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FATTY ACIDS OF SEEDS OF NORTH AMERICAN PEDICILLATE *TRILLIUM* SPECIES

VICTOR G. SOUKUP* and RALPH T. HOLMAN

Herbarium, University of Cincinnati, Cincinnati, OH 45221, U.S.A.; Hormel Institute, University of Minnesota, Austin, MN 55912, U.S.A.

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Key Word Index—*Trillium*; *Paris*; Trilliaceae; seed; elaiosome; fatty acids; myrmecochory.

Abstract—The fatty acid components of the lipids of the seed elaiosomes of a group of pedicillate *Trillium* species differed only slightly from those of the seeds proper. The fatty acids seem to offer no clue to the range of ant activities associated with myrmecochory in this genus.

INTRODUCTION

Approximately 55 species of *Trillium* and *Paris* constitute the Trilliaceae†. *Trillium* occurs both in temperate North America and temperate East Asia while *Paris* is restricted to East Asia with the exception of one species which ranges widely across northern Europe. *Trillium* and *Paris* are vernal scapose herbs which produce one flower per stem. In *Paris*, the flower is pedicillate, but in *Trillium* it may be either pedicillate or sessile, with the latter habit being considered more advanced [1]. Sessile-flowered *Trillium* are restricted to North America. After pollination, the ovary swells greatly to produce a large, usually somewhat angled and/or ridged fruit, which matures in *ca* 3 months, and has few to many seeds.

Most species of *Trillium* and *Paris* are myrmecochorus. Ants carry away diaspores from dehisced fruits [2, 3] and one of us (V.G.S.) has photographed ants in the process of removing seeds from dropped fruits to their nests and at work within dehisced fruits. The reasons for this activity are the large, somewhat gelatinous, fatty-appearing arils, termed elaiosomes, attached to the seeds. Species of *Paris* in Li's Section 7 (*Paris*) [4] produce non-arillate seeds and *T. undulatum* produces seeds with very small elaiosomes. Myrmecochorus behaviour is associated only with those species which produce elaiosomes. The elaiosomes, in some cases larger than the seed proper, are variously coloured, white or pale yellow in *Trillium*, and white, pale greenish white, yellow, or scarlet in *Paris*. The absence of an elaiosome is considered to be the derived condition [4].

Since myrmecochory undoubtedly developed conjointly with ants, it seems quite possible that some unique chemical compound(s) attractive to ants may be involved. It is also possible that since different ants occur in various parts of the wide range covered by *Trillium* and *Paris* that the original chemically attractive compound(s) may have undergone evolutionary changes and that perhaps more than one attracting composition is now involved. The elaiosomes are avidly consumed but the seeds themselves are discarded, often being buried (planted) at some distance from the nest. These observations suggested that a study be undertaken of the seeds of Trilliaceae. Here, we report the fatty acid composition of the total lipids of the elaiosomes and of the seeds proper of a group of pedicillate-flowered *Trillium*.

RESULTS AND DISCUSSION

Nine species of North American pedicillate *Trillium* comprising a representative sampling of this group were examined. Members of the wide-ranging 'erectum complex' [5], *erectum*, *simile*, *vaseyi*, *cernuum*, *rugelli*, *flexipes* and *sulcatum*, which together constitute half of the pedicillate species of eastern North America, dominate the list. The specimens analysed and the sites of the original collections, which cover a large portion of the eastern half of the United States, are shown in Table 1.

Tables 2 and 3 give the fatty acid compositions of the elaiosomes and of the seeds proper. All percentages over 0.1% of acids of known structure are shown.

The fatty acid compositions of the seeds proper and of the elaiosomes are not vastly different from each other, except that the composition of the elaiosomes' fatty acids is much more variable. The virtual absence of $20:1\omega 9$ in the seeds and of 20:0 in the elaiosomes is the biggest difference. The replacement of a portion of the 16:0 with 20:0 and, on the average, lower $18:2\omega 6$ in the seeds as compared to the elaiosomes are also major differences.

The fatty acids tabulated generally account for a high percentage (over 97%) of the total fatty acids. In a few specimens (E3, F1, and F3 elaiosomes, and F4 seed) other fatty acids are present in concentrations > 0.1% but their structures are uncertain. For example, E3 and F1 contain 6.5 and 3.9% of 18:1 acids of unknown double bond position. In F3 elaiosome, there is 4.9% of unidentified unsaturated acids, the major constituent being 2.4% of (probably) a 14:2 acid. Finally in F4 seed, there is 2.1% of a highly unsaturated C_{20} acid. A number of other acids are present in very small quantities, less than 0.1% each. Most

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*To whom correspondence should be addressed.

†Two other genera of North America, *Medeola*, monotypic of eastern NA, and *Scoliopus*, two species of western NA, are usually also placed within Trilliaceae but most evidence suggests they do not belong there.

Table 1. Collection locations of *Trillium* species examined

Species		*Location*
G *T. grandiflorum*	Salisb.	Hocking Co., OH
N *T. nivale*	Riddell	Madison Co., OH
CA *T: catesbaei*	Ell.	Surry Co., NC
U *T. undulatum*	Willd.	Clarion Co., PA
E1 *T. erectum*	L.	Tuscola Co., MI
E2 *T. erectum f. albiflorum*	R. Hoffm.	Sevier Co., TN*
E3 *T. erectum f. albiflorum*	R. Hoffm.	Seiver Co., TN*
F1 *T. flexipes*	Raf.	Jefferson Co., KY
F2 *T. flexipes*	Raf.	Mower Co., MN
F3 *T. flexipes*	Raf.	Winnebago Co., IA
F4 *T. flexipes f. Walpolei*	Farwell	Green Co., OH
V *T. vaseyi*	Harb.	Pickens Co., SC
R1 *T. rugelii*	Rend.	Swain Co., NC
R2 *T. rugelii*	Rend.	Transylvania Co., NC
R3 *T. rugelii*	Rend.	Henderson Co., NC
CE *T. cernuum*	L.	Grand Forks Co., ND

*Two populations *ca* 26 km apart.
Voucher specimens deposited at CINC.

of these are unidentified but lie in the range from C_{14} to C_{22}. Hydrogenation of the mixture of methyl esters of the elaiosome fatty acids shows most of these other acids to be straight chain unsaturated as they are converted to 14:0, 16:0, 18:0 and 20:0. However, the GC retention times of a few peaks remain unchanged and suggest the presence of small quantities of saturated branched chain acids in the mixture.

The most striking difference between the fatty acid compositions of the seeds and of the elaiosomes is the much higher degree of uniformity in the percentages of the individual fatty acids of the seeds. This difference would tend to suggest that the fatty acid composition of the seed has remained fixed during evolution of the genus, while that of the elaiosomes has undergone, and probably still is undergoing, adaptive changes in response to reproductive pressures. It is perhaps noteworthy that in *T. undulatum* with its endozoochorus adapted fruit and small elaiosomes, the fatty acid composition of the elaiosomes is not significantly different from the other species and that the species of the 'erectum complex' show as much dissimilarity from each other as does the entire group of species analysed.

The acyl glycerols of seeds are the depot for storage of energy rich fatty acids which provide carbon atoms for biosynthesis and energy for initial growth during germination. The polyunsaturated acids, 18:2ω6 and 18:3ω3, are commonly found in seed oils, and these may play a role in the initial oxidative processes whereby fatty acids are converted to carbohydrates, because of their ease of peroxidation [6]. In the 16 samples of seed oils of *Trillium* species, the pattern of fatty acids was remarkably constant indicating that the nine species had not evolved divergently with respect to the kinds and proportions of fatty acids stored for germination. Linoleic acid, 18:2ω6, the principal polyunsaturated fatty acid of the seed oil, was 18.1 ± 2.5% of the total fatty acids for the 16 samples.

The fatty acid composition of the elaiosome lipids, in contrast, was much more variable. The 18:2ω6 content of the 16 samples was 26.4 ± 13.1%, ranging from 3.9 to 42.1%, a factor of more than 10-fold. In compensation for the very low linoleic acid in three specimens, increases in oleic acid (CA), palmitic acid (F1) or palmitoleic acid (R1) were found, indicating three different mechanisms of compensation. The function of the elaiosomes is to provide 'bait' for the insect or animal vector in an easily accessible food and energy source, and this can be provided by a broad range of fatty acid patterns. With three exceptions, all specimens were relatively rich in the essential fatty acid, 18:2ω6, which may be a required nutrient for ants. The content of linolenic acid, known to be required by some insects, in elaiosome lipids (1.7 ± 1.3%) is much greater than in the seed lipids (0.26 ± 0.18%), indicating some segregation of this acid into the elaiosomes.

None of the differences between the fatty acids of the elaiosomes and of the seeds would be expected to be responsible for selectively attracting ants to the elaiosomes or for causing them to reject the seeds after the elaiosomes had been consumed. This might suggest that the orginal attraction of ants to the dehisced fruits is via an olfactory mechanism involving some volatile compound secreted by the ripening fruit tissues or even the elaiosomes themselves. In Berg's experiments [2], seeds with attached elaiosomes placed within range of ant nests did not appear to attract ants foraging for food. However, as soon as a foraging ant chanced upon a group of seeds, that ant plus others to whom the location was communicated quickly removed those seeds to the nest. In a few instances, some elaiosomes were consumed at the discovery site. Marshall *et al.* [7] reported the results of experiments in which the seeds of *Viola odorata* or artificial seeds coated with lipid fractions of the elaiosomes of *V. odorata* seeds were exposed to ants. While there seemed to be no volatile attractant present, a diacylglycerol fraction, specifically 1,2-diolein, appeared to be the compound attractive to ants. Of course *Trillium* and *Viola* have different methods of primary dispersal of diaspores; slow dehiscence followed by dropping to the ground versus rapid dehiscence accompanied by explosive ejection, respectively, that the two cases might be expected to not have much in common. Our observations confirm this type behaviour as an ant

Table 2. Fatty acid composition of elaiosomes of eastern North American pedicillate *Trillium* seeds

Species		14:0	14:1	16:0	$16{:}1\omega7$	18:0	$18{:}1\omega9$	$18{:}2\omega6$	$18{:}3\omega3$	20:0	$20{:}1\omega9$	$20{:}2\omega6$	Total %
T. grandiflorum	G	—	—	10.5	2.1	5.8	45.2	22.9	1.9	—	4.6	—	93.0
T. nivale	N	0.5	—	15.1	0.7	3.0	25.6	35.4	5.4	—	9.5	0.5	95.7
T. catesbaei	CA	0.4	0.3	18.4	10.0	0.3	62.2	4.8	0.8	—	1.2	—	98.4
T. undulatum	U	0.4	—	15.2	7.8	3.0	59.6	10.5	1.8	—	0.6	—	98.9
T. erectum	E1	0.3	—	21.6	2.2	0.7	27.3	42.1	2.0	—	1.5	—	97.7
T. erectum	E2	0.2	—	18.3	1.6	0.1	38.6	37.6	0.5	—	1.0	—	97.9
T. erectum	E3	—	—	15.5	8.5	4.2	26.5	32.1	3.5	—	—	—	91.1
T. flexipes	F1	—	—	21.0	20.8	1.2	19.7	30.3	1.2	—	0.6	—	94.8
T. flexipes	F2	0.3	—	17.4	5.4	2.0	34.3	35.9	1.5	0.3	1.7	—	98.8
T. flexipes	F3	0.5	3.2	30.9	2.9	1.7	39.3	7.0	0.5	3.3	0.7	0.1	90.1
T. flexipes	F4	0.1	—	18.7	0.9	1.2	32.7	43.4	0.7	—	1.2	—	98.9
T. vaseyi	V	—	—	20.7	5.3	1.2	36.7	32.4	1.0	—	2.2	—	99.5
T. rugelii	R1	—	—	20.7	42.4	0.4	22.4	3.9	2.0	—	0.3	—	94.7
T. rugelii	R2	—	—	11.9	30.4	0.6	25.6	27.4	1.3	—	1.2	—	98.4
T. rugelii	R3	—	—	11.2	17.8	1.2	42.2	25.0	0.3	—	1.2	—	98.9
T. cernuum	CE	0.5	—	17.3	4.1	1.3	37.9	31.5	1.5	—	2.3	—	96.4

Table 3. Fatty acid composition of eastern North American pedicillate *Trillium* seeds

Species		14:0	16:0	$16{:}1\omega7$	18:0	$18{:}1\omega9$	$18{:}2\omega6$	$18{:}3\omega3$	20:0	$20{:}1\omega9$	Total %
T. nivale	N	0.3	6.0	0.2	1.6	37.9	22.2	0.6	29.0	—	97.8
T. undulatum	U	0.1	7.4	0.2	1.9	37.0	19.2	0.3	31.8	—	97.8
T. erectum	E1	0.1	6.2	0.5	0.7	46.4	18.4	0.4	25.2	—	97.9
T. erectum	E2	—	5.3	0.4	0.7	49.6	17.7	0.3	24.4	—	98.4
T. flexipes	F1	—	5.4	0.6	0.6	44.6	20.0	—	26.9	—	98.1
T. flexipes	F4	0.1	5.5	0.4	0.9	42.1	16.1	0.3	31.6	0.3	97.0
T. vaseyi	V	—	5.7	0.5	0.7	48.1	18.4	—	24.2	—	97.6
T. rugelii	R2	—	5.6	1.6	0.2	51.6	15.2	0.3	23.5	—	98.0
T. rugelii	R3	—	5.5	0.9	0.9	48.7	20.0	0.2	22.2	—	98.4
T. cernuum	CE	0.1	4.9	0.6	0.8	52.7	13.7	0.2	26.7	—	99.7

was observed by V.G.S. to pass a group of seeds five times at distances of less than 5 cm in the course of 10 min of random foraging to distances as great as 110 cm from the seed until on the sixth approach the path crossed the seed location. At this point, the behaviour became orderly and resulted in seed removal. These experiments and observations would suggest that if a volatile attractant is involved, then it is only present in the fruit tissues and serves to bring the ants to the ripe, dehisced or about to dehisce fruits.

These observations also do not explain the rejection of the seed after consumption of the elaiosomes. The answer may lie (partially) in the hardness of the seed as contrasted to the almost gelatinous elaiosome. If the ants cannot effectively attack a smooth hard seed, it may be treated as a pebble and removed from the nest. However, this will, once again, not explain the removal to a distance, often followed by burial of the seed, a manoeuvre not practiced with pebbles. It would appear that the elaiosomes provide an attractive and easily accessible source of energy to the ant and that the seeds containing the embryos are discarded because of their impenetrability, resulting in dispersion.

EXPERIMENTAL

Material. Mature fruits of *Trillium* species were collected in the field from stands of pure species previously collected in flower and sampled for other studies, or were collected from plants in the experimental garden of one author (V.G.S.) in Cincinnati, OH or of Fred Case in Saginaw, MI. Fruits were preserved by being wetted with and stored in a small amount of MeOH in tightly stoppered bottles until analyses could be carried out.

Methods. Seeds with attached elaiosomes were removed from fruits and all adhering portions of the outer and inner tissues of the fruit including the funiculi were carefully cut away. The elaiosomes were then quickly dissected as completely as possible from the seed bodies and placed in small capped test tubes with a small amount of MeOH under dry N_2. The seeds proper were processed by removing the outer skin with minute traces of adhering elaiosome and slicing the pale cream to tan seeds into very small pieces. They also were placed in small capped test tubes with a small amount of MeOH under N_2. 20–30 elaiosomes or 6–10 seeds constituted a specimen (sample).

The fatty acids in the comminuted whole tissues were transesterified (BF_3/MeOH) at room temp. for 2 hr and then at 78° for 3 hr at which point no traces of the original tissues remained. The resulting Me esters were extracted with petrol and after removal of solvents in a stream of N_2, stored under N_2.

The esters were analysed by GC on a WCOT FFAP 50 m × 0.22 mm column (180–220° at 2°/min followed by a final hold at 220°) using He 0.63 ml/min, split ratio 117:1, FID, with injection and detector temps maintained at 270°. Identifications of the Me esters were made by comparison of R_ts and by reduction (Adams catalyst) and subsequent GC.

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