SEASONAL ROOTING CHANGES IN APPLE HARDWOOD CUTTINGS AND THEIR IMPLICATIONS TO NURSERYMEN

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Abstract. Vacuum-extracted root promoting cofactors from M.26 apple hardwood cuttings were found to correlate positively with M.26 seasonal rooting fluctuations. These substances were present throughout the stock plant as well as in previously disbudded cuttings. An abundant phenolic — phloridzin, and a polyphenol oxidase enzyme, also found in the extracted sap appeared to fluctuate in a way which implicated them as precursors of the root promoting co-factors. When the products of this phenolic reaction were added to IBA, rooting was significantly increased in mung bean and apple hardwood cuttings.

REVIEW OF LITERATURE

Traditionally, clonal apple rootstocks have been propagated by stooling or layering (7). Hardwood cuttings collected between October and April are being increasingly used to supplement older techniques (9). Although the hardwood cutting technique has advantages such as higher shoot productivity per plant and decreased labor requirement during summer compared with stooling, its limitation from a management perspective is a marked seasonal variability in rooting.

Typically, M.26 apple hardwood cuttings root moderately well (40% to 90%) during autumn, but between November and early February, depending on the year, there is a sharp decline in rooting (0% to 20%), followed by an equally rapid rooting increase during mid to late winter which remains high (70% to 100%) until late April, when bud break occurs and rooting again decreases (8).

A commonly held view based largely on a loss of rooting in disbudded cuttings is that increasing bud activity in late winter produces root promoting substances which are translocated to the basal end of the cutting and thus stimulate root initiation (15,6,12). Howard (10) later found that the addition of an anti-desiccant to the cut surfaces of disbudded apple, pear and plum cuttings largely nullified the negative effect of disbudding on rooting and further showed that seasonal rooting patterns were unaltered by the presence or absence of buds on hardwood cuttings of apple and plum (8).

Bouillenne and Bouillenne-Walrand (5) hypothesized that phenolic cofactors and auxins stimulate rooting in the presence of a polyphenol oxidase (PPO) enzyme. Past attempts to correlate

seasonal rooting trends with extracted root promoters have been only partially successful (11,6,14,13). This study, therefore, was aimed at finding the cause of seasonal rooting in M.26 apple cuttings using a more physiologically sensitive technique based on vacuum extracted sap rather than extraction with methanol or ethanol as used by other workers. Furthermore, seasonal levels of PPO enzyme and phloridzin, the abundant glucoside in apples (16) were also investigated.

MATERIALS AND METHODS

Hardwood cuttings, 60 cm in size, were taken from severely pruned hedges and treated with 2500 ppm IBA and propagated for four weeks at 20°C bottom heat in a peat/grit compost at intervals through the winter. At the same time, sap was obtained from other cuttings by reducing pressure at their bases while simultaneously cutting back their tips (4).

Sap co-factor content was investigated by paper chromatography and enzyme activity by colorimetric methods using catechol oxidation. Phloridzin was determined by measuring its absorbance at 283 nm after purification by chromatography.

Rooting co-factors were synthesized by reacting a commercial source of polyphenol oxidase enzyme (PPO) with phloridzin and applying it to mung bean or apple cuttings in the presence of 2 ppm IBA, respectively.

RESULTS

Root promoting activity was found in normal as well as disbudded shoots, roots and thick branch framework. Eight discrete areas of activity containing substances which were phenolic in nature, consistently appeared on the chromatogram as determined by the mung bean rooting bioassay (1) (Table 1). There was little or no activity however, when IBA was not added indicating that these substances are auxin co-factors.

Table 1. Mung bean rooting response to apple root and shoot sap in the presence of 2 ppm IBA.

Co-factor chromatogram location (Rf)	Roots per cutting
0→.06	18.3
.06→.20	23.8
.20→.27	18.8
.33→.41	15.4
.45→.51	12.0
.57→.69	18.3
.80→.90	23.6
	Control = 7.9 roots
	LSD 5% = 4.3 roots

Cutting collection and extraction of sap was carried out in conjunction with normal seasonal rooting experiments both in

1977-1978 and 1978-1979. There was a highly significant correlation between the levels of co-factors found in the sap and rooting of cuttings throughout the season (Table 2).

Table 2. Co-factor activity in relation to rooting percentage in M.26 cuttings for 1977-78 and 1978-79.

Date of collection	Percent rooted M.26 cuttings	Co-factor activity (no. of mung bean roots more than controls)
(1977-1978)		
11/10/77	88.8	19.4 (4 cofactors only)
12/15/77	83.8	20.2
1/19/78	38.0	14.5
2/27/78	38.2	16.3
3/20/78	96.3	29.2
4/18/78	92.5	19.8
(1978-1979)		
11/ 7/78	48	50.6 (all 8 cofactors)
12/12/78	58	45.5
1/22/79	22	33.4
2/5/79	64	41.8
2/19/79	84	73.3
3/26/79	86	72.9

PPO activity and phloridzin content increased significantly ($P<0.001$ and $P<0.01$, respectively) in late January and early February 1979, preceding the concurrent rises in M.26 rooting percentages and cofactor activity in the sap (Table 3).

Table 3. Phloridzin content and PPO activity related to cofactor activity and rooting percent of M.26 cuttings.

Date	Phloridzin	PPO	M.26 rooting	Co-factor
11/7/78	12.7	5.8	48	50.6
12/12/78	13.3	5.2	58	45.5
1/22/79	20.8	9.1	22	33.4
2/5/79	23.2	8.6	64	41.8
2/19/79	21.8	8.2	84	73.3
3/26/79	17.7	8.5	86	72.9

The interaction of all three factors: auxin (IBA, 2 ppm); phenolic (phloridzin, 2×10^{-3}M); and active and denatured (boiled) PPO enzyme (400 units tyrosinase), was tested in the mung bean bioassay. Table 4 shows that a dramatic rise in rooting occurred by adding active enzyme to phloridzin and IBA.

Table 4. Mung bean response to polyphenol oxidase, phloridzin and IBA.

Treatment	Mung bean roots/cutting
IBA	7.0
IBA + phloridzin	7.8
IBA + phloridzin + denatured enzyme	9.2
IBA + phloridzin + active enzyme	26.7
	LSD 0.1% = 2.9

When testing these compounds on hardwood cuttings at the

poorer rooting time of year, higher concentrations of the same phenolic glucoside (400 ml of 10^{-2}M) and enzyme (160,000 units tyrosinase) were reacted together and then added to the standard 2500 ppm IBA quick dip. Rooting percentages rose significantly from 20% with IBA alone to 38% with the phloridzin-PPO reaction products plus IBA and root numbers were doubled.

DISCUSSION

Rooting co-factors in vacuum extracted sap showed a strong correlation with seasonal rooting patterns in M.26 hardwood cuttings over 2 years. As co-factor activity was present throughout plant tissues regardless of the presence or absence of buds, it is likely that the source of the seasonal stimulus is in the tissues of the shoot itself. Moreover, shoots isolated from the hedge plant in autumn, placed in cold storage, and propagated subsequently along with field collected cuttings also exhibited typical seasonal rooting trends (2).

The rise in PPO activity and phloridzin content which just preceded the rises in co-factor activity and rooting may indicate a causal relationship whereby PPO and phloridzin are the precursors of the co-factors that influence hardwood cutting rooting. This idea is strengthened by the fact that PPO and phloridzin caused large rooting increases in mung bean cuttings when applied in the presence of IBA. Other similarities between the sap extracted co-factors and synthesized PPO-phloridzin products have been reported by Bassuk, *et al* (3).

The significant rooting improvement of M.26 cuttings treated with the products of a phloridzin-PPO reaction in the presence of IBA at the normally poor rooting time of year (20%, February 1979) was encouraging as little work had been done to test various concentrations or methods of applying these synthesized cofactors.

The implication of this work for nurserymen is that in future they may collect hardwood cuttings at times convenient to themselves and treat them with rooting co-factors to obtain consistently high levels of rooting. The possibility also exists to improve the rooting of difficult-to-root subjects and to alter cold storage practice by not removing cuttings from the hedge until adequate levels of precursors or co-factors have developed.

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BRUCE BRIGGS: How do you get the phloridzin to work? We have tried it a number of years and have not been able to get it to work.

NINA BASSUK: It is not the phloridzin but the products of the enzyme and phloridzin that are the active substances.

BRUCE BRIGGS: Is phloridzin destroyed by heat in sterilization?

NINA BASSUK: No.