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Germination of Apple Pollen as Influenced by Fungicides

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Abstract

Pollen collected from two apple cultivars was tested in the laboratory for germinability in solutions of the fungicides or bactericides which are commonly applied during bloom to control apple diseases. Dodine significantly ($P = 0.01$) reduced 'Jonathan' and 'Golden Delicious' pollen germination. Pollen grains which did germinate, however, produced germ tubes which elongated at the same rate as controls. Streptomycin at 50 ppm had no effect on either germination or germ tube elongation of 'Golden Delicious' pollen but significantly ($P = 0.01$) reduced germination at 100 ppm. All fungicides applied at recommended rates for disease control significantly ($P = 0.01$) reduced apple pollen germination. Benomyl and sulfur affected pollen germination the least while the dithiocarbamate fungicides (zineb, metiran, dinocap-mancozeb) and captan proved most toxic.

Introduction

The most common and serious pathogens affecting apple production and the diseases they cause are *Venturia inaequalis* (scab), *Gymnosporangium* spp. (rusts), *Erwinia amylovora* (fire blight) and *Podosphaera leucotricha* (powdery mildew). These diseases require that fungicide control measures be initiated prior to and continuing through bloom until disease pressure subsides usually during the summer. Fungicide applications during bloom for disease control are currently recommended by many extension plant pathologists.

Several researchers have reported non-target effects of fungicides on germination of various fruit pollens. Rich (5) observed that captan adversely affected apple pollen germination *in vitro* but not *in vivo*. Eaton (1, 2, 3) found that captan reduced *in vitro* sweet cherry pollen germination, *in vitro* strawberry pollen germination, and *in vivo* apple pollen germination. Shawa, et al (6) reported that captan inhibited cranberry pollen germination in *in vitro* tests. The following report details the effects of captan as well as several other commonly applied fungicides on the *in vitro* germination and germ tube elongation of 'Jonathan' and 'Golden Delicious' apple pollen.

Materials and Methods

Nonsprayed 'Jonathan' and 'Golden Delicious' apple blossoms at the balloon stage were randomly picked on 1 May 1974. Anthers were excised by rubbing the blossoms across a 12-mesh screen. They were dried at room temperature (24° C) for 48 hours in culture plates, and this caused them to dehisce. This preparation containing both pollen grains and floral parts was stored in sealed glass vials at −16° C until used.

A germination medium containing 0.3M raffinose and 0.001% boric acid in glass distilled water was prepared (4). Germination medium (0.4 ml) was placed in each of 12 depressions of porcelain plates. Aliquots (0.1 ml) of fungicide or bactericide suspensions in distilled water containing fivefold the desired test rate was added to each depression thereby leaving a final fungicide concentration equivalent to the rates given below. All rates

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are expressed in either pounds per 100 gallons or in parts per million (ppm) of formulated product for better reader understanding. Fungicides examined for their effect on pollen germinability and the rates tested were the following: streptomycin (Agri-Strep 17W, Merc) at 0, 10, 25, 50, 75, and 100 ppm; dodine (Cyprex 65W, American Cyanamid) at 0, 0.25, 0.375, 0.5, 0.75, and 1 pound per 100 gallons; captan (Captan 50W, Stauffer) at 0, 0.5, 1.0, 1.5, 2.0, and 2.5 pounds per 100 gallons; sulfur (Sulfur 90W, Niagara) at 0, 1, 2, 3, 4, and 5 pounds per 100 gallons; benomyl (Benlate 50W, du Pont) at 0, 2, 4, 2 ounces plus 1 quart of superior oil, and 1 quart of superior oil per 100 gallons; zineb (Dithane Z-78 75W, Rohm & Haas) at 0, 0.5, 1.0, 1.5, 2.0, and 2.5 pounds per 100 gallons; metiram (Polyram 80W, Niagara) at 0, 0.5, 1.0, 1.5, 2.0, and 2.5 pounds per 100 gallons; and dinocap-mancozeb (Dikar 80W, Rohm & Haas) at 0, 1, 2, 1 pound + 3 ounces Triton B-1956, 2 pound + 3 ounces Triton B-1956, and 3 ounces Triton B-1956.

Immediately following addition of each test fungicide to the germination medium, pollen was transferred to each depression with a camel hair brush. The pollen, germination medium, and fungicide were mixed with an inoculation needle. The depression plates were immediately placed on moistened Kim Pak cellulose paper pads in covered plastic boxes to reduce evaporation and incubated at room temperature (24° C). Germination was stopped after two hours by the addition of 0.1 ml of 3% gluteraldehyde in 0.005M phosphate buffer at pH 6.8. All plates containing pollen-fungicide-germination medium were frozen at −30° C until examined.

Percent germination and the amount of germ tube elongation was determined by thawing porcelain plates containing treated or nontreated (controls) pollen and observing samples on microscope slides at 40X on a Reichert Visopan viewing microscope. Tube elongation in cm was measured directly from the viewing screen. Controls consisted of pollen added to 0.4 ml of germination medium and 0.1 ml of distilled water. At least 200 pollen grains were examined per fungicide rate tested and all experiments were replicated twice. Fungicide treatments were arranged in a split-plot design with two replications. Treatments were assigned at random to the whole unit in a randomized complete block design; rates were assigned as subunits within each whole unit.

Results

Apple pollen grains are triangular to circular in shape. When germination occurs a single germ tube grows from one of the 3 apices. Germ-tube growth was rapid when no inhibitory compound was present with tubes averaging 4.7 cm long after 2 hours of incubation. The percentage germination was not dependent upon either the number of pollen grains placed in the germination medium or upon any significant alteration of the pH of the germination medium by the fungicides at the rates examined. All results are expressed as percentages of the controls.

Table 1. Effect of different concentrations of streptomycin (Agri-Strep 17W) on *in vitro* germination and germ tube elongation of apple (*Malus domestica* cv. 'Golden Delicious') pollen.

Rate (ppm formulated product)	Germination (% of controls) Golden Delicious	Germ tube growth (% of controls) Golden Delicious
0	100.0	100.0
10	101.5	104.2
25	74.4	87.9
50[1]	75.9	96.2
75	75.9	83.2
100[1]	56.5	71.5
LSD, 1% level	26.9	41.8

[1]Commonly recommended rate or rates to obtain disease control.

Streptomycin, the only bactericide tested, is frequently applied for fire blight control during the bloom period at recommended rates of 50 to 100 ppm. Table 1 shows that streptomycin reduced germinatoin at 100 ppm ($P = 0.01$) but reduction in germ tube elongation of 'Golden Delicious' pollen was not statistically significant at any rate tested.

Dodine commonly applied to control apple scab during the spring (Table 2) significantly ($P = 0.01$)

Table 2. Effect of different concentrations of dodine (Cyprex 65W) on *in vitro* germination and germ tube elongation of apple (*Malus domestica* cv. 'Jonathan' and 'Golden Delicious') pollen.

Rate (lbs. formulated product/100 gal.)	Germination (% of controls) Jonathan	Germination (% of controls) Golden Delicious	Germ tube growth (% of controls) Jonathan	Germ tube growth (% of controls) Golden Delicious
0.00	100.0	100.0	100.0	100.0
0.25[1]	29.4	21.8	111.5	44.5
0.375[1]	11.8	20.6	100.7	29.8
0.50[1]	8.4	11.6	87.2	21.6
0.75	2.5	10.9	110.5	32.6
1.00	4.3	13.1	47.2	40.1
LSD, 1% level	64.5	28.7	95.0	41.8

[1]Commonly recommended rate or rates to obtain disease control.

reduced germination of both 'Jonathan' and 'Golden Delicious' pollen at all rates examined. Dodine, however, appeared to have no statistically significant effect on germ tube growth of 'Jonathan' pollen but had a pronounced effect on 'Golden Delicious' tube elongation even at the lowest rate tested.

Less frequently used fungicides such as captan or sulfur were also phytotoxic to pollen. Captan at the recommended orchard use rate of 2 lb/100 gal. reduced germination of 'Jonathan' pollen by 96% and germination of 'Golden Delicious' pollen by 85% (Table 3). The lowest captan rate tested significantly ($P = 0.01$) reduced germination. Germ tube

Table 3. Effect of different concentrations of captan (Captan 50W) on *in vitro* germination and germ tube elongation of apple (*Malus domestica* cv. 'Jonathan' and 'Golden Delicious') pollen.

Rate (lbs. formulated product/100 gal.)	Germination (% of controls) Jonathan	Germination (% of controls) Golden Delicious	Germ tube growth (% of controls) Jonathan	Germ tube growth (% of controls) Golden Delicious
0.0	100.0	100.0	100.0	100.0
0.5	20.9	43.0	35.4	24.5
1.0	12.2	11.1	31.6	22.0
1.5	10.1	8.3	17.5	18.0
2.0[1]	4.0	14.8	45.3	25.1
2.5	7.9	14.5	27.0	16.2
LSD, 1% level	27.6	28.7	51.9	42.0

[1]Commonly recommended rate to obtain disease control.

elongation was also significantly reduced.

Sulfur reduced pollen germination but was less toxic to 'Golden Delicious' pollen than to 'Jonathan' pollen (Table 4). Significant ($P = 0.01$) re-

Table 4. Effect of different concentrations of sulfur (Sulfur 90W) on *in vitro* germination and germ tube elongation of apple (*Malus domestica* cv. 'Jonathan' and 'Golden Delicious') pollen.

Rate (lbs. formulated product/100 gal.)	Germination (% of controls) Jonathan	Germination (% of controls) Golden Delicious	Germ tube growth (% of controls) Jonathan	Germ tube growth (% of controls) Golden Delicious
0	100.0	100.0	100.0	100.0
1	60.2	83.2	58.0	79.0
2	59.4	81.8	62.5	68.7
3	55.5	85.0	64.7	58.0
4	27.3	83.0	52.3	51.1
5[1]	29.6	69.8	44.3	47.3
LSD, 1% level	21.2	33.0	54.8	42.1

[1]Commonly recommended rate to obtain disease control.

duction of 'Jonathan' pollen germination occurred at rates of 1 lb/100 gal or higher while 'Golden Delicious' pollen was not significantly affected even at rates as high as 5 lb/100 gal. Generally, sulfur had less effect in

preventing germ tube elongation than many other fungicides.

Benomyl (Table 5) at 2 ounces in combination with 1 quart of Superior oil per 100 gallons significantly ($P = 0.01$) reduced pollen germination of both cultivars, although nearly half the pollen grains germinated. Similarly, benomyl at 4 oz per 100 gal significantly reduced germination of both apple cultivars but more than half the pollen grains germinated.

Dithiocarbamate-containing fungicides (zineb, metiram, and dinocap-mancozeb, Tables 6, 7, and 8, respectively) all adversely affected pollen germination and tube elongation in a similar manner. All significantly ($P = 0.01$) reduced pollen germination of both apple cultivars by 85-100% and germ tube growth by 60-100% at rates recommended for

Table 5. Effect of different concentrations of benomyl (Benlate 50W) on *in vitro* germination and germ tube elongation of apple (*Malus domestica* cv. 'Jonathan' and 'Golden Delicious') pollen.

Rate (lbs. formulated product/100 gal.)	Germination (% of controls) Jonathan	Germination (% of controls) Golden Delicious	Germ tube growth (% of controls) Jonathan	Germ tube growth (% of controls) Golden Delicious
0	100.0	100.0	100.0	100.0
2	78.7	72.7	46.4	38.0
4[1]	60.2	56.6	37.1	25.3
2 + 1 qt oil[1]	48.1	33.1	52.3	22.4
1 qt oil	69.1	73.3	66.9	54.5
LSD, 1% level	22.1	28.6	43.7	41.6

[1]Commonly recommended rate or rates to obtain disease control.

Table 6. Effect of different concentrations of zineb (Dithane Z-78 50W) on *in vitro* germination and germ tube elongation of apple (*Malus domestica* cv. 'Jonathan' and 'Golden Delicious') pollen.

Rate (lbs. formulated product/100 gal)	Germination (% of controls) Jonathan	Germination (% of controls) Golden Delicious	Germ tube growth (% of controls) Jonathan	Germ tube growth (% of controls) Golden Delicious
0.0	100.0	100.0	100.0	100.0
0.5	56.2	32.9	44.5	56.7
1.0	14.7	21.9	3.1	31.8
1.5	7.2	5.1	3.6	39.7
2.0[1]	0.6	5.1	36.3	12.9
2.5	0.6	13.5	29.1	25.2
LSD, 1% level	23.1	33.2	43.8	58.9

[1]Commonly recommended rate to obtain disease control.

Table 7. Effect of different concentrations of metiram (Polyram 80W) on *in vitro* germination and germ tube elongation of apple (*Malus domestica* cv. 'Jonathan' and 'Golden Delicious') pollen.

Rate (ozs. formulated product/100 gal.)	Germination (% of controls) Jonathan	Germination (% of controls) Golden Delicious	Germ tube growth (% of controls) Jonathan	Germ tube growth (% of controls) Golden Delicious
0.0	100.0	100.0	100.0	100.0
0.5	3.7	19.3	51.2	25.5
1.0	2.4	8.2	35.2	20.7
1.5	3.7	8.1	31.7	14.4
2.0[1]	0.0	7.0	0.0	10.5
2.5	0.0	1.6	0.0	9.0
LSD, 1% level	83.5	28.3	92.1	43.7

[1]Commonly recommended rate to obtain disease control.

Table 8. Effect of different concentrations of dinocap-mancozeb (Dikar 80W) on *in vitro* germination and germ tube elongation of apple (*Malus domestica* cv. 'Jonathan' and 'Golden Delicious') pollen.

Rate (lbs. formulated product/100 gal.)	Germination (% of controls) Jonathan	Germination (% of controls) Golden Delicious	Germ tube growth (% of controls) Jonathan	Germ tube growth (% of controls) Golden Delicious
0.0	100.0	100.0	100.0	100.0
1.0	2.1	11.2	50.0	11.7
2.0	2.1	7.8	35.7	17.0
1.0 + 3 oz. Triton B 1956	1.4	17.1	29.3	17.9
2.0 + 3 oz. Triton B 1956[1]	1.4	14.0	12.5	20.0
3 oz. Triton B 1956	63.4	80.8	80.6	89.2
LSD, 1% level	54.0	26.8	90.9	45.0

[1]Commonly recommended rate to obtain disease control.

cedar-apple or quince rust disease control.

Discussion

Streptomycin is commonly applied at 4-day intervals during the bloom period to control the blossom blight phase of fire blight. This bactericide had no adverse effect on pollen germination at rates of 75 ppm or less. It would appear, therefore, that this spray can be continued without affecting fruit set.

Other spray materials, frequently applied during bloom, varied in toxicity to apple pollen in these tests. The toxicity was either reflected in reduced germination or in many cases reduced germ tube growth. A comparison of Tables 2-8 shows that the materials least likely to inhibit pollen germination during bloom are benomyl at either the 4-ounce or 2-ounce in combination with Superior oil rate, or sulfur at the 2-pound rate. Benomyl at these rates will control both apple scab and powdery mildew but will not control the rust diseases.

The rust diseases frequently require control measures during bloom. Of the currently recommended fungicides tested, all were severely phytotoxic to pollen under the test conditions employed. Bloom sprays of dithiocarbamate-based fungicides may reduce fruit set and adversely affect yield especially in a year when a limited number of flower buds survive the winter.

Because of the potential reduction in fruit set due to fungicide applications during bloom, growers should avoid fungicide sprays where practical during full bloom. Careful timing of fungicide sprays with an application when the king blossoms are at full pink should provide adequate disease protection until the petal-fall stage when a normal spray program could be resumed.

It must be emphasized that the data presented in this report represents only laboratory results. Generally, under field conditions, more pollen and flowers are present than necessary to insure a full crop. Therefore even though many commonly used fungicides resulted in reduction of germinable pollen under laboratory conditions these same fungicides may not have a measurable effect in reducing fruit set in the field. Studies that are currently under way will investigate the importance of fungicide toxicity to fruit set under field conditions.

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