Stimulation of Germination of Puccinia carthami Teliospores by Polyacetylenes from Safflower

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ABSTRACT

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Volatile polyacetylenes from safflower stimulate germination of teliospores of *Puccinia carthami*. Ten isomers of known C_{13} polyacetylenic hydrocarbons containing from two to five acetylenic bonds were isolated from safflower seedlings and assayed for activity. Exposure of teliospores to 15 ng/cm^3 of 1, 11-tridecadiene-3,5,7,9-tetrayne resulted in 83% germination, whereas controls with no stimulator showed 10% germination. Polyacetylenes with three or two acetylenic bonds were less active, but were present in larger quantities.

Teliospores of *Puccinia carthami* Cda. are stimulated to germinate by substances from safflower (*Carthamus tinctorius* L.) roots, leaf and stem fragments, and by exposure to volatiles from safflower seedlings, stems, or leaves (10, 11). Although germination of teliospores not exposed to volatile substances from plant parts regularly was less than 10%, more than 90% of the spores germinated in 7 days when exposed to seedling volatiles (11).

The object of this study was to isolate and identify volatile compounds from safflower that stimulate germination of the safflower rust teliospores. Knowledge of germination-stimulating compounds is fundamental to understanding interactions in this host-parasite combination, the nature of resistance in safflower to rust and the biological processes involved in germination of these rust teliospores.

MATERIALS AND METHODS

Seed of a commercial cultivar of safflower was used to grow seedlings either in a flask through which air was blown and to which water occasionally was added, or in open trays. Seed was soaked for 2 hr in water and allowed to germinate for 4-5 days. Seedlings were steeped in ether at 20C for 10 min, then the ether solution was decanted, dried, and concentrated by distillation. The residue was divided into compounds extracted by 5% HCl, compounds extracted by 5% KOH, carbonyl compounds extracted by Girard's Reagent T, and compounds that did not react with this reagent. The most active fraction (Table 1) was further fractionated by chromatography on a 5 × 85 cm column of Sephadex LH-20 in methanol. Active fractions were chromatographed on a 2 × 95-cm column of silca gel H (dried at 125 C) with heptane as the eluting solvent. Polyacetylenes started to elute from the column after 600 ml of heptane was used and eluted continuously until 2500 ml of solvent had passed through the column. An ultraviolet (UV) detector was not used to monitor the elution of compounds because intense UV light causes isomerism of double bonds. Polyacetylenes in aliquots of fractions were recognized by their distinctive UV spectra and further characterized by their nuclear magnetic resonance spectra and mass spectra. The purity of the isomers was determined by high-pressure liquid chromatography on a 2-mm × 50-mm Vydak reverse phase column.

Teliospores were collected, washed, and dried as described (11) and utilized in assays to determine the effect of volatile compounds on their germination. Small volumes (10-400 μliters) of ether or heptane solutions containing compounds isolated from safflower were dispensed in microbeakers which were placed in spore germination chambers (11) beneath inverted petri dishes containing spores on water agar. The air space in these chambers was 125-130 cm^3. Solvent was allowed to evaporate before the chambers were closed. Similar volumes of solvent only were assayed for possible stimulation or inhibition of spore germination. Generally, the stimulatory effect of the amount of sample derived from six seedlings was compared to the stimulatory effect of six seedlings. Germination controls consisted of spores on agar and spores on agar in dishes inverted over six seedlings. Two hundred spores were scored in each of two dishes per assay on the 7th day of incubation. Spores were scored as germinated when a four-celled promycelium was produced by one or both cells.

RESULTS

Ether and heptane solvent residues were neither stimulatory nor inhibitory to spore germination. Ether extracts of ground safflower seed, of the water in which the seeds were soaked, and of the ground seed after soaking in water did not stimulate spore germination (Table 1). However, an ether rinse of 4-day-old seedlings was strongly stimulatory. Acidic, basic, and carbonyl compounds extracted from the ether rinse of seedlings were not active stimulators, whereas potent stimulation was shown by the remainder of this extract.

The solvent-free residue from the ether rinse was applied to a 1.8 × 25-cm silicic acid column and eluted with 10% ether in isopentane, ether, and methanol. Assays for activity of the resultant fractions showed 81% spore germination with the nonpolar fraction, 13% with the moderately polar fraction, and 26% with the polar fraction. Only the nonpolar fraction was investigated further.

Chromatography on Sephadex LH-20 concentrated active compounds and eliminated higher molecular weight compounds, notably glycerides. 1, 8, 11, 14-Heptadecatetraene was not eliminated and previously was found to be present in a relatively large amount (4). However, a pure sample of heptadecatetraene had no germination-stimulating activity in our assays. Gas-liquid chromatography of a hydrogenated portion of the active fractions indicated the presence of heptadecane and tridecane. Ultraviolet spectra of various active chromatography fractions were distinctive for polyacetylenes previously isolated from immature safflower seed (8).

The mixture of active compounds was resolved by repeated chromatography on silica gel H. The compounds were characterized by their UV, nuclear magnetic resonance, and mass spectra as polyacetylenes with two to five acetylenic bonds in an open chain of 13 carbon units (R. G. Binder et al., *published*).

In order to assay a known amount of compound, the concentration of a solution was determined from its UV spectrum (8) and aliquots were taken as samples. Assays for stimulatory activity of the polyacetylenes isolated from safflower seedlings (Table 2) indicated that with only 20 ng of the most active compound, more than 50% of the spores germinated. The maximum concentration of this compound in the germination chamber was about 0.15 ng/cm^3.

TABLE 1. Effect of volatile substances from safflower fractions on germination of teliospores of *Puccinia carthami*

Source of volatiles[a]	Germination in 7 days (%)
Ground safflower seed	2
Compounds leached from seed by water	2
Ground seed after soaking in water	6
Ether rinse of seedlings	92
5% HCl extract of ether rinse[a]	1
5% KOH extract of ether rinse[a]	5
Carbonyl compounds from ether rinse[a]	4
Remainder of ether rinse[a]	82
Seedings (6)	95
Agar	4

[a]In these sources the amount of fraction is equivalent to four rather than six seedlings.

DISCUSSION

Twenty-seven polyacetylenes from safflower have been identified previously (2, 3, 5, 6, 8, 12). Several have shown biological activity. At low concentrations, 1, 11-tridecadiene-3, 5, 7, 9-tetrayne and 1-tridecene-3, 5, 7, 9, 11-pentayne strongly inhibit growth of some species of bacteria and fungi (13). Curiously, in this study the same compounds as well as other polyacetylenes from safflower stimulated germination of *Puccinia carthami* teliospores. Two polyacetylenic diols, *trans, trans*-3, 11-tridecadiene-5, 7, 9-triyne-1, 2-diol and *trans*-11-tridecene-3, 5, 7, 9-tetrayne-1, 2-diol inhibit mycelial growth of *Phytophthora drechsleri (2, 3)*] *Recently, cis, trans*-, and *trans*-1, 3, 11-tridecatriene-5, 7, 9-triyne were shown to be potent nematicides (12).

TABLE 2. Effect of polyacetylenes from safflower on germination of *Puccinia carthami* teliospores

Compound	Germination[a] (%) in 7 days at ng/cm^3				
	1,500 (ng/cm^3)	150 (ng/cm^3)	15 (ng/cm^3)	1.5 (ng/cm^3)	0.15 (ng/cm^3)
1-tridecene-3, 5, 7, 9, 11-pentayne			52[b]	33[b]	15[b]
cis-1, 11-tridecadiene-3, 5, 7, 9-tetrayne			66[b]	56[b]	41[b]
trans-1, 11-tridecadiene-3, 5, 7, 9-tetrayne			83[b]	62[b]	57[b]
1,3-tridecadiene-5, 7, 9, 11-tetrayne			32[b]	32[b]	10[b]
cis, cis-1, 3, 11-tridecatriene-5, 7, 9-triyne		50[c]	19[d]	2[c]	
cis, trans-1, 3, 11-tridecatriene-5, 7, 9-triyne		46[c]	12[d]	4[c]	
trans, trans-1, 3, 11-tridecatriene-5, 7, 9-triyne		40[c]	12[d]	7[c]	
trans, cis, trans-1, 3, 5, 11-tridecatetraene-7, 9-diyne		12[c]	4[c]		
cis, trans, trans-1, 3, 5, 11-tridecatetraene-7, 9-diyne	40[c]	12[c]	4[c]		
trans, trans, trans-1, 3, 5, 11-tridecatetraene-7, 9-diyne	60[c]	12[c]	4[c]		

[a]Values are averages of two replications.
[b]Controls: no stimulator 10%; seedlings 87%.
[c]Controls: no stimulator 2%; seedlings 72%.
[d]Controls: no stimulator 5%; seedlings 65%.

Teliospores of *Ustilago* species were stimulated by *n*-nonanol (7).We found that 100 μliters of *n*-nonanol stimulated the germination of 35% of the *Puccinia carthami* teliospores. However, volatiles from six safflower seedlings stimulated 95% of the teliospores to germinate. Nonanol may be present in seedling volatiles, but clearly could not play so significant a role in stimulating spore germination as do the polyacetylenes. We did not attempt to identify volatile aldehydes or alcohols.

The relative activities of the compounds (Table 2) may not indicate their relative importance in providing stimulus to germination of teliospores under field conditions. There is 18 times more 1, 3, 11-tridecatriene-5, 7, 9-triyne than 1, 11-tridecadiene-3, 5, 7, 9-tetrayne in the seedlings and an even greater amount of 1, 3, 5, 11-tridecatetraene-7, 9-diyne. Large amounts of the less active compounds may stimulate more germination than small amounts of the most active compound.

We do not know the mechanism by which the compounds stimulate germination. It is worth noting, however, that some fungi produce polyacetylenes. The six C_{13} polyacetylenes isolated from mycelial cultures of *Fistulina hepatica* (9), of *Fistulina pallida* (1) would be readily derivable from triyne and tetrayne compounds listed in Table 2.

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