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PSORALENS IN SENESCING LEAVES OF *Ruta graveolens*

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Abstract—Concentrations of three furanocoumarins, psoralen, xanthotoxin, and bergapten, were measured on the surface and within mature whole leaves of two groups of *Ruta graveolens* L. late autumn plants, 2 and 6 years old, which contained green, yellow, and dry yellow leaves. Upper green leaves contained higher concentrations of these coumarins than lower green leaves, green leaves contained several times as much as yellow leaves, and dry leaves contained even smaller amounts than yellow ones. The dry yellow leaves contained only a very small percentage of furanocoumarins on the surface, suggesting that extrusion to the surface of yellow leaves was slower or had stopped, while loss from the surface continued. The loss of psoralen was the most dramatic in and on the dry leaves. Bergapten's ratio to the other coumarins increased during senescence. Xanthotoxin was always the predominant furanocoumarin in this species.

Key Words—*Ruta graveolens*, Rutaceae, rue, senescence, furanocoumarins, leaf surface, plant defense.

INTRODUCTION

Senescence, a normal process in a plant (Simon, 1967; Mothes, 1980; Janzen and Waterman, 1986) occurs during tissue ontogenesis (Wiermann, 1980), and is characterized by very complex processes differing from those in juvenile tissues (Woolhouse, 1967). Changes occur both in primary metabolism and structure (Malik, 1987; Fobel et al., 1987; Braber, 1980; Patra and Mishra, 1979) and in secondary metabolism (Mann, 1980; Haslam, 1985). Senescence coin-

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cides with a change in the action of hormones (Osborne, 1968, Audus 1972), which can be involved in regulation of secondary product metabolism (Ebel, 1979).

At the end of the normal senescence process, leaves dry out and fall to the ground, where leakage both from the leaf surface and its interior occurs, followed by decay. Because some secondary products do not undergo ready turnover (Margna and Vainjaero, 1981) and others do (Barz and Hoesel, 1979; Barz and Koester, 1981), it is important to know what compounds are retained in senescent and dry leaves, because such compounds can potentially enter the environment. Although some secondary metabolites appear to accumulate in plant tissues simply as waste products, others are metabolized and therefore do not accumulate. Some that do accumulate can be allelochemicals (Barz et al., 1985; Jacques et al., 1985) influencing hormone action (Hale and Orcutt, 1987) or serving as natural pest and disease control agents. Certain secondary products that have survived the senescing process may be released by the dead plants into the soil (Barz and Weltring, 1985).

Among products of secondary metabolism, furanocoumarins have long been known to act as growth inhibitors or growth retardants (Rodighiero, 1954; Shina-Roy and Chakraborty, 1976; Friedman et al., 1982). We have found psoralens (linear furanocoumarins) on the surface of rutaceous and umbelliferous plants (Zobel and Brown, 1990a), sometimes in amounts of micrograms per gram fresh weight within the green leaves of *Ruta graveolens* and on the leaf surface (Zobel and Brown, 1989). As with other compounds, these coumarins may pass into the soil both from living leaves on the plant (through the action of rain and other physical weathering processes) and dead ones on the ground. Because furanocoumarins have a broad spectrum of biological activity (Murray et al., 1982; Ivie, 1987a,b), the possibility of effects on the growth of neighboring plants cannot be excluded.

The behavior of coumarins during senescence and their possible role in that process have not yet been explored. In this study we have investigated the extent to which psoralens remain on and within the leaves during the process of senescence.

METHODS AND MATERIALS

Plant Material. R. graveolens L. plants, growing outdoors in the soil during the summer growing season of 1988, were used. Leaf samples of 2- and 6-year-old specimens, each containing about 20 shoots, each with about 30 leaves, were collected for analysis November 1, just before the autumn frosts. We wished to obtain all the necessary stages of leaf development on each plant and to avoid the influence of variations in the vegetative period that we had

observed in *Heracleum lanatum* (Zobel and Brown, 1990b), as well as changing environmental conditions, knowing that frost can cause a 100-fold increase in furanocoumarins. On the plant there were four distinct populations of leaves, all mature: group I, upper green, which were smaller in size; II, lower green, which were larger and older; III, lower yellow, which were still turgid, showing a decline in the amount of chlorophyll; and IV, the lowest, yellow, which had dried on the plant.

Two green leaves from each shoot were removed: the third from the shoot apex (called here "upper green") and the third green leaf from the bottom ("lower green"). The upper leaves were not new growth, but small-sized (2–4 cm) mature, "hard" leaves. Lower green leaves (7–12 cm) showed no signs of damage or yellowing. Below the green leaves on the shoot were yellowish leaves, and the lowest location had dry yellow leaves, collected as "lower yellow" and "lower dry" samples, respectively. Separate samples were collected from the 2- and 6-year-old plants, and each of the four different leaf samples, containing 12–14 leaves, was duplicated.

Analyses. The leaves of each sample were weighed as a group and surface coumarins removed from the surface by a brief dipping in almost-boiling water (Zobel and Brown, 1988). After extraction, the dry weights of these leaves were determined for comparison with those of the senescent leaves. Because there were two groups of leaves, dry and turgid, there were alternative ways of expressing concentrations, on the basis of either dry or fresh weight. For comparison with our previous papers, we chose to calculate on the basis of fresh weight, as the values for only one group of leaves (yellow, dry) then had to be adjusted. Groups III and IV were the most similar, because they developed at the beginning of the vegetative period when all the leaves were growing very actively and were of similar size. Thus calculation of fresh weight of group IV was done according to the equation:

$$\frac{\text{Fresh weight of group III}}{\text{Dry weight of group III}} = \frac{\text{Fresh weight of group IV}}{\text{Dry weight of group IV}}$$

The furanocoumarins in the extracts were purified and determined by previously described procedures (Thompson and Brown, 1984; Zobel and Brown, 1988). The error of the method was $\leq 10\%$.

RESULTS

Table 1 compares the sum of the concentrations (Σ values) of psoralen, xanthotoxin, and bergapten (P + X + B).

Whole Leaves. The whole leaves of the younger group of plants contained higher concentrations than the older: 40% (4200 vs. 2500) in the upper leaves

TABLE 1. CONCENTRATIONS[a] OF TOTAL FURANOCOUMARINS IN AND ON THE SURFACE OF *Ruta graveolens* PLANTS

	Younger plants (2-year-old)			Older plants (6-year-old)		
	$\Sigma P + X + B$[b]			$\Sigma P + X + B$		
Leaves	Whole leaf	On surface	On surface (%)	Whole leaf	On surface	On surface (%)
Upper, green[c]	4200 ± 100	830 ± 30	20	2500 ± 100	760 ± 40	30
Lower, green[c]	1400 ± 100	520 ± 50	37	980 ± 60	190 ± 10	19
Lower, yellow[c]	580 ± 10	130 ± 10	23	1100 ± 100	140 ± 10	13
Yellow, dry[c]	580 ± 40	25 ± 2	4.3	680 ± 40	20 ± 2	2.9
Yellow, dry[d]	70 ± 5	3 ± 0.5	4.3	76 ± 5	2.2 ± 0.2	2.9

[a] Mean of two samples.
[b] P: psoralen, X: xanthotoxin, B: bergapten.
[c] μg/g fresh weight.
[d] μg/g dry weight.

and 30% (1400 vs. 980) in the lower green leaves. In both age groups the upper leaves had over twice as much as did the lower leaves, in spite of the fact that all these leaves were mature, differing only in size and location on the shoot.

In the yellowish senescent leaves of the younger plants (becoming senescent but still not dry), the Σ value was about 40% of that of the lower green ones (580 vs. 1400). In the fully dry leaves, this value was still of similar magnitude, indicating that in the course of the drying stage of senescence there was little if any diminution of furanocoumarins. In the yellowish leaves of the older plants, the Σ value was much higher than in the young plants, but in the dry leaves the two values were comparable. In both groups of plants the old, dry leaves, the lowest on the plant, contained a much lower concentration than the younger, upper leaves and the lower, green leaves, although the difference was less pronounced in the older plants. In younger plants the upper green leaves contained over seven times, (and in older plants over three times) the concentration of furanocoumarins as dry ones.

Leaf Surface. In both younger and older plants the total surface concentrations showed similar tendencies, decreasing down the shoot in absolute amounts. Differences between upper green and dry leaves were even more pronounced than in the whole leaf: on the upper green leaves of both age groups, concentrations were >33 times (830/25 and 760/20) as high as on dry ones. In younger plants the percentage of furanocoumarins on the surface decreased toward the base of the shoot from 20% to 4.3%, and on the older plants from 30% to 2.9%. The lower percentage in the case of younger plants reflects the very high

concentration in the whole leaf (4200 μg/g). Except for the upper green leaves, the younger plants had a higher percentage on the surface than the older.

Changes in the individual furanocoumarins during the experiment are shown in Table 2.

Xanthotoxin. Xanthotoxin was the predominant furanocoumarin in each age group, both in the whole leaf and on the surface. Of the three coumarins, xanthotoxin had the highest percentage on the surface only in the case of the lower, yellow leaves of both age groups. In terms of absolute concentrations, the amounts on the surface followed similar trends to those described above for the sum of the three coumarins. In both groups only a small fraction remained on the surface of the lower dry leaves compared to the upper green leaves.

Psoralen. Psoralen concentrations, both in the whole green leaf and on the surface, were always lower than those of xanthotoxin, especially on the surface of the yellow leaves, and throughout the dry leaves. In the whole lower green leaves there were three (200/75) to 10 times (370/35) as much psoralen as in

TABLE 2. CONCENTRATION[a] OF PSORALENS ON SURFACE AND IN WHOLE LEAF OF *Ruta graveolens*

	On surface			In leaf			Whole leaf			On surface (%)		
Leaves	P[b]	X[b]	B[b]	P	X	B	P	X	B	P	X	B
Younger plants												
Upper	280	460	120	1100	1900	600	1300	2300	710			
green	240	440	120	900	1700	520	1200	2200	650	21	20	18
Lower	210	230	54	160	460	220	400	810	280			
green	170	300	70	200	540	180	340	730	240	51	35	23
Lower	3.4	110	30	35	190	240	40	300	270			
yellow	3.0	90	28	29	170	210	30	260	250	9.1	36	11
Lower dry	1.2	21	4.6	20	440	120	23	460	125			
	1.2	19	4.2	20	420	120	19	430	125	5.7	4.5	3.7
Older plants												
Upper	220	450	150	490	990	310	700	1500	470			
green	200	370	130	450	890	290	660	1300	410	31	30	32
Lower	60	85	55	150	500	160	210	610	230			
green	60	75	45	130	480	160	190	530	190	30	14	24
Lower	9.4	100	40	70	700	300	79	700	360			
yellow	9.0	88	34	58	600	300	69	580	330	12	15	11
Lower dry	1.5	14	5.9	30	460	180	30	480	180			
	1.5	12	5.0	26	460	160	27	460	170	5.1	2.3	3.1

[a] μg/g fresh weight; duplicate values shown.
[b] P: psoralen; X: xanthotoxin; B: bergapten.

the yellow, and seven (200/29) to 17 times (370/21) as much as in the dry; on the leaf surface of the younger plants this factor reached 160 (190/1.2).

Bergapten. Bergapten was always found in smaller concentrations than xanthotoxin. Compared to psoralen, it was lower in the upper green leaves, at a similar level in the lower green leaves, but much higher in both yellow and dry leaves. Smaller surface concentrations of bergapten were noted on both upper and lower green leaves, but on yellow and dry leaves the bergapten concentration was several times as high as that of psoralen. Despite lower absolute amounts, the percentage of bergapten on the surface was high—in most cases at least comparable to those of the other two.

The ratios of surface and whole-leaf concentrations of the three coumarins of the upper green leaves and the dry leaves of the same age group are compared in Table 3. This table shows the factors by which concentrations of furanocoumarins are lower in the yellow, dry leaves compared to the upper green, emphasizing the decline in furanocoumarin concentrations from the uppermost leaves to the dry ones at the base. The two groups showed similar tendencies: surface concentrations decreased more than those of the whole leaf, e.g., for younger plants, psoralens decreased on the surface 217 times, but only 60 times in the whole leaf; xanthotoxin 23 times vs 5; bergapten 27 vs 5. Younger plants showed higher ratios of decrease than older ones; the sharpest decrease was observed for psoralen, and the other two were similar. Psoralen appears more susceptible to changes in senescing leaves, showing ratios up to 12 times (60/5) those of xanthotoxin and bergapten.

Ratio of Psoralen to Xanthotoxin and Bergapten. Table 4 shows the proportions of the three furanocoumarins in the leaf and on the leaf surface, with the value for psoralen taken as unity. In both the younger and older plants the concentrations of xanthotoxin were higher than those of psoralen (i.e., >1), most notably in the yellow and dry leaves. Again in both groups, in almost all

TABLE 3. DIFFERENCES[a] IN CONCENTRATIONS OF FURANOCOUMARINS IN GREEN UPPER LEAVES AND DRY LEAVES OF *Ruta graveolens*

	Surface			Whole leaf		
Collection date	P[b]	X[b]	B[b]	P	X	B
November 1						
Younger plants	217	23	27	60	5	5
Older plants	140	32	26	23	3	3

[a] Concentrations of psoralens in and on upper green leaves/concentrations of psoralens in and on yellow dry leaves.
[b] P: psoralen, X: xanthotoxin, B: bergapten.

TABLE 4. RATIOS[a] OF CONCENTRATIONS OF FURANOCOUMARINS IN WHOLE LEAF AND ON SURFACE OF *Ruta graveolens* PLANTS

	Younger plants				Older Plants			
	Whole leaf		Surface		Whole leaf		Surface	
Leaves	X[b]	B[b]	X	B	X	B	X	B
Upper, green	1.8	0.54	1.7	0.46	2.0	0.65	2.0	0.67
Lower, green	2.1	0.71	1.4	0.33	5.9	1.0	1.3	0.83
Lower, yellow	8.0	7.4	31	9.0	8.6	4.6	10	4.0
Lower, dry	21	6.0	17	3.7	16	6.2	8.7	3.6

[a] Based on psoralen = 1.
[b] X: xanthotoxin, B: bergapten.

cases, the variations on the surface were greater than in the whole leaf. In the green leaves of the younger plants, bergapten was lower than psoralen, but in the yellow and dry leaves it was several times as high. In older plants, although bergapten was less in the upper leaves, there was at least as much in the lower, green leaves, and it was several times as high as psoralen in yellow or dry ones. On the surface, bergapten showed the same tendencies in the green leaves of both groups of plants, being lower than psoralen, but again it was much higher than psoralen in both yellow and dry leaves.

In yellow and dry leaves, both xanthotoxin and bergapten were higher than psoralen, but xanthotoxin was more so, by factors of ca. 8–30, both in the whole leaf and on the surface, in contrast to ca. 4–9 times for bergapten. Bergapten on green leaves, however, was in smaller absolute amounts than psoralen, as shown in Table 2. These findings point to very marked changes in concentrations and proportions of particular furanocoumarins during leaf aging.

DISCUSSION

The process of leaf aging is associated with changes in the physiology of the cells and, therefore, in the concentrations of the products stored in them. Senescence can be either a complex natural process, occurring over the course of the vegetative period, or may be caused by environmental changes. In view of the changes in furanocoumarin concentrations known to occur during the vegetative period in *Heracleum lanatum* (Zobel and Brown, 1990b), autumn leaves, all on the same plant, were chosen so that all the leaves would be mature without new growth, and the naturally senescing leaves would exhibit a range of stages simultaneously. In each of the two groups of plants examined, younger

and older, we distinguished four kinds of leaves, using as a marker their color and location on the shoot.

Younger and older plants tended to exhibit the same changes during senescence. Marked diminutions in furanocoumarin concentrations observed both in and on senescing leaves indicated lower production and extrusion rates during senescence. Changes of surface concentrations in each of these four groups of leaves showed the same tendencies: decreasing concentrations of both total furanocoumarins and each individual furanocoumarin in the whole leaf and, even more strikingly, on the surface. This suggests decreased extrusion as one possible explanation for the decline. Whether it is connected with the presumed need for less protection by leaves located lower on the plant must remain only speculation at this point.

The drop in the total concentration of the three furanocoumarins of aging leaves, in the whole leaf, and on the surface, was followed by a decrease in each individual concentration, but not in the same proportions. That of bergapten on the dry leaves was not so extreme as that of psoralen, whose concentrations decreased drastically by factors of >100. Psoralen was the most susceptible compound to changes due to senescence, both in the whole leaf and on the surface. Xanthotoxin always predominated in absolute amounts, but in the range of changes between the upper green leaves and dry ones it was comparable to that of bergapten. Bergapten might be termed a "senescing compound" because it was found in higher concentrations on and within aging callus cells (Zobel and Brown, 1991). We wish to emphasize the importance of visualizing changes in concentrations of particular coumarins as well as their ratios, in the knowledge that they can react synergistically (Berenbaum and Neal, 1985). Further investigations on the coexistence of other coumarins in the plant and on its surface, both qualitative and quantitative, are indicated.

In yellow, dry leaves there remained only a small fraction of furanocoumarins, which could eventually enter the soil, but the absolute amounts were quite substantial (580–680 μg/g fresh weight, or >70 μg/g dry weight). From the surface of the leaves, during the process of senescence, the loss was from 800 to 25 μg/g. More investigation is needed to measure the extent to which these compounds were washed by rain into the soil, vaporized into the air, or degraded chemically or biologically. Also needed is more study to evaluate the influence of changing environmental conditions on surface furanocoumarin concentrations and to distinguish these from genetically dependent changes such as natural physiological aging.

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