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Influence of Photoperiod, Apical Meristem, and Explant Orientation on Axillary Shoot Proliferation of Apple Cultivars in Vitro

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Abstract. **The apple (*Malus domestica* Borkh.) cultivars Empire, McIntosh, Delicious, Triple Red Delicious, and Vermont Spur Delicious were grown on Linsmaier and Skoog (LS) medium containing 4.4 μM BA, 0.5 μM IBA, 1.3 μM GA_3, 87.6 mM sucrose, and 7 g·liter^{-1} Difco Bacto-agar. All cultivars produced significantly more total shoots and shoots >1 cm (usable shoots) on a 16-hr compared to a 24-hr photoperiod. Removing the apical meristem significantly increased both the total and usable shoots only for 'Triple Red Delicious'. Placing explants horizontally on the medium significantly increased the number of total and usable shoots for all cultivars except 'McIntosh'. Internode length was significantly reduced for 'Delicious', 'Triple Red Delicious', and 'Vermont Spur Delicious' and increased for 'Empire' on 24-hr photoperiods. Chemical names used: *N*-(phenylmethyl)-1*H*-purin-6-amine (BA); 1*H*-indole-3-butanoic acid (IBA); gibberellic acid (GA_3).**

Medium components, carbon source, type and source of growth regulators, and environmental conditions are some of the factors that affect shoot proliferation of apple cultivars in vitro (16). Although considerable research has been published on the effects of medium components, carbon sources, and growth regulators, little has been reported on the influence of environmental factors on shoot proliferation. With regard to daylength, 16-hr photoperiods were more productive than 12-hr photoperiods for shoot proliferation of M.26 apple rootstock (13) and 16-hr photoperiods are the most commonly used (16). However, Sriskandarajah et al. (12) found that shoots of 'Delicious' and 'Jonathan' apple from cultures grown with continuous illumination rooted better than those from cultures grown on 16-hr photoperiods.

The hypothesis that apical dominance in plants is due to the inhibiting effect of the apex on the lateral buds, mediated either directly or indirectly by auxin, is well-known (2, 10). Lane (4) showed that removal of the apical tip of pear shoots, followed by placement of the shoot horizontally or inverted in the medium, stimulated growth of axillary buds. Apple shoot-tip explants routinely have been placed horizontally on the medium in our laboratory, but this technique never has been compared quantitatively with vertical placement.

Cultivar differences in growth and rooting are known to exist (16), and previous studies have shown that 'McIntosh' was generally easier to root than 'Delicious' (19), as well as easier to proliferate (R.H.Z. and I.F., unpublished observations). In addition, preliminary trials indicated that 'Empire' also might proliferate and root readily. Since 'Empire' resulted from the cross 'McIntosh' x 'Delicious', we thought that a comparison of these cultivars would be of interest.

This study was conducted 1) to determine the effect of photoperiod, meristem removal, and explant orientation on axillary shoot proliferation of apple cultivars; 2) to determine the comparative shoot proliferation of 'Empire', 'McIntosh', and 'Delicious'; and 3) to compare the in vitro shoot proliferation potential of several 'Delicious' strains.

Materials and Methods

Actively growing shoot tips from 'McIntosh', 'Empire', 'Delicious', 'Triple Red Delicious', and 'Vermont Spur Delicious' apples were obtained from in vitro cultures established using previously published methods (17, 18). They were grown on LS medium (6) supplemented with 4.4 μM BA, 0.5 μM IBA, 1.3 μM GA_3, 87.6 mM sucrose, and 7 g·liter^{-1} Difco Bacto-agar. The pH was adjusted to 5.2 prior to adding agar, and 150 ml of medium was dispensed into 500-ml (80 × 90 mm) glass jars prior to autoclaving for 20 min at 121°C and 1.1 kg·cm^{-2}.

Shoot tips ≈1.5 cm long were collected from the proliferating mother cultures and were explanted in glass-covered jars, which then were sealed with high-cling polyvinylchloride film. One to 2 mm of the shoot apex was removed from one-half of the shoots just before explanting. Ten explants with or without the apical meristem were placed in each jar, with the explants placed onto the medium vertically in half the jars and horizontally in the other half. Twenty shoot tips, except 30 shoot tips for 'Triple Red Delicious', were used for each treatment with individual jars serving as replicates.

Cultures were grown for 4 weeks in 16-hr (culture room) or 24-hr (growth chamber) photoperiods, at a photon flux of about 60 μmol·s^{-1}·m^{-2} provided by warm white fluorescent tubes. Temperature was maintained at a constant 25° ± 1°C.

At the end of the culture period, all shoots were counted: the number of shoots <1 cm (defined as small shoots), shoots between 1 cm and 2.5 cm (medium shoots), and shoots >2.5 cm

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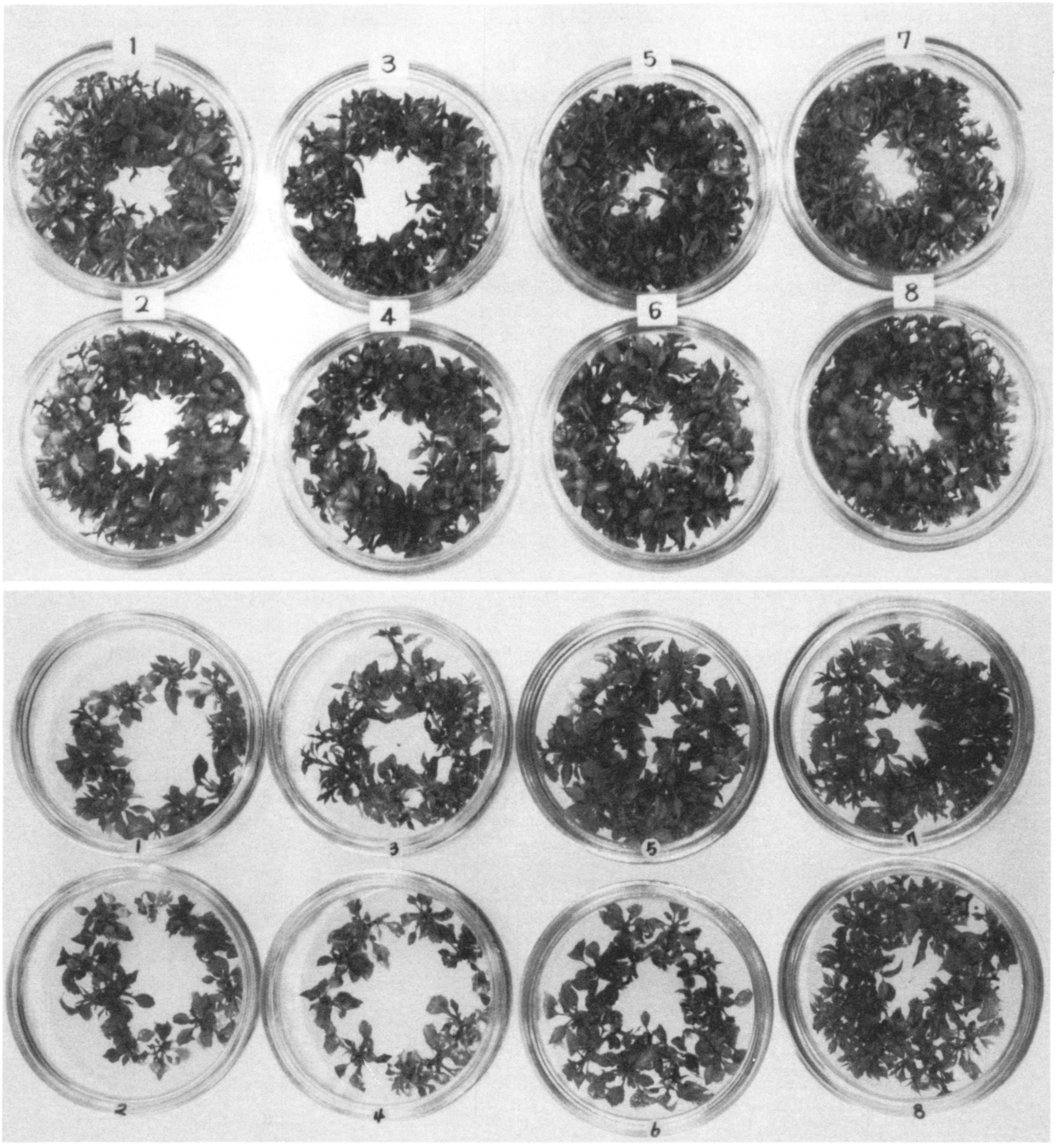

Fig. 1. Shoot proliferation of 'McIntosh' (**top**) and 'Triple Red Delicious' (**bottom**) after 4 weeks of treatment. Treatment codes are: photoperiod—16-hr: 1, 3, 5, and 7; 24-hr: 2, 4, 6, and 8; apical meristem removal—meristem present: 1, 2, 5, and 6; meristem removed: 3, 4, 7, and 8; shoot orientation—vertical: 1, 2, 3, and 4; horizontal: 5, 6, 7, and 8.

(long shoots). Shoots >1 cm were considered usable shoots and the number of total shoots was calculated as the sum of small shoots and usable shoots. Only data on total and usable shoots are presented. Average internode length was calculated by measuring the length of three to five nodes of typical shoots from the node having the youngest fully expanded leaf to the shoot base.

A factorial combination of treatments was used in these experiments. Data were analyzed by analysis of variance using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS)(11).

Results

Cultivar comparison. 'Empire' and 'McIntosh' produced more shoots than any of the 'Delicious' strains, and gross differences among cultivars and treatments were clearly visible (Fig. 1). Little difference was found among 'Delicious' types, although 'Vermont Spur Delicious' tended to be the most prolific and 'Triple Red Delicious' the least prolific. Thus, the potential proliferation of 'Empire' was found to be similar to that of its seed parent, 'McIntosh'.

Photoperiod. The numbers of total shoots and usable shoots

Table 1. Effect of photoperiod on shoot proliferation and elongation of apple cultivars after 4 weeks of culture.

Cultivar	Photoperiod (hr)	Total no. shoots	No. usable shoots	Internode length (mm)
McIntosh	16	17.6	11.6	2.6
	24	12.0	8.2	2.8
		***	**	NS
Empire	16	18.0	9.7	2.6
	24	11.4	6.6	2.9
		***	***	*
Delicious	16	10.5	4.7	2.3
	24	5.2	2.4	1.6
		***	***	**
Triple Red Delicious	16	8.0	2.7	1.9
	24	4.2	1.1	1.6
		***	***	**
Vermont Spur Delicious	16	10.9	5.9	2.4
	24	8.1	3.8	1.9
		**	*	**

NS,*, **, ***Nonsignificant or significantly different at the 5%, 1%, or 0.1% levels, respectively.

Table 2. Effect of spinal meristem removal on shoot proliferation and elongation of apple cultivars after 4 weeks of culture.

Cultivar	Meristem[z]	Total no. shoots	No. usable shoots	Internode length (mm)
McIntosh	+	15.2	10.5	3.0
	−	14.4	9.3	2.5
		NS	NS	**
Empire	+	15.0	8.8	2.9
	−	14.4	7.5	2.6
		NS	*	*
Delicious	+	7.4	3.3	1.9
	−	8.2	3.7	2.0
		NS	NS	NS
Triple Red Delicious	+	5.5	1.7	1.9
	−	6.8	2.6	1.7
		**	**	NS
Vermont Spur Delicious	+	9.1	4.7	2.2
	−	9.8	5.0	2.1
		NS	NS	NS

[z]+ = present, − = removed.
NS, *, **Nonsignificant or significantly different at the 5% or 1% levels, respectively.

were significantly greater on a 16-hr than on a 24-hr photoperiod for all cultivars (Table 1). Internode length was reduced significantly for 'Delicious', 'Triple Red Delicious', and 'Vermont Spur Delicious' and increased for 'Empire' on the 24-hr photoperiod. For 'McIntosh', the internode length was not significantly influenced by photoperiod. A slight leaf chlorosis that developed in the 'Delicious' strains was more severe in the 24-hr than in the 16-hr photoperiod.

Apical meristem removal. Removing the apical meristem significantly increased the total number of shoots and usable shoots of 'Triple Red Delicious', but significantly reduced the number of usable shoots on 'Empire' (Table 2). No effects were found with the other three cultivars. Meristem removal reduced the internode length significantly on 'McIntosh' and 'Empire', but had no effect on the other cultivars.

Explant orientation. The proliferation of total and usable shoots from horizontally placed explants of 'Empire', 'Delicious', 'Triple Red Delicious', and 'Vermont Spur Delicious' was signif-

Table 3. Effect of explant orientation on shoot proliferation and elongation of apple cultivars after 4 weeks of culture.

Cultivar	Explant orientation[z]	Total no. shoots	No. usable shoots	Internode length (mm)
McIntosh	V	14.1	9.1	2.7
	H	15.5	10.7	2.7
		NS	NS	NS
Empire	V	13.6	7.4	2.7
	H	15.8	8.9	2.8
		*	*	NS
Delicious	V	5.4	2.6	1.8
	H	10.3	4.5	2.1
		***	***	NS
Triple Red Delicious	V	3.8	1.4	1.8
	H	8.4	2.9	1.8
		***	***	NS
Vermont Spur Delicious	V	7.6	3.9	2.1
	H	11.4	5.9	2.2
		***	*	NS

[z]V = Vertical; H = horizontal.
NS, *, ***Nonsignificant or significantly different at the 5% or 0.1% levels, respectively.

Table 4. Interaction of photoperiod and explant orientation on total number of shoots and internode length after 4 weeks of culture.

Cultivar	Photoperiod (hr)	Explant orientation		Significance
		Vertical	Horizontal	
		Total no. of shoots		
Delicious	16	6.7	14.3	***
	24	4.0	6.3	
Triple Red Delicious	16	4.9	11.1	**
	24	2.8	5.7	
		Internode length (mm)		
Empire	16	2.5	2.8	*
	24	3.0	2.8	
Triple Red Delicious	16	1.8	2.1	*
	24	1.8	1.4	

*, **, ***Interaction significant at the 5%, 1%, or 0.1% levels, respectively.

icantly greater than from vertically placed ones (Table 3), although the magnitude of the increase was smaller for 'Empire'. Internode elongation was not affected by explant orientation.

Interaction. A significant interaction between photoperiod and explant orientation occurred with two cultivars, both for the total number of shoots and for internode length (Table 4). For 'Delicious' and 'Triple Red Delicious', horizontal placement of the explants had a far greater effect on shoot proliferation on the 16-hr than on the 24-hr photoperiod. In contrast, the effect on internode length was inconsistent, with photoperiod having no effect on horizontally placed explants for one cultivar and vertically placed explants for the other. With 'Delicious', apical meristem removal had no effect on explants grown on the 16-hr photoperiod, but greatly increased total and usable shoots grown with continuous light (Table 5).

Discussion

For all cultivars tested, cultures proliferated under a 16-hr photoperiod produced more shoots than those on a 24-hr photoperiod, as found also for an azalea selection (1). Although a direct comparison of these two photoperiods has not been reported previously for apple shoot proliferation, nearly all reports indicated the use of a 16-hr photoperiod (3, 5, 14–18). No basis could be found here for extending the photoperiod to 24 hr, as has been done for some apple cultivars (12) and other crops (7–9). 'Delicious' and its strains tend to senesce in vitro sooner than 'McIntosh', 'Empire', and most other apple cultivars. This senescence sometimes necessitates the subculture of 'Delicious' and its strains after 3, or even 2, weeks in contrast to the normal schedule of every 4 weeks. The leaf yellowing and necrosis at

Table 5. Interaction of photoperiod and apical meristem status on total and usable shoots after 4 weeks of culture.

Cultivar	Photoperiod (hr)	Apical meristem		Significance
		Present	Removed	
		Total no. of shoots		
Delicious	16	10.7	10.4	*
	24	4.2	6.1	
		No. of usable shoots		
Delicious	16	4.9	4.5	*
	24	1.6	3.0	

*Interaction significant at the 5% level.

the base of the petiole, characteristic of this senescence, increased on the 24-hr photoperiod.

Proliferation of axillary shoots on pear can be increased by decreasing or eliminating apical dominance on the original shoot explant (4). This effect was tested here in apple by removing the apical 1 to 2 mm of half of the shoots and by comparing horizontal vs. vertical orientation of the explants. Removing the apical meristem had virtually no effect on axillary shoot proliferation. However, horizontal placement of the explant on the medium greatly increased shoot proliferation, particularly for 'Delicious', 'Triple Red Delicious', and 'Vermont Spur Delicious'. A smaller increase was found with 'Empire'. It is interesting to note that 'Delicious' and its strains tend to be vigorous, upright trees with generally narrow crotch angles and relatively few lateral branches, especially on young, field-grown trees, indicating that these cultivars have strong apical dominance. On the other hand, 'McIntosh' and 'Empire' tend to be spreading, with more branching and wider crotch angles. In these experiments, 'Empire' responded more like the seed parent 'McIntosh' than its pollen parent 'Delicious'.

Literature Cited

1. Economou, A.S. and P.E. Read. 1982. Effect of light duration and intensity on microcutting production and rooting of a deciduous azalea. Vol. II, 21st Intl. Hort. Congr. 2:1863. (Abstr.)
2. Hillman, J.R. 1984. Apical dominance, p. 127–148. In: M.B. Wilkins (ed.). Advanced plant physiology. Pitman, London.
3. Hutchinson, J.F. 1984. Factors affecting shoot proliferation and root initiation in organ cultures of the apple 'Northern Spy'. Scientia Hort. 22:347–358.
4. Lane, W.D. 1979. Regeneration of pear plants from shoot meristem-tips. Plant Sci. Lett. 16:337–342.
5. Lane, W.D. and J.M. McDougald. 1982. Shoot tissue culture of apple: comparative response of five cultivars to cytokinin and auxin. Can. J. Plant Sci. 62:689–694.
6. Linsmaier, E.M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18:100–127.
7. Lloyd, G. and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. Comb. Proc. Intl. Plant Prop. Soc. 30:421–427.
8. McCown, B. and R. Amos. 1979. Initial trials with commercial micropropagation of birch selections. Comb. Proc. Intl. Plant Prop. Soc. 29:387–393.
9. McCown, B.H. and G.B. Lloyd. 1983. A survey of the response of *Rhododendron* to *in vitro* culture. Plant Cell Tissue Organ Cult. 2:77–85.
10. Phillips, I.D.J. 1969. Apical dominance, p. 163–202. In: M.B. Wilkins (ed.). The physiology of plant growth and development. McGraw-Hill, London.
11. SAS Institute Inc. 1982. SAS user's guide: statistics, 1982 edition. SAS Institute, Cary, N.C.
12. Sriskandarajah, S., M.G. Mullins, and Y. Nair. 1982. Induction of adventitious rooting in vitro in difficult-to-propagate cultivars of apple. Plant Sci. Lett. 24:1–9.
13. Standardi, A. 1979. Indagine preliminare sull influenza della luce nella micropropagazione di alcune specie legnose. Tecniche di Colture In Vitro per la Propagazione su Vasta Scala delle Specie Ortoflorofrutticole. Pistola, Italy. p. 107–118.
14. Werner, E.M. and A.A. Boe. 1980. In vitro propagation of Malling 7 apple rootstock. HortScience 15:509–510.
15. Zimmerman, R.H. 1984. Rooting apple cultivars in vitro: interactions among light, temperature, phloroglucinol, and auxin. Plant Cell Tissue Organ Cult. 3:301–311.
16. Zimmerman, R.H. 1984. Apple, p. 369–395. In: W.R. Sharp, D.A. Evans, P.V. Ammirato, and Y. Yamada (eds.). Handbook of plant cell culture. Vol. 2, Crop species. Macmillan, New York.
17. Zimmerman, R.H. and O.C. Broome. 1980. Apple cultivar micropropagation, p. 54–58. In: Proceedings conference on nursery production of fruit plants through tissue culture—applications and feasibility. USDA Sci. & Educ. Adm., Agr. Res. Results ARR-NE-11.
18. Zimmerman, R.H. and O.C. Broome. 1981. Phloroglucinol and in vitro rooting of apple cultivar cuttings. J. Amer. Soc. Hort. Sci. 106:648–652.
19. Zimmerman, R.H. and I. Fordham. 1985. Simplified method for rooting apple cultivars in vitro. J. Amer. Soc. Hort. Sci. 110:34–38.

CORRIGENDA

In the article "Weight Loss in Sweet Potatoes During Curing and Storage: Contribution of Transpiration and Respiration", by David H. Picha (*J. Amer. Soc. Hort. Sci.* 111:889–892, November 1986), Figs. 1 and 2 were reversed. The correct figures and captions are printed below.

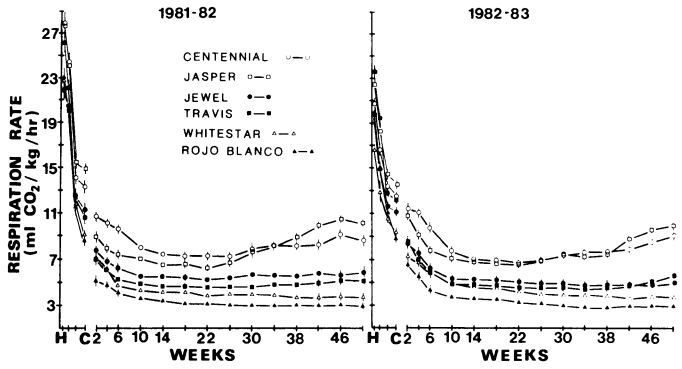

Fig. 1. Respiration rate (fresh weight basis) of 6 sweet potato cultivars at harvest (H), after curing (C), and during 50 weeks of storage at 15.6°C, 90% RH. Each point represents the average of 8 roots. Vertical bars represent SE of the mean and, when absent, fall under the symbol.

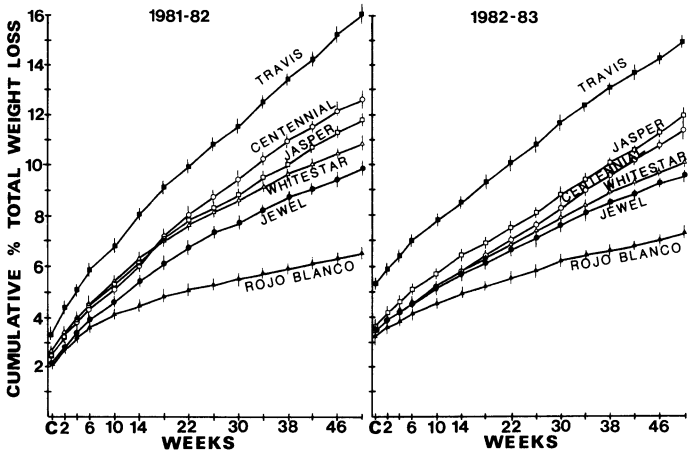

Fig. 2. Cumulative percentage of total weight loss in 6 sweet potato cultivars after curing (C) and during 50 weeks of storage at 15.6°C, 90% RH. Each point represents the average of 60 individual roots. Vertical bars represent SE of the mean.

In the article "Relationship of Harvest Date, Storage Conditions, and Fruit Characteristics to Bruise Susceptibility of Apple", by Joshua D. Klein (*J. Amer. Soc. Hort. Sci.* 112:113–118, January 1987), the last paragraph of Materials and Methods, sixth line, should read "...of filtered extract rapidly with 1 ml of 0.1 M catechol...", not 0.1 ml.