Effects of Selected Fungicides, Insecticides, and Adjuvants on In Vitro Germination of Highbush Blueberry Pollen

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ABSTRACT

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Pollen from highbush blueberry (*Vaccinium corymbosum*) readily germinated on 9% sucrose agar but was inhibited by more than 50% (ED_{50}) when the medium contained 50 μg a.i./ml of captan, benomyl + captan, captafol, ferbam, triforine-EC, diazinon, or X-77. Some pollen still germinated with acephate, captafol, triforine-WP, triforine-F, Triton B-1956, and Tween 20 at a concentration of 5,000 μg/ml. For most chemicals, the ED_{50} value was less than the concentration in spray suspensions at recommended rates. At very low concentrations (0.1 and sometimes 0.5 μg/ml), all chemicals except the combination of benomyl + captan stimulated germination. The emulsifiable concentrate (EC) formulation of triforine was markedly more toxic to pollen than either the wettable powder or flowable formulations, with complete inhibition by the EC formulation at 100 μg/ml. Virtually all of the toxicity of the EC formulation was accounted for by one of the inert ingredients and not triforine.

Additional key word: phytotoxicity

Current disease and insect control recommendations for highbush blueberry (*Vaccinium corymbosum* L.) call for the application of pesticides during bloom when needed (5,6). Numerous researchers have reported on the adverse effects of pesticides on pollen germination. A brief review is given by Church and Williams (4).

Facteau and Chestnut (11) have reported the toxicity of various air pollutants to pollen germination and pollen tube growth in apricot and sweet cherry. There are several reports on the toxicity of adjuvants to pollen (12–14,19,20,23).

Fungicides applied during bloom have reduced fruit set (22) and berry size (3) in cranberry and fruit set in apple (7,17) and increased the incidence of malformed strawberry fruit (1,10). Triforine formulated as an emulsifiable concentrate (EC) was toxic to blueberry pollen but did not reduce either fruit set or berry size when applied during bloom to control the mummyberry disease (*Monilinia vaccinii-corymbosi* (Reade) Honey) (2). On cranberry, the EC formulation of triforine lowered yield but the wettable powder (WP) and the flowable (F) formulation did not (3). In vitro tests showed that the EC was more toxic to pollen than the other two formulations (3).

Precautions are usually taken when insecticides are used during bloom to minimize the risk of killing or repelling bees. There is, however, no information on the toxicity of commonly used insecticides to pollen of highbush blueberry.

The objective of this research was to determine the in vitro toxicity of several fungicides, insecticides, and adjuvants to pollen of highbush blueberry.

MATERIALS AND METHODS

Sucrose agar, containing 18% sucrose

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(w/v) and 5% agar (w/v), was prepared with glass distilled water, autoclaved, cooled to 45 C, and then mixed with equal volumes of aqueous suspensions of various chemicals or sterile glass distilled water. Eighteen milliliters was dispensed into each 9-cm-diameter petri plate. The final concentration of all chemicals is expressed as micrograms or microliters of active ingredient (a.i.) per milliliter of sucrose agar. Prepared plates were used immediately by seeding with fresh pollen.

The chemicals tested were 1) fungicides: benomyl (Benlate 50WP), captan (Captan 50WP), captafol (Difolatan 4F), ferbam (Vancide 95WP), and triforine (EC = CME-74770, 186 g a.i./L; F = CME-10224, 800 g a.i./L; 50WP = CME-10225); 2) insecticides: acephate (Orthene 75S), azinophos-methyl (Guthion 50WP), diazinon (4EC), and malathion (4EC); and 3) adjuvants: thalestol (Triton B-1956, 77%), X-77 (Ortho; alkylarylpolyoxyethylene, glycols, free fatty acids, and isopropanol), and Tween 20 (20 POE [polyoxyethylene sorbitan monolaurate]). The last two adjuvants were considered as 100% active ingredient. Technical-grade triforine and the inert components of CME-74770 were provided by EM Industries (Hawthorne, NY).

Ungerminated pollen was obtained from freshly collected blueberry flowers from a field planting. To seed prepared plates, a newly opened blossom was held by its pedicel with forceps in an inverted position over the agar surface and the forceps were tapped with a pencil. Each plate was seeded with pollen from two blossoms. Seeded plates were incubated at 27 C in darkness for 18 hr unless otherwise noted. Maximum germination of blueberry pollen occurred within 8 hr on 9% sucrose agar in preliminary tests. After incubation, pollen was killed and stained with phenolic rose bengal (1% rose bengal, 5% phenol, and 0.01% $CaCl_2$ [all w/v]). Blueberry pollen is shed as tetrads composed of four pollen grains or cells with each cell capable of producing a pollen tube. The percentage of tetrads with at least one pollen tube and the percentage of pollen cells germinating was determined for 50 tetrads per plate. At least two replicate plates per treatment were included in each experiment. Sections of plates with evenly distributed tetrads were used for counting.

To determine ED_{50} values, chemical concentrations were transformed to $\log_{10}$ and germination was converted first to percentage of the water check and finally to probits. The ED_{50} values were then tested by analysis of variance and means separated by the Student-Newman-Keuls (SNK) test.

RESULTS

Tetrads and cells of highbush blueberry pollen (cultivar Concord) germinated 75 and 33%, respectively, after 18 hr on 9% sucrose agar (water check). Captafol, benomyl + captan, and ferbam were the most toxic to pollen. Each reduced tetrad germination by at least 50% at a concentration < 10 μg/ml (Table 1). The ED_{50} for captan, triforine-EC, diazinon, and X-77 was 50 μg/ml. Pollen cell germination closely paralleled that for tetrads. Some pollen still germinated when the concentration of the following chemicals was 5,000 μg/ml or greater: acephate, captafol, triforine-F, triforine-WP, Triton B-1956, and Tween 20. The ED_{50} values for captan, captan + benomyl, captafol, ferbam, triforine-EC, azinophos-methyl, diazinon, malathion, and X-77 were all lower than the recommended concentrations for use on highbush blueberry.

At a concentration of 0.1 μg/ml, all of the test chemicals except the mixture of benomyl + captan stimulated pollen cell germination of both Bluecrop and Concord pollen (Table 2). They also stimulated tetrad germination in Concord, but stimulation of Bluecrop pollen was not possible because germination in the water check already exceeded 95%.

Of the three formulations of triforine, the EC was the most toxic, completely inhibiting germination of pollen of both cultivars when the concentration reached 100 μg/ml. When each component of the EC formulation of triforine was combined in all possible combinations at the concentration represented by each in a suspension of CME-74770 where triforine was 100 μg/ml, only the emulsifier reduced germination of Bluecrop pollen tetrads and cells ($P \leq 0.001$) (Table 3). This one component accounted for 99.3 and 98.1% of the variation for tetrad and cell germination, respectively.

DISCUSSION

Pollen of highbush blueberry is very sensitive to a number of pesticides and adjuvants commonly used for disease and insect control on this crop. The concentrations of active ingredients in compounds used for disease and insect

Table 1. Effects of pesticide and adjuvant concentration on the germination of highbush blueberry pollen

	Chemical		ED_{50} (μg or μl a.i./ml)		Recommended concentration
Cultivar	Group	Compound	Tetrads	Cells	(μg or μl a.i./ml)
Concord[v] (75,33)[w]	Fungicides	Benomyl	100–500 d[x]	50–100 d	240
		Captan	10–50 c	10–50 c	480
		Benomyl + captan	5–10 b	1–5 a	240 + 480[y]
		Captafol	1–5 a	1–5 a	1,918
		Ferbam	5–10 b	5–10 b	1,366
		Triforine-EC	10–50 c	5–10 b	719
		Triforine-WP	1,000–5,000 e	1,000–5,000 f	719
		Triforine-F	>5,000 f	>5,000 g	719
	Insecticides	Acephate	1,000–5,000 e	1,000–5,000 f	1,798
		Azinophos-methyl	100–500 d	100–500 e	449
		Diazinon	10–50 c	10–50 c	479
		Malathion	100–500 d	100–500 e	749
	Adjuvants	Ortho X-77	10–50 c	10–50 c	1,250
		Triton B-1956	>5,000 f	1,000–5,000 f	301
		Tween 20	>5,000 f	>5,000 g	469
Bluecrop[z] (98,78)[w]	Fungicides	Triforine-EC	10–50 a	5–10 a	719
		Triforine-WP	>10,000 b	1,000–5,000 b	719
		Triforine-F	>10,000 b	>10,000 c	719

[v] Incubated at 27 C in the dark. Maximum concentration tested = 5,000 μg or μl a.i./ml.

[w] Percent germination of tetrads and cells, respectively, on unamended 9% sucrose agar.

[x] Values within columns for each cultivar followed by the same letter are not significantly different ($P \leq 0.01$) according to the Student-Newman-Keuls test.

[y] Each at the indicated concentration.

[z] Incubated at room temperature (23 ± 1.5 C) in the dark. Maximum concentration tested = 10^4 μg a.i./ml.

Table 2. Stimulation of highbush blueberry pollen germination by a very low concentration (0.1 μg or μl a.i./ml) of pesticides and adjuvants

Chemical		Germination (%)		
		Bluecrop[x]	Concord	
Group	Compounds	Cells	Tetrads	Cells
Fungicides	Water check	78 a[y]	75 a	33 a
	Benomyl	–[z]	95 b	53 b
	Captan	–	92 b	50 b
	Benomyl + captan	–	77 a	36 a
	Captafol	–	86 ab	41 ab
	Ferbam	–	93 b	56 b
	Triforine-EC	83 ab	98 b	68 c
	Triforine-WP	89 b	87 b	43 b
	Triforine-F	91 b	89 b	52 b
Insecticides	Acephate	–	87 b	46 b
	Azinophos-methyl	–	90 b	46 b
	Diazinon	–	88 b	44 b
	Malathion	–	94 b	46 b
Adjuvants	Ortho X-77	–	91 b	50 b
	Triton B-1956	–	87 ab	44 b
	Tween 20	–	91 b	45 b

[x] Bluecrop pollen incubated at room temperature (23 ± 1.5 C) in the dark.
[y] Values within columns followed by the same letter are not significantly different ($P \leqslant 0.05$) according to the Student-Newman-Keuls test.
[z] – = Not tested.

Table 3. Effects of the emulsifiable concentrate formulation of triforine (CME-74770) compared with the components of that formulation with and without the emulsifier on the germination of highbush blueberry pollen (cultivar Bluecrop)

Treatment	Germination (%)	
	Tetrads	Cells
CME-74770[a]	0	0
Blank formulation[a]	1	1
Blank formulation without emulsifier[a]	93	67
Water check	93	74

[a] The concentration of triforine is 100 μg a.i./ml.

control generally exceed the in vitro ED_{50} values. Even so, they apparently do not have an adverse effect on yield when applied to blueberries in the field. The EC formulation of triforine when used to control the mummyberry disease did not reduce either fruit set or berry size (2). Of the three formulations of triforine applied to cranberry (*V. macrocarpon* Ait.) twice during bloom, only the EC significantly reduced yield (3). The reduction was due to smaller but not fewer berries.

The in vitro toxicity of the three formulations of triforine to blueberry pollen was the same as that reported for cranberry pollen (3). Others (12,14) have reported that spray adjuvants accentuate the toxicity of the pesticide with which it is combined, leading them to suggest that each component of a pesticide formulation be evaluated separately and in combination for toxicity. When this was done for the EC formulation of triforine, virtually all of the toxicity was accounted for by the emulsifier.

Harley et al (16) demonstrated that Tween 20 had some hormonal activity in the thinning of apples, but it most likely does not act as a pollenicide because of its low toxicity to blueberry pollen. Conversely, the ED_{50} for X-77 was between 10 and 50 μl/ml, exceeding the concentration reported to 1) cause blueberry flower buds and flowers to be more sensitive to cold injury, 2) lower yield, and 3) reduce berry size (21). Fruit size of blueberry (9,18) and cranberry (8,15) is positively correlated with seed number, which reflects the number of ovules fertilized, i.e., the amount of pollen germination.

In vitro testing of pollen to chemicals is a rapid way of identifying those materials that are directly toxic to blueberry pollen. However, field testing is necessary to determine if their use during bloom will adversely affect yield or fruit quality. Materials composing the inert portion of a pesticide formulation may also have biological activity, and the activity may be detrimental to pollen. This inconspicuous yet real form of phytotoxicity needs to be considered when using or testing chemicals on fruit crops where a high level of fruit set is necessary for profitable yields.

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