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LEAF WAX KETONES IN THE GENUS *COINCYA*

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Abstract—Leaf wax ketones in the genus *Coincya* vary between C_{22} and C_{31}, and is dominated by those with an odd number of carbon atoms. The most abundant ketones are C_{25}, C_{27} and C_{29}. The main one, C_{29}, ranges from 57.7% in *C. monensis* subsp. *cheiranthos* var. *setigera* to 21.2% in *C. monensis* subsp. *nevadensis*. A positive correlation between the content of C_{29} and the glaucous character was observed.

INTRODUCTION

The genus *Coincya* Rouy (Brassicaceae) is included in the tribe Brassiceae along with other genera of well-known commercial and nutritional value, such as *Brassica* and *Sinapis* [1]. There are very few reports regarding chemical studies of *Coincya*, these being on lipids from *Coincya* [2–8] and glucosinolate composition [9–11]. In this study, ketone composition of leaf waxes and its relationship with environmental conditions and morphological characteristics was assessed.

The ketones studied here constitute a class of compounds that form a homologous series with saturated, linear aliphatic chains; the position of the carbonyl group was not determined. In plants, they are not as common as alkanes or alcohols. In general, the range of variation is C_{25}–C_{33}, the main ones being C_{29} and C_{31} [12].

Coincya has a distribution centred in the Iberian peninsula (Spain and Portugal) where it is represented by 11 of the 14 taxa currently recognized [13]. It is a very complex taxonomic group with a high phenotypic variation, as well as a great number of intraspecific taxa and synonyms.

We have studied 10 of the 11 taxa present in the peninsula. In var. *recurvata*, populations that suffer special environmental factors (var. *recurvata* from Pirineos and Sintra) or with morphological differences (var. *recurvata* from Granada) have been treated separately in this study. The majority of the taxa are endemic to the peninsula with a very restricted distribution. Table 1 lists the studied taxa, habitat and glaucousness.

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RESULTS AND DISCUSSION

The ketone constituents account for 0.6–12.3% of the total leaf waxes in *Coincya* [4], compared to 36% in Brassica [14] where only C_{29} is present in significant amounts. However, the main ketones in *Coincya* contain from 22 to 31 carbon atoms (Table 2). This chain-length range does not differ much from that observed in other fractions of leaf waxes, such as hydrocarbons (22–31) [5] or alcohols (20–31) [6]. The series is dominated by ketones with an odd number of carbon atoms, as observed for hydrocarbons [5, 15], but not with the corresponding alcohols [6, 16]. For example, the percentage of odd ketones varies between 78.5 in subsp. *nevadensis* and 89.6 in var. *setigera*.

Coincya monensis subsp. *orophila*, one of the most abundant and widespread taxon, has 31% C_{29} ketone (Fig. 1), while the other taxon with a broad distribution, var. *recurvata* s.s., has 29.5% C_{29} ketone.

Glaucous character and high abundance of C_{29} ketones

The highest C_{29} ketone contents are observed in taxa and populations that possess glaucous leaves, suggesting a correlation between glaucousness and ketone content. The taxa with the highest content of C_{29} is var. *setigera* (57.7%). Other taxa with a high content of C_{29} ketones and high glaucousness is *C. rupestris* (Table 2). A comparable correlation has also been made between abundances of C_{29} hydrocarbons and glaucousness [5].

In *Coincya*, glaucousness does not appear to be related to water stress, as found in other plants [17].

Table 1. Habitat and level of glaucousness of *Coincya* species studied

Taxa	Special habitat and/or environmental factors	Glaucousness
C. transtagana	Arid, very low pluviosity	−−
C. longirostra	Semi-arid, low pluviosity	+
C. rupestris		
subsp. *rupestris*	Semi-arid, low pluviosity	++
subsp. *leptocarpa*	Semi-arid, low pluviosity	++
C. monensis		
subsp. *cheiranthos*		
var. *recurvata* s.s.		−−
var. *recurvata* from Pirineos	Alpine, low temperature	−−
var. *recurvata* from Sintra	Very high pluviosity and relative moisture	−−
var. *recurvata* from Granada		+++
var *johnstonii*	Sand dune taxon	−−
var. *setigera*	High pluviosity	+++
subsp. *orophila*		−−
subsp. *puberula*	High pluviosity	−−
subsp. *puberula* bordering with var. *setigera*	High pluviosity	++
subsp. *nevadensis*	Alpine, very low temperature	−−

−− Not glaucous; + slightly glaucous; ++ glaucous; +++ very glaucous.

Taxa that suffer the highest hydric pressure, *C. transtagana* (an arid taxon) and var. *johnstonii* (a sand dune taxon), do not show this character.

Ketone composition in populations of subsp. puberula *bordering with var.* setigera

Plants that belong morphologically to subsp. *puberula*, but border var. *setigera*, exhibit glaucousness, as well as a ketone composition intermediate between both taxa (Fig. 2). This may indicate that these populations represent an intermediate taxon between both taxa, perhaps as a result of hybridization.

Water availability and ketone composition

Smaller contents of C_{29} ketones are present in taxa and populations that support lower hydric pressures, due to the low temperature or to the high pluviosity. The taxon with the lowest percentage of C_{29} ketone is subsp. *nevadensis* (21.2%) where C_{27} is most abundant. In this taxon an increase of smaller, even numbered

Table 2. Leaf wax ketone composition in *Coincya*

Taxa	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	*n**
C. transtagana	0.4±0.1	1.2±0.4	2.4±0.2	15.5±0.3	3.3±0.2	32.2±2.2	5.0±0.1	38.1±1.0	0.8±0.3	1.4±0.7	2
C. longirostra	0.4±0.1	2.9±0.5	3.0±0.8	17.8±4.0	4.0±0.9	31.2±3.5	6.2±0.9	33.0±7.2	0.5±0.1	1.2±0.3	4
C. rupestris											
subsp. *rupestris*	0.3±0.0	2.6±0.0	1.6±0.0	9.0±0.0	2.8±0.0	25.2±0.0	6.8±0.0	48.9±0.0	0.9±0.0	1.9±0.0	1
subsp. *leptocarpa*	0.3±0.1	1.1±0.2	1.1±0.2	9.1±1.9	2.5±0.3	31.6±1.4	5.7±0.5	46.4±3.5	0.6±0.2	1.7±0.6	3
C. monensis											
subsp. *cheiranthos*											
var. *recurvata*	1.0±1.4	2.3±1.4	3.0±1.5	15.8±5.0	5.5±1.6	34.9±5.5	5.5±1.6	29.5±7.4	1.1±0.8	2.7±1.6	14
var. *recurvata* from Pirineos	0.5±0.2	1.7±0.5	3.4±1.2	20.5±6.8	4.8±0.7	35.2±3.5	4.5±0.6	27.0±9.8	0.7±0.3	1.7±0.8	6
var. *recurvata* from Sintra	0.6±0.4	2.0±1.7	3.6±1.0	26.8±7.3	4.0±1.2	29.5±0.7	3.2±0.9	26.5±9.6	1.1±0.0	2.9±0.4	2
var. *recurvata* from Granada	0.4±0.4	1.3±0.8	1.6±0.4	13.2±6.4	2.4±1.3	39.5±12.7	5.1±2.3	35.8±14.2	0.4±0.1	0.6±0.2	2
var. *setigera*	0.3±0.2	0.4±0.1	0.8±0.3	4.5±0.6	2.3±0.4	23.8±2.5	6.0±0.7	57.7±3.1	1.1±0.5	3.2±0.4	6
var. *johnstonii*	0.2±0.0	0.7±0.0	1.4±0.1	10.4±0.2	3.9±0.2	48.9±4.3	3.6±1.0	29.8±4.6	0.2±0.1	1.0±0.7	2
subsp. *orophilia*	0.9±0.6	2.9±0.9	4.6±2.0	20.5±6.5	4.2±1.3	26.6±5.5	5.5±1.8	31.0±7.1	1.1±0.6	2.7±1.8	15
subsp. *puberula*	1.0±0.4	4.1±1.7	3.8±0.8	21.2±1.1	3.9±0.6	29.0±2.6	8.1±4.3	26.2±3.0	0.7±0.3	2.0±0.9	3
subsp. *puberula* bordering with var. *setigera*	0.4±0.3	0.6±0.1	1.2±0.1	7.8±2.0	3.0±0.5	24.4±2.4	6.2±0.8	51.3±2.8	1.8±0.5	3.3±0.4	3
subsp. *nevadensis*	1.6±0.3	2.2±0.3	5.7±1.4	19.0±2.7	7.5±3.1	32.2±3.8	4.9±1.5	21.2±4.1	1.9±2.2	3.9±4.7	4

Results are expressed in percentages±s.d.
*Numbers of populations studied.

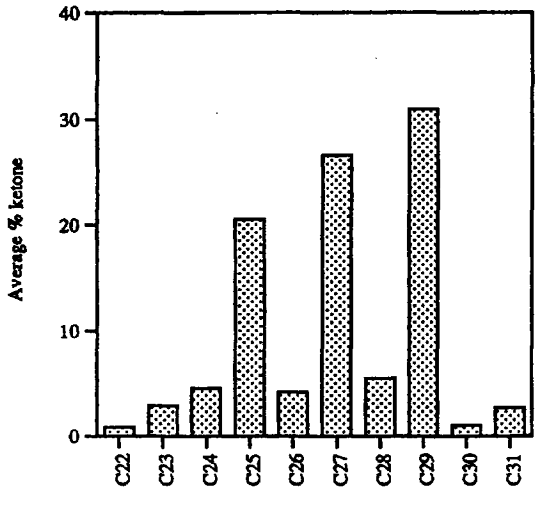

Fig. 1. Average ketone composition in *C. monensis* subsp. *orophila*.

ketones is also observed. Thus, these plants have the highest content of C_{26}, C_{24} and C_{22} ketones, and lowest odd/even relationship of the genera (78.5%/21.6%). The increased amount of shorter chain length ketones with lower fusion point and higher polarity could perhaps facilitate or at least not limit the passage of water through the wax layer. In var. *recurvata* from Pirineos and subsp. *puberula*, the average contents of C_{29} ketones are also below the average of the genera, with 27 and 26.2%, respectively.

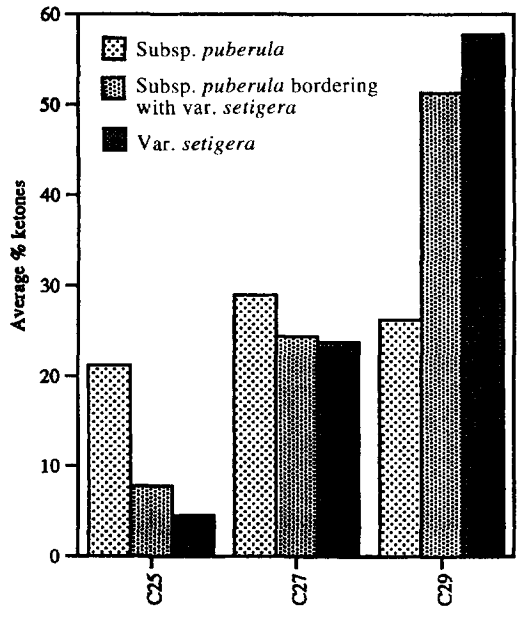

Fig. 2. Average ketone composition of C_{25}, C_{27} and C_{29} compounds in subsp. *puberula* var. *setigera* and populations of subsp. *puberula* bordering with var. *setigera*.

CONCLUSIONS

The ketone composition in *Coincya* shows a positive correlation between C_{29} content and glaucousness.

This character in *Coincya* is independent of the environment and appears in taxa that suffer very different environmental conditions, and not related taxonomically, such as var. *setigera* and *C. rupestris*.

In the wax fractions that form homologous series, with compounds of different chain lengths, i.e. hydrocarbons, ketones, alcohols and acids from esters, an agreement between the biosynthesis routes proposed and chain length of the compounds is observed. In this sense, ketone composition, with predominance of odd chain length molecules, agrees with the hypothesis that they are derived by oxidation from secondary alcohols which are derived from alkanes [18]. Also, hydrocarbons show a predominance of odd molecules since they are derived by decarbonylation of even numbered aldehydes [19, 20]. Although aldehydes are not found commonly in the waxes of plants, they represent an intermediate metabolite in the biosynthesis of hydrocarbons. Alcohols show a predominance of even numbered molecules, since they are derived by reduction of even numbered acyl CoA derivatives [21]. In the same way, even numbered acids from esters are derived by the hydrolysis of even numbered acyl CoA units.

EXPERIMENTAL

Plant material. Waxes were extracted from leaves taken from the medium zone of the basal rosette, from several plants in a population. The number of populations studied for every taxon is shown in Table 2. These populations are distributed in all of the Iberian peninsula, although some taxa have a very local distribution. Field collections were made in spring between 1989 and 1991. Voucher specimens of the populations studied are deposited in the Herbarium of the Department of Vegetal Biology and Ecology at the University of Seville (Spain).

Isolation of epicuticular wax. Leaves were soaked for 30 sec in $CHCl_3$ at room temp. The $CHCl_3$ extract was filtered and concd by rotary evapn.

Purification of wax ketones. Extracted wax was applied to silica gel 60-G TLC plates (0.5 mm thick) and the plates were developed in hexane–Et_2O–HOAc (70:30:1). Ketones were detected with I_2 vapour and identified with the appropriate standards. Ketones were recovered from the adsorbent with Et_2O and analysed by GC. It was assumed that all components had similar

FID responses. Integration of peak areas was effected with an electronic integrator.

Gas chromatography. The instrument was fitted with a FID and a capillary column HP-5 (5% Ph Me silicon, 25 m × 0.32 mm). The injection and detection temps were maintained at 275°. The column temp. was held at 250°.

Peak identification. The components of a population of *C. monensis* susbsp. *cheiranthos* var. *recurvata* were identified by GC-MS (data not shown). The components of the other species were identified by R_t comparison.

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