developed in BE solvent. If any doubt remains, uv spectroscopy should be used to confirm identity.

Examine the plate developed in CA solvent further under long-wave light to determine if aflatoxin is present. The presence of aflatoxin B_1 is indicated by a distinctly blue fluorescence near R_f 0.54, or at the same position as an aflatoxin B_1 standard. Moieties A and B, which are expected slightly below aflatoxins B_1, are usually quite faint and greenish in color and should not be confused with aflatoxins G_1 and G_2. If aflatoxin B_1 is absent, aflatoxins B_2, G_1, and G_2 are not expected. Assignment of any distinctly blue spot near R_f 0.54 to aflatoxin B_1 should be confirmed by cochromatography with an aflatoxin standard. If AME and AOH are present, the CA solvent is recommended for quantitation of the B aflatoxins even though a slight contribution from moieties A and B might occur. If AME and AOH are absent, the BMA solvent could be tried to move moieties A and B above the aflatoxin region and therefore allow for better quantitation.

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Factors Affecting Chemical Stimulation of Uredospore Germination in Pustules of Crown Rust of Oats, Common Corn Rust, Stem Rust of Wheat, and Leaf Rust of Wheat

Richard C. French,* Anne W. Gale, Charles L. Graham, and Howard W. Rines[1]

The stimulatory activity of nonanal and related compounds on uredospore germination, previously reported on *Puccinia graminis* F. sp. *tritici* (stem rust of wheat), also has been observed on *P. coronata* F. sp. *avenae* (crown rust of oats), *P. recondita* (leaf rust of wheat), and *P. sorghi* (common corn rust). The effects of type of compound, concentration, and temperature on germination of uredospores in pustules were examined. Volatile chemical stimulators at concentrations of less than 1 ppm by volume, applied during exposure of rust pustules to dew in a sealed chamber, induced germination of spores of *P. coronata, P. sorghi, P. graminis,* and *P. recondita,* over a temperature range of 15–25°. 1-Nonanal, 1-nonanol, 1-octanol, and 6-methyl-5-hepten-2-one stimulated germination of these rust spores floated on water. Germination in pustules of *P. coronata* and *P. graminis* was induced by all four compounds; spores of *P. sorghi* and *P. recondita* did not respond in the pustule to methylheptenone under the conditions examined. Nonanal was most effective in stimulating germination of uredospores of *P. recondita* in the pustule.

1-Nonanal, an endogenous spore-germination stimulator (French and Weintraub, 1957), was identified in *Puccinia graminis* uredospore distillates. Rines *et al.* (1974) found nonanal and 6-methyl-5-hepten-2-one in *P. graminis* spore distillates and in moist air streams passed through fresh spores. In addition, nonanal was found in distillates of uredospores of *P. coronata, P. sorghi, P. recondita,* and other rusts. Of those rust uredospores studied, only these three species were chemically induced to germinate by methods then in use. Germination of spores of *P. graminis* in the pustule by action of stimulators in a closed chamber under dew-forming conditions had been reported by French and Gallimore (1972b). The object of this research was to examine several species of rust for the capability of being stimulated by nonanal and related compounds, and to check for stimulation of germination over a broad temperature range, so that the limitations of in-pustule germination as a practical technique of disease control might be further evaluated.

Plant Disease Research Laboratory, U. S. Department of Agriculture, Agricultural Research Service, P.O. Box 1209, Frederick, Maryland 21701.
[1] Present address: Department of Botany, University of Georgia, Athens, Georgia 30601.

MATERIALS AND METHODS

Uredospores of *Puccinia coronata* Cda. F. sp. *avenae* (crown rust of oats), *Puccinia recondita* Rob. ex Desm. f.

Table I. Effect of Nonanal, Nonanol, Octanol, and Methylheptenone on Germination of Uredospores of *P. coronata*, *P. recondita*, and *P. sorghi*

	% germination ± standard error											
	P. coronata				*P. recondita*				*P. sorghi*			
Concn, ppm	Nonanal	Nonanol	Octanol	Methyl-heptenone	Nonanal	Nonanol	Octanol	Methyl-hepte-none	Nonanal	Nonanol	Octanol	Methyl-hepte-none
1000	0±0	0±0	0±0	88±1.5	0±0	0±0	0±0	4±1.8			0±0	
500	0±0	0±0	0±0	94±1.8	0±0	1±1.7	2±1.5	85±2.0			.2±0.2	
250	0±0	1±1.0	72±5.6	95±1.6	12±2.5	1±1.0	38±1.6	97±1.0	62±2.9	1±0.8	50±3.9	47±2.0
100	90±3.5	70±2.4	87±1.7	94±1.5	89±2.2	84±1.0	82±4.3	95±0.6	58±2.4	41±5.0	62±4.7	48±6.0
50	96±0.9	91±0.8	88±1.6	71±5.5	86±1.0	92±1.8	82±1.3	96±1.2	69±4.4	51±1.1	61±2.0	53±6.8
25	98±0.4	83±1.9	87±2.1	67±6.0	82±3.3	94±1.6	83±3.0	96±0.5	63±2.0	45±2.9	63±7.3	60±8.5
5	89±3.4	91±0.6	87±6.0	9±3.8	17±4.5	86±5.7	79±1.7	51±13.1	46±7.7	50±3.8	65±1.6	32±4.7
0.5	6±0.5	45±6.1	39±6.1	2±1.9	1±0.4	36±9.4	14±1.5	12±2.6	24±2.5	35±8.5	59±2.8	27±9.3
0.05	4±2.1	1±0.4	22±10.1	1±0.5	6±1.9	9±1.3	4±2.0	31±8.3	17±3.8	32±4.5	37±6.5	23±7.7
0	1±0.6	3±0.9	10±2.8	1±0.3	4±1.2	1±0.3	4±1.0	21±4.8	20±3.0	19±2.8	43±2.5	23±2.7

sp. *tritici* Eriks. (leaf rust of wheat), *Puccinia sorghi* Schw. (common corn rust), and *Puccinia graminis* F. sp. *tritici* (Eriks. & E. Henn.) Guyot (stem rust of wheat) were grown in the greenhouse on Clinton oats, Baart wheat, and Funks 4444 corn and used directly from infected plants. Vials of spores of *P. graminis* from liquid nitrogen storage were heated 5 min at 40° before use in temperature studies with methylheptenone. Spores of *P. sorghi*, also from liquid nitrogen, were used directly for dosage response studies.

Dosage response curves for the various stimulatory compounds were run in 7 × 27 mm glass dishes containing test compounds in 0.5 ml of distilled water. The small dishes were isolated in covered Conway cells. Germination percentage was determined from four counts of 100 spores.

Spores were prefloated not more than 10 min on distilled water in a 5-ml beaker. Enough spores were added by a 3-mm platinum transfer loop to cover the surface of the test solutions uniformly. Tests were run at room temperature (19–22°) in the dark for 90 min and terminated by addition of formaldehyde.

Spores for temperature studies were dispersed on sections of Millipore filter, then transferred to equilibrated 1% water–agar in 50-mm plastic petri dishes with and without 10 ppm (by volume) of 1-nonanol or 250 ppm of 6-methyl-5-hepten-2-one (25 ppm for corn rust) in various incubators from 6 to 30°, ±1° for 2 hr, and then killed by formaldehyde vapor.

Germination in pustule was observed by placing rusted plants grown in 4-in. clay pots in a sealed dew chamber overnight, with stimulatory chemicals on filter paper as previously described (French and Gallimore, 1972b). Effectiveness of treatment was expressed as an estimated percentage of the pustules on the plant that showed whitish cottony germ tubes.

EXPERIMENTAL RESULTS

Stimulation of Germination. The effects of 1-nonanal, 1-nonanol, 1-octanol, and methylheptenone on germination of uredospores of *P. coronata*, *P. recondita*, and *P. sorghi* are summarized in Table I. Spores of *P. coronata* and *P. recondita* were markedly stimulated by nonanol, octanol, nonanal, and methylheptenone, in approximate order of effectiveness. Spores of *P. sorghi* were also stimulated, but not as much as the other rusts, and spore concentrations had to be high, as described above, in order to demonstrate stimulation over controls. Spores of *P. sorghi* may contain less endogenous inhibitor, thus requiring a greater number of spores to be present before control germination is noticeably inhibited. Once a spore concentration showing self-inhibition had been reached, however, all of the compounds tested showed some stimulation. Increased numbers of spores of the other rusts also resulted in lower control germination values for the same reason—more inhibitor present (Macko *et al.*, 1971; Macko and Staples, 1973)—but stimulators were very effective in bringing about excellent germination. In all instances, nonanol and octanol were slightly more effective and active, over a wider concentration range, than nonanal. Methylheptenone was not as effective as nonanal.

Temperature Effects. Stimulation by 10 ppm of nonanol was observed over a wide range of temperatures (6–30°) with *P. coronata*, *P. recondita*, *P. graminis*, and *P. sorghi*. Germ tubes were very short at the extremes of the temperature range, 6 and 30°, where the germ-tube's length was often no greater than its width. Stimulation over the range of 12 to 28 or 30° is shown in Figure 1A-D, for the four rusts.

The response to 250 ppm of methylheptenone (Figure 2A–C), near the optimum concentration for stimulation, was similar for *P. coronata*, *P. recondita*, and *P. graminis*, in that stimulation was observed at virtually all temperatures. Spores of *P. sorghi* were inhibited at all temperatures by 250 ppm of methylheptenone in agar (not shown). At 25 ppm, methylheptenone stimulated at 12, 16, and 20°, but inhibited at 23, 26, and 28°. The same pattern of inhibition at higher temperatures with 25 ppm of methylheptenone in agar was noted with several spore lots of *P. sorghi*. A typical response is shown in Figure 2D. In water, both 250 and 25 ppm of methylheptenone stimulated germination of spores of *P. sorghi* at 20°; at 26°, 250 ppm inhibited and 25 ppm of methylheptenone stimulated. From the standpoint of both temperature and concentration, *P. sorghi* spores appear different from the other three rusts in the nature of the response to methylheptenone. The presence of 1% agar appears to increase inhibition of germination of spores of *P. sorghi* with certain concentrations of methylheptenone at higher temperatures. We have no explanation for this response.

Both nonanol and methylheptenone were examined as spore stimulators over a wide temperature range, with 1% agar as a germination medium. The unstimulated germination values did not always reach the same optima from experiment to experiment, but were usually at adjacent points of the curve. Subtle environmental factors, such as prior exposure to unknown temperatures and moisture regimes, different microbiological contaminants (French *et al.*, 1964; Searles and French, 1964), and previous exposures to endogenous volatile stimulators and inhibitors (French, 1973), which may occur in different areas of the

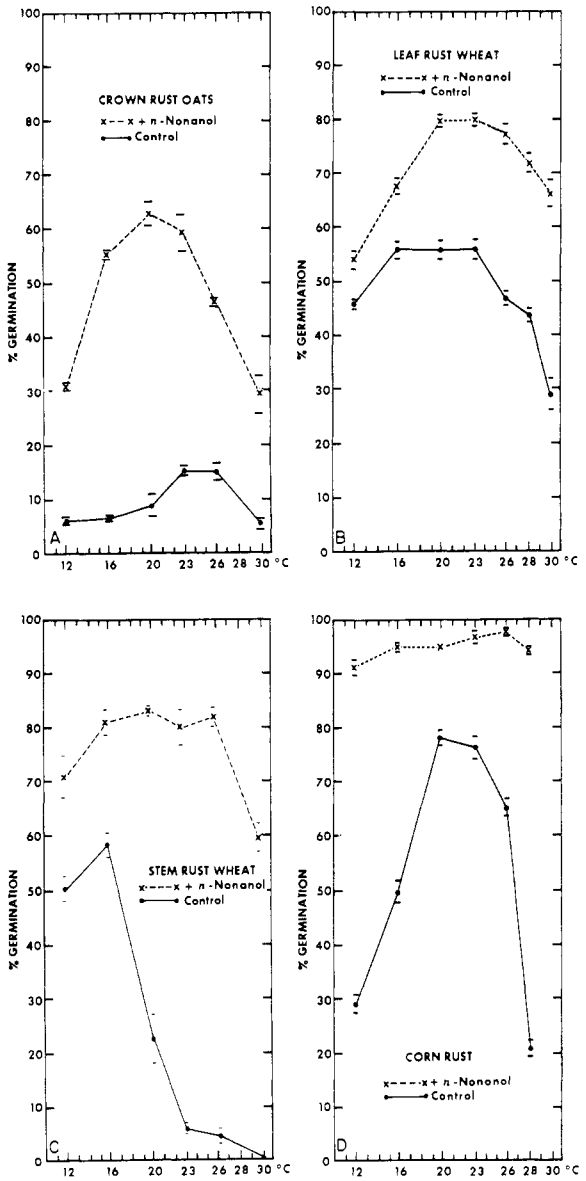

Figure 1. Effect of ±10 ppm of 1-nonanol on 2-hr germination of uredospores of *P. coronata* (A), *P. recondita* (B), *P. graminis* (C), and *P. sorghi* (D) in response to temperature.

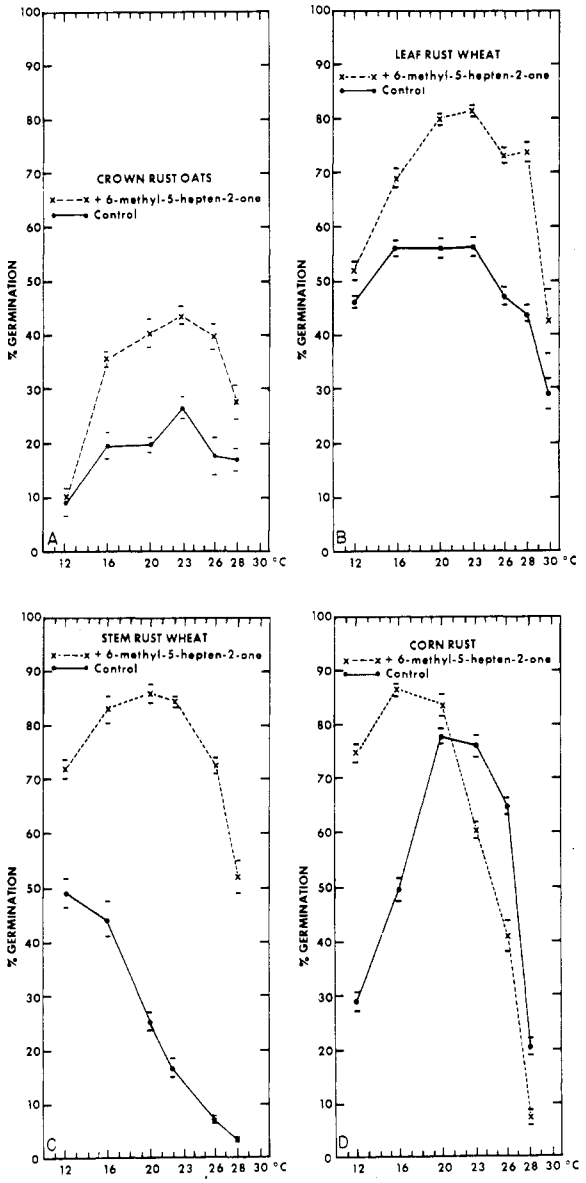

Figure 2. Effect of ±250 ppm of 6-methyl-5-hepten-2-one on 2-hr germination of uredospores of *P. coronata* (A), *P. recondita* (B), and *P. graminis* (C), and of ±25 ppm on *P. sorghi* (D) in response to temperature.

spore lot, all may affect the extent of germination. Depending on the relative humidity of the day, drying or hydration was possible while dry spores dispersed on Millipore filters were being carried to incubators, and this appeared at times to influence subsequent germination. Optima of stimulated germination did not always match those of the unstimulated germination. Maximum increase in germination often occurred at points other than at the unstimulated optimum. In general, stimulated germination was observed over a wide temperature range for spores of *P. coronata*, *P. recondita*, *P. graminis*, and *P. sorghi*.

Germination in Pustules. Uredospores of *P. coronata*, *P. recondita*, and *P. sorghi* all germinated in the pustule when exposed to the appropriate quantity of the proper stimulators. Figure 3 shows results of overnight germination. Massive germination of spores in pustules was noted for each rust. Controls showed no germination in the pustule.

The most effective concentration and temperature ranges over which massive germination could be observed were determined, and an evaluation was made of some of the stimulators as to relative ability to stimulate germination in the pustule. The rusts were not exposed to all available temperatures or over the complete concentration range, because of limited availability of dew chambers and occasional irregularities in the availability of diseased plants.

To determine optimum stimulator dosage, diseased plants were exposed to a concentration range of 10–200 μl of selected compounds in dew chambers. The optimum dose for the three stimulators and two mixtures (Table II) was 100–200 μl. Higher amounts were less effective and sometimes toxic to spores and plants. Assuming complete and immediate volatility, 200 μl of compound in the 220-l. dew chamber would equal 0.9 ppm by volume. The compounds actually diffuse into the atmosphere over a period of hours; hence 0.9 ppm represents a maximum calculated value. With 200 μl of nonanol, some odor could be detected on the filter-paper wick after an overnight exposure of about 16 hr. Buttery *et al.* (1969) have discussed the technical difficulties encountered in measuring nonanal in the vapor form. Occasional stimulation was noted with as little as 20 μl of compound, but 100–200 μl was usually required.

Germination in the pustule was observed over a wide

Table II. Optimal Stimulator Dosage,[a] Temperature Range,[b] Ratio of Successful/Total Number of Exposures,[c] and Maximum Per Cent In-Pustule Germination[d] of Uredospores of *P. coronata*, *P. recondita*, *P. graminis*, and *P. sorghi*

	P. coronata				*P. recondita*				*P. graminis*				*P. sorghi*			
Compd	Opt. dose, μl	Temp range, °C	Succ./ total	Max. % of germ. pustules	Opt. dose, μl	Temp range, °C	Succ./ total	Max. % of germ. pustules	Opt. dose, μl	Temp range, °C	Succ./ total	Max. % of germ. pustules	Opt. dose, μl	Temp range, °C	Succ./ total	Max. % of germ. pustules
Nonanol	100	18–25	22/25	90	200	15–24	7/31	70	100	18–25	15/20	80	200	15–24	16/26	75
Octanol	200	15–24	6/6	90	200	23–24	3/6	25	200	23	3/5	50	200	23–24	5/6	70
Nonanol–octanol, 1:1	200	17–24	9/9	80	200	23	1/3	10	200	17–23	3/3	50	200	17–25	3/3	80
Methylheptenone	200	20–22	4/7	50	0		0/18	0	100	20–25	10/14	80	0		0/13	0
Methylheptenone–nonanol, 1:1	100	20–25	10/12	90	200	19–25	9/18	80	200	18–25	17/18	90	200	20–25	16/20	80

[a] 200 μl of compound, assuming complete volatility, in a 220-l. dew chamber would be 0.9 ppm by volume. [b] Lowest and highest temperatures at which 10% or more of pustules showed germination. [c] Number of exposures in which 10% or more of pustules showed germination per total number of exposures. [d] Maximum percentage of pustules showing germination.

temperature range for the four rusts. The temperatures at which germination occurred in 10% or more of the pustules are shown in Table II. The lower operable limit of the dew chambers was about 15°. At temperatures above 25°, dew was too voluminous for consistent results. For temperatures of 15–25°, germination in the pustule was observed over the same temperature range at which stimulation by 10 ppm of nonanol in 1% agar was evident.

The maximum extent of germination induced by any treatment was in 90% of the pustules. Germination seldom occurred in pustules in areas of dead leaf tissue. Small portions of the plants always seemed to escape deposition of observable dew, and pustules in these areas showed no germination. In other wet areas, pustules often showed no germination. In certain lots of rusted plants, very little germination (much less than 10%, sometimes zero) occurred under otherwise optimum conditions. This is roughly expressed (Table II) by the fraction showing numbers of successful exposures per total exposures.

Of the rust species studied, spores of *P. coronata* and *P. graminis* responded best to stimulators, and over the widest concentration range. Nonanol and octanol were most effective overall, although methylheptenone was very active on spores of *P. graminis*. The mixtures of stimulators used in this study showed no outstanding synergism above that of the more active component alone. Methylheptenone was notable for its complete lack of activity on pustules of *P. recondita* and *P. sorghi*.

Nonanal was not originally considered for extensive testing, because nonanol was 10 times more effective, less toxic, and stimulatory over a wider concentration range (French and Gallimore, 1971). Nonanal was tested later, however, particularly on *P. recondita*, because spores of this fungus responded rather poorly to all the other compounds. Although the spores were stimulated on agar or water by all stimulators tested, nonanal appeared to be more dependable than nonanol for inducing germination of spores of *P. recondita* in pustule. In a separate series of experiments at 22°, diseased plants (*P. recondita*) exposed to 200 μl of nonanal showed positive germination responses in 10% or more of the pustules in 15 of 18 exposures; maximum response was germination in 80% of the pustules. Plants of the same lot, exposed to the same concentration of nonanol, showed only one positive response, germination in 20% of the pustules, in 17 exposures.

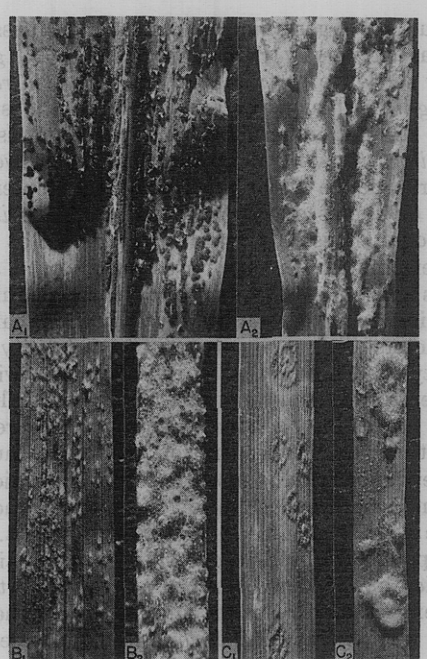

Figure 3. Effect of overnight exposure to dew ± stimulator at 22°: (A1) *P. sorghi* + dew; (A2) dew + 200 μl of 1-octanol; (B1) *P. coronata* + dew; (B2) dew + 200 μl of 1-nonanol; (C1) *P. recondita* + dew; (C2) dew + 200 μl of 1-nonanal.

DISCUSSION

Recent reviews have indicated an accelerating interest in the effects of organic volatiles on biological activity, particularly on the growth of fungi (Fries, 1973; Hutchinson, 1971), and insect alarm and other pheromones (Blum, 1969). Of the two endogenous compounds discussed in this report, nonanal vapor has been reported to stimulate growth of wood-rotting fungi, particularly *Stereum sanguinolentum* Alb. & Schw. ex Fr. and *Daedalea unicolor* Bull. ex Fr. (Fries, 1960, 1961). Methylheptenone has been reported to occur in volatiles from *Ceratocystis* sp. (Birkenshaw and Morgan, 1950; Sprecher, 1964), and also as an alarm pheromone of certain *Iridomyrmex* species of ants (Crewe and Blum, 1971). Nonanal and

methylheptenone have been identified in volatiles from uredospores of *P. graminis,* and nonanal has been found in other species of rust (Rines *et al.,* 1974). An interaction among nonanal–water vapor–liquid water (dew)–inhibitor, which may have profound effects on germination, has previously been described (French and Gallimore, 1972a; French, 1973), and exposure of spores to various proportions of the volatile components from the gaseous inhibitory environment of the pustule through the airborne path to a new infection site may regulate the future course of germination.

One would expect the least likely place for a spore to germinate would be in the pustule in which it was produced, because the highest concentration of endogenous inhibitor is encountered there. Considering the survival of the organism, by analogy with a successful manufacturing and distribution operation, the opening of the spore package of the pathogen before delivery (premature germination) to a potential new address (new host) would be most inefficient and, in fact, disastrous. The volatile stimulators described have the ability at low concentrations to pry open the spore packages and to clog the factories with germinated spores. In other words, live spores must travel to a new receptive host site in order to start another cycle; spores that do germinate prematurely may effectively prevent spread of ungerminated spores in the same pustule by entrapment in the cottony mass of entangled germ tubes.

The induction of germination of spores, en masse, in the pustule was previously demonstrated with *P. graminis,* and is now shown to be true for *P. coronata, P. recondita,* and *P. sorghi.* Although the four stimulators described in this report stimulated germination of the four rust species quite well *in vitro,* some important differences were noted in their performance in the pustule. Methylheptenone was ineffective on spores of *P. sorghi* and *P. recondita* in the pustule. Nonanal, usually inferior to nonanol as a stimulator of germination *in vitro,* was superior to all other compounds with spores of *P. recondita* in the pustule.

In addition to direct stimulatory biochemical effects, physical factors such as vapor pressure, volatility, solubility, fugacity, and interaction of the chemicals with water vapor or aerosol (fog) or dew may have great influence on whether a particular chemical reaches the spores in the pustule in the proper concentration or form to cause stimulation. Dew deposition is essential for germination in the pustule. One would expect diffusion of compounds to the spore to be about the same for the different rusts. That is, if the compound diffused to one species and activated it, other species in the dew chamber at the same time also would be expected to respond. On this basis, the success of nonanal with spores of *P. recondita* in pustule and the failure of methylheptenone with spores of *P. sorghi* and *P. recondita* probably cannot be explained in terms of diffusion factors. Perhaps some subtle difference in the anatomical arrangement of spores in the pustule or in the way the chemical–water vapor–colloidal water droplet is presented to the uredospore is responsible for the differences in reaction.

While germination of spores in the pustule may have no practical value at this time, it does offer a possible new approach to control of the spread of certain rust diseases. All of the rusts examined in this study did germinate in the pustule under certain circumstances. Practical application of minute doses of stimulators as a means of control of disease spread remains subject to overcoming serious technological difficulties. While the technological problems may never be solved, the study of in-pustule germination (germination under least likely circumstances) by consideration of the stimulator–inhibitor–water vapor–liquid water interaction may lead to greater knowledge of spore germination and perhaps to better methods of disease control. The demonstrated extension of chemical stimulation of germination to include three additional rust species, and current research in progress on others, suggest that a broad spectrum of activity for these chemicals exists in the physiology of rust-spore germination.

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