Effect of Some Nonyl Derivatives and Related Compounds on Germination of Uredospores

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The list of compounds which stimulate rust spore germination was extended to include saturated and unsaturated linear hydrocarbons, esters, and nitrogen and sulfur derivatives. Physical property range of stimulators was extended from water-insoluble, volatile compounds to include nonvolatile, water-soluble and insoluble compounds. Nonane, 1-nonene, nonyl mercaptan, 1,9-nonanedithiol, di-octyl sulfide, and *n*-octyl thiocyanate were stimulatory but were noninhibitory at high concentrations. Direct (nonvolatile) test methods indicated stimulation by 1-nonanol as low as 0.04 ppm or 2.8×10^{-7} *M*. The unique physical and chemical properties of certain of the stimulators suggest their possible use to influence spore germination under various environmental situations.

French and Weintraub (1957) reported that *n*-nonanal is an endogenous stimulator of the germination of uredospores of the wheat stem rust organism *Puccinia graminis* var. *tritici* (Eriks & E. Henn.) Guyot. Later, some 30 or more compounds of diverse chemical composition were reported also to be stimulatory (French, 1961). The substances included long-chain fatty aldehydes, alcohols, ketones, many noncyclic and cyclic terpenes, saturated and unsaturated hydrocarbons, and isoprene. Since that report many additional compounds have been found active and are listed herein. Included are a chemically diverse group of nine-carbon compounds which have been studied in some detail in the hope of finding some clue to the mechanism of their stimulatory action.

Most of the compounds tested were highly odoriferous, oily liquids of low solubility in water. The quantity of chemical tested covered a concentration range below complete water solubility to amounts above the saturation point, in which case the excess floated on the surface of water as an oily film. For quantitative measurement of germination activity, a 1-μl Hamilton syringe was used to apply precise volumes of compound directly into water in the center well of the Conway cell. The concentrations were expressed as μl of liquid/2.0 ml distilled water. Use of the syringe eliminated inaccuracies associated with weighing out minute amounts of nonwater-soluble, volatile, oily compounds and attempting quantitative transfer to water with nonaqueous solvents. Levels of concentration below 0.01 μl required dilution with 95% ethanol. The alcoholic solution was added directly to the 2.0 ml of water upon which the uredospores were placed. Appropriate controls were run to compensate for any stimulation due to ethanol, which was inactive. Maximum alcohol concentration used was 0.05%.

The solid compounds (nonvolatile, sulfonates, amides) were weighed and dissolved or suspended in water for direct tests only. The volatile nine-carbon compounds studied were 95% pure or better, as determined by glc analysis.

Information regarding the water solubility of these compounds is not generally available. The Handbook of Chemistry and Physics (1959) indicated that most of these compounds were insoluble. Buttery *et al.* (1969) determined the solubility of nonanal in water as 96 ppm. Davis (1968) found the solubility to be 110 ppm at 30° C. Using the data of Pierotti *et al.* (1959), the calculated water solubility of some of the compounds used is as follows: *n*-nonanal, 111 ppm (25° C); 1-nonanol, 173; 2-nonanone, 176; *n*-nonanoic acid 196; and *n*-nonane 25 (16° C). Kinoshita *et al.* (1958), using surface tension methods, determined the solubility of nonanol at 25° C to be 0.014 wt%, or 140 ppm. Using density values, the following approximate solubilities were calculated in terms of μl/2.0 ml: *n*-nonanal, 0.26 μl/2.0 ml; 1-nonanol, 0.42; 2-nonanone, 0.42; *n*-nonane, 0.07; and *n*-nonanoic acid, 0.44.

RESULTS

Table I lists the compounds tested for stimulatory activity. Of the active compounds, the amines, amides, nitrile, unsaturated linear alcohols, linear hydrocarbons, esters, thiol, dithiol, thiocyanate, sulfide, and sulfonate derivatives have not previously been reported (French, 1961).

Two compounds notable for their lack of stimulatory activity are 2,5-dimethyl-1,5-hexadien-3-ol and 1-octyn-3-ol. 2,5-Dimethyl-1,5-hexadien-3-ol inhibited in the vapor phase at a dilution of $1:10^2$ was inactive at lower concentrations, and had no inhibitory effect on germ tube length. 1-Octyn-3-ol inhibited at $1:10^3$ showed a very weak stimulation of germination at 10^4, but germ tubes were very short, the length being equal to the width of the tube; lower concentrations were ineffective. The lack of or extremely low activity contrasts markedly with other volatile eight-carbon derivatives which were very active and effective over a wide concentration range.

The nonvolatile octyl and nonyl sulfonates had rather weak activity over a narrow concentration range. Germ tube

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length was somewhat depressed at the active concentrations. While *n*-octyl thiocyanate and 1,9-nonanedithiol (both volatile) were very active over a wide concentration range, they also depressed germ tube length even at a dilution of $1:10^7$. Both compounds as well as dioctyl sulfide did not inhibit germination at the highest levels tested, $1:10^2$.

COMPARISON OF C-9 COMPOUNDS

A detailed comparison of the stimulatory ability of various nine-carbon compounds was made. Nine-carbon compounds were selected because of the availability of a diverse assortment and because they are closely related to *n*-nonanal and 9,10-epoxyoctadecanoic acid found in spores (Tullock and Ledingham, 1960). It was supposed that study of the stimulatory capability of these diverse chemical compounds might help explain the nature of the stimulatory response. Table II summarizes the results of tests of these compounds on spore germination.

The concentration range for maximum stimulation was from 0.005 to 1.0 μl for volatile compounds, or 4.6 to 0.03 mg with the nonvolatile solid compounds.

All of the nine-carbon compounds tested were stimulatory except *n*-nonanoic acid. All of the oxygen-containing liquid compounds were inhibitory at 0.5 to 1.0 μl in 2.0 ml water. 1,9-Nonanediol, a nonvolatile solid, inhibited at 10^{-1} *M*. *n*-Nonanamide, a nonvolatile solid, but also very insoluble in water, did not inhibit in a 10^{-1} *M* suspension of the solid.

All compounds, including the methyl and ethyl esters of nonanoic acid, were stimulatory over a rather broad concentration range. Several compounds were not inhibitory. *n*-Nonane, 1-nonene, *n*-nonyl mercaptan, and 1,9-nonanedithiol were not inhibitory at concentrations as high as 10 μl/2.0 ml. *trans*-2-Nonene, however, was completely inhibitory at 10 μl/2.0 ml.

With the exception of 1,9-nonanediol and sodium nonyl sulfonate, none of these nine-carbon compounds had any appreciable degree of water solubility. At high concentration ranges, 1 μl or higher, the oils could be readily seen floating on the water surface. With 10 to 1 μl of nonyl mercaptan, the uredospores accumulated at the oil–water interface, where germination occurred in contact with the oil. In this case, germination occurred at a high percentage, but germ tubes were very short, in many cases the length of the germ tube being only 1–2 times the diameter of the germ tube. Under these conditions spores often had 3 to 4 germ tubes. At lower concentration ranges germ tube growth appeared not to be inhibited; spores germinated at a high percentage. The activity of 1,9-nonanedithiol was very similar to nonyl mercaptan. Since sulfhydryl groups are often toxic to fungi, the lack of toxicity of the sulfur derivatives was most unexpected. At high concentrations multiple germ tubes and restricted germ tube growth were not noted with the other apparently nontoxic stimulators, *n*-nonane, *trans*-2-nonene, and dioctyl sulfide.

STIMULATION AT LOW CONCENTRATIONS

The stimulatory activity of many compounds appeared to extend below the quantity of chemical that could be delivered directly to water by a syringe. The lower limits of stimulatory concentrations of 1-nonanol and *n*-nonanal were examined in detail. When compounds were first dissolved in alcohol before being added to water, no oily material was observable on the water surface, yet high degrees of stimulation could be obtained.

Table I. Compounds Tested for Stimulation of Uredospore Germination

Volatile Stimulatory Compounds (Dilution v/v)

Sulfur derivatives	Alcohols	Ketones
n-Nonyl mercaptan, 10^5	Hexanol, 10^4	2-Heptanone, 10^4
1,9-Nonanedithiol, 10^4	1-Nonanol, 10^5	3-Heptanone, 10^4
n-Octyl thiocyanate, 10^6	2-Nonanol, 10^5	4-Heptanone, 10^4
Di-*n*-octyl sulfide, 10^2	4-Nonanol, 10^4	2,6-Dimethyl-4-heptanone, 10^5
	5-Nonanol, 10^4	
	1-Nonen-3-ol, 10^4	2-Nonanone, 10^5
Nitrile	1-Nonen-4-ol, 10^4	5-Nonanone, 10^4
n-Nonanonitrile, 10^6		
Hydrocarbons	**Aldehydes**	**Amines**
n-Nonane, 10^4	Hexanal, 10^4	Heptylamine, 10^4
1-Nonene, 10^3	Benzaldehyde, 10^4	Octylamine, 10^4
trans-2-Nonene, 10^4	*trans*-Cinnamaldehyde, 10^3	Nonylamine, 10^4

Nonvolatile Stimulatory Compounds (Dilution w/w)—(Direct Tests)

Amides	Sulfonates	Alcohol
Hexanamide, 0.8×10^6	Na *n*-Octylsulfonate, 0.5×10^3	1,9-Nonanediol, 0.6×10^4
Nonanamide, 0.6×10^5	Na *n*-Nonylsulfonate, 0.4×10^3	

Inactive Compounds

Isobutyraldehyde	Hexanolactone	Methylamine
Valeraldehyde	3-Hydroxy-2-butanone	Diethanolamine
Isovaleraldehyde	1-Octyn-3-ol	*n*-Nonanoic acid
2,5-Dimethyl-1,5-hexadien-3-ol	Dimethylamine	1,7-Nonanedioic acid

Table II. Direct Tests of Various Nonyl Derivatives for Germination Stimulation

Compound	Lowest inhibitory concentration	Concentration of maximum stimulation	Lowest stimulatory concentration
Volatile Liquid Compounds (μl compound/2.0 ml water)			
n-Nonanal	0.5	0.02	0.001
1-Nonanol	1.0	0.1	0.0001
2-Nonanol	0.5	0.1	0.01
4-Nonanol	0.5	0.1	0.01
5-Nonanol	0.5	0.1	0.01
1-Nonen-3-ol	1.0	0.1	0.03
1-Nonen-4-ol	1.0	0.5	0.03
2-Nonanone	1.0	0.01	0.01
3-Nonanone	0.5	0.1	0.01
4-Nonanone	1.0	0.05	0.05
5-Nonanone	1.0	0.1	0.01
n-Nonane		1.0	0.5
1-Nonene		1.0	1.0
trans-2-Nonene	10.0	0.1	0.01
n-Nonylamine	0.1	0.05	0.01
n-Nonanonitrile	0.5	0.005	0.0001
Nonanoic acid	0.5		
Methyl nonanoate	0.5	0.05	0.01
Ethyl nonanoate	0.5	0.05	0.01
Nonyl acetate	0.5	0.01	0.01
n-Nonyl mercaptan		0.5	0.005
1,9-Nonanedithiol		0.01	0.001
Nonvolatile Solid Compounds (mg compound/2.0 ml water)			
1,9-Nonanediol	32	0.32	0.032
n-Nonanamide		0.031	0.0031
Na Nonylsulfonate	46	4.6	4.6

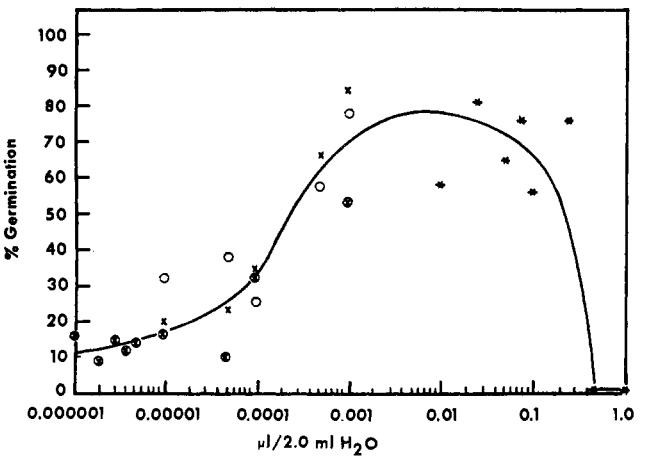

Figure 1. Effect of concentrations of 1-nonanol on uredospore germination. Concentrations below 0.01 μl were prepared by dilution with ethanol. Control germination percentages were as follows: ⊗, 10, *, 13, ○, 20, and ×, 26. Calculated solubility: 173 ppm or 0.42 μl/2.0 ml

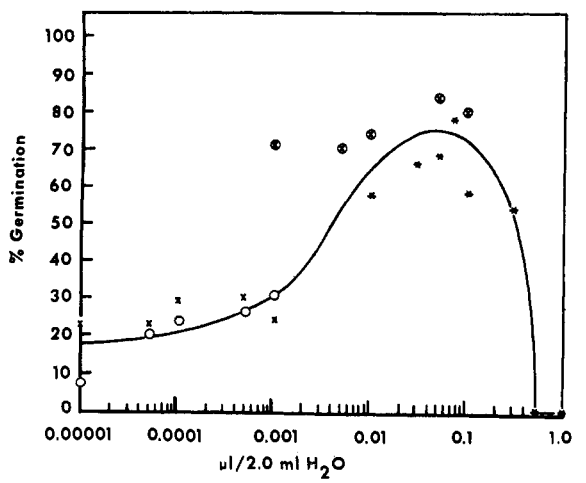

Figure 2. Effect of concentrations of *n*-nonanal on uredospore germination. Concentrations below 0.01 μl were prepared by dilution with ethanol. Control germination percentages were as follows: *, 10, ⊗, 17, ○, 20, and ×, 25. Calculated solubility: 111 ppm or 0.26 μl/2.0 ml

The lower stimulatory limits of *n*-nonanal and 1-nonanol were determined by dissolving the compounds in ethanol and adding carefully measured aliquots into 2.0 ml of water in Conway cells. Stimulation by *n*-nonanol (Figure 1) occurred at a high plateau from 0.25 to 0.0005 μl, with some activity even below 0.0001 μl. At 0.0001 μl the concentration, assuming complete solubility at this low level, was approximately 2.8×10^{-7} *M* or 0.04 ppm. *n*-Nonanal (Figure 2) stimulated at a high level from 0.25 to 0.01 μl, with less activity at 0.001 μl, and possible slight stimulation at lower levels. The alcohol 1-nonanol was appreciably more effective at lower concentrations than the aldehyde *n*-nonanal. Nonanonitrile and octyl sulfocyanate, upon dilution, appeared to be as effective as 1-nonanol.

DISCUSSION

Germination stimulators previously reported were all volatile, mostly with high boiling points and appreciable vapor pressures, and easily detectable by characteristic odors. Compounds included fatty alcohols, aldehydes, ketones, saturated, and unsaturated hydrocarbons, isoprene derivatives, and cyclic compounds. Compounds with 5 to 12 carbons were active.

In this report the list of active compounds is expanded to include volatile esters, mercaptans, sulfides, thiocyanates, nitriles, amines, and amides. Also active were nonvolatile derivatives of stimulatory compounds, the water-soluble 1,9-nonanediol, sodium octyl and nonyl (weak) sulfonates, and water-insoluble *n*-nonanamide. These nonvolatile forms were shown for the first time to be active.

Activity in such diverse groups of chemicals suggests that stimulation of germination does not occur by reaction with one particular enzyme or by enhancement of the concentration of one specific metabolite. All the compounds perhaps could enter into lipid metabolism, or perhaps could displace a naturally occurring substance like *n*-nonanal from an absorbed and inactive site, subsequently making it available to relieve inhibition of the CoA–fatty acid complex, as described by Farkas and Ledingham (1959).

Related compounds have been reported to stimulate growth in other organisms. Crosby and Vlitos (1961) have described long chain fatty alcohols (22 carbons), the terpene phytol, and an unsaturated acid which stimulate tobacco growth. Stowe (1961) has described growth stimulation in pea sections by 12 carbon and longer fats, glycerides, and terpenes such as phytol, and vitamins E and K_1. The diversity of compounds active in stimulating uredospore germination appears to have some features in common with the range of fatty compounds active in stimulating tobacco and pea section growth. Compounds active on uredospores, however, include smaller carbon chain lengths such as isoprene. Nyman (1969) has reported a series of unbranched aliphatic aldehydes to be stimulatory to the growth of the acceleration phase of *Dipodascus aggregatus*, a yeast-like organism. Activity occurred in C-3 to C-11 aldehydes; C-5 and C-12 were inhibitory. *n*-Nonanal was most active; activity was found with 1-nonanol; some activity was found with nonanoic acid, but no activity was found with nonane; and very little activity was found with 2- and 5-nonanones. With uredospores, the C-3 to C-5 aldehydes were not active, while nonane was active. The stimulation of *Dipodascus* thus is different from that of uredospores and offers no clues as to the mechanism of stimulation in uredospores.

The multitude of chemical properties found in the many stimulators has contributed little to any explanation of the mode of action. The lack of activity found with 1-octyn-3-ol and 2,5-dimethyl-1,5-hexadien-3-ol has only further confused the problem. All other eight-carbon compounds tested were active. 1-Nonene-3-ol was active, as were all of the nonyl derivatives except nonanoic acid. At this time one can only point to the chemical stimulation of germination as an interesting curiosity; a ready explanation of the mechanism of action is not forthcoming.

Since the uredospore endogenous germination inhibitor and the mechanism of its action have not yet been determined, the true nature of the stimulation may await further progress in this area. The stimulators may act merely by counteracting this inhibitor, perhaps by influencing cell permeability. It is quite clear that this type of stimulation is rather unique and its mechanism of operation is not subject to an easy uncomplicated solution.

Aside from the interest in mechanism of action, the unique physical and chemical properties of some of the stimulators may qualify them for use as a practical means of controlling

spore germination and hence infection of the host under various environmental conditions.

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