Stimulation of Germination of Uredospores of Stem Rust of Wheat in the Pustule by *n*-Nonanal and Related Compounds

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Germination of uredospores in pustules of infected wheat plants has been induced with nonanal, nonanol, and other rust spore germination stimulators. Stimulator applied during an overnight dew period results in protrusion of white, cottony germ tubes from the pustules, due to the germination of great numbers of spores. The abortive germination and the physical barrier to spore dispersal provided by the germ tubes may provide a possible new means of deterring spread of the rust fungus and, consequently, retard the rate of development of rust epidemics in the field.

Nonanal is a component of the flavor and fragrance of a great variety of natural products ranging from essential oils such as rose, cinnamon, and various citrus (Guenther, 1949) to rancid peanut oil and meat (Iselin, 1948, 1949). Nyman (1969) reported nonanal to be the most active of several related compounds in stimulating the acceleration phase of the growth of the yeast, *Dipodascus aggregatus*. *n*-Nonanal was identified as a constituent of uredospores of the stem rust of wheat organism, *Puccinia graminis* var. *tritici* (Eriks. & E. Henn.) Guyot, and was reported to be an endogenous stimulator of uredospore germination (French and Weintraub, 1957). Many other related compounds have been reported capable of inducing uredospores to germinate, virtually in unison (French, 1961; French and Gallimore, 1971). Pretreatment of spores with nonanol vapor and water vapor may kill spores or stimulate subsequent germination and infection, depending on nonanol concentration (French and Gallimore, 1972). Certain concentrations of nonanal, nonanol, and other active chemicals stimulate germination of spores floated on water, on a water–agar medium, and, (the subject of this report) at the site of spore production, the pustule on the leaf of the living wheat plant. Extensive growth of the germ tubes from the pustule immobilizes the spores and offers possible means of controlling spread of the disease. Some of the active chemicals are effective at great dilution, are relatively inexpensive, and are assumed to be nontoxic, since they have been a part of the human diet for centuries.

MATERIALS AND METHODS

Pustules were exposed to stimulator volatiles on isolated leaf sections in Conway diffusion cells or on intact plants placed in dew chambers at 20°C.

In the former method, sections of Baart wheat leaves containing several pustules of Race 56 spores were placed in the center wells of Conway cells. Suspensions of the various stimulators in water (4.0 ml) were placed in the annulus. Cells were closed with a ground glass plate and placed overnight at 20°C.

To test the effect on intact plants, Baart wheat was grown in pots and inoculated with Race 56, *Puccinia graminis* var. *tritici* (Eriks. & E. Henn.) Guyot. Rusted plants, approximately 3-weeks-old, were placed in dew chambers and a quantity of test compound (0.1 to 0.2 ml) was placed on a dry filter paper disc supported by a glass cylinder placed in the center of the chamber. Pustules were examined after an overnight exposure in the dew chamber.

Germination of spores from the pustule was examined by pressing pustules against 1% water agar and against agar plus 10 ppm (by volume) of 1-nonanol. Germination counts using plain agar gave an estimate of the immediate capacity of spores to germinate. Nonanol agar appeared to give a maximum germination value, indicating whether spores had been damaged during treatment. Nonanol, effective as low as 0.04 ppm, was used in most experiments and in agar mixtures because it is one of the most effective stimulators (French and Gallimore, 1971).

EXPERIMENTAL RESULTS

Experiments in Conway Cells. Uredospores in pustules responded to nonanol in various concentrations in much the same way as do uredospores on agar. Isolated pustules were exposed to the vapor from 1000 ppm, 100 ppm, and 10 ppm nonanol in water in the annuli of Conway diffusion cells. Other pustules were pressed lightly against the surface of 1% water agar containing the same concentrations of nonanol. Pustules in Conway cells were exposed for an overnight period. Agar germination was observed after 2 hr. Results (Table I) indicated inhibition or death of spores at 1000 ppm in both cases. At 100 ppm nonanol, germ tubes were short, and on agar, many ruptured. At 10 ppm spores germinated and germ tubes grew best, and it is this concentration that seems to be most effective for producing the long entangled germ tubes that prevent further dispersal. Both nonanol and nonanal, the endogenous stimulator, induced massive white cottony growth of germ tubes from spores in pustules at 10 ppm.

The massive germination observed with 10 ppm nonanol is shown in Figure 1. The photographs show pustules with and without nonanol. In the pustules in which germination has occurred, the mass of germ tubes appears as a bit of white cotton. Microscopic inspection reveals the typical germ tube appearance of uredospores. No evidence of possible invading fungi, such as mildew, was observed.

Intact Plants. Rusted plants were placed in a 20°C dew chamber (220 l. volume) and exposed to vapors of 100 μl (0.1 ml) nonanal on filter paper. After overnight exposure, virtually all pustules on treated plants were cottony white from the massive germination of spores in the pustules. In another dew chamber, plants not exposed to the stimulator were covered with dew, but no germination was observed and pustules were the usual dark brown color. Results with

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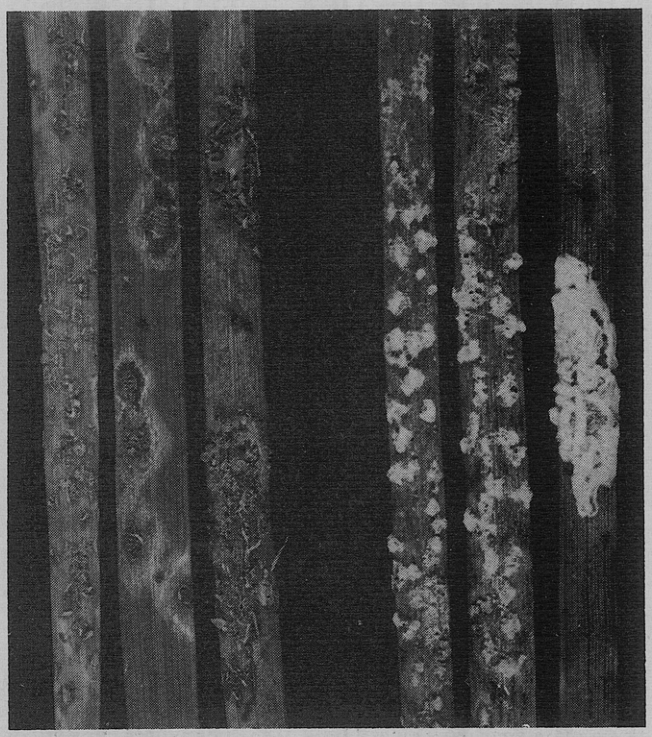

Figure 1. Pustules subjected to dew (left) and dew plus 0.5 ppm of nonanol (right) overnight in dew chamber at 21°C (×2). Photographs made while dew was on leaves

Table I. Effect of Nonanol Concentrations on Germination of Uredospores on 1% Water Agar and in Isolated Pustules

Concentration of nonanol	Germination in pustule[a]	Germination on agar[b]	Germ tube characteristics[c]
1000 ppm	0%	0%	Spores damaged
100	Some germ tubes noted	95	Short, multiple tubes, many ruptured
10	Massive germination	98	Long germ tubes, vigorous growth
0	0	20	Normal

[a] Nonanol suspensions in annuli of Conway cells, overnight germination. [b] Nonanol suspensions in agar, 2 hr germination. [c] Observed on agar.

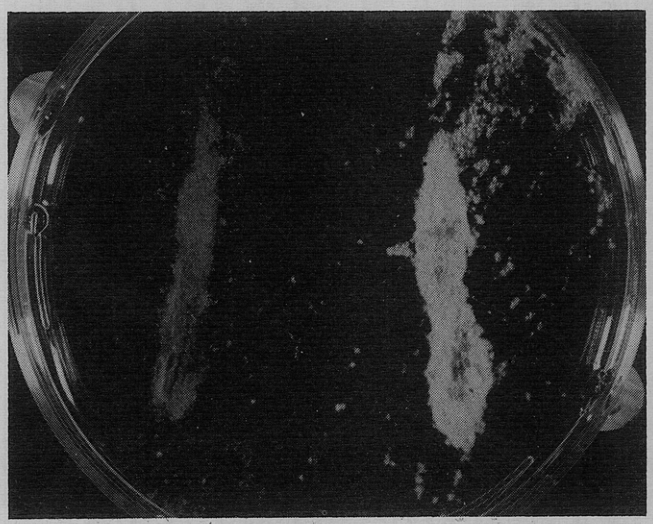

Figure 2. Pustules pressed against 1% agar in Petri plate: (left) pustule pressed against agar just before being photographed, spores ungerminated; (right) pustule pressed against agar, massive germination of spores after 18 hr in dew chamber plus 0.5 ppm of nonanol, 21°C (×2)

Table II. Time Course of Spore Germination in Pustules Exposed to Nonanol

Time, hr	Germinated spores per pustule[a]	Estimated germ tube length, μ	Remarks
1	45, 39, 12, 22, 43	15	
2	19, 47, 8, 55, 71	15–60	
3.5	24, 42, 26, 57, 18	120–180	Some germination visible in pustules
5.5	Almost no transfer of spores to agar because of entangled germ tubes		Germination visible in pustule/light tan color
7	Germination in about 80% of pustules		Germination visible in pustule/light tan color

[a] Pustules were pressed lightly against a 1% water agar surface and germination was determined immediately. Five pustules were counted for each time period and counts represent germinated spores per pustule (20°C).

nonanol were similar and perhaps slightly more effective than with nonanal.

The rate of germination in the pustule was very similar to that of spores on water or agar with nonanol. Infected plants were placed in dew chambers and pustules were sampled periodically by transfer of spores to agar. Five separate pustules were examined and the number of germinated spores per pustule was determined immediately (Table II).

Germination began in about 60 min, at which time germ tube length was approximately half the length of the spore. With increase in time, an increase in germ tube length was noted, but no great increase in number germinated among those spores released to agar was observed. This is undoubtedly caused by the entanglement of germ tubes, which holds most of the spores in the pustule. Only ungerminated spores and those few with untangled germ tubes are freed from the pustule. With increase in time, fewer spores are released. After overnight exposure, virtually no spores are visible or transferable to agar from germinated pustules because of the cottony mass. Spores released from untreated pustules pressed against agar and transferred to a dew chamber with nonanol vapor exhibit, after overnight exposure, a massive white cottony growth resembling germination in the pustule (Figure 2).

Plants with pustules in which germination had occurred were transferred to the greenhouse and observed for further development. Upon drying, germ tubes collapse and the pustule assumes a light tan color. Usually no further sporulation occurs from the pustule in which germination has occurred. After several days, however, a brown ring of spores is produced around the old grayish pustule. This ring is typical development of untreated pustules. When subjected to the stimulator treatment during a dew period, germination occurs in this ring as well. On young infected plants, with spores just beginning to be discharged through the ruptured epidermis, germination occurred only where spores had emerged. No obvious changes were noted in the flecks.

Quantity of Nonanol Required for Stimulation in the Dew Chamber. Rusted wheat plants were placed into a dew

chamber previously free of nonanol or any other known stimulatory chemical. On successive days, different rusted plants were treated with 1.0, 10, 20, 30, and 100 μl of 1-nonanol on filter paper in the center of the chamber, which had a volume of 220 l. Some germination occurred in pustules treated with 1.0 and 10 μl, even on isolated leaves placed in the far corners of the chamber. At the level of 20 μl, massive germination of spores occurred in more than 80% of the pustules on the plant and on isolated leaves in corners, about 20 cm from the source of nonanol. On a volume of liquid per volume of air basis, the concentration of nonanol in the air, assuming complete volatilization (which may not actually occur) was less than 10^{-4} μl of nonanol per cm^3 of air, or less than 0.1 ppm by volume. In a previous paper (French and Gallimore, 1971) we have reported the stimulation of germination by nonanol in water at levels as low as 0.04 ppm. Buttery *et al.* (1969) have discussed the difficulties encountered in measuring concentration of *n*-nonanal and related compounds.

Use of Nonanol Sprays. Suspensions of nonanol–water equivalent to 10,000, 1000, 100, 10, and 0 ppm were prepared and 10-ml portions sprayed on rusted plants placed in plastic bags. Bags were tied and placed overnight in a dew chamber at 20°C.

At 10,000 ppm no germination in the pustule occurred. Pustules were dark brown or black. Upon microscopic examination spores were found to be damaged; some had short germ tubes, less than the diameter of the spore. Abundant germination in pustules was obtained with 10 to 1000 ppm. Pustules had a white or tan cottony appearance, characteristic of massive germination.

Other Active Compounds. More than 60 compounds have been tested in this laboratory and found active in stimulating the germination of uredospores. Only a few have been tested for the ability to stimulate germination in the rust pustule. Of the compounds tested, the following actively induced germination in the pustule: *n*-nonanal, 1-nonanol, octanol, nonyl amine, and 1-nonene. Pustules on intact wheat plants were exposed to the above compounds in a dew chamber. Concentrations were 0.1–0.2 ml, placed on filter paper in a dew chamber (220 l. volume). The following compounds were inactive at the level tested, although all have been strong germination stimulators and nontoxic or noninhibitory even at high concentrations: dioctyl sulfide, octyl thiocyanate, 3-nonanone, and nonyl mercaptan.

DISCUSSION

Germination of uredospores while in the pustule is seldom observed in nature. If common, it would be a detriment to survival of the organism. Germination in the pustule would preclude dissemination of functional spores to new infection sites. The development of long intertwined germ tubes would function somewhat like a cotton plug, restraining young ripening spores and greatly impeding dispersal. The collapsing germ tubes, besides providing a physical barrier to spore dispersal, also may provide entrance for microbial invasion, which could further impede the growth and development of uredospores in the pustule. Induction of germination in the pustule presents a possible new method for controlling the spread of rust in the field, if the germination stimulator could be effectively and economically delivered, and if it could be retained at the site long enough to be effective. The volatile stimulators are effective at very low concentrations, and if delivered to the pustules during a dew period, might provide a good means of greatly reducing the number of spores available for spread of the disease.

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