HRI's Mission:

To direct, fund, promote and communicate horticultural research, which increases the quality and value of ornamental plants, improves the productivity and profitability of the nursery and landscape industry, and protects and enhances the environment.

Lignification Associated with Decreased Adventitious Rooting Competence of English Ivy Petioles[1]

Richard A. Reineke[2], Wesley P. Hackett[3], and Alan G. Smith[4]
Department of Horticultural Science, University of Minnesota
356 Alderman Hall, St. Paul, MN 55108

Abstract

English ivy (*Hedera helix* L.) was used to study the relationship between lignin accumulation and adventitious rooting. Juvenile-phase and three chronological ages of mature-phase petioles were collected from clonal stock plants, analyzed for quantity of vascular lignin, and placed in an *in vitro* rooting assay. Rooting competence was determined by observing the number of roots formed per petiole. The number of roots per petiole differed significantly between juvenile-phase and mature-phase petioles. Lignin accumulation increased during petiole growth and development with chronologically older mature-phase petioles having the greatest amounts of lignin. There was a negative correlation between lignin accumulation and adventitious rooting competence.

Index words: *Hedera helix*, roots, ontogenetic phase, chronological age.

Significance to the Nursery Industry

Many horticultural crop plants possess characters that are only expressed in the mature phase of growth, which necessitates the selection of these characters in the mature phase. For example, the selection of ornamental attributes, growth characteristics, or resistance to biotic or abiotic stresses for many woody perennial crops must be done with plants in the mature-phase. Since many cultivars are genetically heterogeneous, asexual reproduction via rooted stem cuttings is essential to efficient propagation. Diminished adventitious rooting competence of the mature-phase of many horticultural crops limits the potential propagation and production of cultivars with desirable characteristics. Therefore, it is of economic interest to understand phase-related factors, especially those that influence adventitious rooting competence in order to provide improved methods for propagating mature-phase plants and to identify markers for rooting competence.

Introduction

Phase change in woody plants is a gradual progression from juvenile to mature phenotype, wherein competence for flowering is attained and competence for adventitious root formation may be greatly diminished. The process of maturation may occur over a period of decades in some species (8). The lack of adventitious rooting competence in the mature-phase of many species limits the propagation of desirable cultivars.

English ivy (*Hedera helix* L.) is a classic model for studying phase-related characters, as it exhibits distinct phenotypic differences in growth habit, phyllotaxy, leaf shape, flowering competence, and adventitious rooting competence between mature and juvenile plants. Shoots of juvenile-phase English ivy readily form roots, while mature-phase shoots form roots less frequently (6). However, juvenile shoots have preformed root meristems at the nodes, making experimental comparisons between mature and juvenile shoots less useful. Debladed ivy petioles, which lack preformed root meristems, have anatomy and rooting characteristics similar to those of mature and juvenile shoots from which they were obtained. Therefore, they provide an excellent system for studying phase-related differences in adventitious rooting (5).

Auxin and sucrose are necessary for the induction of cell divisions and the growth of root primordia, respectively (5). Root initiation is the morphogenic process in which competent cells respond to an auxin stimulus and undergo organized divisions to form meristems and primordia. For English ivy petioles, the three stages of root initiation are: auxin induction, days one through six; meristem organization, days six through nine; and root elongation, days nine through eighteen (5). Auxin-stimulated cell divisions are localized to phloem parenchyma and inner cortical parenchyma cells in juvenile petioles, while mature-petiole cell divisions occur in analogous cell types and throughout the cortex. Juvenile root primordia form from phloem and cortical parenchyma cells adjacent to vascular bundles. In contrast, the rare root primordia in mature petioles form from cells of new callus without vascular connection.

Woo et al. (14) showed that rooting competence of mature-phase ivy is influenced by chronological age, thus enabling comparative research within the mature-phase, as well as between the phases. Lignin is differentially distributed in the vascular tissue of stems, with greater amounts in mature-phase relative to juvenile-phase English ivy (6). Lignin is deposited as a secondary cell wall component in some differentiated plant cells (10, 13). It has been hypothesized that bands of lignified cells are a physical obstruction to root elongation and emergence, however there is little supportive evidence (1, 3, 6, 11). It is also possible that the presence of lignin has only an indirect association with rooting competence. Biochemical or physiological factors related to the accumulation of vascular lignin may be responsible for the phase-differentiated competence (9). The hypothesis that a negative correlation exists between adventitious rooting competence and the quantity of lignification of *in vitro*-cultured petioles of English ivy was tested. Results establish a correlation between lignin quantity and rooting competence. These data may be used as a practical marker for assessing the root-

[1]Received for publication June 9, 2002; in revised form September 20, 2002. This research was support in part by the Minnesota Agricultural Experiment Station. Paper No. 981210031. Also funded by the Binational Agricultural Research and Development Fund. BARD, P.O. Box 6, Bet Dagan 50250, Israel. We wish to thank Ms. Faye Propsom for help in statistical analysis.

[2]Graduate Student Assistant.

[3]Professor Emeritus.

[4]Associate Professor. To whom reprint requests should be addressed.

ing competence of cuttings and provide insight into the factors controlling development of adventitious roots.

Materials and Methods

Genetically identical clones of mature and juvenile-phase stock plants of a single English ivy clone were maintained in a greenhouse with sodium metal halide lamps, 14-hour days, and 400 µmol/m²/s. Day/night air temperature setpoints were 21C (70F) for heating and 26C (79F) for venting. Plants were irrigated with a 0.02 g/liter (0.0007 oz/1.1 qt) Peters Calmag 15N–5P–15K fertilizer solution (Scotts-Sierra, Marysville, OH). Mature plants were grown three to a pot in 19-liter (5 g, #5) plastic nursery containers, and juveniles were grown doubly in 15.2-cm (6 in) clay pots. Stock plants were arranged randomly in the greenhouse.

Three different chronological ages of mature petioles were collected from nodal positions one (Mat1), four (Mat4), and seven (Mat7) of mature shoots with 10 to 15 total nodes. Mat1 was the most basal and chronologically oldest. From juvenile shoots, petioles were collected from the third or fourth nodal positions. All four petiole types were excised from the lamina and held in deionized water for 15 min. The petioles were surface sterilized in a solution of 0.5% sodium hypochlorite and two drops of Tween 80 for 15 min, after which they were triple rinsed in sterile deionized water.

The basal 1.0 cm (0.4 in) of each petiole base was excised and used for lignin quantification. Hand sections about 0.1 mm (0.004 in) thick were made from the distal end of the petiole base and placed on a glass slide. Three drops of a phloroglucinol solution 0.2 g (0.0007 oz) phloroglucinol/0.05 liter (0.053 oz) 70% ethanol) were applied to the sections for 5 min, after which three drops of concentrated hydrochloric acid were applied (4). Lignified areas of the sections stained red after 2 to 3 min. Excess liquid was blotted, three drops of glycerin were applied, and a cover slip was mounted.

Preparations were viewed in brightfield using a Nikon Eclipse E800 photomicroscope. Digital images were collected with a CoolCam liquid-cooled, three chip color CCD camera (Cool Camera Company, Decatur, GA) and captured to a 486DX2 personal computer using Image Pro Plus version 1.3 software (Media Cybernetics, Silver Spring, MD).

Digital image analysis was performed using the NIH Image program (U.S. National Institutes of Health, http://rsb.info.nih.gov/nih-image/). The program was used to quantify the areas of total vascular lignin, phloem lignin, and the total section area from each digitally stored section image. Total vascular lignin includes xylem lignin and phloem lignin.

In order to culture petioles in a vertical orientation, petiole holders were constructed by making 12 uniformly-spaced 3-mm holes in Magenta B-caps (Magenta Corporation, Chicago, IL). The lids were placed in 100 mm (4 in) × 25 mm (1 in) sterile petri plates that contained two Whatman #1 filter papers. 3.8 ml (0.12 oz) of modified Romberger medium with 1.0-mM naphthalene acetic acid per petri plate was added (7). A single explant per petiole (consisting of the basal 2.0 cm (0.8 in), after removal of the 1.0 cm (2.5 cm) explant for lignin quantification) was randomly placed in the petiole holder. Each experiment consisted of six petri plates with three replicates of each petiole type per plate, in a completely randomized design. A total of 18 petiole explants of each type was analyzed in each experiment. The experiment was replicated twice over time. After 24 hr the petioles were transferred to modified Romberger medium without auxin for the duration of the rooting assay. To prevent drying each petri plate was sealed with a single wrap of Parafilm. All petri plates were placed randomly in a single growth chamber at constant 21C (70F), 16 h photoperiod, and irradiance of 200 µmol/m²/s from fluorescent lamps.

After 28 days the petioles were examined and the number of roots per petiole recorded. These data were compared to lignin area measurements from stained sections of the corresponding petiole base to test for a correlation between roots per petiole and the quantity and anatomical location of lignin. Analyses of variance and Pearson correlations were performed using SPSS 8.0 (SPSS, Inc., Chicago, IL).

Results and Discussion

English ivy provided an excellent model system to investigate the relationship between the ability to develop adventitious roots and the accumulation of lignin. Petioles from juvenile-phase and each chronological age of mature-phase English ivy had analogous anatomy, but differed in the relative amounts of lignification (Fig. 1). The four petiole types showed obvious differences in the levels of lignification, which was visualized, by the dark staining regions, after phloroglucinol staining.

Juvenile-phase petioles had greater competence to form adventitious roots relative to all ages of mature-phase petioles when competence was measured as roots per petiole (Fig. 2A). Juvenile-phase petioles also differed significantly in total lignin and phloem lignin levels from most chronological ages of mature-phase petioles (Fig. 2B and 2C). The only exception was that juvenile-phase and Mat7 had statistically equivalent levels of phloem lignin. These data indicate a close association between phase, rooting competence, and lignification. Juvenile-phase petioles had no phloem lignin, and more roots per petiole and significantly lower levels of total lignin than mature-phase petioles. Mature-phase petioles as a group had fewer roots per petiole and significantly higher levels of total lignin and phloem lignin (Fig. 2A, 2B, and 2C). These data indicate a phase-dependent lignification that is correlated with a decrease in adventitious rooting.

The phase-dependent difference in adventitious rooting is consistent with previous reports (5, 14). Girouard (6) reported similar data with regard to rooting competence and lignin quantity between stems of mature-phase and juvenile-phase English ivy. However, these experiments are complicated by the presence of preformed root meristems in many juvenile-phase stems. The accumulation of greater quantities of lignin in mature-phase English ivy may be a response to increased stress due to its orthotropic growth habit, requiring greater quantities of lignin for support. In comparison, juvenile-phase plants have a plagiotropic habit, requiring little support, so they may require less lignin. Greater rooting competence of juvenile-phase plants may help to establish new juvenile-phase plants.

Comparisons of lignin quantity and rooting competence in mature-phase petioles of different chronological ages permitted observations without the influence of phase. The number of roots per petiole did not differ statistically among the three chronological ages of mature-phase petioles (Fig. 2A). However, there was trend toward fewer roots per petiole as chronological age increased. The level of total lignin and phloem lignin per petiole decreased with decreasing chronological age among mature-phase petioles (Fig. 2B and 2C). Although the level of total lignin was similar for Mat1 and

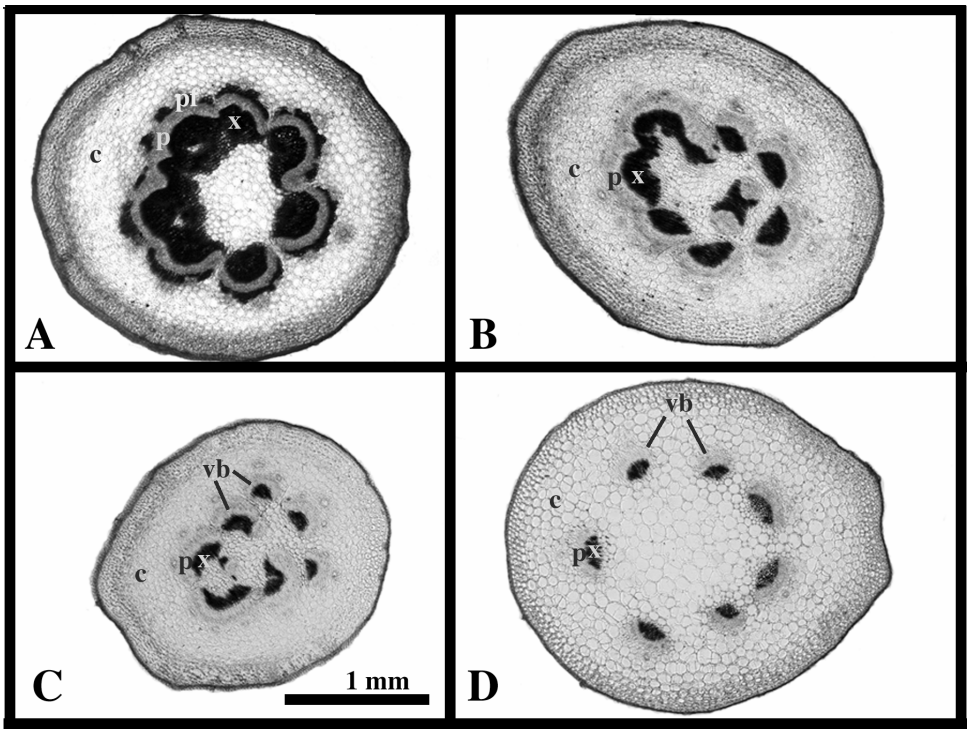

Fig. 1. Representative photomicrographs of cross sections of leaf petioles of English Ivy Mat1 (A), Mat4 (B), Mat7 (C), and juvenile (D), stained for lignin with phloroglucinol. Cortex (c), phloem (p), phloem fiber (pf), vascular bundle (vb), and xylem (x). The bar in panel C represents one mm for each of the micrographs.

Mat4 and the level of phloem lignin was similar for Mat4 and Mat7, the total and phloem lignin accumulation was correlated to the chronological age of the petiole, older petioles generally having more area of lignified cells. There was a significant negative correlation between total and phloem lignin accumulation and the number of roots formed per petiole (Table 1).

The lack of differences in numbers of roots per petiole among the three chronological ages of mature-phase petioles was unexpected, because Woo et al. (14) and Sanchez et al. (12) demonstrated that chronologically younger, mature-phase petioles had greater number of roots per explant and a higher rooting percentage than older, mature-phase petioles. The nodal difference between the two chronological ages in the Woo et al. (14) and Sanchez et al. (12) was no more than six nodes, coming from two to three leaves above and below the youngest fully expanded laminae. The present study also had a maximum nodal difference of six nodes, from Mat1 to Mat7. However, the shoots from which the petioles were collected were different. Woo et al. (14) and Sanchez et al. (12) collected petioles from actively growing shoots, where it was possible to get petioles from two to three nodes above the youngest fully expanded laminae. In the present study, the shoot meristems had ceased activity with ten to fifteen nodes, and petioles near the youngest fully expanded lamina would be near node ten and too short for the *in vitro* rooting assay. Only Mat7 was long enough, and its laminae were nearly always fully expanded. Another difference was that Woo et al. (14) counted root primordia in stained hand sections at day 16 under a microscope, rather than macroscopically visible roots at day 28.

The increase in lignification during chronological aging of mature-phase petioles was largely due to the accumulation of lignin in the phloem (Fig. 2D). The ratio of phloem lignin to total lignin increased from Mat7 to Mat1. The increased lignification of the phloem is significant because phloem cells respond to auxin via cell divisions. Some of these divisions result in organized root meristems, which are a first step in adventitious root development (12). The accumulation of lignin in cells that participate in adventitious root formation, and the associated reduction in root formation, support the hypothesis that lignification is negatively correlated with rooting competence.

Analysis of these data using Pearson correlations clearly demonstrates a negative correlation between total and phloem lignin per petiole and the number of roots per petiole (Table 1). The correlation is significant at the 0.01 level of significance. Therefore, for English ivy, the accumulation of lignin is a good marker for a petiole's ability to form adventitious roots.

The accumulation of lignin may prevent cells from dividing and forming organized root meristems. It is also possible that phloem lignin has an indirect effect on the development of root meristems, influencing the competence of cells biochemically rather than physically. High endogenous levels of phenolic compounds, especially cinnamic acid, a precursor in lignin synthesis, were found to be negatively correlated with rooting competence in hardwood cuttings of wax flower, *Chamaelaucium uncinatum* Schauer (2). These phenolic compounds are substrates for lignin production and would be present in areas where lignin is accumulated. Although phloem lignin is negatively associated with rooting competence in mature-phase petioles, it is likely that other factors are involved in determining rooting competence.

This research has established a negative correlation between the accumulation of lignin and adventitious rooting

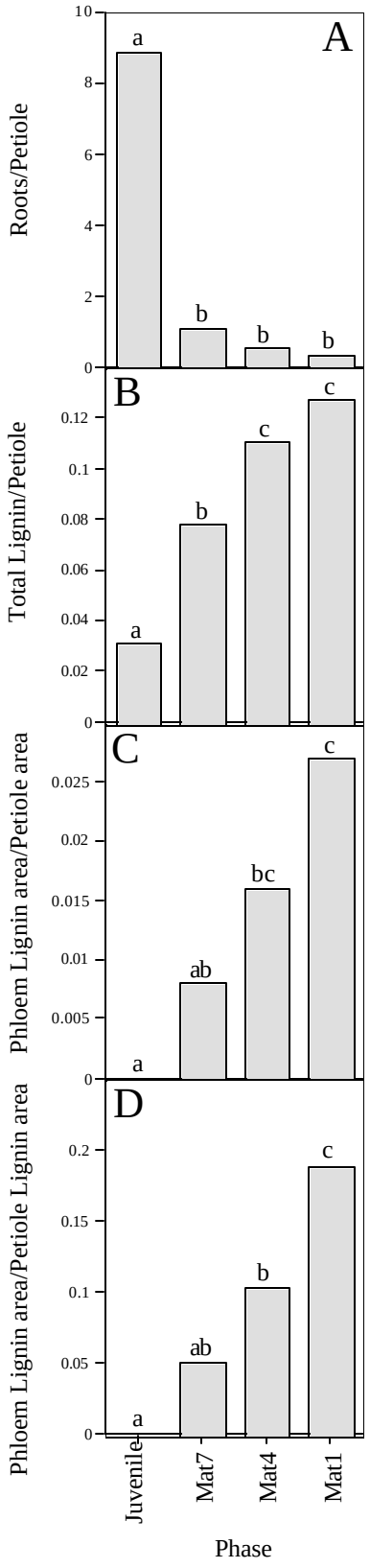

Figure 2. (A) Number of roots per petiole; (B) Total lignin per petiole (area of total lignin divided by the area of the petiole); (C) Phloem lignin per petiole (area of phloem lignin divided by the area of the petiole); (D) Ratio of phloem lignin to total lignin in juvenile-phase and three chronological ages of mature-phase petioles. Significant differences determined at the p = 0.05 using Tukey's HSD, indicated by different letters above the bars.

Table 1. Pearson correlations among number of roots per petiole (Rt/Pt); total lignin per petiole area (Lig/Pt); phloem lignin per petiole area (Plig/Pt).

Variable	Rt/Pt	Lig/Pt	Plig/Pt
Rt/Pt	1.00	–0.623**[z]	–0.427**
Lig/Pt	1.00	0.860**	
Plig/Pt	1.00		

[z]**Indicates correlation is significant at the 0.01 level (2-tailed).

competence in petioles of English ivy. Whether the presence of phloem lignin has a direct physiological effect or is an indirect marker is not known. However, the determination of lignification level may be a useful tool to predict a cutting's ability to form adventitious roots. Future work will investigate the utility of this marker for other vegetatively propagated crops and how manipulation of lignin accumulation affects rooting competence.

Literature Cited

1. Beakbane, A.B. 1969. Relationships between structure and adventitious rooting. Proc. Inter. Plant Prop. Soc. 19:192–201.

2. Curir, P., S. Sulis, F. Mariani, C.F. Van Sumere, A. Marchesini, and M. Dolci. 1993. Influence of endogenous phenols on rootability of *Chamaelaucium uncinatum* Schauer stem cuttings. Scientia Hort. 55:303–314.

3. Davies, F.T., J.E. Lazarte, and J.N. Joiner. 1982. Initiation and development of roots in juvenile and mature leaf bud cuttings of *Ficus pumila* L. Amer. J. Bot. 69:804–811.

4. Faulkner, G. and W.C. Kimmins. 1975. Staining reactions of the tissue bordering lesions induced by wounding, tobacco mosaic virus, and tobacco necrosis virus in bean. Phytopathology 65:1396–1400.

5. Geneve, R.L., W.P. Hackett, and B. Swanson. 1988. Adventitious root initiation in debladed petioles from the juvenile and mature-phases of English ivy. J. Amer. Soc. Hort. Sci. 113:630–635.

6. Girouard, R.M. 1967. Initiation and development of adventitious roots in stem cuttings of *Hedera helix*. Can. J. Bot. 45:1877–1886.

7. Hackett, W.P. 1970. The influence of auxin, catechol, and methanolic tissue extracts on root initiation in aseptically cultured shoot apices of the juvenile and adult forms of *Hedera helix* . J. Amer. Soc. Hort. Sci. 95:398–402.

8. Hackett, W.P. 1985. Juvenility, maturation, and rejuvenation in woody plants. HortRev. 7:109–155.

9. Hackett, W.P., J.R. Murray, H-H. Woo, R.E. Stapfer, and R. Geneve. 1990. Cellular, biochemical, and molecular characteristics related to maturation and rejuvenation in woody species. p. 147–152 *In*: R. Rodriguez, R.S. Tames, and D.J. Durzan (Editors). Plant Aging: Basic and Applied Approaches. Plenum Press, New York.

10. Northcote, D.H. 1989. Control of plant cell wall biogenesis. p. 1–15 *In*: N.G. Lewis and M.G. Paice (Editors). Plant Cell Wall Polymers: Biogenesis and Biodegradation. Toronto, Ontario, Canada.

11. Sachs, R.M., F. Loreti, and J. De Bie. 1964. Plant rooting studies indicate sclerenchyma tissue is not a restricting factor. Calif. Ag. 18:4–5.

12. Sanchez, M.C., A.G. Smith, and W.P. Hackett. 1995. Localized expression of a proline-rich protein gene in juvenile and mature ivy petioles in relation to rooting potential. Physiol. Plant. 93:207–216.

13. Sederoff, R., and H-M. Chang. 1991. Lignin Biosynthesis. p. 263–285 *In*: M. Lewin and I.S. Goldstein (Editors). Wood Structure and Composition. Marcel Dekker, Inc., New York.

14. Woo, H-H., W.P. Hackett, and A. Das. 1994. Differential expression of a chlorophyll a/b binding protein gene and a proline rich protein gene in juvenile and mature-phase English ivy (*Hedera helix*). Physiol. Plant. 92:69–78.