Final Scientific Report**BARD Project Number:** IS-4181-08**Date of Submission of Report:** 1.1.2013**Project Title:** Application of pre-storage short anaerobiosis to alleviate superficial scald and bitter pit in Granny Smith apples**Investigators:**

Position in Proposal	Name	affiliated institution
1) Principal Investigator (PI)	Edna Pesis	Dept. of Postharvest Science of Fresh Produce, the Volcani Center, ARO, ISRAEL
2) co-PI	Elizabeth J Mitcham	Dept. of Plant Sciences, UC Davis, CA 95616, USA
3) Collaborating	Susan E Ebeler	Dept. of Viticulture and Enology, UC Davis, USA
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Keywords: low oxygen; ethylene; α -farnesene; reactive oxygen species; aroma volatiles**Abbreviations:** LO₂, low oxygen; MHO, 6-methyl-5-heptene-2-one; ROS, reactive oxygen species,**Budget:** IS: \$ 150,000

US: \$ 150,000

Total: \$ 300,000

Signature**Principal Investigator (PI)
Institution**

Signature**Institution's Authorizing Official, Principal**

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1		1	2
Submitted, in review, in preparation	1		3	4
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts	3		3	6
Not refereed (proceedings, reports, etc.)	3	3	2	7

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Revital Sabban-Amin – Volcani, Israel

Andrew Macnish – Davis, CA

Malkeet Padda – Davis, CA, 662-07-2060

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		2	1	3
Longer Visits (Sabbaticals)				

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

There is increased demand for high quality fruit produced and marketed with reduced chemical inputs to minimize toxic effects on human health and the environment. Granny Smith (GS) apple quality is reduced by two major physiological disorders, superficial scald and bitter pit (BP). These disorders cause great loss to apple growers worldwide. Superficial scald is commonly controlled by chemical treatments, mainly the antioxidant diphenylamine (DPA) and/or the ethylene action inhibitor, 1-methylcyclopropene (1-MCP). Both chemicals are ineffective in controlling bitter pit incidence. We proposed to investigate the beneficial use of non-chemical, abiotic stress with low O₂ (LO2) applied for 10d at 20°C on GS apple fruit. During the project we expanded the treatment to more apple cultivars, Golden Delicious (GD) and Starking Delicious (SD) and another pome fruit, the pear. Apple and pear have similar physiological disorders that develop during cold storage and we examined if the LO2 treatment would also be effective on pear. Application of 0.5% LO2 atmosphere for 10d at 20°C or 500ppb 1-MCP at 20°C prior to cold storage at 0°C, was effective in reducing superficial scald in GS apple. Moreover, LO2 pretreatment was also effective in reducing bitter pit (BP) development in California GS and Israeli GD and SD apples. The BP symptoms in GS from California were much more prominent, so the effect of LO2 was more dramatic than the effect on the Israeli cvs. GD and SD, nevertheless the LO2 treatment showed the same trend in all cultivars in reducing BP. The LO2 and 1-MCP -treated fruit exhibited lower levels of ethylene, α -farnesene and its oxidation product, 6-methyl-5-hepten-2-one (MHO), as determined by SPME/GC-MS analysis. In addition, LO2 pretreatment applied to California Bartlett or Israeli Spadona pears was effective in reducing superficial scald, senescent scald and internal breakdown after 4 m of cold storage at 0°C. For GS apple, low-temperature storage resulted in oxidative stress and chilling injury, caused by increased production of superoxide anions which in turn led to the generation of other dangerous reactive oxygen species (ROS). Using confocal laser-scanning microscopy and H₂O₂ measurements of apple peel, we observed ROS accumulation in control fruit, while negligible amounts were found in LO2 and 1-MCP treated fruit. Gene-expression levels of ROS-scavenging enzymes were induced by the various pretreatments: catalase was induced by LO2 treatment, whereas Mn superoxide dismutase was induced by 1-MCP treatment. We assume that LO2 and 1-MCP pretreated fruit remained healthier due to reduced production of ethylene and reactive oxygen substances, such as MHO, during cold storage. The LO2-treated apple exhibited greener peel and firmer fruit after 6 m of cold storage, and the fruit had high crispiness leading to high taste preference. In both pear cultivars, the LO2 treatment led to a reduction in internal breakdown and browning around the seed cavity. We tested the LO2 pre-storage treatment on a semi-commercial scale that would be applicable to a small organic grower by sealing the fruit within the plastic field bins. The treatment was most effective with a continuous flow of nitrogen through the bins; however, a single 6 hour flush of nitrogen was also fairly effective. In addition, we determined that it was very important to have the oxygen levels below 0.5% for approximately 10 days to achieve good scald control, not counting the time required to reduce the oxygen concentration. Our LO2 technology has been proven in this project to be effective in reducing several physiological disorders developed in pome fruit during cold storage. We hope that our non-chemical treatment which is friendly to the environment will be used in the near future for the organic apple and pear industry. The next step should be an analysis of the cost-benefits and commercial feasibility.

Achievements

Low-O₂ stress at 20°C (LO₂) for 10 d in Granny Smith (GS) apples prior to cold storage was effective in reducing superficial scald development in apple fruit during 6-8 months cold storage plus one week of shelf life. The effectiveness of LO₂ application, which is a non-chemical treatment, was quite similar to the commercial-chemical treatment of the antioxidant - DPA, and the ethylene inhibitor, 1-MCP (App. 1, 2, 6). Using confocal laser-scanning microscopy, fluorometry and H₂O₂ measurements of apple peel, we were able to detect reactive oxygen species (ROS) accumulation in control GS fruit, while negligible amounts were found in LO₂ or 1-MCP-treated fruit. The high effectiveness of LO₂ and 1-MCP treatments is probably due to the fact that this pretreatment reduced ethylene and α -farnesene production, and formation of α -farnesene oxidation products, leading to less ROS accumulation which enhanced peel damage in GS apples (App. 1, 2, 7).

Due to the success in eliminating superficial scald with LO₂ treatment in GS apples, we expanded the LO₂ treatments to other apple cultivars, Starking Delicious (SD) and Golden Delicious (GD) grown in Israel and to pear fruit, Bartlett in California and Spadona in Israel.

Another reason we expanded our treatments to other apple cultivars was to further study the effects of LO₂ on bitter pit (BP). Californian GS apples are highly susceptible to BP and the LO₂ treatment was found to be effective in reducing BP incidence. However, while GS apples grown in Israel do not suffer from BP symptoms, SD and GD do suffer from low levels of BP. Control of BP by LO₂ is quite amazing because for many years it was believed that growth conditions, especially calcium deficiency, are responsible for this physiological disorder (App. 4). We suggest that elimination of ROS by the LO₂ treatment (App. 2, 7) and the higher levels of ethanol in the LO₂-treated fruit (App. 1, 6, 9) may keep membrane integrity that reduced BP symptoms.

LO₂ treatment applied for 7d at 20C prior cold storage was found to be effective in reducing superficial scald appearance in Bartlett pear grown in California and senescent scald in Spadona pears grown in Israel. The effectiveness of LO₂ in reducing scald in pears as in apple, likely occurs *via* the same mechanism of reduction of ethylene and α -farnesene production. In addition, the LO₂ treatment, which caused lower ethylene production during cold storage, led to a reduction in internal breakdown and browning around the seed cavity in both pear cultivars (App. 5, 8). This fact is very significant since the anti-scald chemicals, such as diphenylamine

(DPA) or ethoxyquin, which are used commercially, are very efficient in preventing superficial scald, but not effective against internal breakdown in pear.

Using GC-MS and SPME technology we measured aroma volatiles in apple and pear peel. The LO₂ prestorage treatments resulted in an increase in ethanol concentration which was correlated to the O₂ concentration and to the efficacy for control of scald and bitter pit as well as maintenance of fruit quality. The accumulation of α -farnesene and MHO was delayed by pre-storage LO₂ treatments, is in agreement with scald control (App. 1, 2, 3, 6, 7, 8, 9).

Some changes in the aroma volatiles profile were observed after the LO₂ treatments in both apples and pears. In Californian GS apples, total volatile production by apples exposed to semi-commercial LO₂ treatments increased during subsequent cold storage, but there was no difference between the various LO₂ treatments in the pattern or abundance of total volatiles (App. 9). On the other hand, in Israel in LO₂-treated GS and GD apples the levels of total volatiles and hexyl esters was lower than in control fruit similar to the levels in 1-MCP treated fruit (App. 3, 6). The difference in volatiles production between Israel and California is probably because in Israel the fruit was exposed to lower O₂ levels.

The LO₂ treatments were also effective in maintaining apple quality during air storage, all apple cultivar tested were firmer with greener skin color and with high crispiness leading to better taste. The treatments that provided the lowest oxygen and the highest ethanol content had the greatest effect in maintaining firmness and green skin color (App. 1, 3, 6, 9).

The effectiveness of LO₂ in reducing scald in pears as in apple, likely occurs *via* the same mechanism of reduction of ethylene and α -farnesene production. In addition, the LO₂ treatment, which caused lower ethylene production during cold storage, led to a reduction in internal breakdown and browning around the seed cavity in Bartlett and Spadona pears (App. 5, 8).

The low-O₂ stress provides a simple non-chemical means to maintain apple and pear quality that could be suitable for organic fruit markets. Treatments could be applied in standard control atmosphere (CA) rooms or on a small scale using field bins and a source of nitrogen for flushing. The fruit appear to tolerate this treatment very well, with little risk of injury from the very low O₂ or high CO₂ concentrations that can develop.

Agriculture Impact

Granny Smith apple: In both Israel and California, the LO₂ treatment was successful in keeping the quality of GS apples without superficial scald for more than 6 months (4 months

under semi-commercial conditions) in regular cold storage without using chemicals, such as the antioxidant – DPA or the inhibitor of ethylene – 1-MCP. The explanation for reduction in superficial scald by LO₂ treatment is quite clearly via a reduction of ethylene production leading to less production of α -farnesene and its oxidation product MHO, and prevention of ROS accumulation (App. 1, 2, 6, 7, 9). Moreover, in California the LO₂ treatment dramatically reduced another physiological disease, BP. Reduction in BP symptoms by LO₂ treatment is quite astonishing, and we still don't have a full explanation as to how it works. One hypothesis is that less ROS accumulation prevents BP symptom development. Second possibility is that maybe the ethanol produced by the LO₂ treatment maintained membrane stability. Another possibility is a shift in calcium localization to favor calcium levels in the apoplast, perhaps due to stress induced growth regulator activity (App. 4).

Starking Delicious apple: LO₂ treatment successfully maintained the quality of SD apples for more than 6 months in regular cold storage without using chemicals such as DPA or 1-MCP. The SD apples in Israel do not exhibit severe superficial scald, but they suffer from some BP symptoms. SD apples grown in Israel reacted very well to LO₂ treatment and exhibited much less superficial scald and BP symptoms (App. 4).

Golden Delicious apple: The LO₂ treatment on GD apples grown in Israel was also very effective in maintaining fruit quality. LO₂ treated apples maintained better firmness compared to control and 1-MCP treated fruit after 6 m cold storage at 0°C plus one week shelf life. In the case of Golden Delicious, the LO₂ treatment kept the fruit greener and firmer than 1-MCP (App. 3). The fruit was also more crispy than any of the other treatments. Crispiness is a very important feature, especially in GD apples which lose their crispiness during storage (App. 3). The Israeli Golden Delicious apple does not suffer from superficial scald, but suffers from a low BP incidence. Interestingly, the LO₂ treatment reduced BP symptoms to a minimum, while 1-MCP did not reduce them (App. 4).

Bartlett pear: LO₂ treatment successfully maintained the quality of Bartlett pear fruit grown in California for 4.5 months in regular cold storage without using chemicals such as DPA or 1-MCP. One of the main problems of Bartlett pear is that it suffers during cold storage from internal breakdown and pulp browning in addition to superficial scald. These disorders usually appear after 3 ½ months of cold storage. The LO₂ pretreatment successfully overcame all these disorders (App. 8).

Spadona pear: We were successful at storing Spadona pears for 4.5 months in regular cold storage without using chemicals such as ethoxyquin or 1-MCP (app. 5). The Spadona pear grown in Israel suffer from senescent scald exhibited as black spots on the peel and internal breakdown symptoms. The LO2 pretreatment succeeded to overcome these disorders (App. 5). Moreover, while 1-MCP treatment can cause irreversible ripening inhibition in pears because of very low ethylene accumulation, in the case of LO2 treatment the levels of ethylene were reduced, but not to negligible levels and therefore the fruit ripened normally (App. 5, 8).

Difficulties

There are some challenges in making this LO2 technology applicable to the industry. The industry that is interested in such technology is the organic industry, who do not have access to most chemicals such as DPA or 1-MCP, and which is a relatively small industry. In the lab it is quite simple to apply LO2 treatments in sealed containers and to reach the very low oxygen levels that are necessary for the success of the treatment. However, application of the LO2 technology on a large scale is quite complicated because of the lack of sealed rooms for short durations of 7-10 days after harvest. The industry would have to invest large amounts of capital (which they do not have) in creating such sealed room that will serve mainly the organic market. It may be possible to use the sealed bin strategy with either a continuous slow flush or a single flush, but this would take more time and labor to accomplish and the benefits will need to be weighed against the costs.

Details of cooperation:

During the first year of the project there was a joint meeting at the Sixth International. Postharvest Conference in Antalya, Turkey (2009). Dr. Pesis met the Co-PI, Dr. Elizabeth Mitcham and her postdoctoral scientist, Dr. Malkeet Padda, who worked on the project at UC Davis. We discussed together the results we had obtained thus far and next steps.

During the second year of this project, Dr. Edna Pesis visited the lab in Davis to meet the Co-PI., Dr. Elizabeth Mitcham, as well as Dr. Sue Ebeler, Ph.D. student, Sergio Tonetto de Freitas and Dr. Andrew Macnish Project Scientist, who replaced Dr. Malkeet Padda who left for a job in industry. We shared results from our year 2 activities and formulated a plan for the third year, in light of our new findings. The timing of Dr. Pesis' visit also corresponded with the removal of apples from 6.5 m of cold storage. We examined together the shelf life quality of these apples, and discussed how to optimize the experimental protocols.

During the third year, Dr. Pesis visited again the lab in Davis and met with Dr. Mitcham, Dr. Ebeler and Dr. Macnish. We discussed together the results and saw together the fruit that was coming out of cold storage after 6 m..

One of the main differences between the two labs that influenced the results was the application of the LO₂ treatment. In the Volcani Center, we used a water trough to close the 700L chamber which contained the plastic totes, while in UC Davis the plastic fruit totes were placed in a sealed plastic tent, which probably was not sealed enough, so the O₂ level was too high and it influenced the results.

List of Publications:

Refereed

- Pesis, E., Ebeler S.E., Tonetto SF, Padda, M., Mitcham. E.J. (2010). Short anaerobiosis period prior to cold storage alleviates bitter pit and superficial scald in Granny Smith apples. *J. Sci. Food Agric.* 90: 2114-2123. **Joint**
- Sabban-Amin, R., Feygenberg, O., Belausov, E., Pesis E. (2011). Low-oxygen and 1-MCP pretreatments delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples. *Postharvest Biol. Technol.* 62: 295-304. **Israeli**
- Pesis, E., Feygenberg, O., Goldenberg, L., Ben Arie, R. (2013). Application of low oxygen stress and 1-MCP prior to cold storage for improvement of 'Golden Delicious' apple quality. *Postharvest Biol. Technol.* (in preparation). **Israeli**
- Pesis, E., Sabban-Amin R., Feygenberg, O., Ben Arie, R. Mitcham E. (2013). Bitter pit in apples reduced by postharvest treatment of low O₂ stress prior cold storage. *J Amer Hort Sci* (in preparation). **Joint**
- Feygenberg, O., Goldenberg L., Ben Arie, R., Pesis, E. (2013). Inhibition of chilling injury expressed as senescent scald and internal browning in Spadona pear by LO₂ and 1-MCP treatment. *Postharvest Biol. Technol.* (in preparation). **Israeli**
- Goldenberg L., Feygenberg, O., Ben Arie, R., Pesis, E. (2013). Effect of LO₂ and 1-MCP on Granny Smith quality and volatiles production after regular air cold storage. *J. Sci. Food Agric.* (in preparation) **Israeli**

Not Refereed

- Feygenberg, O., Ben-Arie, R., Sabban-Amin, R., Pesis E. (2010). Short low oxygen treatment to reduce superficial scald in apples. *Alon Hanotea* 64: No. 10. 34-39. (in Hebrew). **Israeli**
- Macnish, A., Padda, M., Biasi B., Ebeler, S.E, Pesis, E., Mitcham, E.J. 2010. Control of storage scald and bitter pit in Granny Smith apples with pre-storage low oxygen exposure. In: Mitcham, EJ, C. Crisosto, F. Zakharov, and JM Labavitch, Eds., Report to Northeast 1036 Multistate Research Project. **Joint**
- Macnish, A., Padda, M., Biasi B., Ebeler, S.E., Pesis, E., Mitcham, E.J. 2011. Control of storage scald and bitter pit in Granny Smith apples with pre-storage low oxygen exposure. In: Mitcham, EJ, C. Crisosto, F. Zakharov, and JM Labavitch, Eds., Report to Northeast 1036 Multistate Research Project. **Joint**
- Pesis, E., Feygenberg, O., Goldenberg, L., Sabban-Amin, R. (2012). Superficial scald symptoms in Granny Smith apples associated with reactive oxygen species (ROS) accumulation. *Proc. Fla. State Hort. Soc.* 2012 (*in press*). **Israeli**

Pesis, E., Feygenberg, O., Goldenberg, L., Sabban-Amin, R., Ebeler, S.E., Mitcham, E.J., Ben Arie, R. (2012). The role of low oxygen pre-storage treatment in reducing chilling injuries of deciduous fruit. Proceedings of the 7th CIGR Section VI International Technical Symposium “Innovating the Food Value Chain ”Stellenbosch, South Africa (*in press*). **Joint**

Macnish, A., Ebeler, S.E, Mitcham, E.J. (2013). Application of pre-storage short anaerobiosis to alleviate superficial scald and bitter pit in ‘Granny Smith’ apples. Bard Final Report. (Appendix 9). **USA**

Abstracts

Pesis, E., Ebeler, S., Mitcham, E. (2008). Low oxygen pretreatment inhibited ripening and reduced superficial scald in Bartlett pear. Postharvest Unlimited 2008. Berlin, (Nov 2008), Germany, Bornimer Agratechnische Berichte, Heft 64:106. **Joint**

Pesis, E., Ibanez, A.M., Ebeler, S. Dandekar, A., Mitcham, E. (2009). Prevention of superficial scald symptoms in apples by using various approaches. 6th Inter. Conf. Postharvest Sci. Antalya, (April 2009) Turkey, pg. 111. **Joint**

Sabban-Amin, R., Belausov, E. Feygenberg, O., Ben Arie, R. Pesis, E. (2010). Are reactive oxygen species (ROS) the cause of superficial scald in apples? 28th Inter. Hort. Sci. (Aug. 2010) Lisbon, Portugal. Pg. 107. **Israeli**

Pesis E. (2012). Prevention of cold storage damages in apple and pear by low oxygen atmosphere or 1-MCP pretreatment. Florida State Horticultural Society (FSHS) (June 2012) Delray Beach, Florida. pg. 27, HP-16. **Israeli**

Feygenberg, O., Goldenberg L., Ben-Arie, R., Pesis E. (2012). Application of pre-storage short anaerobic treatment to improve postharvest quality of ‘Spadona’ pear. American Society for Horticultural Science (ASHS) (July 2012) Miami, Florida. Abst. Pg. 18. **Israeli**

Pesis, E., Feygenberg, O., Sabban-Amin, R., Ebeler, S.E., Mitcham, E.J., Ben Arie, R. (2012). The Role of Low Oxygen Pre-Storage Treatment in Reducing Chilling Injuries of Deciduous Fruit. 7th CIGR Section VI Inter. Tech. Symp. “Innovating the Food Value Chain” Stellenbosch, South Africa. pg. 320 (Nov. 2012). **Joint**

List of Appendices

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2. Sabban-Amin, R., Feygenberg, O., Belausov, E., Pesis E. (2011). Low-oxygen and 1-MCP pretreatments delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples. Postharvest Biol. Technol. 62: 295-304. Israeli	12
3. Pesis, E., Feygenberg, O., Goldenberg, L., Ben Arie, R. (2013). Application of low oxygen stress and 1-MCP prior to cold storage for improvement of 'Golden Delicious' apple quality. Postharvest Biol. Technol. (in preparation). Israeli	13
4. Pesis, E., Sabban-Amin R., Feygenberg, O., Ben Arie, R. Mitcham E. (2013). Bitter pit in apples reduced by postharvest treatment of low O ₂ stress prior cold storage. J Amer Hort Sci (in preparation). Joint	26
5. Feygenberg, O., Goldenberg L., Ben Arie, R., Pesis, E. (2013). Inhibition of chilling injury expressed as senescent scald and internal browning in Spadona pear by LO ₂ and 1-MCP treatment. Postharvest Biol. Technol. (in preparation). Israeli	36
6. Goldenberg L., Feygenberg, O., Ben Arie, R., Pesis, E. (2013). Effect of LO ₂ and 1-MCP on Granny Smith quality and volatiles production after regular air cold storage. J. Sci. Food Agric. (in preparation) Israeli	45
7. Pesis, E., Feygenberg, O., Goldenberg, L., Sabban-Amin, R. (2012). Superficial scald symptoms in Granny Smith apples associated with reactive oxygen species (ROS) accumulation. Proc. Fla. State Hort. Soc. 2012 (in press). Israeli	57
8. Pesis, E., Feygenberg, O., Goldenberg, L., Sabban-Amin, R., Ebeler, S.E., Mitcham, E.J., Ben Arie, R. (2012). The role of low oxygen pre-storage treatment in reducing chilling injuries of deciduous fruit. Proceedings of the 7th CIGR Section VI International Technical Symposium "Innovating the Food Value Chain" Stellenbosch, South Africa (in press). Joint	68
9. Macnish, A., Ebeler, S.E, Mitcham, E.J. (2013). Application of pre-storage short anaerobiosis to alleviate superficial scald and bitter pit in 'Granny Smith' apples. Bard Final Report. USA	76

Appendix 1

Pesis, E., Ebeler S.E., Tonetto SF, Padda, M., Mitcham. E.J. (2010). Short anaerobiosis period prior to cold storage alleviates bitter pit and superficial scald in Granny Smith apples. J. Sci. Food Agric. 90: 2114-2123. **Joint**
Separate PDF

Appendix 2

Sabban-Amin, R., Feygenberg, O., Belausov, E., Pesis E. (2011). Low-oxygen and 1-MCP pretreatments delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples. Postharvest Biol. Technol. 62: 295-304. **Israeli**
Separate PDF