

Project Title: New active ingredients for pear superficial scald control (PR-19-103)

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Report Type: Final Project Report

Total Project Request for Year 1 Funding: \$84,894

Total Project Request for Year 2 Funding: \$86,893

Total Project Request for Year 3 Funding: \$89,036

Agency Name: USDA-ARS, In-house project

Cost-sharing: \$105,946/3 yrs.

Notes: In-house project with complimentary objectives. Funds for storage maintenance and costs (\$8000/yr), supplies and materials (\$3000/yr), travel (\$5000/yr), and 0.1 FTE (PI, co-PI) and 0.05 FTE (technical).

WTFRC Budget

Item	2019	2020	2021
Salaries			
Benefits			
Wages			
Benefits			
RCA Room Rental		6695	6695
Shipping			
Supplies			
Travel			
Plot Fees			
Miscellaneous			
Total		6695	6695

Budget 1

Co PI 2: Carolina Torres

Organization Name: Washington State University**Contract Administrator:** Anastasia Mondy**Telephone:** 916-897-1960**Contract administrator email address:** arcgrans@wsu.edu**Station Manager/Supervisor:** Chad Kruger**Station manager/supervisor email address:** ckruger@wsu.edu

Item	2019	2020	2021
Salaries	52,196	53,679	55,290
Benefits	17,198	17,714	18,246
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous (Fruit purchase)	3000	3000	3000
Plot Fees			
Total	72,394	74,393	76,536

Budget 2**Primary PI:** David Rudell**Organization Name:** USDA-ARS**Contract Administrator:** Chuck Myers and Sharon Blanchard**Telephone:** 510-559-5769 (CM), 509-664-2280 (SB)**Contract administrator email address:** Chuck.Myers@usda.gov, Sharon.Blanchard@usda.gov

Item	2019	2020	2021
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	1000	1000	1000
Travel			
Miscellaneous*	11,500	4805	4805
Miscellaneous			
Plot Fees			
Total	12,500	5805	5805

Footnotes: One-eighth instrument service contract

OBJECTIVES:

1. Test squalane-based formulation(s) for scald control of 'd'Anjou' pear.
2. Determine mode of action of this new active ingredient.
3. Determine any quality impacts and control of other appearance-related defects.

Goals and Activities for the next year:

Project Year 3 goals are to confirm scald control properties and impacts on fruit finish of squalane (E7 formulation) using multiple orchards and harvest maturities from different growing regions. Scald control efficacy of delayed drenches of up to 3 months is being tested on additional orchards. Control of CO₂-related disorders using squalane (E7 formulation) is being evaluated. Analysis of scald control mechanism using squalane is expected to be completed.

SIGNIFICANT FINDINGS:

1. Formulations containing squalane reduced or eliminated superficial scald of 'd'Anjou'.
2. Control using squalane emulsions was comparable with ethoxyquin drenches.
3. Squalane (E7 formulation) emulsion drenches can impact peel degreening.
4. Squalane is the active ingredient in these formulations.

METHODS

Equipment and Cooperative Summary: Fruit quality assessment, fruit chemistry analyses using analytical instrumentation (gas and liquid chromatography-mass spectrometry), and tissue cryopreservation will be performed using facilities currently in place at ARS-TFRL, Wenatchee. Storage experiments will be conducted in TFRL in-house CA chambers.

Outreach (Deliverables are summarized under "Anticipated Products" Table 1): Aside from reports to the WTFRC, new information will be disseminated through presentations at industry meetings and at professional conferences, and by publications in industry publications and peer-reviewed journals. Dr. Torres will continue to interface with crop protectant providers interested in her product.

Objective 1: Test squalane-based formulation(s) for scald control of 'd'Anjou' pear

Year 1: Superficial scald control using the existing formulation and other formulations containing squalane needed to be demonstrated on 'd'Anjou'. In Year 1, we tested the previously established rate on 'd'Anjou' pears from an orchard in each of the Hood River, Yakima, Wenatchee, and Okanogan regions. We harvested 1296 fruit twice [2 weeks (early) and 1 week (late) before commercial harvest] from external canopies and double that from the Wenatchee location. Fruit were transported to TFRL, initial fruit quality evaluated, and 432 drenched 0.5% squalane formulation (E7), 432 drenched with 2000 ppm ethoxyquin, and 432 drenched with washed with water.

Additional pears (36 fruit/treatment/storage duration) from each location were drenched with 3 concentrations of another emulsion containing 0.5%, 1%, and 2% squalane, Triton X-100, and water.

Year 2: Repeated Year 1 harvest protocol from the same Hood River and Wenatchee locations. We added a 1.0 % E7 squalane treatment to test if scald control is improved without phytotoxicity at a higher rate. Another activity, testing the scald control efficacy of E7 treatments during 0.6% O₂ CA, were performed by placing pears in CA at harvest, treating one group with 0.5% E7 immediately and after 1, 2, and 3 months of storage. All scald evaluations will be on 100 pears per treatment.

Year 3: In Year 3, our focus has been on finishing our examination of the squalane emulsion on pears from multiple locations and maturities from the Hood River and Wenatchee Valley areas. Pears were harvested around commercial maturity from 4 locations around Hood River and 3 locations in the Wenatchee Valley, with 1 location in the Wenatchee Valley harvested at 5 different maturities. Pears from one Wenatchee Valley and one Hood River location are represented in all 3 years of the project.

Pears were treated with 0.5% squalane (E7 formulation) or 2000 ppm ethoxyquin immediately after harvest.

Storage and quality analysis. Pears from both harvest from every orchard as well as those treated with the Triton formulation (Years 1 and 2) were stored in commercial CA rooms (33°F; 1% O₂, 1.5 % CO₂) for 3, 6, or 8 months (Years 1-3), respectively. Pears from the Wenatchee location were also stored in air (33°F) for 3, 6, or 8 months (Years 1 and 2). For the delayed squalane trial (Years 2 and 3), pears were stored in TFRL CA chambers (33°F; 1.0% O₂, 0.5% CO₂) for 8 months.

Disorder and quality analysis. Scald incidence and severity as well as phytotoxicity and fruit quality are being evaluated upon removal from storage as well as after 7 and 14 at 68 °F (if intact) days of storage. Fruit quality and maturity was evaluated on all treatments at all sampling periods using fruit weight, I_{AD}, °Hue (green to yellow), firmness, soluble solids, starch index, titratable acidity, and whole fruit ethylene production. In Year 2, pears from each treatment will be peeled for subsequent metabolic analysis.

Objective 2: Determine mode of action of this new active ingredient

Year 1: Peel from pear from the Wenatchee location that were drenched with 0, 0.5, 1, and 2 % squalane emulsion formulated with Triton X-100 and stored in CA was sampled at 3, 6, and 8 months for chemical analysis to determine the mode of action of squalane.

Year 2: Test for scald control activity of other ingredients. Squalane in formula E7 was replaced with the same concentration of soybean oil (triacylglyceride) and tested alongside squalane-based E7 on pears harvested from both locations. Oleic acid, soybean oil, and squalane were formulated with Triton X-100 to confirm any scald-control properties using a dose response test. Peel will also be sampled at multiple pullouts from air and 3, 6, and 8 months CA from pears treated as in activities under objective 1. All scald control evaluations are performed on over 100 pears per treatment.

Year 3: Metabolic analysis of peel sampled from Year 2.

Objective 3: Determine any quality impacts and control of other appearance-related defects

Year 1: 180 pears (from each of 2 harvests) from the Yakima location were selected for an experiment looking into how antioxidant (ethoxyquin) and squalane treatments impact peel injury caused by elevated CO₂ in storage. 36 fruit (per treatment) at each harvest were left untreated or treated with 2000 ppm ethoxyquin (drench), 2000 ppm DPA (drench), 1% squalane/oleic acid emulsion (drench), or 2% squalane/Triton X-100 emulsion (drench). Pears are stored at 33°F, 0.5% O₂, 5% CO₂ to check for peel injury related to CO₂ sensitivity. Pear appearance will be evaluated at 6 months.

Year 3: CO₂ injury reduction by squalane (E7 formulation) is under evaluation. d'Anjou pears were harvested from Wenatchee Valley locations at around commercial maturity. Pears were treated with 0.5% squalane (E7 formulation) or 2000 ppm ethoxyquin immediately after harvest. Pears in TFRL CA chambers (33°F; 0.5% O₂, 5% CO₂) for 3, 6, or 8 months.

RESULTS AND DISCUSSION

Drench properties and immediate impacts on appearance and finish

Ripeness and other fruit quality attributes were not impacted by any of the treatments in either year. All emulsifiable concentrate formulations, applied as drenches, spread nicely on the fruit surface. The E7 formulation dried more slowly than the Triton X-100 formulation, but residue quickly

disappeared leaving no trace. None of the treatments have negatively impacted fruit quality. None of the formulations tested in Year 1 developed any detectable symptoms of phytotoxicity.

There was also no phytotoxicity in Year 2 associated with the principal squalane (E7) emulsion at any applied rate. The highest rate of squalane (4 mL L⁻¹) formulated with Triton X-100 caused minor darkened lenticels on pears from both locations. Oleic acid formulated in Triton X-100 caused more severe staining of the peel. Both formulations were merely meant to test control mechanisms and are not proposed for use in fruit production. In Year 3, there has not been peel damage associated with any treatment as of 3 months.

Wenatchee		Hue°					
Treatments	Storage duration (months+weeks, 33F+68F)						
	At Harvest	3M	6M	6M+2W	8M	8M+2W	
T1 - Control	113.5	112.0 a	109.1 a	95.7 c	109.7 a	95.6 bc	
T2 - 0.5% E7 (Squalane)	113.5	111.9 a	109.7 a	101.9 ab	111.3 a	101.2 a	
T3 - 1% E7 (Squalane)	113.5	108.6 a	111.8 a	103.8 a	111.2 a	101.0 a	
T4 - 0.5% E7 (Soybean oil)	113.5	112.3 a	110.3 a	97.8 bc	110.8 a	98.8 ab	
T5 - Ethoxyquin (2000ppm)	113.5	112.9 a	111.6 a	94.1 c	110.9 a	93.2 c	

Hood River		Hue°					
Treatments	Storage duration (months+weeks, 33F+68F)						
	At Harvest	3M	6M	6M+2W	8M	8M+2W	
T1 - Control	113.0	108.0 a	105.1 a	90.5 c	106.0 a	92.1 c	
T2 - 0.5% E7 (Squalane)	113.0	108.8 a	108.4 a	97.1 b	106.9 a	102.3 a	
T3 - 1% E7 (Squalane)	113.0	108.9 a	108.4 a	103.6 a	106.5 a	97.5 b	
T4 - 0.5% E7 (Soybean oil)	113.0	110.9 a	108.4 a	94.9 bc	103.6 a	93.2 c	
T5 - Ethoxyquin (2000ppm)	113.0	110.4 a	108.2 a	91.9 bc	104.8 a	94.1 bc	

Different superscripts within the column are significantly different at $p < 0.05$ by Tukey's HSD test.

Figure 1. Squalane (E7) drench reduced d'Anjou peel degreening after both 6 and 8 months CA storage in year 2. Squalane drenches when formulated with Triton X-100 had no impact on color. Squalane did not impact color in Year 1 in any formulation. A lower hue angle indicates more yellow than green peel in this evaluation.

Pear peel remained greener in pears treated with the squalane (E7) emulsion during ripening following 6 and 8 months of CA storage in Year 2. This is best indicated by the hue angle where a lower value indicates, in this case, peel that is more yellow (Figure 1). There was no color difference among any other treatments in Year 2, including squalane drenches formulated with Triton X-100. There was no difference of peel color among treatments during Year 1.

Scald control using emulsifiable concentrates containing squalane

In Year 1, out of 4 orchards, superficial scald only developed on pears from the Wenatchee and Hood River orchards following 6 months CA. Superficial scald development and etiology followed expected patterns on pears from the orchards providing a realistic testing scenario for these ingredients. Scald was not present upon removal from CA, even after 8 months, only developing and worsening over the 14 days at 68 °F (simulated retail shelf). Scald incidence was generally greater Hood River for the location, although it diminished more on pears harvested on the second harvest compared with Wenatchee. Scald did not develop on air stored fruit before it ripened *to the point of spoiling*.

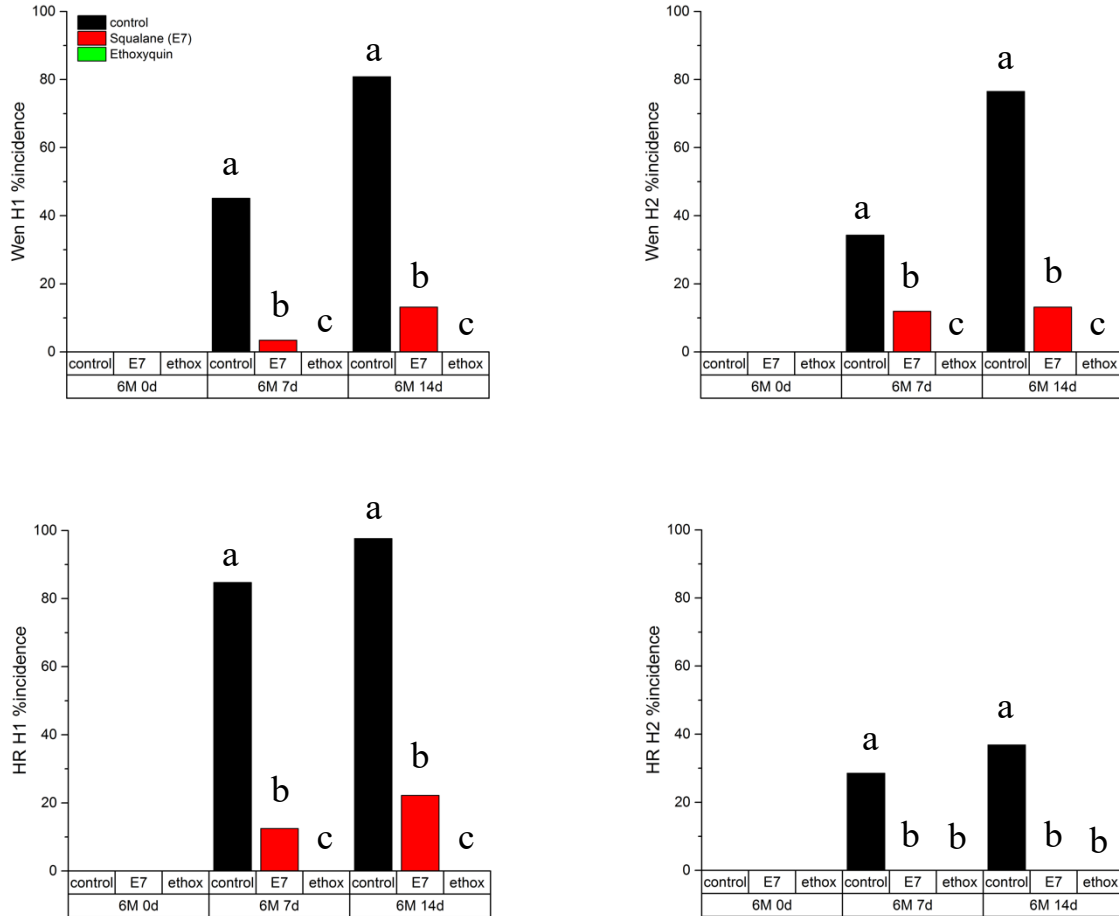


Figure 2. Squalane (red) and ethoxyquin (green) emulsions control or reduce scald compared to untreated (black) ‘d’Anjou’ pears. Pears harvested twice from Hood River (HR) and Wenatchee (Wen) were stored for 6 months in CA (33°F; 1% O₂, 1.5 % CO₂) and superficial scald rated at 0, 7, and 14 d at 68 °F. Significance was tested using a pooled z-test. Different lower-case letters within each group indicate different scald incidence among treatments at that rating period.

Our existing squalane emulsifiable concentrate (E7), applied at a rate of 0.5%, controlled or reduced scald to varying degrees depending upon orchard, harvest maturity, and storage duration (Figures 2 and 3). Ethoxyquin (2000 ppm) drench-controlled scald in most cases except pears harvested from Hood River at the early date, stored 8 months, and held at 68 °F + 14 d. Much less scald also

developed on ethoxyquin treated pears from Wenatchee by 8 months storage plus 7 days at 68°F on this treatment.

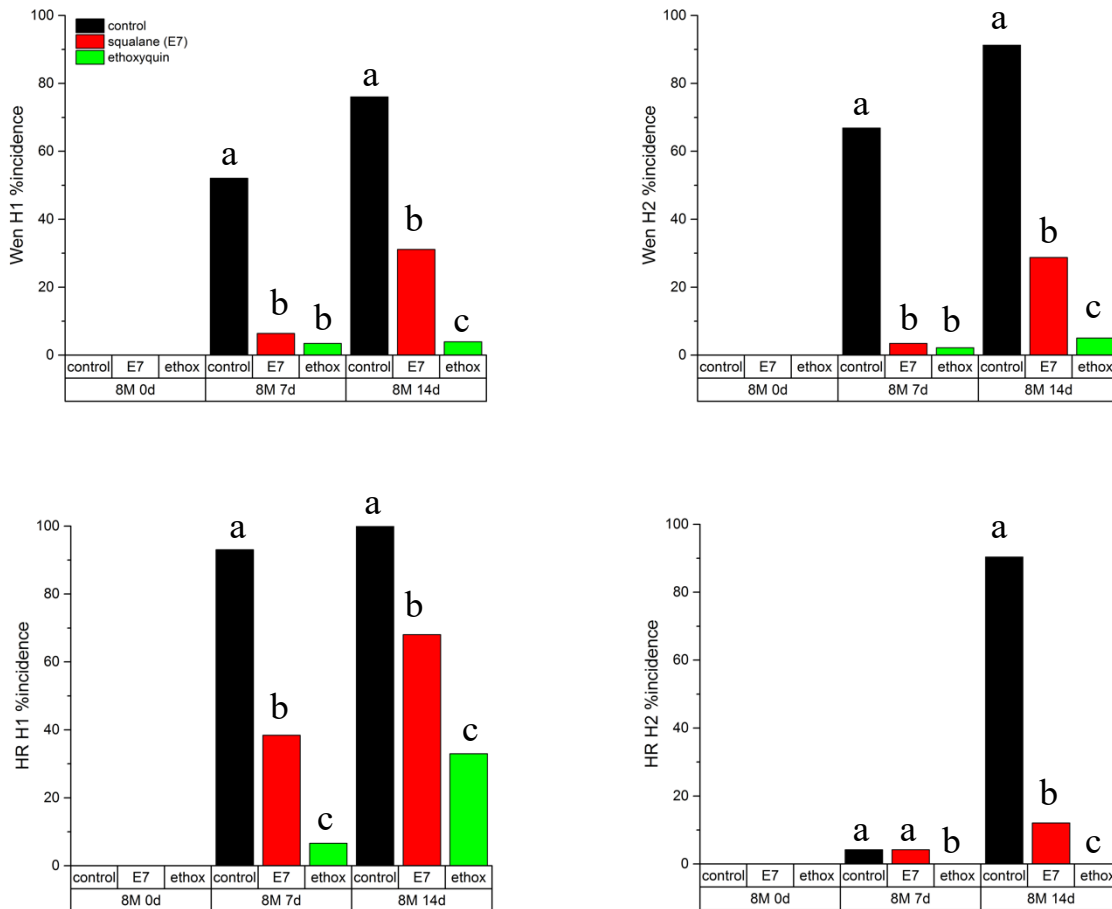


Figure 3. Squalane (red) and ethoxyquin (green) emulsions control or reduce scald incidence compared to untreated (black) ‘d’Anjou’ pears. Pears harvested twice from Hood River (HR) and Wenatchee (Wen) were stored for 8 months in CA (33°F; 1% O₂, 1.5 % CO₂) and superficial scald rated at 0, 7, and 14 d at 68 °F. Significance was tested using a pooled z-test. Different lower-case letters within each group indicate different scald incidence among treatments at that rating period.

While control using squalane (E7 formulation) drenches at the 0.5 % rate was not equal to that of the ethoxyquin, incidence was significantly reduced in all but the most severe cases where ethoxyquin was also inadequate (Hood River, Harvest 1, 8M CA). E7 reduced scald incidence to same levels as ethoxyquin on both harvests from the Wenatchee location following 8M CA + 7d and below 14% after 6 or 8M +7 d in every other instance except Hood River, H1, 8M + 7d. E7 controlled scald on pears stored for 6 months from Hood River, H2.

In Year 2, scald only developed on pears from the Hood River location. As in Year 1, scald began to develop during post-storage ripening after 6 months and was more severe after 8 months CA storage. Scald was reduced or eliminated by both rates of squalane (E7 formulation) drench to the same extent as the 2000 ppm ethoxyquin drench (Table 1). Soybean oil (substituted for squalane in E7) drench also controlled scald but only following 6 months CA.

Table 1. Squalane (E7 formula) drench controlled superficial scald of d’Anjou pears harvested from the Hood River area as effectively as ethoxyquin in Year 2. Pears were stored 3, 6, or 8 months in 1.5% O₂:1% CO₂ at 33°F and ripened for up to 14 d at 68°F. Scald was also controlled when squalane was substituted with soybean oil in this formulation after 6 M CA storage but not after 8 months. Pears harvested in the Wenatchee Valley in Year 2 did not develop scald.

Treatment	Superficial scald incidence (%)								
	Storage duration								
	3M	3M+7d	3M+14d	6M	6M+7d	6M+14d	8M	8M+7d	8M+14d
control	0	0	0	0	0	85 a	0	78 a	100 a
0.5% squalane (E7)	0	0	0	0	0	6 b	0	0 b	6 d
1% squalane (E7)	0	0	0	0	0	0 b	0	0 b	22 c
0.5% soybean oil (E7)	0	0	0	0	0	0 b	0	67 a	83 b
Ethoxyquin (1000 ppm)	0	0	0	0	0	2 b	0	0 b	9 cd

Significance was tested using a pooled z-test (n=54, p<0.05). Different lower-case letters within each group indicate different scald incidence among treatments at that rating period.

Squalane mode of action in superficial scald control

The squalane (E7) emulsion controls d’Anjou superficial scald. However, it is conceivable, given the nature of the chemicals, that inactive ingredients in the E7 formulation may have a role in scald control. Developing an emulsifiable formulation from the E7 formulation without squalane to test whether squalane is the principal active ingredient was not impossible. Our goal was to establish if squalane is the sole active ingredient in this formulation and determine a mode(s) of action.

To begin to determine if squalane was the sole ingredient actively controlling scald, in Year 1, we formulated a simple emulsion by mixing squalane at concentrations of 0, 0.5, 1.0, and 2 mL L⁻¹ with Triton X-100, a surfactant. Emulsions using this formulation appeared stable and complete (no oil droplets on the surface of the drench). While control was evident, especially at the 2 mL L⁻¹ rate, following both 6 and 8M storage and both 7 and 14d on pears from both locations, a dose response was not universally observed (see Year 2 report). Differences of formulation may influence efficacy of the squalane resulting in the observed differences of scald control. Also, even though instability of the emulsion was not obvious, variability of fruit or solution temperature may have impacted efficacy. However, given the many instances of dose-driven scald control in these tests, we can assume there is some relationship with squalane and scald control.

In Year 2, we extended our analysis to include, where possible, emulsifiable concentrates of the principal inactive ingredient (oleic acid) with Triton X-100. We also substituted soybean oil (triglyceride) for squalane in both the E7 formulation as well as with the Triton X-100 to approximate the “oiliness” of the squalane formulations, to test if that may be a scald control mechanism. While squalane formulations reduced or eliminated scald, soybean oil only reduced scald following 6 months CA and only the E7 formulation was effective (Table 2). Soybean oil easily formed more stable emulsions than squalane in the E7 or Triton X-100 emulsions. It is still possible that a higher concentration of soybean oil may afford more scald control but not at the comparable rate to squalane. A second dose-response study of squalane formulated with Triton X-100 using a greater concentration range (up to 4% squalane) yielded a clearer dose response effect on scald incidence than in Year 1. This was further supported by a comparison of 0.5% and 1% squalane (E7) emulsions where the 1% provided more scald control (Table 1). Evidence-to-date indicates that squalane is the

active ingredient controlling scald in these formulations, although the coating effect of higher rates of soybean oil than those tested may afford some scald reduction and should be tested.

Table 2. d’Anjou pear superficial scald reduction by squalane emulsion formulated with Triton X-100 is dose dependent. Pears were stored 6 or 8 months in 1.5% O₂:1% CO₂ at 33°F and ripened for up to 14 d at 68°F. Only timepoints where scald was present are reported. Oleic acid (an inactive ingredient in formula E7) and soybean oil did not impact scald incidence when formulated with Triton X-100.

Treatment	Superficial scald incidence (%)		
	Storage duration		
	6M+14d	8M+7d	8M+14d
Control (5 mL/L Triton)	15 a	80 ab	96 ab
Control (7.5 mL/L Triton)	0 b	61 bc	85 c
1 mL/L Squalane (5 mL/L Triton)	6 ab	78 ab	96 ab
2 mL/L Squalane (5 mL/L Triton)	0 b	46 c	81 c
4 mL/L Squalane (7.5 mL/L Triton)	0 b	28 d	72 c
1 mL/L Soybean oil (5 mL/L Triton)	0 b	61 bc	100 a
2 mL/L Soybean oil (5 mL/L Triton)	6 ab	65 bc	87 bc
4 mL/L Soybean oil (7.5 mL/L Triton)	0 b	59 c	87 bc
1 mL/L Oleic acid (5 mL/L Triton)	4 ab	61 bc	94 abc
2 mL/L Oleic acid (5 mL/L Triton)	0 b	87 a	98 ab
4 mL/L Oleic acid (7.5 mL/L Triton)	9 a	59 c	91 bc

Significance was tested using a pooled z-test (n=54, p<0.05). Different lower-case letters within each group indicate different scald incidence among treatments at that rating period.

Delayed treatment with squalane emulsion following storage in ULO CA controls scald

Short-term storage of pears before packing is often necessary during harvest when packing lines are fully committed. In air or conventional CA storage, scald mitigation treatments should be applied as rapidly as possible to be most effective. However, in apples this period can be extended using ultra-low oxygen (ULO; 1% or less O₂) conditions. A preliminary experiment was performed in Year 2 to determine if squalane (E7) emulsion would be effective following up to 3 months of ULO-CA. Pears were placed immediately into ULO CA (1.0% O₂: 0.5% CO₂) following harvest. Trays of fruit were drenched with 0.5% squalane (E7) emulsion or 2000 ppm ethoxyquin at 0, 1, 2, and 3 months, air dried, and immediately placed back into ULO-CA for a total of 8 months. Appearance, scald risk assessment chemicals (CTOL), and quality was evaluated at 8 months + 7 d. This preliminary study indicated that scald was reduced or controlled following delayed treatments under these conditions (Figure 4). CTOL levels (scald risk assessment) indicated lower scald risk that was later reflected by reduced scald incidence. An expanded version of this experiment is being performed in Year 3.

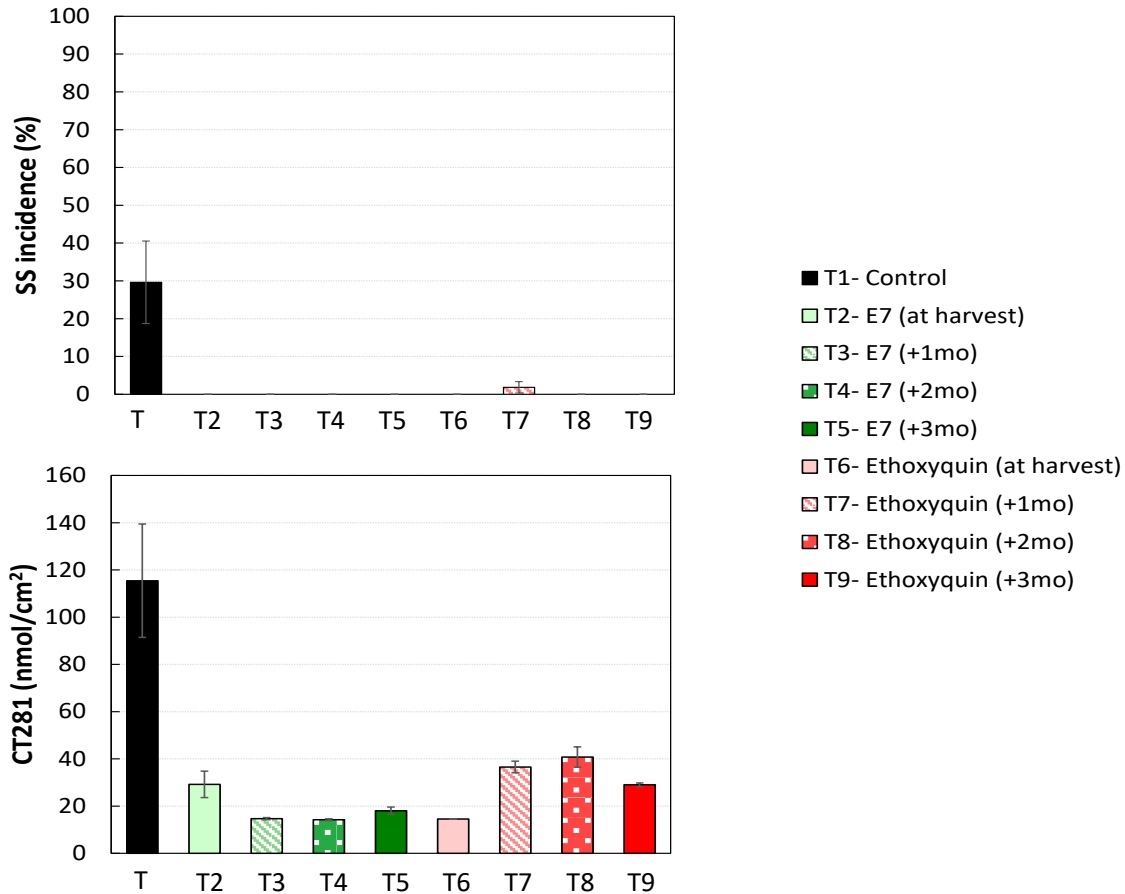


Figure 4. Scald control was effective even if squalane (E7 formula) and ethoxyquin drenches were delayed up to 3 months for d'Anjou pears stored in ULO CA (1.0% O₂:0.5% CO₂). CTOL (CT281) (scald risk indicator) levels at 8 months were reduced by both active ingredients. Pears were stored for 8 months at 33°F and ripened 7 d at 68°F.

Conclusions

Squalane-based emulsions reduced or eliminated scald. Reduction of scald using squalane (E7 formulations) drenches was as or nearly as efficient at controlling scald as ethoxyquin during ripening following 8 months of CA storage. Delaying drenching treatment with squalane (E7) or ethoxyquin up to 3 months during ULO CA (1.0 % O₂: 0.5 % CO₂) was as effective at controlling scald during ripening following 8 months storage as immediate treatment. The primary formulation did not cause any phytotoxicity at any of the effective rates. Peel degreening was reduced by squalane treatment in one out of two years. Beyond understanding that squalane can control scald, the mechanism for control is not known. Studies of mechanism indicate that squalane is the primary active ingredient in the E7 formulation as oleic acid (inactive ingredient) did not control scald.

Project Title: New active ingredients for pear superficial scald control (PR-19-103)

Executive Summary

Keywords: pear, cold chain, fruit finish, superficial scald, squalane, scald control

Abstract: With diminishing market acceptability of ethoxyquin, new tools and strategies are required to control d'Anjou superficial scald that do not have negative impacts on eating quality. A drench containing squalane was effective for controlling superficial scald of Packham's Triumph is also as or nearly as effective at controlling scald of d'Anjou as ethoxyquin following 8 months of conventional CA storage. Furthermore, delaying drenching up to 3 months in ULO CA conditions was as effective at controlling scald as drenching immediately following harvest. No negative impacts on other appearance or quality attributes were indicated when using effective rates of the principal formulation. Our evaluation of mechanism indicates squalane is the active ingredient, although more work using this type of formulation and soybean oil or other triglycerides is warranted given incomplete results. Commercialization of the squalane emulsion for this purpose is underway.

Project outcome:

1. A drench that controls d'Anjou superficial scald with similar efficacy as ethoxyquin without negatively impacting appearance or quality.

Significant Findings:

1. Formulations containing squalene reduced or eliminated superficial scald of 'd'Anjou'.
2. Control using squalane emulsions was comparable with ethoxyquin drenches.
3. Squalane (E7 formulation) emulsion drenches can impact peel degreening.
4. Squalane is the active ingredient in these formulations.

Future Directions:

1. Test other application methods such as fogging in storage or orchard spray application.
2. Test different CA atmosphere conditions that can be used effectively with delayed squalane application to control scald.
3. Further evaluation of soybean oil and other triglycerides formulated similarly to squalane as scald control drenches.
4. Find additional pear scald control strategies that can be used in a variety of regulatory conditions.