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A positive correlation between endogenous root-inducing cofactor activity in vacuum-extracted sap and seasonal changes in rooting of M.26 winter apple cuttings

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SUMMARY

A synergistic response was observed between the endogenous stimulus responsible for improved rooting of M.26 apple cuttings in late winter and spring and treatment with IBA. A water-soluble extract from sap was also synergistic with IBA when tested on mung bean cuttings *in vitro*, and separation by paper chromatography gave four areas which were tentatively identified as rooting cofactors. The natural seasonal rooting response of cuttings was closely correlated with the root-promoting activity of the sap extract, the intensity of which was partly associated with changes in available sap and partly with changes in concentration. Rooting cofactor activity was found in sap extracted from annual shoots, older woody framework and roots. Removing axillary buds in the autumn did not alter the general rooting trend when shoots were subsequently propagated as cuttings but the late winter rise in rooting was not maintained for as long as in normal cuttings. Cofactor activity was generally similar in normal and disbudded cuttings. Cuttings isolated from the stockplant in late January and taken into cold storage also showed improved rooting when propagated in early February similar to that of cuttings retained on the hedge. Subsequent samples, while showing a clear improvement over January-propagated cuttings, failed to maintain the level of the controls which were not cold stored. The combination of disbudding and prior isolation also failed to prevent a late winter improvement in rooting, but this was smaller than in normal cuttings removed from the stockplant at the time of propagation.

SEASONAL fluctuations in the rooting of leafless woody cuttings collected between October and April limit the time available for most effective propagation, so presenting a management problem for nurserymen. Fluctuations in the rooting of IBA-treated Crab C and M.26 apple and Myrobalan B plum cuttings are characterized by relatively low rooting during midwinter, sometimes preceded by moderate autumn rooting and invariably followed by a strong peak in late winter and spring (Howard, 1965, 1968, 1980).

Methanol extracts of *Hedera helix* and *Hibiscus rosa-sinesis* were shown to contain root-promoting substances termed cofactors because they synergize response to IAA (Hess, 1964a and b). Attempts to explain seasonal fluctuations in rooting by changes in cofactor activity have been only partly successful. Lanphear and Meahl (1963) found a weak correlation in juniper cuttings. Shirzad and Miles (1977) found no correlation between seasonal trends and endogenous root-promotors in the K14 apple rootstock, while Tustin (1976) found a positive relationship between rooting in M.12 apple rootstock cuttings and an endogenous IAA-like substance, but only a partial correlation with cofactor level. Fadl and Hartmann (1967) obtained extracts from pear buds in which increased root-promoting activity preceded that of cuttings in the late winter. In all these cases ethanol or methanol was employed as the extraction agent.

Many plants exhibit improved rooting as

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spring approaches and it has been suggested that this is determined by growth factors emanating from the developing vegetative buds. This hypothesis was based mainly on the decreased rooting of various plants in the spring when their axillary buds had been removed. In some cases the plants used in such experiments bore preformed roots, e.g., *Salix, Populus, Ribes* and *Vitis* spp. (van der Lek, 1925 and 1934) and the effects may have been on root emergence rather than initiation.

The rooting of cuttings of M.26 apple was not adversely affected by disbudding if the wounds were protected with an antidesiccant such as petroleum jelly. Furthermore, chilled and unchilled cuttings, with buds capable of sprouting or not sprouting respectively, rooted equally well (Howard, 1968).

Subsequently, doubt has been cast on the interpretation of results from disbudding experiments through the demonstration that moisture changes arising from disbudding or wounding cuttings cause large effects on rooting (Howard, 1980). General evidence that bud activity is not responsible for improved rooting comes from the observation that in the late-leafing M.26 apple the rise in rooting preceeds visible bud-burst by about two months.

The aim of the studies reported here was to investigate further the endogenous factors influencing rooting in apple cuttings by assessing sap extracts, not hitherto used, by means of a revised mung bean bioassay (Bassuk and Howard, 1981) and to investigate also the distribution of cofactor activity within the M.26 stockplant and the effect of disbudding on cofactor activity and rooting.

MATERIALS AND METHODS

Propagation conditions

One-year shoots of M.26 apple rootstock were cut from hard-pruned hedges with secateurs as close as possible to the swollen shoot base, and at right-angles to the long axis. Shoots were *c.* 8 mm in diameter at the basal internode and were shortened distally to 60 cm. Replicates of 10 cuttings were prepared by apportioning relatively thick and thin shoots between each one. Six replicates per occasion or treatment were typically used.

Indolylbutyric acid (IBA) was applied to the basal 1 cm of the cuttings by dipping for 5 s in a 2500 ppm (1.2×10^{-2} M) solution in 50% aqueous acetone. Excess liquid was shaken off and cuttings were left to dry for about 30 min before they were rooted in a cool building, in bins containing a mixture of equal parts by volume of coarse peat and grit to a depth of 25 cm. The cuttings' bases were situated *c.* 4 cm above electric cables with a loading of 160 W m^{-2}, which provided heat regulated by a resistance thermometer to give a basal temperature of *c.* 20°C. Propagation was for a standard four-week period on all occasions.

Cuttings were disbudded if required, either in mid-December 1978 while still attached to the stockplant, or at collection in mid-January 1979. All but the one or two flat proximal buds previously associated with the basal rosette of leaves were removed by a shallow scooping action using a narrow-bladed scalpel and the 30 or so wounds on each shoot were treated with petroleum jelly. The length of shoot retained on the stockplant was slightly greater than the 60 cm later to be used as the cutting. When cuttings were isolated from the stockplant prior to being rooted they were enclosed in polyethylene bags and held at 1°C in a commercial cold store.

Cuttings for each seasonal sample were collected from pre-identified and randomized hedge sections and planted in pre-identified randomized bin positions. On removal, the numbers of rooted cuttings, the numbers of roots per cutting and extent of callus or dead tissue were recorded as appropriate. Analyses of variance were carried out on rooting data and least significant differences and correlation coefficients were computed as required.

Extraction procedure

Cuttings, branch framework and roots for sap samples were collected on the day following each propagation from an adjacent hedge plant. Sap was extracted using a technique described by Bollard (1953), in which bark was stripped from the proximal 5 cm of cuttings or other material with a razor blade and the ends re-cut. They were then inserted through holes in rubber bungs to give an airtight fit and the bung placed in a metal cylinder so that the cutting's base projected into the mouth of a glass vial held in a lower bung at the other end of the cylinder. By means of a side arm, the cylinders were connected in sets through a manifold to a vacuum pump. When pressure was reduced to 625–700 mm Hg, sap dripped from the cut base into the collecting vials, assisted by repeatedly cutting off 0.5 cm sections from the

distal end of the shoots or roots at *c.* 10-s intervals. Sap from six to nine cuttings was combined to form one replicate, its volume recorded, and stored at −20°C. The initial fresh weight, residual fresh weight after extraction and oven dried weight of each cutting replicate were also recorded. Other material was treated similarly.

A volume of sap equivalent to 50 g residual fresh weight of tissue was evaporated to *c.* 0.2 ml under a stream of nitrogen while being heated on a water bath at 50°C and then spotted for separation by paper chromatography.

Methanol extracts were obtained from samples of 10 cuttings by chopping *c.* 1 cm lengths into a volume of 80% aqueous methanol equal to the ml-equivalent of twice the cuttings' fresh weight (g). After storing for 24 h at 3°C the first extract was decanted and the process repeated before combining and then removing the alcohol under vacuum at 25°C in a rotary evaporator. The aqueous residues were centrifuged and water-soluble material freeze-dried and stored at −20°C. The dry weight of extracted material was recorded.

Chromatography

Vacuum-extracted sap equivalent to 50 g residual fresh weight was spotted on Whatman 3MM paper and developed by descending chromatography with *n*-butanol-acetic acid – water (63:10:27) after the solvent ran to within *c.* 5 cm of the end of the paper. The chromatogram was air-dried for 24 h and cut into transverse sections corresponding to highly reproducible fluorescent and absorbent bands made visible when the chromatograms were fumed with ammonia, or viewed under longwave UV light (365 nm). The boundaries of these bands were marked lightly with pencil while under UV immediately after fuming and the sections cut out for use in the mung bean bioassay.

Mung bean bioassay

Extract activity was tested in the presence of 2 ppm IBA with hypocotyl cuttings of mung bean (*Vigna radiata* L. Wilczek) seedlings, using four cuttings per 5-ml vial as a replicate. The test solution was replenished by distilled water every three days while cuttings were held for a period of seven days at 700 lm ft^{-2} and 16 h photoperiod (21.2 W m^{-2} PAR) with an ambient air temperature of 23°C. Emerged roots were counted and treatment responses expressed as increased root numbers over the control. Preliminary investigations of factors affecting the rooting of mung bean cuttings were described previously (Bassuk and Howard, 1981).

RESULTS

Rooting

Two general patterns of seasonal rooting were obtained from 1974 to 1978. In 1974–75, 1977–78 and 1978–79 a relatively high autumn rooting level was followed by poor rooting in late January or early February, followed by a rapid improvement during February, March and early April, after which a fall-off in rooting was associated with leaf development. Root numbers per rooted cutting closely followed the percentage rooting curves and the results are shown for 1978–79 (Figure 1). During the winters of 1975–76 and 1976–77 rooting was generally low in the autumn, progressively improving until the following March, when it declined in 1976 but not in 1977.

In 1976–77 cuttings were propagated with and without IBA treatment. The influence of time of year was synergistic with the IBA response. Without IBA, differences due to time were not significant, whereas following IBA treatment a significant improvement in rooting occurred from 10 January onwards (Table I). In a parallel study with the more difficult-to-root M.9, 2% rooting in early January was raised to 11% with IBA, while rooting in late February was raised to 40%.

Sap extraction

Between 2 and 6 ml of sap were extracted per

TABLE I
Percent rooting of M.26 during 1976–77 with and without IBA treatment

2500 ppm IBA	8 November	8 December	10 January	31 January	21 February	21 March	18 April
−	0	3	5	7	10	17	10
+	13	28	57	53	53	64	69

LSD ($P < 0.05$) = 21.3

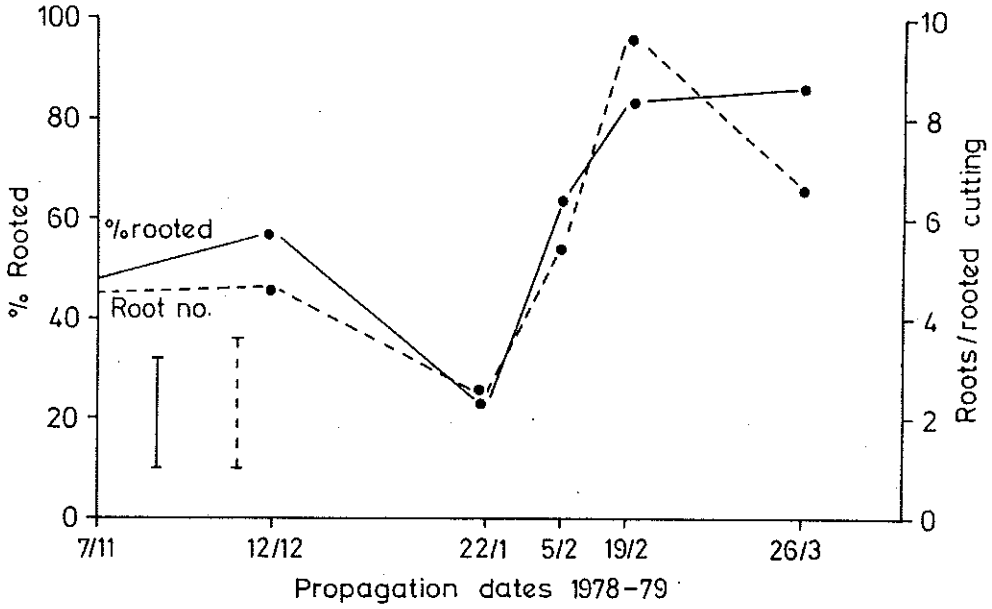

FIG. 1
Seasonal rooting in M.26 cuttings 1978–79. Bars = LSD ($P < 0.05$)

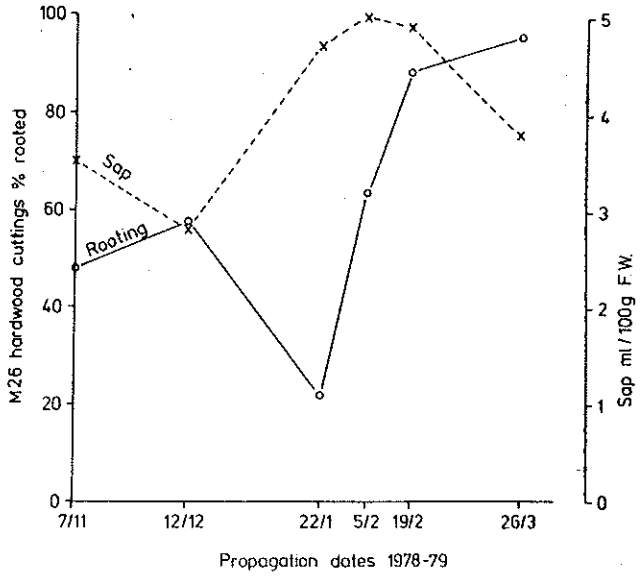

FIG. 2
Vacuum-extracted sap (ml per 100 g FW) in relation to rooting 1978–79

100 g residual fresh weight of cuttings, the amount being positively correlated with cutting thickness. The amount of sap extracted generally declined from autumn to spring in 1976–77 ($P<0.05$) but increased in 1977–78 over the same period ($P<0.05$). In 1978–79 there was an initial decrease followed by a marked increase ($P<0.001$) and then a reduction to about the initial amount. Common to all three years was a peak in volume of extractable sap during

February which preceded the maximum level of rooting. Results are shown for 1978–79 (Figure 2).

The water content of cuttings represented slightly less than half the fresh weight, with a maximum of about 10% being extractable as sap. Because of the relatively large and constant amount of non-extractable water, the percentage of sap to remaining water showed a trend with time generally similar to that in Figure 2 for sap itself (Table II).

Rooting activity in vacuum-extracted sap

Sap taken from cuttings at a time of year when they rooted readily (26 March 1979) was chromatographed and the chromatogram divided into 12 unequal segments reflecting the boundaries of fluorescent and absorbent bands detectable under UV light. Following assay by the mung bean test in the presence of 2 ppm IBA, standard errors for R_f values bordering each segment were calculated to obtain a measure of reproducibility using data from seven chromatograms. Variability between segment R_f was low indicating that the segments were discrete (Figure 3).

The strongest cofactor root-promoting activity was contained in the first three segments, tentatively designated 1A, 1B and 1C. 1A included a dark line coincident with the origin and also contained a faint white fluorescence. 1B fluoresced with a light blue colour, and 1C was slightly absorbent with a light orange-brown colour visible in normal light. The fourth segment showed a

TABLE II
Water characteristics of M.26 cuttings at different dates in 1978–79

	7 November	12 December	22 January	2 February	19 February	26 March
Dry matter g per 100 g FW	56.0	51.6	53.5	52.0	54.4	53.7
Vacuum-extracted sap ml per 100 g FW	3.3	2.8	4.5	4.8	4.7	3.8
Non-vacuum extracted water g per 100 g FW	40.7	45.6	41.9	43.2	40.9	42.4
Sap as % total water at constant DW	7.6	5.9	9.7	10.0	10.4	8.3

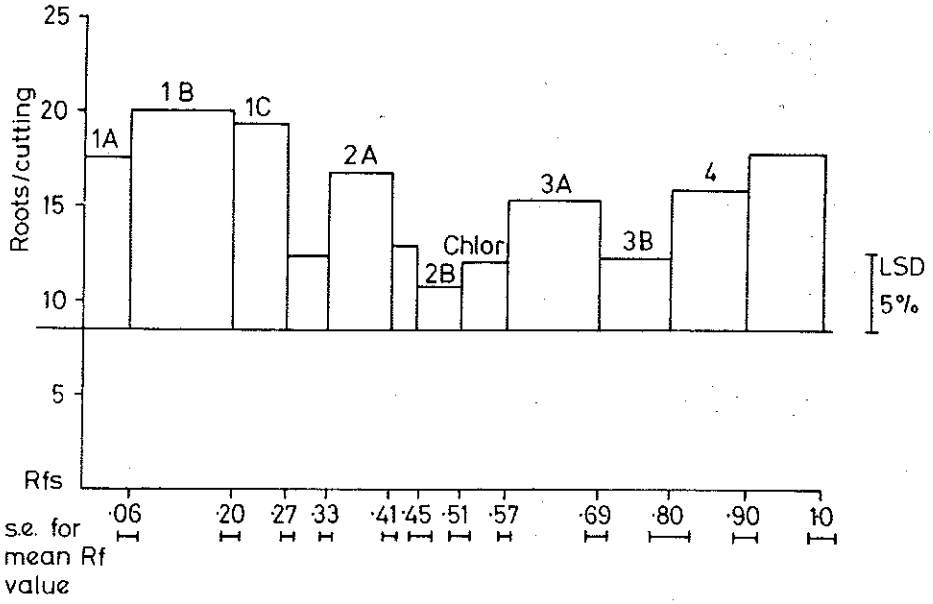

FIG. 3
Mung bean responses to chromatographed vacuum-extracted sap

bright turquoise fluorescent band which was not significantly active. Segments 5 and 7 (2A and 2B) were both orange in visible light and faintly absorbent under UV. The orange colours were enhanced by fuming the strips with ammonia. 2A was consistently active but 2B was not consistently so. Segment 6 fluoresced faintly purple under UV and its colour was enhanced by ammonia fuming. Segment 8 (chlor.) fluoresced a bright blue-green colour when fumed with ammonia under UV, ran in a similar position and had a similar colour to chlorogenic acid and was mildly but not significantly active in promoting rooting. Segment 9 (3A) fluoresced bright blue with ammonia and was a significantly ($P<0.05$) active root promotor. The substance in the tenth segment (3B) ran to the same R_f as did phloridzin and showed the characteristic yellow-brown absorbent spot under UV which was enhanced and deepened in colour by ammonia fumes. When examined with a UV spectrophotometer, the eluate from 3B absorbed at 283 nm and stained orange in visual light when the segment was sprayed with diazotized *p*-nitroaniline, both characteristic of phloridzin. The eleventh segment (4) fluoresced with a faint bluish-purple colour which was enhanced slightly by fuming with ammonia. The activity of this area was inconsistent as was that of the last segment. Whatman 3MM paper contained a root-promoting factor which occasionally accumulated at the solvent front so that activity there may not have been due to the sap alone.

Synergism between sap activity and IBA

Sap from cuttings collected on 19 February 1979 was tested for root-promoting activity in the mung bean bioassay both with and without added IBA. A volume of sap corresponding to 50 g residual fresh weight was spotted on Whatman 3MM paper, developed and divided into 12 sections. Each paper segment was placed in a 5-ml vial and eluted with either distilled water or a solution of 10^{-5} M (2 ppm) IBA in distilled water and assayed for root promotors. Three replicate vials of four cuttings were used for each chromatogram section. Only the first chromatogram section, corresponding to cofactor 1A, caused a significant ($P<0.001$) rooting response in water with an increase of three roots over the control. In the presence of IBA, seven sap components significantly ($P<0.001$) increased the rooting of mung bean cuttings through a synergistic response (Figure 4).

Relationships between sap activity and rooting of cuttings

In 1977–78 four replicates of sap from every propagation date were analysed and compared with M.26 rooting. The activity of four individual

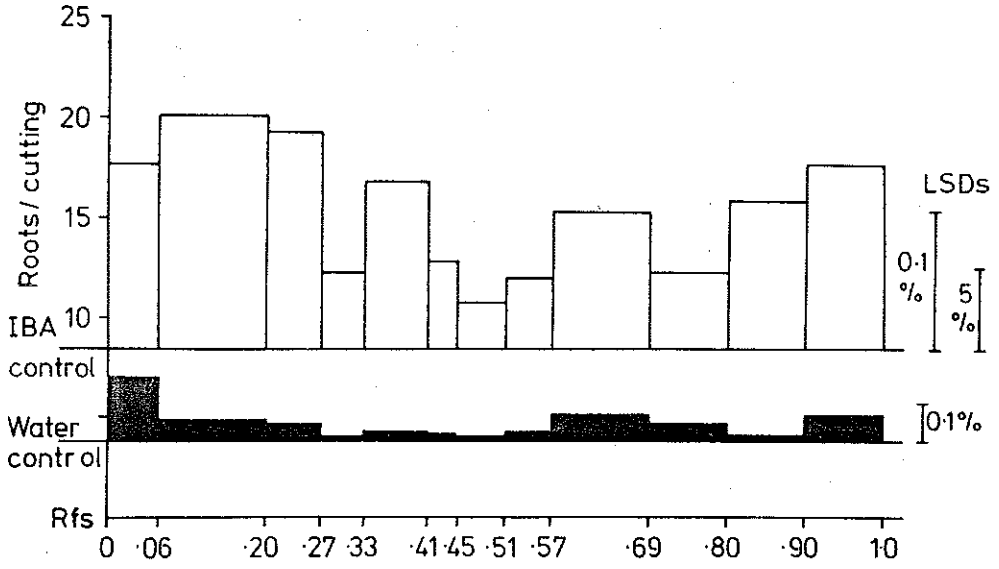

FIG. 4
Mung bean responses to chromatographed vacuum-extracted sap with (open) and without (solid) 2 ppm (10^{-5}M) IBA

cofactors (see below) and the total activity were measured. Total cofactor activity was closely correlated with M.26 rooting especially during the lowest rooting period and the sharp rise immediately after (Figure 5). Correlation coefficients between M.26 rooting percentage and the four individual cofactors were also determined. Each cofactor increased in activity during the period of greatest improvement in M.26 rooting from 19 January to 27 February. Cofactor 1B was most closely correlated with rooting ($P<0.001$) and cofactor 3B was also correlated ($P<0.05$). Cofactors 2A and 4 were not significantly correlated.

In 1978–79 all cofactors were compared with seasonal rooting curves. Taken in total, the combined cofactor activity differed with propagation date at a 6.3% significance level and the correlation coefficient for total cofactor activity and M.26 rooting percentages was significant ($P<0.001$) (Figure 6). The same level of significance was obtained for the correlation between cofactor activity and roots per rooted M.26 cutting. Curves for individual cofactors differed with time, 1B again being the most closely correlated with M.26 rooting. The activity of all cofactors increased during the period of greatest improvement of M.26 cuttings between 22 January and 19 February (Table III).

TABLE III
Relative individual cofactor activity (number of mung bean roots greater than control per cutting) in relation to increase in M.26 rooting

	22 January 1979	19 February 1979
Cofactor		
1A	4.4	7.4
1B	3.7	9.8
1C	6.5	9.0
2A	5.7	8.5
2B	2.1	4.6
3A	4.1	7.4
3B	3.7	5.4
4	3.4	9.6

LSD ($P < 0.05$): Vertical comparisons 4.4
Horizontal comparisons 5.2

Comparison of sap with methanol-extract activity

During 1976 and 1977 some 30 tests using methanol extracts failed to show any consistent seasonal differences in cofactor activity. A comparison of activity in methanol extracts and in vacuum-extracted sap collected at a poor M.26 rooting occasion (8 December 1976) and a good rooting time (3 March 1977) showed total cofactor activity to increase relatively more in the sap than in the methanol extract, while a large increase in activity in the apparently important cofactor 1B from sap between these two dates was not obtained in the methanol extract (Table IV).

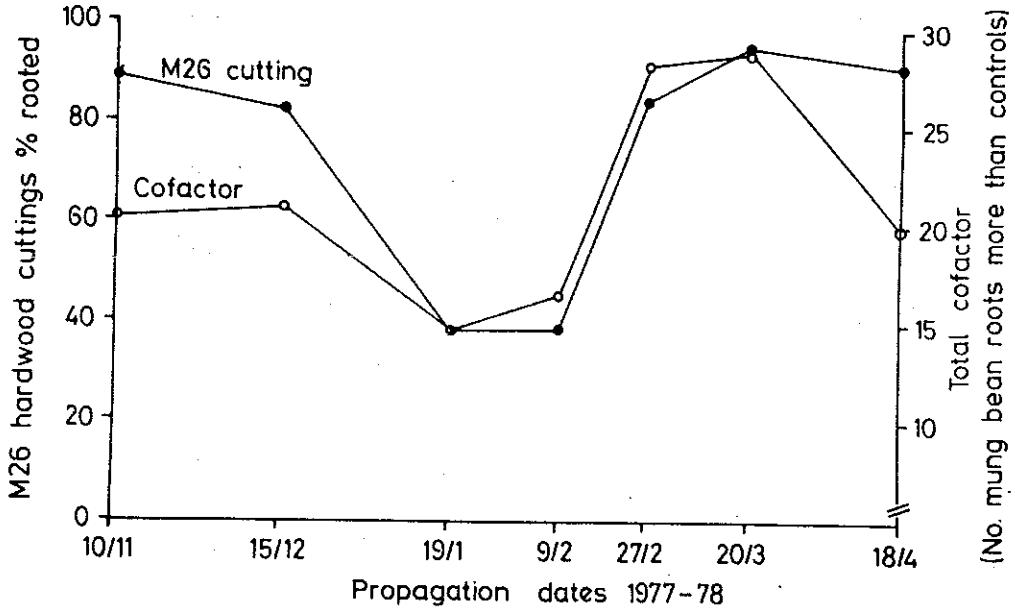

FIG. 5
Cofactor activity in relation to rooting (%) in M.26 cuttings 1977–78

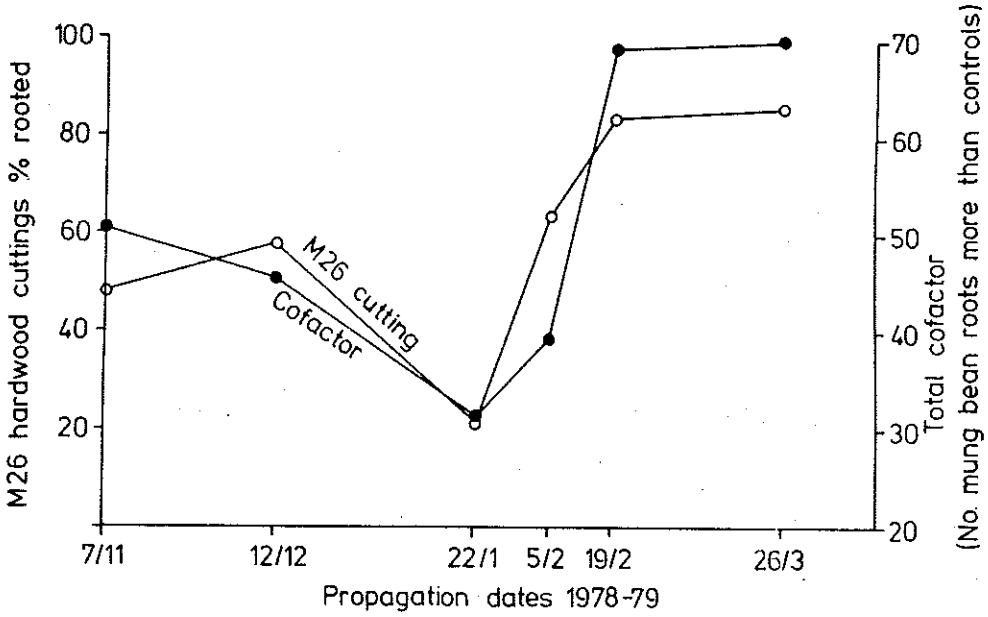

FIG. 6
Cofactor activity in relation to rooting (%) in M.26 cuttings 1978–79
(Correlation coefficient $P < 0.001$)

TABLE IV
Relative cofactor activity (number of mung bean roots greater than control per cutting) from sap (50 g FW equivalent) and methanol extract (0.25 g residual DW equivalent)

Date	Total Cofactor		Cofactor 1B	
	sap	methanol	sap	methanol
8 December 1976	6.8	6.6	5.4	18.1
3 March 1977	10.1	7.2	18.2	14.7
LSD ($P < 0.05$)	2.8	1.4	8.0	4.3

Cofactor activity in branch framework and roots of hedge plants

Sap from sections of the multi-jointed framework below the annual shoot system developed by severe annual winter pruning and from roots 1–2 cm thick was also extracted, chromatographed and bioassayed. The 12 coloured bands described above for sap from annual shoots were again visible when viewed under UV light.

Marked activity was obtained in those areas assigned as cofactors. Cofactors 1A and 1B, 2A, 3A and 4 were again particularly prominent (Table V).

TABLE V
Mung bean response (mean roots per cutting) to sap components from M.26 branch framework and roots (all in the presence of 2 ppm IBA)

Cofactor	R_f	Root sap	Branch sap
1A	0–.06	16.5	20.0
1B	.06–.20	24.3	23.3
1C	.20–.27	22.6	14.9
	.27–.33	9.0	10.1
2A	.33–.41	14.4	16.4
	.41–.45	8.5	10.9
2B	.45–.51	10.4	13.5
Chlor.	.51–.57	8.3	11.3
3A	.57–.69	13.5	22.4
3B	.69–.80	15.1	21.4
4	.80–.90	24.1	23.0
	.90–1.0	7.4	17.3
Control = 7.9		LSD ($P < 0.05$) = 4.3	

Disbudding

Cuttings disbudded in mid-December 1978

were collected in lots of 50 on four subsequent occasions and their rooting compared with normal cuttings. Similar trends for both rooting percentages and roots per rooted cutting were obtained for both types of cutting, namely an initial reduction followed by a sharp improvement during late January and February. However, the level of improvement was not maintained in disbudded cuttings during March (Figure 7). The total activity in sap extracted on three occasions showed a similar trend in total activity to that from normal cuttings (Table VI). Of the individual cofactors, 1B showed the greatest similarity between normal and disbudded cuttings, while 2B, 3A and 4 from disbudded cuttings also increased markedly with time, often to a greater extent than in normal cuttings. Only for chlorogenic acid did disbudding remove most of the activity present in normal cuttings.

TABLE VI
Mean cofactor activity
(number of mung bean roots more than the control)

	22 January	19 February	26 March
Normal	3.7	7.3	8.4
Disbudded	3.4	5.0	9.3

SED 1.02; Treatment × date interaction NS

Isolation

Cuttings propagated from cold store on three occasions in 1979 gave improved rooting in late winter similar to normal cuttings although the improvement was not maintained at its initial level in those previously isolated.

When disbudding was combined with prior isolation a small improvement in rooting occurred but not of the same magnitude as in either the normal or disbudded-only cuttings (Figure 8).

DISCUSSION

In these studies seasonal rooting fluctuations of M.26 cuttings conformed to two patterns. Either medium to high autumn rooting was followed by a low level in midwinter rising rapidly again in spring, or low rooting in the autumn was followed

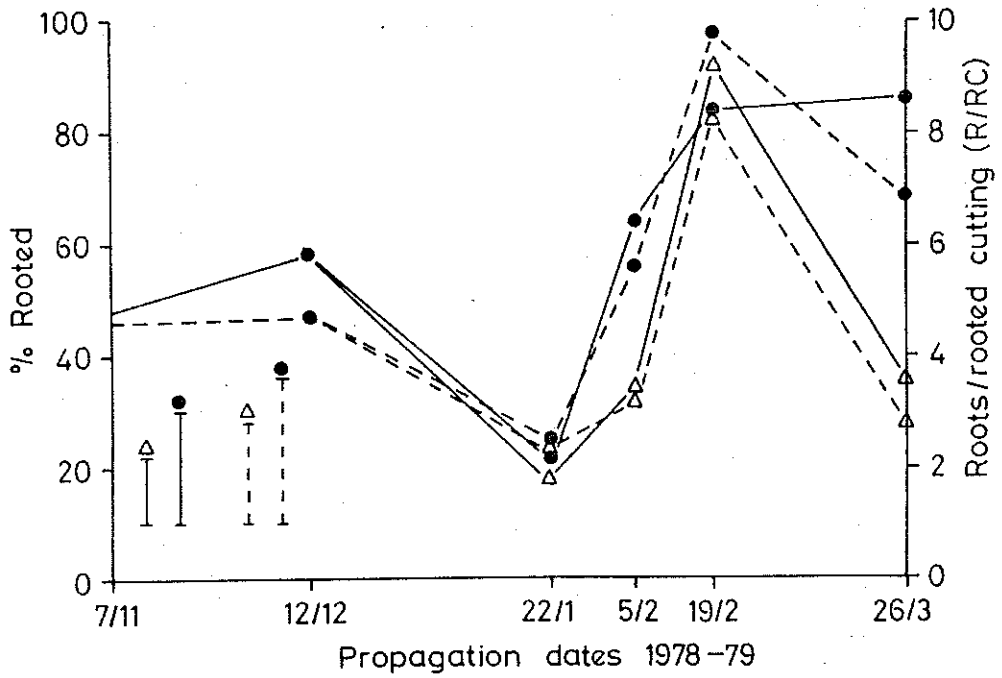

FIG. 7
Seasonal rooting of normal and previously disbudded M.26 cuttings
Bars = LSD ($P < 0.05$)
● —— ● percent normal cuttings; Δ —— Δ percent disbudded cutting;
● --- ● roots per rooted normal cutting; Δ --- Δ roots per rooted disbudded cutting

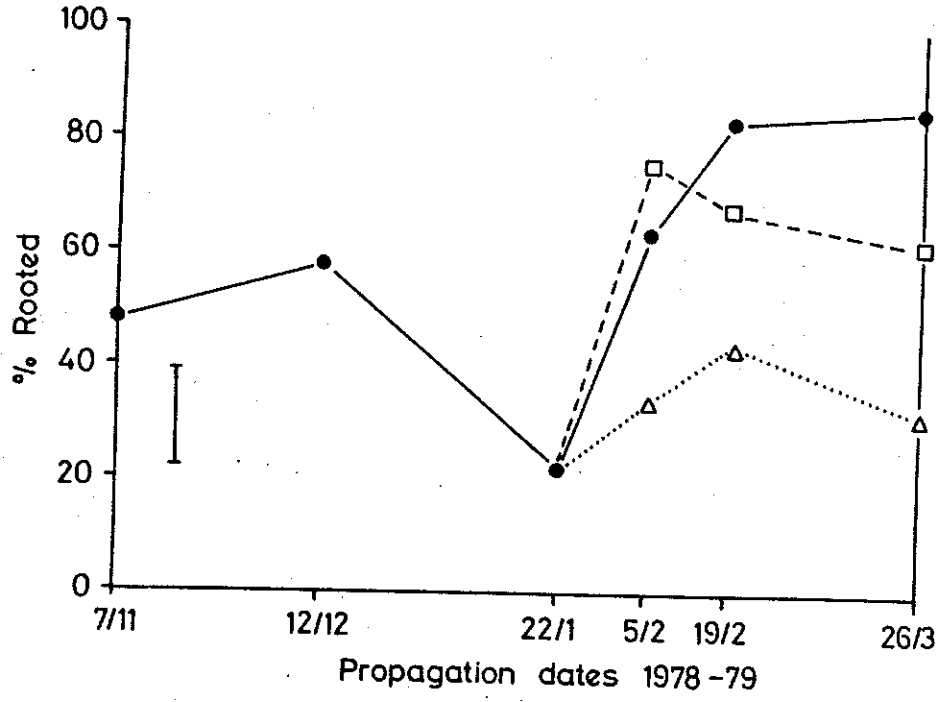

FIG. 8
Seasonal rooting of normal and previously isolated and/or disbudded cuttings (percent)
Bar = LSD ($P < 0.05$)
● —— ● normal; □ – – – □ previously isolated; Δ . . . . Δ isolated and disbudded

by a gradual rise to a late winter and spring peak. These observations support those of Howard (1965, 1968, 1980) for winter cuttings of apple and plum. Winter rooting patterns have been found with some similarities to those observed here in such diverse genera as *Pyrus* (Fadl and Hartmann, 1967), *Olea* (Hartmann and Loreti, 1965) and *Picea* (Tognoni *et al.*, 1977), suggesting that this may be a general response among woody plants caused by a commonplace seasonal phenomenon.

In 1976–77 M.26 cuttings propagated without IBA gave almost no rooting. Although IBA treatment significantly increased rooting in winter and spring, it was less effective in raising rooting percentages during the earlier period of poor rooting. This evidence of seasonal trends being accentuated by IBA is supported by the findings of Lanphear and Meahl (1963) and suggests that there is an endogenous rooting factor in shoots at times of good rooting that acts synergistically with IBA but is absent or less available at periods of poor rooting.

A similar synergism *in vitro* between IBA and extracted cofactors indicated that the mung bean bioassay is relevant to investigations of large leafless cuttings.

Different extraction procedures greatly affected the gross activity of cofactors as measured by the mung bean bioassay. The levels of root-promoting cofactors found in vacuum-extracted sap were highly correlated with the seasonal rooting trends of IBA-treated cuttings. Cofactor 1B was most closely correlated with rooting especially in the period of poor rooting in winter through to spring.

Other workers have found activity in this area near the origin of the chromatogram in water or methanol/ethanol extracts of the following plants: apple (R_f .1–.4, Ashiru and Carlson, 1968); (R_f 0–.2, Lipecki and Dennis, 1972); *Salix* (R_f 0–.1, Kawase, 1970; *Cotoneaster, Euonymus, Ilex, Lonicera, Physocarpus, Symplocos, Taxus* and *Viburnum* (R_f 0–.1, Kawase, 1971).

Methanol extracts from good and poor rooting periods showed few differences, however, and with one exception contained similar high amounts of cofactors. It is interesting that

Tognoni *et al.* (1977) obtained relatively good correlation between seasonal rooting changes in *Picea* cuttings in an aqueous extract but not with methanol extraction.

A basic difference between the extraction procedures used in these studies with M.26 was the higher degree of cell disruption likely to be caused by methanol compared to sap extraction. Comparable levels of response in the mung bean bioassay were produced by a 0.25 g dry weight equivalent of methanol extract and a 50 g residual fresh weight (*c.* 25 g dry weight) equivalent of vacuum-extracted sap. Taking into account that about one-tenth of the shoot's water content is removed during vacuum extraction, while all water- and methanol-soluble contents were supposedly extracted during the methanol procedure, the 10-fold greater gross activity of the methanol extract apparently masked any seasonal fluctuations in cofactor activity. Moreover, methanol extraction of the entire cutting is not likely to relate closely to seasonal differences in cofactor availability at the site of root initiation, whereas the contents of xylem sap may represent more mobile factors within the plant which become available at rooting sites. The apparent importance of the extraction method used for rooting cofactors makes it difficult to interpret studies of seasonal relationships by other workers.

It is not possible from the studies reported here to identify the cause of the increase in rooting cofactors in the annual shoot removed as a cutting, other than to link tentatively the major period of cofactor activity to a prior rise in available extracted sap. In 1978–79 extracted sap volume increased during late December and early January from 5.9 to 9.7% of the total water in the cutting at a constant dry-matter content but it is not clear whether this was due to water moving into the shoot or a withdrawal of water from adjacent tissues into the xylem.

Increase in the volume of sap extracted from 50 g residual tissues was not the main reason for increased activity in the mung bean bioassay or in rooting of M.26 cutting. In 1976–77 a significant fall ($P<0.05$) in extracted-sap volume was associated with a small progressive increase in M.26 rooting and on the two dates that sap was sampled cofactor activity was inversely related to sap volume. In 1977–78 cofactor activity and rooting of M.26 fell until mid-January while extracted-sap volume progressively increased, and in 1978–79 an increase in extracted sap volume in the second-half of December was associated with a continued reduction in rooting and cofactor activity until late January, while a reverse trend developed in February. These results suggest that changes in concentration of cofactor occur which are mainly responsible for the observed bioassay and rooting effects but that the amount of water in the xylem may be important in relation to the development of the highest rooting peak in cuttings during late winter and spring.

Cofactor activity was present in all tissues examined, which suggests that common plant constituents are involved. Increased activity and rooting in late winter did not require the presence of buds and it also appears that sufficient cofactors or their precursors are contained in the shoot when taken into cold store before the normal rooting rise to enable this to occur subsequently. However, there might be an earlier time before which cuttings were not preconditioned.

The fact that cofactor activity continued to rise in disbudded cuttings while rooting fell at the last measurement suggests that the bud contributes a factor necessary to maintain the improved rooting achieved by cofactor or that the decline was due to an artefact. Possible artefacts might include reduced water movement through the shoot due to 'leaky' buds being replaced by petroleum jelly.

These results are taken from a thesis by Nina L. Bassuk, accepted by London University in partial fulfilment of the PhD degree.

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