

# Pollen Tube Growth and Fruit Set in Apple

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**Abstract.** Pollination is an essential prerequisite for the production of many fruit and seed crops, including apple. In apple, successful fertilization requires pollen transfer to the stigma, pollen germination, and successful pollen tube growth resulting in fruit set. Precise selection of the most effective pollinizers for commercial orchards is not possible however, until these processes are more fully understood. The present study was undertaken to compare pollinizers in terms of pollen tube growth and fruit set. On trees of ‘Honeycrisp’, ‘Fuji’, and ‘Gala’ from which bees were excluded, flowers were hand-pollinated using pollen collected from crabapple (‘Ralph Shay’ or *Malus floribunda*), ‘Delicious’ and ‘Golden Delicious’. Flowers were harvested at one, two, three, and four days after pollination (DAP). Pollen source had a significant influence on pollen germination on the stigmatic surface, number of pollen tubes penetrating the stigma, distance of pollen tube growth down the style, and pollen tubes reaching the base of the style. In ‘Honeycrisp’ and ‘Gala’, ‘Golden Delicious’ pollen grew the fastest, followed by ‘Delicious’ and crabapple. Neither ‘Ralph Shay’ nor *Malus floribunda* were effective pollinizers for ‘Honeycrisp’ and resulted in low fruit set suggesting incompatibility may be involved. However, both these crabapples were effective pollinizers for ‘Fuji’ and ‘Gala’. These results indicate that pollen source can have a tremendous impact on pollen tube growth and fruit set. The physiological basis for these effects is not clear, but implications for pollinizer selection are obvious.

Pollination is an essential process for fruit set, fruit growth, fruit quality, and seed set of most apple cultivars. The first step of successful apple pollination is the transfer of pollen to the stigmatic surface (typically vectored by bees) followed by an adhesion of pollen grains to the papilla cells of the stigmatic surface (Dresselhaus and Franklin-Tong, 2013; Selinski and Scheibe, 2014). The deposited pollen hydrates and germinates and then pollen tubes penetrate the stigma and grow down the style. Pollen recognition occurs both on the stigmatic surface and within the style (Dresselhaus and Franklin-Tong, 2013). Once pollen reaches the base of the style, one sperm nucleus enters the egg cell to produce the embryo (resulting in seed set), and the other fuses with the two polar nuclei to produce the endosperm.

Pollen source and temperature have a tremendous influence on the rate of pollen tube growth. Jackson (2003) and Petropoulou and Alston (1998) proposed that the proportion of the stigmatic surface covered by the germinated pollen of apples and pears depends on

the pollen donor and environmental temperature at the time of pollination. They reported that ‘Spartan’ pollen had a higher germination percentage than that of ‘Cox’s Orange Pippin’ at 8 to 10 °C and ‘Idared’ at 14 to 16 °C. Linear correlations between pollen germination on the stigmatic surface and temperature were reported from 13 to 29 °C (Yoder et al., 2009) and from 6 to 33.5 °C (Jefferies and Brain, 1984).

Almost all apple cultivars are reported to be either self-incompatible or semicompatible and require cross-pollination to set fruit in marketable quantities (Garratt et al., 2013; Matsumoto, 2014). For commercial production, at least two cross-compatible cultivars with synchronous flowering are recommended (Garratt et al., 2013; Goldway et al., 2012). Matsumoto et al. (2008) reported a significant decline in fruit set with increasing distance between pollinizer and main cultivars, and suggested pollinizers should be planted not more than 10 m from the target cultivars (Matsumoto et al., 2008).

Pollen source is considered to be one of the most influential factors affecting the fruit set and therefore orchard design. Pollen density on the stigmatic surface is positively correlated with pollen tube growth and fruit growth rate (Zhang et al., 2010). Pollen tube growth down the style has been widely studied, but pollen source effects on pollen tube growth are not well understood. The objective of this study was to determine the impact of pollen source on the pollen tube growth and fruit set. The effects on the seed set, fruit quality, and subsequent return

## Materials and Methods

This experiment was conducted in 2013 and repeated in 2014 at the Samuel G. Meigs Horticulture Facility in Lafayette, IN, USA. Three commercial apple cultivars were used: ‘Honeycrisp’/M.7 planted in 2003, ‘Fuji’/B.9 planted in 2001 and ‘Gala’/B.9 planted in 2001. These cultivars were chosen because of their economic importance and also because they represent a range of genetic potential for biennial bearing, so that the effects on flowering could be compared. Selected flowers of each cultivar were hand-pollinated using one of three pollen sources: crabapple [‘Ralph Shay’ (2013) and *Malus floribunda* (2014)], ‘Delicious’ and ‘Golden Delicious’. Since ‘Ralph Shay’ crabapple was found to be a poor pollinizer of ‘Honeycrisp’ in 2013, it was replaced by *Malus floribunda* in 2014. All cultivars were pollinated over a 2-hour period on the same day.

Temperature data were collected by a weather station located less than one mile from experimental plots. There was little variation in air temperature between 2013 and 2014 during the pollination period and during the days of sample collection, so it seems reasonable to assume any differences between the years were not due to temperature (Fig. 1). Trees were spaced 5.0 × 3.0 m and trained to a vertical axis. General orchard management, including pest management, was according to commercial guidelines for Indiana (Midwest Fruit Workers Group, 2013). Trees were not chemically thinned but were hand thinned to approximate a light commercial crop load to ensure that crop load was not a limiting factor.

The experiment was designed as a completely randomized design where two uniform adjacent trees were selected for each cultivar in late April of 2013. The same trees were used in 2014. Trees were netted in late April, before flower opening, to exclude bees, and thus, prevent cross-pollination. Care was taken to ensure the netting was secure and close visual examination did not observe any pollinators inside the netting. At the tight cluster stage of floral development, a total of 60 flower clusters were randomly selected on each cultivar, distributed between the two selected trees. Of the 60 flowers, 20 were randomly assigned to each of the three pollinizer treatments. There was not an unpollinated control because unpollinated flowers were presumed to result in very low fruit set. At late pink (popcorn) stage, just before the flower completely opened, all anthers were removed to prevent self-pollination, and flowers were hand-pollinated using a small brush. Pollen was applied liberally to ensure the amount of pollen was not limiting. Only king flowers were pollinated for uniformity of the experiment, and all lateral flowers as well as all nontagged flowers on the tested trees were either manually removed or dropped off. Pollen used for the

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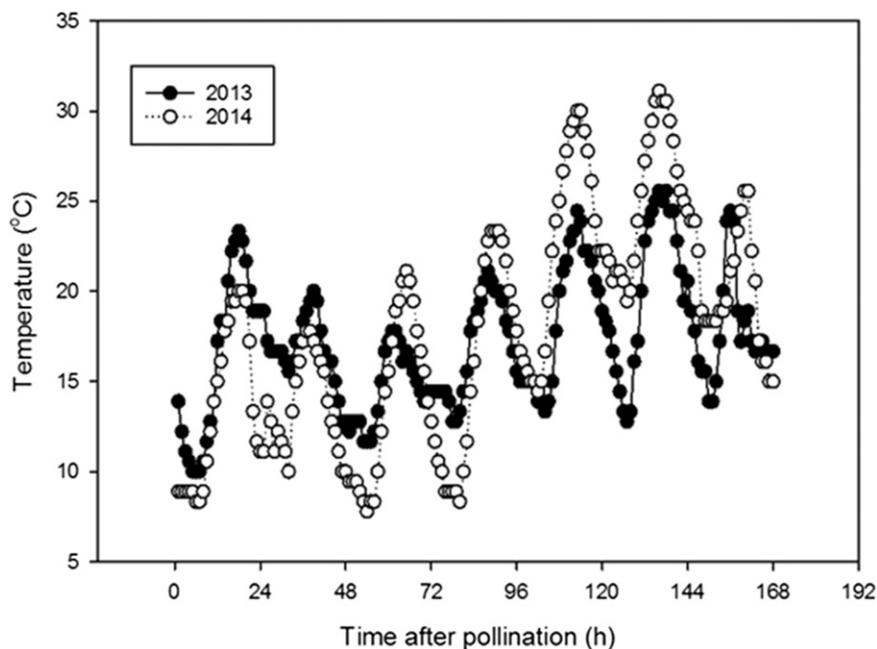


Fig. 1. Hourly air temperature during the pollination period and days of sampling after pollination (flowers were hand-pollinated in 3 May 2013 and 4 May 2014). Data presented are from 3 May to 9 May for each year.

experiment was collected from earlier flowering orchards in southern Indiana (2013) or from branches placed in the greenhouse to force flower opening (2014).

Pollen was tested in the laboratory in petri dishes before pollination to examine viability. Following the methods of Yoder et al. (2009), pollen was placed on a medium of 1% agarose, 10% sucrose, and 10 ppm boric acid at room temperature for 24 h. The proportion of germination was visually observed under a light microscope. All pollen used in these experiments had high germination rates (>80%).

The methods of Yoder et al., (2009) were modified to evaluate pollen germination on the stigmatic surface, number of pollen tubes penetrating the stigma, the length of the longest pollen tube growing down the style, and number of pollen tubes that reached the base of the style. Briefly, five hand-pollinated flowers were collected from each treatment at one, two, three, and four DAP. The flowers were then placed in a solution of 5% sodium sulfite, boiled for 15 min and then refrigerated in the same solution until the time of microscopic examination. Later, five pistils from each flower were detached from the ovary, rinsed with distilled water, and placed in a water-soluble solution of 0.01% aniline blue stain in 0.067 M  $K_2HPO_4$  on microscope slides. Detached pistils were squashed between two microscope slides. The pistils were then incubated in the dark at room temperature for 24 h, and the length of each pollen tube was measured by viewing it in epifluorescence mode through a blue filter (350/50 460/50) using a 10× 0.45 NA objective. The position of each pistil's end was located based on  $xy$  coordinates provided by an encoded, motorized  $xy$  stage (Nikon Ti-S-ER) on a Nikon Ti-E microscope. Length was

defined as the linear distance between each endpoint. Images of pistils were acquired using a 10× 0.45 NA objective and Nikon DS-Ri1 color camera on a Nikon 90i epifluorescence microscope using blue (350/50 460/50) and red (560/40 630/60) filter sets. Individual images of each pistil were manually assembled into a montage (Fig. 2).

The collected data comprised the rating of pollen tube growth on the stigmatic surface (0% to 100% of stigmatic surface covered by germinated pollen), number of visible pollen tubes penetrating the stigma, average length of the longest pollen tube growing down the style, and number of pollen tubes that reached the base of the style (Fig. 2). The proportion of the stigmatic surface covered by germinated pollen was rated on a scale of 0 to 10 based on the visual estimation of the proportion of the stigmatic surface covered where 0 = no germinated pollen tubes visible on the stigmatic surface, 1 = 1% to 10%, 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 the stigmatic surface was covered by germinated pollen tubes (Yoder et al., 2009). The pollen density on the stigmatic surface was rated because earlier work on pear showed this to be related to pollen tube growth (Zhang et al., 2010). The number of pollen tubes that penetrated the stigma and reached the base of style was counted, and the longest pollen tube was measured using the previously indicated software. Maximum growth of pollen tubes down the style was calculated as the length of the longest pollen tube divided by the total length of the style. The number of fruit that set on the tagged flowers was counted and the proportion fruit set calculated as percent fruit set.

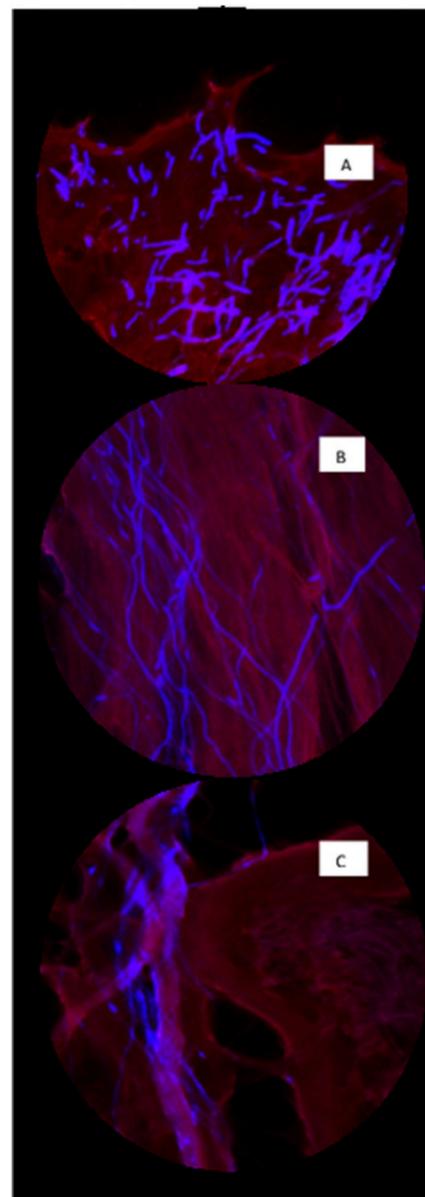


Fig. 2. (A) Pollen tubes germinated on the stigmatic surface, (B) pollen tubes growth down the style, and (C) pollen tubes that reached the base of the style of 'Fuji' flowers.

Statistical analyses included analysis of variance, Tukey multiple range test, and regression analysis using the Statistical Analysis System for PC (SAS 9.4). Means and standard errors of five pistils from five flowers ( $n = 25$ ) per treatment per day are reported.

## Results

Pollen germination percentage on the stigmatic surface increased with time after pollination in all cultivars in both years (data not shown). Of all the pollen sources, crabapple pollen showed the lowest amount of pollen tube growth on 'Honeycrisp' stigmatic surfaces in both years. In 2013, 'Golden Delicious' pollen germination was higher than 'Delicious' on 'Honeycrisp', but in

2014, they performed similarly (Table 1). On 'Fuji' flowers, pollen germination of crabapple was highest in 2013 except at 48 h when 'Golden Delicious' was highest. Pollen tubes of all cultivars grew similarly on 'Fuji' in 2014 except that 'Golden Delicious' exhibited much higher germination at 72 h. On the stigmatic surfaces of 'Gala' flowers, pollen tube growth was highest with 'Delicious' and lowest with 'Ralph Shay' in 2013, but in 2014, *Malus floribunda* crabapple pollen tube growth was the highest. The amount of pollen tube growth on the stigmatic surfaces depended on both male and

female cultivars and their interaction. For instance, 'Golden Delicious' pollen tube growth was highest on 'Honeycrisp' and 'Fuji' in 2013, followed by 'Delicious' and then 'Ralph Shay', whereas in 2014, pollen from 'Delicious' had the highest growth rate on 'Honeycrisp' followed by 'Golden Delicious'.

Significant male × female interactions were found for both years except at 72 h in 2013 and at 24 h in 2014. For instance, the visible germinated pollen of 'Golden Delicious' and 'Delicious' was greater than 'Ralph Shay' on 'Honeycrisp' stigmas on

all sampling dates in 2013. However, *Malus floribunda* was similar to or higher than the other two pollinizers when applied to 'Fuji' and 'Gala' in 2014 (Table 1).

The number of pollen tubes penetrating the stigma showed similar trends to the amount of pollen tube growth with low numbers of 'Ralph Shay' (2013) and *M. floribunda* (2014) pollen tubes penetrating the stigmas of 'Honeycrisp'; however, pollen from both crabapples performed well in this regard on the other cultivars in both years (Table 2). On most dates across the two years, the number of pollen tubes penetrating

Table 1. Effects of pollen source on the rating of visible pollen tube growth on the stigmatic surface of 'Honeycrisp', 'Fuji', and 'Gala' after hand-pollination in 2013 and 2014. Mean values ± SD of 25 observations are reported in each cell.

Male	Rating of visible pollen tube growth on the stigmatic surface (0–10) <sup>z</sup> 2013				Rating of visible pollen tube growth on the stigmatic surface (0–10) <sup>z</sup> 2014			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
	Honeycrisp							
Crabapple <sup>y</sup>	3.6 ± 0.5 b <sup>x</sup>	4.5 ± 0.5 b	1.8 ± 0.4 b	1.5 ± 0.4 c	0.6 ± 0.1 a	0.7 ± 0.5 b	0.8 ± 0.2 b	0.7 ± 0.2 b
Delicious	6.1 ± 0.5 a	4.5 ± 0.5 b	2.3 ± 0.4 b	4.6 ± 0.4 b	0.4 ± 0.1 a	1.5 ± 0.5 a	1.9 ± 0.2 a	2.1 ± 0.2 a
Golden Delicious	6.2 ± 0.5 a	7.1 ± 0.5 a	5.4 ± 0.4 a	7.7 ± 0.4 a	0.6 ± 0.1 a	2.4 ± 0.5 a	1.2 ± 0.1 a	1.4 ± 0.2 a
Significance <sup>w</sup>	***	***	***	***	NS	**	***	***
	Fuji							
Crabapple	5.5 ± 0.5 a	4.4 ± 0.5 b	6.0 ± 0.6 a	5.4 ± 0.5 a	0.6 ± 0.1 a	2.4 ± 0.2 a	1.0 ± 0.4 b	1.0 ± 0.2 a
Delicious	2.4 ± 0.7 b	5.5 ± 0.5 ab	6.3 ± 0.6 a	3.6 ± 0.5 b	0.6 ± 0.1 a	1.2 ± 0.2 a	1.1 ± 0.4 b	2.5 ± 0.2 a
Golden Delicious	5.1 ± 0.5 a	6.5 ± 0.5 a	7.2 ± 0.6 a	3.6 ± 0.6 b	0.5 ± 0.1 a	1.3 ± 0.2 a	4.3 ± 0.4 a	1.3 ± 0.2 a
Significance	***	**	NS	**	NS	NS	***	NS
	Gala <sup>v</sup>							
Crabapple	5.6 ± 0.5 a	2.1 ± 0.3 b	5.1 ± 0.5 a	3.3 ± 0.6 b	0.6 ± 0.1 a	1.0 ± 0.4 a	1.1 ± 0.1 a	3.0 ± 0.3 a
Delicious	3.7 ± 0.5 ab	2.8 ± 0.3 b	5.5 ± 0.5 a	7.1 ± 0.6 a	0.7 ± 0.1 a	1.6 ± 0.4 a	1.3 ± 0.1 a	0.8 ± 0.3 b
Golden Delicious	2.5 ± 0.5 b	7.1 ± 0.3 a	6.8 ± 0.6 a	5.2 ± 0.6 ab	0.5 ± 0.1 a	2.6 ± 0.4 a	1.6 ± 0.1 a	1.0 ± 0.3 b
Significance	***	***	NS	***	NS	NS	NS	***
Male	NS	***	***	***	NS	*	***	*
Female	**	***	**	NS	NS	NS	***	NS
Male × female	***	**	NS	***	NS	***	***	***

<sup>z</sup>Rating of visible pollen tubes germinated on the stigmatic surface scaled 0 to 10 where 0 = no pollen tubes visible on the stigmatic surface; 1 = 1% to 10% stigmatic surface 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% stigmatic surface was covered by pollen tubes.

<sup>y</sup>Crabapple pollen was from 'Ralph Shay' (2013) and *Malus floribunda* (2014).

<sup>x</sup>Statistical differences among male genotypes tested within column.

<sup>w</sup>NS, \*, \*\*, \*\*\* Non-significant; and significant at  $P = 0.05, 0.01, 0.001$ , respectively.

<sup>v</sup>Data of pollen tubes germinated on the stigmatic surface of 'Gala' were transformed by the square root of Y before analysis because data were not normally distributed; however, untransformed means are presented in the table.

Table 2. Effects of pollen source on number of pollen tubes penetrating the stigma, of 'Honeycrisp', 'Fuji', and 'Gala' in 2013 and 2014. Mean values ± SD of 25 observations are reported in each cell.

Male	Number of visible pollen tubes penetrating the stigma 2013				Number of visible pollen tubes penetrating the stigma 2014			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
	Honeycrisp							
Crabapple <sup>z</sup>	11.6 ± 1.0 b <sup>y</sup>	6.1 ± 1.1 b	1.6 ± 0.9 b	5.7 ± 1.2 c	2.6 ± 0.7 a	4.8 ± 1.7 a	8.2 ± 1.5 b	3.0 ± 1.9 b
Delicious	18.2 ± 1.0 a	4.6 ± 1.1 b	3.6 ± 0.9 b	10.3 ± 1.2 b	1.0 ± 0.6 ab	8.6 ± 1.7 a	11.5 ± 1.5 ab	14.9 ± 1.9 a
Golden Delicious	18.2 ± 1.0 a	15.6 ± 1.1 a	13.6 ± 0.9 a	15.0 ± 1.2 a	0.0 ± 0.6 b	8.5 ± 1.7 a	14.6 ± 1.5 a	14.0 ± 1.8 a
Significance <sup>x</sup>	***	***	***	***	**	NS	**	***
	Fuji							
Crabapple	7.2 ± 1.5 a	7.7 ± 1.2 a	5.8 ± 1.3 b	7.0 ± 1.0 a	7.8 ± 1.4 a	17.0 ± 1.2 a	12.0 ± 1.4 a	9.6 ± 1.6 a
Delicious	4.0 ± 1.8 a	10.0 ± 1.3 a	12.2 ± 1.3 a	2.2 ± 1.0 b	6.1 ± 1.4 a	16.0 ± 1.2 a	14.7 ± 1.4 a	14.8 ± 1.6 a
Golden Delicious	8.2 ± 1.4 a	8.7 ± 1.3 a	13.4 ± 1.3 a	6.0 ± 1.2 a	0.0 ± 1.4 b	13.9 ± 1.3 a	14.3 ± 1.4 a	11.2 ± 1.6 a
Significance	NS	NS	***	***	***	NS	NS	NS
	Gala							
Crabapple	11.2 ± 1.4 a	10.5 ± 1.1 b	12.5 ± 0.9 b	8.7 ± 1.4 b	6.4 ± 1.2 a	9.7 ± 1.3 b	11.6 ± 1.3 b	17.6 ± 1.3 a
Delicious	6.0 ± 1.3 b	9.3 ± 1.1 b	16.8 ± 0.9 a	16.2 ± 1.4 a	4.1 ± 1.2 ab	15.2 ± 1.3 a	16.8 ± 1.3 a	9.4 ± 1.3 b
Golden Delicious	9.3 ± 1.3 ab	15.7 ± 1.1 a	19.8 ± 0.9 a	16.8 ± 1.4 a	0.1 ± 1.2 b	11.9 ± 1.3 ab	13.6 ± 1.3 ab	13.6 ± 1.3 ab
Significance	**	***	***	***	***	**	**	***
Male	*	***	***	***	***	*	**	*
Female	***	**	***	***	***	***	NS	NS
Male × female	***	***	***	***	NS	NS	NS	***

<sup>z</sup>Crabapple pollen was from 'Ralph Shay' (2013) and *Malus floribunda* (2014).

<sup>y</sup>Statistical differences among male genotypes tested within column.

<sup>x</sup>NS, \*, \*\*, \*\*\* Non-significant; and significant at  $P = 0.05, 0.01, 0.001$ , respectively.

stigmas was the result of both male and female genotypes and their interaction (Table 2).

Data for the length of the longest pollen tube within the style generally followed the pattern observed for pollen tube growth and pollen tube penetration through the stigma (Table 3). Pollen tube growth increased with time after pollination regardless of male or female genotypes or year. On most sample dates, ‘Ralph Shay’ and *M. floribunda* pollen tubes were shorter than those of both ‘Delicious’ and ‘Golden Delicious’ when applied to ‘Honeycrisp’ flowers in both years and were also shorter in ‘Gala’ in 2013. ‘Delicious’ produced the longest pollen tubes at 72 and 96 h and ‘Ralph Shay’ at 24 and 48 h when applied to ‘Fuji’ flowers in 2014, but in 2013, all male genotypes performed equally with ‘Fuji’ except that ‘Delicious’ was lower

at 24 h and ‘Ralph Shay’ at 24 and 72 h. In ‘Gala’ flowers in 2014, *Malus floribunda* had longer pollen tubes than ‘Delicious’ and ‘Golden Delicious’ at 24 and 96 h, but all pollen sources performed equally at 72 h. As with the number of pollen tubes penetrating the stigma, the length of pollen tubes depended on male and female genotypes and their interaction (Table 3).

The number of pollen tubes that reached the base of the style increased linearly with time after pollination (Table 4). Very few ‘Ralph Shay’ and no *M. floribunda* pollen tubes reached the base of ‘Honeycrisp’ styles (Table 4). In 2013, ‘Golden Delicious’ had more pollen tubes reaching the base of the style than ‘Delicious’ in ‘Honeycrisp’ and ‘Gala’ at 48 h and in ‘Fuji’ at 72 h. However, in 2014, ‘Delicious’ pollen demonstrated superior pollen tube growth in both

‘Honeycrisp’ and ‘Fuji’ at 96 h, while *Malus floribunda* was greatest on ‘Gala’. The number of pollen tubes that reached the base of styles depended on pollen source, cultivar and their interaction. Generally, pollen tube migration through the style took about four DAP to reach the base of the style with exception in 2013; ‘Golden Delicious’ pollen tubes reached the base of the style in ‘Honeycrisp’ and ‘Gala’ in 48 h, and ‘Delicious’ pollen tubes took 72 h in all cultivars. This implies the time period required for fruit set under these conditions.

Fruit set was counted every two weeks. Generally, fruit abscission occurred within two weeks after pollination. Fruit set was lower in ‘Honeycrisp’ pollinated by crabapple regardless of the particular crabapple species used (‘Ralph Shay’ in 2013 and *Malus floribunda* in 2014, Table 5). In

Table 3. Effects of pollen source on pollen tube growth down the style of ‘Honeycrisp’, ‘Fuji’, and ‘Gala’ after hand pollination in 2013 and 2014. Mean values ± SD of 25 observations are reported in each cell.

Male	Avg length of the longest pollen tube (mm) 2013				Avg length of the longest pollen tube (mm) 2014			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
	Honeycrisp							
Crabapple <sup>z</sup>	2.9 ± 0.4 b <sup>y</sup>	7.5 ± 1.0 b	1.1 ± 1.0 b	9.5 ± 0.7 b	1.0 ± 0.2 a	1.5 ± 0.5 b	3.1 ± 0.6 b	4.9 ± 0.7 b
Delicious	7.2 ± 0.4 a	8.2 ± 1.0 ab	9.7 ± 1.0 a	10.8 ± 0.7 ab	0.1 ± 0.2 b	3.2 ± 0.5 a	4.0 ± 0.6 a	10.6 ± 0.7 a
Golden Delicious	5.8 ± 0.4 a	11.3 ± 1.0 a	11.1 ± 1.0 a	13.2 ± 0.8 a	0.0 ± 0.2 b	3.5 ± 0.5 a	5.1 ± 0.6 a	9.7 ± 0.7 a
Significance <sup>x</sup>	***	**	***	***	***	**	*	***
	Fuji							
Crabapple	1.8 ± 0.6 b	3.8 ± 0.8 a	4.1 ± 0.8 b	7.0 ± 1.0 a	2.6 ± 0.3 a	7.9 ± 0.6 a	9.5 ± 0.8 ab	7.4 ± 0.9 b
Delicious	1.1 ± 0.7 b	5.0 ± 0.9 a	10.0 ± 0.8 a	4.3 ± 1.0 a	0.5 ± 0.3 b	5.3 ± 0.6 b	10.2 ± 0.8 a	10.7 ± 0.9 a
Golden Delicious	3.9 ± 0.5 a	5.6 ± 0.8 a	8.0 ± 0.8 a	5.7 ± 1.1 a	0.0 ± 0.3 b	7.0 ± 0.6 ab	7.0 ± 0.8 b	5.3 ± 0.9 b
Significance	***	NS	***	NS	***	**	**	***
	Gala							
Crabapple	4.7 ± 0.5 a	4.2 ± 0.5 b	9.7 ± 0.6 b	6.1 ± 0.9 b	2.7 ± 0.3 a	7.1 ± 0.6 a	10.3 ± 0.7 a	11.9 ± 0.8 a
Delicious	2.9 ± 0.5 b	4.9 ± 0.5 b	11.5 ± 0.6 b	10.6 ± 0.8 a	0.5 ± 0.3 b	4.0 ± 0.6 b	9.8 ± 0.7 a	9.9 ± 0.8 ab
Golden Delicious	3.2 ± 0.5 ab	10.3 ± 0.5 a	14.1 ± 0.7 a	11.3 ± 0.9 a	0.1 ± 0.3 b	6.6 ± 0.6 a	10.0 ± 0.7 a	8.8 ± 0.8 b
Significance	**	***	***	***	***	**	NS	**
Male	*	***	***	**	***	**	NS	***
Female	***	***	***	***	**	***	***	**
Male × female	***	NS	***	***	**	***	**	***

<sup>z</sup>Crabapple pollen was from ‘Ralph Shay’ (2013) and *Malus floribunda* (2014).

<sup>y</sup>Statistical differences among male genotypes tested within column.

<sup>x</sup>NS, \*, \*\*, \*\*\* Non-significant; and significant at  $P = 0.05, 0.01, 0.001$ , respectively.

Table 4. Effects of pollen source on pollen tubes that reached the base of the style of ‘Honeycrisp’, ‘Fuji’, and ‘Gala’ after hand pollination in 2013 and 2014. Mean values ± SD of 25 observations are reported in each cell.

Male	Number of pollen tubes that reached the base of style (2013)				Number of pollen tubes that reached the base of style (2014)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
	Honeycrisp							
Crabapple <sup>z</sup>	0.0 ± 0.0 a <sup>y</sup>	0.0 ± 0.0 b	0.0 ± 0.0 b	1.3 ± 0.9 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 c
Delicious	0.0 ± 0.0 a	0.7 ± 0.6 b	2.0 ± 0.4 a	4.5 ± 0.9 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.1 b	3.6 ± 0.4 a
Golden Delicious	0.0 ± 0.0 a	4.0 ± 0.6 a	3.2 ± 0.4 a	6.9 ± 0.9 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.2 ± 0.1 a	1.4 ± 0.4 b
Significance <sup>x</sup>	NS	***	***	***	NS	NS	**	***
	Fuji							
Crabapple	0.1 ± 0.1 a	0.0 ± 0.0 a	0.5 ± 1.2 b	2.6 ± 0.4 a	0.0 ± 0.0 a	0.3 ± 0.1 a	2.4 ± 0.6 a	2.3 ± 0.7 b
Delicious	0.0 ± 0.0 a	0.2 ± 0.2 a	4.4 ± 1.2 b	0.4 ± 0.4 b	0.0 ± 0.0 a	0.0 ± 0.1 a	1.6 ± 0.6 a	5.5 ± 0.7 a
Golden Delicious	0.2 ± 0.1 a	0.5 ± 0.2 a	10.0 ± 1.2 a	0.2 ± 0.5 b	0.0 ± 0.0 a	0.0 ± 0.0 a	3.2 ± 0.6 a	1.7 ± 0.7 b
Significance	NS	NS	***	***	NS	NS	NS	***
	Gala							
Crabapple	0.3 ± 0.1 a	0.0 ± 0.0 b	1.4 ± 0.9 b	1.4 ± 1.1 b	0.0 ± 0.0 a	0.2 ± 0.1 a	5.5 ± 0.8 a	5.4 ± 0.7 a
Delicious	0.0 ± 0.0 a	0.0 ± 0.0 b	7.5 ± 0.9 a	7.1 ± 1.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	1.6 ± 0.8 b	2.7 ± 0.7 b
Golden Delicious	0.0 ± 0.0 a	4.3 ± 0.6 a	8.2 ± 0.9 a	6.0 ± 1.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	3.1 ± 0.8 ab	3.6 ± 0.7 ab
Significance	NS	***	***	***	NS	NS	***	**
Male	NS	***	***	***	NS	*	**	**
Female	NS	**	***	***	NS	NS	***	***
Male × female	NS	***	**	***	NS	NS	**	***

<sup>z</sup>Crabapple pollen was from ‘Ralph Shay’ (2013) and *Malus floribunda* (2014).

<sup>y</sup>Statistical differences among male genotypes tested within column.

<sup>x</sup>NS, \*, \*\*, \*\*\* Non-significant; and significant at  $P = 0.05, 0.01, 0.001$ , respectively.

2013, ‘Delicious’ pollen resulted in the highest fruit set followed by ‘Golden Delicious’ and crabapple in all cultivars, but in 2014, all pollinizers performed similarly with the exception of *Malus floribunda* crabapple on ‘Honeycrisp’ which resulted in very low fruit set (Table 5).

### Discussion

Although pollen germination on the stigmatic surface and pollen tube growth within the style are both important, the most critical parameter for pollination is the number of pollen tubes reaching the base of the style and fertilizing the egg cell. Temperature (Petropoulou and Alston 1998; Yoder et al., 2009) and pollinizer selection (Albuquerque Jr. et al., 2010) have profound influences on both pollination and fertilization. Higher number of anthers per flower, number of pollen grains per anther, and pollen germination ability (Albuquerque Jr. et al., 2010) are important elements. However, with flowers that were hand-pollinated with excess amount of pollen, we found that pollen source had a significant influence on pollen germination on the stigmatic surface, as well as the growth down the style. For example, germinated ‘Ralph Shay’ and *M. floribunda* pollen covered a lower proportion of ‘Honeycrisp’ stigmatic surfaces compared with ‘Delicious’ and ‘Golden Delicious’ in both years. Conversely, germinated crabapple pollen covered a higher proportion of ‘Fuji’ stigmatic surfaces in 2013 and ‘Gala’ stigmas in 2014 (Table 1). The visualized pollen that germinated on the stigmatic surface of each cultivar was significantly different for each individual pollen source. For instance, ‘Golden Delicious’ pollen germination was highest on ‘Honeycrisp’ in 2013 but was not different in 2014.

It has long been known that pollen tube growth down the style depends on temperature. Yoder et al. (2009) reported that pollen tube growth down the style takes 24–96 h, depending on the temperature. Namikawa (1923) reported 48 h were required for pollen tubes to grow down the entire style and fertilize the egg, whereas Williams et al., (1970) reported 5–7 d. Our results revealed that it can take as short as 48 h for pollen tubes to reach the base of ‘Honeycrisp’ and ‘Gala’ styles and 72 h for ‘Fuji’ styles (Fig. 3). Air temperature during the pollination period and days of samples collection after pollination was generally similar between 2013 and 2014 (Fig. 1); therefore, these are mostly the results of pollinizer compatibility to the cultivar. The reduction in number of pollen tubes that reached the base of the style of ‘Fuji’ and ‘Gala’ at 92 h (Fig. 3) is presumably due to tissues vanishing under the microscope as they aged.

Several studies reported that semi- and cross-incompatibilities have become more prevalent as more interrelated cultivars are grown. Such results could become critical considerations when planning orchards in the future. For instance, Alston (1996) showed

that even though ‘Cox’s Orange Pippin’ and ‘Idared’ are completely cross-compatible, a much lower percentage of fruit set was recorded after hybridization between their progenies. The authors concluded that allele sharing a common ancestor led to semi-incompatibility. In our studies, ‘Ralph Shay’ and *M. floribunda* pollen tubes had the lowest germination and slowest growth in ‘Honeycrisp’ stigmas and styles, and comparatively, fewer pollen tubes reached the base of the style. This may have been a result of semi-incompatibility of ‘Ralph Shay’ and ‘*Malus floribunda*’ crabapples with ‘Honeycrisp’, which resulted in lower fruit set (Table 5). ‘Honeycrisp’ fruit also had lower seed numbers in 2013 when pollinated with ‘Ralph Shay’ compared with other pollen sources (Jahed and Hirst, 2017). Delaplane et al. (2000) reported that closely related apple cultivars, (for example, ‘McIntosh’, ‘Early McIntosh’, ‘Cortland’, and ‘Macoun’) do not cross-pollinate with each other well. Our results did not support this statement, however. We found good pollen tube growth rates of ‘Delicious’ (grandparent) and ‘Golden Delicious’ (parent) in ‘Gala’ styles in both 2013 and 2014. Therefore, the explanation for the semi-incompatibility observed in our studies may be more complex than simple degree of relatedness.

Bessho et al. (2009) evaluated crabapples for their suitability as pollinizers for two commercial cultivars in terms of bloom time, pollen compatibility, seed number, and productivity. They found that 5 out of 19 crabapple cultivars performed well as pollinizers for the Fuji and Tsugaru cultivars. Meanwhile, Das et al. (2011) reported that ‘Manchurian’ crabapple was found to be an effective pollinizer for ‘Oregon Spur Delicious’ apple in terms of fruit set. Our results were generally consistent with these findings. For example, fruit set of ‘Fuji’ was comparatively low in crosses with ‘Ralph Shay’ crabapple in 2013, and relatively high when pollinated by *M. floribunda* in 2014. Delaplane et al. (2000) reported crabapple as an effective pollinizer for a commercial orchard. However, our results showed that ‘Ralph Shay’ and *Malus floribunda* crabapples were not suitable pollinizers because their pollen tubes grew the slowest in ‘Honeycrisp’ styles and fewer pollen tubes reached the base of the styles. The result was that ‘Honeycrisp’ showed low fruit set when pollinated by both crabapples tested here.

### Conclusion

Pollen source had a significant influence on pollen germination on the stigmatic surface, pollen tube growth down the style, and the proportion of pollen tubes that grew to the base of the style. All these aspects of pollen germination and pollen tube growth increased with time after pollination under field conditions. The number of pollen tubes that grew to the base of the style was affected by pollen donor and was varied year-to-year. For ‘Honeycrisp’, as short as 48 h were required

Table 5. Fruit set (%) at harvest time of ‘Honeycrisp’, ‘Fuji’, and ‘Gala’ after hand-pollination with crabapple, ‘Delicious’ or ‘Golden Delicious’ pollen.

Female	Male		
	Crabapple <sup>z</sup>	Golden Delicious	Delicious
		2013	
Honeycrisp	33.33	60.00	66.67
Fuji	33.33	50.00	66.67
Gala	50.00	63.33	86.67
		2014	
Honeycrisp	6.67	53.33	56.67
Fuji	56.67	63.33	80.00
Gala	26.67	83.33	76.67

<sup>z</sup>‘Ralph Shay’ crabapple was used in 2013 and *Malus floribunda* in 2014.

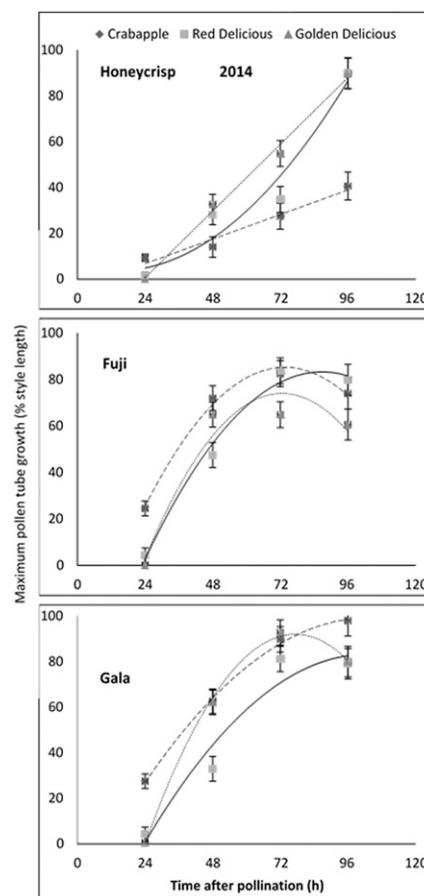


Fig. 3. Effect of pollen source on pollen tube growth of ‘Honeycrisp’, ‘Fuji’, and ‘Gala’ apple in 2014. In ‘Honeycrisp’ styles, *Malus floribunda* pollen tubes grew at  $y = 0.4471x - 4$ ,  $R^2 = 0.9632^{**}$ ; ‘Delicious’,  $y = 0.0123x^2 - 0.3442x + 6$ ,  $R^2 = 0.94^{**}$ ; ‘Golden Delicious’,  $y = 1.2138x - 28.55$ ,  $R^2 = 0.99^{**}$ . In ‘Fuji’ styles, *Malus floribunda* pollen tubes grew at  $y = -0.024x^2 + 3.579x - 46.5$ ,  $R^2 = 0.99^{**}$ ; ‘Delicious’,  $y = -0.02x^2 + 3.549x - 70.77$ ,  $R^2 = 0.98^{**}$ ; ‘Golden Delicious’,  $y = -0.03x^2 + 4.3617x - 84.35$ ,  $R^2 = 0.939^{**}$ . In ‘Gala’ styles, *Malus floribunda* pollen tubes grew at  $y = -0.0115x^2 + 2.3717x - 23.45$ ,  $R^2 = 0.99^{**}$ ; ‘Delicious’,  $y = -0.0134x^2 + 2.7417x - 57.4$ ,  $R^2 = 0.94^{*}$ ; ‘Golden Delicious’,  $y = -0.0325x^2 + 5.02x - 101.82$ ,  $R^2 = 0.99^{**}$ .

for ‘Golden Delicious’ pollen tubes and as long as 96 h were required for ‘Delicious’ and ‘Ralph Shay’ pollen tubes to reach the base of the style, but in ‘Fuji’ and ‘Gala’, a maximum of 72 h were required regardless of pollen source. Two crabapples, ‘Ralph Shay’ and *Malus floribunda*, were ineffective pollinizers for ‘Honeycrisp’ apple resulting in low fruit set. On average, ‘Delicious’ pollen resulted in the highest fruit set, followed by ‘Golden Delicious’ and crabapple. These results highlight the importance of male–female interactions in apple, and understanding such interactions has obvious implications for orchard design.

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