# The Effects of Fungicide Application on Pollen Germination and Tube Growth

**Project No.:** **13-POLL3-Williams**

**Project Leader:** Neal Williams
Associate Professor of Entomology
Department of Entomology and Nematology
UC Davis
One Shields Avenue
Davis, CA 95616-8584
530.752.9358
nmwilliams@ucdavis.edu

**Project Cooperators and Personnel:**
Dr. Claire Brittain, Postdoctoral Research Scholar, and Dr. Eric Mussen, Extension Apiculturist, Department of Entomology, UC Davis

## Objectives:

The objective of the study was to help optimize the timing of fungicide application during almond bloom to minimize potential negative impacts on post-pollination stages of fertilization. Specifically we wanted to investigate how coarse differences in the timing of spray affect pollen viability, stigma receptivity and pollen tube growth in almond. We proposed to test flowers exposed in the field to gain a more accurate reflection of real field-based exposure levels. We tested for differences in the impact of fungicide in flowers that were open when the fungicide was applied, versus those that had yet to open when the application was made.

## Interpretive Summary:

We tested how the exposure of almond flowers to two different fungicides affects pollen germination and pollen tube development in the flowers. Tests compared the effects of exposure on flowers that had already been pollinated and on flowers that were hand pollinated following fungicide application. Our results showed no consistent effects of either of the two fungicides on pollen germination or pollen tube growth. We suggest that our methods can be applied to test other fungicides with the same and different modes of action to explore best timing of application and to reassure growers that the fungicides they are applying are not detrimental to yield through direct effects on pollen germination or pollen tube growth in almond flowers.

## Materials and Methods:

*Exposure of open and unopened flowers*
In a UC Davis orchard, almond branches were covered with mesh pollinator exclusion bags before bloom that prevent access to the flowers by bees but allow air flow. The exclusion bags were placed on trees of two varieties: Nonpareil and Drake, half of which were to be sprayed with a FRAC (Fungicide Resistance Action Committee) group 3 fungicide (a demethylation inhibitor) and the other half with a FRAC group 7 fungicide (a succinate dehydrogenase

inhibitor). Immediately before the first fungicide application on February 14th three quarters of the pollinator exclusion bags were replaced with fungicide exclusion bags. The remaining one quarter of the branches were left unbagged so that the buds and flowers could be exposed to the fungicide. Half of the unbagged branches were on trees sprayed with the FRAC 3 fungicide and the other half were on the trees sprayed with the FRAC 7 fungicide.

Following the first fungicide application, the fungicide exclusion bags were switched back to the pollinator exclusion bags to allow for better air flow around the branches. The open flowers that were exposed to the fungicide application and a set of bagged no-spray control flowers were cut from the branches; their stems placed into mini-vases to maintain their freshness, and brought to the laboratory for hand pollination. The exposed buds were left on the tree and marked so that they could be brought back to the lab for hand pollination once they had opened. Hand pollination was performed in the laboratory using dried honey bee thoraxes mounted on toothpicks as pollen brushes. The pollen was collected from one flower by brushing the thorax over the anthers and then deposited on another flower by touching the stigma with the pollen coated thorax. Prior to hand pollination the anthers were cut off the flower receiving pollen, and the thoraxes were cleaned before use with compressed air. All hand pollination was done by crossing Nonpareil and Drake. For each of the two fungicides, four types of hand pollination crosses were performed (see **Table 1**). Crosses were not performed between the different fungicide types.

**Table 1.** Experimental design of pollination crosses.

| | Control Drake pollen | Fungicide exposed Drake pollen | Control Nonpareil pollen | Fungicide exposed Nonpareil pollen |
|---|---|---|---|---|
| Control Nonpareil stigma | *Hand pollination* | *Hand pollination* | | |
| Fungicide exposed Nonpareil stigma | *Hand pollination* | *Hand pollination* | | |
| Control Drake stigma | | | *Hand pollination* | *Hand pollination* |
| Fungicide exposed Drake stigma | | | *Hand pollination* | *Hand pollination* |

Following the first spray, the marked branches were checked daily, so that any of the exposed buds that had opened could be taken to the laboratory for hand pollination. The second fungicide application was on February 20th. Prior to the spray, approximately half of the remaining bags were removed from the branches. The other bags were replaced with fungicide exclusion bags to act as no-spray controls. After the spray, open flowers that were exposed were taken to the laboratory for hand pollination, along with no-spray control flowers. The same hand pollination crosses were conducted as described above and in **Table 1**. Buds that were exposed were marked and collected for hand pollination once they had opened.

After hand pollination, flowers were placed in microcentrifuge tubes containing 0.5 ml tap-water, avoiding contact of the stigma with the centrifuge-tube wall or water surface. The flowers remained in the tubes for three days at room temperature to allow for pollen tube development. After the three days, the flowers were fixed in FAA (10:7:2:1 ethanol (95 %), $H_2O$, formalin, acetic acid, stored at 4°C) until further processing.

*Exposure of flowers already pollinated*
The second part of the experiment aimed to investigate the effects of fungicide application on flowers that have already been pollinated. In same orchard, the morning before the second spray, we hand pollinated no-spray control flowers as described above using honey bee thoraxes, leaving the flower on the branch. Nonpareil no-spray control flowers were hand pollinated with Drake no-spray control pollen (half on trees to be sprayed with FRAC 3 and half on trees to be sprayed with FRAC 7). Drake no-spray control flowers were hand pollinated with Nonpareil no-spray control pollen (half on trees to be sprayed with FRAC 3 and half on trees to be sprayed with FRAC 7). The flowers were marked and left unbagged during the second fungicide application. As an additional control, Drake and Nonpareil flowers were hand pollinated and then rebagged with fungicide exclusion bags. After the spray, all hand pollinated flowers were marked and rebagged with pollinator exclusion bags. After three days they were taken to the laboratory and fixed in FAA (as described above).

*Examination of pollen tube development*
Once all the flowers had been fixed they were processed in batches of twenty. The flower pistils were boiled in 5% sodium sulfite ($Na_2SO_3$) to soften the tissue and soaked in water for 20 minutes before staining. The pistils were incubated for 12-24 hours in a decolorized staining solution of 0.1% aniline blue dye dissolved for 1 hour in 0.1N $K_3PO_4$. The stained pistils were squashed onto a microscope slide to reveal the pollen tubes. The slides were examined using a fluorescent microscope (Nikon Eclipse 80i with a CFL-FITC filter). For each slide, the numbers of pollen grains, the number of pollen tubes initiating growth at top of the style and the number of pollen tubes reaching the base of the style were counted. 'Pollen germination' was calculated as the number of pollen tubes initiating growth divided by the number of pollen grains and 'pollen tube development' was calculated as the number of pollen tubes reaching the base of the style divided by the number of pollen tubes are the top of the style.

*Statistical Analysis*
The flowers that were hand pollinated in the laboratory and those that were hand pollinated in the field were analyzed separately. For the flowers hand pollinated in the laboratory, pollen germination, pollen tube development and the number of pollen tubes reaching the base of the style were tested to see if they differed between treatments. The four treatments were: CC (control stigma, control pollen), CE (control stigma, exposed pollen), EC (exposed stigma, control pollen) and EE (exposed stigma and exposed pollen). Analysis of variance (ANOVA) was used to test for differences between the treatment groups. The tests were done separately for flowers exposed to the FRAC 3 fungicide and flowers exposed to the FRAC 7 fungicide (the same control data were used for both). ANOVA assumes homogeneity of variance and where necessary the data were log transformed to conform to this assumption. Where a significant difference with treatment was found ($P<0.05$), the Tukey HSD (honest significant difference) test was used to determine which treatments differed significantly.

For the flowers that were pollinated in the field the Wilcoxon-Mann-Whitney test was used to test whether there was a difference in pollen germination, pollen tube development or the number of pollen tubes reaching the base of the style in flowers that were exposed to fungicide following hand pollination and flowers that were not (exposed vs. control).

## Results and Discussion:

In total, 574 flowers were hand pollinated in the laboratory and successfully stained for pollen tube growth; 292 Drake stigmas and 282 Nonpareil. In the field, 192 flowers were hand pollinated and subsequently stained to visualize pollen tube growth; 104 Drake and 88 Nonpareil. The results from the analysis of the flowers hand pollinated in the laboratory are given in **Table 2**. No effect of treatment was found on pollen tube development or the number of pollen tubes reaching the base of the style in either variety when exposed as buds or flowers. There was an effect of treatment on pollen germination in Drake stigmas exposed to the FRAC 7 as buds (**Figure 1a**). No-spray control flowers had significantly greater pollen germination than the flowers whose stigmas were exposed to the FRAC 7 as buds whether they were hand pollinated with no-spray control or exposed pollen (**Figure 1a**). There was also an effect of treatment on pollen germination in Nonpareil flowers exposed to the FRAC 3 as buds (**Figure 1b**). The combination of a control Nonpareil stigma and exposed Drake pollen had lower pollen germination than the other three treatments (**Figure 1b**). Neither of these effects persisted through to differences between treatments in pollen tube development or in the numbers of pollen tubes reaching the base of the style.

**Table 2.** The results (*P* values) of analysis of variance tests, investigating if pollen germination, pollen tube development or the number of pollen tubes reaching the base of the style differed with fungicide exposure. Significant results ($P<0.05$) are given in bold and the differences between treatments are shown in **Figure 1**. ¶Data log transformed for homogeneity of variance.

| Stigma variety | Chemical | Exposure | Pollen germination | Pollen tube development | No. pollen tubes end |
|---|---|---|---|---|---|
| Drake | FRAC 3 | bud | 0.210 | ¶0.166 | 0.337 |
| Drake | FRAC 3 | flower | 0.321 | 0.878 | 0.506 |
| Drake | FRAC 7 | bud | **0.003** | 0.655 | 0.982 |
| Drake | FRAC 7 | flower | 0.994 | ¶0.969 | 0.952 |
| Nonpareil | FRAC 3 | bud | **0.004** | ¶0.176 | 0.115 |
| Nonpareil | FRAC 3 | flower | 0.816 | 0.517 | 0.921 |
| Nonpareil | FRAC 7 | bud | 0.066 | ¶0.089 | 0.224 |
| Nonpareil | FRAC 7 | flower | 0.861 | 0.510 | 0.383 |

**Table 3.** The results (*P* values) of Wilcoxon-Mann-Whitney tests, investigating if fungicide exposure following hand pollination affected pollination of the flowers. Significant results (*P*<0.05) given in bold.

| Stigma variety | Chemical | Pollen germination | Pollen tube development | No. pollen tubes end |
|---|---|---|---|---|
| Drake | FRAC 3 | 0.107 | 0.453 | 0.739 |
| Drake | FRAC 7 | 0.080 | 0.615 | 0.649 |
| Nonpareil | FRAC 3 | 0.190 | 0.417 | 0.324 |
| Nonpareil | FRAC 7 | 0.422 | **0.008** | **0.011** |

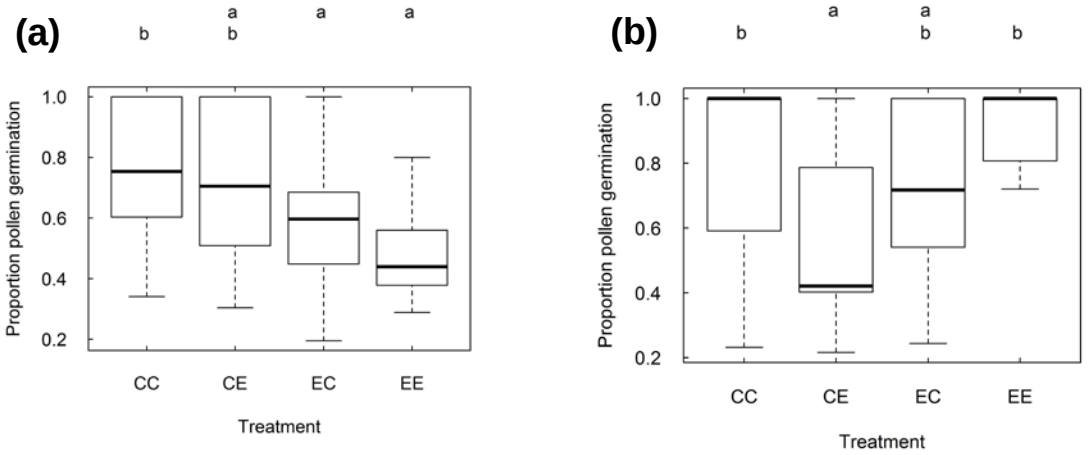


**Figure 1.** The differences in pollen germination for the two cases where a significant effect of treatment was found (**Table 2**). (a) Pollen germination in Drake stigmas and Nonpareil pollen exposed to a FRAC 7 fungicide as buds. (b) Pollen germination in Nonpareil stigmas and Drake pollen exposed to a FRAC 3 fungicide as buds. The treatment letters represent the following: CC=control stigma, control pollen; CE=control stigma, exposed pollen; EC=exposed stigma, control pollen; EE=exposed stigma, exposed pollen. The letters a and b above the boxplots for each treatment represent significant differences between those treatments if no letter is shared. The heavy lines within each box represent the median value and the upper and lower parts of the box the upper and lower quartile respectively.

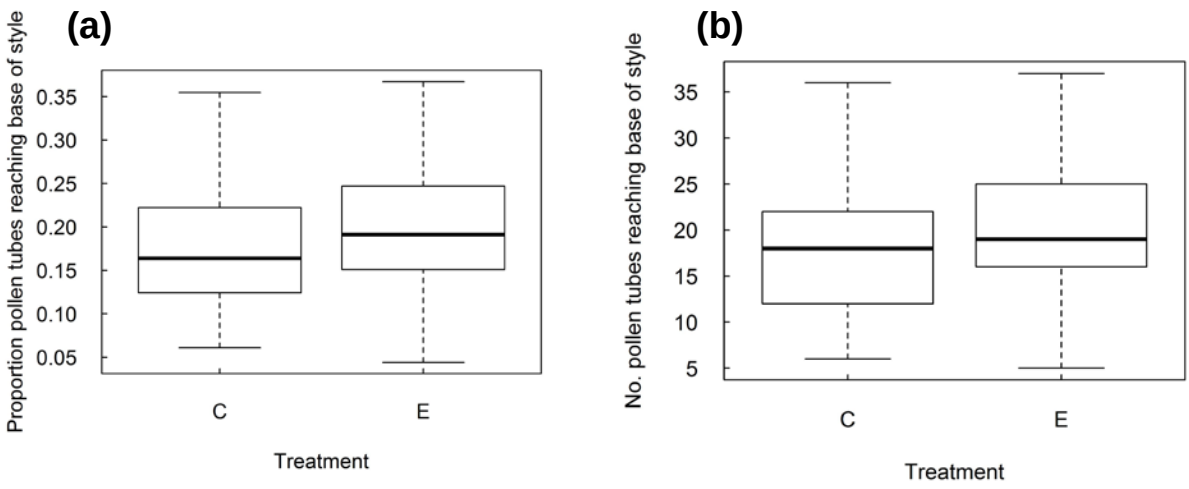


**Figure 2.** The two cases where fungicide exposure following hand pollination significantly affected pollination of the flowers (**Table 3**). Pollen tube development (a) and the number of pollen tubes reaching the base of the style (b) in Nonpareil flowers pollinated with Drake pollen before the application of a FRAC 7 fungicide. C= no spray control flowers that were hand pollinated in the field and then bagged to prevent fungicide exposure. E= Nonpareil flowers that were hand pollinated in the field prior to the application of a FRAC 7 fungicide.

The results from the analysis of flowers hand pollinated in the field are given in **Table 3**. No effects of exposure to the FRAC 3 or the FRAC 7 fungicide were found for Drake stigmas already pollinated. For the Nonpareil stigmas that were pollinated in the field prior to exposure, there was no effect found from the FRAC3 fungicide. There was however a significant difference in pollen tube development and the number of pollen tubes reaching the base of the style in the flowers exposed to the FRAC 7 fungicide. Both the pollen tube development (**Figure 2a**) and the number of tubes reaching the base of the style (**Figure 2b**) were higher in the flowers exposed to the FRAC 7 fungicide following pollination than they were in the no spray control flowers.

The results do not show a consistent effect of fungicide application on the pollination of almond flowers. In the case of exposure to fungicides prior to pollination, there is an indication of a negative effect on pollen germination when the flowers were exposed as buds, but this was not consistent and did not follow through to affect pollen tube development and the number of pollen tubes reaching the base of the style. For the flowers exposed to fungicide after pollination, there was no effect of the fungicide application found in Drake flowers, and in the Nonpareil flowers pollen tube germination and the number of pollen tubes reaching the base of the style was slightly higher in the flowers exposed to the FRAC 7 fungicide. It is possible that some of the other ingredients in the FRAC 7 mixture applied to the flowers are beneficial for pollen tube growth, but this was not supported by the data from the flowers hand pollinated in the laboratory. Our results show no consistent effect of the application of two fungicides to the

pollination of almond flowers. The results were not consistent between the two almond varieties, so different effects may be seen in other varieties. We therefore recommend carrying out similar tests in other popular varieties. While we chose two chemicals that represent two of the more widely used FRAC groups, within each group there are a range of chemicals and surfactants and we do not suggest that our results represent the effects of all fungicides that fall within these groups. We suggest this method can be applied more widely to test for the potential effects of other fungicides on the pollination of almond flowers, both within FRAC groups 3 and 7 and for fungicides with other modes of action.

**Research Effort Recent Publications:**
None.

**References Cited:**
None cited.