Inhibition of Pollen Germination by Volatile Compounds Including 2-Hexenal and 3-Hexenal

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Vapors emitted from macerated tomato leaves inhibit germination of apple pollen. Compounds from the leaves were trapped on Tenax and identified by GC-MS. Authentic samples of the tomato leaf components and other volatiles were bioassayed for inhibition of germination, and the amounts of these compounds in the bioassay dish vapor phase were estimated by direct headspace analysis. The lipoxygenase-lyase-derived volatiles, (*Z*)-3-hexenal and (*E*)-2-hexenal, were important factors in the inhibition of pollen germination. Phenylpropanoid-derived compounds can also inhibit germination, but nonpolar compounds tested, such as terpene hydrocarbons, had little or no effect on apple pollen germination.

INTRODUCTION

It is well-known that volatile compounds occur widely in plants including major organs such as leaves, stems, fruits, flowers, and roots. In many cases the functions of these compounds are unknown, but in edible plant parts they contribute to flavor and in flowers they are responsible for fragrances which are thought to attract pollinators (Pellmyr and Thien, 1986). In several recent studies volatiles were shown to stimulate or inhibit the germination of propagules such as seeds (French and Leather, 1979; Bradow and Connick, 1990; Gardner et al., 1990) and fungal spores (French, 1985). There has been a very limited amount of work on the effect of naturally occurring volatiles on pollen, although French et al. (1979) have shown that synthetic compounds can stimulate germination of pine pollen.

Successful pollination of fruit trees is one of the most important factors limiting crop production. Apple pollen was chosen for study because of the economic importance of the fruit and since the pollen is commercially available on a year-round basis for investigation. Recently, Archbold (unpublished results) tested the effects of total volatiles emitted from several plant tissues on apple pollen germination. It was found that many tissues had no significant effect on germination but that crushed tomato leaves strongly inhibited germination. Subsequent tests indicated that the inhibitory factor was not ethylene, a volatile phytohormone, since a Tenax trap placed in an air stream passing over the crushed leaves removed the inhibition. Tenax adsorbs many organic volatiles but has a low affinity for C_2 compounds such as ethylene (Bertsch et al., 1974).

The objective of the present study was to identify the compounds emitted from macerated tomato foliage responsible for inhibition of apple pollen germination. Also, the effects of additional naturally occurring volatile organics including compounds emitted from apple flowers were evaluated to obtain information on the comparative effect of a wide array of volatile compounds on pollen germination.

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EXPERIMENTAL PROCEDURES

Tomato plants, *Lycopersicon esculentum* L. cv. Mountain Pride, were grown in containers in a greenhouse and outdoors.

Bioassay. Five grams of freshly harvested leaves were macerated without added water in a mortar and pestle and placed around the outside bottom portion of a 5-cm glass Petri dish contained within a 9-cm glass Petri dish (volume 120 mL). Within the smaller dish was placed an approximately 1-cm^3 block of 3% agar. Commercially purchased Red Delicious apple pollen (Antles Pollen Supplies, Inc., Wenatchee, WA) was removed from frozen storage (-20 °C) and hydrated for 20 min with a stream of 100% humidified air. The pollen was then dispersed by brushing it through a screen onto the top surface of the agar block. The top cover of the 9-cm Petri dish was replaced, and the outside edge was wrapped with Parafilm to close the dish. The controls were set up in the same manner except no tomato leaf tissue was added. The bioassays were conducted at room temperature under fluorescent lights for 90 min. At the end of the bioassay period the pollen was examined under a microscope at 40 power and photographs were taken. The total number of pollen grains per microscopic field averaged ca. 100, and the germination in the controls ranged from 20-40%. The percent germination data were normalized to the control, which was set equal to 100%.

In tests with authentic compounds, quantities of 1 μL or less of a chemical were dissolved in low-volatility solvents, namely, 1,2-propanediol (Aldrich) or paraffin oil (Fisher, viscosity 335/350). This permitted reproducible introduction of small quantities of the test compounds into the bioassay system. Most compounds were soluble in 1,2-propanediol except nonpolar compounds such as terpene hydrocarbons, which were soluble in paraffin oil. Ten microliters of the solution of the test compound in the solvent was added to 1 mL of Millipore filtered water in a 1 cm diameter glass cup. The cup was placed in the 9 cm diameter Petri dish along with the agar block containing the pollen as described above. Higher levels of compounds (10 or 100 μL) could be introduced accurately into the bioassay dish directly from a syringe, and thus at these levels an authentic standard without solvent was added to 1 mL of water in the 1-cm cup. The cup and the agar block containing the pollen were placed in the bioassay dish. Six replicates of each concentration of authentic compounds were tested in the bioassay.

Isolation and Identification of Volatiles. Freshly harvested tomato leaves (50 g) were macerated with a mortar and pestle and placed in a 500-mL round-bottom flask. The flask was placed in a water bath at 30 °C, and high-purity compressed air was passed over the sample at a flow rate of 500 mL/min. The air stream with entrained volatiles then passed through a Tenax

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trap (6 cm × 1 cm i.d., 1.5 g) which had been conditioned at 200 °C under a stream of nitrogen. After a sampling period of 2 h, the Tenax was eluted with 30 mL of hexane and the solution was concentrated to approximately 1 mL by using a microstill with a Vigreaux column. Tetrahydronaphthalene was added as an internal standard for quantitation. Three samples of tomato leaves were analyzed separately to obtain quantitative data.

The Tenax-trapped headspace samples were chromatographed on a Hewlett-Packard 5880A GC using a 60 m × 0.32 mm Supelcowax [poly(ethylene glycol)] column operated under the following conditions: injector, 220 °C; oven temperature, 50 °C for 1 min and then 2 °C/min to 220 °C; FID, 240 °C; helium carrier, 31 cm/s.

Mass spectra were obtained with a Hewlett-Packard 5985 GC–MS instrument operated at 70 eV and using the same GC column as above. The solutions obtained from several Tenax samplings were combined and concentrated under a stream of nitrogen prior to mass spectral analysis. Compound identification was based on comparison of the mass spectra of leaf volatiles with those of authentic compounds or literature spectra and GC cochromatography unless otherwise noted.

Direct Headspace Sampling of Vapors in Petri Dishes. For estimation of the quantities of a component in the gas phase of a 9-cm Petri dish (120 mL), a gastight 500-μL syringe was used to withdraw a 250-μL sample of vapor. The dish was modified so that a rubber septum was placed in a hole in the center of the top cover. Samplings were carried out 1 h after the test compound was placed in the dish. The dish also contained an agar block and was set up in exactly the same way as described above for bioassay except no pollen was added. The 250-μL vapor sample was injected directly into a Varian 3700 GC with a 30 m × 0.53 mm DB-5 (methylsilicone) fused silica column operated as follows: injector, 220 °C; column temperature, 50 °C for 5 min and then 3 °C/min to 200 °C; FID, 240 °C; helium, 6 mL/min. Samplings were carried out on at least three separate dishes for each level of compound.

Similar direct sampling was done with 5 g of crushed tomato leaves placed in a 9-cm Petri dish, but it was found that neither hexanal and (*Z*)-3-hexenal nor limonene and β-phellandrene could be separated on the above column. To separate these compounds, a Hewlett-Packard 5880A with a 30 m × 0.53 mm DB-Wax [poly(ethylene glycol)] fused silica column was operated as follows: injector, 220 °C; oven, 50 °C for 5 min and then 5 °C/min to 200 °C; FID, 240 °C; hydrogen carrier, 3 mL/min. All quantitative analyses were repeated at least three times.

FID response factors of compounds were determined in the vapor phase by using a 2-L static dilution bottle (Tekmar Co., Cincinnati, OH) according to the method of Winberry et al. (1988). For (*Z*)-3-hexenal and β-phellandrene the response factors of (*E*)-2-hexenal and limonene were used, respectively.

Compounds. Volatiles were purchased from commercial suppliers or were gifts from Bedoukian Research Inc. (Danbury, CT). (*Z*)-3-Hexenal was synthesized from (*Z*)-3-hexen-1-ol according to the method of Kajiwara et al. (1975). β-Phellandrene for bioassay was extracted from intact tomato leaves by using pentane according to the method of Buttery et al. (1987). The extract was concentrated on a microstill and passed through a silica column to remove oxygenated compounds, and the pentane was allowed to evaporate before the bioassay.

RESULTS AND DISCUSSION

Isolation and Identification of Tomato Leaf Volatiles from Tenax Trapping. Identification of the volatile compounds isolated from macerated tomato leaves with a Tenax trap is presented in Table I. The Tenax trapping allowed the accumulation of sufficient quantities of samples for mass spectral analysis. The results show that several terpenes including β-phellandrene, 2-carene, and limonene were major compounds. This agrees with the work of Andersson et al. (1980), Urbasch (1981), Lundgren et al. (1985), and Buttery et al. (1987), who reported that terpene hydrocarbons were predominant compounds in intact tomato leaves.

In addition, the macerated leaves contained large amounts of C_6 aldehydes, which were formed by the action of lipoxygenase and hydroperoxide lyase on polyunsaturated fatty acids, and additional C_6 compounds that were probably formed from the aldehydes through biochemical reactions [see reviews by Galliard and Chan (1980) and Hatanaka et al. (1987)]. (*Z*)-3-Hexenal is formed initially from linolenic acid and may rearrange by chemical means or by means of an isomerase to (*E*)-2-hexenal. Alternatively, the (*Z*)-3-hexenal may be reduced by alcohol dehydrogenase to (*Z*)-3-hexen-1-ol, and this compound may be subsequently esterified to (*Z*)-3-hexenyl acetate. Hexanal is formed from linoleic acid by lipoxygenase–lyase and may be reduced to hexanol by alcohol dehydrogenase. Buttery et al. (1987) noted that intact leaves produced low levels of lipoxygenase–lyase-derived aldehydes but damaged leaves produced large amounts of these products.

Table I. Identification of Headspace Components of Macerated Tomato Leaves Collected on a Tenax Trap for 2 h

compd	evidence[a]	yield, ng of compd/g of leaves[b]
hexanal	MS, RT	1280 ± 289
(*E*)-2-hexenal	MS, RT	2170 ± 636
(*Z*)-3-hexenal	MS, RT	3610 ± 835
1-hexanol	MS, RT	329 ± 67
(*E*)-2-hexenol	MS, RT	173 ± 47
(*Z*)-3-hexenol	MS, RT	2150 ± 405
(*Z*)-3-hexenyl acetate	MS, RT	49 ± 21
2-carene	MS, RT	1480 ± 795
limonene	MS, RT	1530 ± 836
α-phellandrene	MS, RT	369 ± 160
β-phellandrene	MS[c]	6570 ± 3610
α-pinene	MS, RT	107 ± 33
α-terpinene	MS, RT	338 ± 160
caryophyllene	MS, RT	720 ± 380
α-humulene	MS, RT	138 ± 68
benzyl alcohol	MS, RT	20 ± 5

[a] Identification is based on comparison of mass spectral and GC retention time data of plant components with those of authentic compounds unless otherwise noted. [b] Mean of three analyses ± standard deviation. [c] Spectrum is consistent with that for β-phellandrene reported in intact tomato leaves by Buttery et al. (1987).

Apple Pollen Germination Bioassay and Headspace Measurement of Individual Compounds. Tests with 5 g of crushed Mountain Pride leaves showed that there was no pollen germination in the presence of the tomato leaves at the conclusion of the 90-min bioassay. Following identification of the leaf compounds, individual tomato leaf components as well as other volatiles including compounds from apple flowers, which we identified earlier (Loughrin et al., 1990), were tested for their effects on apple pollen germination. In addition, the levels of the compounds in the bioassay dishes were measured.

Table II presents data for the aliphatic compounds which includes the lipoxygenase–lyase compounds identified. Frequently, past investigations on the effects of volatiles on germination have been based on the amount of compound added to the bioassay system as a liquid or in solution, but in the present work we have included measurements of vapor-phase levels of compounds. Among the factors that affected the amount of compound in the vapor phase were solubility in the solvent/water mixture and vapor pressure. We measured the headspace quantities of compounds in this study to better assess how the vapor-phase level of each compound to which apple pollen was exposed affected germination in the bioassay system. Several of the compounds were inhibitory to pollen germination at the highest levels tested. The two unsaturated aldehydes (*Z*)-3-hexenal and (*E*)-2-hexenal were particularly inhibitory. To make a better comparison among the volatiles, the vapor-phase concentrations of compounds were plotted against the percent germination,

Table II. Volatile Compounds Assayed for Activity against Apple Pollen (*Malus* × *domestica* cv. Red Delicious) in Bioassays[a]

μL of compd added[b]	vapor-phase level, μg of compd/L of air	% germination[c]	μL of compd added[b]	vapor-phase level, μg of compd/L of air	% germination[c]
			Aliphatics		
(*E*)-2-hexenal (T)[d]			(*Z*)-3-hexen-1-ol (A, T)		
0	0	100 ± 9.8a	0	0	100 ± 28a
0.01	1.8 ± 0.5	83 ± 26a	0.1	23 ± 4.0	78 ± 19a
0.1	23 ± 5.0	50 ± 19b	1.0	65 ± 9.2	93 ± 22a
1.0	448 ± 139	0 ± 0c	10.0	671 ± 27	28 ± 14b
(*Z*)-3-hexenal (T)			1-hexanol (A, T)		
0	0	100 ± 19a	0	0	100 ± 18a
0.01	11 ± 0.4	49 ± 11b	0.1	12 ± 2.3	124 ± 47a
0.1	36 ± 8.4	11 ± 14c	1.0	154 ± 41	98 ± 21a
1.0	481 ± 187	0 ± 0c	10.0	1640 ± 45	5 ± 6b
hexanal (T)			(*Z*)-3-hexenyl acetate (A, T)		
0	0	100 ± 14a	0	0	100 ± 19a
0.01	4.1 ± 0.6	125 ± 49a	0.01	2.6 ± 0.7	88 ± 22a
0.1	32 ± 3.5	108 ± 27a	0.1	65 ± 18	90 ± 17a
1.0	1280 ± 187	18 ± 6b	1.0	1020 ± 196	0 ± 0b
(*E*)-2-hexen-1-ol (T)			nonanal		
0	0	100 ± 24a	0	0	100 ± 36a
0.1	6.1 ± 0.3	96 ± 33a	0.01	6.1 ± 1.9	101 ± 20a
1.0	59 ± 22	79 ± 25a	0.1	16 ± 10	104 ± 23a
10.0	901 ± 277	11 ± 13b	1.0	334 ± 60	0 ± 0b
			Aromatics		
benzaldehyde			benzyl acetate (A)		
0	0	100 ± 17a	0	0	100 ± 17a
0.1	24 ± 6.5	94 ± 31a	0.1	5.8 ± 1.0	105 ± 22a
1.0	180 ± 54	34 ± 16b	1.0	75 ± 1.8	8 ± 10b
10.0	1550 ± 89	2 ± 3c	10.0	139 ± 7.5	7 ± 6b
benzyl alcohol (A, T)			methyl benzoate		
0	0	100 ± 12a	0	0	100 ± 19a
1.0	2.6 ± 1.2	72 ± 12a	0.1	21 ± 5.4	89 ± 10a
10.0	10 ± 3.7	96 ± 24a	1.0	136 ± 44	49 ± 14b
100.0	55 ± 25	80 ± 24a	10.0	691 ± 164	16 ± 14c
2-phenylethanol (A)			methyl salicylate		
0	0	100 ± 25a	0	0	100 ± 30a
1.0	<1.4	104 ± 20a	0.1	7.4 ± 1.6	104 ± 21a
10.0	31 ± 8.3	114 ± 18a	1.0	62 ± 8.7	42 ± 22b
100.0	39 ± 2.4	104 ± 30a	10.0	161 ± 46	1 ± 3c
			Terpenoids		
2-carene (T)			caryophyllene (T)		
0	0	100 ± 18a	0	0	100 ± 21a
0.01	11 ± 1.5	89 ± 17a	1.0	15 ± 0.4	97 ± 18a
0.1	87 ± 26	76 ± 23a	10	107 ± 4.1	75 ± 23a
1.0	1040 ± 186	71 ± 21a	100	123 ± 17	89 ± 20a
(D)-limonene (T)			α-humulene		
0	0	100 ± 24a	0	0	100 ± 31a
0.01	3.4 ± 0.5	92 ± 24a	1	19 ± 1.0	90 ± 22a
0.1	39 ± 4.8	89 ± 26a	10	91 ± 11	85 ± 25a
1.0	651 ± 108	98 ± 18a	100	149 ± 65	89 ± 31a
(D)-α-pinene (T)			1,8-cineole		
0	0	100 ± 23a	0	0	100 ± 18a
0.01	3.8 ± 0.3	80 ± 42a	0.01	1.6 ± 0.3	102 ● 28a
0.1	64 ± 19	94 ± 25a	0.1	12 ± 2.5	100 ± 20a
1.0	1350 ± 212	95 ± 19a	1.0	618 ± 41	62 ± 11b
(+)-sabinene			(±)-linalool (A)		
0	0	100 ± 23a	0	0	100 ± 22a
0.01	8.0 ± 2.0	95 ± 49a	0.1	5.5 ± 1.8	121 ± 17a
0.1	178 ± 13	74 ± 50a	1.0	145 ± 12	73 ± 39a
1.0	2360 ● 179	59 ± 18a	10	341 ± 30	24 ± 17b

[a] Pollen was prepared as described under Experimental Procedures. Pollen germination was assessed at the end of 90 min. [b] Delivered as dilutions of compounds in 1,2-propanediol except terpene hydrocarbons, 1,8-cineole, and methyl salicylate for which paraffin oil was used. Ten microliters of solution was floated on the surface of 1 mL of water in separate dish. Compounds at levels of 10 μL and above were added neat to 1 mL of water; controls contained 10 μL of paraffin oil or 1,2-propanediol. Values represent the mean of at least three replications ± standard deviation. [c] Normalized to control germination = 100%. Mean of six replications ± standard deviation. Means followed by the same letter are not different by a LSD at $p = 0.05$. [d] T or A indicates that a compound has been identified in the Tenax-trapped volatiles from tomato leaves or apple flowers.

and curves were fitted to the data. From these plots an estimate of the effective dose, ED_{50}, of each compound that inhibited germination by 50% was made and is presented in Table III. It was found that quadratic

Table III. Estimated ED_{50}s of Compounds Assayed for Activity against Apple Pollen (*Malus* × *domestica* cv. Red Delicious)[a]

compd	model	ED_{50},[b] μg of compd/L of air	R^2
(*Z*)-3-hexenal	quadratic	19 (0.19)	0.9735
(*E*)-2-hexenal	quadratic	25 (0.25)	0.9373
benzyl acetate	quadratic	32 (0.21)	0.9926
methyl salicylate	quadratic	51 (0.34)	0.9937
benzaldehyde	quadratic	130 (1.2)	0.9996
methyl benzoate	quadratic	130 (0.96)	0.9996
nonanal	linear	170 (1.2)	0.9971
linalool	linear	220 (1.4)	0.9758
(*Z*)-3-hexenol	linear	480 (4.8)	0.9299
(*E*)-2-hexenol	linear	500 (5.0)	0.9869
(*Z*)-3-hexenyl acetate	linear	510 (3.6)	0.9957
hexanal	linear	760 (7.8)	0.9749
1,8-cineole	linear	790 (5.1)	0.9987
1-hexanol	linear	850 (8.3)	0.9801

[a] ED_{50}s estimated from plots of vapor-phase concentrations of compounds vs percent pollen germination. [b] Values in parentheses are estimated ED_{50}s expressed as micromoles of compound per liter of air.

equations fit the data better for the more inhibitory compounds and linear models fit better for the remaining compounds, as was indicated by coefficients of determination (R^2).

The unsaturated aldehydes were considerably more inhibitory than the saturated aldehyde hexanal. Examples of other processes in which (*E*)-2-hexenal was found to act as an inhibitory compound include seed germination (Bradow and Connick, 1990; Gardner et al., 1990), fungal spore germination (Major et al., 1960), mycelial growth (Zeringue, 1989), and bacterial growth (Schildknecht and Rauch, 1961). It has been suggested that α,β-unsaturated aldehydes, such as (*E*)-2-hexenal, may be toxic due to formation of addition products with groups such as sulfhydryl or generation of Schiff bases with amino groups of proteins (Schauenstein et al., 1977). (*Z*)-3-Hexenal has been shown to be isomerized readily to (*E*)-2-hexenal in plant tissue (Phillips et al., 1979), and a similar reaction might occur in pollen and contribute to inhibition. Interestingly, comparison of the two saturated aldehydes hexanal and nonanal showed that the C_9 compound was considerably more inhibitory than the C_6 compound when the amount of each compound in the vapor phase was taken into account. Similarly, Gardner et al. (1990) showed that the C_9 unsaturated aldehyde (*E*)-2-nonenal was more inhibitory than (*E*)-2-hexenal to germinating seeds.

Comparison of the inhibition of several aromatic compounds is presented in Table II. Generally the aromatic compounds, especially benzyl acetate, inhibited germination. Benzyl acetate is as inhibitory as the lipoxygenase–lyase compounds when compared on a molar basis as shown in Table III. Comparisons of benzaldehyde with benzyl alcohol showed that the former inhibited pollen germination, whereas benzyl alcohol did not at the levels tested. It can be seen that the vapor levels of alcohols such as benzyl alcohol and 2-phenylethanol were low in the bioassay dishes. Most of the aromatic compounds tested have been found as components of flowers (Bauer and Garbe, 1985).

These compounds are thought to arise from phenylpropanoid metabolism through the β-oxidation of cinnamoyl CoA (Luckner, 1973). It is interesting in view of the probable exposure of pollen to vapor of the floral headspace that several aromatic compounds inhibit pollen germination in the bioassay. Benzyl acetate is one of the most inhibitory compounds, and it was identified as a Tenax-trapped volatile from apple flowers (Loughrin et al., 1990). However, the level of this compound to which apple pollen is exposed during pollination processes is unknown.

The third group of compounds investigated were terpenoids, as shown in Table II. In general, these compounds, especially the hydrocarbons, exhibited little or no inhibition of the apple pollen. Assays of compounds such as 2-carene and limonene, which were major components of tomato leaves, showed no significant inhibition at the highest levels tested, even though the amounts in the vapor phase were relatively high. β-Phellandrene could not be obtained commercially, so it was extracted from tomato leaves as described under Experimental Procedures. This compound at levels 4 times those obtained from macerated tomato leaves (as measured by direct headspace sampling described below) did not inhibit germination. The bioassay yielded 98 ± 5.2% germination for β-phellandrene extract vs 100 ± 6.3% for the control. The leaf extract also contained other hydrocarbons such as 2-carene and limonene, but these already had been shown to have little or no effects.

α-Pinene, caryophyllene, and humulene, which were found in tomato leaves, and sabinene were also bioassayed and showed no effect. The terpenes α-phellandrene and α-terpinene, which were not readily available as pure standards, were minor components of tomato leaves and were not bioassayed. Oxygenated terpenes such as linalool and 1,8-cineole were inhibitory to pollen germination, which was similar to the results with other oxygenated compounds.

The primary purpose of the bioassays used in the present study was to determine the level of volatile compounds that inhibited the germination of the apple pollen and to identify the components of crushed leaves which caused the observed inhibition. In some instances there were indications that certain concentrations of volatile compounds stimulated pollen germination, but the level of variation was too great to draw conclusions concerning the significance of this effect. The apple pollen was at the maximum level of germination in the controls at the time (90 min) when the bioassays were terminated. Effects on stimulation could possibly be seen at earlier time periods after the start of the bioassays prior to the development of extensive germination. Stimulation effects are more difficult to discern than inhibition in bioassays of the type used and could best be confirmed in future tests by lower levels of pollen dispersal and shorter bioassay time periods.

French et al. (1979) found that incorporation of small amounts of certain volatiles such as nonanol into a 2% agar solution stimulated germination of pine species pollen placed on the agar for several days. Generally, this was more effective when the pollen was dispersed on the agar at low rates such as a few grains per 90× microscopic field, which limited the amount of self-stimulation of germination. While self-stimulation of germination was not investigated in the present experiments on apple pollen, an effort was made to maintain a level of pollen dispersal (100 grains/40× field) high enough for reproducible germination counts. In cases where aggregates of multiple grains of pollen occurred, there appeared to be increased germination; however, only germination rates for single grains of pollen were used for the experiments.

French et al. (1979) reported that nonylamine was inhibitory to germination of one species of pine pollen and monoterpene hydrocarbons including α-pinene and limonene were inactive on the pine pollen. Similarly, these monoterpenes were also inactive on apple pollen as noted above.

Direct Headspace Measurement of Tomato Volatiles. Studies were done on the amounts of volatiles present in Petri dishes containing macerated tomato leaves.

Table IV. Direct Headspace Measurement of Levels of Crushed Tomato Leaf Volatiles in Pollen Bioassay[a]

compd	level,[b] μg/L of air	compd	level,[b] μg/L of air
2-carene	29 ± 3	(Z)-3-hexenol	7 ± 1
caryophyllene	6 ± 2	limonene	14 ± 2
hexanal	34 ± 19	β-phellandrene	57 ± 11
(E)-2-hexenal	19 ± 8[c]	α-pinene	7 ± 1
(Z)-3-hexenal	28 ± 15[c]		

[a] Five grams of leaves was ground with a mortar and pestle and incubated for 1 h in a 120-mL Petri dish. [b] Mean of three replications ± standard deviation. [c] Level of tomato leaf component measured in bioassay dish similar to estimated ED_{50} value listed in Table III.

The results obtained by using direct headspace sampling 1 h after the leaves were macerated are shown in Table IV. It can be seen that the levels of (Z)-3-hexenal and (E)-2-hexenal in crushed leaves were in the same range as the ED_{50} values obtained with authentic samples of compounds. (Z)-3-Hexenal and hexanal cochromatographed on the nonpolar (DB-5) column used for direct headspace analysis but were separated and quantitated on the polar (DB-wax) GC column. The amounts of other compounds found in the direct headspace of leaves, such as hexanal and the monoterpenes, were well below inhibitory levels for these compounds.

Iwanami (1984) showed that pollen from a lily species stored soaked in organic solvents such as butanol and pentanol for a number of years maintains its viability. It may be as suggested by the author that pollen grains stored in these solvents become dormant but recover their activity when the solvents are removed. In the present studies, the apple pollen apparently was killed after exposure to crushed tomato leaf vapor since germination of the pollen did not occur up to 24 h after removal of the leaves from the bioassay system.

In general it can be seen that certain volatiles are inhibitory to apple pollen germination. Among several plant tissues tested in preliminary experiments, vapors from tomato leaves were inhibitory and the lipoxygenase–lyase products, namely, (Z)-3-hexenal and (E)-2-hexenal, contribute to the leaf-induced inhibition. Nonpolar compounds such as terpene hydrocarbons, at comparable levels, exhibit little or no inhibition of pollen germination.

Volatile compounds are very widespread in plants. Successful pollination is one of the principal factors limiting production in many horticultural species. The present work indicates some of the types of volatiles that are inhibitory to apple pollen germination. Aromatic compounds such as benzyl acetate, which was inhibitory, occur widely in flowers. Further studies may indicate that these compounds affect germination of pollen in flowers of certain species. With regard to lipoxygenase–lyase products, such as (Z)-3-hexenal and (E)-2-hexenal, these compounds are more closely associated with vegetative tissue. Investigations are needed to determine the possible roles of these compounds in the germination of other propagules such as spores of fungal species that cause diseases on plants.

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