

## Possible Involvement of Gibberellins on Leaf Expansion and Carbohydrate Accumulation in Citrus

### Mögliche Rolle der Gibberelline bei Wachstum und Kohlenhydratspeicherung in Citrusblättern

Carmen Martínez-Cortina, Amparo Sanz and J. L. Guardiola  
(Departamento de Biología Vegetal. Universidad de Valencia, Spain)

#### Introduction

During the spring flush of growth both vegetative shoots and inflorescences are formed in *Citrus*. Some of these inflorescences bear both flowers and leaves (GOLDSCHMIDT and MONSELISE 1972). The leaves from leafy inflorescences are smaller in size (GUARDIOLA et al. 1984) and differ in composition, both organic (SANZ et al. 1987b) and mineral (EMBLETON et al. 1973) as compared to leaves from vegetative sprouts. Some of these differences are related to the competition of nearby developing flowers and fruitlets, but a different type of regulation may also be involved. Early removal of developing reproductive organs had no effect on leaf growth (SANZ et al. 1987a). Further, inflorescence leaves show during June drop a transient accumulation of starch. This accumulation cannot be explained by a source-sink relationship, and was prevented by removal of the developing fruitlet (SANZ et al. 1987b).

Since the effect of the developing fruitlets on starch accumulation could be mimicked by GA<sub>3</sub> application, involvement of hormonal regulation was suggested (SANZ et al. 1987a). To prove this hypothesis, GA-like activity in vegetative and inflorescence leaves was measured from leaf expansion until the end of June drop, and the effect of

fruitlet removal on GA levels in leaves was determined. These GA-levels have been related to differences in leaf growth and in the accumulation of carbohydrates in leaves.

#### Materials and Methods

##### *Flower removal experiments*

On 16-year-old orange trees (*Citrus sinensis* L. Osbeck, cv. Washington Navel) grafted on sour orange (*Citrus aurantium* L.) leaf development in vegetative shoots and leafy inflorescences were compared. A sufficient number of both vegetative sprouts and leafy single-flowered inflorescences were tagged shortly after bud sprouting. In half of the leafy inflorescences the terminal flower bud was manually removed 23 days before bloom, during the early stages of leaf lamina expansion. The mean leaf surface at that time was 3,25 cm<sup>2</sup> as compared to a final value of 15 cm<sup>2</sup>. The different marked shoots were allocated on the same tree with less than 40 shoots tagged per tree. A sample consisted of 50 sprouts picked at random and was kept under refrigeration (0–4°C). The total leaf area was determined with a portable area meter (Li-Cor, Lincoln, NE, USA), and leaves were lyophilized and stored at –20°C.

*Gibberellin applications*

The same layout was used in a separate experiment to study the effect of GA<sub>3</sub> on leaf development in deblossomed inflorescences. These deblossomed inflorescences were immersed for 5–10 seconds in a GA<sub>3</sub> solution (25 mg l<sup>-1</sup>) with a wetting agent (0.1 % nonyl-phenyl polyethylene glycol ether) after flower bud removal and repeated 25 days later. Gibberellins were extracted and partially purified as described by GARCÍA-LUIS et al. (1985). This procedure included solvent partitions at pH 3 and 8, a purification through a PVP column to remove phenolics, and paper chromatography using Whatman 3MM paper developed in an ascending way with a mixture of isopropanol : ammonia : water (80 : 0.1 : 19.9 v/v), prior to activity estimation by the Tan-ginbozu dwarf rice bioassay. Total metabolizable carbohydrates (starch plus soluble sugars) were determined after exhaustive extraction of the powdered material in succession with 80 % ethanol and 35 % HClO<sub>4</sub>, as described by MCCREADY et al. (1950).

**Results**

*Leaf growth*

At the end of leaf expansion, inflorescence leaves had a smaller dry weight and area than vegetative ones (Fig. 1). Differences in the growth rate of leaves rather than in the duration of the growth period caused these differences in leaf area since leaf expansion ceased shortly after flower opening. The initial increase in dry weight of the leaves was closely related to the increase in leaf area. It continued at a smaller rate after the completion of leaf expansion, but the specific leaf area, i. e. ratio of leaf area to total leaf dry weight, was identical for vegetative and inflorescence leaves throughout the experiment. Differences in leaf dry weight and area were unrelated to the presence of the developing flowers and fruitlets. Early removal of developing flower buds did not affect these parameters (Fig. 1).

*Carbohydrate contents*

During June drop, inflorescence leaves accumulated more metabolizable carbohydrates, i. e. starch plus

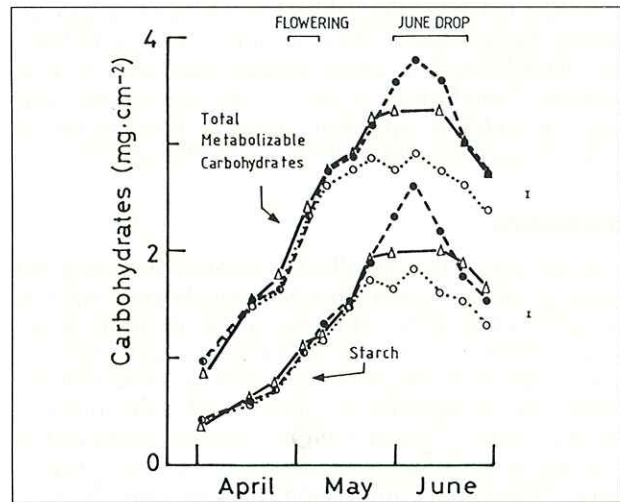


Fig. 2. Changes in starch and total metabolizable carbohydrates in leaves from vegetative shoots (Δ), intact (●) and deblossomed inflorescences (○). S.E. are given as vertical bars.

*Zeitliche Veränderungen des Gehaltes an Stärke und aller umwandelbarer Kohlenhydrate in Laubblättern (Δ), Blättern des Blütenstandes (●) und Blättern des Blütenstandes von denen die Blüten entfernt worden waren (○). Senkrechter Stab: Standardabweichung.*

ethanol-soluble sugars, than vegetative ones (Fig. 2). This effect was due to a transient accumulation of starch, while differences in soluble sugar contents were non significant (data not shown), and was directly related to the presence of the developing fruitlets.

*Effect of exogenous GA<sub>3</sub> on leaf growth and carbohydrate contents*

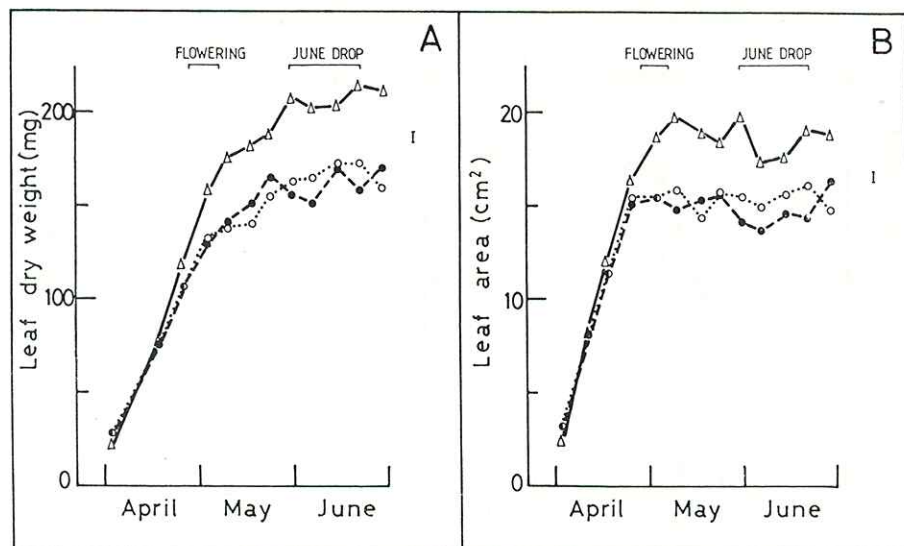
Application of GA<sub>3</sub> to deblossomed inflorescences during the early stages of leaf development enhanced leaf expansion and increased the dry weight of leaves (Fig. 3). The GA<sub>3</sub>-treated leaves accumulated more metabolizable carbohydrates than leaves in untreated inflorescences.

*Endogenous gibberellins*

Overall, total GA<sub>3</sub>-like activity was highest in inflorescence leaves during June drop (Fig. 4). The increase in

Fig. 1. Time-course changes in dry weight (A) and area (B) in leaves from vegetative shoots (Δ), intact (●) and deblossomed inflorescences (○) during leaf expansion and the June drop period. Standard errors (S.E.) are given as vertical bars.

*Zeitliche Veränderungen der Trockenmasse (A) und Blattfläche (B) während des Blattwachstums und des Junifalls, in Laubblättern (Δ), Blättern des Blütenstandes (●) und Blättern von Blütenständen von denen die Blüten entfernt worden waren (○). Senkrechter Stab: Standardabweichung.*



GA<sub>3</sub>-like activity in the inflorescence leaves was dependent on the presence of the developing fruitlets. Gibberellin levels in the leaves from deblossomed inflorescences remained low throughout this period, close to the value found during leaf expansion. It was so until the end of June, when gibberellin levels became similar in all leaves.

## Discussion

Vegetative growth is usually depressed by flowering and fruiting, and competition has been sought as an explanation (BOLLARD 1970). However, none of the above described differences between inflorescence and vegetative leaves, namely leaf expansion and carbohydrate accumulation, may be explained in this manner. On the contrary, the fruit-induced accumulation of metabolizable carbohydrates in the inflorescence leaves is opposite to expectation from a competition among the leaves and the developing fruitlets for carbohydrates, since the presence of the fruit should result in a decrease rather than an increase in leaf carbohydrate levels.

A regulatory role of gibberellins on leaf expansion seems likely from the lower gibberellin levels in inflorescence as compared to vegetative leaves (Fig. 4), and from the enhancement of leaf expansion by exogenous GA<sub>3</sub> both in intact (GUARDIOLA et al. 1980) and deblossomed inflorescences (Fig. 3). Although an absolute proof is lacking, these differences in gibberellin levels seem unrelated to the presence of the developing flower bud since: a) they are established from the very first stages of bud sprouting (GOLDSCHMIDT and MONSELISE 1972, MINGO-CASTEL 1979), and b) leaf growth is unaffected by the elimination of the flower bud (Fig. 1). They may arise from the influence of other plant parts which would thus control not only leaf growth but flower formation, which at this early stage is very sensitive to gibberellins as shown by the inhibitory effect of exogenous gibberellins on flowering (GOLDSCHMIDT and MONSELISE 1972, GUARDIOLA et al. 1982).

As for leaf expansion, both the endogenous contents (Fig. 4) and the response to exogenous GA<sub>3</sub> (Fig. 3) support a regulatory role of gibberellins on carbohydrate accumulation in the leaves during the June drop period, this accumulation being enhanced by high gibberellin levels. In this case, however, there is strong evidence for a direct effect of the fruit on leaf gibberellins since the removal of the flower bud prevents the increase in gibberellin contents found in the leaves from intact inflorescences (Fig. 4). Further, some of the leaf gibberellins may have been transported directly from the fruit, since FERGUSON et al. (1986) have recovered in the nearby leaves up to 20% of the <sup>14</sup>C-GA<sub>3</sub> applied to the fruit, and the main gibberellin in inflorescence leaves during the June drop is the only native gibberellin found in the fruit diffusates (GARCÍA-PAPI and GARCÍA-MARTÍNEZ 1984).

The transport of gibberellins from the fruits to the source leaves has been reported in a few cases for both native (HOAD et al. 1977) and applied gibberellins (FERGUSON et al. 1986, PERETÓ and BELTRÁN 1987), and in one case an effect on phloem transport has been demonstrated (PERETÓ and BELTRÁN 1987).

The effect described here is different since carbohydrate accumulation in, rather than transport from the leaves is stimulated. Although other hormones are transported from the fruit to the source leaves (BRENNER 1987), only gibberellins, when applied exogenously, were capable to enhance carbohydrate accumulation in the leaves (SANZ et al. 1987a), so it seems logical to assume the same role for endogenous gibberellins. At the present moment the role of this transient carbohydrate accumulation on fruit set is not known, but unpublished research by RUIZ-SCHNEIDER and GUARDIOLA demonstrate that when it is not present in the inflorescence the fruit abscises, being one of the earliest indicators of fruitlet abscission.

In conclusion, from the endogenous levels and the response to exogenously applied GA<sub>3</sub> regulatory mechanisms involving gibberellins seem likely to regulate both

Fig. 3. The effect of exogenously applied GA<sub>3</sub> on dry weight (A), area (B) and carbohydrate contents (C) in leaves from deblossomed inflorescences. The arrows indicate the hormone applications. (○) untreated, (◻) GA<sub>3</sub>-treated leaves. S. E. are given as vertical bars.

Wirkung der GA<sub>3</sub>-Behandlung auf die Trockenmasse (A), die Blattfläche (B) und den Kohlenhydratgehalt (C) der Blätter eines Blütenstandes. (◻) behandelt, (○) nicht behandelt. Die Pfeile zeigen die Zeit der Behandlung. Senkrechter Stab: Standardabweichung.

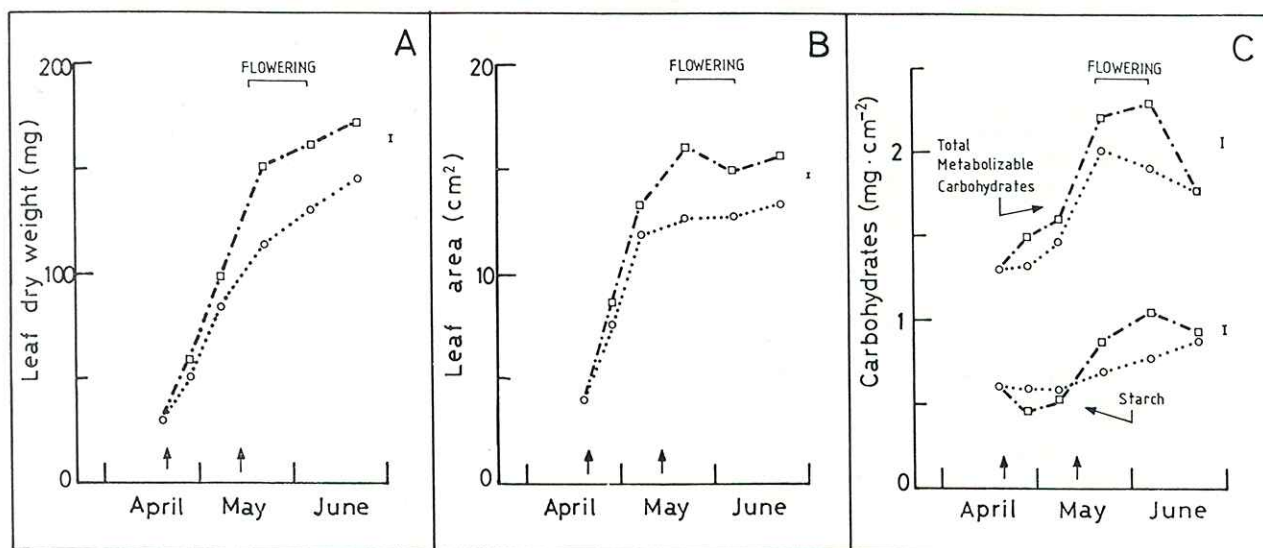
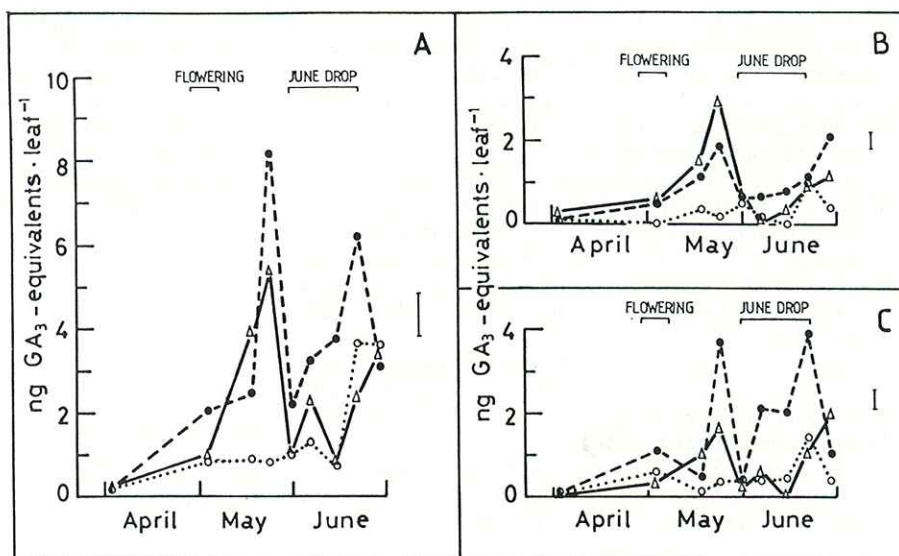


Fig. 4. Time-course changes in total endogenous gibberellin-like substances (A), and components at  $R_f = 0.2 - 0.4$  (B) and  $R_f = 0.8 - 1.0$  (C), in leaves from vegetative shoots ( $\Delta$ ), intact inflorescences ( $\bullet$ ) and deblossomed inflorescences ( $\circ$ ). S. E. are given as vertical bars.

F values for the time of sampling (7,21) and type of leaves (2,21) effects: Total GA contents, 8.2 ( $p \leq 0.001$ ) and 10.7 ( $p \leq 0.001$ ), respectively; component at  $R_f = 0.2 - 0.4$ , 6.4 ( $p \leq 0.001$ ) and 9.2 ( $p \leq 0.01$ ) and component at  $R_f = 0.8 - 1.0$ , 6.8 ( $p \leq 0.001$ ) and 16.9 ( $p \leq 0.001$ ).

Zeitliche Veränderungen des endogenen  $GA_3$ -Äquivalents in Laubblättern ( $\Delta$ ), Blättern des Blütenstandes ( $\bullet$ ) und Blättern des Blütenstandes von denen die Blüten entfernt worden waren ( $\circ$ ). A: Gesamt-Gibberellin; B: Komponente mit  $R_f = 0.2 - 0.4$ ; C: Komponente mit  $R_f = 0.8 - 1.0$ . Senkrechter Stab: Standardabweichung. F-Werte für Zeit (7,21) und Art von Blättern (2,21)

Effekte: Gesamt-GA, 8,2 ( $p \leq 0,001$ ) und 10,7 ( $p \leq 0,001$ ); Komponente mit  $R_f = 0,2 - 0,4$ , 6,4 ( $p \leq 0,001$ ) und 9,2 ( $p \leq 0,01$ ); Komponente mit  $R_f = 0,8 - 1,0$ , 6,8 ( $p \leq 0,001$ ) und 16,9 ( $p \leq 0,001$ ).



leaf expansion and carbohydrate accumulation, both processes being enhanced by gibberellins. The origin of the differences in gibberellin levels between vegetative and inflorescence leaves appear to be different during leaf expansion and June drop, inflorescence leaves having a lower gibberellin content during leaf expansion, and a higher one during the June drop, than vegetative leaves.

### Summary

The endogenous gibberellin-like substances of leaves during their expansion and in the June drop period has been determined and were related to leaf growth and carbohydrate accumulation. In developing leaves gibberellin levels were higher in leaves from vegetative sprouts than in inflorescence leaves, but after leaf expansion was completed the reverse situation was found. Removal of the flowers caused a significant decrease of gibberellin contents in the leaves. Flower removal had no effect on leaf expansion but prevented the transient accumulation of carbohydrates found in the leaves from intact inflorescences during the June drop period. The possible role of the changes in gibberellin contents on leaf expansion and carbohydrate accumulation is discussed.

### Zusammenfassung

Der Gibberellin Gehalt der Blätter während des Blattwachstums und des Fruchtfalls wurde untersucht und in Beziehung gesetzt zum Blattwachstum sowie der Kohlenhydratakkumulation. In noch wachsenden Blättern war der Gibberellin Gehalt im Blütenstand höher als im vegetativen Sproß, aber das Gegenteil stellte sich bei ausgewachsenen Blättern heraus. Die Entfernung der Blüten bewirkte eine signifikante Erniedrigung des Gibberellin Gehaltes im Blütenstand und hatte keinen Einfluß auf die Ausdehnung der Blattfläche, verhinderte aber die vorübergehende Kohlenhydratspeicherung, die in Blättern des intakten Blütenstandes beobachtet wurde. Die mögliche Rolle der Veränderungen des Gibberellin Gehaltes in Blättern wird diskutiert.

### Literature

- BOLLARD, E. G. 1970: The physiology and nutrition of developing fruits. In: The biochemistry of fruits and their products, Vol. 1. Ed. A. C. Hulme. Academic Press, New York. Pp 387-425.
- BRENNER, M. L. 1987: The role of hormones in photosynthate partitioning and seed filling. In: Plant hormones and their role in plant growth and development. Ed. P. J. Davies. Martinus Nijhoff. Dordrecht. Pp 474-493.
- EMBLETON, T. W., W. W. JONES, C. K. LABANAUSKAS and W. REUTHER 1973: Leaf analysis as a diagnostic tool and guide to fertilization. In: The Citrus industry, vol. 3. Ed. W. Reuther. Univ. of California, Berkeley, California. Pp 183-210.
- FERGUSON, L., T. A. WHEATON, F. S. DAVIES and M. A. ISMAIL 1986:  $^{14}C$ -Gibberellic acid uptake, translocation, persistence, and metabolism in grapefruit. J. Amer. Soc. Hort. Sci. 111, 926-932.
- GARCÍA-LUIS, A., M. AGUSTÍ, V. ALMELA, E. ROMERO and J. L. GUARDIOLA 1985: Effect of gibberellic acid on ripening and peel puffing in "Satsuma" mandarin. Sci. Hort. 27, 75-86.
- GARCÍA-PAPI, M. A. and J. L. GARCÍA-MARTÍNEZ 1984: Endogenous growth substances content in young fruits of seeded and seedless clementine mandarin as related to fruit set and development. Sci. Hort. 22, 265-274.
- GOLDSCHMIDT, E. E. and S. P. MONSELISE 1972: Hormonal control of flowering in Citrus and some other woody perennials. In: Plant growth substances 1970. Ed. D. J. Carr. Springer Verlag. Berlin - Heidelberg - New York. Pp 758-766.
- GUARDIOLA J. L., M. AGUSTÍ, M. BARBERÁ and F. GARCÍA-MARÍ 1980: Influencia de las aplicaciones de ácido giberélico durante la brotación en el desarrollo de los agrios. Rev. Agroquim. Tecnol. Aliment. 20, 139-143.
- GUARDIOLA, J. L., F. GARCÍA-MARÍ and M. AGUSTÍ 1984: Competition and fruit set in the Washington navel orange. Physiol. Plant. 62, 297-302.

- GUARDIOLA, J. L., F. GARCÍA-MARÍ and M. AGUSTÍ 1984: Competition and fruit set in the Washington navel orange. *Physiol. Plant.* **62**, 297–302.
- GUARDIOLA, J. L., C. MONERRI and M. AGUSTÍ 1982: The inhibitory effect of gibberellic acid on flowering in Citrus. *Physiol. Plant* **55**, 136–142.
- HOAD, G. V., B. R. LOVEYS and K. G. M. SKENE 1977: The effect of fruit-removal on cytokinins and gibberellin-like substances in grape leaves. *Planta* **136**, 25–30.
- MCCREADY, R. M., J. GUGGOLZ, V. SILVEIRA and H. S. OWENS 1950: Determination of starch and amylose in vegetables. Application to peas. *Anal. Chem.* **22**, 1156–1158.
- MINGO-CASTEL, A. M. 1979: Análisis de fitohormonas en yemas de naranjo Valencia (*Citrus sinensis* L. Osbeck) por cromatografía gaseosa. *Anales I.N.I.A. Serie Producción Vegetal* **9**, 203–222.
- PERETÓ, J. G. and J. P. BELTRÁN 1987: Hormone directed transport during fruit set induced by gibberellins in *Pisum sativum*. *Physiol. Plant.* **69**, 356–360.
- SANZ, A., C. MARTÍNEZ-CORTINA and J. L. GUARDIOLA 1987a: The effect of the fruit and exogenous hormones on leaf expansion and composition in Citrus. *J. Exp. Bot.* **197**, 2033–2042.
- SANZ, A., C. MONERRI, J. GONZÁLEZ-FERRER and J. L. GUARDIOLA 1987b: Changes in carbohydrates and mineral elements in Citrus leaves during flowering and fruit set. *Physiol. Plant.* **69**, 93–98.

Eingegangen: 28. 2. 1989 / 2. 5. 1989.

Anschrift der Verfasser: C. Martínez-Cortina und Dr. A. Sanz, Departamento de Biología Vegetal, Universidad de Valencia, c/o Dr. Moliner, 50. 46100-Burjassot (Valencia). Prof. Dr. J. L. Guardiola, Departamento de Biología Vegetal, Universidad Politécnica de Valencia. Camino de Vera, 14. 46020-Valencia, Spanien.