FINAL PROJECT REPORT
WTFRC Project Number: #439934

Project Title: Temperature Effect on Pollen Germination and Tube Growth in Apples

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Other funding Sources

Agency Name:
Amount awarded:
Notes:

Total Project Funding: **$**93,439.00

Budget History:

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3:** 2007
Salaries	20,000	20,600	21,218
Benefits	7,350	8,845	9,110
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	1,000	1,316	1,000
Travel	0	0	0
Contractual services	1,000	1,000	1,000
	0	0	0
	0	0	0
Miscellaneous	0	0	0
Total	**29,350**	**31,761**	**32,328**

SIGNIFICANT FINDINGS

- Observation have been conducted on pollen tube growth in approximately 10,000 styles of more than 2000 hand-pollinated flowers.
- Our database includes the commercial cultivars Gala, Golden Delicious and Fuji and the pollinizing cultivars Gala and Golden Delicious as well as Manchurian and Snowdrift crabapples and 12 other crabapples (Figs. 1 & 2).
- Results have been combined to formulate pollen tube growth patterns suitable for development and testing of a computer-generated pollen tube growth model for Washington State growers.

Highlights of Year 1 – (2005)

- Pollen tube growth to base of styles on Gala pistils occurred in less than 96 hours after pollination at alternating 55ºF 12-hr light/35ºF 12-hr dark (55/35º lt/dk) with all pollinizers tested.
- Pollen tube growth to base of styles on Gala pistils in tests at 65/40º lt/dk and 75/45º lt/dk showed all with pollen tube growth to end of styles 48 hours after pollination.
- Pollen tubes reached the end of some styles in less than 48 hours after pollination with Manchurian and Snowdrift pollen at 65/40º lt/dk and 75/45º lt/dk test trials. At 55/35º lt/dk pollen tubes reached the end of some Gala styles in less than 96 hours after pollination with Manchurian and Snowdrift pollen (Figs. 3 & 4).
- There were no evident pollen tubes at stylar bases of Golden Delicious pistils pollinated with Golden Delicious pollen in any test.
- Some Golden Delicious pollen tubes growing on Golden Delicious stigmas and in styles developed bulbous ends which appeared to impede growth of tubes. This was not observed with Manchurian and Snowdrift pollen and may be related to pollen incompatibility.
- Stigma receptivity to pollen germination continued to 7 days after full bloom and pollen tubes grew to the end of styles after 96 hours at 55/35º lt/dk. Optimum receptivity occurred 3 days after full bloom with tube growth to the bases in 100% of the styles, 45% after 5 days, and 30% after 7 days.
- Crabapple pollinizers are cultivar/temperature sensitive.

Highlights of Year 2 – (2006)

- In-orchard applications of Liquid Lime Sulfur + Crocker's Fish Oil (LLS+CFO), based on predicted fertilization timing, were shown to be effective for preventing fruit set (Fig. 5).
- Growth progress of pollen tubes in styles affects the success/failure of application of bloom thinner (LLS+CFO, Fig. 6).
- LLS+CFO applied 4 hours (72°F) after hand pollination of 'Golden Delicious' pistils on-tree stopped all growth of pollen tubes into styles from stigmas. Mean temperature for 24-hr period after application was 63°F.
- LLS+CFO applied 24 hours (72°F) after pollination stopped all growth of pollen tubes to base of styles from stigmas. Average temperature for 24-hr period after application was 67°F and it was predicted that ovule fertilization had not yet occurred.
- LLS+CFO applied 48 hours (68°F) after pollination of 'Golden Delicious' pistils in orchard had little or no effect on growth of pollen tubes into styles from stigmas. Mean temperature for 24-hr period after application was 57°F and ovule fertilization had been predicted.
- Mean number of pollen tubes reaching base of 'Golden Delicious' styles sprayed 48 hours after pollination was similar to styles that received no spray treatment.
- LLS+CFO applied 4 hours after pollination of 'Fuji' pistils on detached spurs and placed in 75/45°F (lt/dk) rotation for 48 hours stopped all pollen tube growth from stigmas into styles.
- Controlled temperature/light tests on hand-pollinated 'Gala' and 'Golden Delicious' pistils confirm '06 data.

- Model predictions using 2006 full bloom (FB) and temperature data from three locations in Washington State show a range required for ovule fertilization after pollination: 30 hours (full bloom April 28) at Wenatchee to 102 hours at Omak/Pogue Flats (full bloom April 30, Fig. 7&8).

Highlights of Year 3 – (2007)

- Comparing in-orchard growth of Snowdrift pollen tubes into 7 cultivars, Golden Delicious flowers favored the longest pollen tube growth (6.4 mm) after 24 hours (Fig 9).
- Snowdrift pollen tube growth Red Delicious flowers was 2.0 mm in 24 hours.
- Snowdrift pollen tube growth was intermediate in Braeburn, Pink Lady, Gala, Honeycrisp, and Fuji after 24 hours.
- Pollen tube growth was slower in older flowers, decreasing 50% in flowers pollinated 3, 4, and 5 days after opening compared to those pollinated in the first 2 days later (Fig. 10).
- Thunderchild ranked higher than Snowdrift and Selkirk for number of pollen tubes penetrating Gala stigma bases; a commonly used commercial pollinizer, Manchurian, was low.
- Comparison of mean length of longest pollen tubes in Gala vs Fuji 96 hours after pollination showed equivalent pollen tube growth at 65/40ºF lt/dk and 75/45ºF lt/dk.
- Pollen tubes had reached the base of all Gala and Fuji styles tested at 65/40ºF lt/dk and 75/45ºF lt/dk at 72 hours after pollination.
- There were twice as many pollen tubes reaching base of Gala styles compared to Fuji.

METHODS

Pollen collection: Golden Delicious trees grown in root bags were removed from the orchard in early March and placed in cold rooms to delay flowering, then placed in a greenhouse to induce flowering for harvesting pollen. Branches of Manchurian and Snowdrift crabapples were collected from the field and forced to produce pollen in the greenhouse. Some pollen was also collected from trees in the field. Balloon stage flowers with anthers that had not yet dehisced were harvested for pollen. Anthers were removed from stamens of harvested flowers and allowed to dry overnight at room temperature, then pollen was screened, placed in glass vials and stored at 0°C in a larger jar containing Drierite. Pollen viability was checked on an agar/sucrose/boric acid mixture by incubating it at room temperature for 1 or 2 hours before scoring for germination under the microscope.

General procedures, growth chamber pollination studies: Gala, Fuji or Golden Delicious trees grown in root bags were removed from the orchard row early in March and held in a cold room to delay onset of bloom, then forced in a greenhouse to induce bloom. At late balloon stage, 12 flowers /treatment were selected for the pollination experiment. One day before hand pollination of test flowers, all anthers were removed from test flowers to prevent self-pollination. All other flowers on test trees were removed to prevent cross-pollination and to balance the test blossom distribution. Selected flowers were tagged and pollen was applied to stigmas with a #2 brush. 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 indicated lengths of time, temperature, and lighting. Flowers were removed from trees at indicated times, placed in a solution of 5% sodium sulfite in labeled glass containers, boiled for 15 min., then refrigerated until microscopic examination. Five styles from each of three flowers were detached from the ovary, dipped in fluorescence solution, squashed between microscopic slides, and allowed to incubate 24 hrs before examination with epi-UV light using a Zeiss HBO-50 high pressure mercury vapor light source at 100X. Collected data included abundance of pollen germination/tube growth (0-10) on the stigma surface (rating scale), number of tubes penetrating the stigma base, mean length of the longest pollen tube, mean style length, and number of pollen tubes reaching the base of the style.

General procedures, field pollination studies: Flowers on orchard trees were selected at late balloon stage for field pollination test conducted to examine the effect of a bloom thinning treatments using Liquid Lime Sulfur + Crocker's Fish Oil (LLS+CFO), applied at selected intervals after pollination, on pollen germination and tube growth. Golden Delicious/M.27 root-bagged trees in the field were used for this experiment. Trees were selected for uniformity and divided into four groups. Flowers at full bloom were hand-pollinated with Snowdrift pollen and any unused flowers were removed. Trees were covered with white insect netting until spray applications to prevent pollination from natural sources. Treatments were applied at 4, 24, and 48 hours after pollination. LLS+CFO was applied only once per treatment. Flowers, collected 48 hours after the treatment was applied, were processed and evaluated as indicated above or same for all field experiments. Hourly temperature for the period from application to sample harvest was recorded.

RESULTS & DISCUSSION

Research on the influence of temperature on pollen germination, fertilization, and pollen tube growth on apple cultivars over the past 40 years has been limited. The search for a better understanding of the biological process of pollination and the effect of temperature on pollen tube growth and fertilization was the basis for our research project covering the last 3 years. Previous research (Williams, 1965) on the Effective Pollination Period (EPP) indicated that fertilization would normally take from 5-7 days after pollination for pollen tubes to fertilize the ovule. Our research under controlled temperature and light conditions and in-orchard under normal growing conditions showed the process of flower fertilization occurring in as little as 24 hours after pollen is applied to the stigmatic surface under optimum growing conditions. Under suboptimal growing conditions the same result of flower fertilization can occur in 48 hours or less. These factors are critical to a grower who may be applying a bloom thinner with a mode of action of stopping pollen tubes from growing into the style. Under these parameters, a grower who waits 48 hours after bloom to apply the bloom thinner would be wasting time, money, and material to stop bloom set due to faster than anticipated tube growth and callose plugging that occurs during tube growth down the style. A pollen growth model showing the growth pattern once it is attached to the stigma would give the grower an essential tool to help determine the time frame for application of bloom-thinners. Our research has yielded the data necessary for the development of such a model.

From its inception, this research project has conducted over 20 individual experiments on the effect of temperature on pollen tube growth on the stigma surface, growth into the style, and culminating in the pollen tubes reaching the ovules. We have conducted tests using commercially important cultivars such as Gala, Fuji, and Golden Delicious. We have prepared for hand-pollinated over 2000 flowers. Approximately 10,000 styles have been dissected and evaluated to determine the growth rate of pollen tubes at various times after pollination. We have used as pollen sources for our testing Manchurian and Snowdrift crabapple pollen. We have conducted preliminary studies using other crabapple pollens for tests and some of these pollinizers show promise but will require additional testing before they can be recommended to growers as alternatives to standard pollinizers presently in use in orchards. Our research results are now ready to be implemented into a computer-generated model to project pollen tube growth under actual growing conditions in Washington State orchards. The yearly summary of our project that follows gives a breakdown of some of the more important findings.

In 2004, we found that pollen tubes in "on-tree" flowers grew more rapidly to the base of the style under continuous light in growth chambers than in flowers that were detached in either the dark or light. Based on these results, we believe that reserves mobilized to the flowers by the tree and additional ongoing photosynthesis are important influences in determining the rate of pollen tube growth at various temperatures. These findings led us to believe that in most cases these tests should be conducted on-tree for more reliable and conclusive results.

Our experiments in 2005, conducted on trees under controlled light/dark temperature regimes continued to build on findings in 2004, yielding significant information on the effects of temperature

and light on pollen germination, fertilization, and pollen tube growth on Golden Delicious and Gala. Data from these experiments and subsequent tests have been used to develop a model to predict pollen tube growth and fertilization. Our tests involving several pollinizers and fruiting cultivars show that we cannot generalize pollen germination/tube growth rates to all pollinizer/cultivar combinations. Additional tests under in-orchard field conditions are needed to test the robustness of that modeling program so that growers can use in practice. Any modeling program must be cultivar/pollinizer specific, not an all-encompassing model of one size fits all program.

2006 experiments conducted in temperature and light-controlled growth chambers and in the orchard yielded more useful information. Temperature effect or growth rate of pollen tubes after pollination has a significant impact on optimal timing of bloom thinner applications. Knowledge of growth rate of tubes into styles after pollination is critical for successful bloom-thinning practices. Our tests have shown that delaying applications by one day can result in the bloom thinner LLS+CFO having little or no effect on flower fertilization on earlier pollinated flowers but inhibits the more recently pollinated ones. By understanding pollen tube growth rate after pollination in relation to temperatures, growers may save time and money by reducing sprays or by applications of sprays at optimum thinning times. By developing temperature-based pollen tube growth models, we can follow the development of pollen tubes from pollination of stigmas to fertilization of ovules. These models could take into consideration the mode of action of the type of thinners used.

In 2007 tests comparing Gala vs Fuji, pollen tube growth rate was not significantly different at evaluated temperature and times. Pollen tube growth rate decreased significantly in flowers pollinated 3 or more days after first opening. Average length of pollen tubes growing into styles was approximately 50% less in flowers pollinated 3 days after first opened compared to those pollinated in the first two days after opening. Our tests on flowers pollinated in-orchard showed marked differences in pollen tube growth in the varieties tested. Compatibility and viability tests of several crabapple pollinizers showed significant variability in numbers of pollen tubes growing into styles, which might result in reduced fruit set. Our continued studies in 2007 on the effects of alternating temperatures (35ºF to 75ºF) and light on pollen germination and tube growth have been combined with previous years data to formulate a growth pattern used to calculate pollen tube growth in selected Golden Delicious, Gala, and Fuji apples. At present we are collaborating with the Washington Tree Fruit Research Commission and Vincent Jones of Washington State University Tree Fruit Research and Extension Center in the development of a computer-generated pollen tube growth model for Washington State growers.

Fig. 1 % FRUIT SET 28 DAYS AFTER POLLINATION OF 'GALA' AND 'FUJI' FLOWERS "ON-TREE" IN ORCHARD WITH VARIOUS POLLENS (2005)

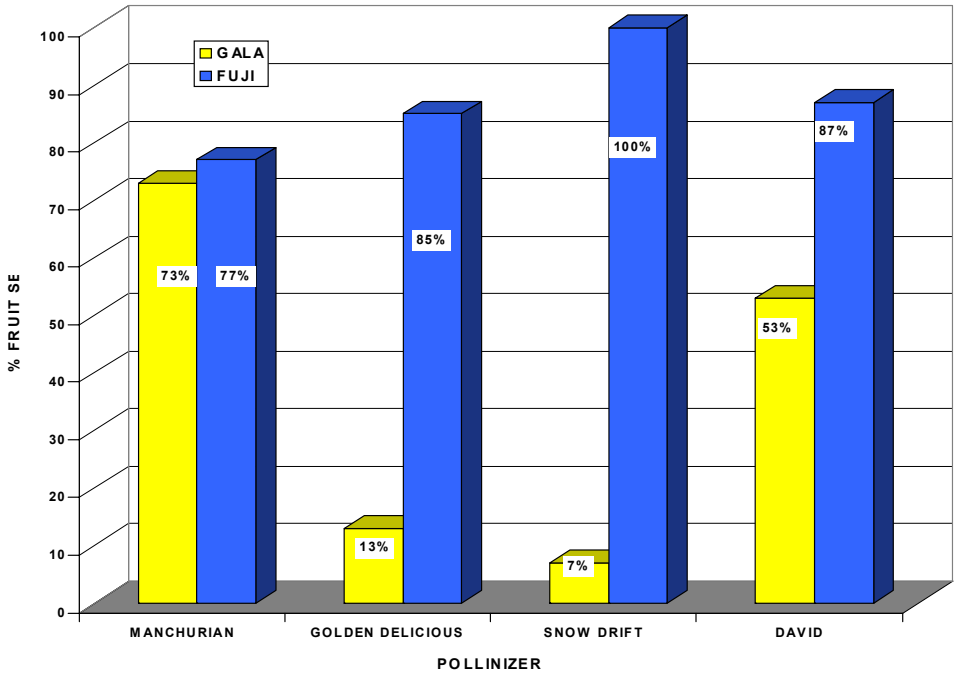


Fig. 2 VIABILITY AND COMPATIBILITY OF SELECTED CRABAPPLE POLLENS APPLIED TO 'GALA'/M.9 FLOWER PISTILS ON DETACHED SHOOTS AND EVALUATED AFTER 12 HOURS AT 75°F(LT) AND 12 HOURS AT 45°F(DK) (2007)

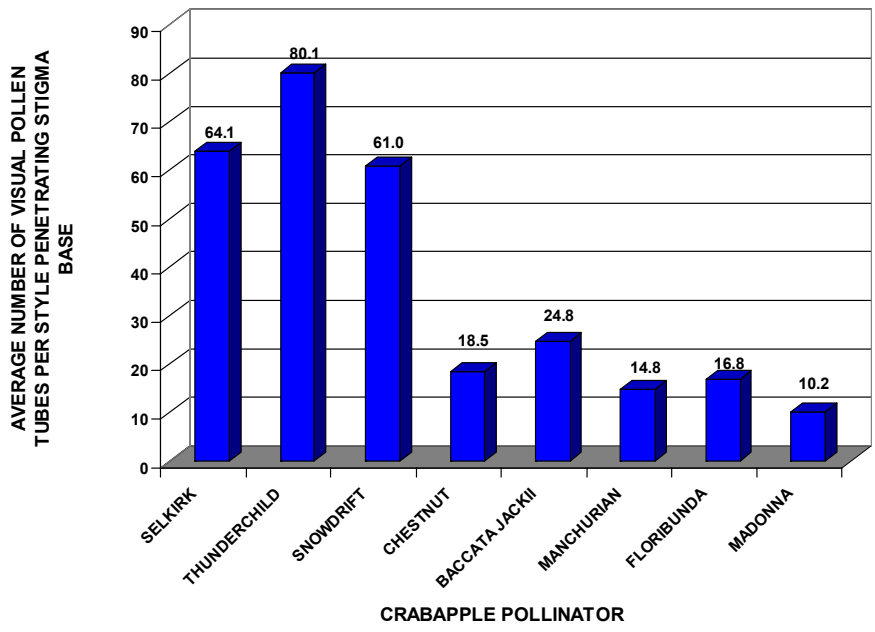


Fig. 3

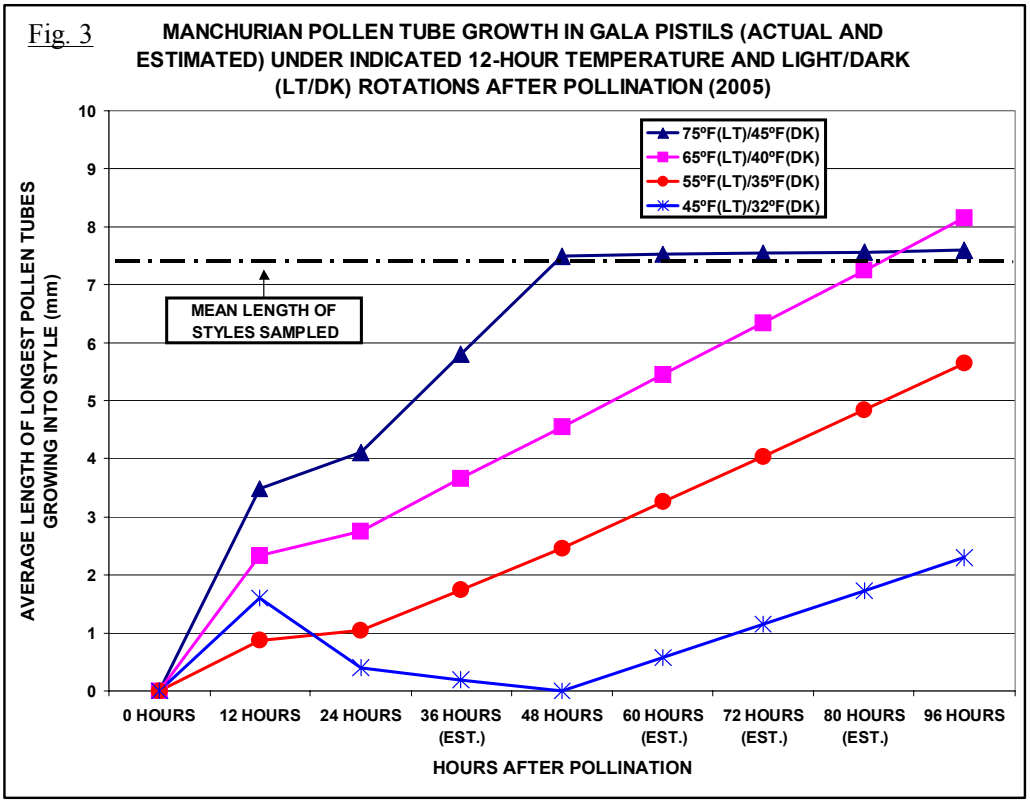

MANCHURIAN POLLEN TUBE GROWTH IN GALA PISTILS (ACTUAL AND ESTIMATED) UNDER INDICATED 12-HOUR TEMPERATURE AND LIGHT/DARK (LT/DK) ROTATIONS AFTER POLLINATION (2005)
75ºF(LT)/45ºF(DK)
65ºF(LT)/40ºF(DK)
55ºF(LT)/35ºF(DK)
45ºF(LT)/32ºF(DK)
MEAN LENGTH OF STYLES SAMPLED
AVERAGE LENGTH OF LONGEST POLLEN TUBES GROWING INTO STYLE (mm)
0
1
2
3
4
5
6
7
8
9
10
0 HOURS
12 HOURS
24 HOURS
36 HOURS (EST.)
48 HOURS
60 HOURS (EST.)
72 HOURS (EST.)
80 HOURS (EST.)
96 HOURS
HOURS AFTER POLLINATION

Fig. 4

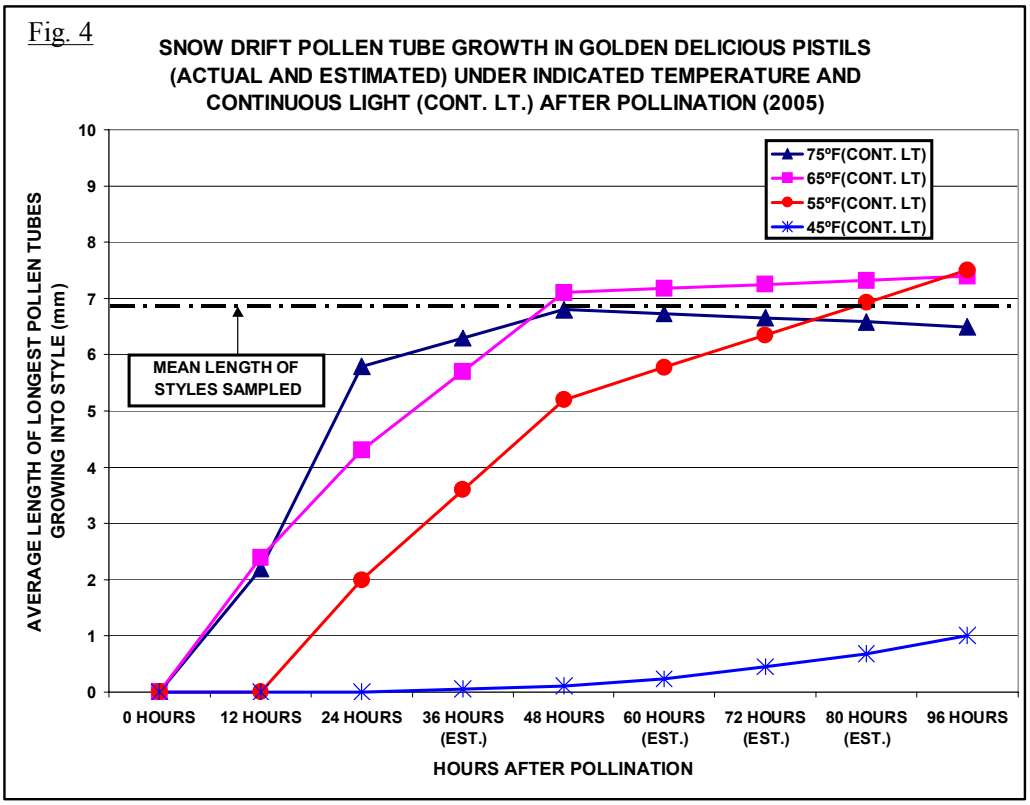

SNOW DRIFT POLLEN TUBE GROWTH IN GOLDEN DELICIOUS PISTILS (ACTUAL AND ESTIMATED) UNDER INDICATED TEMPERATURE AND CONTINUOUS LIGHT (CONT. LT.) AFTER POLLINATION (2005)
75ºF(CONT. LT)
65ºF(CONT. LT)
55ºF(CONT. LT)
45ºF(CONT. LT)
MEAN LENGTH OF STYLES SAMPLED
AVERAGE LENGTH OF LONGEST POLLEN TUBES GROWING INTO STYLE (mm)
0
1
2
3
4
5
6
7
8
9
10
0 HOURS
12 HOURS
24 HOURS
36 HOURS (EST.)
48 HOURS
60 HOURS (EST.)
72 HOURS (EST.)
80 HOURS (EST.)
96 HOURS
HOURS AFTER POLLINATION

Fig. 5

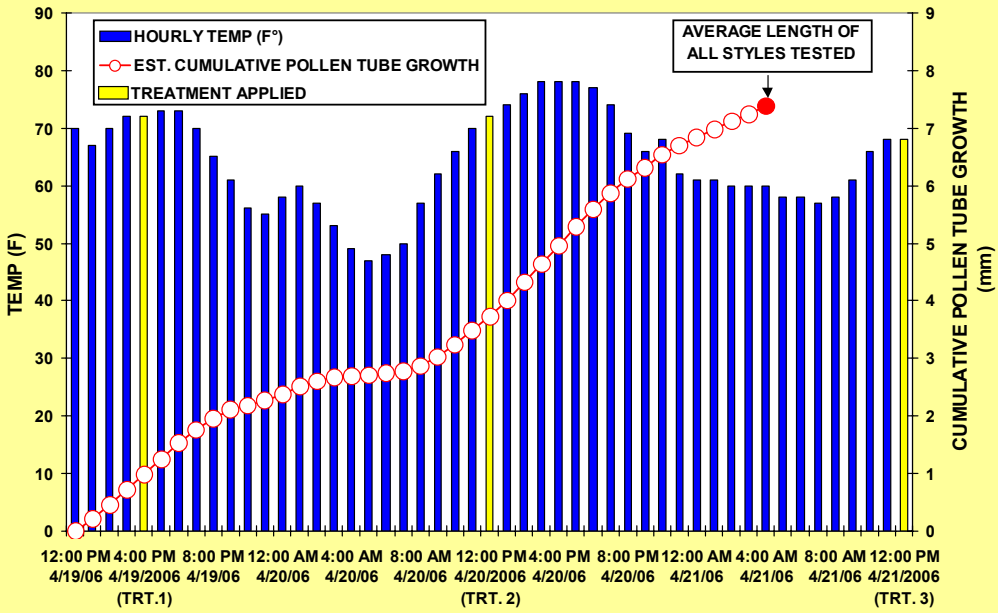

EFFECT OF TIMING OF APPLICATION OF LLS+CFO AT 4, 24, OR 48 HOURS AFTER POLLINATION ON POLLEN TUBE GROWTH IN HAND POLLINATED 'GOLDEN DELICIOUS'/M.27 FLOWER PISTILS IN FIELD (2006)
HOURLY TEMP (F°)
EST. CUMULATIVE POLLEN TUBE GROWTH
TREATMENT APPLIED
AVERAGE LENGTH OF ALL STYLES TESTED
TEMP (F)
CUMULATIVE POLLEN TUBE GROWTH (mm)
12:00 PM 4/19/06 (TRT.1)
4:00 PM 4/19/2006
8:00 PM 4/19/06
12:00 AM 4/20/06
4:00 AM 4/20/06
8:00 AM 4/20/06
12:00 PM 4/20/2006 (TRT. 2)
4:00 PM 4/20/06
8:00 PM 4/20/06
12:00 AM 4/21/06
4:00 AM 4/21/06
8:00 AM 4/21/06
12:00 PM 4/21/2006 (TRT. 3)
HOURLY TEMPERATURE AND ESTIMATED POLLEN TUBE GROWTH FROM POLLINATION TO TIME OF LAST TREATMENT APPLICATION

Fig. 6

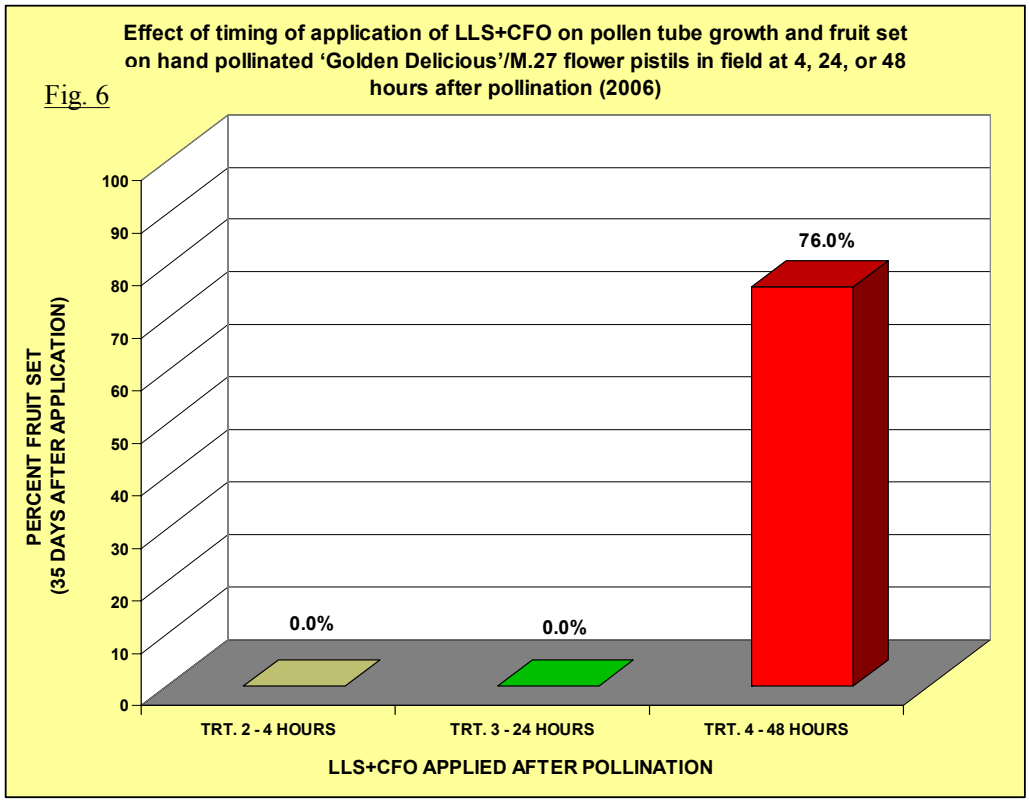

Effect of timing of application of LLS+CFO on pollen tube growth and fruit set on hand pollinated 'Golden Delicious'/M.27 flower pistils in field at 4, 24, or 48 hours after pollination (2006)
PERCENT FRUIT SET (35 DAYS AFTER APPLICATION)
76.0%
0.0%
0.0%
TRT. 2 - 4 HOURS
TRT. 3 - 24 HOURS
TRT. 4 - 48 HOURS
LLS+CFO APPLIED AFTER POLLINATION

Fig. 7

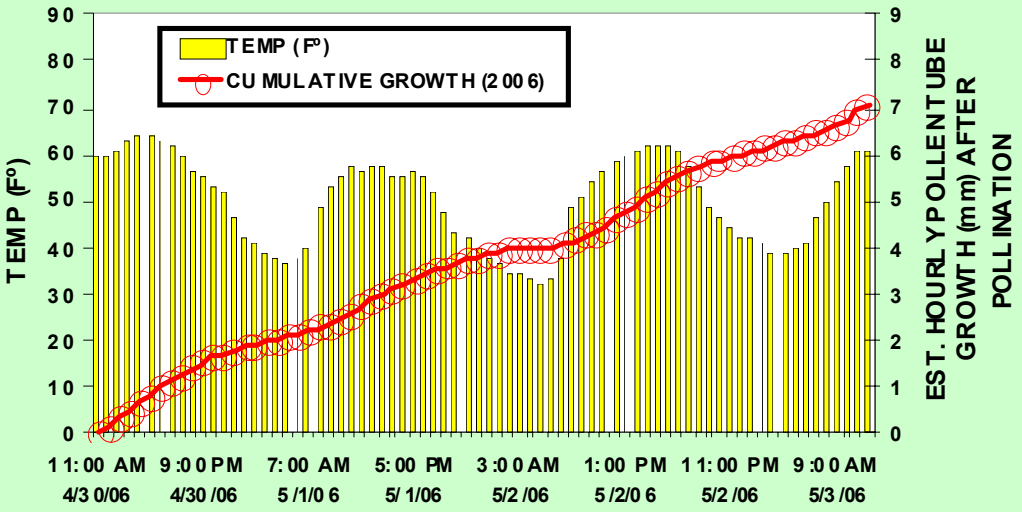

ESTIMATED POLLEN TUBE GROWTH FOR OMAK/POGUE FLATS, WA (2006)
TEMP (F°)
CUMULATIVE GROWTH (2006)
TEMP (F°)
EST. HOURLY POLLEN TUBE GROWTH (mm) AFTER POLLINATION
90
80
70
60
50
40
30
20
10
0
9
8
7
6
5
4
3
2
1
0
11:00 AM 4/30/06
9:00 PM 4/30/06
7:00 AM 5/1/06
5:00 PM 5/1/06
3:00 AM 5/2/06
1:00 PM 5/2/06
11:00 PM 5/2/06
9:00 AM 5/3/06

Fig. 8

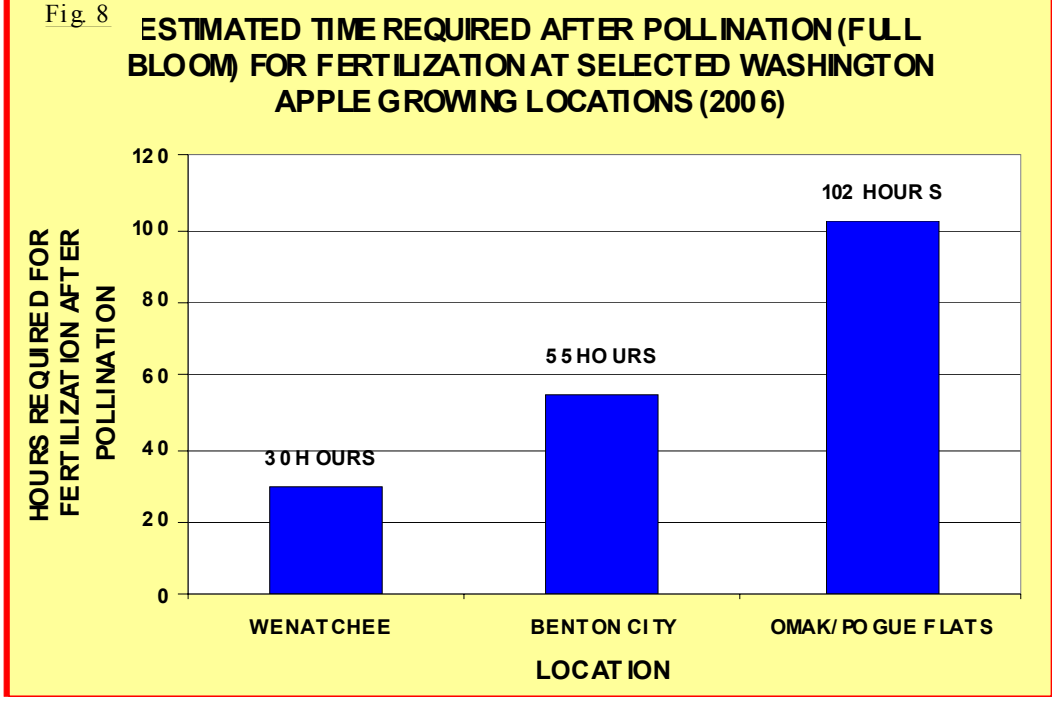

ESTIMATED TIME REQUIRED AFTER POLLINATION (FULL BLOOM) FOR FERTILIZATION AT SELECTED WASHINGTON APPLE GROWING LOCATIONS (2006)
HOURS REQUIRED FOR FERTILIZATION AFTER POLLINATION
120
100
80
60
40
20
0
30 HOURS
55 HOURS
102 HOURS
WENATCHEE
BENTON CITY
OMAK/POGUE FLATS
LOCATION

Fig. 9

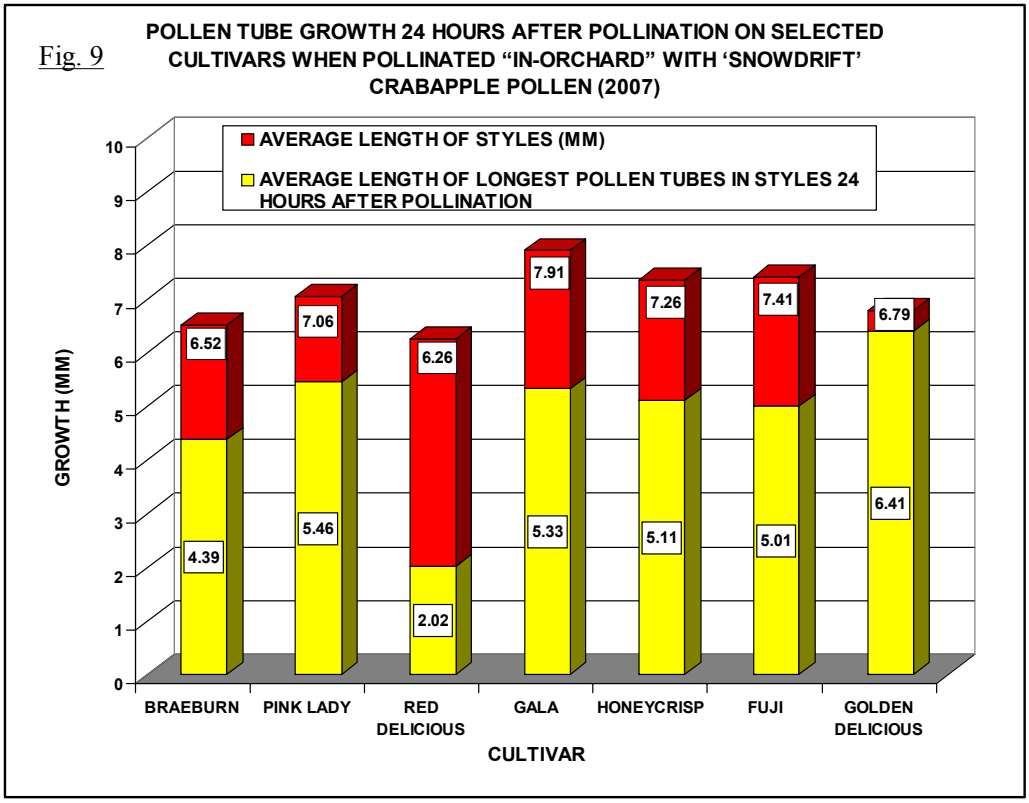
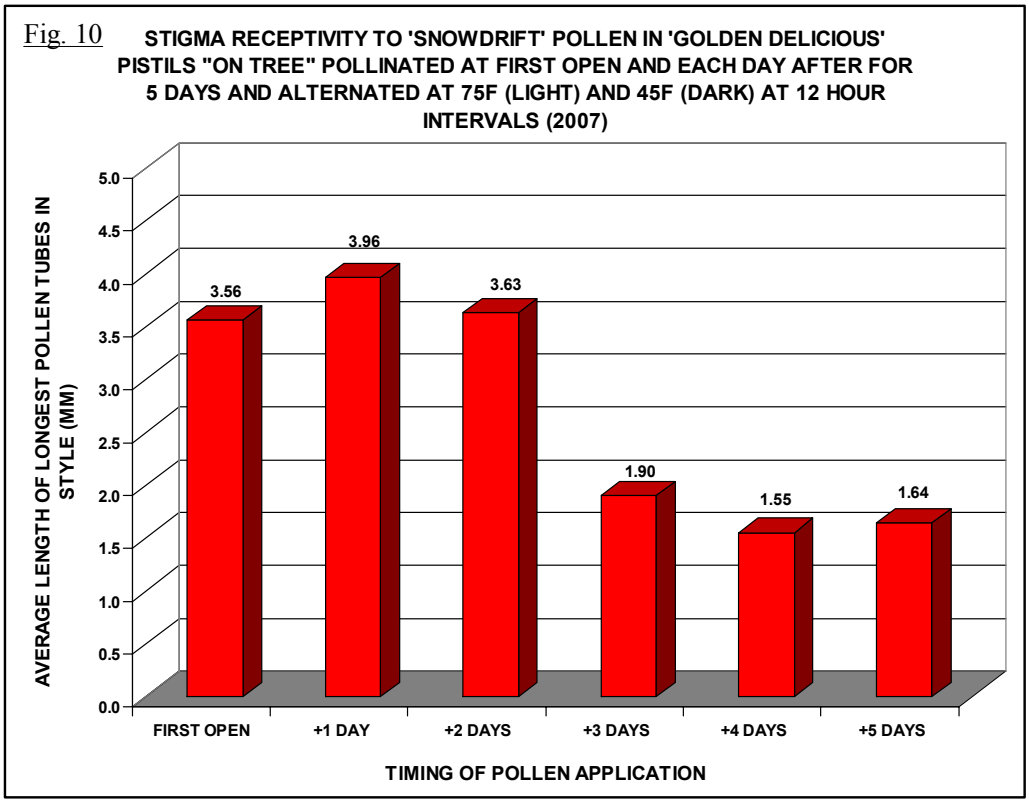

POLLEN TUBE GROWTH 24 HOURS AFTER POLLINATION ON SELECTED CULTIVARS WHEN POLLINATED "IN-ORCHARD" WITH 'SNOWDRIFT' CRABAPPLE POLLEN (2007)
AVERAGE LENGTH OF STYLES (MM)
AVERAGE LENGTH OF LONGEST POLLEN TUBES IN STYLES 24 HOURS AFTER POLLINATION
GROWTH (MM)
0
1
2
3
4
5
6
7
8
9
10
6.52
4.39
7.06
5.46
6.26
2.02
7.91
5.33
7.26
5.11
7.41
5.01
6.79
6.41
BRAEBURN
PINK LADY
RED DELICIOUS
GALA
HONEYCRISP
FUJI
GOLDEN DELICIOUS
CULTIVAR

Fig. 10


STIGMA RECEPTIVITY TO 'SNOWDRIFT' POLLEN IN 'GOLDEN DELICIOUS' PISTILS "ON TREE" POLLINATED AT FIRST OPEN AND EACH DAY AFTER FOR 5 DAYS AND ALTERNATED AT 75F (LIGHT) AND 45F (DARK) AT 12 HOUR INTERVALS (2007)
AVERAGE LENGTH OF LONGEST POLLEN TUBES IN STYLE (MM)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
3.56
3.96
3.63
1.90
1.55
1.64
FIRST OPEN
+1 DAY
+2 DAYS
+3 DAYS
+4 DAYS
+5 DAYS
TIMING OF POLLEN APPLICATION