Deformation of the stem of containerized black spruce seedlings

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Abstract

When containerized black spruce seedlings *(Picea mariana* (Mill.) B.S.P.) are grown rapidly, they often bend over, grow horizontally and become deformed. This phenomenon, commonly referred to as spiralization, has had a severe impact on the containerized seedling industry in Quebec, particularly for producers using heated greenhouses. Research programs at *Universite Laval* and the *Universite du Quebec* a *Chicoutimi* have attempted to determine causes as well as possible solutions. The physiological basis of stem spiralization appears to be associated with a redubed capacity of root systems to convert the amino acid, phenylalanine, into lignin precursors that will permit the stem to lignify in a normal manner. High light levels together with an aerobic root environment were found to stimulate lignin synthesis in black spruce. The development cf spiralized stems was found to result from the sum total of a series of relatively small bending movements as well as minor corrective movements (i.e. 0-15°) measured every 2 to 3 days but integrated over the production cycle. A direct relationship was found between the maximal angle of bending and the risk that the seedling would have a significant defect at the end of the production cycle. A seedling which bent at more than 90° from the vertical at any time nearly always was classified as a defective seedling at the end of the production period. Vector analyses of seedling movements indicated that seedlings tended to move in the direction of the dominant light source.

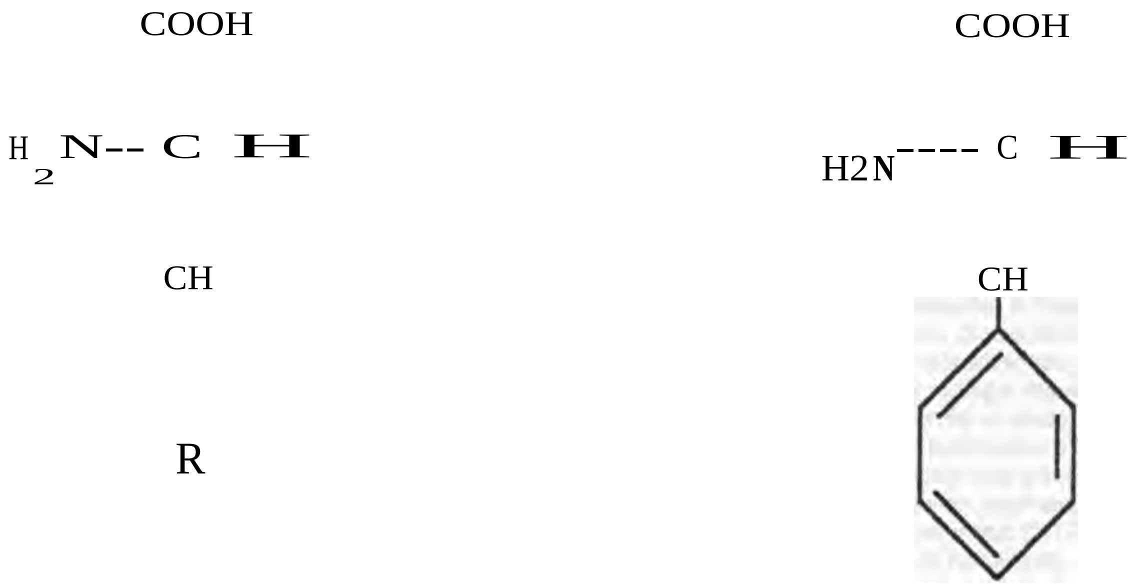
Résumé

*Le **gauchissement** de la tige d'epinette noire.*
Le gauchissement de la tige est un defaut de croissance, provenant dune verse partielle ou complete du semis pendant sa phase de croissance exponentielle. Cette deformation se retrouve principalement sur les semis d'epinette noire (Picea mariana *(Mill.) B.S.P.) en recipients, produits en serre pendant l'hiver. Le gauchissement de la tige **entrene** des pertes importantes pour l'industrie du plant en recipient au Quebec. Les travaux de recherche effectuês a l'Universitó Laval et a l'Universite du Québec a Chicoutimi ont demontre que le gauchissement de la tige etait associa a la diminution du taux de transformation d'un acide amine, le phenylalanine, en un compose qui est un precurseur de la lignine. De plus, it a ate demontre que la combinaison des traitements de forte intensite lumineuse et d'oxygenation du substrat racinaire augmentait la lignification des semis d'epinette noire. La mesure de Tangle et* de *l'orientation de la tige, tous les 2 a 3 jours pendant la periode de production, a demcntre que le gauchissement de la tige resuitait dune sommation de mouvements de faible amplitude (i.e. 0-15°). L'angle de la tige a la fin de la periode de production etait correle* avec */'angle maximal atteint par le semis pendant sa croissance. Ainsi, un semis ayant atteint un angle supêrieur a 90° pendant sa croissance etait presque toujours classe inaccceptable pour le reboisement,* avec *un angle de la tige supêrieur a 15°* a /a *fin de la periode de production. L'analyse vectorielle des mouvements du plant a demontre que l'orientation de la source lumineuse dominante avait un effet significatif sur l'orientation des mouvements de la tige.*

Introduction

The problem of stem spiralization during the production of containerized tree seedlings is most common for black spruce *(Picea mariana* (Mill.) B.S.P.) raised during winter in heated greenhouses. The spiralization occurs when the stem bends over during the exponential growth phase and the seedling begins to grow horizontally. This can result in various kinds of deformations (i.e. stair-steps, bent stems, etc.) that can be grouped together under the name, spiralization. The end result is a population of seedlings of reduced quality. The principal buyer of containerized seedlings in Quebec, the *Ministere de l'Energie et des Ressources,* considers seedlings with an inclination of greater than 15° to he defective and unsuitable for reforestation (Brouillette *et al.* 1987). Thus spiralized seedlings are a significant source of lost revenue for seedling producers. While losses are generally in the range of 5% to 10%, cases as high as 20% have occurred.

Research on various aspects of the spiralization problem of black spruce have been undertaken at two Quebec universities, *Universite Laval* in Quebec City and the *Universite du Quebec* in Chicoutimi. This paper summarizes some of the results of these efforts.



General Form of an Amino Acid

Phenylalanine

Figure 1. Generalized structure of an amino acid with its variable R-group and a specific amino acid, phenylalanine, where the R-group is comprised of a 6-carbon phenolic ring. Each corner of the 6-carbon phenolic ring denotes the location of a carbon atom.

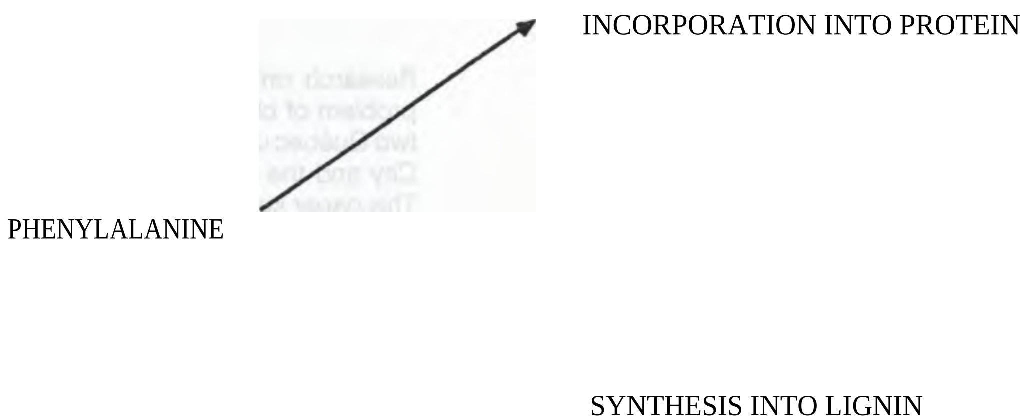


Figure 2. The amino acid phenylalanine can be either incorporated into a protein or used as a substrate for the synthesis of lignin.

Physiological Differences between Spiralized **and Healthy Black Spruce Seedlings** ***(Universite Laval)***

A visual inspection of spiralized black spruce seedlings, particularly the ones that bend over after only 6 to 8 weeks of growth, suggested that the stems have not sufficiently lignified and thus could not support the weight of their rapidly expanding shoot. For this reason, a study was undertaken to determine what physiological differences exist between spiralized and healthy seedlings in respect to lignin metabolism.

Lignin Synthesis – Amino acids are fairly small molecules which contain nitrogen (Figure 1). At one end of the molecule there is a standard arrangement of carbon, nitrogen, hydrogen and oxygen atoms. At the other end is the R-group which designates the part of the molecule which varies so as to form the different individual amino acids. Amino acids can be linked together to form certain kinds of proteins called enzymes. Enzymes are the molecules which are the driving force of plant metabolism, i.e., the equivalent to the role of the engine in an automobile.

One amino acid, named phenylalanine, can either be incorporated into a protein like the other amino acids or can be transformed into lignin (Figure 2). The uniqueness of phenylalanine comes from the fact that its R-group, the variable part of an amino acid, is a ring of 6 carbon atoms. This ring is very hard and very stable and thus gives lignin (i.e. wood) its unique properties. Lignin is formed when a large number of these carbon rings attach together.

Methodology – One-hundred-and-forty-four spiralized and 144 healthy eight-week-old black spruce seedlings were fed the amino acid phenylalanine which had been labelled with a radioactive carbon molecule, ^{14}carbon. The normal non-radioactive form of carbon is ^{12}carbon. In one treatment, the radioactive phenylalanine was incorporated during 6 hours by the root systems of intact seedlings (both spiralized and healthy) and in the second treatment, the root systems were cut off and the incorporation was done directly into stems. After the 6 hour incorporation period, seedlings were placed in a growth chamber. After 24, 48 and 72 hours, the seedlings were removed from the growth chamber and the percentage of ^{14}0 found in lignin and in protein in the stems was determined.

Results and Discussion – When the ^{14}C-phenylalanine was incorporated by roots, healthy seedlings partitioned a significantly greater percentage of their ^{14}0 to lignin than spiralized seedlings (Figure 3a) while spiralized seedlings partitioned a significantly greater percentage cf ^{14}C to protein than healthy seedlings (Figure 4a). When the phenylalanine was incorporated directly into stems, however, the percentage of ^{14}0 partitioned to lignin (Fig. 3b) and to protein (Fig. 4b) was essentially the same for both spiralized and healthy seedlings. Furthermore, the ^{14}0 percentages obtained after stem incorporation were similar to those obtained for spiralized seedlings which had incorporated the phenylalanine through roots.

Thus it appears that reduced lignin synthesis is indeed a physiological reason for the spiralization problem of black spruce. Furthermore, the root system appears to be the metabolic source of the problem since it appears that the roots of spiralized seedlings do not metabolize phenylalanine into lignin precursors that can be converted to lignin in the stem. In regards to the metabolism of lignin, therefore, spiralized seedlings behave as if they did not have a root system at all.

Effects of Light Level and Root Aeration on Lignification *(Universite Laval)*

Since lack of lignin synthesis was shown to be a physiological reason for spiralization, we wished to determine what kind of enviromental conditions might increase lignin synthesis in black spruce seedlings.

In this experiment, black spruce seedlings were grown for 10 weeks in a greenhouse with photoperiods extended to 18 hours with *HPS* lamps. Half of the seedlings were covered with a shade cloth during this 10-week period that reduced incoming radiation by 29%. The other half received full light. After the 10-week period was over, the peat moss was carefully removed from the root systems of 32 seedlings from each light treatment and the seedlings were allowed to incorporate radioactive ^{14}C-phenylalanine for 6 hours. After the incorporation, the seedlings were placed in beakers with their root systems submerged in distilled water and returned to the greenhouse. Ambient air was bubbled through half the beakers and nitrogen gas through the other half of the beakers. Thus the root systems of half the seedlings were in an aerobic environment while the other half were in an anaerobic environment. Half of each of these beakers were placed under the shade cloth and the other half exposed to

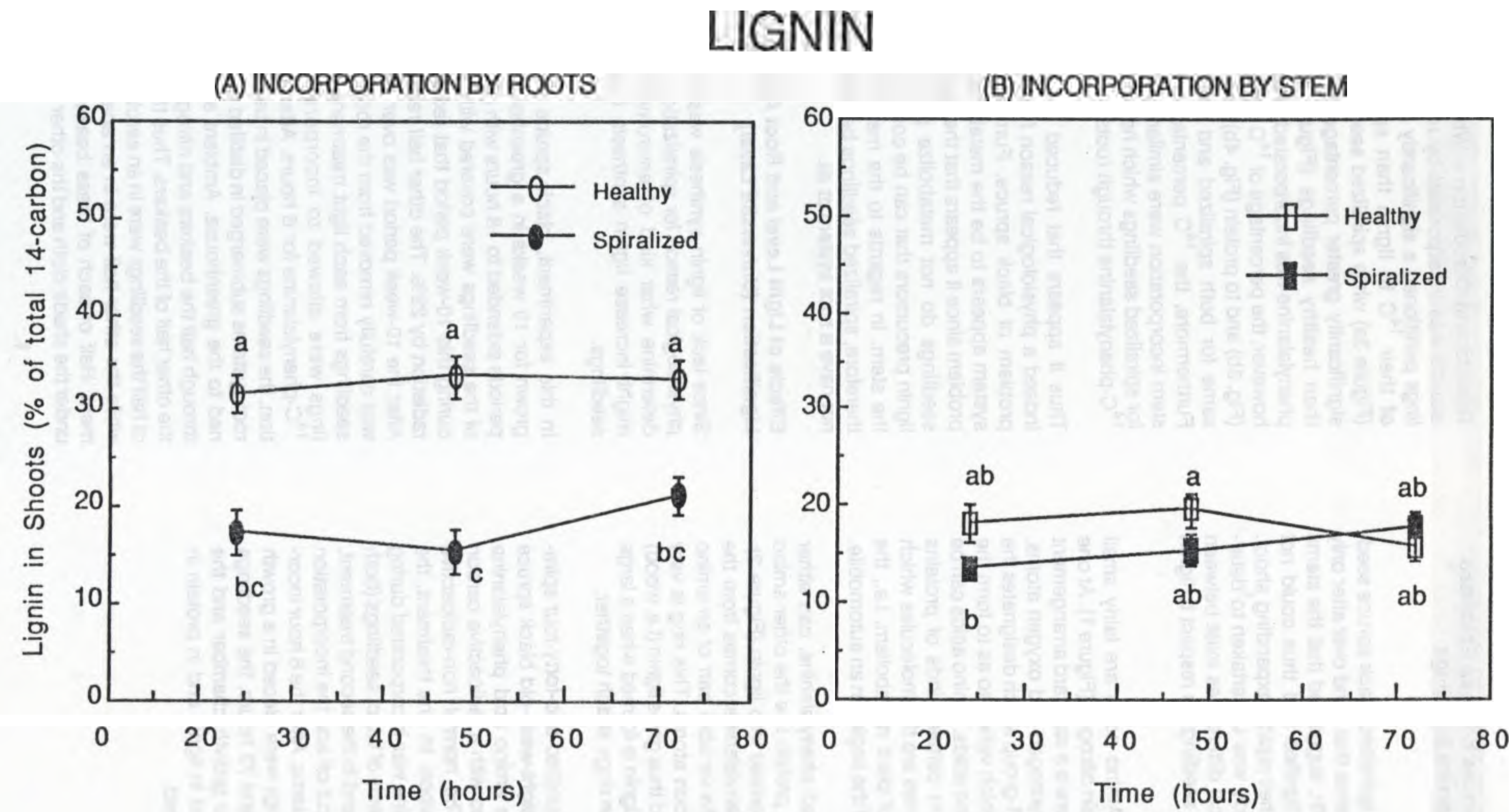


Figure 3. Percentage of ^{14}C partitioned to lignin in spiralized and healthy black spruce seedlings; 24, 48 and 72 hours after incorporation of ^{14}C-phenylalanine by (a) roots or (b) stems. Each point denotes the mean of 24 seedlings, bars denote standard errors. Points associated with different letters are significantly different at $p < 0.05$.

PROTEIN

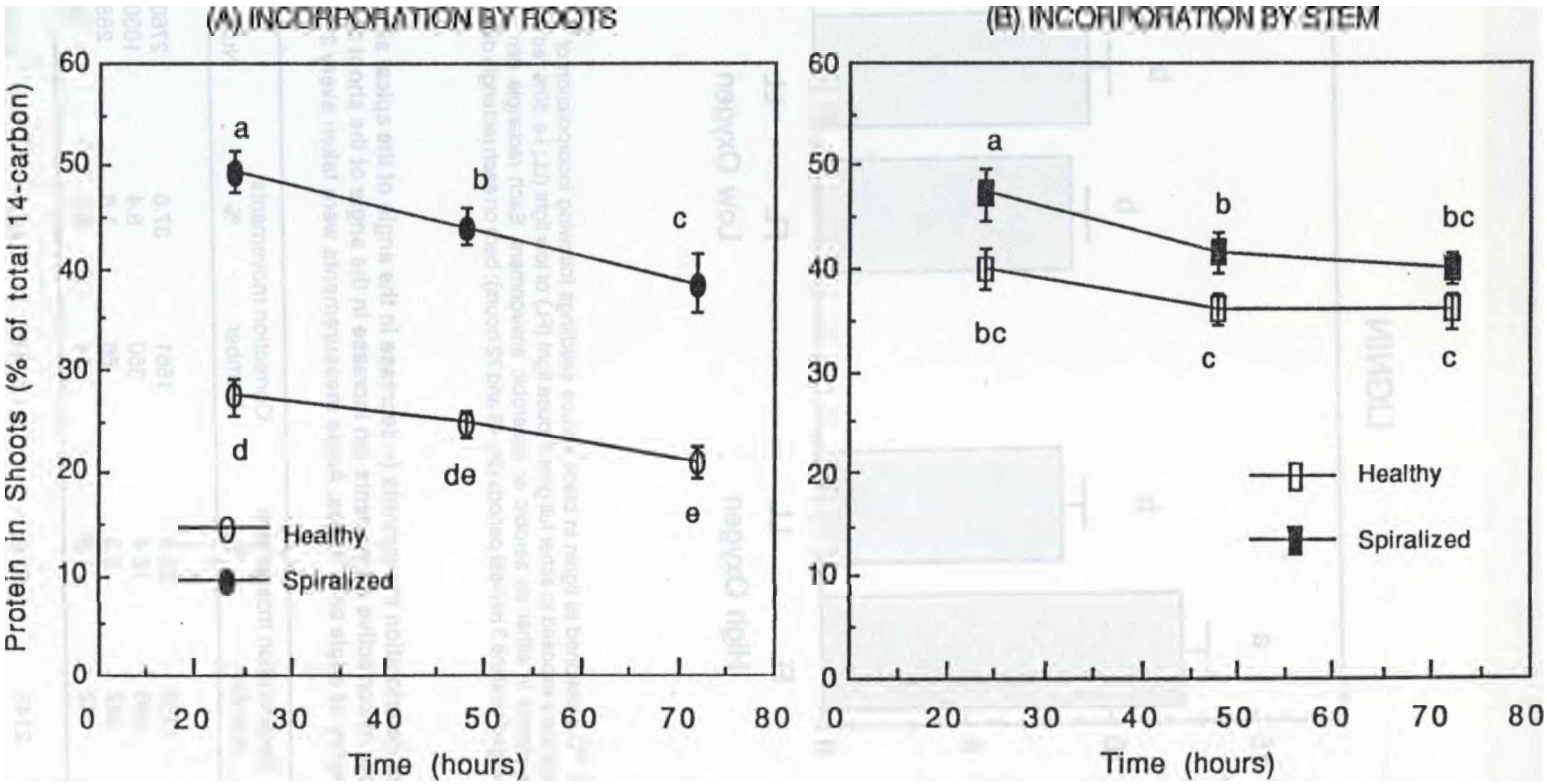


Figure 4. Percentage of ^{14}C partitioned to protein in spiralized and healthy black spruce seedlings; 24, 48 and 72 hours after incorporation of ^{14}C-phenylalanine by (a) roots or (b) stems. Each point denotes the mean of 24 seedlings, bars denote standard errors. Points associated with different letters are significantly different at $p < 0.05$.

Table 2. Relationship between the maximum angle obtained by a seedling during the production period and the final angle of the seedling at the end of the production period

Final angle	Maximum angle 0-15°	16-30°	31-45°	46-60°	61-90°	91-120°	120°	Total
	Number of seedlings							
0- 15°	16	19	12	13	6	2	0	68
16- 30°		5	7	1	3	4	0	20
31- 45°			8	7	5	3	1	24
46- 60°				6	5	8	0	19
61- 90°					4	6	3	13
91-120°						8	3	11
>120°							5	5
Total	16	24	27	27	23	31	12	160

full light. Thus we had a 2 by 2 factorial with light level and root environment as the two factors. Seedlings were harvested after 24, 48 and 72 hours and the percentage of the total ^{14}C found in lignin was determined.

Results and Discussion — Seedlings placed in the aerobic root environment under full light partitioned a greater percentage of their ^{14}C to lignin than the other three treatments (low light - aerobic, full light - anaerobic, and low light - anaerobic) (Fig. 5). Carbon partitioning to lignin was increased 41% in relation to the other 3 treatments. This indicates that both high light levels and an aerobic root environment are important for increasing the lignin in black spruce seedlings and thus might be useful in reducing the occurrence of spiralization.

Effects of Short-Term Movements and the Direction of the Light Source on Seedling Development
(Universite du Quebec a Chicoutimi)

The following results come from two experiments, the first conducted during autumn 1987 - winter 1988 and the second conducted in winter 1989. The greenhouse was oriented east-west along its long axis. A central aisle separated the northern seedling beds, referred to as NORTH, from the southern seedling beds, referred to as SOUTH.

During the first experiment, the artificial light sources were high-pressure sodium *(HPS)* 400 W lamps suspended two at a time along the central aisle. The high light intensity plots had a photon flux density of 3500 lux (50 y mole of light m^{-2} s^{-1}) while the low light intensity plots received about 200 lux (3 y mole of light m^{-2} s^{-1}). Each main plot was divided into two subplots, one on the south side of the aisle and the other on the north side.

For the second experiment all the plots were placed on the south side of the central aisle and artificial lighting of low intensity (200 lux or 3 iimole of light $m^{-2}s^{-1}$) was furnished by fluorescent 40 W bulbs placed directly over the seedlings.

One-hundred and sixty black spruce seedlings were followed during the first experiment and 240 seedlings for the second experiment. The type, the angle and the orientation cf the defects of each seedling were measured every 2 to 3 days.

Results and Discussion — The summary of all the movements which were observed during the 1987-88 experiment are shown in Table 1. The majority of the spiralization movements for a seedling as well as fora population of seedlings are of weak intensity. It is the summation of the accumulation of small movements which finally results in a spiralized seedling. The majority (66.4%) of all movements did not reach 15° while 91.2% of the movements were less than 30°. Furthermore, bending movements were of greater amplitude than corrective movements. The average bending movement was 16.2° while the average corrective movement was 11.8°.

For the 1987-88 experiment, measures on a total of 160 seedlings indicated that there was a direct relationship between the maximum angle attained by a seedling at any given time during the production cycle and the probability that it would have a significant defect at the end of the production period (Table 2). Only 2 of the 43 seedlings which attained an angle of greater than 90° during the production period were able to correct themselves such that they were not defective at the end of production (189 days). Only 10 of these 43 seedlings corrected themselves to less than 45°. Of the 68 seedlings which were not defective (i.e. less than 15°) at the end of the production period, only 2 had obtained an angle of greater than 90° during their growth period. For the seedlings which did not surpass 30° during the production period, 87.5% were under the 15° threshold at the end of the production period (Table 2).

The seedlings had a tendancy to bend in the direction of the dominant light source, whether this was natural (the sun) or artificial. In the case of artificial lighting, the light intensities had to be more intense than those used just to prevent dormancy. Figure 6 represents the vectorial addition of all defects of all seedlings. The system of coordinates corresponds to the directions north, east, south and west. The longer the vector line, the greater the angle of the defect. The four treatments are shown; low light - north side, high light - north side, low light - south side, and high light - south side. It is clear that the defects are preferentially oriented towards the source of the dominant light source (Figure 6). Figure 7 represents a similar vector diagram for the 3 repetitions of the 1989 experiment where artificial lighting was of weak intensity. These seedlings all tended to bend in a southernly direction, i.e. towards the source of the dominant light source. Thus it appears that phototropism plays an important role in the bending movements of black spruce seedlings in the greenhouse environment. These movements which can result in a significant bending angle can also result in a permanent defect once the stem has lignified.

General Conclusions

1. The spiralization of black spruce seedlings appears to be caused by a reduction in the amount of carbon partitioned to lignin during seedling development.

2. The metabolic problem appears to be located in the roots although this does not mean that the aboveground environment cannot be indirectly affecting root metabolism.

3. High light levels and an aerobic root environment appear to promote the partitioning of carbon to lignin in seedling stems.

4. Seedlings make regular movements but these movements are usually of low amplitude. The sum of these small movements, however, can result in a spiralized seedling.

5. If a stem has a defect angle of greater than 90 ° at any given stage of development, these seedlings will most likely be permanently spiralized at greater than 15 ° at the end of the production cycle.

6. The movements of the stem are orientated toward the direction of the dominant light source.

7. High light levels located directly above seedlings together with an aerobic root environment should limit the spiralization of black spruce seedlings while still maintaining rapid growth rates.

Reference

BROUILLETTE, J.-G., J.-P. TETREAULT and J. LORTIE, 1987. *Guide d'êvaluation de la qualite des plants de reboisement produits en recipients.* Ministére de l'Energie et des Ressources, Service de la regeneration forestiere. 50 p.

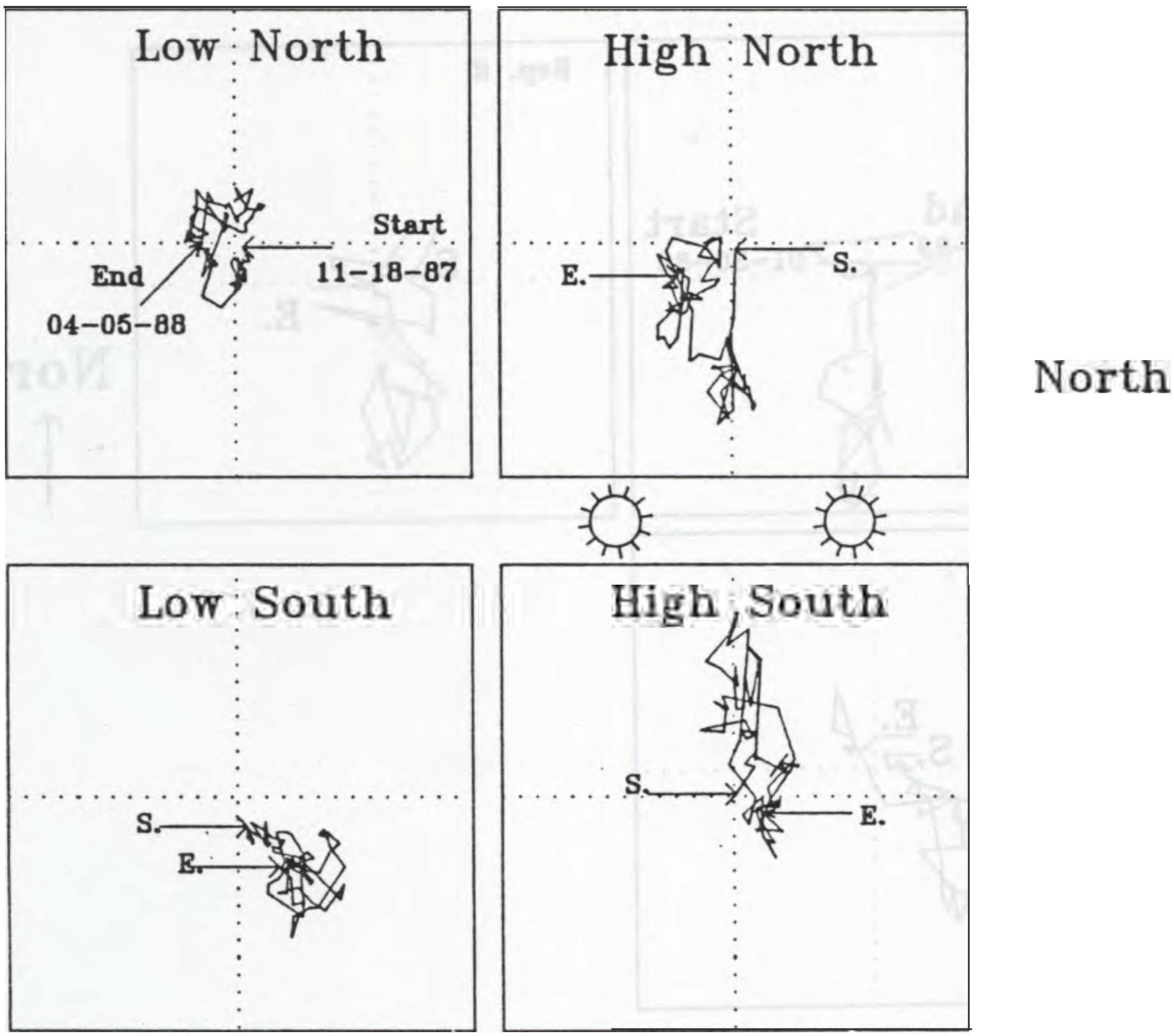


Figure 6. Vectorial addition of seedling bending defects by light treatment (high or low) and by position in the greenhouse (north or south side) for the 1987-88 experiment. The longer the line, the greater the sum of the bending angles. The direction of the line, on the other hand, indicates the orientation (i.e. north, south, etc.) of the movement.

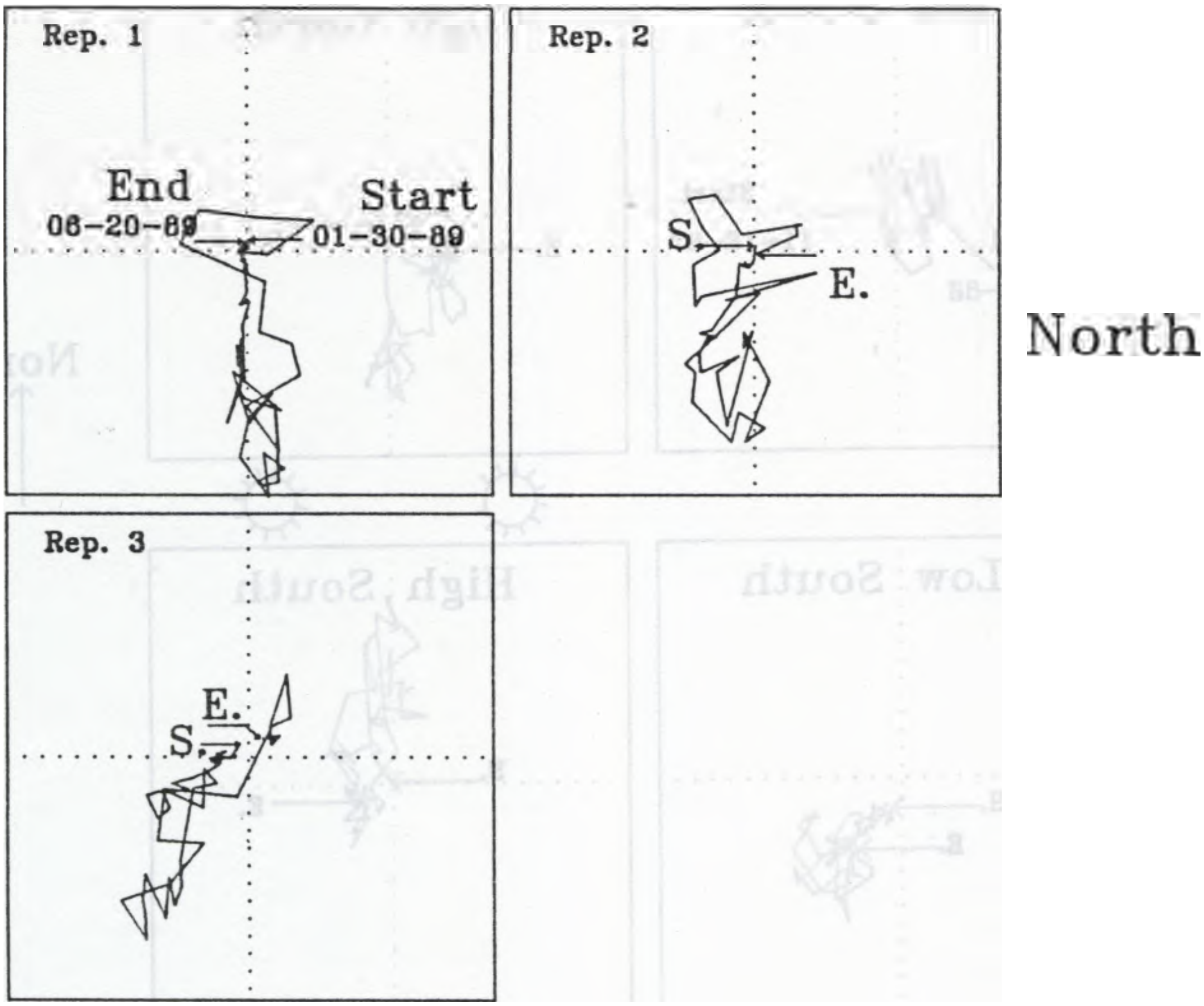


Figure 7 Vectorial addition of seedling bending defects for each replication of the 1989 experiment according to its position in the greenhouse (north or south side). No differences in artificial lighting were imposed. The longer the line, the greater the sum of the bending angles. The direction of the line, on the other hand, indicates the orientation (i.e. north, south, etc.) of the movement.