# Volatiles Emitted during the Sexual Stage of the Canada Thistle Rust Fungus and by Thistle Flowers

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Fragrance volatiles responsible for the sweet aroma produced when Canada thistle [*Cirsium arvense* (L.) Scop.] plants are infected with the systemic sexual stage of the Canada thistle rust [*Puccinia punctiformis* (Strauss) Roehl.], a prospective biological control agent for that weed, have been identified by capillary gas chromatography/mass spectrometry (GC/MS) after thermal desorption from Tenax. The four major peaks in the chromatogram were identified as benzaldehyde, phenylacetaldehyde, phenethyl alcohol, and indole, at average relative molar concentrations of 0.05, 0.85, 0.44, and 1.00, respectively. The known insect-attracting properties of these compounds may aid cross-fertilization of the fungus. Healthy Canada thistle flowers emitted these same compounds, except indole, plus methyl salicylate. Phenylacetaldehyde was the most concentrated fragrance volatile of the flowers.

## INTRODUCTION

The strong aroma produced during the sexual stage of the Canada thistle rust organism is one of the most striking characteristics of this pathogen, and in the past, reference to the odor was incorporated in its formal Latin name. Buller (1950), an authority on the life cycle of this fungus, preferred *Puccinia suaveolens* as the name of the Canada thistle rust organism because "suaveolens" means "sweet-smelling". He credited the name "suaveolens" to C. H. Persoon, 1799. Buller, when referring to the scented nectar, stated "...(The) very numerous pycnidia of the fungus give out so strong a scent that a group of infected thistle in a pasture may be detected by their odor before one has observed them with the eye". The name *Puccinia punctiformis* (Strauss) Roehl. was recently introduced by Cummins (1978) and is generally accepted at this time.

Volatile compounds related to flavors and aromas have been shown to have important biological effects, such as the stimulation of fungal spore and weed seed germination (French, 1985), allelochemical effects such as the inhibition of crop seed germination (Bradow and Connick, 1990; Connick et al., 1989), and activity as insect pheromones and attractants (Blum, 1969; Metcalf, 1987).

The yellow-orange pycnia of this obligate parasite may emit the flowerlike aroma to attract insects for cross-fertilization. During this stage of infection there is also an elongation of the infected thistle shoot. After fertilization, aroma production ceases, and the fungus produces large quantities of dark brown aeciospores that can be dispersed to new infection sites. The infected shoot is devastated and seldom flowers, suggesting that this fungus could be used as a biological control agent for Canada thistle [*Cirsium arvense* (L.) Scop.], a noxious, perennial weed that infests the northern United States and Canada.

The objectives of this study were to identify and compare the volatile chemical components from systemically infected shoots bearing pycnia and from healthy Canada thistle flowers. These compounds may have useful biological activity that could be exploited in the effort to control Canada thistle.

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## MATERIALS AND METHODS

**Plant Material.** Thistle plants were grown from root cuttings in soil in 10-cm clay pots in the greenhouse. To obtain infected plants, stimulated teliospores of *P. punctiformis* were spread on the surface of the buds of root cuttings. Teliospores were previously floated 6 days at 18 °C, in darkness, on a 15–25 μL/L aqueous suspension/solution of a hexane extract of steam-distilled thistle roots. This extract contained a volatile chemical that stimulated teliospore germination, starting at 7 days after exposure (French et al., 1988). Infected shoots bearing yellow-orange pycnia that emitted a strong, sweet odor appeared after 5 or more weeks (French et al., 1987).

**Trap Tubes.** Tenax trap tubes consisted of Pyrex glass tubes (84 mm length × 9 mm o.d. × 1 mm wall thickness) (Tek Lab, Baton Rouge, LA), each packed with 0.10 g of Tenax GC (60/80 mesh) porous polymer adsorbent held in place near the center with glass wool plugs. Tubes were preconditioned at 220 °C (24 h, 15 mL/min He). A mark was scribed near one end of each tube to designate the inlet for sample volatiles. The tubes were reversed for subsequent thermal desorption so that volatiles were backflushed from the tube into the GC.

**Trapping Volatiles from Infected Plants.** Pots, each containing a fragrant, systemically infected thistle plant (30–46 cm tall), were wrapped with aluminum foil so as to restrict diffusion of extraneous volatiles, if any, from the soil. Four pots were placed in a 61 × 30.5 cm diameter, 50-L glass jar covered by a plastic lid with a 2.5-cm center hole. A Tenax trap tube was attached to a vacuum line by using 6.4 mm i.d. gum rubber tubing and was suspended close to the plant foliage. Air was drawn through the tube at approximately 100 mL/min for 8 h; ambient temperature was 20–26 °C. Two replications were run by using separate sets of infected plants. Additionally, volatiles from a group of seven plants with infected shoots (uninfected portions were removed) were also trapped, as before. The control samples consisted of trapped volatiles from two separate groups of four uninfected plants each. Experiments were conducted in a laboratory that was relatively free from volatile contaminants.

**Trapping Volatiles from Thistle Flowers.** A group of six 76 cm tall and a group of six 122 cm tall potted thistle plants bearing 33 and 50 open, fragrant flowers, respectively, were each arranged with the shoots and flowers squeezed into the open end of an inverted glass jar (34.3 cm tall × 10.8 cm i.d.). Sexual differences in flower morphology were ignored in this study. A Tenax tube attached to a vacuum line was suspended through

a 2.5-cm hole in the inverted bottom of the jar, near the flowers. Approximately 100 mL/min of air was drawn through the tube by vacuum for 8 h. Another collection of volatiles was made from cut, 15–25-cm stems bearing a total of 213 open flowers. The cut stems were placed in a 31 cm tall × 31 cm diameter, 25-L glass jar containing 1 L of tap water plus 15 g of activated carbon (to trap extraneous volatiles in the water). The jar was covered with a plastic lid with a 2.5-cm hole in the center, and volatiles were trapped by using a Tenax tube, as previously described. A control sample consisted of volatiles trapped from six potted thistle plants with tightly closed flower buds.

All samples were analyzed 5–7 days after collection of volatiles. In the interim, the Tenax tubes were sealed with Teflon and stored in a freezer.

**GC/MS Analysis.** Volatiles collected from rust-infected Canada thistle and from healthy thistle flowers were thermally desorbed from Tenax trap tubes in an external, closed inlet system (Scientific Instrument Service, River Ridge, LA) (Legendre et al., 1979) interfaced with a Perkin-Elmer Sigma 300 GC/Finnigan MAT Model 705 B ion trap detector (ITD). The capillary GC column was 50 m × 0.31 mm (i.d.) coated with a 0.52-μm film of cross-linked 5% phenyl methyl silicone (Hewlett-Packard Ultra-2).

Volatiles were desorbed from a Tenax trap tube at 200 °C (3 min, 10 mL/min He) in the external inlet unit and collected on the head of the capillary column, which was held at −30 °C by dry ice in a wire basket placed in the column oven (Dupuy et al., 1985). The injector valve was switched to the vent position; the oven temperature was raised at 6 °C/min to 30 °C, held for 5 min, and raised at 3 °C/min to 250 °C. The GC injector was at 200 °C, GC/ITD helium pressure was 12 psi, the split ratio was 20:1, and the ITD (version 3.00 software with automatic gain control; autotuned) was set for full scan (45–300 amu), 1-s scan time, 80-min acquisition.

A tentative identification of the sample volatiles resulted from a computerized search of the NBS/EPA mass spectral library. Authentic compounds (Aldrich Chemical Co.) were then injected into the GC/ITD in the same manner as the thistle-related volatiles, and their mass spectra were entered into a user-generated library for rapid searching and comparison. Compounds were considered to be positively identified if their retention times and mass spectra closely matched those obtained for authentic compounds.

To determine the relative molar concentration of the trapped volatiles, methanol solutions containing a range of known concentrations of each of the identified compounds from the *P. punctiformis* infected thistle were injected (1 μL) and analyzed under the GC/ITD conditions already described. The average peak area per mole was determined for each component and, with peak area data from the Tenax-trapped samples, used to calculate relative molar concentrations.

## RESULTS AND DISCUSSION

Volatile aroma compounds emitted during the sexual stage of the Canada thistle rust fungus infection and by healthy thistle flowers were trapped and concentrated at ambient temperature on Tenax solid adsorbent. This trapping method excludes artifacts that usually accompany maceration or extraction techniques and increases the probability that detected compounds are emitted under natural conditions (Cole, 1980). Care was taken during the handling of plants to minimize cell damage, which can release volatile degradation products.

Chromatograms of volatiles from rust-infected thistle plants contained four major peaks (Figure 1A). These peaks were identified as benzaldehyde, phenylacetaldehyde, phenethyl alcohol (benzeneethanol; 2-phenylethanol), and indole (Table I). Volatiles trapped from uninfected thistles (controls) contained no detectable phenylacetaldehyde or phenethyl alcohol, and only trace amounts of benzaldehyde and indole. The relative molar concentrations of the trapped volatiles were determined (Table II). Indole was the major component, followed closely by phenylacetaldehyde; phenethyl alcohol concentration was about half those concentrations. Benzaldehyde was the least concentrated component identified.

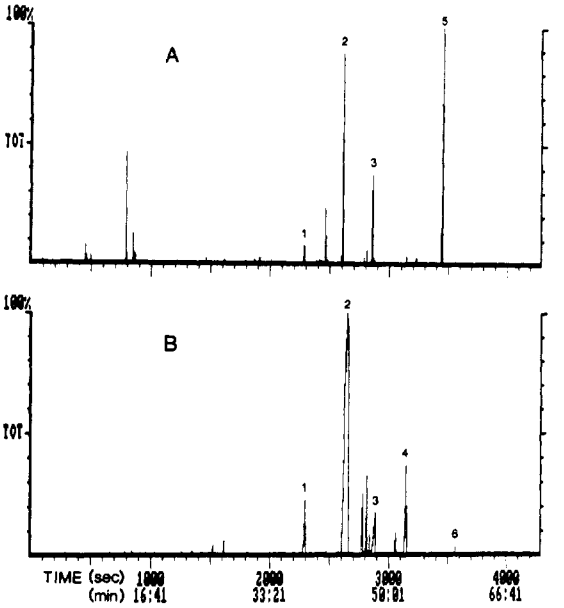


**Figure 1.** Total ion current capillary GC/ion trap chromatograms of Tenax-trapped, thermally desorbed volatiles collected from the air surrounding (A) infected Canada thistle, during the sexual stage of *P. punctiformis* [peak assignments: (1) benzaldehyde, (2) phenylacetaldehyde, (3) phenethyl alcohol, and (5) indole; unlabeled peaks were found also in the uninfected thistle control sample] and (B) Canada thistle flowers [peak assignments: (1) benzaldehyde, (2) phenylacetaldehyde, (3) phenethyl alcohol, (4) methyl salicylate, (6) methyl 2-methoxybenzoate; unlabeled peaks remained unidentified]. Analysis procedures and conditions are described in the text.

**Table I. Volatiles Emitted during the Sexual Stage of Canada Thistle Rust Fungus Infection**

| compd | MW | GC retention time, s | |
|---|---|---|---|
| | | sample | known |
| benzaldehyde | 106 | 2244 | 2261 |
| phenylacetaldehyde | 120 | 2561 | 2562 |
| phenethyl alcohol | 122 | 2808 | 2812 |
| indole | 177 | 3392 | 3386 |

**Table II. Relative Molar Concentrations of Volatiles**

| compd | relative molar concn | |
|---|---|---|
| | systemically rusted thistle | flowers |
| benzaldehyde | 0.05 | 0.10 |
| phenylacetaldehyde | 0.85 | 1.00 |
| phenethyl alcohol | 0.44 | 0.11 |
| indole | 1.00 | |

Phenylacetaldehyde was, surprisingly, detected only in trace amounts in the trapped volatiles when Carbotrap, a graphitized carbon black adsorbent, was used instead of Tenax under the trapping and thermal desorption conditions of this study. This observation was confirmed with authentic samples. The mildly basic nature of Carbotrap may have catalyzed an aldol condensation of phenylacetaldehyde during thermal desorption.

The vegetative stage of the Canada thistle rust fungus occurs primarily on the leaves and can be recognized by the formation of discrete dark brown pustules from which urediniospores are released to be carried by winds to new infection sites. Damage to the host at this stage is minimal. During the sexual phase of the life cycle, mycelial growth becomes somewhat coordinated with shoot growth. As the

Table III. Volatiles Emitted by Canada Thistle Flowers

| compd | MW | GC retention time, s | |
|---|---|---|---|
| | | sample | known |
| benzaldehyde | 106 | 2248 | 2261 |
| phenylacetaldehyde | 120 | 2579 | 2562 |
| phenethyl alcohol | 122 | 2822 | 2812 |
| methyl salicylate | 152 | 3079 | 3074 |
| methyl 2-methoxybenzoate | 166 | 3495 | 3503 |

shoot elongates, the fungus proliferates systemically, surrounding the stem and covering the petioles and abaxial surfaces of the leaves with thousands of fragrant, yellow-orange pycnia.

Bailiss and Wilson (1967) have shown that systemically infected thistle shoots have higher concentrations of gibberellins and auxins (indole-3-acetic acid) than noninfected plants. This increase in growth hormones may be responsible for the rapid, etiolated shoot growth. It is possible that the indole found in the volatiles from such infected shoots may originate from increased levels of auxin in the shoots, perhaps via the shikimate pathway.

There was a striking similarity in the components identified in thistle flowers compared with those of infected thistle shoots and leaves. The chromatogram contained only about six or seven peaks of significant size (Figure 1B). Five components were identified: benzaldehyde, phenylacetaldehyde, phenethyl alcohol, methyl salicylate (methyl 2-hydroxybenzoate), and methyl 2-methoxybenzoate (trace) (Table III).

Therefore, three of the four volatile compounds (indole being the exception) associated with the rust fungus were also prominent components of flower aroma. Phenylacetaldehyde was clearly the major component, present at 10 times the concentrations of benzaldehyde and phenethyl alcohol (Table II). The sample collected from open flowers on cut stems gave essentially the same chromatogram as those from flowers on intact plants. Trace quantities of benzaldehyde and methyl salicylate were detected in the control sample collected from plants with tightly closed buds.

All the identified volatiles are structurally similar mono- or disubstituted phenyl compounds. They have well-known flavor, fragrance, and insect attractant properties (Fenaroli, 1975; Metcalf, 1987). Benzaldehyde, phenylacetaldehyde, phenethyl alcohol, indole, and methyl salicylate were found in elder flowers and/or leaves (Velisek et al., 1981). Phenethyl alcohol, a major component of rose fragrance, is synthesized in rose petals from L-phenylalanine (Zaprometov, 1978). Benzaldehyde and phenylacetaldehyde are also produced by roses. Phenylacetaldehyde is a very effective attractant of moths (Cantelo and Jacobson, 1979). Phenethyl alcohol is an attractant of onion and seedcorn flies (Ishikawa et al., 1983) and Japanese beetles (Schwartz and Hamilton, 1969). Indole attracts flies (Mulla et al., 1977), the western corn rootworm, and the striped cucumber beetle (Andersen and Metcalf, 1986).

The volatiles associated with *P. punctiformis* have been produced by other fungi. Indole was isolated from a black mold (*Aspergillus niger*) (Bau, 1981), and phenethyl alcohol was found in cultures of *Candida albicans* (Lingappa et al., 1969). Benzaldehyde, phenylacetaldehyde, phenethyl alcohol, and indole were produced by some strains of *Aspergillus clavatus* (Seifert and King, 1982).

The flowerlike fragrance emitted during the sexual stage of the Canada thistle rust facilities cross-fertilization by attracting insects, much like the fragrance from thistle flowers attracts insects for cross-pollination. Canada thistle plants are dioecious (male or female); thus, cross-pollination is necessary for seed production. Systemic infection of thistle in the field (at least in central Maryland) usually occurs several weeks before flowering. The volatile chemical signals emitted by the systemically rusted thistle shoots or by the flowers undoubtedly indicate to insects, including thistle predators, the location of thistle plants. The beetle *Ceutorhynchus litura* (Fab.), for example, which feeds on Canada thistle and may aid in the dispersal of *P. punctiformis* (Maw, 1976; Peschken and Beecher, 1973; Peschken and Wilkinson, 1981), might be so attracted. The use of the individual chemical components or mixtures of these scents may be useful in luring increased numbers of predatory insects of thistle to patches of the weed earlier in the season, when the plants are younger and more tender and thus more susceptible to damage. As far as we could determine, *P. punctiformis* is the only rust noted for its fragrant aroma. The fungus is an obligate parasite, only infecting Canada thistle, and therefore would not damage other plants, including valuable crops, if developed as a biological control agent.

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**Registry No.** Benzaldehyde, 100-52-7; phenylacetaldehyde, 122-78-1; phenethyl alcohol, 60-12-8; indole, 120-72-9; methyl salicylate, 119-36-8.