

In vitro morphogenesis of grapevine (*Vitis vinifera* L.) originated from anticipated or latent buds

by

M. ADRIAN, J. C. FOURNIOUX and R. BESSIS

Institut Universitaire de la Vigne et du Vin, Laboratoire des Sciences de la Vigne, Université de Bourgogne, Dijon, France

S u m m a r y : While in outdoor-grown vines shoots originate from latent buds, grapevine shoots from microcuttings cultured *in vitro* are produced by the anticipated bud. The latter shoots show physiological and morphological features of juvenility. This study was carried out to obtain more conform *in vitro* grapevine shoots. Latent buds were induced to develop *in vitro*. Shoots produced by latent buds had more juvenile features than those produced by anticipated buds. New information on the control of juvenility of grapevines *in vitro* is presented.

K e y w o r d s : conformity of propagation, bud, *in vitro* culture, juvenility, micropropagation.

Introduction

In vitro propagated cuttings of *Vitis vinifera* show different "juvenile" characters similar to those found in plantlets, i.e. a spiral phyllotaxy and a decrease or even a disappearance of tendrils (BERNARD and MUR 1979; FAVRE and GREANAN 1979; GREANAN 1979, 1982 a and b; MULLINS *et al.* 1979; NOZERAN *et al.* 1982; FOURNIOUX and BESSIS 1986). This can lead to serious problems at the time of return to the vineyard. Indeed, even if plants quickly develop a distichous phyllotaxy with tendrils, some juvenile morphological features still persist such as more jagged and hairy leaves, stem colouration by anthocyanins and often a great decrease of fertility (GREANAN 1982 a and b, 1984; BESSIS 1986; CANCELLIER and COSSIO 1988), i.e. ampelographic characteristics are different. This "lack of conformity" of these clones makes them useless with regard to grapevine improvement which uses many technologies related to *in vitro* culture.

In grapevine, anticipated and latent buds form the axillary complex at each node (Fig. 1). The anticipated bud is situated in the axil of the leaf; it has only one prophyll and is able to develop during the season of its formation. The latent bud is in the axil of the prophyll of the anticipated bud, it has two prophylls, contains inflorescences and is characterized by a dormancy period during which growth is retarded until the following year. This structure has been precisely described by BUGNON and BESSIS (1968). In the vineyard, primordial bunches are produced by shoots developed by latent buds. According to BERNARD and MUR (1979) and BERNARD (1980), the anticipated buds of *in vitro* microcuttings develop, leading to an inhibition of the development of latent buds (NIGOND 1961). Bunches of shoots previously obtained *in vitro* resemble those produced by anticipated shoots in the vineyard. The aim of this study

was to compel the latent bud (instead of the anticipated) to grow *in vitro* in order to obtain more conform shoots.

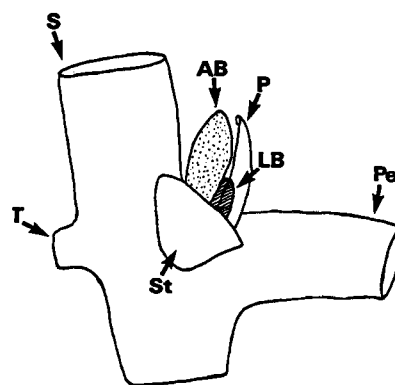


Fig. 1: The axillary complex of a microcutting (schematic). LB: latent bud, AB: anticipated bud, P: prophyll of the anticipated bud, S: stem, Pe: petiole, T: tendril, St: stipules.

Material and methods

Plant material: Two different sorts of single bud cuttings from *Vitis vinifera* L. cv. Pinot noir (clone 113), were used: herbaceous cuttings removed from shoots obtained from hardwood cuttings (hereafter referred to as R1 cuttings) and cuttings from plants produced *in vitro* after several subcultures (hereafter referred to as Rn cuttings). Hardwood cuttings used for R1 cuttings were collected in winter and kept in a greenhouse. R1 cuttings were sterilized with calcium hypochlorite (70 g l^{-1} for 20 min) and rinsed by sterile distilled water before introduction *in vitro*.

R1 and Rn cuttings with an axillary bud and a 10 mm section of internode were planted on a nutrient medium (20 ml) developed by MUR (unpubl., details: FOURNIOUX

and BESSIS 1993). Each cutting was planted in a 25 mm x 250 mm culture tube covered by a metal cap enabling free gas exchange between the atmosphere and the container (FOURNIOUX and BESSIS 1986) before being incubated at constant conditions (24 ± 2 °C with a 16 h photoperiod).

Methods: With R1 and Rn cuttings 4 main experiments were carried out, called A, B, C and T (A1, B1, C1 and T1 for R1 material and An, Bn, Cn and Tn for Rn material, respectively), differing by the bud type forced to grow or by the time at which the latent bud was compelled to grow (Fig. 2). When a cutting was introduced into a tube, its latent bud had already some young leaves (generally 4 on a R1 cutting and one on a Rn cutting) but it developed until the anticipated bud had reached its "one-extended-leaf-stage". The latent bud then had 2 (R1 cutting) or 2 to 4 (Rn cutting) new young leaves (FOURNIOUX 1995). Consequently, the latent bud may behave differently, depending on its development when it is forced to grow.

Cuts were made just above the first prophyll from the base of the shoot, forcing the latent bud to grow.

In order to determine shoot phyllotaxy, transverse sections of apices were made. Apices were drawn, fixed in FAA (ethanol 50 % : acetic acid : formol 40 % = 18:1:1, v/v/v) and dehydrated in tertial butylic alcohol before paraffin inclusion. 8 μ m serial sections were made using a microtome (Reichert Jung Mod 1140/Autocut); they were stained with Safranin/Fast-Green.

Shoot length and number of leaves and roots were recorded weekly. They were expressed in number of days from placement of the cutting in tubes for experiment T, from dissection day for A and from section day for B and C. After 70 d of culture, plantlets were taken out of their tubes in order to determine leaf blade size (length and width) and internode length in the median part (i.e. between the 4th and 7th leaf from the base). In addition, tendril-bearing node frequency was determined according to their position on the axis.

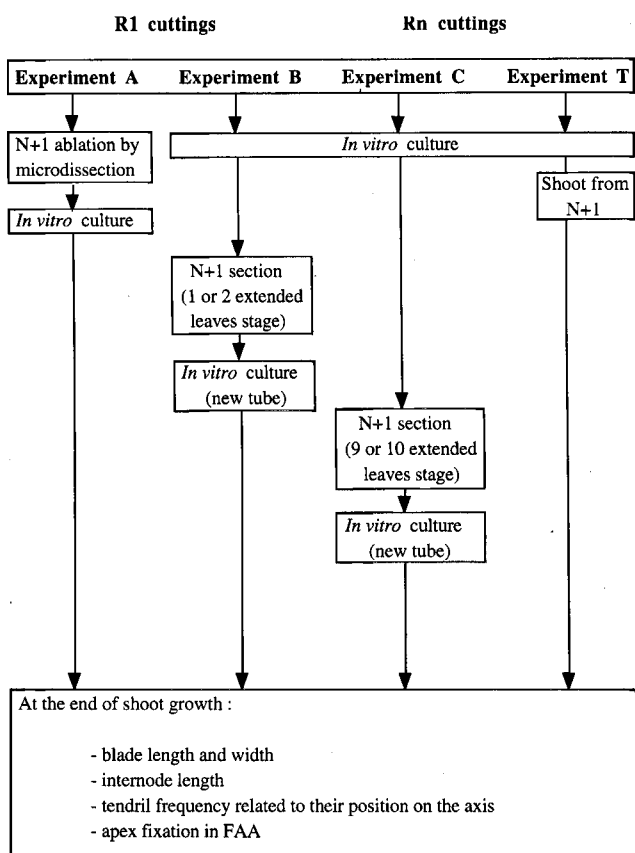


Fig. 2: Schedule of experiments.

Each experiment was carried out with 24 tubes and repeated three times. Experiment T was the control: microcuttings were directly planted so that the anticipated bud grew. In experiments A, B and C the anticipated bud was removed at different stages. To induce the development of the latent bud, the anticipated bud was removed by microdissection before introduction of microcuttings *in vitro* (experiment A). Other experiments were directed to the stage at which the shoot was sectioned: either at the "one-" or "two-extended-leaf-stage" (experiment B) or at the "nine-" or "ten-extended-leaf-stage" (experiment C).

Results

Blade size and internode length: For R1 as well as for Rn material, internode length was almost identical for T, A and B shoots and slightly higher in C (Table). There was no significant difference in leaf size. In all cases, blade width values were higher than those of blade length.

Shoot growth: A1, B1 and C1 shoot growth was substantially faster than that of T1 shoots (Fig. 3). The same is true for the number of leaves and roots. However,

Table

Mean blade size and mean internode length for the median part of shoots. A1, B1, C1: *in vitro* material derived from hardwood cuttings; An, Bn, Cn: material derived from *in vitro* plants; T1, Tn: control

Experiment	Mean internode length (cm)	Mean leaf blade length (cm)	Mean leaf blade width (cm)
T1	1.2 ± 0.4	1.7 ± 0.3	2.2 ± 0.5
A1	1.1 ± 0.2	1.8 ± 0.3	2.3 ± 0.4
B1	1.1 ± 0.3	1.8 ± 0.4	2.2 ± 0.5
C1	1.4 ± 0.3	1.8 ± 0.3	2.0 ± 0.4
Tn	1.3 ± 0.3	2.1 ± 0.3	2.2 ± 0.4
An	1.1 ± 0.2	2.0 ± 0.4	1.9 ± 0.4
Bn	1.3 ± 0.4	1.9 ± 0.3	2.0 ± 0.4
Cn	1.4 ± 0.5	2.0 ± 0.4	2.1 ± 0.4

there was no significant difference between An, Bn, Cn and Tn shoots neither with respect to shoot length nor to the number of leaves and roots. An analysis of the length of shoots developed by latent buds related to their stage of enforced growth was also carried out (data not shown). There was almost no difference between the R1 and Rn

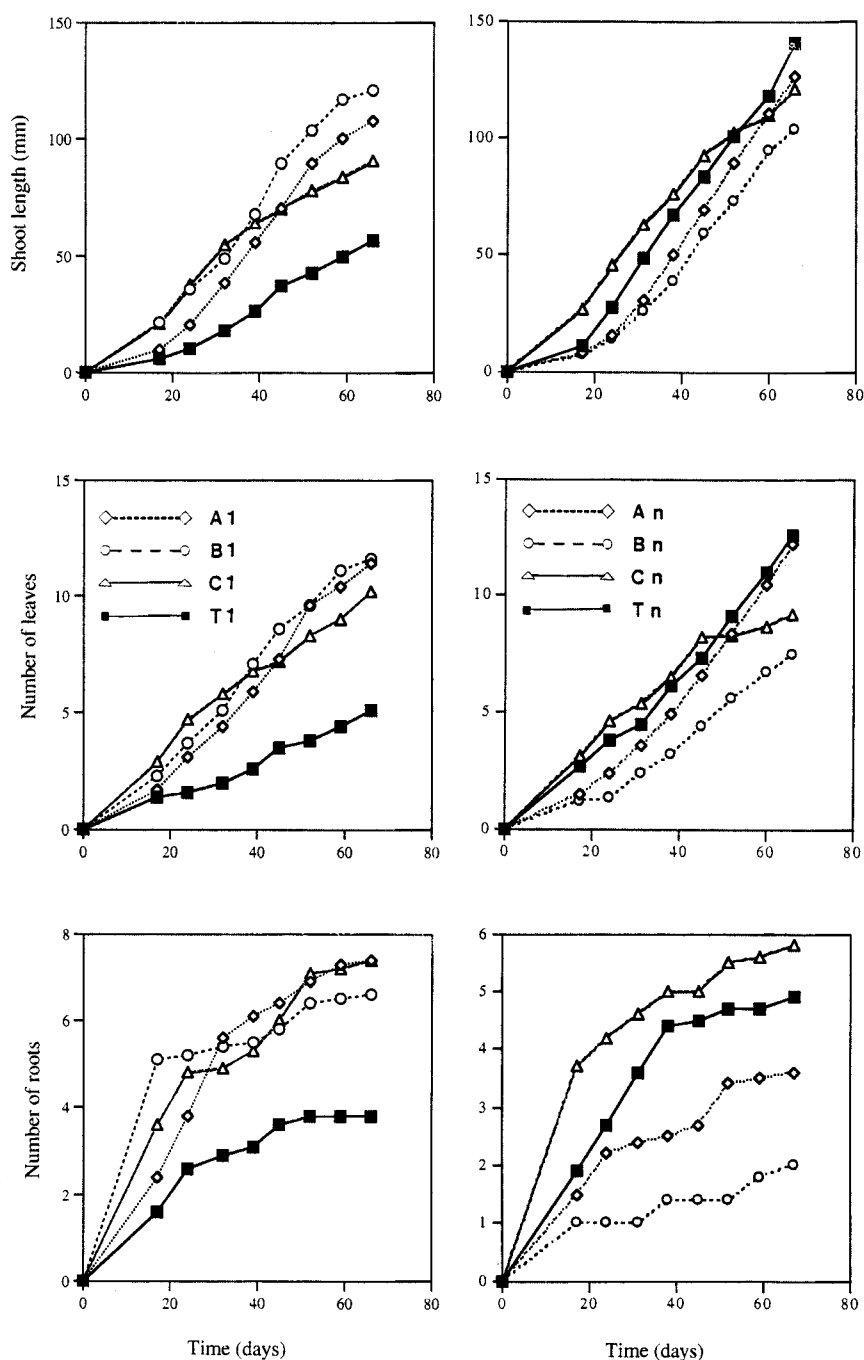


Fig. 3: Development of shoot length and number of leaves and roots of A1, B1, C1 plants (left side) and of An, Bn, Cn, plants (right side). T1 and Tn: control plants; A1, B1, C1; An, Bn, Cn: see Table.

material. Consequently, the fact that latent buds were forced to grow earlier or later did not change their growth rate.

Tendrils according to their position on the axis: Similar results were obtained for A1 and T1 shoots: the first tendrils appeared at low nodes (3 or 4) in a low frequency. Then, in the median part of shoots, they were present in higher frequency (40 to 60%). The first tendrils were observed at higher insertions and lower frequency for B1, and particularly C1 shoots (inferior to 30%) (Fig.4). Tendril frequency was higher for Tn shoots than for An, Bn, and especially Cn shoots (none of them exceeded 12%). Moreover, they

appeared at a higher node for An, Bn and Cn shoots (mainly An and Cn: node 10) than for Tn shoots. Many nodes were without any tendril (only 3 for Cn shoots)(Fig. 5). No relation was found between the frequency of tendril rhythm N0-N1-N2 (BOUARD 1966) and any of the 4 experiments.

The results indicate an increase of tendril frequency from the base to the median part of the shoots and a decrease to the apex. To a certain extent, it seems that the more delayed growth is enforced on the latent bud, the lower are tendril frequency and number of tendril-bearing nodes (principally for Rn material). Moreover, there were more tendrils on R1 than on Rn material. This confirms the idea that rejuvenation is largely due to the successive

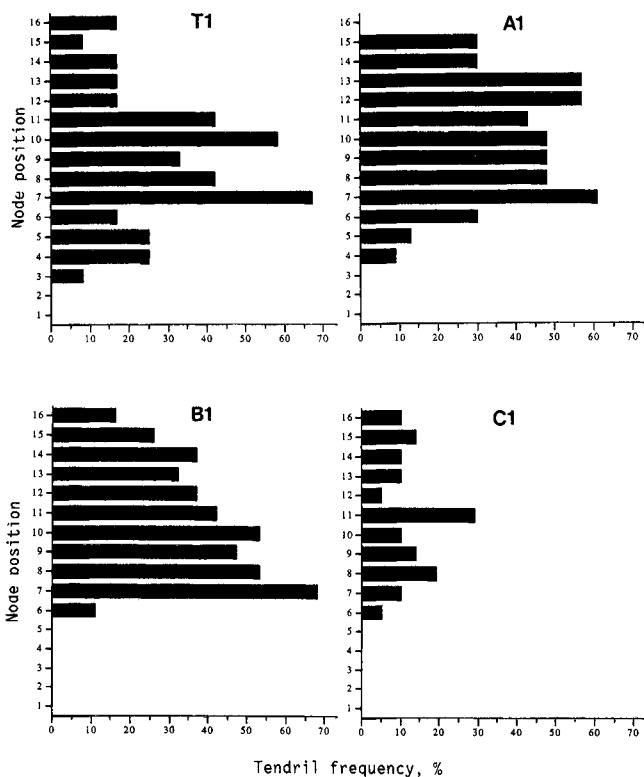


Fig. 4: Tendril frequency of A1, B1, C1 and T1 (control) shoots related to their position on the axis. A1, B1, C1: see Table.

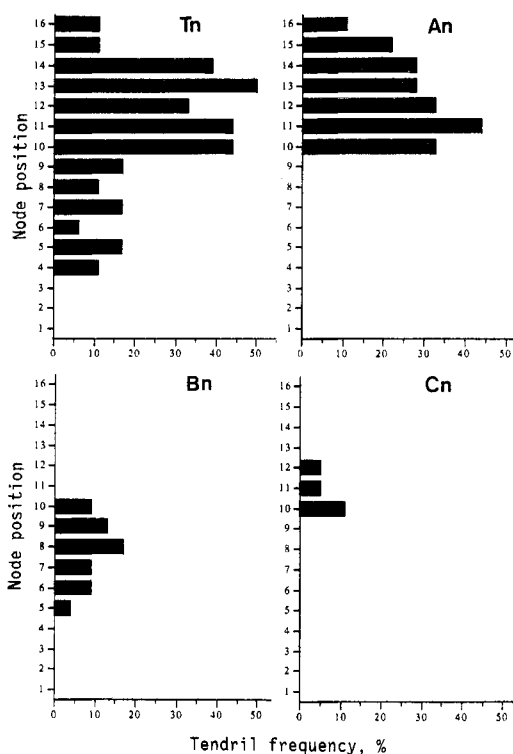


Fig. 5: Tendril frequency of An, Bn, Cn and Tn (control) shoots related to their position on the axis. An, Bn, Cn: see Table.

subcultures (MULLINS *et al.* 1979 and FOURNIOUX and BESSIS 1990). Finally, A and T shoots had about the same number of tendrils which was higher than that of B and C shoots.

Phyllotaxy: The microscopic examination of serial transverse sections of apices showed a distichous phyllotaxy for all the shoots (Fig. 6, left) except for Cn whose phyllotaxy was almost 2/5 (Fig. 6, right).

Discussion

As soon as the latent bud is no longer inhibited by the anticipated bud, it will grow. There is no dormancy as in the vineyard. This may be due to *in vitro* culture with constant ambient conditions. The experiments enabled us to

point out a new system of tendril formation control. It seems that the more delayed growth is enforced on the latent bud, the more rejuvenated it is by *in vitro* culture, in particular in relation to Rn material, which is already rejuvenated by several subcultures. By keeping in mind the meristem miniaturization theory, we think that the meristem is so small that it is not able to produce tendrils any longer while leaf formation is still possible.

From these experiments it is assumed that the later the latent bud is forced to grow, the more rejuvenated are the shoots produced, especially in the case of Rn material. Only Cn shoots show almost a 2/5 phyllotaxy and they have the lowest tendril frequency. It seems that shoots originated from the latent buds rejuvenate more quickly *in vitro* than anticipated ones. Acclimation of the plants originating from latent buds has been achieved, we are now studying their morphology.

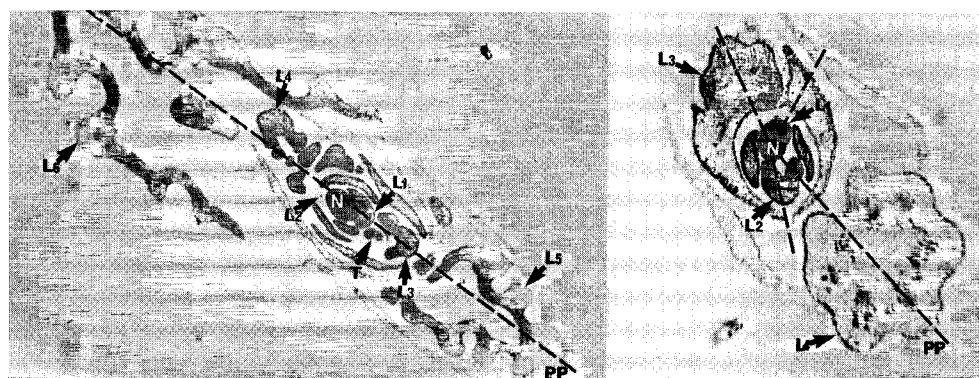


Fig. 6: Transverse sections of a Tn shoot (left) and a Cn shoot (right). N: axis; L1-L6: leaves; T: tendril; PP: phyllotaxial plan.

Conclusion

For the first time, these experiments show that it is feasible to force latent buds to develop *in vitro* at different stages. These results are encouraging in the sense that they show that *in vitro*, as well as in the vineyard, latent and anticipated shoots are different, not only physiologically (intensity of shoot growth) but also morphologically (tendrill formation). Unfortunately, this method has no effect upon the juvenility markers used. Indeed, although R1 latent shoots exhibit faster growth than anticipated ones, this is not true for Rn. Rn shoots show a lower tendrill production. In fact, the latent bud is more rapidly rejuvenated by the several subcultures than the anticipated. The occurrence of morphological juvenility markers *in vitro* is now better understood and this work describes a method to favour juvenility.

Acknowledgements

We would like to thank S. DEBORD and S. KINDT for their technical advice.

References

- BERNARD, A. C.; 1980: Contribution à l'étude de la biologie et des méristèmes des Vitacées. Thèse Univ. Montpellier, France.
- -; MUR, G.; 1979: Observations sur l'organogenèse des bourgeons de plants de *V. vinifera* cultivés *in vitro*. Ann. Amélior. Plantes. **29**, 311-323.
- BESSIS, R.; 1986: Grape vine physiology: the contribution of culture *in vitro*. *Experientia* **42**, 927-933.
- BOUARD, J.; 1966: Recherches physiologiques sur la vigne et en particulier l'aouïtement des sarments. Thèse Univ. Bordeaux, France.
- BUGNON, F.; BESSIS, R.; 1968: Biologie de la Vigne. Acquisitions Récentes et Problèmes Actuels. Masson et Cie., Paris.
- CANCELLIER, S.; COSSIO, F.; 1988: Risultati di osservazioni su un clone di „corvina veronese“ (*Vitis vinifera* L.) moltiplicato *in vitro*. Riv. Viticol. Enol. **3**, 2-10.
- FAVRE, J. M.; GREANAN, S.; 1979: Sur la production de vrilles, de fleurs et de baies chez la vigne cultivée *in vitro*. Ann. Amélior. Plantes **29**, 247-252.
- FOURNIOUX, J. C.; 1995: Facteurs de l'édification de la tige de *Vitis vinifera* L. dans différentes conditions de culture. Thèse Univ. de Dijon, France.
- -; BESSIS, R.; 1986: Influence de la teneur en gaz carbonique sur la morphogenèse de la vigne en culture *in vitro*. Can. J. Bot. **64**, 2608-2616.
- -; - -; 1990: Eléments du contrôle de la morphogenèse de la vigne *in vitro*: sympodisation. Can. J. Bot. **68**, 841-851.
- -; - -; 1993: Use of carbon dioxide enrichment to obtain adult morphology of grapevine *in vitro*. Plant Cell Tissue Organ Cult. **33**, 51-57.
- GREANAN, S.; 1979: Possibilités d'élimination des modifications foliaires, apparues sur la variété Grenache N. après un passage prolongé en culture *in vitro*. Progr. Agric. Viticol. **96**, 152-157.
- -; 1982 a: Implications fondamentales et appliquées de conséquences de la culture *in vitro* de *Vitis vinifera* L. Thèse Univ. Orsay, France.
- -; 1982 b: Quelques réflexions à propos des modifications morphogénétiques consécutives à la culture *in vitro* de la vigne (*Vitis vinifera* L.). Ann. Sci. Nat. Bot. Biol. Veget. Sér. **13** (4), 135-146.
- -; 1984: Polymorphisme foliaire consécutif à la culture *in vitro* de *Vitis vinifera* L. *Vitis* **23**, 159-174.
- MULLINS, M. G.; NAIR, Y.; SAMPET, P.; 1979: Rejuvenation *in vitro*: induction of juvenile characters in an adult clone of *Vitis vinifera* L. Ann. Bot. **44**, 623-627.
- NIGOND, J.; 1961: Contribution à l'étude de la dormance de la vigne sous le climat du Languedoc. Thèse 3ème cycle, Univ. Paris, France.
- NOZERAN, R.; DUCREUX, G.; ROSSIGNOL, L.; 1982: Réflexions sur les problèmes de rajeunissement chez les végétaux. Bull. Soc. Bot. Fr. **139**, Lettres Bot. **2**, 107-130.

Received February 22, 1996