

***FINAL REPORT**

TITLE: ALTERNATIVE APPLE CROP THINNING STRATEGIES

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PROJECT SUMMARY

Thinning the apple crop during the bloom and immediate post bloom period is absolutely essential to ensure large fruit size, superior fruit quality, and reliable annual cropping. Fundamental approaches to evaluating potential new alternatives to thinning were studied in a revised project for 2003. The focus of the effort was to affect fruit crop set during the bloom period by interrupting the pollination, fertilization or early fruit set period. This could be accomplished by killing pollen, preventing or stopping pollen germination, killing pistils thereby preventing fertilization, or limiting carbohydrate supply by limiting photosynthesis (Fig 1). All of these conditions would result in fruit drop and thereby reduced fruit set.

The focus of studies was to develop a fundamental understanding of pollen and fertilization biology of apples *in vivo* and in the field, and to study photosynthesis suppression of model vegetative plants in greenhouse conditions thereby potentially limiting carbohydrate supply for fruit set.

OBJECTIVES:

1. Investigate the potential of alternative apple thinning chemical with three different modes of action:
 - a. Prevent pollination and fertilization of developing seeds
 - b. Photosynthetic inhibitors that may cause fruitlet abscission
 - c. Stimulate internal ethylene causing embryo abortion and abscission of fruitlets
2. Field test promising chemical treatments (planned for 2004)

ACCOMPLISHMENTS AND SIGNIFICANT FINDINGS

This project was initially proposed as a three-year project and partially and conditionally funded for one year. Thus, in this final report, the accomplishments and activities of 2003 are presented. Because of amended budgets, the project was revised to focus on laboratory and model system studies to understand the biology of fruit thinning and to screen potential thinning chemicals.

The strategies used were to prevent pollination by preventing flowers from opening, preventing anthesis, preventing pollen germination, killing germinated pollen, killing pistils to prevent fertilization, and/or prevent fruitlets from developing by limiting carbohydrate supply or generating internal ethylene causing fruit set (Fig 1).

During this past year we have developed *in vitro* systems for pollen germination and growth, and pistil viability to test potential alternative thinning agents. A number of potential osmotic agents, pH modifying solutions, salts, biological oils and extracts have been screened in the *in vitro* systems. We found that the *in vitro* pistil tests correlated to fruit set in field tests and was a reliable indices of thinning potential.

Vegetative model plants of apple were used to study the impact of potential thinning agents on photosynthesis and growth as a means of screening materials which may cause reductions in fruit set by limiting carbohydrate supply.

Vegetative model plants of apple to assess treatments which may produce internal ethylene production thereby stimulating seed abortion and fruit abscission have not been successful at this point due to instrumentation and calibration difficulties. This method is still being pursued.

The objective of developing methods and beginning screening of a number of chemicals, biological extracts and oils, and other compounds which may be useful for thinning was accomplished and the methods are now ready to be fully employed. These methods will lead to identification of potential alternative thinning methods to be used for field treatments in future studies.

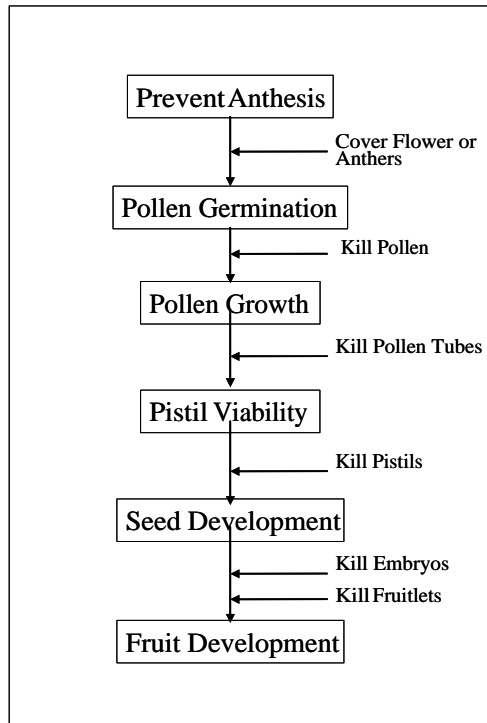


Figure 1. Overview of strategies for preventing pollination, fertilization and/or fruit set during the bloom period of apple. This figure also demonstrates the line of study by which potential thinning chemicals will be screened. Chemicals will be screened in a sequence 1) as pollenicides, 2) as pistilicides, 3) as photosynthetic inhibitors, and/or 4) as embryocides or abscission agents via ethylene.

METHODS AND RESULTS

Objective 1. Evaluation of Potential Thinning Agents - Model Plant Studies

Objective 1.A Preventing flower pollination and fertilization.

A. Inhibition of flower opening and anthesis.

Three concentrations of a natural lecithin polymeric film (NPF) were sprayed on apple 'Gala' and 'Pink Lady' flowers on mature trees under field conditions. All three concentrations significantly reduced the number of flowers opening and thereby reduced fruit set. Fruits remaining on limbs treated with the NPF were larger than on limbs of untreated control although there was no difference in soluble sugars, firmness or russet. The higher density and concentrations of NPF caused significant foliage burn (see Pn studies below).

B. Inhibition of pollen germination and pollen tube growth.

Pollen germination and growth were studied *in vitro* by applying apple pollen (Firm Yield Pollen Services, 'Golden Delicious') to an agar media (15/l) with sucrose as a carbon source (150g/l) with 10ml agar placed in 9cm petri dishes, then placed in controlled temperature incubators (25± 1 C), and observing at 4, 8, 12, 24, 48, and 72 hours. Studies were later condensed to evaluate treatments at 4, 12, and 24 hrs as there was little treatment effect in preliminary studies after 24 hrs. Treatments were applied to duplicate or triplicate dishes with 3-5 individual field readings (90x

magnification) made for each plate. Data collection included percent of pollen germinated, and a rating of pollen tube vigor on a scale of 0-5 (0 - no growth, 5 - vigorous pollen tube growth).

Test materials were applied either prior to application of pollen to evaluate prevention of germination, or after pollen had germinated and grown for 24 hrs to evaluate lethality to pollen germ tubes. Pollen germination or germinated pollen may be killed by several different effects; 1) osmotic effects causing cellular desiccation, 2) pH effects causing cellular disruption or improper conditions for metabolism, 3) saponified fatty acids and lipids (soaps and natural oils) causing membrane disruption.

Because pollen and pistil viability may be affected by the chemical properties of potential thinning agents, a series of preliminary tests were conducted to determine the effects of osmotic tension, solution pH, and electrical conductivity (dielectric salt solution), on pollen and pistils.

1. Effect of osmotic stress on pollen germination and growth.

Pollen *in vitro* as described above were exposed to a range of solution osmotic tensions strengths (0 to 5 MPa) created by mixing concentrations of polyethylene glycol (PEG; MW = 10,000).

Pollen germination was significantly reduced with 24 hrs by osmotic tensions of ≥ 3 MPa.

2. Effect of electrically conductive salt concentration on pollen germination and growth.

Pollen *in vitro* as described above were exposed to a range of solution electrical conductivities of a dielectric salt solution from 19mv (water) to 100 mv (10% NaCl solution). Pollen germination was reduced to less than 10% at 62mv EC and 0% at ≥ 100 mv EC at 24 hrs.

3. Effect of solution pH on pollen germination and growth.

A. Solution pH was increased by adding concentrations of NaOH from 0 - 10% creating a pH range of 7.0 - 13.8. Solution pH was decreased by adding HCl water creating a pH range of 7.0 - 2.0.

Pollination germination was reduced by more than 50% at solution pH ≤ 4.0 and ≥ 10.0 and completely inhibited at solution pH ≤ 3.0 and ≥ 11.0 . It is interesting to note that the a 1% solution of calcium polysulfide (lime-sulfur) has a pH of 11.1

4. Solution Characteristics of potential thinning agents with osmotic tension, pH or salt content as potential modes of action.

The solution characteristics of osmotic tension, pH, and electrical conductivity at concentrations of 0, 0.25, 0.5, 1.0, 2, 5, and 10% were measured for the following potential organic thinners which could have mode of action as pollenicides or pistilicides: calcium polysulfide (lime sulfur), CaCl_2 , CuSO_4 , $\text{C}_2\text{H}_4\text{O}_2$ (acetic acid), NaOH, NaCl, $\text{Na}_2\text{S}_2\text{O}_5$, $\text{K}_2\text{S}_2\text{O}_7$, KHSO_4 , and KHCO_3 . These compounds at concentration resulted in a range of characteristics with osmotic tension ranging from 0.6 to - 70 MPa, to pH ranging from 2.3 to 13.8, and EC ranging from -380 to 0 to 330 mv.

All future compounds with potential thinning activity will have these chemical characteristics studied to 1) determine appropriate solution concentrations, and 2) to understand potential mode of action.

5. Effect of various osmotic, salts, and pH modifying solutions on pollen germination and growth.

Solutions of calcium polysulfide (lime sulfur), CaCl_2 , CuSO_4 , $\text{C}_2\text{H}_4\text{O}_2$ (acetic acid), NaOH, NaCl, $\text{Na}_2\text{S}_2\text{O}_5$, $\text{K}_2\text{S}_2\text{O}_7$, KHSO_4 , and KHCO_3 were mixed in distilled water at concentrations of 0, 0.25, 0.5, 1.0, 2, 5, and 10% and applied to pollen growing *in vitro*. Pollen germination and growth were observed at 4, 12, and 24 hrs. All of the following experimental summaries are of observations of pollen germination and growth at 24 hrs.

Lime-sulfur at concentrations $\geq 0.25\%$ completely inhibited pollen germination.

CaCl_2 applied at $\geq 5\%$ reduced pollen germination and at $\geq 7\%$ completely inhibited pollen germination.

CuSO₄ caused a 75% reduction in pollen germination at 0.25% and completely inhibited pollen germination and growth at concentrations of $\geq 0.50\%$.

Acetic acid completely inhibited pollen germination and growth at concentrations $\geq 0.50\%$.

NaOH inhibited pollen germination and growth at concentrations $\geq 0.50\%$.

Na₂S₂O₅ at concentrations $\geq 0.25\%$ completely inhibited pollen germination.

K₂S₂O₅ at concentrations $\geq 0.25\%$ completely inhibited pollen germination.

The concentration effects of other K- and P-salts and osmotic agents have not been analyzed as of this date.

6. Effect of various biologically derived oils and extracts on pollen germination and growth.

A series of tests have been conducted or are in process. Solutions studied include fish oil (CFO), bioneem, methyl jasmonate, etc. Solution concentrations of 0 (water control), 0.25, 0.5, 1.0, 2, 5, and 10% were mixed and applied to pollen growing on an agar support in petri dishes. Pollen germination and growth were measured at 4, 12, and 24 hrs after application.

Methyl jasmonate at 1% concentration reduced pollen germination by 80% and completely inhibited pollen germination and growth at concentrations of $\geq 2.0\%$.

Data analyses on bioneem, and other plants extracts and oils, etc. are pending at this time.

C. Effects of various osmotics, salts, and pH modifying solutions on pistil viability.

1. Effect of osmotic stress on pistil viability - *in vitro*

Pistils of apple several cultivars were excised and placed on 8.2mm dia. filter paper in 9mm petri dishes. Each dish contained 25-35 individual pistils. After placing pistils on paper supports, 3.5 ml of solution was pipetted onto the paper. Petri dishes were then placed in controlled temperature incubators (25 ± 1 C), and observed under stereo-dissecting scope at 4, 8 12, 24, 48, and 60 hours. All treatments were applied to triplicate petri dishes. Pistils were rated as alive, damaged or dead based upon color, water soaked appearance, or total oxidative destruction (darkening), respectively.

A range of solution osmotic tensions strengths (0 to 5 MPa) were created by mixing concentrations of polyethylene glycol (PEG; MW = 10,000). Osmotic concentrations of ≥ 3.0 MPa, resulted in 25% or less pistil survival; solution osmotic tension of ≥ 5.0 killed pistils.

2. Effects of solution characteristics (EC, and pH), various osmotic agents, salts, organic acids, biologically derived oils and extracts on pistil viability - *in vitro*

A series of additional *in vitro* pistil tests were planned but a method of storing pistils for treatment failed. Experiments will be resumed in January, 2004, after limbs which have been cut and cold-stored to satisfy physiological dormancy requirements are forced to flower in a greenhouse. Flowering limbs will either 1) have pistils excised for additional *in vitro* studies, or for *in vivo* studies of short-term (5 - 7 day) treatment response on the detached, flowering limbs.

3. Effect of osmotic stress on pistil viability and fruit set - *in vivo*.

Limbs of apple ('Gala', 'Jonagold', and 'Arkansas Black') had individual clusters tagged prior to king bloom. All lateral fruit were removed leaving only a single fruit per spur. Daily for 3 days as the king blooms opened, they were hand pollinated by applying 'Golden Delicious' pollen to pistils via a brush. Twenty-four hours after first pollination, flowers were sprayed with a low pressure hand sprayer applying a range of solution osmotic tensions strengths (0 to 5 MPa) treatment created by mixing concentrations of polyethylene glycol (PEG; MW = 10,000). Solution treatments were applied to 6 replicate limbs per cultivar with approximately sample flowers per replicate. Pistils were rated 48 hr after treatment. Fruit drop/set was counted at 5 weeks after treatment.

Solution osmotic tensions of ≥ 4 MPa resulted in little or no pistil survival. Pistil survival and fruit set were related in a nonlinear manner as the untreated control with 100% pistil survival had 70 - 80% ultimate fruit set. There was a significant correlation between predicted pistil mortality *in*

vitro and observed fruit thinning *in vivo* validating *in vitro* pistil studies as a useful model system to study potential fruit set. Solution osmotic tension of ≥ 2.5 MPa was necessary to reduce fruit set to levels one half that of the control or 35-40% total fruit set. At harvest, fruit weight varied with treatment and was related to the thinning effect of the treatments; the control (water) treatment which had approximately 70% fruit set had the smallest fruit, significantly smaller than any other osmotic treatment. There was no effect on soluble solids content, but firmness improved with thinning irrespective of the changes in fruit size. Treatments did not effect russet ratings.

The use of the *in vitro* pistil viability tests will allow us to screen a large number of chemicals and biological extracts for their effect on pistils and seed fertilization. Better methods have been established for storing and using pistils allowing us to extend our testing season by several months (starting about mid-January and continuing until about mid-May).

Objective 1.B. Transient photosynthetic inhibitors that may cause fruitlet abscission

Vegetative, single-shoot clonal trees of apple were grown in 4.1L pots under greenhouse conditions. When trees were approximately 15-20cm tall, treatments of various compounds that may inhibit photosynthesis by physical or biochemical means were applied to trees. The third to fifth unfolded leaf from the apex was tagged and used for gas exchange measurements of photosynthesis (P_n), evapotranspiration (Et), and stomatal conductance (gs). Gas exchange was measured prior to treatment (day 0) and 1, 3, 5, 10, and 14/15 days after treatment.

A. Effect of various osmotic tension solutions on apple leaf gas exchange

The effects of osmotic tension of solutions on gas exchange characteristics was studied by applying a range of solution osmotic tensions strengths (0 to 5 MPa) created by mixing concentrations of polyethylene glycol (PEG; MW = 10,000) in water.

Osmotic solution tensions ≥ 1.0 MPa significantly reduced all gas exchange characteristics within one day of application. When leaves were treated Solution concentrations of ≥ 3.0 MPa, gas exchange never recovered within 15 days, however at solution tension ≤ 1.0 MPa did recover to within 90% of control by 7 days. Leaf burn from marginal to severe was noted at higher solution tensions.

B. Effect of potential thinning chemicals on apple leaf gas exchange.

1. Calcium polysulfide (lime-sulfur) Lime sulfur was applied at concentrations of 1, 2, 4, and 8%. All concentrations resulted in a significant reduction in gas exchange within 1 day after treatment by 10-15% of the control. Trees treated with solution concentrations of 1 and 2 percent recovered by 7 days while trees treated with higher solution concentrations did not recover completely.

A study of the effect of repeat applications of lime-sulfur is not reported as instrumentation failed during the study and data were determined not to be reliable.

2. Fish Oil (CFO)

Crocker's fish oil was applied at concentrations of 1, 2, 5, and 10%. All solutions reduced gas exchange 20-40% within one day of application. Trees treated with a 1% solution had recovered similar to controls within 14 days; those treated with a 2% solution were within 15% of the control. Leaves treated with higher concentrations had declining gas exchange to about 40-50% of the control by 7 days and had little or no recovery by 14 days.

3. Natural polymeric films

A soy-derived natural polymeric film in three composition treatments was applied to young apple trees. All three treatments reduced photosynthesis compared to the control. The lowest concentration and molecular weight treatment exhibited its greatest reduction with 1 day of treatment and had recovered to control levels of gas exchange within 14 days of treatment. The highest concentration and molecular weight material did not achieve its maximum photosynthetic reduction

until 5 days after treatment and suppressed photosynthesis by more than 40%. The gas exchange of these treated leaves did not recover to within 20% of the control within 14 days.

DISCUSSION

After revision due to budget constraints, the 2003 studies were focused on developing, testing, and using model systems to screen and test potential alternative thinning agents. As a result, reliable *in vitro* tests for pollen germination and growth, and excised pistils were established. Fundamental information about solution chemistry affecting pollen germination and pistil viability were developed. When solution characteristics resulted in osmotic tensions ≥ 3.0 MPa, had a solution pH ≤ 4.0 or ≥ 11.0 , or had high EC salt content, pollen germination was inhibited, pollen tube growth stopped, and pistil viability terminated. As a result of these findings, we are now evaluating a range of chemicals at specific concentrations that meet those criteria. Similar studies for saponified lipids (soaps) which may cause membrane disruption or leakage, are also in progress. Potassium salts and biological oils are being formulated in the lab to create such soaps. Lastly, a range of other plant extracts are currently being tested.

The use of small, vegetative model trees as a means of assessing chemicals that may cause transient photosynthetic suppression and growth retardation have proven successful. This system is now being used to test chemicals after the pollen and pistil tests.

One of the surprising findings was a test of a soy-based, natural lecithin polymeric film which may prevent flowers from opening and anthesis, and which also inhibits photosynthesis. Under limited field test of individual spurs, this was effective at preventing fruit set. Larger scale tests of these products are planned for the future.

All this information will be used to formulate possible treatments for test in field conditions.

BUDGET

Requested in 2003 and Spent

Item	Original Request Year 2 - 2003	Received for 2003
Total Requested	\$13,300	\$10,000

Current Year Breakdown

Research Specialist ¹ - 50% FTE Salary and Benefits	\$6,200	\$6,200
Research Assistance ² - 25% FTE Salary and Benefits	\$3, 100	\$ 1,300
Supplies ³	-	\$1,000
Equipment	-	-
Greenhouse rental and operations ⁴	\$ 1,500	\$1500
Travel ⁵	\$ 2,500	\$ 0

¹ To support salary for Research Specialist (RS) in charge of project at the University of Arkansas; the RS is assigned 50% time to this project for project management, data collection, and analysis.

² To hire a part-time assistance for trials, greenhouse management, and data entry.

³ Supplies include purchase of fruit trees for model plant studies, pots, growing media, etc.

⁴ Greenhouse rental and operations.

⁵ Travel of PI to research sites WA and attend related meetings.

Contribution by University of Arkansas Agricultural Experiment Station

The University of Arkansas Agriculture Experiment station will contribute to the support of the project by A) paying PI scientist salary for the work, B) supporting graduate student assistantship (one ½-time assistantship in 2002;) assigned to the project, C) some miscellaneous supplies (equipment repair, computer repair, etc.) and D) contributing over-head costs.

It was anticipated that this project occupied approximately 10% of the PI research scientist (Rom) annual appointment (40% research appointment) - or 25% of total research obligation of PI research time (approximate contribution \$7,000). The graduate research assistant assigned to this project is a 50% FTE appointment (approximate contribution \$18,500 per year) for a period Aug. 2002 through Aug. 2004.

For this project, additional instrumentation for gas exchange was purchased and instrument repair was conducted. For ethylene determination, new GC columns, gases, and additional equipment was purchased. Charges for all related instrument repair, instruments, and supplies was \$3100 in 2003.

Because the WTFRC does not pay for institutional over-head, the estimated contribution of overhead of 30.3% and internal matching funds required for the project is \$3030 for the year 2003.

The estimated total contribution to this project by the UA is \$31,630 per year.