FINAL REPORT

PROJECT TITLE: Alternative crop thinning strategies
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Organization: University of Arkansas

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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 begun in 2004 and concluded in 2005. 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. 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 vitro* 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, two as bloom thinning strategies, and one as a post-bloom thinning strategy.
    a. Prevent pollination and fertilization of developing seeds by pollencidal activity of chemicals,
    b. Prevent pollination and fertilization of developing seeds by pistilical activity of chemicals,
    c. Photosynthetic inhibitors that may cause fruitlet abscission

SIGNIFICANT FINDINGS

This project was initially proposed as a two-year project.This report presents the accomplishments and activities of the revised 2004 and 2005 project as was funded in those years. Because of amended budgets and with counsel from the WTFRC, 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 pollen germination, killing germinated pollen, killing pistils to prevent fertilization as bloom-thinning strategies, and to prevent fruitlets from developing by limiting carbohydrate supply due to temporary inhibition of photosynthesis thereby causing reduced fruit set. Following are accomplishments and significant findings during the past two years of funding.

- Studies were conducted to develop an assay of pollen germination *in vitro* and affects of potential thinning agents on pollen germination and germ tube vigor
- Studies were conducted to developed an assay of excised pistils *in vitro* and the affects of potential thinning agents on pistil viability/mortality
- Studies were conducted to correlate *in vitro* studies to field thinning performance. Model studies correlated (r^2 = 0.74 to 0.86) to field thinning.
- Twenty five studies were conducted to test concentration effects of potential bloom thinners on pollen germination and pollen tube growth.
- Twenty five studies were conducted to test concentration effects of potential bloom thinners on pistil viability.
- Seventeen tests of seven concentrations of essential oils representing chemical groups of monoterpenes, monoterpenol esters, sesqueterpines, sesqueterpine phenols, phenolic esters, and oil oxides were tested for *in vitro* pistilicidal effects. Of those tested, all killed pistils at concentrations of 1.0% or less and many in the range of 0.1 to 0.025 %.
- Solution pH was significantly quadratically related to pollen germination and pollen tube growth (r^2 = 0.42, 0.48) with pH less than 4.2 or greater than 9.6 eliminating pollen germination and pollen tube growth.
- Solution electrical conductivity (EC) was significantly negatively quadratically related to pollen germination and pollen tube growth (r^2 = 0.68, 0.68) with an EC > than 200 mV eliminating pollen germination and pollen tube growth.
- Solution water potential (MPa) was significantly negatively quadratically related to pollen germination and pollen tube growth (r^2 = 0.19, 0.22) with an solution water potentials less than 3.5 Mpa eliminating pollen germination and pollen tube growth.
- Solution pH was quadratically related to pistil viability with pH less than 3.8 and greater than 10.0 killing pistils.
- Solution electrical conductivity was significantly although weakly related to pistil viability with solution EC greater than 250 mV killing pistils.
- Solution water potential was significantly related to pistil viability with solution water potentials less than -4.0 Mpa killing pistils.
- Nine studies of 47 selected treatments were conducted to screen chemicals for transient inhibition of photosynthesis. Of those, 15 chemicals caused a 20% or greater inhibition lasting three or more days. Additional studies of concentration effects of these chemicals are in process.

METHODS

Prevent Flower Fertilization by Chemical Effects on Pollen and Pistils - Potential Bloom Thinners

Objective 1A. Pollenicidal Activity of Potential Bloom Thinning Chemicals (Table 1).

The purpose of this series of studies was to develop an *in vitro* assay for laboratory assessment of potential bloom-thinning chemical treatments.

Pollen germination and growth were studied *in vitro* by applying commercially purchased 'Golden Delicious' apple pollen (Firm Yield Pollen Services) 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 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). Experiments were repeated three to five times.

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 including 1) osmotic effects causing cellular dessication, 2) pH effects causing cellular disruption or improper conditions for metabolism, 3) salt effects as indicated by solution electrical conductivity, and 4) saponified fatty acids and lipids (soaps and natural oils) causing membrane disruption.

Because pollen 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 using polytethylene gylcol as an osmotic source, sodium chloride as a salt source, hydrchloric acid as a low pH source, and sodium hydroxide as a high solution pH source. Data from these standard tests were related to solution characteristics of test solutions. Solution characteristics were determined for all test solutions.

Treatment solutions were tested at concentrations of 0.0, 0.1, 0.2, 0.5, 10.0% v/v.

Objective 1B. Pistilicidal Activity of Potential Bloom Thinning Chemicals (Tables 1, 3).

The purpose of this series of studies was to develop an *in vitro* assay of treatments which may cause pistil death as visually rated by colored-oxidation. The assay would be used to screen potential bloom thinning chemicals. Further, it was proposed to relate the *in vitro* results to field performance.

Limbs of 'Gala' were collected from the field and put into a cold room to complete physiological dormancy requirements. Limbs were removed from storage, recut, placed in water and forced to bloom in a greenhouse. Prior to bloom and anthesis, flowers were cut, opened and pistils excised. Excised pistils were place on a moistened (distilled water) borosilicate (glass) paper in a sterile petri dish. Ten pistils were placed in each dish. After dishes were prepared, 0.5ml of test solutions were added to the dish by micropipette. Pistils were observed at 2, 4, 8, 24, 48, and 72 hours after treatment and rated for oxidative browning and apparent viability on a scale of 0 (no damage) to 5 (dark oxidation, dead, shriveled pistils) using a photographic standard developed in preliminary studies. Data from several preliminary studies indicated that observation after 24 hours was sufficient and the last two observation periods were discontinued.

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Using control osmotic agents (PEG), salts (sodium chloride), and pH varied solutions (acid and base), treatments were applied to individual flowers on columnar trees in a greenhouse and to individual spurs in an orchard to evaluate effects of solution characteristics on fruit set. Greenhouse and field observation of pistils, and fruit set after 40-60 days was related to *in vitro* observations.

Treatments were typically applied at concentrations of 0.0, 0.1, 0.2, 0.5, 10.0 %. Several studies were repeated with concentrations at lower concentrations (0.0, 0.01, 0.025, 0.05, 0.1, 0.2, 0.5% v/v).

Objective 1A/B. Effects of Potential Bloom Thinning Agents on Fruit Set in the Field (Table 2).

Based upon observations from several pollencidal and pistilicidal studies described above, several chemicals were selected for model field studies. Individual spurs of 'Gala' were tagged prior to full bloom. At full bloom, individual spur clusters (flowers and leaves) were sprayed with chemicals using a high pressure, low volume solution atomizer. Twenty replicate spurs of each treatment were treated. After 24-48 hours, pistil oxidation was rated. Fruit set as fruits/cluster were counted 40-60 days after bloom. Fruit set correlated significantly with rated pistil oxidation. These studies were conducted in both April 2004, and 2005.

Objective 1C. Transient Reduction in Gas Exchange and Growth Suppression

The purpose of these studies were to determine the effects of various potential post-bloom thinning chemicals on the leaf gas exchange and growth model vegetative trees grown in a greenhouse. 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 (Pn), 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. All treatments will be tested at 2% concentrations. However, if warranted, some treatments may be repeated at lower or higher concentrations if data from preliminary or previous studies indicate. Each study utilized a water-spray control. Data were expressed as percent of control gas exchange. Solution characteristics (pH, osmotic tension, EC) were determined for all test solutions.

Objective 1A/B. Effects of Potential Post-Bloom Thinning Agents on Fruit Set in the Field (Table 5).

Based upon observations from several effects on transient suppression of gas exchange in studies described above, several chemicals were selected for model field studies. Individual spurs of 'Gala' were tagged prior at full bloom. At 15 days after petal fall, individual spur clusters (fruitlets and leaves) were sprayed with chemicals using a high pressure, low volume solution atomizer. Eleven replicate spurs of each treatment were treated. Fruit set as fruits/cluster were counted 60 days after treatment. This study was conducted in April 2005.

Results and Discussion

This study determined that potential bloom thinning chemicals could be screened using several means. First, a determination of treatment solutions chemical properties, particularly the pH, the electrical conductivity, and osmostic tension may indicate that chemicals could be pollenicidal or pistilicidal due to there chemical (not necessarily metabolic) effects on pollen and pistil viability. Chemicals could be screened *in vitro* using pollen which can be stored for relatively long periods, and pistils from limbs held in storage. This greatly enhances the chemcial-thinner testing season. It was noted that pollen germination did significantly decline in storage after six to eight months and studies were discontinued until fresh pollen was obtained. Pistils excised from stored limbs and testing on forced in a greenhouse expanded the screening period from January through May compared to a relatively narrow one to two week window in the field.

Our pollenicidal and pistilicidal *in vitro* assays correlated well to small scale studies on excised limbs in the greenhouse, columnar trees in the greenhouse, and trees in the field. Thus, the two year effort provided valuable information on pollen and pistil biology and provided convenient tool for screening potential bloom thinning chemicals.

Using these methods, more than 40 selected chemicals were tested at five to twelve concentrations. From those chemicals, approximately 25 have potential thinning activity and should be field tested for efficacy and potential undesirable phytotoxicity symptoms.

The model vegetative tree studies to evaluate the effect of potential post-bloom thinners on transient gas exchange was a much more difficult task. Experiments took 20-30 days in length. At times anomalous data, not that unusual with gas exchange studies in greenhouse conditions, necessitated repeating experiments. Of 47 treatments tested at specific concentrations (Table 4), only 15 treatments reduced photosynthesis by 20% or more for more than three days. Follow-up studies indicated a strong concentration response of some chemicals. Further, only a very few chemicals resulted in significantly reduced growth of the trees (dry matter accumulation).

The clear next step is to ramp-up treatments to field-scale application in orchards. Several treatments will need to be determined for concentration effects, specific timing effects, interaction with other chemicals, particularly adjuvants, and variability which may be induced by orchard condition, age, cultivar and seasonal effects. However, it is felt a good first step at developing new screening methods and identifying potential chemical thinners was accomplished. This work will continue pending continued funding.

BUDGET

Following is the budget as obtained and spent during the project. Funds were used primarily for personnel and supplies to conduct the studies.

Item	Year 1 - 2004	Year 2 - 2005
Total Received	**$10,000**	**$10,000**
Project Techniciant[1] - 25% FTE Salary and Benefits	$6,500	$6,500
Supplies[2]	$1500	$1500
Equipment	$1500	$1500

Greenhouse operations[3]	$ 500	$ 500

[1] To support salary for Project Technician (PT) in charge of project at the University of Arkansas; the PT is assigned 25% time to this project for project management, data collection, and analysis.
[2] Supplies include purchase of fruit trees for model plant studies, pots, growing media, petri dishes, acquisition of chemicals, etc,. Equipment cost included maintenance and parts for gas exchange system, parts for measuring solution characteristics, particularly osmotic tension.
[3] Greenhouse operations.

Contribution by University of Arkansas

The University of Arkansas Agriculture Experiment station contributed to the support of the project by A) paying PI scientist salary for the work, B) supporting graduate student assistantship (one 1/4-time assistantship in 2004-2005;) assigned to the project, C) some miscellaneous supplies (equipment, computing, etc.) and D) contributing over-head costs. An undergraduate student received a departmental grants ($1000) to be used for hourly wage to complete part of this project as part of an study-project. .

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 25% FTE appointment (approximate contribution $9,250 per year) for a period Jan 2003-Dec 2004.

For this project, additional instrumentation for gas exchange was purchased and instrument repair was conducted. 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 approx. $3000 for each year of the project.

The estimated total contribution to this project by the UA was greater than $19,000 per year of the project.

Table 1 . Summary results of 24 studies of potential bloom thinning compounds screened at seven concentrations (0.0, 0.25, 0.5, 1.0, 2.0, 5.0, and 10.0 %) for *in vitro* on 'Golden Delicious' apple pollen and excised 'Golden Delicious' pistils. The lethal concentration was that dose which reduced pollen germination by more than 80% or resulted in a pistil damage rating of 4 or greater (0-5 scale) after 24 hours. All experiments were repeated with five replications.

Type/Class of thinners	Chemical Name	Chemical Formula	Form	Lethal Concentration (%)	
				Pollen	Pistil
Osmotic Agents	Potassium Bicarbonate	KHCO3	Granular	0.25	1.0
	Potassium Bisulfate	K2SO4	Crystal	0.50	0.25
	Potassium Iodide	KI	Granular	2.0	2.0
	Potassium meta-Bisulfate	K2S2O5	Crystal	5.0	0.25
	Sodium meta-Bisulfate	Na2S2O5	Crystal	0.25	0.25
	Potassium Sulfate	K2SO4	Crystal	0.25	-
	Sodium Chloride	NaCl	Crystal	2.0	5.0
	Sodium Hydroxide	NaOH	Cyrstal	0.25	0.25
	Calcium Chloride	CaCl2	Solution (10%W/V)	5.0	10.0
	Ferric Sulfate	Fe2(SO4)3	Powder	0.25	0.25
	Calcium Polysulfide	CaSx	Solution	0.25	0.25
	Ammonium Sulfate	(NH4)2(SO4)	Crystal	0.5	2.0
Acids	Acetic Acid	CH3COOH	Solution (10%W/V)	0.25	5.0
	Citric Acid	C6H8O7	Solution (1.000 M)	0.25	0.25
	Salicylic Acid	HO.C6H4.COOH	Crystal	0.25	0.25-
	Cinnamic Acid	C6H5CH=CHCO2H	Crystal	0.25	0.25
	Oxalic Acid	HOOCCOOH	Solution (10%W/V)	0.25	0.25
	Glutamic Acid	HOOCCH2CH(NH2)COOH	Crystal	0.25	2.0
Oils	Soybean Oil		Liquid	>10.0	>10.0
	Canola Oil		Liquid	>10.0	>10.0
	Clove Oil		Liquid	0.25	0.25
Others	Copper Sulfate	CuSO4	Solution (10%W/V)	0.25	0.25
	Methyl Jasmonate	C13H20O3	Solution	0.25	-
	Sodium Hypochlorite	NaOCl	Solution (10%W/V)	0.25	0.25

Table 2. Effect of selected potential bloom thinning agents applied to 'Gala' flower clusters at full bloom on pistil damage 24-48 hrs after treatment and fruit set 40 days after petal fall. Data represent 20 individual replicate spurs for each treatment.

Treatment	Concentration (%)	Pistil Damage Rating (0-5)	Fruit Set (% of control)
Water	100	1.3 j	100.0a
C6H8O7	0.25	1.8 ghi	100.1a
Soybean Oil	1.00	1.6 ij	97.5a
HOOCCH2CH(NH2)COOH	2.00	1.9 fghi	94.5a
Fe2(SO4)3	0.5	2.1 fgh	88.9ab
KHCO3	2.00	2.2 f	82.8abc
Fe2(SO4)3	0.25	2.1 fg	76.9abc
CH3COOH	0.5	2.0 fgh	58.0cd
C6H8O7	0.5	1.9 fghi	58.0cd
CaSx (Lime-Sulfur)	2.0	1.7 hi	37.1de
HOOCCOOH	1.00	4.4 bc	35.1de
C6H5CH=CHCO2H	1.00	2.8 e	31.8de
K2S2O5	2.00	3.5 d	26.2e
Na2S2O5	2.00	3.6 d	24.3e
CuSO4	0.5	3.6 d	21.1e
HO.C6H4.COOH	1.00	4.1 c	12.8e
(NH4)2(SO4)	2.00	4.7 ab	11.5e
KI	5.00	4.8 a	8.9e
KHSO4	1.00	3.1 e	8.5e

Table 3. Results of 17 screening studies with seven concentrations (0.0, 0.01, 0.025, 0.05, 0.1, 0.2, 0.5 % v/v) of essential oils as potential bloom thinning chemicals on excised 'Gala' pistil viability rating (0-5 scale) *in vitro*. Lethal concentration was determined by a dose-response that caused complete pistil death (rating $\geq$ 4.0 of 5). Each study was repeated three times with 10 pistils per replication.

Treatment	Minimum Concentration to Kill Pistils (%)
Cedarwood oil	0.01
Cinnamon oil	0.01
Tea tree oil	0.01
Ginger oil	0.01
Eucalyptus oil	0.05
Clove oil	0.025
Fir needle oil	0.05
Ylang ylang III oil	0.025
Spruce oil	0.025
Lavender oil	0.025
Lemon oil	0.05
Camphor oil	0.05
Pine needle oil	0.05
Cypress oil	0.10
Grapefruit oil	0.10
Tangerine oil	0.20
Black pepper oil	0.50

Table 4. Summary results of nine studies including 47 treatments of potential post bloom thinners on gas exchange inhibition of apple. Each study was repeated with 6-10 replications using a complete random block design.

Treatment	Concentration (%)	Max A Inhibition (%)	Max Et Inhibition (%)	Max gs Inhibition (%)
Control-H2O	**0**	**0**	**0**	0
Soybean Oil	4	37	22	59
Canola oil	2	4	1	0
Corn oil	2	2	2	0
Crocker's fish oil	2	6	8	19
Soybean oil	2	2	3	0
Linseed oil	2	12	0	0
Cedarwood oil	2	7	24	38
Cinnamon oil	2	9	8	**7**
Pepper oil	2	9	0	3
Clove oil	0.025	26	8	16
Clove oil	0.05	5	0	16
Clove oil	0.125	2	0	0
Clove oil	0.25	3	0	0
Clove oil	2	100	100	100
Lime sulfur	2	5	10	15
Lime sulfur	8	31	14	34
Lime sulfur + soybean oil	**4**	42	19	61
Lime sulfur + soybean oil	**6**	21	4	6
Lime sulfur + soybean oil	**8**	18	15	56
Ferric sulfate	2	19	9	14
Ammonium sulfate	2	24	17	10
Copper sulfate	2	6	4	8
Copper sulfate	2	18	18	24
Potassium bisulfite	5	37	23	53
Potassium sulfate	0.25	7	7	15
Potassium sulfate	0.5	0	0	3
Potassium sulfate	1	1	5	8
Potassium sulfate	2	3	0	6
Potassium sulfate	2	4	3	5
Potassium sulfate	2	29	13	20
Potassium bicarbonate	5	48	16	3
Sodium Sulfite	2	0	7	19
Sodium chloride	0.5	18	14	**7**
Sodium chloride	2	8	16	37
Acetic acid	2	8	5	8
Citric acid	0.25	20	13	19
Citric acid	0.5	34	20	21
Citric acid	1	27	18	25
Citric acid	2	5	4	11

Treatment	Concentration (%)	Max A Inhibition (%)	Max Et Inhibition (%)	Max gs Inhibition (%)
Citric acid	2	15	2	29
Salicylic acid	2	6	13	21
Oxalic acid	2	0	6	9
Catechin	2	36	8	3
Coumarin	2	10	0	6
Chitosan	2	27	9	5
Thymol	2	33	6	9

Table 5. Effect of selected potential post-bloom thinners applied to individual spurs of 'Gala' 15 days after petal fall on individual cluster fruit set relative to control (water sprayed) clusters 60 days after treatment.

Treatment	Concentration (%)	Fruit Set (% of Control)
Control Water	100	100.0
Clove Oil	0.1	73.0
Clove Oil + Dormant Oil	0.1 1.0	75.7
Cedarwood Oil	0.25	77.4
Potassium Sulfate	0.5	71.3
Copper Sulfate	0.5	107.8
Ammonium Sulfate	1.0	81.7
Oxalic Acid	1.0	84.0
Lime Sulfur + Crocker Fish Oil	2.0 2.0	70.4
2.5% Cinnamon Oil	2.5	100.0