FRUITING CUTTINGS: REVISED METHOD FOR PRODUCING TEST PLANTS OF GRAPEVINE CULTIVARS

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ABSTRACT

The inflorescences of hardwood cuttings of the grapevine usually shrivel and die soon after bud burst. The original method developed by Mullins (1) for producing small fruiting cuttings for use as experimental plants has now been improved. Cuttings are rooted in a heated container (26°C at bases of cuttings) in a cold room (4°C). Buds remain dormant. After four weeks the rooted cuttings are transferred to the glasshouse or to growth rooms (27°C day and 22°C night, 16 h photoperiods, irradiance 60 Wm^{-2}). At bud burst, leaves basal and adjacent to inflorescences are removed as soon as accessible and the shoot tip is excised. These treatments promote inflorescence growth. A lateral shoot is permitted to grow from an axillary bud of one of the defoliated nodes on the main axis. When four to six leaves have been produced the lateral shoot is tipped and disbudded. Development of the bunch to maturity is supported by the foliage of the lateral shoot. Véraison occurs at approximately 12 weeks from bud burst and ripe fruit is produced in 16 to 18 weeks. The ripe berries of test plants are about half normal size but seed is highly germinable. A list is given of cultivars which are easy, intermediate or difficult to propagate as test plants.

Some of the technical difficulties inherent in experimental work on flowering and fruiting in the grapevine, such as the large size of fruiting plants and the annual occurrence of the crop, can be overcome by using miniaturized plants of the commercially-important cultivars. A method was developed by Mullins (1) for production of fruiting test plants from hardwood cuttings. These test plants are raised from cold-stored (4°C) dormant canes and can easily be accommodated in standard glasshouses and controlled-environment cabinets. The use of test plants overcomes the dependence of research on fruiting upon field-grown material and the natural seasons, and a supply of fruiting plants can be produced on a year-round basis.

The original method has been substantially modified and improved over the years and extensive use has been made of fruiting cuttings in grapevine research in this Department and elsewhere. Details of the improved method have not been published hitherto.

MATERIALS AND METHODS

Uniform hardwood cuttings (four to six nodes) are collected at pruning time from well-ripened dormant canes. The cuttings are sealed in plastic bags and stored under refrigeration (4°C) until required.

The production of fruiting cuttings is founded on two basic procedures — pre-rooting and pruning. First, the cuttings are propagated by a technique which ensures that formation of adventitious roots precedes bud burst. Cuttings are grown in a thermostatically-controlled heated container (26°C at the bases of the cuttings) in a cold room (4°C). Perlite is used as the rooting medium (80 to 100 mm above the heating coil) and it is kept moist by spraying with water on alternate days. Roots are produced by the cuttings (Fig. 1A) but the buds remain dormant (Fig. 1B). This treatment is termed "pre-rooting". Pre-rooted cuttings are planted in pots containing a mixture of perlite, vermiculite and peat (6:3:1), and are transferred to the glasshouse or to growth rooms.

Next, the leaves borne proximal to, and adjacent to, the inflorescence(s) are removed as soon as they are accessible at bud burst (Fig. 1C, 1D). Later, the shoot tip is excised (Fig. 2A) so that an inflorescence is in a terminal position (Fig. 2B) on the defoliated shoot. More than one inflorescence may be retained if required. Finally, a lateral shoot is permitted to grow from one of the axillary buds proximal to the inflorescence. This shoot provides the leaves to support the subsequent growth of the bunch (Fig. 2C, 2D). Véraison, the first appearance of anthocyanin, occurs in colored grapes at approximately 12 weeks from bud burst and ripe fruit is produced in 16 to 18 weeks.

Nutrients are provided on alternate days by irrigation with half-strength Hoagland solution. Further details are given in Results.

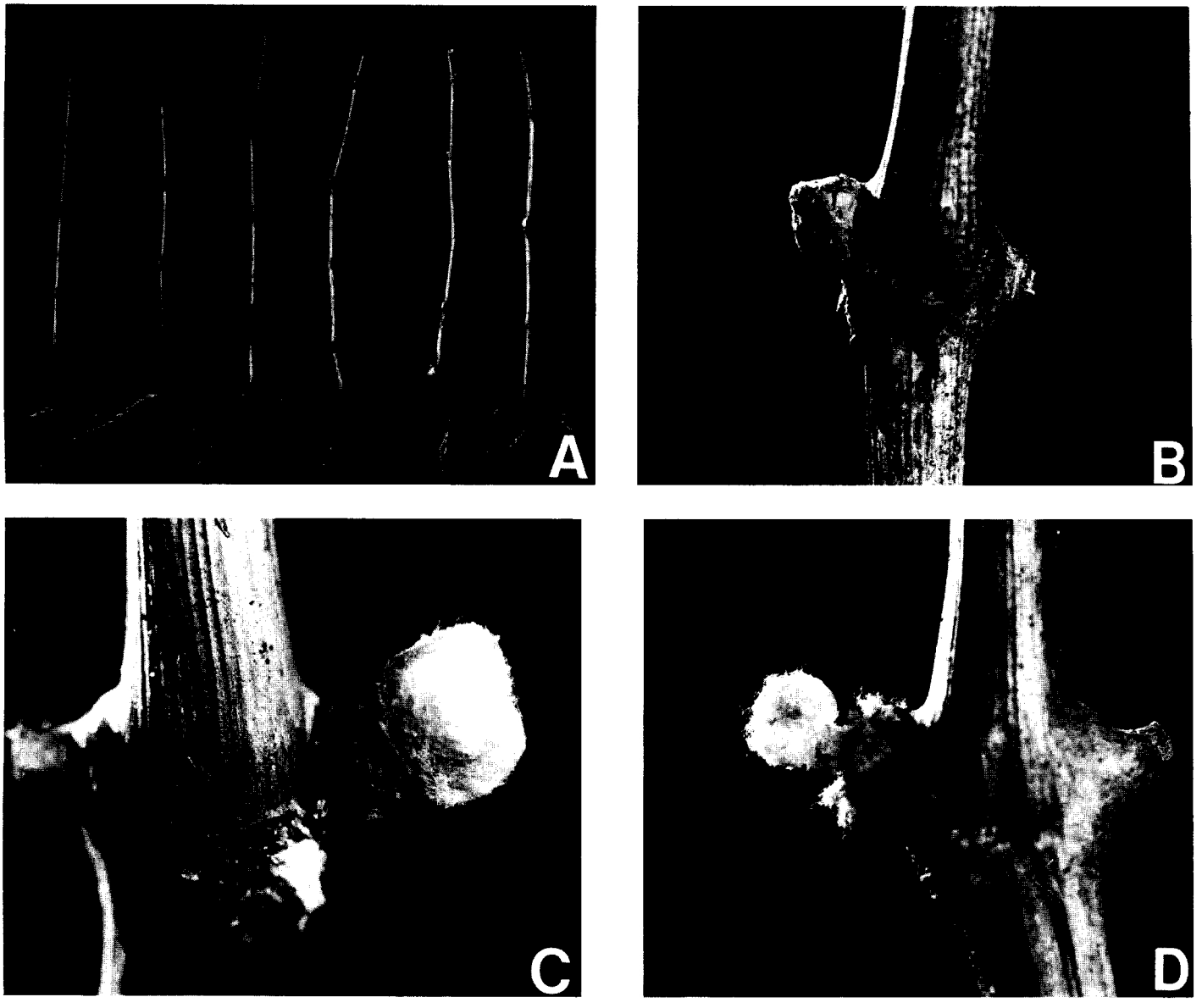

Fig. 1. A, four-node cuttings of Cabernet Sauvignon showing typical root development on removal from heated container (27°C) after four weeks; B, dormant bud in a rooted cutting; C, stage of bud burst at which leaves must be removed to ensure retention of inflorescences. Photographed 10 days after planting; and D, the same bud as in 1C photographed after removal of basal leaves. Note the inflorescence.

RESULTS

Effects of time of pre-rooting on retention of inflorescences: Cuttings of Cabernet Sauvignon were treated in the heated container in the cold room for 2, 3, 4, 5 or 6 weeks and were then grown in a temperature-controlled glasshouse (27°C day, 22°C night) with natural illumination.

Upon removal from the cold room, cuttings which had been treated for three weeks or longer all bore four to six roots. Prolonged treatment of cuttings in the heated container (four to six weeks) gave plants with longer roots than short-term treatment (Table 1). The standard leaf and shoot tip removal treatment was applied at bud burst and 25 days later measurements were made of the maximum length of inflorescences. Well-grown inflorescences were born by cuttings which were pre-rooted for three to six weeks and which possessed roots when planted in the glasshouse. Without exception, the inflorescences of rootless cuttings (two weeks' treatment) shrivelled and died within 25 days of bud burst. The success rate in producing test plants, as indicated by the percentage of plants with inflorescences at anthesis, was low (40 to 50%) even in pre-rooted plants, and there was much variation in the number of flowers per inflorescence (600 to 1200).

In a related set of experiments cuttings were treated with Indole butyric acid (IBA) by the quick-dip method (1500 to 2000 ppm) before planting in the heated container. IBA-treatment induced large numbers of roots but there was no significant improvement in inflorescence growth and fruit-set as compared with untreated cuttings.

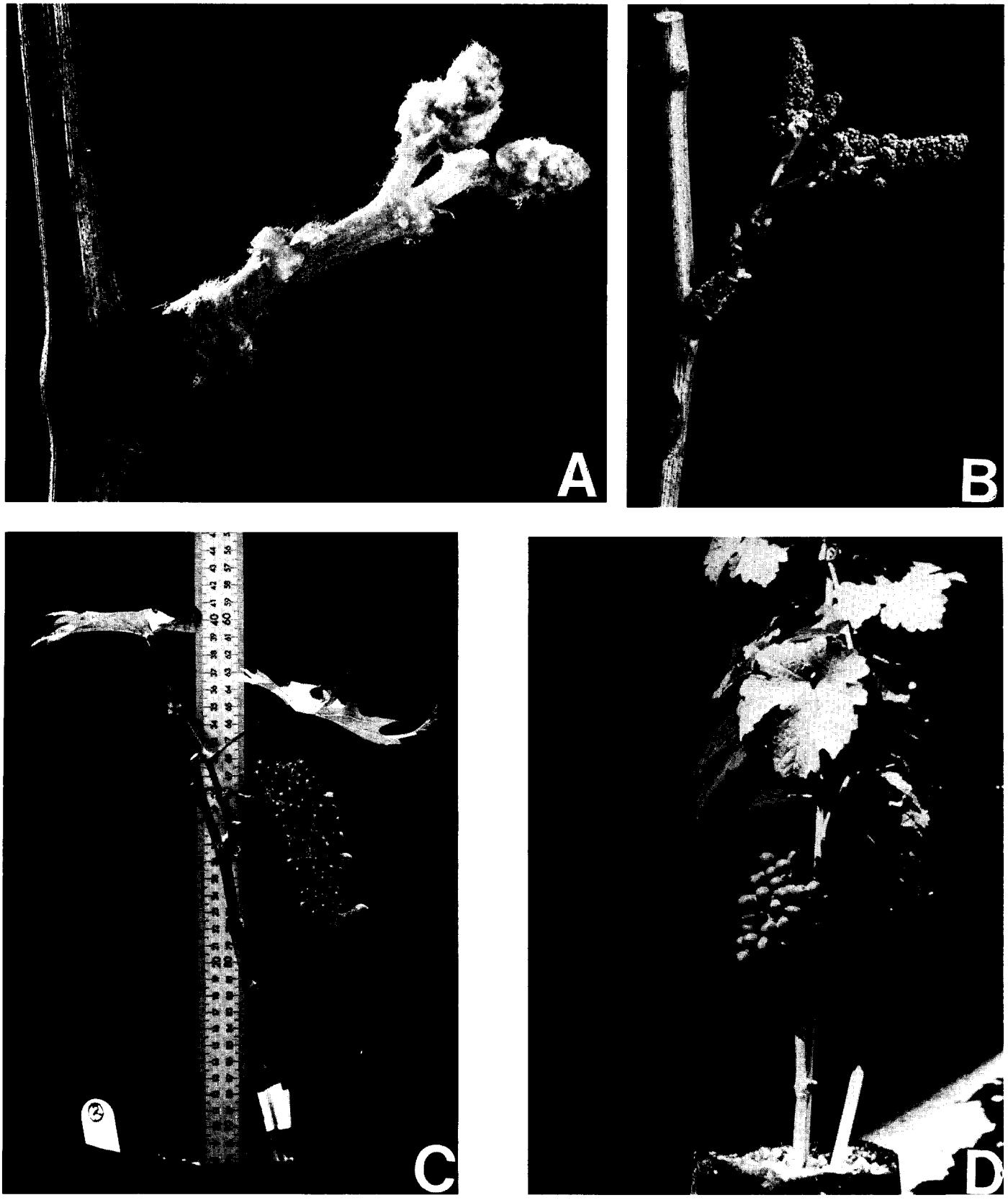

Fig. 2. A, three days after removal of basal leaves. Note that the shoot tip has also been removed so as to promote the growth of the inflorescence; B, 20 days after leaf removal (cv. Grenache); C, a test plant of cv. Cabernet Sauvignon with developing bunch after 45 d of leaf removal. Few leaves are retained in the plant after fruit set; and D, a test plant of cv. Cabernet Sauvignon with a ripe bunch of grapes. Photographed 16 weeks after bud burst.

Table 1. Effect of time of pre-rooting on inflorescence growth.

Time in heated container	No. of roots (> 3 mm)	Max. length of roots (mm)	Max. length of inflorescence (mm)[a]	% plants with inflorescence surviving to anthesis
2 weeks	—	—	—	—
3 weeks	4.7 ± 1.3	23 ± 3	56 ± 8	40
4 weeks	4.5 ± 0.2	56 ± 4	48 ± 5	40
5 weeks	6.2 ± 0.8	79 ± 10	60 ± 5	50
6 weeks	4.6 ± 0.6	61 ± 4	58 ± 1	50

[a]Measured 25 days after bud burst.

Effect of temperature on inflorescence growth and fruit-set: Uniform pre-rooted cuttings (four weeks) of Cabernet Sauvignon were grown in individual temperature-controlled units in a research glasshouse. There were four temperature regimes, i.e., 25°C, day/19°C night, 27°/22°, 30°/25° and 33°/28°C. The experiments were made in spring and early summer (October-November) and plants were grown with natural illumination. The standard defoliation and pruning treatments were applied at bud burst. Bud opening and the initial growth of inflorescences were more rapid at 33°/28°C than at lower temperatures, but subsequent growth of inflorescences at 33°/28° was abnormal and they shrivelled and died soon after anthesis (Table 2). There were only minor differences in inflorescence growth and fruiting among plants grown at 24°/19°, 27°/22° and 30°/25°C. Greatest numbers of berries were borne on plants grown at 27°/22°C (about 60). The berries from all the fruiting cuttings were small, about half the normal size, and they contained either one or two seeds. (The normal seed number is four).

Table 2. Effect of temperature on fruiting of test plants of Cabernet Sauvignon.

	Temperature regimes (°C)				L.S.D.
	24/19	27/22	30/25	33/28	P = 0.05
Days to bud burst	12.8	9.8	9.7	4.3	5.2
Days to flower separation	20.0	14.8	14.4	8.7	6.4
Days to anthesis	37.7	33.3	27.8	—	3.7
No. of plants with inflorescences surviving at anthesis (out of 25)	21	22	19	—	—
Mean length of inflorescence 25 d after bud burst (mm)	53.9	56.3	58.0	—	n.s.
Mean No. of berries per bunch (> 5 mm diameter)	42.08	58.14	48.2	—	7.8

Effect of light on inflorescence development and fruiting: Cabernet Sauvignon cuttings were pre-rooted as before and grown in a temperature controlled glasshouse (27°C day and 22°C night) up to the stage of bud burst. Immediately after removal of the shoot tip and basal leaves the plants were allocated to five treatments comprising differing growing conditions: 1) Glasshouse with natural illumination (spring and summer), 2) Glasshouse - shades applied to inflorescences and leaves, 3) Growth Cabinet with continuous illumination, 4) Growth Cabinet with short days (8 h), and 5) Growth Cabinet with long days (16 h). In all treatments the temperature was maintained at 27°C day and 22°C night. The Growth Cabinets were illuminated with a mixture of fluorescent and incandescent lights and the irradiance at the level of the shoot tips was maintained at 60 Wm^{-2}.

The inflorescences of plants which were subjected either to shading treatments or to continuous illumination did not survive at anthesis. In all the other treatments, survival of inflorescences was in excess of 70%. Fruit production by cuttings was greatest with photoperiod of 16 h (Table 3).

Table 3. Effects of light on inflorescence retention and fruiting.

	Natural illumination (spring/summer)	Growth cabinet	
		Short day (8 h)	Long day (16 h)
No. cuttings with inflorescences at anthesis (out of 25)	18	21	22
Mean No. berries per bunch (> 5 mm diameter)	49.5 ± 4.7	54.1 ± 8.5	64.9 ± 10.6

Effects of disbudding and defoliating the newly-formed lateral shoots on fruit production in pre-rooted, pruned cuttings of Cabernet Sauvignon: In the production of fruiting test plants the apex and the leaves of the main axis are removed and inflorescence development is supported by the foliage of a lateral shoot which arises from a proximal (defoliated) node.

Cuttings of Cabernet Sauvignon were pre-rooted (four weeks) and were defoliated and pruned at bud burst in the usual way. The plants were grown in a controlled environment cabinet (27°C, irradiance 40 to 60 Wm^{-2}, photoperiod 16 h).

At seven days before anthesis, when the lateral shoots each bore seven to nine expanded leaves, the plants were made into four groups. One of the groups was a control in which no further manipulations were applied to the plants. Plants of the other three groups were defoliated so that the lateral shoots bore 2, 4, or 6 leaves, respectively. At the same time, all axillary buds on the lateral shoot were removed and the apex was excised. The object of this treatment was to prevent regrowth at defoliated nodes and to prevent the production of new leaves.

In these experiments the greatest production of fruit was found in cuttings in which the lateral shoots bore four leaves. Cuttings with two or six leaves did not differ significantly in their fruiting but all treatments were greatly superior to the control in which elongation of the lateral shoot and production of new leaves was allowed to proceed unchecked (Table 4).

Germination of seed from test plants: Seed lots of Grenache were obtained from a commercial winery and from fruit from the Viticulture Research Station, NSW Department of Agriculture, Griffith. Germination in this normally grown open-pollinated material was compared with the germination of seed from test plants of Grenache grown in a controlled environment

Table 4. Effects of leaf number on the newly-formed lateral shoot on fruiting of test plants of Cabernet Sauvignon.[a]

No. leaves	No. ripe berries
Control	11.6 ± 2.1
2 leaves	70.4 ± 13.0
4 leaves	98.5 ± 11.4
6 leaves	71.0 ± 12.0

[a]Mean of five replicates.

cabinet (Table 5). In further experiments open-pollinated seed was collected from the fruit of a hybrid grapevine *Vitis vinifera* × *V. rupestris*. The vine concerned is primarily a male but it produces a few bunches in some seasons. Germination of this seed was compared with that of seed from test plants grown from cuttings from the same grapevine. The inflorescences of the test plants were feminized by cytokinin treatment according to the method of Negi and Olmo (5). In all experiments the germinability of seed from test plants was greatly superior to that of seed from conventionally grown grapevines (Table 5).

Table 5. Seed germination: comparison of seed from test plants with seed from normally grown grapevines.

	No. seeds sown	% germination
Grenache		
Normal vines		
Commercial source	120	23
Research station	100	36
Test plants	75	61
V. vinifera × *V. rupestris*		
Normal vines	68	61
Test plants	225	82

Seeds were stratified (4°C) in moist sand for six weeks and were germinated (27°C) in a peat:perlite mixture (1:1). Records were taken four weeks after sowing.

The influence of genotype on ease of production of fruiting test plants: Experience with test plants is that the technique is more reliable in some cultivars than in others. Three groups can be distinguished: "Easy", (> 50% of cuttings produce bunches), "Intermediate" (30 to 50% of cuttings produce bunches), and "Difficult" (< 30% of cuttings produce bunches). This classification (Table 6) applies only to cuttings from canes which have been cold stored for less than 12 months. In the second year of storage the success rate is halved, and the inflorescences of cuttings which have been stored for three years are seldom retained.

Table 6. Influence of genotype on ease of production of fruiting test plants.

Easy - greater than 50% cuttings produce fruit	Intermediate - 30-50% of cuttings produce fruit	Difficult - Less than 30% of cuttings produce fruit
Palomino	Shiraz	Sultana
Cabernet Sauvignon	Riesling	Muscat Gordo
Grenache	Italia	Kattakourgan (♀)
Traminer	Muscat Ottonell	Grenache × Cabernet Sauvignon (♀)
Villard noir	Black Monukka	
Villard blanc	Red Emperor	Cabernet Sauvignon × Sultana (♀)
Campbell's Early	Chardonnay	
V. rupestris × *V. vinifera*	Semillon	Sumull × Cabernet Sauvignon (♀)
Johannes Seyve 23-416	*V. longii* (♀)	Sideritis
(*V. rupestris* × *V. cinerea*) × *V. vinifera*	Sumull × Cabernet Franc (♀)	
V. cinerea (♀)		
V. cinerea (♂)[a]		
V. longii (♂)[a]		
V. rupestris (♂)[a]		
V. riparia (♂)[a]		

[a]Feminized by cytokinin.

DISCUSSION

The production of fruiting test plants is based on treatments which prevent the shedding of inflorescences (1). Most of the lateral (latent) buds on dormant one-year-old vine canes contain inflorescence primordia. Rooted cuttings have the potential to produce bunches of grapes but this potential is seldom realized because inflorescence growth usually stops soon after bud burst. In cuttings the inflorescence fails to elongate and growth and development of the flowers is attenuated. These incompletely-developed inflorescences shrivel and drop off within a few weeks of bud burst.

Information on the hormonal control of inflorescence growth in the grapevine is now available which explains the failure of inflorescence development in cuttings. Differentiation of flowers from the inflorescence primordia occurs at the time of bud burst and is a cytokinin-requiring process (2,3,4,11). In an established grapevine, cytokinin is synthesized in roots (9) and is translocated to the developing inflorescence in the ascending sap (9,10). In cuttings, bud burst precedes the formation of a root system and cytokinin, from reserves in the cane (8), is a limiting factor to inflorescence differentiation. Moreover, expanding leaves are stronger sinks for endogenous cytokinin than the young inflorescences (3,4). In short, atrophy of inflorescences in vine cuttings is a result of cytokinin deficiency.

Inflorescences of grapevine cuttings are retained when treated with exogenous cytokinin (2) but this method of producing fruiting plants introduces confounding factors into experiments concerned with endogenous compounds or synthetic growth regulators. In the original method, and in its subsequent modifications, manipulation of growing conditions and pruning are used to promote the development of inflorescences.

It is likely that the pre-rooting treatment (Table 1) augments the endogenous cytokinin supply to the expanding bud and that removal of expanding leaves and apices causes cytokinin to be diverted to inflorescence growth. Together these treatments permit the production of bunches of grapes by vine cuttings.

The environment under which the pre-rooted cuttings are grown has a marked effect on inflorescence and fruit production. The most favorable conditions include a day/night temperature of 27°C/22°C, long days (16 h) and a high light intensity (Tables 2, 3). As at the time of bud burst, leaf growth and shoot growth in the

established cutting have an inhibitory effect on bunch development and a second defoliation treatment is required to ensure a worthwhile yield of fruit (Table 4).

The mature fruits produced by test plants are small, about half normal size, but the seeds produced have a high degree of germinability (Table 5). A similar finding was supported by Ottenwaelter et al. (6). Test plants of vinifera cultivars have been used as a source of pollen (7) but their use as female parents in grape breeding, although feasible, is limited by the practical difficulties encountered in emasculating the small flowers, and by the poor response of certain genotypes to pre-rooting and pruning (Table 6).

Fruiting cuttings are very useful tools in viticultural research but they are contrived, if not artificial, plants in which gross changes are made to the normal temporal and spatial relationships of vegetative growth and fruit growth. Accordingly, much care is needed in the interpretation of experiments with test plants, especially in the extrapolation of results to the vineyard situation.

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