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Effects of Initial Oxygen Stress Treatments in Low Oxygen Modified Atmosphere Storage of 'Granny Smith' Apples[1]

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***Abstract*. 'Granny Smith' apples (*Malus domestica* Borkh.) harvested over 7 weeks were not affected by alcoholic taints and were firmer, greener and less susceptible to scald and core flush following storage in 1.0% CO_2 and 1.5% O_2 (1.0:1.5) at −0.5°C as compared to present commercial modified atmosphere (MA) storage at 3.5% CO_2:3.5% O_2. Storage response was optimized by harvesting fruit with a starch-iodine maturity index of 5 to 6. With lower starch-iodine indices the incidence of core flush was increased, and at higher indices the potential for scald development increased substantially. Exposure of fruit to an initial O_2 stress at or below 0.5% for 9 days gave additional benefits in scald control, fruit firmness and color over continuous storage at 1.0:1.5. If O_2 was totally excluded by a 14-day treatment with continuous flow-through of O_2-free nitrogen, core flush and alcoholic tainting increased, but scald decreased. Core flush following initial flow-through nitrogen treatments was also significantly increased by the effect of postharvest diphenylamine calcium chloride treatment or by an increase in storage temperature from 0° to 4°.**

The storage life of 'Granny Smith' apples in modified atmosphere (MA) storage is limited by the development of internal core flush, superficial scald and loss of flesh firmness and skin greenness. With a wider international acceptance of diphenylamine (DPA) treatments scald is no longer a problem, but skin residue limits may restrict the use of drench concentrations which are needed when scald susceptibility is acute.

Core flush and associated flesh browning were effectively reduced with low O_2 storage (O_2 between 1.0% and 1.5%) and by maintaining CO_2 at less than 1% (8). Core flush was also reduced when temperature was kept above 1°C, especially with stepwise cooling from 4° to 0° during the first 6 weeks of storage, although these treatments accelerated softening and yellowing (8).

Advantages gained from the use of low O_2 storage with other apple cultivars have been reported for 'Cox's Orange Pippin' (4, 5) and 'Delicious' (1). However, core flush was increased by an initial 7 day storage period in N_2 followed by normal MA (3) or by continuous MA at under 1% O_2 (1), and use of these low O_2 storage methods was considered by Fidler and North (6) to be close to a minimum O_2 theshold resulting in alcohol accumulation to a level causing permanent tainting. However, provided alcohol accumulation in fruit tissue during anaerobiosis did not exceed about 220 mg 100 g^{-1} most 'Cox's Orange Pippin' and 'Golden Delicious' could be salvaged after aeration at 14°C (6, 7, 10). There was also a relationship of 3000:1 between the level of ethyl alcohol expressed in mg 100 g^{-1} in the fruit tissue and the concentration of alcohol expressed in mg $liter^{-1}$ in the vapor of the storage atmosphere (10).

In the present trials we compared low O_2 storage and commercial MA storage (2.5–3.0% CO_2:2.5–3.5% O_2) at 0°C to establish which MA method was more suited for 'Granny Smith' apple storage. We determined the effect of harvest time on fruit storage at these 2 MA levels. We also studied the effects of exposure time to initial O_2 stress treatments between 0.2% and 0.5% and the effects of re-establishing the low O_2 atmosphere by controlled venting or air flushing following initial O_2 stress treatments. Finally, we wished to ascertain the effects of initial O_2 stress treatments of 0% O_2, or O_2 at 0.2 to 0.5% where these treatments were given at storage temperatures of 0° and 4°.

Materials and Methods

Apples were harvested from orchards in the outer Melbourne metropolitan and Mornington Peninsula orchard district of southern Victoria, a relatively cool growing district in Australia.

Fruit treatment units were obtained by selecting 10 fruit of commercial sizes from each of 10 trees. The total quantity of fruit harvested from each tree did not exceed half the crop ensuring representative samples in size and quality at each harvest. A subsample of 20 fruit was taken at each harvest in each trial to assess starch-index values as a maturity indicator on a scale of 10 following the method used by Beattie et al. (2).

Trial 1 (1978). Commercial MA (3.5% CO_2:3.5% O_2) was compared with low O_2 (1.0% CO_2:1.5% O_2) storage where the fruit stored was picked at different stages of maturity. There were 8 harvests made at weekly intervals commencing on March 23, taken from 4 orchard replicates. The MA atmospheres were established within 7 days of the last harvest when fruit core temperature was −0.5°C.

Trial 2 (1978). One harvest was made on April 14 from 4 orchard replicates. Modified atmosphere conditions were established within 7 days of harvesting. Adjustment to low O_2 conditions following initial O_2 stress treatments at 0.2%–0.5% O_2 for 0, 3, or 9 days were by 24 hr air flushing followed by O_2 pulldown or by controlled venting to increase O_2 to the desired level.

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Trial 3 (1979). Fruit were harvested on April 18 from 4 orchard replicates, and the effects of maintaining initial O_2 stress treatments were compared by either flow-through or static MA techniques; temperature was 4°C. Two different initial O_2 stress treatments using the flow-through technique were obtained by continuously passing pre-mixed N_2 either O_2-free or containing 0.5% O_2, through 1100 liter gas-tight cabinets at a rate of 0.7 liter hr^{-1} kg^{-1} of fruit. Initial O_2 stress treatments using the static technique were obtained by diluting the MA O_2 with N_2 either O_2-free or containing 0.5% O_2. Subsequent retention of these atmospheres required O_2 monitoring twice daily and N_2 or air was added as required. Actual gas levels obtained with the flow-through technique were constant, but with the static technique variability at 0% O_2 was to 0.2% and at 0.5% O_2 was between 0.3% and 0.7%. Atmosphere pulldown to 1.0% CO_2:1.5% O_2 required 7 days and initial O_2 stress treatments of 14 days were then given. Low O_2 MA followed these initial O_2 stress treatments and was achieved by controlled venting with outside air to raise O_2 to 1.5%. Ethanol and acetaldehyde concentrations in the storage atmospheres were measured in 2.5 ml samples by gas chromatography.

In Trial 4 (1979). An initial O_2 stress treatment at 0.5% O_2 was applied by static technique to fruit harvested on April 18 from 4 orchard replicates, and this reverted to low O_2 MA by controlled venting with outside air to 1.0% CO_2:1.5% O_2. Storage at these MA conditions was either at 0° or 4°C, and in Trials 3 and 4 half the fruit were stored either undrenched or post-harvest drenched in DPA 1000 mg $liter^{-1}$ plus $CaCl_2$ 3.0% (w/v), commercial flake grade material, immediately after harvest.

All MA atmospheres apart from those with initial O_2 stress obtained by the flow-through technique were obtained initially by diluting the O_2 in gas tight MA cabinets with commercial grade N_2 to achieve the desired O_2 levels. CO_2 was removed by placing 100 g $Ca(OH)_2$ per 20 kg fruit inside sealed cabinets and by use of adjustable flow external $Ca(OH)_2$ scrubbers. Because of low level leakage in some MA cabinets, regular N_2 flushing or introduction of CO_2 was necessary to maintain levels. O_2 infiltration from leakage did not exceed 0.1% daily and occurred mainly at times of barometric pressure change. Gas levels were monitored twice daily for the first 30 days and daily thereafter. CO_2 and O_2 analyses were done manually using a paramagnetic O_2 analyzer and an infra-red CO_2 analyzer.

Fruit were removed from MA storage in mid-December and assessed for color, firmness, flavor tainting and wastage level after a simulated marketing treatment at 18°C for 10 days. Skin color was rated on a scale of 0–4 by reference to Maerz and Rea-Paul color dictionary (9). Greenest apples scoring 0 matched plate 18, L-9, and yellowest apples scoring 4 matched plate 18, K-4. Flavor was rated by 3 judges to detect tainting, and scores given were 10 for severe tainting and 1 for no tainting. Firmness was measured in kg using a hand held Effegi penetrometer with an 11 mm plunger and 1 pared punch on 20 fruit. Scald and core flush were expressed as a percentage of fruit affected. Relevant data were statistically analyzed and effects and interactions were tested by analysis of variance. Mean separation was by the method of least significant difference (LSD) at the 5% and 1% level.

Results

Effects of MA treatments on fruit from different harvests (Trial 1). Starch-iodine scores at harvest were comparable on fruit used in both MA treatments (Table 1). Scald wastage exceeded 90% where starch-iodine scores exceeded 9 on April 10 if fruit was in low O_2 MA or 5.5 on April 17 if fruit was in commercial MA.

Table 1. Effects of modified atmosphere storage conditions and time of harvest on scald and core flush in 'Granny Smith' apples after storage at -0.5°C.

Modified atmosphere treatment	Harvest date	Starch index (0-10)	Scald (%)	Scald (angles)[z]	Core flush (%)	Core flush (angles)[z]
CO_2%:O_2% 3.5:3.5 (commercial MA)	Mar. 23	10.0	100	(1.571)	6.2	(0.252)
	Mar. 28	10.0	100	(1.571)	2.2	(0.148)
	Apr. 3	10.0	100	(1.571)	0	(0.000)
	Apr. 10	9.8	98	(1.522)	6.1	(0.249)
	Apr. 17	5.6	96	(1.356)	29.7	(0.576)
	Apr. 27	4.4	52	(0.811)	21.4	(0.480)
	May 3	3.5	27	(0.549)	36.4	(0.648)
	May 11	0	1	(0.097)	38.6	(0.671)
1.0:1.5 (low O_2 MA)	Mar. 23	10.0	100	(1.571)	4.2	(0.208)
	Mar. 28	10.0	100	(1.571)	1.9	(0.139)
	Apr. 3	9.7	99	(1.533)	0.2	(0.047)
	Apr. 10	9.2	97	(1.423)	1.1	(0.107)
	Apr. 17	5.5	60	(0.882)	1.1	(0.107)
	Apr. 27	4.7	12	(0.352)	3.9	(0.199)
	May 3	4.0	0	(0.000)	4.9	(0.214)
	May 11	1.5	0	(0.000)	5.6	(0.238)
LSD 5%				(0.259)		(0.243)

[z]Arcsine square root of the percentage transformation.

Fruit harvested with starch-iodine scores less than 5.6 at harvest on or after April 17 were progressively less susceptible to scald at progressively later harvest dates under both storage techniques but in this fruit scald was significantly reduced by storage in low O_2 MA compared with commercial MA. The incidence of core flush was also related to storage atmosphere and maturity as measured by starch index, but in a different way. Core flush was significantly increased as fruit became more mature and showed starch-iodine values lower than 5.6, or if harvesting was as early as March 23, but this occurred only where storage was in commercial MA. In low O_2 MA core flush was not significantly different at any time of harvesting.

In this trial, MA conditions were not established until after the last harvest. The slower O_2 pulldown with earlier harvested fruit may have affected their storage performance. Since low O_2 in MA storage reduced scald and core flush, it is possible that a quicker O_2 pulldown may have reduced these disorders even further than is shown in Table 1.

Fruit were firmer and greener ($P<0.01$) after low O_2 storage than after commercial MA storage, and these storage effects were greater than those associated with harvested date. Flavor scores were not affected by MA treatments.

Effects of exposure time to, and air venting after, initial O_2 stress treatments with low O_2 (MA) storage (Trial 2). The mean starch-iodine score at harvest was 5.5 which corresponded to the stage of maturity in Trial 1 where scald susceptibility was declining and fruit were becoming susceptible to core flush.

Where single factor effects were examined, the differences between the 2 initial O_2 stress treatments (0–0.2% or 0.4–0.7% O_2) did not differentially affect fruit color, flavor or firmness but scald was significantly reduced under the initial O_2 stress of 0 to 0.2% ($P<0.05$).

Treatments which did affect fruit firmness were exposure time to initial O_2 stress and the method of obtaining transition from initial O_2 stress and these effects were additive. Increasing exposure time to initial O_2 stress increased firmness as also did monitored venting compared with air flushing (Table 2). Interactions between exposure time to initial O_2 stress and transition from initial O_2 stress to low O_2 at 1.0% CO_2:1.5% O_2 occurred with the other

Table 2. Effects of exposure time to initial O_2 stress treatments and the method of re-establishing atmospheres to low O_2 MA storage on fruit flavor, color and scald.[z]

Exposure time to initial O_2 stress[y] (Days)	MA storage regime after initial O_2 stress treatment: Air flushing then O_2 pull-down to 1.0% CO_2:1.5% O_2			Monitored air venting to 1.0% CO_2:1.5% O_2		
	Color (0-4)	Firmness (kg)	Scald[x] (%)	Color (0-4)	Firmness (kg)	Scald[x] (%)
0	1.87 AB[w]	6.6 B	51.7 A	1.80 A*[w]	6.7 A	30.9 A*
3	2.00 A	6.8 AB	24.3 AB	1.50 AB*	7.0 B*	10.0 A
9	1.50 B	7.0 A	12.9 B	1.25 B*	7.2 B*	1.2 B*

[z]Cooled to 0°C within 3 days of harvest before initial O_2 stress treatments were given.
[y]O_2 level maintained by the static technique during initial O_2 stress treatment.
[x]Arcsine square root of the percentage transformation.
[w]Values significantly different by LSD after analysis of variance at 1% within columns and 5% (*) across columns.

2 factors measured, scald, and color, and these are presented in Table 2. These data show a 9-day initial O_2 stress treatment is required to affect green color retention, although there appears to be a tendency for the effect to be more marked under subsequent monitored venting than under subsequent air flushing. Scald was reduced with increasing exposure time to initial O_2 stress. This effect was more marked and total scald levels were lower where low O_2 storage was re-established by monitored air venting rather than by air flushing. Beneficial effects in retaining color also occurred after initial O_2 stress treatments if low O_2 storage was re-established by monitored venting. Core flush levels in all treatments were below 5% and data were not analyzed.

Effects of a static or flow-through technique for initial O_2 stress treatments (Trial 3). Mean starch-iodine score at harvest was 7 indicating that this fruit was harvested in a condition of high scald susceptibility and moderate core flush susceptibility.

There was less scald after a 14-day initial O_2 stress treatment at 0% than at 0.5% O_2, and this level of scald control was further improved when the initial O_2 stress treatment was obtained by the flow-through technique (Table 3). However, even with these specialized MA conditions, scald control to levels under 1% was only obtained with DPA as a pre-storage treatment.

After storage and simulated marketing, no fruit tainting occurred unless the fruit had been drenched with DPA plus $CaCl_2$ (Table 3). All drenched fruit was tainted where the initial O_2 stress was at 0% and was maintained by the flow-through method.

Table 3. Effects on scald and fruit tainting from different methods of achieving initial O_2 stress treatments prior to continuous low O_2 storage where fruit were stored with or without DPA + $CaCl_2$ treatments.[z]

Method of achieving initial O_2 stress	Initial O_2 stress (%)	Postharvest drenching treatment: Undrenched		1000 mg l^{-1} DPA + 3% $CaCl_2$	
		Taint score (1-10)[y]	Scald (%)[x]	Taint score (1-10)[y]	Scald (%)[x]
Static	0.0	1.0	34.5 A[w]	2.6 B**[w]	0.5**
	0.5	1.0	91.1 B	1.7 A	0.6**
Flow-through	0.0	1.0	8.2 C	10.0 D**	0.4
	0.5	1.0	56.6 A	4.8 C**	0.3**

[z]Cooled to 4°C within 3 days of harvesting, stored in low O_2 until May 1, then given initial O_2 stress treatments for 14 days followed by continuous low O_2 storage.
[y]Untainted fruit score 1.
[x]Arcsine square root of the percentage transformation.
[w]Values significantly different by LSD test after analysis of variance at 1% within columns and 1% (**) across columns.

Increasing the initial O_2 concentration from 0% to 0.5% using the flow-through method significantly reduced tainting and changing the method of maintaining the initial O_2 stress to the static method led to a further reduction in tainting at an initial O_2 stress of 0% and again when the concentration of O_2 was increased to 0.5%. The levels of ethanol and acetaldehyde in the storage atmospheres on May 18 and July 2, 3 days and 48 days after the conclusion of O_2 stress treatments, are tabulated in Table 4. In general terms, the ethanol and acetaldehyde levels on May 18 conform with the level of tainting in drenched fruit but undrenched fruit was stored in the same container and was not tainted. It is not known whether the ethanol and acetaldehyde were produced by both drenched and undrenched fruit with only drenched fruit producing a taint or whether only the drenched fruit produced ethanol and acetaldehyde.

Retention of green color and firmness and the incidence of core flush were unaffected by the method of applying the initial O_2 stress treatment. Undrenched fruit were greener on removal from MA storage and after 7 days at 20°C where initial O_2 stress was at 0.5% (Table 5). Fruit were also firmer following initial O_2 stress at 0.5%, and this effect was greatest on undrenched fruit after 7 days at 20° (Table 5). DPA + $CaCl_2$-drenched fruit were firmer and greener than undrenched fruit regardless of the level of initial O_2 stress both on removal from store and after 7 days. Core flush was increased by reduction of O_2 from 0.5% to 0% during initial O_2 stress treatment, and this effect was accentuated further by DPA + $CaCl_2$ drenching.

Effects of temperature during low O_2 storage (Trial 4). Fruit which had been DPA + $CaCl_2$ drenched remained firmer during storage at both 0° and 4°C (Table 6). On the other hand, without drenching, fruit stored at 4° was softer than fruit stored at 0°. The softer fruit from the 4° storage was also more yellow but the color of all other treatments was comparable (Table 6). DPA + $CaCl_2$ drenching reduced the level of scald from more than 80% to under

Table 4. Effects of different methods of achieving initial O_2 stress treatments prior to continuous low O_2 storage on ethanol and acetaldehyde concentration in the storage atmosphere.[z]

Method of achieving initial O_2 stress	Initial O_2 stress (%)	Acetaldehyde ($\mu g\ l^{-1}$) May 18	July 2	Ethanol ($\mu g\ l^{-1}$) May 18	July 2
Static	0.0	9.3	4.1	9.8	3.7
	0.5	8.9	8.6	6.0	3.6
Flow-through	0.0	57.2	12.3	51.0	5.4
	0.5	22.3	11.1	11.4	2.8

[z]Cooled to 4°C within 3 days of harvesting, stored in low O_2 until May 1 then given 14 days initial O_2 stress followed by reversion to low O_2 storage on May 15.

Table 5. Effects of postharvest drenching and initial O_2 stress treatments prior to continuous low O_2 storage on core flush, and on fruit ground color, and firmness at removal from storage and after 7 days at 20°C.[z]

Initial O_2 stress (%)	Postharvest drenching treatment									
	Undrenched					1000 mg l^{-1} DPA + 3% $CaCl_2$				
	Color (1-4)		Firmness (kg)		Core flush (%)[y]	Color (1-4)		Firmness (kg)		Core flush (%)[y]
	Removal	7 days	Removal	7 days		Removal	7 days	Removal	7 days	
0	1.5 A[x]	2.9 A	6.4	5.6 A	31.7 A	1.0**[x]	1.2**	6.4 a	6.1 a**	77.8 A**
0.5	1.3 B	1.4 B	6.6	6.2 B	4.3 B	1.1**	1.1**	7.0 b*	6.6 b*	42.4 B**

[z]Cooled to 4° C within 3 days of harvesting, then given initial O_2 stress treatments for 14 days from April 25 followed by continuous low O_2 storage at 1.0% CO_2:1.5% O_2.
[y]Arcsine square root of the percentage transformation.
[x]Values significantly different by LSD test after analysis of variance at 5%, 1% due to O_2 level and at 5% (*), 1% (**) due to drenching treatment.

Table 6. Effects of storage temperature and postharvest drenching treatments on the incidence of scald and core flush and on fruit ground color and firmness. MA storage at 1.0% CO_2:1.5% O_2 followed a 14-day initial O_2 stress treatment at 0.5% O_2.

Temperature during MA storage (°C)	Postharvest drenching treatment							
	Undrenched				1000 mg l^{-1} DPA + 3% $CaCl_2$			
	Color (1-4)	Firmness (kg)	Core flush[z] (%)	Scald[z] (%)	Color (1-4)	Firmness (kg)	Core flush[z] (%)	Scald[z] (%)
0	1.1 a[y]	6.8 a	4.6	80.4	1.1	7.3*[y]	9.4 A	0.4**
4	1.3 b	6.3 b	2.1	91.1	1.1*	7.5**	52.8 B**	0.6**

[z]Arcsine square root of the percentage transformation.
[y]Values significantly different by LSD test after analysis of variance at 5%, 1% due to temperature and at 5% (*), 1% (**) due to drenching.

1%. Increasing storage temperature from 0° to 4° did not affect scald but increased core flush following DPA + $CaCl_2$ treatments (Table 6). Flavor taints were not evident at 0°, but occurred at 4° for fruit which had received DPA + $CaCl_2$ treatments.

Discussion

Our trials have shown that storage under a MA of 1.0% CO_2:1.5% O_2 (low O_2 storage) has given advantages over commercial MA storage at 3.5% CO_2:3.5% O_2 in the control of scald and core flush and the retention of fruit firmness and green color in 'Granny Smith' apples. These results support those of Fidler and North in experiments with 'Cox's Orange Pippin' apples (5, 6). However, in Trial 3, permanent tainting occurred following an initial 14-day period in N_2 during storage at 4°C, but this effect was dependent on the use of a DPA + $CaCl_2$ drench. The ethanol concentration in the atmosphere, where severe flavor tainting occurred, was 53 μg liter^{-1}, which is calculated to be about 160 mg 100 g^{-1} of fruit. This level is less than the 220 mg 100 g^{-1} which caused permanent tainting on 'Cox's Orange Pippin' which were stored without DPA + $CaCl_2$ drench (10).

Although the 'Granny Smith' apples in Trial 1, harvested between April 10–17, were of a similar maturity to those in Trial 2, as judged by starch-iodine test, the incidence of scald was higher in Trial 1 under low O_2 storage (Table 1) than in Trial 2 where initial O_2 stress preceded low O_2 storage (Table 2). Though initial O_2 stress treatments reduced scald, these treatments tended to increase core flush particularly following initial O_2 stress treatments at 0% O_2 rather than 0.5% O_2. This is clearly shown in Trial 3 where core flush on fruit not treated with DPA + $CaCl_2$ exceeded 31% following initial O_2 stress at 0% O_2 but was only 4.3% following initial O_2 stress at 0.5% O_2 (Table 5). By comparison, where fruit of approximately similar maturity were stored without initial O_2 stress in Trial 1 core flush was under 3% (Table 1).

We consider that optimum storage conditions for 'Granny Smith' require initial O_2 stress at 0.5% for 9–14 days followed by MA storage at 1.0% CO_2:1.5% O_2. We have shown that with this storage technique DPA + $CaCl_2$ drenches can be used effectively, provided storage temperature is at 0°C (Table 6). Further work is required to establish whether the concentration of DPA + $CaCl_2$ can be reduced to give adequate control of scald without the undesirable effects on core flush and tainting which occurred where initial O_2 stress was at 0% O_2 or where temperature of storage was 4°.

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