JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE
VOLUME 48, NUMBER 3, JULY, 1973

FRAGRANCE ANALYSES OF *TRILLIUM LUTEUM AND TRILLIUM CUNEATUM* (Liliaceae)

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ABSTRACT

The flower fragrances of *Trillium cuneatum* and *T. luteum* were analyzed by means of gas chromatography. A total of 12 different compounds were detected, only two of which occurred in both taxa. Four compounds were specific to *T. luteum*, six to *T. cuneatum*. The identity of only one compound, the terpene alcohol linalool, was determined. This is the major fragrance component of *T. luteum* and is believed to be the compound responsible for the lemony odor so characteristic of the flowers of the plant. Taxonomic relationships of *T. luteum* and *T. cuneatum* are discussed.

INTRODUCTION

Despite the abundance, widespread distribution and relatively large, often showy flowers of species of *Trillium*, little is known concerning the nature of pollination and the species identity of pollen vectors. While the flowers of some of the species have been long known to emit a disagreeable odor and to attract flies, others produce an agreeable fragrance not obviously attractive to specific insects. The flowers of still others are apparently odorless. The odors reported include spicy, sweet, musky, rose, lemon, aminoid and foetid, but nowhere is information available on the chemistry of any of the compounds.

The present report is a chromatographic comparison of the fragrance compounds of the flowers of two closely related kinds of *Trillium* of eastern United States (subgenus Phyllantherum Raf.), the taxonomic treatment of which has been the subject of disagreement. The chemical data constitute information believed to be useful in investigations, not only taxonomic but biological, in the sense of providing a basis for and an impetus to future works along the lines of pollination ecology and hybridization.

MATERIALS AND METHODS

Plants of *Trillium luteum* and *T. cuneatum* were collected near Ozone, Cumberland County, and Nashville, Davidson County, Tennessee, respectively, in the early spring of 1969. The plants were transplanted to a greenhouse at the University of Miami where they soon flowered and flower fragrance analyses were performed by the first author. Following anthesis flower fragrances were obtained by one of the following two methods. By the first method intact scapes were individually enclosed within airtight plexiglass chambers (box-like in design) the outlets of which were equipped with swage lock fittings and self-sealing septa. This procedure minimized damage to the plants and permitted sampling to be performed throughout the flowering period. By the second method whole flowers were removed from the plants and several of these placed within airtight glass bottles, the tops of which were also equipped with swage lock fittings and self-sealing septa. Although the latter procedure did not afford the advantages of repeated sampling over a period of several days, it did allow sampling over a period of several hours and permitted a comparatively more concentrated sample to be obtained.

The chambers were equilibrated for 30 minutes to allow the internal atmosphere to become as fully saturated with fragrance compounds as possible. Samples of the fragrance-containing atmosphere were extracted in 10 cc volumes with the use of a gas-tight syringe.

Analyses were performed isothermally at oven temperatures of 70°, 100°, 130° and 160°C with an F & M model 810 dual flame gas chromatograph equipped with a 1:1 effluent splitter. At each temperature a 10cc test sample was injected into each of two different 6 ft. x ¼ in. stainless steel columns. One of the columns was packed with 3% carbowax 20M on 80-100 mesh Diatoport S, the other with 10% Lac 446 (diethylene glycol adipate) on 80-100 mesh Chromosorb W. The effluent splitter permitted the compounds to be examined olfactorally as they eluted from the column. However, the extremely small quantity of each compound in the injected air sample precluded its collection for use in other analytical procedures. Quantitative measurements were obtained for each sample from an integrator trace and expressed as a percentage of the injected sample. Fragrance components were identified by calculation of relative retention times of the peaks using the standards: β-pinene at 70°, 2-phenylethylacetate at 100°, ethyl benzoate at 130°, and 2-phenylethanol at 160°C.

RESULTS

Peaks obtained on the tracings were assigned arbitrary numbers on the basis of increasing relative retention times and increasing temperatures. Certain peaks with high relative retention times at 70° as well as those with low values at 130°C could also be detected at 100°C. Such peaks were assumed to be identical on the basis of similar size, similar elution odor, and projected decrease or increase in relative retention time accompaning the specific temperature increase or decrease, respectively. Equivalence of peaks on the two types of columns was judged on the basis of the same criteria. Repeated analyses using the techniques described indicated that the floral fragrances of *Trillium luteum* and *T. cuneatum* consist of a total of 12 different compounds. The relative retention time and percentage composition of each compound are given in Table 1.

The floral fragrance of *Trillium luteum* was found to consist of six different chemical compounds. Of the six compounds detected, peak 11 was the major component, accounting for slightly over 70 percent of the total fragrance. This was also the only compound that had a detectable odor as it eluted from the column. This odor was distinctly lemon-like. Comparison of the relative retention times of the fragrance compounds of *Trillium luteum* against retention times of the standards used suggested that peak 11 was probably the terpene

Table 1: Relative retention time and percentage composition of chemical compounds in injected samples of the floral fragrances of Trillium luteum and Trillium cuneatum.

Temp.	Peak No.	Relative Retention Time Carbowax	Lac 466	% Composition in T. luteum	T. cuneatum
70°	1	0.42	0.50	3.2	
	2	0.48	0.57		11.6
	3	0.65	0.63	9.4	36.3
	4	0.85	--		3.7
	5	1.01	1.00		10.6
	6	1.33	1.31	1.9	4.6
	7	1.61	1.82		6.7
	8	2.23	--	2.4	
100°	6	0.35	--		
	8	0.51	--		
	9	1.36	1.14		
	11	2.08	1.79		
130°	9	0.40	0.38	12.2	
	10	0.51	--		12.4
	11	0.55	0.52	70.9	
	12	0.75	--		14.1

Note: Peaks 6 and 8 were detected at both 70° and 100°; peaks 9 and 11 at 100° and 130°C.

alcohol linalool. This possibility was checked by augmentation. For this purpose head space samples of standard linalool were run on both columns at 130°C. The relative retention times and odor of linalool as it eluted from the column were found to be identical to that of peak 11. By augmentation a sample of the flower fragrance was enriched with a small amount of standard linalool (head space sample) and this mixture analyzed. An increase in the size of the peak was obtained. This result, without appearance of a shoulder, was taken as a good indication that linalool was the major fragrance component. As a further check, the temperature was dropped to the next lowest standard level (100°C) and the augmentation procedures repeated. (cf., Table 1). The fragrance compound and the standard linalool were found to have identical relative retention times on both columns, and enrichment again produced a single enlarged peak without a shoulder.

It was concluded from these data that linalool is the major fragrance compound produced by the flowers of *Trillium luteum*, and is the compound responsible for the lemony fragrance which the flowers produce. The other compounds produced by the flowers probably alter slightly the odor of linalool inasmuch as the fragrance produced by the flowers smells slightly sweeter than the odor of pure linalool.

The floral fragrance of *Trillium cuneatum* was found to consist of eight compounds. The major compound, peak 3, accounted for approximately 36 percent of the total fragrance. This peak did not, however, have a detectable odor as it eluted from the column, nor did any of the other compounds that were present. Of the eight compounds detected, only one possessed a relative retention time similar to that of any available standard compound. Peak 5 had a relative retention time very similar to that of β-pinene. It was not possible to equate peak 5 with β-pinene because the standard β-pinene had a very distinct and easily detected odor as it eluted from the chromatographic column even when used in quantities comparable to those of peak 5.

The fragrance profiles of the two species were quite different. Only two compounds, peaks 3 and 6, were found to occur in both, but the quantities of each of the two compounds were noticeably different. Both peaks 3 and 6 occurred in *Trillium cuneatum* in quantities about three times greater than those quantities found to occur in *T. luteum*.

Discussion

Trillium luteum (Huhl.) Harb. is distributed in the Ridge and Valley and southern Appalachian provinces of eastern Tennessee, Kentucky, western North Carolina and northwestern Georgia. The flowers are yellowish green to bright yellow, relatively uniform in color and emit a pleasant lemon-like fragrance. The geographical range of *T. luteum* (Fig. 1) is essentially surrounded, except to the northeast, by that of the comparatively widespread and closely related *T. cuneatum* Raf.

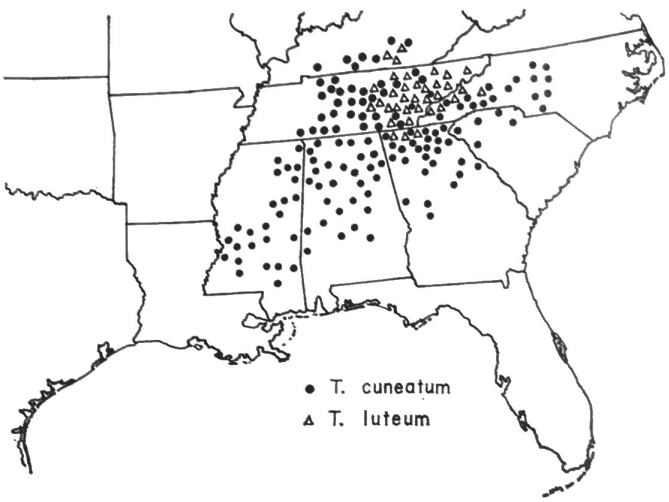

Fig. 1: Distribution of *Trillium luteum* and *T. cuneatum*.

Trillium cuneatum (excluding *T. luteum* consists of highly variable populations of individuals distributed geographically (Fig. 1) over the Blue Ridge and Piedmont provinces, ranging from the Carolinas southwestward across Georgia, Alabama and Mississippi and northward through Tennessee into the Highland Rim of Kentucky. The flowers are brown-purple or maroon, sometimes greenish, greenish yellow, rarely yellow, highly variable in color, and emit a sweet spicy fragrance. Plants with greenish yellow flowers are in the minority in this species, and individuals with yellow flowers are rare indeed. There is some evidence that flowers maroon in early anthesis often become greenish in age.

The close morphological similarities of these two plants have naturally led to disagreement not only as to identification, but to taxonomic treatment as well. *Trillium luteum* has been variously treated as a species and as a geographical variety of *T. cuneatum*.

While differences in flower fragrances are useful in distinguishing *Trillium luteum* and *T. cuneatum*, they may also provide clues to possible hybridization between these taxa. Freeman (1969), in his taxonomic treatment of sessile-flowered species of *Trillium*, has examined populations including putative hybrids of these taxa