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Abstract

Storage of the 'Nijisseiki' cultivar of Japanese pears was studied over three seasons for periods up to 36 weeks at 0°C. Storage in 50 pm thick low-density polyethylene (LDPE) bags at 0°C considerably delayed yellowing in all experiments, even after fruit was removed to 20°C for 1 week at the end of storage. The addition of an ethylene absorbent made from potassium permanganate on aluminium oxide (Purafil II) further delayed yellowing. Carbon dioxide levels in both treatments varied, but were generally in the range 2-3%. Oxygen levels remained high, generally 16-19%. In bags without Purafil, ethylene levels rose slightly during storage and were generally about 0.15 pi I 1. When Purafil was included in the bags, the ethylene level was reduced 10-fold or more. A sensory test indicated that the use of LDPE bags and ethylene absorbent resulted in fruit with better eating quality than fruit stored in air. Disorders over the 3-year investigation were low even after long-term storage. The use of polyethylene bags reduced the severity of flesh browning, and flesh spot decay was virtually absent. Tire use of bags increased the severity of core browning. Inclusion of an ethylene absorbent in bags reduced the severity of disorders, particularly core browning. Treatment of the fruit with 1 -methylcyclopropene (1-MCP), before or during storage, resulted in higher ethylene levels in the polyethylene bags. At the concentrations used, 1-MCP did not improve the storage of 'Nijisseiki' compared to the use of polyethylene bags with Purafil II.

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Effects of polyethylene bags, ethylene absorbent and 1-methylcyclopropene on the storage of Japanese pears

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SUMMARY
Storage of the 'Nijisseiki' cultivar of Japanese pears was studied over three seasons for periods up to 36 weeks at 0°C. Storage in 50 μm thick low-density polyethylene (LDPE) bags at 0°C considerably delayed yellowing in all experiments, even after fruit was removed to 20°C for 1 week at the end of storage. The addition of an ethylene absorbent made from potassium permanganate on aluminium oxide (Purafil II) further delayed yellowing. Carbon dioxide levels in both treatments varied, but were generally in the range 2–3%. Oxygen levels remained high, generally 16–19%. In bags without Purafil, ethylene levels rose slightly during storage and were generally about 0.15 $\mu l\, l^{-1}$. When Purafil was included in the bags, the ethylene level was reduced 10-fold or more. A sensory test indicated that the use of LDPE bags and ethylene absorbent resulted in fruit with better eating quality than fruit stored in air. Disorders over the 3-year investigation were low even after long-term storage. The use of polyethylene bags reduced the severity of flesh browning, and flesh spot decay was virtually absent. The use of bags increased the severity of core browning. Inclusion of an ethylene absorbent in bags reduced the severity of disorders, particularly core browning. Treatment of the fruit with 1-methylcyclopropene (1-MCP), before or during storage, resulted in higher ethylene levels in the polyethylene bags. At the concentrations used, 1-MCP did not improve the storage of 'Nijisseiki' compared to the use of polyethylene bags with Purafil II.

Shen (1980) reported that pears, possibly *Pyrus pyrifolia* and *Pyrus calleryana*, have been grown in the wet lowlands of China for 3,000 years. Several *Pyrus* species have been hybridised by Japanese plant breeders to remove stone cells found in some earlier cultivars. The new cultivars produce fruit of very high quality and of wide acceptance (Johnson, 1986). One of the early cultivars, 'Nijisseiki', also called 'Twentieth Century' (Iwahori, 2001) has been grown in Australia for several decades.

Japanese pears are now marketed in Australia under the Japanese name for pear, 'Nashi'. While, in Japan, fruit is consumed at harvest or soon after, in Australia fruit is cold stored for several months. The fruit is 'eating ripe' at harvest, and because of this, is readily damaged. It becomes yellow early in storage and develops a number of physiological disorders (White *et al.*, 1990). The industry wishes to extend the storage life and ultimately export 'Nashi' to Northern Hemisphere countries during their off-season.

There are few reports on storage of the 'Nijisseiki' cultivar or on 'Nashi' generally. A number of preliminary studies determined that many post-harvest treatments, known to affect other pome fruit had little effect on 'Nashi'. These studies included temperature variations during storage and addition of mineral salts and are not discussed further. Storage in modified atmospheres, however, had a marked effect on green colour retention and disorders. These treatments are evaluated more fully in this paper.

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MATERIALS AND METHODS

Harvest of fruit

The experimental unit was 20 fruit. All fruit were obtained from Shepparton in Northern Victoria (the main production area in Australia) and transported 700 km by road to the University. Fruit was cooled and stored within 2-3 d of harvest. To widen the range of conditions, fruit was harvested weekly over a 4-week period. Harvesting began during the first week in February (a few d before commercial harvest) and continued using different blocks during the commercial 3-week harvesting period. There was no sign of yellowing at first harvest, but slight yellowing became evident as the harvest period proceeded.

Fruit storage

Fruit was cooled upon arrival at the University, and then packed in 450 mm × 800 mm low-density polyethylene (LDPE) bags (50 μm thick). Dishes containing 150 g of an ethylene absorbent made from potassium permanganate on an aluminium oxide carrier (Purafil II, Purafil Inc., U.S.A.) were included in some bags. Purafil was placed in small dishes among the fruit and the bags were then sealed with tape. Storage was at 0°C for (up to) 36 weeks in each of 3 years. Fruits used for sensory testing were stored for 31 weeks at 0°C, and then moved to 20°C for 1 week before the test was carried out.

Measurement of atmospheric composition

Carbon dioxide, oxygen and ethylene levels in the bag atmospheres were measured several times during

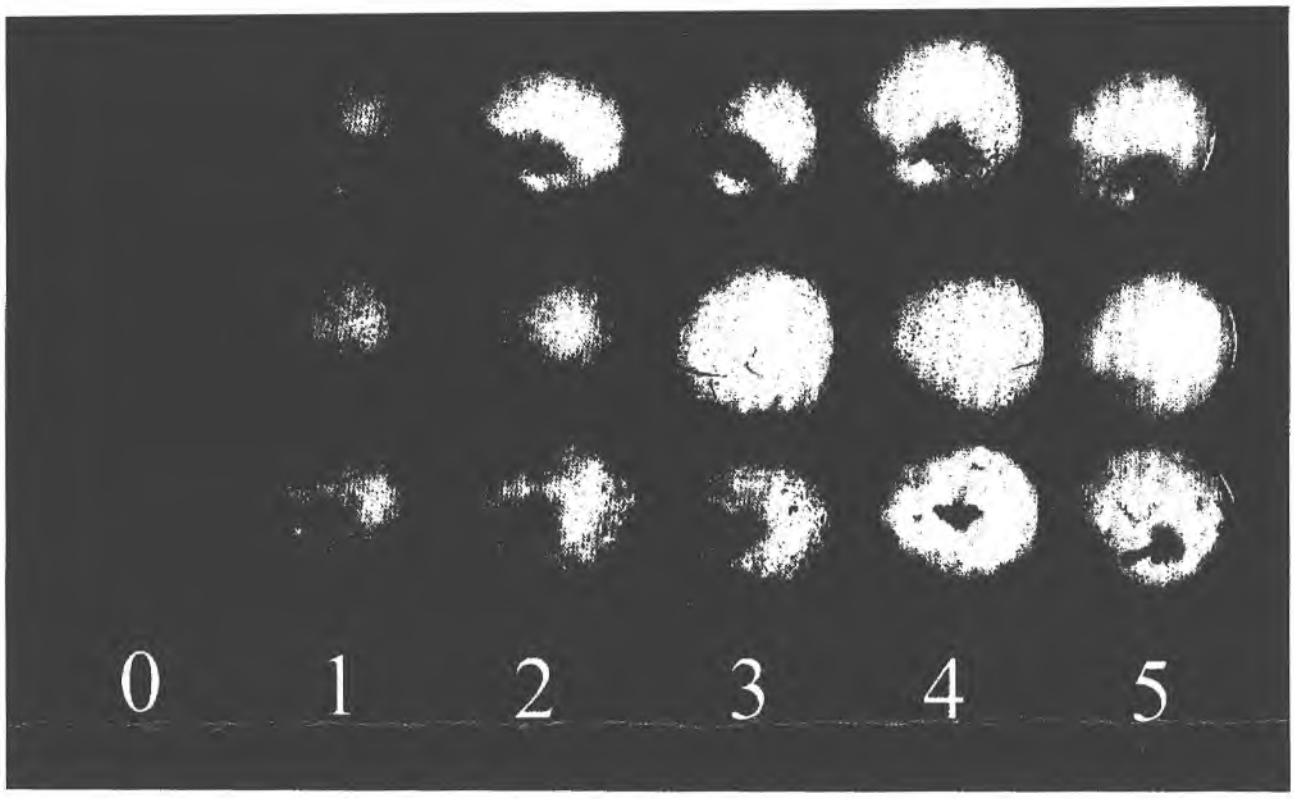

FIG. 1
Visual colour scores used for 'Nashi' (Japanese pears). 0 = deep green, 1 = green, 2 = more green than yellow, 3 = more yellow than green, 4 = yellow and 5 = deep yellow.

storage by gas chromatography. Oxygen and carbon dioxide were measured using a gas chromatograph (Series 350, Gow-Mac, USA) fitted with a thermal conductivity detector. Oxygen was determined using a 1.8 m stainless steel column packed with Molecular Sieve Type 5A (30–60 mesh). Carbon dioxide was measured with a 3.7 m stainless steel column packed with Porapak Q. Ethylene was measured with a gas chromatograph (Series 3300, Varian, USA) fitted with a flame ionisation detector and a 1.8 m stainless steel column packed with activated aluminium oxide (80–100 mesh).

Atmospheric composition data were analysed using analysis of variance and Duncan's Multiple Range Test.

Colour scoring and chlorophyll determination

Delaying colour change is considered to be of prime importance in the long-term storage of 'Nashi'. Physical and chemical methods were first considered for determining colour change. However, a non-destructive method for assessment of colour was selected that allowed colour to be measured without removing samples from their storage environment. This allowed regular inspections throughout the entire storage period. Fruit were scored on a 6-point scale: deep green, green, more green than yellow, more yellow than green, yellow and deep yellow (Figure 1). These colour scores were allocated ratings of 0–5 inclusive, respectively. Mean scores at each sampling time were calculated by multiplying the number of fruit with each score, adding the values obtained and dividing by the total number of fruit. Mean scores were used in the statistical analyses, which involved analysis of variance as well as regression analysis. To relate colour scores to chlorophyll content, chlorophyll was extracted according to Moran and Porath (1980). The fruit skin was removed with a vegetable peeler and discs of skin were cut with an 8 mm borer. The minimum amount of flesh was included in the sample. Skin discs weighing approximately 1 g, from three fruit in each colour grade, were placed in 10 ml N,N-dimethylformamide at 0°C in total darkness. After 24 h, chlorophyll *a* and *b* levels were determined by measuring absorbance at 603 nm, 647 nm and 664 nm and substituting these values into the chlorophyll equations developed by Moran (1982). Total chlorophyll was calculated by addition of chlorophyll *a* and *b* values. Linear regression was used to relate visual colour scores to chlorophyll content.

Application of 1-methylcyclopropane

After experiments revealed that removal of ethylene from the storage atmosphere was beneficial, fruit was also treated with 1-methylcyclopropane (1-MCP). This substance had been reported to inhibit the effects of ethylene in fruit (Fan and Mattheis, 1999; Feng *et al.*, 2000; Rupasinghe *et al.*, 2000; Watkins *et al.*, 2000; DeEll *et al.*, 2002). In the first season, 1-MCP in the form of Agrofresh (Rohm and Haas, Australia) was applied at a concentration of 1µl l^{-1}. To generate the required concentration of 1-MCP gas, 1.9 mg Agrofresh powder was dissolved in 30 µl water at 40°C in a small Eppendorf tube. The mixture was immediately placed into 28 l drums at 0°C along with the fruit to be treated. After

exposure for 24 h, the fruit was transferred from the drums into LDPE bags and stored at 0°C. Untreated fruit was also held for the same time in similar drums. In the second season, 1-MCP was applied at 0°C or at 22°C, using either 90 µl l^{-1} 1-MCP or 180 µl l^{-1} 1-MCP. In the case of fruit treated at 22°C, the beaker containing the 1-MCP formulation was removed after 24 h. The fruit was then placed at 0°C. The 1-MCP formulation was not removed from the bags of fruit treated at 0°C and remained inside until the conclusion of the experiment. Thus 0°C fruit were exposed to 1-MCP for a longer time.

Assessment of physiological disorders

After up to 36 weeks of storage at 0°C, the fruit was removed. The polyethylene bags were opened and the fruit was held at 20°C for 1 week before being examined for wastage. Each fruit was cut four times equatorially and disorders were given a 4-point weighted score for severity, on a scale of 0–4 where 0 = no disorder, 1 = slight, 2 = moderate and 4 = severe disorder. The mean scores were then calculated (as for colour) and these values were used for statistical analysis. Statistical differences were determined between treatments using an error mean square calculated from a two-way analysis of variance.

Sensory testing

In one season, fruit was held in polyethylene bags with and without the ethylene absorbent, together with control fruit not stored in bags. After 31 weeks storage at 0°C, the bags were opened and the fruit was held at 20°C for 1 week. Fruit was then scored, by a 15-member taste panel, for crispness, juiciness and overall quality on a scale 1–12, where 1 = very poor quality and 12 = excellent quality.

RESULTS AND DISCUSSION

Relationship between colour score and chlorophyll levels

The relationship between total chlorophyll content and colour scores is shown in Figure 2. Colour scores were clearly related to each of the values for chlorophyll. If y = colour score and x = total chlorophyll, a regression of log (y + 0.08) on x showed a strong linear relationship with an R^2 value of 97.0%. This gives the relation between x and y as:

$$y = 6.42\exp(-0.0403x)-0.08$$

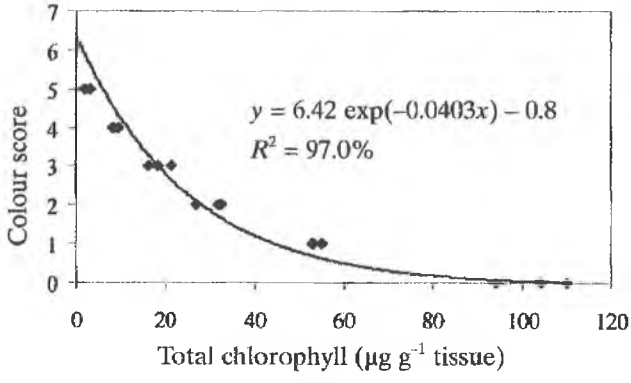

FIG. 2
Relationship between total chlorophyll content (µg g^{-1} tissue) and visual colour score of 'Nashi'.

The visual scoring system thus gave a good indication of the loss of chlorophyll as well as the loss of green colour.

There was a marked and rapid loss of chlorophyll initially, as fruit became less green, then the rate of chlorophyll loss slowed, as fruit became more yellow. As the 'Nashi' changed from deep green to green (scores 0 to 1) around 40% of the initial chlorophyll content was lost, decreasing from 99 µg g^{-1} skin tissue to 59 µg g^{-1} skin tissue. Between green (1) and more green than yellow (2), the fruit lost a further 23% of its chlorophyll content and contained 36 µg g^{-1} skin tissue. Fruit that was more yellow than green (3) contained 19 µg g^{-1} skin tissue, yellow (4) fruit contained only 7 µg g^{-1} skin tissue, and deep yellow (5) fruit had no detectable chlorophyll content.

Effect of ethylene absorbent

When fruit from various treatments were examined for colour at 0°C, treatment differences became evident after about 5 weeks in storage. Packing 'Nashi' in polyethylene bags consistently delayed the loss of green colour. Packing an ethylene absorbent with the fruit in bags usually further delayed the loss of green colour. The regressions of colour score on storage time for storage in bags with and without the ethylene absorbent, or with

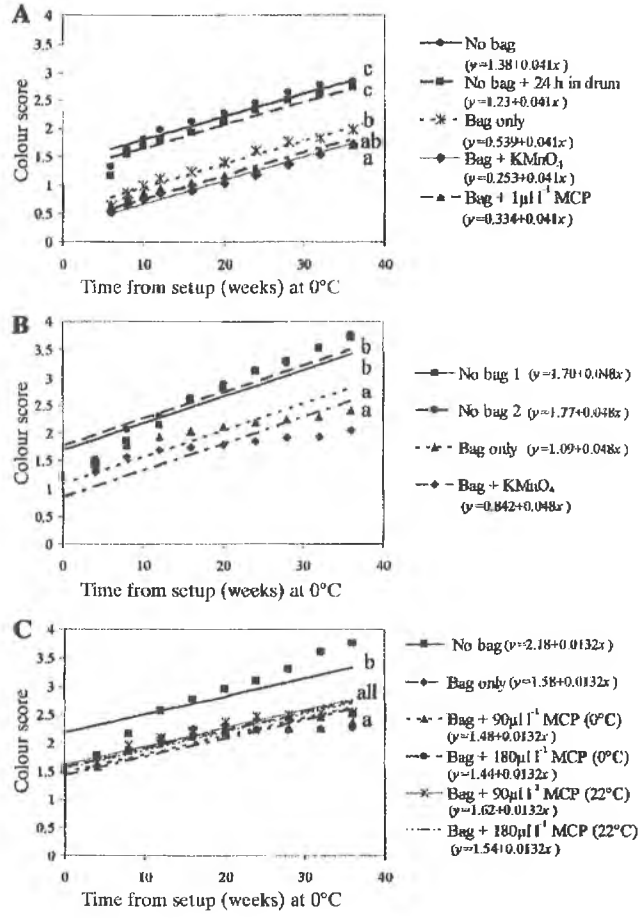

FIG. 3
Effect of polyethylene bags alone, with ethylene absorbent or 1-MCP on the colour scores of 'Nashi' held at 0°C over 36 weeks. Panel A, 'Nashi' stored in polyethylene bags with or without ethylene absorbent or 1-MCP; Panel B, 'Nashi' stored in polyethylene bags with or without ethylene absorbent; and Panel C, 'Nashi' stored in polyethylene bags with or without 1-MCP applied at 0°C or 22°C. Different letters indicate significant differences between regression equations calculated for each treatment in each experiment ($P < 0.05$).

TABLE I
Mean colour of 'Nashi' stored in polyethylene bags with or without ethylene absorbent ($KMnO_4$) or 1-MCP after storage for 36 weeks at 0°C then 1 week at 20°C

Experiment	Treatment	Colour Score
1 (Year 1)	No bag	3.8 a[1]
	No bag + 24 h in drum	3.6 ab
	Bag only	3.5 b
	Bag + $KMnO_4$	2.8 c
	Bag + 1 $\mu l\ l^{-1}$ MCP	3.2 d
2 (Year 2)	No bag 1	4.4 a
	No bag 2	4.5 a
	Bag only	3.3 b
	Bag + $KMnO_4$	2.7 c
3 (Year 2)	No bag	4.4 a
	Bag only	3.2 bc
	90 $\mu l\ l^{-1}$ MCP (0°C)	3.1 bc
	180 $\mu l\ l^{-1}$ MCP (0°C)	3.3 b
	90 $\mu l\ l^{-1}$ MCP[2] (22°C)	3.3 b
	180 $\mu l\ l^{-1}$ MCP[2] (22°C)	3.0 c

[1]Different letters between treatments (within experiments) indicate significant differences ($P < 0.05$).
[2]Fruit treated with 1-MCP for 24 h at 22°C, then at 0°C for 36 weeks.

and without 1-MCP are shown in Figure 3. Both bag-, and bag-plus-ethylene absorbent-treated fruits became less green after removal from 0°C, but they remained greener than untreated fruit (Table I).

Atmospheric composition in bags

The concentrations of carbon dioxide, oxygen and ethylene in all bags were measured several times during storage (Table II). In bags without another treatment, carbon dioxide remained low (generally less than 3%) during storage. Oxygen levels were commonly above 17%, and levels were similar in all bag treatments. Ethylene levels were generally less than 0.2 $\mu l\ l^{-1}$. The use of an ethylene absorbent considerably reduced the ethylene to levels at or below the limit of detection (< 0.005 $\mu l\ l^{-1}$). Carbon dioxide was usually slightly lower in bags with permanganate.

Effect of 1-MCP application

In the first season, fruit stored in bags with 1-MCP were significantly greener at the end of storage than fruit stored in bags alone (Table I). However, in the second season, applying 1-MCP either before or during storage, followed by storage in polyethylene bags, did not

TABLE II
Mean atmospheres in polyethylene bags, each with 20 fruit during storage at 0°C for 36 weeks

Experiment	Treatment[1]	C_2H_4 ($\mu l\ l^{-1}$)	CO_2 (%)	O_2 (%)
1 (Year 1)	Bag only	0.10 a[2]	2.0 a	18.0 a
	$KMnO_4$	0.01 a	2.3 a	17.4 a
	1 $\mu l\ l^{-1}$MCP	0.23 b	2.2 a	17.4 a
2 (Year 2)	Bag only	0.14 a	3.1 a	16.4 a
	$KMnO_4$	0.01 b	2.5 b	17.4 b
3 (Year 2)	Bag only	0.12 a	2.7 a	18.0 a
	90 $\mu l\ l^{-1}$ MCP (0°C)	1.41 bc	2.7 a	17.8 a
	180 $\mu l\ l^{-1}$ MCP (0°C)	1.86 b	2.5 a	17.9 a
	90 $\mu l\ l^{-1}$ MCP[3] (22°C)	1.05 cd	2.0 b	18.7 a
	180 $\mu l\ l^{-1}$ MCP[3] (22°C)	0.64 d	2.1 b	18.5 a

[1]All treatments were for a minimum of 4 replicates and 3 analyses..
[2]Different letters between atmospheres (within experiments) indicate significant differences ($P < 0.05$).
[3]Fruit treated with 1-MCP for 24 h at 22°C, then at 0°C for 36 weeks.

significantly improve colour retention compared to fruit stored in bags alone. Surprisingly, treatment of fruit with the ethylene inhibitor 1-MCP resulted in ethylene levels in the bags that were generally higher than when no 1-MCP was applied (Table II). Thus we appear to have an inhibitor of ethylene action that apparently increases ethylene production during storage in polyethylene bags at 0°C.

Incidence of disorders

Although the 'Nijisseiki' cultivar is prone to a number of disorders (White *et al.*, 1990), the incidence of disorder was not high. The mean scores for most treatments were generally low (i.e., less than 1, slight severity) even after long-term storage (Table III). The modified atmospheres and 1-MCP treatments affected disorders differently. Core browning seemed to be the most important disorder. This was increased by the modified atmosphere (similar to core flush in apples, where high carbon dioxide increases the disorder (Scott *et al.*, 1962)). Ethylene absorption reduced core browning, a similar finding to Scott and Wills (1974), who showed that lowering ethylene levels resulted in a decrease in brown heart (a form of core browning) in 'William Bon Cretien' pears. Flesh browning was increased by storage in bags with or without 1-MCP, but not by storage in bags with the ethylene absorbent. Flesh spot decay, a physiological

TABLE III
Mean disorder scores after storage at 0°C for 36 weeks followed by 1 week at 20°C for fruit stored in polyethylene bags with ethylene absorbent or treated with 1-MCP, or controls

Experiment	Treatment[1]	Flesh Browning	Core Browning	Flesh Spot Decay	Brown Heart
1 (Year 1)	No bag	0.00 a[2]	1.00 a	0.70 c	
	No bag + 24 h in drum	0.00 a	0.87 a	0.66 c	
	Bag only	0.33 b	2.24 b	0.31 a	
	Bag + $KMnO_4$	0.11 a	0.91 a	0.69 c	
	Bag + 1 $\mu l\ l^{-1}$1-MCP	0.12 ab	1.97 b	0.47 b	
2 (Year 2)	No bag 1	0.44 b	0.26 b		
	No bag 2	0.38 b	0.28 b		
	Bag only	1.56 c	0.00 a		
	Bag + $KMnO_4$	0.11 a	0.31 b		
3 (Year 2)	No bag	0.03 a	0.32 a	0.31 b	0.00 a
	Bag only	0.01 a	0.98 b	0.08 a	0.00 a
	90 $\mu l\ l^{-1}$ MCP (0°C)	0.61 b	0.67 ab	0.00 a	0.06 a
	180 $\mu l\ l^{-1}$ MCP (0°C)	0.72 b	0.52 ab	0.04 a	0.04 a
	90 $\mu l\ l^{-1}$ MCP[3] (20°C)	0.65 b	0.52 ab	0.00 a	0.06 a
	180 $\mu l\ l^{-1}$ MCP[3] (20°C)	0.39 ab	0.29 a	0.16 ab	0.06 a

[1]Each experiment contained 4 replicates.
[2]Different letters between treatments (within experiments) indicate significant differences ($P < 0.05$).
[3]Fruit treated with 1-MCP for 24 h at 22°C, then at 0°C for 36 weeks.

TABLE IV
Sensory properties of 'Nashi' stored in polyethylene bags with or without ethylene absorbent for 31 weeks at 0°C followed by 1 week at 20°C

Treatments	Mean Values[1]		
	Juiciness	Crispness	Overall preference
No bag	7.1 a[2]	2.6 a	2.4 a
Bag only	7.5 a	5.1 a	7.8 b
Bag + $KMnO_4$	11.6 b	10.6 b	9.8 b

[1]Values are the mean of 15 replicates.
[2]Different letters between treatments indicate significant differences ($P < 0.05$).

disorder not caused by microorganisms as the name suggests (White *et al.*, 1990), occurred with low incidence and severity, but was observed in several experiments to be absent in polyethylene bag storage. Brown heart, a well-known form of carbon dioxide injury in pears (Hall and Scott, 1977) was observed in some preliminary experiments, but was not detected in this series of experiments except when fruit was treated with 1-MCP (Table III). The optimum level of carbon dioxide is probably low, but this level needs to be determined to avoid brown heart. It is not known what effect oxygen levels have on 'Nijisseiki'. Scott (unpublished) has studied the effects of oxygen on apples and pears and believes that levels of oxygen close to those in air have little if any effect on the storage life of these fruit. Simple modified atmospheres with about 2% carbon dioxide, with or without ethylene absorption, appear to be of considerable value for extending the storage life of 'Nashi'. The retention of green skin colour is increased with these treatments, which are simple to develop and control. On the basis of these initial studies, it is recommended that carbon dioxide levels be kept at about 2% to avoid any increase in core browning. Although fruit maturity or harvest date were not included as a factor in these experiments, it was observed each year that fruit harvested first stored better than fruit harvested later. Maturity at harvest may therefore be worth further investigation.

Sensory testing

The results of the sensory testing showed that fruits that were stored in bags with an ethylene absorbent were significantly juicier and crisper than fruits stored either in bags without the absorbent, or without a bag (Table IV). Adding an ethylene absorbent resulted in fruit that was still highly acceptable, even after 31 weeks storage.

Commercial implications

In areas where 'Nashi' are stored in lug boxes, bushel boxes or their equivalent, the use of polyethylene bags plus an ethylene absorbent would be worth further study, as this is a simple storage method. In Australia, where all 'Nashi' storage is in bins, work on simple 'ventilated' storage developed originally by Kidd and West (1927) in the UK would be worth studying. This form of storage, initially called gas storage, is simple to control. The entry of air is controlled to regulate the carbon dioxide level. The oxygen level is usually 21% minus the carbon dioxide level. Such conditions, with 2% carbon dioxide and 19% oxygen, would approximate to the conditions in the polyethylene bags. These atmospheres are easy to develop and would not require generators or scrubbers, as used during low-oxygen storage of pome fruit. Such low oxygen atmospheres may further improve storage, but this needs to be shown to be of economic value.

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