

Production and Performance of the Native Newfoundland Plant Species *Alnus viridis*
subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale* for Restoration
(Granite Canal, South-Central Newfoundland, Canada)

By:

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ABSTRACT

To offset loss of fish habitat, Newfoundland and Labrador Hydro (currently Nalcor Energy) constructed the Granite Canal Fish Habitat Compensation Facility (FHCF). In conjunction with construction the riparian zone was vegetated with a non native hydroseed mixture and later with native species including *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale*. Due to a lack of information on the use of native species for riparian restoration in Newfoundland, research was directed at developing propagation protocols, tracking survival/growth and developing recommendations for future work. The results indicate stratification is not required for germination of *A. viridis* subsp. *crispa* or *B. papyrifera* but was required for *C. stolonifera* and increased germination of *M. gale*. Cuttings and live stakes of *A. viridis* subsp. *crispa* and *B. papyrifera* rooted poorly, whereas *C. stolonifera* and *M. gale* rooted well. During field trials overall survival was high and the incidence of herbivory was low. Treatment of plants with a commercial mycorrhizal fungi inoculant did not result in enhanced growth however growth media had a significant effect on growth. All species except *Cornus* were not negatively affected by a non-native hydroseed mixture.

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List of Abbreviations

ANOVA	Analysis of Variance
DFO	Department of Fisheries and Oceans
FHCF	Fish Habitat Compensation Facility
IBA	Indole Butyric Acid
MUN	Memorial University of Newfoundland
NLH	Newfoundland and Labrador Hydro
US	United States
USDA	United States Department of Agriculture

Chapter 1: General Introduction

1.1 Background

In August of 2003 Newfoundland and Labrador Hydro (NLH, currently Nalcor Energy) officially opened the Granite Canal Hydroelectric Generating Station (N 48°11' 42.2", W 56°47'36.6") located approximately 85 km south-west of the town of Millertown via dirt road, (Figure 1.1). The Granite Canal Station added an additional 40 megawatt generating capacity to the existing Bay d'Espoir Hydroelectric development taking advantage of the 40 m head difference between Granite Lake and Meelpaeg reservoir (Hurst, 2003). Associated with the development was the diversion of water from the existing Granite Canal into a new 1.9 km power canal, construction of a concrete intake, penstock, power house and 1.4 km tailrace canal (Hurst, 2003). The diversion of water away from the existing Granite Canal into the new power canal resulted in the loss of 45,000 m² of spawning and rearing habitat for *Salmo salar* L. (ouananiche, also known as landlocked Atlantic salmon) and *Salvelinus fontinalis* Mitchill (brook trout). Under Section 35(1) of the *Fisheries Act*, habitat alteration, disruption or destruction of fish habitat is prohibited (Government of Canada, 1985). However, under Section 35(2) of the *Act*, habitat alteration, disruption or destruction may be allowed under conditions authorized by the Minister or under regulations made by the Governor in Council (Government of Canada, 1985). To allow the project to proceed, the Minister of Fisheries and Oceans Canada authorized the destruction of the fish habitat downstream of the diversion on the condition that Newfoundland and Labrador Hydro undertake the

construction of a Fish Habitat Compensation Facility (FHCF), (Figure 1.2). This facility consists of a network of meandering streams which has a total length of 3.3 km and provides habitat suitable for spawning and rearing of ouananiche and brook trout (Hurst, 2003). To ensure the effectiveness of the created fish habitat there was a need to provide suitable physical and biological components such as suitable water depths and shoreline vegetation (Dalley et al., 2004).

The FHCF is located in south-central Newfoundland within the Central Barrens subregion of the Maritime Barrens ecoregion. The topography in the area is generally gently rolling ground moraine scattered with glacial erratics and dominated by barrens interspersed with areas of peat bog and forest (Government of Newfoundland and Labrador, 2011). The dominant soils in the area are relatively dry brown soils which contain mostly inorganic material (humo ferric podzols), (Government of Newfoundland and Labrador, 2011).

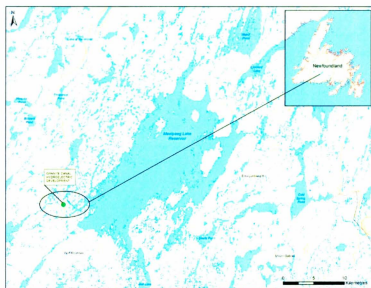


Figure 1.1: Location of the Granite Canal Site in south-central Newfoundland

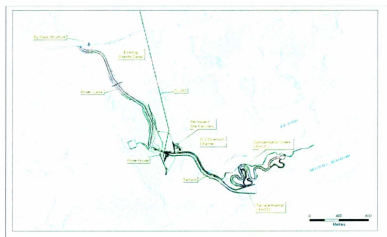


Figure 1.2: The Granite Canal Hydroelectric Development and Fish Habitat Compensation Facility (Figure courtesy of Newfoundland and Labrador Hydro)

Dominant vegetation is a mixture of *Abies balsamea* (L.) Mill. (balsam fir) and *Picea mariana* (Mill.) B.S.P. (black spruce) interspersed with stands of *Betula papyrifera* Marsh (paper or white birch) and *Larix laricina* (DuRoi) K. Koch (larch). There are also numerous bog, fen and barren areas which contain the shrub species *Betula michauxii* Spach and *Betula pumila* L. (species of dwarf birch), *Vaccinium angustifolium* Ait. (blueberry), *Vaccinium vitis-idaea* L. (partridge berry), *Empetrum* spp. (crowberry), *Rosa nitida* Willd. (bog rose), *Chamaedaphne calyculata* (L.) Moench (leather leaf) and *Myrica gale* L. (sweet gale). Within the general area there are also numerous areas where mineral soils have been exposed due to previous development associated with the Bay d'Espoir hydroelectric development. These previously disturbed areas are dominated by extensive *Alnus viridis* (Villars) DC subsp. *crispa* (Ait.) Turrill (green alder) thickets. A number of less common shrub species can also be found in disturbed areas, including a variety of *Salix* spp. (willow species), *Spiraea latifolia* (Ait.) Borkh. (meadow sweet) and *Cornus stolonifera* Michx. (Red-osier dogwood).

The climate of the Granite Canal area (Burnt Pond weather station, located approximately 40 km west of the study site) has an average July/August temperature of 14.6°C; average January/February temperature of -10.2°C and average yearly precipitation of 1437.5 mm of which 26% occurs as snow. Average daily temperature is 2.2°C with extreme summertime highs of 31.0°C and winter lows of -39.5°C recorded (Government of Canada, 2006).

Riparian zones are important to, and are influenced by, terrestrial and aquatic ecosystems (Osborne and Kovacic, 1993). In terrestrial ecosystems riparian zones

provide enhanced habitat for wildlife (Oelbermann and Gordon, 2000); provide refugia for small mammals and nesting sites for small birds (Naiman and Décamps, 1997); and support greater species diversity and abundance than upland sites (Doyle, 1990; La Rue et al., 1994). Riparian zones have also been shown to be effective for removing pollutants from runoff such as sediment (Lee et al., 2000; Hook, 2003), nutrients (Vought et al., 1994; Clausen et al., 2000) and pesticides (Naiman and Décamps, 1997). Vegetated riparian zones also provide organic matter for stream biota (Anderson and Sedell, 1979; Maloney and Lamberti, 1995; Naiman and Décamps, 1997; Oelbermann and Gordon, 2000; Muto et al., 2009), regulate stream temperatures (Johnson and Jones, 2000; Johnson, 2004), and stabilize river banks (Smith, 1976; Beeson and Doyle, 1995; Naiman and Décamps, 1997).

Due to bank instability and slumping at the FHCF, a mixture of non-native hydroseed was initially applied until native species could be procured. Unfortunately the hydroseed provided forage for *Rangifera tarandus* L. (caribou) and *Alces alces* L. (moose) which resulted in a further loss of bank stability at some locations (B. Sellars, pers. comm.)

NLH realized that the quantity of native plants required for revegetation was not available and plants would have to be produced from seeds and/or cuttings. Moreover, the knowledge necessary to produce these species in sufficient quantities was not readily available within Newfoundland. To accomplish their revegetation goals using native species, NLH partnered with Memorial University of Newfoundland's Botanical Garden (MUN Botanical Garden). MUN Botanical Garden was experienced with the propagation

of native plant species, provided suitable propagation facilities and possessed scientific expertise to accomplish the task. This partnership resulted in a Memorandum of Understanding that MUN Botanical Garden procure, produce and install 100,000 native plants for revegetation activities. The partnership also provided funds for public educational programs, and financial support for this Master's project.

The goal of this Master's project was to fill the inherent regional (Newfoundland and Labrador) knowledge gaps relating to the propagation and performance of native plant species. Propagation protocol development focused upon seed based and vegetative propagation of the native plant species *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale*. These species are indigenous to the project site and are commonly found within, or in close proximity to, riparian zones throughout Newfoundland. Performance monitoring included assessing the establishment, survival and growth of these species under a variety of field conditions and experimental treatments at the Granite Canal site. The propagation and field trial results were used to develop recommendations for future restoration work using these species. These recommendations fill the knowledge gaps for *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale* but may also serve as a starting point for the development of propagation and establishment protocols for other restoration species.

The thesis is organized into four chapters. Chapter 1 (this chapter) provides an overview of the project by outlining the natural environment in the project area and providing background into the development of the project. The chapter also discusses the evolution of the science of restoration ecology and the process of ecological restoration.

There is also discussion of Canadian laws which require restoration and habitat preservation (primarily related to fish habitat and riparian zones). Finally, the chapter culminates with a discussion of environmental changes (chemical and biological) associated with disturbance and provides discussion of the challenges that are faced when using native plant species for restoration activities. Chapter 2 focuses on the development of propagation protocols of the four native plant species (*A. viridis* subsp. *crispa*, *B. papyrifera*, *C. stolonifera* and *M. gale*) through a series of experimental treatments of seed and cuttings.

Chapter 3 outlines the effects of field conditions encountered at the FHCF upon the establishment growth and survival of the species. In particular, a number of field experiments were conducted to determine 1) the effects of competition from hydroseed upon native species growth, 2) effect of large mammal herbivory upon native species, 3) the effectiveness of commercially available mycorrhizal fungi inoculants for providing enhanced growth of native species and 4) the effect of growth media upon the growth of native species. The final chapter, Chapter 4 provides a general summary of research results provides and provides suggestions for future work.

1.2 Laws

In Canada, federal and provincial laws have been developed to reduce the impacts of development on the natural environment. Acts such as the *Canadian Environmental Assessment Act* (federal) and the *Environmental Protection Act* (within the province of Newfoundland and Labrador) ensure that the potential environmental effects of a project

are minimized. The goal of this legislation is to ensure sustainable development through the identification and mitigation of potential environmental effects prior to project commencement. Mitigation measures include fundamental assessment of the project location (i.e., could relocating the project result in diminished environmental effects?); assessment of project alternatives (i.e., could the project be conducted in an alternative manner which can decrease the environmental impacts?); assessment of opportunities to compensate for lost habitat (i.e., construction or enhancement of habitat to balance the habitat lost) and assessments of project effects upon the socio economic environment. In addition, standard mitigation measures are implemented (e.g., erosion control measures such as silt fencing) to reduce the effects of the project. If all mitigation measures are implemented and substantial residual environmental effects remain, either Act may allow regulatory authorities to stop the project from proceeding.

The *Fisheries Act* prohibits the alteration, disruption or destruction of fish habitat and prohibits the deposit of any substance into a waterbody which may be detrimental to fish or fish habitat (Government of Canada, 1985). In 1986 the Canadian Department of Fisheries and Oceans (DFO) adopted a policy of no net loss of fish habitat. In particular this policy outlines an approach whereby any fish habitat that is altered, disrupted or destroyed through construction or development must be repaired or reconstructed. The *Act* also requires financial assurances to be provided by the proponent to ensure that rehabilitation/compensation can be performed in the event the proponent fails to carry out rehabilitation/compensation activities (Fisheries and Oceans Canada, 2009). Please note that in April 2012 the Government of Canada announced that changes to both the

Fisheries Act and the *Canadian Environmental Assessment Act* will be forthcoming. As of the time that this thesis was written these changes were not enacted therefore any changes are not reflected within.

1.3 Ecological/Chemical Impacts of Anthropogenic Disturbance

Anthropogenic disturbances can lead to a number of changes to the natural environment including the direct loss of habitat (Ballard et al., 1988; Quétier and Lavorel, 2011; Syphard et al., 2011) and wildlife avoidance of areas of development (Ballard et al. 1988; Forman and Alexander, 1998; Mahoney and Schaefer, 2002). The physical loss of habitat and avoidance of certain areas leads to habitat fragmentation which may lead to higher densities of animals in smaller areas which in turn may result in higher incidence of predation (Ballard et al., 1988).

While anthropogenic disturbance has effects upon the local fauna they can also have major effects upon the local flora and its ability to re-establish after disturbance. The most important effects of disturbance upon the re-establishment of vegetation are those which affect soil composition. Soil can be thought of as a living entity which is essential for the functioning of terrestrial ecosystems (Lal, 1999). Undisturbed soil contains a number of microbial, plant and animal populations which are essential to its functioning. Lal (1999) has stated that a single teaspoon of fertile soil can contain over 9 billion organisms. Soil microbes such as bacteria and fungi break down organic matter releasing nutrients which can be absorbed by plant roots and function on the development of soil structure (Ros et al., 2004). Mycorrhizal fungi associations further enhance a

nutrient absorption of plants by forming symbiotic relationships which effectively increase the root surface area of the plant (Davies et al., 2000; Hart et al., 2003; Klironomos, 2003; Piotrowski et al., 2004). In addition, soil macro invertebrates such as worms and insects also aid in the breakdown of organic matter and aerate soils allowing increased gas exchange and water infiltration (Lavelle et al., 2006).

Typically, when an area is disturbed as a result of development, the top layers of organic and mineral based soil are removed or are compacted so that the soil cannot function normally. Removal of the overburden is usually required to expose a suitable base material for construction activities (e.g., road construction, mining and construction of physical works). These activities can leave behind a substrate with low soil moisture and nutrient content, high substrate temperature and chemical toxicity (Mallik and Karim, 2008). Construction activities often results in the mixing of soil horizons (organic layer, topsoil, mineral soil, subsoil) leading to reduced soil quality (Shukla et al., 2004; Landis et al., 2005). In the case of disturbances associated with mining, quarry operations and other large construction projects (e.g., hydroelectric developments) there may be large piles of spoil, tailings and other waste materials which are devoid of organic matter (Kramer et al., 2000; Rydgren et al., 2011). While topsoil placement and addition of organic matter may promote the establishment of vegetation, adequate establishment may be hindered due to high acidity or alkalinity of the underlying waste material, presence of metals in soil and reduction in the abundance of soil microbes which aid in nutrient cycling (Pitchel and Salt, 1998; Sydnor and Redente, 2000). Compaction causes a loss of pore spaces between soil particles which in turn results in a soil which is poorly aerated

and restricts the movement of soil organisms (Whisenant, 1999). Compacted soils have less available water, have oxygen limitations and have disruptions in the cycling of nutrients (Whisenant, 1999). Ros et al. (2004) have shown that human trampling over a 29 day period had resulted in a significant decrease of vegetation cover, species diversity, basal respiration and enzymatic activity (provides an indication of biogeochemical cycling capacity of the soil).

1.4 Restoration Ecology

The increased focus on environmental repair has led to the development of a new branch of ecology termed restoration ecology, which is the science of restoring ecosystems (Sarr et al., 2004). Associated with restoration ecology is the concept of ecological restoration which includes the principles of restoration ecology but also includes the human sciences, natural sciences, politics, technology, economics and culture (Higgs, 2005). Initially, restoration ecology was focused upon the return of an ecosystem to a historical or indigenous state (Wagner et al., 2000) but recently the focus has shifted to the development of a natural self sustaining ecosystem (Halle, 2007). While ecological restoration has grown immensely over the last few decades there are opponents to the idea that humans can restore ecosystems. Katz (1995) is one such opponent citing that ecological restoration is just another example of how humanity tries to control the natural world. While this may be true, it is humanity's past control and adaptation of the natural world that has primarily resulted in the need for restoration activities. Advocates for restoration ecology recognize the need to hasten the process of

restoration but caution that factors relating to climate change such as temperature fluctuations, precipitation patterns, weather patterns and sea level changes may influence the success and direction of ecosystem restoration (Harris et al., 2006). Furthermore, Halle (2007) points out that restoration activities must be ecosystem specific and restoration strategies must be implemented in the context of former land use and existing surroundings. Similar to Halle, Hobbs et al. (2009) further builds on the idea that it may not be realistic to return an ecosystem to its pre disturbance condition. Particularly, Hobbs et al. (2009) suggest restoration practitioners need to evaluate restoration goals in the context of the current ecosystem state. Moreover they suggest that ecosystems may fall into three categories i) where the restoration of a historic ecosystem may be useful and achievable, ii) where restoration of the historic system is not possible but restoration of some key structure and function can be achieved or, iii) an area where biological and physical changes have resulted in the development of a novel ecosystem which is unlikely to return to the historic system as a result of restoration thresholds (Hobbs et al, 2009). Restoration practitioners would therefore need to carefully evaluate which category an altered ecosystem falls into, through the identification of barriers to restoration, in order to determine realistic restoration goals.

The development of restoration ecology has led to a set of terminology including restoration, rehabilitation, reclamation and remediation (Box 1.1). With the advent of this new terminology comes a sometimes obscure and unclear usage of the terminology. In addition to the interchangeable use of the terminology, restoration has been defined differently through the years. Initially, the definition of restoration focused on the return

to exact pre-disturbance conditions (Whisenant, 1999). However, more recently ecological restoration has been defined as the process of assisting the recovery of damaged, degraded or destroyed ecosystems (Hobbs, 2007). The changing definition of restoration further outlines that restoration ecology is an evolving field.

Box 1.1: Restoration ecology related terminology

Restoration: Restoration is defined as the return of an ecosystem to its pre-disturbance state both structurally and biologically. Restoration requires an in depth knowledge of pre-disturbance species composition, ecosystem processes and the intricate interactions of biological and physical processes (Dunster and Dunster, 1996).

Rehabilitation: Rehabilitation, unlike restoration, does not seek to return the site to its pre-disturbance state but rather tries to make degraded land useful again by creating a functional ecosystem which has different land use and species composition than the pre-disturbance condition (Dunster and Dunster, 1996; Whisenant, 1999).

Reclamation: Reclamation falls somewhere between restoration and rehabilitation in that it seeks to recreate a functionally stable ecosystem which contains similar species as the ecosystem prior to disturbance (Whisenant, 1999).

Remediation: A broad definition of remediation is to make good or to rectify. Remediation focuses on the repair process and gives no indication of the final end point (Bradshaw, 2002).

The terminology used when referring to the repair of damaged ecosystems may impart different meaning to the task at hand. Early definitions of restoration imply that an ecosystem be returned to its pre-disturbance condition in all facets whereas both reclamation and rehabilitation do not adhere to this guideline. True restoration would therefore require an in depth knowledge of ecological interactions, species interactions, environmental processes and may require substantial capital investment. To that end Choi (2007) suggests that the majority of ‘restoration projects’ are more akin to rehabilitation. It is evident that in the situation of severe disturbances such as mine sites, restoration is

unlikely to occur within an adequate time frame. Therefore rather than restoring sites like these it is likely that rehabilitation would better suit the situation whereby a suite of plant species better adapted to the site conditions are used rather than species native to the pre-disturbance condition.

Although the distinction between the terminologies may be obscure, it is clear that the development of restoration ecology and ecological restoration highlights society's increase in environmental awareness, responsibility and willingness to attempt to hasten the process of restoration. Overall restoration activities must take into account prior land use; the nature of ecosystem degradation; climatic changes that may influence the ecosystem; and the political, social and economic environment under which restoration activities are undertaken. Furthermore the completion of a restoration project should not be viewed as the end of restoration but rather as the starting point of natural restoration.

1.5 Challenges of Restoration: Supply of Native Species and Revegetation Practices

One of the major problems that NLH faced in establishing vegetation at Granite Canal was the lack of available suitable native plant species. Within Newfoundland and Labrador there is a single nursery, the provincial tree nursery in Wooddale, which specializes in the production of native plant species. Here, production is primarily restricted to five tree species *Picea mariana*, *P. glauca* (Moench) Voss (white spruce), *L. laricina*, *Pinus strobus* L. (white pine) and *P. resinosa* Ait (red pine). While some native species may be available, pioneer species such as *A. viridis* subsp. *crispa* are unavailable as nursery stock in Newfoundland. *A. viridis* subsp. *crispa* are usually thought of as a

weed species by many and are actively removed or killed when they become established. However, it is the 'weedy nature' (i.e. highly adaptable and able to survive under a variety of conditions) that commonly results in *A. viridis* subsp. *crispa* being one of the first woody plant species to colonize disturbed sites.

A regular practice for treating disturbed sites (e.g., road sides) in the province is to apply a hydroseed mixture of non-native species as 'quick fix' for erosion protection. This is also common practice in other areas (Mallik and Karim 2008; Bochet et al. 2010; Grant et al. 2011). The lack of requirements for the use of native plants in revegetation projects has led to a lack of available native root stock and seed. However, in recent years there has been considerable effort and expenditures by the United States Government to investigate and utilize native species for revegetation and restoration activities. Specifically, the Western Federal Lands Highway Division of the Federal Highway Administration and the USDA Forest Service developed a partnership which focuses on using native plants for restoration activities (Landis et al., 2004). Similarly, the US Forest Service recently adopted a national native plants policy which requires that genetically appropriate plant material be used for revegetation activities (Grant et al., 2011). Parks Canada has developed a guidance document titled "Principles and Guidelines for Ecological Restoration in Canada's Protected Natural Areas" which suggests, but does not require, the use of genetic material native to the area. The development of programs/guidelines which promote, or even better, require the use of natives for restoration activities will, over time, result in filling the knowledge gaps with respect to the propagation and overall utilization of native species. Adoption of policies by

government, and best management practices by industry that require widespread use of native species would ensure the development of efficient seed collection and propagation protocols by restoration practitioners.

Two general hypotheses were explored in this study 1) The native plant species *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera*, and *Myrica gale* can reliably be propagated using standard nursery practices, and 2) Native plant species can be used successfully for restoration projects within Newfoundland.

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Co-Authorship Statement

All manuscripts in this thesis were co-authored with Dr. Wilf Nicholls and Dr. Yolanda Wiersma. In all instances I was the principal contributor to project design and proposal, implementation of the field research component, data analysis and manuscript preparation. It is anticipated that Chapters 2 and 3 will be submitted to the Journal of Restoration Ecology for publication.

Chapter 2: Production of *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale* for Habitat Restoration

Abstract

Prior to this study, protocols for producing native plant species for riparian restoration projects were not readily available. This research fills the void by providing seed based and vegetative propagation protocols for the native species *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale*. Seed based propagation included variation of the length of stratification prior to sowing and exposure to vegetative smoke (smoke produced by burning plant material). Stratification studies resulted in increased percentage germination for *Cornus* and *Myrica* whereas smoke increased percentage germination of *Myrica*. Vegetative propagation studies included variation in rooting media, cutting length, rooting conditions and timing of the collection of cutting material. *Alnus* and *Betula* failed to root unless semi-hardwood material was used. Semi-hardwood cuttings under mist invariably produced the greatest rooting percentage in all species. Vegetative propagation studies using live stakes resulted in the complete failure of *Alnus* and *Betula* to root whereas both *Myrica* and *Cornus* rooted with and without rooting hormone application. Overall, *A. viridis* subsp. *crispa* and *B. papyrifera* were better suited to seed propagation without stratification, whereas *C. stolonifera* and *M. gale* were propagated using either seed based propagation with stratification or vegetative propagation.

2.1 Introduction

2.1.1 Challenges of Restoration using Native Species

The lack of suitable native plants has been cited by numerous authors as a reason for the continued reliance upon non-native species for revegetation activities (Mallik and Karim, 2008; Bochet et al., 2010 and Grant et al., 2011). Richards et al. (1998) indicate that although the development of technology allowing the use of native plants has progressed for some species, there is still a lack of knowledge and technology for many important restoration species. In addition, while commercial nurseries have developed successful methods of propagating some native species, the information is often not readily available to other practitioners (Harrington et al., 1999).

In NL, the provincial tree nursery is the only nursery that specializes in mass production of native plant species. However, production is restricted to forestry species. In many cases the species suited to restoration following anthropogenic disturbances are pioneer species which can inhabit and thrive in harsh conditions. For example, *Alnus viridis* subsp. *crispa*, a shrub species which is of little value to the forestry and horticultural industries in Newfoundland and Labrador, is often one of the first shrub species to colonize disturbed sites. Within the province the species is often thought of as a “weedy” species and most interaction with the species centers around its removal and eradication.

Should restoration ecologists successfully secure a nursery to produce native species for restoration the restoration ecologist and the nursery are faced with two tasks:

- 1) Sourcing suitable plant material (seeds, cuttings or rhizomes) for propagation.
- 2) Determination of suitable regional specific propagation protocols for each species.

The first task involves the collection of seed, cuttings or rhizomes from the wild which can be labour intensive and time consuming. The second task involves determining how best to propagate a species to maximize yield per unit effort (i.e., produce the greatest number of plants per unit area of greenhouse/nursery space). While the literature can provide some information on the propagation of some native species, this information is often not regionally specific.

One of the major factors which affect the success of seed based propagation is seed dormancy. Dormancy ensures that seed does not germinate at the wrong time of the year and subject seedlings to unfavourable conditions (Macdonald 1986; Whisenant, 1999). Dormancy may be physical or physiological. Physical dormancy occurs as a result of a hard or waxy seed coat (Macdonald 1986). Physiological dormancy results from insufficient embryo development or for biochemical reasons such as the incomplete digestion of fats, proteins, and complex compounds found within the seed; or due to the presence of chemical inhibitors (Macdonald 1986). Plant propagators have used several methods to overcome physical and physiological dormancy including physical scarification of the seed coat, hot water soak, acid scarification, cool moist stratification, warm moist stratification, early seed collection (before the onset of dormancy) and chemical soak (Macdonald 1986). Dormancy is a complex process which can be mediated by environmental conditions such as temperature during seed development, moisture/humidity during development and as a result of harvesting and storage

conditions (e.g., drying seed too rapidly) as well as heredity (Schopmeyer, 1974a). It is expected that a period of cool moist stratification would result in an increase in germination percentage over unstratified seeds as stratification would mimic the regional climate (i.e. a cool moist spring).

Vegetative smoke has been reported by numerous authors to break dormancy in a number of plant species (Brown and Van Staden, 1997; Light et al., 2002; Pérez-Fernández and Rodríguez-Echeverría, 2003; Flematti et al., 2004; Razanamandranto et al., 2005). While the ability of smoke to promote germination has been recognized for a number of years, the compound which promoted germination was not known until Flematti et al. (2004) identified the compound as the butenolide 3-methyl-2H-furo[2,3-c]pyran-2-one.

While the actual mechanism as to how smoke breaks seed dormancy is unknown it is thought to be complex (Razanamandranto et al., 2005). Razanamandranto et al. (2005) suggest that smoke may act through a number of pathways including scarification of the external and subdermal cuticle thereby increasing seed coat permeability; hormone like effects that trigger changes which lead to germination; activation of pH dependent growth regulators (e.g., nitrite, gibberellic acid, potassium cyanide); or overcoming the light requirement for germination. The ability of smoke to break dormancy has been observed in North America, Australia, South Africa and Europe within a variety of families (Brown and Van Staden, 1997; Van Staden et al., 2000; Pérez-Fernández and Rodríguez-Echeverría, 2003). Although the plant families in this study were not listed, the wide range of listed families suggest that the mechanisms of action may be fundamental in

nature and may apply to numerous other species from fire dependent ecosystems. Generally, the boreal forest across Canada, and within Newfoundland, are part of a fire dependent system (Weber and Stocks, 1998). Thus, it is expected that exposure of seeds to vegetative smoke will result in increased germination percentage over seed not exposed to vegetative smoke.

In addition to seed propagation, nurseries often use vegetative propagation to produce plants for revegetation activities. Common nursery vegetative propagation methods use cuttings taken from established plants to produce a clone of the parent plant. Macdonald (1986) provides a comprehensive overview of vegetative propagation using cuttings. Live staking is vegetative propagation method that uses large dormant cuttings of plant species which easily root without hormone treatment and do not require controlled conditions (i.e., greenhouse environment). Gray and Sotir (1996) provide an overview of vegetative propagation using live stakes.

Rooting success can depend on a number of factors including the health of the plant from which cuttings are collected, the timing of collection, juvenility and rooting media used (Macdonald, 1986). Some species can root at any time of year whereas others will only root if cutting material is collected at a particular time. Dirr and Heuser (2006) use the flowering crabapple (*Malus spp.*) as an example, citing that the cuttings taken earlier in the growing season (July) rooted in high percentage but cuttings taken later did poorly. Juvenility implies collecting cutting material from younger source plants (i.e., the 'younger' a plant is, the more likely a cutting will root). Dirr and Heuser (2006) give an example of the Katsura tree in which 100% of cuttings collected from one year old

seedlings rooted but cuttings from a 15-20 year old tree failed to root. The rooting media can also have significant effects upon rooting. Specifically, the media needs to retain moisture but drain well enough so as not to promote the development of conditions that cause the cutting to rot (Macdonald, 1986).

The primary goal of this research is to develop suitable and cost effective seed based and vegetative propagation protocols for the native plant species *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale* which may be used for future restoration and revegetation work. Furthermore this work will provide regionally specific (Newfoundland) propagation protocols for these species and will fill existing knowledge gaps.

2.1.2 Description and Goals of Current Work

Seed based propagation primarily focused on the breaking of seed dormancy either through cool moist stratification or exposure of seeds to vegetative smoke. Vegetative propagation investigated the effect of rooting media, cutting length, timing of cutting collection and rooting conditions upon the rooting success of nodal cuttings. Variation in the rooting media was used to determine if the standard media (1:1 Promix[®] - perlite) used by nurseries such as Memorial's Botanical Garden could be replaced with a more cost effective substitute. The thought behind variation in cutting length was that success of rooting was similar between short and long cuttings then more cuttings could be produced from the same quantity of plant material. Dirr and Heuser (2006) have

indicated that the timing of cutting collection can be critical for successful rooting in some species; therefore this study investigated rooting success in the context of the timing of cutting material collection.

Macdonald (1986) describes the use of misting systems and the use of a polyethylene enclosed propagation beds for the rooting of cuttings as standard nursery practice. Both methods were investigated to determine the appropriate method for each species. Assuming similar success the latter method could be used by nurseries to reduce overhead and production costs. The use of live stakes was also investigated as it serves as a way of plant production that does not require any specialized propagation facilities. Based upon the research goals and the investigation of the various treatments a number of hypotheses were developed as follows:

Seed Propagation

H_{1a}: The odds of germinating will be higher for stratified seed compared to unstratified seed for each species.

H_{1b}: The odds of germinating will be higher for smoked treated seed compared to untreated seed for each species.

Vegetative Propagation-Nodal Cuttings

H₀: The rooting media used will have significant effects upon the odds of rooting for each species (Promix®-Perlite>Peat-Sand>Sand).

Macdonald (1986) provides a listing of propagation media which regularly contain peat that have been successfully used by nurseries. Thus the ranking of the peat based media (Promix®-Perlite and Peat-Sand) above pure sand (Macdonald does not cite pure sand as a successful media). Macdonald's list includes peat-perlite (Promix®-Perlite in this case) and peat-sand media however Promix® is a sterile potting media which is pH balanced, contains perlite, vermiculite (another component of some of the media listed by Macdonald), macronutrients, micronutrients and a wetting agent thus it is ranked above the peat-sand media.

H₀: The odds of rooting will not be significantly different between 7.5 cm and 15 cm cuttings for each species.

H₀: Wood type will have a significant effect upon the odds of rooting (Semi-hardwood>dormant spring hardwood>dormant winter hardwood>actively growing hardwood).

Based upon the available propagation information (below) for the target species, the use of semi-hardwood (softwood for *Alnus*) was common hence it is anticipated that the odds of rooting would be highest for semi-hardwood cuttings. Macdonald (1986) indicated that research on difficult to root species suggested that one of the peak times to encourage rooting of hardwood cuttings is a few weeks prior to bud burst. Therefore it is anticipated that the odds of rooting for dormant spring hardwood would rank immediately below semi-hardwood for odds of rooting. Macdonald (1986) also indicated that cuttings

taken after bud break may have insufficient root development to support the top growth. Therefore it is anticipated that dormant hardwood cuttings would have greater odds of rooting than actively growing hardwood cuttings but would have lower odds of rooting than either semi-hardwood or dormant spring hardwood cuttings.

H₀: The odds of rooting under misted conditions will be significantly higher than cuttings rooted under high humidity conditions for each species.

Vegetative Propagation-Live Stakes

H₀: The odds of rooting will be significantly higher for hormone treated stakes compared to stakes not treated with rooting hormone.

2.1.3 Species Description and Current Propagation Knowledge

Alnus viridis subsp. *crispa* - Green Alder

Alnus viridis subsp. *crispa* is a common thicket forming, deciduous, shrub species which thrives within many disturbed areas of the province of Newfoundland and Labrador. Once established, the species can spread rapidly due to a well-developed root system and production of copious amounts of wind dispersed seed (Nickel et al., 2001). *A. viridis* subsp. *crispa* is a relatively short lived species reaching sexual maturity at 4-6 years of age and rarely living beyond 20 years (Brousquet et al., 1987). Members of the genus *Alnus* form symbiotic associations with nitrogen fixing actinomycete bacterial species of the genus *Frankia* (Nesme et al., 1985; Batzli et al., 2004; Huguet, 2004) allowing the species to thrive in nitrogen deficient soils.

Reproduction is sexual with annual viable seed yield of up to 9.5 million seeds per hectare annually (Farmer et al., 1985). It is unclear whether cool moist stratification is required for germination of the species since Farmer et al. (1985) successfully germinated seed with and without stratification. Schopmeyer (1974) has suggested that members of the genus germinate readily without stratification, but for seed lots displaying dormancy, stratification is recommended. Sound seed production can be variable between seed lots (Schopmeyer, 1974) and can even be variable between isolated groupings of plants (Farmer et al., 1985). Farmer et al. (1985) determined that light is required for germination unless seeds undergo a period of cool moist stratification. Therefore, it is predicted that in a stratification experiment germination percentage would be higher for seeds which have undergone stratification.

Vegetative propagation information on *A. viridis* subsp. *crispa* is not readily available. However, Dirr and Heuser (2006) and Schrader and Graves (2000) provide accounts of successfully rooting other members of the genus including *A. cordata* Loisel, *A. glutinosa* L. Gaertn, *A. incana* (L.) Moench and *A. maritima* (Marsh.) Muhl. ex Nutt. Thus, it is expected that it will be possible to root this species as a number of other members of the genus have been successfully rooted.

Betula papyrifera - Paper Birch

Betula papyrifera is a common deciduous tree species found throughout the province of Newfoundland and Labrador and around the world. The species quickly colonizes exposed mineral soils and can thrive in areas of nutrient deficient soils (Campbell and Hawkins, 2004). Reproduction is sexual with annual sound seed yield of wind dispersed winged seed up to 36 million seeds per acre (Clennett and Sanderson, 2002). The proportion of viable seeds is influenced by seed production such that a higher proportion of seed is viable in years with high seed production compared to years of low seed production (Brinkman, 1974a). Brinkman (1974a) and Bevington (1986) indicate that germination is mediated by exposure to adequate light and therefore stratification is not a requirement. However, while stratification may not be required, Bevington and Hoyle (1981) have suggested that exposure of seed to a stratification period increases sensitivity to light and can promote germination under low or even no light conditions. Therefore it is predicted that for this species germination percentage would be higher for seeds which have undergone stratification. Dirr and Heuser (2006) provide species specific information for vegetative propagation of the species. They indicate that cutting material used for rooting must just begin to firm (transitioning from softwood to semi hardwood). Wounded 15-20 cm long cuttings were treated with 0.8% indole byrutric acid talc powder and placed in a peat-sand media. However, the conditions under which rooting took place (e.g., misting) were not indicated. Thus, one would expect that rooting percentage would be highest for soft/semi-hardwood cuttings in a peat based media.

Cornus stolonifera- Red-Osier Dogwood

Cornus stolonifera is a common thicket-forming world-wide deciduous shrub found throughout the insular portion of the province and in southern and central regions of Labrador (Ryan, 1995). Reproduction is both sexual and asexual through layering, stolon development, shoot production from underground roots and through regeneration of new shoots from below areas of the plant that have sustained damage (Crane, 1989).

Peterson (1953); Brinkman (1974b); Haeussler and Coates (1986); Harrington et al. (1999); and Dirr and Heuser (2006) all suggest long cold stratification periods (60-90 days) are necessary for consistent germination. Acharya et al. (1991) found that the species exhibits variable germination between populations and between years. However, tetrazolium tests have revealed that the species displays a high proportion of viable seed (>90%; Acharya et al. 1991). Therefore it is predicted that for this species germination percentage would be higher for seeds which have undergone 60-90 days of stratification.

Dirr and Heuser (2006) indicate that the species is easy to root. Particularly, they indicate cuttings collected in June-July into early fall, treated with 0.1% IBA solution, placed under mist in perlite:peat media resulted in 90-100% rooting. Thus, it is expected that rooting percentage would be highest for soft/semi-hardwood cuttings in a peat based media under mist. There are numerous cultivars of *C. stolonifera* and production of this plant is well understood. *C. stolonifera* cultivars are standard stock at many woody plant nurseries. Propagation of these cultivars is by cuttings, therefore vegetative propagation in the species is well understood. However, there are no NL cultivars therefore rooting protocols may be different here.

Myrica gale- Sweet Gale

Myrica gale is a common thicket-forming deciduous shrub species found throughout Newfoundland and Labrador to approximately 57 degrees north (Ryan, 1995). The species is commonly found within a variety of wet habitats (Ryan, 1995). Reproduction is both sexual and asexual through rhizomes (Skene et al., 2000).

As with *C. stolonifera*, germination in the species appears to benefit from stratification. Dirr and Heuser (2006) indicate that seeds germinated well with 3 months of stratification at 4.4°C whereas Schwintzer and Ostrofsky (1989) indicate that stratification increased germination. Skene et al. (2000) also indicate that seeds harvested later into the winter produced greater germination than earlier harvested seed. Skene et al. (2000) also indicate that seeds of sweetgale germinate best if floated on water at 5°C for several weeks. While stratification promotes germination, *Myrica gale* seeds also require extended light exposure before germinating (Schwintzer and Ostrofsky, 1989; Skene et al., 2000; Dirr and Heuser, 2006). Therefore one would predict that for this species germination percentage would be greatest for seeds which have undergone long stratification periods (e.g., 90 days).

General information regarding vegetative propagation of the species is available. Skene et al. (2000) indicate that the species can be propagated by stem cuttings, root division or through the transplantation of suckers, but no propagation protocol is provided. Dirr and Heuser (2006) have indicated that semi-hardwood cuttings of a related species (*Myrica cerifera* L.) treated with 1-1.5% IBA solution, in a peat perlite media

under mist rooted 90%, whereas rooting of winter cuttings was poor. Thus, based upon the rooting results of *M. cerifera* it is expected that rooting percentage would be highest for semi-hardwood cuttings in a peat based media.

2.2 Methods

Propagation experiments were conducted at Memorial Universities Botanical Garden nursery facility located at 306 Mount Scio Road in St. John's, Newfoundland, Canada. This facility includes adjacent greenhouses with an attached header house, coldhouse located at the rear of the greenhouse/header house and numerous holding beds for potted and bare root stock.

2.2.1 Seed Propagation

Seed Collection

Seeds of all species were collected between late summer and fall 2005. *A. viridis* subsp. *crispa* and *B. papyrifera* seeds were collected from the Granite Canal area in early November. Drupes of *C. stolonifera* were collected in early September from the eastern Avalon Peninsula. Drupes of *C. stolonifera* were soaked in water and hand macerated (ground against each other by hand) to remove the fleshy exocarp. Seeds of *M. gale* were harvested from the eastern Avalon in early November and separated by gentle rubbing between one's hands.

Seed Treatment - Stratification

Stratification consisted of mixing seeds with moistened 00-silica sand followed by placement into Ziploc® bags with numerous pin holes for gas exchange. The Ziploc® bags were then placed into a household refrigerator maintained at 3-5°C for a predetermined stratification period (see Table 2.1). Following stratification treatment, seeds were removed from the fridge, rinsed to remove the silica sand, and sown into seed

starter flats containing moistened Promix®-BX, covered by a thin layer of vermiculite, and placed onto a heated sand frame maintained at 22°C. The number of germinations was then recorded every 2-4 days by marking each seedling with a toothpick. While initially the duration of stratification periods was set at 30, 60 and 90 days, the stratification period was slightly longer for all cases due to logistical reasons such as a lack of availability of space over the heated sand frame (Table 2.1).

Table 2.1: Stratification treatment levels used prior to sowing of *A. viridis* subsp. *crispa*, *B. papyrifera*, *C. stolonifera* and *M. gale*. Stratification was at 3-5°C and seed were placed in perforated Ziploc bags mixed with moistened 00-silica sand.

Treatment	Number of Seeds Sown	Days in Stratification	Number of Days Germinations Recorded
Fresh Seed (No Stratification)	384	0	119
30 Days Cool Moist Stratification	384	36	65
60 Days Cool Moist Stratification	384	65	71
90 Days Cool Moist Stratification	384	103	66

Seed Treatment - Smoke

Dry refrigerator stored unstratified seeds of *Alnus*, *Betula*, *Cornus* and *Myrica* were soaked in water for 48 hours to imbibe. Seeds were then sown as for stratification tests (above) but were not covered with vermiculite. Seeds (384 of each species per treatment) were placed into the smoke box and subjected to smoke exposure of 0 minutes (control), 10, 30, 60 and 180 minutes. Seeds were removed from treatment, covered with vermiculite, lightly watered, placed on the heated sand frame and germinations counted every 2 to 4 days for a 65 day period. Smoke was produced by heating alder catkins on an

electric range element contained in a barbeque fire box. The smoke then passed through flexible aluminum ducting into a box containing the seeds (Figure 2.1).

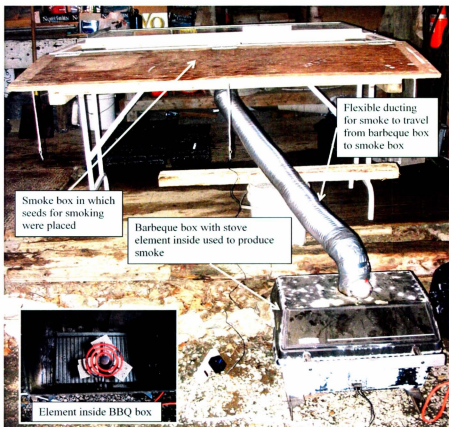


Figure 2.1: Setup used for the smoking of native plant seeds. Smoke was produced by heating *A. viridis* subsp. *crispa* catkins in an aluminum pan placed over a stove element. Smoke passed from barbeque box to the smoke through flexible ducting.

Seed Viability Testing

To compare the proportion of seeds that germinated during treatments (stratification and smoke) to the proportion of seeds that were actually viable, seed viability testing was undertaken. Viability of dry refrigerator stored unstratified seeds was tested by the tetrazolium test and cut seed viability test. For tetrazolium testing and cut seed viability testing, seeds of each species were first soaked in tap water for 24 hours to imbibe. Then for each test, one hundred randomly selected seeds of each species were longitudinally bisected using a scalpel (razor blade for *Cornus*). One half of each bisected seed was placed into a glass vial containing tap water until all seeds were cut.

Following the tetrazolium testing procedure outlined by Macdonald (1986) bisected seeds were placed into a 1% solution of tetrazolium chloride, incubated in the dark (inside a covered cardboard box) at room temperature within the header house of the Botanical Garden nursery facility for 15-18 hours. After incubation the seeds were individually scored as viable (metabolically active) or non-viable based upon the staining pattern. As indicated by Macdonald (1986) the presence of a pink-red colour indicated viability whereas unviable seeds remained unstained. While Macdonald recommended 24 hour incubation the staining of an initial seed lot of each species produced staining within 15-18 hours.

For the cut seed viability test seeds were scored as viable or non-viable based on embryo colour and texture. If the bisected embryo was a white colour without areas of brown tissue, and if the embryo had a firm (not hard) texture it was scored as viable.

2.2.2 Vegetative Propagation

Vegetative propagation of native species within this study used nodal cuttings and live staking. Cutting material was collected from along the sides of the Trans Canada Highway within an approximately 8 km² area west of St. John's, Newfoundland.

Dormant hardwood (late fall/winter), actively growing hardwood (summer), semi-hardwood (late summer/early fall) and dormant hardwood (spring) cuttings of *A. viridix* subsp. *crispa*, *B. papyrifera*, *C. stolonifera* and *M. gale* were collected during 2005 and subjected to several treatments. Treatments included the use of three different rooting media (1:1 Promix®-Perlite, 1:1 Peat-Sand and Sand), variation of cutting length (7.5 cm or 15 cm) and variation in rooting conditions (mist or high humidity) as outlined in Table 2.2. Collection of cutting material in the field consisted of taking only sections of plant material near the apex of a branch or stem. The goal was to select the newest growth as suggested by Macdonald (1986), while reducing damage to the plant as a result of the collection of cuttings. At the nursery cuttings of each species were cut to length (15 cm or 7.5 cm), sterilized in a 5% bleach-water solution (50 mL bleach/L solution + 1 drop dish detergent/L), thoroughly rinsed and blotted dry. Cuttings were dipped into the liquid rooting hormone Stim Root 10000 (1% Indole-3-butyric acid) as per the manufacturer's directions, stuck to a depth of half the cutting length into each of the media and placed into the respective rooting treatment. Tools (cutting boards, beakers and other glassware), cutting preparation surfaces and nursery containers were washed in a bleach/soap solution prior to cutting preparation. Throughout the process of cutting preparation surfaces were regularly treated with a mist of 70% isopropyl alcohol. Cutting blades used

for each group or species of cuttings were regularly dipped into 70% isopropyl alcohol prior to and periodically during usage. The misting treatment utilized in this study is similar to that described by Macdonald (1986) and utilized a Mist-A-Matic (E.C. Geiger Inc.) system to provide intermittent mist with a heating coil providing a constant bottom heat of 22°C. High humidity treatments consisted of two different setups. The greenhouse high humidity treatment consisted of a closed chamber over top of a heated sand frame. A soaker hose installed in the sand frame was connected to the Mist-A-Matic system so that the sand in the sand frame remained wet to maintain high humidity within the chamber. The coldhouse (unheated greenhouse) high humidity treatment consisted of an open topped chamber which was covered with a polyethylene top. The chamber was constructed over top of an unheated sand frame. A soaker hose installed in the sand frame was connected to a timer that delivered water 4 times per day for 5 minutes to keep the sand moist and maintain high humidity. The greenhouse high humidity treatment was used for dormant winter hardwood, semi-hardwood and dormant spring hardwood cuttings whereas the cold house humidity treatment was used for actively growing hardwood cuttings. The cold house humidity treatment was used for actively growing hardwood cuttings due to high summertime greenhouse temperatures. Cuttings remained in each treatment for 7-8 weeks, were removed, fertilized and allowed to grow under greenhouse conditions for 4-9 weeks before being evaluated for rooting.

One hundred dormant hardwood stakes (basal diameter of 1-2 cm and greater than 40 cm long) of *A. viridis* subsp. *crispa*, *B. papyrifera*, *C. stolonifera* and *M. gale* were field collected for live staking. At the nursery, stakes were shortened to 40 cm and

separated into two groups of 50 stakes per species. One group was treated with Stimroot 10 000 as per the manufacturer's directions while the other group remained untreated. Stakes were then taken to an outside holding bed where they were stuck 30cm into the soil (10 cm remained above the soil surface) and the soil firmed around each stake. Stakes remained in this holding bed for approximately 17 weeks until they were dug up and evaluated for rooting. Rooting was scored as with nodal cuttings in that there had to be at least 3 roots over 1cm long to be considered rooted.

Table 2.2: Treatments under which nodal cuttings of *A. viridis* subsp. *crispa*, *B. papyrifera*, *C. stolonifera* and *M. gale* collected at different times of the year were rooted. Treatments included the use of different length cuttings (7.5 and 15cm), under different growing conditions (misted and high humidity) in different cutting mixes (1:1 Promix®-Perlite, 1:1 Peat-Sand and Sand). Sample sizes for each treatment are included in brackets***.

Cutting Material	Treatment Duration (weeks)	Rooting Condition	1:1 Promix®-Perlite*				Cutting Mix 1:1 Peat-Sand**				Sand**			
			Humidity		Mist		Humidity		Mist		Humidity		Mist	
Dormant Hardwood (Fall-Winter)	8	Length (cm)	7.5 (36)	15 (36)	7.5 (33)	15 (33)	7.5 (36)	15 (36)	7.5 (33)	15 (33)	7.5 (36)	15 (36)	7.5 (33)	15 (33)
Actively Growing Hardwood	8	Length (cm)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)
Semi-hardwood	7	Length (cm)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)
Dormant Hardwood (Spring)	7	Length (cm)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)	7.5 (50)	15 (50)

* Considered to be a sterile media therefore was not microwaved

** Sterilized by heating in a microwave for 10 minutes on high power

***Sample sizes were increased from 36 and 33 for Dormant Hardwood (Fall-Winter) to 50 for remaining humidity and mist treatments due to increased availability of nursery containers and space

2.2.3 Data Analyses

Data collected during this study were analyzed using Minitab® Statistical software Version 16.0. For stratification and smoked seeds germination tests Binary Logistic Regression was used to evaluate the odds of germination and determine if there were significant differences between treatment groups. Binary Logistic Regression was also used to evaluate the odds of rooting of nodal cuttings and live stakes and to determine if there were significant differences between cutting treatment groups. Binary Logistic Regressions were completed in the event trial format using the Logit link function.

Binary Logistic Regressions were evaluated by first observing the table outlining tests of terms with more than one degree of freedom (i.e., model terms with three or more levels). If the p -value for any model term was below the level of significance ($\alpha=0.05$) the odds of individual levels were compared. To evaluate model terms with only one degree of freedom (i.e., model terms with two levels) the statistic generated by the test that all slopes are zero was first evaluated. If the p -value for this statistic was below the level of significance ($\alpha=0.05$) the Z-value and its respective p -value for each term was evaluated for significance. The explanatory factors (model terms) used for each analysis are outlined in Table 2.3.

Table 2.3: Explanatory factors (model terms) used for each binary logistic regression analysis of various propagation treatments.

Analysis	Explanatory Factor(s)
Seed Stratification Tests	Length of stratification period (0, 30, 60 or 90 days)
Smoked Seed Tests	Duration of smoke exposure (0, 10, 30, 60 or 180 minutes)
Vegetative Propagation- Nodal Cuttings	Media (1:1 Promix®-perlite, 1:1 peat-sand, sand) Cutting length (7.5cm and 15cm) Rooting conditions (mist and high humidity) Wood type (dormant winter hardwood, actively growing summer hardwood, semi hardwood and dormant spring hardwood) Interaction terms: media*cutting length, media*rooting conditions, media*wood type, length*rooting conditions, length*wood type and rooting conditions*wood type
Vegetative Propagation- Live Stakes	Treated with rooting hormone or not treated

2.3 Results

2.3.1 Seed Propagation

Germination of *A. viridis* subsp. *crispa* seed ranged from 38-43.5% while germination of *B. papyrifera* seed ranged from 0.8-2.6%, (Figure 2.2). Germination of *C. stolonifera* seed ranged from 0-80.2% while germination of *M. gale* ranged from 1.3-15.9%, (Figure 2.2). Binary logistic regression results indicated that the odds of germination for *A. viridis* subsp. *crispa* or *B. papyrifera* are not significantly different with stratification ($\chi^2=2.40$, $df=3$, $p=0.494$ and $\chi^2=4.10$, $df=3$, $p=0.251$, respectively). *C. stolonifera* showed a significant difference in the odds of germination with stratification ($\chi^2=40.88$, $df=3$, $p<<0.001$). The odds of germinating with 60 or 90 days stratification were equal, but were 2.43 (OR=2.43, 95% CI= 1.76-3.37, $p<<0.001$) times higher than those with 30 days stratification. *M. gale* showed a significant difference in the odds of germination with stratification ($\chi^2=46.52$, $df=3$, $p<<0.001$). The odds of germinating with 30, 60 and 90 days stratification were 4.39 (95% CI 1.64-11.75, $p=0.003$), 14.32 (95% CI 5.69-36.07, $p<<0.001$) and 11.09 (95% CI 4.37-28.16, $p<<0.001$) times higher

compared to seeds without stratification, respectively.

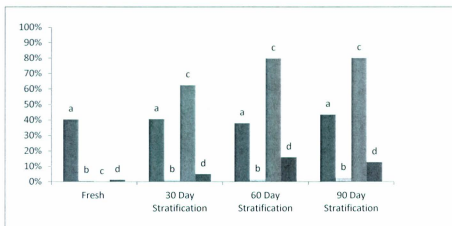


Figure 2.2: Percent germination of seed of *A. viridis* subsp. *crispa* (a), *B. papyrifera* (b), *C. stolonifera* (c) and *M. gale* (d) under various cool moist stratification periods. Stratification was at 3-5°C and seed were placed in perforated Ziploc bags mixed with moistened 00-silica sand.

Germination of Smoke Treated and Untreated Seed

Germination for *Alnus viridis* subsp. *crispa* seed exposed to vegetative smoke ranged from 38.3-48% (Figure 2.3). Germination for both *Betula* and *Cornus* were consistently low across all treatments (0.5-2.1% for *Betula* and 0-0.5% for *Cornus*), (Figure 2.3). Germination for *Myrica* seeds ranged from 2.3-19.0%, with a general trend that germination percentage increased with duration of exposure to smoke (Figure 2.3). Binary logistic regression results indicate that the odds of germination change with duration of smoke exposure for *Alnus* ($\chi^2=10.11$, $df=4$, $p=0.039$) but odds ratios show that germination is not significantly different for seeds with 0, 10, 60 and 180 minutes of smoke exposure (OR=0.98, 95% CI 0.73-1.31, $p=0.882$; OR=1.01, 95% CI 0.76-1.35,

$p=0.941$; OR=1.07, 95% CI 0.8-1.42, $p=0.658$, respectively). However, the odds of germinating after exposure to 30 minutes of smoke were 1.45 times higher (95% CI 1.09-1.93, $p=0.011$) than for seeds that were not stratified. Binary logistic regression results for both *Betula* and *Cornus* indicate that the odds of germination do not change significantly with duration of smoke exposure ($\chi^2=5.59$, df=4, $p=0.232$ and $\chi^2=0.0000059$, df=4, $p=1$, respectively). Smoke exposure had a significant effect upon the odds of germination for *Myrica* seeds ($\chi^2=69.26$, df=4, $p<<0.001$). Specifically, the odds of germination were 2.53 (95% CI=1.15-5.58, $p=0.021$), 3.79 (95% CI=1.78-8.06, $p=0.001$), 2.66 (95% CI=1.21-5.82, $p=0.015$) and 9.82 (95% CI=4.84-19.96, $p<<0.001$) times higher for seeds exposed to 10, 30, 60 and 180 minutes smoke exposure respectively.

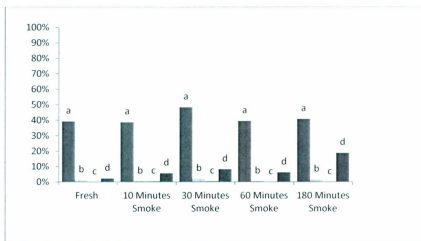


Figure 2.3: Percent germination of seed of *A. viridis* subsp. *crispa* (a), *B. papyrifera* (b), *C. stolonifera* (c) and *M. gale* (d) after exposure to various duration of vegetative smoke. Seeds were removed from dry cool (3-5°C) storage and soaked in water for 48 hours prior smoke treatment.

Seed Viability Testing

Tetrazolium and cut seed viability testing indicated that viability for *Cornus* was the highest of the four species followed by *Alnus*, *Myrica* and *Betula* (Table 2.4). The tetrazolium test consistently scored viability higher than the maximum germination percent observed during either (stratification or smoke) germination test (10%, 2.4%, 15.8% and 10% higher for *Alnus*, *Betula*, *Cornus* and *Myrica*, respectively). On the other hand the cut seed viability test indicated that viability was slightly lower (within 1% for all species) than the maximum germination percent observed during germination tests for all species except *Cornus*. For *Cornus* the cut seed viability test scored viability higher than tetrazolium viability test or either germination test (Table 2.4).

Table 2.4: Seed viability of *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale* under various viability test methods.

Species	Tetrazolium	Viability Testing Method (% Viable)		
		Cut Seed	Maximum %	Maximum %
			Germination of Stratified/Unstratified Seed	Germination of Smoked/Unsmoked Seed
<i>Alnus viridis</i> subsp. <i>crispa</i>	58.0	47.0	43.5	48.0
<i>Betula papyrifera</i>	5.0	2.0	2.6	2.1
<i>Cornus stolonifera</i>	96.0	99.0	80.2	0.5
<i>Myrica gale</i>	29.0	18.0	15.9	19.0

2.3.4 Vegetative Propagation

Nodal Cuttings

Misted semi hardwood cutting material invariably produced the highest rooting percentage for all species (Table 2.5). *Alnus* and *Betula* cuttings rooted poorly when the plant material was either dormant hardwood or actively growing hardwood, with the only appreciable rooting occurring with semi hardwood cuttings (Table 2.5). The highest rooting percentage for *Alnus*, *Betula* and *Cornus* was observed with 15 cm cuttings whereas the highest rooting percentage for *Myrica* was observed with 7.5 cm cuttings (Table 2.5). Rooting percentage was highest for *Alnus* cuttings in sand media for both lengths whereas for *Betula* 1:1 Promix®-Perlite and 1:1 Peat-Sand produced the highest rooting percentage for 7.5 cm and 15 cm cuttings respectively (Table 2.5). Misted and high humidity *Cornus* cuttings of both lengths (15 cm and 7.5 cm) all had the highest rooting percentage in 1:1 Promix®-perlite rooting media (Table 2.5). Misted cuttings of *Myrica* had their highest rooting percentages in 1:1 peat-sand media for both lengths (7.5 cm and 15 cm), (Table 2.5). Under high humidity conditions the greatest rooting percentage for 7.5 cm cuttings was in 1:1 Promix®-perlite, and for 15 cm cuttings the greatest rooting percentage was in sand (Table 2.5).

The results of the binary logistic regression for *Alnus* indicated that none of the model terms (main effects or interaction terms) had a significant effect upon the odds of rooting. Results of the binary logistic regression for *Betula* indicated that the interaction terms of media *cutting length and rooting conditions *wood type were significant

($\chi^2=7.04$, $df=2$, $p=0.030$ and $\chi^2=13.44$, $df=3$, $p=0.04$, respectively). Odds ratio tests showed that this interaction was particularly significant between 1:1 Promix®-perlite media and 15cm long cuttings (OR=0.69, 95% CI 0.2-0.06, $p=0.011$). Although there was a significant interaction effect detected between rooting conditions and wood type, it was not possible to attribute this effect to a specific wood type-rooting condition interaction. For *Cornus* there were significant interactions for media*length ($\chi^2=13.20$, $df=2$, $p=0.001$), media*rooting condition ($\chi^2=18.77$, $df=2$, $p<<0.001$), length*wood ($\chi^2=10.21$, $df=3$, $p=0.017$) and rooting condition*wood ($\chi^2=166.72$, $df=3$, $p<<0.001$). There were also significant main effects for media ($\chi^2=6.25$, $df=2$, $p=0.044$), wood type ($\chi^2=44.08$, $df=3$, $p<<0.001$), rooting conditions ($Z=-3.39$, $df=1$, $p=0.001$) and length ($Z=6.29$, $df=1$, $p<<0.001$) but due to the interaction effects it was difficult to interpret the results of the binary logistic regression.

Table 2.5: Rooting percentage of nodal cuttings of *A. viridis* subsp. *crispa*, *B. papyrifera*, *C. stolonifera* and *M. gale* collected at different times of the year under various rooting treatments. Treatments included the use of different length cuttings (7.5 and 15cm), different growing conditions (misted and high humidity) and in different cutting mixes (1:1 Promix®-Perlite, 1:1 Peat-Sand and Sand).

Species	Cutting Material	15cm Mist			7.5 cm Mist			15cm Humidity			7.5cm Humidity		
		1:1 Pro- Per*	1:1 Peat- Sand	Sand	1:1 Pro- Per*	1:1 Peat- Sand	Sand	1:1 Pro- Per*	1:1 Peat- Sand	Sand	1:1 Pro- Per*	1:1 Peat- Sand	Sand
<i>A. viridis</i> subsp. <i>crispa</i> ***	Dormant Hardwood (Winter)	0	0	0	0	0	0	0	0	0	0	0	0
	Growing Hardwood	0	0	0	2	2	10	0	0	0	0	0	0
	Semi-Hardwood	30	30	40	26	18	32	0	0	0	2	0	0
	Dormant Hardwood (Spring)	0	0	0	0	0	0	0	0	0	0	0	0
<i>B. papyrifera</i> **	Dormant Hardwood (Winter)	0	0	3.03	0	0	0	0	0	0	0	0	0
	Growing Hardwood	2	0	2	0	0	4	0	2	2	2	0	2
	Semi-Hardwood	14	42	32	36	32	24	0	0	0	0	2	2
	Dormant Hardwood (Spring)	0	0	0	0	0	0	0	0	2	0	0	0
<i>Cornus stolonifera</i> **	Dormant Hardwood (Winter)	24.2	3.03	12.12	16.7	8.3	0	54.5	61.1	18.2	8.3	5.6	8.3
	Growing Hardwood	70	42	26	44	16	30	50	46	40	46	24	30
	Semi-Hardwood	92	84	68	74	52	60	24	22	26	2	10	22
	Dormant Hardwood (Spring)	60	60	48	76	32	24	70	62	64	66	28	54
<i>Myrica gale</i> ***	Dormant Hardwood (Winter)	3.0	9.1	9.1	2.78	0	0	0	18.2	30.3	0	22.2	0
	Growing Hardwood	34	62	18	90	84	66	2	4	30	84	72	56
	Semi-Hardwood	38	96	92	74	98	94	6	78	48	26	30	50
	Dormant Hardwood (Spring)	36	58	60	22	38	26	38	80	94	30	28	38

*Promix®-Perlite Rooting Media

** Samples sizes were 50 for all treatments except dormant hardwood (winter) cuttings when sample sizes were 33 for Mist treatment and 36 for Humidity treatment.

For *Myrica* there were significant interactions for media*length ($\chi^2=49.41$, $df=2$, $p<<0.001$), media*rooting condition ($\chi^2=15.87$, $df=2$, $p<<0.001$), media*wood ($\chi^2=64.44$, $df=6$, $p<<0.001$), length*wood ($\chi^2=198.60$, $df=3$, $p<<0.001$) and rooting condition*wood ($\chi^2=124.17$, $df=3$, $p<<0.001$). There were also significant main effects for wood type ($\chi^2=48.53$, $df=3$, $p<<0.001$) and length ($Z=2.90$, $df=1$, $p=0.004$) but due to the interaction effects it was difficult to interpret the results of the binary logistic regression.

To accommodate for the interactions between model terms for *Cornus* and *Myrica*, main effect terms were evaluated separately. The odds of rooting, and goodness of fit were determined for each wood type, length and media irrespective of rooting condition (humidity or mist). Similarly, the odds of rooting, goodness of fit were determined for each wood type, rooting condition (mist or humidity) and media irrespective of length.

When main factor terms were evaluated separately there were a number of significant differences in the odds of rooting in various media for wood type and lengths for *Cornus*. Similarly, significant differences were also observed for wood type and rooting conditions for various media (Table 2.6). The odds of rooting of 15 cm hardwood-fall/winter cuttings of *Cornus* were significantly different between rooting media with the odds of rooting in Promix®-perlite 1.39 times that of 1:1 peat-sand whereas the odds of rooting in sand were 0.38 times that of rooting in 1:1 peat-sand (Table 2.6). For growing hardwood-summer the odds of rooting in various media were significantly different at either 7.5 cm or 15 cm length with the greatest odds of rooting in Promix®-perlite media for each length (Table 2.6). In the case of rooting success for

semi-hardwood the odds of rooting were not significantly different in either media at either length (Table 2.6). Dormant hardwood-spring cuttings showed a significant difference in the odds of rooting in different media for 7.5 cm cuttings but not 15 cm cuttings. In particular, the odds of rooting were 5.71 times greater in Promix®-perlite and 1.49 times greater in sand compared to 1:1 peat-sand (Table 2.6).

When main factor terms for wood type, rooting condition and media were evaluated there were significant differences in the odds of rooting in various media under both rooting conditions for all wood types, except for semi hardwood or growing hardwood-summer rooted under high humidity conditions (Table 2.6). Cuttings rooted under misted conditions had higher odds of rooting in Promix®-perlite regardless of wood type (4.14, 3.25, 2.3, 2.49 for dormant hardwood-fall/winter, growing hardwood-summer, semi-hardwood or dormant hardwood-spring respectively) whereas the odds of rooting in sand were similar to or slightly less than the odds of rooting in peat-sand media (1, 0.95, 0.84, 0.66 for dormant hardwood-fall/winter, growing hardwood-summer, semi-hardwood or dormant hardwood-spring respectively). With respect to cuttings rooted under humidity conditions the odds of rooting for dormant hardwood-fall/winter cuttings in Promix®-perlite media were similar to the odds of rooting in peat-sand media (0.93) but the odds of rooting in sand were substantially lower (0.32) than the odds of rooting in peat-sand (1). (Table 2.6). However, for dormant hardwood-spring cuttings the odds of rooting under humidity conditions were greater for cuttings in Promix®-perlite (2.6) and sand (1.76) compared to 1:1 peat-sand (1). (Table 2.6).

Table 2.6: Evaluation of main effects terms for *Cornus stolontifera*. Main effects evaluated by determining rooting odds and goodness of fit for wood type, length and media irrespective of rooting condition (left side of table). Odds of rooting and goodness of fit also determined for wood type, rooting condition and media irrespective of length (right hand side of table).

Wood Type	Length						Rooting Condition					
	15cm			7.5cm			Mist			Humidity		
	OR 1:1 Promix [®] - Perlite	OR 1:1 Peat- Sand	OR 1:1 Sand	OR 1:1 Promix [®] - Perlite	OR 1:1 Peat- Sand	OR 1:1 Sand	OR 1:1 Promix [®] - Perlite	OR 1:1 Peat- Sand	OR 1:1 Sand	OR 1:1 Promix [®] - Perlite	OR 1:1 Peat- Sand	OR 1:1 Sand
Dormant Hardwood- Fall/Winter	1.39	1	0.38	1.91	1	0.58	4.14	1	1	0.93	1	0.32
	G=10.48, df=2, p=0.005			G=3.54, df=2, p=0.170			G=9.51, df=2, p=0.009			G=8.73, df=2, p=0.013		
Growing Hardwood	1.91	1	0.63	3.27	1	1.71	3.25	1	0.95	1.71	1	1
	G=15.01, df=2, p=0.001			G=14.72, df=2, p=0.001			G=22.75, df=2, p<<0.001			G=4.68, df=2, p=0.096		
Semi- Hardwood	1.22	1	0.79	1.36	1	1.55	2.3	1	0.84	0.78	1	1.66
	G=2.44, df=2, p=0.296			G=2.29, df=2, p=0.318			G=10.41, df=2, p=0.05			G=4.35, df=2, p=0.113		
Dormant Hardwood- Spring	1.19	1	0.81	5.71	1	1.49	2.49	1	0.66	2.6	1	1.76
	G=1.71, df=2, p=0.426			G=38.20, df=2, p<<0.001			G=21.84, df=2, p<<0.001			G=11.04, df=2, p=0.004		

When main factors were evaluated separately there were several significant differences in the odds of rooting in various media for wood type and length for *Myrica*. Similarly, significant differences were also observed for wood type and rooting conditions for various media (Table 2.7). For dormant hardwood-fall/winter, the odds of rooting 7.5 cm and 15 cm cuttings were significantly different between rooting media ($G=14.05$, $df=2$, $p=0.001$; $G=13.81$, $df=2$, $p=0.001$, respectively). The odds of rooting in the Promix®-perlite and sand were 0.11 and 0.00, respectively compared to the peat-sand media for 7.5 cm cuttings. The odds of rooting were 0.1 for Promix®-perlite and 1.55 for sand compared to peat-sand (1) for 15 cm cuttings (Table 2.7). The odds of rooting for cuttings of growing hardwood-summer were also significantly different in the different rooting media for 7.5 cm and 15 cm lengths (Table 2.7). In particular, the odds of rooting in Promix®-perlite was 1.89 and in sand the odds were 0.44 times that of rooting in peat-sand media at 7.5 cm length, and 0.45 and 0.64 times that of rooting in peat-sand media for Promix®-perlite and sand respectively at 15 cm length (Table 2.7). For semi-hardwood cuttings the odds of rooting were significantly different in various media for 7.5 cm and 15 cm cuttings ($G=10.54$, $df=2$, $p=0.005$ and $G=99.77$, $df=2$, $p<<0.001$, respectively). For both cutting lengths the odds of rooting in Promix®-perlite were lower than in peat sand (0.56 and 0.04 for 7.5 cm and 15 cm cuttings respectively), whereas the odds of rooting in sand were 1.45 and 0.35 for 7.5 cm and 15 cm cuttings respectively compared to peat-sand (Table 2.7). The odds of rooting for 15 cm dormant hardwood-spring cuttings were significantly different between rooting media with the odds of cuttings rooting in Promix®-perlite lower (0.26) and the odds of rooting in sand greater

(1.5) compared to peat-sand media. When main factor terms for wood type, rooting condition and media were evaluated, there were significant differences in the odds of rooting in various media under both mist and humidity conditions for all wood types except for misted dormant hardwood-fall and growing hardwood-summer under humidity conditions (Table 2.7). In all cases where there were significant differences in the odds of rooting between the various media, the odds of rooting in Promix®-perlite were less than that of rooting in peat-sand (Table 2.7). Similarly, the odds of rooting were all lower for cuttings in sand media except for dormant hardwood-spring cuttings grown under humidity conditions (Table 2.7).

Table 2.7: Evaluation of main effect terms for *Myrica gale*. Main effects evaluated by determining rooting odds and goodness of fit for wood type, length and media irrespective of rooting condition (left side of table). Odds of rooting and goodness of fit also determined for wood type, rooting condition and media irrespective of length (right hand side of table).

Wood Type	Length						Rooting Condition					
	15cm			7.5cm			Mist			Humidity		
	OR 1:1 Promix [®] - Perlite	OR 1:1 Peat- Sand	OR 1:1 Sand	OR 1:1 Promix [®] - Perlite	OR 1:1 Peat- Sand	OR 1:1 Sand	OR 1:1 Promix [®] - Perlite	OR 1:1 Peat- Sand	OR 1:1 Sand	OR 1:1 Promix [®] - Perlite	OR 1:1 Peat- Sand	OR 1:1 Sand
Dormant Hardwood- Fall/Winter	0.1	1	1.55	0.11	1	0	0.66	1	1	0	1	0.67
	G=13.81, df=2, $p=0.001$			G=14.05, df=2, $p=0.001$			G=0.27, df=2, $p=0.873$			G=21.82, df=2, $p<<0.001$		
Growing Hardwood- Summer	0.45	1	0.64	1.89	1	0.44	0.6	1	0.27	1.23	1	1.23
	G=6.07, df=2, $p=0.048$			G=18.78, df=2, $p<<0.001$			G=20.59, df=2, $p<<0.001$			G=0.690, df=2, $p=0.708$		
Semi- Hardwood	0.04	1	0.35	0.56	1	1.45	0.04	1	0.41	0.16	1	0.82
	G=99.77, df=2, $p<<0.001$			G=10.54, df=2, $p=0.005$			G=67.97, df=2, $p<<0.001$			G=38.47, df=2, $p<<0.001$		
Dormant Hardwood- Spring	0.26	1	1.5	0.71	1	0.96	0.44	1	0.82	0.44	1	1.65
	G=37.78, df=2, $p<<0.001$			G=1.38, df=2, $p=0.502$			G=8.24, df=2, $p=0.016$			G=21.27, df=2, $p<<0.001$		

Live Staking

Live staking completed at MUN Botanical Garden showed that *Cornus stolonifera* and *Myrica gale* were able to root with and without the use of rooting hormone. *Alnus viridis* subsp. *crispa* and *Betula papyrifera* live stakes failed to root regardless of whether stakes were treated with rooting hormone (Figure 2.4).

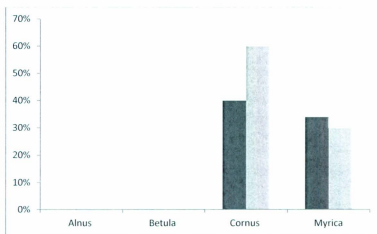


Figure 2.4: Rooting success of live stakes of *A. viridis* subsp. *crispa*, *B. papyrifera*, *C. stolonifera* and *M. gale* treated with rooting hormone (black) and without rooting hormone (gray). Stakes were collected in the spring while dormant and placed in treatment for 17 weeks and evaluated for rooting.

Live stakes of *C. stolonifera* without hormone treatment had a higher rooting percentage (60%) than treated stakes (40%). However, for sweetgale the reverse was true, rooting was higher for hormone treated stakes (34%) compared to untreated stakes (30%). When the results were analyzed by Binary Logistic Regression there was a

significant difference in the odds of rooting for hormone treated and non-treated stakes of *C. stolonifera* ($Z=-1.99$, $df=1$, $p=0.047$) but not for *M. gale* ($Z=0.43$, $df=1$, $p=0.668$). The odds of rooting with hormone treatment were 0.44 (95% CI= 0.20-0.99) of that without treatment for *C. stolonifera* stakes.

2.4 Discussion

In general the results of this study indicate that stratification prior to sowing is not required for *Alnus viridis* subsp. *crispa* or *Betula papyrifera*, but is required for *Cornus stolonifera* and results in increased germination for *Myrica gale*. Exposure of each species to plant smoke only resulted in increased germination of *M. gale*. Vegetative propagation (cuttings and live stakes) results were generally poor for *A. viridis* subsp. *crispa* and *B. papyrifera* whereas they were appreciably better for *C. stolonifera* and *M. gale*. Overall *A. viridis* subsp. *crispa* and *B. papyrifera* appear to be better suited to seed based propagation. *C. stolonifera* can be reliably produced by seed based or vegetative propagation methods based upon the high germination and rooting percentages observed. Whereas *M. gale* can also be produced by either seed or by vegetative cuttings.

2.4.1 Seed Propagation

Germination Tests of Stratified and Unstratified Seed

Alnus viridis subsp. *crispa*

Stratification did not result in a significant increase in the percent germination for *Alnus* during this study. Related to this is an observation made by MUN Botanical Garden staff as part of the larger revegetation project relating to the germination of *Alnus* without stratification. Prior to the start of this study, Garden staff collected *Alnus* seed in the fall at Granite Canal. The seed was stored until spring before being sown and

germination was poor. Similarly, during this study the seed bearing *Alnus* catkins were collected in the fall at Granite Canal. However, some of the catkins were wet since they were collected on a rainy day. Upon returning to the Garden the wet catkins were spread in the greenhouse (maintained at 20°C) to dry which resulted in the observation of seed germinating within the female catkins. Immediate sowing of seeds (with no storage or stratification) resulted in much higher (almost 50%) germination. These observations suggest that the species does not require a cool moist stratification period for germination. Observations made as part of this study and by Botanical Garden staff are consistent with the findings of Farmer et al. (1985), who showed that germination was almost complete for unchilled seeds under a 16 hour photoperiod at temperatures between 20-30°C. However, Nichols (1934), Schopmeyer (1974b) and Dirr and Heuser (2006) did suggest that a period of stratification may be necessary for germination. Particularly, Nichols (1934) indicated that germination increased from 2.5% without refrigeration to 40% with refrigeration. While Farmer (1985) reported almost complete germination for viable unchilled seeds, the proportion of viable seeds was low with mean sound seed percentages of 20% and 14% from each of the two sample populations studied. Farmer (1985) also noted a high variability (range from 1-48% and 0-42% within the two sample areas) in the proportion of sound seed within clumps of alder. Benowicz et al. (2000) also found that there was also high variability in the germination capacity of sitka alder (*A. sinuata* (Regel) Rydb.) and suggested that this appears to be a common occurrence for members of the genus. Tetrazolium and cut seed viability tests concur with the results of Farmer (1985), in that almost all seed scored as viable germinated without stratification.

Particularly, tetrazolium testing and cut seed viability testing conducted scored 58% and 47% of seed as viable respectively, whereas actual germination of fresh (unstratified) seed was 40.4%.

Betula papyrifera

Betula seeds had a consistently low germination percentage (Figure 2.2). Viability testing by tetrazolium test and cut seed test also revealed a low viability (5% and 2% viability, respectively). Brinkman (1974a) indicated that seed viability is influenced by seed production and that during years of high seed production a higher proportion of seed is viable. In addition, Bevington (1986) indicated that the production of viable seeds is influenced by factors such as climate, mother tree (i.e. genetic origin) and site. Bjorkbom (1971) reported that 53-86% seeds collected for his study were viable, whereas Brinkman (1974a) reported variation in germination ranging from 11-87% which were appreciably higher than the viability (2-5%) and germination percentage (0.5-2.6%) observed for this study. Stratification did not have a significant effect upon germination of *Betula* seed. Brinkman (1974a) and Bevington (1986) both indicated that germination is mediated by exposure to adequate light and therefore stratification is not a requirement. However, while stratification may not be required, Bevington (1986) has suggested that exposure of seed to a stratification period increases sensitivity to light and can promote germination under lower light levels than seeds that have not been subject to stratification. While percent viability and germination percentage were both low there was agreement between viability and germination (greatest viability was only 2.4% higher than the greatest

germination percentage) suggesting that viability, not dormancy, led to the low percentage germination.

Cornus stolonifera

Germination percentage rapidly increased with an increase in stratification period from 0 to 30 to 60 days (0, 62.5% and 79.9% germination, respectively) but increased only slightly (increased by 0.3%, to 80.2%) when stratification period was increased to 90 days. These results suggest that the species requires 60-90 days stratification for maximum germination. The results of this study are supported by the results of Acharya et al.(1991) who observed average germinations of 65% and 41% for 1984 and 1985 seed lots using 30 day cool moist stratification, whereas Peterson (1953) observed 87% germination with 90 days stratification. Acharya et al. (1991) also found that germination ability varied between populations and over years. While germination was variable in all years, a tetrazolium test conducted on the 1986 seed revealed high viability (greater than 90% viable), (Acharya et al., 1991). The current study found 96% and 99% viability in tetrazolium and cut seed viability testing, respectively and 80.2% maximum germination. Therefore the results of this study and the Acharya et al. (1991) study suggest that the species may produce highly viable seed lots (e.g., >90% viable) even though germination ability may be substantially lower.

Myrica gale

Based upon the results of germination tests it is evident that stratification enhances germination of the species since germination increased from 1.3% for unstratified seed to 15.9% for seed undergoing 60 days stratification. Dirr and Heuser (2006) indicate that *Myrica gale* seeds germinated well with 3 months of stratification at 4.4°C. Similarly, Schwintzer and Ostrofsky (1989) indicate that stratification increased germination by 26-164% over the 17.8% germination observed without stratification. Skene et al. (2000) also indicated that seeds harvested later into the winter produced greater germination than earlier harvested seed suggesting that stratification has occurred while the seed is still attached to the plant. Skene et al. (2000) also indicate that seeds of sweetgale germinate best if floated on water at 5°C for several weeks.

While stratification promotes germination (germination increased from 1.3% with no stratification to a maximum of 15.9% germination after 60 days stratification). Dirr and Heuser (2006), Schwintzer and Ostrofsky (1989) and Skene et al. (2000) all indicated that *Myrica gale* requires extended light exposure before germination will occur. Even though seeds were regularly exposed to light (natural daily light dark cycle) and may have been subjected to some supplemental lighting in the greenhouse there may have been insufficient light to promote maximum germination. In particular, germination tests were conducted at a time of the year (late fall into winter-approximately 8-9 hours of daylight) when the daylight hours were substantially shorter than when natural germination occurs (approximately 15-16 hours of daylight). Seeds of *M. gale* were also

covered with vermiculite after sowing which may have impeded the exposure of seeds to light and therefore resulting in reduced germination.

Germination Tests of Smoked and Unsmoked Seed

Percentage germination of *Alnus* and *Betula* seeds after exposure to vegetative smoke was not statistically different than seeds not exposed to smoke. While not statistically significant, germination of *Alnus* and *Betula* seeds was greatest after 30 minutes smoke exposure, then germination began to decrease. Pérez-Fernández and Rodríguez-Echeverría (2003) and Razanamandranto et al. (2005) have indicated that excess smoke exposure can result in an inhibiting effect upon germination. This effect may explain why after 30 minutes of smoke exposure there was a general trend of reduced germination in *Alnus* and *Betula*.

Exposure of *Cornus* and *Myrica* to vegetative smoke did not result in a statistically significant increase in germination for *Cornus* whereas smoke exposure resulted in a statistically significant increase in germination for *Myrica*. Viability testing by tetrazolium test (29% viable) and cut seed test (18% viable) indicated that smoke treatment potentially broke seed dormancy of the species since germination of unstratified seed was 19% after 180 minutes of smoke exposure. Since germination was increasing at the end of the test (180 minutes exposure to smoke), longer duration exposure may produce even greater germination.

2.4.2 Vegetative Propagation

Nodal Cuttings

Alnus viridis subsp. *crispa*

In the current study the greatest rooting success was for semi-hardwood cuttings in a sand media treated with a 10 000 ppm IBA liquid hormone and subjected to misted conditions (40% rooting). Dirr and Heuser (2006) have provided information on the rooting of cuttings from several *Alnus* species. Particularly, Dirr and Heuser (2006) indicate that *A. cordata* (Loisel) Duby., *A. glutinosa* (L.) Gaertn and *A. incana* (L.) Moench cuttings rooted 25%, 64% and 65% respectively when treated with 8000 ppm IBA talc with wounding. However, the type of wood utilized (e.g., softwood) was not specified nor were the conditions specified under which rooting took place. Schrader and Graves (2000) provide a propagation protocol for rooting softwood cuttings of *A. maritima*. Cuttings were collected from two areas, subjected to two hormone concentration levels (1000 ppm and 8000 ppm IBA), stuck in a perlite rooting media and subjected to intermittent mist. Rooting success was variable for location and rooting hormone concentration such that at one location rooting success was 57% and 68% for 1000 ppm and 8000 ppm IBA respectively whereas, at the other location rooting percent was 32% and 29% at 1000 ppm and 8000 ppm IBA respectively (Schrader and Graves, 2000). The rooting percentage of *Alnus viridis* subsp. *crispa* in the current study is comparable to the results observed by Schrader and Graves (2000), although different species were used.

Betula papyrifera

Of the four wood types utilized for experiments only semi-hardwood under misted conditions showed appreciable rooting (14-42%) with the highest rooting in peat-sand media. For other wood types the highest rooting success for the species was 4% (growing hardwood). Dirr and Heuser (2006) indicate that timing is critical for successful rooting of *Betula* and that shoots must still be active with the base of the cutting just becoming firm (semi-hardwood). Cuttings should be 15-20 cm and should be given a long shallow wound prior to treatment with rooting hormone. Dirr and Heuser (2006) report good results using a 2000 ppm IBA solution but report 100% rooting using 8000 ppm IBA-talc powder and indicate that a mixture of peat and sand is a suitable rooting media. The collection of cutting material 1-2 weeks later resulted in no rooting (Dirr and Heuser, 2006). Dirr (1977) reported 50% rooting of cuttings treated with 20 ppm IBA for 24 hours. While the rooting percentage in this study was not as high as that reported by Dirr and Heuser (2006), the results were consistent with their findings.

Cornus stolonifera

Rooting percentage was highest (92%) for late summer-early fall (semi-hardwood) cuttings in Promix® (a peat based potting soil)-perlite media under misted conditions. Dirr and Heuser (2006) indicate that *Cornus* cuttings collected from June into early fall, treated with 1000 ppm IBA solution, in peat-perlite media, under mist give 90-100% rooting. Furthermore Dirr (1977) had 90% success for cuttings treated with 1000 ppm IBA/50% alcohol any time leaves are present. Dirr (1977) also reported a rooting

success of 90-100% of hardwood cuttings placed immediately in the field. The results of this study are consistent with those of Dirr and Heuser (2006).

Myrica gale

In the current study, semi-hardwood cuttings treated with a 1% IBA solution under mist in a peat sand media had the highest rooting percentage (98%) for *M. gale*. Dirr and Heuser (2006) have indicated that the semi-hardwood cuttings of a related species, the southern wax myrtle (*M. cerifera* L.), treated with 1-1.5% IBA solution, in a peat perlite media under mist rooted 90% whereas the rooting of winter cuttings was poor.

Live Staking

In the current study the complete failure of *Alnus* or *Betula* live stakes to root suggests that either species is not suitable for live staking. Whereas the rooting of live stakes of *Cornus* and *Myrica* (with and without hormone treatment) suggest that either species may be suitable for live staking. A review of the literature produced no references which outlined the use of *Alnus*, *Betula* or *Myrica* for live staking. Gray and Sotir (1996) provide a list outlining plant species which are suitable for stabilizing unstable slopes and eroding soils. This list also assesses the ability of each species to root from cuttings. *A. viridis* subsp. *crispa* is not listed whereas *B. papyrifera* is listed as having poor rooting ability. While *A. viridis* subsp. *crispa* is not included, *Alnus rubra* Bong. (red alder) is listed but rooting ability is poor. The listing of a member of the genus *Alnus* and *B.*

papyrifera as having a poor rooting ability by Gray and Sotir (1996) agrees with the current study. The failure of hardwood cuttings to root during greenhouse trials further indicates the poor rooting ability of *Alnus* and *Betula*. Gray and Sotir (1996) list *C. stolonifera* as having very good rooting ability. Similarly, Lewis (2000) and Barrett et al. (2006) indicate that *Cornus* is a suitable species for live staking. Barrett et al. (2006) used dogwood (*Cornus spp.*) and willow (*Salix spp.*) for a stream bank restoration project in 1999. An assessment of survival in 2004 revealed 74% and 39% overall survival of stakes located in the upper and lower river bank respectively (Barrett et al., 2006).

2.5 Conclusion

While there are often knowledge gaps in the literature relating to the production of native plant species, it is possible to develop suitable propagation protocols for many species. The use of standard nursery practices (e.g., use of a variety of means of stratification and seed pre-treatments to break dormancy, rooting of cuttings in a variety of media or variation of the length of cuttings) will help plant propagators 'get the ball rolling'. Even though knowledge gaps pertaining to a particular species may be present, the available literature can provide information on other members of the genera or even family which may help to direct propagation experiments. When propagation protocols for a particular species are available from the literature, propagators may be faced with a protocol developed in a different geographical setting requiring adjustment in the timing of seed/cutting collection. Standard practices and the literature provide the base for the development of propagation protocols but repeated experimentation will refine the protocols leading to cost effective and efficient production of native species. Through the use of standard nursery practices and the available literature, it was possible to develop regionally specific (Newfoundland) propagation protocols for the native species *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale*.

Even though regionally specific propagation protocols were developed the statistical analyses did not provide a clear indication as to whether a number of null hypotheses could be supported or falsified due to significant interactions between model terms. In particular, determinations on the most appropriate rooting media for each

species, cutting length, or rooting treatment were not able to be clearly made due to interactive effects. In the case of wood type, it was possible to determine that the null hypothesis (H_0 : Wood type would have a significant effect on the odds of rooting) could be falsified for *Alnus* and *Betula* but could not be supported or falsified for *Cornus* or *Myrica* due to significant interaction. It was possible to make determinations that stratification is not required for *Alnus* or *Betula* since the null hypothesis was falsified (H_0 : The odds of germination will be higher for stratified seed compared to unstratified seed of each species). Conversely for *Cornus* and *Myrica* the null hypothesis is supported. With respect to smoke exposed seeds it was possible to support or falsify the null hypothesis that smoke exposed seeds have higher odds of germinating (*Myrica* was the only species where the null hypothesis was supported, whereas it was falsified for the remaining species). Similarly, for live stakes the null hypothesis that hormone treated live stakes would have higher odds of rooting was supported for *Cornus* and falsified for *Myrica* (no live stakes of *Alnus* or *Betula* rooted).

Even when suitable propagation protocols are available, propagators are faced with the problem of securing a supply of native plant material for propagation. Currently, to my knowledge, there are no local suppliers of native seed or cutting material within the province therefore the propagator must undertake collection of seed and cuttings in the wild. While the collection of seed and cutting material for species such as *Alnus viridis* subsp. *crispa* is not overly labour intensive (due to the formation of dense thickets and production of copious amounts of seed), others such as *Cornus stolonifera* have much

reduced seed production and thicket size, requiring substantially longer time to collect seed/cutting material.

As indicated previously, the lack of suitable native species was cited by Mallik and Karim (2008), Bochet et al. (2010) and Grant et al. (2011) as a reason for the continued reliance upon non-native species. Harrington et al. (1999) have also cited the lack of information sharing between plant propagators as another reason that native species are not used. It is hoped that studies like this one will help to make information on the propagation of native species more available to other revegetation and restoration practitioners. Furthermore, promoting the use of native species and developing an industry which produces native species will require industry and government involvement through the adoption of policies and practices that require the use of native species.

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Chapter 3: Performance of *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale* in a Central Newfoundland Habitat Restoration Project

Abstract

The establishment and growth of four native plant species (*Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale*) at a fish habitat compensation facility, constructed in Newfoundland, was tested under various scenarios. Scenarios included determining if competition from previously applied non-native hydroseed would affect establishment and survival; whether large mammal herbivory may reduce the success of revegetation efforts in the area; whether the use of commercially available mycorrhizal fungi inoculants facilitate establishment and growth of plants and whether the growth media may affect establishment and growth. Growth of native species was significantly higher when grown in 5-inch pots filled with compost and sunk into the soil compared to those in ground, whereas hydroseed density had minimal effect. Throughout the project, the incidence of herbivory was low (approximately 3% of plants were browsed) within herbivory plots established at the site and overall. Inoculation with mycorrhizal fungi had no significant effect upon any of the species in either media. There were significant media effects for each of the species. The results suggest that the species used in this study would be suitable for riparian

restoration projects elsewhere in a variety of soil types and even in the presence of non native hydroseed.

3.1 Introduction

To restore vegetative cover to disturbed areas, restoration ecologists must find ways to overcome site conditions which may be inhospitable to plants. One important factor to consider when planning restoration is to determine what the environmental conditions are at the onset of restoration. Factors such as soil composition (e.g., peat, topsoil or base construction materials such as sand gravel and cobble), nutrient levels, pH, presence of phytotoxic compounds and moisture regime can be important in determining additional efforts required to overcome barriers to vegetation establishment and for selecting appropriate species.

Methods to overcome inhospitable soil conditions include the use of amendments to increase soil nutrients (fertilizer), adjust pH (lime), stabilize phytotoxic compounds (metal immobilizing agents) and add organic matter. The addition of beneficial soil microorganisms (bacteria, mycorrhizal fungi) and soil macro-organisms (worms) can help to restore soil functions such as nutrient cycling. The addition of lime and fertilizer has been practiced at metal contaminated sites which typically have low pH and low nutrient levels making the establishment of vegetation difficult (Lautenbach, 1987). Similarly, the use of metal immobilizing agents at mine sites has successfully reduced plant uptake and the movement of metals through the soil (Vangronsveld et al., 1997). Organic amendments have also been used for restoration activities to add organic matter and/or nutrients to deficient soils. A variety of amendments have been utilized including soil transfer (Helm and Carling, 1993); wood chips and straw mulch (Petersen et al.,

2004); pulp sludge (Carpenter and Fernandez, 2000); manure (Munshower, 1994); compost (De Ona et al., 2008); sewage sludge (Ferrer et al., 2011); and mixtures of thermal treated organic contaminated soils, papermill sludge and compost (Séré et al., 2008).

A common practice used to re-establish vegetation is to stockpile the topsoil overlying the work area and reapply once construction activities are completed. While prolonged stockpiling of topsoil in deep piles can cause reductions in soil quality, stockpiling in shallow piles for short periods of time can have little effect upon soil quality (pH and mineral content), (Strohmayer, 1999). While Abdul-Kareem and McRae (1984) indicate that mycorrhizal activity and earthworm biomass can be reduced in stockpiled soils, they suggest that biological activity rapidly recovers upon topsoil reapplication.

This study compared the survival and growth of the native species *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale* in a revegetation project under a variety of site conditions. The project included an investigation into the effect of competition from non-native hydroseed species previously established at the site upon survival and growth of the native species. Another aspect of the project involved assessing the potential effect of large mammal herbivory upon survival and growth of these species through the use of pair-wise plots with and without herbivore exclusion structures. Finally, the project included an investigation into the effect of various growth media in conjunction with and without mycorrhizal fungi inoculation upon the survival and growth of the native species. At the FHCF there were three dominant substrates

encountered, a mixture of topsoil and other overburden material that was placed for revegetation purposes (termed granite mix); a gravelly-sandy soil comprised of post construction material (termed spoil) and a naturally occurring purely peat based soil (termed peat).

To explore the effects of these treatments several hypotheses were explored including:

Hydroseed Competition

H₀: Growth rates will be higher for plants in sparse hydroseed than for plants in heavy hydroseed. The rationale was that areas of sparse hydroseed have reduced above and below ground competition between hydroseed species and native species.

H₀: Growth rates will be higher for plants in 5" fiber pots than for plants in ground. The use of fiber pots allows native species to produce an adequate density of roots before being subjected to root competition from hydroseed species since the pots break down over approximately one growing season. Thus, the fiber pots essentially eliminate below-ground root competition during the establishment and early growth stages of the native plants.

Herbivory

H₀: Plants isolated from browsing will have higher growth rates than those not isolated from browsing.

Media/Mycorrhizal Inoculation

H₀: Growth media will have significant effects upon plant growth rate (Growth rate greatest for all species in granite mix, followed by spoil, then peat).

H_a: Mycorrhizal fungi inoculation will enhance plant growth (growth rates will be higher in plants inoculated with mycorrhizal fungi).

Mycorrhizal inoculation is another way that the establishment of plants at restoration sites can be enhanced. This association benefits the host plant through an increase in the surface area available for the absorption of soil nutrients, increased resistance to soil pathogens, increased drought tolerance and decreased soil toxicity (Morrison et al., 1993). The potential for the use mycorrhizal inoculants to enhance plant growth has been recognized for horticultural, forestry and restoration purposes (Dodd and Thomson, 1994). As a result companies such as Premier Tech Biotechnologies have developed mycorrhizal inoculants which can be utilized to inoculate commercial crop species and have the potential to be utilized for restoration and revegetation activities. Thus, it is expected that the use of mycorrhizal fungi inoculants would result in higher growth rates for inoculated plants.

While fertilizers can help in the establishment of vegetative cover it can be problematic in that the effect can be short term (Petersen et al., 2004), require multiple applications (Bloomfield et al., 1982; Helm and Carling, 1993) and when applied in excess can leach from soils into nearby watersheds leading to eutrophication (Harriman, 1978). As an alternative, the use of species which form symbiotic associations with

nitrifying bacteria can be utilized to gradually increase soil nitrogen and organic matter. A common practice involves the use of hydroseed containing seeds of species from the Fabaceae which form associations with nitrifying bacteria. As an example, in Newfoundland, standard hydroseed mixtures include seeds of *Lotus corniculatus*, *Trifolium repens*, *Phleum pratense* and *Festuca rubra*. These species establish well under a variety of conditions, add soil nitrogen (*L. corniculatus* and *T. repens* are members of the Fabaceae), add soil organic matter and help to stabilize soils. Even though hydroseed can provide a rapid covering over areas requiring revegetation, it can pose problems for the establishment of native species. In particular, hydroseed species are a mixture of rapidly growing non-native species that once established can compete with native species for resources (Ashe and Barton, 1995; Matesanz et al., 2006). Ashe and Barton (1995) have shown that species diversity was lower on plots where hydroseed was applied compared to un-hydroseeded plots, suggesting that the non-native species in the hydroseed mix compete with native species for soil resources and space. Therefore, it is anticipated that hydroseed species would compete with native species. Given that the non-native species in the hydroseed mixture may compete for soil nutrients and for available light, therefore it is predicted that the growth rate of native species would be lower in the presence of heavy hydroseed.

Another factor which restoration ecologists may need to consider is herbivory. In areas with dense populations of herbivores there is a potential of significant reduction in the success of revegetation. Davis and Coulson (2010) indicated that during a revegetation study areas subjected to browsing had mortalities of over 25% whereas in

areas where herbivores were excluded mortality was zero. Herbivores can also influence the species composition, biomass and production in heavily browsed areas (Connor, 1999). Large herbivores also create unfavourable conditions for plant establishment through trampling, decreased soil fertility, soil compaction and increased exotic species abundance (Heckel et al., 2010). Large Newfoundland herbivore species such as *Alces alces* and *Rangifer tarandus* have been shown to influence plant regeneration. *A. alces* (moose) browsing has been reported to influence soil composition, nutrient cycling and even forest succession (Connor, 1999). Intense browsing by dense populations of *A. alces* within certain areas of the province has led to the failure of regeneration of mature *A. balsamea* stands (Gosse et al., 2011). Heavy browsing has also resulted in a shift from feathermoss seedbeds to seedbeds dominated by grasses and non-native species, thus hindering balsam fir germination (Gosse et al., 2011). Furthermore, moose exclusion studies by McLaren et al. (2009) indicate that when browsing pressure is removed, broadleaf trees and shrubs quickly regenerate to the detriment of *A. balsamea*. Manseau et al. (1996) indicated grazing and trampling by *R. tarandus* resulted in significantly lower cover by lichens and a significantly higher proportion of bare soil in forage areas compared to areas not foraged.

At the Granite Canal Fish Habitat Compensation Facility (FHCF) the topsoil layer was stockpiled prior to construction. After construction the topsoil was placed along the banks of the FHCF to provide a suitable base for the establishment of vegetation. The banks of the FHCF were then hydroseeded with a mixture of *L. corniculatus*, *T. repens*, *P. pratense* and *F. rubra* to provide vegetative cover for bank stabilization. The

hydroseeded area became attractive to *R. tarandus* and *A. alces* which resulted in extensive browsing, trampling and loss of bank stability. Through the use of herbivore exclusion measures, one would anticipate that growth rates would be greater for plants within exclusion areas.

3.2 Materials and Methods

3.2.1 Study Area

Research was conducted at the Granite Canal Fish habitat Compensation Facility (FHCF) (N 48°11' 42.2", W 56°47' 36.6") located in south-central Newfoundland approximately 85 km south-west of the town of Millertown via dirt road (Figure 1.1).

Field Plot Layout

At the Granite Canal FHCF, 24 - 7 x 2 m plots were laid out to determine the effects of hydroseed density (competition), herbivory, mycorrhizal associations and substrate upon establishment, growth and survival of these native species. A total of 21 individually labeled plants (6 *A. viridis* subsp. *crispa*, 6 *B. papyrifera* and 6 *C. stolonifera* and 3 *M. gale*) grown over the previous winter and spring at MUN Botanical Garden were randomly planted into each of the 24 plots. *A. viridis* subsp. *crispa* and *B. papyrifera* plants were produced from seed whereas *C. stolonifera* and *M. gale* were produced from nodal cuttings. Plants were installed in mid June 2005 with and growth/survival parameters recorded in early July, early August and mid-September 2005 and in the following year in mid-June, mid-July and mid-August. Soil samples were also collected from each plot from approximately 5-10 cm below the soil surface. Five subsamples were collected from random locations within each plot and combined to form as a single sample. Samples from 11 of the plots were analyzed for soil pH, carbon, nitrogen, phosphorus, potassium, calcium and magnesium at the provincial soils lab. A composite

sample of the compost collected from Memorial University's Botanical Garden used to fill fiber pots was also submitted for soil analysis.

Survival and Growth Measurement

Survival was a measurement of whether the plant was alive or dead. Growth measurement was comprised of several measurements, including basal stem diameter (mm), length of longest branch (cm), number of nodes along longest branch and number of branches. In addition to growth measurements, the overall plant health and degree of herbivore (large mammal) damage were scored according to pre-set criteria (see Table 3.1). The change in plant health and herbivory between initial and final measurements was included in the statistical models to account for changes in growth which may be the result of a change in health or as a result of herbivory.

Table 3.1: Scoring of plant health and herbivory

Scale	Health	Herbivory
1	Dead. Stem dry and easily cracks/Stem cracked off at ground level	Whole plant browsed with no nodes remaining.
2	Leaves very discoloured (whole leaf) or absent. Stem 'flexible' and does not crack	Plant browsed at main stem but at least one node remains.
3	Leaves moderately discoloured. Over half of leaf surface discoloured.	Slight browsing. Tips browsed or bark chewed.
4	Leaves slightly discoloured (Leaves slightly yellow, orange or brown edges).	No sign of browsing.
5	Very healthy (No leaf discolouration/spots).	No sign of browsing.

3.2.3 Competition

The effect of hydroseed density was tested by placing plots in areas with sparse (less than 50% coverage by hydroseed) and dense hydroseed (greater than 50% coverage). Plants were planted either directly into the soil or were potted into 5" fiber pots containing compost from Memorial University's Botanical Garden that were then sunk into the soil. In total there were 8 plots (2 hydroseed density x 2 planting condition treatments with 2 plots per treatment) testing the effect of hydroseed density on growth.

3.2.4 Herbivory

To test the degree of herbivory, plots (7 m x 2 m) were laid out in areas where herbivores were sighted by Nalcor Energy personnel while conducting work at the site during previous years. Herbivore exclusion plots where plants were enclosed in a plastic mesh tube (Vexar) and control plots (plants not enclosed in Vexar) were established, side by side, at two locations where herbivores had been observed by Nalcor Energy personnel (four plots in total).

3.2.5 Substrate and Mycorrhizal Fungi Inoculation

Replicate pairwise plots were established in each of the three substrates (12 plots in total). Plants in the treatment plot were inoculated with a commercially available mycorrhizal fungi inoculant (MYKE®) produced by Premier Tech Biotechnologies. *A.*

viridis subsp. *crispa*, *C. stolonifera* and *M. gale* were inoculated with the dry granular Pro-AN-1 inoculant containing *Glomerus intraradices* Schenck and Smith, and *Psilolithus tinctorius* (Pers.) Coker and Couch propagules whereas *B. papyrifera* plants were inoculated with the liquid inoculum of *Laccaria bicolor* (Maire) P.D. Orton as directed by the manufacturer. Initially, a hole approximately twice the diameter of the root ball and the same depth of the root ball was dug. In each hole the required amount of granular inoculant was placed in the bottom of the hole, the rootball slightly loosened by hand, the plant installed and the soil firmed around the plant. For *B. papyrifera* a the planting hole was dug as described, the rootball slightly loosened, the liquid inoculum was applied directly to the rootball, the plant installed and the soil firmed around the plant. For plots where plants were not inoculated the procedure was repeated but without the addition of the inoculant.

3.2.6 Data Analyses

Data collected during this study were analyzed using Minitab® Statistical software Version 16.0. The General Linear Model was used to carry out statistical tests. Residuals were examined for normality, independence and homogeneity to ensure that the statistical test assumptions were not violated. In situations where sample size was small ($n < 30$), p -value was close to the level of significance and assumptions were violated, the data were randomized to provide a more accurate approximation of the p -value. P -values generated using analysis of variance (ANOVA) were used to determine if differences in sample means were significant when alpha was 5%. Interpretations were not made for random

plot effects included in the models except for herbivory plots where there were differences in media between replicates (i.e. media in replicate 1 was peat whereas the media in replicate 2 was granite mix). Minitab® was also used to generate descriptive statistics for each test. Years were evaluated separately since measurements were not taken within the same timeframe in both years (July-September in 2005 and June-August in 2006). A Pearson correlation analysis showed significant positive correlation between the measurements used to quantify growth. Therefore, for brevity basal diameter growth rate and height growth rate were the only parameters analysed. Plants which died within either year were eliminated from the analyses for that year. Growth rates for basal stem diameter (mm/day) and height (cm/day) of each species were evaluated based upon treatment conditions.

The explanatory factors (model terms) used for each analysis are as follows. For competition explanatory factors included Pot treatment (whether the plant was in a 5" Fiber Pot or directly in the Ground), Hydroseed treatment (Heavy or Sparse) Health-Rate, Herb-Rate as well as the interaction term between the factors in the Pot and Hydroseed treatments. The explanatory factors for herbivory analyses included Plot treatment, Vexar treatment, Health-Rate and Herb-Rate. The explanatory factors for Media/Mycorrhizal Inoculation included Plot treatment (whether the plant was inoculated with MYKE® or not), Media treatment (Granite Mix, Peat or Spoil), Health-Rate, Herb-Rate and the interaction term MYKE®*Media. In instances where there was no change in either Health-Rate or Herb-Rate between initial and final measurement in either year, the term

was removed from the model. Table 3.2 provides an overview of the model terms included for each analysis along with a word description of the model terms.

Table 3.2: Description of model terms used for the analysis of each experimental treatment

Field Treatment	Model Term	Word Description of Model Term
Competition	Pot Treatment	Whether plant was in a 5" fiber pot sunk into the ground directly planted into the ground.
	Hydroseed Treatment	Whether the plant was located in a plot with sparse or heavy hydroseed.
	Health-Rate	The change in plant health between initial and final growth measurements.
	Herb-Rate	The change in the level of herbivory between initial and final growth measurements.
	Pot Treatment*Hydroseed Treatment	Interaction term between pot treatment and hydroseed treatment.
Herbivory	Plot Treatment	Whether the plant was in replicate plot 1 or 2 since the media in replicate plots was different.
	Vexar Treatment	Whether the plant was enclosed in a plastic mesh tube (Vexar) to exclude herbivores from browsing.
	Health-Rate	The change in plant health between initial and final growth measurements.
	Herb-Rate	The change in the level of herbivory between initial and final growth measurements.
Media/Mycorrhizal Inoculation	Plot Treatment	Whether the plant was inoculated with mycorrhizal fungi inoculant (MYKE®).
	Media Treatment	Whether the plant was in a plot containing Granite Mix, Peat or Spoil).
	Health-Rate	The change in plant health between initial and final growth measurements.
	Herb-Rate	The change in the level of herbivory between initial and final growth measurements.
	MYKE*Media	Interaction term between plot treatment and media treatment

3.3 Results

3.3.1 General Results (Survival and soil analysis results)

Survival

It is evident from Figure 3.1 that the initial overall survival was high with 98.4% of the plants installed at the FHCF alive by July 2005 (approximately two weeks after planting).

Survival remained high throughout the summer of 2005 for *Alnus*, *Betula* and *Cornus* (93.7%, 95.8% and 92.4% survival by the end of the final 2005 field visit in September). *Myrica* on the other hand experienced a steady decline in survival throughout the summer (97.2%, 86.1% and 79.2% in July, August and September respectively), (Figure 3.1).

During the initial field visit in 2006 all species had experienced substantial winter mortality (for purposes of this study, mortality is defined as the death of the aerial portion of the plant), (Figure 3.1). *Alnus* and *Myrica* experienced an additional 5.5% mortality whereas *Betula* experienced 0.7% mortality and *Cornus* actually had a reduction in the number of dead plants by 1.3% (2 plants had begun to regrow from below ground) by the final field visit in August. Overall survival was 76.4%, 81.9%, 87.5%, 58.3% for *Alnus*, *Betula*, *Cornus* and *Myrica* respectively, whereas overall average survival was 76.0%.

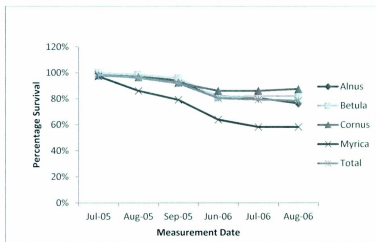


Figure 3.1: Percentage survival by species of individually tagged plants at Granite Canal FHCF.

Soil

From Table 3.3 it is evident that soil nutrients (macro and micro) display some variability across soil samples collected at experimental plots at the FHCF.

Table 3.3: Results of soil analysis of samples taken at the Granite Canal FHCF and compost used in 5" fiber pots

Granite Canal Plot	Soil pH	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	N (%)	C (%)
Peat-1	3.7	11	8	37	7	0.98	51
Compost in 5" Pots	5.6	363	705	4839	770	0.59	9.92
Spoil-1	4.8	153	7	77	8	0.14	1.21
Spoil-2	6.2	111	13	220	12	0.13	0.49
Gran-Mix*-1	4.6	63	35	288	112	0.31	10.6
Gran-Mix*-2	6.7	91	32	2321	66	0.28	4.54
Comp-1-HH	5	123	19	127	14	0.15	1.85
Comp-1-SH	6.7	115	37	2174	51	0.21	4.22
Comp-2-HH	5.7	98	23	1679	77	0.26	6.97
Comp-2-SH	6.6	81	25	2201	42	0.24	3.75
Herb-1	6.9	94	38	1763	45	0.18	2.81
Herb-2	3.6	16	38	95	68	0.99	47.6

* Granite Mix

3.3.2 Competition

Growth rates for basal stem diameter (mm/day) and height (cm/day) of each species were evaluated based upon treatment conditions.

Alnus viridis subsp. *crispa*

In 2005 the interaction term for Pot treatment*Hydroseed treatment was slightly above the level of significance ($p=0.077$) and sample size was large for basal stem diameter growth rate. Although p is greater than 0.05, Snedecor and Cochran (1980) suggest that when F is much greater than 1 and p is close to the level of significance ($\alpha=0.05$) the means across one factor should be compared within each level of the other

factor. Analysis of treatment levels 5" Pot and In Ground separately indicate no significant effect of either level upon basal diameter growth rate. Height growth rate was significantly different whether plants were in a 5" Pot or In Ground ($F_{1,36}=89.18$, $p<<0.001$). Average growth rates were 0.3249 cm/day for plants in 5" pots compared to 0.00393 for plants in ground, (Figure 3.2).

In 2006 there was as significant effect upon growth rate for both basal stem diameter and height whether the plant was in a 5" fiber pot or in ground ($F_{1,31}=41.38$, $p<<0.001$ and $F_{1,31}=4.20$, $p=0.049$ respectively), (Figure 3.3). Herb-Rate also had a significant effect upon basal stem diameter growth rate ($F_{1,31}=6.45$, $p=0.016$). There was a significant effect of Heavy or Sparse Hydroseed upon the height growth rate ($F_{1,31}=4.29$, $p=0.047$) with mean growth rates of 0.5463cm/day and 0.291cm/day in heavy and sparse hydroseed respectively.

Betula papyrifera

In 2005 there was as significant effect upon growth rate for both basal stem diameter and height whether the plant was in a 5" fiber pot or in ground ($F_{1,37}=15.57$, $p<<0.001$ and $F_{1,37}=19.25$, $p<<0.001$, respectively). Average growth rates for plants in 5" fiber pots were 0.0143 mm/day and 0.0142 cm/day for diameter and height respectively, whereas growth rates for plants in ground were 0.00076 mm/day and -0.00279 cm/day, respectively (Figure 3.2). Negative growth rates for height may be attributed to a reduction in height as a result of browsing or as a result of shoot dieback (height was recorded to the top of live growth).

In 2006 there was a significant interaction term (Pot treatment*Hydroseed treatment) for height growth rate and the interaction term for diameter growth rate ($p=0.076$) was near the level of significance, therefore growth rates of plants in heavy and sparse hydroseed were compared within each level of the Pot treatment factor (plant in 5" pot or in ground). When the diameter growth rates for treatment levels of heavy and sparse hydroseed were run separately there were no significant effects of either level on the growth rate. For the height growth rate there was a significant effect of whether the plant was in a 5" pot or in the ground ($F_{1,12}=7.52$, $p=0.022$) for plants in heavy hydroseed. While the effect was significant, the sample size was small (<30) and the assumption of homogenous residuals was violated therefore the data were randomized as a precaution (randomized p -value=0.020). When height growth rate was evaluated for sparse hydroseed there was a significant Health-Rate effect rate ($F_{3,7}=8.83$, $p=0.009$). (Figure 3.3).

Cornus stolonifera

In 2005 the interaction term for Pot treatment* Hydroseed treatment was slightly above the level of significance ($p=0.081$) for basal stem diameter growth rate. While sample size was large (46 measurements), the means across one factor were compared within each level of the other factor since, F was much greater than 1 ($F=3.22$) and one of the terms of the interaction has an F-value much greater than the other term (F for Pot treatment was 35.51 and for Hydroseed treatment was 1.61), (Snedecor and Cochran 1980).

Analysis of Pot treatment levels separately indicated no significant effect of either level upon basal stem diameter growth rate. There was a significant effect of hydroseed treatment upon diameter growth rate for plants in ground ($F_{1,17}=5.51, p=0.031$). There was a significant effect upon height growth rate whether the plant was in a 5" fiber pot or in ground ($F_{1,37}=9.98, p=0.03$), (Figure 3.2). The other explanatory factors did not have a significant effect upon either diameter or height growth rates.

In 2006 there was a significant interaction term (Pot treatment* Hydroseed treatment) for diameter growth rate but there was no significant effect of either explanatory term upon height growth rate. Therefore the basal stem diameter growth rate was analysed separately for hydroseed treatment levels. When the growth rate for heavy hydroseed was run separately p was close to the level of significance, sample size was small and the assumption of homogenous errors was violated therefore randomization was undertaken to get a more accurate p -value ($F_{1,14}=5.05, p=0.060$, by randomization). Effects of explanatory factors were not significant for plants in sparse hydroseed (Figure 3.3).

Myrica gale

In 2005 the interaction term Pot treatment*Hydroseed treatment was near the level of significance ($F_{1,9}=5.06, p=0.051$) for basal stem diameter growth rate. Therefore the means across one factor (Hydroseed treatment) were compared within each level of Pot treatment (5" Pots and In Ground).

Analysis of plants in 5" Pots was problematic due to reduced degrees of freedom. However, the F-ratios and p-values were calculated manually using the sequential sums of squares. The results indicated that there were no significant effects of either treatment level upon basal stem diameter growth rate for plants in 5" Pots. While effects were not significant, it should be noted that due to reduced degrees of freedom for the error term (3) there may be substantial error in the calculated p-values due to the low power of the analysis. Basal stem diameter growth rates of In Ground plants were not significantly affected by either explanatory factor. Height growth rate was not affected by either explanatory factor. However, for the explanatory factor Herb-Rate the *p*-value was close to the level of significance, sample size was small and errors were not normal. Therefore the data were randomized with the results indicating a non-significant effect of Herb-Rate on the growth rate for height ($F_{2,9}=3.84, p=0.062$).

In 2006 there was a significant effect of whether the plant was in heavy or sparse hydroseed for basal stem diameter growth rate ($F_{1,9}=11.82, p=0.007$). The factors Health Rate and Pot treatment also had p-values near the level of significance but due to a small sample size ($n=16$) and violation of the assumption of homogenous errors randomization was required. Randomization resulted in a significant effect of Health Rate upon basal diameter growth rate ($F_{2,9}=5.19, p=0.043$) while the effect of whether plants were in 5" Pots or In Ground was not significant (Figure 3.3). No significant effect of either explanatory factor was observed for height growth rate.

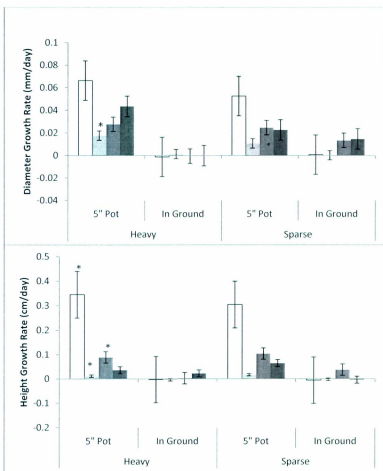


Figure 3.2: Mean basal stem diameter (top) and height (bottom) growth rates (± 1 SE) for *A. viridis subsp. crispa* (white), *B. papyrifera* (light gray), *C. stolonifera* (dark grey) and *M. gale* (black) plants grown in 5" Pot or In ground under Heavy and Sparse Hydroseed density, 2005. * indicates a significant difference between treatment groups. Negative growth rate in upper chart is from measurement error (widest part of stem not measured). Negative growth rate in lower chart is due to browsing and/or dieback.

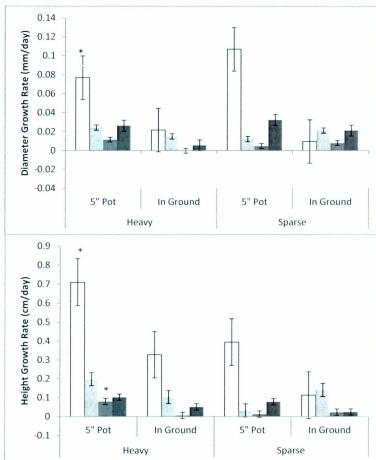


Figure 3.3: Mean basal stem diameter (top) and height (bottom) growth rates (\pm ISE) for *A. viridis* subsp. *crispa* (white), *B. papyrifera* (light gray), *C. stolonifera* (dark grey) and *M. gale* (black) plants grown in 5" Pot or In ground under Heavy and Sparse Hydrosed density, 2006. * indicates a significant difference between treatment groups.

3.3.3 Herbivory

Incidence of Herbivory

Although it was originally thought that browsing by *R. tarandus* and *A. alces* could have significant effects upon the success of the vegetation establishment of the FHCF it appeared that browsing has played a relatively minor role. Within the herbivory plots the incidence of browsing was low (2.4%) with only two incidents of herbivory recorded. Similarly, overall incidence of browsing was low (3.0%) with 12 instances of browsing attributed to *R. tarandus* and *A. Alces* (evidenced by a jagged appearance to the browsed stem) with an additional 3 instances not able to be identified.

Growth Rates

Alnus viridis subsp. *crispa*

In 2005, there was no significant effect of either explanatory factor upon growth rate for basal stem diameter for *Alnus*, but there was a significant effect of whether the plant was enclosed in Vexar ($F_{1,15}=6.27$, $p=0.024$) on height growth rate (mean without Vexar = -0.0170, mean with Vexar = 0.0243), (Figure 3.4). However, error assumptions appear to be violated as a result of a single outlier. Since p was close to the level of significance and the sample size was small the analysis was re-run with the single outlier removed to determine if the outlier affected the decision. After re-running the analysis the decision remained unchanged ($F_{1,14}=7.99$, $p=0.013$). Results for 2006 were reversed with a significant effect observed for basal stem diameter growth rate, but not height growth

rate, for the explanatory factor Vexar/No Vexar ($F_{1,12}=5.25$, $p=0.041$, Mean without Vexar = 0.01506, Mean with Vexar = 0.02967).

Betula papyrifera

For *B. papyrifera* there was no significant effect observed of either explanatory factor upon growth rate for basal stem diameter or height in 2005. However in 2006 there was a significant effect of Plot on basal stem diameter growth rate for *B. papyrifera* ($F_{1,18}=18.56$, $p=0.001$), (Figure 3.5). There appeared to be a significant effect of Vexar/No Vexar upon the height growth rate but p was close to the level of significance, sample size was small and the assumption of homogenous residuals appeared to be violated, primarily by a single outlier. The outlier was removed and the analysis re-run to determine if the outlier would affect the decision. After re-running the analysis the decision changed, therefore the data were randomized to get a more accurate estimate of p ($F_{1,12}=18.56$, $p=0.035$, by randomization).

Cornus stolonifera

In 2005 there was a significant effect of Plot on basal stem diameter and height growth rates ($F_{1,19}=17.35$, $p=0.001$ and $F_{1,19}=11.71$, $p=0.004$, respectively), (Figure 3.4). Whereas in 2006 there was a significant plot effect observed for height growth rate ($F_{1,10}=6.55$, $p=0.028$).

Myrica gale

There was a significant effect upon growth rate for basal stem diameter whether the plant was enclosed in Vexar or not enclosed in Vexar observed in 2005 ($F_{1,4}=8.52$, $p=0.043$, mean growth rate with Vexar was 0.01296mm/day, mean growth rate without Vexar 0.00648mm/day. In 2006 there was no significant effect of either explanatory factor for basal stem diameter or height growth rate.

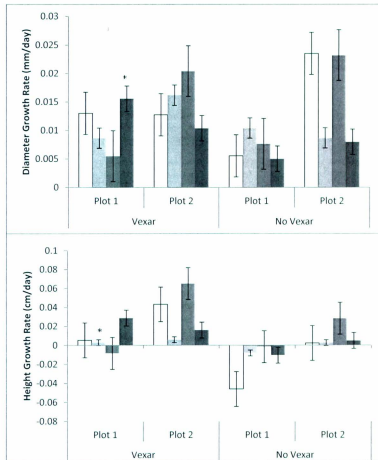


Figure 3.4: Mean basal stem diameter (top) and height (bottom) growth rates (± 1 SE) for *A. viridis* subsp. *crispa* (white), *B. papyrifera* (light gray), *C. stolonifera* (dark grey) and *M. gale* (black) plants grown in Plot 1 or 2 and enclosed in Vexar or not enclosed in Vexar, 2005. * indicates a significant difference between treatment groups. Negative growth rates may be explained by either herbivory or vegetative die-back. When die-back occurred the height was recorded to the top of live portion of the stem.

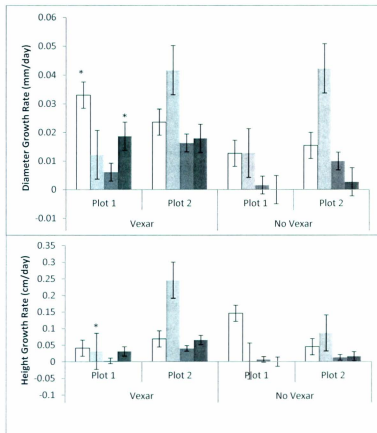


Figure 3.5: Mean basal stem diameter (top) and height (bottom) growth rates (± 1 SE) for *A. viridis* subsp. *crispa* (white), *B. papyrifera* (light gray), *C. stolonifera* (dark grey) and *M. gale* (black) plants grown in Plot 1 or 2 and enclosed in Vexar or not enclosed in Vexar, 2006. * indicates a significant difference between treatment groups.

3.3.4 Media/Mycorrhizal Inoculation

Growth rates for basal stem diameter (mm/day) and height (cm/day) of each species were evaluated using ANOVA based upon treatment conditions outlined in Table 3.3.

Alnus viridis subsp. *crispa*

There was as significant effect of Media upon the basal stem diameter growth rate ($F_{2,52}=3.49, p=0.038$), while neither explanatory factor had a significant effect on height growth rate in 2005 (Figure 3.6). In 2006, the interaction term Media*MYKE[®] was near the level of significance, therefore as per Snedecor and Cochran (1980) levels of the interaction were evaluated separately. When growth rates of MYKE[®] inoculated and un-inoculated plants were evaluated separately there was a significant media effect for inoculated and un-inoculated plants ($F_{2,19}=12.61, p<<0.001$ and $F_{2,19}=5.17, p=0.016$, respectively). There was also a significant effect of media upon height growth rate ($F_{2,42}=13.63, p<<0.001$).

Betula papyrifera

No explanatory factor had a significant effect upon growth rate for basal stem diameter or height in 2005. However, in 2006 there were significant media effects for basal stem diameter and height g rowth rates ($F_{2,52}=37.72, p<<0.001$ and $F_{2,52}=21.95, p<<0.001$, respectively), (Figure 3.7).

Cornus stolonifera

There was a significant effect of media upon both basal stem diameter and height growth rates in 2005 ($F_{2,53}=18.66$, $p<<0.001$ and $F_{2,53}=5.78$, $p=0.005$), (Figure 3.6) and 2006 ($F_{2,53}=6.90$, $p=0.002$ and $F_{2,53}=4.68$, $p=0.013$), (Figure 3.7).

Myrica gale

The interaction term (MYKE[®]*Media) was significant for basal stem diameter growth rate ($F_{2,16}=4.21$, $p=0.034$) but not for height growth rate in 2005. Therefore the analysis of MYKE[®] inoculated and un-inoculated plants was run separately. There was a significant media effect for MYKE[®] inoculated plants ($F_{2,6}=5.27$, $p=0.048$), but not for un-inoculated plants. There was no significant effect observed for either explanatory factor upon basal stem diameter or height growth rate in 2006.

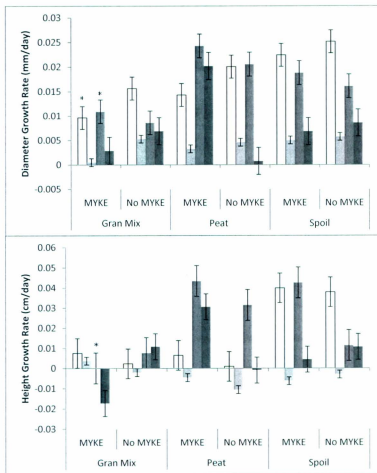


Figure 3.6: Mean basal stem diameter (top) and height (bottom) growth rates ($\pm 1SE$) of MYKE[®] Inoculated and Non Inoculated *A. viridis* subsp. *crispa* (white), *B. papyrifera* (light gray), *C. stolonifera* (dark grey) and *M. gale* (black) plants grown in Granite Mix, Peat and Spoil, 2005. * indicates a significant difference between treatment groups. Negative growth rates may be explained by either herbivory or vegetative die-back. When die-back occurred the height was recorded to the top of live portion of the stem.

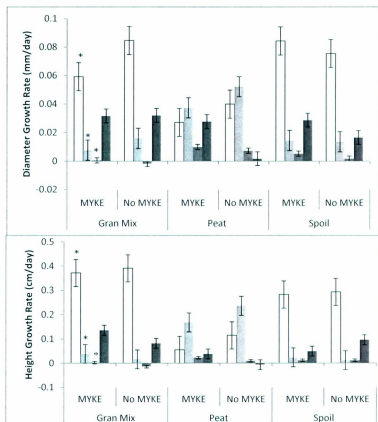


Figure 3.7: Mean basal stem diameter (top) and height (bottom) growth rates ($\pm 1SE$) of MYKE[®] Inoculated and Non Inoculated *A. viridis* subsp. *crispa* (white), *B. papyrifera* (light gray), *C. stolonifera* (dark grey) and *M. gale* (black) plants grown in Granite Mix, Peat and Spoil, 2006. * indicates a significant difference between treatment groups. Negative growth rates in upper panel are due to measurement error (not measuring widest portion of stem). Negative growth rates in bottom panel may be explained by either herbivory or vegetative die-back. When die-back occurred the height was recorded to the top of live portion of the stem.

3.4 Discussion

Given the relatively high survival (78.5% overall) observed during this study the native species *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale* are suitable for the restoration of riparian zones within Newfoundland. Generally, all species except *Cornus* are not negatively affected by competition from heavy hydroseed comprised of non native grass and legume species. Herbivory played a minimal role on the success of revegetation with only three percent of all plants showing evidence of browsing. While the use of a commercial mycorrhizal fungi inoculant was not suitable for use with these species the planting media had a significant effect upon the growth.

3.4.1 General Results (Survival and Soil Analysis Results)

Survival

The large mortality experienced for *M. gale* suggests that the species may not be suitable for revegetation projects. However, the species is known to be closely associated with water (it is commonly found partially submerged) therefore it may be necessary for future revegetation efforts utilizing the species to plant it in close proximity to water. Conversely *C. stolonifera*, another species closely associated with water, experienced the least mortality of either species suggesting that the species may be able to thrive under a variety of moisture regimes. The increase in percentage of live plants of *C. stolonifera* by

the end of the 2006 field season indicates the plant's ability to regenerate from below ground when conditions are suitable. The fact that *A. crispa* experienced the second highest amount of mortality was unexpected since the species is known as a pioneering species which colonizes recently disturbed sites. Similar to *Cornus*, there were incidents when the aerial portion of the plant appeared dead but re-sprouted from the root crown.

Soil Analyses

The variability in nutrient levels between replicate plots indicated that within a single media nutrient levels may be a factor. As an example, the granite mix media was comprised of topsoil stripped from the construction area, stockpiled and reapplied for revegetation activities. While the granite mix media came from the same stockpile, variability in nutrient levels between granite mix plots was observed (e.g., Ca is almost 10X higher in plot 2 than plot 1). This variability in soil nutrient levels may influence other analyses. For example, higher soil nutrient levels in a MYKE[®] inoculated plot adjacent to an un-inoculated plot could result in the detection of a significant effect of MYKE[®] inoculation but may be due to differences in soil nutrient levels.

Thus for future work, multiple composited soil samples could be collected from each designated plot area to determine if overall soil nutrient levels are similar. Alternatively, collection and analysis of soil samples prior to the establishment of plots could help to ensure that soils in pairwise plots have similar characteristics. Pairwise experimental plots could also be constructed so that each plot has the same soil depth and filled with soil with the same nutrient, pH and carbon levels.

3.4.2 Competition

A number of the analyses indicated that there was a significant effect of whether plants were in ground or in a 5" pot. When an effect was significant, plants in 5" pots invariably had higher growth rates than plants in ground. This suggests that the 5" pot reduces root competition between potted plants and non-native hydroseed species within the plots. However, the pots were filled with compost from Memorial University's Botanical Garden rather than using a soil similar to that of the competition plots (e.g., Granite Mix). The issue that this presents is that the compost media had higher levels of macro-nutrients (nitrogen, phosphorous and potassium), micro-nutrients (calcium and magnesium) and carbon than any of the competition plots for which soil samples were analyzed. The elevated nutrient and carbon levels may have resulted in the higher growth rates observed for plants in 5" pots. Ideally the media used to fill the 5" pots should have been the same material (Granite Mix) placed along the banks of the FHCF so that nutrient and carbon levels would be similar. In the absence of increased carbon and nutrient levels the significantly higher growth rates observed for plants in 5" pots could be attributed to the elimination of root competition. While the null hypothesis (increased growth for plants in 5" pots) was supported by the results, the real reason for the enhanced growth may be a result of the elimination of root competition, as a result of elevated soil carbon and nutrient levels or some combination of the two.

With respect to the potential effect of competition between native plants and hydroseed in plots, there were only two analyses which indicated significant differences between growth rates of plants in heavy and sparse hydroseed. One case was for C.

stolonifera where growth rates were higher in sparse hydroseed compared to heavy hydroseed and the other was for *A. viridis* subsp. *crispa* where the opposite was true. The null hypothesis (H_0 : Growth rates would be higher for plants in sparse hydroseed than for plants in heavy hydroseed) was falsified for three of the four species but was supported by *C. stolonifera* suggesting that the species may be susceptible to competition from hydroseed species. Alternatively, differences in soil nutrient levels between areas with heavy and sparse hydroseed may have contributed to differences in growth. As an example calcium and potassium levels within soils with sparse hydroseed were higher than those with heavy hydroseed. With respect to the remaining species it does not appear that hydroseed density has had an effect on the growth of these native species. An explanation for this may be that the size of the plants used in the competition plots may have resulted in little aerial competition between native plants and hydroseed species since most of the plants were of sufficient height so that they were not excessively shaded by hydroseed species.

3.4.3 Herbivory

The overall lack of browsing by *R. tarandus* and *A. alces* upon native species suggests that browsing may have had little effect upon revegetation success. Alternatively, the lack of browsing may be a result of low density of animals within the area during the study period. The overall low instance of browsing (3.0%) and lack of browsing within the herbivory plots (2.4%) suggest that the area is not a regular foraging

location. Browsing may have been the result of opportunistic individuals passing through the area. The infrequent browsing of plants may also be explained by the small size of most plants and low density of study plants within the area. Within the established plots, the average density was 1.5 plants per square meter (all species combined). Spacing between plots may have also affected the frequency of browsing (in some cases distance between plots was in excess of 100 m but may be less than 5 m). The combination of the small size of individual plants and the spacing between plants and plots may have resulted in reduced search efficiency by herbivores. De Knecht et al. (2007) report that when forage plant density was high, herbivores spend more time foraging in an area, whereas in areas of low plant density herbivores spent less time foraging in an area. Miller et al. (2007) suggest that plants with few stems may be browsed less than those with many stems. Marell et al. (2002) indicate that *R. tarandus* selected sites with higher green biomass of birch and willow species as forage areas. While the incidence of browsing was low for this two year study the development of larger multi-stemmed plants and increased green biomass in subsequent years may result in increased incidence of browsing.

An additional factor that may have influenced the lack of browsing is avoidance of the general area of the hydroelectric development site. The development of the hydroelectric site has led to an alteration of the habitat from vegetated barrens with pockets of forest to a relatively large barren area (several hectares) devoid of vegetation due to the construction of the development and placement of waste rock removed from the power canal and tailrace canal. The general location of the FHCf may also play a role

in reduced browsing such that the facility is essentially surrounded on three sides by water (Meelpaeg Reservoir to the south, RR Pond to the east and the tailrace canal to the west) and the generation facility and associated infrastructure is located to the north. It has been documented by Mahoney and Schaefer (2002) that *R. tarandus* exhibit avoidance behaviours of developed areas. Similarly, Ballard et al. (1988) also indicated that *A. alces* exhibit avoidance of developed areas.

When there was a significant Plot effect, the growth rate was higher for plants in Plot 2 (Plot 1 contained Granite Mix whereas Plot 2 contained Peat). This effect may be explained by differences in soil characteristics in the plots. In particular soil nitrogen, carbon and magnesium levels were 5.5, 16.9 and 1.51 times higher respectively in Plot 2 compared to Plot 1.

When there was a significant Vexar/No Vexar effect observed, plants enclosed in Vexar had higher growth rates than plants not enclosed in Vexar. These results support the null hypothesis that plants enclosed in Vexar would have enhanced growth due to the exclusion of herbivory by large herbivores. However, this hypothesis was formulated in the context of herbivore exclusion but the incidence of browsing was so low in the herbivory plots (2.4%) that the observed effect was not likely as a result of browsing. A possible explanation for differences in growth rate may be due to microclimates created through the use of the mesh tubes. As suggested by Johnson and Okula (2006) Vexar enclosures may have provided shade and reduced evapo-transpiration rates. Furthermore Vexar may have reduced the direct exposure of plants to wind. When plots with plants enclosed in Vexar were visited early in the morning, dew was noted on the Vexar mesh.

The mesh may have served to collect water (dew) and direct it to the soil below the Vexar tube. Similarly, during rainfall events the Vexar mesh may have acted as a funnel to intercept incoming rain drops and direct the water to the soil below.

3.4.4 Media/Mycorrhizal Inoculation

While *Alnus viridis* subsp. *crispa* (Malloch and Malloch, 1981; Massicotte 1985), *B. papyrifera* (Keane and Manning, 1988; Jones et al., 1997), *C. stolonifera* (Malloch and Malloch, 1981) and *Myrica gale* (Rose, 1980; Harley and Harley, 1987; Skene et al., 2000) can form mycorrhizal associations, the results of this study indicate that mycorrhizal inoculation had no effect upon growth rates of the target species in any of the media tested (Granite Mix, Peat or Spoil) (i.e. the null hypothesis of enhanced plant growth with mycorrhizal inoculation was falsified).

Inoculation of some members of the *Alnus* genus with arbuscular mycorrhizal fungi of the genus *Glomus* has been shown to result in increased growth and nutrient supply during early seedling establishment but ectomycorrhizal species become dominant after the initial established phase (Roy et al., 2007). Roy et al. (2007) indicate that of the natural mycorrhizal fungi species associated with *Alnus*, none were members of the genus *Glomus*. However, *Laccaria bicolor* (the species utilized for inoculation of *B. papyrifera*) is included as one of the species which associate with *Alnus*. Nelson (1987) indicated that cuttings of *C. stolonifera* Flaviramea and *M. gale* inoculated with *G. intraradices* were infected with *Glomus* 20 weeks after inoculation, suggesting that infection of the species

by *G. intraradices* is possible. However, Berliner and Torrey (1989) found no infection of *M. gale* by *G. intraradices* in the field or during greenhouse trials using inoculated local soil. The lack of infection of either species suggests that either *G. intraradices* is not suited to the local climate or there is incompatibility between plant and mycorrhizal species used in this experiment.

The other mycorrhizal species which is found in the MYKE® Pro-AN-1 inoculant is *Pisolithus tinctorius*. The species is known to have a southern temperate distribution (G. Warren, pers. comm.). This southern temperate distribution suggests that the species may not be suited to the climate of the region, and the Granite Canal area. Alternatively it is possible that *P. tinctorius* is incompatible with the study species.

The ectomycorrhizal fungus *Laccaria bicolor* was used to inoculate *B. papyrifera*. *L. bicolor* is a common species found within boreal forests and is known to infect members of the *Betula* genus. Jonsson et al. (2001) successfully inoculated *Betula pendula* Roth with *L. bicolor*. However, the species has not been collected in Newfoundland but a closely related species *L. laccata* (Scop. ex Fr.) Berk and Br. is widespread throughout the province (G. Warren pers. comm.). Similar to the other mycorrhizal species there may be plant-mycorrhizal fungi incompatibility between *B. papyrifera* and *L. bicolor*. Alternatively, since the species has not been collected in Newfoundland it may not be adapted to the local climate.

While the mycorrhizal-host association is often viewed as beneficial for the host, Johnson et al. (1997) suggest that the association is a continuum that falls somewhere between a mutualist association and parasitic association which is driven by

environmental conditions (e.g., nutrient levels). Comparisons of growth rates between inoculated and un-inoculated plants indicate that inoculation was not successful, however following the parasitism-mutualism hypothesis suggested by Johnson (1997) it is possible that inoculation was successful but the environmental conditions were not suitable for inoculation to benefit the host plants. Future investigations into the use of mycorrhizal inoculants should also include an examination of plant roots to confirm mycorrhizal associations.

While mycorrhizal inoculation had no effect upon growth rates, media regularly had a significant effect on growth. Growth rates were lowest for *Alnus* and *Betula* in peat. This is not atypical since *Alnus* are primarily found in soils with a sandy, gravelly or rocky texture (Matthews, 1992) whereas *Betula* are typically found on well drained but moist soils (Ryan, 1995). Growth rates for *Cornus* were highest in peat when media effects were significant. In general the species grows best on moist rich soils but does grow on a variety of soils (Crane, 1989). Crane (1989) indicates that growth on gravel and organic soils is fair to poor; while growth on sand, sandy loam, loam and clay-loam is good. The greatest growth on peat does not seem to fit with the preferences outlined by Crane (1989), however the peat substrate was the one with the highest moisture content and highest nitrogen content of either media. This suggests that the species may be able to thrive in atypical substrates given a suitable moisture regime and adequate nitrogen supply. The higher growth rates of *Cornus* in spoil (post construction material composed of gravelly-sandy soil) also suggest that the species may inhabit atypical conditions such as gravel media and reduced moisture availability if nutrient levels are suitable. In

particular, the higher phosphorous levels in spoil compared to granite mix appears to have promoted higher growth rates.

For *Myrica*, the only time a significant media effect was detected was when MYKE[®] inoculated and un-inoculated plant basal diameter growth rates were evaluated separately. In particular, growth rates were greatest in peat, followed by spoil and granite mix. High growth rates in peat may be expected since the species thrives in wet areas, including areas of peat based soil, whereas the increased growth rate in Spoil over Granite Mix may be due to higher phosphorous levels.

3.5 Conclusion

The restoration of disturbed sites is challenging since there may be conditions which are inhospitable for the establishment of plants. These conditions may be overcome through the use of a variety of methods (e.g., fertilization, organic soil amendments, pH adjustment and use of metal immobilizing agents). The use of cover crops which contain species which fix atmospheric nitrogen has also been common practice to help in soil development. The inoculation of soils with microbial populations (mycorrhizal inoculants or through the addition of beneficial bacterial populations) has also been practiced to help hasten the process of soil development. At the Granite Canal site stockpiling of topsoil and reapplication, use of hydroseed and addition of mycorrhizal fungi was practiced to help provide an environment suitable for plant establishment. While it has been documented that non-native hydroseed species may potentially compete with native species, this study found no clear evidence of competition between native species and non-native hydroseed species. While the use of mycorrhizal inoculants proved inadequate it remains an emerging field which has shown success elsewhere. Future development of inoculant production processes and continued refinement may lead to the use of native strains of mycorrhizal fungi suited to local climates. Herbivory has been shown to be an important driver of forest ecology. Intense herbivory can lead to ecosystem level changes and changes to plant assemblages (Gosse et al. 2011). While herbivory was not a factor in determining the success of the Granite Canal revegetation project (at least in the first two years) it is important to be cautious when discounting the

role that herbivory may play for successful revegetation and restoration. When in the planning stages restoration ecologists should plan for potential herbivory related effects. As an example unpalatable plant species may be selected in areas where there are large populations of herbivores. Overall careful pre-project planning, knowledge of site conditions and an understanding of the possible mechanisms of failure will help to ensure the success of restoration projects.

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Chapter 4: Overview and Recommendations for Future Work

4.1 Overview

Restoration projects which plan to use native vegetation are often faced with several issues which include the lack of availability of native plant suppliers, lack of suitable propagation protocols for native species and having to overcome site conditions which may not be suitable for plant growth. The lack of native plant suppliers is a major reason for the continued use of non-native species for revegetation activities. Although technology and research into the production of native species has progressed, there are still knowledge gaps for many species suitable for restoration. Even though propagation protocols for some species may be developed by individual nurseries, this information is usually not readily available and is often regionally specific.

In addition to securing native plants for restoration activities restoration ecologists are faced with overcoming site conditions which may be inhospitable to plants. Specifically, factors such as the material make-up of the soil at the site (e.g., peat, topsoil or base construction materials such as sand gravel and cobble), nutrient levels, pH, presence of phytotoxic compounds and moisture regime can be important in determining additional efforts required to overcome barriers to vegetation establishment and for selecting appropriate species. At sites where non-native hydroseed has been applied to provide a rapid vegetative cover and to initiate soil building processes, native species may have to compete with hydroseed species for resources. In areas frequented by large

herbivores the success of revegetation efforts may also be reduced due to heavy browsing pressure.

The goal of this research was two-fold: (1) to try to bridge knowledge gaps specific to the production of four targeted native species (*Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale*) found throughout Newfoundland and Labrador and; (2) to investigate the effects of various site conditions upon the establishment and growth of these species in a restoration project.

Propagation

Seed based and vegetative propagation (nodal cuttings and live stakes) was carried out for each species. Seed based propagation suggests cool moist stratification is not required for *A. viridis* subsp. *crispa* or *B. papyrifera* whereas stratification is required for *C. stolonifera* and enhanced germination of *M. gale* occurs with stratification. Exposure of unstratified seeds of each species with plant based smoke was inconclusive for *Alnus* and *Betula*, smoke did not promote germination of *C. stolonifera*, but there was a general trend of increased germination of *M. gale*. Vegetative propagation using nodal cuttings of each species produced best results for semi-hardwood cutting material rooted under misted conditions. In general, 15 cm cuttings rooted better than shorter 7.5 cm cuttings. Rooting of cuttings in various media was a factor but was linked with the type of wood used for cuttings. However, overall rooting of *Cornus* was better in Promix®-Perlite Media whereas for *Myrica* rooting was better in Peat-Sand media. Live stakes treated and

not treated with rooting hormone of *Alnus* and *Betula* failed to root whereas *Cornus* and *Myrica* rooted with and without hormone treatment.

Field Trials

Field trials included assessments of competition, large mammal herbivory, media and mycorrhizal fungi inoculation on plant growth. Competition from non-native hydroseed did not have a significant effect upon establishment and plant growth over the duration of this study. The incidence of large mammal herbivory was low (approximately 3% of plants were browsed). The use of the MYKE® brand of mycorrhizal fungi inoculants did not affect plant growth at the project site, whereas there were significant effects of media upon plant growth.

Overall the revegetation of the Granite Canal FHCF was successful. Based on the overall high survival rate it is evident that restoration was well underway by the end of this project. The results of this study suggest that the restoration of disturbed sites is entirely possible using native species when species specific protocols are followed. With the continued development and refinement of propagation protocols for a variety of species it will be possible to develop restoration using natural assemblages of plant species. As an example, the development of native grass and herbaceous seed mixtures will be able to provide a rapid natural vegetative cover rather than relying on the non-native hydroseed species. The use of these seed mixtures along with propagated woody species would allow revegetation using native species. The biggest obstacle to the use of native species for restoration is the lack of a reliable, cost-effective supply. This lack of

supply is a result of the lack of requirements for their use during restoration and revegetation activities.

4.2 Future Work

An integral part of expanding the availability and use of native species for future restoration activities is the development of suitable propagation protocols for a variety of species. This should be a major part of the focus of future work. Where propagation methods already exist it may be possible to refine methods to make production more efficient.

Another direction for broad scale research to focus is on understanding the inter-relatedness of restoration practices with the ecology of a disturbed site. In particular, restoration ecologists must develop an understanding of how to implement restoration practices which lead to the establishment of natural processes. As an example, restoration activities may place topsoil and install plants but restoration ecologists need to know how to guide the re-establishment of nutrient and carbon cycling processes before the restored area can be self-sustaining system comprised of native species. An important part of determining suitable restoration practices is to understand why previous restoration projects have failed. To develop this understanding regular long term monitoring of restoration projects is necessary. Monitoring would allow restoration ecologists detect factors that may lead to restoration failure. Regular monitoring would also allow restoration ecologists to adaptively manage restoration projects and take corrective measures before restoration failure.

The use of mycorrhizal fungi as part of restoration projects is one such way to help with the re-establishment of soil processes. While unsuccessful for this study, further

experimentation with mycorrhizal inoculants is encouraged especially if inoculants can be derived from local mycorrhizal species. Additional work should also undertake confirmatory tests to determine if inoculation is successful by sampling several individuals of each plant species for mycorrhizal infection. Further work should also be completed to determine minimum plant sizes to be used to vegetate previously hydroseeded areas so that natives are not under excessive competitive pressure for light resources.

An integral part of expanding the use of native species for restoration activities is the continued development of suitable and efficient propagation protocols for native species by government agencies, industry and academia. Furthermore, once protocols are developed they need to be readily available for restoration ecologists. Overall, the lack of availability of native species has led to the use of non-natives which in turn results in the lack of a need to develop an industry which specializes in the production of native species. To develop such an industry provisions must be made either through government regulations requiring the use of native species, adoption of best management practices which utilize native species by industry (e.g., mining companies using native species as part of mine closure planning) and through continued research that highlights the benefits, both short and long term, of using native species.

Appendix A: Recommendations for Restoration Projects Using Native Plant Species

General Recommendations

An integral part of a successful restoration project is early and proper planning. Many construction projects are planned and completed with specific restoration activities as an afterthought. In Newfoundland and Labrador there is a lack of a regular availability of suitable native species for revegetation activities, therefore proper planning will be required to engage nurseries to produce suitable species. In particular, revegetation planning should commence at least one year in advance of restoration activities. This lead time will allow for the collection of plant propagules (seed, cutting materials and/or rhizomes); plant propagation; and a period of growth under nursery conditions. Furthermore, a multiple year lead time could allow the production of larger more robust plants which may have enhanced survival over smaller plants. This additional lead time could also allow further experimentation and fine tuning of propagation methods for more efficient production.

When planning restoration activities restoration ecologists need to have an idea of what the site conditions will be prior to the onset of restoration activities. As an example, metal tailings disposal areas often have high metal concentrations and low pH, both of which create barriers to plant establishment. Therefore restoration activities must be planned with these conditions in mind and use amendments which counteract the effects of adverse site conditions.

The selection of appropriate species will be guided by several factors including availability of a particular species, site specific conditions and the availability of propagation information for species of interest. Some species may already be

commercially available. As an example pioneering species such as *Alnus viridis* subsp. *crispa* are shade intolerant but are tolerant of marginal soils low in nutrients typical of many disturbed sites. Another factor which needs to be considered is whether information on the propagation of the species is available. The availability of propagation information ensures less of a need to determine suitable propagation conditions resulting in more cost effective and efficient plant production.

Research Specific Recommendations

The following includes a number of recommendations, based upon the results of this study, for future work utilizing the species *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale*.

Propagation

Seed

Alnus viridis subsp. *crispa*

-Sow as soon after collection without cool moist stratification pretreatment.

Betula papyrifera

-Sow without cool moist stratification pretreatment.

Cornus stolonifera

-Prior to sowing ensure cool moist stratification at 3-5°C in moistened media for 60-90 days for best germination.

Myrica gale

- Prior to sowing ensure cool moist stratification at 3-5°C in moistened media for 60-90 days for best germination.
- Expose unstratified seeds to 180 minutes vegetative smoke to promote germination.

Vegetative Propagation

Alnus viridis subsp. *crispa*

- Misted semi-hardwood cuttings under mist in sand media produce good rooting results.
- Longer 15cm cuttings root better than 7.5cm cuttings.
- After sticking place cuttings onto a heated sand frame maintained at 22°C.
- Species is not suitable for live staking.

Betula papyrifera

- Semi-hardwood cuttings under mist in 1:1 peat-sand media produce good rooting results.
- Longer 15cm cuttings root better than 7.5cm cuttings.
- After sticking place cuttings onto a heated sand frame maintained at 22°C.
- Species is not suitable for live staking.

Cornus stolonifera

- Semi-hardwood cuttings under mist in Promix[®]-perlite media produce good rooting results.
- Rooting throughout year possible but results variable.
- Longer 15cm cuttings root better than 7.5cm cuttings.

- After sticking place cuttings placed onto a heated sand frame maintained at 22°C.
- Species is suitable for live staking with and without hormone treatment.

Myrica gale

- Semi-hardwood cuttings under mist in 1:1 peat-sand produced the highest rooting percentage.
- Cuttings 15cm and 7.5cm long both root well.
- After sticking cuttings placed onto a heated sand frame maintained at 22°C.
- Species is suitable for live staking with and without hormone treatment.

Herbivory

Herbivory does not have an appreciable effect upon the success for revegetation using the target species, suggesting the use of herbivore exclusion measures is not required for revegetation projects. However, spacing and small plant size may reduce the 'chance' of an herbivore finding individual plants. Higher plant densities and the presence of larger plants may increase the occurrence of browsing.

Mycorrhizal Fungi Inoculation with MYKE®

Difficult to determine if mycorrhizal inoculation suitable as data could not conclusively indicate as suitable/unsuitable. However, preliminary tests indicate that the use of MYKE® brand of mycorrhizal fungi inoculant utilizing the species *Glomerus intraradices*, *Pisolithus tinctorius* and *Laccaria bicolor* did not result in enhanced growth over the two year study period for target plant species within south central Newfoundland.

Media

Media utilized for planting of *Alnus* could include a sandy/gravel soil or topsoil but does not do well in peat based soils, whereas *Betula*, *Cornus* and *Myrica* can tolerate mineral based soils, topsoil or peat based soils.

Competition with Non-Native Hydroseed Species

The use of non-native hydroseed to provide rapid vegetative cover does not affect the growth of targeted native species. The use of appreciably sized planting stock (plants that are taller than hydroseed species at the time of planting) may help reduce aerial competition from hydroseed.

Practical Recommendations for Nursery Scale Production

The following includes a number of practical recommendations for nursery scale production of the target species *Alnus viridis* subsp. *crispa*, *Betula papyrifera*, *Cornus stolonifera* and *Myrica gale* based on lessons learned while carrying out this study.

Alnus viridis subsp. *crispa*

Seed propagation would be more suitable than vegetative propagation from cuttings. Given the time and effort required to collect cuttings and the difficulty in rooting cuttings this method of production would be inefficient. Rather the collection of copious amounts of seed for this species is simple and germination, while not near complete, is reliable. The sowing of multiple seeds per unit cell (i.e., pot) with post germination thinning (i.e., removal of individuals so that each unit cell contains a single plant) would ensure reliable nursery scale production.

Betula papyrifera

Seed propagation would be more suitable than vegetative propagation from cuttings. Similar to *Alnus* the collection of cutting material can be labor intensive for little rooting success. The collection of seed for this species is relatively simple but it may be difficult to collect seed from larger trees due to their height. Also, from experience the density of seed bearing trees is lower for *Betula* as compared to *Alnus* (e.g., most individuals of *Alnus* bear seeds whereas the same is not true for *Betula*). Therefore the collection of seed from *Betula* is more labour intensive than for *Alnus*. Similar to *Alnus*, the sowing of

multiple seeds per unit with post germination thinning would ensure reliable nursery scale production.

Cornus stolonifera

This species is suitable for seed based or vegetative propagation due to good seed germination and rooting of cuttings. For *Cornus* the selection of a propagation method chosen may be based upon the availability of seed or cutting material. As an example some thickets of *Cornus* may produce large amounts of seed bearing fruit while some may produce little seed or none at all in a given year. If seed is readily available I would suggest that propagation by seed would be less labour intensive and more efficient. Due to high germination percentage sowing of a single seed, or at most two seeds, per unit cell should result in a viable plant within each unit cell. Sowing of more than two seeds per cell would only result in reduced plant production. If seed is not readily available then the use of cuttings will be required.

Myrica gale

This species is suitable for seed based or vegetative propagation due to decent seed germination and good rooting of cuttings. As for *Cornus*, the selection of a propagation method for *Myrica* may be based upon the availability of seed or cutting material. If seed is readily available I would suggest that propagation by seed would be less labour intensive and more efficient. However, the germination percentages observed during this study were substantially lower than observed for *Cornus*, therefore the sowing of

multiple (three to four) seeds per cell may be necessary. When seed is not available then the use of cuttings will be required. While cuttings are more labor intensive the high rooting percentages observed ensure efficient production.



