Plant Growth Facility (PGF)

Compression Wood Formation in a Microgravity Environment

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Abstract

In a microgravity environment aboard the Space Shuttle Columbia Life and Microgravity Mission STS-78, compression wood formation and hence altered lignin deposition and cell wall structure, was induced upon mechanically bending the stems of the woody gymnosperms, Douglas fir (*Pseudotsuga menziesii*) and loblolly pine (*Pinus taeda*). Although there was significant degradation of many of the plant specimens in space-flight due to unusually high temperatures experienced during the mission, it seems evident that gravity had little or no effect on compression wood formation upon bending even in microgravity. Instead, it apparently results from alterations in the stress gradient experienced by the plant itself during bending under these conditions. This preliminary study now sets the stage for long-term plant growth experiments to determine whether compression wood formation can be induced in microgravity during phototropic-guided realignment of growing woody plant specimens, in the absence of any externally provided stress and strain.

Introduction

The woody gymnosperms, loblolly pine (*Pinus taeda*) and Douglas fir (*Pseudotsuga menziesii*) represent two very important commercial plant species used today in pulp and paper manufacture and for lumber applications. 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 (*1*). A very deleterious feature in woody plant development, however, occurs when their stems are displaced from a vertical alignment (*2*). 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 (2). 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 its *p*-coumaryl alcohol content. 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.
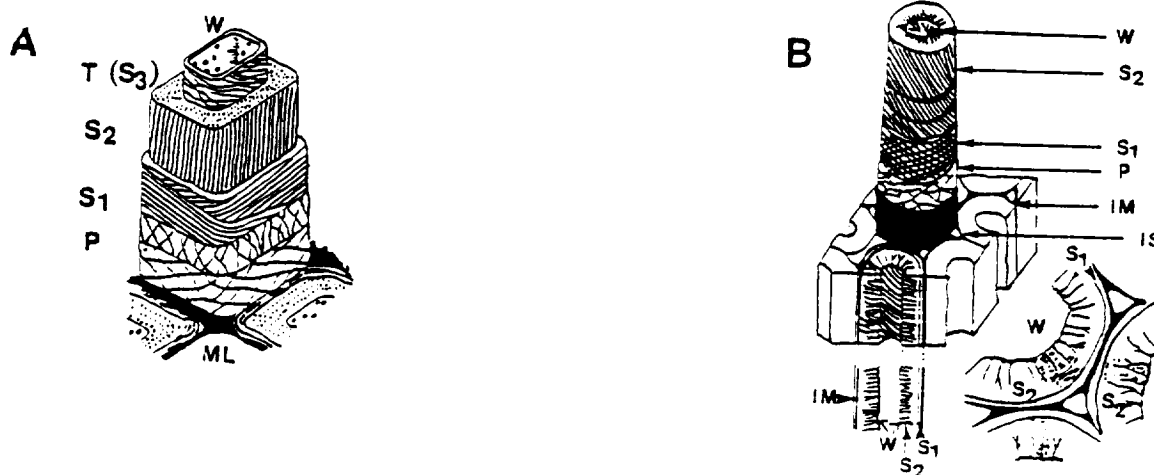

Figure 1. Schematic model of the cell wall structure of A: softwood tracheids and hardwood libriform fibers and B: typical compression wood tracheids (redrawn from references *3* and *2*, respectively).

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.

Results and Discussion

In the first objective of LMS Mission on STS-78, 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 the preferred experimental design to test whether compression wood formation occurs in microgravity, 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 had 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 it would be the ideal experiment (Figure 2).
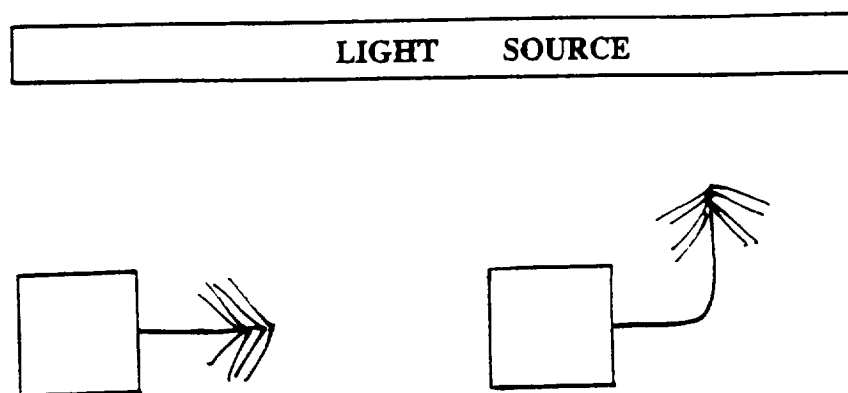

Figure 2. Preferred conformation for studying compression wood formation in microgravity.

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 Shuttle Flight experiments. Consequently, we developed an alternative system for examining whether compression wood formation could be engendered in microgravity over the STS-78 flight duration (17 days).

This involved bending (at both 45° and 90°) loblolly pine and Douglas fir plants (see Figure 3) 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 and 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 gels 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 about 10 months of developing the necessary protocols, 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 Kennedy Space Center, and 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.

Next, the astronauts, Jean-Jacques Favier and Susan Helms 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 orbit in microgravity. Selected plants were harvested and sectioned (both upright vertical controls and oriented specimens, at days 10 and 13, respectively) under shuttle flight laboratory conditions, in preparation for light microscopy analyses.

With all preparations satisfactorily completed, Shuttle Columbia (STS-78) was launched on June 22, 1996, from Kennedy Space Center. The space flight experiment, while answering the question of whether compression wood formation occurred under such conditions, was not without difficulty. The Shuttle cabin temperatures were higher than any recorded previously (> 29°C), this having 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. As can be seen, 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 the

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temperatures experienced. This was established to be the case (data not shown), and a future publication will describe the entire preparations and space-flight experiment and its results in full detail (4).

Many authorities would 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 high enough to ensure that the organisms can still respond to it, or much 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 hence 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, but without introduced mechanical stresses such as by bending.

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.

Concluding Remarks

It is now established that woody gymnosperm plants, such as Douglas fir, when placed in off-vertical configuration in microgravity, relative to the light source, respond by forming compression wood. This is proposed to be due to the influences of an internal stress gradient within the plant. It now needs to be established if woody angiosperm would form comparable reaction wood under these conditions. This adds to the steadily growing body of knowledge associated with general phenylpropanoid metabolism and cell wall development (5- 36). It also needs to be proven whether reaction (compression) wood would result if plants were able to realign their stems without the influence of harnessing e.g., by phototropic responses over longer time periods (months, years). This will be an important experiment to conduct in Space Station Alpha.

Acknowledgments

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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.
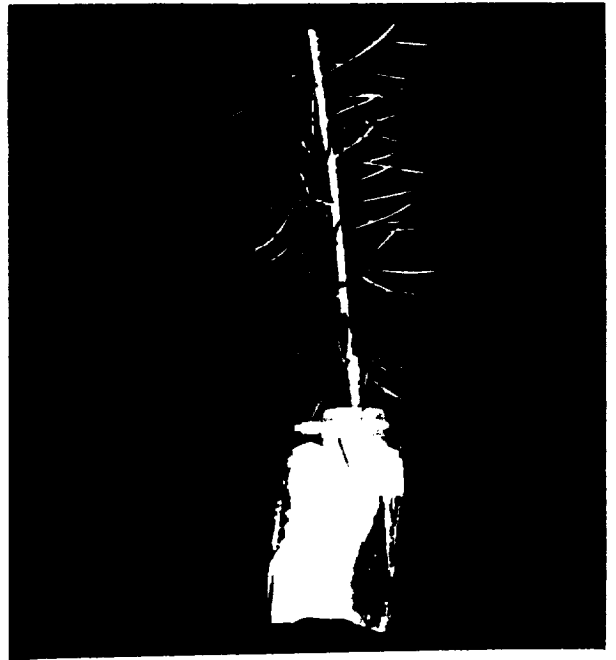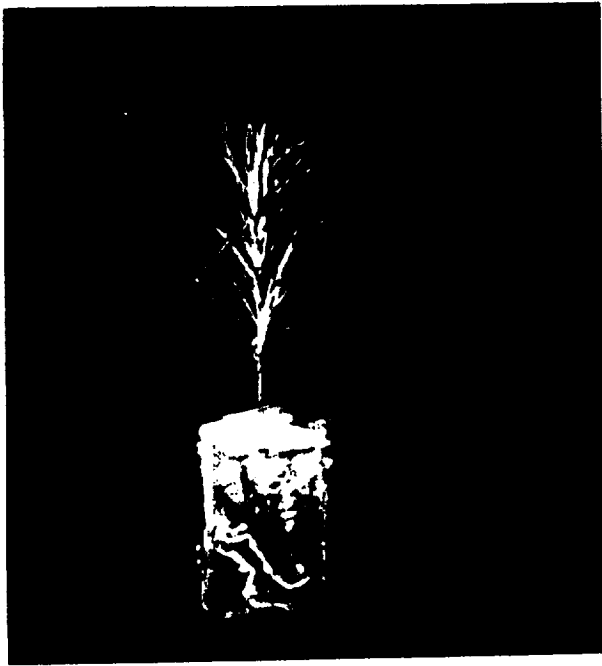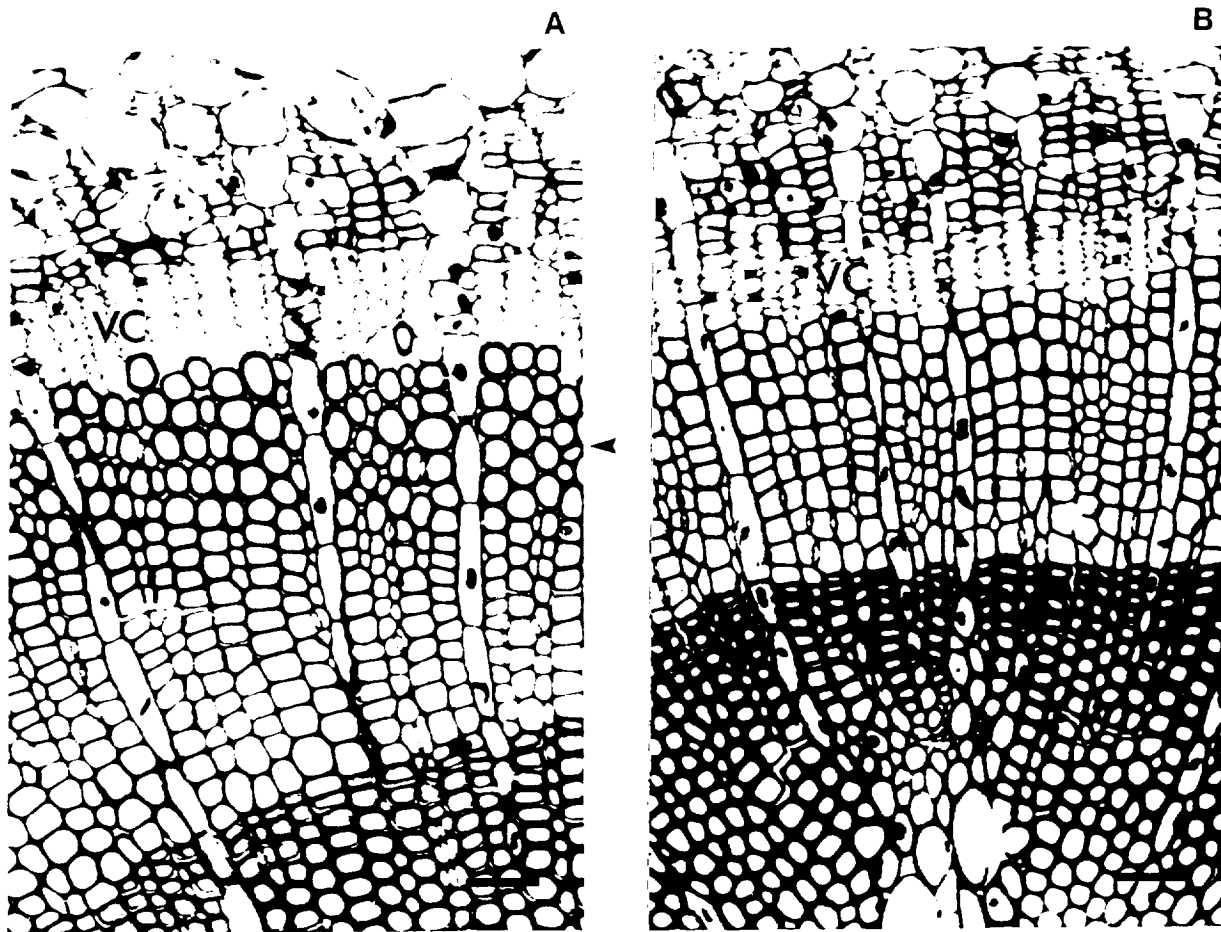
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Figure 5.   Nutrient pack and PGC.

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.
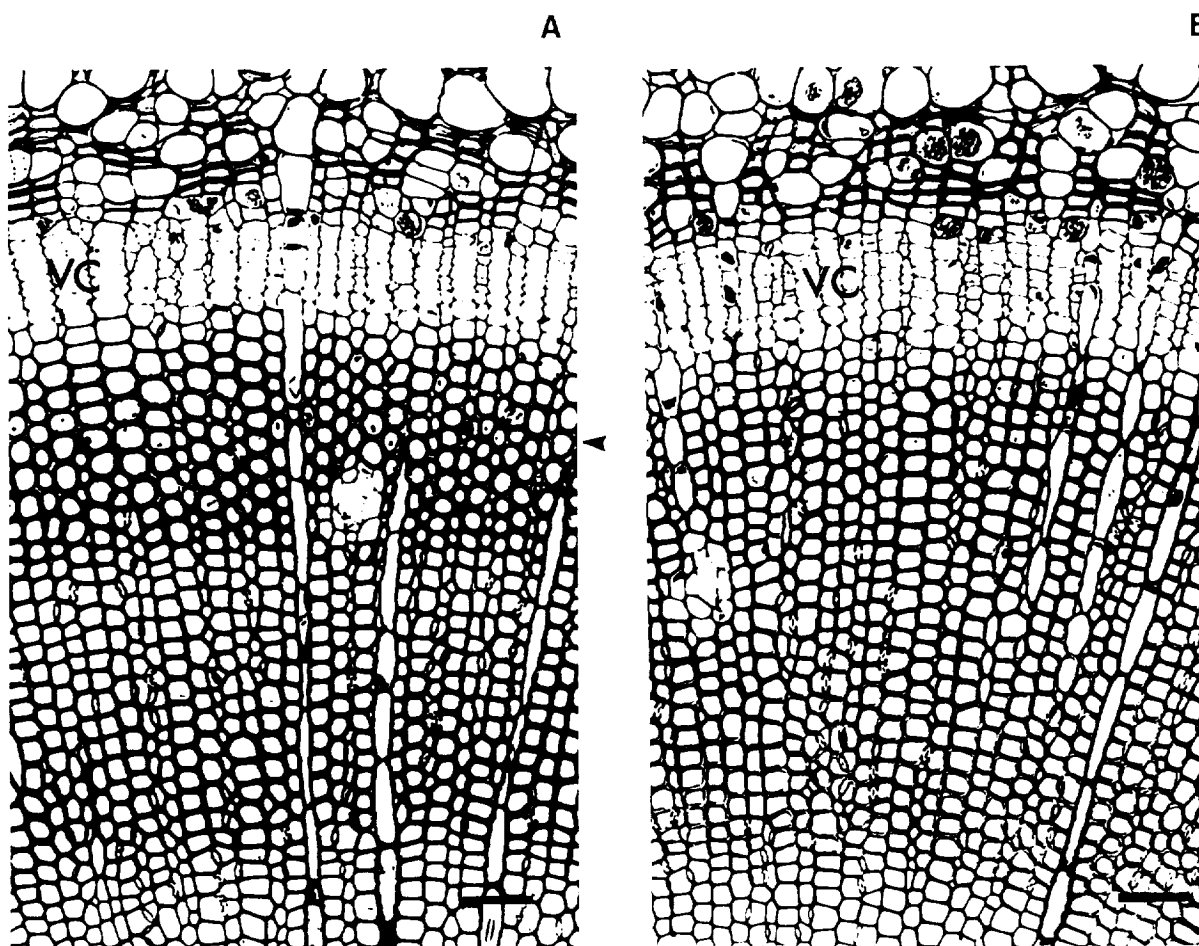
Figure 7.   Light microscopy cross-sections (310X) of Douglas fir (*Pseudotsuga menziesii*) seedlings. STS-78; flight; 16 days; 45° orientation. **A:** Reaction wood and **B:** Opposite Wood. Arrow shows compression wood.
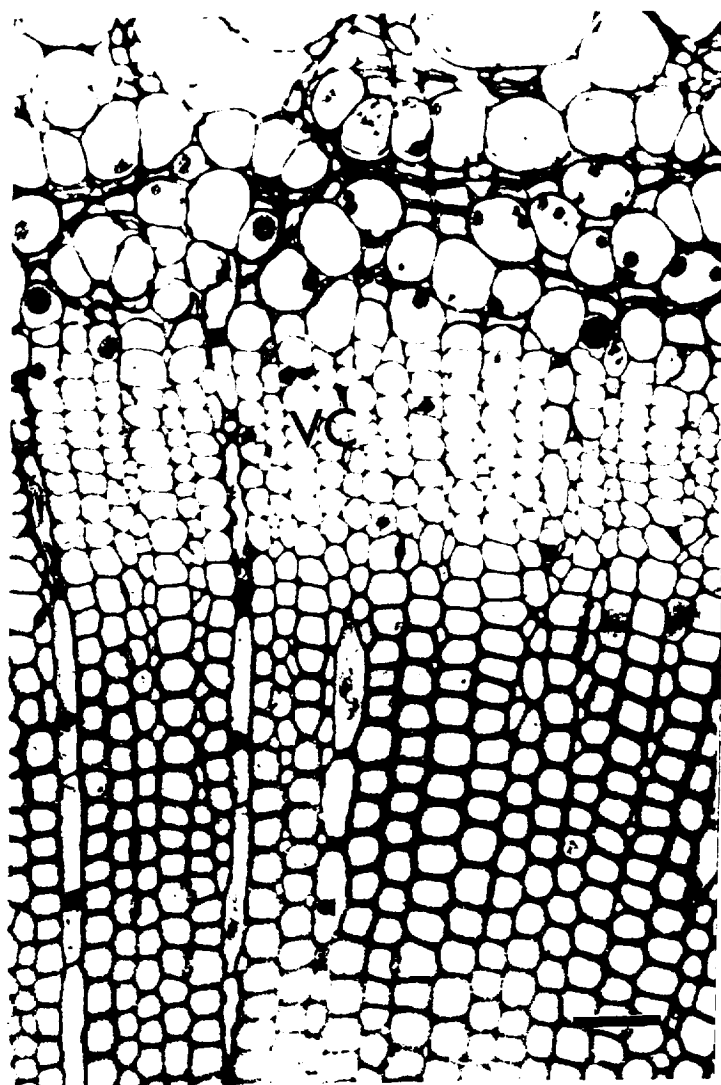
Figure 8.   Light microscopy cross-sections (310X) of Douglas fir (*Pseudotsuga menziesii*) seedlings. STS-78; flight; 16 days; vertical orientation.
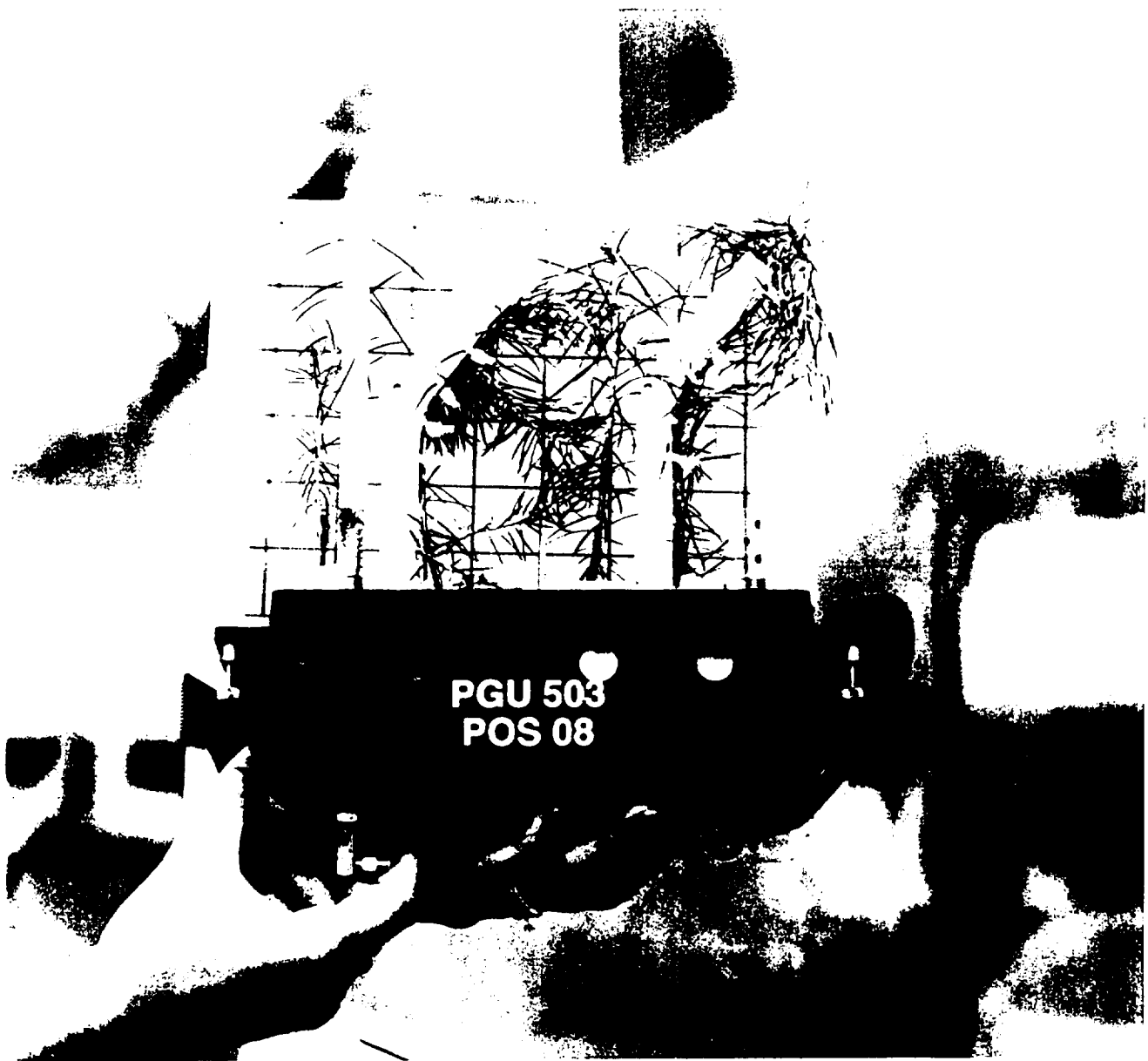
Figure 9.   Harness used to keep the seedlings at a 45° angle

Figure 10.   Plant Growth Chamber.