Rooting of Apple, Rhododendron, and Mountain Laurel Cuttings from Stock Plants Etiolated under Two Temperatures

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Abstract. Dormant stock plants of apple (*Malus domestica* Borkh.) rootstocks M.26 and Ottawa 3; *Rhododendron* 'Britannia', 'Purple Splendour', and 'Unknown Warrior'; and mountain laurel (*Kalmia latifolia* L.) 'Ostbo Red' and seedlings were forced to grow at 18 or 28 °C in continuous darkness or 14-h photoperiods. Etiolated shoots were then acclimated to light with or without aluminum foil wrapped around their bases to keep the bases etiolated. Shoots forced in diurnal light were neither etiolated nor wrapped and served as controls for the etiolation treatments. Compared to controls, wrapping etiolated stems improved rooting of M.26 (60% vs. 82%) and 'Ottawa 3' (81% vs. 97%) apple and of 'Britannia' (76% vs. 90%) and 'Unknown Warrior' (80% vs. 91%) rhododendron. Etiolation improved rooting percentage of 'Unknown Warrior' regardless of wrapping. Regardless of etiolation, forcing 'Ottawa 3' at 18 °C improved rooting percentage (92% vs. 74%) and roots per cutting (12 vs. 7) compared to forcing at 28 °C. Etiolated mountain laurel cuttings generally rooted best at 18 °C; control cuttings rooted best at 28 °C.

Etiolation promotes adventitious root formation on stem cuttings of many woody species (Anderson, 1981; Bassuk et al., 1984; Davis and Potter, 1983; Delargy and Wright, 1979; Gardner, 1937; Harrison-Murray, 1981; Herman and Hess, 1963; Maynard and Bassuk, 1987, 1991a, 1991b; Patience and Alderson, 1984). Generally, among these studies, the most effective treatment began by constructing a light barrier (black) and allowing shoots to develop in darkness or heavy shade, resulting in etiolated new growth. While still etiolated, the basal portion of the new shoot was wrapped with opaque material to maintain localized etiolation while the apical portion was acclimated to light and developed chlorophyll. After a period of acclimation, new shoots were excised so that the base of the initially etiolated shoot was now the base of the cutting; this would be the basal end of the wrapped portion for wrapped stems. For some species/

cultivars, etiolation without wrapping improved rooting percentage substantially (Anderson, 1981; Blakesley et al., 1992; Patience and Alderson, 1984), sometimes as much as etiolation plus wrapping (Bassuk et al., 1984; Harrison-Murray, 1981; Maynard and Bassuk, 1987, 1991a, 1991b). Thus, the efficacy of etiolation and wrapping should be determined for individual taxa (Maynard and Bassuk, 1991b), especially since wrapping stems to maintain localized etiolation is labor intensive.

Using a light barrier increases the temperature inside the barrier. Temperature differences across light barriers reached 6 °C for a ventilated barrier (Patience and Alderson, 1984) and 7 to 15 °C for nonventilated barriers (Blakesley et al., 1992; Patience and Alderson, 1984). Such temperature differences not only affected rooting, they constituted an uncontrolled variable (Patience and Alderson, 1984). The objectives of this study were to determine if temperature during etiolation affects rooting of the selected plant material and if initial shoot etiolation affects rooting of selected plant material even if wrapping, to maintain localized etiolation at the cutting base, is omitted.

Materials and Methods

A factorially designed experiment including two stock plant temperatures and three etiolation treatments for each cultivar/species was done twice. About 80-cm-tall, 1-year-old, stooled M.26 and Ottawa 3 apple rootstocks, 10- to 20-cm-tall, 2-year-old, containerized 'Britannia', 'Purple Splendour', and 'Unknown Warrior' rhododendron plants, and 15- to 30-cm-tall, 2-year-old 'Ostbo Red' (Expt. 1) or seedlings (Expt. 2) of mountain laurel in con-

tainers were obtained from commercial nurseries. The rhododendron and 'Ostbo Red' mountain laurel were propagated by tissue culture. All plants had overwintered outside until they were purchased, fulfilling the necessary chilling requirements. Apple rootstocks were pruned to a height of 15 cm and potted in 1.8-L containers with a medium of 1 peat : 1 perlite : 1 loam soil (by volume). Rhododendron plants remained in the 2.6-L pots from the nursery; mountain laurel plants were repotted in 2.6-L pots with the peat-perlite-soil medium. Each experiment included 60 stock plants of each apple rootstock and 20 stock plants of each rhododendron cultivar and mountain laurel at each temperature.

To force budbreak, stock plants were transferred to a relatively warm environment starting 5 Mar. (Expt. 1) and 5 Apr. 1985 (Expt. 2). To promote etiolated growth, plants were placed in two insulated light-tight cabinets (2.4 × 1.2 × 1.2 m) housed in an unheated shed (<18 °C); a thermostatically controlled heater maintained the air inside the cabinets at 18 or 28 ± 2 °C (monitored by thermocouple/recorder). The cabinets were ventilated with outside air to lower humidity and discourage growth of foliar/stem pathogens. To promote nonetiolated growth (controls), plants were placed in controlled environment chambers at 18 or 28 ± 2 °C, with a 14-h photoperiod of 250 μmol·m⁻²·s⁻¹ (measured with a quantum flux meter, model LI-185; LI-COR, Lincoln, Neb.) from cool-white fluorescent and incandescent lamps.

Because the rate of stem elongation at a given temperature varied even within cultivars, the duration of dark exposure was adjusted to produce shoots of suitable length. Plants of apple and rhododendron remained in darkness at 28 °C for 15 to 22 d (Expt. 1) and 10 to 17 d (Expt. 2). The corresponding durations at 18 °C were 20 to 26 d (Expt. 1) and 18 to 24 d (Expt. 2) because stem elongation was slower at 18 than at 28 °C and slower in March than in April. Stock plants of mountain laurel developed more quickly and were removed from darkness 3 to 4 d earlier than other plants.

After removing plants from darkness, stems of half the population of etiolated shoots were selected randomly and their bases were wrapped with aluminum foil, while the other half remained unwrapped. The length of the wrapped zone was standardized for apples and mountain laurel at 4 and 3 cm, respectively. Rhododendron shoot length varied widely; some shoots were only 1.5 cm long below the leaves, and the entire 1.5 cm was wrapped. Many rhododendron shoots (50%, 35%, and 17% for 'Britannia', 'Unknown Warrior', and 'Purple Splendour', respectively) were at least 7 cm long and one-half to two-thirds of the length of these shoots was wrapped. Etiolated stock plants were allowed to acclimate and develop normal leaves and stems under gradually decreasing shade in a glasshouse. Environmental conditions during acclimation were as follows: 23 °C day/18 °C night and solar irradiance gradually was increased over 2 weeks by removing layers of neutral density shade cloth; initial and final shade were 95%

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and 0%. Nonetiolated plants were treated the same (except they were never wrapped).

After acclimation for 6 weeks (apple) or 10 weeks (rhododendron and mountain laurel), softwood (apple) or semihardwood (rhododendron and mountain laurel) cuttings were obtained by excision at the basal end of the wrapped stems or from the analogous part of the nonwrapped stems. For a given genus, the acclimation period began when the last plant was removed from darkness. The total number of cuttings varied (Table 1); cuttings were distributed equally among treatments, ranging from ≈ 30 for Ottawa 3 apple to more than 70 for 'Purple Splendour' rhododendron. If present, second-flush stem growth was removed. The base of each rhododendron and mountain laurel cutting was wounded by removing two longitudinal strips of bark (2 to 4 mm long) at the base and then dipped into Hormodin III (0.8% indolebutyric acid in talc) (Merck & Co., Rahway, N.J.). Cuttings were inserted in flats (46 $\times$ 37 $\times$ 6.5 cm) containing a medium of 1 peat : 1 perlite (by volume) and placed under a tent of clear 0.1-mm polyethylene with intermittent mist (8 s every 15 min for 14 h daily) in a glasshouse (27 °C day/22 °C night).

Cuttings were evaluated twice for rooting (after 6 and 14 weeks for apple and 12 and 20 weeks for rhododendron and mountain laurel). Cuttings were scored as rooted if there was at least one root >5 mm long for apple, or if there was at least one root >10 mm long for rhododendron and mountain laurel. After the first evaluation, unrooted cuttings were returned to the rooting medium to be evaluated at the second scoring; rooted cuttings were discarded. Rooting percentages were calculated as the sum of rooted cuttings from both scorings per 100 cuttings prepared. Quality of rooting was measured only at the first evaluation, and for apple, as the number of roots >5 mm long per rooted cutting. For rhododendron and mountain laurel, quality of rooting was measured as the maximum horizontal diameter of the rootballs; only cuttings with rootballs >20 mm across were included.

Statistical analyses were run separately for

the two experiments and combined. There were no significant differences between the two experiments; therefore, results from the two experiments were pooled. No attempt was made to pool the data for the clone and seedling mountain laurel. Analysis of variance was run for each cultivar; the model included temperature (18 and 28 °C) and etiolation treatments (control, etiolated, and etiolated + wrapped) as main effects, and interaction between temperature and etiolation treatment (SAS Institute, 1987).

Results

Apple. After rooting for 14 weeks, etiolation combined with wrapping increased the rooting percentage for the two apple rootstocks compared to the untreated control (Table 1). The lower forcing temperature (18 °C) increased the rooting percentage of Ottawa 3 cuttings with or without etiolation. If data for Ottawa 3 in Table 1 are averaged across all three etiolation treatments, the rooting percentage at 18 °C was 92% compared to 74% at 28 °C. Compared to controls, the combination of etiolation and wrapping increased the number of roots per cutting for both apple cultivars (Table 2). Forcing temperature significantly affected root quality only for Ottawa 3; root counts were higher at 18 than 28 °C for all three etiolation treatments (F test, $P \leq 0.05$). When the data for Ottawa 3 were averaged across all three etiolation treatments, 12 roots formed per cutting at 18 °C compared to 7 at 28 °C (data not tabulated).

Rhododendron. After rooting for 20 weeks, etiolation combined with wrapping increased the rooting percentage for 'Britannia' and 'Unknown Warrior' rhododendron compared to the untreated control (Table 1). Also, for 'Unknown Warrior', etiolation without wrapping increased the rooting percentage. At 20 weeks, etiolation did not significantly affect the rooting percentage of 'Purple Splendour'. However, at 12 weeks, the combination of etiolation and wrapping stimulated rooting of this cultivar (80% vs. 64% and 63% for the control and nonwrapped treatments respec-

tively, $P \leq 0.05$; temperature data pooled). At the first evaluation (6 weeks for apple and 12 weeks for the other taxa), etiolation affected rooting percentage of only one other taxon, 'Unknown Warrior'. The control of 'Unknown Warrior' rooted 38% compared to 67% for nonwrapped and 66% for wrapped cuttings, $P \leq 0.001$ (temperature data pooled). Compared to controls, the combination of etiolation and wrapping increased the rootball size for 'Britannia' and 'Unknown Warrior' at the first evaluation (Table 2). Etiolation without wrapping increased rootball size for 'Britannia'.

Mountain laurel. Forcing temperature interacted with etiolation treatment of 'Ostbo Red' and seedling mountain laurel so that control cuttings rooted best at 28 °C while nonwrapped etiolated cuttings rooted best at 18 °C, $P \leq 0.05$. For wrapped etiolated cuttings of mountain laurel, only seedlings rooted best at 18 °C, $P = 0.05$ (Table 1).

All taxa. Compared to controls, rooting percentage increased when etiolation with wrapping increased quality of rooting, as expressed by number of roots per cutting and rootball size (compare Tables 1 and 2, although these observations are from different evaluation times).

Discussion

The apple rootstock Ottawa 3 is considered difficult to propagate from cuttings (Osborne, 1983); however, control (nonetiolated) cuttings of Ottawa 3 rooted well. Also, the normally difficult-to-root rhododendron cultivar Britannia and mountain laurel rooted better than expected. Therefore, our intention of comparing difficult-to-root and easy-to-root taxa was only partially realized. Apparently, undefined factors increased the ease of rooting for controls of these plants. Such factors could include stock plant condition, forcing, or relatively low light during early stem growth.

While it is well documented that etiolating or blanching stems can promote adventitious root formation, the rooting responses to such treatments differ among species and among

Table 1. Rooting percentages of stem cuttings from plants forced to grow in darkness (etiolated, E) or in diurnal light (control, C) at two temperatures. Etiolated stems were either left nonwrapped (NW) or wrapped (W) with aluminum foil. Percentage data are the total number of cuttings rooted after 14 weeks (apple) or after 20 weeks (rhododendron and mountain laurel) per 100 cuttings prepared. Data from expts. 1 and 2 were pooled.

Species and cultivar	Total (no.)	Cuttings						F test C vs. E, NW C vs. E, W Temp Interaction between temp and etiolation			
		Rooted (%)									
		Control		Etiolated nonwrapped		Etiolated and wrapped					
				Growing temp (°C)							
				18	28	18	28				
Apple											
M.26	331	56	63	56	54	83	82	NS	*	NS	NS
Ottawa 3	192	91	71	86	58	100	94	NS	*	*	NS
Rhododendron											
Britannia	275	75	77	78	79	90	91	NS	*	NS	NS
Purple Splendour	441	91	94	88	85	92	94	NS	NS	NS	NS
Unknown Warrior	288	76	84	91	89	92	91	*	*	NS	NS
Mountain laurel											
Ostbo Red	255	53	71	67	38	69	61	NS	NS	NS	*
Seedlings	203	19	38	51	27	55	33	NS	NS	NS	*

NS, *Nonsignificant or significant at $P \leq 0.05$, respectively.

Table 2. Quality of rooting of stem cuttings from plants forced to grow in darkness (etiolated) or diurnal light (control). Quality was evaluated at 6 weeks for apple and at 12 weeks for rhododendron and mountain laurel. Data were pooled from expts. 1 and 2 and from both temperature treatments.

Species and cultivar	No. of cuttings	Control	Etiolated nonwrapped	Etiolated and wrapped
<i>Roots per cutting (no.)</i>				
Apple				
M.26	109	4 b ^a	4 b	11 a
Ottawa 3	106	7 b	10 ab	13 a
<i>Rootball diameter (mm)</i>				
Rhododendron				
Britannia	134	56 b	67 a	66 a
Purple Splendour	305	72 ^{ns}	72	75
Unknown Warrior	164	68 b	72 b	83 a
Mountain laurel				
Ostbo Red	41	34 ^{ns}	39	41
Seedlings	33	43 ^{ns}	46	36

^aMean separation within rows by Ryan-Einot-Gabriel-Welsch multiple-range test at $P \leq 0.05$.

related cultivars (Bassuk et al., 1984; Davis and Potter, 1983; Maynard and Bassuk, 1987, 1991b). In our study, etiolation with wrapping improved rooting (compared to controls) of all taxa, except 'Purple Splendour' rhododendron and mountain laurel, although the ease of rooting of the 'Purple Splendour' controls made improvement difficult. Also, as reported by others (Maynard and Bassuk, 1987, 1991b), our experiments emphasized the variability among taxa in efficacy of maintaining localized etiolation at the base of etiolated stems during acclimation to light. Etiolation without wrapping increased rooting percentage for 'Unknown Warrior' and rootball size for 'Britannia' similar to that of etiolation plus wrapping, but these were the only taxa affected by this treatment.

Compared to controls, when etiolation with wrapping improved rooting quality at the first evaluation (Table 2), rooting percentage increased at the second evaluation (Table 1), indicating that early or vigorous root formation was associated with high rooting percentage. The increased rooting percentage due to etiolation combined with wrapping at the first evaluation for 'Purple Splendour' and 'Unknown Warrior' also indicates early rooting was promoted by this treatment for some taxa, a finding consistent with another study of rhododendron (Maynard and Bassuk, 1991b).

The time needed to acclimate etiolated rhododendron shoots is necessarily many weeks, because shoots grow in spring, and

cuttings root best if they are taken in summer (Wells, 1954). Thus there was considerable time during the long acclimation period to destroy any rooting promotion effect due to etiolation, particularly if the base of the cutting was not kept etiolated. Remarkably, some species or cultivars, including rhododendron, retain this etiolation effect even if nonwrapped stems are left to acclimate for 4 to 8 weeks (Maynard and Bassuk, 1987, 1991a, 1991b) or 10 weeks, as in the present work.

Our results of temperature effects during etiolation differ from those of Patience and Alderson (1984) for *Syringa vulgaris* L. (lilac). Their positive effect of 25 °C compared to 15 °C is contrary to our findings in which the lower forcing temperature (18 °C compared to 28 °C) promoted rooting of Ottawa 3 apple and of etiolated mountain laurel, except the wrapped 'Ostbo Red' cuttings. The higher forcing temperature (28 °C) did not promote rooting of any etiolated stems. The extended acclimation period in our experiments, 6–10 weeks as opposed to 1–3 days for lilac, may account for this difference.

If light barriers designed to promote etiolated shoot growth are exposed to the sun, the temperature inside the barrier can get much higher than ambient air (Blakesley et al., 1992; Patience and Alderson, 1984). The present study suggests that this temperature increase may adversely affect rooting of etiolated cuttings. Regardless of the forcing temperature, etiolation combined with wrapping improved

rooting of most taxa, but etiolation without wrapping was much less effective.

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