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Screening of Nonanal and Related Volatile Flavor Compounds on the Germination of 18 Species of Weed Seed

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Eighteen species of weed seed were tested in light and dark for germination responses to 28 volatile compounds that have been shown previously to stimulate fungal spore germination. Most of these chemicals occur naturally as components of flavors and fragrances. Seeds of curly dock (*Rumex crispus*) and red sorrel (*Rumex acetosella*) were stimulated by nonanenitrile, octyl thiocyanate, 2-nonanol, 2-nonanone, and other compounds. Inhibition was the most common response observed and was expressed by two or more compounds on nine species. Formative effects such as inhibition of radicle, swelling of radicle, swelling and splitting of seed, and production of exudate were observed. The swelling of smartweed seed, forming a turgid spherical body, induced by nonanal and other aldehydes, and the excretion of a gel, induced by citral and related compounds in morningglory, were particularly noteworthy.

Nonanal and 6-methyl-5-hepten-2-one were identified in uredospores of *Puccinia graminis* f. sp. *tritici* and other rusts (French and Weintraub, 1957; Rines et al., 1974). Nonanal or β-ionone, and related flavor components including terpenes, stimulated germination of spores in 24 fungal species, belonging to seven genera: *Puccinia*, *Uromyces*, *Coleosporium*, *Melampsora*, *Ustilago*, *Urocystis*, and *Penicillium* (French, 1961; French et al., 1975b, 1978). Stimulation of germination of pine pollen was also observed (French et al., 1979). The broad spectrum of activity of these volatile, naturally occurring flavor compounds, some of which are also insect pheromones, suggested testing other propagules for stimulation. This report summarizes the results from testing 28 compounds on the germination of 18 species of weed seed.

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MATERIALS AND METHODS

Seed used in this study were obtained from commercial sources. Because of space limitations in the desiccators used to contain the volatile chemicals, seed were tested in two groups as follows: group I—velvetleaf (*Abutilon theophrasti* Medic.), redroot pigweed (*Amaranthus retroflexus* L.), johnsongrass [*Sorghum halepense* (L.) Pers.], curly dock (*Rumex crispus* L.), wild mustard [*Brassica kaber* (DC.) L.C. Wheeler var.], giant foxtail (*Setaria faberi* Herrm.), jimsonweed (*Datura stramonium* L.), Pennsylvania smartweed (*Polygonum pensylvanicum* L.); group II—common morningglory [*Ipomoea purpurea* (L.) Roth], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], green foxtail [*Setaria viridis* (L.) Beauv.], lambsquarters (*Chenopodium album* L.), quackgrass [*Agropyron repens*

Table I

aldehydes	alcohols	ketones
A. saturated		
butyraldehyde	nonanol	2-heptanone
hexanal	2-nonanol	2-octanone
octanal	3-nonanol	2-nonanone
nonanal	4-nonanol	
	5-nonanol	
B. unsaturated		
citral	1-nonen-3-ol	6-methyl-5-
	1-hexyn-3-ol	hepten-2-one
	1-octyn-3-ol	
C. cyclic		
benzaldehyde		β-ionone

sulfur derivatives	nitrogen derivatives
nonyl mercaptan	nonanenitrile
octyl thiocyanate	nonylamine
dimethyl sulfoxide	
nonyl isothiocyanate	

acid and ester	miscellaneous
nonanoic	lime oil
methyl nonanoate	

(L.) Beauv.], common ragweed (*Ambrosia artemisiifolia* L.), red sorrel (*Rumex acetosella* L.), canada thistle [*Cirsium arvense* (L.) Scop.], common purslane (*Portulaca oleracea* L.), buckhorn plantain (*Plantago lanceolata* L.).

The chemical compounds used in this study are volatile oily liquids, fat soluble, sparingly soluble in water, and most have boiling points above that of water. Some may be adsorbed on container surfaces. Consequently, precise concentrations of compounds are difficult to determine. The primary objective of this investigation was to search for biological activity among the test compounds. The tests were performed empirically, in the confined atmosphere of 10-L desiccators, over a 2.5–3 log range in the levels of volatile oily liquids studied.

Seed were counted by an electronic seed counter (Old Mill Co., Savage, MD) or manually. Two replicates of 50 seed were placed on Whatman No. 3 filter paper in open 10-cm plastic petri dishes. Three milliliters of distilled deionized water was added, and dishes were uniformly distributed in 10-L desiccators lined with filter paper and containing 1.36 L of water in the base to maintain a high humidity level. Compounds were added by pipet to 11-cm Whatman No. 1 filter paper disks in the center of the desiccator. Paper disks were supported on a 25-mL graduated cylinder by a cotton swab thrust through the center of the paper. Tests were made in light and dark at 25 °C. Light chambers were set on a 12-h light–dark cycle. Seed receiving dark treatments were placed on the filter paper in the darkroom under a green safelight.

At 3 days, filter papers with residual chemicals were removed, and germinated seed were counted and discarded. Counts were again made at 10 days and percent germination was determined. Formative effects and other growth responses were also noted.

For group I, levels of test compounds used were 0, 0.01, 0.1, and 1.0 mL. Since 1.0-mL amounts of compounds were very inhibitory, the highest dosage was reduced to 0.5 mL in the second series. Hence, for group II, levels were 0, 0.01, 0.1, and 0.5 mL.

The compounds tested are given in Table I.

EXPERIMENTAL RESULTS

Inhibition of Germination. The most common effect of the 28 volatile compounds on the 18 species of weed seed was inhibition of germination (Table II), particularly at the highest concentrations. Maximum inhibition was determined for each compound from the range of concentrations studied: 0.01, 0.1, 0.5, or 1.0 mL of compound per 10 L desiccator. For each chemical, inhibition of germination of 50 or more percentage points below the germination percentage of controls was included in Table II, expressed as L or D, inhibition for light or dark, respectively. Values less than 50 percentage points below controls were deleted.

Seed of five species, johnsongrass, smartweed, velvetleaf, morningglory, and ragweed were not inhibited.

Seed of four species, wild mustard (53%, hexanal), canada thistle (50%, 5-nonanol), common purslane (67%, 3-nonanol), and crabgrass 50%, 4-nonanol) were inhibited in light only by one compound.

In most species, inhibition was greatest in seed exposed to light; with jimsonweed, curly dock, red sorrel, all inhibition was in seed germinated in the light. Inhibition could not be determined in those seed exposed to darkness, since few seed germinated in the dark. Germination of giant foxtail was inhibited only in the dark by hexanal, nonanal, and citral. Germination of green foxtail and lambsquarters was inhibited by many compounds, in both light and dark. Pigweed, green foxtail, and buckhorn plantain were inhibited by 22, 16, and 15 of the 28 compounds, respectively, and by compounds in all major classes of chemical substituents examined.

Stimulation of Germination. Of the 18 species tested, only nine showed stimulation of 20 or more percentage points above controls. Six of these responded to only one of the 28 compounds, as follows: giant foxtail, 20%, dimethyl sulfoxide, dark; quackgrass, 25%, dimethyl sulfoxide, dark; johnsongrass, 24%, benzaldehyde, light; lambsquarters, 25%, octyl thiocyanate, dark; buckhorn plantain, 38%, 2-nonanone, light; and green foxtail, 20%, nonylamine, light. Pigweed responded to two compounds, benzaldehyde, 26% light, and 6-methyl-5-hepten-2-one, 21% dark. Curly dock was stimulated by several secondary alcohols, plus octyl thiocyanate, benzaldehyde, 2-nonanone, 2-octanone, and particularly by nonanenitrile. Germination of curly dock was very low in the dark, and all stimulation observed was in dark germination only (Table III).

Red sorrel was stimulated by 16 of the 28 compounds; stimulation was variable, occurring in light and/or dark. Maximum stimulation of dark germination in the screening tests was induced by nonanenitrile, followed by octyl thiocyanate, 5-nonanol, and 2-nonanone. In light, nonanenitrile, 2-nonanol, 4-nonanol, and 2-octanone were most effective (Table III).

Confirmatory tests of 2-nonanone, octyl thiocyanate, and nonanenitrile verified the stimulatory activity observed on curly dock and red sorrel (Table IV).

Curly dock was stimulated at 3 days by vapors from 0.1 mL of 2-nonanone. At 10 days, 0.1 and 0.5 mL of 2-nonanone were stimulatory. Octyl thiocyanate stimulated at all three levels, even as low as 0.01 mL. Nonanenitrile stimulated at 0.1 and 0.5 mL, and maximum stimulation, 93% (vs. 13.6%, control), was observed at 0.5 mL.

Red sorrel was stimulated at 10 days by all three compounds. Nonanenitrile and 2-nonanone stimulated at 0.1 and 0.5 mL. Maximum stimulation again was observed with nonanenitrile at 0.5 mL. Octyl thiocyanate was stimulatory at all three levels.

Growth or Formative Effects. Besides the inhibition or stimulation of germination, many of the compounds were observed to affect growth of the seedlings in various ways. The most common effect was inhibition in length of the radicle. Radicles of red sorrel, lambsquarters, green

Table II. Maximum Inhibition of Germination of 18 Species[a] of Weed Seed by Two or More of 28 Compounds at 10 Days, 25 °C, in Light and Dark, over the Concentration Range of 0.01, 0.1, 0.5 mL (or 1.0 mL*) Volatile Compound/10 L, Desiccator[b]

	giant foxtail*	pigweed*	jimsonweed*	curly dock*	red sorrel	lambsquarters	buckhorn plantain	green foxtail	quackgrass
aldehydes									
butyraldehyde		LD						LD	
hexanal	D	LD		L					
octanal		L		L	L	D	L	LD	
nonanal	D	LD		L					
citral	D	LD	L			LD	L	LD	
benzaldehyde		L	L	L	L	LD	L	LD	
alcohols									
nonanol		LD					LD	LD	
2-nonanol		L				L	L	LD	L
3-nonanol		L		L	L	LD		LD	
4-nonanol		L	L			LD		LD	D
5-nonanol		L			L	L		LD	LD
1-nonen-3-ol		LD		L		LD		LD	
1-hexyn-3-ol		LD		L	L	LD	D	LD	
1-octyn-3-ol		LD	L	L	L	L	D	LD	L
ketones									
2-heptanone		LD					L		
2-octanone		LD		L			L	D	
2-nonanone		L	L					LD	
6-methyl-5-hepten-2-one		L	L				L		
β-ionone		D					L		
acid and ester									
nonanoic									
methyl nonanoate		L				LD	L	LD	D
S derivatives									
nonyl mercaptan									
nonyl isothiocyanate									
octyl thiocyanate		L					L		L
dimethyl sulfoxide									
N derivatives									
nonanenitrile							L	L	
nonylamine		LD		L	L	LD	L	LD	
miscellaneous									
lime oil		L							

[a] Species inhibited less than 50%: johnsongrass, smartweed, velvetleaf, morningglory, ragweed. (Wild mustard, common purslane, canada thistle, and crabgrass inhibited by only one of 28 compounds. Cited in text.) [b] L = 50% or more inhibition of control germination in light; D = 50% or more inhibition of control germination in dark. Levels of inhibition below 50% deleted.

foxtail, and curly dock were inhibited by 18, 15, 14, and 12 compounds, respectively, of the 28 compounds tested.

Some compounds induced exudation of various types. Nonylamine was associated with a yellowish exudate in nine species, 1-nonanol and methyl nonanoate with whitish exudates in four of the 18 species. Fourteen chemicals induced a gelatinous exudate from morningglory; four and three chemicals induced whitish exudates from lambsquarters and buckhorn plantain, respectively.

Aldehydes, alcohols, and an ester inhibited morningglory germination. The blackened radicles usually were accompanied by the formation of a clear gelatinous exudate. Citral appeared to be the most effective compound in inducing formation of gel. The exudate is assumed to be nonstarchy since it did not react with iodine.

In supplemental experiments, morningglory seed were exposed in desiccators for 3 days to citral over a concentration range of 0.01 to 1.0 mL. The percentage of seed producing gel increased from less than 1 to 11% or more with increasing levels of citral. Germination correspondingly decreased from 10 to 5% (Table V).

Blackening of seed was common with buckhorn plantain, occurring with 15 of the 28 chemicals tested, and activity was observed in all categories except sulfur derivatives.

Other responses noted included swelling of radicles, effects on root hair formation, stimulation of growth, and swelling and splitting of seed.

Swelling and splitting of seed was noted particularly with lambsquarters, morningglory, and smartweed, in which eight, seven, and six compounds, respectively, of the 28 were effective. Aldehydes, alcohols, and nonanoic acid and its ester caused splitting in lambsquarters. The range of effective chemical types was more diverse with morningglory.

Some chemicals, mostly aldehydes, induced swelling in smartweed seed, often without rupturing the integument or producing exudate. Occasionally the seed coat was split completely apart by the swollen embryo plus integument, which had formed a swollen sphere about 3 mm in diameter. In separate experiments, smartweed seed were exposed to nonanal for 16 h, then placed on moist filter paper. After 4 weeks, up to 40% of the smartweed seed were split. This particular lot of seed did not germinate over 10%, and none of the chemical treatments were effective in stimulating germination above this level.

DISCUSSION

Previous studies with uredospores of the fungus causing the stem rust of wheat disease, *Puccinia graminis* f. sp. *tritici*, showed nonanal, 1-nonanol, nonyl mercaptan, and nonanenitrile to be very effective germination stimulators (French and Gallimore, 1971). Nonanal had also been identified in volatiles from viable spores and spore distillates (French and Weintraub, 1957; Rines et al., 1974).

Table III. Optimum Stimulation[a] of Germination over Control Values of *Rumex acetosella* and *R. crispus* at 10 Days 25 °C in Light and Dark[b]

	% stimulation[a]			
	curly dock		red sorrel	
	light	dark	light	dark
aldehydes				
butyraldehyde				24 (7, 0.5)
hexanal				23 (7, 0.5)
octanal				33 (10, 0.1)
nonanal			29 (54, 0.1)	
citral				
benzaldehyde		68 (11, 0.1)		
alcohols				
nonanol				
2-nonanol		62 (9, 1.0)	39 (47, 0.1)	33 (10, 0.1)
3-nonanol		68 (25, 0.1)		
4-nonanol		53 (14, 1.0)	38 (52, 0.1)	45 (19, 0.1)
5-nonanol		63 (10, 1.0)	32 (60, 0.1)	53 (19, 0.5)
1-nonen-3-ol		67 (14, 0.1)	20 (49, 0.01)	
1-hexyn-3-ol				
1-octyn-3-ol				
ketones				
2-heptanone				20 (13, 0.5)
2-octanone		31 (14, 0.1)	36 (47, 0.1)	41 (7, 0.1)
2-nonanone		73 (6, 1.0)	30 (62, 0.1)	48 (16, 0.01)
6-methyl-5-hepten-2-one			33 (51, 0.1)	35 (20, 0.5)
β-ionone				
acid, ester				
nonanoic				
methyl nonanoate				
S derivatives				
nonyl mercaptan			28 (39, 0.5)	
nonyl isothiocyanate				36 (21, 0.1)
octyl thiocyanate		41 (9, 1.0)		59 (13, 0.5)
dimethyl sulfoxide				
N derivatives				
nonanenitrile		84 (6, 0.1)	59 (34, 0.1)	62 (4, 0.1)
nonylamine				
miscellaneous				
lime oil				

[a] Percent stimulation = maximum percent germination resulting from treatment with volatile chemical − % germination of controls. The first figure in parentheses gives the % germination of the controls; the second, the most effective volume (mL) of the compound tested. [b] Concentration range: 0.01, 0.1, and 1.0 mL of volatile compound/10 L desiccator for curly dock, 0.01, 0.1, and 0.5 mL/10 L desiccator for red sorrel. Values under 20% deleted.

Table IV. Effect of Nonanenitrile, Octyl Thiocyanate, and 2-Nonanone on Percent Germination of Curly Dock and Red Sorrel Scored at 3 and 10 Days, in Dark at 25 °C (Six Replicates of 50 Seeds)

dosage and compound (mL/10 L desiccator)	% germination			
	curly dock		red sorrel	
	3 days	10 days	3 days	10 days
control	7.0	13.6	2.4	9.0
0.01 2-nonanone	6.6	14.4	1.6	10.6
0.1	25.0**[a]	73.0**	8.4	49.0**
0.5	12.6	76.4**	0.6	36.6**
0.01 octyl thiocyanate	13.0	49.6**	6.4	43.0**
0.1	9.0	60.4**	4.0	67.4**
0.5	7.4	62.6**	1.6	67.4**
0.01 nonanenitrile	9.4	18.0	3.6	17.6
0.1	15.4	63.4**	7.6	73.0**
0.5	22.4	93.0**	8.0	79.6**

[a] (**) significant at 0.01 level of probability.

Table V. Effect of Citral on Germination and Formation of Gel in Morningglory Seed at 3 Days (Six Replicates of 50 Seeds)[a]

dosage of citral, mL/10 L desiccator	% germination	% seeds with gel
control	10.0	0.8
0.01	9.5	2.5
0.1	4.0*	8.0**
0.25	3.8**	9.2**
0.5	4.3*	12.7**
1.0	5.3	11.0**

[a] (*) significant at 0.05 level of probability; (**) significant at 0.01 level of probability.

Many compounds were active, but 1-nonanol, with a terminal hydroxyl group, thus a primary alcohol, was most active.

Among 18 species of weed seed, two species of *Rumex*, *R. crispus* and *R. acetosella*, were effectively stimulated. From these preliminary screening data, an interesting generic preference for certain compounds is apparent for both *Rumex* species, as compared to uredospores of *Puccinia graminis* f. sp. *tritici*, as well as a species preference between the two *Rumex* species.

Neither *Rumex* species responded to a terminal hydroxyl group or primary alcohol, as 1-nonanol; however, both were stimulated by the secondary alcohols, the 2-, 4-, or 5-nonanols, or 1-nonen-3-ol, and the 2-ketones, 2-octanone and 2-nonanone. In most cases, the nine-C derivatives were somewhat more effective than the eight-C.

Red sorrel was slightly stimulated by four-, six-, eight-, and nine-C linear aldehydes, but not by the cyclic benzaldehyde, which stimulated curly dock. Red sorrel was also stimulated by 6-methyl-5-hepten-2-one and nonyl mer-

captan, which were without effect on curly dock. These observations are based on our screening data and must be considered tentative until definitive structure–activity studies can be made.

The mechanism of action postulated for the stimulation of uredospores of *Puccinia graminis* f. sp. *tritici* by nonanal or nonanol has been that of overcoming an endogenous inhibitor, methyl-cis ferulate, identified by Macko et al. (1971). In uredospores of the bean rust organism, *Uromyces phaseoli*, another endogenous inhibitor, methyl-cis-3,4-dimethoxy cinnamate (Macko et al., 1970, 1972), is overcome by β-ionone, the most effective compound of many tested (French et al., 1977).

Another type of biological activity for some of the fatty alcohols has been reported by Sinohara (1973), who found that 1-octanol was the most effective of the one–eight C alcohols in inducing de novo formation of glucose dehydrogenase in dormant spores of *Aspergillus oryzae*. Octanol is almost as active as nonanol as a stimulator of fungal spores germination (French et al., 1975a). Similarly, Feofilova and Arbuzov (1975) reported that β-ionone, another rust spore stimulator (French et al., 1977), induced de novo formation of carotogenic enzymes in *Blakeslea trispora*.

In this connection, it is interesting to note the effects of nonanal and other aldehydes on the swelling phenomenon of smartweed and the effects of citral and other compounds on the formation and excretion of gels in morningglory seed. As previously mentioned in fungal spores, perhaps certain enzyme systems are being activated in seeds. With smartweed, an end product accumulates which greatly increases osmotic intake of water. With morningglory, an increase in gel-forming enzymes may occur. In both cases the growth process appears to be bypassed. Research on these effects is in progress.

While our studies of the 28 volatile flavor compounds on 18 species of weed seed have not shown effects on all species, several responses are worthy of note and further study, particularly the stimulatory effects of nonanenitrile, octyl thiocyanate, and 2-nonanone. Perhaps these compounds could be used to induce premature germination, under conditions in which the seedlings could not survive, such as just before onset of winter. Compounds such as nonanal and citral, and related chemicals, might be used to develop methods for inactivating species like smartweed and morningglory by inducing swelling or exudation, short-circuiting the growth process, and promoting microbial destruction of the seed. Such speculative possibilities suggest the value of continued screening for activity in compounds related to the flavor group, and of a detailed study of structure–activity relationships and mechanism of action. Besides the organoleptic responses induced by all of the compounds, some of them have previously shown activity as insect pheromones, as inducers of de novo enzyme synthesis, and as stimulators of fungal spore and pollen germination. This study shows that some of the flavor compounds also stimulate germination of seed of two *Rumex* species.

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Volatile Constituents of Some Unifloral Australian Honeys

Anthony D. Graddon, James D. Morrison,* and John F. Smith

A study has been made of the volatile constituents of some unifloral Australian honeys, using a gas chromatograph–mass spectrometer–computer system. The extracts of honey volatiles prove to be complex mixtures of at least 100 compounds. A surprising range of hydrocarbons and oxygenated compounds are present, some of which may be unique to the floral sources.

Honey has played a part in the diet of mankind since the earliest recorded times. Honeys are often very distinctive, each country producing varieties which are sometimes highly prized. Australia, with a flora somewhat different from the rest of the world, produces several rather unusual honeys.

The composition of honey has been studied extensively. Most of the studies reported in the literature have been directed toward quality control and establishing standards for the honey industry and these studies have usually been

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