

Fungicide Impacts on Almond Pollen Germination and Tube Elongation Through Pistils

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Abstract

Almond (*Prunus dulcis* ((Miller)) D. Webb) pollen development was studied after exposure to fungicides. Freshly shed pollen was applied to the surface of hanging drops of germination medium containing commercially important fungicides and to stigmas of excised pistils that had been treated with fungicides 24 hours earlier. Pollen tube growth through the lower styles was evaluated using fluorescent microscopy. Stigmas still damp with fungicides interfered with pollen germination. Once the stigmatal surfaces dried, the pollen germinated and tube elongation through the styles appeared normal. Fungicides tested were azoxystrobin (Abound[®]), captan (Captan[®]), fenhexamid (Elevate[®]), trifloxystrobin (Flint[®]), myclobutanil (Rally[®]), iprodione (Rovral[®]), cyrodinil (Vangard[®]), and ziram (Ziram[®]).

Keywords

Prunus dulcis, azoxystrobin, captan, fenhexamid, trifloxystrobin, myclobutanil, iprodione, cyrodinil, ziram

Introduction

Nut production in almonds depends upon transfer by honey bees of viable, compatible pollen between healthy flowers (Micke, 1996). Many fungal diseases of almonds, such as brown rot, blossom rot, shot hole and anthracnose, are controlled by annual treatment with fungicides just previous to, during, or immediately following bloom (Flint, 2002). Detrimental effects of fungicides on pollen germination (Eaton, 1961; Church and Williams, 1978; Redalen, 1980; Marcucci *et al.*, 1983; Bristow and Windom, 1987; Watters and Sturgeon, 1990; Wetzstein, 1990, 2003) and pollen tube growth (Marcucci *et al.*, 1983; Abbott, *et al.*, 1991; He *et al.*, 1996) have been demonstrated for commercially important plants. This study was conducted to determine if fungicides, in current or anticipated use on almonds, interfere with pollen germination or tube growth.

Materials and methods

Branches with unopened flowers were pruned from two early, two mid, and two late blooming varieties of almond trees (Ne Plus Ultra and Peerless, Nonpareil and Carmel, and Butte and Mission, respectively) growing in commercial orchards. The cuttings, which were collected prior to applications of fungicides, were clipped underwater and stored in tap water under laboratory conditions.

After 24 h, freshly opened blossoms were severed from the branches with a razor blade. Flowers serving as pistil donors had their sepals, petals, and anthers removed by squeezing and tearing the tissues away from the perimeter of the nectar cup with a pair of fine forceps. The pistils, along with the remaining flower parts, were floated on 2 mL of 5% sucrose in 5 mL wells of tissue culture plates. Plate covers were placed over the wells diagonally, to maintain humidity and positioned so as not to contact the stigmata. For the first 24 h, plates containing the pistils were stored at room temperature under a large plastic box to keep the humidity relatively high. Then, the plates were held partially covered for the remainder of the experiment.

Freshly opened pollen donor blossoms were excised from the stored cuttings. The filaments were grasped in forceps and pulled from the blossoms to obtain stamens. Stamens were stored over night in small, plastic petri dishes with the top ajar while they dehisced. Remaining open blossoms were removed from the branches and discarded daily.

Pollen germination

Microscope slides (7.5 X 2.5 cm) were prepared with a single 0.5 mL drop of distilled water, germination fluid (9 g sucrose, 10 mg boric acid and 10 mg calcium nitrate in 91 mL distilled water), or fungicide solution (Table 1) containing 11 g sucrose, 11 mg boric acid and 11 mg calcium nitrate in 100 mL of distilled water. Experimental dosages were calculated from recommended field dosages (grams or kilograms of formulated fungicide per hectare in 934.6 L of water) currently used in blossom disease control. Approximately 100 grains of pollen from each almond variety were applied to each droplet by grasping three filaments bearing dehisced anthers and touching them to the surface of the drop. Two slides were inverted over a single 5 cm Petri dish containing 10 ml distilled water. Pollen grains were examined with a dissecting

microscope after 2 and 24 h at room temperature. Lengths of pollen tubes were estimated in numbers of pollen grain diameters.

Pollen tube growth

In eight of nine replicates, stigmatic surfaces on five floating pistils were treated with fungicides (Table 1) and allowed to dry. Field dose concentrations of each fungicide, dissolved in distilled water, were applied directly to the stigmatic surface with a 1 μ l plastic loop. The small droplet of treatment solution covered the stigma surface and either moved down into the plant tissue or was absorbed from the edge of the stigma a minute later with a small piece of blotting paper.

Twenty-four h later, the stigmas were dusted with compatible pollen collected the previous day. Pollen was applied by tapping three different anthers against the stigmatic surface. At least 100 pollen grains were deposited on each stigma. For the ninth replicate, pollen was applied in the same manner immediately following application of the treatment solutions, while the stigmas were still damp.

Five days after pollination, the styles were severed just above the ovaries, placed in distilled water, autoclaved for 5 minutes, blotted dry, stained in aniline blue (0.005g aniline blue, 3.28g $K_3PO_4 \cdot XH_2O$, 100 mL of distilled H_2O - from O'Brien and McCully) for 30 min, blotted again, placed on microscope slides, squashed and then mounted in 65% sucrose. Lower stylar regions (Embree and Foster, 1999) were examined under 100X magnification with a Nikon Microphot SA compound microscope with an EPI-Fluorescence attachment. A mercury lamp provided illumination through an EX 450-490 excitation filter and BA 520 barrier filter.

results

Pollen from all varieties germinated at 90% or greater (except Butte = 70%) in synthetic medium (Table 2). In 24 h, pollen tubes had lengthened to 20 times the diameter of the pollen grains. Nonpareil and Butte pollen failed to germinate in distilled water, and pollen tubes of varieties that did germinate had tube lengths about half the length attained in germination fluid. Six of the eight fungicides completely inhibited germination of almond pollen when they were added to the germination fluid. Partial inhibition occurred in the presence of Rovral (10-50% germination, except Nonpareil = 0%) and tube lengths were similar to, or shorter than, those in distilled water. All pollen types germinated (15-40%) and tubes grew to a limited extent (1-12 diam) in Ziram. Some tubes were distorted or had very thin diameters.

Twenty to eighty-five pollen tubes typically grew through the lower stylar area of untreated pistils and pistils that were treated with distilled water or field concentrations of the experimental fungicides 24 hr before application of pollen. Results were more variable when pollen was applied while the stigmas were still damp with water or test solutions (ninth replicate), with counts ranging between 2 and 15 tubes.

Discussion

Evidence from the *in vitro* hanging drop experiment and the ninth replicate of the *in situ* pollen tube growth trials indicate that all of the tested fungicides, while in solution, reduced almond pollen germination and tube growth. These results are very similar to those reported by Marcucci, *et al.* (1983) and He, *et al.* (1996), but we tested current generation fungicides.

The length of almond pollen tube growth in germination fluid, compared to growth in distilled water, suggests that tubes utilize nutrients from the germination fluid or that osmotic pressures

are more suitable for tube development in media containing sucrose. The limited growth the tubes in media containing fungicides suggest that fungicides may interfere with nutrient uptake or pollen metabolism. Additionally, distorted and abnormally thin tubes were seen only with Ziram. It is possible that the very thin tubes actually had ruptured, as reported by He, *et al.* (1996).

Since our germination and pollen tube growth studies were not conducted on media containing agar, it can not be determined whether we would have seen, for almond pollen, inhibitory effects on germination similar to those reported by Eaton (1961) for cherries, Church and Williams (1978) and Watters and Sturgeon (1990) for apples, Redalen (1980) for raspberries, Bristow and Shawa (1981) for cranberries, Bristow and Windom (1987) for blueberries, Wetzstein (1990) for pecans, Abbott et al. (1991) for muskmelon and He *et al.* (1996) for *Tradescantia virginiana*.

Thus, our second set of tests, involving germination on, and pollen tube growth through, the dried layer of fungicides on stigmata more nearly demonstrated what would be expected to happen in an orchard situation (*in vivo*). These studies suggest that field application of many of the tested fungicides might interfere with germination of pollen, but only for a short period of time before the chemical either was absorbed into the stigmatal tissue or evaporated to dryness. If the fungicides caused stigmatic damage in 24 h, as reported by Wetzstein (1990) and Weiguang et al. (2003) for pecan and almond, it was not severe enough to interfere with almond pollen germination and growth of pollen tubes to the ovary. With freshly opened blossoms, the loss of a day should not affect commercial pollination significantly. However, since Vezvaei and Jackson (1995) determined that almond blossoms lose receptivity fairly quickly (10% after the first day, 55% after the second), applications of the tested fungicides could interfere with

fertilization of older blossoms and possibly lead to crop loss. Growers should be advised to apply fungicides pre-bloom or try to delay fungicide treatments for as long as possible during bloom.

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Table 1.

Fungicide Concentrations Used in Germination Medium

<u>Product</u>	<u>Field Dose</u>	<u>Laboratory Dose</u>
azoxystrobin	355.6 ml/378.4 liters	0.094 ml/100 ml
captan	3.6 kg /378.4 liters	0.960 g /100 ml
fenhexamid	454.0 g /378.4 liters	0.120 g /100 ml
trifloxystrobin	85.1 g /378.4 liters	0.023 g /100 ml
myclobutanil	227.0 g /378.4 liters	0.060 g /100 ml
iprodione	473.0 ml/378.4 liters	0.125 ml/100 ml
cyrodinil	284.0 g /378.4 liters	0.075 g /100 ml
ziram	3.6 kg/378.4 liters	0.960 g /100 ml

Table 2.

Almond Pollen Germination

In Distilled Water				
<u>Pollen Variety</u>	<u>% Germination</u>		<u>Length of Pollen Tubes</u>	
	<u>2 hr</u>	<u>24 hr</u>	<u>2hr</u>	<u>24 hr</u>
Ne Plus	40	90	3X	8-10X
Peerless	10	12	3X	8-10X
Nonpareil	0	0		
Carmel	20	20	3X	8-10X
Butte	0	0		
Mission	50	75	3X	8-10X
In Germination Medium				
<u>Pollen Variety</u>	<u>% Germination</u>		<u>Length of Pollen Tubes</u>	
	<u>2 hr</u>	<u>24 hr</u>	<u>2hr</u>	<u>24 hr</u>
Ne Plus	99	99.9	7X	20X
Peerless	75	90.0	6X	20X
Nonpareil	50	90.0	4X	20X
Carmel	90	99.9	6X	20X
Butte	20	70.0	3X	20X
Mission	99	99.9	7X	20X

In Germination Medium plus Fungicides

Pollen Variety	% Germination		Length of Pollen Tubes	
	2 hr	24 hr	2hr	24 hr
Ne Plus				
Rovral	50	50	2-6X	10X
Ziram	25	25	1X	1X
Peerless				
Rovral	5	10	2-3X	6X
Ziram	1	15	1.5X	2-3X
Nonpareil				
Ziram	40	40	3X	8X (very thin)
Carmel				
Rovral	20	33	6X	6-8X
Ziram	25	25	2-3X	3-6X(distorted)
Butte				
Ziram	1	15	4X	4-12X (very thin)
Mission				
Rovral	5	10	6X	8X
Ziram	0	25	1X	1X