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COMPONENTS OF OIL OF TANSY (*TANACETUM VULGARE*) THAT REPEL COLORADO POTATO BEETLES (*LEPTINOTARSA DECEMLINEATA*)

W.R. SCHEARER[1]

Department of Chemistry, Lehigh University, Bethlehem, PA 18015

ABSTRACT.—The steam distillate of fresh leaves and flowers of tansy, *Tanacetum vulgare*, was found to be strongly repellent to Colorado potato beetles, *Leptinotarsa decemlineata*. Liquid chromatography detected 56 compounds present. Identification of compounds was by gc/ms. The major components, camphor (30%) and umbellulone (25%), show the plant to be a previously unreported chemotype. A commercial oil of tansy was also found to be highly repellent to Colorado potato beetles; gc/ms analysis found bornyl acetate (74%) to be the major component. To determine the active compounds of the oils, bioassays were run using Colorado potato beetles as detectors for gc and tlc. To determine the relative strengths of repellency, an olfactometer was constructed using potato beetles as detectors. The strongest repellents found were: 1,8-cineole, bornyl acetate, *p*-cymene, γ-terpinene, and camphor.

In the past 20 years, the concept that some secondary plant products can act as repellents or feeding deterrents in herbivore-plant interactions has become widely accepted (1-6). Since early times, many plant materials have been used as insect repellents by mankind to protect himself and his clothing (7), and there have been claims that some plants contain repellents sufficiently potent to repel insects from neighboring plants (8). Recently, Matthews (9,10) reported the results of field trials showing that the aromatic perennial, tansy (*Tanacetum vulgare* L, Compositae), produces a significant decrease in the number of Colorado potato beetles (*Leptinotarsa decemlineata* Say) on nearby potato plants (*Solanum tuberosum* L) relative to potato plants at a distance. The volatile compounds in tansy apparently repel the beetles from the vicinity. Another recent report shows that an aqueous extract of tansy is a feeding deterrent for several kinds of beetles (11). Tansy has also been used medically as an anthelmintic and as a flavoring, but it appears to be relatively toxic (12, 13). We became interested in identifying those volatile substances present in tansy that might account for the repellent properties.

A survey of the chemical literature found more than 100 papers published since 1878 on tansy, with approximately 100 compounds reported to be present. A major finding is that the volatile oil of tansy is not consistent in composition from all plants, but that more than 30 chemotypes of tansy have been reported, each of which has a different compound as the major component of the volatile oil (14,15). As has been found for many other species, a single plant produces an oil with relatively constant composition, but plants from different geographic areas produce oils of different composition apparently because of genetic differences (16,17).

To be certain that we were working with a repellent chemotype, we obtained samples of the same plants studied by Matthews (9,10) and used them for the work reported in this paper.

In this paper, we show that compounds obtained from tansy are repellent to Colorado potato beetles, identify the major compounds in two chemotypes of tansy using gc/ms, and rank the compounds in order of strength of repellency toward Colorado potato beetles by means of an olfactometer. We also report a new chemotype of tansy.

RESULTS AND DISCUSSION

Steam distillation of fresh leaves and flowers of tansy produced a fragrant oil in a

[1]Present address: Department of Chemistry, Dickinson College, Carlisle PA 17013.

0.22% yield. Two simple bioassays confirmed that this oil was highly repellent to Colorado potato beetles. In the first test, an eyedropper containing a drop of the oil was brought near a beetle on a desk top. At a distance of about 3 cm, the beetle would suddenly stop walking flatten its antennae against its head, rear up on back legs, spit "tobacco," turn, and rapidly leave the area. This repellent behavior was characteristic and repeatable. An eyedropper of deionized H_2O provoked no response.

In a second bioassay, a sheet of glossy paper was coated lightly with soot. Then one drop of the oil of tansy was placed on the sheet. After allowing several beetles to roam freely on the sheet, one would observe beetle footprints fairly evenly distributed on the paper except in a circle approximately 6 cm in diameter around the drop of oil where few or no marks occurred.

Liquid chromatography of the oil detected 56 components. Identification of the compounds present was done by gc/ms, by comparison of gc retention times with authentic compounds, and by reference to earlier studies of tansy oil (14, 18, 19). The results are presented in Table 1 with the compounds listed in their order of elution from a Carbowax 20M column. Identification of additional components has proven difficult because of the small amounts present and extensive peak overlap.

TABLE 1. Composition of Fresh Oil of Tansy

Compound	% Present	Identification
tricyclene	0.1	ms
α-pinene	0.4	ms, rt
camphene	2.5	ms, rt
sabinene	6.0	ms
α-terpinene	0.1	ms, rt
1,8-cineole	5.1	ms, co
γ-terpinene	0.5	ms, co
p-cymene	1.0	ms, co
sabinol acetate	0.2	ms
unidentified	1.4	
camphor	29.6	ms, rt
unidentified	0.3	
unidentified	0.3	
terpinen-4-ol	1.5	ms
umbellulone	24.7	ms
borneol	0.8	ms, rt
sesquiterpene hydrocarbon	8.3	ms
carvone	0.7	ms, co
at least 12 unidentified components	8.3 (total)	
valeranone	0.4	ms
thymol	6.8	ms, rt

ms=mass spectroscopy
rt=comparison of retention time with authentic sample co=co-chromatography with authentic sample

This plant is a new chemotype because apparently no oil of tansy with both camphor and umbellulone as major components has been previously reported.

The Lapine Scientific Co. sells a product called "Oil of Tansy." This product was found to be strongly repellent to Colorado potato beetles by the eyedropper bioassay. Gc/ms showed it to have a different composition from that of the oil we had obtained from fresh plants. The composition is shown in Table 2 with the compounds listed in their order of elution from a Carbowax 20M column.

TABLE 2. Composition of a Commercial Oil of Tansy

Compound	% Present	Identification
tricyclene	0.2	ms
α-pinene	0.6	ms, rt
camphene	1.8	ms, rt
3-carene	0.2	ms, rt
myrcene	0.1	ms, rt
phellandrene	0.9	ms
α-terpinene	0.3	ms, rt
limonene	3.5	ms, rt
1,8-cineole	1.8	ms, rt
γ-terpinene	0.4	ms, rt
p-cymene	1.7	ms, rt
α-thujone	1.2	ms, rt
β-thujone	4.8	ms, rt
camphor	1.7	ms, rt
unidentified	1.3	
bornyl acetate	73.5	ms, rt
β-caryophyllene	4.5	ms, rt
borneol	0.2	ms, rt
sesquiterpene hydrocarbon	0.7	ms

ms=mass spectroscopy
rt=comparison of retention time with authentic sample

Several bioassays were set up to determine the active components. First, Colorado potato beetles were used as a detector for a gas chromatograph. The typical repellent behavior of the beetles indicated the repellent compounds. The test is difficult to run accurately because the beetles must be walking towards the gas stream to get an effect and their unpredictable walking pattern makes it difficult to get consistent results. On four runs using the fresh oil of tansy, camphor and thymol were found to give a repellent effect. On three of the four runs a repellent response was obtained from sabinene, terpinene-4-ol, umbellulone, and two unidentified peaks that eluted between carvone and thymol during gc on a Carbowax 20M column.

In a second bioassay, beetles were used as a "visualization agent" for tlc. The strongest repellent effect for the fresh oil of tansy was found at the highest Rf region. The strong repellent effect of this region was confirmed by cutting the tlc sheet and testing the pieces in an olfactometer. Extraction and analysis by gc/ms found these compounds present: camphene, sabinene, 1,8-cineole, camphor, and an unidentified sesquiterpene hydrocarbon that eluted between borneol and carvone during gc on a Carbowax 20M column.

The above two bioassays suggest which compounds are most active at the natural concentration. The results are based on a combination of the inherent repellency, volatility, and the amount of each compound present. To assess more accurately the repellent strength of the components of the oil by eliminating the effects of differing amounts and to determine a ranking by strength of repellency of the various materials in this work, an olfactometer study was undertaken. An olfactometer (20) was constructed which allows beetles a choice of two air streams, both of which have the same temperature, humidity, and velocity, but only one contains the vapor of the compound being tested. This bioassay was chosen to make comparisons under more standardized conditions that would suggest materials likely to be successful in field testing as repellents. The concentration of the compounds in the air stream vary according to the volatility of the compounds similar to the usual situation in field testing. The oils, plant materials, and the compounds identified in the oil that were readily available from chemical sup-

ply houses were tested in the olfactometer. The results are expressed by an index used by Willis and Roth (21) and modified by Chamberlain (22). This index is positive if the substance is repellent and negative if the substance is an attractant.

Index = (Number of beetles at control air stream minus number at sample stream) × 100/ (total number of beetles multiplied by number of observations)

Table 3 lists the results of the olfactometer study. These compounds appear to be the strongest repellents: 1,8-cineole, bornyl acetate, *p*-cymene, γ-terpinene, and camphor. Further work may show that some of the smaller unidentified components also contribute significantly to the repellent effect of the oil. Whether or not any of these compounds is effective as a repellent for protecting potato plants from potato beetles when used under agricultural conditions must be determined, of course, under actual field conditions.

TABLE 3. Ranking Substances by Decreasing Level of Repellency towards Potato Beetles as Determined by Olfactometer

Substance Tested	Index
1,8-cineole	63
Lapine oil of tansy	63
bornyl acetate	56
p-cymene	54
fresh tansy oil	51
γ-terpinene	51
camphor	51
Rf 0.86-1.0 region of tlc of fresh oil	47
camphene	46
carvone	45
α-pinene	44
fresh tansy leaf	44
3-carene	16
thymol	12
freeze dried tansy leaf	11
Rf 0.43-0.86 region of tlc of fresh oil	10
Rf 0-0.43 region of tlc of fresh oil	7
β-caryophyllene	2
uncrushed potato leaf	−25

EXPERIMENTAL

MATERIALS.—Plants of *T. vulgare* were harvested in early September 1981, from plantings by Diane Matthews at the Organic Gardening and Farming Research Center, R 1, Box 323, Kutztown, Pennsylvania 19530. The original seed was obtained from Cyrus Hyde of the Wellsweep Herb Farm, 317 Mt. Bethel Rd., Port Murray, New Jersey 07865.

A sample specimen of the plant has been deposited at the United States National Herbarium, Smithsonian Institution, Washington, DC 20560. The sheet number is 2975791. Dr. Harold Robinson of the Institution has verified the identification as *T. vulgare*. A sample is also on deposit at the Dickinson College Herbarium, Carlisle, Pennsylvania.

L. decemlineata were raised on potato plants by Diane Matthews of the Organic Gardening and Farming Research Center. The original beetles were obtained from J.H. Lashomb of Rutgers University who collected them in New Jersey.

The commercial oil of tansy was obtained from LaPine Scientific Co., Norwood, New Jersey.

ISOLATION OF VOLATILES FROM *T. VULGARE*.—Freshly harvested leaves, flowers, and small stems

were steam distilled and the distillate extracted with 35°-60° petroleum ether. Evaporation under vacuum at room temperature yielded 2.1 g of oil from the original 940 g of plant material (0.22%).

INSTRUMENTATION.—A liquid chromatogram was obtained on a Varian 5000 hplc starting with 50% MeOH-H_2O and increasing to 100% MeOH at 1%/min. A Partisil pxs 10/25 C-8 column, 20 cm×4.6 mm, was used with the uv detector at 212 nm and AUFS 2. Flow rate was 1 ml/min and the sample size was 10 ul.

A Finnigan 4000 gc/ms/ds system was used with a 10% Carbowax 20M on Chromosorb W column, 4.5 m×3.2 mm. The carrier gas was helium at 30 ml/min, and the sample size was 1 ul. Temperature ramp was 3°/min from 65° to 200°. The percent composition was recorded directly from the electronic integrator without correction. Retention times were obtained on a Perkin-Elmer Sigma 3B gc with flame ionization detector using the same gc conditions.

EYEDROPPER BIOASSAY.—The procedure was similar to that of Eisner (5). The potato beetles responded at about 3 cm.

SOOTY PAPER BIOASSAY.—A sheet of glossy paper (22×28 cm) was coated lightly with soot by holding it in an air-deficient Bunsen burner flame. A drop of fresh oil of tansy and a drop of H_2O as control were placed about 10 cm apart on the sheet. After placing an 8-cm high glass restraining wall around the sheet, three adult, unstarved potato beetles were allowed to roam freely for 30 min. Footprints were found evenly distributed over the sheet except clearly in a circle about 6 cm in diameter around the drop of oil, where almost no footprints occurred. Two replications gave essentially the same results.

GC BIOASSAY.—The flame ionization detector on a Perkin-Elmer Sigma 3B gc were turned off and the cover removed for easy access to the outlet port. After injecting fresh oil of tansy, an open Petri dish containing three adult potato beetles was continually moved so that the outlet gas stream was directed head-on to at least one beetle at all times. The beetle must be walking for any effect to be seen. When the typical repellent behavior was observed, a mark was made immediately on the recorder paper. After each run, the marked paper was superimposed on a chromatogram obtained with the flame ionization detector on. The marks located the active peaks.

TLC BIOASSAY.—Fresh oil of tansy was spotted on silica gel tlc sheets (Eastman Kodak Chromagram™ with fluorescent indicator) and developed with 10% EtOAc in hexane. The components of the oil were found to be distributed along the length of the sheet after visualizing with iodine vapor or observing the quenching of the fluorescence under uv light.

After drying briefly in air to remove solvents, the tlc sheet was placed so that two potato beetles had access to walking on the sheet. Observation of the repellent behavior and of the location of walking indicated the location of repellent compounds was mostly at the highest Rf region. The bioassay was repeated three times, allowing the beetles to roam approximately 5 min on each.

A fresh chromatogram was prepared and cut into three pieces: Rf 0-0.43, 0.43-0.86, and 0.86-1.0. Each piece was extracted with petroleum ether (35°-60°). Each extract was then concentrated and injected into the gc under the usual conditions so that the peaks could be identified by comparison with gc/ms experiment above.

Another chromatogram was prepared and cut as above. The pieces were evaluated in the olfactometer described below to obtain a quantitative comparison of repellent properties.

OLFACTOMETER STUDY.—The olfactometer was a modification of the device described by Bongers (20). Compressed air was filtered, then warmed and humidified to near 100% humidity by bubbling through water at 33°-36°. The air stream was split into two streams with one stream passing through the sample container. The sample was the pure compound or the material as listed in Table 3. The control stream passed through an empty container. All tubing was glass or Teflon. The beetle test arena was constructed of a 1000-ml beaker, well lighted with an incandescent bulb. The sample and control air streams rose vertically in laminar flow via clusters of parallel tubing. The arena was covered with opaque paper except for the top, which was open for viewing and exhaust of air streams. Air temperature in the arena was 28°-30°. Air flow through each half was maintained at 2.1 liters/min with a flowmeter. The walls of the arena were lightly coated with silicone grease to discourage climbing. The sample was switched to the other air stream half-way through each run to eliminate any bias from instrument orientation. Test insects were mature Colorado potato beetles raised on potato plants and were not starved prior to testing. Two beetles were used in each run. Every 15 sec the number of beetles on the sample half of the arena was recorded. A minimum of 70 readings was taken for each sample.

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