C. In this combination of treatments, ozone effectively sanitised grapes and reduced the amount of inoculum residing on the fruit surface, while *M. albus* provided long lasting suppression of grey mould that developed from incipient infections which resided inside the berries and were protected from the killing action of ozone, but the durability of this induction was not investigated. Ozone treatment also reduced decay on berries inoculated with *R. stolonifer* before or after treatment with ozone, indicating a possible induction of resistance in berries. Both biocontrol and ozone treatments can be used on "organic" labelled products.

Chemical treatments

GRAS substances

Bicarbonate and carbonate salts

SBC ($NaHCO_3$, baking soda) and sodium carbonate (SC) (Na_2CO_3, soda ash) are common food additives allowed with no restrictions and are generally regarded as safe (GRAS) by the United States Food and Drug Administration . They have also been found to be useful in controlling plant pathogens, and for many years have been especially useful in the citrus industry [79–83]. A 90% reduction in blue mould caused by *P. italicum* in oranges occurred when fruits were treated with a 3 or 4% SC solution at 45°C. SBC applied at room temperature at 2–4% reduced decay caused by *P. italicum* by more than 50% [81]. The effect of the SBC and SC treatments is primarily fungistatic because the fungal spores were not killed and spore germination was only delayed. Germinating spores seem to be more readily killed by SBC than non-germinating spores [84]. A 2% SBC solution killed germinating *P. digitatum* spores in citrus wounds [82]. Control of green mould caused by *P. digitatum* was significantly improved by following treatment with a 3% SBC or SC solution with applications of the antagonist *Pseudomonas syringae* strain ESC 10 (the active ingredient in BioSave 10; JetHarvest Solution, Longwood, FL) [83]. Several isolates of *Bacillus subtilis* were evaluated for control of green mould and blue mould on citrus fruits. While one of the antagonist isolates or SBC alone reduced decay to 10–20%, the combination resulted in complete control of both diseases [80].

Control of *P. expansum* and *Alternaria alternata* on pears was significantly increased when the antagonists *C. laurentii* or *Trichosporon pullulans* were combined with 2% SBC [85]. *C. laurentii*, with and without SBC, provided more effective control of both pathogens than *T. pullulans*, and in combination with SBC, completely eliminated *A. alternata* decay on pears. The antagonist *Pantoea agglomerans* CPA-2 combined with SC and SBC reduced incidence of green mould from approximately 90% among control oranges to 55% and 25%, respectively after treatment with SC and SC + the antagonist, and 60% and 40%, respectively after treatment SBC and SBC + the antagonist [86*]. A 2% solution of SBC combined with *M. pulcherrima* significantly reduced blue mould of apple fruits caused by *P. expansum*. However, there was no significant effect when SBC was combined with *C. laurentii* although the combination tended to result in smaller lesions [26**]. In a pilot test, 'Golden Delicious" apples were wound-inoculated with *P. expansum* and treated with various combinations of SBC and two antagonists (*M. pulcherrima, C. laurentii*) and stored in CA (1.5% O_2 and 2.0% CO_2) [43**]. SBC alone reduced decay but was much more effective when combined with the antagonists. In combining SBC or SC with any antagonist, it is important that the antagonist is compatible with the carbonate solution and able to proliferate in fruit wounds in their presence.

Calcium

Postharvest decay caused by fungal pathogens in apples was reduced by applications of calcium solutions [87*, 88]. This early work involved active infiltration, either by pressure or vacuum infiltration, of $CaCl_2$ solutions directly into apple fruits. In this case, the mechanism by which increased tissue calcium reduces decay and maintains firmness may be related to calcium ions in the cell wall [89]. Calcium-induced resistance to fungal pathogens is attributed to a process making the cell wall less accessible to fruit-softening enzymes or to cell wall degrading enzymes produced by fungal pathogens [90–92]. Therefore, calcium reduces decay mainly by increasing resistance of the fruit rather than any direct effect on the pathogen. In a study emphasising this type of resistance mechanism and showing the beneficial effect of combining calcium solutions and biocontrol, apple fruits were pressure infiltrated (103 kPa, 3 min) with a 4% solution of $CaCl_2$, stored for 6 months at 1°C, and then wound inoculated with *P. expansum* or with *P. expansum* plus the antagonist *P. syringae* [93*]. The antagonist alone reduced the incidence of decay by approximately 40% while fruits treated with calcium alone had 53% less decay. Fruits treated with both the antagonist and the calcium solution had 88% less decay than the untreated apples. More recently, research has emphasised the direct effect of calcium solutions by inhibiting spore germination and growth [94*]. A study was conducted to determine the effect of salt solutions combined with yeast antagonists (*Candida* sp.) in reducing postharvest decay of apples caused by *B. cinerea* and *P. expansum* [95]. Fruit wounds were treated with a suspension of *Candida* sp. in 0–2% solutions of $CaCl_2$ and then inoculated with either of the two pathogens. Biological control of both pathogens was enhanced when wounds were treated with the antagonist in a 2% solution of $CaCl_2$. The effect of calcium on the biocontrol effectiveness of these strains of *Candida* sp. was thought to result from an interaction by the pathogen with the yeast or

its metabolic products in the wound site. Another study, also indicating that calcium reduces fungal infection through direct inhibition of fungal spore germination and growth, was conducted using 2% calcium solution, two antagonistic yeasts, *Candida guilliermondii* and *Pichia membranefaciens,* and *R. stolonifer* causing decay on peach and nectarine fruits [96]. The addition of calcium resulted in lower spore germination rates and slower growth of germ tubes *in vitro*, as well as in lower disease incidence and smaller lesion diameters compared with the yeast antagonists alone. The addition of the calcium solution also allowed the use of lower concentrations of the antagonist without reducing control achieved with the antagonist alone at the higher concentration. Similarly, the two antagonistic yeasts, *Candida guilliermondii* and *Pichia membranefaciens*, in combination with a 2% calcium solution were evaluated for the control of apple decay caused by *P. expansum* [97]. Results were similar to the previous study [96] in that the addition of the calcium solution resulted in lower spore germination rates and slower germ tube growth *in vitro*, as well as in lower disease incidences and severity compared with the yeast treatment alone.

An investigation was undertaken to evaluate the antagonist *C. laurentii* for its activity in reducing postharvest grey mould of pear caused by *B. cinerea* [98]. Suspensions of *C. laurentii* at concentrations of 10^6–10^9 CFU/mL were prepared in distilled water or in 2% $CaCl_2$ solutions. The suspensions were applied into fruit wounds followed by inoculation with *B. cinerea.* The efficacy of *C. laurentii* was significantly enhanced by the addition of the calcium solution which allowed a reduction in the antagonist concentration from 10^9 CFU/mL to 10^8 CFU/mL without reducing control of the decay [96]. Another study was designed to evaluate the feasibility of the combined application of an antagonist, *Aureobasidium pullulans*, and 1% $CaCl_2$ in controlling naturally occurring postharvest decay of sweet cherry [99]. These naturally occurring decays were mainly caused by *B. cinerea*, *A. alternata* and *Monilinia laxa*. Using postharvest dips of cherry in a calcium solution or the antagonist, the calcium treatment reduced decay incidence by up to 33%, the antagonist alone by up to 44% and the combination of the calcium solution and the antagonist reduced decay incidence by up to 70%. Once again, it was concluded that the addition of the calcium solution directly inhibited the pathogens and enabled the antagonist to better compete with the pathogens. The biocontrol activity of *P. membranifaciens* against anthracnose rot caused by *C. acutatum* on loquat fruits was enhanced by the addition of the 2% calcium solution and resulted in significantly improved decay control compared with treatment by the antagonist or calcium alone [100]. In addition, a combination of the antagonist with the 2% $CaCl_2$ solution induced higher activities of two defence related enzymes, chitinase and β-1,3-glucanase, and inhibited spore germination and germ tube elongation of *C. acutatum* more than the antagonist or $CaCl_2$ alone. The addition of $CaCl_2$ also enhanced the efficacy of a series of antagonists in controlling postharvest decay of table grapes [101]. In all of the above studies, the calcium solution had no negative effects on the antagonist's populations.

Ethanol

Ethanol occurs naturally in many foods and is considered an approved preservative for many. Its efficacy in reducing fungal decay by postharvest pathogens is probably the result of protein denaturation, especially those of mitochondrial membranes [102]. On lemons inoculated with *P. digitatum,* immersion in solutions of 10–20% ethanol at 32, 38 and 44°C effectively controlled decay on fruits stored at 20°C for 3 weeks [103]. At 50°C, all treatments, including water, reduced incidence of decay to less than 5%. Little additional enhancement occurred when ethanol concentrations exceeded 10%. In a second study, a combination of hot water and ethanol (10%) at immersion temperatures of 46 or 50°C for 2.5 min. reduced the postharvest decay of naturally inoculated peaches and nectarines caused by *M. fructicola* and *R. stolonifer* from 82.8% to 33.8% and 24.5%, respectively [104]. Ethanol (50%) applied before harvest to organically grown strawberries significantly reduced the incidence of postharvest grey mould caused by *B. cinerea* [105]. The antagonist *Saccharomyces cerevisiae* combined with ethanol controlled grey mould caused by *B. cinerea* on apples and pear [106*]. Biocontrol activity of *S. cerevisiae* was strongly affected by the addition of ethanol. Ethanol at a concentration of 22% was toxic to the antagonist but completely inhibited spore germination of *B. cinerea*. On the other hand, ethanol at 16% enhanced antagonistic activity against the pathogen. On lemons, immersion of fruits inoculated with *P. digitatum* in 10% ethanol at 45°C followed by an application of the biocontrol yeast *Candida oleophila* reduced the incidence of green mould by 95% (equivalent an imazalil treatment) compared with a 50% reduction by the yeast alone [107].

Silicon

Silicon is a major inorganic constituent of plants [108*] and its exogenous application has been shown to reduce disease severity of fungal infections [109, 110]. The mechanism by which silicon reduces disease is thought to involve eliciting biochemical defence reactions in the plant [108*, 111]. *C. laurentii* combined with silicon (2%) was more effective in controlling decay caused by *A. alternata* or *P. expansum* on jujube fruits than silicon alone or silicon combined with the yeast *Rhodotorula glutinis* [112]. After 7 days at 20°C, the only treatment that eliminated the incidence of decay caused by *P. expansum* and reduced decay caused by *A. alternata* by 80% was a combination of silicon and *C. laurentii*. Silicon or either antagonist alone or silicon in combination with *R. glutinis* reduced the incidence of decay by no more than approximately 20%. There was no effect of silicon on the populations of either antagonist. In a more thorough study on sweet cherry, fruits treated with silicon (1% sodium metasilicate) alone had 63.4% and 86.6% lower incidence of decay caused by *P. expansum* and *M. fructicola*, respectively, and was more effective than the antagonist (*C. laurentii*) alone, which reduced incidence of decay by either pathogen by

about 50% after incubation at 20°C for 3 days [113**, 114]. However, the combination of silicon and *C. laurentii* completely suppressed decay by both pathogens. Silicon treatment stimulated populations of the antagonist, strongly inhibited spore germination, germ tube elongation and growth of the pathogens, and induced a significant increase in the activities of phenylalanine ammonia lyase, polyphenoloxidase, and peroxidase in sweet cherry. It was concluded that the improvement in decay control with silicon may be associated with the increased population density of the antagonist, its direct phytotoxicity to the pathogens, and the elicitation of biochemical defence responses in fruits.

Nisin

Nisin, a broad spectrum pore-forming bacteriocin, is produced by lactic acid bacteria that are often found on produce [115]. It is active against many gram-positive bacteria [116]. Nisin is the only commercially available bacteriocin recognised as a safe and legal biological food preservative by the United States Food and Drug Administration. The addition of 4% nisin to the antagonist *C. oleophila* suspension, used for treating apples, eliminated grey mould and reduced blue mould decay by 93%, while the antagonist alone reduced decay by 68% and 67%, respectively [117*]. Nisin enhanced the antagonistic activity of *C. oleophila* in the wounds of apples by inhibiting spore germination and restriction of the growth of germ tubes of both pathogens, which favoured the antagonist and therefore enhanced biocontrol.

Elicitors of host defences and retardants of senescence

Chitosan and harpin

Chitosan (poly ß-(1→4)*N*-acetyl-D-glucosamine) is a deacetylated form of chitin, which is commonly obtained by a chemical process from crustacean shells [118]. It has widespread applications in agriculture, food, medicine and environmental remediation. In addition to its antimicrobial activity it can induce resistance in plants [119–121]. The antimicrobial activity of chitosan and its derivatives (eg, glycolchitosan or carboxy-methyl chitosan) depends mainly on the degree of deacetylation, pH of the medium, temperature and the presence of food components. Yeasts appear to be more sensitive than bacteria, but reductions in bacterial populations as high as 5 log were reported [122, 123]. Chitosan and its derivatives can be used as an antimicrobial film on strawberries, raspberries, peach, kiwifruit, Japanese pear, cucumber, bell papers, longan fruit, banana and mango to extend the shelf-life of these produce [120, 124–129]. Because of its antimicrobial activity, chitosan and its derivatives must be screened for their effect on biocontrol agents and the most appropriate concentrations determined before they can be used as a combined treatment [130]. Combining glycochitosan at 0.2% with the antagonist *Candida saitoana* resulted in control of green mould of oranges and lemons equivalent to an imazalil treatment [130]. Pretreatment of lemon with SC prior to application of the yeast with glycolchitosan further improved control of green mould. The glycolchitosan/antagonist treatment reduced the incidence of grey mould and blue mould of apple from 100% in controls to 23% and 25%, respectively. On both apple and citrus fruits, the combined treatment was always superior to the individual treatments. Chitosan at a concentration of 0.1% reduced germination of *P. expasum* conidia by ~ 50% but did not inhibit populations of the antagonist *C. laurentii* in apple wounds [131]. Combining chitosan with *C. laurentii* reduced incidence of blue mould of apples from 100% to 14.0%, while treatments with chitosan or the antagonist alone reduced decay incidence by 87.5% and 48.4%, respectively [131]. The antagonistic activity of this yeast against *P. expansum* on pear was enhanced greatly (from 40% to 6% decay) when the yeast was grown in culture media supplemented with 1% chitin powder [132*]. Similarly grown yeast grew better in pear fruit wounds and had much higher chitinase activity compared with the yeast grown in unamended media. The culture filtrate also had higher chitinase activity and significantly reduced severity of blue mould.

Chitosan at 1% induced resistance in 'Red Delicious' apple [121]. The effects were strongest 96 h after treatment, and higher on fresh than on CA-stored fruits. The reduction of decay by the combination of chitosan with *C. saitoana* was greater than either treatment alone. In the same study, combining the yeast with harpin treatment also resulted in superior control of blue mould [133**].

Despite its antimicrobial activity, chitosan and its derivatives can be combined with yeast biocontrol agents to improve control of fruit decay. The dual action of antimicrobial activity and induced resistance necessitates the careful selection of the optimal concentrations for each antagonist/pathogen/host system. The coating properties of chitosan can provide a venue for good distribution of a biocontrol agent and has already resulted in the development of a product "Bio-Coat" that showed promise in commercial trials [118, 134, 135*].

Methyl jasmonate and salicylic acid

Jasmonic acid (JA), its methyl ester methyl jasmonate (MeJA) and salicylic acid (SA) are signalling molecules occurring in a wide variety of plants and play important roles in plant growth, development and responses to environmental stresses, including defence reactions against pathogen invasion [136*, 137]. As applications of SA primes cells for rapid expression of defence genes upon invasion by pathogens, the activity of applied JA and MeJA depends on the subsequent action of ethylene [138]. There can be signal cross-talk between SA- and JA-induced pathways resulting in additive or synergistic effects [139*]. Activity of the key enzymes, such as chitinase, ß-1,3-glucanase, phenylalanine ammonia-lyase, peroxidase or super oxide dismutase, involved in defence reactions, was often stimulated in fruits following application of these growth regulators [140*–143]. Application of MeJA or SA induced resistance to various pathogens in different fruits, including resistance to brown rot (caused by *M. fructicola*) and blue mould (caused by *P. expansum*) in sweet

cherry [142, 144**]; fungal decay in papaya [145], anthracnose (caused by *Colletotrichum cocodes*) in tomatoes [146], anthracnose (caused by *C. acutatum*) in loquat fruits [147], green mould (caused by *P. digitatum*) in grapefruits [148]), and apple decay [149].

The application of MeJA and SA in combination with various antagonists resulted in control of fruit decay that was superior to individual treatments on a variety of fruits. On pears, combining the antagonist *R. glutinis* with MeJA (200 µM) resulted in a 4.2% incidence of blue mould compared with 7.5%, 14.6% and 20.8% for *R. glutinis*, MeJA and control treatments, respectively, on naturally-infected fruits stored for 2 months at 4°C followed by 15 days at 20°C [150]. On peach, combining *C. laurentii* with MeJA (200 µM) allowed a reduction in antagonist concentration from $1x10^8$ CFU/mL to $5x10^7$ CFU/mL without a resulting reduction in control of brown rot (caused by *M. fructicola*) or blue mould (caused by *P. expansum*) (143). This could significantly lower the cost of the antagonist treatment. MeJa (10 µM) reduced germination of *C. accutatum* spores from 96% to 29%, and growth of germ tubes from 6.1 to 2.3 µm *in vitro*; however, it did not reduce anthracnose lesions on loquat fruits when used alone and when used in combination with the yeast antagonist, *P. membranifaciens,* reduced decay incidence from 100% to only 84% after 6 days at 20°C [151*].

A combination of SA (10 µg/mL) with *C. laurentii* significantly improved control of blue mould on apple [152] and blue and grey mould on pears [153]. The effect of SA was most pronounced when fruits were inoculated 48 or 72 h after the treatment, again indicating induction of resistance in fruits. SA (0.5 mM) combined with *C. laurentii* or *R. glutinis* improved control of blue mould on sweet cherry [140*], and combined with *P. membranefaciens* or *R. glutinis* improved control of brown rot and grey mould, respectively, on peach [141, 154]. Proteome analysis of peach exposed to SA and *P. membranefaciens* suggests that antioxidant and pathogenesis-related proteins, and enzymes associated with sugar metabolism were involved in the induction of resistance by these treatments [155].

Although the efficacy of many of these combined treatments in reducing fruit decay is still below commercially acceptable levels, the fact that MeJA and SA induced resistance in fruits and sometimes had a direct effect on various pathogens, indicates their potential for use together with biocontrol treatments. Additional tests, with more effective antagonists on a larger scale, are needed to reveal the full potential of these treatment combinations.

Growth regulators

The physiological state of the fruit determines its natural resistance to decay. As the fruit ripens and senesces, its innate and inducible resistance declines [156**]. Plant growth regulators such as indole-3-acetic acid (IAA), gibberellins and cytokinins are effective retardants of senescence and promote resistance to fruit decay [157–161]. The combination of IAA with *C. laurentii*, one of the most common biocontrol agents, reduced the incidence of blue and grey mould on pears by 18.7% and 14.7% of the yeast treatment alone, respectively, compared with an incidence of 100% and 80% in the control fruits, respectively. [162]. The same combination of treatments on apples resulted in the reduction of blue mould and grey mould by about half of the yeast treatment alone [163, 164*]. However, the overall efficacy of the treatments on apple was relatively low and did not exceed a 50% reduction compared with the pathogen only control. IAA had no antifungal activity at 20 µg/mL and induced resistance in apple 48 h after application [164*] On both apple and pear, the IAA treatment stimulated catalase and peroxidase, as well as superoxide dismutase on apple and polyphenol oxidase on pear, indicating that IAA can induce resistance in fruits. Gibberellic acid had no direct effect on *P. expansum* or *C. laurentii* at 2,000 µg/mL, but in combination with the yeast it reduced the incidence of blue mould decay of apples to 8.3% compared with 45.9% and 100% with the yeast alone and control treatments, respectively [165]. The cytokinin N^6-benzyladenine, perhaps the most effective senescence-retarding growth regulator, also enhanced blue mould control on *C. laurentii*-treated apple and pears at concentrations of 20 and 1,000 µg/mL, respectively [166, 167]. The incidence of decay was reduced from 37.5% on apple treated with the antagonist alone to 4.9% in combination with N^6-benzyladenine [166], and from more than 70% on pear with the antagonist alone to about 10% in combination with N^6-benzyladenine [167].

Concentrations of N^6-benzyladenine as high as 2,000 µg/mL did not inhibit *C. laurentii* growth in apple and pear wounds, but germination of *P. expansum* conidia in potato dextrose broth was reduce by approximately 10% and 80% at N^6-benzyladenine concentrations of 20 µg/mL and 200 µg/mL, respectively [166]. The beneficial effect of these growth regulators was shown for only one antagonist but it can be reasonably expected that many others may benefit from these combinations as well. Cytokinins and gibberellins are on the United States Environmental Protection Agency's approved biopesticide list for postharvest applications and combining them with microbial antagonists could be easily be put into practice without significant developmental and equipment expense [168].

Low doses of fungicides

Application of low doses of fungicides with biocontrol agents may serve several purposes. It may: broaden the spectrum of activity of the treatment (eg, fungicide may provide eradicative effect); manage fungicide resistance (eg, rotation with fungicides with different modes of action to which pathogens did not develop resistance); bring the efficacy of the biocontrol treatment to commercially acceptable levels; and reduce the amount of biocontrol agent applied without compromising decay control, making biocontrol treatment more economical. Yeast antagonists vary in sensitivity to fungicides

and generally are very sensitive to triazoles and dithiocarbamates [169]. Their tolerance to low doses of fungicides must be established not only *in vitro* but also on fruits as the MIC may not be the same in both tests.

Combining *Pichia guilliermondii* (US-7) with 200 pm of thiabendazole (TBZ) reduced the incidence of citrus decay equivalent to the commercial treatment of 2,000 ppm TBZ [170]. The decay control in the combined treatment was more consistent than with TBZ treatment alone. Similarly, application of the commercial product Aspire, based on the antagonistic yeast *C. oleophila*, in combination with 200 ppm TBZ, resulted in a reduction of blue and green mould incidence of citrus equivalent to the conventional fungicide treatment [171**]. The combined treatment also controlled sour rot (*Geotrichum candidum*) a disease that cannot be controlled with the fungicide alone. *C. infirmo-miniatus* (YY6) and *C. laurentii* (HRA5) in combination with 15 ppm TBZ reduced blue mould of apples as effectively as a conventional treatment with TBZ at 525 ppm [172]. The same antagonists in combination with 20 ppm iprodione controlled brown rot (caused by *M. fructicola*) of cherries equivalent to the full dose, 525 ppm, of the fungicide. Similarly, integrating *C. laurentii* with 104 ppm of TBZ improved control of grey mould (caused by *B. cinerea*) on apples [169], and the application of *C. laurentii* in combination with 25 ppm imazalil or 50 ppm kresoxim-methyl resulted in less decay caused by *A. alternata* and *M. fructicola* than when the treatments were applied separately on jujube fruits stored in a CA of 10% O_2 + 0% CO_2 at 0^{o}C [173]. Blue mould of pears caused by a TBZ-sensitive *P. expansum* isolate was also completely controlled by a combination of TBZ at 100 ppm with either *C. infirmo-miniatus* (YY6), *R. glutinis* (HRB6) or *C. laurentii* (HRA5), and blue mould control by *Pseudomonas syringae* (BioSave 110) or *C. oleophila* (Aspire) combined with 100 ppm of TBZ was equal to control with 569 ppm of TBZ, the highest allowed rate, in packinghouse trials [174]. Sequential application of TBZ immediately after harvest followed 6 weeks later by BioSave 110 provided excellent decay control after 5 months of cold storage on nonwounded pears containing natural inoculum [175**]. This approach may contribute to the suppression of the development of resistance to fungicides in pathogens. The addition of the fungicide cyprodinil at 20 ppm to *P. syringae* (used in BioSave 100) treatment allowed a reduction in the concentration of the antagonist from 1 x10^{8} CFU/mL to 6 x 10^{7} CFU/mL without reducing the effectiveness of blue mould control on 'Empire' and 'McIntosh' apples. This greatly reduced the cost of the treatment and may be helpful in the development of a management strategy for benzimidazole (TBZ)-resistant populations of *P. expansum* [176*].

Nutrients and nutrient analogues

The effectiveness of biocontrol on fruits depends on the balance between the antagonist and the pathogen. This balance could be easily tilted to the antagonist's advantage if higher populations of the antagonist are applied to the fruit, if antagonist populations or the mechanism of biocontrol on fruits are boosted by addition of nutrients, or growth of the pathogen is inhibited by exogenously applied substances. Although applying higher populations of the antagonist is the simplest way to achieve this, it is also the most expensive. Thus, various attempts have been made to use nutrients to stimulate antagonist populations or increase the mechanism of biocontrol, or nutrient analogs have been employed to inhibit pathogen growth. To enhance decay control activity, applied nutrients must benefit the antagonist to a much greater extent than the pathogen. Fruits are rich in carbon but poor in nitrogen sources, therefore, applications of nitrogenous substances with antagonists were very effective in improving biocontrol [177*, 178]. Combining L-asparagine and L-proline (80 mM) with *P. syringae* increased populations of the antagonist by as much as 1 log unit in the wounds of mature 'Golden Delicious' apples, and resulted in an additional reduction of the incidence of blue mould of apples from 41% to 0% and from 75% to 20%, respectively [177*]. L-serine and L-aspartic acid at a concentration of 4 mM combined with *Candida sake* reduced lesion size of blue mould decay on apples from 9 mm to 0 mm and from 4 mm to 0 mm, respectively [178]. Interestingly, 5 mM ammonium molybdate reduced the growth of *C. sake* in apple and pear wounds and at the same time improved control of blue mould, grey mould, and Rhizopus rot [178, 179*]. Apparently the beneficial effect of this compound comes from its ability to inhibit pathogen growth, as it alone significantly reduced fruit decay. Interest in using this compound to enhance biocontrol on fruit is expanding and the beneficial effects were also reported in other systems on pear, cherry and jujube fruits [180–182]. At a concentration of 1 mM it reduced growth of the antagonist *R. glutinis* in a media but not in pear fruit wounds, and enhanced control of blue mould by reducing disease incidence from 52% when using the antagonist alone to 11% in the combined treatment [182]. On jujube fruit, at a concentration of 15 mM, in combination with *C. laurentii* and *R. glutinis,* it reduced incidence of blue mould by from 40% to ~2% and from 57% to ~20%, respectively [181]. Also on cherry fruit, at a concentration 5 mM combined with *C. laurenti* and *P. membranefaciens,* it significantly improved control of brown rot, and the incidence of decay was less on fruits in CA than in the air storage [180].

The nutrient analog 2-deoxy-D-glucose (2-DOG) can be absorbed by yeast and filamentous fungi but they cannot metabolise it, so it accumulates in cells, and retards growth [183]. The degree of inhibition depends on the intracellular concentration. A combined treatment of 2-DOG at 2 mg/mL with the antagonist *Sporbolomyces roseus* allowed more than a 10-fold reduction in the concentration of the antagonist applied to fruits without diminishing the level of control of blue mould on apples and pears [184*]. A combination of 2-DOG with *P. syringae* was also very effective; no lesions developed on apples and only small lesions on pears. A concentration of 1 mg/mL 2-DOG combined with *C. sake* enhanced biocontrol of blue mould on pears by 64% [178]. A 2

mg/mL concentration of 2-DOG combined with *C. saitoana* improved control of decay on apples and various citrus fruits, often to the level obtained with commercially used fungicides [185].

Both nutrients and nutrient analogs can be very useful in enhancing biocontrol of fruit decay. Their application, however, could be more suitable as a part of a developed antagonist formulation than as a separate treatment.

Conclusions

There is tremendous potential for improving control of postharvest decay by combining biological control with other alternatives to conventional fungicide treatments. Biological control is the core treatment and can be combined with one or more alternative treatments in one integrated system. The combined treatments are not as versatile as fungicides, and are often specific to a host/pathogen interaction. The efficacy of the treatments was often comparable with conventionally used fungicides, and there was no negative effect on fruit quality. More large-scale tests are needed to validate the feasibility of implementing these combined treatments under commercial conditions. The combination of biocontrol with low doses of fungicides will remove the treatment from the organic domain, but, in addition to improving decay control, it may be helpful in retarding the development of resistance to fungicides. Most of the treatments used in combination with biocontrol could be easily adapted to current packinghouse practices, but some, eg, hot air treatment, will require making significant modifications. Cost effectiveness analyses are needed to determine the practical usefulness of these combined treatments.

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Papers of Interest had been classified as:
*Marginal importance
**Essential reading.

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