**University of Alberta**

# Impact of seedling characteristics, outplanting time, and container size on early establishment of aspen (*Populus tremuloides*) on reclamation sites

by

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# Dedication

To my mom Gloria Amanda and my brother Victor Leonardo who have always been there for me. To the loving memory of my father Victor Efren, whose dedication and life example has been and will be part of me through my life.

## Abstract

Aspen seedlings often suffer from transplant shock after outplanting. The influence of seedling characteristics and time of outplanting (spring, summer and fall) on field performance was examined on mining reclamation sites. Seedling characteristics were modified by inducing premature bud set using blackout (B), artificial growth retardants (H) and naturally reduced photoperiod (C). Some seedlings were also shortened by clipping their shoots (CL). Seedling characteristics were also manipulated by growing them in two container sizes and moving them outside the greenhouse at different times of the growing cycle. Results indicated that stock types with high root to shoot ratio (RSR) and root reserves (TNC) that were outplanted in the spring or fall had greater growth and reduced dieback. Seedlings moved earlier outside the greenhouse showed also better height growth, while container size had little effect, as long as RSR and root TNC were high.

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# List of Abbreviations

| | |
|---|---|
| ANOVA | Analysis of Variance |
| B | Blackout stock type |
| C | Conventional stock type |
| CDC | Crop Diversification Center |
| CL | Clipped stock type |
| H | Hormone stock type |
| LFH | Litter Fibric Humid (Surface mineral soil) |
| LSD | Least Significant Difference Test |
| RSR | Root to Shoot Ratio |
| TNC | Total Non-structural Carbohydrates |
| 615A | Container size with a volume of 340 $cm^3$ |
| 415D | Container size with a volume of 170 $cm^3$ |
| 5-20 | Stock moved outside at 5 cm and shoot growth terminated at 20 cm |
| 15-20 | Stock moved outside at 15 cm and shoot growth terminated at 20 cm |
| 35-35 | Stock moved outside at 35 cm and shoot growth terminated at 35 cm |

# Chapter 1. General Introduction

Anthropogenic activities such as extraction of natural resources by surface mining have impacted large areas all over the world. In order to access the resources in surface mining, the vegetation, soil and subsoil layers that overly the resource have to be removed. In Alberta two major resources are currently exploited using surface mining: coal and oil sands. Alberta possesses 70% of coal resources in Canada and about 50% of Alberta's land area contains coal formations (Government of Alberta - Environment, 2011) with most of the exploitation based on surface mining. Oil sands deposits comprise the third largest proven crude oil reserve in the world, with a surface mineable area of 4800 $Km^2$ (Government of Alberta - Oil sands, 2011). Both of these natural resources are extracted in Alberta on lands that are covered with boreal forest, wet lands, and/or small scale grasslands.

The boreal forest biome located in a northern circumpolar band extending across subarctic latitudes of Russia, Scandinavia and North America occupies 35% of Canada's total land area (Government of Canada, 2002). In Alberta, the boreal forest covers about 50% of the province (Government of Alberta - Tourism, parks and recreation, 2011) comprising pure stands of trembling aspen or mixed wood stands of deciduous trees such as trembling aspen, balsam poplar and paper birch mixed with conifer species such as white spruce, black spruce and balsam fir (Rowe, 1972).

In Alberta, mined areas are required to be reclaimed to fully self-sustaining ecosystems with a land capability similar to pre-disturbance conditions (Oil Sands Vegetation Reclamation Committee, 1998). Although there are no natural analogues, these highly disturbed sites roughly correspond to early stages of primary succession; therefore, if these sites are allowed to recover through natural regeneration processes this could be very slow. In order to speed up the re-establishment of forests on these sites, the early development of a tree canopy is crucial as it will exclude non-desirable early successional ruderal non-native and native species. Among them, bluejoint grass (C*alamagrostis canadensis* Michx), fireweed (*Epilobium angustifolium* L) (Landhäusser and Lieffers, 1998; Maundrel and Hawkins, 2004), yellow toadflax (*Linaria vulgaris* Mill.) (Sutton et al., 2007), canada thistle (*Cirsium arvens L.),* dandelion (*Taraxacum officinale* G. H. Webber ex Wiggers), ox-eye daisy (*Leucanthemum vulgare* L.) and persian darnel (*Lolium persicum)* (Cole et al., 2007). A quick developing canopy will help with soil development, especially the litter (LFH) layers and contribute to nutrient and carbon cycling in the developing soil (Klinka et al., 1990).

The history of reclamation in the oil sands region is relatively short. Reclamation started in the 1970s on the Suncor and Syncrude oil sands leases. Initially, reclamation involved the seeding of agronomic grasses and legumes such as awnless bromegrass (*Bromus inermis* Leyss), creeping red fescue (*Festuca rubra L.)*, and alfalfa (*Medicago sativa L.)* to control soil erosion. Later on, native and non-native tree and shrub species were added with varying success. At that time, the operators recognized that competition between agronomic species and trees played a strong role in tree performance and the use of perennial agronomic species was discontinued in forest land reclamation. Then, nursery crops such as barley (*Hordeum vulgare L)* and oat (*Avena sativa*) were added and showed good control of soil erosion without significant competing effects over tree species (Oil Sands Vegetation Reclamation Committee, 1998). In 1998, government regulations changed and required that only native species be used in forest reclamation (Oil Sands Vegetation Reclamation Committee, 1998). As a result tree species such as jack pine (*Pinus banksiana*), white spruce (*Picea glauca*) and aspen (*Populus tremuloides* Michx), and shrubs such as wild rose (*Rosa acicularis)*, raspberry (*Rubus strigosus)*, gooseberry (*Ribes setosum*), saskatoon (*Amelanchier alnifolia)* and chokecherry (*Prunus virginiana*) have been planted. Over time, reclamation success has varied and many sites seeded with the agronomic grasses and legumes are still dominated by those species. Therefore, in order to re-establish functional and diverse forest ecosystems, site capture and quick development of a tree-canopy is critical.

Aspen is an early successional, invasive and fast growing native species to North America. This tree is the most widely distributed species in North America, ranging from Newfoundland and Labrador to British Columbia in Canada, parts of United States and scattered locations in Mexico (Little, 1971). This tree species predominantly regenerates vegetatively through the production of root suckers (Perala, 1978) which develop from lateral root systems located 2-10 cm below the soil surface (Steneker, 1976: DeByle et al., 1985) after fire or harvest disturbance have killed the above ground portion of trees. Suckers are genetically identical to the parent root system (Steneker, 1976). Aspen also produces abundant light seeds and therefore creates opportunities for rapid long-distance dispersal through wind. Natural aspen seedling establishment requires mineral soil with no weed competition, moderate soil temperature especially in soils blackened after a fire disturbance and high and constant soil moisture for germination and growth in the first growing season (Maini and Cayford, 1968; Kay, 1993). Recent studies have suggested that aspen establishment from seeds is infrequent (Elliot and Baker, 2004; Romme et al., 2005). Even though natural regeneration through suckering or seeds is possible, it has limitations for forest land reclamation.

The production of aspen seedlings in nurseries is a reliable method to obtain large quantities of seedlings. Aspen can be grown as containerized and bareroot seedling stock. Bareroot planting stock is either seeded directly into nursery beds or it is seeded in containers, grown in the greenhouse for one growing season, stored frozen, then transplanted in nursery beds and grown for an additional year (plug+1) (Benson and Dubey, 1972; Williams and Hanks, 1994). Seedlings are mechanically lifted in the fall and the large root system and shoot are pruned and then stored frozen (-3°C) until outplanting the following spring.

In Alberta, the nursery production of aspen seedlings is dominated by container grown planting stock. Aspen seedlings are started in May by sowing the seeds into containers filled with peat-vermiculite growing media. These seedlings are grown for 10 to 11 weeks in greenhouses under controlled conditions, moved outside and grown for 6 weeks until reaching the desired root collar diameter , pruned to a height of 40 cm, lifted (removing seedlings including the growing medium from the container (Sutton and Tinus, 1983)), placed in plastic bags, boxed and either planted in late summer/early fall or stored frozen at -3°C during the winter for outplanting next spring.

After outplanting, aspen seedlings often suffer from transplant shock or planting check (van den Driessche et al., 2003; Martens et al., 2007). Planting check is defined as slow growth and survival of seedlings within the first three years after planting (Sutton and Tinus, 1983; Watson, 1986), thereby delaying establishment and site capture. Several experiments on aspen seedlings using different fertilization and irrigation regimes (van den Driessche et al., 2003) and others involving vegetation control (Reighard et al., 1985; Shepperd and Mata, 2005) have shown little initial height growth improvement after outplanting. This could indicate that the poor growth of the outplanted aspen seedlings might be related to morphological and physiological characteristics of the seedling stock rather than the planting site limitations alone.

Seedlings planted on reclamation sites have to cope with a wide variety of mechanical and environmental stresses (Wilson and Jacobs, 2006). Seedlings that establish more successfully in the field can be considered high quality seedlings (Mattsson, 1996). Seedling research frequently describes the form or structure of seedlings (morphological characteristics) (Thompson, 1985) by measuring height, diameter, root volume, fresh weight, bud size, first order lateral roots, root to shoot ratio (RSR) (Thompson, 1985; Lloret et al., 1999; Jacobs et al., 2004); however, these factors have not always been successfully linked to seedling field performance (Jacobs et al., 2005b).

Physiological characteristics can also be used to characterize seedling quality (Haase, 2008). Their measurement; however, can be laborious and requires expensive equipment, limiting their operational use (Mattsson, 1996). Some of these physiological characteristics include electrolyte leakage, enzymatic activity, water potential, water conductance, mineral nutrition, chlorophyll fluorescence and total non-structural carbohydrates (TNC) (Mattsson, 1996; Haase, 2008).

Most seedling quality research has focused on seedling morphology and physiology based on conifers; very little information is available for deciduous seedlings. Some of the information on deciduous broadleaf seedling quality that is available in the literature is on bareroot seedlings of species such as northern red oak (*Quercus rubra* L.), white oak (*Quercus alba* L.), black cherry (*Prunus serotina* Ehrh) and green ash (*Fraxinus pennsylvanica* Marsh) (DuPlissis et al., 2000; Jacobs et al., 2005a; Jacobs et al., 2005b). Only published work by van den Driessche et al., (2003) and Martens et al., (2007) provides more detailed information on seedling height, diameter, and TNC reserves for aspen seedling stock.

Martens et al., (2007) observed that naturally establishing aspen seedlings had higher TNC reserves and higher RSR in the first year after germination compared to nursery grown stock. One year after planting, these seedlings had much greater height growth, no shoot dieback and better survival than nursery stock. RSR measures the balance between the water absorbing area (roots) of the seedlings and the transpirational area (shoot) (Haase, 2008) while TNC reserves measure the concentration of sugars and starches in plant tissues.

Sugars are metabolically active carbohydrates involved in the biosynthetic and maintenance activities and starches are reserve carbohydrates used by seedlings. Starches can be converted to sugars when photosynthesis is not enough to supply energy for respiratory and growth needs (Marshall, 1985). High TNC reserves in seedlings before planting might therefore be a good indicator of potential for survival, establishment, and growth after outplanting (Davis and Jacobs, 2005). Aspen seedlings that had higher RSR and TNC performed better in field conditions and will most likely be better in capturing reclamation sites (Martens et al., 2007; Landhäusser unpublished); however this has not been tested.

Currently nurseries produce tall seedlings in large containers because large seedlings of conifers usually show higher growth potential than smaller seedlings (Alm, 1982; Thiffault, 2004). However, the tall seedlings produced under the conventional nursery settings also have low RSR (approximately 1). Previous research has shown that aspen seedlings can also successfully be grown in nursery settings to achieve much higher RSR (Landhäusser unpublished); however, these seedlings are much shorter and require no pruning. Currently minimum specification and targets of aspen nursery planting stock are not based on research conducted on aspen seedlings and it is important to relate seedlings characteristics to outplanting success. In addition, large containers are expensive, as they use significant more greenhouse space and resources when seedlings are grown and they are more expensive to plant. Therefore it would be interesting to determine if seedlings with high RSR and TNC can also be grown in smaller containers reducing the reforestation costs while not compromising seedling quality.

In the boreal climate, aspen seedlings are currently outplanted at three different times of the year in the field. As a result seedlings will be different in morphological and physiological conditions during these planting times, especially in shoot dormancy. Tree nurseries induce dormancy in aspen seedlings by inducing budset. This can be accomplished by increasing physiological stress through shortening of day length, cooler root temperatures (Landhäusser and Lieffers, 1998), reduction of N and other nutrients, and/or through the application of a shoot growth retardant hormone (e.g. paclobutrazol) (Landhäusser et al., unpublished).

Spring planted seedlings are fully dormant, planted when soil moisture conditions are favourable and have a full growing season for establishment. Summer planted seedlings are semi-hardened (budset of shoot) but outplanted with green leaves towards the end of summer. Fall planted seedlings are fully dormant but have not received all the chilling requirements during cold storage. Dormant seedlings are more resistant to stress during lifting, storage and planting than non-dormant seedlings (McKay, 1996). As a result, planting time plays a significant role in seedling outplanting performance. For example, studies in conifers (Western larch (*Larix occidentalis* Nutt.)) showed fall planted seedlings performed better than spring planted seedlings (Barber, 1989). In temperate hardwoods (*Fraxinus americana* L*., Juglans nigra* L*., Liriodendron tulipifera* L*., Prunus serotina* Ehrh*.,Quercus alba* L., *and Quercus rubra* L.) no clear difference in planting performance was found (Seifert et al., 2006); however, in aspen the effect of time of planting is unknown.

## 1.2 Overall thesis objectives

The aim of my research was to determine in a field experiment how planting time affects the establishment and growth of seedlings of *Populus tremuloides* (aspen) and how characteristics such as RSR and root TNC influence seedling outplanting performance. In a second study, I explored the response of size of aspen seedlings and the timing of when seedlings were exposed to outdoor conditions and shoot growth termination. In addition, I investigated whether desirable seedling characteristics such as high RSR and TNC concentrations can be achieved in seedlings grown in smaller containers and whether the outplanting performance is affected by container size.

## References

Alm, A.A. 1982. The status of container planting programs in the Northern United States. 2. Lake States-Minnesota, Wisconsin, Michigan. In: Scarratt, J.B.; Glerum, C.; Plexman, C.A., eds. Proceedings, Canadian Containerized Tree Seedling Symposium; 1981 September 14-16; Toronto, ON. COJFRC Symp. Proc. O-P-10. Sault Ste. Marie, ON: Canadian Forestry Service, Great Lakes Forest Research Centre, pp. 63-65.

Barber, H. 1989. Planting western larch: A comparison of stocktypes and season of planting in Northeast Washington. Tree Planter's Notes. 40, 20-24.

Benson, M.K., Dubey, D. 1972. Aspen seedling production in a commercial nursery. Genetics and Physiology Notes, Number 12, Institute of Paper, Appleton, Wisconsin. 7p.

Cole, D. E., King, J. R., Oyarzun, D. A., Dietzler, T. H.. McClay, A. S. 2007. Experiences with invasive plant management and ecology in Alberta. Can. J. Plant. Sci. 87, 1013–1022.

Davis, A., Jacobs, D. 2005. Quantifying root system quality of nursery seedlings and relationship to outplanting performance. New Forest, 30, 295–311.

DeByle, N. V., Winokur, R.P (eds), 1985. Aspen: ecology and management in the western United States. USDA Forest Service, General Technical Report RM-119. Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO. 283 p.

DuPlissis, J., Xiwei, Yin., Baughman, M.J. 2000. Effects of site preparation, seedling quality, and tree shelters on planted northern red oaks. Staff Paper Series No. 141 College of Natural Resources and Minnesota Agricultural Experiment Station. University of Minnesota St Paul, Minnesota, (accessed 05.16.2011 at http://www.forestry.umn.edu/prod/groups/cfans/@pub/@cfans/@forestry/documents/asset/cfans_asset_184452.pdf).

Ellitot, G.P., Baker, W.L. 2004. Quaking aspen (*Populus tremuloides* Michx) at treeline: A century of change in the San Juan Mountains, Colorado, USA. J. Biogeo. 31, 733-745.

Government of Alberta – Environment, 2011. Coal mines, (accessed 05.10.2011 at http://environment.alberta.ca/02251.html).
Government of Alberta-Oil Sands, 2011. The Facts, (accessed 05.10.2011 at http://oilsands.alberta.ca/FactSheets/The_Facts_v5_FINAL.pdf).

Government of Alberta - Tourism, parks and recreation, 2011. Alberta's Boreal Forest Natural Region, (accessed 19.05.2011 at http://www.tpr.alberta.ca/parks/heritageinfocentre/naturalregions/borealforest.aspx).

Government of Canada, 2002. The Atlas of Canada - 5th Edition, (accessed 07.08.2011 at http://atlas.gc.ca).

Haase, D.L. 2008. Understanding forest seedling quality: Measurements and interpretation. Tree Planter's Notes*.* 52, 24-30*.*

Jacobs, D.F., Wilson, B.C., Davis, A.S. 2004. Recent trends in hardwood seedling quality assessment. In: Riley, L. E.; Dumroese, R. K.; Landis, T. D., tech coords. National proceedings: Forest and Conservation Nursery Associations—2003; 2003 June 9–12; Coeur d'Alene, ID; and 2003 July 14– 17; Springfield, IL. Proc. RMRS-P-33. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, (accessed 05.16.2011 athttp://www.rngr.net/publications/proceedings/2003/PDF.2004-06-08.4952).

Jacobs, D.F, Gardiner, E.S; Salifu, K.F.; Overton, R.P; Hernandez, G., Corbin, M.E.; Wightman, K.E., Selif, M.F. 2005a. Seedling quality standards for bottomland hardwood afforestation in the lower Mississippi river alluvial valley: preliminary results. In: Dumroese, R. K.; Riley, L. E.; Landis, T. D., tech. coords. 2005. National proceedings: Forest and Conservation Nursery Associations—2004; 2004 July 12–15; Charleston, NC; and 2004 July 26–29; Medford, OR. Proc. RMRS-P-35. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, (accessed on 05.16.2011 at http://www.fs.fed.us/rm/pubs/rmrs_p035/rmrs_p035_009_016.pdf )

Jacobs, D.F., Salifu, K.F., Seifert, J.R. 2005b. Relative contribution of initial root and shoot morphology in predicting field performance of hardwood seedlings. New Forest. 30, 235–251.

Kay, C. 1993. Aspen seedlings in recently burned areas of Grand Teton and Yellowstone National Parks. Northw. Sci. 67, 94-104.

Klinka, K; Wang, Q., Carter, R.E. 1990. Relationships among humus forms, forest floor nutrient properties and understory vegetation. For. Sci. 36, 564-581.

Landhäusser, S.M and Lieffers, V.J. 1998. Growth of *Populus tremuloides* in association with *Calamagrostis canadensis*. Can. J. For. Res. 28, 396-401.

Little, E. L., Jr. 1971. Atlas of the United States trees. Volume 1. Conifers and important hardwoods. Misc. Publ. 1146. Washington, DC: U.S. Department of Agriculture, Forest Service. 320 p.

Lloret, F.; Casanovas, C.,Penuelas, J. 1999. Seedling survival of Mediterranean shrubland species in relation to root:shoot ratio, seed size and water and nitrogen use. Funct. Ecol. 13, 210-216.

Maini, J. S., Cayford J. H. (eds), 1968. Growth and utilization of poplars in Canada. Department of Forestry and Rural Development, Forestry Branch, Departmental Publication 1205. Ottawa, ON. 257 p.

Marshall, J.D. 1985. Carbohydrate status as a measure of seedling quality, in: Duryea, M. L. (ed).. Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Workshop held October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis, pp. 49-58.

Martens, L.A., Landhäusser, S.M., Lieffers, V.J. 2007. First-year growth response of cold-stored, nursery-grown aspen planting stock. New Forest. 33, 281–295.

Mattsson, A. 1996. Predicting field performance using seedling quality assessment. New Forest. 13, 223–248.

Maundrell, C., Hawkins, C. 2004. Use of an aspen overstory to control understory herbaceous species, Bluejoint grass (*Calamagrostis canadensis*), and Fireweed (*Epilobium angustifolium*). North . J. Appl. For. 21,74-79.

Mckay, H.M. 1996. A review of the effect of stresses between lifting and planting on nursery stock quality and performance. New Forest. 13, 363–393.

Oil Sands Vegetation Reclamation Committee, 1998. Guidelines for reclamation to forest vegetation in the Athabasca oil sands region. ISBN 0-7785-0411-5. 56 p.

Perala, D. A. 1978. Aspen, sucker production and growth from outplanted root cuttings. Research Note NC-241. North Central Forest Experiment Station. Forest Service –USDA. 4 p.

Reighard, G.L; Howe, G., Hanover, J.W. 1985. Effects of chemical weed control and seedling planting depth on survival and growth of aspen. Tree Planter's Notes. 36, 3-7.

Romme, W.H.; Turner, M.G.; Tuskan, G.A., Reed, R.A. 2005. Establishment, persistence and growth of aspen (*Populus tremuloides*) seedlings in Yellowstone National Park. Ecology. 86, 404-418.

Rowe, J.S. 1972. Forest regions of Canada. Publication No. 1300. Ottawa: Canadian Forestry Service, Department of the Environment. 172 p.

Seifert, J.R., Jacobs, D.F., Selig, M.F. 2006. Influence of seasonal planting date on field performance of six temperate deciduous forest tree species. For. Ecol. Manage. 223, 371-378.

Shepperd, W.D., Mata, S.A. 2005. Planting aspen to rehabilitate riparian areas: A pilot study. USDA Forest Service RMRS-RN-26. 5 p.

Steneker, G.A. 1976. Guide to silvicultural management of trembling aspen in the Prairie Provinces. North. For. Res. Cent., Edmonton, Alberta. Information Report NOR-X-164. 8 p.

Suttton, R.F., Tinus, R.W. 1983. Root and root system terminology. Supplement to Forest Science. Forest Science Monograph 24. 137 p.

Sutton, J.R., Stohlgren, T.J., Beck, K.G. 2007. Predicting yellow toadflax infestations in the flat tops wilderness of Colorado. Biol. Invasions. 9, 783–793.

Thiffault, N. 2004. Stock type in intensive silviculture: A (short) discussion about roots and size. Forest. Chron. 80, 463-468.

Thompson, B. E. 1985. Seedling morphological evaluation – What you can tell by looking. In: Duryea, M. L., ed. Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Oregon State University, Corvallis, pp 59-71.

Watson, G.W. 1986. Cultural practices influence root development for better transplant success. J. Environ. Hort. 4, 32–34.

van den Driessche, R; Rude, W., Martens, L. 2003. Effect of fertilization and irrigation on growth of aspen (*Populus tremuloides* Michx.) seedlings over three seasons. For. Ecol. Manage. 186, 381-389.

Williams, R.D., Hanks, S.H. 1994. Hardwood nursery guide. Agricultural Handbook Number 473, United States Department of Agriculture, Forest Service, Washington, D.C. 78 p.

Wilson, B.C., Jacobs, D.F. 2006. Quality assessment of temperate zone deciduous hardwood seedlings. New Forest. 31, 417–433.

# Chapter 2. Effect of stock type and planting time on field performance of aspen (*Populus tremuloides* Michx.) seedlings in reclamation areas

## 2.1 Introduction

Aspen (*Populus tremuloides* Michx) is a widely distributed tree species native to North America. Its natural range extends from Alaska in the west, across Canada into the north-eastern United States and in higher elevations in the south-western areas of the United States and northern Mexico (Fowells, 1965; Little, 1971). This species is considered relatively drought tolerant, fast growing and early successional, making it an ideal species for reclamation of disturbed sites. Currently in the boreal forest region of Canada, most aspen reclamation programs use nursery-grown seedlings. However, the outplanting success of aspen seedlings has been limited, because seedlings often suffer from transplant shock and have several years of slow growth after outplanting (Rietveld, 1989; Martens et al., 2007). Developing seedlings with characteristics appropriate for stressed conditions such as high root to shoot ratios and planting them at the most suitable time might improve the establishment success of seedlings in mine reclamation sites in the boreal forest.

In the past, seedling quality was assessed based upon height, shoot diameter, and terminal bud size (Sutton, 1979; Chavasse, 1980;

Thompson, 1985; Navarro Cerrillo et al., 2006); however, it has been recognized that these factors are not always associated with field performance (Jacobs et al., 2005). Other studies have shown a negative correlation between seedling initial height growth and field performance in northern red oak seedlings (*Quercus rubra* L) (Thompson and Schultz, 1995) as well as a negative relationship between survival and taller trees in sawtooth oak *(Quercus acutissima* Carruth) seedlings (Hashizume and Han, 1993). These studies confirm that a tall seedling (e.g. low root to shoot ratio) might not have the best outplanting success. Therefore, characteristics such as root to shoot ratio (RSR) and total non-structural carbohydrate (TNC) reserves might be more meaningful seedling quality characteristics but there is limited information on these for aspen seedling stock. Martens et al. (2007) observed that root systems of naturally regenerating aspen seedlings had very high TNC reserves after the first growing season and this was tightly coupled with high RSR. These natural seedlings grew much better in height in the following growing season compared to other planting stock produced by nurseries (Martens et al., 2007). It is known that conifer seedlings with high levels of TNC in roots were able to grow more new roots upon transplanting (Grossnickle, 2005). TNC reserves are also important for maintaining respiration from the time of lifting until restarting photosynthesis after outplanting (Marshall, 1985). Root to shoot ratio and TNC in aspen seedlings can be increased by terminating shoot elongation early during nursery production (Landhäusser et al., unpublished).

The selection of planting time is generally based upon local climatic conditions and/or operational constraints such as planter availability. Spring planting uses fully dormant seedlings that have been stored frozen over winter and are easy to handle. In the spring, site conditions are generally moist (shortly after snowmelt), and seedlings have a full growing season for growth and root system development before the following winter. Summer planting uses seedlings that have set a terminal bud and stopped height growth or are top-pruned. These seedlings are considered to be “hot” planted because they have green leaves at time of planting. This allows for expansion of the root system after outplanting, prior to shoot growth in the following spring (Taylor and Dumbroff, 1975; Good and Corell, 1982). Fall planting uses fully dormant seedlings and eliminates the need for prolonged frozen storage but seedlings do not have time to develop a root system and create contact with the surrounding soil. Different planting times have been investigated with conifers (Barber, 1989; Dierauf, 1989; Adams et al., 1991) and temperate hardwoods (Seifert et al., 2006), but effects of planting time in aspen have not been tested.

Generally nursery-grown aspen seedlings are sown in early May, grown over the summer, and depending on the planting time, shoot dormancy is initiated artificially through shortening of day length (use of blackout cloths) for the summer plant or naturally through shortened day length and cooler nights in the fall; these seedlings can be used for a late fall planting or a spring planting the following year. For the spring planting, the fall-lifted seedlings are stored frozen over winter at -3°C for up to seven months. As an alternative method, shoot dormancy can be induced through the application of a growth retardant hormone to nursery seedling stock (Landhäusser et al., unpublished).

The objectives of this study were to determine the effect of planting time and stock types on establishment and growth of aspen seedlings and how seedling characteristics such as root to shoot ratio and root reserves influence outplanting performance on reclaimed open-pit mining sites.

## 2.2 Materials and methods

### 2.2.1 Planting stock production

The aspen seed used in this study was collected from two open pollinated seed sources, Edmonton (53°34′N 113°31′ W, 668 m asl) and Fort McMurray (56°43′ N 111°22 W, 370 m asl). Seeds were sown mid-May into 615A Styroblock ™ (Beaver Plastics Ltd, Acheson, Alberta) containers. Each seedling cavity had a volume of 340 $cm^3$ (6 cm in diameter and 15 cm deep). Seedlings were grown in a mixture of peat and vermiculite (9 to 1 by volume). Seedlings were germinated under greenhouse conditions at a commercial tree nursery (Smoky Lake

Forest Nursery, Smoky Lake; Alberta 54° 6' N: 112° 28' W, elevation 598 m asl). During germination the greenhouse had a mean temperature of 21°C, with a minimum of 18°C and maximum of 28°C; relative humidity was maintained at greater than 70%. After seeding, styroblocks were irrigated using multiple mists per day for 4 weeks. Fertilization began 4 weeks after seeding using a solution of 83 ppm of N, 76 ppm of P, 160 ppm of K, and chelated micronutrients; nutrients were applied with every watering. Seedlings were moved to outside conditions after 8 or 10 weeks depending on the stock types produced (Table 2-1). Once outside, the fertilization regime was changed to 54 ppm of N and 95 ppm of K, but P remained at 76 ppm; this was done to limit height growth. Seedlings continued to be fertilized with every watering cycle over the next 12 weeks.

The study was also repeated in time, with planting stock being produced in 2008 and in 2009. In 2008, both the Edmonton and Fort McMurray seed sources were used. In 2009 only the Fort McMurray seed source was used because the Edmonton seed showed poor germination success. Approximately 3200 seedlings were grown each year. Prior to moving seedlings outside, styroblocks were assigned to three different treatments to create the three different stock types with different root to shoot ratios and root TNC reserves. The three stock types were named after the treatments used to terminate shoot growth: blackout (B), hormone (H), and no treatment to terminate shoot growth prematurely, herein called conventional (C). Treatments were applied on the dates shown in Table 2-1. Only in 2009 we added another stock type during the summer plant; this stock is currently produced as summer stock at nurseries. This stock type was grown under conventional conditions but the terminal shoot was clipped (CL), to reduce total height, at the end of the growing cycle prior to shipping.

For the B stock type, budset was induced after 8 weeks of growth. These seedlings were moved outside and subjected to an artificial shortening of day length for 7 consecutive days, by covering them with a black plastic tarp for a portion of the day to shorten the photoperiod to 8 hours from the ambient 17 hours. The same treatment was repeated again two weeks later. In the hormone (H) stock type, premature budset was induced after seedlings had been moved outside after 8 weeks of growth in the greenhouse. Seedlings were treated with the plant growth regulator paclobutrazol (Bonzi®, Syngenta, North Carolina, USA). Paclobutrazol is absorbed by roots and shoots and inhibits gibberellin biosynthesis (Hedden and Graebet, 1985) reducing internode expansion and apical dominance. This growth regulator was applied to roots by soaking the styroblocks in a water bath with a concentration of 5 ml of Bonzi per L of water (0.02 g of paclobutrazol / Liter of water).

For the C stock type, seedlings were moved outside from the greenhouse after 11 weeks and grown until they hardened off. The CL stock type was sown in April of 2009, grown for 12-13 weeks in the greenhouse then moved outside and grown for 5 more weeks. Then seedlings were pruned to a height of 40 cm for shipping and outplanting in summer of 2009.

### 2.2.2 Planting time

After moving all stock types to outside conditions, about 1000 (a third) seedlings were lifted after 13 to 15 weeks of growth for the summer outplanting treatment in the third week of August. The remaining seedlings stayed outside and were allowed to set bud naturally. Another third of the seedlings were lifted at the end of September for the fall outplanting treatment. The remaining third of seedlings were lifted in November and stored frozen at -3 °C until the following spring for the spring outplanting treatment. A more detailed description of the timeline of stock type production and application of treatments is provided in Table 2-1.

### 2.2.3 Planting areas

Seedlings were outplanted in three different areas in central and northern Alberta, Canada. One area was located at the Genesee coal mine near Warburg, Alberta (53°10′ N, 114°19′ W, 820 m asl) and the other two on reclamation sites on the Suncor and the Syncrude oil sands mining leases close to Fort McMurray, Alberta (56°43′ N 111°22 W, 370 m asl). Climatic data for the duration of the study were obtained from the West Central Airshed Society for the Genesee planting area and for the two planting areas in Fort McMurray, from a meteorological station on the Suncor lease located close to both reclamation sites. Precipitation and mean air temperature in winter and summer are reported for Genesee and Fort McMurray in 2008, 2009 and 2010 in Table 2-2.

The reclamation prescription and soil placement were different for the three planting areas. At Genesee coal mine 1 m of subsoil was placed over sodic spoils and then capped with 20 cm of salvaged topsoil. The site had been used as an agricultural reclamation area with alfalfa grown for three years. Prior to planting the sites, the alfalfa was killed using herbicide (Glyphosate, Roundup, Monsanto, St Louis - Missouri, USA) and then the site was rototilled. After planting of the aspen seedlings, perforated plastic mulch (90 x 90 cm) (Arbortec Industries Ltd, Mission-British Columbia, Canada) was placed around each seedling to suppress the establishment of noxious weeds that are common in this agricultural area.

At the Suncor planting area, 1 m of subsoil was placed over saline sodic overburden and this was then capped with a 30 cm layer of peat-mineral mix (70% muskeg – 30% mineral). This site was prepared for reclamation in 2007. Syncrude planting area was reclaimed placing 50 cm of peat – mineral mix over overburden. No plastic mulch was used around the seedlings as the spread of agronomic weeds in these boreal forest sites was not an issue.

In each planting year (2008 and 2009) seedlings were lifted and planted at three different planting times: summer, fall and spring (Table 2-3). Summer and fall planted seedlings required no storage and were lifted from the styroblocks a few days prior to planting. Seedlings planted in the spring of 2009 and 2010 were lifted in late November and stored frozen for about 5 months at -3°C inside waxed cardboard boxes. Seedlings were planted by hand at a spacing of 0.85 × 0.85 m at Genesee and 1 × 1 m at Fort McMurray.

For each planting year (2008, 2009) a separate site was selected at each of the three planting areas (Genesee, Suncor and Syncrude). Each planting site for a particular year was approximately 1000 $m^2$. Sites were divided into 72 plots randomly assigned to 9 treatment combinations, three planting times (summer, fall, spring) and three stock types (B, H, C) and replicated 8 times. Each plot consisted of 16 seedlings (subsamples) of the same treatment combination; thus making the plot the experimental unit in this study. In 2008 the total number of planted seedlings was 1152 seedlings per site. The general setup and design was the same for both planting years; however, the clipped stock type (CL) was added for the summer 2009 planting time only; this added 8 extra plots for a total of 80 randomly assigned experimental plots in the 2009 planting year for both planting areas. The design of the study was completely randomized. All sites were surrounded by a buffer row of aspen seedlings.

In all planting areas and sites, 12 soil samples each were taken from the top 20 cm of soil. Soil texture was estimated by the particle size method using a graduate cylinder and hydrometer (Carter and Gregorich, 2008). Soil texture at the Genesee coal mine was classified as a silty clay loam with 28% clay, 52.5% silt and 19.5% sand. At Suncor the soil texture was classified as a sandy clay loam with 25% clay, 27% silt and 48% sand. At Syncrude the soil texture was classified as a sandy loam with 11% clay, 9% silt and 80% sand. Soils were analysed for $K^+$, $Na^+$, $Mg^{2+}$ and $Ca^{2+}$ concentrations using the 1M $NH_4OAc$ method (Page, 1982), for $NO_3^-$ and $NH_4^+$ using the 2N KCl method (Jones, 2001), and for $PO_4^{3-}$ with the Kelowna method (Carter and Gregorich, 2008). Total N and total P were analysed with the Kjeldahl digestion method (Carter and Gregorich, 2008). Summary data are provided in Table 2-4.

### 2.2.4 Seedling measurements

Prior to planting, 10 seedlings of each stock type were randomly selected for destructive sampling of pre-planting conditions. After measuring height and diameter, roots were carefully washed to remove the substrate. Root volume was measured using the water displacement method (Harrington, 1994). Shoots, roots, and leaves (only for the summer stock) were separated and dried at 70°C for 2 to 3 days and their dry mass determined. Water soluble sugars and starch in root and shoot tissues were determined from dry tissue samples ground to 40-mesh with a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA). Water soluble sugars were extracted using hot ethanol (80%) and concentrations measured colorimetrically using the phenolsulfuric acid method. Remaining starch in residues were solubilised with sodium hydroxide and hydrolyzed using an enzyme mixture of α-amylase (ICN 190151, from *Bacillus licheniformis*) and amyloglucosidase (Sigma A3514, from *Aspergillus niger*) and then measured colorimetrically using peroxidase glucose-o-dianisidine solution (Sigma Glucose Diagnostic Kit 510A) (Chow and Landhäusser, 2004).

After planting, initial height (height at time of planting) was measured on all planted seedlings in the spring of 2009 and 2010. In the spring of 2009, after the first winter and prior to the first full growing season, two seedlings of each stock type planted in summer and fall of the previous year were excavated to determine whether root growth had occurred during the previous partial growing season. No root growth occurred in the fall planted seedlings, while some minor root growth occurred in the summer planted seedlings. However the root growth was not large enough to significantly affect  root mass after the first full growing season (see below). At the end of each first full growing season

(August 2009 and 2010), total height (from ground level to top of terminal bud) was measured. Seedlings planted in 2008 were re-measured in 2010 at the end of the second full growing season. Shoot growth was determined by measuring the length of the longest shoot. To determine shoot dieback, the distance from the ground to the top shoot with leaves was measured and subtracted from the initial height measurement. All planted seedlings were measured for initial height, total height, shoot growth and shoot dieback. Mortality was calculated as the ratio of the number of trees dead per stock type after the 2009 and 2010 growing seasons divided by the total number of trees planted of each stock type and expressed as a percentage.

To measure more detailed information for both planting years, two seedlings of the 16 seedlings planted in each plot were excavated after the first growing season, for a total of 16 seedlings for each treatment combination. Diameter, root growth, and leaf dry mass were measured. Diameter growth and root growth were determined by subtracting the diameter and root mass at the end of the growing season from the average initial diameter and root mass of the 10 seedlings determined from seedlings measured prior to planting. Leaf dry mass, was only measured in the 2009 planting year. Leaves were collected in late summer, dried at 70°C for 2 to 3 days and their dry mass determined.

### 2.2.5 Experimental design and Data analysis

The study was designed as a completely randomized design, with three stock types (B, H, C) and three planting times (spring, summer, fall), with 8 replicates each. The study was repeated over two consecutive years, 2008 and 2009 (planting years), in three different planting areas (Genesee, Suncor and Syncrude). In the 2009 planting year and only during the summer planting time a fourth stock type (CL) was added for comparative reasons only.

The effect of stock types and planting times on the growth variables measured was tested by analysis of variance (ANOVA) using the GLM procedure of SAS (SAS 9.2, SAS Institute, Cary, NC). Prior to analyses, the variables were examined for normality (Shapiro-Wilk test) and homogeneity of variances (Levene test). Planting areas (Genesee, Suncor, Syncrude) and planting years (2008, 2009) were analyzed separately. The outplanting performance and responses of seedlings planted at Suncor and Syncrude planting areas were very similar, therefore only Suncor data is presented but we considered these responses representative of both reclamation sites in Fort McMurray. The data on outplanting performance of seedlings at Syncrude is summarized and attached in appendix A and B.

Variables not conforming to normality or homogeneity of variances were transformed. At Genesee, for the 2008 planting site, root growth was transformed with $Log_{10}$ and shoot dieback was transformed with

$Log_{10}(x+1)$ and root growth with square root. At Suncor 2008 and 2009 planting sites, no variables were transformed. For data presentation in graphs, non-transformed means were used. Simple linear regression was used to explore the impact of RSR and root TNC on the outplanting performance of seedling in the different planting areas.

A significance level of $\alpha=0.05$ was used for all analyses. When significant treatment effects were detected, differences among means were determined by the multiple comparison LSD test.

## 2.3 Results

### 2.3.1 Stock type characteristics prior to planting

Overall, the conventional stock type (C) was 45 cm tall – twice as tall as seedlings of the blackout (B) and hormone (H) stock type (22 cm). The H stock type tended to have the highest root volumes while stock types planted in summer tended to have the lowest root volumes. The H stock type overall had the highest root to shoot ratio (RSR) with 3.6, followed by the B stock type with 2.6 and the C stock type with a RSR of 1 ($P<0.001$). The H stock type also had the highest root TNC concentrations compared to the B and C stock types, while shoot TNC concentrations showed little difference among stock types. Regardless of stock type, TNC in shoots was highest in the fall planting time (mean of 18.9% for B, H and C), lower in the spring planting time (16.7%) and most variable in the summer planting time with 15.5% for B and 16.7%

for H and only 8.4% for C stock type. Generally TNC reserves in roots were about two times higher than in shoots. Shoot TNC reserves were lower in the summer planting time. This was driven by very low shoot TNC reserves in C (8.4%). Overall, shoot TNC reserves were not that different among planting times. Root TNC in spring and fall was high among all stock types (~38%). On the other hand, summer-planted seedlings had lowest root TNC reserves, particularly in the C stock type (14.5%) (Table 2-5). Overall, the differences between stock types and planting times for seedlings grown in the 2008 and 2009 planting year were very similar, therefore only 2008 pre-planting characteristics were shown. For the summer planting time in 2009, the clipped CL stock type closely resembled the C stock type.

### 2.3.2 Planting year of 2008

For seedlings planted in 2008, seedling mortality after two growing seasons was less than 3% by the end of 2010 and was not different between stock types, planting times and the planting areas of Genesee and Fort McMurray (data now shown). After the first growing season at Genesee, differences in height growth were relatively small; however the C stock only grew 27 cm in height when planted in the spring while the other stock types across the three planting times grew 44 cm on average (Figure 2-1 A). This resulted in a significant statistical interaction between planting time and stock type (P=0.003); however, this difference was not noticeable anymore after the second growing season as there were no significant differences in height growth among the different stock types (P=0.793). In the second growing season all seedlings, regardless of planting time and stock type, grew approximately 104 cm. There were no differences in diameter growth between the stock types and planting times in either growing season (Figure 2-1 B). After the first winter, the C stock type planted either in the summer or the fall had about 7 times greater shoot dieback than the other stock types; however, dieback was not different from the other stock types when the C stock type was planted in the spring (Figure 2-1 C). This resulted in a significant interaction between planting time and stock type (P=0.001). Root growth after the first growing season was not affected by planting time (P=0.345) or stock type (P=0.104) (Figure 2-1 D).

At Fort McMurray, seedlings of the H stock type grew more in height than the other stock types with an average 16.3 cm, followed by B with 11.8 cm and C with 6.7 cm (P=0.001). Height growth of fall and spring planted seedlings was very similar, with 12.8 cm and 13.2 cm respectively, while height growth in summer planting was lowest with 8.9 cm (P=0.001). Lowest height growth in the summer planting time was mainly driven by the height growth of the C stock type with only 3.7 cm (Figure 2-2 A). Differences in growth due to stock type (P=0.029) and planting time (P=0.039) were still observed after the second growing season. In the 2009 year, height growth of the H seedlings was 13.8 cm followed by B with 11.9 cm and C with 11.2 cm. Among planting times, height growth in fall planted seedlings was 13.8 cm, spring was 12.6 cm and summer was 10.5 cm.  The C stock type had the lowest height growth with 7.9 cm when planted in the summer. There were no differences in diameter growth among stock types when seedlings were planted in spring (average of 1.8 mm); in both the summer and fall plantings, however, the H stock diameter growth was better than in the other stock types, resulting in a significant interaction between planting time and stock types (P=0.028) (Figure 2-2 B). This interaction was no longer evident after the second growing season (P=0.344). The C stock type planted in the summer or fall had more shoot dieback (0.5 cm) than the B and H stock types (0.2 cm on average) (P=0.003); however, in general it was was low (Figure 2-2 C). Overall, there were no differences in root growth as a result of stock types (P=0.751) and planting times (P=0.327) (Figure 2-2 D); however, root growth of the H stock type planted in spring was lowest with 1.2 g.

**2.3.3 Planting year of 2009**

Seedlings planted in summer and fall of 2009 and spring of 2010 also had low mortality (<3%) after the first growing season. By the end of 2010 there were no differences among stocks types, planting times, and the planting areas of Genesee and Fort McMurray (data now shown). Although not tested, the CL stock type had a mortality rate of 7%. The H stock type had the greatest height growth; overall, height growth of the H stock type was 53.8 cm, 46.0 cm for B stock and 32.9 cm for C stock (P<0.001) (Figure 2-3 A) while the CL stock had slightly higher growth than the C stock type. The C stock type had the lowest diameter growth among stock types (P=0.016) but this was especially true when planted in summer (5.6 mm) and spring (6.2 mm). The diameter growth of the C stock type planted in the fall was not different from other stock types that were planted in the same time (7.8 mm); this resulted in a significant interaction between planting time and stock type (P=0.011) (Figure 2-3 B). Shoot dieback was highest in the C stock type with 3.1 cm compared to the other two stock types (P=0.001). The clipped (CL) stock type, however, had much higher dieback (14.2 cm) (Figure 2-3 C). Overall, the three stock types planted in the fall had similar dieback (0.8 cm), while the C stock type had higher dieback in summer (5.1 cm) and spring (3.0 cm) resulting in a significant interaction between planting time and stock type (P=0.005) (Figure 2-3 C). Root growth was larger in the fall planting time (9.3 g), followed by spring (8.4 g) and was lowest in the summer planting time (6.3 g) (P=0.034). Among stock types, C stock type had less root growth (5.9 g) compared to the H and B stock types which averaged 9.1 g (P=0.007) (Figure 2-3 D). H and B stock types carried 35% more leaf dry weight than the C stock type (5.2 g) (P=0.003) (Figure 2-3 E).

At Fort McMurray, seedlings of the H stock type had greatest height growth (15.4 cm), followed by the B stock type (8.2 cm) and the C stock type (5 cm) (P=0.001). However, when planted in the summer the B stock type had the lowest height growth (4.9 cm) resulting in a significant interaction between stock type and planting time (P=0.002) (Figure 2-4 A). Overall, diameter growth was highest in seedlings planted in the fall (1.8 mm) (P=0.001) compared to 1.1 mm for the seedlings planted in the summer and spring. The H stock type grew 1.43 mm in diameter, compared to 1.37 mm for C and 1.11 mm for B (Figure 2-4 B) (P=0.038). Much of this difference in growth in the H and C stock types was driven by seedlings planted in the fall. Shoot dieback of H stock type (0.4 cm) was the lowest among all of the stock types (P=0.001) (Figure 2-4 C), while the CL stock type had much higher dieback (6.1 cm). There was a tendency for increased root growth of the B and C stock types especially when planted in the fall with 1.71 and 1.73 g respectively (Figure 2-4 D). Leaf dry weight was highest in seedlings that had been planted in the fall (1.20 g), followed by spring (1.12 g) and summer (0.67 g) (P=0.001) (Figure 2-4 E). At Fort McMurray, the field performance of CL stock type was the poorest among all stock types for the five response variables reported, with height growth of 1.7 cm, diameter growth of 0.4 mm, shoot dieback of 6.1 cm, root growth of 0.6 g and leaf dry weight of 0.4 g (Figure 2-4).

## 2.4. Discussion

Seedlings planted in either fall or spring performed better than seedlings planted in the summer. All stock types, when planted in summer, had greater amounts of shoot dieback and performed poorest compared to the other planting times. Summer seedlings were planted the year before the next full growing season; this did give them an opportunity for root growth prior to onset of winter. Summer planting, however, still had overall lower planting performance than planting in the fall or spring. This poor performance could be the result of lower moisture availability and higher soil/air temperatures during the summer months when seedlings were planted. At this time the seedlings had green leaves, which could have made them more prone to dessication and water stress after outplanting, likely reducing photosynthesis and the ability to accumulate root reserves for the following growing season (Rietveld, 1989; Martens et al., 2007). This is crucial in a time when roots need to adapt to the soil environment of a transplant site. Stock types with higher RSR and root TNC reserves such as B and H performed much better than the C and CL stocktypes that had lower RSR and root TNC reserves. The H stock type, although much shorter in stature at the time of planting, showed consistently higher height and diameter growth among the three stock types regardless of planting year and time. The results, however, were most striking on the Fort McMurray sites.

Overall, the B stock type had the second best growth performance; however, RSR and root TNC concentrations were lower than in the H stock type. This could perhaps be attributed to the lower efficacy and uniformity of shoot growth termination using a blackout cloth compared with the use of hormonal growth retardants. Our hormone-treated aspen seedlings did not show growth inhibition after outplanting, which has been observed for jack pine (*Pinus banksiana* Lamb), red pine (*Pinus resinosa* Ait.) and eastern larch (*Larix laricina* (Du Roi) K. Koch) bareroot nursery seedlings after treating with paclobutrazol (Rietveld, 1988). This could perhaps be the result of comparing gymnosperms with angiosperms or it could be the result of the dose concentration; Rietveld treated his seedlings with a higher concentration of paclobutrazol (4g /L). The H seedlings likely performed better because of their higher capacity to take up water (larger root volume) and their high TNC reserves, which have been found to be important in newly establishing seedlings (Wilson and Jacobs, 2006; Farmer 1978) as an energy source between planting and restart of photosynthesis (Marshall, 1985; Carlson and Miller, 1990; Landhäusser and Lieffers, 2002). Further, the smaller H and B seedling could potentially take advantage of its shorter shoot size to cope with the greater transpirational demands imposed on the root system due to proportional higher root mass (Ritchie, 1984).

Overall the CL and C seedlings had more shoot dieback compared to the H and B stock types. In particular the CL stock type seedlings had significant dieback in the shoot prior to leaf flush, which led to reduced height growth in the first summer after planting. This result is somewhat surprising because previous studies on aspen have reported that cutting back aspen seedlings at time of planting did not affect planting success or height growth, at least on bare-root seedlings (Borset, 1960). A study done in water oak (*Quercus nigra* L.) actually showed a 52% increase in height growth after the first 2 years on clipped seedlings compared to unclipped seedlings (Adams, 1985). Poor performance and higher shoot dieback in the CL and C aspen seedlings in our study could be explained by their relatively low TNC reserve concentrations which potentially led to reduced root and shoot growth which in turn could lead to water stress and reduced photosynthesis (Carlson and Miller, 1990). TNC reserves are especially critical in shoot regrowth after pruning (Kozlowski, 1991). The lifting and planting time of the summer stock coincides with a phenological stage of low carbohydrate storage (Table 2-5; Kozlowski and Pallardy, 2002). Overall, low TNC concentrations appear to make the stock types planted in summer more susceptible to planting stress than either the fall or spring planted stock.

Outplanting performance of stock types also was different depending on planting area. Seedlings planted at Genesee grew 3 to 4 times more in height than at Fort McMurray. The Genesee site likely had better seedling growth because of soil nutrient conditions and the use ofplastic mulch.  Some notable differences in the soil nutrients at the sites was that Genesee had double the $PO_4^{3-}$ and 44 times the $NO_{3-}$ levels of the Suncor site (Table 2-4); both of these nutrients are important for aspen growth (Strong and La Roi, 1985; van den Driessche et al., 2003; Vitousek and Howarth, 1991). Also, this planting area had been used for alfalfa production for several years. The plastic mulch probably reduced water evaporation (Allen et al., 1998; Mamkagh, 2009) and increased soil temperature, which might have amplified N mineralization (Truax and Gagnon, 1992). The greater percentage of sand in the soil at both Fort McMurray sites was outside of the optimum range of the textural class for aspen (Steneker, 1976), which may have negatively affected growth; however, even with these site conditions, H seedlings grew much better than other stock types, particularly the C stock type.

As can be expected overall growth performance was not the same between the two planting years. Differences were particularly evident at Genesee where average diameter growth was 12 mm in the first planting year and 7 mm in the second planting year. The high precipitation (Table 2-2) and accumulation of water beneath the plastic mulch in the 2010 growing season could have caused stressful conditions for seedling growth. Some seedlings showed symptoms of oxygen deprivation (blue root tips). These symptoms are well linked to reduced growth performance in aspen (Landhäusser et al., 2003). However, the H and B seedlings (particularly those planted in fall) still performed better and had lower shoot dieback than the C and CL stock types. This reason for better performance in the H and B might be explained by their higher TNC reserves before planting, which allowed seedlings affected by oxygen deprivation to better withstand the quick depletion of TNC reserves (Silva et al., 2009).

## 2.5 Conclusions

This study suggests that short aspen seedlings with high RSR and TNC concentrations and that are well hardened will perform much better in the field particularly on the sites with stressful conditons – too dry on both the Suncor and Syncrude sites and flooded on Genesee in 2009. This is supported by the strong relationship between initial RSR and height growth ($P<0.001$; $R^2= 0.42$) and root TNC and height growth ($P<0.001$; $R^2= 0.21$) for both years at the Suncor site, and the height growth on the flooded site at Genesee in 2009 (RSR $P<0.001$; $R^2=0.33$; root TNC $P<0.001$; $R^2=0.08$). However, in 2008 when growing conditions were good at Genesee, there was no relationship detectable.

Generally, spring and fall planting times appear to be the most suitable planting times for outplanting of aspen seedlings on forest reclamation sites. These seedlings had better height, diameter and root growth, grew more leaves and had a reduced chance of shoot dieback compared to seedlings lifted and planted in the summer.

Clipping containerized aspen seedlings before planting for a summer plant can not be recommended as these seedlings performed very poorly.

In order to facilitate the interpretation of the results of this experiment, I developed a matrix summarizing the results of the impact of seedling characteristics and planting times on outplanting performance (Table 2-6).

**Table 2-1.** Time line of stock type production and shoot growth termination treatments for planting stock in 2008 and 2009.

| Stock type | Planting year [a] 2008 | 2009 |
|---|---|---|
| Blackout (B) | **Edmonton seed source.** Seeded on May 23. Moved outside on July 24.<br>Blackout on: July 29 and August 19.<br>**Fort McMurray seed source.** Seeded on May 23. Moved outside on July 24.<br>Blackout on: July 28 and August 18. | **Fort McMurray seed source** [b]<br>Seeded on May 12.<br>Moved outside on July 14.<br>Blackout on: July 27 and August 18. |
| Hormone (H) | **Edmonton seed source**<br>Hormone application on: July 15.<br>Moved outside on July 24.<br>**Fort McMurray Seed source**<br>Hormone application on: July 24.<br>Moved outside on July 24. | **Fort McMurray seed source**<br>Hormone application on June 23. Moved outside on July 14. |
| Conventional (C) | **Edmonton seed source**<br>Moved outside on August 4.<br>**Fort McMurray seed source**<br>Moved outside on August 4. | **Fort McMurray seed source**<br>Moved outside on July 28. |
| Clipped (CL) | N/A | **Fort McMurray seed source**<br>Seeded on April 12<br>Moved outside on July 21.<br>Clipping August 18. |

[a] The seedlings produced in 2008 were evaluated for outplanting success after first (fall 2009) and second growing season (fall 2010). The seedlings produced in 2009 were evaluated only after first growing season (fall 2010).

[b] In 2009, Edmonton seed did not germinate well, therefore it was removed. Only the Fort McMurray seed source was used.

**Table 2-2.** Winter (September (previous year) to April) / summer (May to August) precipitation and mean air temperatures for 2008, 2009 and 2010 at Genesee and Suncor/Syncrude planting areas.

| **Planting area** | **Planting year** | | | | | |
|---|---|---|---|---|---|---|
| | **2008** | | **2009** | | **2010** | |
| | Precipitation (mm) | Mean Air Temperature (°C) | Precipitation (mm) | Mean Air Temperature (°C) | Precipitation (mm) | Mean Air Temperature (°C) |
| Genesee | 87.6/262.2 | -1.4/15.1 | 102.3/217.5 | -3.4/14.3 | 306.9/288.9 | -0.3/13.8 |
| Suncor/ Syncrude | 26.2/305 | -9.7/16.2 | 131.8/229 | -5.8/14.4 | 24.4/219 | -2.6/15.4 |

**Table 2-3.** Planting dates for 2008 and 2009 planting years.

| Planting time | Planting year | |
|---|---|---|
| | **2008** | **2009** |
| Seeded on | May 23, 2008 | May 12, 2009 |
| Summer | August 20, 2008 | August 24, 2009 |
| Fall | September 25, 2008 | September 28, 2009 |
| Spring | May 4, 2009 | May 10, 2010 |

**Table 2-4.** Means and (standard deviation) of nutrient concentrations from surface soils collected at Genesee, Suncor and Syncrude planting areas.

| Planting area | $Na^+$ | $K^+$ | $Mg^{2+}$ | $Ca^{2+}$ | $NH4^+$ | $NO_3^-$ | $PO_4^{3-}$ | Total N | Total P |
|---|---|---|---|---|---|---|---|---|---|
| Genesee | 30.9 **ab** (13.26) | 194.2 **a** (26.74) | 475.3 **a** (67.81) | 2779.2 **b** (118.81) | 14.2 **a** (2.11) | 78.7 **a** (30.41) | 32.1 **a** (5.82) | 4.1 **ab** (0.79) | 1.8 **a** (0.74) |
| Suncor | 57.6 **a** (31.14) | 67.7 **b** (24.47) | 543.8 **a** (34.88) | 5357.2 **ab** (682.20) | 12.8 **a** (1.46) | 1.8 **b** (0.67) | 18.3 **b** (6.42) | 2.9 **b** (1.06) | 1.1 **ab** (0.96) |
| Syncrude | 7.7 **b** (2.54) | 65.6 **b** (19.69) | 273.4 **b** (44.43) | 6376.0 **a** (423.70) | 14.9 **a** (3.59) | 5.0 **b** (8.25) | 17.8 **b** (5.58) | 9.7 **a** (6.36) | 0.7 **b** (0.40) |

Numbers in a column followed by the same letter are not significantly different (n=3 for $Na^+$, $K^+$, $Mg^{2+}$,$Ca^{2+}$; n=6 for $NH^{4+}$, $NO3^-$ ,$PO_4^{3-}$, Total N and Total P)

$Na^+$, $K^+$, $Mg^{2+}$, $NH^{4+}$, $PO_4^{3-}$ and Total P were analysed with multiple comparison LSD test.

$Ca^{2+}$, $NO3^-$ and Total N analysed with Kruskal-Wallis analysis of variance on ranks and multiple comparison Tukey test.

Values of $Na^+$, $K^+$, $Mg^{2+}$,$Ca^{2+}$, $NH^{4+}$, $NO3^-$ and $PO_4^{3-}$ are expressed in mg/Kg of oven dried soil.

Values of Total N and Total P are expressed in mg/L of soil.

**Table 2-5.** Means and (standard deviation) of pre-planting characteristics of aspen seedlings from Edmonton and Fort McMurray seed sources that were grown in 2008 and 2009. The clipped (CL) stock type was not compared statistically with other stock types (n=10). Numbers in a column followed by the same letter are not significantly different (LSD test).

| Planting time | Stock type | Height (cm) | Root Volume (ml) | Root to Shoot ratio | Shoot TNC (%) | Root TNC (%) |
|---|---|---|---|---|---|---|
| Summer | Blackout | 22.80 **c** (4.27) | 3.02 **c** (0.79) | 1.98 **d** (0.78) | 15.45 **e** (2.41) | 24.88 **d** (5.36) |
| | Hormone | 21.31 **c** (3.84) | 4.50 **b** (1.76) | 2.64 **c** (1.24) | 16.71 **cd** (2.54) | 26.97 **d** (3.18) |
| | Conventional | 46.91 **a** (3.80) | 2.52 **c** (0.56) | 0.57 **f** (0.11) | 8.42 **f** (1.78) | 14.52 **e** (2.45) |
| | Clipped (2009 only) | 40.60 (1.13) | 1.50 (0.67) | 0.69 (0.21) | 8.27 (2.59) | 15.91 (4.29) |
| Fall | Blackout | 22.05 **c** (2.34) | 4.43 **b** (0.94) | 2.79 **bc** (0.57) | 18.25 **b** (1.51) | 37.15 **bc** (2.83) |
| | Hormone | 21.63 **c** (2.56) | 6.00 **a** (1.32) | 4.22 **a** (0.83 ) | 18.32 **b** (1.82) | 39.92 **a** (3.22) |
| | Conventional | 42.73 **b** (4.78) | 4.64 **b** (1.14) | 1.14 **e** (0.17) | 20.04 **a** (1.57) | 37.06 **bc** (2.63) |
| Spring | Blackout | 22.63 **c** (6.12) | 4.46 **b** (0.88) | 3.12 **b** (1.02) | 15.94 **de** (1.02) | 35.34 **c** (4.13) |
| | Hormone | 22.78 **c** (2.74) | 6.15 **a** (1.62) | 4.03 **a** (0.84) | 16.63 **cd** (1.11) | 38.82 **ab** (2.77) |
| | Conventional | 45.43 **a** (3.28) | 5.82 **a** (1.04) | 1.26 **e** (0.27) | 17.39 **bc** (0.90) | 35.00 **c** (4.70) |

**Table 2-6.** Matrix summarizing the results of this field trial to facilitate decision making on what to plant and when. Based on the results stock types are ranked from best overall performance at the top to worst, while planting time is ranked from best left to worst right.

| | Planting time | | | |
|---|---|---|---|---|
| **Stock type** | **Fall** | **Spring** | **Summer** | |
| **Hormone** | 3 | 3 | 3 | 9 |
| **Blackout** | 3 | 3 | 2 | 8 |
| **Conventional** | 2 | 2 | 1 | 5 |
| **Clipped** | N/A | N/A | 0 | 0 |
| | 7 | 7 | 5 | |

**Ranking system**

3 = Preferred. Seedling performance is expected to be best.
2 = Acceptable. Seedling performance is expected to be good.
1 = Potential. Seedling performance will be dependent on climatic, topographical and planting substrate conditions allowing for continued water supply for seedlings.
0 = Avoid. Seedlings performance is expected to be the poor.
N/A = Not evaluated in this study

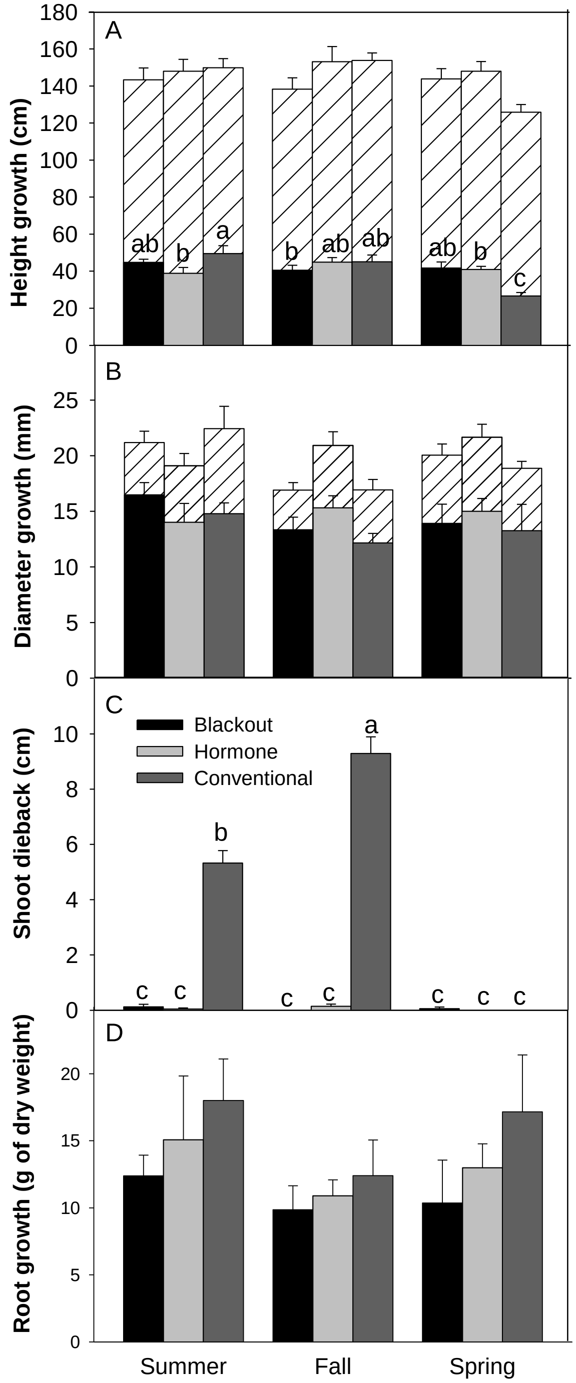


**Figure 2-1.** Height growth (A), Diameter growth (B), Shoot dieback (C) and Root growth (D); for blackout (B), hormone (H) and conventional (C) stock types planted in summer and fall of 2008 and spring of 2009 at Genesee. Solid colors represent the first full growing season (2009) while hatched bars represent height or diameter growth during the second growing season (2010). Data were collected in fall of 2009. Bars with the same letter are not significantly different (upper case letters for 2010 and lower case for 2009 growing season) (LSD test, p=0.05).

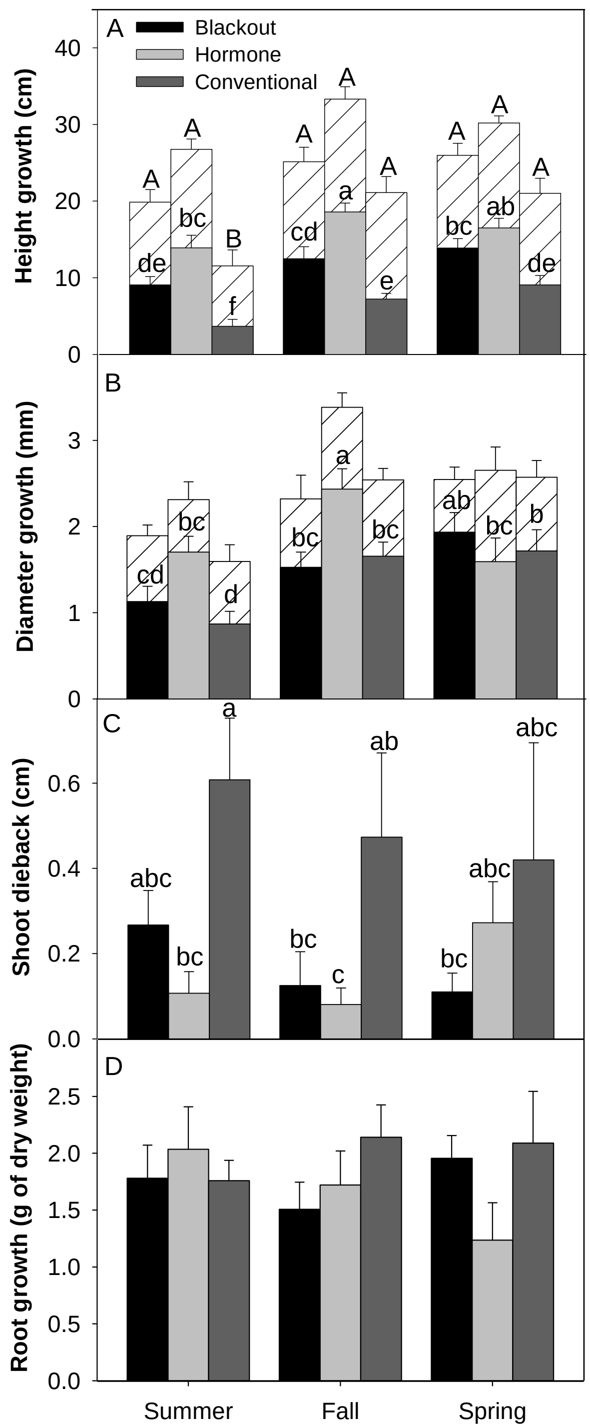


**Figure 2-2**. Height growth (A), Diameter growth (B), Shoot dieback (C) and Root growth (D); of blackout, hormone and conventional stock types planted in summer and fall of 2008 and spring of 2009 at Suncor. Solid colors represent the first growing season (2009) while hatched bars represent height or diameter growth in the second growing season (2010). Data were collected in fall of 2009. Bars with the same letter are not significantly different (LSD test, p=0.05).

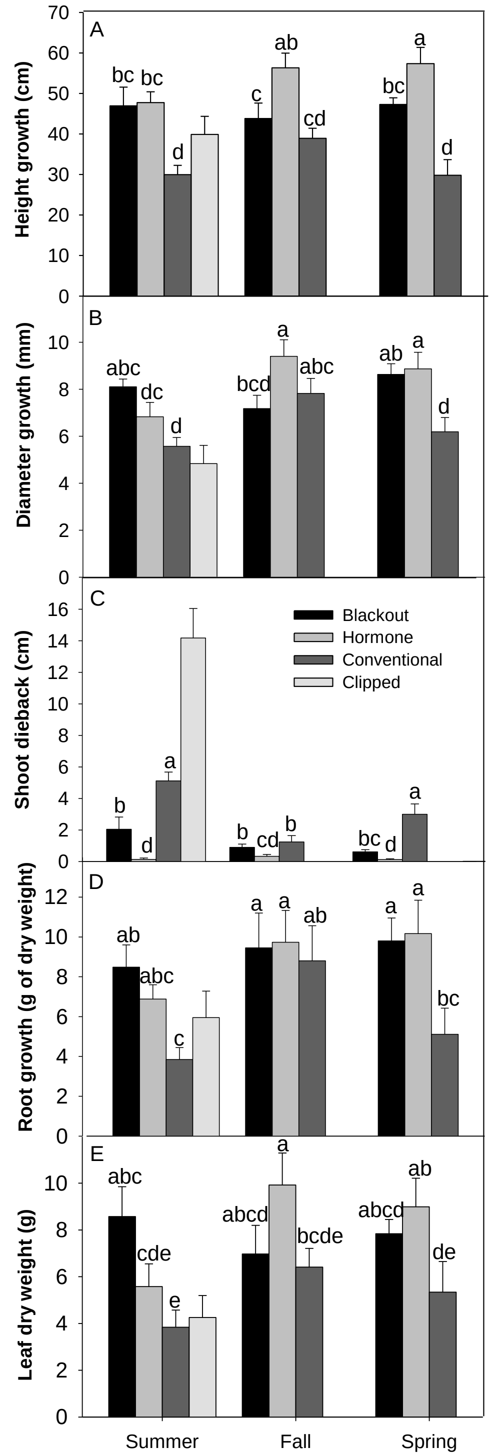


**Figure 2-3.** Height growth (A), Diameter growth (B), Shoot dieback (C), Root growth (D) and Leaf dry weight (E) of blackout, hormone, conventional and clipped stock types planted in summer, and fall of 2009 and spring 2010 at Genesee. Data were collected in fall of 2010. Bars with the same letter are not significantly different (LSD test, p=0.05).

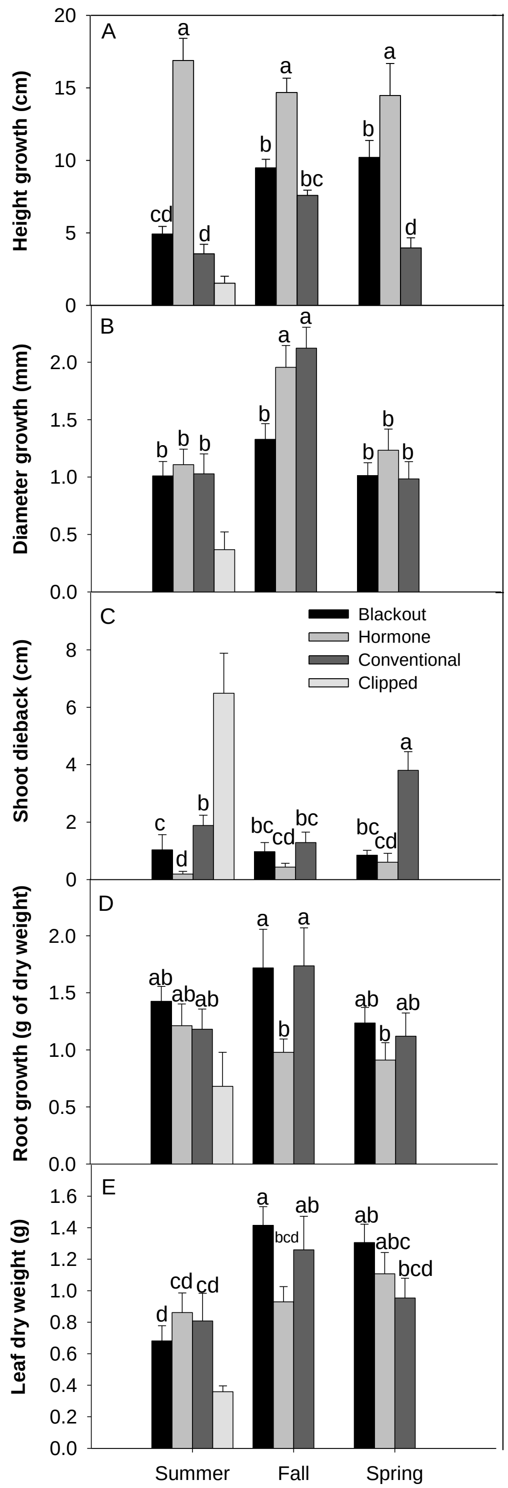


**Figure 2-4.** Height growth (A), Diameter growth (B), Shoot dieback (C), Root growth (D) and Leaf dry weight (E) of blackout, hormone, conventional and clipped stock types planted in summer and fall 2009 and spring 2010 at Suncor. Data were collected in fall of 2010. Bars with the same letter are not significantly different (LSD test, p=0.05).

## References

Adams J. C., 1985. Severe top pruning improves water oak seedling growth. In: Shoulder, E., ed. Proceedings, 3rd Biennial Southern Silvicultural Research Conference; 1984 Nov. 7-8; Atlanta, GA. Gen. Tech Rep SO-54 USDA Forest Service. New Orleans, pp. 1-3.

Adams, D.L., Graham, R.T., Wenny, D. M., 1991. Effect of fall planting on survival and growth of three coniferous species of container seedlings in Northern Idaho. Tree Planter's Notes. 42, 52-55.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration - Guidelines for computing crop water requirements. In: FAO Irrigation and Drainage Paper 56. FAO Food and Agriculture Organization of the United Nations, Rome, (accessed 04.06.2011 at: http://www.fao.org/docrep/X0490E/x0490e00.htm#Contents).

Barber, H., 1989. Planting western larch: A comparison of stocktypes and season of planting in Northeast Washington. Tree Planter's Notes. 4, 20-24.

Borset, O., 1960. Silviculture of aspen. Scottish Forestry. 14, 68-80.

Carlson, W.C., Miller, D.E., 1990. Target seedling root system size, hydraulic conductivity, and water use during seedling establishment. In: Rose, R.; Campbell, S.J.; Landis, T. D., eds. Proceedings Western Forest Nursery Association; Roseburg, OR, General Technical Report RM-200, Department of Agriculture Forest Service, Rocky Mountain Forest and Range Experiment Station Fort Collins, CO: U.S, pp. 53-65.

Carter, M.R., Gregorich, E.G., 2008. Soil sampling and methods of analysis, second ed. CRC Press, Taylor & Francis, Boca Raton.
Chavasse, C.G.R., 1980. Planting stock quality: A review of factors affecting performance. New Zeal. J. For. 25, 144–171.

Chow, P.S., Landhäusser, S.M., 2004. Method for routine measurements of total sugar and starch content in woody plant tissues. Tree Physiol. 24, 1129 –1136.

Dierauf, T. 1989. Early planting, over-winter storage, and late planting of white pine seedlings. Virginia Department of Forestry. Occasional Report 83, pp. 1-7.

Farmer, R.E Jr., 1978. Seasonal carbohydrate levels in roots of Appalachian hardwood planting stock. Tree Planter's Notes. 29, 22–24.

Fowells, H.A., 1965. Silvics of forest trees of the United States. U.S. Dep. Agric. Handb. No. 271.Washington, (accessed 05.30.2011 at http://www.na.fs.fed.us/spfo/pubs/silvics_manual/volume_2/populus/tremuloides.htm).

Good, G.L., Corell, T.E., 1982. Field trials indicate the benefits and limits of fall planting. Am. Nurseryman. 155, 31-34.

Grossnickle, S.C., 2005. Importance of root growth in overcoming planting stress. New Forest. 30, 273–294.

Harrington, J. T., Mexal, J. G., and Fisher, J. T., 1994. Volume displacement provides a quick and accurate way to quantify new root production. Tree Planters' Notes. 3, 121-124.

Hashizume, H., Han, H., 1993. A study on forestation using large-size *Quercus acutissima* seedlings. Hardwood Res. 7, 1–22.

Hedden, P., Graebet, J.E., 1985. Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. J. Plant Growth Regul. 4, 111 – 122.

Jacobs, D.F., Salifu, K.F., Seifert J.R., 2005. Relative contribution of initial root and shoot morphology in predicting field performance of hardwood seedlings. New Forest. 30, 235-251.

Jones, J.B Jr, 2001. Laboratory guide for conducting soil tests and plant analysis. CRC Press, 2001, pp. 122-124.

Kozlowski, T.T.1991. Effects of environmental stresses on deciduous trees. In: H. A. Mooney, E. Pell & W. E. Winner,eds. Response of plants to multiple stresses. Academic Press, San Diego, California, pp. 391-411.

Kozlowski, T.T., Pallardy, S.G. 2002. Growth control in woody plants. Academic Press, San Diego. 641 p.

Landhäusser, S.M., V.J. Lieffers., 2002. Leaf area renewal, root retention and carbohydrate reserves in a clonal tree species following aboveground disturbance. J. Ecol. 90, 658-665.

Landhäusser, S.M., Silins U., Lieffers, V.J., Liu, W., 2003. Response of *Populus tremuloides*, *Populus balsamifera*, *Betula papyrifera*, and *Picea glauca* seedlings to low soil temperature and waterlogged soil conditions. Scan. J. For. Res. 18, 391-400.

Little, E. L., Jr. 1971. Atlas of United States Trees. Washington, D. C.: U. S. Department of Agriculture, Forest Service. 1146. 9 p.

Mamkagh, A.M.A., 2009. Effect of tillage time and plastic mulch on growth and yield of okra (*Abelmoschus esculentus*) grown under rain fed conditions. Int. J. Agric. Biol. 11, 453–457.

Marshall, J.D., 1985. Carbohydrate status as a measure of seedling quality. In: Duryea, M. L.,ed. Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Workshop held October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis, pp. 49-58.

Martens, L.A., Landhäusser, S.M., Lieffers, V.J., 2007. First-year growth response of cold-stored, nursery-grown aspen planting stock. New Forest. 33, 281–295.

Navarro Cerrillo, R.M., Retamosa, M.J., López, J., del Campo, A., Salmoral, L., 2006. Nursery practices and field performance for the endanger Mediterranean species *Abies pinsapo* Boiss: 5-year results. Ecol. Eng. 27, 93–99.

Page, A.L., 1982. Methods of soil analysis: Part 2 –Chemical and microbiological properties. Second ed. Madison, American Society of Agronomy, pp. 416-418.

Rietveld, W. 1988. Effect of paclobutrazol on conifer seedling morphology and field performance. In: Landis, T.D., tech coord. Proceedings, combined meeting of the Western Forest Nursery Associations August 8-11, Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, pp. 19-23.

Rietveld W.J., 1989. Transplanting stress in bareroot conifer seedlings: its development and progression to establishment. North. J. Appl. For. 6, 99–107.

Ritchie, G. A., 1984. Chapter 23: Assessing seedling quality. In: Duryea, M.L., and Landis, T.D. (Eds.), Forest Nursery Manual: Production of bareroot seedlings. Martinus Nijhoff/Dr W. Junk

Publishers. The Hague/Boston/Lancaster, Forest Research Laboratory, Oregon State University. Corvallis, pp. 243-259.

Seifert, J.R., Jacobs, D.F., Selig, M.F., 2006. Influence of seasonal planting date on field performance of six temperate deciduous forest tree species. For. Ecol. Manag. 223, 371–378.

Silva, C., Piedade, M., Franco, A., Carvalho, J , Junk, W., 2009. Adaptive strategies to tolerate prolonged flooding in seedlings of floodplain and upland populations of *Himatanthus sucuuba*, a Central Amazon tree. Aquat. Bot. 90, 246–252.

Steneker, G.A., 1976. Guide to silvicultural management of Trembling Aspen in the Prairie Provinces. North. For. Res. Cent., Edmonton, Alberta. Information Report NOR-X-164, pp. 1-8.

Strong, W.L., La Roi, G.H., 1985. Root density—soil relationships in selected boreal forests of central Alberta, Canada. For. Ecol. Manag. 12, 233-251.

Sutton, R. F. 1979. Planting stock quality and grading. For. Ecol. Manag. 2, 123–132.

Taylor, J.S., Dumbroff, E.B.,1975. Bud, root, and growth regulator activity in *Acer saccharum* during dormant season. Can. J. Bot. 53, 321-331.

Thompson, J.R., Schultz, R.C., 1995. Root system morphology of *Quercus rubra* L. planting stock and 3-year field performance in Iowa. New Forest. 9, 225–236.

Thompson, B.E., 1985. Chapter 6: Seedling morphological evaluation - what you can tell by looking. In: Durvea, M. L.,ed. Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Workshop held October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis, pp. 59-71.

Truax, B., Gagnon, D., 1992. Effects of straw and black plastic mulching on the initial growth and nutrition of butternut, white ash and bur oak. For. Ecol. Manag. 57, 17-27.

van den Driessche, R., Rude, W., Martens, L., 2003. Effect of fertilization and irrigation on growth of aspen (*Populus tremuloides* Michx.) seedlings over three seasons. For. Ecol. Manag. 186, 381–389.

Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry. 13, 87-115.

Wilson, B.C., Jacobs, D.F., 2006. Quality assessment of temperate zone deciduous hardwood seedlings. New Forest. 31, 417–433.

# Chapter 3. Impact of timing of shoot termination and container size on aspen seedling quality and outplanting performance

## 3.1 Introduction

Aspen is a widespread North American tree species native to the boreal forest (Perala, 1990). Its relative drought tolerance (Lieffers et al., 2001) and fast growth rates (Heilman et al., 1996) make it very useful in the reclamation of disturbed land. Aspen is also a desirable tree species for tree improvement as it has a strong genetic component to growth, wood quality, insect and disease resistance (Navratil et al.,1990; Einspahr et al., 1968; Einspahr and Winton, 1976). As production of aspen growing stock from vegetative propagation is difficult (Haapala et al., 2004; Hartmann et al., 2002; Yu et al., 2001; Snedden et al., 2010), seeding is considered an easier means of establishment. Therefore seedlings are generally used in nursery production of aspen, unlike the clonal propagation of other *Populus* species in the *Tacamahaca* and *Aigeiros* sections (Eckenwalder, 1996). Little research, however, has been conducted on quality of aspen planting stock and its subsequent outplanting performance (Martens et al., 2007). Currently, aspen container stock is grown in greenhouses settings in stress free conditions until they achieve the required height and shoot diameter; once target sizes are achieved, they are semi hardened for either late summer planting or fully hardened for freezer storage and planting the following spring (Burr, 1985). After outplanting, aspen planting stock often shows reduced survival and a period of slow growth (i.e. planting check or transplant shock), especially in the first year (van den Driessche et al., 2003), indicating that seedling quality at time of planting plays a significant role.

Morphological variables such as seedling shoot height and diameter, root to shoot ratio, and physiological variables such as root growth potential, photosynthesis and chlorophyll content are currently used to estimate seedling quality (Sutton 1979; Ritchie, 1984; Puttonen, 1996; Grossnickle, 2000). However, minimum specification and targets of nursery planting stock are based on experiences and research conducted mostly on conifer seedlings. High carbohydrate reserves and mineral nutrient content in nursery stock have been considered good indicators of seedling quality (Stape et al., 2001) and increased stress resistance after outplanting (Ritchie, 1982; Puttonen, 1986). Therefore high levels of stored carbohydrate reserves may be particularly important for dormant deciduous seedlings that rely on stored reserves to initiate leaf area and new root growth after outplanting (Krueger, 1967; Loescher et al., 1990).

Seedling characteristics such as RSR and TNC concentrations can be modified in aspen by inducing shoots to prematurely terminate growth (Landhäusser et al., unpublished). Termination of shoot growth can be achieved through various processes; however, the application of growth retardants such as paclobutrazol proofed effective at producing uniform timing of bud set (Landhäusser et al, unpublished). Growth retardants have been widely used in agriculture with diverse applications such as antilodging in rice (Lürssen, 1988; Oshio and Izumi, 1986); reducing growth in apple trees (Steffens, 1988); and increasing shoot thickness, accelerating root formation and early fruit yield in tomato seedlings (Berova and Zlatev,  2000). In trees they have been used to reduce growth of *Eucalyptus nitens* seedlings (Williams et al., 1999) and enhance height growth and chlorophyll content in seedlings of silver maple (*Acer saccharinum*) (Bai and Chaney, 2000).

The selection of container size also plays an important role in aspen seedling production. Larger containers allow for greater root volume to be filled and generally create larger rooting systems and larger seedlings (Kinghorn, 1974; Alm, 1982). After outplanting lodgepole pine (*Pinus contorta* Loudon) seedlings grown in large containers were taller and had more roots (Endean and Carlson, 1975), cork oak (*Quercus suber* L) seedlings had higher root growth (Chirino et al., 2008), and Italian stone pine (*Pinus pinea)* seedlings had greater stem diameter and nutrient content at the end of the growing season as well as overall better field performance (Dominguez-Lerena et al., 2006). A main constraint of growing hardwood species (such as aspen) in containers with smaller volume is the tight spacing between seedlings, which can generate significant shading among seedlings in the nursery trays; however, this could be avoided by slowing height growth and leaf development through the growing conditions.

In addition, by exposing aspen seedlings to outside conditions earlier during their establishment, stopping height growth earlier, and growing them in containers with smaller volumes and higher bed density, the costs of seedling production could be reduced through lower space requirements and reduced greenhouse operation costs. On the downside, smaller containers will likely result in smaller root volume and smaller seedlings. However, we hypothesize that if root to shoot ratio (RSR) and total non-structural carbohydrate (TNC) concentrations are high in these smaller seedlings, outplanting performance might not be negatively affected.

Two related data sets were collected to examine the timing of transfer of aspen seedling stock to outside conditions and the height seedlings attained at bud set. First, we grew seedlings in large containers and repeatedly sampled the growing stock for developmental changes in root and shoot development and root TNC accumulation over the nursery period. In the second study, we varied the time of transfer and seedling height at bud set but we also grew the stock in both large and small containers. In this second data set, we evaluated seedling characteristics at the end of the nursery period and the success of these different stock types on outplanting performance.

## 3.2 Materials and methods

### 3.2.1 Early nursery conditions

All seedlings were grown from seed collected from open pollinated seed sources near Edmonton (53°34′N 113°31′ W, elevation 668 m asl). Seeds for both of the experiments described below were sown the first week of June 2009 into styroblock containers (Beaver Plastics Ltd, Acheson, Alberta). All cavities were filled with a growing medium of peat-vermiculite mixture (9 to 1 volume). Seedlings were established under greenhouse conditions at the University of Alberta with average temperature of 25°C, relative humidity of 50% and 20 h daylight photoperiod using artificial lightning. All seedlings were watered daily. After two weeks, seedlings were thinned, leaving the tallest seedling. Fertilization started the same week as thinning with one application of 1 g/L of a commercial fertilizer 10-52-10 N-P-K. Two weeks later seedlings were fertilized once with a solution of 2 g/L of 28-14-14 to encourage rapid root growth and from then on, they were fertilized every two weeks with a solution of 2 g/L of 15-30-15. All three commercial fertilizers contained chelated micronutrients and were manufactured by Plant Products Co, Brampton, Ontario.

### 3.2.2 Developmental changes in roots, shoots and TNC in the nursery

Seedlings for this study were sown in 615A styroblocks (45 cavities, 340cm$^3$ (6 cm diameter and 15 cm deep), density of 213 seedlings/m$^2$)) (Beaver plastic, Edmonton, Alberta). Seedlings were divided into three groups and moved outside at three times, when they had reached a height of about 5 cm (four weeks after seeding) (July 4), 15 cm (six weeks after seeding) (July 22), and 35 cm (nine weeks after seeding) (August 6). In addition shoot growth was artificially terminated when seedlings had reached a height of 20 cm (only for seedlings moved outside at a height of 5 and 15 cm) and 35 cm (only for seedlings moved outside at 35 cm). Height growth was then terminated by applying the growth retardant paclobutrazol (Bonzi®, Syngenta, North Carolina, USA). This chemical was applied by soaking the styroblocks in a water bath for about 3 minutes until water filled the whole cavity with a concentration of 5 ml per L of Bonzi (0.02 g of paclobutrazol/Liter of water). Hereafter the height when seedlings were moved outside and the height when shoot growth was terminated are defined as “stock”. As a result three stocks (5-20, 15-20 and 35-35) were created; however, the combination of container size and stock is referred to as “stock type”.

Starting four weeks after seeding, 10 seedling of each of the three stocks (5-20, 15-20 and 35-35) were sampled every 3 to 4 weeks until

November 13, 2009 (Table 3-1). Several variables were measured on each collection date. Leaf dry weight was determined in the first 4 sampling dates (until August 19) before leaf senescence. Seedling root systems were carefully washed to remove the substrate and shoot height measured. Shoots, roots and leaves were dried 2 to 3 days at 70°C after which their dry weight was taken. Root to shoot ratios (RSR) were calculated as the ratio of root dry weight to shoot dry weight (excluding leaves). Total non-structural carbohydrate (TNC) reserves were determined in root and shoots (without leaves) separately. Samples were ground to 40-mesh with a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA), soluble sugars were extracted from tissue samples using hot ethanol (80%) and their concentration measured colorimetrically using phenolsulfuric acid. Remaining starch in the residues was later solubilised by sodium hydroxide and hydrolyzed using an enzyme mixture of α-amylase (ICN 190151, from *Bacillus licheniformis*) and amyloglucosidase (Sigma A3514, from *Aspergillus niger*) and then measured colorimetrically using peroxidase glucose-o-dianisidine solution (Sigma Glucose Diagnostic Kit 510A) (Chow and Landhäusser, 2004).

### 3.2.3 Evaluation of stock types

For this study seedlings were grown in both 615A styroblocks (45 cavities, 340 $cm^3$ (6 cm diameter and 15 cm deep, density of 213 seedlings/m$^2$) and in 415D styroblocks (77 cavities, 170 cm$^3$ (4 cm in diameter and 15 cm deep, density of 364 seedlings/m$^2$) (Beaver plastic, Edmonton, Alberta). Similar to the first study, seedlings were started in the greenhouse and moved outside when the stock in the 615A cavities reached a height of 5 cm (four weeks after seeding), 15 cm (six weeks after seeding), or 35 cm (nine weeks after seeding). Shoot growth was terminated by applying the shoot growth retardant paclobutrazol (see above for more details on application) when seedlings had reached a target height of 20 and 35 cm which was approximately 7 and 9 weeks after seeding, respectively. Dates were similar to the first experiment. As a result, 3 stocks were created for each of the two container sizes for a total of 6 stock types. The first number (615A or 415D) represents the container size, followed by height when seedlings were moved outside and the height when paclobutrazol was applied (Table 3-2). Seedlings were kept outside until lifted November 13, 2009 and stored frozen at -3°C to be outplanted in May 2010.

A sample of 10 seedlings of each stock type was taken after cold storage in April 2010. Seedling height and diameter at the root collar were measured. Root systems were carefully washed to remove the substrate. Root volume was determined using the water displacement method (Harrington et al., 1994). Shoots and roots were dried at 70°C and then shoot and root dry weights were determined as well as root to shoot ratios (RSR). Total non-structural carbohydrate (TNC) reserves in root and shoots were determined using the same methods described previously.

### 3.2.4 Outplanting

In the spring of 2010, 80 seedlings of each of the two container sizes and three stock types were planted at the Crop Diversification Center (CDC) North Edmonton (53°34′N 113°31′ W, elevation 668 m asl). Precipitation during the growing season (May 1 to August 31 of 2010) was 243 mm and mean air temperature was 13.5°C. Soil texture was a silty loam, rototilled before planting and subsequently covered with plastic mulch on April 20, 2010 to suppress growth of agronomic weeds. Planting was done by hand and seedlings were spaced at 50 cm with an inter-row distance of 1.2 m. Seedlings were planted in a completely randomized block design with 6 blocks, each block contained 10 plots for each of the 6 stock types (total 60 plots). Each plot was planted with 8 seedlings (subsamples) each.

After outplanting initial seedling height was measured on all seedlings in the first week of May 2010. In the last week of August 2010, total seedling height and the length of the longest shoot were measured. In addition, 20 seedlings of each treatment combination were excavated and root length of the three longest roots, leaf dry weight, diameter, shoot dry weight, and root dry weight determined. New growth of seedling diameter, shoot and root growth were determined by subtracting the initial average measurements taken in April 2010 after cold storage from the fall 2010 measurements. Shoot dieback was defined as death of the terminal developed at the nursery. Mortality and shoot dieback were evaluated by counting the number of trees that were dead and measuring the length of stem that had died back after the first growing season.

### 3.2.5 Experimental design and data analysis

The first study was analyzed as a 3 $\times$ 7 factorial design with 3 stocks (5-20, 15-20 and 35-35) and 7 collection times (4, 7, 9, 11, 16, 20 and 22 weeks). At each collection time the response variables height, root dry weight, RSR, root TNC, shoot TNC and leaf dry weight were fitted with a linear mixed-effects model using the statistical software R (R Development Core Team, 2008). This model uses the functions *lme* to allow the use of time as a random variable and *varIdent* to allow a different variance structure for each level of the random variable (time). Both functions are contained in the nlme package in R (Pinheiro et al., 2010). Once data were fitted, response variables were analyzed using analysis of variance (ANOVA) procedures in R. Differences among means of pre-planting characteristics of seedlings planted were determined by the multiple comparison LSD test.

The effect of container volume, and height when seedlings where moved outside and height when shoot growth was terminated on the characteristics at time of planting were analysed with ANOVA using the MIXED procedure of SAS (SAS 9.2, SAS Institute, Cary, North Carolina). Growth variables were examined to ascertain that the variables were normally distributed (Shapiro-Wilk test) and variances were homogeneous (Levene test) before running the ANOVA. No variables were transformed. When significant treatment effects were detected, differences among means were determined by pair-wise comparisons made with the Bonferroni correction.

The outplanting performance at CDC north was designed as completely randomized block design with 6 blocks and 10 treatment plots randomly assigned to each block. Each treatment plot consisted of 8 seedlings (subsample). The effect of container size, and height when seedlings where moved outside and height when shoot growth was terminated on the variables measured was analysed with ANOVA using the MIXED procedure of SAS. Growth variables were tested for normality and homogeneity of variances using the methods described before. No variables were transformed. A significance level of $\alpha = 0.05$ was utilized for all analyses. When significant treatment effects were detected, differences among means were calculated with the same approach described before.

## 3.3 Results

### 3.3.1 Developmental changes in roots, shoots and TNC

Overall, the trajectory of height growth of the 5-20 and 15-20 stock over time was different from that of the 35-35 (both $P=0.001$). After applying the growth retardant at a seedling height of 20 cm, seedlings in both the 5-20 and 15-20 grew on average 17 cm before they terminated shoot growth at week 11, while in the 35-35 stock type the application of the growth retardant resulted in only additional height growth of 8 cm by week 11 (Figure 3-1 A). The root development of the three stocks was not different (all $P>0.309$). Root dry weight in the 5-20 and 15-20 stock was 0.8 g at week 9 while in the 35-35 stock it was 0.4 g. After that time, root dry weight in all three stocks sharply increased and caught up until reaching 4.4 g by week 16. During the measured period, root dry weight reached a maximum at week 22 with an average of 5.3 g (Figure 3-1B). The development of RSR of the 5-20, 15-20 and 35-35 stocks in time was different among all three stocks (all $P<0.005$). Between week 4 and 11 average RSR increased only 0.2 in the 35-35 stock, while in the 5-20 and 15-20 it increased by 1.0. Between mid-August (week 11) and mid-September (week 16) RSR significantly increased in all stocks. RSR in 5-20, 15-20 and 35-35 stocks increased by 1.5, 1.3, and 1.1 respectively; however, by week 16 the RSR of the 35-35 stock was still consistently lower than in the other two stocks. As a result at week 22, RSR in 5-20 and 15-20 stocks was 3.4 or 26 % higher than in the 35-35 with 2.5 (Figure 3-1C).

The development of leaf dry weight of the 15-20 stock over time was different from that of the 35-35 stock ($P=0.049$) but not different from that of the 5-20 ($P=0.565$). Until week 9, leaf dry weight was not different among the three stocks with an average of 1.3 g. Leaf dry weight only changed between the three stocks at week 11. Starting that week, leaf dry weight of 35-35 stock was 2.3 g or 37 % higher than in the 5-20 and 15-20 (Figure 3-1D).

Root TNC in 5-20 and 15-20 stocks increased much earlier in the season than in the 35-35 (both $P<0.011$). Rapid accumulation of root TNC in the 5-20 and 15-20 stocks started at week 7 while in the 35-35 it was delayed two weeks. Root TNC in 35-35 stock was less than in the 5-20 and 15-20 stocks until week 16. By this week, root TNC in the 35-35 stock (35.5%) caught up and surpassed the other two stock types; however, by week 22, root TNC was not different among stocks with an average of 35.6 % (Figure 3-1E). Similarly to root TNC, shoot TNC in 5-20 and 15-20 stocks started to accumulate earlier than in the 35-35 (both $P=0.001$). Shoot TNC in 5-20 and 15-20 stocks rapidly increased at week 7 while in the 35-35 it took two more weeks. Stem TNC in 35-35 stock was less than in 5-20 and 15-20 stocks until week 16. By this week, shoot TNC in 35-35 was very similar to the other two stocks with

18.1 % and by week 22 was not different with an average of 20% (Figure 3-1F).

### 3.3.2 Impact of container volume and stock type

Overall, seedlings grown in 615A container were larger than seedlings grown in 415D containers. The average height of seedlings grown in 615A containers was 32.4 cm compared to 24.7 cm in the 415D (P=0.001); however, the 615A-35-35 stock type produced the tallest seedlings with 37.4 cm compared to the other 5 stock types which were on average 26.8 cm. This resulted in a significant interaction between container size and stock (P=0.043). Lowest shoot dry weight was found in the 15-20 stock with 25.9 cm, followed by 5-20 with 27.4 cm and 35-35 with 32.4 cm (P=0.001). Diameter of seedlings grown in 615A container size was 5.6 mm compared to 4.3 mm in the 415D (P=0.001). Shoot dry weight of 615A-35-35 stock type was highest with 2.1 g, while for the other stock types shoot dry weight was on average 1 g. This resulted in a significant interaction between container size and stock (P=0.006). Overall, shoot dry weight of seedlings grown in 615A container size was 1.5 g compared to 0.8 g in the 415D (P=0.001). Seedlings of the 5-20 stock had the lowest shoot dry weight with 1 g, nearly followed by 15-20 with 1.1 g and greatest in 35-35 with 1.5 g (P=0.001).

Root dry weight of seedlings grown in 615A container size was greatest in the 15-20 and 35-35 stock (on average 3.6 g), while root dry weight of seedlings grown in 415D container size was the least in the 35-35 stock (1.5 g), resulting in a significant interaction of container size and stock (P=0.006). Root dry weight was highest in seedlings grown in in 615A container size with 3.2 g compared to 1.9 g in 415D (P=0.001). Seedlings of 5-20 stock had the lowest root dry weight with 2.2 g, followed by 35-35 with 2.6 g and 15-20 with 2.9 g (P=0.03). RSR of 5-20 stock grown in 415D container size decreased when these seedlings were grown in 615A container size shifting from 2.8 to 2.1, resulting in a significant interaction of container size and stock (P=0.010). Overall RSR of 35-35 stock was lowest with 1.7, while in 5-20 and 15-20 it was 2.5 and 2.7 respectively. On average, root volume was highest in seedlings grown in the 615A container size with10.3 $cm^3$ compared to 6.3 $cm^3$ in 415D (P=0.001).

Root TNC differences were relatively small, seedlings of 5-20 stock grown in 615A container size had the lowest root TNC with 25.5 % while for the other 5 stock types root TNC was on average 30 %, resulting in a borderline interaction term between container size and stock (P=0.054). Few differences in shoot TNC were found. Seedlings of 35-35 stock grown in 615A container size had the lowest shoot TNC with 13.9 %, while the average shoot TNC of the other 5 stock types was 17.4 %.

This resulted in a significant interaction of container size and stock (P=0.017) (Table 3-3).

### 3.3.3 Outplanting

Although there were large differences in initial sizes between the different seedling stock types grown in the two containers sizes, container size had no impact on overall final field performance. However, seedlings that were moved outside earlier and had their shoot growth terminated earlier (5-20 and 15-20 stock) grew 65.7 cm, approximately 10 cm more in height than the 35-35 stock which grew 56 cm (P=0.004). Nevertheless, container size did not influence height growth (P=0.419) (Figure 3-2). All other measured variables (diameter growth, total height, root length, leaf dry weight, shoot growth, root growth and shoot dieback) did not show any differences among stock types.

Regardless of stock type, seedlings had on average a total height of 87.7 cm, root length of 91.5 cm, leaf dry weight of 26.2 g and had a diameter growth of 8.2 mm, shoot mass of 31.7 g and root dry weight of 22.5 g. Overall, seedlings had little shoot dieback (0.5cm) and overall mortality was 0% (data not shown).

## 3.4 Discussion

Moving aspen seedlings to outside conditions earlier in their development in combination with an early termination of shoot growth resulted in an immediate accumulation of TNC reserves in the seedlings. TNC reserves appear to increase quickly once seedlings have terminated shoot growth while root mass continues to increase throughout the growing season. However, because all of the seedlings were allowed a long period of growth after termination of shoot growth, root TNC was similar in all treatments by the end of growth cycle. It is noteworthy that in seedlings allowed to grow taller under the more conventional nursery settings, root growth never caught up relative to shoot size (reduced RSR) (see also Chapter 2). In this study, however, we found almost no differences in RSR among all stock types when grown in different soil volumes, which is similar to Endean and Carlson (1975) who observed little difference in RSR in lodgepole pine (*Pinus contorta* Dougl) seedlings grown in different container volumes.

Generally container size is known to play a significant role in seedling characteristics and outplanting performance (Davis and Jacobs, 2005); however, much of the work is based on research in conifer planting stock. Our study indicated that aspen grown in smaller container sizes will produce smaller seedlings during the nursery phase. This response has also been found in other species such as Interior spruce (*Picea glauca*) (Sutherland and Newsome, 1988; Simpson, 1991); Douglas fir (*Pseudotsuga menziesii),* Western hemlock (*Tsuga heterophylla* ) and Sitka spruce (*Picea sitchensis*) (Arnott and Bedows,1982); and Deodar cedar (*Cedrus deodara*), Loblolly pine (*Pinus taeda),* Japanese black pine (*P. thunbergi),* Red pine, (*P. resinosa),* Scotch pine, (*P. sylvestris),* Afghan pine (*P. eldarica),* Chinese pistache (*Pistacia chinensis),* Shumard oak (*Quercus shumari)* (Appleton and Whitcomb, 1983). Interestingly, our aspen stock differences in seedlings size did not translate into large differences in field performance. This observation contradicts what most studies have found. For example Arnott and Bedows (1982) showed that initially taller seedlings of Douglas-fir, western hemlock and Sitka spruce had greater growth and remained taller after the first and subsequent five growing seasons; likewise Sutherland and Newsome (1988) reported that interior spruce seedlings grown in large containers were taller than seedlings grown in smaller containers even after 5 growing seasons and Simpson (1991) described the same response with Sitka spruce after 2 growing seasons.

Moving seedlings outdoors early and/or treating them with a height growth retardant (paclobutrazol) also created shorter seedlings compared to conventional-grown aspen seedlings (see Chapter 2). Both factors likely exerted an influence on seedling characteristics at the same time and are therefore difficult to be separated. For example, seedlings of black spruce (*Picea mariana* Mill) (Paterson, 1996) and loblolly pine *(Pinus taeda* L.) (Boyer and South, 1984; Retzlaff, 1990) grown outdoors were shorter than seedlings grown indoors. In our study, these smaller seedlings provided less shading on neighbor seedlings especially when grown in small containers, thereby explaining some of the differences in height, RSR, root and shoot TNC at time of planting of 5-20 and 15-20 stock types.

On the other hand the 35-35 stock showed large differences in height and shoot TNC prior to outplanting, which were not reflected in planting performance. Perhaps, root TNC was a better predictor of field performance as well as the adaptation of seedlings to outdoor conditions. It is known that root TNC reserves can be critical after outplanting and initial establishment until roots multiply to explore site resources (Wilson and Jacobs, 2006). Besides, seedlings moved to outdoor conditions earlier are expected to be better acclimatized to the natural conditions and have higher survival and better growth performance (Paterson, 1996). In our study, height growth was the only variable which differed among stock types after outplanting and was higher in seedlings moved outside and treated early with paclobutrazol. Enhanced height growth was shown after one growing season in Loblolly pine (*Pinus taeda* L) seedlings when subjected to a longer hardening period (Mexal et al., 1979). In our study however, the shorter stock tended to catch up with taller stock at the time of planting and thus, there was little difference in total height by the end of the first summer. This indicates that shorter stock could be used just as effectively as the taller stock, as long as it is hardened enough and had high RSR. In addition, seedlings grown in the 615A container size offered no real advantage over the 415D which is a less expensive stock both for nursery production and handling.

Although this study did not quantify the costs associated with seedling production, it can be assumed that by growing aspen seedlings in smaller containers and moving them outside the greenhouse earlier, the costs associated with lighting, heating, ventilating and controlling humidity in greenhouses will be reduced as the cost of natural gas, trays (styroblocks) and boxes are considered significant (Chaudhary, 2006). Smaller seedlings can also be boxed more efficiently, will take up less room in cold storage and are easier for planters to carry and plant.

Though, mortality in our study was not observed, mortality of seedlings grown outside can be considerably lower than when grown inside. Retzlaff (1990) found that loblolly pine (*Pinus taeda* L) seedlings grown outdoors had lower mortality during nursery production than seedlings grown indoors; however, growing seedlings outdoors may make them also susceptible to damage through climatic conditions such as early and late frosts as well as hail, heavy rain and winds.

## 3.5 Conclusions

In this study moving seedlings outside much earlier did not increase root TNC concentrations or RSR in seedling stock. However, there was an indication that the prolonged exposure to conditions inside the greenhouse had a negative impact on future growth performance. Overall planting shorter seedlings that were acclimated to outside conditions performed very well and are thought to withstand more stressful conditions such as drought (Close et al., 2005; van den Driessche, 1991); however we did not test this in this study.

Both experiments suggest that aspen seedlings grown in smaller container volumes and moved outside with their shoot growth terminated, produced the same RSR and TNC reserves compared to seedlings in larger containers. In addition, they performed very similar when outplanted on sites with good growing conditions.

**Table 3-1.** Sampling dates for stocks 5-20, 15-20 and 35-35 grown in 615A container size.

| Sampling date (Year 2009) | Weeks after seeding |
|---|---|
| July 4 | 4 |
| July 22 | 7 |
| August 6 | 9 |
| August 19 | 11 |
| September 24 | 16 |
| October 23 | 20 |
| November 13 | 22 |

**Table 3-2.** Treatment combinations used in the various experiments and outplanting assessment.

| Stock types | Container size | Height when seedlings were moved outside (cm) | Height when shoot growth was terminated (cm) |
|---|---|---|---|
| 615A-5-20 | 615A | 5 | 20 |
| 615A-15-20 | 615A | 15 | 20 |
| 615A-35-35 | 615A | 35 | 35 |
| | | | |
| 415D-5-20 | 415D | 5 | 20 |
| 415D-15-20 | 415D | 15 | 20 |
| 415D-35-35 | 415D | 35 | 35 |

**Table 3-3.** Means and (standard deviation) of seedling characteristics of 6 stock types at time of planting (n=10). Numbers in a column followed by the same letter are not considered significantly different (Bonferroni correction)

| Stock type | Height (cm) | Diameter (mm) | Shoot dry wt (g) | Root dry wt (g) | RSR | Root Volume ($cm^3$) | Root TNC (%) | Shoot TNC (%) |
|---|---|---|---|---|---|---|---|---|
| 615A-5-20 | 30.40 **b** (3.43) | 5.12 **b** (0.58) | 1.19 **bc** (0.41) | 2.47 **b** (0.96) | 2.12 **bc** (0.60) | 8.15 **b** (3.79) | 25.52 **b** (7.74) | 17.11 **a** (1.56) |
| 415D-5-20 | 24.45 **d** (1.86) | 4.39 **ab** (0.94) | 0.76 **d** (0.42) | 1.96 **bc** (0.76) | 2.84 **a** (0.70) | 6.22 **bc** (3.48) | 29.90 **ab** (4.02) | 18.43 **a** (2.23) |
| 615A-15-20 | 29.41 **bc** (1.73) | 5.77 **a** (0.66) | 1.33 **b** (0.45) | 3.64 **a** (0.59) | 2.96 **a** (0.83) | 11.46 **a** (2.68) | 30.44 **a** (4.44) | 17.49 **a** (1.09) |
| 415D-15-20 | 22.31 **d** (2.64) | 4.36 **ab** (0.39) | 0.88 **cd** (0.15) | 2.16 **bc** (0.57) | 2.46 **ab** (0.52) | 7.36 **bc** (2.09) | 29.81 **ab** (4.26) | 17.40 **a** (1.82) |
| 615A-35-35 | 37.36 **a** (2.89) | 5.93 **a** (0.70) | 2.10 **a** (0.67) | 3.61 **a** (0.88) | 1.76 **c** (0.30) | 11.20 **a** (3.50) | 31.54 **a** (3.67) | 16.74 **a** (1.35) |
| 415D-35-35 | 27.46 **c** (1.88) | 4.19 **ab** (0.58) | 0.87 **cd** (0.25) | 1.49 **c** (0.74) | 1.66 **c** (0.51) | 5.29 **c** (2.81) | 28.20 **ab** (4.41) | 13.91 **b** (4.02) |

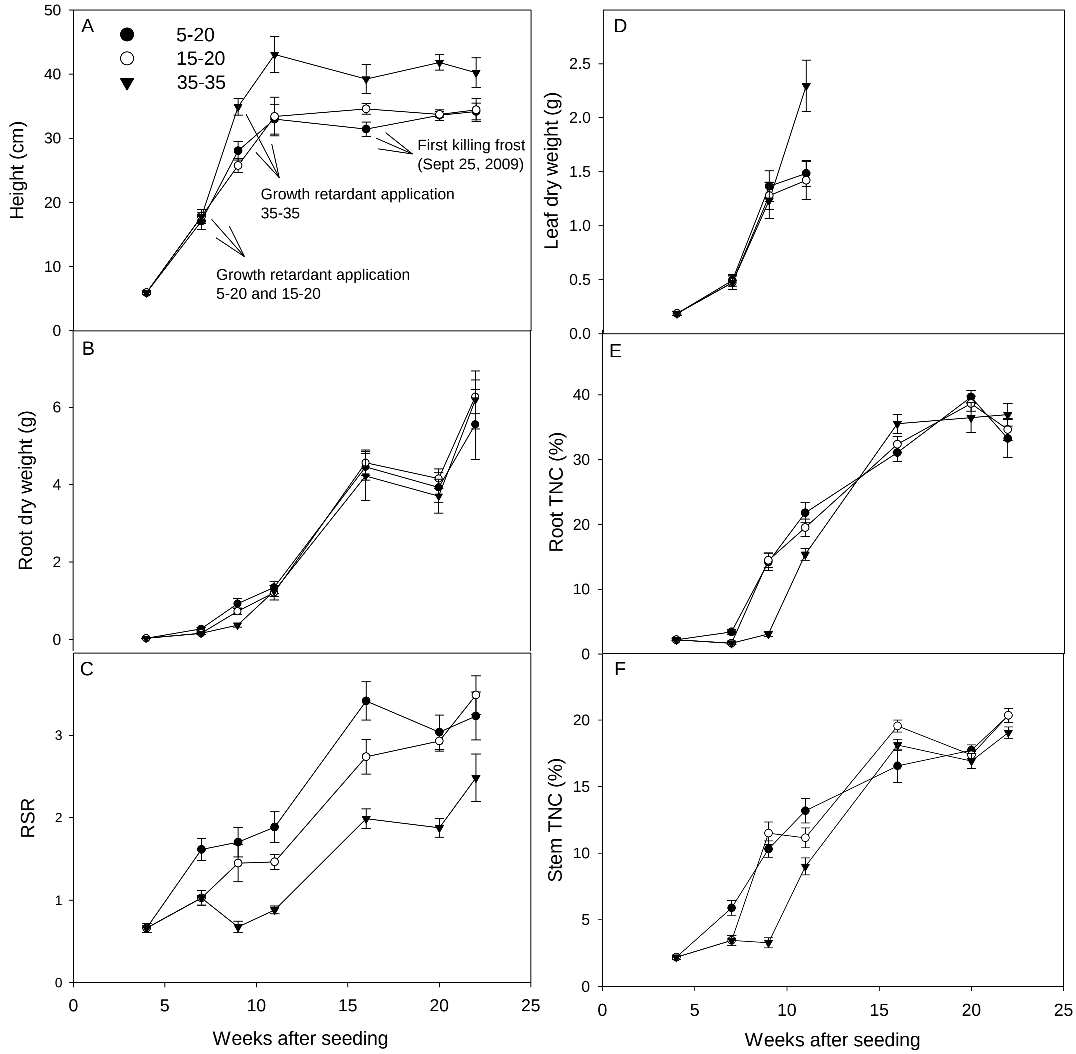


**Figure 3-1.** (A) Height, (B) Root dry weight, RSR (C), Leaf dry weight (D), Root TNC (E) and Shoot TNC (F) development of aspen seedlings in response to the timing of transfer of aspen seedling stock to outside conditions and the height seedlings attained at bud set.

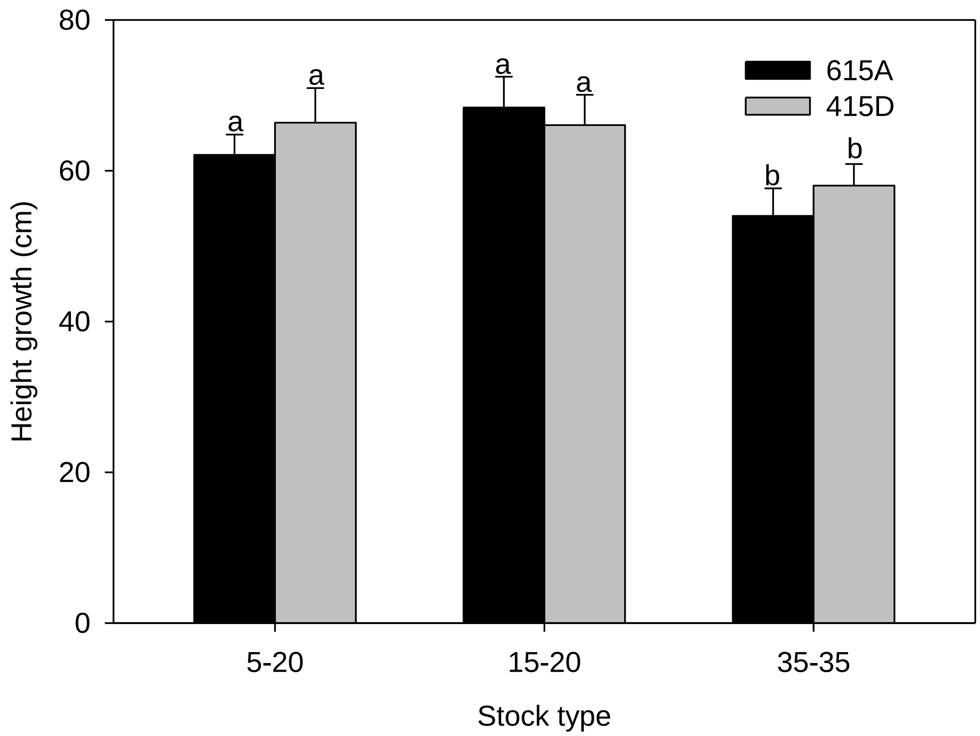


**Figure 3-2.** Height increment of aspen seedlings of 6 stock types after one growing season.

## References


Alm, A.A. 1982. The status of container planting programs in the Northern United States. 2. Lake States-Minnesota, Wisconsin, Michigan. In: Scarratt, J.B.; Glerum, C.; Plexman, C.A., eds. Proceedings, Canadian Containerized Tree Seedling Symposium; 1981 September 14-16; Toronto, ON. COJFRC Symp. Proc. O-P-10. Sault Ste. Marie, ON: Canadian Forestry Service, Great Lakes Forest Research Centre, pp. 63-65.

Appleton, B.L., Whitcomb, C.E. 1983. Effects of container size and transplanting date on the growth of tree seedlings. J. Environ. Hort. 1, 89-93.

Arnott, J.T., Beddows, D. 1982. Influence of styroblock container size on field performance of Douglas-fir, Western Hemlock, and Sitka Spruce. Tree Planter's Notes. 3, 31-34

Bai, S. J. and Chaney, W. R. 2000. Treatment of silver maple trees with gibberellin synthesis inhibitors influences growth of progeny seedlings. PGRSA Quarterly. 3, 115-126

Berova, M., Zlatev, Z. 2000. Physiological response and yield of paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.) Plant Growth Regul. 30, 117–123.

Boyer, J.N., South, D.B. 1984. A morphological comparison of greenhouse-grown loblolly pine seedlings with seedlings grown outdoors. USDA Forest Service, Tree Planter's Notes. 3, 15-18.

Burr, K.E. 1985. Greenhouse production of Quaking Aspen seedlings. In: Intermountain Nurseryman's Association Meeting, Fort Collins, Colorado, August 13-15, pp. 31-37.

Chaudhary, G.N. 2006. The economics of production and marketing of greenhouse crops in Alberta. Economics Unit Economics and Competitiveness Division. Alberta Agriculture, Food and Rural Development, (accessed 19.05.2011 at http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex4369/$file/821-59.pdf).

Chirino, E., Vilagrosa, A., Hernandez, E.I., Matos, A., Vallejo, V.R. 2008. Effects of a deep container on morpho-functional characteristics and root colonization in *Quercus suber* L. seedlings for reforestation in Mediterranean climate. For. Ecol. Manag. 256, 779–785.

Chow, P.S., Landhäusser, S.M. 2004. Method for routine measurements of total sugar and starch content in woody plant tissues. Tree Physiol. 24, 1129 –1136.

Close, D.C., Beadle, C.L., Brown, P.H. 2005. The physiological basis of containerized tree seedling ‘transplant shock’: a review. Australian Forestry. 2, 112–120.

Davis, A.S., Jacobs, D.F. 2005. Quantifying root system quality of nursery seedlings and relationship to outplanting performance. New Forests. 30, 295–311.

Dominguez-Lerena,S; Herrero Sierra, N., Carrasco Manzano, I., Ocana Bueno, L., Penuelas Rubira, J.L., Mexal, J.G. 2006. Container characteristics influence *Pinus pinea* seedling development in the nursery and field. For. Ecol. Manag. 221, 63–71.

Eckenwalder, J.E. 1996. Systematics and evolution of *Populus*. In: R.F. Stettler, H.D. Bradshaw, Jr., P.E. Heilman, and T.M. Hinckley, eds. Biology of *Populus* and its implications for management and conservation. NRC Research Press, National Research Council Canada, Ottawa, Ontario, pp. 7-32.

Einspahr, D.W., Benson M.K.,Peckham, R. 1968. Wood property variation in *Populus*. In: Proceedings of the Eighth Lake States Forest Tree Improvement Conference; Res. Pap. NC-23. St. Paul, MN; U.S. Forest Service, North Central forest Experiment Station, pp. 24-27.

Einspahr, D W., Winton, L.L., 1976. Genetics of Quaking Aspen. Aspen Bibliography. Paper 5026.

Endean, F., Carlson, L.W. 1975. The effect of rooting volume on the early growth of lodgepole pine seedlings. Can. J. For. Res. 5, 55-60.

Grossnickle, S.C. 2000. Ecophysiology of Northern Spruce species: The performance of planted seedlings. NRC Research Press. 409 p.

Haapala, T., Pakkanen, A., Pulkkinen, P. 2004. Variation in survival and growth of cuttings in two clonal propagation methods for hybrid aspen (*Populus tremula* × *P. tremuloides*). For. Ecol. Manag. 193, 343–354.

Harrington, J. T., Mexal, J. G., and Fisher, J. T. 1994. Volume displacement provides a quick and accurate way to quantify new root production. Tree Planter’s Notes. 4, 121-124.

Hartmann H.T., Kester, D.E., Davies, F.T. Jr . 2002. Hartmann and kester’s plant propagation: principles and practices, 7th edn. Prentice-Hall, Upper Saddle River, NJ, pp. 278–694

Heilman, P.E., Hinckley, T.M., Roberts, D.A., Ceulemans, R. 1996. Production physiology. In: R.F. Stettler, H.D. Bradshaw, Jr., P.E. Heilman, and T.M. Hinckley, eds. Biology of *Populus* and its implications for management and conservation. Part II Chapter 18. NRC Research Press. National Research Council of Canada, Ottawa, ON, pp. 459-489.

Kinghorn, J. M. 1974. Principles and concepts in container planting. In: R. W. Tinus, W. I. Stein, and W. E. Balmer, eds. Proc. North Amer. Container. For. Tree Seedling Symp. Aug. 26-29, 1974. Great Plains Agric. Council Publ. Denver, Colorado No. 68, pp. 8-18.

Krueger, K. W. 1967. Nitrogen, phosphorus, and carbohydrate in expanding and year-old Douglas-fir shoots. For. Sci. 13, 352-356.

Lieffers, V.J., Landhäusser, S. M., Hogg, E. H. 2001. Is the wide distribution of Aspen a result of its stress tolerance? In: Sheppperd, W.D., Binkley, D., Bartos, D.L., Stohlgren, T.J., Eskey, L.G., comps. Sustaining aspen in western landscapes: Symposium proceedings; 13-15 June 200; Grand Junction, CO. Proceedings RMRS-P-18. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, pp. 311-324.

Loescher, W.H., McCant, T., Keller, J.D. 1990. Carbohydrate reserves, translocation, and storage in woody plant roots. HortScience. 3, 274-281.

Lürssen K. 1988. Triazole plant growth regulators: Effects and mode of action. In: Berg D. and Plempel M. (eds), Sterol Biosynthesis Inhibitors. Ellis Horwood Ltd., Chichester, England, pp. 305–320.

Martens, L.A., Landhäusser, S.M., Lieffers, V.J. 2007. First-year growth response of cold-stored, nursery-grown aspen planting stock. New Forests. 33, 281–295.

Mexal, J.G., Timmis, R., Morris, W.G. 1979. Cold-hardiness of containerized loblolly pine seedlings. Southern J. Appl. Forestry. 3, 15-19.

Navratil, S., Bella, I.E., Peterson, E.B. 1990. Silviculture and management of aspen in Canada: the western Canada scene. In: R.D. Adams, editor. Proceedings of the Aspen Symposium ‘89, July 25-27, 1989, Duluth, Minnesota. U.S. Department of Agriculture Forest Service, St. Paul, Minnesota, USA, General Technical Report NC-140, pp. 39-60.

Oshio, H., Izumi, K., 1986. S-3307, a new plant growth retardant. Its biological activities, mechanism and mode of action. In: Plant Growth Regulators in Agriculture. Food and Fertilizer Technology Center Book Series. 34, pp. 198-208.

Paterson, J., 1996. Growing environment and container type influence field performance of Black Spruce container stock. New Forest. 13, 329–339.

Perala, D.A. 1990. *Populus tremuloides* Michx.: quaking aspen. In: Silvics of North America. Vol. 2. Hardwoods. *Edited by* R.M. Burns and B.H. Honkala. USDA For. Serv. Agric. Handb. 654, pp. 555–569.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, S and the R Development Core Team. 2010. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3, pp. 1–97.

Puttonen, P. 1986. Carbohydrate reserves in *Pinus sylvestris* seedling needles as an attribute of seedling vigor. Scand. J. For. Res. 1, 181–193.

Puttonen, P. 1996. Looking for the “silver bullet” – Can one test do it all? New Forest. 13. 9-27.

R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0 (athttp://www.R-project.org).

Retzlaff, W.A., Miller, A. E., Allen, R. M. 1990. Comparison of container-grown *Pinus taeda* L. seedlings raised outdoors and in a growth chamber in sunlight. New Forests. 4, 223—230.

Ritchie, G. A. 1982. Carbohydrate reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Can. J. For. Res. 12, 905-912.

Ritchie, G.A. 1984. Chapter 23: Assessing seedling quality. In: Duryea, M. L., Landis, T.D, eds. Forest Nursery Manual: Production of bareroot seedlings. Martinus Nijhoff/Dr W. Junk Publishers. The

Hague/Boston/Lancaster, for Forest Research Laboratory, Oregon State University. Corvallis. 386 p.

Simpson, D.G. 1991. Growing density and container volume affect nursery and field growth of interior spruce seedlings. Northern J.  Appl. Forestry. 8, 160-165.

Snedden, J.; Landhäusser, S.M.; .Lieffers, V.J., Charleson, L.R. 2010. Propagating trembling aspen from root cuttings: impact of storage length and phenological period of root donor plants. New Forest. 39, 169–182.

Stape, J.L., Goncalves, J.L.M., Goncalves, A.N. 2001. Relationships between nursery practices and field performance for *Eucalyptus* plantations in Brazil – A historical overview and its increasing importance. New Forest. 22, 19-41.

Steffens, G. L. 1988. Gibberellin biosynthesis inhibitors; comparing growth-retarding effectiveness on apple. - J. Plant Growth. Regul. 7, 27-36.

Sutherland, C., Newsome, T. 1988. Field performance of five interior spruce stock types with and without fertilization at time of planting. USDA For. Serv. Gen. Tech. Rep. RM-167, pp 195-198.

Sutton, R. E., 1979. Planting quality and grading. For. Ecol. Manag. 2, 123-132.

van den Driessche, R. 1991. Influence of container nursery regimes on drought resistance of seedlings following planting. I. Survival and growth. Can. J. For. Res. 21, 555-565.

van den Driessche, R., Rude, W., Martens, L. 2003. Effect of fertilization and irrigation on growth of aspen (*Populus tremuloides* Michx.) seedlings over three seasons. For Ecol. Manag. 186, 381-389.

Williams, D.R., Ross, J.J., Reid, J.B., Potts, B.M. 1999. Response of *Eucalyptus nitens* seedlings to gibberellin biosynthesis inhibitors. Plant Growth Regul. 27, 125–129.

Wilson, B.C., Jacobs, D.F. 2006. Quality assessment of temperate zone deciduous hardwood seedlings. New Forests. 31, 417–433.

Yu, Q.B., Mantyla, N., Salonen, M. 2001. Rooting of hybrid clones of *Populus tremula* L. x *P. tremuloides* Michx. by stem cuttings derived from micropropagated plants. Scand. J. For. Res. 16, 238–245.

# Chapter 4. General conclusions

## 4.1 Research summary

My thesis aimed to evaluate the effect of seedling characteristics, planting time and container size in establishment and growth of aspen seedlings in mining reclamation areas one and two years after outplanting. In order to do this, two studies were performed. In the first experiment, the outplanting performance of different stock types (blackout, hormone, conventional and clipped) of aspen seedlings planted at different times of the year (summer, fall, spring) in three mining reclamation areas (Genesee, Suncor and Syncrude) was assessed. In the second experiment, we evaluated the developmental changes in height, root dry weight, root to shoot ratio (RSR), leaf dry weight and total non-structural carbohydrates (TNC) in roots and shoots of aspen seedlings subjected to different timing of transfer to outside conditions and height attained at bud set. These seedlings were grown in two different container sizes (340 and 170 $cm^3$). Morphological and physiological characteristics of seedlings grown in both containers were measured before planting as well as their outplanting performance after one growing season.

There were large differences in seedling morphological and physiological characteristics among the stock types produced for the first study. The H stock type had the highest RSR (3.6) while RSR of B, C and CL were 28%, 72% and 81% lower than H stock type respectively. Particularly, all stock types lifted for the summer planting had low RSR and root TNC compared to stock types lifted for the fall and spring planting. Summer stock was lifted during the growing season, which gave the fall and spring stock an added month for root growth and TNC accumulation. Interestingly, the time of lifting did not affect shoot growth likely because seedlings of all stock types at that time had already set bud either artificially using paclobutrazol application (H) or blackout treatment (B) or naturally under outside conditions (C and CL).

After outplanting the spring or fall planted H and B stock types showed the best establishment and early growth performance at all three planting areas. These stock types had the greatest height growth, diameter growth and leaf dry weight after one growing season. However, differences in growth between the H and B stock types and the C and Cl stock types were more striking at the two Fort McMurray planting areas. In contrast, differences among stock types planted at Genesee were much smaller, most likely because of the generally superior growing conditions at Genesee. I expected that seedlings with high RSR and TNC would perform better, as seedlings with high RSR have a relatively larger root system to explore for site resources (Jacobs et al, 2005) while seedlings with high TNC can access their reserves during establishment and speed the establishment of leaf area

(Landhäusser and Lieffers, 2002). In fact, seedlings with higher TNC reserves produced more leaf area than seedlings with low reserves.

Height growth, diameter growth and leaf dry weight at Genesee were about 3-fold, 6-fold, and 7-fold, respectively higher than Suncor and Syncrude. At Genesee, there was only 1.1% more height growth in H and B stock types than C, while at the Fort McMurray planting areas differences in height growth were larger (12.6%). At the Genesee planting area, height growth increased significantly in the second growing season with a 3- fold increase compared to the first growing season, indicating that the seedlings had established (overcome planting check) and were entering the exponential growth phase. In contrast, at the Fort McMurray planting areas height growth in the second growing season was very similar to the previous year's growth. It can be speculated that the better growth performance at Genesee is likely the result of higher soil nutrient content. Further, the use of plastic mulch to suppress competition and to retain soil moisture and the finer texture of the soil might have given seedlings an advantage at Genesee compared to the two Fort McMurray planting areas.

Terminal shoot dieback was greater in the C and CL stock types ranging between 0.6 and 6 cm for C and 6 to 14cm for Cl depending on the planting area. C stock type had greater shoot dieback at Genesee than Suncor; however its magnitude was proportional to height growth. On the other hand, shoot dieback of CL stock type was highest regardless of planting area. It can be speculated that the shoot dieback of seedlings of C and Cl stock type was caused by their low TNC reserves and their physiological condition. Clipping of Cl stock type caused a reduction in TNC reserves. These TNC reserves allowed little shoot regrowth after outplanting (Kozlowski, 1991).

Seedling response to time of planting varied. Overall height growth was better for the spring and fall planting times. Shoot dieback of summer planted C and Cl stock type was the highest, indicating that shoots might have been damaged after outplanting, which might relate to the only partially dormant shoot tissue. Then height growth could have been affected by the production of multiple leader shoots. At the Genesee planting area, in the likely less stressful conditions, seedlings did not respond to the timing of planting, indicating that the stock type characteristics are not as important in less stressed sites. On the other hand the seedlings planted in the summer at the two Fort McMurray planting areas were stunted, growing 31% less than seedlings planted either in fall or spring. This effect was still noticeable in the height growth after the second growing season.

Overall, the H stock type with the highest RSR and root TNC concentrations outperformed all other stock types, particularly in the two Fort McMurray planting areas, confirming that high RSR and root TNC are important characteristics for seedlings to do well in stressful conditions (Rietveld, 1989; Seifert et al, 2006).

In my second study, I found that seedlings moved outside the greenhouse accumulated root TNC earlier, likely because their height growth was reduced and therefore fewer reserves were used to grow new tissues. In these seedlings root TNC and RSR had reached a maximum earlier than in seedlings that were moved outside later (at week 9). The latter seedlings did not catch up to the RSR of seedlings moved outside earlier at the end of the first growing season; however, root TNC reserves were similar.

In another study I tested whether aspen seedlings grown under the same treatment conditions but in smaller containers will also produce similar RSR ratios and root TNC as the above seedlings. Although I found that seedlings grown in the larger container size were taller and had higher diameter, shoot dry weight, root dry weight and root volume than seedlings grown in the smaller containers, the RSR and root TNC were not different. With that in mind I outplanted all these seedlings and found that after one growing season none of the variables such as diameter growth, total height, root length, leaf dry weight, shoot growth, and root growth were different among the different container sizes and stock types. I therefore conclude that regardless of container size, seedlings with high RSR and root TNC will perform well. However, it is not clear yet whether these seedlings will also perform similarly under more stressful conditions as found in the two Fort McMurray planting areas (Chapter 2).

## 4.2 Management implications

Currently, most criteria for aspen seedling quality are based on parameters such as height and diameter which appear to be poor measurements to assess seedling outplanting performance. In fact, seedlings that had greater diameter and height were more likely to perform poorly than seedlings with shorter stature.  Most likely RSR and root TNC are better predictors of aspen seedling quality.  Nurseries should be assessing these variables as measures of quality of stock. Seedlings that show less planting check will be better suited to re-establish a forest canopy on a reclamation site and it appears that seedling with high RSR and root TNC showed better early outplanting performance.  These seedlings characteristics in combination with outplanting in spring or fall could expedite the early development of a tree canopy.

I do not recommend that seedlings be planted in the summer months when the shoot has not been fully hardened (Table 2-6). However, if there was plenty of summer rain and site conditions are appropriate for holding soil moisture for root development, summer plants might be possible.  Under high stress conditions, summer planted seedlings are likely more susceptible to desiccation and planting check, even though they are able to grow roots immediately after planting and prior to the dormant season (Taylor and Dumbroff, 1975; Good and Corell, 1982).

Clipping of seedlings cannot be recommended as an artificial means to improve RSR. Clipped seedlings had low TNC reserves and performed poorly on all sites; however, this could also be related to the fact that we had only clipped seedlings that were planted in the summer. Therefore clipping of dormant stock still requires additional testing. Treating seedlings with a hormone produced very uniform budset and appears to eliminate the need for clipping seedlings (Landhäusser et al. unpublished).

Aspen seedlings can be moved outside as early as 4 weeks after seeding, contrary to usual nursery practices of a minimum of 10 weeks. At the same time, it appears that seedlings can also have their shoot growth terminated at week 11 without affecting seedling quality. In addition growing aspen seedlings in small containers is feasible and could be combined with nursery practices such as moving them outside the greenhouse earlier and terminating their shoot growth with paclobutrazol to harden them. Together, these practices can greatly diminish some nursery production costs. Overall costs of heating and cooling, as well as sources of artificial light or shading with blackouts for lighting could be reduced (Landis, 1995).

Both of my studies showed that high RSR and root TNC concentrations are better seedling characteristics to evaluate the quality of aspen planting stock than height and diameter. High values of these characteristics can be also attained in smaller container size. Small seedlings occupy less space and more seedlings can be produced per unit greenhouse space and more can be stored per box, reducing frozen storage and shipping costs. Finally the planting costs of smaller stock will be lower as planters would be more efficient with smaller plug sizes. These reduced costs could translate into lower costs for the companies interested in forest reclamation, which could result in an increase in future planting densities without increasing the overall costs per hectare.

At roughly 10,000 shoots per hectare and after just two growing seasons, the seedlings planted at Genesee had achieved almost full canopy closure. The shade generated continued to suppress weed competition after the plastic mulch had been removed in 2011. This accomplishes one of the first steps in establishing tree cover on moving surface mined sites towards forested ecosystems. On the stressful Fort McMurray sites, the much slower growth rates will delay establishment making seedlings vulnerable to other agents. Further research is necessary to investigate the impact limiting site factors, such as nutrition and drought.

## 4.3 Future research

My two studies are the first that combine aspen seedling quality, time of the year when seedlings are outplanted, and the impact of nursery operations such as container size where seedlings are grown. Only two previous studies (Martens, 2006; Landhäusser et al., unpublished) examined morphological and physiological seedling characteristics (seedling quality) and related them to outplanting performance. Due to the importance of these studies, continuous monitoring of the long-term effects of planting time and stock type on the growth and development of a tree canopy should continue. Perhaps the initial better performance of stock types with high RSR and root TNC reserves might not be visible 5 years after planting. As already mentioned, partial canopy closure was accomplished after two growing seasons at Genesee. Canopy closure will affect soil nutrients, soil and air temperature and it has the potential to exclude invasive species (Man and Lieffers 1999) providing a habitat for later successional tree and understory species. Hence future monitoring of seedling establishment from these species which might have been in soil or germinated from seeds carried from neighbor sites would indicate the recruitment of a forest ecosystem closer to the predisturbed one.

The treatment of aspen seedlings with paclobutrazol in order to stop shoot growth has proven to be effective (Landhäusser et al, unpublished). In chapter 3, I treated aspen seedlings with paclobutrazol as early as week 7; however they stopped shoot growth at week 11 after elongating 13 cm on average. Seedlings treated with paclobutrazol performed better (H stock type), however the underlying physiological changes as a result of the hormone treatment are not fully understood; thus more research on the physiological impacts and the dosage and method of application is needed. It would be useful to test higher and lower concentrations of paclobutrazol than the one used in these studies and if soaking the styroblocks or directly spraying the seedlings with paclobutrazol as method of application could accomplish shoot cessation at a desired target height.

The outplanting of aspen seedlings grown in small containers subjected to an early move to outside conditions and shoot growth terminated in forest reclamation sites with limiting conditions such as Fort McMurray has to be investigated. I planted these seedlings in a site with excellent growing conditions for plant growth (good nutrient status, good drainage); however planting performance could be significantly different in a site with limiting conditions.

## References

Good, G.L. and Corell, T.E. 1982. Field trials indicate the benefits and limits of fall planting. Am. Nurseryman. 155:31-34.

Jacobs, D.F., Salifu, K.F., Seifert J.R., 2005. Relative contribution of initial root and shoot morphology in predicting field performance of hardwood seedlings. New Forest. 30, 235-251

Kozlowski, T.T.1991. Effects of environmental stresses on deciduous trees. In: H. A. Mooney, E. Pell & W. E. Winner, eds. Response of plants to multiple stresses. Academic Press, San Diego, California. pp 391-411.

Landhäusser, S.M., V.J. Lieffers., 2002. Leaf area renewal, root retention and carbohydrate reserves in a clonal tree species following aboveground disturbance. J. Ecol. 90, 658-665.

Landis , T.D. 1995. Chapter 4. Environmental Controls and Seedling Production Equipment. Volume one: Nursery Planning, Development and Management. In: The Container Tree Nursery Manual. USDA Agricultural Handbook 674.

Man, R. and V.J. Lieffers. 1999. Are mixtures of aspen and white spruce more productive than single species stands? Forestry Chronicle 75:505–513.

Martens, Lee Anthony. Response of aspen (Populus tremuloides Michx.) seedlings to cold storage, root aeration and watering regime. MS thesis. University of Alberta, Edmonton, 2006. Print

Rietveld, W.J., 1989. Transplanting stress in bareroot conifer seedlings: Its development and progression to establishment. North. J. Appl. For. 6, 99– 107.

Seifert, J.R; Jacobs, D.F. and Selig, M.F. 2006. Influence of seasonal planting date on field performance of six temperate deciduous forest tree species. For. Ecol. Manag. 223: 371–378.

Taylor, J.S. and Dumbroff, E.B. 1975. Bud, root, and growth regulator activity in *Acer saccharum* during dormant season. Can. K. Bot. 53: 321-331.

# APPENDIX A

**Table A1.** Means and (standard deviation) of seedling growth of seedlings planted in summer and fall of 2008 and spring of 2009 at Syncrude after first growing season. n=128 for total height, height growth and shoot dieback and n=16 for the other variables. Numbers in a column followed by the same letter are not considered significantly different (LSD test, p=0.05).

| | | First growing season | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Planting time** | **Stock type** | **Total height (cm)** | **Height growth (cm)** | **Shoot dieback (cm)** | **Diameter growth (mm)** | **Root growth (g)*** | **Shoot dry weight (g)** | **RSR** |
| Spring | Blackout | 28.85 **d** (1.20) | 9.93 **c** (2.12) | 0.14 **b** (0.14) | 1.71 **ab** (0.81) | 1.14 (0.60) | 2.65 **c** (0.49) | 1.71 **ab** (0.47) |
| | Hormone | 29.55 **d** (2.33) | 12.34 **b** (2.37) | 0.20 **b** (0.45) | 2.06 **a** (0.82) | 1.20 (0.73) | 2.99 **bc** (0.67) | 1.79 **a** (0.24) |
| | Conventional | 42.46 **a** (2.32) | 3.99 **ef** (1.35) | 0.28 **b** (0.28) | 1.17 **bc** (0.32) | 0.95 (0.29) | 4.22 **a** (0.90) | 1.23 **cd** (0.21) |
| Summer | Blackout | 24.70 **e** (1.91) | 4.13 **ef** (0.92) | 0.35 **b** (0.76) | 0.76 **c** (0.27) | 0.71 (0.16) | 1.57 **d** (0.17) | 1.48 **bc** (0.15) |
| | Hormone | 24.49 **e** (1.67) | 6.82 **d** (1.24) | 0.07 **b** (0.06) | 0.81 **c** (0.54) | 0.90 (0.42) | 1.51 **d** (0.40) | 1.94 **a** (0.38) |
| | Conventional | 38.11**b** (4.51) | 3.16 **f** (1.20) | 0.99 **a** (0.96) | 0.83 **c** (0.50) | 0.99 (0.30) | 2.76 **bc** (0.30) | 1.03 **d** (0.26) |
| Fall | Blackout | 27.96 **d** (1.79) | 7.49 **d** (1.50) | 0.09 **b** (0.17) | 1.41 **b** (0.46) | 0.94 (0.23) | 2.46 **c** (0.25) | 1.82 **a** (0.23) |
| | Hormone | 33.27 **c** (1.68) | 14.22 **a** (1.83) | 0.07 **b** (0.10) | 2.26 **a** (0.66) | 1.43 (0.58) | 3.40 **b** (0.98) | 1.88 **a** (0.24) |
| | Conventional | 44.03 **a** (2.59) | 4.84 **e** (1.13) | 0.21 **b** (0.35) | 1.47 **b** (0.51) | 1.32 (0.43) | 4.92 **a** (0.58) | 1.08 **d** (0.12) |

*Root growth was not statistically significant

**Table A2**. Means and (standard deviation) of seedling growth of seedlings planted in summer and fall of 2008 and spring of 2009 at Syncrude after second growing season (n=128). Numbers in a column followed by the same letter are not considered significantly different (LSD test, p=0.05)

| | | **Second growing season** | | |
|---|---|---|---|---|
| **Planting time** | **Stock type** | **Total height (cm)** | **Height growth (mm)** | **Diameter growth (mm)*** |
| Spring | Blackout | 36.70 **def** (3.14) | 8.31**bcd** (1.89) | 0.75 (0.64) |
| | Hormone | 38.24 **cd** (5.08) | 9.34 **bc** (4.74**)** | 0.73 (0.50) |
| | Conventional | 48.38 **a** (4.65) | 8.21 **bcd** (3.06**)** | 0.75 (0.30) |
| Summer | Blackout | 32.68 **f** (4.77) | 9.25 **bc** (2.90) | 0.72 (0.28) |
| | Hormone | 33.61 **ef** (3.72) | 9.38 **bc** (2.54) | 0.68 (0.43)) |
| | Conventional | 42.23 **bc** (4.04) | 5.49 **d** (2.00) | 0.77 (0.23) |
| Fall | Blackout | 37.49 **de** (4.21) | 9.72 **b** (3.25) | 0.53 (0.20) |
| | Hormone | 46.23 **ab** (4.92) | 12.95 **a** (3.92) | 0.73 (0.36) |
| | Conventional | 49.73 **a** (5.12) | 6.54 **cd** (3.20) | 0.55 (0.21) |

*Diameter growth was not statistically significant.

**Table A3.** P values of variables measured at Syncrude planting area of seedlings planted in summer and fall of 2008 and spring of 2009 after first growing season.

| | First growing season | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Total height*** | **Height growth** | **Shoot dieback** | **Diameter growth** | **Root growth*** | **Shoot dry weight*** | **RSR** |
| Planting time | **0.001** | **0.001** | **0.033** | **0.001** | 0.101 | **0.001** | 0.334 |
| Stock type | **0.001** | **0.001** | **0.017** | **0.004** | 0.552 | **0.001** | **0.001** |
| Interaction | **0.003** | **0.001** | 0.094 | 0.084 | 0.743 | 0.057 | 0.125 |

*Total height, root growth and shoot dry weight in first growing season were transformed using Log10.

**Table A4.** P values of variables measured at Syncrude planting area of seedlings planted in summer and fall of 2008 and spring of 2009 after second growing season.

| | **Second growing season** | | |
|---|---|---|---|
| | **Total height** | **Height growth** | **Diameter growth** |
| Planting time | **0.001** | 0.178 | 0.434 |
| Stock type | **0.001** | **0.004** | 0.922 |
| Interaction | 0.077 | 0.144 | 0.872 |

# APPENDIX B

**Table B1.** Mean and (standard deviation) of seedlings planted in summer and fall of 2009 and spring 2010 at Syncrude after first growing season. n=128 for total height, height growth and shoot dieback and n=16 for the other variables. Numbers in a column followed by the same letter are not considered significantly different (LSD test, p=0.05).

| Planting time | Stock type | Total height (cm) | Height growth (cm) | Shoot dieback (cm) | Diameter growth (mm) | Root growth (g) | Shoot dry weight (g) | RSR | Leaf dry wt (g) |
|---|---|---|---|---|---|---|---|---|---|
| Spring | Blackout | 37.05 **bc** (2.12) | 7.26 **bc** (1.18) | 0.40 **bc** (0.48) | 1.42 **b** (0.53) | 1.93 **b** (0.78) | 2.02 **b** (0.58) | 1.84 **b** (0.51) | 1.19 **a** (0.22) |
| | Hormone | 32.70 **ed** (3.74) | 14.82 **a** (3.21) | 0.36 **c** (0.44) | 1.72 **b** (0.54) | 1.58 **b** (0.63) | 1.27 **cd** (0.39) | 2.91 **a** (1.47) | 1.01 **b** (0.39) |
| | Conventional | 39.47 **b** (3.55) | 6.75 **c** (2.05) | 2.95 **a** (2.09) | 1.81 **b** (0.80) | 1.76 **b** (0.50) | 2.19 **b** (0.61) | 1.24 **c** (0.14) | 1.19 **a** (0.58) |
| Summer | Blackout | 30.43 **ef** (1.41) | 3.45 **d** (1.43) | 0.24 **c** (0.31) | 0.82 **c** (0.16) | 1.61 **b** (0.19) | 1.04 **d** (0.21) | 1.95 **b** (0.28) | 0.52 **d** (0.24) |
| | Hormone | 24.87 **g** (3.13) | 8.87 **b** (2.33) | 0.34 **c** (0.29) | 0.97 **c** (0.43) | 1.17 **b** (0.97) | 0.81 **e** (0.43) | 2.16 **b** (0.11) | 0.68 **bd** (0.28) |
| | Conventional | 35.06 **cd** (2.10) | 1.39 **e** (0.78) | 1.33 **b** (1.48) | 0.82 **c** (0.33) | 0.82 **c** (0.38) | 1.08 **d** (0.27) | 1.23 **c** (0.31) | 0.37 **d** (0.34) |
| | Clipped* | 29.54 (5.89) | 1.22 (1.29) | 6.83 (5.42) | 0.75 (0.32) | 0.97 (0.24) | 2.32 (0.12) | 0.72 (0.19) | 0.38 (0.10) |
| Fall | Blackout | 39.40 **b** (2.27) | 7.36 **bc** (1.60) | 0.43 **bc** (0.48) | 1.86 **b** (0.87) | 2.67 **a** (1.42) | 2.58 **ab** (0.78) | 1.76 **bc** (0.25) | 1.30 **a** (0.35) |
| | Hormone | 30.00 **f** (1.91) | 12.55 **a** (1.79) | 0.35 **c** (0.25) | 2.24 **a** (0.19) | 1.38 **b** (0.62) | 1.44 **c** (0.17) | 2.04 **b** (0.56) | 0.95 **b** (0.16) |
| | Conventional | 46.34 **a** (2.86) | 8.26 **bc** (1.30) | 0.56 **bc** (0.59) | 2.14 **a** (0.33) | 2.32 **a** (0.72) | 3.12 **a** (1.07) | 1.19 **c** (0.20) | 1.12 **a** (0.42) |

*Clipped stock type was not compared statistically with the other three stock types.

**Table B2.** P values of variables measured at Syncrude planting area of seedlings planted in summer and fall of 2009 and spring 2010 after first growing season.

| | Total height | Height growth [a] | Shoot dieback | Diameter growth | Root growth | Shoot dry weight [b] | RSR | Leaf dry weight |
|---|---|---|---|---|---|---|---|---|
| Planting time | **0.001** | **0.001** | **0.013** | **0.001** | **0.001** | **0.001** | 0.160 | **0.001** |
| Stock type | **0.001** | **0.001** | **0.001** | 0.126 | **0.001** | **0.001** | **0.001** | 0.290 |
| Interaction | **0.000** | **0.002** | **0.003** | 0.809 | **0.027** | 0.324 | 0.200 | 0.107 |

[a] Height growth was transformed with square root

[b] Shoot dry weight was transformed with Log10