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FATTY ACIDS AND DIACYLGLYCEROLS FROM ELAIOSOMES OF SOME ANT-DISPERSED SEEDS

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Key Word Index—*Jeffersonia diphylla*; Berberidaceae; *Sanguinaria canadensis*; Papaveraceae; *Trillium sessile*, Trilliaceae; *Dicentra cucullaria*; Furnariaceae; elaiosomes; fatty acids; diacylglycerols.

Abstract—Lipids were isolated from the elaiosomes of four ant-dispersed herbaceous plants (*Jeffersonia diphylla*, *Sanguinaria canadensis*, *Trillium sessile*, *Dicentra cucullaria*) found in Northern Virginia. Fatty acid methyl esters and trimethysilyl derivatives of diacylglycerols were separated and identified by capillary GC/MS. Oleic acid and palmitic acid are significant components of the lipid fractions from all four species, and 1,2-diolein is the major diacylglycerol of *Jeffersonia diphylla*. These results support previous studies which had found oleic acid in elaiosomes and suggested 1,2-diolein to be an ant attractant.

INTRODUCTION

Seed and fruit dispersal by ants is a familiar phenomenon in certain shrublands in South Africa [1] and Australia [2, 3], and in the herbaceous understory of temperate forests in Europe and North America [4, 5]. Ants are attracted to seeds bearing a structure, known as an elaiosome, usually attached to the surface of the seed coat. Plant species from over 60 families have been recorded as being ant-dispersed, and local herbaceous floras in North America are often dominated by ant-dispersed plants [5]. Elaiosomes attract workers from a large number of ant species, and the ants appear to distinguish among elaiosome-bearing seeds of *Asarum canadense*, *Jeffersonia diphylla*, *Sanguinaria canadensis* and *Viola papilionacea*, when presented with choices, mainly by seed size relative to the worker's ability to carry the seed [6]. Rockwood (unpublished results) found 15 species of ants attracted to seeds of *S. canadensis*, *V. papilionacea* and *Trillium sessile*, in Loudoun County, Virginia, while Beattie and Culver [4] found nine species of ants carried seeds when they tested 10 elaiosome-bearing seed species in West Virginia. Hanzawa *et al.* [7] used nine ant species in their study of directed dispersal of *Corydalis aurea* seeds in Crested Butte, Colorado.

Many studies have shown that the ants carry the diaspores to their nests where the elaiosome is cut off and eaten or fed to larvae. The seed itself is not consumed, but is carried to another location in or near the nest. The potential benefits of ant dispersal for the plants have been reviewed by Beattie [8]. Recent support for the idea of 'directed dispersal' is described by Hanzawa *et al.* [7].

Elaiosomes are generally thought to contain several classes of lipids. Bresinsky [9] found that only the lipid component of elaiosomes elicited a positive response from ants. Marshall *et al.* [10] investigated the chemical basis for ant dispersal using *V. odorata* seeds and the ant *Aphaenogaster rudis*. Bioassays of the elaiosome revealed that the principal attractant appeared to be a diacylglycerol. The major fatty acid present was oleic acid and the authors suggested that 1,2-diolein was the attractant. As part of our ongoing investigation of the biochemistry of ant–plant interactions, we report here on chemical analyses of the elaiosomes from four ant dispersed North American species.

RESULTS AND DISCUSSION

The fatty acid composition of the elaiosomes from *J. diphylla*, *S. canadensis*, *T. sessile* and *D. cucullaria* is summarized in Table 1. Oleic acid was abundant in all four species, as was palmitic acid. Linoleic acid was present in all species but in lesser amounts. Palmitoleic acid and stearic acid were not present in *D. cucullaria*, and linolenic acid was found only in *J. diphylla* and *T. sessile*.

Triacylglycerols were the major component of the elaiosomes in all four species. Elaiosomes of *S. canadensis*, *T. sessile*, and *D. cucullaria* contained small amounts of diacylglycerols, insufficient to perform further analyses. Only *J. diphylla* elaiosomes contained significant quantities of diacylglycerols, the major one being 1,2-diolein.

Marshall *et al.* [10] identified oleic acid as the major fatty acid present in the elaiosome of *V. odorata*. They isolated diacylglycerols by TLC but did not determine which compounds were present, but performed bioassays with several standard diglycerides. Of those tested, 1,2-diolein was the most strongly attractive to ants. Our finding that 1,2-diolein is the major diglyceride in elaiosomes of *J. diphylla* lends support to the hypothesis that this diglyceride is an ant attractant.

Bresinsky [9] had suggested that ricinoleic acid was the ant attractant in Old World ant-dispersed plants. Marshall *et al.* [10], however, found that bioassays for ricinoleic acid were negative. We found no evidence for the presence of ricinoleic acid in elaiosomes of any of the four plant species we analysed

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Table 1 Fatty acid composition (%) of di- and triacylglycerol fractions isolated from elaisomes of few plant species

	Diacylglycerols	Triacylglycerols			
Fatty acids	*J diphylla*	*J diphylla*	*S canadensis*	*T sessile*	*D cucullaria*
16 0	23 8	17 3	14 0	23 0	31 9
16 1	7 1	7 5	7 4	6 5	—
18 0	3 6	8 4	2 6	3 3	2 7
18 1	47 1	55 1	73 2	45 0	52 5
18 2	17 2	1.3	1 7	16 1	6 1
20 0	- -	1 7	—	-	3 6
18 3	0 8	5 5	—	5 2	3 2
Unidentified compounds	0 5	3 1	1 2	1 0	—

Our results show that oleic acid and palmitic acid are significant components of the lipid fractions of elaiosomes from four Virginia species, and that 1,2-diolein is the major diacylglycerol (50 1%) of *J diphylla* with smaller quantities (16 3%) of the 1-palmitoyl, 2-oleyl compound. Future studies may reveal whether there is a specific compound in the elaiosomes of all ant-dispersed species which serves as the ant-attractant

EXPERIMENTAL

Seeds of *J diphylla* (L.) Persoon *S canadensis* L., and *T sessile* L were collected from a roadside forest along route 15 near the Point of Rocks bridge over the Potomac River in Loudoun County, Virginia during June and early July, 1987 Seeds of *D cucullaria* (L.) Bermh were collected on May 7, 1987 near Stone Bridge of the Manassas Battlefield Park, Prince William County, Virginia Seeds were removed from their pods and stored at −80° until ready for processing Elaiosomes were sepd from seeds and extd in $CHCl_3$–MeOH (2 1) at 20° for 48 hr [10, 11]. The $CHCl_3$ phase was washed ×3 with H_2O (1 4) and the $CHCl_3$ phases combined and evapd to dryness under N_2

Polar and nonpolar fractions were resolved on Sep-Pak C_{18} columns with $CHCl_3$, $CHCl_3$–MeOH (1 1) and MeOH as eluents Lipid frs were sepd by TLC (silica gel H) with petrol –Et_2O–HOAc (90.10 1) and hexane–Et_2O–HOAc (40 10.1) Spots were visualized with UV after spraying with 2',7'-dichlorofluorescein Di- and triacylglycerols were scraped from the plates, extracted with $CHCl_3$ and Me esters of fatty acids prepd using BF_3–MeOH as follows [12–14] 2 ml of BF_3–MeOH was added to lipids in 2 ml petrol and heated at 100° for 10 min The reaction was stopped by adding 1 ml of H_2O The upper layer was removed and the petrol evapd The residue was dissolved in CH_2Cl_2 for GC-MS analysis. Sepn of 1 μl injections was achieved on a 15 m × 0 25 mm i d WCOT SP-2330 fused silica capillary column, 1 100 split ratio, 190° with He linear velocity 20 cm/sec and inj 220°, detector 250° Peaks were identified by MS and relative amounts of fatty acid Me esters were calculated using response factors derived from repeated injections of standard mixts

TMSi derivatives of diacylglycerols isolated by TLC were prepd as follows [15, 16] diacylglycerols were mixed with Sylon HTP (Supelco-3038) under N_2 for 30 min at 21° [17] The solvent was evapd with N_2 and the residue dissolved in *ca* 2 ml of petrol After centrifugation, the solid residue was removed by decantation, and the solvent evpd TMSi derivatives of diacylglycerols in CH_2Cl_2 were detected by FID as described above at 250° with inj and detector at 270 and 300°, respectively, He linear velocity 30 cm/sec Peaks were identified by comparison with ref standards and GC/MS analysis of similar mixts.

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