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# Cutting Propagation of Coniferous Forest Trees in Québec

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Mass cutting propagation of forest species is widely used in many different countries for reproducing elite material from the best controlled crosses. The production of coniferous plants from cuttings has continued to increase since 1989, when the Cutting Propagation Centre at the Pépinière forestière de Saint-Modeste (Saint-Modeste Forest Nursery) was established. White spruce (Picea glauca [Moench] Voss), black spruce (P. mariana [Mill.] B.S.P.), Norway spruce (P. abies [L.] Korst.) and hybrid larch (Larix × marschlinsii Coaz.) are now propagated at the centre using two unique and complementary systems (Bouturathèques and double-walled enclosures) developed by the Ministère des Ressources naturelles et de la Faune du Québec (MRNFQ). A collaboration between the researchers at the Direction de la recherche forestière (Forest Research Directorate) and practitioners at the Direction générale des pépinières et des stations piscicoles (Nurseries and Fish Farms Directorate), has led to refined cultural scenarios to meet the demands of each species (stock plant culture, rooting of cuttings and transplanting geared toward the production of large-sized plants). The integration of somatic embryogenesis, development of new production scenarios, and characterization of controlled crosses are presently receiving particular attention. In 2007, the provincial objective is to produce, via cutting propagation, 5.15 million of the 150 million conifer seedlings destined for reforestation in Québec.

### INTRODUCTION

For almost 20 years, cutting propagation has been used to produce reforestation stock at the operational scale in the province of Québec (Canada). Elite plant material, originating from conifer breeding programs, is selected for rapid growth and high wood quality. The cuttings are taken from stock plants grown from seeds of the best controlled crosses. In 1989, the Cutting Propagation Centre opened at the Pépinière forestière de Saint-Modeste, in the Lower Saint Lawrence region of the province of Québec, where a unique multiplication system, the Bouturathèque (Fig. 1a), was conceived and developed by the Ministère des Ressources naturelles et de la Faune du Québec (MRNFQ) (Vallée et Noreau, 1990). In 1998, an outdoor double-walled enclosure system (Fig. 1b) was developed to complement the Bouturathèques (Tousignant and Rioux, 2002, Tousignant et al., 2007). In 2007, the provincial objective is to produce, via cutting propagation, 5.15 million of the 150 million conifer seedlings destined for reforestation in Québec.



**Figure 1.** The two systems at the Cutting Propagation Centre at the Pépinière forestière de Saint-Modeste: (a) general view of one of the Bouturathèques; (b) outside view of one of the double-walled enclosures. (Photos M.-A. Grenier, MRNF and P. Lemay, Direction de la recherche forestière).

# BLACK SPRUCE AND BOUTURATHÈQUES

Bouturathèques are four-level mini-greenhouses used for rooting, each equipped with artificial lighting. They are located in climate-controlled rooms to maximize control of environmental conditions (Fig. 1a). Bouturathèques are described in detail by Vallée and Noreau (1990) and Tousignant et al. (1996). Their principal advantage is that they are very space efficient and permit year-round production. Rooting occurs independently of outside climate and at controlled conditions of relatively low light intensity (20 to  $25 \,\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> PAR at plant level, 20-h photoperiod at 20 °C). This is slightly above the light compensation point. According to Yue and

Margolis (1992), the light compensation point of black spruce softwood cuttings rooting in this system varies with temperature, from 5  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> at 5 °C to 27  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> at 30 °C. In comparison, the saturation point varies between 300 and 400  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>.

Although propagation of cuttings of forest conifers was practiced on a large scale elsewhere in the world (Ritchie, 1991), and rooting of the species of particular interest to us had already been studied (Armson et al., 1980; Kleinschmit, 1992; Mason, 1989; Tognoni et al., 1977), examples of closed-case rooting systems similar to the Bouturathèques were non-existent at the time. The absence of references forced us to conduct our own trials and adapt the available knowledge to meet our needs.

The first species propagated by the program was black spruce (*Picea mariana* [Mill.] B.S.P.). Stock plants are grown in greenhouses under artificial lighting and an 18-h photoperiod, which permits cuttings to be harvested year-round. Black spruce is well adapted to this type of continuous culture. Over a 2-year period, 12 cuttings per stock plant can be harvested every 10 weeks (up to five times a year).

Softwood black spruce cuttings are collected semi-lignified branches, once elongation has ceased and tissue water content (cutting fresh mass / cutting dry mass) has decreased to approximately 70% (Tousignant, 1995). Our research has permitted us to standardize the level of lignification by instituting an original type of short-day treatment. This has greatly improved the quality of the cuttings at the time of harvest. In effect, by reducing the photoperiod to which the stock plants are exposed (8 h of light for 9 consecutive days), approximately 2 weeks before harvesting, shoot growth is slowed and lignification is initiated. The system used to create total darkness is relatively simple. An opaque cover is rolled or unrolled, as necessary, over a portable support, from 4:00 pm to 8:00 am. Three to 6 days after the initial light conditions are re-established, the cuttings are harvested. The systematic pruning of the stock plants following cutting collection eliminates any residual effect of the short-day treatment and immediately induces growth of new lateral branches on the black spruce stock plants. However, repeated short day treatments contribute to aging of the stock plants, thus limiting their useful life to about 2 years.

Terminal shoots, 4 to 7 cm in length, are harvested with scissors. These cuttings are propagated in 45–110 multipot-type containers (45 cavities of 110 cm<sup>3</sup>). The substrate used is a mix of horticultural grade perlite and long-fibre blond peat moss (1 : 1, v/v). Three cuttings are inserted into each cavity (Tousignant and Rioux, 1996), in holes preformed by a template designed for the purpose. The bases of the cuttings are buried to a depth of 1.5 cm below the substrate surface. The work is done in an assembly line around a table equipped with superimposed conveyors, permitting a very satisfactory level of quality and yield be obtained.

A team of 28 people can handle approximately 100,000 cuttings per day. Usually, 1 million black spruce cuttings are collected and rooted during each harvesting period. The average yield per person is approximately 3,500 cuttings per work day (7.15 h), including collection, propagation, and installation in the Bouturathèques.

Our trials with black spruce have shown that the application of a hormone (a foliar spray of K-indolebutyric acid, K-IBA), can significantly increase the growth of the root mass and number of roots per cutting, though it has little effect on rooting percentage. A weak aqueous solution of K-IBA (4  $\mu$ g/cavity) is sprayed on the cuttings during the rooting period (2<sup>nd</sup> to 4<sup>th</sup> week after cutting harvest), using the Bouturathèque misting robot. This has proven to be an inexpensive application method.

# WHITE SPRUCE, NORWAY SPRUCE, HYBRID LARCH, AND DOUBLE-WALLED ENCLOSURES

Each species is different and requires a specially adapted cultural scenario. It is difficult to maintain continuous growth of white spruce (*P. glauca* [Moench] Voss) and Norway spruce (*P. abies* [L.] Korst.) under greenhouse conditions and these species are, therefore, less compatible with the Bouturathèque production system than is black spruce. Similarly, hybrid larch (*Larix* × *marschlinsii* Coaz.), with its long needles, requires lower cultural densities than do the spruces, which is easier to attain under outside conditions than in a Bouturathèque. Therefore, to optimize the use of propagation space, a double-walled enclosure system was installed in 1998 to complement the existing system (Tousignant et Rioux, 2002; Tousignant et al., 2007). The facilities are comparable to certain European models with traditional misting systems (Fig. 1b). Our work has focused on the optimisation and adaptation of stock plant production scenarios, the control of environmental variables during rooting and the cultural scenarios best adapted to the requirements of each individual species (container, substrate, irrigation, fertilisation, hardening, etc.).

White spruce and Norway spruce stock plants are seeded in containers and grown under tunnel or greenhouse conditions. After a year, they are transferred outside. Cuttings are harvested from actively growing stock plants in early July of their second growing season. The cuttings are lateral shoots with an optimal length of 5 to 7 cm and a precise degree of lignification (water content  $\leq 72$  %). The (2+0) stock plants are grown using the standard cultural techniques for containerized large-sized seedlings. After yielding an average of 13 cuttings, the seedlings used for stock plants are delivered to planting sites the following spring. The operational yield for cutting harvest, in terms of cuttings handled by each person per day, is approximately 4,800 to 6,500. The rigidity of the stems, as well as a different organisation of the work area, explains the superior yield with respect to that achieved for black spruce.

Hybrid larch stock plants are individually seeded and grown in a greenhouse during their first growing season. Under forced conditions, the stock plants begin their active growing season at the end of February of the following year, so that cuttings can be harvested in May, June, and, if necessary, July. The reproduction factor is of the order of 60-deliverable cuttings per stock plant. In general, the stock plants are kept for a maximum of 2 years.

White spruce, Norway spruce, and hybrid larch cuttings are grown in the same type of substrate and container as those used for black spruce. An automatic robot and misting jets humidify the air to avert water stress. The cuttings are then placed in the double-walled enclosures.

Even though it may appear to be relatively simple at first, the propagation of softwood spruce and hybrid larch cuttings is a complex and delicate operation. Its success necessitates the optimization and precise control of many environmental variables, notably light quality and intensity, temperature, relative humidity of the air, and vapour pressure deficit (VPD). The double-walled enclosures developed by the MRNFQ permit optimal rooting conditions to be maintained, thanks to the two white semi-transparent polyethylene covers that reduce light intensity, a central misting line that helps avoid high temperatures while maintaining relative humidity at close to saturation, and a misting robot that applies fine water droplets onto the needles of the cuttings. The rooting percentage of cuttings harvested from 1-year-old stock plants varies from 85% to 90%. Contrary to the propagation of black spruce, rooting hormones are not used for these species. For all conifer species propagated in Québec, cuttings are transplanted in the nursery to produce large-sized plants (Figs. 2a, 2b, 2c). The planting of large-sized seedlings (height  $\geq$ 35 cm) is currently used at an operational scale as one of the possible alternatives to the application of herbicides in Eastern Canada (Jobidon et al., 1998; Lamhamedi et al., 1998). Most of the cuttings are transplanted into containers with large volume cavities (> 300 cm<sup>3</sup>) (Fig. 2a). The remainder are transplanted as bareroot stock (Fig. 2b). In general, conifer cuttings are grown for 2 years before being delivered to reforestation sites. They are submitted to the same 25 quality standards as seedlings, in terms of size, morphology, root system quality, and foliar nutrient concentration (N  $\geq$  1.7%).







**Figure 2.** Nursery production areas for the cultivation of transplanted conifer cuttings at the Pépinière de Saint-Modeste: (a) container culture; (b) bareroot culture; (c) large-sized white spruce plants from cuttings, ready for delivery to reforestation. (Photos by D. Tousignant and N. Robert, Direction de la recherche forestière).

The demand for black spruce plants produced from cuttings has declined from 3 million in 2003 to about 500,000 for the 2009 planting season. In contrast, the demand for white spruce and hybrid larch, which are faster growing species, is rapidly increasing. Use of the Bouturathèques is less intensive than it once was, since three harvests per year are sufficient to meet production objectives. On the other hand,

in response to the increasing demand for white spruce and hybrid larch plants produced from cuttings, use of the double-walled enclosures has increased. In light of the results obtained at Pépinière Saint-Modeste and in response to this increasing demand, new propagation facilities (double-walled enclosures) were set up in 2006 at two provincial government forest nurseries, in Berthierville and Grande-Piles, for the propagation of white spruce cuttings.

# **FUTURE TRENDS**

The use of dormant cuttings is currently under study, and should result in a better use of our specialised work force by distributing the workload over different periods of the year. We also think that we can reduce the cultural period for certain types of production to 1 year. In addition, the use of Jiffy<sup>®</sup> pellets will permit us to mechanize the transplant into multipots for the rest of the growing cycle.

An important study is presently being conducted to characterize the white spruce full-sib families used for cutting propagation (Lamhamedi et al., 2007). These families all result from controlled crosses. We hope to better target the behaviour of the stock plants according to this multiplication method. The results of these studies should permit the optimization of the cutting propagation process, from seeding of the stock plants to the delivery of plants produced from cuttings.

The use of somatic embryogenesis to produce white spruce stock plants has been tested over the last few years and has proved successful (Lamhamedi et al. 2000, 2003; Tremblay and Lamhamedi, 2006). However, we currently only produce the plants necessary for clonal testing. In 3 to 5 years time, when the results of the testing currently being done by forest geneticists are known, we envisage producing stock plants by somatic embryogenesis. For this, the best clones will be chosen using selection criteria similar to those presently used for controlled crosses. This material is presently conserved in the cryoconservation facilities at Saint-Modeste. Our preliminary results showed that somatic white spruce and Norway spruce seedlings can be used as stock plants. We observed a great genetic variability in rooting ability among cuttings collected from different clones of Norway spruce produced by somatic embryogenesis. The presence of this variability indicates that the potential exists to select elite clones for practical applications in nursery cutting programs.

The results of the first somatic embryogenesis trials using hybrid larch were excellent. Adjusting the production scenarios should result in the use of hybrid larch stock plants produced by somatic embryogenesis in our laboratory at Saint-Modeste. Currently, the first clonal selections have been identified by forest geneticists. Additional recommendations will be made following the next clonal tests. A clone bank is conserved in the cryogenic facilities at Saint-Modeste. More than 5,000 stock plants are currently under production by somatic embryogenesis, representing 12 clones. Beginning in 2009, we plan to produce 25,000 stock plants per year. Once reproduced, these plants will enable us to produce 1 million plants per year from cuttings.

#### CONCLUSION

This short overview of the forest tree reproduction program established by MRNFQ, principally at the Pépinière forestière de Saint-Modeste, is a good illustration of the efforts put forward by the province of Québec to be at the forefront of wide-scale

reproduction of forest conifers. The technical, technological, and scientific aspects essential to the refinement of this program have necessitated much research in the domain of plant propagation. We will continue our research and trials in close collaboration with our colleagues from the Direction de la recherche forestière (MRNFQ). The improvement in the performance of our reproduction methods for forest conifers is the ultimate goal of our efforts.

# LITERATURE CITED

- Armson, K.A., M. Fung, and W.R. Bunting. 1980. Operational rooting of black spruce cuttings. J. For. 78 (June):341–343.
- Jobidon, R., L. Charette, and P.-Y. Bernier. 1998. Initial size and competing vegetation effects on water stress and growth of *Picea mariana* (Mill.) BSP seedlings planted in three different environements. For. Ecol. Mgt. 103:295–308.
- Lamhamedi, M.S., F. Colas, D. Tousignant, and M. Rioux. 2007. Characterization and multi-criteria selection of families for the mass cutting propagation of white spruce (*Picea glauca*) in Québec, p. 64. In: Beardmore, T. L. et J. D. Simpson (eds.). Recent advances in seed physiology and technology. Proceedings, IUFRO Tree Seed Symposium, meeting of IUFRO Research Group 2.09.00. Fredericton (Nouveau-Brunswick), July 18–20, 2006.
- Lamhamedi, M.S., H. Chamberland, and F.M. Tremblay. 2003. Epidermal transpiration, ultrastructural characteristics and net photosynthesis of white spruce somatic seedlings in response to in vitro acclimatization. Physiol. Plant.18:544–561.
- Lamhamedi, M.S., H. Chamberland, P.-Y. Bernier, and F.M. Tremblay. 2000. Clonal variation in morphology, growth, physiology, anatomy and ultrastructure of container-grown white spruce somatic seedlings. Tree Physiol. 20: 869–880.
- Lamhamedi, M.S., P.-Y. Bernier, C. Hébert, and R. Jobidon. 1998. Physiological and growth responses of three types of containerized *Picea mariana* seedlings outplanted with and without vegetation control. For. Ecol. Mgt. 110:13–23.
- Mason, W.L. 1989. Vegetative propagation of hybrid larch (*Larix × eurolepis* Henry) using winter cuttings. Forestry 62 (Suppl.):189–198.
- Ritchie, G.A. 1991. The commercial use of conifer rooted cuttings in forestry: A world overview. New For. 5:247–275.
- Tognoni, F., M. Kawase, and A. Alpi. 1977. Seasonal changes in rootability and rooting substances of Picea glauca cuttings. J. Amer. Soc. Hort. Sci. 102(6):718–720.
- Tousignant, D., and M. Rioux. 1996. Choosing the best container size and rooting substrate for bulk propagation of black spruce [Picea mariana (Mill.) B.S.P.] cuttings. 14<sup>th</sup> North American Forest Biology Workshop, Sainte-Foy (Québec), June 16–20:39.
- Tousignant, D., and M. Rioux. 2002. Le bouturage des résineux à la Pépinière de Saint-Modeste (Québec, Canada) : 10 ans de recherche, de développement et d'innovations, p. 65-86. In: Verger, M. (ed.). Multiplication végétative des ligneux forestiers, fruitiers et ornementaux. Proceedings [CD-ROM]. Montpellier, France : CIRAD-INRA, Troisième rencontre du groupe de la Sainte-Catherine, 22–24 Oct. 2000, Orléans, France.
- Tousignant, D. 1995. Relation entre la teneur en eau des boutures d'épinette noire et leur enracinement en bouturathèque. Ministère des Ressources naturelles, Direction de la recherche forestière. Note de recherche forestière 66:7. <a href="http://www.mrnf.gouv.gc.ca/publications/forets/connaissances/recherche/Tousignant-Denise/Note66.pdf">http://www.mrnf.gouv. gc.ca/publications/forets/connaissances/recherche/Tousignant-Denise/Note66.pdf</a>>
- Tousignant, D., M.S. Lamhamedi, F. Colas, M. Rioux, P. Lemay, and N. Robert. 2007. New technological developments in cutting propagation for increased forest productivity in Quebec. Ministère des ressources naturelles et de la faune, Carrefour de la recherche forestière, Québec, Québec, Canada. 19–20 Sept. 2007, 6 p. <a href="http://www.mrnf.gouv.qc.ca/publications/forets/connaissances/recherche/Tousignant-Denise/Carrefour-stand-anglais-1-6.pdf">http://www.mrnf.gouv.qc.ca/publications/forets/connaissances/recherche/Tousignant-Denise/Carrefour-stand-anglais-1-6.pdf</a>
- **Tousignant, D., P. Périnet,** and **M. Rioux.** 1996. Black spruce cutting propagation at the Pépinière de Saint-Modeste. Gouvernement du Québec, Ministère des Ressources naturelles. RN96-3004.

- Tremblay, L., and M.S. Lamhamedi. 2006. Embryogenèse somatique au ministère des Ressources naturelles et de la Faune du Québec : Du laboratoire au site de plantation. Des plants et des Hommes 9(3):6–11. <a href="http://www.mrnf.gouv.qc.ca/publications/forets/connaissances/recherche/Lamhamedi-Mohammed/DesPlants-des-Hommes-9–3–6–11.pdf">http://www.mrnf.gouv.qc.ca/publications/forets/connaissances/recherche/Lamhamedi-Mohammed/DesPlants-des-Hommes-9–3–6–11.pdf</a> >
- Vallée, G., and R. Noreau. 1990, La « bouturathèque » : système de bouturage compact hors serre. Ministère de l'Énergie et des Ressources, Direction de la recherche, Note de recherche forestière 41.
- Yue, D., and H.A. Margolis. 1992. Photosynthesis and dark respiration of black spruce cuttings during rooting in response to light and temperature. Can. J. For. Res. 23:1150–1155.