

1 **In vitro culture establishment and multiplication of the *Prunus* rootstock**  
2 **'Adesoto 101' (*P. insititia* L.) as affected by the type of propagation of the**  
3 **donor plant and by the culture medium composition**

4

5 Pilar Andreu & Juan A. Marín\*

6 Pomology. Estación Experimental de Aula Dei (CSIC), Apartado 202, 50080

7 Zaragoza, Spain

8 \*Author for correspondence (Tel. +34 976 716128; Fax: +34 976 716145; E-  
9 mail: jmarin@eead.csic.es)

10

11 **Abstract**

12 The establishment of new in vitro cultures from mature woody plants is often a  
13 difficult task due to the little growth of initial explants. Since the explant origin  
14 plays an important role, in this work the effect of the origin of the explants  
15 (micropropagated or conventionally propagated plants) on both establishment  
16 and multiplication of the in vitro cultures has been studied using different culture  
17 media. Best results during establishment were obtained with explants taken  
18 from micropropagated plants. The multiplication rate of new cultures was  
19 strongly affected by the type of propagation of the mother plants. Thus, while  
20 the cumulative number of shoots increased sharply in cultures originated from  
21 micropropagated plants, cultures originated from cutting-derived plants showed  
22 only a moderate increase. Culture medium composition influenced the  
23 multiplication rate. After 9 subcultures, a significantly lower number of shoots  
24 was found on QL medium than on MS or on WP. The positive effect of  
25 micropropagation of donor plants on the establishment and multiplication of new

26 in vitro cultures is discussed in terms of a possible reinvigoration during in vitro  
27 culture.

28

29 **Keywords:**

30 Apparent rejuvenation, reinvigoration, micropropagation, cuttings, culture  
31 medium composition

32

33 **Introduction**

34 Woody plants raise frequent propagation difficulties when using conventional  
35 techniques. Tissue culture can relieve this problem since it has been reported  
36 that plants may acquire higher rooting capabilities after continuously  
37 subculturing in vitro (Howard et al., 1989; Jones and Hadlow, 1989; Webster  
38 and Jones, 1989; Hammatt and Grant, 1993; Grant and Hammat, 1999).

39 Besides, this type of 'rejuvenation' can influence other aspects of plant  
40 propagation, such as the ability to initiate new healthy in vitro cultures. During  
41 the establishment of new cultures from mature plants, the explants often show  
42 slow growth and low survival rates; however, juvenile explants taken from  
43 young grafts displayed better in vitro growth than those taken from adult cashew  
44 plants (Thimmappaiah et al., 2002), and had higher multiplication rates in *Fagus*  
45 (Meier and Reuther, 1994). This different performance of juvenile vs. mature  
46 explants has been related with the contents of phenolic compounds in chestnut  
47 (Mato et al., 1994). Thus, rejuvenation has been used to facilitate in vitro culture  
48 of explants from mature plants, mainly grafting buds into juvenile rootstocks  
49 (Pliego-Alfaro and Murashige, 1987; Meier and Reuther, 1994; Sanchez et al.,  
50 1997; Thimmappaiah et al., 2002). Similarly, a partial rejuvenation was also

51 obtained by intensive pruning, what stimulates the sprouting of the basal buds  
52 that could retain juvenile characters, increasing both in vitro proliferation and  
53 maintenance of culture lines of filbert (Diazsala et al., 1994), as well as affecting  
54 the endogenous polyamine contents in hazelnut leaves and buds (Rey et al.,  
55 1994).

56

57 No direct relation between growth regulators and rejuvenation was found, when  
58 they were included in the culture medium (see George, 1993, for a review).

59 However, both internal concentration and external applications of growth  
60 regulators were related with juvenile traits. Thus, juvenile tissues contain higher  
61 IAA levels as a consequence of high concentrations of auxin protectors (Mato et  
62 al, 1994). On the other hand, exogenously applied cytokinins improved in vitro  
63 performance of mature explants of chestnut in terms of establishment,  
64 multiplication and rooting (Sanchez et al., 1997).

65

66 Recently, different factors of *Prunus* micropropagation have been studied as the  
67 effect of subculture frequency (Grant and Hammatt, 1999), the effect of different  
68 carbohydrates (Harada and Muray, 1996; Nowak et al., 2004), the comparison  
69 of different iron sources in the culture medium (Molassiotis et al., 2003), the  
70 effect of different combinations of growth regulators (Pruski et al., 2000), the  
71 application of mycorrhiza for pathogen protection, and the performance of  
72 micropropagated plants after their transfer to soil (Hammerschlag and Scorza,  
73 1991; Hammat, 1999; Marín et al., 2003).

74

75 Different culture media have been used in *Prunus* with a genotype-dependent  
76 response as in apricot and almond (Perez-Tornero and Burgos, 2000;  
77 Channuntapipat et al., 2003). In addition to media composition, the  
78 concentration of salts may play an important role, as in cherry, in which half  
79 concentration MS macronutrients resulted in more growth than full or double  
80 concentration (Ruzic et al., 2003). Nevertheless, culture medium can affect in  
81 vitro growth in different ways, depending on the culture stage, so it would be  
82 interesting to study the effect of commonly used culture media in a particular  
83 *Prunus* species along the micropropagation phases.

84

85 Here we studied the effect that previous micropropagation of donor plants has  
86 on obtaining suitable explants to establish new in vitro cultures. This study  
87 confirmed that micropropagated plants could acquire this juvenile character, as  
88 it was previously stated for rooting capability. We compared the effect of the  
89 type of propagation of pruned mother plants (cuttings vs. micropropagation) on  
90 the establishment and multiplication of new in vitro cultures in three different  
91 culture media.

92

### 93 **Materials and methods**

94 One-node explants of the *Prunus* rootstock 'Adesoto 101' (*Prunus insititia* L.)  
95 were taken in spring from trees propagated either by cuttings or by  
96 micropropagation that had been severely pruned every winter. After washed in  
97 running tap water, the surface of the explants was disinfected with HgCl<sub>2</sub>  
98 (0.05%) for 15 min and then rinsed 3 times in sterile distilled water. Explants (48  
99 one-node explants per treatment) were placed on 15 ml medium contained in

100 33 ml glass tubes sealed with polypropylene caps. Three kinds of medium were  
101 used: MS medium (Murashige and Skoog, 1962), WP (Lloyd and McCown,  
102 1980), and QL (Quoirin and Lepoivre, 1977). All three media were  
103 supplemented with 0.5  $\mu$ M IBA , 5  $\mu$ M BA, 30 g-l-1 sucrose, and 7 g-l-1 agar  
104 (Bacto-agar, Difco, Fisher Scientific). The pH was adjusted to 5.6 before  
105 autoclaving. Explants were cultured at 22° C under a photoperiod of 16h of  
106 cool-white fluorescent light (35  $\mu$ molxm-2xs-1). The explants were examined  
107 weekly and those that exhibited healthy expanding leaves were scored and the  
108 percentages of established cultures, after an initial period of 8 weeks, were  
109 recorded. Subsequently, shoots that arose from nodes were transferred onto 30  
110 ml fresh medium in 100 ml glass culture vessels (Sigma Chemical Co., St. Louis  
111 MO, USA). As a result, a variable number of culture lines were multiplied  
112 depending on the combination of treatments. Thus, 16,14 and 16 culture lines  
113 were maintained and multiplied respectively for MS, WP and QL when the  
114 cultures were originated from trees propagated by cuttings, whereas they raised  
115 to 20, 25 and 22 culture lines from micropropagated trees as average. Every  
116 culture line, derived from a growing node, was identified and transplanted to a  
117 fresh medium at 4-weeks intervals. New shoots were cut off and placed again in  
118 the same medium, and the number of shoots was scored. A cumulative number  
119 of shoots per line after 9 subcultures was obtained as a measurement of the  
120 multiplication rate, while the percentage of lines per treatment that showed  
121 continuous growth after that period indicated the survival of the culture lines.  
122 The whole experiment was repeated three times on different dates.

123

124 Data analysis

125 A completely randomized design with two treatments (type of propagation of  
126 donor plants and culture medium composition) and three repetitions was  
127 applied. Two-factors analysis of variance (ANOVA) and Duncan's multiple  
128 range test were performed to analyse the cumulative number of shoots at the  
129 9th subculture, as well as the transformed percentages (arcsine transformation)  
130 of both, the establishment of new cultures and the survival of culture lines.  
131 SPSS statistical software (SPSS Inc., Chicago, USA) was used.

132

### 133 **Results**

#### 134 Establishment of new culture lines in vitro

135 Most of the nodes exhibited growth soon after the culture initiation, showing  
136 some bud swelling and leaf expansion. The number of explants that showed  
137 healthy growth increased sharply during the first 30-40 days in all culture media  
138 and plant origin, and then continued displaying a slight increase or, in some  
139 cases a decrease, as in cutting derived cultures on either WP or QL (Figure 1).  
140 Buds taken from micropropagated plants grew faster even in a higher amount  
141 (up to 63.9% of explants at the end of the initial phase) than those taken from  
142 plants propagated by cuttings (up to 43.8%) in all three culture media tested,  
143 (Figure 1) and this effect was statistically significant ( $P < 0.05$ , Table 1).  
144 Therefore, the initial growth of explants during the establishment of in vitro  
145 culture was significantly affected by the type of propagation of mother plants.  
146 On the other hand, culture medium composition affected the percentage of  
147 establishment of new cultures in vitro, since it was higher in WP than in QL or  
148 MS (Table 1), either in micropropagated or in cutting derived cultures. However,  
149 these differences were not statistically significant (Table 1).

150

#### 151 Multiplication of culture lines

152 The cumulative number of shoots of each combination of treatments increased  
153 with time in all cases but at different rates (Figure 2). The propagation technique  
154 of donor plants influenced shoot production during the multiplication phase  
155 since micropropagated derived cultures produced more shoots than cutting-  
156 derived cultures in any subculture of the multiplication phase. Cultures derived  
157 from micropropagated trees produced more shoots than those derived from  
158 cuttings and this effect was maintained in every culture medium (Figure 2, Table  
159 1). After 9 subcultures the statistical analysis of the multiplication rate (as the  
160 number of cumulated shoots per culture line) showed significant differences  
161 between micropropagated and cutting-derived cultures ( $P \leq 0.001$ , Table 1).  
162 Cultures derived from micropropagated trees produced an overall average of  
163 17.3 shoots per culture line, while cultures derived from cutting-trees produced  
164 only 9.9 shoots per culture line. On the other hand, the composition of the  
165 culture media had also an effect on shoot production, thus, explants cultured in  
166 MS or WP media developed significantly more shoots than explants cultured in  
167 QL ( $P < 0.01$ , Figure 2, Table 1), with 17.0, 13.5 and 8.5 shoots per culture line  
168 respectively, as overall averages, while no significant differences were found  
169 between MS and WP following Duncan's multiple range test.

170

#### 171 Survival of culture lines

172 While most of the established cultures continued growing during the  
173 multiplication phase, the growth of some culture lines declined and they  
174 eventually died, mainly when the culture lines derived from trees propagated by

175 cuttings and cultured on MS or QL (Table 1). However, differences in the  
176 percentages of survival of the culture lines (an overall average of 64.7% in  
177 cutting-derived culture lines vs. 85.5% in micropropagation-derived cultures)  
178 were not statistically significant.

179

180 No significant interaction between the method of propagation and the  
181 composition of the culture medium has been found either in the culture  
182 establishment or in the multiplication and survival of the culture lines.

183

184

## 185 **Discussion**

186 The data contained in this work clearly indicate that the type of propagation of  
187 the donor plants affected the establishment of new cultures and the  
188 multiplication rates of the culture lines. Plants obtained by micropropagation  
189 were a better source of explants to establish new in vitro cultures than those  
190 propagated by cuttings. The positive effect of micropropagation suggests that  
191 the formerly micropropagated Adesoto 101 plants remained apparently  
192 rejuvenated after being transferred to soil and affected their in vitro cultures  
193 thereafter. To our knowledge, this has not been described for the establishment  
194 of new cultures; however, an apparent rejuvenation that improved rooting  
195 capability of cuttings had been observed in micropropagated apple, pear, cherry  
196 and plum trees, as well as in rhododendron plants (Howard et al., 1989; Jones  
197 and Webster, 1989; Marks, 1991; Webster and Jones, 1992; Grant and  
198 Hammat, 1999).

199

200 Partial rejuvenation, or re-invigoration, is related with a period of culture under  
201 in vitro conditions (Howard et al, 1989; Devries and Dubois, 1994) affecting  
202 growth and development of tissues; thus, the number of shoots per culture and  
203 the ability of shoots to produce adventitious roots increased with the age of a  
204 culture line in the cherry rootstock F12/1 (Hammatt and Grant, 1993; Grant and  
205 Hammat, 1999), but the length of the culture period to induce juvenile  
206 characters is not predetermined, since a variable number of subcultures for  
207 different culture lines was required, as in an adult clone of grape (Mullins et al.,  
208 1979). These changes can be associated with physiological differences already  
209 described between juvenile and adult tissues, thus, the polypeptide contents  
210 appeared to reflect the ontogenetic age of chestnut tissues (Amomarco et al.,  
211 1993), and higher polyphenol contents were found in juvenile tissues of  
212 chestnut (Mato et al., 1994). Furthermore, juvenile tissues of grape showed a  
213 lower concentration of abscisic acid than adult tissues (Langilier and Fournioux,  
214 2000). However, in vitro culture affects not only the acquisition of juvenile traits,  
215 but also mature traits as it was described for in vitro induced flowering, under  
216 certain conditions, after a long-term culture of pear shoots (Harada and Murai,  
217 1998). This apparent paradox may be explained since separate features of  
218 juvenility are supposed to be independently controlled, as pointed out by  
219 George (1993) using the different characteristics of juvenility reported in  
220 different species that have been micropropagated.

221

222 The composition of the culture medium has influenced the growth of new in vitro  
223 cultures, and this effect is caused by the salt composition of the media since the  
224 rest of the components remained unchanged. WP was the medium that

225 promoted a better establishment of the cultures, but MS supported higher  
226 multiplication rates. This effect seems to be related with an optimization of  
227 different phases of micropropagation with different culture media. In contrast,  
228 MS was better than WP for both explant establishment and multiplication of  
229 chokecherry (*Prunus virginiana* L.) (Zhang et al., 2000), and a similar effect was  
230 found in the culture establishment of mature wild cherry (Hammatt and Grant,  
231 1997). Culture medium performance also depends on the genotype, thus, in  
232 almond AP medium performed better in the establishment of cultures of the  
233 cultivar Nonpareil, whereas MS medium was preferred for the cultivar Ne Plus  
234 Ultra (Channuntapipat, 2003).

235

236 Intensively pruned plants derived from micropropagation or propagation cuttings  
237 were used in this work, and this pruning treatment has possibly improved the  
238 establishment and multiplication of explants taken from adult plants, which  
239 showed here relatively high values. It was reported that intensive and repetitive  
240 pruning promoted physiological changes that affected the endogenous  
241 polyamine content in hazelnut leaves and buds (Rey et al., 1994) and that these  
242 changes were related with juvenility and rejuvenation.

243

244 In conclusion, micropropagated plants are a better source of explants than  
245 plants propagated by cuttings to initiate and multiply new in vitro cultures. This  
246 can be due to a possible partial rejuvenation of in vitro propagated plants. On  
247 the other hand, the growth and development of explants in culture is affected by  
248 the salt composition of the culture medium in a different way depending of the  
249 micropropagation phase.

250

251 **Acknowledgements**

252 This research was made possible by financial support of Spain's CICYT grants  
253 no. AGF98-0277-C04-01, AGL2001-2414-C04-01 and AGL 2002-03231, and  
254 CONSI+D –DGA P012/2001.

255

256 **References**

- 257 Amomarco, J.B., Vidal, N., Vieitez, A.M., Ballester, A., 1993. Polypeptide  
258 Markers Differentiating Juvenile and Adult Tissues in Chestnut. *J. Plant*  
259 *Physiol.* 142, 117-119.
- 260 Channuntapipat, C., Sedgley, M., Collins, G, 2003. Micropropagation of almond  
261 cultivars Nonpareil and Ne Plus Ultra and the hybrid rootstock Titan x  
262 Nemaguard. *Sci. Hort.* 98, 473-484.
- 263 Devries, D.P., Dubois, L.A.M., 1994. Re-Invigoration of Clonal Rose Rootstocks  
264 by Sustained Micropropagation. *Gartenbauwissenschaft* 59, 81-85.
- 265 Diazsala, C., Rey, M., Rodriguez, R., 1994. Temporary Modification of Adult  
266 Filbert Proliferation Capacity by Sequential Subcultures - Intensive  
267 Pruning as a Pre-Treatment for In vitro Reinvigoration. *J. Hort. Sci.* 69,  
268 673-678.
- 269 Grant, N.J., Hammat, N., 1999. Increased root and shoot production during  
270 micropropagation of cherry and apple rootstocks: effect of subculture  
271 frequency. *Tree Physiol.* 19, 899-903.
- 272 George, E.F. 1993. Plant propagation by tissue culture. Part 1. The technology.  
273 Exegetics Ltd. Edington. 574 pp.

274 Hammatt, N., 1999. Delayed flowering and reduced branching in  
275 micropropagated mature wild cherry (*Prunus avium* L.) compared with  
276 rooted cuttings and seedlings. *Plant Cell Rep.* 18, 478-484.

277 Hammatt, N., Grant N.J., 1993. Apparent Rejuvenation of Mature Wild Cherry  
278 (*Prunus avium* L) During Micropropagation. *J. Plant Physiol.* 141, 341-  
279 346.

280 Hammatt, N., Grant, N.J., 1997. Micropropagation Of Mature British Wild  
281 Cherry. *Plant Cell Tiss. Org. Cult.* 47, 103-110.

282 Hammerschlag, F. A., Scorza, R., 1991. Field performance of micropropagated,  
283 own-rooted peach trees. *J Amer. Soc. Hort. Sci.* 116: 1089-1091.

284 Harada, H., Murai, Y., 1996. Micropropagation Of *Prunus mume*. *Plant Cell*  
285 *Tiss. Organ Cult.* 46, 265-267.

286 Harada, H., Murai, Y., 1998. In Vitro Flowering On Long-Term Subcultured Pear  
287 Shoots. *J. Hort. Sci. Biotech.* 73, 225-228.

288 Howard, B.H., Jones, O.P., Vasek, J., 1989. Long-term improvement in the  
289 rooting of plum cuttings following apparent rejuvenation. *J. Hort. Sci.* 64,  
290 147-156.

291 Jones, O.P., Hadlow, W.C.C., 1989. Juvenile-like character of apple tree  
292 produced by grafting scions and rootstocks produced by  
293 micropropagation. *J. Hort. Sci.* 64, 395-401.

294 Jones, O.P., Webster, C.A., 1989. Improved rooting from conventional cuttings  
295 taken from micropropagated plants of *Pyrus communis* rootstocks. *J.*  
296 *Hort. Sci.* 64, 429-434.

297 Langilier, P., Fournioux, J.C., 2000. Endogenous abscisic acid in juvenile and  
298 adult grape (*Vitis vinifera* L. cv. Pinot noir). *Vitis* 39, 47-48.

299 Lloyd, G., McCown, B., 1980. Commercially feasible micropropagation of  
300 mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc. Int.  
301 Plant Propag. Soc. 30, 421-427.

302 Marín. J.A., Castillo, M., García, E., Andreu, P., 2003. Field performance of  
303 grafted fruit-tree rootstocks was not affected by micropropagation Acta  
304 Hort. 616, 295-299.

305 Marks, T.R., 1991. Rhododendron cuttings. I. Improved rooting following  
306 'rejuvenation' in vitro. J. Hort. Sci. 66, 103-111.

307 Mato, M.C., Mendez, J., Vazquez, A., 1994. Polyphenolic auxin protectors in  
308 buds of juvenile and adult chestnut. Physiol. Plant. 91, 23-26.

309 Meier, K., Reuther, G., 1994. Factors controlling micropropagation of mature  
310 *Fagus sylvatica*. Plant Cell Tiss. Org. Cult. 39, 231-238.

311 Molassiotis, A. N., Dimassi, K., Therios, I., Diamantidis, G., 2003. Fe-EDDHA  
312 promotes rooting of rootstock GF-677 (*Prunus amygdalus* x *P-persica*)  
313 explants in vitro. Biol. Plant. 47, 141-144.

314 Mullins, M.G., Nair, Y., Sampet, P., 1979. Rejuvenation in vitro: Induction of  
315 juvenile characters in an adult clone of *Vitis vinifera* L. Ann. Bot. 44, 623-  
316 627.

317 Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio  
318 assays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.

319 Nowak, B., Mieczynski, K., Hudy, L., 2004. Sugar uptake and utilisation during  
320 adventitious bud differentiation on in vitro leaf explants of 'Wegierka  
321 Zwykla' plum (*Prunus domestica*). Plant Cell Tiss. Organ Cult. 76, 255-  
322 260.

323 Perez-Tornero, O., Burgos, L., 2000. Different media requirements for  
324 micropropagation of apricot cultivars. *Plant Cell Tiss.Organ Cult.* 63, 133-  
325 141.

326 Pliego-Alfaro, F., Murashige, T., 1987. Possible rejuvenation of adult avocado  
327 by graftage onto juvenile rootstocks in vitro. *HortScience* 22, 1321-1324.

328 Pruski, K. W., Lewis, T., Astatkie, T., Nowak, J., 2000. Micropropagation of  
329 chokecherry and pincherry cultivars. *Plant Cell Tiss. Organ Cult.* 63, 93-  
330 100.

331 Quoirin, M., Lepoivre, P., 1977. Etude de milieux adaptes aux cultures in vitro  
332 de Prunus. *Acta Hort.* 78, 437-442.

333 Rey, M., Diazsala, C., Rodriguez, R., 1994. Effect of repeated severe pruning  
334 on endogenous polyamine content in hazelnut trees. *Physiol. Plant.* 92,  
335 487-492.

336 Ruzic, D., Saric, M., Cerovic, R., Culafic, L., 2003. Contents of macroelements  
337 and growth of sweet cherry rootstock in vitro. *Biol. Plant.* 47, 463-465.

338 Sanchez, M.C., Ballester, A., Vieitez, A.M., 1997. Traitements de  
339 rajeunissement de chataigniers adultes. *Ann. Sci. For.* 54, 359 – 370.

340 Thimmappaiah, Shirly, R.A., Sadhana, P.H., 2002. In vitro propagation of  
341 cashew from young trees. *In Vitro Cell. Dev. Biol.-Plant* 38, 152-156.

342 Webster, C.A., Jones, O.P., 1989. Micropropagation of the apple rootstock M.9:  
343 effect of sustained subculture on apparent rejuvenation in vitro. *J. Hort.*  
344 *Sci.* 64, 421-428.

345 Webster, C.A., Jones, O.P., 1992. Performance of Field Hedge and Stoolbed  
346 Plants of Micropropagated Dwarfing Apple Rootstock Clones with  
347 Different Degrees of Apparent Rejuvenation. *J. Hort. Sci.* 67, 521-528.

348 Zhang, Z., Dai, W.H., Cheng, Z.M., Walla, J.A., 2000. A shoot-tip culture  
 349 micropropagation system for chokecherry. J. Environ. Hort. 18, 234-237.

350

351 Table 1. Percentages of both in vitro culture establishment and culture line  
 352 survival, and cumulated number of shoots per culture line of in vitro cultures of  
 353 the clonal rootstock Adesoto 101 (*Prunus insititia*) grown on three culture media  
 354 and initiated from explants taken from micropropagated or from cutting-derived  
 355 trees. Each value is the average of three separate experiments.

356

Propagation method	Culture Medium	Establishment (%)	Cumulated shoots per culture line	Culture line survival (%)			
Cuttings	MS	25.0	11.7	55.0			
	WP	43.8	11.4	84.2			
	QL	28.1	5.1	50.1			
Micropropagation	MS	37.5	22.4	87.7			
	WP	63.9	15.7	94.5			
	QL	54.5	12.0	68.9			
ANOVA		F-value	P-value	F-value	P-value	F-value	P-value
Propagation method		6.3	0.017*	15.6	0.001***	3.8	0.060
Culture Medium		2.9	0.066	6.5	0.005**	2.9	0.069
Method x Medium		0.17	0.840	1.1	0.332	0.45	0.640

357

358

359

360

361

362

363

364

365

366 Figure captions

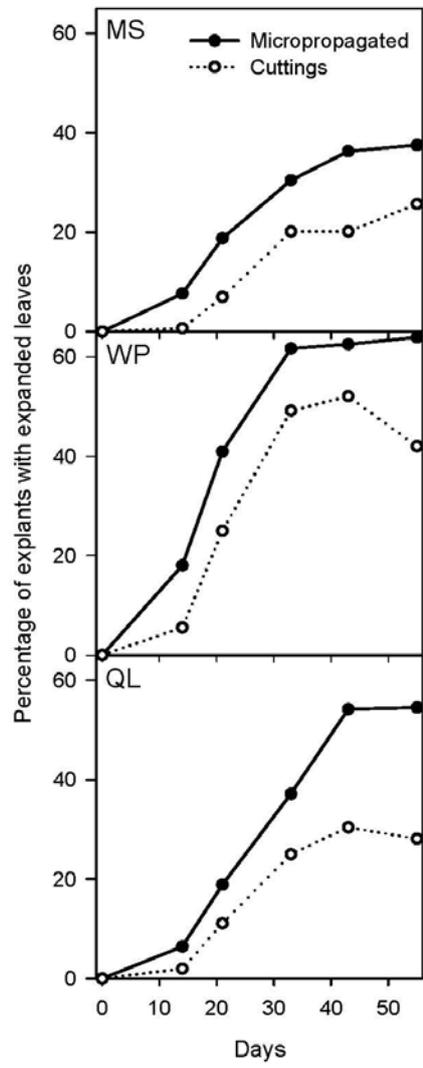
367

368 Figure 1. Evolution of the percentages of one-node explants grown on three  
369 culture media, showing expanding leaves during the establishment of new in  
370 vitro cultures of the clonal rootstock Adesoto 101 (*Prunus insititia*) previously  
371 micropropagated or propagated by cuttings.

372

373 Figure 2. Evolution of the cumulative number of shoots per treatment during  
374 nine subcultures on three culture media, and initiated from explants taken from  
375 micropropagated or from cutting-derived trees of the clonal rootstock Adesoto  
376 101 (*Prunus insititia*).

377



378

379

