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# Pollen Tube Growth in Apple: A Review

Thomas M. Kon[1] and James R. Schupp

**Abstract**

Certain aspects of apple pollination biology have received significant attention in the literature due to direct impacts on orchard productivity, such as incompatibility and floral longevity. Until recently, studies of the biochemical and genetic mechanisms of pollen tube growth were limited to model plant species, such as *Arapidopsis*, *Lilium*, and *Nicotania*. This review is focused on recent discoveries in pollen tube growth of apple.

**Additional index words:** stigma receptivity, pollination, flower structure, self-incompatibility, *Malus xdomestica*

While general reviews regarding apple pollination (Jackson, 2003; Ramírez and Davenport, 2013) and floral morphology and anatomy (Pratt, 1988) are available, an apple-specific review with emphasis on pollen tube growth is justified, based on recent discoveries. As one of the most intensively studied cellular systems, there have been numerous advances in our understanding of pollen tube growth in model plant species (Cheung and Wu; 2008; Franklin-Tong, 1999; Krichevsky et al., 2007; Taylor and Hepler, 1997). Recently, several papers specific to the reproductive biology of apple have been contributed, which corresponds with the publication of the genomic sequence of apple (Velasco et al., 2010).

*Pollen tube growth on the stigmatic surface.* As pollen germinates on the stigmatic surface, it initiates the fastest plant cell growth that is currently known (Taylor and Helper, 1997). Pollen growth is strictly tip based, much like that of fungal hyphae or root hairs. A steep calcium gradient in the pollen tube tip has been observed (Gu et al., 2015). TGase, a calcium dependent protein cross-linking enzyme, was shown to play a role in tip growth and cell wall building of apple pollen tubes (Di Sandro et al., 2010). TGase inhibitors blocked apple pollen tube growth, while incorporation of fluorescent mammalian TGase increased pollen tube length and germination. As pollen tubes grow, the cytoplasm is physically separated from the rest of the pollen tube by callose plugs. The area behind the callose plug is often vacuolated, while the cytoplasm is located near the apex of the pollen tube. Vacuolation may be a mechanism to maintain turgor pressure (Beauzamy et al., 2014), or pH at the pollen tube tip. While turgor pressure is likely involved in pollen tube growth, it is unclear if it is the driving force in the rapid growth of pollen tubes (Beauzamy et al., 2014). Pollen tubes have oscillatory growth patterns, and cytoplasmic streaming has been observed in the pollen tube apex (Cheung and Wu, 2008).

Major tree fruit species have a wet-type stigma that is comprised of globular papillae (Helsop-Harrison and Shivanna, 1977). Prior to anthesis, these cells are turgid. Papillae start to lose turgidity at the late balloon stage and begin to collapse at full bloom (Losada and Herrero, 2012). As papillae collapse, the cellular contents are emitted and a secretion occupies intercellular spaces. The stigmatic secretion is primarily composed of polysaccharides (49.6%) and proteins (45.9%). These constituents are likely present as glycoproteins (Pusey et al., 2008). Losada and

[1] Corresponding author: Department of Plant Science, Fruit Research and Extension Center, Pennsylvania State University, Biglerville, PA 17307

1st Place recipient – U.P. Hedrick Award

Herrero (2012) characterized stigmatic receptivity at different phenological stages, ranging from tight cluster to anthesis. Maximal receptivity was observed at anthesis (Losada and Herrero, 2012; Sheffield et al., 2005). Stigmatic receptivity accompanied an increase in arabinogalactan-protein levels in the stigmatic secretion, which may play a role in cell wall building and orientation of pollen tubes (Losada and Herrero, 2012).

In pomology textbooks, the stigmatic surface is considered to be a distinctive structure that is located at the terminus of the style. Martin and Yocum (1918) made the following observation, "The style is grooved just below the stigma, the groove being almost a millimeter in length." Furthermore, the presence of a stylar groove was observed on multiple apple and pear cultivars, and this structure originates at the stigma and terminates proximal to the nectar cup (see Fig. 1; Spinelli et al., 2005). The stylar groove is a direct continuation of the stigmatic surface, and is comprised of functional papillae. While pollen can germinate in the stylar groove, several layers of parenchymatous cells and vascular tissue act as a barrier to the inner style, thereby preventing pollen tube penetration (Cresti et al., 1980). *Erwina amylovora* readily colonized the stylar groove in laboratory conditions (Spinelli et al., 2005). The secretion from the paplillate tissue provides an excellent medium to support microbial populations (Pusey et al., 2008), including *E. amylovora* and fire blight antagonists such as *Pantoea agglomerans*.

*Pollen tube growth in the style.* The inner style, referred to as transmitting tissue, is comprised of cells that have abundant cytoplasm, are rich in organelles, have large Golgi vesicles, and act as a conduit between the stigma and the ovary (Cresti et al., 1980). The transmitting tissue of the style is wide near the stigmatic surface, and begins to taper near the base of the style. Secretions of these cells are thought to aid in pollen tube growth by providing proteins and polysaccharides (Pratt, 1988). Coupled with reduced

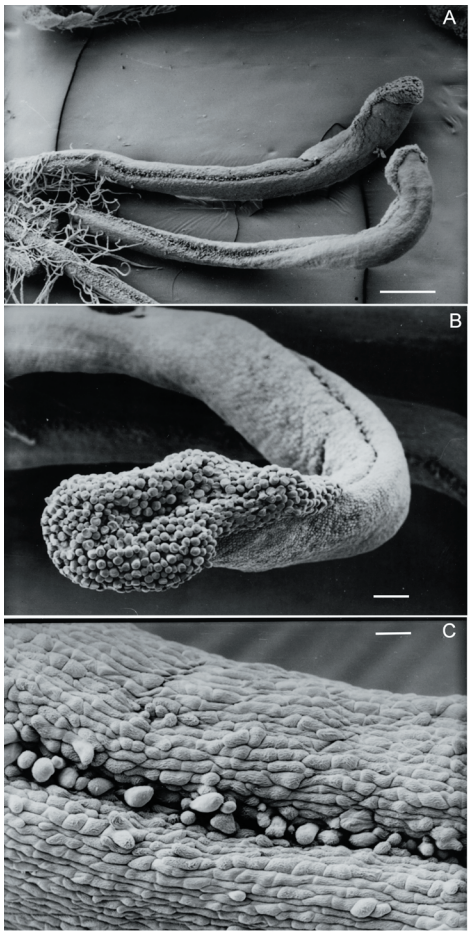


**Fig. 1.** Morphology of the apple pistil investigated by SEM. (a) Micrograph of pistils (cv. Golden Delicious) from a newly opened flower with a stylar groove originating from the stigma and present along the pistil. The groove epidermis is completely occupied by stigmatic papillae. On the left, at the base of the style, surrounded by multicellular trichomes, the number of stigmatic papillae is higher and the groove is wider than at the distal part of the stigma. Scale bar = 500 µm. (b) Close-up of the apple stigma. The stigma is bilobe and covered by turgid, columnar papillae. The stylar groove originates directly from the stigma. Scale bar = 100 µm. (c) Part of the stigmatic papillae covering the apple stylar groove. The papillae on the internal and external surfaces of the groove are distinguishable from the epidermal cells by their columnar shape. No morphological differences between the stylar and stigmatic papillae were detected. Scale bar = 25 µm.
*Figure reproduced from Spinelli et al. (2005) with kind permission from Springer Science and Business Media.*

transmitting tissue area, there is a concomitant basipetal reduction in the availability of resources that aid pollen tube growth, particularly extensins and polysaccharides, inducing competition among pollen tubes (Losada and Herrero, 2014). The rate of pollen tube growth is rapid in stylar tissue as compared to that of the stigmatic surface and the ovary. 'Gala' pollen tubes grew 87 $\mu m \cdot h^{-1}$ on the stigmatic surface, while pollen tubes travelled 177 $\mu m \cdot h^{-1}$ in styles of 'Golden Delicious' (Losada and Herrero, 2014). The observed accelerated growth rate was suggested to be the result of an autotrophic growth habit on the stigma compared to a heterotrophic growth habit in the style. Extensins and insoluble polysaccharides in the transmitting tissue were depleted in pollinated blossoms, but these compounds were abundant in unpollinated blossoms. This suggests that pollen tubes utilized the aforementioned compounds as resources (Losada and Herrero, 2014). Pollen tubes reached the base of the style in two waves, but it is unclear if early or late pollen tubes have a higher potential of fertilizing the ovules (Losada and Herrerro, 2014). Child (1966) also observed "leader tubes" that grew <1 mm ahead of the vast majority of pollen tubes in the style.

In a stigma excision experiment, Beaumont (1927) alluded that pollen tubes had the capability to pass from one locule to another since high seed numbers were observed in fruit where multiple stigmas had been removed. Despite this early evidence, apples were thought to be imperfectly syncarpic, meaning that the transmitting tissue of each carpel leads to a corresponding locule (Cresti et al., 1980; Pratt, 1988). Anvari and Stösser (1984), hand pollinated varying numbers of stigma (1-5) and evaluated the effect on fruit set, fruit shape, and seed number. Fruit set and shape was not affected by the number of stigmas that were pollinated, and seed number was only reduced in the event that only one stigma was pollinated. "Basal gaps" in the carpels were attributed to the capability of pollen to enter other locules to fertilize ovules (Anvari and Stösser, 1984). Sheffield et al. (2005) observed perfect syncarpy in 'Summerland McIntosh'. The transmitting tissue of the styles was fused above the ovaries, and pollen tubes from any style could enter any locule. While perfect syncarpy has not been confirmed in additional cultivars, future study may be warranted since fruit shape and quality may be impacted due to gynoecium structure (Sheffield et al., 2005).

Location of planting, cultivar, strain, and year can influence style length (Yoder et al., 2013). Williams (1965) suggested that 5 to 7 d was required for pollen tubes to reach the style base; however, Child (1966) and Yoder et al. (2009) reported that pollen tubes could reach the style base in 1 to 3 days, and growth rate was strongly influenced by temperature. Losada and Herrero (2013b) observed pollen tubes at the base of the style in 3 d, but pollen tubes did not enter the ovule until 6 d after pollination in field conditions. Pollen tube growth rates of several apple cultivars have been determined and modeled (Yoder et al., 2013). Maternal cultivar, temperature, and style length are inputs in the model, and evaluations of paternal cultivar effects on pollen tube growth rate are underway (DeLong et al., 2015).

*Self-incompatibility system*. Self-fertilization of apple is inhibited by a gametophytic self-incompatibility (GSI) system which is controlled by a single multi-allelic locus. Incompatibility of apple has received much attention in the literature due to direct impacts on production and breeding. Ramírez and Davenport (2013) reviewed the GSI system of apple, and Broothaerts et al. (2004) compiled the S-genotypes of 150 cultivars. The gene MdCBL5, was identified as a calcium signaling factor that is involved in the self-incompatibility response (Gu et al., 2015). Additionally, some of the genes (Yuan et al., 2014), proteins (Meng et al., 2014a), and cellular components (Meng et al., 2014b) involved in S-RNase transport have been identified.

*Pollen tube growth in the ovary.* Pollen tube growth in the ovary is poorly under-

stood (Herrero, 1992), though the ovary may have a significant role in the pistil-pollen interaction. Dong et al. (1998) isolated 15 pollination-induced genes from apple ovaries within 48 h of the pollination event. Induced genes were members of two general groups: 1) growth and development and 2) stress/defense responses. GA treatment alone induced five of six stress related genes. In general, pollination and subsequent fertilization of the ovule(s) is a requisite for the formation of apple fruit. While true parthenocarpy has been observed in apple, parthenocarpic (seedless) apple cultivars are rare and are of no commercial importance. Use of $GA_{4+7}$ + 6-benzyladenine induced parthenocarpic fruit set, and can be utilized as a rescue treatment in apple when applied proximal to a spring frost event (McArtney et al., 2014).

Unpollinated blossoms do not increase in diameter or weight, but size differences were observed in pollinated blossoms 6-7 days after bloom (Greene et al., 2013; Losada and Herrero, 2013b). Cell production increased sharply between 8-11 DAFB in pollinated blossoms, while there was no observed cell production in unpollinated blossoms (Malladi and Johnson, 2011). Cell production data corresponded with the expression of cell cycle genes.

Williams (1965) developed the concept of the Effective Pollination Period (EPP) to estimate the longevity of apple blossom receptivity. A review summarized ~40 years of EPP-based experiments of several tree fruit crops, and the potential factors that contribute to the duration of the EPP (Sanzol and Herrero, 2001). Flower position within the apple inflorescence may influence the 1) duration of stigmatic receptivity, and 2) the ability for pollen grains to adhere to the stigmatic surface. Stigmas of 'Golden Delicious' king blossoms were receptive for a shorter period of time when compared to lateral blossoms, however, greater numbers of pollen grains adhered to the stigmas of king blossoms after hand pollination (Losada and Herrerro, 2013a). Differences between king and lateral blossoms may increase the opportunity for pollinator visitation and subsequent reproductive success based on environmental conditions in a given year.

## Summary

Significant progress has been made in the understanding of apple pollen tube growth *in vivo*. Genetic techniques have been used to identify and catalogue the S-locus of many commonly planted apple cultivars and pollinizers, which has utility in breeding efforts. Advances in microscopy, cellular staining, and genetics has enabled researchers to make significant and rapid progress. Current projects are underway to create models that estimate pollen tube growth rates of several commercially important apple varieties, which can be used as a timing aid for blossom thinner applications. Projects are underway to screen crabapples with the goal of finding improved pollinizers. Work should be continued to determine if genetic and biochemical pathways are conserved between model plant species and economically important crops, such as apple. Additional work is needed to elucidate pollen tube growth in the ovary.

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