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LEAF HYDROCARBONS OF *PHACELIA* SPECIES (HYDROPHYLLACEAE)

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Key Word Index—*Phacelia*; Hydrophyllaceae; leaf alkanes.

Abstract—Whole leaf hydrocarbons of six species of *Phacelia* were investigated for their variability among natural Californian populations, and for adaptive significance of individual hydrocarbon constituents in dry habitats. The hydrocarbon composition within a single taxon varied so much from one natural habitat to another as to render alkane composition impractical for taxonomic determinations between species of this genus. Large differences also occurred between wild populations and their greenhouse-grown counterparts. Nonacosane accumulated in high amounts in the driest habitats of *P. distans*.

INTRODUCTION

In the genus *Phacelia*, which has been known to cause dermatitis in humans [1], it has been different to separate some species from each other (e.g. *P. tanacetifolia* Benth. from *P. distans* Benth.) because of parallelisms existing in their morphology [2, 3]. In our quest for chemical markers which could delineate such species, alkanes were found in abundance in *Phacelia* taxa growing in diverse habitats in California. Alkanes have been used previously for taxonomic determination [4–6]. In this study, they seemed to offer an additional chance to examine possible environmental effects upon their deposition.

The role of these wax constituents is not truly known. Since they appear mainly on the exterior surfaces of higher plants, they do not seem to be utilized as an energy source even under adverse conditions. However, they increase resistance to abrasion which facilitates the penetration of pathogens and toxic materials. Due to their non-wetting nature, they also provide a defense against pathogenic microorganisms. Primarily, however, it has been assumed that they play an important role in plant water economy by reducing cuticular transpiration to a minimum. There is no clear correlation, however, between surface waxes and xeromorphic adaptation, a fact which has been interpreted to mean that wax has little survival value [7].

Investigation in both the composition of soluble cuticular lipids and the water permeability of cuticular membranes showed large variability in the permeability between and within four years of testing of a grown *Citrus aurantium* L. tree. Thus, no relationship between composition of soluble cuticular lipids and water permeability could be formulated [8]. However, an adaptive significance of whole-leaf *n*-nonacosene to dry habitats in *Clarkia tembloriensis* Vasek was suggested [9]. The xerophytic habitats of various locations in our study offered an opportunity to investigate the hydrocarbon composition versus habitat (Table 1).

Table 1. Harvest sites of *Phacelia* species

Locality	Elevation (m)	Average precipitation (cm/yr)	Site
Bonsal	130	40	Lush coastal sage brush
Death Valley	1700	2.5–5	Johnson Canyon Desert
Deep Canyon	320	2.5–7.6	Desert floor
Fallbrook Creek	400	40	Mesic area near creek
Grand Terrace	550	20.5–35.5	Sage scrub
Joshua Tree N.M.	1370	5–6	Dry upper desert
Mitchells Cavern	1677	20	*Pinus monophylla* community
Morongo Valley	610	25	Creosote community
North San Bernardino	430	25	Mesic, near creek
Reche Canyon	630	35	Dry chaparral
Red Mountains	1390	10	Upper high desert, creosote community
Phacelia Sanctuary	854	10	High desert, sandy area in creosote community
Torrey Pines	76	30–40	Coastal chaparral
Willow Glen	1500	40	Mesic area near stream

RESULTS AND DISCUSSION

In total leaf wax composition of various species of *Phacelia*, the *n*-paraffins from C_{23} to C_{34} were found with the odd carbon-numbered members usually predominating (Table 2). Quantitative differences in paraffins were present between species as well as among the various populations within species, harvested in different ecological habitats. The largest divergences, however, were observed between wild populations and their greenhouse-grown counterparts.

The paraffins varying most widely were C_{27}, C_{29}, C_{31} and C_{33}. Heptacosane and nonacosane were present in higher amounts in all plants from wild habitats of *Phacelia distans* Benth., *P. tanacetifolia* Benth., *P. cicutaria* Greene., *P. vallis-mortae* Voss., and *P. ramosissima* var. *australitorales* Munz as compared to their greenhouse-grown counterparts. The exceptions were for the City Creek (foothills north of San Bernardino) and Fallbrook collections of *P. ramosissima* Dougl. ex Lehm. var. *suffrutescens* Perry., which had lesser amounts of heptacosane. Both collection sites, however, had a far milder and more humid microclimate than the other collection sites. This is in agreement with an alkane study on *Pedilanthus macrocarpus* harvested from geographically separated regions, where the significant quantitative variations were thought to reflect variation in climatic influence [10].

A similar decrease in heptacosane, nonacosane, hentriacontane and tritriacontane in greenhouse-grown plants was reported in *Antirrhinum majus* [11]. Our data on *Phacelia* show, however, an increase for hentriacontane in all greenhouse-grown taxa as compared to all the habitats of a given species, and an increase for tritriacontane for all greenhouse-grown taxa as compared to their wild collection sites except the Fallbrook collection site of *P. ramosissima* var. *suffrutescens* (Table 2). For *P. cryptantha* Greene. only seeds were procured at Mitchell's

Table 2. *n*-alkanes of *Phacelia* leaves

	Chain length												
	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	I_{33}	C_{33}	C_{34}
P. distans													
Bonsal	0.79	0.45	2.14	1.01	9.94	2.55	34.99	5.79	30.83	4.44	2.84	2.84	1.30
Deep Canyon	1.02	0.27	4.97	1.52	16.11	2.88	36.26	5.11	24.20	2.94	2.25	2.25	0.16
Sunnymead	0.77	0.43	3.76	1.40	16.00	3.10	34.18	6.02	25.94	4.14	2.34	1.78	0.07
Morongo Valley	0.93	0.40	2.56	1.10	14.76	2.94	44.39	4.49	22.03	2.51	1.75	2.04	0.02
Joshua Tree	0.85	0.45	2.55	1.06	15.21	3.02	41.58	5.09	23.22	2.81	1.74	1.74	0.59
Reche Canyon	0.84	0.46	5.68	1.60	23.42	3.20	33.14	7.30	18.16	3.68	1.83	0.52	0.11
Reche C. Greenh.	1.00	0.74	2.10	1.02	5.99	2.18	26.00	5.73	36.63	5.24	6.28	6.56	0.51
P. tanacetifolia													
Red Mountain	1.06	1.18	3.07	1.30	24.12	2.60	35.89	3.19	19.33	3.78	2.06	2.30	0.05
Sanctuary	0.90	0.35	5.23	1.31	35.83	2.20	36.00	2.05	12.69	0.99	1.17	1.06	0.15
Sanct. Greenh.	0.51	0.41	1.98	0.98	13.03	2.93	34.91	5.32	27.61	4.17	3.06	3.34	1.67
P. cicutaria													
Fallbrook	1.85	0.92	3.08	1.78	9.50	3.53	19.44	7.65	30.05	10.64	5.24	5.21	2.38
Willow Glen	1.62	0.93	3.22	1.59	7.75	3.01	18.99	6.87	32.47	10.58	6.02	3.71	1.83
Willow Greenh.	1.43	0.87	3.13	2.79	6.11	3.47	14.43	6.53	33.04	12.39	5.67	7.10	2.97
P. vallis-mortae													
Death Valley	2.47	0.81	4.76	2.45	13.26	4.05	28.54	7.21	22.32	5.63	1.17	5.93	1.32
Mitchells Cavern	1.31	0.77	2.97	1.08	7.77	2.97	27.58	6.49	33.15	6.69	2.70	5.76	0.69
Mitchells Greenh.	0.81	0.46	1.40	0.77	5.40	2.21	22.36	5.98	38.00	4.70	2.68	14.78	0.38
P. cryptantha													
Mitchells Greenh.	1.26	0.11	2.06	0.76	4.51	1.03	8.21	3.70	38.45	11.42	9.44	18.88	0.11
P. ramosissima (var. *suffrutescens*) Parry													
Bonsal	1.44	1.00	3.69	1.69	14.20	3.09	26.31	6.38	35.23	1.10	2.29	2.28	1.30
Sunnymead	1.41	0.63	4.75	1.52	12.49	2.59	22.22	8.73	31.27	8.09	4.54	1.49	0.21
North San Bernardino	1.05	0.33	3.88	1.62	8.67	2.33	19.86	7.04	38.30	8.21	4.67	2.31	1.60
Fallbrook	1.58	0.53	2.27	1.01	8.04	2.52	24.04	6.40	37.18	6.78	4.55	4.87	0.16
Grand Terrace	0.55	0.28	3.03	0.74	11.00	2.63	24.00	10.89	31.58	8.38	3.97	1.97	0.93
Grand Ter. Greenh.	0.48	0.49	2.61	2.60	9.34	2.87	15.74	6.23	40.38	9.56	4.72	4.70	0.19
P. ramosissima (var. *australitoralis*)													
Torrey Pines	1.80	0.72	3.94	1.76	8.59	3.42	24.90	8.23	33.11	6.51	3.40	3.40	0.16
Torrey Pines Greenh.	1.45	0.60	2.41	1.75	6.22	2.41	16.42	6.37	41.58	10.09	5.47	4.87	0.30
P. ramosissima (var. *australitoralis* × var. *suffrutescens*)													
Greenhouse	0.74	0.46	1.65	0.92	7.43	2.58	15.25	5.75	42.18	9.55	6.63	6.63	0.18
Greenhouse	1.12	0.34	3.03	1.24	6.98	2.02	14.78	5.21	44.38	8.14	4.17	8.37	0.15
5% "A"-LSD	0.9	0.6	1.5	1.0	2.7	1.4	3.7	1.8	3.9	2.1	1.6	1.8	0.7

No. of plants per sample = 40.

Cavern, California, and no other live material was available. The composition cited in Table 1 is of greenhouse-grown material. Amounts of C_{27} and C_{31} fall into similar ranges, with C_{29} low and C_{33} high.

Previous work [9] suggested that nonacosane has adaptive value in dry habitats. We found the highest deposition of C_{29} in *P. distans* Benth., which was collected in Morongo Valley and in Joshua Tree National Monument under the most xerophytic conditions. In *P. tanacetifolia* collected at the desert site of Red Mountain, some gene flow with *P. distans* was later established to exist. In retrospect, this gene flow may have been the influential factor in reducing heptacosane to intermediacy and increasing hentriacontane and tritriacontane to near intermediacy between the pure desert *P. tanacetifolia* collected at the Phacelia santuary and *P. distans*.

In the greenhouse-grown hybrids of *P. ramosissima* var. *australitoralis* × *P. ramosissima* var. *suffrutescens*, hentriacontane and tritriacontane were present in a greater amount than in either parent.

While this study did not produce satisfactory markers for species delimitation, it showed that whole-leaf hydrocarbons differ quantitatively in different natural populations of the same species and that differences also exist between populations grown in the greenhouse as well as in the wild. Nonacosane occurred in high amounts in those populations of *P. distans* which were growing in the driest natural habitats.

EXPERIMENTAL

For alkane analysis, healthy leaves of about 3 months growth before anthesis from 40 field- and 20 greenhouse-grown plants per habitat (Table 1) were harvested on various field trips in late March to April 1974 and in 1975. For the hybrid study of *P. ramosissima* var. *suffrutescens*, only two plants were available. The leaf material was air-dried and coarsely milled. 200 g were extracted continuously with 2000 ml petroleum (bp 30–60°), and the resulting extract was evaporated to near dryness. The residue was dissolved in hot Me_2CO, decolorized (charcoal), filtered and then cooled to 0°. All solids that precipitated were collected and the IR spectrum obtained was indicative of alkanes. A small sample was dissolved in hexane and 20 μl injected into a Varian 1520 C gas chromatograph with TC detector. The oven temp. was programmed from 100–340° at 2-min intervals using a 183 cm × 0.64 cm o.d. stainless steel column packed with 10% UC W-98 coated on 80–100 mesh gas Chrom Q. The carrier gas was helium with 60 ml/min flow rate; injector temp. was 150°; detector temp., 350°. The alkanes eluted in distinct areas and were identified by comparison with reference samples and retention times. The areas under the peaks were converted into percent composition using a Varian 475 Digital Integrator.

The leaf material of 40 field plants was pooled together in order to get sufficient alkane. Although the standard deviation from among individuals is thus not available, some indication of their cumulative effect can be obtained by examining the standard deviation between the bulked averages obtained from similar habitats. Errors were approximated and values calculated, assuming averages of 40 plants. Differences between any two locations exceeding the above defined value should be considered different at the 5% level.

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