FINAL PROJECT REPORT

Project Title: Mechanisms and practical solutions to control scald of pears

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Other funding sources
none

Total Project Funding: 117,062

Budge History:

Item	2017	2018	2019
Salaries	20,222[1]	20,829	21,454
Benefits	1,950[2]	2,009	2,069
Wages	10,744[3]	11,066	11,398
Benefits	1,074[4]	1,107	1,140
Equipment			
Supplies	3,500[5]	3,500	3,500
Travel	500[6]	500	500
Miscellaneous			
Total	37,990	39,011	40,061

Footnotes:
[1]Postdoctoral Research Associate: 1/2 FTE. 3% increase is factored into Year 2 and 3.
[2]OPE: 1/3 FTE. 4% increase is factored into Year 2 and 3.
[3]Wages: 800hr for a Biological Science Tech. at $13.43/hr. 3% increase is factored into Year 2 and 3.
[4]OPE: 10% of the wage, with a 3% annual increase.
[5]Supplies: maintaining cold storage and CA storage rooms, buying fruit, gases (helium, nitrogen, hydrogen, air, and standard gases), gas tank rental, and chemicals.
[6]Travel: field trips to packinghouses and orchards.

OBJECTIVES

1. Understand completely the physiological mechanisms of scald development; understand growing season conditions and harvest maturity effects on the natural antioxidant capacity associated with the oxidation of α-farnesene into conjugated trienols (CTols) and therefore scald susceptibility of Anjou pear.
2. Study commercially-feasible methods for controlling scald of susceptible Anjou pear; the potential of the combination treatments of Harvista/ReTain + ethoxyquin + low-O_2.
3. Study the potential of Lovastatin and naturally-occurring, food-grade antioxidants mixed with edible coatings as alternatives to ethoxyquin for controlling scald of Anjou pear.
4. Develop pre- and postharvest practices to reduce Anjou pear storage losses due to scald.

SIGNIFICANT FINDINGS

- Physiological mechanism of scald development. The reduction of α-farnesene and increase in CTols during storage are associated with superficial scald development.
- Higher ACU in Anjou pears reduced superficial scald by increasing antioxidant metabolites, antioxidant enzymes, and total antioxidant capacity.
- Pre-harvest Ca spray increased fruit calcium content, antioxidant metabolites, antioxidant enzymes, and total antioxidant capacity of Anjou pears and resulted in a slight reduction in superficial scald after 4 and 5 months in storage.
- Sunlight exposure prior to harvest delayed the accumulation of α-farnesene and CTols, and inhibited the development of superficial scald development.
- Harvest maturity affected scald development. More mature fruit developed more scald.
- Regardless of rate and timing, NAA application did not affect superficial scald. After 4 months of storage, all NAA treatments had relatively high incidence of superficial scald, with no significant differences between treatments and the untreated control.
- O_2 concentrations of 0.5 and 1% inhibited the development of superficial scald, with 0.5% O_2 stored fruit remaining free of scald over the entire storage period, whereas 2% O_2 stored fruit developed 68% scald at 6 months and increased to 90% after 10 months.
- AVG applied 1 WBH at 60 or 120 ppm resulted in reductions in scald compared to the untreated control after 3 and 5 months of RA storage. Incidence of superficial scald of all treatments increased through the storage period and reached 100% by month 7.
- Harvista at 120 g/acre applied 10 d before harvest to 'Anjou' pears reduced scald development compared to the control after 4 and 6 months of RA storage.
- Pears treated with AVG at 60 and 120 ppm and stored at 0.8 % O_2 could were scald free following 10 months of storage.
- AsA did not affect scald development, while Lovastatin at rates of 2 and 5 % provided greater effects on reducing scald incidence than ethoxyquin treatment.

RESULTS

<u>1. Understand completely the physiological mechanisms of scald development</u>

α-Farnesene metabolism

For Anjou pear harvested at 16-15 lbs. and stored in regular-air (RA) at 30 °F, the incidence of superficial scald after 7 d at 68 °F remained relatively low through three months of storage but increased thereafter (Fig 1). α-Farnesene showed marked increase from one month of storage, peaking at three months and then declining. CTols concentration showed a similar pattern but peaked one month later than α-farnesene and preceded the large increase in scald.

Fruit with varied accumulated cold units (ACU) on fruit scald susceptibility

Fruit with ACU ranging from 12-316 at commercial harvest maturity was collected from 5 orchards located at elevations ranging from 500 to 2,000 ft. and placed in RA for up to 6 months. After 2-6 months in storage, fruit with ACU at 227 and 316 had lower incidence of superficial scald than fruit with ACU at 15 or 118 (Fig. 2). Higher ACU significantly reduced the production of α-farnesene and CTols and maintained relatively high levels of TP, TFO, SOD, CAT, APX, GR, DPPH, and FRAP after 5 months of storage (Table 1).

Fruit with varied Ca concentration on fruit scald susceptibility

$CaCl_2$ was applied at 3.47 pound/acre (100 gal/acre, Ca rate at 0.15%) 6 times from 1 month after full bloom to 1 week before harvest. Fruit were collected at commercial harvest maturity, then placed in RA for up to 5 months. Superficial scald and fruit physiological and biochemical responses were evaluated after 2, 3, 4, and 5 months of storage. Pre-harvest Ca sprays resulted in higher calcium content of fruit flesh at harvest, but no significant difference in fruit skin Ca content (Table 2). Ca sprays resulted in a slight reduction in superficial scald after 4 and 5 months in storage (Fig. 3). Additionally, Ca treated fruit showed trends towards reduced α-farnesene, and CTols as the storage duration increased. Ca sprays resulted in an increase in the antioxidant metabolite TP but no difference in TFO, or the antioxidant enzymes SOD and CAT or APX (Table 3). GR was higher in Ca treated fruit as was FRAP. DPPH showed no response to Ca treatment.

Different sunlight exposure on fruit scald susceptibility

Bagged and unbagged fruit were collected at commercial harvest maturity and placed in RA storage. Fruit were evaluated at harvest, and 2, 3, 4, and 5 months after storage. Scald was evaluated on the blushed (sun-exposed) and shaded portions of the fruit peel. The blushed peel of unbagged fruit had no incidence of scald throughout the 5-month storage period, while the shaded peel developed 0, 8, 89 and 100% scald incidence at 2, 3, 4, and 5 months, respectively. Bagged fruit developed 0, 39, 95, and 100% scald incidences at 2, 3, 4, and 5 months storage, respectively. There were no significant differences in α-farnesene concentration of blushed and shaded peels of unbagged fruit from harvest through 5-month storage periods (Fig. 4). Both had the highest level of α-farnesene at 3 to 4 months of storage. In bagged fruit, the concentration of α-farnesene was similar to that of unbagged fruit at harvest but was higher by 2 months of storage, and peaked earlier and at a much higher level. The blushed peel of unbagged fruit had the lowest concentration of CTols throughout the experimental period. The blushed and shaded peels of unbagged fruit accumulated the highest CTols concentrations at 4 months, while the bagged fruit reached the highest level of CTols 1 month earlier. The shaded peels of unbagged fruit and peels of bagged fruit had similar CTol concentrations at 4 and 5 months of storage. Compared to the shaded peels and bagged fruit, the blushed peel remained higher TP, TFO, SOD, APX, GE, DPPH, and FRAP after 5 months of storage (Table 4).

Influence of harvest maturity on fruit scald susceptibility

Anjou pears harvested at 15.9-13.9 lbs. developed less superficial scald than fruit harvested at 13.9-11.8 lbs. after 3-4 months of RA storage and 3-7 months in CA at 30 °F following 7 days at 68 °F (Fig. 5). Anjou pears harvested at 13.9-11.8 lbs. had relatively high incidence of superficial scald development after 3-4 months in RA storage and 5-6 months in CA storage following 7 d at 68 °F. For fruit stored in RA, all harvest maturities developed nearly 100% incidence of scald by 5 months of storage. Scald incidence of all harvest maturities of CA stored fruit increased up to 9 months of storage.

Influence of NAA on fruit scald susceptibility

NAA was applied at 20 ppm 1 and 2 WBH and 40 ppm 1, 2, and 3 WBH. At harvest, fruit were placed in RA storage. Non-treated and NAA-treated fruit were evaluated after 2, 3, and 4 months of RA storage plus 7 d at 68 °F. After 4 months of storage, all NAA treatments had relatively high incidence of superficial scald, with no significant differences between treatments and the untreated control (Fig. 6). α-Farnesene content and CTols content of NAA-treated fruit and the untreated control increased during storage, with no significant differences between treatments and untreated control.

2. Commercially-feasible methods for controlling scald of susceptible Anjou pear.

Effects of low-O_2 CA on fruit scald susceptibility

Anjou pears were harvested at commercial harvest maturity from MCAREC, and after 2 d of cold storage, fruit were loaded into gas-tight cabinets. The cabinets were flushed with purified nitrogen and then CO_2 concentration was adjusted to < 0.5% by adding hydrated lime. O_2 concentration was adjusted to 2.0, 1.0, or 0.5% within 6 d of sealing the cabinets. Air-stored fruit was placed in storage at 30 °F. After 6, 8, and 10 months of storage, fruit were removed and held at 68 °F for 7 days. Superficial scald incidence of air stored fruit was 100% following 6 months of cold storage (Fig. 7). The 2% O_2 stored fruit developed 68% scald at 6 months and increased to 90% after 10 months. The lower O_2 concentrations of 0.5 and 1% inhibited the development of scald, with 0.5% O_2 stored fruit remaining free of superficial scald over the entire storage period. Ethylene production in air-stored fruit decreased from 5 to 1 ng kg^{-1} s^{-1}, while respiration rate increased from 11 to 13 µg CO_2 kg^{-1} s^{-1} from month 6 to 10. Low O_2 treatments had lower ethylene production and respiration rates at month six, then increased thereafter. The ethylene production rate of 2% O_2 stored fruit increased, reaching a maximum level of 7 ng kg^{-1} s^{-1} after 8 months of storage, before decreasing to 4 ng kg^{-1} s^{-1}. The ethylene production rate of 0.5 and 1% O_2 stored fruit increased between 6 and 10 months of storage, reaching 3 and 5 ng kg^{-1} s^{-1}, respectively. Total antioxidant capacity of air and CA stored fruit decreased during ripening. Reducing O_2 concentration from 21% to 0.5% inhibited the reduction of total antioxidant capacity, but no significant differences were observed among 2, 1 and 0.5% O_2 treatments. The effect of low O_2 treatments on α-farnesene content were similar to those on ethylene production. CTols of air and CA stored fruit gradually increased, and the 0.5 and 1% O_2 stored fruit had lower CTols contents than other treatments. These results indicate that reducing O_2 concentration can reduce superficial scald of Anjou pears with 1 and 0.5% O_2 providing nearly complete and complete control.

Effect of pre-harvest Retain on fruit scald susceptibility

AVG (Retain) was applied at 60 ppm 1 WBH and 120 ppm applied 1 and 2 WBH. After harvest, fruit were placed in RA storage. Fruit were evaluated after 1, 3, 5, and 7 months of RA storage plus 7 d at 68 °F. Incidence of superficial scald of all treatments increased through the storage period and reached 100% by month 7 (Fig. 8). After 3 (Fig. 8, right picture) and 5 months of RA storage AVG applied 1 WBH at 60 or 120 ppm provided reductions in scald compared to the untreated control.

Effects of pre-harvest Harvista on fruit scald susceptibility

Harvista (1-MCP) was applied at 120 g/acre 10 d before harvest. Fruit was collected at commercial harvest maturity and 4 days later and placed in RA storage. Fruit was evaluated after 3, 4, 6, and 8 months of storage. Based on reductions of FF and green color (data not shown), Harvista extended the harvest window by 3-4 days. During 4-6 months of storage, both Harvista and delay-harvest pears had significantly lower superficial scald incidence (Fig. 9).

Effects of the combination of pre-harvest AVG + ethoxyquin + 0.8 % O_2 CA on fruit scald susceptibility

AVG was applied at 60 and 120 ppm 1 WBH. Fruit was harvested at commercial harvest maturity from MCAREC, and after 2 d of cold storage fruit from each treatment was treated with 1000 ppm ethoxyquin, and then loaded into gas-tight cabinets. The cabinets were flushed with purified nitrogen and then O_2 concentration was adjusted to 0.8 % within 6 d. Fruit were evaluated after 6 and 10 months of storage. Regardless of AVG treatment, decreasing O_2 level from 2.3 to 0.8 % prevented development of superficial scald following 10 months of storage (Fig. 10).

3. The potential of Lovastatin and naturally-occurring, food-grade antioxidants mixed with edible coating as alternative to ethoxyquin for controlling scald of Anjou pear

Anjou pears were harvested at 14.23 lbs and treated with Lovastatin at rates of 1, 2, and 5% or ascorbic acid (AsA) at rates of 1, 2, and 5% for 10 min, and then stored in RA storage for 4 and 6 months. Compared to the untreated fruit, AsA did not affect superficial scald after 4 and 6 months of storage (Fig. 11). Conversely, Lovastatin at rates of 2 and 5 % significantly reduced superficial scald, with better control than ethoxyquin. We intended to test the combination of 5% Lovastatin with Semperfresh and Chitosan, but Lovastatin failed to dissolve in Semperfresh or Chitosan and no results of these combinations are available.

4. Develop pre- and postharvest practices to reduce Anjou pear storage losses due to scald.

In this study, we investigated both preharvest and postharvest practices that may provide reductions in scald development. The effects of environmental factors such as sunlight exposure and ACU may be set at harvest, and therefore outside the control of growers, but they may be managed to some degree through inventory control at the packing house (e.g. ship low ACU fruit first). We determined that there are benefits of high antioxidant capacity in fruit to reducing scald development. This finding suggests that practices that increase this capacity should be optimized. Pre-harvest Ca application and harvest at optimum maturity both contribute to fruit with high antioxidant capacity and low scald during storage. Preharvest application of Retain and Harvista helped reduce scald at optimal RA storage time. Excessive storage time or extending the harvest window resulted in reduced efficacy of ReTain and Harvista for controlling scald. Decreasing the O_2 level in CA storage from 2.0 to 1.0 % provided better scald control. Decreasing the O_2 level to < 0.8 % when combined with pre-harvest ReTain treatment provided an additional reduction in scald incidence, but pears failed to ripen.
Our results with Lovastatin indicate that it may be an alternative to ethoxyquin for controlling scald. Additional study is needed, however, to optimize its use in combination with other coating formulations.

Table 1. Total polyphenols (TP), total flavonoids (TFO), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, and ferric reducing antioxidant power (FRAP) of flesh tissue in pears with varied ACU after 5 months of storage plus 7 d at 68 °F.

	TP (mg g^{-1})	TFO (mg g^{-1})	SOD (U g^{-1})	CAT (U g^{-1})	APX (U g^{-1})	GR (U g^{-1})	DPPH (mM g^{-1})	FRAP (mM g^{-1})
ACU 12	0.5 b	0.5 c	0.9 cd	0.9 b	81 c	5 b	1.9 b	0.3 c
ACU 15	0.6 b	0.6 c	1.0 c	0.8 b	65 e	6 b	1.7 c	0.5 b
ACU 118	0.6 b	0.6 c	0.8 d	0.6 c	77 d	5 b	1.3 d	0.2 c
ACU 227	0.8 a	0.7 b	1.4 a	1.3 a	93 a	8 a	2.2 a	0.6 a
ACU 316	0.8 a	0.9 a	1.2 b	1.2 a	89 b	8 a	2.3 a	0.7 a

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

Table 2. Effects of Ca treatment on calcium content of skin and flesh tissue in pears at harvest.

	Calcium content (ppm)	
	Skin tissue	Flesh tissue
Control	377.63 a	1258.53 b
Ca treatment	396.60 a	1401.00 a

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

Table 3. TP, TFO, SOD, CAT, APX, GR, DPPH radical scavenging capacity, and FRAP of flesh tissue in pears with and without Ca treatment after 5 months of storage plus 7 d at 68 °F.

	TP ($mg\ g^{-1}$)	TFO ($mg\ g^{-1}$)	SOD ($U\ g^{-1}$)	CAT ($U\ g^{-1}$)	APX ($U\ g^{-1}$)	GR ($U\ g^{-1}$)	DPPH ($mM\ g^{-1}$)	FRAP ($mM\ g^{-1}$)
Control	0.6 b	1.2 a	1.4 a	0.9 a	78.0 a	9 b	2.5 a	1.4 b
Ca treatment	0.8 a	1.1 a	1.5 a	1.1 a	68.0 a	14 a	2.8 a	2.2 a

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

Table 4. TP, TFO, SOD, CAT, APX, GR, DPPH radical scavenging capacity, and FRAP of flesh tissue in blushed and shaded of unbagged pears and whole peel of bagged fruit after 5 months of storage plus 7 d at 68 °F.

	TP ($mg\ g^{-1}$)	TFO ($mg\ g^{-1}$)	SOD ($U\ g^{-1}$)	CAT ($U\ g^{-1}$)	APX ($U\ g^{-1}$)	GR ($U\ g^{-1}$)	DPPH ($mM\ g^{-1}$)	FRAP ($mM\ g^{-1}$)
Blushed peel	1.1 a	1.3 a	1.9 a	0.6 a	124 a	14 a	3.4 a	1.2 a
Shaded peel	0.6 b	0.8 b	1.0 b	0.6 a	48 b	6 b	1.8 b	0.8 b
Bagged pears	0.5 b	0.7 b	0.7 c	0.5 a	46 b	5 b	1.7 b	0.7 b

Data within columns with different letters are significantly different by Fisher's protected LSD test at $P < 0.05$.

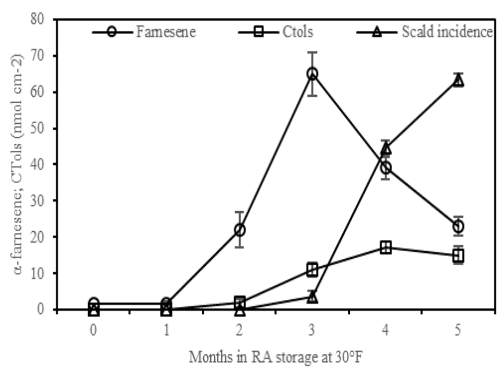

Fig. 1. α-Farnesene, conjugated trienols (CTols), and superficial scald incidence of Anjou pear following 7 d at 68 °F during 5 months storage in regular-air (RA) at 30 °F. Values are means ± standard deviation.

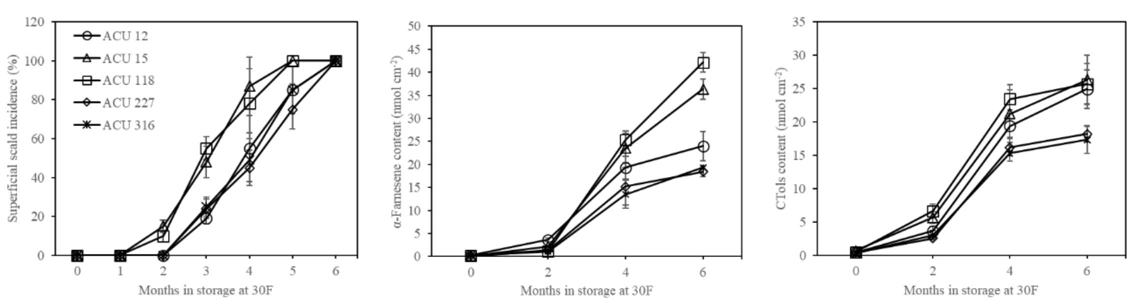

Fig. 2. Effects of varied ACU on superficial scald incidence, α-farnesene, and CTols of Anjou pears during 6 months of RA storage at 30 °F.

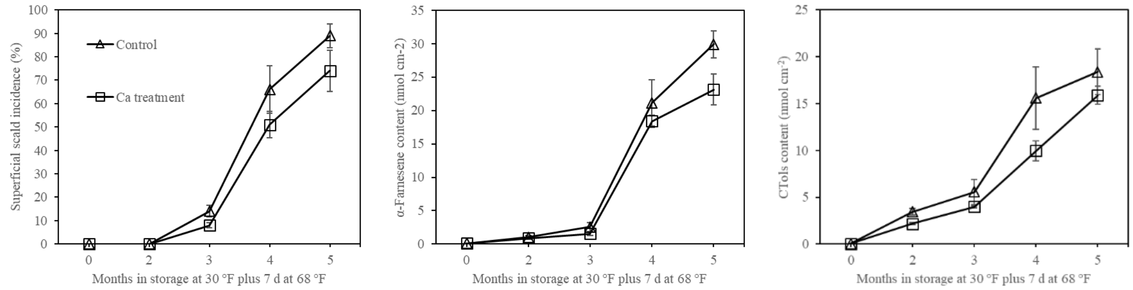

Fig. 3. Effects of Ca treatment on superficial scald incidence, α-farnesene, and CTols of Anjou pears during 5 months of RA storage at 30 °F.

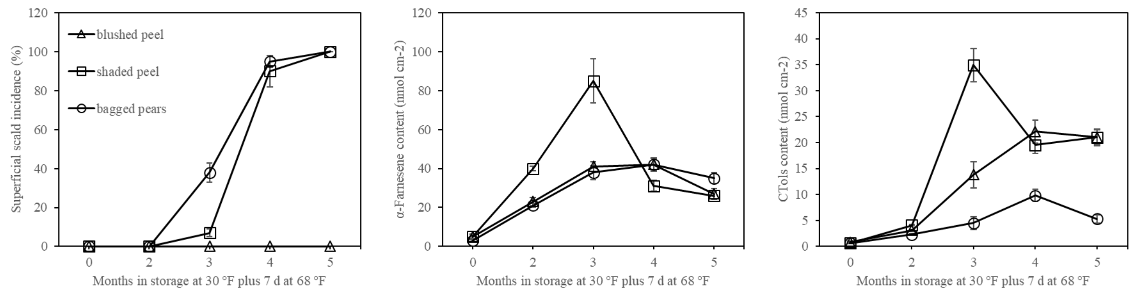

Fig. 4. Effects of sunlight exposure on superficial scald incidence, α-farnesene, and CTols of Anjou pears during 5 months of RA storage at 30 °F.

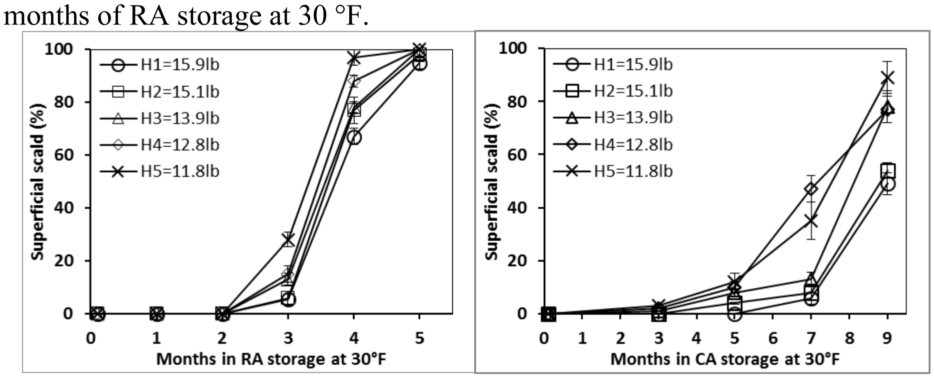

Fig. 5. Effects of harvest maturity on superficial scald incidence of Anjou pear following 5 months of RA storage at 30 °F or 9 months of CA storage at 30 °F, both plus 7 d at 68 °F.

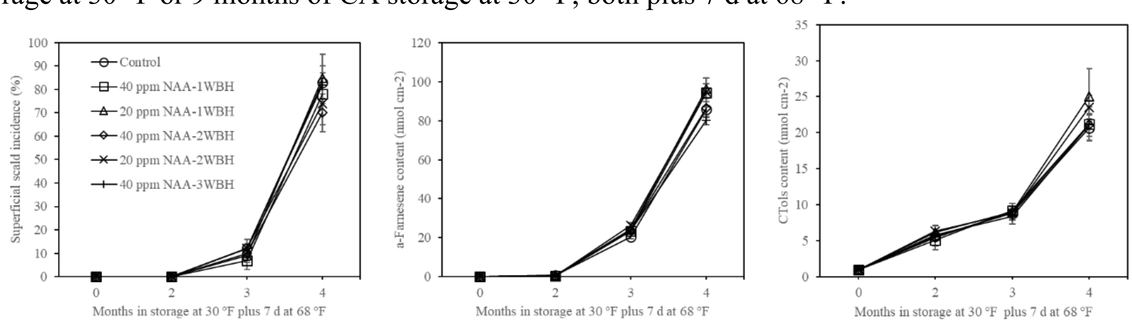

Fig. 6. Effects of pre-harvest NAA applied at 20 or 40 ppm 1, 2, or 3 weeks before commercial harvest on superficial scald incidence, α-farnesene, and CTols of Anjou pears during 4 months of RA storage at 30 °F.

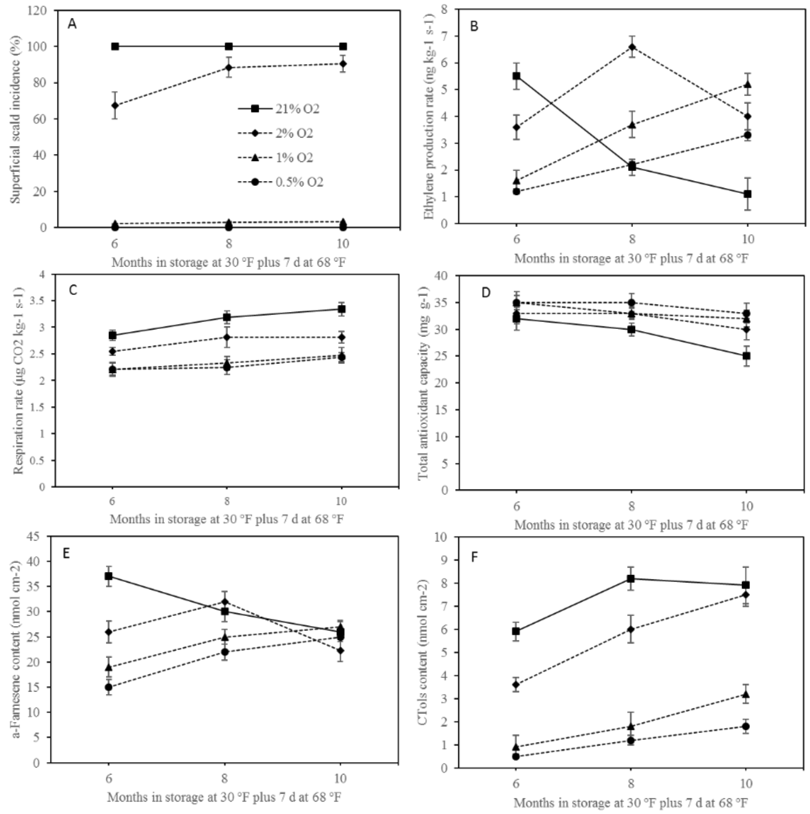

Fig. 7. Effects of different O_2 regimes on superficial scald incidence, EPR, RR, total antioxidant capacity, α-farnesene content, and CTols content of Anjou pears during 10 months of storage at 30 °F. O_2 concentrations were 21 % (air), 2 %, 1 %, and 0.5 % with CO_2 < 0.05 %.

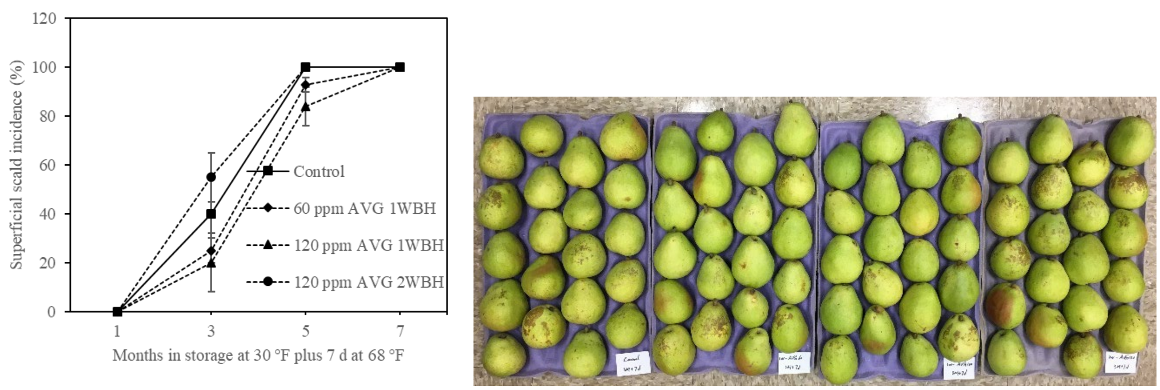

Fig. 8. Effects of preharvest treatments of AVG at rate of 60 and 120 ppm applied at 1 and 2 weeks before commercial harvest on superficial scald of Anjou pears during 7 months of RA storage at 30 °F.

Fig. 9. Effects of preharvest Harvista applied 10 d before commercial harvest on superficial scald incidence of Anjou pears during 8 months of RA storage at 30 °F.

Fig. 10. Effects of preharvest 60 and 120 ppm AVG applied 1 w before commercial harvest on superficial scald incidence of Anjou pears during 10 months of CA storage (2.3 and 0.8% O_2) at 30 °F.

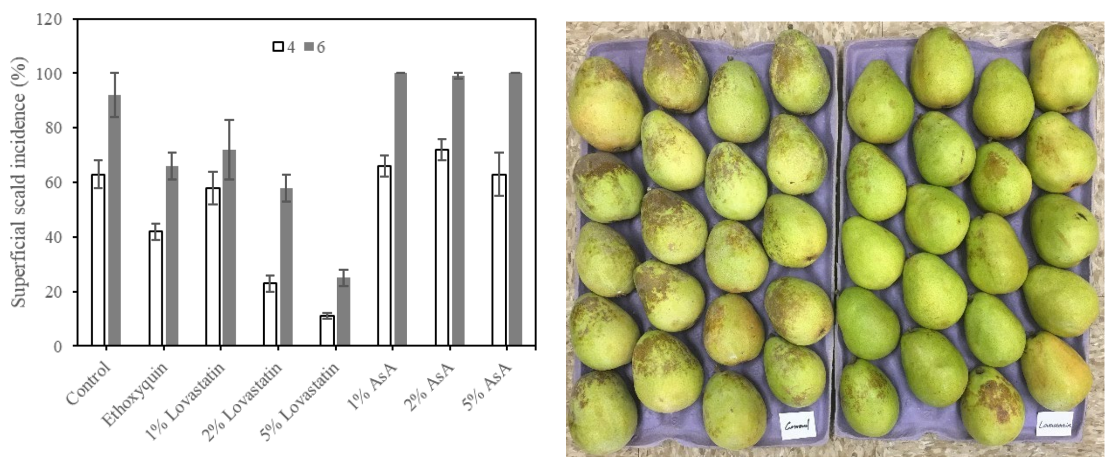

Fig. 11. Effects of 1-5 % Lovastatin and 1-5% ascorbic acid on superficial scald incidence of of Anjou pears after 4 and 6 months of RA storage at 30 °F.

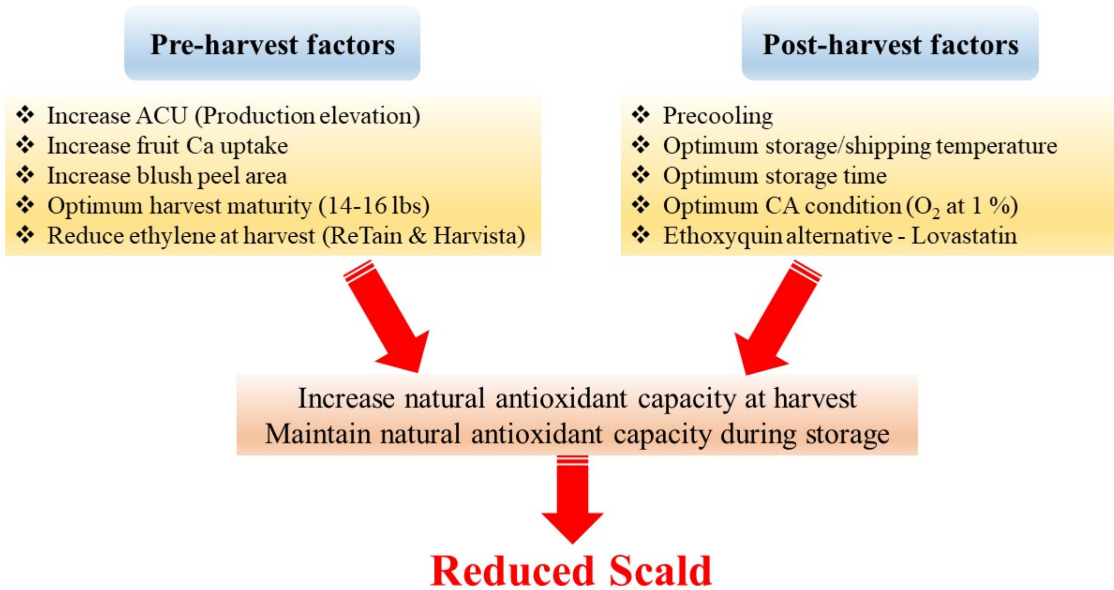

Fig. 12. Summary of pre- and postharvest practices for reducing scald susceptibility by increasing the natural antioxidant capacity in fruit.

EXECUTIVE SUMMARY

Project Title: Mechanisms and practical solutions to control scald of pears

Keywords: Anjou Pears; Scald control; Antioxidant; Practice

Abstract: Understanding of the mechanisms and pre- and postharvest factors affecting scald development are necessary for developing procedures to reduce scald loss. This project found that the reduction of α-farnesene and increase in CTols are associated with the development of scald. Antioxidant metabolites played important roles in controlling scald. High ACU, high fruit Ca content, sunlight exposure, and optimum harvest maturity resulted in high antioxidants at harvest and reduced scald. Optimizing practices in ReTain, Harvista, O_2 regimes, and Lovastatin coating provided a great scald control than ethoxyquin application alone.

Controlling superficial scald of Anjou pears relies on the pre-storage application of the antioxidant ethoxyquin. This practice, however, does not provide adequate control because significant storage losses due to scald continue to occur in some years. Additionally, the European Union has withdrawn authorization for plant protection products containing ethoxyquin. Therefore, alternatives to ethoxyquin application for scald control are needed. Ethylene biosynthesis triggers and enhances the synthesis of α-farnesene in the fruit peel with subsequent induction of scald development. Greater understanding of the mechanisms and pre- and postharvest factors affecting scald development are necessary for developing procedures to reduce losses due to scald.

In this project, we confirmed that the reduction of α-farnesene and increase in CTols during storage are associated with the development of superficial scald. We also showed that antioxidant metabolites in storage played important roles in controlling scald. Furthermore, we found that preharvest environmental conditions such as ACU, fruit Ca content, sunlight exposure, and harvest maturity impacted antioxidant metabolites at harvest. Significant findings include:

- Pears with low ACU had higher scald incidence due to the lower antioxidant metabolites.
- Pre-harvest Ca application increased fruit Ca content, antioxidant metabolites, antioxidant enzymes, and total antioxidant capacity and reduced scald in storage.
- Sunlight exposure delayed the accumulation of α-farnesene and CTols, and inhibited the development of scald by increasing antioxidants and related enzymes at harvest.
- More mature fruit developed more scald.

Additionally, we found that scald development was influenced by pre- and post-harvest practices. Significant findings include:

- Pre-harvest NAA application did not affect scald in storage.
- AVG applied at 60 and 120 ppm 1 week before harvest significantly reduced scald incidence during 3-5 months of RA storage, but not beyond 5 months.
- AVG applied 2 weeks before harvest did not affect scald.
- Harvista application at 120 g/acre 10 d before harvest extended harvest window for 4 d and controlled scald for 4 months.
- Reducing storage O_2 level from 2 to 1 or 0.5% resulted in complete control of scald over 10-month storage period.
- Storage O_2 at 0.8 % plus AVG at 60 and 120 ppm prevented scald in ethoxyquin treated fruit for 10 months.
- AsA did not affect scald development.
- Lovastatin applied at 2 and 5 % provided significant reductions in scald incidence.

Optimizing these practices and conditions should provide a greater degree of scald control than ethoxyquin application alone.