Strategy for Combining Heat Treatment, Calcium Infiltration, and Biological Control to Reduce Postharvest Decay of 'Gala' Apples

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Abstract. The viability of *Penicillium expansum* Link conidia in sporulating culture declined rapidly when exposed to 38 °C, and when conidia were exposed to 38 °C prior to inoculation of apple fruits (*Malus ×domestica* Borkh.), the resulting lesions were smaller than those on fruit inoculated with nonheated conidia. 'Gala' apples were heated after harvest (38 °C for 4 days), pressure infiltrated with a 2% solution of CaCl_2 , or treated with the antagonist *Pseudomonas syringae* van Hall, alone or in combinations to reduce postharvest decay caused by *Penicillium expansum*. After up to 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*, antagonist, or Ca. Parallel lots of heat-treated and nonheated fruit that were either infiltrated or not infiltrated with Ca were stored up to 6 months. They were then inoculated with *P. expansum* alone, or with the antagonist followed by *P. expansum*. Prior heat treatment did not influence lesion size. Calcium alone, the antagonist alone, and heat plus Ca all reduced the incidence of decay by ≈25%, whereas heat plus the antagonist reduced it by 70%. Calcium plus the antagonist or Ca plus the antagonist and heat reduced decay incidence by 89% and 91%, respectively. The integrated strategy of heat-treating fruit, followed by Ca infiltration and then treatment with an antagonist, may be a useful alternative to controlling postharvest decay with fungicides.

Fungicides, when properly used, offer a high level of control of postharvest pathogens in most cases. However, alternatives to chemical control are being sought because of the growing concern of consumers, either real or perceived, that chemical residues on the surface of fruit can create health problems, as well as the increasing resistance of many fungi to commonly used fungicides. The ultimate goal of the research is to use alternatives, either alone or in combination, to reduce our dependency on fungicides.

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Prestorage hot air treatment of apples has promise as a method to reduce storage decay. Exposing 'Golden Delicious' apples to 38 °C for 4 d reduced decay caused by *Penicillium expansum* Link in fruit inoculated after 6 months of storage at 0 °C (Sams et al., 1993). More recently, decay caused by *Botrytis cinerea* Pers:Fr. or *P. expansum* was virtually eliminated in stored 'Golden Delicious' apples heat-treated at 38 °C after inoculation with the fungus (Fallik et al., 1995; Klein et al., 1997).

Increasing the Ca concentration of apple flesh by direct postharvest application also reduces postharvest decay caused by *B. cinerea*, *Glomerella cingulata* (Stoneman) Spauld. & Schrenk, and *P. expansum* (Conway and Sams, 1983; Conway et al., 1991). The Ca concentration necessary to reduce decay in fruit is higher than can be gained with standard fertilization practices. Therefore, direct post-

harvest application is the most successful method of achieving the desired concentration (Conway, 1982).

A mechanism proposed for this increased resistance involves the binding of Ca ions to pectins in the cell wall. Apple fruit cell walls with higher concentrations of Ca are more resistant to maceration by polygalacturonase produced by *P. expansum* than cell walls lower in Ca (Conway et al., 1988). Increasing the Ca content of apples also maintains fruit firmness (Sams and Conway, 1984) and reduces storage disorders, since many of these disorders are related to tissue Ca concentration (Shear, 1975).

A more recent alternative to the use of postharvest fungicides involves biological control of postharvest pathogens (Janisiewicz, 1988, 1998; Korsten et al., 1994; Wilson and Wisniewski, 1989). Decay caused by *B. cinerea* and *P. expansum* has been controlled on apples and pears (*Pyrus communis* L.) by bacterial and yeast antagonists, both under laboratory and large-scale storage tests (Chand-Goyal and Spotts, 1996; Janisiewicz and Marchi, 1992; Janisiewicz et al., 1994; Roberts, 1990). Additionally, commercially acceptable levels of control have been achieved in some instances (Janisiewicz and Marchi, 1992; Roberts, 1990). *Pseudomonas syringae* van Hall (strain ESC-11) is sold commercially under the names BioSave-11 and BioSave-110 (EcoScience Corp., Orlando, Fla.), and is currently being used to control decay on apples and pears caused by *B. cinerea*, *Mucor piriformis* Fischer, and *P. expansum* (Janisiewicz and Marchi, 1992; Janisiewicz and Jeffers, 1997).

The alternatives to chemical control are generally less effective than many fungicides. The effect of external factors on biological control (performance margin) is generally greater than for fungicides. For example, as fruit mature, higher concentrations of the antagonist must be used to achieve the same level of control as on immature fruit (unpublished data). One of the major problems with using Ca infiltration as a decay resistance factor has been the inability to predict the amount of Ca absorbed and the resulting surface discoloration if too much is absorbed (Conway et al., 1994a). While heat treatment virtually eliminates decay if fruit are inoculated prior to heating, it has little effect when applied after inoculation (Klein et al., 1997).

A combination of the above three methods may be more effective than any one alone, and the different methods may complement one another to help minimize the shortcomings of each. Combining heat and Ca treatments was found to be more effective than either alone under certain circumstances (Conway et al., 1994b; Klein et al., 1997; Sams et al., 1993) and combining Ca infiltration with a biocontrol agent was more effective than the individual treatments alone (Janisiewicz et al., 1998).

The objective of this study was to combine heat treatment, Ca infiltration, and the antagonist *P. syringae* strain ESC-11 to develop an integrated strategy to reduce postharvest decay of 'Gala' apple fruit caused by *P. expansum*.

Materials and Methods

Fruit. 'Gala' apples were harvested on 8 Sept. 1997 in the preclimacteric stage from a commercial orchard in Pennsylvania and randomized prior to treatment. Ethylene production was $<2 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{s}^{-1}$ and the climacteric rise in CO_2 production had not yet begun. Firmness was 88.2 N, soluble solids concentration 12.6%, and starch 6.6 as measured by the Cornell Scale of 1 to 8.

Heat treatment. Fruit were placed in tray-packed boxes with perforated polyethylene bags as liners and then heated in a thermostatically controlled ($\pm 1^\circ\text{C}$) walk-in chamber. Apples were heated at 38°C for 4 d and the relative humidity was maintained at $>85\%$ in the chamber. Storage conditions were monitored with a hygrothermograph (Belfort Instrument Co., Baltimore).

Calcium treatment. 'Gala' apples were pressure-infiltrated (3 min at 103 kPa) with a $0.14 \text{ mol} \cdot \text{L}^{-1}$ solution of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. Following treatment, the fruit were allowed to air-dry and then were either stored immediately at 1°C or given further treatment prior to storage. Fruit not heat treated were Ca-infiltrated within 2 d of harvest. Heat-treated fruit were placed in the heat chamber within 2 d of harvest, removed after 4 d, allowed to cool to 24°C for $\approx 24 \text{ h}$, and then Ca infiltrated.

Total calcium content. Calcium content of the apple tissue at the depth of the inoculation wound was determined by removing the peel and outer flesh of the entire fruit to a depth of 2 mm with a mechanical peeler. This layer was discarded. The next 2 mm of flesh was removed, again with the mechanical peeler, and used for analysis. After being removed, the fruit flesh was frozen immediately in liquid N_2 , freeze-dried, and ground. From each sample, 1 g of dried material was ashed, dissolved in 5 mL of $2 \text{ mol} \cdot \text{L}^{-1}$ HCl, and filtered. The samples were then analyzed for Ca content by inductively coupled plasma emission spectrometry. The flesh from four apples made up one sample and five samples were analyzed for each treatment.

Pathogen. The pathogen used was a very aggressive isolate of *P. expansum* (MD-8) from our collection, which had been previously isolated from a decayed apple in storage. It was maintained on potato-dextrose-agar (PDA) and continued virulence was assured by periodic transfers through apple. The conidial suspension used for fruit inoculations was prepared as previously described (Janisiewicz and Marchi, 1992).

Effect of heat on the pathogen. To determine the direct effect of heat on viability and pathogenicity of *P. expansum* conidia, a 10-d-old culture of the pathogen growing on PDA in a petri plate was held at 38°C . After 24, 48, 72, and 96 h, a small section of the culture was removed, the conidia were harvested in sterile distilled water containing 0.05% Tween-20 (polyoxyethylene sorbitan monolaurate) and the concentration of the conidial suspensions adjusted to either 10^4 , 10^5 , or 10^6 spores/mL using a haemocytometer. To determine the viability of the conidia, 0.10 mL of each of the

three conidial concentrations was spread with a glass rod over the surface of PDA plates. The plates were then placed in a 22°C incubator and evaluated daily for colony growth. The viability of the conidia was determined by the number of colonies on the plate. Three plates were used per conidial concentration. To determine the effect of heat on pathogenicity, 'Gala' apples which had been in storage at 1°C for 6 months were removed, placed at 24°C for $\approx 24 \text{ h}$, inoculated with *P. expansum* conidia that had previously been exposed to 38°C as above, incubated at 20°C for 7 d, and rated for decay as described below. There were three replicates of a single apple per treatment, and each fruit was wounded and inoculated at three sites.

Antagonist. The antagonist used was *P. syringae* isolate ESC-11, which is commercially used in BioSave-11 and BioSave-110. This isolate was grown in 50 mL of nutrient yeast-dextrose-broth (NYDB) medium in 250-mL Erlenmeyer flasks on a rotary shaker at 150 rpm at 26°C . Following incubation for 24 h, the cells were harvested by centrifugation at 7000 g, for 10 min, resuspended in water, and the concentration adjusted to $5.4 \times 10^8 \text{ CFU/mL}$ with a spectrophotometer.

Fruit inoculation. To test the effect of heat on *P. expansum* pathogenicity and host resistance, wounded apples were inoculated with *P. expansum* just prior to heat treatment (38°C , 4 d). Following heat treatment, fruit were evaluated after incubation at 24°C for 7 d or after 3 and 6 months storage at 1°C . To determine the effects of the treatment combinations, heat-treated fruit were allowed to cool to 24°C and then pressure infiltrated with the Ca solution, allowed to air dry, and placed in storage at 1°C . A similar inoculation protocol was followed when the fruit were inoculated 3 and 6 months after storage, except that, in some treatments, inoculation with the antagonist preceded inoculation with *P. expansum* conidia. Each apple was wounded twice on the same side by removing a section of tissue 3 mm in diameter and 3 mm in depth. One wound was 15 mm above the equator of the fruit toward the stem end and the other 15 mm below the equator directly under the first wound. Each wound was inoculated with 25 μL of the *P. syringae* suspension, allowed to air dry ($\approx 1 \text{ h}$), and then inoculated with 20 μL of a conidial suspension (5×10^4 conidia/mL) of *P. expansum*. There were 15 fruit per replicate and three replicates per treatment. The fruit were incubated on fruit tray packs in plastic boxes at 24°C for 7 d, and the incidence and diameter of the lesions determined. The fruit were arranged in a randomized block design.

Antagonist recovery after heat treatment. The effect of heat on the growth or activity of the antagonist was tested by wound-inoculating the fruit with the antagonist prior to heat treatment at 38°C . Separate lots of fruit were removed from the heated chamber 24, 48, 72, and 96 h after inoculation for recovery studies. As a control, a similar lot of fruit was inoculated with the antagonist, placed at 26°C and removed after the same times in storage

as the heat-treated fruit. Recovery of the antagonist from the wounds was conducted according to the procedure described previously (Janisiewicz, 1996). The colonies on the plates were counted with a Cobanna III laser counter (Spiral Biotech, Bethesda, Md.) after incubation for 24 h at 24°C . The counts of CFU per wound were determined using the BEN 4.0 program (Spiral Biotech). There were three fruit with three wounds per fruit for each removal time.

Statistical analysis. Analysis of variance was performed on decay development data using the general linear models procedure (PROC GLM) of the Statistical Analysis System based on type III sums of squares and a randomized complete-block design with factorial treatments (SAS Institute, 1989). The minimum significant differences for separation of the means for the 3 and 6 month data for heat, Ca, antagonist treatments, and their combinations were determined with the Bonferroni (Dunn) *t* test.

Results

Effect of heat on the pathogen. Heat treatment of the *P. expansum* culture at 38°C reduced conidia survival 10-fold after only 24 h and well over 100-fold after the 96 h exposure (Fig. 1). During the same time period, there was little change in the viability of the conidia taken from the culture maintained at 22°C (data not shown). Pathogenicity of heat-treated *P. expansum* conidia on apples decreased and the difference was more apparent on fruit inoculated with lower conidial concentrations (Fig. 2). Exposure to 38°C for 96 h reduced lesion size on fruit inoculated with 10^4 , 10^5 , or 10^6 conidia/mL by 92%, 67%, and 25%, respectively. The decline in lesion size with the increasing duration of the heat treatment was more rapid on the fruit inoculated with 10^4 conidia/mL than on those inoculated with 10^5 conidia/mL. Only after 72 h exposure was there a significant decline in lesion size on fruit inoculated with 10^6 conidia/mL.

Effect of heat on the antagonist. Heat treatment reduced 10,000-fold the populations of the antagonist, *P. syringae*, in wounds, whereas the populations of the antagonist on apples incubated at 26°C over 96 h were not adversely affected (Fig. 3).

Effect of treatments on lesion size and incidence of decay. Fruit inoculated with *P. expansum* before heat treatment and stored at 20°C for 7 d or at 1°C for 3 or 6 months, followed by incubation at 20°C for $\approx 24 \text{ h}$, had no decay lesions (Fig. 4). Similarly, no lesions developed on any combination treatment of *P. expansum*, heat, antagonist, or Ca (data not shown). In comparison, decay lesions of *P. expansum* on inoculated, nonheated fruit were very extensive and difficult to measure accurately after 3 and 6 months in storage, and even though many were over 85 mm in diameter, they were reported as $\approx 85 \text{ mm}$ (data not shown).

Another lot of heat-treated or control fruit that were either infiltrated or noninfiltrated with Ca were removed after 3 or 6 months in storage at 1°C and inoculated with *P. expansum*

Fig. 1. Effect of heat treatment (38 °C) for various periods of time on the viability of *Penicillium expansum* conidia. A sporulating culture of *P. expansum* was exposed to 38 °C, after which the conidia were harvested and spread-plated on PDA. Error bars indicate standard error of the means.

Fig. 2. The effect of heat treatment (38 °C) for various time periods prior to inoculation of fruit on lesion development in 'Gala' apples inoculated with different concentrations of *Penicillium expansum* conidia. The regression lines were generated from equations: $Y = 11.50 - 3.06X$ with $R^2 = 0.90$, $P > F = 0.01$, for 10^4 conidia/mL; $Y = 18.90 - 3.08X$ with $R^2 = 0.99$, $\text{Prob} > F = 0.01$, for 10^5 conidia/mL; and $Y = 21.43 - 1.50X$ with $R^2 = 0.64$, $P > F = 0.10$, for 10^6 conidia/mL.

Fig. 3. Effect of temperature on the recovery of the antagonist *Pseudomonas syringae* isolate ESC11 from wounds in 'Gala' apples. Wounded apples were inoculated with the antagonist and incubated at the indicated temperatures for various periods of time prior to recovery. Error bars indicate standard error of the means ($n = 3$).

only or with the antagonist, *P. syringae*, followed by inoculation with *P. expansum*. Treatments with heat, Ca or antagonist significantly reduced lesion size and incidence of decay (Table 1). Heat treatment resulted in a significantly greater reduction of decay on antagonist-treated fruit, but not on fruit not treated with the antagonist. Nonheated and heat-treated fruit inoculated with the pathogen alone after 3 months of storage did not differ in incidence of decay. This indicates that heat treatment had little residual effect (Fig. 5A). Ca treatment alone significantly reduced the incidence of decay by 24%. The antagonist alone reduced decay incidence by 40%, which was similar to the 44% reduction obtained by combining heat and Ca treatments. Combining heat with the antagonist reduced incidence of decay by 59%, and Ca plus the antagonist, with and without heat, resulted in a reduction of 98% and 95%, respectively. Reduction in lesion size also varied considerably with treatment, but a given treatment reduced lesion size and incidence of decay similarly (data not shown).

After 6 months in storage, the relative effectiveness of the various treatments in reducing the incidence of decay and lesion diameter was similar to that for the fruit stored for 3 months. The incidence of decay was just over 90% for both the nontreated fruit and the heat-treated fruit (Fig. 5). Ca alone, the antagonist alone, and heat plus calcium all reduced the incidence of decay by $\approx 25\%$, whereas heat plus the antagonist reduced incidence of decay by 70%. Ca plus the antagonist with and without heat treatment reduced decay incidence by 91% and 89%, respectively. As with incidence of decay, heat treatment did not affect lesion diameter (data not shown).

Calcium concentration in fruit. Infiltration with a $0.14 \text{ mol} \cdot \text{L}^{-1}$ solution of CaCl_2 resulted in fruit with a significantly higher Ca content ($46.86 \text{ mmol} \cdot \text{kg}^{-1}$ dry weight) than that of nontreated fruit ($8.69 \text{ mmol} \cdot \text{kg}^{-1}$) and fruit that were heated before infiltration ($37.87 \text{ mmol} \cdot \text{kg}^{-1}$). However, infiltration of heated significantly increased tissue Ca concentration.

Discussion

Our results for the effects of heating on fungal conidia germination and the severity of decay on apples inoculated with heated conidia are similar to those of Fallik et al. (1995). They concluded that decay reduction caused by heating of 'Golden Delicious' apples inoculated with *P. expansum* is mainly due to a direct effect of high temperature on the fungus, but that the formation of an anti-fungal substance in the heated peel may also play a role. Decay developed more slowly on heated than on nonheated apples, and almost no decay developed on fruit wounded before heating but inoculated at the end of the heat treatment (Lurie et al., 1998). In our work with apples wounded and inoculated after 3 and 6 months of storage, any antifungal compound may have decreased to ineffective levels, and therefore the residual effect on decay was marginal.

After both 3 and 6 months in storage, no decay developed on apples that had been inoculated before heat treatment. However, decay lesions on nonheated fruit were extensive and were difficult to measure accurately. Similar results occurred on 'Golden Delicious' fruit inoculated with *B. cinerea* prior to heat treatment (Klein et al., 1997). There was only a marginal residual effect on decay of heat treatment alone if 'Gala' fruit were inoculated 3 or 6 months after storage in our study and little effect on 'Golden Delicious' in previous work (Klein et al., 1997). Prestorage heat treatment of pear fruits at similar temperatures prior to wounding and inoculation with various postharvest pathogens, including *B. cinerea* and *P. expansum*, also did not control decay (Spotts and Chen, 1987).

Heat treatment after inoculation of the wounded fruit with *P. syringae* significantly reduced the population of this antagonist, making it less effective. Since the concentration of the antagonist in these types of wounds must be $\approx 6.0 \times 10^6$ CFU/wound to effectively reduce decay (Janisiewicz and Marchi, 1992), the population of 2.5×10^4 remaining after heat treatment would be ineffective. Control of blue mold with the *P. syringae* antagonist was significantly greater when applied to heated than to nonheated apples. In other work, heat treatment also enhanced the effectiveness of an antagonist, a strain of *Pseudomonas glathei* Zolg and Ottow, in controlling postharvest decay of oranges caused by *Penicillium digitatum* Sacc. (Huang et al., 1995). The biocontrol ability of this antagonist was improved greatly when the inoculated fruits were incubated at 30 °C for 24 h before being moved to 25 °C. Heat treatment at 30 °C stimulated the multiplication of the antagonistic bacteria, but retarded spore germination of the pathogen. This allowed the bacteria to become well established at wound sites on fruit before germination of conidia and infection by the pathogen. The heat treatment of apples at 38 °C for 4 d was either too long or the temperature too high for biocontrol to occur. A shorter period of heat treatment at higher temperature, however, was less effective in controlling decay of apples (Klein et al., 1997; Lurie et al., 1998).

The exact mechanism by which *P. syringae* reduces decay is not clear, although nutrient/space competition is a strong possibility (Janisiewicz and Bors, 1995; Janisiewicz and Marchi, 1992). A similar mode of action has been suggested for biocontrol by yeast antagonists on apples (Wilson and Chalutz, 1989). Combining an antagonist with Ca infiltration

may allow lower concentrations of both to be used while still maintaining effective control of decay (Janisiewicz et al., 1998). This can reduce the potential for Ca injury and may compensate for the declining effectiveness of biocontrol as the fruit mature.

Combining the antagonist treatment with Ca infiltration reduced the incidence of decay and lesion size more effectively than either treatment alone after 3 and 6 months in storage. This agrees with the results of Janisiewicz et al. (1998) with 'Delicious' apples. Biocontrol of *B. cinerea* and *P. expansum* on apple by two strains of *Candida* sp. was also enhanced by the addition of 0.14 mol·L⁻¹ Ca salts to the antagonist suspension (McLaughlin et al., 1990), which allowed the use of lower concentrations of the antagonist without losing effectiveness. However, in that study the beneficial effect of Ca was attributed to the effect on the antagonists and not on the resistance of fruit to decay.

Development of a strategy which combines several alternative control measures may require a specific treatment order. Since heat treatment negatively affected the antagonist, the antagonist must be applied after heat treatment. Heating very effectively reduces the inoculum level of the pathogen, thereby reducing inoculum pressure and enhancing the control provided by Ca infiltration or the antagonist. As the inoculum concentration de-

creases, the relative effectiveness of cell wall-bound Ca in reducing decay increases (Conway et al., 1987), as does the effectiveness of the antagonist, which may allow a lower concentration of the antagonist to be used (Janisiewicz, 1987).

Heating changed the pattern of epicuticular wax in 'Golden Delicious' apples (Lurie et al., 1996; Roy et al., 1994). The epicuticular wax of heat-treated fruit did not have the same network of deep cracks observed on nonheated fruit. This apparent obstruction of the cracks due to a possible recrystallization or 'melting' of the wax platelets hinders infiltration of CaCl₂ into the fruit. Heating 'Gala' fruit reduced subsequent infiltration of CaCl₂. These results suggest that fruit should be treated with Ca prior to heat treatment so more Ca solution can enter the fruit. However, Ca infiltration followed by heating resulted in Ca injury at a Ca concentration much lower than that which would injure non-heat-treated fruit (Conway et al., 1994b). Thus, Ca infiltration should follow heat treatment to reduce the possibility of injury. Fruit tissue Ca concentration can be increased to sufficient levels by infiltration following heat treatment (Klein et al., 1997; Roy et al., 1994).

The presence of Ca did not negatively affect the populations of the antagonist (Janisiewicz et al., 1998). Therefore, the antagonist can be applied to the fruit immedi-

Fig. 4. Effect of heat treatment (38 °C, 4 d) on incidence of decay caused by *Penicillium expansum* in 'Gala' apples after storage for 0, 3, or 6 months. Fruit were inoculated with the pathogen before heat treatment.

Table 1. Summary of the significant effects of heat treatment (inoculated after storage), Ca infiltration, storage duration (months), and antagonist (antag) and pathogen inoculation on lesion size and incidence of decay on 'Gala' apples after storage at 1 °C for 3 and 6 months.

Source	df	Lesion size			df	Decay incidence		
		Mean square	F value	P > F		Mean square	F value	P > F
Heat	1	0166.76	5.27	0.0218	1	2.68	18.63	0.0001
Ca	1	24820.48	784.35	0.0001	1	44.95	312.17	0.0001
Antag	1	36417.36	1150.82	0.0001	1	94.43	659.36	0.0001
Heat × antag	1	0122.14	3.86	0.0497	1	0.64	4.47	0.0347
Ca × antag	1	4437.18	140.22	0.0001	1	0.52	3.60	0.0581
Month × heat × antag	1	0230.02	7.27	0.0071	1	0.97	6.75	0.0095
Heat × Ca × antag	1	0468.02	14.79	0.0001	1	1.97	13.64	0.0002

ately after Ca treatment. Since Ca is applied to apples in a solution under pressure and the antagonist is applied as a spray under commercial conditions, the antagonist should be applied following Ca infiltration to prevent it from being washed from the fruit.

Most postharvest pathogens are present on the fruit surface during harvest, but cause decay only after the tissue is wounded or softens sufficiently to permit pathogen penetration and infection. While heat treatment is phytosanitary in that it significantly reduces the pathogen population on the fruit surface and the possibility of infiltration of conidia into the fruit, it provides little residual protection. The residual protection provided by Ca and the antagonist adds to the control provided by the heat treatment. Ca not only protects the cell wall from maceration by pectic enzymes produced by the fungus, but also maintains fruit firmness and reduces physiological malady as well. The presence of the antagonist complements this residual Ca resistance. The strategy of heat-treating fruit, followed by Ca infiltration and then treatment with an antagonist may be a useful alternative to fungicides for postharvest control of decay.

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Fig. 5. The effects of heat treatment (H), calcium infiltration (C), antagonist application (A), and combinations thereof on incidence of decay of 'Gala' apples by *Penicillium expansum* (P). After heat treatment followed by calcium infiltration, the fruit were stored for 3 or 6 months at 1 °C. After removal from storage, the fruit were placed at 24 °C, wounded, and then inoculated with *P. expansum* alone or with the antagonist and *P. expansum*. Fruit were evaluated for incidence of decay 7 d after inoculation. Con indicates nontreated fruit.

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