

## From pollination to fertilization in fruit trees

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### Abstract

The phase that elapses from pollination to fertilization is re-examined giving special attention to pollen pistil interaction in compatible matings. Pollination induces an activation of the pistil. A number of changes take place in the different tissues of this organ that appear to support male gametophyte development and to assist fertilization. Thus pollination induces stigma secretion, the release of starch from the transmitting tissue and prolongs embryo sac viability. It appears that even those pollen grains that do not achieve fertilization have a synergistic role supporting others to do so.

The pistil also has an effect on pollen tube growth. Pollen tube growth along the pistil is not continuous, accelerations and decelerations take place depending on the different tissues they traverse. The fact that pollen tube growth is heterotrophic, at the expenses of the pistil reserves, and that these reserves are not continuously produced confers the pistil with a role controlling pollen tube growth kinetics.

### 1. Introduction

The programic phase, determined as the time that elapses from pollination to fertilization [30], is a period during which there is considerable interaction between male and female tissues [19]. Furthermore, the fact that fertilization is mediated through a pollen tube is emerging as a uniquely adapted system for this interaction [20, 35]. However, we still know very little on the physiological mechanisms that control these interactions.

Much work has been devoted to the study of the pollen grain and to the development of the male gametophyte [27]. Likewise, stigma, style and ovule development have been studied. However, the study of pollen pistil interaction has been mainly orientated to incompatible matings [20]. In compatible crosses it has been stated that the pistil has a chemotropic [41, 34] and trophic role [28, 15] in the control of pollen tube growth, but little other physiological work has been done to study the effect of the pistil on pollen tube development, conversely the effect of actively growing pollen tubes on pistil development has received little attention.

Here the process from pollination to fertilization is reexamined in fruit trees giving special attention to pollen pistil interaction.

### 2. The pistil offers support and controls pollen tube growth

#### 2.1 Pollen germination and pollen tube growth in the style

The stigma is a papillate surface that offers an adequate media for pollen germination; before maturation papillae are turgid cells and as the stigma matures the cells degenerate and a secretion is produced [13, 15]. Pollen tube structure and mode of growth at the stigma is very similar to pollen tube growth *in vitro* [15] since during this phase the pollen tubes grow autotrophically at the expense of the pollen grain reserves [17].

When the pollen tubes enter the style there is an acceleration of growth [16] that is accompanied by a change from autotrophic to heterotrophic metabolism [17]. The style contains abundant starch reserves which disappear as the pollen tubes grow through the stylar tissue [15].

#### 2.2 Pollen tube growth in the ovary region

While much work has been done regarding pollen

germination at the stigma [19] or concerning pollen tube growth in the style [51], there is a paucity of data regarding pollen tube growth in the ovary region [27]. It has been tacitly assumed that, once the pollen tubes enter the ovary, fertilization was a straightforward process. However, working with fruit tree species, we have observed that in the ovary a number of interactive processes are set up from the arrival of the pollen tubes until fertilization occurs [14]. In peach (*Prunus persica*), the time between the arrival of the pollen tubes at the base of the style until fertilization takes place is variable, in some cases it may even exceed the time required by the pollen tubes to grow down the style [13].

When the pollen tubes reach the base of the style and enter the ovary they meet the obturator which is a placental protuberance connecting the style with the ovule micropyle. An histochemical study of this structure [1] reveals that when the pollen tubes arrive at the obturator they stop growing and growth is not resumed until five days later. On the arrival of the pollen tubes the obturator cells are full of starch reserves but five days later starch disappears from these cells and a secretion that stains for carbohydrates and proteins is produced. Concomitant with the production of this secretion growth of the pollen tubes is resumed on the obturator. The fact that pollen tubes appear to require this secretion to grow reinforces the idea that pollen tube growth along the pistil is heterotrophic [13, 15]. However, a major difference exists between growth in the style and on the obturator. While in the transmitting tissue starch digestion is triggered by pollination and only occurs in compatible matings [15], on the obturator this process is independent of pollination and appears to be a maturative stage of the pistil for it takes place in a similar way in pollinated and unpollinated flowers [1].

Once the pollen tubes have passed along the obturator, callose starts to accumulate on this structure [1]. This mechanism confers the obturator a critical role in controlling pollen tube penetration into the ovary since it acts as a bridge either connecting or isolating the ovary to the style. Thus, pollen tube growth is not possible before the secretion phase, neither is it possible later once the obturator degenerates. This mechanism, apart from having a role in pollen tube growth control,

may play a significant part in preventing infection. A different mechanism relating to the control of pollen tube entrance into the ovary has been described in *Zea mays* [21] in which the first pollen tubes entering the ovary induce a loss of turgidity in the cells at the base of the style which prevents other pollen tubes from entering this region.

The presence of an obturator has been reported in a number of unrelated species, but, despite its early description [24], attention has only recently been given to its function. The presence of transfer cells in this region [23] had led to Peterson et al. [39] to hypothesize that such cells could play a role in pollen tube guidance and this hypothesis has been further supported by other workers [44, 46]. It is unclear, however, if a mechanism similar to the one observed in peach [1] operates in these species too, **certainly such a mechanism could regulate pollen tube access to the ovule.**

Once the pollen tubes have traversed the obturator a further delay of three days takes place in peach [13] before the pollen tube enters the micropylar exostome and after a further three days fertilization occurs. Further study is necessary to provide an explanation for these later delays to pollen tube growth. However, the fact that pollen tubes may wander before entering the ovule suggests that, like at the obturator, further maturation of the ovule may be necessary before the pollen tubes can penetrate and affect fertilization. A chemotropic factor in this phase has been suggested in other species [22, 32, 45], since there is an absence of an anatomical guide for entry into the micropyle [12].

It has tacitly been assumed that a chemotropic stimulus may be necessary for pollen tube directional growth [50] and further work has demonstrated an effect of the pistil on pollen tube directionality [37]. However, directional pollen tube growth, along the transmitting tissue and the obturator, could be determined by following the path of available reserves [13]. The chemotropic effect might therefore be only necessary for the pollen tubes to penetrate the ovule [32].

### 2.3 Pollen tube kinetics

The rate of pollen tube growth through the pistil is not constant. In peach these rate variations are produced by differences induced by the pistil struc-

tures through which the pollen tubes have to grow. An acceleration of growth has been previously recorded when the pollen tubes enter the transmitting tissue from the stigma surface, and this acceleration has been associated with a change from autotrophic to heterotrophic metabolism [2, 16, 36]. A deceleration has been recorded when the pollen tubes enter the ovary region. Pollen tubes stop before they traverse the obturator and their growth again stops before entering the micropyle. The stop at the obturator can be explained by a lack of resources to nurture the pollen tubes when they reach this structure, since once these cells produce their characteristic secretion, pollen tube growth resumes [1]. However, more attention needs to be given to the next stop the tube exhibits before entering the micropyle.

In peach a lag phase occurs between the arrival of the pollen tubes at the base of the style and fertilization. While the first pollen tubes reach the base of the style 7 days after pollination, the first fertilized ovules cannot be seen until 19 days after pollination. Similarly a long delay between pollination and fertilization has been reported in gymnosperms and also in some angiosperms [54]. It would be worth investigating if a situation, like the one described here for peach, could be extended to other species, since some changes might have to take place in the pistil before fertilization can occur.

Since the first detailed study concerning the effect of temperature on pollen tube growth [5], much attention has been paid to this factor in fruit trees [31, 53]. While it is clear that temperature does have an effect on pollen tube growth kinetics, the effect of the pistil on pollen tube growth cannot be overlooked, since it plays a major role controlling pollen tube growth kinetics. The pistil, far from being a passive structure that supports pollen tube growth, plays an active role controlling growth [13]. At anthesis, maturation of the pistil in peach is not complete, maturation occurs during the flower life in a basipetal way, starting at the stigma and proceeding along the style down to the ovule. Maturation implies a number of secretory processes, along the pistilar tract, specially suited for opening the way to the passage of pollen tube. This secretion is followed by cell degeneration which confers a further role to the pistil: that of closing the access down, once the pollen tubes have passed

through. This process, apart from regulating pollen tube passage, may be involved in preventing infection.

### 3. Pollination affects the pistil prior to fertilization

#### 3.1 Pollination activates the pistil

On pollination, a 'wave' of cytoplasmic and biochemical activity precedes the pollen tubes along the length of the pistil, and is capable of inducing activities in various tissues of this organ. This is evidenced by an increase in polysome number [15], RNA changes [8], and alterations in levels of protein synthesis [6]. Furthermore, changes in sugar levels [47], starch [15] and the rate of respiration [29] are also induced by pollination.

Likewise this biochemical activation has a reflection in a number of changes that appear devised to promote the development of the male gametophyte and to assist fertilization. In some species the stigma releases a post-pollination exudate [26, 43, 49] that promotes pollen germination. In others, pollination stimulates the transmitting tissue to release carbohydrates which are later used to support pollen tube growth [48, 15]. Furthermore, it would seem that even those pollen grains destined not to fertilize ovules play an important part in assisting other pollen grains to do so. For example, a population effect at germination has been recorded for a large number of pollens [4], where large accumulations of grains have a synergistic effect on germination, and, following mixed pollinations, incompatible tubes can prime the stigma and style to permit the growth of a small number of compatible tubes [16]. Pollination is also clearly capable of stimulating development within the ovary, preparing it for fertilization [10, 38, 18].

In relation to this, an effect of the pollen tubes on the ovule has been recorded in pear (*Pyrus communis*) [18]. Pollen tubes, while not altering embryo sac maturation, delay embryo sac degeneration. Thus, in a cross-pollinated flower, this degeneration is postponed by about ten days, extending the period over which a successful fertilization can take place. This extension of embryo sac viability is accompanied by an elongation of the embryo sac itself. These two phenomena are initiated two weeks before fertilization takes place.

This must result in a dramatic extension of the period at which the ovule is receptive to compatible pollen, and thus increase the effective pollination period [52] and, in turn, the chances that fertilization will take place.

### 3.2 *Could the pollination stimulus be hormonally mediated?*

From the earliest investigations [9] it was clear that ovary growth and development could be stimulated by pollination and application of pollen extracts. One of the essential ingredients of these pollen extracts was identified by Gustafson [11] as auxin and, subsequently, synthetic auxins have been shown to have similar effects. In pears treating unpollinated flowers with gibberellic acid ( $GA_3$ ) induces similar changes to pollination [18]. Moreover, the reverse effect is produced when treating pear flowers with the gibberellin antagonist, paclobutrazol (PP333) [7]. It is tempting to put forward that active  $GA_3$  secreted by the pollen tubes may move towards the ovule since pollen is known to be a rich source of  $GA_3$  [3] and, interestingly, the period of rapid pollen tube growth is known to coincide with a conversion of a number of gibberellic acids into the more active polar forms, mainly  $GA_3$  [25]. It thus seems quite possible that active  $GA_3$ , secreted by the elongating pollen tubes, may be translocated to the ovule.

Alternatively the observed effect on embryo sac life might not be an exclusive effect of  $GA_3$ , but rather a consequence of a general activation of the pistil. For example Sedgley and Buttrose [42] have recorded a 24 hour increase in embryo sac life in watermelon after treatment with the synthetic auxin naphthalene-1-acetic acid. The situation is further complicated by the fact that activation of the ovary tissue appears to be one of the first manifestation of fruiting, whether it is induced by pollination or parthenocarpy [33]. In this context, prolonged embryo sac viability might only reflect some aspects of this activation in that a generally heightened metabolic state of the ovule could delay embryo sac degeneration. A similar model has been put forward by Williams [52] to explain an extended period of meristematic activity at the nucellus which leads to prolonged embryo sac life in nitrogen fertilized apple flowers.

Whether extension of embryo sac viability is mediated by  $GA_3$ , or is a response to a general activation of the ovary, it does extend the period in which the ovule is receptive to the pollen tube, thus increasing the chances that fertilization will take place.

## 4. Conclusion

The evidence discussed here suggests that control mechanisms are present in the pistil that regulate male-female interaction. While some of these mechanisms appear devised to assist fertilization, others appear as barriers, or difficulties, the gametophytes have to overcome. Furthermore, since pollen tubes depend on pistil secretions for nutrients, together with the fact that stylar secretion is sequentially produced, suggests that synchronous pollen tube growth with pistil maturation is required for successful fertilization [13].

Some of these events, for example the stigma release of a post pollination exudate [26, 43] that promotes germination, or the transmitting tissue release of carbohydrates which are later used to support pollen tube growth [15], or the prolongation of embryo sac viability, are induced by pollination [18]. Other events however, appear independent of pollination, for example the obturator secretion that opens the passage to the ovule [1].

Those events induced by pollination require only that pollen arrives at the stigma and therefore even those pollen grains destined not to fertilize ovules play an important part in assisting other pollen grains to do so. As suggested by Herrero and Dickinson [16] such mechanisms serve to underline the economy and degree of adaptation of the angiosperm reproductive process.

In conclusion evidence is emerging on how the pollen and the pistil interact and affect each other. A number of mechanisms appear devised to regulate such interaction. A complete knowledge of these mechanisms and their significance is needed to fully understand the fertilization process.

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