Pergamon

PII: S0031-9422(96)00570-5

Phytochemistry, Vol. 44, No. 5, pp. 825–831, 1997

0031-9422/97 $17.00+0.00

PHENOLOGICAL CHANGES IN PRIMARY AND SECONDARY CHEMISTRY OF REPRODUCTIVE PARTS IN WILD PARSNIP

ARTHUR R. ZANGERL,* ELLEN S. GREEN, RICHARD L. LAMPMAN† and MAY R. BERENBAUM

Department of Entomology, University of Illinois at Urbana-Champaign, 320 Morill Hall, 505 S. Goodwin, Urbana, IL 61801, U.S.A.; †Illinois Natural History Survey, Center for Economic Entomology, Medical Entomology Program, 607 E. Peabody Dr. Champaign, IL 61820, U.S.A.

(*Received in revised form* 29 *July* 1996)

Key Word Index—*Pastinaca sativa*; Apiaceae; wild parsnip; furanocoumarins; terpenes; fatty acids; protein; ascorbic acid; myo-inositol.

Abstract—Few studies have documented the developmental profile of both primary and secondary metabolites of plants; we set out to construct such a profile, in order to determine the extent to which primary and secondary metabolites covary. Primary and secondary chemistry have been documented for buds, female flowers and green fruits of wild parsnip *Pastinaca sativa*. A distinct qualitative shift in secondary chemistry occurs over the course of development. Mono- and sesquiterpenes were abundant in buds but absent from female flowers and green fruits. Furanocoumarins, which were found in all organs, were present at low concentration in buds, at intermediate concentrations in female flowers, and at highest concentrations in fruits. Among the primary metabolites, developmental shifts were, for the most part, quantitative. Soluble protein and fatty acid content declined with development. A qualitative change in fatty acid composition was observed in that linolenic acid in buds was replaced by petroselinic acid in fruits. Overall variation in primary metabolites was rarely correlated with variation in secondary metabolites: of the 233 possible correlations, only 25 were significant.

INTRODUCTION

For over a century, chemicals in plants have been categorized according to their function. Primary metabolites are those constituents involved in carrying out basic physiological demands of daily life (e.g. proteins, fatty acids, carbohydrates), including such fundamental processes as photosynthesis and respiration. These compounds tend to be widespread, even universal, in occurrence and include amino acids, sugars, fatty acids, and vitamins [1]. In contrast, secondary metabolites play no role in basic physiological processes; rather, these compounds serve an ecological function and are involved in defending plants against microbes, herbivorous insects, and other enemies [1]. Biosynthetically, primary and secondary compounds are inextricably linked in the sense that all secondary compounds are derived from primary metabolic pathways. One of the distinguishing features of secondary metabolites is their variability; whereas primary metabolites are almost universal constituents of plant tissue, secondary metabolites are highly idiosyncratic in their distribution [1]. Within a plant, secondary substances are not uniformly distributed among tissues, within a population, secondary substances are not uniformly distributed among individuals, and, within a genus, secondary compounds are not uniformly distributed among species [1]. Certain substances, however, that by virtue of their structure and biosynthetic origin might be considered primary metabolites, display the same kind of idiosyncratic distribution that classical secondary metabolites display. There are several possible explanations of such variation in distribution and abundance in primary metabolites. An adaptive explanation is that physiological demands for growth and photosynthesis vary in time and space. In reproductive structures, for example, green buds are photosynthetic whereas flowers, lacking chlorophyll, are nonphotosynthetic. Alternatively, primary metabolite variation may result from coordinated regulation of secondary metabolic pathways. Many of the secondary metabolic pathways are inducible by herbivory, infection, or mechanical damage—that is to say, such environmental stresses elicit *de novo* biosynthesis of protective compounds. Enhanced availability of common precursors within these inducible pathways may result in incidental overproduction of primary metabolites. Finally, primary metabolites may, in certain cir-

* Author to whom correspondence should be addressed.

cumstances, serve secondary roles; these compounds may contribute to the defence of the plant against herbivores and pathogens by reducing digestibility or palatability [2].

To date, there have been few studies of the developmental profile of both primary and secondary chemistry. We set out to construct such a profile, with the object of determining the degree to which primary and secondary metabolites vary independently of each other. We chose for this study the wild parsnip *Pastinaca sativa*, a biennial weed introduced from Europe and extensively naturalized throughout the United States, where it is typically found growing along roadsides, in old fields, and in wastelands [3]. This plant was selected because there exists a wealth of information about the chemistry of the plant and the ecological function of many of these chemicals [4]. The phytochemistry of this wild parsnip is simpler than that of most relatives in the family Apiaceae, species of which can contain 100 or more essential oil constituents [5].

Among the known groups of secondary chemicals found in parsnips are furanocoumarins, which have demonstrable antifeedant and phototoxic properties against a broad range of herbivores [6] these are; phenylpropanoids, e.g. myristicin, which is toxic to certain insects and is a synergist of furanocoumarin toxicity [4]; monoterpenes, which are known attractants for pollinators and also antimicrobial agents [7]; sesquiterpenes, which are toxic and deterrent to insects; and fatty acid esters, which are dietary toxins for certain lepidopterous larvae. Significant variation is known to exist in levels of furanocoumarins in this plant [8, 9] and developmental differences exist in concentrations as reproductive organs mature [10]. Myristicin and also octyl acid esters have been shown to vary among individuals in occurrence and concentration [11]. No comparable information exists on the levels of variation in primary metabolites in this plant—protein, fatty acids, vitamins or carbohydrates.

RESULTS AND DISCUSSION

A substantial portion of the variation in both primary and secondary metabolites was due to differences among plants (Table 1). Significant between-plant variation was found for all but two components, stearic acid in buds and flowers, and palmitolactone in flowers. Both primary and secondary metabolites changed dramatically in concentrations as reproductive structures developed from buds to fruits (Figs 1 and 2). Among the secondary compounds, monoterpenes, sesquiterpenes, and myristicin, which are abundant constituents in parsnip leaves [12], were also abundant in buds (Fig. 1). However, these compounds declined rapidly with development and many were absent in female flowers and fruits. In contrast, there was a several-fold increase in octyl acetate, octyl butyrate, and furanocoumarins. Two previously unreported sesquiterpenes were identified in our samples—cubebene and bergamotene. Ontogenetic changes in primary chemicals also occurred; soluble protein and fatty acid content declined with development (Fig. 2). The fatty acid linolenic acid is abundant in buds but is absent in female flowers and green fruits; another C18 fatty acid, petroselinic acid (6-octadecenoic acid), appears to replace linolenic acid in green fruits (Table 1).

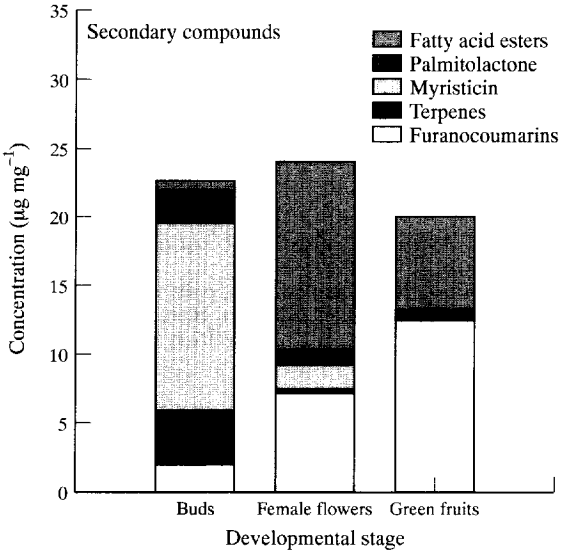

Fig. 1. Concentrations of secondary metabolites in buds, female flowers and half-filled green fruits of wild parsnip.

To evaluate the degree to which developmental changes among metabolites are coordinated, we calculated product–moment correlations between metabolites at each stage of development. The large number of correlations calculated (510) precludes reporting all of them; however, there are several patterns worth noting. As might be expected, correlations within

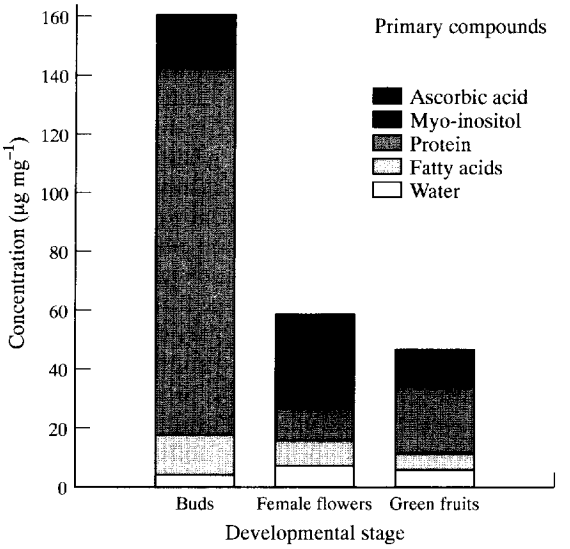

Fig. 2. Concentrations of primary metabolites in buds, female flowers and half-filled green fruits of wild parsnip.

Table 1. Composition of buds, female flowers and half-filled green fruits of wild parsnip. All values are in $\mu g\ mg^{-1}$ unless otherwise indicated. Sample sizes were 50 (fruits), 49 (female flowers) and 48 (buds) with the exception that bud protein and myo-inositol estimates were based on 47 samples

	Buds			Flowers			Fruits		
Compound	Mean	S.D.	P^1	Mean	S.D.	P^1	Mean	S.D.	P^1
Bergamotene[2]	0.93	1.77	**	Not detected			Not detected		
Cubebene[2]	1.00	0.55	**	Not detected			Not detected		
Caryophyllene[2]	0.27	0.49	**	Not detected			Not detected		
Farnesene[2]	1.09	0.59	**	Not detected			Not detected		
cis-Ocimene[2]	0.38	0.26	**	Not detected			Not detected		
trans-Ocimene[2]	0.20	0.15	**	0.16	0.15	**	Not detected		
Octyl acetate	Not detected			2.98	2.39	**	1.51	1.84	**
Octyl butyrate	0.58	0.67	**	10.9	4.43	**	5.48	4.25	**
γ-Palmitolactone[2]	2.35	1.00	**	1.04	0.46	0.08	0.64	0.23	**
Imperatorin	0.39	3.36	na	1.84	1.11	na	3.40	1.29	na
Bergapten	0.56	0.53	na	0.72	0.31	na	2.76	1.40	na
Psoralen	1.18	0.25	na	0.46	0.24	na	0.58	0.22	na
Isopimpinellin	0.08	0.06	na	0.27	0.15	na	0.65	0.39	na
Xanthotoxin	0.78	0.02	na	3.84	1.68	na	4.66	1.73	na
Sphondin	0.08	0.20	na	0.07	0.05	na	0.22	0.21	na
Myristicin	13.8	6.9	**	1.84	1.10	**	Not detected		
Protein	125	42	**	10.3	15.6	**	23.0	16.0	**
Palmitic acid	2.70	0.50	*	2.06	0.49	**	1.11	0.26	**
Linoleic acid	5.90	1.80	**	6.00	1.46	**	3.33	0.84	**
Linolenic acid	4.60	2.97	**	Not detected			Not detected		
Petroselinic acid	0.38	0.22	**	0.42	0.12	**	0.85	1.00	**
Stearic acid	0.66	0.18	0.25	0.50	0.13	0.07	0.42	0.12	**
Myoinositol	18.0	11.0	**	30.6	15.6	**	3.0	1.67	**
Ascorbic acid	0.29	0.15	**	1.60	1.28	**	0.53	0.44	**
Water ($mg\ mg^{-1}$)	3.98	0.29	na	7.30	1.00	na	5.10	0.92	na

[1]From one-way analysis of variance, asterisks indicate significant differences between plants: $**P < 0.001$, $*P < 0.01$. Fifty individual plants were sampled for each stage of development.

[2]$\mu g\ mg^{-1}$ in hexadecane equivalents.

na (not analysed) denotes analyses that did not include duplicate sampling and therefore could not be analysed for differences among plants.

biosynthetic pathways were generally positive and significant (Table 2). Of the 42 correlations between furanocoumarins, 35 were positive and the remainder were not significant. Among the 22 correlations between fatty acids, 20 were significant; 18 of these were positive and two were negative. The two negative correlations were between linolenic acid and other C18 fatty acids in buds, suggesting that the replacement of linolenic by other fatty acids, evident in later developmental stages, begins in the bud stage. The associations between metabolites of the terpenoid biosynthetic pathway were less consistent. The sequiterpene bergamotene was negatively correlated with two other sesquiterpenes, farnesene ($r = -0.29$, $P = 0.044$) and cubebene ($r = -0.357$, $P = 0.011$). Cubebene and farnesene were positively correlated ($r = 0.350$, $P = 0.013$), and the only other sequiterpene, caryophyllene, was correlated only with the monoterpene *cis*-ocimene ($r = 0.466$, $P < 0.001$). The two monoterpenes abundant in wild parsnip, and presumably having a similar biosynthetic pathway, *cis*- and *trans*-isomers of ocimene, were also positively correlated ($r = 0.377$, $P = 0.007$). Nine other correlations between terpenes were not significant.

γ-Palmitolactone, a 16-carbon component that is not commonly found in essential oils [12], was not consistently correlated with any single biosynthetic pathway (Table 2). In buds, for example, γ-palmitolactone was positively correlated with three terpenes (ocimene, farnesene, and cubebene) but was not correlated with any of the furanocoumarins. In contrast, γ-palmitolactone was positively correlated with two furanocoumarins (imperatorin and xanthotoxin) in flowers and with five furanocoumarins (imperatorin, bergapten, psoralen, xanthotoxin, and sphondin) in fruits. The only other correlations involving γ-palmitolactone were positive ones with certain fatty acids (linolenic and petroselinic acid in buds, the same two fatty acids and palmitic acid in flowers, and palmitic and linoleic acids in fruits). Presumably, γ-palmitolactone and palmitic acid are biosynthetically related; both compounds have saturated long-chain structures and irradiation of palmitic acid results in the formation of γ-palmitolactone [13].

Table 2. Significant product–moment correlations ($P < 0.05$) between constituents in wild parsnip buds, female flowers and half-filled green fruits

Constituents	Correlation		
	Buds	Flowers	Fruits
Between furanocoumarins			
Imperatorin and bergapten	0.595	0.582	0.569
Imperatorin and psoralen	0.389	0.486	0.450
Imperatorin and isopimpinellin	0.479	ns	ns
Imperatorin and xanthotoxin	0.379	0.539	0.539
Imperatorin and sphondin	ns	0.366	ns
Bergapten and psoralen	0.888	0.744	0.585
Bergapten and isopimpinellin	0.659	0.423	0.377
Bergapten and xanthotoxin	0.849	0.824	0.682
Bergapten and sphondin	0.546	0.426	0.497
Psoralen and isopimpinellin	0.579	ns	ns
Psoralen and xanthotoxin	0.913	0.617	0.737
Psoralen and sphondin	0.604	0.359	0.427
Isopimpinellin and xanthotoxin	0.716	0.447	ns
Isopimpinellin and sphondin	0.475	ns	ns
Xanthotoxin and sphondin	0.733	0.429	0.375
Between terpenes			
cis-Ocimene and *trans*-ocimene	0.377	nd	nd
cis-Ocimene and caryophyllene	0.466	nd	nd
trans-Ocimene and caryophyllene	0.357	nd	nd
Bergamotene and farnesene	−0.287	nd	nd
Bergamotene and cubebene	−0.357	nd	nd
Farnesene and cubebene	0.349	nd	nd
Between fatty acids			
Palmitic and linoleic	0.501	0.974	0.950
Palmitic and linolenic	0.282	nd	nd
Palmitic and petroselinic	0.402	0.828	0.491
Palmitic and stearic	0.482	0.452	0.756
Linoleic and linolenic	−0.633	nd	md
Linoleic and petroselinic	0.716	0.856	0.589
Linoleic and stearic	0.414	0.471	0.720
Linolenic and petroselinic	−0.0391	nd	nd
Linolenic and stearic	ns	nd	nd
Petroselinic and stearic	0.459	0.542	ns
Correlations with palmitolactone			
cis-Ocimene	0.300	nd	nd
Farnesene	0.450	nd	nd
Cubebene	0.452	nd	nd
Linoleic	0.508	0.443	0.339
Linolenic	−0.409	nd	nd
Petroselinic	0.477	0.430	ns
Palmitic	ns	0.417	0.287
Imperatorin	ns	0.338	0.373
Bergapten	ns	ns	0.443
Psoralen	ns	ns	0.418
Xanthotoxin	ns	0.229	0.511
Sphondin	ns	ns	0.294
Water	0.321	ns	ns
Myo-inositol	−0.458	ns	ns

ns, not significant; nd, one of the constituents was not detected.

Table 3. Significant product–moment correlations ($P < 0.05$) between primary and secondary metabolites in wild parsnip buds, female flowers and half-filled green fruits

Buds	
cis-Ocimene and palmitic acid	−0.287
Caryophyllene and linoleic acid	−0.299
Cubebene and linolenic acid	−0.279
Cubebene and myo-inositol	−0.316
Farnesene and petrolselinic acid	0.331
Octyl butyrate and myo-inositol	0.282
Female flowers	
cis-Ocimene and linoleic acid	−0.490
cis-Ocimene and petroselinic acid	−0.399
cis-Ocimene and stearic acid	−0.487
Octyl acetate and stearic acid	−0.427
Fruits	
Octyl acetate and protein	−0.422
Octyl acetate and stearic acid	−0.289
Octyl butyrate and myo-inositol	0.323
Palmitic acid and imperatorin	0.361
Palmitic acid and bergapten	0.288
Palmitic acid and xanthotoxin	0.300
Linoleic acid and imperatorin	0.361
Linoleic acid and bergapten	0.351
Linoleic acid and isopimpinellin	0.320
Linoleic acid and xanthotoxin	0.339
Petroselinic acid and isopimpinellin	0.577
Stearic acid and imperatorin	0.462
Stearic acid and xanthotoxin	0.337
Bergapten and ascorbic acid	−0.322
Isopimpinellin and myo-inositol	−0.524

Variation in primary metabolites (protein, fatty acids, ascorbic acid, and myo-inositol) was rarely correlated with variation in secondary metabolites. Discounting correlations with γ-palmitolactone, only six of the 107 correlations between primary and secondary constituents in buds, four of the 70 correlations in flowers, and 15 of the 56 correlations in fruits were significant (Table 3). As evidence of possible competition between primary and secondary pathways, six of the seven significant correlations between terpenes and fatty acids in buds and flowers were negative (Table 3); both of these pathways require the precursor acetate [14]. There was also evidence of competition within pathways; the fatty acid ester, octyl acetate, was negatively correlated with stearic acid in both female flowers and fruits (Table 3). Most of the significant correlations in fruits involved positive correlations between fatty acids and furanocoumarins (10 correlations). These positive correlations are unlikely to have a biosynthetic basis. Although synthesis of both furanocoumarins and fatty acids requires acetate [14], synthesis of furanocoumarins also requires products from the phenylpropanoid pathway [14]. In general, primary metabolites in wild parsnip are therefore no less likely to vary over the course of development than are secondary metabolites. This extensive variation, however, occurs largely independently of the variation in secondary metabolites.

Although not necessarily applicable to every constituent, there is an apparent pattern of change characterizing the development of reproductive structures from buds to green fruits. Whereas early stages are associated with high levels of primary metabolites and volatile secondary metabolites, later stages are characterized by low levels of primary metabolites and high levels of non-volatile secondary metabolites. This pattern is consistent with the changing nature of interaction with plant-consuming insects over the course of development. Buds and flowers in this insect-pollinated species [15] are dependent upon mutualistic visitors; volatile secondary compounds and elevated levels of primary metabolites, which in most cases function as insect nutrients, serve to increase the attractiveness of these structures to mutualists. In contrast, fruits, which are dorso-ventrally flattened, winged, and presumably wind-dispersed in this species, gain no benefit from insect visitation. Thus, reduced levels of primary nutrients and elevated levels of highly insecticidal secondary compounds both contribute to decreasing losses to herbivores. Overall, in *P. sativa*, variation in levels of both primary and secondary metabolites is consistent with regulation in response to the ecological needs of the plant at each stage of development.

EXPERIMENTAL

We collected all samples from plants growing at the University of Illinois Phillips Tract research area located in Champaign County 6 km northeast of the Urbana campus. Buds, female flowers and half-filled green fruits were cut with a razor blade from primary umbels of 50 plants. To avoid potential effects of repeated sampling of individual plants on chemistry, samples of each stage of development were collected from different plants; a total of 150 plants were sampled (50 per stage of development). Samples were placed in 1.5 ml Eppendorf tubes and placed in liquid nitrogen. At the laboratory, the tubes were weighed, opened, and dried in a lyophilizer. Each tube was reweighed to quantify the amount of H_2O in the samples and then sealed and stored at −80° until analysis. In total, 24 primary and secondary compounds were quantified for each sample. To determine whether there is phenotypic variation among plants for both primary and secondary metabolites, we analysed two aliquots from each sample. Exceptions to this two-aliquot procedure were the analyses of furanocoumarins, which have previously been shown to exhibit ample genetic and phenotypic variation [16, 17], and H_2O content. We did not subdivide the original sample for analysis of water content out of concern that the amount of material in each subsample might not be sufficient for analysis of all of the secondary and primary metabolites.

Secondary metabolites. Furanocoumarins were extracted from pre-weighed (approx. 10 mg) powdered samples in Eppendorf tubes with 200 μl of EtOAc for 1 hr at room temp. The tubes were spun at 12 200 *g* for 10 min in a microcentrifuge to remove particulates; 10 μl of the extract was analysed by HPLC (Alltech Absorbosphere™ silica column, 15 cm × 4.6 mm i.d., 5 μm particle size; mobile phase, 55:42:3 cyclohexane–isopropyl ether–n-BuOH butanol, 1.5 ml/min^{-1}; detection, 254 nm). A separate aliquot of powdered sample was weighed (5–9 mg) and extracted in Eppendorf tubes with 250 μl of hexane containing hexadecane (internal standard) at 80° for 2 hr. Tubes were centrifuged as before and 1 μl of extract was analysed by GC for quantification of terpenes, palmitolactone, and myristicin (Alltech, SE-30, 30 m capillary column, i.d., 0.32 mm, film thickness 0.25 μm, temperature programme 60°, 1 min, 5° min^{-1}, 200°, 5 min). Because of the similarity in mass spectra of related sesquiterpenes, we were unable to identify the specific isomers of farnesene, caryophyllene, cubebene, and bergamotene. Of the remaining extract, 75 μl was transferred to a fresh tube for fatty acid analysis. The pellet consisting of ground tissue and solvent was stored at −80° for protein and sugar analysis.

Primary metabolites. The 75 μl of hexane extract was dried *in vacuo* and methyl derivatives of the fatty acids were prepared by adding 0.5% sulphuric acid in MeOH to the residue and incubating the mixture at 80° for 1 hr. To this residue was added 400 μl of distilled water and 300 μl of hexane, containing hexadecane as an int. standard. The mixt. was vortexed for 10 s at 12 200 *g* and an aliquot (100 μl) of the hexane-soluble components was removed for GC analysis (1 μl injection on the column previously described; triple ramp temperature programme: 120°, 1 min, 3° min^{-1}, 170°, 1° min^{-1}, 180°, 1 min, 10° min^{-1}, 200°). Fatty acid methylester standards (Sigma) were used to generate calibration curves.

The pellet remaining after the initial hexane extraction was dried under vacuum and extracted for 1 hr at room temp. with 150 μl distilled water. The samples were then centrifuged as before to remove particulates and 5 μl of the extract was mixed with 1 ml of BioRad reagent inside a disposable microcuvette for quantification of soluble protein. A standard calibration curve for soluble protein was constructed with ribulose bisphosphate carboxylase (Sigma). Another 40 μl portion of the aqueous extract was dried under vacuum and 200 μl of Tri-Sil Z (Pierce) was added to the residue and allowed to react with the sugars for 15 min at 60°. One microlitre of the derivatized sample was then analysed by GC (same column, temperature programme: 60°, 0 min, 10° min^{-1}, 290°C). Derivatized myo-inositol standards were prepared from myo-inositol (Sigma) and used to construct standard curves.

Ascorbic acid was extracted directly from weighed, powdered sample with an aq. soln containing 0.1% TCA and 0.2% sodium metabisulphite (preservative). From this extract, 10 μl were analysed by RP-HPLC (Alltech Altima C-18 column, 250 mm × 4.6 mm i.d., 5 μm particle size; mobile phase, 0.05 M sodium acetate, 1.5 ml min^{-1}; detection, 263 nm). Authentic ascorbic acid (Sigma) was used to prepare a standard curve.

After all of the chemical analyses were completed, the original sample collection tubes were emptied, washed, oven-dried, and reweighed to determine, by subtraction, the dry weight of the original sample. Water content was expressed in milligrams water per milligram dry weight.

Identification of sugars, fatty acids, and hexane-extractable secondary metabolites was made by GC–MS, consultation of previously published analyses of wild parsnip [12, 18] and, where possible, by comparison with authentic standards. In GC analyses for which standards were unavailable, amounts of compounds were calculated as equivalents of hexadecane. All concentrations were calculated on the basis of dry sample weight.

cis-*Ocimene*: GC–MS, 70 eV, 136[M]$^+$ (1), 93 (100), 91 (47), 41 (43), 39 (42), 92 (41), 79 (41), 77 (36), 43 (21).

trans-*Ocimene*: GC–MS, 70 eV, 136[M]$^+$ (7), 93 (100), 91 (62), 41 (61), 79 (51), 39 (49), 77 (46), 80 (41), 43 (35).

Caryophyllene: GC–MS, 70 eV, 204[M]$^+$ (4), 41 (100), 91 (62), 93 (55), 79 (54), 69 (49), 133 (48), 105 (35), 77 (31).

Bergamotene: GC–MS, 70 eV, 204[M]$^+$ (2), 119 (100), 93 (98), 91 (49), 69 (49), 107 (41), 77 (40), 79 (38), 55 (36).

Farnesene: GC–MS, 70 eV, 204[M]$^+$ (1), 41 (100), 69 (95), 93 (51), 79 (22), 67 (22), 39 (21), 91 (16), 55 (16).

Cubebene: GC–MS, 70 eV, 204[M]$^+$ (15), 161 (100), 105 (63), 91 (61), 41 (56), 81 (49), 79 (48), 119 (36), 77 (32).

γ-Palmitolactone: GC–MS, 70 eV, 85 (100), 41 (50), 43 (46), 55 (43), 69 (26), 57 (26), 56 (25), 83 (21).

A voucher specimen of *Pastinaca sativa* L. was collected by Mark Carroll (no. 1) and was deposited in the Herbarium at the University of Illinois.

Acknowledgements—We thank Mark Carroll, Colin Favret, Christine Andrasz, Tanya Hanlon and Amy Hanlon for assistance with collections of plant material in the field. This research was supported by a grant from the National Science Foundation (DEB-9509826).

REFERENCES

1. Berenbaum, M. R., *Oxford Surveys in Evolutionary Biology*, **7**, 285.
2. Berenbaum, M. R., *Journal of Chemical Ecology*, 1995, **21**, 925.

3. Fernald, M. L., *Gray's Manual of Botany*, 8th edn. American Book Company, New York, 1950.
4. Berenbaum, M. R., *Recent Advances in Phytochemistry*, 1985, **19**, 139.
5. Heywood, V. H., ed. *The Biology and Chemistry of the Umbelliferae.* Academic Press, New York, 1971.
6. Berenbaum, M. R., *Archives of Biochemistry and Physiology*, 1995, **29**, 119.
7. Harrewijn, P., Minks, A. K. and Mollema, C., *Chemoecology*, 1994/1995, **5/6**, 55.
8. Zangerl, A. R. and Berenbaum, M. R., *Ecology*, 1990, **71**, 1933.
9. Zangerl, A. R. and Berenbaum, M. R., *Ecology*, 1993, **74**, 47.
10. Nitao, J. K. and Zangerl, A. R., *Ecology*, 1987, **68**, 521.
11. Stahl, E. and Kubeczka, K.-H., *Planta Medica*, 1979, **37**, 49.
12. Kubezcka, K.-H. and Stahl, E., *Planta Medica*, 1979, **31**, 173.
13. Vajdi, M. and Nawar, W. W., *Journal of the American Oil Chemists' Society*, 1979, **56**, 906.
14. Robinson, T., *The Organic Constituents of Higher Plants.* Cordus Press, North Amherst, MA, 1991.
15. Bell, C. R., in *The Biology and Chemistry of the Umbelliferae*, ed. V. H. Heywood. Academic Press, New York, 1991, p. 93.
16. Berenbaum, M. R., Zangerl, A. R. and Nitao, J. K., *Evolution*, 1986, **40**, 1215.
17. Zangerl, A. R., Berenbaum M. R. and Levine, E., *Journal of Heredity*, 1989, **80**, 404.
18. Kleiman, R. and Spencer, G. F., *Journal of the American Oil Chemists' Society*, 1982, **59**, 29.