Prune Pollination and Fruit Set

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Problem and its Significance:

Forecasted in October as 50% below the 2006 crop, the 2007 dried plum crop yield appears to be similar to the prune crops of 2004 and 2005. Like 2004 and 2005, the temperature during bloom was extremely warm with daytime temperatures often reaching into the 80s Fahrenheit. Final fruit production is based largely on fruit set which in turn is dependent on a series of successful reproductive processes. Therefore, the disruption of any one of the reproductive events may result in lower fruit set.

Literature spanning many years suggests temperature plays a role in determining nearly all successful reproductive processes. Previous work on other *Prunus* crops examining temperature effects on these reproductive events show high temperatures may adversely effect pollen germination and tube growth as well as ovule development and longevity.

While temperature has been studied in regards to its effects on prune reproductive biology, the data was elucidated using the 'Italian' variety as well as others not grown in California. Given the current climate situation and data showing the effects of temperature on reproductive processes vary even between cultivars, we have conducted an investigation looking at different temperature parameters on pollen germination, tube growth and ovule longevity in prune flowers of varieties grown in California.

Objectives:

Our objective in this project is to determine temperature parameters that affect pollen germination, pollen tube growth and fruit set in prune flowers. The first objective is to generate temperature responses for pollen germination and pollen tube growth for 'French' and 'Muir Beauty' and to use these regressions to interpolate temperature optima and elucidate supraoptimal responses. We also conducted field experiments designed to elucidate temperature responses in vivo. Finally, we initiated a preliminary experiment to determine the efficacy of using irrigation to moderate high termperatures during bloom.

Completing the final year of a proposed two-year study, we have conducted two years of *in vitro* studies allowing us to compare pollen germination and tube growth in 'French Improved' and 'Muir Beauty' prune cultivars in response to temperature. Adding to this data, we completed *in vivo* experiments allowing for the examination of 'French Improved' pollen tube kinetics and dynamics. In our pollen tube kinetics study, we calculated the percentage of flowers with pollen tubes at various points in the style and the percentage of the style traveled by the longest pollen tube. For our dynamics investigation, we quantified the number of pollen tubes at the base of each style several days following bloom. After developing the methodologies for studying 'French

Improved' ovule senescence last year, circumstances did not allow us to determine ovule viability in 'French Improved' prune. From the two-year study, we have modeled the temperature effects on germination and tube growth in 'French Improved' in addition to determining pollen tube kinetics and dynamics under a range of temperatures.

Methods:

After developing an *in vitro* system for determining pollen germination and pollen tube growth responses to temperature in year one, we ran a second set of similar experiments to add additional replications to our model. Freshly collected pollen was incubated on an agar-solidified medium consisting of 1.0 mM Calcium Chloride, 1.0 mM Boric Acid, 10% Sucrose and 1.2% Agar in petri plates maintained on a temperature gradient bar. The temperature gradient bar consists of a meter-long aluminum bar with channels cut into both ends. Polyethylene glycol (antifreeze) solutions are pumped through the channels. The solution at one end is cooled by a chilling unit and the solution at the other end of the bar is heated with a submersible heater. When the bar reaches equilibrium, petri plates with media are brought to temperature equilibrium on the bar and inoculated with pollen. Pollen tube growth is stopped by fixation after three hours and the plates are evaluated for pollen germination percentages and pollen tube growth. Pollen tubes are measured from digital photomicrographs using image analysis software. The data from each replication was fitted to a parabolic regression (second order polynomial) with optima found at the vertex (-b/2a) and summarized using SAS Version 9.1 Software.

In vivo experiments were conducted in two treatments and one control. In one treatment, a single 'French Improved' tree was enclosed and covered with polyethylene sheeting allowing for the accumulation of heat. Using another 'French Improved' tree in the same growing block, two micro-sprinklers on each side of the tree irrigated for up to 5 midday hours with 40L/hr of water. Temperature was monitored with data loggers placed in appropriate locations approximately 2.5m high in the enclosed and irrigated space as well as an adjacent ambient tree that served as a control. Flowers were hand pollinated as they emerged over several days. Pistils were collected at two-day intervals following pollination. The pistil samples were fixed in ethanol-acetic acid solution. Style portions were softened and squashed to reveal pollen tubes. The squashed styles were stained with alkaline aniline blue, a fluorescent stain for callose, a pollen tube cell wall component. Stained samples were observed in a fluorescence microscope, and pollen tube growth in several portions of the style was determined for pollen tube kinetic and dynamic studies. Ovary portions were dissected to remove the ovules. After using a series of stains and filters, we were not successful in staining ovules to determine viability in primary ovules.

Results:

In Vitro Pollen Germination and Tube Growth Responses to Temperature.

Pollen germination regressions for 'French Improved' and 'Muir Beauty' pollen are shown in Figure 1. Pollen tube lengths for both cultivars are shown in Figure 2.

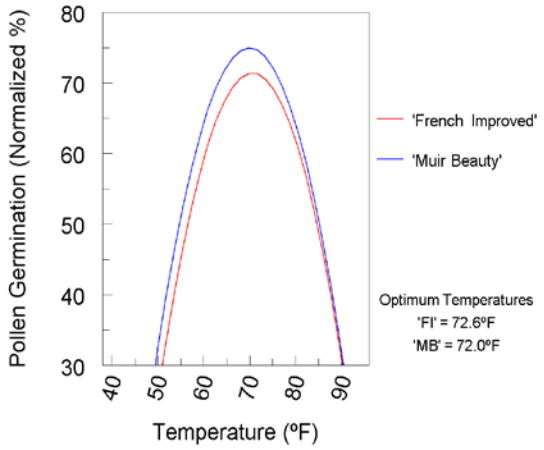


Figure 1. Regression curve for 'French Improved' and 'Muir Beauty' prune pollen germination vs. temperature. R=0.84 for 'French Improved' and 0.83 for 'Muir Beauty'.

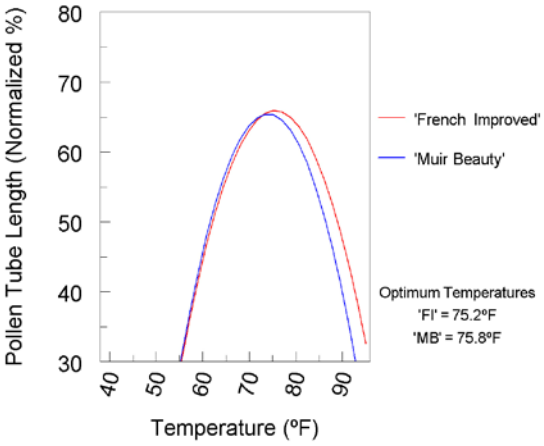


Figure 2. Regression curve for 'French Improved' and 'Muir Beauty' prune pollen tube length vs. temperature. R=0.41 for 'French Improved' and 0.38 for 'Muir Beauty'.

With the second year's data added to the first, the temperature optima for germination and tube growth range from 72 to 75.8ºF in 'French Improved' prune and fall off sharply at extremely hot temperatures.

Table 1. Optimum temperatures for pollen germination and pollen tube growth determined from the regression curves shown in Figs 1-2.

	Cultivar	Optimum
Pollen Germination	'French Improved'	72.6 ºF
Pollen Germination	'Muir Beauty'	72 ºF
Pollen Tube Growth	'French Improved'	75.2 ºF
Pollen Tube Growth	'Muir Beauty'	75.8 ºF

In vivo Pollen Tube Growth Kinetics and Dynamics for 'French Improved' prune.

The tree enclosed in polyethylene sheeting is shown in Figure 3.

Figure 3. Experimental 'French Improved' prune tree at Wolfskill Experimental Orchard in Winters, CA.

The tree used for the microsprinkler irrigation treatment is shown in Figure 4.

Figure 4. Experimental 'French Improved' prune tree at Wolfskill Experimental Orchard in Winters, CA. Microsprinkler irrigation began at midday and lasted until 5 PM on days when temperatures at the level of the sensor (as indicated) reached into the upper 70s/lower 80s Fahrenheit.

Figures 5-7 show temperature conditions for the ambient and heated temperature tree in the enclosure as well as the tree subjected to microsprinkler irrigation.

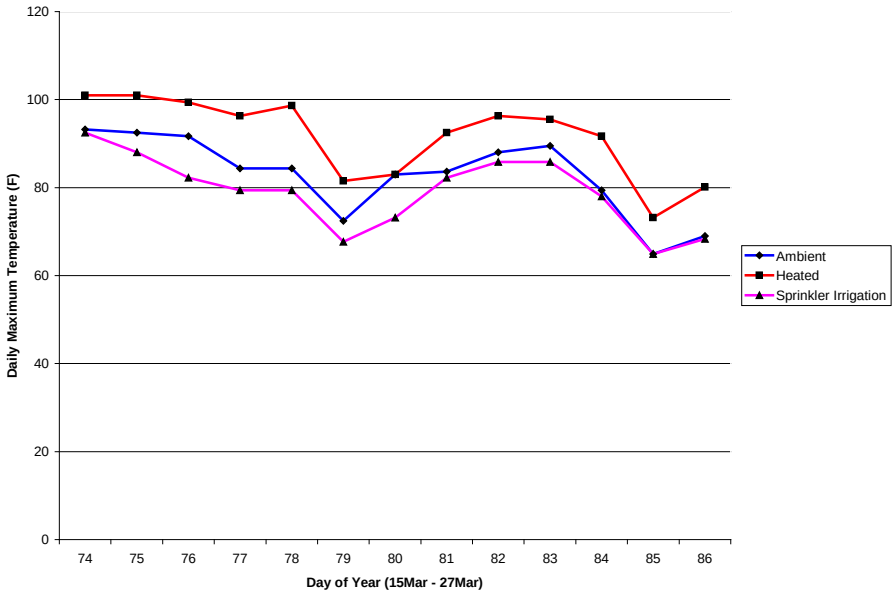


Figure 5. Maximum daily temperatures over the course of the experiments for ambient, microsprinkler irrigation and heated temperature treatments.

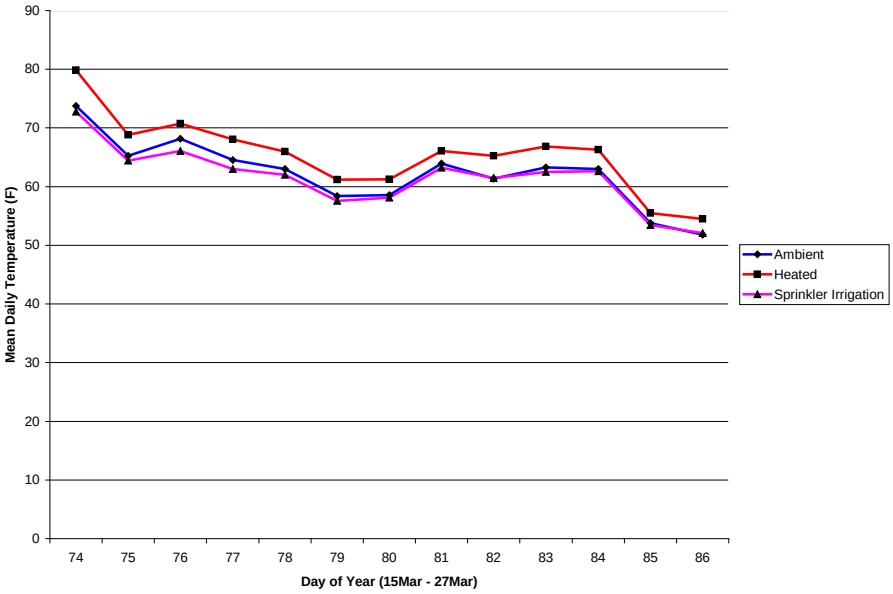


Figure 6. Mean daily temperatures over the course of the experiments for ambient, microsprinkler irrigation and heated temperature treatments.

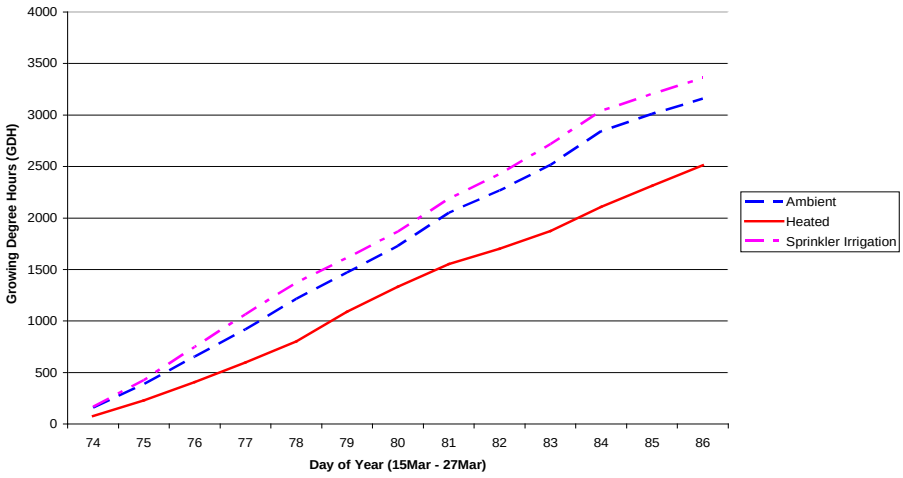


Figure 7. Accumulated heat units over the course of the experiments for ambient, microsprinkler irrigation and heated temperature treatments.

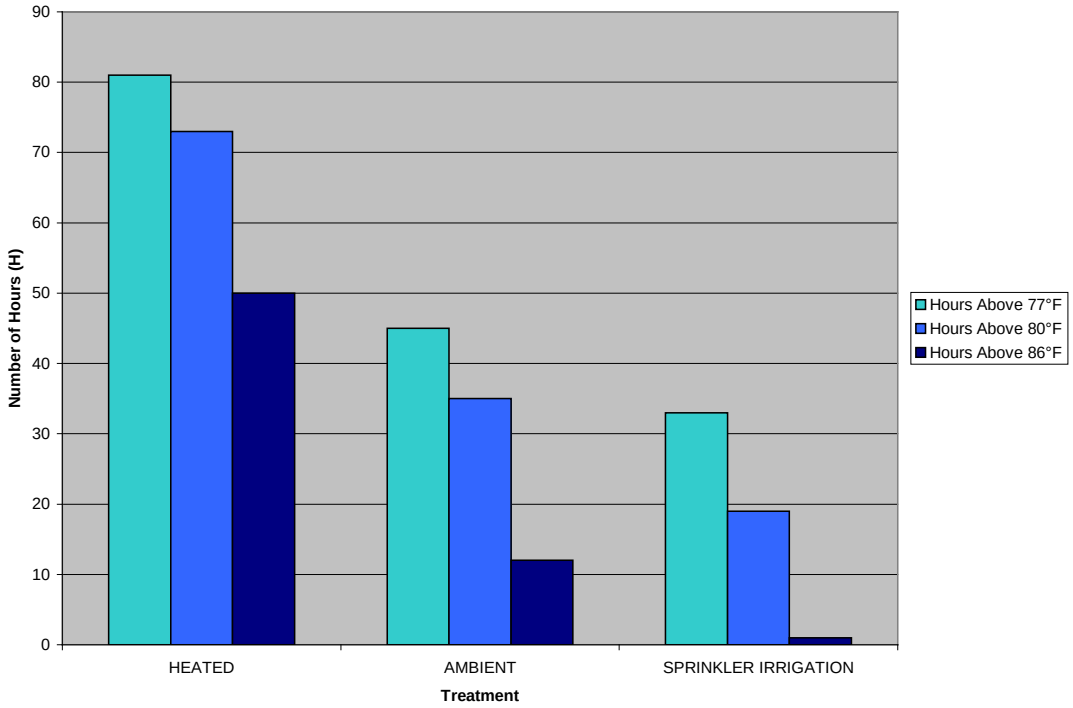
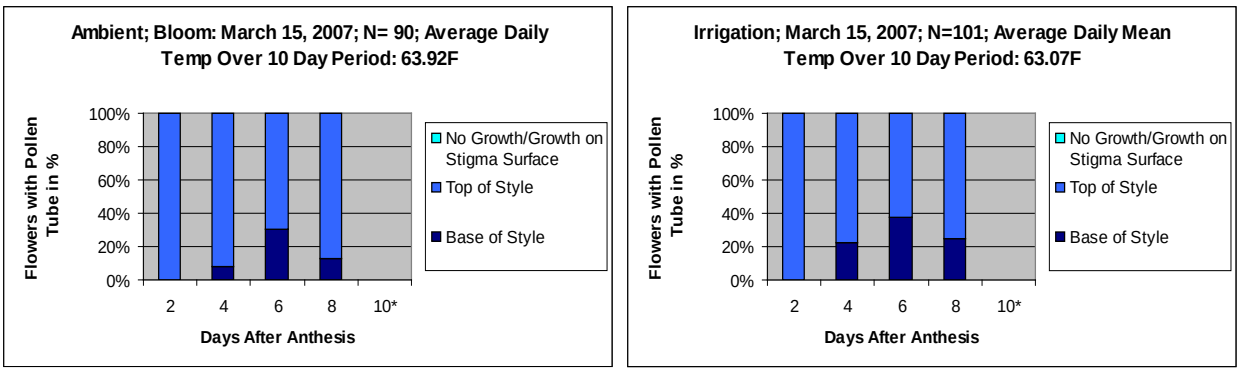


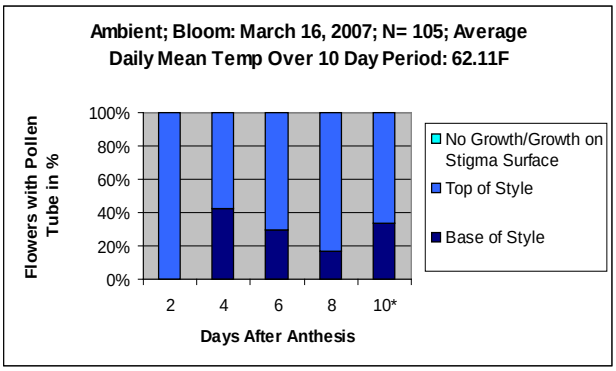
Figure 8. Number of Hours Above Several Temperature Thresholds for Heated, Ambient and Sprinkler Irrigation Treatments totaling (13) Days of Bloom.

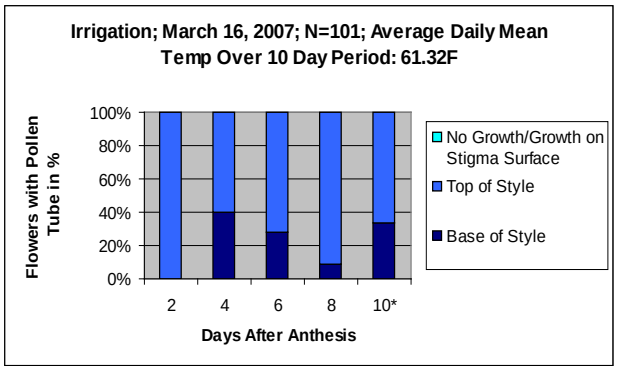
Ambient Conditions resembled those of 2004 and 2005 where daytime temperatures remained in the 80s Fahrenheit and high temperatures reached into the 90s Fahrenheit for several days after bloom. The sprinkler irrigation treatment received the least amount of hours over 77° Fahrenheit and subsequently, the most growing degree hours. Pollen tube growth was rapid in the ambient and microsprinkler irrigation temperature regimes. Within two days, tube growth germinated through into the style. By four days after bloom, tubes reached the bottom of the style.



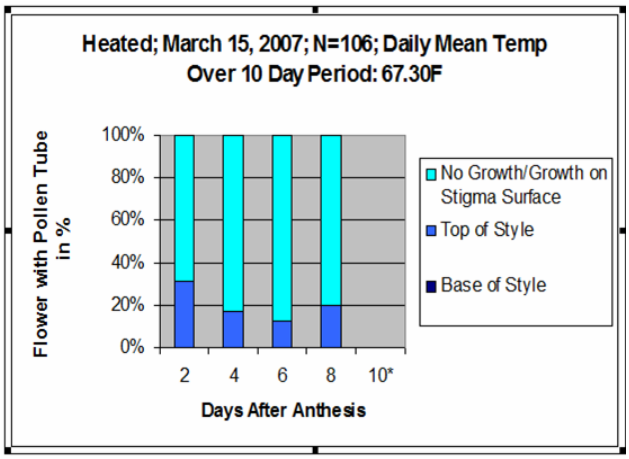
Figures 2 and 10. Flowers blooming March 15, 2007 with pollen tube growth *in vivo* under ambient (left) and microsprinkler irrigation (right) conditions. Data is shown as the percentage of flowers with pollen tubes at various stages: no growth/growth on the stigma surface, growth into the top of the style and growth through to the base of the style at four

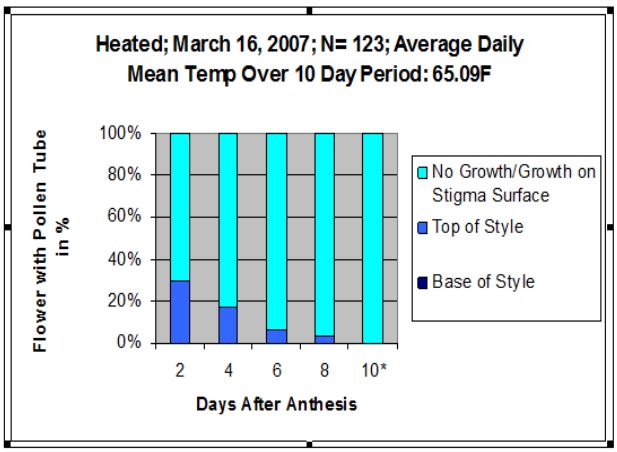
increments after bloom. Time is shown as the days after anthesis with (*) indicating a lack of results.





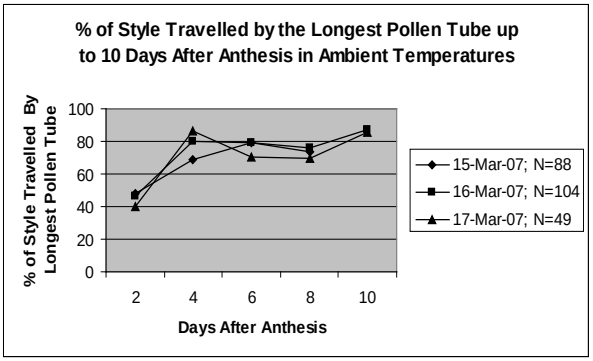
Figures 11 and 12. Flowers blooming March 16, 2007 with pollen tube growth *in vivo* under ambient (left) and microsprinkler irrigation (right) conditions. Data is shown as the percentage of flowers with pollen tubes at various stages: no growth/growth on the stigma surface, growth into the top of the style and growth through to the base of the style at four increments after bloom. Time is shown as the days after anthesis with (*) indicating a lack of results.

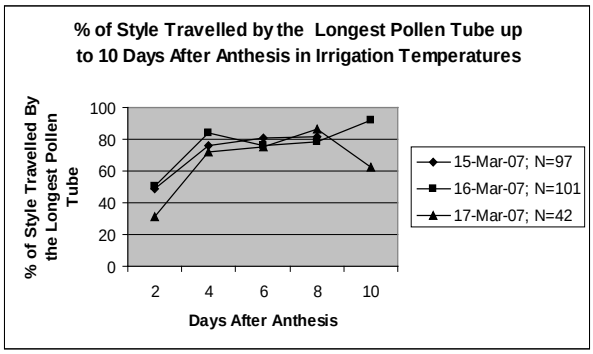


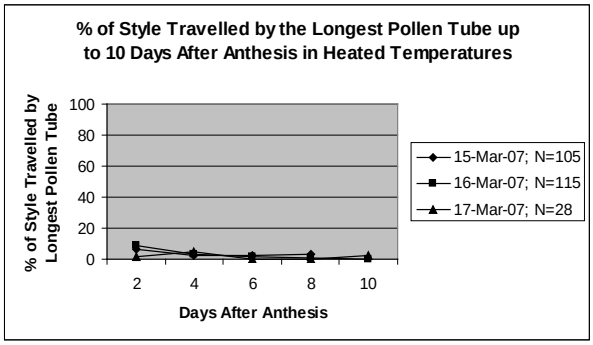


Figures 13 and 14. Flowers blooming March 15, 2007 (left) and March 16, 2007 (right) with pollen tube growth *in vivo* under heated conditions. Data is shown as the percentage of flowers with pollen tubes at various stages: no growth/growth on the stigma surface, growth into the top of the style and growth through to the base of the style at four increments after bloom. Time is shown as the days after anthesis with (*) indicating a lack of results.

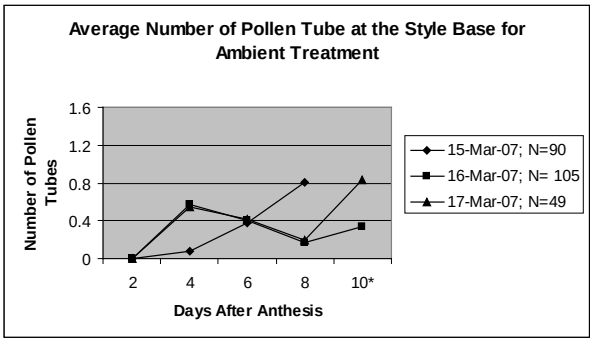
Pollen tube growth is not supported in the flowers from the trees in the heated treatment. Pollen tubes grow into the top of the style but do not reach the base (Figures 13 and 14).

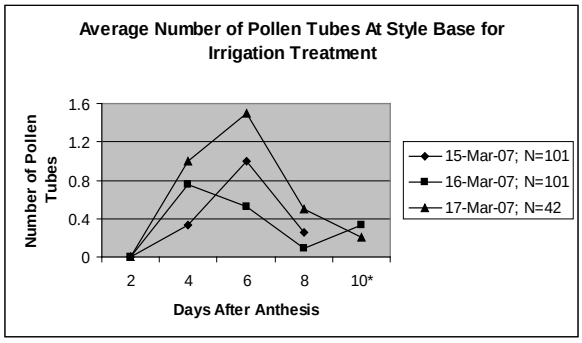






Figures 15, 16 and 17. Pollen tube growth *in vivo* for ambient (top left), microsprinkler irrigation (top right) and heated (bottom) treatments. Data are shown as the percentage of the style traveled by the longest pollen tube over the three days of bloom. Time is shown for five increments and represents the number of days after anthesis.





Figures 18 and 19. Pollen tube growth in vivo for ambient (left) and microsprinkler irrigation (right) treatments. Data are shown as an average number of pollen tubes reaching the base of the style over the three days of bloom. Time is shown at five increments and represents the number of days after anthesis.

For the ambient and microsprinkler irrigation treatments, growth begins immediately into the top of the style (Figures 9-12) and transverses nearly 50% of the style (Figures 15 and 16). With time and successful pollination, the percentage of flowers with tubes at the base increases (Figures 9-12) as does the average length traveled by the longest tube

(Figures 15 and 16). While the average number of pollen tubes reaching the base of the style at ambient and microsprinkler irrigation temperatures is variable over the 10 day period following bloom, there is also a general increase in number with time after pollination (Figures 18 and 19). This response is typical of viable pollen tubes which show visible aniline-blue fluorescence as they grow through the style.

For the heated temperature treatment, tube growth began in a seemingly normal manner. The first day of bloom showed tubes growing into the top of the style but the following time increments after bloom showed no pollen tubes present at the base of the style (Figures 13 and 14). The kinetics study indicates on average no more than 10% growth (Figure 17) through the style at any time following bloom. Unlike the ambient and microsprinkler irrigation treatments, a dynamics study could not be performed on those flowers exposed to heated temperatures because we did not encounter aniline-blue fluorescence at the style base of any flower. Under these circumstances, the lack of aniline-blue fluorescence is indicative of dying pollen tubes.

Tentative Conclusions

'French Improved' germination and pollen tube growth are negatively affected by high and extremely high temperatures after bloom. Adding a second year of data, the temperature optima for pollen growth are approximately 72-76ºF as inferred from the regression equations for the *in vitro* germination (Figure 1) and growth (Figure 2) experiments (Table 1). These optima are approximately 3-5ºF lower than stated last year due to the added data dictating a better fit to a second order regression curve.

Germination and tube growth rapidly fall off above the optima. Mean daily temperatures for the heated temperature treatment were near the optimal range of 72-76ºF (Figure 6) for the first couple of days of bloom before falling below these temperatures. Nighttime temperatures lowered this daily mean temperature but daily highs reached or exceeded these temperatures every day in the heated treatment and for 10 and 11 days of the 13 day period of the experiment in the microsprinkler irrigation and ambient treatments, respectively.

Our results suggest that daily maximum temperatures at or above the optimum for pollen tube growth correlate with reduced *in vivo* pollen tube growth. It appears that supraoptimal temperatures not only slow pollen germination and tube growth at high temperatures but prolonged extreme temperatures may irrevocably inhibit these processes. Our results suggest this growth inhibition may occur in conjunction with approximately 6 hours per day of supraoptimal temperatures (Figure 8).

Preliminary results also suggest that high temperatures can be moderated by afternoon microsprinkler irrigation during the bloom period. Flowers exposed microsprinkler irrigation during the bloom period accumulated additional growing degree hours (Figure 7) in addition to fewer hours per day of supraoptimal temperatures (Figure 8). Additional research is needed to better determine if microsprinkler irrigation affects temperature in

the canopy in a variety of orchard environments and if so, do irrigated flowers show significantly higher rates of pollen tube germination and growth.