Effects of Temperature and the Combination of Liquid Lime Sulfur and Fish Oil on Pollen Germination, Pollen Tube Growth, and Fruit Set in Apples

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Abstract. Effects of temperature and the combination of liquid lime sulfur (LLS) and fish oil (FO) applied during bloom on pollen germination and pollen tube growth in flowers and fruit set were examined in apples (*Malus × domestica* Borkh.). Percent germination of pollen of ‘Manchurian’ crabapples and ‘Golden Delicious’ apple flowers on the stigmatic surface of ‘Golden Delicious’ pistils increased with increasing temperature from 13 to 29 °C in the first 24 and 48 h after pollination, respectively, but not thereafter. Pollen tube growth rate in the style increased quadratically with increasing temperature from 13 to 29 °C. ‘Manchurian’ was a more effective pollenizer of ‘Golden Delicious’ than was ‘Golden Delicious’ pollen. For example, at 24 or 29 °C, some ‘Manchurian’ pollen tubes grew to the base of ‘Golden Delicious’ styles by 24 h after pollination. On the other hand, no ‘Golden Delicious’ pollen tube grew to the base of a ‘Golden Delicious’ style regardless of temperature and time. Pollen tube growth rate in the style increased with increasing day/night temperature from 7/0 to 24/7 °C. The time required for pollen tubes to grow to the base of styles decreased with increasing day/night temperature from 13/2 to 24/7 °C. Only ≈36 h was required for pollen tubes to grow to the base of style at 24/7 °C, whereas pollen tubes grew very slowly and no pollen tubes grew to the base of style at 7/0 °C regardless of pollen source. LLS + FO, applied 4 or 24 h after pollination, inhibited pollen germination, pollen tube growth in the style, fertilization, and fruit set, but it had no effect when applied 48 h after pollination. These results suggest that LLS + FO applied at this bloom stage causes flower or fruit abscission most likely by inhibiting pollen germination, pollen tube growth in the style, and fertilization.

Chemical thinning is one of the most effective measures to improve apple (*Malus domestica* Borkh.) fruit size, color and quality at harvest, increase return bloom the next year, reduce biennial bearing, and increase the profitability of commercial apple orchards. Blossom thinning is superior to post-bloom thinning because it can be done earlier, thus having a greater effect on fruit size and return flowering (Greene, 2002). Blossom thinners usually reduce fruit set by preventing pollination and fertilization through damaging the anthers, stigma, and style of flowers or inhibiting pollen tube growth in the style of flowers and are applied when some, but not all, flowers are fertilized (Fallahi and Willemsen, 2002; Greene, 2002;

McArtney et al., 2006; Williams and Edgerton, 1981). However, blossom thinning results are extremely variable and it is difficult to predict final fruit set because we do not know how environmental factors, especially temperature, affect pollen germination, pollen tube growth, fertilization and fruit set, and optimum timing for application (Fallahi and Willemsen, 2002; Greene, 2002).

Several reports have documented that temperature is the most important factor affecting pollen germination and pollen tube growth rate in pear (Mellenthin et al., 1972; Vasilakakis and Porlingis, 1985), sour cherry (Cerovic and Ruzic, 1992), avocado (Sedgley and Annells, 1981), and almond (Socias i Company et al., 1976). In apple, Williams (1970) also studied the influence of temperature on pollen tube growth and ovule longevity, but used only detached flowers of cultivars that are no longer of economic importance to the U.S. apple industry. It has been found that pollen tubes grew more

rapidly to the base of styles in “attached” flowers than flowers that were detached and placed on artificial media (Yoder and Combs, unpublished data). Furthermore, temperatures studied by Williams (1970) were lower than typically experienced during the bloom period (7 to 15 °C). Better understanding of pollen tube growth after pollination under a wide range of temperatures will facilitate better-informed decisions relative to apple crop load management. The purposes of this investigation were to 1) study the effect of temperature on apple pollen germination and pollen tube growth in styles; and 2) evaluate effects of liquid lime sulfur (LLS) + fish oil (FO) at different times after pollination on apple pollen germination, pollen tube growth, and fruit set in the field.

Materials and Methods

General. All pollination studies were conducted on intact flowers on trees. ‘Gala’/M.9, and ‘Golden Delicious’/M.27 trees grown in 19-L root bags (Lacebark Inc., Stillwater, OK) at Alson H. Smith, Jr. Agricultural Research and Extension Center, Winchester, VA. Trees were removed from the orchard early in March and held in a cold room to delay onset of bloom and then forced to bloom in a greenhouse. At late pink stage (just before flowers opened), flowers were selected for the pollination experiment and, 1 d before hand-pollination, all anthers were removed from test flowers to prevent self-pollination. All other flowers on test trees were removed to prevent crosspollination and balance flower distribution on the trees. Selected flowers were tagged for hand-pollination. Pollen used for experiments was either fresh pollen harvested the year of the experiment or the previous year’s pollen stored in a freezer at –12 °C. Before use, all pollen was tested for percent germination after 2 h at 21 °C on a medium containing agarose (10 g/L), sucrose (100 g/L), and boric acid (10 mg/L) using the methods of Williams and Maier (1977). Pollen was applied to stigmas with a No. 2 brush. Trees were then placed in temperature-controlled rooms under a high-pressure sodium (HPS) 1000-W lamp (Sunlight Supply, Inc., Vancouver, WA) with irradiance ≈600 μmol·m⁻²·s⁻¹ at the tree upper canopy for indicated photoperiod, temperature, and lighting conditions. All photosynthetic photon flux measurements were made at the top of the trees with a quantum sensor (LI-190SB; LI-COR Biosciences, Lincoln, NE).

Pollen tube examination. The method of Embree and Foster (1999) was modified to determine pollen germination on the stigmatic surface, number of pollen tubes penetrating through the stigma, and number of pollen tubes that grew to the base of the style. Briefly, flowers were removed from trees at indicated times, placed in a solution of 5% sodium sulfite in labeled glass containers, boiled for 15 min, and then refrigerated until microscopic examination. Fifteen styles from three flowers were detached from the ovary,

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Table 1. Effects of temperature on tube growth of 'Manchurian' crabapple pollen and 'Golden Delicious' apple pollen in 'Golden Delicious' pistils on tree (2004).^z

Temperature (°C)	Rating of pollen tube growth on stigma surface (0–10) ^y			Number of pollen tubes penetrating through the stigma			Number of pollen tubes that grew to the base of the style			Fruit set (%) ^x
	24 h	48 h	96 h	24 h	48 h	96 h	24 h	48 h	96 h	
Manchurian crabapple pollen										
13	0.9	2.7	5.2	2.9	18.1	30.4	0	0.3	3.1	75
18	0.5	4.6	5.5	1.6	32.9	41.2	0	4.3	10.0	100
24	1.9	3.7	3.3	9.2	29.1	22.2	1.8	7.7	7.6	75
29	3.7	4.0	4.7	26.5	25.1	35.6	0.3	5.3	5.5	75
Significance										
Linear	***	NS	NS	***	NS	NS	NS	***	NS	NS
Quadratic	NS	NS	NS	NS	*	NS	NS	***	***	NS
Golden Delicious apple pollen										
13	1.0	1.9	5.2	0.4	3.0	11.0	0	0	0	0
18	2.6	5.4	7.0	4.8	10.5	22.9	0	0	0	0
24	4.1	6.0	5.3	15.9	22.1	32.0	0	0	0	0
29	5.0	6.8	5.9	16.1	22.7	25.9	0	0	0	0
Significance										
Linear	***	***	NS	***	***	**	NS	NS	NS	NS
Quadratic	NS	*	NS	NS	*	*	NS	NS	NS	NS

^zMean of 15 observations.

^yRating of visible stigma surface area covered by pollen tube growth. Scale 0 to 10: 0 = no pollen tubes visible on the surface; 1 = 1% to 10% area covered; 2 = 11% to 20%; 3 = 21% to 30%; 4 = 31% to 40%; 5 = 41% to 50%; 6 = 51% to 60%; 7 = 61% to 70%; 8 = 71% to 80%; 9 = 81% to 90%; and 10 = 91% to 100% of surface area covered by pollen tubes.

^xFruit set was determined 96 h in the growth rooms and 24 d in the field after pollination.

NS, *, **, ***Nonsignificant or significant at $P = 0.05$, 0.01, or 0.001, respectively.

Fig. 1. Effects of temperature on pollen tube growth of (A) 'Manchurian' crabapple and (B) 'Golden Delicious' apple in 'Golden Delicious' pistils on a tree in light in 2004. 'Manchurian' pollen tube growth: at 13 °C, $Y = 0.2780 + 0.0746X$, $R^2 = 0.92^{**}$; at 18 °C, $Y = 9.0597/[1 + e^{-(X-35.2889)/6.2184}]$, $R^2 = 0.99^{**}$; at 24 °C, $Y = -0.0824 + 0.2517X - 0.0016X^2$, $R^2 = 0.99^{**}$; at 29 °C, $Y = 9.000/[1 + e^{-(X-24.7618)/0.9520}]$, $R^2 = 0.98^{**}$. Legend in B applies to A. Data are means $\pm$ SE ($n = 15$).

rinsed with distilled water, and placed in a water-soluble fluorescence solution of 0.01% Aniline Blue stain in 0.067 M K_2HPO_4 on microscope slides. Styles were then squashed between two microscope slides and allowed to incubate 24 h in the dark at room temperature before examination with epi-ultraviolet light using a Zeiss HBO-50 high-pressure mercury vapor light source (OSRAM GmbH, Aushurg, Germany) at 100 $\times$. Collected data included rating of pollen germination/tube growth on the stigmatic surface (0% to 100% of visible stigmatic surface covered), number

of visible tubes penetrating the base of the stigma, mean length of the longest pollen tube, mean style length, and number of visible pollen tubes reaching the base of the style.

Expt. 1: Effects of temperature on 'Manchurian' crabapple and 'Golden Delicious' apple pollen tube growth in 'Golden Delicious' pistils. Dormant 8-year-old 'Golden Delicious'/'M.27 trees were removed from the orchard in early Mar. 2004, held in a cold room, and then placed in a greenhouse to stimulate growth and blooming. Sixty king flowers at the late pink stage were selected on

each tree, tagged, and emasculated. Thirty of the emasculated flowers were pollinated using a brush with 'Golden Delicious' pollen harvested in 2004, and the other 30 flowers were pollinated with 'Manchurian' crabapple pollen harvested in 2003. Remaining flowers on test trees were removed 1 d before hand-pollination to prevent crosspollination. Trees were randomly assigned to temperature-controlled rooms set at constant air temperatures of 13, 18, 24, or 29 °C. The light source in the chambers was HPS lamps, as indicated previously, with a 24-h day. Three flowers per single tree per test temperature were collected for each temperature and each pollen source 24, 48, and 96 h after pollination. To validate the technique for fertilization of hand-pollinated flowers, fruit set of 16 pollinated flowers was recorded 24 d after pollination. The styles were processed and stained as indicated previously. The stigmatic surface area covered by pollen tube growth was visually rated on a scale of 0 to 10 in which 0 = no pollen tubes visible on the surface; 1 = 1% to 10% area covered; 2 = 11% to 20%; 3 = 21% to 30%; 4 = 31% to 40%; 5 = 41% to 50%; 6 = 51% to 60%; 7 = 61% to 70%; 8 = 71% to 80%; 9 = 81% to 90%; and 10 = 91% to 100% of surface area covered by pollen tubes. The number of pollen tubes penetrating the stigma, average length of longest pollen tubes in the styles, mean style length, and number of pollen tubes growing to the base of the styles were recorded.

Expt. 2: Effects of selected day/night temperatures on 'Golden Delicious' apple and 'Manchurian' and 'Snowdrift' crabapple pollen tube growth in 'Gala' pistils. Dormant 4-year-old 'Gala'/'M.9 trees growing in root bags were removed from the orchard in early Mar. 2005, held in a cold room, and later moved to a greenhouse to force bloom. Forty-five flowers at the late pink stage were selected on each tree, tagged, and emasculated. The

emasculated flowers were pollinated using a brush with pollen of either 'Golden Delicious' apple or 'Manchurian' or 'Snowdrift' crabapple harvested in 2005. Trees were randomly assigned to day/night temperatures of 7/0, 13/2, 18/4, or 24/7 °C. The light source in the chambers was HPS lamps from 0600 to 1800 HR and completely dark from 1800 to 0600 HR. Three flowers were collected from a single test tree for each temperature and pollen source 12, 24, 48, and 96 h after pollination. They were processed and evaluated as indicated previously.

Expt. 3: Effects of selected day/night temperatures on 'Golden Delicious' apple and 'Manchurian' and 'Snowdrift' crabapple pollen tube growth in 'Golden Delicious' pistils. Dormant 9-year-old 'Golden Delicious'/M.27 trees growing in root bags were removed from the orchard in early Mar. 2005, held in a cold room, and later placed in a greenhouse. Forty-five flowers at the late pink stage were selected on each tree, tagged, and emasculated. The emasculated flowers were pollinated using a brush with pollen of 'Golden Delicious' apple and 'Manchurian' and 'Snowdrift' crabapples harvested in 2005. Trees were randomly assigned to day/night temperatures of 7/0, 13/2, 18/4, or 24/7 °C. The light source in the chambers was HPS lamps from 0600 to 1800 HR and completely dark from 1800 to 0600 HR. Three flowers were collected from a single test tree for each temperature and pollen source 12, 24, 48, and 96 h after pollination and processed and evaluated as indicated previously.

Expt. 4: Effects of selected day/night temperatures on 'Manchurian' crabapple pollen tube growth in 'Golden Delicious' pistils. Dormant 10-year-old 'Golden Delicious'/M.27 trees growing in root bags were removed from the orchard in early Mar. 2006, held in a cold room, and then placed in a greenhouse. Twenty-one flowers at the late pink stage were selected on each tree, tagged, and emasculated. The emasculated flowers were pollinated using a brush with pollen of 'Manchurian' crabapples harvested in 2005. Trees were randomly assigned to day/night air temperatures of 13/2, 18/4, or 24/7 °C. The light source in the chambers was HPS lamps from 0600 to 1800 HR and completely dark from 1800 to 0600 HR. Three flowers were collected from a single test tree for each temperature and each pollen source 12, 24, 36, 48, 72, and 96 h after pollination, placed in a 5% sodium sulfite solution, and stored in a refrigerator until processing and assessment as described previously.

Expt. 5: Effects of timing of application of liquid lime sulfur and fish oil on pollen tube growth and fruit set in 'Golden Delicious' apple in the field in 2006. Twelve 10-year-old 'Golden Delicious'/M.27 trees in an orchard row were grouped into four blocks of three trees in a randomized complete block design on 23 Apr. 2006. Thirty flowers at full bloom stage were tagged on each tree and pollinated with 'Snowdrift' crabapple pollen using a brush; remaining flowers were removed. To prevent additional pollination from natural pollen sources in the field, trees were covered

with white insect netting until application of LLS and FO (Crocker's Fish Oil, Inc., Quincy, WA) with a low-pressure hand wand sprayer. One tree in each block was sprayed with the combination of LLS (2%) and FO (2%) 4, 24, or 48 h after pollination. Flowers were sampled 48 h after application of LLS and FO. Pollen germination and tube growth on the stigmatic surface, number of pollen tubes penetrating through the stigma, average length of longest pollen tubes in the styles, mean style length, and number of pollen tubes that grew to the base of the style were determined as described previously. Fruit set of 15 flowers per tree was recorded 35 d after pollination.

Statistical analyses. Growth room Expts. 1 through 4 were conducted and analyzed as completely randomized designs with data from 15 replicate styles per treatment/collection interval. The in-field bloom thinning experiment was conducted as a randomized complete block design with four blocks. Statistical analyses included analysis of variance, Duncan's multiple range test, and regression analysis using Statistical Analysis Systems Software for PC (SAS Inst., Cary, NC).

Results

Expt. 1: Effects of temperature on 'Manchurian' crabapple and 'Golden Delicious' apple pollen tube growth in 'Golden Delicious' pistils, 2004. The rating of pollen tube growth by 'Manchurian' crabapple and 'Golden Delicious' apple pollen on the stigmatic surface of 'Golden Delicious' pistils

Table 2. Effects of various day/night temperature on tube growth of 'Golden Delicious' apple pollen and 'Manchurian' and 'Snowdrift' crabapple pollen in 'Gala' pistils on tree (2005).²

Day/night temp (°C)	Rating of pollen tube growth on stigma surface (0–10) ³				Number of pollen tubes penetrating through stigma				Number of pollen tubes growing to the base of style			
	12 h	24 h	48 h	96 h	12 h	24 h	48 h	96 h	12 h	24 h	48 h	96 h
Manchurian crabapple pollen												
7/0	3.3	3.1	3.8	5.1	23.3	2.1	0.0	39.1	0.0	0.0	0.0	0.0
13/2	3.5	4.2	4.5	4.6	12.3	21.4	36.3	48.8	0.0	0.0	0.0	1.9
18/4	3.5	5.2	6.7	6.1	31.9	51.7	76.9	76.2	0.0	0.0	0.2	8.2
24/7	3.3	4.0	5.4	4.1	27.8	42.7	59.1	45.7	0.0	0.0	3.7	12.1
Significance												
Linear	NS	NS	***	NS	NS	***	***	NS	NS	NS	***	***
Quadratic	NS	*	*	NS	NS	**	***	***	NS	NS	*	NS
Snowdrift crabapple pollen												
7/0	3.3	2.5	5.0	5.3	0.4	10.9	19.9	10.5	0.0	0.0	0.0	0.0
13/2	5.5	4.9	6.2	5.9	19.4	9.1	51.2	49.5	0.0	0.0	0.0	1.2
18/4	6.1	6.3	7.8	8.2	51.0	58.9	93.5	103.3	0.0	0.0	0.7	11.5
24/7	3.9	4.9	5.1	5.3	28.9	52.0	54.1	53.8	0.0	0.0	3.5	15.2
Significance												
Linear	NS	***	NS	NS	***	***	***	***	NS	NS	***	***
Quadratic	***	***	***	***	***	NS	***	***	NS	NS	*	NS
Golden Delicious apple pollen												
7/0	3.6	3.1	4.9	6.5	16.8	9.1	0.3	23.2	0.0	0.0	0.0	0.0
13/2	3.4	3.9	6.3	6.8	8.3	9.6	51.0	78.3	0.0	0.0	0.0	1.4
18/4	4.1	4.4	6.4	6.0	22.9	26.6	67.9	73.5	0.0	0.0	0.1	14.6
24/7	4.3	5.2	5.9	5.5	39.0	47.2	70.6	55.4	0.0	0.0	8.9	17.1
Significance												
Linear	NS	***	NS	**	***	***	***	***	NS	NS	***	***
Quadratic	NS	NS	*	NS	**	NS	***	***	NS	NS	***	NS

²Mean of 15 observations.

³Rating of visible stigma surface area covered by pollen tube growth. Scale 0 to 10: 0 = no pollen tubes visible on the surface; 1 = 1% to 10% area covered; 2 = 11% to 20%; 3 = 21% to 30%; 4 = 31% to 40%; 5 = 41% to 50%; 6 = 51% to 60%; 7 = 61% to 70%; 8 = 71% to 80%; 9 = 81% to 90%; and 10 = 91% to 100% of surface area covered by pollen tubes.

NS, *, **, ***Nonsignificant or significant at $P = 0.05$, 0.01 , or 0.001 , respectively.

increased with increasing temperature from 13 to 29 °C in the first 48 h after pollination, and rating of pollen tube growth by 'Manchurian' crabapple increased in the first 24 h, but neither was affected by temperature thereafter (Table 1). There was either a linear, quadratic, or no relationship between the number of 'Manchurian' pollen tubes penetrating through the stigma of 'Golden Delicious' pistils and temperature 24, 48, or 96 h after pollination, respectively. However, the number of 'Golden Delicious' pollen tubes penetrating the stigma of 'Golden Delicious' pistils increased with increasing temperature from 13 to 29 °C 24, 48, and 96 h after pollination. Pollen tube growth rate in the style also increased with increasing temperature from 13 to 24 °C, but decreased slightly when temperature increased from 24 to 29 °C regardless of pollen source (Fig. 1A–B). Some 'Manchurian' pollen tubes had grown to the base of 'Golden Delicious' styles 24 h after pollination at 24 or 29 °C but not at 13 or 18 °C. The number of 'Manchurian' pollen tubes growing to the base of the 'Golden Delicious' styles increased quadratically with increasing temperature from 13 to 29 °C 48 and 96 h after pollination. On the other hand, no 'Golden Delicious' pollen tube grew to the base of a 'Golden Delicious' style regardless of temperature. Fruit set for 'Golden Delicious' flowers pollinated with 'Manchurian' crabapple pollen was 100% at 18 °C and was 75% at the other temperatures. With 'Manchurian' crabapple pollen, fruit set at 13 °C was 75%, although it took more than 100 h for the pollen tubes to reach the base of the style at this temperature (Fig. 1A). None of the 'Golden Delicious' flowers pollinated with 'Golden Delicious' pollen set fruit.

Expt. 2: Effects of selected day/night temperatures on 'Golden Delicious' apple and 'Manchurian' and 'Snowdrift' crabapple pollen tube growth in 'Gala' pistils, 2005. Temperature did not affect the rating of 'Golden Delicious' pollen tube growth on the 'Gala' stigmatic surface 12 h after pollination (Table 2). The rating of 'Golden Delicious' pollen tubes on the stigmatic surface increased linearly and quadratically with increasing day/night temperature from 7/0 to 24/7 °C 24 and 48 h after pollination, respectively, but it decreased linearly with increasing temperature 96 h after pollination. The rating of 'Manchurian' pollen tube growth on the 'Gala' stigmatic surface increased quadratically with increasing day/night temperature from 7/0 to 24/7 °C 24 and 48 h after pollination, but it was unaffected by temperature 12 or 96 h after pollination. The rating of 'Snowdrift' pollen tube growth on the stigmatic surface increased quadratically with increasing day/night temperature from 7/0 to 24/7 °C over the 96-h observation period.

The number of pollen tubes penetrating through 'Gala' stigmas increased with increasing day/night temperature from 7/0 to 24/7 °C regardless of pollen source, except for the number of 'Manchurian' pollen tubes 12 h after pollination. Pollen tube

growth rate in 'Gala' styles also increased with increasing day/night temperature from 7/0 to 24/7 °C regardless of pollen source (Fig. 2A–C). When day/night temperature was 18/4 or 24/7 °C, no pollen tube grew to the base of styles until 48 h after pollination. No pollen tube grew to the base of styles at a day/night temperature of 7/0 °C regardless of pollen source and time, whereas it took ≈ 96 h for pollen tubes to grow to the base of style at 13/2 °C. The number of pollen tubes growing to the base of styles increased with increasing day/night temperature from 7/0 to 24/7 °C 48 and 96 h after pollination.

Expt. 3: Effects of selected day/night temperatures on 'Golden Delicious' apple and 'Manchurian' and 'Snowdrift' crabapple pollen tube growth in 'Golden Delicious' pistils, 2005. The rating of 'Golden Delicious' pollen tube growth on the 'Golden Delicious' stigmatic surface increased linearly with increasing day/night temperature from 7/0 to 24/7 °C 12 and 24 h after pollination and increased quadratically with increasing temperature thereafter (Table 3). The rating of 'Manchurian' and 'Snowdrift' pollen tube growth on 'Golden Delicious' stigmatic surfaces increased quadratically

Fig. 2. Effect of day/night temperature on pollen tube growth of (A) 'Golden Delicious' apple, (B) 'Manchurian' crabapple, and (C) 'Snowdrift' crabapple in 'Gala' pistils in 2005. 'Golden Delicious' pollen tube growth: at 7/0 °C, $Y = 0.5825 + 0.0044X$, $R^2 = 0.06^{NS}$; at 13/2 °C, $Y = -0.4000 + 0.0617X$, $R^2 = 0.97^{**}$; at 18/4 °C, $Y = 0.1725 + 0.0869X$, $R^2 = 0.99^{***}$; at 24/7 °C, $Y = 0.0185 + 0.2176X - 0.0013X^2$, $R^2 = 0.99^*$. 'Manchurian' pollen tube growth: at 7/0 °C, $Y = 0.2675 + 0.0165X$, $R^2 = 0.36^{NS}$; at 13/2 °C, $Y = -0.0500 + 0.0575X$, $R^2 = 0.99^{***}$; at 18/4 °C, $Y = 0.7025 + 0.0794X$, $R^2 = 0.97^{**}$; at 24/7 °C, $Y = 0.2908 + 0.2197X - 0.0015X^2$, $R^2 = 0.97^*$. 'Snowdrift' pollen tube growth: at 7/0 °C, $Y = 0.1782 + 0.0528X - 0.0004X^2$, $R^2 = 0.88^{NS}$; at 13/2 °C, $Y = -0.3025 + 0.0640X$, $R^2 = 0.98^{**}$; at 18/4 °C, $Y = 0.3225 + 0.0910X$, $R^2 = 0.97^{**}$; at 24/7 °C, $Y = 0.4385 + 0.2145X - 0.0014X^2$, $R^2 = 0.98^*$. Legend in C applies to all figures. Data are means $\pm$ SE ($n = 15$).

Table 3. Effects of temperature on tube growth of 'Golden Delicious' apple pollen and 'Manchurian' and 'Snowdrift' crabapple pollen in 'Golden Delicious' pistils on tree (2005).^z

Day/night temp (°C)	Rating of pollen tube growth on stigma surface (0–10) ^y				Number of pollen tubes penetrating through stigma				Number of pollen tubes growing to the base of style			
	12 h	24 h	48 h	96 h	12 h	24 h	48 h	96 h	12 h	24 h	48 h	96 h
Manchurian crabapple pollen												
7/0	0.1	0.0	1.0	0.8	0.0	0.0	1.9	1.3	0.0	0.0	0.0	0.0
13/2	2.2	2.1	3.2	3.5	4.7	6.0	11.7	20.8	0.0	0.0	0.0	0.5
18/4	0.8	1.9	1.4	4.2	0.6	7.8	7.7	36.7	0.0	0.0	0.3	4.0
24/7	0.9	1.3	1.7	3.4	2.5	6.1	14.9	31.9	0.0	0.0	0.8	10.5
Significance												
Linear	NS	**	NS	***	NS	*	**	***	NS	NS	**	***
Quadratic	**	***	*	***	NS	NS	NS	***	NS	NS	NS	*
Snowdrift crabapple pollen												
7/0	0.1	0.2	0.2	1.1	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
13/2	2.5	2.6	3.1	4.3	1.5	5.3	18.9	22.9	0.0	0.0	0.0	0.1
18/4	2.5	1.7	4.9	7.7	15.4	6.3	49.7	76.1	0.0	0.0	0.9	4.9
24/7	1.3	1.3	2.1	2.9	6.9	5.9	19.1	27.7	0.0	0.0	1.4	7.4
Significance												
Linear	NS	NS	**	**	*	**	**	***	NS	NS	***	***
Quadratic	***	***	***	***	NS	*	***	***	NS	NS	NS	NS
Golden Delicious apple pollen												
7/0	0.5	0.7	1.3	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13/2	1.6	2.3	1.5	4.9	1.0	0.5	0.1	14.7	0.0	0.0	0.0	0.0
18/4	1.0	1.2	3.5	5.9	0.6	0.2	8.0	13.3	0.0	0.0	0.0	0.0
24/7	1.3	2.7	2.5	6.5	3.5	3.8	5.1	26.7	0.0	0.0	0.0	0.0
Significance												
Linear	*	**	**	***	***	***	***	***	NS	NS	NS	NS
Quadratic	NS	NS	*	***	*	*	NS	NS	NS	NS	NS	NS

^zMean of 15 observations.

^yRating of visible stigma surface area covered by pollen tube growth. Scale 0 to 10: 0 = no pollen tubes visible on the surface; 1 = 1% to 10% area covered; 2 = 11% to 20%; 3 = 21% to 30%; 4 = 31% to 40%; 5 = 41% to 50%; 6 = 51% to 60%; 7 = 61% to 70%; 8 = 71% to 80%; 9 = 81% to 90%; and 10 = 91% to 100% of surface area covered by pollen tubes.

NS, *, **, ***Nonsignificant or significant at $P = 0.05$, 0.01, or 0.001, respectively.

with increasing day/night temperature from 7/0 to 24/7 °C over the 96-h observation period. Very few pollen tubes penetrated the stigma of 'Golden Delicious' pistils when day/night temperature was at 7/0 °C regardless of pollen source and time. The number of pollen tubes penetrating the stigma increased with increasing day/night temperature from 7/0 to 24/7 °C over the 96-h observation period except for the number of 'Manchurian' pollen tubes 12 h after pollination. Pollen tube growth rate in 'Golden Delicious' styles also increased with increasing day/night temperature from 7/0 to 24/7 °C regardless of pollen source (Fig. 3A–C). No 'Golden Delicious' pollen tubes grew to the base of a 'Golden Delicious' style regardless of temperature and time. No 'Manchurian' or 'Snowdrift' crabapple pollen tubes grew to the base of style until 48 h after pollination when day/night temperature was 18/4 or 24/7 °C. No 'Manchurian' or 'Snowdrift' pollen tubes grew to the base of style at a day/night temperature of 7/0 °C, whereas it took ≈96 h for the pollen tubes to grow to the base of a style at 13/2 °C. The number of 'Manchurian' or 'Snowdrift' pollen tubes growing to the base of styles increased with increasing day/night temperature from 7/0 to 24/7 °C 48 and 96 h after pollination.

Expt. 4: Effects of selected day/night temperatures on 'Manchurian' crabapple pollen tube growth in 'Golden Delicious' pistils, 2006. The rating of 'Manchurian' pollen tube growth on the 'Golden Delicious' stigmatic surface, number of pollen tubes penetrating through the stigma, and pollen

tube growth rate in styles increased with increasing day/night temperature from 13/2 to 24/7 °C over the 96-h observation period (Table 4). It took ≈36 h for 'Manchurian' pollen tubes to grow to the base of styles at a day/night temperature of 24/7 or 18/4 °C, whereas 72 h was required for the pollen tubes to grow to the base of styles at 13/2 °C. The number of pollen tubes growing to the base of styles increased with increasing day/night temperature from 13/2 to 24/7 °C 36, 48, 72, and 96 h after pollination.

Expt. 5: Effect of timing of application of liquid lime sulfur and fish oil on pollen tube growth and fruit set in 'Golden Delicious' apples in field in 2006. The rating of 'Snowdrift' pollen tube growth on the 'Golden Delicious' stigmatic surface and the number of pollen tubes penetrating the stigma were reduced by application of LLS + FO (Table 5). Pollen tube growth in styles was inhibited by LLS + FO applied 4 or 24 h after pollination, but was unaffected by LLS + FO when applied 48 h after pollination. Average style length ranged from 6.9 to 7.9 mm. No pollen tubes grew to the base of a style and no fruit set occurred in the 'Golden Delicious' apple trees treated with LLS + FO 4 h or 24 h after pollination. By contrast, there was no difference between the number of pollen tubes growing to the base of styles in the untreated control and LLS + FO applied 48 h after pollination, and 76% of 'Golden Delicious' apple flowers set fruit when pollinated with 'Snowdrift' pollen and treated with LLS + FO 48 h after pollination.

Discussion

Williams (1965, 1970) reported that it takes ≈5 to 7 d for apple pollen tubes to grow to the base of styles. However, our results showed that temperature affected pollen tube growth rate in the style, thereby influencing the time required for pollen tubes to grow to the base of styles, fertilization, and fruit set. For example, it took only 24 h for 'Manchurian' crabapple pollen tubes to grow to the base of styles at constant temperatures of 24 and 29 °C or 48 h at 13 and 18 °C. Under various day/night temperature conditions, the time required for pollen tubes to grow to the base also decreased with increasing temperature. Pollen tubes grew very slowly and no pollen tubes grew to the base of styles at 7/0 °C regardless of pollen source. Approximately 72 h was required for pollen tubes to grow to the base of styles at 13/2 °C, whereas only ≈36 or 48 h was enough for pollen tubes to grow to the base of styles at 18/4 or 24/7 °C. With a daily high and low temperature of ≈23/10 °C, application of LLS + FO 4 or 24 h after pollination under natural field conditions effectively inhibited pollen tube growth, reduced the number of pollen tubes growing to the base of styles, and eliminated fruit set, but the application was not effective when delayed until 48 h after pollination. These data provide further evidence to support the statement that less than 48 h is required for pollen tubes to grow to the base of the style at high day/night temperature. These results suggest that application timing is critical to the success of blossom

Fig. 3. Effect of day/night temperature on pollen tube growth of (A) 'Golden Delicious' apple, (B) 'Manchurian' crabapple, and (C) 'Snowdrift' crabapple in 'Golden Delicious' pistils in 2005. 'Manchurian' pollen tube growth: at 7/0 °C, $Y = -0.0500 + 0.0058X$, $R^2 = 0.93^{**}$; at 13/2 °C, $Y = -0.1525 + 0.0381X$, $R^2 = 0.96^{**}$; at 18/4 °C, $Y = -0.3400 + 0.0683X$, $R^2 = 0.96^{**}$; at 24/7 °C, $Y = 8.1473/(1 + e^{-(x-35.5372)/11.9979})$, $R^2 = 0.99^{**}$. 'Snowdrift' pollen tube growth: at 7/0 °C, $Y = -0.0525 + 0.0031X$, $R^2 = 0.78^{*}$; at 13/2 °C, $Y = 0.1800 + 0.0317X$, $R^2 = 0.82^{*}$; at 18/4 °C, $Y = 0.0725 + 0.0852X$, $R^2 = 0.96^{**}$; at 24/7 °C, $Y = -0.3877 + 0.1799X - 0.0010X^2$, $R^2 = 0.95^{*}$. Legend in A applies to all figures. Data are means $\pm$ SE (n = 15).

thinning because blossom thinners are applied after some, but not all, flowers have been fertilized (Greene, 2002; McArtney et al., 2006; Williams and Edgerton, 1981). Delaying application by 1 d can result in failure of the bloom thinners because they would have little or no effect on fertilization and fruit set of earlier pollinated flowers. Research with additional cultivars and other temperatures is needed to establish temperature-based pollen tube growth models to predict optimum timing for growers to spray blossom thinners.

The precise mode of action of LLS and FO as a blossom thinner is not clear (McArtney et al., 2006). LLS or sulfur not only affects pollen germination and increases the percentage of newly opened flowers having no

pollen tubes in the style (MacDaniels and Furr, 1930; McArtney et al., 2006), but also suppresses leaf photosynthesis in apples (Hyre, 1939; McArtney et al., 2006; Noordijk and Schupp, 2003). In our study, LLS + FO applied 4 or 24 h after pollination inhibited pollen germination and pollen tube growth in the style and resulted in no fertilization and no fruit set. These results suggest that LLS + FO applied over the bloom period causes flower or fruit abscission mainly through inhibiting pollen germination, pollen tube growth in the style, and fertilization. This suggestion is supported by the fact that LLS + FO applied 48 h after pollination had no influence on fruit set, although leaf photosynthesis might also be inhibited by LLS + FO (McArtney et al., 2006).

Our results demonstrate that pollen germination on the stigmatic surface and the number of pollen tubes penetrating the stigma comparably increased with increasing temperature after self- or crosspollination in apples. However, the pollen tube growth was arrested in the style and no pollen tube grew to the base of styles after self-pollination in 'Golden Delicious' apples regardless of temperature and time. Consistent failure of 'Golden Delicious' pollen tubes to reach the base of the 'Golden Delicious' styles under the range of conditions tested here raises doubts about the popular perception that 'Golden Delicious' is partially self-fertile. These results are consistent with a previous report that the pollen tube growth is inhibited within the style after self-pollination in apples (De Nettancourt, 1977). Self-incompatibility of apple is controlled by S-RNase gene encoding ribonucleases, which are located within the S-locus (De Nettancourt, 1977).

In conclusion, pollen germination on the stigmatic surface, the number of pollen tubes penetrating through the stigma, pollen tube growth rate in the style, and the number of pollen tubes growing to the base of styles increased with increasing temperature in the range tested. The time required for pollen tubes to grow to the base of styles decreased with increasing temperature. LLS + FO applied over the bloom period causes flower or fruit abscission primarily by inhibiting pollen tube growth in the style and fertilization. Better understanding of the effects of temperature on these processes and more attention to actual temperatures during the bloom period will improve the accuracy of postpollination application timing, thereby providing more reliable bloom-thinning results.

Literature Cited

- Cerovic, R. and D. Ruzic. 1992. Pollen tube growth in sour cherry (*Prunus cerasus* L.) at different temperatures. *J. Hort. Sci.* 67:333-340.
- De Nettancourt, D. 1977. Incompatibility in angiosperms, p. 28-57. In: Frankel, R., G.A.E. Gal, and H.F. Linskens (eds.). *Monographs on theoretical and applied genetics*. Springer, Heidelberg, Germany.
- Embree, C.G. and A. Foster, Jr. 1999. Effects of coatings and pollenicide on pollen tube growth through the stigma and style of 'McIntosh' apple blossoms. *J. Tree Fruit Prod.* 2:19-32.
- Fallahi, E. and M.W. Willemsen. 2002. Blossom thinning of pome and stone fruit. *HortScience* 37:474-477.
- Greene, D.W. 2002. Chemicals, timing, and environmental factors involved in thinner efficacy on apple. *HortScience* 37:477-481.
- Hyre, R.A. 1939. The effect of sulfur sprays on the photosynthesis and transpiration of apple leaves. *N.Y. Agr. Expt. Sta. Mem.* 222.
- MacDaniels, L.H. and J.R. Furr. 1930. The effect of dusting-sulfur upon the germination of pollen and the set of fruit of the apple. *Bul. Cornell Univ. Agr. Expt. Sta.* 499.
- McArtney, S., J. Palmer, S. Davies, and S. Seymour. 2006. Effects of lime sulfur and fish oil on pollen tube growth, leaf photosynthesis and fruit set in apple. *HortScience* 41:357-360.

Table 4. Effects of various day/night temperature of 'Manchurian' crabapple pollen tube growth in 'Golden Delicious' pistils on tree (2006).^z

Day/night temp (°C)	Hours after pollination					
	12 h	24 h	36 h	48 h	72 h	96 h
	Rating of pollen tube growth on stigma surface (0–10) ^y					
13/2	0.9	1.2	1.9	2.1	3.0 a ^x	2.7 b
18/4	2.8	1.9	4.6	3.1	3.8 a	4.0 a
24/7	4.7	4.6	5.0	6.7	— ^w	—
Significance						
Linear	***	***	***	***		
Quadratic	NS	*	**	**		
	Number of pollen tubes penetrating through stigma					
13/2	3.5	7.2	11.7	14.2	30.0 b	25.8 b
18/4	22.9	18.9	48.1	41.3	48.5 a	56.2 a
24/7	48.3	54.0	56.0	81.0	—	—
Significance						
Linear	***	***	***	***		
Quadratic	NS	NS	***	NS		
	Average length of longest pollen tubes in style (mm)					
13/2	0.7	0.8	2.3	3.1	4.7 b	5.0 b
18/4	1.9	2.4	4.9	5.4	6.4 a	7.5 a
24/7	3.1	4.2	6.6	7.3	—	—
Significance						
Linear	***	***	***	***		
Quadratic	NS	NS	**	NS		
	Number of pollen tubes growing to the base of style					
13/2	0	0	0.0	0.0	0.3 b	0.5 b
18/4	0	0	0.9	0.8	8.8 a	14.4 a
24/7	0	0	11.0	26.8	—	—
Significance						
Linear	NS	NS	***	***		
Quadratic	NS	NS	***	***		

^zMean of 15 observations.

^yRating of visible stigma surface area covered by pollen tube growth. Scale 0 to 10: 0 = no pollen tubes visible on the surface; 1 = 1% to 10% area covered; 2 = 11% to 20%; 3 = 21% to 30%; 4 = 31% to 40%; 5 = 41% to 50%; 6 = 51% to 60%; 7 = 61% to 70%; 8 = 71% to 80%; 9 = 81% to 90%; and 10 = 91% to 100% of surface area covered by pollen tubes.

^xMean separation within column by Duncan's new multiple range test at $P = 0.05$.

^wAll styles had pollen tubes reaching the base of styles 48 h after pollination. No further evaluations were conducted thereafter.

NS, *, **, ***Nonsignificant or significant at $P = 0.05$, 0.01, or 0.001, respectively.

Table 5. Effects of timing of application of liquid lime sulfur (LLS) at 2% + fish oil (FO) at 2% on 'Snowdrift' pollen tube growth and fruit set in 'Golden Delicious' apple in the field (2006).^z

Treatment	Timing of application (HAP) ^y	Rating of pollen tube growth on stigma surface (0–10) ^{w,x}	Number of pollen tubes penetrating through stigma	Avg length of longest pollen tubes in style (mm)	Number of pollen tubes growing to the base of style	Avg style length (mm)	Fruit set (%)
Control	—	5.7 a ^y	43.7 a	7.4 b	17.4 a	7.4 a	— ^u
LLS + FO	4	0.8 d	0.0 d	0.0 d	0.0 b	6.9 b	0.0 b
LLS + FO	24	2.6 c	8.9 c	0.7 c	0.0 b	7.0 b	0.0 b
LLS + FO	48	3.8 b	35.8 b	7.9 a	16.2 a	7.9 a	76.0 a

^z'Golden Delicious' apple flowers were pollinated with 'Snowdrift' crabapple pollen; pollen tube growth was determined 48 h after application of LLS and FO, and fruit set was determined 35 d after pollination.

^yHours after pollination.

^xMean of three observations.

^wRating of visible stigma surface area covered by pollen tube growth. Scale 0 to 10: 0 = no pollen tubes visible on the surface; 1 = 1% to 10% area covered; 2 = 11% to 20%; 3 = 21% to 30%; 4 = 31% to 40%; 5 = 41% to 50%; 6 = 51% to 60%; 7 = 61% to 70%; 8 = 71% to 80%; 9 = 81% to 90%; and 10 = 91% to 100% of surface area covered by pollen tubes.

^uMean separation within columns by Duncan's new multiple range test at $P = 0.05$.

^vNontreated control trees were inadvertently thinned before fruit set data were recorded.

- Mellenthin, W.M., C.Y. Wang, and S.Y. Wang. 1972. Influence of temperature on pollen tube growth and initial fruit development in 'd'Anjou' pear. *HortScience* 7:557–559.
- Noordijk, H. and J. Schupp. 2003. Organic post bloom apple thinning with FO and lime sulfur. *HortScience* 38:690 (abstr.).
- Sedgley, M. and C.M. Annells. 1981. Flowering and fruit-set response to temperature in the avocado cultivar 'Hass'. *Sci. Hort.* 14:27–33.
- Socias i Company, R., D.E. Kester, and M.V. Bradley. 1976. Effects of temperature and genotype on pollen tube growth in some self-incompatible and self-compatible almond cultivars. *J. Amer. Soc. Hort. Sci.* 101:490–493.
- Vasilakakis, M.D. and I.C. Porlingis. 1985. Effect of temperature on pollen germination, pollen tube growth, effective pollination period, and fruit set of pear. *HortScience* 20: 733–735.
- Williams, M.W. and L.J. Edgerton. 1981. Fruit thinning of apples and pears with chemicals. U.S. Dept. Agr. Bul. 289, Washington, DC.
- Williams, R.R. 1965. The effect of summer nitrogen applications on the quality of apple blossom. *J. Hort. Sci.* 40:31–41.
- Williams, R.R. 1970. Factors affecting pollination in fruit trees, p. 193–207. In: Luckwill, L.C. and C.V. Cutting (eds.). *Physiology of tree crops*. Academic Press, London, UK, and New York, NY.
- Williams, R.R. and M. Maier. 1977. Pseudo-compatibility after self-pollination of the apple Cox's Orange Pippin. *J. Hort. Sci.* 52:475–483.