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THE CAUSES OF RARITY OF BLUNT-LOBED WOODSIA
AT THE NORTHERNMOST MARGIN OF ITS DISTRIBUTION

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BY
MATTHEW WILD

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LES CAUSES DE LA RARETÉ DE LA WOODSIE À LOBES ARRONDIS
À LA PÉRIPHÉRIE NORD DE SON AIRE DE DISTRIBUTION

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PRÉSENTÉE
COMME EXIGENCE PARTIELLE
DU DOCTORAT EN BIOLOGIE

PAR
MATTHEW WILD

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RÉSUMÉ

Au Canada, un nombre élevé d'espèces de fougères ne se trouve que dans des habitats très restreints et spécialisés et n'ont qu'un faible nombre de populations connues, contenant seulement quelques individus. Ceci est reflété par le nombre élevé de fougères par rapport aux autres plantes vasculaires que l'on retrouve sur les listes d'espèces en péril. Un exemple de ceci est la woodsie à lobes arrondis (*Woodsia obtusa*), qui, bien que très commune vers le centre de son aire de répartition, est extrêmement rare dans la partie septentrionale de son aire de répartition dans le sud du Canada, où elle est connue à partir de seulement huit populations contenant peu d'individus. Des travaux récents ont montré que de l'habitat propice est disponible pour cette espèce à l'intérieur et à proximité de ses populations existantes au Canada. Par conséquent, il semblerait que, contrairement au paradigme qui suggère que les fougères sont principalement limitées par la disponibilité de l'habitat propice en raison des faibles limitations à la dispersion et à l'établissement, la woodsie à lobes arrondis est limitée dans sa capacité à disperser ses spores et/ou à recruter des individus et/ou à survivre en tant que sporophyte, puisque ces limitations sont les seules explications probables de non-saturation de l'habitat propice. Par une série d'expériences de terrain et de laboratoire, nous tentons de déterminer si une ou plusieurs des étapes du cycle de vie de la woodsie à lobes arrondis sont affectées négativement par certains facteurs environnementaux à la partie septentrionale de son aire de répartition, ce qui aurait comme effet de limiter la capacité de l'espèce à saturer l'habitat propice et donc d'établir plus de populations que l'on en retrouve actuellement. Ces expériences sont séparées en trois volets : i) la dispersion, ii) le recrutement des gamétophytes et des sporophytes et iii) la survie du sporophyte.

Pour mesurer la dispersion des spores, nous avons recueilli des échantillons de sol à l'intérieur et autour (jusqu'à 50 mètres) de deux populations de l'espèce et nous les avons incubées dans des conditions propices pour favoriser la germination des spores et le développement ultérieur des gamétophytes. Des spores viables de woodsie à lobes arrondis ($n = 638$) ont germé dans plus de la moitié des échantillons de sol prélevés (69/130). Sur les 638 spores qui ont germé, 571 provenaient des sols situés à l'intérieur de cinq mètres du centre des populations, montrant un patron très inégal (leptokurtique) de dispersion des spores. Bien que nos résultats montrent que des spores viables se dispersent dans l'habitat propice, un manque apparent de recrutement *in situ* suggère que certains facteurs, notamment les taux faibles d'humidité dans l'habitat xérique de la woodsie à lobes arrondis au Canada, limite l'établissement de nouveaux individus, réduisant ainsi la dispersion fonctionnelle à un minimum.

Pour le recrutement des gamétophytes et sporophytes, des spores de woodsie à lobes arrondis ont été recueillies dans deux populations. Ces spores ont ensuite été semées dans des conditions contrôlées au laboratoire afin de mesurer les facteurs affectant le recrutement du gamétophyte et du sporophyte. Les paramètres testés étaient les suivants: le pH, l'intensité de la lumière, l'humidité initiale, l'arrosage ultérieur et la densité des spores. Pour le recrutement des gamétophytes, un pH acide est un facteur limitant, et l'arrosage et la lumière réduite avaient un effet positif faible, mais significatif. Pour les sporophytes, des niveaux extrêmes de pH (4 et 8,5) limitent le recrutement, et les deux traitements d'eau (humidité initiale et arrosage hebdomadaire) augmentent significativement le recrutement. Les résultats suggèrent que les faibles niveaux d'eau observés dans les habitats naturels de la woodsie à lobes arrondis au Canada limitent le recrutement des gamétophytes et, surtout, le recrutement des sporophytes. Ceci suggère que la niche de l'espèce est très réduite au Canada comparativement à celle observée dans le centre de son aire de répartition.

Pour la survie des sporophytes, des sporophytes de six mois cultivés en serre ont été transplantés dans des parcelles expérimentales dans deux populations naturelles et la survie de ces sporophytes a été suivie pendant quatre ans. Dans chaque parcelle, des données environnementales ont été recueillies. Des modèles de régression logistique multivariée ont été construits pour expliquer quels facteurs environnementaux ont significativement affecté la survie. La survie des sporophytes transplantés était plus élevée au parc Frontenac (27,4%), où l'ouverture du couvert forestier était la plus importante (13,5%). Après quatre ans, tous les individus survivants produisaient des sporanges. Les principaux facteurs environnementaux favorables à la survie des sporophytes sont un haut niveau de luminosité et une faible quantité de litière.

Ces études expérimentales ont révélé un certain nombre de facteurs limitants agissant à des degrés différents à toutes les étapes du cycle de vie de la woodsie à lobes arrondis. Toutefois, le principal facteur expliquant la rareté de la woodsie à lobes arrondis au Canada semble être les différences observées entre les niches des deux générations de son cycle de vie (gamétophyte et sporophyte). En fait il semblerait que, contrairement à la plupart des exemples tirés de la littérature, que dans ce cas, les niches du gamétophyte et du sporophyte sont très différentes, et montrent très peu de chevauchement. Les résultats montrent que le recrutement du gamétophyte et du sporophyte requièrent de l'eau, mais que les quantités d'eau nécessaires pour ces deux types de recrutement sont rarement trouvées dans les habitats xériques où les sporophytes adultes de la woodsie à lobes arrondis ont le plus de succès.

Bien que l'exploitation de niches différentes à des étapes distinctes d'un cycle de vie soit un phénomène relativement commun chez les espèces animales mobiles (par exemple les amphibiens et les insectes), ceci s'avère un énorme handicap pour une espèce sessile, signifiant que pour s'établir un individu doit se trouver au bon endroit au bon moment pour que les deux générations de son cycle de vie puissent bénéficier

de conditions favorables. Cela pourrait potentiellement expliquer la rareté de plusieurs espèces de fougères (ou d'autres taxons sessiles à deux générations distinctes) à la limite de leur aire de distribution, et pourrait être au moins en partie, sinon entièrement responsable du patron de rareté observé chez de nombreuses espèces de fougères.

Mots clés : Woodsie à lobes arrondis, dispersion, établissement, rareté, survie, théorie de la niche

ABSTRACT

In Canada, a relatively large number of fern species occur in highly restricted and specialized habitats and have low numbers of known populations containing few individuals. This is reflected by the high ratios of ferns versus other vascular plants occurring on local lists of vulnerable, threatened and endangered species. An example of this is Blunt-lobed Woodsia (*Woodsia obtusa*), which, although common throughout most of its distributional range, is extremely rare in the northernmost part of its range in southern Canada, where it is known from only eight populations containing relatively few individuals. Recent work has shown that suitable habitat is available for the species within, and in proximity to, its extant populations in Canada. Therefore, it would appear that, contrary to the common paradigm, which suggests that ferns are mainly limited by the extent of available suitable habitat due to few limitations to dispersal and establishment, Blunt-lobed Woodsia is somehow limited in its capacity to disperse and/or recruit new individuals and/or survive as established individuals, as such limitations are the only likely explanation for empty available and suitable habitat. Here with a series of field and laboratory experiments, we attempt to identify if one or more of Blunt-lobed Woodsia's life cycle stages are being negatively affected by some environmental factor at the northernmost part of its range, thereby limiting the species' capacity to saturate suitable, available habitat, and therefore to establish more populations than are currently found. These experiments are divided into three parts: i) dispersal, ii) gametophyte and sporophyte recruitment, and iii) sporophyte survival.

To measure spore dispersal we collected soil samples from the immediate vicinity (up to 50 meters) of two northern populations and incubated them in suitable conditions to promote spore germination and subsequent gametophyte development. Viable Blunt-lobed Woodsia spores (n=638) germinated from over half of the collected soil samples (69/130). Of the 638 germinated spores, 571 were from soils located within five meters of the population centres, showing a highly skewed (leptokurtic) pattern of spore dispersal. Although our results show that viable spores of Blunt-lobed Woodsia are dispersing to available habitat, an apparent lack of *in situ* recruitment suggests that some factor, most likely lack of moisture in its xeric habitat in Canada, is limiting the establishment of new individuals, thereby reducing functional dispersal to a minimum.

For gametophyte and sporophyte recruitment, spores of *Woodsia obtusa* were collected from two populations. These spores were then sown in controlled conditions in the laboratory to measure which factors affected gametophyte and sporophyte recruitment. Tested parameters were: medium pH, light intensity, initial humidity, subsequent watering and spore density. For gametophyte recruitment, an acidic pH

was limiting, and watering and reduced light had a small, but significant, positive effect. For sporophytes, extreme pH levels (4 and 8.5) limited recruitment, and both water treatments (initial humidity and weekly watering) significantly increased recruitment. Results suggest that the low levels of water observed in Blunt-lobed *Woodsia*'s natural habitats in Canada do limit gametophyte and, especially, sporophyte recruitment. This suggests that the species' niche is much reduced in Canada compared to that observed in the center of its distributional range.

For sporophyte survival, six month old greenhouse grown sporophytes were transplanted into experimental plots in two study populations and sporophyte survival was monitored for the following four years. In each plot, environmental data were also collected. Multivariate logistic regression models were built to explain which environmental factors significantly affected survival. Survival of transplanted sporophytes was highest at Frontenac Park (27.4 %), where canopy openness was greatest (13.5 %). After four years, all surviving plants were producing sporangia, thus reproducing. The principal environmental factors beneficial to survival were high levels of light and low amounts of litter.

These experimental studies revealed a number of limitations acting to different degrees at each stage of the fern life-cycle, however, the main factor in explaining Blunt-lobed *Woodsia*'s rarity in Canada appears to be the difference between the niches of the two generations of its life-cycle (gametophyte and sporophyte). In fact it would appear, contrary to most examples from the literature, that in this case, the niches of the gametophyte and that of the sporophyte are very different, and possibly overlap very little. Results show that to be successful, both gametophyte and sporophyte recruitment require water, however, the amounts of water required for both types of recruitment are rarely found in the xeric habitats in which Blunt-lobed *Woodsia* sporophytes are most successful.

Although exploiting different niches for separate stages of a life-cycle is a relatively common occurrence in motile animal species (e.g. amphibians, insects), it is a huge handicap for a sessile species, meaning that to be successful an individual must find itself in the right place at the right time for both generations of its life-cycle to benefit from suitable conditions. This could likely be an explicative factor for the rarity of many fern species (or other sessile taxa with two distinct generations) at the limits of their distribution range, and could be at least partly, if not mostly responsible for the rarity pattern observed in numerous fern species.

Keywords: Blunt-lobed *Woodsia*, dispersal, establishment, rarity, survival, niche theory

CHAPTER I

GENERAL INTRODUCTION AND CONTEXT

1.1 Context

In Canada, a relatively large number of fern species occur in highly restricted and specialized habitats and have low numbers of known populations containing few individuals. This is reflected by the high ratios of ferns versus other vascular plants occurring in local lists of vulnerable, threatened and endangered species (e.g.: Argus and White, 1977; Argus and White, 1983; Bouchard *et al.*, 1983; Labrecque and Lavoie, 2002; Wild *et al.*, 2006; CDPNQ, 2008).

Compared to angiosperms, the distribution of ferns is widely reported to be more directly related to climate and substratum (Barrington, 1993; Given, 1993; Marquez *et al.*, 1997). This assertion, coupled with the frequently repeated (but rarely tested) premises that homosporous ferns are not limited by dispersal (Tryon, 1970; 1986) or establishment (Smith, 1972; 1993), due to their production of numerous small, self-fertilizing spores, suggests that the distribution of ferns is mostly determined by factors of climate and habitat, or microclimate and microhabitat (Marquez *et al.*, 1997).

Given the aforementioned hypotheses, the absence of individuals of a fern species from a site should be associated with adverse local habitat or microhabitat factors. However, previous work (Wild, 2003; Wild and Gagnon, 2005) suggests that this is not always the case. In summary, these studies compared biotic and abiotic habitat data from plots containing individuals of five locally rare fern species (*Asplenium rhizophyllum* L., *Asplenium ruta-muraria* L., *Asplenium scolopendrium* var. *americanum* (Fernald) Kartesz & Gandhi, *Pellaea atropurpurea* (L.) Link, and *Woodsia obtusa* subsp. *obtusata* Sprengel) with data from nearby plots without any individuals. For all five study species, comparisons revealed no significant differences between the plots containing individuals and those without. These results

suggest that the patchy distributions observed for these rare fern species are not due to lack of available habitat, as suitable habitat seems to be locally available.

Therefore, it would appear that, contrary to the common paradigm, they are somehow limited in their capacity to disperse and/or establish new individuals and/or survive as established individuals, as such limitations are the only likely explanation for empty available and suitable habitat.

Based on the above, I decided to investigate the dispersal, establishment and survival limitations of Blunt-lobed *Woodsia* (*Woodsia obtusa* subsp. *obtusa*) at the northernmost part of its distributional range in southern Quebec and Ontario. This requires the identification of the limiting stage or stages in the species' life cycle, using experimental tests of dispersal, establishment and survival. This should allow us to answer the question: In the specific case of Blunt-lobed *Woodsia* in Canada, what exactly causes its rarity, and on a more general level, how can this be transposed to other species showing similar patterns of rarity (ie. other ferns, other species limited to a specific bedrock, etc.)?

1.2 Rarity

Before commencing the study of a rare species, one should address the definition of rarity. Many authors have attempted to define biological rarity. Perhaps the most elegant definition comes from Reveal (1981), who states: "...rarity is merely the current status of an extant organism which, by any combination of biological or physical factors, is restricted either in numbers or area to a level that is demonstrably less than the majority of other organisms of comparable taxonomic entities." Several different schemes have also been proposed to categorize the forms of rarity. Of note are Rabinowitz's (1986) seven-class scheme based on local population size (small vs. large), geographic range (narrow vs. wide) and habitat specificity (restricted vs. broad), and Fiedler and Ahouse's (1992) four-class scheme based on spatial

distribution (narrow vs. wide) and taxon persistence (short vs. long). A lot of the proposed schemes have abundance and range size as the two common denominators, but many other variables have been suggested, including habitat specificity, taxonomic distinctness and persistence through ecological or evolutionary time (Gaston, 1994). Notwithstanding these attempts to clarify the concept of rarity, the determination of a given species' "rarity status" can often be arduous. Species can be common in parts of their distributional range and rare in others. This presents an interesting dilemma. Why put effort into the conservation of a species that is locally rare but common and secure elsewhere? Ecologists are divided on the importance of this question. On the one hand, species that are not globally endangered can be perceived as being of lesser conservation significance than species that are, if the perspective taken is the survival of species. On the other hand, it has been suggested that isolated peripheral populations are important reservoirs of genetic material, because they are adapted to more extreme conditions, which may increase their chance of survival in times of environmental stress, or allow expansion into new territory (Lesica and Allendorf, 1995). I subscribe to the latter view, and believe that detailed studies of the ecology and distribution of any plant, rare or common, can potentially benefit our understanding of many other species.

The fact that species tend to show a pattern of abundance which is highest in the centre of their ranges, and that declines gradually toward its boundaries has often been shown (Brown, 1984 and references therein). Several studies have been undertaken on the specific ecologies and dynamics of peripheral populations in the northernmost range of their known distribution. Seminal work was accomplished in this respect on populations of *Tilia cordata* Mill. in northwestern England (Pigott and Huntley, 1978; 1979; 1981). More recent examples include studies on trees (*Pinus rigida* Mill.: Meilleur *et al.*, 1997, and *Acer rubrum* L.: Tremblay *et al.*, 2002), shrubs (*Fumana procumbens* (Dunal) Gren. & Godr.: Bengtsson, 1993, and *Rhus aromatica*

Ait.: Nantel and Gagnon, 1999), annual herbs (*Floerkea proserpinacoides* Willd.: McKenna and Houle, 2000), perennial herbs (*Helianthus divaricatus* L.: Nantel and Gagnon, 1999), ferns (*Trichomanes speciosum* Willd.: Rumsey *et al.*, 1999), and bryophytes (several spp.: Hedderson, 1992). The aforementioned studies arrive at various conclusions regarding the causes of relative rarity for these species at their northern distributional limit. These causes include lack of suitable habitat (*P. rigida*), infrequent sexual recruitment (*A. rubrum*), low reproductive capacity of adult individuals (*F. procumbens*), increased demographic variability and local extinction probability (*R. aromatica* and *H. divaricatus*), infrequent long-distance dispersal events and highly variable conditions among years (i.e. high environmental stochasticity) (*F. proserpinacoides*), low spore production with little recruitment of sporophytes (*T. speciosum*), and limited dispersal potential (bryophytes).

This gives rise to the central question of this study: What causes rarity in the specific case of Blunt-lobed *Woodsia* in the northernmost part of its range? Before addressing causality, the following section provides information on what is known of the biology, the distribution and the ecology of the study species.

1.3 Study species

1.3.1 Description

Blunt-lobed *Woodsia* (*Woodsia obtusa*) is a small to medium sized fern with fronds up to 60 cm long and 15 cm wide (Figure 1.1). It is characterized by the blunt, rounded lobes of its fronds, which are retained late into the fall. The leaf stalk (rachis) is straw-coloured, occasionally darker at its base, and is not articulate but relatively brittle. The blade is coarsely cut and evidently 2-pinnate with the proximal pinnules of the lower pinnae usually shallowly lobed or merely dentate. In the field, Blunt-

lobed Woodsia is most often confused with Fragile Fern (*Cystopteris fragilis*) but it has a stiffer aspect, glands and scales on the axes and veins, as well as opaque stipes. Blunt-lobed Woodsia comprises two cytotypes that are often treated as subspecies because they show subtle morphological and ecological distinctions, and tend to have different distributions. Tetraploid populations (subsp. *obtusa*) are found throughout the eastern part of its range, commonly occurring on limestone. The diploid (subsp. *occidentalis*) is found near the western edge of the species range, usually on sandstone and granitic substrates. It has been hypothesized that Blunt-lobed Woodsia might be an autopolyploid derived from *W. oregana* (Brown, 1964). However, isozyme and spore ornamentation studies indicate that these species are not closely related, and the discovery of a diploid cytotype of Blunt-lobed Woodsia suggests a different origin for this taxon. Blunt-lobed Woodsia has been known to hybridize with *W. oregana* subsp. *cathcartiana* (B. L. Robinson) Windham to form the sterile tetraploid hybrid known as *W. × kansana* Brooks (Flora of North America Editorial Committee, 1993).

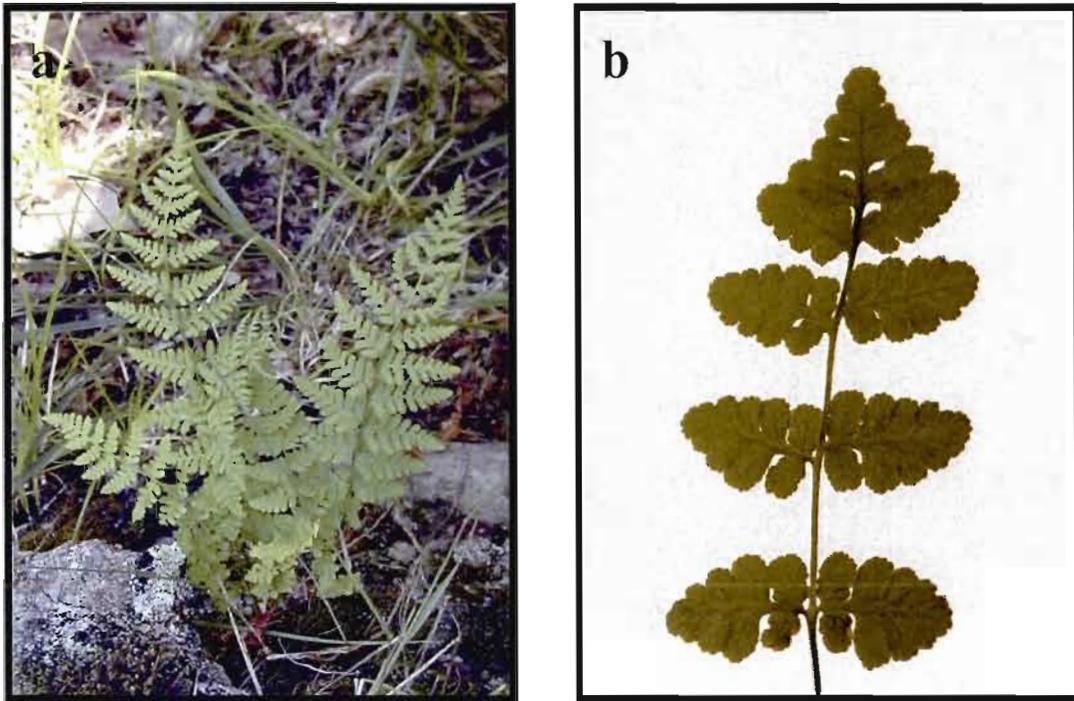


Figure 1.1. Blunt-lobed Woodsia. a) habit; b) frond.

1.3.2 Habitat

All Canadian populations of Blunt-lobed Woodsia are found in the Great Lakes-St. Lawrence Forest Region (Rowe, 1972) and all are located on calcareous rock slopes (marble, dolomite, limestone) with a southern aspect. The habitat of the species is generally forested, consisting of scattered, relatively small trees (10 to 15m high; 10 to 20 cm diameter breast height). Canopy openness ranges from fairly closed (~7% in Gatineau Park, QC) to moderately open (~20% in Frontenac Park, ON). One population is atypically found in a very open site on a rock outcrop in an abandoned field. Shallow soils over bedrock and the overall xeric nature of the sites are the major cause for the small stature of the trees, although trees appear to be relatively young at several sites, indicating some recent disturbance (i.e. harvesting of stems, fire, etc.).

Generally associated tree species are Sugar Maple (*Acer saccharum*), Red Oak (*Quercus rubra*), White Oak (*Q. alba*), White Ash (*Fraxinus americana*) and Ironwood (*Ostrya virginiana*). The shrub layer has low cover on sites where Sugar Maple and Red Oak dominate (Quebec sites). Poison Ivy (*Rhus radicans*), and Prickly Gooseberry (*Ribes cynosbati*) are the most frequent shrub species on these sites. In all Ontario sites (although to a lesser degree for the Frontenac Park population), Red Oak and White Oak dominate, Eastern Red Cedar (*Juniperus virginiana*) is relatively common, and shrubs such as Fragrant Sumac (*Rhus aromatica*) and Downy Arrow-wood (*Viburnum rafinesquianum*) are abundant. The herb layer is very well developed and is most often dominated by Pennsylvania Sedge (*Carex pensylvanica*). Other commonly found associated herbaceous species are Bottlebrush Grass (*Elymus hystrix*), Bluestem Goldenrod (*Solidago caesia*), Marginal Shield Fern (*Dryopteris marginalis*), and Herb-Robert (*Geranium robertianum*). Data from six Canadian sites show an average soil depth of 3.5 cm (range: 0.5 - 9.5 cm) on an average slope of 43° (range: 26 - 88°) under an average 82% closed canopy (range: 40 - 96%) (Wild and Gagnon, 2005). Soil data collected from Canadian Blunt-lobed Woodsia populations show a pH range of 5.9 to 7.1, with an average of 6.6, and a high average soil calcium content of almost 10 000 ppm (Wild and Gagnon, 2005).

Although in some populations the habitat of Blunt-lobed Woodsia shows signs of minimal disturbance over the past several years, no site appears to have been dramatically modified. The sites are generally on relatively steep rock faces or bedrock outcrops, unsuitable for development or agriculture. Most sites are located within abandoned or rarely used areas that are not likely to suffer from direct human disturbance (COSEWIC, 2007).



Figure 1.2. Typical *Woodsia obtusa* habitat in Canada; shallow soils over south-facing, calcareous bedrock.

1.3.3 Distribution

Blunt-lobed *Woodsia* occurs throughout the eastern United States, occurring in all states east of the 100th parallel (except North Dakota and South Dakota), reaching north to south-eastern Canada (Ontario and Quebec) and extending south to south-central Texas and northern Florida (Figure 1.3). It is generally not found on the Atlantic coastal plain (Brown, 1964). However, the species is extremely rare in Canada, occurring in only eight known populations containing no more than a few hundred individuals each, whereas further south, towards the main part of its distributional range (see Figure 1.3), it is extremely common. For example, on their 26 ha study site in Iowa, Peck *et al.* (1990) estimated *W. obtusa* to account for almost 30% of the entire fern abundance (10000 plants out of a total of 35300). These

discrepancies can largely be explained by the fact that *W. obtusa* reaches the northernmost limit of its range in southern Canada.



Figure 1.3. Distribution map of Blunt-lobed Woodsia in North America (modified from Flora of North America Editorial Committee (1993)).

The extreme northern limit of this species' range just reaches into southern Ontario and Quebec; in fact, no Canadian populations are situated more than 100 km from the US border. The first Canadian report of Blunt-lobed Woodsia was from Missisquoi County in southern Quebec in 1936 by Belval and Raymond (Cinq-Mars, 1969). The Canadian extent of occurrence for this species is estimated to be approximately 14 000 km². This is based on an area, triangular in shape, ranging from Frontenac Park, ON., north to Gatineau Park, QC, and east to the Quebec populations at Saint-Armand/Frelighsburg. This amounts to less than 1% of the species' global range. The species' area of occupancy in the Canadian range is estimated to be less than 1 km². Of the eight known populations, four occur in Ontario and four in Quebec (Figure 1.4).



Figure 1.4. Location of the eight known Canadian populations of Blunt-lobed Woodsia.

The four Quebec populations are found in two distinct regions, separated by several hundred kilometres. Three of these populations are found on dolomite in Missisquoi County, the other one occurs on marble bedrock slopes along the Eardley Escarpment in Gatineau Park.

All four populations in Ontario are found on the Frontenac axis, a southern extension of the Precambrian shield, extending south across the Ottawa River to the Rideau Lakes area, through the Gananoque and Brockville area, and crossing the St. Lawrence River to connect with the Adirondacks in the United States. Three populations occur within a few kilometres of each other along the north shores of Big Rideau Lake and Sand Lake near Westport. The other is located in Frontenac Park, approximately 20 km southwest from the others.

1.3.4 Status

NatureServe ranks *W. obtusa* subsp. *obtusa* as G5 (globally secure) and as N5 (nationally secure) in the US. It is ranked "at risk" in the following states: Florida, Maine and Michigan (S1, critically imperilled); Delaware, New Hampshire and Rhode Island (S2, imperilled); and Vermont (S3, vulnerable). In Canada it is ranked as N1, and both S1 in Ontario and Quebec (NatureServe, 2006) (Figure 1.5). Blunt-lobed Woodsia is listed as threatened in Canada and is on Schedule 1 of the federal Species at Risk Act (SARA). In Ontario, the species is listed as Endangered and is protected under the Endangered Species Act (S.O. 2007, Chapter 6). As a listed species, Blunt-lobed Woodsia is given consideration under Ontario's Provincial Policy Statement and Provincial Parks Act. In Quebec, the species has recently (August 2005) been legally designated threatened under the *Loi sur les espèces menacées ou vulnérables* (L.R.Q., c. E-12.01).

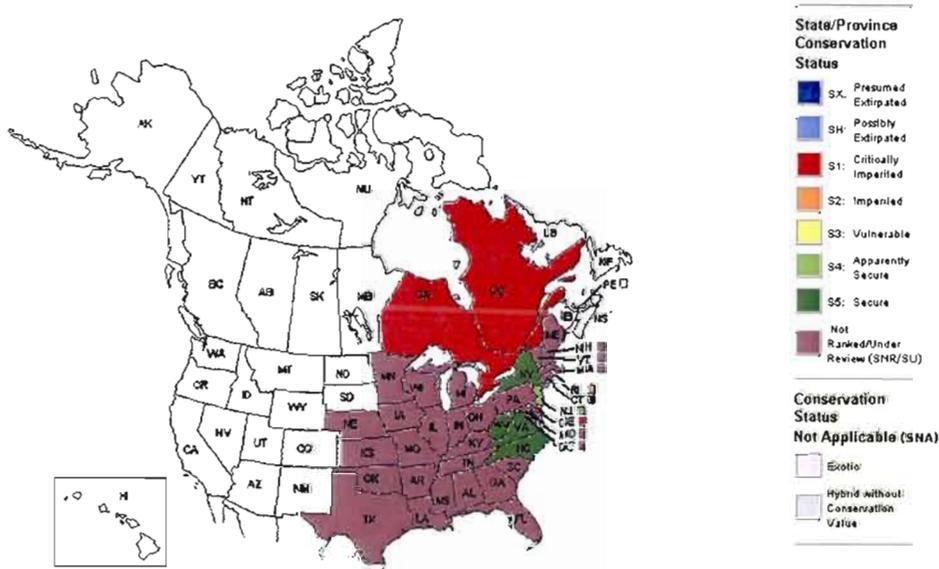


Figure 1.5. NatureServe rankings for Blunt-lobed Woodsia in North America (modified from NatureServe (2006))

1.3.5 Biology

Most of the general biological knowledge for this species comes from Brown's *A monographic study of the fern genus Woodsia* (1964). More recently, research on habitat characteristics and available habitat has been undertaken in Canadian populations. Results from these studies have been reported in Wild (2003), Wild and Gagnon (2005) and COSEWIC (2007).

1.3.5.1 Life cycle and reproduction

Blunt-lobed *Woodsia* is a perennial homosporous fern species. Spore production occurs annually towards the end of summer and beginning of fall. This fern is known to be capable of producing high quantities of spores; Peck *et al.* (1990) estimated 60 million spores per individual in Iowa. In culture, spores take from six to 15 days to germinate and the production of sporophytes takes from three to five months (Brown, 1964). This species is not known to reproduce asexually, although (as is the case for most homosporous pteridophytes), bisexual gametophytes can be produced and self fertilize, resulting in a sporophyte that is 100% homozygous (Peck, 1980).

1.3.5.2 Physiology

Although there is evidence that biologically the species may have weedy characteristics (Brown, 1964), it is quite possible that water limitation plays a large role in limiting the number and size of Blunt-lobed *Woodsia* populations in Canada. Bryan and O'Kelley (1967) showed that an absence of calcium in their growing substrate precluded the formation of archegonia, antheridia and sporophytes, thereby demonstrating the necessity for soil with high calcium content.

1.4 Causality

According to Gaston (1994), attempting to understand the causes of rarity for any particular taxon can be approached in three different ways. One approach is to compare the biology of rare taxa with related common congeners (e.g. Kunin and Gaston, 1993; Bevill and Louda, 1999). Another possible approach is to search for correlations between species' abundances or geographic range sizes and any plausible factor that might cause rarity (e.g. Thompson *et al.*, 1998; 1999; Gaston *et al.*, 2000). The third option is the autoecological approach, or the experimental investigation of the ecological and biological parameters of an individual taxon in order to determine what traits limit its distribution and abundance. Though widely accepted as the most thorough method for an individual species, this approach has sometimes been criticized as generating a limited payoff, i.e. an intensive effort is required in exchange for results pertaining solely to an individual species (Gaston, 1994; Murray *et al.*, 2002). However, it is quite possible that the results obtained by this method can be extrapolated to plants with similar ecologies; in this case other ferns. This is suggested by Grime (1985) who, in addressing detailed autoecological studies, claims: "... it seems reasonable to conclude that useful generalizations can be applied not only at the level of individual species, genera and families but, perhaps surprisingly, in relation to *the ecology of pteridophytes as a whole.*" (italics in original). In this study, I propose this type of autoecological approach to attempt to explain what causes the rarity of Blunt-lobed *Woodsia* in southern Canada.

Determining what causes rarity is complicated by the fact that it may result from a combination of numerous factors. The possibilities I envisage for the rarity of Blunt-lobed *Woodsia* in the northernmost part of its range appear in Figure 1.6. A detailed explanation and discussion of each category follows, with emphasis on those categories that pertain to my hypotheses.

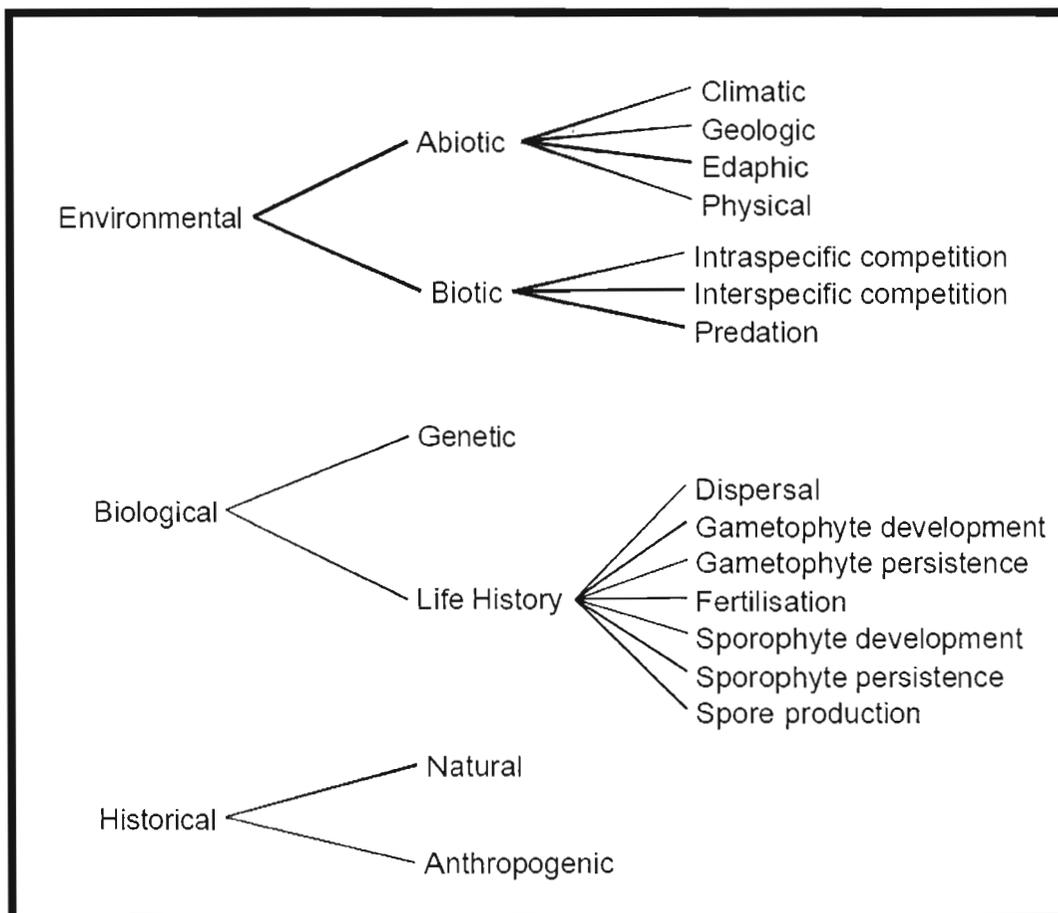


Figure 1.6: Possible causes for the rarity of Blunt-lobed Woodsia in Canada

1.5 Possible causes for the rarity of Blunt-lobed Woodsia in Canada

1.5.1 Environmental factors

1.5.1.1 Abiotic

Abiotic environmental factors include climatic, geologic, edaphic and physical variables. Climate is definitely a limiting factor for *W. obtusa* in Canada. This is evident when we consider that in Canada, all known populations are found on south-facing slopes under semi-open, mostly deciduous canopy in xeric conditions (COSEWIC, 2007), whereas further south, *W. obtusa* occurs on north-facing slopes

under dense deciduous canopy cover in mesic conditions (Greer *et al.*, 1997). Although this suggests that a favourable, warm microclimate is required in order for this species to exist in the northern part of its range, it does not explain the absence of the species in habitats with the same microclimate conditions in areas adjacent to extant populations. A similar argument can be made for geology; that it is limiting at a large scale, in the sense that without high levels of calcium in the underlying bedrock (i.e. marble, limestone, dolomite, etc.), *W. obtusa* does not occur. This does not explain why, at a local scale (over the same rock substrate), it is not found more frequently. As for edaphic and physical variables, although the niche of *W. obtusa* appears relatively restricted in Canada, our previous work suggests that these are not limiting, as we observed unoccupied microhabitat with suitable edaphic and physical conditions for *W. obtusa* in the immediate vicinity of extant populations (Wild, 2003; Wild and Gagnon, 2005).

One explanation for the patchiness of known Blunt-lobed *Woodsia* populations in Canada could be linked to a lack of water in its habitats at the northernmost limit of its distribution. When comparing habitat attributes, it is obvious that the habitat in Canada is very different from where the species is found further south. This lack of water and how it affects the different stages of the life cycle of the species is the basis of the main hypothesis of this study and is developed in the section on the species' life history.

1.5.1.2 Biotic

Possible biotic causes of rarity include both intra- and interspecific competition, and predation. Intraspecific competition is known to occur in natural fern populations, among sporophytes (Page, 1979; Watson and Vazquez, 1981), among gametophytes (Tryon and Vitale, 1977; Schneller *et al.*, 1990) and between the two (Munther and Fairbrothers, 1980). Based on the distance between individuals in Canadian populations of *W. obtusa*, relative to the actual size of the plants, it is unlikely that

intraspecific competition is a factor for sporophytes. Interactions among gametophytes are usually related to the production of antheridiogen (Schneller *et al.*, 1990). Antheridiogen is a gametophyte-released hormone that stimulates nearby gametophytes to develop antheridia only, leading to an increase in cross fertilization (FNA, 1993). Although Peck and Peck (1986) showed an antheridiogen effect in *W. obtusa*, they also found sporeling production to be density-independent, which suggests that intraspecific competition among gametophytes is not a factor. As for competition between sporophytes and gametophytes, it has been shown that some fern sporophytes release phenolic compounds resulting in lower germination rates and inhibited growth of gametophytes (Munther and Fairbrothers, 1980). However, even if *W. obtusa* has the potential to be autotoxic, I suspect that the sparseness of these populations and their xeric nature would reduce the effect of water-soluble phenols to a negligible level. Interspecific competition can also affect both sporophyte and gametophyte generations (Page, 1979), although in a low plant cover habitat, such as the calcareous rock habitat studied here, interspecific competition is expected to have little effect. Indeed, data collected in Canadian *W. obtusa* populations show a low richness and cover of both vascular and non-vascular plant species (Wild, 2003). In the case of predation, it has been found that significantly fewer phytophagous insects (only 465 documented species) attack ferns compared to flowering plants (over 357000 species). This equates to a ratio of approximately 1 species of insect for every 19 species of fern ($465 / 9000 = 1:19.35$) as opposed to more than 1 species of insect per flowering plant ($357000 / 275000 = 1:0.77$) (Hendrix, 1980). As for grazing herbivores, ferns appear to be generally unpalatable to herbivores (Page, 1979), and no evidence of grazing on *W. obtusa* individuals, even in areas with high White-tailed deer (*Odocoileus virginianus*) density has been reported.

1.5.2 Historical factors

Historical factors can be of natural or anthropogenic origin, and need to be considered at two scales: the large scale, where they affect a species' "extent of occurrence" (*sensu* Gaston, 1994) and the local scale, where they affect a species' "area of occupancy" (*sensu* Gaston, 1994). An example of a large-scale effect was introduced by Willis (1922), with his age-area hypothesis, where range size is a direct function of species' age. While this may be true, and responsible for the overall range of *W. obtusa* in North America, this would not appear to be a causative factor for its sparse distribution in Canada, as apparently suitable, but empty sites fall well within the species' "extent of occurrence". Possible natural factors affecting distribution at a small scale include time since last disturbance and successional stage of extant vegetation. Anthropogenic causes to species' distributions can be attributed to the effects of pollution, habitat modification and/or destruction, and direct harvesting. Pollution has been shown to have a negative effect on some fern species (Ashenden *et al.*, 1990; Lawrence and Ashenden, 1993), but is unlikely to have any significant impact on Canadian populations of *W. obtusa*, relatively remote from large urban or industrial pollution sources, at least no more so than on their southern counterparts. As for habitat modification, the extreme rockiness and strong slopes of the sites where *W. obtusa* occurs in Canada generally precludes it from development.

Harvesting of ferns for commercial purposes is negligible in North America. They have few practical uses, and are of relatively slight economic importance (Lellinger, 1985). Amateur collecting has been blamed for causing the rarity of several species in Europe, especially in Britain where the "pteridomania" craze of the 19th century caused wide damage to the native fern flora (Allen, 1969). One well documented example is that of the Oblong woodsia (*Woodsia ilvensis* (L.) R. Brown) (Dyer *et al.*, 2001). In North America, however, collection is considered to have had a negligible impact on natural fern populations. Historical events are among the most difficult to

deduce. According to Gaston (1994), they may frequently account for the tally of species for which causality cannot be readily determined. Based on this, no direct attempt to evaluate historical factors will be made in this study, but their effect will be retained as an alternative hypothesis.

1.5.3 Biological factors

1.5.3.1 Genetics

The study of genetics has long been a large part of pteridology (Wagner, 1974), generating a large amount of literature (e.g. Klekowski, 1979; Haufler, 2002 and references therein). Much of the research on fern genetics has specifically addressed homosporous ferns and their ability to inbreed extensively through bisexual gametophytes. Because of the complications that the fern life cycle imparts on genetics, separate terms are needed to describe homosporous breeding systems. Klekowski (1979) suggests the terms intergametophytic crossing, intergametophytic selfing and intragametophytic selfing. Intergametophytic crossing is the equivalent of true outcrossing, where an antherozoid from the gametophyte of one sporophyte fertilises an archegonium on the gametophyte from another sporophyte. Intergametophytic selfing occurs when fertilisation is achieved between two gametophytes from the same sporophyte, and intragametophytic selfing, when fertilisation occurs between antheridia and archegonia of a same gametophyte. The latter results in a sporophyte that is 100% homozygous. This capacity for absolute inbreeding has important implications for fern genetics (Haufler, 2002). Another genetically important factor in pteridophytes is their propensity for polyploidy, with estimates for ferns ranging from 30% to as high as 95% (Soltis and Soltis, 2000). Polyploids are reputed to have increased vigour, increased ability to tolerate cold and an enhanced capacity for colonisation relative to diploids (Rabe and Haufler, 1992). Soltis and Soltis (2000) present the results from several studies on the potential for intragametophytic selfing of polyploid ferns, concluding that the available evidence

for ferns, while limited, suggests reduced inbreeding depression in polyploids. Although it is probable that the isolated populations of *W. obtusa* in Canada are the result of a single spore colonizing event, implying complete homozygosity, it is likely that the negative components of inbreeding, such as inbreeding depression and the related effects of deleterious recessive genes, are countered by the polyploid nature of *W. obtusa* occurring in these populations, as suggested by Soltis and Soltis (2000).

1.5.3.2 Life history

The life history of an organism is the history of changes it undergoes from inception or conception, to death; in other words, its life cycle. Figure 1.7 illustrates the typical life cycle of a homosporous fern divided into seven sequential steps: i) spore dispersal; ii) spore germination; iii) gametophyte development; iv) fertilisation; v) sporophyte development; vi) sporophyte persistence; vii) spore production.

Theoretically, each of these stages has the potential to be affected by a limiting factor in fern species. In the following step-by-step analysis of the fern life cycle, I will consider spore germination (ii) and gametophyte development (iii) as one step (gametophyte recruitment) and fertilisation (iv) and subsequent sporophyte development (v) as another step (sporophyte recruitment).

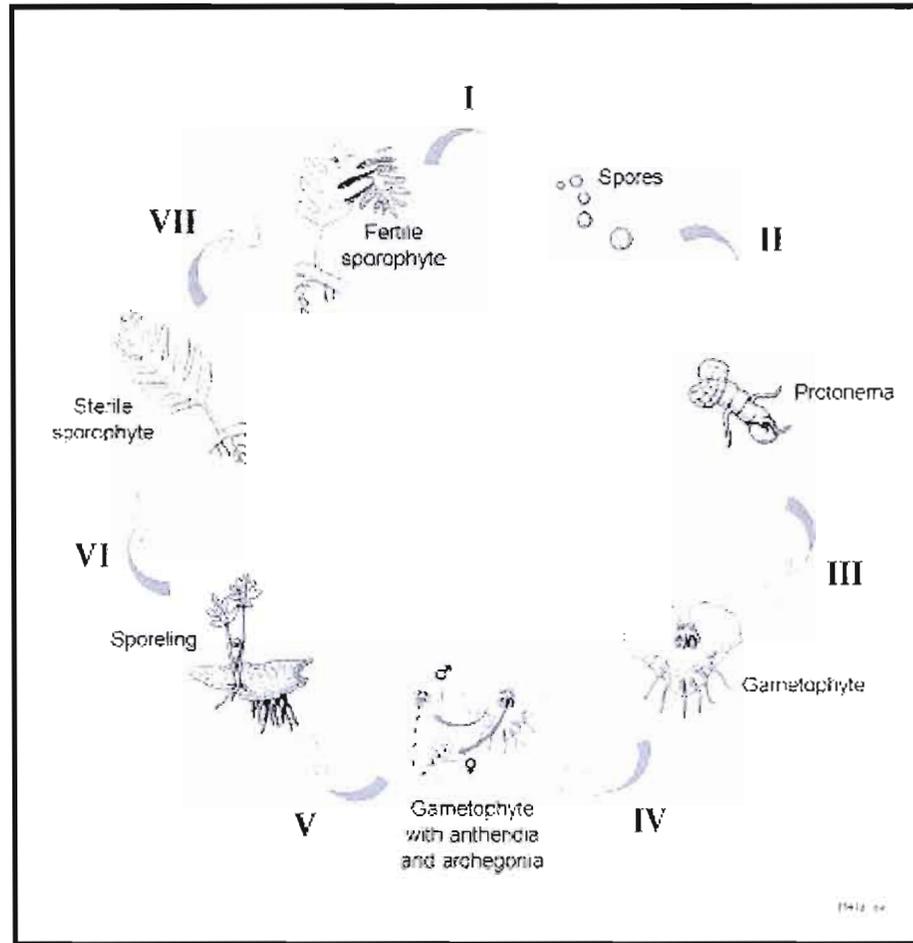


Figure 1.7. Typical life cycle of a homosporous fern

Dispersal

Dispersal is not considered to be a limiting factor for ferns. This claim originates from biological aspects of ferns in general and from biogeographical data of specific fern species. Biological aspects include the astronomical number of spores produced, their small size and surprisingly long viability (Page, 1979). Biogeographical data is based on certain species' large distributional ranges (Tryon, 1970; Smith, 1972), the rapid colonisation of naturally occurring virgin areas (Page, 1979 and references therein) and examples of disjunct fern populations suspected to have arisen through long-distance dispersal of spores (Bouchard *et al.*, 1977; Cousens, 1981; Wagner and Rouleau, 1984; Brunton, 1986; Kato, 1993). Most of the reasons stated above are

based on anecdotal evidence, relatively little empirical work has attempted to actually quantify fern spore dispersal. The objective of Chapter III of this thesis is to quantify Blunt-lobed *Woodsia* spore dispersal at a local scale.

Gametophyte recruitment

The effects of varying conditions on spore germination have been investigated for several fern species (e.g. Pangua *et al.*, 1994; Kiss and Kiss, 1998; Quintanilla *et al.*, 2000). In their recent study on spore germination in *Cheilanthes feei* Moore, Nondorf *et al.* (2003) varied levels of pH, temperature, light intensity, light quality and moisture, concluding that although spores germinated under a wide variety of conditions, optimal conditions for spore germination and for subsequent protonemal growth overlap narrowly. Spore germination for *W. obtusa* was quantified by Peck *et al.* (1990) where, under suitable conditions, they observed over 82% germination for spores sown on natural substrates and 93% for those sown on agar. As for gametophyte development, Watson and Vasquez (1981) suggest that ferns that do not reproduce asexually produce gametophytes of greater vigour than ferns capable of asexual reproduction, implying some sort of trade-off between sexual and asexual reproduction. Interestingly, this is also reflected in data collected by Peck *et al.* (1990), where out of a possible 14 species, they found naturally occurring gametophytes of only six. Out of these six, four are not known to reproduce asexually. *Woodsia obtusa* is included among these four species. Based on the above, it would appear that, as long as conditions are suited, the gametophytic stage of *W. obtusa* is not a limiting factor. However, I suspect that the conditions in Canadian populations of Blunt-lobed *Woodsia* are limiting on gametophyte recruitment. Chapter IV of this thesis examines the effects of different environmental conditions on gametophyte recruitment through a series of *in vitro* experiments.

Sporophyte recruitment

In ferns, sporophyte recruitment occurs when a sporeling (young sporophyte) arises from a gametophyte. Most homosporous ferns require water for sporophyte recruitment, as antherozoids need a film of water in order to travel from antheridia to archegonia. Exceptions to this are sometimes encountered in xeromorphic species such as cheilanthoid ferns (Pteridaceae: Cheilanthoidae), which are capable of apogamy. In the most common type of apogamy, fertilization is not required for production of a sporophyte, which instead originates directly from the tissues of the gametophyte. Apogamy is not known to occur in *W. obtusa*, and therefore, I suspect that the low levels of water encountered in the xeric environment of its northernmost habitats greatly affect this stage of its life cycle. Another factor that could potentially limit sporophyte recruitment in fern populations is the “isolate potential” of spores and gametophytes. Spore isolate potential is calculated as the percentage of isolated spores producing sporophytes, and gametophyte isolate potential as the percentage of mature isolated gametophytes producing sporophytes. In their study on Midwestern fern populations, Peck *et al.* (1990) determined isolate potential for *W. obtusa* spores at 66% and gametophyte isolate potential at 80%. It has been suggested that a high percentage of spore germination, a high percentage of bisexuality of isolated gametophytes and the apparent absence of a genetic load in *W. obtusa* all contribute to favour successful sexual reproduction in local habitat (Peck and Peck, 1986). It is expected that this potential also exists in Canadian populations, but I suspect it is limited by environmental factors encountered in their northern habitat. The effect of various environmental factors on Blunt-lobed *Woodsia* sporophyte recruitment is addressed in Chapter IV of this thesis.

Sporophyte persistence

It is important to underline here that the fern life cycle is made up of two distinct and very different phases: the long-lived, iteroparous sporophyte and the short-lived, semelparous gametophyte. This poses somewhat of a problem, called by Page (1979)

“an ecological handicap”. Simply put, this implies that the sporophyte can only occur in a habitat that has previously proved itself suitable for a gametophyte. Intuitively, this suggests that if a small, thalloid non-vascular gametophyte was able to grow, then a large, leafy, vascular sporophyte should also be able to. Therefore, I do not expect this stage to be limiting for *W. obtusa*. In order to verify this, sporophytes can be transplanted to areas where the species does not occur. Long-term growth and survival of these individuals will indicate that some other life history stage is limiting the presence of the species in these areas. Chapter V of this thesis looks at the long term survival of transplanted Blunt-lobed Woodsia individuals into apparently suitable habitat.

Spore production

Blunt-lobed Woodsia is known to be capable of producing high quantities of spores; Peck *et al.* (1990) estimated 60 million spores per individual of *W. obtusa* in Iowa. However, low seed production has been recognised as a factor in limiting the distribution of species towards the periphery of their ranges (Pigott and Huntley, 1978; 1981), and differential spore production has also been shown to occur in a fern species along an environmental gradient (Arens, 2001). This is not expected to be limiting in Blunt-lobed Woodsia as previous observations has shown similar spore production in Canadian populations of the species (M. Wild, unpublished data), and hundreds of plants have been produced from single fronds of the species (see Chapter V).

1.6 Why is Blunt-lobed Woodsia rare in Canada? A general hypothesis

The Canadian habitat of Blunt-lobed Woodsia is semi open sites, on flat areas with shallow soils and frequent bedrock outcrops, or on steep south-facing cliffs, or cliff crests, where outcrops are also frequent (Wild *et al.*, 2005; COSEWIC, 2007). The nature of the bedrock is always calcareous (dolomite, marble). These characteristics

are quite different from those occurring in the habitat of the species within the central part of its range, which are described as mesic (Brown, 1964; Carlquist *et al.*, 1997). Also, Blunt-lobed *Woodsia* does not appear to have a strict association with calcareous bedrock within the central part of its range (FNA, 1993).

In Canada, the habitat of Blunt-lobed *Woodsia* has a warmer and dryer microclimate than the adjacent mesic forests, where Sugar Maple (*Acer saccharum*) dominates. A warmer microclimate in temperate latitudes de facto creates a dryer microclimate, evident on south-facing steep slopes, where above average solar radiation is received, with its effect on evapotranspiration, combined with the physical effect of the slope which rapidly evacuates any rainfall. Why would Blunt-lobed *Woodsia* occur in such a warmer/dryer microhabitat/microclimate at the northern edge of its range in southern Canada? This is not unusual and has often been observed in other plant species (Red Oak, Ginseng, Sugar Maple, etc.). The ultimate cause of this phenomenon, for Blunt-lobed *Woodsia* and other species, may perhaps be that snow cover is of shorter duration, or the growing season longer, in warmer microclimates, and more similar in duration to what they are further south, nearer to the centre of the distributional range of these species.

It has been proposed that the niches of plants become more restricted, narrower, near the margins of their distribution range (Brown, 1984). How would this concept transfer to the two distinct generations in the life cycle of ferns? In the gametophyte generation, small, ephemeral and hardly ever noticed, male gametes (antherozoids) require a film of water to swim from one end of the gametophyte towards female organs (archegonia) situated at the other end in order to achieve fertilisation. Fertilisation leads to creation of the sporophyte generation which produces spores. Successful spore germination produces a gametophyte. Indeed, we find that niche/habitat differences between the gametophyte form and the sporophyte form of

fern species have been documented (Watson and Vazquez, 1981; Kingston and Hayes, 2005).

If two separate niches exist, how different are they? Assuming, from the literature (eg. Watson and Vazquez, 1981; Kingston and Hayes, 2005), that they are, our hypothesis is that they will vary individualistically along the environmental gradient from the centre of the range to its margins, and in Blunt-lobed *Woodsia*'s particular case to the northern margin of its range. Furthermore, these two separate niches could diverge along this gradient, potentially resulting in a very narrow overlap at the northern margin of the species' range. That an overlap of these two niches exists is however an absolute requirement in order for both fern life forms to physically co-occur, and thus for reproduction to occur. A narrow overlap of both niches could be the cause of rarity of *Woodsia obtusa* in southern Canada, with sporophytes surviving and growing best in warmer/dryer microclimates/habitats, whereas gametophytes must occur in more moist and shaded habitats, with a less warm and dry microclimate, in order for fertilisation to occur.

The occurrence of two niches is not a concept unique to ferns. It has been observed in trees, where the "regeneration niche" is much different (germination of seeds and establishment of seedlings) than the niche of mature forest trees (Grubb, 1977). Nor is it unique to the plant world, as animals often have two distinct life stages in their life cycle (larval and adult stages), such as in the case of insects, where the larval stage may even be aquatic (i.e. mosquito, dragonfly, mayfly, etc.).

This general hypothesis is the underpinning of this thesis, whose objective is to determine the nature of the limitations preventing Blunt-lobed *Woodsia* from being as abundant in southern Canada as it is near the centre of its range. To achieve this objective, I have used field and laboratory experiments in order to determine in which part of the life cycle of Blunt-lobed *Woodsia* are limiting factors occurring to reduce

the species' success in southern Canada. In Chapter II, I explore the general rarity of fern taxa compared to other plants, and discuss several of the most likely causes for this rarity. In Chapter III, I address the role played by limitations in spore dispersal as a potential cause of rarity for Blunt-lobed *Woodsia* and test the hypothesis that although there are viable spores in soils surrounding extant Blunt-lobed *Woodsia* populations in Canada, a highly leptokurtic distribution pattern of spores greatly limits the long-distance dispersal potential for the species. In Chapter IV, I carry out a series of laboratory experiments aimed at identifying potential limiting factors at the spore germination stage of Blunt-lobed *Woodsia*, as well as at the gametophyte stage, which determines fertilisation success and sporophyte production. The hypothesis that water is the main environmental factor limiting recruitment is tested, which would explain why both gametophyte and sporophyte recruitment are naturally low in this species because of the effect of the low levels of water found in the xeric habitat of Blunt-lobed *Woodsia* in Canada. In Chapter V, I address limiting factors to sporophyte survival and growth, up to spore production, in Blunt-lobed *Woodsia*'s Canadian habitat with a four-year sporophyte field transplantation experiment and test the hypothesis that once established, Blunt-lobed *Woodsia* sporophytes are not limited by the xeric nature of their habitat at the northern limit of the species' distribution range.

It is important to note that the *ex situ* experiments presented in this thesis (Chapters III and IV), were not an attempt to quantify absolute values of the different parameters tested (pH, light, H₂O) that the species requires, but rather which, if any, of these environmental factors have a limiting effect on the various life stages of Blunt-lobed *Woodsia*. The experimental ranges of the environmental variables were selected based on previous studies on species with similar ecologies to Blunt-lobed *Woodsia* and in some cases represent extremes aimed at detecting a significant response.

CHAPTER II

WHY ARE FERNS REGULARLY OVER-REPRESENTED ON STATE AND PROVINCIAL RARE PLANT LISTS?

Matthew Wild, Daniel Gagnon, and André Bouchard

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Abstract

Several recent studies have suggested that rare species are not randomly distributed throughout plant taxa. This would appear to apply to North American ferns, which are frequently over-represented on local lists of rare plant species. However, such lists often paint a skewed portrait of the true situation because of our tendency to recognize the rarity of well-known and charismatic species while ignoring that of lesser-known, or less appreciated species. In order to verify if this over-representation of ferns is a real and consistent trend throughout local floras in North America, we used data from what we consider to be the most complete and objective available database: NatureServe Explorer (<http://www.natureserve.org/explorer/>). We compiled data on total vascular plant species, total fern species as well as rare vascular plant species and rare fern species for each North American subnational (Canadian province or US state) flora. Rare species were defined as those belonging to one of NatureServe's "at risk" categories. The null hypothesis that the contribution of rare ferns to total rare species did not differ from their contribution to the total vascular flora was assessed using χ^2 . Out of 64 subnational floras, we obtained significantly higher values than expected in 28 cases, and significantly lower in only one case. Similar trends hold true for individual fern families. These tendencies could be related to several factors of anthropogenic, biological, climatological, evolutionary, and geographical origin. However, we believe that the main reason is related to scale, namely the geopolitical units at which rarity is often studied. Our results illustrate one of the problems of a parochial approach to conservation, where the perceived rarity of an entire taxon is exaggerated because of the scale at which rarity is addressed.

Key Words: Aspleniaceae, Dryopteridaceae, Ferns, Monilophytes, NatureServe, Ophioglossaceae, Pteridaceae, Rarity

2.1 Introduction

Several recent studies have investigated whether rare species are randomly distributed throughout plant taxa (Edwards, 1998; Grytnes *et al.*, 1999; Edwards & Westoby, 2000; Mills, 2003; Pilgrim *et al.*, 2004). If they are, then the percentage of a given taxon in the rare flora should be proportionate to its percentage in the total flora. Attempts have been made to understand why this is often not the case and why certain plant families contain more (or less) than their fair share of rare species (Schwartz, 1993; Schwartz & Simberloff, 2001; Lozano & Schwartz, 2005). Unfortunately, these studies have seldom addressed any specific fern families, and never ferns as a group. In fact, although aspects of North American fern distribution have been the focus of numerous studies, both at a broad scale (Wagner, 1972; Kramer, 1993), and a small scale (Richard *et al.*, 2000; Karst *et al.*, 2005; Wild & Gagnon, 2005), there have been few studies on fern rarity *per se*. This is relatively surprising when one considers the high ratios of ferns versus other vascular plants occurring in local lists of vulnerable, threatened and endangered species in North America. For example, in the province of Quebec (Canada), Labrecque and Lavoie (2002) identify 375 rare species of which 27 are ferns, equating to 7.2%. This is almost double the proportion of the entire vascular flora of the province which consists of ferns (108/2862 (3.8%)). This is a tendency that can be found over and over again in lists of rare plants throughout continental North America (eg. Bouchard *et al.*, 1983; Argus & Pryer, 1990; Oldham, 1999; Young & Weldy, 2003).

This begs the question: Are these numbers representative of biological reality or are they tainted by some form of bias? Subjective bias has often been reported as a recurring problem in the designation of rare species, where highly visible, charismatic taxa are more likely to be designated than lesser-known or less appreciated taxa. This is obvious when considering numbers of designated vascular plants versus non-vascular (Burgman, 2002), and perhaps even more so when one considers the number

of insects currently recognized as threatened with extinction (0.9%) compared with the number of birds (10.8%) or mammals (11.2%) (May *et al.*, 1995). This type of problem can be compounded when local authorities on certain taxa help increase the chances of these taxa being designated as rare or at risk (Burgman, 2002).

In an attempt to circumvent these problems, we compiled data from NatureServe (<http://www.natureserve.org/>), also known as the Heritage or Nature Conservancy rankings. The NatureServe protocol is a classification system developed initially by The Nature Conservancy which uses trained experts who evaluate quantitative data and make intuitive judgments about species vulnerability (Regan *et al.*, 2004). The classification scheme considers number of occurrences; population size; extent of occurrence; area of occupancy; short- and long-term trends in population size and geographic range; scope, severity and imminence of threats; number of appropriately protected and managed occurrences; and intrinsic vulnerability (Andelman *et al.*, 2004). As of May 2006, 17384 species of vascular plants were indexed in their North American database (excluding exotics, subspecies and varieties). Rankings are available at three scales, a global scale (G), national scale (N) and subnational scale (S). For a detailed description of the NatureServe system see Master (1991).

Recent studies have attempted to quantify the objectivity of this type of ranking and justify its use in a conservation context (Andelman *et al.*, 2004; O'Grady *et al.*, 2004; Regan *et al.*, 2004; 2005). For example, in their comparison of different ranking methods for identifying species at risk on U.S. Forest Service land, Andelman *et al.* (2004) conclude that the Heritage protocols may be the most suitable of existing protocols. Furthermore, Regan *et al.* (2004) describe the NatureServe database as the most comprehensive and current database for at risk species in the United States and Canada.

Here, using data retrieved from NatureServe Explorer, we attempt to verify whether ferns really do have consistently more rare species than what we would expect if rarity was randomly distributed throughout plant taxa, and if so, to attempt to explain why this is the case.

2.2 Methods

All data were retrieved from NatureServe's online database (<http://www.natureserve.org/explorer/>) during the month of May 2006.

Classification of plants by NatureServe follows Kartesz (1994; 1999). Groups analyzed were ferns, or monilophytes (*sensu* Pryer *et al.* 2004) (including leptosporangiate ferns, horsetails, marattioid ferns, ophioglossoid ferns and whisk ferns), as well as all fern families with more than 40 species occurring in the North American flora: Dryopteridaceae (140 spp.); Pteridaceae (106 spp.); Aspleniaceae (70 spp.); and Ophioglossaceae (47 spp.). Subspecies, varieties, and populations were excluded from all analyses, as were exotics.

In each subnational unit (U.S. state or Canadian province or territory), we counted total numbers of vascular plant species, fern species, "at risk" vascular plant species, and "at risk" fern species. This allowed us to calculate ratios of ferns to plants and "at risk" ferns to "at risk" plants for each state, province and territory. The same was done for the four individual fern families.

Classification of "at risk" species was based on the definition used by NatureServe, where, to be considered at risk at the subnational level (U.S. states and Canadian provinces and territories), a species must be classified as SX, SH, S1, S2, or S3. Table 2.1 shows the definition for each rank used by NatureServe.

The null hypothesis that the percentage of a given taxon in the "at risk" flora would be proportionate to its percentage in the total flora was assessed using χ^2 .

2.3 Results

According to the NatureServe data, 519 fern species occur in North America of which 145 are found in Canada and 510 in the United States (402 in continental U.S.). Of these 519 species, 397 (76.5%) are considered "at risk" in at least one of the states/provinces in which they occur (SX, SH, S1, S2, or S3), leaving 122 (23.5%) considered secure throughout their range. A total of 147 (28.3%) are considered globally "at risk" (GX, GH, G1, G2, or G3). Of these 147, 113 are endemic to only one state/province (90 of which are Hawaiian).

Out of 64 subnational floras, "at risk" ferns rank significantly higher than expected in 28 cases (χ^2 , $p < 0.05$) (Fig. 2.1). Only one was significantly lower than expected, and that was the particular case of Hawaii. Similar trends hold true for the individual fern families analyzed separately (Fig. 2.2). Results for all analyzed taxa are summarized in Table 2.2.

2.4 Discussion

However one looks at these data, it appears evident that ferns are indeed over-represented on subnational lists of rare species. Several factors could potentially lead to these results. Here, we group these factors into anthropogenic, biological, climatic, evolutionary, and geographical considerations.

2.4.1 Anthropogenic considerations

One can argue that crypticity could play a part in increasing a group's number of rare species by creating an undersampled group. However, although ferns could be

considered cryptic at first glance, they are a relatively small group with which field botanists tend to be familiar. As Wagner (1972) stated more than 30 years ago "Their popularity with researchers and field botanists has caused the pteridophyta to be well collected and represented in herbaria", adding that the finding of a new disjunct population was to be considered an event. Therefore, we do not believe undersampling of fern populations to be a causative factor for the results obtained here.

Although the above attests to their relative popularity in North America, pteridophytes have never garnered the interest here that they did during the period of Pteridomania during the 19th century in the UK, where some fern species were driven to extinction or near-extinction through collection (Allen, 1969). Although some fern species are reported to have been overcollected in North America (ie. Hand Fern (*Cheiroglossa palmatum* (Linnaeus) C. Presl) in Florida), there is no reason to believe that the widespread results observed here are related to overcollecting or harvesting.

2.4.2 Biological considerations

A common life-history trait could be responsible for increased rarity throughout a taxon. It has been suggested that two important traits that help contribute to rarity are poor dispersal ability and lower overall reproductive effort (Kunin & Gaston, 1993), neither of which readily applies to ferns. Furthermore, a recent meta-study of 94 traits across 54 studies concludes that although the "outcome is overwhelmingly for the studies to differ from one another in their findings", the one trait that decreased rarity fairly consistently was high seed production (Murray *et al.*, 2002). This is certainly not a problem for ferns, well known for their ability to produce extremely high numbers of spores.

Another possible causative biological trait is seed size. Hodgson's (1986) study across 11 angiosperm families concludes that average seed size ("moderately small") is the best indicator for families containing low numbers of rare species. He found that the two families containing the highest ratios of rare species had, on average, either minute seeds (Orchidaceae: mean 0.01 mg) or very large seeds (Fabaceae: mean 3.7 mg). If it is possible to equate fern spores to angiosperm seeds, then this could explain the patterns observed here, as fern spore size is clearly at the small end of the scale.

Page (2002) suggests seven aspects of pteridophyte biology that could possibly be considered as intrinsic limitations to their ecological success: 1) The handicap of an independent gametophyte stage; 2) Single growing-point limitations of sporophyte architecture; 3) Slow plant growth rates; 4) Intolerance of widely fluctuating conditions; 5) Poorly controlled evaporative potential; 6) Uncontrolled high reproductive commitment; 7) Need to 'return to the water to breed'. We believe that the first and last of these limitations would be the most likely to contribute to high rarity rates, as they directly impact the establishment and recruitment of new individuals. In fact, both of these limitations have been qualified as the Achilles' heel of the pteridophytes (Page, 2002), and have led to pteridophytes being described as the amphibians of the plant world (Page, 1985; 2002). However, Page (2002) also enumerates twelve recurring advantaging strategies that can mitigate, tolerate, and sometimes positively exploit, the effects of the seven limitations in a number of ways.

2.4.3 Climatic considerations

The outlier in Figure 1 suggests that our results could also be influenced by climatic factors. The only state with significantly lower numbers of rare ferns than expected, Hawaii, is also the only state with a purely tropical climate. Is it possible that

continental North American ferns are rare simply because they are out of their element or climatic optimum?

When one considers the fact that more fern species occur in the 28 337 km² of Hawaii (152 spp.) and in the 170 451 km² of Florida (141 spp.) than in the 181 440 km² of New England (88 spp.), and as many that occur in the 10 million km² of Canada (145 spp.), it appears evident that climate must have some influence on fern distribution in North America. As is the case for many organisms, diversity for ferns is much higher in the tropics. This was elegantly illustrated for homosporous fern species by Tryon (1986). Although this would suggest that climate is the leading cause for the high numbers of ferns found in Hawaii, it is likely that its insular nature is also involved. It has long been recognized that most island floras consist of a high proportion of pteridophytes (Tryon, 1970; Smith, 1972). Kornas (1993) gives some striking examples of this, such as the case of Madagascar with 500 species of ferns compared to all of tropical Africa with 600 species from an area fifty times larger. The putative cause in the creation of these disharmonic floras is the ease with which long distance dispersal occurs in ferns. Interestingly, this tendency is reflected in our results, where four of the five state/provinces with the highest proportion of ferns in their floras are islands or peninsulas (Hawaii, Newfoundland, Nova Scotia and Prince Edward Island).

2.4.4 Evolutionary considerations

Although ferns have long been believed to be an evolutionary primitive group, current knowledge suggests that the major lineage of ferns, the polypods, evolved following the radiation of the angiosperms (Schneider *et al.*, 2004). In a study on the evolutionary and taxonomic aspects of commonness and rarity in the flora around Sheffield, UK, Hodgson (1986) found a negative correlation between the

preponderance of rare species in a family and its degree of evolutionary advancement. However, Schwartz (1993) tested Hodgson's hypothesis that evolutionary advancement is associated with commonness at the family level; he studied dicotyledon families from nine regions of North America, and concluded that although there appears to be taxonomic affiliations with rarity, taxonomic advancement does not predict these affinities. Given these contradicting results, it is difficult to make a case for evolutionary advancement (or lack of) contributing to our results.

2.4.5 Geographical considerations

Although it may appear counterintuitive, it is possible that the high dispersability of fern spores could account for the patterns observed here. In fact, it is quite easy to imagine a high level of disjunction, for which ferns are well known (Bouchard & Hay, 1976; Bouchard *et al.*, 1977; Cousens, 1981; Wagner & Rouleau, 1984; Brunton, 1986), creating increased rarity at the subnational level. Figure 2.3 illustrates a theoretical example of this, where two plant species are found in 25 populations each. Whereas one species is found in six units, in only two of which it would be designated as rare, the other is spread thinly across the 25 sampling units, creating a rare species in 15 of the 16 units in which it occurs. This type of distribution pattern causes what is known as suffusive rarity (Schoener, 1987). Suffusive rarity applies to widespread species that are rare throughout most of their geographic range. A recent study by Mills and Schwartz (2005) shows that suffusively rare species are significantly more likely to be seedless vascular plant species (ferns, horsetails, quillworts, club mosses and whisk ferns) than expected. This suggests that our results could be an artifact of political boundaries, and therefore not necessarily indicative of rarity at a larger scale. Several examples of widespread North American fern species tend to support this suggestion. These

include *Botrychium oneidense* (Gilbert) House, which has a national US rank of N4 (apparently secure) and occurs in 23 states, but is considered "at risk" in 16 states; *Asplenium bradleyi* D.C. Eat., also ranked as N4, which occurs in 19 states and is considered "at risk" in 16 of them; and *Woodsia alpina* (Bolton) Gray, which has a rank of N4 in Canada, occurs in 12 provinces and territories and is considered "at risk" in a total of nine. Furthermore, ferns are significantly under-represented amongst the globally "at risk" vascular plants in North America (χ^2 , $p < 0.05$), strongly suggesting that our results are scale-dependent.

2.5 Conclusion

Our results suggest that at the subnational level North American ferns are indeed rarer than would be expected if rarity was randomly distributed throughout plant taxa. Although there are several inherent properties of ferns that could potentially explain this pattern, we suspect that the potential impact of these factors is compounded by the high dispersability of ferns, leading to patterns of suffusive rarity, and by the subnational scale at which rarity was studied here. Although this type of parochial approach is the one most often used in conservation planning (Rodrigues & Gaston, 2002) and in funding allocation for conservation (Hunter & Hutchinson, 1994), the merits of such an approach have been debated (Hunter & Hutchinson, 1994; Dudley, 1995). We believe that our results illustrate one of the problems of a parochial approach to conservation, where the perceived rarity of an entire taxon is exaggerated because of the scale at which rarity is addressed.

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2.7 References

- Allen, D. E. 1969. The Victorian fern craze: A history of Pteridomania. Hutchinson, London.
- Andelman, S. J., Groves, C., and Regan, H. M. 2004. A review of protocols for selecting species at risk in the context of US Forest Service viability assessments. *Acta Oecologica* **26**: 75-83.
- Argus, G. W. and Pryer, K. M. 1990. Rare vascular plants in Canada - our natural heritage. Canadian Museum of Nature, Ottawa.
- Bouchard, A. and Hay, S. 1976. *Thelypteris limbosperma* in eastern North America. *Rhodora* **78**: 552-553.
- Bouchard, A., Barabé, D., and Hay, S. 1977. An isolated colony of *Oreopteris limbosperma* (All.) Holub in Gros Morne National Park, Newfoundland, Canada. *Naturaliste Canadien* **104**: 239-244.
- Bouchard, A., Barabé, D., Dumais, M., and Hay, S. 1983. Les plantes vasculaires rares du Québec. National Museums of Canada, Canadian Museum of Nature, *Syllogeus* **48**: 1-79.
- Brunton, D. F. 1986. Status of the southern maidenhair fern, *Adiantum capillus-veneris* (Adiantaceae), in Canada. *Canadian Field-Naturalist* **100**: 404-408.
- Burgman, M. A. 2002. Are listed threatened species really at risk? *Australian Journal of Botany* **50**: 1-13.
- Cousens, M. I. 1981. *Blechnum spicant* - Habitat and vigor of optimal, marginal, and disjunct populations, and field observations of gametophytes. *Botanical Gazette* **142**: 251-258.
- Dudley, J. P. 1995. Bioregional parochialism and global activism. *Conservation Biology* **9**: 1332-1334.
- Edwards, W. 1998. Rarity within taxonomic lineages and the use of taxa above the level of species. *Ecography* **21**: 625-629.
- Edwards, W. and Westoby, M. 2000. Families with highest proportions of rare species are not consistent between floras. *Journal of Biogeography* **27**: 733-740.

- Grytnes, J. A., Birks, H. J. B., and Peglar, S. M. 1999. The taxonomic distribution of rare and common species among families in the vascular plant flora of Fennoscandia. *Diversity and Distributions* **5**: 177-186.
- Guo, Q. F., Kato, M., and Ricklefs, R. E. 2003. Life history, diversity and distribution: a study of Japanese pteridophytes. *Ecography* **26**: 129-138.
- Hodgson, J. G. 1986. Commonness and rarity in plants with special reference to the Sheffield flora. 3. Taxonomic and evolutionary aspects. *Biological Conservation* **36**: 275-296.
- Hunter, M. L. and Hutchinson, A. 1994. The virtues and shortcomings of parochialism - Conserving species that are locally rare, but globally common. *Conservation Biology* **8**: 1163-1165.
- Karst, J., Gilbert, B., and Lechowicz, M.J. 2005. Fern community assembly: The roles of chance and the environment at local and intermediate scales. *Ecology* **86**: 2473-2486.
- Kartesz, J. T. 1994. A synonymized checklist of the vascular flora of the United States, Canada, and Greenland. 2nd edn. 2 vols. Timber Press, Portland, OR.
- Kartesz, J. T. 1999. A synonymized checklist and atlas with biological attributes for the vascular flora of the United States, Canada, and Greenland. Synthesis of the North American flora Version 1.0 [computer program] (ed. by J.T. Kartesz and C.A. Meacham). North Carolina Botanical Garden, Chapel Hill, NC.
- Kornas, J. 1993. The significance of historical factors and ecological preference in the distribution of African pteridophytes. *Journal of Biogeography* **20**: 281-286.
- Kramer, K. U. 1993. Distribution patterns in major pteridophyte taxa relative to those of angiosperms. *Journal of Biogeography* **20**: 287-291.
- Kunin, W. E. and Gaston, K. J. 1993. The biology of rarity - Patterns, causes and consequences. *Trends in Ecology and Evolution* **8**: 298-301.
- Labrecque, J. and Lavoie, G. 2002. Les plantes vasculaires menacées ou vulnérables du Québec. Gouvernement du Québec, ministère de l'environnement. Direction du patrimoine écologique et du développement durable, Québec.
- Lozano, F. D. and Schwartz, M. W. 2005. Patterns of rarity and taxonomic group size in plants. *Biological Conservation* **126**: 146-154.

- Master, L. L. 1991. Assessing threats and setting priorities for conservation. *Conservation Biology* **5**: 559–563.
- May, R. M., Lawton, J. H., and Stork, N. E. 1995. Assessing extinction rates. *Extinction rates* (ed. by J.H. Lawton and R.M. May), pp. 1-24, Oxford University Press, Oxford.
- Mills, M. H. 2003. Correlates of rarity in the flora of North America: Life histories, habitats, and geographic distributions. Ph.D. Thesis, University of California, Davis.
- Mills, M. H. and Schwartz, M. W. 2005. Rare plants at the extremes of distribution: broadly and narrowly distributed rare species. *Biodiversity and Conservation* **14**: 1401-1420.
- Murray, B. R., Thrall, P. H., Gill, A. M., and Nicotra, A. B. 2002. How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology* **27**: 291-310.
- NatureServe Explorer. (2005) available from <http://www.natureserve.org/explorer/index.htm> (accessed May 2006)
- O'Grady, J. J., Burgman, M. A., Keith, D. A., Master, L. L., Andelman, S. J., Brook, B. W., Hammerson, G. A., Regan, T., and Frankham, R. 2004. Correlations among extinction risks assessed by different systems of threatened species categorization. *Conservation Biology* **18**: 1624-1635.
- Oldham, M. J. 1999. Natural Heritage Resources of Ontario: Rare vascular plants. 3rd edn. Natural Heritage Information Centre, Ontario Ministry of Natural Resources, Peterborough, Ontario.
- Page, C. N. 1985 Pteridophyte biology, the biology of the amphibians of the plant world. *Proceedings of the Royal Society of Edinburgh Section B: Biology* **86B**: 439–442.
- Page, C. N. 2002. Ecological strategies in fern evolution: a neopteridological overview. *Review of Palaeobotany and Palynology* **119**: 1-33.
- Pilgrim, E. S., Crawley, M. J., and Dolphin, K. 2004. Patterns of rarity in the native British flora. *Biological Conservation* **120**: 161-170.

- Pryer, K. M., Schuettpeltz, E., Wolf, P. G., Schneider, H., Smith, A. R., and Cranfill, R. 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on the early Leptosporangiate divergences. *American Journal of Botany* **91**: 1582-1598.
- Regan, T. J., Master, L. L., and Hammerson, G. A. 2004. Capturing expert knowledge for threatened species assessments: a case study using NatureServe conservation status ranks. *Acta Oecologica* **26**: 95-107.
- Regan, T. J., Burgman, M. A., McCarthy, M. A., Master, L. L., Keith, D. A., Mace, G. M., and Andelman, S. J. 2005. The consistency of extinction risk classification protocols. *Conservation Biology* **19**: 1969-1977.
- Richard, M., Bernhardt, T., and Bell, G. 2000. Environmental heterogeneity and the spatial structure of fern species diversity in one hectare of old-growth forest. *Ecography* **23**: 231-245.
- Rodrigues, A. S. L. and Gaston, K. J. 2002. Rarity and conservation planning across geopolitical units. *Conservation Biology* **16**: 674-682.
- Schneider, H., Schuettpeltz, E., Pryer, K. M., Cranfill, R., Magallon, S., and Lupla, R. 2004. Ferns diversified in the shadow of angiosperms. *Nature* **428**: 553-557.
- Schoener, T. W. 1987. The geographical distribution of rarity. *Oecologia* **74**: 161-173.
- Schwartz, M. W. and Simberloff, D. 2001. Taxon size predicts rates of rarity in vascular plants. *Ecology Letters* **4**: 464-469.
- Schwartz, M. W. 1993. The search for pattern among rare plants - Are primitive species more likely to be rare. *Biological Conservation* **64**: 121-127.
- Smith, A. R. 1972. Comparison of fern and flowering plant distributions with some evolutionary interpretations for ferns. *Biotropica* **4**: 4-9.
- Tryon, R. 1970. Development and evolution of fern floras of oceanic islands. *Biotropica* **2**: 76-84.
- Tryon, R. 1986. The biogeography of species, with special reference to ferns. *The Botanical Review* **52**: 117-156.
- Wagner, W. H. 1972. Disjunctions in homosporous vascular plants. *Annals of the Missouri Botanical Garden* **59**: 203-17.

- Wagner, W. H. and Rouleau, E. 1984. A western holly fern, *Polystichum X scopulinum*, in Newfoundland. *American Fern Journal* **74**: 33-36.
- Wild, M. and Gagnon, D. 2005. Does lack of available suitable habitat explain the patchy distributions of rare calcicole fern species? *Ecography* **28**: 191-196.
- Young, S. M. and Weldy T. W. 2003 New York rare plant status list. New York Natural Heritage Program, Albany, NY.

Table 2.1: Definitions for interpreting NatureServe conservation status ranks at the subnational level. SX, SH, S1, S2 and S3 categorize “at risk” species. (modified from <http://www.natureserve.org/explorer/>)

Category		Definition
SX	Presumed Extirpated	Species is believed to be extirpated from the nation or state/province. Not located despite intensive searches of historical sites and other appropriate habitat, and virtually no likelihood that it will be rediscovered.
SH	Possibly Extirpated (Historical)	Species occurred historically in the state/province, and there is some possibility that it may be rediscovered. Its presence may not have been verified in the past 20-40 years. A species could become SH without such a 20-40 year delay if the only known occurrences in a state/province were destroyed or if it had been extensively and unsuccessfully looked for. The SH rank is reserved for species for which some effort has been made to relocate occurrences, rather than simply using this status for all elements not known from verified extant occurrences.
S1	Critically Imperiled	Critically imperiled in the state/province because of extreme rarity (often 5 or fewer occurrences) or because of some factor(s) such as very steep declines making it especially vulnerable to extirpation from the state/province.
S2	Imperiled	Imperiled in the state/province because of rarity due to very restricted range, very few populations (often 20 or fewer), steep declines, or other factors making it very vulnerable to extirpation from the state/province.
S3	Vulnerable	Vulnerable in the state/province due to a restricted range, relatively few populations (often 80 or fewer), recent and widespread declines, or other factors making it vulnerable to extirpation.
S4	Apparently Secure	Uncommon but not rare; some cause for long-term concern due to declines or other factors.
S5	Secure	Common, widespread, and abundant in the state/province.

Table 2.2: Summary of results obtained for all analyzed taxa showing total number of species for each taxon, and in how many states/provinces rare fern numbers were significantly higher and lower than expected ($p < 0.05$).

Taxon	N spp.	Number of states/provinces in which rare fern numbers	
		were significantly higher than expected	were significantly lower than expected
Monilophytes	519	28	1
Dryopteridaceae	140	13	1
Pteridaceae	106	17	1
Aspleniaceae	70	23	1
Ophioglossaceae	47	26	0

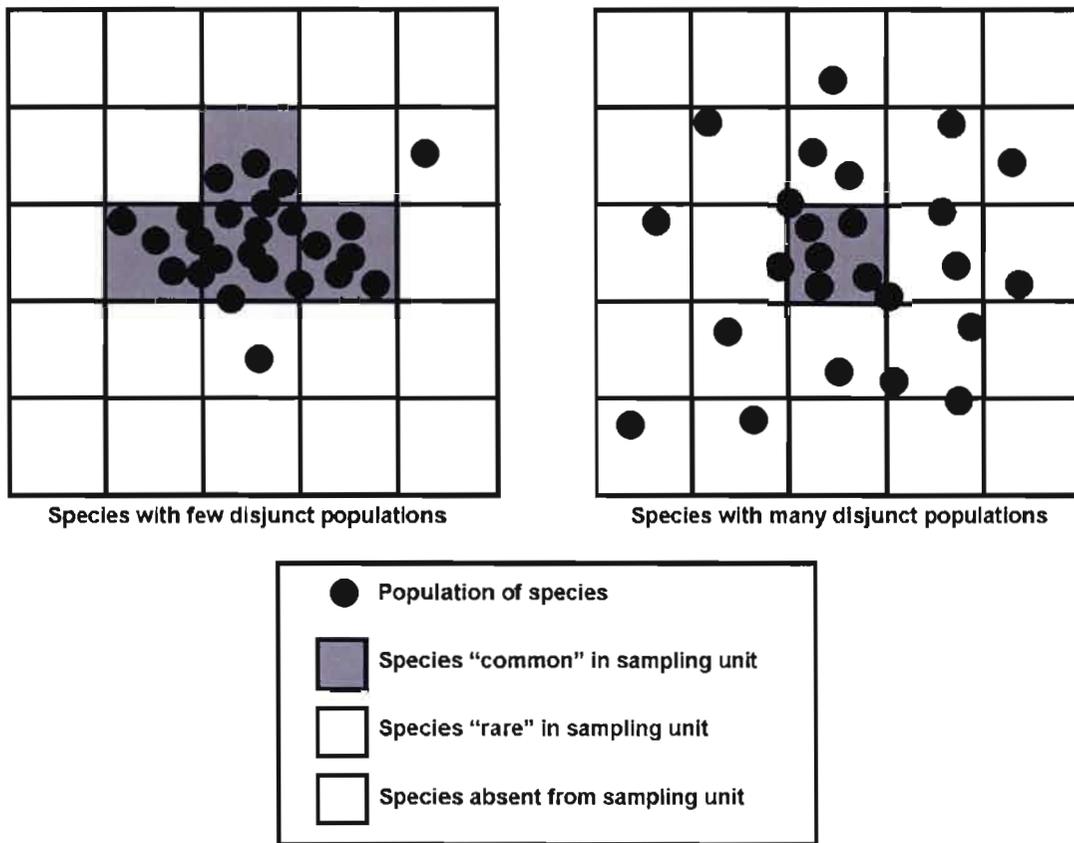


Figure 2.3: Theoretical comparison of two species with 25 known populations in a matrix of 25 sampling units. The species with infrequent disjunctions is "common" in 4 states and "rare" in 2. The highly disjunct species is "common" in 1 state and "rare" in 15.

CHAPTER III

EVIDENCE FROM THE SOIL SPORE BANK OF THE FERN *WOODSIA OBTUSA* AT
THE NORTHERN LIMIT OF ITS RANGE SUGGEST RECRUITMENT LIMITATIONS
IN OTHER LIFE STAGES

Matthew Wild, Daniel Gagnon and André Bouchard

Abstract

Although common throughout most of its distributional range, Blunt-lobed *Woodsia* is extremely rare in the northernmost part of its range in southern Canada, where it is known from only eight populations containing relatively few individuals. Recent work has shown that suitable habitat is available for the species within, and in proximity to, its extant populations in Canada, but very little recruitment appears to be taking place in these populations. Here we attempt to verify if spore dispersal is a limiting factor for this species at the northern periphery of its range. We collected soil samples from the immediate vicinity (up to 50 meters) of two northern populations and incubated them in suitable conditions to promote spore germination and subsequent gametophyte development. Viable Blunt-lobed *Woodsia* spores (n=638) germinated from over half of the collected soil samples (69/130). Of the 638 germinated spores, 571 were from soils located within five meters of the population centres, showing a highly skewed (leptokurtic) pattern of spore dispersal. Although our results show that viable Blunt-lobed *Woodsia* spores are dispersing to available habitat, an apparent lack of *in situ* recruitment suggests that some factor, most likely lack of moisture in its xeric habitat in Canada, is limiting the establishment of new individuals, thereby reducing functional dispersal to a minimum.

Keywords: Blunt-lobed *Woodsia*, dispersal, gametophyte, recruitment, spore bank

3.1 Introduction

Blunt-lobed Woodsia, or Blunt-lobed cliff-fern (*Woodsia obtusa* subsp. *obtusa* (Sprengel) Torrey: Dryopteridaceae), is a homosporous fern found in eastern North America. The species is very common in most of the eastern United States, and has even been described as “weedy” by Brown (1964). However, at the northernmost limit of its distribution in southern Canada, it is only known from a total of eight sites (four in Quebec and four in Ontario), containing a total of approximately 1350 individuals (COSEWIC, 2007). Blunt-lobed Woodsia is considered rare in Quebec (Bouchard *et al.*, 1983; CDPNQ, 2008), in Ontario (Argus and White, 1977), as well as nationally in Canada (Argus and Pryer, 1990). Its low number of sparsely distributed populations have earned the species a NatureServe rank of N1 (critically imperiled) in Canada, provincial ranks of S1 (critically imperiled) in both Quebec and Ontario (NatureServe, 2006), and has led to it being designated “Threatened” by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2006) and listed on Schedule 1 of the federal Species at Risk Act (S.C. 2002, c. 29). In Quebec, the species is legally designated threatened under the *Loi sur les espèces menacées ou vulnérables* (L.R.Q., c. E-12.01) and in Ontario, the species is listed as Endangered and is protected under the Endangered Species Act (S.O. 2007, Chapter 6).

Homosporous fern distribution has previously been suggested to be mostly determined by factors of climate and habitat (or microclimate and microhabitat) due to relatively low limitations in ferns for dispersal and establishment (Tryon, 1970; Tryon, 1986; Marquez *et al.*, 1997). This would imply that at a local scale, habitat availability is the principal factor limiting Blunt-lobed Woodsia in Canada. However, recent work (Wild and Gagnon, 2005) suggests that suitable habitat is available to the species within, and in proximity to, existing populations, but that factors affecting some stage of its life cycle are limiting its distribution. Here, we attempt to verify if spore dispersal is a limiting factor for this species by confirming the presence of a

viable spore bank in the soil surrounding extant populations of Blunt-lobed *Woodsia* in Canada.

Although a lot less studied than seed banks, several studies have been undertaken on soil spore banks. While most of these have examined the relative contributions to the soil spore bank of multiple fern species (eg. Leck and Simpson, 1987; Milberg, 1991; Dyer and Lindsay, 1992; Simabukuro *et al.*, 1999; Ranal, 2003; Ramirez-Trejo *et al.*, 2004; Hock *et al.*, 2006), or bryophyte species (During and ter Horst, 1983; Bisang, 1996; During *et al.*, 1987), relatively few have targeted or attempted to quantify specific fern species in spore banks (Hamilton, 1988; Penrod and McCormick, 1996; Schneller and Holderegger, 1996). Most homosporous ferns, including *W. obtusa*, annually produce non-chlorophyllous spores that can potentially stay viable for several years (Milberg, 1991; Dyer and Lindsay, 1992) allowing them to remain in the spore bank until conditions are suitable for spore germination and subsequent gametophyte growth. Depending on where a spore disperses to, several outcomes are possible: the recruitment of a new individual within the population, the migration from one population to another, or the establishment of a new population (Werth and Cousens, 1990). However, all of these outcomes are dependent on not only the spore dispersing but, equally as important, the spore must also germinate and subsequently develop into a viable gametophyte able to produce a sporophyte.

Seeing as homosporous ferns can produce bisexual gametophytes, there are three types of fertilization possible: 1) cross fertilization between two gametophytes derived from different sporophytes (intergametophytic crossing), 2) cross-fertilization between two gametophytes derived from the same sporophyte (intergametophytic selfing), and 3) self-fertilization of the same gametophyte (intragametophytic selfing) (Klekowski, 1979). The latter results in a sporophyte that is 100% homozygous, implying a higher frequency of recessive, deleterious traits which could potentially lead to inbreeding depression. This could apply to Blunt-lobed *Woodsia* as it has been

shown to have high isolate potential (i.e. the ability to produce bisexual gametophytes capable of intragametophytic selfing) (Peck, 1985). However, although it is highly probable that the isolated populations of Blunt-lobed *Woodsia* in Canada are the result of a single spore arrival, implying complete homozygosity, negative components of inbreeding are likely countered by the tetraploid nature of the individuals occurring in these populations (Windham, 1993), as suggested by Soltis and Soltis (2000).

We know of no work that has directly studied the presence of Blunt-lobed *Woodsia* spores in the soil, but previous work on the species by Peck *et al.* (1990), has shown it to be very fecund, contributing over 40% of the total fern spore crop in a 26 ha study area in Iowa, USA. Peck (1980) estimated that individual plants produce 60 million spores annually and it was the most frequently observed species of gametophyte during his study. This contrasts with Canadian populations where, although annual spore production per individual appears similar (M. Wild unpubl. data), no naturally occurring gametophytes have been observed despite extensive searches every year from 2000 to 2006. This begs the following questions: To what extent are viable spores present in soils surrounding extant populations of Blunt-lobed *Woodsia* in Canada? How abundant are they and what is their spatial distribution with respect to extant spore producing sporophytes?

We hypothesize that there are viable spores in soils surrounding extant Blunt-lobed *Woodsia* populations in Canada, but that a highly skewed (leptokurtic) distribution pattern of spores greatly limits the long-distance dispersal potential for the species.

3.2 Methods

To address this question we adopted a method used to verify the presence of viable fern spores in soil samples (e.g. Milberg, 1991; Penrod and McCormick, 1996). The method consists of a series of soil samples taken from the vicinity of extant populations, incubated in suitable conditions for spore germination and subsequent gametophyte development.

Soil samples were collected in October 2003 from areas surrounding two Canadian populations of Blunt-lobed *Woodsia*. One population is located in Gatineau Park, Quebec (N 45° 28', W 75° 50'); the other is in Frontenac Park, Ontario (N 44° 33', W 76° 29'). At each site, soil samples were collected along eight transects radiating from the mean centre of the study population at distances of 0, 1, 2, 5, 10, 20, 30, 40 and 50 meters, for a total of 130 samples (65 per population). Mean population centres were calculated from the average X/Y coordinates of extant Blunt-lobed *Woodsia* individuals in each study population (n=38 in Gatineau Park, n=49 in Frontenac Park). All extant individuals in the study populations were located within five meters of this mean. Soil samples were collected to a depth of 2 cm using a bulb planter with an approximate diameter of 7 cm, and placed individually in sealed plastic bags. Samples were left in total darkness at 4°C until needed.

In March 2004, the soil samples were homogenised in their bags, and a 1 cm deep (approximately 12.5 cm³) layer of soil from each sample was placed on top of 4 cm (approximately 50 cm³) of microwave-sterilised sand in randomly located individual compartments of a standard 72-cell plug tray (4 cm cell diameter). In the remaining empty seven cells in each tray, microwave-sterilised potting soil was placed on the sand to serve as a control for contamination from airborne spores during the experiment (no contamination was observed). Eight extra cells (in a separate tray) containing microwave-sterilised sand and potting soil were also sown with spores

taken directly from Blunt-lobed *Woodsia* fronds. These would serve as a comparison for identifying Blunt-lobed *Woodsia* gametophytes emerging from study samples. All trays were placed in a Conviron EF7H growth chamber (Controlled Environments Limited, Winnipeg, Canada) set to a 16h/8h day/night cycle. Temperature was set to 24°C day/20°C night, and relative humidity was ambient. A mixture of fluorescent and incandescent lamps supplied a PAR of approximately 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Cultures were watered weekly with distilled water.

As fern spores contained in the samples germinated and developed into gametophytes, they were carefully removed, isolated on microwave-sterilised potting soil in individual compartments of 288-cell plug trays, and kept growing until it was possible to positively identify the gametophyte, or subsequent sporophyte, as being a Blunt-lobed *Woodsia* individual or an individual of another fern species (Kaur and Chandra, 1973; Peck, 1980). Any bryophyte or non-fern vascular plant growth appearing in the cultures was removed immediately in order to minimize interspecific competition. Gametophyte emergence monitoring was stopped after six months, at which time no fresh gametophyte growth had been observed for several weeks. Isolated gametophytes (and subsequent sporophytes) were kept growing for an additional six months until all individuals had been positively identified as Blunt-lobed *Woodsia* or another species.

A simple power regression was fitted to the data of the cumulative percent of total spore germination from both study populations using Microsoft Office Excel (2003).

3.3 Results

Blunt-lobed *Woodsia* spores germinated in 69 of the 130 soil samples collected from both populations. Forty-four of these 69 (63.7%) were from locations originating from within five meters of the population centres. The total number of Blunt-lobed

Woodsia gametophytes identified was 638, of which 571 (89.5%) were from soils collected from within five meters of the populations centre. Figures 3.1 and 3.2 illustrate the sharp decline of germinated spores as sampling distance from centre of population increases. Although the abundance of spores drastically decreased as distance from population centre increased, at least one spore germinated at all distances sampled in both populations. Cumulative germination averages from both populations (Figure 3.3) show the negative exponential relationship between abundance of germinated spores and distance from the population centre ($R^2=0.91$). This regression shows that, on average, 83% of germinated spores are likely to occur within 5m, 93% within 10m, and 97% within 20m of the population centre.

Figures 3.4 and 3.5 represent the pattern and abundance of germinated Blunt-lobed Woodsia spores in soils from both study populations. Figures 3.4a and 3.5a show the extent of sampling up to 50 meters, and Figures 3.4b and 3.5b show the centre of the sampling area where the vast majority of germinated spores were found.

3.4 Discussion

Our results confirm the existence of a viable Blunt-lobed Woodsia spore bank surrounding extant Canadian populations, and that spore germination and subsequent gametophyte development can be induced when placed in suitable conditions. The fact that no gametophytes have been observed in Canadian populations, despite extensive searches, implies that even though apparently suitable habitat is located in proximity to extant populations (Wild and Gagnon, 2005), and that viable spores are effectively dispersing into this habitat, some factor is limiting either spore germination or subsequent gametophyte establishment.

Although Blunt-lobed Woodsia sporophytes seem to survive and grow relatively well in the xeric habitat (i.e. steep south-facing slopes and shallow soils) at the

northernmost limit of its distributional range, natural recruitment in this habitat appears to be minimal. The sub-optimal nature of the Canadian habitat of the species seems evident when compared to habitat descriptions from further south in its distributional range: "...*Woodsia obtusa* occurred in habitats with high moisture, pH, nutrient, and canopy cover values" (Greer *et al.*, 1997), and "*Woodsia obtusa* [...] is more mesic than the others [woodsias] and this fact is borne out not only by geographical distribution and habitat observations but by the water requirements of potted specimens..." (Brown, 1964).

The two most often mentioned requirements for fern recruitment are light for spore germination (Raghavan, 1992; Pérez-García *et al.*, 2007), and water for subsequent gametophyte development and fertilisation (Page, 1985; 2002). Both of these factors are likely to be affecting recruitment in Canadian populations of Blunt-lobed *Woodsia*. A litter layer composed of Sugar Maple (*Acer saccharum* Marsh) leaves, and the slowly decomposing leaves from several species of the Fagaceae, Red Oak (*Quercus rubra* L.), White Oak (*Quercus alba* L.), and American Beech (*Fagus grandifolia* Ehrh.) (Côté and Fyles, 1994), probably affects light availability for spore germination, and as mentioned above, water availability is definitely limited in these habitats, thus affecting gametophyte development and fertilisation. Further studies on spore germination and gametophyte development in controlled conditions would help elucidate which of these limiting factors, and at which precise life stage, is having the greatest effect on recruitment in Canadian Blunt-lobed *Woodsia* populations.

The independent, relatively fragile, gametophyte stage has been mentioned as being somewhat of a handicap for ferns in general (Page, 2002), and also as a possible explanation for the overabundance of fern species appearing on rare plant lists throughout North America (Wild *et al.*, 2006). However, Peck (1985) stated that Blunt-lobed *Woodsia*'s high germination rate, uniform normal development, high percentage of bisexual isolated gametophytes, and absence of genetic load should

favour the establishment of new populations. Based on our results, we do not believe easy establishment to be the norm for Canadian populations, where there is an apparent lack of recruitment *in situ*.

This lack of recruitment is compounded by the fact that long distance spore dispersal in the species would appear to be an infrequent event in itself, as observed in our study, where only 5.8% (35/638) of total spores germinated were found in soils collected from distances over 10m from the population centre, as well as in Peck's (1980) study where he failed to find any Blunt-lobed *Woodsia* spores dispersing more than 10m from a spore source. A highly skewed, or leptokurtic pattern of spore dispersal, as observed here (Figure 3.3), where a large majority of released spores fall in the immediate vicinity of spore producing individuals, has also been shown in other fern species (Peck *et al.*, 1990; Penrod and McCormick, 1996). The relative rarity and fragmented nature of Blunt-lobed *Woodsia* habitat in south eastern Canada suggests that the probability of a migration event to new habitats, or any type of rescue effect between populations (i.e. metapopulation dynamics), is unlikely for the species. This implies that the few known populations are in a precarious situation, and that even a small scale disturbance event could lead to local extinctions and the loss of populations.

We also observed some distinct differences in dispersal patterns between the two study populations. The Gatineau Park population (Figure 3.4) is located on a steeper slope (avg. slope 53.1°) than the Frontenac Park population (Figure 3.5) (avg. slope 33.1°). This has the effect of less spores dispersing upslope at Gatineau Park. Also, vegetation cover is different between the two sites, Gatineau Park has a higher canopy cover than Frontenac (93.3% vs. 79.9%), but it also has a less developed herbaceous layer. Previous studies (Raynor *et al.*, 1976; Peck, 1980; Peck *et al.*, 1990) have shown that understory vegetation can have an important effect on spore dispersal. Although the spatial pattern of spore dispersal observed in the Frontenac

population (less unidirectional and more spores sampled outside of 5 m [Figure 3.5a]) suggests that it would have better chances of spreading to empty suitable habitat, this advantage is probably offset by the fact that the site of the extant population in Frontenac Park is located on a small rock outcropping found in a matrix of unsuitable habitat (open, sedge prairie), and suitable sites in Frontenac are situated at some distance (> 50 m) from the potential source, whereas in Gatineau Park, the study population is situated on a large escarpment in a continuum of apparently suitable habitat

Other fern species found in the soil spore bank and which germinated from the samples were, in descending order of abundance: Fragile Fern (*Cystopteris fragilis* (L.) Bernh.), Marginal Wood Fern (*Dryopteris marginalis* (L.) A. Gray), Maidenhair Spleenwort (*Asplenium trichomanes* L.), Rock Polypody (*Polypodium virginianum* L.) and Purple-stem Cliff Brake (*Pellaea atropurpurea* (L.) Link). Observed numbers range from hundreds in the case of *C. fragilis* to one individual of *P. atropurpurea*. These observations correspond well with the relative frequencies of these fern species found in the study locations, where *C. fragilis* is by far the most common species, and *P. atropurpurea* is very rare. This also corroborates previous descriptions of coexisting fern species in Blunt-lobed Woodsia habitat in Canada (Lafontaine, 1973; Brunton and Lafontaine, 1974; Britton, 1977).

3.5 Conclusion

The results of this study, along with the clustering of naturally occurring populations around the St-Armand/Frelightsburg area (Quebec) and the Westport/Great Rideau Lake area (Ontario) point to the existence of a local dispersal potential, although weak, for *Woodsia obtusa* in Canada. It could also be argued that some occasional long distance dispersal events are possible, as evidenced by the isolated populations of Gatineau Park (Quebec) and Frontenac Park (Ontario). However, in spite of this

dispersal potential, very few *Woodsia obtusa* populations exist in Canada, even though the presence of suitable habitat and microhabitat does not appear to be lacking (Wild 2003; Wild and Gagnon 2005). Taken together, this evidence points to a limiting factor that is acting elsewhere within the life cycle of the fern, most probably at the critical stages of gametophyte recruitment, sexual reproduction or sporophyte recruitment. We suggest that water availability, in the xeric Canadian habitat, be investigated experimentally as a possible limiting factor for recruitment in Canadian Blunt-lobed *Woodsia* populations, at any or all of these critical life stages.

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3.7 References

- Argus, G. W. and Pryer, K. M. 1990. Rare Vascular Plants in Canada: Our Natural Heritage. National Museums of Canada, Canadian Museum of Nature, Ottawa, Ontario.
- Argus, G. W. and White, D. J. 1977. The rare vascular plants of Ontario. National Museums of Canada, Canadian Museum of Nature, Syllogeus **14**: 1-63.
- Bisang, I. 1996. Quantitative analysis of the diaspore banks of bryophytes and ferns in cultivated fields in Switzerland. *Lindbergia* **21**: 9-20.
- Bouchard, A., Barabé, D., Dumais, M., and Hay, S. 1983. Les plantes vasculaires rares du Québec. National Museums of Canada, Canadian Museum of Nature, Syllogeus **48**: 1-79.
- Britton, D. M. 1977. The fern *Woodsia obtusa* (Spreng.) Torrey in Ontario. *Canadian Field-Naturalist* **91**: 84-85.
- Brown, D. F. M. 1964. A monographic study of the fern genus *Woodsia*. *Beichefte zur Nova Hedwigia* **16**: 1-154.
- Brunton, D. F. and Lafontaine, J. D. 1974. An unusual escarpement flora in western Quebec. *Canadian Field-Naturalist* **88**: 337-344.
- Centre de données sur le patrimoine naturel du Québec (CDPNQ). 2008. Les plantes vasculaires menacées ou vulnérables du Québec. 3^e édition. Gouvernement du Québec, ministère du Développement durable, de l'Environnement et des Parcs, Direction du patrimoine écologique et des parcs, Québec.
- COSEWIC. 2007. Update COSEWIC Status Report on Blunt-lobed *Woodsia* (*Woodsia obtusa*). Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario.
- Côté, B. and Fyles, J. W. 1994. Leaf litter disappearance of hardwood species of southern Quebec: interaction between litter quality and stand type. *Ecoscience* **1**: 322-328.
- During, H. J. and ter Horst, B. 1983. The diaspore bank of bryophytes and ferns in chalk grassland. *Lindbergia* **9**: 57-64.

- During, H. J., Brugués, M., Cros, R. M., and Lloret, F. 1987. The diaspore bank of bryophytes and ferns in the soil in some contrasting habitats around Barcelona, Spain. *Lindbergia* **13**: 137-149.
- Dyer, A. F. and Lindsay, S. 1992. Soil spore banks of temperate ferns. *American Fern Journal* **82**: 89-122.
- Farrar, D. R. 1990. Species and evolution in asexually reproducing independent fern gametophytes. *Systematic Botany* **15**: 98-111.
- Greer, G. K., Lloyd, R. M., and McCarthy, B. C. 1997. Factors influencing the distribution of pteridophytes in a southeastern Ohio hardwood forest. *Journal of the Torrey Botanical Society* **124**: 11-21.
- Greer G. K. and McCarthy B. C. 1999. Gametophytic plasticity among four species of ferns with contrasting ecological distributions. *International Journal of Plant Sciences*, **160**: 879-886.
- Hamilton, R. G. 1988. The significance of spore banks in natural populations of *Athyrium pycnocarpon* and *A. thelypteroides*. *American Fern Journal* **78**: 96-104.
- Hock, Z., Szövényi, P., and Tóth, Z. 2006. Seasonal variation in the spore bank of ferns in grasslands on dolomite rock. *Plant Ecology* **187**: 289-296.
- Kaur, S. and Chandra, S. 1973. Morphology of prothallus of *Woodsia obtusa*. *Phytomorphology* **23**: 171-175.
- Klekowski, E. J. Jr. 1979. The genetics and reproductive biology of ferns. *The Experimental Biology of Ferns* (ed. by A. F. Dyer), pp. 133-169. Academic Press, London.
- Lafontaine, J. D. 1973. Range extension of the blunt-lobed woodsia (*Woodsia obtusa* (Speng.) Torr.) (Polypodiaceae), in Canada. *Canadian Field-Naturalist* **87**: 56.
- Leck, M. A., and Simpson, R. L. 1987. Spore bank of a Delaware River freshwater tidal wetland. *Bulletin of the Torrey Botanical Club* **114**: 1-7.
- Marquez, A. L., Real, R., Vargas, J. M., and Salvo, A. E. 1997. On identifying common distribution patterns and their causal factors: a probabilistic method applied to Pteridophytes in the Iberian Peninsula. *Journal of Biogeography* **24**: 613-631.

- Microsoft Office Excel. 2003. Part of Microsoft Office Professional Edition 2003.
- Milberg, P. 1991. Fern spores in a grassland soil. *Canadian Journal of Botany* **69**: 831-834.
- NatureServe. 2006. NatureServe Explorer: An online encyclopedia of life [web application]. Version 6.1. NatureServe, Arlington, Virginia. Available from <http://www.natureserve.org/explorer> [accessed 27 April 2007].
- Page, C. N. 1985. Pteridophyte biology, the biology of the amphibians of the plant world. *Proceedings of the Royal Society of Edinburgh Section B: Biology* **86B**: 439-442.
- Page, C. N. 2002. Ecological strategies in fern evolution: a neopteridological overview. *Review of Palaeobotany and Palynology* **119**: 1-33.
- Peck, C. J. 1985. Reproductive biology of isolated fern gametophytes. Ph.D. dissertation, Department of Botany, Iowa State University, Ames, IA.
- Peck, J. H. 1980. Life history and reproductive biology of the ferns of Woodman Hollow, Webster County, Iowa. Ph.D. dissertation, Department of Botany, Iowa State University, Ames, IA.
- Peck, J. H., Peck, C. J., & Farrar, D. R. 1990. Influences of life-history attributes on formation of local and distant fern populations. *American Fern Journal* **80**: 126-142.
- Penrod, K. A. and McCormick, L. H. 1996. Abundance of viable hay-scented fern spores germinated from hardwood forest soils at various distances from a source. *American Fern Journal* **86**: 69-79.
- Pérez-García, B., Mendoza-Ruiz, A., Sanchez-Coronado, M. E., and Orozco-Segovia, A. 2007. Effect of light and temperature on germination of spores of four tropical fern species. *Acta Oecologica* **32**: 172-179.
- Raghavan, V. 1992. Germination of fern spores. *American Scientist* **80**: 176-185.
- Ramirez-Trejo, M. d. R., Perez-Garcia, B., and Orozco-Segovia, A. 2004. Analysis of fern spore banks from the soil of three vegetation types in the central region of Mexico. *American Journal of Botany* **91**: 682-688.
- Ranal, M. A. 2003. Soil spore bank of ferns in a gallery forest of the ecological station of Panga, Uberlândia, MG, Brazil. *American Fern Journal* **93**: 97-115.

- Raynor, G. S., Ogden, E. C., and Hayes, J. V. 1976. Dispersion of fern spores into and within a forest. *Rhodora* **78**: 473-487.
- Sato, T. and Sakai, A. 1981. Cold tolerance of gametophytes and sporophytes of some cool temperature ferns native to Hokkaido. *Canadian Journal of Botany* **59**: 604-608.
- Schneller J. J. and Holderegger, R. 1996. Soil spore bank and genetic demography of populations of *Athyrium filix-femina*. *Pteridology in perspective*. (ed. by J. M. Camus, M. Gibby and R. J. Johns), pp. 663-665. Royal Botanic Gardens, Kew, UK.
- Simabukuro, E. A., Begovacz, A., Esteves, L. M., and Felipe, G. M. 1999. Fern spore bank at Pedregulho (Itirapina, Sao Paulo, Brazil). *Revista Brasileira de Biologia* **59**: 131-139.
- Soltis, P. S. and Soltis D. E. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences, USA* **97**: 7051-7057.
- Tryon, R. 1970. Development and evolution of fern floras of oceanic islands. *Biotropica* **2**: 76-84.
- Tryon, R. 1986. The biogeography of species, with special reference to ferns. *Botanical Review* **52**: 117-156.
- Watson, P. J. and Vazquez, M. 1981. Comparative ecology of *Woodsia scopulina* sporophytes and gametophytes. *American Fern Journal* **71**: 3-9.
- Werth, C. R. and Cousens, M. I. 1990. Summary: The contributions of population studies on ferns. *American Fern Journal* **80**: 183-190.
- Wild, M. 2003. Have five calcicolous fern species of different habitats saturated all local habitat available to them? M.Sc. Thesis, Université du Québec à Montréal, Montreal, Qc, Canada.
- Wild, M. and Gagnon, D. 2005. Does lack of available suitable habitat explain the patchy distributions of rare calcicole fern species? *Ecography* **28**: 191-196.
- Wild, M., Gagnon, D., and Bouchard, A. 2006. Why are ferns regularly over-represented on state and provincial rare plant lists? *Diversity and Distributions* **12**: 749-755.

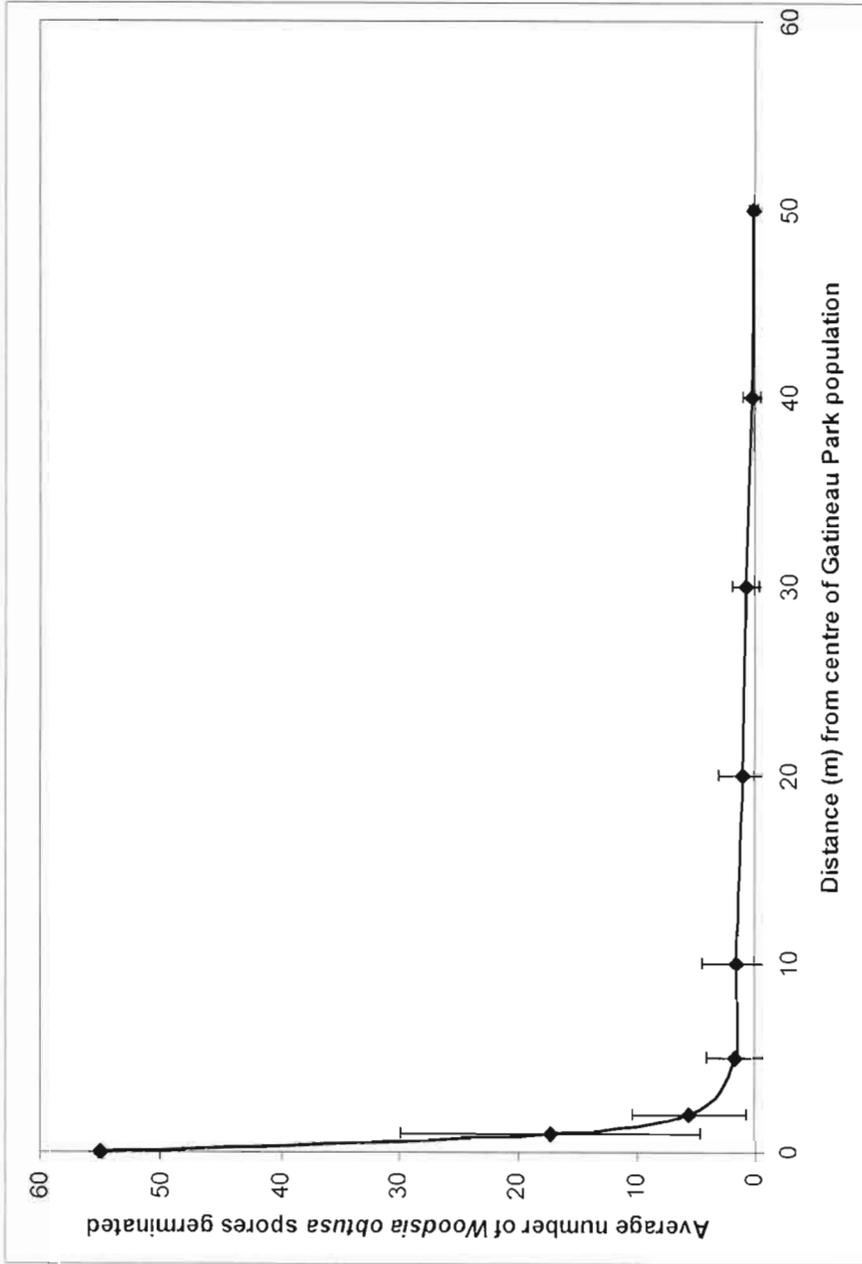


Figure 3.1: *Woodisia obtusa* spores germinated in relation to sampling distance from centre of the Gatineau Park population. n=1 at 0 meters, n=8 at all other distances. Error bars represent standard deviation.

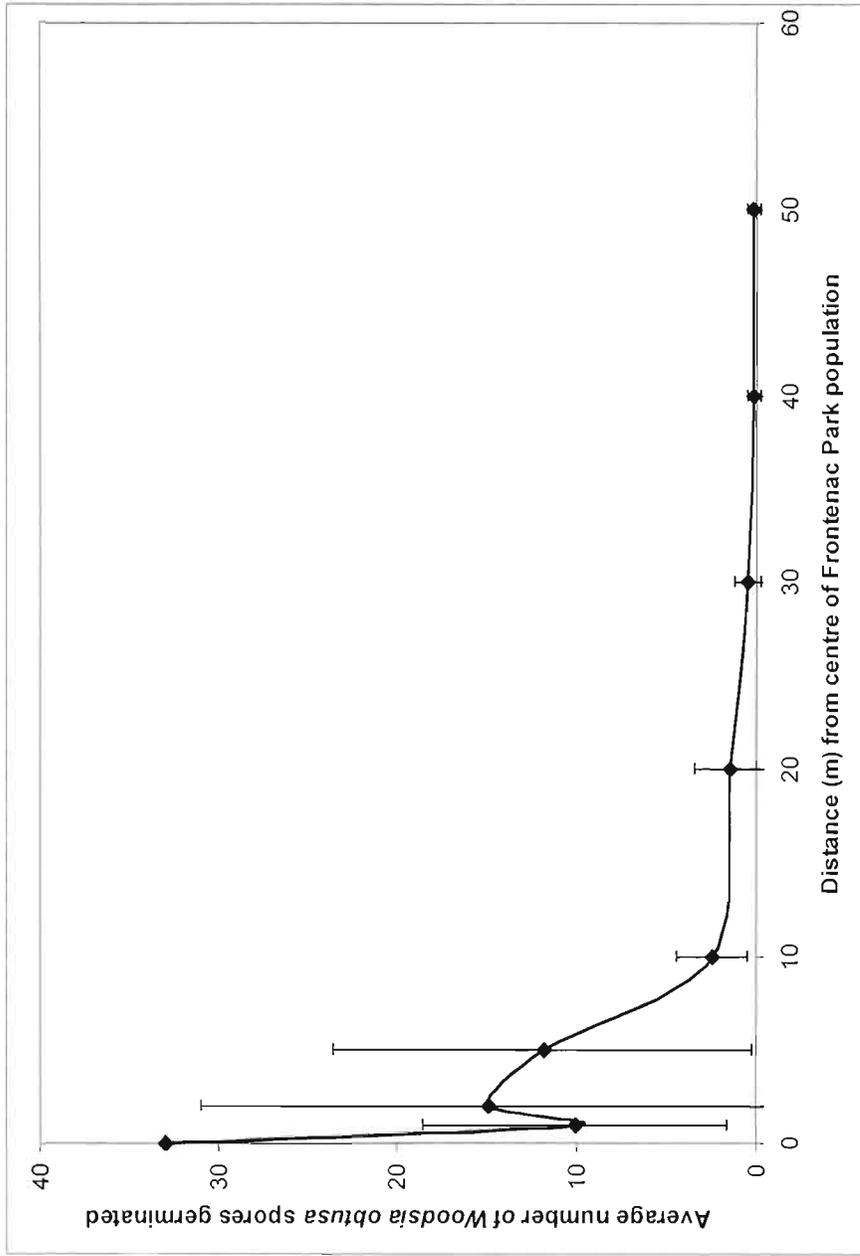


Figure 3.2: *Woodisia obtusa* spores germinated in relation to sampling distance from centre of the Frontenac Park population. n=1 at 0 meters, n=8 at all other distances. Error bars represent standard deviation.

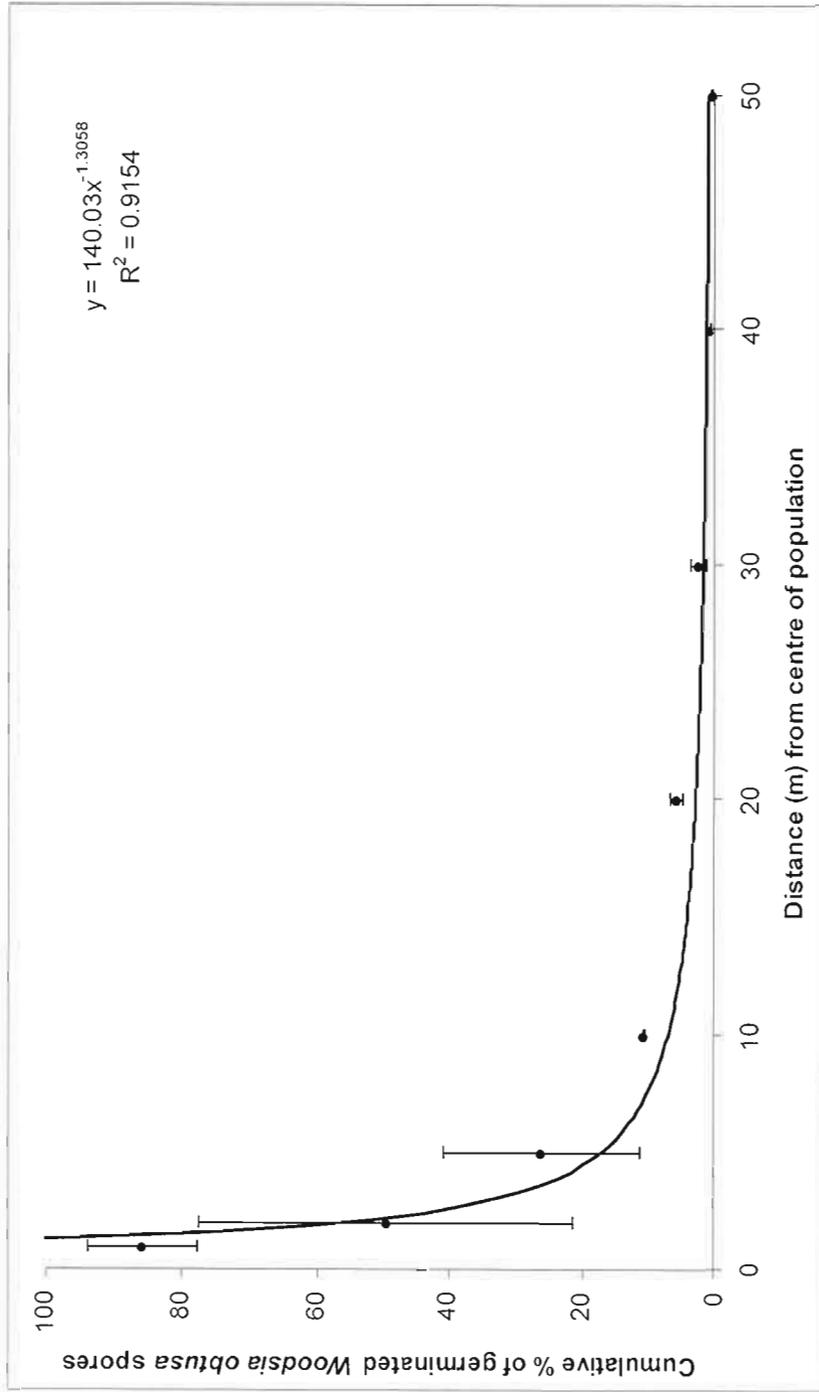


Figure 3.3: Cumulative percent of *Woodisia obtusa* spore germination in Gatineau Park and Frontenac Park study populations in relation to sampling distance from centre of population. Average data represented by points, error bars represent standard deviation; regression represented by solid line.

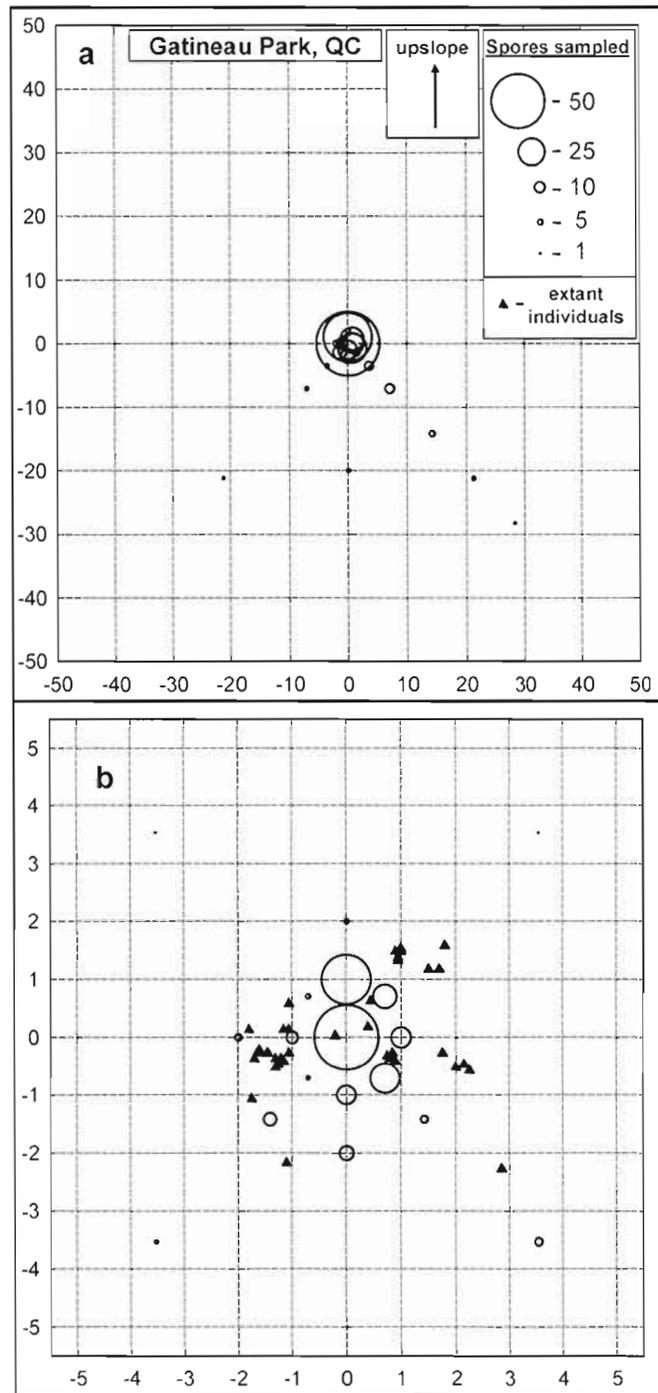


Figure 3.4: Pattern and abundance of *Woodsia obtusa* spores germinated in soils collected in the Gatineau Park population (4a: 0-50m scale; 4b: 0-5m scale). Triangles indicate locations of extant *W. obtusa* plants. Circles represent the locations where spores germinated. The size of these circles corresponds to the number of spores germinated.

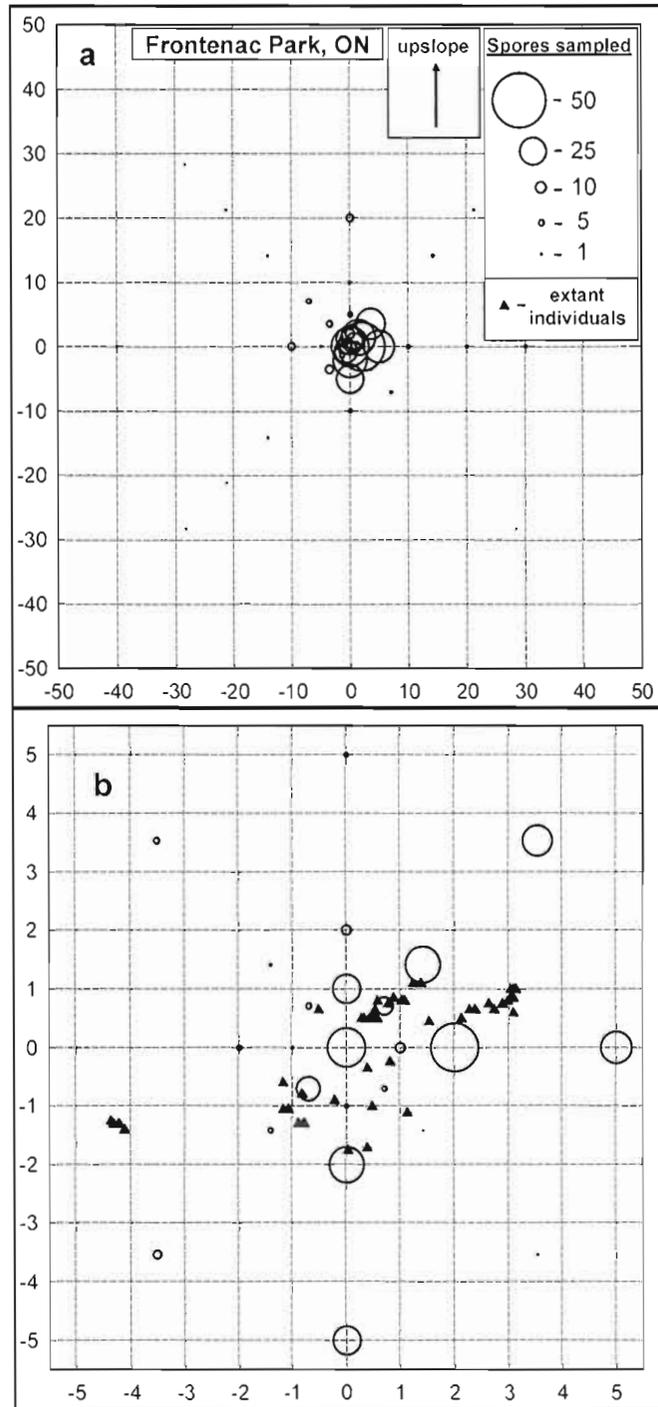


Figure 3.5: Pattern and abundance of *Woodsia obtusa* spores germinated in soils collected in the Frontenac Park population (5a: 0-50m scale; 5b: 0-5m scale). Triangles indicate locations of extant *W. obtusa* plants. Circles represent the locations where spores germinated. The size of these circles corresponds to the number of spores germinated.

CHAPTER IV

THE XERIC NATURE OF BLUNT-LOBED *WOODSIA* HABITAT AT THE
NORTHERN EDGE OF ITS RANGE LIMITS SPOROPHYTE RECRUITMENT

Matthew Wild and Daniel Gagnon

Abstract

Many species show patterns of abundance which are highest in the centre of their ranges and that decline gradually toward its boundaries, and various conclusions regarding the causes of relative rarity for these species at their distributional limit have been presented. The underlying question of this study is what causes rarity of the fern *Woodsia obtusa* at the northernmost part of its range? More specifically, is it gametophyte and/or sporophyte recruitment which is limiting the species in its habitat in Canada? With a series of laboratory experiments, we attempt to verify the hypothesis that water is the main environmental factor limiting recruitment and that both gametophyte and sporophyte recruitment are naturally low in northern populations because of the effect of low levels of water found in the xeric habitat of Blunt-lobed *Woodsia* in Canada. Spores of *W. obtusa* were collected in August 2007, from two populations. These spores were then sown in controlled conditions to measure which factors affected gametophyte and sporophyte recruitment. Tested parameters were: medium pH, light intensity, initial humidity, subsequent watering and spore density. For gametophyte recruitment, an acidic pH was limiting, and watering and reduced light had a small, but significant, positive effect. For sporophytes, extreme pH levels (4 and 8.5) limited recruitment, and both water treatments (initial humidity and weekly watering) significantly increased recruitment. Results suggest that the low levels of water observed in Blunt-lobed *Woodsia*'s natural habitats in Canada do limit gametophyte and, especially, sporophyte recruitment. This suggests that the species' niche is much reduced in Canada compared to that observed in the center of its distributional range. There is also strong evidence that niche requirements for the two separate stages of the Blunt-lobed *Woodsia*'s life-cycle (gametophyte and sporophyte) are dissimilar, and that this dissimilarity increases as one reaches the periphery of the species' range. The xeric nature of the habitat occupied by Blunt-lobed *Woodsia* in Canada contributes to very low sporophyte recruitment rates, thereby severely reducing overall species niche size. This reduction of niche size is compounded by the fact that niche overlap is needed by a species with two independent life stages, and that this overlap is extremely reduced in northern populations of Blunt-lobed *Woodsia*. We believe that this is also a likely explanation for other fern species showing similar patterns of rarity near the margins of their range

4.1 Introduction

The fact that species tend to show patterns of abundance which are highest in the centre of their ranges and that decline gradually toward its boundaries has often been described (Brown 1984 and references therein). Several studies have been undertaken on the specific ecologies and dynamics of peripheral populations in the northernmost range of their known distribution. Seminal work was accomplished in this respect on populations of *Tilia cordata* Mill. in northwest England (Pigott and Huntley, 1978; 1979; 1981). More recent examples include studies on trees (*Pinus rigida* Mill.: Meilleur *et al.*, 1997, and *Acer rubrum* L.: Tremblay *et al.*, 2002), shrubs (*Fumana procumbens* (Dunal) Gren. & Godr.: Bengtsson, 1993, and *Rhus aromatica* Ait.: Nantel and Gagnon, 1999), annual herbs (*Floerkea proserpinacoides* Willd.: McKenna and Houle, 2000), perennial herbs (*Helianthus divaricatus* L.: Nantel and Gagnon, 1999), ferns (*Trichomanes speciosum* Willd.: Rumsey *et al.*, 1999), and bryophytes (several species.: Hedderson, 1992). The aforementioned studies arrive at various conclusions regarding the causes of relative rarity for these species at their northern distributional limit. These causes include lack of suitable habitat (*P. rigida*), infrequent sexual recruitment (*A. rubrum*), low reproductive capacity of adult individuals (*F. procumbens*), increased demographic variability and local extinction probability (*R. aromatica* and *H. divaricatus*), infrequent long-distance dispersal events and highly variable conditions among years (*F. proserpinacoides*), low spore production with little recruitment of sporophytes (*T. speciosum*), and limited dispersal potential (bryophytes). This gives rise to the underlying questions of this study: What causes rarity in the case of Blunt-lobed *Woodsia* in the northernmost part of its range? More specifically, is it gametophyte and/or sporophyte recruitment which is limiting this fern species in its xeric habitats in Canada?

At a larger scale, the species' rarity at the northern limit of its distributional range is most likely related to climate and habitat (COSEWIC, 2007). That climate is a limiting factor appears evident when we consider that in Canada, all known populations are found on south-facing slopes under semi-open, mostly deciduous canopy in xeric conditions (Wild and Gagnon, 2005; COSEWIC, 2007), whereas further south, *W. obtusa* occurs on north-facing slopes under dense deciduous canopy cover in mesic conditions (Greer *et al.*, 1997). Also, it would appear that an absolute prerequisite for the species' presence is calcareous bedrock; all Canadian populations occur in shallow soils over marble, dolomite or limestone (Wild and Gagnon, 2005; COSEWIC, 2007). Again this is at odds with descriptions from elsewhere in the species' range, where it does not appear to be an obligate calcicolous species, being described as "found on a variety of substrates including both granite and limestone" (Flora of North America Editorial Committee, 1993). Although this suggests that a favourable, warm microclimate with underlying calcareous bedrock is required in order for this species to exist in the northern part of its range, it does not explain why there is uncolonized habitat with the same microclimate and habitat conditions in areas adjacent to extant populations (Wild and Gagnon, 2005).

The typical life cycle of a homosporous fern can be divided into seven sequential stages: i) spore dispersal; ii) spore germination; iii) gametophyte development; iv) fertilisation; v) sporophyte development; vi) sporophyte persistence; vii) spore production (Figure 4.1). Here, we consider spore germination (ii) and gametophyte development (iii) as a single step: gametophyte recruitment; and fertilisation (iv) and subsequent sporophyte development (v) as another single step: sporophyte recruitment.

Theoretically, each of the fern life cycle stages has the potential to be the stage at which limiting factors will operate. However, previous work has shown that spore dispersal (Chapter III) and sporophyte persistence (Chapter V) do not appear to be

limiting for the species, and that spore production in Canadian populations is very similar to that observed in populations further south (M. Wild, unpub. data; Peck *et al.*, 1990). Although previous work has shown that dispersal is effectively taking place and that viable spores are present in soils taken from what appears to be suitable habitat (Chapter III), no naturally occurring gametophytes or young sporophytes have been found despite many searches in all known Canadian populations of Blunt-lobed *Woodsia*. Also, the clustering of Blunt-lobed *Woodsia* populations around Westport/Great Rideau Lake area (Ontario) and around the St-Armand/Frelighsburg area (Quebec) suggests that a local dispersal potential exists for the species in Canada. It could also be argued that long distance dispersal events are possible, as evidenced by the more isolated populations of Frontenac Park (Ont.) and Gatineau Park (Que.). However, in spite of this dispersal potential, or capacity, very few *Woodsia obtusa* populations exist in Canada, even though the presence of suitable habitat and microhabitat also does not appear to be lacking (Wild, 2003; Wild and Gagnon, 2005). All of this evidence points to one or several limiting factors that are acting elsewhere within the life cycle of the fern, most probably at the critical stages of gametophyte recruitment or sporophyte recruitment.

There also exists the possibility that differences between the niches of Blunt-lobed *Woodsia*'s life cycle stages could be limiting the species at its range margins. If, as previous work suggests, sporophytes survive and grow best in warmer/dryer microclimates/habitats, whereas gametophytes need more moist and shaded habitats, in order for fertilisation to occur, then a narrow overlap of the two niches could contribute to the species' rarity in southern Canada.

The occurrence of two niches is not a concept unique to ferns. It has been observed in trees, where the "regeneration niche" is much different (germination of seeds and establishment of seedlings) than the niche of mature forest trees (Grubb, 1977). Nor is it unique to the plant world, as animals often have two distinct life stages in their

life cycle (larval and adult stages), such as in the case of insects, where the larval stage may even be aquatic (i.e. mosquito, dragonfly, mayfly, etc.).

Here, with a series of laboratory experiments, we attempt to verify the hypothesis that gametophyte and/or sporophyte recruitment are the life stages where limiting factors are operating for this species. We hypothesize that water is the main environmental factor limiting recruitment and that both gametophyte and sporophyte recruitment are naturally low in this species because of the effect of low levels of water found in the xeric habitat of Blunt-lobed *Woodsia* in Canada.

4.2 Materials and methods

4.2.1 Study species

Blunt-lobed *Woodsia*, or Blunt-lobed cliff-fern (*Woodsia obtusa* subsp. *obtusa* (Sprengel) Torrey: Dryopteridaceae), is a homosporous fern found in eastern North America. Although the species is common throughout much of its distributional range, it is only known to occur in eight sparsely populated sites at the northern periphery of its distribution (southern Canada), these eight occurrences (four in southern Quebec and four in southern Ontario) contain a total of approximately 1350 individuals (COSEWIC, 2007). The low number of small populations has earned the species a NatureServe rank of N1 (critically imperilled) in Canada, provincial ranks of S1 (critically imperilled) in both Quebec and Ontario (NatureServe, 2009), and has led to it being listed as “Threatened” under Canada’s Species at Risk Act, Quebec’s *Loi sur les espèces menacées et vulnérables* and Ontario’s Endangered Species Act. Blunt-lobed *Woodsia* appears on rare plant lists in Quebec (Bouchard *et al.*, 1983; Labrecque and Lavoie, 2002; CDPNQ, 2008), Ontario (Argus and White, 1983), and Canada (Argus and Pryer, 1990).

4.2.2 Spore collection and sterilization

Fertile fronds of *W. obtusa* were collected in August 2007, from two Canadian populations (Gatineau Park, QC and Frontenac Park, ON) and stored in a sealed paper envelope inside a plastic bag containing a silica desiccant, at room temperature. A month later, dried fronds from each fern population were combined together and crushed in a mortar with a pestle to release the spores. Double-distilled water was added and the suspension was filtered twice through a 60- μm nylon mesh. The spores were kept soaked overnight in a distilled water centrifuge tube (Sarstedt). Water was removed after a centrifugation at 1000 g for 3-4 min. The spores were surface-sterilized with a 5% (v/v) solution of commercial bleach (5.25% NaOCl) containing 0.1% Tween-20 for 5 min, and then rinsed 3 times with sterile double-distilled water by centrifugation, then re-suspended in double distilled water. Spore density was estimated by counting the number of spores in a 50 μl sample under the microscope (5 replicates). The sterilized spore suspension was divided equally into 4 centrifuge tubes that were completed with the inorganic C-Fern medium (Hickok and Warne, 1998) at one of four pH values: 4.0, 5.5, 7.0 and 8.5. The spores were washed twice with the same culture medium.

4.2.3 Factors affecting gametophyte recruitment

The experiment was conducted from October 2007 to November 2007 in 60 x 15 mm Petri dishes (Sarstedt) containing 8 ml of C-Fern medium solidified with 1% Bacto-Agar (Figure 4.2a) in a growth chamber (Conviron) with a 16 hour / 8 hour photoperiod, a temperature of 24°C and 50% ambient humidity. The effects of the following factors on spore germination and gametophyte development were studied with 3 Petri dishes of each treatment as replicates: - pH: 4.0, 5.5, 7.0 or 8.5; - light intensity: 20 or 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; - weekly watering: 0 or 0.5 ml of sterile double distilled water added once a week; - spore density: 160 or 1600 spores/ml (3

replicates x 4 pHs x 2 light intensities x 2 watering treatments x 2 densities = 96 experimental units). Spores were uniformly sowed on the agar surface using a cell spreader. Gametophyte recruitment was calculated by counting the number of cordate gametophytes (Figure 3.3) per Petri dish after four weeks. The statistical significance of the effects of factors on gametophyte recruitment was determined using ANOVA followed by Tukey-Kramer means comparison tests (JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007).

4.2.4 Factors affecting sporophyte recruitment

The experiment was conducted from October 2007 to January 2008 in small jars containing 50 ml of cleaned and autoclaved sand (Figure 4.2b). Factors evaluated were: - pH: 4.0, 5.5, 7.0 or 8.5; - weekly watering: 0 or 0.5 ml of sterile double distilled water added once a week with a sprayer; - spore density: 160 or 1600 spores/ml; - initial substrate moisture level: wet or 'dry' (6 replicates x 4 pHs x 2 watering treatments x 2 densities x 2 initial moistures = 192 experimental units). The volume of liquid culture medium was adjusted to 8 ml (dry) and 15 ml (wet) to reproduce the initial moisture level in the sand substrate. 1 ml of the sterilized spore suspension was added to each jar, thus slightly increasing initial humidity. The cultures were monitored after three months in the growth chamber (16 hour / 8 hour photoperiod, light intensity of $100 \mu\text{mol.m}^{-2}.\text{s}$, temperature of 24°C , 50% ambient humidity) for the appearance of sporophytes. The statistical significance of the effects of factors on sporophyte recruitment was determined using ANOVA followed by Tukey-Kramer means comparison tests (JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007).

4.3 Results

4.3.1 Gametophyte recruitment

There were no statistically significant differences in gametophyte recruitment obtained between high (1600 spores/ml) density and low (160 spores/ml) density. Results illustrated (Figure 4.4) are those obtained with high density sowing. These results show that pH 4 has a limiting effect, which is compounded by a lack of water. Recruitment at all other pH levels is statistically identical. Within each pH level tested, a similar trend is seen, with both watering and reduced light having a positive effect on gametophyte recruitment. Although these differences are statistically significant [water: $F=41,45$; $df=1$; $P<0.0001$; light: $F=50,72$; $df=1$; $P<0.0001$], the overall effect of adding water and decreasing light is weak, only increasing recruitment by an average of 3.4%. On average, excluding the apparently limiting pH 4 treatment, just over 20% of total sown spores developed into gametophytes.

4.3.2 Sporophyte recruitment

There were no statistically significant differences in sporophyte recruitment obtained between high (1600 spores/ml) and low (160 spores/ml) sowing densities. The results illustrated (Figure 4.5) are those obtained with high density sowing. At acidic pH levels (pH 4) as well as at the most alkaline tested (pH 8.5), very little sporophyte production was observed (< 1% of spores sown developed into sporophytes). The highest sporophyte recruitment was obtained at pH 5.5. As for the results of the watering treatments, similar trends can be noticed at pH 5.5. and pH 7, where the highest recruitment is achieved with initial moisture as well as subsequent watering (3.8 to 6.9% recruitment), intermediate sporophyte recruitment was achieved with either initial moisture or subsequent watering treatments (2.5 to 4.7% recruitment),

and the lowest recruitment rates occurred when there were neither initial moisture or subsequent watering (1.4 to 1.9% recruitment).

4.4 Discussion

Although, once established, Blunt-lobed *Woodsia* sporophytes thrive and produce viable spores in northern populations (Wild and Gagnon, 2005; COSEWIC, 2007; Chapter V), our results suggest that the lack of observed recruitment in the field is most likely due to the effect of low levels of available water for the recruitment of both gametophytes and sporophytes, although the effect is strongest on sporophyte recruitment. As mentioned earlier, there are apparent differences between the northern habitat of Blunt-lobed *Woodsia* and that found further south. These differences are evident when comparing northern habitat specifics to published habitat descriptions from further south in its distributional range, such as: "...*Woodsia obtusa* occurred in habitats with high moisture, pH, nutrient, and canopy cover values" (Greer *et al.*, 1997), and "*Woodsia obtusa* [...] is more mesic than the others [Woodsias] and this fact is borne out not only by geographical distribution and habitat observations but by the water requirements of potted specimens..." (Brown, 1964). These differences in occupied habitats for the species, notably the obvious difference in available water, could explain the small size of northern populations because of the limiting effect of lack of water on recruitment. Lack of water has a small but significant effect on gametophyte recruitment, but it had a larger and very significant effect on sporophyte recruitment.

4.4.1 Gametophyte recruitment

Gametophyte recruitment includes spore germination and subsequent gametophyte development. Contrary to our hypothesis, low levels of water did not have an important effect on gametophyte recruitment. The effects of varying conditions on

spore germination have been investigated for several fern species (e.g. Pangua *et al.*, 1994; Kiss and Kiss, 1998; Quintanilla *et al.*, 2000) with varied results. For example, in a study on spore germination in *Cheilanthes feei*, Nondorf *et al.* (2003) varied levels of pH, temperature, light intensity, light quality and moisture, concluding that although spores germinated under a wide variety of conditions, optimal conditions for spore germination and for subsequent protonemal growth overlap narrowly (spores germinated in most moisture levels and did well in saturated conditions, but protonema did poorly in saturated conditions). Potential spore germination for *W. obtusa* was quantified by Peck *et al.* (1990) who observed over 82% germination for spores sown on natural substrates and 93% for those sown on agar. As for gametophyte development, Watson and Vasquez (1981) suggest that ferns that do not reproduce asexually produce gametophytes of greater vigour than ferns capable of asexual reproduction, implying some trade-off between sexual and asexual reproduction. Interestingly, this is also reflected in data collected by Peck *et al.* (1990), where out of a possible 14 species, they found naturally occurring gametophytes of only six. Out of these six, four, including *Woodsia obtusa*, are not known to reproduce asexually. The results obtained here support the suggestion that Blunt-lobed *Woodsia* gametophytes are, in fact, relatively vigorous, and able to withstand environmental extremes, including a relative lack of moisture.

Although significantly lower gametophyte recruitment was obtained at pH 4, this is not a naturally occurring pH in northern Blunt-lobed *Woodsia* populations, and it is extremely unlikely that it is a natural limiting factor for the species in Canada. Also, although both increased light levels and lack of watering had significantly negative effects, these effects may not be strong enough for these factors to be important limiting factors in gametophyte recruitment. Light intensity has previously been shown to affect gametophyte recruitment, including the possibility of a photoinhibition effect on spore germination (Raghavan, 1989; Viviani and Randi, 2008) and gametophyte growth (Raghavan, 1989).

Our results suggest little effect on recruitment of gametophytes among the factors tested here. The fact that no naturally occurring gametophytes have been observed in Canadian populations of Blunt-lobed *Woodsia* could simply be due to the fact that their small size and ephemeral nature make locating fern gametophytes in the field a very difficult task.

4.4.2 Sporophyte recruitment

Sporophyte recruitment includes fertilisation and subsequent sporophyte development. Most homosporous ferns require water for fertilisation, as antherozoids need a film of water in order to travel from antheridia to archegonia. Exceptions to this are sometimes encountered in xeromorphic species such as cheilanthoid ferns (Pteridaceae: Cheilanthoidae), which are capable of apogamy (Klekowski, 1969). In the most common type of apogamy, fertilisation is not required for production of a sporophyte, which instead originates directly by parthenogenesis from the tissues of the gametophyte. Apogamy is not known to occur in *W. obtusa*, and therefore, it is expected that the low levels of water encountered in the xeric environment of its northernmost habitats greatly affect this stage of its life cycle. Interestingly, one of the ferns commonly found with Blunt-lobed *Woodsia* in Canada is an obligate apogamous species, Purple Stem Cliff-brake (*Pellaea atropurpurea*), suggesting that their shared habitat is suited to this type of strategy requiring little or no water for reproduction.

Our results confirm our hypothesis by clearly illustrating a strong dependence on water for sporophyte recruitment, where significantly more sporophyte recruitment was observed in those treatments with combined initial substrate humidity and weekly watering compared to treatments with solely initial water or weekly watering. The least amount of sporophyte recruitment was observed in the treatment with no

initial humidity of the substrate and no weekly watering. We suggest that it is this last combination of treatments which most closely replicates those conditions found in natural populations of Blunt-lobed *Woodsia* at the northern limit of its distribution in southern Canada, although it is probable that the conditions in these populations are actually dryer than those studied here.

4.4.3 Niche effects

Coupled with our previous work on sporophyte persistence (Chapter V) and the long-time survival of apparently viable populations in Canada (COSEWIC, 2007), the results obtained here suggest that the conditions required for gametophyte and sporophyte recruitment possibly overlap only narrowly with conditions required for sporophyte persistence. A difference in the ecology of gametophytes versus that of sporophytes has been noted before for other fern species. Watson and Vazquez (1981) compared the ecologies of *Woodsia scopulina* gametophytes and sporophytes and found both spatial and temporal differences in habitat factors between the two, thereby suggesting different niche requirements for each life stage. This contradicts previous authors who have claimed that sporophytes and gametophytes have the same ecological requirements (eg. Nayar and Kaur, 1971). Any such difference in niche factors is likely to be more limiting as a species reaches the limit of its distribution range. Niche theory suggests that a species' occupied niche is likely to become smaller as one approaches the margins of its distributional range (e.g. Lennon *et al.*, 2002; Murphy and Lovett-Doust, 2007). This can be explained by increased biological constraints in marginal populations, and is part of the explanation behind the "abundant-centre-distribution" theory (Brown, 1984). Our observations seem to confirm this with Blunt-lobed *Woodsia*, where the marked difference between the habitat occupied by Blunt-lobed *Woodsia* in Canada compared to where it is found towards the centre of its range would appear to be related to water availability

(Brown, 1964; Greer *et al.*, 1997) resulting in a smaller, more constrictive niche in populations at the northern range limit.

For an organism with two independent life-stages, as is the case for ferns with their independent gametophyte and sporophyte stages, this type of niche reduction can have very important repercussions. Figure 4.6 illustrates a theoretical representation of the above model comparing an organism with only one independent life stage with an organism (such as a fern) with two independent life stages with different niches. In this example we assume a reduction in niche size from the fundamental niche (100%) to an occupied niche 50% smaller at the center of the organism's range and 90% smaller at its margin. For a sessile organism with two independent life stages, it is the overlap between the two stage's niches that allows survival. In this case, for a species with two independent life stages showing similar niche reduction, the overlap at the center of the organism's range that remains is 41% of its fundamental niche but only 2% at the range margin. This model illustrates the cumulative negative effect of a species having two independent life stages with differing niches, as niche size decreases nearing the margins of the distribution range.

4.5 Conclusion

To answer the question of what causes the rarity of Blunt-lobed *Woodsia* in Canada, we interpret our results as showing that the xeric nature of the habitat occupied by Blunt-lobed *Woodsia* in Canada contributes to very low sporophyte recruitment rates, thereby severely reducing overall species niche size. This reduction of niche size is compounded by the fact that niche overlap is needed by a species with two independent life stages, and that this overlap would appear to be extremely reduced in northern populations of Blunt-lobed *Woodsia*. We believe that this is also a likely explanation for other fern species showing similar patterns of rarity near the margins of their range (Chapter II (Wild *et al.*, 2006)).

4.6 Acknowledgements

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4.7 References

- Argus, G. W. and Pryer, K. M. 1990. Rare vascular plants in Canada, our natural heritage. Canadian Museum of Nature. Ottawa.
- Argus, G. W. and White, D. J. 1977. The rare vascular plants of Ontario. *Syllogeus* **14**: 1-63.
- Bengtsson, K. 1993. *Fumana procumbens* on Oland - population-dynamics of a disjunct species at the northern limit of its range. *Journal of Ecology* **81**: 745-758.
- Bouchard, A., Barabé, D., Dumais, M., and Hay, S. 1983. Les plantes vasculaires rares du Québec. *Syllogeus* **48**: 1-79.
- Brown, D. F. M. 1964. A monographic study of the fern genus *Woodsia*. J. Cramer, Weinheim.
- Brown, J. H. 1984. On the relationship between abundance and distribution of species. *American Naturalist* **124**: 255-279.
- Centre de données sur le patrimoine naturel du Québec (CDPNQ). 2008. Les plantes vasculaires menacées ou vulnérables du Québec. 3e édition. Gouvernement du Québec, ministère du Développement durable, de l'Environnement et des Parcs, Direction du patrimoine écologique et des parcs, Québec.
- COSEWIC 2007. COSEWIC assessment and update status report on the Blunt-lobed *Woodsia* *Woodsia obtusa* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa.
- Flora of North America Editorial Committee. 1993. *Flora of North America: North of Mexico*. vol. 2. Pteridophytes and gymnosperms. Oxford University Press, New York.
- Greer, G. K., Lloyd, R. M., and McCarthy, B. C. 1997. Factors influencing the distribution of Pteridophytes in a southeastern Ohio hardwood forest. *Journal of the Torrey Botanical Society* **124**: 11-21.
- Grubb, P. J. 1977. The maintenance of species-richness in plant communities: The importance of the regeneration niche. *Biological Reviews* **52**: 107-145.

- Hedderson, T. A. 1992. Rarity at range limits - Dispersal capacity and habitat relationships of extraneous moss species in a boreal Canadian National Park. *Biological Conservation* **59**: 113-120.
- Hickok, L. G. and Warne, T. R. 1998. C-Fern Manual. Carolina Biological Supply Company, Burlington, NC.
- JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007.
- Kiss, H. G. and Kiss, J. Z. 1998. Spore germination in populations of *Schizaea pusilla* from New Jersey and Nova Scotia. *International Journal of Plant Sciences* **159**: 848-852.
- Klekowski, E. J. 1979. The genetics and reproductive biology of ferns. *In*: The experimental biology of ferns. Dyer, A. F. *Ed.* Academic Press, New York.
- Labrecque, J. and Lavoie, G. 2002. Les plantes vasculaires menacées ou vulnérables du Québec. Gouvernement du Québec, ministère de l'environnement. Direction du patrimoine écologique et du développement durable, Québec.
- Lennon, J. J., Kunin, W. E., Corne, S., Carver, S., and Van Hees, W. W. S. 2002. Are alaskan trees found in locally more favourable sites in marginal areas? *Global Ecology and Biogeography* **11**: 103-114.
- McKenna, M. F. and Houle, G. 2000. Under-saturated distribution of *Floerkea proserpinacoides* Willd. (Limnanthaceae) at the northern limit of its distribution. *Ecoscience* **7**: 466-473.
- Meilleur, A., Brisson, J., and Bouchard, A. 1997. Ecological analyses of the northernmost population of pitch pine (*Pinus rigida*). *Canadian Journal of Forest Research* **27**: 1342-1350.
- Murphy H. T. and Lovett-Doust, J. 2007. Accounting for regional niche variation in habitat suitability models. *Oikos* **116**: 99-110.
- Nantel, P. and Gagnon, D. 1999. Variability in the dynamics of northern peripheral versus southern populations of two clonal plant species, *Helianthus divaricatus* and *Rhus aromatica*. *Journal of Ecology* **87**: 748-760.
- NatureServe. 2009. NatureServe Explorer: An online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. Available <http://www.natureserve.org/explorer>. (Accessed: June 9, 2010).

- Nayar, B. K. and Kaur, S. 1971. Gametophytes of homosporous ferns. *Botanical Review* **37**: 95-396.
- Nondorf, S. L., Dooley, M. A., Palmieri, M., and Swatzell, L. J. 2003. The effects of pH, temperature, light intensity, light quality, and moisture levels on spore germination in *Cheilanthes feei* of southeast Missouri. *American Fern Journal* **93**: 56-69.
- Pangua, E., Lindsay, S., and Dyer, A. 1994. Spore germination and gametophyte development in 3 species of *Asplenium*. *Annals of Botany* **73**: 587-593.
- Peck, J. H., Peck, C. J., and Farrar, D. R. 1990. Influences of life-history attributes on formation of local and distant fern populations. *American Fern Journal* **80**: 126-142.
- Pigott, C. D. and Huntley, J. P. 1978. Factors controlling distribution of *Tilia cordata* at northern limits of its geographical range. 1. Distribution in northwest England. *New Phytologist* **81**: 429-441.
- Pigott, C. D. and Huntley, J. P. 1980. Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. 2. History in northwest England. *New Phytologist* **84**: 145-164.
- Pigott, C. D. and Huntley, J. P. 1981. Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. 3. Nature and causes of seed sterility. *New Phytologist* **87**: 817-839.
- Quintanilla, L. G., Pajaron, S., Pangua, E., and Amigo, J. 2000. Effect of temperature on germination in northernmost populations of *Culcita macrocarpa* and *Woodwardia radicans*. *Plant Biology* **2**: 612-617.
- Raghavan, V. 1989. *Developmental biology of fern gametophytes*. Cambridge University Press, Cambridge, U.K.
- Rumsey, F. J., Vogel, J. C., Russell, S. J., Barrett, J. A., and Gibby, M. 1999. Population structure and conservation biology of the endangered fern *Trichomanes speciosum* Willd. (Hymenophyllaceae) at its northern distributional limit. *Biological Journal of the Linnean Society* **66**: 333-344.
- Tremblay, M. F., Bergeron, Y., Lalonde, D., and Mauffette, Y. 2002. The potential effects of sexual reproduction and seedling recruitment on the maintenance of red maple (*Acer rubrum* L.) populations at the northern limit of the species range. *Journal of Biogeography* **29**: 365-373.

- Viviani, D. and Randi, Á. M. 2008. Effects of pH, temperature and light intensity on spore germination and growth analysis of young sporophytes of *Polypodium lepidopteris* (Pteridophyta, Polypodiaceae). *Rodriguésia* **59**: 751-760.
- Watson, P. J. and Vazquez, M. 1981. Comparative ecology of *Woodsia scopulina* sporophytes and gametophytes. *American Fern Journal* **71**: 3-9.
- Wild, M. and Gagnon, D. 2005. Does lack of available suitable habitat explain the patchy distributions of rare calcicole fern species? *Ecography* **28**: 191-196.
- Wild, M., Gagnon, D., and Bouchard, A. 2006. Why are ferns regularly over-represented on state and provincial rare plant lists? *Diversity and Distributions* **12**: 749-755.

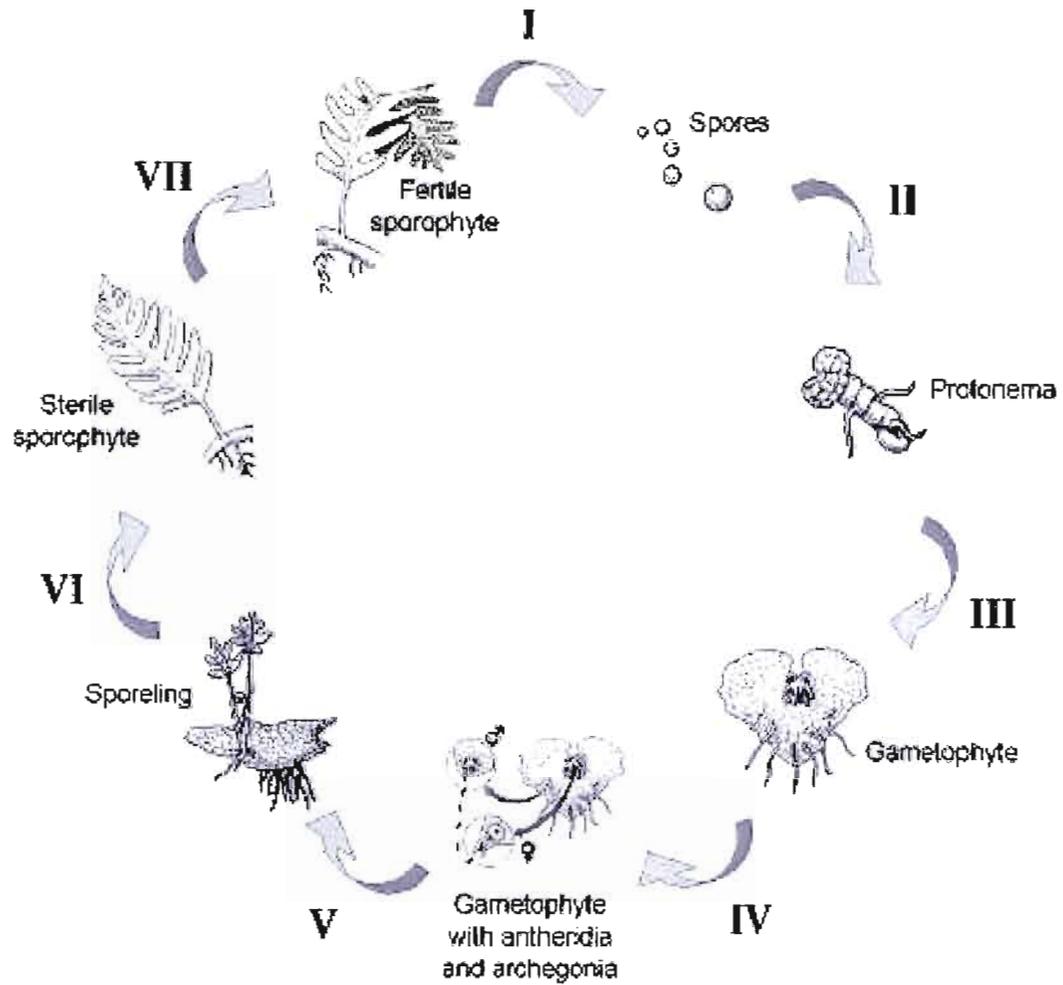


Figure 4.1. Typical life cycle of a homosporous fern species.

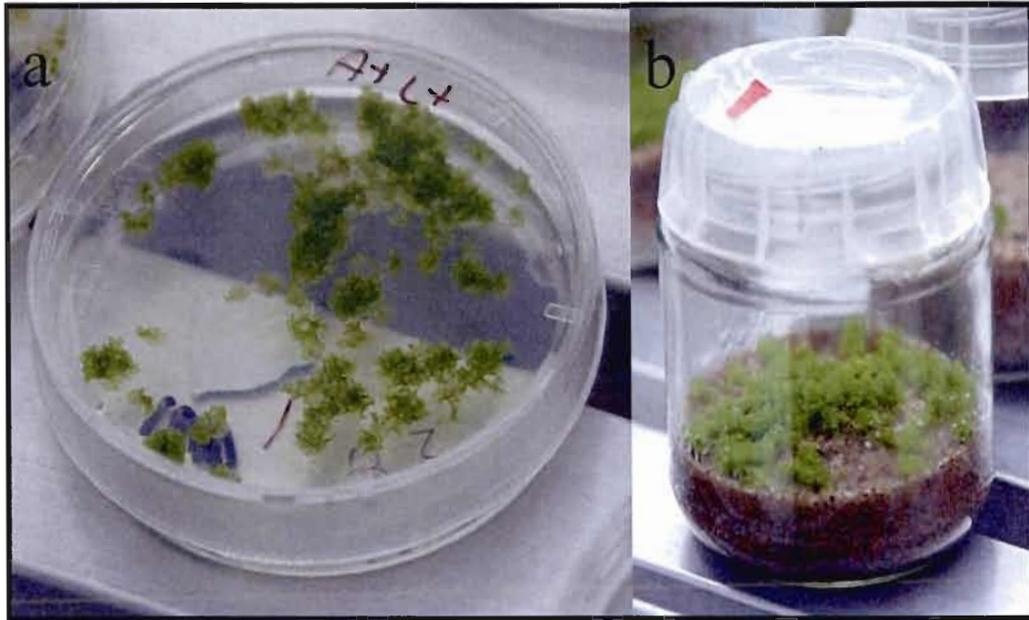


Figure 4.2. Containers used for *Woodsia obtusa* recruitment experiments: a) Petri dish used for gametophyte recruitment experiment; b) jar used for sporophyte recruitment experiment.



Figure 4.3. A cordate gametophyte of *Woodsia obtusa*

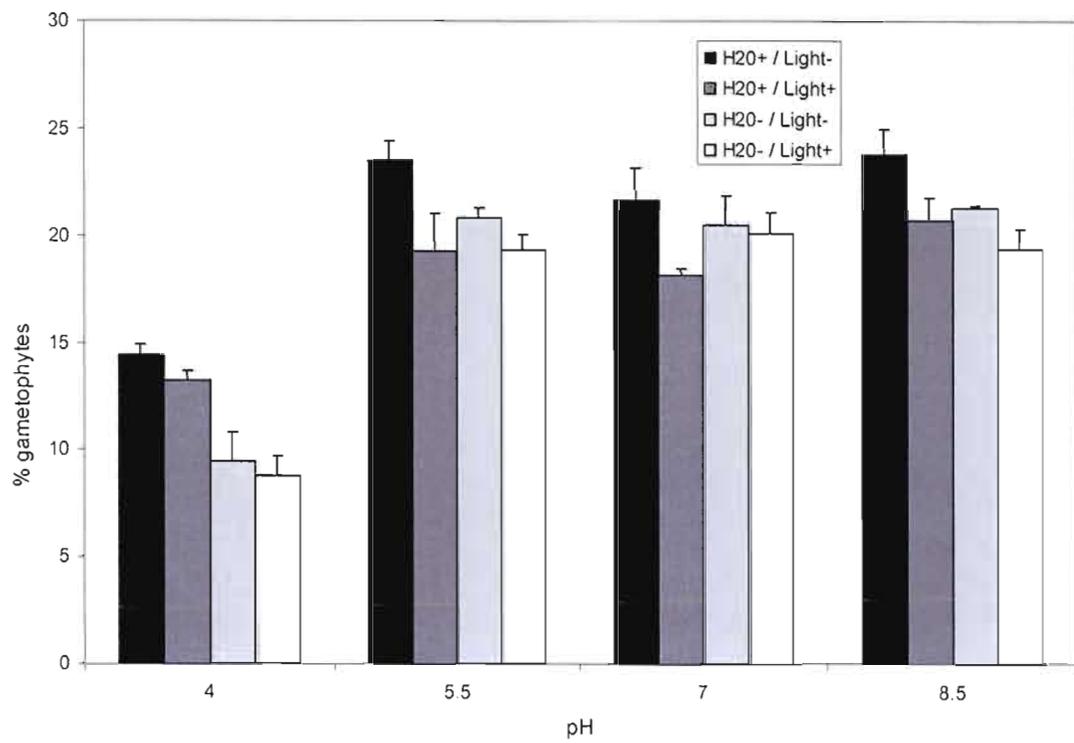


Figure 4.4. Effects of medium pH, moisture level and light level on *Woodsia obtusa* gametophyte recruitment using an initial spore density of 1600 spores/ml x 1 ml.

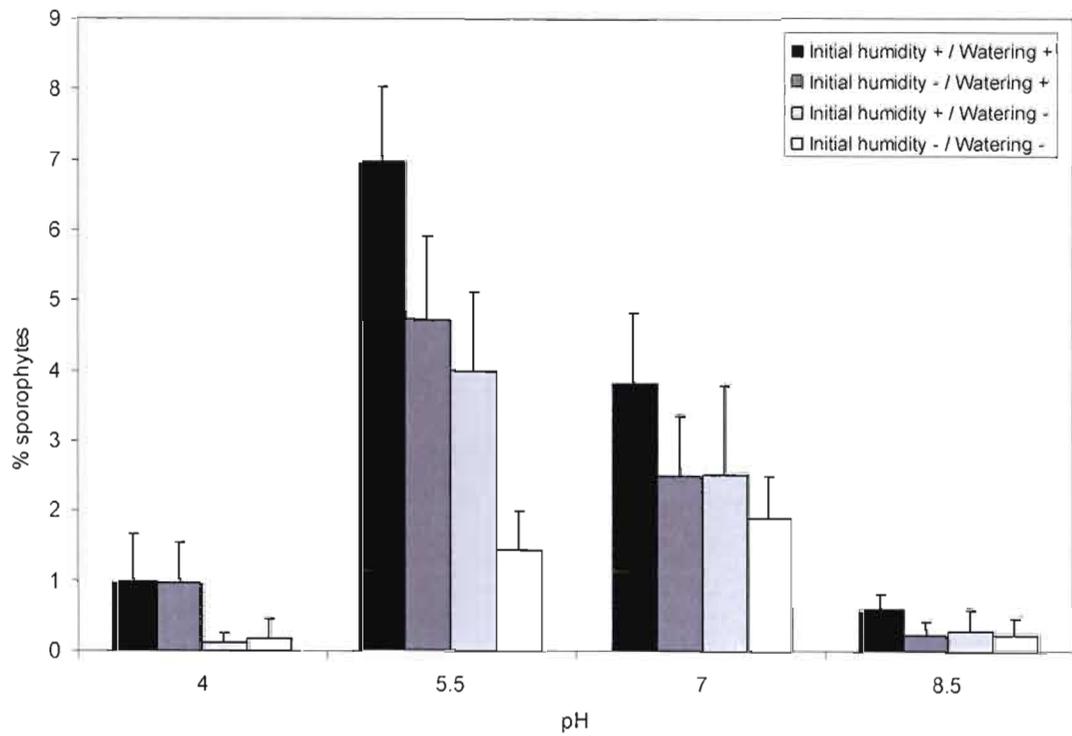


Figure 4.5. Effects of medium pH and four moisture levels on *Woodsia obtusa* sporophyte recruitment using an initial spore density of 1600 spores/ml x 1 ml.

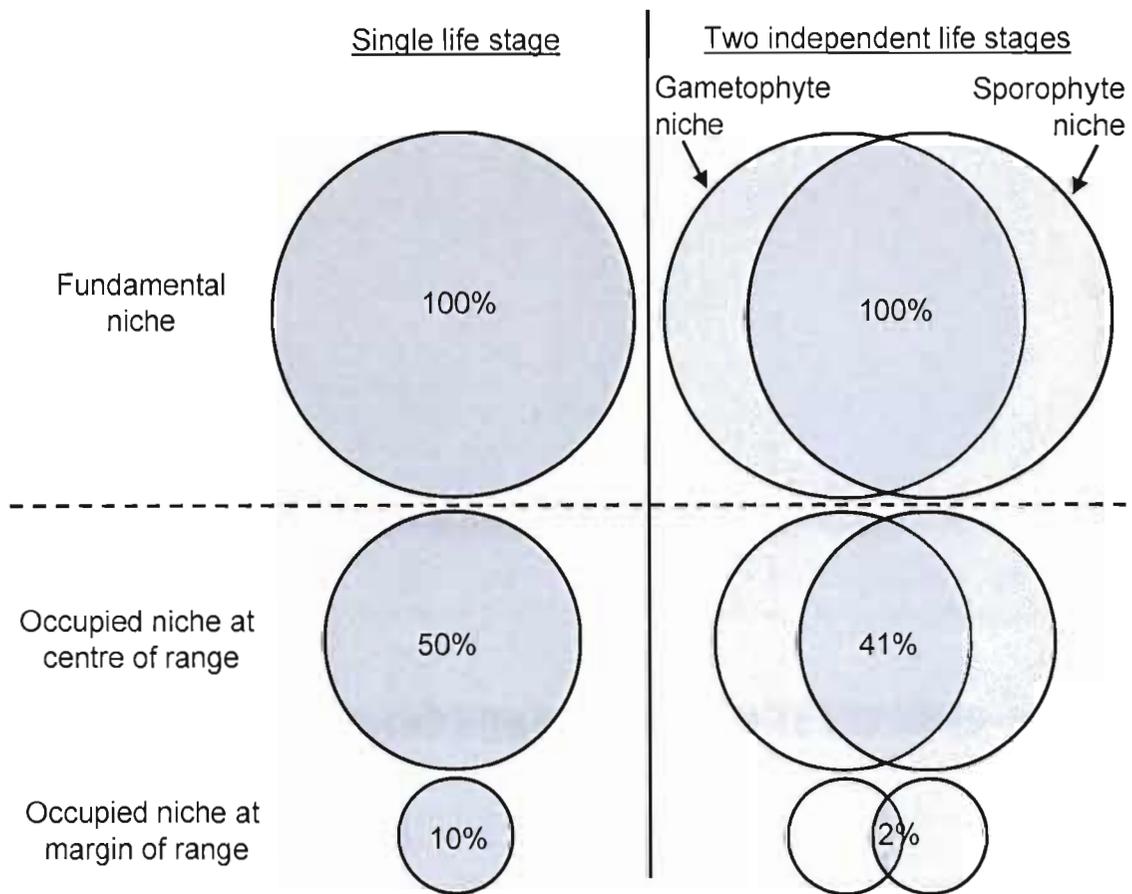


Figure 4.6. Theoretical model showing the effect of reducing niche size for a sessile organism with two independent life stages whose occupied niches differ partially.

Chapter V

SURVIVAL AND GROWTH OF BLUNT-LOBED WOODSIA
SPOROPHYTES DOES NOT LIMIT THE PERSISTENCE OF
POPULATIONS OF THE SPECIES AT THE NORTHERN
PERIPHERY OF ITS RANGE

Matthew Wild and Daniel Gagnon

Abstract

Research to explain the rarity of Blunt-lobed *Woodsia* in Canada has shown that although suitable habitat is locally available for the species, and that dispersal of spores into this habitat does not appear to be limiting, very little recruitment is observed in this habitat, suggesting a limitation at some stage of the species' life cycle. There also appears to be some differences between the realised niche of gametophytes of the species and that of its sporophytes. In this paper we use a four-year sporophyte field transplantation experiment to address the question whether the long term survival of sporophytes could be limiting the persistence of populations of Blunt-lobed *Woodsia* at the northern periphery of the species' distribution range. Six month old greenhouse grown sporophytes were transplanted into experimental plots in two study populations and sporophyte survival was monitored for the following four years. In each plot, environmental data were also collected. Multivariate logistic regression models were built to explain which environmental parameters significantly affected survival. Survival of transplanted sporophytes was highest at Frontenac Park (27.4 %), where canopy openness was greatest (13.5 %). After four years, all surviving plants were producing sporangia, thus fertile. The principal environmental factors beneficial to survival were high levels of light and low amounts of litter. From a conservation viewpoint, these results will also benefit reintroduction efforts for the species by defining suitable habitat for species survival. We suggest that no population restoration efforts, with transplanted sporophytes, be attempted on sites with less than 10 % canopy openness.

5.1 Introduction

Research to explain the rarity of Blunt-lobed *Woodsia* in Canada has shown that suitable habitat is locally available for the species (Wild, 2003; Wild and Gagnon, 2005) and that dispersal of spores into this habitat does not appear to be limiting (Chapter III). However, very little recruitment is observed in this habitat, suggesting a limitation at some stage of the species' life cycle. There also appears to be some differences between the realised niche of gametophytes of the species and that of sporophytes (Peck *et al.*, 1990; Greer *et al.*, 1997; Wild, 2003; Wild and Gagnon, 2005). Experimental work also suggests that these differences play an important role in the rarity of the species at the northernmost limit of its distribution range (Chapter IV).

The fact that the fern life cycle is made up of two distinct and very different phases, the short-lived, semelparous gametophyte, and the long-lived, iteroparous sporophyte, poses somewhat of a problem, called "an ecological handicap" by Page (1979). Simply put, this implies that the sporophyte can only occur in a habitat that has previously proved itself suitable for a gametophyte. Intuitively, this suggests that if a small, thalloid, non-vascular gametophyte managed to grow, then a large, leafy, vascular sporophyte should also be able to grow. However, as mentioned above, our previous results suggest that the two life stages have somewhat different optima for growth and survival (Chapter IV), and this difference probably plays a role in the rarity of the species at the northern periphery of its range. This is mainly caused by the very low levels of *in situ* recruitment in xeric habitats. Any such limitation on recruitment rates would be greatly compounded if, once established, survival rates were also low.

In this paper we use a four-year sporophyte field transplantation experiment to address the question whether the survival of sporophytes in their natural habitats could also be limiting the persistence of Blunt-lobed *Woodsia* populations at the northern periphery of the species' range. If long-term growth and survival of these individuals is shown, this will reinforce our previous findings that it is at some other life history stage, namely the natural recruitment event of sporophytes, that environmental factors are limiting the presence of Blunt-lobed *Woodsia* at the northern limit of its distribution range.

5.2 Methods

5.2.1 Study species

Blunt-lobed *Woodsia*, or Blunt-lobed Cliff Fern (*Woodsia obtusa* subsp. *obtusa* (Sprengel) Torrey: Dryopteridaceae), is a homosporous fern found in eastern North America. Although the species is common throughout much of its distributional range, it is only known to occur in eight sparsely populated sites at the northern periphery of its distribution (southern Canada); these eight occurrences (four in southern Quebec and four in southern Ontario) contain a total of approximately 1350 individuals (COSEWIC, 2007). The low number of small populations has earned the species a NatureServe rank of N1 (critically imperilled) in Canada, provincial ranks of S1 (critically imperilled) in both Quebec and Ontario (NatureServe, 2009), and has led to it being listed as "Threatened" under Canada's Species at Risk Act, Quebec's *Loi sur les espèces menacées et vulnérables* and Ontario's Endangered Species Act. Blunt-lobed *Woodsia* appears on rare plant lists in Quebec (Bouchard *et al.*, 1983; Labrecque and Lavoie, 2002; CDPNQ, 2008), Ontario (Argus and White, 1983), and Canada (Argus and Pryer, 1990).

5.2.2 Species' habitat

All Canadian populations are found in the Great Lakes-St. Lawrence Forest Region (Rowe, 1972) and all are located on calcareous rock (marble, dolomite, limestone) with a southern aspect. The habitat of the species is generally forested, consisting of scattered, relatively small trees (10 to 15 m high; 10 to 20 cm diameter breast height). Canopy openness ranges from fairly closed (~7% in Gatineau Park, QC) to fairly open (~20% in Frontenac Park, ON). Shallow soils over bedrock and the overall xeric nature of the sites are the major cause for the small stature of the trees, although trees appear to be relatively young at several sites, indicating some recent disturbance (i.e. harvesting of stems, fire, etc.). Generally associated tree species are Sugar Maple (*Acer saccharum*), Red Oak (*Quercus rubra*), White Oak (*Q. alba*), White Ash (*Fraxinus americana*) and Ironwood (*Ostrya virginiana*). Poison Ivy (*Rhus radicans*), Prickly Gooseberry (*Ribes cynosbati*), Fragrant Sumac (*Rhus aromatica*) and Downy Arrow-wood (*Viburnum rafinesquianum*) are the most frequent shrub species on these sites. The herb layer is most often dominated by Pennsylvania Sedge (*Carex pensylvanica*). Other commonly found associated herbaceous species are Bottlebrush Grass (*Elymus hystrix*), Bluestem Goldenrod (*Solidago caesia*), Marginal Shield Fern (*Dryopteris marginalis*), and Herb-Robert (*Geranium robertianum*). A recent study in six Canadian sites shows an average soil depth of 3.5 cm (range: 0.5 - 9.5 cm) on an average slope of 43° (range: 26 - 88°) under an average 18 % canopy openness (range: 4 – 60 %) (Wild and Gagnon, 2005). Recent soil data collected from Canadian Blunt-lobed Woodsia populations shows a pH range of 5.9 to 7.1, with an average of 6.6, and a high soil calcium content of almost 10000 ppm on average (Wild and Gagnon, 2005).

5.2.3 Spore collection and sporophyte production

Spores were collected from the two study populations in the summer of 2002 by collecting a few fronds bearing sporangia in each population. These fronds were dried in newspaper sheets in a plant press. The spores extracted from the dry fronds were sown into separate sowing flats (for each population) containing sterilised soil which were placed in a greenhouse and allowed to germinate. Once gametophytes had been produced and fertilisation had taken place, sporophytes were placed in individual cells in 144-cell seedling trays. These sporophytes were kept growing for six months.

5.2.4 Experimental plots

In the spring of 2003, in areas surrounding the two study populations (between 30 m and 300 m distant from existing individuals), several potential transplant areas were identified. Selection of these areas was based on a visual appearance similar to locations containing extant individuals (slope, aspect, existing vegetation, presence of bare rock). These transplant areas were divided into 1 m² square plots. From each 1 m² square plot the following data were collected: average surface slope (3 measurements), average soil depth (5 measurements), canopy openness (hemispherical photograph of the canopy was taken with a digital camera (Nikon Coolpix 950) equipped with a fisheye lens (Nikon FC-E8), and analysed using the Gap Light Analyzer software package (version 2.0, Frazer *et al.*, 1999)), average vascular plant height, vascular plant richness, bryophyte and rock cover (Braun-Blanquet cover classes), and a soil sample. For the soil sample, 5 small sub-samples were taken per plot, homogenised, and subsequently analysed for pH, major cations (Ca, K, Mg), cation exchange capacity (CEC), NO₃, NH₄ and PO₄. Soil pH was determined with a glass electrode pH-meter from 2:1 water:soil solution. Cations and CEC were extracted with BaCl₂ and analysed following the methods of Hendershot *et*

al. (1993). NO_3 and NH_4 were extracted with KCl and analysed with a Tecator FIAStar 5020 analyser (Maynard and Kalra, 1993). Phosphorus extraction methods followed McKeague (1978).

The six month old sporophytes were transplanted to these experimental plots in July of 2003. In order to avoid genetic contamination of these populations, only plants produced from local spores were used at each site. All individuals were planted in groups of 10 as illustrated in Figure 5.1. In Gatineau Park a total of 220 sporophytes were transplanted into 22 experimental plots (21-24 July 2003) and in Frontenac Park, 190 were transplanted into 19 plots (28-31 July 2003). All litter accumulation was removed from a 60 cm X 20 cm area prior to planting. In all cases, plantings were oriented on the same axis as the slope of the ground surface. In each plot, the group of five plants toward the lower end of the slope was watered weekly (50 ml/individual) for a period of eight weeks following planting (August and September 2003). At each watering, all plants were monitored for survival.

Each summer for the next four years (2004-2007), during the months of July or August, survival was monitored. Survival was counted as successful if fresh green aerial shoots were located on an individual. Following the fourth year of monitoring (2007) a litter sample was taken from a 20cm x 20cm area at the centre of the experimental plot, air dried in the laboratory and weighed.

5.2.5 Data analysis

To ensure normality, data were transformed for all variables by subtracting the parametric mean and dividing by the sample standard deviation. Before beginning multivariate logistic regression analyses, univariate analysis of each variable was undertaken. Variables which were not significant at $p < 0.25$ were excluded from the

model. $P < 0.25$ was used because more traditional levels such as 0.05 can fail in identifying variables known to be important (Hosmer and Lemeshow, 2000). Remaining variables were checked for multicollinearity and for those pairs of variables with a significant correlation > 0.9 , the least significant variable (based on univariate analysis) was removed. A survival model was then fitted using stepwise logistic regression including all retained variables for both study populations combined. Similar models were also fitted using data from each study population individually. Variables retained to build the full model were: dry litter weight, canopy openness, surface slope, soil depth, soil Mg, soil cation exchange capacity (CEC), average vascular plant height, vascular plant richness (n of species) and rock cover. Variables used to build the Frontenac model were dry litter weight, canopy openness, soil Mg, and vascular plant richness. Variables used to build the Gatineau model were dry litter weight, pH, soil Ca, NO_3 and PO_4 . All data analyses were done with JMP, Version 7 (SAS Institute Inc., Cary, NC, 1989-2007).

5.3 Results

During the initial eight-week monitoring period in 2003, only two of the 410 introduced plants did not survive; both were in Gatineau Park. In both cases it appeared that a physical disturbance had uprooted the plants, probably due to animal activity (likely White-tailed deer (*Odocoileus virginianus*)). During this time, and through until the end of the experiment, there was no significant difference in survival rates for plants that had been watered during the initial monitoring period and those that did not receive water.

The following spring, in 2004, significant differences in survival rates were recorded between the two study populations. In Gatineau Park, only a total of 20 individuals (9%) had survived their first year of transplantation, compared with 93 in Frontenac Park (49%). This trend continued throughout the duration of the experiment with only

9 (4.1%) individuals still alive in Gatineau Park and 52 (27.4%) in Frontenac Park after four years, in 2007. This trend is just as marked when counting the number of successful plots (at least one fern still alive out of 10 transplanted) versus unsuccessful plots. In this case, a total of 3/22 (13.6%) plots were successful in Gatineau Park whereas 12/19 (63.2%) were successful in Frontenac Park (Table 1).

When considering all plots, from both study sites, the principal factor explaining survival was higher light (canopy openness) ($P < 0.001$; Wald statistic = 10.856) and the second explaining factor was lower litter quantity ($P < 0.005$; Wald statistic = 3.249). In the case of Frontenac Park, the principal factor explaining Blunt-lobed *Woodsia* sporophyte survival was lower litter quantity ($P < 0.1$; Wald statistic = 3.645), and a second slightly significant factor was lower soil Mg ($P < 0.1$; Wald statistic = 2.731). In Gatineau Park, the sole explaining factor for sporophyte survival was higher soil pH ($P < 0.1$; Wald statistic = 2.666). However, because of the very low sporophyte survival in Gatineau Park, this result could be a statistical artefact.

5.4 Discussion

When data from both sites are combined, higher light and lower litter quantity are the two significant factors explaining Blunt-lobed *Woodsia* sporophyte survival. A higher amount of light reaching the understory, because of greater canopy openness, is certainly the most important factor of long term sporophyte survival and growth. Light available in the understory is therefore an important limiting factor for maintenance of Blunt-lobed *Woodsia* populations in Canada. However, it is possible that the heat generated by the increased light is more important than the light itself. Indeed, this increased radiation, compounded by slope orientation (all Canadian populations located on sites with generally south-facing slopes), creates a warmer and dryer microclimate.

The litter factor is mostly related to the fact that Gatineau Park, where very few individuals survived, has a higher average canopy cover than Frontenac Park (4.4% canopy openness vs. 13.5% canopy openness) and that higher quantities of litter were observed in Gatineau Park, more than double the amount found in Frontenac Park. This seems to suggest that low light is correlated with more litter. Because these two variables are only weakly correlated (Pearson Correlation coef. = - 0.342) at a large scale (among site scale: e.g. higher canopy cover in Gatineau Park = greater quantity of litter; opposite at Frontenac Park) this implies that other factors also contribute to litter accumulation. Indeed, at a small scale (plot scale) microtopography results in small concave areas in which litter accumulates independently of canopy cover directly over the plot (M. Wild, personal observation).

The fact that litter is the most significant variable in the Frontenac Park model suggests that apart from the differences between the two study sites, litter does in fact play a role in limiting sporophyte survival. There have been many studies on the effects of litter on the establishment and early survival of plants (eg. Ahlgren and Ahlgren, 1981; Molofsky and Augspurger, 1992; Lopez-Barrera and Gonzalez-Espinosa, 2001; Maruyama *et al.*, 2004; Rotundo and Aguiar, 2005; Scarpa and Valio, 2008). However, most of the previous work has been on gymnosperms or angiosperms, and we are not aware of any studies directed at the relationship between litter and fern establishment and/or survival in the literature. Rotundo and Aguiar (2005) have proposed that litter effects on seedlings can be of a physical, biological or chemical nature, and also that these effects can be positive or negative. For Blunt-lobed *Woodsia*, litter appears to have a negative effect on the survival rate of young sporophytes, which could be compounded by the persistence of litter because of its low rate of decomposition in a dry ecosystem (Meentemeyer, 1978). Our results suggest that litter accumulation, particularly of recalcitrant oak leaves, has a negative physical impact on the young and fragile sporophytes of Blunt-lobed *Woodsia*.

The sharp drop in survival between the initial monitoring period (July to October 2003) and the following year (July 2004) (Figure 5.2) is likely due to the phenomenon known as transplant shock. This effect is frequently observed in transplantation experiments and can be due to several factors, including the small size (and young age) of the transplants, the lack of a hardening off period, and the environmental conditions at the time of transplanting. That this is the main reason behind these initial low rates of survival is corroborated by the fact that during the following years of monitoring, the survival rate was relatively constant.

All surviving individuals in this study were producing spores by the end of the third year of monitoring (2006), therefore indicating a successful outcome to the species' life cycle. This is one of the most, if not the most important benchmark for transplantation success in population restoration of endangered plant species (Menges, 2008).

As well as contributing to explain the rarity of the species in Canada, our results can also serve in the identification of the best suited habitat to direct reintroduction efforts for Blunt-lobed Woodsia, as suggested in the National Recovery Strategy for Blunt-lobed Woodsia in Canada (Environment Canada, in press). A species such as Blunt-lobed Woodsia (*Woodsia obtusa*), which is known in Canada from only eight small populations, could benefit greatly from population size augmentation resulting from the introduction of transplanted individuals. At the moment, most Canadian populations contain fewer than 200 individuals and are contained within small areas (COSEWIC, 2007). This means that a single, isolated, stochastic event (tree-fall, landslide, etc) could result in the loss of an entire population. However, as is the case for many species which would benefit from reintroductions (Fahselt, 2007), very little was known on the exact habitat requirements for Blunt-lobed Woodsia, and experimental studies were required before attempting a large scale reintroduction programme. Based on the results of this study, we suggest that no restoration or

population augmentation efforts, with transplanted sporophytes, be attempted on sites with less than 10 % canopy openness. The results obtained in this and our other studies (Chapters III and IV) should provide a sound basis for the conservation management of the species.

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5.6 References

- Ahlgren, C. E. and Ahlgren, I. F. 1981. Some effects of different forest litters on seed germination and growth. *Canadian Journal of Forest Research* **11**: 710-714.
- Argus, G. W. and Pryer, K. M. 1990. *Rare Vascular Plants in Canada: Our Natural Heritage*. National Museums of Canada, Canadian Museum of Nature, Ottawa, Ontario.
- Argus, G. W. and White, D. J. 1977. The rare vascular plants of Ontario. National Museums of Canada, Canadian Museum of Nature, *Syllogeus* **14**: 1-63.
- Bouchard, A., Barabé, D., Dumais, M., and Hay, S. 1983. Les plantes vasculaires rares du Québec. National Museums of Canada, Canadian Museum of Nature, *Syllogeus* **48**: 1-79.
- Centre de données sur le patrimoine naturel du Québec (CDPNQ). 2008. Les plantes vasculaires menacées ou vulnérables du Québec. 3e édition. Gouvernement du Québec, ministère du Développement durable, de l'Environnement et des Parcs, Direction du patrimoine écologique et des parcs, Québec.
- COSEWIC. 2007. Update COSEWIC Status Report on Blunt-lobed Woodsia (*Woodsia obtusa*). Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario.
- Environment Canada. In press. Recovery Strategy for Blunt-lobed Woodsia (*Woodsia obtusa*) in Canada. *Species at Risk Act* Recovery Strategy Series. Environment Canada, Ottawa.
- Fahselt, D. 2007. Is transplanting an effective way of preserving vegetation? *Canadian Journal of Botany* **85**:1007-1017.
- Frazer, G. W., Canham, C. D., and Lertzman, K. P. 1999. Gap light analyzer (GLA): imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs. Users Manual and Program Documentation, Version 2.0, Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York.
- Greer, G. K., Lloyd, R. M., and McCarthy, B. C. 1997. Factors influencing the distribution of pteridophytes in a southeastern Ohio hardwood forest. *Journal of the Torrey Botanical Society* **124**: 11-21.

- Hendershot, W. H., Lalande, H., and Duquette, M. 1993. Ion exchange and exchangeable cations. *In: Soil sampling and method of analysis*, M.R. Carter, Ed. Lewis Publishers, Boca Raton.
- Hosmer, D. W. and Lemeshow, S. 2000. Applied Logistic Regression. John Wiley & Sons, Inc. New York
- JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007.
- Labrecque, J. and Lavoie, G. 2002. Les plantes vasculaires menacées ou vulnérables du Québec. Gouvernement du Québec, ministère de l'environnement. Direction du patrimoine écologique et du développement durable, Québec.
- López-Barrera F. and González-Espinosa, M. 2001. Influence of litter on emergence and early growth of *Quercus rugosa*: A laboratory study. *New Forests* **21**: 59-70.
- Maruyama, R., Maruyama, M., and Konno, Y. 2004. Effects of understory vegetation and litter on the establishment of *Abies sachalinensis* and *Picea jezoensis* seedlings in a conifer forest in Hokkaido, northern Japan. *Japanese Journal of Ecology* **54**: 105-115.
- Maynard, D. G. and Kalra, Y. P. 1993. Nitrate and exchangeable ammonium nitrogen. *In: Soil Sampling and Methods of Analysis*. Carter, M. R. Ed. Lewis Publishers, Boca Raton, USA.
- McKeague, J. A. 1978. Manuel de méthodes d'échantillonnage et d'analyse des sols. Comité de pédologie, Ottawa, Canada.
- Meentemeyer, V. 1978. Macroclimate and lignin control of litter decomposition rates. *Ecology* **59**: 465-475.
- Menges, E. S. 2008. Turner review no. 16. Restoration demography and genetics of plants: When is a translocation successful? *Australian Journal of Botany* **56**: 187-196.
- Molofsky, J. and Augspurger, C. K. 1992. The effect of leaf litter on early seedling establishment in a tropical forest. *Ecology* **73**: 68-77.
- NatureServe. 2006. NatureServe Explorer: An online encyclopedia of life [web application]. Version 6.1. NatureServe, Arlington, Virginia. Available from <http://www.natureserve.org/explorer>

- Page, C. N. 1979. Experimental aspects of fern ecology. *In: The experimental biology of ferns*. Dyer, A. F. *Ed.* Academic Press, New York.
- Peck, J. H., Peck, C. J., and Farrar, D. R. 1990. Influences of life-history attributes on formation of local and distant fern populations. *American Fern Journal* **80**: 126-142.
- Rotundo, J. L. and Aguiar, M. R. 2005. Litter effects on plant regeneration in arid lands: a complex balance between seed retention, seed longevity and soil-seed contact. *Journal of Ecology* **93**: 829-838.
- Rowe, J. S. 1972. *Forest Regions of Canada*. Publication No. 1300. Canadian Forestry Service, Department of the Environment. Ottawa.
- Scarpa, F. M and Valio, I. F. M. 2008. Relationship between seed size and litter effects on early seedling establishment of 15 tropical tree species. *Journal of Tropical Ecology* **24**: 569-573.
- Wild, M. 2003. Have five calcicolous fern species of different habitats saturated all local habitat available to them? M.Sc. Thesis, Université du Québec à Montréal, Montreal, Qc, Canada.
- Wild, M., and Gagnon, D. 2005. Does lack of available suitable habitat explain the patchy distributions of rare calcicole fern species? *Ecography* **28**: 191–196.

Table 5.1. Average survival of *Woodsia obtusa* transplants and average environmental factor values measured for Gatineau Park and Frontenac Park study populations. Final column indicates those values showing a significant difference (Chi2 = $p < 0.05$) between study populations.

Variable		Gatineau Park	Frontenac Park	Chi2 p<0.05
Survival	plots	13.6%	63.2%	*
	individuals	4.1%	27.4%	*
Litter	(g. dry weight)	24.25	11.95	*
Light	(% openness)	4.39	13.48	*
Angle	(degrees)	32.08	23.92	*
Soil depth	(mm)	39.26	49.78	
pH		6.17	6.13	
Ca	ppm	6620.50	3676.24	*
K	ppm	147.45	184.74	
Mg	ppm	409.37	346.38	
CEC		36.78	21.67	*
NO ₃	ppm	13.81	1.40	
NH ₄	ppm	11.57	16.02	
PO ₄	ppm	13.23	19.67	

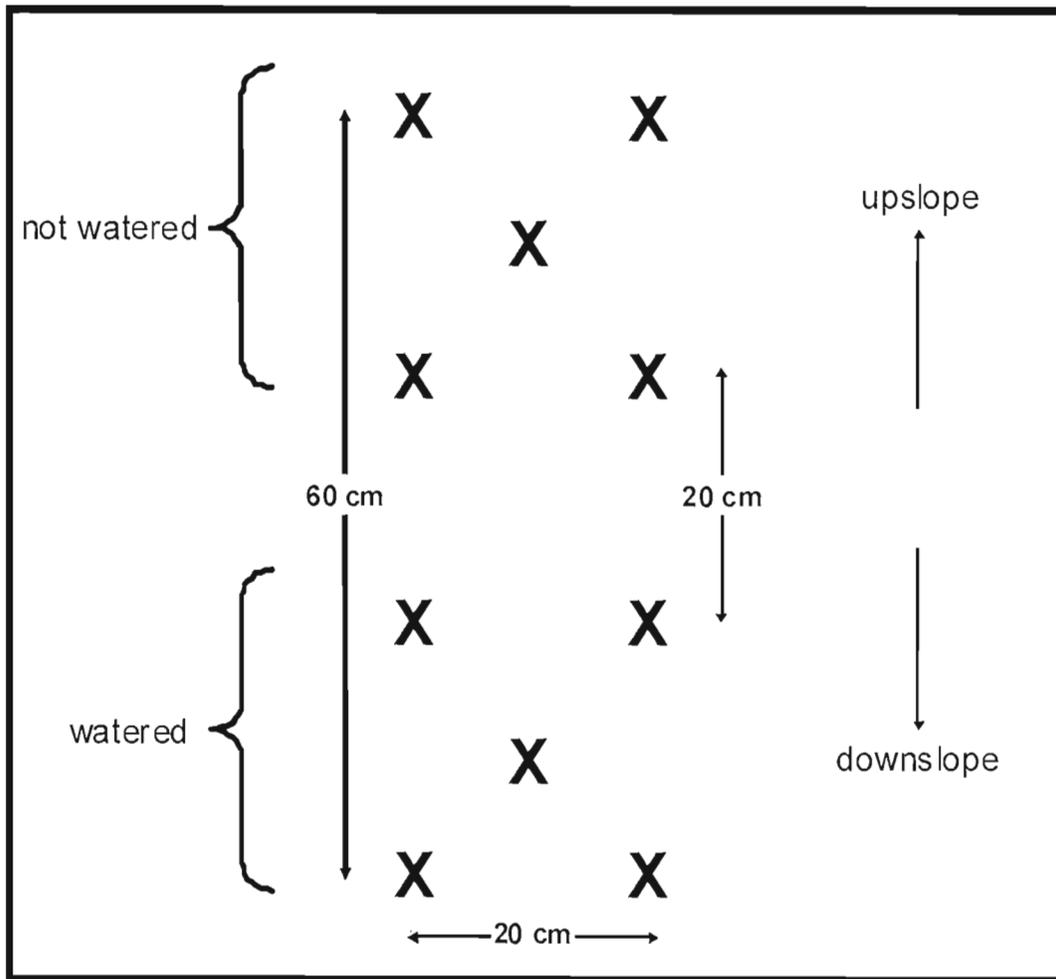


Figure 5.1: Distribution of transplanted *Woodsia obtusa* individuals in transplant plots.

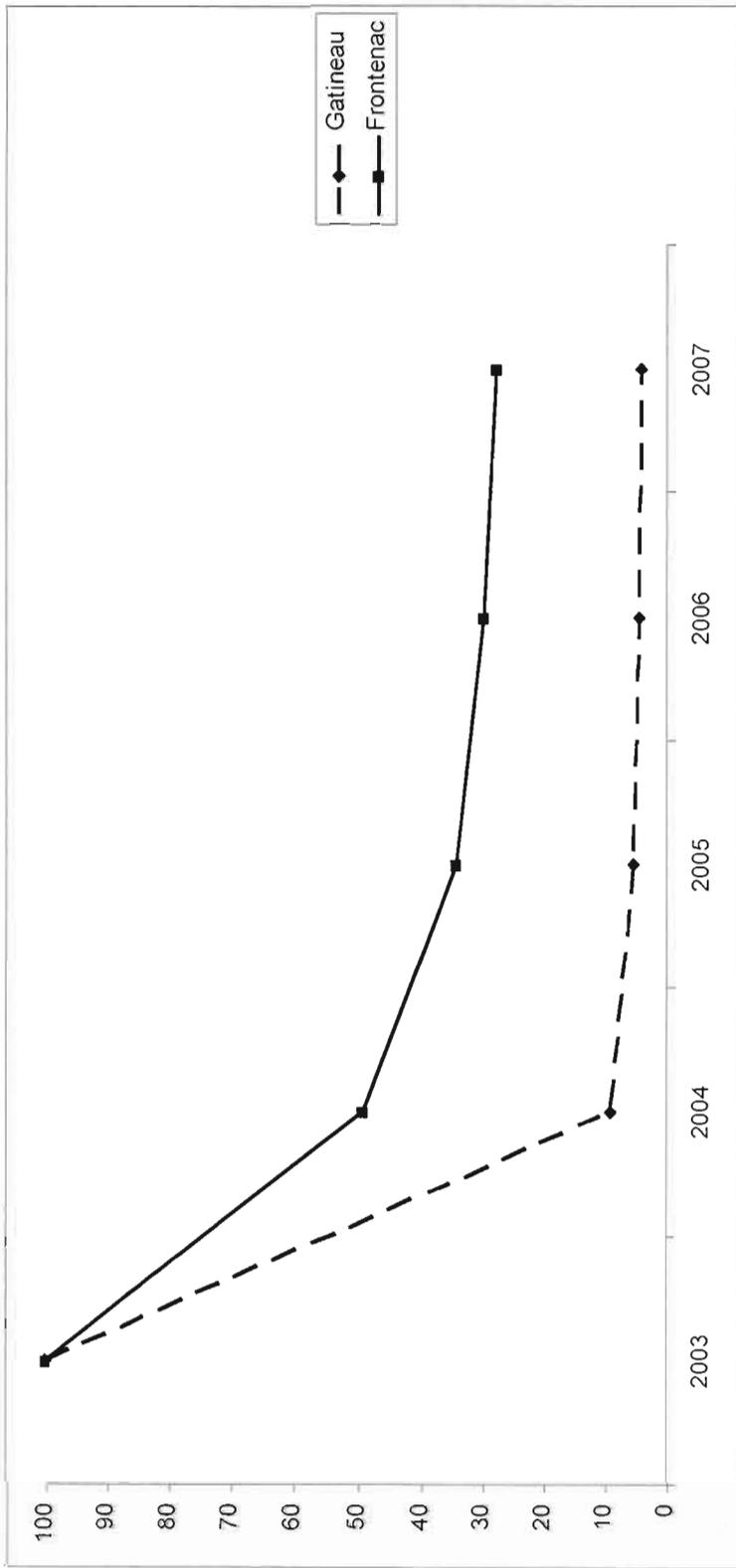


Figure 5.2. Percent survival of transplanted *Woodsia obtusa* individuals from 2003 to 2007 in both experimental sites. Initial $n = 220$ in Gatineau Park and 190 in Frontenac Park.

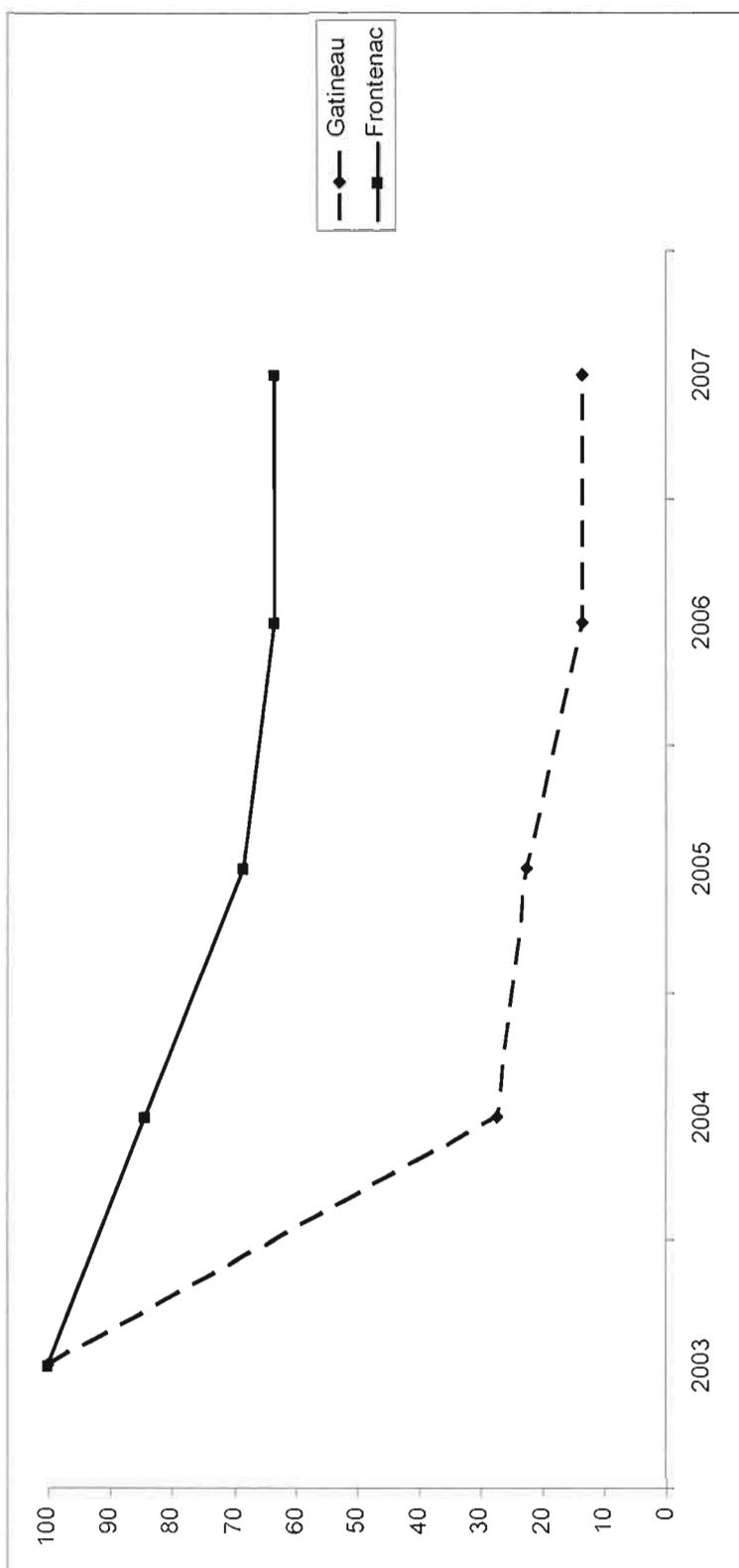


Figure 5.3. Percent of successful *Woodisia obtusa* transplant plots (containing at least one surviving individual from 2003 to 2007). Initial $n = 22$ in Gatineau Park and 19 in Frontenac Park.

CHAPTER VI
GENERAL CONCLUSION

The principal objective of this thesis was to explain the causes behind the rarity of Blunt-lobed *Woodsia* at the northernmost limit of its distribution range in southern Canada. Although climate definitely plays a role in limiting the northward distribution of this species, and bedrock is definitely a factor at the landscape scale, my previous research has shown much suitable, yet empty, habitat in close proximity to extant Canadian populations (Wild and Gagnon, 2005). This suggests that something other than climate and geology is limiting the species. This is unexpected, seeing as most of the literature suggests that ferns tend to saturate suitable habitat because of their lack of limitations to dispersal and establishment. There are many published examples of studies aimed at identifying the causes of a species' rarity, and numerous reasons have been proposed as being the explaining factors causing rarity for these species. However, only a few of these studies have addressed the particular case of the homosporous fern life-cycle. This thesis is an attempt to identify if one or more of Blunt-lobed *Woodsia*'s life-cycle stages were being negatively affected by some environmental factor at the northernmost part of its range, thereby limiting the species' capacity to saturate suitable, available habitat, and therefore to establish more populations than are currently found.

Following an introduction (Chapter I) presenting the context of this thesis and its supporting theory, Chapter II is a study on the general rarity of fern taxa compared to other plants, where several of the most likely causes for this relatively widespread rarity are discussed. The current parochial approach to conservation is argued against, and is proposed as being a plausible artificial cause for the observed above average rarity of ferns. Chapters III through V are experimental studies aimed at identifying potential limiting factors for Blunt-lobed *Woodsia* and, if present, at which life-cycle stage they are acting. Although these experimental studies revealed a number of

limitations acting to different degrees at each stage of the fern life-cycle (Table 6.1), what I believe to be the main factor in explaining Blunt-lobed *Woodsia*'s rarity in Canada is the difference between the niches of the two generations of its life-cycle. In fact it would appear, contrary to most examples from the literature, that in this case, the niches of the gametophyte and that of the sporophyte are very different, and possibly overlap very little (Figure 6.1). Undoubtedly, the differences between the niches of gametophytes and sporophytes are easier to detect at the margin of the range of fern species. I have shown that to be successful, both gametophyte and sporophyte recruitment require water (Chapter III). However, the amounts of water required for both types of recruitment are rarely found in the xeric habitats in which Blunt-lobed *Woodsia* sporophytes are most successful (Chapter IV).

Table 6.1. Synthesis of the findings of this thesis

Life-cycle stage	Chap.	Research findings	Probable level of effect on BLW rarity in Canada
Dispersal	III	- A highly skewed leptokurtic dispersal pattern suggests possible, but very infrequent, long distance dispersal	very low
Gametophyte recruitment	IV	- Increased water and less light had small but significant positive effects	low
Sporophyte recruitment	IV	- Increased water had a highly significant positive effect	very high
Sporophyte survival	V	- Increased light and less litter had highly significant effects	high

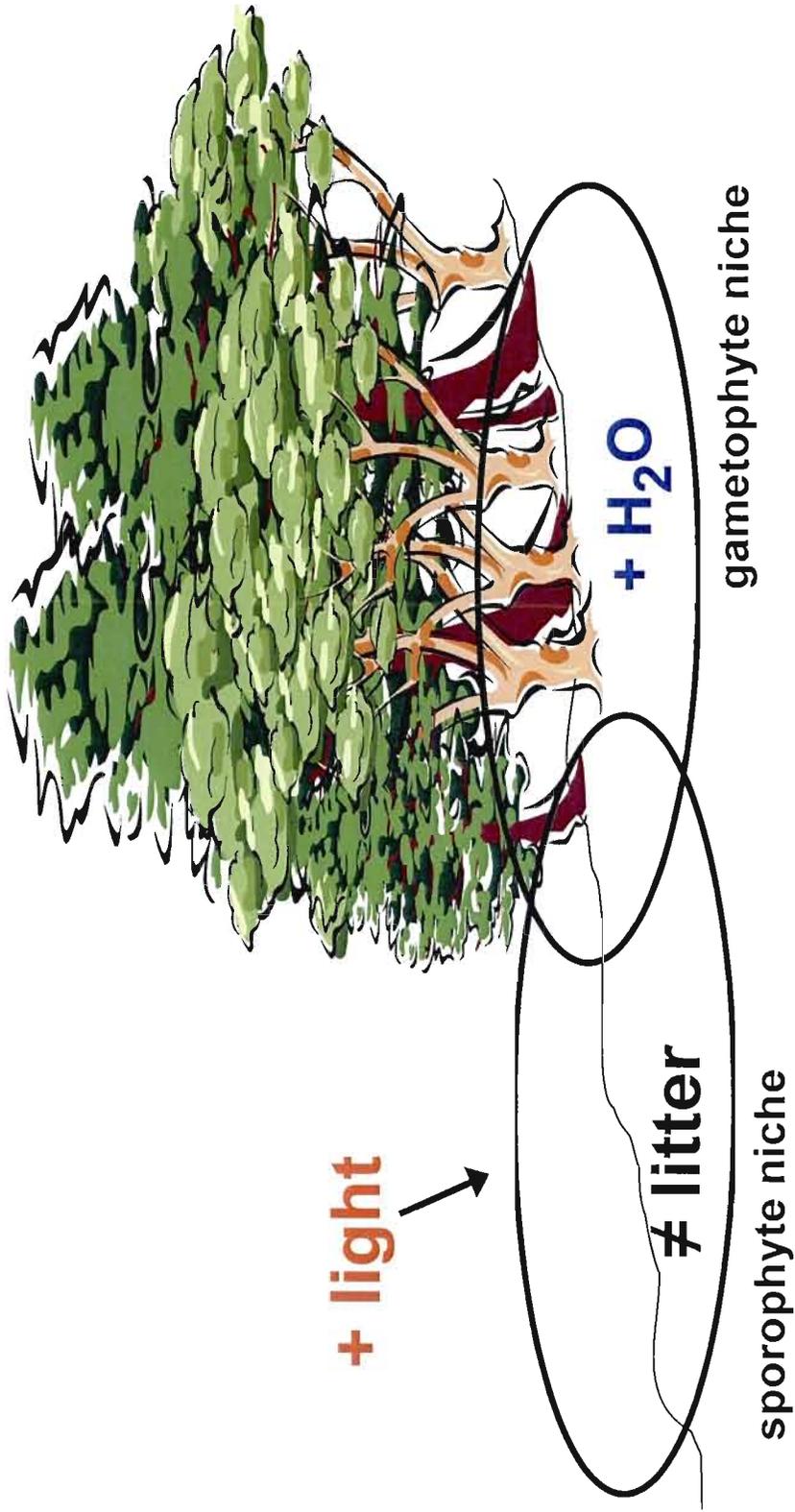


Figure 6.1. Illustration showing the differences between the sporophyte niche (increased light, decreased litter and soil moisture) and the gametophyte niche (increased moisture, decreased light and increased litter) for Blunt-lobed Woodsia. Theoretically, individuals can only survive and grow in the small overlap between the two niches.

Although exploiting different niches for separate stages of a life-cycle is a relatively common occurrence in motile animal species (e.g. amphibians, insects), it is a huge handicap for a sessile species, meaning that to be successful an individual must find itself in the right place at the right time for both generations of its life-cycle to benefit from suitable conditions. Even though this has been shown to occur in a few fern species before (see references in Chapter IV), it is not believed to be a common occurrence. However, we believe it could likely be an explaining factor for the rarity of many fern species (or other sessile taxa with two distinct generations) at the limits of their distribution range. It is likely that this is limited to land-plants, as most other taxa considered as sessile (sponges, corals, brachiopods, etc) also have a motile generation, and therefore are less likely to suffer the consequences of different niches for different life stages. In order to validate the hypothesis that this is a common occurrence for fern species, similar studies to the one conducted here could be undertaken with other species. To really evaluate the difference between niches for each generation, *ex situ* studies, such as the *in vitro* work done here on the gametophyte generation, could be undertaken simultaneously on gametophytes and sporophytes. This would allow for a more direct comparison between the two.

The benefits gained from this type of study are twofold. First, the theoretical implications can be applied to any number of species with similar ecologies, such as other rare species at the margins of their distribution or other rare fern species. Second, specific knowledge on the ecology of the study species can serve at numerous levels, such as conservation or active management.

As mentioned previously, the theoretical implications of this thesis are mainly related to the fact that I believe to have identified a major factor in determining the high rarity levels of fern species, especially at range margins. Although Chapter II lists several possible mechanisms that could cause frequent fern rarity, and concludes that

the principal reason is probably the fragile gametophytic stage combined with the scale at which rarity is often studied, the specific work on Blunt-lobed *Woodsia* presented in Chapters III through V suggests that it is in fact the discrepancies observed between the niches of the separate life stages that most likely explain the species' rarity. This is quite possibly applicable to many rare fern species, but it has not been identified as an explanation for fern rarity before. Future research along these lines is important in order to increase our understanding of what I believe to be an important finding.

As for conservation implications, in the case of the results obtained on Blunt-lobed *Woodsia*, they could go a long way in assisting the recovery of this provincially (Quebec and Ontario, both provinces where it occurs) and federally designated species at risk. This could be achieved by using the results from Chapter V to direct conservation efforts by aiding in the selection of micro sites for transplantation of sporophytes to increase the number of individuals in a small population (augmentation), re-establish an extirpated population (reintroduction), or relocate a highly threatened population (transplantation). The *in vitro* techniques developed in Chapter IV help give specific information on how to produce numerous sporophytes from only a few fertile fronds. Again, this information could be very useful for the conservation (both *in situ* and *ex situ*) of Blunt-lobed *Woodsia*.

BIBLIOGRAPHY

- Allen, D. E. 1969. The Victorian fern craze: a history of pteridomania. Hutchinson, London.
- Arens, N. C. 2001. Variation in Performance of the Tree Fern *Cyathea caracasana* (Cyatheaceae) Across a Successional Mosaic in an Andean Cloud Forest. *American Journal of Botany* **88**: 545-551.
- Argus, G. W. and Pryer, K. M. 1990. Rare vascular plants in Canada, our natural heritage. Canadian Museum of Nature. Ottawa.
- Argus, G. W. and White, D. J. 1977. The rare vascular plants of Ontario. *Syllogeus* **14**: 1-63.
- Ashenden, T. W., Bell, S. A., and Rafarel, C. R. 1990. Effects of nitrogen-dioxide pollution on the growth of 3 fern species. *Environ. Pollut.* **66**: 301-308.
- Barrington, D. S. 1993. Ecological and historical factors in fern biogeography. *Journal of Biogeography* **20**: 275-279.
- Bengtsson, K. 1993. *Fumana procumbens* on Oland - population-dynamics of a disjunct species at the northern limit of its range. *Journal of Ecology* **81**: 745-758.
- Bevill, R. L. and Louda, S. M. 1999. Comparisons of related rare and common species in the study of plant rarity. *Conservation Biology* **13**: 493-498.
- Bouchard, A., Barabe, D., and Hay, S. 1977. Isolated colony of *Oreopteris limbosperma* (All) Holub in Gros Morne National Park, Newfoundland, Canada. *Naturaliste canadien* **104**: 239-244.
- Bouchard, A., Barabé, D., Dumais, M., and Hay, S. 1983. Les plantes vasculaires rares du Québec. *Syllogeus* **48**: 1-79.
- Brown, D. F. M. 1964. A monographic study of the fern genus *Woodsia*. J. Cramer, Weinheim.
- Brown, J. H. 1984. On the relationship between abundance and distribution of species. *American Naturalist* **124**: 255-279.

- Brunton, D. F. 1986. Status of the southern maidenhair fern, *Adiantum capillus-veneris* (Adiantaceae), in Canada. *Canadian Field-Naturalist* **100**: 404-408.
- Bryan, A. L. and O'Kelley, J. C. 1967. The influence of replacing calcium with strontium on the development of *Woodsia obtusa*. *American Fern Journal* **57**: 27-31.
- Carlquist, S., Schneider, E. L., and Yatskievitch, G. 1997. SEM studies on vessels in ferns. 1. *Woodsia obtusa*. *American Fern Journal*. **87**: 1-8.
- Centre de données sur le patrimoine naturel du Québec (CDPNQ). 2008. Les plantes vasculaires menacées ou vulnérables du Québec. 3e édition. Gouvernement du Québec, ministère du Développement durable, de l'Environnement et des Parcs, Direction du patrimoine écologique et des parcs, Québec.
- Cinq-Mars, L. 1969. L'habitat du *Carex laxiculmis* Schwein. et du *Woodsia obtusa* (Spreng.) Torr. à Frelighsburg (Missisquoi), Québec, *Naturaliste canadien* **96**: 157-165.
- COSEWIC. 2007. COSEWIC assessment and update status report on the blunt-lobed woodsia *Woodsia obtusa* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa.
- Cousens, M. I. 1981. *Blechnum spicant* - Habitat and vigor of optimal, marginal, and disjunct populations, and field observations of gametophytes. *Botanical Gazette* **142**: 251-258.
- Dyer, A., Lindsay, S., and Lusby, P. 2001. The fall and rise of the Oblong woodsia in Britain. *Botanical Journal of Scotland* **53**: 107-120.
- Fiedler, P. L. and Ahouse, J. J. 1992. Hierarchies of cause: toward an understanding of rarity in vascular plant species. *In*: Conservation biology: the theory and practice of nature conservation, preservation, and management. Fiedler, P. L. and Jain, S. K. *Eds.* Chapman and Hall, New York.
- Flora of North America Editorial Committee. 1993. Flora of North America : North of Mexico. vol. 2. Pteridophytes and gymnosperms. Oxford University Press, New York.
- Gaston, K. J. 1994. *Rarity*. Chapman & Hall, London.

- Gaston, K. J., Blackburn, T. M., Greenwood, J. J. D., Gregory, R. D., Quinn, R. M., and Lawton, J. H. 2000. Abundance-occupancy relationships. *Journal of Applied Ecology* **37**: 39-59.
- Given, D. R. 1993. Changing aspects of endemism and endangerment in Pteridophyta. *Journal of Biogeography* **20**: 293-302.
- Greer, G. K., Lloyd, R. M., and McCarthy, B. C. 1997. Factors influencing the distribution of Pteridophytes in a southeastern Ohio hardwood forest. *Journal of the Torrey Botanical Society* **124**: 11-21.
- Grime, J. P. 1985. Factors limiting the contribution of Pteridophytes to a local flora. *Proceedings of the Royal Society of Edinburgh Section B- Biological Sciences* **86**: 403-421.
- Grubb, P. J. 1977. The maintenance of species-richness in plant communities: The importance of the regeneration niche. *Biological Review* **52**: 107-145.
- Haufler, C. H. 2002. Homospory 2002: an odyssey of progress in pteridophyte genetics and evolutionary biology. *Bioscience* **52**: 1081-1093.
- Hedderson, T. A. 1992. Rarity at range limits - Dispersal capacity and habitat relationships of extraneous moss species in a boreal Canadian National Park. *Biological Conservation* **59**: 113-120.
- Hendrix, S. D. 1980. An evolutionary and ecological perspective of the insect fauna of ferns. *American Naturalist* **115**: 171-196.
- Kato, M. 1993. Biogeography of ferns - Dispersal and vicariance. *Journal of Biogeography* **20**: 265-274.
- Kingston, N. and Hayes, C. 2005. The ecology and conservation of the gametophyte generation of the Killarney Fern (*Trichomanes speciosum* Willd.) in Ireland. *Biology and Environment: Proceedings of the Royal Irish Academy* **105B**: 71-79.
- Kiss, H. G. and Kiss, J. Z. 1998. Spore germination in populations of *Schizaea pusilla* from New Jersey and Nova Scotia. *International Journal of Plant Sciences* **159**: 848-852.
- Klekowski, E. J. 1979. The genetics and reproductive biology of ferns. *In: The experimental biology of ferns*. Dyer, A. F. Ed. Academic Press, New York.

- Kunin, W. E. and Gaston, K. J. 1993. The biology of rarity - Patterns, causes and consequences. *Trends in Ecology & Evolution* **8**: 298-301.
- Labrecque, J. and Lavoie, G. 2002. Les plantes vasculaires menacées ou vulnérables du Québec. Gouvernement du Québec, ministère de l'environnement. Direction du patrimoine écologique et du développement durable, Québec.
- Lawrence, P. A. and Ashenden, T. W. 1993. Effects of acidic gases and mists on the reproductive capability of 3 fern species. *Environmental Pollution* **79**: 267-270.
- Lellinger, D. B. 1985. A field manual of the ferns & fern-allies of the United States & Canada. Smithsonian Institution Press, Washington, D.C.
- Lesica, P. and Allendorf, F. W. 1995. When are peripheral populations valuable for conservation? *Conservation Biology* **9**: 753-760.
- Marquez, A. L., Real, R., Vargas, J. M., and Salvo, A. E. 1997. On identifying common distribution patterns and their causal factors : a probabilistic method applied to Pteridophytes in the Iberian Peninsula. *Journal of Biogeography* **24**: 613-631.
- McKenna, M. F. and Houle, G. 2000. Under-saturated distribution of *Floerkea proserpinacoides* Willd. (Limnanthaceae) at the northern limit of its distribution. *Ecoscience* **7**: 466-473.
- Meilleur, A., Brisson, J., and Bouchard, A. 1997. Ecological analyses of the northernmost population of pitch pine (*Pinus rigida*). *Canadian Journal of Forest Research* **27**: 1342-1350.
- Munther, W. E. and Fairbrothers, D. E. 1980. Allelopathy and autotoxicity in 3 eastern North-American ferns. *American Fern Journal* **70**: 124-135.
- Murray, B. R., Thrall, P. H., Gill, A. M., and Nicotra, A. B. 2002. How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology* **27**: 291-310.
- Nantel, P. and Gagnon, D. 1999. Variability in the dynamics of northern peripheral versus southern populations of two clonal plant species, *Helianthus divaricatus* and *Rhus aromatica*. *Journal of Ecology* **87**: 748-760.
- NatureServe. 2006. Web site: (<http://www.natureserve.org/explorer/index.htm>). [accessed September 2006].

- Nondorf, S. L., Dooley, M. A., Palmieri, M., and Swatzell, L. J. 2003. The effects of pH, temperature, light intensity, light quality, and moisture levels on spore germination in *Cheilanthes feei* of southeast Missouri. *American Fern Journal* **93**: 56-69.
- Pangua, E., Lindsay, S., and Dyer, A. 1994. Spore germination and gametophyte development in 3 species of *Asplenium*. *Annals of Botany* **73**: 587-593.
- Page, C. N. 1979. Experimental aspects of fern ecology. *In*: The experimental biology of ferns. Dyer, A. F. *Ed.* Academic Press, New York.
- Peck, J. H. 1980. Life history and reproductive biology of the ferns of Woodman Hollow, Webster County, Iowa. Ph.D. dissertation, Department of Botany, Iowa State University, Ames, IA.
- Peck, C. J. and Peck, J. H. 1986. Sexuality and isolate potential of isolated *Woodsia obtusa* gametophytes in culture and field. *American Journal of Botany* **73**: 739.
- Peck, J. H., Peck, C. J., and Farrar, D. R. 1990. Influences of life-history attributes on formation of local and distant fern populations. *American Fern Journal* **80**: 126-142.
- Pigott, C. D. and Huntley, J. P. 1978. Factors controlling distribution of *Tilia cordata* at northern limits of its geographical range. 1. Distribution in northwest England. *New Phytologist* **81**: 429-441.
- Pigott, C. D. and Huntley, J. P. 1980. Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. 2. History in northwest England. *New Phytologist* **84**: 145-164.
- Pigott, C. D. and Huntley, J. P. 1981. Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. 3. Nature and causes of seed sterility. *New Phytologist* **87**: 817-839.
- Quintanilla, L. G., Pajaron, S., Pangua, E., and Amigo, J. 2000. Effect of temperature on germination in northernmost populations of *Culcita macrocarpa* and *Woodwardia radicans*. *Plant Biology* **2**: 612-617.
- Rabe, E. W. and Haufler, C. H. 1992. Autopolyploidy and its contribution to fern genetics and evolution: Evidence from a maidenhair mutant. *In*: Fern horticulture: past, present and future perspectives: the Proceedings of the International Symposium on the Cultivation and Propagation of Pteridophytes. Ide, J. M., Jermy, A. C., and Paul, A. M. *Eds.* Intercept, Andover, Hants, U.K.

- Rabinowitz, D., Cairns, S., and Dillon, T. 1986. Seven forms of rarity and their frequency in the flora of the British Isles. *In: Conservation biology : the science of scarcity and diversity*. Soulé, M. E. *Ed.* Sinauer Associates, Sunderland, Mass.
- Reveal, J. L. 1981. The concepts of rarity and population threats in plant communities pp. 41-47. *In: L.E. Morse & M.S. Henifin (eds.), Rare plant conservation: Geographical data organization*. New York: New York Botanical Garden.
- Rowe, J. S. 1972. Forest Regions of Canada. Publication No. 1300. Canadian Forestry Service, Department of the Environment. Ottawa.
- Rumsey, F. J., Vogel, J. C., Russell, S. J., Barrett, J. A., and Gibby, M. 1999. Population structure and conservation biology of the endangered fern *Trichomanes speciosum* Willd. (Hymenophyllaceae) at its northern distributional limit. *Biological Journal of the Linnaean Society* **66**: 333-344.
- Schneller, J. J., Haufler, C. H., and Ranker, T. A. 1990. Antheridiogen and natural gametophyte populations. *American Fern Journal* **80**: 143-152.
- Smith, A. R. 1972. Comparison of fern and flowering plant distributions with some evolutionary interpretations for ferns. *Biotropica* **4**: 4-9.
- Smith, A. R. 1993. Phytogeographic principles and their use in understanding fern relationships. *Journal of Biogeography* **20**: 255-264.
- Soltis, P. S. and Soltis, D. E. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences, USA*. **97**: 7051-7057.
- Thompson, K., Gaston, K. J., and Band, S. R. 1999. Range size, dispersal and niche breadth in the herbaceous flora of central England. *Journal of Ecology* **87**: 150-155.
- Thompson, K., Hodgson, J. G., and Gaston, K. J. 1998. Abundance range size relationships in the herbaceous flora of central England. *Journal of Ecology* **86**: 439-448.
- Tremblay, M. F., Bergeron, Y., Lalonde, D., and Mauffette, Y. 2002. The potential effects of sexual reproduction and seedling recruitment on the maintenance of red maple (*Acer rubrum* L.) populations at the northern limit of the species range. *Journal of Biogeography* **29**: 365-373.

- Tryon, R. 1970. Development and evolution of fern floras of oceanic islands. *Biotropica* **2**:76-84.
- Tryon, R. 1986. The biogeography of species, with special reference to ferns. *Botanical Review* **52**:117-156.
- Tryon, R. M. and Vitale, G. 1977. Evidence for antheridogen production and its mediation of a mating system in natural populations of fern gametophytes. *Botanical Journal of the Linnaean Society* **74**: 243-249.
- Wagner, W. H. 1974. Pteridology - 1947-1972. *Annals of the Missouri Botanical Garden* **61**: 86-111.
- Wagner, W. H. and Rouleau, E. 1984. A western holly fern, *Polystichum X scopulinum*, in Newfoundland. *American Fern Journal* **74**: 33-36.
- Watson, P. J. and Vazquez, M. 1981. Comparative ecology of *Woodsia scopulina* sporophytes and gametophytes. *American Fern Journal* **71**: 3-9.
- Wild, M. 2003. Have five calcicolous fern species of different habitats saturated all local habitat available to them? Université du Québec à Montréal, Montreal, Qc, Canada.
- Wild, M. and Gagnon, D. 2005. Does lack of available suitable habitat explain the patchy distributions of rare calcicole fern species? *Ecography* **28**: 191-196.
- Wild, M., Gagnon, D., and Bouchard, A. 2006. Why are ferns regularly over-represented on state and provincial rare plant lists? *Diversity and Distributions* **12**: 749-755.
- Willis, J. C. 1922. Age and area: a study in geographical distribution and origin of species. The University Press, Cambridge, U.K.