Volatiles from Germinating Canada Thistle Seed and Root Cuttings That Stimulate Germination of Teliospores of the Canada Thistle Rust Fungus, *Puccinia punctiformis*

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Volatiles collected from germinating Canada thistle (*Cirsium arvense* L.) seeds stimulated germination of the teliospores of the Canada thistle rust organism, *Puccinia punctiformis*. As thistle seed germination increased to 92% in 7 days, germination of teliospores on 1% agar test plates exposed to the volatiles in the germination chamber increased from 0% to 50%. Seedling volatiles collected on Tenax columns increased germination up to 31% after 7 days. Volatiles from undamaged germinating seeds represent normal, endogenous products. Two tridecylpolyacetylene compounds isolated from safflower (Binder et al., 1977) stimulated germination of the teliospores of *P. punctiformis*. Volatiles collected from root cuttings of Canada thistle, which may become infected when root buds are inoculated with teliospores (French and Lightfield, 1990), also stimulated teliospore germination. Secondary shoots from seed inoculated with teliospores were systemically infected after 5 weeks. This information may be useful in manipulating the Canada thistle rust fungus for use as a biocontrol agent.

Keywords: *Noxious weeds; fungal spores; stimulatory aroma; biocontrol; C_{13} polyacetylenes*

An extract of steam distilled Canada thistle roots [*Cirsium arvense* (L.) Scop.] stimulates the germination of teliospores of the Canada thistle rust fungus [*Puccinia punctiformis* (Strauss) Roehl.] (French et al., 1988). The teliospores germinate and produce up to eight basidiospores which may initiate a systemic, devastating infection in thistle shoots. This pathogen, an obligate parasite, is being considered as a possible biocontrol agent for Canada thistle, an important noxious weed. Many of the specific details of the life cycle of the Canada thistle rust fungus are not known, hence the biochemical properties and the chemical identity of the germination stimulator are being studied to learn how they relate to the mechanism of infection.

This paper describes the production of stimulatory volatiles from germinating Canada thistle seed and from sprouting root cuttings and presents evidence for the first time of the systemic infection of shoots from seeds inoculated with teliospores of *P. punctiformis*. It also describes stimulatory activity from 13-carbon polyacetylene compounds isolated and identified by Binder et al. (1977) from safflower (*Carthamus tinctorius* L.), previously shown to be active on teliospores of the safflower rust fungus, *Puccinia carthami*.

The specific objectives of this research were (1) to demonstrate that living propagules of Canada thistle (seedlings and root cuttings) generate volatile substances which stimulate germination of teliospores of *P. punctiformis*, (2) to collect such substances for bioassay, analysis, and identification, (3) to examine compounds from related plants (safflower) which have been reported to stimulate teliospores of the safflower rust fungus, and (4) to determine the susceptibility of germinating thistle seed to systemic infection from teliospores of *P. punctiformis* applied to the seed.

MATERIALS AND METHODS

Volatiles from Germinating Thistle Seed. In exploratory experiments, teliospores of *P. punctiformis* (Strauss) Roehl. were placed on 1% agar in 1.5 × 0.6-cm plastic dishes which were placed on 1% agar in the center of 3.5-cm plastic Petri plates. Thistle seeds, previously soaked in 100 mL of 0.001 M gibberellic acid under aeration in a gas washing bottle overnight and rinsed with tap water, were placed around the outside edge of the 1.5-cm plastic dishes, using 0, 1, 2, 5, 10, and 25 seeds per plate, replicated three times. The plates were incubated in the dark at 18 °C for 14 days, at which time the extent of seed and teliospore germination was determined.

Unlike domesticated crop plants such as safflower, which usually germinate readily and more or less in unison, Canada thistle seeds may have varying degrees of dormancy. Gibberellic acid (Aldrich Chemical Co., Inc., Milwaukee, WI) was used to synchronize germination, so that the seeds would be producing radicles, undergoing cell expansion, and producing volatiles in unison, thereby maximizing the amount for collection, bioassay, and analysis. Volatile production could then be related to physiological events in the germination process. Thistle seeds (7.5 g) were soaked 24 h in aerated 0.001 M gibberellic acid to synchronize germination. Volatiles were collected on 1.7 g Tenax GC 60/80 mesh (Alltech Associates, Inc., Deerfield, IL) (Buttery et al., 1982) in 2.0 × 15.0-cm glass columns. After soaking, seeds were washed and placed on Whatman No. 1 filter paper covering a 30 × 9.7-cm glass plate. The plate was inserted into a horizontal 33 × 11-cm glass cylinder and tap water was added to cover the edges of the filter paper. Moist air was drawn from a 15 × 25-cm glass beaker lined with filter paper and containing tap water, through the cylinder containing the seeds and tap water, past test plates, and through an activated Tenax column by house vacuum (Figure 1). Flow rate was 100–125 mL/min. Test plates were 1.6 × 0.6-cm plastic dishes filled with 1% agar and inoculated with teliospores of *P. punctiformis*. Plates were incubated at 18 °C in darkness and germination counts were made at 7 and 14 days. Test plates and Tenax columns were replaced every 24 h for up to 7 days.

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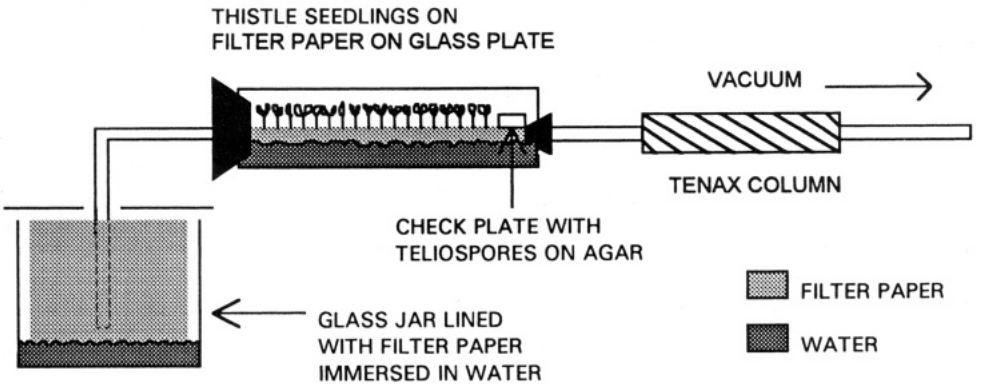

Figure 1. Diagram of apparatus for collecting volatiles from germinating thistle seeds.

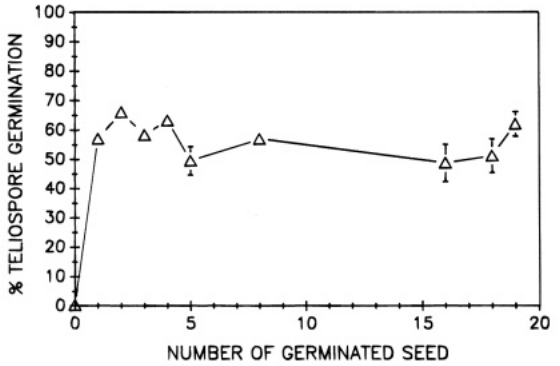

Figure 2. Fourteen-day germination response of teliospores of *P. punctiformis* to volatiles generated by germinating thistle seeds. Spores were incubated at 18 °C in darkness. Data points represent the average of three replications of 4 × 100 teliospores ± SE.

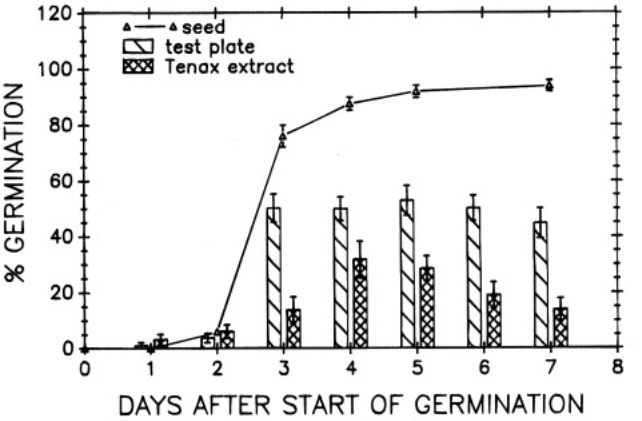

Figure 3. Germination response of teliospores to volatiles from 7.5 g of germinating thistle seeds. Teliospores on test plates were exposed to volatiles in the germination chamber for 24-h periods and then incubated at 18 °C in darkness for 7 days. Extracts from Tenax columns represented 24-h collections of volatiles. Data points are the average of 8 × 100 counts ± SD, for 10 μL of extract (= 2.0% of total extract).

Tenax columns were extracted with 200 mL redistilled, peroxide-free ethyl ether, which was distilled off on a hot water bath to 3 mL. Hexane (5 mL) was then added, distilled to 2 mL, then further concentrated to 0.5 mL at 0 °C under nitrogen gas. Extracts from Tenax columns were bioassayed on 1% agar in 5-cm plastic petri plates. Samples (10 μL) were added by syringe to a teliospore–isopentane (0.2 mL) suspension as the spores were dispersed on agar. The isopentane evaporated in less than 1 min. Plates were placed in darkness at 18 °C and germination was counted (8 × 100 spores at 100× magnification) at 7 and 14 days. Data from one of three similar experiments are presented.

Root Cuttings. Root cuttings (2–4 cm) were made from the washed roots of 4–6-month-old thistle plants grown from root cuttings in 10-cm pots in the greenhouse. The cut ends were dipped in melted paraffin to control contaminants.

Open plastic petri plates with teliospores on 1% agar were inverted over single root cuttings in a 2-cm furrow in six 10-cm pots of moist soil. These were held 7 days in darkness at 16 °C. Another group of six plates was placed over similar pots of soil without root cuttings, under the same conditions. Teliospore germination counts (4 × 100 spores) were made at 7 days.

To collect volatiles, 144 root cuttings (64.1 g) were placed on filter paper on the plate inside the glass cylinder in a manner similar to that used for collecting seed volatiles. New test plates were inserted into the germination chamber daily. Volatiles were collected on a Tenax column for the entire 7-day period. Bioassay of the concentrated extract was carried out over a range of 10–1000 μL/L and germination tests were performed as described above. Data from one of three similar experiments are presented.

Bioassay of Compounds from Safflower. Volatiles produced by germinating safflower seeds had been found to stimulate germination of teliospores of *P. punctiformis* (unpublished data). Therefore, polyacetylene compounds previously isolated and identified by Binder et al. (1977) from safflower (*Carthamus tinctorius* L.) and found to stimulate germination of teliospores of *Puccinia carthami* Cda. were tested for activity on teliospores of *P. punctiformis*. Canada thistle and safflower are both members of the Cynarea tribe of the Compositae family. The compounds (0.002–2 μg, log concentration) in hexane were added to suspensions of teliospores in 0.2 mL of isopentane on the surface of 1% agar in 5-cm plastic Petri plates as described above. Three replications per concentration were made and 400 spores were counted per replication. Statistical significance was determined using Waller's grouping, performed on arc sine transformed data, alpha = 0.01.

Seed Inoculation. Thistle seeds in a 23-mm glass dish were covered with an isopentane suspension of teliospores of *P. punctiformis* (4 mg/2 mL). The seeds were gently agitated as the isopentane evaporated, depositing the teliospores on the seed. Seeds were individually transferred to a seedling tray with 200 2-cm holes in the polystyrene plastic. Holes were filled with a peat/perlite mixture. Seeds were planted 1 cm deep, one seed per hole. The tray was placed in a large steel pan with water at 16 °C in the dark for 2 weeks and then transferred to the greenhouse. After the seedlings emerged, the tray was placed on a larger flat of soil over gravel. Seedlings were checked weekly for infection. Infected plants were removed to make room for those remaining.

RESULTS

Volatiles from Germinating Seeds. After 14 days at 18 °C, the volatiles produced from one seed were enough to stimulate teliospore germination 56% (controls 0.25%) (Figure 2). Maximum germination was 65% with two seeds, but greater numbers of germinated seeds did not increase teliospore germination.

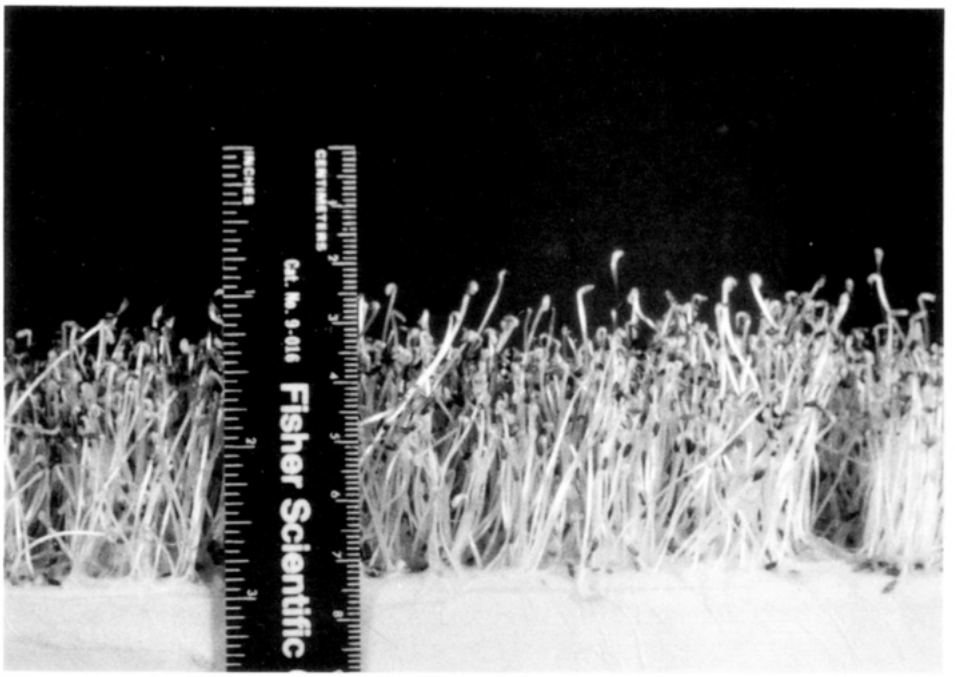

Figure 4. Fourteen-day thistle seedlings after removal from germination chamber, showing uniformity of germination and growth.

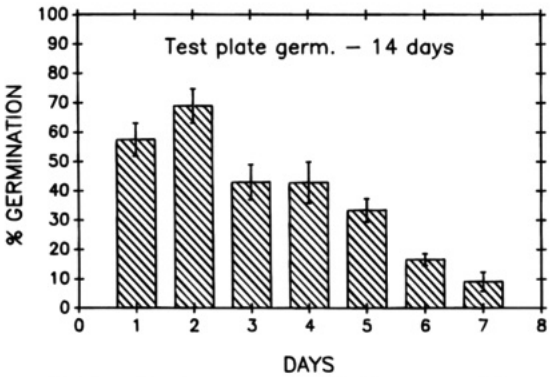

Figure 5. Germination response of teliospores of *P. punctiformis* on test plates exposed to volatiles produced by 144 thistle root cuttings. Teliospores were exposed for 24-h periods during the first 7 days of experiment. Germination was assessed after 14 days at 18 °C in darkness. Data points represent the average of 8 × 100 spores ± SD. Control germination was 0%.

Table 1. Effect of Volatiles[a] Collected from 144 (64.1 g) Sprouting Thistle Root Cuttings on Germination of Teliospores of *P. punctiformis*

concn (μL)	% germination ± SE	concn (μL)	% germination ± SE
0	0 ± 0	0.5	39.7 ± 2.53
0.05	10.7 ± 0.75	1.25	55.1 ± 1.95
0.125	11.2 ± 0.92	2.5	60.2 ± 2.13
0.25	22.1 ± 2.13	5.0	67.6 ± 1.23

[a] Volatiles were collected for 7 days on Tenax column, extracted with ethyl ether, concentrated, and bioassayed. Germination was determined after incubation for 14 days, 18 °C, in darkness. 0.05 μL = 0.01% of total extract.

During the collection of volatiles, the germination of Canada thistle seeds increased from 5% at day 2 to 76%, 87%, 92%, and 93% at days 3, 4, 5, and 7, respectively (Figure 3). Average seedling height increased from 2 mm at day 4 to 10 mm at day 7. Seed germination was fairly uniform (Figure 4) and seedling height at 14 days was approximately 3.5 cm. Germination of teliospores on the test plates, which were located near the exit port of the germination chamber and exposed to much of the atmosphere passed over the seeds, increased from 3% on day 2 to 50% for days 3, 4, 5, 6, and dropped to 44% on day 7. Stimulation from volatiles collected and eluted from Tenax columns (10 μL of extract) increased from a low level of 3 and 6% germination at 2 and 3 days to 13 and 31% at days 3 and 4, then decreased to 13% at day 7. Control germination was 0% at 7 days. Production of volatiles, as indicated by test plate germination and bioassay of column extracts, began with the onset of germination and appeared closely related to seed germination and initial growth of the seedlings (Figure 3).

Root Cuttings. Volatiles produced by single root cuttings in open moist soil stimulated teliospore germination 37.5% ± 4.2 SD, the average of 4 × 100 spore counts from each of six cuttings in separate pots, after 7 days. Germination values from six plates without root cuttings was 0% at 7 days.

Volatiles from root cuttings also stimulated germination of teliospores on test plates in the germination chamber (Figure 5). Exposure to volatiles for 1 and 2 days stimulated germination 57% and 69%. Germination declined each following day to less than 10% for the seventh day. Control germination was 0%. Volatiles extracted from the Tenax column stimulated germination (Table 1), the percentage increasing from 10% at 0.05 μL to a maximum of 67% at 5 μL after 14 days (controls 0%).

Bioassay of Safflower Compounds. The compounds from safflower were previously isolated and identified by Binder et al. (1977). Three of the C_{13} polyacetylene compounds were isomers having three double bonds at carbons 1, 3, 11 and three triple (acetylenic) bonds at the 5, 7, and 9 positions. Two were isomers with two double bonds at carbons 1 and 11 and four triple bonds at the 3, 5, 7, and 9 carbons. The isomers differed in cis–trans configurations at carbons 3 and 11. All of the tridecyl acetylene compounds from safflower stimulated germination of teliospores of *P. punctiformis* (Table 2). The compounds with four acetylenic bonds were slightly more active than the rest. Concentrations as low as 0.002 μg of trideca-1,11(*E*)-diene-1,3,7,9-tetrayne stimulated germination 24% (con-

Table 2. Effect of 0.002–2.0 μg Log Concentrations of Five Tridecylpolyacetylene Compounds from Safflower on Germination of Teliospores of *P. punctiformis* (18 °C, Dark, 14 Days)[a]

	% germination			
	0.002 μg	0.02 μg	0.2 μg	2.0 μg
trideca-1,3(*Z*),11(*Z*)-triene-5,7,9-triyne	7.4 ij	32.4 f	53.0 d	67.6 abc
trideca-1,3(*E*),11(*E*)-triene-5,7,9-triyne	1.0 jk	16.1 h	35.4 f	63.1 c
trideca-1,3(*Z*),11(*E*)-triene-5,7,9-triyne	1.8 jk	5.5 ijk	24.3 g	48.8 de
trideca-1,11(*E*)-diene-3,5,7,9-tetrayne	24.4 g	64.3 bc	70.6 ab	71.3 a
trideca-1,11(*Z*)-diene-3,5,7,9-tetrayne	11.9 hi	43.8 e	63.7 c	72.9 a
control germination 0.5% *k*				
stimulated control germination 70.7% ab[b]				

[a] Means followed by the same letter are not significantly different as determined by Waller's grouping, performed on arc sine transformed data, alpha = 0.01. Assays were carried out on 5 mL of 1% agar, three replicates/each concentration, 400 spores counted per replicate. Compounds in hexane were added to suspensions of teliospores in 0.2 mL of isopentane on the agar surface and mixed thoroughly. Solvents evaporated in less than 1 min, depositing spores and compounds evenly on agar surface. [b] Stimulated control = 50 μL/L hexane extract of steam-distilled thistle roots.

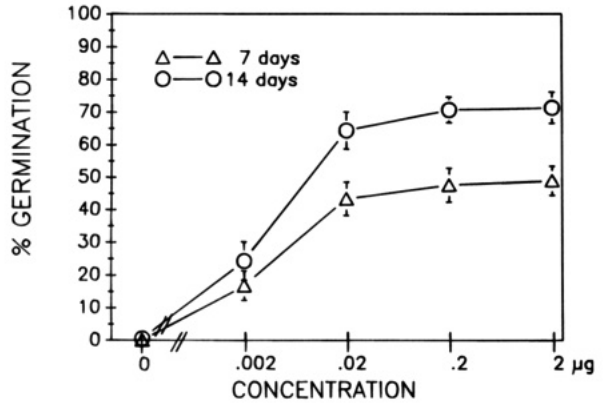

Figure 6. Germination response of teliospores of *P. punctiformis* to trideca-1,11(*E*)-diene-3,5,7,9-tetrayne, after incubation at 18 °C in darkness. Data points represent the average of three replications of 4 × 100 spores.

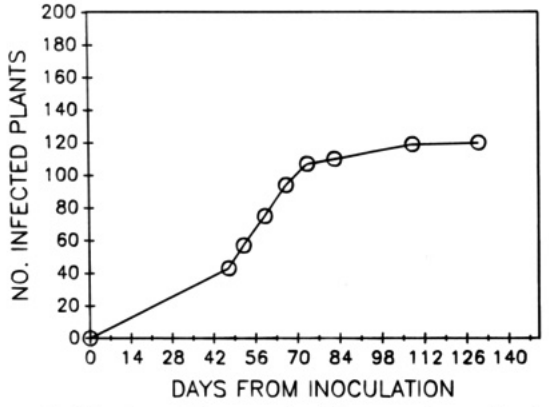

Figure 7. Number of days required for symptoms of systemic infection to appear after inoculated Canada thistle seeds were planted. Seeds were held at 16 °C in darkness for 2 weeks before transfer to the greenhouse.

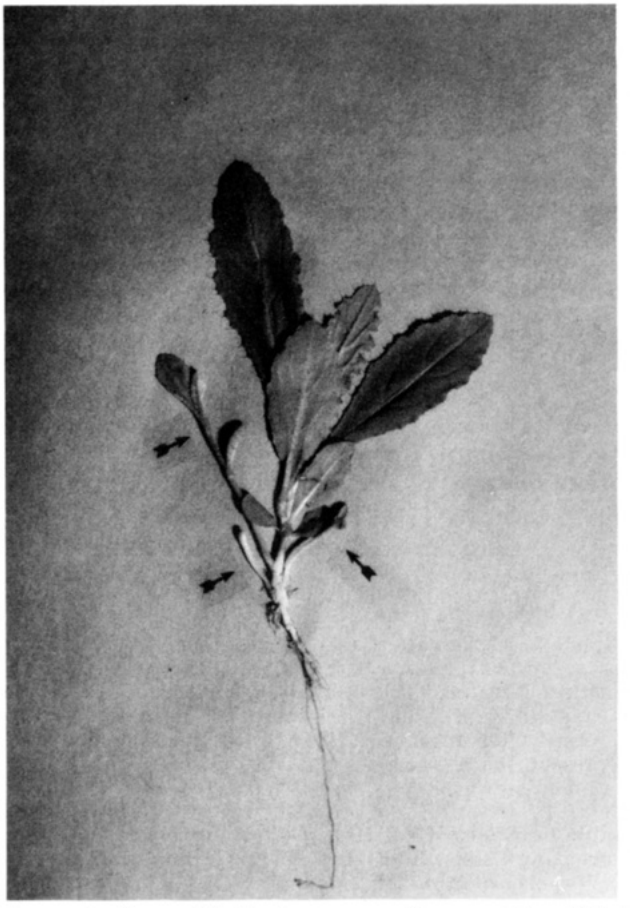

Figure 8. Young thistle plant grown from seed inoculated with teliospores of *P. punctiformis*. Note uninfected primary shoot and three young systemically infected secondary shoots (arrows).

trol 0%) at 14 days. Maximum germination with this compound was 71% at 2 μg at 14 days (Figure 6).

Inoculated Seed. Sprouts from inoculated seed started to appear above the soil surface at 14 days. The first sign of infection was observed at 47 days (Figure 7). The primary shoot produced from the seed was healthy. Secondary shoots were systemically infected (Figure 8). As many as three infected secondary shoots on one plant were observed. The number of infected plants reached a plateau at 72 days. At 130 days, 122 of 200 plants (61%) were infected. Both Canada thistle seeds and buds of root cuttings inoculated with teliospores become infected with the rust. The first sprout from the seed, and the first shoot from an inoculated root cutting are not infected. Subsequent shoots in both propagules are infected with the devastating systemic phase of the Canada thistle rust fungus.

DISCUSSION

Volatile emanations from germinating Canada thistle seeds stimulated germination of teliospores of the fungus which causes Canada thistle rust, a potential biocontrol agent for this noxious weed. Active production of volatiles commenced at germination and increased as the sprouts grew to 5 mm. The stimulatory volatiles produced by germinating seeds are naturally occurring products of germination and not artifacts produced by mechanical injury from cutting or by heat

injury during steam distillation of thistle roots. One of our samples of teliospores, which has been kept at 4 °C for 7 years, germinates 0% on agar. In the presence of stimulator germination may increase to 60% at 14 days. The C_{13} polyacetylene compounds provide teliospores of *P. punctiformis* as well as those of *P. carthami* with a potent "GO" signal initiating a series of events which may lead to infection of the hosts.

Systemic infection of Canada thistle shoots can be induced in germinating seeds or sprouting buds of root cuttings by inoculating them with teliospores. We have previously reported systemic infection from root cuttings by placing teliospores on root buds (French and Lightfield, 1990). We do not know if or how this may occur in nature. If teliospores must be delivered somehow to underground root buds for infection to take place, how do they get there? Are they washed down into the soil in the vicinity of the root bud or carried by some insect or other animal? Likewise, how would teliospores reach the seed? In either case, the likelihood of the teliospore reaching either target seems remote. Yet both the germinating seed and the root cutting with the expanding bud are generating volatile GO signals to teliospores. Other mechanisms must exist.

Germ tubes of spores of *Gigaspora gigantea* have been reported to be attracted to volatile chemical signals given off by seedling roots (Koske, 1982; Gemma and Koske, 1988). Perhaps germ tubes of teliospores of *P. punctiformis* can grow through the soil toward expanding root buds or germinating seeds. Other than long germ tubes occasionally observed with certain thistle root extracts, we have seen no evidence to support the idea that such growth occurs. Such studies would be virtually impossible in soil. In Petri plates, expanding root buds or germinating thistle seeds have not been found to attract or influence the direction of germ tube growth from teliospores.

As shown in this research for the first time, germinating seeds and root cuttings produce volatiles which stimulate teliospore germination. This may be of great importance in initiating the systemic phase of the Canada thistle rust and may be useful in adapting this pathogen for use as a biocontrol agent. The chemical identities of the active C_{13} polyacetylene compounds from Canada thistle seeds and roots are described in detail in the following paper (Binder and French, 1994).

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