DETERMINING THE ECOLOGICAL MECHANISMS OF FOREST ENCROACHMENT WITHIN THE ASPEN PARKLAND OF WESTERN CANADA

by

Rodrigo Lastra

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Department of Biological Sciences

University of Manitoba

Winnipeg

Trembling aspen – *Populus tremuloides* Michx.

ABSTRACT

The encroachment of woody species into grassland and savanna ecosystems has been well documented since the early 1800s. Elucidating mechanisms that regulate ecological interactions within systems is critical to understanding processes that drive change. Within the parkland ecoregion of western Canada, trembling aspen (*Populus tremuloides* Michx: Salicaceae) has been increasing in dominance. Aspen encroachment is best explained not by single mechanism, but rather by a number of interacting ecological factors. In this study I examine the ecological consequences of clonal biology in aspen as a means to explain persistence and observed tree-grass ratios within grassland savannas of Riding Mountain and Prince Albert National Parks, Canada. Results suggest that aspen stands cycle between a "stable" phase characterized by a dense mature canopy, and an "unstable" phase characterized by canopy breakup and increased regeneration from root suckers (Chapter 2). It is during this unstable phase that clonal encroachment is likely to occur. Within these mature stands, different-aged ramets promote persistence by maximizing the developmental variation in ecologically adaptive traits (Chapter 3). The survival of ramets is also dependent on the interaction between environmental conditions and maintaining functional physiological integration with the genet, this is especially true for ramets establishing on the leading edge of a clone (Chapter 4). Finally chapter 5 examines the consequences of variation in adaptive ecological relevant traits among individuals based on differences in vigor among aspen clones in relation to the production of defensive compounds. Findings demonstrate a high degree of variation in leaf phenolic glycosides production among clones. In all instances, vigorous clones were significantly higher in levels of phenolic glycosides compared with dieback clones, suggesting that some individuals may be predisposed to undergo density-independent mortality. This implies that one of the key mechanisms regulating population dynamics, community interactions and biodiversity may be related to intrinsic adaptive differences in susceptibility among individuals. These results clearly demonstrate that biotic and abiotic damaging agents rarely act upon populations in a uniform manner. Constraints imposed by internal biologic factors and contingencies set by random interactions with environmental factors results in a loss of uniformity in form and function among individuals.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank L. Adams, F. Daayf, T. Giroux, A. Hadrami, R. Lindroth, L. O'Bordovich, K. Orr, W. Vanderschuit, and J. Weir for their valuable help with various aspects of my project. I would also like to sincerely thank my committee: Dr. Bruce Ford, Dr. David Lobb, Dr. Jim Teller, and Dr. John Wilmshurst.

I would finally like to extend a very special thanks to my advisor, Dr. Norm Kenkel, whose mentoring, guidance and vast knowledge of nature and its complexities enriched my experience as a graduate student.

Funding was generously provided by Natural Sciences and Engineering Research Council of Canada, and Parks Canada, as well as a Fellowship and the University of Manitoba's Duff Roblin Graduate Fellowship.

THIS WORK WOULD NOT HAVE BEEN POSSIBLE WERE IT NOT FOR THE UNWAVERING SUPPORT OF MY FAMILY

I would like to dedicate this to them:

My Parents – Miguel Angel and Marcela, *for teaching me the meaning of hard work, committment and sacrifice*

My Wife – Jane, *for being my foundation, my beacon and my best friend*

My Children – Emily Jane and Matias Ambrossi, *for reminding me every day of the little things that truly matter in life*

TABLE OF CONTENTS

LIST OF FIGURES

FIGURES

LIST OF TABLES

TABLES

CHAPTER 1

INTRODUCTION

"Of the prairie, not much need be said; they are flat, covered with a fine growth of grass, and interposed with "bluffs" which are gradually disappearing before the hungry fire. If prairie-fires had been by some means arrested fifty years since, Manitoba would to-day have been a densely-wooded, instead of a prairie country."

R. Miller Christy (1887)

Notes on the Botany of Manitoba, Journal of Botany, London and Foreign

1.1 Historical perspective

British naturalist Robert Miller Christy's 1887 description and insight on the biogeography of southern Manitoba was important on a number of counts. First, it exemplified the non-static view of the natural world that was beginning to emerge towards the end of the 19th century (Hult 1881; Cowles 1899; reviewed in Cowles 1911). Second, it emphasized the critical role of "prairie-fires" in shaping the changing mosaic of the prairies. Third, it was consistent with earlier accounts that characterized the frontier of the Northern Great Plains as a vast undulating sea of "verdure", interspersed by "bluffs" of trees (Curtis 1959; Bird 1961). Within 40 years, much of this vast frontier had changed dramatically. The rapid expansion of agriculture and urban development altered the character of this natural landscape, while also removing the influence of natural processes such as fire and the modifying effects of grazers. The isolated “bluffs” of trees that were once kept “in check” by frequent fires (and thus often restricted to river valleys) expanded, encroaching the last refuges of untouched prairie and reducing the quality of forage lands throughout the region.

The prairie landscape described by Christy most likely corresponds to the southern extent of the aspen parkland in Manitoba. This region is a transitional biome separating the boreal mixed-wood forest to the north and the Great Plains to the south (Bird 1930; Bailey and Wroe 1974). As the name suggests, the aspen parkland is characterized by the presence of trembling aspen (*Populus tremuloides* Michx: Salicaceae), a dominant clonal tree species recognized for its extensive ecological and geographical distribution as well as its high genetic diversity (Barnes 1966; Mock et al. 2008). Trembling aspen, unlike other member of the genus *Populus*, is able to tolerate the drier conditions typical of the parkland. It does so through physiological adaptations that reduce the effects of moderate soil moisture stress, such as greater stomatal sensitivity (Lieffers et al 2001, see § 1.3.3).

The aspen parkland is considered a northern savanna ecosystem (Bird 1930; Archibold 1999), characterized by a scattered distribution of isolated groves of trees that break up an otherwise continuous grassland landscape (Archibold et al. 1996). Soils of this region are generally classified as calcareous organic-matter-rich Black Chernozems (i.e., black grassland soils; Fuller and Anderson 1993), which indicates both a relatively recent post-glacial arrival of forest and long-term persistence of prairie vegetation (Bird 1961).

Most of the early written accounts of southern Manitoba depict a landscape in flux (**Figure 1.1**). The description by Henry Youle Hinds (1859:46) of the changing mosaic of the parkland just west of the Brandon Hills underscores the dynamic and fluctuating nature of this region:

> *"...of the aspens and willows which then covered the country, nearly all disappeared [in a 20 year period]...the face of the country had changed, the aspen forest had been burnt and no vestige remained".*

Robert Christy's observations of southern Manitoba during the late 1880s came after a series of similar dry periods extending back to the early 1860s, which resulted in a concomitant increase in fire frequency (Sauchyn and Beaudin 1998). Thus the extent of open grasslands that characterized much of the aspen parkland savanna during the late 1800s cannot be interpreted as a "natural" condition, but rather one contingent on climate and the frequency and intensity of disturbance. Forest expansion is favored during wetter, cooler climatic periods when the frequency and intensity of fire are reduced; conversely, grassland expansion occurs during the periods of aridity when severe fires are more common. Variability in climate and disturbance cycles creates conditions that permit the long-term persistence of both grasslands and forests; it also implies that the parkland savanna is an inherently dynamic system (Baudena et al. 2010). Human settlement through much of the region resulted in the loss or severe dampening of most of the critical factors regulating this forest-grassland dynamic (Bird 1961).

The objective of this chapter is to discuss the dynamics of tree-grass interaction in the aspen parkland of western Canada. More specifically, I summarize and discuss the factors that determine contemporary patterns of coexistence between neighboring species functional groups (trees and grasses) in the absence of fire. I do this by presenting a case for vegetation change along the northern fringe of the Great Plains of North America, and by synthesizing existing mechanistic explanations.

1.2 Toward the coalescence of tree-grass coexistence theories: resolving the conundrum of woody plant encroachment

The invasion of grassland communities by woody (tree and shrub) species has been documented since the early 1800s (Brown and Archer 1999; Polley et al. 2003). Woody plant encroachment of grasslands is a pervasive global phenomenon (Throop and Archer 2008) that has generated a great deal of research (Loehle et al. 1996; Van Auken 2000; Jurena and Archer 2003; Stokes and Archer 2010). Forest encroachment has been documented in savanna ecosystems throughout in the world (e.g. Africa: Dublin 1979, Schwartz et al. 1996; Australia: Bowman et al. 2001; North America: e.g., Cottam and Stewart 1940, Burkhardt and Tisdale 1976, Van Auken 2000, Briggs et al. 2002; South America: Hoffman and Haridasan 2008). Most research effort has focused on understanding critical ecological processes, in order to better manage and conserve remaining native grasslands and economically important pastures (Scholes and Archer 1997).

While the theoretical framework of tree-grass coexistence models is well developed (Sankaran et al. 2004), the integrative maturity that comes with the development of an overarching general theory is lacking (Scheiner and Willig 2008). Because savannas are so geographically widespread and ecologically diverse, generalities regarding mechanisms of tree-grass coexistence are difficult to formulate outside a given biogeoclimatic zone (Archer 1990). Proposed mechanisms of woody encroachment often suffer from limitations imposed by the inherent complexity that results from historical contingencies and chance events. Results inconsistent with predictions from coexistence models may arise from functional differences in the responses of species to environmental conditions. In addition, historical contingencies may initiate cascading effects that alter the trajectories predicted by deterministic processes (*sensu* Clark et al. 2004).

Understanding the ecological processes that regulate forest-grassland coexistence provides the basis for the elucidation of critical mechanisms of forest-grassland dynamics. Climate and disturbance history are the primary factors regulating savanna ecosystem dynamics (Arno and Gruell 1983), so it is hardly surprising that these factors form the basis of contemporary, empirically based coexistence models. Classic equilibrium and ecological stability models (*sensu* May 1974) have been used to further develop a theoretical understanding of forest-grassland coexistence and dynamics (reviewed in Sankaran et al. 2004). The following three general models are used:

1) Equilibrium Models: These models assume that the ratio of tree to grass vegetation in savannas is fixed, determined by competitive interactions among species, and independent of changes in environmental conditions (Scholes and Archer 1997; Sankaran et al. 2004). In these models, differences in ecologically relevant traits among species provide the mechanistic explanation for long-term stability. Equilibrium models are predicated on the competitive exclusion principle, since spatial or temporal partitioning of resources among species is considered the principal

mechanism ensuring long-term co-existence and stability (Walter 1971; Walker and Noy-Mier 1982).

2) Disequilibrium Models: These models assume that the long-term coexistence of trees and grasses is not possible. Under this scenario, these ecosystems shift between two possible fixed states (forest or grassland) in response to changes in environmental drivers (e.g. altered fire regime; Sankaran et al. 2004).
3) Non-Equilibrium Models: These models assume that savanna ecosystems are in a constant state of flux. In these models dynamic changes in the tree to grass ratio are contingent upon variation in environmental conditions, as well as the frequency and intensity of disturbances (Higgins 2000). These factors promote the long-term coexistence of trees and grasses by eliminating the ecological consequences of stable environmental conditions (Chase 2007).

It should be noted that tree-grassland coexistence models were developed to provide an understanding of the stabilizing landscape-level factors regulating forest-grassland dynamics. By contrast, May's (1974) models were originally developed to explain patterns of species diversity as a function of equilibrium and stability ("diversity beget stability", sensu Elton 1958). When species-level mechanisms have been invoked in tree-grass coexistence models, they have done so only to disentangle the inherent complexity of population-level studies (e.g., Clark 2010, Stokes and Archer 2010).

All three models have some relevance to forest-grassland dynamics in the aspen parkland. As discussed below, model support is dependent on the specific scenarios or mechanisms examined.

Support for Equilibrium Models — Equilibrium-based models of forest-grassland coexistence in aspen parkland focus on the clonal biology of aspen, and on limitations to aspen invasion imposed by grass competition and edaphic factors. The ability of ramets to "average" soil conditions through their connected lateral root system has been cited as a major factor favouring aspen encroachment (Maini 1960). However, the "competitive advantage" of clonal integration in aspen has recently been questioned (Peltzer 2002). Equilibrium-based models propose that spatial partitioning of resource capture minimizes competition between tree and grassland vegetation (Higgins et al. 2000), under the assumption that competition from grasses limits tree species recruitment. While a negative relationship between grass abundance and the emergence and survival of tree seedlings has been demonstrated (e.g. Landhäusser et al. 2007; Hoffman and Haridasan 2008), structural interference by grass litter (rather then direct root competition from grasses) explains the relationship.

Equilibrium models invoke partitioning of resource space as an explanation for long-term co-existence. Such models have limited relevance in regions where environmental conditions are variable (as in the aspen parkland), since such variability negates competitive exclusion (Cahill et al. 2008, Lamb and Cahill 2008).

Support for Disequilibrium Models — In many forest encroachment studies, recurrent fire is assumed to be the critical factor controlling tree invasion into grasslands (Bond and Keeley 2005). Under a scenario of active fire suppression, a shift from a grassland or open savanna state towards a woodland state occurs (Arno and Gruel 1983). The assumed importance of fire forms the basis of grassland management, i.e. the need to introduce fire to restore "ecological integrity" (Sentar 1992). This scenario fits well with the disequilibrium model, at least as a means of generating management-based hypotheses. However, disequilibrium models may have limited merit to the long-term dynamics of aspen parkland. Aspen encroachment is determined not by fire frequency *per se* but rather fire intensity (reduction in stand-replacing fires), which is highly invariant on the landscape (Johnson 1992). Clonal recruitment in aspen is highest under a scenario of short interval fires of low to moderate intensity (Perala 1990; Weir and Johnson 1998), particularly those occurring early or late in the growing season when cooler, wetter conditions reduce fire intensity (Simonson and Johnson 2005).

Aspen parkland studies indicate that changes in environmental conditions and disturbance history lead not to community replacement (as predicted by the disequilibrium model), but rather to long-term co-existence (Hogg and Hurdle 1995). Although arid conditions (invariably coupled with regular, intense fires) have historically favored the expansion of grasslands at the expense of forests, aspen has nevertheless persisted in the parkland ecosystem for thousands of years (Ally et al. 2008). Similarly, cooler and moister conditions do not result in the exclusion of grasslands. Variability in environmental conditions precludes these ecosystems from moving to a "fixed" end point as predicted by disequilibrium models.

Support for Non-equilibrium Models — Most studies of tree-grassland dynamics are consistent with the predictions of non-equilibrium models (Staver et al. 2009). The aspen parkland, for example, is an inherently dynamic system prone to fluctuations in forest to grassland ratios in response to the "wax and wane" of climate and disturbance regimes at decadal time-scales (Scholes and Archer 1997). Although trembling aspen cover has increased as a result of fire suppression (Köchy and Wilson 2001), environmental and/or species-level factors that limit aspen growth and survival have impacted encroachment rates.

Non-equilibrium models implicitly recognize that ecological processes are determined not by a single mechanism, but rather by a large number of interacting factors (Higgins et al. 2000). This recognition does not require an increase in model complexity, but rather a conceptual understanding of how proposed mechanisms can fit into a pre-existing framework. For example, aspen recruitment/encroachment is a density-dependent process, regulated by a decline in the mature canopy that stimulates secondary recruitment (Chapter 3; Cumming et al. 2000). Changes in environmental factors can either amplify or dampen this biological process. Environments are inherently stochastic (*sensu* May 1974), and this stochasticity results in fluctuations in the relative abundances of organisms. This is consistent with non-equilibrium models, and serves as a parsimonious explanation for the long-term co-existence of species (Sankaran et al. 2004).

Demographic bottleneck models, which are special cases of non-equlibrium models, are based on any "top-down" disturbance that limits the recruitment and/or survival of trees; examples of such disturbances include drought, mammalian herbivory, and fire frequency/intensity (Staver et al. 2009). Such disturbance may affect only a portion of the population (e.g. mammalian herbivory, which affects only seedling and saplings; Higgins et al. 2000), or all demographic stages (e.g. fire). Overcoming demographic bottlenecks is central to explaining the success of trees in an otherwise grass-dominated system. Environmental fluctuations and disturbance regime affect ecological interactions and determine the relative proportions of trees and grasslands. For example, if critical thresholds in temperature and/or precipitation are exceeded over an extended period of time (e.g. severe protracted drought), sudden changes in community structure and composition can occur. However, severe environmental conditions are not a requirement of demographic bottleneck models; changes in community structure and composition can also occur under moderate but prolonged environmental shifts.

1.2.1 Reconciling co-existence models

Recent theoretical advances in ecology have come from integrating seemly disparate concepts, and by taking into consideration scale, contingencies and chance events (e.g. "diversity and stability debate"; Ives 2007). Advances in our understanding of forest-grassland co-existence have come from incorporating multiple conceptual models of co-existence, using mechanistic factors such as intraspecific variation, environmental heterogeneity, scale and chance events. All of these factors have an important role in the the complex dynamics of savannas.

Environmental 'bottom-up' models of co-existence — Edaphic-based models state that long-term co-existence of forest and grasslands is determined by strong vegetation-soil relationships (e.g. grassland communities occur on grassland soils; *sensu* Jenny 1941). Soil parent material is known to determine organic soil development and associated vegetation; in central Saskatchewan, the resilience and persistence of aspen forest and grassland communities reflects variation in the distribution of soil parent material (Bélanger and Pinno 2008). Forest stands occurring on moderately-drained glacio-lacutrine sites are more resilient to changes in environmental conditions and disturbance frequency than those on well-drained sites.

Facilitative Models Revisited —The debate in community ecology as to whether patterns of biodiversity are consistent with niche-based models (Tilman 1982), or dispersal–based "neutral" assembly models (Hubbell 2001), may be reaching a resolution (Chave et al. 2002; Chase 2005). When contingencies in local environmental conditions are considered, the development of community structure may shift between these two seemingly disparate assembly models (Chase 2007). With respect to encroachment by woody species, recent studies have indicated that during the initial colonization phase strong environmental selection pressures favour the recruitment of "stress tolerators" (Stokes and Archer 2010). As conditions change and environmental selection pressures ease, colonization shifts from a niche-driven process towards a dispersal-mediated one. This model invokes mechanisms of facilitation (Connell and Slatyer 1977) to explain positive feedbacks in woody encroachment, incorporating niche differentiation during the initial colonization stage and both demographic bottleneck effects and neutral processes at the post-establishment phase (Stokes and Archer 2010).

In the aspen parkland, woody species encroachment results in a gradual change in vegetation structure, composition, and edaphics (Fuller and Anderson 1993). For example, aspen encroachment is followed by the proliferation of woody shrubs such as *Symphoricarpos occidentalis* Hook. (western snowberry) that require relatively mesic conditions to establish and grow (Cameron 1975; Esser 1975). Other facilitative models incorporate changes in forest structure as a positive feedback mechanism that favors stability. For example, tree encroachment results in increased canopy light interception, reducing understory vegetation productivity. This lowers fuel loads, which in turn reduces both fire frequency and intensity (Hennenberg et al. 2006; Banfai and Bowman 2007).

1.2.2 The consequences of individual-level variation of ecologically relevant traits

Functional differences in ecologically relevant traits among species are central to classic competition-based models of coexistence. In recent years, attention has also focused on the importance of individual-level variation of traits (a central tenet of evolutionary biology) in promoting biodiversity-coexistence. Indeed, it is claimed that treating species as “variant entities” largely negates the importance of deterministic niche-based models (Hubbell 2001). Intraspecific variation in ecological response has been used to explain the coexistence of tree species (Clark 2010), and such ideas could be extended to explain long-term persistence of both forest and grassland vegetation in savanna ecosystems. The concept of individual-level variation of ecological relevant traits has also been used to explain intraspecific differences in susceptibility to hazards (O'Reilly-Wapstra et al. 2004; O'Reilly-Wapstra and Iason. 2007; Diner et al. 2009).

Incorporating variation among individuals (rather than a "mean" population response) can overcome the limitations of general "top-down" and "bottom-up" theories of coexistence. For example, the strict determinism of demographic bottleneck (non-equilibrium) models can be overcome by considering intraspecific variation in ecological traits. Intraspecific variation in survivorship, which is a characteristic feature of all species, has direct implications for savanna ecosystem dynamics. Tree encroachment into grasslands is well documented, but it does not occur uniformly across the landscape; some areas increase in forest cover, while others remain stable or even recede. These differences may reflect intraspecific variation in ecologically relevant traits (e.g. colonization ability, susceptibility to disturbances). For example, numerous studies have noted considerable spatial variation in the growth and mortality of aspen clones (e.g., Maini 1960; Barnes 1966; Cameron 1975). It is therefore important to examine the ecologically relevant traits that promote encroachment of trembling aspen into grasslands.

1.3 On the biological characteristics related to tree encroachment: a review of trembling aspen (*Populus tremuloides* Michx.) biology

The biological characteristics of woody species determine their ability to encroach into grasslands. Successful encroachers can generally tolerate moderate drought conditions, herbivory, and fire, and are capable of propagating both sexually and asexually under harsh growing conditions (Scholes and Archer 1997). The following review focuses on the biology of trembling aspen, with specific emphasis to the parkland savanna of western Canada.

1.3.1 Phylogeny of the Genus Populus

Trembling aspen (*Populus tremuloides* Michx.: Salicaceae, Section Populus; 2*n* = 38) is the most widely distributed tree species in North America. Six sections and 33 species are recognized within the genus *Populus* (Eckenwalder 1996; Hamzeh and Dayanandam 2004). The six sections are demarcated according to natural hybridization barriers (**Table 1.1**).

It is speculated that trembling aspen attained its vast ecological amplitude and genetic diversity by hybridizing with now-extinct taxa in the section Populus. The ancestral taxon of the genus *Populus* is hypothesized to have belonged to the section Abaso. This ancestral taxon, which was likely a tropical species, entered the fossil record about 60 million years BP and vanished approximately 29 million years BP. Members of the section Populus first occur in the North American fossil record approximately 20 million years BP (Eckenwalder 1996).

Table 1.1. The six sections and 33 species of the genus *Populus* (Eckenwalder 1996; Hamzeh and Dayanandan 2004). Bolded species are indigenous to Manitoba, Canada.

SECTION	**SPECIES**
ABASO:	*P. mexicana, P. guzmanantlensis*
TURANGA:	*P. euphratica, P. pruinosa, P. ilicifolia*
LEUCIODES:	*P. lasiocarpa, P. glauca, P. heterophylla*
AEGIROS:	*P. nigra,* ***P. deltoides****, P. fremontii, P. sargentii, P. angulata*
TACAMAHACA:	*P. laurifolia, P. ciliata, P. szechuanica, P. yunnanensis,* *P. suaveolens, P. simonii, P. laurifolia* ***P. balsamifera****, P. trichocarpa, P. angustifolia*
POPULUS:	*P. alba, P.tremula, P. adenopoda, P. gamblei,* *P. sieboldii, P. simaroa, P. davidiana* *P. monticola,* ***P. grandidentata, P. tremuloides***

1.3.2 Brief description of Aspen

Trembling aspen is a disturbance-dependent, clonal tree species. The species quickly re-establishes following disturbance (e.g. fire) from the perennial "bud bank" along its extensive, long-lived lateral rooting system. The clonality of aspen may be central to explaining its considerable ecological tolerance over a vast geographic range (Peterson and Peterson 1992).

Sexual reproduction — Aspen has long been considered to be functionally asexual (Maini 1960), but recent work in western North America indicates that reproduction from seed is more common than once thought (Mock et al. 2008). Trembling aspen, like all species of the genus *Populus,* is dioecious (separate male and female plants). Male and female clones are usually well mixed in forest stands, with a sex ratio close to 1:1 (Grant and Mitton 1979). Flowers occur in catkins that emerge early in spring (early May in Manitoba), before the flush of leaves; this facilitates wind-dispersal of pollen. Pollen grains have a thin exine layer that lacks a protective coating (i.e. sporopollenin), making it sensitive to dry atmospheric conditions (Maini 1960). Seed production in aspen is considerable, exceeding one million seeds by 20 years of age. The seeds are small (approximately 6 mm in length) and light (approximately 0.2 mg), facilitating long-distance wind dispersal. Production is highly variable, ranging from episodic (typical of clones within forest stands) to continuous (isolated, open-grown clones). Germination success can be low, since the seeds are non-dormant and not resistant to high temperature or low moisture levels. Seeds that fail to germinate immediately decay rapidly (Maini 1960, Eckenwalder 1996). However, more recent studies have shown that germination rates can be high in the foothills of Alberta and many parts of the boreal forest (Landhäusser et al. 2010)

Variation of clone size — Clone size in trembling aspen is determined by a number of interacting factors, including genet age, ramet production, root growth, genetics, competition, and environmental effects (Steneker 1973; Mock et al. 2008).

'Pando', the largest aspen clone recorded to date, occurs along the western limit of the species' range in Utah. It covers approximately 44 ha, with an estimated stem density of 1175 ramets ha^{-1} (DeWoody et al. 2008). There is a general trend of increasing clone size from eastern to western North America; this trend is most apparent for isolated clones (Mitton and Grant 1996). Factors thought to determine this longitudinal gradient in clone size include disturbance history (more frequent fires in the west – rejuvenates aspen clones), genet age (western clones are older), and competition among clones and with other tree species (Perela 1990).

In forest stands, trembling aspen clone size is constrained by competitive interactions with both intraspecific neighbors and other tree species (Barnes 1966). Forest clones in Riding Mountain National Park (near Lake Audy) range in size from < 40 m^2 to 15,000 m^2 (0.004 to 1.5 ha), although 44% of clones are < 40 m^2 in size (Steneker 1973). Clone size is determined by many factors, including the initial density of aspen individuals (genet density), genetic variation (some clones allocate more resources towards growth), sex differences, soil nutrient status (particularly soil calcium and potassium availability), and edaphic conditions (Kemperman and Barnes 1976). The lateral growth of forest clones only occurs once neighboring clones experience significant stem dieback (Kemperman 1977). This finding suggests that clonal boundaries along grassland edges may have higher rates of root-sucker initiation than those adjacent to another clone, or other tree species (Steneker 1973).

Variation in stem size and productivity — In Canada, aspen attains its maximum productivity and size in the boreal forest region, with stems often exceeding 25 meters in height and 25 cm in diameter (Peterson and Peterson 1992). By contrast, aspen occurring in the parkland savanna of western Canada are less productive; growth is stunted, with stems seldom exceeding 10 meters in height and 15 cm in diameter (Perela 1990; *personal observation*). The productivity of parkland aspen stands is likely limited by soil moisture (Maini 1972) and nutrients, particularly calcium, potassium, phosphorus and nitrogen (Gifford 1967). Moisture stress is not limited to drought, as water-logged soil conditions are also detrimental to aspen growth and survival.

Clonal and vegetative longevity — Aspen shoots (ramets: suckers and mature stems) are relatively short-lived (about 100 years, maximum 150 years), but longevity of the individual (genet) is often much greater. Estimating the exact age of clones is challenging, but recent investigations using molecular markers have found that clones in southwest Alberta range in age from 200 to 9,652 years (Ally et al. 2008).

Variation in leaf tissue within a clone — Trembling aspen leaves are simple, alternate, stipulate, and petiolate (transversely flattened). The genus *Populus* has heterophyllous leaves: the first-formed (pre-formed) leaves develop toward the end of the growing season and expand early in the following spring, whereas neo-formed leaves undergo leaf bud initiation and expansion in late spring and eventually replace the pre-formed leaves (Eckenwalder 1996). Neo-form leaves have greater photosynthetic rates, and can allocate greater resources to the production of defensive compounds following damage (Stevens and Lindroth 2005).

1.3.3 External factors influencing aspen health and survival

Mammalian herbivory — Trembling aspen is susceptible to damage from a variety of mammals, including elk (*Cervus canadensis*), moose (*Alces alces*), white-tailed deer (*Odocoileus virginianus*), black bear (*Ursus americanus*), porcupines (*Erethizon dorsatum*), beaver (*Castor canadensis*), snowshow hares (*Lepus americanus*), ruffed grouse (*Bonasa umbellus*), and a number of small rodent species. Wildlife damage can result in the loss of photosynthetic tissue (e.g., defoliation) and/or severe to moderate stem damage caused by chewing, clawing and antler rubbing. In western North America, mature aspen stems can be severely damaged by elk feeding on aspen bark, especially in wintering grounds (Kay 1997). Ungulate browsing can result in a temporary reduction in root-sucker regeneration during periods of excessive browsing, but root-sucker production is promoted when browsing intensity is low to moderate. Browsing damage is thought to stimulate sucker initiation by reducing apical dominance (Jones et al. 2009).

Insect pests — Insects are not considered a lethal damaging agent to aspen, as full recovery from even the most severe outbreaks is possible. Over 300 species of insects are known to affect aspen within Canada alone (Perala 1990). The most important group of insect pests are the defoliators such as forest tent caterpillar (*Malacosoma disstria* and M. *californicum*) and large aspen tortrix (*Choristoneura conflictana*), and the wood-boring insects such as the poplar borer (*Saperda calcarata*) and the bronze poplar borer (*Agrilus liragus;* Worrall 2010). Bark wounds (e.g. galleries) resulting from wood-borers can increase the susceptibility to other more serious damaging agents (Peterson and Peterson 1992). Forest tent caterpiller, aspen tortix and poplar borer have been noted throughout the Riding Mountain parkland (personal observation). It should also be noted that forest tent caterpiller can play a role in stimulating aspen root suckers following a defoliation event (Vic Lieffers *pers. Comm.*).

Fungal pathogens — Whereas mammalian herbivory and insect pests are general not considered lethal to aspen, fungal pathogens can be. Indeed, aspen decline (morality of the individual clone) is almost invariably attributable to fungal pathogens. One of the most serious is cytospora canker (*Valsa sordida*), which can result in the complete failure of root-sucker regeneration (thus killing the clone). This fungal pathogen has been implicated as a key biotic agent causing aspen decline throughout the species' range (Debyle and Winokur 1985; Worrall et al. 2008). Any agent resulting in bark damage or branch dieback can initiate an outbreak of this serious canker infection.

1.3.4 Internal factors influencing aspen health and survival

Considerable differences in aspen vigour occur over the distributional range of the species. In some regions fire suppression has resulted in a decline in stand health, which is thought to be associated with age-dependent increases in susceptibility to insects and/or fungal pathogens (Rehfledt et al. 2009, Worrall et al. 2010). By contrast, in the parklands of central Canada fire suppression has promoted encroachment of aspen in grasslands and open savannas (Köchy and Wilson 2001).

Trembling aspen stands in the parkland savanna consists of both dieback and vigorous clones. Aspen loss (dieback events) is occurring in southern Alberta, where the combined effects of prolonged drought and severe insect defoliation events have negatively impacted all members of the population (Hogg et al. 2008). Aspen health is more variable in the boreal mixed wood forests of Manitoba and Saskatchewan; the dry atmospheric conditions characteristic of the western portion of the parkland are less evident in Manitoba and eastern Saskatchewan (Hogg et al. 2002). Stand-level variation in edaphic and species adaptive traits therefore play a much more important role in regulating aspen dynamics in these forests. In most areas of western Manitoba, aspen stands include a mixture of dieback and vigorous clones (Hogg et al. 2002).

Variation of adaptive traits — Trembling aspen shows high genetic diversity (Cheliak and Dancik 1982; Stevens et al. 2007; Mock et al. 2008). Corresponding variation of ecologically relevant plant traits may explain variation in survivorship among individuals in a population. Genetically determined differences in clone size, shoot initiation, flowering time, polyphenolic compound production, and phenology of both pre-formed leaves and neo-formed leaves all reflect genetic and developmental variability among clones (Eckenwalder 1996; Kemperman 1977; Kemperman and Barnes 1976). The ecological consequences include differences in susceptibility among clones to numerous environmental factors, including herbivory and abiotic stressors (McGarth et al. 2010). Genetic variation can also occur as new ramets are produced through the process of somatic mutation, resulting in a "genetic mosaic" within a clone (Ally et al. 2008). The ecological consequences of inter-ramet genetic variation are unknown.

1.3.5 Species adaptive traits that enhance encroachment potential.

The ability of aspen ramets to survive and grow in the drought-prone grasslands of the parkland is contingent upon critical species adaptive traits. I discuss here three critical ecologically adaptive traits that enhance aspen encroachment.

Clonal integration — The extensive and integrated lateral rooting system of aspen is critical to its long-term success in the aspen parkland and the boreal mixed wood forest (Maini 1960). Clonal integration averages the spatial heterogeneity of limiting resources in the soil, thereby permitting the clone to exploit patchy resources (de Kroon and Knops 1990; Peltzer 2002). The connected root system also allows mineral nutrients, hormones, water and fixed carbon to be transported between developing and mature stems (ramets). Connections among ramets may persist for 50-80 years (DesRochers 2000). Clonal integration therefore enables aspen to establish in areas that might otherwise be considered too harsh. Although the original connectivity may be lost as the root system ages, connections may be re-established via root grafting (Sandberg 1951, Lieffers et al. 2001).

Water conservation — A critical adaptive trait of encroaching species is the ability to tolerate moderate soil moisture stress. Physiological integration is one of several ecologically relevant traits that enhances aspen clone fitness; others include water use efficiency and leaf morphological characteristics. Stomatal conductance in aspen is sensitive to changes in leaf water potential (more sensitive than other *Populus* species); stomatal conductance is significantly reduced in hot and dry weather (Lieffers et al. 2001). The species is able to conserve soil water, and minimize the likelihood of stem cavitation, by maintaining remarkably consistent transpiration rates over a wide range of atmospheric conditions. In addition, the flattened leaf petioles promote “fluttering”, which cools the leaves as much as 2 – 4^0C and thus improves CO_2 uptake in warm and dry weather (Maini 1960; Lieffers at al. 2001). An additional adaptive response of aspen to dry environments is reduction in maximum height growth, which lowers maintenance costs associated with respiration (Peterson and Peterson 1992).

Although aspen is well adapted to growing in drought-prone areas, phenotypic variation results in clones with comparatively high stomatal conductance. Individual clones with higher stomatal conductance are at greater risk of rapid dieback under prolonged drought conditions (Lieffers et al. 2001). Variability of ecologically relevant traits, when combined with environmental variation, results in differential performance among individual clones.

Plant secondary metabolites — Trembling aspen produces a vast array of secondary products, including condensed tannins and phenolic glycosides (both of which are stored in stem and leaf tissue) as well as coniferyl benzoate, which is found only in flowering buds (Lindroth and Hwang 1996). These phenolic products are derived from shikimic acid and aromatic amino acids via the shikimic acid pathway. The allocation of phenolic glycosides and condensed tannins is related to stem development; phenolic glycoside production is highest in root-suckers less than 5 years old, whereas production of condensed tannins continues to increase with age (Donaldson et al. 2006).

The most important secondary products are the phenolic glycosides salicortin and tremulacin, which are considered effective broad-spectrum chemical deterrents against a host of "inciting" biotic factors that include mammalian herbivores, insect pests and fungal pathogens. The efficacy of these phenolic compounds is concentration-dependent, since there is an inverse relationship between phenolic glycoside concentration and consumption rate or palatability (Stevens and Lindroth 2005). Variation in phenolic glycoside production among clones is determined by both genetics and environmental interactions; there is a strong relationship between the vast genetic diversity of aspen clones (e.g., Cheliak and Dancik 1982) and phenolic glycoside production (Lindroth et al. 2007). It has been hypothesized that this variation of phenolic glycoside production may have important implications on the population dynamics of the species.

1.3.6 Persistence of aspen at different developmental stages

The local persistence of species is related to risk factors associated with critical developmental stages. I begin with a discussion on the root system of aspen.

Root system — Aspen roots are the longest-lived portion of the species, and the least vulnerable state (Perela 1990). Aspen roots are classified into two general types, sinker (vertical) roots and lateral (horizontal) roots. The sole function of sinker roots is to acquire water and nutrients. The function of lateral roots is more complex, as they may give rise to additional sinkers as well as vegetative shoots or "root suckers" (Pregitzer and Friend 1996). The ability of aspen to successfully encroach into grasslands is facilitated by the species' ability to reproduce vegetatively; indeed, the ultimate biological unit of aspen "invasion" is its lateral root system (Barnes 1966; Schier 1975; Perala 1979). The spatial pattern of encroachment is therefore determined by the extent, persistence and growth of the lateral root system. In some cases, aspen "encroachment" is nothing more than the re-establishment of suckers from an existing lateral root system (Shier 1975).

A single aspen clone can have numerous shoot initiation events over the course of its lifetime (Maini 1960). Given the potential longevity of aspen clones (Ally et al. 2008), the massive lateral root system has been referred to as a perennial "bud bank" (Kemperman 1977). This bud bank remains largely dormant provided that the clone maintains a sufficient leaf area in the canopy (Landhäusser and Lieffers 2002).

Under favorable conditions, stand establishment and subsequent clonal growth (via root-suckering) occurs. In the specific case of aspen encroachment into grasslands, growth of the root system can be constrained by numerous factors such as root severing by burrowing animals, soil compaction, soil moisture stress, and physical barriers imposed by the distribution of excessively-drained soils (Cameron 1975).

The survival of the clone is ultimately determined by survival of the root system. The term "aspen dieback" refers to mortality of mature stems (but not the root system), whereas "aspen decline" refers to the mortality of the individual. Root death can occur following severe and chronic dieback of stems, or from a multitude of biotic and abiotic factors that adversely affect the root system (see review in Frey et al. 2004). For example, anaerobic soil conditions resulting from standing water can cause rapid death of roots (Perela 1990). One of the critical factors influencing root survival in aspen is carbon supply. A constant source of fixed carbon in the form of nonstructural carbohydrates is required to maintain a positive carbon budget within the root system. Given the high respiratory costs associated with maintaining a large root system, any decline in clonal carbon reserves can result in root death. If the root system of aspen is starved of a carbon source for at least four years, respiration costs will result in death of the entire clone (W. D. Shepperd, *pers. comm.*). This can occur following any process that results in the prolong loss of canopy leaf area (e.g., defoliation, or stem mortality), but can be reversed upon re-establishment of the canopy from root-suckers.

Initiation of shoot primordia (sucker initiation) — The initiation of shoot primordia from lateral roots is determined by a multitude of abiotic and biotic factors (Schier 1973).

Shoot initiation occurs in response to stand-level disturbances such as fire, logging or severe defoliation (Perela 1990, Cumming et al. 2000). Mature stem mortality triggers a hormone-mediated response that initiates shoot primordia development. This process is regulated through the production and degradation of growth hormones. Although uncertainties remain regarding the exact role of hormones (e.g. auxin and cytokinin) in shoot primordia initiation or inhibition, it is generally accepted that cytokinin directly or indirectly promotes the initiation of shoot primordia. Auxin, on the other hand, tends to inhibit the initiation of shoot primordia (Schier and Zasada 1973). Auxin-mediated shoot inhibition (“dormancy”) is maintained along a given lateral root for as long as an adjacent stem is alive (Schier 1973). Loss of apical dominance, resulting from the death of mature stems following disturbance (e.g. windstorms, fire, logging, fungal infection), reduces the auxin to cytokinin ratio and so promotes prolific suckering.

Once the shoot primordia develops, subsequent growth and performance depend on soil temperature, root carbohydrate reserves, and soil nutrients (Fraser et al. 2002; Landhäusser and Lieffers 2002; Mundell et al. 2008). Soil temperature strongly regulates the growth rate of shoot primordia. Optimal shoot initiation occurs in warm soils (20^{o}C), with no shoot development occurring at temperatures below 10^{o}C (Maini 1961; Fraser et al. 2002). If initiated late in the growing season, most shoot initials remain in a dormant state or fail to emerge from the soil (Schier 1973; Schier and Campbell 1980). Growth is also highly dependent on root carbohydrate reserves (Schier and Zasada 1973). Indeed, such reserves are critical to the re-establishment of aspen stands following disturbance.

Growth of the shoot primordia: recruitment stage — Once a shoot initial emerges from the soil it is termed a root sucker, shoot or ramet. The newly emerged aspen ramet is initially dependent on the parent root for resources (water, nutrients and fixed carbon), but this dependency declines rapidly as the shoot becomes photosynthetically independent. In most clones, the functional connection of a ramet with its parent root is reduced by 10 years of age (Sandberg 1951). In older ramets, physiological integration functions primarily as a means to allocate carbon reserves obtained from stems (leaves and bark photosynthesis) towards the roots (Landhausser and Lieffers 2002). In mature stems, the carbohydrates needed for canopy leaf flush in the spring are mobilized from reserves stored in large crown branches (Landhäusser and Lieffers 2003).

Young aspen ramets (< 5 years of age) are susceptible to numerous demographic bottleneck processes; they can experience damage or mortality from a wide range of biotic and abiotic factors (Perela 1990). As such, it is the most vigorously defended stage in terms of secondary defensive compounds (Lindroth and Hwang 1996).

1.4 Conceptual and theoretical framework of the thesis

The theoretical underpinnings of this thesis are based on a well-established framework of contemporary tree-grass coexistence models as they apply to aspen biology. The genesis of this project came from the need to understand the principal factors regulating aspen encroachment within remnant fescue grasslands of Riding Mountain National Park (RMNP) and Prince Albert National Park (PANP). Historic studies conducted by Blood (1966) and Trottier (1974) in RMNP revealed extensive expansion of trembling aspen into neighboring grasslands during the early and mid 20th century. All grasslands in the park were impacted, and both studies cited the cessation of cattle grazing and fire suppression policies in the early half of the 20th century as factors contributing to the increase in tree cover. Later work by Slogan (1997) documented long-term changes in grasslands based on permanent study plots established by Trottier (1974). It was found that some grassland areas declined by as much as 10% over a 22-year period (1973 to 1995). These results are consistent with other studies undertaken in the aspen parkland and Northern Great Plains ecoregions of western Canada (Southern Saskatchewan: Maini 1960; Prince Albert National Park: Cameron 1971).

The aim of this thesis is to elucidate the mechanisms regulating coexistence of aspen forest and grasslands in parkland savanna, specifically by building on previous work in order to better understand the ecology of aspen encroachment. The four original research chapters in this thesis are in manuscript form. **Chapter 2** begins by describing the spatial pattern of aspen encroachment. Most studies of tree encroachment into grasslands have implicitly assumed that forest "invasion" is uniform. Within the grasslands of RMNP, one of the most striking features is the strong variability in patterns of forest encroachment. Trembling aspen clones show considerable variability in health, with some showing signs of dieback and others displaying more vigorous growth. As a result, some regions have experienced significant forest expansion whereas in other (neighboring) regions forest boundaries have remained static or even receded over time. The objective of **Chapter 3** is to describe the spatial pattern of root-suckering within four isolated aspen clones. Long-term temporal analysis examining mortality and individual ramet growth rates were explored in order to determine age/size class related susceptibility.

The final two chapters (Chapters 4 and 5) examine the "individual susceptibility hypothesis", which states that variation in ecologically relevant traits among clones influences the impact of demographic bottlenecks within a population. **Chapter 4** examines the ecological consequences of clonal integration in dieback and vigorous clones located in three edaphically distinct grassland sites. The objective is to determine variation in the long-term survival of ramets following lateral root severing, and to what extent moderate soil moisture stress and clone health affects ramet survival. **Chapter 5** examines the causes of strong variability in vigor among aspen clones. The objective is to determine whether intraspecific variation in the production of defensive plant secondary products can explain differences in health (vigorous versus dieback) among aspen clones. I hypothesize that low foliar concentrations of phenolic glycosides may predispose some individuals to chronic stress, leading to differential mortality within populations.

5.1 Research Objectives:

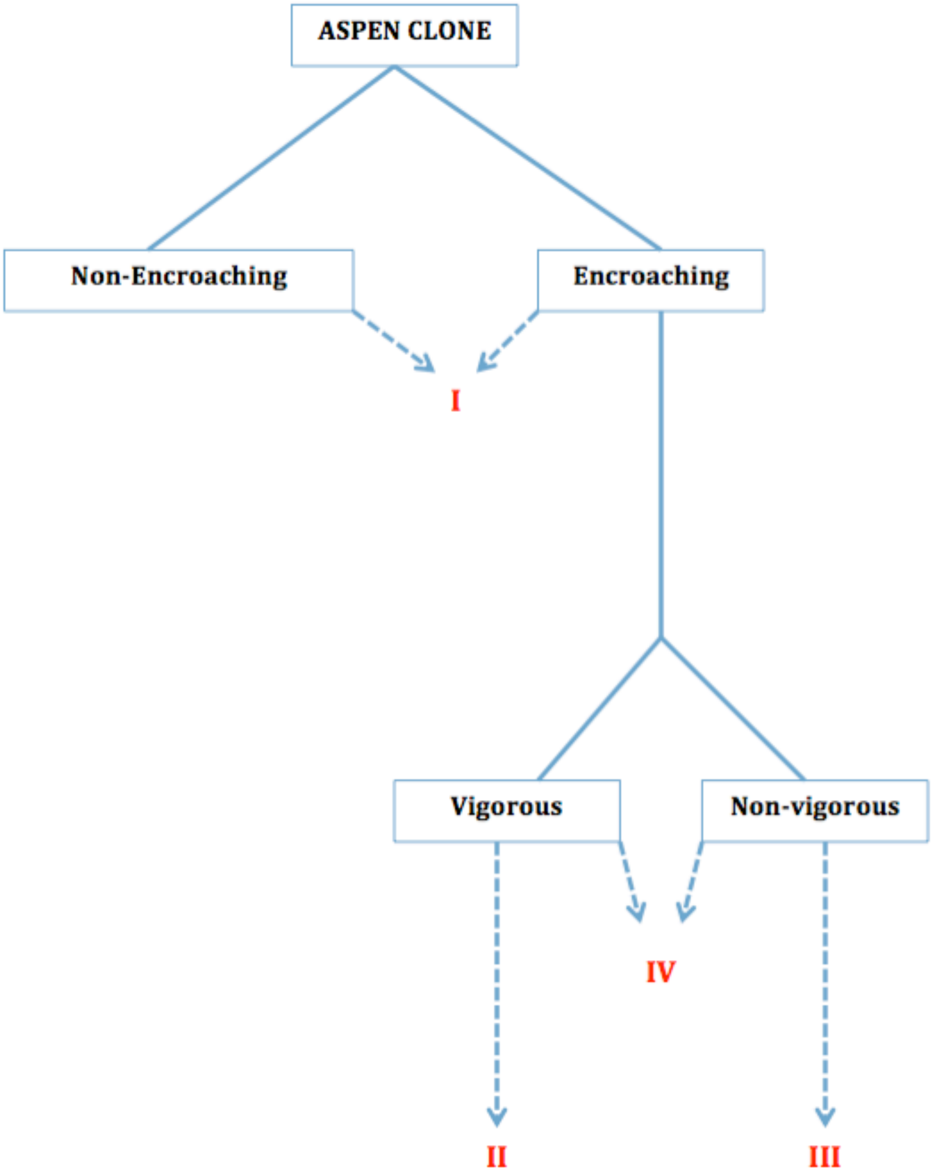
The objectives of this study are to elucidate the biological mechanisms that drive aspen invasion within the fescue grasslands of Riding Mountain and Prince Albert National Park. The four objectives are derived to address clone encroachment and vigor

I. TO ASSESS THE CAUSES AND CONSEQUENCES OF CLONAL ENCROACHMENT

II. TO ASSESS THE SIGNIFICANCE OF CLONAL INTEGRATION

III. TO DETERMINE THE GROWTH AND MORTALITY RATES OF ASPEN ROOT SUCKERS

IV. TO DETERMINE THE CAUSAL FACTOR(S) THAT EXPLAIN VARIATION IN CLONE VIGOR



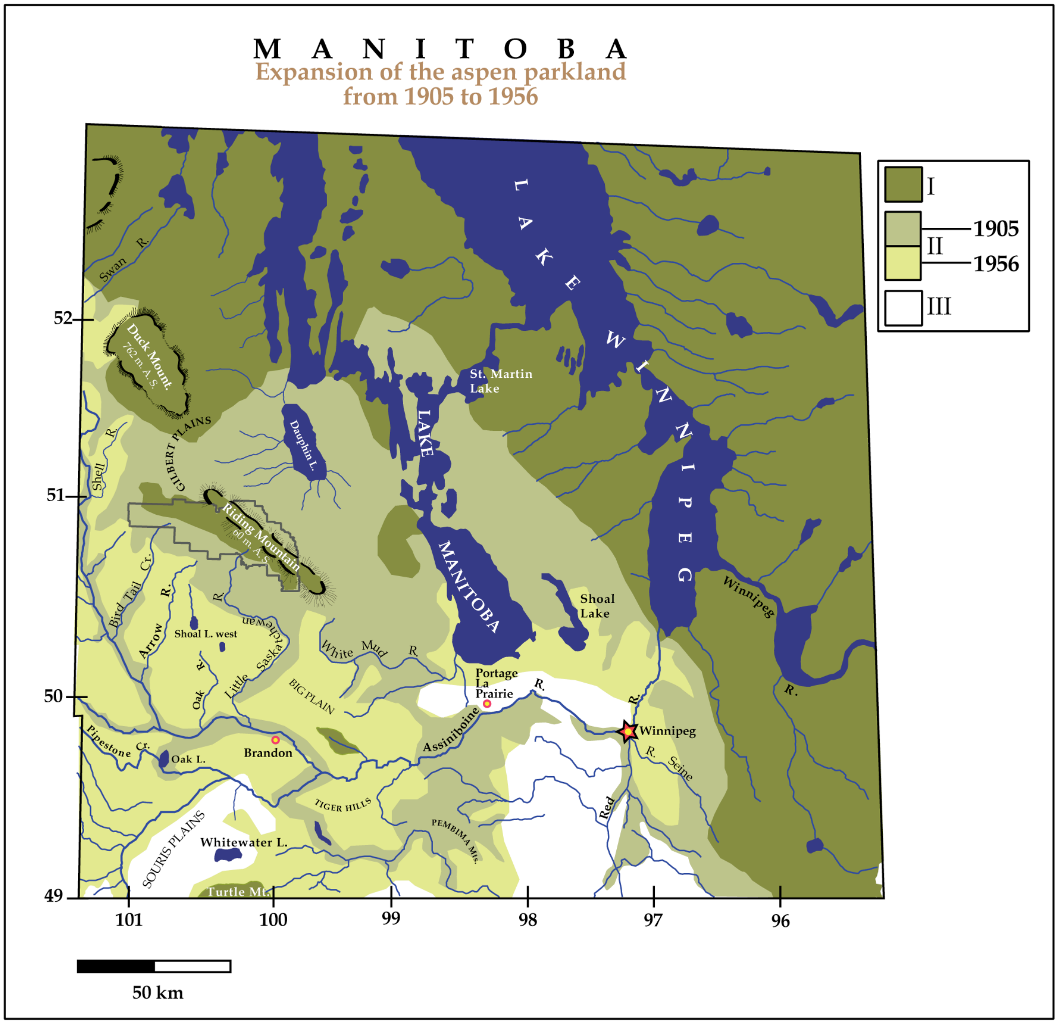


Fig. 1a. Map of southern Manitoba depicting coverage of grassland (I), aspen parkland (II) and boreal forest biomes in 1905 (Modified from Bird 1961).

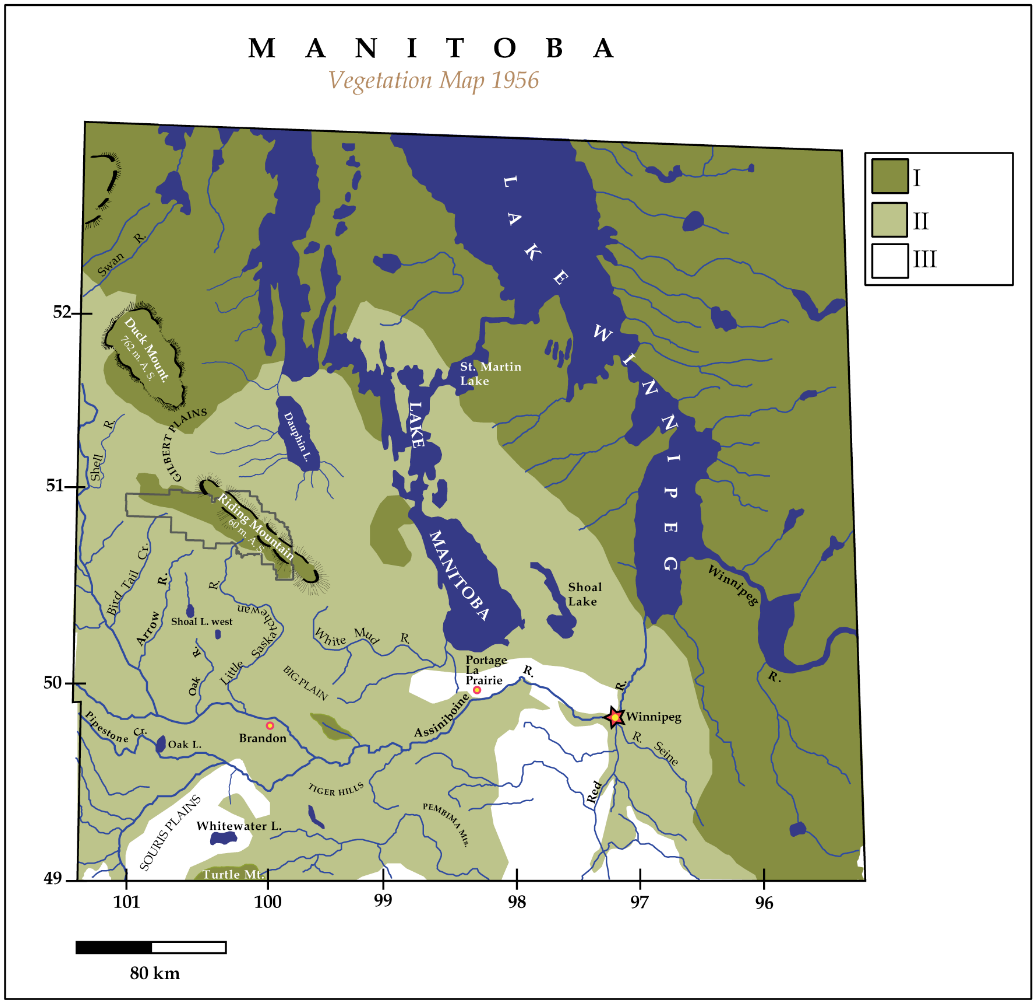


Fig. 1b. Map of southern Manitoba depicting coverage of grassland (I), aspen parkland (II) and boreal forest biomes in 1956 (Modified from Bird 1961).

CHAPTER 2

THE ECOLOGICAL CAUSES AND CONSEQUENCES OF ASPEN ENCROACHMENT WITHIN NOTHERN FESCUE GRASSLANDS

2.1. INTRODUCTION

Trembling aspen (*Populus tremuloides* Michx.), a species of considerable genetic variability (Mock et al. 2008), has the widest geographic and ecological range of any North America tree (Mitton and Grant 1996). The ecological success of trembling aspen has been attributed to its clonal biology, particularly its ability to re-establish from root suckers following any disturbance that results in the partial or complete loss of above-ground biomass (Schier 1975; Shepperd et al. 2001; Landhäusser and Lieffers 2002; Fraser et al. 2004). It is this "legacy effect" of a perennial clonal "bud bank" that ensures the long-term regeneration potential of aspen (Gradowski et al. 2010).

The production of aspen root suckers is initiated by the release of apical dominance following stem mortality. This loss of apical dominance stimulates the initiation of root suckering via a hormone-mediated response within the clone; subsequent development is determined by various environmental factors, including soil temperature, soil nutrient levels, and light availability (Fraser et al. 2002; Landhäusser et al. 2006). Under appropriate environmental conditions, stem loss results in extremely rapid and prolific sucker production; in the first season following clear-cutting, root sucker densities can exceed 70,000 stems ha^{-1} (Crouch 1983; Mundell et al. 2008; Lennie et al. 2009). While the ability of aspen to regenerate from root suckers following large "catastrophic" disturbances (e.g. stand-replacing fire, clear-cutting) is well established, recent studies indicate that pulses in stand regeneration following the formation of canopy gaps (i.e. mortality of individual stems, or a small number of stems) results in the persistence of trembling aspen in later-successional forests (Kurzel et al. 2007). In boreal aspen stands in Alberta, small canopy gaps showed a 15-fold increase in root sucker densities compared to areas with a continuous canopy (Cumming et al. 2000). Similarly, mature stem loss attributable to severe storm damage resulted in a 20-fold increase in root sucker density (Peltzer and Wilson 2006). These results indicate that any physical disturbance – at any spatial scale – that results in stem loss will initiate vigorous root sucker production (Shier and Campbell 1978). This clonal regeneration cycle (sucker initiation following release from apical dominance) will continue for as long as the clone remains alive and healthy. Given that some aspen clones can live for 1,000 years or more (Ally et al. 2008), this mortality-regeneration cycle is a critically important mechanism explaining the self-replacing capacity and persistence of aspen clones. Perhaps surprisingly, the ecological consequences of this mechanism are often ignored or downplayed in models of forest stand dynamics that include trembling aspen (e.g. Bergeron 2000, and references therein).

There is considerable variation in the overall health and vigor of trembling aspen stands in North America. Throughout the drier, western distributional range of the species (Arizona, Utah, Colorado, Montana, southern Alberta), nearly half of the extant stands are under going synchronous and rapid decline (Hogg et al. 2008; Wolken et al. 2009; Worrall et al. 2008, 2010; Rehfeldt et al. 2009). Fire suppression is cited as the principal factor leading to these "decadent" aspen stands. As such stands age they become increasingly susceptible to the cumulative effects of various stressors (e.g. prolonged drought and mammalian herbivory), which reduce their health and vigor. These "weakened" stands are highly susceptible to systemic stem fungal infection (e.g. *Cytospora* canker; Worrall et al. 2008), which is considered the last stage in a tree mortality cycle (Manion 1991). Fungal canker infections interfere with the mortality-regeneration cycle by inhibiting root sucker production, resulting in "decadent" non-regenerating stands and eventual clone death.

The situation is very different in those regions of continental North America that are less susceptible to prolonged drought (Saskatchewan, Manitoba). Here, the persistence and expansion of aspen stands (i.e. encroachment) in savanna ecosystems and rangeland has been documented since the late 1800s (Bird 1961; Bailey and Wroe 1974; Köchy and Wilson 2001; Kulakowski et al. 2004). In these regions, grassland management practices have focused on controlling aspen encroachment through repeated controlled burning and/or root trenching (Simonson and Johnson 2005). Fire suppression is thought to be one of several factors leading to the encroachment of trembling aspen into grassland habitat (native grasslands as well as economically important pastures and rangelands). Trembling aspen encroachment is part of a global ecological phenomenon of proliferation of woody species into open grasslands and savannas (Brown and Archer 1999). The loss or dampening of natural disturbances (particularly fire), which have historically regulated the long-term co-existence of grassland and forest vegetation, has been cited as the critical driver contributing to the expansion of woody species (Dublin 1979; Schwartz et al. 1996; Scholes and Archer 1997; Van Auken 2000; Bowman et al.

2001). Elucidating the causes of aspen encroachment and the ecological consequences on understory biodiversity has important management and restoration implications.

The degree of aspen encroachment is highly variable across the landscape; some areas increase in forest cover over time, while others forest cover remains stable or even recedes (Maini 1960; Barnes 1966; Cameron 1975). This variation may reflect stand-level differences in the mortality–regeneration cycle. Elucidating the biological mechanisms regulating sucker production (both within and among clones) is critical to our understanding of the dynamics of aspen encroachment. Such information will also improve our approaches to aspen "management" (i.e. controlling encroachment), through consideration of the natural cycle of mortality-regeneration that is characteristic of this clonal species. Although studies have considered the mortality-regeneration cycle to explain the persistence of aspen in later-successional forest stands (Cumming et al. 2000; Kurzel et al. 2007), none have directly used this approach to examine aspen encroachment within grasslands.

The objective of this study is to determine the relationship between patterns of aspen encroachment ("encroaching" versus "non-encroaching" forest edges) and the mortality-regeneration cycle of trembling aspen, and to examine how aspen encroachment affects the composition, structure and diversity of aspen savanna plant communities. I hypothesize that a reduction in stem density (mortality of mature stems, loss of apical dominance) facilitates both stand persistence (self-replacement) and encroachment of aspen suckers into grasslands. I also hypothesize that the mortality-regeneration cycle of trembling aspen has predictable effects on the composition and diversity of savanna plant communities.

2.2 METHODS AND MATERIALS

STUDY SITE

The study was conducted in four forest-grassland sites, two in Prince Albert National Park (PANP), Saskatchewan (53° 35' - 54° 20' N, 106° 00' - 106° 45' W), and two in Riding Mountain National Park (RMNP), Manitoba (50° 30' - 51° 01' N, 99° 33' - 101° 06' W; **Figure 2.1**). PANP is located approximately 300 km west and 280 km north of RMNP. Both of these Canadian National Parks occur within the Mixed-wood Section of the Boreal Forest Region (B18a), which extends from west-central Manitoba to central Alberta (Rowe 1972). Upland forests of the boreal mixed-wood section contain a mixture of deciduous trees (primarily trembling aspen, with white birch and balsam poplar) and coniferous tree species (primarily white spruce, with jack pine, and balsam fir). PANP and RMNP have similar macroclimates: mean annual temperature of 0.4° versus 0.1° C, and mean annual precipitation of 467 versus 508 mm (29 year means (1971-2000), Environment Canada 2009). Approximately two-thirds of the total annual precipitation falls as rain between the months of May and September.

The four study sites occur in aspen parkland savannas, characterized by plains rough fescue grasslands interspersed with groves of trembling aspen. The grassland-forest boundary in these savannas varies from abrupt (distinct grassland and forest communities) to ecotonal (a mixed grassland-forest community). **Table 3.1** summarizes the environmental features of the four study areas.

STUDY DESIGN

Initial field observations of the four study sites indicated considerable intra-site variation in the extent of trembling aspen encroachment into rough fescue grasslands. Some areas showed little or no encroachment, while others showed significant aspen encroachment. Encroachment is defined as the presence of a broad ecotonal boundary characterized by aspen regeneration (aspen suckers < 1.2 m high). Aspen regeneration within these ecotone areas is typically episodic, producing a distinct recruitment profile of recent regeneration (small, young ramets) along the grassland edge and older regeneration (larger ramets) along the edge of mature forest stands (**Figure 2.2**). Lateral root excavations at the four study sites indicated that aspen regeneration was entirely from root suckers connected to clonal "parent" trees; there was no evidence of establishment from seed. Non-encroachment areas showed an abrupt transition from forest and grassland, with the forest edge consisting only of mature ramets (> 3 m in height).

Within each of the four study areas, forest-grassland edges were selected that were either "encroaching" (dynamic, ecotonal) or "non-encroaching" (static, non-ecotonal), as defined above. Encroaching and non-encroaching edges were spatially paired (> 50 m and < 100 m apart) in order to minimize the confounding effect of differences in site conditions (edaphic, microclimatic, biotic). Forest stands adjacent to excessively drained alluvial gravel beds were excluded, since such environments uniformly impede forest advancement (Cameron 1975). Only sites located along the edge of a continuous forest stand were enumerated; isolated aspen groves (tree "islands") were not considered. Areas showing evidence of severe mammalian browsing of aspen suckers were also avoided.

A total of 17 “encroaching” and “non-encroaching” forest pairs (34 sites) were enumerated: five pair at Baldy Lake (RMNP), five pair at Deep Lake (RMNP), four pair at Westrom Flats (PANP), and three pair at Area 3 (PANP). At each site, a 10 x 10 m plot was located in the mature “core” aspen forest (containing the largest and oldest stems), and another 10 x 10 m plot along the forest edge. The following data were recorded in each plot: ramet diameter at 1.37 m height (DBH; diameter at breast height), ramet status (live, standing dead, or fallen dead), and canopy height. At each of the 34 sites, stand age was estimated by determining the age of the largest diameter aspen. Ages were estimated from counts of finely sanded tree cores, which were obtained by coring the bole at its base using a standard increment borer. Not all stands could be aged in this way, since the central portion of some boles had rotten.

Preliminary investigations indicated that at all sites, stems in the mature “core” aspen stands were always > 12 cm DBH and > 6 m in height. Based of this finding, the ramets encountered in this study were classified as follows:

Mature (initial post-disturbance cohort): > 12 cm DBH, > 6 m height.

Regenerating (secondary cohort): < 12 cm DBH, < 6 m height.

Additional vegetation data were obtained from the five paired study sites at Baldy Lake (RMNP), in order to examine changes in the composition, structure and diversity of savanna plant communities along the grassland-forest gradient. Within each of the 10 x

10 m "core" forest and forest edge plots, eight 1 x 1 m quadrats were located in a 2 x 4 grid. An additional 10 x 10 m plot was established in "core" grassland habitat (beyond the extent of aspen encroachment and/or shading), and sampled in the same way (**Figure 2.3**). Within each quadrat, percent cover of each plant species was estimated to the nearest 10%. Quadrat data were then used to determine mean species cover values for each of the 30 replicates (5 sites x 2 encroachment types x 3 habitat types).

DATA ANALYSIS

ASPEN STEM DENSITY AND ENCROACHMENT

Densities of live and dead (standing and fallen boles) mature stems in the mature "core" forests of encroaching and non-encroaching stands were compared using standard paired t-tests (Zar 1999). Differences in root sucker (regeneration) densities between the ecotones and "core" forests of encroaching and non-encroaching stands were compared using two-way analysis of variance (Zar 1999).

The relationship between mature aspen stem density (DBH > 12.5 cm) and the probability of aspen encroachment was investigated statistically using logistic regression (Gotelli and Ellison 2004). In this analysis stem density in each of 34 "core" forest sites (17 "encroachment", 17 "non-encroachment") is the independent *x*-variable, while aspen encroachment is the discrete response variable *y* (1 = encroachment, 0 = non-encroachment). Logistic regression parameters (estimated using maximum likelihood functions) include the y-intercept (ßo) and the logistic regression coefficient ($ß_1$), which quantifies the rate at which the logistic curve rises to the maximum value of $y = 1$ (Gotelli and Ellison 2004). The null hypothesis (H_o: $ß_1 = 0$) was tested using the log-likelihood χ^2 statistic. Parameter estimates and graphs were generated in R using the Generalized Linear Models (glm{stats}) package (version 2.9.2; R Development Core Team 2009) and Data Desk 6.3 (Data Description Inc. Ithaca NY). Statistical test examining the fit of the model and the significance of model parameters were conducted using the *binary logistic* module in SPSS 16.0.1 (SPSS inc. 2007).

FOREST ENCROACHMENT AND COMMUNITY HETEROGENEITY, COMPOSITION AND DIVERSITY

A series of analyses were undertaken to investigate variation in species composition and diversity across the six site groups at Baldy Lake: three habitat types (grassland, ecotone and forest), in both "encroaching" and "non-encroaching" areas. Each site group was replicated five times, for a total of 30 sites. A total of $S = 68$ plant species were encountered at these 30 sites.

Trends in plant species composition across the 30 sites were examined using principal co-ordinate analysis (Gower 1966). This ordination method, a generalized version of principal component analysis, uses a matrix of among-sites distances to summarize compositional variation among sites. Inter-site distances were determined using the Jaccard dissimilarity index (Legendre and Legendre 1998), based on species presence-absence:

$$d_{i,j} = [b + c] / [a + b + c]$$

where $d_{i,j}$ is the distance (range 0 – 1) between sites i and j. In the equation, a is the number of species present in both sites i and j, b is the number of species present in site i but absent in site j, and c is the number of species present in site j but absent in site i. The analysis was performed using the SYNTAX package (Podani 2001).

The dispersion pattern of the 30 sites in two-dimensional ordination space was used to summarize compositional similarity among and within each of the six site groups. The centroid of each group was determined, and the residual distance of each site to its respective centroid was measured (*betadisper* {vegan} package in R, version 2.9.2; R Development Core Team 2009). The mean residual distance for a given group is a measure that group's compositional heterogeneity (a larger mean value indicating higher group heterogeneity). This measure corresponds closely to Whittaker's (1972) index of beta diversity. Finally a multivariate test for homogeneity based on the spread of points around each centroid was used to examine statistical differences in compositional heterogeneity among groups were determined using a multivariate analogue of the Levene homogeneity test (Van Valen 1978; Anderson 2006a,b). The approach is based on a standard analysis of variance (ANOVA) in which differences in residual distances are compared over two or more groups. F-ratios are calculated in the usual way, but corresponding *P*-values are determined random permutations of the residual distances (Anderson 2006a). The randomization approach was used since it makes no assumptions about the distribution of the residuals. The test was performed using PERMDISP (*available online*), a FORTRAN computer program written by Marti Anderson (2004). The *P*-values were determined from 999 random permutations of the observed data.

Cluster analysis was used to classify the 68 species into “ecological groups” (Kenkel 1986). Sum of squares agglomerative clustering (Ward 1963; Legendre and Legendre 1998), based on the Jaccard presence-absence dissimilarity index, was used to delineate five species ecological groups. The relationship between these five species groups and the six site groups was examined using concentration analysis (Feoli and Orlóci 1979). This method examines inter-relationships of an ordered (5 species x 6 site groups) table. Within each of the 30 blocks of the table, the number of occurrences of species in the ecological group *i* within site group *j* is recorded; the result is a 5 x 6 table of frequencies, which are then adjusted to equal block size (Feoli and Orlóci 1979). The total contingency chi-square of the adjusted table is additively partitioned using correspondence analysis (Legendre and Legendre 1998). This ordination method produces a simultaneous ordination of the six site groups and five species ecological groups, thus summarizing group inter-relationships. All analyses were performed using the SYNTAX package (Podani 2001).

Variation in species diversity among the six site groups was examined using rank-abundance plots (Magurran 2004). In addition, the following diversity measures were determined for each site group:

S = species richness (number of species).

N_1 = effective species richness (Hill 1973).

E_{21} = Hill’s evenness ratio (Alatalo 1981).

Analyses were conducted in R using the *rankabundance* {BiodiversityR} and *diversity* {Vegan} packages, version 2.9.2 (R Development Core Team 2009).

2.3 RESULTS

ASPEN STEM DENSITY AND ENCROACHMENT

Mature stem densities (both live and dead) for the four study areas, for both “encroaching” and “non-encroaching” stands, are summarized in **Table 2.2**. Preliminary analyses revealed that variation in the density of mature stems was statistically similar across all four study areas; summaries are therefore presented over all 17 sites. In “core” forest, the mean density of live mature stem in “non-encroaching” stands was 1765 stems/ha, significantly greater than the mean density of 924 stems/ha in “encroaching” stands ($t_{16} = 8.88$, $P < 0.001$). However, total (live + dead) stem densities of “encroaching” and “non-encroaching” stands were not significantly different (1982 vs. 1876 stems/ha; $t_{16} = 1.23$, $P = 0.24$). This indicates that “encroaching” and “non-encroaching” stand densities were similar in the past, but that “encroaching” stands have undergone much greater stem mortality (standing and fallen dead stems) in recent years. These trends were consistent across all the 17 study sites.

The “encroaching” forest stands had significantly greater ramet regeneration that “non-encroaching” stands, in both the “core” forest (1141 versus 265 stems/ha) and the grassland-forest ecotone (2882 versus 441 stems/ha). The interaction term (encroaching/non-encroaching x edge/forest) is statistically significant ($F_{1,64} = 35.5$, $P <$

0.001), indicating disproportionately greater regeneration ramet density along the edges of "encroaching" compared to "non-encroaching" stands (**Figure 2.4**).

Together, these results suggest that aspen encroachment is initiated following "break-up" (stem mortality) of the initially established cohort. The release of apical dominance following stem mortality results in the production of aspen suckers from pre-existing lateral roots, both within the "core" forest (stand perpetuation) and the grass-forest ecotone (stand encroachment). Logistic regression confirms that stem density is a significant determinant of the likelihood of encroachment ($ß_1$= -0.0063; $\chi_1^2 = 33.6$, $P <$ 0.001; **Figure 2.5**). The stem density parameter $ß_1$ is inversely related to forest encroachment, indicating that encroachment probability declines as the density of mature stems increases (Odds Ratio = 0.993 ± 0.004). The overall predictive accuracy of the logistic regression model is 88.2%.

FOREST ENCROACHMENT AND COMMUNITY HETEROGENEITY, COMPOSITION AND DIVERSITY

The first axis of the principal coordinate analysis ordination reflects a trend in species composition from grasslands to ecotone to closed forest vegetation (**Figure 2.6**). The "encroaching" and "non-encroaching" sites have similar grassland and ecotone vegetation, as reflected by their similar positions in two-dimensional ordination space. However, the forest vegetation of "encroaching" sites is highly distinct from that of "non-encroaching" sites; it is floristically intermediate between ecotone and "non-encroaching" forest sites.

Compositional heterogeneity was lowest for the grassland sites and “non-encroaching” forest sites, and highest for the ecotone and “encroaching” forest sites. This is reflected in the results of the dispersion permutation ANOVA (**Figure 2.7**). These results indicate that sites with more uniform environments (grasslands, closed forests) are compositionally homogeneous, whereas more variable environments (ecotones, discontinuous canopy forests) are compositionally heterogeneous.

The five species ecological groups delineated by cluster analysis are summarized in **Table 2.3** Species group A includes mostly common species occurring in all regions except for closed-canopy forests; these species include northern bedstraw (*Galium boreale* L.), strawberry (*Fragaria virginiana* Duchesne), and blue-grass (*Poa pratensis* L.). Group B includes species found mainly in grassland habitats (occasionally ecotone habitats), including awned wheat-grass (*Agropyron subsecundum* (link) Hitchc.), bluebell bellflower (*Campanula rotundifolia* L.), Seneca snakeroot (*Polygala senega* L.), and hoary pucoon [*Lithospermum canescens* (Michx.) Lehm.]. Group C contains species restricted to grassland habitats; these include blue lettuce (*Lactuca pulchella* DC), reflexed locoweed (*Oxytropis deflexa* (Pall.) DC.) and sand violet (*Viola adunca* J.E. Smith). Group D contains less common species found in both grassland and ecotone habitats, including June grass (*Koeleria macrantha* (Ledeb.) Schult.), false melic grass (*Schizachne purpurascens* (Torrey) Swallen) and cinquefoil (*Potentilla arguta* L.). Group E contains species that are largely restricted to forest habitats, including sedges (*Carex* spp.), rice grass (*Oryzopsis asperifolia* Michx.), bracted orchid (*Habenaria viridis* (L.) R. Br.)) and fringed loosestrife (*Lysimachia ciliata* L.).

The relationships between the five ecological groups and the six site groups are summarized in **Figure 2.8**. The total contingency chi-square is highly significant ($\chi_{20}^{2} = 22.13$, $P < 0.001$), indicating strong correspondence between the species and site groups. The first ordination axis, which summarizes 87.4% of the total contingency chi-square, reflects a gradient from grassland to ecotone to closed forest. Species groups B and C are most strongly associated with the grassland sites, whereas species group E is strongly associated with the forested sites. Group A, which contains ubiquitous species, occurs near the centre of the ordination diagram. Group D includes species found in both the grassland and ecotone sites.

Species richness is highest in the grassland and ecotone sites (range of $S = 42$ to 51), as is the effective species richness (range of $N_1 = 18.03$ to 22.66; **Figure 2.9; Table 2.4**). The "encroaching" forest stands (which have lower mature stem density than non-encroaching stands) also have relatively high species richness ($S = 36$) and effective species richness ($N_1 = 19.14$). By contrast, the "non-encroaching" closed forest stands (high mature stem density), have much lower species richness ($S = 18$) and effective species richness ($N_1 = 4.14$). Species evenness is also much lower in "non-encroaching" closed forest stands ($E_{21} = 0.500$) compared to "encroaching" forest stands ($E_{21} = 0.737$) and the grassland and ecotone sites (range of $E_{21} = 0.606$ to 0.679). These results indicate that the low-light conditions characteristic of closed forest stands ("non-encroaching" forest) reduces community diversity and evenness. Reduced canopy stem density (characteristic of "encroaching" forest stands) increases species diversity and evenness, presumably by creating light conditions more conducive to the establishment of light-demanding grassland species.

2.4 DISCUSSION

In the following discussion a distinction is made between "declining" and "dieback" aspen clones. Although these terms are sometimes used interchangeably (Manion 1981), in this study aspen dieback refers to mortality of mature canopy stems or ramets (but not the clonal genet) whereas aspen decline refers to gradual decline in the health of a clonal genet leading to eventual mortality.

The initiation of aspen root suckering following mature stem dieback is well documented in the literature (Maini 1960; Doucet 1989; Frey et al. 2003). This secondary aspen recruitment is determined by changes in both physiological and environmental factors that occur during canopy dieback. Physiological factors affecting sucker regeneration potential and growth rate include release from apical dominance following mature stem mortality (altering the ratio of auxin to cytokinin, Schier 1972; 1973), and the amount of stored non-structural carbohydrate reserves present in the root system (Landhäusser and Lieffers 1997). An important environmental consequence of canopy breakup is higher incident solar radiation to the forest floor, which raises the soil temperature beyond the critical threshold (> 12°C) necessary to initiate root suckering. Another factor determining secondary recruitment success is root density, which is positively correlated with stand productivity (density and total basal area; Graham et al.

1963). Together these factors determine the probability of successful sucker initiation and growth following canopy dieback.

My results indicate that the degree of canopy dieback (reduction in mature stem density) is a reliable predictor of aspen encroachment. The alteration of forest stand structure – resulting from dieback of the mature canopy – initiates recruitment of aspen root suckers, both within the mature forest stand (resulting to stand regeneration) and along the forest-grass boundary (resulting to forest encroachment). Within closed forest stands this mortality-regeneration cycle can result in trembling aspen forming self-replacing stands, even in the absence of recurrent catastrophic disturbance (Cumming et al 2000; Kurzel et al. 2007). Any process that results to stem mortality (including canopy breakup in mature stands) promotes vigorous root sucker initiation, provided that the root system is healthy. I found that partial canopy dieback resulted in a four-fold increase in root sucker density within established forest stands, and an eight-fold increase along the forest-grass boundary. Similar increases in sucker density along forests edges following disturbance to mature aspen stands have been reported in southern Saskatchewan (Peltzer and Wilson 2006). The extent of root-sucker production is dependent on root density and health, as well as the degree of canopy dieback. Complete stem mortality (following a catastrophic disturbance such as fire of logging) results in synchronous root-sucker initiation, usually at very high densities (Peterson and Peterson 1992). Conversely, my results indicate that the gradual breakup of a mature aspen canopy results in continuous root sucker initiation, at relative modest densities (see also Cumming et al. 2000).

The dynamics and persistence of aspen clones is dependent on many factors, the most important being the mortality-regeneration cycle that facilitates rapid re-establishment following mature stem mortality. This regeneration process is under direct physiological control, reflecting changes in the ratio of growth hormones (mainly cytokinin and auxin) in the roots and associated stems (Schier 1973; Schier and Zasada 1973). Stem mortality results in increased cytokinin levels, which in turn break the dormancy of root sucker buds along the pre-existing lateral roots. The density of initiated root suckers is strongly influenced by the availability of soil nitrogen, and in particular nitrates (Min et al. 1998; Légaré et al. 2005; Landhäusser et al. 2010). Soil nitrate is taken-up preferentially by aspen clones, and nitrate is critical to the production of cytokinins (Landhäusser et al. 2010). In my study, inter-site variation in root sucker density (in both encroaching and non-encroaching forest stands) may be attributable to differences in soil nitrate levels. In maturing aspen stands, mineralization rates increase and nitrification rates decline over time; nitrification rates are restored only following a stand-replacing fire. In the absence of fire, low rates of soil nitrification may reduce aspen regeneration during canopy breakup (Jerabkova and Prescott 2007). In addition, the low soil nitrate to ammonium ratio characteristic of mature aspen stands may create conditions more favorable to the establishment of competing species (e.g. white spruce, *Picea glauca*).

My results indicate that the likelihood of aspen encroachment increases significantly when stand density is < 1400 stems ha^{-1}, which corresponds to the threshold density distinguishing "stable" and "breakup" stands (Peterson and Peterson 1992). Reduced mature stem density may also result in a positive feedback, leading to greater stem mortality within stands (Worrall et al. 2008). Increased regeneration following mortality of mature stems has a number of important implications for aspen population dynamics. In the absence of competing tree species (i.e. pure stands), stand regeneration from aspen suckers following breakup of an earlier cohort ensures long-term persistence of the species (Cumming et al. 2000).

The success of aspen stand regeneration is strongly dependent on the cause of stem dieback. Density-dependent aspen mortality occurs during the self-thinning stage that follows stand initiation; self-thinning mortality is the direct result of intra-specific competition (Peterson and Peterson 1992). During the self-thinning stage, secondary recruitment (i.e. the initiation of root suckering) is very rare (Perela 1990). Density-independent mortality, which typically occurs at later stages of stand development (i.e. following self-thinning), is attributable to three factors: stem age, biotic stressors, and “random” mortality resulting from severe environmental events (e.g. severe windstorm, acute drought, fire; Lieffers et al. 2001). The timing of “aging” mortality in physiologically mature stems is genetically variable, but generally occurs by ages 60-80 (Peterson and Peterson 1992; Meng et al. 2008). Stem age mortality often promotes stand regeneration from root suckers, provided that the clone is healthy (Cumming et al. 2000). Biotic stressors also result in stem dieback, and some can also lead to clonal decline (i.e. mortality of the individual). Repeated ungulate browsing suppresses root-sucker production and growth, but the clone generally recovers once browsing pressure is reduced (Kauffman et al. 2010). Conversely, systemic stem infection (fungal or viral) often leads to a lack of root sucker regeneration and clone decline; aspen clones rarely recover from severe fungal infection (Peterson and Peterson 1992). The most common fungal mortality agents are cytospora canker (*Valsa sordida* (Nitschke)), *Armillaria* root rot, venturia shoot blight (*Venturia macularis* (Fr.) E. Müll. & Arx), and aspen trunk rot (*Phellinus tremulae* (Bondartsev) Bondartsev & Borisov *in* Bondartsev) (Debyle and Winokur 1985; Worrall et al. 2008). These fungal agents result in rapid canopy dieback, and some (particularly cytospora canker) lead to clone decline by attacking the root system (and thus the ability of the clone to produce root-suckers). Although stem fungal infections (aspen trunk rot and cytospora) were noted within my study area, they were not common.

The results of this study suggest that mature aspen stands cycle between a “stable” (density-dependent) phase characterized by a dense mature canopy, and an “unstable” (density-independent) phase characterized by canopy breakup (stem dieback) and increased regeneration from root suckers. In the absence of inter-specific competition from other woody species, aspen clones may undergo both canopy replacement (stand perpetuation) and encroachment into grasslands (stand expansion) during the “unstable” phase (Barnes 1966; Peltzer and Wilson 2006). However, stand regeneration from root suckers may be less successful (or entirely lacking) when competing woody species are present, particularly when these competitors cast a deep shade. In mixed-wood boreal stands, the colonization of conifers (e.g. white spruce or balsam fir) beneath an established aspen canopy can reduce or negate aspen sucker regeneration, leading to canopy replacement by conifers (Bergeron 2000). In the parkland region, shrubs such as beaked hazel (*Corylus cornuta* Marsh.) can dramatically reduce root sucker densities during stand breakup, resulting in a shrub-dominated community (Mundell et al. 2007).

My results also indicate that the switch from a “stable, non encroaching” (density-dependent) to an “unstable, encroaching” (density-independent) mature aspen stand strongly affects plant community composition and diversity. A dense, stable aspen canopy casts a deep shade, reducing species diversity and strongly determining species composition. The plant community in such stands is dominated by a few species adapted to low light conditions, such as sedges (*Carex* spp.), rice grass (*Oryzopsis asperifolia* Michx.), bracted orchid (*Habenaria viridis* (L.) R. Br.) and fringed loosestrife (*Lysimachia ciliata* L.). In “unstable” stands, reduced stem density (attributable to stem dieback) increases the amount of light reaching the forest floor. This in turn increases plant community composition and diversity, as species adapted to higher light levels are capable of growing beneath the newly opened canopy (e.g. june grass [*Koeleria macrantha* (Ledeb.) Schult]) and wild peavine (*Lathyrus venosus* Muhl.)). These “unstable” stands are also much more heterogeneous (higher beta-diversity), which likely reflects the greater environmental variation (e.g. in light levels) that results following canopy breakup.

Community heterogeneity (beta-diversity) was also high at the forest-grassland ecotone. These ecotonal areas included both grassland and forest habitat species, as well as species that were most commonly encountered in transitional ecotone habitats (e.g. June grass , false melic grass (*Schizachne purpurascens* (Torrey) Swallen) and white cinquefoil (*Potentilla arguta* (Pursh)). My results indicate that changes in environmental conditions following forest encroachment strongly affect plant community composition and structure. Prior to forest encroachment, the grassland community is stable, homogeneous (low beta-diversity) and niche-assembled. Forest encroachment alters environmental conditions (e.g. increased shading), favoring colonization by ecotonal and forest habitat species. The result is a dispersal-assembled community, with altered community composition and greater community heterogeneity (higher community “patchiness”). Similar shifts in community composition and structure have been observed in other savannas systems experiencing woody encroachment (Stoke and Archer 2010).

In conclusion, differences in the degree of canopy dieback at the landscape-level may explain observed spatial variation in aspen encroachment in the western range of trembling aspen (Schwarz and Wein 1997). In the absence biotic stressors such as fungal pathogen infection, the aspen mortality-regeneration cycle is an effective mechanism for ensuring long-term persistence of the species (Gradowski et al. 2010). Although the ecological consequences of aspen clonal biology are often downplayed, they are critical to achieving a conceptual and practical understanding of the persistence and dynamics of trembling aspen stands throughout the species’ range.

2.5 ACKNOWLEDGEMENTS

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant to N.C. Kenkel, Parks Canada and a University of Manitoba's Duff Roblin Graduate Fellowship to R.A. Lastra. I gratefully acknowledge Parks Canada for their financial and logistic support throughout this study. In particularly, I would like to thank Wybo Vanderschuit (RMNP), Jeff Weir (PANP), John Wilmshurst (Parks Canada Service Center), Tina Giroux and Lloyd O'Brodovich. I'm also indebted to Kelly Orr and Peter Sinkins for their assistance with fieldwork.

2.6 REFERENCES

Alatalo, R.V. 1981. Problems in the measurement of evenness in ecology. Oikos 37:199-204.

Ally, D. K. Ritland, and S.P. Otto. 2008. Can clone size serve as a proxy for clone age? An exploration using microsatellite divergence in *Populus tremuloides.* Molecular Ecology 17:4897-4911.

Anderson, M.J. 2004. PERMDISP: a fortran computer program for permutational analysis of multivariate dispersions (for any two-factor ANOVA design) using permutation tests. Department of Statistics, University of Auckland, New Zealand. <http://www.stat.auckland.ac.nz/~mja/Programs.htm>

Anderson, M.J. 2006a. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62:245-253.

Anderson, M.J., K.E. Ellingsen, and B.H. McArdle. 2006. Multivariate dispersion as a measure of beta diversity. Ecological Letters 9:683-693.

Bailey, A. W., and R. A. Wroe. 1974. Aspen invasion in a portion of the Alberta parklands. Journal of Range Management 27:263-266.

Barnes, B.V. 1966. The clonal growth habit of American Aspens. Ecology 47:439-447.

Barnes, B. V. 1969. Natural variation and delineation of clones of *Populus tremuloides* and *P. grandidentata* in north Lower Michigan. Silvae Genetica 18:130-142.

Bergeron, Y. 2000. Species and stand dynamics in the mixedwoods of Quebec's southern boreal forest. Ecology 81:1500-1516.

Bird, R.D. 1961. Ecology of the aspen parkland of Western Canada. Can. Dept. Agric. Publ. 1066. 155 pages.

Bowman, D. M. J. S., A. Walsh, and D. J. Milne. 2001. Forest expansion and grassland contraction within a Eucalyptus savanna matrix between 1941 and 1994 at Litchfield National Park in the Australian Monsoon Tropics. Global Ecology and Biogeography 10:535-548.

Brown, J.R., and S. Archer. 1999. Shrub invasion of grassland: Recruitment is continuous and not regulated by herbaceous biomass or density. Ecology 80:2385-2396.

Cameron, T.F. 1975. Fescue grasslands and associated communities in Prince Albert National Park, Saskatchewan. Parks Canada Report, Ottawa. 190 pages.

Crouch, G.R. 1983. Aspen regeneration after commercial clearcutting in southwestern Colorado. Journal of Forestry 83:316-319.

Cumming, S.G., F.K.A. Schmiegelow, and P.J. Burton. 2000. Gap dynamics in boreal aspen stands: is the forest older than we think? Ecological Applications 10:744-759.

Debyle, N.V. and R.P. Winokur (editors). 1985. Aspen: ecology and management in the western United States. Gen, Tech. Rep. RM-119. Fort Collins, CO: USDA Forest Service, Rocky Mountain Forest and Range Experiment Station. 283 pages.

Doucet, R. 1989. Regeneration silviculture of aspen. Forestry Chronicle. 65:23-27.

Dublin, H. 1979. Vegetation dynamics in the Serengeti-Mara ecosystem: The role of Elephants, fire and other factors. In Serengeti: Dynamics of an ecosystem. Sinclair, A. R. E., and M. Norton-Griffith (Editor) Pages 71-90. Chicago, University Press.

Feoli, E. and L. Orlóci. 1979. Analysis of concentration and detection of underlying factors in structure tables. Vegetatio 40:49-54.

Fraser, E.C., V.J. Lieffers and S.M. Landhäusser. 2004. Wounding of aspen roots promotes suckering. Canadian Journal of Botany 82:310-315.

Gilbert, E.F. 1966. Structure and development of somac clones. American Midland Naturalist 75:432-445.

Gower, J.C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53:315-328.

Gradowski, T., V.J. Lieffers, S.M. Landhäusser, D. Sidders, J. Volney and J.R. Spence. 2010. Regeneration of *Populus* nine years after variable retention harvest in boreal mixedwood forests. Forest Ecology and Management 259:383-389.

Graham, S.A., R.P. Harrison, and C.E. Westell. 1963. Aspens: phoenix trees of the Great Lakes region. University of Michigan Press, Ann Arbor, Mich.

Gunn, A., G.W. Scott, D. Sept, and L. Carbyn. 1976. Prescribed controlled burn study of the fescue grasslands in Prince Albert National Park. Progress Report No. 1. Canadian Wildlife Service, Edmonton 117 pages.

Hill, M.O. 1973. Diversity and evenness: a unifying notion and its consequences. Ecology 54:427-432.

Hogg, (Ted), E.H., J.P. Brandt, and M. Michaelian. 2008. Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Canadian Journal of Forest Research 38:1373-1384.

Jerabkova, L., and C.E. Prescott. 2007. Post-harvest soil nitrate dynamics in aspen- and spruce-dominated foreal forests. Forest Ecology and Management 242: 209-216.

Kauffman, M.J., J.F. Brodie, and E.S. Jules. 2010. Are wolves saving Yellowstone's aspen? A landscape-level test of a behaviorally mediated trophic cascade. Ecology 91: 2742-2755.

Kenkel, N.C. 1986. Structure and dynamics of jack pine stands near Elk Lake Ontario: a multivariate approach. Canadian Journal of Botany 64:486-497.

Köchy, M., and S.D. Wilson. 2001. Nitrogen deposition and forest expansion in the northern Great Plains. Journal of Ecology 89:807-817.

Kulakowski, D., T.T. Veblen, and S. Drinkwater. 2004. The persistence of quaking aspen (*Populus tremoiludes)* in the Grand Mesa area, Colorado. Ecological Applications 14: 1603-1614.

Kurzel, B.P., T.T. Veblen and D. Kulakowski. 2007. A typology of stand structure and dynamics of quaking aspen in northwestern Colorado. Forest Ecology and Management 252:176-190.

Landhäusser, S.M. and V.J. Lieffers. 1997. Seasonal changes in carbohydrate storage and regrowth in rhizomes of and stems of four boreal forest shrubs. Scandinavian Journal of Forest Research 12:27-32.

Landhäusser, S.M. and V.J. Lieffers. 2002. Leaf area renewal, root retention and carbohydrates reserves in a clonal tree species following above-ground disturbance. Journal of Ecology 90: 658-665.

Landhäusser, S.M., V.J. Lieffers, and T. Mulak. 2006. Effects of soil temperature and time of decapitation on sucker initiation of intact Populus tremuloides root systems. Scandinavian Journal of Forest Research 21:299-305.

Landhäusser, S.M. W. Xianchong, V.J. Lieffers, and P.S. Chow. 2010. Nitrate stimulates root suckering in trembing aspen (*Populus tremuloides).* Canadian Journal of Forest Research 40:1962-1969.

Leaky, R.R.B. 1985. The capacity for vegetative propagation in tree. In Cannell, M.G.R., and J.E. Jackson (eds.) Attributes of trees as crops plants. Abbotts Ripton, Institute of Terrestrial Ecology, 110-133 pages.

Légaré, S., Y. Bergeron, and D. Pare. 2005. Effects of aspen (*Populus tremuloides*) as a companion species on the grwith of black spruce (*Picea mariana*) in southerwestern boreal forest of Quebec. Forest Ecology and Management 208:211-222.

Legendre, P. and L. Legendre. 1998. Numerical Ecology. 2nd edition Elsevier, Amsterdam, the Netherlands.

Lennie, A.D., S.M. Landhäusser, V.J. Lieffers, and D. Sidders. 2009. Regeneration of aspen following partial and strip understory protection harvest in boreal mixedwood forests. Forest Chronicle 85:631-638.

Maini, J.S. 1960. Invasion of grasslands by *Populus tremuloides* in the Northern Great Plains. Ph.D. thesis, University of Saskatchewan. 231 pages

Manion, P.D. 1991. Tree disease concepts. 2nd ed. Prentice-Hall Inc., Upper Saddle River, N.J. 402 pages..

Magurran, A.E. 2004. Measuring biological diversity. Blackwell, Oxford, UK.

Min, X, M.Y. Siddiqi, R.D. Guy, A.D.M. Glass, and H.J. Kronzucker. 1998. Induction of nitrate and nitrate reductase activity in trembling aspen and lodgepole pine. Plant, Cell and Environment 21:1039-1046.

Mitton, J.B., and M.C Grant 1996. Genetic variation and the natural history of quaking aspen. Bioscience 46:25-31.

Mock, K. E., A. Rowe, M. B. Hooten, J. Dewoody, and V. D. Hipkins. 2008. Clonal dynamics in western North American aspen (*Populus tremuloides*). Molecular Ecology 17:4827-4844.

Mundell, T.L.., S.M. Landhäusser, and V.J. Lieffers. 2008. Root carbohydrate and aspen regeneration in relation to season of harvest and machine traffic. Forest Ecology and Management 255:68-74.

Peltzer, D.A., and S.D. Wilson. Hailstorm damage promotes aspen invasion into grassland. Canadian Journal of Botany 84:1142-1147.

Peterson, E.B., and N.M. Peterson. 1992. Ecology, management and use of aspen and balsam poplar in the prairie provinces. Forest Canada Northwest Region Report No. 1, Edmonton. 252 pages.

Podani, J. 2001. SYN-TAX 2000. Computer programs for data analysis in ecology and systematics, Budapest, Hungary.

Pothier, D., F. Raulier, and M. Riopel. 2004. Ageing and decline of trembling aspen in Quebec. Canadian Journal of Forest Research 34:1251-1258.

R Development Core Team. 2009. R: a language and environment for statistical computing. Version 2.9.2. R foundation for Statistical Computing, Vienna, Australia. http://www.R-project.org

Rehfeldt, G.E., D.E. Ferguson, N.L. Crookston. 2009. Aspen, climate, and sudden decline in western USA. Forest Ecology and Management 258:2353-2364.

Rowe, J.S. 1972. Forest regions of Canada. Dept. Env., Canadian Forest Service, Ottawa, Publication No. 1300. 72 pages.

SPSS, 2007. Release 16.0.1 for the Mac. SPSS, Chicago, Illinois, USA.

Schier, G.A. 1972. Apical dominance in multishoot cultures from aspen roots. Forest Science. 18:147-149.

Schier, G.A. 1973. Origin and development of aspen root suckers. Canadian Journal of Forest Research 3:45-53.

Schier, G.A. 1975. Deterioration of aspen clones in the middle rocky mountains. USDA forest Service Research Paper INT-170.

Schier, G.A. and R.B. Campbell. 1978. Aspen sucker regeneration following burning and clearcutting on two sites in the Rocky Mountains. Forest Science 24:303-308.

Schier, G.A. and A.D. Smith. 1979. Sucker regeneration in a Utah aspen clone after clearcutting, partial cutting, scarification and girdling. USDA For.Serv.Rep. INT-253.

Scholes, R.J., and S.R. Archer. 1997. Tree-grass interactions in Savannas. Annual Review of Ecology and Systematics 28:517-544.

Schultz, M., D. Walker and R. Lastra. 2007. Brush encroachment of pasturelands in Manitoba, Saskatchewan and Alberta. Saskatchewan Agriculture and Food. 29 pages.

Schwartz, D., H. de Foresta, A. Mariotti, J. Balesdent, J. P. Massimba, and C. Girardin. 1996. Present dynamics of the savanna-forest boundary in the Congalese Mayombe: a pedological, botanical and isotopic (^{13}C and ^{14}C) study. Oecologia 106:516-524.

Shepperd, W.D., D.L. Bartos, and S.A. Mata. 2001. Above- and below-ground effects of aspen clonal regeneration and succession to conifers. Canadian Journal of Forest Research 31:739-745.

Simonson, J.T., and E.A. Johnson. 2005. Development of the cultural landscape in the forest-grassland transition in southern Alberta controlled by topographic variable. Journal of Vegetation Science 16:523-532.

Stokes, C.J. and S. R. Archer. 2010. Niche differentiation and neutral theory: an integrated perspective on shrub assemblage in a parkland savanna.

Trottier, G.C. 1985. Evaluation of the prescribed burn study, Prince Albert National Park. Canadian Wildlife Service Report, Edmonton. 139 pages.

Trottier, G.C. 1986. Distribution of rough fescue, *Fescue hallii*, grassland by livestock grazing I, Riding Mountain National Park, Manitoba. Can. Field-Naturalist 100:488-495.

Van Auken, O. W. 2000. Shrub invasions of the North American semiarid grasslands. Annual Review of Ecology and Systematics. 31:197-215.

Van Valen, L. 1978. The statistics of variation. Evolutionary Theory 4:33-43.

Ward, J. 1963. Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association 58: 236-244.

Whittaker, R. H. 1972. Evolution and measurement of species diversity. Taxon 21:213-251.

Wolken, J.M, V.J. Lieffers, S.M. Landhäusser, T. Mulak. 2009. Spring frost and decay fungi are implicated in suppressing aspen re-growth following partial cleaning in juvenile stands. Annals of Forest Science 66:805-813.

Worrall, J.J., L. Egeland, T. Eager, R.A. Mask, E.W. Johnson, P.A. Kemp, and W.D. Sheppard. 2008. Rapid mortality of *Populus tremuloides* in southwestern Colorado, USA. Forest Ecology and Management 255:686-696.

Worrall, J.J., S.B. Marchetti, L. Egeland, R.A. Mask, T. Eager, B. Howell. 2010. Effects and etiology of sudden aspen decline in southwestern Colorado, USA. Forest Ecology and Management. 260:638-648.

Zar, J.H. 1999. Biostatistical Analysis, 4th edition. Prentice-Hall, Upper Saddle River, New Jersey, USA.

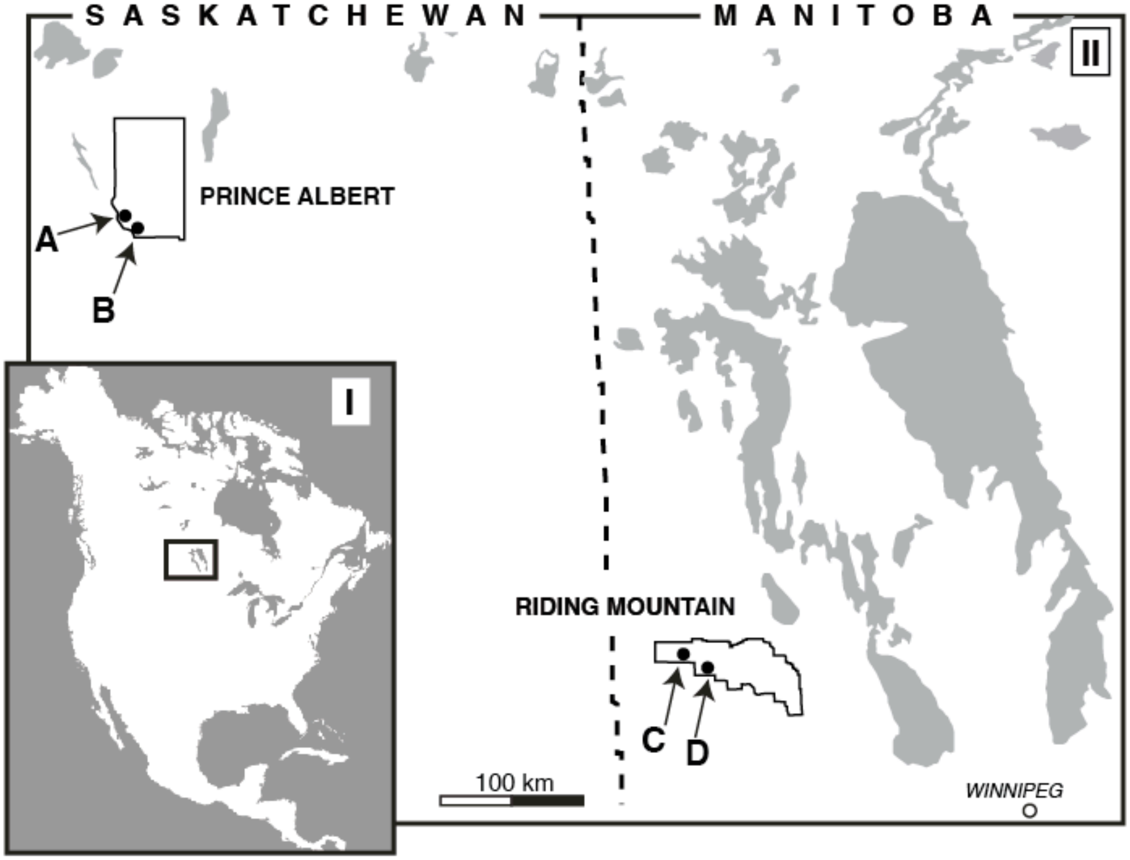


Fig. 2.1. Study area map. I) Inset map shows location of study region in North America. II) Location of Riding Mountain National Park (Manitoba), and Prince Albert National Park (Saskatchewan). Where A = Westrom Flats, B = Area 3, C = Deep Lake grassland, and D = Baldy Lake meadow.

Table 2.1 Summary of study sites.

	RMNP		**PANP**	
Annual Precipitation (mm)	508		467	
Annual Temp (°C)	0.1		0.4	
Location	Baldy Lake Meadow	Deep Lake Grassland	Westrom Flats	Area 3
Latitude	50° 47' 01.09" N	50° 52' 54.27" N	53° 36' 06.77" N	53° 35' 41.69" N
Longitude	100° 35' 43.40" W	100° 50' 56.76" W	106° 31' 00.87" W	106° 25' 29.81" W
No. Sites (pairs)	*5*	*4*	*3*	*5*
Elevation (m asl)	640	618	512	544
Area (ha)	90	45	65	30
Relief	Gently rolling	Steeply rolling	Flat	Gently rolling
Fire	Pre-1940	Pre-1940	1982 (control)	1982 (control)

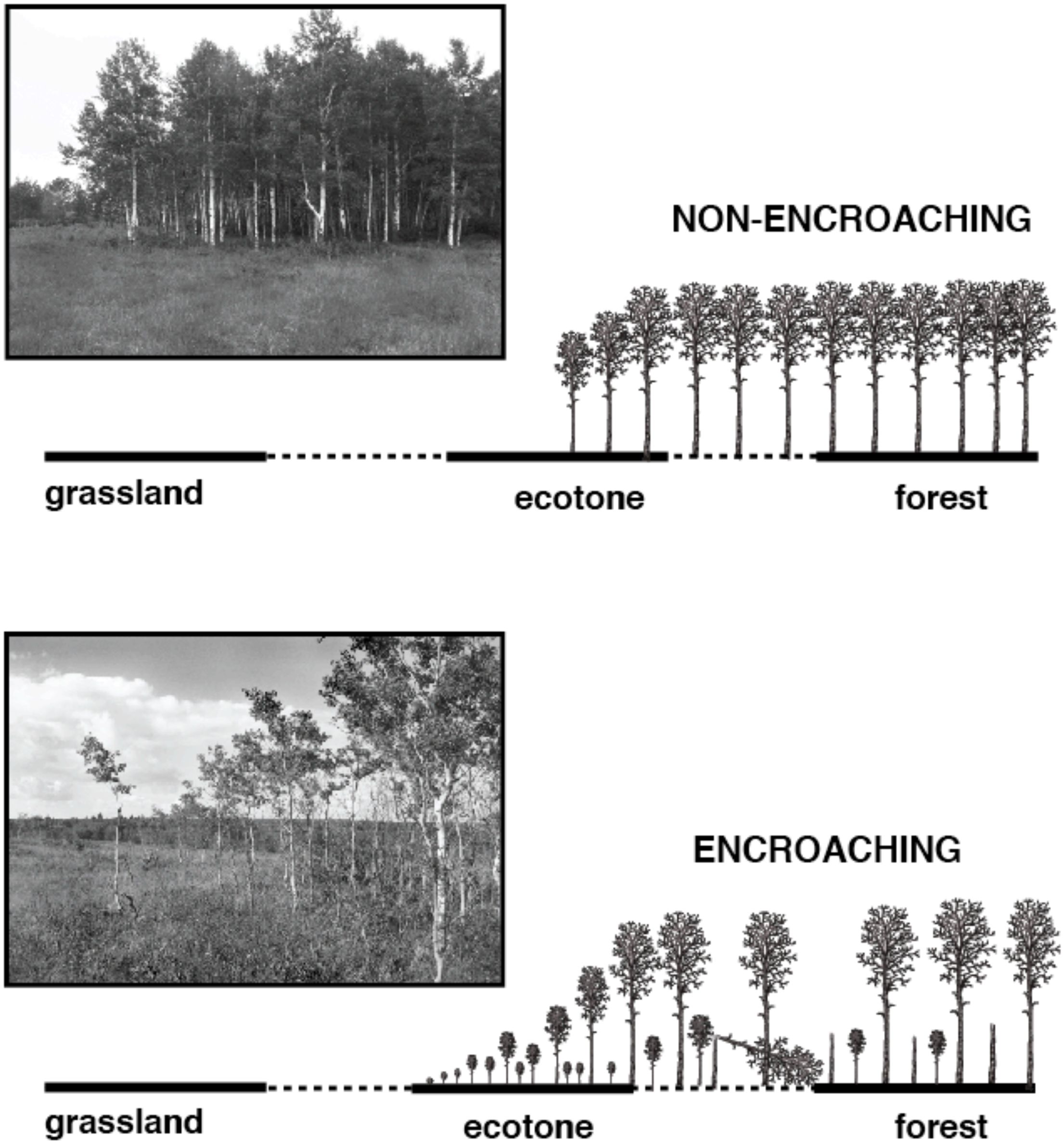


Fig. 2.2. Examples showing closed canopy (non-encroaching) and open canopy (encroaching) aspen stands. Corresponding images were taken in Baldy lake grassland, Riding Mountain Naitonal Park, Manitoba, Canada (July 2004; Photo credit: R. Lastra).

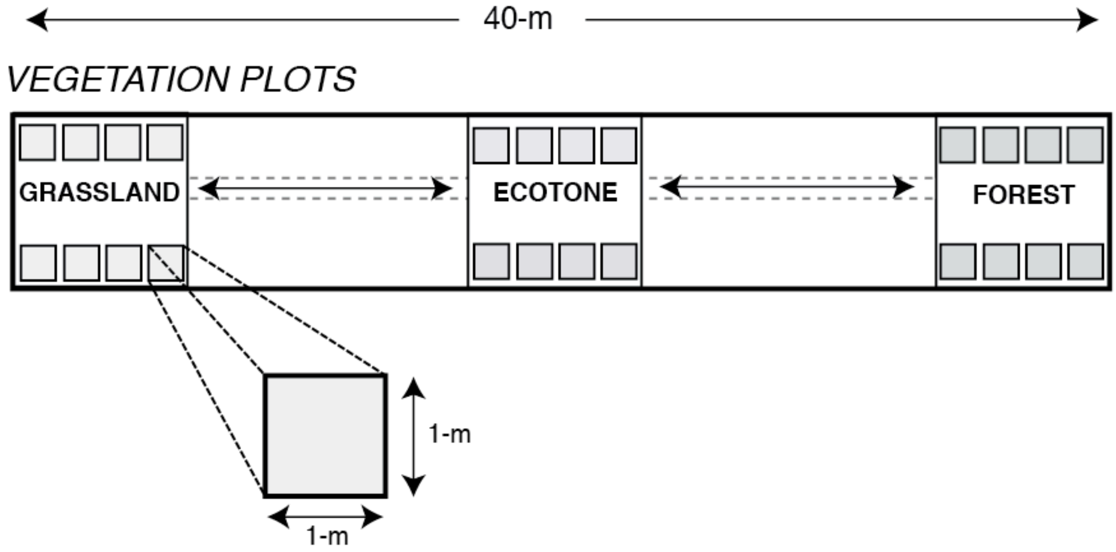


Figure 2.3. Sample design; 40 m line transects were placed extending from grassland to core forest habitat. Each transect was paired (encroaching and non-encroaching) within 50 m from one another. Forest vegetation quadrats (1 x 1 m): 8 vegetation quadrats were enumerated within each ten, 10 x 10 m plots (grassland, ecotone and forest plots); total of 240,1 x 1 m quadrats. The vegetation survey was conducted in Baldy Lake meadow; 5 paired (encroaching and non-encroaching) grassland-forest transects were used.

Table 2.2. Summary of mean (+/- Standard Error) canopy density (stems per ha) for the mature stems (> 12 cm DBH); Live and Live + Dead aspen stems.

	NON-ENCROACHING **Core Forest**	**ENCROACHING** **Core Forest**
Live stems **	1765 +/- 89	924 +/- 60
Live + Dead stems	1876 +/- 81	1982 +/- 58

** = significant difference between encroaching and non-encroaching stands (Paired t-test: $P < 0.001$)

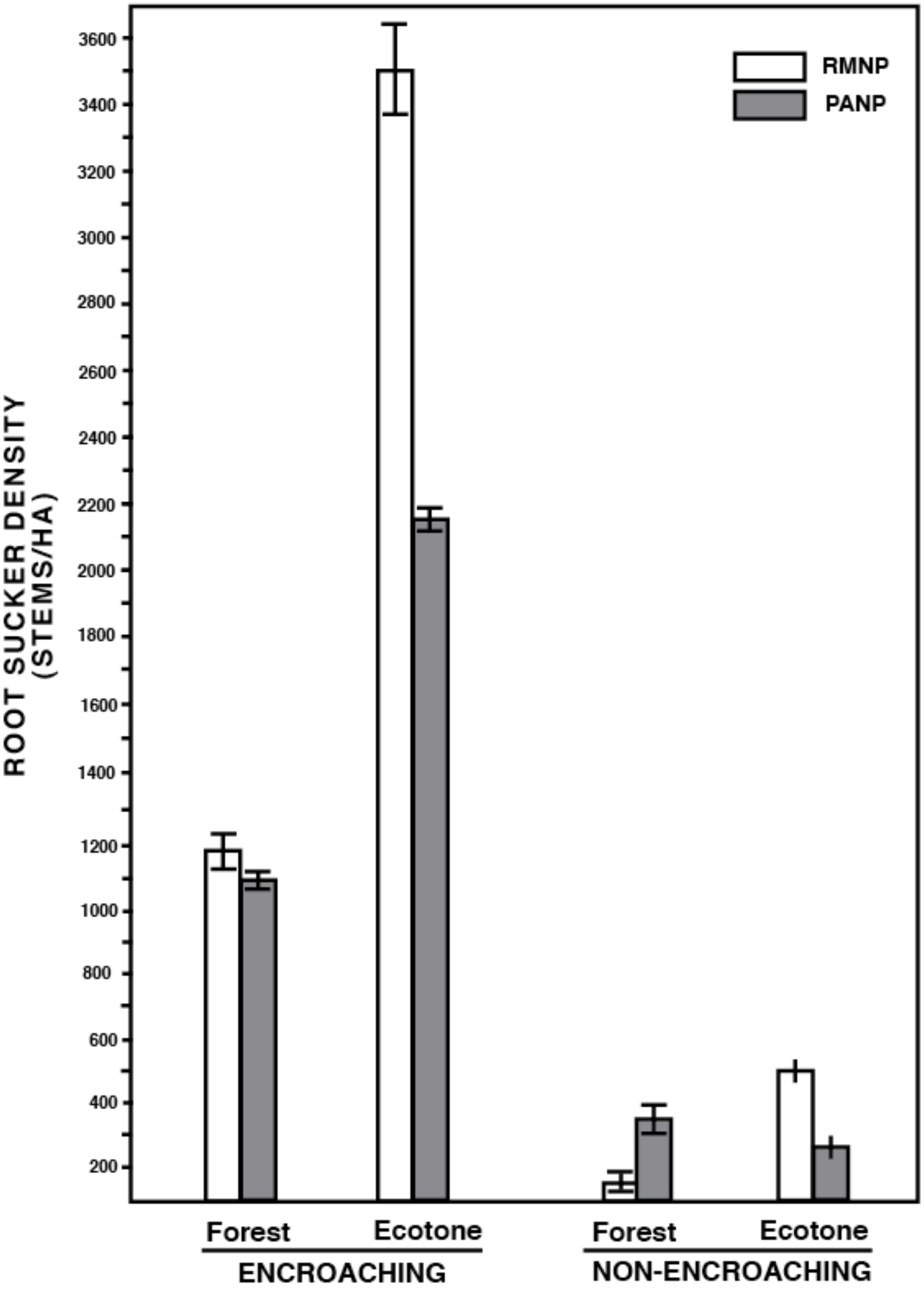


Fig. 2.4. Mean (+/- S.E.) stem density of aspen root suckers (0.25 - 1 m; < 2.5 DBH [diameter at breast height]) within non-encroaching (closed canopy) and encroaching (open canopy) forests.

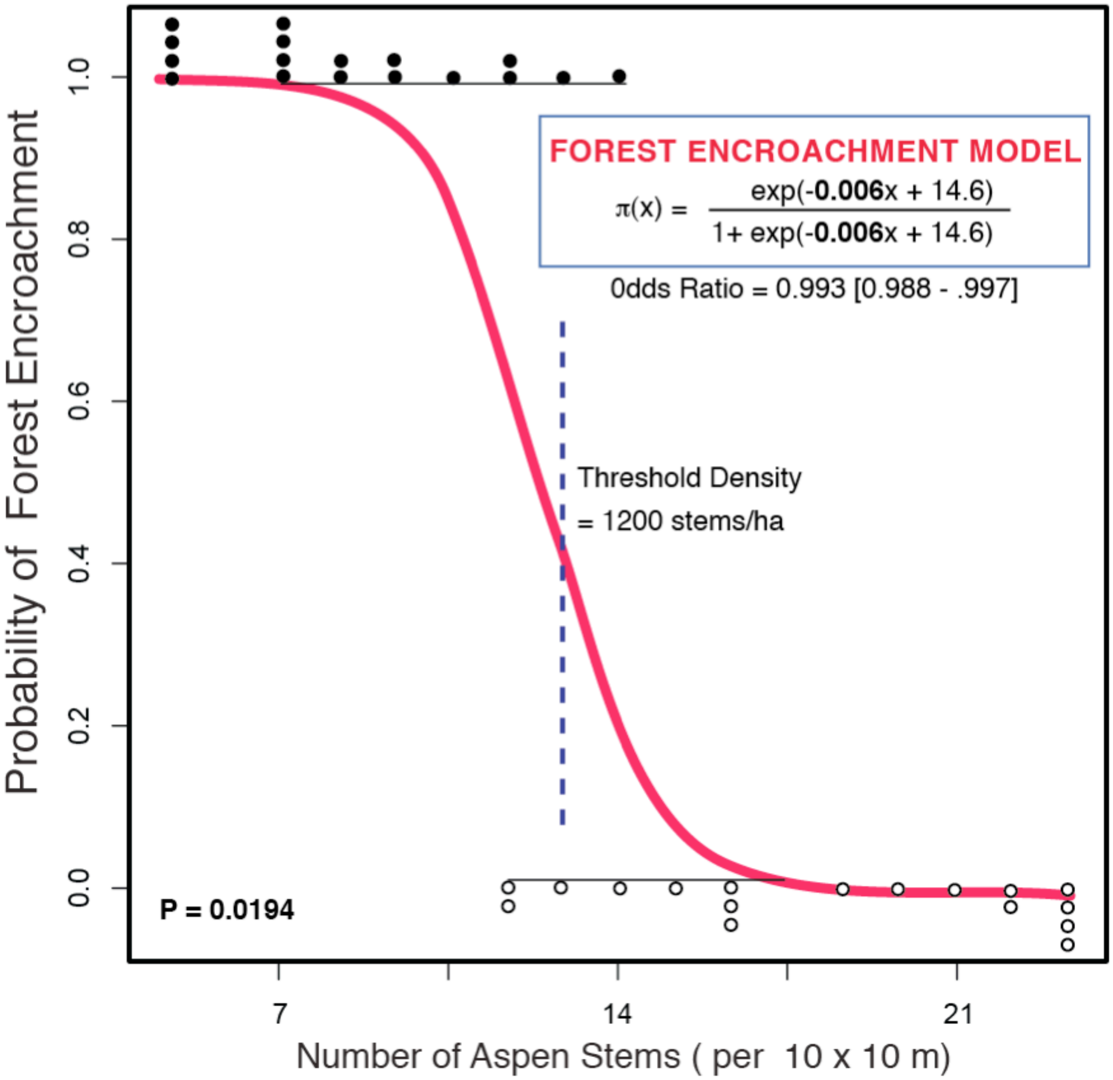


Fig. 2.5. The probability of trembling aspen encroachment as a function of the number of mature (≥ 12.5 cm DBH) stems (per 10 x 10 m). The model indicates that for every increase in live stem number there is a decrease in the probability of forest encroachment (odds = 0.993; b1 = -0.0063, Wald statistics = 5.5, *P* = 0.0194). The overall predictive accuracy of this model is 88.2%. Open and closed circles indicate the frequency distribution of stems within non-encroaching and encroaching forest stands.

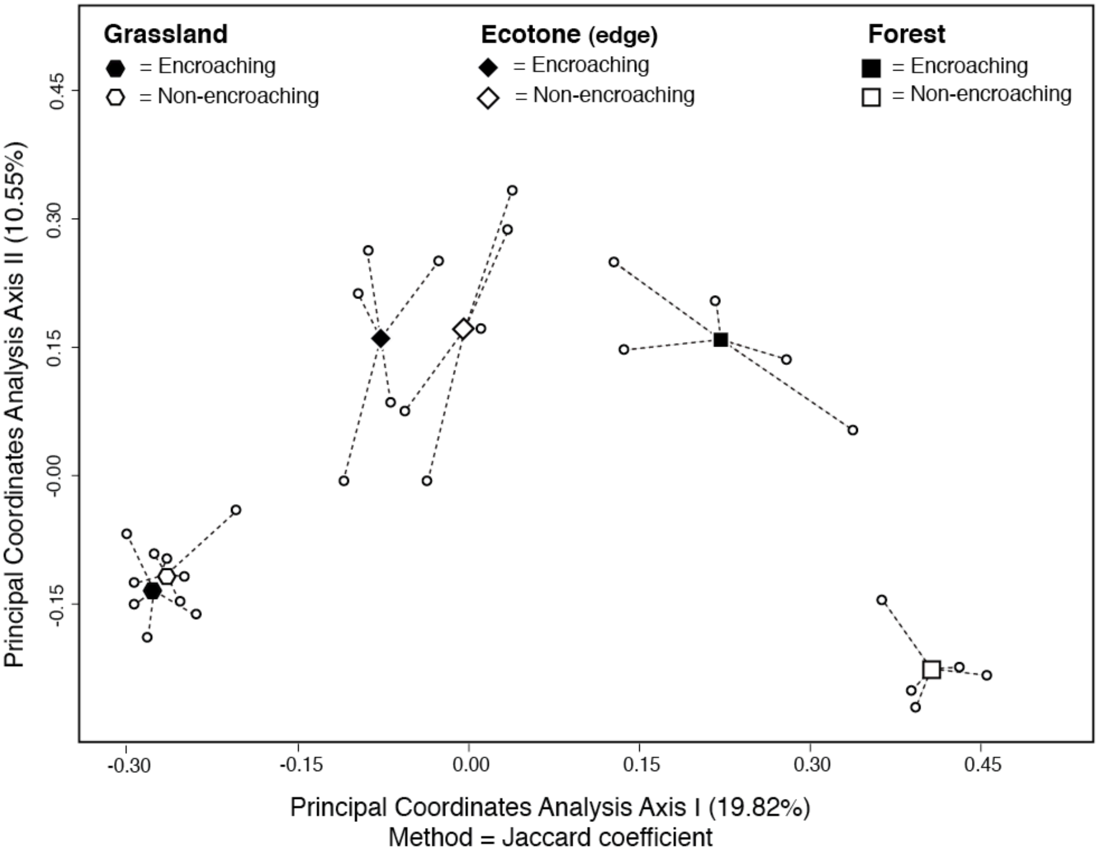
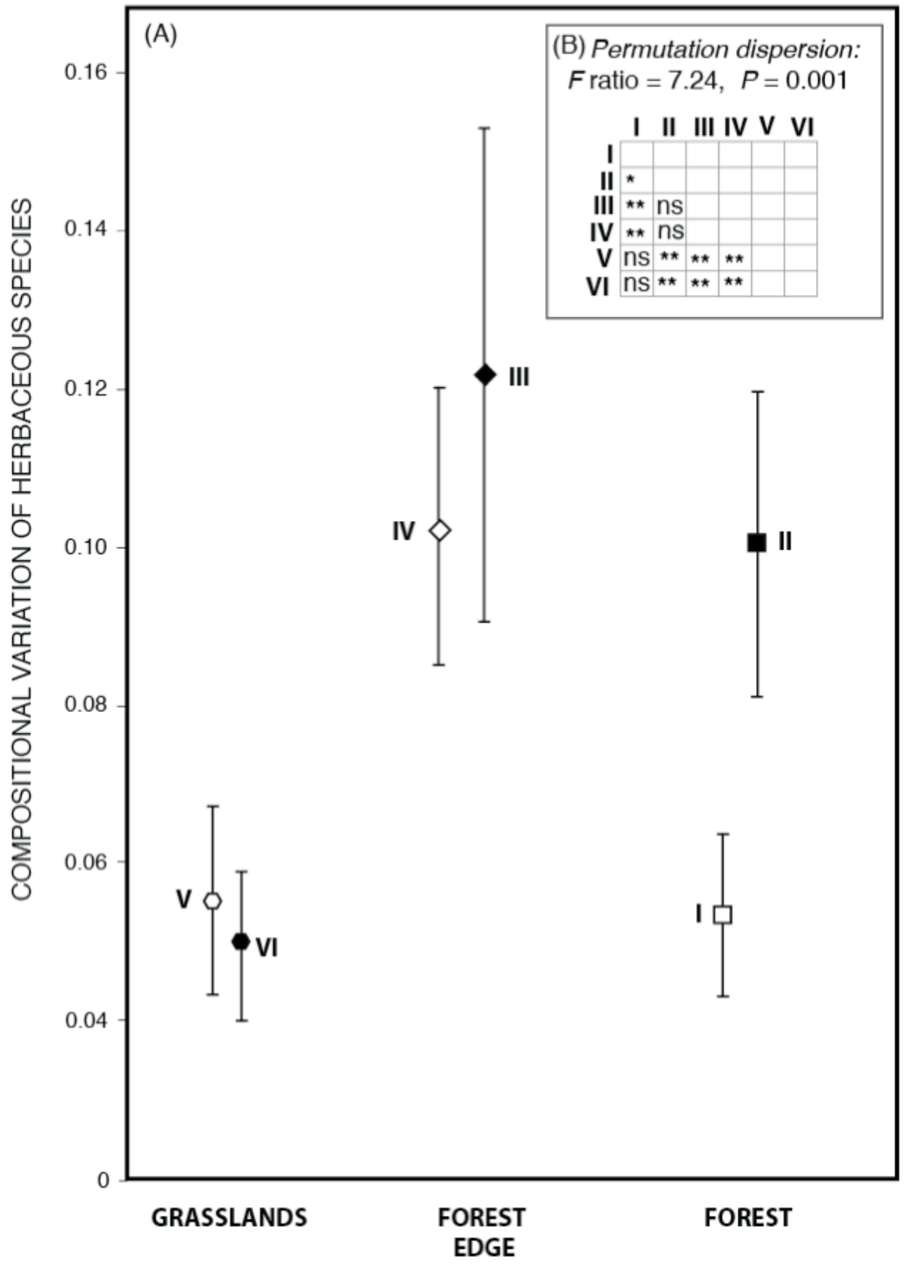


Fig. 2.6 Principal Coordinates Analysis (PCoA; Method = Jaccard Coefficient) model of understorey beta-diversity among forest, ecotone (edge) and grassland plots. Model examines species turnover across encroaching (black) and non-encroaching (open) sites; the centroid for each group is illustrated by the convergence of line connecting the five data points (each point represents 8, 1 x 1 m plot). Points depict the spatial coordinates of PCoA object scores using Jaccard's 'presence-absence' measure of similarity. The spread of points represents the degree of species turnover (beta-diversity) or dissimilarity among the groups. Non-encroaching forest plots have low relative species turnover compared to ecotone plots.

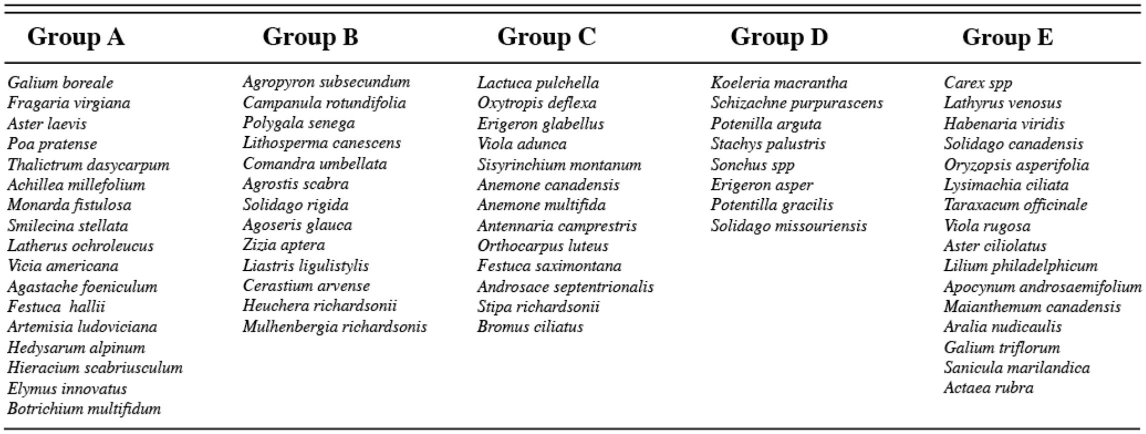


(B) *Permutation dispersion:*
F ratio = 7.24, *P* = 0.001

	I	II	III	IV	V	VI
I						
II	*					
III	**	ns				
IV	**	ns				
V	ns	**	**	**		
VI	ns	**	**	**		

Fig. 2.7. (A) Average distance to centroid among the 6 vegetation groups (+/- S.E., n = 5); distances are based on euclidean measures derived from the PCoA (method = Jaccard's measure of dissimilarity) biplot ordination scores. Averages closely correspond to Whittakers (1960) beta diversity index ($\beta w = \alpha/\gamma - 1$); Black symbols = encroaching sites, White symbols = non-encroaching. (B) Results from the Permutation of Dispersion ANOVA (PERMANOVA) based on 999 randomizations (* $P \leq 0.05$; ** $P < 0.01$; ns = not significant) .

Table 2.3. Species list of the five vegetation groups derived from sums of squares agglomerative clustering. Order of species corresponds to their relative frequencies (highest to lowest). Group A = "ubiquitous" species common in all sites except non-encroaching forest plots; Group B = Grassland and ecotone species; Group C = grassland species; Group D = Forest and ecotone; Group E = Forest.



Group A	**Group B**	**Group C**	**Group D**	**Group E**
Galium boreale	*Agropyron subsecundum*	*Lactuca pulchella*	*Koeleria macrantha*	*Carex spp*
Fragaria virgiana	*Campanula rotundifolia*	*Oxytropis deflexa*	*Schizachne purpurascens*	*Lathyrus venosus*
Aster laevis	*Polygala senega*	*Erigeron glabellus*	*Potenilla arguta*	*Habenaria viridis*
Poa pratense	*Lithosperma canescens*	*Viola adunca*	*Stachys palustris*	*Solidago canadensis*
Thalictrum dasycarpum	*Comandra umbellata*	*Sisyrinchium montanum*	*Sonchus spp*	*Oryzopsis asperifolia*
Achillea millefolium	*Agrostis scabra*	*Anemone canadensis*	*Erigeron asper*	*Lysimachia ciliata*
Monarda fistulosa	*Solidago rigida*	*Anemone multifida*	*Potentilla gracilis*	*Taraxacum officinale*
Smilecina stellata	*Agoseris glauca*	*Antennaria camprestris*	*Solidago missouriensis*	*Viola rugosa*
Latherus ochroleucus	*Zizia aptera*	*Orthocarpus luteus*		*Aster ciliolatus*
Vicia americana	*Liastris ligulistylis*	*Festuca saximontana*		*Lilium philadelphicum*
Agastache foeniculum	*Cerastium arvense*	*Androsace septentrionalis*		*Apocynum androsaemifolium*
Festuca hallii	*Heuchera richardsonii*	*Stipa richardsonii*		*Maianthemum canadensis*
Artemisia ludoviciana	*Mulhenbergia richardsonis*	*Bromus ciliatus*		*Aralia nudicaulis*
Hedysarum alpinum				*Galium triflorum*
Hieracium scabriusculum				*Sanicula marilandica*
Elymus innovatus				*Actaea rubra*
Botrichium multifidum				

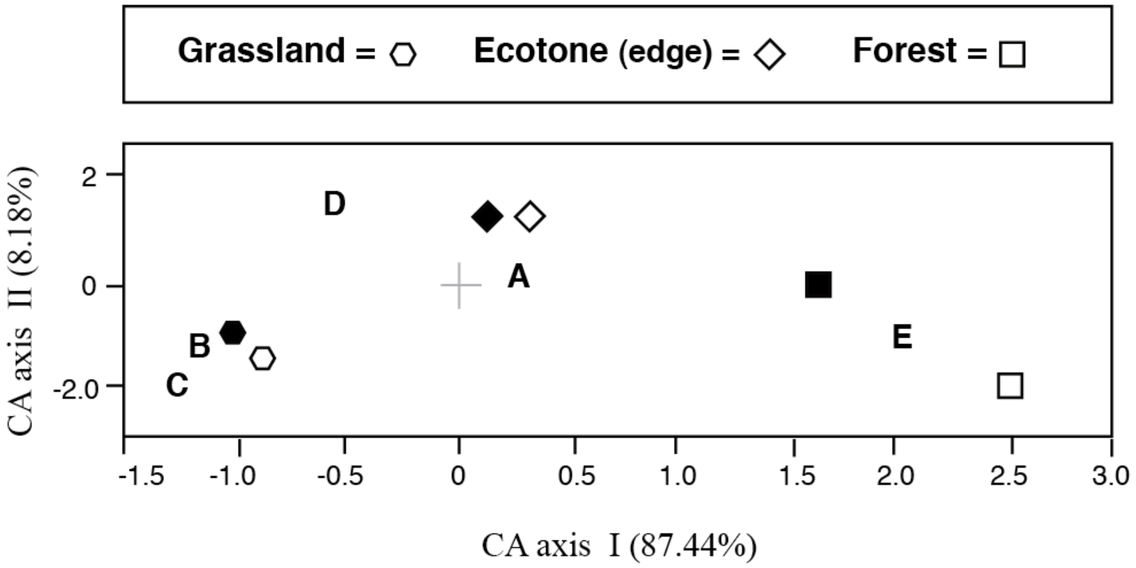


Fig. 2.8. Concentration Analysis depicting the inter-relationship among the six site groups (forest, ecotone and grassland sites that are encroaching [black symbols] or non-encroaching [open symbols]) and five species ecological groups (symbols A = ubiquitous species group; B = grassland and ecotone; C = grassland; D = ecotone; E forest). The species groups were derived using sums of squares clustering (measure of association = Jaccard’s similarity index). The analysis performs a correspondence analysis (CA) on a 5 x 6 table of species frequencies (adjusted to block size).

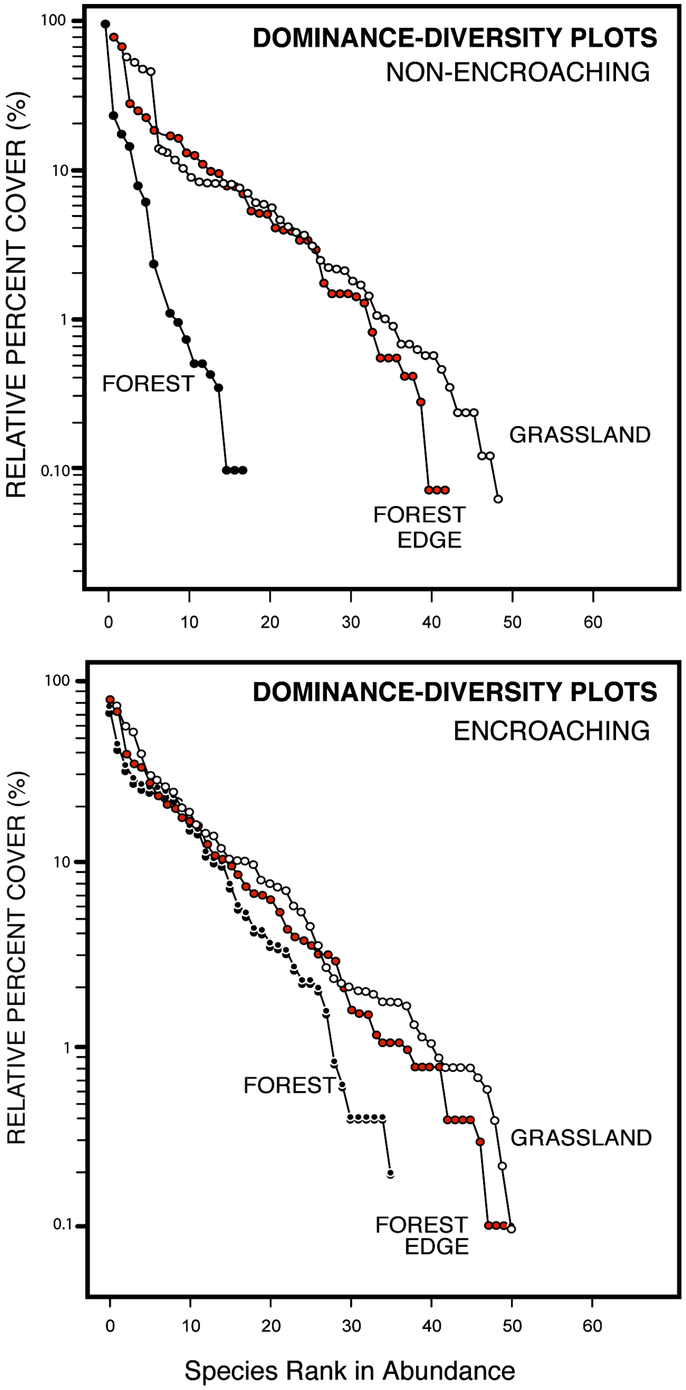


Fig. 2.9. Species rank abundance plots for non-encroaching and encroaching forest stands.

Table 2.4. Summary of biological diversity indices (S, N_1, E_{21}) for encroaching and non-encroaching forest, ecotone (forest edge) and grassland plots.

	NON-ENCROACHING	**ENCROACHING**
	Core Forest	
Species richness (S)	18	36
Effective species richness (N_1)	4.14	19.14
Hill's evenness ratio (E_{21})	0.50	0.737
	Ecotone	
Species richness (S)	42	51
Effective species richness (N_1)	18.11	20.92
Hill's evenness ratio (E_{21})	0.619	0.679
	Grassland	
Species richness (S)	47	50
Effective species richness (N_1)	18	23
Hill's evenness ratio (E_{21})	0.606	0.64

CHAPTER 3

THE SPATIAL AND TEMPORAL PATTERN OF STEM GROWTH AND MORTALITY FOR A CLONAL TREE SPECIES OVER SEVEN YEARS

3.1 INTRODUCTION

Long-term species dynamics is determined by complex interactions involving internal and external factors acting upon age-structured populations (Harper 1977; Rees and Ellner 2009). In clonal plants, the age (or size) structure of ramets is often ignored when examining population-level processes; instead, it is assumed that the clone consists of a single cohort of even-aged ramets. The process of self-thinning (density-dependent mortality) of ramets is examined to explain demographic changes within clones (Hutchings 1974; Westoby 1984), while processes that produce variation in individual (genet) performance are examined to understand long-term species dynamics (Rivano et al. 2010). These two processes are usually considered independently. For multi-cohort clones, however, variation in ramet age (or size) can strongly affect the density-independent survivorship (Jerling 1988). The growth rate and survivorship of ramets determine the fitness and size (number of ramets) of a clonal genet (Hartnett and Bazzaz 1985; Hosaka et al. 2005, 2008). The demographics of individual genets (i.e. the clonal ramets that make up the genet) are therefore critical to our understanding of the population dynamics of long-lived clonal plants.

Trembling aspen (*Populus tremuloides* Michx.) is the most widely distributed and genetically diverse tree species in North America (Mock 2008). The considerable phenotypic variation among aspen genets, coupled with the species' ability to propagate clonally from a root-sucker bud bank, are thought contribute to the ecological success of trembling aspen throughout its range (Kulakowski et al. 2004; Gradowski et al. 2010). The observed variation in genet size in trembling aspen populations likely reflects inter-specific variation in rates of establishment, clonal expansion and ramet dieback (Mock 2008).

In established aspen clones, dieback of the initial canopy cohort promotes the initiation and establishment of root suckers, resulting in the development of a multi-aged (and multi-sized) stand (Cumming et al. 2000). Age-structured (and size-structured) ramet assemblages undergo differential growth and mortality rates, with important consequences on population dynamics (Hosaka et al. 2008). Very little long-term data are available on the demography of established aspen clones, however.

In this study, I examine the spatial and temporal patterns of ramet growth and mortality in four established dieback aspen clones, over a seven-year period (2003 – 2009). My objectives are to describe the spatial patterns of live and dead ramets within a clone, and to determine ramet growth and mortality rates for each of the four clones over a seven-year period. I hypothesize that mortality will be greater, and relative growth rates lower, for older "suppression" ramets compared to younger, faster growing ramets.

3.2 METHODS AND MATERIALS

STUDY SITE

The study was conducted in Baldy Lake meadow (50° 47' 01.09" N, 100° 35' 43.40" W; 640 m a.s.l.), which is located in the west-central region of Riding Mountain National Park (RMNP), Manitoba, Canada. The study area is a typical aspen parkland savanna, dominated by mature and regenerating stands of trembling aspen (*Populus tremuloides*) interspersed with wetlands and northern fescue prairie grasslands (Trottier 1986). The regional climate is characterized by warm, short summers and long, cold winters. Data from the nearby town of Wasagaming (29-year means, 1971-2000; Environment Canada 2009) provides a climatic summary. Mean total annual precipitation is approximately 400 mm, and the two wettest months are June (mean = 84.5 mm) and July (mean = 75.3 mm). The mean annual temperature is 0.1°C, with mean monthly temperatures varying from –19.6°C in January to 16.5°C in July.

STUDY DESIGN AND DATA COLLECTION

SELECTION AND MAPPING OF CLONES — Four isolated aspen clones (i.e. single genets completely surrounded by grassland) were selected for mapping. All four clones were in the “dieback” stage: most of the mature (first cohort) ramets had died, and a dense second cohort of much younger ramets (initiated through root-sucker recruitment) had established. For all clones, regular and repeated browsing of ramets by ungulates was noted. The clones varied in both aerial extent (ranging from 382 to 935 m^2) and number of ramets (**Table 3.1**).

The four aspen clones were mapped between July 3 and Aug 30, 2003. Each clone was enclosed within a square plot (ranging from 10 x 10 m to 50 x 50 m, depending on clone aerial extent), and the four corners marked with a steel rebar pole hammered into the ground. All aspen ramets (live, standing dead, and decomposing boles) were marked with flagging tape prior to measurement. The spatial location of each marked ramet was determined using a total station with an infrared beam and a reflector (Topcon GPT-1003 Prismless Total Station, Surya Topcon, Ltd). The total station was located in one corner of the square plot, and the spatial position of ramets determined using relative angle (± 1^{o}) and distance (± 0.1 m). Spatial coordinates (X-Y plane) were determined using the angular equations:

X-coordinate (m) = sin [angle (radians) x distance (m)]

Y-coordinate (m) = cos [angle (radians) x distance (m)]

RAMET GROWTH AND MORTALITY — All living ramets (*n* = 1477 over the four clones) were monitored each summer over a six-year period (2003 to 2009) to determine mortality status (alive or dead). For ramets with a stem diameter ≤ 10 cm in 2003, ramet height (to the nearest cm) and basal diameter (to the nearest mm) were measured in 2003, and again in either 2009 (for ramets still alive in 2009) or in the year the ramet was first recorded as dead.

DATA ANALYSIS

SPATIAL PATTERN OF CLONAL RAMETS — All four aspen clones were broadly elliptical in shape, but with major axes oriented in different directions. For comparison purposes, the point configuration (point = ramet location) of each clone was oriented along its respective major and minor axes using principal component analysis (PCA; Legendre and Legendre 1998). This method rotates the two-dimensional configuration by maximizing linear variation along the first or major axis. The rotation is rigid, so that the relative point positions are unchanged; only the orientation of the configuration is altered. The result is a projection of the coordinate system, in which locations are expressed relative to the major and the minor axes. These projections were used to derive three diameter-class elliptical boundaries for each clone: (a) entire clone, all ramets; (b) ramets > 2.5 cm diameter; (c) ramets > 5 cm diameter.

The relative distance of each ramet from the center of the clone ellipse (0 = clone center, 1 = clone ellipse edge) was determined, and variation in ramet density was quantified by dividing the entire clone ellipse into five radially equidistant concentric sub-ellipses: 0.0 – 0.2; 0.2 – 0.4; 0.4 – 0.6; 0.6-0.8; and 0.8 – 1.0. For each clone, ramet density was determined for each of these five sub-ellipses.

RELATIVE GROWTH RATES OF CLONAL RAMETS — Within each clone, mean annual relative growth rates (Hunt 1978) of newly recruited clonal ramets (≤ 1 cm diameter in 2003) were determined using both stem height and basal diameter data. The following equations were used:

Relative Growth Rate: Stem Height

$$RGR_H = [\ \ln H_t - \ln H_o\ ] / t$$

Relative Growth Rate: Stem Basal Diameter

$$RGR_D = [\ \ln D_t - \ln D_o\ ] / t$$

In these equations, H_o = stem height (cm) in 2003, D_o = stem basal diameter (cm) in 2003, H_t = stem height (cm) after t years, and D_t = stem basal diameter (cm) after t years. Note that $t = 6$ for ramets that survived to 2009; for ramets dying between 2004 to 2008, $t < 6$ (e.g. $t = 4$ for ramets recorded as dead in 2007). The units for both RGR_H and RGR_D are: cm cm^{-1} yr^{-1}. The Student t-test (Legendre and Legendre 1998) was used to compare RGR values between ramets dying during the study period ($t < 6$) and those that survived to 2009 ($t = 6$). These tests were used to determine whether the growth rates of dying ramets were significantly less than those of surviving ramets.

Changes in the relative growth rate of ramets with age were examined by computing RGR_D as a function of initial stem diameter (in 2003). The RGR_D (mean ± standard deviation) was computed over seven diameter classes (note the logarithmic scale):

(1) 0.18 – 0.32 cm; (2) 0.32 – 0.56 cm; (3) 0.57 – 1.00 cm; (4) 1.01 – 1.78 cm;
(5) 1.79 – 3.16 cm; (6) 3.17 – 5.62 cm; (7) 5.63 – 10.00 cm.

In this analysis, ramets were pooled over the four clones to increase sample size. Ramet height data were not used in this analysis, since many of the larger ramets (> 1 cm diameter in 2003) were browsed; this rendered stem height a poor measure of ramet growth.

CLONE DEMOGRAPHY: SURVIVAL ANALYSIS — Kaplan-Meier survival analysis was used to describe mortality rates among the four clones, and to examine temporal patterns in mortality among two diameter size classes (≤ 2.5 cm and > 2.5 cm) within each clone. Survival rates were determined within each size class using time (72 month period) as a covariate (Kaplan-Meier survival curves; Kaplan and Meier 1958). Survival analyses were conducted using the Survival Package in R 9.2.9 (R development Core Team 2009).

3.3 RESULTS

SPATIAL PATTERN OF CLONAL RAMETS — In all clones, the largest live and dead stems (> 5.0 cm diameter) were located near the clone center, while intermediate-sized ramets (2.5 – 5.0 cm) were more widely dispersed (**Figures 3.1 – 3.4**). The smallest ramets (< 2.5 cm diameter) were generally found throughout the clone. In all four clones, the peripheral ramets (i.e. those near the clone boundary) belonged to the smallest size class. These results indicate that the dieback of mature canopy stems (> 5 cm diameter) promotes the recruitment of aspen suckers, both within the initial clonal boundary (as defined by the largest stem diameter class) and beyond (resulting in expansion of the clone).

The spatial distribution of new sucker recruitment (smallest diameter class) varied among clones (**Figure 3.5**). In the two largest clones (“A” and “B”), the most recently recruited ramets were found throughout the clone (i.e. center and periphery). Conversely, in the two smaller clones (“C” and “D”) there is little recruitment at the center of the clone (i.e. beneath mature ramets). In all four clones, intermediate and large diameter ramets (> 2.5 cm diameter) occured in the central portion of the clone, but were uncommon or absent from the clone periphery (**Figure 3.5**). Results for ramet height confirm this general trend (**Figure 3.6**). The density of tall ramets (> 1.5 m height) was highest near the center of the clone and declined toward the periphery. By contrast, short ramets (≤ 1.5 m height) occurred at high density throughout the four clones; the only exception to this was clone “C”, where no small ramets were found at the center of the clone. Together, these results demonstrate that aspen encroachment (recruitment of young ramets beyond the initial clone boundary) occurs following mature stem dieback.

RELATIVE GROWTH RATES OF CLONAL RAMETS — Mean relative growth rates (RGR_H and RGR_D) of the recently recruited ramets (≤ 1 cm diameter) were similar over all four clones (**Table 3.1**). On average, live ramets (those surviving from 2003 – 2009) more than doubled in size (both height and diameter) over the six years (RGR = 0.115 cm cm^{-1} yr^{-1} corresponds to a 100% increase in size over six years). Relative growth rates were highest in clones "B" and "D", and lowest in clone "C". Pooled over all four clones, ramets on average increased in diameter by 115% ($RGR_D = 0.128$ cm cm^{-1} yr^{-1}) and in height by 140% ($RGR_H = 0.145$ cm cm^{-1} yr^{-1}) over six years.

The relative growth rates of dying ramets (i.e. those not surviving until 2009) were significantly lower than surviving ramets, for all clones (t-tests, $P < 0.001$ for all tests). Over all clones, the dying ramet relative growth rate averaged $RGR_D = 0.085$ and $RGR_H = 0.098$, corresponding to increases of only 66% and 80% respectively over six years. These results indicate that dying ramets undergo an extended period of reduced growth before they die – a slow, lingering death rather than a sudden one. This suggests that biotic processes (e.g. competition or disease) are more important than catastrophic events (e.g. physical damage such as stem girdling) in determining young ramet mortality.

The relative diameter growth rate of ramets declined rapidly with stem size (**Figure 3.7**). On average, newly recruited ramets (≤ 1 cm diameter) more than doubled their stem diameters over six years ($RGR_D = 0.144 – 0.146$). Older (i.e. larger) ramets grew much more slowly, however, with relative growth rates declining log-linearly as a function of size. Ramets in diameter class 3.1 – 5.7 cm have a $RGR_D = 0.053$, or about one-third that of newly recruited ramets. For the largest diameter class (5.7 – 10 cm), the $RGR_D = 0.012$ is less than one-tenth that of newly recruited ramets.

CLONE DEMOGRAPHY: SURVIVAL ANALYSIS — Mortality patterns varied considerably among the four aspen clones (**Figure 3.8**). For clone “A”, over half of the large stems (> 2.5 cm diameter) died between 2003 and 2009 compared to < 10% of small stems (≤ 2.5 cm diameter), a result that is highly statistically significant ($\chi_1^2 = 118$, $P < 0.001$). Most of the large stems died within the first two years of the study (2004 and 2005), whereas mortality of smaller stems was more consistent from year to year. For clone “B”, mortality of small and large stems was similar and not statistically significant ($\chi_1^2 = 1.3$, $P = 0.256$); over the six years, total mortality was 43% for small, and 53% for large stems. Similar results were obtained for clone “C” ($\chi_1^2 = 1.3$, $P = 0.256$), although total mortality was somewhat lower: 31% for small stems, and 21% for large ones. For clone “D”, the mortality of large stems was high (totaling 78%), with most death occurring in the last two years of the study (2008 and 2009). Mortality of smaller stems (totaling 29%) was significantly lower ($\chi_1^2 = 39.6$, $P < 0.001$).

3.4 DISCUSSION

The results of this long-term study indicate that aspen clones undergo clonal expansion following canopy dieback; in all four clones, secondary root-sucker recruitment occurred both beneath the existing canopy (i.e. clone re-establishment) and beyond it (i.e. clone expansion). Secondary recruitment of root-suckers is known to result from a loss of apical dominance following canopy dieback (e.g. Cumming et al. 2000). This method of clonal expansion implies that the clone's root system extends well beyond the mature canopy, i.e. into adjacent grassland habitat. However, in aspen the rate of clonal expansion following mature stem dieback is a complex and highly variable process, controlled by both external (e.g. edaphic conditions, interspecific competition) and internal factors (e.g. intaspecific genetic variability, hormonal suppression of root-sucker production).

Determining the multitude of factors affecting the growth and survival of individual ramets (genetically identical members of a clone) is critical to understanding clonal persistence (Hosaka et al. 2008). In this study, the youngest, newly recruited ramets (< 1 cm diameter) had the highest mean relative growth rate; over six years, these stems more than doubled in size. For larger ramets, relative growth rates decline log-linearly with increasing ramet size. This decline in relative growth rates with increasing stem age has been observed for other tree species (e.g. Philip 1994), and has been posited as a mechanism to rapidly overcome size inequalities within stands (Dolezal et al. 2004).

Newly recruited ramets that died during the study period (2003-2009) had significantly lower relative growth rates (prior to mortality) than did surviving ones. This indicates that ramet productivity systematically declines prior to mortality (Peterson and Peterson 1992), suggesting that biotic processes (e.g. competition or disease) are the primary determinants of mortality in these ramets (Franklin et al. 1987).

Survival analysis revealed considerable variation in ramet mortality, both among clones and between size-classes. Two of the four clones showed significantly higher mortality for larger stems (> 2.5 cm diameter) compared to small ones. For these two clones, large stem mortality exceeded 50% over six years, while only 10-30% of small stems died over the same period. Total mortality over six years ranged from 10.8% (clone "A") to 46.2% (clone "B"), and there were no discernible patterns in the timing of mortality (e.g. in clone "A" most of the larger ramets died in 2003 and 2004, whereas in clone "B" most died in 2008 and 2009). Together, these results indicate that clone demography is being driven by endogenous (e.g. genotype, clone development stage; Frey et al. 2004) rather than exogenous processes (e.g. climatic extremes, insect pests; Hogg et al. 2002, 2008).

Regular ungulate browsing of regenerating ramets was observed in all four clones. Intermediate-sized ramets (2.5 – 5 cm diameter), which display much of their foliage at ungulate "browse height", are particularly vulnerable to herbivore damage (De Jager et al. 2009). Ungulate herbivore damage initiates a positive feedback, in which browsed ramets become increasingly susceptible to regular and repeated herbivore browsing. Repeated browsing reduces ramet productivity, which in turn limits both stem height and diameter growth. Repeated and regular browsing also results in the loss of strong apical dominance, resulting in multi-stemmed, stunted ramets with little or no vertical growth. These older, stunted ramets also produce lesser amounts of chemicals that deter herbivores (Donaldson et al. 2006; Lindroth et al. 2007), further increasing browsing pressure. The decline in relative growth rate with ramet size may reflect the detrimental effects of herbivory on the productivity of intermediate-sized ramets. The reduction in photosynthetic capacity resulting from ungulate browsing also increases the overall respiratory burden on the clone (Lieffers et al. 2001). The strong browsing pressure observed in these clones may account for the high levels of mortality, and low relative growth rates, observed in the clonal ramets. Ungulate herbivory may also limit the ability of these clones to re-establish a full and tall canopy, since few of the regenerating ramets will have the opportunity to grow high enough to “escape” the browse zone.

Differential mortality of clonal ramets may have important ecological consequences on the persistence of aspen clones (Hosaka et al. 2008). While clonal ramets are genetically identical, developmental variation in multi-aged aspen clones can result in functional differences among shoots (Brandt et al. 2003). For example, biotic stressors such as susceptibility to ungulate herbivory, insect defoliations, and fungal pathogens tend to disproportionately affect larger ramets (e.g. Ives et al. 1973; Peterson and Peterson 1992; Hogg et al. 2002; Donaldson et al. 2006). As a consequence, disease contagion within a forest stand is best modeled by taking into consideration stem densities within critical size-classes (e.g. Franklin et al. 1987). In a clonal species, temporal partitioning of ramets (size or age classes) promotes the survival probability of a given ramet, increasing overall plant fitness. Not unlike genetic diversity within a population, diversity in size-structure (or age-structure) promotes persistence by maximizing the developmental variation in ecologically adaptive traits (Donaldson et al. 2006). Such a mechanism overcomes a well-established limitation of clonality: the functional phenotypic uniformity of ramets within a spatially and temporally heterogeneous environment (reviewed in Stuefer 1996). In the context of aspen biology, a clone consisting of even-aged ramets would be considered to be phenotypically uniform, whereas a clone consisting of different-aged ramets is functionally diverse in terms of adaptive traits. Thus developmental differences in traits among ramets can be scaled up to the level of a clone, and may explain the ecological success of the species.

3.5 ACKNOWLEDGEMENTS

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant to N. C. Kenkel, Parks Canada and a University of Manitoba's Duff Roblin Graduate Fellowship to R. A. Lastra. I would like to thank Wybo Vanderschuit and Dr. John Wilmshurst for help with various aspects of this study as well as Kelly Orr.

3.6 REFERENCES

Brandt, J.P., H.F. Cerezke, K.I. Mallet, W.J.A. Volnet, and J.D. Weber. 2003. Factors affecting trembling aspen (*Populus tremuloides* Michx.) health in Alberta, Saskatchewan, and Manitoba, Canada. Forest Ecology and Management 178:287-300.

Cumming, S.G., F.K.A. Schmiegelow, and P.J. Burton. 2000. Gap dynamics in boreal aspen stands: is the forest older than we think? Ecological Applications 10:744-759.

De Jager, N.R., J. Pastor, and A.L. Hodgson. 2009. Scaling the effects of moose browsing on forage distribution, from the geometry of plant canopies to landscapes. Ecological Monographs 79:281-297.

Dolezal, J., P. Stastna, T. Hara, and M. Srutek. 2004. Neighborhood interactions and environmental factors influencing old-pasture succession in the Central Pyrenees. Journal of Vegetation Science 15:101-108.

Donaldson, J.R., M.T. Stevens, H.R. Barnhill, and R.L. Lindroth. 2006. Age-related shifts in leaf chemistry of clonal aspen (*Populus tremuloides*). Journal of Chemical Ecology 32:1415-1429.

Franklin, J.F. , H.H. Shugart, M.E. Harmon. 1987. Tree death as an ecological process. Bioscience 37:550-556.

Gradowski, T., V.J. Lieffers, S.M. Landhäusser, D. Sidders, J. Volney and J.R. Spence. 2010. Regeneration of *Populus* nine years after variable retention harvest in boreal mixedwood forests. Forest Ecology and Management 259:383-389.

Harper, J. L. 1977. Population Biology of Plants. Academic Press, London.

Hartnett, D.C., and F.A. Bazzaz. 1985. The genet and ramet population dynamics of *Solidago canadensis* in an abandoned field. Journal of Ecology 73:407-413.

Hogg, E.H., J.P. Brandt, and B. Kochtubajda. 2002. Growth and dieback of aspen forests in northwestern Alberta, Canada, in relation to climate and insects. Canadian Journal of Forest Research 32:823-832.

Hogg, E.H., J.P. Brandt, and M. Michaelian. 2008. Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Canadian Journal of Forest Research 38:1373-1384.

Hosaka, N., S. Gomaz, J. F. Stuefer, and D.F. Whigham. 2005. The ecological significance of clonal growth in the understory tree, Pawpaw (*Asimina triloba*). Northeastern Naturalist 12:11-22.

Hosaka, N., N. Kachi, H. Kudoh, J. F. Stuefer, and D.F. Whigham. 2008. Patch structure and ramet demography of the clonal tree, *Asimina triloba*, under gap and closed-canopy. Plant Ecology 197:219-228.

Hunt, R. 1978. Plant growth analysis. Edward Arnold, London. 67 pages.

Hutchings, M. J. 1974. Weight-density relationships in ramet populations of clonal perennial herbs, with special reference to the -3/2 power law. Journal of Ecology 67: 21-33.

Ives, W.G.H., J.J. Lawrence, J.K. Robins. 1973. Prairie region. Pages 74-81 *in* Annual report of the forest insect and disease survey, 1972. Environment Canada, Canadian Forest Service, Ottawa, Ontario.

Jerling, L. 1988. Clone dynamics, population dynamics and vegetation pattern of Glaux maritime on a Baltic sea shore meadow. Vegetatio 74:171-185.

Kulakowski, D., T.T. Veblen, and S. Drinkwater. 2004. The persistence of quaking aspen (Populus tremuloides) in the Grand Mesa area, Colorado. Ecological Applications 14: 1603-1614.

Legendre, P. and L. Legendre. 1998. Numerical Ecology. 2nd edition. Elsevier, Amsterdam. 853 pages.

Lieffers, V. J., S. M. Landhäusser, and E. H. Hogg. 2001. Is the wide distribution of aspen a result of its stress tolerance? In Sustaining aspen in western landscapes: Symposium proceedings June 13-15, 2001, Grand Junction, Colorado. Edited by W. D. Sheppard, D. L. Binkley, D. Bartos, T. J. Stohlgren, and L.G. Eskew. U.S. Dep. Agric. For. Serv. Rocky Mt. For. Range Exp. Stn. Proc. RMRS-P-18. pages 311-323.

Lindroth, R. L., J. R. Donaldson, M. T. Stevens, and A. C. Gusse. 2007. Browsing quality in quaking aspen (*Populus tremuloides*): effects of genotype, nutrients, defoliation, and coppicing. Journal of Chemical Ecology 33:1049-1064.

Mock., K.E., A. Rowe, M.B. Hooten, J. Dewood and V.D. Hipkins. 2008. Clonal dynamics in western North American aspen (*Populus tremuloides*). Molecular Ecology 17:4827-4877.

Peterson, E.B., and N.M. Peterson. 1992. Ecology, management and use of aspen and balsam poplar in the prairie provinces. Forest Canada Northwest Region Report No. 1, Edmonton. 252 pages.

Philip, M.S. 1994. Measuring trees and forests. 2nd edition. CABI, New York. 310 pages.

R Development Core Team. 2009. R: a language and environment for statistical computing. Version 2.9.2. R foundation for Statistical Computing, Vienna, Australia. http://www.R-project.org

Rees, M., and S.P. Ellner. 2009. Integrak projection models for populations in temporally varying environments. Ecological Monographs 79:575-594.

Rivano F., M. Martinez, V. Cevallos, and C. Cilas. 2010. Assessing resistance of rubber tree clones to *Microcyclus ulei* in large-scale clone trails in Ecuador: a less time-consuming field method. Eur. J. Plant Pathology 126:541-552.

Stuefer, J.F. 1996. Potential and limitations of current concepts regarding the response of clonal plants to environmental heterogeneity. Vegetatio 127:55-70.

Trottier, G.C. 1986. Distribution of rough fescue, *Fescue hallii*, grassland by livestock grazing I Riding Mountain National Park, Manitoba. Canadian Field Naturalist 100:488-495.

Westoby, M. 1984. The self-thinning rule. Advances in Ecological Research 14:167-225.

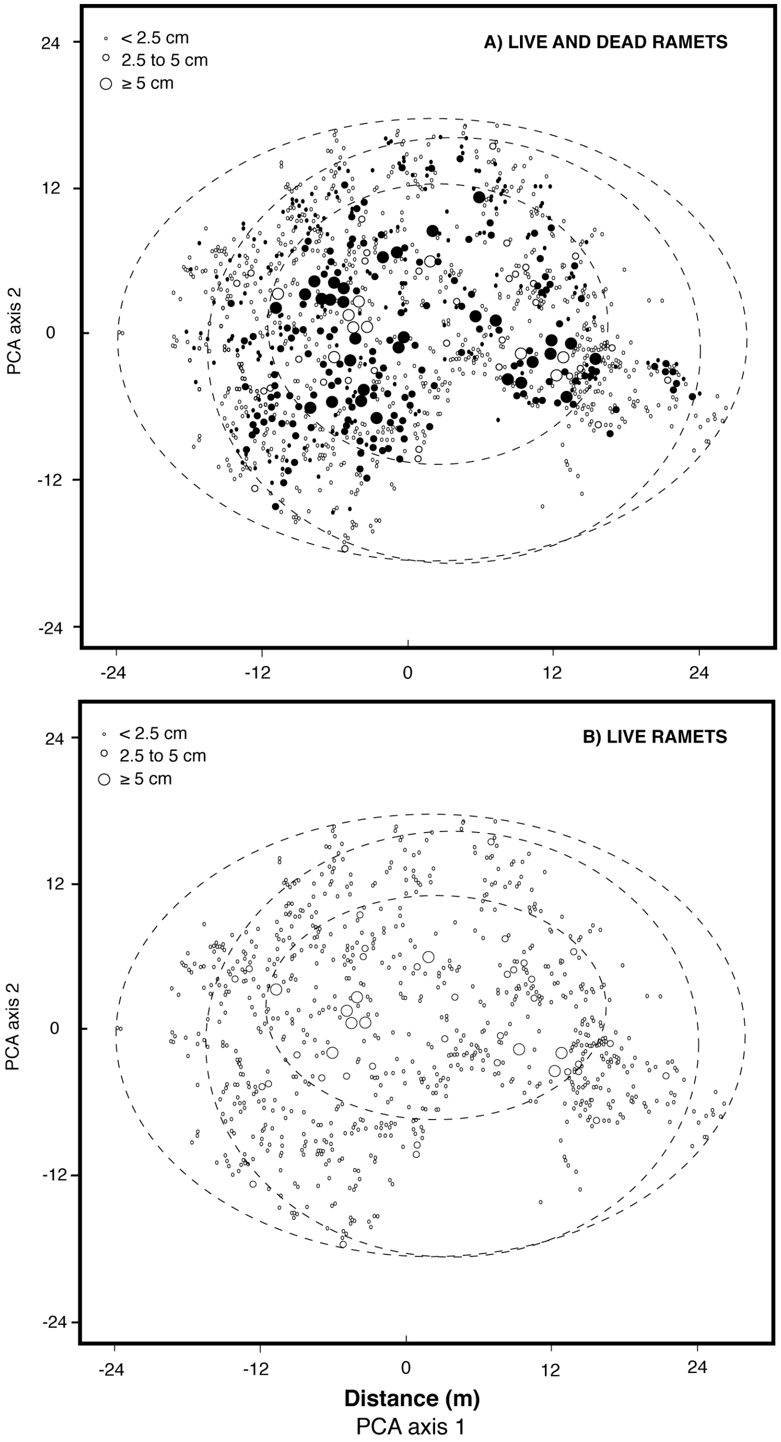


Fig. 3.1 Clone A: 1a) Spatial distribution of live (open) and dead (closed) ramets of an isolated aspen clone, represented in three diameter (cm) size classes (see legend) at the begining of the study (2003). 1b) Spatial distribution of live stems (2003). Ordination axis is expressed in deviations (metres) from the centroid of an ellipse. Dotted ellipses represents diameter size boundaries.

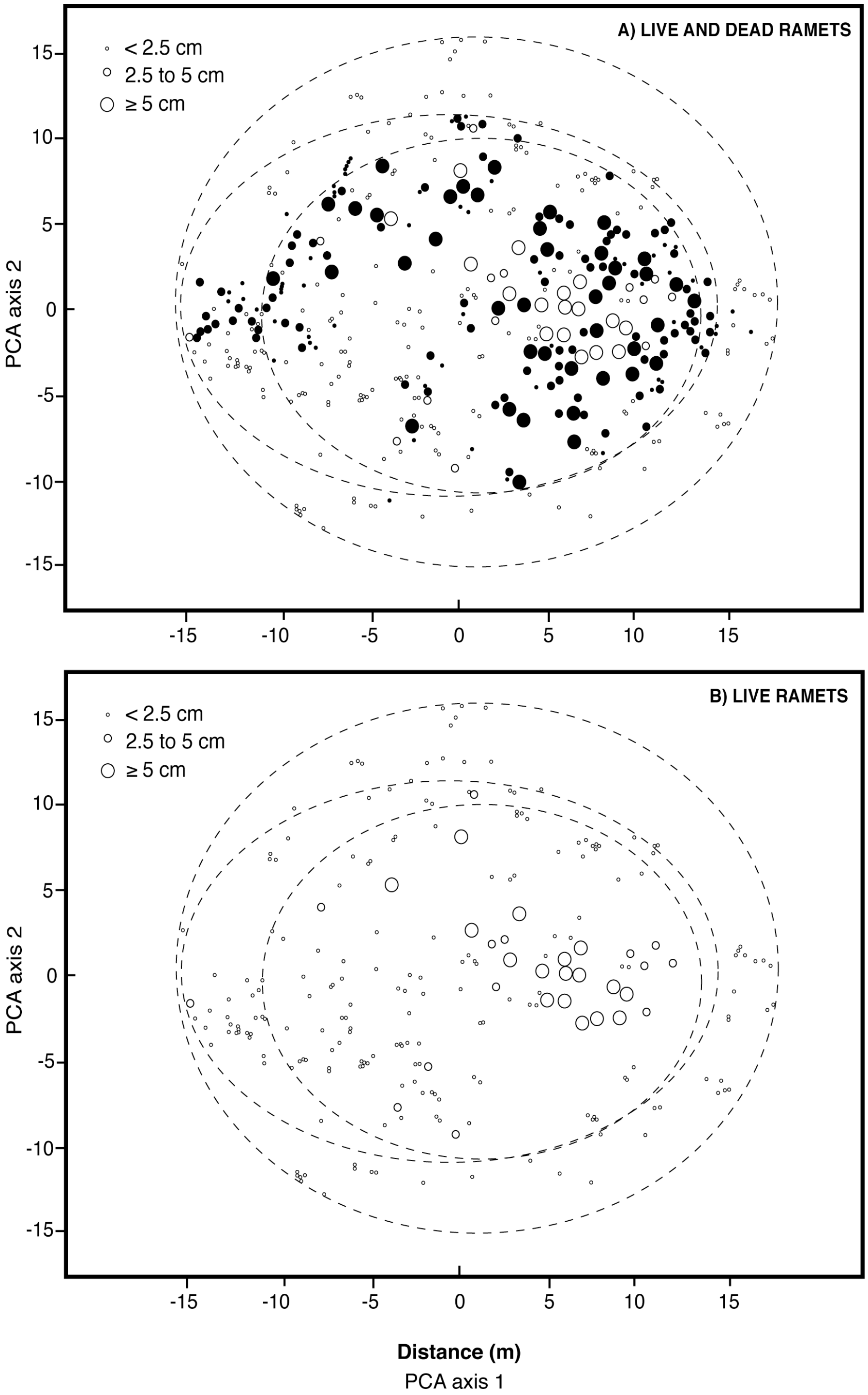


Fig. 3.2 Clone B: 1a) Spatial distribution of live (open) and dead (closed) ramets of an isolated aspen clone, represented in three diameter (cm) size classes (see legend) at the begining of the study (2003). 1b) Spatial distribution of live stems(2003). Ordination axis is expressed in deviations (metres) from the centroid of an ellipse. Dotted ellipses represents diameter size boundaries.

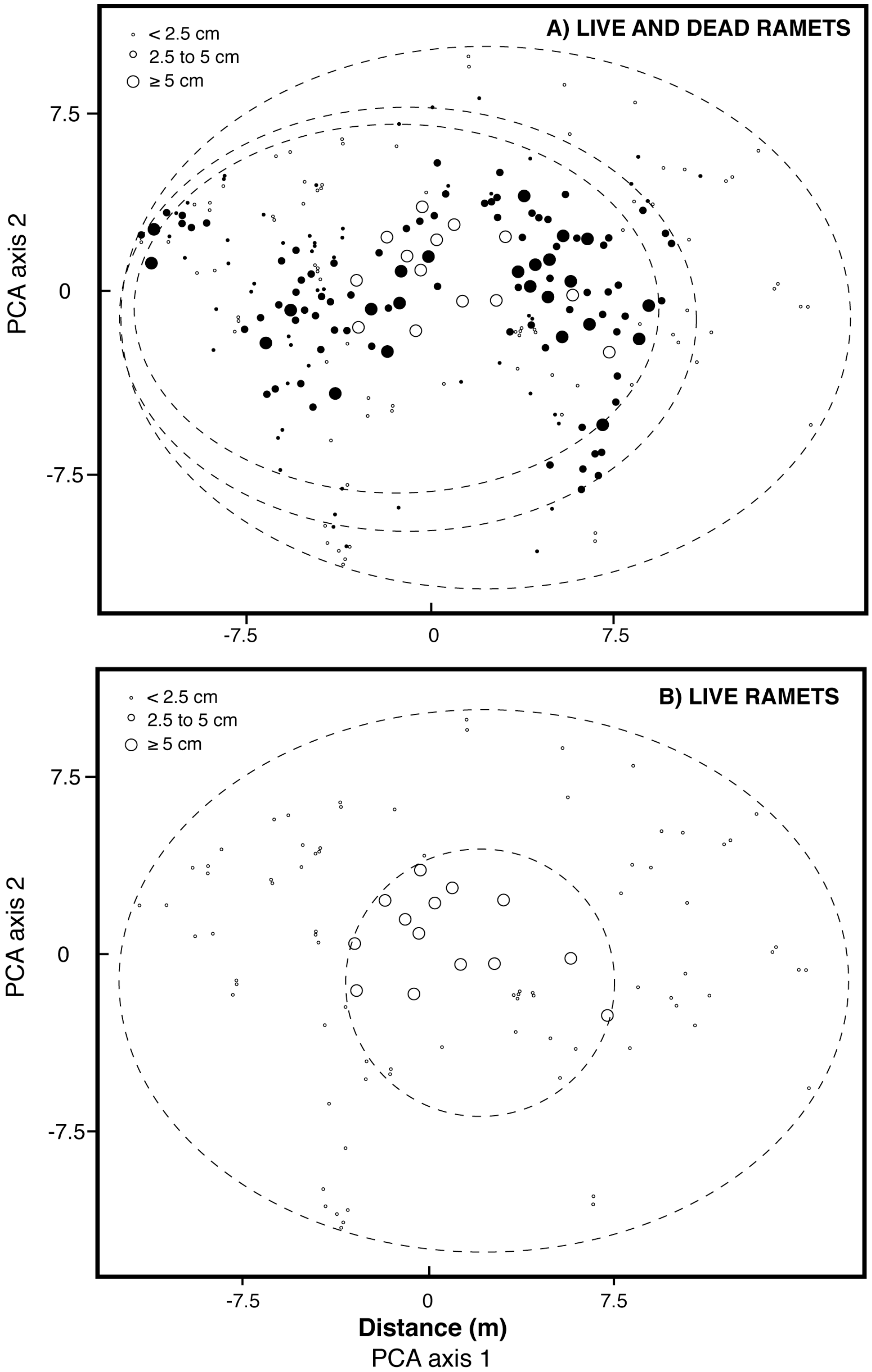


Fig. 3.3 Clone C: 1a) Spatial distribution of live (open) and dead (closed) ramets of an isolated aspen clone, represented in three diameter (cm) size classes (see legend) at the begining of the study (2003). 1b) Spatial distribution of live stems (2003). Ordination axis is expressed in deviations (metres) from the centroid of an ellipse. Dotted ellipses represents diameter size boundaries.

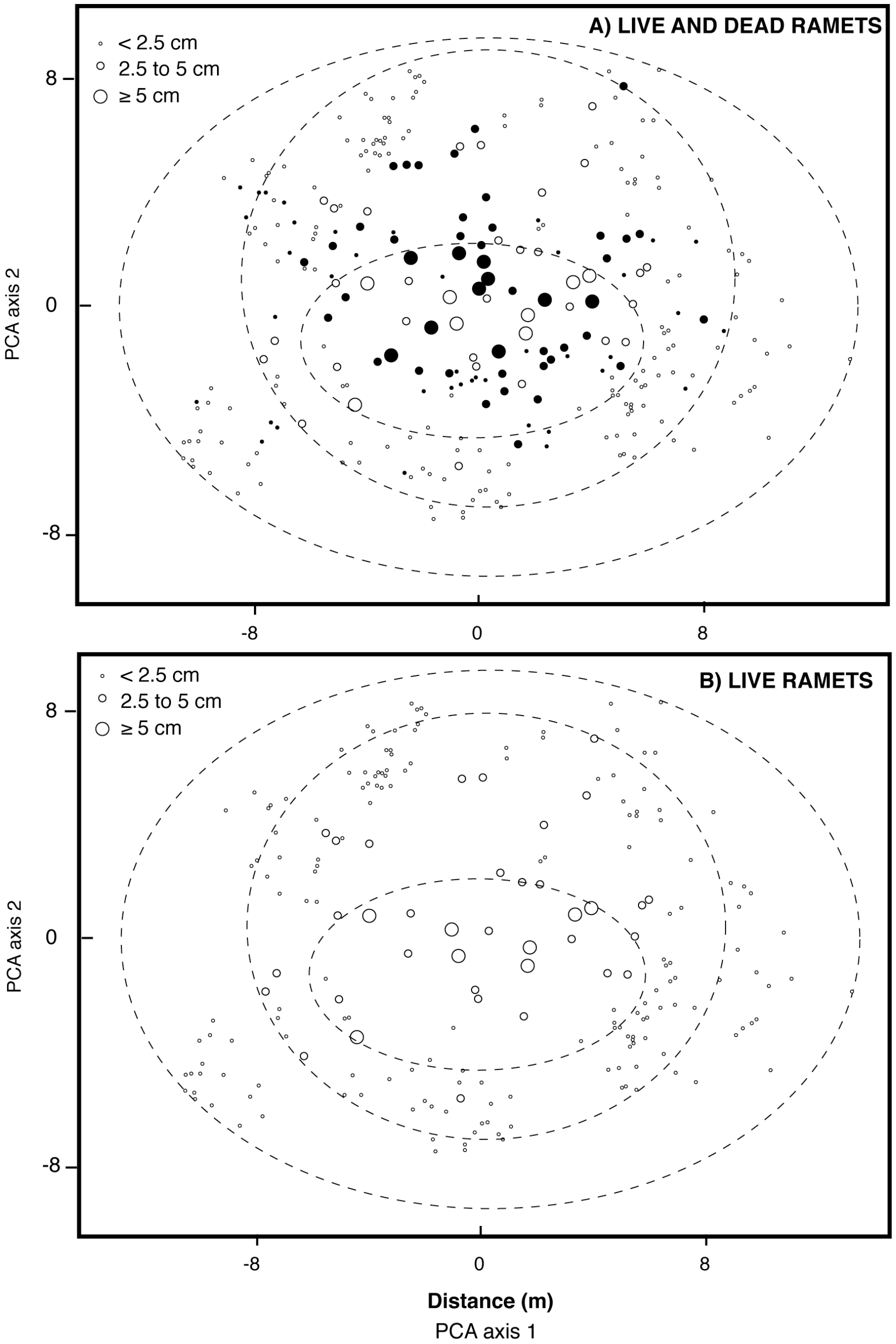


Fig. 3.4 Clone D: 1a) Spatial distribution of live (open) and dead (closed) ramets of an isolated aspen clone, represented in three diameter (cm) size classes (see legend) at the begining of the study (2003). 1b) Spatial distribution of live stems(2003). Ordination axis is expressed in deviations (metres) from the centroid of an ellipse. Dotted ellipses represents diameter size boundaries.

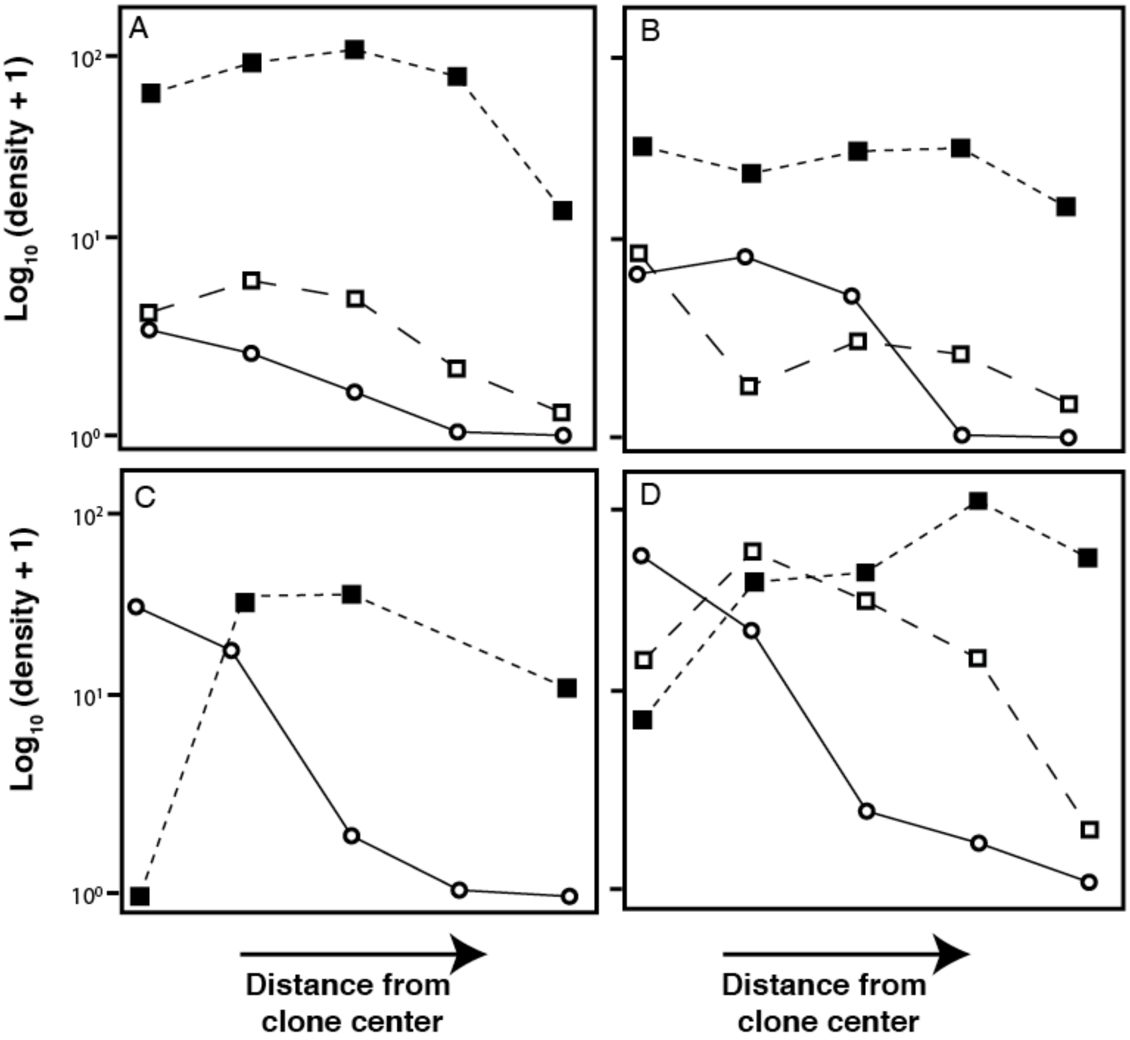


Figure 3.5. Stem diameter size class profile (clone center to edge) for clones A, B, C, and D. Where the solid squares indicate diameter classes < 2.5 cm, open squares diameter classes between 2.5 – 5 cm and open circles indicating diameter size classes > 5 cm.

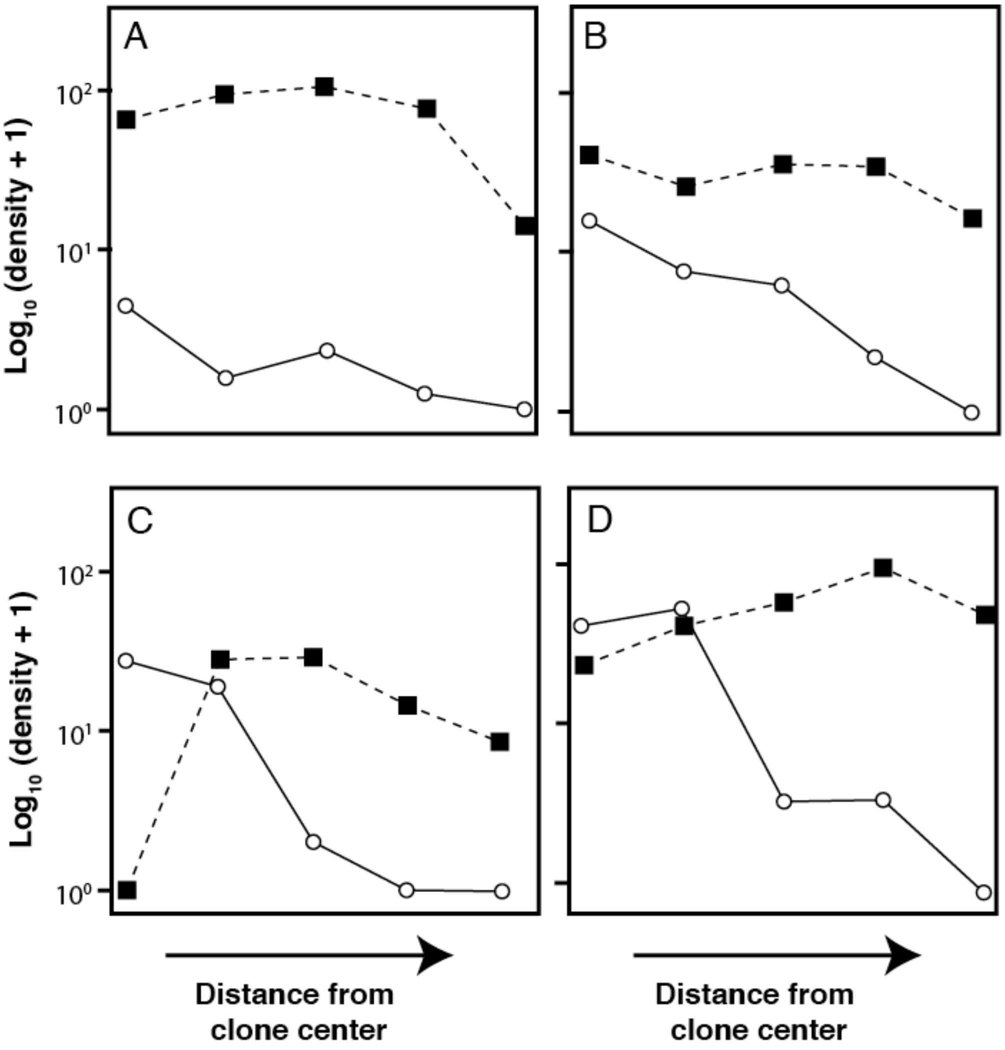


Figure 3.6. Stem height class profile (clone center to edge) for clones A, B, C, and D. Where the solid squares indicate height classes > 1.5 m and open circles indicate height classes < 1.5 m.

Table 3.1. Mean (+/-1 SD) relative height and basal diameter (cm) growth rates (cm cm^{-1} yr^{-1}) summarized using only aspen ramets with diameter ≤ 1 cm (in 2003). Growth rates were based on 6 years of data (2003 – 2009).

	CLONES				
	A	**B**	**C**	**D**	**POOLED**
DEAD					
RGR (height)	0.068 (*0.045*)	0.101 (*0.037*)	0.081 (*0.028*)	0.08 (*0.03*)	0.085 (*0.04*)
RGR (diameter)	0.103 (*0.054*)	0.113 (*0.034*)	0.079 (*0.026*)	0.088 (*0.034*)	0.098 (*0.04*)
N	26	51	30	34	141
LIVE					
RGR (height)	0.121 (*0.048*)	0.145 (*0.027*)	0.123 (*0.008*)	0.139 (*0.011*)	0.128 (*0.04*)
RGR (diameter)	0.147 (*0.026*)	0.163 (*0.07*)	0.120 (*0.010*)	0.138 (*0.09*)	0.145 (*0.03*)
N	453	125	75	132	785

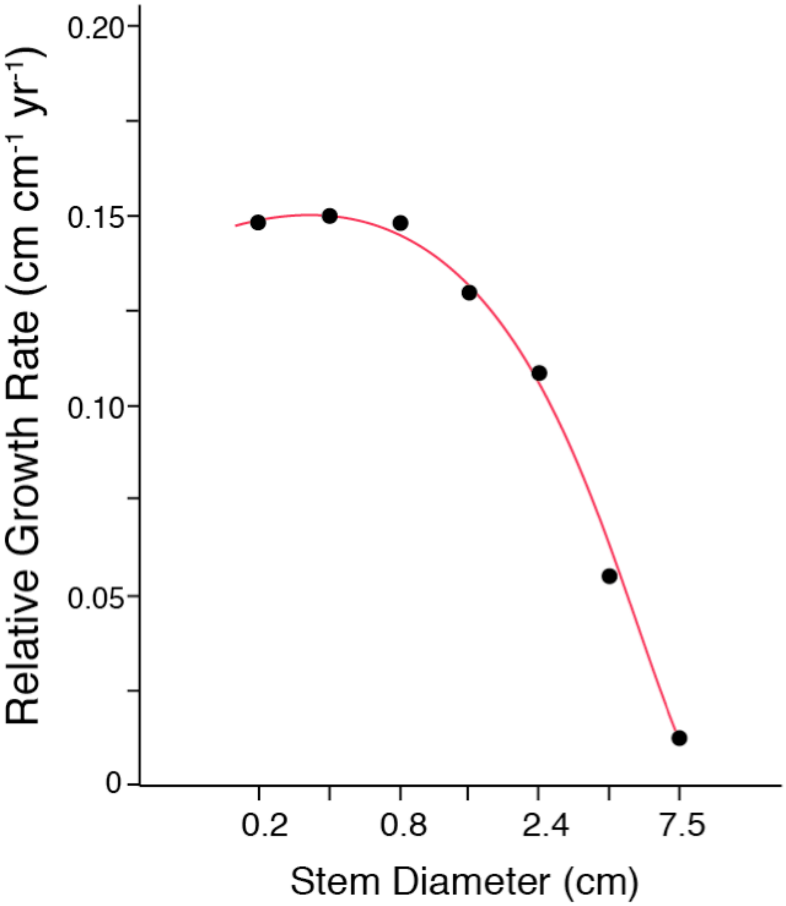


Figure 3.7. Relative diameter growth rate (cm cm^{-1} yr^{-1}) as a function of initial 2003 diameter measurements. Data represents RGR_D for 6 yrs (2003 – 2009). Dashed line indicates minimum RGR_D required to double stem diameter over 6 years.

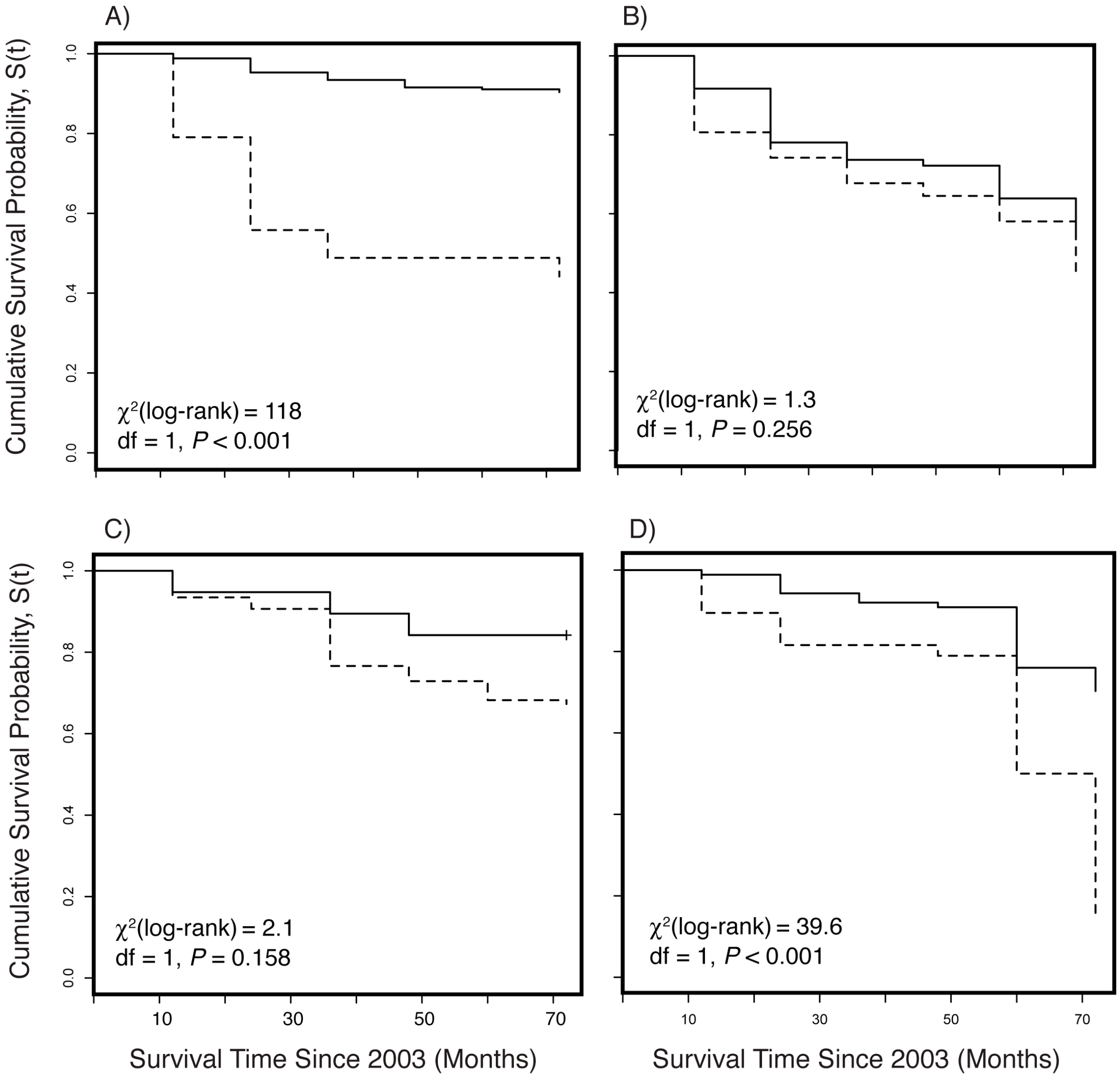


Figure 3.8. Kaplan-Meier [K-M] survival curves, [S(t)] as a function of diameter size class for clones A, B, C, and D. Solid line indicates stem diameter classes ≤ 2.5; dotted line > 2.5 cm. Statistical comparison (log-rank test) between the GK-M survival curves indicates that clone A and D are the only ones that significantly different.

CHAPTER 4

THE ECOLOGICAL SIGNIFICANCE OF CLONAL INTEGRATION IN ASPEN

4.1 INTRODUCTION

In clonal plant species, the physiological integration of inter-connected modules (ramets) has been posited as an adaptive strategy that “buffers environmental variation” (Chesson and Peterson 2002) by enhancing the sharing of resources (Qureshi and Spanner 1971; Pitelka and Ashmum 1985; Caldwell and Pearcy 1994; Hutchings and Wijesinghe 2008). The translocation of resources within plants has been shown to benefit fitness (production, relative growth rate and survival), and to alter ecological interactions via the translocation of hormones, defense signals and pathogens (Stuefer et al. 2004; Gómez et al. 2007; Matsushita et al. 2010). Indeed, the evolutionary and ecological consequences of clonal biology are well established in the literature (Harper 1977). However, numerous authors have pointed out that less attention has been placed on the ecological consequences of clonal “disintegration”, defined as the breaking of connections among ramets that results in physiological independence (Stuefer 1997; Hay and Kelly 2008).

Although physiological integration has its benefits, the maintenance of a network of inter-connected clonal ramets can also have significant energetic and health costs (e.g. high respiratory costs of connective tissues, and increased risk of disease transfer; Stuefer et al. 2004; Hay and Kelly 2008). The disintegration (or “fragmentation”) of a single clone into smaller subunits has been documented in most clonal species (Harper 1977). The mortality of inter-ramet connections is considered a coordinated physiological response to a multitude of environmental and biological factors (Hay and Kelly 2008). In general, physiological dependence declines substantially once a ramet develops its own root system (Lei 2010). In mature clones, the loss of physical (and functional) inter-ramet connections may have important ecological implications, on both the dynamics of ramet making up the clone and overall clone persistence.

Trembling aspen (*Populus tremuloides* Michx.) is the most common clonal tree species in North America. Numerous studies have noted that the clonal biology of aspen may play an important role in explaining the long-term persistence of this species. A number of investigators have described the clonal rooting system of aspen (e.g. Day 1944; Gifford 1966), but relatively few studies have directly examined the ecological consequences of physiological integration in the genus *Populus* (DesRochers and Lieffers 2001; Peltzer 2002; Wiehle et al. 2009). Trembling aspen is subject to clonal disintegration, i.e. the natural severing of ramets root connections (Lieffers et al. 2001). Physiological connectivity of ramets may be lost as the root system ages (Sandberg 1951), or through direct physical damage to roots resulting from belowground herbivory (Cantor and Whitman 1989). More recent investigations have found that fragmentation can result in a clone becoming a “genetic mosaic”, the result of somatic mutations within new root-suckers (Ally et al. 2008).

The loss of physiological integration of clonal ramets can have detrimental effects on survivorship, both by reducing the sharing of limiting resources and by impeding stress signaling within the clone (Gómez et al. 2007; Stuefer et al. 2004; Poor et al. 2005). In the only comprehensive study of clonal integration in trembling aspen, Peltzer (2002) found that severing inter-ramet connections had no effect on their subsequent growth and survivorship. Peltzer's (2002) results cannot be generalized, however, since the experiment was conducted at only one site. Additional studies are needed to determine the relative importance of ramet physiological integration – for example, does clonal integration become more important in more stressful habitats?

The objective of this study is to examine the interaction of environmental variability and loss of physiological integration the survivorship, relative growth rates, and above-ground (leaf) productivity of clonal ramets (Hay and Kelly 2008). I hypothesize that the severing of functional root connections in aspen will negatively impact ramet fitness, and that this effect will be most pronounced in more stressful environments.

4.2 METHODS AND MATERIALS

STUDY SITES

The study was conducted in three distinct forest-grassland ecotone sites located in Riding Mountain National Park (RMNP; 50°31'59"-51°01'19" N, 99°33'9"-101°05'19" W; 2,978 km^2), Canada: (1) Grasshopper Valley meadow, (2) Baldy Lake meadow, and (3) Deep Lake grassland. The sites were approximately 20 km apart, and occur along a west to east gradient of increasing soil moisture availability (**Figure 4.1**). This moisture gradient reflects differences in both local soil drainage and topography (**Table 4.1**). The westernmost site is Deep Lake, which consists of rolling grasslands on topographically steep slopes (ranging form 30-40%, or ~ 20° slope; **Figure 4.2a**). The aspen clones at the Deep Lake site occur on the well-drained upper slopes. The central site, Baldy Lake meadow, consists of meadows of grassland and aspen groves on gently rolling slopes (typically 5%). The easternmost site is Grasshopper Valley, which is topographically flat (slopes 2% or less). The aspen clones at Grasshopper Valley typically occur in low-lying micro-depressions (**Figure 4.2b**).

In addition to topographic considerations, the relative soil moisture availability at the three study sites was determined using the following edaphic factors:

1. Soil Texture Analysis: Particle size analyses were based on three 150 g soil cores collected at each of the selected aspen clones. Soil texture was determined for each soil core using the hydrometer method (Bouycous 1951).
2. Degree of Stoniness: Stoniness was determined using the soil cores, as well as the soil profile (see below). The "degree of stoniness" was quantified on ordinal scale, based on a visual classification of sorted particles between 2 and 64 mm in diameter (pebbles; Soil Classification Working Group 1998).
3. Surficial Deposits: These data were obtained from surficial geology maps for the Province of Manitoba.
4. Soil profile: A one-meter deep soil profile was dug in a representative area at each of the three study sites. Soils were classified using the Canadian System of Soil Classification (Soil Classification Working Group 1998).

These data, summarized in **Table 4.1**, confirm a gradient of increasing soil moisture stress from east (Grasshopper Valley) to west (Deep Lake). All sites were classified as parkland savanna, with trembling aspen (*Populus tremuloides* Michx.) as the dominant tree species and plains rough fescue (*Festuca hallii* (Vasey) Piper) the dominant graminoid.

Riding Mountain National Park is characterized by warm, short summers and long, cold winters. Twenty-nine years of climatic data (1971-2000), compiled from the town of Wasagaming, RMNP (50°39.3' N, 99°56.51' W), were used to summarize annual rain and precipitation norms (Environment Canada 2009). Total annual rainfall averaged 398 mm, with June and July being the two wettest months (mean monthly rainfall = 84.5 and 75.3 mm respectively). Mean annual temperature was 0.1°C, and the mean monthly temperature ranged from –19.6°C in January to 16.5°C in July.

SELECTION OF CLONES AND RAMETS — Three isolated aspen clones were selected within each of the three study sites. Only vigorous "healthy" clones were chosen; i.e. clones showing no evidence of stem dieback or decline. The area of each clone was estimated using radial measurements taken from the center of each clone. These measurements were later used to determine the outer area of each clone (defined as 40% of the outer radial distance). Only the outer area of each clone was considered when selecting experimental ramets. Between July 18 and August 28, 2003 a total of 180 aspen ramets were selected, 20 ramets per clone (10 for root-severing treatment and 10 for non-severed controls); see **Table 4.2**. All selected ramets were ≤ 1.5 m in height and not damaged by ungulate browsing. Once selected, ramets were tagged and their initial size (basal stem diameter and height) and leaf productivity (total leaf count) measured.

EXPERIMENTAL DESIGN — Within a given clone, aspen root-suckers were paired (severed or control treatments, 3 – 6 m apart) based on stem height, basal diameter, herbivore damage, and distance from forest edge. Each pair was then randomly assigned to either the severed or non-severed (control) treatment levels. The direction of the "parent" (distal) root was determined for each ramet by carefully excavating the surface litter and soil. The distal and proximal ends of the horizontal root associated with the ramet were then carefully excavated (**Figure 4.3, Figure 4.4**). Care was taken to minimize disturbance to the feeder and the main lateral roots. The horizontal root of non-severed (control) ramets was left intact. For severed treatment ramets, the horizontal root was cut 50 cm from the ramet base using pruning shears. The shear blades were dipped in 70% isopropyl alcohol after each cut to prevent contamination. A 15 x 50 cm section of plastic lawn edging was then placed on either end of the severed root sections to prevent clonal re-attachment (root grafting). The excavated soil and litter layers were then carefully replaced.

RAMET PRODUCTIVITY — The productivity of each ramet was determined by counting the total number of leaves, which provides a reliable estimate of total photosynthetic capacity. Leaf counts were made in the summer of 2003 (prior to experimental root severing) and again in the summer of 2004. The change in the number of leaves between 2003 and 2004 was used to quantify gain (increase in 2004) or loss (decrease in 2004) in ramet productivity resulting from the experimental manipulations.

SURVIVAL AND HEIGHT GROWTH DATA — A survival census (i.e. noting whether experimental ramets had died), and height measurements, were conducted annually from 2003 to 2008 (i.e. six years of data). The exception was Grasshopper Valley, where a prescribed burn (conducted May 5th, 2005) killed all the experimental ramets. One year later (summer of 2006), a new set of 60 ramets (regenerating root-suckers following the burn) was selected and enumerated. Height and survival data for the Grasshopper Valley site are therefore available for only three years (2006-2008).

STATISTICAL ANALYSES

RAMET PRODUCTIVITY — Changes in total leaf count between 2003 (prior to root severing) and 2004 (one year following root severing) was used to quantify productivity changes in the root-severed and control ramets. Analysis of covariance (ANCOVA), using a randomized block design (blocking by paired ramets: one control and one root-severed), was employed to test for statistical differences between the severed and control treatments (Zar 1999, Quinn and Keough 2002). Initial leaf count (2003, prior to severing) was used as the covariable. The analysis was performed on the 2004 leaf counts (i.e. one year following the severing treatment).

Differences in leaf count and stem heights for 2005 to 2008 were analyzed using paired t-tests (Legendre and Legendre 1998).

RELATIVE GROWTH RATES — Height measurements on all ramets were performed annually between 2003 and 2008. The relative growth rate (Hunt 1978) of each ramet was determined as:

$$\mathrm{RGR} = [\ \ln H_p - \ln H_o\ ] \,/\, [\ T_p - T_o\ ]$$

where H_o = height in year T_o (2003) and H_p = height in year T_p (2008 for surviving ramets; otherwise, the year the ramet was first recorded as dead). The two-sample t-test

(Legendre and Legendre 1998) was used to test for differences in the relative growth rates of control (non-severed) and treatment (root-severed) ramets.

SURVIVAL ANALYSIS — Survival analysis was used to examine time-dependent differences in mortality between severed and control ramets (Kaplan-Meier survival curves; Kaplan and Meier 1958). The study uses censored data (right-censoring method), since not all ramets had died by the end of the experiment. Results are expressed as the cumulative probability of survival [$S(t)$] over time, where $0 \leq S(t) < 1$. Differences in ramet mortality will result in survivorship curves that diverge over time, the significance of which is tested used the chi-squared log-rank statistic (Jager et al. 2008). This non-parametric test considers the entire survey period, not just differences in survivorship at a single arbitrary point in time. The method is considered a highly robust statistical test for censored survivorship data (Pyke and Thompson 1986, 1987). Survival analyses were conducted using the Survival Package in R 9.2.9 (R development Core Team 2009).

4.3 RESULTS

RAMET PRODUCTIVITY — At all sites, ramet productivity (leaf production) was adversely affected in the year following root severing (**Figure 4.5**). In the control ramets (excavated, but not root-severed), leaf counts were statistically similar in 2003 and 2004: Deep Lake, mean = 236 ± 30 to 245 ± 30; Baldy, mean = 182 ± 15 to 173 ± 14; Grasshopper, mean = 230 ± 20 to 244 ± 21. By contrast, the root-severed ramets showed a considerable decline leaf counts between 2003 and 2004: Deep Lake, mean = 225 ± 38 to 160 ± 28; Baldy, mean = 166 ± 14 to 112 ± 13; Grasshopper, 224 ± 16 to 208 ± 14. For all sites, the root-severed ramet leaf counts were significantly lower than those of control ramets (ANCOVA: **Table 4.3**). Differences in leaf production between control and root-severed treatments corresponded to the soil moisture gradient, being greatest at the driest site (Deep Lake: mean difference = 70), intermediate at the central site (Baldy: mean difference = 45) and least at the most mesic site (Grasshopper Valley: mean difference = 30); see **Figure 4.5**. These results suggest that physiological clonal integration may be more important to maintaining ramet productivity in more stressful environments.

RELATIVE GROWTH RATES — At all three sites, the relative (height) growth rates of control (non-severed) ramets were significantly greater than the root-severed ramet growth rates (**Figure 4.6**). The difference in mean growth rate was smallest for the driest site (Deep Lake: control = 0.048 cm cm^{-1} yr^{-1} *vs*. root-severed = 0.038 cm cm^{-1} yr^{-1}; t_{52} = -1.830, $P = 0.036$), intermediate for the central site (Baldy Lake: control = 0.088 cm cm^{-1} yr^{-1} *vs*. root-severed = 0.050 cm cm^{-1} yr^{-1}; t_{53} = -6.516, $P \leq 0.001$), and greatest for the most mesic site (Grasshopper Valley: control = 0.115 cm cm^{-1} yr^{-1} *vs*. root-severed = 0.059 cm cm^{-1} yr^{-1}; t_{50} = -9.637, $P \leq 0.001$). The results for the Grasshopper Valley site are not directly comparable to the other two sites, however, since they are based on year-old, vigorous post-fire root suckers that were measured over a shorter time period (2006-2008). At the other two sites, the enumerated ramets were not of post-fire origin and much older, and were measured over a longer time period (2003-2008).

For all three sites, the results indicate the importance of physiological integration (inter-ramet root connections) in aspen suckers; relative height growth rates are significantly reduced following the severing of lateral root connections. This result holds for both older, established ramets (Deep Lake and Baldy Lakes sites), as well as for newly established, vigorous post-fire ramets (Grasshopper Valley). The very large reduction in mean relative growth rate of root-severed ramets at the Grasshopper Valley site suggests that post-fire root suckers are especially dependent on physiological integration to obtain supplementary resources for rapid post-disturbance establishment.

SURVIVAL ANALYSIS — The survivorship results indicated that root-severing has less of an effect on ramet mortality that on productivity (number of leaves produced) and relative height growth rates (**Figure 4.7**). The driest site, Deep Lake, showed a significant time-dependent difference in survivorship between root-severed ramets and control (non-severed) ramets (χ_1^2 (log-rank) = 6.5, $P = 0.011$). Nearly half (47%) of root-severed ramets died over the five years of the study, compared to only 17% (5 of 30) of the control (non-severed) ramets. At the intermediate Baldy Lake site, nearly 37% (11 of 30) root-severed ramets died, compared to only 17% (5 of 30) of the control ramets over five years (χ_1^2 (log-rank) = 3.0, $P = 0.085$). Only two years of data (2006-2008) are available for the Grasshopper Valley site. During this time, 3 of 30 root-severed ramets died, compared to just one of the control (non-severed) ramets; this difference was not statistically significant (χ_1^2 (log-rank) = 1.1, $P = 0.299$).

4.4 DISCUSSION

This study provides empirical evidence for the benefits associated with maintaining functional physiological integration in young aspen ramets established along the leading edges of clones. In general, the performance of aspen ramets was adversely affected following the severing of root connections: leaf productivity, relative height growth rate, and survivorship were significantly reduced in most cases. A number of studies have reported significant decreases in the growth, productivity, and survivorship of clonal ramets following severing of inter-ramet connections (e.g. Hartnett and Bazzaz 1985; Wang et al. 2009; Chen et al. 2010; Xu et al. 2010; Rioloa et al. 2010). This suggests that physiological integration of shared resources among ramets enhances the invasive ability of clonal species (Pysek 1997; Otfinowski and Kenkel 2008). In aspen, functional root connections are thought to buffer ramets against adverse environmental conditions during forest invasion, and to give ramets a developmental advantage over seedlings of similar age (Barnes 1966; Schier and Zasada 1973; Frey et al. 2003). Root physiological integration also promotes the transduction of signaling hormones between mature stems and the lateral root system, initiating root suckering following canopy disturbance (Cumming et al. 2000). Other studies have directly demonstrated that clonal integration significantly enhances overall plant fitness (Amsberry et al. 2000).

Productivity and clonal integration

Leaf production in aspen ramets is regulated by total non-structural carbohydrate (TNC) reserves stored in the neighboring parental root system (Johansson 1993), as well as reserves stored in aerial stems (Landhäusser and Lieffer 2003). The TNC levels in roots at the beginning of the growing season strongly affect the initiation of new root-suckers, the growth of established stems, and annual leaf production. Levels of TNC fluctuate seasonally in response to variation in the supply and demand for carbon. Carbohydrate levels generally peak toward the end of the growing season, as root reserves are replenished from photosynthates contributed by ramets (Landhäusser and Lieffer 2003; Snedden et al. 2010).

My results indicate that annual leaf production is adversely, and significantly, affected by root severing. This reduction in leaf productivity was most pronounced at Deep Lake, the driest of my three study sites. Soil moisture stress results in decreased aspen photosynthetic rates by reducing leaf stomatal conductance (Lieffers et al. 2001), which in turn lowers the re-allocation of carbohydrates from ramets back to the roots. Root severing enhances this deficit, by limiting the mobilization of root resources in the spring during leaf flush (Snedden et al. 2010). Root severing also significantly reduced leaf production at the least moisture-stressed site (Grasshopper Valley), although the effect was less pronounced; this suggests that clonal integration is less critical in less stressful environments.

Experimental severing of lateral roots mimics natural root system fragmentation, which is known to impede ramet access to clonal resources (Zahner and Debyle 1965; Fraser et al. 2004). Given that the mobilization of carbohydrate reserves in spring is critical for leaf production (Snedden et al. 2010), it follows that any factor that isolates a ramet's root segment from the rest of the clone will reduce performance. In forest management of aspen stands, mechanical root damage has been found to increase both root-sucker initiation and total leaf area (Farmer 1962; Fraser et al. 2004). However, in these studies mechanical damage does not sever and isolate individual ramets from the lateral root system.

Effects on relative growth rates and survivorship

The relative height growth and survivorship of aspen ramets were both negatively impacted by root severing. It is well known that ramet growth in aspen is highly dependent on TNC mobilized from the parent root system (e.g. Schier and Zasada 1973). In this study, the root-severing treatment occurred in the early half of summer, when TNC root reserves are low compared to later in the growing season (Landhäusser and Lieffers 2003; Frey et al. 2003; Snedden et al. 2010). Root severing greatly reduces the supply of TNC root reserves, reducing ramet growth and leaf productivity in subsequent years. This reduction in productivity also limits the replenishment of TNC reserves to the remaining root system, which further reduces growth (DesRocher and Lieffers 2001; Landhäusser and Lieffers 2003).

Results from this study indicate that relative growth rates varied widely across sites. The mean relative height growth rate of non-severed ramets was lowest at the dryest site (Deep Lake), and highest at the wettest site (Grasshopper Valley). Interestingly, the reduction in growth rate following root severing showed the opposite trend: height growth was reduced by only 21% at Deep Lake, compared to 43% at Baldy Lake and 49% at Grasshopper Valley. The comparatively small reduction in growth at Deep Lake likely reflects the adverse conditions: growth is compromised as much by lack of moisture as by limited access to lateral root reserves. The large reduction in growth following root severing observed at Grasshopper Valley may reflect the relative youth, and the post-fire origin, of these ramets. It is known that young, actively growing aspen ramets are more dependent on the parental root system than are older ramets (Peterson and Peterson 1992).

Previous studies of root damage by fossorial mammals have found that the growth and survivorship of aspen ramets increases significantly following the experimental removal of these root herbivores (Cantor and Whitham 1989). This suggests that the loss of root biomass (DesRocher and Lieffers 2001), and/or the severing of connective root tissue, reduces the translocation of critical resources to ramets during the growing season (Frey et al. 2003).

Peltzer (2002) also found that the aspen ramet growth rates and survivorship declined following root severing, but concluded that this did not affect competitive interactions with interspecific neighbors. While interspecific interactions were not investigated in this study, my results clearly demonstrate that reductions in the relative performance of root-severed ramets are site-dependent: leaf productivity and survivorship (though not height growth) are more negatively affected as soil moisture stress increases.

The reduction in growth rate following root severing may increase a ramet's susceptibility to damaging agents (pests and pathogens), which may eventually lead to mortality (Worrall et al. 2008). The relationship between reduced growth rate and increased mortality is well established for trees (reviewed by Franklin et al. 1987). My results are consistent with this relationship: the loss of leaf area (reduced leaf count) following the severing treatment results in a systematic reduction in relative growth rate, which in turn compromises ramet survivorship.

Costs and Benefits of Clonal Integration

The experimental severing of aspen roots mimics nature, since inter-ramet connections are normally lost as a stand ages (Sandberg 1951). Although natural root grafting may occur in older clones (Jelínková et al. 2009), ramets typically become increasingly isolated over time and may produce a clonal “genetic mosaic” as the stand ages (Ally et al. 2008). Results from my study indicate that physiological integration is beneficial to the growth and survivorship of regenerating and encroaching aspen ramets. My results also suggest that the benefits of physiological integration are greatest in more stressful environments, and in recently established post-fire ramets. As an aspen clone ages, the adaptive advantage of clonal integration may be lost. Indeed, the natural disintegration of larger clones into smaller subunits has been posited as a strategy to minimize the contagion of damaging agents (Stuefer 2004 et al.; Hay and Kelly 2008), thus ensuring persistence of the clone.

4.5 ACKNOWLEDGEMENTS

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant to N. C. Kenkel, Parks Canada and a University of Manitoba's Duff Roblin Graduate Fellowship to R. A. Lastra. I would like to thank Wybo Vanderschuit and Dr. John Wilmshurst for help with various aspects of this study as well as Kelly Orr.

4.6 REFERENCES

Ally, D., K. Ritland, and S.P. Otto. 2008. Can clone size serve as a proxy for clone age? An exploration using microsatellite divergence in *Populus tremuloides*. Molecular Evolution 17:4887-4911.

Amberry, L., M.A. Baker, P.J. Ewanchuk, and M.D. Bertness. 2000. Clonal integration and the expansion of *Phragmites australis*. Ecological Applications 10:1100-1118.

Barnes, B.V. 1966. The clonal growth habit of American aspens. Ecology 47:439-447.

Bouycous, G.J. 1951. A recalibration of the hydrometer method for making mechanical analysis of soil. Agronomy Journal. 43:434-438.

Caldwell, M.M. and R.W. Pearcy (eds) 1994. Exploitation of environmental heterogeneity by plants. Academic Press, Inc. San Diego.

Cantor, L.E., and T.G. Whitman 1989. Importance of belowground herbivory: pocket gophers may limit aspen to rock outcrop refugia. Ecology 70:962-970.

Chen Jin-Song, L. Ning-Fei, D. Ming. 2010. Clonal integration improves the tolerance of *Carex praeclara* to sand burial by compensatory response. Acta Oecologica 36:23-28

Chesson, P., and A.G. Peterson. 2002. The quantitative assessment of the benefits of physiological integration in clonal plants. Evolutionary Ecology Research 4:1153-1176.

Charpentier, F. Mesleard, and J.D. Thompson. 1998. The effects of rhizome severing on the clonal growth and clonal architecture of *Scirpus maritimus*. Oikos 83:107-116.

Day, M.W., 1944. The root system of aspen. American Midland Naturalist 32:502-509.

DesRocher, A. and V.J. Lieffers 2001. Root biomass of regenerating aspen (*Populus tremuloides*) stands of different densities in Alberta. Canadian Journal of Forest Research 31:1012-1018.

DesRocher, A. and V.J. Lieffers 2001. Course root system of mature aspen (*Populus tremuloides*) in declining stands in Albereta, Canada. Journal of Vegetation Science 12: 355-360.

Farmer, R.E. 1962. Aspen root sucker formation and apical dominance. Forest Science 8:403-410.

Franklin, J.F. , H.H. Shugart, M.E. Harmon. 1987. Tree death as an ecological process. Bioscience 37:550-556.

Fraser, E.C., V.J. Lieffers and S.M. Landhäusser. 2004. Wounding of aspen roots promotes suckering. Canadian Journal of Botany 82:310-315.

Frey, B.R., V.J. Lieffers, S.M. Landhäusser, P.G. Comeau, and K.J. Greenway. 2003. An analysis of sucker regeneration of trembling aspen. Canadian Journal of Forest Research 33:1169-1179.

Gifford, F. G. 1966. Aspen studies on three sites in Northern Utah. American Midland Naturalist 75:132-133.

Gómez, S., V. Latzel, Y. M. Verhurst, J.F. Stuefer. 2007. Costs and benefits of induced resistence in a clonal plant network. Oecologia 153:921-930.

Harper, J.L. 1977. Population biology of plants. Academic Press, New York.

Hartnett, D.C., and F.A. Bazzaz. 1985. The integration of neighborhood effects by clonal genets of *Solidago canadensis*. Journal of Ecology 73:415-428.

Hay, M.J.M., and C.K. Kelly. 2008. Have clonal plant biologists got it wrong? The case for changing the emphasis to disintegration. Evolutionary Ecology 22:461-465.

Hutchings, M.J., D.K. Wijesinghe. 2008. Performance of a clonal species in patchy environments: effects of environmental context on yield at local and whole-plant scales. Evolutionary Ecology 22:313-324.

Jelínková H., T. Francine, and A. DesRochers. 2009. Molecular and dendrochronological anaylsis of natural root grafting in *Populus tremuloides* (Salicaceae). American Journal of Botany 96:1500-1505.

Johansson, T. 1993. Seasonal changes in contents of root starch and soluble carbohydrates in 4-6 year old *Betula pubescens* and *Populus tremula.* Scandinavian Journal of Forest Research 8:94-106.

Kaplan, E.L. and P. Meier. 1958. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 53:457-481.

Landhäusser, S.M., and V.J. Lieffers. 2003. Seasonal changes in carbohydrates reserves in mature northern *Populus tremuloides* clones. Trees 17:471-476.

Lei, S.A. 2010. Benefits and costs of vegetative and sexual reproduction in perennial plants: A review of the literature. Journal of the Arizona-Neveda Acadamy of Science 42: 9-14.

Legendre, P. and L. Legendre. 1998. Numerical Ecology. 2nd edition. Elsevier, Amsterdam. 853 pages.

Lieffers, V.J., S.M. Landhäusser, and E.H. Hogg. 2001. Is the wide spread distribution of aspen a result of its stress tolerance. USDA Firest Service Proceedings RMRS-P-18.

Matsushita, M., N. Tomaru, D. Hoshino, N. Nishimura, and S-I. Yamamoto. 2010. Factors affecting the production, growth, and survival of sprouting stems in the multi-stemmed understory shrub *Lindera tribola.* Botany 88:174-184.

Otfinowski, R., and N.C. Kenkel. 2008. Clonal integration facilitates the proliferation of smooth brome clones invading northern fescue prairies. Plant Ecology 199:235-242.

Peltzer, D.A. 2002. Does Clonal integration improve competitive ability? A test using aspen (*Populus tremuloides* [Salicaceae]) invasion into prairie. American Journal of Botany 89:494-499.

Pennings, S.C., and R.M. Callaway. 2000. The advantage of clonal integration under different ecological conditions: A community-wide test. Ecology 81:709-716.

Peterson, E.B., and N.M. Peterson. 1992. Ecology, management and use of aspen and balsam poplar in the prairie provinces. Forest Canada Northwest Region Report No. 1, Edmonton, 252 pages.

Pitelka, L.F., and J.W. Ashmum. 1985. Physiology and integration of ramets in clonal plants.- In: Jackson, J.B.C., L.W. Buss, and R.E. Cook (eds), Population and evolution of clonal organisms. Ytale University Press, New Haven, 399-435 pages.

Poor, A., C. Herchock, K. Rosella, and D.E. Goldberg. 2005. Do physiological integration and soil heterogeneity influence the clonal growth and forage of *Schoenoplectus pungens*. Plant Ecology 181:45-56.

Pyke, D.A and J.N. Thompson. 1986. Statistical analysis of survival and removal rate experiments. Ecology 67:240-245.

Pyke, D.A and J.N. Thompson. 1987. *Erratum*. Ecology 68:232.

Pysek, P. 1997. Clonality and plant invasion: can a trait make a difference? In de Kroon H. J. van Groenendal (eds) The ecology and evolution of clonal plants. Backhuys Publishing, Leiden.405-427 pages.

Quinn, G.P., and M.J. Keough. 2002. Experimental design and data analysis for biologists. Sixth edition. Cambridge University Press. 537 pages.

Qureshi, F.A., and D.C. Spanner. 1971. Unidirectional movement of tracers along the stolon of *Saxifraga sarmentosa.* Planta 101:133-146.

R Development Core Team. 2009. R: a language and environment for statistical computing. Version 2.9.2. R foundation for Statistical Computing, Vienna, Australia. http://www.R-project.org

Renkema, K.N. S.M. Landhäusser, and V.J. Lieffers. 2009. Suckering response of aspen to traffic-induced-root wounding and the barrier-effect of log storage. Forest Ecology and Managament 258:2083-2089.

Roiloa, S. R., S. Rodríguez-Echeverría, E de la Peña, and H. Freitas. 2010. Physiological integration increases survival and growth of the clonal invader *Carpobrotus edulis*. Biological Invasions 12:1815-1823.

Sandberg, D. 1951. The regeneration of quaking aspen by root suckering. M.Sc. thesis, School of Forestry, University of Minnesota.

Schier, G.A. and J.C. Zasada. 1973. Role of carbohydrate reserve in the development of root suckers in *Populus tremuloides*. Canadian Journal of Forest Research 3:242-250.

Stuefer, J.F. 1997. Division of labour in clonal plants? On the response of stoloniferous herbs to environmental heterogeneity to environmental heterogeneity. PhD thesis. Utrecht University, Utrecht.

Stuefer, J.F., S. Gómez and T. Van Mölken. 2004. Clonal Integration beyond resource sharing: implications for defense signaling and disease transmission in clonal plant networks. Evolutionary Ecology 18:647-667.

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

Soil Classification Working Group. 1998. The Canadian System of Soil Classification. Agric. and Agri-Food Canada. Publication 1646. 187 pages

Trottier, G. C. 1986. Distribution of rough fescue, *Festuca hallii*, grassland by livestock grazing in Riding Mountain National Park, Manitoba. Canadian Field-Naturalist 100:488-495.

Wang N., F. Yu, P.X. Li, W.M. He, J. Liu, G.L. Yu, Y.B. Song, and M. Dong. 2009. Clonal integration supports the expansion from terrestrial to aquatic environments of the amphibious stoloniferous herb *Alternanthera philoxeroidses*. Plant Biology 11:483-489.

Wiehle, M., P. Eusemann, N. Thevs, and M. Schnittler. 2009. Root suckering in *Populus euphratica* (Euphrates poplar, Salicaceae). Trees 23:991-1001.

Worrall, J.J., L. Egeland, T. Eager, R.A. Mask, E.W. Johnson, P.A. Kemp, and W.D. Sheppard. 2008. Rapid mortality of *Populus tremuloides* in southwestern Colorado, USA. Forest Ecology and Management 255:686-696.

Xu, Cheng-Yuan, S.Schooler, and R.D.Van Klinken 2010. Effects of clonal integration and light availability on the growth and physiology of two invasive herbs. Journal of Ecology 98:833-844.

Zahner, R., and N.V. DeByle. 1965. Effect of pruning the parent root on growth of aspen suckers. Ecology 46: 373-375.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, New Jersey.

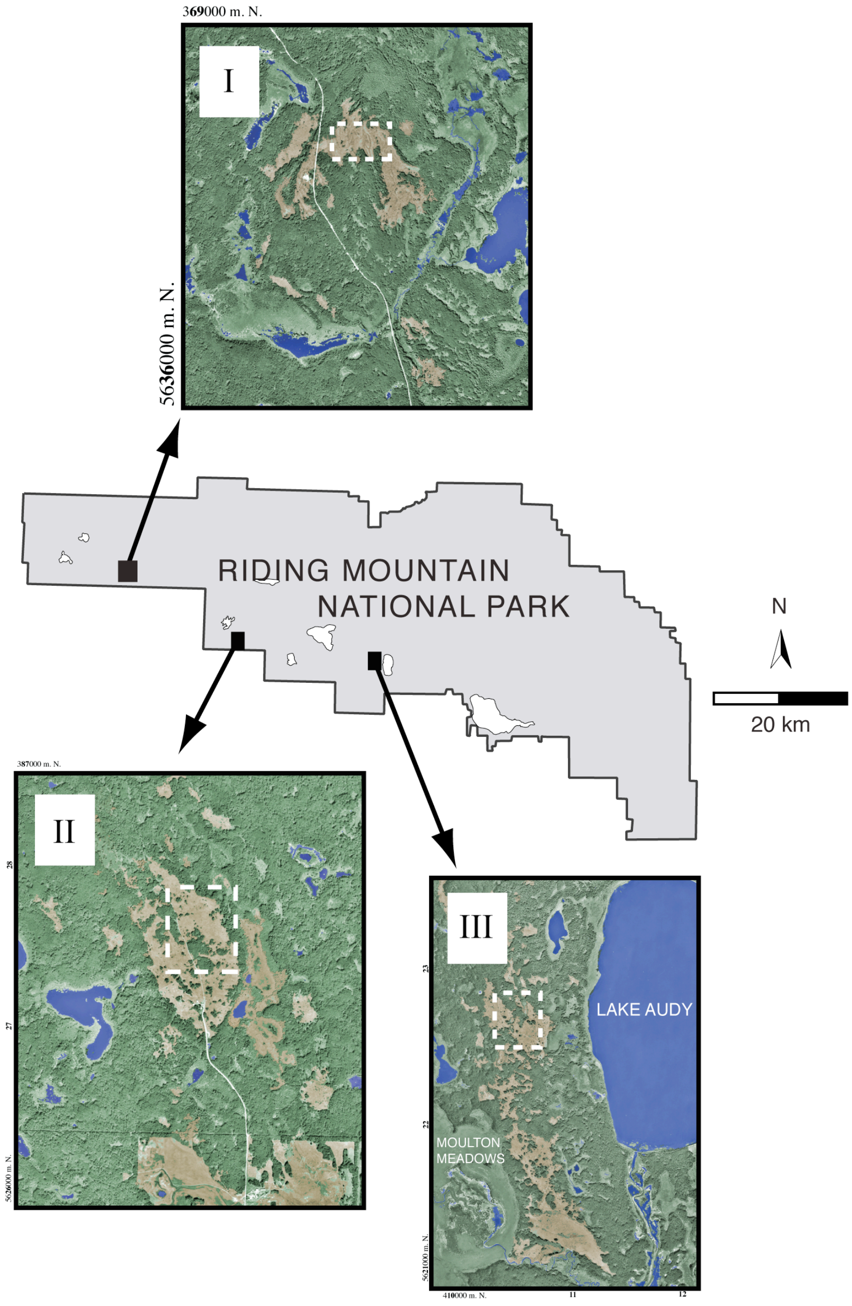


Fig. 4.1. Distribution of the three study locations (Deep Lake (I), Baldy Lake (II), Grasshopper Valley (III)) in Riding Mountain National Park, Manitoba, Canada.

Table 4.1. Summary of soil and topographic characteristics of each study site in Riding Mountain National Park, Manitoba Canada. Soil moisture classes were assigned based local drainage, soil texture and parent material. Soil profile information were determined from 1 m deep soil pits. Percent Slope was determined for each clone; aspect refers to the cardinal direction (where w = west) of the forest edge where treatment and control stem were located.

SITE	CLONE SIZE (ha)	% SLOPE	ASPECT	SURFICIAL DEPOSITS	SOIL TEXTURE	STONINESS	SOIL PROFILE NOTES
I - Deep Lake	0.15 0.08 0.04	15 35 45	W W S	Alluvium and outwash deposits	Loamy Sand Sandy Loam Sandy Loam	Very Stony Moderately Stony Moderately Stony	A Horizon: Dark grey sandy loam, < 3 cm thick C Horizon: Light grey, coarse sand and gravel
II - Baldy Meadow	0.12 0.09 0.04	5 2 5	S N NE	Alluvium and outwash deposits	Sandy Loam Sandy Loam Sandy Loam	Moderately Stony Moderately Stony Moderately Stony	A Horizon: Dark grey sandy loam, > 10 cm thick C Horizon: Light grey, coarse sand and gravel
III - Grasshopper Valley	0.19 0.08 0.06	2 2 2	N N E	Glaciolacustrine deposits	Sandy Clay Loam Sandy Clay Loam Sandy Clay Loam	Slightly Stony None None	A Horizon: V. Dark grey loam, > 18 cm thick C Horizon: Grey clay loam, iron stained and mottled

Table 4.2. Summary of severed treatment experiment (RMNP).

Clone	**UTM Location (Nad 87 Zone14)**	**Number of stems (treatment + control)**
	BALDY LAKE 1	
I	387,430m E; 5,626,905m N	20
II	387,495m E; 5,627,276m N	20
III	387,439m E; 5,627,413m N	20
	DEEP LAKE PRAIRIE	
IV	369,856m E; 5,638,249m N	20
V	369,851m E; 5,638,216m N	20
VI	370,095m E; 5,638,266m N	20
	GRASSHOPPER VALLEY	
VII	410,080m E; 5,622,351m N	20
VIII	410,501m E; 5,622,555m N	20
IX	410,554m E; 5,621,544m N	20
		180

Fig. 4.2. Topographic variation between the A) Deep Lake (dry parkland savanna) and B) Grasshopper valley (mesic parkland savanna).

Fig. 4.3. Excavated and severed lateral root of a *Populus tremuloides* root-sucker.

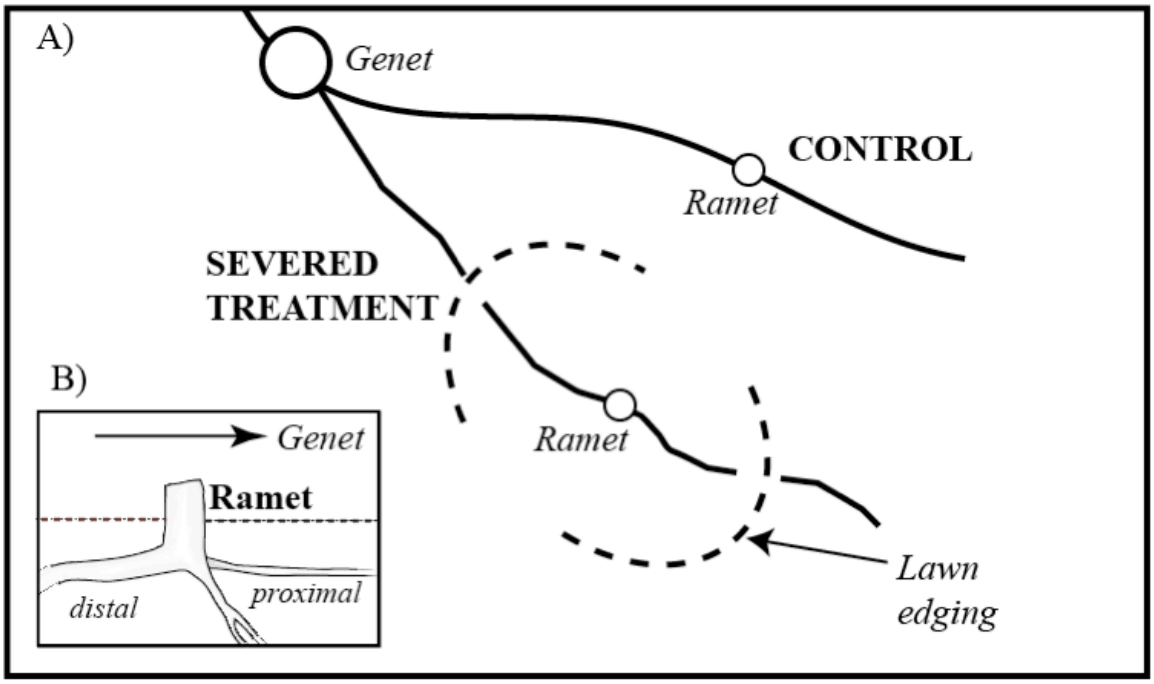


Figure 4.4. A) Illustration of experimental design; B) Schematic of ramet and horizontal root system depicting the orientation of distal (parental) and proximal roots with respect to the parent (genet) stem.

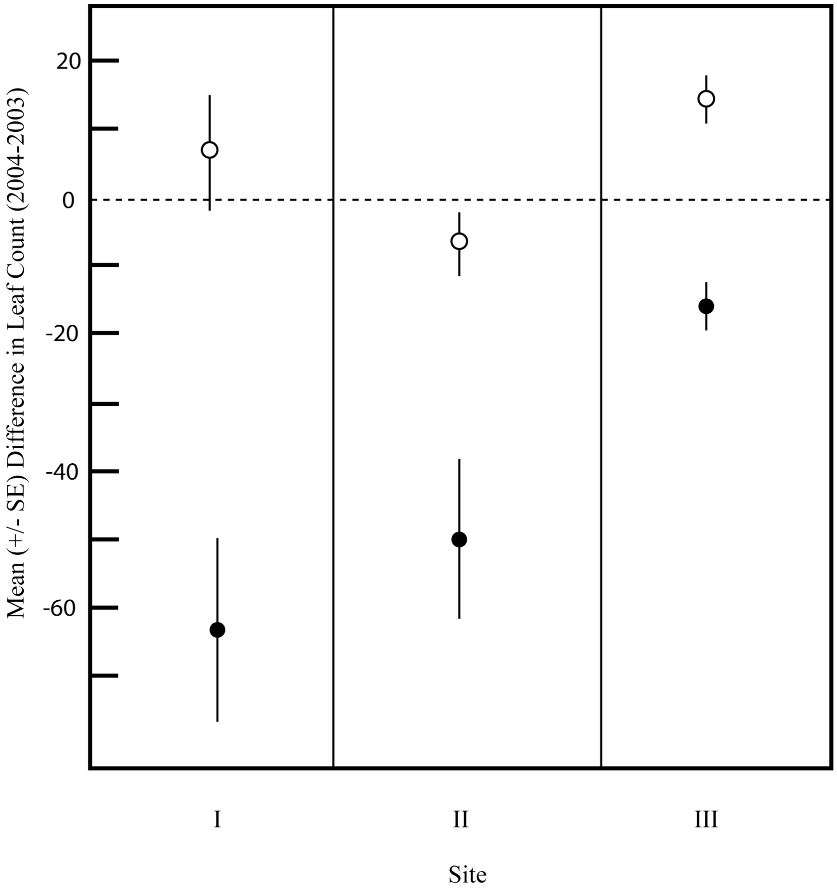


Fig. 4.5. Mean difference in leaf count for severing and control treatments. All treatments were significantly different (ANCOVA: $P < 0.0001$). Open circles represent control, closed circles represent severed treatments.

Table 4.3. Analysis of covariance (ANCOVA) for the effect of aspen clonal root severing on the difference in leaf count between 2003 and 2004.

Source	*df*	Difference in Leaf Count	
		MS	*F*
Deep lake (I)			
Treatment	1	71879.7	36.12 ***
Error	22	1990.07	
Baldy (II)			
Treatment	1	22819.1	24.2 ***
Error	20	942.94	
Grasshopper Valley (III)			
Treatment	1	13242	28.5 ***
Error	27	383.70	

*** $P < 0.0001$

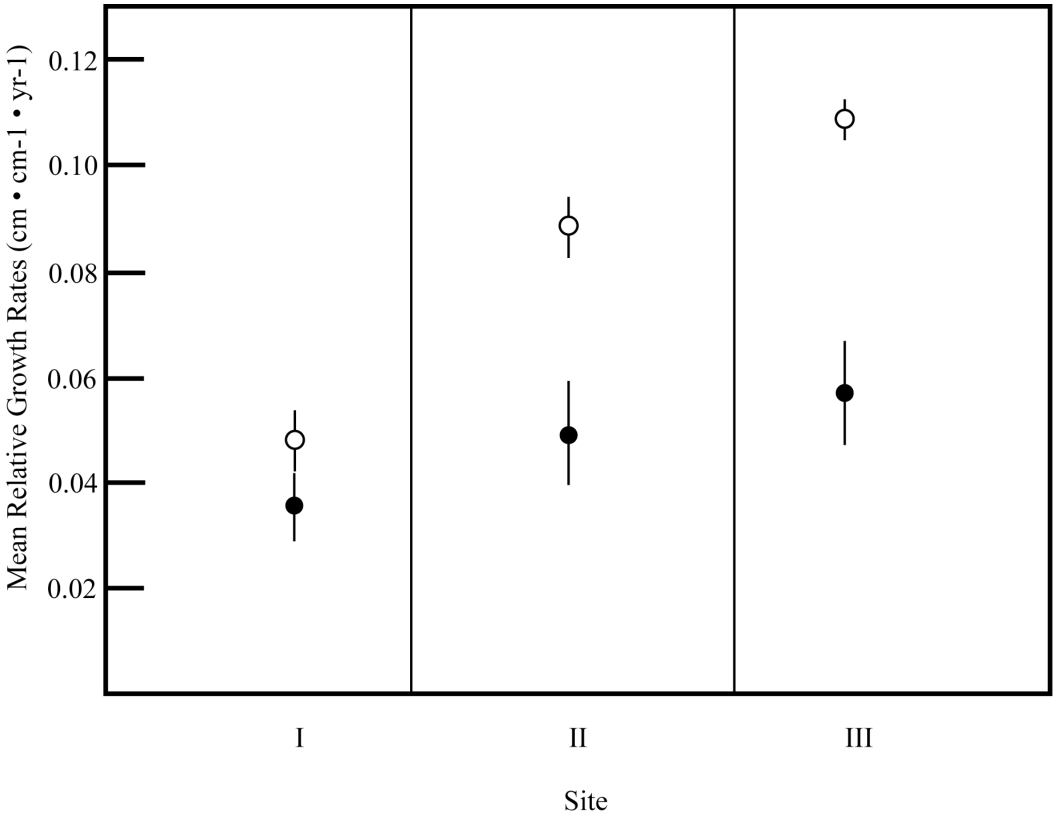


Fig. 4.6. Mean relative height growth (cm/cm/yr) for severed (closed circles) and non-severed (open circles) aspen ramets. results are presented with respect to study sites, ordered from dry to mesic parkland savanna. Where Site 1 = Deep Lake, Site 2 = Baldy Lake, and Site 3 = Grasshopper Valley. Differences in mean RGR between the severed treatment and control were significant across the three sites (two sample t-test: site I, $P = 0.0364$, sites II and III, $P \leq 0.0001$).

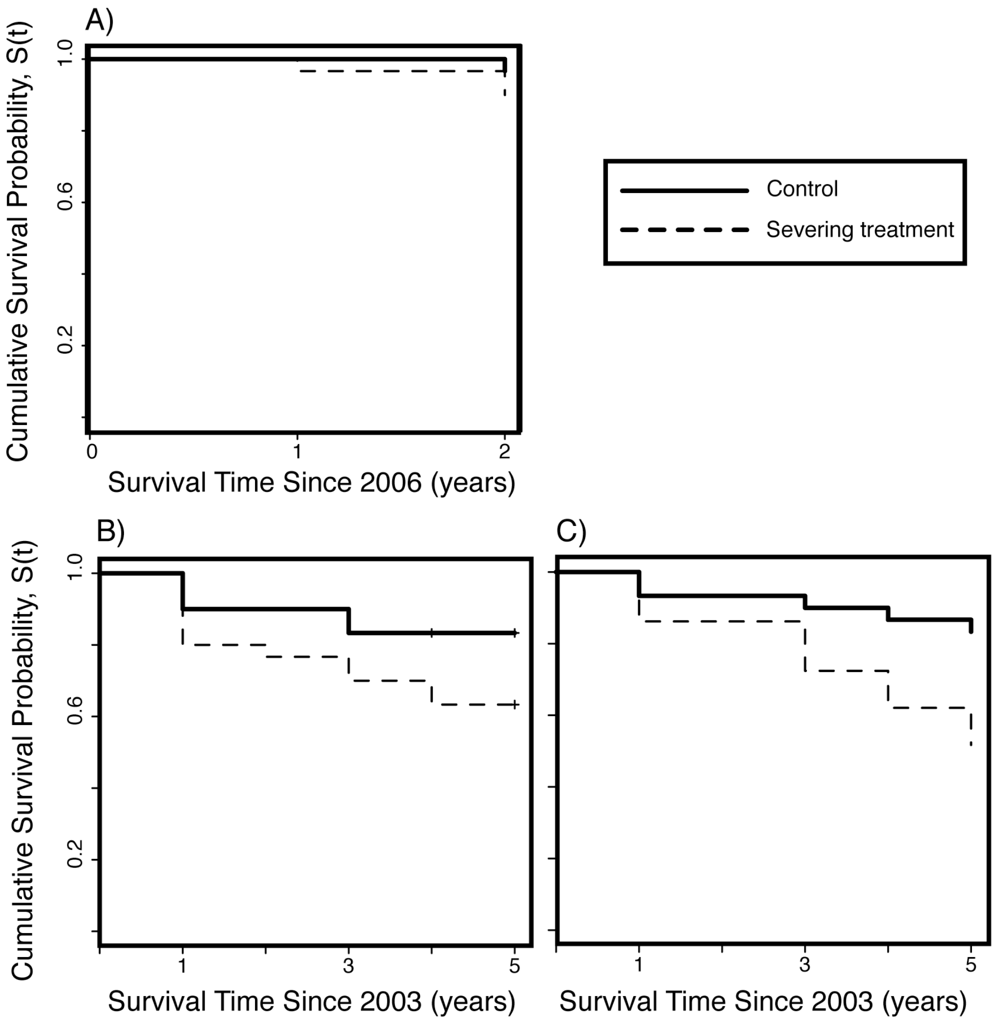


Fig. 4.7. Survival curves (Kaplan-Meier [K-M] survival curve, S(t)) as a function of treatment level (severed and non-severed (control) ramets). Study was conducted for from 2003 to 2008, except in Grasshopper Valley where mortality data was collected between 2006 to 2008 (Prescribe burn in 2005 destroyed the initial samples). A) survival curve for Grasshopper Valley (2-yrs). There was no significant difference between survival rate of controls (1/30 dead) and severed (3/30 dead) ramets (c2(log-rank) = 1.1 df = 1, P = 0.299). B) survival curve for Baldy Lake (5-yrs). There was no significant difference between survival rate of controls (5/30 dead) and severed (11/30 dead) ramets (c2(log-rank) = 3 df = 1, *P* = 0.0847). C) Deep Lake (5-yrs). There was a significant difference between survival rate of controls (5/30 dead) and severed (14/30 dead) ramets (c2(log-rank) = 6.5 df = 1, *P* = 0.0109).

CHAPTER 5

INTRASPECIFIC VARIATION OF PHENOLIC GLYCOSIDES AND ITS EFFECTS ON INDIVIDUAL RESISTANCE, AND SUSCEPTIBILITY OF *POPULUS TREMULOIDES*

5.1 INTRODUCTION

Unraveling the factors that influence the growth and survivorship of a species is critical to advancing a biological understanding of population dynamics (Schoener 1971; May 1974; Mueller-Dombois 1987). Classical ecological models are founded on a theoretical framework in which site contingencies (e.g., the chance occurrence of an event that has a cascading effect on a system), environmental factors (e.g., niche models), and biotic interactions (e.g., competition models) are the principal drivers affecting long-term species dynamics. These models consider the mean phenotypic response of a species, while ignoring or treating as “noise” any variation about the mean (species as “invariant entities”, Hubbell 2001: 328). Such an approach is justifiable given that the scale of most ecological theory ranges from the species to ecosystems levels of biological organization. As Clark et al. (2003:17) note:

> “*Individual variation violates the assumptions of most ecological theory and almost all classical statistical models used to test it*”.

While such models provide important insights regarding the deterministic effects of biological and environmental interactions on species growth and survivorship (e.g. niche-based theory), it is increasingly recognized that the outcome of such interactions also depends on a critical axiom of biology: the importance of variation in ecologically relevant traits among individuals of a species (Scheiner and Willig 2008). As a consequence, recent research has focused on the ecological implications of phenotypic variation among individuals, and on the role of phenotypic variation in determining model outcomes (Clark et al. 2003; Kotowska et al. 2010).

Numerous environmental factors affect the growth, demographics and mortality of trembling aspen (Bartos and Campbell 1998; Frey et al. 2004; Brown et al. 2006). Population-level, synchronous trembling aspen dieback has been observed throughout its distributional range, but especially at the northern edge of the Great Plains in Canada (Hogg et al. 2008) and in the western United States (Shepperd et al. 2006). Many researchers have proposed that prolonged and severe drought is the main environmental factor leading to dieback (e.g. Bailey and Wroe 1974; Sexton et al. 2006; Hogg et al. 2008; Leonelli et al. 2008; Thibault and Brown 2008), especially when coupled with mammalian herbivory and defoliating insect outbreaks (Cooke and Roland 2007; Worrall et al. 2008). Severe drought is an extreme environmental event that adversely affects all members of a population once a critical biological threshold is crossed (Denny et al. 2009). Such extreme events are particularly devastating to populations at or near the periphery of a species' distributional range (Bridle et al. 2010).

Over much of the central (i.e. non-peripheral) range of trembling aspen, the synchronous mortality events ("diebacks") characteristic of peripheral populations are relatively uncommon; instead, highly asynchronous and spatially variable patterns of density-independent mortality are typical. These variable mortality patterns are attributable in part to corresponding variation in ecologically relevant species traits; the environmental-biological determinacy of niche-based modeling cannot fully characterize (nor predict) spatial-temporal variation in ecological pattern (MacAurthur 1972; Brown 1995). Although intra-specific variation in trembling aspen traits is well documented (e.g., Barnes 1969, 1975; Namroud et al. 2006), the effects of such variation on stand structure, dynamics, and persistence are often overlooked (Kanaga et al. 2008). Trembling aspen stands show considerable variation in clone size, growth rate, morphology (e.g. branching pattern, leaf shape), phenology, and overall vigour or "health" (Shier and Campbell 1980; Shields and Bockheim 1981; Suzuki et al. 1999; Kashian et al. 2007), even in areas where differences in the physical environment are negligible (Megan et al. 2008). This variation is a reflection of the relatively high genetic diversity of trembling aspen (Stevens et al. 1999; Mock et al. 2008), and may explain its ability to tolerate a wide variety of habitats (Yeh et al. 1995) and its broad eco-geographic range (Jelinski and Cheliak 1992).

It is well established that variation in plant resistance traits confers population-level protection against herbivores, pests and pathogens (e.g. Browning and Frey 1969; Dimock et al. 1976; Lawler et al. 1998; Mousseau et al. 2000; O'Reilly-Wapstra et al. 2004, 2007). This phenotypic variation also affects ecological interactions, which in turn determine species recruitment and mortality patterns (Richards et al. 2005). A critically important resistance trait in most plant species is the production of physiologically active secondary metabolites (Camp 1949; Harborne 1994). The "raison d'être" of plant secondary metabolites (Fraenkel 1959) extends well beyond the co-evolutionary implications of insect-plant interactions (Ehrlich and Raven 1964); they play an important ecological role as allelopathic agents (Whittaker and Feeny 1971), in alleviating photo-damage (Close and McArthur 2002), and especially in “defending” plants against herbivores, insect pests and pathogens (reviewed in Harborne 1994; Hartmann 2007).

In many tree species, it has been found that a higher concentration of secondary metabolites results in increased resistance to herbivores, pests and/or pathogens (e.g. *Pseudotsuga:* Midock et al. 1976; *Betula*: Palo 1985, Reichardt et al. 1984; *Pinus*: Snyder 1992, Rehill et al. 2006, O’Reilly-Wapstra and Iason 2007; *Eucalyptus*: Lawler et al. 1998, O’Reilly-Wapstra et al. 2002, 2004, Moore and Foley 2005, Bergvall and Leimer 2005). Within the Salicaceae (Willow) Family, considerable intra-specific variability in the production of phenolic metabolites (Palo 1984) has been reported (*Salix*: Roche and Fritz 1997, Fields and Orion 2006, Stolter 2008; *Populus*: Whitham et al 1996, Donaldson et al. 2005, Osier and Lindroth 2001, 2006, Diner et al. 2009, Häikiö et al. 2009). In trembling aspen, the production of secondary phenolics (specifically coniferyl benzoate, condensed tannins, and phenolic glycosides) shows considerable intra-specific variation (see review by Philippe and Bohlman 2007). Ecologically, the most important compounds in trembling aspen are the phenolic glycosides (e.g. tremulacin); these are broad-spectrum chemical deterrents against mammalian herbivores (Hemming and Lindroth 1999; Wooley et al. 2008; Diner et al. 2009), insect pests (Osier and Lindroth 2001; Donaldson and Lindroth 2008), and fungal pathogens (Holeski et al. 2009). The production of these compounds is regulated by the modifying influence of external (environmental) factors acting on inherent (constituent or genetic) differences among individuals (Stevens and Lindroth 2005). Regardless of the source of intra-specific variation (i.e. whether inherent or environmentally induced), functional differences in ecologically relevant plant resistance traits may have important implications on population structure and dynamics (Agrawal et al. 1999).

The objective of this study is to determine the extent to which intra-specific variation in levels of phenolic metabolites can explain differences in growth and survival among individuals (clones) of trembling aspen. I hypothesize that a lower foliar concentration of phenolic metabolites predisposes an individual to chronic stress (*sensu* Franklin et al. 1987), resulting in differential mortality within populations. Numerous studies have demonstrated that phenolic glycoside levels vary greatly among individuals, but this variation has not been directly linked to intra-specific differences in resistance, susceptibility or survival. In this study, I forge a direct link between secondary metabolite production and individual performance by addressing and answering the following questions: [1] To what extent do differences in environmental (soil moisture) stress affect phenolic glycoside production in aspen clones?; [2] How strong is the relationship between phenolic glycoside production and vigour ("health") among individual clones?; [3] To what extent do "dieback" and "vigorous" clones differ in their productivity, and does this difference suggest a tradeoff between growth and biochemical defense?; [4] Is the phenolic glycoside concentration of an aspen ramet a useful predictor of its probability of mortality?

5.2 METHODS AND MATERIALS

STUDY SITE

This study was conducted in three forest-grassland ecotone sites located in the west-central region of Riding Mountain National Park (RMNP), Manitoba, Canada (**Fig. 5.1**). The Park constitutes the extreme southeast region of the Mixed-wood Section of the Boreal Forest Region (B18a; Rowe 1972). The study area is dominated by mature and regenerating stands of trembling aspen (*Populus tremuloides*) interspersed with wetlands and northern fescue prairie grasslands (Trottier 1986). The regional climate is characterized by warm, short summers and long, cold winters. Data from the nearby town of Wasagaming (29-year means, 1971-2000; Environment Canada 2009) provides a climatic summary. Mean total annual precipitation is approximately 400 mm, and the two wettest months are June (mean = 84.5 mm) and July (mean = 75.3 mm). The mean annual temperature is 0.1^{o}C, with mean monthly temperatures varying from –19.6^{o}C in January to 16.5^{o}C in July.

ASSESSING AMONG-SITE DIFFERENCES — To test the hypothesis that the allocation of resources toward secondary metabolites (defensive compounds) in aspen is greater in more stressful environments, I sampled individual clones from three study sites along a soil moisture "stress" gradient (**Table 5.1**). The sites, each approximately 20 km apart, occurred along a gradient of increasing soil moisture stress from east to west. The easternmost site (III = Grasshopper Valley) is topographically flat with moderately drained, loamy soils. The central site (II = Baldy Lake) has a gently rolling topography with well-drained, somewhat gravelly sandy-loam soils. The westernmost site (I = Deep Lake) has a steeply rolling topography with excessive drained, gravelly-stony sandy soils. At each site, soil moisture content in late summer was determined at the edge of each of four aspen clones (8 replicates per clone), using a time-domain reflector (Hydrosense, Campbell Scientific Inc., Logan UT). Measurements were based on *in-situ* incubation of surface mineral soil (0 – 10 cm depth). Measurements were made over two sampling periods (August 5 – 8, 2004 and August 12 - 16, 2006), which were preceded by at least one week without rain. Values for the two sampling periods were not significantly different and therefore pooled. Percent volumetric soil moisture was significantly different among sites ($F_{2,93} = 31.2$, $P < 0.001$): site I = 5.8 ± 1.98%, site II = 8.7 ± 1.82%, site III = 10.3 ± 2.92%, (**Table 5.1**).

Assessing intraspecific variation in clone health — To test the hypothesis that intra-specific variation in phenolic glycoside production accounts for differences in vigor or "health" among aspen clones, I enumerated four similar-sized "vigorous" and four "dieback" clones at each of the three study sites ([4 + 4] x 3 = 24 clones in total). All vigorous and dieback clones were spatially paired (> 50 m apart, but < 200 m apart) to minimize variation in edaphic and microclimatic conditions across replicate clones. Aspen clones were classified as either "vigorous" or "dieback" using a set of clearly-defined visual criteria (Figure 5.2):

Vigorous Clones: These clones have low mortality of mature stems (i.e. few "snags"). The smaller stems (ramets < 2 m tall) are not (or only lightly) browsed by mammals, and show limited pest and/or pathogen damage. The physiognomic profile of vigorous clones is “dome-shaped”; this is consistent with continuous recruitment of ramets.

Dieback Clones: These clones have moderate to high mortality of mature stems (i.e. numerous “snags”). The smaller stems (ramets < 2 m tall) show signs of heavy browsing by mammals, as well as pest and/or pathogen damage. The physiognomic profile of dieback clones is “stair-step” shaped; this is consistent with discontinuous recruitment of ramets and/or vertical suppression of intermediate age classes.

IDENTIFICATION OF ASPEN GENOTYPES —Trembling aspen is a clonal tree species, capable of producing thousands of genetically identical “trees” (ramets; Maini 1960). In any experimental investigation, it is critically important to ensure that the enumerated clones are distinct individuals, i.e. that each experimental replicate is genetically distinct. In the absence of genetic data, I used three methods to recognize individual clones:

(1) Spatially isolated aspen clones were sampled; clones within or immediately adjacent to the continuous forest matrix were avoided. This proved quite straightforward, since the three selected study sites were northern fescue grasslands consisting of isolated forest patches (aspen groves) of varying sizes within a continuous prairie-shrub matrix. I selected only relatively small (< 200 m^2), dome-shaped forest patches to increase the likelihood that they were genetically distinct individuals (Maini 1960).

(2) Selected clones were ≥ 50 m from any adjacent clone, and the grassland surrounding the selected clone (the ≥ 50 m “buffer zone”) was entirely free of aspen regeneration.

(3) I used phenotypic cues to confirm clonal uniformity, i.e. to ensure that all ramets in my selected clones were genetically identical. For each clone, flowers from individual

ramets were examined in spring to ensure that they were all the same sex. I also used leaf phenology as an indicator of clonal uniformity: ramets (stems) of a given clone have identical leaf phenology, but there is considerable inter-clonal variation in the timing of spring bud break and leaf-out, and in the timing of autumn coloration and leaf-fall (Maini 1960). Foresters have long used phenology as a reliable indicator of the extent of aspen clones within forest stands; adjacent stems showing simultaneous leaf-out (and/or leaf-fall) are deemed to be ramets from a single clone. A recent study noted a near one-to-one correspondence in aspen clone identification using leaf phenology versus genetic markers (Diner et al. 2009), confirming the reliability of the leaf phenology approach. In this study, I made careful observations of leaf phenology for all 24 clones, in both May (leaf-out) and September (leaf-fall), to ensure clonal uniformity. In all cases, all stems in selected study clones had identical leaf phenology, confirming that they are from the same individual.

assessing growth and mortality — to test the hypothesis that variation in phenolic glycoside concentration (a resistance trait) can influence population demographics, i undertook a repeated measure study of ramet growth and mortality within each of the 24 study clones. in june 2004 i permanently tagged eight random selected young ramets (< 1.5 m in height) within each of the 24 study clones, and enumerated the 192 ramets twice yearly (june and september) over four years (2004 to 2007). the following measurements were made on each ramet: growth status (live vs. dead), stem height and basal diameter (to nearest cm), and number of branch nodes. in addition, ungulate browsing severity was computed as the ratio of browse-damaged to total branches per ramet, expressed as a percentage.

Variation in ramet physiognomy (branch structure and crown architecture) was examined by enumerating actively growing, mature ramets (ranging in age from 8 – 19 years) from four “vigorous” and four “dieback” clones located in Baldy Lake meadow (site II). Eight ramets were enumerated with each clone in the summer of 2007, for a total sample size of 64 ramets. For each ramet, the height and basal diameter of the main stem, and length and basal diameter of each all branches, were measured. Branch volume was determined using the simple allometric equation (cone volume):

$$\Sigma\ [\pi\, r^2\, L\,]\,/\,3$$

where L = branch length and r = radius of branch at base. The summation is over all branches. The volume of the main stem was determined using the same equation (L =

stem height and r = basal radius). Each ramet was also photographed to obtain a physiognomic profile, and then cut at the base to determine their age.

COLLECTION AND CHEMICAL ANALYSIS OF LEAF TISSUE— Four healthy leaves were collected (by cutting at the petiole base) from upper stem branches of each ramet; all leaves were collected over a five-day period (June 12 – 16, 2005). The freshly collected leaves were immediately placed in a cooler and transported to the University of Manitoba. For each ramet, the four leaves were freeze-dried, combined and ground to a powder, and stored at -20°C.

Standard high-performance liquid chromatography (HPLC; Lindroth and Pajutee 1987; Lindroth et al. 1993) was used to determine salicortin and tremulacin concentrations in the collected leaf tissue. Salicortin and tremulacin constitute over 90% of total phenolic glycoside content (by mass) of aspen leaves (Lindroth and Koss 1996). Extraction procedure began by suspending 500 mg of powdered leaf tissue in 5 ml of 80% ice-cold methanol, and shaking the suspension for four hours (Lindroth and Pajutee 1987). The suspension was then centrifuged at 6,000 revs/min/min for three minutes to isolate and extract the supernatant; this was repeated three times. Gallic acid (1 ml, 0.05 mg/ml) was then added as an internal standard, followed by a concentration of the supernatant using a rotovap (Rotavapror R 110, Büch1 Laboratorium, Flawil, Switzerland). The remaining aqueous solution contained various pigments, lipids and phenolics. The pigments were removed using petroleum ether, and the phenolics isolated using ethyl acetate. The phenolic-rich supernatant was then dried to a powder under a nitrogen stream. Soluble phenolics were analyzed by reverse-phase, high-pressure liquid chromatography (HPLC), using a Waters 2695 separation module coupled with a Water 996 photodiode array detector. Forty milliliters of each phenolic extract were injected onto an RP-18 (5 µm) lichrospher 100 column, and eluted at a flow rate of 1 ml min^{-1} with a gradient of acetonitrile / 0.1% H_3PO_4 in water; run time was 65 minutes (gradient: ACH3CN Bacid H_20g 220to370). Data were analyzed using Empower 2.0 (Waters, Ville-Saint-Laurent PQ, Canada). Compounds were identified based on their retention time, their characteristic UV spectra, and through co-elution with salicortin and tremulacin purified standards.

STATISTICAL ANALYSES

TWO-WAY ANALYSIS OF COVARIANCE — A two-way, fixed-effect analysis of variance model was developed to examine variation in phenolic glycoside concentrations across two statistical treatments: soil moisture stress (three levels: Sites I-III), and clone health (two levels: dieback and vigorous). Individual clones were the experimental replicates (four replicates for each of the 3 x 2 = 6 treatment combinations), and were used to determine the experimental error. Intra-clonal variation in phenolic glycoside concentration (eight ramets per replicate clone) was included in the model as the sampling error, i.e. it was not included in the calculation of F-values and associated probabilities (Ostle and Malone 1988). The data analyzed were total phenolic glycosides (tremulacin and salicortin) expressed as percent dry weight (Osier and Lindroth 2001).

Initial analysis of the data revealed a statistically significant negative relationship between phenolic glycoside concentration and ramet age ($P < 0.0001$), as noted by other researchers (e.g. Donaldson et al. 2006). I removed the potentially confounding effect of differences in ramet age by modifying my statistical model, incorporating ramet age as a covariate in a two-way analysis of covariance (ANCOVA).

SURVIVAL ANALYSIS — Survival analysis was used to examine differences in mortality between "vigorous" and "dieback" clonal ramets, over a four-year period. survival rates between treatments, using time as a covariate (Kaplan-Meier survival curves; Kaplan and Meier 1958, Zens and Peart 2003). Repeated-measure data on ramets from two sites (Deep Lake and Baldy Lake grasslands) were used in the survival analysis (64 vigorous and 64 dieback clonal ramets). The third site (Grasshopper Valley grassland) was not included in the analysis, since a prescribed burn in the spring of 2005 killed all marked ramets.

Factors (covariates) that influence time to death in the K-M survival analysis were examined using a Cox proportional hazards regression model. Logistic regression was used to generate a predictive model relating chemical resistance to survival. Data were recorded over a 50-month period starting June 2004, samples were measured once in the spring and once in the fall. Site one was excluded since it was burned in the spring of 2005.

Time-to-death of dieback and vigorous clonal ramets was examined using the Kaplan-Meier product limit procedure (Kaplan and Meier 1958; Zens and Peart 2003).

This method uses both uncensored (time of death of ramets) and censored data (ramets still alive at the end of the study) to generate survival curves, in which the cumulative probability of survival [$S(t)$] over time is graphed. At the beginning of the study $S(t) = 1$, declining monotonically over time ($0 \leq S(t) < 1$) as mortality occurs. Differences in ramet survival between dieback and vigorous ramets results in their respective survival curves diverging over time. A test of the statistical significance of survival curve divergence is provided by a chi-squared log-rank statistic; this test considers the entire survey period, not just the difference in survival at an arbitrary point in time (Jager et al. 2008).

A Cox proportional hazards regression model (Cox 1972; Cox and Oakes 1984; "*coxph survival*" library in R) is used to examine the effects of one or more covariate(s) on time-to-death. I used this semi-parametric regression model to complement the Kaplan-Meier product limit procedure. Whereas Kaplan-Meier simply examines the mortality as a function of time, the Cox model determines the "risk factor" associated with mortality by relating phenolic glycoside concentration to the hazard rate of the population (Hasbrouck et al. 1992). In my study, the "hazard" $h_i(t)$ of a given ramet is the instantaneous risk of mortality at time t conditional on its survival to time t (McCullagh 1980). This is modeled as the product of the baseline hazard experienced by all individuals (the time-dependent function, $h_0(t)$) and the exponential of the covariate (phenolic glycoside concentration):

$$h_i(t) = h_0(t) \exp (\beta_1 x_{i1})$$

The regression coefficient β_1 measures the relative influence of the covariate on the instantaneous mortality risk. The interpretation of β is based on the risk or hazard ratio

$HR = e^{\beta}$. HR is interpreted as the relative hazard of one group (1 = dieback) on another (0 = vigorous), and is constant. HR values that significantly depart from unity indicate an increasing (HR > 1) or decreasing (HR < 1) risk of mortality associated with the covariate(s). HR = 1 ($\beta_1 = 0$) indicates no effect or equal hazard of mortality for both groups.

Logistic Analysis — Logistic regression (generalized linear models procedure "*logit function*" in R) was used to develop a model to predict ramet "fate" over the four-year study period (categorical variable, live vs. dead) as a function of leaf phenolic glycoside concentration (continuous variable). Logistic regression uses maximum likelihood to estimate model parameters (Everitt and Hothorn 2006; Quinn and Keough 2002). The method fits a logistic curve that relates a continuous covariate to a categorical variable. The general logistic equation is:

$$\pi(\mathrm{x}) = \exp(\beta_0 + \beta_1\, x_1) \;/\; 1 - \exp(\beta_0 + \beta_1\, x_1)$$

where $\pi(\mathrm{x})$ is the probability of ramet mortality at phenolic glycoside concentration x, and β_1 is the rate of change in $\pi(\mathrm{x})$ as a function of x. The predicted mortality probability is thus expressed as a continuous function of phenolic glycoside concentration. Mortality probability can be expressed as probability odds ratio OD, the odds of the event occurring divided by the odds of it not occurring:

$$\mathrm{OD} = \pi(\mathrm{x}) \;/\; [1-\pi(\mathrm{x})] = \exp(\beta_1)$$

The interpretation of OD is dependent on its departure from unity; if OD = 1, the covariate (phenolic glycoside concentration) has no effect on mortality or survival. OD > 1 is the per unit increase in ramet mortality probability per unit increase in phenolic glycoside concentration (e.g. OD = 3 indicates a three-fold increase in the likelihood of mortality per unit increase in phenolic concentration). Likewise, OD < 1 indicates that the mortality probability declines as phenolic glycoside concentation increases. The residual deviance or likelihood ratio (LR) χ^2 statistic ($G^2 = -2 \ln \Lambda$) was used to test the significance of the logistic model (H_0: $\beta = 0$; Quinn and Keough 2002).

All statistical analyses were conducted using R 9.2.9 (R development Core Team 2009), DataDesk 6.2 (Data Description Inc. Ithaca New York, USA), and/or SPSS 16.0 program (SPSS, Chicago Illinois, USA).

5.3 RESULTS

PHENOLIC GLYCOSIDE CONCENTRATION, SITE MOISTURE STRESS, AND CLONE "HEALTH"

Phenolic glycoside concentrations showed considerable variation among the 24 sampled aspen clones, ranging from 2 – 18% dry weight (**Figure 5.3**). At all three study sites, phenolic glycoside concentration was much higher in "vigorous" than "dieback" clones (**Figure 5.3**). For both "vigorous" and "dieback" clones, foliar phenolic glycoside concentration generally increased with increasing moisture-stress, as was highest at the driest site (I, Deeplake Lake grassland; **Figure 5.4**). Two-way analysis of covariance (with ramet age as the covariate) showed that site-level differences in phenolic glycosides accounted for 10% of the explained variance in the data (ANCOVA: $F_{2,18} = 4.17$, $P = 0.03$; **Table 5.2**). The most significant source of variation (about 50%) was clone "health" (ANCOVA: $F_{1,18} = 70.4$, $P < 0.0001$; **Table 5.2**), with "vigorous" clones averaging nearly double the concentration of phenolic glycosides of "dieback" clones (**Figure 5.4**). The interaction of site and "health" was not statistically significant (ANCOVA: $F_{2,18} = 3.05$, $P = 0.07$; **Table 5.2**). Most of the error in the data was associated with variation among clones (experimental error: 34.4% variance explained); within-clone error was low (sampling error: 2.3% variance explained), indicating that phenolic glycoside concentrations are similar among ramets of the same clone (genet).

PHENOLIC GLYCOSIDE CONCENTRATION AND BROWSING DAMAGE

Browsing damage to ramets ranged from 0% (no browsing) to 100% (all branches browsed). There was a statistically significant difference in ramet browsing damage between "dieback" and "vigorous" clones ($t_{190} = 26.8$, $P < 0.001$; **Figure 5.5**); browsing damage averaged 69.7 ± 19.4% for dieback clones, versus only 8.9 ± 10.7% for vigorous clones. Differences in phenolic glycosides concentration among ramets explained much of the variation in browsing damage: there was a significant negative correlation between browsing and total phenolic glycoside concentration (Pearson $r^2 = -0.65$, $P < 0.0001$; **Figure 5.5**).

Ramets of "dieback" clones (low phenolic glycoside concentration) are stunted and have a dense branch structure, indicating that they are heavily browsed. Conversely, "vigorous" clone ramets (high phenolic glycoside concentration) have a more typical tree form, with a well-defined main trunk and regular branching (**Figure 5.6a**). Total woody stem volume (main stem plus branches) of "vigorous" and "dieback" clone ramets is similar over all ages (**Figure 5.6b**), averaging approximately 500 cm^3 at age 10 and 1500 cm^3 at age 20. Allocation of woody tissue to branch volume differs strongly between "vigorous" and "dieback" clones, however; unbrowsed "vigorous" allocate 11% of total aboveground volume to branches, versus 52% in browsed "dieback" clones, over all age classes (**Figure 5.6c**).

PHENOLIC GLYCOSIDE CONCENTRATION, RAMET MORTALITY AND CLONE VIGOR

Between June 2004 and September 2007 (50 months) nearly 50% of monitored "dieback" clone ramets died, compared to only 14% of ramets from "vigorous" clones

(**Figure 5.7**). Kaplan-Meier survival analysis indicated that the difference in the mortality rates between vigorous and dieback clones is statistically significant (log-rank: $\chi_1^2 = 18.6$, $P < 0.001$). The proportional hazard ratio (Cox regression: $\beta = 4.4$, HR = 81.5) confirms that mortality risk is substantially greater for ramets of "dieback" clones.

Logistic regression analysis indicated that phenolic glycoside concentration predicts ramet mortality with 71% accuracy (**Figure 5.8**), and that the relationship is highly significant statistically (likelihood-ratio statistic: $\chi^2_{127} = 32.3$, $P < 0.001$). Ramets with high phenolic glycoside concentration (which are almost invariably from "vigorous" clones) have a lower mortality risk; for every unit increase in phenolic glycoside concentration, the odds of survival increase by OD = 0.76.

5.4 DISCUSSION

The results of this study are consistent with previous studies concluding that higher concentrations of phenolic glycosides in trembling aspen reduce foraging by mammalian herbivores (e.g., Bailey et al. 2004; Lindroth et al. 2007; Wooley et al. 2008). My study goes further, by demonstrating that the overall clone "health" (vigorous versus dieback) is directly associated with phenolic glycoside production: clones producing high amounts of phenolic glycosides are able to regenerate successfully and show limited browsing by ungulate herbivores, whereas clones with low levels of phenolic glycosides suffer from "dieback", show limited regeneration, and are heavily browsed by ungulate herbivores.

I found that phenolic glycoside production increases with decreasing soil moisture availability (increasing environmental stress), indicating that site-level variation in environmental conditions influences resource allocation to carbon-based secondary metabolites. Hale et al. (2005) also found that foliar phenolic glycoside concentration in aspen increases under moderate moisture stress.

The growth-differentiation balance hypothesis (Loomis 1932) states that any factor that limits growth (e.g. moderate moisture stress) relative to the rate of carbon fixation results in greater accumulation of leaf carbon and carbon-based plant secondary metabolites (Herms and Mattson 1992; reviewed by Koricheva et al. 1998). However, under severe and prolonged drought both growth and carbon assimilation are affected, resulting in a reduction in carbon-based defenses (Hale et al. 2005).

The functional tradeoff between growth and secondary metabolite production is well documented in the literature (Mopper and Whitham 1992; Hale et al. 2005). Secondary metabolite production does not necessarily incur additional metabolic costs, since the pathway used in phenolic production is the same as that used in protein synthesis (i.e. growth). Plants capable of acquiring more energy have greater growth rates, and can therefore more easily replace damaged tissues by allocating resources towards growth – a tolerance mechanism. Since soil moisture stress slows the growth rate of aspen (Shields and Bockheim 1981), secondary metabolite production is predicted to increase under stress as individuals shift from a tolerance to a resistance-based defensive mechanism (Bryant et al. 1983; Coley et al. 1985). Even so, my results indicate considerable genotypic variation in secondary metabolite production by trembling aspen; even in the most stressful soil moisture environment (site I, Deep Lake) where allocation to secondary metabolites is greatest, intraspecific variation in metabolite production is high.

Numerous studies have demonstrated that differences in secondary metabolite production within local populations are genetically determined (Hwang and Lindroth 1997; Holeski et al. 2009). My results indicate that a local population process, clone "dieback", is strongly predicted by phenolic glycoside concentration; vigorous clones had significantly higher levels of these defensive compounds than did dieback clones. The phenolic glycoside concentrations of my vigorous clones were similar to levels known to reduce foraging by mammalian browsers (Wooley et al. 2008).

In trembling aspen, changes in phenolic glycoside concentration may also result from an adaptive inducible defense response, i.e. a phenotypic response to defoliation in which phenolic glycoside production is altered. The induction of phenolic glycosides production in trembling aspen varies mainly by genotype, but may also vary with edaphic conditions (Stevens and Lindroth 2005). Inducible change in aspen is a delayed response; phenolic glycoside production often declines in the year following a major defoliation event ("inducible susceptibility", Stevens and Lindroth 2005). The opposite effect has also been observed, however; the leaf phenolic content of newly emerged leaves has been shown to increase following severe frost-defoliation (St. Claire 2009).

Ungulate browsing on trembling aspen initiates a positive feedback loop, in which the likelihood of repeated selective browsing increases in subsequent years (Danell et al. 1994; De Jager et al. 2009). I have shown that repeated mammalian browsing of "dieback" clonal ramets suppresses vertical growth, resulting in the development of a dense, highly branched architecture. This herbivore-induced alteration in ramet architecture greatly increases the amount of shoot and leaf biomass within the browsing zone (Shipley 2007). The cycle of repeated selective browsing may be the result of genetic variation in secondary metabolite production: clonal ramets with lower levels of secondary metabolites may be predisposed to greater browsing pressure. Alternatively, a given ramet may be initially browsed by chance, resulting in an induced susceptibility response (i.e. reduction in secondary metabolite production the following year; Steven and Lindroth 2005) that increases ramet palatability and so promotes further browsing. In practice, both genetic predisposition and induced susceptibility may be important. Regardless of the exact mechanism, repeated browsing increases a ramet's susceptibility to further damage by progressively lowering its resistance (Donaldson et al. 2006; Lindroth et al. 2007).

Both genotypic and environmental factors can result in low production of defensive plant secondary metabolites in aspen ramets, predisposing them to repeated browsing. Repeated ungulate herbivore browsing alters the crown architecture of ramets by reducing height growth and increasing branch density within the browse-zone (< 2 m height). Given the high cost of producing carbon-based defensive compounds, most plants produce such compounds only when needed, i.e. during the most vulnerable life-history stages (Strauss and Agrawal 1999). In trembling aspen, phenolic glycoside production is highest in the first few years, but declines considerably after five years of growth (Donaldson et al. 2006). In the absence of browsing, ramet branches typically occur above the browse-zone height within five years (i.e., before phenolic glycoside production begins to decline). Conversely, the branch system of repeatedly browsed ramets is dense, and the branches remain within the browse-zone for many years. After five years, these accessible branches begin to produce leaves with low levels of phenolic glycosides, reinforcing the cycle of repeated ungulate browsing. This positive feedback in ungulate browsing reduces clone vigour, and may explain the variation is clone "health" among aspen clones in my study sites.

My logistic models indicate that intraspecific variation in phenolic glycosides is an important predisposing factor leading to ramet mortality, and by extension to decline of aspen clones. With few exceptions (e.g. severe drought, fire), tree mortality is the result of chronic (long-term) rather than acute (short-term) processes. Mortality results from the complex interaction of intrinsic and extrinsic factors that operate in a hierarchical manner, in what Franklin et al. (1987) refer to as a "mortality spiral". The nature and magnitude of stress factors are necessary, but not sufficient, determinants of mortality; historical contingencies and chance effects may be just as important (Mueller-Dombois 1987). An individual subjected to a given stress (e.g. drought, frost, mammalian herbivory, insect pests, fungal pathogens) is "weakened", making it increasingly susceptible to other stressors that cause further weakening. Such individuals are caught in a mortality spiral, with the probability of mortality increasing over time with each successive stressor. The tree disease model proposed by Manion (1991) also recognizes the synergistic reinforcing effects of stressors, but it also considers phenotypic variation within populations as an important predisposing mortality factor. In trembling aspen, lower production of phenolic glycosides may predispose an individual to repeated browsing. In turn, browsing stress renders the individual more susceptible to other stressors (e.g. insect pests or fungal pathogens), further weakening the clone and increasing its mortality risk.

In trembling aspen, individuals with low levels of phenolic glycosides have correspondingly higher levels of crude protein content, since polyphenolic and protein synthesis share the common precursor phenylalanine ammonia lyase (Margna 1977; Jones and Hartley 1999). This polyphenolic-protein metabolic tradeoff is common throughout the plant kingdom, and is considered a pleiotropic effect (Meyer et al. 2006; Bragazza and Freeman 2007). This metabolic tradeoff could play an important role in foraging behaviour, given that aspen palatability is likely influenced by a multitude of factors that could include crude protein content. For example, clonal variation in the rate of protein loss during the growing season may explain why deer selectively browse protein-rich aspen leaves in autumn (Tew 1970). However, given the metabolic tradeoff between protein synthesis and phenolic compound content, it is equally possible that mammalian herbivores avoid phenolic-rich ramets and that greater protein intake is an indirect benefit (Stolter 2008).

Given that variation in phytochemical defensive traits is largely genetically determined, why does a seemingly deleterious trait (low phenolic glycoside production) persist in trembling aspen populations? There are three possibilities:

(1) Trembling aspen is an obligate out-crosser, but sexual reproduction (seed germination and establishment) may be “functionally absent” for large periods of time given that clonal propagation is so common (Maini 1960). When clonal propagation predominates, both adaptive and non-adaptive traits will persist in the population.

(2) The ecological and genetic consequences of higher phenolic glycoside production are evident only during early life-history stages (< 5 year old ramets), when herbivore selection pressure is greatest; older ramets show little variation in leaf phenolic glycoside levels (Steven et al. 2007; Harding et al 2009). Young clonal ramets may

"resist" browsing by allocating more resources to secondary metabolites (and thus less to growth), or "escape" browsing by allocating resources to rapid height growth (made possible by reducing the allocation to secondary metabolites). Spatial and temporal variation in environmental conditions may alternately select for and against these "resist" and "escape" growth strategies, perpetuating intraspecific variability in phenolic glycoside production.

(3) The degree of browse selection by herbivores is a function of herbivore density and food availability. During periods of very high herbivore density (and/or low food availability), all individuals in the population have an equal probability of being browsed (Donaldson and Lindroth 2008). At very low herbivore densities, herbivores have little impact on plants. Herbivores are more selective at moderate densities and/or when the food supply is high relative to demand (Bergvall and Leimer 2005; Wooley et al. 2008). Under such conditions, ramets with low levels of phenolic glycosides are much more likely to be browsed. High levels of phenolic glycosides are advantageous when herbivore densities are moderate, but not when herbivores are absent or occur at very low or very densities (Bryant et al. 1983). Natural fluctuations in herbivore densities will therefore perpetuate clonal variation in phenolic glycoside production.

In conclusion, my results demonstrate that intraspecific variation of phenolic glycoside production is correlated with clone "health": dieback clones consistently produce low amounts of phenolic glycosides, while vigorous clones contain amounts known to deter mammalian herbivores (Wooley et al. 2008). This finding will improve our understanding of the intrinsic and extrinsic factors influencing the complex dynamics of trembling aspen populations.

5.5 ACKNOWLEDGEMENTS

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant to N. C. Kenkel, Parks Canada and a University of Manitoba's Duff Roblin Graduate Fellowship to R. A. Lastra. We would like to thank Kelly Orr for help in the field. We gratefully acknowledge Parks Canada and Riding Mountain for financial and logistical support. We would also like to thank Adam Gusse and Dr. Richard Lindroth who generously provided HPLC standards (salicortin and tremulacin), preliminary advice on methods and for processing and verifying samples (Department of Entomology, University of Wisconsin-Madison). We express our deep gratitude to Lorne Adam, and Dr. Abdelbasset El Hadrami, for providing lab space, equipment, invaluable training and assistance on the "art" of plant secondary metabolite extraction. We would like to thank Dr. Sylvie Renault who graciously provided Acetonitrile (ACN) at a time when there was a global shortage of this critical HPLC solvent. We also thank Wybo Vanderschuit for help with various aspects of this study.

5.6 LITERATURE CITED

Agrawal, A. A., P. M. Gorski, and D. W. Tallamy. 1999. Polymorphism in plant defense against herbivory: constitutive and induced resistance in *Cumumis sativus*. Journal of Chemical Ecology 25:2285-2304.

Altermatt, F., and D. Ebert. 2008. Genetic diversity of Daphnia magna populations enhances resistance to parasites. Ecology Letters 11:918-928.

Bailey, A. W., and R. A. Wroe. 1974. Aspen invasion in a portion of the Alberta parklands. Journal of Range Management 27:263-266.

Bailey, J. K., J. A. Schweitzer, B. J. Rehill, R. L. Lindroth, G. D. Martinsen, and T. G. Whitham 2004. Beavers as molecular geneticists: a genetic basis to the foraging of an ecosystem engineer. Ecology 85:603-608.

Bailey, J. K., S. C. Wooley, R. L. Lindroth, and T. G. Whitham. 2006. Importance of species interactions to community heritability: a genetic basis to trophic interactions. Ecological Letters 9:78-85.

Balmer, O., S. C. Stearns, A. Schötzau, and R. Brun. 2009. Intraspecific competition between co-infecting parasite strains enhance host survival in African trypanosomes. Ecology 90:2267-3378.

Barnes, B. V. 1969. Natural variation and delineation of clones of *Populus tremuloides* and *P. grandidentata* in north Lower Michigan. Silvae Genetica 18:130-142.

Barnes, B. V. Phenotypic variation of trembling aspen of western North America. Forest Science 21:319-328.

Bartos, D. L., and R. B. Campbell Jr. 1998. Decline of quaking aspen in the interior west, examples from Utah. Rangelands 20:17-24.

Bergvall, U. A. and O. Leimer. 2005. Plant secondary compounds and the frequency of food types affect food choice by mammalian herbivores. Ecology 86:2450-2460.

Bragazza, L. and C. Freeman. 2007. High nitrogen availability reduces polyphenol content in Sphagnum peat. Science of the Total Environment 377:493-443.

Bridle, J. R., J. Polechová, M. Kawata, and R. K. Butlin. 2010. Why is adaptation prevented at ecological margins? New insights from individual-based simulations. Ecology Letters 13:485-494.

Brown, James. 1995. Macroecology. University of Chicago Press. Chicago.

Browning, J. A., and K. J. Frey. 1969. Multiline cultivars as a means of disease control. Annual Review of Phytopathology 7:355-382.

Bryant, J. P., F. S. Chapin, and D. R. Klein. 1983. Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357-368.

Camp, W. H. 1949. Cinchona at high altitudes in Ecuador. Brittonia 6:394-430.

Cheliak, W. M., and B. P. Dancik. 1982. Genetic diversity of natural populations of a clone forming tree *Populus tremuloides.* Canadian Journal of Genetics and Cytology. 24:611-616.

Clark, J. S., J. Moham, M. Dietze, and I. Ibanez. 2003. Coexistence: how to identify trophic trade-offs. Ecology 84:17-31.

Close, D. C., and C. McArthur. 2002. Rethinking the role of many plant phenolics – protection from photodamage not herbivores? Oikos 99:166-172.

Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. Science 230:895-899.

DeGabriel, J. L., B. D. Moore, W. J. Foley, and C. N. Johnson. 2009. The effect of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecology 90:711-719.

Denny, M. K., L. J. H. Hunt, L. P. Miller, and C. D. G. Harley. 2009. On the prediction of extreme ecological events. Ecological Monographs 79:397-421.

Dimock, E. J. II, R. R. Silen, and V. E. Allen. 1976. Genetic resistance in Douglas-fir to damage by snowshoe hare and back-tailed deer. Forest Science 22:106-121.

Diner, B. D. Berteaux, J. Fyles, and R. L. Lindroth. 2009. Behavioral archives link the chemistry of trembling aspen to food choice of North American porcupine.Oecologia 160:687-695.

Donaldson, J. R., M. T. Stevens, H. R. Barnhill, and R. L. Lindroth. 2006. Age-related shifts in leaf chemistry of clonal aspen (*Populus tremuloides*). Journal of Chemical Ecology 32:1415-1429.

Donaldson, J. R. E. L. Kruger, and R. L. Lindroth. 2006. Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*). New Phytologist 169:561-570.

Donaldson, J. R., and R. L. Lindroth. 2008. Effects of variable phytochemistry and budbreak phenology on defoliation of aspen during a forest tent caterpiller outbreak. Agriculture and Forest Entomology 10:399-410.

Ehrlich, P., and P. Raven. 1964. Butterflies and plants: a study in coevolution. Evolution 18:586-608.

Elton, S. Charles. 1958. The ecology of invasions by animals and plants. Methuen & Co., London.

Erwin, E. A., M. G. Turner, R. L. Lindroth, and W.H. Romme. 2001. Secondary plant compounds in seedling and mature aspen (*Populus tremuloides*) in Yellowstone National Park, Wyoming. American Midland Naturalist 145:299-308.

Everitt, B., and T. Hothorn. 2006. A handbook of statistical analysis using R. Chapman & Hall/CRC.

Fields, M. J., and C. M. Orians. 2006. Specificity of phenolic glycoside induction in willow seedlings (*Salix sericea*) in response to herbivory. Journal of Chemical Ecology 32:2647-2656.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford University Press. Oxford, England.

Fornoni J. P. L. Valverde, and J. Nunez-Farfan. 2004. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. Evolution 58:1696-1704.

Frankham, R., and K. Ralls. 1998. Inbreeding leads to extinction. Nature 392:441-442.

Franklin, J. F., H. H. Shugart, and M. E. Harmon. 1987. Tree death as an ecological process. BioScience 37:550-556.

Fraenkel, G. 1959. The raison d'être of secondary plant substances. Science. 129:1466-1470.

-------------- 1969. Evaluation of our thoughts on secondary plant substances. Entomologia Experimentalis et Applicata 12: 473-486.

Frey, B. R., V. J. Lieffers, E. H. (Ted) Hogg, and S. M. Landhäusser. 2004. Predicting landscape patterns of aspen dieback: mechanisms and knowledge gaps. Canadian Journal of Forest Research 34: 1379-1390.

Gleason, H. A., 1926. The individualistic concept of the plant association. Bulletin of the Torrey Botanical Club 53:7-26.

Guglielmo, C. G., W. H. Karasov and W. J. Hjakubas. 1996. Nutritional costs of a plant secondary metabolites explain selective foraging by ruffled grouse. Ecology 77:1103-1115.

Häikiö, E., M. Makkonen, R. Julkubeb-Tiitto, J. Sitte, V. Freiwald, T. Silfver, V. Pandey, E. Beuker, T. Holopainen, and E. Oksanen. 2009. Performance and secondary chemistry of two hybrids aspen (*Populus tremula* L. *x Populus tremuloides* Michx.) Clones in long-term elevated ozone exposure. Journal of Chemical Ecology 35:664-678.

Halden, J. B. S. 1932. The causes of evolution. Longmans, Green & Co., London.

Hale, B. K., D. A. Herms, R. C. Hansen, T. P. Clausen, and D. Arnold. 2005. Effects of drought stress and nutrient availability on dry matter allocation, phenolic glycosides, and rapid induced resistence of poplar to two lymantrid defoliators. Journal of Chemical Ecology 31:2601-2620.

Harbourne, J. B. 1982. Introduction to Ecological biochemistry. 2nd ed. Academic Press, New York, NY.

Harper, J. L. 1967. A Darwinian approach to plant ecology. Journal of Ecology 55:247-270.

Hemming, J. D. C., and R. L. Lindroth. 1995. Intraspecific variation in aspen phytochemistry: effects on performance of gypsy moth and forest tent caterpillars. Oecologia 103:79-88.

Hemming, J. D. C., and R. L. Lindroth. 1999.Effects of light and nutrient availability on aspem: growth, phytochemistry, and insect performance. Journal of chemical ecology 25:1687-1714.

Herms, D. A., and W. J. Mattson. 1992. The dilemma of plant: to grow or defend. Quarterly Review of Biology. 67:283-335.

Hogg, E. H., J. P. Brandt, and M. Michaelian. 2008. Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Canadian Journal of Forest Research 38:1373-1384. .

Holeski, L. M., A. Vogelzang, G. Stanosz, and R. L. Lindroth. 2009. Incidence of *Venturia* shoot blight in aspen (*Populus tremuloides* Michx.) varies with tree chemistry and genotype. Biochemical Systematics and Ecology 37:139-145.

Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton University Press.

Hutchnson, G. E. 1957. Concluding remarks. Cold Spring Harbor Symp. Quantitative Biology 22:415-427.

Jelinski, D. E., and W. M. Cheliak. 1992. Genetic diversity and spatial subdivision of *Populus tremuloides* (Salicaceae) in a heterogeneous landscape. American Journal of Botany 79:728-736.

Johnson, M. T. J., and A. A. Agrawal. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). Ecology 86:874-885.

Johnson, M. T. J. 2008. Bottom-up effects of plant genotype on aphids, ants, and predators. Ecology 89:145-154.

Jones, C. G., and S. E. Hartley. 1999. A protein competition model of phenolic allocation. Oikos 86:27-44.

Kanaga, M. K., R. J. Ryel, K. E. Mock, and M. E. Pfrender 2008. Quantitative-genetic variation in morphological and physiological traits within a quaking aspen (*Populus tremuloides*) population. Canadian Journal of Forest Research 38:1690-1694.

Karban, R. 1987. Effects of clonal variation of the host plant, interspecific competition, and climate on the population size of a folivorous thrips. Oecologia 74:298-303.

Kashian, D. M., W. H. Romme, and C. M. Regan. 2007. Reconciling divergent interpretations of quaking aspen decline on the northern Colorado Front Range. Ecological Applications 17:1296-1311.

Kotowska, A. M., J. F. Cahill Jr, and A. B. Keddie. 2010. Plant genetic diversity yields increased plant productivity and herbivore performance. Journal of Ecology 98:237-245.

Koricheva, J., S. Larsson, E. Haukioja and M. Keinänen. 1998. Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. Oikos 83:212-226.

Lawler, I. R., W. J. Foley, B. M. Eschler, D. M. Pass, and K. Handasynde. 1998. Intraspecific variation in Eucalyptus secondary metabolites determines food intake by folivorous marsupials. Oecologia 116:160-169.

Lawrence, R., B. M. Potts, T. G. Whitham. 2003. Relative importance of plant ontogeny, host genetic variation, and leaf age for a common herbivore. Ecology 84:1171-1178.

Leonelli, G., B. Denneler, and Y. Bergeron. 2008. Climate sensitivity of trembling aspen radial growth along a productivity gradient in northeastern British Columbia, Canada. Canadian Journal of Forest Research 38:1211-1222.

Lindroth, R. L., and S. –Y. Hwang. 1996. Clonal variation in foliar chemistry of quaking aspen. Biochemical Systematics and Ecology. 24:357-364.

Lindroth, R. L., and P. A. Koss. 1996. Preservation of salicaceae leaves for phytochemical analysis: further assessment. Journal of Chemical Ecology. 22:765-771.

Lindroth, R. L., and M. S. Pajutee. 1987. Chemical analysis of phenolic glycosides: art, facts, and artifacts. Oecologia 74:144-148.

Lindroth, R. L., K. K. Kinney, and C. L. Platz. 1993. Responses of deciduous trees to elevated CO_2: productivity, phytochemistry and insect performance. Ecology 74:763-777.

Lindroth, R. L., J. R. Donaldson, M. T. Stevens, and A. C. Gusse. 2007. Browsing quality in quaking aspen (*Populus tremuloides*): effects of genotype, nutrients, defoliation, and coppicing. Journal of Chemical Ecology 33:1049-1064.

Loomis, W. E. 1932. Growth-differentiation balance vs. carbohydrate-nitrogen ratio. Proceeding of the American Society of Horticulture. 29:240-245.

MacArthur, R. H. 1972. Geographical Ecology. Harper and Roe. New York.

Maini, J. S. 1960. The invasion of grasslands by *Populus tremuloides* in the North Great Plains. Ph.D. thesis. University of Saskatchewan. 231 pages.

Margna, U. 1977. Control at the level of substrate supply-an alternative in the regulation of phenylpropanoid accumulation in plant cells. Phytochemistry 16:419-426.

Manion, P. D. 1991. Tree disease concepts. 2nd ed. Prentice-Hall Inc., Upper Saddle River, N.J.

May, R. M. 1974. Biological populations with non-overlapping generations: stable points, stable cycles, and chaos. Science 186:645-647.

Meier, C. L., and W. D. Bowman. 2008. Phenolic-rich leaf carbon fractions differentially influence microbial respiration and plant growth. Oecologia 158:95-107.

Meyer, S., Z. G. Cerovic, Y. Goulas, P. Montpied, S. Demoted-Mainard, L. P. R. Bidel, I. Moya and E. Dreyer. 2006. Relationships between optically assessed polyphenols and chlorophyll contents, and leaf mass per area ratio in woody plants: a signature of the carbon-nitrogen balance within leaves? Plant, Cell and Environment 29:1338-1348.

Mock, K. E., A. Rowe, M. B. Hooten, J. Dewoody, and V. D. Hipkins. 2008. Clonal dynamics in western North American aspen (*Populus tremuloides*). Molecular Ecology 17:4827-4844.

Moore, B. D., and W. J. Foley. 2005. Tree use by koalas in a chemically complex landscape. Nature 435:488-490.

Mopper, S., and T. G. Whitham. 1992. The plant stress paradox: effects on pinyon sawfly sex ratios and fecundity. Ecology 73:515-525.

Mousseau, T.A., B. Sinervo, and J. Endler. 2000. Adaptive genetic variation in the wild. Oxford University Press, Oxford.

Mueggler, W. F. 1989. Age distribution and reproduction on intermountain aspen stands. Western Journal of Applied Forestry 4:41-45.

Mueller-Dombois, D. 1987. Natural dieback in forests: Causes and Consequences. Bioscience 37:575-583.

Nyman, T., and R. Julkenen-Tiitto. 2005. Chemical variation within and among six northern willow species. Phytochemistry 66:2836-2843.

O'Reilly-Wapstra, J. M, C. McArthur, and B. M. Potts. 2002. Genetic variation in resistance of *Eucalyptus globules* to marsupial browsers. Oecologia 130:289-296.

O'Reilly-Wapstra, J. M, C. McArthur, and B. M. Potts. 2004. Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. Functional Ecology 18:677-684.

O'Reilly-Wapstra, J. M, G. R. Iason. 2007. The role of genetic and chemical variation of *Pinus sylvestris* seedlings in influencing slug herbivory. Oecologia 152:82-91

O'Reilly-Waptra, J. M., J. R. Humphreys and B. M. Potts. 2007. Stability of genetic-based defensive chemistry across life stages in a *Eucalyptus* species. Journal of Chemical Ecology 33:1876-1884.

Osier, T. L., and R. L. Lindroth. 2001. Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. Journal of Chemical Ecology 27: 1289-1313.

Osier, T. L., and R. L. Lindroth. 2006. Genotype and environment determine allocation to and cost of resistance in quaking aspen. Oeologia 148:293-303.

Ostle, B. 1963. Statistics in research: basic concepts and techniques for research workers. Iowa State University Press. 585 pages

Palo, T. R. 1984. Distribution of birtch (*Betula* spp.), willow (*Salix* spp.) and poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. Journal of Chemical Ecology 10:499-520.

Palo, R. T. 1985. Chemical defense in birch: inhibition of digestibility in ruminants by phenolic extracts. Oecologia 68:10-14.

Pereira, D. M., P. Valentão, J. A. Pereira and P. B. Andrade. 2009. Phenolics: from chemistry to biology. Molecules 14:2202-2211.

Petermann, J. S., A. J. F. Fergus, C. Roscher, L. A. Turnbull, A. Weigelt, and B. Schmid. 2010. Biology, chance, or history? The predictable reassembly of temperate grassland communities. Ecology 91:408-421.

Philippe, R. N. and J. Bohlmann. 2007. Poplar defense against insect herbivores. Canadian Journal of Botany. 85:1111-1126.

Quinn, G. P., and M. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge.

R Development Core Team. 2009. R: a language and environment for statistical computing. Version 2.9.2. R foundation for Statistical Computing, Vienna, Australia. http://www.R-project.org

Rehill, B., T. Whitham, G. Martinsen, J. Scheitzer, J. Bailey, and R. L. Lindroth. 2006. Developmental trajectories in cottonwood phytochemistry. Journal Chemical Ecology. 32:2269-2285.

Reichardt, P. B., J. P. Bryant, B. R. Mattes, T. P. Clausen, F. S. Chapin III, and M. Meyer. 1990. Winter chemical defense of Alaskan balsam poplar against snowshoe hares. Journal of Chemical Ecology 16:1941-1959.

Richards, C. L., S. C. Pennings, and L. A. Donovan. 2005. Community-wide patterns of phenotypic variation in salt marsh plants. Plant Ecology 176:263-273.

Roche, B. M., and R. S. Fritz. 1997. Genetics of resistance of *Salix sericea* to a diverse community of herbivores. Evolution 51:1490-1498.

Scheiner, S. M., and M. R. Willig. 2008. A general theory of ecology. Theoretical Ecology 1:21-28.

Schoener, T. W. 1971. Theory of feeding strategies. Annual Review of Ecology and Systematics 2:369-404.

Schweitzer, J. A., M. D. Madritch, J. K. Bailey, C. J. LeRoy, D. G. Fischer, B. J. Rehill, R. L. Lindroth, A. E. Hagerman, S. C. Wooley, S. C. Hart, and T. G. Whitham. 2008. From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a Populus model system. Ecosystems 11: 1005-1020.

Seehausen, O. 2007. Chance, historical contingency and ecological determinism jointly determine the rate of adaptive radiation. Heredity 99: 361-363.

Shepperd, W. D., P. C. Rogers, D. Burton, D. L. Bartos. 2006. Ecology, biodiversity, management, and restoration of aspen in the Sierra Nevada. Gen. Tech. Rep. RMRS-GTR-178. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Shields, W. J., and J. G. Bockheim. 1981. Deterioration of trembling aspen clones in the Great Lakes region. Canadian Journal of Forest Research 11:530-537.

Shier, G. A. and R. B. Campbell. 1980. Variation among healthy and deteriorating aspen clones. USDA For. Serv. Res. Pap. INT-264.

Smilanich, A. M., L.A. Dyer, J. Q. Chambers and M. D. Bowers. 2009. Immunological cost of chemical defence and the evolution of herbivire diet breadth. Ecological Letters 12:612-621.

Shipley, L. A., 2007. The influence of bite size on foraging at larger spatial and temporal scales by mammalian herbivores. Oikes 116:1964-1974.

Snyder, M. A., 1992. Selective herbivory by Albert's squirrel mediated by chemical variability in ponderosa pine. Ecology 73:1730-1741.

Stevens, M. T., M. C. Turner, G. A. Tuskan, W. H. Romme, L. E. Gunter, and D. M. Waller. 1999. Genetic variation in postfire aspen seedlings in Yellowstone National Park. Molecular Ecology. 8:1769-1780.

Stevens, M. T., and R. L. Lindroth. 2005. Induced resistance in the intermediate growth of aspen (*Populus tremuloides*). Oecologia 145:298-306.

Stevens, M. T., D. M. Waller, and R. L. Lindroth. 2007. Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency. Evolutionary Ecology. 21:829-847.

Stolter, C. 2008. Intra-individual plant response to moose browsing: feedbacks loops and impacts on multiple consumers. Ecological Monographs 78:167-183.

Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends in Ecology and Evolution 14: 179-185.

St. Clair, S. B., S. D. Monson, E. A. Smith, D. G. Cahill, and W. J. Calder. 2009. Altered leaf morphology, leaf resource dilution and defense chemistry induction in frost-defoliated aspen (*Populus tremuloides*). Tree Physiology 29:1259-1268.

Suzuki, K., H. Suzuki, D. Binkley, and T. J. Stohlgren. 1999. Aspen regeneration in the Colorado Front Range: differences at local and landscape scales. Landscape Ecology 14:231-237.

Tew, R. K. 1970. Seasonal variation in the nutrient content of aspen foliage. The Journal of Wildlife Management. 34:475-478.

Trottier, G. C. 1986. Distribution of rough fescue, *Festuca hallii*, grassland by livestock grazing in Riding Mountain National Park, Manitoba. Canadian Field-Naturalist 100:488-495.

Turner, B.L. 1970. Molecular approaches to populational problems at the intraspecific level. Pages 187-205 *in* Harbourne, J. B., (ed.) Phytochemical phylogeny: proceedings of the phytochemical society symposium, Bristol April 1969. Academic Press. New York.

Underwood, N., and M. D. Rausher. 2000.The effects of host-plant genotype on herbivore population dynamics. Ecology 81:1565-1576.

Wagner, H. 1979. Phenolic compounds in plants. Biochemistry and plant phenolics. Recent Advances in Phytochemistry 12:589-616.

Whitham, T. G., K. D. Floate, D. M. Gregory, E. M. Driebe, and P. Keim. 1996. Ecological and evolutionary implications of hybridization: *Populus*-herbivore interactions. Pages 247-275 *in* Stettler, R. F., H. D. Bradshaw, Jr., P. E. Heilman, and T. M. Hinckley, editors. Biology of *Populus* and it implications for management and conversation. NRC Research Press, Ottawa.

Whittaker, R. H., and P. P. Feeny. 1971. Allelochemics: chemical interactions between species. Science 171:757-770.

Wooley, S. C., S. Walker, J. Vernon, and R. L. Lindroth. 2008. Aspen decline, aspen chemistry, and elk herbivory: are they linked? Rangelands 30:17-21.

Yeh, F. C., D. K. X. Chong, and R-C Yang. 1995. RAPD variation within and among natural populations of trembling aspen (*Populus tremuloides* Michx.) from Alberta. Journal of Heredity 86: 454-460.

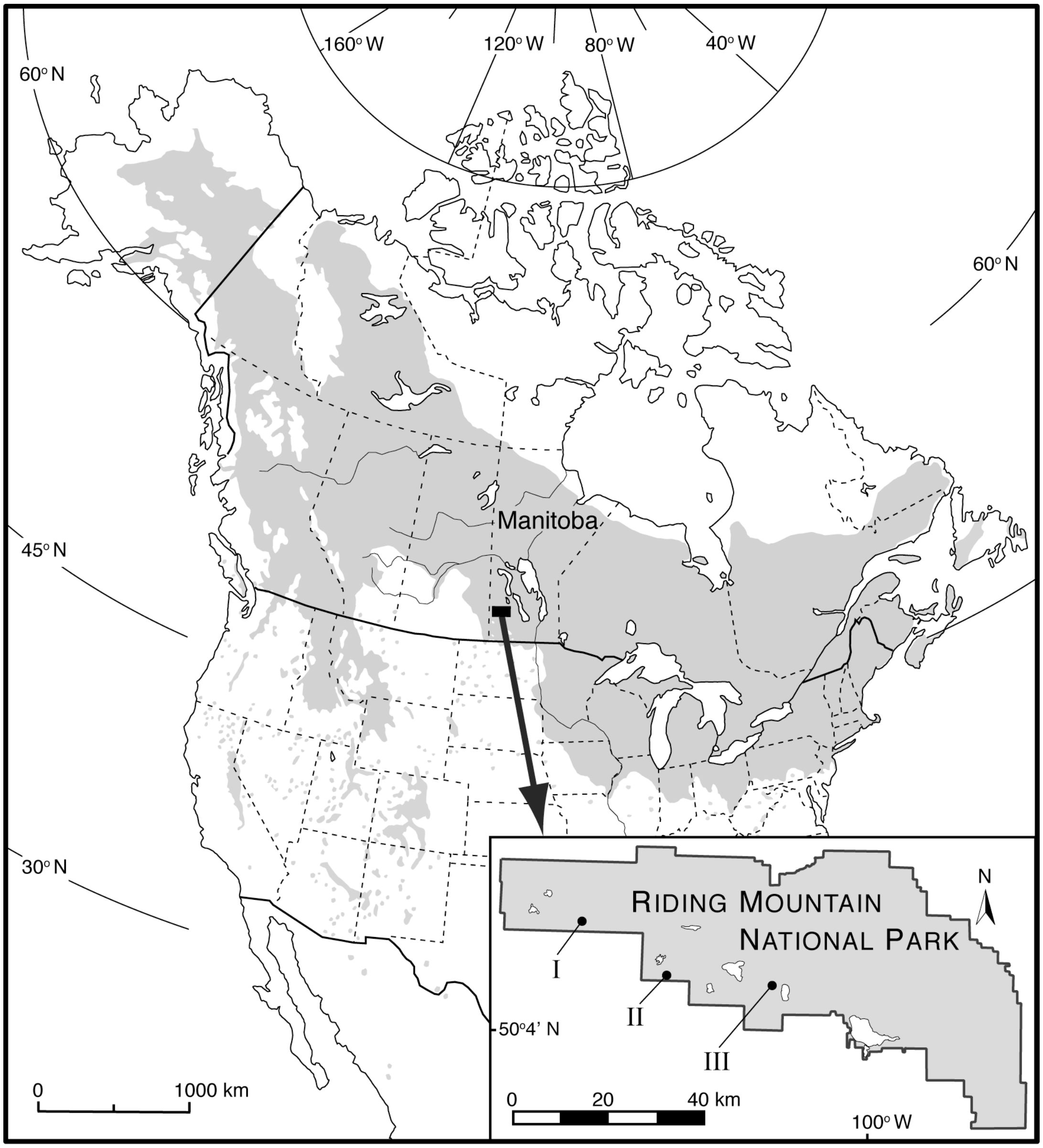


Fig. 5.1. Natural distribution of *Populus tremuloides* across North America (gray shading; modified from Perala 1990). Study area map (lower inset) showing the distribution of the three study locations (I, II, III) in Riding Mountain National Park, Manitoba, Canada.

Table 5.1. Summary of study area, Riding Mountain National Park.

SITE	VOLUMETRIC SOIL MOISTURE CONTENT (%)	SURFICIAL DEPOSITS	SOIL TEXTURE	STONINESS
I - Deep Lake	5.8 +/- 1.98	Alluvium and outwash deposits	Sandy Loam	Very Stony - Moderately Stony
II - Baldy Meadow	8.7 +/- 1.82	Alluvium and outwash deposits	Sandy Loam	Moderately Stony
III - Grasshopper Valley	10.3 +/- 2.92	Glaciolacustrine deposits	Sandy Clay Loam	Slightly Stony

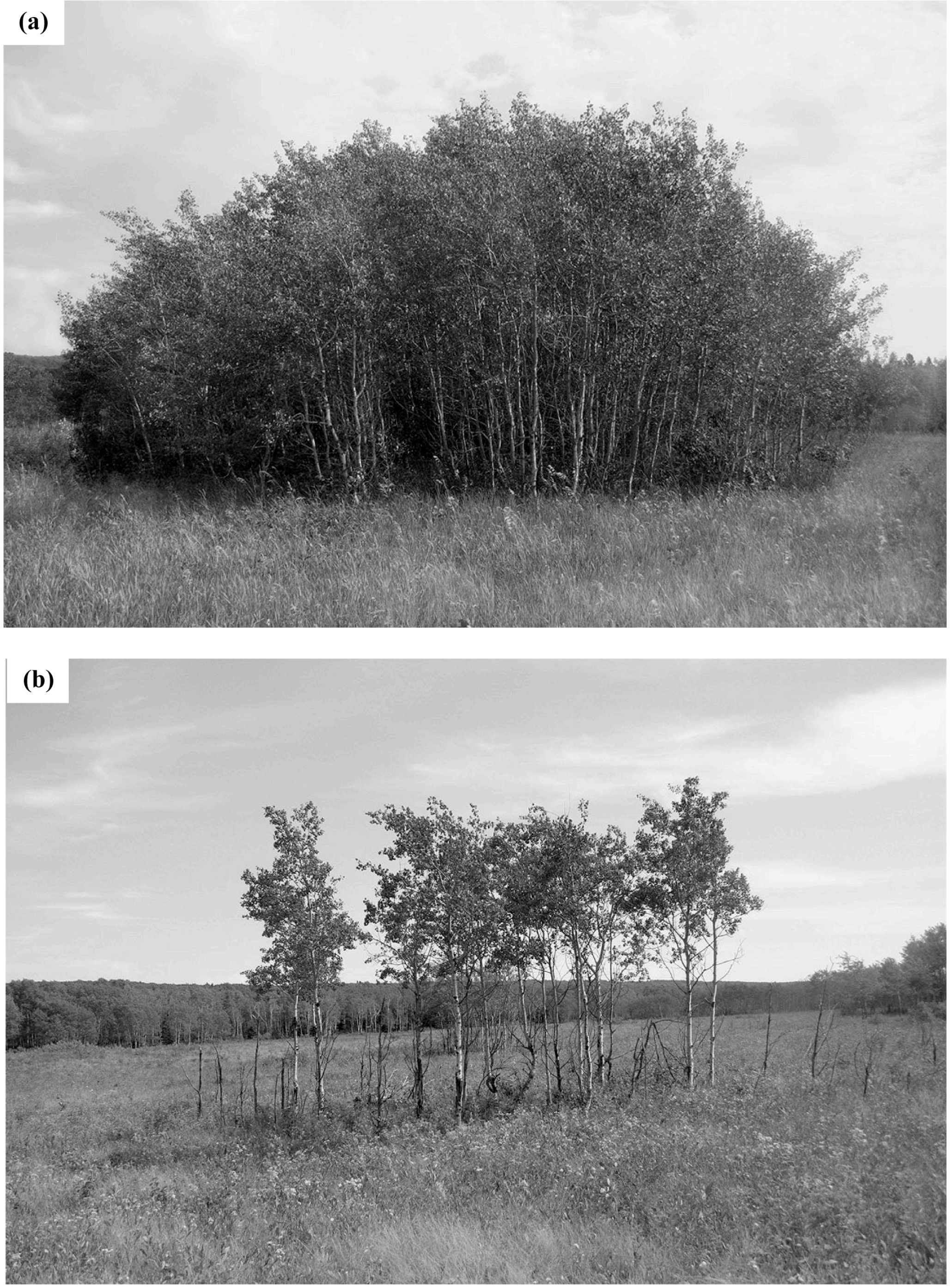


Figure 5.2 Representative aspen clones depicting a vigorous (A) and dieback (B) clones. Clones were located in Baldy Grassland (II) Riding Mountain National Park. Photo credit: R. Lastra

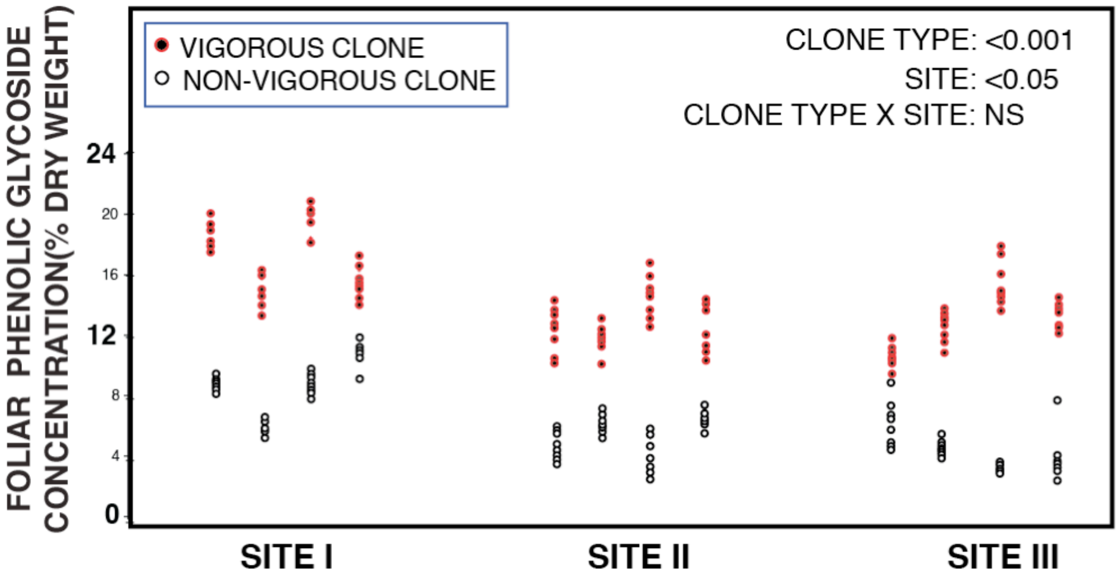


Fig. 5.3. Intraspecific variation of phenolic glycosides (Salicortin + Tremulacin) among 24 aspen clones (12 vigorous, and 12 non-vigorous "dieback") . Leaf samples were haphazardly collected from 8 young ramets (< 8 years of age) within each clone (raw values shown).

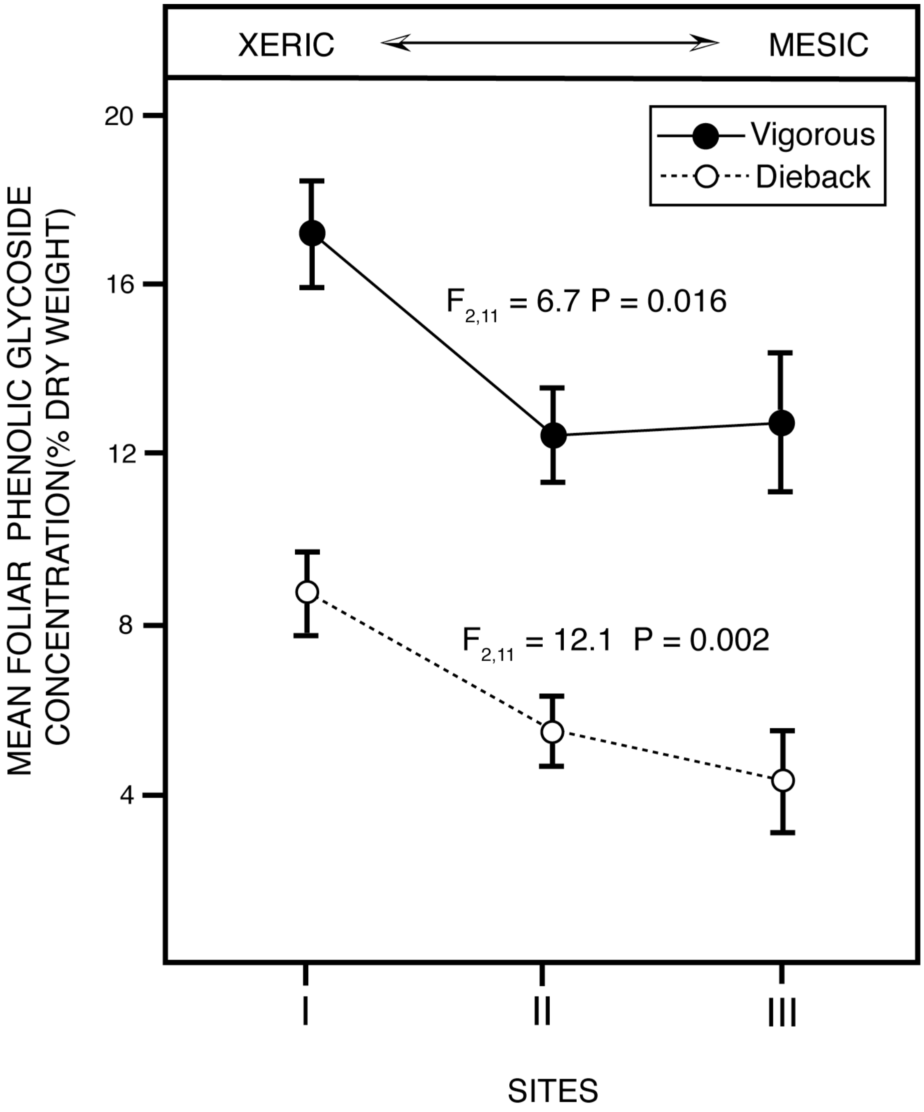


Fig. 5.4. Foliar phenolic glycoside concentrations (mean +/- 1 S.E., n = 4 clones per site) across three sites.

Table 5.2. Two-way fixed factor ANCOVA (with partitioning of the error term) results of the differences in foliar phenolic glycoside levels (tremulacin + salicortin; % Dry Weight) between dieback and vigorous aspen (*Populus tremuloides* Michx.) clones. Sources of variation were examined within populations and among populations. Ramet age was used as the covariate in this linear model.

		Phenolic glycoside (% DW)			
Source of variation	df	MS	*F*	*P*	PEV
Treatment	1	1314.88	**70.40**	**≤ 0.0001**	49.8
Site	2	77.76	**4.17**	**0.03**	10.5
Treatment x Site	2	57.08	3.06	0.07	3.0
Experimental error (clones)	18	18.68			34.4
Sampling error (ramets)	168	1.24			2.3

Notes: The F ratios for both fixed factors and the interaction term use the partitioned experimental error mean square (MS) in the demoninator. Significant terms ($P < 0.05$) are shown in boldface. PEV = Percent explained variance (Gotelli and Ellison 2004).

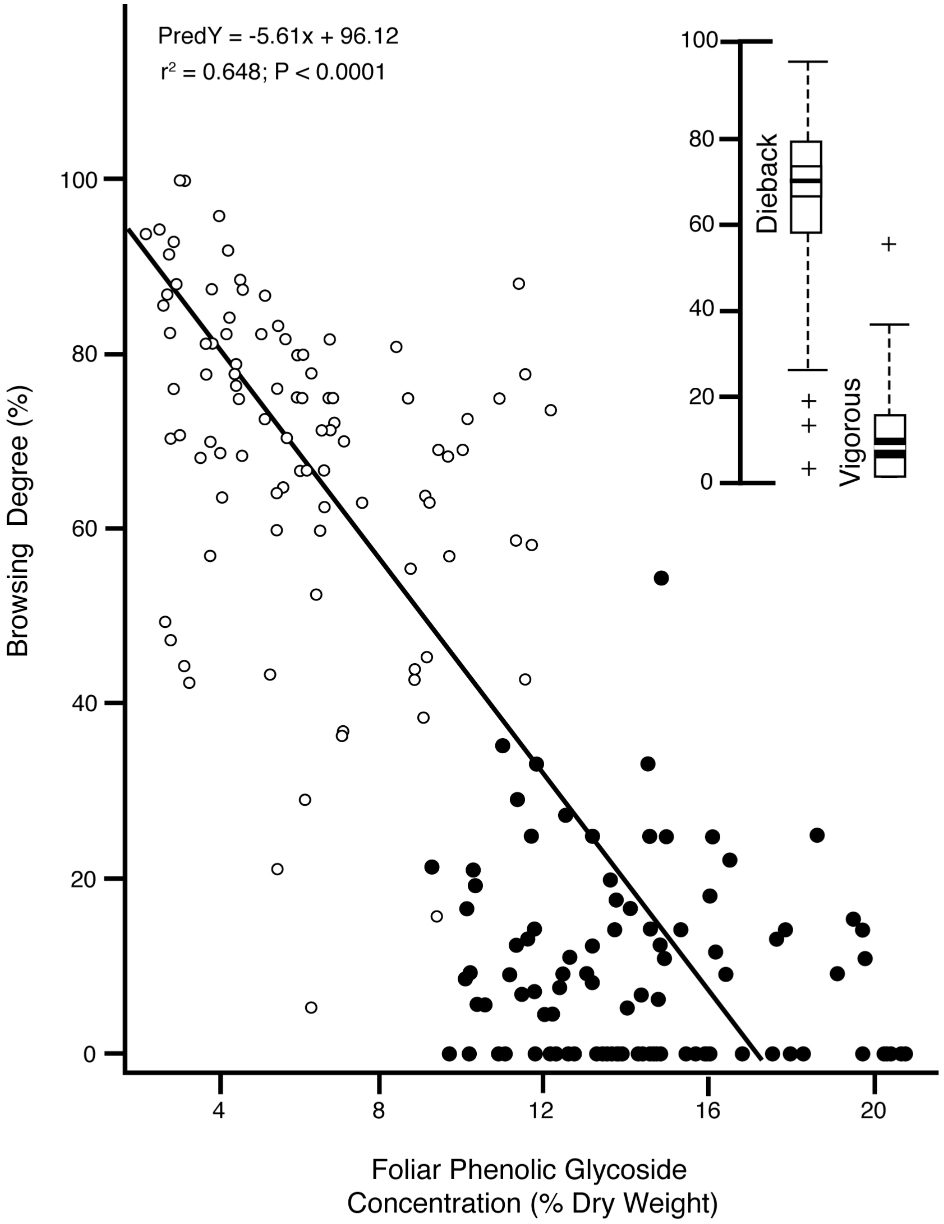


Fig. 5.5. Regression of ramet browsing and foliar phenolic glycoside concentration. 65% of the total variance is explained by the regression model ($r^2 = 0.65$); the regression line is significant, $P < 0.0001$. This relationship should not be interpretated as causal. Phenolic glycoside concentration is a risk factor that predisposes individuals to increased chances of herbivory when levels are low. The inset boxplot (upper right) indicates that differences in browsing between treatments are significant ($P < 0.001$).

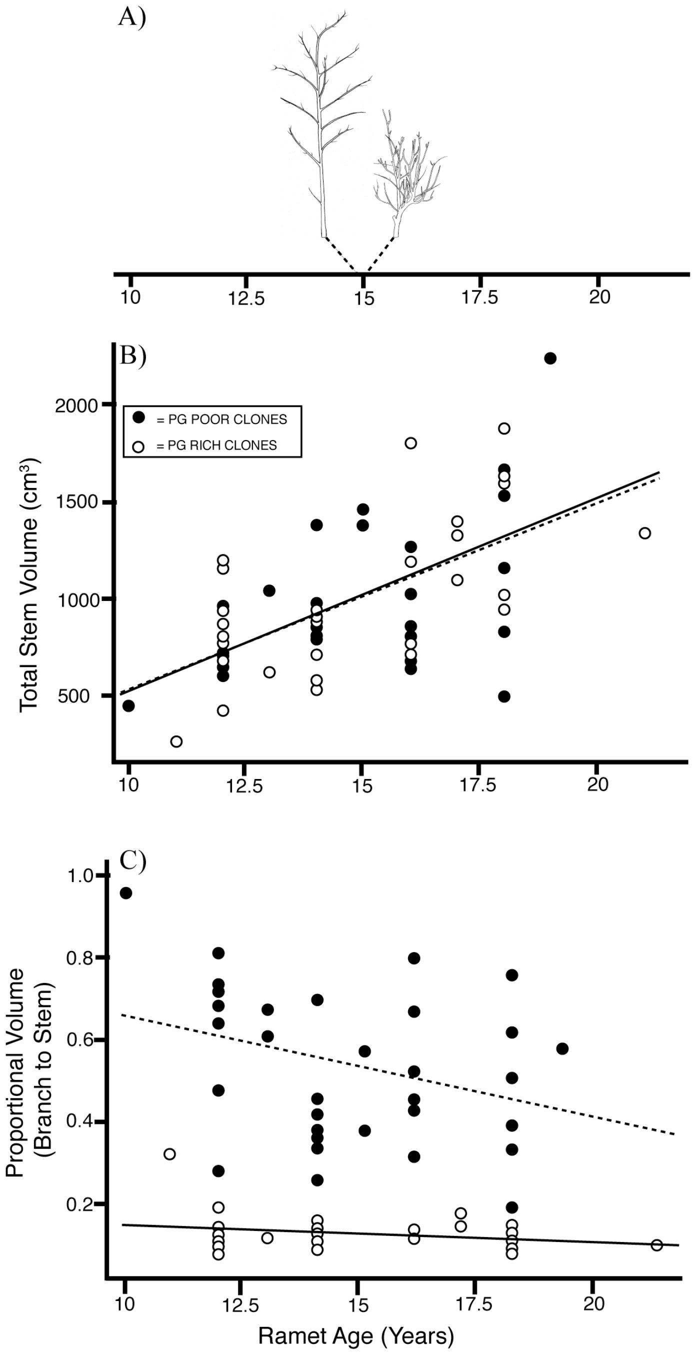


Fig. 5.6. A) Stem profiles of an aspen root-sucker at 15 years of age; drawing depict changes in stem sizing and branch density as a function of browsing intensity B) Relationship between total stem volume (= Basal area * Height (h)) age (years). C) Proportional volume (ratio of total branch volume (= S [p r2 L] / 3)) + stem volume and ramet age (years). Both figures are blocked by aspen health; phenolic glycoside (PG) rich (vigorous; n = 32) and PG poor (dieback; n = 32) clones.

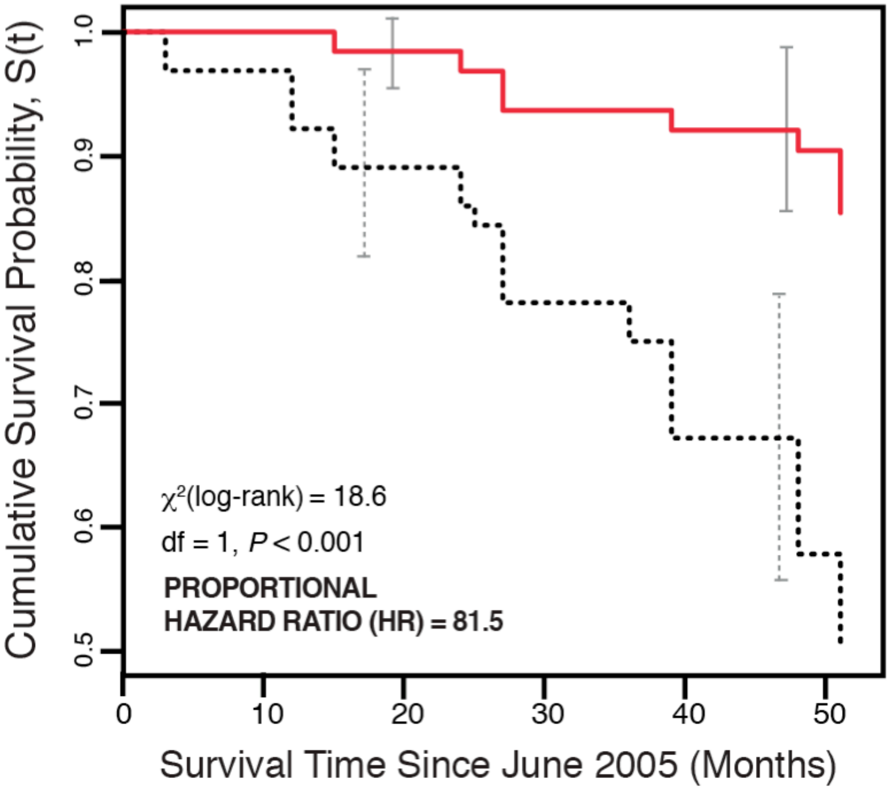


Fig. 5.7. Mortality curves (Kaplan-Meier [K-M] survival curve, S(t)) of phenolic glycoside (PG) rich (vigorous; n = 64; solid line) and poor (dieback; n = 64; dashed line) aspen clones located in sites I and II (site III was omitted from the analysis since it was burned in the spring of 2005; M (proportional mortality) = proportion of ramets that died between 2005 and 2008 (No. dead in 2008 / No. alive in 2005)). Statistical comparison (log-rank test) between the GK-M survival curves of PG rich and poor clones indicate a significant difference in survival pattern between the two treatments. Vertical bars represent 95% confidence interval.

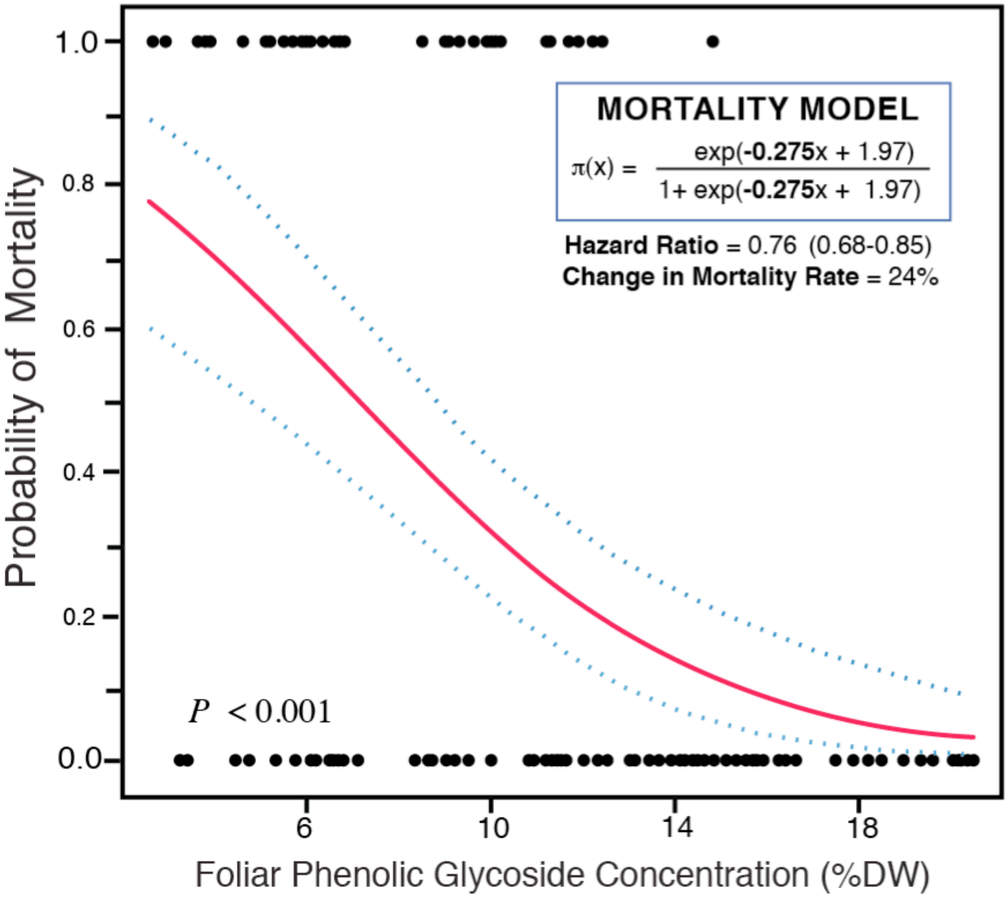


Fig. 5.8. The probability of mortality related to foliar phenolic glycoside (PG) concentration among aspen ramets. The overall predictive accuracy of this model is 71%. The likelihood ratio c2 statistic indicates that the slope coefficient (β1) of the independent variable PG is significant (G2 = - 2ln(L) = -32. 3; P < 0.001). The solid line illustrates the expected probability mortality increases as the concentration of phenolic glycosides decrease. The dotted lines show the 95% confidence interval of expected mortality probability. The model predicts that for ever unit increase in PG the probability of mortality decreases by a 3/4 (hazard ratio). The likelihood of mortality increased by 24% (CMR). At low concentrations of PG the probability of mortality is 77% while at higher concentrations the probability of mortality is < 1%. The logistic mortality-risk model indicates that leaf PG concentration influences mortality probability ($r^2 = 0.20$), it should not be interpreted as causal relationship. Clones with low levels of PG have a greater mortality probability when exposed to a cumulative set of biotic and abiotic hazards compared to clones with higher PG levels. NOTE: r^2-value = 1 - [ln(Lm/Lo)]; where Lm = likelihood of the full model, Lo = likelihood of constant (reduced model).

CHAPTER 6

A SYNTHESIS ON ASPEN ENCROACHMENT

Understanding the ecological and biological processes regulating the function of ecosystems is a principal aim of ecology, and one of the main goals of ecosystem management. An inherent feature of all natural ecosystems is that population dynamics is driven by complex ecological processes (May 1974). It is increasingly recognized that chance events provide the rules, not the exceptions, in explaining ecosystem dynamics (Hubbell 2001). Chance events modify the "predictable" trajectories of ecosystems, giving rise to a myriad of complex spatio-temporal patterns and processes. In the case of forest encroachment, deciphering critical ecological and biological processes that regulate the dynamics of trees and grassland systems requires a holistic, theoretical approach (Clark et al. 2004).

The ecological processes governing aspen clonal dynamics are ultimately determined by complex interactions with numerous biotic and environmental damaging agents over the life of the clone. Any one of these probabilistic interactions can initiate a cascade of events (Franklin et al. 1987) that alter further interactions, resulting in either rapid decline or recovery of the individual.

Despite the predictions of equilibrium models (e.g. Walker and Noy-Mier 1982; Sankaran et al. 2004), aspen population dynamics is not dependent solely on stand replacing disturbances. In the absence of strong inter-specific competition, aspen stands can persist through root-sucker regeneration following the release of apical dominance when mature canopy trees die (Cumming et al. 2000). My observations (see **Chapter 2**) of forest-grassland edges in Riding Mountain and Prince Albert National Parks revealed that aspen canopy breakup resulted in the expected establishment of root-suckers beneath the former canopy (clone regeneration), but also root-sucker production and establishment beyond the clone and into adjacent grassland habitat (clone invasion). It is this second "flush" of root-sucker regeneration that results in clonal expansion. It is also this stage of development which juvenile stems are investing photosynthates towards secondary defenses and translocating carbon from the parental rooting system for maintenance and growth.

The population dynamics of aspen clones is variable, and numerous potential trajectories are possible. Following a stand-replacing disturbance (typically a catastrophic fire), vigorous root-sucker production re-established a dense cohort that undergoes self-thinning as the clone grows (Shier and Campbell 1978). Once the stand matures (after 60-80 years), the initial even-aged ramet cohort enters the "dieback" phase. Break-up of the initial canopy results in a second flush of sucker recruitment and establishment, leading to the eventual development of clone consisting of multi-aged ramets (Mundell et al. 2008; Lennie et al. 2009; **Chapter 2**). At any time, this developmental pattern can be disrupted by any of a large number of probabilistic events. Inter-specific competition from coniferous species may prevent the successful establishment of secondary aspen root-suckers, resulting in canopy replacement and the death of aspen clones (Bergeron 2000; Frelich 2002). Alternatively, pest infestations or infection by stem fungal pathogens (e.g. *Cytospora* canker) can lead to clonal decline and mortality (Worrall et al. 2008). Stem cankers can interfere with hormone signal transduction by girdling the stem, thus impeding root-sucker production following breakup of the initial aspen cohort (**Fig. 6.1**). The incidence and severity of *Cytospora* outbreaks increases as a function of drought stress and leaf defoliation stress (Guyon et al 1996). Regular and repeated ungulate herbivory can adversely affect the normal development of aspen root-suckers, resulting in a clone consisting of heavy-browsed stems (see **Chapter 5**).

My research indicates that aspen ecology is best explained not by a single mechanism, but rather by a large number of interacting ecological factors (Higgins et al. 2000). Aspen encroachment or invasion is simply a single outcome of a complex non-equilibrium based process. Within the parkland ecoregion, aspen has coexisted with grasslands for centuries (Bird 1961). The extent of its dominance cycles in conjunction with changing environmental conditions (Baily and Wroe 1974). One of the major contributions of this dissertation emphasizes that biological and structural variation within aspen stands can be used to explain the persistence, and dynamics of this clonal species. Results of my research, characterizing differences in canopy mortality and root-sucker production (**Chapter 2**), long-term survivorship of ramets (**Chapter 3**), the consequences of physiological integration across different environments (**Chapter 4**) and the intraspecific variation in susceptibility of individuals (**Chapter 5**), clearly demonstrates that biotic and abiotic damaging agents rarely act upon populations in a uniform manner. Constraints imposed by internal biologic factors and contingencies set by random interactions with environmental factors results in a loss of uniformity in form and function among aspen clones.

GENERAL CONCLUSIONS

In **Chapter 2**, differences between encroaching and non-encroaching forest edges within four fescue grasslands was attributed to differences in density-independent mortality. The onset of mature canopy dieback might have been triggered by a multitude of gap-level processes within stands such as wind-throw, or other weather related factors (see Peltzer and Wilson 2006). The result is a single forest stand that is proliferating by means of root-suckering, extending the ecotonal boundary of its forest edges into neighboring grasslands; the other is a stable non-encroaching stand. This implies that aspen encroachment is a manifestation of periodic ramet generation within a clone. Episodes of forest encroachment coincide with a loss of abrupt ecotonal grass-forest boundaries. Boundaries become diffuse as the mature canopy deteriorates. It should be noted that one of the main differences between aspen stands in the parkland and those encountered in the US south-west is the reduced frequency of protracted drought events that happen to coincide with severe pest and pathogen outbreaks (Hogg et al. 2008; Wolken et al. 2009; Worrall et al. 2008, 2010; Rehfeldt et al. 2009). Changes in climate are expected to have adverse effects on the persistence of aspen by increasing the severity of pest and pathogen outbreaks (Hogg 2001; Hogg et al 2002). Such events would impede this natural root-sucker generation cycle leading to rapid dieback and eventual decline of affected clones.

The fact that in most grassland sites encroachment is occurring during canopy breakup implies that management efforts seeking to restore biodiversity of grasslands and open savannas will be most effective during this period. Conversely within stable, non-encroaching stands, the boundary between grass-forest is sharp. This is reflecting in the abrupt changes in understory species diversity.

Chapter 3, examined the dynamics of four aspen clones. Results form this study support the general findings of **Chapter 2**. The loss of mature aspen stems results in episodic expansion of pre-existing clonal boundaries. Clones undergoing extensive dieback respond by increasing production towards root-sucker generation. However, the long term survivorship of newly formed ramets varies dramatically. Nearly all stems > 1cm diameter experienced a significant reduction in mean relative growth rates. As stems increased in size and age the cumulative effects of stem damage from herbivores was evident. After 7 years there were significant decreases in survivorship among all the monitored ramets within each clone. This suggests that although secondary recruitment following the loss of the mature canopy leads to a flush of root-sucker recruitment, environmental constraints imposed by biotic agents limits the success of new ramets. One of the major findings of this study is that size inequalities within clones leads to differences in susceptibility to damage and mortality among ramets. This has previously been posited as a persistence mechanism within clones, assuming that the intensity of biotic stressors changes over time (Dolezal et al. 2004).

Chapter 4 examined the consequences of physiological integration among root-suckers established along the edge of encroaching clones. My results provides evidence to support the hypothesis that clonal integration is critical for the survivorship of aspen ramets invading neighboring grasslands. Findings from severing experiments suggest that this general dependency on the parental rooting system increases as environmental conditions becomes more stressful (Hartnett and Bazzaz 1985; Wang et al. 2009; Chen et al. 2010; Xu et al. 2010; Rioloa et al. 2010).

Finally **Chapter 5** directly examined the consequences of variation in adaptive ecological relevant traits among individuals. In this study I examined differences in vigor among aspen clones in relation to the production of defensive compounds (phenolic glycosides). Findings of this study demonstrate a high degree of variation in leaf phenolic glycosides production among clones. A significant amount of this variation was accounted for by differences in clone vigor (within population: *individual susceptibility hypothesis*), with a smaller amount related to environmental differences (among populations). In all instances, vigorous clones were significantly higher in levels of phenolic glycosides compared with dieback clones, suggesting that some individuals may be predisposed to undergo density-independent mortality. In addition, there were significant differences in survivorship between vigorous and dieback clones. This has important ecological implications, because it implies that one of the key mechanisms regulating population dynamics, community interactions and biodiversity may be related to intrinsic adaptive differences in susceptibility among individuals. Elucidating the various factors involved in the persistence and dynamics has important ecological and management applications. Results of my research will contributes to our understanding of the intrinsic and extrinsic factors that influence the complex dynamics of trembling aspen.

A BRIEF NOTE ON THE ECOLOGY AND MANAGEMENT OF ASPEN

Discussion regarding the dynamics of aspen clones and their interaction with nieghboring grassland communities would be amiss if one were to ignore the significant impacts of human settlement since the mid-1800s. Although there is little to debate regarding the link between decreasing fire frequency, woody plant encroachment and degraded biodiversity and ecosystem function of grasslands. Surprising little attention has been given to anthropogenically induced ecosystem change and the state of a critically important species such as trembling aspen; a species which by all accounts defines an ecosystem. Results from **chapter 5**, clearly indicates statistical differences among clones with respect to genetically determined chemical defenses. Two possible scenerios can be posited based on shifting selection pressures within a population: i) increased fire frequencies and low ungulate browing intensity would favor genets with rapid stem growth; or ii) as the mean return interval of fires and browsing intensity both increase, selection pressures will favor genets that allocate more towards defense rather than growth. If the persistence of aspen clones is based on their ability to sustain periods of root sucker intiation following canopy breakup (**chapter 2**), then the long term persistance of aspen clones is expected to decline as fire frequencies increase. This has important ecological implication as fast growing poorly defended clones will be under greater susceptability to biotic and abiotic stressors compared to clones which allocate more towards defence. This suggests that the ecological impacts of 20th century fire suppresion go beyond the degredation of grassland biodiversity to extend towards other ecoregions. The loss of frequent fires throughout the aspen parkland of Riding Mountain and Prince Albert National parks has contributed to the degeneration of aspen clones.

This despite the fact that most of these stands are at varying stages of encroachment (Figure 6.2). A return to a more climatically determined fire regime would be important not only as a means to help restore native grassland communities (following decades of fire surpression) but also to improve the condition of aspen clones within the parkland ecoregion of western Canada.

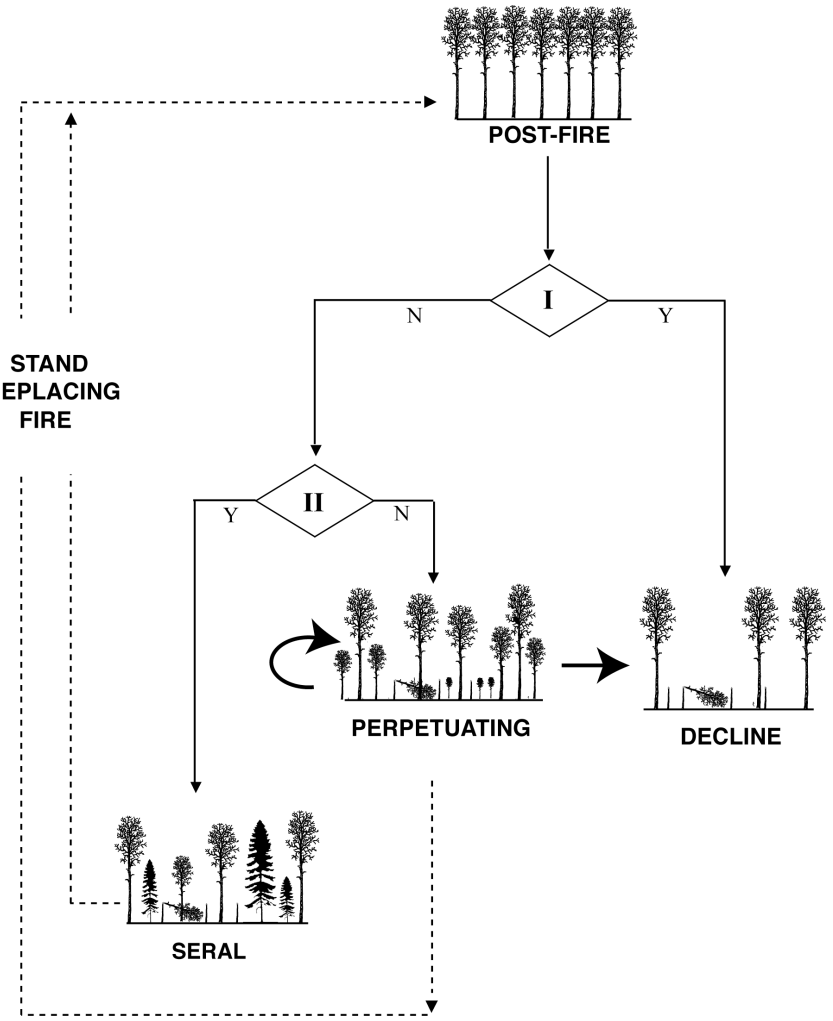


Figure 6.1. Trembling aspen (*Populus tremuloides* Michx.) stand persistence model. The persistence of aspen is contingent on the presence of conspecific competators (e.g., white spurce, **II**) and on predisposing factors (e.g. phenotypic variation on defensive compounds) that increase the likelihood of biotic agents (e.g. fungal infections, **I**) that could lead to clone death (decline). Healthly clones undergo waves of continuous root-sucker recruitment in response to the release of apical dominance as mature canopy stems dieback. Its during this phase that clone expansion (encroachment) occurs.

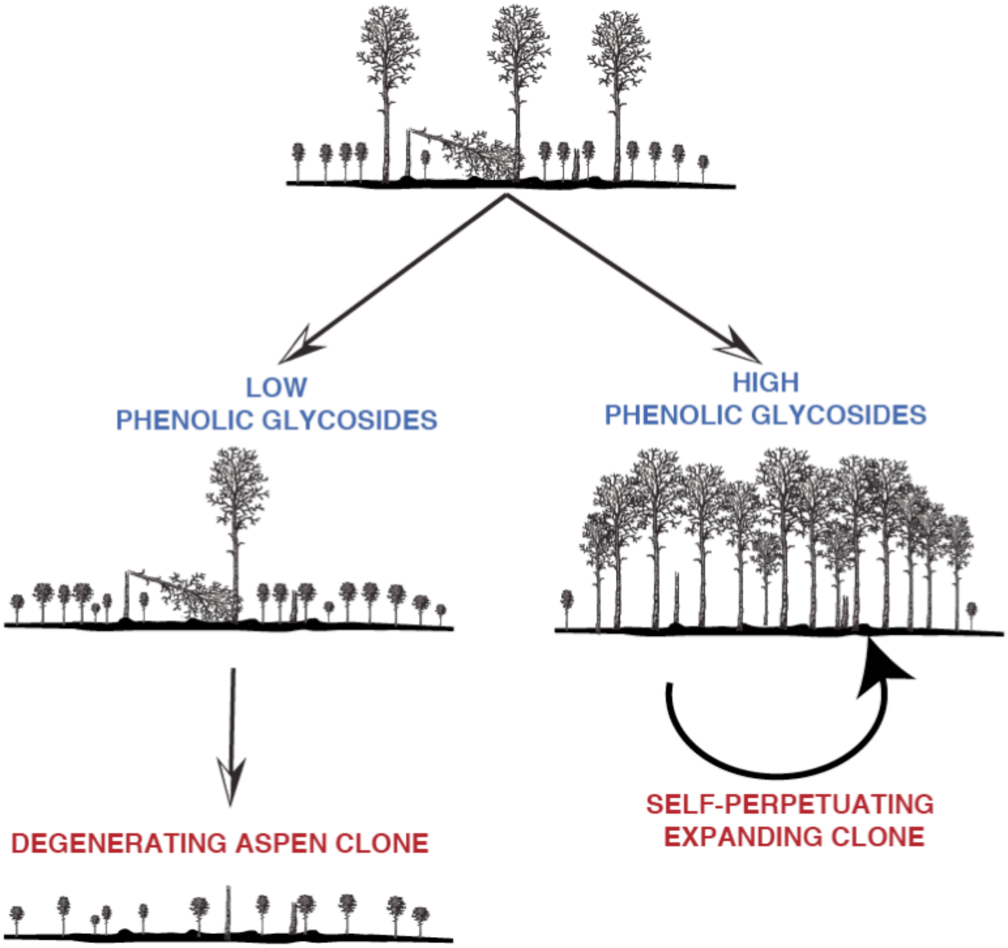


Figure 6.2. Aspen clone dynamics conceptual model. In the abence of fire, stand canopies will begin to break up around 80 – 100 years of age (top), initiating root suckering. Two trajectories are possible based on differences in inherent allocation of leaf phenolic glycosides. Clones that allocate a high concentration of leaf phenolic glycosides will establish vigorous self-perpetuating stands. Clones that do not allocate resources towards defence are under greater suspectability to become degenerating aspen clones.

GENERAL REFERENCES

Ally, D., K. Ritland, and S.P. Otto. 2008. Can clone size serve as a proxy for clone age? An exploration using microsatellite divergence in *Populus tremuloides*. Molecular Evolution 17:4887-4911..

Ally, D., K. Ritland, and S.P. Otto. 2010. Aging in a long-lived clonal tree. PLoS Biology 8:1-8.

Anderson, H.G. and A.W. Bailey. 1980. Effects of annual burning on grasslands in the aspen parkland of east-central Alberta. Canadian Journal of Botany. 58:985-996.

Archibold, O.W., E.A. Ripley, and D.L. Bretell. 1996. Comparison of the microclimates of a small aspen grove and adjacent prairie in Saskatchewan. American Midland Naturalist. 136: 248-261.

Archibold, O. W. 1999. The aspen parkland of Canada. Pages 406-416 In Anderson, R. C., J. S. Fralish, and J. M. Baskin (editors). Savannas, barrens, and rock outcrop plant communities of North America. Cambridge University Press.

Archer, S. 1990. Development and stability of grass/woody mosaics in a subtropical savanna parkland, Texas, U.S.A. Journal of Biogeography 17: 435-462.

Arno, S.F., and G.E. Gruell. 1983. Fire history at the forest-grassland ecotone in southwestern Montana. Journal of Range Management 36: 332-336.

Bailey, A. W., and R. A. Wroe. 1974. Aspen invasion in a portion of the Alberta Parklands. Journal of Range Management 27: 263-266.

Banfai, D.S., and D. Bowman. 2007. Drivers of rain-forest boundary dynamics in Kakadu National Park, northern Australia: a field assessment. Journal of Tropical Ecology 23: 73-86.

Barnes, B.V. 1966. The clonal growth habit of Amercian aspens. Ecology 47:439-447.

Baudena, M., F. D'Andrea, and A. Provenzale. 2010. An idealized model for tree-grass coexistence in savannas: the role of life stage structure and fire disturbances. Journal of Ecology 98:74-80.

Bélanger, N., and B. D. Pinno. 2008. Carbon sequestration, vegetation dynamics and soil development in the boreal transition ecoregion of Saskatchewan during the Holocene. Cantena 74:65-72.

Bergeron, Y. 2000. Species and stand dynamics in the mixed woods of Quebec's southern boreal forest. Ecology 81: 1500-1516

Bird, R.D. 1930. Biotic communities of the aspen parkland of central Canada. Ecology 11: 356-442.

Bird, R.D. 1961. Ecology of the aspen parkland of western Canada: in relation to land use. Research branch Canada Department of Agriculture, Ottawa. Contribution No. 27. 155 pp.

Blood, D. A. 1966. The *Fesctuca scabrella* association of Riding Mountain National Park, Manitoba. Canadian Field Naturalist 80:24-32.

Bond, W.J. and J.E. Keeley. 2005. Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. TRENDS in Ecology and Evolution 20:387-394

Bowman, D. M. J. S., A. Walsh, and D. J. Milne. 2001. Forest expansion and grassland contraction within a Eucalyptus savanna matrix between 1941 and 1994 at Litchfield National Park in the Australian Monsoon Tropics. Global Ecology and Biogeography 10:535-548.

Briggs, J. M., G.A. Hoch, and L. C. Johnson. 2002. Assessing the rate, mechanisms, and consequences of conversion of tallgrass prairie to *Juniperus virginiana* forest. Ecosystems 5:578-586.

Brown, J.R., and S. Archer. 1999. Shrub invasion of grassland: Recruitment is continuous and not regulated by herbaceous biomass or density. Ecology 80:2385-2396.

Brown, K. J., J. S. Clark, E. C. Grimm, J. J. Donovon, P.G. Mueller, B. C. S. Hansen, and I. Stefanova. 2005. Fire cycles in North America interior grasslands and their relation to prairie drought. PNAS 102: 8865-8870.

Browing, D.M., S.R. Archer, G.P. Asner, M.P. McClaren, and C.A. Wessman. Woody plants in grasslands: post-encroachment stand dynamics. Ecological Applications 18: 928-944.

Burkhardt, J. W., and E.W. Tisdale. 1976. Causes of juniper invasion in southern Idaho. Ecology. 57:472-484.

Cahill, J. F. Jr., S. W. Kembel, E. G. Lamb, and P. A. Keddy. 2008. Does phylogenetic relatedness influence the strength of competition among vascular plants. Perspectives in plant ecology, evolution and systematics 10:41-50.

Cameron, T.F. 1975. Fescue grasslands and associated communities in Prince Albert National Park, Saskatchewan. Parks Canada Report, Ottawa. 190 pages.

Campbell, I.D., W. M. Last, C. Campbell, S. Clare, and A. McAndrews. 2000. The late Holocene paleohydrology of Pine Lake Alberta: a comparison of proxy types. Journal of Paleolimnology 24:427-441.

Chase, J.M. 2005. Towards a really unified theory for metacommunties. Functional Ecology 19:182-186.

Chase, J.M. 2007. Drought mediates the importance of stochastic community assembly. PNAS 104:17430-17434.

Chave, J., H. Muller-Landau, S.A. Levin. 2002. Comparing classical community modules: theoretical consequences for patterns of diversity. American Naturalist 159:1-23.

Cheliak, W.M., and B.P. Dancik. 1982. Genetic diversity of natural populations of a clone forming tree *Populus tremuloides*. Canadian Journal of Genetics and Cytology. 24: 611-616.

Clark, J.S., J. Moham, M. Dietze, and I. Ibanez. 2003. Coexistence: how to identify trophic trade-offs. Ecology 84: 17-31.

Clark, J.S., S. LaDeau, and I. Ibanez. 2004. Fecundity of trees and the colonization-competition hypothesis. Ecological Monographs 74: 415-442.

Clark, J.S. 2010. Individual and the variation needed for high species diversity in forest trees. Science 327:1129-1132.

Connell, J.H., and R.O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. American Naturalist 111:1119-1114.

Cottam, W.P. and G. Stewart. 1940. Plant succession as a result of grazing and of meadow desiccation by erosion since settlement in 1862. J. For. 38:613-626.

Cowles, H.C. 1899. The ecological relations of the vegetation on the sand dunes of Lake Michigan. Parts 1-4 Botanical Gazette. 27:95-117, 167-202, 2851-308, 361-391.

Cowles, H.C. 1911. The causes of vegetation cycles. Botanical Gazette 51:161-183.

Cumming, S.G., F.K.A. Schmiegelow, and P.J. Burton. 2000. Gap dynamics in boreal aspen stands: is the forest older than we think? Ecological Applications 10:744-759.

Curtis, J.T. and R.P. McIntosh. 1951. An upland forest continuum in the prairie-forest border region of Wisconsin. Ecology 32: 476-496.

Curtis, J.T. 1959. The vegetation of Wisconsin: An ordination of plant communities. Madison, Wisc. 657 pages.

De Kroon, H., and J. Knops. 1997. Habitat exploration through morphological plasticity in two chalk grassland perennials. Oikos 59:39-49.

DesRocher, A. 2000. Aspen (Populus tremuloides Michx.) clonal root dynamics and respiration. Ph.D. thesis. University of Alberta. 102 pages.

DeWoody, J., C.A. Rowe, V.D. Hipkins, and K.E. Mock. 2008. Pando lives: molecular genetic evidence of a gient aspen clone in central Utah. Western North American Naturalist. 68:493-497.

Dix, R.L. 1961. A history of biotic and climatic changes within the North American Grassland. Contribution No. 331. Dept. of Plant Ecology, Univ. of Saskatchewan.

Diner, B. D. Berteaux, J. Fyles, and R. L. Lindroth. 2009. Behavioral archives link the chemistry of trembling aspen to food choice of North American porcupine. Oecologia 160:687-695.

Donaldson, J.R., M.T. Stevens, H.R. Barnhill, and R.L. Lindroth. 2006. Age-related shifts in leaf chemistry of clonal aspen (*Populus tremuloides*). Journal of Chemical Ecology 32:1415-1429.

Dublin, H. 1979. Vegetation dynamics in the Serengeti-Mara ecosystem: The role of Elephants, fire and other factors. In Serengeti: Dynamics of an ecosystem. Sinclair, A. R. E., and M. Norton-Griffith (Editor) Pages 71-90. Chicago, University Press.

Eckenwalder, J.E. 1996. Systematics and evolution of *Populus*. In Biology of *Populus* and its implications for management and conservation. (Editors) Stettler, R.F., H.D. Bradshaw, P.E. Heilman, and T.M. Hinckley. NRC Research Press, Ottawa, Ontario, Canada. 539 pp.

Elton, C.S. 1958. The Ecology of Invasions by Animals and Plants. London, Methuen.

Esser, L.L. 1995. *Symphoricarpos occidentalis*. In: Fischer, W.C. compiler. The fire effects information system [database]. Missoula, Mt: U.S.D.A., Forest Service, Intermountain Research Station, Intermountain Fire Sciences Laboratory.

Frelich, L.E. 2002. Forest dynamics and disturbance regimes: studies from temperate evergreen-deciduous forests. Cambridge University Press, Cambridge. 266 pages.

Frey, B. R., V. J. Lieffers, E. H. (Ted) Hogg, and S. M. Landhäusser. 2004. Predicting landscape patterns of aspen dieback: mechanisms and knowledge gaps. Canadian Journal of Forest Research 34: 1379-1390.

Fuller, L.G. 1989. Effects of forest invasion on grassland soils of the aspen parkland. M.Sc. thesis, University of Saskatchewan.

Fuller, L.G., and D.W. Anderson. 1993. Changes in soil properties following forest invasion of Black soils of the Aspen Parkland. Canadian Journal of Soil Science 73:613-627.

Gifford, G.F. 1967. The influence of growth media, temperature and light intensities on aspen root and top growth. USDA Forest Service Research Note INT-67.

Grant, M.C., and J.B. Mitton. 1979. Elevational gradients in adult sex ratios and sexual differentiation in vegetative growth rates of *Populus tremuloides* Michx. Evolution 33: 914-918.

Gunn, A., G.W. Scotter, D. Sept and L. Carbyn. 1976. Prescribed controlled burn study of fescue grasslands in Prince Albert National Park. Progress report 1. Canadian Wildlife Service. 117 pages.

Guyon, J.C. W.R. Jacobi and G.A. McIntyre. 1996. Effects of environmental stress on the development of cytospore canker of aspen.

Hennenberg, K.J., F. Fischer, K. Kouadio, D. Goetze, B. Orthmann, K.E. Linsenmair, F. Jeltsch, and S. Porembski. 2006. Phytomass and fire occurance along forest-savanna transects in the Comoe National Park, Ivory Coast. Journal of Tropical Ecology 22: 303-311.

Higgins, S.I, W.J. Bond, and W.S.W. Trollope. 2000. Fire, resprouting, and variability: a recipe for grass-tree co-existence in savanna. Journal of Ecology 88:213-229.

Hind, H. Y. 1859. A preliminary and general report on the Assiniboine and Saskatchewan exploring expedition. Queen's printer Toronto.

Hogg, E.H. and P.A. Hurdle. 1995. The aspen parkland in Western Canada: A dry-climate analogue for the future boreal forest? Water, Air and Soil Pollution 82:391-400.

Hogg, E. H., J. P. Brandt, and B. Kochtubajda. 2002. Growth and dieback of aspen forests in northwestern Alberta, Canada, in relation to climate and insects. Canadian Journal of Forest Research 32: 823-832.

Hogg, E. H., J. P. Brandt, and M. Michaelian. 2008. Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Canadian Journal of Forest Research 38: 1373-1384.

Hoffman, W., and M. Haridasan. 2008. The invasive grass, *Melinis minutiflora*, inhibits tree regeneration in a Neotropical savanna. Austral Ecology 33: 29-36.

Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton University Press.

Hult, R. 1881. Försök till analytisk behandling af växtformationerna. Meddel. af Soc. Faun. Flor. Fenn. 8:1-156.

Ives, A.R. 2007. Diversity and stability in ecological communities. *In* Theoretical Ecology. 3rd Edition. Edited by May, R. and A. McLean. Oxford University Press.

Jenny, H. 1941. Factors of soil formation: a system of quantitative pedology. Dover Publication Inc. New York.

Johnson, E.A. 1992. Fire and vegetation dynamics: studies from the North American boreal forest. Cambridge studies in ecology. Cambridge University Press, Cambridge, UK.

Jurena, P. N., and S. Archer. 2003. Woody plant establishment and spatial heterogeneity in grasslands. Ecology 84: 907-919.

Kemperman, J.A. 1977. Aspen clones: Development, variability and identification. Ministry of Natural Resources. Division of Forests. Forest research information paper No. 101. 11 pages.

Klassen, R.W. 1979. Pleistocene geology and geomorphology of the Riding Mountain and Duck Mountain areas, Manitoba-Saskatchewan. Geological Survey of Canada. 52 pages.

Köchy, M. and S.D. Wilson. 2001. Nitrogen deposition and forest expansion in the northern Great Plains. Journal of Ecology 89:807-817.

Kupsch, W.O. 1969. Radiocarbon-dated organic sediment near Hebert, Saskatchewan. American Journal of Science 258:282-292.

Lamb, E.G., and J.F. Cahill. 2008. When competition does not matter: grassland diversity and community composition. The American Naturalist 171:777-787.

Laird, K. R., S. C. Fritz, K. A. Maarch, and B. F. Cumming. 1996. Greater drought intensity and frequency before AD 1200 in the Northern Great Plains, USA. Nature. 384: 552-554.

Landhäusser, S.M., and V.J. Lieffers. 2002. Leaf area renewal, root retention and carbohydrate reserves in a clonal tree species following above-ground disturbance. Journal of Ecology 90:658-665.

Landhäusser, S.M., and V.J. Lieffers. 2003. Seasonal changes in carbohydrate reserves in mature northern *Populus tremuloides* clones. Trees 17:471-476.

Landhäusser, S.M., T.L. Mulak, and V.J. Lieffers. 2007. The effect of roots and litter of *Calamagrostis canadensis* on root sucker regeneration of Populus tremuloides. Forestry 80:481-488.

Landhäusser, S.M., D. Deshaies, and V.J. Lieffers. 2010. Disturbance faciliates rapid range expansion of aspen into higher elevations of the Rocky Mountains under a warming climate. Journal of Biogeography 37:86-76.

Lindroth, R. L., and S. –Y. Hwang. 1996. Clonal variation in foliar chemistry of quaking aspen. Biochemical Systematics and Ecology. 24: 357-364.

Lindroth, R. L., J. R. Donaldson, M. T. Stevens, and A. C. Gusse. 2007. Browsing quality in quaking aspen (*Populus tremuloides*): effects of genotype, nutrients, defoliation, and coppicing. Journal of Chemical Ecology 33:1049-1064.

Lieffers, V. J., S. M. Landhäusser, and E. H. Hogg. 2001. Is the wide distribution of aspen a result of its stress tolerance? In Sustaining aspen in western landscapes: Symposium proceedings June 13-15, 2001, Grand Junction, Colorado. Edited by W. D. Sheppard, D. L. Binkley, D. Bartos, T. J. Stohlgren, and L.G. Eskew. U.S. Dep. Agric. For. Serv. Rocky Mt. For. Range Exp. Stn. Proc. RMRS-P-18. pages 311-323.

Livingston, B. E. 1903. The distribution of upland plant societies of Kent county, Michigan. Botanical Gazatte 35:36-55.

Loehle, C, L. Bai-Lian, and R. C. Sundell. 1996. Forest spread and phase transitions at forest-prairie ecotones in Kansas, U.S.A. Landscape Ecology 11:225-235.

McGarth, J.M., D.F. Karnosky, and E.A. Ainsworth. 2010 Spring leaf flush in aspen (*Populus tremuloides*) clones is altered by long-term growth at elevated carbon dioxide and elevated ozone concentration. Environmental Pollution 158:1023-1028.

May, R.M. 1974. Stability and complexity models in ecology. 2nd Edition. Princeton University Press. Princeton, NJ.

MacArthur, R. H., and E. O. Wilson. 1967. the theory of island biogeography. Princeton University Press, Princeton New Jersey, USA.

Maini, J. S. 1960. The invasion of grasslands by *Populus tremuloides* in the North Great Plains. Ph.D. thesis. University of Saskatchewan. 231 pages.

Maini, J. S. 1972. Silvics and ecology in Canada. In aspen symposium proceedings. USDA For. Serv. Gen. Tech. Rep. NC-1. Pages 67-73.

Mock, K. E., A. Rowe, M. B. Hooten, J. Dewoody, and V. D. Hipkins. 2008. Clonal dynamics in western North American aspen (*Populus tremuloides*). Molecular Ecology 17:4827-4844.

Mundell, T.L., S.M. Landhausser, and V.J. Lieffers. 2008. Root carbohydrates and aspen regeneration in relation to season of harvest and machine traffic. Forest Ecology and Management 255:68-74.

O'Reilly-Wapstra, J. M, C. McArthur, and B. M. Potts. 2004. Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. Functional Ecology 18:677-684.

O'Reilly-Wapstra, J. M, G. R. Iason. 2007. The role of genetic and chemical variation of *Pinus sylvestris* seedlings in influencing slug herbivory. Oecologia 152:82-91

Peltzer, D. A. 2002. Does clonal integration improve comteitive ability? A test using aspen (*Populus tremuloides* [Salicaceae]) invasion into prairie. American Journal of Botany 89:494-499.

Perala, D.A. 1979. Clone expansion and competition between quaking and bigtooth aspen suckers after clearcutting. USDA Forest Service Research Paper NC-201.

Perala, D.A. 1990. *Populus tremuloides* Michx. – quaking aspen. *In* Silvics of North America. Vol. 2. Hardwoods. *Edited by* R.M. Burns and B.H. Honkala. U.S. Dep. Agric. Agric. Handbook 654. pages 555-569.

Pettapiece, W. W., and M. A. Zwarich. 1970. Micropedological study of a Chernozemic to Grey wooded sequence of soils in Manitoba. Journal of Soil Science 21:138-145.

Polly, W.H., H.B. Johnson, and C.R. Tischler. 2003. Woody invasion of grasslands: evidence that CO_2 nerichment indirectly promotes establishment of *Prosopis grandulosa*. Plant Ecology. 164:84-94.

Pregitzer, K.S., and A.L. Friend. 1996. The structure and function of *Populus* root systems. *In* Stettler, R.F., H.D. Bradshaw, Jr., P.E. Heilman and T.M. Hinckley (eds.). Biology of *Populus*. NCR Research Press, Ottawa. 539 pages.

Rehfeldt, G.E., D.E. Ferguson, and N.L. Crookston. 2009. Aspen, climate, and sudden decline in western USA. Forest Ecology and Management 258: 2353-2364.

Ritchie, R.C. 1969. Absolute pollen frequencies and carbon-14 age of a section of Holocene lake sediment from the Riding Mountain area of Manitoba. Canadian Journal of Botanty 47:1345-1349.

Ritchie, R.C., and G.A. Yarranton. 1978. Patterns of change in the late-Quaternary vegetation of the Western Interior of Canada. Canadian Journal of Botnay 56:2177-2183.

Rowe, J.S. 1955. Factors influencing white spruce reproduction in Manitoba and Saskatchewan. Technical note no. 3. Department of Northern Affairs and National Resources, Forestry Branch. Ottawa, Canada. 27 pages.

Sandberg, D. 1951. The regeneration of quaking aspen by root suckering. M.Sc. thesis, School of Forestry, University of Minnesota.

Sankaran, M., J. Ratnam, and N.P. Hanan. 2004. Tree-grass coexistence in savannas revisited insights form an examintion of assumptions and mechanisms invoked in existing models. Ecology Letters 7:480-490.

Sauchyn, D.J. and A.B. Beaudoin. 1998. Recent environmental changes in the southwestern Canadian Prains. Canadian Geographer 42:337-353.

Scheiner, S.M., and M.R. Willig. 2008. A general theory of ecology. Theoretical Ecology 1:21-28.

Schier, G.A. 1973. Origin and development of aspen root suckers. Canadian Journal of Forest Research 3: 45-53.

Schier, G.A. 1975. Deterioration of aspen clones in the middle rocky mountains. USDA forest Service Research Paper INT-170.

Schier, G.A., and R.B. Campbell. 1980. Variation among healthy and deteriorating aspen clones. USDA Forest Service Research Paper INT-264.

Scholes, R.J., and S.R. Archer. 1997. Tree-grass interactions in Savannas. Annual Review of Ecology and Systematics. 28:517-544.

Schwartz, D., H. de Foresta, A. Mariotti, J. Balesdent, J. P. Massimba, and C. Girardin. 1996. Present dynamics of the savanna-forest boundary in the Congalese Mayombe: a pedological, botanical and isotopic (^{13}C and ^{14}C) study. Oecologia 106:516-524.

Sentar Consultants Ltd. 1992. The Riding Mountain National Park: A literature review of historic timber and forest fire activity. Parks Canada 21 pages.

Simonson, J.T., and E.A. Johnson. 2005. Development of the cultural landscape in the forest-grassland transition in southern Alberta controlled by topographic variables. Journal of Vegetation Science 16:523-532.

Slogan, J. R. 1997. Long-term vegetation dynamics of plains rough fescue (*Festuca hallii*) in Riding Mountain National Park, Manitoba. M.Sc. University of Manitoba, Winnipeg, Manitoba. 177 pages.

Sole, R. V., and J. Bascompte. 2006. Self-organization in complex ecosystems. Monographs in population biology 42. Princeton University Press. Princeton.

Staver, A.C., W.J. Bond, W.D. Stock, S.J. van Rensburg, and M.S. Waldram. 2009. Browsing and fire interact to suppress tree density in an African savanna. Ecological Applications 19: 1909-1919.

Steneker, G.A. 1973. The size of trembling aspen (*Populus tremuloides* Michx.) clones in Manitoba. Can. J. For. Res. 3: 472-478.

Stevens, M. T., and R. L. Lindroth. 2005. Induced resistance in the intermediate growth of aspen (*Populus tremuloides*). Oecologia 145:298-306.

Stevens, M. T., D. M. Waller, and R. L. Lindroth. 2007. Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency. Evolutionary Ecology. 21:829-847.

Stokes, C. J., and S. R. Archer. 2010. Niche differentiation and neutral theory: an integrated perspective on shrub assemblages in a parkland savanna. Ecology 91:1152-1162.

Swetnam, T.W., C.H. Baisan, P.M. Brown, A.C. Caprio and R. Touchan. 1990. Late Holocene fire and climate variability in Giant Sequoia groves. Bulletin of the Ecological Society of America. 71:342-360.

Throop, H.L. and S.R. Archer. 2008. Shrub (*Prosopis velutina*) encroachment in a semidesert grassland: spatial-temporal changes in soil organic carbon na d nitrogen pools. Global Change Biology 14:2420-2431.

Tilman, D. 1982. Resource competition. Princeton University Press. Princeton, N.J.

Trottier, G.C. 1974. Range studies, Riding Mountain National Park. Vols. I and II. Unpubl. Report. Parks Canada, RMNP.

Trottier, G.C. 1986. Distribution of rough fescue, *Fescue hallii*, grassland by livestock grazing I Riding Mountain National Park, Manitoba. Canadian Field Naturalist. 100:488-495.

Van Auken, O. W. 2000. Shrub invasions of the North American semiarid grasslands. Annual Review of Ecology and Systematics. 31:197-215.

Vogl, R.J. 1974. effects of fire on grasslands. *In* Fire and ecosystems. Eds. Kozlowski, T.T. and C.E. Ahlgren. Academic press, New York. 542 pages.

Walker, B.H. and I. Noy-Meir. 1982. Aspects of stability and reliance of savanna ecosystems, In: Ecology of tropical Savannas. Editors, Walker, B.J., and B.H. Huntley. Springer-Verlag, Berlin. 556-590 pages.

Wier, J.M.H, and E.A. Johnson. 1998. Effects of escaped settlement fires and logging of foresr composition in the mixedwood boreal forest. Canadian Journal of Forest research 28: 459-467.