Stimulation of Uredospore Germination of *Puccinia helianthi* and *Uromyces vignae* by Aromatic Nitriles and Related Flavorlike Compounds

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A variety of aldehydes, ketones, alcohols, nitriles, and esters, mostly aromatic and ranging from volatile oils to nonvolatile, water-soluble compounds, stimulated germination of uredospores of *Puccinia helianthi*, the causal agent of sunflower rust, and *Uromyces vignae*, the causal agent of cowpea rust. β-Ionone stimulated uredospores of both species and was used as a standard for comparing the relative effectiveness of compounds. Of the 28 compounds most active on *P. helianthi*, 17 were aromatic nitriles. Benzonitrile in the gaseous phase stimulated uredospore germination in pustules of rusted sunflower plants. An aqueous solution of 3-cyanophenol induced germination in pustules when sprayed on rusted sunflower plants. Of nine compounds most active on uredospores of *U. vignae*, five were aromatic nitriles. Application of an aqueous solution of 1000 ppm of 4-cyanobenzaldehyde to rusted cowpea plants induced some germination in pustules. The aromatic nitriles are a group of compounds shown for the first time to stimulate uredospore germination.

In previous research, several chemicals have been reported most effective in stimulating the germination of certain groups of species of rusts. The most stringent requirement for being considered "most effective" was that the compound be capable of inducing uredospores in rust pustules to germinate en masse when diseased plants were placed in dew chambers with the proper quantity of volatile compound. Although many chemicals stimulated germination of spores floated on water, only a few induced spores to germinate in pustules (French et al., 1975a, 1977) where spores would be expected to contain the maximum amount of self-inhibitors.

The ability to stimulate germination in pustules has been shown previously for *n*-nonanal, identified as an endogenous component of uredospores of *Puccinia graminis* var. *tritici* and other species (French and Weintraub, 1957; Rines et al., 1974). *n*-Nonanal was active on *P. graminis* var. *tritici* and *Puccinia coronata*. β-Ionone had similar activity on uredospores of *Uromyces phaseoli* (French et al., 1977). Although not effective on spores in pustules, 5-methyl-2-hexanone has been shown to be most effective in stimulating uredospores of *Uredo eichhorniae* (Charudattan et al., 1981), *Puccinia punctiformis*, and *Puccinia chondrillina*, pathogens of the noxius weeds, water hyacinth (*Eichhornia crassipes*), Canada thistle (*Cirsium arvense*), and rush skeleton weed (*Chondrilla juncea*) (French, 1983) respectively. This research describes several new stimulators of uredospores of *Puccinia helianthi* and *Uromyces vignae*. These compounds include aromatic flavorlike compounds and related nitriles.

MATERIALS AND METHODS

Uredospores for testing were grown on the appropriate hosts in the greenhouse. Uredospores of *P. helianthi* Schw. Schr. were produced on sunflower, *Helianthus annuus* L., cv. Sputnik-71. Uredospores of *Uromyces vignae* Barcl. were produced on cowpea, *Vigna sinensis* (L.) Endl., cv. California.

Germination tests were run as previously described (French et al., 1977). Nearly all of the compounds were obtained from the Aldrich Chemical Co., and purity was assumed to be that listed in the catalog. Compounds were tested at 0, 10, 25, 50, 100, 250, 500, and 1000 ppm in microdishes in the center of Conway diffusion cells. Liquid compounds in microliter amounts were added directly to 2.0 mL of distilled water in the center of Conway diffusion cells by Hamilton syringe. Solid compounds were dissolved or suspended in water at 1000 mg/L and diluted to the proper concentration. A 0.5-mL portion of the 2.0-mL sample in the center well was transferred to a 22 mm diameter microdish placed in the center well. The microdish was used to reduce the number of uredospores required, through release of endogenous inhibitor, to give germination values of controls close to zero, so that maximum stimulatory activity could be expressed. Uredospores (ca. 2.5 mg) were placed on the surface of the liquid in the microdish and dispersed uniformly with a sterile inoculating loop. The Conway diffusion cell, covered with a 7.5 cm square wet ground glass lid, served as an individual isolation chamber for each concentration. Germination tests were run at 22 °C for 2.5 h, in the dark. Germination values were compared to that of a standard stimulator, β-ionone, at 10 μL/L, and the rating was calculated as follows:

$$\text{rating} = \frac{\%\ \text{germ.(most effective concn.)} - \%\ \text{germ.(controls)}}{\%\ \text{germ.(10 } \mu\text{L/L } \beta\text{-ionone)} - \%\ \text{germ.(controls)}} \times 100 \quad (1)$$

To test for germination of spores in pustules, rusted plants were placed in a 220-L dew chamber at 22 °C with 0.1–0.2 mL of the volatile liquid to be tested placed on a 9-cm filter paper disk supported on a 250-mL graduated cylinder. Compounds that are water-soluble solids were dissolved in water at 500–1000 mg/L and sprayed on the rusted plants, and the plants were placed in the dew chamber before the solution evaporated.

RESULTS

Early in these studies β-ionone was found to be an effective stimulator of germination of uredospores of both *P. helianthi* and *Uromyces vignae*; hence, this compound was used as the standard stimulator for both species and for calculating the ratings. A rating of 50 indicated the compound was 50% as effective as β-ionone. A rating of 125 indicated the compound was more stimulatory than β-ionone, etc. The ability of 101 compounds to stimulate uredospore germination was compared to that of the β-

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ionone standards. For uredospores of *P. helianthi*, the mean ± SD of the 101 water controls = 1.63 ± 2.35 and of the 101 β-ionone controls = 73.21 ± 14.2. For uredospores of *U. vignae*, the mean ± SD of 101 water controls = 1.52 ± 3.14 and for 101 β-ionone controls = 71.06 ± 12.3.

In the following discussion of activity, the rating of a compound, from Table I, appears in parentheses following the name of the compound.

P. helianthi. *Aliphatic Compounds.* Linear and branched aldehydes and alcohols had little activity on uredospores of *P. helianthi*. Linear ketones with six to nine carbons were only slightly active. Of those tested, 5-nonanone was most active with a rating of 78 (Table I). Branched ketones, including 5-methyl-2-hexanone, which was most effective on *P. punctiformis*, *P. chondrillina* (French, 1983), and *Uredo eichhorniae* (Charudattan et al., 1981), were inactive. The linear nitriles hexanenitrile (96), nonanenitrile (77), and decanenitrile (82) were very active on *P. helianthi*.

Aromatic or Cyclic Compounds. (*1*) *Alcohols.* The simplest cyclic alcohol, phenol, was active (91). Among the benzyl alcohols, the 2-hydroxy (107) and 4-hydroxy (73) derivatives were active, whereas the 3-hydroxybenzyl alcohol was not. The 3-methoxy- (85) and 4-methoxybenzyl (56) alcohols were active. Disubstituted hydroxy and methoxy compounds were inactive. The branched and unsaturated compounds, carveol and eugenol, were inactive.

(*2*) *Aldehydes.* Among cyclic aldehydes, benzaldehyde (64) was moderately active. Most of the hydroxyl, cyano, and methoxyl (anisaldehydes) monosubstituted benzaldehydes (Table I) had activity close to or better than that of the β-ionone standard. Of the chloro-substituted benzaldehydes, only 4-chlorobenzaldehyde was active (81). Of the methyl-substituted derivatives (tolualdehydes), only 2-methyl (49) and 4-methyl (53) were moderately active. The dihydroxy derivatives were not very active. Benzaldehyde derivatives with various combinations of hydroxyl and methoxyl, including 4-hydroxy-3-methoxybenzaldehyde (vanillin) (101), were active, as was the disubstituted 3,4-dimethoxybenzaldehyde (100). The trisubstituted compound, 4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde), also was active (77).

(*3*) *Esters.* Methyl salicylate (95), benzyl acetate (52), methyl 2,4-dihydroxybenzoate (57), and methyl 3,5-dihydroxybenzoate (63) varied from active to moderately active. Methyl 3,4-dimethoxybenzoate (1) and methyl mandelate (4) were inactive.

(*4*) *Ketones.* Acetophenone (92), α-irone (65), piperitone (89), and *l*-carvone (81) were active; *d*-carvone (55) was less active than the *l* isomer. Menthone (61), phorone (51) and α-ionone (58) were moderately active; isophorone (24) and benzophenone (2) were much less active.

(*5*) *Nitriles.* Most of the cyclic nitriles were equal to or better than β-ionone in stimulating germination of uredospores of *P. helianthi*. Benzonitrile (120), benzyl cyanide (104), 3-methoxybenzonitrile (107), 4-methoxybenzonitrile (anisonitrile) (125), and the amino derivatives 2-aminobenzonitrile (anthranilonitrile) (164) and 3-amino- (104) and 4-aminobenzonitrile (151) were all very active. Benzoyl cyanide (0) and mandelonitrile (0) were inactive. Cinnamonitrile (100), 4-methoxycinnamonitrile (100), 2-cyanophenol (163), 3-cyanophenol (171), and 4-cyanophenol (123) also were very active. Of the nitro-substituted benzonitriles, 4-nitrobenzonitrile (77) was active.

Many of the bicyclic or heterocyclic nitriles were active. Compounds such as 5-cyanoindole (95), 3-indolylacetonitrile (177), and 1-cyanonaphthalene (113) were very active. 1-Methoxy-2-indanol (60) was moderately active. 2-Furonitrile (26) and safrole (36) were only slightly active.

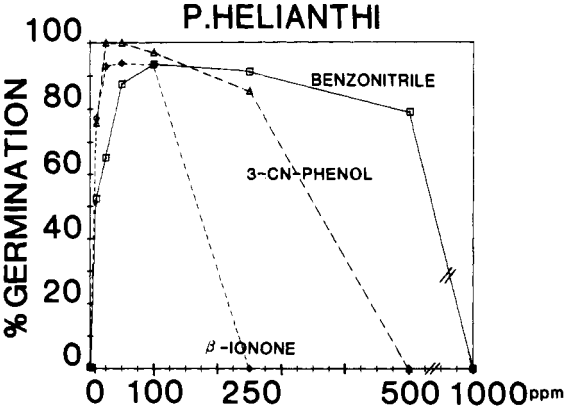

Figure 1. Effect of 0–1000 ppm of β-ionone (◇), benzonitrile (□), and 3-cyanophenol (△) on germination of uredospores of *P. helianthi* at 22 °C, $2^1/_2$ h.

Of the compounds listed in Table I, these 28 had ratings of 90 or better: 3-hydroxybenzaldehyde, 3-cyanobenzaldehyde, 4-cyanobenzaldehyde, 3-methoxybenzaldehyde, 4-methoxybenzaldehyde, 2-hydroxy-5-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 2-hydroxybenzyl alcohol, benzonitrile, benzyl cyanide, 3-methoxybenzonitrile, 4-methoxybenzonitrile, 2-aminobenzonitrile, 3-aminobenzonitrile, 4-aminobenzonitrile, 5-cyanoindole, 3-indolylacetonitrile, 1-cyanonaphthalene, cinnamonitrile, 4-methoxycinnamonitrile, phenol, 2-cyanophenol, 3-cyanophenol, 4-cyanophenol, methyl salicylate, acetophenone, and hexanenitrile.

This active group included compounds with nitrile, hydroxyl, aldehyde, methoxy, amino, ester, and carbonyl functional groups. Eighteeen of the 28 compounds (64%), including those found most active, were nitriles. Twenty-one compounds were equal to or better than the β-ionone standard, 15 (71%) of which were nitriles, and all of these were cyclic. Anisaldehydes (ortho, meta, and para), benzonitrile, benzyl cyanide, several methoxy and amino benzonitriles, 5-cyanoindole, and the 2-cyano-, 3-cyano-, and 4-cyanophenols were very effective.

Benzonitrile, 3-cyanophenol, and 2-aminobenzonitrile were the most effective of all the compounds tested on uredospores of *P. helianthi* because they were effective over a broad concentration range (Figure 1). Benzonitrile was effective from 10 to 500 ppm and 3-cyanophenol from 10 to 250 ppm. The latter was more water soluble than either benzonitrile or β-ionone. Both cyano derivatives were active over a much broader concentration range than was the β-ionone standard.

When 0.2 mL of benzonitrile was placed on filter paper suspended in a 220-L dew chamber with rusted sunflower plants, massive germination of spores in the pustules was induced, as shown in Figure 2. Pustules, normally dark brown from masses of ungerminated uredospores, were cottony white after treatment because of the great numbers of germ tubes produced. This response was also observed when rusted plants were sprayed with 1000 ppm of 3-cyanophenol or 2-aminobenzonitrile (Figure 2).

Uromyces vignae. *Aliphatic Compounds.* The linear aldehydes or alcohols tested did not stimulate appreciably the germination of uredospores of *U. vignae* (Table I). However, of the three to nine carbon linear methyl ketones, the six C (81), seven C (82), eight C (90), and nine C (86) (Table I) were about equally stimulatory. Methyl or 2-ketones were most active. Compounds with the carbonyl at other than the 2-position, such as 3-nonanone (48),

Table I. Effect of Various Flavorlike Chemicals on Germination of Uredospores of *Puccinia helianthi* and *Uromyces vignae*, Compared to 10 μL/L β-Ionone as Standard (Temperature 22–23 °C, 2.5 h)

compound	*P. helianthi* concn, ppm[a]	*P. helianthi* rating[b]	*U. vignae* concn, ppm[a]	*U. vignae* rating[b]
aliphatic compounds				
ketones				
2-propanone	25	0	1000	0
2-butanone	50	2	500	0
2-pentanone	500	8	250	5
2-hexanone	500	41	100	81
2-heptanone	250	28	100	82
3-heptanone	250	35	100	0
4-heptanone	100	49	100	0
2-octanone	100	49	50	90[c]
2-nonanone	100	54	25	86
3-nonanone	250	41	50	48
5-nonanone	100	78	100	3
5-methyl-2-hexanone	100	25	25	90[c]
6-methyl-5-hepten-2-one	25	0	25	13
2,6-dimethyl-4-heptanone	500	26	100	60
nitriles				
hexanenitrile	250	96[c]	100	59
nonanenitrile	100	77	25	62
decanenitrile	25	82	50	6
aromatic or cyclic compounds				
alcohols				
phenol[d]	250	91[c]	25	14
catechol[d]	10	0	10	0
carveol	100	34	250	0
eugenol	10	0	25	0
2-hydroxybenzyl alcohol[d]	500	107[c]	250	0
3-hydroxybenzyl alcohol[d]	1000	4	500	4
4-hydroxybenzyl alcohol[d]	1000	73	500	4
2-methoxybenzyl alcohol	100	27	10	0
3-methoxybenzyl alcohol	500	85	100	24
4-methoxybenzyl alcohol	1000	56	500	54
3-hydroxy-4-methoxybenzyl alcohol[d]	1000	11	50	0
4-hydroxy-3-methoxybenzyl alcohol[d]	1000	6	25	22
aldehydes				
benzladehyde	250	64	50	12
monosubstituted				
2-hydroxybenzaldehyde (salicylaldehyde)	25	61	10	26
3-hydroxybenzaldehyde[d]	100	107[c]	100	18
4-hydroxybenzaldehyde[d]	250	88	50	68
aldehydes				
trisubstituted				
4-hydroxy-3,5-dimethoxybenzaldehyde[d] (syringaldehyde)	50	77	50	76
other				
isocyclocitral	10	0	10	14
cinnamaldehyde	100	40	1000	0
2-pyridinecarboxaldehyde	250	32	25	69
esters				
methyl salicylate	10	95[c]	25	19
benzyl acetate	100	52	50	39
methyl 2,4-dihydroxybenzoate[d]	100	57	10	0
methyl 3,5-dihydroxybenzoate[d]	1000	63	100	59
methyl 3,4-dimethoxybenzoate[d]	10	1	10	0
methyl mandelate[d]	10	4	10	0
ketones				
cyclohexanone	100	6	1000	24
cycloheptanone	50	29	10	0
acetophenone	25	92[c]	25	34
benzophenone[d]	10	2	10	26
d-carvone	50	55	25	18
l-carvone	100	81	100	0
hydroquinone[d]	10	0	25	0
α-ionone	25	58	25	104[c]
β-ionone				
α-irone	25	65	100	83
menthone	10	61	1000	0
1-phenyl-2-butanone	25	0	25	16
phorone	100	51	50	0
isophorone	25	24	50	6
piperitone	10	89	25	1
nitriles				
benzonitrile	100	120[c]	50	62
benzyl cyanide	50	104[c]	50	67
benzoyl cyanide	10	0	10	0
3-methoxybenzonitrile	10	107[c]	25	96[c]
4-methoxybenzonitrile (anisonitrile)[d]	100	125[c]	25	102[c]
2-aminobenzonitrile (anthranilonitrile)[d]	100	164[c]	100	72
3-aminobenzonitrile[d]	250	104[c]	100	84
4-aminobenzonitrile[d]	100	151[c]	25	94[c]
2-nitrobenzonitrile[d]	50	31	10	0

3-cyanobenzaldehyde[d]	250	98[c]	25	101[c]	3-nitrobenzonitrile[d]	50	30	25	0
4-cyanobenzaldehyde[d]	100	104[c]	25	100[c]	4-nitrobenzonitrile[d]	50	77	10	0
2-chlorobenzaldehyde	50	0	10	0	mandelonitrile	10	0	10	0
3-chlorobenzaldehyde	25	3	10	0	cinnamonitrile	10	100[c]	50	36
4-chlorobenzaldehyde[d]	100	81	50	52	4-methoxycinnamonitrile	10	100[c]	10	57
2-methylbenzaldehyde (*o*-tolualadehyde)	50	49	25	61	2-cyanophenol[d]	50	163[c]	10	13
3-methylbenzaldehyde (*m*-tolualdehyde)	100	13	25	78	3-cyanophenol[d]	25	171[c]	50	75
4-methylbenzaldehyde (*p*-tolualdehyde)	250	53	50	98[c]	4-cyanophenol[d]	100	123[c]	25	80
2-methoxybenzaldehyde (*o*-anisaldehyde)[d]	100	78	100	15	heterocyclic or bicyclic				
3-methoxybenzaldehyde (*m*-anisaldehyde)	250	117[c]	100	83	5-cyanoindole[d]	10	95[c]	25	82
4-methoxybenzaldehyde (*p*-anisaldehyde)	250	122[c]	100	86	2-furonitrile	500	26	250	2
disubstituted					3-indolylacetonitrile	50	177[c]	50	1
2,3-dihydroxybenzaldehyde[d]	50	21	10	0	1-cyanonaphthalene[d]	10	113[c]	10	0
2,4-dihydroxybenzaldehyde[d]	50	43	10	0	safrole	50	36	25	58
2,5-dihydroxybenzaldehyde[d]	10	0	10	0	1-methoxy-2-indanol	1000	60	100	6
3,4-dihydroxybenzaldehyde[d]	10	0	10	0					
2-hydroxy-3-methoxybenzaldehyde (*o*-vanillin)[d]	50	57	25	7					
2-hydroxy-4-methoxybenzaldehyde[d]	100	88	25	64					
2-hydroxy-5-methoxybenzaldehyde	50	96[c]	10	0					
3-hydroxy-4-methoxybenzaldehyde[d]	500	45	25	13					
4-hydroxy-3-methoxybenzaldehyde (vanillin)[d]	250	101[c]	100	4					
3,4-dimethoxybenzaldehyde[d]	250	100[c]	25	0					

[a] μL/L for liquids, mg/L for solids; most effective concentration over the range 0–1000 ppm. [b] Rating = [% germ.(most effective concn) − % germ.(controls)]/[% germ.(10 μL/L β-ionone) − % germ.(controls)] × 100. [c] Compounds with ratings of 90 or greater. [d] Solid compounds at room temperature.

5-nonanone (3), 3-heptanone (0), and 4-heptanone (0), were much less active. 5-Methyl-2-hexanone (90) also was active.

Among the linear nitriles tested, hexanenitrile (59) and nonanenitrile (62) were active. Decanenitrile (6) was much less active.

Aromatic or Cyclic Compounds. (*1*) *Alcohols.* Among the cyclic alcohols tested, only 4-methoxybenzyl alcohol (54) showed moderate activity.

(*2*) *Aldehydes.* Of the monosubstituted benzaldehydes, the 2-methyl- (61), 3-methyl- (78), and 4-methylbenzaldehydes (98) (*o*-, *m*-, and *p*-tolualdehydes, respectively) were active. The 3-methoxy- (83) and 4-methoxybenzaldehyde (86) (*m*- and *p*-anisaldehydes, respectively), 4-chlorobenzaldehyde (52), and 4-hydroxybenzaldehyde (68) were active. 3-Cyanobenzaldehyde (101) and 4-cyanobenzaldehyde (100) were very active and equal to the β-ionone standard. Only 2-hydroxy-4-methoxy-benzaldehyde (64) of the disubstituted benzaldehydes was active. Benzaldehydes with 2-hydroxy, 3-hydroxy, 2-chloro, 3-chloro, and 2-methoxy substituents had very little activity. Syringaldehyde (76), which is trisubstituted, also was active.

(*3*) *Esters.* Of the aromatic esters tested, only methyl 3,5-dihydroxybenzoate (59) had moderate activity.

(*4*) *Ketones.* α-Ionone (104) and α-irone (83) were the only active cyclic ketones. All others had ratings less than 50.

(*5*) *Nitriles.* Of the cyclic nitriles tested, benzonitrile (62), benzyl cyanide (67), 2-aminobenzonitrile (72), 3-aminobenzonitrile (84), 4-aminobenzonitrile (94), 5-cyanoindole (82), 4-methoxycinnamonitrile (57), 3-cyanophenol (75), and 4-cyanophenol (80) were active. 4-Methoxybenzonitrile (102) (anisonitrile) and 3-methoxybenzonitrile (96) were as good as, or better than, the β-ionone standard (100). The other cyclic nitriles had little activity in stimulating the germination of uredospores of *U. vignae*.

In general, uredospores of *U. vignae* were stimulated by a much smaller number of compounds than were those of *P. helianthi*.

Of all the compounds tested on uredospores of *U. vignae* (Table I), the following nine compounds had ratings of 90 or higher: 3-cyanobenzaldehyde, 4-cyanobenzaldehyde, 4-methylbenzaldehyde, 3-methoxybenzonitrile, 4-methoxybenzonitrile, 4-aminobenzonitrile, 2-octanone, 5-methyl-2-hexanone, and α-ionone. Of these nine, seven were cyclic or aromatic and two were linear ketones. Five compounds (55%) had nitrile functional groups. 4-Cyanobenzaldehyde was an effective stimulator and was effective over a wider range than the standard, β-ionone (Figure 3). Methyl 3,5-dihydroxybenzoate was not as stimulatory but was active in the range of 10–250 ppm. It was not inhibitory at 1000 ppm.

Rusted cowpea plants sprayed with an aqueous solution of 1000 ppm of 4-cyanobenzaldehyde and placed in a dew chamber overnight developed white cottony pustules due to germination of the spores in the pustules (Figure 4). However, germination was not as extensive as that observed with benzonitrile on sunflower rust. This was the first time any germination in pustules was observed with *U. vignae*.

Although uredospores of both *P. helianthi* and *U. vignae* were stimulated to germinate by β-ionone, each species responded differently to most other compounds. In contrast to uredospores of *U. phaseoli*, which responded preferentially to 2-heptanone and to 2-ketones generally (French et al., 1977), those of *P. helianthi* were not responsive to 2-ketones and of the linear ketones responded

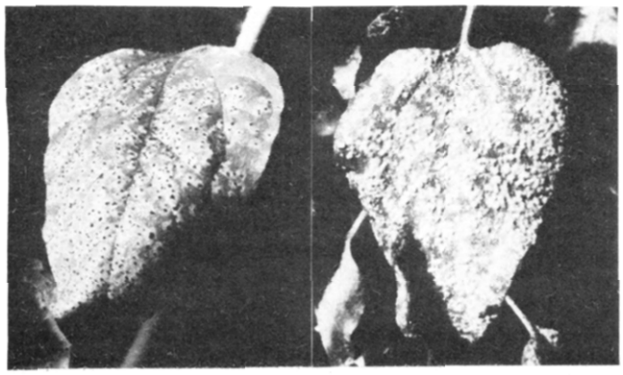

Figure 2. Leaves of rusted sunflower plants: (left) control, exposed overnight to dew at 22 °C, showing no germination; (right) exposed overnight at 22 °C to dew plus vapor from 0.2 mL of benzonitrile on a filter paper disk (estimated concentration ca. 2 ppm), showing massive germination of uredospores in the pustules.

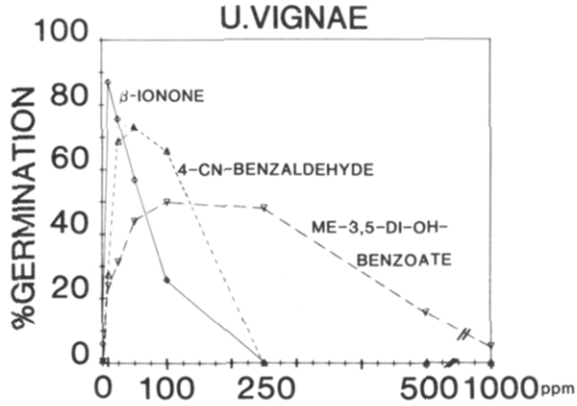

Figure 3. Effect of 0–1000 ppm of β-ionone (◇), 4-cyanobenzaldehyde (△), and methyl 3,5-dihydroxybenzoate (▽) on germination of uredospores of *Uromyces vignae* at 22 °C, $2^1/_2$ h.

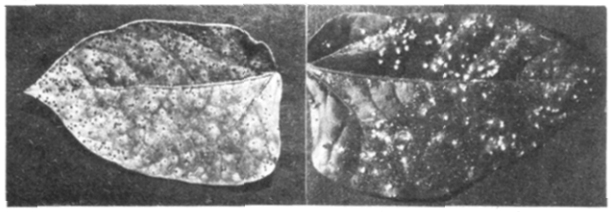

Figure 4. Rusted cowpea plants sprayed and placed in dew chambers for 18 h at 22 °C: (left) with distilled water (control); (right) with 1000 ppm of 4-cyanobenzaldehyde.

best to 5-nonanone. Uredospores of *U. vignae* were more like those of *U. phaseoli*, responding actively to 2-ketones or methyl ketones, but rather uniformly over the 6–9 C range, and did not respond to other than 2-ketones.

In considering the aromatic structures active on uredospores of both rust species, we found that 1,3- or 1,4-disubstitution of the benzene ring with –CHO, –OH, –CN, –OMe, or $–NH_2$ groups was most common, with one substituent of the two being –CN, the cyano group.

Only five compounds, 3-cyanobenzaldehyde, 4-cyanobenzaldehyde, 3-methoxybenzonitrile, 4-methoxybenzonitrile, and 4-aminobenzonitrile, were among the most active on both species. All of these were nitriles, solids at room temperature, and were more water soluble than most of the compounds tested.

DISCUSSION

Previous research has shown that fungal spore germination can be chemically stimulated in 27 species of 7 genera: *Puccinia*, *Uromyces*, *Urocystis*, *Coleosporium*, *Ustilago*, *Melampsora*, and *Penicillium*. Of over 100 flavorlike compounds tested, nonanal, β-ionone, and 5-methyl-2-hexanone (Charudattan et al., 1981; French, 1983; French et al., 1975a,b, 1978; French and Gallimore, 1971) have been most effective with certain groups of fungal species. Seeds of curly dock (Rumex *crispus*) and red sorrel (*Rumex acetosella*) were stimulated by nonanenitrile and 2-nonanone (French and Leather, 1979). Some of the naturally occurring flavorlike compounds tested in this research included *p*-anisaldehyde (occurring in anise and vanilla extracts, e.g.), benzyl cyanide (garden cress) ("The Merck Index", 1976), benzonitrile (cocoa aroma), methyl salicylate (wintergreen, raspberry), styrene (cranberry, grape), vanillin and syringaldehyde (lignin), and α-irone (orris root, raspberry) (Furia and Bellanca, 1975).

Although many compounds may stimulate germination of uredospores floated on water, or an agar, very few are active on spores in pustules. The stimulation of germination of spores in pustules is unique and has been observed in a limited number of species with only certain specific compounds. Previously nonanal or nonanol has been reported to be active with spores of *P. graminis* var. *tritici*, nonanal on *Puccinia recondita* and *Puccinia coronata*, nonanol for *Puccinia rubigo-vera* (French et al., 1975b), and β-ionone for *Uromyces phaseoli* (French et al., 1977). Benzonitrile can now be included as effective on *P. helianthi* and 3-cyanophenol as the first compound effective in a spray technique to achieve in-pustule germination. 4-Cyanobenzaldehyde was somewhat less effective in inducing in-pustule germination of uredospores of *Uromyces vignae*, but it is the only compound found to do so in this species. The standard, β-ionone, did not induce germination in pustules of either *P. helianthi* or *U. vignae*.

Benzonitrile, along with nonanal, nonanol, β-ionone, and 5-methyl-2-hexanone, may be considered to represent key compounds most effective in stimulating uredospore germination in certain groups of species of rust spores.

The importance of these stimulators lies in their ability to initiate germination in unision with low concentrations of transient chemicals that have been part of the human diet for centuries. In this report we have demonstrated that some less volatile, more water-soluble aromatic nitriles can be sprayed on rusted plants of sunflower and cowpea to stimulate massive germination of uredospores in pustules. The ability to induce such germination, almost at will, could be used to cause premature or precocious germination at an inopportune time for survival or to make the spores more amenable to control using fungicides. On the other hand, if the stimulatable spore were that of a biocontrol fungus, pathogenic on a noxious weed, for example, stimulators might be used to obtain maximum germination for the initial phase of a biocontrol epidemic. At the present time the practicality for such use has yet to be demonstrated.

Although organic nitriles have been shown to be very effective in stimulating germination of uredospores of sunflower and cowpea rusts and the germination of curly dock and red sorrel seeds (French and Leather, 1979), such compounds are not known to occur in these propagules. Relatively little information concerning the natural occurrence of organic nitriles is available. However, cyanogenic glycosides and cyanolipids are known to occur in over 2000 plant species, in over 100 plant families (Seigler, 1981), including ferns, gymnosperms, monocotyledenous, and dicotyledenous angiosperms, and in insects and other animals. In fact, benzoyl cyanide and mandelonitrile, inactive on uredospores of *P. helianthi* and *U. vignae*, have

been reported to occur in the defense secretions of certain millipedes (Conner et al., 1977; Duffy et al., 1977).

Many of the naturally occurring cyanogenic compounds are difficult to isolate because they are labile. In many cases there has been no occasion or reason to look for certain nitriles in plants. The demonstrated biological activity of the nitriles shown in this report may stimulate investigations of this type.

On the other hand, many of the phenolic compounds are known to occur widely in the plant kingdom. Many of the active compounds, such as vanillin, might arise in plant residues resulting from the degradation of lignin. They may have insect pheromonal properties or allelopathic activity and stimulate or inhibit germination of propagules, including seeds and spores of various types. In earlier reports we have shown nonanal and 6-methyl-5-hepten-2-one to occur in uredospores of various rusts (French and Weintraub, 1957; Rines et al., 1974). It is hoped that future research may permit us to look for active stimulatory compounds in these organisms now known to respond markedly to certain aromatic compounds, many of which already are known to occur naturally.

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Registry No. 2-Propanone, 67-64-1; 2-butanone, 78-93-3; 2-pentanone, 107-87-9; 2-hexanone, 591-78-6; 2-heptanone, 110-43-0; 3-heptanone, 106-35-4; 4-heptanone, 123-19-3; 2-octanone, 111-13-7; 2-nonanone, 821-55-6; 3-nonanone, 925-78-0; 5-nonanone, 502-56-7; 5-methyl-2-hexanone, 110-12-3; 6-methyl-5-hepten-2-one, 110-93-0; 2,6-dimethyl-4-heptanone, 108-83-8; hexanenitrile, 628-73-9; nonanenitrile, 2243-27-8; decanenitrile, 1975-78-6; phenol, 108-95-2; catechol, 120-80-9; carveol, 99-48-9; eugenol, 97-53-0; 2-hydroxybenzyl alcohol, 90-01-7; 3-hydroxybenzyl alcohol, 620-24-6; 4-hydroxybenzyl alcohol, 623-05-2; 2-methoxybenzyl alcohol, 612-16-8; 3-methoxybenzyl alcohol, 6971-51-3; 4-methoxybenzyl alcohol, 105-13-5; 3-hydroxy-4-methoxybenzyl alcohol, 4383-06-6; 4-hydroxy-3-methoxybenzyl alcohol, 498-00-0; benzaldehyde, 100-52-7; *o*-vanillin, 148-53-8; 2-hydroxy-4-methoxybenzaldehyde, 673-22-3; 2-hydroxy-5-methoxybenzaldehyde, 672-13-9; 3-hydroxy-4-methoxybenzaldehyde, 621-59-0; vanillin, 121-33-5; 3,4-dimethoxybenzaldehyde, 120-14-9; syringaldehyde, 134-96-3; isocyclocitral, 432-24-6; cinnamaldehyde, 104-55-2; 2-pyridinecarboxaldehyde, 1121-60-4; methyl salicylate, 119-36-8; benzyl acetate, 140-11-4; methyl 2,4-dihydroxybenzoate, 2150-47-2; salicylaldehyde, 90-02-8; 3-hydroxybenzaldehyde, 100-83-4; 4-hydroxybenzaldehyde, 123-08-0; 3-cyanobenzaldehyde, 24964-64-5; 4-cyanobenzaldehyde, 105-07-7; 2-chlorobenzaldehyde, 89-98-5; 3-chlorobenzaldehyde, 587-04-2; 4-chlorobenzaldehyde, 104-88-1; *o*-tolualdehyde, 529-20-4; *m*-tolualdehyde, 620-23-5; *p*-tolualdehyde, 104-87-0; *o*-anisaldehyde, 135-02-4; *m*-anisaldehyde, 591-31-1; *p*-anisaldehyde, 123-11-5; 2,3-dihydroxybenzaldehyde, 24677-78-9; 2,4-dihydroxybenzaldehyde, 95-01-2; 2,5-dihydroxybenzaldehyde, 1194-98-5; 3,4-dihydroxybenzaldehyde, 139-85-5; methyl 3,5-dihydroxybenzoate, 2150-44-9; methyl 3,4-dimethoxybenzoate, 2150-38-1; methyl mandelate, 771-90-4; cyclohexanone, 108-94-1; cycloheptanone, 502-42-1; acetophenone, 98-86-2; benzophenone, 119-61-9; *d*-carvone, 2244-16-8; *l*-carvone, 6485-40-1; hydroquinone, 123-31-9; α-ionone, 127-41-3; β-ionone, 79-77-6; α-irone, 79-69-6; menthone, 89-80-5; 1-phenyl-2-butanone, 1007-32-5; phorone, 504-20-1; isophorone, 78-59-1; piperitone, 89-81-6; benzonitrile, 100-47-0; benzyl cyanide, 140-29-4; benzoyl cyanide, 613-90-1; 3-methoxybenzonitrile, 1527-89-5; anisonitrile, 874-90-8; anthranilonitrile, 1885-29-6; 3-aminobenzonitrile, 2237-30-1; 4-aminobenzonitrile, 873-74-5; 2-nitrobenzonitrile, 612-24-8; 3-nitrobenzonitrile, 619-24-9; 4-nitrobenzonitrile, 619-72-7; mandelonitrile, 532-28-5; cinnamonitrile, 4360-47-8; 4-methoxycinnamonitrile, 28446-68-6; 2-cyanophenol, 611-20-1; 3-cyanophenol, 873-62-1; 4-cyanophenol, 767-00-0; 5-cyanoindole, 15861-24-2; 2-furonitrile, 617-90-3; 3-indolylacetonitrile, 771-51-7; 1-cyanonaphthalene, 86-53-3; safrole, 94-59-7; 1-methoxy-2-indanol, 71720-52-0.

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