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Registry No. COB, 125172-14-7; 3-CHO-CB (isomer 1), 125172-15-8; 3-CHO-CB (isomer 2), 125196-92-1; 3′-C=O-COB, 125172-16-9; 2′-COH-COB, 125172-17-0; 3′-CHOH-COB (isomer 1), 125172-18-1; 3′-CHOH-COB (isomer 2), 125172-29-4; 3′-C=O-CB, 125172-19-2; CB, 125172-20-5; 1′-CH_2OH-COB, 125172-21-6; 4′-CH_2OH-COB, 125172-22-7; 2′-COH-triol, 125172-23-8; 3′-CHOH-triol, 125172-24-9; 4′-CH_2OH-CB, 125172-25-0; 1′-CH_2OH-CB, 125172-26-1; AOB, 125172-27-2; AB, 125172-28-3; NADPH, 53-57-6.

Evaluation of Volatile Compounds on the Germination of Seventeen Species of Weed Seeds

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Seventeen species of weed seeds were evaluated for their germination response to twelve naturally occurring, or related, volatile compounds. Germination of curly dock (*Rumex crispus*) was stimulated in darkness by 0.01 and 0.1 mL of methyl salicylate/10 L of air. Styrene, safrole, 2-cyanopyridine, and 5-methyl-2-hexanone also stimulated the dark germination of curly dock. Red sorrel (*Rumex acetosella*) dark germination was 67% (controls 3.4%) when exposed to styrene vapor. Methyl salicylate and benzyl acetate stimulated johnsongrass (*Sorghum halepense*) germination in darkness and light. Inhibition of germination was the usual response in a majority of tests. Vinylpyridines inhibited germination of most of the species tested.

Several naturally occurring volatile compounds stimulate the germination of some weed seeds (French and Leather, 1979) and also stimulate the germination of rust spores, including rusts of weeds (French et al., 1986). French (1985) reviewed the bioregulatory action of volatile flavor compounds on fungal spores and other propagules such as pine pollen and seeds, citing examples of germination stimulation and inhibition, induction of formative or morphological changes, and enzyme induction by this group of compounds. Recently Bradow and Connick (1988a,b) described the inhibitory activity of some volatile allelochemicals from the residues of *Amaranthus palmeri* on tomato, onion, and carrot seed germination. As part of our continuing effort to study mechanisms of controlling propagule germination, we investigated the effects of 12 volatile compounds on the germination of seeds of 17 weed species. These compounds were selected because of previously demonstrated biological activity in other propagules including morphological changes and stimulation of germination.

Table I. Weed Seeds Exposed to Volatile Compounds

common name	genus–species	Bayer code[a]
buckhorn plantain	*Plantago lanceolata* L.	PLALA
Canada thistle	*Cirsium arvense* L. (Scop.)	CIRAR
common lambsquarters	*Chenopodium album* L.	CHEAL
common purslane	*Portulaca oleracea* L.	POROL
common ragweed	*Ambrosia artemisiifolia* L.	AMBEL
curly dock	*Rumex crispus* L.	RUMCR
giant foxtail	*Setaria faberi* Herrm.	SETFA
green foxtail	*Setaria viridis* L. (Beauv.)	SETVI
jimsonweed	*Datura stramonium* L.	DATST
johnsongrass	*Sorghum halepense* L. (Pers.)	SORHA
Pennsylvania smartweed	*Polygonum pensylvanicum* L.	POLPY
quackgrass	*Agropyron repens* L. (Beauv.)	AGRRE
red sorrel	*Rumex acetosella* L.	RUMAA
redroot pigweed	*Amaranthus retroflexus* L.	AMARE
tall morningglory	*Ipomoea purpurea* L. (Roth.)	PHBPU
velvetleaf	*Abutilon theophrasti* Medik.	ABUTH
wild mustard	*Sinapis arvensis* L.	SINAR

[a] Five-letter standardized computer code for common weeds (Bayer, 1983).

MATERIALS AND METHODS

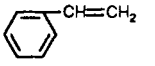
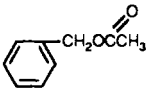
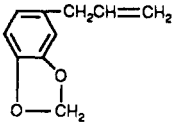
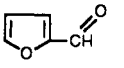
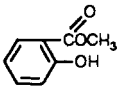
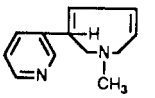
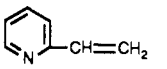
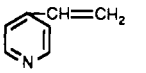
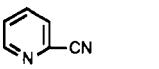
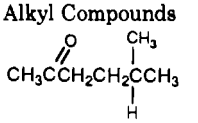
Dormant weed seeds (Table I) were afterripened to provide a germination level of 50% or less in control treatments at 25 °C with a 12-h photoperiod. At this level, inhibition or stimulation of germination could be detected. Seeds that required light for activation germinated less than 5% in the dark. Initially, two replicates of 50 seeds were placed on Whatman No. 3 filter paper in open 10-cm plastic Petri dishes. A 3-mL portion of distilled water was added, and the dishes were randomly distributed in 10-L desiccators lined with filter paper resting in water to maintain high humidity. Within the desiccators, an 11-cm filter paper disk was supported on a 25-mL graduated cylinder by a cotton swab thrust through the paper (French and Leather, 1979).

The primary objective of this investigation was to search for biological activity. Volatile compounds were applied to the filter paper wick at 0.5, 0.1, or 0.01 mL. Because of variations in vapor pressure, solubility in water, and surface absorption of compounds, precise concentrations were not determined. Tests were performed empirically in the confined atmosphere of 10-L desiccators. Each chemical was tested with combinations of 17 weed species × three concentrations × dark/light (17 × 3 × 2 = 102). Compounds (Table II) included nicotine, an aroma compound in tobacco and common insecticide for aphids, three compounds with ethylene moieties (vinylbenzene and two vinylpyridines), and pyridine, which constitutes one ring of the nicotine molecule. The cyclic nitrogen compounds were previously shown to alter metabolic activity in rust uredospores by inducing germination and/or vacuolation (French and Wilson, 1981). Nicotine also induced vacuolation in dormant teliospores of Canada thistle rust (*Puccinia punctiformis*), sunflower rust (*Puccinia helianthi*), and cowpea rust (*Uromyces vignae*) (French, 1988). 5-Methyl-2-hexanone was most effective in stimulating germination of urediniospores of Canada thistle rust (*P. punctiformis*) (French, 1983). The compounds used had various degrees of toxicity; thus, manipulations were carried out in a fume hood.

Tests were made in dark and light at 25 °C. Light chambers were set on a 12-h dark–light (18.8 $\mu E \cdot m^{-2} \cdot s^{-1}$) cycle. All manipulations for the tests in dark chambers were performed under a green safelight in a dark room. After 3 days, the filter paper wicks were removed and germinated seeds counted and discarded. Desiccators were sealed, and germination was allowed to continue to 10 days.

Upon completion of the initial evaluation with all compounds, a second test was performed to confirm any observed germination stimulation. Six replications of 50 seeds were exposed to compounds as previously described. Germination was analyzed by the Student's *t*-test and significance determined by nonoverlapping confidence levels at $P = 0.05$.

Table II. Volatile Compounds Evaluated for Their Effects on the Germination of Weed Seeds

name	formula	occurrence[a]
	Cyclic Compounds	
styrene (vinylbenzene)		cranberry, grape, parsley
benzyl acetate		jasmine, hyacinth, gardenia
safrole		sassafras root, camphor, nutmeg
furfural		tobacco leaves, lavender, eucalyptus oil
methyl salicylate		wintergreen oil, cherry, apple juices
	Nitrogen-Containing Cyclic Compounds	
nicotine		tobacco
pyridine		*Atropa belladonna*, coffee, tobacco
2-vinylpyridine		
4-vinylpyridine		
4-cyanopyridine		
	Alkyl Compounds	
5-methyl-2-hexanone		
trans-2-hexen-1-ol	$CH_3CH_2CH_2CH{=}CHCH_2OH$	raspberry aroma, valencia orange juice

[a] Furia and Bellanca, 1979.

RESULTS AND DISCUSSION

Inhibition of Germination. Inhibition of germination was the most common response of the weed seeds to the compounds in the initial evaluation (Table III). The vinylpyridines were extremely toxic to the seeds, and no germination occurred at concentrations of 0.5 and 0.1 mL of compound/10 L of air. An exception was Canada thistle, which germinated 1–2% in light with 4-vinylpyridine at 0.1 mL/10 L of air (data not shown). 4-Vinylpyridine inhibited germination in 69% of the initial tests (in 70 of a total of 102 tests) while 2-vinylpyridine inhibited germination in 54% of these tests (Table III). Furfural, benzyl acetate, and *trans*-2-hexen-1-ol inhibited germination in 60, 53, and 50% of the initial tests, respectively, but the extent of inhibition by these compounds was less than that of the vinylpyridines. The extensive inhibition was similar to that

Table III. Number of Tested Weed Seed Species with Altered Germination following Exposure to Volatile Compounds

compound	concn, mL/10 L air	dark		light	
		inhib	stim	inhib	stim
methyl salicylate	0.01	1	1	6	2
	0.1	6	3	11	2
	0.5	9	1	12	0
styrene	0.01	2	0	1	0
	0.1	3	1	3	2
	0.5	4	2	5	1
safrole	0.01	2	1	5	2
	0.1	5	1	5	1
	0.5	5	1	7	0
furfural	0.01	6	1	6	0
	0.1	10	1	13	0
	0.5	11	0	15	0
benzyl acetate	0.01	3	0	1	1
	0.1	9	2	13	2
	0.5	12	0	16	0
nicotine	0.01	0	0	1	1
	0.1	2	0	2	1
	0.5	3	0	5	1
pyridine	0.01	4	0	1	0
	0.1	8	0	5	0
	0.5	9	0	10	0
2-vinylpyridine	0.01	4	0	7	0
	0.1	11	0	11	0
	0.5	11	0	11	0
4-vinylpyridine	0.01	10	0	12	0
	0.1	10	0	14	0
	0.5	10	0	14	0
2-cyanopyridine	0.01	1	1	2	0
	0.1	9	1	6	1
	0.5	10	0	10	0
5-methyl-2-hexanone	0.01	2	1	3	1
	0.1	5	1	5	1
	0.5	7	1	10	0
trans-2-hexen-1-ol	0.01	5	0	2	0
	0.1	11	0	11	0
	0.5	22	0	11	0

reported earlier for 28 volatile compounds (French and Leather, 1979).

Stimulation of Germination. Methyl salicylate was the most stimulatory to weed seed germination of all chemicals tested (Tables III and IV). Curly dock dark germination was 25% more than controls after 3 days in 0.1 mL of methyl salicylate/10 L of air and increased to 85% more than controls at 10 days (Table V). Germination of this species was also significantly stimulated in the light; germination at 3 days was 50, 78, and 86% for the control, 0.01 and 0.1 mL of methyl salicylate/10 L of air, respectively, and 54, 88, and 93%, respectively at 10 days. These results agree with previous reports for this compound and weed species (French et al., 1986).

The dark germination of curly dock was also stimulated by styrene, safrole, 2-cyanopyridine, and 5-methyl-2-hexanone (Table V). The 10-day germination was highest at 0.5 mL/10 L of air for these compounds except 2-cyanopyridine, which caused the greatest germination at 0.1 mL/10 L of air. At 0.5 mL/10 L of air (data not shown), 2-cyanopyridine inhibited germination of curly dock. Curly dock appears to be the most sensitive to volatiles. Curly dock seeds were stimulated to germinate by nonanenitrile, octyl thiocyanate, 2-nonanol, and 2-nonanone as reported previously by French and Leather (1979). The mechanism of this stimulation of curly dock germination in the dark is not understood but may involve membrane perturbations and/or phytochrome control of germination (Taylorson and Hendricks, 1973, 1979).

Red sorrel seeds have similar germination requirements as curly dock. In the initial evaluation, red sorrel

Table IV. Species of Weed Seed Stimulated To Germinate by Volatile Compounds

compound	concn, mL/10 L air	dark	light
methyl salicylate	0.01	RS[a]	JG, JW
	0.1	RS, CD, JG	JG, JW
	0.5	CD	
styrene	0.01		
	0.1	RS	RS, GF
	0.5	RS, CD	RS
safrole	0.01		VL, QG
	0.1	CD	VL
	0.5	CD	
furfural	0.01	JG	
	0.1	JG	
	0.5		
benzyl acetate	0.01		WM
	0.1	CD, JG	RS, JG
	0.5		
nicotine	0.01		WM
	0.1		WM
	0.5		WM
2-cyanopyridine	0.01	CD	
	0.1	CD	RS
	0.5		
5-methyl-2-hexanone	0.01	GF	CD
	0.1	CD	CD
	0.5	CD	

[a] Abbreviations: RS = red sorrel; JG = johnsongrass; JW = jimsonweed; CD = curly dock; GF = giant foxtail; VL = velvetleaf; QG = quackgrass; WM = wild mustard.

Table V. Dark Germination of Curly Dock Exposed to Volatile Compounds[a]

volatile concn, mL/10 L air	day 3, %	day 10, %
	Methyl Salicylate	
0.0	2.0 a	2.0 a
0.01	10.0 b	11.0 b
0.1	27.4 c	87.0 c
	Styrene	
0.0	13.6 a	26.0 a
0.01	14.6 ab	29.4 a
0.1	24.4 b	43.6 b
0.5	13.0 a	54.4 b
	Safrole	
0.0	2.6 a	24.0 a
0.01	6.6 ab	29.6 a
0.1	5.0 ab	43.6 b
0.5	11.0 b	44.0 b
	2-Cyanopyridine	
0.0	1.6 a	14.0 a
0.01	12.4 b	19.0 ab
0.1	11.0 b	33.6 b
	5-Methyl-2-hexanone	
0.0	2.4 a	21.6 a
0.01	2.0 a	21.4 a
0.1	5.4 a	28.4 a
0.5	1.4 a	53.0 b

[a] Values in a column for each chemical having the same letters are not significantly different as determined by nonoverlapping confidence limits at the 0.05 level.

was stimulated by methyl salicylate (0.01 and 0.1 mL/10 mL of air, dark), benzyl acetate (0.1 mL/10 L of air, light), and 2-cyanopyridine (0.1 mL/10 L of air, light). Because of variability in germination, subsequent tests failed to show significant stimulation. Stimulation of red sorrel germination was evident at 10 days in dark and light with 0.1 and 0.5 mL of styrene/10 L of air (Table VI).

Germination of johnsongrass seeds was stimulated by low levels of methyl salicylate and benzyl acetate in both

Table VI. Germination of Red Sorrel following Exposure to Styrene Vapors[a]

concn, mL/10 L air	dark day 3, %	dark day 10, %	light day 3, %	light day 10, %
0.0	0.6 ab	3.4 a	27.6 b	49.4 a
0.1	2.0 b	27.0 b	10.6 a	82.6 c
0.5	0.0 a	67.0 b	7.6 a	69.0 b

[a] Values in a column having the same letters are not significantly different as determined by nonoverlapping confidence limits at the 0.05 level.

Table VII. Germination of Johnsongrass Seeds Exposed to Volatile Compounds[a]

concn, mL/10 L air	dark day 3, %	dark day 10, %	light day 3, %	light day 10, %
	Methyl Salicylate			
0.0	2.0 a	5.0 a	8.0 a	8.6 a
0.01	13.0 b	19.4 b	31.0 b	38.0 b
0.1	1.6 a	23.0 b	40.6 b	63.0 c
	Benzyl Acetate			
0.0	1.0 a	2.4 a	6.0 a	12.4 a
0.01	4.0 ab	13.6 b	15.0 ab	27.0 ab
0.1	6.6 b	18.4 b	18.6 b	34.4 b

[a] Values in a column for each chemical having the same letters are not significantly different as determined by nonoverlapping confidence limits at the 0.05 level.

dark and light (Table VII). Stimulation of this species peaked in the light with 0.1 mL of methyl salicylate/10 L of air. The stimulation of germination by methyl salicylate suggests this compound should be further tested as a biological weed control agent. The control strategy would include stimulation of weed seed germination in the fall season with subsequent winter kill of the weed seedlings.

Formative Effects. In many tests where germination was delayed, radicle elongation was also inhibited. A common response was increased radial growth in the zone of elongation, or "spade root" as a result of abnormal root tip growth. These and other formative effects were similar to those reported previously (French and Leather, 1979).

An unusual response was the production of a blue pigment in the cells of the zone of elongation of morningglory radicles during exposure to 2-cyanopyridine. The pigment was produced in dark and light germinated seeds, and at all three concentrations of the chemical. Subsequent experiments have shown that 2-cyanopyridine induced the blue pigment in red-, white-, and blue-flowered varieties, indicating no probable relationship with flower pigment. We have not characterized this blue pigment nor determined the mechanism of this response.

In this research we have shown biological action, including inhibitory, stimulatory, and formative effects in another group of aroma compounds, mostly related to nicotine. The results are similar to those previously reported (French and Leather, 1979). In our first report, the active inhibitory compounds included 2-heptanone, 2-octanone, and 2-nonanone, which inhibited germination of a number of species tested. Connick et al. (1987, 1989) have since identified these compounds in the volatile emanations from Palmer amaranth residues. These compounds have inhibited field germination of tomato, onion, and carrot seed, demonstrating possible allelopathic or ecological effects. Bioactive compounds in the aroma group are different from the usual germination stimulators such as gibberellin. They are volatile, and the effects may be quite transient and hence difficult to measure in a biological system. Evaluating such compounds is one way to establish bioactivity, and knowing the natural occurrence of such compounds, inferences may then be made leading to allelopathic involvement. We have identified new activity in a group of volatile compounds. These include inhibitory, stimulatory, and formative properties. Further research may reveal many types of biological responses initiated at fatty organic/aqueous interfaces by volatile, diffusable, aroma compounds.

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Registry No. Styrene, 100-42-5; benzyl acetate, 140-11-4; safrole, 94-59-7; furfural, 98-01-1; methyl salicylate, 119-36-8; nicotine, 54-11-5; pyridine, 110-86-1; 2-vinylpyridine, 100-69-6; 4-vinylpyridine, 100-43-6; 4-cyanopyridine, 100-48-1; 5-methyl-2-hexanone, 110-12-3; *trans*-2-hexen-1-ol, 928-95-0.