Sustainable approaches to control postharvest diseases of apples

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1 Introduction

An increase in apple production and a growing consumer market starting at the beginning of the last century created the necessity for storing apples for extended periods of time. At that point, there was little information on apple ripening, storage or the diseases that developed in storage. Heavy losses from physiological disorders and decays caused by fungi were often experienced. Although direct assessments were not made, the losses can be estimated to be between 20 and 50% (Kader, 1992). Losses as high as 15% and 22.6% to various decays on apples in storage were still reported from Ireland and France, respectively, in the late 1960s (Bondoux, 1967; Swinburne, 1970) and even more recently, 5% losses were reported due to apple blue mould (caused by *Penicillium expansum*) at the New York terminal market (Cappelini et al., 1987). Total postharvest losses are difficult to determine and they are often underestimated because, to be complete, they should encompass losses occurring during storage, handling, transportation, retail and at the consumer level (Moline, 1984). Nevertheless, the need to reduce these losses has been great.

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Control of postharvest diseases and disorders of apples went through several stages during the past century. Initially, studies were conducted on the effects of harvest time, respiration after harvest, and storage at low temperature and high humidity on apple quality and decay development (Culpepper and Caldwell, 1927; Eustace, 1908; Morse, 1908). Maintaining apple quality under different storage conditions, and the effects of these conditions on physiological disorders such as Jonathan spot and soft scald, apple scald or internal breakdown (Plagge and Gerhardt, 1930; Plagge and Maney, 1924, 1925) dominated early research. During this period, postharvest decays of apples from several countries were described in various monographs, which were summarized by Kidd and Beaumont (1924) and Rose (1924). This was followed by a comprehensive monograph on postharvest diseases of apples, pear and quinces (Rose et al., 1933). At that time, the control recommendations for the major postharvest decays of apples; blue mould, grey mould (caused by *Botrytis cinerea*), brown rot (caused by *Monilinia* spp.) and bull's-eye rot (caused by *Neofabraea malicorticis, N. perennans* and *N. alba*), emphasized careful handling to prevent apple bruising and prompt cold storage after harvest (Wright and Smyth, 1954). Recommendations for brown rot and bull's-eye rot also included orchard sprays with sulphur and Bordeaux mixtures, respectively. The first chemical reported to successfully control decays on harvested apples was sodium chloro-2-phenylphenate, applied as a disinfecting wash (English, 1948; English et al., 1948). It was recommended for postharvest use on apples in the second edition of the monograph by Rose et al. (1951). From then onward, fungicides have been routinely recommended for drenching fruit before storage, and several biocides, mainly chlorine and acetic acid, for use on packing lines and in the sterilization of contaminated bins to reduce conidia concentration of pathogens which resulted in significant reductions in losses (Eckert and Kolbezen, 1964; Eckert and Ogawa, 1988; Hardenburg and Spalding, 1972; Harvey, 1978; Pierson, 1966; Rosenberger, 2001, 2009; Sholberg, 1998b; Smith, 1962; Sutton et al., 2014; Watkins et al., 2014). Later, drenches with hot water, calcium or biocontrol agents were also recommended for suppression of blue mould decay (Hansen et al., 2010; Sutton et al., 2014; Watkins et al., 2014). The control measures against newly emerging diseases important in the western United States, for example Sphaeropsis rot and Phacidiopycnis rot, include pre- and postharvest application of fungicides as well as the removal of cankers and twigs with dieback symptoms (Kim and Xiao, 2008; Xiao and Kim, 2008).

Extensive reliance on fungicides to control postharvest fruit decays during the past several decades has exposed the limitations of this approach. This has been demonstrated by the development of resistance to fungicides in major pathogens and the increasing difficulties in developing new fungicides (Baraldi et al., 2003; Bertrand and Saulie-Carter, 1978; Kim and Xiao, 2010; Li and Xiao, 2008; Rosenberger and Meyer, 1981; Spot and Cervantes, 1986; Xiao and Kim, 2010; Xiao et al., 2014). The extensive use of fungicides also raises concerns about their effect on the environment by leaving residues in the air, soil and water, and the effect on non-target microorganisms (Andrews and Kenerley, 1978; Lamichhane et al., 2016; Vorstermans and Creemers, 2011). The potential negative effects on human health, especially for fungicide applicators and consumers ingesting fungicide residues with fruits, have resulted in social pressure demanding a reduction in the use of fungicides (Bertolini, 2008; Harris, 2013; Janisiewicz, 2013; Kader, 1992; Kennedy et al., 1998; Kuchler et al., 1996; Vorstermans and Creemers, 2011; Xiao and Boal, 2009). Thus, the search for alternative approaches that are more sustainable in controlling postharvest diseases of fruits has intensified during the past three decades (Bertolini and Pratella, 2008; Kennedy et al., 1998; Palou et al., 2016). The main focus has been on exploring the

usefulness of various natural products, food additives and substances generally regarded as safe (GRAS), as well as physical treatments (Janisiewicz and Conway, 2010; Palou et al., 2016), biological control (Janisiewicz and Korsten, 2002; Janisiewicz, 2013) and natural fruit resistance either by induction in harvested fruit or by exploration in breeding programmes (Janisiewicz and Conway, 2010; Janisiewicz et al., 2008, 2016; Johnson et al., 1997; Jurick et al., 2011; Stevens et al., 2005). Most of these approaches are inadequate by themselves; however, when used in combination they may rival fungicide treatments. In this chapter we will describe various alternative approaches to synthetic fungicides for controlling postharvest diseases of apples that are more sustainable and safer to human health and the environment.

2 Natural plant-derived products

2.1 Plant volatile compounds

Several plant volatile compounds have been evaluated for their effect against major postharvest pathogens of apples. The most effective include acetaldehyde, hexanal and *trans*-2-hexenal.

2.1.1 Acetaldehyde

Acetaldehyde (Aa) occurs naturally in fruits and vegetable (Nurtsen, 1970). Aa vapour was shown to effectively control blue mould on 'Red Delicious' and 'Golden Delicious' apples (Stadelbacher and Prasad, 1974). Exposure of *P. expansum* conidia to Aa vapour at a concentration of 0.5% for 180 min at 21°C prevented conidia germination on potato dextrose agar (PDA) medium and reduced decay on puncture inoculated apples by 94%. No phytotoxicity was observed. Shorter exposures at higher concentrations were equally effective without any phytotoxicity. There was a direct correlation between the concentration and the exposure time. However, the effectiveness of this compound was only demonstrated at 21°C and RH 65% and not under cold storage conditions. Although this is a naturally occurring compound and it can be produced by some yeasts, the potential for using it to control fruit decays has diminished, as it has been classified as a Group 1 carcinogen (Secretan et al., 2009).

2.1.2 Hexanal and *trans*-2-hexanal

Hexanal is a precursor of the esters that provide a characteristic apple aroma (Pillard, 1986). Fumigation with 100 ppm hexanal completely inhibited *P. expansum* and *B. cinerea* growth on PDA medium during a 48 h exposure interval; however, growth resumed after additional incubation in ambient air (Song et al., 1998). Concentrations of 450 ppm were required for complete prevention of growth after removal from the treatment and subsequent incubation in the air for 120 h. In a test on wounded 'Golden Delicious' apples treated with 8–12 μmol/L hexanal, almost all fruit developed lesions that were about half the size of the control treatment at 22°C (Fan et al., 2006). At 4°C with 5–7 μmol/L concentrations, the incidence of blue mould was reduced by 33% after 6 weeks of storage. To make this treatment commercially feasible, Sholberg and Randall (2007) combined the preharvest application of a fungicide, cyprodinil, with hexanal fumigation at

low rates (2–3 mg/L) for 24 h immediately after harvest. This treatment reduced infections on wounded apples from 10% to 1%.

Fumigation treatment of apples with *trans*-2-hexenal vapours at 12.5 µL/L for 24 h and incubation for 7 days at 20°C reduced the incidence of apple blue mould between 50% and 98%, depending on cultivar, without any negative effect on fruit quality (Neri et al., 2006b). This was the most effective compound out of several plant volatiles tested (Neri et al., 2006a). This compound also inhibited conidial germination of *Neofabraea alba*, the causal agent of lenticel rot of apples *in vitro*, but had little effect on controlling decay (Neri et al., 2009).

2.1.3 Allyl isothiocyanates

Volatile allyl isothiocyanates (AITC) are products of the enzymatic hydrolysis of glucosinolates that occur in many plants, especially in crucifers (e.g. sinigrin). They were shown to be inhibitory to germination and mycelial growth of *P. expansum* (Delaquis and Sholberg, 1997; Mari et al., 1993) and reduced the incidence of blue mould on pears by as much as 90% after 24 h exposure to an AITC-enriched atmosphere (Mari et al., 2002). No phytotoxic effects were observed. Delaying treatment for 24–48 h after inoculation with *P. expansum* also significantly reduced decay. Pre-storage treatment with AITC has been suggested as a possible application. It is very likely that this treatment will also be effective on apples.

2.2 Alkylresorcinols

Alkylresorcinols (AR) are a group of phenolic lipids that naturally occur in plants, including fruit (mango) and grains, and exhibit antimicrobial activity that may be involved in resistance to various diseases (Droby et al., 1987; Hassan et al., 2007; Landberg et al., 2008; Reiss, 1989). Rye bran is rich in AR and has been used to extract pure AR for testing its effectiveness against various fungi including apple pathogens *P. expansum* and *N. perennas* (causing bull's-eye rot) (Dey et al., 2013; Tahir et al., 2014). AR applied as an emulsion spray reduced spore germination and mycelial growth of both fungi by 50% on PDA medium, and blue mould between 17% and 52%, and bull's-eye rot between 31% and 78%, depending on cultivar of apples stored at 2°C for 12 weeks. Although this compound looks promising because no negative effect on apple skin appearance and organoleptic quality was apparent, more work is needed especially on effective concentrations on different apple cultivars in long-term storage.

2.3 Essential oils

Essential oils (EO) are obtained from plant materials mainly by steam distillation in commercial production; however, they can be also obtained by fermentation, pressing, and cold or hot enfleurage (Van de Braak and Leijten, 1999). There are a few thousand known EO of which approximately 300 are used predominantly as fragrances, flavours and pharmaceuticals. The antimicrobial properties of some EO have been known for a long time and their potential for application in foods has been widely investigated (see review by Burt, 2004; Deans and Ritchie, 1987). The phenols eugenol, thymol and carvacrol, and terpenoids menthol and eucalyptol have been most widely studied for their application in foods (Serrano et al., 2008). Results from studies on the use of EO for postharvest

control of decays on fruits and vegetables have been mixed. Although tests *in vitro* often indicated good antifungal activity, tests on fruit are less impressive. Postharvest application of eugenol, formulated with ethoxylate or Tween 80, to several apple cultivars was ineffective in controlling lenticel infection caused by *Phlyctema vagabunda*, blue mould (*P. expansum*), grey mould (*B. cinerea*) and brown rot (*Monilinia fructigena*), and when heated to 50°C, these treatments induced phytotoxicity and cuticle damage occurred. However, eugenol formulated with soy lecithin reduced the diseases without significant cuticle damage (Amiri et al., 2008). EO from several Mediterranean plants inhibited grey mould and blue mould development on apples, with emulsions of savoury and thyme oils at 1% being the most effective and having greater efficacy on 'Granny Smith' and 'Red Chief' than on 'Golden Delicious' (Lopez-Reyes, 2010). In general, the efficacy of the EO declined with length of storage and was dependent on cultivar. Thus, for the treatment to be effective during extended storage, it would have to be applied repeatedly. Garlic extract (40% and 50%) applied to apples reduced blue mould and grey mould more in a curative application than in a protective application, and in combination with 1% clove oil, further increased the efficacy of the protective application on the three apple cultivars tested (Daniel et al., 2015).

It appears that the potential usefulness of EO for controlling postharvest diseases of apples is restricted to applications combined with other alternative treatments, often with limited activity, that can result in an additive or synergistic effect that provides commercially adequate control (Janisiewicz and Conway, 2010; Mari et al., 2003; Spadaro and Gullino, 2014; Usall et al., 2015). The balance between the effective concentration of EO and phytotoxicity is a significant limitation to this approach. Before EO can be used commercially for controlling postharvest diseases, more research must be conducted on determining the relationships between an effective EO concentration (used individually or

Figure 1 Portable automated bin drencher developed for testing various decay control treatments applied to apples and pears before storage. A small reservoir tank allows for testing small volumes on an entire bin. This is particularly important with biocontrol agents, where limited amounts are available because they are often grown in small fermenters under laboratory conditions (Janisiewicz and Peterson, 2005).

in mixtures), exposure time, formulation and phytotoxicity. Also, the effect of EO on the organoleptic qualities of different cultivars of apples must be determined.

3 GRAS substances and sanitizers

3.1 Bicarbonate salt

Sodium bicarbonate (SBC, baking soda) was recommended for controlling postharvest decays of citrus fruits almost a century ago (Barger, 1928); however, with the advent of synthetic fungicides, its use has diminished. A renewed interest in this compound has occurred recently through demonstrations of its effectiveness on apples in combination with newly developed alternative methods, in particular biological control, hot water and hot air, and calcium chloride treatments, and also with other treatments on citrus, peaches and cherries (see review by Janisiewicz and Conway, 2010). On 'Golden Delicious' apples, treatment with 0.3% or 1.0% SBC significantly reduced blue mould, but not bitter rot, after 4 months of storage at 0°C followed by 14 days at 20°C (Conway et al., 2004). In a subsequent study, 2% SBC greatly improved control of blue mould when combined with two antagonists (see Section 8), *Cryptococcus laurentii* and *Metschnikowia pulcherrima*, applied either individually or in combination (Conway et al., 2005). In commercial trials, drenching bins Fig. 1 containing artificially wounded 'Golden Delicious' apples treated with 2% SBC reduced incidence of blue mould decay from 28 to 18% and from 72 to 35% after 5.5 months storage at ~1°C in CA (1.5% O_2, 2.0% CO_2) in two consecutive years, respectively (Janisiewicz et al., 2008b). In a drop inoculation test on wounded 'Golden Delicious' apples, 2% SBC reduced the incidence of grey mould and blue mould by 70% and 29%, respectively; in combination with the antagonist *Candida oleophila,* no grey mould developed and blue mould incidence was only ~2% (Droby et al., 2003). The reduction of postharvest decays on apples and other fruits under various conditions after treatment with SBC testifies to its usefulness, especially in combination with other alternative treatments, and as a cheap GRAS substance, it should be given serious consideration for wide commercial applications.

3.2 Calcium

Treatment of apples with calcium chloride ($CaCl_2$) after harvest reduces both physiological disorders such as bitter pit, internal breakdown and fruit softening (Bangerth et al., 1972; Conway et al., 2002; Mason et al., 1975; Reid and Padfield, 1975; Scott and Wills, 1979) and decays such as blue mould, grey mould and bitter rot (caused by *Colletotrichum gloeosporioides,* telomorph *Glomerella cingulata*) (Conway et al., 1991). The beneficial effect of calcium is attributed to increasing resistance in the fruit by making cell walls less accessible to fruit-softening enzymes or cell-wall-degrading enzymes produced by the pathogen rather than any direct effect it may have on the pathogens (Conway et al., 1988; Grant et al., 1973; Knee, 1978). Pressure infiltration of 'Golden Delicious' apples at 103 kPa with 2% $CaCl_2$ was more effective than dipping or vacuum infiltration (Conway, 1982), and after storing apples for 6 months at 0°C, inoculation with the pathogens and subsequent incubation at 20°C for up to two weeks, the incidence of bitter rot, grey mould and blue mould were reduced by 70%, 59% and 37%, respectively (Conway et al., 1991). Similar $CaCl_2$ treatments of 'Gala' apples stored at 1°C for 6 months reduced

blue mould by 38%, and in combination with an antagonist, *Pseudomonas syringae*, by ~80% (Conway et al., 1999). Calcium treatment combined with many other antagonists and heat treatments increased control of postharvest decays on various fruits (see review by Janisiewicz and Conway, 2010); dipping apples in $CaCl_2$ has been practised in some commercial packinghouses. However, to obtain the best results in reducing postharvest decays, concentrations of calcium in the tissue should be increased to a higher level, which can be achieved only by pressure infiltration (Conway, 1982). The uptake of calcium is affected by differences in cultivars, fruit maturity, growing season and even the orchard, creating the potential for peel injury if too much calcium is infiltrated, which complicates the use of this treatment (Conway et al., 2002).

3.3 Chitosan

Chitosan is derived mostly from the chitin of outer shell of crustaceans, mainly shrimp, crab and krill, by partial N-deacetylation (Sanford, 1989). Its structure is very similar to cellulose and, like cellulose, is not water soluble, which limits its application. Chitosan is approved as a food additive in the United States and has been used, for example, in food preservation, processing or deacidification of fruit juices (Zhang et al., 2011). It has wide antimicrobial activity and has been shown to inhibit the development of various fungal structures and postharvest decays of fruits, including those caused by *B. cinerea* and *P. expansum* on apples (El-Ghaouth et al., 2000a,b; Rabea et al., 2003; Yang et al., 2012; Yu et al., 2012; Zhang et al., 2011). It has also been shown to induce resistant responses against postharvest pathogens in apples and other fruits (Bautista-Banos et al., 2006; de Capdeville et al., 2002). A 2% aqueous suspension of chitosan reduced the lesion sizes of blue mould decay by as much as 85% on 'Red Delicious' when applied in a drop application test to intact fruits which were later wounded and inoculated with the pathogen. Glycol chitosan, a water-soluble form of chitosan, when used alone as the bioactive coating at 0.5%, reduced the incidence of blue mould and grey mould decay of 'Red Delicious' apples by 30%, and in combination with a biocontrol agent *Candida saitoana*, by 80% and 84%, respectively (El-Ghaouth et al., 2000a,b). The use of glycol chitosan as a bioactive coating is an attractive approach, because it has higher wettability, which allows for a uniform spread on fruit upon drying resulting in adequate adhesion, cohesion and durability to function properly. This may be especially beneficial when applying in combination with other treatments to increase the efficacy of control. It also eliminates the need for combining chitosan with surfactants, such as Tween 80 (Choi et al., 2002). Growing the biocontrol agent *Rhodotorula mucilaginosa* (a yeast) in nutrient yeast dextrose broth medium amended with 0.5% chitosan increased biocontrol activity of the yeast against postharvest pathogens of strawberries by stimulating its chitinase activity that degrades the pathogens' mycelial cell walls (Zhang et al., 2014). It will be worthwhile to determine if this approach would also work with antagonists in an apple system; for this to be commercially valuable, the antagonists will also have to be amenable to large-scale fermentation production systems.

3.4 Other chemicals

The potential of other substances such as silicon, selenium, potassium sorbate, nisin or ethanol has been explored for controlling pathogens causing postharvest diseases of apple. However, either low efficacy limits their usefulness or there is a lack of appropriate

follow-up tests on fruit to determine their potential effectiveness after the original *in vitro* research was published (Fadda et al., 2015; see review by Janisiewicz and Conway, 2010; Wu et al., 2014, 2016).

3.5 Acetic acid vapour and peracetic acid

Sholberg and Gauce (1995) were the first to demonstrate control of blue mould on wounded 'Golden Delicious' apples by fumigation with acetic acid (AA). Sholberg (1998b) also tested the effectiveness of several other short-chain organic acids against postharvest decays; however, AA was the most effective against *P. expansum* and other postharvest pathogens. Concentrations of 2.7 mg AA/L were sufficient to completely control the disease under laboratory conditions. High relative humidity in storage increased the effectiveness and consistency of the control at 5 and 20°C. In a large-scale test with naturally infected fruit that was fumigated with AA and then stored in air for 3 months at 1°C, a 50% reduction in decay was observed compared to the control (Sholberg et al., 2001). Unfortunately, AA treatment may cause lenticel burning that increases in severity with the number of fumigation cycles, and varies with the quantity of fruit, container type and apple cultivar. Thus, the fumigation with AA can be safely used only for sanitization of picking bins and large storage rooms (Sholberg, 2004, 2009).

Peracetic acid, which is derived from the treatment of AA with hydrogen peroxide, is also considered to be a good sanitizer, and has been successfully used on 'Braeburn' apples to reduce artificial contamination with *E. coli* O157:H7. Reductions in the bacterial population increased as the exposure time increased from 5 to 15 min at which point a 5-log reduction was achieved (Wisniewsky et al., 2000). No visible changes in the appearance or texture of the apples were observed after the treatment, however apples were rinsed with water to remove peracetic acid residues. At a concentration of 80 ppm, peracetic acid reduced *P. expansum* conidia by no more than 2-log units on the surface of various apple cultivars artificially inoculated with the fungus (Salomão et al., 2008). Such a reduction may not be adequate for effective decay control, especially when higher concentrations of pathogen spores occur in the handling system.

3.6 Chlorine and ozone

Chlorinated and ozonated water have been used as postharvest treatments for fruits and vegetables because of their biocidal activity; their usefulness in controlling fruit decays is limited mainly to dump tanks and flume water (Horovitz and Cantalejo, 2014; Salomão et al., 2008; Smilanick, 2003; Suslow, 1997). Both can inhibit spore germination of the major fruit decaying fungi; however, they are strongly effected by organic matter and are not effective in controlling decays originating from wound infection (Spotts and Cervantes, 1992; Spotts and Peters, 1980). Both are more useful as sanitizers against bacterial and viral contamination of produce (Suslow, 1997, 2004). Research from the 1940s indicates the usefulness of 1–3 ppm ozone gas as a sanitizer to lower spore counts on the walls of storage rooms and packaging more so than for direct control of apple decays where a concentration of 3.25 ppm may cause injury to fruit (see review by Horovitz and Cantalejo, 2014). There are some reports on the usefulness of ozone gas to control fruit diseases, for example it was reported that the shelf-life of apples was extended for several weeks by exposure to 5–6 mg/m^3 ozone gas for four hours every

day, but no follow-up research has been conducted after this initial report (Bazarova, 1982). Ozone gas is toxic to humans and special protection is needed for workers as the time-weighted average exposure for an 8 h work day is only 0.1 ppm according to the federal exposure limit (Smilanick, 2003). A postharvest dip of apples infected with flyspeck and sooty blotch in chlorine (800 ppm for 7 min) followed by 30 sec brushing and a rinse, resulted in an increase of Extra Fancy-grade apples from 25% to 55% and 100% for 'Jonathan' and 'Golden Delicious', respectively (Batzer et al., 2002). In the same study, treatment with a mixture of hydrogen peroxide and peracetic acid (80 ppm for 7 min) was less effective. As much of apple handling in packinghouses is in water, chlorine is often added to kill spores of fungal pathogens causing fruit decays and also to protect against contamination with food-borne pathogens (Kelley, 2004; Suslow, 2004). As pH and organic content have significant effects on the antimicrobial activity of chlorine special equipment must be used to maintain the desirable concentration of active chlorine.

4 Heat treatment

Heat treatments of apples before storage have been developed for controlling pathogens and insects, enhancing fruit quality by slowing fruit ripening and senescence, and reducing storage disorders (Klein and Lurie, 1990; Lurie, 2001; Lurie et al., 1998; Porritt and Lidster, 1978; Smith and Lay-Yee, 2000). In controlling postharvest decays, the main effect is directly on the pathogens, although there may be some defence reactions induced in apples (Conway et al., 1999; Lurie, 1998; Maxin et al., 2012a,b). Heat may be applied to apples as hot dry air, a hot water dip, or a short hot water rinse and brushing (Burchill, 1964; Fallik, 2004; Fallik et al., 2001; Klein and Lurie, 1990; Porritt and Lidster, 1978). Heat treatments alone are only partially effective in reducing fruit decays on apples kept for long periods of time in cold storage; however, in combination with other treatments, for example calcium infiltration, SBC and biological control, can provide a commercially acceptable level of control (Conway et al., 1999, 2005; Leverentz et al., 2000). Results from heat treatments with respect to the efficacy of decay control, sensitivity to heat injury or the effect on fruit quality are often cultivar dependent, which puts an additional burden on the commercial development of this treatment.

4.1 Hot air

The most commonly reported hot air treatment of preclimacteric apples is 38°C for four days. This temperature and duration of exposure effectively controlled blue mould from natural infection (Porritt and Lidster, 1978) and artificial inoculation with *P. expansum* or *B. cinerea* (Conway et al., 1999; Fallik et al., 1995; Klein et al., 1997). This treatment also reduced fruit softening and respiration on 'Anna' and 'Granny Smith' as well as enhanced the colour and overall quality of 'Golden Delicious' apples without causing heat injury after several months storage at 0 or 1°C (Fallik et al., 2001; Klein and Lurie, 1990; Porritt and Lidster, 1978; Sams et al., 1993). Hot air treatment changed the structure of the epicuticular wax of 'Golden Delicious' apples, removing the typical wide network of deep cracks, resulting in lower penetration of $CaCl_2$ during dip or pressure infiltration (Roy et al., 1994; Lurie et al., 1996). This should be taken into consideration when using an integrated

approach that includes hot air prior to treatment with $CaCl_2$ (Sams et al., 1993; Conway et al., 1999). When wounded 'Red Fuji' and 'Gala' apples were subjected to hot air treatment (38°C, 4d) and subsequent inoculation with *P. expansum*, *B. cinerea* or *C. acutatum*, wound healing occurred, resulting in significant decay reduction on 'Red Fuji' but not on 'Gala' apples (Shao et al., 2010).

4.2 Hot water dips, vapour and rinsing with brushing

The earliest experimentation with heat treatment for controlling postharvest decays of apples was conducted on 'Cox's Orange Pippin' infected with *Gloeosporium* spp. (Burchill, 1964; Edney and Burchill, 1967). Gloeosporium rot from natural infection was reduced to negligible levels by dipping apples in 45°C water for 6 minutes and storing at 2.8°C for 5 months (Burchill, 1964). Higher temperatures caused fruit damage, and temperatures lower than 40°C were ineffective. In subsequent studies, the effectiveness of hot water and steam vapour were compared on 'Cox's Orange Pippin' artificially inoculated with the fungus. Again, 45°C was the most effective temperature in controlling decay without causing fruit injury, and the hot water dip treatment appeared to be slightly more effective than the steam vapour treatment. Delaying the heat treatment for 3–9 days after inoculation increased the effectiveness of the treatments. This delay allowed spores to germinate, making the fungus more susceptible to the heat treatment. Treatment of organically grown 'Topaz' apples with hot water at 53°C for 2 min reduced *Gloeosporium* spp. decay from 56.5% to 2.9% and from 94.4% to 15.7% after 4 and 6 months of storage at 1°C, respectively (Trierweiler et al., 2003). Sensory evaluation of heated and non-heated fruit showed no differences except on the appearance of the apple because of browning of the peel, indicating that further refinement of the treatment is necessary for apples designated for fresh market. Hot water dips at 50°C for 3 min were very effective in reducing decays on 'Elstar' apples inoculated with conidia of *Neonectria galligena* and *P. expansum,* and to a lesser extent on apples inoculated with *B. cinerea* and stored at 2°C for several weeks (Maxin et al., 2012a). Heat treatment, in addition to a direct effect on pathogen viability, also reduced decay on 'Elstar' apples inoculated with *P. expansum* after heat treatment, indicating the activation of a defence response in the fruit. Skin damage on this cultivar begins to occur at 51°C. On 'Ingrid Marie' and 'Pinova' apples, this treatment was also effective against decays caused by natural infection of *N. alba*, *N. perennans*, *M. fuctigena*, *C. acutatum*, *Phacidiopycnis washingtonensis, Cladosporium* spp. and *N. galligena* after 3 months of storage at 2°C followed by 18 days at 18°C.

Hot water rinsing has been shown to be effective in protecting other fruits and vegetables from postharvest decays (Fallik, 2004). Fallik et al. (2001) compared hot air (38°C, 4 d) with hot water rinsing and brushing (55°C, 15 sec) and found both treatments to be equally effective in controlling blue mould on naturally infected or artificially inoculated with *P. expansum* 'Golden Delicious' apples stored for 4 months at 1°C followed by 10 days at 20°C. However, hot water rinsing and brushing at 60°C for 20 sec increased decays caused by *P. expansum*, *Mucor* spp., *Phoma exigua* and *P. washingtonensis* on 'Ingrid Marie' and 'Pinova' apples, which was attributed to increased sensitivity of the fruit due to heat damage (Maxin et al., 2012b).

Despite problems with heat damage and the need to establish optimal conditions for individual cultivars, short hot water dipping is an attractive treatment as part of an integrated system because it could be relatively easily adopted for application on packing lines for treatment of fruit before, during and after storage.

5 Controlled atmosphere

The research on controlled atmosphere (CA) storage of apples with reduced O_2 and elevated CO_2 dates back to 1920 with the pioneering work by F. Kidd and C. West in England, followed in the 1930s by R. Smock in the United States (see Olsen, 1986; updated 2010). A fungistatic effect of CA on postharvest pathogens of apple such as *P. expansum* or *B. cinerea* was first observed *in vitro* (Littlefield et al., 1966; Wells and Uota, 1970), and then demonstrated on strawberries where oxygen levels of 0.25% were effective in reducing decay caused *by B. cinerea* but not by *Rhizopus stolonifer* (Couey et al., 1966). As it became apparent that apples stored in CA senesce less and have higher quality than those stored in air, parameters for atmosphere composition in CA

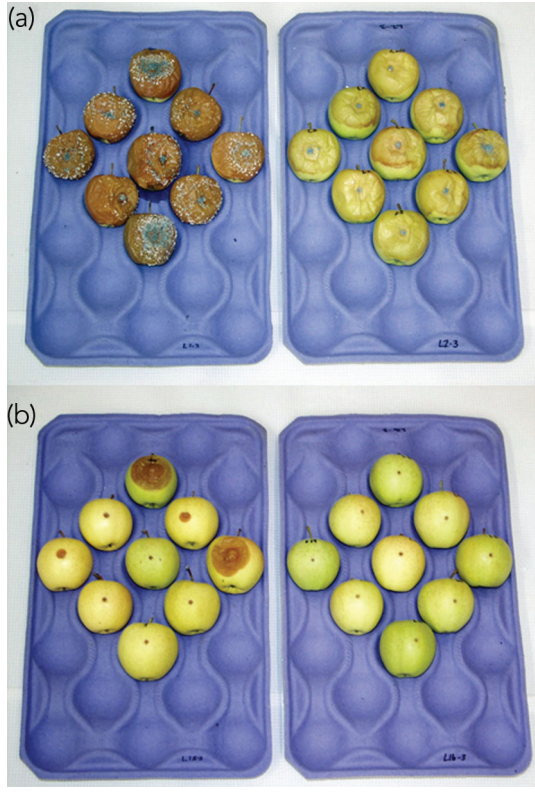


Figure 2 Blue mould development on 'Golden Delicious' apples wound-inoculated with *Penicillium expansum* alone (control treatment) (a) or in combination with a mixture of two antagonists and sodium bicarbonate (2% w/v) (b), and stored in air (trays on the left) or in CA (trays on the right) storage for 4 months at 1°C. Note slightly reduced and lighter in colour decay symptoms on control treatment apples stored in CA in comparison with apples stored in the air. Complete decay control was achieved on apples protected with the antagonist mixture and sodium bicarbonate and stored in CA, while apples with the same treatments but stored in air still exhibited some decay (Conway et al., 2007; Janisiewicz, 2013).

storage were developed for individual cultivars (Drake, 1999; Olsen, 1986). Anecdotal evidence indicated that apples stored in CA had less decay than those stored in air, which agrees with general observations that apples exhibiting a lesser degree of senescence are less susceptible to disease. Later it was shown that CA storage suppresses postharvest diseases by both extending the period of host resistance and by the direct fungistatic effect on the pathogens (Sommer, 1985). Sitton and Patterson (1992) showed conclusively that storing 'McIntosh', 'Golden Delicious' and 'Delicious' apples in CA with CO_2 at 2.8% reduced the development of decay caused by *B. cinerea*, *P. expansum* and *Pezicula malicorticis* after 61 days at 0°C and maintained high fruit quality. Low O_2 had limited fungistatic activity and at levels above 3% appears to favour decay development. Storing organic 'Topaz' apples in CA (1% O_2, 3% CO_2) at 1°C reduced *Gloeosporium* decay from 56.5% to 24.1% and from 94.4% to 41.4% after 4 and 6 months of storage, respectively (Trierweiler, 2003).

Levels of decay control on apples stored in CA have not been adequate to rely on this approach alone for controlling decays in commercial storage. Instead, CA can be used as an additional tool that could be easily combined with other alternative treatments for combating postharvest decays (Conway et al., 2007; Tahir et al., 2009; Fig. 2). To achieve optimal decay control by CA treatment without compromising fruit quality, different parameters such as the cultivar, physiological age of the fruit, susceptibility to physiological disorders such as superficial scald, soft scald, brown core, internal browning, and susceptibility to low O_2 and high CO_2 injuries, as well as prevailing pathogens in the region and length of storage should be considered.

6 Irradiation with UV-C

The application of UV-C irradiation, in addition to having induced resistance activity at low irradiation levels of 7.5 kJ m^{-2} (see section on induced resistance), has been shown to have direct germicidal activity against postharvest pathogens such as *B. cinerea*, *C. gloeosporioides* and *Cryptosporiopsis perennans* (now *Neofabraea perennans*, causing bull's-eye rot) (Bartnicki et al., 2010; Marquenie et al., 2002; Stevens et al., 1996, 2005). The germicidal effect was shown to be greatly enhanced against *B. cinerea* and *C. acutatum* by including a dark period of four hours immediately after irradiation (Janisiewicz et al., 2015, 2016b). It is presumed that this prevents activation of the light-induced repair mechanism for repairing DNA damaged during exposure of the fungus to UV-C. Irradiation of apples with UV-C may serve two purposes, a reduction of food-borne bacterial pathogens and control of fruit decays caused by fungi. Exposing the surface of 'Red Delicious' apples artificially inoculated with *Escherichia coli* O157:H7 to a UV-C dose of 24 mW cm^{-2} reduced populations of this bacterium by 3.3-log units (Yuan et al., 2004). Under commercial conditions, a UV-C dose of 0.0069 kJ m^{-2} reduced bull's-eye rot on artificially inoculated 'Fuji' and 'Gala' apples by 56% and 69%, respectively, and on naturally infected apples by 54% and 85%, respectively (Bartnicki et al., 2011). In the same study, UV-C treatment was as effective as a hot water (50°C) spray for 12 sec. UV-C treatment of apples on sorting lines has been used occasionally in packinghouses before experimentally proven efficacy (Janisiewicz, personal observations); however, with the new information, its use will only increase due to its relatively high efficacy, adaptability to current practices and presumed compatibility with other alternative treatments (Wilson et al., 1997).

7 Natural sources of resistance

7.1 Induction of host defence mechanisms

Plants have evolved complex coordinated mechanisms to defend themselves against fungal, bacterial and insect pathogens. Innovative studies in plant defence have been conducted in the model plant *Arabidopsis thaliana* due to its short life cycle, genetic tractability, and the broad molecular toolkit developed. Over the past 10 years, fundamental scientific breakthroughs in plant innate immunity, induced resistance and preformed defences have been translated from *Arabidopsis* to fruit and vegetable crops with the end goal of achieving sustainable control. The bulk of these investigations have focused on physical and chemical aspects to prime host plant defences and induce resistance. Here we discuss apple fruit defences induced by UV-C irradiation, heat treatment, wound responses, antioxidant signalling and systemic acquired resistance (SAR) inducers (Terry and Joyce, 2004).

Earlier mentioned physical treatments of apple fruit may also induce host defences and have largely entailed the use of low hermetic doses of UV-C and heat treatments (i.e. hot air or water). Hot air treatment at 38°C for 4 days was shown to be cultivar dependent on its impact on wound healing and defence protein activity (Shao et al., 2010). Hot water treatment of apples at 45°C revealed levels of blue mould control from 30 to 100% and was accompanied by the induction of genes involved in heat shock that may also mediate resistance (Spadoni et al., 2015). Low hermetic doses of UV-C reduced disease incidence and severity of bitter rot and *Alternaria* rot on 'Golden Delicious' apples by 70% and 40%, respectively (Stevens et al., 1996, 2005; Wilson et al., 1994). Positioning apples with the stem end towards the irradiation source induced resistance to *C. gloeosporioides* more than those positioned on their side, or with two-side rotation, suggesting the presence and concentration of signal transduction receptors located at the stem end of the fruit (Stevens et al., 2005). While these tactics independently provided varying levels of control they have not been adopted by industry; however, they could be combined with other alternatives to achieve higher levels of control. Both heat and UV-C treatments not only induced resistance in the host but, as mentioned previously, also have biocidal effects on the pathogen. It is possible that the resistance genes induced by heat and UV-C can be activated in commercial apple varieties like 'Gala', 'Fuji' and 'Honeycrisp' using molecular approaches. These inducible defence pathways could be amplified in the absence of the stimulus (i.e. UV-C and heat) to create higher levels of resistance to decay in existing commercial apple varieties.

Treatment of apple fruit with chemical inducers that activate plant defence pathways had been studied mainly using salicylic acid (SA), jasmonate (JA), β-amino butyric acid and ethylene. A study by Quaglia et al. (2011) showed that apple fruit treated with methyl jasmonate, β-amino butyric acid or acibenzolar-*S*-methyl (SA analogue) induced the accumulation of pathogenesis-related (PR) proteins in apple, but was found to be ineffective against *P. expansum* infection. However, the antimicrobial activity of these compounds and their direct effects on fungal growth were demonstrated. It is possible, therefore, that the timing or intensities at which the host response was elicited may be altered to provide a better means of control in the future.

Molecular genetic studies using pathogen-inoculated fruit corroborates findings from physiological investigations using SAR inducers and shows that apple defence mechanisms

are complex and multi-factorial. For example, Yin et al. (2013) showed that resistance in apple fruit to *Diplocarpon mali* involves the production of reactive oxygen species (ROS), SA/JA signalling and PR protein expression, which are hallmarks of plant defence. It is clear that more fundamental research in priming host defence responses using chemical elicitors is needed in apple. In-depth investigations combining the use of genomic, metabolomic and proteomic approaches may uncover primary pathways, master regulators or potent chemical inducers of resistance that can lead to defence reactions resulting in higher levels of resistance to postharvest decay.

Apples are hand harvested and stored for extended periods of time, during which fruit may succumb to bruises, fingernail scratches, stem punctures and stem-end pulls that are the main route of infection for major pathogens such as *Penicillum* spp. and *B. cinerea*. Numerous investigations concerning the role of wound responses and physical barriers to prevent decay in apple have been conducted as a result. Lakshininarayana et al. (1987) demonstrated wounds healing in two different apple cultivars, at a pre-climacteric stage of maturity, which resulted in higher levels of phenolic compounds, tannins, lignins and callose. Ultimately, healed wounds were more resistant to *B. cinerea* and *P. expansum* than fresh wounds due to the formation of physical barriers that prevented pathogen establishment. More recent studies by Torres et al. (2003) have shown the important role of hydrogen peroxide (H_2O_2) synthesis and antioxidant enzyme production in resistance to blue mould of immature 'Golden Delicious' apples. Their results indicate that harvest date and fruit maturity play pivotal roles in modulating resistance. Additional studies by Su et al. (2011) and Vilanova et al. (2014) showed that wound-induced activation of H_2O_2 accompanied by antioxidant enzyme activity and lignification decreased with host maturity indicating that host metabolism in apple fruit is an important component of resistance to decay and may be a future target for manipulation in the host to mitigate decay.

7.2 Genetic sources of resistance and their application for postharvest decay control

Resistance to disease and insects has been routinely considered an important component of apple breeding programmes worldwide. However, the focus of these efforts has been towards field production with little attention towards resistance to postharvest decay (Janisiewicz et al., 2008). The genus *Malus* is composed of 27 wild species of which *M. sieversii* Lebed. is considered the progenitor of the domesticated apple (Juniper et al., 1999). Central Asia is the centre of origin for apples, and wild *Malus sieversii* forests are still present in Kazakhstan (Dzhangaleiv, 2003). In the 1990s, the USDA conducted several collecting trips for wild apple germplasm resulting in the Kazak collection in Geneva, New York (Forsline et al., 2003). This wild apple collection includes a wide variety of horticultural traits, represents a wide array of genetic diversity and has been the focus of identifying sources of resistance to physiological disorders (scald, drought), field diseases (fire blight, scab) and postharvest decay (blue mould, bitter rot).

The Kazak wild apple germplasm collection has been evaluated for resistance to two of the most economically important postharvest decay pathogens in the United States, *P. expansum* and *C. acutatum* (Janisiewicz et al., 2008a; Jurick II et al., 2011). These pioneering studies revealed previously undocumented levels of resistance to blue mould and bitter rot, and in some cases, immunity to both diseases. These studies uncovered that resistance to both pathogens is rare and that the distribution of phenotypes with regard to decay tolerance is skewed towards susceptibility. They also showed that

accessions resistant against one pathogen (i.e. *P. expansum*) can be susceptible to the other (i.e. *C. acutatum*) and vice versa. Results from these studies have reinforced the utility of the wild apple germplasm collection for the identification of resistance to blue mould and bitter rot, which are currently lacking in commercial apple cultivars (Spotts et al., 1999). Phenotypic data from these studies are being used by breeders to find quantitative trait loci (QTL) associated with resistance to blue mould and bitter rot and to develop DNA-based markers (Norelli, 2013). Such tools can assist breeders in screening crosses to incorporate resistance into new varieties against postharvest decay pathogens to help abate decay (Iwata et al., 2016).

Time course studies of wound responses in wild apple accessions with varying levels of resistance have shown that resistance is positively correlated with the speed of the wound response that prevents the pathogen from infecting the host and causing decay (Janisiewicz et al., 2016a). No decay developed, even from wounds inoculated with pathogen concentration of 10^5 conidia/mL, on most resistant/immune accessions. Histochemical analysis of the wound area revealed that high levels of ROS were detected immediately after wounding in both resistant/immune and highly susceptible accessions, and that callose and lignin/suberin play minor roles in the defence response. These studies indicate that durable resistance in physiologically mature wild apples is governed by several mechanisms. This presents new challenges in explaining the mechanisms of resistance as some of these results, especially with regard to ROS, are contrary to the earlier reports in the literature for cultivated apples. Since it appears that a constitutive resistance/immunity exists in some accessions, profiles of phenolic compounds from these apples may be the first logical step to explore and are currently underway by Janisiewicz and colleagues.

Conducting omics-based studies including comparative genomics, transcriptome sequencing, metabolomics and proteomics to discover molecular regulators, biochemical pathways and DNA-based markers mediating resistance in wild apple fruit to postharvest decay is essential to explain the basis of the observed resistance. They can be followed by comparative/functional genomics to explain the genetic mechanisms and their regulators which can then be transferred to existing commercial cultivars to make them resistant to decay. A two-pronged approach for creating new apple cultivars using molecular markers for disease resistance and genome-editing technologies to modify existing cultivars would result in less decay and increased fruit quality. DNA-based markers for resistance, utilized in marker-assisted breeding, could be used by breeders to make new apple cultivars with superior disease resistance. Genome-editing technologies involving CRISPR/Cas9 (Hsu et al., 2014; Ledford, 2015; van der Oost et al., 2009) may be used to manipulate molecular regulators and edit promoters of apple fruit defence genes to enhance decay resistance in popular apple cultivars. With forthcoming fundamental developments in determining mechanisms and regulators of host resistance, application of these breakthroughs will give rise to new possibilities for controlling postharvest decays, and are limited only by the scope of scientific creativity, imagination and resources.

8 Biological control

Biological control of postharvest diseases of temperate, subtropical and tropical fruits has been the most widely studied alternative to synthetic fungicides during the past three decades. Research conducted in more than two dozen countries resulted in

several commercial products originally developed mostly for apples such as Bio-Save® (a.i. *Pseudomonas syringae*), Boni Protect (a.i. *Aureobasidium pullulans*), Candifruit (a.i. *Candida sake*), Nexy (a.i. *Candida oleophila*), Pantovital (a.i. *Pantoea agglomerans*), and Yield Plus (a.i. *Cryptococcus albidus*) and for citrus fruits, Aspire (a.i. *Candida oleophila*) (Droby et al., 1998; EFSA, 2012; Janisiewicz and Jeffers, 1997; Jijakli et al., 1993; Leibinger et al., 1997; Nunes et al., 2002; Usall et al., 2001). As more information is accumulated on the efficacy of some of these products the registration has expanded to other fruits and vegetables.

Bio-Save® is the only biocontrol product currently being used for control of postharvest decays on apples and pears in the United States, and is a good example for a case study (Janisiewicz and Jeffers, 1997; Janisiewicz and Marchi, 1992). Aspire™, based on *Candida oleophila*, is the other registered product; however, it was taken off the market three years after its introduction in 1995. Bio-Save® use has been increasing since its large-scale introduction in 1995, and the original registration for postharvest use on apples, pears and citrus fruit has been expanded to cherries, potato and sweet potato, with registrations for additional commodities pending (http://jetharvest.com/; Janisiewicz, personal information). This product has been very effective in controlling blue mould of apples (Janisiewicz and Jeffers, 1997). In three out of four large-scale trials, on wounded and artificially inoculated with *P. expansum* conidia 'Golden Delicious' and 'Delicious' apples, it reduced the incidence of blue mould decay to below 4% compared with controls where decay incidence ranged from 40% to 92% (Janisiewicz and Jeffers, 1997). The reduction did not differ from standard 0.05% thiabendazole (TBZ) treatment. In the fourth test (conducted as a first in the series), the decay incidence on Bio-Save® treated apples was 29% and 96% on control. In all four trials, control by an unformulated *P. syringae* antagonist suspension was no different from TBZ treatment. Selection of this antagonist is a result of a massive

Figure 3 An example of the enhancement of blue mould biocontrol on 'Golden Delicious' apples with an antagonist mixture. Apples were wound-inoculated with *Penicillium expansum* and treated with either suspensions of individual yeast antagonists (ST1-A24 or ST4-E14, upper boxes) or a mixture of these antagonists (ST1-A24 x ST4-E14). The antagonists were mixed in equal proportions and the total biomass of the mixture was equal to the biomass in treatment with an individual antagonist suspension.

screening process of microorganisms isolated from apple and pear fruit that lead to finding several very effective bacterial and yeast antagonists (Janisiewicz et al., 1987; Janisiewicz and Roitman, 1988). Currently there are more than two dozen programmes worldwide working on the development of potential antagonists for biological control of postharvest decays on various fruits primarily using bacteria and yeasts isolated from the fruit surfaces (Janisiewicz and Korsten, 2002; Mari et al., 2014). Selecting a screening method in a search for the antagonists may predetermine the mechanism of biocontrol by which an antagonist controls the pathogen. For example, *in vitro* dual culture plate method, which selects an antagonist based on its ability to inhibit growth of pathogen mycelium on agar media, selects for antagonists producing inhibitory compounds such as antibiotics, siderophores (compounds sequestering iron) or lytic enzymes (e.g. chitinase) (Calvente et al., 1999; Chen et al., 2016; Di Francesco et al., 2016; Janisiewicz and Roitman, 1988). A membrane method may be used to select antagonists colonizing different fungal structures including appressoria, which may be important for fungi such as *Colletotrichum* spp. or *Monilinia* spp. that have a latent infection stage on the fruit (Janisiewicz et al., 2011). A direct screening on fruit, although much more laborious, results in the selection of antagonists with potentially different mechanisms of biocontrol, which, in addition to those previously mentioned, may include competition for limited nutrients and space, direct parasitism, induced resistance or interference with pathogenicity factors (Di Francesco et al., 2016; Manso and Nunes, 2011; Zhang et al., 2016). Thus, it is important to use more than one method to increase the pool of antagonists with different mechanisms of biocontrol. Some antagonists may operate by more than one mechanism. Antagonists producing antibiotics are often very effective but may be difficult to commercialize because of potential problems with registration. However, they may lead to development of effective fungicides; for example, *Pseudomonas cepacia* (now *Burkholderia cepacia*) has been shown to be a very effective antagonist against blue mould and grey mould on apples and pears, grey mould on strawberries, brown rot on peaches and nectarines and blue mould on citrus in postharvest applications (Janisiewicz and Roitman, 1988; Smilanick et al., 1993). It produces an antibiotic compound identified as pyrrolnitrin (Roitman et al., 1990), which alone was also very effective in controlling blue mould and grey mould of apples, grey mould of strawberries and raspberries, blue mould on lemons, and *B. cinerea* on rose flowers (Janisiewicz et al., 1991; Takeda et al., 1990; Goulart et al., 1992; Hammer et al., 1993; Janisiewicz and Roitman, 1990). Several analogues of this compound have been produced and evaluated for disease control activity, and one of them, fludioxonil, is currently one of the most widely used postharvest fungicides against fruit decays worldwide.

For biocontrol agents to succeed in controlling decays originating from wound infections they must be good wound colonizers, the main court of entry for major apple pathogens. This may have additional benefits by protecting wounds against potential food-borne pathogens such as *E. coli* O157:H7 which can grow exponentially in apple wounds (Janisiewicz et al., 1999a). *P. syringae* (used in Bio-Save®) prevented *E. coli* from increasing its populations in apple wounds (Janisiewicz et al., 1999b). Biocontrol effectiveness can be increased by using mixtures of mutually compatible antagonists (Janisiewicz and Bors, 1995; Fig. 3), adding nutrients that are more beneficial to antagonists than to the pathogen they control (Janisiewicz et al., 1992), or growing antagonists in media that promote their survival and mechanism of biocontrol (Abadias et al., 2001; Zhang et al., 2014; Zeng et al., 2015).

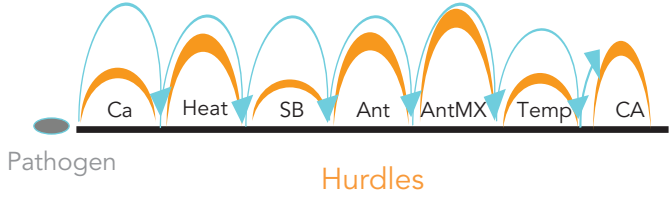
Many different yeasts isolated from various habitats, in addition to those mentioned previously, have been evaluated for biocontrol activity against postharvest decays of

apple. Unfortunately, after initial reports, few have been developed for commercial use. There may be several reasons for this, including poor performance of a biocontrol agent in more stringent tests, poor prospects for mass production and/or formulation of the biocontrol agent, concerns about human safety, lack of long-term commitment by the investigators and the reluctance of granting agencies to fund such long-term projects. Judging from the products on the market, the development of biological control against postharvest decays of fruit takes approximately ten years from inception to commercial production (Janisiewicz, 1998).

Large-scale commercial application of the biological control against postharvest disease of apples revealed its limitations. Although in many cases biocontrol can be as effective as fungicide treatments, it has inherent limitations such as lack of eradicative activity, more rapid decline in performance on ripe fruit and sensitivity to postharvest practices including high temperatures in drying tunnels, incompatibility with antioxidants (DPA, ethoxyquin) or chlorine residues on fruit handled in chlorinated water before storage (e.g. pre-sizing). Thus, biological control can be applied as the stand-alone treatment only when precautions are taken considering these limitations; otherwise it should be used as the main treatment in integrated control with other alternatives to synthetic fungicides described in previous sections (Di Francesco and Mari, 2014; Janisiewicz, 2013; Janisiewicz and Conway, 2010; Mari et al., 2014).

9 Integrated control

There is increasing agreement among investigators working with control of postharvest decays of fruits that replacing synthetic fungicides with a single alternative treatment may provide adequate control but only under some specific circumstances; in most situations it will be inadequate because of the limitations of the alternative treatments. Much of the recent research has been focused on developing integrated control that either encompasses biological control as the pillar in combination with other compatible treatment(s), or is just a simple combination of alternative treatments (see reviews by Di Francesco and Mari, 2014; Janisiewicz and Conway, 2010; Palou et al., 2016; Romanazzi et al., 2016; Usall et al., 2015). This approach is well represented by the hurdles concept



Ca = calcium, Heat = 38C for 4 d, SB = sodium bicarbonate, Ant= antagonist, AntMX = antagonist mixture; Temp = low storage temperature, CA = controlled atmosphere storage

Figure 4 Hurdle concept for controlling postharvest diseases on pome fruits. With each additional treatment (hurdle) reducing fruit decay by a certain amount, an additive or even synergistic effect can be achieved resulting in complete control (Janisiewicz, 2008).

developed for apples (Janisiewicz, 2008, 2013; Janisiewicz et al., 2008b; Fig. 4), which follows the original idea developed for food preservation (Leistner, 2000). Here, each additional treatment reduces the incidence/severity of the decay by a certain percentage, which may be additive or synergistic, and eventually results in the pathogen not being able to overcome the final hurdle, resulting in control of the fruit decay. Figure 4 represents apple treatments described in earlier sections, but the combination of treatments can vary as long as the treatments are mutually compatible and do not cause fruit damage (Di Francesco and Mari, 2014; Errampalli and Brubacher, 2006; Janisiewicz and Conway, 2010; Palou et al., 2016).

10 Future trends and conclusions

The need for sustainable and safe protection methods against postharvest decays of apples, and a rapidly growing organic market require the development of alternative approaches to synthetic fungicides. Advances made during the past two decades indicate that we are on the verge of developing new approaches that require integration of several alternative methods for apple decay control under a broad range of conditions. The extent of implementation of these methods will depend on the market forces as consumer demand for apples free of synthetic pesticides is growing rapidly and the supply of organic apples is often inadequate. As some of the alternatives, such as biological control, have been used in commercial packinghouses to satisfy the most urgent needs, transition to an integrated approach with a wider spectrum of activity and less limitations is expected to occur in the near future. The discovery of resistance to postharvest apple decays among the wild apple germplasm, for the first time, gives hope for the ultimate, genetic solution to achieve long-lasting durable resistance. This approach, however, will take a considerable amount of time and will require the long-term commitment of researchers and supporting institutions.

11 Where to look for further information

Much of the general information about controlling postharvest diseases of apples can be found on the websites of university extension services from major apple growing regions in the United States such as Washington State (http://postharvest.tfrec.wsu.edu), New York (http://www.scaffolds.entomology.cornell.edu), Pennsylvania (http://extension.psu.edu/plants/tree-fruit/diseases/apple-diseases) and Oregon (http://extension.oregonstate.edu/yamhill/search/postharvest%252Bapple/0/4). The second edition of Compendium of Apple and Pear Diseases and Pests, published by The American Phytopathological Society, includes postharvest diseases and their control. Most of the above sources provide information on control of postharvest apple decays that is focused on the use of fungicides. Agricultural Handbook No. 376, *Market Diseases of Apples, Pears and Quinces*, published by U.S. Government Printing Office in 1971, describes major postharvest diseases, except those described more recently, and also includes information on other decay control approaches including sanitation and cultural practices. The Research Institute of Organic Agriculture in Switzerland (FiBL) website (http://www.fibl.org/en/homepage.htm) provides

in-depth information on alternatives to synthetic fungicides, and various educational institutions such as the University of Hohenheim in Stuttgart (https://www.uni-hohenheim.de/eur-organic), University of Göttingen in collaboration with the University of Kassel-Witzenhausen in Göttingen (https://www.uni-goettingen.de/en/100751.html) and EUR-Organic consortium (http://www.eur-organic.eu/) offer individual courses and graduate degrees in organic agriculture. The latter also includes the websites of various educational and research organizations focused on sustainable agricultural practices.

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