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The Action of Lecithin and Calcium Dips in the Control of Bitter Pit in Apple Fruit[1]

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Abstract. **Addition of 1% lecithin (phosphatidyl choline) to 4% Ca dips increased internal CO_2 levels and decreased O_2 levels in fruit of apple (*Malus domestica* Borkh.) stored at 3°C. This effect was greater than that found with lecithin alone. At 18°, respiration and ethylene production by the fruit were reduced slightly by Ca, but to a much greater extent by lecithin. A further reduction of the production of both gases was found with Ca and lecithin. The climacteric rise was also delayed by lecithin treatments. At 3°, ethylene production was both delayed and reduced by lecithin treatments, but no influence on CO_2 production was detected. Although differences in Ca concentration of the fruit caused by the addition of lecithin to Ca could not be detected when bulked samples of fruit were analysed, use of ^{45}Ca showed that both the initial rate of Ca uptake and the final Ca content of the fruit flesh after 6 weeks at 3° were increased by lecithin. The beneficial effects of lecithin plus Ca in bitter pit control probably result from rapid modification of gas exchange together with increased Ca uptake.**

The effectiveness of post-harvest calcium dips for the treatment of bitter pit in apples can be enhanced by including lecithin in the dip solutions (5, 7). It has been suggested that lecithin might increase Ca uptake into the fruit (5), or might modify fruit skin porosity resulting in an increase in internal CO_2 and a decrease in internal O_2 concentrations (7).

Lecithin increased the initial rate of Ca uptake into South African 'Golden Delicious' fruit (12). However, in both this fruit and New Zealand (7) and English (3) 'Cox's Orange Pippin' fruit, no differences in the final Ca content of fruit dipped in $CaCl_2$ alone or with lecithin were measurable after cool storage. Sharples et al. (7) suggested, therefore, that a major effect of lecithin was the development of modified atmospheres within the fruit. Early work (10) demonstrated that skin coatings of castor oil and shellac decreased skin porosity, slowed the rate of ripening and reduced bitter pit incidence in apple fruit. A combination of the commercial xanthan gum "Keltrol" with Ca increased internal CO_2 concentrations in English 'Cox's Orange Pippin' fruit and reduced bitter pit (3). However, only a slight increase in internal CO_2 levels was detected in fruit dipped in lecithin and Ca, and lecithin alone had no effect on bitter pit.

There is still interest in the use of skin coatings as a practical treatment for the control of bitter pit. We have attempted to resolve the question of whether lecithin acts through modification of gas atmospheres, or through facilitation of Ca uptake into the fruit.

Materials and Methods

Fruit which had received no Ca sprays during the growing season were harvested from 'Cox's Orange Pippin' apple trees at the DSIR Research Orchard, Appleby, Nelson, and graded to size 65–75 mm diameter, average weight 150 g. Unless otherwise indicated, all data refer to fruit picked on February 20, 1981.

Five replicates of 20 fruit per treatment were randomly selected, placed in net bags, and dipped for 1 min at ambient temperature in the following solutions: water, 4% (w/v) $CaCl_2$ (commercial flake, 78%), 1% (w/v) lecithin or 1% lecithin plus 4% $CaCl_2$. All solutions contained 0.5% (w/v) Na_2CO_3, necessary for lecithin dispersion (7) and 0.025% (v/v) Agral (ICI Australia) as a wetting agent. The fruit were stored at 3°C in air for 6 weeks and then at ambient temperatures for 1 week. Plugs (~ 7 mm diameter, 0.5 cm thick) of cortical tissue were taken from just inside the skin of an equatorial slice of the fruit. One plug per fruit was taken from each replicate of 20 fruit, bulked, and analysed for Ca, Mg, and K according to the methods of Turner et al. (11).

For measurement of CO_2 and ethylene production and internal levels of CO_2 and O_2, further replicates of fruit were dipped under the same conditions and in the same solutions as above. Internal atmospheres of 4 fruit per treatment were measured immediately after removal from 3°C at 7, 14, 28 and 42 days after treatment, by the methods of Reid et al. (6). CO_2 and ethylene were measured by taking gas samples at 1 or 2 day intervals from duplicate bulk samples of 16 fruit per treatment placed in respiration jars (flow rate 15 liter hr^{-1}) at 3° and 18°. Gases were measured by gas chromatography, CO_2 and O_2 using thermal conductivity, and ethylene using photoionization detectors, respectively. An experiment conducted in 1979 is also reported, in which CO_2 and ethylene production of 5 individual fruit per treatment were measured. All procedures were the same as for bulked samples except that the flow rates through the respiration jars were 1 l hr^{-1}.

For measurement of Ca uptake, fruit were dipped for 1 min in 4% $^{45}CaCl_2$ (260 MBl μmol^{-1} Ca) plus 0.025% Agral, or in 4% $^{45}CaCl_2$ and 1% lecithin plus 0.025% Agral. After the dip solution had dried, fruit were stored at 3°C and 6 fruit per treatment were sampled after 3, 7, 14, 28, and 42 days. Each fruit was cut longitudinally and a 1 cm diameter plug of tissue was removed

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from the core outwards from each half. Plugs were divided into skin, and flesh segments of length 0–6, 6–12, 12–18 and >18 mm from the skin inwards. Segments from each pair of plugs were combined to give 6 replicates for each segment per treatment. The tissue was weighed directly into scintillation vials and ashed for 12 hr at 500°. The ash was taken up in 0.5 ml 0.2 N HCl, 5 ml of toluene scintillant were added, and the samples were counted by liquid scintillation spectrophotometry.

Results

Internal atmospheres. Within 1 week of treatment, lecithin dips resulted in lower internal O_2 concentrations and higher internal CO_2 concentrations than that for apple fruit at 3°C treated only with water or Ca (Fig. 1). There was a smaller but consistent difference between the lecithin and calcium/lecithin treatments, the differences being 0.9% and 1.0% for O_2 and CO_2, respectively.

CO_2 and ethylene production. At 18°C Ca caused some reduction in the maximum production of both CO_2 and ethylene but lecithin reduced CO_2 and ethylene production by approximately 58% and 43%, respectively (Fig. 2a, b). The maximum production of both gases was reduced approximately a further 5% by the Ca/lecithin treatment.

The initiation of the climacteric rise as indicated by CO_2 and ethylene production was also delayed by lecithin. On the basis of ethylene production, the climacteric was delayed by 2, 4 and 6 days by Ca, lecithin and Ca/lecithin treatments, respectively (Fig. 2b). In a bulked sample of fruit it is possible for a few fruit to alter the timing of the climacteric rise by ripening early. Results for individual fruit (Table 1) confirm the influence of lecithin on delaying the climacteric.

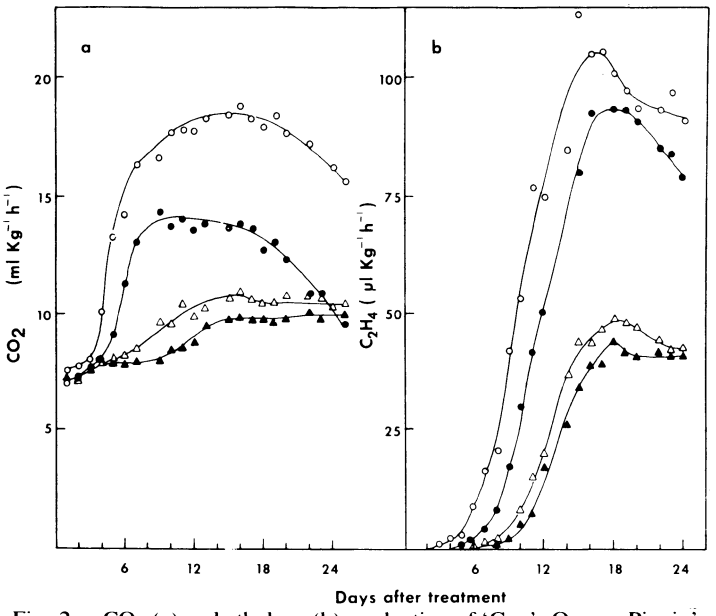

Fig. 2. CO_2 (a) and ethylene (b) production of 'Cox's Orange Pippin' fruit at 18°C after dipping in water (○), calcium (●), lecithin (Δ) and calcium plus lecithin (▲). Each point represents the mean of 2 bulked samples of 16 fruit.

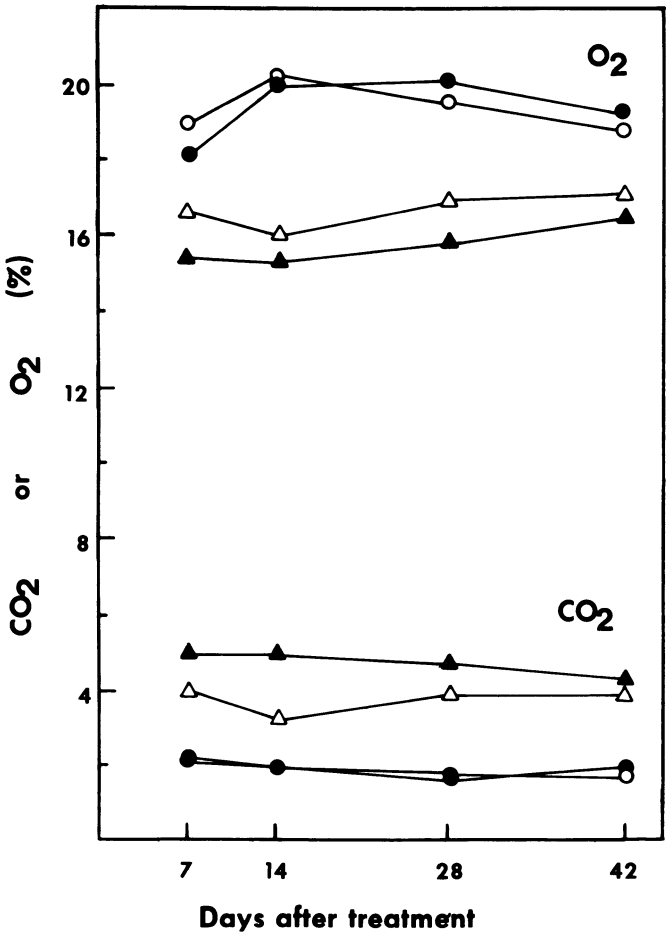

Fig. 1. Internal concentrations of CO_2 and O_2 in 'Cox's Orange Pippin 'fruit during storage at 3°C after dipping water (○), calcium (●) lecithin (Δ) and calcium plus lecithin (▲). Each point represents the mean of 4 fruit. Average pooled estimate of SE for 0_2 values is ±0.62; for CO_2 values, ±0.34.

At 3°C, no climacteric rise in respiration was evident. At this temperature, there were no significant differences in CO_2 production among treatments, the mean rates (ml CO_2 kg^{-1} hr^{-1}) over the storage time being: control 2.17±0.48, Ca 2.00±0.56, lecithin 1.99±0.74, Ca/lecithin 2.08±0.56. Ethylene production of fruit in all treatments increased over the 6 week storage period (Fig. 3). Lecithin caused a reduction in total ethylene production and a delay in the onset of the ethylene rise similar to that occurring at 18°. A further reduction and delay in ethylene production was evident with Ca/lecithin.

Calcium uptake. There was rapid movement of Ca (as monitored by ^{45}Ca) into the outer 6 mm of the fruit cortex during the first 7 days after treatment (Fig. 4). In the presence of lecithin the rate of Ca movement was greater and a higher equilibrium value was reached; thus after 7 days, the difference between treatments was 52.7, and after 42 days 80 nmol Ca g^{-1} fresh weight Ca treatment alone increased the cortical Ca concentration of the apple cortex after 42 days from 368 to 499 nmol Ca g^{-1} fresh weight, and the addition of lecithin increased this further to 579 nmol g^{-1} fresh weight. Although movement of Ca beyond the outer 6 mm of cortical tissue was only significant after 14 days (Fig. 4), the rate of movement here was also greater in the pre-

Table 1. Days from treatment for 'Cox's Orange Pippin' fruit to reach climacteric rise as indicated by CO_2 and ethylene production. Fruit were harvested March 8, 1979, and treated on March 9, 1979.

Treatment	Days to climacteric rise	
	Range (n=5)	Mean
Water	2–4	2.8
4% $CaCl_2$	2–4	3.2
1% lecithin	3–6	4.2
1% lecithin + 4% $CaCl_2$	3–7	5.6

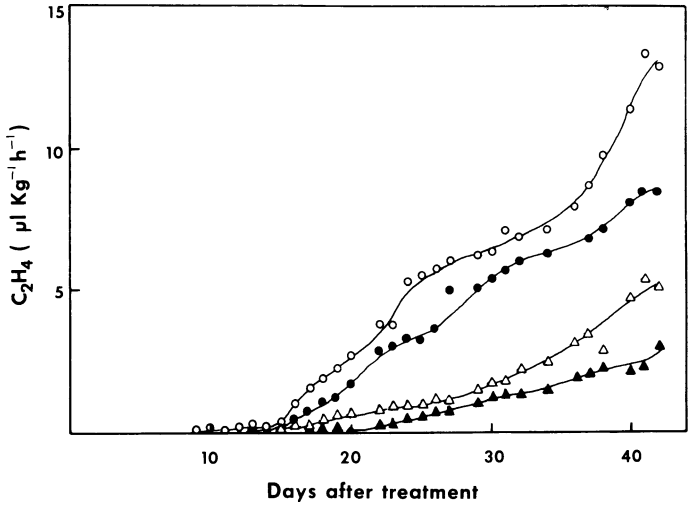

Fig. 3. Ethylene production of 'Cox's Orange Pippin' fruit at 3°C after dipping in water(○), calcium (●), lecithin (Δ) and calcium plus lecithin (▲). Each point represents the mean of 2 bulked samples of 16 fruit.

sence of lecithin. Movement of Ca beyond 12 mm was negligible during the experimental period. The Ca content of the peel disks immediately after treatment was 1143 and 2032 nmol g^{-1} fresh weight for the Ca and Ca/lecithin treatments, respectively, the average difference between treatments being approximately 80% during the course of the experiment.

Although lecithin increased ^{45}Ca uptake, we did not detect any increase of Ca in the fruit by measurements of endogenous Ca levels in bulked samples of cortical plugs from 20 fruit treated with Ca and lecithin dips. Ca concentrations of outer cortical tissue following water and lecithin treatments were, after 42 days of fruit storage, 335 and 365 nmol Ca g^{-1} fresh weight respectively. The Ca dip increased this level to 734 nmol g^{-1} fresh weight. There was no significant difference between the Ca and Ca/lecithin (646 nmol g^{-1}) treatments.

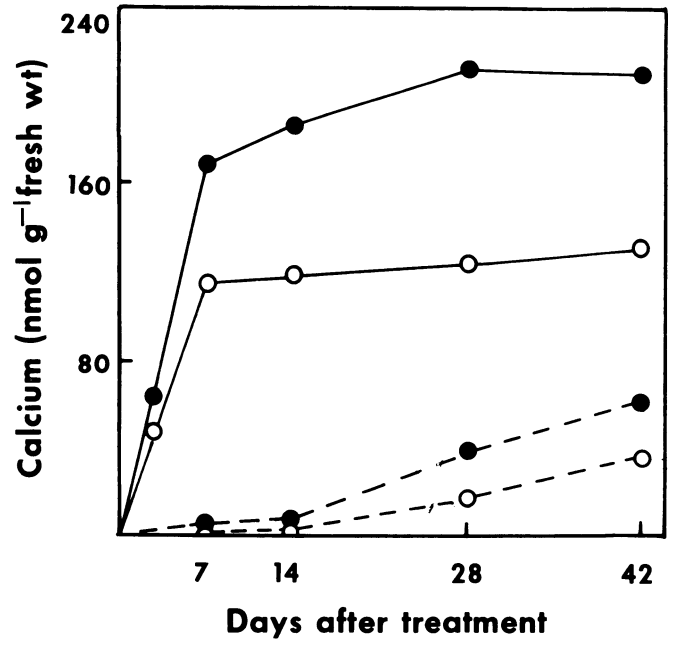

Figure 4. Calcium uptake, as monitored by ^{45}Ca, into 'Cox' Orange Pippin' fruit treated with calcium (○) and calcium plus lecithin (●) in the 0–6 mm (———) and 6–12 mm (— — —) segments of the fruit fresh. Fruit were stored at 3°C. Each point represents the mean of 6 fruit. Averaged pooled estimated of SE for 0–6 mm data is ± 16.22; for 6–12 mm data, ± 2.88.

The effectiveness of lecithin in reducing bitter pit was dependent upon the Ca concentration. The combination of 4% $CaCl_2$ and lecithin reduced pit from 35% to 2%, but a 2% $CaCl_2$/lecithin combination only reduced pit to the same levels achieved by 4% $CaCl_2$ alone, i.e. 12%

Discussion

The reduction of CO_2 and ethylene production by Ca has been demonstrated in a number of fruit (1, 9, 13). In 'Cox's Orange Pippin' fruit at 18°C, the time before the onset of the climacteric was delayed, and the production of CO_2 and ethylene were reduced by Ca, but the reduction was small compared with that achieved by lecithin alone or combined with Ca. At 3°, there was no effect caused by lecithin or Ca on respiration, but ethylene production was reduced in a pattern similar to that at 18°. Hopkirk and Wills (2) also found no effect on respiration but a reduction of ethylene production in apple fruit by lecithin at 0°.

Lecithin alone or with Ca maintained higher internal CO_2 levels and lower internal O_2 levels. Such a modification of fruit atmosphere is similar to that resulting from other skin coatings such as oils, waxes and thickeners (3, 4, 10). A modification of the internal atmosphere results from changed diffusion properties of the fruit skin, and the subsequent slowing down of ripening is associated with maintenance of green color and fruit firmness (4, 8, 10). However, associated with these benefits is a tendency for some fruit to develop alcoholic flavors (7). With the Ca/lecithin treatment we detected only a small number of alcoholic off-flavors in fruit when they were left at ambient temperatures; such development therefore seems minimal.

Sharples et al. (7) suggested that Ca might act to increase the "coating" influence of lecithin by forming bridges between phosphatidyl choline molecules. The resulting network of molecules may be more effective in modifying the gas atmosphere of the fruit. However, Ca/lecithin caused only a small change beyond that of lecithin on internal atmospheres, and CO_2 and ethylene production of apple fruit. Improved bitter pit control by combining Ca and lecithin suggests that the action of Ca and lecithin on bitter pit must be more than a modification of the gas atmosphere of the fruit. [For example, lecithin alone reduced pit by 17% (5) or not at all (7), whereas the addition of Ca to lecithin improved pit control by a further 20% (5) and 46% (7).] We have also found that the use of higher concentrations of Ca with the same concentration of lecithin further reduced bitter pit incidence.

We demonstrated an influence of Ca beyond that associated with gas exchange when lecithin accelerated Ca uptake into the fruit, and increased the final levels of Ca in the flesh. The increase in the rate of Ca uptake was high in the first week after treatment. The difference associated with lecithin could be especially important at this time in the establishment of higher Ca concentrations at the site of potential bitter pit lesions. The 0–6 mm segment of the flesh has the lowest endogenous concentration of Ca (unpublished data) and this may account for the slow appearance of ^{45}Ca in the tissue interior to this segment. The stimulatory effect of lecithin was probably due to retention of higher Ca concentrations on the skin surface. Also, because of the binding of Ca and lecithin, there is possibly a more even spread of Ca on the surface. Thickeners such as cornflour and xanthan gums have also been

shown to retain Ca on the fruit surface, resulting in increased Ca levels in the flesh (3).

de Villiers and Hanekom (12) also found increased Ca uptake when radioactively-labelled Ca was used. We and others (3, 7), were not able to detect differences in total Ca content of the fruit flesh with the lecithin treatments. The increased Ca levels measured with ^{45}Ca, although physiologically significant, are probably masked by the variation in endogenous Ca levels in the fruit. For example, adding lecithin to the Ca dip increased Ca in the flesh by about 15% above that resulting from the Ca dip alone, and we have found variations of Ca concentrations of this order in analyses of bulked samples of fruit tissue.

This work indicates that the greater efficiency of dips containing both Ca and lecithin for the control of bitter pit may result primarily from enhanced Ca uptake in fruit with modified atmospheres. The rapidity of both effects may be an important feature of the treatment. The immediate response to change in gas diffusion may be a lowered rate of fruit metabolism. The increase in rate of Ca uptake should reinforce these beneficial effects on the incidence of the disorder.

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