FINAL PROJECT REPORT
WTFRC Project # AH-03-308

Project Title:	Efficacy and physiological effect of oil/lime sulfur combinations
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Cooperators:
AppleEye, Mustang and Baird Orchards in WA
Sunny Slope Orchard in PA
Prospect Hill Orchard in NY
Singer Farms in NY

Objectives:

Research has demonstrated that apple can be thinned by photosynthetic inhibition caused by shading or sub-lethal doses of herbicides (Byers, et al., 1990). In the era preceding modern pesticides the use of lime sulfur was known to reduce apple fruit set (Burrell, 1945). In Japan, where lime sulfur is a registered thinner, two applications are typically made, the first at full bloom, and the second at petal fall (Koike and Ono, 1998). Burrell (1945) noted that lime sulfur at petal fall or shortly after caused the greatest reduction in fruit set. Conversely, Byers et al. (1990) showed that applying photosynthetic inhibitors closer to June drop caused more thinning than earlier timings.

Recent research in both WA and NY has shown that a tank mix of Crocker's Fish oil and liquid lime sulfur (FOLS) is an effective thinner when applied during bloom. Research conducted in N.Y. in 2001 and 2002 demonstrated that post-bloom sprays of FOLS were even more effective than sprays applied during bloom. If, as we suspect, photosynthetic inhibition is the primary cause of thinning, then post-bloom sprays should provide greater efficacy. These studies were conducted to evaluate the effect of timing on FOLS thinning efficacy and photosynthetic responses.

A second question focuses on alternatives to fish oil. There are several concerns with fish oil when used in this thinning mixture. Phytotoxicity is one concern. Apple growers have a reluctance to utilize this thinner because of its expense and repulsive odor. Research to date has been conducted using oil from a single small source in Washington State. The purity and consistency of fish oil from other sources is unknown. Fish oil may function as a surfactant and penetrant, and it may also have a direct thinning effect. A second objective of these studies was to evaluate the efficacy of several surfactants and oils in combination with lime sulfur for thinning apples.

The objectives of these studies were:
- To evaluate the effect of timing of fish oil / liquid lime sulfur (FOLS) applications on fruit set, size and quality, photosynthesis and leaf anatomy of apple.
- To evaluate the effect of alternatives to fish oil in combination with liquid lime sulfur on thinning efficacy and physiological responses of apple trees.
- To evaluate the effect of spray volume and concentration on FOLS efficacy and safety.

Significant Findings:
- FOLS sprays reduced photosynthesis (Pn) rates shortly after treatment, with recovery evident within four to five days in WA. Recovery of Pn following FOLS sprays in eastern sites was much slower.
-Double sprays of FOLS were effective for consistent thinning in eastern orchards, while three sprays were necessary in WA.
-Post-bloom FOLS sprays were more effective in the east, while FOLS sprays during bloom were better in WA.
-Spacing three FOLS sprays at a 4 day interval thinned Washington State Gala more effectively than longer or shorter intervals (2004).
-Tank mixing LS with fish oil or soybean oil provided more thinning of Gala fruit than dormant (petroleum) oil, while LS alone was ineffective (2004).
- FOLS reduced the size of leaf epidermal cells, palisade cells, and spongy mesophyll cells in leaves of Gala trees in N.Y. State in 2004, but had no effect on leaf anatomy in either WA or N.Y. in 2005.
- Spraying FOLS at 200 gallons per acre was slightly more effective than at 100 or 50 gallons per acre. Concentrating spray materials in lower spray volumes did not give a greater response.

Methods:
Timing and Spray Interval Studies:

To evaluate the effect of post-bloom applications of FOLS, treatments were made using different timings and different intervals. In 2003 the treatments in both N.Y. and WA consisted of single and double applications of FOLS beginning at PF and extending through 21 days after petal fall. One triple spray was applied in WA starting at PF and repeated at seven day intervals. Based upon the 2003 results, the 2004 protocol was adjusted to start treatments at 80% full bloom in WA, with two repeat sprays at two, four, or eight day intervals. Similar studies were conducted in N.Y. and PA, using two applications of FOLS at the same intervals.

Fruit set was evaluated after June drop. Yield was recorded in eastern studies, and fruit size and quality were evaluated at all sites using standard methods.

Leaves were measured for Pn activity 48 hours after spraying and thereafter until treatment differences dissipated. Leaf phytotoxicity ratings were taken three days after treatment using a 1-5 scale. Data were gleaned from anatomical examination of Gala leaves harvested in Geneva, New York from trees treated with FOLS and from untreated trees in 2004, and from both N.Y. and WA studies in 2005.

Evaluation of Alternatives to Fish Oil:

The thinning efficacy of lime sulfur was evaluated by itself, and with either a non-ionic or an organosilicone surfactant, or in combination with various oils. Petroleum- and vegetable-based oils were compared to Crockers fish oil. Oils were applied at 2% (vol:vol), and LS was applied at 2.5%. Surfactants were applied according to the respective labels. All treatments were applied with an air-blast or Prop Tech sprayer.

Fruit set, yield, fruit size, fruit color and russet were evaluated. Fruit size distribution was determined in N.Y. and PA, using commercial grading equipment. Leaf damage was rated.

Spray Volume and Concentration Studies:

Studies were conducted in 2003 and 2004 to evaluate the effect of spray concentration and volume on FOLS efficacy. Spray volumes of 50, 100, and 200 gallons per acre were applied and the materials either concentrated to apply the same amount of materials per acre, or not concentrated so as to apply the same amount of materials per 100 gallons of spray mix.

Results and Discussion:
Timing and Spray Interval Studies:

Post-bloom applications of FOLS were not as effective in Washington as in the eastern states. Over all the experiments in the three years of this project, post-bloom oil and lime sulfur treatments were effective in reducing fruit set in just 14% of the treatments applied in WA. This contrasts with the outcomes in N.Y. and PA over the same period, where 70% of the post-bloom treatments reduced set. The low incidence of successful post-bloom thinning in WA also contrasts sharply with bloom thinning trials in WA, were oil and lime sulfur treatments thinned in 61% to 74% of the cases, depending on which oil was used (WTFRC data, 1999-2004).

Evaluation of photosynthesis following treatment in 2003 showed that FOLS reduced net photosynthesis for about 4-5 days following treatment in WA (Figure 1). We theorized that the effects of the single and double timings on Pn were too short in duration to impact fruit set, and the fairly rapid Pn recovery rate suggested that greater thinning efficacy may have been obtained by shortening the intervals between applications to less than seven days.

Following review of the 2003 results, we adjusted the timing study in WA to include treatments that began at 80% bloom, and increased the number of sprays to three to see if we could increase efficacy. In 2004, three sprays of FOLS at 4 day intervals starting at 80% bloom gave the greatest thinning response, and fruit size was increased by FOLS spray protocols that started at 80% bloom and were repeated at either 2- or 4-day intervals. Unfortunately, results in 2005 were not consistent enough to confirm this outcome.

Our results suggest that, if photosynthetic inhibition is important to cause thinning, an interval of about four days between sprays may prove to be the most effective in WA, because it suppresses photosynthesis over the longest time period and without allowing recovery of Pn between sprays. Based upon our results, post-bloom FOLS protocols have been less effective in WA than in eastern U.S. trials, and haven't increased fruit size as well as earlier timings.

WA orchards receive higher light intensity than do eastern orchards, which may explain why photosynthetic inhibition is less effective as a thinning mechanism here. The total amount of light received by WA orchards may raise the threshold for thinning efficacy with photosynthetic inhibitors. Reducing Pn to 50% the level of the untreated trees for four or five days under high light conditions, as our treatments did, may not reduce the carbohydrate supply below the fruit thinning threshold under such conditions. High light intensity in WA results in thicker leaf cuticle development (Table 1), which may also lessen FOLS efficacy by limiting chemical uptake.

While this research has confirmed that FOLS efficacy isn't limited to the bloom period, the bloom period appears to still be the best application window for WA, as it includes not only photosynthetic inhibition but also the effect of FOLS as a blossom thinner. While we had hoped that our research would increase the utility of FOLS to western growers by extending the effective application window beyond petal fall, as occurs in eastern U.S. orchards, this appears not to be the case.

Leaf Anatomy:

Leaf anatomy analysis in N.Y. showed no obvious external or internal phytotoxic changes in cell or organ structure between controls and FOLS leaves in 2004. Parameters measured are illustrated in Figure 2. The lower photosynthetic capacity of FOLS-treated spur leaves appeared to relate to differences in leaf anatomy. FOLS treated primary spur leaves had smaller epidermal cells, smaller cells in the palisade layers, and thinner cells in the spongy mesophyll region (Table 1). Bourse leaves were much less affected by FOLS than were the primary leaves. The influence of primary spur leaves is important for fruit set and early fruit growth, but diminishes once shoot leaves, such as bourse leaves fully emerge and begin to contribute to assimilation. Additional anatomical work in 2005 was conducted to see if primary spur leaves are consistently more impacted by FOLS sprays than are shoot leaves, which, if it were the case, would provide insight into why the earlier timings seem to have been more effective than later timings in WA.

The anatomy component of the overall project was designed to test whether anatomical differences accompany or help explain differences in fruit thinning between FOLS and non-sprayed control trees, especially through FOLS' possible negative impact on photosynthesis or through phytotoxic effects on leaves. We did see some phyotoxiciy (stippling) of leaves treated with FOLS in both WA and NY, but it was inconsistent as to position on the leaf or to position within the canopy. We hypothesized that we would see more limited cuticle development in FOLS-treated leaves compared to controls, especially in NY State, where normally cooler weather and decreased light intensity compared to WA State could limit cuticle development. Although cuticle thickness did not vary much between spray treatments, WA spur leaves did develop a thicker cuticle than did NY leaves (Table 1). This difference may partially explain the extra thinning effectiveness of FOLS sprays seen in NY compared to WA. The lower photosynthesis readings found for FOLS-treated spur leaves in NY (Figure 1) did not appear to affect leaf anatomical features a day or two after treatment. There also did not appear to be a carry over effect of such treatments as discovered in the anatomical analysis of NY leaves 1 month post bloom. The FOLS treatment at bloom time in WA also did not appear to affect leaf structure as seen in the 1-month post bloom collection. We did not have a bloom time collection of WA spur leaves to see whether there was a short-term affect on leaf anatomy just after spraying the younger spurs. We saw little evidence for this in the NY situation. Because so much of the effect of FOLS appears to impact the biochemical/physiological mechanism operating during photosynthesis, any future study of the effect of FOLS should include more detailed study of chloroplast ultrastructure using electron microscopy.

Fish Oil Alternatives:

Applications of lime sulfur alone were not effective for thinning apples in any of our experiments. Following inconclusive results in WA in 2003, tank mixing LS with either fish oil or soybean oil provided the most thinning activity in both WA trials in 2004. Petroleum oil plus LS also provided a moderate amount of thinning. Individual fruit size was greatest with soybean oil+ LS at both locations, while petroleum oil+LS also increased fruit size at one of the locations in 2004. None of the oil+lime sulfur treatments were effective in WA in 2005. Fruit russet has not been a problem with any of the oil and lime sulfur combinations

Combining lime sulfur with surfactants was shown to be ineffective in two years in NY. The addition of petroleum-based oil with lime sulfur, such as dormant oil and JMS Stylet oil, has been consistently effective. In several of our studies the petroleum oil tank mixes have resulted in the best fruit size, despite slightly less reduction in fruit set. It would seem that the challenge in eastern orchards is to use caution not to inhibit Pn so long as to hamper fruit growth, while in WA the challenge is to get a strong enough reduction in Pn so as to achieve thinning.

Spray Volume and Concentration:

As with oil alternatives, the discussion on spray volume and concentration must be based upon results in eastern orchards due to the lack of significant results in WA. Regression analysis showed that the greater the spray volume of water the greater the thinning efficacy of FOLS sprays (Figure 3). This would suggest that dilute sprays provide better coverage of flowers and young spur leaves giving greater effect. The rate of lime sulfur and fish oil did not affect thinning performance of this combination at either 2X or 4X spray volumes.

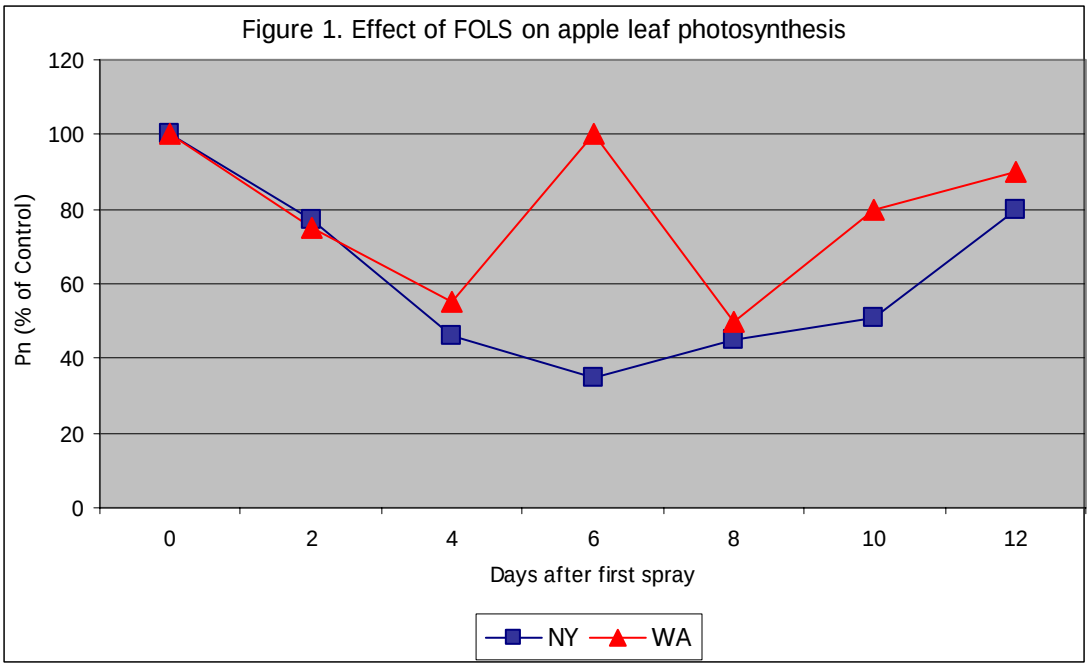

Table 1. Comparison of means for Gala primary spur leaves of control trees and trees treated with FOLS or FOLS+Shade in New York or with FOLS in Washington State in 2005.

Location and Date	Treatment	Pn: Net Photosyn-thesis[1]	Upper Cuticle Thick. (μm)[2]	Upper Epid. Cell Thick. (μm)	Upper Palisade Cell Ht. (μm)[3]	Upper Palisade Chloro-plast L. (μm)[4]	Lower Palisade Cell Ht. (μm)[5]	Lower Palisade Chloro-plast L. (μm)	Spongy Meso-phyll Thick. (μm)[6]	Lower Epid. Cell Thick. (μm)	Total Leaf Thick. (μm)
New York 19 May	Control	12.1 ± 0.7	0.70 ± 0.08	12.5 ± 0.8	36.7 ± 2.6	3.4 ± 0.1	37.6 ± 2.2	3.6 ± 0.1	118 ± 7	12.1 ± 0.7	217
	FOLS	5.6 ± 1.1	0.73 ± 0.08	12.8 ± 0.9	36.5 ± 2.1	4.2 ± 0.1	36.1 ± 2.1	4.6 ± 0.1	119 ± 5	11.8 ± 0.9	217
	FOLS+Shade	8.5 ± 2.1	0.70 ± 0.08	12.0 ± 0.7	34.9 ± 1.7	4.0 ± 0.1	34.5 ± 1.7	4.2 ± 0.1	111 ± 6	11.3 ± 0.5	204
New York 21 June	Control	*	0.58 ± 0.03	13.4 ± 0.4	48.4 ± 1.1	4.8 ± 0.1	47.6 ± 1.28	5.2 ± 0.1	133 ± 4	12.4 ± 0.4	254
	FOLS	*	0.63 ± 0.03	13.5 ± 0.4	45.5 ± 1.7	4.8 ± 0.1	43.0 ± 1.6	5.2 ± 0.1	140 ± 6	11.6 ± 0.2	254
	FOLS+Shade	*	0.57 ± 0.04	13.3 ± 0.4	45.5 ± 0.9	4.8 ± 0.1	42.9 ± 1.0	5.4 ± 0.1	120 ± 6	12.2 ± 0.6	234
Washington 1 June	Control	9.5 ± 0.4	0.85 ± 0.17	15.7 ± 0.7	36.9 ± 3.2	4.2 ± 0.1	30.2 ± 2.4	4.2 ± 0.2	90 ± 6	12.4 ± 0.7	186
	FOLS	7.6 ± 1.2	0.78 ± 0.15	15.4 ± 0.1	38.1 ± 2.0	4.4 ±0.1	34.0 ± 1.4	4.1 ± 0.2	106 ± 5	12.2 ± 0.7	205

[1]Pn measured as µmol/m2/s.
[2]Thickness of waxy covering on cells on upper leaf surface.
[3]Vertical height of photosynthetic cell layer just below the epidermal cell layer.
[4]A measure of the size and capacity of the chloroplast to produce photosynthate or to store starch.
[5]Vertical height of photosynthetic cell layer just below the upper palisade cell layer.
[6]Vertical thickness of the aerated photosynthetic tissue filling the lower half of the leaf's thickness.
*Not recorded for samples leaves in NY in the June collection.

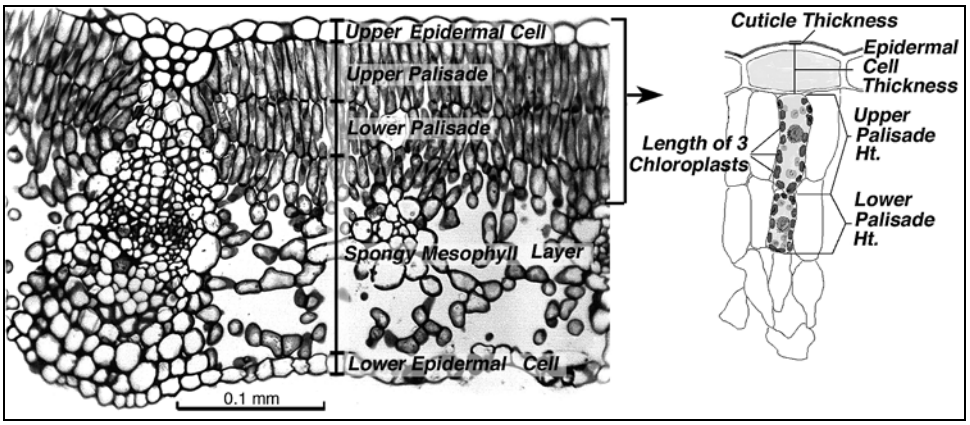

Figure 2. Anatomical parameters measured for each leaf processed for histological examination.

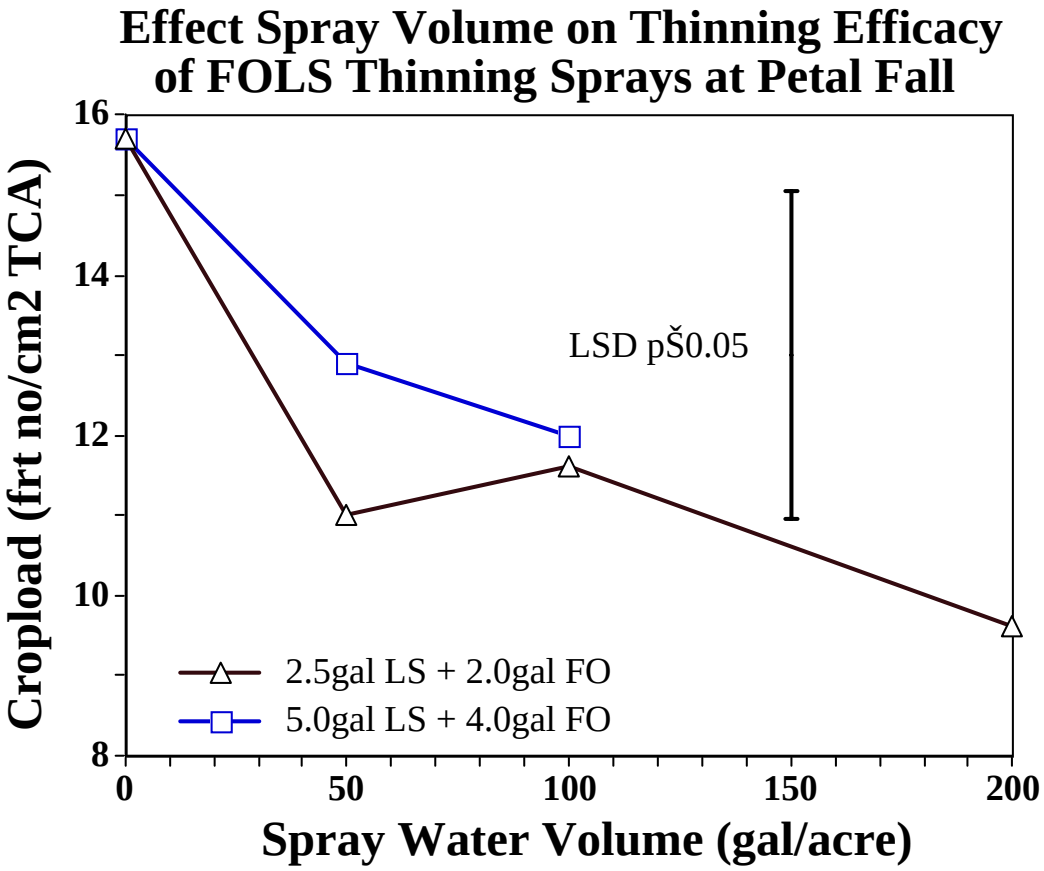

Figure 3. Effect of FOLS spray volume on cropload of Gala/M.9 apple trees from sprays applied at bloom.

Literature Cited:

Burrell, A. B. 1945. Practical use of our newer knowledge of apple scab control. Proc. 90th NY State Hort. Soc. pp. 9-16.

Byers, R.E., J.A. Barden, R.F. Polomski, R.W. Young and D.H. Carbaugh. 1990. Apple thinning by photosynthetic reduction. J. Amer. Soc. Hort. Sci. 115: 14-19.

Koike, H., and T. Ono. 1998. Optimum crop load for apples in Japan. Compact Fruit Tree 31:13-16.

Budget:

Project Title: Efficacy and Physiology of Oil / Lime Sulfur Apple Thinners.
PI: Jim Schupp
Project duration: 2003-2005
Current year: 2005
Project total (3 years): $54,000

Item	Year 1(2003)	Year 2 (2004)	Year 3 (2005)
Salaries	6165	0	0
Benefits	2362	0	0
Wages	4680	10,000	11,206
Benefits	1793	4100	4594
Equipment	0	0	0
Supplies	3000	2200	2200
Travel	0	1700	0
Miscellaneous	0	0	0
Total:	18,000	18,000	18,000