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Plant Volatiles Inhibit Pollen Germination of Apple and Other Species

Douglas D. Archbold, Thomas R. Hamilton-Kemp, and John H. Loughrin

Department of Horticulture and Landscape Architecture, University of Kentucky, Lexington, KY 40546-0091

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French et al. (1979) reported that volatile compounds similar to those known to affect fungal spore germination in vitro affected germination of *Pinus* spp. pollen. Our objectives in this preliminary work were to screen plant-derived volatile compound mixtures, floral and vegetative, to determine if they influenced germination of pollen from several fruit species.

Apple *(Malus domestica* Borkh. 'Red Delicious' and 'Golden Delicious'), sweet cherry *(Prunus avium* L. 'Van'), and plum *(P. domestica* L. 'Friar') pollen (Antles Pollen Supplies, Wenatchee, Wash.) were stored at -20C over anhydrous $CaCl_2$. Aliquots of stamen were rehydrated in 13 × 100-mm test tubes at room temperature and 100% relative humidity for 30 min. The tubes were shaken to facilitate pollen release; pollen grains were captured on a camel hair brush and dispersed across the surface of a l-cm^3 block of 3% agar (Sigma, St. Louis) by gently moving the brush across a fine mesh screen. The agar block was placed in an uncovered 5-cm glass petri dish contained within a 9-cm glass petri dish. Selected tissues were then placed around the perimeter of the 5-cm dish, and the cover was placed on the 9-cm dish and sealed with parafilm. The sealed dishes were maintained in the laboratory in darkness at ambient temperature. Each treatment was replicated three times and experiments were repeated at least twice.

Pollen germination was assessed in the presence of the following tissues (number used): tomato *(Lycopersicon esculentum* Mill.) leaflets (five), cucumber *(Cucumis sativus* L.) leaves (one), chrysanthemum [*Dendranthema* × *grandiflorum* (Ramat.) Kitamura] leaves (five), strawberry *(Fragaria* × *ananassa* Duch.) leaflets (five), rose *(Rosa* spp.) petals (10), and 'Red Delicious' apple flowers (five). The leaves and rose petals were collected from greenhouse-grown plants in March and April, and the apple flowers were collected from field-grown trees 'at full bloom. With the exception of the rose petals, all tissues were tested either intact or macerated by light grinding in a mortar and pestle. In addition, to determine if ethylene released upon maceration had an effect, intact tomato leaves were dipped in 1000 ppm 2-chloroethyl phosphonic acid (ethephon) before bioassay.

After 2 h, microphotographs (× 40) of the pollen were taken. Four fields from each agar block were photographed. Total and germinated pollen grains were counted from the photographs, recording only single grains on the agar surface. Grains were classified as germinated when the pollen tube length exceeded the diameter of the grain. Percent germination values were derived for each treatment and tested by analysis of variance. After determining that data transformation was not necessary, treatments means were compared by Dunnett's test.

Intact leaves from the tested species did not affect apple pollen germination (Table 1). However, with the exception of chrysanthemum leaves, macerated leaves inhibited 'Red Delicious' apple pollen germination. Neither apple flowers, macerated or intact, nor rose petals influenced apple pollen germination (data not shown). The pollen of 'Golden Delicious' apple, 'Van' sweet cherry, and 'Friar' plum were also inhibited by the volatiles produced by macerated tomato leaves (Table 2). Thus, the response was not species-specific.

Exposure to elevated levels of ethylene failed to affect pollen germination (data not shown); thus, the response to volatiles produced by macerated leaves was not due to ethylene. Ethylene generation by the ethephon-treated leaves at levels three times that of the macerated tissues was confirmed by GC-FID analysis (data not shown). Ethylene has been reported to have no effect or to promote germination in other species (Buchanan and Biggs, 1969; Sfakiotakis et al., 1972). High CO, levels, which may have developed in the enclosed dishes containing macerated leaf tissues, have been reported to stimulate tulip pollen germination (Sfakiotakis et al., 1972), although stimulation was absent in this study. Although low O_2 levels may have influenced the responses, subsequent bioassay work with pure volatile compounds (i.e., no leaf tissue) revealed that the macerated tomato leaf volatiles Z-3-hexenal and E-2-hexenal were inhibitory (Hamilton-Kemp et al., 1991). Thus, altered ethylene, CO_2, and O_2 levels in the dishes were not solely responsible for the observed inhibition of pollen germination. Stimulatory effects similar to those described by French et al. (1979) were not observed. Our work did not separate inhibitory effects on germination from effects on tube elongation.

Table 1. Inhibition of 'Red Delicious' apple pollen germination by leaf tissue as influenced by maceration.

Species	Maceration	Germination (%)
Control		56
Tomato	–	57^{NS}
	+	34*
Control		66
Cucumber	–	60^{NS}
	+	46*
Control		67
Chrysanthemum	–	63^{NS}
	+	60^{NS}
Control		65
Strawberry	–	70^{NS}
	+	46*
Control		57
Apple	+	25*

^{NS},*Nonsignificant or significant by Dunnett's test, $P < 0.05$, control vs. treatment.

Table 2. Inhibition of 'Red Delicious' and 'Golden Delicious' apple, 'Van' sweet cherry, and 'Friar' plum pollen germination by volatiles from macerated tomato leaves.

		Germination (%)	
Species	Cultivar	Control	Volatiles
Apple	Red Delicious	35	19*
	Golden Delicious	45	12*
Sweet cherry	Van	22	2*
Plum	Friar	25	8*

*Significant within each species and cultivar by analysis of variance F test, $P = 0.05$.

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