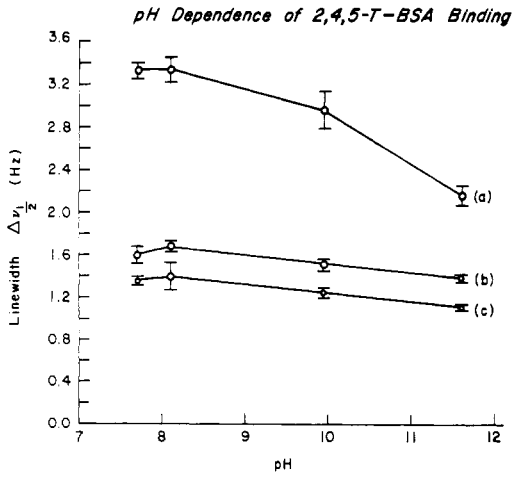

Figure 4. Effect of pH on binding of 2,4,5-T (10 mg/ml) to BSA (4 mg/ml) in D_2O at 29°.

activity. This may lead to some error when calculating K_p. Secondly, the potassium salt of the herbicide may produce some ion pairs. The calculation of T_2 using line width is not very accurate and thus T_2 measurements using the spin–echo method may be preferred.

The line-width changes in 2,4,5-T resonance peaks at a fixed BSA concentration in solutions of different pH are shown in Figure 4. The decrease in line width with increasing pH may be explained on the basis of a decrease in binding at higher pH values. Such a decrease may be due to denaturation of the protein at higher pH values.

The ^1H NMR results of herbicide–BSA binding indicate that the CH_2 protons are more affected by the binding than the ring protons, suggesting that they are closer to the binding site. This is in agreement with the earlier investigation of the binding of phenoxyacetic acid and proteins (Fischer and Jardetzky, 1965). It is interesting to note that this is in contrast with the binding of the chlorinated hydrocarbon bis(*p*-chlorophenyl)acetic acid with BSA, where the binding involves the benzene ring (Haque et al., 1974). Such behavior may be due to the large difference in the hydrophobic characteristics of chlorinated hydrocarbons and these herbicides.

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Differences in Germination Response of Spores of Several Species of Rust and Smut Fungi to Nonanal, 6-Methyl-5-hepten-2-one, and Related Compounds

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Uredospores of the following rust species were stimulated to germinate by nonanal or 1-nonanol and/or 6-methyl-5-hepten-2-one (methylheptenone): *Puccinia arachidis, P. coronata, P. hieracii, P. parca, P. pelargonii-zonalis, P. polysora, P. recondita, P. rubigo-vera, P. sorghi, Uromyces phaseoli, Coleosporium ipomoeae,* and *Melampsora abietis-canadensis.* Uredospores of *Puccinia helianthi* were not stimulated by these compounds, but were stimulated by cinnamaldehyde and β-ionone, as were some of the above. Aeciospores of *Puccinia podophylli* were stimulated by nonanol. Teleutospores of *Ustilago avenae, U. maydis,* and *U. tritici* were stimulated by nonanol. Striking differential responses to the five compounds were observed with certain species. The stimulatory action of nonanal and related compounds has been extended thus far to include members of four fungal families—the Pucciniaceae, Coleosporiaceae, Melampsoraceae, and Ustilaginaceae; five genera—*Puccinia, Uromyces, Coleosporium, Melampsora,* and *Ustilago;* and 19 species.

An endogenous spore germination stimulator from uredospores of *Puccinia graminis* Pers. f. *tritici* Eriks. & E. Henn. was first reported in 1957 (French et al.) and identified as *n*-nonanal (pelargonaldehyde) (French and Weintraub, 1957). More recently, nonanal was identified in volatiles collected directly from fresh uredospores, and in distillates of uredospores of *P. coronata* Cda., *P. recondita* Rob. ex Desm., *P. sorghi* Schw., *P. helianthi* Schw., *P. striiformis* West., and *Uromyces phaseoli* (Reben.) Wint. (Rines et al., 1974). In addition, 6-methyl-5-hepten-2-one (methylheptenone) was identified in *P. graminis* var. *tritici* as the stimulatory volatile that diffused from uredospores floated on water. Nonanal and more than 60 related compounds have been reported to stimulate germination of uredospores of *P. graminis* var. *tritici* (French, 1961, 1973; French and Gallimore, 1971). Chemical stimulation

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of germination of uredospores of *P. coronata, P. recondita,* and *P. sorghi* was reported previously (French et al., 1975). This report describes the chemical stimulation of germination of various rust and smut spores, collected around Frederick, Md., or others held in culture at the Plant Disease Research Laboratory.

MATERIALS AND METHODS

Routine tests for stimulatory activity on spore germination were carried out in 5-cm plastic petri plates on 1% water agar ± 10 ppm of 1-nonanol or 250 ppm of methylheptenone. Tests were carried out in the dark at room temperature, until maximum germination was observed. The time required for germination ranged from 90 min to 24–48 hr, depending on the type of spore.

When dosage response curves were determined, spores were dispersed on the surface of a minimum volume of distilled water and transferred by platinum loop to 2 ml of water + compound in the center of Conway cells or to 0.5 ml in microdishes placed in the center of Conway cells covered with ground glass plates. Tests were run at room temperatures ranging from 18 to 26°. Quantities of spores great enough to induce self-inhibition were used; with many species, the entire surface area of the test solution was covered with spores.

Spores were collected from the field and used directly or were cultured on greenhouse-grown plants and collected just before use. Some spores were from liquid nitrogen storage.

The chemical compounds were added directly to water or agar with a microsyringe. Extremely low concentrations were diluted with a minimum amount of redistilled methanol. The use of a diluent was avoided whenever possible. Germination was counted directly on agar plates or by transference of loops of spores to glass slides. Averages of 400 spores, counted by two observers, were usually obtained for each treatment and reported with standard error.

EXPERIMENTAL RESULTS

Germination tests have indicated (Table I) spores of 12 species of *Puccinia,* 1 of *Uromyces,* 1 of *Coleosporium,* 1 of *Melampsora,* and 3 of *Ustilago* to be stimulated by chemicals commonly found in natural products known as essential oils. These five genera represent four families—the Pucciniaceae, Coleosporiaceae, Melampsoraceae, and Ustilaginaceae; and three types of spores—uredospores, aeciospores, and teleutospores. Maximum activity ranged from 0.5 to 250 ppm of compound. High concentrations invariably inhibited or damaged the spores, sometimes penetrating them and rendering them hyaline.

The data in Table I were rated for effectiveness by arbitrary assignment of a plus to chemically treated germination values above 30% that were at least twice that of the controls. Chemical treatments that did not meet this criterion were assigned zero.

Some remarkable differences were noted in the response of certain species to the different test chemicals. Whereas most spores responded positively to nonanal or nonanol and methylheptenone, *P. hieracii* (Roehling) Mart. and *Uromyces phaseoli* were stimulated only by methylheptenone. On the other hand, *P. pelargonii-zonalis* Doidge and *C. ipomoeae* (Schw.) Burri responded to nonanal, but not to methylheptenone. Though nonanol was active on all three species of *Ustilago,* only *U. maydis* (DC.) Cda. was strongly stimulated by both nonanol and methylheptenone. *P. helianthi* responded to neither compound.

We searched some of the many compounds stimulatory to *P. graminis* f. *tritici* to find one active on *P. helianthi. trans*-Cinnamaldehyde and β-ionone stimulated germination of uredospores of *P. helianthi,* as well as *P. coronata, P. recondita,* and *P. pelargonii-zonalis* (Table I). Then we determined dosage response curves of four compounds, nonanal and methylheptenone (endogenous to *P. graminis* f. *tritici*) and β-ionone and *trans*-cinnamaldehyde on a group of five species with unique responses, *P. helianthi, P. pelargonii-zonalis, Melampsora abietis-canadensis* C. A. Ludwig ex Arth., *Coleosporium ipomoeae,* and *Uromyces phaseoli.* Responses of *P. graminis* f. *tritici* (previously reported stimulated by all four compounds) were included for comparison. Results (Figure 1) indicate a strong positive response of all species in the group except *M. abietis-canadensis* to β-ionone at 25 or 100 ppm. *trans*-Cinnamaldehyde stimulated *P. helianthi* at 50 ppm, *P. pelargonii-zonalis* at 25 ppm, and *M. abietis-canadensis* at 100 ppm, but inhibited *U. phaseoli* at 25 ppm or higher. Nonanal (100 ppm) stimulated only *P. pelargonii-zonalis* and *C. ipomoeae.* Methylheptenone stimulated spores of *Uromyces phaseoli* and *M. abietis-canadensis,* but none of the other three species in this group.

Some stimulation of *P. pelargonii* by methylheptenone was noted if the test period was extended from 4 hr to overnight, but the extent of stimulation was not equal to that of nonanal in 4 hr. Response to chemical stimulators differed strikingly in certain species of fungal spores.

DISCUSSION

Many compounds (more than 60 reported in several previous articles) have been reported active in stimulating the germination of uredospores of *P. graminis* f. *tritici* (French, 1961; Searles and French, 1964; French and Gallimore, 1971). Some unsaturated 3-ols, whose properties seem no different from those of active compounds, for some unknown reason were inactive. A similar pattern of inactivity toward certain chemical stimulators is noted among some of the species tested, notably *P. helianthi, P. pelargonii-zonalis, P. hieracii, P. sorghi, C. ipomoeae, M. abietis-canadensis,* and *Uromyces phaseoli.*

In a previous report (Rines et al., 1974) we found no stimulatory action by methylheptenone on bean rust, *Uromyces phaseoli;* on corn rust, *P. sorghi;* or on sunflower rust, *P. helianthi.* As indicated in Table I, stimulatory activity by methylheptenone can be shown in both bean rust and corn rust when the spore concentration is greatly increased to a level yielding very low control germination. The greater spore load implies a greater concentration of endogenous inhibitor, hence reduced germination in control samples. Demonstration of stimulation in the species listed in this report suggests the existence of other endogenous inhibitors, similar to those elucidated by Macko and associates (Macko et al., 1970, 1971, 1972), which can be overcome by endogenous stimulators, such as nonanal, methylheptenone, or related, but as yet unidentified, naturally occurring compounds.

Rines et al. (1974) reported the occurrence of nonanal in distillates of *P. graminis, P. recondita, P. striiformis, P. sorghi, P. helianthi,* and *Uromyces phaseoli.* Although found in *P. helianthi* and *U. phaseoli,* nonanal has not been observed to stimulate their germination. Methylheptenone was found in *P. graminis* f. *tritici* and *P. striiformis.* Other compounds did not occur in quantities great enough in our extracts for positive identification, and large quantities of all the species of spores studied were not readily available for extensive analysis.

The endogenous inhibitor originally described by Allen (1955) was identified by Macko and co-workers (Macko et al., 1970, 1971, 1972) as methyl *cis*-ferulate for *P. graminis* f. *tritici.* A similar endogenous inhibitor, methyl *cis*-3,4-dimethoxycinnamate, was isolated from uredospores of *U. phaseoli.* β-Ionone and *trans*-cinnamaldehyde, of the four stimulators studied in detail, are structural analogs of these endogenous cinnamate-based inhibitors. Although both compounds stimulated *P. helianthi, trans*-cinnamaldehyde inhibited *U. phaseoli.*

Table I. Stimulation of Fungal Spore Germination by Nonanal and Related Compounds

Family and species	Host	Germ. time, hr	(ppm/vol); % germination ± SE, treated/control; rating[b] Nonanal	Nonanol	Methylheptenone	*trans*-Cinnamaldehyde	β-Ionone
Puccineaceae (uredospores)							
Puccinia arachidis	Peanut	3.5	(100); $\frac{80 \pm 4.6}{3 \pm 1.3}$; +		(100); $\frac{98 \pm 0.9}{6 \pm 1.1}$; +		
P. coronata	Oats	1.5	(25); $\frac{98 \pm 0.4}{1 \pm 0.6}$; +		(250); $\frac{96 \pm 1.6}{1 \pm 0.3}$; +	(50); $\frac{96 \pm 0.4}{1 \pm 0.4}$; +	(50); $\frac{78 \pm 3.8}{1 \pm 0.4}$; +
P. helianthi	Sunflower	3.5	(25); $\frac{15 \pm 2.4}{3 \pm 1.5}$; 0		(25); $\frac{5 \pm 1.5}{1 \pm 0.4}$; 0	(50); $\frac{77 \pm 5.5}{9 \pm 2.3}$; +	(25); $\frac{95 \pm 1.5}{9 \pm 2.3}$; +
P. hieracii	Dandelion	1.5	(50); $\frac{8 \pm 1.0}{12 \pm 2.6}$; 0		(25); $\frac{64 \pm 5.8}{12 \pm 2.6}$; +	(25); $\frac{12 \pm 1.3}{19 \pm 1.8}$; 0	(25); $\frac{38 \pm 4.4}{19 \pm 1.8}$; +
P. parca[a]	Black bindweed	2.0		(10); $\frac{97 \pm 0.5}{35 \pm 11.6}$; +	(250); $\frac{85 \pm 4.0}{35 \pm 11.6}$; +		
P. pelargonii-zonalis	Geranium	4.0	(100); $\frac{65 \pm 3.2}{0}$; +		(100); $\frac{7 \pm 1.6}{0}$; 0	(25); $\frac{35 \pm 4.1}{0.2 \pm 0.2}$; +	(25); $\frac{77 \pm 2.0}{0.2 \pm 0.2}$; +
P. podophylli[a] (aeciospores)	May apple	24		(10); $\frac{97 \pm 1.4}{3 \pm 0.5}$; +			
P. polysora	Corn	2.0		(5); $\frac{64 \pm 4.8}{18 \pm 3.3}$; +	(25); $\frac{82 \pm 2.5}{18 \pm 3.3}$; +		
Puccinia sp.	Orchard grass	1.5		(5); $\frac{34 \pm 5.7}{3 \pm 0.5}$; +	(50); $\frac{61 \pm 3.5}{27 \pm 3.5}$; +		
P. recondita	Wheat	1.5	(100); $\frac{89 \pm 2.2}{4 \pm 1.2}$; +		(250); $\frac{98 \pm 1.0}{21 \pm 4.8}$; +	(100); $\frac{68 \pm 4.2}{3 \pm 0.7}$; +	(50); $\frac{55 \pm 4.8}{3 \pm 0.7}$; +
P. rubigo-vera	Rye	1.5	(50); $\frac{52 \pm 1.9}{10 \pm 1.3}$; +	(0.5); $\frac{59 \pm 7.7}{14 \pm 1.9}$; +	(100); $\frac{52 \pm 1.7}{14 \pm 1.9}$; +		
P. sorghi	Corn	1.5	(50); $\frac{69 \pm 4.4}{20 \pm 3.0}$; +		(25); $\frac{60 \pm 8.5}{23 \pm 2.7}$; +	(25); $\frac{63 \pm 8.3}{31 \pm 3.4}$; +	(50); $\frac{36 \pm 4.3}{31 \pm 3.4}$; 0
Uromyces phaseoli	Bean	4.0	(250); $\frac{6 \pm 1.4}{0}$; 0		(100); $\frac{85 \pm 1.0}{9 \pm 3.3}$; +	(25); $\frac{2 \pm 0.3}{29 \pm 3.6}$; 0	(25); $\frac{90 \pm 3.4}{29 \pm 3.6}$; +
Coleosporiaceae							
Coleosporium ipomoeae	Morning glory	3.0	(100); $\frac{62 \pm 5.6}{0}$; +		(500); $\frac{1 \pm 0.4}{0}$; 0	(50); $\frac{14 \pm 2.2}{8 \pm 0.9}$; 0	(100); $\frac{34 \pm 1.2}{8 \pm 0.9}$; +
Melampsoraceae							
Melampsora abietis-canadensis	Aspen	1.5	(25); $\frac{8 \pm 2.4}{4 \pm 0.7}$; 0		(250); $\frac{62 \pm 0.5}{4 \pm 0.7}$; +	(100); $\frac{41 \pm 6.1}{6 \pm 1.2}$; +	(10); $\frac{22 \pm 4.1}{17 \pm 1.2}$; 0

Ustilaginaceae (teleutospores)					
Ustilago avenae	Oats	24	(5); $\frac{69 \pm 3.6}{18 \pm 1.9}$; +	(5); $\frac{17 \pm 3.7}{18 \pm 1.9}$; 0
U. maydis	Corn	24	(25); $\frac{44 \pm 5.1}{5 \pm 0.8}$; +	(125); $\frac{74 \pm 2.4}{5 \pm 0.8}$; +
U. tritici	Wheat	24	(5); $\frac{74 \pm 1.3}{19 \pm 1.4}$; +	(5); $\frac{32 \pm 2.1}{19 \pm 1.4}$; 0

[a] Tested on 1% agar. [b] A plus rating is arbitrarily assigned to chemically treated germination values above 30% that are at least twice that of the controls. Chemicals that did not meet this criterion were assigned zero rating.

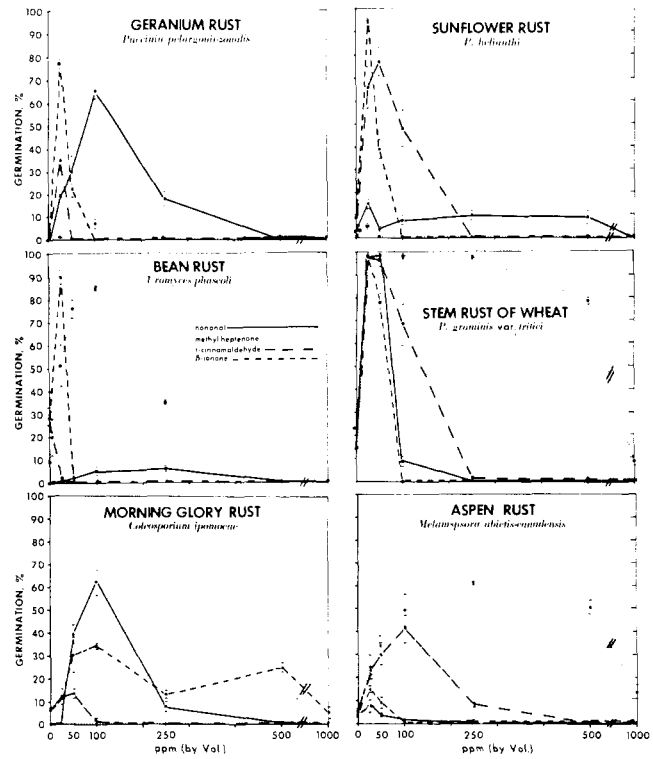

Figure 1. Germination response of uredospores of six rust species to 0–1000 ppm of 1-nonanal, methylheptenone, *trans*-cinnamaldehyde, and β-ionone.

Stimulatory action has been found particularly effective in certain species of rusts whose spores may be induced to germinate in the pustule. The rusted plants, after exposure to low concentrations of effective stimulators in dew chambers, have a cottony white mass of germ tubes covering the pustules. Germination in pustule has previously been reported for *P. graminis* f. *tritici* (French and Gallimore, 1972), *P. recondita, P. coronata,* and *P. sorghi* (French et al., 1975), and has been observed in *P. rubigo-vera* (DC.) Wint. (on rye), *Uromyces phaseoli,* and a species of *Puccinia* on orchard grass (unpublished).

Many of the compounds stimulatory to spore germination are also insect pheromones. Table II is a list of compounds that are active as pheromones in ants, termites, or bees (Blum, 1969) and which also stimulate uredospore germination (*P. graminis* f. *tritici*).

An interesting relationship exists between a group of economically important leaf-cutting ants, the attines (with alarm pheromones that are also fungal spore stimulators), and fungi used as food by the group (Weber, 1972). The fungus gardens maintained by these ants are usually a monoculture of a particular fungus species kept relatively contaminant-free by the ants, suggesting some sort of control mechanism of inhibitory or antibiotic compounds in ant secretions. At the same time, the growth of the host fungus is stimulated by ant secretions, suggesting the presence of specific stimulatory or nutritionally beneficial compounds. Nonanal has been reported stimulatory to the growth of several species of wood rotting fungi (Fries, 1960, 1961, 1973). Methylheptenone has been identified as a volatile product of other wood-rotting fungi, *Ceratocystis* sp. (Birkinshaw and Morgan, 1950; Sprecher, 1964a,b). Further elucidation of these relationships could lead to more specific biological control tools involving both insects and fungi.

All of the active compounds have been found in various natural products, particularly in essential oils or perfumes. They have been recognized mainly for their ability to elicit an olfactory response, particularly as components of flavors

Table II. Spore-Stimulating Insect Pheromones[a]

Compound	Insect species
6-Methyl-5-hepten-2-one	*Conomyrma pyramicus* (Roger)
	Dolichoderus scabridus (Roger)
	Iridomyrmex conifer (Forel)
	I. detecus (F. Smith)
	I. nitidiceps (Andre)
	I. rufoniger (Lowne)
	Liometopum microcephalum Panz.
	Tapinoma nigerrimum (Nyl.)
2-Heptanone	*Apis mellifera* L.
	Trigona postica Latreille
	T. tubiba F. Smith
	Azteca spp.
	Conomyrma pyramicus (Roger)
	Iridomyrmex pruinosus (Roger)
2-Hexenal	*Crematogaster africana* Mayr
2-Nonanone	*Trigona postica* Latreille
	T. tubiba F. Smith
Citronellal	*Acanthomyops claviger* (Roger)
	Lasius spathepus Wheeler
Citral	*Lestrimelitta limao* (F. Smith)
	Acanthomyops claviger (Roger)
Limonene	*Drepanotermes rubriceps* (Frogg)
	Myrmicaria natalensis F. Smith

[a] Pheromone information from Blum, 1969.

or fragrances detectable by humans. Their action as insect pheromones or spore germination stimulators represents a different type of biological activity for these compounds, although the biochemical mechanism may be similar "in essence" to that of olfaction. As shown by this research, stimulator–inhibitor balance, previously shown in terms of nonanal–methyl ferulate for *P. graminis* f. *tritici*, occurs in a rather broad range of spore types, as evidenced by the action of nonanal. Related stimulators suggest the probable presence of similar stimulator–inhibitor balances or switches, controlled in certain species on the stimulator or "on" side of the switch by methylheptenone, β-ionone, *trans*-cinnamaldehyde, or other unknown endogenous activators. They are probably paired with as yet unidentified inhibitors, perhaps similar to methyl ferulate and related compounds, on the "off" side of the switch.

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