FINAL REPORT
WTFRC Project # AH-02-209

Title: *In-vitro,* Greenhouse, and Field Evaluation of Potential Apple Bloom Thinning Agents

Subtitle: Pollen Germination and Tube Growth of Apple as Affected by Temperature and Cultivar in *in-vitro* Media and Detached Pistils

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Introduction:

R. R. Williams (1965) and Byers et al. (unpublished data prior to 2004), used detached flowers in all studies on the influence of various temperatures on pollen germination and growth. Our data in 2004 indicated a major flaw in conclusions drawn from research using detached flowers as reported by R.R. Williams (1970) and reproduced in Westwood's 1978 book. Apparently reduced carbon balance and perhaps many other negative influences occur when the flower is detached from the tree. We believe procedures to study the influences of all temperatures on pollen tube growth in the laboratory should use only "on-tree" flowers under controlled conditions where the tree foliage is in continuous light or 12 hrs light/12 hrs dark, especially when comparing temperatures at 85ºF or greater.

Objectives: Our overall goal (for 2002-2004) was to develop a better understanding of pollen germination, pollen tube growth, and the influence of bloom thinners given under a range of environmental conditions. The specific objectives of these studies were:

1) To determine the influence of temperature on pollen germination and growth in styles under constant temperatures in the range of 35ºF to 95ºF. Commercially important pollen sources such as 'Gala', 'Golden Delicious', 'Fuji', 'Pink Lady' and/or 'Delicious' cultivars; and/or crab apples 'Snow Drift' or 'Manchurian' were to be used.

2) To develop a model for pollen tube growth during a typical diurnal flowering period in a programmable growth chamber or switching to and from constant temperatures to determine the growth pattern to pollen tube growth as temperatures change.

3) To screen for potentially new pollination/fertilization inhibitors using *in-vitro*, greenhouse and field techniques described by Embree and Foster (1999) and Wolf and Byers (2001, unpublished). Among the many potential thinning agents, we emphasized chemicals that were acceptable under the new Final Rules of the Organic Food Act as listed by the Organic Materials Review Institute.

4) Attempted to use a biological test (seed germination/growth) that would approximate pollen tube growth in the fluctuating temperatures and correlate it with pollen tube growth in styles in the orchard environment. Ideally, a simple germination test started

anytime during bloom could provide an indicator on pollen growth without microscopic examination of pollen tubes in styles.

5) Finally, we proposed to conduct limited field studies to further evaluate selected bloom thinning compounds that would have shown promise in greenhouse and or *in-vitro* studies. These field studies would be necessary to determine effective application rates and potential phytotoxicities to fruit and foliage.

2004 Objectives:

- To study the influence of constant temperatures and/or alternating temperatures (from 55ºF to 85ºF) typically experienced in the field.

- To research "on-tree" pollen germination and pollen tube growth ('Golden Delicious', 'Manchurian') in "on- tree" 'Golden Delicious' pistils using continuous and/or alternating light and temperature regimes in the laboratory.

- To determine the minimum time for pollen tubes to grow to the pistil base at optimum temperatures for 'Golden Delicious' vs 'Manchurian' pollen.

- Compare results of data from 2003 using "detached" flowers and 2004 "on-tree" flowers.

Significant Findings:

- 'Manchurian' pollen tube growth reached base of styles in less than 48 hours at 55ºF, 65ºF, 75ºF, or 85ºF.

- 'Golden Delicious' pollen tube growth failed to reach base of styles after 96 hours at 55ºF, 65ºF, 75ºF, or 85ºF.

- On-tree studies showed increased pollen germination and tube growth rate as temperatures increased from 55ºF to 75ºF. At 85ºF (the highest temperature) tube growth rate was only slightly inhibited.

- "On tree" stigmas that were hand-pollinated with 'Golden Delicious' pollen germinated at higher rate than 'Manchurian' pollen. Pollen tubes growing into style from stigma was similar in numbers. However, pollen tube growth from the base of stigma to the style base was drastically reduced in comparison to 'Manchurian'.

- The growth of pollen tubes down the style appeared to be more inhibited by pollen cultivar used on the pistil but temperature was an important factor as well.

Materials and Methods:

Expt. 1. Effect of temperature and light on pollen tube growth at 96 hours in 'Golden Delicious' pistils pollinated '*on-tree*' with 'Golden Delicious' pollen (2004). 'Golden Delicious' trees in root bags were removed from orchard early March and placed in cold rooms to delay bloom until used. Selected trees were removed from cold rooms and placed in greenhouse to induce bloom. Once bloom on trees reached late balloon stage flowers were selected (12 flowers/trt.) for pollination test.

One day before hand pollination of flowers, all anthers were removed from test flowers to prevent cross-pollination. Also all other flowers on trees were removed to prevent cross-pollination of test flowers. On the day of pollination, selected flowers were tagged for identification and hand pollinated with pollen harvested 2004 by using a painters' brush to apply pollen to stigmas of flowers. Trees were then placed in temperature controlled rooms under HPS 1000 watt lamp (approx. 600 $\mu mols \cdot m^{-2}s^{-1}$ at the tree upper canopy) for designated lengths of time, temperature, and lighting. At conclusion of temperature/light test, flowers were removed from trees and placed in labeled glass containers in a solution of 5% sodium sulfite. Flowers were then boiled for a time of 15 minutes. Flower samples were then placed in refrigerator for storage until time of microscopic examination. Three flowers were examined for each temperature. Stigmas were detached from the ovary, separated into the five stylets, soaked in dye over night, squashed between microscopic slides, allowed to incubate for an additional 24 hrs before examination with epi-UV light using a Zeiss HBO-50 high pressure mercury vapor light source at 100X. A rating of pollen density/field (0-10) on each stylet stigma surface, germinated pollen tubes on the surface, number of pollen tubes penetrating the stigma base, the average length of the longest pollen tube, the average length of the stylets, and number of pollen tubes reaching the end of the stylet.

Expt. 2. Effect of temperature and light on pollen tube growth of 'Manchurian' crabapple and'Golden Delicious' in 'Golden Delicious' pistils on tree for 24 and 48 hours (2004). 'Golden Delicious' trees in root bags were removed from orchard early March and placed in cold rooms to delay bloom until used. Selected trees were removed from cold rooms and placed in greenhouse to induce bloom. Once bloom on trees reached late balloon stage flowers were selected (12 flowers/trt.) for pollination test. One day before hand pollination of flowers on all anthers were removed from test flowers to prevent cross-pollination. Also all other flowers on trees were removed to prevent cross-pollination of test flowers. On the day of pollination, selected flowers were tagged for identification and hand-pollinated with 'Golden Delicious' pollen harvested 2004 and 'Manchurian' crabapple pollen harvested 2003 by using a painters' brush to apply pollen to stigmas of flowers. Trees were then placed in temperature controlled rooms under HPS 1000 watt lamp (approx. 600 $\mu mols \cdot m^{-2}s^{-1}$ at the tree upper canopy) for designated lengths of time, temperature, and lighting. At conclusion of temperature/light test, flowers were removed from trees and placed in labeled glass containers in a solution of 5% sodium sulfite. Flowers were then boiled for a time of 15 minutes. Flower samples were then placed in refrigerator for storage until time of microscopic examination. Three flowers were examined for each temperature. Stigmas were detached from the ovary, separated into the five stylets, soaked in dye over night, squashed between microscopic slides, allowed to incubate for an additional 24 hrs before examination with epi-UV light using a Zeiss HBO-50 high pressure mercury vapor light source at 100X. A rating of pollen density/field (0-10) on each stylet stigma surface, germinated pollen tubes on the surface, number of pollen tubes penetrating the stigma base, the average length of the longest pollen tube, the average length of the stylets, and number of pollen tubes reaching the end of the stylet.

Expt. 3. Effect of temperature and light on pollen tube growth of Manchurian crabapple and Golden Delicious in Golden Delicious pistils on tree for 24, 48, and 96 hours in continuous light (2004). 'Golden Delicious' trees in root bags were removed from orchard early March and placed in cold rooms to delay bloom until used. Selected trees were removed from cold rooms and placed in greenhouse to induce bloom. Once bloom on trees reached late balloon stage flowers were selected for pollination test. One day before hand pollination of flowers all anthers were removed from test flowers to prevent cross-pollination. Also all other flowers on trees were removed to prevent cross-pollination of test flowers. On the day of pollination, selected flowers were tagged for identification and hand pollinated with 'Golden Delicious' pollen harvested 2004 and 'Manchurian' crabapple pollen harvested 2003 by using a painters' brush to apply pollen to stigmas of flowers. Trees were then placed in temperature controlled rooms under HPS 1000 watt lamp (approx. 600 $\mu mols \cdot m^{-2}s^{-1}$ at

the tree upper canopy) for designated lengths of time, temperature, and lighting. At conclusion of temperature/light test, flowers were removed from trees and placed in labeled glass containers in a solution of 5% sodium sulfite. Flowers were then boiled for a time of 15 minutes. Flower samples were then placed in refrigerator for storage until time of microscopic examination. Three flowers were examined for each temperature. Stigmas were detached from the ovary, separated into the five stylets, soaked in dye over night, squashed between microscopic slides, allowed to incubate for an additional 24 hrs before examination with epi-UV light using a Zeiss HBO-50 high pressure mercury vapor light source at 100X. A rating of pollen density/field (0-10) on each stylet stigma surface, germinated pollen tubes on the surface, number of pollen tubes penetrating the stigma base, the average length of the longest pollen tube, the average length of the stylets, and number of pollen tubes reaching the end of the stylet. An additional 15 flowers per treatment were hand-pollinated and left on tree for fruit set evaluation. These were evaluated 24 days after pollination.

Results and discussion

Expt. 1. Influence of laboratory temperatures and light on 'Golden Delicious' pollen tube growth after 96 hours using '*on-tree*' 'Golden Delicious' pistils and harvested pollen (2004). When comparing 55ºF, 65ºF and 75ºF, the average length of longest pollen tubes in styles showed significantly more growth at 75ºF continuous 24 hr light and/or at alternating 75ºF (12 hr light /55ºF 12 hr dark) than at either 65ºF or 85ºF. A significant reduction in average number of pollen tubes penetrating stigma base occurred only at 85ºF continuous light (Table 1). High temperature (85ºF) may have reduced pollen tube growth rate. Continuous light at lower temperatures of 75ºF and 65ºF were not inhibitory (Table 1). No pollen tubes grew to the base of styles in any of the temperature/light ranges tested which suggests some degree of incompatibility of 'Golden Delicious' pollen in 'Golden Delicious' pistils. Pollen tube growth and density on stigma surface after 96hrs appeared to be similar in all temperature/light ranges tested (Table 1).

Expt. 2. Effect of temperature and light on pollen tube growth of 'Manchurian' crabapple and 'Golden Delicious' in 'Golden Delicious' pistils '*on-tree*' for 24 and 48 hours (2004). 'Golden Delicious' pistils pollinated with 'Manchurian' pollen and tested at 65ºF in continuous light for 48 hours showed pollen tubes growing to base of styles in 80% of styles examined (Fig. 1). 'Golden Delicious' pollen on 'Golden Delicious' pistils resulted in no tubes reaching base of styles at any of the tested parameters. Alternating temperature and light levels resulted in pollen tubes from 'Manchurian' pollen reaching style base in only 20% of those examined compared to 'Golden Delicious' pollen tubes not reaching the base in any styles examined (Fig. 1). Reduced temperature and light levels light levels appear to reduce pollen tube growth (Table 2) with 'Manchurian' pollen but not in 'Golden Delicious'.

Expt. 3. Effect of temperature and light on pollen tube growth of 'Manchurian' crabapple and 'Golden Delicious' in 'Golden Delicious' pistils *on-tree* for 24, 48, and 96 hours in continuous light (2004). Under microscopic examination of 'Golden Delicious' pistils pollinated '*on-tree*' with 'Manchurian' crabapple or 'Golden Delicious' pollen, visible pollen tube rating on stigma (Table 3) showed a higher concentration of 'Golden Delicious' pollen tubes growing on stigmatic surface than that of 'Manchurian' pollinated stigmas after 24 hours at all test parameters. This pattern continued through all tests except for tests at 55ºF/48HR and 55ºF/96HR where Manchurian pollen tubes had a slightly higher rating. Length of pollen tubes growing in styles (Fig. 3) are significantly longer in 'Manchurian' pollinated styles than in those pollinated with 'Golden Delicious' pollen. Styles pollinated with 'Manchurian' pollen resulted in pollen tube growth to base of in all tests but 55ºF/24HR and 65ºF/24HR (Fig. 2). The same test using 'Golden Delicious' pollen resulted in none of the pollen tubes reaching the base of styles at any test parameter. Flowers pollinated and left on

tree for fruit set evaluation resulted in flowers pollinated with 'Manchurian' pollen (Fig. 4) setting fruit on 75%-100% of flowers in tests at all temperatures. None of the flowers pollinated with 'Golden Delicious' pollen set fruit (Fig. 4).

Conclusions

Because pollen tubes can traverse the entire style at 75ºF in less than 24 hours and in less than 48 hours at non-optimum temperatures of 55ºF, 65ºF, and 85ºF if flowers are "on-tree". Our data indicated a major flaw in conclusions drawn from research using "detached" flowers (as reported by R.R. Williams (1970 and reproduced in Westwood's 1978 book, and Byers et al. unpublished data prior to 2004.). We believe that using "on-tree" flowers is essential to determining the effective pollination period. We believe procedures to study temperature influences in the laboratory should use only "on tree" flowers under conditions where the tree foliage is in continuous light or in 12 hrs light/12 hrs dark, especially when comparing to temperatures at 85ºF or greater.

After the transfer of pollen to the stigma, the influence of fluctuating temperatures are among several factors that affect fertilization and fruit set. Williams (1970) showed that low temperatures significantly slowed pollen tube growth, and that the optimum growth was different for different cultivars. Williams (1970) studies were conducted on English cultivars that are not of economic importance in the U.S; and temperatures studied were typically lower than that in the bloom period in the U.S. (7^{O} C to 15^{O} C). Since William's work in 1970, little has been published on the influence of temperature on apple pollen germination, pollen tube growth, fertilization, and fruit set. Comparisons of pollinating cultivars and crab apples should be further researched using "on-tree" flowers under controlled light and temperature conditions.

Literature Cited

Embree C. G. and A. Foster. 1999. Effects of coatings and pollenicides on pollen tube growth through the stigama and style of 'McIntosh" apple blossoms. Journal of Tree Fruit Production. 2:19-32.

Williams, R.R. 1970. Factors affecting pollination in fruit trees. *In Physiology of tree crops*. 193-208. Ed. L.C. Luckwill and C.V. Cutting. Academic Press, New York and London.

Additional references are available on request.

Budget: *In-vitro*, Greenhouse, and Field Evaluation of Potential Apple Bloom Thinning Agents

PI(s): **Dr. Ross Byers**

Project duration: **2002-04**

Year	**2002**	**2003**	**2004**
Item	Year 1	Year 2	Year 3
Salary	3,700	30,700	15,000
Benefits	259	2,419	4,500
Supplies	1,000	1,840	500
Total	**4,959**	**34,959**	**20,000**

Table 1. Effect of temperature and light on pollen tube growth at 96 hours in 'Golden Delicious' pistils pollinated on tree with 'Golden Delicious' pollen (2004).

Time	Light/Temperature[z]	Pollen tube density on stigma (visual rating; 0-10)[y] (Apr 04)	Average number of pollen tubes penetrating stigma base (Apr 04)	Average length of longest pollen tube (mm) (Apr 04)	Average number of pollen tubes at end of styles (Apr 04)	Average length of styles (mm) (Apr 04)
96 HR	65ºF (Continuous light)	6.73 a[x]	27.13 a	1.27 b	0 a	6.42 b
96 HR	65ºF (12 HR Continuous light) + 65ºF (12 HR Dark)	6.97 a	30.05 a	1.57 b	0 a	7.02 ab
96 HR	75ºF (Continuous light)	5.53 a	37.33 a	2.93 a	0 a	8.25 a
96 HR	75ºF (12 HR Continuous light) + 75ºF (12 HR Dark)	6.73 a	37.45 a	2.81 a	0 a	8.24 a
96 HR	85ºF (Continuous light)	6.00 a	6.07 b	1.37 b	0 a	7.00 ab
96 HR	85ºF (12 HR Continuous light) + 85ºF (12 HR Dark)	5.33 a	26.88 a	1.53 b	0 a	8.07 a

[z]Pollen viability test (1 hr at room temperature-70ºF) = 65% (visual estimate). Media= Agarose= 10g/L; sucrose= 100g/L; boric acid= 10mg/L.
[y]Pollen tube density rating: 0= no visible pollen tubes ; 10= heavy concentration of pollen tubes.
[x]Mean separation within columns by Duncan's New Multiple Range Test ($P \leq 0.05$).

Table 2. Effect of temperature and light on pollen tube growth of 'Manchurian' crabapple and 'Golden Delicious' in 'Golden Delicious' pistils on tree for 24 and 48 hours (2004).

Pollinator	Time[z] +Temperature +Light exposure	Pollen tubes in stigma (visual rating 0-10)[y]		Average number of pollen tubes penetrating stigma base		Average length of longest pollen tube (mm)		Average number of pollen tubes at end of styles		Average length of styles (mm)	
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Manchurian Crabapple	65ºF (Continuous Light)	0.47	4.60	1.60	32.93	1.27	8.02	0	4.33	8.41	8.71
Golden Delicious	65ºF (Continuous Light)	2.53	5.05	4.80	10.47	0.36	0.48	0	0.00	8.78	8.44
Manchurian Crabapple	12 HR @(65ºF) (Continuous Light) + 12 HR @(55ºF) (Dark)	2.13	4.25	9.80	23.20	1.77	5.83	0	0.50	7.35	7.62
Golden Delicious	12 HR @(65ºF) (Continuous Light) + 12 HR @(55ºF) (Dark)	1.47	3.07	3.13	6.62	0.48	0.62	0	0.00	8.03	7.51

[z]Pollen viability test (2 hr at room temperature-70ºF) = % (visual estimate). Media= Agarose= 10g/L; sucrose= 100g/L; boric acid= 10mg/L.
[y]Visual rating scale : 0 = no pollen grains or tubes visible; 10 = large number of pollen grains or tubes visible.

Table 3. Effect of temperature and continuous light on pollen tube growth of 'Manchurian' crabapple and 'Golden Delicious' in 'Golden Delicious' pistils on tree for 24, 48, and 96 hours in continuous light (2004).

Temperature[z] Time	Pollen tubes in stigma (visual rating 0-10)[y]		Average number of pollen tubes penetrating stigma base		Average length of longest pollen tube in style (mm)		Average number of pollen tubes reaching style base		Average length of styles (mm)	
	G. Del.	Manch.	G. Del.	Manch.	G. Del.	Manch.	G. Del.	Manch.	G. Del.	Manch.
13ºC (55ºF) 24 HR	1.00	0.93	0.40	2.92	0.09	1.53	0.0	0.0	7.89	8.41
13ºC (55ºF) 48 HR	1.90	2.73	2.98	18.13	0.39	5.22	0.0	0.3	7.83	8.52
13ºC (55ºF) 96 HR	5.18	5.20	11.00	30.43	0.71	6.89	0.0	2.95	7.61	7.93
18ºC (65ºF) 24 HR	2.60	0.48	4.80	1.60	0.36	1.27	0.0	0.0	8.78	8.41
18ºC (65ºF) 48 HR	5.05	4.60	10.47	32.93	0.48	8.02	0.0	4.3	8.44	8.71
18ºC (65ºF) 96 HR	7.00	5.50	22.90	41.20	1.17	9.06	0.0	10.0	8.60	9.06
24ºC (75ºF) 24 HR	4.07	1.93	15.90	9.20	0.83	4.81	0.0	1.8	8.50	8.23
24ºC (75ºF) 48 HR	6.00	3.73	22.07	29.07	1.37	8.45	0.0	7.7	8.92	9.05
24ºC (75ºF) 96 HR	5.27	3.30	32.00	22.23	2.01	9.20	0.0	7.5	8.74	9.20
29ºC (85ºF) 24 HR	5.00	3.52	16.13	24.82	0.67	2.79	0.0	0.3	8.51	8.25
29ºC (85ºF) 48 HR	6.80	4.00	22.67	25.07	1.01	9.41	0.0	5.3	9.28	9.41
29ºC (85ºF) 96 HR	5.93	4.73	25.93	35.60	1.45	8.59	0.0	5.5	9.22	8.59

[z]Pollen viability test (2 hr at room temperature-70ºF) = % (visual estimate). Media; Agarose= 10g/L; sucrose= 100g/L; boric acid= 10mg/L

.[y]Visual rating scale : 0 = no pollen grains or tubes visible; 10 = large number of pollen grains or tubes visible.

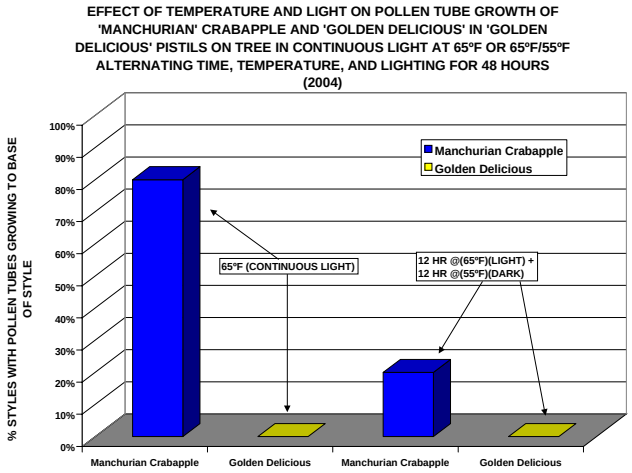

Figure 1

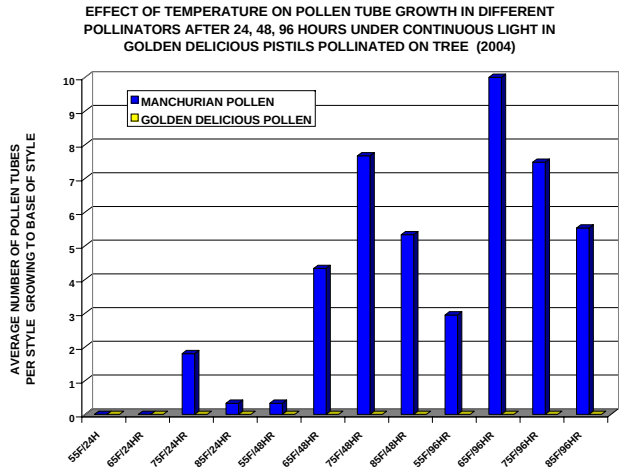

Figure 2

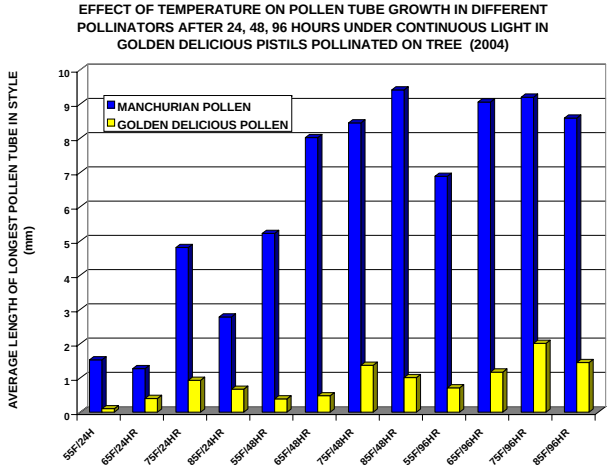

Figure 3

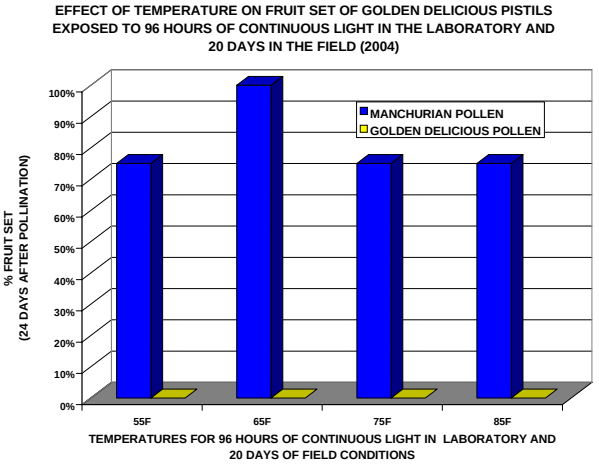

Figure 4