Effect of Pretreatment with Stimulator and Water Vapors on Subsequent Germination and Infectivity of Uredospores of Puccinia graminis var. tritici

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ABSTRACT

Exposure of dry spores to vapors of volatile stimulators in the absence of water vapor had no effect on subsequent germination. Pretreatment of spores in the presence of water vapor with high concentrations of vapors of polar stimulators (*n*-nonanal, *n*-nonanol) reduced subsequent germination and infectivity to zero and damaged spores. Hydration with low concentrations of nonanal and nonanol stimulated germination and infectivity above that of spores exposed to water vapor alone. Hydration in the presence of vapors of the nontoxic hydrocarbon stimulators, even at 10 μliters/4.0 ml water, resulted in subsequent stimulation of germination. Concentration of nonanol, which normally stimulates germination of spores on water, inhibited germination and damaged dry spores when applied in the presence of water vapor as a 2-hr pregermination (hydration) treatment. Exposure of inoculated wheat leaves to short pretreatments with high concentrations of polar stimulators in a moist atmosphere prevented or greatly reduced infection.

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A large number of volatile compounds are capable of stimulating uredospore germination (4). Of these, *n*-nonanal, or pelargonaldehyde, was identified as coming from distilled uredospores (7). However, a product of enzymatic action, nonanol, was also stimulatory (9). Neither has been obtained directly from live spores, although unidentified stimulatory aldehydes were obtained when airstreams were passed through large quantities of uredospores (5). Exposure of uredospores to still or moving moist (100% relative humidity [RH]) air for 2 to 24 hr was also effective in increasing germination of certain spore lots (10). Passing of a moist airstream (100% RH, comparable to hydration) through a mass of spores for several hours increased the yield of stimulatory volatiles. French (5) earlier suggested that enhanced germination of hydrated spores resulted from stimulator released during exposure of spores to the saturated atmosphere.

If high concentrations of water vapor trigger the release of the volatile substances that stimulate germination, then spores exposed to such volatile substances alone should show enhanced germination when transferred to a favorable environment. During exposure, spores would be activated or would accumulate enough volatile compound on the spore to dissolve into the aqueous medium in quantities great enough to stimulate germination. French & Gallimore (6) showed that as little as 0.04 ppm *n*-nonanol or 0.4 ppm *n*-nonanal stimulated germination. We tested *n*-nonanal as a substitute for water vapor in the hydration treatment. Uredospores, therefore, were exposed to water vapor, stimulator vapor, and a mixture of both to determine whether stimulator alone could explain the enhanced germination.

MATERIALS AND METHODS.–Greenhouse-produced uredospores of the stem rust organism, *Puccinia graminis* var. *tritici* (Eriks. & E. Henn.) Guyot, Race 56, retrieved from liquid nitrogen storage were used in most experiments. Such spores are designated cold dormant (1), and usually were used without a heat treatment to break dormancy. In some cases, freshly harvested uredospores were used. Uredospores were dispersed on clean glass cover slips in a settling tower and exposed to vapors in glass desiccators or in Conway diffusion cells covered with ground glass lids. Spores were transferred to small dishes of 1% agar placed in Conway diffusion cells, or the cover slips plus spores were immersed in 2.0 ml distilled water in the center well of a Conway cell. After incubating for 90 min at 20 C, spores were killed with formaldehyde, and per cent germination was determined from an average of 400 spores.

For infectivity studies, paired pots of 1-week-old Baart wheat were inoculated with spores in a settling tower. Pairs were split and placed in separate dew chambers at 20 C for stimulator treatment during the dew period. Inoculated plants in dew chambers were treated with stimulators to study effect on infectivity. Plants in one dew chamber were subjected to *n*-nonanol or *n*-nonanal. Stimulators (0.1 ml or 100 μliters) were placed on filter paper and suspended above the plants in the center of the chamber. After an overnight period, plants were transferred to the greenhouse. After 10 days, the number of pustules per leaf was determined for 10 leaves from plants in each of 10 or more pots. Average pustule values were determined and compared to estimated concentrations of stimulator in air. Inoculated plants were also pretreated with stimulator vapor, with and without water vapor, in large (9L) desiccators for short periods before being placed in the dew chamber with stimulator vapor. Concentration of stimulator, which diffused from filter paper wicks in desiccators or dew chambers, was calculated, assuming complete volatilization. Stimulator effects on spores of inoculated plants were expressed in terms of infectivity (pustules/leaf).

Because the solubilities of some of the compounds

used were not known, all quantities used were usually expressed in terms of μliters of compound per 4.0 ml water. The highest quantities used were greater than the solubility, and the insoluble portion floated on the water surface. Approximate solubilities calculated from data of Buttery et al. (2), Davis (3), and Pierotti et al. (8) at 25 C for the following compounds were: *n*-nonanal, 0.52 μliter/4.0 ml water (96 ppm); *n*-nonanol, 0.83 (173 ppm); 2-nonanone, 0.84 (176 ppm); *n*-nonanoic acid, 0.88 (196 ppm). At 16 C, the solubility of *n*-nonane was 0.14 μliter/4.0 ml (25 ppm). The term "stimulator", as used in this report, refers to a diverse group of predominantly volatile compounds (6) that are capable, at some concentration, of stimulating the germination of uredospores in direct contact with liquid water. The unique germination responses (some toxic or inhibitory) to be described result from pretreatment of "dry" spores with various ratios of stimulator vapor and water vapor before the spores are placed in contact with liquid water.

RESULTS.–*Exposure to stimulator vapor.*–The effect of pretreatment with volatile stimulators on germination was tested by the exposure of uredospores to a *n*-nonanal-saturated atmosphere for as long as 180 min before placement in an environment suitable for germination. Subsequent germination behavior of both cold-dormant and heat-activated spores was unaffected by the *n*-nonanal vapor treatment. Germination of cold-dormant spores was not affected by *p*-cymene, a hydrocarbon chemically less reactive than the aldehyde, nonanal.

Exposure to stimulator plus water vapor.–Spores were placed in a 100% RH atmosphere, in an *n*-nonanal atmosphere, and in a mixture of both. Subsequent germination tests (Fig. 1) revealed the

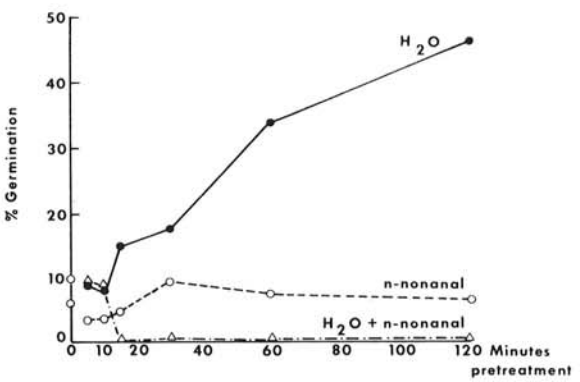

Fig. 1. Exposure of dry uredospores of *Puccinia graminis* var. *tritici* to a saturated atmosphere of nonanal, with and without water vapor, for varying time intervals up to 120 min before start of germination period.

usual enhancement of germination by exposure to water vapor, and no significant response at all to *n*-nonanal vapor. The combination of water and *n*-nonanal vapor, however, reduced germination to zero after 15-min exposure. Exposure to *p*-cymene and water vapor appeared merely to cancel the stimulatory effect of the water vapor, and complete inhibition was not observed. Occasionally on very humid days, apparently dry spores pretreated with an atmosphere of *n*-nonanal were completely inhibited. When anhydrous calcium chloride was added to absorb water vapor, the extreme inhibition did not occur.

The inhibitory effect of the *n*-nonanal and water vapor treatment was independent of temperature. Over the range of 18 to 33 C, germination was zero at six temperature values, 3 C apart.

Concentration of stimulator plus water vapor.–Experiments in which length of time of exposure to volatile substances is employed are in effect concentration studies in which the ultimate concentration of a test substance is determined by its diffusion and deposition on the spore. Concentration effect was also examined by placing varying concentrations of *n*-nonanal in water in the outer ring of Conway cells. With 2-hr pretreatments, the nonanal-water vapor mixtures with concentrations of 1 and 10 μliters *n*-nonanal/4.0 ml water reduced germination to zero (Table 1). The uredospores also appeared damaged. A stimulation above that of the hydrated water control was noted at 0.1 μliter and 0.01 μliters/4.0 ml.

The same general pattern was obtained with *n*-nonanol (Table 2). Pretreatment with concentrations of *n*-nonanol over 0.5 μliter/4.0 ml prevented germination. Germination with 0.01 μliters/4.0 ml was slightly higher than the hydrated controls. Toxicity was noted above and below the saturation point, ca. 0.84 μliters/4.0 ml. Exposures of spores to vapors of either 10, 1, or 0.5 μliters *n*-nonanol/4.0 ml water stimulated spores floated immediately on water; spores exposed to these vapors for 2 hr before being placed on water failed to germinate, and appeared damaged. Cover slips without spores, exposed to nonanol-water vapor, adsorbed only enough *n*-nonanol to stimulate, but not enough to inhibit germination, when immersed in 2.0 ml distilled water and inoculated with untreated spores. This indicates that a quantity of *n*-nonanol great enough to inhibit germination and damage the spores could not have diffused to the cover slip alone during the hydration period. Thus, high concentrations of nonanol were not responsible for failure of spores to germinate. A change in spore

TABLE 1. Effect of 2-hr pretreatment with vapors of nonanal-water concentrations on subsequent germination of spores of *Puccinia graminis* var. *tritici*

Concentration of *n*-nonanal, 4.0 ml H_2O	% Germination
μliter	
0	16.3
0.01	51
0.1	53
1.0	0
10.0	0

TABLE 2. Effect of a 2-hr *n*-nonanol and water vapor pretreatment, compared to *n*-nonanol treatment during germination[a] of uredospores of *Puccinia graminis* var. *tritici*

Concentration of *n*-nonanol/4.0 ml water	% Germination	
	2-hr pretreatment of spores, germination in nonanol-free atmosphere	Nonanol treatment during germination, no pretreatment
μliters		
10	0[b]	14
1	0[b]	56
0.5	1[b]	76
0.1	47	88
0.01	65	58
Control, nonhydrated[c]	3.4	
Control, hydrated[d]	50	

[a] Germination on water in Conway cells.
[b] Damaged.
[c] No nonanol.
[d] Hydrated 2 hr, no nonanol.

metabolism or membrane permeability in response to nonanol-water vapor is a more likely explanation.

Exposure to noninhibitory stimulators.—French & Gallimore (6) reported several stimulators which apparently do not inhibit germination at high concentrations. Uredospores were subjected to their vapors in the presence of water vapor, and the results were compared with the responses to nonanal and nonanol (Table 3). *n*-Nonanal and *n*-nonanol were very toxic. Pretreatment with vapors of the noninhibitory hydrocarbon stimulators, nonane, 1-nonene, and nonyl mercaptan was completely nontoxic, and even stimulated germination. The most polar compounds appeared to be most toxic. Oxygen functional groups in the terminal positions were the most toxic; oxygen in the carbonyl group at carbon two in 2-nonanone was somewhat less toxic. *n*-Nonyl amine was toxic. Dry spores were less sensitive to all stimulator vapors. Vapors of noninhibitory stimulators and the stimulators toxic at high concentrations were all much less effective during pretreatment in the absence of water vapor.

Infectivity studies.—The results of treatment of inoculated wheat plants with stimulator and water vapors were similar to the effects on spores on glass cover slips. Results of treatments are summarized in Table 4.

The presence of nonanol or nonanal during the dew period greatly enhanced subsequent pustule development. The spores used for inoculation were from storage in liquid nitrogen and were cold dormant. The effect of stimulator was that of breaking dormancy and greatly increasing germination.

When inoculated plants were pretreated with both stimulator and water vapors, spores were apparently damaged, and infection was completely prevented during the dew period. Pretreatment of inoculated plants with stimulator vapor alone had no detrimental effect on later pustule development. As with spores

TABLE 3. Germination of uredospores of *Puccinia graminis* var. *tritici* with pretreatment consisting of 2-hr exposure to stimulatory volatiles[a]

Water vapor	% Germination	
	10 *μ*liters compound/4.0 ml water	10 *μ*liters compound, dry
A. With		
Control	38	23
n-nonanol	0	
2-nonanol	0	
4-nonanol	1	
2-nonanone	3	
5-nonanone	1	
Nonane	83	
1-nonene	79	
B. With and without		
Controls	6	2
Nonyl mercaptan	51	1
1-nonene	27	13
Nonyl amine	0	2
n-nonanal	0	0.5

[a] A and B with different spore lots.

TABLE 4. Effect of exposure of inoculated plants to stimulator and water vapors on subsequent pustule (*Puccinia graminis* var. *tritici*) development

Pretreatment (in desiccator)	Dew chamber treatment	Avg pustules/ leaf
None	Dew	3.4
None	Dew + 0.45 ppm nonanol	13.5
13.3 ppm nonanol + water vapor (6 hr)	Dew + 0.45 ppm nonanol	0
None	Dew	1.0
None	Dew + 0.45 ppm nonanal	5.6
13.3 ppm nonanol + water vapor (3 hr)	Dew + 0.45 ppm nonanal	0
None	Dew	1.0
None	Dew + 0.45 ppm nonanal	4.5
0.13 ppm nonanol + H_2O vapor	Dew + 0.45 ppm nonanal	4.1
1.33 ppm nonanol + H_2O vapor	Dew + 0.45 ppm nonanal	5.7
13.3 ppm nonanol + H_2O vapor	Dew + 0.45 ppm nonanal	0
13.3 ppm nonanol $-H_2O$ vapor	Dew + 0.45 ppm nonanal	4.0

on glass slides, spores on wheat plants exposed to stimulator and water vapors together were severely damaged to the extent that no infection occurred.

DISCUSSION.—It is perhaps unrealistic to expect the dry spore to respond to pretreatment with dry nonanal vapor, since it is not mobilizing metabolic machinery prior to the onset of germination. In the presence of water vapor, however, spores have a higher moisture content; hence, enzyme systems are more receptive to substrates and to stimulators which may activate metabolic paths blocked by inhibitors. Low concentrations of stimulators in water vapor probably operate in this way to increase germination. In fact, endogenous *n*-nonanal, released from spores in very humid atmospheres, may diffuse throughout the population to activate the entire lot. Evidence has been presented here for the first time that spores in humid atmospheres can be pretreated with stimulator vapors, including endogenous nonanal, to increase subsequent germination. The authors consider this as further evidence of a role for nonanal in the stimulation of germination induced by hydration.

The deleterious effect of high concentrations of polar stimulator vapors with water vapor is not understood. Perhaps the association has some unusual devastating effect on membrane permeability, an effect which apparently is missing with nonpolar stimulators. The physical state of water in contact with the spore apparently controls the nature of the response to polar stimulator vapor, inasmuch as the interaction with water vapor may yield a toxic effect, but with liquid water, stimulation of germination occurs. In the absence of water vapor, stimulator vapor has little effect.

The varied effects on germination and infectivity of certain water-stimulator vapor combinations may be of some practical importance in control of the rust disease. As an aid to study of the disease, infectivity of hosts may be increased with proper concentrations of stimulator vapors under conditions conducive to dew formation. Although high concentrations of polar stimulators and water vapors have completely suppressed pustule formation on inoculated plants in the laboratory, it is doubtful that such treatment could be made technically feasible under field conditions at this time.

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