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VARIATION IN SEED FURANOCOUMARIN CONTENT WITHIN THE WILD PARSNIP (*PASTINACA SATIVA*)

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Key Word Index—*Pastinaca sativa*; Umbelliferae; parsnip; furanocoumarins; imperatorin; bergapten; isopimpinellin; xanthotoxin; sphondin; vittae; within-plant distribution.

Abstract—Furanocoumarins are restricted within the ripe seeds of *Pastinaca sativa*, the wild parsnip, to the vittae or oil tubes. The furanocoumarin content and composition of these organs vary with their location. Vittae on the inner or commissural side of the mericarp contained over 90% of the total furanocoumarin content of the seed; the relative abundance of the five furanocoumarin components also varies with seed side. The location of the seed within an umbel does not appear overall to affect furanocoumarin characteristics; however, the location of the seed among umbels is associated with significant differences in furanocoumarin content. The absolute amount of furanocoumarin present in seeds of primary and secondary umbels was approximately twice that of seeds in tertiary umbels. In the case of the wild parsnip, then, furanocoumarin distribution is not homogeneous, either through the plant or within the plant part in which it is localized.

INTRODUCTION

As Janzen [1] states, "a list of Latin binomials feeding on other Latin binomials carries almost no information when it is remembered that the secondary-compound chemistry of two different plant parts on the same plant is much more likely to be different than the same." This is particularly the case as regards selective feeding in herbivorous insects; due to their small size, insects are capable of perceiving considerably finer detail in the distribution of secondary chemicals than is customarily resolved with traditional phytochemical analyses. As part of a study examining the interactions between wild parsnip (*Pastinaca sativa*) and its associated insect herbivores, we used a highly sensitive high pressure liquid chromatographic technique to determine within-plant distribution of furanocoumarins, secondary chemicals known to influence insect feeding behaviour and physiology [2–5], in a single plant part—the ripe seed.

The ripe seeds of wild parsnips are fed upon by a variety of insect herbivores [6], particularly by hemipterous insects capable of considerable tissue selectivity in feeding; for example, Flemion *et al.* [7] demonstrated that *Lygus lineolaris*, the tarnished plant bug (a major pest on cultivated and wild parsnip), can insert and manoeuvre its stylets so as to feed selectively on embryo tissue almost exclusively. We examined furanocoumarin distribution as a function both of seed location within the plant and of within-seed anatomy. The wild parsnip fruit is a schizocarp, comprised of two seedlike carpels or mericarps suspended from a carpophore, itself an elongation of the floral axis. At maturity, the fruit splits and the two seeds separate from one another. As in most umbellifers, the seeds are ribbed and in among the ribs are vittae or oil tubes; it is in these tubes in which furanocoumarins are reported to be localized [8]. On the outer face there are four vittae and on the inner or commissural face there are generally two somewhat larger vittae.

Seeds in umbellifers are borne in umbrella-like inflorescences usually consisting of a number of subcomponents or umbellets. The wild parsnip is centripetally protandrous [9] and the outer umbellets on a single umbel develop first and are followed by the central umbellets. Each parsnip plant generally consists of a single terminal or primary umbel and several lateral umbels, classified by the number of branch points off the main stem. There are phenological differences among umbel types—the primary umbel usually matures first [10] followed by secondary umbels and lastly tertiary or higher order umbels. There are functional differences as well; proportionately fewer flowers are perfect with increasing umbel order, with the result that the probability of seed set declines with increasing umbel order [10]. In view of both phenological and functional differences associated with seed location, we evaluated seeds for furanocoumarin content with respect to umbel order (primary, secondary or tertiary), position within an umbel (central vs peripheral umbellets) and with respect to position within a fruit (commissural or noncommissural side of seed coat).

RESULTS AND DISCUSSION

As reported previously [8], we found no trace of furanocoumarins in any part of the seed other than in the vittae; endosperm, embryo and testa were all devoid of detectable furanocoumarins. The position of the seed within the fruit appears to affect furanocoumarin distribution. Over 90% of the total furanocoumarin content of the seed is present in the two inner vittae; the four outer vittae on the noncommissural side consistently contained less than 10% of the total. The relative composition of furanocoumarins differed as well; while isopimpinellin predominated on the noncommisural face, xanthotoxin predominated on the commissural face (Table 1). The position of the seed within the umbel did not, with one

Table 1. Mean furanocoumarin content and proportional composition of the commissural and non-commissural face of the seed coat in *Pastinaca sativa*

Seed face	Total	Imperatorin	Bergapten	Isopimpinellin	Xanthotoxin	Sphondin
Commissural	27.8	9.0 (32.4)	5.3 (19.1)	1.6 (5.8)	11.4 (41.0)	0.4 (1.4)
Non-commissural	3.3	0.8 (24.2)	0.6 (18.2)	1.0 (30.3)	0.8 (2.4)	0.1 (3.0)
ANOVA	***	*** (***)	** (***)	** (***)	*** (***)	*** (NS)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS = not significant. Content is expressed as μg/seed; percentages are in parentheses.

Table 2. Mean furanocoumarin content and proportional composition in seeds of *Pastinaca sativa* from central and peripheral umbellets

Umbellet	Total	Imperatorin	Bergapten	Isopimpinellin	Xanthotoxin	Sphondin
Central	25.4	12.8 (50.4)	3.2 (12.6))	2.8 (11.0)	6.2 (24.4)	0.4 (1.6)
Peripheral	24.7	12.7 (51.4)	3.2 (13.0)	2.9 (11.7)	5.9 (23.9)	0.4 (1.6)
ANOVA	NS	NS (NS)	NS (NS)	NS (NS)	NS (NS)	NS (NS)

NS = not significant. Content is expressed as μg/seed; percentages are in parentheses.

exception, appear to be associated overall with differences in furanocoumarin content or composition (Table 2). In the primary umbel of one individual, seeds in the peripheral umbellets contained 80% more furanocoumarins than did seeds from central umbellets ($p < 0.05$; t-test) (Table 3). The composition of the furanocoumarins present differed as well within this particular umbel.

Table 3. Differences in furanocoumarin content in umbellets within the primary umbel (μg/seed, five seeds from each region)

Region	IMP	BER	ISO	XAN	SPH	TOT
Centre	5.5	0.9	1.3	2.2	0.07	10.1
Periphery	8.2	2.4	2.2	5.2	0.16	18.3

The furanocoumarin content of seeds varied with umbel order; while the absolute amounts of furanocoumarin present in seeds of primary and secondary umbels did not differ, they were approximately twice as great as that in seeds from tertiary umbels (Table 4). There are differences in seed weights among umbels (ANOVA of seed weights $p < 0.0001$)—tertiary seeds at an average of 2.48 mg/seed are smaller than both primary and secondary umbel seeds ($p < 0.005$) and secondary umbel seeds at 3.44 mg/seed are smaller than primary umbel seeds at 4.00 mg/seed ($p < 0.045$). Differences in seed weights do not, however, necessarily account for differences in furanocoumarin content among umbels. In this study, while seeds from secondary umbels are significantly smaller than those from primary umbels, they contain equivalent amounts of furanocoumarins on a dry weight basis.

Natural products such as furanocoumarins have been advanced as protective agents in plants against herbivores and pathogens. In order to evaluate the ecological significance of these compounds, it is important to document the distribution and variation within a plant as it is perceived by prospective plant enemies. In the case of the wild parsnip, furanocoumarin distribution is not homogeneous throughout the plant, nor is it homogeneous throughout the plant part in which it is localized. The significance of this within-plant variation has yet to be demonstrated with respect to defense against herbivores; in that differences in furanocoumarin distribution correspond to functional and structural differences among plant parts, both extrinsic factors (such as herbivores) and intrinsic factors (such as physiological processes relating to seed maturation) may be important in determining that distribution.

Table 4. Mean furanocoumarin content and proportional composition in seeds of *Pastinaca sativa* from primary, secondary and tertiary umbels

Umbel order	Total	Imperatorin	Bergapten	Isopimpinellin	Xanthotoxin	Sphondin
1°	27.3	11.1 (40.7)	4.5 (16.5)	3.3 (12.1)	7.8 (28.6)	0.5 (1.8)
2°	29.5	11.8 (40.0)	5.0 (16.9)	3.1 (10.5)	9.2 (31.2)	0.4 (1.7)
3°	14.5	5.9 (40.7)	2.2 (15.1)	1.8 (12.4)	4.5 (31.0)	0.2 (1.4)
ANOVA	***	*** (NS)	*** (NS)	** (NS)	*** (NS)	*** (NS)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS = not significant. Content is expressed as μg/seed; percentages are in parentheses.

EXPERIMENTAL

Furanocoumarin extraction and separation were by HPLC as described in ref. [11]. Within-seed variation was assessed by soaking ten primary umbel seeds in H_2O for 24 hr and subsequently dissecting the seed into commissural seed coat, non-commissural seed coat and embryo plus endosperm. All other analyses were conducted on intact seeds. Within-umbel variation was assessed by sampling individually five seeds from each region from each of three plants; in one plant a primary umbel was sampled and in the other two plants a secondary umbel was sampled. Between-umbel samples were five-seed pooled samples from each of 20 primary, secondary and tertiary umbels and the quantities are expressed in terms of average μg/seed. Effects of location between umbels, within umbels and within seeds on furanocoumarin content and relative composition were evaluated by one-way analysis of variance. Statistical analyses of proportional composition were performed on arcsine transformed proportions.

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