with 0.5% IBA in February resulted in 71% rooting. Good rooting was achieved in June and August, but almost no rooting occurred during April, October, and December. The rooting percentage of adult cuttings rooting was highest during June and August, with little or no rooting during February, April, October, and December.

Lack of rooting during April, October, and December was associated with the absence of foliage on the cutting. Cuttings made during February were dormant, but had received adequate chilling for growth. Buds began growth within 2 weeks when placed in the greenhouse. Cuttings made during April already had young shoot growth, but when they were placed in the greenhouse the shoots rapidly abscised even though the cutting remained alive during the 90 days in the propagation area. Cuttings in June and August retained a portion of their foliage, possibly because they were more mature. Senescence had begun in October and cuttings defoliated soon after being placed under mist and insufficient chilling had been received by December to allow sufficient shoot growth. Thus, cuttings had foliage in February, June, and August and these were the only treatments in which significant rooting occurred.

Results of this study indicate that a 1% solution of IBA was the optimum concentration for root promotion in juvenile cuttings. Foliage seems to be necessary for rooting to occur; therefore, a root-promoting substance must be synthesized in the leaves. Juvenile cuttings root much more readily than adult cuttings, with the February propagation date producing the greatest amount of rooting and the most vigorous root system.

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Net Photosynthesis and Dark Respiration of Apple Leaves Are Not Affected by Shoot Detachment[1]

J. A. Barden, J. M. Love, P. J. Porpiglia, R. P. Marini, and J. D. Caldwell
Department of Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Additional index words. stomatal resistance, *Malus domestica*

Abstract. **Net photosynthesis (Pn) rates of greenhouse-grown apple leaves were unaffected for at least 24 hours after shoot detachment. With shoots detached from orchard trees, overnight holding did not affect Pn rates nor stomatal resistance. Use of detached shoots for Pn and dark respiration (Rd) determinations in apple leaves was concluded to be a valid technique.**

Several techniques have been used to determine net photosynthesis (Pn) of apple leaves but potential problems exist with each. Enclosing an entire tree, as done by Heinicke & Childers (6) and Sirois et al. (15) becomes impractical where multiple replications are necessary. Other problems include difficulty in characterizing the photosynthetically active radiation (PAR) level, determining leaf area, and providing adequate and uniform air movement within the canopy. Heinicke (7) devised a mobile lab system which enabled him to use single leaves on trees in the orchard. Seeley and Kammereck (14) developed an elaborate system which enclosed a branch and provided control of climatic variables. Even with this elaborate system, data collection would be most difficult during rainy weather as occurs in humid climates. Young, container-grown trees have been widely used because they can be readily transported to a lab and Pn of an individual attached leaf can be determined repeatedly during the season (5). However, results with such trees may not be extrapolated directly to mature orchard trees because of obvious differences such as tree age, cropping, environment, and pesticide applications.

We have recently investigated the possibility of using leaves or shoots excised in the orchard and brought to the lab. This technique offers several advantages over those described above: 1) the elimination of many technical problems associated with a mobile system, 2) the potential for collecting samples from a wide geographical area to be brought to the lab, 3) the reproducible environmental conditions in the lab, and 4) lack of dependence on good weather.

Detached leaves or shoots have been used in Pn studies with a range of plants, but most researchers have expressed no concern for the possibility that detached parts may not function normally (1,3,9,10,11,12,13). Nevins and Loomis (11) reported that under conditions of low relative humidity and intense light, sugar beet leaves showed a decline in Pn immediately upon detachment. Criswell and Shibles (4) found that detached oat leaves maintained a stable Pn rate for 20-40 min. at 61% relative humidity (RH) but then declined. At 46% RH, the decline in Pn was almost immediate. With white spruce and balsam fir branches, Pn rates did not differ between intact and excised branches (2). Crews et al. (3) used detached peach shoots and indicated no problems with this technique.

In our initial attempts to use excised apple leaves, we determined Pn of intact leaves in a system previously described (5). The leaf was removed from the chamber, excised under water, and Pn determined immediately. In other cases, leaves were excised the following morning and Pn determined. In either case, Pn

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rates after excision were variable. Some reached the pre-excision level and remained stable while others declined before or soon after reaching this level. We concluded that the use of individual detached leaves of apple in our system was not dependable.

The objective of the experiments reported here were to determine: 1) effects of shoot detachment on Pn, 2) effects of holding detached shoots several hours, 3) effect of removing the terminal ½ of a detached shoot on Pn and Rd, 4) effects of pre-conditioning on Pn of detached shoots, 5) effect of detachment on stomatal resistance.

Greenhouse experiments. One-year-old trees of 'Stark Golden Delicious'/sdlg and 'Starking Delicious'/sdlg were grown in 3.7 liter containers in a polyethylene greenhouse in which the ends were covered with screenwire. All trees were trained to a single shoot and given uniform watering, fertilization, and pest control. Five single tree replicates were used in each of 3 experiments to determine the effect of shoot detachment on Pn rates of individual leaves in August. In expt. 1 we used 4 'Delicious' and 6 'Golden Delicious', in expt. 2, 10 'Delicious', and in expt. 3, 10 'Golden Delicious'.

For each experiment trees were taken to the lab and pre-treatment Pn determined on the 10th leaf below the shoot apex. Shoots were then excised near the base of the shoot from 5 replicates with hand shears, and immediately placed in flasks of water. After 1 hr elapsed, the initial post-treatment determination of Pn was made on both excised and control shoots. The materials were then held in the lab (temperature 21°C) until the following morning when the final determination was made.

There were no significant differences (α = .05) between leaves on intact vs. detached shoots (Table 1). From these data we conclude that not only are detached shoots suitable for Pn determinations, but also that holding for 24 hr after detachment does not affect Pn rates.

Field expt. 1. A current season's shoot may be 100 cm in length and is difficult to transport and handle in the laboratory. An experiment was conducted to determine the effect of removing the terminal portion of a shoot on the Pn and Rd of the oldest leaf on the shoot. Four shoots were cut at dusk and immediately placed in water from each of six 13-year-old 'Starking Delicious'/Malling Merton (MM) 111 trees. Two shoots from each tree were left intact while the distal half was removed from the other 2. The shoots were transported to the laboratory and Pn and Rd were determined the following morning. As previously described (5) Pn was determined at a PAR level of 1100μEm^{-2}sec^{-1} at 25-30°C and an air flow rate of 3 liters min^{-1}. Rd was measured in darkness at 18-21° with an air flow rate of 3 liters min^{-1}. The design was a randomized complete block with subsampling with trees serving as blocks. The analysis of variance indicated that Pn and Rd were not affected by removal of the distal half of the shoot (data omitted).

Table 1. Net photosynthesis (Pn) of apple leaves on 1-year container grown trees before and after shoot detachment: greenhouse expts.

Expt.[z]	Treat.	Pn ($mgCO_2dm^{-2}hr^{-1}$) Prior to shoot detachment	1 hr after shoot detachment	1 day after shoot detachment
1	Control	24.4[y]	23.1	18.8
	Detached	22.2	21.9	18.1
2	Control	18.6	18.5	19.6
	Detached	19.1	20.2	17.3
3	Control	22.9	18.9	20.7
	Detached	22.4	21.4	21.3

[z]Expt. 1: 2 replicates 'Delicious', 3 of 'Golden Delicious'; expt. 2: 5 replicates of 'Delicious'; expt. 3: 5 replicates of 'Golden Delicious'.

[y]Paired means do not differ significantly at the 5% level.

Field expt. 2. A randomized complete block design was used to study the influence of short-term preconditioning treatments on Pn and Rd of 'Starking Delicious' apple leaves. From each of 5 trees similar to those described above, 4 shoots were cut, immediately placed in water, and randomly assigned to one of the following treatments:

1. cut at 6:00 PM and held in lab overnight at 21°C.
2. same as #1, with all but the 2 oldest leaves removed.
3. cut at 6:00 PM, but shoots were left in the orchard overnight (minimum temperature of 13°C).
4. cut at 6:30 AM, and brought to the lab immediately.

Following the overnight preconditioning, Pn and Rd were determined. Treatments had no effect on Pn or Rd (Table 2). Stomatal resistance (R_s) measured before and after Pn determinations did not differ among treatments (data omitted).

Field expt. 3. Stomatal resistance (R_s) measurements were made with a LI- COR LI65 Diffusion Resistance Autoporometer on intact spur leaves on the periphery of 'Stayman'/MM 111 trees in the field between 7 and 8:30 PM (dusk). Spurs were then cut, immediately placed in flasks of water, and transported to the lab. Spurs were held on an east-facing window sill overnight at 20°C. At 3 additional times R_s was determined on the same leaves: just prior to and after Pn determination (8-12 AM, next day), and immediately after dark respiration (Rd) measurement.

Stomatal resistance was similar at times 1, 2, and 4 with lower readings immediately after removal from the Pn chamber (Table 3). Detachment appears to have had minimal effects on stomatal resistance.

Table 2. Influence of short-term preconditioning on Pn and Rd of 'Delicious' apple leaves.

Treatment[z]	Pn[y]	Rd[y]
1	21.5	1.8
2	19.8	1.5
3	22.7	2.0
4	20.3	1.4
	ns[x]	ns

[z]Treatments: 1: cut at 6 PM and held in lab overnight at 21°C; 2: same as 1 with all but the two oldest leaves removed, 3: same as 1 but shoots left in the orchard overnight (min. temp. of 13°C), 4: cut at 6:30 AM.

[y]Expressed as mg $CO_2dm^{-2}hr^{-1}$.

[x]ns = not significant.

Table 3. Stomatal resistance (R_s)[z] of 'Stayman'/MM 111 leaves in the orchard and at 3 times after spur detachment.

Time[y]	R_s (sec cm^{-1}) on following dates: May 20-21	July 3-4
1. Prior to detachment	4.7 a[x]	5.9 a
2. Prior to Pn	2.9 ab	1.5 ab
3. After Pn	0.1 b	0.3 b
4. After Rd	5.4 a	4.5 a

[z]All readings temperature corrected to 25°C.

[y]Time as follows: 1: 7-8:30 PM in field, 2: 8-12 AM next day in lab, 3 & 4: same times as #2, but after Pn and Rd determinations respectively.

[x]Means separation within columns by Friedman's test, 5% level (8).

We speculate that an individual leaf has a limited "pool" of water and is much more readily stressed under conditions of high transpiration, whereas a leaf attached to a shoot has a larger "pool" of water and thus additional resistance to moisture stress.

From our earlier experience with individual detached leaved and the types of data reported herein, we conclude that the use of detached apple leaves is

not dependable with our type of system, but that detached shoots offer a sound technique which opens up various possibilities for field Pn studies without the necessity for a mobile lab.

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In Vitro Propagation of Malling Merton Apple Rootstocks [1,2]

Iona Snir and Amnon Erez

Institute of Horticulture, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel

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Abstract. **Rapid propagation of the apple rootstocks Malling Merton (MM) 104, MM 106 and MM 109 (*Malus* sp.) achieved was by shoot tip proliferation using a Murashige and Skoog basal medium with 1 mg/liter 6-benzylamino purine (BA) and 1 mg/liter indolebutyric acid (IBA). Improved proliferation was achieved by using a liquid medium. Two media were selected for rooting: the first contained IBA for root initiation and the second was without IBA but plus 0.25% activated charcoal for improved root development. Plantlets were successfully transferred to soil.**

Recent developments in high-density planting systems in apple orchards led to the need for a low-cost nursery stock. One approach is propagation of the apple using tissue culture methods (1, 4, 5, 6, 7, 9, 10, 12). Jones et al. (7) developed an *in vitro* propagation system for apple that incorporated the phenolic compounds phloroglucinol and phlorizin which was used with the rootstocks East Malling (M) 7 and M 26. This study was initiated to facilitate propagation of Malling Merton rootstocks which are in short supply in Israel; quarantine regulations limit the importation of rooted material.

Source plants were kept in a shaded greenhouse (40% of neutral shade) during spring and early summer. Two to 3 cm explants from apical shoot tips of the apple rootstocks were excised, sterilized, and placed on the proliferation medium in 2.5x10cm test tubes. The method of disinfection and the first medium were similar to those of Jones et al. (7), except that phloroglucinol was omitted after no advantage to it was found in preliminary experiments.

The plant hormones used (per liter) were: 1 mg BA, 1 mg of IBA, and 0.1 mg of gibberellic acid (GA_3). The medium was solidified by 0.7% Bacto-Agar. The test tubes were kept under Gro-Lux lamps in a growth chamber at 26°C with a 16-hr photoperiod and irradiance intensity of 600 $\mu W/cm^2$ in the visible range; air humidity was kept at 70%.

After 4 to 8 weeks on this medium, the newly developed shoots were cut and cuttings of 2 to 3 cm each were transferred to the same fresh medium and the development of new shoots was continued. Three weeks later the plant material was transferred to the same medium but in a jar (6x15 cm). In these jars the shoots elongated, more shoots were grown from axillary buds, and in 4 weeks the jar was crowded with apple shoots (Fig. 1A). The long shoots were taken for rooting and the small ones for repeated proliferation. These small shoots were cut and placed individually in groups of 10 per 100 ml flask with 10 ml of liquid medium. The flasks were shaken on an orbital shaker under a 16-hr (light), 8-hr (dark) regime for 4 days.

A faster growth rate was observed, in comparison with a solid medium, possibly due to an increased absorption of hormones and nutrients from the medium, not only through the shoot's basal end but through its entire surface. Another possibility is that agar contains toxic components for some plant systems (8). In addition, rapid vegetative development in the axillary buds. When these shoots were transferred back to a solid medium, intense proliferation was evident. A 5-fold multiplication rate per month was achieved with this system.

The long shoots (about 2 cm) which were taken for rooting were placed on the root initiation medium after they were wounded on both sides with a scalpel. The medium contained half-strength Murashige and Skoog minerals (11), 2% sucrose, 0.8 mg/liter thiamin-HCl, 100 mg/liter myo-inositol, 1 mg/liter 3-indolebutyric acid (IBA), and 0.7% Bacto-Agar. The pH was adjusted to 5.8, before addition of agar and before autoclaving. After 6 to 8 days, when root initials were seen, the shoots were transferred to the root developing medium. This medium was identical to the former one except that IBA was omitted and 0.25% activated charcoal was added. Shortening of the IBA treatment period to only 6 – 8 days

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