Stimulation of Germination of Teliospores of *Uromyces appendiculatus* by Volatile Aroma Compounds

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More than 100 aroma compounds were tested for activity in stimulating germination of teliospores of *Uromyces appendiculatus*, the fungus that causes bean rust disease. Preliminary volatility tests, in which teliospores on agar were exposed briefly to gaseous emanations from 10 μL of compounds, were useful in quickly selecting stimulatory compounds for more detailed study. Of the various aldehydes, alcohols, esters, ketones, and isothiocyanate derivatives tested, several aldehydes and esters were effective. Isobutyraldehyde (55%), isovaleraldehyde (42%), and furfural (37%) stimulated germination. The esters methyl isobutyrate (60%), propyl propionate (54%), allyl butyrate (44%), and furfuryl propionate (28%) also stimulated germination. The most effective compounds showed maximum activity at 14 days, 19 °C, over the concentration range 25–250 μL/L, in alternating light and darkness. Chemical stimulators may facilitate studies of the sexual cycle of the fungus, which starts with the germination of the teliospores, and may help in the development of resistant bean varieties or lead to new methods of controlling the disease.

INTRODUCTION

Dormant or overwintering fungal spores carry fungi through periods of unfavorable environmental conditions and often germinate very slowly. Teliospores of *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus* [syn. *U. phaseoli* (Reben) Wint.] function in this manner and help to make bean rust disease a worldwide problem in bean production (Stavely and Pastor-Corrales, 1989). Teliospores of *U. appendiculatus* require a dormant period before they germinate (Gold and Mengden, 1983a; Harter et al., 1935; Groth and Mogen, 1978). Gold and Mendgen (1983b) reported germination of these teliospores after 9–48 months of storage at 4 °C and 70% relative humidity when subsequently exposed to a suitable environment. Storage at −18 or 20 °C severely reduced germination, and alternating periods of light and darkness were required for germination.

Many volatile aroma compounds have been reported to stimulate germination of nondormant fungal spores and certain weed seeds as well (French, 1985, 1992). A few volatile compounds have stimulated germination of teliospores. These include a group of tridecyl ethylenic and acetylenic hydrocarbons, active on teliospores of *Puccinia carthami* (Binder et al., 1977; Klisiewicz, 1973), and dodecyl isothiocyanate, active on *Puccinia punctiformis* (French, 1990).

In the research being reported, more than 100 volatile aroma compounds, including aldehydes, alcohols, ketones, esters, isothiocyanates, and heterocyclic compounds, were screened for activity in stimulating the germination of teliospores of *U. appendiculatus*. Previous research has indicated that several species of rust urediniospores or teliospores or weed seed could be activated or stimulated to germinate by brief exposures to the volatile emanations from small amounts of certain aroma chemicals (French, 1985, 1992). The objectives of this research were (1) to find a method for reducing the germination time of teliospores, (2) to test the usefulness of brief exposures of spores to volatiles from aroma compounds as a rapid and reliable way to select germination stimulators, and (3) to study the expanding role of aroma compounds as activators of propagule germination, in this instance, dormant teliospores of the bean rust fungus.

MATERIALS AND METHODS

Teliospores of the bean rust fungus, *U. appendiculatus* (Pers.: Pers.) Unger, formerly *U. phaseoli* (Pers.) G. Wint., were grown on bean plants, *Phaseolus vulgaris* L., cultivar Pinto 111, previously inoculated with race 43 (Stavely, 1984), and grown in the greenhouse in 10-cm pots. Teliospores were collected using a vacuum microcyclone device (Lange et al., 1958) and then cleaned by brushing them through a No. 325 (44 μm) screen. They were stored in screw-capped vials at 4 °C.

Bioassay Techniques. Teliospore germination tests were made on agar in plastic Petri plates. Approximately 2 mg of teliospores was withdrawn from the vial by glass capillary tube and suspended in 2.0 mL of 2-methylbutane (isopentane). Aliquots (0.2 mL) of this suspension were transferred to the surface of 5 mL of 1% water agar, with or without compounds, in 5-cm plastic Petri plates. The solvent evaporated within 90 s and distributed the teliospores evenly over the agar surface.

Volatility Tests. To expedite the search for effective stimulators, teliospores of *U. appendiculatus* on agar were exposed briefly to volatiles from various aroma compounds. Ten microliters of each pungent oily liquid was placed on a 10-mm filter paper disk placed in the center of the underside of a Petri dish lid and closed with the empty bottom of the plate. The lids with chemicals were placed for 2 min over agar plates inoculated with teliospores and then were replaced by the original lids. Plates were held at 18–20 °C in alternating light and darkness for up to 28 days. Compounds were tested in groups of 12, with one control. Six dozen compounds were tested in this manner.

Dosage Response Tests. Direct tests over the concentration range 10–1000 μL/L were made on selected, potentially active compounds. These were measured by 1-μL Hamilton syringe, mixed thoroughly in 5 mL of 1% warm agar, and poured into 5-cm plastic Petri plates, which were then inoculated with teliospores as described previously.

Chemicals Tested. Most of the compounds tested occur naturally in various flavors or fragrances. Some, however (e.g.,

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Table I. Chemicals Tested in the Volatile Phase for Ability To Stimulate Germination of Teliospores of *U. appendiculatus*

	% stimulation[a]		% stimulation[a]
esters		heterocyclic compounds	
ethyl propionate	3	2-acetylpyrrole	0
ethyl butyrate	9	2-acetylfuran	0
ethyl pentanoate	8	2-acetyl thiophene	0
ethyl hexanoate	7	2-acetylthiazole	0
ethyl heptanoate	0	2-acetylpyridine	0
ethyl octanoate	0	2-acetylpyrazine	0
methyl butyrate	16	2-methylpyrazine	0
propyl butyrate	18	2,3-dimethylpyrazine	0
isopropyl butyrate	0	3,5-dimethylpyrazine	0
hexyl butyrate	0	2,6-dimethylpyrazine	0
allyl butyrate	13	2,3,5-trimethylpyrazine	0
benzyl butyrate	0	2,3,5,6-tetramethylpyrazine	0
furfuryl propionate	20	thiazole	0
furfuryl butyrate	3	4-methylthiazole	0
furfuryl pentanoate	0	4,5-dimethylthiazole	0
furfuryl hexanoate	0	2,4,5-trimethylthiazole	0
furfuryl heptanoate	0	2-isobutylthiazole	0
furfuryl octanoate	0	2-ethoxythiazole	0
methyl tiglate	0	2-ethylpyrazine	0
propyl tiglate	0	2,3-diethylpyrazine	0
isopropyl tiglate	0	3-ethyl-2-methylpyrazine	0
hexyl tiglate	0	2-methoxypyrazine	0
allyl tiglate	1	2-methoxy-3-methylpyrazine	0
benzyl tiglate	1	2-isobutyl-3-methoxypyrazine	0
methyl isobutyrate	15		
aldehydes		ketones	
benzaldehyde	0	2-heptanone	0
citral	0	β-ionone	0
isobutyraldehyde	6	6-methyl-5-hepten-2-one	0
isovaleraldehyde	13	5-methyl-2-hexanone	2
nonanal	0	2-nonanone	0
salicylaldehyde	0		
valeraldehyde	2		
isothiocyanates		miscellaneous	
butyl isothiocyanate	0	benzonitrile	0
dodecyl isothiocyanate	0	1-nonanol	0
hexyl isothiocyanate	0	limonene	6
phenyl isothiocyanate	0	nonylamine	0
phenylethyl isothiocyanate	0	nonyl mercaptan	4
		safrole	0

[a] % stimulation = % germination of chemically treated teliospores − % germination of controls. The average of controls = 1.71 ± 0.81 SE.

furfural), may be toxic or hazardous or have foul odors at high concentrations, and safety precautions should be taken when using them. Twenty-four esters and 24 heterocyclic compounds (Table I) were tested in the volatile phase. Compounds active in the volatile phase were then tested in 1% agar at concentrations of 0, 10, 25, 50, 100, 250, 500, and 1000 μL/L. Additional compounds, previously shown to be active with other propagules, or structurally related to active compounds, also were tested and are listed in Table I or II. The most promising active compounds were tested several times and in replicated experiments. Germination counts (8 × 100) were made microscopically at 100× at weekly intervals from 7 to 28 days.

Temperature Tests. The most active compounds, methyl isobutyrate and isobutyraldehyde, were tested over a temperature range by placing plates with teliospores, with and without stimulator, on a thermogradient plate (previously described) (French and Lightfield, 1990) with temperatures ranging from 7 to 31 °C. Teliospores were placed in 15 h of light (40-W cool white fluorescent tube; 8.0 microeinstein m^{-2} s^{-1}) and in constant darkness. Plates in darkness were wrapped in aluminum foil. Total germination time was 14 days, at which time the plates were placed in formaldehyde vapor to stop germination. Germination percentage was determined as described above.

RESULTS

Of the compounds tested in the volatile phase (Table I), none of the heterocyclics, isothiocyanates, or ketones showed appreciable activity. Several esters, including furfuryl propionate (20%), propyl butyrate (18%), methyl butyrate (16%), methyl isobutyrate (15%), and allyl butyrate (13%), stimulated germination more than 10% above controls. Isovaleraldehyde (13%) was the most active of the aldehydes and other miscellaneous compounds tested, including compounds previously shown to be active on other propagules.

Some of the compounds most active in the volatile test were retested over a 10–1000 μL/L concentration range in agar. Some compounds structurally related to those shown to be active also were tested. Among the most active esters were (Table II) methyl isobutyrate (60% at 100 μL/L), propyl propionate (54% at 250 μL/L), allyl butyrate (44% at 100 μL/L), and furfuryl propionate (28% at 25 μL/L). Isobutyraldehyde (55% at 100 μL/L), furfural (37% at 25 μL/L), and isovaleraldehyde (34% at 100 μL/L) were most active among the aldehydes tested.

Most of the compounds stimulated germination at levels of 25–100 μL/L but inhibited it at higher concentrations. Isobutyraldehyde (Figure 1A) and furfural (Figure 1B) stimulated teliospore germination at 25–250 and 10–25 μL/L, respectively. Furfuryl propionate was stimulatory at 10–25 μL/L (Figure 1D). Methyl isobutyrate (Figure 1C) stimulated at 25–1000 μL/L, a much broader concentration range than the other compounds, and it was less inhibitory.

Isobutyraldehyde and methyl isobutyrate, at 100 μL/L (Figure 2), stimulated teliospore germination over the temperature ranges 17–23 and 13–23.5 °C, respectively. Maximum germination with isobutyraldehyde was 35% at 19 °C and with methyl isobutyrate 40% at 18.5 °C at 14 days in 15 h of light. Teliospores in light without

Table II. Maximum Stimulatory Activity of Various Aroma Compounds on Germination of Teliospores of *U. appendiculatus* Tested on 1% Agar over the Concentration Range 10–1000 μL

	μL/L	% stimulation[a]		μL/L	% stimulation[a]
aldehydes			esters		
butyraldehyde	50	29	methyl butyrate	100	11
isobutyraldehyde	100	55	methyl isobutyrate	100	60
hexanal		0	methyl nonanoate		0
2-hexenal	25	2	methyl valerate		0
octanal	25	10	ethyl butyrate	500	9
nonanal	250	7	ethyl isovalerate	100	3
2-nonenal	50	11	ethyl pentanoate	1000	13
2,4-nonadienal	25	2	ethyl hexanoate	100	5
decanal		0	propyl acetate	50	1
2,4-decadienal	25	4	propyl propionate	250	54
2-methylvaleraldehyde		0	propyl butyrate	100	27
isovaleraldehyde	100	34	allyl butyrate	100	44
valeraldehyde	50	19	butyl formate	250	14
dodecanal		0	butyl butyrate	250	12
citral		0	furfuryl acetate	10	3
furfural	25	37	furfuryl propionate	25	28
phenylacetaldehyde	50	2			
alchols			isothiocyanates		
1-butanol		0	octyl isothiocyanate	25	2
1-octanol	100	2	nonyl isothiocyanate	10	1
1-nonanol	50	19	decyl isothiocyanate		0
2-nonanol		0	dodecyl isothiocyanate	250	1
3-nonanol	250	10			
4-nonanol		0	ketones		
5-nonanol		0	2-nonanone	250	2
1-decanol	25	13			
2-decanol	50	12			
dodecanol	25	1			
furfuryl alcohol	10	1			

[a] % stimulation = % germination of chemically treated teliospores − % germination of controls. The average of controls = 2.18% ± 0.5 SE.

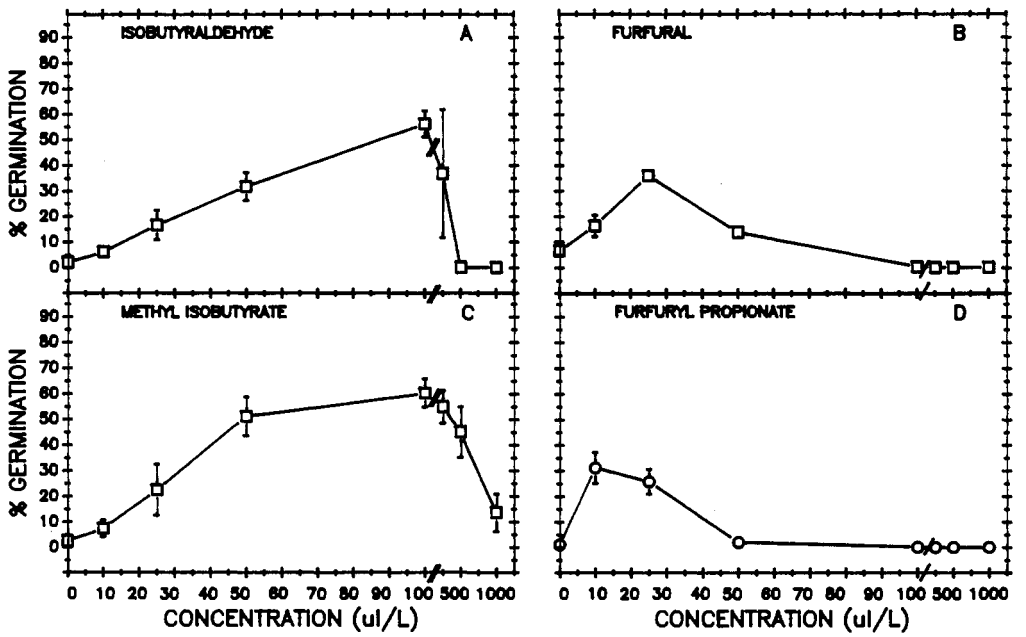

Figure 1. Stimulation of germination of teliospores of *U. appendiculatus* by (A) isobutyraldehyde, (B) furfural, (C) furfuryl propionate, and (D) methyl isobutyrate. Tests were performed over the concentration range of 0–1000 μL/L, 14 days, alternating light and darkness, 18–20 °C. Results show the average of duplicate experiments. Vertical bars = ± SD.

stimulator germinated from 16 to 23.5 °C. Maximum germination was 7%, also at 18.5 °C. Stimulated teliospores in darkness germinated 2% at 23 °C and those without stimulator, 0% at 23 °C. Germination in constant light or darkness was 0%.

DISCUSSION

The volatile exposure test was useful in selecting likely candidates for stimulatory activity. Four of the six compounds rating over 10% in the volatile test (Table I) also were among the most active, over 30% stimulatory, in the dosage response test (Table II). Some compounds were not tested by both techniques, but all of those active were aldehydes or esters having the furfuryl, isovaleryl, isobutyryl, butyrl, or propionyl structures. All have at least one carbonyl oxygen and four to seven carbon atoms.

The volatile test is based on the observation that several propagules, including several species of urediniospores stimulated by different volatile chemical compounds, teliospores of a rust species, and a species of weed seed

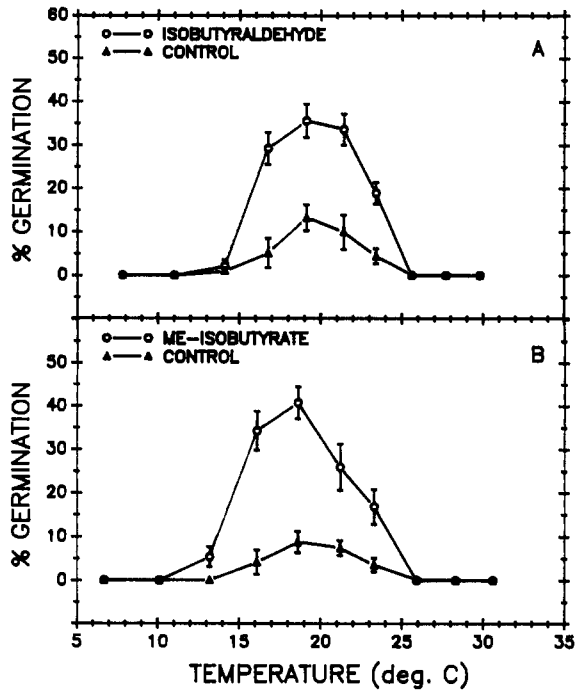

Figure 2. Effect of temperature on stimulation of germination of teliospores of *U. appendiculatus* by (A) isobutyraldehyde (100 μL/L) and (B) methyl isobutyrate (250 μL/L) at 14 days, 15 h of light. Vertical bars = ±SD.

(*Rumex crispus* L.) (French, 1992; French et al., 1986), have been reported to respond to volatile aroma compounds in a very short period of time compared to the actual germination time. For example, urediniospores of different species exposed to volatiles from 10 μL of nonanal, β-ionone, and benzonitrile for 10 s germinated en masse at the end of the normal germination times of 90 and 120 min. The source of the volatiles could be removed after a brief exposure and was not required during the remainder of the germination time. A single exposure of spores to volatiles for 2 min represented a considerable savings in preparation and bioassay time.

The active aldehydes or closely related derivatives have been reported to be stimulatory to other propagules. Isobutyraldehyde and butyraldehyde were among compounds that stimulated *Verticillium dahliae* (Gilbert and Griebel, 1969). Isovaleraldehyde and other related compounds stimulated germination of sclerotia of *Sclerotium rolfsii* (Beute and Rodriguez-Kabana, 1979; Linderman and Gilbert, 1969). Isovaleraldehyde was one of several compounds identified in stimulatory distillates of alfalfa (*Medicago sativa* L.) hay. Isovaleric acid, closely related to isovaleraldehyde, stimulated germination of the spores of *Agaricus bisporus* (Lösel, 1964; Rast and Stauble, 1970). Furfural was previously reported to stimulate the germination of oospores of *Peronosclerospora sorghi* (French and Schmitt, 1980), which causes the downy mildew disease in sorghum (*Sorghum bicolor* L.).

Urediniospores of the bean rust fungus (*U. appendiculatus*) are markedly stimulated by β-ionone and several other volatile ketones, including 6-methyl-5-hepten-2-one (French et al., 1977). 6-Methyl-5-hepten-2-one, along with nonanal, has been found in urediniospores of the bean rust fungus and in several other species (Rines et al., 1974). β-Ionone placed on a filter paper wick in a dew chamber along with rusted bean plants stimulated germination of urediniospores en masse, in the pustules, and after an overnight exposure the brown pustules resembled white bits of cotton because of the masses of germ tubes on the leaves (French, 1992). This occurs at a calculated dose of less than 1 μL/L. β-Ionone, however, did not stimulate germination of the teliospores of *U. appendiculatus*. Dodecyl isothiocyanate, which stimulated teliospores of *P. punctiformis* (Canada thistle rust) (French, 1990), and volatiles from germinating safflower seed containing several 13-carbon acetylenic compounds that stimulated germination of teliospores of *P. carthami* (Binder et al., 1977; Klisiewicz, 1973) did not stimulate teliospores of *U. appendiculatus*.

Thus, the teliospores of *U. appendiculatus* represent yet another example of a propagule that can be triggered to germinate by a narrow range of chemical structures normally classified in the aroma category. It is not yet known if any of these compounds are produced naturally by the bean plants that are susceptible to the disease. Gold and Mendgen (1983b) reported stimulation of germination of teliospores of *U. appendiculatus* by volatile emanations from bean plants.

In summary, we have found several compounds to be active in stimulating germination of the overwintering teliospores of the bean rust fungus, *U. appendiculatus*. Chemical stimulators should be useful in facilitating study of the sexual cycle of the fungus, which begins with the germination of the teliospore, and thus may be of help in genetic studies of resistance or susceptibility or may lead to new techniques of disease control.

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