Screening Thermal Shock as an Apple Blossom Thinning Method. I. Stigmatic Receptivity, Pollen Tube Growth, and Leaf Injury in Response to Temperature and Timing of Thermal Shock

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Abstract. The use of short-duration applications of thermal energy (thermal shock; TS) as an apple blossom thinning strategy was investigated. Effects of TS temperature and timing on stigmatic receptivity, pollen tube growth *in vivo*, and visible leaf injury were evaluated in multiple experiments on ‘Crimson Gala’. TS treatments were applied to blossoms and spur leaves using a variable temperature heat gun. TS temperatures $\geq 86^\circ\text{C}$ had a strong inhibitory effect on pollen tube growth on the stigmatic surface and in the style. TS temperatures $>79^\circ\text{C}$ reduced average pollen tube length to less than the average style length. Timing of TS treatment (0 or 24 hours after pollination) was not an influential factor, indicating that effective TS temperatures reduced pollen tube growth up to 24 hours after the pollination event. The onset of thermal injury to vegetative tissues occurred at similar TS temperatures that inhibited pollen tube growth *in vivo*. Excessive leaf injury ($>33\%$) was observed at 95°C , suggesting relatively narrow differences in thermal sensitivity between reproductive and vegetative tissues. Inconsistent TS temperatures and/or responses were observed in some experiments. Ambient air temperature may have influenced heat gun output temperatures and/or plant susceptibility. While results suggest some promise, additional work is required to validate and further develop this concept.

There has been a significant effort to develop new blossom thinners for apple over the past 35 years. Approximately 150 chemistries and multiple iterations of tractor-mounted and hand-held mechanical thinners were evaluated (Kon and Schupp, 2018). Although existing blossom thinners may re-

duce fruit set, there are negative consequences associated with chemical and mechanical blossom thinners, such as erratic responses from year-to-year (Byers, 1997; Webster and Spencer, 1999), a chronic reduction in spur leaf photosynthesis (McArtney et al., 2006), and chemical or mechanical injury to vegetative structures (Byers, 1997; Kon et al., 2013). Spur leaves are important in promoting fruit growth, thus injury to these tissues had negative impacts on fruit size, fruit set, and fruit mineral content (Ferree and Palmer, 1982). A key to developing effective blossom thinners for apple is to limit injury to these important vegetative tissues.

The application of short-duration thermal treatments to plant canopies is not a novel concept. Thermal treatments were applied to weeds via hot water, steam (Leon and Ferreira, 2008), flaming, infrared (Ascard, 1998), and microwave (Brodie et al., 2012). Many thermal weed control methods were effective in weed injury/mortality and inhibition of weed seed germination. However, thermal weed control has not been a cost-effective measure as compared with conventional herbicides, and weed species differ in their thermal tolerance (Leon and Ferreira, 2008).

The possibility of using short-duration applications of thermal energy (TS) as a blossom thinning method has not been investigated. Apple blossoms are sensitive to temperature stress. Apple styles and ovules are more sensitive to freezing temperatures when compared with surrounding floral tissues (Palmer et al., 2003). Pistils of apple blossoms at pink to full bloom can be killed at -3.9 to -1.7°C with only 1 h of exposure (Ballard et al., 1981). Temperature stress in plants is a complex interaction of the intensity, duration, and rate of temperature change (Wahid et al., 2007). As demonstrated with an ornamental lily, pollen tube growth *in vivo* was temporarily arrested with 10-s exposure to 45°C (Pierson et al., 1993). Unlike leaves and pistils, mature pollen does not have heat tolerance mechanisms and is very sensitive to heat stress (Snider and Oosterhuis, 2011).

Heat stress effects on plant sexual reproduction has received considerable attention in the literature (Snider and Oosterhuis, 2011; Wahid et al., 2007; Zinn et al., 2010). Although increased ambient temperatures decreased the length of the effective pollination period (EPP), it is unknown if TS influences EPP of fruit trees. High temperatures increased the rate of apple pollen tube growth but reduced the longevity of ovule and stigma life span/receptivity. Stigmatic receptivity can be a limiting factor in the length of the EPP (Sanzol and Herrero, 2001). Because the purpose of the stigma is to adhere pollen grains and support pollen grain hydration and germination, damaging the stigma may result in reduced fruit set. Elevated air temperatures can reduce carbohydrate availability in pistils, resulting in reduced pollen tube growth and poor fruit set (Snider and Oosterhuis, 2011).

The onset of thermal injury to leaf tissue can occur over a very narrow temperature range. For example, there was no observable injury to soybean leaves that were subjected to a hot water bath of 53°C for 1 min, but exposure to 54°C caused chlorosis of leaf tissue and 55°C caused necrosis (Daniell et al., 1969). Lethal temperatures caused disintegration of cellular membranes. TS could present a new method of blossom thinning apple, if application(s) prevents fertilization of later blossoms and spur leaf injury is minimal.

The purpose of this work was to determine 1) effects of TS treatments on the duration of stigmatic receptivity, 2) effects of TS temperature and timing on pollen tube growth *in vivo* and visible spur leaf injury, and 3) if TS has potential as a crop load management strategy.

Materials and Methods

Trials were conducted in 2014 and 2015 at the Pennsylvania State University’s Fruit Research and Extension Center in Biglerville, PA. ‘Crimson Gala’/‘M.9’ trees planted in 2011 at $0.9 \times 3.7\text{-m}$ spacing were used for all experiments. Trees were managed according to standard commercial practices (The Pennsylvania State University, 2018).

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Expt. 1: TS effects on stigmatic receptivity of 'Crimson Gala' apple. Ninety spurs on 2- to 3-year-old wood were selected on 30 trees when the king blossom was at late balloon stage. All lateral flowers were removed. Selected king flowers were depetaled, emasculated, and pollinators were excluded from spurs with a bag made of spunbonded row-cover material to prevent unregulated pollination. Treatments were randomly assigned to solitary blossoms and flagged. On the following day, a range of TS treatments was applied to apple blossoms. A variable temperature heat gun set to an air flow rate of 0.50 m³·min⁻¹ was used to apply all treatments (8988-20; Milwaukee Electric Tool Corporation, Brookfield, WI). A gas-powered generator supplied electricity to the heat gun in the field. A data-logging thermocouple (EL-GFX-TC; Lascar Electronics Inc., Erie, PA) was used to monitor the output temperature of the heat gun. The thermocouple probe was attached to the heat gun 2 cm away from the heat gun outlet. For a given treatment, a nominal heat value was set on the heat gun, and actual thermal output was recorded and reported. After the heat gun temperature stabilized, all replicates of a given heat treatment were applied in ascending order. The start and stop times were recorded. The heat gun was positioned perpendicular to the calyx when heat treatments were applied to the pistil. Distance of the heat gun aperture from the pistil and duration of application were held constant (2 cm and 2 s, respectively). Mean, SD, and variance was determined for each level of temperature. Descriptive statistics of TS treatments is provided in Table 1. In all subsequent tables and figures, mean output temperatures are presented as the explanatory variable.

Fifteen blossoms were hand pollinated with 'Rome' pollen using a painter's brush on 0, 2, 4, 6, 8, and 10 d after treatment. Blossoms were collected 24 h after pollination, placed in labeled vials containing 5% sodium sulfite, and stored at 4 °C until analysis.

Table 1. Expt. 1: Descriptive statistics for thermal shock output temperatures applied to 'Crimson Gala' in 2014 and 2015.²

Treatment ID	Mean (°C)	SD
2014		
1	15 ^a	—
2	48 ^a	0.5
3	66	0.4
4	76	0.4
5	86	0.8
2015		
1	19	—
2	48	3.9
3	63	4.2
4	78	3.4
5	87	3.6

²Treatments were applied on 3 May 2014 and 29 Apr. 2015. In both years, all treatments were applied within 1 h.

^aControl treatment = ambient air temperature at timing of treatment.

^xAll heat treatments were applied with a variable temperature heat gun for 2 s. Distance from the heat gun aperture and the pistil was 2 cm.

ysis. A modified version of the method of Embree and Foster (1999) was used to visualize pollen tube growth. Before microscopic examination, samples were autoclaved at 121 °C for 10 min to soften tissues. Blossoms were rinsed with distilled deionized water and the style was removed with a scalpel at the junction with the hypanthium. Styles were rinsed, separated, and soaked in a water-soluble fluorescence solution of 0.01% Aniline Blue stain in 0.067 M K₂HPO₄ on a microscope slide. Styles were squashed between two microscope slides and were incubated overnight at room temperature. Samples were observed using fluorescence microscopy at ×100 (BX51; Olympus Optical Co., Tokyo, Japan). A high-pressure mercury vapor light source and ultraviolet/DAPI long-pass filter cube was used (part 19000; Chroma Technology Corp., Bellows Falls, VT). Style damage was visually rated (1–6 scale, see Fig. 1; 1 = no visible injury; 2 = trace to 10% style damaged; 3 = 11% to 25% style damaged; 4 = 26% to 50% style damaged; 5 = 51% to 75% style damaged; 6 = 76% to 100% style damaged). Pollen tubes that germinated on the stigma, and entered the style were counted. The percentage of stigmas and styles that supported pollen tube growth was calculated.

Expt. 2: TS effects on pollen tube growth in vivo. Eighty 'Crimson Gala' spurs were selected using the same criteria described previously. Selected king blossoms were emasculated and excluded from pollinators. On the following day, all blossoms were hand pollinated with 'Rome' pollen using a painter's brush. Using the methods described in Expt. 1, TS treatments were applied to apple blossoms at two distinct timings: 1) 1 h before pollination, and 2) 24 h after pollination. Descriptive statistics of TS treatments for 2014 and 2015 are provided in Table 2. In all subsequent tables and figures, mean output temperature is presented as the explanatory variable. Blossoms were harvested 120 h after pollination and placed in a labeled vial containing 5% sodium sulfite and stored at 4 °C. Samples were prepared using the previously described methods.

Pollen density on the stigmatic surface was visually rated using a 0 to 10 scale (0 = no pollen tubes visible on the surface and 10 = 91% to 100% of surface covered by pollen tubes) described in Yoder et al. (2009). Pollen tubes that entered the style were counted. Longest pollen tube length and style length were measured with an ocular micrometer.

Expt. 3: TS effects on visible spur leaf injury. In 2015, 30 flowering spurs were randomly selected. Spurs were manipulated to permit unobstructed airflow to test leaves. At each spur, all blossoms were removed and three fully expanded spur leaves were selected and the rest were removed. Treatments were randomly assigned to spurs and were flagged. Using the methods described in Expt. 1, TS treatments were applied to persisting spur leaves at each spur. Spur leaf blades were held in a fixed position perpen-

dicular to the heat gun aperture using forceps. The adaxial surface of the leaf was treated and all treatments were applied within 1 h. Descriptive statistics of TS treatments are provided in Table 3. Visual injury to spur leaves was visually rated 7 d after treatment and the percentage of leaf tissue that exhibited injury (0% to 100%) was estimated.

Statistical analysis. All experiments had a completely randomized design. Two factors were evaluated in Expt.1, temperature and pollination day, and were structured in a factorial arrangement. Five levels of temperature (quantitative variable) and six pollination days (qualitative variable) were tested: a total of 30 treatments. The experiment was replicated three times.

Two factors were evaluated in Expt. 2: temperature and timing, and were structured in a factorial arrangement. Ten levels of temperature and two levels of timing were tested; a total of 20 treatments. The experiment was replicated four times. In Expt. 3, 10 levels of temperature were evaluated and the experiment was replicated three times.

The PC version of SAS (version 9.3; SAS Institute, Cary, NC) was used for all statistical analysis. Where appropriate, analysis of variance was used to test main effects and interactions and regression analysis was conducted via PROC GLM.

Results and Discussion

Expt. 1: TS effects on stigmatic receptivity of 'Crimson Gala' apple. In both years, TS temperature influenced stylar injury (Fig. 1) and pollen tube growth responses (Table 4). However, the effect of pollination day and the interaction between temperature and pollination day were not significant. We recognize that time (pollination day) is an influential factor in the duration of floral longevity (Sanzol and Herrero, 2001). Maximal stigmatic receptivity of apple blossoms occurred at anthesis, and coincided with a loss in turgidity of stigmatic papillae (Losada and Herrero, 2012; Sheffield et al., 2005). Secretions from the stigma support pollen adhesion, germination, and growth. Floral tissues are ephemeral and receptivity of apple stigmas diminished with later pollination days (Losada and Herrero, 2013). In this experiment, a total loss of stigmatic receptivity was observed on pollination day 8 or 6 (2014 and 2015, respectively), across all treatments. However, parametric tests assume equal variance among samples. In 2014, responses on pollination days 8 and 10 violated assumptions of equal variance, and were not included in tests of main effects and interactions (data not presented). Similarly, in 2015, blossoms pollinated on days 8 and 10 abscised before collection, and blossoms collected on pollination day 6 violated assumptions of equal variance and were not included in tests of main effects and interactions. There was no interaction between temperature and pollination day, indicating that slopes for each pollination day did not differ. This suggests that thermal injury to floral

tissue and the subsequent reduction in pollen germination, and growth was acute. Therefore, linear and quadratic models were evaluated for each response variable using temperature as a predictor variable.

Chemicals can have detrimental effects on the morphology of the stigma, potentially accelerating papillae collapse (Yi et al., 2003). As TS temperature increased, a curvilinear increase in visible injury to stylar tissue was observed in both years (Table 5). The onset of this injury corresponded with reduced pollen germination on the stigmatic surface and pollen tube growth in the stylar tissue. Similarly, chemical blossom thinners can cause oxidative injury to apple styles, and stylar injury ratings correlated well with

reduced pollen tube growth and/or fruit set (Janoudi and Flore, 2005; Rom and McFerson, 2003).

The highest level of TS temperature tested in each year (86 and 87 °C) had a strong inhibitory effect on pollen tube growth on the stigmatic surface and in the style. When compared with ambient temperatures (control), these temperatures reduced the number of germinated pollen tubes on the stigmatic surface by 90% and 88% (2014 and 2015, respectively). Similarly, the highest level of TS temperature reduced numbers of pollen tubes that entered the style to 0.6 or 1.5 (2014 and 2015, respectively), compared with >15 pollen tubes in the control. In the style, pollen tube growth occurs in the transmitting tissue,

a longitudinal sector of specialized cells, which provides proteins and polysaccharides to support pollen tube growth (Cresti et al., 1980; Pratt, 1988). Damage to stigmatic papillae or the transmitting tissue may be detrimental to pollen tube growth and subsequent fruit set. In theory, fruit set may result if only one pollen tube fertilized one ovule. However, reduced pollen load resulted in poor fruit and seed set (Janse and Verhaegh, 1993). Most apple flowers have five carpels with two to four ovules per carpel (Pratt, 1988; 10–20 ovules per blossom). Sheffield et al. (2005) observed perfect syncarpy ‘Summerland McIntosh’, because stylar transmitting tissue was fused above the ovaries, and pollen tubes from any style could enter any locule. For potential of a full complement of seeds, however, at least 10 pollen tubes must enter the style and fertilize the ovules (McArtney et al., 2006). Blossoms with limiting pollen tube number were observed at TS temperatures 66 °C and >78 °C in 2014 and 2015, respectively (Table 5).

As TS temperature increased, a quadratic reduction in the proportion of stigmas and styles that supported pollen tube growth was observed. Low TS temperatures did not reduce the percentage of receptive stigmas below 93%. Even at high temperatures, the percentage of receptive stigmas was 28% or 50% in 2014 and 2015, respectively. Quantifying stigmatic receptivity as the percentage of stigmas that were capable of supporting pollen germination may be somewhat misleading. The germination of one pollen grain resulted in classifying a stigma as being receptive. When screening chemical blossom thinners, pollen tube growth in the upper style was significantly reduced, but not totally eliminated with effective chemicals (Embree and Foster, 1999). The highest level of TS temperature tested in each year (86 and 87 °C) reduced the percentage of styles that

Fig. 1. Examples of visible injury to stylar tissue due to thermal shock. Style damage was visually rated using a 1 to 6 scale: 1 = no visible injury; 2 = trace to 10% style damaged; 3 = 11% to 25% style damaged; 4 = 26% to 50% style damaged; 5 = 51% to 75% style damaged; 6 = 76% to 100% style damaged. From left to right, styles were rated as follows: 1, 3, 5, and 6.

Table 2. Expt. 2: Descriptive statistics for thermal shock treatments applied to ‘Crimson Gala’ in 2014 and 2015.^z

Treatment ID	Timing (HAP)	2014		2015	
		Mean output temp (°C)	SD	Mean output temp (°C)	SD
1	0	15	—	12	—
2	0	36	0.4	35	1.4
3	0	43	0.6	43	2.0
4	0	51	0.3	55	2.8
5	0	54	0.5	61	2.7
6	0	60	0.4	64	2.4
7	0	63	1.5	66	3.0
8	0	64	4.6	72	2.1
9	0	66	3.4	79	1.9
10	0	68	4.5	84	1.4
11	24	14	—	18	—
12	24	27	0.3	39	1.5
13	24	37	0.9	52	2.4
14	24	47	0.4	62	1.9
15	24	49	1.0	67	2.8
16	24	55	1.7	73	3.0
17	24	58	1.8	77	2.4
18	24	63	2.3	84	1.9
19	24	67	1.1	89	1.8
20	24	70	1.9	95	1.4

^zControl treatment was the ambient air temperature at timing of treatment. With the exception of the controls, all heat treatments were applied with a variable temperature heat gun for 2 s. Distance from the heat gun aperture and the pistil was 2 cm. At each timing, all treatments were applied within 1 h. Timing 0 was applied on 5.3.14 and 24 was applied on 5.4.14. HAP = hours after pollination.

contained pollen tubes to less than 20%. Assuming a five-carpel pistil, less than one style supported pollen tube growth per flower at these temperatures.

Table 3. Expt. 3: Descriptive statistics for thermal shock output temperature applied to ‘Crimson Gala’ spur leaf tissue in 2015.^z

Treatment ID	Mean (°C)	SD
1	28	—
2	60	3.4
3	64	3.6
4	67	3.9
5	73	4.0
6	79	4.3
7	80	4.1
8	86	2.7
9	92	2.9
10	95	3.0

^zControl treatment was the ambient air temperature at timing of treatment. With the exception of the control, all heat treatments were applied with a variable temperature heat gun for 2 s. Distance from the heat gun aperture and the leaf was 2 cm.

Expt. 2: TS effects on pollen tube growth in vivo. When using the variable temperature heat gun to apply TS, consistent nominal output temperatures were set in each year, and actual thermal output was recorded with a data-logging thermocouple. Because the air source was not supplied, ambient air temperature and/or relative humidity appeared to affect the actual output temperature of the heat gun despite using the same nominal heat settings in each year (refer to Table 2). Therefore, the range of TS temperatures tested differed in each year.

In both years, TS temperature influenced pollen tube growth responses, whereas timing and the interaction term were not significant (Table 6). In 2014, TS temperature had a weak linear relationship with pollen density ($P = 0.0039$; $r^2 = 0.10$) and the number of pollen tubes that entered the style ($P = 0.0347$; $r^2 = 0.06$) (Fig. 2). The weakness and trend of these relationships is likely explained by the limited high range of TS

temperatures evaluated. In 2014, the highest temperature evaluated was 70 °C (Table 2). In Expt. 1, pollen tube germination and growth were not consistently reduced/inhibited at <86 °C. This suggests the range of TS temperatures evaluated in 2014 were too low to have an influence of practical significance on pollen germination and growth in vivo.

In 2015, the interaction between TS temperature and timing was significant for pollen density ($P = 0.0006$) and the number of pollen tubes entering style ($P = 0.0371$). TS treatments applied 24 hours after pollination (HAP) provided ample time for pollen to germinate, and begin to grow down the style. Pollen grains germinated by 2 h after pollination and penetrated the style by 10 h (Losada and Herrero, 2014). A curvilinear reduction in pollen density and number of pollen tubes entering the style was observed when TS was applied at 0 HAP. Less than 10% of the stigma was covered in germinated

Table 4. Main effects and interactions of thermal shock temperature and pollination date on stylar browning rating, pollen tube growth on the stigma, pollen tube growth in the style, percentage of receptive stigmas, and the percentage of styles supporting pollen tube growth of ‘Crimson Gala’ in 2014 and 2015.^z

Response variable	Significance ($P > F$)		
	Temperature	Pollination day	Interaction
	2014 ^y		
Stylar browning	<0.0001	0.8869	0.4139
No. pollen tubes on stigmatic surface	<0.0001	0.4277	0.6256
No. pollen tubes in style	<0.0001	0.0707	0.2201
Receptive stigmas	<0.0001	0.8569	0.2515
Styles supporting pollen tubes	<0.0001	0.2913	0.5049
	2015 ^x		
Stylar browning	<0.0001	0.2579	0.7730
No. pollen tubes on stigmatic surface	0.0004	0.3845	0.7243
No. pollen tubes in style	0.0018	0.3110	0.5306
Receptive stigmas	0.0021	0.9978	0.9367
Styles supporting pollen tubes	<0.0001	0.6717	0.9500

^zExperiment was a completely randomized design with a factorial treatment structure (5 quantitative temperatures $\times$ 6 quantitative pollination days).

^yTo avoid violating assumptions of equal variance, data from pollination days 8 and 10 were omitted from the data set (2014).

^xTo avoid violating the assumptions of equal variance, data from pollination days 6, 8, and 10 were omitted from the data set (2015).

Table 5. Effects of thermal shock temperature on stylar browning rating, pollen tube growth on the stigma, pollen tube growth in the style, percentage of receptive stigmas, and percentage of styles supporting pollen tube growth of ‘Crimson Gala’ in 2014 and 2015.^z

Temperature (°C)	Stylar Browning Rating (1–6) ^y	No. pollen tubes on stigma	No. pollen tubes in style	Receptive stigmas (%) ^x	Styles supporting pollen tubes (%) ^w
	2014				
15	1.3	26.1	15.6	95	80
48	1.6	21.3	10.8	95	87
66	1.6	20.5	9.1	93	75
76	2.5	8.8	4.1	55	41
86	3.7	2.6	0.6	28	13
Significance	Q	Q	L	Q	Q
<i>P</i> value	<0.0001 ^v	<0.0001	<0.0001	<0.0001	<0.0001
<i>r</i> ²	0.51	0.44	0.37	0.59	0.55
	2015				
19	1.5	29.2	18.9	98	93
48	1.5	32.5	22.6	100	91
63	1.6	26.2	15.9	98	89
78	2.1	21.4	12.6	98	71
87	3.2	3.4	1.5	50	18
Significance	Q	Q	Q	Q	Q
<i>P</i> value	<0.0001	0.0002	0.0016	<0.0001	<0.0001
<i>r</i> ²	0.60	0.34	0.26	0.43	0.58

^zMeans of five observations.

^yStyle damage was visually rated (1–6 scale; 1 = no visible injury; 2 = trace; 3 = 25% style damaged; 4 = 50% style damage; 5 = 75% style damage; 6 = 100% style damaged).

^xReceptive stigmas = (no. of stigmas supporting pollen germination/no. stigmas)*100.

^wStyles supporting pollen tubes = (no. of styles supporting pollen germination/no. styles)*100.

^v*P* value is for the model: $n = 60$ and 45 in 2014 and 2015, respectively.

L = linear model; Q = quadratic model.

Table 6. Main effects and interactions of thermal shock temperature and pollination date on pollen density on the stigma, no. of pollen tubes that entered the style, and length of the longest pollen tube of 'Crimson Gala' in 2014 and 2015.^{z,y}

Response variable	Significance ($P > F$)		
	Temperature	Timing	Interaction
2014			
Pollen density	0.0022	0.1345	0.2181
No. pollen tubes entering style	0.0201	0.4866	0.9234
Length of longest pollen tube	0.0058	0.7816	0.8709
2015			
Pollen density	<0.0001	0.0026	0.0006
No. pollen tubes entering style	<0.0001	0.0501	0.0371
Length of longest pollen tube	<0.0001	0.2785	0.2733

^zAll heat treatments were applied with a variable temperature heat gun for 2 s. Distance from the heat gun aperture and the pistil was 2 cm.

^yExperiment was a completely randomized design with a factorial treatment structure [10 temperatures (quantitative) $\times$ 2 timings (qualitative)].

Fig. 2. Effects of thermal shock temperature and timing on pollen density (A and B) and the number of pollen tubes that enter the style (C and D) in 2014 and 2015. All heat treatments were applied with a variable temperature heat gun for 2 s. Distance from the heat gun aperture and the pistil was 2 cm. A visual rating scale was used to quantify pollen density on the stigma: 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%; 10 = 91% to 100% of surface covered by pollen tubes. Vertical bars represent the SE. HAP = hours after pollination.

pollen (pollen density rating = 1) and <10 pollen tubes entered the style at temperatures ≥ 79 °C.

Blossoms in this experiment remained in situ on the tree for 5 d before sampling; however, pollen tubes did not reach the style base in 2014 (Fig. 3). Although pollen tubes can grow to the style base in less than 48 h, cool temperatures reduced pollen tube growth rates (Yoder et al., 2009). Cool spring temperatures were observed in 2014 (mean daily temperature = 14.6 °C), and pollen tubes did not have sufficient time to grow to the style base before collection. The weak relationship between TS temperature and

pollen length ($r^2 = 0.09$) in 2014, may be explained in part by inhibition of all pollen tube growth caused by cool spring temperatures. In addition, the narrow range of actual TS temperatures applied in 2014 may have been a function of the cool ambient temperatures. Influential data points were observed at the higher temperatures (≥ 63 °C) evaluated in 2014.

Warmer ambient temperatures occurred during bloom in 2015, and we observed pollen tubes at the style base after the 5-d incubation period (mean daily temperature = 19.4 °C). A curvilinear reduction in pollen tube length was observed with increased TS

temperature in 2015. TS temperatures greater than 79 °C reduced pollen tube length to less than the average style length. Application timing and the interaction of TS temperature and timing did not affect pollen tube length in either year. The absence of an interaction between TS timing and temperature is important and may have practical implications. One of the most consistent apple blossom thinners, lime sulfur (calcium polysulfide), was effective in reducing pollen tube growth up to 24 h after treatment (Yoder et al., 2009). Blossom thinning is a time-sensitive operation, so the ability of a blossom thinner to reduce or inhibit pollen tube growth over a

Fig. 3. Effects of thermal shock temperature on the average length of the longest pollen tube in 2014 and 2015 (A and B, respectively). All heat treatments were applied with a variable temperature heat gun for 2 s. Distance from the heat gun aperture and the pistil was 2 cm. HAP = hours after pollination.

Fig. 4. Effect of thermal shock temperature on visible spur leaf injury in 2015. The percentage of spur leaf tissue that exhibited injury was estimated visually. Visible injury to leaf tissue was not observed with TS treatments below 67 °C, and was not included in analysis. All heat treatments were applied with a variable temperature heat gun for 2 s. Distance from the heat gun aperture and the pistil was 2 cm.

wide period of time is very desirable, because the number of applications may be reduced (Schmidt and Elfving, 2007). Future research should consider the efficacy of TS at later timings and/or in relation to pollen tube growth models (Yoder et al., 2013). Timing TS application with predictive models may increase the repeatability of results and increase the precision of thinning outcomes.

Expt. 3: TS effects on visible spur leaf injury. Visible injury to leaf tissue was not

observed with TS treatments below 67 °C, and was not included in analysis. As TS temperature increased, a curvilinear increase in leaf tissue that exhibited visible injury was observed (Fig. 4). In general, injury to spur leaf tissue is undesirable. Spur leaf injury thresholds have been determined, by excision or mechanical wounding of spur leaf area. Hall and Ferree (1976) found that net photosynthesis (Pn) was unaffected by a 10% removal of individual apple leaves, but higher rates of leaf removal reduced Pn. Ferree and Palmer (1982) demonstrated that removal of >33% of spur leaf area reduced fruit set, fruit size, and mineral nutrition. We observed >10% visible spur leaf injury at 86 °C, and >33% injury at 95 °C. The onset of visible leaf injury occurred over a relatively narrow temperature range, which accords with results of Daniell et al. (1969). In this experiment, minor leaf injury (<10%) occurred at temperatures that reduced or inhibited pollen tube growth in vivo. However, reductions in Pn may occur without visible symptoms (Byers et al., 1990). Future work should investigate TS effects on Pn and determine if there is a relationship between visible leaf injury and leaf function.

Conclusions

This was exploratory work to determine the potential of a previously untested crop

load management strategy. A series of small-scale trials using a variable temperature heat gun to apply various TS output temperatures to solitary blossoms and spur leaves was conducted. Specifically, a range of TS temperatures that reduced apple pollen tube growth in vivo was identified. TS temperatures ≥ 86 °C had a strong and consistent inhibitory effect on pollen tube growth on the stigmatic surface and in the style. At effective temperatures, TS effects were acute and did not influence the duration of the stigmatic receptivity. In addition, pollen tube growth was reduced up to 24 h after the pollination event, TS temperatures >79 °C reduced pollen tube length to less than the average style length. Although minimal spur leaf injury (<10%) occurred at 86 °C, unacceptable leaf injury (>33%) was observed at 95 °C. Although our data show that TS was effective in reducing pollen tube growth in vivo, the onset of visible injury to leaf tissue occurred at similar temperatures. Additional work is required to validate and further develop this concept.

Some challenges in using forced heated air as a TS delivery system were identified. Much like chemical thinning, environmental effects appeared to influence the efficacy of the treatment. The likely influence of the environment on heat gun output temperatures is problematic and resulted in application of lower output temperatures than expected. Although not considered in this study, the initial temperature of tissues, relative humidity, wind speed, and physical structure of the tissues may influence the amount of heat energy transferred by convection (Hamer, 1985; Tsilingiris, 2008). Additional application considerations, such as duration of treatment (Kon et al., 2020) and canopy distance from the heat source, would influence thermal transfer to reproductive or vegetative tissues of apple. Use of forced heated air is a relatively inefficient method of heat transfer. More efficient methods of heat transfer, such as infrared or steam, should be considered in future research. However, given the relatively narrow difference in thermal sensitivity between reproductive and vegetative tissues, use of inefficient methods of heat transfer may be advantageous to limit injury to nontarget tissues.

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