Effects of Controlled Atmosphere and Calcium Infiltration on Decay of Delicious Apples

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ABSTRACT

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Delicious apples were harvested, sorted for uniformity, and divided into three 120-fruit lots. One lot was further divided and treated with 0, 2, 4, or 8% solutions of $CaCl_2$ by pressure infiltration (68.95 kPa). All three lots were wound-inoculated with a conidial suspension of *Penicillium expansum*. The $CaCl_2$-treated fruit were then transferred to storage at 0 C; the other two lots were placed in either 3% O_2 and 2% CO_2 or 1% O_2 controlled-atmosphere (CA) storage at 0 C. After 3 mo, the $CaCl_2$-treated fruit were 5–19% firmer and had 29–47% less area of decay than the control (0% $CaCl_2$). The 3% O_2- and 2% CO_2-treated fruit and the 1% O_2-treated fruit had 44 and 46% less area of decay and were 12 and 20% firmer, respectively, than the control fruit. After storage, all fruit were held at 20 C for 3 days. The rate of decay at 20 C was slower in the 8% $CaCl_2$-treated fruit than in either the CA-stored or control fruit.

Additional key words: low-oxygen storage, postharvest

Various postharvest treatments as well as modified atmospheres have been shown to influence losses caused by decay of stored fruit. Apples with increased calcium content resulting from postharvest treatments were shown more resistant to decay by *Penicillium expansum* Link ex Thom (5). The level of decay is determined by the amount of calcium that can be successfully taken into the fruit, and calcium uptake by the fruit depends on the method of treatment. In a previous experiment on the effect of postharvest calcium treatment on decay caused by *P. expansum*, the concentration of calcium in the flesh of apples dipped in calcium chloride ($CaCl_2$) increased as the concentration of the solution increased but not enough to reduce decay (6). Vacuum infiltration of a 12% $CaCl_2$ solution doubled the calcium content of fruit compared with a dip treatment in the same solution and resulted in 30% less area of decay. Pressure infiltration of the 12% $CaCl_2$ solution increased the calcium content of fruit to more than twice that resulting from vacuum infiltration, and treated fruits had 50% less decay area than untreated fruits.

Controlled-atmosphere (CA) storage has been shown beneficial to apples in preventing or delaying the onset of storage disorders (20). Internal breakdown, bitter pit, and softening have been reduced significantly in CA storage. The effect of CA on decay of stored fruit seems to vary depending on the particular fungus involved. Yackel et al (24) found that the effect of CA on growth and development of various decay-causing fungi, including *P. expansum*, was variable and temperature-related. Borecka (4) indicated that the growth of *P. expansum* on apples was retarded under CA conditions of 3% O_2 and 5% CO_2 but was only slightly retarded at 3% O_2 and 0% CO_2 when held at 4 C. Other workers (21) observed a retardation of lesion development caused by *P. expansum* on Golden Delicious apples at 2.5% O_2 and 5% CO_2 at 0 C. Earlier workers (14,16) also indicated that the development of decay caused by *P. expansum* was inhibited much more effectively in CA than in normal cold storage.

Low-O_2 storage also has been shown useful in delaying the onset of storage disorders of apples (11,19). However, 1% O_2 concentrations must be controlled within ±0.1% and the apples must be cooled to 4 C before establishing the low-O_2 concentrations to avoid anaerobic respiration (15). As with regular CA, low-O_2 effects on host-parasite interactions seem to depend on the host and pathogen involved (10). Parsons et al (18) found that atmospheres of 1 or 0% O_2 greatly reduced decay of peaches caused by *Rhizopus stolonifer* (Ehrenberg ex Fries) Lind or *Monilinia fructicola* (Wint.) Honey. Parson et al (17) also found that decay of mature-green tomatoes caused by *Rhizopus* spp. and *Alternaria* spp. was significantly less after storage in 3% O_2. Lockhart and Eaves (13) indicated that there was greater decay of mature-green tomatoes at 0% O_2 than at 2.5% O_2. The decay-causing organisms investigated in this study were *Rhizopus* spp., *Fusarium* spp., *Alternaria* spp., and bacteria. Aharoni and Lattar (1) showed that atmospheres containing 2.5 or 5% O_2 greatly reduced decay development in Shamouti oranges caused by *Alternaria citri* Ell. & Pierce, *P. digitatum* Sacc., *P. italicum* Wehmr., and *Diplodia natalensis* P. Evans. Lipton (12) found that at 10 C, 2% O_2 reduced the incidence of aerial mycelium by 50% on radishes with lesions caused by *Peronospora parasitica* Pers. ex Fr.

Because both CA and increased tissue calcium can affect decay development, the objective of this experiment was to compare the effectiveness of postharvest calcium treatment and CA (either 3% O_2 and 2% CO_2 or 1% O_2) in reducing decay of Delicious apples caused by *P. expansum*

MATERIALS AND METHODS

Delicious apples were harvested from a commercial orchard. The fruits were randomized, sorted for uniformity, and divided into three 120-fruit lots. One lot was further divided and pressure-infiltrated (68.95 kPa) for 2 min with laboratory-grade USP calcium chloride ($CaCl_2$, 76%) made up as 0, 2, 4, or 8% solutions. There were 25 fruits per treatment. After treatment, this lot of fruits was placed on Kraft paper and allowed to drain for 4 hr. All three lots of fruits were then wound-inoculated with *P. expansum* as described previously (6). After 18 hr at 0 C, the two lots of fruits to be stored in CA were placed in the appropriate chambers in the same storage room (0 C) as the $CaCl_2$-treated fruits. Stainless steel chambers (220-L) were used as test chambers. The desired CA (3% O_2-2% CO_2) was obtained initially by flushing the sealed chambers containing the fruit with N_2 and adding CO_2. Airflow through the chambers was adjusted to provide the O_2, and a flow of N_2 from a cylinder was used to adjust the CO_2

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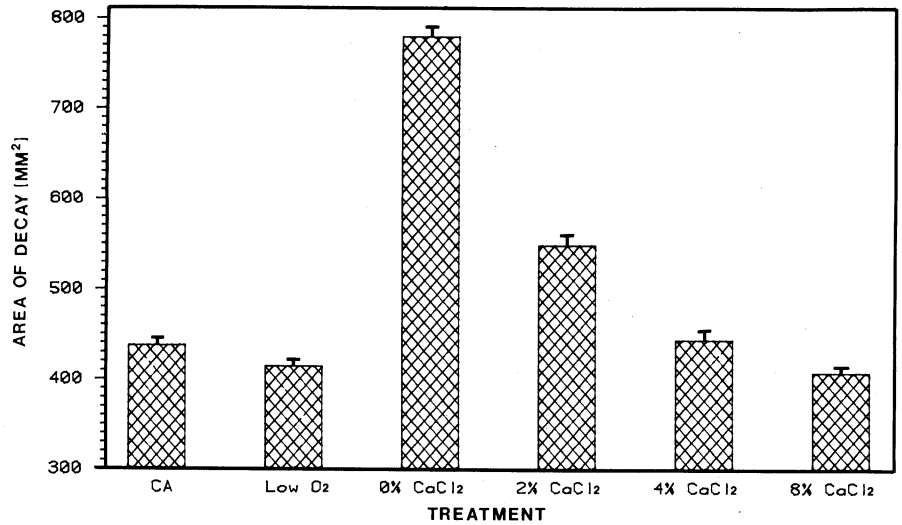

Fig. 1. Relationship between area of decay and fruit treatment. Treatments include controlled atmosphere (CA) (3% O_2 and 2% CO_2), low oxygen (1% O_2), and calcium chloride ($CaCl_2$) solutions. Bars indicate standard errors of means.

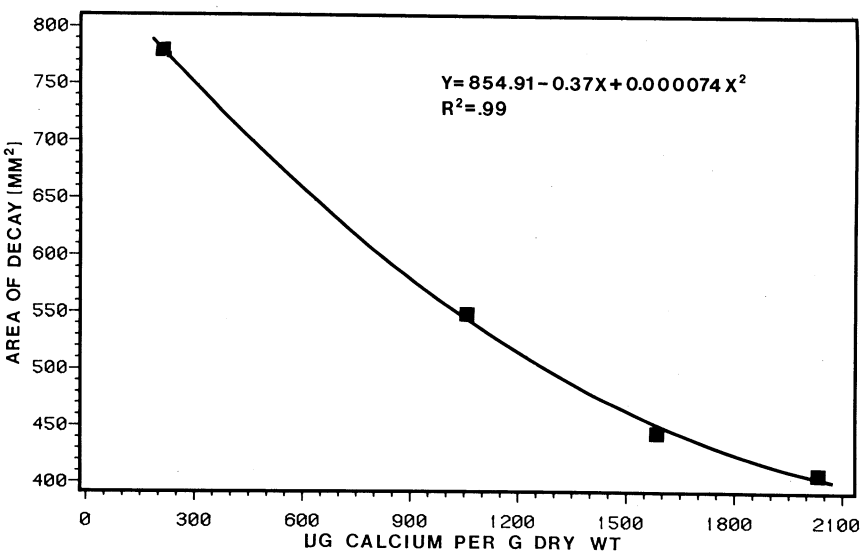

Fig. 2. Relationship between area of decay and calcium concentration of apple tissue when fruit were pressure-infiltrated with calcium chloride ($CaCl_2$). Data points indicate the concentration of the $CaCl_2$ solutions (0, 2, 4, or 8%) from left to right, respectively.

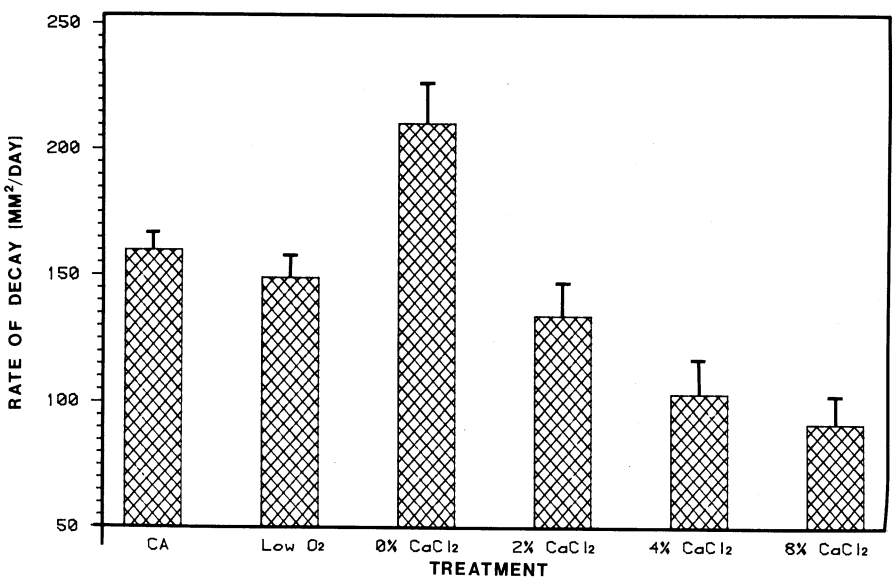

Fig. 3. Relationship between rate of decay and fruit treatment. Treatments include controlled atmosphere (CA) (3% O_2 and 2% CO_2), low oxygen (1% O_2), and calcium chloride ($CaCl_2$) solutions. Bars indicate standard errors of means.

concentration when necessary. The flow rates varied from about 0.1 to 10 L/hr, which did not exceed three gas volume changes per chamber per week. The gas concentrations were monitored with an Orsat-type analyzer and averaged 3.05% O_2 and 1.95% CO_2 during the test. Purafil to absorb ethylene and water to maintain relative humidity were placed in trays in the chambers. The desired atmosphere was established within 3 days.

The low-O_2 (1%) atmosphere was established in the same type of stainless steel chamber as was the normal CA. The desired 1% O_2 atmosphere was premixed with N_2 as the filler gas. The chambers containing the fruits were first flushed with N_2. The flow rate needed to maintain the 1% O_2 atmosphere varied from 0.1 to 10 L/hr and again did not exceed three gas volume changes per chamber per week. The O_2 concentration was also analyzed with an Orsat-type analyzer and averaged 0.93% O_2 and 0% CO_2 during the investigation. The low-O_2 chambers also contained Purafil, water, and dehydrated lime to absorb CO_2. The fruits were in the desired O_2 atmosphere within 36 hr after being placed in the chambers.

Decay lesions on untreated (0% $CaCl_2$ at 0 C) fruits were monitored until the diameters of lesions averaged about 32 cm. The time interval in storage for lesions to develop to this point was about 3 mo. At this time, all fruits were removed from storage, rated for decay severity and firmness, and analyzed for calcium content as described previously (6). Decay severity was measured on 25 fruits per treatment, and fruit firmness was measured on 15 fruits per treatment. For calcium analysis, the flesh from three apples made up one sample, and three samples from each treatment were analyzed. Apples rated for decay were then placed at 20 C, and decay severity was measured again after 3 days to determine the relative rates of decay.

RESULTS

CA and calcium treatments were effective in both retarding decay and maintaining fruit firmness. Decay in the fruits (Fig. 1) stored in 3% O_2 and 2% CO_2 or 1% O_2 CAs was 44 and 46% less, respectively, than in untreated (fruits neither treated with $CaCl_2$ nor stored in CA) fruits. The area of decay in fruits infiltrated with 8% $CaCl_2$ was 47% less than in untreated apples. Lower concentrations of $CaCl_2$ retarded decay to a lesser extent. Area of decay was inversely correlated to the calcium content of the fruit among the $CaCl_2$ treatments (Fig. 2). The calcium content of apple flesh was similar to that found in previous work (6) and ranged between 200 $\mu g/g$ in the untreated fruits to 2,000 $\mu g/g$ in the fruits treated with the 8% $CaCl_2$ solution. Fruits stored in CA had essentially the same calcium concentration in the flesh as untreated fruits.

The rate of decay of apples over 3 days at 20 C after removal from CA and cold storage is shown in Figure 3. Since decay caused by *P. expansum* spreads spherically from the point of origin, the rate of advance of the pathogen through the tissue can be reliably estimated from measurements of the external dimensions of the lesion. Because the growth rate of the pathogen is slower at low temperature, there is probably a lag period in growth corresponding to the interval from when the fruits are removed from 0 C until the fruits warm to 20 C. As the calcium concentration of the fruit flesh is increased, the rate of decay decreases, so that the most effective treatment in retarding decay at 20 C after storage was infiltration of 8% $CaCl_2$.

After 3 mo of storage, the fruits in 3% O_2 and 2% CO_2 or 1% O_2 were 12 and 20% firmer, respectively, than untreated fruits (Fig. 4). Fruits treated with an 8% $CaCl_2$ solution were 19% firmer, whereas lower concentrations of $CaCl_2$ maintained firmness to a lesser extent. No evidence of fruit injury was observed with any of the treatments.

DISCUSSION

In previous work (5–7) comparing the amount of decay on fruits taken from storage (0 C), either treated with $CaCl_2$ or held under low-O_2 conditions, apples were inoculated after storage to determine the effectiveness of the various treatments in retarding decay over a 7-day period after storage. This period would simulate movement of fruits in marketing channels in which no effort would be made to maintain the low temperature (0 C) required to retain fruit quality. This procedure determined, in effect, the ability of the apples to resist decay in the condition in which they came from storage. In this study, apples were inoculated before being placed in storage. This procedure would simulate fruits inoculated through mechnical injuries (cuts and bruises sustained as a result of harvesting and handling operations), thus determining the ability of the fruits to resist decay under the conditions in which they were stored, either in air or CA and at 0 C. The pathogen, then, not only had to overcome the physical barrier presented by the cell wall of the fruit but had to do so in atmospheres and at temperatures that may not be optimal for fungal growth.

Although fruit firmness was maintained and decay retarded to a similar degree with the higher concentrations of $CaCl_2$ and in the CAs, the mechanisms by which these results were attained are seemingly different. The level of decay retardation in stored apples achieved with calcium treatment is related to the amount of $CaCl_2$ taken up by the fruit (5). Calcium from postharvest treatments enters the fruit through epidermal openings, primarily lenticels (3), and leakage experiments suggest that localization of native calcium and of calcium originating from postharvest treatment were the same (23). Because calcium-treated apples remain firmer than low-calcium fruit (2), calcium appears to stabilize the cell wall and maintain fruit firmness by resisting degradation by enzymes occurring naturally in the fruit. Calcium-induced changes in the cell wall may also render the cell wall more resistant to enzymes produced by fungal pathogens, and this in turn slows penetration by the fungus and decreases decay.

Investigations on the effect of CA on growth and development of postharvest pathogens indicate that the effect is quite variable and tends to be temperature-related (24). A definite interaction has been shown between CA and storage temperature. The effectiveness of CA usually increases as the temperature is reduced, leading to the conclusion that to control postharvest pathogens, CA should be used with the lowest storage temperature that would be acceptable for the commodity in question. For *P. expansum* in particular, both sporulation and mycelial growth in CA was significantly reduced as the temperature approached 2 C. CA may also reduce the activity of pectolytic enzymes, but this effect also varies with species (8). In addition to the effect low-temperature CA has on retarding growth and sporulation by suppressing metabolic activities of the fungus, it may also influence disease development by delaying senescence of the host. This would make it somewhat more resistant to decay, because as fruit mature and soften, they become more susceptible (9).

Low O_2, especially at concentrations of 1% or less, can significantly reduce growth, sporulation, and germination in most postharvest fungi, probably through its effect on oxidative enzymes (10). As with CA, the reduction of O_2 needed to retard growth and sporulation varies among species (10), and the effect may be temperature-dependent as well. Wells (22), working with *R. stolonifer*-infected strawberries stored in low-O_2 atmospheres at 15 C, found that pectic enzyme activities were directly related to the extent of decay on the berries in each atmosphere. Highest activities occurred in extracts from berries held in normal atmospheres, and lowest activities occurred in extracts from berries held in 0% O_2. Polygalacturonase activity from extracts of berries held at 1% O_2 was one-half that found in extracts from berries held at normal atmospheres. Because low-O_2 storage also tends to delay senescence and maintain fruit firmness (11), the improved physiological condition of the host during storage may also have been responsible for retarding fungal growth.

Although either increasing the calcium content of fruit before storage or placing them in the proper CA conditions retards decay effectively, several factors must be considered in determining the best storage regime for a given situation. The cost of treating fruits with calcium and placing them in conventional cold storage is much less than that of constructing CA facilities, and the resulting decay reduction and firmness retention is similar. However, a cultivar such as Golden Delicious may be somewhat more susceptible to calcium injury (6) than one such as Delicious (5), so CA storage might be more effective for Golden Delicious, whereas either would be acceptable for Delicious. As an additional benefit, the calcium treatment continues to slow the rate of decay somewhat even after the fruits are removed from storage and placed in marketing channels.

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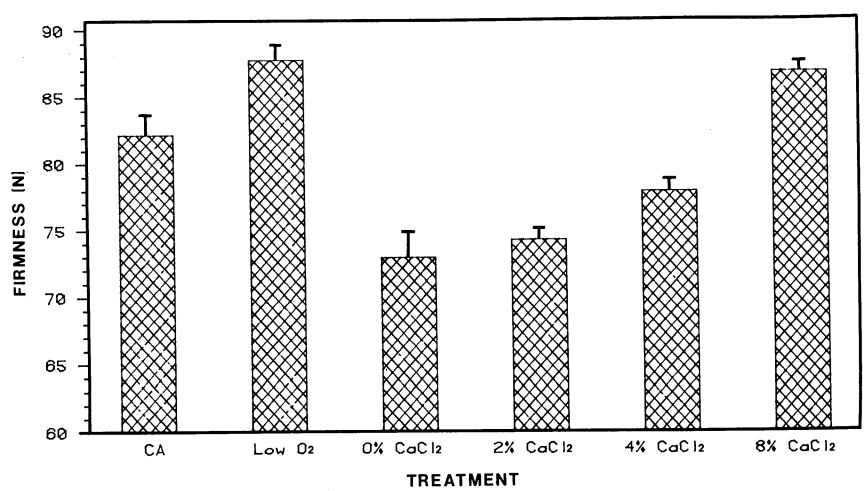

Fig. 4. Relationship between fruit firmness and treatment. Treatments included controlled atmosphere (CA) (3% O_2 and 2% CO_2), low oxygen (1% O_2), and calcium chloride ($CaCl_2$) solutions. Bars indicate standard errors of means.

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