ORIGINAL PAPER

Chemical Composition and Nutritional Characteristics of the Seed Oil of Wild Lathyrus, Lens and Pisum Species from Southern Spain

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Received: 21 October 2008 / Revised: 9 January 2009 / Accepted: 13 January 2009 / Published online: 4 February 2009 © AOCS 2009

Abstract The fatty acid composition of the seed oil of 19 wild legume species from southern Spain was analyzed by gas chromatography. The main seed oil fatty acids ranged from $C_{14:0}$ to $C_{20:0}$. Among unsaturated fatty acids, the most abundant were linoleic, oleic and linolenic acids, except for Lathyrus angulatus, L. aphaca, L. clymenum, L. sphaericus and L. nigricans where $C_{18:3}$ contents were higher than $C_{18:1}$ contents. Palmitic acid was the most abundant saturated acid in studied species, ranging from 11.6% in Lathyrus sativus to 19.3% in Lens nigricans. All studied species showed higher amounts of total unsaturated fatty acids than saturated ones. Among studied species, the ω 6/ ω 3 ratio was variable, ranging from 2.0% in *L. nigri*cans to 13.8% in L. sativus, there being eight species in which the ω 6/ ω 3 ratio was below 5. The fatty acids observed in these plants supports the use of these plants as a source of important dietary lipids.

Keywords Lathyrus · Lens · Pisum · Fatty acids · Seed oils · Legumes

Introduction

Legumes are, together with cereals, the main plant sources of proteins in human diets. In particular, legumes have a high content of good quality proteins [[1\]](#page-5-0), playing an

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important role in the nutrition of many countries, especially in developing ones. However, legume intake has decreased in recent decades in many Western countries [\[2](#page-5-0)], despite the fact that both the chemical and the nutritional composition of some legumes, such as beans, also include dietary fibre, carbohydrates and a low content of saturated fats.

In recent years, health benefits of legumes consumption have been recognized and related to legume components, such as fibre, proteins or some minor compounds, such as certain lipids, polyphenols or bioactive peptides [[3\]](#page-5-0). These health promoting effects have been related to the prevention of diseases like diabetes mellitus, coronary heart diseases or colon cancer [[4\]](#page-5-0). In particular, the cardio-protective effect of pulses may be due to the synergistic effect of bioactive peptides or free fatty acids.

Fats and oils are an important component of the human diet, both as a source of energy and as carbon building blocks. Major sources of plant oils in the human diet are soy, canola, palm, peanut and sunflower. While oil from plants such as palm and coconut are rich in short chain saturated fatty acids, those of soybean and canola are rich in the more healthy polyunsaturated fatty acids. With a few exceptions, such as soybean and peanut, legumes have a low content of seed lipids [\[5](#page-5-0)], but have a high proportion of unsaturated fatty acids, which are of interest from a nutritional and functional point of view [\[6](#page-5-0)]. The main fatty acids in legume seed lipids are palmitic, oleic, linoleic and linolenic acids, with variable contents depending on the studied species [\[7](#page-6-0)]. In recent years, unsaturated and polyunsaturated fatty acids are the object of increasing interest due to their health promoting activity related to the observed reduction of cardiovascular diseases associated with their ingestion [\[8](#page-6-0)]. For example, the regular consumption of foods rich in ω -3 long chain polyunsaturated fatty acids has multiple positive health benefits. Some

authors have suggested that the capacity of legumes to decrease the glycemic index and blood cholesterol is due to their favourable seed fatty acid composition [[9\]](#page-6-0). Therefore, although present in low amounts, the fatty acids of the seed oil of legumes may have health promoting effects beyond their nutritional characteristics.

Lathyrus, Lens and Pisum genera belong to the tribe Fabeae Rchb. [[10\]](#page-6-0). These plants, as in the case of many other legumes, are adapted to grow under drought stress conditions and they can grow on poor soils due to their high biological nitrogen fixation rate [\[11](#page-6-0)]. Some of the species studied are broadly cultivated, such as *Pisum sat*ivum and Lens culinaris for human consumption, whereas some Lathyrus species are locally cultivated as forage crops for livestock feeding [\[12](#page-6-0)].

In these species, as in the majority of legumes, oil seed contents are low. For example, in different cultivars of P. sativum the oil seed content ranged from 0.76% to 3.95% [[13\]](#page-6-0). Similar contents were observed for L. culinaris (3.16%) [\[14](#page-6-0)], Lathyrus maritimus (1.1%) and Lathyrus sativus (1.2–1.3%) [[15\]](#page-6-0).

The fatty acid composition of cultivated species such as Lens culinaris [[14\]](#page-6-0) and Pisum sativum [\[15](#page-6-0)] has been studied previously, together with some Lathyrus species, such as L. sativus [\[16](#page-6-0)], L. aphaca [\[17\]](#page-6-0), L. annuus, L. hirsutus, L. pratensis, L. setifolius and L. sphaericus [[18–20\]](#page-6-0).

In the last few decades a large amount of the world phytodiversity has been lost. The reason is that farmers have substituted local varieties and species with commercial varieties which have a high yield and a genetic uniformity. However, the conservation of biodiversity may be an important factor for their development, especially in developing countries. To recover and maintain this biodiversity, a diversification of plant species is necessary, and this can be achieved by increasing our knowledge of local plants. In the present work, 19 species from three taxonomically related genera have been studied. Wild populations of these species were collected in Southern Spain. To our knowledge, this is the first time that wild populations of the selected species have been studied from a seed oil fatty acid composition point of view. The aim of the present research was to analyze and compare the fatty acid composition of selected species, in order to determine whether they show a more favourable seed oil composition compared with commercial varieties from both the nutritional and functional point of view.

Materials and Methods

Material

Fully matured seed samples were collected from wild populations located in Andalusia (southern Spain). The seeds were collected from ten specimens in a given population and stored at -20 °C until the fatty acid composition of the seed oil was determined. Fatty acids standards were purchased from Sigma (#1892, #1894, #1898) (Tres Cantos, Madrid, Spain). All other reagents were of analytical grade.

Determination of Fatty Acid Composition

Fatty acid composition of seed oils was determined by gas chromatography as methyl esters according to Garcés and Mancha [\[21](#page-6-0)] with modifications. Seeds were ground with a domestic blender (190 W power) (Moulinex, Barcelona, Spain). About 1 mL of methylathion solution (methanol, 39%; sulphuric acid, 5%; dimethoxipropane, 5%; toluene, 2%) and heptane (1 mL) were added to 50 mg of seed flour and incubated at 85° C for 50 min. The upper phase (1 mL) was taken to dryness under nitrogen and redissolved in 50 μ L of heptane. About 2 μ L of this solution were taken for the analysis of fatty acid methyl esters by gas chromatography. Previously seed oil from one sample of Lens culinaris was extracted with hexane in a soxhlet extractor for 9 h. Analysis of fatty acid methyl esters of this oil yielded same composition as the direct analysis of the seed flour as described by Garcés and Mancha $[21]$ $[21]$.

An HP 5890 series II gas chromatograph equipped with a HP Carbowax 20 M capillary column (25 m length and 0.2 mm ID) was employed. Hydrogen was used as carrier gas for the gas chromatography analysis at a pressure of 2 kg/cm². Temperatures of injector, detector and oven were 225 °C, 250 °C and 170 °C, respectively. Fatty acids methyl esters were identified by comparison with standards.

Statistical Analysis

Results are expressed as the mean values \pm standard deviation of several samples except for species with only one population. The data were statistically analyzed by one way analysis of variance (ANOVA). Means were compared by Tukey's test; significance was accepted at 5% level $(p \le 0.05)$. Cluster analysis of different taxa was performed using PRIMER-pc program, employing the Bray– Curtis index of dissimilarity [\[22](#page-6-0)]. The dissimilarity index was transformed to the index of similarity $(1 -$ dissimilarity index \times 100).

Result and Discussion

The main seed oil fatty acids in the studied legumes ranged from $C_{14:0}$ to $C_{20:0}$ with a predominance of unsaturated fatty acids of the series C_{18} (Fig. [1\)](#page-2-0). Among saturated fatty acids, palmitic acid $(C_{16:0})$ was the most abundant in all

Fig. 1 Average fatty acid composition in studied Lathyrus, Lens and Pisum species. Results are the average of the different species studied in each genera \pm standard deviation, except for *Pisum* where the average \pm standard deviation correspond to the different P. sativum populations studied

studied species with contents ranging from 11.6% in Lathyrus sativus to 19.3% in Lens nigricans (Table [1](#page-3-0)). Significant differences ($p < 0.001$) in palmitic acid contents were observed among L. nigricans and seven species of Lathyrus with lesser amounts of this acid (L. amphicarpos, L. angulatus, L. annuus, L. ochrus, L. sativus, L. setifolius and L. tingitanus). Another saturated fatty acid relatively abundant in legumes is stearic acid $(C_{18:0})$. Among studied species, the highest content of this fatty acid was observed in L. sativus with 9.1%, which shows significant differences ($p \le 0.001$) with respect to most of the remaining species (Table [1](#page-3-0)). A negative correlation between the $C_{16:0}$ and $C_{18:0}$ fatty acids contents was observed. Therefore, species with a low $C_{18:0}$ content tend to have a higher amount of $C_{16:0}$ $C_{16:0}$ $C_{16:0}$ (Table 1). This is probably because $C_{16:0}$ is the biochemical substrate for the biosynthesis of $C_{18:0}$. Consumption of foods rich in saturated fatty acids from 12 to 16 carbon lengths is positively associated with low-density lipoprotein and cardiovascular disorders, whereas $C_{18:0}$ is considered neutral in this respect $[23]$ $[23]$. Thus, $C_{18:0}$ is less hypercholesterolemic than $C_{16:0}$ [\[24](#page-6-0)]. Therefore, species with a low ratio $C_{14:0} + C_{15:0} + C_{16:0}/C_{18:0}$ would be more appropriate for human nutrition. The lowest ratio was observed in some species, which have been grown traditionally (*L. sativus* and L. ochrus), and in two of the nine non cultivated Lathyrus species (L. amphicarpos and L. setifolius). The highest ratio was observed in two Lens species (L. culinaris and L. nigricans), the former being usually used for human consumption (Table [2](#page-4-0)). Others saturated fatty acids, such as $C_{14:0}$, $C_{15:0}$ and $C_{20:0}$, were found in lesser concentrations and with similar values in all species examined.

Among unsaturated fatty acids, the most abundant in decreasing order were linoleic acid $(C_{18:2})$, oleic acid $(C_{18:1})$ and linolenic acid $(C_{18:3})$ (Table [1\)](#page-3-0), except for *Lathyrus* angulatus, L. aphaca, L. clymenum, L. sphaericus and Lens nigricans in which $C_{18:3}$ values were higher than $C_{18:1}$ contents. The C_{18:2} contents ranged from 38.0% in L. nigricans to 60.1% in Lathyrus cicera. The $C_{18:2}$ content in the latter species was significant different ($p \le 0.001$) from those observed in L. annuus, L. latifolius, L. nigricans and *P. sativum,* which showed $C_{18:2}$ percentages below 42%.

Regarding $C_{18:1}$, the highest percentages were observed in L. latifolius with 28.8%. Seven of the studied species had $C_{18:1}$ contents significantly lower ($p \le 0.001$) than those reported for L. latifolius. In most of the species studied, the quantity of $C_{18:1}$ was lower than $C_{18:2}$, and therefore the $C_{18:1}/C_{18:2}$ ratio is low (Table [2\)](#page-4-0), as with other legumes [\[15](#page-6-0), [20](#page-6-0)]. A negative correlation ($r^2 = 0.61$) between these two fatty acids was observed, which supports previous findings on *Lathyrus* [\[18](#page-6-0)], probably because $C_{18:1}$ is the substrate for the biosynthesis of $C_{18:2}$.

The $C_{18:3}$ contents ranged from 4.0% in *Lathyrus sativus* to 18.9% in Lens nigricans. The highest content of $C_{18:3}$ was observed in L. nigricans (18.9%), although average values were usually lower. Thus, $C_{18:3}$ content in L. nigricans was significantly different ($p \le 0.001$) with respect to all the species, except for Lathyrus filiformis and Lens lamottei.

All studied species had higher contents of total unsaturated fatty acids (TUFAs) than saturated ones (TSFAs) (Table [2\)](#page-4-0). These results are in accordance with those obtained by other authors in species of these genera [\[15–18](#page-6-0), [20](#page-6-0)] and in other legumes [\[6](#page-5-0)]. TSFAs contents oscillate between 21.3% in Lens culinaris and 31.2% in Lathyrus sphaericus (Table [2](#page-4-0)). Significant differences were observed between L. sphaericus, the species with the highest TSFAs contents, and L. culinaris, P. sativum, Lathyrus clymenum, L. latifolius, L. ochrus, L. sativus, L. setifolius and L. tingitanus. Therefore, L. sphaericus possess the lowest amount of TUFAs (68.8%), which significantly differs from the above mentioned taxa.

Although L. culinaris possesses the highest ratio $C_{14:0} + C_{15:0} + C_{16:0}/C_{18:0}$, it is also the species with the lowest TSFA contents. Lathyrus sativus, the species with the lowest $C_{14:0} + C_{15:0} + C_{16:0}/C_{18:0}$ ratio, also had below average TSFA with 23.6%. Other species with a low $C_{14:0} + C_{15:0} + C_{16:0}/C_{18:0}$ ratio and low TSFA contents were Lathyrus ochrus and Lathyrus setifolius. In contrast, other species with a low C_{14:0} + C_{15:0} + C_{16:0}/C_{18:0} ratio have high TSFA contents, as is the case with Lathyrus amphicarpos.

Polyunsaturated fatty acids such as $C_{18:2}$ and $C_{18:3}$ not only contribute to a healthy diet but are also essential fatty acids for humans. Considering the negative effects on

Table 1 Fatty acid composition (%) of the seed oil of studied Lathyrus, Lens and Pisum species (mean \pm s.e.) **Table 1** Fatty acid composition (%) of the seed oil of studied *Lathyrus*, *Lens and Pisum* species (mean \pm s.e.)

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n*

 number of populations studied * $p\lt 0.05$, ** $p\lt 0.01$, *** $p\lt 0.001$

Table 2 Different parameters based on the fatty acid composition of the seed oil of studied Lathyrus, Lens and Pisum species

Species	\boldsymbol{n}	$C_{14:0}$ + $C_{15:0}$ + $C_{16:0}/C_{18:0}$	TUFAs**	TSFAs**	TUFA _s / TSFAs	$PUFAs***$	MUFAs***	PUFA _s / MUFAs	$C_{18:1}/$ $C_{18:2}$	ω 6/ ω 3
Lathyrus amphicarpos	3	2.0	72.2 ± 0.2^{ab}	27.8 ± 0.2^{ab}	2.6	$59.2\,\pm\,4.2^\mathrm{abc}$	$13.0 \pm 4.0^{\text{abcd}}$	4.5	0.2	7.7
Lathyrus angulatus	$\overline{4}$	3.8	$73 \pm 1.8^{\rm ab}$	27.0 ± 1.8^{ab}	2.7	$61.6 \pm 2.4^{\rm abc}$	$11.4 \pm 1.6^{\rm abc}$	5.4	0.2	4.6
Lathyrus annuus	6	4.6	$73.3 \pm 1.6^{\rm ab}$	26.7 ± 1.6^{ab}	2.7	53.6 \pm 1.8 ^{ab}	19.7 ± 2.3^{bcd}	2.7	0.5	3.9
Lathyrus aphaca	τ	4.5	$71.3 \pm 0.6^{\rm ab}$	$28.7 \pm 0.6^{\rm ab}$	2.5	$65.5 \pm 1.1^{\rm bc}$	$5.8 \pm 1.1^{\rm a}$	11.3	0.09	6.6
Lathyrus cicera	$\overline{7}$	3.3	72.2 ± 2.2^{ab}	27.8 ± 2.2^{ab}	2.6	$59.7 \pm 2.6^{\rm abc}$	$12.5 \pm 1.9^{\rm abc}$	4.8	0.2	8.5
Lathyrus clymenum	$\overline{7}$	3.7	74.5 ± 1.9^b	$25.5 \pm 1.9^{\rm a}$	2.9	$67.9 \pm 2.0^{\circ}$	6.6 ± 1.4^{ab}	10.2	0.1	7.7
Lathyrus filiformis	$\mathbf{1}$	4.0	75.0 ^{ab}	25.0 ^{ab}	3.0	52.1 ^{abc}	22.9 ^{abcd}	2.3	0.5	3.3
Lathyrus hirsutus	3	4.9	72.3 ± 3.6^{ab}	27.7 ± 3.6^{ab}	2.6	$55.2 \pm 5.6^{\rm abc}$	17.1 ± 7.2 ^{abcd}	3.2	0.3	6.8
Lathyrus latifolius	5	6.0	$74.9 \pm 2.9^{\rm b}$	$25.1 \pm 2.9^{\rm a}$	3.0	45.1 ± 13.6^a	$29.8 \pm 10.9^{\rm d}$	1.5	0.7	8.3
Lathyrus ochrus	$\overline{4}$	2.6	75.1 ± 3.2^b	$24.9 \pm 3.2^{\rm a}$	3.0	$61.0 \pm 8.6^{\rm abc}$	14.1 ± 6.2 ^{abcd}	4.3	0.2	9.9
Lathyrus pratensis	$\overline{4}$	4.4	74.3 ± 2.4^{ab}	25.7 ± 2.4^{ab}	2.9	$61.0 \pm 2.4^{\rm abc}$	$13.3 \pm 4.4^{\text{abc}}$	4.6	0.2	6.7
Lathyrus sativus	2	1.5	76.4 ± 0.9^b	$23.6 \pm 0.9^{\rm a}$	3.2	$58.5 \pm 1.9^{\rm abc}$	$17.9 \pm 1.1^{\text{abcd}}$	3.3	0.3	13.8
Lathyrus setifolius	3	2.5	74.8 ± 1.0^b	$25.2 \pm 1.0^{\rm a}$	3.0	$61.4 \pm 1.5^{\rm abc}$	$13.4 \pm 1.0^{\rm abc}$	4.6	0.2	7.2
Lathyrus sphaericus	5	3.1	68.8 ± 2.6^a	31.2 ± 2.6^b	2.2	$61.0 \pm 2.2^{\rm bc}$	7.8 ± 1.4^{ab}	7.8	0.2	4.6
Lathyrus tingitanus	$\overline{7}$	2.3	$73.9 \pm 1.5^{\rm b}$	$26.1 \pm 1.5^{\rm a}$	2.8	$56.0 \pm 6.9^{\rm abc}$	17.9 ± 7.2 ^{abcd}	3.1	0.4	7.1
Lens culinaris	$\mathbf{1}$	7.8	78.7^{b}	$21.3\pm^{a}$	3.7	52.0 ^{abc}	$26.7^{\rm abcd}$	1.9	0.6	4.8
Lens lamottei		4.9	76.4 ^{ab}	$23.5\pm^{ab}$	3.2	58.7abc	17.7 ^{abcd}	3.3	0.4	3.1
Lens nigricans	3	7.0	73.7 ± 0.2^{ab}	26.3 ± 02^{ab}	2.8	$57.0 \pm 3.7^{\rm abc}$	$16.7 \pm 3.9^{\text{abcd}}$	3.4	0.4	2.0
Pisum sativum	$\overline{4}$	3.2	75.9 ± 1.6^b	$24.1 \pm 1.6^{\rm a}$	3.2	50.3 ± 8.9^{ab}	25.6 ± 9.9 cd	1.9	0.6	4.9

Data are expressed as the average \pm standard deviation. Different small letters indicate significant differences between values in the same column (Tukey's test)

n number of populations studied

TUFAs total unsaturated fatty acids

TSFAs total saturated fatty acids

TUFAs/TSFAs total unsaturated fatty acids/saturated fatty acids ratio

PUFAs total polyunsaturated fatty acids

MUFAs monounsaturated fatty acids

PUFAs/MUFAs polyunsaturated fatty acids/monounsaturated fatty acids ratio

 ω 6/ ω 3 linoleic acid/linolenic acid ratio

** $p < 0.01$, *** $p < 0.001$

human health of saturated fatty acids, the FAO [\[25](#page-6-0)] recommend that the relation TUFAs/TSFAs should be between 0.84% and 1.16%. This ratio ranged from 2.2% in Lathyrus sphaericus to 3.7% Lens culinaris (Table 2), which are above the recommendations established by the FAO.

In addition to the total amounts of unsaturated fatty acids it is also important to establish the relative proportion of each fatty class. Thus, a diet rich exclusively in polyunsaturated fatty acids (PUFA) is not recommended if the proportion between ω -6 and ω -3 fatty acids is not balanced [\[26](#page-6-0)]. $C_{18:2}$ and $C_{18:3}$ are the precursors of eicosanoids (prostaglandins, thromboxanes, and leukotrienes) with opposite effects in the human body. Eicosanoids derived from $C_{18:2}$, such as arachidonic acid, increase the risk of cardiovascular diseases [\[27](#page-6-0)]. In contrast, eicosapentaenoic

and docosahexaenoic acids derived from $C_{18:3}$ have opposite effects. Thus, the hypolipidemic, antithrombotic, and antiinflammatory effects of ω 3 fatty acids have been extensively reported [[28\]](#page-6-0). In this regard, research suggests that a high ω 6/ ω 3 ratio may contribute to an increase in cardiovascular diseases [\[29](#page-6-0)]. According to the FAO [\[30](#page-6-0)], this ratio should be between 5/1 and 10/1, although some authors suggest that a 4/1 proportion is better, since an increase in $C_{18:3}$ improves the health of people suffering from asthma or arthritis [\[26](#page-6-0), [31](#page-6-0)]. The optimal proportion may also vary in function of the severity of the illness and the genetic predisposition $[26]$ $[26]$. Among the studied species, the ω 6/ ω 3 ratio ranged from 2 in L. nigricans to 13.8 in L. sativus (Table 2). Eight species had an ω 6/ ω 3 ratio below 5 and therefore a more balanced composition of polyunsaturated fatty acids. Thus, although Lathyrus

Fig. 2 Clustering based on the seed oil fatty acid composition of studied legumes, according to the Bray–Curtis similarity index $(1 -$ dissimilarity index \times 100)

sativus showed a good composition with respect to the TSFA contents and $C_{14:0} + C_{15:0} + C_{16:0} / C_{18:0}$ proportion, it had the worst ω 6/ ω 3 ratio.

The analysis of similarity shows that all species have an affinity higher than 82% based on the fatty acid composition of the seed oil (Fig. 2). However, the taxa are divided into two well-defined groups. Group A includes P. sativum, the genus Lens and Lathyrus latifolius, L. annuus and L. filiformis. In this group, L. culinaris, P. sativum and L. latifolius show an affinity of over 90%. Group B includes the remainder of the studied Lathyrus. In this group L. aphaca and L. clymenum show the highest dissimilarity. Both species have the lowest and the highest proportions of $C_{18:1}$ and $C_{18:2}$ respectively, and therefore the $C_{18:1}/C_{18:2}$ parameter is the lowest of studied species and the proportion PUFAs/MUFAs is the highest, being over 10 in both species (Table [2\)](#page-4-0). Although in other cases fatty acid composition has been taxonomically useful to resolve species, in this case it fail to resolve the studied genera into separated clusters.

Dietary fat is a major factor in determining the longterm fatty acid composition of adipose tissue. Therefore, the fat composition of the diet rather than the amount of fat intake is more important to determine the risk factors for heart disease. The role of $C_{18:3}$ in human nutrition is important after long-term dietary intake. Thus, although most legumes act to displace fat from the diet due to their low oil contents, the good balance in the proportion TSFA/ TUFA and ω -6/ ω -3 fatty acids of the studied legumes would, in the long term, help to correct this current balance in the western diet.

An advantage of the intake of $C_{18:3}$ over ω -3 rich fish oils is the intake of the associated amounts of vitamin E absent from fish oils. Besides, it has been observed that the processing and cooking of legumes do not affect the fatty acid composition, while antinutritional compounds present in legumes are removed or degraded [[32,](#page-6-0) [33\]](#page-6-0).

Conclusion

Most of the studied species had a good fatty acid composition according to the FAO requirements. This composition is in accordance with the plant nature of the samples, with a high content of unsaturated acids and a good proportion of ω 6/ ω 3. Moreover, some species cultivated locally have a fatty acid composition as good as that observed in widely grown pulses, like P. sativum. Thus, the good fatty acid composition, together with the high nutritional quality of other components of these pulses, like proteins, may be useful for the reevaluation of these plants and the extension of their cultivars. This reevaluation will result in conservation of phytodiversity and better human nutrition.

Acknowledgments This work was supported by grant AGR-711 from the Junta de Andalucía (Spain). Thanks are due to Alvaro Villanueva and Carlos de la Osa for technical assistance.

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