PRUNE POLLINATION AND FRUIT SET

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PROBLEM AND ITS SIGNIFICANCE

Prune fruit set was generally poor in 2004 and 2005. Problems with fruit set were correlated with high temperatures during the bloom period. There is little data in the literature on temperature parameters affecting prune pollination and fruit set. What data exist suggest that high temperatures can lead to markedly reduced fruit set by adversely affecting pollen tube growth and ovule longevity. It is clear from the literature that there are great differences among cultivars in responses to temperatures. Unfortunately, the scant literature on the subject is based on cultivars not grown in the California prune industry.

OBJECTIVES

Our objective in this project is to determine temperature parameters that affect pollen germination, pollen tube growth and ovule longevity in prune flowers. This is proposed as a two-year project. The first year's objectives focused on generating temperature data for pollen germination and pollen tube growth for 'French' and 'Muir Beauty'. We also began developing methodologies for studying ovule senescence for 'French' prune. For the second year, we intend to repeat the pollen experiments, adding other cultivars and selections as appropriate. We also plan to conduct experiments designed to elucidate temperature responses for ovule viability. The resulting data should be useful for creating a model for temperature effects on pollination and fruit set in prune.

METHODS

We developed an *in vitro* system for determining pollen germination and pollen tube growth responses to temperature. Pollen was incubated on an agar-solidified medium 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 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 resulting data were fitted to second order polynomial regressions.

In vivo experiments were conducted by enclosing a single 'French Improved' tree in an enclosure covered with polyethylene sheeting. A 1400W greenhouse heater was used to raise the temperature in the enclosure above ambient. Two temperature regimes were used based on relative proximity to the heater. Temperature and relative humidity were monitored with data loggers place at appropriate locations in the enclosed space and in 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 the top and basal portions of the style was determined. Ovary portions were dissected to remove the ovules. We were not successful in staining ovules to determine viability and intend to continue on this work over the coming months.

RESULTS

In Vitro Pollen Germination and Tube Growth in Response to Temperature.

Pollen germination regressions for 'French Improved' and 'Muir Beauty' pollen are shown in Figures 1 and 2.

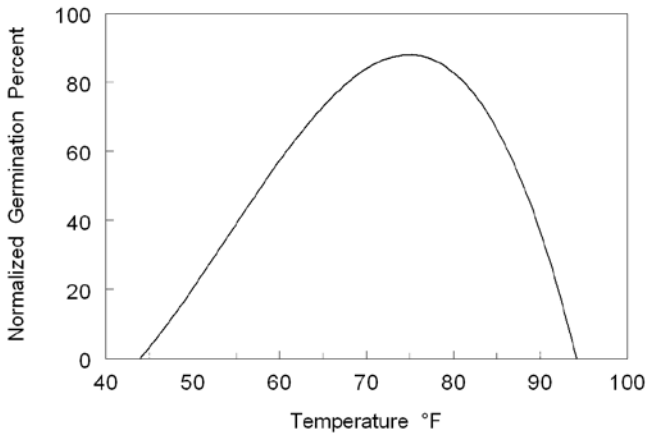

Figure 1. Regression curve for 'Improved French' prune pollen germination vs. temperature. R=0.86.

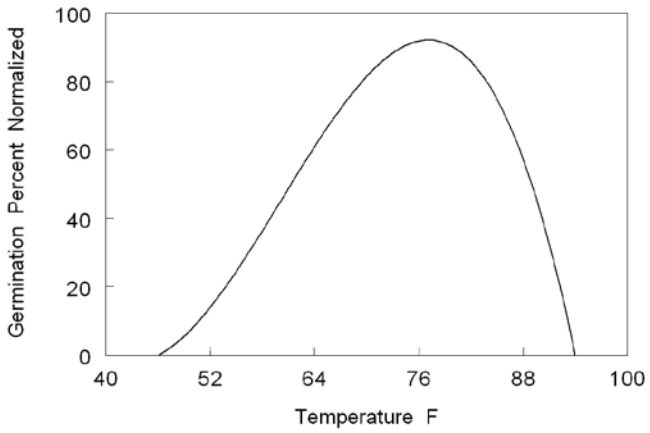

Figure 2. Regression curve for 'Muir Beauty' prune pollen germination vs. temperature. R=0.91**.**

Pollen tube growth responses to temperature are shown in Figures 3 and 4.

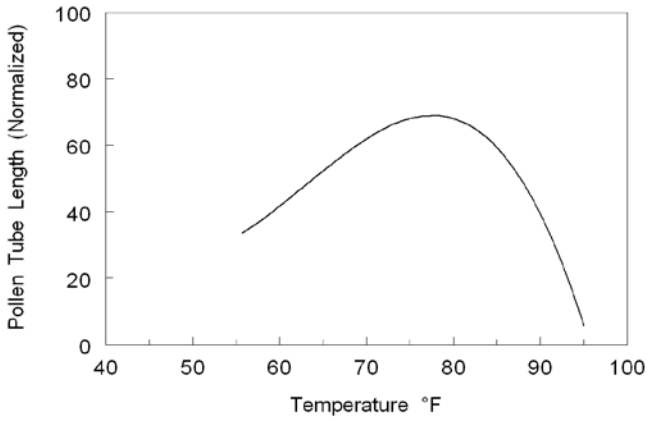

Figure 3. Regression curve for pollen tube growth for 'French Improved' prune pollen vs. temperature. R=0.40**.**

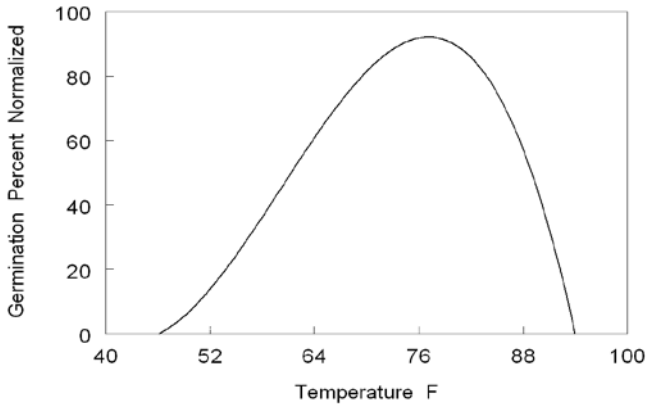

Figure 4. Regression curve for pollen tube growth for 'Muir Beauty' pollen vs. temperature. R=0.25.

Temperature optima for germination and tube growth range from 75 to 78ºF (Table 1) and both fall off sharply at temperatures above these optima.

Table 1. Optimum temperatures for pollen germination and pollen tube growth determined from the regression curves shown in Figs 1-4.

	Cultivar	Optimum
Pollen Germination	'French Improved'	75 ºF
Pollen Germination	'Muir Beauty'	77 ºF
Pollen Tube Growth	'French Improved'	77 ºF
Pollen Tube Growth	'Muir Beauty'	78 ºF

In Vivo Pollen Tube Growth.

The tree enclosed in polyethylene sheeting is shown in Figure 5 below.

Figure 5. Experimental 'French Improved' prune tree at Wolfskill Experimental Orchard in Winters, CA.

Figures 6-8 show temperature conditions for the ambient tree and the two temperature regimes (high and medium-high) in the enclosure.

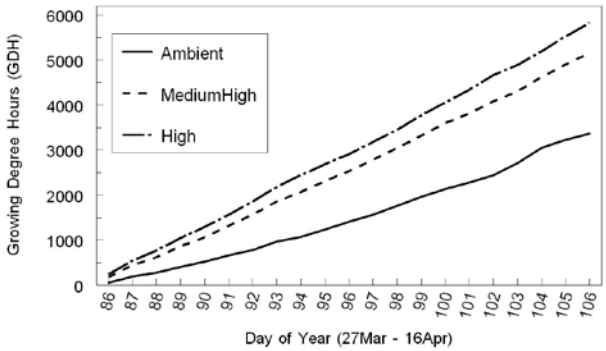

Figure 6. Accumulated heat units over the course of the experiments for ambient and the two temperature treatments.

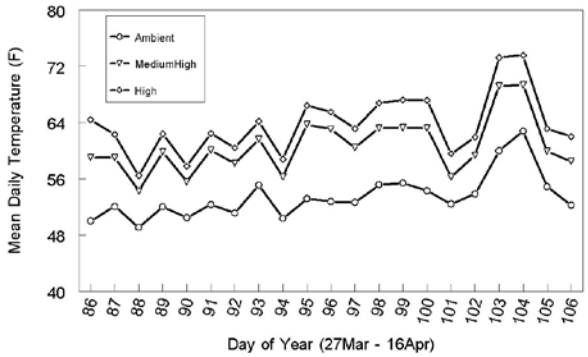

Figure 7. Mean daily temperatures over the course of the experiments for ambient and the two temperature treatments.

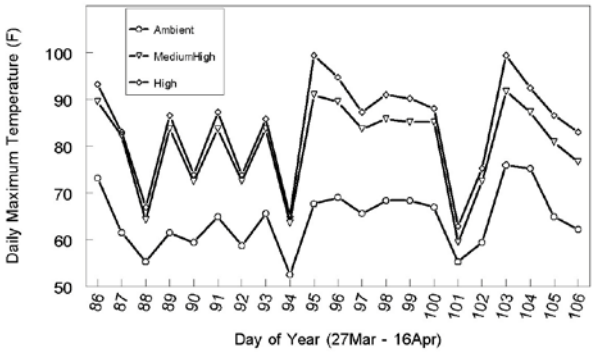

Figure 8. Maximum daily temperatures over the course of the experiments for ambient and the two temperature treatments.

Ambient conditions were more closely normal for the season compared to the two previous years. Pollen tube growth under ambient temperature conditions was as expected: Tube growth began in the upper portions of the style and progressed through the style to the top of the ovary in approximately 6 to 8 days (Figure 9).

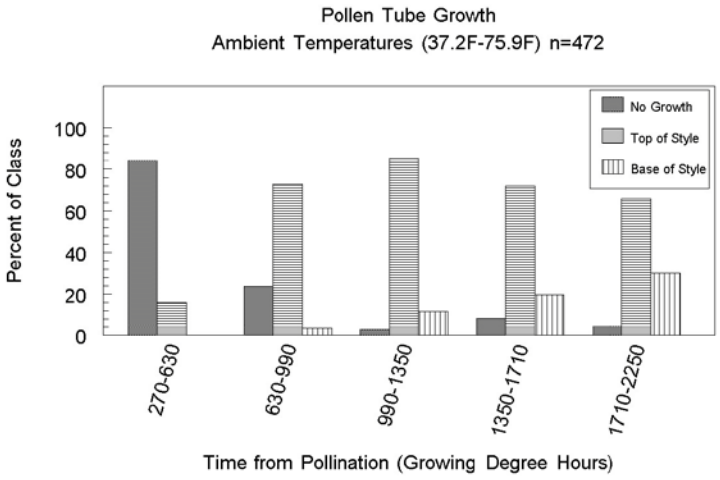

Figure 9. Pollen tube growth *in vivo* under ambient temperature conditions. Data are shown as percent of styles showing no growth, growth into the top of the style and growth to the base of the style for five time increments following pollination. Time is shown as

growing degree hours and data are expressed as the percent of styles in each time increment.

By contrast, flowers from the trees receiving the medium-high and high temperature treatments did not support normal pollen tube growth (Figures 10, 11).

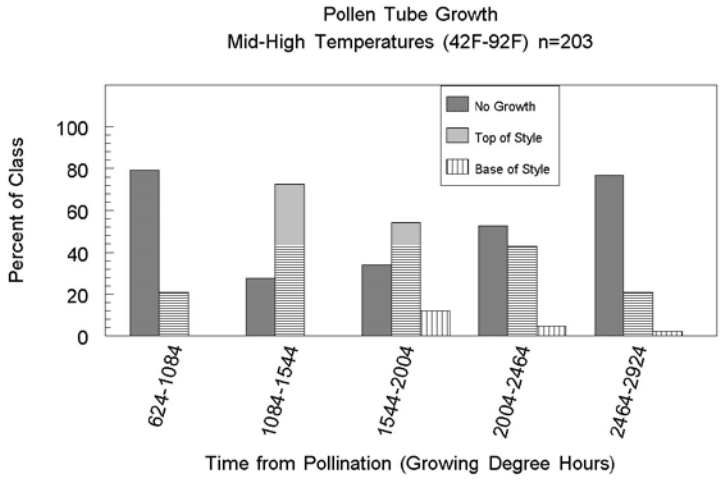

Figure 10. Pollen tube growth *in vivo* for the medium-high temperature treatment. Data are shown as percent of styles showing no growth, growth into the top of the style and growth to the base of the style for five time increments following pollination.

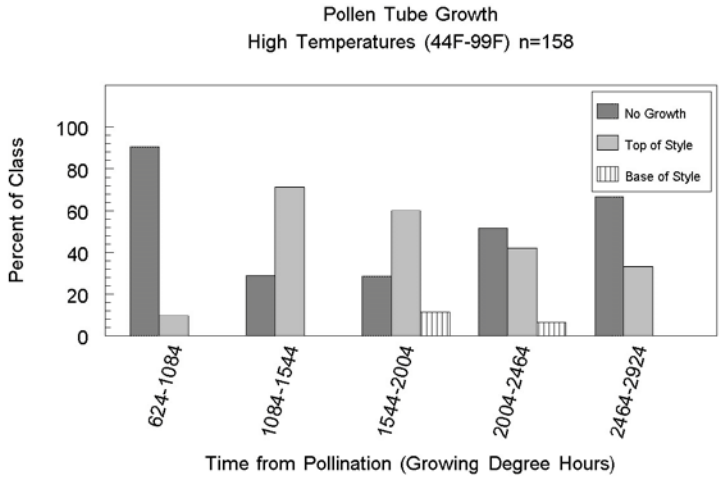

Figure 11. Pollen tube growth *in vivo* for the high temperature treatment. Data are shown as percent of styles showing no growth, growth into the top of the style and growth to the base of the style for five time increments following pollination.

For the medium-high treatment, tube growth began in a generally normal manner into the top of the style but few pollen tubes were present at the base of the style and frequency of pollen tubes at both locations declined with increasing time after pollination (Figure 10). The same response was seen in the high temperature treatment, where fewer tubes were present at the style base (Figure 11). Note that for the ambient temperature treatment the number of tubes at the top of the style is essentially constant once tube growth has begun,

and the number at the base increases with time after pollination. This is the expected response as the living pollen tubes which show visible analine-blue fluorescence remain in the style. In the elevated temperature treatments, the number of pollen tubes showing positive staining decreased with time both at the top and the base of the style. This indicates to us that pollen tubes were dying under these conditions.

SUMMARY AND TENTATIVE CONCLUSIONS

These results indicate that pollen tube growth and pollen germination are adversely affected by high temperatures. Temperature optima for pollen growth are approximately 75-78ºF as inferred from the regression equations for the *in vitro* germination (Figures 1, 2) and growth (Figures 3, 4) experiments (Table 1). Germination and growth fall off sharply at temperatures above the optima. Mean daily temperatures for the two elevated temperature treatments were below the optimal range of 75-78ºF (Figure 7) but daily highs exceeded that level for 15 days of the 20 day period of the experiment in both of the elevated temperature treatments. Results suggest that at supraoptimal temperatures, pollen tube growth does not merely slow, as it does at suboptimal temperature. It appears that growth may be irrevocably inhibited at high temperatures. Further research is needed to determine if the irrevocable growth inhibition requires sustatined exposure to supraoptimal temperatures and if so for how long.