



# Investigating the impact of fungicide application on pollen germination and tube growth



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## Background

Fungicide application to prevent infection is an integral part of best practice in almond cultivation. However, we lack robust information on how fungicide application, specifically:

- + pollen viability
- + stigma receptivity
- + pollen tube growth

We will test flowers exposed in the field to gain a more accurate reflection of how real field-based exposure levels impact flower fertilization. We do not know how the timing of the fungicide application relative to pollen deposition and flower stage at application may affect the impacts of the fungicide on fertilization.



## Aims

- Quantify whether fungicide exposure in the field affects the fertilization of almond flowers through impacts on pollen, stigmas or both.
- Test whether flowers still in bud during fungicide application are affected in the same way as open flowers.
- Investigate if the timing of pollen deposition in relation to application of the fungicide affects the impact of fungicide on the fertilization of almond flowers.

## Methods

The project will be conducted at the UC Davis Plant Pathology experimental orchards, where fungicide efficacy trials are ongoing.

### Part 1.)

#### Impacts of fungicide application on pollen and stigmas in flowers exposed when open and as buds.

Shortly before application we will place fungicide exclusion bags around branches of two almond varieties in the orchard to allow us access to control and exposed flowers. Hand crosses will be performed in the field on open flowers (Table 1) using a consistent amount of pollen. The flowers will be left for 3 days and then removed and processed in the laboratory (Fig. 1). The same crosses (Table 1) will be conducted but using flowers that were in the bud stage during application and have subsequently opened. Again, the flowers will be left for 3 days and then processed in the laboratory (Fig. 1).

**Table 1.** Hand pollination treatments using flower exposed to fungicide and bagged unexposed flowers

	Unexposed variety a pollen	Fungicide exposed variety a pollen	Unexposed variety b pollen	Fungicide exposed variety b pollen
Unexposed variety b stigma	<i>hand pollination (control)</i>	<i>hand pollination (pollen)</i>		
Fungicide exposed variety b stigma	<i>hand pollination (stigma)</i>	<i>hand pollination (pollen &amp; stigma)</i>		
Unexposed variety a stigma			<i>hand pollination (control)</i>	<i>hand pollination (pollen)</i>
Fungicide exposed variety a stigma			<i>hand pollination (stigma)</i>	<i>hand pollination (pollen &amp; stigma)</i>

### Part 2.)

#### Timing of the application in relation to pollination.

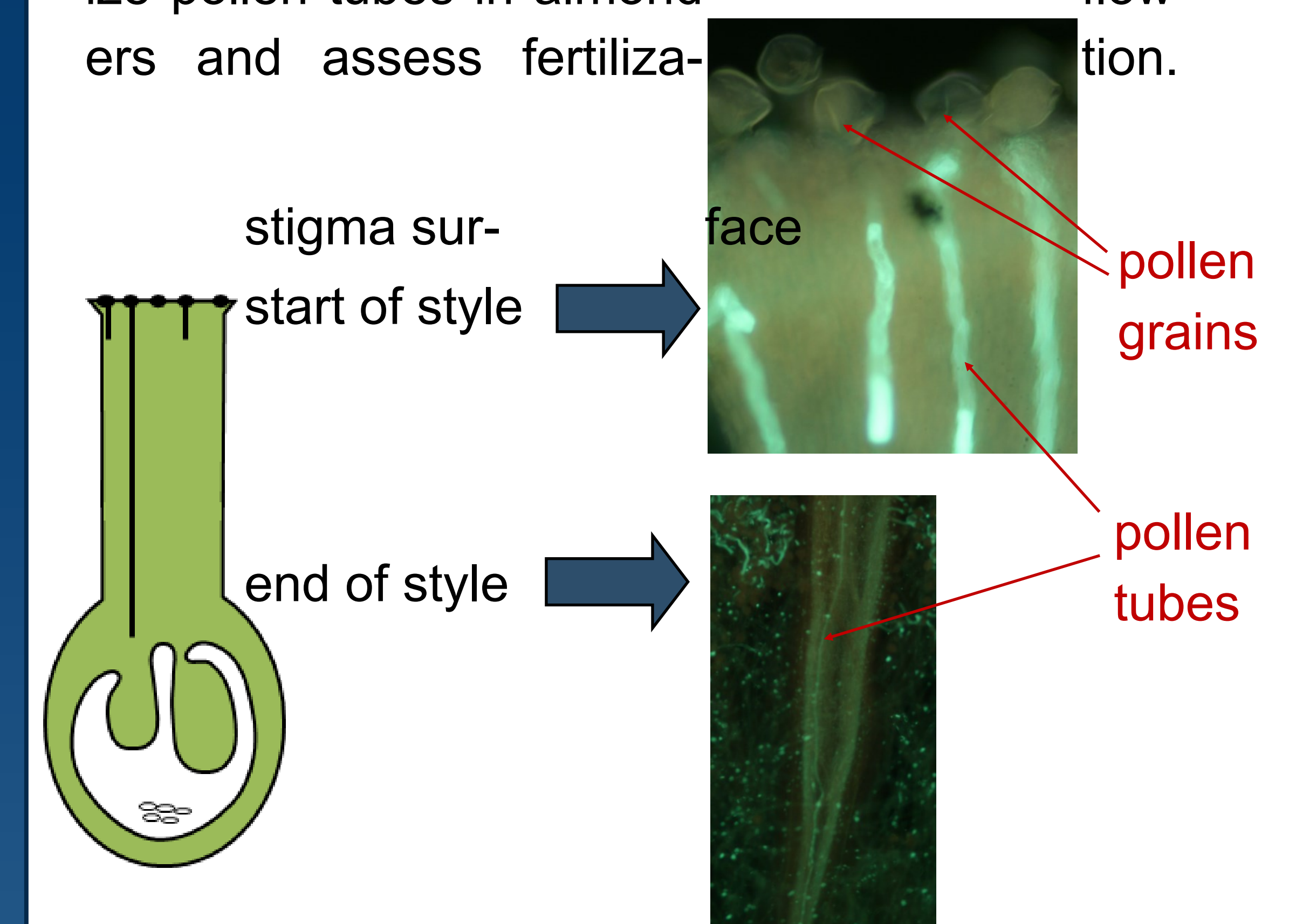
One group of flowers will be hand cross pollinated in the field approximately 1 day before fungicide application. A second group will be hand pollinated in the field just before fungicide application. Following the fungicide application the two groups of flowers will be left in the field for 2 and 3 days respectively and then the flowers will be removed and processed in the laboratory (Fig. 1).

### Part 3.)

#### Visualizing pollen grain numbers and pollen tube growth

To view pollen germination and pollen tube growth the pistils will be softened and then soaked in aniline blue dye. A fluorescent microscope will be used to (Fig. 1) count the number of pollen grains on the stigma, the number of pollen tubes initiating growth (pollen germination) and the number of pollen tubes reaching the base of the style (indicator of fertilization).

**Figure 1.** Using fluorescent microscopy to visualize pollen tubes in almond flowers and assess fertilization.



## Outputs

- We will be able to analyze the impact of a fungicide application at actual exposure levels on pollen germination and pollen tube growth.
- We will be able to do this for flowers that were open during fungicide application, and flowers that were still in bud.