CORRELATION OF PHENOLICS WITH ETIOLATED AND LIGHT-GROWN SHOOTS OF *Carpinus betulus* STOCKPLANTS

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Abstract Phenolic compounds are believed to play an important role in adventitious root formation, though the mechanism by which phenolics act is still largely unknown. In this study, changes in methanol-extractable phenolic compounds were characterized during the early growth of light-grown shoots and etiolated shoots of *Carpinus betulus* L. 'Fastigiata'. Two dimensional thin-layer chromatography of stem extracts produced twenty-eight fluorescent spots, many of which were tentatively identified as specific phenolics or belonging to a particular class of phenolic compounds. Changes in phenolic compounds were followed for 7 weeks in light-grown shoots or 4 weeks in previously etiolated and then light-grown shoots. Etiolated shoots initially had fewer phenolic compounds, but by the second week of exposure to light had a phenolic composition similar to light-grown shoots at week 0. Some of the phenolic compounds in light-grown shoots disappeared after 7 weeks.

Key words: adventitious root formation, *Betulaceae*, European Hornbeam, phenolics

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INTRODUCTION

European hornbeam (*Carpinus betulus* L.) is a desirable tree for landscape planting. Propagation of *C. betulus* by seed is inconsistent, and grafting is labor intensive and not always successful. Cutting propagation has proven most reliable, though rooting success varies among cultivars (3). Extensive work on the cutting propagation of *C. betulus* 'Fastigiata' has been undertaken by Maynard (8).

Stockplant etiolation (the exclusion of light) and stem banding during bud break and initial growth of new shoots increase the rooting of *C. betulus* softwood stem cuttings (8, 9). Maynard (8) found that rooting percentages of etiolated stem cuttings remained high through 12 weeks of greening while rooting of light-grown stem cuttings decreased rapidly in that time, such that etiolation extended the time for which rooting remained at an acceptable level.

Some phenolic compounds are believed to affect adventitious root formation by preventing the degradation of auxin, by forming auxin-phenol complexes, or by acting as rooting inhibitors (6, 14). Extractable phenolic compounds have been found to differ between etiolated and light-grown stem tissues (7, 13), and have been correlated with the success of rooting of stem cuttings.

This study observed the changes in phenolic compounds during the greening of etiolated shoots and growth of light-grown shoots of *C. betulus* 'Fastigiata', and correlated these changes and the rooting potential of cuttings from etiolated versus light-grown stockplants.

MATERIALS AND METHODS

One-year-old ramets of *C. betulus* 'Fastigiata' propagated by stem cuttings the previous season and placed in cold storage (5°C) in December, 1989, were removed over a four week period in February, 1990. The plants were repotted into 12.7 cm diameter plastic containers in a medium of 1 sandy loam soil:1 spaghnum peat:2 perlite (by vol.) and grown in a greenhouse with a 31°C day / 11°C night temperature regime. At bud break, half of the stock plants were etiolated as described by Maynard and Bassuk (9). Briefly, plants were placed in a black cloth enclosure until the etiolated shoots reached 4-6 cm in length, which took about 7 d, and were then allowed to turn green gradually by exposing the plants to increasing light levels over 7-10 d. Shoots from both light-grown and etiolated plants were collected for chemical analysis at weekly intervals, up to week 7 for light-grown plants and week 4 for etiolated plants. Week 0, the start of sampling, was designated as the day the etiolated stockplants were first removed from the black cloth enclosure, and when light-grown shoots were 4-6 cm in length. Light-grown shoots were greened from budbreak, and thus exposed to light about 7 d longer than etiolated shoots.

The phenolic compounds of excised stems were extracted twice with 1 ml absolute MeOH at ~70°C for 10 min. The crude extracts were dried under N_2 and redissolved in 80% MeOH. Fifty mg fresh weight equivalents (FWE) aliquots of each crude extract sample were spotted near one corner of a thin-layer chromatography (TLC) sheet (cellulose without fluorescent indicator, 20 cm x 20 cm, Eastman Kodak #13255, Rochester, NY), and chromatographed in the first dimension using n-butanol:acetic acid:H_2O (BAW; 4:1:2.2 by vol.) and in the second dimension using 15% aqueous acetic acid.

Additional procedures to aid in the identification of phenolic compounds used stems from light-grown

stockplants collected from weeks 0 and 3. Acid hydrolysis (10) allowed for the identification of free phenolic acids (aglycones). After MeOH extraction, a 100 mg FWE sample was hydrolyzed in 1 ml 2N HCl for 45 min at ~95°C. Aglycones were taken up in excess diethyl ether, dried under N_2, redissolved in 80% MeOH and chromatographed two dimensionally (2-D) in BAW and 15% acetic acid or 2-D in BAW and Forestal (acetic acid:H_2O:HCl, 30:3:10 by vol.). A procedure described by Steck (12) was used for the separation and identification of free forms of caffeic, p-coumaric, and ferulic acid and their derivatives, which behave similarly in common chromatography solvents.

Phenolics on the chromatograms were examined under ultraviolet (UV) light and were then fumed with ammonia (NH_3) and examined under UV or white light to note color changes characteristic of certain classes of phenolic compounds (5, 10). For comparison, phenolic standards were chromatographed and evaluated by the same methods used for extracted material.

Relative concentrations of phenylalanine and tyrosine were compared between etiolated and light-grown tissues at the onset of the experiment using 50 mg FWE samples spotted on Whatman 3MM chromatography paper and co-chromatographed (1-D in BAW) with the corresponding standards. These chromatograms were then sprayed with ninhydrin and heated for 3 min at 105°C.

RESULTS AND DISCUSSION

The 2-D chromatographic separation of methanol extracts from *C. betulus* shoots (in BAW and 15% acetic acid) consistently yielded a pattern of up to 28 spots which varied in quantity and intensity over the first 4 weeks of greening etiolated and first 7 weeks of light-grown shoot growth (Figure 1). In the first two weeks, fewer phenolic compounds were present in extracts of greening etiolated shoots than of light-grown shoots. Phenylalanine, the major precursor to phenolic synthesis, was present in visually detectable levels on the chromatogram of extracts of etiolated shoots at the end of the etiolation treatment (before greening, week 0), but not in light-grown material. Not until the second week of greening (3 weeks after budbreak) was the pattern of spots from extracts of etiolated shoots similar, in intensity or number of compounds separated, to that of light-grown shoots at week 0. Phenolic compounds in light-grown material began to decrease in number and intensity by week 7. Several compounds (such as #1, #3, #19, and #22) were present in extracts of light-grown and etiolated shoots at week 0.

Several of the compounds were tentatively identified (summarized in Table 1) by comparing their colors and R_fs to phenolic standards and published data. Identities of several of the classes of different phenolic compounds (ie. flavonols, catechins, and hydroxycinnamic acids) were speculated based on the fact that the colors of aglycones (standards) and their derivatives are often similar, even though their relative mobilities are usually different. Several aglycones which have been previously identified in *C. betulus* (1), including myricetin, quercetin, and kaempferol (three flavonols commonly found in plants) and caffeic acid (a hydroxycinnamic acid) were identified after acid hydrolysis of crude extracts. Compounds #3, #5, #6, #10, #16, and #19 were not identified, but were similar to each other in color.

Correlations between these phenolic compounds and their effect on adventitious root formation can be made based on rooting and anatomy data of similar material collected by Maynard (8). It has been suggested that phenolic

compounds may be important in the rooting process by interacting directly with 1H-indole-3-acetic acid (IAA) and the process of adventitious root formation, or by acting as precursors to lignin formation (6). Hydroxycinnamic acids which are converted to cinnamyl alcohols are important precursors to lignin. Doud and Carlson (4), Schmidt (11), and Maynard (8) each found that etiolated stems were less lignified and contained fewer sclereids than light-grown stems. The formation of sclereids in putative root initiation loci has been correlated with a decrease in the adventitious root formation on cuttings of both *Tilia tomentosa* Moench. (11) and *C. betulus* (8), presumably by usurping available initiation sites. Maynard (8) found that the xylem of light-grown *C. betulus* shoots sampled 7-14 d after budbreak was becoming lignified and sclereids were forming, whereas it was not until 4-6 weeks of greening that substantial lignification began to occur in initially etiolated shoots. Hydroxycinnamic acids were present in light-grown shoots at week 0, about 7 d after budbreak, but did not appear in initially etiolated shoots until 2-3 weeks of greening. This lag in hydroxycinnamic acid production seems to correlate well with the lag in lignin formation in initially etiolated shoots, and speculates a possible role these hydroxycinnamic acids might play in the formation of lignin and the ability of cuttings from etiolated stockplants to have a higher success of rooting for a longer period of time than light-grown stockplants. The disappearance of hydroxycinnamic acids in light-grown shoots by week 7 might represent their incorporation into lignin or other secondary plant products.

Overall, there were few direct correlations of the presence or absence of phenolic compounds and the success of rooting. The complement of phenolics present in etiolated shoots at week 2 was similar to the phenolics present in light-grown shoots at week 0. The success of rooting was nearly identical for both light-grown and etiolated stem cuttings at week 2 (87.5% and 90.5%, respectively). However, by week 6 in light-grown shoots, when most phenolics were still similar to week 2, rooting of light-grown shoots had decreased to 50%.

It has been proposed that the presence or absence of specific phenolic compounds may have a profound effect on the rate of success of adventitious root formation, by acting either as rooting promoters or as rooting inhibitors. Ortho-dihydroxyphenolics are generally regarded as being more effective than other phenolics in inhibiting the oxidation and destruction of IAA) by the auxin oxidase complex (6, 14). Chlorogenic acid, a common plant o-dihydroxyphenolic, was present early in the growth of both light-grown and etiolated *C. betulus* shoots, and decreased in light-grown shoots at a time when rooting success was also decreasing (week 6, 50% rooting). Several of the compounds identified as possibly being flavonols or hydroxycinnamic acids (which may also be o-dihydroxyphenolics) followed similar patterns. Ellagic acid derivatives may be rooting inhibitors of cuttings from light-grown chestnut (*Castanea*) stockplants (13). In this study ellagic acid (compound #1) was present in both etiolated and light-grown shoots, and did not correlate well with rooting success.

The most apparent effect of light during new shoot growth was the lag in phenolic compound production in previously etiolated shoots relative to light-grown shoots sampled at a similar time period. The changes in phenolic compounds in both greening etiolated and light-grown tissues paralleled the anatomical changes noted by Maynard (8). The correlations between the changes in phenolic compounds observed in this study and the changes in rooting potential and stem anatomy observed by Maynard (8) suggests a need for further research to examine the activity of phenolic compounds in both etiolated and light-grown plant material.

LITERATURE CITED

1. Bate-Smith, E.C. 1962. The phenolic constituents of plants and their taxonomic significance. J. Linn. Soc. (Bot.) 58:95-174.
2. Challice, J.S. and A.H. Williams. 1966. Paper chromatographic separation and behaviour of the cis- and trans-isomers of cinnamic acid derivatives. J. Chromatog. 21:357-362.
3. Dirr, M.A. and C.W.Heuser, Jr. 1987. The reference manual of woody plant propagation: From seed to tissue culture. Varsity, Athens, Ga., p 100.
4. Doud, S.L. and R.F. Carlson. 1977. Effects of etiolation, stem anatomy, and starch reserves of root formation of layered *Malus* clones. J. Am. Soc. Hort. Sci. 102:487-491.
5. Harborne, J.B. 1984. Phytochemical Methods. Chapman and Hall, New York, 288 p.
6. Haissig, B.E. 1986. Metabolic processes in adventitious rooting of cuttings. *In* New Root Formation of Plants. Ed. M.B. Jackson. Martinus Nijhoff Pub., Boston, pp 141-190.
7. Herman, D.E. and C.E. Hess. 1963. The effect of etiolation upon the rooting of cuttings. Proc. Int. Plant Prop. Soc. 13:42-62.
8. Maynard, B.K. 1990. Physiological and anatomical factors associated with the etiolation response in cutting propagation. Ph.D. thesis, Cornell University, Ithaca, NY.
9. Maynard, B.K. and N.L. Bassuk. 1987. Stockplant etiolation and blanching of woody plants prior to cutting propagation. J. Am. Soc. Hort. Sci. 112(2):273-276.
10. Ribereau-Gayon, P. 1972. Plant Phenolics. Oliver and Boyd, Edinburgh, 254 p.
11. Schmidt, G. 1986. Effect of etiolation on the histological structure of the stem of *Tilia tomentosa* Moench. Folia Dendro. 13:217-262.
12. Steck, W. 1967. On the identification of some naturally occurring hydroxycinnamic acid derivatives. Anal. Biochem. 20(3):553-556.
13. Vieitez, F.J. and A. Ballester. 1988. Effect of etiolation and shading on the formation of rooting inhibitors in chestnut trees. ØYTON 48 (1/2):13-19.
14. Wilson, P.J. and J. van Staden. 1990. Rhizocaline, rooting co-factors, and the concept of promoters and inhibitors of adventitious rooting - a review. Annals of Botany 66:479-490.

Figure 1: Relative intensities of the compounds separated by two-dimensional chromatography of methanol extracts of *C. betulus* stem segments.

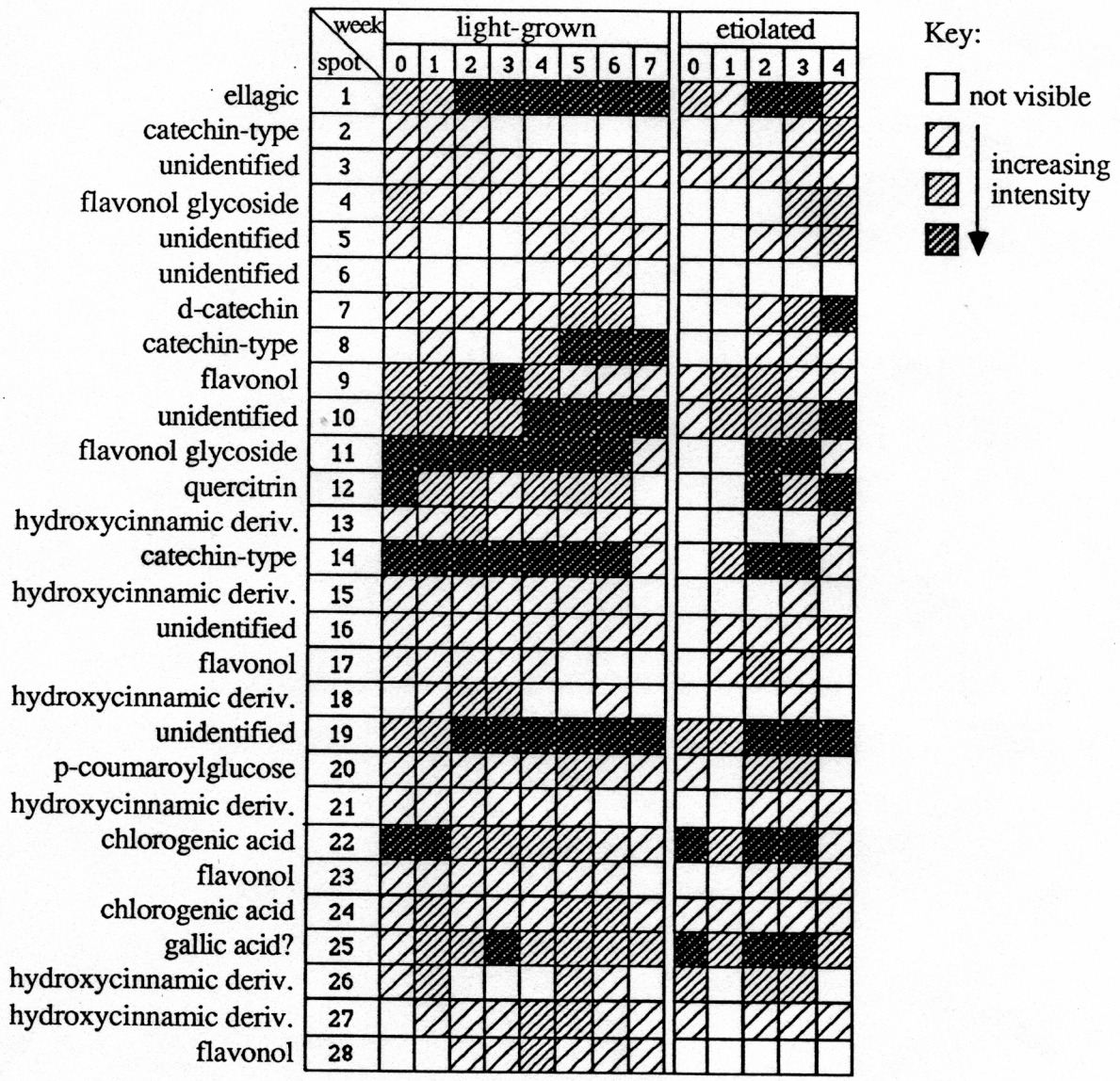

Table 1: Tentative identification and characteristics of compounds from separated crude extracts of *C. betulus* shoots.

TLC spot #	Tentative identification	R_f BAW	R_f 15% AA	Source/procedure for Identification
1	ellagic acid	.30	.02	B
2	catechin type	.50	.05	A
3	unidentified	.47	.16	-
4	flavonol glycoside	.54	.22	5, A, C
5	unidentified	.80	.12	-
6	unidentified	.73	.21	-
7	d-catechin	.64	.34	B
8	catechin type	.37	.32	A
9	flavonol type	.37	.39	5, A, C
10	unidentified	.62	.40	-
11	flavonol glycoside	.71	.41	5, A, C
12	quercitrin	.81	.44	5, B, C
13	hydroxycinnamic derivative	.78	.47	5, 10, A, C
14	catechol	.89	.47	B
15	hydroxycinnamic derivative	.87	.51	5, 10, A, C
16	unidentified	.58	.51	-
17	flavonol type	.67	.54	5, A, C
18	hydroxycinnamic derivative	.45	.62	5, 10, A, C
19	unidentified	.63	.65	-
20	p-coumaroylglucose	.75	.64	2, 12, B
21	hydroxycinnamic derivative	.81	.69	5, 10, A, C
22	trans-chlorogenic acid	.69	.70	2, 12, B
23	flavonol type	.81	.78	5, A, C
24	cis-chlorogenic acid	.68	.80	2, 12, B
25	gallic acid conjugate	.37	.80	A, C
26	hydroxycinnamic derivative	.83	.86	5, 10, A, C
27	hydroxycinnamic derivative	.62	.87	5, 10, A, C
28	flavonol type	.27	.80	5, A, C

Key to procedures:

A - color characteristics under UV and after fuming with NH_3

B - comparisons with standards (R_f and color changes)

C - comparison to identified products of acid hydrolysis