Synergism between Environmental Variation and the Biology of Three Saxicolous Lichens: *Arctoparmelia centrifuga, Xanthoparmelia viriduloumbrina* and *X. cumberlandia*

By

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ABSTRACT

Saxicolous lichens on exposed bedrock are subjected to desiccation stress and intense light levels. Members of the genera *Xanthoparmelia* and *Arctoparmelia* are common foliose lichens on the Precambrian Shield, produce abundant sexual structures, and form part of the bedrock communities. The general goal of this thesis was to better understand the influence of community and underlying geology on three saxicolous lichens: *Arctoparmelia centrifuga, Xanthoparmelia viriduloumbrina* and *X. cumberlandia.* More specific goals were further examined in five chapters to investigate: 1) life history strategies of the three species, 2) a trade-off between fecundity and fungal secondary metabolite production; 3) an effect of substratum element composition on previously defined communities and lichen biology, 4) substratum preferences of *Xanthoparmelia* species, and 5) the photobiont guild hypothesis of the three species in a preliminary study. Field collections of lichens and environmental data were made in four locations on the Precambrian Shield in Manitoba and Ontario. Secondary metabolites were determined by digitally enhanced thin-layer chromatography. Fecundity was measured by number of apothecia, ascospores, and percent germination. Elements in rock samples were quantified by aqua-regia digest and inductively coupled plasma optical emission spectroscopy analysis and light microscopy was used to observe and quantify fungal germination and growth. The results showed eighty-one lichen species comprising three lichen communities; mossy rock, grassy rock, and treed rock communities. Lichen communities and fecundity were used to characterize life history strategies as competitive for *Arctoparmelia centrifuga,* stress tolerant for *Xanthoparmelia viriduloumbrina,* and ruderal generalist for *Xanthoparmelia cumberlandia.* A potential trade-off was reported for *X. cumberlandia* between sexual fecundity and a secondary metabolite. Substratum preferences were found at the genus level and element differences at the

species level. Experimental evidence further supported geological preferences for the three species. Finally, the photobiont guild hypothesis could not be supported by this preliminary work. This research provides a broad overview of ecological and biological patterns found in *Arctoparmelia* and *Xanthoparmelia* species. The research forms a foundation for further studies in substratum preference and life history characterization in lichens. It can be further applied to habitat suitability modelling which may be valuable for phylogenetic context or in conservation biology of lichens.

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Thesis, the publication status of chapters, and the role of each coauthor in each chapter

Chapter	Author	Contributions of each author	Article status
1	Chris Deduke	I reviewed the literature, organized the chapter, and wrote the chapter.	Not to be published.
2	Chris Deduke Tom Booth Michele D. Piercey-Normore	I performed the experiment, collected the data, performed the analysis and wrote the chapter. T. Booth helped with field work, discussed results and recommended literature. MPN generated the original idea with me, guided the process and edited the chapter.	Published in Botany 92(10): 723-735.
3	Chris Deduke Michele D. Piercey-Normore	I conducted the experiment, collected the data, performed the analysis of the data and wrote the chapter. MPN helped form the original idea, mentored the process, and edited the chapter.	Published in the Byrologist 117 (3): 290-296.
4	Chris Deduke Norm M. Halden Michele D. Piercey-Normore	I collected the material, performed statistical analysis of the data and wrote the chapter. NHM checked rock classifications, helped interpret and discussed results. MPN guided the process and edited the chapter.	To be submitted to an appropriate journal.
5	Chris Deduke Michele D. Piercey-Normore	I performed the experiment, collected and analyzed the data and wrote the chapter. MPN colloborated on the original idea, mentored the process, and edited the chapter.	In press in Fungal Biology.
6	Chris Deduke Michele D. Piercy-Normore	I performed PCR and sequencing, collected the data, analyzed the results and wrote the chapter. MPN guided the process,	Partially combined with Chapter 5 and in press in Fungal Biology.

		helped troubleshoot PCR, interpretation of results and edited the chapter.	
7	Chris Deduke	I summarized the research, reviewed the literature and wrote the chapter.	Not to be published.

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CHAPTER 1

General Introduction and Literature Review

1.1 Introduction

Exposed rock outcrops, such as those found underlying the Precambrian Shield of the boreal forest in North America, may provide a harsh environment for the survival of plants, fungi, and other organisms. Plants and fungi in these exposed environments are subjected to higher light levels and desiccation stress than those in more sheltered habitats partially because of small amounts of accumulated soil resulting in sparse vegetation cover (Clayden and Bouchard 1982; Hawksworth and Rose 1977). Saxicolous (rock inhabiting) lichens are one of the most well adapted members of these rock outcrops. Saxicolous lichens such as *Arctoparmelia centrifuga* (L.) Hale, *Xanthoparmelia viriduloumbrina* (Gyelnik) Lendemer and *X. cumberlandia* (Gyelnik) Hale compete for space before plants and other organisms colonize an area (Woolhouse et al. 1985). Lichen thalli are comprised of the fungal partners (mycobiont) from which they are named and the photosynthetic partners (photobiont; green algae and/or cyanobactia), which form symbiotic associations (Nash 2008a; Tehler and Wedin 2008). Lichens, especially crustose lichens, are among the first colonizers of rock substratum (Brock 1973) because they are capable of coping with light damage and low water availability (BeGora and Fahselt 2001; Kranner 2002; Kranner et al. 2008). They play an important role in weathering rock, creating soils and enhancing nutrient cycling in these habitats through fixing substratum and atmospheric sources of nutrients (Ascaso et al. 1976b; Nash 2008b; Williams and Rudolph 1974), which facilitates the colonization by vascular plants. The mechanisms by which lichens

interact with rock substrata have been studied (Adamo and Violante 2000; Chen et al. 2000) and include mechanical and chemical mechanisms.

Research on the lichen flora in Manitoba began with work by Macoun (1902), Bisby and Buller (1922), McClure (1943) and Dix (1950). Further floristic studies include those by J.C. Ritchie in the 1950's and 1960's (Ritchie 1956, 1957, 1959, 1960a, 1960b, 1960c) and Kershaw (1974, 1977, 1978). More recently, the lichen flora has been studied by Frego and Staniforth (1986), Miller (2000), Kotelko et al. (2008), Piercey-Normore (2003, 2005, 2006a, 2008, 2010) and Toni and Piercey-Normore (2013). Floristic studies that include saxicolous lichens are uncommon (Frego and Standiforth 1986; Ritchie 1956; Toni and Piercey-Normore 2013). Community level work regarding saxiclous lichens is even less common and has been understudied in Manitoba (Fontaine et al. 2014; Toni and Piercey-Normore 2013). Ahti (1977) provides a summary of saxicolous lichen communities where the work applies to the circumpolar boreal forest in general.

In addition to the identification of saxicolous lichen communities, a better understanding of the synergism among members of the community, their biology, and the environment is needed. The assignment of life history strategies to species under the competitive, stress and ruderal (CSR) theory (Grime 1977, 2001) provides a framework for understanding this synergism. The CSR model uses nutrient availability and disturbance to explain the different life history strategies, with competitive species existing in high nutrient environments and low disturbance areas; stress tolerant species are in habitats with low nutrient availability and low disturbance; and ruderal species are considered to inhabit high nutrient environments in highly disturbed habitats (low nutrient and high disturbance habitats were not considered by Grime (1977, 2001), but were addressed by Pugh (1980). This work built upon the original dichotomy

of r (rate) and K (carrying capacity) selection theory by MacArthur and Wilson (1967). Rate referred to species that had a high quantitative output of offspring, whereas carrying capacity denotes species that have low quantitative but high qualitative reproductive output. Ruderal species are comparable to r selection species with short life spans and high reproductive output, competitive species are located in the middle between the two extremes and stress tolerant species are comparable to K selection species with long lives and low reproductive output (Grime 2001). This framework has been expanded to include bryophytes (During 1979), fungi (Pugh 1980) and more broadly to lichens (Roger 1990; Topham 1977), despite Grime's (1977, 2001) initial generalization of lichens as stress-tolerant. Using growth rates, growth forms, reproductive strategies, morphology and other characteristics, individual species can be classified according to life history strategies and relative to other ecological processes, such as succession (Ahti 1977; During 1979; Frego and Staniforth 1986; Roger 1990; Topham 1977).

Growth and reproduction, and also life history strategies, may be influenced by the community around a species. Community may therefore result in trade-offs by species trying to meet competing energetic demands of growth and reproduction, which forms part of Grime's theory of life history strategies (Grime 1977, 2001). Environment would influence these trade-offs by shaping the limitations of a species based on nutrient availability, disturbance, and other factors. As previously mentioned, exposed bedrock habitats are subject to high light levels and desiccation stress while providing a nutrient pool that is chemically bound in minerals. In addition, species must compete for space, have protection against predators (Gauslaa 2005; Pentecost 1980), and have sufficient growth and reproductive capacity to adapt to the habitat conditions. Trade-offs, an increase in one characteristic that corresponds with a decrease in another characteristic, have been reported between growth and reproduction in *Lobaria*

pulmonaria (L.) Hoffm. (Gauslaa 2006), but this relationship has been found to be variable in other lichens (Hestmark et al. 2004a), especially in *X. cumberlandia* (Jackson et al. 2006; Pringle et al. 2003) with the correlation between thallus size and number of sexual structures. The trade-off between secondary metabolite production or carbon based secondary metabolites (CBSM) and vegetative growth of the thallus has not been extensively studied in lichens (Bidussi et al. 2013). In addition, the allocation of secondary compounds between different "tissue" types within the lichen thallus, such as somatic and reproductive tissues, has been rarely studied (Hyvärinen et al. 2000; Liao et al. 2010). The allocation of CBSM between different thallus tissues revolves around the role of secondary metabolites in protection against environmental factors specifically affecting those tissues, which is called the optimal defence theory (Hyvärinen et al. 2000; Rhoades 1979). The trade-off between secondary metabolites and sexual fecundity may be exhibited because *Arctoparmelia* and *Xanthoparmelia* species produce a number of secondary metabolites and reproduce sexually through apothecia production. Optimal allocation of secondary metabolites could help alleviate trade-off pressures because production and storage of secondary metabolites might be limited to specific areas of the lichen thallus (Rhoades 1979). Another theory to explain secondary metabolite production is through the carbon-nutrient balance hypothesis which states that excess carbon, as a result of an imbalance in carbon and nutrients, would lead to an increase in carbon-based secondary metabolites (Bryant et al. 1983). This argument also suggests that higher nutrient levels and low carbon input will decrease quantities of CBSMs (Bryant et al. 1983). This hypothesis is controversial (Koricheva 2002a) but it may help to explain patterns in the production of secondary metabolites in some lichens.

The pH of the rock substratum colonized by saxicolous lichens is among the most important variables influencing saxicolous lichen growth (Lisci et al. 2003). Lichens may break

down the rock and absorb elements into their thalli for nutrients, and some secondary metabolites may help manage acidity tolerance of the substrata (Clark et al. 1999, 2001; Hauck and Jürgens 2008). Alternatively, toxic or unfavourable elements may also be present in the substratum and must be managed by the lichen to avoid negative effects. *Xanthoparmelia* species have been found to grow on different rock types and some of the secondary compounds produced in the medulla have been shown to affect element chelation from rocks and minerals at different rates (Ascaso et al. 1976a; Giordani et al. 2002; Iskandar and Syers 1972). The photobionts may also be influenced by the substratum, and evidence suggests that some photobionts may form ecological guilds (Peksa and Škaloud 2011; Rikkinen et al. 2002) where the best adapted alga was selected by the mycobiont for the particular environment. In contrast, the photobiont choice may be based on the phylogenetic relationships of the fungi (Otálora et al. 2010) or some photobionts may be selected by both epiphytic and terricolous lichens (Elvebakk et al. 2008; Myllys et al. 2007; Stenroos et al. 2006). Alternatively, the photobiont may be selected by the mycobiont because it forms the best genetically compatible photobiont out of all those photobionts available in the habitat (Yahr et al. 2006). Most of the research to date showing contrasting evidence is based on cyanobacterial photobionts and not green algae. Based on these conclusions, it is possible that both the mycobionts and green algal photobionts may show preferences for substratum and habitat, providing new insights into lichen adaptation and symbiotic associations.

1.2 Thesis Goals and Objectives

The general goal of this thesis is to better understand the influences that community and geology have on three saxicolous lichens: *Arctoparmelia centrifuga*, *Xanthoparmelia*

viriduloumbrina and *Xanthoparmelia cumberlandia* regarding their life history strategies, sexual fecundity, secondary metabolites, substratum preferences and photobiont selectivity. This goal will be addressed by specific objectives in five chapters:

1) An investigation of the life history strategies of *A. centrifuga*, *X. viriduloumbrina* and *X. cumberlandia* relative to their surrounding communities formed Chapter 2. More specifically, the objectives were (*i*) to describe the lichen communities around the three study species using a species similarity index and environmental variables, (*ii*) to determine which environmental variables influence fecundity of the study species, and (*iii*) to characterize each study species according to a life history strategy.

2) An examination of evidence for a trade-off between fecundity and CBSM within the thallus of *A. centrifuga*, *X. viriduloumbrina* and *X. cumberlandia* was undertaken in Chapter 3, by *(i)* examining correlations among measures of fecundity, and *(ii)* examining correlations between fecundity and CBSM.

3) An investigation of the influence of elemental composition and broadly defined rock types on previously defined lichen communities (Chapter 2) and the biology of *A. centrifuga, X. viriduloumbrina* and *X. cumberlandia* was studied in Chapter 4. This was accomplished through *(i)* comparing the variation in elemental composition and rock types among lichen communities, and *(ii)* by examining the effect of elemental composition on percent cover, fecundity (average numbers of apothecia, ascospores, and percent germination) of each species.

4) Substrate preferences of *X. viriduloumbrina* and *X. cumberlandia* were investigated in Chapter 5 by comparing percent germination and percent cover between two species of lichen fungi, and among five treatments of growth media containing defined crushed rock substrata.

5) Chapter 6 was a preliminary investigation of the photobiont partners of *A. centrifuga*, *X. viriduloumbrina* and *X. cumberlandia* using algal actin gene sequences by: (i) testing the selectivity of the fungi; (ii) testing the photobiont guild hypothesis for green algae with substratum types and (iii) testing the photobiont guild hypothesis for green algae with lichen community types.

Finally, a summary of findings and conclusions about the research was addressed in Chapter 7 producing a model showing the complexity of the relationships and future potential avenues of research as a result of these findings.

1.3 Literature Review

1.3.1 Geological history of Manitoba

The Laurentide Ice Sheet covered the province of Manitoba, extending southwest into Saskatechwan and Alberta, south into North Dakota and Minnesota, and southeast into Ontario at its maximum 75,000 years ago (Andrews and Barry 1978) before its retreat approximately 13,000 years ago (Teller and Leverington 2004). The glacial lake, Lake Agassiz, replaced the retreating ice sheet, which covered an area of approximately 1.6×10^6 km^2 (Teller and Leverington 2004). The current geomorphology of Manitoba is significantly influenced by the recession of Lake Agassiz. As the lake drained northeastward towards its final drainage basin of Hudson Bay (Teller and Leverington 2004), leaving behind outcrops of Ordovician and Silurian limestones and dolostones. After a period of glaciation, these sedimentary outcrops were covered in glacial sediments and ground moraine when the glacier melted. This area is known as the Western Canada Sedimentary Basin and covers about 40% of the province (Manitoba Geology 2014). The remaining 60% of the exposed bedrock in Manitoba is the Precambrian

Shield (Government of Manitoba 2014), which is divided into three regions with the Superior Province in the east, the Trans-Hudson Orogen in the north and the Superior Boundary Zone between the two regions near Thomson, Manitoba (Fig 1.1; Manitoba Geology 2014).

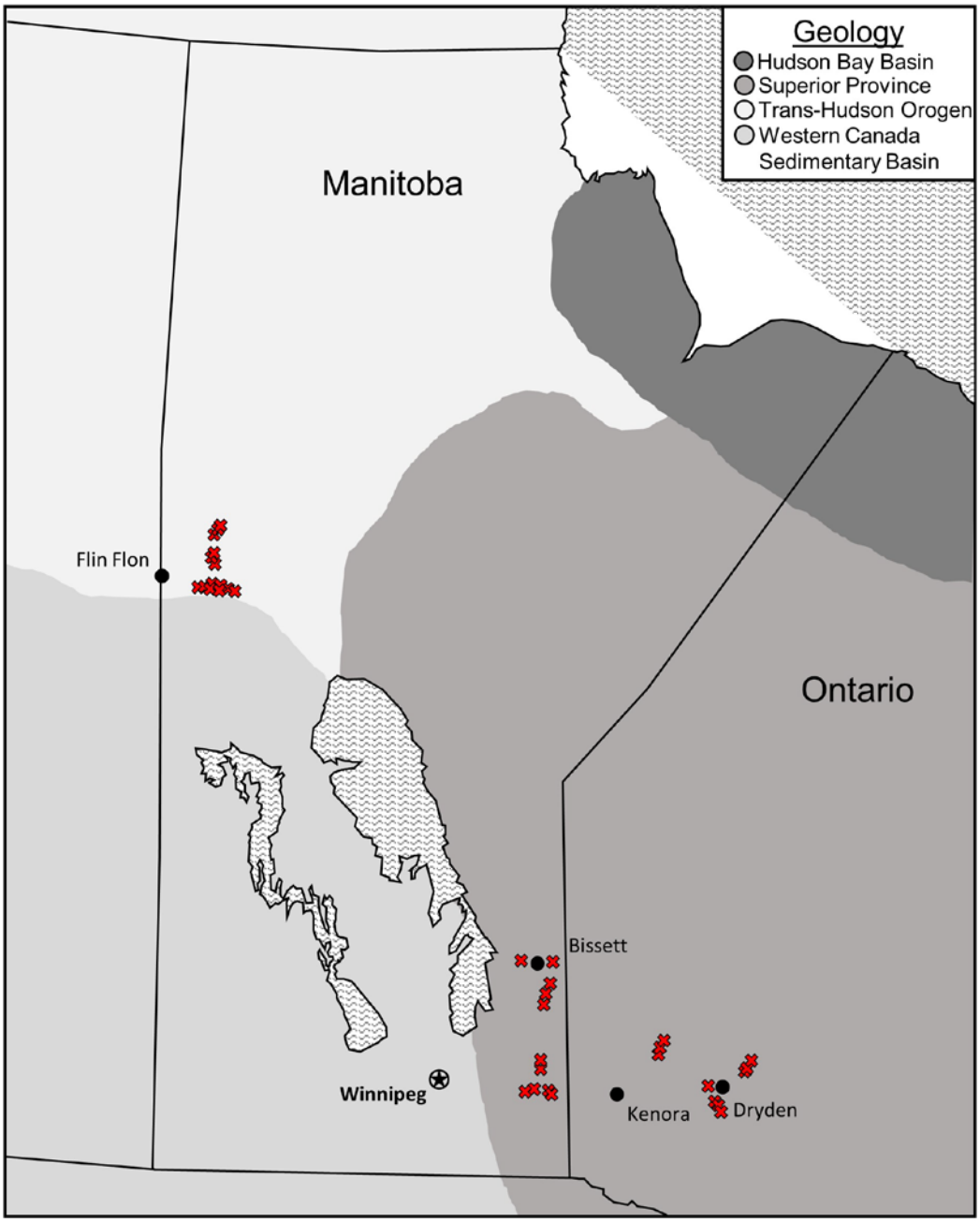

Figure 1.1. A map of Manitoba showing the geological provinces found in the provinces of Manitoba and part of Ontario. This map was created using reference material from Douglas (1973), Manitoba Geology (2014), Ontario Ministry of Mines and Northern Development (1991) and the University of Regina (2006). Red Xs represent transect locations of field collections. For a complete list of transects and location data, see Appendix B.

The geology of Manitoba is highly variable where the Superior Province is subdivided into eleven regions (domains) comprised of three rock suites: a) granitoids and gneisses; b) granite-greenstone belts; and c) metasedimentary rocks (Mantioba Geology 2014). The Trans-Hudson Orogen is the remains of the oceanic subduction zones between two ancient contintenal plates (cratons) known as the Superior Province in the east and the Churchill Province to the northwest. This geological area is divided into ten regions (domains) within Manitoba and it includes many different kinds of lithologies represented by a highly variable suite of rock types including volcanic, plutonic, sedimentary, and metasedimentary rocks (Manitoba Geology 2014). The Superior Boundary Zone represents the transition zone between the Superior Province and Trans-Hudson Orogen and is found between the Western Sedimentary Basin to the south and the Hudson Bay Basin (sedimentary) to the northeast. It is comprised of granitic, mafic and metamorphic rocks (Manitoba Geology 2014). This variable geology provides substratum for many saxicolous lichens but the relationship between geology and saxicolous lichens is unknown.

1.3.2 Saxicolous lichens

Saxicolous lichens form part of many communities of lichens and other organisms on the Precambrian Shield. After glaciation, colonization of the exposed rock was thought to begin with pioneer crustose lichens and other organisms (Ahti 1977). Foliose lichens succeed crustose lichens in these habitats by competing for space and by overgrowing the crustose lichens (Ellis et al. 2007; Plitt 1927). Many species are known to prefer specific habitats but can be found in less than ideal habitats if competition is lacking (Armstrong 1982; Bates 1978; Lawrey 1981). These less than ideal habitats may contain variable conditions such as rock types, element composition,

changes in slope, aspect, light availability, and inadequate water retention capabilities. Certain saxicolous lichen species may be present on some types of rock and absent or rare on other types of rocks (Pentecost 1980). These differences may result from chemical composition and water retention properties of the rocks studied (Bates 1978). Kerr et al. (1989) suggested that lichens may further alter the chemistry and water retention properties of the rock types on which they grow to create more favourable habitats. Goward et al. (1994) noted that *Arctoparmelia centrifuga* was found on both acidic and basic rock types. Rock type has been shown to be a significant factor in lichen habitat selection (John et al. 1990). Saxicolous lichens are important members of their ecological communities because they are responsible for weathering rocks and creating soil (Ascaso et al. 1976b; Williams et al. 1974). Furthermore, rock lichens contribute to the habitat development for other species in their immediate environment such as endolithic (de los Ríos et al. 2002) and other organisms.

1.3.3 Study species

The symbiotic nature of lichens refers to the interaction between a fungal mycobiont and algal/cyanobacterial photobiont. Taxonomically, lichens are classified according to the fungal species and the sexual structures of lichens represent the fungal component of the thallus. The saxicolous genera, *Arctoparmelia* and *Xanthoparmelia*, are ascomycete fungi and produce apothecia. These genera were reclassified from one genus (*Xanthoparmelia)* to two separate genera (*Arctoparmelia* and *Xanthoparmelia*) due to the presence of a polysaccharide, lichenan, within their hyphal walls (Elix et al. 1992). *Arctoparmelia* contains *Cetraria*-type lichenan while the *Xanthoparmelia* species contain a *Xanthoparmelia*-type lichenan (Common 1991). The difference in lichenan is apparent with various types of iodine staining, the most effective of

which is Metzler's Reagent. When Metzler's Reagent is applied to the cell wall of the hyphae, *Cetraria*-type lichenan produces an orange color and *Xanthoparmelia*-type lichenan changes to a deep red colour (Elix 1993). Lichenan may also be of interest to the medical community as an antitumour agent as it was shown to have 100% remission rate in mice with implanted tumours (Nishikawa 1974).

Arctoparmelia centrifuga is a yellowish-green foliose lichen that grows close to the substratum. The thallus commonly dies in the center, forming concentric rings of young thalli and lobes with exposed rock in the center. The species lacks both soredia and isidia and the underside of the lichen is white or pale brown with dark (brown to black) rhizines (attachment organs). It reproduces sexually, resulting in apothecia that are reddish to dark brown (Thompson 1984) with a thallus wall. The diagnostic secondary compounds produced by *A. centrifuga* include alectoronic acid, atranorin, usnic acid, and an unidentified aliphatic acid (Culberson 1970). *Xanthoparmelia viriduloumbrina* is a yellowish-green foliose lichen that is loosely attached to the substratum. The lichen produces larger lobes than *A. centrifuga* and the upper surface of the lobes are emaculate (without maculae, which are thallus color patterns resulting from breaks in the algal layer under the thallus cortex; Brodo et al. (2001)) and shiny. The species does not produce isidia or soredia, and the lower surface is pale brown to darker brown and has many brown rhizines. Pycnidia (conidiospore producing structures) are commonly found on this species. It also reproduces sexually resulting in apothecia (Hale 1990). The secondary compounds include salazinic, consalazinic, lobaric (+/-), and usnic acids (Hale 1990). *X. cumberlandia* is morphologically similar to *X. viriduloumbrina*, although it has a thicker black outline around the margin of the lobes and has more angular lobe edges. *X. viriduloumbrina* has strap-like, and square to rounded lobe edges. Identification keys chemically distinguish between

the two species using the K test where the medulla will quickly turn blood red when salazinic acid is present, while the medulla will slowly turn a yellow to orange-red when sticitic acid is present (Brodo et al. 2001). *X. cumberlandia* contains stictic, constictic, norstictic and usnic acids and belongs to a different chemotype than *X. viriduloumbrina* (Hale 1990).

Arctoparmelia centrifuga is a circumpolar arctic saxicolous species found in Russia, northern Europe and North America, including northern Manitoba (Andreev et al. 1996; Clayden 1992; Thomson 1984). *X. viriduloumbrina* is an emaculate species (Lendemer 2005), possessing upper cortical spotting caused by a discontinous algal layer underneath (Brodo et al. 2001) occuring in eastern North America, similar to the maculate species *X. stenophylla* (Ach.) Ahti & Hawksworth. *X. stenophylla* is known as *X. somloënsis* (Gyeln.) Hale in older keys before it was renamed in 2005 (Ahti et al. 2005). *X. somloënsis* is a circum-temperate species found from Canada to Mexico and across Europe, into Asia (Hale 1990). *X. cumberlandia* is a widely distributed species, located throughout North and South America, Europe and southern Africa (Giordani et al. 2002; Hale 1990). All three species (*A. centrifuga*, *X cumberlandia*, and *X. viriduloumbrina*) grow on the Precambrian Shield in North America, prefer siliceous rocks (Brodo et al. 2001), and are common in the province of Manitoba where their ranges overlap. All species belong to the family Parmeliaceae and have been collected together on rock outcrops. Prior to 1986, all three species were classified within the genus *Xanthoparmelia* (Hale 1986), where *Arctoparmelia centrifuga* was formerly known as *Xanthoparmelia centrifuga* (L.) Hale.

Species distribution and diversity may be influenced by geological composition (John et al. 1990). The geology of Manitoba varies where southwestern Manitoba consists of sedimentary rocks including sandstones, limestone and dolomite (Manitoba Geology 2014). The rest of the province is underlain by Precambrian Shield and is largely made up of granite and gneiss. The

Trans-Hudson Orogen shows a mix of igneous, metamorphic and sedimentary rock types (Manitoba Geology 2014). While the pH of the rock substratum is the most important selection criteria for lichen colonization (Lisci et al. 2003), all three study species occur on acidic, non-calcareous rock (Hale 1967). The calcareous substrata (limestone and dolostone) of the Western Sedimentary Basin has a pH distinct from that of the silicious Precambrian Shield. The igneous and metamorphic rocks tend to be characterized by a greater variety of mineral assemblages. These types of rocks can be identified using physical characteristics including foliation, mineralolgy and mineral habit (Klein and Dutrow 2007). Some of the secondary metabolites found in these three species have been shown to influence pH of surrounding fluids and the breakdown of minerals depending on the type of metabolite (Ascaso et al. 1976a; Hauck and Jürgens 2008; Iskandar and Syers 1972). Other environmental conditions may also influence the type and quantity of the secondary compounds produced (Leuckert et al. 1990). *Arctoparmelia centrifuga* has an associated chemospecies that lacks usnic acid sometimes acknowledged as *A. aleuritica* (Nyl.) Hale but this name has not been formally recognized (Esslinger 2014). Usnic acid production and pH regulation has also been shown to limit photobiont growth (Bačkor et al. 1998).

1.3.4 Reproduction and the photobiont

Sexual reproduction is important because it increases the genetic variability of a species (Barton and Charlesworth 1998). Sexual reproduction also has a prominent role in the life histories of *Arctoparmelia centrifuga*, *Xanthoparmelia viriduloumbrina* and *X. cumberlandia* because they produce abundant apothecia from sexual reproduction and they lack isidia and soredia, two common methods of vegetative reproduction. Sexual reproduction allows lichens to

be more adaptable to environments that are prone to disturbances or instability (Hedenås et al. 2000; Milton et al. 1984) by shuffling alleles resulting in population genetic variation. Latitude is known to play a role in reproductive patterns of some species (Lawrey 1980b) where sexual or vegetative reproduction may be preferred depending on habitat conditions. Since all three species, *Arctoparmelia centrifuga*, *Xanthoparmelia viriduloumbrina* and *X. cumberlandia*, lack soredia and isidia, vegetative propagation is limited to fragmentation. Lichens are known to produce sexual or vegetative propagules at a given location due to investment in growth or other biological aspects (Bowler and Rundel 1975). Competition between species may cause some species to invest more in growth, which results in outgrowing other species and dominance in the community. *Xanthoparmelia conspersa* is known to employ this strategy to outgrow lichens such as *Parmelia saxatalis* (L.) Ach. and *Parmelia glabratula* ssp. *fuliginosa* (Lamy) Nyl. (syn. *Melanelixia fuliginosa* (Fre. ex Duby) Blanco et al.) (Armstrong 1982).

The species of algae that associate with lichen fungi vary within and between species of lichen fungi. This may depend on genetic compatibility between partners and/or adaptation to environmental conditions. A single species of fungus may associate with a single algal species or more than one algal species. On the other hand several fungal species may associate with the same or different algal species. Variability in algal association with three *Cladonia* species suggested both genetic compatibility and environmental influence on the algal choice (Piercey-Normore 2006) where *C. multiformis* G. Merr. selected a single algal haplotype regardless of the geographic location and other algal haplotypes present in the area. Algae may also be shared or segregated among different fungal species in the same habitat (Doering et al. 2009) where four lichen fungi on a single tree associated with the same algal species. A high level of algal species sharing among fungal partners indicates a low level of algal selectivity, and a low level of algal

sharing indicates a high level of selectivity. Lichens are capable of dispersing great distances (Harmata et al. 1991) and vegetative propagules may carry both partners making them available for lichenization with other partners whereas ascospores must reassociate with an alga to form a lichen thallus. The formation of a lichen by ascospores from sexual reproduction, is limited by the availability of photobiont partners across great distances (Lawrey 1980b) and the level of selectivity by the fungal partner. Environmental factors, such as rock type, may also play a role in limiting the availability of algae in a given location; and therefore, in the selection of photobionts (Romeike et al. 2002). Patterns of selectivity and distribution were observed for Parmeliaceae and Lecanoraceae lichens growing on tree bark in Europe (Beck et al. 1998; Hauck et al. 2007a). Three types of lichen compatible photobionts have been found on rocks; chlorococcoid, trentepohlioid, and cyanobacterioid photobionts (Rambold et al. 1998). *Arctoparmelia centrifuga* and the *Xanthoparmelia* species have chlorococcoid photobionts, which are in the genus *Trebouxia* (Ahmadjian 1993a; Leavitt et al. 2013; Nash et al. 2002).

Photobionts in the genus *Trebouxia* are known to be free-living in nature (Bubrick et al. 1984; Sanders and Lücking 2002) but have been rarely reported suggesting that most of the *Trebouxia* photobionts are found within lichen associations. If lichens undergo sexual reproduction and dispersal of ascospores, the ascospore must form a partnership with a compatible algal species before the lichen thallus can be formed. The algal species that forms a partnership with *A. centrifuga* and *X. viriduloumbrina* are not well studied. *Xanthoparmelia stenophylla* is known to associate with *Trebouxia arboricola* Puymaly and *T. gigantea* (Hildreth& Ahmadjian) Gärtner (Ahmadjian, 1993a). *Arctoparmelia centrifuga* is known to associate with *Trebouxia* sp. but the species is unknown (Thompson 1984). *X. cumberlandia* has

been shown to associate with *T. impressa* Ahmadjian and with photobionts within the *T. gigantea/T. arboricola* clade (Leavitt et al. 2013).

1.3.5 Sexual fecundity

Sexual fecundity may be regarded as the quantitative sexual reproductive output produced by an organism or population, measured through number of sexual propagules. Ascospore viability may reflect sexual fecundity of a lichen by representing a measure of success of reproduction. Ascospore production may be influenced by environment and thereby the geographic distribution (Lawrey 1980b) with temperate lichen species displaying mixed reproductive strategies. Foliose and fruticose lichens have a larger number of species with asexual vegetative structures (isidia and soredia) than crustose lichens (Bowler and Rundel 1975; Piercey-Normore 2005). However, it is uncommon for a single species to have both asexual and sexual structures (Bowler and Rundel 1975). Lichens that rely on sexual reproductive ascospores for dispersal have been shown to have a more limited distribution relative to asexually reproducing lichens (Bowler and Rundel 1975). As a result, it might be expected that the number and viability of apothecial and spore production shows fluctuations across the geographic range of the species. Geographic distribution may play a role in distribution of male and female gametophores in some species of bryophytes. For example, the male sexual structures of *Bryopteris diffusa* (Sw.) Nees are sporadically dispersed and are absent from Central America and the West Indies specimens (Gradstein 1994). In areas where the male structure is lacking, the species must reproduce asexually. Another measure of fecundity, viability of asexual propagules, can also be affected by latitude. The observed pattern with latitude may also apply to vascular plants such as *Daucus carota* L., also known as wild carrot, which produces fewer viable

offspring in southern latitudes than northern latitudes due to greater insect damage in southern latitudes (Lacey 1984). While the reduction of viability in the wild carrot was reported to be environmentally based, other species may also show similar reasons for variation in viability. Studies involving habitats with colder temperatures and arctic conditions showed that sexual reproduction in macrolichens is less important in these locations and is replaced by macrolichens that produce soredia, asexual progopules containing both symbionts, or lichens that diserpse through fragmentation (Bowler and Rundel 1975; Lindsay 1977; Thomson 1972). In contrast, Fahselt et al. (1989) showed that sexual reproduction commonly occurs in macrolichens in polar regions and is not diminshed by the colder climate.

Spore number and germination rates are not the only ways of measuring sexual fecundity in lichens. A more direct method to testing sexual fecundity is to count the number of sexual structures (apothecia) on a thallus. A larger number of apothecia present on the thallus suggests that a larger number of ascospores are available for release by the asci but these are successful only if they are viable and land in a suitable habitat near a compatible alga. The size of the lichen thallus has been thought to influence the number of apothecia present on the thallus. However, the relationship between growth and number of apothecia is controversial. Some studies suggest a correlation exists between thallus size and the number of apothecia (Pringle et al. 2003; Ramstad and Hestmark 2001), which included *X. cumberlandia*. Conversely, a follow-up study on *X. cumberlandia* and *X. coloradoensis* (Gyelnik) Hale showed that thallus size is not a reliable indicator of fecundity in different environments, nor does thallus size account for all of the variation in apothecia production (Jackson et al. 2006). As a result, the use of thallus size would not be the most accurate indicator for sexual fecundity.

1.3.6 Secondary metabolites

Secondary metabolites may be derived from primary metabolism and typically correspond with particular life stages of the fungi (Keller et al. 2005). They are not considered essential for the survival of the organism, as they are not required for growth and development (Elix 1996; Keller et al. 2005) but they may be used for other purposes. Several theories provide potential explanations for the evolution of secondary metabolite production. Secondary metabolites were originally considered to be waste products derived from primary metabolism that served a detoxification function in the organism (Hartmann 2007). Further research showed that secondary metabolism was non-essential for growth and development but served an essential role in the evolutionary adaptation and acclimation to changing environments (Hartmann 2007). Various functions of secondary compounds may include antimicrobial, allelopathic and antiherbivore activities (Lawrey 1986). Antimicrobial studies showed that some secondary compounds can deter gram positive soil bacteria (Lawrey 1989). Allelopathic studies include the inhibition of crustose lichen ascospore germination by lichen secondary metabolites (Whiton and Lawrey 1984), inhibition of ascospore germination in *Cladonia cristatella* Tuck. and *Sordaria fimicola* (Roberge ex Desm.) Ces & De Not. (Whiton and Lawrey 1982), inhibition and delayed germination of moss spores (Lawrey 1977), effects upon hypocrealean fungal growth (Lawrey et al. 1994), and inhibition of the growth of *Sclerotinia sclerotiorum* (Lib.) de Bary and *Ophiostoma novo-ulmi* ssp. *americana* Brasier (Kowalski et al. 2011). Studies on the antiherbivore nature of secondary compounds have provided mixed results. Antiherbivore studies on slug predation showed that *Xanthoparmelia cumberlandia* produced stictic acid as a defense against slug feeding (Lawrey 1980a, 1984). Conversely, some secondary compounds are thought to be beneficial to herbivores by providing calcium to oribatid mites that ingest *X.*

conspersa (Lawrey 1980c) and by aiding in the digestive process by caribou when they consume caribou lichens (Palo 1993). Production of secondary compounds is thought to occur solely within the fungal partner. This assumption has been questioned by Honegger (1992) who suggests the photobiont also influences the production of secondary compounds through carbohydrates provided to the mycobiont. Research on lichen photobionts showed that photobionts are capable of producing four types of carbohydrates where lichen fungi in the Parmeliaceae receive carbohydrates in the form of ribitol from *Trebouxia* (Feige and Jensen 1992).

Based on the literature, it is expected that saxicolous lichens will show variation in life history and community composition resulting in some specificity to the species composition. Greater differences should be discovered between *Arctoparmelia* and *Xanthoparmelia* based on the large genetic differences between fungal genera, in addition to influences by ecology, variability in geological substratum and photobiont selectivity. It is the intention of the authour that this thesis will provide new insights into the boreal ecology of saxicolous lichens and an additional means of organising a diverse array of previous research from different fields into life history strategies for lichens, while concentrating on community and geological research.

CHAPTER 2

Lichen fecundity on the Precambrian Shield: an alternative life history strategy approach

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2.1 Abstract

Symbiotic interactions are widespread in nature, but the relationship between life history, fecundity, and habitat specificity has been underexplored. This study investigated the life history strategies of foliose saxicolous lichens relative to their surrounding communities. Macrolichens were collected from 39 transects in Manitoba and Ontario. Frequency and percentage of ground cover of macrolichens, environmental variables, and numbers of apothecia and ascospores were recorded. Lichen assemblages were characterized using species similarity in a cluster analysis and ordination methods. The assemblages were further defined as communities using analysis of variance of the biotic variables among assemblages. Lichen life history strategies were inferred from community features, lichen fecundity, and morphological features. A general linear model determined which environmental variables may have influenced fecundity. The 81 species of macrolichens present in three lichen communities differed in composition, with low species richness in the open mossy rock community, moderate in the grassy rock community, and high in the treed rock community. Three foliose saxicolous lichens dominated particular communities, and the life history strategy was characterized as competitive for *Arctoparmelia centrifuga*, stress tolerant for *Xanthoparmelia viriduloumbrina*, and ruderal generalist for *Xanthoparmelia cumberlandia*. The proportion of sexual and asexual reproductive propagules for macrolichens

showed uniformity between communities despite a significant difference in species richness. The study provides insights into the ecology of saxicolous lichens growing in the boreal forest, the characterization of lichen communities, and it shows how morphologically similar lichens can exhibit different life history strategies.

2.2 Introduction

Reproduction and colonization in lichens has been studied with respect to sexual and vegetative reproduction under various ecological or substratum conditions (Ellis and Coppins 2007; Gauslaa 2006; Hestmark et al. 2004b). Ellis and Coppins (2007) showed evidence for a shift from sexual to vegetative communities with a change in aspen bark age and pH. Mixed reproductive strategies in *Parmotrema* species led Lawrey (1980) to hypothesize that the mixed strategies may ensure genetic variability and facilitate lichenization. If sexual reproduction is important for allelic diversity to allow adaptation to changing conditions (Pauls et al. 2013), then habitats with high levels of change or disturbance would be expected to have species with rapid life cycles, high allelic diversity through sexual reproduction, and high reproductive output to respond to the changing habitat. Relaxed allelic diversity through vegetative reproduction may be the result of an investment in methods to compete for space and nutrients (During 1979; Grime 1977). More stable habitats might be expected to support species with less allelic diversity than those in disturbed habitats. The exposed rock outcrops of the Precambrian Shield in North America have habitats that vary from disturbed to stable and should have species that reflect the variation in habitats.

Arctoparmelia Hale and *Xanthoparmelia* (Vainio) Hale are genera of appressed foliose lichens (Hale 1986, 1990), where the name of the lichen refers to the fungal species (Tehler and

Wedin 2008). While these saxicolous macrolichens form a conspicuous component of the Precambrian Shield, their biology and role in the habitat is not well understood. Significant knowledge gaps are present in their community structure, geological preferences, photobiont selection, and life history strategies. Knowledge of the abiotic components of the environment, such as water availability, light and temperature exposure, and interspecific competition is essential to understanding their life history strategies (Bjelland 2003; Ranius et al. 2008). The slow-growing nature of saxicolous lichens (Hestmark et al. 2004b), the age and composition of the bedrock substrate, and the presence of these lichens in a wide variety of exposed habitats (Cutler et al. 2008; Hauck et al. 2007b; Kitayama et al. 2009; Payette et al. 1989; Rees and Juday 2002) suggest that life history strategy would play a key role in adaptation. Relative to other plants and fungi, lichens have been generalized as stress tolerant (Grime 1977), but the 17,000 known species of lichen-forming fungi (International Union for Conservation of Nature 2010; Kirk et al. 2008) exhibit a wide range of variation in life history strategies, including mechanisms for competition (Armstrong and Welch 2007), growth rates, and reproductive strategies (Büdel and Scheidegger 2008). Characterization of saxicolous lichen communities and life history strategies will form the basis for further research on their adaptive abilities and provide insights into species diversity and ecosystem function.

Life history strategy refers to the features of a life cycle, including morphology, reproduction, and abundance or growth rates, that influence the way in which an organism interacts with its environment and reflects survival strategies. Life history strategies form a key component of the Competition, Stress, and Ruderal (CSR) theory (Grime 1977), which posits that vascular plants may be competitors in crowded environments, they may tolerate stressful

conditions such as low nutrient levels, and they may be ruderal early-colonizing plants that often colonize disturbed habitats. Pugh (1980) added two more strategies for fungi — escapers and survivors — to address the ability of many fungi to tolerate high stress and frequently disturbed (or extreme) environmental conditions. During (1979) categorized bryophytes, which share many habitats with lichens, into six life history strategies. He included life span, sexual and vegetative (asexual) reproduction, age of first reproduction, spore characteristics, and growth form to categorize species into ecological roles. Topham (1977) and Rogers (1990) applied the CSR theory to lichens, but Topham (1977) focused on habitat types while Rogers (1990) focused primarily on species growth rates and morphology. Each approach relates life history features to habitat types and has particular merits that might be applied to foliose saxicolous lichens to better understand the ecology of lichen communities.

The goal of this study was to investigate the life history strategies of three foliose saxicolous lichens in the genera *Arctoparmelia* and *Xanthoparmelia* relative to their surrounding communities. More specifically, the objectives were (*i*) to describe the lichen communities around the three study species using a species similarity index and environmental variables, (*ii*) to determine which environmental variables influence fecundity of the study species, and (*iii*) to characterize each study species according to a life history strategy.

2.3 Materials and Methods

2.3.1 *Study location and experimental design*

The study sites were located in northern Ontario (93°15'–92°09'W, 50°11'–49°37'N), southern Manitoba (95°36'–95°23'W, 50°02'–49°50'N; 95°44'–95°17'W, 51°01'–50°34'N), and northern Manitoba (101°34'–101°22'W, 55°00'–54°36'N). A preliminary survey of the

Precambrian Shield found that lichens belonging to the genera *Xanthoparmelia* and *Arctoparmelia* were abundant on rock outcrops. The most common members of these genera became the focus of this study. Transects were subsequently selected based on the presence of Jack pine ridges that were large enough for a 40 m transect in a given location, accessibility, and on the presence of *Xanthoparmelia* and *Arctoparmelia* thalli. Thirty-nine 40 m transects were positioned on selected bedrock outcrops in Manitoba and Ontario during 2010 and 2011. A total of five 1 m × 1 m quadrats were placed every 10 m on each transect, beginning at 0 m. The upper left corner of the quadrat was placed over the nearest thallus of *Xanthoparmelia*, or *Arctoparmelia*, perpendicular to the transect, resulting in a total of 195 quadrats. This method ensured that at least one study lichen was present within the designated quadrat. These lichens were selected as the focus of this study because they are common on the Precambrian Shield, are well studied, and are relatively free of taxonomic issues (Lendemer 2005; Thomson 1993), making these good subjects for ecological studies. All *Arctoparmelia* and *Xanthoparmelia* specimens collected from the quadrats were returned to the laboratory for identification and fecundity determinations. Vouchers from all other lichen samples were also collected for identification. *Arctoparmelia* species were identified using Hale (1986) and Brodo et al. (2001). The *Xanthoparmelia* species were identified using Hale (1990), Thomson (1993), and Lendemer (2005) with reference to Brodo et al. (2001) and Hinds and Hinds (2007). Keys used for other species included those from Thomson (1984), Goward et al. (1994), and Goward (1999).

2.3.2 *Environmental variables*

The percent cover of environmental variables included two abiotic variables, exposed weathered rock (rock that is uncolonized by macroscopic organisms) and bare earth (loose soil

lacking macroscopic organisms), and seven biotic variables, including living vegetation (bryophytes, graminoids, and herbaceous and woody plants), ground litter (deciduous leaves and coniferous needles and cones that have fallen to the ground), deciduous or coniferous woody debris, and lichen litter (lichens as epiphytes on twigs, bark, or cones). Percent ground cover of all lichens and the biotic and abiotic variables were recorded from each 1 m × 1 m quadrat by visually estimating the ground cover. Biotic variables represented the amount of living material in a quadrat, which could provide an indication of the amount of shelter from light and wind, nutrient levels, or moisture retention in the habitat. Abiotic variables, such as weathered rock and bare earth, were measured to understand (*i*) the degree of openness of the habitat and (*ii*) the amount and type of potential substratum available for colonization by these species.

For this study, an assemblage represents lichens in a collection of transects with similar species compositions. A community refers to the broader combination of lichen assemblage and the biotic component of the surroundings, including mosses, graminoids, herbaceous and woody plants, and other biotic variables indicating the presence of surrounding trees. Rock and soil substrates refer to abiotic components of the environment and were not included in the community. Species richness, calculated using the presence data within each assemblage, represents the total number of species for each defined assemblage. Species richness is a component of species biodiversity, which can be used to calculate the influence of a species on an ecosystem (Naeem et al. 1994). Understanding the function of species in an ecosystem can help clairfy their roles, species redundancy (identical roles for multiple species in the same ecosystem) and their relative importance to an ecosystem (Walker 1992). This analysis can be used as a starting point to address these sorts of questions, but are beyond the scope of this paper.

2.3.3 *Life history features*

The life history features measured include species abundance, percent ground cover, and fecundity. The percent ground cover of species and percent ground cover of environmental variables were averaged for each transect and calculated for each of the assemblages. The frequency of occurrence of a species was calculated as the number of quadrats in each transect in which the species occurred. The mean species frequency was calculated from the sum of individual transect frequencies that fell within each of the three identified assemblages. Lichen diversity was represented by the presence and absence of a species in each quadrat, by the mean frequency per transect, or by the percent ground cover of a species per quadrat. From transects grouped in the cluster analysis, a comparison was made between assemblages using the mean species richness and derived standard deviation.

Fecundity refers to the fitness of an organism, which can be inferred by counting the number of reproductive structures. Since reproductive structures are too small to count in the field, the apothecia, which are the products of sexual reproduction, can be used as an indication of sexual reproductive output (Pringle et al. 2003). Since the number of apothecia is inferred to be a measure of the reproductive effort of a sexually reproducing lichen, the sexual fecundity was inferred in this study through the number of mature apothecia produced per thallus. It is generally assumed that number of apothecia correlates with numbers of ascospores released from mature apothecia of selected thalli, but this cannot be tested under field conditions. Therefore, this study also examined the number of ascospores released by apothecia under sterile laboratory conditions as a second fecundity measure.

The number of mature apothecia for each thallus was recorded in the field, and species identifications were confirmed in the laboratory. Apothecia were considered mature based on

cross sections and morphology descriptions by Bellemere and Letrouit-Galinou (2000), and they were excluded from the data collection if they were immature or over-mature. Immature apothecia were conical in shape, exhibiting a small hymenial layer concealed by developing thalline walls and lacked mature asci with spores. Over-mature apothecia showed signs of algal degradation of the hypothecium and had empty asci. Evidence of degradation was a flattening out of the apothecial margins resulting in the loss of the bowl-shaped apothecium and darkening of the hymenial surface caused by bleeding of epihymenial pigmentation. The mature apothecia used in this study had bowl-like structures, an abundant algal layer in the surrounding thalline walls, light brown hymenial layers, and mature asci containing ascospores (Bellemereand Letrouit-Galinou 2000).

Mature apothecia were used for culturing by spore rains to determine the number of ascospores released by the apothecia, the second measure of fecundity. Following a modified protocol developed by Kofler (1970), two apothecia were affixed to each lid of 100 mm × 15 mm polystyrene sterile petri dishes (Fisherbrand) using Vaseline. Malt yeast agar (0.5 g agar, 0.5 g malt, 0.05 g yeast, and 25 mL water per plate; Sigma) was used as a growth medium for the spores (Stocker-Wörgötter 2002). Spores were counted once per week for a 20-week period using a dissecting microscope (Leica MZ 6) at 40× magnification. A 10 × 10 unit grid was place within the right eyepiece, and counts were derived from within 10 consecutive grid squares representing 1/10 of the total area.

2.3.4 *Morphological features*

Lichen growth forms were categorized as crustose, squamulose, appressed foliose, leafy foliose, fruticose *Cladonia*, and fruticose *Cetraria*. Crustose lichens are those lichens that are

attached directly to the substratum by the entire undersurface of the thallus, and do not include the squamulose *Cladonia* spp. Squamulose lichens are *Cladonia*-type lichens without podetia. Appressed foliose lichens are foliose lichens that have many rhizines and maintain close contact with the substratum. Leafy foliose lichens are foliose lichens that are weakly attached by rhizines or maintain contact with the substratum from a single point such as a holdfast. Fruticose *Cladonia* lichens have upright branched or unbranched podetia present. Fruticose *Cetraria* lichens have a strap-shaped branched thallus with very few points of attachment to the substratum. Growth forms were used as indications of the exposure and amount of moisture in a habitat.

The ability of lichens to fix nitrogen was also used as an indication of the type of habitat. If a habitat contains more lichens that fix nitrogen it would be expected to provide more nutrients to the organisms in the habitat. Nitrogen-fixing lichens were recorded as those with cyanobacteria as their primary photobiont and those that have cephalodia-containing cyanobacteria but green algae form the primary photobiont. Percent cover values for each growth form and nitrogen-fixing lichen were calculated by summing the total percent cover for each category in each quadrat and transect.

2.3.5 *Statistical analysis*

A Jaccard's index of species similarity was calculated from a contingency table of species presence and absence for all 39 transects. The data were analysed using the error sum of squares cluster method (Ward's Method) to produce a dendrogram showing the similarity between transects. Jaccard's index has been shown to be compatible with Ward's Method (Fichet and Le Calve 1984; Gower and Legendre 1986; Plasse et al. 2007). An indicator species analysis

provided an objective selection of the species clusters to define the lichen assemblages. The indicator species analysis was run in PC-ORD (version 6.08; McCune and Mefford 2011). A broad categorization with a small number of clades was chosen to maintain sufficient sample size for analyses. A contingency table was used as a primary matrix, with eight different assemblage classifications sequentially ordered 2 through 9 (10 produced an error due to a clustering equal to 1 transect) for the 39 transects based on the clustering in the dendrogram. A phi coefficient method was used, as suggested for binary data (Tichy and Chytry 2006). A Monte Carlo test determined the significance of the species indicator values. Two selection methods were used, the lowest mean *p* value and the highest number of significant indicators to determine the number of clusters (McCune and Grace 2002). The overall rank was determined by summing the rank for lowest mean *p* and highest number of significant indicators and dividing by 2, the number of tests. Both lowest mean *p* values and highest number of significant indicator species are the two ways recommended to objectively determine optimum number of clusters (McCune and Grace 2002). The results produced three clusters as the best objective classification for defining the assemblages and the results of this cluster analysis served as the antecedent to define each of the three lichen assemblages (equivalent to clusters) for all subsequent analyses (Dufrene and Legendre 1997).

Principal Coordinates Analysis (PCoA) was conducted on the 84 macrolichens found in this study (81 species and three genera; *Candellaria* sp., *Cladonia* sp., and *Stereocaulon* sp.). Lichens were scored as present or absent for 39 transects, and PCoA was chosen to explore the relationship between species and the assemblages because of the binary data set and non-Euclidean distance measure (Jaccard's distance matrix). Environmental variables, abiotic

or biotic, were not explored in the PCoA analysis. PCoA does not make assumptions about the response to environmental variables by species, it can be performed on nonlinear data, and it produces less distortion of data than other methods (McCune and Grace 2002). A PCoA was run in PC-ORD (version 6.08; McCune and Mefford 2011) using Jaccard's distance matrix (Mueller-Dombois and Ellenberg 1974), and scores for response variables (species) were calculated using weighted averaging.

The relationship between measured environmental variables and lichen abundance, coded by transects within assemblages, was examined using Canonical Correspondence Analysis (CCA). CCA was chosen to test the multiple regression relationships between the selected environmental variables and the abundance of lichens. CCA has been deemed to be appropriate for species data sets (McCune and Grace 2002; Peck 2010) and was performed using PC-ORD (version 6.08; McCune and Mefford 2011). The primary data matrix was composed of lichen abundance data, which were calculated for each species, with five being the maximum value (maximum number of quadrats per transect). Twenty-six species were eliminated from the analysis because they were present in less than 5% of transects (Kenkel 2006). The second data matrix represented the cumulative percent cover data for seven environmental variables. Data matrices were log transformed to produce unimodal distributions for environmental variables. A randomization test (Monte Carlo Test) was run to determine the significance of the ordination with a null hypothesis that no relationship existed between the matrices.

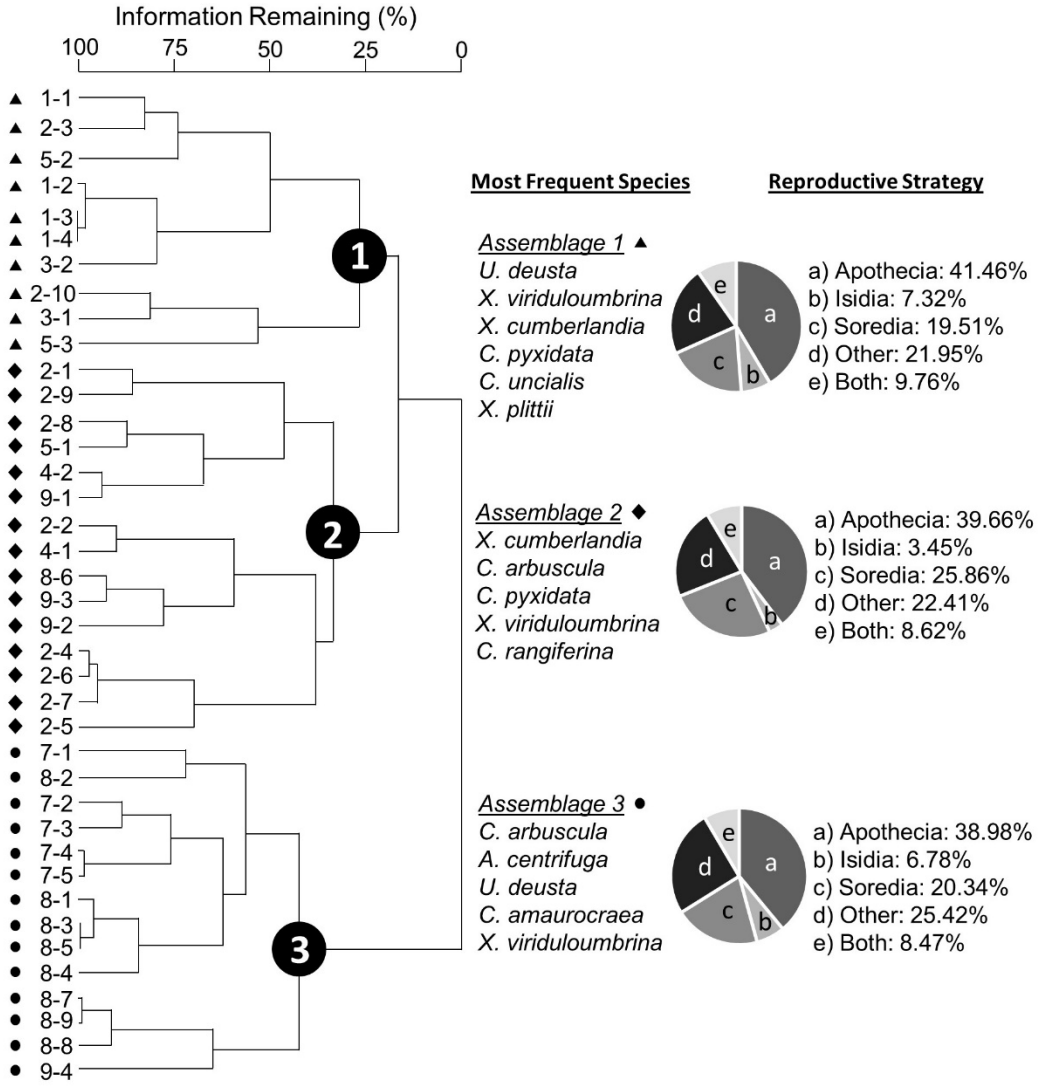

Figure 2.1. Dendrogram showing relationships between lichen assemblages based on Jaccard's Index of Similarity. Numbers in the left column of the dendrogram represent the site and transect numbers (Sites: 1 and 3, Whiteshell area; 2, Ontario; 4 and 5, Nopiming area; and 7, 8 and 9, Flin Flon area). The five most frequently occurring lichen species (last two tied for the 5th position in assemblage 1) are listed to the right of the assemblage. A pie chart showing proportion of reproductive strategies used by lichens within each assemblage is shown using shades of gray (a, apothecia present; b, isidia present; c, soredia present; d, other present and; e, the presence of both sexual and vegetative reproductive structures). "Other" denotes asexual reproductive methods such as fragmentation. The percentage values for pie chart are located beside the figure.

The general linear model (GLM) was implemented with apothecia (an indicator of fecundity) as the response variable and the environmental variables as the independent variables. The number of apothecia was recorded by quadrat, summing the numbers from all thalli together to compare with other quadrats. Percent ground cover of all variables and number of apothecia were log transformed. Half of the percent cover variables still produced non-normal distributions, but likelihood of normality increased with increasing sample size. JMP 10.0.2 (SAS Institute, Cary, North Carolina) was used to calculate the coefficient of determination (R^2) and the unexplained variance or error sum of squares (SSE). Akaike's Information Criteria (AIC), Corrected Akaike's Information Criteria (AIC_c), the level of empirical support for model *i* (Δ_i), and the probability of each model *i* ($weight_i$) were all calculated in Microsoft Excel. Variables with a frequency of less than 80% occurrence were removed to improve predictive ability. Variables that had a Pearson's correlation coefficient (r) > 0.7 were removed to eliminate collinearity. The models for the three study species based on their high frequencies in the transects (Fig. 2.1) resulted in *Arctoparmelia centrifuga* (n = 26) with 7 variables, *Xanthoparmelia cumberlandia* (n = 73) with 6 variables, and *Xanthoparmelia viriduloumbrina* (n = 62) with 5 variables that met the selection criterion.

For the comparison of environmental variables among transects, data were transformed according to the recommendations of Zar (2010). A square-root transformation was applied to frequency data sets, which were analysed by a Kruskal–Wallis test with mean frequency per quadrat as the continuous variable and assemblage as the categorical variable, with a Steel–Dwass rank test to determine the pairwise significance within the data sets. An arcsine transformation followed by a (π/180) multiplier for percent ground cover data were analysed by a Kruskal–Wallis test using mean percent ground cover per quadrat as the continuous variable and

assemblage as the categorical variable and by a Steel–Dwass rank test to determine the pairwise significance within the data sets. The fecundity indicators were analysed using log (x + 1) transformation of the number of apothecia and ascospores released from each apothecium to produce normal distributions. The hypothesis that the variation in sexual fecundity was similar between assemblages was tested using one-way ANOVA (analysis of variance) with fecundity (numbers of apothecia and spores) as the dependent variables and assemblage as the independent variable.

2.4 Results

2.4.1 *Lichen assemblages*

The indicator species analysis produced three clusters as the best solution for broad assemblage classification (Table 2.1). Three clusters produced the second lowest mean *p* value (0.3855), compared with eight clusters (0.3822), and the highest number of significant indicators (25). Classification based on these criteria resulted in three lichen assemblages that are also shown in a dendrogram, which produced clusters based on similarity among the species composition within each transect (Fig. 2.1). The three assemblages, 1, 2, and 3, were defined by 27%, 31%, and 43% information remaining, respectively (Fig. 2.1). One or more study species was among the five most frequent lichens in each assemblage, since quadrats were chosen based on the presence of *Xanthoparmelia* or *Arctoparmelia* thalli. *Xanthoparmelia viriduloumbrina* is the most frequent of the three study species in assemblage 1, second only to *Umbilicaria deusta*; *X. cumberlandia* is most frequent in assemblage 2; and *A. centrifuga* is the most frequent of the study species in assemblage 3, following *Cladonia arbuscula*. The proportions of reproductive strategies employed by all lichens is represented by a pie chart adjacent to each assemblage (Fig.

2.1), and shows that about half of the species reproduce sexually in each lichen assemblage. For species that contain apothecia, assemblage 1 has 51.2%, assemblage 2 has 48.3%, and assemblage 3 has 47.5% (Fig. 2.1). From a total of 81 species distributed among three lichen assemblages (Table 2.2), 28 species produce only apothecia, 24 species produce only vegetative reproductive structures (19 sorediate species and five isidiate species), nine species produce both sexual and vegetative structures, and 20 species used other inferred strategies such as fragmentation.

The relationship among species compositions in the three assemblages is shown by a PCoA with three axes explaining 40.15% of the cumulative variance (Fig. 2.2). Axis 1 explained 19.33%, axis 2 explained 12.82%, and axis 3 explained 8.0% of the variance. All three assemblages are separated from each other.

The relationship between species and assemblages, constrained by environmental variables, was explained by two axes of the CCA ordination representing 18.2% of the total variation (Fig. 2.3). Axis 1 explained 12.8% and axis 2 explained 5.4% of the variation in the data. A Monte Carlo randomization test rejected the null hypothesis that there is no relationship between the species and environmental data (Table 2.3). Correlation values for both axes were 0.906 and 0.923 (Table 2.3). The ordination showed the three lichen assemblages and vector lines representing the extent and direction of the correlations for environmental variables. Assemblage 1 is located primarily in the negative region of axes 1 and 2. Assemblage 2 is located with the greatest representation in the negative region along axis 1 and both negative and positive regions along axis 2. Assemblage 3 has very little overlap with assemblage 1 and is found in the positive region along axis 1 and the positive and negative regions along axis 2. The environmental variables that correlated the strongest with these two axes are weathered rocks,

herbaceous and woody plants, woody debris, lichen litter, mosses, and graminoids. Weathered rock shows the strongest correlation with assemblage 2. The remaining environmental variables all correlate most strongly with assemblage 3.

Table 2.1. Results of the indicator species analysis for choosing the optimum number of clusters based on the lowest mean p value and highest number of significant indicators. Overall rank was determined by averaging the ranks of mean *p* values and number of significant indicators.

No. of clusters	Mean *p* value	Rank	No. significant Indicators	Rank	Overall Rank
2	0.4302	7	19	4	6
3	0.3855	2	25	1	1
4	0.4388	8	17	7	8
5	0.4298	6	13	8	7
6	0.4231	5	18	5	5
7	0.4099	4	18	5	4
8	0.3822	1	21	2	1
9	0.3885	3	21	2	3

Note: Rankings for each analysis are shown to the right of results with cumulative overall cluster rank shown in the far right column.

The mean species richness differed significantly among lichen assemblages (Table 2.4). Assemblage 1 had the lowest mean species richness with 12.0 species (41 species in total), assemblage 2 had 16.5 species (58 species in total), and assemblage 3 had 21.8 species (59 species in total). The assemblage 3 had significantly greater species richness than the other two assemblages.

2.4.2 *Assemblages and environmental variables define communities*

Percent ground cover of environmental variables showed significant differences among the three assemblages (Table 2.4; Fig. 2.4). Percent cover of weathered rock was significantly higher in assemblages 1 and 2 than assemblage 3 (Fig. 2.4A). Mosses had greater percent cover in assemblage 1 (open mossy rock community) than assemblage 3 (Fig. 2.4A). Percent cover of graminoids was significantly higher in assemblage 2 (the grassy rock community; Fig. 2.4A) than the open mossy rock community (assemblage 1) and assemblage 3. Herbaceous and woody plant percent cover was greatest in the grassy rock community (assemblage 2) and assemblage 3 (Fig. 2.4A). Percent cover of tree indicating variables (pine litter, woody debris, and lichen litter) was significantly higher in assemblage 3 (treed rock community) than the other two communities.

Percent cover of crustose lichens was significantly greater in the treed rock community than the open mossy rock community (Fig. 2.4B). Percent cover of appressed foliose lichens was significantly higher in the open mossy rock community than the other communities (Fig. 2.4B). Percent cover of the leafy foliose lichens and the fruticose lichens was significantly higher in the treed rock community than the other two communities (Fig. 2.4B).

Four cyanobacterial species (*Peltigera*) and seven cephalodiate species (*Stereocaulon*) are reported. *Stereocaulon tomentosum* and *S. saxatile* were the two most common *Stereocaulon* spp. in the study while others included *S. dactylophyllum*, *S. grande*, and *S. rivulorum*. The percent ground cover of the genus *Stereocaulon* was significantly highest in the grassy rock and treed rock communities (Fig. 2.4C). The genus *Peltigera* comprised two commonly occurring species, *Peltigera rufescens* and *P. malacea*, while other less common species included *P. didactyla* and *P. horizontalis*. The percent cover of *Peltigera* was significantly higher in the grassy rock community than the open mossy rock community (Fig. 2.4C).

Table 2.2. List of species collected in all locations in this study showing the species code used in Figure 2.2, and the assemblages in which each species was found.

Code	Species Name	Assemblage	Code	Species Name	Assemblage
Ace	*Arctoparmelia centrifuga* (L.) Hale	2, 3	Csy	*Cladonia symphycarpia* (Flörke) Fr.	2
Asu	*Arctoparmelia subcentrifuga* (Oxner) Hale	3	Ctr	*Cladonia trassii* Ahti	2
Bru	*Baeomyces rufus* (Hudson) Rebent.	3	Cturg	*Cladonia turgida* Hoffm.	1, 2
-	*Candalariella* species* Müll. Arg.	1	Cun	*Cladonia uncialis* (L.) F. H. Wigg.	1, 2, 3
Cer	*Cetraria ericetorum* subsp. *reticulum* (Räsänen) Kärnefelt	2, 3	Cve	*Cladonia verticillata* (Hoffm.) Scharer	2, 3
Cis	*Cetraria islandica* (L.) Ach.	2, 3	Fni	*Flavocetraria nivalis* (L.) Kärnefelt & Thell	3
Cac	*Cladonia acuminata* (Ach.) Norrlin	2	Mdi	*Melanelia disjuncta* (Erichsen) Essl.	3
Cam	*Cladonia amaurocraea* (Flörke) Schaerer	1, 2, 3	Mhe	*Melanelia hepatizon* (Ach.) Thell	3
Car	*Cladonia arbuscula* (Wallr.) Flowtow	1, 2, 3	Mpa	*Melanelia panniformis* (Nyl.) Essl.	3
Cbor	*Cladonia borealis* S. Stenroos	1, 2, 3	Mso	*Melanelia sorediata* (Ach.) Goward & Ahti	2, 3
Cbot	*Cladonia botrytes* (K. G. Hagen) Willd.	3	Mst	*Melanelia stygia* (L.) Essl.	3
Cca	*Cladonia cariosa* (Ach.) Sprengel	2, 3	Min	*Melanohalea infumata* (Nyl.) O. Blanco et al.	2, 3
Cce	*Cladonia cenotea* (Ach.) Schaerer	2, 3	Pfr	*Parmelia fraudans* (Nyl.) Nyl.	3
Cch	*Cladonia chlorophaea* (Flörke ex. Sommerf.) Sprengel	1, 2, 3	Pom	*Parmelia omphalodes* (L.) Ach.	2, 3
Ccoc	*Cladonia coccifera* (L.) Willd.	1, 2, 3	Psa	*Parmelia saxatalis* (L.) Ach.	1, 3
Ccon	*Cladonia coniocraea* (Flöke) Sprengel	1, 2, 3	Psu	*Parmelia sulcata* Taylor	3
Ccor	*Cladonia cornuta* (L.) Hoffm.	1, 2, 3	Pdi	*Peltigera didactyla* (With.) J. R. Laundon	2
Ccrisp	*Cladonia crispata* (Ach.) Flowtow	2, 3	Pho	*Peltigera horizontalis* (Hudson) Baumg.	2
Ccrist	*Cladonia cristatella* Tuck.	1, 2, 3	Pma	*Peltigera malacea* (Ach.) Funck	3
Cde	*Cladonia deformis* (L.) Hoffm.	3	Pru	*Peltigera rufescens* (Weiss) Humb.	2
Cfi	*Cladonia fimbriata* (L.) Fr.	1, 2, 3	Phi	*Phaeophyscia hispidula* (Ach.) Essl.	1, 2
Cgrac	*Cladonia gracilis* (L.) Willd.	2, 3	Psc	*Phaeophyscia sciastra* (Ach.) Moberg	2
Cturb	*Cladonia gracilis* subsp. *turbinate* (Ach.) Ahti	1, 2, 3	Pca	*Physcia caesia* (Hoffm.) Fürnr.	2
Cgray	*Cladonia grayi* G. Merr ex. Sandst.	2, 3	Pph	*Physcia phaea* (Tuck.) J. W. Thomson	2

Cmaci	*Cladonia macilenta* Hoffm.	1	Psu	*Physcia subtilis* Degel.	1, 2
Cmacr	*Cladonia macrophylla* (Schaerer) Stenh.	1, 2, 3	Rch	*Rhizoplaca chrysoleuca* (Sm.) Zopf	3
Cmi	*Cladonia mitis*† Sandst.	1	Sda	*Stereocaulon dactylophyllum* Flörke	1, 2, 3
Cmu	*Cladonia multiformis* G. Merr.	1, 2, 3	Sgr	*Stereocaulon grande* (H. Magn.) H. Magn.	1, 2, 3
Coc	*Cladonia ochrochlora* Flörke	3	Spa	*Stereocaulon paschale* (L.) Hoffm.	1, 2, 3
Cph	*Cladonia phyllophora* Hoffm.	1, 2, 3	Sri	*Stereocaulon rivulorum* H. Magn.	1
Cpl	*Cladonia pleurota* (Flörke) Schaerer	1, 2, 3	Ssa	*Stereocaulon saxatile* H. Magn.	1, 2, 3
Cpo	*Cladonia pocillum* (Ach.) Grognot	1, 2, 3	-	*Stereocaulon* species* Hoffm.	1, 2
Cpy	*Cladonia pyxidata* (L.) Hoffm.	1, 2, 3	Sto	*Stereocaulon tomentosum* Fr.	1, 2, 3
Cra	*Cladonia rangiferina* (L.) F. H. Wigg.	1, 2	Ude	*Umbilicaria deusta* (L.) Baumg.	1, 2, 3
Cre	*Cladonia rei* Schaerer	2	Uma	*Umbilicaria mammulata* (Ach.) Tuck.	2, 3
Csc	*Cladonia scabriuscula* (Delise) Nyl.	2	Umu	*Umbilicaria muehlenbergii* (Ach.) Tuck.	1, 2, 3
-	*Cladonia* species* P. Browne	1, 2, 3	Uto	*Umbilicaria torrefacta* (Lightf.) Schrader	3
Csq	*Cladonia squamosa* Hoffm.	1, 2	Xan	*Xanthoparmelia angustiphylla* (Gyelnik) Hale	1, 3
Cste	*Cladonia stellaris* (Opiz) Pouzar & Vězda	2, 3	Xco	*Xanthoparmelia conspersa* (Erhh. ex. Ach.) Hale	1
Csty	*Cladonia stygia* (Fr.) Ruoss	3	Xcu	*Xanthoparmelia cumberlandia* (Gyelnik) Hale	1, 2, 3
Csub	*Cladonia subulata* (L.) F. H. Wigg.	2	Xpl	*Xanthoparmelia plittii* (Gyelnik) Hale	1, 2
Csul	*Cladonia sulphurina* (Michaux) Fr.	3	Xvi	*Xanthoparmelia viriduloumbrina* (Gyelnik) Lendemer	1, 2, 3

* - Denotes identification to genus.

†- Also known as *Cladonia arbuscula* subsp. *mitis* (Sandst.) Ruoss.

2.4.3 *Distribution and fecundity of the study species*

The percent ground cover of each of the study species was significantly different between assemblages (Table 2.4). *Arctoparmelia centrifuga* had the highest percent cover in the treed rock community, *Xanthoparmelia viriduloumbrina* was significantly greatest in the open mossy rock community, and *X. cumberlandia* showed the greatest percent cover in both the open mossy rock and the grassy rock communities (Fig. 2.4). Within the open mossy rock community, *X. viriduloumbrina* was found to have significantly greater percent cover than *X. cumberlandia* (H = 8.923, d.f. = 195, 1, p = 0.0028). The grassy rock community showed that the significantly greatest percent cover belonged to *X. cumberlandia*, followed by *X. viriduloumbrina* and finally *A. centrifuga* (H = 72.468, d.f. = 195, 2, p = <0.0001). *Arctoparmelia centrifuga* was found to have the significantly greatest percent cover in the treed rock community. The *Xanthoparmelia* species showed a significant difference between lichens, with *X. viriduloumbrina* being greater than *X. cumberlandia* but both were significantly less than *A. centrifuga* (H = 33.171, d.f. = 195, 2, p = <0.001).

The three study species showed a significant difference between the number of apothecia produced and the number of ascospores released (Table 2.4). *Xanthoparmelia cumberlandia* and *A. centrifuga* produced significantly more apothecia per thallus than *X. viriduloumbrina*. Ascospore production was significantly higher in *A. centrifuga* than the *Xanthoparmelia* species. The three study species also showed significant differences among communities with respect to numbers of apothecia and ascospores (Table 2.4). *Xanthoparmelia viriduloumbrina* showed significantly higher apothecia production in the grassy rock community than the open mossy rock community (F = 3.746, d.f. = 62, 2, p = 0.0294). *Xanthoparmelia cumberlandia* did not show individual differences in number of apothecia between the three communities. Ascospore production was found to be significant in *X. cumberlandia* (Table 2.4) where the open mossy

rock community produced significantly more ascospores than the grassy rock community, which also produced significantly more ascospores than the treed rock community (F = 16.167, d.f. = 68, 2, p = <0.0001). In a comparison between study species and communities, *X. cumberlandia* showed significantly higher apothecia growth than *X. viriduloumbrina* in the open mossy rock community (F = 4.598. d.f. = 44, 1, p = 0.0378). Ascospore production showed no difference between *Xanthoparmelia* species (F = 0.061, d.f. = 44, 1, p = 0.8057). The grassy rock community showed no difference between species for either apothecia or ascospore production (F = 0.061, d.f. = 61, 1, p = 0.8057; F = 0.061, d.f. = 61, 1, p = 0.8057; respectively). *Arctoparmelia centrifuga* showed greater apothecia production than either *Xanthoparmelia* species in the treed rock community (F = 5.504, d.f. = 53, 2, p = 0.0069). Ascospore production was greatest in *A. centrifuga*, followed by *X. viriduloumbrina*, and finally *X. cumberlandia* with the lowest level of spore production (F = 23.618, d.f. = 53, 2, p <0.0001).

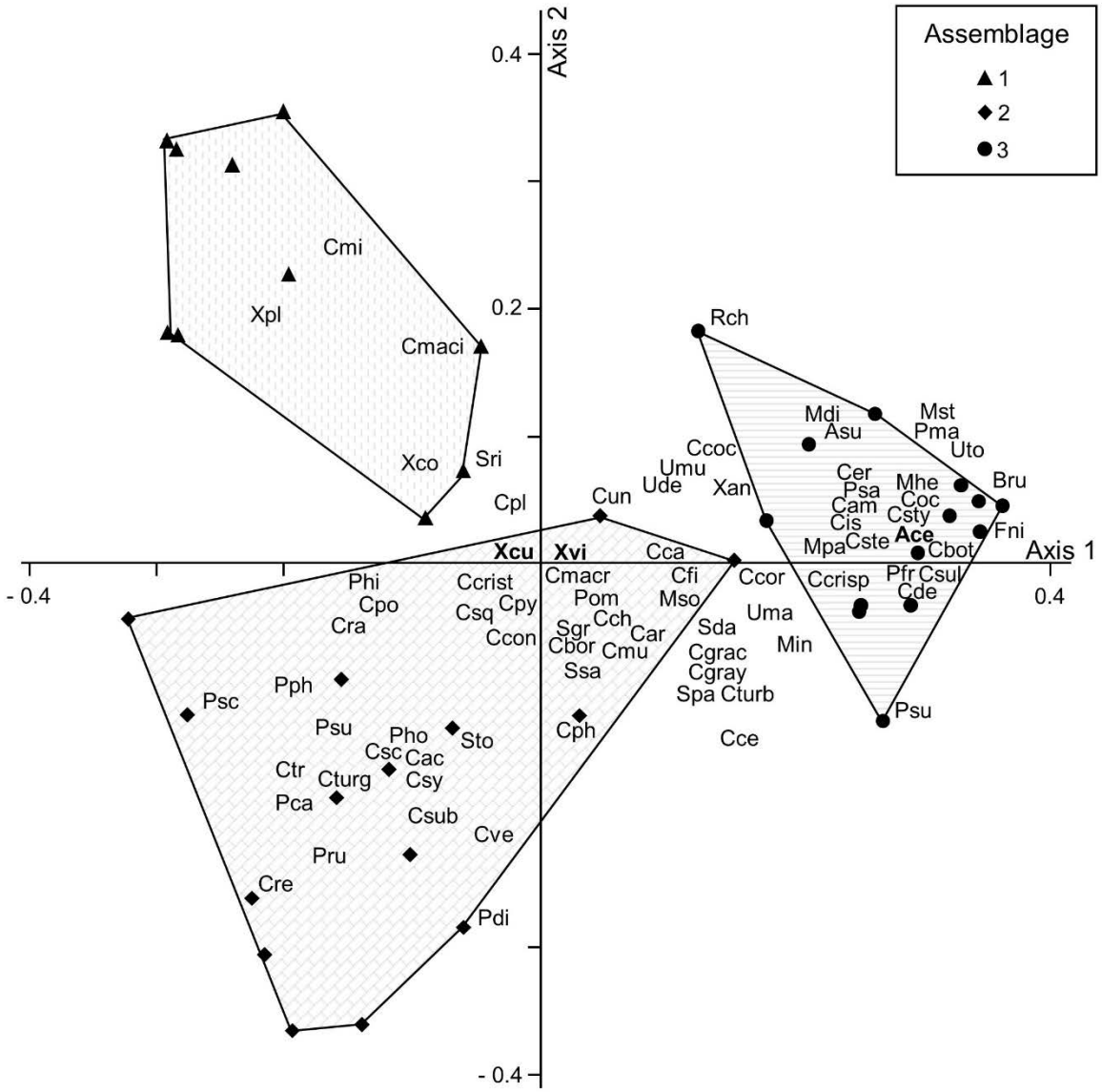

Figure 2.2. Biplot of lichen species and transects within three species assemblages for axes 1 and 2 of the Principle Coordinates Analysis. Species are represented by the first letter of the genus and the two first letters of the species epithet (see Table 2.2).

2.4.4 *Effect of environmental variables on apothecia production*

Two GLM models for *A. centrifuga* showed strong support for apothecia production as an indicator of fecundtity with the best model showing the relationship between percent cover of *A. centrifuga* thallus interacting with the absence of *C. arbuscula* thalli (Table 2.5). The positive relationship between percent cover of *A. centrifuga* thallus and crustose lichens was the next best model. Nine GLM models for *X. cumberlandia* showed strong support for apothecia production with the absence of crustose lichens and the increasing percent cover of *X. cumberlandia* as the two best models. The presence of weathered rock, fruticose lichens, and mosses also factored into GLM models for *X. cumberlandia*. The overall explanatory power of these models was low for *X. cumberlandia,* because the models could only account for a maximum of 4.8% of the variance in the 9 models. Three GLM models for *X. viriduloumbrina* showed strong support for apothecia production with the increasing percent cover of *X. viriduloumbrina* interacting with the absence of weathered rock or the presence of crustose lichens as the best models. Only models with a $\Delta_i \leq 2$ were recorded due to the large number of models generated for each species (Johnson and Omland 2004). Eight variables were used for *A. centrifuga* resulting in twenty-four tests. Six variables were chosen for each *Xanthoparmelia* species, with twelve tests run for each species.

2.5 Discussion

2.5.1 *Three lichens resemble ruderal, stress-tolerant, and competitive strategies*

The three foliose saxicolous lichens in this study may be described as ruderal, stress tolerant, and competitive relative to the communities they dominate. Because of the high frequency and percent cover of *X. viriduloumbrina* in the open mossy rock community (Figs. 2.1

and 2.5), with high exposure of weathered rock and mosses and lowest percent cover of all vascular plants and nitrogen-fixing lichens (Fig. 2.4), and because of its lower apothecia production than *X. cumberlandia* ($F = 4.598$, d.f. = 1, $p = 0.0378$), it is proposed that *X. viriduloumbrina* is a stress tolerator. The vegetative dominance of *X. cumberlandia* in the grassy rock community (Figs. 2.1 and 2.5) with conditions suggesting moderate exposure and moisture (stress) levels compared with the open mossy rock and treed rock communities (Fig. 2.4), and its having the second highest dominance and high apothecia production in a more exposed community (open mossy rock) (Fig. 2.1), indicate that *X. cumberlandia* represents a ruderal species. Lastly, the frequency of *A. centrifuga* in the treed rock community, which has the greatest species diversity, is a highly competitive environment, and the abundance of *A. centrifuga* in this competitive environment, along with its high reproductive output, suggests that *A. centrifuga* is a competitor.

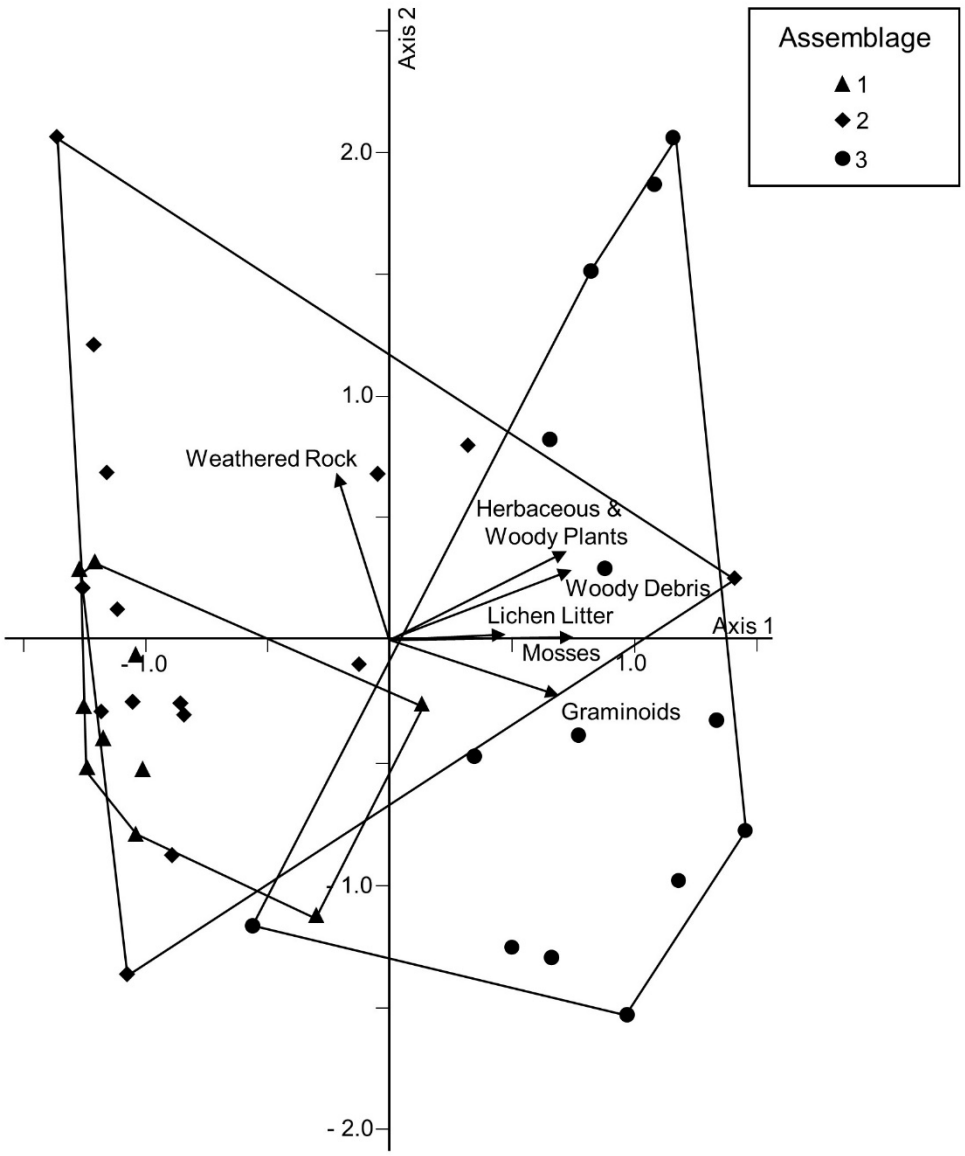

Figure 2.3. Biplot of environmental variables and transects within species assemblages for axes 1 and 2 of the Canonical Correspondence Analysis. Vector arrows indicate the extent of influence of the correlation for the environmental variables shown.

Table 2.3. Results of the Canonical Correspondence Analysis (CCA) showing eigenvalues, variance in species data, cumulative variance in species data and Pearson correlation values for the first three axes.

CCA Statistics	Axis 1	Axis 2	Axis 3
Eigenvalue	0.282	0.119	0.093
Variance	12.8	5.4	4.2
Cumulative Variance	12.8	18.2	22.4
Pearson Correlation	0.906	0.923	0.818
Monte Carlo Test		$p = 0.005$	
Environmental variable*	Axis 1	Axis 2	Axis 3
Weathered Rock	-0.228	0.703	-0.417
Mosses	0.766	0.011	0.295
Graminoids	0.714	-0.225	-0.324
Herbaceous and Woody Plants	0.744	0.381	-0.339
Pine Litter	-0.203	0.303	0.720
Woody Debris	0.757	0.303	0.339
Lichen Litter	0.495	0.024	0.554

Note: The result of the Monte Carlo test for determining the null hypothesis for no relationship between species and environmental data is shown.
*Correlation values for all seven environmental variables is shown for each of the first three axes.

Xanthoparmelia viriduloumbrina was the most common of the three study lichens in the open mossy rock community and is considered to be stress tolerant. The high amount of weathered rock and mosses in the community, along with low amounts of vascular plants (graminoids, herbaceous and woody plants) and few indicators of large trees and shrubs (pine litter, woody debris, lichen litter) (Fig. 2.4) may indicate an exposed habitat in an early ecological succession of species (Burbanck and Platt 1964; Burbanck and Phillips 1983; Oosting and Anderson 1939). Cohabitation with mosses may allow the lichen to retain moisture when the mosses absorb moisture, but at the same time it would be necessary to withstand desiccating conditions when the mosses dehydrate in the exposed habitat. These exposed habitats also have low levels of vascular plants preventing the retention of moisture, which suggests that organisms in this community must be able to tolerate desiccation and persist in water-stressed environments, indicating that *X. viriduloumbrina* may be stress tolerant. *Xanthoparmelia viriduloumbrina* was consistent with the expectation of high ground cover and low sexual reproductive output (During 1979) in this open mossy rock community and had significantly reduced apothecia production when compared with that of *X. cumberlandia*.

Table 2.4. Summary of results for the hypothesis that the three assemblages (independent variable) are different based on variables of environmental features, lichen growth form, and nitrogen fixing lichens as defined in the methods.

Kruskal-Wallis Test			
Variable	*H*	df	*p*
Species Richness	19.788	195, 2	<0.0001
Environmental Variables (percent ground cover)			
Weathered rock	52.859	195, 2	< 0.0001
Mosses	10.102	195, 2	0.0060
Graminoids	26.421	195, 2	< 0.0001
Herbaceous and woody plants	21.454	195, 2	< 0.0001
Pine litter	30.413	195, 2	< 0.0001
Downed woody debris	46.109	195, 2	< 0.0001
Lichen litter	57.467	195, 2	< 0.0001
Growth form			
Crustose	5.806	195, 2	0.0549
Appressed foliose	26.707	195, 2	< 0.0001
Leafy foliose	40.128	195, 2	< 0.0001
Fruiticose-*Cladonia*	53.915	195, 2	< 0.0001
Nitrogen Producing Lichens			
Stereocaulon spp.	49.775	195, 2	< 0.0001
Peltigera spp.	8.341	195, 2	0.0150
Study Species			
A. centrifuga	107.068	195, 2	< 0.0001
X. viriduloumbrina	20.871	195, 2	< 0.0001
X. cumberlandia	44.354	195, 2	< 0.0001

ANOVA			
Sexual Fecundity	*F*	Df	*p*
Apothecia			
Shield lichen species	7.363	2, 158	0.0009
A. centrifuga	2.197	1, 28	0.1500
X. viriduloumbrina	3.746	2, 62	0.0290
X. cumberlandia	3.981	2, 68	0.0230
Ascospores			
Shield lichen species	12.026	2, 158	< 0.0001
A. centrifuga	<0.001	1, 28	0.9770
X. viriduloumbrina	0.536	2, 62	0.5880
X. cumberlandia	16.167	2, 68	< 0.0001

Note: The percent cover, apothecia and ascospore production results of the three study species are shown. *p* values less than 0.0500 are significant.

The number of apothecia was considered to be one of the best indicators of fecundity in lichens (Pringle et al. 2003), and is feasible for field studies since ascospore output is difficult to measure in the field. High apothecial numbers correlated with high percent cover of crustose lichens and low amounts of weathered rock in the GLM model, which indicates more vegetated habitats (Fig. 2.4) and potentially greater retention of moisture in the grassy rock community.

Xanthoparmelia cumberlandia is the most abundant species in the grassy rock community and the second most abundant appressed foliose lichen in the open mossy rock community, both of which contain significant amounts of uncolonized weathered rock and mosses, but the grassy rock community contains more short vascular plants (graminoids, herbaceous and woody plants) than the open mossy rock community. The presence of short vascular plants in the grassy rock community allows the retention of some moisture in the habitat, but it also has high light levels (and potential for desiccating conditions) as in the open mossy rock community. The ability of *X. cumberlandia* to flourish in both communities may suggest that it is a generalist, but at the same time it must be able to generate sufficient allelic variation through sexual reproduction to withstand the range of conditions. Grime (1977) describes species that have high vegetative mass and high reproductive output but predominate in exposed and disturbed habitats to be ruderals. *Xanthoparmelia cumberlandia* is one of the most frequent species in the open mossy rock community and accounts for about 10% of cover. To have such a high percent cover compared with other lichens and a high reproductive output indicates that this is an early colonizer (Booth et al. 1988; During 1979; Grime 1977; Pugh 1980; Rogers 1990; Topham 1977). The presence of graminoids and dicots in the grassy rock community suggests that the community is in a later successional stage than the open

mossy rock community (Burbanck and Phillips 1983; Burbanck and Platt 1964). This later ecological succession is supported by the presence of lichens that tend to grow in more stable, sheltered environments, such as *Cladonia phyllophora* and *C. gracilis* subsp. *turbinata* (Ahti and Oksanen 1990), and the establishment of reindeer lichens, such as *C. arbuscula* and *C. rangiferina*, which are known to have higher growth rates than others (Den Herder et al. 2003; Pegau 1968) and are also early invaders of disturbed areas (Ahti 1977; Ahti and Oksanen 1990). *Stereocaulon saxatile* in this community may be tolerant of conditions that adversely affect other lichens, such as competition with grasses and herbaceous plants (Gilbert and Fox 1985). Higher nutrient levels in this community are supported by the presence of nitrogen-fixing lichens, particularly *Peltigera rufescens*, which provides nitrogen for the immediate environment through leaching from the thallus by rainfall (Millbank 1982; Millbank and Olsen 1986). The greater species richness of lichens, the introduction of nitrogenous lichens, and the percent cover of faster growing non-lichenized vegetation, resulting in changes to nutrient availability (nitrogen), greater shade (plants), and water availability (plants), all indicate that the grassy rock community is transitional. *Xanthoparmelia cumberlandia* showed the greatest output of ascospore production and is classified here as a ruderal species.

Arctoparmelia centrifuga is the most common of these three lichens in the treed rock community. *Arctoparmelia centrifuga* is considered a chionophytic species (snow-loving) that prefers mesic saxicolous habitats (John 1989; Ritchie 1956), along with *Cladonia stellaris*, a climax community species (Auclair 1985; Foster 1985b*;* Kershaw 1977), and others that also prefer mesic to wet habitats, such as *Peltigera malacea*, *Flavocetraria nivalis*, *Melanelia stygia*, and *Parmelia saxatilis*. Typical boreal forest lichens, including *Cladonia amaurocraea*, *C. arbuscula*, *C. cornuta*, *C. deformis*, *C. gracilis*, *C. stellaris*, *C. stygia*, *C. uncialis*, *P. saxatilis*,

Umbilicaria deusta, and *U. muehlenbergii* (Foster 1985a; Johnson 1981), had high percent ground cover in this community. Nitrogen-fixing lichens, including five members of the genus *Stereocaulon* and one member of the genus *Peltigera,* were also common in this community, suggesting the community is rich in nutrients. The moderate growth rate of *A. centrifuga* (20–30 mm/year; Zotz and Schleicher 2003) compared with crustose (1–20 mm/year; Sancho et al. 2007) and foliose lichens, including *Xanthoparmelia* (5–40 mm/year; Armstrong 2004; Benedict and Nash 1990; Sancho et al. 2007), suggests it may be a competitive species because of its above average rate of growth. The presence of both usnic acid and atranorin in the cortex of *A. centrifuga* (but see Clayden 1992) may suggest a superior competitive advantage over other lichens with a single cortical compound, where usnic acid may serve as a light screen (BeGora and Fahselt 2001; McEvoy et al. 2007a, 2007b), while atranorin reflects light (Solhaug et al. 2010) to allow effective algal photosynthesis in shaded environments, allowing the species to be more tolerant of variable light conditions. The ability for frequent growth, high biomass, and high sexual output indicates that this species is highly competitive. Additionally, the GLM for this species suggests that apothecia production may be best correlated with the absence of the most frequent species in the community, *C. arbuscula*, and its own percent cover or by increased crustose lichen presence. *Cladonia arbuscula* is a fruticose lichen that cannot be overgrown easily by a foliose lichen like *A. centrifuga*, but can be indicative of a diminishing rock outcrop because *C. arbuscula* is a terricolous lichen, requiring the accumulation of soil.

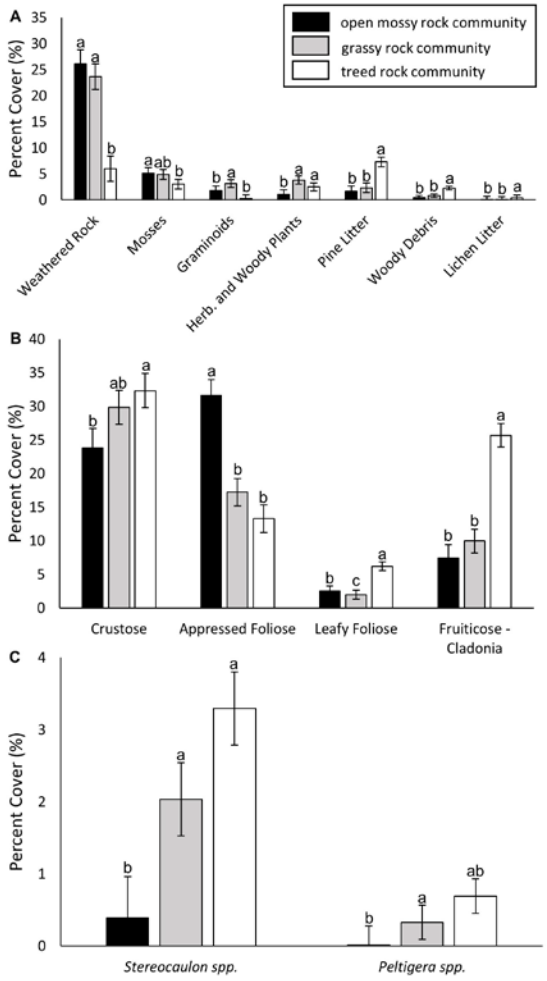

Figure 2.4. Comparison of the average percent ground cover and standard deviation for environmental variables (A), lichen growth forms (B), and nitrogen-fixing species (C) between the three lichen assemblages, as determined by the Kruskal-Wallis test. Different lowercase numbers indicate significant difference between assemblages, as determined by the Steel-Dwass rank test ($p < 0.05$).

2.5.2 *Communities and species diversity*

The environment, including substrata and climate, plays a role in the species distribution (Culberson and Culberson 1967; Rizzi and Giordani 2013), which was also thought to influence species composition and richness on rock outcrops (Toni and Piercey-Normore 2013). The low macrolichen richness in the open mossy rock community is consistent with other studies of saxicolous lichens (Bates 1975; Nash 1972; Ritchie 1956; Toni and Piercey-Normore 2013). The higher species richness in the treed rock community was consistent with that of other studies in sheltered forests (Clayden and Bouchard 1983; Morneau and Payette 1989). Differences between the open mossy community and the treed rock community, as indicated by vegetation and lichen species diversity, could be indicative of the communities representing distinct stages in ecological succession in the boreal forest.

Table 2.5. Comparison of GLMs for best predicting the increasing sexual fecundity (as represented by apothecia production) for each study species, showing the number of samples (n), number of parameters in the model (+2) (k), sum of squares error (SSE), Akaike's information criteria correction (AIC_c), measure of model relative to best model (Δ_i), and the probability of each model (weight). The interaction term is denoted by "x" between the two interacting variables.

	n	k	R^2	SSE	AIC_c	Δ_i	$weight_i$
Arctoparmelia centrifuga model							
Percent cover (*A. centrifuga*) (+) x Percent cover (*C. arbuscula*) (-)	26	4	0.44	3.07	-45.6	0.0	0.4128
Percent cover (*A. centrifuga*) (+) x Crustose lichens (+)	26	4	0.407	3.256	-44.1	1.5	0.1921
Xanthoparmelia cumberlandia model							
Crustose lichens (-)	73	3	0.03	21.627	-82.458	0.0	0.167
Percent cover (*X. cumberlandia*) (+)	73	3	0.023	21.777	-81.953	0.5	0.130
Percent Cover (*X. cumberlandia*) (+) + Crustose lichens (-)	73	4	0.048	21.222	-81.597	0.9	0.109
Weather Rock (+)	73	3	0.018	21.884	-81.595	0.9	0.108
Percent Cover (*X. cumberlandia*) (+) + Fruiticose *Cladonia* lichens (+)	73	4	0.048	21.226	-81.584	0.9	0.108
Mosses + Crustose lichens (-)	73	4	0.041	21.381	-81.053	1.4	0.083
Fruiticose *Cladonia* lichens (+)	73	3	0.009	22.081	-80.941	1.5	0.078
Crustose lichens (-) x Fruiticose *Cladonia* lichens (+)	73	4	0.039	21.43	-80.886	1.6	0.076
Mosses (+)	73	3	0.004	22.189	-80.585	1.9	0.065
Xanthoparmelia viriduloumbrina model							
Percent cover (*X. viriduloumbrina*) (+) x Weathered rock (-)	62	4	0.129	8.928	-114.409	0.0	0.347
Percent cover (*X. viriduloumbrina*) (+) x Crustose lichen (+)	62	4	0.128	8.94	-114.324	0.1	0.333
Percent cover (*X. viriduloumbrina*) (+)	62	3	0.07	9.531	-112.574	1.8	0.137

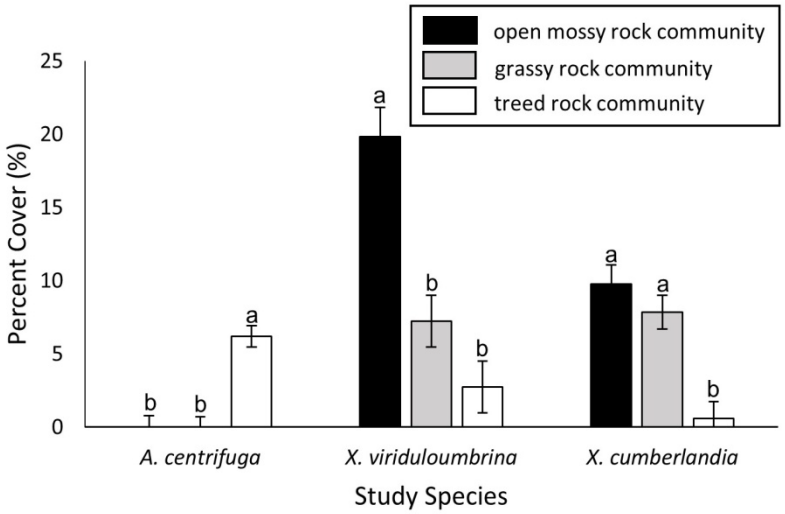

Figure 2.5. Comparison of the percent ground cover for *Arctoparmelia centrifuga*, *Xanthoparmelia viriduloumbrina* and *X. cumberlandia* among three communities, as determined by the Kruskal-Wallis test. Different lowercase numbers indicate significant differences between communities using the Steel-Dwass rank test ($p < 0.05$).

In conclusion, this study showed that saxicolous lichens were categorized into distinct species assemblages that correspond with biotic variables, forming communities. More significantly, the three saxicolous lichens belonging to the genera *Xanthoparmelia* and *Arctoparmelia* have been shown to exhibit ruderal (*X. cumberlandia*), stress-tolerant (*X. viriduloumbrina*), and competitive (*A. centrifuga*) life history strategies despite similarities in growth form, thallus, and spore morphology. Application of life history strategies to lichens was primarily based on habitat characters in this study; however, growth as percent cover and fecundity as the number of apothecia per thallus were consistent with the proposed strategies based on previous literature. Further studies that include preference for substratum may reveal additional features characterizing lichen strategies. A greater understanding of lichen fecundity and life history strategies may provide better insights into ecological patterns in lichens.

2.6 Acknowledgments

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CHAPTER 3

A potential trade-off with stictic acid improves ascospore viability in *Xanthoparmelia cumberlandia*

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3.1. Abstract

Saxicolous lichens are slow growing symbiotic associations that colonize exposed bedrock outcrops that many plants can have difficulty surviving and still face other pressures such as desiccation, excess light, and herbivory. The goal of this paper was to explore trade-offs between fecundity and carbon based secondary metabolite (CBSM) production within the thallus of three species of Rock-shield and Ring lichens, *Arctoparmelia centrifuga*, *Xanthoparmelia viriduloumbrina* and *X. cumberlandia*. Fecundity was measured by numbers of apothecia per thallus, number of spores per apothecium and percent spore germination, and it was compared with the quantity of the major CBSMs produced by the thallus. Results showed negative relationships between stictic acid and all three measures of fecundity for *X. cumberlandia*, which are consistent with trade-offs. When thallus and apothecium quantities of CBSMs were compared, stictic acid was the only CBSM with levels in the apothecium that were significantly higher than those in the thallus supporting a function that may protect ascospores against herbivory. Other CBSMs are hypothesized to have functions associated with the thallus and UV protection of green algae.

3.2. Introduction

Plants and lichens use energy and resources to address physiological demands in response to environmental change. If the resources are limited, a trade-off, or reallocation of resources to one demand at the expense of another, may result in a negative correlation (Rhoades 1979). Trade-offs may occur between the production of carbon based secondary metabolites (CBSM) and primary biological processes such as growth and reproduction (Koricheva 2002b). However, results from some studies have been mixed, sometimes showing negative or positive correlations between phenotypic features, and sometimes showing no correlations between features (Cornelissen et al. 2009; Read et al. 2009). The complex nature of tradeoffs may be explained if plants allocate resources to more than one defensive trait simultaneously, for example, if multiple environmental stressors are acting on the lichen (e.g., protection from herbivory in reproductive structures and UV light in photosynthetic tissue (Agrawal and Fishbein 2006; Read et al. 2009). In addition, if a defensive trait is inducible or highly specific, mixed results might be expected. When allocation of CBSM occurs between different lichen structures, such as the cortex and the medulla of a thallus (Elix and Stocker-Wörgötter 2008), or the soredia and the thallus of *Lobaria scrobiculata* (Asplund et al. 2010), they may have different functions in these structures, which supports the Optimal Defense Theory (ODT, Hyvärinen et al. 2000).

The ODT is based on assumptions that plant defenses (e.g., secondary metabolites) are beneficial by enhancing fitness when herbivores are present, but they may also be costly by allocating resources to the tissue under attack. The allocation of resources is proportional to the value of the tissue in terms of the fitness of the plant (Rhoades 1979). For example, the secondary metabolite meta-scrobiculin was present only in soredia of the lichen, *Lobaria scrobiculata*, and was found to have antiherbivory

properties (Asplund et al. 2010), supporting the ODT. Other studies have examined herbivory in lichens in relation to secondary compounds (Asplund 2011; Gauslaa 2005; Pöykkö et al. 2005). It is reasonable to assume that other biotic or abiotic stressors, such as mineral toxicity or excess UV light and temperature, may also induce allocation of resources to defense reactions if the stressors are severe enough. Secondary metabolites also have protective roles such as anti-microbial activities, allelopathic and hydrophobic properties, and others (reviewed in Huneck 1999). While the presence of usnic acid in apothecia (Culberson et al. 1993; Liao et al. 2010) suggests it might protect developing ascospores from UV light damage, little is known about the relationship between CBSM quantities in apothecia and spore viability, which would relate directly to mycobiont fecundity. If protection from excess biotic or abiotic changes is important for survival of the lichen, a trade-off may exist between production of secondary metabolites and fecundity.

The lichens, *Arctoparmelia centrifuga*, *Xanthoparmelia viriduloumbrina*, and *X. cumberlandia* inhabit rock outcrops on the Precambrian Shield in North America, along with communities of other species (Deduke et al. 2014 [Chapter 2]). They produce abundant apothecia and they produce CBSM that are specific and are almost always present in the thallus (except see Clayden 1992 for *A. centrifuga*). The abundance of apothecia and the consistent production of CBSM in these species allow a quantitative comparison to be made for measures of fecundity and CBSM quantities, in species where size may (Pringle et al. 2003) or may not (Jackson et al. 2006) be related to fecundity. The major CBSM produced by *A. centrifuga* are alectoronic acid, atranorin and usnic acid; *X. cumberlandia* produces stictic, norstictic and usnic acids; and *X. viriduloumbrina* produces usnic, consalazinic and salazinic acids. Usnic acid and atranorin are thought to

have light screening properties (Armaleo et al. 2008; BeGora and Fahselt 2001; McEvoy et al. 2006a; Solhaug et al. 2009, 2010), the stictic acid complex is thought to have antiherbivory properties (Asplund et al. 2009; Gauslaa 2005; Lawrey 1980a) and the salazinic acid complex is thought to have antioxidant properties (Gaikwad et al. 2012). These three species of lichens colonize the rock surface and overlap in their distributions (Brodo et al. 2001; Hale 1990; Lendemer 2005; Thomson 1984), exhibiting different life history strategies (Deduke et al. 2014 [Chapter 2]). *Xanthoparmelia cumberlandia* was classified as a ruderal colonizer of a wide range of habitats, *X. viriduloumbrina* as stress tolerant inhabiting exposed habitats and *A. centrifuga* as a competitive species. While evidence supported these life history strategies (Deduke et al. 2014 [Chapter 2]), and fecundity in plants and vegetative reproduction in lichens is known to be at the expense of vegetative growth (Álvarez-Cansino et al. 2010; Gauslaa 2006; Gerber 1990), the evidence for a correlation between growth and sexual reproduction in lichens remains controversial (Hestmark et al. 2004b; Jackson et al. 2006; Pringle et al. 2003).

If the function of the CBSM in the apothecia is protection from light, then the CBSM quantity in the apothecia should increase as spore viability increases, but thallus CBSM will not change. However, if the CBSMs are using resources that would otherwise be used for spore production, then CSBM (in both thallus and apothecia) and spore viability will have a negative correlation. The goal of this paper was to explore whether a trade-off exists between fecundity and CBSM within the thallus of three lichens. This was accomplished by: 1) examining correlations among measures of fecundity; and 2) examining correlations between fecundity and CBSM.

3.3. Materials and Methods

3.3.1. *Study design and sampling*

Thallus specimens of *Arctoparmelia centrifuga*, *Xanthoparmelia viriduloumbrina* and *X. cumberlandia* were collected from Manitoba and Ontario during 2010 and 2011 as in Deduke et al. (2014 [Chapter 2]). Briefly, four locations on the Precambrian Shield were selected in Manitoba and Ontario, Canada (near Flin Flon, Nopiming Provincial Park, White Shell Provincial Park, Manitoba; and the Kenora-Dryden area of Northern Ontario). The sites were uniform and were chosen based on the presence of a Jack pine ridge with sparse underbrush. The ridge was large enough for the 40 m transect that was laid along the longest exposure and each transect was at least 0.5 km apart. A 1 x 1 m quadrat was placed on every 10 m interval (0 m, 10 m, 20 m, 30 m and 40 m) with the closest *Xanthoparmelia* or *Arctoparmelia* thallus in the upper left corner of the quadrat. Collections of specimens of the three species were made from each of five quadrats for each of 39 transects. Thallus samples including apothecia were returned to the lab for culturing, identifications and thin layer chromatography. Lichens were identified using keys from Brodo et al. (2001), Hale (1990) and Hale (1986).

3.3.2. *Culturing and fecundity measures*

Numbers of apothecia on each thallus were counted in the field. Apothecia classified as mature were counted and defined according to Bellemère and Letroiut-Galinou (2000). The fungal symbiont was cultured using the spore rain method developed by Kofler (1970) and modified by suspending the apothecium upside down on the underside of the upper lid in two

equidistant positions and allowing the spores to be released and germinate on the solid media, rather than attempting to manipulate the spores using a brush or liquid suspension. The solid media used was malt yeast agar (0.5 g agar; 0.5 g malt; 0.05 g yeast and 25 mL water per plate; Sigma, Stocker-Wörgötter 2002). Numbers of ascospores were counted every week beginning seven days after the plates were prepared using a dissecting microscope (Leica MZ6) with a 10 x 10 grid placed in the eyepiece. All spores within one row of 10 squares on the grid were counted and placed among the densest cluster of ascospores on the agar plate. Each week the count was repeated, in potentially a different location on the agar but always within the densest area of spores. Maximum values for number of ascospores and number of spores germinated were recorded within the first five weeks because over time as spores were released, their numbers increased and then as the spores began to germinate the numbers decreased as culture biomass increased and overgrew the densest areas of ascospore release. The maximum number of spores released represents the total reproductive output of a thallus. Ascospore viability was calculated by dividing the maximum number of ascospores germinated by the maximum number of ascospores produced in a given time period.

3.3.3. *Thin layer chromatography*

Digitally Enhanced-Thin Layer Chromatography (DE-TLC) was performed as described by Manthorpe and Lockley (2013), Hess (2007) and in Deduke et al. (2012) following the TLC procedures of Orange et al. (2001) in solvent A (Toluene 180 mL; Dioxane 45 mL; Glacial Acetic Acid 5 mL) modified from Culberson (1972). Twenty spots of acetone were used for each sample at the origin on the TLC plate, for a total of 46 mL of acetone extract (Deduke et al. 2012) per sample. The surface area and intensity of identified metabolites was measured using

Digimizer (Version 4.0.0. MedCalc Software, Ostend, Belgium, 2005–2014), which provided a relative comparison of quantity in pixels between samples. TLC plates were compared with the same standards usnic acid (Sigma-Aldrich, Oakville, Ontario, Canada) and known Rf classes. Thallus samples were weighed to 5 mg. A subset of thalli from each species that had mature apothecia was selected to compare the CBSM quantities between apothecia and thalline samples. There were 13 samples of *Arctoparmelia centrifuga*, six samples of *Xanthoparmelia viriduloumbrina*, and 33 samples of *X. cumberlandia* for the apothecial comparison. DE-TLC was repeated on each mature apothecium. Apothecia were weighed and results were corrected to the 5 mg standard used for thallus samples. Consistency between TLC plate measurements was examined by comparing the significance between 10 (23 mL), 20 (46 mL) and 30 (69 mL) quantified spots of *X. cumberlandia* acetone extract containing stictic acid to test the reliability of Digimizer. Three plates were used, each with three replicates of 10 (23 mL), 20 (46 mL) and 30 (69 mL) spots for a total of nine spots for each plate. Spot quantities were compared within and between plates to determine accuracy. Correlation between the volume of extract and number of pixels was done for all CBSM. Reliability of TLC quantification was tested and found to be consistent when larger volumes were used (46 and 69 mL). Intra-plate comparisons between the three plates showed that DE-TLC pixel values representing quantity are significantly correlated with one another ($r = 0.967$, d.f. = 9, $p < 0.0001$; $r = 0.981$, d.f. = 9, $p < 0.0001$; and $r = 0.970$, d.f. = 9, $p < 0.0001$ for plates one through three , respectively). Kruskal-Wallis analysis showed significance differences between spot quantities (H =7.2, d.f. = 2, $p = 0.0273$; $H = 7.2$, d.f. = 2, p =0.0273; and $H = 7.2$, d.f. = 2, $p = 0.0273$ for plates one through three, respectively). Comparisons between all the plates across all spots was also a significant correlation ($r = 0.853$, d.f. = 27, $p < 0.0001$) and Kruskal-Wallis results for the difference between spot quantities (H =

21.009, d.f. = 2, $p < 0.0001$), showing that this method is reliable. In addition the correlation between the volume of extract and number of pixels was significant for all CBSM (*A. centrifuga*: atranorin, r = 0.976, d.f. = 9, p < 0.0001; alectoronic acid, r = 0.992, d.f. = 9, p < 0.0001; *X. cumberlandia*: norstictic acid, r = 0.884, d.f. = 9, p = 0.0016; usnic acid, r = 0.962, d.f. = 9, p < 0.0001; *X. viriduloumbrina*: consalazinic acid, r = 0.964, d.f. = 9, p < 0.0001; r = 0.982, d.f. = 9, p < 0.0001; usnic acid, r = 0.94, d.f. = 9, p < 0.0001).

3.3.4. *Data analysis*

Data were transformed using a logarithmic transformation (x+1) to reduce the skewedness of the distribution and tested for normality (Zar 2010). Data were analysed using JMP (Version 10.0.2, 64 bit edition, SAS Institute Inc., Cary, North Carolina, 2014). Kruskal-Wallis was used to compare means in fecundity measures (number of apothecia, number of ascospores released, number of ascospores germinated, and spore viability) and quantity of secondary metabolites among i) species, and ii) between thallus and apothecia. Steel-Dwass tests (Day and Quinn 1989) were then used to determine significant differences among the pairs of means. Regression was used to examine relationships among measures of sexual fecundity as the dependent variables and CBSM quantities as the independent variables. A Bonferonni correction was included to indicate significant relationships corrected for multiple comparisons. Kruskal-Wallis and correlation tests were also used on spot tests to determine the accuracy of Digimizer for the DE-TLC results.

3.4 Results

3.4.1. *Relationships among measures of fecundity*

Xanthoparmelia viriduloumbrina and *X. cumberlandia* showed positive relationships between the number of apothecia produced per thallus and the number of ascospores released per apothecium (Table 3.1). *Xanthoparmelia cumberlandia* was the only species of the three studied that showed a significant relationship between the number of apothecia produced by the thallus and the maximum number of ascospores germinated per apothecium. While none of the species showed a relationship between spore viability and number of apothecia, all species showed positive relationships between number of ascospores released and number of ascospores germinated.

Arctoparmelia centrifuga produced a significantly higher number of germinated ascospores ($H = 10.274$, d.f. = 2, 157, $p = 0.0059$) compared to either species of *Xanthoparmelia* (Fig. 3.1). However, *X. cumberlandia* showed greater spore viability than either *A. centrifuga* or *X. viriduloumbrina* ($H = 12.218$, d.f. = 2, 157, $p = 0.0022$, Fig. 3.1).

Table 3.1. Relationship between fecundity measures showing results of pairwise regression between numbers of apothecia per thallus, maximum number of ascospores released per apothecium, maximum number of ascospores germinated and ascospore viability (percent). Significance is determined at $p < 0.05$ and indicated by an asterisk and a Bonferroni correction of $p < 0.004$ (12/0.5) is indicated by two asterisks.

Maximum no. ascospores released/apothecium				
No. apothecia ×	*F*	d. f.	*p*	R^2
A. centrifuga	0.3301	28	0.5705	0.012
X. viriduloumbrina	4.1541	61	0.046*	0.066
X. cumberlandia	11.4284	68	0.0012*/**	0.148
Maximum no. ascospores germinated				
No. apothecia ×	*F*	d. f.	*p*	R^2
A. centrifuga	0.0675	28	0.7971	0.003
X. viriduloumbrina	3.1547	61	0.0809	0.051
X. cumberlandia	16.3882	68	0.0001*/**	0.199
Ascospore viability				
No. apothecia ×	*F*	d. f.	*p*	R^2
A. centrifuga	0.24	28	0.6283	0.009
X. viriduloumbrina	3.8207	61	0.0554	0.061
X. cumberlandia	1.9208	68	0.1704	0.028
Maximum no. ascospores released/apothecium				
Maximum no. ascospores germinated ×	*F*	d. f.	*p*	R^2
A. centrifuga	145.7223	28	<0.0001*/**	0.849
X. viriduloumbrina	240.9543	61	<0.0001*/**	0.803
X. cumberlandia	195.4953	68	<0.0001*/**	0.748

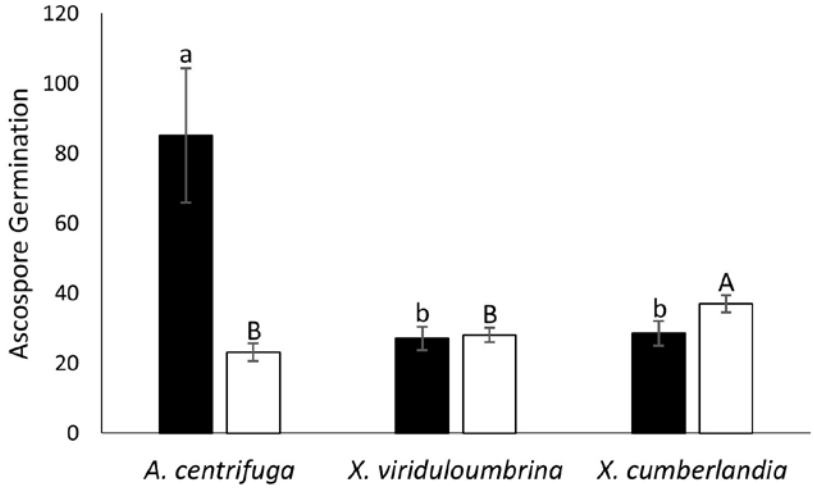

Figure 3.6. Comparison of ascospore germination for three lichens, showing the maximum number of ascospores germinated per apothecium (number of spores; black bars), and the proportion of ascospores germinated per apothecium (percent; white bars). Maximum number of ascospores germinated and proportion of ascospores germinated are the dependent variables. Lichen species are the independent variables. Different lower case letters indicate significant differences among number of ascospores between species, and different upper case letters indicate significant differences among percent germination between species at $p = 0.05$.

3.4.2. *Trade-off between sexual fecundity and secondary metabolites*

Trade-offs were assumed when a negative relationship occurred between the CBSM in the thallus and a measure of fecundity. One such trade-off was found for *Arctoparmelia centrifuga*, where a negative relationship was found between maximum spore production per apothecium and the quantity of atranorin produced by the thallus (Table 3.2). Three significant negative relationships were also found in *Xanthoparmelia cumberlandia* for each of apothecia production, maximum number of ascospores released, and maximum number of ascospores germinated with the quantity of stictic acid. All other comparisons showed no significant relationships (Table 3.2). When the Bonferroni correction was applied to the multiple comparisons all except one comparison was significantly different.

The quantity of secondary metabolites was compared between the apothecium and the thallus within each species. In all cases except stictic acid in *Xanthoparmelia cumberlandia*, the quantity of compound in the thallus was greater than (or equal to) that in the apothecium. *Arctoparmelia centrifuga* showed significantly higher amounts of all three types of compounds in thallus tissue than in apothecia (Fig. 3.2A). *Xanthoparmelia viriduloumbrina* showed significantly higher levels of usnic acid and consalazinic acid in thallus tissue than in apothecia (Fig. 3.2B), but salazinic acid showed no difference between the two tissue types. *Xanthoparmelia cumberlandia* showed a significantly higher amount of stictic acid in the apothecium than the thallus but there was no difference in the other two metabolites (Fig. 3.2C).

Table 3.2. Relationship between fecundity indicators (number of apothecia, number of spores, and percent germination; dependent variables) and the thallus secondary metabolite for each lichen species (independent variables), showing *F* statistic, d.f., and *p* value. Significance was determined at $p < 0.05$ and indicated by an asterik. The negative symbol (-) beside the *p* value indicates that the relationship is negative. Bonferonni correction of $p < 0.0018$ (27/0.05) is indicated by two asterisks.

A. centrifuga	Usnic Acid			Atranorin			Alectoronic Acid		
Fecundity	*F*	d. f.	*p*	*F*	d. f.	*p*	*F*	d. f.	*p*
No. apothecia	0.0288	28	0.8667	0.5974	28	(-) 0.4465	0.6965	28	0.4116
No. ascospores (Maximum)	0.1798	28	0.6571	5.3673	28	(-) 0.0287*	0.9897	28	(-) 0.3290
Germination (%) (Maximum)	0.7043	28	0.4090	2.3215	28	0.1397	-0.1695	28	(-) 0.6840

X. viriduloumbrina	Usnic Acid			Consalazinic Acid			Salazinic Acid		
Fecundity	*F*	d. f.	*p*	*F*	d. f.	*p*	*F*	d. f.	*p*
No. apothecia	2.4705	61	0.1214	0.0022	61	0.9626	0.1038	61	(-) 0.7485
No. ascospores (Maximum)	1.1765	61	0.2825	0.0018	61	0.9659	0.1873	61	(-) 0.6668
Germination (%) (Maximum)	0.2686	61	0.0606	0.9721	61	(-) 0.3282	0.014	61	0.9062

X. cumberlandia	Usnic Acid			Norstictic Acid			Stictic Acid		
Fecundity	*F*	d. f.	*p*	*F*	d. f.	*p*	*F*	d. f.	*p*
No. apothecia	0.6654	68	(-) 0.4176	0.0233	68	0.8791	5.2081	68	(-) 0.0257*
No. ascospores (Maximum)	0.5772	68	(-) 0.4501	0.3915	68	(-) 0.5337	5.6352	68	(-) 0.0205*
Germination (%) (Maximum)	1.4743	68	(-) 0.2290	0.2402	68	(-) 0.6257	10.5905	68	(-) 0.0018* /**

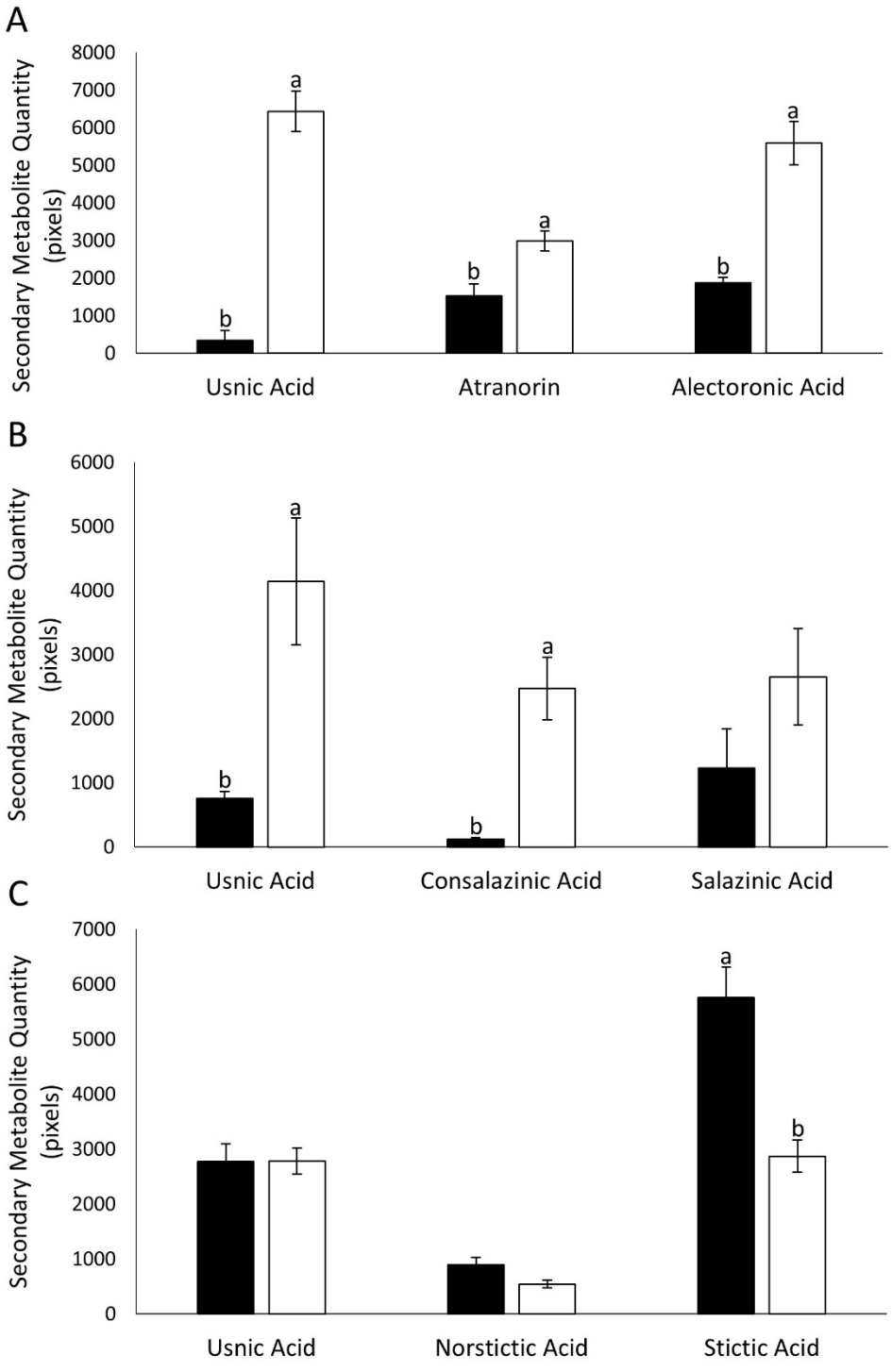

Figure 3.2. Comparison of secondary metabolite quantities between apothecia (black bars) and thallus tissues (white bars). **A.** *Arctoparmelia centrifuga*. **B.** *Xanthoparmelia viriduloumbrina*. **C.** *Xanthoparmelia cumberlandia*. Different lower case letters indicate significant differences between tissues at $p = 0.05$.

3.5. Discussion

3.5.1. *Trade-off between secondary metabolites and sexual fecundity*

The strong negative relationship between stictic acid and three indicators of fecundity for *Xanthoparmelia cumberlandia* suggests that sexual reproduction may occur at the expense of the production of stictic acid. Stictic acid is a medullary compound, which is known to have an anti-herbivory function (Asplund 2011; Gauslaa 2005; Lawrey 1980a). If the lichen shows low stictic acid production because it is producing greater numbers of apothecia and ascospores, it may have a reduced level of protection against herbivores. Since stictic acid is hypothesized to be a defensive compound (Asplund et al. 2009), the reduction in stictic acid in this study may be a response to low herbivory stresses resulting in carbon allocation to growth of reproductive structures and spore production. Another potential trade-off is inferred between atranorin and number of spores. The significant increase in atranorin, with the decrease in number of ascospores in *Arctoparmelia centrifuga*, cannot be considered a response to herbivory because atranorin has been shown to lack herbivore deterring properties (Gauslaa 2005; Hesbacher et al. 1996). As a cortical compound, atranorin is thought to reflect visible light, preventing photoinhibition (Solhaug et al. 2010) by the photobiont beneath the cortex (BeGora and Fahselt 2001; Solhaug et al. 2009). Resources may be allocated to atranorin production, reducing the number of ascospores. On the other hand, if the function of a CBSM is to protect developing spores from excess UV light such as usnic acid in the hymenium of an apothecium (Culberson et al. 1993; Liao et al. 2010), the CBSM may contribute to fecundity rather than detract from it.

The negative relationship between sexual fecundity and stictic acid was further explored because thallus levels of CBSM and the numbers of apothecia and ascospores revealed strong relationships. Even though a causal relationship was inferred in order to propose a trade-off between fecundity and CBSM production, they may not necessarily be causal relationships

(Knops et al. 2007). Further study would be needed to confirm the causal relationship. This study showed that all the comparisons between thalline and apothecial secondary metabolites revealed thallus levels of secondary metabolites that were higher than or equal to the apothecial levels, except for one. The single comparison with apothecial levels higher than the thallus levels was stictic acid in *X. cumberlandia*, suggesting that a function of stictic acid may be to protect the fungal sexual structures from herbivory. The stictic acid difference in this study is contrasted with the findings of Hesbacher et al. (1996), where atranorin and alectoronic acid quantities did not differ between thalline tissue with and without apothecia in *Tephromela atra*. Usnic acid was reported to be present near the green algal cells and the ascospores within the apothecia of several *Cladonia species* (Culberson et al. 1993; Liao et al. 2010), supporting a light protection function for usnic acid (Armaleo et al. 2008; McEvoy et al. 2006a). However, the precise location of either stictic acid or usnic acid within the apothecia of the lichens in this study is not known (e.g., epihymenium or apothecial wall) and may reflect species-specific needs or production by certain types of tissue (Marie et al. 2013).

3.5.2. *Exploring possible functions of CBSM*

Higher thalline quantities for usnic acid and norstictic acid were similar to those found in Asplund et al. (2010) for *Lobaria scrobiculata*, where the Optimal Defense Theory (ODT; Rhoades 1979) was supported for meta-scrobiculin in the soredia. The ODT may be a species-specific response, because meta-scrobiculin was present in soredia but not thallus tissue in *L. scrobiculata*. However, similar comparisons were done for *Lobaria pulmonaria* (Asplund et al. 2010), where stictic acid was found to be equally distributed between reproductive and non-reproductive tissues. Unlike *L. scrobiculata*, *Xanthoparmelia cumberlandia* does not contain

meta-scrobiculin, but the compound may be substituted for stictic acid to play the same role in anti-herbivory defense of ascospores in the apothecia.

The five secondary metabolites that were higher in the thallus than the apothecia may play other roles to protect the photobiont. *Arctoparmelia centrifuga* produced high thalline quantities of alectoronic acid, which is thought to have some antimicrobial capabilities (Gollapudi et al. 1994). Higher quantities of usnic acid in the thallus would be expected if protection of the photobiont from excess UV light is a main function (Armaleo et al. 2008; McEvoy et al. 2006a). Consalazinic acid, along with salazinic acid and atranorin, has been shown to possess antioxidant activities (Gaikwad et al. 2012). The greater presence of consalazinic acid in the thallus may help mitigate oxidative stress caused by the photosynthetic activity of green algae in the thallus, which is the first partner to be damaged by oxidative stress (Kong et al. 1999). While the quantity of salazinic acid was not statistically different between thallus and apothecium, it has been shown to be influenced by temperature (Hamada 1982). Similarly, norstictic acid quantities were not statistically different between thallus and apothecia in *X. cumberlandia*. The CBSMs, which are in higher quantities in the thallus or show no statistical difference between thallus and apothecia, may protect the photobiont from light or other abiotic factors (Lawrey 1989).

In conclusion, this study provides evidence for a trade-off in *Xanthoparmelia cumberlandia* between levels of fecundity and the quantity of stictic acid produced by the thallus. However, further study is needed to confirm causality of the relationship. It also suggests that stictic acid in the apothecia provides protection against herbivory for the developing ascospores, resulting in higher levels of viability. The study also provides evidence to suggest that the other secondary metabolites, usnic acid, consalazinic acid, salazinic acid, atranorin,

alectoronic acid and norstictic acid, may play a role in defense against abiotic factors such as UV light, temperature, antioxidants, etc., directed toward the photobiont for these three species of lichens. While the function of some of these metabolites has been shown in other studies, further experimental studies are required to determine their function in these species.

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CHAPTER 4

Comparing element composition of rock substratum with lichen communities and fecundity of *Arctoparmelia* and *Xanthoparmelia* species.

***This chapter will be submitted to an appropriate journal.**

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4.1 Abstract

Substratum plays an important role in saxicolous lichen communities, providing a stable growth surface for long-term attachment and an influence on relative species abundance. Saxicolous lichen communities around *Xanthoparmelia* and *Arctoparmelia* were previously characterized using thallus cover on the substratum and lichen fecundity. However, it was not known if the substratum influenced the lichen fecundity. The objectives of this paper were to compare variation in whole-rock element concentrations among lichen communities, and to examine the correlation between elemental composition and percent cover and fecundity (numbers of apothecia, ascospores, and percent germination) of each of three lichens *Arctoparmelia centrifuga*, *Xanthoparmelia viriduloumbrina* and *X. cumberlandia*. Percent cover, number of apothecia, ascospores, and percent germination were compared with the rock type and 21 elements from 37 transects. Element concentrations varied among communities but the grassy rock community had significantly higher concentrations of elements than the mossy or treed rock communities. These lichens showed genus specific relationships with rock types where *Xanthoparmelia* was more frequent on granitic rocks, while *A. centrifuga* was more frequently

found growing on mafic metavolcanic rocks. Sexual fecundity results were mixed for the three study species, with correlations for the *Arctoparmelia* and *Xanthoparmelia* species and particular elements. This study provides insight into the complexity of the relationship between geological composition of the substratum and saxicolous lichen biology.

4.2 Introduction

Saxicolous (rock inhabiting) crustose and foliose lichens facilitate the weathering (breakdown) of rock surfaces resulting in a release of ions from the substratum (Adamo et al., 1993; Chen et al., 2000; Jones et al., 1981; Lee and Parsons, 1999). As the rock substratum weathers, elements from the substratum may be taken up within the thallus (Clark et al., 2001, 1999), or they may be chelated producing biominerals (Lee and Parsons, 1999; Prieto et al., 1997). Therefore, it is recognized that the element composition may influence lichen species and even communities, resulting in habitats that may be specific to assemblages of species (Bates, 1978; Clayden and Bouchard, 1983; Favero-Longo et al., 2004; Giordani et al., 2002). Species assemblages may be further influenced by metal tolerance, toxicity and pH changes (Ascaso and Galvan, 1976; Pawlik-Skowrońska and Bačkor, 2011; Sarret et al., 1998; Tyler, 1989), or elements that serve as micro and macronutrients (Puckett and Finegan, 1980).

The life history strategies and community types of three species, *Arctoparmelia centrifuga* (L.) Hale, *Xanthoparmelia cumberlandia* (Gyelnik) Hale, and *X. viriduloumbrina* (Gyelnik) Lendemer were previously characterized (Deduke et al., 2014 [Chapter 2]) showing *A. centrifuga* is a competitor in a treed rock community, *X. cumberlandia* is a ruderal which predominated in open mossy and grassy rock communities, and X. *viriduloumbrina* is stress tolerant and predominant in an open mossy rock community on the Precambrian Shield in North

America. The Precambrian Shield is the exposed bedrock that covers a large area of northern North America and is variable in terms of its mineralogy, structure, and element composition. While the life history strategies of the three lichens have been linked with type of community (Deduke et al., 2014 [Chapter 2]), the attachment and weathering by rhizines with the rock substratum suggests that the substratum may also influence the species and their biology. Therefore, the purpose of this paper was to investigate the influence of whole-rock elemental composition on previously defined lichen communities and the biology of *A. centrifuga*, *X. viriduloumbrina* and *X. cumberlandia*. The specific objectives were 1) to compare variation in elemental concentrations in the rock substrata among lichen communities, and 2) to examine the correlation between elemental composition of the rock substrata and percent cover and fecundity (apothecia, ascospores, and percent germination) of each lichen species.

4.3 Methods and Materials

4.3.1 *Sampling and experimental design*

Lichen samples were collected from thirty-nine locations in northern Ontario (N50°11'-49°37', W93°15'-92°09'), southern Manitoba (N50°02'-49°50', W95°36'-95°23'; N51°01'-50°34', W95°44'-95°17'), and northern Manitoba (N55°00'-54°36', W101°34'-101°22') during June to August in 2010 and 2011. The criteria for selecting sites included the presence of Jack pine ridges on the Precambrian Shield containing *Xanthoparmelia* and *Arctoparmelia* thalli, and reasonable accessibility (Deduke et al., 2014 [Chapter 2]). Five 1m x 1m quadrats were located 10m apart, along each of thirty-nine 40m transects. Presence and percent cover were recorded for *A. centrifuga, X. viriduloumbrina* and *X. cumberlandia* in each quadrat. Lichens were identified using Hale (1990, 1986) and Lendemer (2005). The habitats were characterized by open rock

ridges dominated by Jack pine trees and other less dominant trees such as aspen, white birch and black spruce in moist areas. The three lichen communities were present within the broader habitat of the ridges described in detail in Deduke et al. (2014 [Chapter 2]). Briefly, the open mossy rock community (n=10) was characterized by high exposure to light and percent cover of mosses, the grassy rock community (n=15) had less exposure and high percent cover of graminoids, and the treed rock community (n=14) was characterized by high percent cover of pine litter, woody debris and lichen litter which indicated more canopy cover from trees. The open mossy rock and grassy rock communities had higher percent cover of exposed rock than the treed rock community. The grassy rock and treed rock communities had high percent cover of herbaceous and woody plants (Deduke et al., 2014 [Chapter 2]).

The rock substratum underlying the transects was comprised of granitic and mafic metavolcanic rocks typical of granite-greenstone terrane. The northern Ontario sites were composed of Archean aged rocks in the Superior Province, ranging from granite, granodiorite, gneiss, metavolcanic and metasedimentary rocks (Geology Ontario, 2013). Further west, the Superior Province underlies the southern Manitoba sites, with Archean aged granite and gneiss (Manitoba Geology, 2015). Northern Manitoba sites were located within the Flin Flon Belt of the Trans Hudson Orogen. This is a highly variable geological zone underlain by younger Proterozoic aged rocks comprised of mafic and felsic metavolcanic rock, granite and gneiss (Manitoba Geology, 2015).

Two rock samples were collected from each end of the thirty-nine transects by removing a 100 cm^2 portion of the substratum using a hammer and chisel. Homogeneity was determined if both rock samples belonged to the same rock type. Two transects in northern Manitoba had a heterogeneous substratum along the length of the transect and were eliminated from this study.

One sample from each pair of samples, showing the greatest amount of unexposed surface area, was chosen to represent each transect. Although rock samples could only represent the elemental composition from the 100 cm^2 portion removed from the bedrock, homogeneity of the rock along the length of the transect was inferred. Rock samples were collected between June and July 2012. Samples were dried, fragmented, and an unexposed portion of the rock sample was sent to the Activation Labs (Thunder Bay, Ontario) for AR-ICP (Aqua Regia – Inductively Coupled Plasma) analysis to measure 37 elements. The standard procedure at the Activation Labs was followed where samples were weighed to 0.5 g, crushed, homogenized and digested in heated aqua regia (AR; Actlabs, 2014). After digestion, samples were diluted with deionized water and analyzed using an Inductively Coupled Plasma (ICP) method (Actlabs, 2014). After receiving the results of the analysis, 16 elements were determined to be at or below the detection limits of the AR-ICP method according to the values presented in the quality control results and were excluded from analyses (aluminum, antimony, arsenic, barium, beryllium, bismuth, boron, cadmium, gallium, mercury, molybdenum, thallium, titanium, tungsten, uranium, and zirconium). The 21 elements that were included in the analyses for this study were silver (Ag; ppm); calcium (Ca; %); cobalt (Co; ppm); chromium (Cr; ppm); copper (Cu; ppm); iron (Fe; %); potassium (K; %); lanthanum (La; ppm); magnesium (Mg; %); manganese (Mn; ppm); sodium (Na; %); nickel (Ni; ppm); phosphorus (P; %); lead (Pb; ppm); sulphur (S; %); scandium (Sc; ppm); strontium (Sr; ppm); tellurium (Te; ppm); vanadium (V; ppm); yttrium (Y; ppm); and zinc (Zn; ppm). These elements were chosen because they met quality control standards and produced concentrations above detection limits of the AR-ICP analysis.

Rock samples were classified into three broad categories based on mineralogy, texture and structure, as granitic (igneous; n=20), mafic metavolcanic (metamorphic; n=14) and

metasedimentary (sedimentary; n=3) rock (Klein and Dutrow, 2007). Granitic rocks have mineral grain sizes typically in excess of 3 mm, dominant colours are white and pink, and contain primarily feldspar and quartz, as well as small amounts of biotite, amphibole, and other minerals. Mafic metavolcanic rocks have fine grain mineral sizes less than 1 mm and are dark in color. They are composed primarily of metamorphosed basalt and contain variable amounts of plagioclase and amphibole. Metasedimentary rocks also contain fine-grained minerals (average between 1 and 3 mm) and were dark in color. The metasedimentary rocks have a greater proportion of micaceous minerals (biotite and muscovite) and are more foliated (layered) compared with the granite and the mafic metavolcanic rocks.

4.3.2 *Measurements of fecundity*

Sexual fecundity was measured for *A. centrifuga*, *X. viriduloumbrina* and *X. cumberlandia* using number of apothecia, ascospores and percent germination from Deduke et al. (2014 [Chapter 2]) and Deduke and Piercey-Normore (2014 [Chapter 3]). Some of the fecundity data from Deduke et al. (2014 [Chapter 2]) were examined by addressing new questions in this study on element composition of the substratum as a separate variable from lichen communities. The relationship between fecundity and element composition has never been explored. Briefly, the number of apothecia per thallus was counted for each of the 195 quadrats and the number of ascospores released from each apothecium under controlled lab conditions was estimated (Deduke et al., 2014 [Chapter 2]). The thalli used in the lab experiments included 26 for *A. centrifuga*, 56 for *X. viriduloumbrina* and 65 for *X. cumberlandia.* Maturity of the apothecia was estimated according to Bellemère and Letroiut-Galinou (2000), and the numbers of apothecia counted and spores released were limited to mature apothecia to avoid variation from over or

under mature apothecia. For spore release, a total of 294 apothecia were rehydrated, washed and affixed to petri-plate covers using a procedure modified from Kofler (1970). The modification was that two apothecia were suspended upside down on the underside of the upper lid allowing the spores to be released and germinate on the solid media, rather than physical separation of the spores using a brush or liquid suspension. Petri-plates contained malt-yeast agar (0.5 g agar; 0.5 g malt; 0.05 g yeast in 25 mL water per plate; Sigma) (Stocker-Wörgötter, 2002). As ascospores were released from the apothecia onto the medium, they were counted weekly over a five week period using a dissecting microscope (Leica MZ 6) set to 40x magnification as in Deduke et al. (2014). A 10 x 10 grid was placed in the right eyepiece and a 1 x 10 grid was counted twice. The first count included the number of spores found within the 1 x 10 grid. The second count recorded the number of spores from the first count that had germinated, which was indicated by the presence of at least one germ tube (Deduke and Piercey-Normore, 2014 [Chapter 3]; Deduke et al., 2014 [Chapter 2]). The number of ascospores and the number of germinated ascospores used in the analyses was the maximum number recorded over the five week period of culturing. This value represents the largest number of spores to be released from an apothecium. Percent germination values were determined by dividing the maximum number of germinated ascospores from the maximum number of ascospores counted.

4.3.3 *Data analysis*

The characterization of lichen communities was based on a previous study that produced a cluster analysis separating transects to represent lichen assemblages (Deduke et al., 2014 [Chapter 2]; McCune and Grace, 2002; Peck, 2010). The three resulting lichen assemblages were then characterized as communities using additional environmental variables. The communities

were classified as open mossy rock (low species richness), grassy rock (moderate species richness) and treed rock (high species richness) communities (Deduke et al., 2014 [Chapter 2]). The effect of the communities on the study species was examined in the previous study (Deduke et al., 2014 [Chapter 2]) and integrated into this manuscript where appropriate. Life history strategies of the three study species were characterized in the same study using communities along with fecundity indicators. Fecundity was indicated by the number of mature apothecia and the number of ascospores released from the apothecia. The life history strategies were ruderal for *X. cumberlandia*, stress tolerant for *X. viriduloumbrina* and competitive for *A. centrifuga*.

Element concentration, study species percent cover and sexual fecundity data were transformed using a log transformation (x+1) and tested for normality (Zar, 2010). Multivariate non-metric multidimensional scaling (NMS) was used to identify trends in elemental composition and visualize the relationships between lichen communities and rock types. The NMS matrix included the element composition within the transects, which were coded with the nominal variables, community and rock type. NMS ordinations were calculated using PC-ORD (version 6.08; McCune and Mefford, 2011). NMS ordinations were chosen because they assume only monotonicity in data, they do not assume distribution shapes in data and they do not distort data like other free ordinations (McCune and Grace 2002; Peck, 2010). All NMS ordinations were tested to determine the ideal number of axes using a minimum stress test and a Monte Carlo randomization to ensure significance, and ordinations were checked for monotonicity. Univariate tests were performed with JMP 10.0.2 (SAS Institute, Cary, NC). Two-way ANOVA tests were used to determine interactions between rock type and species with fecundity variables as response variables. Kruskal-Wallis and Steel-Dwass tests were used to compare the means between the pair-wise relationships. Spearman's rank order correlation was used to analyze the

correlation between elemental concentrations, percent cover, and sexual fecundity for *A. centrifuga*, *X. viriduloumbrina* and *X. cumberlandia*. Since some analyses required multiple comparisons, a Bonferroni correction was included to show a corrected alpha level for the 21 elements analyzed. This corrected alpha level ($p \leq 0.002$) has been included where necessary. Correlations with substrate chemistry were made in this study with limitations imposed by the availability of rock type within the studied transects resulting in a heterogeneous sample size between rock types (granitic rock = 20; mafic metavolcanic rock = 14; and metasedimentary rock = 3).

4.4 Results

The NMS ordination for elemental concentrations of 37 transects showed that two dimensions provided the best ordination of the data (Fig. 4.1). The minimum stress value for the ordination was 10.302 and it was significant ($p = 0.004$). The ordination represents 96.1% of the total variance (r^2). The horizontal (primary) axis represents most of the variation ($r^2 = 0.908$) and the vertical (secondary) axis accounts for some of the remaining variation in the data ($r^2 = 0.053$). Joint plots were used to display vectors of the elements showing the direction and strength of the element data in the main matrix. The primary axis represents the overall relationship between element abundance, with the majority of element vectors clustering toward the right side of the ordinations, except Pb, which is more plentiful toward the left end of the primary axis. The NMS ordination showing some overlap in rock type (Fig. 4.1A) is based on the same analysis as Fig. 4.1B, but the elements are compared by rock types rather than communities. The left end of the ordination with lower element quantity is dominated by granitic rock. Mafic metavolcanic and metasedimentary rocks cluster near the right side of the plot where elements are abundant. The

small degree of overlap between granitic and metasedimentary rocks suggest the metasedimentary rocks share more similarities in element abundance with the mafic metavolcanic rocks than with granitic rocks. There was strong overlap between communities (Fig. 4.1B), but the open mossy rock and treed rock communities showed a larger number of sites in the left end of the primary axis with lower concentrations of elements, and the grassy rock community showed a larger number of sites in the right end of the axis with greater concentrations of elements. Proportion of rock types are shown for each community (pie charts in Fig. 4.1B) where the open mossy rock community is composed entirely of granitic rock. The grassy rock and treed rock communities show all three rock classes: granitic, mafic metavolcanic and metasedimentary.

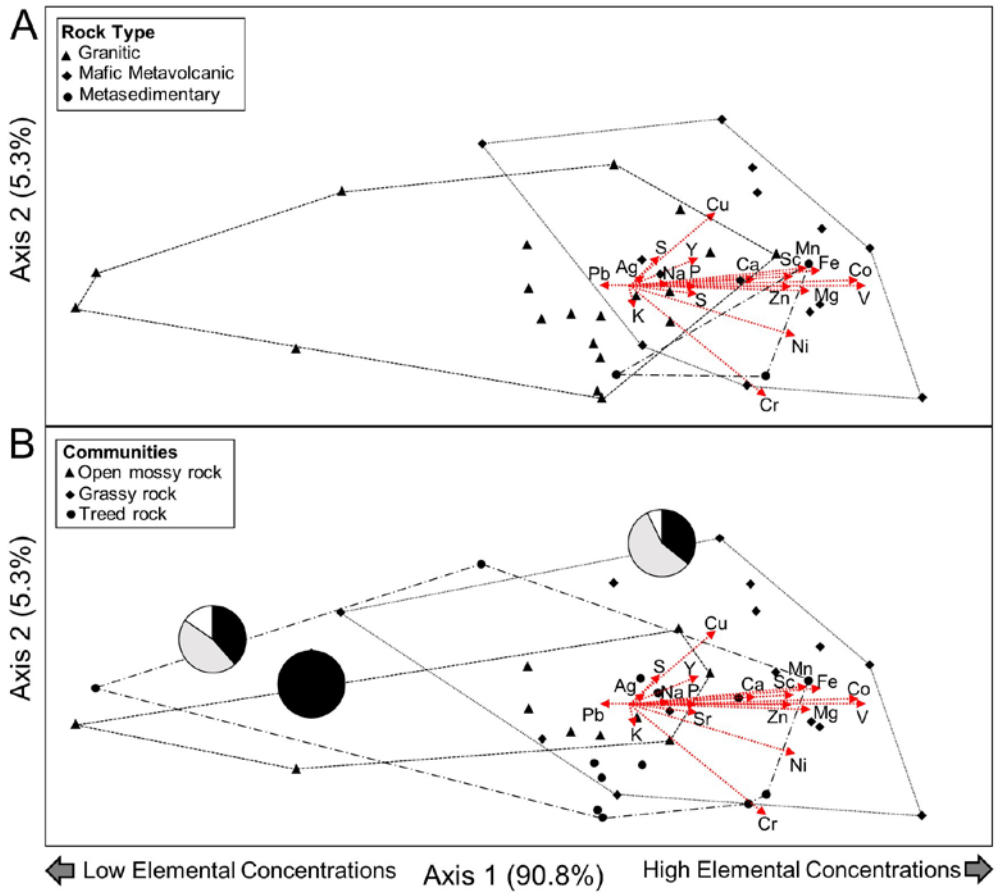

Figure 4.1. NMS ordination showing the element composition among the nominal variables A. rock types and B. lichen communities. Each ordination compared the relationship between element type and quantity and coded for nominal variable. The pie charts in B show the relative distribution of rock types for each of the three communities, black shows Granitic, gray shows Mafic Metavolcanic, and white shows Metasedimentary. Red vector lines represent elemental concentrations relative to coded nominal variables.

A comparison of elements among rock types shows 10 elements with significant differences at a standard alpha of $p = 0.050$, and four of those significant to a corrected alpha of $p = 0.002$ (Table 4.1). A Bonferroni corrected alpha has been included for comparison, but a limitation of using a Bonferroni correction is the potential for underreporting significant results (Moran, 2003). Granitic rock had higher concentrations of La but it also had the lowest concentrations of some other elements. Mafic metavolcanic rock had the highest levels of Ca, Co, Cr, Fe, Mg, Mn, S, Sc and V. Metasedimentary rock had an equally high quantity of Mg to mafic metavolcanic rock (Table 4.1) but the concentrations of other elements were not different from either rock type. A comparison of elements among lichen communities showed only nine elements with significant differences in quantity among the three lichen communities at a significance of $p = 0.050$ (Table 4.2). The grassy rock community showed significantly higher concentrations in six of the nine elements while others were variable (Table 4.2).

Table 4.1. Element concentrations within each of the three rock types reported for this study showing mean and SE (standard error) for each element and n representing the number of samples analysed for each rock type. The asterisk indicates that the Kruskal-Wallis is significant at $p \leq 0.050$ and the lower case letters indicate significant differences using the Steel-Dwass test. A Bonferroni correction was calculated for all 21 elements and set at $p \leq 0.002$ and indicated by two asterisks.

Element (unit, detection limit)	Granitic Rock (Mean ± SE; n=20)	Mafic Metavolcanic Rock (Mean ± SE; n=14)	Metasedimentary Rock (Mean ± SE; n=3)
Ag (ppm, 0.2)	0.22 ± 0.02	0.24 ± 0.02	0.19 ± 0.05
Ca (%, 0.01) *	0.53 ± 0.20 b	1.65 ± 0.24 a	0.25 ± 0.52 ab
Co (ppm, 1)**	7.19 ± 2.27 b	22.42 ± 2.71 a	20.66 ± 5.87 ab
Cr (ppm, 1) *	22.59 ± 30.01 b	97.00 ± 35.87 a	88.00 ± 77.50 ab
Cu (ppm, 1)	16.35 ± 9.51	51.35 ± 11.36	52.00 ± 24.55
Fe (%, 0.01) **	1.94 ± 0.53 b	5.67 ± 0.64 a	4.05 ± 1.38 ab
K (%, 0.01)	0.34 ± 0.08	0.27 ± 0.10	0.60 ± 0.21
La (ppm, 10) *	35.94 ± 7.01 a	12.77 ± 8.38 b	13.66 ± 18.11 ab
Mg (%, 0.01) **	0.58 ± 0.27 b	2.03 ± 0.33 a	1.85 ± 0.71 a
Mn (ppm, 5) *	283.30 ± 70.69 b	716.71 ± 84.49 a	467.33 ± 182.52 ab
Na (%, 0.001)	0.06 ± 0.01	0.11 ± 0.01	0.04 ± 0.41
Ni (ppm, 1)	12.88 ± 10.81	43.78 ± 12.92	32.66 ± 27.92
P (%, 0.001)	0.05 ± 0.01	0.05 ± 0.01	0.03 ± 0.02
Pb (ppm, 2)	4.14 ± 0.54	2.49 ± 0.65	3.32 ± 1.40
S (%, 0.01) *	0.01 ± 0.03 b	0.12 ± 0.03 a	0.01 ± 0.08 ab
Sc (ppm, 1) **	3.49 ± 1.13 b	9.78 ± 1.35 a	14.66 ± 2.92 ab
Sr (ppm, 1)	42.80 ± 10.41	42.35 ± 12.44	11.00 ± 26.88
Te (ppm, 1)	1.74 ± 0.21	1.49 ± 0.25	2.66 ± 0.54
V (ppm, 1) *	44.65 ± 17.02 b	129.21 ± 20.34 a	115.66 ± 43.94 ab
Y (ppm, 1)	8.65 ± 1.59	10.14 ± 1.80	6.33 ± 3.89
Zn (ppm, 2)	7.90 ± 1.26	10.28 ± 1.51	6.00 ± 3.26

Table 4.2. Comparison of elements between lichen communities using a Kruskal-Wallis test with significance at $p \leq 0.05$ and noted with an asterisk. A Bonferroni correction was calculated for all 21 elements and set at $p \leq 0.002$. A significant finding was noted with two asterisks. Inter-community variation was determined using a Steel-Dwass test with a "greater than" symbol representing the community with significantly higher values. Communities are represented by the open mossy rock community (mossy), the grassy rock community (grassy) and the treed rock community (treed). Means and standard error are presented for the 21 elements for all three community types.

Element	*H*	d.f.	*p*	Mossy Rock (n =10)	Grassy Rock (n = 14)	Treed Rock (n = 13)
Ag (ppm) (Mossy > Treed)*	6.20	2	0.045	0.25 ± 0.03	0.25 ± 0.02	0.19 ± 0.02
Ca (%) (Grassy > Mossy & Treed)*	10.96	2	0.004	0.41 ± 0.28	1.68 ± 0.24	0.54 ± 0.25
Co (ppm) (Grassy > Mossy)*	10.77	2	0.005	6.40 ± 3.40	22.29 ± 2.87	11.08 ± 2.98
Cr (ppm)	2.46	2	0.292	22.90 ± 43.08	91.43 ± 36.41	43.56 ± 37.79
Cu (ppm) (Grassy > Mossy & Treed)*	8.69	2	0.013	13.90 ± 13.37	55.29 ± 11.30	22.23 ± 11.72
Fe (%) (Grassy > Mossy & Treed)*	8.98	2	0.011	2.02 ± 0.80	5.54 ± 0.68	2.53 ± 0.70
K (%)	4.86	2	0.088	0.43 ± 0.12	0.23 ± 0.10	0.39 ± 0.10
La (ppm) (Mossy > Grassy & Treed)*	8.88	2	0.012	55.00 ± 8.79	14.35± 7.43	14.46 ± 7.71
Mg (%) (Grassy > Mossy)*	6.82	2	0.033	0.60 ± 0.42	1.89 ± 0.36	1.04 ± 0.37
Mn (ppm)	3.51	2	0.173	304.70 ± 108.56	664.00 ± 91.75	366.08 ± 95.21
Na (%)	0.37	2	0.830	0.07 ± 0.02	0.11 ± 0.02	0.07 ± 0.02
Ni (ppm)	3.85	2	0.146	9.60 ± 15.35	43.57 ± 12.97	20.23 ± 13.46
P (%)	2.26	2	0.324	0.06 ± 0.01	0.07 ± 0.01	0.04 ± 0.01
Pb (ppm)	2.64	2	0.227	4.10 ± 0.77	3.92 ± 0.65	2.46 ± 0.68
S (%) (Grassy > Mossy & Treed)*	10.14	2	0.006	0.01 ± 0.05	0.131 ± 0.04	0.01 ± 0.04
Sc (ppm)	3.06	2	0.217	3.80 ± 1.94	8.21 ± 1.64	7.54 ± 1.70
Sr (ppm)	2.75	2	0.253	20.00 ± 14.33	41.14 ± 12.11	54.31 ± 12.57
Te (ppm)	0.08	2	0.959	1.60 ± 0.31	1.71 ± 0.26	1.84 ± 0.27
V (ppm)*	6.47	2	0.039	35.90 ± 23.78	135.57 ± 20.10	60.92 ± 20.86
Y (ppm)	0.70	2	0.706	11.10 ± 2.10	9.36 ± 1.77	7.08 ± 1.84
Zn (ppm)	1.35	2	0.510	45.60 ± 14.05	77.21 ± 11.88	46.70 ± 12.33

The three species, *Arctoparmelia centrifuga*, *Xanthoparmelia viriduloumbrina* and *X. cumberlandia,* were present on all three rock types but in different proportions (Fig. 4.2). *Arctoparmelia centrifuga* was found most often on mafic metavolcanic rock followed by granitic and metasedimentary rock. The *Xanthoparmelia* species were more frequent on granitic rocks compared to mafic metavolcanic rocks.

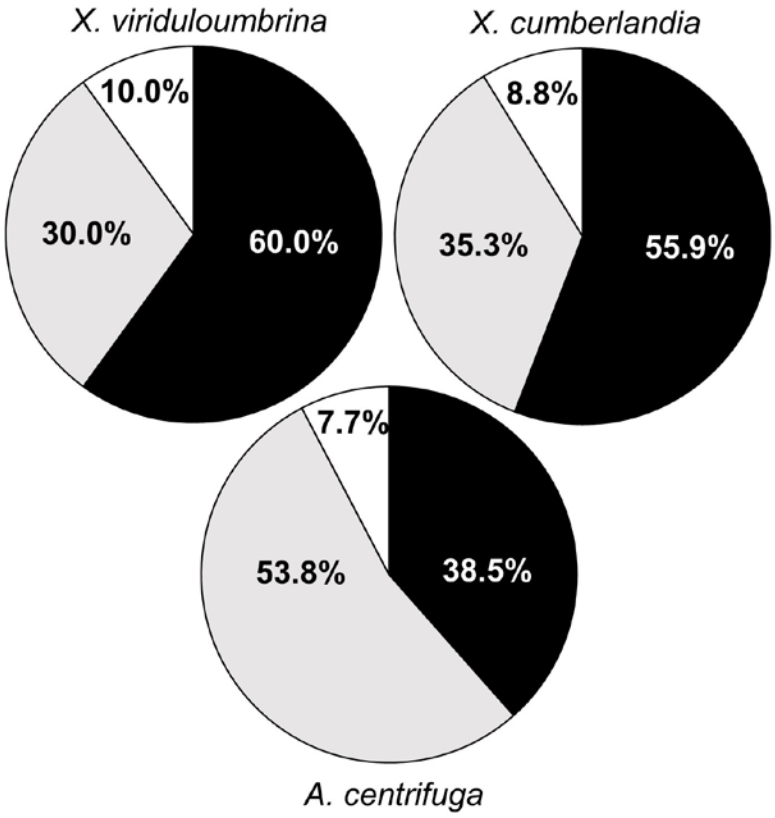

Figure 4.2. Species substrate preference based on presence in a transect for *X. viriduloumbrina* (n = 30), *X. cumberlandia* (n = 34) and *A. centrifuga* (n = 13). Granitic (black), Mafic Metavolcanic (gray) and Metasedimentary (white) rock types.

The percent thallus cover on the substratum varied for each species with rock type (Fig. 4.3A) showing a similar trend as frequency but the differences were not as great. The number of apothecia per thallus varied with both species and rock type (Fig. 4.3B). The average number of ascospores produced varied by species but *A. centrifuga* produced the largest numbers except on metasedimentary rock (Fig. 4.3C). The percent germination of ascospores was greater in *X. cumberlandia* but did not vary by rock type (Fig. 4.3D). The effect of rock type, species and their interaction on measures of lichen sexual fecundity (apothecia, ascospores, and percent germination) was examined by two-way ANOVA to determine whether differences could be attributed to the substratum. The results showed a significant interaction between rock type and species, which affected the number of ascospores and percent germination (Table 4.3). The effect of community, species and their interaction on measures of lichen sexual fecundity was examined by two-way ANOVA to determine whether differences could be attributed to the community. The results showed significant interactions between community and species affecting percent cover, number of ascospores, and percent germination (Table 4.3).

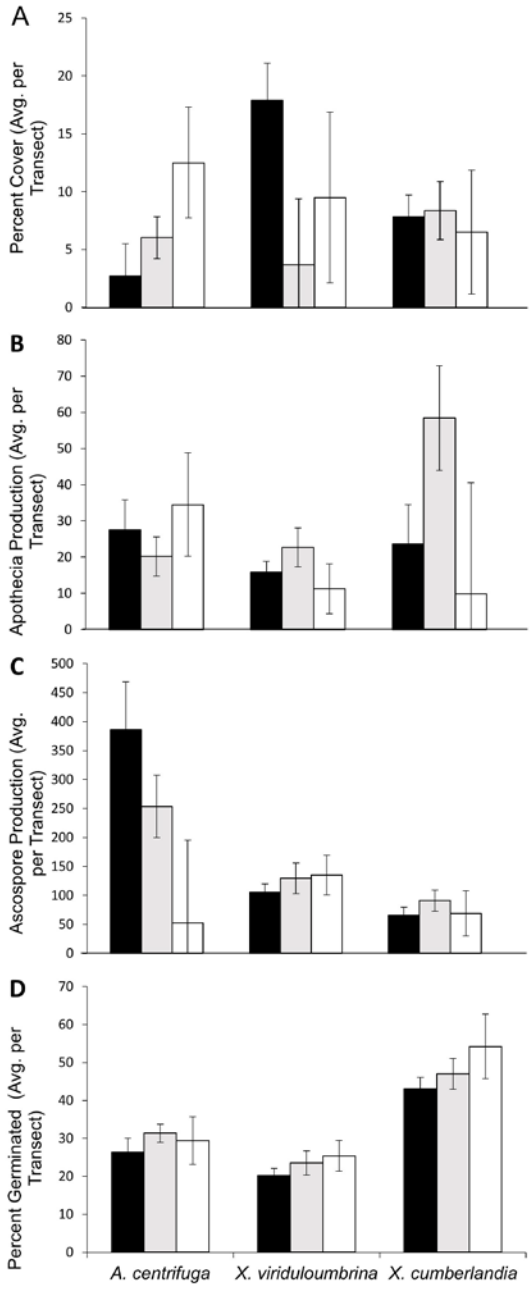

Figure 4.3. Mean and standard error values for average percent cover per quadrat and average apothecia, ascospore and percent germination per thallus for *A. centrifuga*, *X. viriduloumbrina* and *X. cumberlandia*. Species are shown by rock type: Granitic (black), Mafic Metavolcanic (gray) and Metasedimentary (white). Number of transects per species: *A. centrifuga* (Granitic = 3, Mafic Metavolcanic = 7 and Metasedimentary = 1), *X. viriduloumbrina* (Granitic = 16, Mafic Metavolcanic = 5 and Metasedimentary = 3) and *X. cumberlandia* (Granitic = 16, Mafic Metavolcanic = 9 and Metasedimentary = 2).

Table 4.3. Effect of rock type and species (top) and community and species (bottom) on measures of percent ground cover and lichen sexual fecundity (number of apothecia, ascospores, and percent germination) based on two-way ANOVAs with significance at $p \leq 0.050$.

Two-Way ANOVA	*F*	d.f.	*p*
Rock Type			
Percent Cover (Avg. per quadrat)			
Rock Type	1.05	2	0.357
Species	2.44	2	0.096
Rock Type* Species	2.23	4, 57	0.077
Apothecia (Avg. per thallus)			
Rock Type	2.14	2	0.127
Species	1.88	2	0.162
Rock Type*Species	2.21	4, 57	0.092
Ascospores (Avg. per thallus)			
Rock Type	0.37	2	0.690
Species	20.19	2	<0.001
Rock Type*Species	11.12	4, 57	<0.001
Percent Germination (Avg. per thallus)			
Rock Type	1.97	2	0.149
Species	41.91	2	<0.001
Rock Type*Species	22.00	4, 57	<0.001
Community			
Percent Cover (Avg. per quadrat)			
Community	12.46	2	<0.001
Species	3.89	2	0.026
Community*Species	8.59	4, 57	<0.001
Apothecia (Avg. per thallus)			
Community	2.21	2	0.119
Species	1.37	2	0.262
Community*Species	2.14	4, 57	0.088
Ascospores (Avg. per thallus)			
Community	0.12	2	0.885
Species	16.89	2	<0.001
Community*Species	10.90	4, 57	<0.001
Percent Germination (Avg. per thallus)			
Community	5.68	2	0.006
Species	48.17	2	<0.001
Community*Species	26.40	4, 57	<0.001

Correlations between thallus coverage and fecundity with element concentrations in the substratum varied for each of the three lichen species, including a Bonferonni correction (Table 4.4). While 21 elements were examined, only four elements showed significant correlations for *A. centrifuga*, five elements showed significant correlations for *X. viriduloumbrina*, and seven elements showed significant correlations for *X. cumberlandia*, all at a significance of $p = 0.050$. *Arctoparmelia centrifuga* showed the average number of apothecia per thallus was lower in areas with greater concentrations of Mg and Sr; and the percent germination was higher in areas with higher concentrations of La and Mn. For *X. viriduloumbrina* percent thallus cover was lower in areas with high concentrations of Sr, but it was positively correlated with high levels of La. The average number of ascospores per thallus was positively correlated with Mn and Y and percent germination was negatively correlated with high levels of Na. *Xanthoparmelia cumberlandia* showed that percent cover was greater with higher levels of Ag but it was lower with high levels of Sr. The average number of apothecia per thallus was positively correlated with Co, Fe, Mn, and Y while the percent germination was positively correlated with Ni.

Table 4.4. Correlation coefficients between element concentration and each of percent cover (average per quadrat), and fecundity (average number of apothecia, ascospores and percent germination per thallus) for each species based on Spearman's Rho. Significant relationships are $p \leq 0.050$ and denoted by an asterisk. A Bonferroni correction was calculated for all 21 elements and set at $p \leq 0.002$. A significant finding for the Bonferroni correction was noted with two asterisks. See text for element abbreviation.

Element		Fecundity		
	Percent cover	No. apothecia	No. ascospores	Percent germination
A. centrifuga (n = 11)				
La	0.105	0.032	- 0.463	0.632*
Mg	- 0.287	- 0.674*	- 0.077	0.119
Mn	0.127	- 0.046	- 0.036	0.727*
Sr	- 0.156	- 0.661*	0.367	- 0.046
X. viriduloumbrina (n = 24)				
La	0.410*	0.053	0.142	- 0.217
Mn	- 0.040	0.143	0.411*	- 0.121
Na	0.094	- 0.134	0.184	- 0.406*
Sr	- 0.412*	- 0.412	- 0.008	- 0.130
Y	0.124	0.062	0.514*	- 0.071
X. cumberlandia (n = 27)				
Ag	- 0.429*	0.230	0.340	- 0.060
Co	- 0.080	0.392*	0.241	0.160
Fe	0.210	0.463*	0.364	0.089
Mn	0.293	0.514*	0.329	0.210
Ni	- 0.218	0.044	- 0.059	0.395*
Sr	- 0.408*	- 0.040	- 0.247	0.057
Y	0.228	0.442*	0.322	0.118

4.5 Discussion

This study showed four major findings. The first finding was that eight elements showed significant differences between predefined saxicolous lichen communities. Secondly, a difference was found in type of rock substratum between *Arctoparmelia* and *Xanthoparmelia* genera, with greater frequency of *Arctoparmelia* occurring on mafic metavolcanic rocks and both *Xanthoparmelia* species occurring more frequently on granitic rocks. Thirdly, rock substratum was found to have a variable influence on sexual fecundity for *Arctoparmelia* and *Xanthoparmelia* species. Finally, correlations were found between particular element concentrations and each of the *Arctoparmelia* and *Xanthoparmelia* species based on percent cover and sexual fecundity.

4.5.1 *Species correlations with the substratum*

The higher frequency of *A. centrifuga* on mafic metavolcanic separated it from the *Xanthoparmelia* species but this could not be supported by percent cover (Table 4.3) nor with a further breakdown of the elements (Table 4.4). Similarly, the high frequency of both *Xanthoparmelia* species on granitic rock was not consistent with the percent cover of the species on rock substratum (Table 4.3). Leavitt et al. (2011a, 2011b) reported that broad ecological trends could not explain some species differences but Benedict and Nash (1990) showed that *X. linolea*, a western chemotype of *X. viriduloumbrina* (Lendemer, 2005), preferred irregular rock substrata and *X. cumberlandia* preferred more smooth unweathered rock substrata. Similarly, Golm et al. (1993) reported that *X. cumberlandia* was a poor colonizer on smooth unweathered granite tombstones compared to older weathered ones. Since texture was not examined in this

study, a similar comparison could not be made. However the positive correlations with substratum type in this study suggest that element composition may also play a role.

The composition of the underlying geology of the Precambrian Shield for these transects is comprised primarily of silicate minerals, defined by their crystalline structure and element composition (Klein and Dutrow, 2007). The natural weathering rate of silicate minerals varies (White and Brantley, 1995), but lichens were shown to accelerate the weathering process using physical and chemical means (Aghamiri and Schwartzman, 2002; Jackson and Keller, 1970; Paradise, 1997; Stretch and Viles, 2002). Lichens such as *Xanthoparmelia*, may benefit from weathering rock substratum by accumulating ions derived from the substratum in the thallus for storage and nutritional benefit (Clark et al., 2001; 1999). Biotite, along with other micaceous and feldspar minerals are among the best studied regarding lichen weathering effects (Chen et al., 2000; Lee and Parsons, 1999; Wierzchos and Ascaso, 1996) and provide some of the plant nutrients such as K, Mg, and Fe, as well as a non-nutrient Al, which can be absorbed by lichen fungi. Ascaso and Galvan, (1976) showed that *X. cumberlandia* has the ability to release ions from silicate and feldspar minerals, which is consistent with the high frequency of *X. cumberlandia* on granitic rocks in this study. While lichen fungi may extract elements from the substratum, the quantity and availability of the elements extracted must also be considered to determine if they are beneficial to the lichen.

The positive correlation between each of La and Ag, which are potentially toxic elements in the substratum, and percent cover in each of *X. viriduloumbrina* and *X. cumberlandia,* respectively, may be explained if the lichen preferred an element or mineral in granite that was correlated with La and Ag. It may also be explained by a tolerance to La or Ag accumulation in the thallus, such as described for some mycorrhizal fungi (Chen and Zhao, 2007) and some

lichens (Clark et al., 2001; 1999). The toxicity of La to some fungi (Mu et al., 2006) may be ameliorated by increased oxalic acid production, such as in *Aspergillus niger* to form a complex with the ions (Talburt and Johnson, 1967). If *Xanthoparmelia* species are tolerant of La and Ag, they may have a competitive advantage in those environments. This can be tested if the level of La and Ag were also measured in the lichen thallus to determine if the levels are similar with those in the substratum. Ag has been shown to have toxic effects similar to those produced by Cu and Hg (Puckett, 1976), which include lowered photosynthetic rates in algae, and potassium and mannitol leakage in fungi (Burton et al., 1981; Puckett, 1976; Slawson et al., 1990). Despite the adverse effects that would target both partners of the symbiosis, *X. cumberlandia* was abundant on substratum with high levels of Ag. Even though these positive correlations with percent cover are reported, the thallus content not measured in this study.

4.5.2 *Lichen fecundity and element composition of the substratum*

At least one measure of sexual fecundity was correlated in all three species with element composition (Table 4.4). The positive correlation of Fe in *X. cumberlandia* with average number ascospores per thallus may be explained if Fe contributed to ferricrocin, a storage product derived from extracellular Fe. Ferricrocin was shown to be important for conidial and ascospore production in *A. nidulans* (Eisendle et al., 2006) where ferricrocin was deficient, conidial germination was delayed and cleistothecia and ascospore production were inhibited. This study showed a positive correlation between fecundity and Mn in all three species. Mn is important for fungal growth and production of sexual structures (Barnett and Lilly, 1966; Zonneveld, 1975). The strong positive correlation between the level of Mn (a plant nutrient) and La (a toxin) in the substrate and the ability for the ascospores of *A. centrifuga* to germinate may suggest that these

elements or other unmeasured elements that associate with these elements are important for viability of the spores during sexual reproduction (Barnett and Lilly, 1966; Zonneveld, 1975). Conversely, two strong negative correlations of Mg and Sr with apothecia production in *A. centrifuga* may result from these elements directly or others associated with them that were not measured in this study. While these correlations do not imply causation, they suggest a relationship with specific elements may exist, which would need further study.

While ascospore production seems to be influenced by community and rock type, percent germination is influenced only by rock type (Table 4.3), and apothecia production is not influenced by either variable. This may be interpreted if apothecia are merely the vehicles by which products of sexual reproduction can develop and disperse (controlled by inherent interactions after sexual reproduction has been initiated), but the ascospores and their viability are determined by the interaction between the substratum chemistry and the surrounding community of biotic and abiotic variables. If the surrounding community influences environmental features such as humidity (Tibell, 1992), development may also be affected. The genetic makeup of the lichen fungus may also play a role in fecundity where *X. cumberlandia* produces fewer ascospores but with higher percent germination than other species (Deduke et al., 2014 [Chapter 2]). While there was more percent germination on metasedimentary than granite for both *Xanthoparmelia* spp. there were more apothecia produced per thallus on mafic metavolcanic than the other two rock types, suggesting a complex relationship among these variables.

4.5.3 *Community relationship with element composition*

Since saxicolous lichens are capable of accumulating elements through aerial precipitation and pollution (Purvis et al., 2000), the substratum may play a less important role in community structure. The grassy rock community was thought to be a stage in succession between more exposed mossy rock to heavily vegetated treed communities (Deduke et al. 2014 [Chapter 2]) and in boreal forests (Ahti, 1977; Ahti and Oksanen, 1990; Bergeron and Dubuc, 1989). The higher levels of elements in the grassy rock community (Table 4.2) may suggest that either the underlying substratum is most suitable to a grassy community or that the grassy vegetation contributes to the element composition through leachates. Since the analysis was performed on a relatively unweathered portion of the rock (see methods), the presence of vegetation should not contribute to the element composition. If vegetation contributed to the composition of the elements, then the treed rock community with a greater amount of vegetation (Deduke et al., 2014 [Chapter 2]) would be expected to have a larger quantity of elements to serve as plant nutrients, but the treed community had a low quantity of elements. The origin of the elements might be more accurately determined if a comparison was made between the vegetation and the rock.

Levels of the potentially toxic elements Ag and La were higher in the more exposed mossy rock community, which is underlain by granitic rock. Elements necessary for plant growth such as Cu, Fe, Mn, Ni and S are typically less abundant in granite (Garraway and Evans, 1984; Jennings, 1995; Mengel and Kirkby, 1982) offering a nutrient poor environment relative to mafic metavolcanic environments. If the low nutrient level in the mossy rock community prevents further succession of plant species, then these communities might show a cyclic succession with a narrow range of species rather than being part of a broad successional pattern. Lichens are known to undergo cyclic successional processes (John, 1989) influenced by microclimatic

factors like humidity (Tibell, 1992), competition for space (Armstrong and Welch, 2007; Pentecost, 1980), and extraction of nutrients from the substratum (Puckett and Finegan, 1980). Generally, the ecological succession of species on rock substratum in boreal communities is thought to be influenced by lichen weathering, which may build a layer of soil to become colonized by terricolous lichens, mosses, vascular plants and eventually trees (Ahti, 1977; Ahti and Oksanen, 1990; Bergeron and Dubuc, 1989). The results in this study raise hypotheses about cyclic succession in these communities.

In conclusion, while the frequency of occurrence on rock type could separate the two species of *Xanthoparmelia* from *Arctoparmelia centrifuga*, the whole-rock element composition could not separate them. The percent cover of the *Xanthoparmelia* species was positively correlated with two potentially toxic elements and negatively correlated with Sr suggesting substratum conditions required for growth of both species (in terms of biomass coverage on the substratum) may be similar. However, some correlations between element composition and fecundity could be made, which suggest that the elements may play a role in fecundity and; therefore, hypotheses were proposed for further testing. The grassy rock community had a significantly higher abundance of many elements than the mossy or treed rock communities implying that cyclic succession in these communities may occur. Furthermore, the clustering of elements can be more broadly classified as rock types, which makes the knowledge of rock type more applicable to inferring geological preferences in field studies. Knowledge of the rock type preferences may further facilitate taxonomic investigations for the genus *Xanthoparmelia* and related genera. This study provided valuable insights into the complexity of the roles played by community (environmental factors) and geological composition of the substratum in the biology of three saxicolous lichens.

4.6 Acknowledgements

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CHAPTER 5

Substratum preference of two species of *Xanthoparmelia*

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5.1 Abstract

The germination of ascospores produce fungal hyphae that are directly exposed to the substratum before the formation of a symbiotic lichen association. The mineral composition of a rock substratum encountered by saxicolous lichen fungi may vary, providing a patchy environment for fungal growth. Distribution of saxicolous lichens has been previously examined but the effects of substratum preference on fungal growth have been understudied. The objectives of this study were to compare ascospore germination and growth rates for two species of *Xanthoparmelia* using media supplemented with different types of crushed rock. Mature apothecia from *X. cumberlandia* and *X. viriduloumbrina* were collected, prepared for ascospore release on solid media, and subjected to culturing conditions using five treatments, which include water agar supplemented with either crushed granodiorite, basalt, mica schist, dolostone, or malt yeast agar as the control. Ascospore germination and mycelial growth were recorded using a dissecting microscope. Results indicate that *X. cumberlandia* exhibited a species specific substratum preference for the mica schist medium, but *X. viriduloumbrina* did not show a preference under the conditions in this study. Ascospore germination failed to progress beyond

the initial swelling and protrusion stage on the dolostone treatment. In general, *X. cumberlandia* grew faster than *X. viriduloumbrina* until 5 to 6 weeks, providing evidence to support a ruderal life history strategy for *X. cumberlandia*. The slow growth of *X. viriduloumbrina* supports a stress tolerant life history strategy. This study provided insights into the establishment of lichen thalli at early stages of growth, which depend on the success of ascospore germination and the suitability of the substratum for further lichen development.

5.2 Introduction

Colonization of a substratum by a lichen is critical at the early stages of propagule dispersal and the formation of an interaction between the fungal and algal symbionts that make up a lichen. Lichens can reproduce by vegetative reproduction, which includes propagules such as fragments of the thallus, isidia, or soredia where both symbionts are present within each propagule. Sexual reproduction in lichens occurs by the mycobiont resulting in ascospores released into the environment, while sexual reproduction by the photobiont is thought to be absent while in the lichen thallus (Büdel and Scheidegger 2008). The sexual propagules of the mycobiont must come into contact with a suitable photobiont, and the two symbionts will undergo a recognition process resulting in the resynthesis of a new lichen thallus (Honegger 2008). The effect of substratum pH on resynthesis of a lichen thallus was recently examined in *Cladonia rangiferina* (Athukorala and Piercey-Normore in press) showing reduced ability to resynthesize at a high pH level. After synthesis and development of the lichen thallus, the attachment organs must be able to penetrate the substratum and the elements of the substratum must be advantageous and/or nontoxic to allow the lichen to persist on the substratum.

The thallus in *X. viriduloumbrina* (Gyelnik) Lendemer and *X. cumberlandia* (Gyelnik) Hale is comprised of an upper and lower surface with rhizines on the lower surface that securely

attach the thallus to the rock substratum. The rhizines penetrate the rock surface and contribute to the weathering of the rock (Chen et al. 2000). The weathering results in elements absorbed through the fungal hyphae affecting the physiology of the lichen symbionts. Although the pH of the rock substratum is considered the most important aspect of lichen colonization (Lisci et al. 2003), the texture, water retention, and elemental composition must also play roles in long-term colonization as well as during the initial synthesis of the lichen symbiosis. Previously, the life history strategy of *Xanthoparmelia viriduloumbrina* was shown to be stress tolerant and *X. cumberlandia* was a ruderal species (Deduke et al. 2014 [Chapter 2]). The life history strategies of these saxicolous species imply colonization on exposed rock where the ascospore germination and the synthesis of the lichen association are subject to harsh environmental conditions. The environmental parameters that usually affect ascospore germination are oxygen, pH and temperature (Cotter 1981; Cotter and Raper 1968a, 1968b) but these have not been examined for *Xanthoparmelia* species.

The genus *Xanthoparmelia* is a large genus of foliose lichens that primarily grows on hard acidic rock (Hale 1967) with some species growing on soil (Hale 1990). *X. viriduloumbrina*, previously known as *X. somloënsis* (Lendemer 2005), was reported on acidic rocks like granite or schist, with some occurrences on basalt (Giordani et al. 2002). *X. cumberlandia* was primarily on basalts (Giordani et al. 2002) and mafic metavolcanic rock (Deduke et al. in review [Chapter 4]). *X. viriduloumbrina* showed positive correlations with rare earth elements, while *X. cumberlandia* showed correlations with Fe and Mn (Deduke et al. in review [Chapter 4]) suggesting elements in the substratum may influence colonization. However, if other features of the substratum that vary, such as water retention or texture, were eliminated

as confounding variables, insights into the effect of elemental composition on fungal growth may be gained.

The general goal of this study was to investigate the substratum preferences of *X. viriduloumbrina* and *X. cumberlandia* by removing a confounding variable of substratum water retention. More specifically, the objectives were to compare germination and percent cover between two species of lichen fungi, and among five treatments of growth media containing defined crushed rock. The third species, *Arctoparmelia centrifuga*, was not included in this chapter because there was insufficient specimen material available for experimentation.

5.3 Materials and Methods

5.3.1 *Specimen collection and experimental design*

Five specimens of *X. viriduloumbrina* were collected from locations along Highway 44 in Manitoba (N50°02'-49°50', W95°36'-95°23') and five specimens of *X. cumberlandia* were collected from locations along Highway 1 in northern Ontario (N50°11'-49°37', W93°15'-92°09'), based on accessibility and prevalence of the species in these areas. Mature apothecia were used as described in Bellemère and Letroiut-Galinou (2000). Taxonomic identification of the two species was confirmed using Hale (1990) and Lendemer (2005).

The experimental design consisted of two apothecia for each of five biological replicate petri-plates for each of five treatments (granodiorite, dolostone, basalt, mica schist, and malt yeast agar as the control) for each of two species *X. viriduloumbrina* and *X. cumberlandia*. The spacing between apothecia was distant enough in each petri plate to allow for a zone of an absence of ascospores, so ascospores from each apothecium can be counted separately. Apothecia were washed and prepared using a modified protocol of Kofler (1970), and were

attached to the undersurface of the petri-plate lids using Vaseline. After one week the media surface was examined for ascospores, and the petri-plate lids with the apothecia were replaced to prevent apothecia from becoming a potential source of contamination. Petri plates were incubated at a temperature of 20^{o}C and 24 hour darkness.

5.3.2 Data collection

Numbers of ascospores and germinated ascospores were counted twice a week, beginning after the first week for a total of eight weeks using a dissecting microscope (Leica MZ6), with a 10x10 grid placed in the right eye piece. The edges of the grid were marked on the petri plate lid to ensure accuracy with subsequent recounting. Ascospores were counted in a single 1x10 row based on largest numbers of ascospores released using 40x magnification. Three sequential counts were done for each row for the number of ascospores, the number of germinated ascospores, and the percent cover of mycelium for each grid cell. Ascospores were considered germinated when they showed physical changes by the protrusion of a germ tube (Cotter 1981). Percent cover of mycelium (radial growth) was used to determine growth of the mycelium. The method using liquid media and dry weight measurements or measuring the turbidity of the liquid culture (Retrepo and Jiménez 1980) was not considered for this study because these are destructive methods and would not be representative of these fungi in their natural environment. The advantage of the method used in this study on solid media was less unnatural and more representative of fungal growth on a solid substratum as in nature. Morphological observations and photographs could also be taken over a period of time. Photos were taken at week 8.5 using a dissecting microscope (Olympus SZH) at 40x magnification. The camera was an Olympus QColour 5 and imaging software was QCapture (version 3.1.1, Qimaging, 2007).

5.3.3 Culturing and substratum preparation

Rock samples were collected from previous study locations within the boreal forest in Manitoba and northern Ontario (Deduke et al. 2014 [Chapter 2]). Granodiorite was collected from an outcrop along Highway 307, near Brereton Lake, Manitoba (N49˚ 56’ 11.88”; W95˚ 31’ 06.80"). Dolostone was collected along Highway 39 near Cranberry Portage, Manitoba (N54˚ 30' 25.77"; W101˚ 09' 29.25"). Basalt was collected from an outcrop along Sherridon road, Manitoba (N54˚ 41’ 17.56”; W101˚ 31’ 38.89”). Mica schist was collected from an outcrop along Highway 314, north of Finger Lake, Manitoba (N54˚ 46’ 14.98”; W95˚ 17’ 38.49”). Rock samples were sent to Activation Labs (Thunder Bay, Ontario) for AR-ICP analysis (Aqua Regia – Inductively Coupled Plasma). The rock samples were weighed to 0.5 g and crushed to a size where 95 % of the rock powder passed through a 105 μm aperture/150 mesh (Actlabs 2014) and the powder from each of granodiorite, dolostone, basalt and mica schist were returned for preparation with culture media. The culture media consisted of water agar with crushed rock (0.25 g rock powder and 0.5 g agar in 25 mL water) for the rock treatments and malt yeast agar for the control (0.5 g agar; 0.5 g malt; 0.05 g yeast in 25 mL water; Stocker-Wörgötter 2002). Twenty-five elements measured in the AR-ICP analysis included: silver (Ag; ppm); aluminum (Al; %); barium (Ba; ppm); calcium (Ca; %); cobalt (Co; ppm); chromium (Cr; ppm); copper (Cu; ppm); iron (Fe; %); potassium (K; %); lanthanum (La; ppm); magnesium (Mg; %); manganese (Mn; ppm); sodium (Na; %); nickel (Ni; ppm); phosphorus (P; %); lead (Pb; ppm); sulphur (S; %); scandium (Sc; ppm); strontium (Sr; ppm); tellurium (Te; ppm); titanium (Ti; %); vanadium (V; ppm); yttrium (Y; ppm); zinc (Zn; ppm) and zirconium (Zr; ppm).

The rock types, granodiorite, basalt, and mica schist, were identified prior to grinding using characteristics including mineral gain-size, foliation, presence of particular mineral assemblage and cleavage planes (Klein and Dutrow 2007). Dolostone was additionally identified with the observation of a weak bubbling reaction when acetic acid was applied, along with elevated levels of Mg from sample analysis (Klein and Dutrow 2007). The samples of granodiorite, basalt and mica schist are subsamples found within the broadly classified groups of granitic, mafic metavolcanic and metasedimentary rocks used in Deduke et al. (in review [Chapter 4]).

5.3.4 Data Analyses

Element quantities and percent cover data were transformed using a log (x+1) conversion to minimize the variance in the data, based on Zar (2010). Principal component analysis (PCA) was chosen as an ordination method for element concentration data because the quantities are linearly related to one another, it is a non-binary data set and it is not species based (Peck 2010). Non-parametric Kruskal-Wallis and Steel-Dwass tests were done to test the significance of percent cover (as dependent variables) between culture treatments (as independent variables).Time series experiments are uncommon for fungal growth experiements but was chosen to avoid destructive dry weight sampling, mimic a solid medium which is a what these fungi grow on as opposed to a liquid media and to be able to record morphological changes using photography. Mycelial fungi, including lichenized fungi, follow a growth curve that contains a lag phase, log phase and a decelearation phase when grown in culture (Trinci 1969, Koch 1975). Two time periods (weeks 3.5 and 6.0) were chose to compare both species in this study based on their overall growth curves. Week 3.5 was chosen for both species because it occurs early in the

ascospore germination, showing differences in germination. It represents the first log phase for *X. cumberlandia* and the initial log phase for *X. viriduloumbrina*. Week 6.0 was chosen for comparison because it gave time for ascospore germination and development to occur in both species and represents the log phase for both species. Two additional time periods (weeks 2.5 and 7.5) were chosed for *X. cumberlandia* because this species exhibited staggered growth times between sets of treatments. Week 2.5 was chosen for *X. cumberlandia* because it represents the lag phase for this species. Week 7.5 was chosen for *X. cumberlandia* because it represents a second log phase for particular treatments for this species. An additional time (week 7.0) was selected for *X. viriduloumbrina* because it represents the log phase specific to this species. The resulting times for the two species are: *X. cumberlandia* - week 2.5 (early lag phase), week 3.5 (first log phase/species comparison), week 6.0 (log phase/ species comparison), week 7.5 (second log phase); and *X. viriduloumbrina* - week 3.5 (lag phase/species comparison), week 6.0 (early log phase/species comparison), and week 7.0 (log phase).The growth curves of percent cover show the result of mean and standard error (SE) for each time at which the spores were counted. Kruskal-Wallis and Steel-Dwass tests were used to compare variation among the mean percent cover at the different time points.

5.4 Results

5. 4.1 Elemental composition of rock types

The elemental composition and defined rock substrata in the PCA shows the primary axis which explains 57.83% of the variation in the data, and the secondary axis accounts for an additional 30.04% (Fig. 5.1). Dolostone was at the positive end of the primary axis due to higher presence of Ca, Mg and Sr, while granodiorite and mica schist were found at the negative end of

the primary axis. Basalt was centrally located along the primary axis, but was negatively located along the secondary axis. Dolostone contained higher amounts of Ca, Mg and Sr than the other rock types (Table 5.1). Granodiorite was higher than other rock types in Cu, La, Ni and Te. Mica schist had larger amounts of Al, Ba, Cr, and K. Basalt was found to have the greatest quantities of Fe, Mn, Na, Sc and V (Table 5.1).

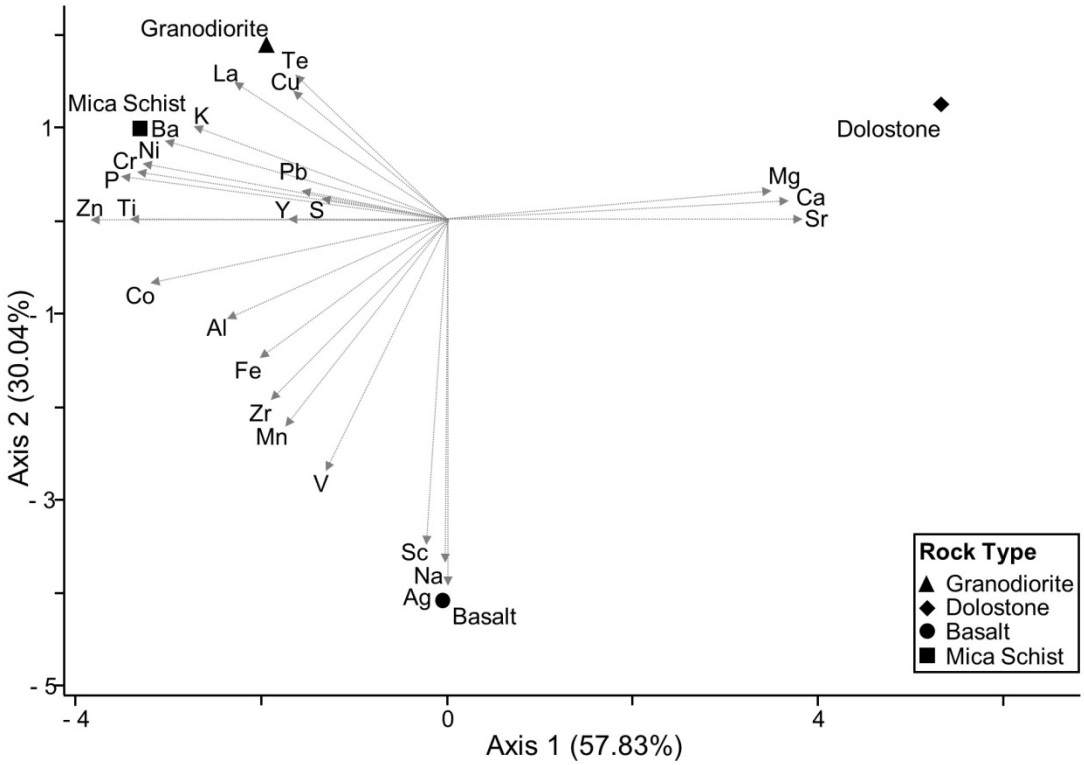

Figure 5.1. PCA showing the relationship among the elements in each of the four rock types used in this study.

Table 5.1. Quantities of the 25 elements within each of the four rock types used as media supplements in this study.

Element	Granodiorite	Dolostone	Basalt	Mica Schist
Ag (ppm)	< 0.2	< 0.2	0.2	< 0.2
Al (%)	0.81	0.06	1.85	2.06
Ba (ppm)	78	< 10	22	100
Ca (%)	0.35	17.4	1.54	0.11
Co (ppm)	8	< 1	11	12
Cr (ppm)	72	2	25	99
Cu (ppm)	33	3	3	17
Fe (%)	1.38	0.19	3.52	3.42
K (%)	0.5	0.03	0.06	0.75
La (ppm)	40	< 10	< 10	33
Mg (%)	0.86	12.6	1.34	1.43
Mn (ppm)	332	157	552	427
Na (%)	0.058	0.024	0.236	0.029
Ni (ppm)	32	1	10	30
P (%)	0.053	0.005	0.022	0.06
Pb (ppm)	2	< 2	< 2	3
S (%)	< 0.01	< 0.01	< 0.01	0.03
Sc (ppm)	4	< 1	12	3
Sr (ppm)	9	39	17	11
Te (ppm)	3	1	< 1	2
Ti (%)	0.13	< 0.01	0.08	0.1
V (ppm)	27	< 1	68	39
Y (ppm)	13	< 1	7	5
Zn (ppm)	39	2	30	57
Zr (ppm)	5	< 1	11	9

5.4.2 Observations of mycelial growth

The growth medium containing granodiorite produced different morphological forms of mycelial mats between the two species (Fig. 5.2). *X. cumberlandia* produced growth patches with clearly separated hyphal branches (Fig. 5.2A) but *X. viriduloumbrina* produced patches with more extensive and dense hyphal branching (Fig. 5.2F). Ascospore germination and mycelial growth for both species on media supplemented with dolostone showed no growth. Some ascospores showed swelling followed by the formation of a small bulge, presumably where a germ tube would emerge. However, no germ tube was produced and ascospores never developed beyond this point nor acquired any additional size (Fig. 5.2B and 5.2G). The media supplemented with basalt produced mycelial patches in both species with small dense hyphal branching (Fig. 5.2C and 5.2H). The media supplemented with mica schist produced large numbers of small patches in *X. cumberlandia* that had well defined central points (Fig. 5.2D) and more extensive and large patches of dense hyphal branching in *X. viriduloumbrina* (Fig. 5.2I). Finally, the MY control produced well-defined patches of growth for both species, with clearly separated hyphal branching (Fig. 5.2E and 5.2J). While *X. viriduloumbrina* had larger number of patches than *X. cumberlandia*, there was also a larger number of ungerminated ascospores in this species (Fig. 5.2J).

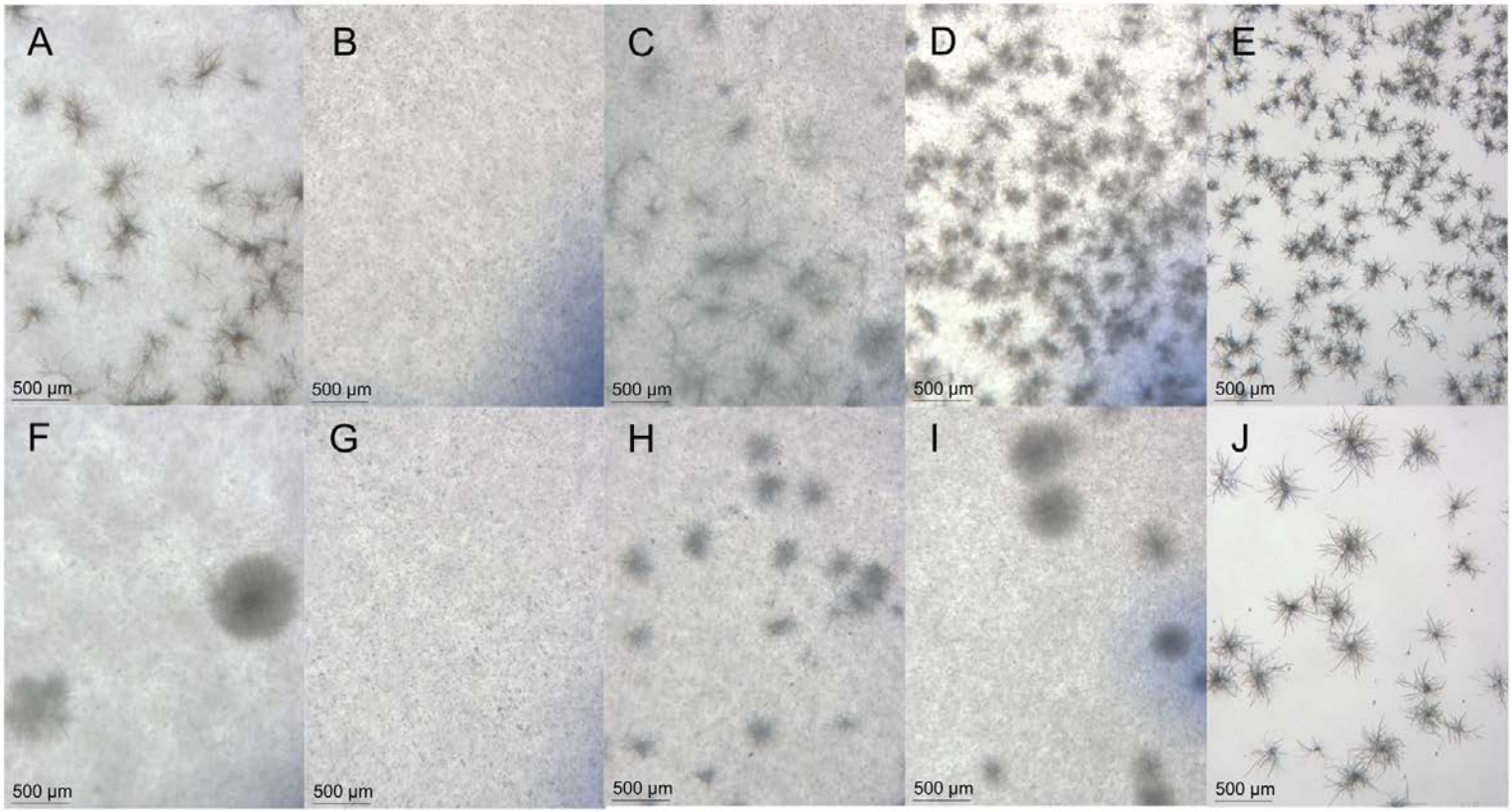

Figure 5.2. Mycelial growth after 8.5 weeks on each of the five treatments for each of two species, *X. cumberlandia* (A-E) and *X. viriduloumbrina* (F-J). The treatments were Granodiorite (A and F); Dolostone (B and G); Basalt (C and H); Mica Schist (D and I); and Malt Yeast Agar Control (E and J). All photos were taken at 40x magnification. Black bar represents 500 µm.

5.4.3 Comparison of growth between treatments within each species

The patterns of growth rates over time were different between treatments where *X. cumberlandia* showed a 10-12% cover in the first 3 weeks and then an increase on the mica schist after 3 weeks up to 70% coverage of the grid (Fig. 5.3A). Growth on the mica schist levelled off after 6.5 to 7 weeks. The other treatments showed a gradual increase (granodiorite, basalt and the control) or no increase (dolostone) in growth. *X. viriduloumbrina* showed growth covering 10% or less of the grid until 4.5 to 5 weeks when it began to increase in 3 of 5 treatments but it did not surpass 40% coverage (Fig. 5.3B). *X. cumberlandia* showed significant differences in growth between treatments (Table 5.2). Mycelial percent cover on mica schist was greater than the control, dolostone and basalt across all time points. Mica schist produced significantly more growth in *X. cumberlandia* than all other treatments and dolostone produced significantly less growth than all other treatments. *X. viriduloumbrina* showed no significant difference between any of the treatments at week 3.5 (Table 5.2). *X. viriduloumbrina* grew significantly better on the granodiorite treatment compared to the other four treatments at weeks 6 and 7. Mycelial growth on both granodiorite and the control were significantly greater than growth on dolostone.

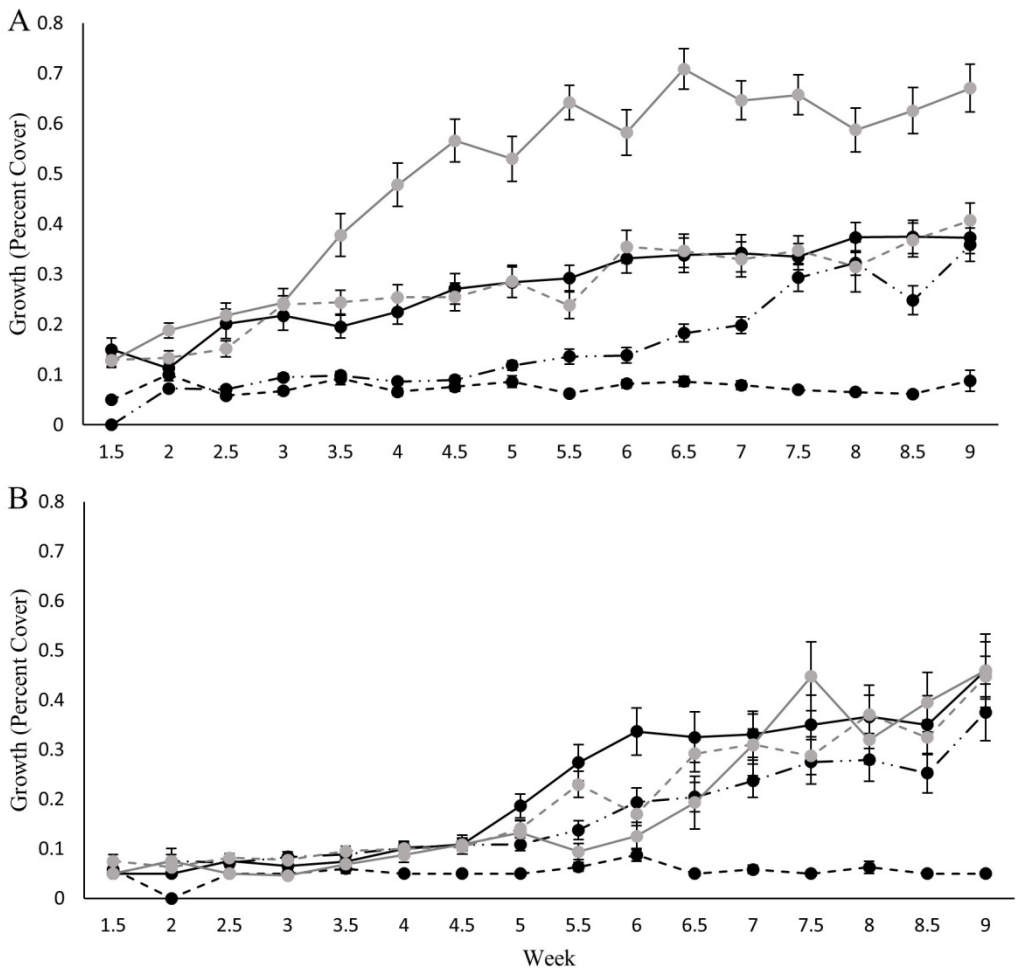

Figure 5.3. Growth of mycelia for A) *X. cumberlandia* and B) *X. viriduloumbrina* treatments from weeks 1.5 to 9. Granodiorite (solid black line), Dolostone (black dash line), Basalt (black dotted line), Mica Schist (solid grey line) and Malt Yeast Agar Control (grey dash line).

Table 5.2. Comparison of percent cover of mycelial growth between treatments for *X. cumberlandia* and *X. viriduloumbrina* at different time intervals using a Kruskal-Wallis test. Rank indicates the significance based on the Steel-Dwass test where different letters indicate a significant difference. A Bonferroni correction of $p < 0.0125$ (4/0.05) is indicated by an asterisk for *X. cumberlandia*. A Bonferonni correction of $p < 0.0167$ (3/0.05) is indicated by an asterisk for *X. viriduloumbrina*.

Species	Treatment	Rank	*H*	*p*
X. cumberlandia				
Week 2.5	Granodiorite	ab	49.45	< 0.0001*
	Dolostone	c		
	Basalt	c		
	Mica Schist	a		
	MYA control	b		
Week 3.5	Granodiorite	b	60.85	< 0.0001*
	Dolostone	c		
	Basalt	c		
	Mica Schist	a		
	MYA control	ab		
Week 6.0	Granodiorite	b	89.64	< 0.0001*
	Dolostone	c		
	Basalt	c		
	Mica Schist	a		
	MYA control	b		
Week 7.5	Granodiorite	b	96.82	< 0.0001*
	Dolostone	c		
	Basalt	b		
	Mica Schist	a		
	MYA control	b		
X. viriduloumbrina				
Week 3.5	Granodiorite		4.49	0.3430
	Dolostone			
	Basalt			
	Mica Schist			
	MYA control			
Week 6.0	Granodiorite	a	18.1	0.0012*
	Dolostone	b		

	Basalt	b		
	Mica Schist	b		
	MYA control	b		
Week 7.0	Granodiorite	a	14.3	0.0006*
	Dolostone	b		
	Basalt	ab		
	Mica Schist	ab		
	MYA control	a		

5.4.4 Comparison of growth between species within each treatment

The patterns of radial growth over time were different between species in the same treatment (Fig. 5.4). *Xanthoparmelia cumberlandia* produced more mycelium on granodiorite than *X. viriduloumbrina* initially, and both growth curves merged at about 5.5 weeks with maximum cover of 40%. Growth of both species fluctuated on dolostone over the 9 weeks with a negligible increase in mycelium reaching no more than 10% cover. Both species showed very similar growth in basalt with a steady increase over 9 weeks reaching a maximum of about 30% cover at week 9. *Xanthoparmelia cumberlandia* showed an increase in growth on mica schist after 3 weeks reaching a maximum of about 70% cover after 6.5 weeks. In contrast, *X. viriduloumbrina* remained below 10% cover on mica schist until about week 6 when it began to increase reaching no more than 40% cover. Lastly, *X. cumberlandia* showed greater percent cover than *X. viriduloumbrina* in the control medium until about week 7 when the growth curves for both species merged, and reached a maximum of 40% cover at 9 weeks.

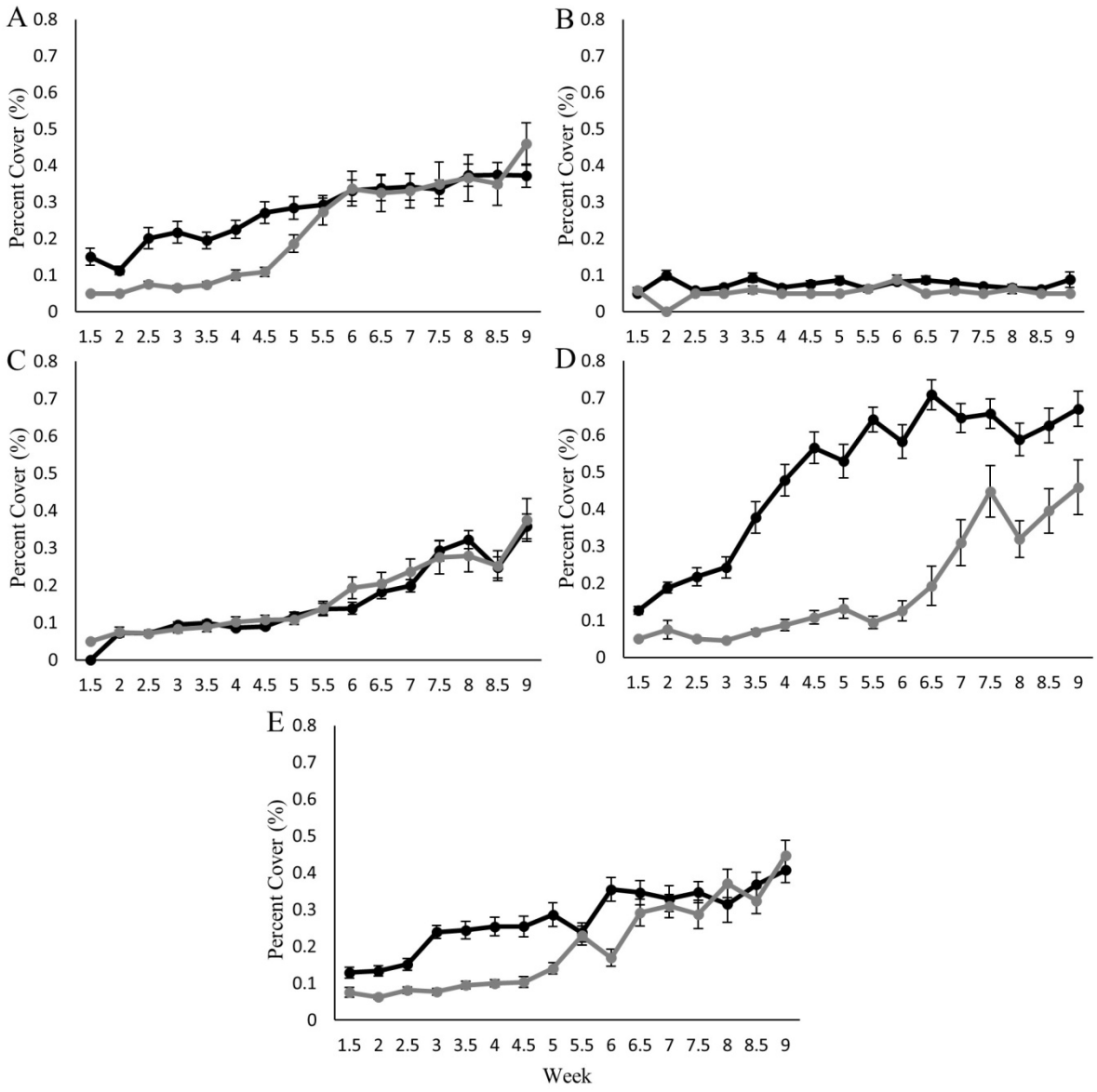

Figure 5.4. Comparison in mycelial growth for *X. cumberlandia* (black line) and *X. viriduloumbrina* (grey line) on five treatments: A) Granodiorite; B) Dolostone; C) Basalt; D) Mica Schist; and E) Malt Yeast Agar. Significance test was conducted at the week 6 interval.

Statistical comparison of growth rates for the two *Xanthoparmelia* species showed significant differences at both 3.5 and 6 weeks for some treatments but not for all treatments (Table 5.3). *Xanthoparmelia cumberlandia* showed more growth than *X. viriduloumbrina* for granodiorite, mica schist and the control (malt yeast agar) at 3.5 weeks (Table 5.3). There was no difference between species for dolostone and basalt (Table 5.3). The treatments for granodiorite, dolostone and basalt showed no significant differences in mycelial growth at 6 weeks (Table 5.3). Mycelial growth on the mica schist treatment and the control (malt yeast agar) showed significantly greater growth for *X. cumberlandia* than for *X. viriduloumbrina* at week 6 (Table 5.3).

Table 5.3. Comparison of growth (percent cover of mycelium) between species (*X. cumberlandia* and *X. viriduloumbrina*) for each of the five treatments (Granodiorite, Dolostone, Basalt, Mica Schist and Malt Yeast Agar Control) at weeks 3.5 and 6 using Kruskal-Wallis and Steel-Dwass tests. Significance of $p < 0.05$ was used. A Bonferonni correction of $p < 0.005$ (10/0.05) is indicated by an asterisk.

Treatment	*X. cumberlandia*	*X. viriduloumbrina*	*H*	*p*
Week 3.5				
Granodiorite	A	B	15.993	< 0.0001*
Dolostone			1.6609	0.1975
Basalt			0.9824	0.3216
Mica Schist	A	B	31.363	< 0.0001*
MYA control	A	B	23.930	< 0.0001*
Week 6.0				
Granodiorite			0.0587	0.8085
Dolostone			0.3965	0.5289
Basalt			2.4973	0.114
Mica Schist	A	B	24.2131	< 0.0001*
MYA control	A	B	18.9379	< 0.0001*

5.5 Discussion

5.5.1 Species-specific substratum preference

This study showed evidence to support species-specific substratum preference for one species of *Xanthoparmelia* but not the other species. *Xanthoparmelia cumberlandia* showed a strong growth preference for the mica schist treatment while *X. viriduloumbrina* showed no preference for the treatments in this study. A previous study showed that *X. cumberlandia* produced greater thallus coverage, higher quantity of stictic acid, and more apothecia (higher fecundity) on Fe and Al rich rock substrata (Deduke et al. in review [Chapter 4]), elements found in biotite, which is a mineral common in mica schist. If Al, Fe and Mg are important to the growth and development of sexual structures in *X. cumberlandia* (Deduke et al. in review [Chapter 4]), and stictic acid facilitates their uptake by fungal hyphae (Ascaso et al. 1976a), substratum changes such as rock type or mineral size that influence element composition, would influence fecundity of *X. cumberlandia*. Mineral size may influence the ability for lichens to sequester elements from the rock substratum (St John et al. 1983). Even though basalt and mica schist share many elements, particularly Fe and Al, the mineral size is different. Basalt has small aphanitic (not visible to the naked eye) minerals while the mica schist has larger phaneritic (visible to the naked eye) minerals, organized in layers (foliation). Granodiorite, like the mica schist, has phaneritic minerals. Rocks composed of phaneritic or aphanitic minerals would comprise a substratum mosaic on which fungi grow, creating patches of both unfavourable and favourable elements. The crushing of rocks in this study homogenized the naturally formed mineral mosaic in some samples, allowing germinating ascospores equal access to all elements in the rock. As rock samples were crushed less than 105 μm, variability in fungal growth caused by mineral grain-size was eliminated from this study making results potentially inconsistent with

those in nature. Previous studies have tried to address growth and reproductive capacity in *X. cumberlandia* (Jackson et al. 2006; Pringle et al. 2003), but they did not account for the influence of the rock substratum. This study, along with Deduke et al. (in review [Chapter 4]) suggests that specific substratum type may act as a confounding variable for addressing questions about growth and reproductive output in these species.

Even though *X. viriduloumbrina* showed a preference for granodiorite after 6 to 7 weeks, its growth was slower than that of *X. cumberlandia* in all treatments examined except basalt and after 6 weeks in granodiorite (Fig. 5.3) suggesting that *X. viriduloumbrina* is inherently a slower growing species. *Xanthoparmelia viriduloumbrina* was found more commonly on rock with La and Pb (Deduke et al. in review [Chapter 4]) which are associated with granodiorite and mica schist (Fig. 5.1). However, the dense growth with increased ramification of *X. viriduloumbrina* hyphae in granodiorite and mica schist treatments (Fig. 5.3) is consistent with the toxic effects of La (Mu et al. 2006), but the growth pattern was not present in *X. cumberlandia*. This may be explained if levels of La in the treatment were not toxic or if *X. viriduloumbrina* was more sensitive than *X. cumberlandia* to the effects of La or other elements. On the other hand, fungal growth is generally regulated to maximize hyphal length when suitable nutrients are present (Paustian and Schnürer 1987) but lateral expansion of fungal hyphae is considered a secondary growth stage (St John et al. 1983) and occurs after length extension. Lateral growth would allow the fungus to increase surface area into previously unexplored space and extract nutrients. The exhibition of different growth patterns by each species in the same treatments may indicate that the two species have separate nutrient requirements or tolerances, further supporting different life history strategies.

5.5.2 Life history strategies and substratum preferences

The faster growth rate of *X. cumberlandia* over *X. viriduloumbrina* in three of the five treatments up to week 7 (Fig. 5.3) supports the contention that *X. cumberlandia* is a ruderal species and X. *viriduloumbrina* is a stress tolerant species (Deduke et al. 2014 [Chapter 2]). A fast relative growth rate is indicative of a ruderal species, while stress tolerant species are characterized by slow growth rates (Grime 2001). The faster growth of *X. cumberlandia* than *X. viriduloumbrina* across treatments with different element compositions (Fig. 5.1) may reflect a genetic difference between the species rather than environmental influence. The remarkable growth on mica schist indicates that *X. cumberlandia* may prefer the element composition and mineral size of the mica schist substratum. The previous suggestion that *X. cumberlandia* is a poor colonizer on granite surfaces (Giordani et al. 2002; Golm et al. 1993), may be explained if *X. cumberlandia* begins as a fast growing species, such as on granodiorite (Fig. 5.4A), and then becomes slower than other species, and becoming outcompeted in nature. However, the slow grow rate of *X. viriduloumbrina* on both element rich and poor substrata (Fig. 5.4) supports a stress tolerant life history strategy (Grime 2001) even though La was present at about one third its toxicity level (Mu et al. 2006) in granodiorite and mica schist (Table 5.1). The initial slow growth may also make *X. viriduloumbrina* a better competitor than *X. cumberlandia*, according to Grime (2001) because faster growing species are prone to quickly exploit their resource pools and invest in reproductive output; whereas stress tolerant species grow slower and are more persistent in their environments.

Both species showed similarly poor growth on the dolostone treatment, supporting the observations that these species do not grow on calcium rich substrata (Rizzi and Giordani 2013), but it is more likely that the total pH of the substratum, not the Ca content alone, determines the

ability to grow (Ascaso et al. 1976a; Iskandar and Syers 1972). However, the pH of biotite, granite and basalt was influenced by the addition of two secondary metabolites, stictic acid and salazinic acid (Iskandar and Syers 1972). Stictic acid, which is produced by *X. cumberlandia*, was reported to be better at chelating Ca in biotite, Ca and Mg in granite, and Ca and Mg in basalt (Iskandar and Syers 1972), while salazinic acid produced by *X. viriduloumbrina*, was better at chelating Al in solution from biotite and Fe and Al in solution from basalt. As a result, the secondary metabolite chemistry may influence pH conditions and the ability of each species to grow on substrata with different element compositions. Substrata that are more felsic, such as granodiorite, favour salazinic acid species like *X. viriduloumbrina*, but the production of salazinic acid may buffer the effects of the substrata. Substrata that are more basic, like mica schist, favour stictic acid species like *X. cumberlandia* but the production of stictic acid may buffer the effects of more felsic substrata. Substratum chemistry and the buffering effect of secondary metabolite production in these lichens may generate diversity while driving speciation in the *Xanthoparmelia* genus.

In conclusion, while *X. cumberlandia* showed a preference for mica schist agar, *X. viriduloumbrina* did not show a preference for any of the treatments. *Xanthoparmelia cumberlandia* grew faster than *X. viriduloumbrina* until week 5 to 6 when it slowed down relative to *X. viriduloumbrina*. The results support the previous classification of a ruderal life history strategy for *X. cumberlandia* and a stress tolerant life history strategy for *X. viriduloumbrina*. The different patterns of growth on granodiorite and mica schist treatments, may suggest different nutrient acquisition strategies or sensitivity to some elements such as La, and further support different life history strategies. While these controlled laboratory conditions cannot reflect the substratum variability found in nature, studies such as this one provide insights

into the establishment of lichen thalli at early stages of growth, which depend on the success of ascospore germination and the suitability of the substratum for further lichen development. Knowledge of ascospore germination and substratum type can lead to a better understanding of ecological preferences, habitat adaptation, and the potential for lichenization in the natural environment.

5.6 Acknowledgements

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CHAPTER 6

Notes on the photobionts of some *Arctoparmelia* and *Xanthoparmelia* species

This chapter has been submitted in combination with Chapter 5 to the journal *Fungal Biology

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6.1 Abstract.

The lichen symbiosis requires compatible fungal and algal partners to synthesize a thallus. Recent studies show that some lichenized photobionts may be specific to particular habitat or substrates, forming ecological guilds. Three study species, *Xanthoparmelia viriduloumbrina*, *X. cumberlandia* and *A. centrifuga*, were examined in this preliminary study to test the ecological guild hypothesis and to identify the photobiont. Algal actin sequences for 16 specimens and additional samples from Genbank were aligned and analysed by Bayesian and maximum parsimony to produce a phylogenetic tree and by haplotype network analysis. Photobionts associated with all three lichen-forming fungal species were closely related to *Trebouxia jamesii/T. simplex*, which is a new report for *A. centrifuga.* The finding confirms previous reports for the *Xanthoparmelia* species. *Xanthoparmelia viriduloumbrina* sequences and haloptypes showed low levels of specificity. Community type and geographic distribution of the photobiont showed no support for the ecological guild hypothesis. Further study may provide a better understanding of photobiont identity and algal selectivity in these lichens.

6.2 Introduction

Lichens such as *Xanthoparmelia cumberlandia, X. viriduloumbrina,* and *Arctoparmelia centrifuga* are sexually reproducing foliose lichens that grow on exposed bedrock and produce abundant apothecia. Wind dispersal of ascospores after sexual reproduction is thought to allow the spores to be dispersed further than asexual propagules (Walser 2004). However, the formation of a lichen thallus requires that fungal ascospores encounter a suitable photobiont in a suitable habitat or a new lichen thallus will fail to be established (Büdel and Scheidegger 2008). If photobionts form ecological guilds reflecting an adaptation to particular habitats and substrata (Beck 1999; Blaha et al. 2006; Helms 2003; Peksa and Škaloud 2011; Rikkinen et al. 2002), the algal species associated with lichen fungi could be predicted based on the substrata regardless of the method of reproduction by the lichen. Saxicolous species of the *Xanthoparmelia* and *Arctoparmelia* genera show substratum preference (Brodo et al. 2001; Deduke et al. in review [Chapter 4]; Deduke and Piercey-Normore in review [Chapter 5]; Giordani et al. 2002), which may be influenced by pH of the substratum (Lisci et al. 2003). While photobionts alone were shown to be influenced by pH (Blaha et al. 2006; Helms 2003; Peksa and Škaloud 2011), both pH and temperature affected the symbionts of *Cladonia rangiferina* (Athukorala and Piercey-Normore In press).

Xanthoparmelia species associate with members of *Trebouxia* spp. (Ahmadjian 1993a; Leavitt et al. 2013) but the identity of the photobiont species that associates with the more distantly related *Arctoparmelia centrifuga* (Crespo et al. 2010; Thell et al. 2012) is not known (Nash et al. 2002). *Xanthoparmelia viriduloumbrina* associates with *Trebouxia arboricola* and *T. gigantea* (Ahmadjian 1993a) while *X. cumberlandia,* being less selective than *X. viriduloumbrina,* is known to associate with the same species in addition to *T. gelatinosa/T.*

impressa (Leavitt et al. 2013). Algal selectivity is defined as the frequency of association in a lichen symbiosis depending on the availability of algae in the surrounding environment. Algal specificity relates to the taxonomic and phylogentic relatedness of photobionts in a lichen symbiosis (Honegger 2008; Yahr et al. 2006). Since photobionts with both species of *Xanthoparmelia* are closely related in the same genus (Leavitt et al. 2013) they are expected to be shared between *X. viriduloumbrina* and *X. cumberlandia*. Additionally, since these three saxicolous lichens have been reported to grow on various types of rock substratum (Brodo et al. 2001; Cloutis 1995; Deduke et al. in review [Chapter 4]; Giordani et al. 2002) the photobionts may further vary according to the rock type if the ecological guild hypothesis is supported. The purpose of this paper was to gain some preliminary insights into the identity and relationship among photobionts associated with three saxicolous lichen species and whether algal selection can be predicted by the rock substratum.

6.3 Materials and Methods

6.3.1 Collections

Lichen samples were collected from transects in northern Manitoba (N101˚34’ to N101˚22’, W55˚00’ to W54˚36’), southern Manitoba (N95˚36’ to N95˚23’, W50˚02’ to W49˚50’) and northern Ontario (N93˚15’ to N92˚09’, W50˚11’ to W49˚37’), based on the methods in (Deduke et al. 2014 [Chapter 2]). Samples were collected from the Precambrian Shield in these locations and brought back to the laboratory for identification. Ten samples of *A. centrifuga*, three samples of *X. viriduloumbrina* and three samples of *X. cumberlandia* were used in this study and were deposited in the cryptogam division of the University of Manitoba Herbarium (WIN). The three communities were defined as the open mossy rock, the grassy rock

and the treed rock communities (Deduke et al. 2014 [Chapter 2]). Rock samples were collected from field transects using a 5 lb mallet and rock chisel to remove a 10cm x 10cm block of exposed rock substratum. Rock features used in classification were foliation, mineralogy, mineral abundance and texture (Klein and Dutrow 2007). Rocks were classified into four types, used in Deduke et al. (in review [Chapter 4]): granitic rock, mafic metavolcanic rock, metasedimentary rock and a mixed sample containing granitic and mafic metavolcanic rock (for two transects that had two distinct rock types present in the transect).

6.3.2 DNA extraction, PCR, and sequencing

DNA was extracted from 5.0 mg thallus portions of the younger lobes from 16 of the same samples used in Deduke and Piercey-Normore (2014 [Chapter 3]). DNA extraction was done using cetyltrimethylammonium bromide (CTAB) extraction buffer and a modified protocol from Grube et al. (1995). Thallus samples had been previously cleaned of debris and the secondary metabolites were extracted and identified following Orange et al. (2001) with solvent A (Toluene 180 mL; Dioxane 45 mL; Glacial Acetic Acid 5 mL). The isolated DNA was further cleaned using a silica bead purification protocol (#K0513B, Fisher Scientific, Ottawa, Canada) following the manufacturer's instructions. DNA pellets were resuspended in sterile distilled water and the algal actin gene was amplified by polymerase chain reaction (PCR). Amplification of the algal actin gene was performed using two forward and two reverse actin gene primers. Because amplification and sequencing of the actin gene in these species was problematic, amplification using two internal primers was performed from the PCR product of the external primers. The external primers are: Act-1T-NS-1F (CAATCACCAGGCTAGATCTG) for the forward and Act-4T-NS-1R (GTTAATACAGCTGCACCTG) for the reverse. The internal

primers were: Act-1T-NS-2F (CTAGACTACGAGCAAGAGCT) for the forward and Act-4T-NS-2R (CGWCGTATGATGCCGTGACA) for the reverse. Actin primers were based on sequences derived from the algal-specific primers Act1T and Act4T (Kroken and Taylor 2000). PCR using the external primers consisted of 10-50 ng of DNA, 1X PCR Buffer (50 mM KCL, 100 mM Tris-HCl [pH 8.3]), 0.5 uM of each dNTPs, 0.5 µM each of two primers (forward and reverse), 0.8 µM of $MgCl_2$ and 0.5 µM of Taq DNA polymerase (Invitrogen, Burlington, ON, Canada). PCR was amplified in a T100 Thermal Cycler (Bio-Rad, Mississauga, ON, Canada). Amplification conditions for the original algal-specific primers had an initial denaturing temperature of 94˚C for 3 mintues, followed by 30 cycles of denaturing at 94˚C for 1 minute, annealing at 56˚C for 30 seconds and an extension at 72˚ for 45 seconds. Amplification conditions for the algal actin gene from the photobiont of *Xanthoparmelia* using the nested primers were the same as the algal-specific primers but the annealing temperature was at 58˚C. Amplification conditions for the actin gene from *Arctoparmelia centrifuga* were identical to the conditions used for the algal-specific primers. PCR product was agarose gel-purified with a Wizard Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. DNA was subsequently quantified on 1% agarose gels and stained using ethidium bromide.

PCR products were cycle sequenced following the manufacturer's instructions using BigDye v.3.1 (Applied Biosystems, Foster City, CA, USA). Precipitation and cleanup of the PCR product followed the EDTA protocol using the manufacturer's instructions and was resuspended in Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) prior to sequencing. Sequencing was done using a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences were edited using Sequencher (v. 4.8, Gene Codes

Corporation, Ann Arbour, MI, USA) and aligned manually using SeAl (Sequencer Alignment Editor, v. 2.0a11, University of Oxford, UK).

6.3.3 Data Analysis

The algal phylogeny was inferred using the algal actin gene sequenced from 16 thallus samples (Table 6.1). An additional 14 sequences were retrieved from NCBI Genbank as reference sequences for *Trebouxia simplex* and *T. jamesii*, which are considered to be conspecific (Friedl, 1989; Table 6.1). All nucleotide sequences generated in this study have been deposited in GenBank (Table 6.1). The phylogenetic analysis was performed using two methods, maximum parsimony and Bayesian analysis. Maximum parsimony (MP) was performed using PAUP 4.0 (Swofford 2003) with tree bisection and reconnection branch swapping, heuristic searches with 1000 random addition replicates, and bootstrap searches of 500 re-samplings (Felsenstein 1985). Bootstrap values greater than 70 are reported on the tree. One sequence (*Asterochloris erici*, AB080314) was assigned as an outgroup. *Asterochloris erici* was chosen because *Asterochloris* used to belong to the *Trebouxia* genus but was found to differ based on morphological, genetic and lichenized associations (Rambold et al. 1998; Piercey-Normore and DePriest 2001; DePriest 2004; Škaloud and Peksa 2008b). Specimens of the lichen containing *A. erici* (associated with *Cladonia cristatella*) was found growing in the same 1m x 1m quadrat among the three study species (Deduke et al. 2014 [Chapter 2]). Bayesian analysis was performed using MrBayes (v. 3.2.2 x64, Ronquist et al. 2012) based on the results of the model selection program jModelTest (v.2.0, Posada 2008). The model used was the K80 + G (gamma distribution) model (Kimura 1980), with 1,500,000 generations and a burnin frequency of 25%. Since the species are closely related and the sequence variation is low, a haplotype network was

created using TCS (v.1.21, Clement et al. 2000) with gaps set to missing data and a 90% connection limit. Two sequences (*Asterochloris erici*, AB080314; Uncultured *Trebouxia* photobiont, DQ086099) were removed from the haplotype analysis because the sequences fell outside the clade with a 95% bootstrap value in the phylogenetic tree (Fig. 6.1).

Table 6.1. Algal specimens used in this study including collection number, source of collection and accession number for GenBank.

Species	Collection no.	Source of collection	Accession no.
Arctoparmelia centrifuga	AC 7-2-0-3	Canada, Manitoba, Payuk Lake	KP322556
Arctoparmelia centrifuga	AC 7-2-3-3	Canada, Manitoba, Payuk Lake	KP322557
Arctoparmelia centrifuga	AC 7-4-0-3	Canada, Manitoba, Payuk Lake	KP322558
Arctoparmelia centrifuga	AC 8-1-0-1	Canada, Manitoba, Sherridon Road	KP322559
Arctoparmelia centrifuga	AC 8-3-2-2	Canada, Manitoba, Sherridon Road	KP322560
Arctoparmelia centrifuga	AC 8-5-1-1	Canada, Manitoba, Sherridon Road	KP322561
Arctoparmelia centrifuga	AC 8-7-0-3	Canada, Manitoba, Sherridon Road	KP322562
Arctoparmelia centrifuga	AC 8-8-0-2	Canada, Manitoba, Sherridon Road	KP322563
Arctoparmelia centrifuga	AC 8-9-1-1	Canada, Manitoba, Sherridon Road	KP322564
Arctoparmelia centrifuga	AC 9-3-0-1	Canada, Manitoba, Highway 10	KP322565
Xanthoparmelia cumberlandia	XC 1-3-3-2	Canada, Manitoba, Rennie	KP322566
Xanthoparmelia cumberlandia	XC 2-1-1-2	Canada, Ontario, Red Lake	< 200 BP
Xanthoparmelia cumberlandia	XC 2-3-2-1	Canada, Ontario, Red Lake	KP322567
Xanthoparmelia viriduloumbrina	XV 2-2-2-2	Canada, Ontario, Red Lake	KP322568
Xanthoparmelia viriduloumbrina	XV 2-3-3-1	Canada, Ontario, Red Lake	KP322569
Xanthoparmelia viriduloumbrina	XV 2-9-3-1	Canada, Ontario, Dryden	KP322570
Trebouxia simplex	GenBank	Muggia *et al.* 2010	HM046943
Trebouxia jamesii	GenBank	Fernández-Mendoza *et al.* unpublished	HM573593
Trebouxia jamesii	GenBank	Fernández-Mendoza *et al.* unpublished	HM573595
Trebouxia jamesii	GenBank	Fernández-Mendoza *et al.* unpublished	HM573598
Trebouxia jamesii	GenBank	Fernández-Mendoza *et al.* unpublished	HM573600
Trebouxia jamesii	GenBank	Fernández-Mendoza *et al.* 2011	GQ375388
Trebouxia jamesii	GenBank	Fernández-Mendoza *et al.* 2011	GQ375393
Trebouxia jamesii	GenBank	Fernández-Mendoza *et al.* 2011	GQ375407
Trebouxia jamesii	GenBank	Fernández-Mendoza *et al.* 2011	GQ375309
Trebouxia jamesii	GenBank	Domascke and Printzen unpublished	GQ375410
Trebouxia jamesii 'vulpinae'	GenBank	Taylor and Kroken unpublished	AY005404
Uncultured	GenBank	Piercey-Normore 2006	DQ086099

photobiont			
Uncultured photobiont	GenBank	Piercey-Normore 2006	DQ086100
Asterochloris erici	GenBank	Yamamoto *et al*. 2003	AB080314

6.4 Results

The results of the Baysian analysis and maximum parsimony trees were found to be consistent. The sequence alignments were a maximum of 268 characters long and the gene region belonged to the actin type I gene (according to GQ375388, NCBI GenBank). The Bayesian phylogenetic tree shows that algal sequences from all specimens in this study cluster with *T. jamesii* and *T. simplex* with 95% bootstrap support and greater than 90% posterior probability (Fig. 6.1). The algae from all three species, *A. centrifuga*, *X. cumberlandia* and *X. viriduloumbrina* were scattered throughout the 95% cluster. The algal sequences from *A. centrifuga* samples 8-1-0-1 and 7-4-0-3 formed a strongly supported (98% bootstrap; > 90% posterior probability) nested cluster with four samples of *T. jamesii* and one of *T. simplex* (Fig. 6.1). Two algal sequences from *X. viriduloumbrina* (2-2-2-2 and 2-3-3-1) formed a strongly supported (>90% posterior probability) cluster separate from the other sequences. The remaining algal sequences from *A. centrifuga*, *X. viriduloumbrina, X. cumberlandia* and an uncultured *Trebouxia* photobiont from *Evernia mesomorpha* collected in southern Manitoba (Piercey-Normore 2006b) were unresolved within the larger cluster.

The rock types and community types are scattered throughout the tree, except for the two sequences from *A. centrifuga* (7-4-0-3 and 8-1-0-1) in the nested cluster that are from treed communities and the two clustered sequences of *X. viriduloumbrina* (2-2-2-2 and 2-3-3-1) which were collected from granitic substrata. The geographic location of the algal sequences are also scattered throughout the tree except for the two sequences of *A. centrifuga* (7-4-0-3 and 8-1-0-1) and *X. viriduloumbrina* (2-2-2-2 and 2-3-3-1).

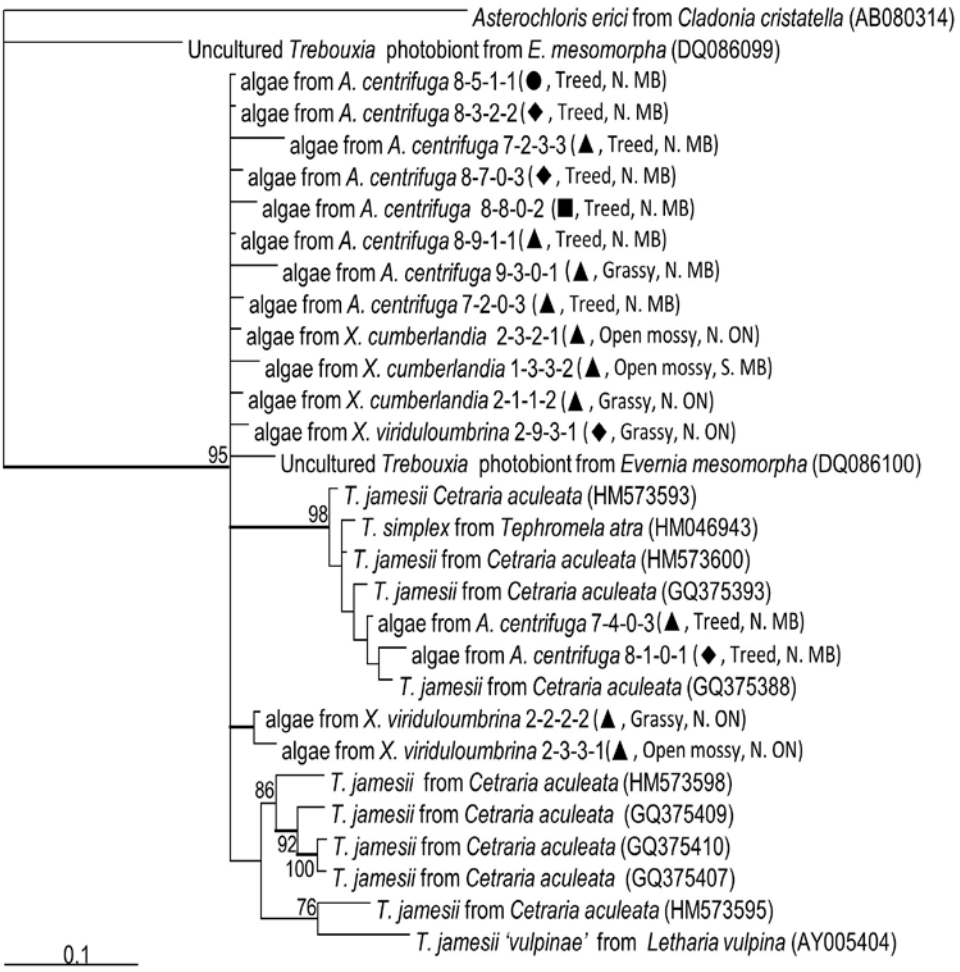

Figure 6.1. Bayesian phylogenetic tree showing evolutionary relationships among the algae associated with three fungal species using the actin gene. The substratum type, community type and geographic location are indicated on the tree. Other algal actin sequences are included from specimens of lichenized *Evernia mesomorpha*, *Cetraria aculeata*, *Letharia vulpina* and *Tephromela atra*. The outgroup is from an algal actin sequence of lichenized *Cladonia cristatella*. The substratum type is indicated by the shapes with granitic rock indicated by a black triangle; mafic metavolcanic rock indicated by a black diamond; metasedimentary rock indicated by a black circle; and granitic and mafic metavolcanic rock indicated by a black square. The community type is indicated by Open mossy for open mossy rock community; Grassy for grassy rock community; and Treed for treed rock community as in Deduke et al. (2014 [Chapter 2]). Geographic location is indicated by N. MB for northern Manitoba; S. MB for southern Manitoba and N. ON for northern Ontario. Values on the branches indicate the bootstrap values greater than 70% from the maximum parsimony analysis. The posterior probability values are indicated by thick black lines (> 95%).

The haplotype network is comprised of 24 distinct haplotypes (Fig. 6.2) and showed a pattern of relationships similar to the phylogenetic tree. There were two haplotypes that had more than one individual making up the haplotype (larger circles). These two haplotypes were from *A. centrifuga* (8-3-3-2, 8-5-1-1, 8-7-0-3 and 8-9-1-19) and *X. cumberlandia* (2-1-1-2 and 2-3-2-1). The majority of algal actin haplotypes were closely related with the exception of two *A. centrifuga* haplotypes ((7-4-0-3 and 8-1-0-1) blue circles in the far left lineage). These two *A. centrifuga* photobiont haplotypes were closely related to the algae, *T. simplex* (gray circle) and *T. jamesii* (brown circle), which were associated with the lichen fungi, *Tephromela atra* and *Cetraria aculeata*, respectively. Rock substratum showed no apparent association with algal haplotype relationships or fungal species.

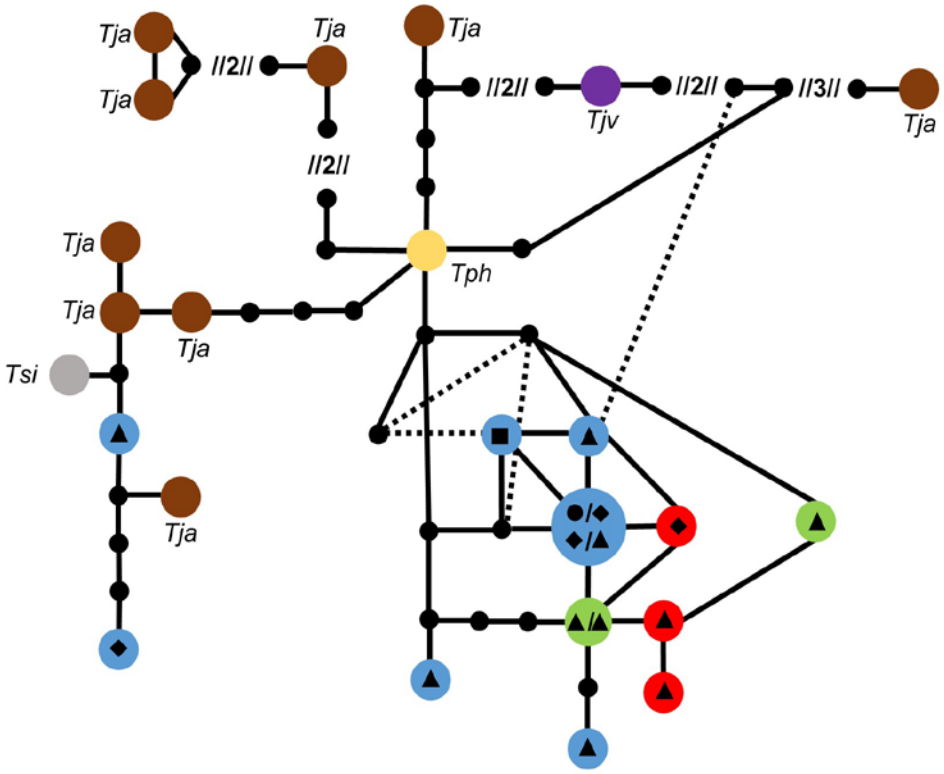

Figure 6.2. Haplotype network showing the results of the algal actin gene for lichenized photobionts in *A. centrifuga* (blue), *X. viriduloumbrina* (red), *X. cumberlandia* (green), *E. mesomorpha* (yellow), *C. aculeata* (brown), *Letharia vulpina* (purple), and *Tephromela atra* (gray). The substratum type is indicated by the shapes overlaying the blue, red and green dots, with granitic rock indicated by a black triangle; mafic metavolcanic rock indicated by a black diamond; metasedimentary rock indicated by a black circle; and granitic and mafic metavolcanic rock indicated by a black square. The double slash with numbers indicate the number of differences between haplotypes. Black circles interconnected by lines indicate differences between haplotypes. The dashed lines represent differences between distantly haplotypes in the network. *Tja* represents *Trebouxia jamesii*; *Tjv* represents *T. jamesii 'vulpinae'*; *Tsi* represents *T. simplex*.

6.5 Discussion

The algal partner that associates with each of the three lichen fungal species in this study is closely related to *T. jamesii* and *T. simplex* (Fig. 6.1). This is consistent with findings from Leavitt et al. (2013) for *X. cumberlandia* and from Ahmadjian (1993a) for *X. viriduloumbrina* (renamed from *X. somloënsis* in Lendemer 2005). The identity of the alga associated with *A. centrifuga* is a new finding, with the photobiont only previously thought to belong to the *Trebouxia* genus (Nash et al. 2001). *Arctoparmelia centrifuga* associates with algal species closely related to *Trebouxia jamesii* and *T. simplex*, which fall within the *Trebouxia gigantea/T.arboricola* clade (Leavitt et al. 2013).

This study also suggests no specificity between the photobiont and the three fungal species despite the large phylogenetic difference between the genera *Xanthoparmelia* and *Arctoparmelia* (Blanco et al. 2006; Crespo et al. 2007; Crespo et al. 2010; Thell et al. 2012). The genus *Arctoparmelia* was placed among the hypogymnioid type of lichens within the Parmeliaceae because of features in the medulla, secondary compounds (with the exception of usnic acid), and a different type of the polysaccharide, lichenan, in the wall of the fungal hyphae (Blanco et al. 2004), which are different from those in *Xanthoparmelia*. Because of these physiological differences in the two genera, the photobiont may have also been expected to be separated. However, Beck et al. (1998) showed that algae classified as *T. jamesii* associate with both parmelioid and hypogymnioid type lichens.

The photobiont guild hypothesis (Ohmura et al. 2006; Peksa and Škaloud 2011; Rikkinen et al. 2002; Yahr et al. 2006) was not supported by the findings in this study. Even though the distribution of rock substratum was diverse, ranging from acidic rock types such as granite or schist to more basic rock types such as basalts (Figs. 6.1 and 6.2; Deduke et al. in

review [Chapter 4]; Giordani et al. 2002), the algal sequence did not correspond with rock type. Two sequences of *X. viriduloumbrina* (2-2-2-2 and 2-3-3-1) were different from the majority of sequences based on a posterior probability value of 99% (Fig. 6.1). These samples also showed similar substrate preferences for granitic rock and were closely related haplotypes (Fig. 6.2).

Community type was not separated in the phyologenetic tree (Fig. 6.1). The two separated *X. viriduloumbrina* photobionts (2-2-2-2 and 2-3-3-1) are geographically close together but were in different communities, the open mossy rock and grassy rock communities (Deduke et al. 2014 [Chapter 2]). These communities are associated with open granite outcrops with small amounts of vegetation compared to the treed rock community with denser vegetation (Ahti and Oksanen 1990; Burbanck and Platt 1964; Burbanck and Phillips 1983; Deduke et al. 2014 [Chapter 2]; Oosting and Anderson 1939). These two exposed communities are also associated with more direct sunlight due to less deciduous and coniferous tree cover (Deduke et al. 2014 [Chapter 2]). If the environment influenced the available algal pool and hence the fungal selction of the photobiont, community type would have separated with photobiont in the tree. Peksa and Škaloud (2011) showed consistent results where clades of *Asterochloris* separated based on altitude and substratum type. Piercey-Normore (2004) showed species-specific photobiont selectivity and specificity for three species of *Cladonia* in similar geographic locations to this study. Larger sample sizes are needed from photobionts associated with all three fungal species to better understand the relationships between photobionts, geographic location, substratum and community types.

In conclusion, this study identified the *Trebouxia* photobiont for *A. centrifuga* by similarity of the actin sequence to known sequences of *T. jamesii*. It further corroborated previous findings that photobionts for the *X. viriduloumbrina* and *X. cumberlandia* are closely

associated with *T. jamesii* and *T. simplex.* While the actin gene sequence is not known to be the best gene for species determination or barcoding in algae (Buchheim et al. 2011; Saunders and Kucera 2010; Saunders and McDevit 2012), it provides an indication of similarity in identity and has been used to infer phylogenetic relationships in other studies (Fernández-Mendoza et al. 2011; Muggia et al. 2010; Piercey-Normore 2006b; Piercey-Normore 2009; Taylor and Kroken 2000). Other suggested genes include the nuclear ribosomal large subunit (LSU) in the D2/D3 regions as a general eukaryotic barcode and the translation unstable factor for the protein EF-Tu 1 (*tufA*) for green algae (Saunders and McDevit 2012). Photobionts did not show strong preferences for substratum or community type suggesting no support for a photobiont guild for these three lichens. This study provides preliminary evidence for a low level of selectivity among these lichen species based on fungal species, geographic region, and type of substratum. However, this study is also limited by small sample sizes and more research is needed to understand algal selection in these saxicolous lichens. A more appropriate sample size would be minimally 300 samples per species for a landscape level study according to estimates using the rarefaction method for determining optimal sample sizes (Werth 2010).

6.6 Acknowledgements

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CHAPTER 7

Final Discussion and Conclusion

This thesis represents a synergistic model between numerous abiotic and biotic factors influencing the lichen symbiosis (Fig. 7.1). The model conveys aspects of the CSR theory, trade-offs through the Optimal Defence Theory and Carbon Nutrient Balance Hypothesis and the Ecological Guild Hypothesis. The model shows a complex, interconnected network of inputs and outputs revealing novel findings, which help to explain *Arctoparmelia* and *Xanthoparmelia* predominance in exposed bedrock outcrops and their interactions with the surrounding environment. Inputs to the model include the combination of substratum composition, pool of free-living algae, community composition surrounding the lichen resulting in *Arctopamerlia* and *Xanthoparmelia* thalli which express the complex symbiosis with specific secondary metabolites, sexual fecundity levels, and photobiont selectivity as outputs. The result of this information is characterization of life history strategies representing an overview of how these particular lichens interact with their surroundings. While the model was created using saxicolous lichens, the components of the model may be used to address other lichen species in their habitats with their unique biology. The model accounts for the interactions between various abiotic and biotic factors affecting the lichen symbiosis, but does not present the relative weight of the factor. Given the multitude of interactions, it is expected that particular factors will exert greater or lesser influence on the lichen symbiosis. It is also expected that given a different habitat or species of lichen, the weights of the particular factors will not remain consistent between models. Substratum influences the fungal establisment before the formation of a lichen thallus, the potential photobiont partner, the thalline synthesis and combined coevolutionary relationships.

Community, in part, determines the life history strategy, the competitors and allies. The biotic environmental variables influence humidity, light levels and temperature among other important factors. Internally, the fungus regulates nutrient uptake from the substratum while the photobiont produces photosynthates. These inputs are controlled by genetic make-up and the environmental conditions to meet the biological demands of growth, reproduction and defence, sometimes resulting in potentially competing demands due to resource scarcity.

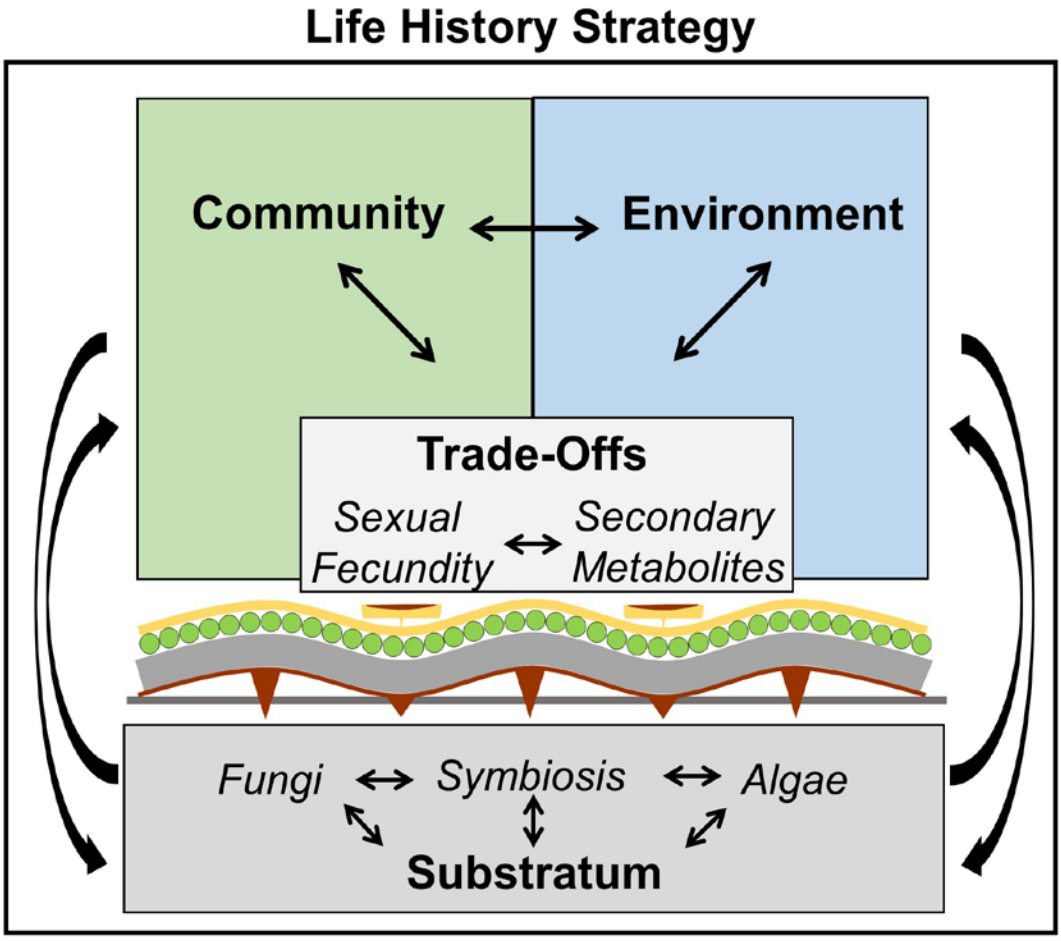

Figure 7.1. A model representing the synergistic interaction between abiotic and biotic factors that influence the lichen symbiosis in an environment. A characterization of life history strategy and effects on the symbionts can be derived using data from the ecological community, environmental variables, lichen biology, and composition of the substratum.

This study showed that rock outcrops on the Precambrian Shield in Manitoba and northern Ontario form distinct lichen assemblages based on lichen diversity and species composition of a community focused around a single genus (Chapter 2). The three species assemblages are distinct and significantly different from one another, further corresponding with communities comprised of lichen assemblages as well as surrounding biotic and abiotic features (Chapter 2). These communities reflect previously known successional trajectories for outcrop habitats (Ahti 1977; Ahti and Oksanen 1990; Burbanck and Phillips 1983; Burbanck and Platt 1964; Oosting and Anderson 1939) and correspond with the middle to late stages of colonization and succession of rock outcrops for saxicolous lichens (Ahti 1977). The earlier stage of colonization, which was not covered in this study, is thought to be dominated by crustose lichens. Further research into the ecology of crustose lichens would be expected to describe more clearly the transition from crustose to foliose and fruticose lichen dominated habitats.

The application of life history strategies to each of three lichen species, despite them being morphologically and taxonomically similar, with quantifiable variables was unusual and now forms a basis on which other species can be characterized. The life history strategies in these lichens further build on an established CSR theory by Grime (1977, 2001), and focus on individual species and their characters. *Arctoparmelia centrifuga*, *Xanthoparmelia viriduloumbrina* and *X. cumberlandia* were classified as competitor, stress tolerant and ruderal generalist, respectively (Chapter 2), which refined the knowledge of life history strategies for lichens beyond the generalized stress tolerant strategy as suggested for all lichens (Grime 2001). Differences in life history strategies were based on fecundity (germinating ascospores, number of apothecia and ascospores), thallus percent cover of the substratum, secondary metabolites, and community composition. The use of this approach opens new directions for characterizing life

history strategies in lichens, paralleling similar strategies applied to fungi, non-vascular and vascular plants (During 1979; Grime 1977, 2001; Pugh 1980). A comprehensive analysis of life history strategies was produced by During (1979) for bryophytes, where species were classified according to a bryophyte specific system summarizing large amounts of research in anatomy and physiology, growth, sexual and asexual reproduction, life span and ecology. The resulting classification created a species profile that can be used to address more complex questions on their ecological roles, allows for comparative studies among species, and it forms a foundation for further studies on species-at-risk. Previous approaches have been applied in a limited scope to lichens, concentrating on differences in growth rates and growth forms at the family level and on habitat type (Rogers 1990; Topham 1977), but not relating life history to measurable variables as in this thesis.

The proposed life history strategies in this thesis were further strengthened by evidence for trade-offs in *X. cumberlandia* because the patterns of sexual fecundity and secondary metabolite production were consistent with known patterns for the ruderal life history strategy. The use of secondary metabolites to characterize life history strategies also allowed support for the Optimal Defence Theory in *X. cumberlandia* because higher quantities of sticitic acid were found in apothecial tissue compared to thalline tissue (Chapter 3). The larger amount of stictic acid in a reproductive tissue implies the tissue is being protected where stictic acid has been shown to possess potential anti-herbivory qualities (Gauslaa 2005; Lawrey 1980a). Ruderal species are known as fast growing (Grime 2001) and secondary metabolites are shown to provide a number of benefits to a lichen including defence against herbivory and other environmental factors (Lawrey 1986). Further research may lead to testing the allocation and quantification of

stictic acid between the "tissue" types with a broader sampling of species and measurable habitat variables.

The effect of the rock substratum on lichen communities and their biology (Chapter 4), where the *Xanthoparmelia* species were more often found on felsic rock types (granitic and metasedimentary rocks), while *Arctoparmelia centrifuga* occured more often on the mafic metavolcanic rock substratum, is a new and innovative research direction in lichenology. These findings coincide with previous research for *X. viriduloumbrina* (syn. *X. somloënsis*, Lendemer 2005) with its prevalence on felsic rocks (Giordani et al. 2002), but the findings were more complex for *X. cumberlandia*. In this study, *X. cumberlandia* occurred more often on granitic than mafic metavolcanic rock. Previous studies suggested that *X. cumberlandia* was not well suited for growth on granitic substratum (Giordani et al. 2002; Golm et al. 1993), as it has been found more often on mafic substrata (Giordani et al. 2002). In this study, *X. cumberlandia* was found on a high proprotion of mafic metavolcanic rock compared to *X. viriduloumbrina*. Elemental analysis showed that the two species of *Xanthoparmelia* were associated with different suites of elements. Percent cover for *X. viriduloumbrina* correlated postively with La and negatively with Sr, while ascospore production was positively correlated with Mn and Y. In contrast, *X. cumberlandia* has higher percent cover on transects positively correlated with Ag and negatively correlated with Sr. Apothecia production was positively correlated with Co, Fe, Mn and Y.

Rock type and elemental abundance showed some additional relationships with secondary metabolite quantities. The significant effect of rock type and species on the quantity of usnic acid (Table A.1) may be supported if usnic acid helps control acid tolerance of saxicolous lichens (Hauck and Jürgens 2008). Additionally, rock types have different pH levels resulting

from their element and mineral contents, with usnic acid showing different levels of success in extracting elements from rocks and minerals (Ascaso and Galvan 1976a; Iskandar and Syers 1972). The overall importance of this difference in usnic acid production relative to genera/species and rock type could be explained by metal homeostasis (the ability to continue normal cellular functioning without experiencing metal toxicity (Hauck 2008)), which is thought to be controlled by secondary metabolites. This is supported by other research showing that lichens bio-accumulate metal ions in their thalli and store these ions in different locations within the lichen thallus (Clark et al. 1999, 2001). Other secondary metabolites have been shown to have the ability to control the cellular uptake of metal elements, including atranorin, but it has not been tested for usnic acid (Hauck and Huneck 2007). If usnic acid is also responsible for metal homeostasis, the species-specific correlations found with different metal elements (Table A.2) would result from the mineral composition of the substratum. It would also explain some of the variation in usnic acid production found for *X. viriduloumbrina* between granitic and mafic metavolcanic rock (Fig. A.1). Metal homeostasis could also be applied to the secondary metabolites norstictic and stictic acid found in *X. cumberlandia* because these compounds have also been shown to extract elements from rocks and minerals (Ascaso and Galvan 1976a; Iskandar and Syers 1972), exhibit significant differences in quantity based on rock type (Figure A.1), and correlate with different elements (Table A.2).

The mycobiont preference for substratum using ascospores and crushed rock samples (Chapter 5) is another novel study that contributes to the understanding of the adaptability of these three species to the rock substratum and serves as a model for studies in other species. *Xanthoparmelia viriduloumbrina* grew fastest on granodiorite rock during the middle stages of growth but, in general, it grew equally well on different substrata. *Xanthoparmelia cumberlandia*

grew significantly faster than *X. viriduloumbrina* and it grew best on mica schist. The fast growth in culture on a non-preferred substratum may suggest that other environmental factors play a role in the growth of *X. viriduloumbrina.* Faster growth of *X. cumberlandia* is consistent with the life history classification for ruderal species (Chapter 2). Slower growth lends support to the stress tolerant classification of *X. viriduloumbrina*. The phylogenetic relationship among *Xanthoparmelia* species, which has over 800 species (Crespo et al. 2007) is currently being examined by other researchers where the use of chemotypes was considered for organizing the species (Leavitt et al. 2011a). Substratum preference and ecology were not strongly addressed in previous studies (Leavitt et al. 2011a, 2011b); however, the application of life history strategies and substratum preference based on the results from this thesis may provide further discriminatory power for organizing this large and convoluted genus.

The preliminary findings on the identification and selection of the photobionts of *A. centrifuga*, *X. viriduloumbrina* and *X. cumberlandia* contributed to an understanding of the role of the photobiont, which was interpreted in this study to be part of the environment, and its potential influence on the mycobiont (Chapter 6). The results show that photobionts of all three lichens were closely related to *Trebouxia jamesii*, corresponding with previous studies of the *Xanthoparmelia* photobionts (Ahmadjian 1993a; Leavitt et al. 2013; Lendemer 2005), but identification of the photobiont for *A. centrifuga* was a new finding. The photobiont guild hypothesis (Peksa and Škaloud 2011; Rikkinen et al. 2002) was not supported by this study, but the small sample size may have limited the ability to make meaningful interpretations. The clustering of the *Xanthoparmelia* and *Arctoparmelia* photobionts was not consistent with that of substratum or community type except for two *X. viriduloumbrina* sequences (Chapter 6). Although these comparative studies have previously focused on the actin gene for *T. jamesii*,

actin has been found to have two distinct paralog clades in algae with significantly different evolutionary rates, undermining the assumption that it is a highly conserved genetic marker (Wu et al. 2009). Phylogenetic relationships outside of this species group have used the ITS gene (Leavitt et al. 2013), but may not be variable enough as it has been proposed as a barcoding marker for algae (Buchheim et al. 2011). An algal based inventory using an appropriate marker would provide valuable baseline information on the large number of species in the Umbilicariaceae and Parmeliaceae families that prefer acid rock substrates (Hale 1967). In addition, a similar use of herbarium samples with collection and habitat information may also address theories about algal selectivity, specificity and ecological guilds in a number of lichen genera across a large geographic area.

In conclusion, the results of this thesis show relationships between the life history strategies, biology, ecological communities and substratum preferences for *A. centrifuga*, *X. viriduloumbrina* and *X. cumberlandia*. *A. centrifuga* is a competitive species that prefers treed rock communities, producing abundant ascospores and growing on mafic metavolcanic rocks. *X. viriduloumbrina* is stress tolerant, a slow growing generalist which prefers granitic rocks high in La and Pb. Finally, *X. cumberlandia* is a fast growing ruderal species that prefers grassy rock communities underlain by substratum abundant in Fe and Mn. Furthermore, substratum low in Fe and Mn could potentially result in elevated quantities of stictic acid, which would result in a significant decrease in sexual fecundity in *X. cumberlandia*. The model describing the synergistic interactions between environmental and biological variables can also be applied to other lichens and their habitats. The development of life history strategies for specific lichens, is a novel approach to an old question which can only result in better representation of lichen biology and ecology. This relatively new focus on community and substratum questions in lichens can

address further questions on colonization, competition, algal selectivity and specificity, coeveolution and the adaptability of lichen symbioses in general.

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Appendix A: Effects of Rock Type and Element Composition on Lichen Secondary Metabolites

Table A.1. Relationship between each of rock type and species with quantity of usnic acid based on a two-way ANOVA.

Two-Way ANOVA			
	F	d.f.	*p*
Usnic Acid			
Rock Type	3.2355	3	0.0240
Species	33.4096	2	<0.0001
Rock Type*Species	18.8156	5, 151	<0.0001

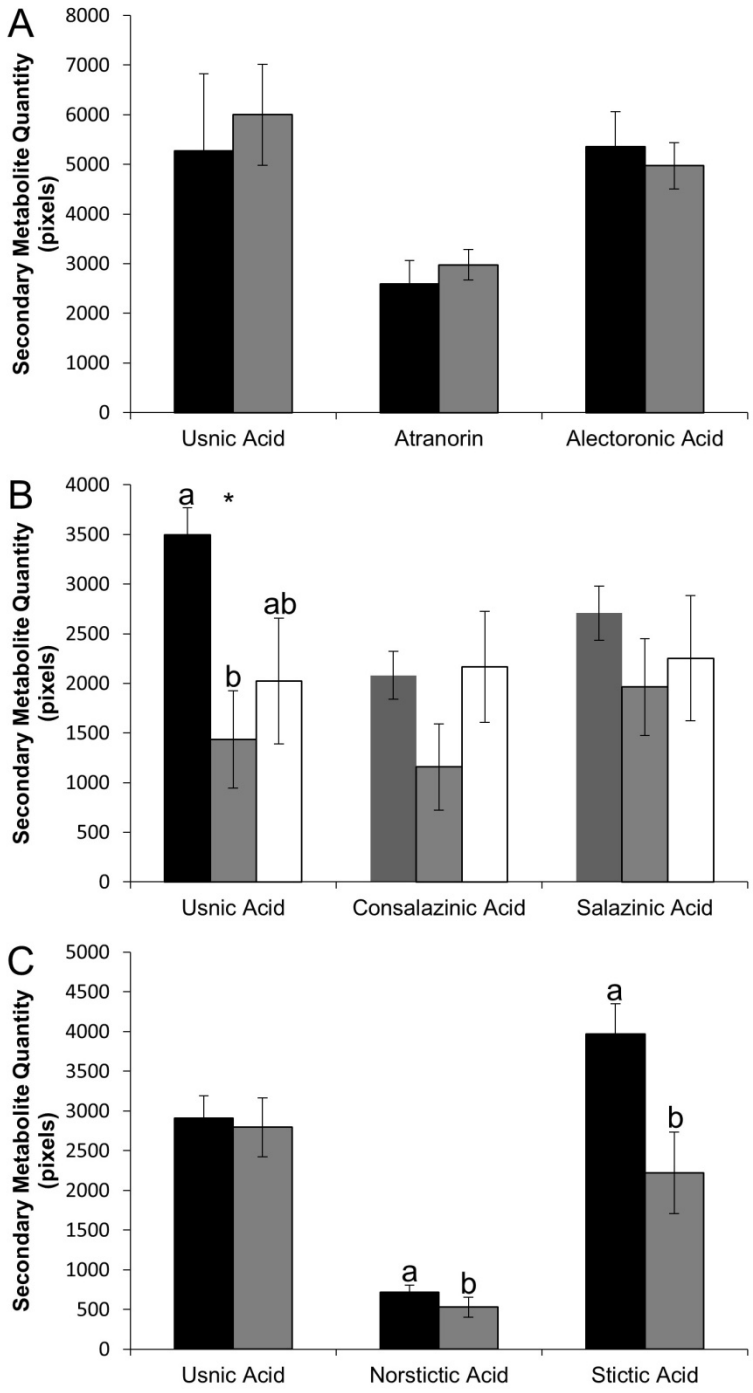


Figure A.1. Comparison between secondary metabolite quantities and rock types for transects with *Arctoparmelia centrifuga* (A); *Xanthoparmelia viriduloumbrina* (B) and *X. cumberlandia* (C) using a Kruskal-Wallis test. Rock types are Granitic (Black); Mafic Metavolcanic (Grey); and Metasedimentary (White). Rock types were excluded if their n < 2. Lower case letters denote significant differences based on a Steel-Dwass test. A Bonferroni correction of $p < 0.016$ (3/0.5) was used and significance is indicated by an asterisk.

Table A.2. Correlations between element concentration and secondary metabolite quantities for each species based on Spearman's Rho. Significant relationships are $p \leq 0.05$ and denoted by an asterisk. A Bonferroni correction was calculated for all 21 elements and set at $p < 0.002$. A significant finding for the Bonferonni correction was noted with two asterisks. See text for element abbreviation. N.A. indicates that there was no variation present for this element.

Element	Secondary Metabolites		
A. centrifuga (n = 11)	Usnic Acid	Atranorin	Alectoronic Acid
Ag	N.A	N.A	N.A
Ca	-0.5103	-0.4601	0.0820
Co	-0.1139	0.1230	-0.3098
Cr	0.0273	0.2455	-0.2636
Cu	-0.5435	-0.3146	-0.0477
Fe	0.3000	0.2091	-0.0818
K	0.4018	0.6073*	0.1370
La	0.7265*	0.6107*	-0.0316
Mg	-0.0137	0.1777	-0.0774
Mn	0.5909	0.3091	-0.0364
Na	-0.1273	-0.1818	-0.4000
Ni	0.0319	0.3280	-0.2415
P	0.4364	0.5818	0.2909
Pb	-0.0792	-0.1320	-0.1426
S	-0.5756	-0.4172	-0.2852
Sc	0.3582	0.3117	-0.1256
Sr	-0.4954	-0.4312	0.0092
Te	0.6080*	0.3518	0.4020
V	-0.0636	0.2091	-0.2273
Y	0.7127*	0.5839	0.3724
Zn	0.3000	0.3727	0.0364
X. viriduloumbrina (n=24)	Usnic Acid	Consalazinic Acid	Salazinic Acid
Ag	0.1756	0.0154	0.4765
Ca	-0.2005	-0.2392	-0.1444
Co	-0.5646*	-0.2367	-0.1647
Cr	-0.5107*	0.0270	-0.1901
Cu	-0.1338	-0.2127	-0.0362
Fe	-0.6591* /**	-0.4791*	-0.0200
K	-0.3900	0.1819	0.0422
La	0.3576	0.3935	0.4250*
Mg	-0.6401* /**	-0.2440	-0.1722
Mn	-0.2740	-0.2087	0.1692
Na	-0.4675*	-0.1722	0.0078
Ni	-0.3993	0.0545	-0.2794
P	0.0113	0.2003	0.0849
Pb	0.3200	0.3069	0.0192
S	-0.1260	-0.3235	-0.0815
Sc	-0.5397*	-0.1955	0.0958
Sr	-0.2574	-0.1304	-0.4365
Te	-0.0469	0.3421	-0.0368
V	-0.7253*	-0.3041	-0.1986

	/**		
Y	0.0066	0.0953	0.4011
Zn	-0.3650	-0.0078	0.0605

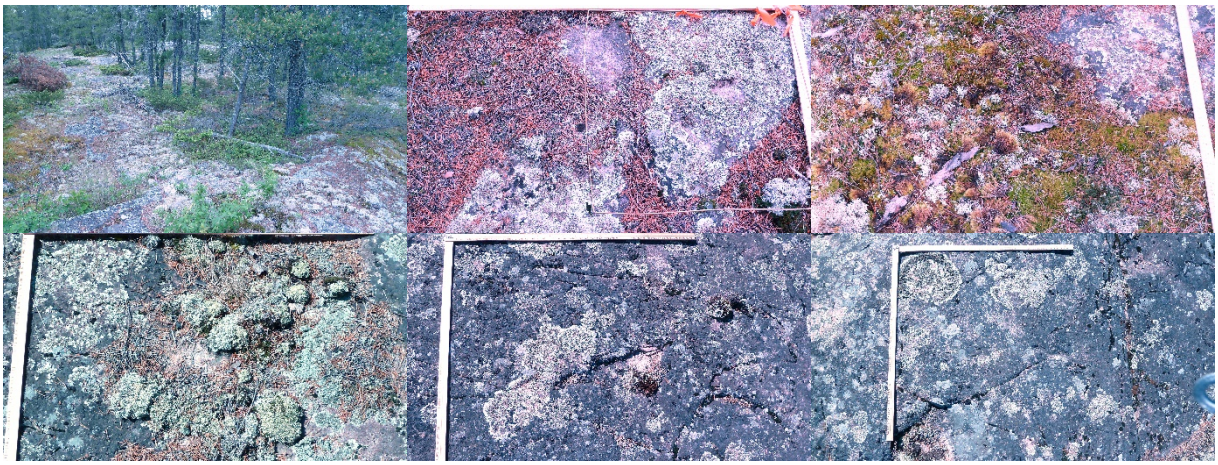
X. cumberlandia (n = 27)	Usnic Acid	Norstictic Acid	Stictic Acid
Ag	-0.3001	0.1945	-0.2558
Ca	-0.1673	-0.3929*	-0.4433*
Co	-0.1153	-0.4024*	-0.5459*
Cr	-0.1906	-0.3726	-0.2715
Cu	-0.1185	-0.2665	-0.2352
Fe	-0.1862	-0.2784	-0.6494*
			/**
K	0.2933	0.1995	0.3006
La	-0.0285	0.2700	0.1198
Mg	-0.0339	-0.4137*	-0.4852*
Mn	-0.1578	-0.1551	-0.5538*
Na	0.0241	0.0834	0.1670
Ni	-0.1260	-0.1606	-0.2326
P	-0.0596	0.1530	-0.1557
Pb	-0.1377	-0.0170	0.0088
S	-0.2552	-0.1264	-0.4248*
Sc	-0.1352	-0.3130	-0.4069*
Sr	-0.1579	-0.3506	-0.2596
Te	0.2429	-0.0556	0.1257
V	-0.2334	-0.2835	-0.5071*
Y	-0.0990	0.1934	-0.2820
Zn	-0.0156	-0.0156	-0.1130

Appendix B: Location Information of Study Transects From Manitoba and Ontario Used in This Thesis

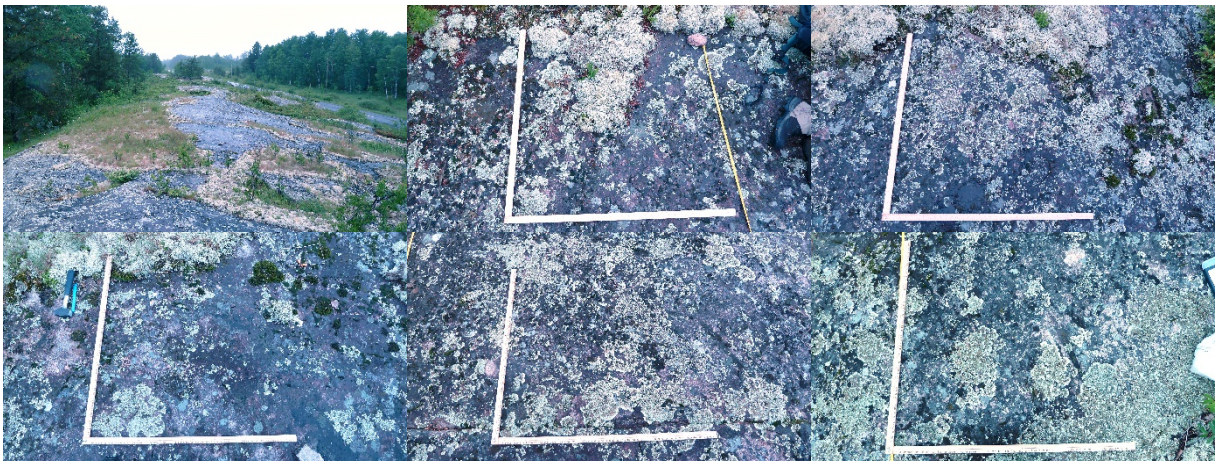
Transect	Latitude/Longitude	GPS Coordinates (NAD 83) ~ 6 m error	Geographic Location	Community Type (Chapter 2)
1.1	49°50'13.96"N 95°23'11.05"W	15 U 0328405 5523266	Southern Manitoba	Open mossy rock
1.2	49°51'11.00"N 95°29'17.35"W	15 U 0321148 5525266	Southern Manitoba	Open mossy rock
1.3	49°51'54.22"N 95°35'52.51"W	15 U 0313305 5526869	Southern Manitoba	Open mossy rock
1.4	49°50'19.93"N 95°23'47.80"W	15 U 0327677 5523747	Southern Manitoba	Open mossy rock
2.1	50°11'47.90"N 93°12'30.32"W	15 U 0485124 5560515	Northern Ontario	Grassy rock
2.2	50°10'0.93"N 93°15'11.06"W	15 U 0481926 5557221	Northern Ontario	Grassy rock
2.3	50° 6'36.00"N 93°15'40.30"W	15 U 0481351 5547879	Northern Ontario	Open mossy rock
2.4	49°57'40.09"N 92° 9'30.55"W	15 U 0560357 5534649	Northern Ontario	Grassy rock
2.5	49°57'28.83"N 92°10'58.58"W	15 U 0558607 5534282	Northern Ontario	Grassy rock
2.6	49°56'5.65"N 92°14'8.56"W	15 U 0554839 5531669	Northern Ontario	Grassy rock
2.7	49°37'47.35"N 92°45'40.48"W	15 U 0517242 5497501	Northern Ontario	Grassy rock
2.8	49°40'50.55"N 92°53'33.46"W	15 U 0507746 5503137	Northern Ontario	Grassy rock
2.9	49°45'13.25"N 92°54'51.09"W	15 U 0506181 5511248	Northern Ontario	Grassy rock
2.10	49°47'8.39"N 93° 4'39.16"W	15 U 0494418 5514803	Northern Ontario	Open mossy rock
3.1	50° 2'6.77"N 95°30'30.03"W	15 U 0320376 5545564	Southern Manitoba	Open mossy rock
3.2	49°56'13.18"N 95°31'1.97"W	15 U 0319373 55434667	Southern Manitoba	Open mossy rock
4.1	51° 2'7.61"N 95°44'34.03"W	15 U 0307702 5657352	Southern Manitoba	Grassy rock
4.2	51° 0'3.88"N 95°24'23.05"W	15 U 0331158 5652704	Southern Manitoba	Grassy rock
5.1	50°46'15.43"N 95°17'38.32"W	15 U 0338250 5626866	Southern Manitoba	Grassy rock
5.2	50°40'42.07"N	15 U 0332630	Southern	Open mossy rock

	95°22'8.45"W	5616378	Manitoba	
5.3	50°34'19.17"N 95°26'16.17"W	15 U 0327380 5606071	Southern Manitoba	Open mossy rock
7.1	54°37'53.38"N 101°32'0.40"W	14 U 0336475 6056738	Northern Manitoba	Treed rock
7.2	54°38'29.10"N 101°30'41.77"W	14 U 0337924 6057791	Northern Manitoba	Treed rock
7.3	54°37'45.86"N 101°33'5.20"W	14 U 0335305 6056548	Northern Manitoba	Treed rock
7.4	54°38'26.06"N 101°33'24.85"W	14 U 0334998 6057803	Northern Manitoba	Treed rock
7.5	54°38'8.15"N 101°33'16.31"W	14 U 0335131 6057244	Northern Manitoba	Treed rock
8.1	54°41'1.10"N 101°30'26.58"W	14 U 0338364 6062478	Northern Manitoba	Treed rock
8.2	54°41'34.64"N 101°31'17.26"W	14 U 0337494 6063547	Northern Manitoba	Treed rock
8.3	55° 1'8.38"N 101°27'2.17"W	14 U 0343329 6099654	Northern Manitoba	Treed rock
8.4	55° 0'32.78"N 101°26'58.93"W	14 U 0343348 6098552	Northern Manitoba	Treed rock
8.5	54°59'11.43"N 101°27'30.50"W	14 U 0342699 6096058	Northern Manitoba	Treed rock
8.6	54°52'2.57"N 101°28'26.46"W	14 U 0341236 6082841	Northern Manitoba	Grassy rock
8.7	54°50'42.63"N 101°28'48.76"W	14 U 0340751 6080385	Northern Manitoba	Treed rock
8.8	54°49'26.68"N 101°27'59.88"W	14 U 0341540 6078007	Northern Manitoba	Treed rock
8.9	54°39'56.29"N 101°29'4.20"W	14 U 0339768 6060423	Northern Manitoba	Treed rock
9.1	54°36'25.03"N 101°22'44.82"W	14 U 0346342 6053659	Northern Manitoba	Grassy rock
9.2	54°38'40.48"N 101°23'39.66"W	14 U 0345501 6057878	Northern Manitoba	Grassy rock
9.3	54°38'55.11"N 101°34'56.81"W	14 U 0333383 6058761	Northern Manitoba	Grassy rock
9.4	54°39'19.80"N 101°32'39.78"W	14 U 0335866 6059434	Northern Manitoba	Treed rock

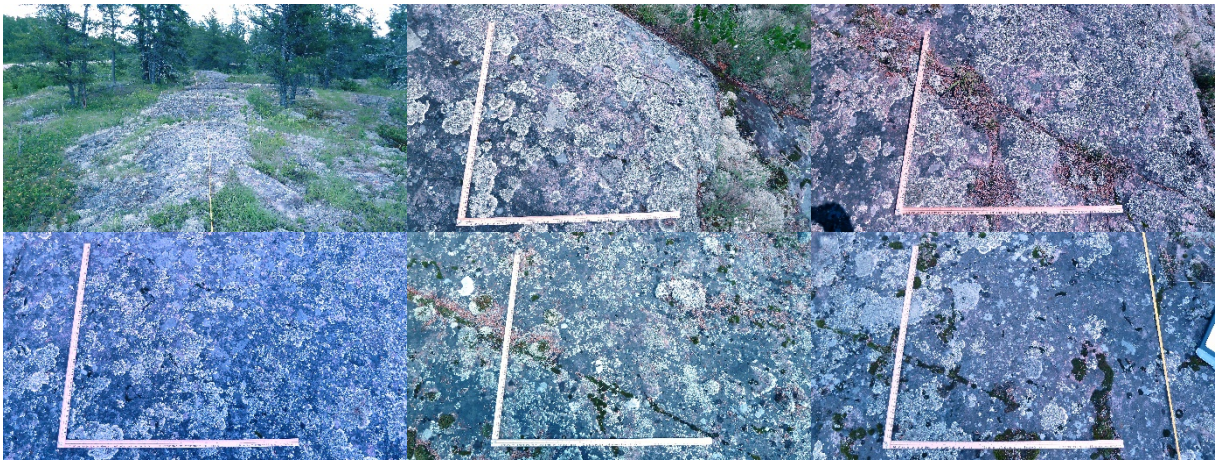
Appendix C: Site and Quadrat Photos Taken From Study Transects Listed in Appendix B



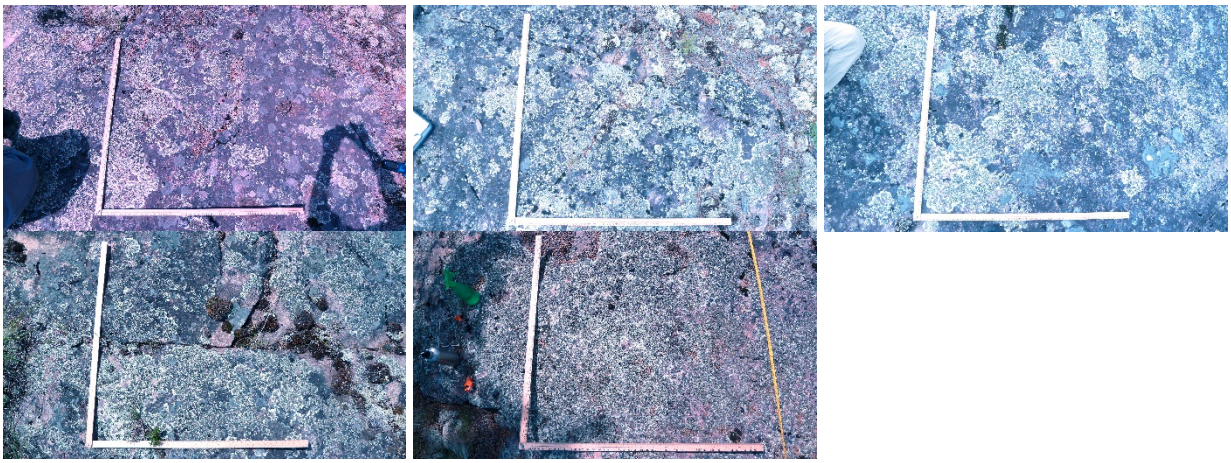
Transect 1.1: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.



Transect 1.2: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.



Transect 1.3: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.



Transect 1.4: Quadrat 0m, 10m, 20m, 30m, and 40m. Site photo missing.

Transect 2.1: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 2.2: Site photo; Quadrat 0m, 10m, 30m, and 40m. Missing quadrat 20m.

Transect 2.3: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 2.4: Missing site photo; Missing quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 2.5: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 2.6: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

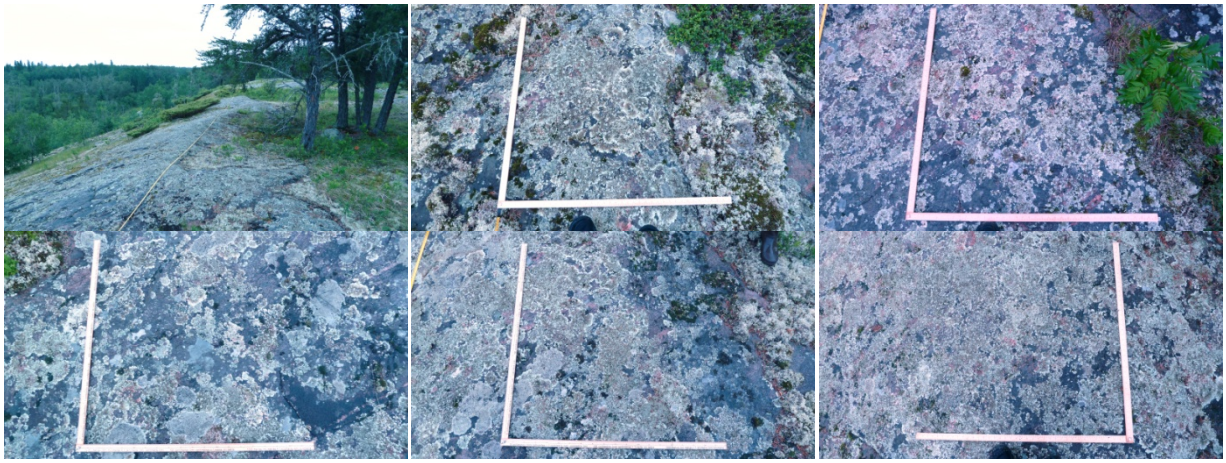
Transect 2.7: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 2.8: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 2.9: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

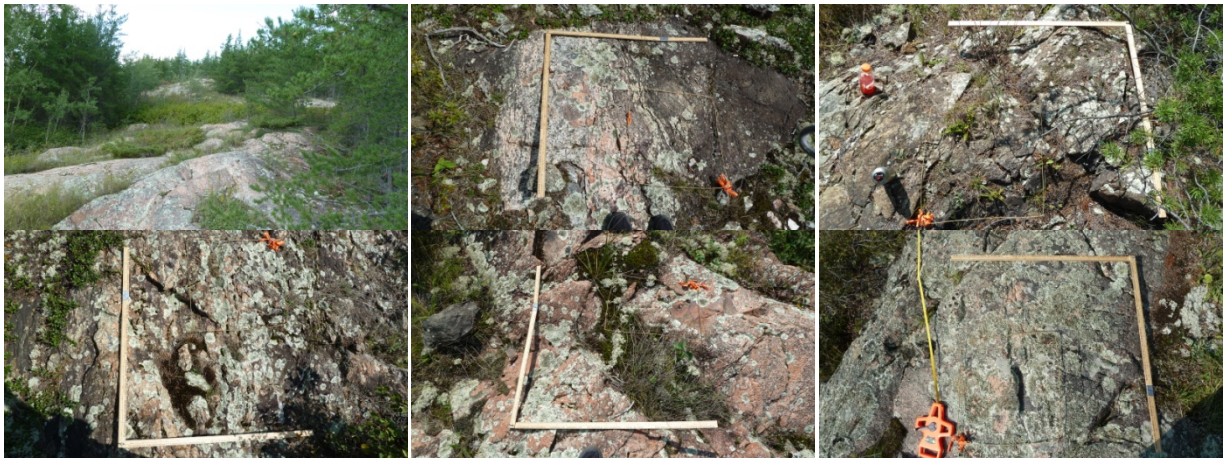
Transect 2.10: Site photo; Quadrat 0m, 10m, 30m, and 40m. Missing quadrat 20m.

Transect 3.1: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

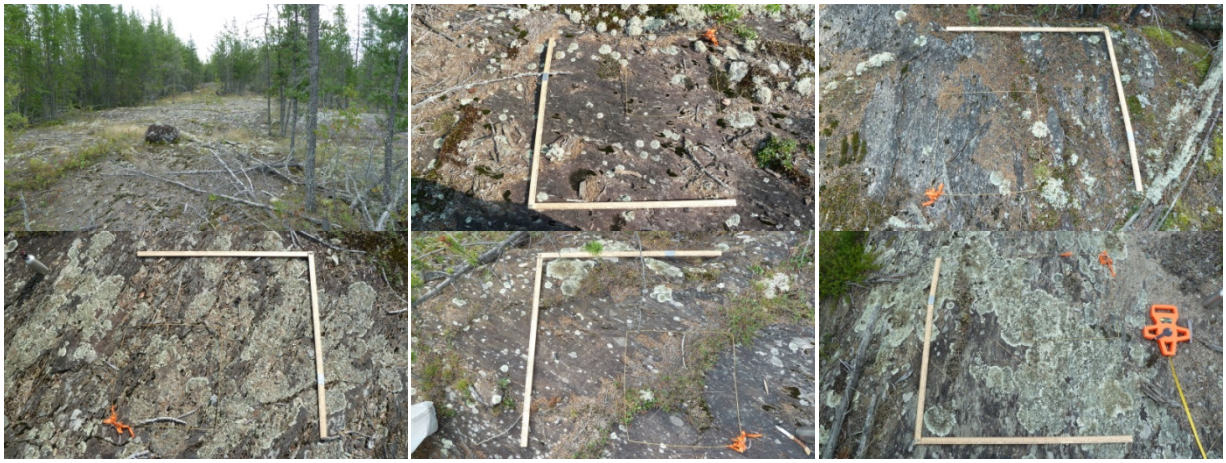


Transect 3.2: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 4.1: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.



Transect 4.2: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.



Transect 5.1: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 5.2: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 5.3: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

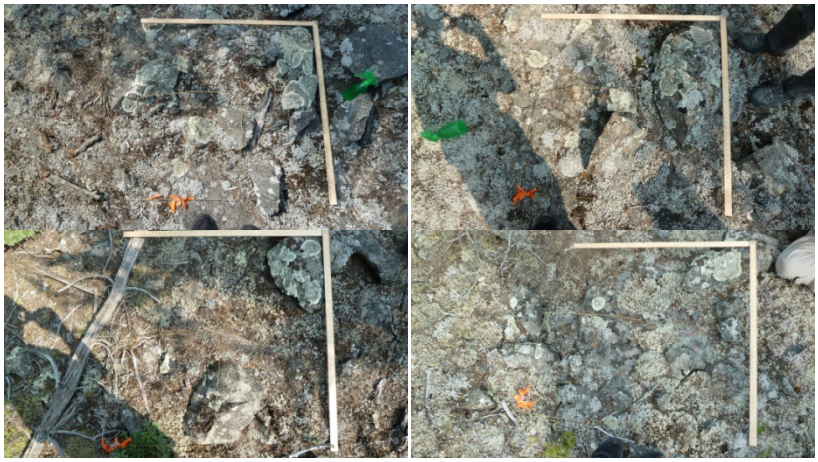
Transect 7.1: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 7.2: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 7.3: Quadrat 0m, 10m, 20m, 30m, and 40m. Missing site photo.

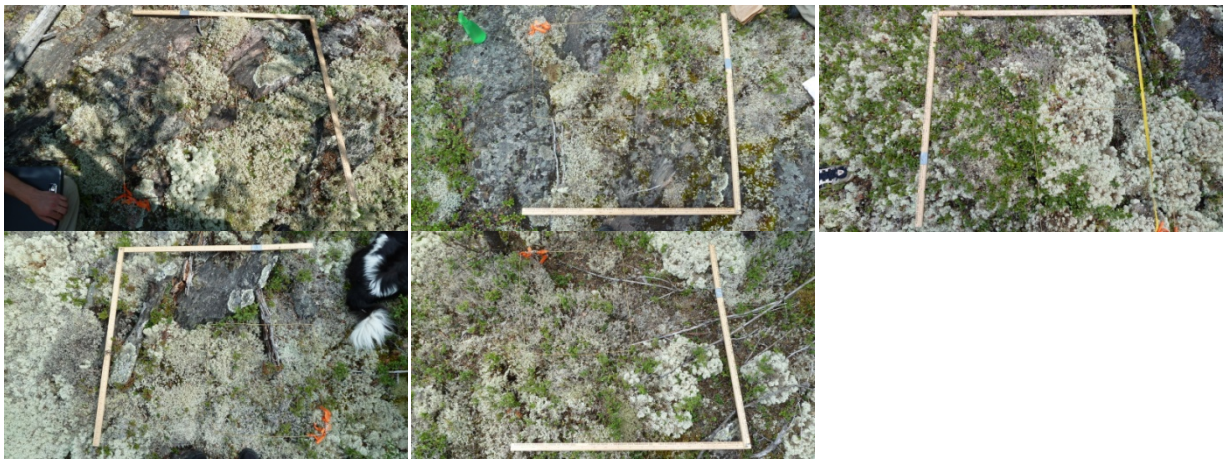
Transect 7.4: Quadrat 0m, 10m, 20m, 30m, and 40m. Missing site photo.

Transect 7.5: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.



Transect 8.1: Quadrat 0m, 10m, 20m, 30m, and 40m. Missing site photo.

Transect 8.2: Quadrat 0m, 10m, 20m, 30m, and 40m. Missing site photo.

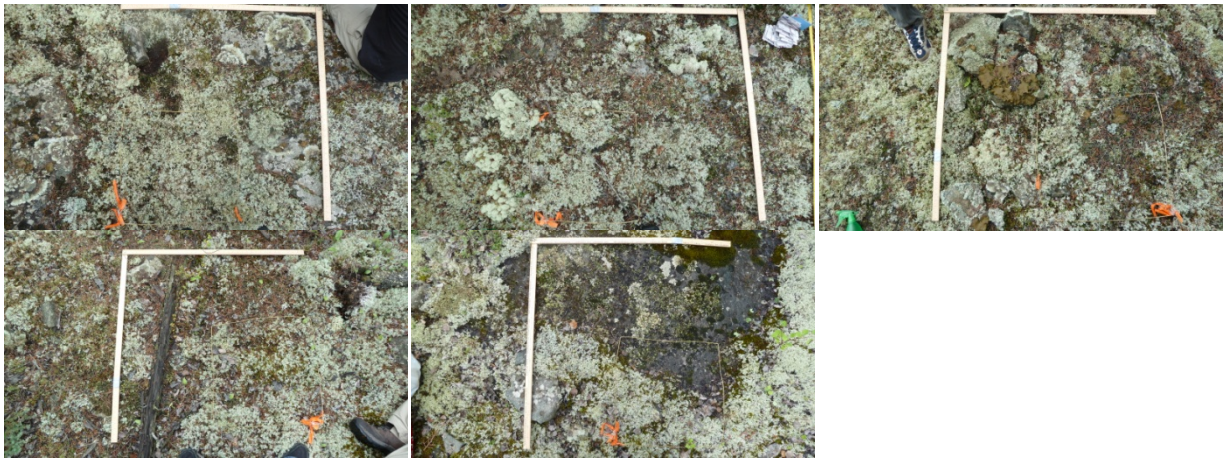


Transect 8.3: Quadrat 0m, 10m, 20m, 30m, and 40m. Missing site photo.

Transect 8.4: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 8.5: Quadrat 0m, 10m, 20m, 30m, and 40m. Missing site photo.

Transect 8.6: Quadrat 0m, 10m, 20m, and 30m. Missing site photo. Missing quadrat 40m photo.



Transect 8.7: Quadrat 0m, 10m, 20m, 30m, and 40m. Missing site photo.

Transect 8.8: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 8.9: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 9.1: Quadrat 0m, 10m, 20m, 30m, and 40m. Missing site photo.

Transect 9.2: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Transect 9.3: Quadrat 0m, 10m, 20m, 30m, and 40m. Missing site photo.

Transect 9.4: Site photo; Quadrat 0m, 10m, 20m, 30m, and 40m.

Appendix D: Summary of Element Data Analyzed By Actlabs Used in Chapter 4 Ecology Study

Analyte Symbol	Ag	Al	As	B	Ba	Be	Bi	Ca	Cd	Co	Cr	Cu	Fe	Ga	Hg	K	La	Mg
Unit Symbol	*ppm*	*%*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*%*	*ppm*	*ppm*	*ppm*	*ppm*	*%*	*ppm*	*ppm*	*%*	*ppm*	*%*
Detection Limit	*0.2*	*0.01*	*2*	*10*	*10*	*0.5*	*2*	*0.01*	*0.5*	*1*	*1*	*1*	*0.01*	*10*	*1*	*0.01*	*10*	*0.01*
1.1	0.3	0.85	4	10	37	0.5	2	0.4	0.5	5	35	12	1.83	10	1	0.13	35	0.59
1.2	0.2	1.11	2	10	136	0.5	2	0.83	0.5	14	27	39	3.61	10	1	0.76	162	1.04
1.3	0.2	0.73	2	10	55	0.5	2	0.15	0.5	6	5	7	1.95	10	1	0.51	10	0.51
1.4	0.4	0.67	2	10	74	0.5	2	0.21	0.5	5	22	9	1.46	10	1	0.43	25	0.55
2.1	0.2	0.91	2	10	111	0.5	2	0.42	0.5	6	12	2	2.08	10	1	0.43	35	0.5
2.2	0.2	0.99	2	10	13	0.5	2	1.38	0.5	18	46	109	5.4	10	1	0.13	10	0.9
2.3	0.2	0.92	2	10	50	0.5	2	0.53	0.5	6	3	8	2.01	10	1	0.11	130	0.52
2.4	0.5	3.83	15	10	31	0.5	2	2.75	0.8	50	46	185	10.9	10	1	0.03	10	2.32
2.5	0.3	3.17	6	10	50	0.5	2	0.9	0.5	35	39	36	13.2	10	1	0.09	10	2.6
2.6	0.2	6.56	2	10	16	0.7	4	4.79	0.5	43	830	2	8.42	20	4	0.01	15	8.01
2.7	0.2	1.88	2	10	267	0.5	2	0.71	0.5	13	23	19	3.06	10	1	1.19	19	1.05
2.8	0.2	1.61	2	10	20	0.5	2	1.1	0.5	40	1	77	6.11	10	1	0.05	10	1.2
2.9	0.5	3.52	2	10	67	0.5	2	1.16	0.5	15	5	71	8.77	10	1	0.11	12	1.81
2.10	0.2	0.35	2	10	10	0.9	2	0.02	0.5	1	1	2	0.28	10	1	0.12	10	0.03
3.1	0.3	1.21	2	10	206	0.5	2	0.79	0.5	11	16	16	4.27	10	1	0.94	80	0.9
3.2	0.4	0.73	2	10	65	0.5	2	0.59	0.5	7	88	40	1.41	10	1	0.41	30	0.89
4.1	0.2	0.16	2	10	41	0.5	2	0.43	0.5	4	1	15	0.65	10	1	0.08	26	0.05
4.2	0.2	0.8	2	10	24	0.5	2	0.5	0.5	4	2	3	1.79	10	1	0.06	10	0.23
5.1	0.2	1.49	6	10	49	0.5	3	0.07	0.5	7	70	14	2.52	10	1	0.28	13	0.99
5.2	0.2	0.56	2	10	20	0.5	2	0.04	0.5	2	4	1	0.77	10	1	0.3	18	0.19
5.3	0.2	1.24	2	10	58	0.5	2	0.53	0.5	7	28	5	2.6	10	1	0.6	50	0.73
7.1	0.2	0.8	2	10	76	0.5	2	0.73	0.5	6	24	5	1.4	10	1	0.24	21	0.61
7.2	0.2	0.2	2	10	14	0.5	2	0.02	0.5	1	1	2	0.37	10	1	0.08	10	0.08
7.3	0.2	1.24	3	10	21	0.5	2	1.69	0.5	17	44	104	2.01	10	1	0.05	10	0.75
7.4	0.2	1.07	5	17	63	0.5	2	0.49	0.5	6	43	2	1.58	10	1	0.14	10	0.76
7.5	0.2	0.99	2	10	29	0.5	2	0.5	0.5	6	42	2	1.63	10	1	0.09	17	0.79

8.1	0.2	1.37	2	10	20	0.5	5	0.35	0.5	8	22	19	2.72	10	1	0.03	10	1.22
8.2	0.2	0.76	2	10	14	0.5	4	0.39	0.5	2	4	2	2.92	10	1	0.05	10	0.31
8.3	0.2	1.91	2	10	112	0.5	2	0.17	0.5	19	97	1	4.08	10	1	1.38	27	1.63
8.4	0.2	1.12	2	10	174	0.5	2	0.2	0.5	9	47	2	1.98	10	1	0.8	25	0.47
8.5	0.2	2.1	2	10	608	0.5	2	0.2	0.5	21	144	7	4	10	1	1.34	18	1.53
8.6	0.2	2.37	2	10	308	0.5	2	2.39	0.5	20	7	40	6.26	10	1	0.53	10	1.29
8.7	0.2	1.81	2	10	115	0.5	2	0.92	0.5	8	18	6	2.78	10	1	0.5	10	1.55
8.8	0.2	1.71	2	10	45	0.5	3	0.36	0.5	9	3	16	3.42	10	1	0.20	10	0.95
8.9	0.2	1.29	3	10	65	0.5	2	0.86	0.5	7	29	2	1.7	10	1	0.21	10	0.76
9.1	0.2	2.49	2	10	43	0.5	2	2.87	0.5	25	144	117	4.12	10	1	0.1	10	1.75
9.2	0.2	2.17	3	10	155	0.5	2	1.87	0.5	20	18	103	5.58	10	1	0.26	24	1.10
9.3	0.2	6.66	2	11	35	0.5	2	3.49	0.5	23	54	57	5.2	10	1	0.14	10	3.64
9.4	0.2	2.94	38	15	45	0.5	2	0.5	0.5	34	50	135	5.65	10	1	0.19	10	3.03

(continued...)

Analyte Symbol	Mn	Mo	Na	Ni	P	Pb	S	Sb	Sc	Sr	Ti	Te	Tl	U	V	W	Y	Zn
Unit Symbol	*ppm*	*ppm*	*%*	*ppm*	*%*	*ppm*	*%*	*ppm*	*ppm*	*Ppm*	*%*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
Detection Limit	*5*	*1*	*0.001*	*1*	*0.001*	*2*	*0.01*	*2*	*1*	*1*	*0.01*	*1*	*2*	*10*	*1*	*10*	*1*	*2*
1.1	281	1	0.104	8	0.046	2	0.01	2	3	69	0.1	1	2	10	29	10	5	45
1.2	475	1	0.096	19	0.166	6	0.03	2	5	35	0.16	1	2	10	75	10	18	71
1.3	245	7	0.083	6	0.04	2	0.01	2	2	9	0.17	1	2	10	37	10	3	38
1.4	223	1	0.061	7	0.051	2	0.01	2	2	18	0.15	1	2	10	29	10	4	52
2.1	288	1	0.062	6	0.046	6	0.01	2	2	17	0.17	5	2	10	31	10	7	44
2.2	416	1	0.219	19	0.04	2	0.07	2	13	8	0.18	1	4	10	298	10	8	34
2.3	259	1	0.049	4	0.041	6	0.01	2	3	26	0.16	2	2	10	27	10	9	35
2.4	1160	1	0.022	44	0.076	4	0.88	2	10	54	0.7	2	2	10	241	10	9	250
2.5	1660	1	0.047	27	0.09	2	0.08	4	16	21	0.51	1	2	10	290	10	20	118
2.6	1280	1	0.02	297	0.126	5	0.01	2	22	131	0.01	1	3	10	161	10	9	110
2.7	416	1	0.067	19	0.05	2	0.01	2	2	28	0.18	2	2	10	46	10	10	61
2.8	304	1	0.076	4	0.032	2	0.32	2	5	31	0.18	2	2	10	285	10	3	42

2.9	1330	1	0.03	6	0.073	2	0.17	2	8	28	0.25	1	2	10	57	10	18	164
2.10	54	1	0.039	1	0.008	6	0.01	2	1	2	0.01	1	2	10	1	10	2	11
3.1	478	1	0.082	6	0.127	6	0.01	2	8	13	0.1	3	2	10	90	10	29	91
3.2	354	1	0.092	29	0.065	2	0.01	2	3	12	0.11	2	2	10	31	10	14	36
4.1	211	1	0.025	2	0.016	15	0.03	2	1	31	0.01	1	2	10	2	10	2	24
4.2	281	1	0.037	1	0.038	4	0.01	2	3	25	0.1	3	2	10	13	10	12	20
5.1	330	1	0.029	20	0.042	6	0.01	2	2	4	0.04	2	2	10	29	10	3	46
5.2	113	1	0.03	2	0.016	5	0.01	2	1	2	0.04	2	2	10	7	10	4	23
5.3	565	1	0.083	14	0.038	4	0.01	2	10	14	0.17	2	2	10	33	10	23	54
7.1	244	1	0.036	15	0.057	3	0.01	2	2	104	0.12	2	2	10	31	10	5	40
7.2	52	1	0.033	1	0.006	2	0.01	2	1	3	0.01	1	2	10	4	10	1	8
7.3	343	1	0.115	25	0.031	2	0.06	2	8	41	0.22	1	2	10	80	10	9	21
7.4	191	1	0.057	21	0.073	4	0.01	2	2	111	0.06	2	2	10	34	10	3	46
7.5	225	1	0.043	22	0.067	2	0.01	2	2	180	0.08	1	2	10	34	10	3	47
8.1	440	1	0.106	5	0.018	2	0.02	2	8	10	0.09	1	2	10	37	10	6	48
8.2	580	1	0.094	1	0.024	2	0.01	2	8	10	0.06	4	2	10	12	10	15	23
8.3	536	1	0.102	46	0.057	3	0.01	3	11	10	0.2	2	2	10	93	10	9	96
8.4	524	1	0.055	22	0.032	2	0.01	2	4	13	0.16	1	2	10	44	10	8	42
8.5	244	1	0.071	53	0.056	2	0.01	2	13	6	0.24	4	2	10	135	10	9	85
8.6	947	1	0.218	6	0.096	3	0.01	2	15	16	0.11	2	2	10	176	10	15	86
8.7	289	1	0.067	14	0.046	2	0.02	2	7	37	0.12	1	2	10	73	10	12	26
8.8	527	1	0.049	1	0.041	2	0.01	2	6	9	0.11	1	3	10	15	10	10	36
8.9	263	1	0.049	13	0.05	4	0.01	2	3	158	0.12	2	3	10	32	10	5	50
9.1	461	1	0.282	92	0.081	2	0.14	2	7	112	0.25	1	2	10	111	10	8	47
9.2	450	2	0.080	13	0.155	4	0.29	2	7	116	0.15	2	2	10	116	10	18	80
9.3	180	1	0.37	24	0.012	2	0.06	4	8	79	0.13	1	2	10	149	10	1	34
9.4	828	1	0.04	25	0.02	2	0.01	2	29	23	0.01	2	2	10	183	10	7	75

(continued...)

Analyte Symbol	Zr
Unit Symbol	*ppm*
Detection Limit	*1*
1.1	8
1.2	6
1.3	7
1.4	9
2.1	8
2.2	7
2.3	5
2.4	9
2.5	11
2.6	3
2.7	12
2.8	4
2.9	14
2.10	6
3.1	2
3.2	8
4.1	8
4.2	12
5.1	6
5.2	13
5.3	13
7.1	7
7.2	18
7.3	8
7.4	6
7.5	5
8.1	18

8.2	5
8.3	27
8.4	13
8.5	8
8.6	6
8.7	23
8.8	9
8.9	6
9.1	2
9.2	6
9.3	1
9.4	4

Appendix E: Summary of Element Data Analyzed By Actlabs Used in Chapter 5 Laboratory Study

Analyte Symbol	Ag	Al	As	B	Ba	Be	Bi	Ca	Cd	Co	Cr	Cu	Fe	Ga	Hg	K	La	Mg
Unit Symbol	*ppm*	*%*	*ppm*	*Ppm*	*ppm*	*ppm*	*ppm*	*%*	*ppm*	*ppm*	*ppm*	*ppm*	*%*	*ppm*	*ppm*	*%*	*Ppm*	*%*
Detection Limit	*0.2*	*0.01*	*2*	*10*	*10*	*0.5*	*2*	*0.01*	*0.5*	*1*	*1*	*1*	*0.01*	*10*	*1*	*0.01*	*10*	*0.01*
Granodiorite	0.19	0.81	1.99	9.99	78	0.49	1.99	0.35	0.49	8	72	33	1.38	9.99	0.9	0.5	40	0.86
Dolostone	0.19	0.06	1.99	10	9.99	0.49	1.99	17.4	0.49	0.9	2	3	0.19	9.99	0.9	0.03	9.99	12.6
Basalt	0.2	1.85	1.99	9.99	22	0.49	7	1.54	0.49	11	25	3	3.52	9.99	0.9	0.06	9.99	1.34
Mica Schist	0.19	2.06	1.99	9.99	100	0.49	4	0.11	0.49	12	99	17	3.42	9.99	0.9	0.75	33	1.43

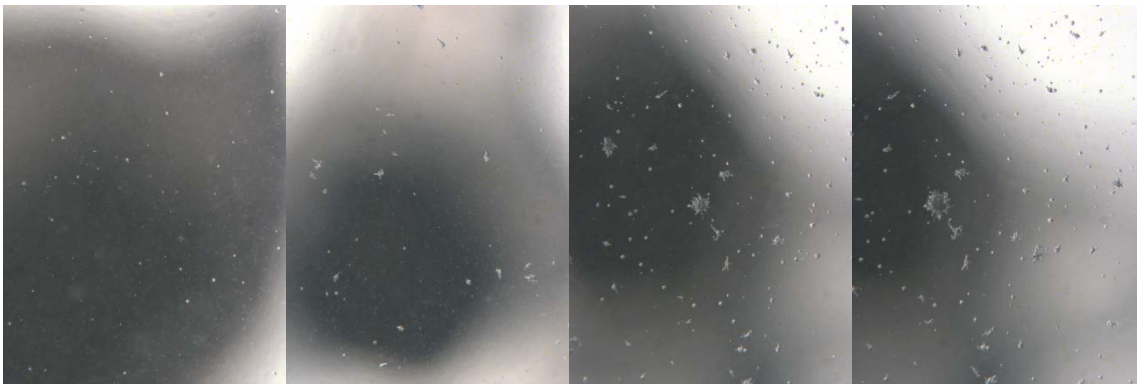
(continued…)

Analyte Symbol	Mn	Mo	Na	Ni	P	Pb	S	Sb	Sc	Sr	Ti	Te	Tl	U	V	W	Y	Zn
Unit Symbol	*ppm*	*ppm*	*%*	*ppm*	*%*	*ppm*	*%*	*ppm*	*ppm*	*ppm*	*%*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
Detection Limit	*5*	*1*	*0.001*	*1*	*0.001*	*2*	*0.01*	*2*	*1*	*1*	*0.01*	*1*	*2*	*10*	*1*	*10*	*1*	*2*
Granodiorite	332	0.9	0.058	32	0.053	2	0.009	1.99	4	9	0.13	3	1.99	9.99	27	9.99	13	39
Dolostone	157	0.9	0.024	1	0.005	1.99	0.009	1.99	0.9	39	0.009	1	1.99	9.99	0.9	9.99	0.9	2
Basalt	552	0.9	0.236	10	0.022	1.99	0.009	1.99	12	17	0.08	0.9	1.99	9.99	68	9.99	7	30
Mica Schist	427	1	0.029	30	0.06	3	0.03	1.99	3	11	0.1	2	1.99	9.99	39	9.99	5	57

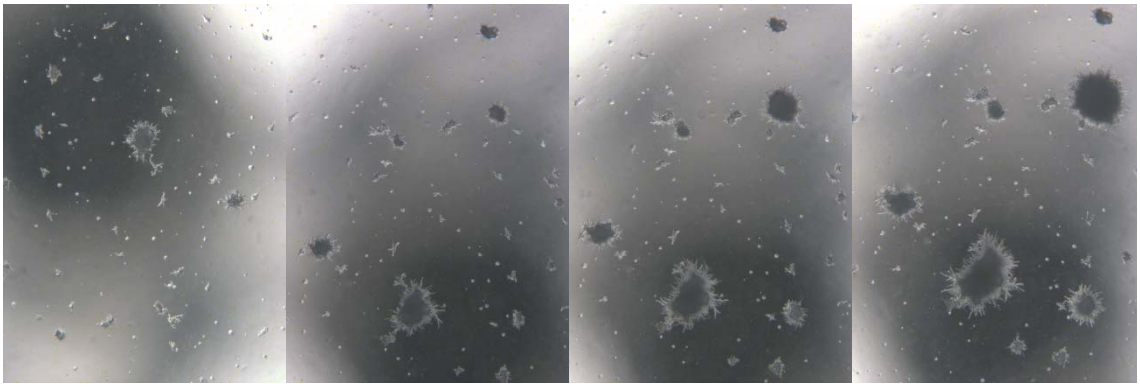
(continued...)

Analyte Symbol	Zr
Unit Symbol	*ppm*
Detection Limit	*1*
Granodiorite	5
Dolostone	0.9
Basalt	11
Mica Schist	9

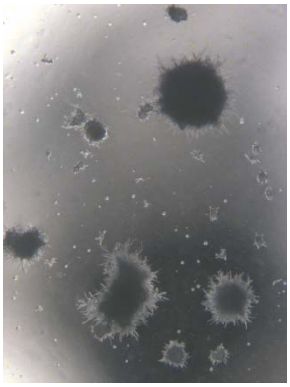
Appendix F: Photos of *Xanthoparmelia cumberlandia* Showing Changes in Growth on Malt Yeast Agar Over a 9 week Period



X. cumberlandia plate D2 40x (100%): Weeks 1-4.



X. cumberlandia plate D2 40x (100%): Weeks 5-8.



X. cumberlandia plate D2 40x (100%): Week 9.

Appendix G: Algal Actin Gene Alignment of *Arctoparmelia centrifuga* (Ac), *Xanthoparmelia cumberlandia* (Xc) and *X. viriduloumbrina* (Xv) Sequences Used in Chapter 6

```
          1---5----10---15---20---25---30---35---40---45---50---55---60
Ac7-2-0-3 -----CTGGCCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAG---GA--------TT
Ac7-2-3-3 TTCACCTGGCCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGACGCTGCAGGT
Ac7-4-0-3 TTCACCTGGCCGTCAGGTAGCTCATAGTTTTTCTCAATGGTGGAGCTTGAAGCTGCAGTT
Ac8-1-0-1 TTCACCTGGCCATCAGGTAGCTCATAGTTTTTCTCAATGKTGGAGCTTGAAGCTGCAGTT
Ac8-3-2-2 TTCACCTGGCCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGACGCTGCAGTT
Ac8-5-1-1 TTCACCTGGCCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGACGCTGCAGTT
Ac8-7-0-3 TTCACCTGGCCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGACGCTGCAGTT
Ac8-8-0-2 TTCACCTGGCCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGACGCTGCAGTT
Ac8-9-1-1 TTCACCTGGCCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGACGCTGCAGTT
Ac9-3-0-1 TTCACCTGGCCGTCAGGT-GYT-ATAGTTYTTCTCAATGGTGGAGCTTGARWCYGCAGTT
Xc1-3-3-2 TTCACCTGGCCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGACGCTG-AG--
Xc2-1-1-2 TTCACCTGGTCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGACGCTGCAGT-
Xc2-3-2-1 TTCACCTGGTCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGACGCTGCAG--
Xv2-2-2-2 ----CCTGGTCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGASGARG-AGDD
Xv2-3-3-1 TTCACCTGGYCGTCAGGTAGCTCATAGTTCTTCTCAATGGTGGAGCTTGAAVAAGMAGDD
Xv2-9-3-1 TTCACCTGGCCGTCAGGTAGVTCATAGTTCTTCTCAATGGTGGAGCTTGACGCSGMAGTT

(continued…) 61--65---70---75---80---85---90---95---100--105--110--115--120
Ac7-2-0-3 GCAAGCTCTTGCTCGTAGTCTAGAG----------AAGCTTCTCCTTGATGTCACGCACA
Ac7-2-3-3 GCAAGCTCTTGCTCGTAGTCTAGCGCCACATACGCAAGCTTCTCCTTGATGTCACGCACA
Ac7-4-0-3 GCAAGCTCTTGCTCGTAGTCTAGAGCCACATACGCAAGCTTCTCCTTGATGTCGCGCACA
Ac8-1-0-1 GCA-GCTCTTGCTCGTAGTCTAGAGCCACATACGCAAGYTTCTCCTTGATGTCGCGCACA
Ac8-3-2-2 GCAAGCTCTTGCTCGTAGTCTAGMGCCACATACGCAAGCTTCTCCTTGATGTCACGCACA
Ac8-5-1-1 GCAAGCTCTTGCTCGTAGTCTAGCGCCACATACGCAAGCTTCTCCTTGATGTCACGCACA
Ac8-7-0-3 GCAAGCTCTTGCTCGTAGSCTAGCGCCACATACGCAAGCTTCTCCTTGATGTCACGCACA
Ac8-8-0-2 GCAAGCTCTTGCTCGTAGTCTAGCGCCACATACGCAAGWTTCTCCTTGATGTCACGCACA
Ac8-9-1-1 GCAAGCTCTTGCTCGTAGTCTAGCGCCACATACGCAAGCTTCTCCTTGATGTCACGCACA
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Ac9-3-0-1 GCAAGCTYTTGCTTGTAGTCTAGCGCCACATACGCAAGCTTCTCCTTGATGTCACGCACA
Xc1-3-3-2 GCAAGCTCTTGCTCGTAGTCTAGMGCCACATACGCAAGCTTCTCCTTGATGTCACGCACA
Xc2-1-1-2 GCAAGCTCTTGCTCGTAGTCTAGMGCCACATACGCAAGCTTCTCCTTGATGTCACGCACA
Xc2-3-2-1 -CAAGCTCTTGCTCGTAGTCTAGMGCCACATACGCAAGCTYCTCCTTGATGTCACGCACA
Xv2-2-2-2 GCAHGCHCTTGCTCGTAGTCTAGCGCCACATACGCAAGCTTCTCCTTGATGTCACGCACA
Xv2-3-3-1 GCAAGCTCTTGCTCGTAGTCTAGCGCCACA---GCAAGCTTCTCCTTGATGTCACGCACA
Xv2-9-3-1 GCAAGCTCTTGCTCGTAGTCTAGCGCCTCATACGCAAGCTTCTCCTTGATGTCACGCACA

(continued…) 121-125--130--135--140--145--150--155--160--165--170--175--180
Ac7-2-0-3 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTMYYCWMRMTCTGTCAGAATCTGCAGTCAT
Ac7-2-3-3 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCAGTCAT
Ac7-4-0-3 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCAGTCAT
Ac8-1-0-1 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTMAGAATCTGCAGTCAT
Ac8-3-2-2 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCAGTCAT
Ac8-5-1-1 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCAGTCAT
Ac8-7-0-3 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCAGTCAT
Ac8-8-0-2 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCAGTCAT
Ac8-9-1-1 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCAGTCAT
Ac9-3-0-1 ATTTCACGCTTTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCAGTCAT
Xc1-3-3-2 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTC-GAATCTGC------
Xc2-1-1-2 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTG-------
Xc2-3-2-1 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCWSYCAT
Xv2-2-2-2 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCHKAATCTGCAGTCAT
Xv2-3-3-1 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCAGTCAT
Xv2-9-3-1 ATTTCACGCTCTGCTGTGGTGGTGAAACTGTAACCACGCTCTGTCAGAATCTGCAGTCAT

(continued…) 181-185--190--195--200--205--210--215--220--225--230--235--240
Ac7-2-0-3 TACAGGTGTTGYTGCASATTGGCATGAAAGATTCTGCTCTGCAAACWAACCCWGGTGGAC
Ac7-2-3-3 TACAGGTGTTGCTGCAGGTTGGCATGAAAAATTCTGCTYTGCAAACAAACCCAGGKGGAM
Ac7-4-0-3 CACAGGTGTTGTTGCAGGTTGGCATGAAAAACTCTACTTTGCAAACAGT--CAGGTGGAC
Ac8-1-0-1 CACAGGTGTTGTTGCAGGTTGGCATGAAAAATTCTACTTTGCAAACAGT-CC-GGTGGAC
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Ac8-3-2-2 TACAGGTGTTGCTGCAGATTGGCATGAAAGATTCTGCTCTGCAAACAAACCCAGGTGGAC
Ac8-5-1-1 TACAGGTGTTGCTGCAGATTGGCATGAAAGATTCTGCTCTGCAAACAAACCCAGGTGGAC
Ac8-7-0-3 TACAGGTGTTGCTGCAGATTGGCATGAAAGATTCTGCTCTGCAAACAAACCCAGGTGGAC
Ac8-8-0-2 TACAGGTGTTGCTGCAGATTGGCATGAAAGATTCTGCTCTGCAAACAAACCCAGGTGGAC
Ac8-9-1-1 TACAGGTGTTGCTGCAGATTGGCATGAAAGATTCTGCTCTGCAAACAAACCCAGGTGGAC
Ac9-3-0-1 TACAGGTGTTGCTGCAGATTGGCATGAAAGATTTTGCTCTGCAAACMAACCCAGGTGGAC
Xc1-3-3-2 ----GGTGTTGCTGCAGATTGGCATGAAARATTCTGCTYTGCAAACARWCCCGKGGRGRM
Xc2-1-1-2 ------------------------------------------------------------
Xc2-3-2-1 YACAGGTGTTGCTGCAGATTGGCATGAAARAYTCTRCTYTGCAAACAAACMCRKGKRSWM
Xv2-2-2-2 TACAGGTGTTGYTGCAGATTGGCATGAAARATTCTGCTYTGCAAACAAACCCAGGKGGAC
Xv2-3-3-1 TACAGGTGTTGYTGCAGATTGGCATGAAARAYTCTRCTYTGCAAACAAACCCRKGKGGAA
Xv2-9-3-1 TACAGGTGTTGCTGCAGATTGGCATGAAAGATTCTGCTCTGCAAACAAACCCAGGTGGAC
```

(*continued…*)

```
241-245--250--255--260--265
Ac7-2-0-3 TAAATACAGACGGTACCATTAGCAAGA
Ac7-2-3-3 AAAATACAGAGGGCACCATTARCGAGA
Ac7-4-0-3 TAAATATAGACGGTACAACTAGCAGGA
Ac8-1-0-1 TAAATATAGACGGTAMCAMTAGCAAGA
Ac8-3-2-2 TAAATACAGACGGTACCATTAGCAAGA
Ac8-5-1-1 TAAATACAGACGGTACCATTAGCAAGA
Ac8-7-0-3 TAAATACAGACGGTACCATTAGCAAGA
Ac8-8-0-2 TAAATACAGACGGTACCATTAGCASGT
Ac8-9-1-1 TAAATACAGACGGTACCATTAGCAAGA
Ac9-3-0-1 TAAATACAGACGGTACCATTACCAAGA
Xc1-3-3-2 TAWATAKASASGAYAMCWWTARSRAGA
Xc2-1-1-2 ---------------------------
Xc2-3-2-1 WAAATACASASGWYACCWWTARSRAGA
Xv2-2-2-2 TAAATACAGACGGYACCWTTARSRAGA
Xv2-3-3-1 TAWATACASACGGYACCWTTARSRAGA
Xv2-9-3-1 TAAATACAGACGGTACCATTAGCAAGA
```

Appendix H: Manuscript Permissions

Manuscript Chapter 2

Deduke, C., Booth, T., Piercey-Normore, M.D. 2014. Lichen fecundity on the Precambrian Shield: an alternative life history approach. Botany 92: 723-735.

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Manuscript Chapter 3

Deduke, C., Piercey-Normore, M.D. 2014. A potential trade-off with stictic acid improves ascospore viability in *Xanthoparmelia cumberlandia*. Bryologist 117: 290-296.

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C. Deduke and M.D. Piercey-Normore. 2014. A potential trade-off with stictic acid improves ascospore viability in Xanthoparmelia cumberlandia. The Bryologist, 117(3): 290-296. DOI: http://dx.doi.org/10.1639/0007-2745-117.3.290. http://www.bioone.org/doi/full/10.1639/0007-2745-117.3.290.

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Combined Submitted Manuscripts Chapter 5 and 6

Deduke, C., Piercey-Normore, M.D. Substratum preference of two species of *Xanthoparmelia*.

Fungal Biology (In Press).

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