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DISTRIBUTION OF CYCLOPROPENOID FATTY ACIDS IN MALVACEOUS PLANT PARTS

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Key Word Index—Malvaceae; roots; fatty acids; cyclopropenoid; sterculate; malvalate; fungi.

Abstract—Fatty acid compositions were determined for vegetative plant parts of seven species in seven genera of Malvaceae. In all cases, sterculate and malvalate were most concentrated in the roots. These cyclopropenoid fatty acids made up larger proportions of neutral lipid fractions than of phospholipids. Concentrations of the two compounds were within the range known to inhibit growth of some fungi.

INTRODUCTION

Sterculate and malvalate, cyclopropenoid fatty acids (CPE) found in seed oils of plants allied with the order Malvales [1–5] and of some gymnosperms [6, 7], are occasionally reported as constituents of vegetative plant parts. Within the Malvaceae, CPE have been identified in leaves of *Jasminum auriculatum* [8], in leaves, stems and roots of several *Malva* species [9, 10], and in roots of flowering or fruiting *Hibiscus esculentus* [11]. They are also known to occur in roots of *Sterculia foetida* (Sterculiaceae) [12, 13] and *Ceiba pentandra* (Bombacaceae) [13], while high levels have been observed in leaves of one gymnosperm, *Gnetum gnemon* (Gnetaceae) [6]. Some negative reports are also available [10–13].

We have hypothesized that, in addition to their role as storage products, CPE may serve as antifungal agents [14]. Dry weight accumulation by one test organism, *Ustilago maydis*, was inhibited by *ca* 50% by 30 μM methyl CPE, a concentration at which extreme stearate accumulation was also apparent in both membrane and storage lipids. Oleate, the product of the desaturation presumably blocked by CPE, could overcome the effects of CPE on dry weight [14]. These observations prompted questions concerning both the amounts of CPE available in vegetative plant parts and the degree to which endogenous oleate might negate their effects on potential pathogens. In this paper, we report the fatty acid compositions of neutral and phospholipid fractions from species representing seven Malvaceous genera.

RESULTS AND DISCUSSION

Fatty acid compositions of neutral and phospholipids from the Malvaceous species examined are presented in Table 1. In all species analysed, CPE were most concentrated in the neutral lipids of roots. CPE in this fraction ranged from 17 to 63%, or 0.2 to 11.5 mg per g dry weight. Leaf CPE, on the other hand, failed to exceed 2.6% of neutral lipid fatty acids, while proportions of CPE in petiole and stem neutral lipids fell between those in leaf blades and roots. In seed oils, CPE are concentrated in triacylglycerols [1]. If this is the case in vegetative tissues, these percentage data may reflect a high glycolipid to triacylglycerol ratio in neutral lipids from photosynthetic tissues. However, it should be noted that digalactosyl diacylglycerols from immature *Lavatera olbia rosea* seeds are reported to contain 3.7% CPE [9]. The CPE gradients shown in Table 1 were also evident on a dry weight basis.

Phospholipids invariably contained lower proportions of CPE than did the corresponding neutral lipid fractions. Root tissues again ranked as the best CPE sources, with phospholipid fatty acids containing 2 to 17% CPE. This was paralleled within cotton seeds, in which the radicle contained the highest concentrations of both neutral and phospholipid CPE. Fisher and Cherry [15] have likewise reported CPE gradients in cotton seeds, with maximum CPE at the radicle tip.

Malvalate was the dominant CPE in stem and root neutral lipids, but was often supplanted in this role by sterculate in phospholipids and in leaf neutral lipids. Dihydrosterculate occurred in almost all fractions, including phospholipid fractions with comparatively little CPE, whereas the peak comigrating with dihydromalvalate was detected only at low levels and in fractions concentrated in CPE. The proportions of dihydromalvalate observed were similar to the 0.1–0.5% reported for seed oils [2].

The Malvaceae investigated in this study resembled most other species for which data are available. The presence of CPE in roots but not in leaves and stems is characteristic of both *Sterculia foetida* and *Ceiba pentandra* [12, 13]. CPE are absent from leaves of the Bombacaceous *Brachychiton acerifolius* and *B. populneus* [10], and they are relatively low in amount in leaves of *Jasminum auriculatum* [8] and some *Malva* species [9, 10]. *Hibiscus esculentus* lacks CPE in all vegetative parts apart from the roots, in which they appear only after the formation of flower buds [11]. The production of CPE by roots of the *Hibiscus* species used in this study was not

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dependent on flowering; however, it may be significant that *H. esculentus* is placed in genus *Abelmoschus* by some authors [16]. The only species within the order Malvales for which available data are inconsistent with the pattern described here are certain *Malva* species. Figures for leaf CPE in *M. parviflora* range from 5 [9] to 20% [10]; roots of this species contain *ca* 8% CPE [9]. Some variation may be due to environmental effects, since winter leaves of both *M. alcea* var. *fastigiata* and *M. sylvestris* contain higher proportions of CPE than do summer leaves [9]. Winter contents of CPE in these plants also exceed those found in leaves during this study. The *M. neglecta* plants for which analyses are reported here were collected in May while in bloom.

The root, the major site of CPE deposition in the Malvaceous species analysed, is exposed to a wide range of soil fungi. In this study, total root CPE ranged from 0.4 to 11.6 mg per g dry weight, or 0.13 to 14 μmol per g fresh weight. Higher amounts would be available in radicles from germinating cotton seeds. Within healthy cotton radicles [17] and perhaps in roots as well, much of this CPE is sequestered in lipid bodies; phospholipid CPE would presumably be associated with membranes. These intracellular locations, the hydrophobic nature of CPE and the possibility that CPE are not evenly distributed among root tissues make it difficult to assess the actual concentrations of sterculate and malvalate available during cell penetration or death due to fungal invasion. If it is assumed that CPE are evenly distributed in the plant cell following exposure to fungal or plant lipases, and that one g fresh weight is roughly equivalent to one ml of water, CPE concentrations could range from 130 μM to 14 mM, concentrations well above the 30 μM treatments affecting growth of *Ustilago maydis* sporidia and *Rhizoctonia solani* hyphae [14].

The above figures suggest that efficient uptake of Malvaceous root cell lipids by a fungal hyphal tip could result in accumulations of CPE in the fungus sufficient to

Table 1. Fatty acid composition (wt %) of Malvaceous plant parts

Plant part		Fatty acid*										(mg/g dry wt)
		14:0	16:0	18:0	18:1	18:2	18:3	MLV	STC	DHS	DHM	
Abutilon pictum												
Leaf blades	P	0.4	36.5	1.6	6.2	29.6	25.6	—	—	—	—	4.7
	N	0.8	11.7	1.0	2.0	17.5	67.0	—	—	—	—	14.4
Petioles	P	2.0	32.4	2.3	9.6	36.0	17.6	—	—	—	—	1.5
	N	2.2	18.2	2.0	6.6	27.0	44.0	—	—	—	—	2.6
Stems	P	2.2	31.0	1.4	10.9	40.2	14.2	—	—	—	—	1.4
	N	1.5	17.9	1.2	9.4	40.8	29.2	—	—	—	—	2.8
Roots	P	1.4	28.8	1.8	8.8	47.0	9.9	0.9	1.0	0.4	—	1.9
	N	1.2	18.5	1.4	8.6	36.5	15.2	10.6	6.7	1.0	—	2.4
Alcea rosea												
Leaf blades	P	0.4	35.6	3.4	5.1	16.5	37.4	—	0.8	0.8	—	3.5
	N	1.0	12.4	2.6	1.6	6.4	73.8	0.5	0.9	0.8	—	14.5
Petioles	P	0.6	30.8	5.0	8.5	24.6	29.8	0.2	0.2	0.4	—	2.3
	N	1.2	15.6	3.5	5.8	16.4	47.0	7.6	2.2	0.8	—	2.5
Roots	P	1.0	31.2	4.6	8.6	29.0	14.2	2.6	4.2	4.6	—	1.7
	N	0.1	9.9	3.4	3.0	9.6	5.6	49.6	13.5	4.7	0.8	18.3
Anisodontea x *hypomandalum*												
Leaves	P	0.5	37.3	1.9	8.7	17.4	33.9	—	0.3	—	—	4.5
	N	0.4	9.4	1.0	3.4	6.0	79.7	—	0.1	—	—	18.4
Stems	P	1.6	27.3	6.7	21.6	23.8	19.2	—	—	—	—	1.0
	N	4.2	15.6	5.0	13.0	15.0	46.6	—	—	0.6	—	1.0
Roots	P	1.1	31.6	6.6	15.6	11.9	13.3	7.0	10.3	2.6	—	1.2
	N	2.5	24.0	6.3	10.6	9.1	15.1	20.8	7.6	3.5	0.4	0.8
Gossypium hirsutum												
O hr cotyledons	P	0.6	22.0	3.8	19.4	54.4	—	—	—	—	—	11.3
	N	1.1	26.6	3.0	17.6	51.6	tr	tr	—	—	—	277.2
O hr hypocotyls	P	0.4	25.7	4.0	12.4	51.9	1.6	0.1	1.0	2.8	—	13.2
	N	0.9	28.7	3.8	14.7	36.8	0.5	6.3	4.4	3.8	—	204.4
O hr radicles	P	1.0	27.1	2.3	7.0	50.0	5.0	0.6	1.5	5.4	—	21.2
	N	1.0	27.1	3.3	9.2	27.0	1.3	20.2	5.5	5.2	0.3	184.9
Leaf blades	P	0.6	47.1	2.5	7.6	21.0	21.2	—	—	—	—	4.1
	N	0.8	14.0	1.4	3.3	8.1	71.4	0.5	0.6	0.1	—	12.0
Petioles	P	2.3	37.0	4.4	8.4	30.4	17.4	—	—	—	—	0.5
	N	1.5	16.6	2.4	5.2	18.5	46.0	6.6	2.6	0.6	—	2.5
Stems	P	0.7	39.0	2.4	7.6	34.9	15.2	—	—	—	—	1.6
	N	0.8	16.0	2.2	6.4	24.6	33.4	12.2	4.0	0.4	—	3.0
Roots	P	0.8	35.2	2.2	12.4	28.3	15.2	2.2	2.8	0.7	—	1.3
	N	0.9	14.4	1.2	13.0	15.0	11.2	32.6	11.0	0.6	0.1	4.5

Table 1. *Continued*

Plant part		Fatty acid*										(mg/g dry wt)
		14:0	16:0	18:0	18:1	18:2	18:3	MLV	STC	DHS	DHM	
Hibiscus rosa-sinensis												
Leaves	P	0.7	37.8	5.9	10.0	28.0	16.1	0.4	0.4	0.6	—	2.1
	N	0.4	7.6	2.4	3.4	33.8	49.5	1.8	0.8	0.4	—	11.8
Stems	P	1.4	34.6	6.6	14.5	35.6	7.3	—	—	—	—	0.9
	N	1.0	7.8	1.8	9.2	45.4	24.2	6.8	3.1	0.6	—	2.6
Roots	P	0.7	32.7	4.5	9.7	36.4	5.0	4.2	5.4	1.3	—	1.4
	N	0.8	8.0	2.3	7.2	25.3	6.2	23.2	25.1	2.0	—	2.9
Malacothamnus hallii												
Leaves	P	0.6	31.7	4.0	6.2	19.6	37.5	—	—	0.5	—	1.7
	N	0.6	10.6	6.2	2.4	8.4	62.2	0.2	2.4	7.0	—	10.7
Stems	P	1.7	28.2	2.9	9.6	35.8	20.4	0.4	0.6	0.5	—	1.1
	N	2.6	16.5	9.0	6.4	21.8	29.7	5.7	1.7	6.4	—	1.7
Roots	P	1.5	29.0	3.0	7.5	32.0	17.8	3.8	3.8	1.8	—	1.6
	N	3.2	22.7	2.8	5.6	22.2	17.8	17.8	4.9	2.6	0.4	1.0
Malva neglecta												
Leaf blades	P	0.4	33.8	2.9	3.1	20.5	39.1	0.1	—	—	—	6.0
	N	0.6	9.8	1.3	1.4	5.8	79.6	1.4	0.1			34.5
Petioles	P	1.1	29.4	2.9	7.2	29.0	29.9	0.2	0.2	0.2	—	6.2
	N	0.9	8.8	1.5	2.4	10.6	72.2	3.2	0.5	tr	—	6.0
Stems	P	1.0	28.4	2.7	8.4	28.8	28.4	0.8	0.8	0.8	—	5.7
	N	1.2	13.6	2.1	3.4	17.6	47.6	11.4	2.4	0.8	—	3.8
Roots	P	0.7	30.7	2.8	7.0	19.0	30.8	2.3	5.2	1.5	—	2.8
	N	0.7	10.7	1.4	3.2	8.8	20.2	42.9	10.6	1.4	—	4.3

*MLV = malvalate, STC = sterculate, DHS = dihydrosterculate, DHM = dihydromalvalate; figures presented here are averages of two or more analyses.

P = phospholipids, N = neutral lipids.

inhibit stearate desaturation and, in some cases, fungal growth. However, the possibility also exists that the plant fatty acid pool will meet the structural needs of the fungus, rendering the fungal desaturase superfluous. Of particular concern is oleate, which ameliorates the effects of 30 μM CPE on *U. maydis* when given at 30 μm [14]. Ratios of CPE to 18:1 in roots of the seven species examined ranged from 1.2 to 17. Further studies are needed to determine the responses of soil fungi to natural CPE- containing mixtures.

EXPERIMENTAL

Plant material. *Abutilon pictum* (Gillies ex Hook. & Arn.) Walp., *Anisodontea* x *hypomandarum* (Sprague) Bates, *Gossypium hirsutum* L., *Hibiscus rosa-sinensis* Linn. f. *variegatus* and *Malacothamnus hallii* (Eastw.) Kearney were greenhouse grown. *Alcea rosea* (L.) Cav. and *Malva neglecta* Wallr. were collected locally. *A. pictum*, *G. hirsutum* and *M. neglecta* were harvested at the flowering stage, *A. rosea* in the rosette stage.

Leaves, stems and washed roots were frozen, lyophilized and ground to pass a 20 mesh screen. If petiole length equalled or exceeded leaf blade length, pètioles and blades were analysed separately. For seed part studies, unimbibed, delinted *G. hirsutum* seeds were dehulled and dissected into radicle, hypocotyl and cotyledons. Seed parts were then flash frozen and lyophilized.

Lipid analysis. Samples of 100–200 mg dry weight were homogenized in $CHCl_3$ and extracted in 100 ml $CHCl_3$–MeOH (2:1) for one hr at room temp. Extn mixts supplemented with an int. std containing tripentadecanoin (1.05 mg/ml) and diheptadecanoyl phosphatidylcholine (1.33 mg/ml) were filtered, dried and transferred to 1.2 g BioSil A columns. A fraction containing neutral lipids and any sulpholipid present was eluted with 12 ml $CHCl_3$ followed by 36 ml Me_2CO [18]. Phospholipids were then eluted from the columns with MeOH. Each fraction was dried and transesterified with 0.5 M NaOMe [19].

Fatty acid Me esters were identified by their GC R_t relative to those of authentic standards. *Sterculia foetida* and *Hibiscus syriacus* oils served as sources of sterculate and malvalate, respectively. Hydrogenation of these seed oil Me esters [20] provided dihydrosterculate and dihydromalvalate qualitative standards.

Fatty acid Me esters were quantitated by GC on a 28.2 cm × 0.16 cm glass column packed with 3% SP-2100 [21]. The injection temp was 220°, the FID temp 240°, the column temp 180° and the He carrier 29 ml/min. The ratio of oleate, linoleate, linolenate and dihydromalvalate, compounds which co-migrated on the packed column, was determined on a 60m × 0.25 mm i.d. fused silica capillary column with a 1 μM film of SPB-1. This column also permitted resolution of stearate from an unidentified contaminant present in some samples and migrating below the Me ester band on silica gel 60F-254 plates developed with petrol–Et_2O (17:3). The column temp was 210°, inj temp. 220°, FID temp 240°, He carrier flow 0.63 ml/min and split ratio 40:1. Make-up gas was supplied at 30 ml/min. In samples containing high dihydromalvalate to 18:1 ratios, the identity of dihydromalvalate could be verified by its R_t at 160° on a 30 m × 0.25 mm fused silica capillary column with a 0.25 μM coating of SP-2330.

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