ATO

Registration of 1-MCP treatments on Tentation apples

Report of experiments storage season 2002-2003

August 2003

Confidential

J.P.J. de Wild
E.C. Otma
T.R. Lammers
M.G. Staal

WAGENINGEN UR

ATO

Registration of 1-MCP treatments on Tentation apples

Report of experiments storage season 2002-2003

August 2003

Confidential

J.P.J. de Wild
E.C. Otma
T.R. Lammers
M.G. Staal

ATO B.V.
Agrotechnological Research Institute
Bornsesteeg 59
P.O. Box 17
6700 AA Wageningen
The Netherlands
Tel: +31.0317.475024
Fax: +31.317.475347

Contents page

Summary

Experiments on 1-methylcyclopropene (1-MCP, SmartFresh™) were carried out by ATO for AgroFresh Inc. and the Greenery to test the efficacy of 1-MCP on 'Tentation' apples in the storage season 2002-2003.
For conducting post-harvest treatments of agro-products, ATO has an official recognition to perform efficacy evaluation trials. This research was done following the rules of this recognition.

Apples were harvested at the optimum picking date and also 14 days later at a more mature stage. Treatments with 1-MCP started two days after harvest. Apples were treated with 312 and 625 ppb 1-MCP.

Subsequently apples were stored in normal air (cooling) or in ULO conditions (low O_2, high CO_2). Quality was determined after 2, 4 an 6 months of air storage and after 4, 6 and 8 months of ULO storage. A shelf-life period of 14 days was simulated after each storage.

Positive effects of 1-MCP were found on retention of firmness, colour and titratable acids and prevention of scald (normal harvest). There were no important effects of 1-MCP on soluble solids content. There were no clear differences between 312 and 625 ppb 1-MCP treatment of Tentation.

1-MCP can be advantageous for ULO (CA) storage.
Treatment with 1-MCP was only necessary for firmness retention of air-stored apples. Firmness loss of ULO-stored control apples was absent or very small, even during 14 days shelf-life. In many cases, 1-MCP resulted in greener apples and higher acidity. The disorder scald did not occur in ULO stored apples.

1-MCP can be used to replace ULO during 4 or 6 months storage period, but only for normal harvest.

1-MCP can facilitate both air and ULO storage of apples that are harvested at a more mature stage.
For the (extremely) late harvested Tentation, there was no big firmness loss of control apples. Application of 1-MCP resulted in apples of even better firmness and often greener colour and higher acidity.

1-MCP improves the shelf-life of ULO-stored apples: 1-MCP inhibited yellowing and led to a higher acidity.

1-MCP prevented development of scald in apples of normal harvest but 1-MCP could not prevent scald in (extremely) late harvested apples.

1 Introduction

To test the efficacy of 1-MCP on apples, experiments were performed on apple cultivar 'Tentation' in the storage season 2002-2003.
The following objectives were formulated before the start of the experiments:

- To reveal the efficacy of 1-MCP treatments on various quality aspects of CA (ULO) stored apples. In other words: does 1-MCP improve CA storage?
- To reveal the efficacy of 1-MCP treatments on various quality aspects of apples in comparison to CA (ULO) storage. In other words: can 1-MCP be used to replace CA under certain conditions?
- Does 1-MCP facilitate the storage of apples harvested at a more mature stage?
- Does 1-MCP improve the shelf-life of CA(ULO)-stored apples?
- Does 1-MCP inhibit development of scald in Tentation apples?

To find an answer to these questions, apples were harvested at the optimum picking date and also 14 days later at a more mature stage. The treatments of 1-MCP started 2 days after harvest. Subsequently apples were stored in normal air (only cooling) or in ULO conditions (low O_2, high CO_2). Quality of Tentation apples was determined after 2, 4, 6 and 8 months of storage and after a simulated shelf-life period of 14 days. The experiments were done following the rules of formal recognition.

2 Formal recognition

For conducting post-harvest treatments of agro-products, ATO has an official recognition to perform efficacy evaluation trials. This research was done following the rules of this recognition.

3 Materials and methods

Harvest
Apples were harvested at their optimal harvest date (commercial advice) and also 14 days after the optimal harvest date. Harvest took place on September 30th and October 14th. Apples were picked from trees that had not been strip-picked before. For the late harvest of Tentation this was an uncommon situation. Tentation was from an orchard in Tholen, province Zeeland, The Netherlands (south-west). After harvest, apples were placed in a temperature controlled room at 1.0 °C.

Sorting and randomisation
1 or 2 days after harvest, apples were sorted and randomised. Apples that were damaged or very small or very large were removed.

1-MCP treatment
Apples were treated during 24 hours with 312 or 625 ppb 1-MCP at 1.0 °C. Treatments started two days after harvest. Treatment dates are given in table 1. Also control apples (0 ppb 1-MCP) were included in the experiments.

Table 1. Harvest dates, 1-MCP treatment dates and start of air/ULO storage for Tentation (all dates were in year 2002).

	Harvest date	Treatment date	Start air or ULO storage
Tentation normal harvest	Sept 30th	Oct 2nd	Oct 4th
Tentation late harvest	Oct 14th	Oct 16th	Oct 18th

To generate 1-MCP levels around the apples, 1-MCP (0.14%) as provided by AgroFresh Inc. was used. The 1-MCP treatments were performed in plastic covers (1.23 m^3 free volume, 100-110 kg apples). In this system airtight plastic is used to cover the fruits which are placed in crates on a pallet. Lime (±10 litre) was placed in each cover to prevent CO_2 accumulation. There was no significant decline in O_2 or rise in CO_2 during treatments. Also in each cover a closed bottle with the desired amount of dissolved 1-MCP was placed. Covers were closed tightly at the top end by a rope and tape. The air inside the covers was circulated by a pump.
1-MCP treatments started by opening the bottles. The flexibility of the cover allowed opening of the bottles from outside the cover.
Control apples were placed in a separate room to prevent any possible contact with 1-MCP.

1-MCP concentration and preparation
The active ingredient of 1-MCP is 0.14%. The free volume inside each pallistore cover was calculated to be 1.23 m^3. The following solutions were made:
- 312 ppb 1-MCP: 0.62 g powder + 98 ml water (ratio product:water 160)
- 625 ppb 1-MCP: 1.23 g powder + 197 ml water (ratio product:water 160)

For preparing each solution, a bottle (1 l) was filled with the correct amount of powder. The flask was closed with a lid containing a septum. Air was drawn from the closed flask with a syringe. The air volume taken out was equal to the volume of water that was added subsequently. The water (demineralised, ± 20 °C) was added with a syringe. The lid was then wrapped with parafilm. The solution was shaken by hand several times until all the powder had dissolved.

Storage conditions
During and after the 1-MCP treatment all apples were kept under ambient air at 1 °C for 1-2 days. Subsequently part of the apples were stored under ambient air (cooling) and part of the apples under standard CA conditions (ULO, 1.2% O_2 and 2.0% CO_2). Dates are given in table 1. Relative humidity during storage was 95-100%. Temperature, relative humidity and ULO conditions were comparable to standard Dutch storage conditions.
Gas conditions were monitored every hour. All apples were stored in containers. Within each container, apples from the same cultivar and harvest date were stored. In order to expose apples from different 1-MCP treatments to equal temperature/gas conditions and to reach 2 replicates, apples from the different 1-MCP treatments were stored together in the containers. The number of replicates was 2 (containers).

Quality measurements
Initial quality of apples was measured within two days after harvest. Initial quality of each harvest was measured on 2 samples of 20 apples.
After storage, apples were sampled for quality measurements. A sample consisted of 20 apples. Again 2 samples (replicates) of 20 apples were measured from each treatment. Assessment dates are given in Table 2.

Table 2. Assessment dates for Tentation directly after storage and after storage plus shelf-life.

Storage duration	Tentation cooling	Tentation ULO
2 months	Dec 3th 2002	
2 months + shelf-life	Dec 17th 2002	
4 months	Feb 3th 2003	Feb 3th 2003
4 months + shelf-life	Feb 17th 2003	Feb 17th 2003
6 months	April 1th 2003	April 1th 2003
6 months + shelf-life	April 15th 2003	April 15th 2003
8 months		May 27th 2003
8 months + shelf-life		June 10th 2003

Directly after storage, apples were measured on firmness, rot and scald. After a simulated distribution period of 14 days, measurements on more quality aspects were performed.
The simulation was performed in a temperature controlled room at 18 °C and 75-80% RH. Measurements were done on firmness (individual apples), colour (individual apples), content of soluble solids (sugars, mixed sample), titratable acidity (mixed sample), and external and internal disorders. Firmness was measured with a fruit texture analyser (Güss, electronic measuring system). Colour was measured visually using a colour chart (Unifruco Research Services LTD/Agricura) with a scale from 0.5 to 5 where 0.5=green and 5=yellow. The content of soluble solids was measured with a digital refracto-meter (ATAGO, PR-1 brix-meter). Titratable acidity was analysed with an automatic titrator using 0.1 M NaOH. Rot included core fungus, rot, and stalk rot.

Extra origins
To study the effect of 1-MCP on scald, late harvested apples of two extra origins were included in the experiments.

Statistical analysis
Data were analysed statistically without transformation. Different treatments were analysed for significant differences by analysis of variance (ANOVA) with the statistical package Genstat. When significant differences were found, comparisons between pairs of data were made using the least significant differences between means (LSD) at a significance level of 95%. ANOVA was not performed for % apples with disorders, as residuals were not normally distributed.

Summary of experimental set-up

Apple variety:	Tentation
Harvest dates:	optimal, late
Treatment:	24 h. at 1.0 °C
1-MCP concentrations:	0, 312 and 625 ppb, starting 2 days after harvest
Storage gas conditions:	ambient air, ULO
Storage temperature:	1.0 °C
Sampling dates:	2, 4, 6 months for air storage 4, 6, 8 months for ULO
Measurement dates:	0, 14 (days after storage)
Measurements:	firmness, colour, sugars, acidity, external and internal disorders
Apples per measurement:	40 (2 replicates * 20 apples)

4. Results Tentation

4.1 Tentation, initial quality

The mean firmness of Tentation at the start of the experiments was 69.7±0.9 N (mean ± standard deviation) for normal harvest and 66.1±0.1 N for late harvest. The mean colour was 2.7±0.2 and 3.4±0.1 respectively. The mean content of soluble solids was 13.2±0.1 and 14.4±0.5 °brix respectively. The mean % titratable acidity was 1.20±0.06 and 0.94±0.02 respectively.

4.2 Tentation, 2 months storage

Firmness of control apples (0 ppb 1-MCP) of normal harvest had decreased during 2 months air storage (Fig. 1A). Treatment with 1-MCP completely prevented this decrease and also prevented firmness loss during 14 days shelf-life. The apparent increase in firmness during shelf-life is probably the consequence of some dehydration of the apples.
While firmness at harvest was 69.7±0.9 N, the firmness after 2 months air was 64.6±0.4, 70.4±0.3 and 70.1±0.8 for control, 312 ppb and 625 ppb 1-MCP respectively.

Also firmness loss of late harvested apples during air storage and subsequent shelf-life was completely prevented by 1-MCP (Fig. 1B).
Firmness at harvest was 66.1±0.1 N, firmness after storage was 61.9±0.6, 65.4±1.4 and 66.7±0.7 N for control, 312 ppb and 625 ppb 1-MCP respectively.

Yellowing was inhibited by 1-MCP for normal harvest (Fig. 2).

There were no clear effects of 1-MCP on soluble solids content (Fig. 3).

In general, 1-MCP treatments resulted in higher titratable acidity (Fig. 4).

There were no clear effects of 1-MCP on % apples with rot (Fig. 5).

No scald had developed during storage (0 days shelf-life) (Fig. 6). After 14 days shelf-life, scald was found in both normal and late harvested apples. 1-MCP treatment could only prevent scald development of normal harvest.

In general there were no clear differences between 312 ppb and 625 ppb 1-MCP treatments.

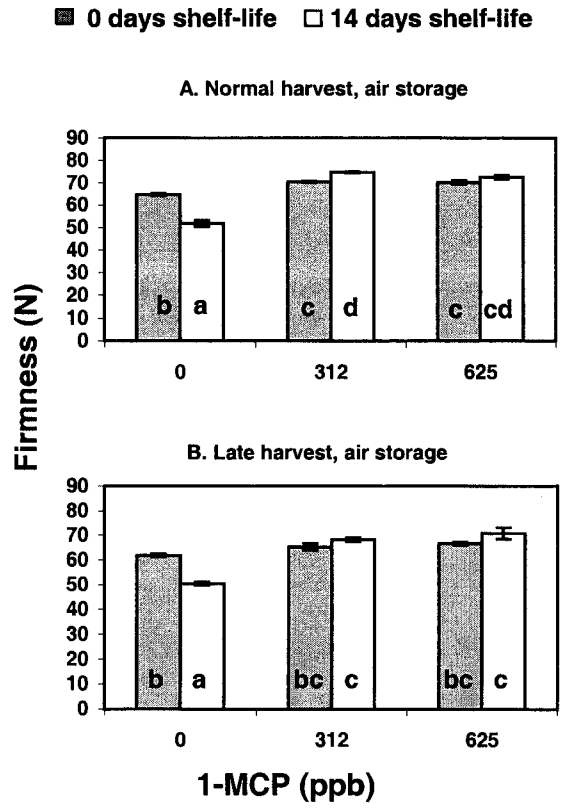

Fig. 1. Firmness of Tentation after 2 months of storage. Vertical bars indicate ± std of the mean of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

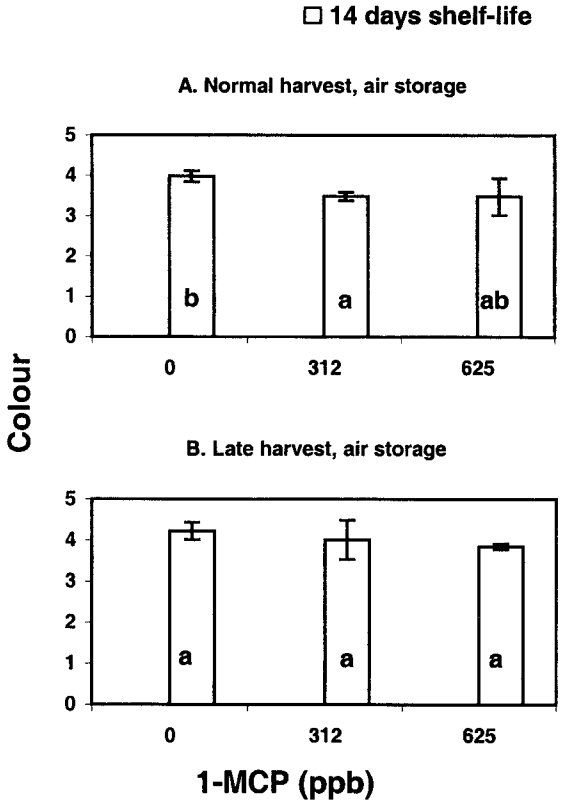

Fig. 2. Colour of Tentation after 2 months of storage. Vertical bars indicate ± std of the mean of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

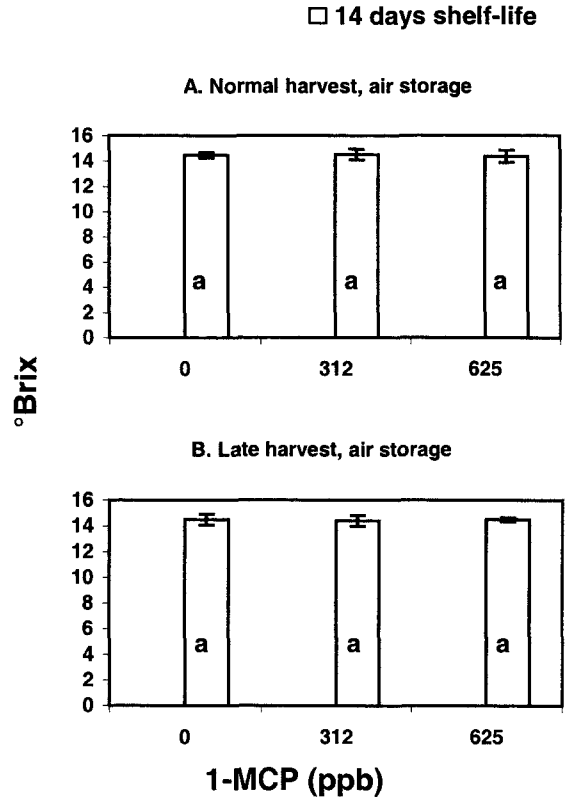

Fig. 3. Soluble solids content of Tentation after 2 months storage. Vertical bars indicate ± std of the mean of 2 replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

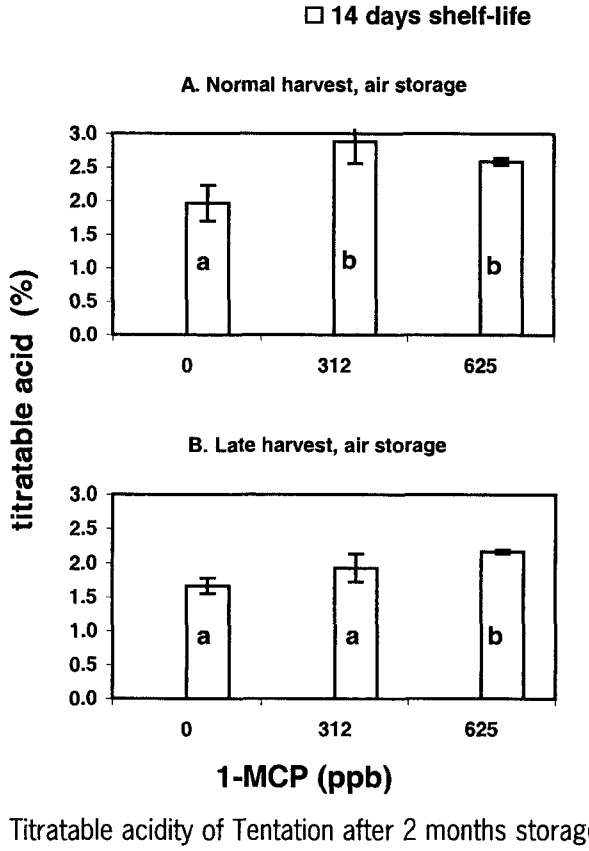

Fig. 4. Titratable acidity of Tentation after 2 months storage. Vertical bars indicate ± std of the mean of 2 replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

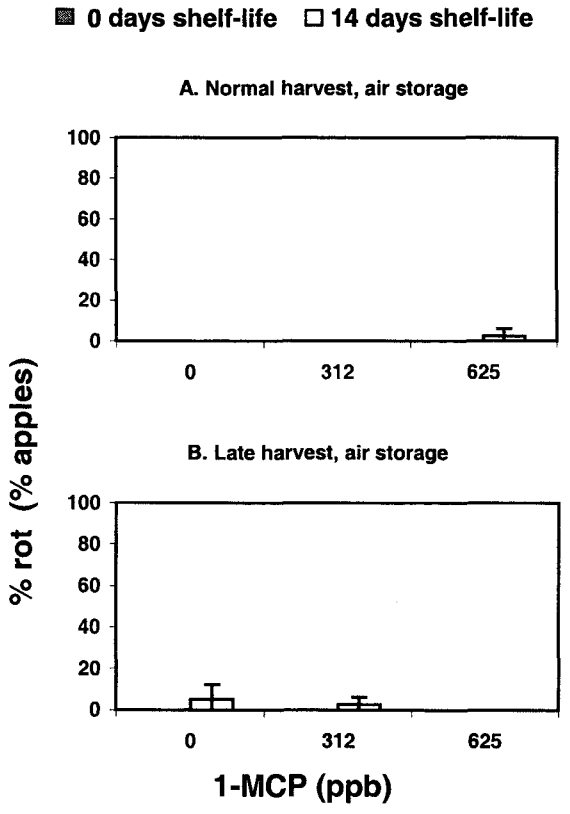

Fig. 5. % apples with rot of Tentation after 2 months storage. Vertical bars indicate ± std of the mean of 2 replicates.

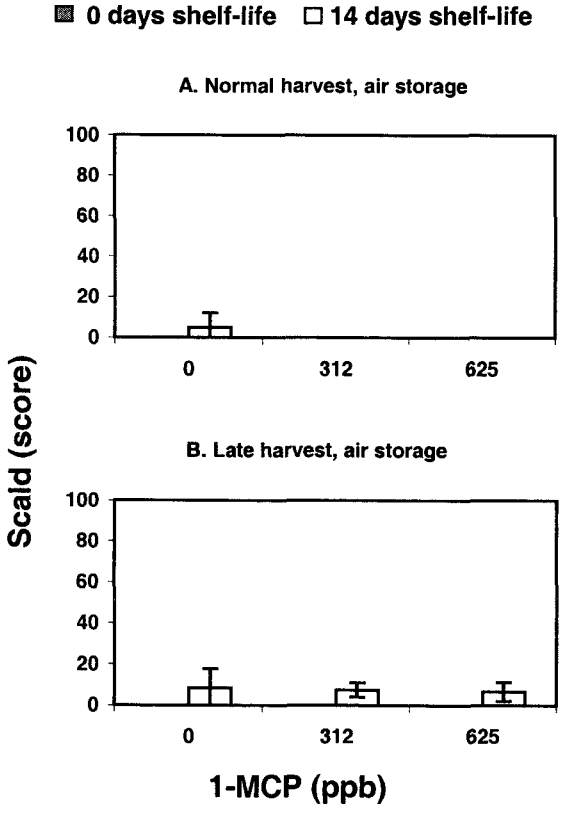

Fig. 6. Index for scald (% of maximum) of Tentation after 2 months storage. Vertical bars indicate ± std of the mean of 2 replicates.

4.3 Tentation, 4 months storage

Firmness loss of normal harvested apples, during air storage and subsequent shelf-life, was completely prevented by 1-MCP (Fig. 7A). Firmness of 1-MCP treated apples after air storage (0 or 14 days shelf-life) was equal to firmness of control apples after ULO (Fig. 7C).
While firmness at harvest was 69.7±0.9 N, the firmness after 4 months air was 57.8±1.4, 71.3±0.2 and 70.9±0.2 for control, 312 ppb and 625 ppb 1-MCP respectively.

Also firmness loss of late harvested apples during air storage and subsequent shelf-life was completely prevented by 1-MCP (Fig. 7B). Firmness of 1-MCP treated apples after air storage + 14 days shelf-life was better than firmness of control apples after ULO + 14 days shelf-life (Fig. 7D).
Firmness at harvest was 66.1±0.1 N, firmness after storage was 56.6±0.3, 66.3±0.3 and 66.8±0.8 N for control, 312 ppb and 625 ppb 1-MCP respectively.

Firmness loss during ULO and subsequent shelf-life was very small (Fig. 7C and 7D). An effect of 1-MCP was only found for shelf-life of late harvest (Fig. 7 D).

1-MCP treatments inhibited yellowing for normal harvest after air storage and for late harvest after ULO (Fig. 8).

There was no important effect of 1-MCP on soluble solids content (Fig. 9).

In all cases 1-MCP treatments resulted in higher titratable acidity (Fig. 10).

There were no clear effects of 1-MCP on development of rot (Fig. 11).

Scald was only found for air storage, not for ULO. For normal harvest, severe scald developed during shelf-life which was prevented by 1-MCP (Fig 12A). For late harvest (which was extremely late), scald already developed during storage (0 days shelf-life) which was promoted by 1-MCP. However after 14 days of shelf-life, 1-MCP treatment resulted in less scald than the control (Fig 12B).

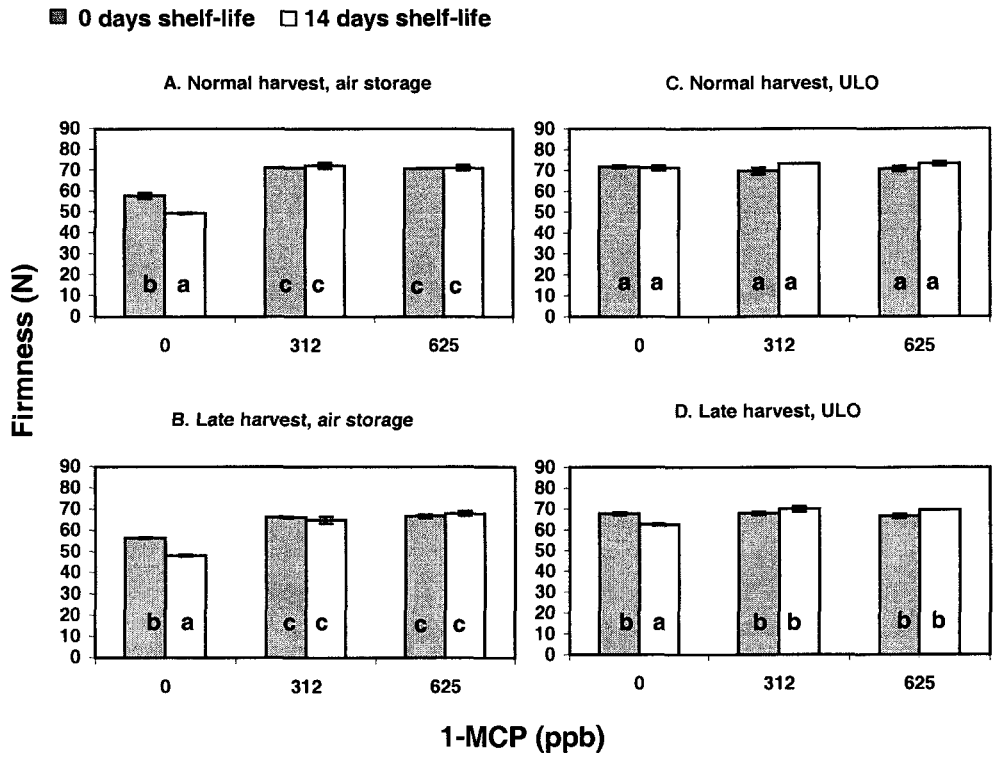

Fig. 7. Firmness of Tentation after 4 months of storage. Vertical bars indicate ± std of the mean of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

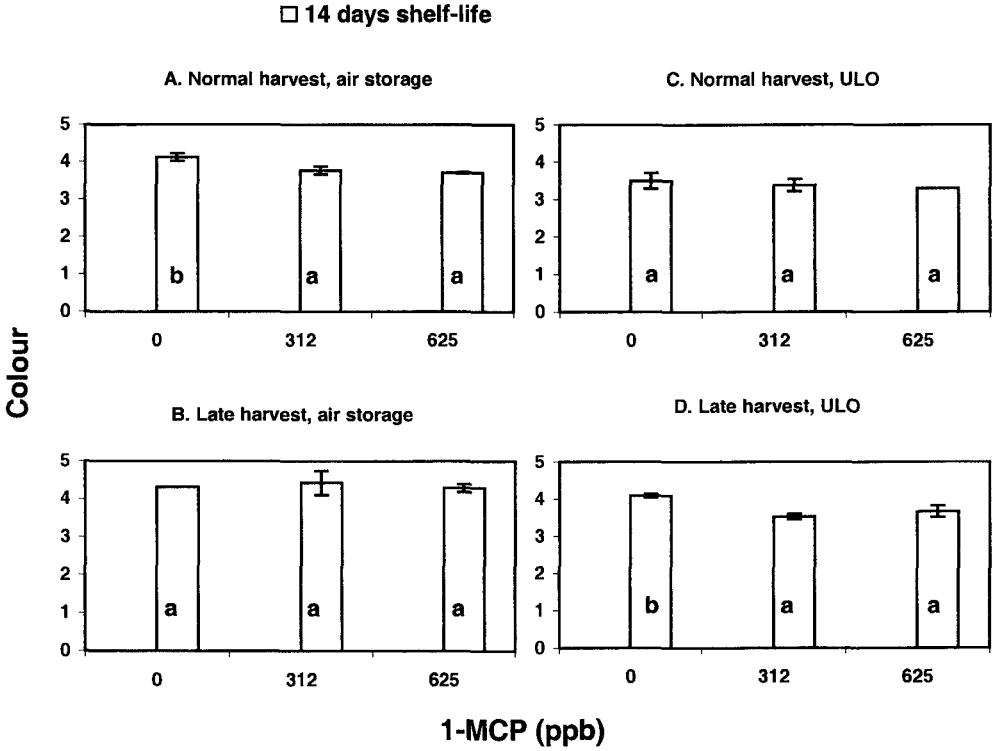

Fig. 8. Colour of Tentation after 4 months of storage. Vertical bars indicate ± std of the mean of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

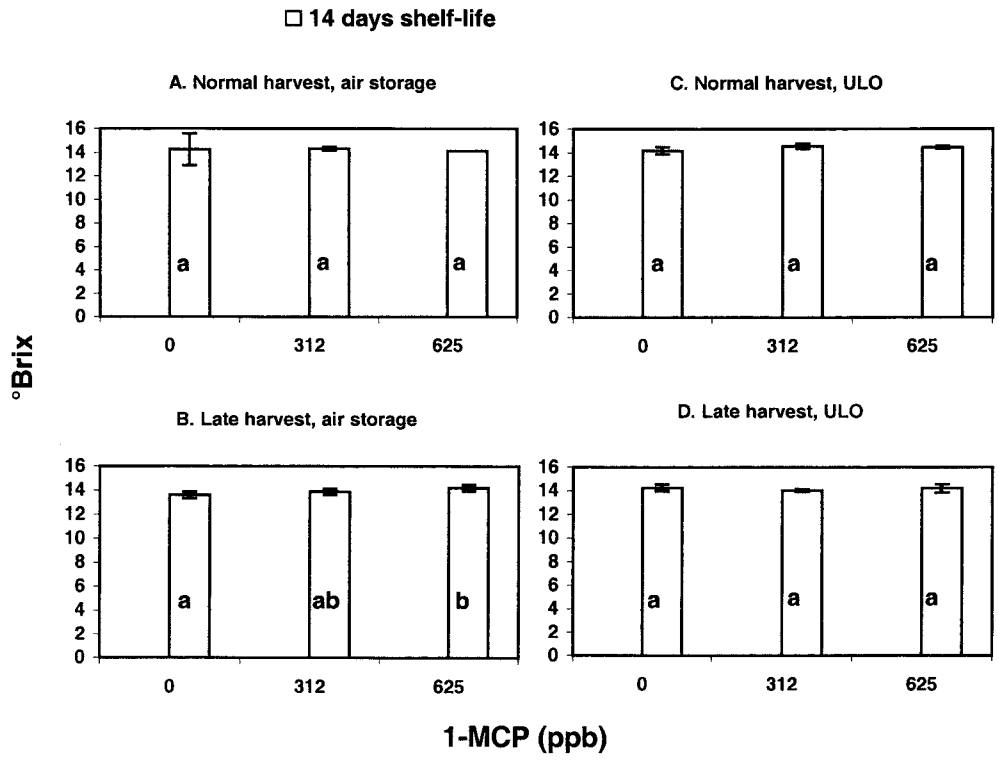

Fig. 9. Soluble solids content of Tentation after 4 months storage. Vertical bars indicate ± std of the mean of 2 replicates Columns within a chart marked with a same letter are not statistically different (P=0.05).

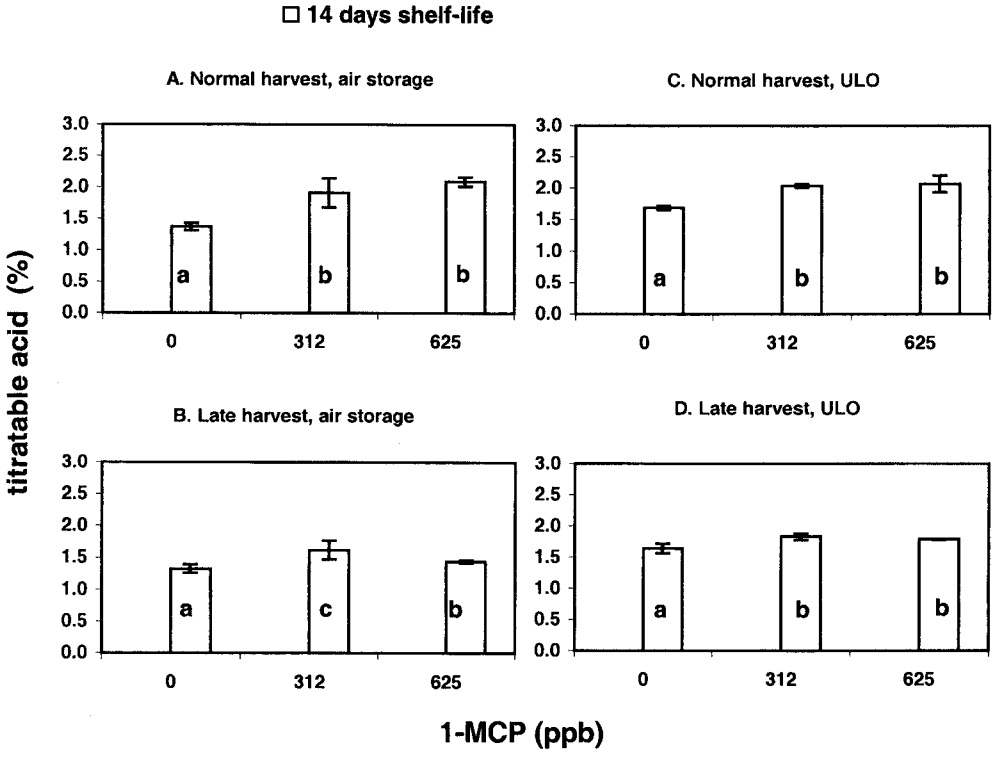

Fig. 10. Titratable acidity of Tentation after 4 months storage. Vertical bars indicate ± std of the mean of 2 replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

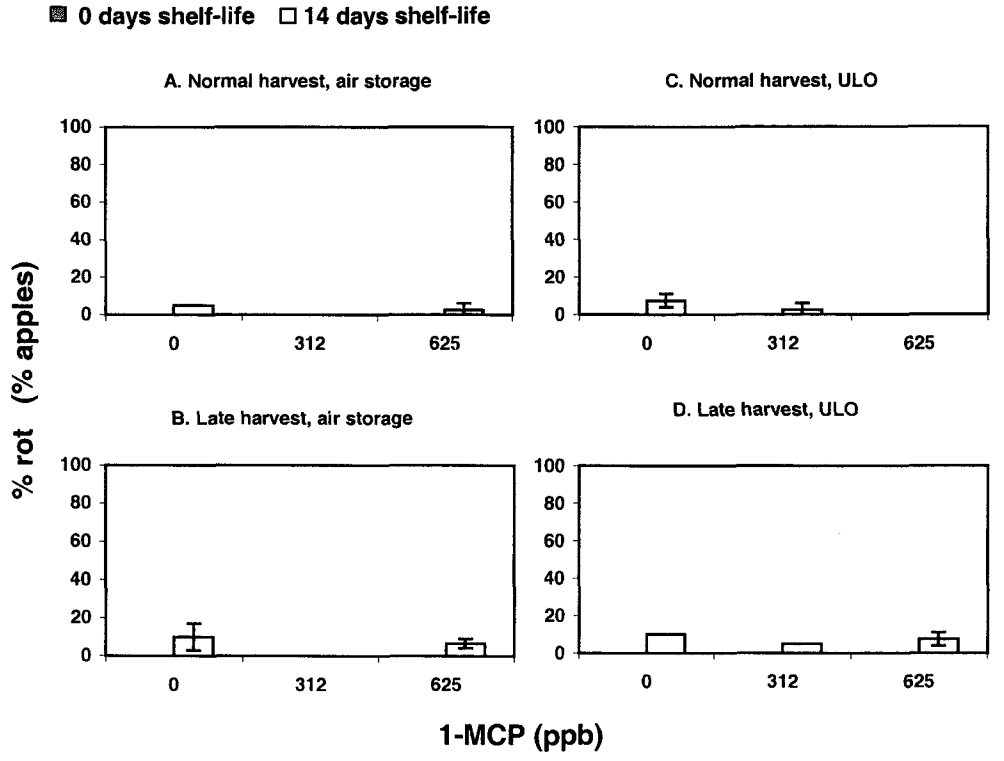

Fig. 11. % apples with rot of Tentation after 4 months storage. Vertical bars indicate ± std of the mean of 2 replicates.

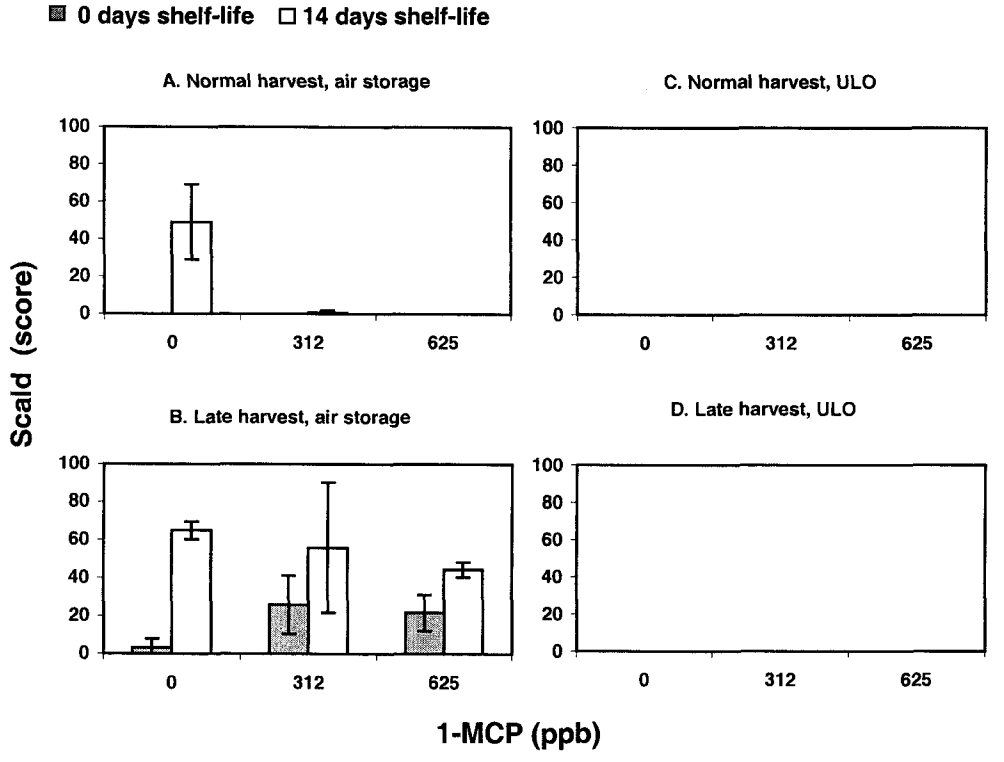

Fig. 12. Index for scald (% of maximum) of Tentation after 4 months storage. Vertical bars indicate ± std of the mean of 2 replicates.

4.4 Tentation, 6 months storage

Apples of late harvest developed severe rot after 6 months air storage. For these apples, quality parameters other than % rot and scald are not given.

Firmness loss of normal harvested apples, during air storage and subsequent shelf-life, was completely prevented by 1-MCP (Fig. 13A). Firmness of 1-MCP treated apples after air storage (0 or 14 days shelf-life) was equal to firmness of control apples after ULO (Fig. 13C)
While firmness at harvest was 69.7±0.9 N, the firmness after 6 months air was 53.4±0.2, 70.8±0.8 and 70.7±0.7 for control, 312 ppb and 625 ppb 1-MCP respectively.

Firmness loss during ULO and subsequent shelf-life was very small, 1-MCP was not necessary for firmness retention (Fig. 13C and 13D).

Yellowing was inhibited by 1-MCP in all cases (Fig. 14).

1-MCP caused higher soluble solids content in some cases but effects were small (Fig. 15).

1-MCP treatments resulted in higher titratable acidity for normal harvest after air storage and for late harvest after ULO (Fig. 16).

For air storage of normal harvest, 1-MCP inhibited the development of rot during shelf-life (Fig. 17A) which was related to inhibition of scald (Fig. 18A).
For air storage of late harvest severe rot occurred (Fig. 17) which was also related to scald (Fig. 18B). In this case there was no effect of 1-MCP.
Less rot developed after ULO storage (Fig. 17C and 17D). There was no clear effect of 1-MCP.
Scald was only found for air storage, not for ULO (Fig. 18C and 18D).

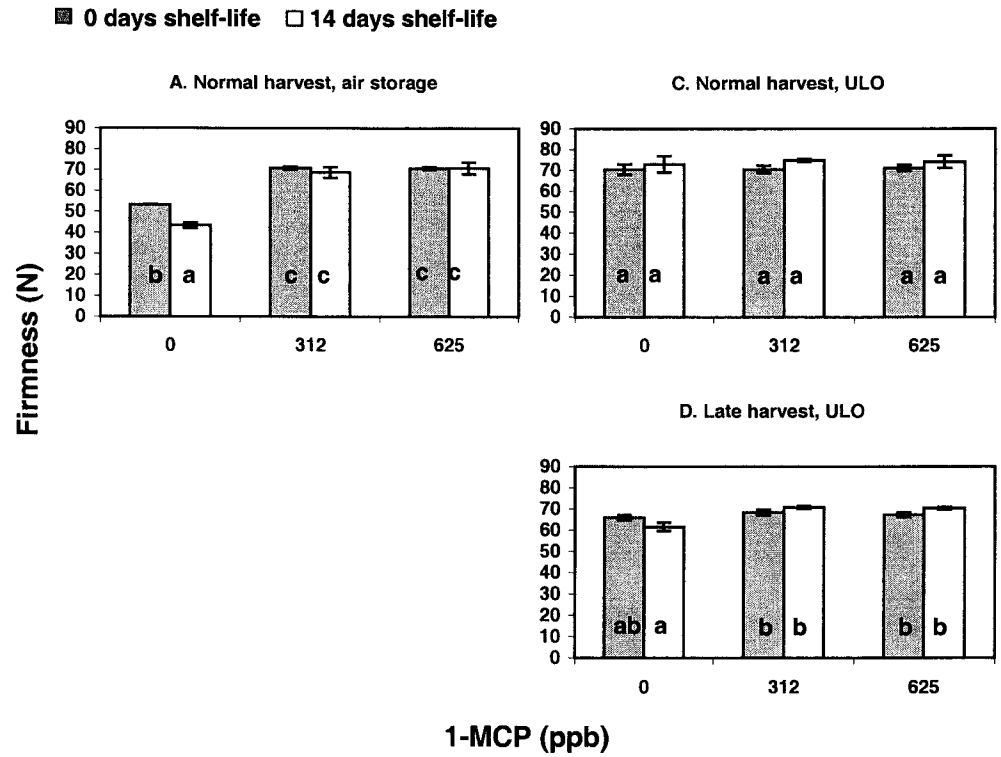

Fig. 13. Firmness of Tentation after 6 months of storage. Vertical bars indicate ± std of the mean of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

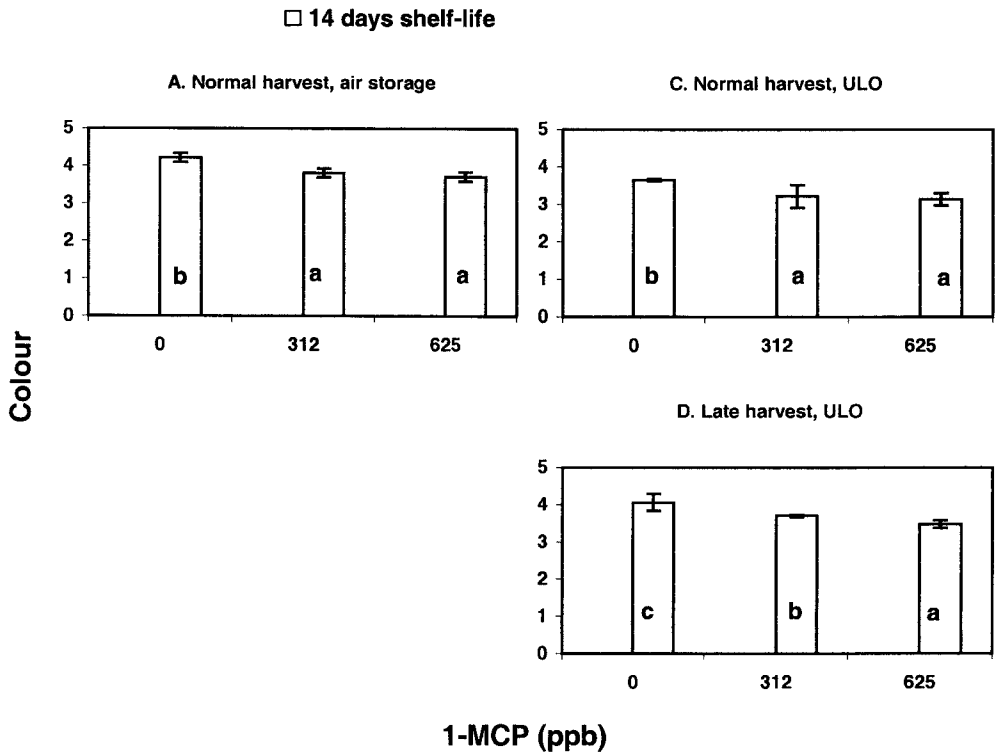

Fig. 14. Colour of Tentation after 6 months of storage. Vertical bars indicate ± std of the mean of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

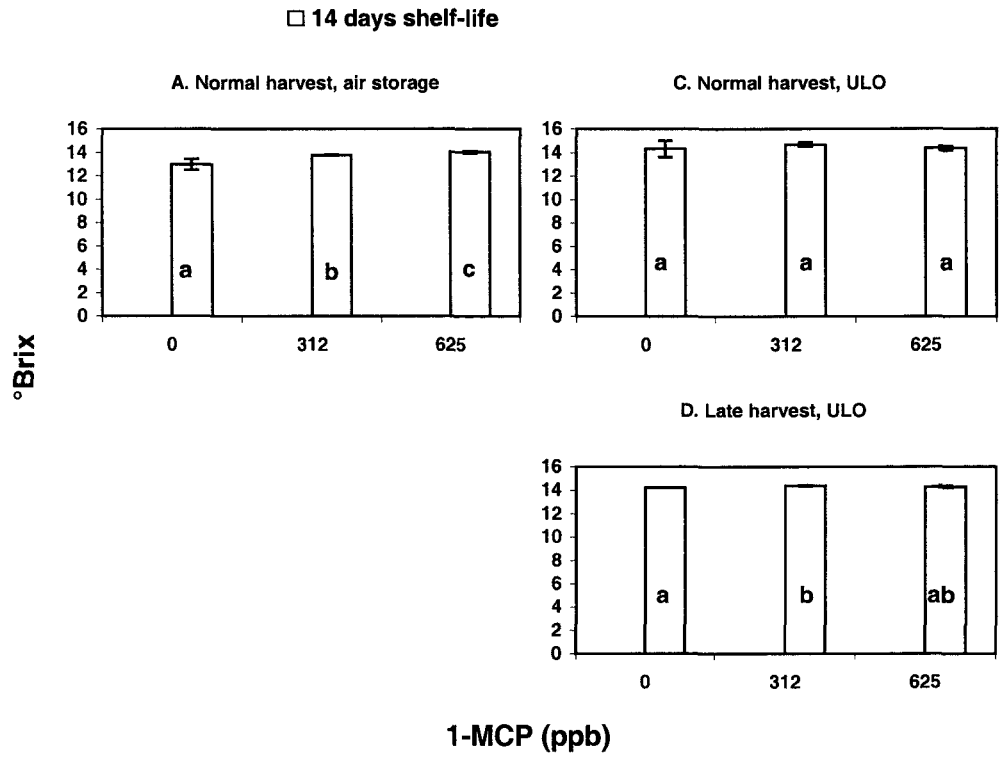

Fig. 15. Soluble solids content of Tentation after 6 months storage. Vertical bars indicate ± std of the mean of 2 replicates Columns within a chart marked with a same letter are not statistically different (P=0.05).

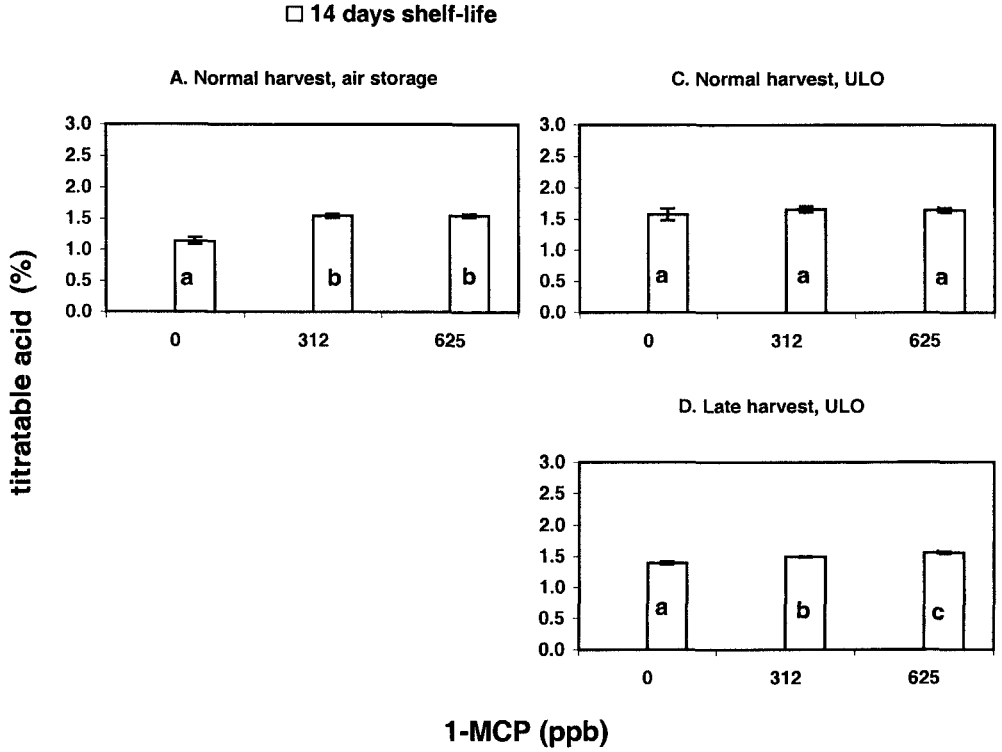

Fig. 16. Titratable acidity of Tentation after 6 months storage. Vertical bars indicate ± std of the mean of 2 replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

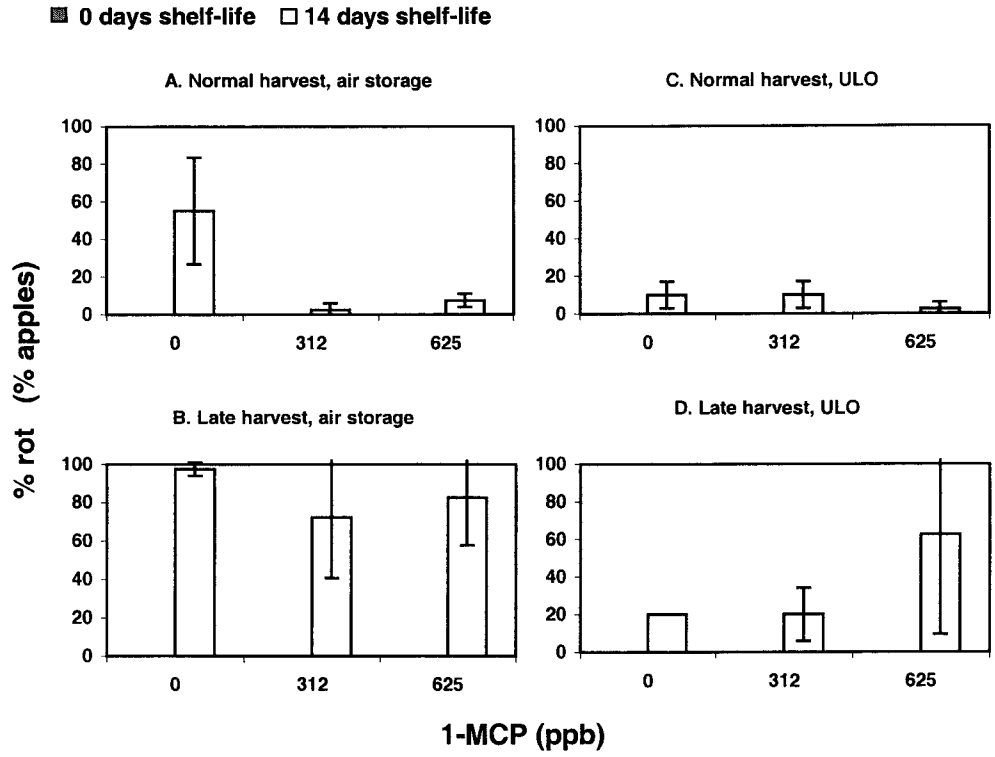

Fig. 17. % apples with rot of Tentation after 6 months storage. Vertical bars indicate ± std of the mean of 2 replicates.

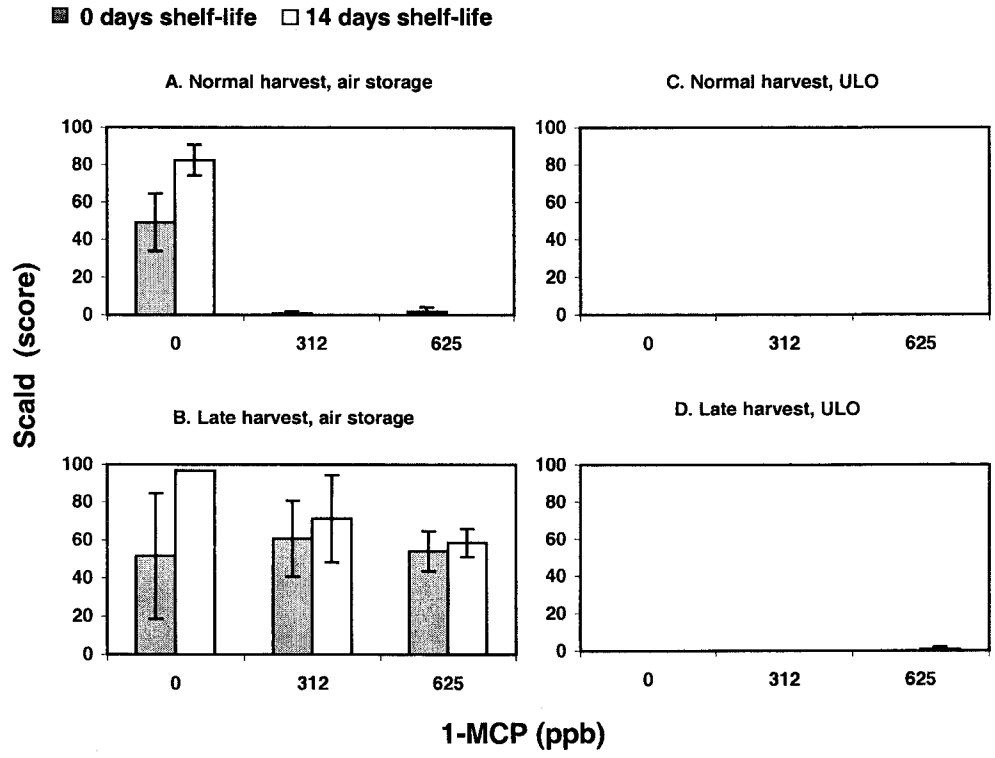

Fig. 18. Index for scald (% of maximum) of Tentation after 6 months storage. Vertical bars indicate ± std of the mean of 2 replicates.

4.5 Tentation, 8 months storage

Control apples did not show firmness loss during ULO and subsequent shelf-life, 1-MCP was not necessary for firmness retention (Fig. 19).
While firmness at harvest was 69.7±0.9 N, the firmness after 8 months ULO was 70.9±1.2, 71.6±0.5 and 72.2±1.0 for control, 312 ppb and 625 ppb 1-MCP respectively.

Yellowing was inhibited by 1-MCP, however not always statistically significant (Fig. 20).

In contrast to 6 months storage, 1-MCP caused lower soluble solids content in some cases, but again effects were small (Fig. 21).

1-MCP treatments resulted in higher titratable acidity (Fig. 22).

There were no clear effects of 1-MCP on rot development (Fig. 23).

Scald was only found scarcely for late harvest, which was not prevented by 1-MCP (Fig. 24).

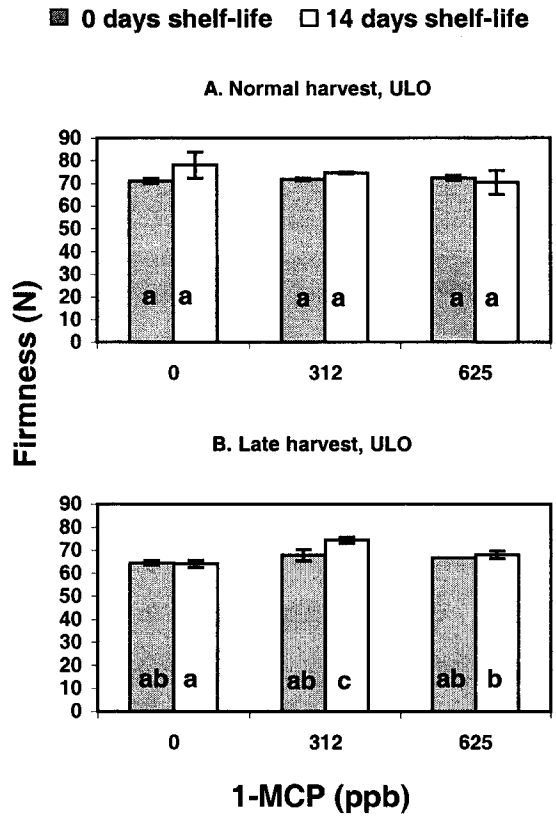

Fig. 19. Firmness of Tentation after 8 months of storage. Vertical bars indicate ± std of the mean of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

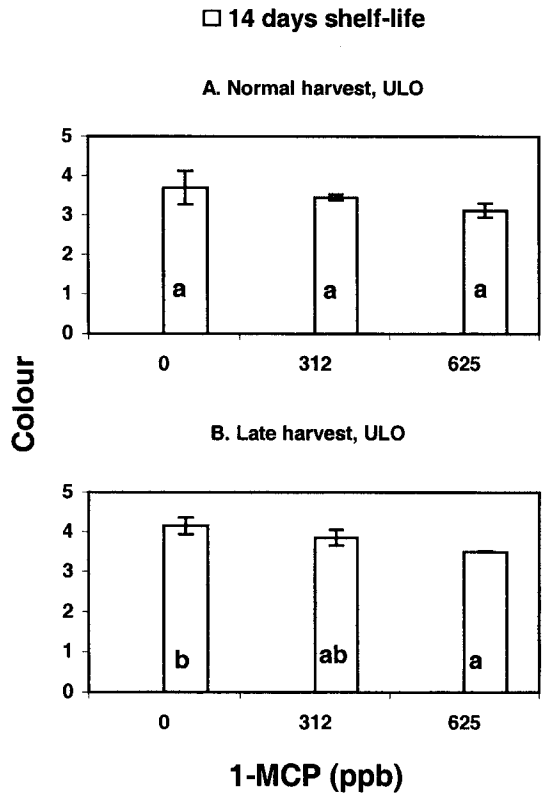

Fig. 20. Colour of Tentation after 8 months of storage. Vertical bars indicate ± std of the mean of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

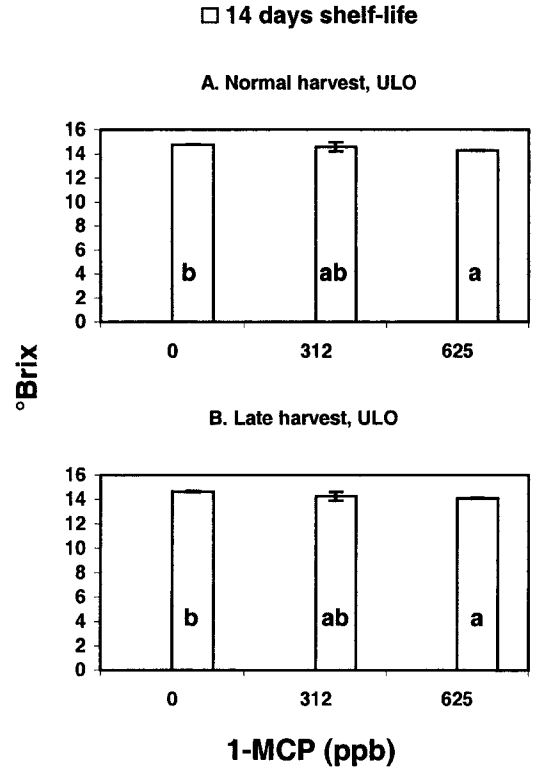

Fig. 21. Soluble solids content of Tentation after 8 months of storage. Vertical bars indicate ± std of the mean of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

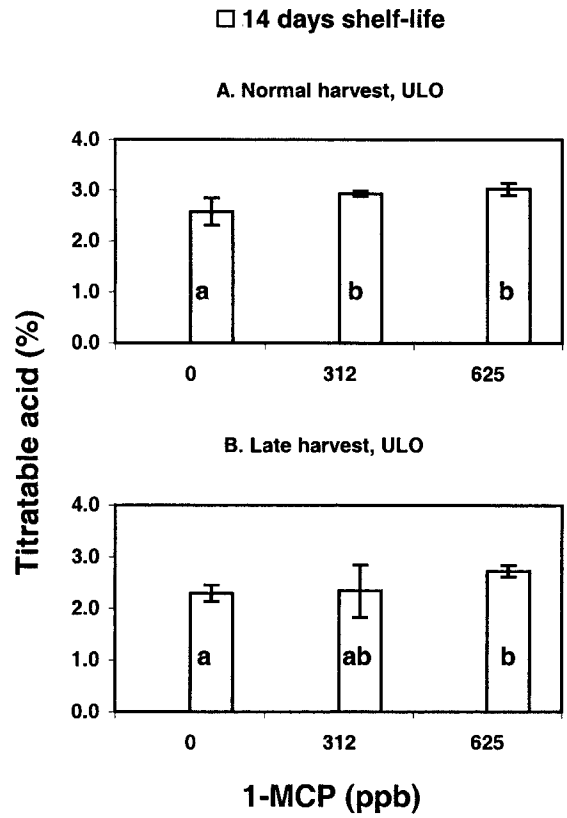

Fig. 22. Titratable acidity of Tentation after 8 months of storage. Vertical bars indicate ± std of the mean of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

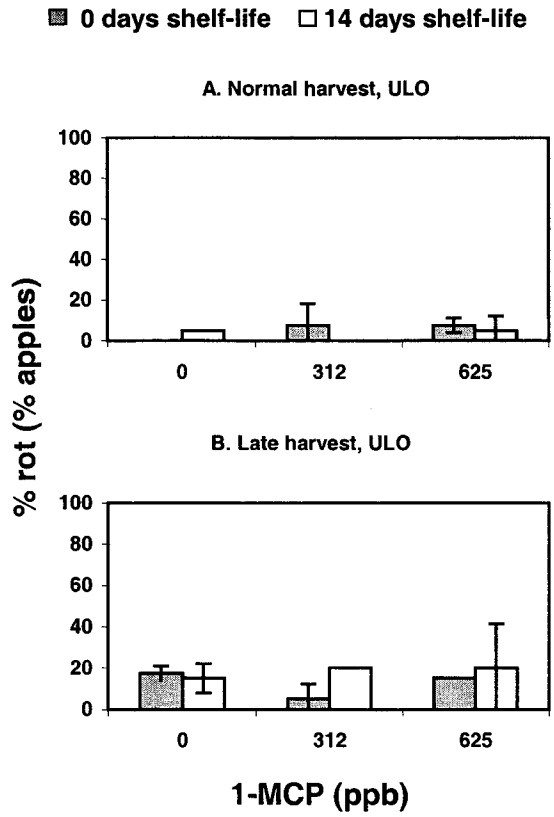

Fig. 23. % apples with rot of Tentation after 8 months of storage. Vertical bars indicate ± std of the mean of two replicates.

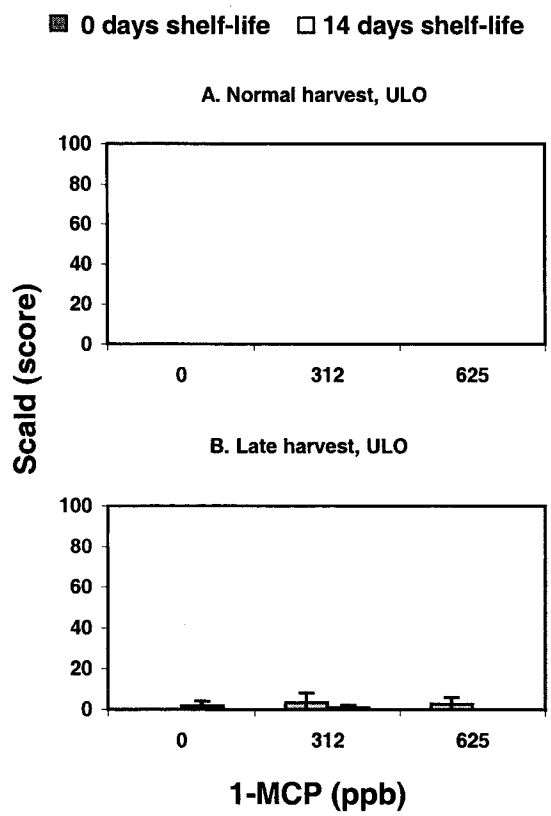

Fig. 24. Index for scald (% of maximum) of Tentation after 8 months of storage. Vertical bars indicate ± std of the mean of two replicates.

5 Tentation, extra origins

For 2 extra origins, Tentation apples of extreme late harvest were stored. These apples were picked ±14 days after the normal harvest date from trees that had not been strip-picked before. In practice, this is an uncommon situation. Assessments were done after 6 months storage (Fig. 25) and after 8 months storage (Fig. 26).
As shown in the main experiment, 1-MCP could not prevent the development of scald in these (extreme) late picked apples.

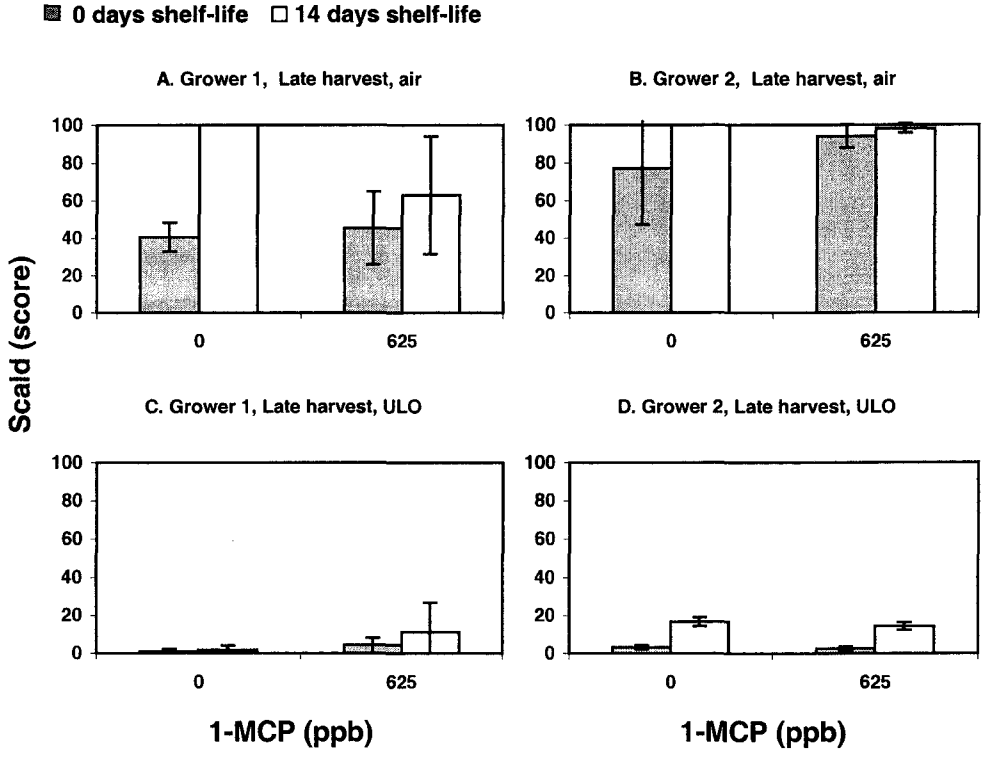

Fig. 25. Index for scald (% of maximum) of late harvested Tentation after 6 months of storage for 2 different growers. Vertical bars indicate ± std of the mean of two replicates.

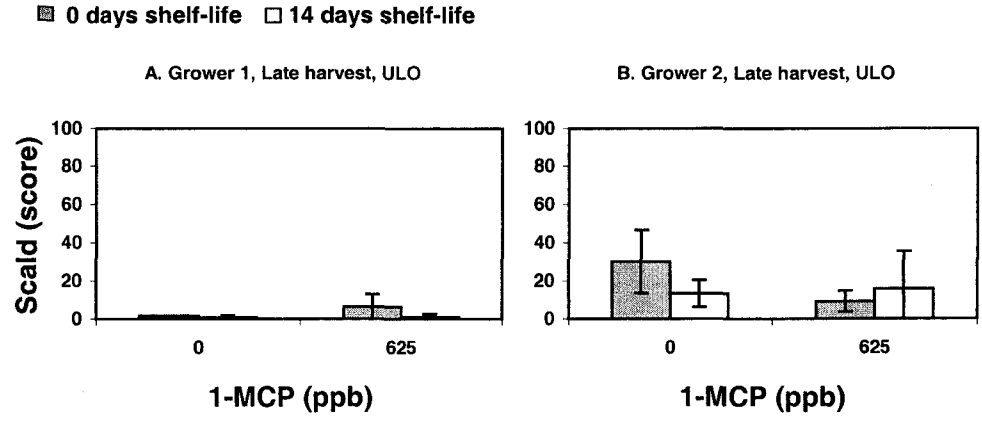

Fig. 26. Index for scald (% of maximum) of late harvested Tentation after 8 months of storage for 2 different growers. Vertical bars indicate ± std of the mean of two replicates.

6 Conclusions

1-MCP was only necessary for firmness retention during air storage but not for ULO storage. Positive effects were found on retention of colour, retention of titratable acids and prevention of scald (normal harvest). There were no important effects of 1-MCP on soluble solids content.
Both 312 and 625 ppb 1-MCP were tested, but there were no important differences between these concentrations.

Conclusions on basis of the five objectives as formulated at the start of the experiments:

- ***1-MCP can improve CA (ULO) storage***
  Firmness loss of Tentation apples during ULO storage and subsequent shelf-life was small. In this case 1-MCP was not necessary for firmness retention. In many cases, 1-MCP resulted in greener apples and higher acidity. There were no important effects of 1-MCP on soluble solids content or % rot. The disorder scald did not occur in ULO stored apples.
- ***1-MCP can be used to replace CA (ULO) under certain conditions***
  1-MCP could be used to replace ULO during 4 and 6 months storage, but only for normal harvest. After storage + 14 days shelf-life, the firmness of 1-MCP treated apples from air storage was equal to the firmness of untreated apples from ULO. For the (extremely) late harvest, results obtained by ULO were better than for 1-MCP without ULO. This was due to the occurrence of scald (and consequently rot) which was prevented by ULO but not by 1-MCP.
- ***1-MCP facilitates the storage of apples harvested at a more mature stage***
  Air storage:
  Firmness of control apples of late harvest was still acceptable after 4 months storage plus shelf-life, but application of 1-MCP resulted in apples of even better firmness. 1-MCP inhibited yellowing and led to a higher acidity. However, 1-MCP could not prevent the development of scald which was shown for apples of 3 origins.
  ULO storage:
  Firmness of control apples of late harvest was still acceptable after 8 months storage plus shelf-life, but 1-MCP resulted in apples of even better firmness, greener colour and higher acidity.
- **1-MCP improves the shelf-life of CA(ULO)-stored apples**
  Firmness loss during shelf-life was not a problem. 1-MCP inhibited yellowing and led to a higher acidity.
- **1-MCP inhibits development of scald**
  In apples of normal harvest, scald only occurred in air storage. In this case, 1-MCP prevented the development of scald. 1-MCP could not prevent scald in (extremely) late harvested apples.