Appendix 1

Lignin Formation and the Effects of Gravity:

A New Approach

NAG 100164

Progress Report: June 1995 - March 1997

by

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Summary

During this grant funding period, two aspects of considerable importance in the enigmatic processes associated with lignification have made excellent progress. The first is that, even in a microgravity environment, compression wood formation, and hence altered lignin deposition, can be induced upon mechanically bending the stems of woody gymnosperms. It now needs to be established if an organism reorientating its woody stem tissue will generate this tissue in microgravity, in the absence of externally applied pressure. If it does not, then gravity has no effect on its formation, and instead it results from alterations in the stress gradient experienced by the organism impacted.

The second area of progress involves establishing how the biochemical pathway to lignin is regulated, particularly with respect to selective monolignol biosynthesis. This is an important question since individual monomer deposition occurs in a temporally and spatially specific manner. In this regard, the elusive metabolic switch between *E*-*p*-coumaryl alcohol and *E*-coniferyl alcohol synthesis has been detected, the significance of which now needs to be defined at the enzyme and gene level. Switching between monolignol synthesis is important, since it is viewed to be a consequence of different perceptions by plants in the gravitational load experienced, and thus in the control of the type of lignification response. Additional experiments also revealed the rate-limiting processes involved in monolignol synthesis, and suggest that a biological system (involving metabolite concentrations, as well as enzymatic and gene (in)activation processes) is involved, rather than a single rate-limiting step.

The Progress Report for the currently funded NASA project covers the time-frame from June 1995 to March 1997 (i.e., over a twenty month duration). The two main objectives include (i) to determine whether compression wood formation occurs in microgravity, and to establish the time-course of its [potential] induction at both 1*g* and in microgravity, respectively, and (ii) to develop methodology to determine the rate-limiting step(s) involved in formation of the monomeric lignin precursors from phenylalanine, including how the corresponding monolignols are transported to the plasma membrane and how the various metabolites and enzymes are organized at the subcellular level.

Progress to both goals, in this fundamentally important area of gravitational plant biology, are well in hand and proceeding smoothly. Individual progress to each objective is described below.

1. Whether Compression Wood Formation Occurs in Microgravity or not.

The woody gymnosperms, loblolly pine (*Pinus taeda*) and Douglas fir (*Pseudotsuga menziesii*) represent two very important commercial plant species used today. Understanding how to optimize both the quality and the texture of their woods (secondary xylem) is an important biotechnological goal, particularly with the trends towards moving to 'fast-growing' plantations as sources of fiber and wood. A very deleterious feature in wood development, however, occurs when their stems are displaced from a vertical alignment. This results in such plants realigning their growth processes, via some presumed gravitactic response, in order to restore vertical alignment to the photosynthetic canopy. This is attained by specific cells in the stem, which were originally programmed to form 'normal' xylem, being induced to undergo formation of a specialized reinforcement tissue trivially known as reaction wood (compression wood in gymnosperms). Formed only in woody plants, it buttresses the stem which results in a concomitant, yet slow, vertical realignment of the photosynthetic canopy as growth continues. Gymnosperm reaction wood consists of two distinct regions, namely compression wood formed at the underside of leaning stems and branches, and opposite wood formed at the upperside, respectively. In compression wood, such as in loblolly pine (*Pinus taeda*), tracheids are shorter than normal (10-40% less), and have rounded outlines with frequently distorted tips and thicker cell walls, which results in a specific gravity approximately twice that of normal wood. From an anatomical viewpoint, these differ from normal tracheids as follows: The S_1 layer of compression wood is thicker than 'normal' wood, the S_2 layer is deeply fissured, and there is no S_3 layer (Figure 1). Further, compression wood has a cellulose with a lower degree of polymerization and which is less crystalline, whereas its lignin is both significantly higher in amount and with a large increase in the *p*-coumaryl alcohol content (discussed below). Significant differences in cellulose microfibril angles are also observed, strongly indicating that the microtubule assembly/orientation is also affected, i.e., from 35.1° to 22.8° depending upon whether the tissue is 'normal' or 'compression' wood. Opposite wood, on the other hand, has longer tracheids of a squared or rectangular shape, with the S_2 layers being unusually thick with transverse helical patterns. In opposite wood, the relative proportions of the plant cell wall polymers (lignin, cellulose and hemicelluloses) appear to be unchanged with respect to 'normal' wood.

In the first objective of the study, we wished to establish whether compression wood formation, which is believed to result as a consequence of alterations in the perceived gravitational vector acting on the entire plant, is formed in microgravity.

With the proposed STS-78 mission (June-July 1996) planned for 17 days, several ground-based base-line experiments were first carried out prior to embarking upon the space-flight experiment.* These included: (i) establishing that compression wood formed within the time-frame of the proposed flight duration and (ii) definition of experimental protocols for initiating experiments, when the Shuttle was in orbit, that would normally result in compression wood formation at 1 *g*.

As described below, this involved a very comprehensive series of experiments (with corresponding controls) at the preflight stage.

In the context of experimental design to test whether compression wood formation occurs in microgravity, however, the best approach would be to grow woody plants horizontally, relative to the light source, in microgravity. Then, some months later, when reorientation of the stem and photosynthetic canopy would have occurred, to examine the resulting xylem cells for compression wood formation. In such an experiment, there would be no imposed external stress on the plants (e.g., by mechanical means due to bending), and hence would be the ideal experiment (Figure 2).

But such an experiment is not possible until the onset of Space Station Alpha, since the time-frame required (number of months) for such reorientations to occur is too short for the Shuttle Flight experiments. Consequently, we developed an alternate system for examining whether compression wood formation could be engendered in microgravity over the STS-78 flight duration (17 days).

As shown in Figure 3, this involved bending (at both 45° and 90°) loblolly pine and Douglas fir plants at 1 *g* for different time intervals (1, 3, 5, 7 and 14 days). After these different time periods, the plants were restored to a vertical alignment, and after a total of 14 days were analyzed for the onset and development of compression wood formation. Under these conditions, it was established that for both loblolly pine and Douglas fir, compression wood formation could readily be detected after 3 days of initial bending with an additional 4 days of further growth when restored to an upright position (Figure 4).

The next technical development required was to be able to grow the plants in the NASA supplied plant growth chambers (PGC's), whose light, temperature, and nutrient provision abilities are quite limited. For example, temperatures can only be *raised* if they fall below the ambient temperature of the Space Shuttle cabin, i.e., they lack cooling capability. Moreover, nutrients and water cannot be supplied during flight, a technical difficulty that NASA is still trying to overcome. Nevertheless, we devised conditions for the satisfactory growth of both loblolly pine and Douglas fir plants in the PGC, using Nutrient Packs (agar gel containing requisite water and nutrients) developed previously in my laboratory for space-flight studies. As shown in Figure 5, this enabled the facile growth of the plants under the light, temperature and humidity levels typical of Shuttle flights.

Following 10 months of sustained effort, the basic experimental approach had, therefore, been devised to explore whether compression wood formation could be formed in microgravity.

With progress to this point in hand, two major technical hurdles needed still to be resolved. The first included conducting a Payload Verification Test (PVT) by transporting the plants to

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* As an aside, our experiment received very significant coverage in both local and international newspapers, as well as being reported on CNN and CBC (Canadian Broadcasting Corporation) television channels. Indeed, school students such as eleven year-old Erin Riley (Florida), repeated the ground-based components of the study and described their results at different Science Fairs. Ms Riley, for example, won first place awards/prizes in school, county and state Science Fairs. A significant part of this national and international interest arose from the economic importance of establishing how compression wood formation occurs, since it is deleterious in terms of lumber quality/pulp and paper production.

Kennedy Space Center, and with the able assistance of Deborah Vordermark and Rena Thompson, then growing the plants under conditions expected for the Shuttle flight. These experiments were conducted over 18 days with plants orientated at 45° after 2 days, then harvested, sectioned and chemically fixed (2.5% glutaraldehyde, 2% paraformaldehyde) on days 11 and 17. Each sectioned tissue was then subjected to subsequent light microscopy examination. The results obtained are illustrated in Figure 6. As can be seen, the characteristic reaction wood is clearly visible.

Next, the astronauts, Jean Jacques Favier and Susan Helms were trained by us at KSC and Johnson Space Center. They were shown how to put specific loblolly pine and Douglas fir plants into the correct (45°) orientation, this being ultimately planned to occur following 2 days of growth in microgravity. Selected plants were harvested and sectioned (both upright controls and oriented specimens, at days 10 and 13, respectively) under shuttle flight laboratory conditions, in preparation for light microscope analyses.

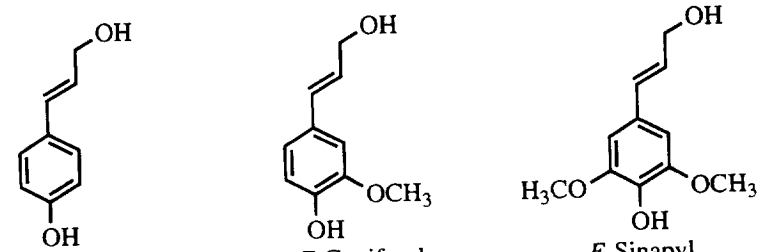
With these training sessions and preparations completed, Shuttle Columbia (STS 78) was launched on June 22, 1996, from Kennedy Space Center, with the N.G. Lewis team being present at both KSC and Dryden (in case of a California landing and recovery). The space flight experiment, while answering the question of whether compression wood formation occurred under such conditions, was not without difficulty. Although the bending experiments were initiated in space, the Shuttle cabin temperatures were higher than any recorded previously (> 29°C),which in average had a deleterious effect on the growth of several specimens. Nevertheless, those still in obviously good conditions (i.e., containing new growth) were harvested, sectioned and fixed both in space (at days 10 and 13), with the remainder harvested, sectioned and fixed upon recovery after the 17 day flight. The results obtained are shown in Figure 7. Under the conditions employed, both sets of plants (i.e., microgravity and 1 *g* grown) were essentially identical, all forming compression wood when orientated at 45°. On the other hand, compression wood formation did not occur when either plants were placed in a vertical configuration (Figure 8). Note also that the experiments had to be repeated several times post-flight (under flight conditions) to verify that the difficulties experienced were, in fact, due to high temperature. This was established to be the case (data not shown).

Most authorities might have expected compression wood not to be formed if the gravitational vector was removed. Thus, to account for its formation in space, either the microgravity influence is still large enough to ensure that the organisms can still respond to it, or more likely, the effect of mechanical loading (by harnessing as shown in Figures 9 and 10) overrides the gravitactic responses (i.e., due to overlapping signal transduction, perception and response mechanisms). Put in another way, even in microgravity, the plants can make appropriate corrections to alleviate the stress gradient introduced by bending, thereby forming compression wood. Indeed, this is why the next experiment on Space Station Alpha now needs to be conducted, i.e., where the plants can reorientate over longer periods (Figure 2), but without introduced mechanical stresses such as by bending. In this way, we will absolutely establish if compression wood can form, or not, in microgravity. This initial experiment is, however, important since it demonstrates that under the correct conditions, it can be formed in microgravity.

Work is also currently in progress, using a freeze-fracture approach, to examine the cellulose microfibril orientation of the space flight plant tissues, in order to determine if the effects on cell wall organization were altered in microgravity in either the newly formed compression wood or normal xylem cells.

2. To develop methodology to determine the basic processes involved in lignification: Definition of rate-limiting step(s) involved in formation of the monomeric lignin precursors from phenylalanine; how the corresponding monolignols are transported to the plasma membrane and how the various metabolites and corresponding enzymes are organized at the subcellular level

Lignin biosynthesis is very poorly understood in terms of the following: how its induction and regulation is achieved; how selective monomer deposition (heterogeneity) occurs in the cell wall, and in specific tissues, such as leading to compression wood, and how the polymer assembly (phenolic coupling and polymerization) is orchestrated *in vivo* (see proposal). The lignins are primarily formed from the monolignols, *E*-*p*-coumaryl alcohol, *E*-coniferyl and *E*-sinapyl alcohols. Gymnosperm 'normal' woody secondary xylem has lignin which is primarily *E*-coniferyl alcohol derived, and to a lesser extent, *E*-*p*-coumaryl alcohol, with ratios of each typically approximating 8:1, respectively. However, the deposition of these monomers occurs in a selective temporal and spatial manner, where *E*-*p*-coumaryl alcohol is involved in the early stages of lignin formation, primarily being deposited into the middle lamella region. *E*-Coniferyl alcohol, on the other hand, is mainly deposited into the secondary wall. The lignins are also biosynthesized, following deposition of the cell wall constituents, cellulose and hemicelluloses, i.e., when the entire architecture of the wall has already been laid out. Importantly, however, the actual process of lignification occurs within the cell walls at precise (or specific) sites in the middle lamellae and the primary wall, i.e., at points far removed from the plasma membrane. On the other hand, during compression wood formation (as discussed above), the entire process of cell wall assembly is massively altered, with its lignin now being derived primarily from *E*-*p*-coumaryl alcohol.



E-*p*-Coumaryl alcohol, **1**

E-Coniferyl alcohol, **2**

E-Sinapyl alcohol, **3**

Figure 11 shows the overall biochemical pathway from phenylalanine to the monolignols. Interestingly, very little is known about the regulatory points in the pathway, and, curiously, essentially every step has been described as key. Fortunately, this question can now truly be determined, since we have a *Pinus taeda* cell suspension culture system which can be induced to lignify if exposed to 8% sucrose. It has the advantage that when an H_2O_2 scavenger, KI, is added, the cells no longer lignify but yet still maintain their ability to biosynthesize the monolignols, *E*-*p*-coumaryl and *E*-coniferyl alcohols, in ratios approximating their relative amounts in 'normal xylem'. Thus, the pathway can now, for the very first time, be analyzed in its entirety.

Hence, over the last twenty months, experiments were designed to probe the question of how regulation of the biochemical pathway to lignin is achieved. This first required the following:

the synthesis of all possible intermediates in the pathway (or purchase of the few commercially available) whether in their free, CoA ester or glucoside forms. Each of the pathway metabolites were then separated by HPLC, in order that the entire biochemical pathway could be simultaneously analyzed. Additionally, LC-MS (electron impact or chemical ionization) was carried out, in order to verify the structures of each metabolite, and to establish their MS fragmentation patterns.

With this background, the following has been completed in order to understand and define the basic processes involved in lignification.

2.1. Phenylpropanoid-nitrogen recycling during lignification.

Since circa 30-40% of all plant material is of phenylpropanoid origin, which in turn is derived from phenylalanine, an efficient mechanism of 'recycling' the equimolar amounts of ammonium ion released intracellularly must be in effect. Thus with combined use of HPLC, ^{15}N NMR and GC-MS spectroscopies, the mechanism for recycling of ammonia during phenylpropanoid metabolism in lignifying *P. taeda* (loblolly pine) cell cultures, sweet potato and suberizing potato (*Solanum tuberosum*) tubers was investigated. The use of enzyme inhibitors was necessary to elucidate the mechanism, with metabolite accumulations being consistent with the known mode of action of L-AOPP, MSO, and AZA; namely, inhibition of PAL, glutamine synthetase, and glutamate synthase, respectively. It was found that the ammonium ion released was *not* made available for general amino acid/protein synthesis. Rather, it was rapidly recycled back to phenylalanine via a PAL/GS/GOGAT system, thereby providing an effective means of maintaining active phenylpropanoid metabolism with no additional nitrogen required (Figure 12). Specifically, the ammonium ion generated during active phenylpropanoid biosynthesis is first incorporated in the δ-amide of glutamine, followed in turn by the α-amino position of glutamate, which then acts as an amino donor for the aromatic amino acids, arogenate, and phenylalanine. This PAL/GS/GOGAT nitrogen cycle explains how optimal use is made of the plant's available nitrogen, so that active phenylpropanoid metabolism and lignification can continue, even at low nitrogen levels.

2.2 Metabolic flux studies and selective monolignol formation.

The question of metabolic flux was investigated in terms of post-phenylalanine metabolites into all possible derivatives, which accumulated either intra- or extra-cellularly in *P. taeda* cells and cell media. *P. taeda* cells were induced to biosynthesize the monolignols, *E*-coniferyl and *E*-*p*-coumaryl alcohol, as before, by treatment with 8% sucrose in the presence of 20 mM KI. Different levels of phenylalanine were then exogenously supplied (from 0 to 40 mM, in 5 mM increments) in order to ensure that the induced pathway was not substrate-limited.

Several critically important findings were made by both isolating and quantifying the levels of *all* metabolites from phenylalanine to the monolignols. Metabolites were measured, both intra- and extracellularly, at different time intervals over the time-frame of 0 to 48 hours. First, only trace levels of the CoA esters, the corresponding aldehydes and monolignols were noted intracellularly, with no glycosides of the monolignols being observed. On the other hand, essentially only three metabolites, *E*-cinnamic, *E*-*p*-coumaric, and *E*-caffeic acids, built up in the cells, whose amounts varied depending upon phenylalanine availability. Over a 12 hour time period, for example, the levels of *E*-cinnamic acid increased more than 10 fold [at 10 mM phenylalanine], but then dropped off to only a two-fold increase [at 40 mM phenylalanine]. *E*-*p*-coumaric acid levels behaved in a similar manner, with increases of 10 fold [at 10 mM phenylalanine] being noted at 12 h, but this decreased to about 20% if 40 mM phenylalanine was added. Levels of *E*-caffeic acid, on the other hand, increased four-fold [at 10 mM phenylalanine], but then also reduced markedly at higher phenylalanine concentrations. By contrast, no changes in the trace levels of intracellular monolignols or other metabolites were noted. Thus, these initial

metabolic flux experiments revealed unambiguously that the regulatory steps in lignin biosynthesis involve not only phenylalanine ammonium lyase, but the effects of different phenylalanine concentrations, and the subsequent metabolism of the corresponding cinnamic acids, i.e., which represent "slow" or "limiting" steps for further conversion into monolignols.

In a related study, we have systematically varied the time of subculturing, and phenylalanine, sucrose and KI concentrations. We have identified conditions which resulted in the very significant discovery of a metabolic switch, whereby *E*-*p*-coumaryl alcohol formation is switched to that of *E*-coniferyl alcohol synthesis, and vice versa. [See Figure 13.] These results now set the stage to comprehensively define, for the first time, how the pathway is truly regulated and how selective monolignol formation is controlled. As indicated above, our results reveal more complex interactions than just a single "rate-limiting step or steps." Instead, the integration of differing metabolite levels (including potential enhancers and feedback inhibitors), variable enzymes activities (induced and non-induced) and differing transcript levels are presumed to be involved. These will be defined in the coming months.

In this context, we are also establishing the subcellular organization of both metabolites and enzymes involved in the post-phenylalanine pathway. Conditions have now been defined for organelle fractionation (vacuoles, ER, Golgi, etc.) which are being assayed for specific enzyme involvement, Additionally, vesicles are being isolated in order to prove unambiguously the nature of the monomer(s) undergoing transport into the cell wall. This is necessary in order to obtain a comprehensive view of the lignification process.

2.3. Lignin and suberin forming processes. In addition to these studies, other experiments related to this investigation have been conducted with suberin forming tissue. Although its aromatic domain was claimed to be lignin, it was shown to be hydroxycinnamate derived, consisting of (*p*-coumaryl)feruloyl tyramine residues.

We have also recently placed considerable emphasis on understanding the biochemical processes involved in phenolic coupling. As discussed in the proposal, an enzyme (trivially described as a dirigent protein) has been isolated, which confers specificity to oxidase catalyzed coupling (e.g., by laccase). Various homologues have also been identified, which we propose are specifically involved in the lignin assembly mechanisms. This work was highlighted in Science recently (Appendix 2) and represents the basis for much of the attached proposal. The first of these dirigent protein has been isolated, its gene cloned and the corresponding recombinant protein obtained using a *Spodoptera*/baculovirus system.

Future work.

The remaining phase of the current study will be to complete the analysis of the Space Shuttle flight experiment, as well as complete the work on metabolic flux and partitioning of the biochemical processes involved in monolignol (and hence lignin) biosynthesis. This is essential if the pathway to the lignin polymers are to be both understood, together with the role or impact of gravity thereon.

Published manuscripts, manuscripts in preparation and work currently underway.

Publications which have appeared in print describing various aspects of the project are summarized below, together with others which are either in print or submitted. Several of these papers represent commitments by N.G. Lewis to various book chapters, but still nevertheless encompass work carried out under NASA sponsorship.

Eberhardt, T.L., Bernards, M., He, L., Davin, L.B., Wooten, J.B. and Lewis, N.G. 1993 Lignification in Cell Suspension Cultures of *Pinus taeda* During Early States of Cell Wall Thickening. J. Biol. Chem. **268:**21088-21096.

Lewis, N.G. and Davin, L.B. 1994 Lignans and Neolignans: A Phytochemical Treasure. Polyphénols Actualités **10:**17-19.

Lewis, N.G. and Davin, L.B. 1994 Evolution of Lignin and Neolignan Biochemical Pathways. *In* "Evolution of Natural Products" (Nes, W.D., ed.), ACS Symposium Series No. 562, pp. 202-246.

Lewis, N.G., Kato, M., Lopes, N. and Davin, L.B. 1995 Lignans: Diversity, Biosynthesis and Function. *In* "Chemistry of the Amazon: Biodiversity, Natural Products and Environmental Concerns" (Seidl, P.R., Gottlieb, O.R. and Kaplan, M.A., eds.), ACS Symposium Series No. 588, American Chemical Society, Washington, DC, pp. 135-167.

Bernards, M.A., Lopez, M.L., Zajicek, J. and Lewis, N.G. 1995 Hydroxycinnamic Acid-derived Polymers Constitute the Polyaromatic Domain of Suberin. J. Biol. Chem. **270:**7382-7386.

Nose, M., Bernards, M.A., Furlan, M., Zajicek, J., Eberhardt, T.L. and Lewis, N.G. 1995 Towards the Specification of Consecutive Steps in Macromolecular Lignin Assembly. Phytochemistry **39:**71-79.

Razal, R.A., Ellis, S., Lewis, N.G. and Towers, G.H.N. 1996 Nitrogen Recycling In Phenylpropanoid Metabolism. Phytochemistry **41:**31-35.

Bernards, M.A. and Lewis, N.G. 1996 Suberin: An Hydroxycinnamic Acid-Derived Polymer. Polyphénols Actualités **14:**4-6.

van Heerden, P., Towers, G.H.N. and Lewis, N.G. 1996 Nitrogen Metabolism in Lignifying *Pinus taeda* Cell Cultures. J. Biol. Chem. **271:**12350-12355.

Singh, S., Lewis, N.G. and Towers, G.H.N. 1997 Interactions Between Nitrogen and Phenylpropanoid Metabolism: Detection of a Novel Nitrogen Cycle in Plants. Polyphénols Actualités **15/16:**16-19.

Davin, L.B., Wang, H.-B., Crowell, A.L., Bedgar, D.L., Martin, D.M., Sarkanen, S. and Lewis, N.G. 1997 Stereoselective Bimolecular Phenoxy Radical Coupling by an Auxiliary (Dirigent) Protein Without an Active Center. Science **275:**362-366.

Towers, G.H.N., Singh, S., van Heerden, P. and Lewis, N.G. 1997 Integrating Nitrogen and Phenylpropanoid Pathways: A New Nitrogen Cycle in Plants and Fungi. *In* "Lignin and Lignan Biogenesis" (Lewis, N.G. and Sarkanen, S., eds.), ACS Symposium Series, American Chemical Society, Washington, DC (in press).

He, L., Heyenga, G.A., Levine, H.G., Davin, L.B., Krikorian, A.D. and Lewis, N.G. 1997 Cellulose Microfibril Organization and Secondary Wall Thickening Patterns of Wheat (*Triticum*

aestivum L.) Primary Roots Developed in Microgravity. Phytochemistry (XXXX). [C.A. Ryan Special Issue]

Heyenga, G., Davin, L.B., Brown, C. and Lewis, N.G. 1997 A Solid-State Nutrient Support System for Long-Term Plant Culture in Microgravity Conditions (submitted).

Gang, D.R., Dinkova-Kostova, A.T., Davin, L.B. and Lewis, N.G. 1996 The Lignan Branch: Cloning and Expression of (+)-Pinoresinol/(+)-Lariciresinol Reductase. *In* "Lignin and Lignan Biosynthesis" (Lewis, N.G. and Sarkanen, S., eds.), ACS Symp. Ser. (XXXX).

Davin, L.B. and Lewis, N.G. 1996 Stereoselective Coupling in Lignin/Lignan Formation. *In* "Lignin and Lignan Biosynthesis" (Lewis, N.G. and Sarkanen, S., eds.), ACS Symp. Ser. XXX, American Chemical Society (XXXX).

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Bernards, M.A. and Lewis, N.G. 1997 The Aromatic Domain of Suberin. Phytochemistry (submitted).

Pearce, G.A., Marchand, P.A., Griswold, J., Lewis, N.G. and Ryan, C.A. 1997 Accumulation of Feruloyltyramine and *p*-Coumaryltyramine in Tomato Leaves in Response to Wounding (submitted).

Chang, M., Davin, L.B., Bedgar, D.L. and Lewis, N.G. 1997 Compression Wood Formation in Microgravity (manuscript in preparation).

van Heerden, P., Anterola, A.M., Nose, M., Zuiches, J., Davin, L.B. and Lewis, N.G. 1997 Lignification in *Pinus taeda* Cell Suspension Cultures: Towards Defining Regulatory Steps in Monolignol Biosynthesis (submitted).

Lewis, N.G. and Sarkanen, S. 1997 Lignin and Lignan Biosynthesis. American Chemical Society XXX (19 chapters).

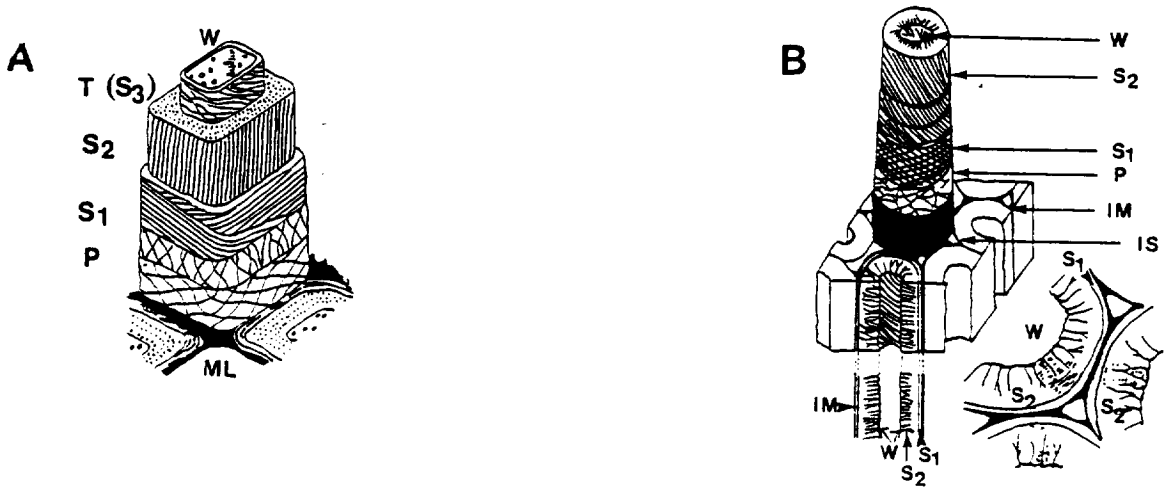

Figure 1. Schematic model of the cell wall structure of **A**: softwood tracheids and hardwood libriform fibers and **B**: typical compression wood tracheids.

ML: Middle lamella, P: Primary cell wall, S_1: Secondary wall 1, S_2: Secondary wall 2, $T(S_3)$: Tertiary wall, W: Warty layer, IS: Intercellular space, IM: Intercellular material.

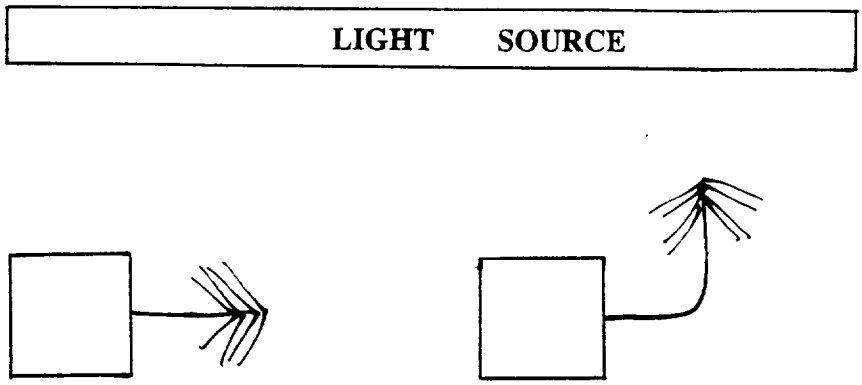

Figure 2. Optimal conformation for studying compression wood formation in microgravity.

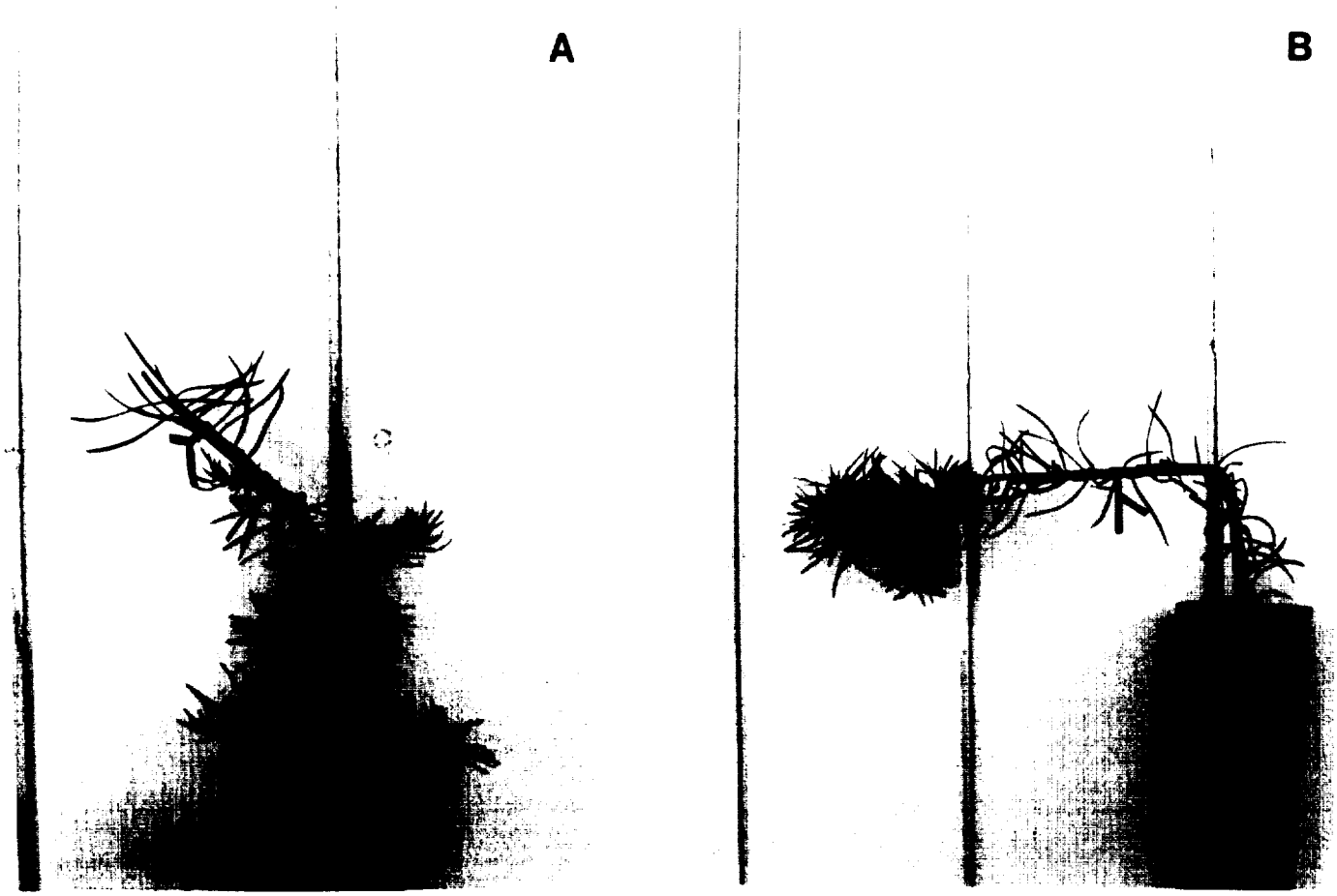

Figure 3. Douglas fir (*Pseudotsuga menziesii*) seedlings grown at **A:** 45° and **B:** 90°.

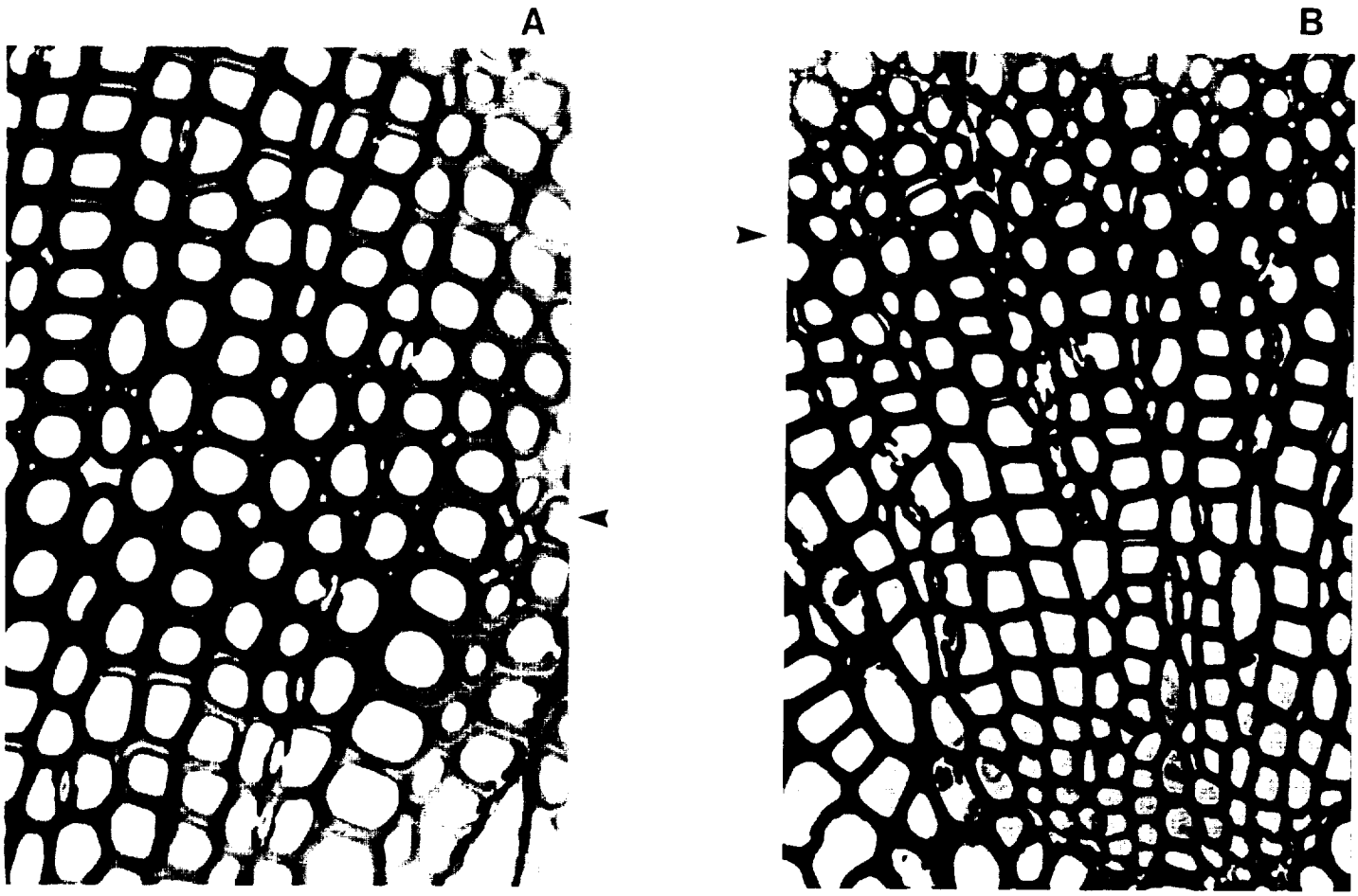

Figure 4. Light microscopy cross-sections of Douglas fir (*Pseudotsuga menziesii*) seedlings bent for 3 days, then allowed to further grow for an additional 4 days in an upright position. **A:** 45° orientation and **A:** 90° orientation. Arrow shows compression wood.

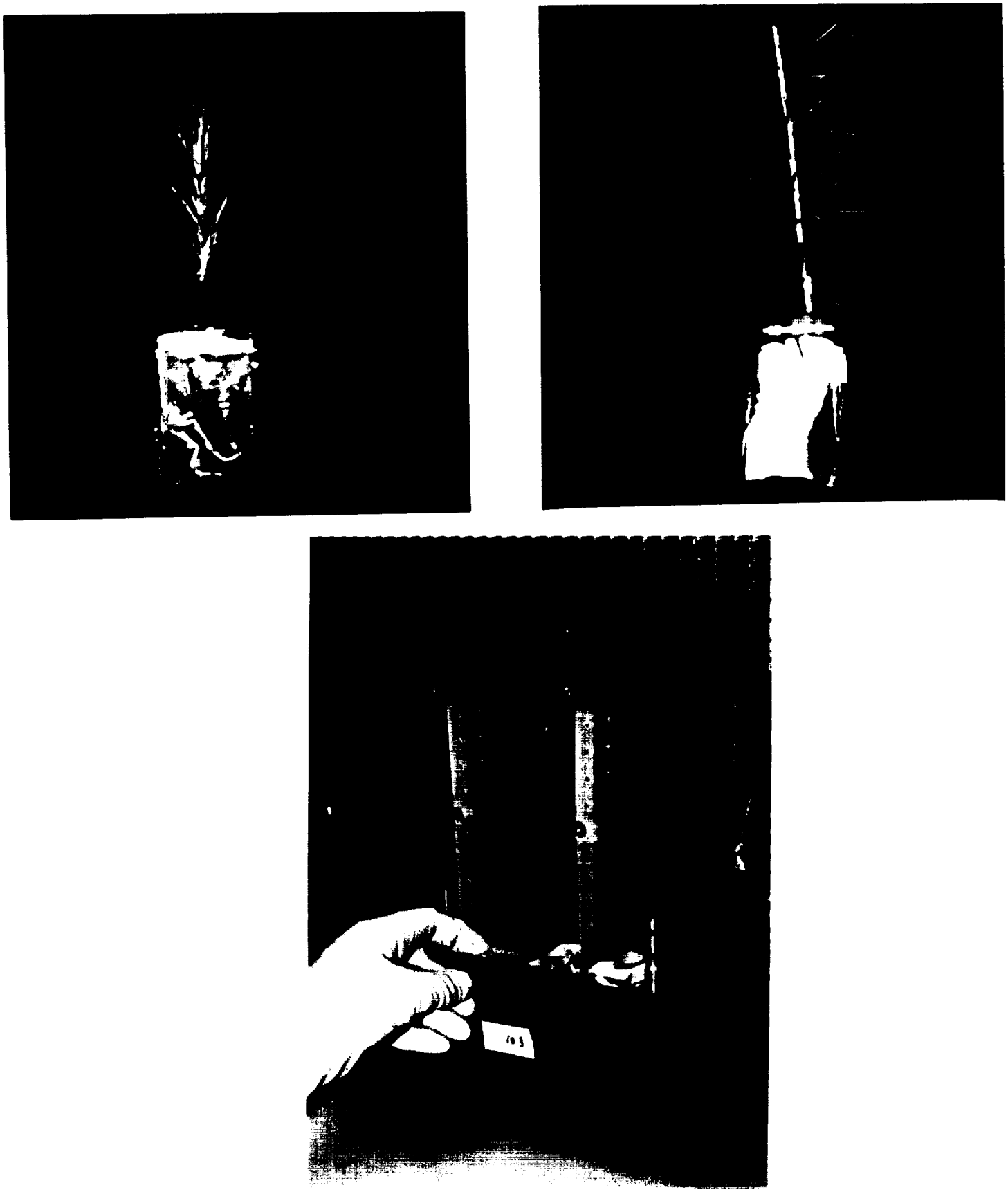

Figure 5. Nutrient pack and PGC.

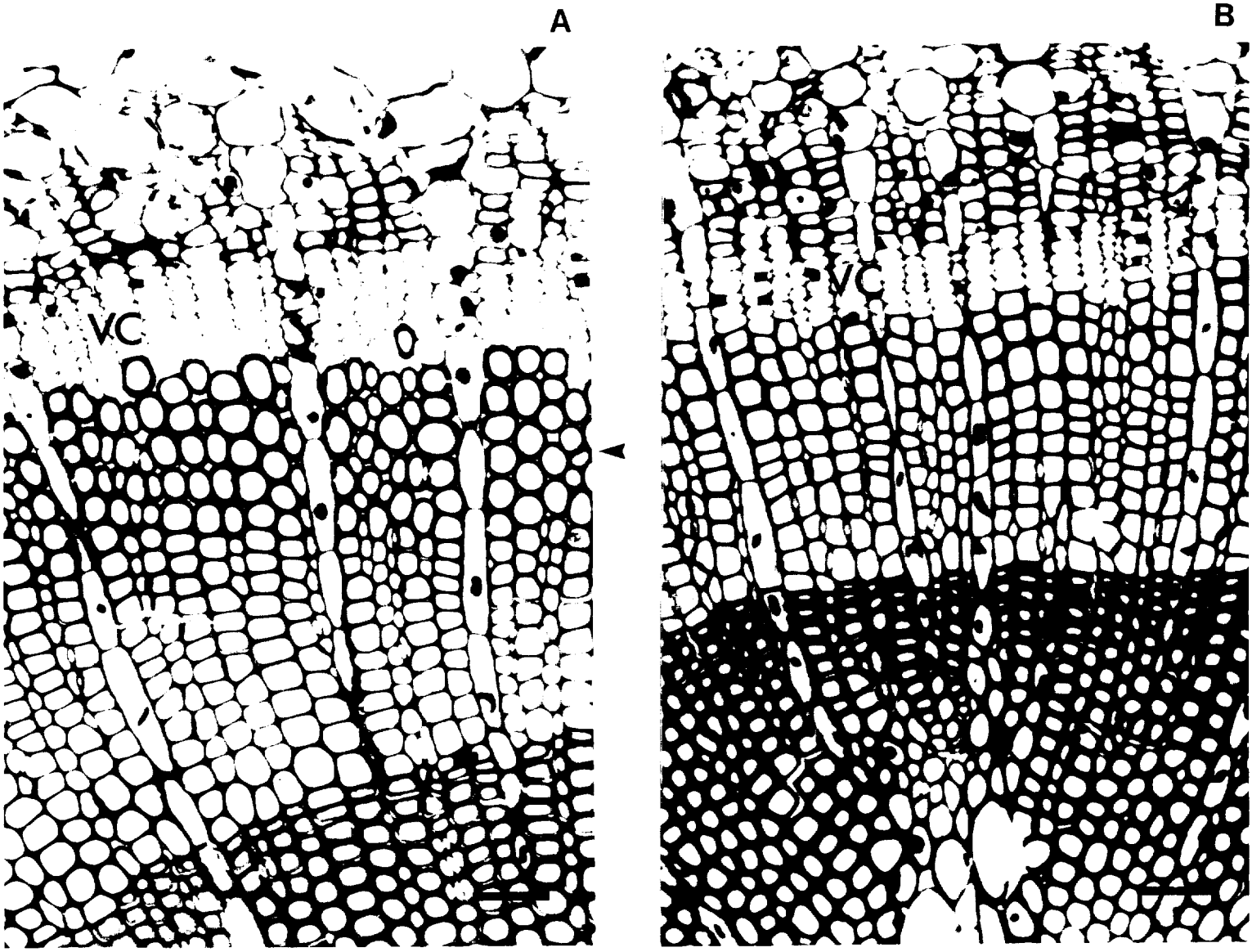

Figure 6. Light microscopy cross-sections (310X) of Douglas fir (*Pseudotsuga menziesii*) seedlings. Payload Verification Test; 15 days; 45° orientation. **A:** Reaction wood and **B:** Opposite Wood. Arrow shows compression wood.

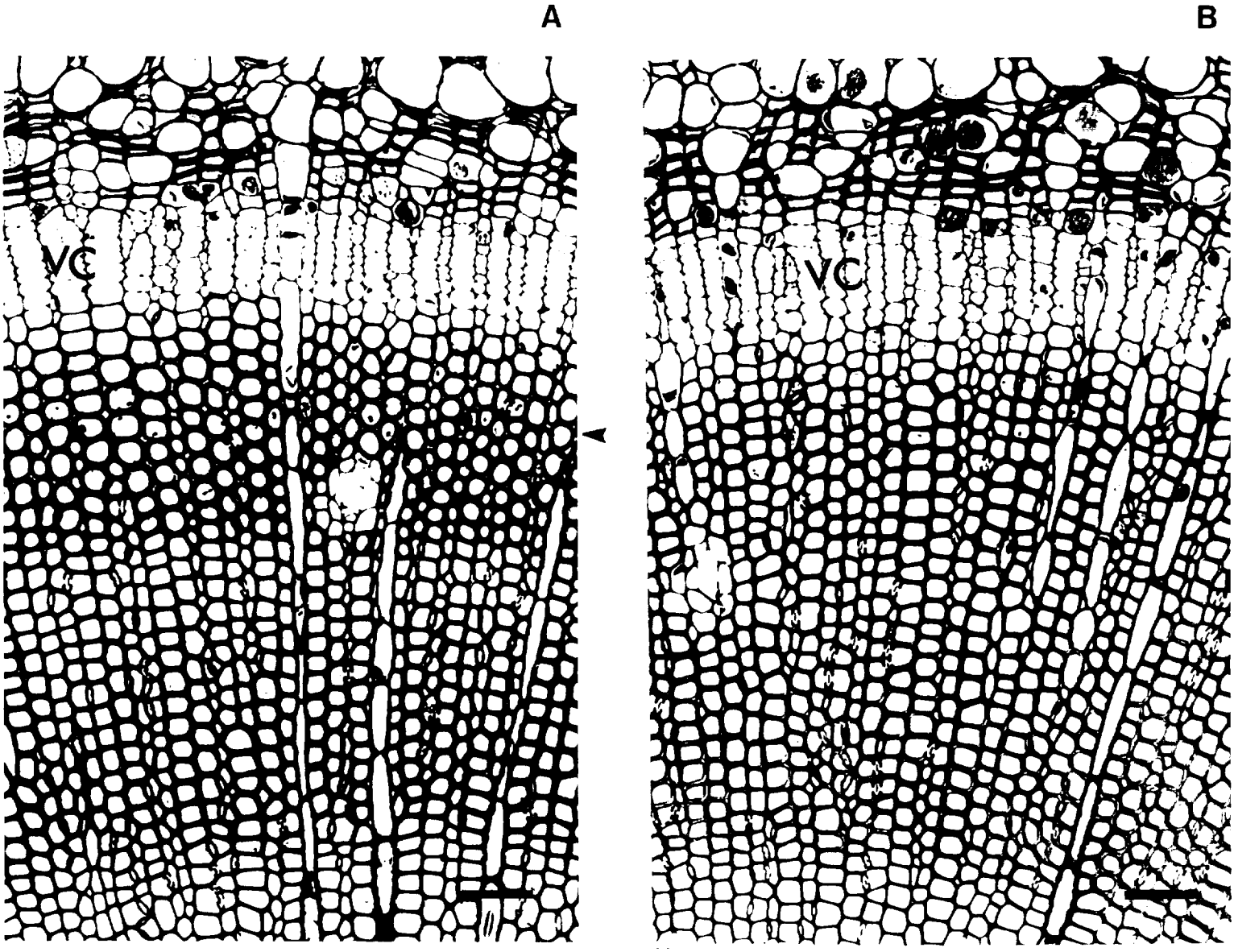

Figure 7. Light microscopy cross-sections (310X) of Douglas fir (*Pseudotsuga menziesii*) seedlings. STS-78; flight; 16 days; 45° orientation. **A:** Reaction wood and **B:** Opposite Wood. Arrow shows compression wood.

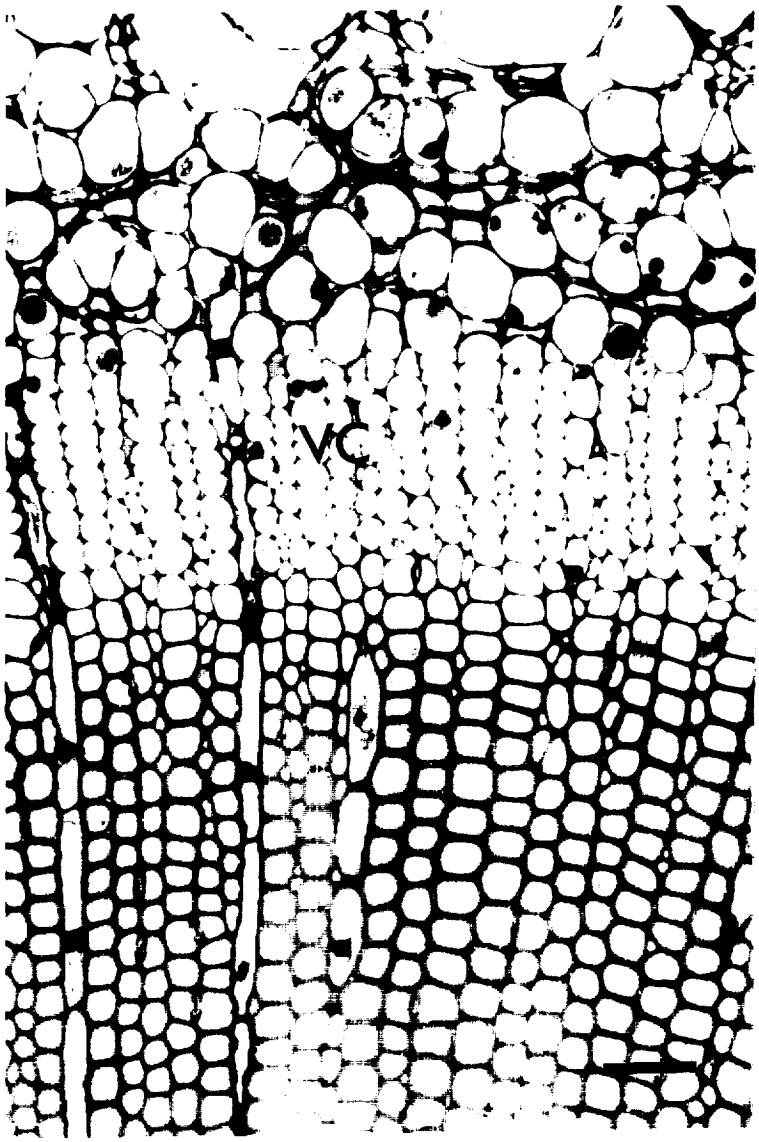

Figure 8. Light microscopy cross-sections (310X) of Douglas fir (*Pseudotsuga menziesii*) seedlings. STS-78; flight; 16 days; vertical orientation.

Figure 9. Harness used to keep the seedlings at a 45° angle

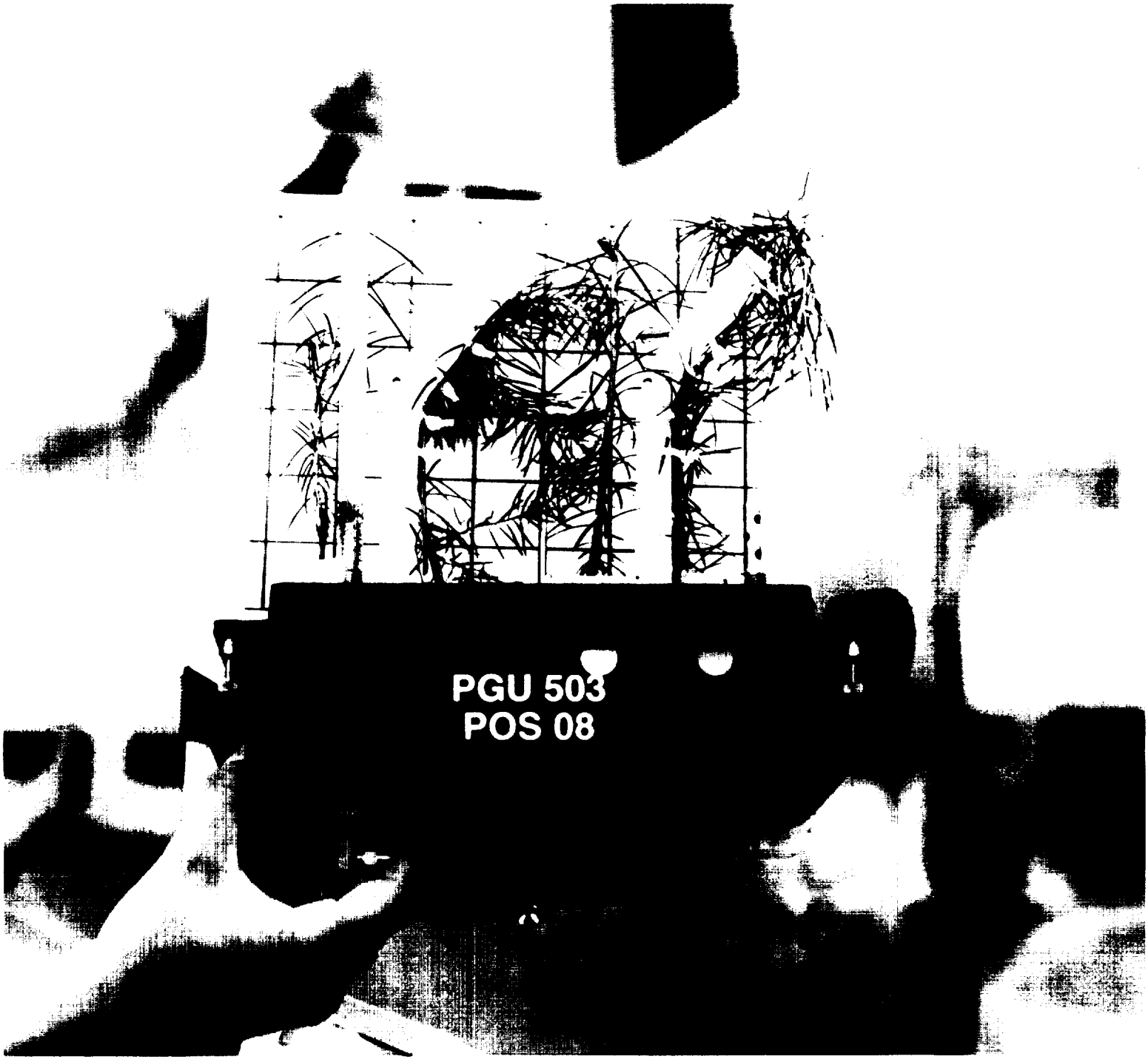

Figure 10. Plant Growth Chamber.

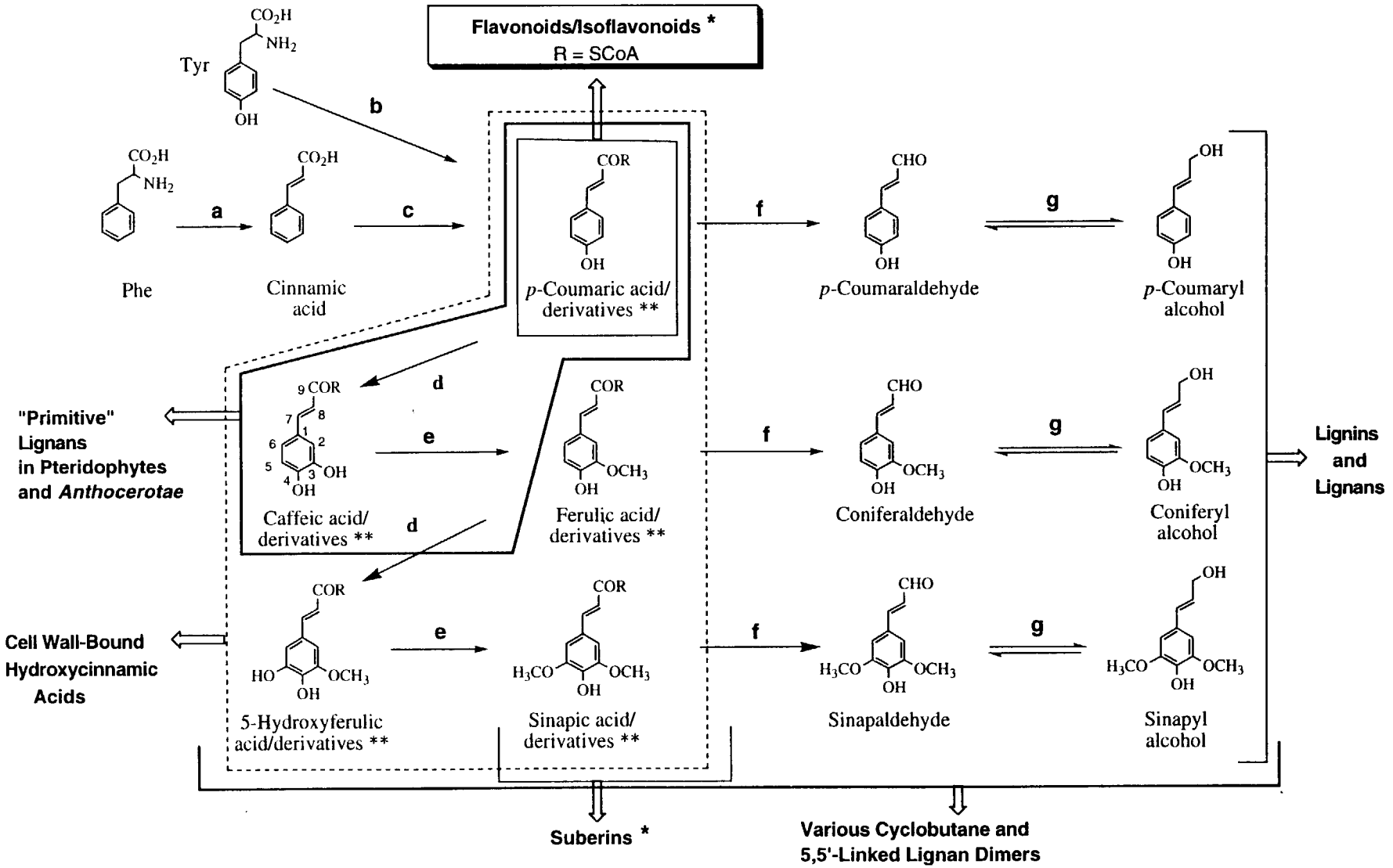

Figure 11. Proposed Biosynthetic Pathway to Monolignols and Related Phenylpropanoid (Acetate) Metabolites.
a. Phenylalanine ammonia-lyase, **b.** Tyrosine ammonia-lyase (mainly in grasses), **c.** Cinnamate-4-hydroxylase, **d.** Hydroxylases, **e.** *O*-Methyltransferases, **f.** Cinnamoyl-CoA:NADP oxidoreductases, **g.** Cinnamyl alcohol dehydrogenases, **h.** Bimolecular phenoxy radical coupling reactions (leading to either dimer or polymer formation).
(Note: * plus acetate pathway for both flavonoids and suberins
** R = OH for free acids and R = SCoA for CoA esters.)

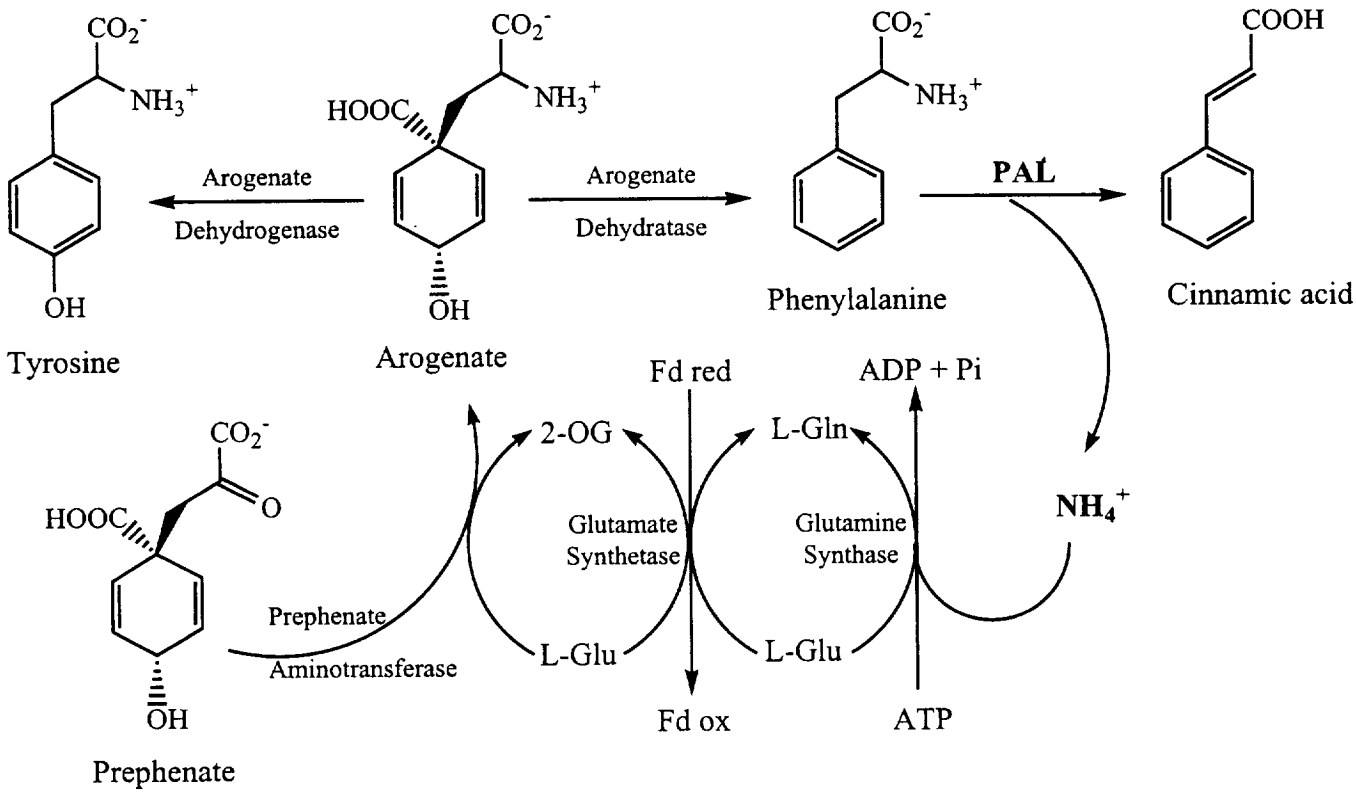

Figure 12. Nitrogen recycling in *Pinus taeda* during active phenylpropanoid metabolism.

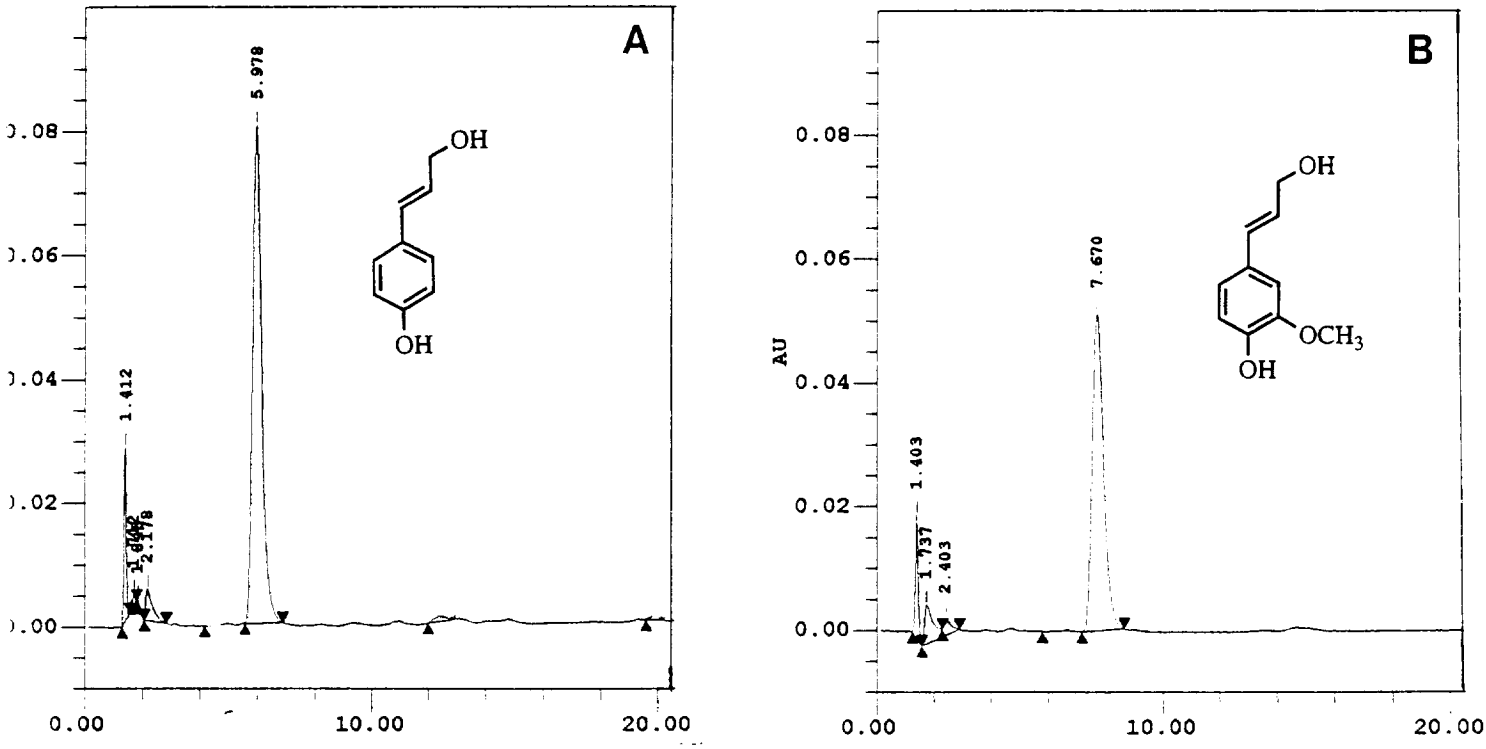

Figure 13. Selective formation of **A.** *E-p*-coumaryl alcohol and **B:** E-coniferyl alcohol in *Pinus taeda* cell lines.