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Stock plant etiolation and stem banding effect on the auxin dose-response of rooting in stem cuttings of *Carpinus betulus* L. 'Fastigiata'

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Abstract. Experiments were undertaken to determine the effect of stock plant etiolation and stem banding, prior to cutting propagation, on the auxin dose-response of rooting in *Carpinus betulus* L. 'Fastigiata' stem cuttings. Stock plants were forced in a greenhouse, etiolated for 10 days and banded with black, light-tight Velcro™ for 8 weeks. Indole-3-butyric acid was applied to cuttings at concentrations ranging from 0 to 79 mM. Rooting percentages and numbers increased to a peak response at 20 mM in light-grown and 40 mM in etiolated shoots, followed by an inhibition at higher concentrations for all except etiolated and banded shoots. Cuttings prepared from shoots which had been etiolated or banded rooted better than controls at low and optimal IBA concentrations. Cuttings from shoots receiving both etiolation and banding also yielded higher rooting percentages and more roots per rooted cutting. Furthermore, etiolation and banding reduced the sensitivity of cuttings to supra-optimal auxin-induced inhibition of adventitious root initiation.

1. Introduction

Auxins are indispensible to the vegetative propagation of most woody plant species. The synthetic auxin used most frequently in rooting stem cuttings is indole-3-butyric acid (IBA). The rooting response of stem cuttings to exogenously applied IBA varies considerably with shoot maturation (softwood, semi-hardwood, hardwood), phenological age (juvenile, mature) and stock plant nutrition [3]. The auxin dose-response curve for rooting a particular species will usually show an increase in rooting with increasing auxin concentration up to an optimum, followed by an inhibition of rooting or symptoms of phytotoxicity at supra-optimal levels of auxin. Changes in the sensitivity of cuttings to applied auxin are important both practically and for an understanding of the physiological events governing root

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formation. Applying the optimal exogenous auxin concentration is often the key to successfully propagating a difficult-to-root species.

Several studies have examined the sensitivity of rooting to auxin, and correlated differential sensitivity with rooting capacity. Welander and Snygg [10] showed that the adult form of 'A2' apple rootstock, cultured in vitro, possessed a higher IBA optimum for root number and was less affected by high concentrations of IBA than the juvenile form. The adult 'A2' also contained one-half or less the amount of extractable IAA as juvenile 'A2'. In a comparison of the rooting of four apple cultivars, Lane and McDougald [7] observed that the clonal rootstock 'MM.111' rooted to the same degree as cultivars 'M.26' and 'M.27' (85% rooting), but at a 3-fold higher auxin level. Conversely, in the same study the scion cultivar 'Macspur' rooted maximally at the same optimal auxin concentration as 'M.26' and 'M.27', but showed a lower capacity for rooting (58% vs. 85%). In an investigation of the differential in vitro rooting responses of 'M.9' (difficult-to-root) and 'M.26' (easy-to-root) apple rootstock cultivars, James [5] found that 'M.26' responded maximally at a 10-fold lower IAA concentration than 'M.9', with both cultivars yielding the same number of roots at their respective optima. This suggested a reduction in the affinity to IAA of processes controlling the rooting of 'M.9', but not a reduction in the overall response capacity. In terms of rooting percentage, 'M.26' was inhibited more by supra-optimal levels of IAA while sub-optimal and optimal IAA concentrations produced similar responses in both cultivars.

The analysis of rooting using dose-response curves represents an opportunity for the study of stock plant light-exclusion treatments on root initiation. In previous studies with *Carpinus betulus* we noted differential responses of root formation to stock plant etiolation and/or stem banding treatments [9]. A natural extension of this work was a determination of how this differential rooting response varied with the level of exogenously applied auxin. The objectives of the present work were to characterize the rooting response of *C. betulus* 'Fastigiata' stem cuttings to a range of exogenously applied IBA, and to determine the relative influences of stock plant etiolation and stem banding on this response.

2. Materials and methods

2.1 Forcing of stock plants

Dormant grafted stock plants of *C. betulus* 'Fastigiata' were potted in late fall into 30 L plastic containers in a medium of 1 sandy loam soil: 1 sphagnum peat: 1 perlite (v/v/v) and placed in cold storage for 12 wks at 4 + 1°C. Stock plants were then forced in a 20°C greenhouse. Incandescent lamp (100W, supplying ~2 μmoles m^{-2} s^{-1} at canopy level) were used from 4:00 PM–12:00 PM to extend the natural photoperiod to ~16 hrs. Stock plants were fertilized weekly with 200 mg 20N–10P–20K.

Within 2–4 wks stock plants began to break bud, at which time half of the stock plants were enclosed in a structure of black cloth draped over wire, with a distance of 0.5–1.0 m maintained between the plants and black cloth. Stock plants to be forced in full sun were left uncovered. The etiolation enclosure excluded > 99% of incident light until developing shoots reached a length of 5–10 cm, which required 10 d. At this time, banding was applied to half of the shoots selected at random throughout the crown of each stock plant. Stems were banded by sandwiching the base of new shoot growth between the 'wool' and 'hooks' of 2.5 cm wide black Velcro™ strips firmly pressed onto the stem, such that they would not slip from that position after application. Light-grown shoots were banded at the same time as etiolated shoots. After banding, etiolated shoots were allowed to green for 8 wks. In terms of actual light exposure, light-grown shoots were exposed to light 10 d longer than etiolated shoots.

2.2 Harvesting and rooting of cuttings

Cuttings were harvested by severing shoots at the base of the new growth. Shoots taken from positions throughout the stock plant crown were prepared, by removing terminal growth, to yield a cutting 7–10 cm long with 3 leaves. Banded shoots were cut immediately proximal to the Velcro™ band and the bands removed. A 2 × 2 factorial of etiolation and banding treatments included light-grown, non-banded shoots (controls); light-grown, banded shoots (banded); etiolated shoots which had greened (etiolated); and etiolated and banded shoots, which yielded a green cutting with a continuously etiolated base (etiolated and banded). Immediately after harvesting, cuttings were immersed for 10 min in an aqueous solution of 0.25 g l^{-1} (w/v) Captan 50WP, placed in polyethylene bags, and stored at ~5°C for one hour before sticking. Cuttings were then treated with a 5-sec dip of the basal 1 cm using 0, 1.2, 2.5, 3.7, 4.9, 9.8, 20, 39, 59 or 79 mM IBA in 50% aqueous ethanol. The cuttings within each hormone treatment were then distributed randomly into seven replicate groups of ten cuttings each prior to insertion to a depth of ~2 cm in a 13 cm deep rooting medium of 2 perlite: 1 sphagnum peat (v/v) in a randomized design.

Cuttings were rooted under mist (6 sec every 8 min from 6:00 AM–8:30 PM), with incandescent lamps (100W, supplying ~4 μmoles m^{-2} s^{-1} at

bench level) used from 4:00 PM–12:00 PM each day to extend the natural photoperiod to ~ 16 hrs.

2.3 Rooting evaluation

Cuttings were considered rooted if they possessed one or more roots > 1 mm in length. Rooting percentage, the number of roots per rooted cutting and root length were recorded after 40 d. The distance from the cutting base to the first emerged root (DFR) also was measured, as an indicator of auxin toxicity to the root initiation process. Percentage data were arcsine transformed [15] and analyzed by regression as a continuous response to log[IBA] and by analysis of variance as factorial responses to stock plant etiolation and banding [14].

3. Results

Rooting percentages, root numbers and length increased up to an optimum IBA concentration and declined at higher auxin concentrations. Two replications of the experiment yielded very similar results; only the rooting percentage data of the second replication are presented herein (Figure 1). More cuttings prepared from etiolated shoots rooted than those from light-grown shoots in response to 0–2.5 mA IBA. The rooting of etiolated and banded shoots increased to 74% at 2.5 mM IBA, while the rooting of light-grown shoots remained low (34–39%). The half-maximal response of etiolated shoots was exceeded at between 1.2 and 2.5 mM IBA, while light-grown stems reached this response at 3.7 mM IBA. The maximal rooting response of light-grown banded shoots peaked sharply at a lower IBA concentration (9.8–20 mM) than etiolated and banded shoots, which exhibited a broader peak over 20 to 70 mM IBA and were virtually unaffected by the higher concentrations of IBA. The highest rooting capacity of non-treated cuttings (83% at 20 mM IBA) was increased by either stock plant treatment to between 90 to 100%. Etiolation improved rooting by an average of 14% ($p = 0.001$). The rooting percentage data were adequately fit to quadratic functions of log[IBA] (Figure 1).

The response of root numbers to IBA and stock plant treatments was similar to that of rooting percentages. The number of roots per rooted cutting (RRC) of cuttings from etiolated shoots peaked at 59 mM IBA with 6.2 ± 0.5 RRC and showed no inhibition at high IBA, while the RRC of light-grown cuttings peaked at 39 mM IBA with 6.0 ± 0.6 RRC and then declined to 4.7 ± 0.4 RRC in response to > 39 mM. Cuttings from banded

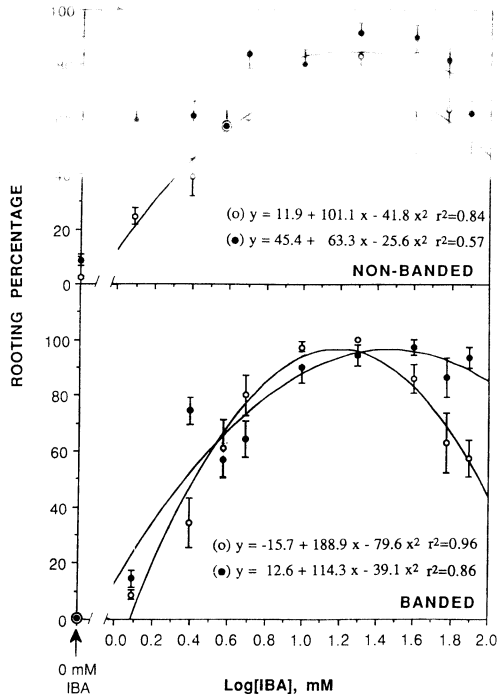

Fig. 1. Rooting percentage versus IBA dose-response of non-banded or Velcro™ banded shoot cuttings of *Carpinus betulus* 'Fastigiata' which were either light-grown (open circle) or initially etiolated (closed circle). Rooting was assessed after 40 d and data fit to a quadratic function of log[IBA], excluding the 0 mM IBA response.

shoots produced more roots (5.0 ± 0.2) than light-grown shoots (3.6 ± 0.1; $p = 0.001$) across the range of exogenous IBA.

The root lengths of cuttings from treated shoots reached 7.0 ± 0.3 mm at 2.5 to 4.9 mM IBA and were inhibited by higher IBA concentrations (4.0 ± 0.1 mm at ⩾ 39 mM IBA). Light-grown cuttings were less responsive to low IBA and produced their longest roots (6.4 ± 0.3 mm) in the range of 20 to 79 mM IBA.

The distances from the cutting base to the first emerged root (DFR) increased with IBA concentration. The DFR of light-grown and etiolated shoots were similar at low IBA (3.3 ± 0.2 mm), while at IBA levels > 20 mM the DFR of etiolated shoots was nearly double that of light-grown shoots (12.8 ± 1.4 vs. 7.0 ± 0.9 mm, respectively; $p = 0.006$). Banding, however, reduced DFR from 5.5 ± 0.4 to 4.5 ± 0.3 mm ($p = 0.03$).

4. Discussion

The rooting percentage response of stem cuttings of *C. betulus* 'Fastigiata' to increasing levels of exogenously applied IBA followed a dose-response curve which increased with increasing concentrations of applied auxin to a peak response and thereafter declined. Similar responses to applied auxin have been observed across a spectrum of auxin-related phenomena [2, 12], and are characteristic of the auxin dose-response of rooting in tissue-cultured *Malus* [5, 6] and in the mung bean rooting bioassay [8].

The maximal rooting response was increased by both stock plant etiolation and stem banding. Stock plant etiolation also dramatically increased the initial rooting percentage response to auxin. Etiolation followed by banding yielded cuttings which rooted the best overall, because of initially higher rooting, a broad peak response, and higher rooting at supra-optimal IBA levels. Our data suggest an increased auxin responsiveness of cuttings taken from stems which have developed in the absence of light, either through etiolation for the first few days of growth, or by continuous blanching of the stem by banding. Such treatments also reduced the requirement for exogenous auxin in the promotion of adventitious root formation. Howard [4] also noted that etiolation and banding increased the rooting response of *Malus* 'M.9' to IBA, though rooting percentage responses were not saturated in the range of auxin (0–12 mM) investigated.

The auxin inhibition of rooting typically involves the discoloration and necrosis of the treated tissues, rooting at a greater distance from the cutting base, and even death of the cutting [1, 10, 13, 16]. The physiological mechanisms of rooting inhibition by high doses of auxin are not known. Ethylene is generally considered to be a causal factor [2], though Mulkey et al. [11] showed that the auxin-induced inhibition of maize root growth was independent of ethylene produced by the treated tissues. In the present study the basal rooting of etiolated shoots was more sensitive to the phytotoxic effects of IBA. However, banding reduced DFR, possibly by promoting the early initiation of roots on intact stems, such that banded cuttings were more prone to root despite the adverse effects of supra-optimal auxin. Banding similarly reversed the auxin-induced increase in DFR observed in a study of rooting in softwood cuttings of *Acer griseum* Pax. [10].

Our data indicate that the optimal rooting of softwood cuttings of *C. betulus* 'Fastigiata' occurs on banded cuttings treated with IBA in the range of 10 to 20 mM. The increased sensitivity to sub-optimal IBA levels, broader peak response, and decreased inhibition of etiolated shoots at high IBA would indicate that etiolation is preferable, though not essential, as an additional stock plant treatment.

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