PMUSER

Proceedings of Manitoba's Undergraduate Science and Engineering Research

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INSIDE

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Letter from the Editor-in-Chief

I am pleased to announce University of Manitoba's inaugural issue of the Proceedings of Manitoba's Undergraduate Science and Engineering Research (PMUSER). PMUSER is a peer-reviewed research journal that allows students to submit their undergraduate level research for publication. Through the various facets of science and engineering, University of Manitoba offers countless research opportunities for students to get involved. In addition to conferences and poster competitions, our goal for this journal is to provide undergraduate researchers another medium to showcase and share their research accomplishments. Whether it be part of an honours thesis, summer research studentship, or a co-op work term, students now have a unique opportunity to exhibit their research discoveries. Furthermore, PMUSER's mission is intended to promote the development of effective scientific communication. This journal entices students to communicate with a clear, concise, honest, and elegant writing style which is a valuable skill and asset to develop for higher academia. On behalf of the PMUSER Editorial Board, we hope you enjoy PMUSER's inaugural issue filled with the fascinating research conducted by University of Manitoba's undergraduates.

John Titus

PMUSER Founder & Editor-in-Chief

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About

The Proceedings of Manitoba's Undergraduate Science and Engineering Research journal is an online open-access refereed journal published annually and hosted by the University of Manitoba. The journal accepts research or review manuscripts written by undergraduate students from any science related faculties including, but not limited to Engineering, Science, Human Ecology, Environment, Earth, and Resources, Agriculture and Food Sciences, Nursing and Medicine. Upon submission, each manuscript undergoes a blind peer-review process by two undergraduate students from a pool associated with the respective research area.

Focus and Scope

The focus of the Proceedings of Manitoba's Undergraduate Science and Engineering Research journal is three fold. The circulation of a peer-reviewed undergraduate journal will provide a chance for students to receive recognition for their research in a wide range of scientific disciplines. The undertaking of writing a journal manuscript will further enhance the understanding of the significance of their respective projects in a broader context. This, in turn, will foster a tremendous growth in their intellectual development outside of the classroom setting, and serves as preparation for a career in research.

Editorial Board

Profile of Dr. Digvir S. Jayas

1. When did you start to develop an interest in Engineering?

Dr. Digvir S. Jayas, *Vice President (Research and International)*

I grew up on a mixed farm in India. Being on the farm, I was helping first and then fixing farm machinery and other systems. Therefore, my interest in engineering probably developed when I was four or five years old however, my real decision was made when I entered grade 9 because I chose physical sciences for my high school years. Main reason for choosing physical sciences was my strong love for mathematics instilled in me by my grandfather. He strongly believed that if someone is strong in mathematics then he/she should have no trouble in any field. From a very early age, he gave me reasonably complex mathematical problems to solve in my head while simultaneously helping him with farm work. In other words I became multi-tasker at an early age.

2. Tell us about the path you took to get to where you are.

After completing my grade 12 education from a high school in Mant (my home town), I joined the G.B. Pant University of Agriculture and Technology in Pantnagar, India to obtain my bachelor's degree in agricultural engineering (1980) followed by master's in agricultural engineering from the University of Manitoba in 1982 and joining the University of Saskatchewan in 1982 for my PhD study. I began working at the University of Manitoba as an Assistant Professor in 1985, while completing my Ph.D., which I received in 1987. I rose through academic ranks quickly becoming an Associate Professor in 1989 and Professor in 1993. I was selected to become Head of the Department of Biosystems Engineering in 1997, the Associate Dean (Research) in the Faculty of Agricultural and Food Sciences in 1999, and Associate Vice-President (Research) in 2001. I also served as Interim Director of the Richardson Centre for Functional Foods and Nutraceuticals during this appointment. In 2009, I was appointed as Vice-President (Research) and in 2011 my portfolio was expanded to include international relations to become Vice-President (Research and International) at the University of Manitoba.

3. Briefly tell us about your research - what role(s) do undergraduate students play in it (if any)?

My love for mathematics continued in my research. During both masters and doctoral studies a component of the work involved the simulation of the flow of gases through porous media (bulk grain). My continuously funded NSERC Discovery Grant program (since 1986) has been on mathematical modeling of stored-grain ecosystems. A stored-grain bulk is a man-made ecological system in which deterioration of the stored product results from interactions among physical, chemical, and biological factors. The important factors are: temperature, moisture, carbon dioxide (CO_2), oxygen (O_2), grain characteristics, microorganisms, insects, mites, rodents, birds, geographical location, and granary structure. Heat, moisture, and CO_2 are produced during the deterioration of grain; and heat, moisture, and gas transfer occur simultaneously in stored-grain ecosystems. The predicted abiotic factors (temperature, moisture content, and gas concentration) interact with biotic factors (grain, insects, mites, fungi, and bacteria) in a grain bin at any geographic location. The interactions between biotic and abiotic factors make the mathematical modelling of stored-grain ecosystems a challenging problem. My

team is the first group in the world to develop three-dimensional mathematical models of heat, moisture, and CO_2 transfer in grain bulks.

My overall research program has focused on reducing the qualitative and quantitative losses in grains. In addition to enhancing the understanding of and managing the interactions among biotic and abiotic variables in the grains, I have done research on: (i) developing soft X-ray and thermal imaging-based techniques to detect low levels of hidden infestation by insects in grains; (ii) developing machine vision and hyperspectral imaging based solutions for detecting fungal, sprout, midge and beetle damage in grains; (iii) understanding the mechanism of flow of air through grain using computed tomography to develop a prototype grain dryer which forces air horizontally and dries grain more uniformly with less energy compared to currently used near-ambient air dryers which force air vertically through grain. Through the execution of the overall research program, (i) my team has significantly advanced the scientific knowledge in the field, (ii) has developed innovative engineering solutions to problems encountered during drying, handling and storing of grains and (iii) has shared our knowledge with designers of grain drying, handling and storage systems and with farmers and storage managers in Canada and around the world.

4. What does it take to be a successful Engineer?

To be a successful engineer, one has to be passionate about the project or program being pursued at a given time. Being able to listen and value the opinion of others with respect and dignity along with being willing to work collaboratively with people from different disciplines and backgrounds helps in being a broad-minded engineer to solve complex problems.

5. Describe your overall lifestyle (work-life balance, family time, leisure time...etc).

I probably do not do a good job at work-life balance in a traditional sense but for me my work became my hobby. To me a hobby is what gives one the most pleasure and no number of hours are enough for that hobby. Most of my free-time is devoted to volunteering for technical and professional organizations. Occasionally, I exercise, watch movies, listen music, write poems and travel for leisure.

6. What advice would you give to undergraduate students currently working in your area of Engineering?

Love wholeheartedly and be passionate about whatever you are working on. Life is too short to waste on projects one does not like. If the job you currently have does not give you complete joy, then look for another job.

7. What advice would you give to undergraduate students that are looking for an Engineering job?

Try different jobs until you find a perfect match. It could the first job or some other number. Ask for help when in doubt. You are never alone.

8. What kind of jobs can undergraduate students, who have research experience in your field of Engineering, have upon graduation?

They can certainly pursue graduate study at the masters or doctoral level but research experience gives them skills in carefully stating a problem, planning a systematic approach to solve the problem by collecting and interpreting data. These skills are transferable to almost any job in the industry, government or academia. Also, as our economy transforms from "resource economy" or "knowledge economy" demand for people with research and development exposure will grow exponentially.

9. How do you foresee the future of your specific field of Engineering?

The future for "bio" engineers, engineers who understand biology, is very bright. Many

problems require knowledge of biology for their solutions. Bio-based problems are more complex due to inherent variability and inhomogeneity of biological systems than physical systems. Engineering properties of biological materials are also more complex than physical materials. This is further illustrated by the fact that many engineering disciplines are being modified by incorporating biology in the curriculum such as biochemical engineering, biomechanical engineering, and biomedical engineering.

Profile of Dr. Georg Hausner

Dr. Georg Hausner, Associate Professor (Microbiology)

1. When did you start to have an interest in Microbiology, or Science in general?

In High school I always I had a love for English, Chemistry and Biology. Once I came to the realization that my actual poetry writing skills were to say "rather limited" I pursued Chemistry and to a larger extend Biology at University. In my second year I had a 'great' prof that turned me on to the world of fungi and I never looked back. I was fortunate that I was able to take a wide variety of courses during my undergraduate years, such as courses dealing with Plants, Fungi, Algae, Genetics, Plant and Animal physiology, Chordates, Parasitology etc. Ultimately I found that molecular biology, genetics and evolution unifies all living organisms on this planet.

2. Tell us about the path you took to get to where you are.

My path was a "see where it takes me approach" i.e. I had no clue(s)! After graduating I spent the summer working on the family farm but eventually I decided that I should do something. So I showed up at the office of Dr. James Reid (formerly of the Department of Botany at the University of Manitoba) in 1985 to see if he had a position for a student. My Master's project dealt with aspects of fungal physiology and my PhD dealt with fungal taxonomy/systematics and fungal evolution using molecular markers and phylogenetics. My postdoctoral work (Michigan State University) dealt with fungal genetics. I worked as a research associate/research scientist in Agriculture Canada research stations in Morden (MB) and Lethbridge (AB) working on developing molecular markers that might provide disease resistance against fungal diseases in flax and potato. Although I enjoyed working on these projects I found the environment restrictive with regards to pursuing my own interests so I accepted a term position with the University of Calgary and eventually (2000) a tenure track position at the University of Manitoba. So depending on your goals, careers in science can be a long term investment.

3. Briefly tell us about your research? What role(s) do undergraduate students play in it?

I primarily work on species of *Ophiostoma*, *Grosmannia* and *Ceratocystis*; these are fungal genera that include many forest pathogens and so-called blue-stain fungi. As pathogens some of these species can have significant impact as they will stunt, or in many instances kill trees. This obviously has an even more dramatic impact, for example: (1) on the environment (trees do represent a sink for CO_2 and on many other forms of life that depend on trees as a habitat); (2) on industry [forestry such as pulp & paper or lumber/construction; or the potential use of trees as a source for cellulose that can be converted into biofuels by microorganisms]; (3) on Urban forests (Dutch Elm Disease – caused by members of the *O. ulmi* species complex); (4) on bioremediation efforts where trees are used to stabilize eroded areas or trees are used to sequester potential soil pollutants. Maintaining healthy forests is vital to our environment and economy, thus understanding the biology and biodiversity of the pathogens that can damage this valuable resource is extremely important. These fungi are also becoming increasingly more problematic due to the arrival of new bark-beetle species that vector these fungi; in the case of the Mountain Pine Beetle the blue-stain fungi actually make it possible for the beetle to successfully overcome the trees resistance mechanisms. This beetle (with "help" of some *Grosmannia* and *Ophiostoma* sp.) is

currently devastating forests in British Columbia.

So my research efforts can be summarized as follows:

1. Developing rapid molecular tools (sequencing the rDNA ITS region) and data bases that allow for rapid identification of forest pests (species of Ophiostomatales).

2. Characterizing mitochondrial genomes of blue-stain fungi to understand the persistence of genetic elements (such as plasmids, mobile elements) that may have implications in genetic diseases and thus allow for the identifying of naturally occurring hypovirulents (can infect trees but do not cause symptoms of the disease) strains that could be used in biocontrol strategies. Recently my work on Chestnut Blight (caused by *Cryphonectria parasitica*) in collaboration with Bertrand and Fulbright (Michigan State University) showed that mtDNA plasmids and a "defective" group II intron can be implicated in manifesting hypovirulence in *C. parasitica*.

3. Characterizing group I and group II introns plus bioprospecting for novel DNA cutting enzymes (so called homing endonucleases) among the fungi. Potential homing endonuclease genes are over expressed in *E. coli* in order to confirm DNA cleavage activity and determine the target and cleavage sequences. Recently we uncovered several twintrons, elements composed of mobile introns inserting within other mobile introns, and we are now interested on how these twintrons evolved and how they splice and move.

Over the years all aspects of my research program has involved undergraduate students either as "Summer students" or as "Project students". The key is to expose students to variety of techniques and topics and find out their potential and their strengths. Ultimately science is a discipline that requires creativity and self-motivation.

4. What does it take to be a successful scientist?

Hard work, persistence and not being afraid of failure are the prerequisite for being a scientist. Ultimately you have to be your own harshest critic and always be your own "devils" advocate to challenge yourself. You also have to be successful in communicating your findings both verbally and in writing. Publish or perish is certainly applicable to science.

5. Describe your overall lifestyle (work-life balance, family time, leisure time? etc).

Maintaining a funded research program, publishing papers, training research personal (undergraduate and graduate students), teaching and service to the University and Scientific community can be a 24/7 undertaking. There is no easy way to balance all aspects of life except to realize that one cannot get everything done and that one needs "down" time. I do enjoy gardening (indoors and outside), cooking, and I enjoy listening to music (from classical to heavy metal).

6. What advice would you give to undergraduate students currently working in research?

Do you have passion for research? Do you look forward to getting up in the morning to get to the laboratory? Does failure raise your level of curiosity and make you work harder? My advice if you said yes to the previous questions – research will ultimately reward you. We all have strength and weaknesses – recognized the latter and built on the first. Never be afraid to challenge yourself; science is extremely competitive so you have to thrive to be the best in what you do. Be careful with regards to "overspecialization" – you might be the "best" in the field but the only job out there is taken and that person will not retire for the next 20 years. Make sure you have training in core skills that can be applied to other areas.

7. What advice would you give to undergraduate students that are looking for a research job?

Get as much experience as possible by getting summer positions in research laboratories and yes grades count as you need to know the background of the area you wish to work in and to be competitive for summer jobs. Read as much as you can on the science topics that interests you. Ultimately be willing to relocate and to continuously learn new ideas and techniques. Science is rapidly changing so it is easy to be left behind.

8. What kind of jobs can undergraduate students, who have research experience in your field, have upon graduation?

Research experience gained in my research group includes basic molecular biology skills, genetics, plus some applied bioinformatics, and mycology. These are core skills that can be applied to many other research areas. Former students from my program currently work in agricultural, medical, and mycological research environments (Industry, Government and University). Some have moved on to enter Medical Schools.

9. How do you foresee the future of Microbiology or life science?

There will always be a need for scientists and with microbes being of concern to human health, relevant to bioremediations, biotechnology, biofuels, food industry, and agriculture (forestry etc.) there should be many opportunities. I am still optimistic that there is a bright future for the life sciences – but one has to keep an almost global view to pursue opportunities. As stated above be flexible in changing locations and areas of research, expect to continuously upgrade yourself by learning new techniques and by staying current on various scientific topic.

The response of *Scenedesmus quadricauda* and *Selenastrum capricornutum* to glyphosate toxicity (Roundup® formulation) with cellular growth and chlorophyll-a synthesis as endpoints

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Abstract

Glyphosate is a commonly-used agricultural herbicide which enters freshwater sources and risks affecting non-target aquatic organisms, including algae. In this study, lab cultures of *Scenedesmus quadricauda* and *Selenastrum capricornutum* were inoculated with glyphosate (Roundup® formulation) to determine its impact on cellular growth and chlorophyll-a (Ch-a) synthesis. A concentration of 10 mg/L of glyphosate significantly inhibited growth and Ch-a synthesis in *S. quadricauda* and *S. capricornutum*. Moreover, concentrations ranging from 0 to 3 mg/L of glyphosate did not affect cellular growth or Ch-a synthesis in either species. Finally, a concentration of 6 mg/L of glyphosate did initially reduce the growth of *S. quadricauda*, but growth recovered and Ch-a concentrations were high. In the case of *S. capricornutum*, growth and Ch-a synthesis were low, and pheophytin concentrations were significantly elevated relative to the control at 6 mg/L of glyphosate. Based on these results, *S. capricornutum* was more sensitive to glyphosate than *S. quadricauda*; this was likely due to differences in the surface area to volume ratios between the species. In the future, glyphosate toxicity should be studied in greater detail by conducting mesocosm studies within the natural aquatic environment.

Keywords: Glyphosate, green algae, Roundup®, cellular growth, chlorophyll

Introduction

Glyphosate (N-phosphonomethylglycine) is one of the world's most used and fastest growing agricultural herbicide (Baylis, 2000). It targets grasses, sedges, and broad-leaved weeds (Goldsborough & Brown, 1988); therefore, water bodies surrounded by agricultural activity are at risk of glyphosate contamination through wind drift, surface run-off, or direct overspray (Peterson et al., 1994). Roundup® is a common commercial herbicide consisting of glyphosate, formulated as isopropylamine salt (IPA salt), and polyethoxylated tallow amine (POEA) as surfactant (Tsui & Chu, 2003). Initial absorption of glyphosate depends on the surfactant to facilitate entry into the plant (Cranmer & Linscott, 1991). Within a cell, glyphosate disrupts aromatic acid biosynthesis, which reduces protein synthesis and causes cellular death (Vera et al., 2010). High glyphosate concentrations also cause chlorosis, which is the insufficient production of Ch-a (Shikha & Singh, 2004); lack of Ch-a reduces photosynthesis, causing death of the organism.

Glyphosate is reaching aquatic sources within Manitoba and is potentially harming algae and other organisms that are dependent on the algae for nutrient cycling, oxygen production, and food (Beck, 1987; Kent & Caux, 1995). In this study, the green algal species, *Scenedesmus quadricauda* and *Selenastrum capricornutum,* were chosen to evaluate the toxicity of glyphosate. Algae are important bio-indicator species due to their sensitivity and high occurrence within the aquatic ecosystem (Tsui & Chu, 2003). Algal death resulting from glyphosate toxicity could affect the entire aquatic ecosystem due to their significance as a food source to organisms at higher trophic levels (Kent & Caux, 1995).

Previous studies have found that high glyphosate concentrations inhibit cellular growth and Ch-a synthesis in green algal species (Peterson et al., 1994; Saenz, DiMarzio, Alberdi, & Tortorelli, 1997; Wong, 2000; Shikha & Singh, 2004; Vendrell, de Barreda Ferraz, Sabater, & Carrasco, 2009). Wong (2000) found that 2 mg/L or more significantly inhibited Ch-a synthesis and cellular growth. Other studies have also found that algal species vary in sensitivity to glyphosate (Peterson et al., 1994; Perez

et al., 2007; Vendrell et al., 2009; Vera et al., 2010). Peterson et al. (1994) tested the effects of glyphosate on *S. quadricauda* and *S. capricornutum* and found that *S. capricornutum* exhibited greater percent inhibition (18%) compared to *S. quadricauda* (3%). In another study, Rojickova-Padrtova and Marsalek (1999) found that *S. capricornutum* was more sensitive to toxic contaminants compared to *S. quadricauda*, and suggested that these findings were the result of differences in the surface area: volume (SA:V) ratios between the two species.

The objective of this study was to determine toxicity and sensitivity differences between *S. quadricauda* and *S. capricornutum* by measuring cellular growth rate and final Ch-a concentrations as endpoints. We hypothesized that glyphosate would affect cellular growth and Ch-a synthesis in both algal species. Similar to Wong (2000), we predicted that glyphosate would cause inhibition at high concentrations (>2 mg/L). We also hypothesized that there would be sensitivity differences between the green algal species; specifically, *S. capricornutum* would be more sensitive to glyphosate than *S. quadricauda.*

Results

A concentration of 10 mg/L of glyphosate inhibited cellular growth and Ch-a synthesis in *S. quadricauda* (Table 1; Fig. 2A). Initially, the growth rate was negative subsequent to glyphosate inoculation at 3 and 6 mg/L, but the effect of glyphosate lessened and living cells continued to grow and reproduce at a positive rate.

Based on these results, cellular growth rates between glyphosate treatments were statistically different ($F_{4,15}$: 16.32; p = 0.0001). Cellular growth at 0, 1, 3, and 6 mg/L of glyphosate was significantly different from growth at 10 mg/L ($p < 0.025$), and growth at 3 mg/L of glyphosate was statistically different from growth at 6 mg/L (p = 0.006).

Unlike *S. quadricauda*, the growth rate of *S. capricornutum* was negative at 6 mg/L of glyphosate (-55417 cells/mL/day), and at 10 mg/L of glyphosate, cells continued to die at a negative rate (-329167 cells/mL/day) until day nine, at which point growth was completely inhibited (Fig 2B). Based on these results, growth rates between glyphosate treatments were statistically different ($F_{4,15}$: 44.97; p = 0.0001). Mean growth at 0, 1, 3, and 6 mg/L of glyphosate was significantly different from growth at 10 mg/L (p = 0.0001), and growth at 1 mg/L of glyphosate was different from growth at 6 mg/L (p = 0.0490).

Ch-a concentrations of *S. quadricauda* were highest at 6 mg/L of glyphosate (194 µg/L) (Fig. 3A). At 1 and 3 mg/L of glyphosate, Ch-a levels were greater (141 and 121 µg/L) compared to respective pheophytin concentrations (72 and 93 µg/L), and at 10 mg/L, only pheophytin was present (173 µg/L). Despite these differences, chlorophyll and pheophytin concentrations were not statistically different between glyphosate treatments.

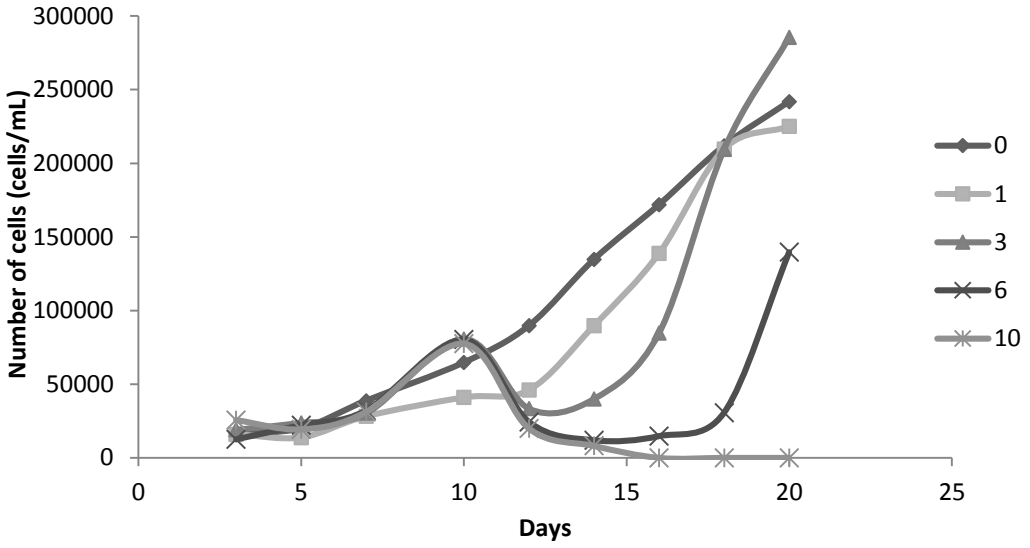
A

Table 1. Mean cellular growth (cells/mL/day ± SE, n = 20) of S*. quadricauda* and *S. capricornutum*, as affected by glyphosate (A).

Glyphosate concentration (mg/L)	Mean cellular growth (cells/mL/day)*	
	Scenedesmus quadricauda	***Selenastrum capricornutum***
Control (0)	19079 ± 4243^{ab}	22292 ± 23002^{ab}
1	23907 ± 4021^{ab}	47292 ± 30468^{a}
3	33669 ± 3301^{a}	30208± 10100^{ab}
6	12469 ± 3701^{b}	-55417 ± 12677^{b}
10	-5000 ± 2437^{c}	-329167± 32050^{c}

* Concentrations marked by the same letter are not statistically different.

A



B

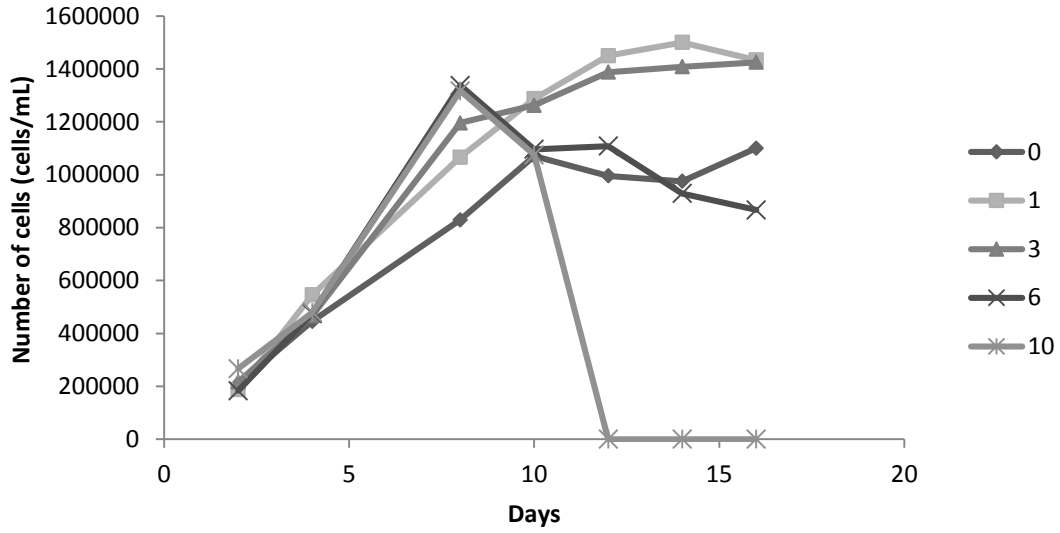
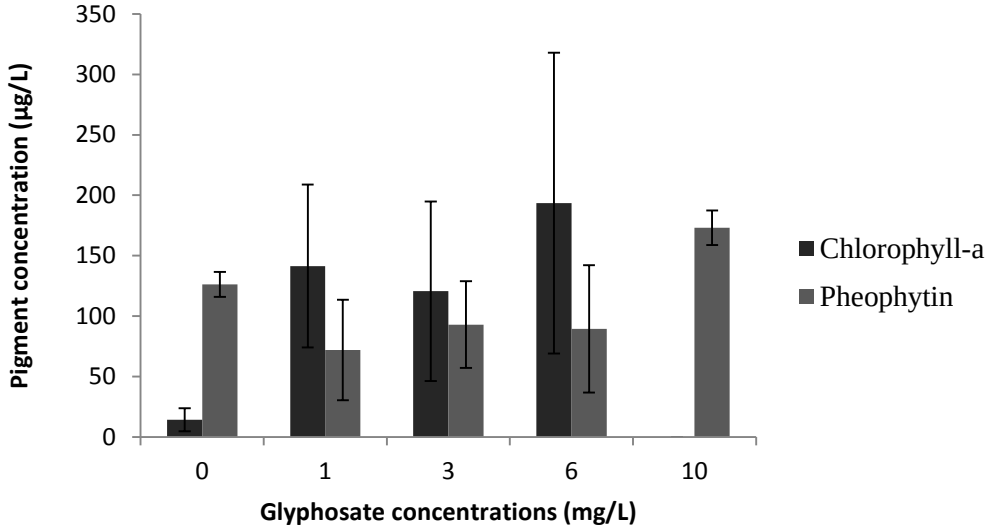


Figure 2. Number of algal cells through time (cells/ mL, n = 20). Glyphosate (0, 1, 3, 6, and 10 mg/L) was added at day twelve for *S. quadricauda* (A) and day eight for *S. capricornutum* (B). Standard error bars were removed for clarity.

At 0, 1, and 3 mg/L of glyphosate, Ch-a levels of *S. capricornutum* were greater (281, 362, and 241 µg/L) compared to respective pheophytin concentrations (11, 29, and 35 µg/L) (Fig. 3B). At 6 mg/L of glyphosate, pheophytin concentrations were greater (128 µg/L) than Ch-a (18 µg/L), and at 10 mg/L of glyphosate, only pheophytin was present (96 µg/L). After analyses, we found that Ch-a ($F\text{-ratio}_{4,15} = 9.63$; p

= 0.0005) and pheophytin ($F_{4,15}$= 13.07; P = 0.0001) concentrations were statistically different between glyphosate treatments; Ch-a concentrations at 0, 1, and 3 mg/L of glyphosate were significantly different from Ch-a at 10 mg/L ($p < 0.0366$), and Ch-a at 1 mg/L of glyphosate differed from Ch-a at 6 mg/L ($p = 0.0025$). Pheophytin concentrations at 0, 1, and 3 mg/L of glyphosate were significantly different from pheophytin at 6 mg/L ($P < 0.0027$), and pheophytin at 0 and 1 mg/L of glyphosate differed from pheophytin at 10 mg/L ($P < 0.0129$).

A



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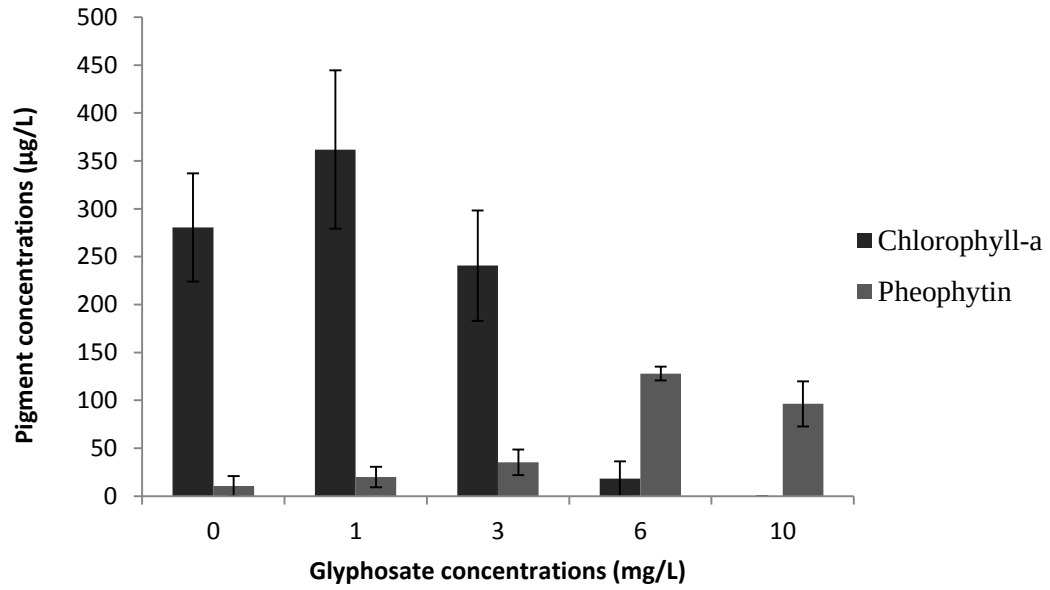

Figure 3. Chlorophyll-a and pheophytin concentrations (µg/L ± SE, n = 20) of *S. quadricauda* (A) and *S. capricornutum* (B). Ch-a was determined when growth approached stationary phase (maximum cellular yield).

Discussion and Conclusion

We had predicted that a concentration greater than 2 mg/L of glyphosate would inhibit cellular growth and Ch-a synthesis in both species. Concentrations of 1 and 3 mg/L of glyphosate did not affect growth or Ch-a synthesis in either species. In a similar study, Peterson et al. (1994) also found that glyphosate was relatively non-toxic at the expected environmental concentration (EEC) of 2.848 mg/L.

At 6 mg/L of glyphosate, cellular growth was affected in both algal species (Fig. 2). In *S. quadricauda*, cellular growth was less subsequent to glyphosate inoculation, but once glyphosate molecules broke-down and toxicity weakened, living cells grew and reproduced at a positive rate (Fig. 2A). This phenomenon could be attributed to the properties and half-life of glyphosate. In water, the half-life of glyphosate is 4.2 days, after which it is degraded by bacteria or incorporated into algal biomass (Vera et al., 2010). A short half-life could justify the initial and abrupt toxicity of glyphosate and explain why Ch-a concentrations were high at final yield (Fig. 3A). For *S. capricornutum*, growth was reduced and pheophytin levels were high relative to the control at 6 mg/L of glyphosate (Fig. 2B and 3B). These results support our second hypothesis; we found that *S. capricornutum* was more sensitive to glyphosate than *S. quadricauda*. Previous studies have found that sensitivity will vary based on the SA:V ratios of the organisms (Pirszel, Pawlik, & Skowronski, 1995; Lei, Hu, Wong, & Tam, 2007). Lei et al. (2007) measured a SA:V ratio of 0.89 μm^{-1} for *S. quadricauda* and 1.82 μm^{-1} for *S. capricornutum*; the greater the SA:V ratio of an organism, the greater its exposure to the surrounding environment (Pirszel et al., 1995). Dvorakova, Rojickova-Padrtova, and Marsalek (1999) also suggested that sensitivity differences could be attributed to the morphology (cell size and shape, colony formation, etc.), cytology (cell wall and intracellular structure), physiology (growth, nutrient uptake, metabolic rate, etc.) or genetics of the organisms.

Finally, we found that a concentration of 10 mg/L of glyphosate significantly inhibited cellular growth and Ch-a synthesis in both species. In similar studies, Saenz et al. (1997) found that concentrations of 12.5 and 25 mg/ L of glyphosate significantly reduced cellular growth in *S. quadricauda*, and Wong (2000) found that a concentration of 20 mg/L of glyphosate completely inhibited cellular growth in *S. quadricauda*. In another study, Tsui and Chu (2003) found that a concentration of 5.81 mg/L of glyphosate partially inhibited (50%) cellular growth in *S. capricornutum*, suggesting that a concentration near 10 mg/L of glyphosate would completely inhibit growth. It has been suggested that glyphosate causes growth inhibition by means of the shikimate pathway enzyme, 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS) (Tesfamariam, Bott, Cakmak, Roemheld, & Neumann, 2009). The inhibition of EPSPS stops aromatic and amino biosynthesis, reducing protein synthesis and the overall growth of the algae (Vera et al., 2010).

Ch-a synthesis of *S. quadricauda* and *S. capricornutum* was also inhibited at 10 mg/L of glyphosate (Fig. 3). These results were comparable with the study of Wong (2000), who found that 2 mg/L of glyphosate affected Ch-a synthesis, whereas a concentration of 20 mg/L of glyphosate completely inhibited Ch-a synthesis in *S. quadricauda*. In another study, Bozeman, Koopman, and Bitton (1989) found that 7.8 mg/L of glyphosate partially inhibited (50%) Ch-a synthesis in *S. capricornutum*, suggesting that a concentration near 10 mg/L of glyphosate would completely inhibit Ch-a synthesis. The inhibition of Ch-a synthesis reduces photosynthesis, resulting in cellular death (Shikha & Singh, 2004).

Throughout our study, we encountered issues with variability between the treatment replicates. Dvorakova et al. (1999) proposed that the formation of 2-, 4- or 8-celled coenobia of *S. quadricauda* causes lack of uniformity and results in counting variability. Dvorakova et al. (1999) also stated that *S. quadricauda* has the tendency to stick to flask walls. In contrast, Blaise (1993) considered *S. capricornutum* a good test species because it is unicellular and non-motile. Skulberg (1967) also reasoned that *S. capricornutum* is ideal to test because it has little morphological variability. However, we found that *S. capricornutum* was difficult to count because of its small size, which made it difficult to determine whether cells were living or dead under the microscope. Based on these issues, we suggest using methods other than cell counts to determine algal biomass. The American Water Works Association (2010) stated that cell aggregations increase the variability between replicate samples and every subsample taken from the original flask increases the probability of error. Alternative methods, such as CO_2 uptake and ash-free dry mass might be more appropriate indicators of algal biomass (American Water Works Association, 2010).

Other studies have detected no greater than 1 mg/L of glyphosate in Manitoba freshwater systems (Beck, 1987; Goldsborough & Brown, 1993); however, direct spray or concentrated spills of glyphosate could

affect algal species and influence organisms that rely on algae as food. To further understand the toxicity of glyphosate, outdoor mesocosm studies should be implemented since the effects of glyphosate could be influenced by abiotic factors such as light availability and temperature associated with seasonal change (Pesce et al., 2009). More studies should also focus on the toxicological effects of POEA and other surfactants that are combined with glyphosate.

Materials and Methods

Treatments

We purchased isolated cultures of *S. quadricauda* and *S. capricornutum* from Carolina Biological Supply (Burlington, NC, USA). These species were selected based on their common use in batch culture studies and their varying sensitivity to glyphosate toxicity (Peterson et al., 1994; Saenz et al., 1997; Wong, 2000). We also purchased commercial Roundup® manufactured by Monsanto Chemical Co. (St. Louis, MO, USA) (540 g/L of glyphosate as active ingredient) from Ag Advantage Ltd. (Marquette, MB, Canada). For a previous experiment, we applied treatments ranging from 0, 0.01, 0.1, 1, and 10 mg/ L of glyphosate, but found that most of these concentrations did not affect *S. quadricauda* and *S. capricornutum*. Therefore, we chose additional treatment levels to determine the threshold concentration at which growth and Ch-a synthesis were inhibited: 0 (control), 1, 3, 6 and 10 mg/L. All treatment levels were replicated four times to account for variability.

Culturing techniques

A batch culture medium provides a finite source of nutrients to growing algae (Andersen, 2005). The medium of choice for this study was Chu no.10 (40 g/L Ca $(NO_3)_2$, 5 g/L K_2HPO_4, 25 g/L $MgSO_4 \cdot 7H_2O$, 20 g/L Na_2CO_3, 20 g/L Na_2SiO_3, and 0.8 g/L $FeCl_3$) because it is often used to culture freshwater green algae (Chu, 1942; Stein, 1973). We added hydrochloric acid (HCl) to acidify the medium to a suitable pH (7.00-7.50) for algal growth (Chu, 1942). We then dispensed 100 mL of the medium into 20 autoclaved Erlenmeyer flasks (250 ml) and added the appropriate algal solution (3 ml).

The cultured algae were stored in a growth chamber to ensure that light and temperature were kept consistent throughout the course of the experiment. The particular chamber maintained a regular temperature of 22 ± 0.3° C and a light-dark cycle of 16 to 8 hours. Typically, algae are light-saturated at an intensity of 250 µmol/m^2/s (i.e. represents the point at which light is no longer limiting the rate of photosynthesis) (Andersen, 2005); therefore, we maintained a light intensity of approximately 250-300 µmol/m^2/s. We also randomized the placement of flasks within the chamber between density counts so that each flask received approximately equal exposures of light.

Cell count procedures and growth rate analysis

Before counting a sample, we shook the flasks to ensure the algal cultures were homogenized. We then pipetted a small algal sample to fill both counting chambers of a Neubauer Improved haemocytometer (C.A. Hausser and Son, Philadelphia, PA, U.S.A) and used a phase-contrast compound microscope (Leitz Diaplan) to count the cells within the grid regions of the counting chambers. Once cell count reached exponential phase (Fig. 1), we inoculated the cultured algae with glyphosate. After inoculation, we continued counting until cellular growth approached stationary phase (maximum cellular yield).

To determine glyphosate toxicity after inoculation, we calculated the slope of exponential growth by linear regression using JMP® 10.0.1 (SAS Institute Inc., Cary, NC, U.S.A.). This was accomplished by plotting day versus cell count for each treatment. All the assumptions of a linear regression were tested prior to analysis. If the data did not follow parametric assumptions, we still considered the linear regression to be robust enough to account for deviations, which were likely the result of a small sample size.

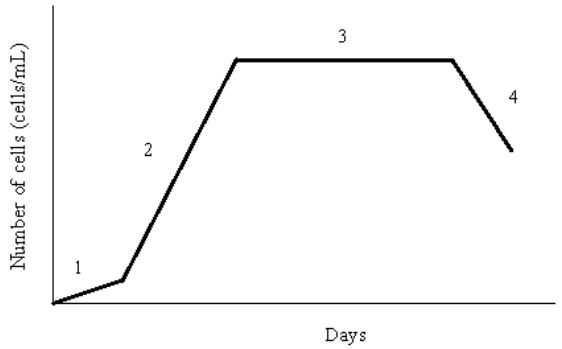

Figure. 1. Growth curve representing algal growth through time (days). The curve comprises of the lag phase (1), log or exponential phase (2), the stationary phase (3), and the death phase (4).

Chlorophyll-a analysis

Ch-a concentrations (µg/L) were analyzed when cellular growth approached stationary phase. We passed the culture medium of each flask through a vacuum filter system using Whatman GF/C filter papers. We used these particular filter papers because they were a suitable size (1.2 µm) for collecting our algal species. After filtration, we pipetted the algal samples into plastic cuvettes and loaded them into a spectrophotometer, which read the absorbance values of all pigment samples. Absorbance was measured at both 665 and 750 nm for all samples. A wavelength of 665 nm represented the peak absorbance of Ch-a and a wavelength of 750 nm accounted for contaminants outside the typical wavelength of Ch-a. We then added (0.1 mL) diluted hydrochloric acid (0.02 M) to the cuvettes in order to destroy all Ch-a within the samples. After one hour, we measured absorbance readings again at 665 and 750 nm to determine the amount of pheophytin (break-down product of Ch-a) within the samples. Finally, we calculated Ch-a and pheophytin concentrations based on the formulae described by Marker, Crowther, and Gunn (1980).

Statistical Analysis

The significance between glyphosate treatments and mean cellular growth rates, final Ch-a, and pheophytin concentrations was tested in JMP® using a one-way analysis of variance test (ANOVA) followed by a post-hoc Tukey test (p-value ≤ α = 0.05). We tested the assumptions of the ANOVA prior to analysis. If the data did not follow parametric assumptions, we still considered the ANOVA to be robust enough to account for deviations, which were likely the result of a small sample size.

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Impacts on declining moose populations in southeastern Manitoba

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Abstract

Moose (*Alces americanus*) populations in eastern and central North America have declined in many parts of their southern habitat range. Many potential impacts have been suggested as contributing to moose declines, including changing habitat disturbance regimes and enhanced disease transmission through increasing deer populations. We examined factors affecting moose in Game Hunting Area (GHA) 26 in southeastern Manitoba, an important traditional hunting area where moose populations have declined substantially, by comparing provincial aerial survey data with features of the landscape. Moose were more likely to be found in areas with high logging (>25%) and recent forest fires (within the past 30 years), indicating that moose respond favorably to habitat disturbances. The presence of roads did not affect the likelihood of moose presence; however moose populations were negatively impacted by white-tailed deer (*Odocoileus virginianus*). We used model selection to determine the variables most important for predicting the presence of moose in GHA 26. The best model included the presence of deer, logging, and forest fires. Among the variables considered, deer presence had the highest relative importance. This study suggests that to increase moose numbers, controlled burns and potential logging areas should be considered as ways to produce new habitat and plant growth for moose in southern Manitoba. Managing the deer population may also help control the effect of the deer brain worm (*Parelaphostrongylus tenuis*) on the moose population in GHA 26.

Keywords: *Alces americanus*, forest fire, logging, *Parelaphostrongylus tenuis*, white-tailed deer

Introduction

Moose (*Alces americanus*) are important herbivores in the boreal forest ecosystem and help maintain forest structure by consuming specific plant species (McInnes et al., 1992). Moose are important prey for wolves when smaller ungulates are not available, and moose that are subject to non-predatory mortality are an essential source of food for scavenging wolves and other carnivores (Forbes & Theberge, 1992). Furthermore, moose are important economically, with important consumptive and non-consumptive value to humans. Moose make up a significant portion of people's diets in many regions. In parts of Alaska about 7,300 moose are harvested each year and about 100kg of moose meat is eaten annually per person (Titus et al., 2009). However, moose populations in southern ranges in North America have declined in recent decades, with several explanations proposed for the cause of the decline (Lankester, 2001).

One important factor determining moose abundance and distribution is the white-tailed deer (*Odocoileus virginianus*). White-tailed deer carry a meningeal parasite, the deer brain-worm (*Parelaphostrongylus tenuis*), that also affects moose living in the same area (Schmitz & Nudds, 1994). The deer brain-worm causes neurological diseases in moose, which can be fatal (Gilbert, 1973). Whitlaw and Lankester (1994) found that the density of moose was inversely related to the density of deer in 83 Wildlife Management Units in Ontario. The highest moose densities were found where the deer densities were <4 per km^2 and the lowest moose densities were observed where the highest mean intensity of *P. tenuis* in deer feces was found (Whitlaw & Lankester, 1994). Moose populations may also be affected by major roads, which cause habitat loss, high noise levels and direct moose mortality (Laurian et al., 2008). Moose are known to be attracted to roads to avoid insects and because of sodium rich vegetation that is created by the addition of salt to icy roads (Laurian et al., 2008). Dussault et al. (2007) showed that moose consider areas near highways as low-quality habitat by using moose-vehicle accident data and Global Positioning System (GPS, a satellite-based navigation system that provides time and location information) collars that recorded the number of road crossings for 47 moose. Roads also allow hunters to have access to moose habitats.

Logged forest areas are generally known to have high moose densities because of new forest conditions and growth (Leavesley, 2010). In one Quebec population, moose densities increased by 25% solely because of a logged area that was harvested 10 years prior (Potvin et al., 2005). However, logging also allows road access to hunters, which can increase moose mortality (Crichton et al., 2004). Regions inflicted by forest fires are likely to attract moose as they can forage on new plant growth in the burned areas after a forest fire (MacCracken & Viereck, 1990). In Alaska, areas with high fire frequency had on average a 10% increase in moose density each year within a 10-year period, as heavily burned areas produced a higher amount of new browse (Shenoy et al., 2011).

Southern Manitoba has experienced a dramatic drop in moose numbers in recent years. For example, the moose population in Game Hunting Area (GHA) 26 in southeastern Manitoba has declined by 47 percent over a four year period, from 1,553 individuals (95% CI:1,300 - 1,807) in 2006 to 823 individuals (95% CI: 675 - 972) in 2010 (Leavesley, 2010). To understand the cause of this decline, we examined several factors that possibly affect moose presence in the area, including the presence of deer, roads, logging and forest fires. We predicted the presence of deer and roads would negatively affect the presence of moose, and logging and recent forest fires would positively affect moose populations. We expected deer presence would be particularly important for moose because of the high prevalence of the deer brain-worm in this area; it is estimated that 80% of the deer are infected (Manitoba Conservation, unpubl. data). The declining moose population is a concern because moose are an important ungulate species that regulate boreal habitats and are essential sources of food for many predator species, including humans.

Results

Deer presence and row (i.e., latitude) were highly correlated (r_s=0.59, p<0.0001), with lower pairwise correlations between all other predictor variables. Moose presence was positively correlated with high logging (r_s=0.17, p=0.0009) and forest fire presence (r_s=0.27, p<0.001), negatively correlated with deer (r_s=-0.45, p<0.001), and unrelated to road presence (r_s=-0.08, p=0.103) (Figure 3).

The full logistic regression model, including all the predictor variables, was highly significant (R^2=0.263, χ^2=142.7, p<0.0001) and produced similar results for individual predictors. The presence of deer (χ^2=4.60, p=0.0320), high logging (χ^2=3.86, p=0.0495) and fire (χ^2=3.89, p=0.0485) significantly affected moose presence, as did both row (χ^2=21.81, p<0.0001) and column (χ^2=29.47, p<0.0001). The presence of roads was unrelated to the presence of moose (χ^2=0.82 p=0.364).

The best model for explaining the presence of moose within our study area included the presence deer, logging and forest fire (Table 1). We also found some support (ΔAIC<2) for the full model (with roads), the model with just deer and logging, and the model with just deer and fire (Table 1). Comparing the relative importance of the predictor variables, deer presence had the highest relative importance (0.77), followed by logging (0.69), fire (0.62), and road presence (0.33).

A

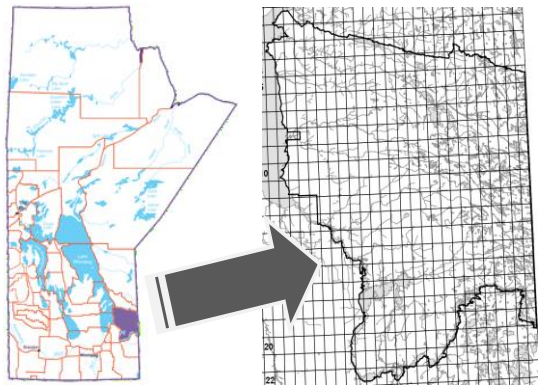

Figure 1. A map of Manitoba with GHA 26 highlighted in purple (left) and a map of GHA 26 with the grid system overlaid onto the region (right)(A).

A

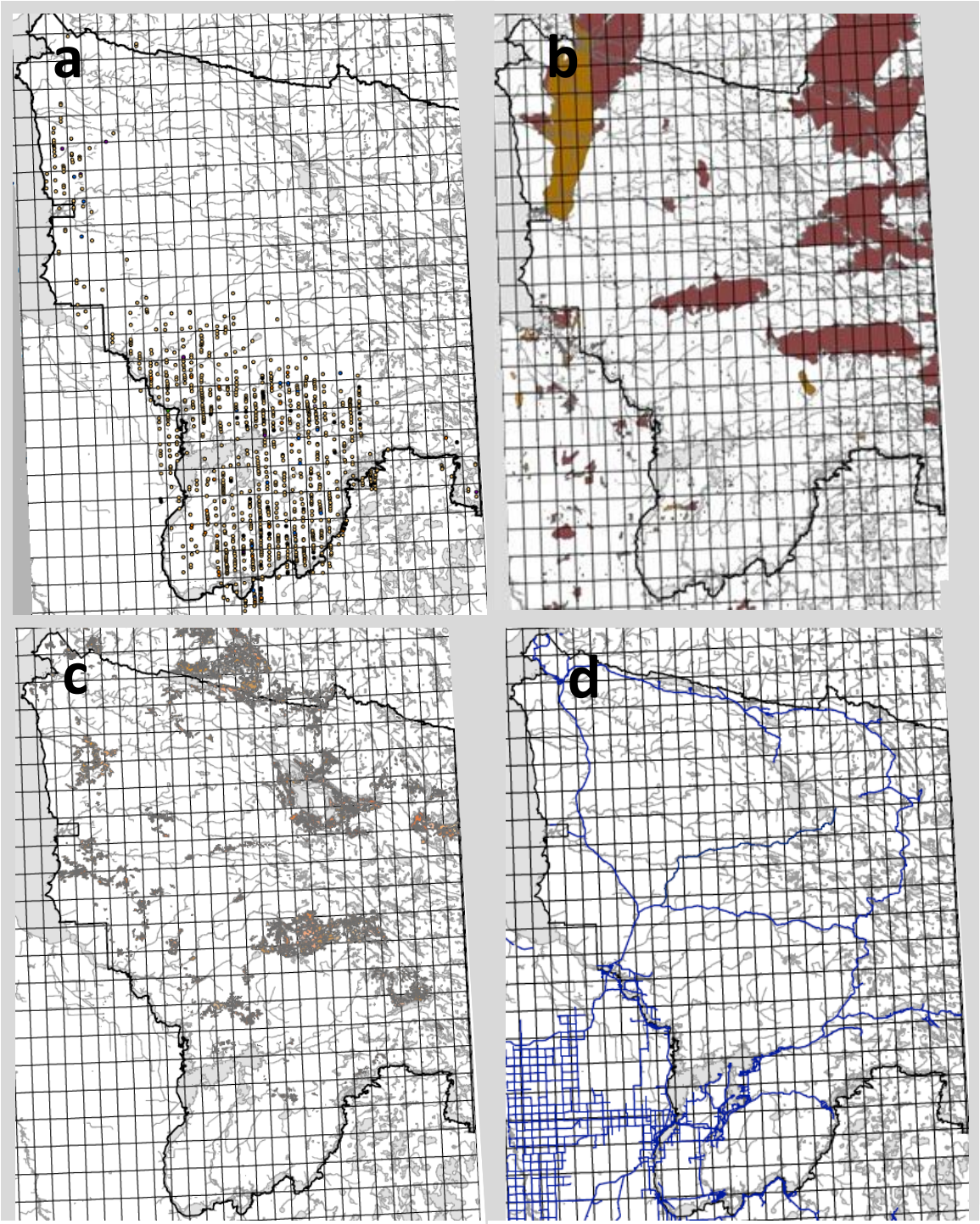

Figure 2. Maps of GHA 26 with the grid system and locations of the four independent variables: a) white-tailed deer (2010), b) forest fires (since 1980), c) logged areas (since 1983), and d) major roads (A).

A

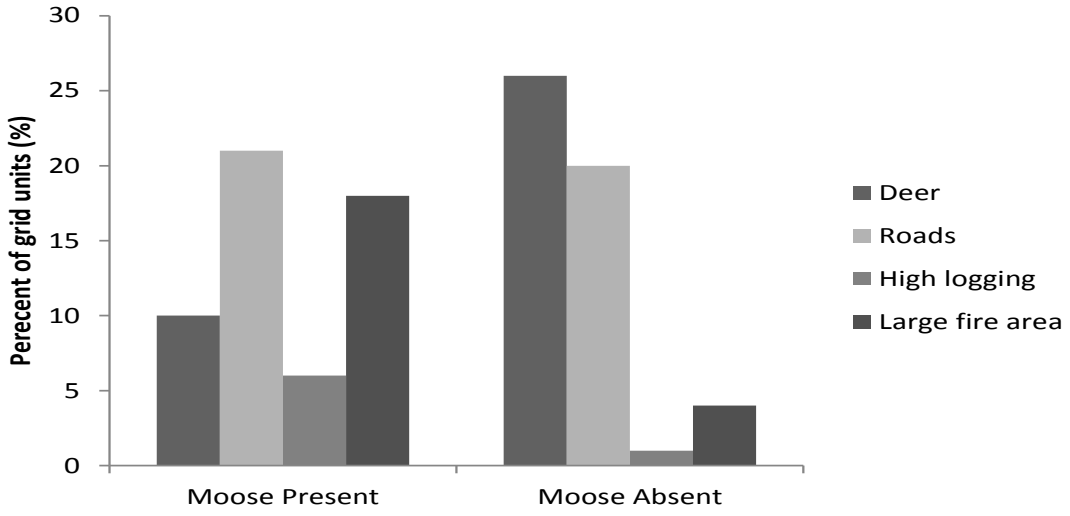

Figure 3. Probability of encountering each predictor variable in grid units with or without moose present (N=397) (A).

Table 1. Comparison of logistic regression models using Akaike's Information Criteria (AIC), showing the top ten models for explaining the presence of moose. Row and column variables were also included in each model to control for spatial autocorrelation. The coefficient of determination (R^2), loglikelihood, sample size (N), number of parameters in the model (K), uncorrected AIC (AIC), delta AIC (ΔAIC), and the probability of each model (weight) are shown (A).

A

Model Variables	R^2	Loglikelihood	N	K	AIC	ΔAIC	Weight
deer, fire, logging	0.2614	200.415	397	7	414.830	0.000	0.2323
deer, roads, fire, logging	0.2630	200.002	397	8	416.005	1.174	0.1291
deer, logging	0.2553	202.084	397	6	416.168	1.338	0.1190
deer, fire	0.2551	202.128	397	6	416.257	1.426	0.1138
fire, logging	0.2531	202.668	397	6	417.337	2.506	0.0663
deer	0.2448	203.851	397	5	417.701	2.871	0.0553
deer, roads, fire	0.2558	201.931	397	7	417.862	3.031	0.0510
deer, roads, logging	0.2558	201.949	397	7	417.897	3.067	0.0501
fire	0.2481	204.041	397	5	418.083	3.253	0.0457
logging	0.2473	204.260	397	5	418.521	3.690	0.0367

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Discussion and Conclusion

Moose were more likely to be seen in areas that have been subject to habitat disturbances (logging or fires) and in areas where deer are absent (Figure 3). Whitlaw and Lankester (1994) also showed that moose tend to occupy areas where deer are absent. Additionally, areas that have been logged have seen increased moose presence 10 years after harvesting (Potvin et al., 2005) and annual increases in moose presence have also been noted in severely burned areas (Shenoy et al., 2011), agreeing well with the findings of this study. Contrary to our expectation, however, the presence of a major road was not an important influence on moose presence.

Deer presence had a greater impact on moose presence than any other variable, as predicted. The deer brain worm is thought to strongly impact moose populations (Schmitz & Nudds, 1994), but increasing deer numbers could also introduce other factors such as other parasites and competition for resources (e.g., habitat and food) that could negatively affect moose. Deer presence and moose presence should be examined together in relation to habitat (fields, swamps, forests, etc.) to examine potentially confounding influences on the relationship between these two variables, such as associated habitat types.

It is important to recognize that the stratification data represent a small window of time, and that the visibility of moose and tracks may depend on snow conditions, temperature, weather conditions, and habitat. For example, tracks are easier to see in bright sunlight and moose are easier to see in overcast conditions (K. Leavesley, pers. comm.). Moose also move around more in cold weather to keep warm, making more tracks in the snow. Very thick bush cover can prevent surveyors from sighting animals or tracks located underneath (Gasaway et al., 1986). Moose and tracks are much easier to see in open, post forest fire and logged areas. In 2010, the stratification flights started after a 48 hour fresh snowfall and the weather was mainly clear with a sun and cloud mix, providing good conditions for visibility of both tracks and moose (Leavesley 2010). Furthermore, our study used a very large sample size increasing its reliability, and our results were highly significant based on statistical tests.

Declining moose presence in GHA 26 is a concern because of ecological and predatory relationships. This study demonstrated that deer are likely the key factor affecting moose presence in GHA 26, which suggests that the deer population may need to be controlled to decrease the spread of *P. tenius* to moose. Currently in GHA 26, muzzle loader and rifle deer seasons are extended and extra deer tags are available to hunters to reduce the deer population. Controlled burns in certain regions in GHA 26 would greatly increase the amount of new plant growth available to moose in the area and increase their presence. Potential areas that would be good for logging should be assessed to create new plant growth and habitat for moose. Further studies could also focus on different habitat types, food sources, predators, hunting pressures and weather conditions in GHA 26 that might affect moose presence. This study suggests that conservation measures could help recovery of the declining moose presence in GHA 26.

Materials and Methods

Data on the distribution of moose and deer in the study area were collected by Manitoba Conservation in Lac du Bonnet, Manitoba, using aerial surveys of the 7,702 km^2 study area, employing the Modified Gasaway-Lynch Method (Leavesley, 2010; Gasaway et al., 1986). This technique involved stratification flights flown along prearranged transect lines in a north-south orientation using a Cessna 337 (a safe and efficient aircraft commonly used for wildlife surveys) at ~400ft above the ground and ~90 mph. Surveys for moose and deer were done during winter (February 2010) for greater sightability of the animals and tracks in the snow (Gasaway et al., 1986), and any observed moose or deer sightings, tracks or cratering by deer were noted and recorded as GPS locations (Leavesley, 2010). Moose and tracks could be easily distinguished because moose are much larger and leave a diamond-shaped imprint in light snow, while deer tracks resemble ski tracks and do not leave a diamond shape (Oswald, 1997). Moose overall walking patterns are meandering while deer travel in distinctive directional patterns, leaving paths in the snow (Oswald, 1997). Deer also jump in deep snow, leaving distinct blotch patterns, whereas moose do not (Oswald, 1997).

Locations for major roads were determined using regional highway maps and then shape files were made. Logged areas were determined by taking aerial photos from a fixed wing aircraft and GPS locations were recorded. The photos were used to create shapefiles. Each year a fixed wing aircraft flew the perimeter of recent forest fires to record GPS locations and shapefiles were made. All the GPS locations and shapefiles were superimposed on a map of GHA 26 that was overlaid by a grid in ArcGIS Desktop 10.0, a geographic information system (GIS) commonly used for analysing aerial survey data and producing maps, The grid squares or units were 3.5 x

5.5 km (19.25 km²), with n=397 grid units covering the entire study area (Figure 1).

Five different maps of GHA 26 were created for the study; one for the dependent variable (moose presence) and one for each independent variable (presence of deer, roads, logging and forest fires; Figure 2). For each grid unit we determined if moose were present (a binary variable) based on sightings of either animals or tracks, since thick canopy cover can prevent surveyors from sighting animals underneath (Gasaway et al., 1986). We also determined the presence of each independent variable on each grid unit. For deer presence we included deer sightings and tracks as well as evidence of cratering (digging through the snow to feed on vegetation). Roads were recorded as present if any road ran through any portion of the grid unit. We used logging data collected from 1983-2010 and forest fire data from 1980-2010 because valuable habitat to moose is expected to decline sharply 20-30 years after a disturbance (Manitoba Conservation, pers. comm.). We considered grid units that were highly affected by logging or fires as distinct from grid units that were only slightly affected. Delong and Tanner (1996) considered large fires and logged areas to be greater than five square kilometers. Since 25% of a grid unit was 4.8 km², we considered grid units to have low forest fire or logging presence when < 25% of the grid unit was covered and high forest fire or logging presence when ≥25% of the grid unit was covered.

We used a series of logistic regression models to test which variables best predicted moose presence. To control for spatial autocorrelation we added row and column variables to each model (reflecting latitude and longitude, respectively). First, we used the Spearman's rank order correlation (r_s) to check the predictor variables for multicollinearity. We considered $r_s > 0.7$ or $r_s < -0.7$ as highly correlated. We also examined the correlations between the dependant and independent variables, to understand associations between them. We then ran each logistic regression model and used model selection to rank them based on Akaike's Information Criteria (AIC). We used uncorrected AIC due to the large sample size, and also calculated the Akaikie weight of each model (the probability of being the best model of those considered). Finally, we calculated relative importance of each predictor variable by summing the weights of all models containing that variable to determine which independent variable had the biggest influence on moose presence.

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Mycological diversity on Jack Pine and Black Spruce bark by Payuk Lake, Manitoba

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Abstract

Fungi provide an essential role to the ecosystem they inhabit by decomposing dead organic material; however, they have been studied little in Northern Manitoba. This is seen in Payuk Lake; located within a chain of lakes that make up the headwaters of the Grass River. This region is currently under consideration for conservation. The objectives of this study were to investigate the mycological diversity of Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) at Payuk Lake, MB, and to examine the differences in mycological diversity between heights of tree trunks and between Jack Pine (*Pinus banksiana*) and Black Spruce *(Picea mariana*). Bark samples were collected from three heights on Jack Pine and Black Spruce trees along the ridges of Payuk Lake. The bark samples were dried and placed in moist chambers for three weeks and their characteristics were described via the use of the dissecting and compound light microscopes. A total of 18 organisms were cultured from the bark samples, nine of which were classified as myxomycetes and nine as zygomycetes. Two-way cluster analysis showed that the most common organisms according to abundance were zygomycetes, and the uncommon were myxomycetes. Moreover, cluster analysis showed distinct clustering between tree species and heights. Shannon's diversity index was not significant between Jack Pine and Black Spruce, but trends were evident. The diversity of fungi, and other organisms, could be large and should be further investigated to better understand the importance of the Payuk Lake region, which would help conserve the headwaters of the Grass River.

Keywords: Conservation, fungal diversity, Grass River, Payuk Lake, myxomycetes

Introduction

Fungi are heterotrophic organisms that have a critical role in all ecosystems. Fungi decompose organic debris to be used in the nutrient cycles of the ecosystem and are important food sources for many animals, including humans (Kendrick, 2000). The "macroscopic" groups of fungi, the Basidiomycota (club fungi) and Ascomycota (sac fungi) are most well-known; however, the "microscopic" groups of fungi are equally important (Kendrick, 2000). Zygomycetes, the bread molds, colonise substrates rich in carbon and are important decomposers of rich organic materials (Kendrick, 2000). Zygomycetes also form symbiotic relationships with the roots of economically important trees, such as pine and spruce, and are found worldwide. Myxomycetes, or the slime molds, are found on moist substrata, such as wood and litter (Schnittler et al. 2006), which is filled with bacteria. They can be found in all types of habitats including alpine regions (Roniker & Roniker, 2009), dry deserts (Wrigley de Basanta et al., 2008), various forest types, and aquatic ecosystems (Lindley et al., 2007).

Both groups of microscopic fungi have been known to grow on trees (Kendrick, 2000). Bark provides a good microhabitat for many fungi. Bark characteristics, such as texture and pH, vary between tree species. Different bark textures allow for the retention of water and other nutrients that may be essential for the survival of many fungi. In addition to, bark pH and moisture influences the distribution of fungi between tree species as seen along different heights of tree trunks (Everhart et al., 2008), since different species exhibit different tolerances to pH and water content. While these organisms have been greatly studied, the diversity of Zygomycetes and Myxomycetes of Northern Manitoba has been studied little (Bisby et al., 1938), and little is currently known about the bark inhabiting fungi of this region.

Payuk Lake is a post-glacial lake nestled within the boreal forest of Northern Manitoba. It is a part of a

chain of lakes in the Mystik Creek system, and is one of the many important water bodies in the headwaters of the Grass River (T. Booth, personal communication, 23 April 2012). Payuk Lake is the last lake in the chain, before the start of the Grass River. The Grass River is protected within the Grass River Provincial Park, and is an important river system ecologically. The Grass River Provincial Park protects the river system, as well as preserves the geological transition between the Churchill River Uplands and the Manitoba Lowlands within the boreal forest (Manitoba Conservation, 2012). The park also serves to protect the natural habitat of the endangered woodland caribou. The park prides itself for the pristine, clear waters of the Grass River which is protected by the Clean Environment Commission (Manitoba Conservation, 2012). The Mystik Creek system, however, has been decreasing in water quality over the years (T. Booth, personal communication, 23 April 2012), due to mining and misuse of the water through recreation. The continued pollution of Mystik Creek and its interconnected lakes could eventually harm the Grass River region, affecting not just the caribou, but plants and other animals as well. Locals around the Flin Flon area are currently trying to extend the conservation boundaries of the Grass River Provincial Park to include the Mystik Creek system (T. Booth, personal communication, 23 April 2012). Investigation of the plants, fungi, lichens, and animals within this region will be used to justify the extension of the park boundaries.

The objectives of this study were 1) to observe the diversity of Zygomycetes and Myxomycetes on Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) at Payuk Lake, MB, 2) to examine the difference in mycological diversity between Jack Pine and Black Spruce , and 3) to examine the change in diversity with respect to height within Jack Pine and Black Spruce populations.

Results

There were 18 cultured organisms present after the three week culturing period (Table 1). Six organisms had a plasmodium and the rest had hyphae present. Nine of the organisms had fruiting structures and spores, while the rest were sterile (Table 1).

Shannon's Diversity Index was not significantly different between Jack Pine and Black Spruce (Figure 2 A), or within either Jack Pine or Black Spruce. However there were opposite trends within the two tree species. Within Jack Pine, Shannon's Diversity Index showed a decrease in diversity from the bottom height to the top height while the opposite was true within Black Spruce (Figure 2 B-C).

Two-way cluster analysis between organisms present and bark samples of Jack Pine and Black Spruce revealed two distinct clusters within the organisms and four distinct clusters between bark samples (Figure 3). The organisms clustered by commonness, with organisms 1, 5, 7, and 13 being most common (Figure 3 cluster B), and the rest being uncommon (Figure 3 cluster A). All myxomycetes present except organism 7 clustered within the uncommon group, with organism 7 clustering in the common group.

Cluster I of the bark samples contained only Black Spruce samples, with all heights present, and Cluster II contained only Jack Pine samples, with all heights present (Figure 3). Cluster III had two distinct sub-clusters, one containing only Black Spruce top samples (Figure 3 cluster III.1), and one containing only Jack Pine samples with all heights present (Figure 3 cluster III.2). Sub-cluster IV.1 had only Black Spruce bottom samples and sub-cluster IV.2 clustered a mix of both Jack Pine and Black Spruce, with all heights present (Figure 3).

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Table 1. Morphological characteristics of cultured organisms found on Jack Pine and Black Spruce bark from Payuk Lake, Manitoba.

Organism	Overall Colour	Main Strcture	Main Structure Colour	Septation	Branching	Main Structure Tips	Cell Walls	Fertility	Spore Size (units)	Spore Shape	Spore Colour	Number of Cells per Spore	Spore Texture	Spore Walls
1	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
2	Orange	Plasmodium	Hyaline to Orange	Aseptate	No	Blunt	Single	Fertile	5	Circular	Clear	1	Smooth	Single
3	Bright Red	Plasmodium	Red	Aseptate	No	Blunt	Single	Fertile	6	Circular	Clear	1	Smooth	Single
4	Yellow Green and	Plasmodium	Yellow	Aseptate	No	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
5	White	Hyphae	Light Green	Septate	No	Blunt	Single	Fertile	2	Circular	Clear	1	Smooth	Single
6	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
7	Black	Plasmodium	Hyaline	Septate	No	Blunt	Single	Fertile	5	Circular	Brown	1	Ridged	Single
8	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
9	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
10	White	Hyphae	Hyaline	Septate	Yes	Tapering	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
11	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
12	Light Pink-Orange	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
13	Grey-Clear	Hyphae	Light Brown	Septate	Yes	Tapering	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
14	Light Green	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Sterile or Immature	N/A	N/A	N/A	N/A	N/A	N/A
15	White	Hyphae	Hyaline	Septate	Yes	Blunt	Single	Fertile	1	Circular	Clear	1	Smooth	Single
16	White and Brown	Hyphae	Hyaline to Light Brown	Septate	No	Blunt	Single	Fertile	6 long, 2 wide	Oval/ Ellipsoid	Clear	1 to 2	Smooth	Single
17	Brown	Plasmodium	Brown	Aseptate	No	Blunt	Single	Fertile	6	Circular	Light Brown	1	Rough	Single
18	Yellow-Grey	Plasmodium	Yellow-Grey	Aseptate	No	Blunt	Single	Fertile	5	Circular	Clear	1	Smooth	Single

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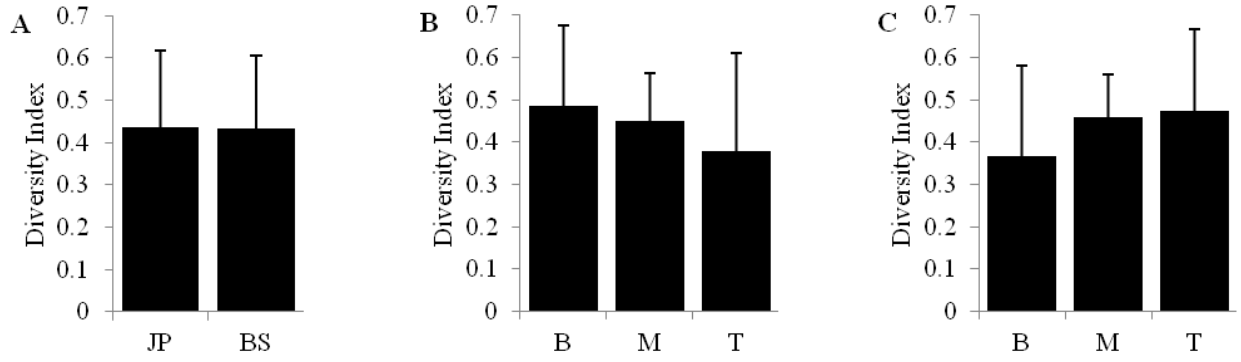

Figure 1. A comparison of Shannon's Diversity Index on Jack Pine (JP) and Black Spruce (BS), showing standard deviations. A: Differences between JP and BS, n = 18. B: Differences in height above ground (B = 0 m, M = 0.65 m, T = 1.3 m) of JP, n = 6. C: Differences in height above ground (B = 0 m, M = 0.65 m, T = 1.3 m) of BS, n = 6 (A).

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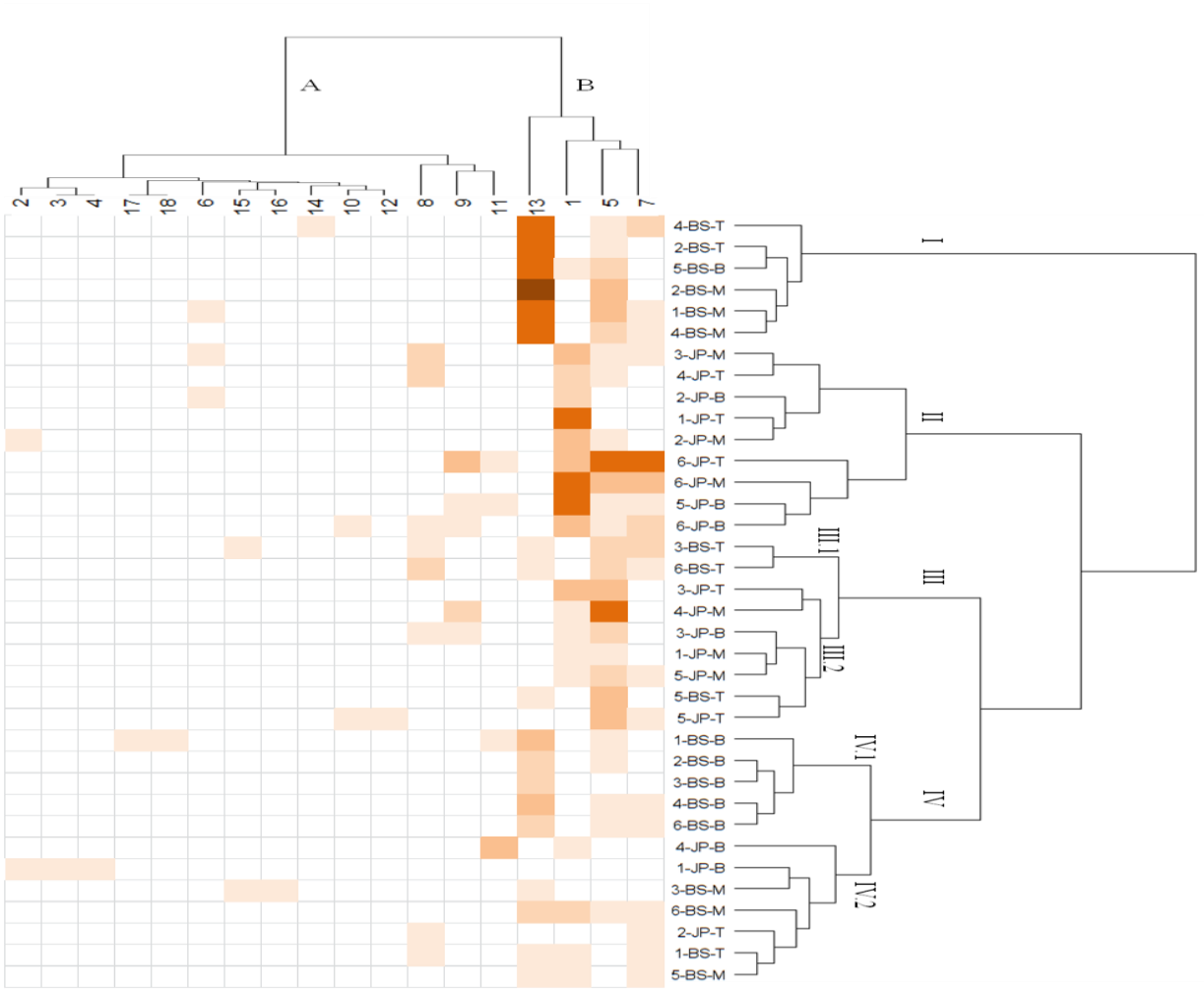

Figure 2. Two-way clustering of cultured organisms (1-18) and bark samples (transect #, tree species, height) of Jack Pine (JP) and Black Spruce (BS) from three heights (B = 0 m, M = 0.65 m, T = 1.3 m above ground) (A).

Discussion and Conclusion

Historically, little has been known about the mycological diversity of northern Manitoba (Bisby et al., 1938), and little is currently known about the bark inhabiting fungi of this region. Organisms 2, 3, 4, 7, 17, and 18 had plasmodia and were considered to be myxomycetes (Table 1). Organism 16 may have been an ascomycete due to the presence of well-developed asci and cup-like appearance. The other organisms were either zygomycetes or ascomycete anamorphs, though due their immature or sterile form cannot be completely determined (Table 1).

The relationship with tree species and height indicating the presence and absence of the organisms is quite interesting. The most common organisms present were either zygomycetes or anamorphs (Figure 3 cluster B). Organism 13 was found only on Black Spruce, with the highest frequencies in the top and middle heights (Figure 3 cluster I) and some occurrences in the bottom height (Figure 3 cluster IV.1), which could be a result of higher pH in Black Spruce bark compared to Jack Pine (Dix and Webster, 1995). Organism 1 was found almost exclusively within the lower height (Figure 3 cluster II, IV.2). Dix and Webster (1995) suggest that fungal species tend to colonise substrata with either a low or high pH. Organisms 5 and 7 were found to occur in both Jack Pine and Black Spruce samples, with no preference to height (Figure 3 cluster I, II, III), which could suggest these organisms tolerated a range of pH conditions or other environmental conditions.

The effect of pH on growth occurs alongside multiple factors and cannot be the sole cause of fungal substrate preference (Dix and Webster, 1995). Another factor that could have resulted in organism preference of one tree species is moisture content, as determined by the texture of the bark (Dix and Webster, 1995). Black Spruce bark had more scaly bark than Jack Pine, which had very flaky, layered bark. The different texture of the barks could result in varying moisture content as water is trapped within the layers. Rougher, scalier bark would be able to hold more moisture than smoother, more appressed bark (Dix and Webster, 1995). Since organism 13 only occurred on Black Spruce bark, it could be suggested that it has a higher tolerance for lower moisture content than the other common organisms and therefore could grow on this particular bark. It can be further suggested that organism 1 has a higher tolerance to higher moisture content than the other organisms and is able to grow on Jack Pine bark. Organisms 5 and 7 could have a moderate tolerance for either moisture content and could grow on either Jack Pine or Black Spruce bark.

The exact clustering of the bark samples was also caused by the presence of uncommon organisms. Within cluster A, the uncommon organisms showed two clustering patterns. The uncommon anamorphs and zygomycetes (Figure 3 cluster A) showed no distinct preference for either tree species or height category, except for organism 6 which only occurred in the middle heights of both Jack Pine and Black Spruce. This preference may be a result of a balance of many factors such as pH, moisture, and other factors. The myxomycetes (organisms 2, 3, 4, 17, and 18) clustered only in bottom samples, with organisms 2, 3, and 4 occurring on Jack Pine and organisms 17 and 18 present on Black Spruce. The preference to the bottom height may be due to different pH and moisture content as compared to the other heights in both Jack Pine and Black Spruce. Also, myxomycetes feed on bacteria, which are abundant in soil (Kendrick, 2000). Being closer to the ground would ensure a plentiful food supply to survive. The myxomycete, organism 7, occurred on all the heights in both tree species, suggesting that this organism may have a different feeding strategy or is more adapted to varying environmental conditions.

Shannon's diversity index showed opposite height trends in Jack Pine and Black Spruce. Diversity was higher on the bottom of Jack Pine than the top, and lower on the bottom of Black Spruce than the top (Figure 2). This trend is confirmed by the cluster analysis (Figure 3) where all the Black Spruce bottoms formed a cluster characterised by low organism frequencies (Figure 3 cluster IV.I). Although, Jack Pine did not form as distinct a cluster as the Black Spruce, the Jack Pine bottoms generally displayed higher organism frequencies than the other Jack Pine heights in all clusters (Figure 3). Jack Pine bottoms may have had higher frequencies of organisms due to a more suitable environment, higher nutrient levels or interactions of environmental factors. Diversity differences between heights on the Black Spruce may be due to differences in bark texture. At the bottom, the bark was less flaky and more tightly compressed. While, higher up the bark, it was found to be flakier, allowing for water and nutrients to be trapped. This is similar to the flaky bark found on all heights of the Jack Pine trees.

In summation, the mycological diversity on Jack Pine and Black Spruce at Payuk Lake Manitoba has been investigated and 18 morphological organisms have been described, though not identified. There appears to be some preference between Jack Pine and Black Spruce bark, as well as with height above ground within Jack Pine and Black Spruce. Further investigation of the mycological diversity should be undertaken with other tree species, such as White Spruce, Tamarack, and Aspen, and among the lakes within the Mystic Creek/Grass River headwaters.Also, molecular identification and phylogenetic characterisation of the fungi at Payuk Lake would complement the research into the diversity and provide a complete collection of the fungi in this area. Finally, continued research and understanding of the diversity within this area could help to extend the conservation area of the Grass River Provincial Park. By understanding the diversity in this area, we can infer and further investigate the environmental changes in this ecosystem and how it may be affected by the tourism and recreational development, which can be used to protect the headwaters of this important river.

Materials and Methods

Site Description

Payuk Lake is a lake in Northern Manitoba nestled within the Canadian Shield. The study sites around Payuk Lake were characterised by boreal forest dominated by Jack Pine (*Pinus banksiana*), Black Spruce (*Picea mariana*), and White Spruce (*Picea glauca*). Other trees present included White Birch (*Betula papyrifera*), Balsam Fir (*Abies balsamea*) and Trembling Aspen (*Populus tremuloides*). The dominant shrubs present were Alder (*Alnus* spp.) and Willow (*Salix* spp.). In addition to this, common herbs included Common Juniper (*Juniperus communis*), Blueberry (*Vaccinium myrtilloides*), Bearberry (*Arctostaphylos uva-ursi*), Red Osier Dogwood (*Cornus sericea*), and Twinflower (*Linnaea borealis*). Grass and moss species were also present. Finally, common ground-dwelling lichens present were *Cladonia* spp., *Cladonia stellaris*, *Cladonia rangiferina*, *Cladonia arbuscula*, *Stereocaulon* spp., *Xanthoparmelia* spp., and *Umbilicaria* species.

GIS

Initial study sites were selected in the open source software Quantum GIS v. 1.7.0 Wroclaw (Quantum GIS Development Team, 2012), using forest inventory maps (Manitoba Land Initiative 2004). Transect locations were randomly selected in appropriate forest types (Jack Pine (JP) ≥ 10%, Black Spruce (BS) ≥ 10%) along the shores of Payuk Lake and the coordinates were imported onto the GPS (Garmin GPSmap 76C x, datum WGS 84). Twelve sites were randomly selected, though only 6 suitable sites visited were to be sampled. This allowed for some error in the accuracy of the 8-year-old forest inventory.

Field Methods

Study sites were reached by canoe and the GIS forest compositions confirmed. The GPS coordinates were recorded (Figure 1). The overall site vegetation was recorded. A 50 m transect was laid down following the ridge to minimise the change in slope. The closest Jack Pine and Black Spruce to the transect were sampled every 10 m. Each tree was sampled at three heights (B = 0 m, M = 0.65 m, T = 1.3m) on the north side of the tree. Bark samples at each height were collected by scraping off the outer bark with a pocket knife and placed into paper bags for transport back to the lab. A 10 cm x 10 cm area of bark was collected for each sample.

Culturing

Bark samples were air dried in the lab for two days (Lindley et al., 2007). Deep dish petri plates were labelled and filled with 10 tsp of fine-grain vermiculite. The vermiculite was soaked overnight with sterile distilled water. The bark samples were placed in their respective dishes to minimise overlapping and to cover the surface of the vermiculite (Lindley et al., 2007). The petri dishes were covered to act as moist chambers, and placed in a temperature controlled room at 15°C with the lights remaining on. The samples were cultured for three weeks and observed at the end of the three weeks under the dissecting microscope for mycological growth. If growth was present, the organism was described using the features observed under the dissecting scope and compound light microscope.

Data Analysis

Shannon's Diversity Index (Shannon, 1948), measure of α diversity including the evenness of the samples, was calculated by summing the numbers of organisms present for each height category together for each transect so that a single diversity index was calculated for each height of each tree species in each transect. Shannon's Diversity was compared between tree species and among heights with a Student's t-test and ANOVA in Minitab 14 Student Edition (Minitab 14 Statistical Software, 2010), with α = 0.05. Two way cluster analysis using Euclidean distances and Ward's method of clustering was performed between the

organisms present and the bark samples using R (R Development Core Team, 2009).

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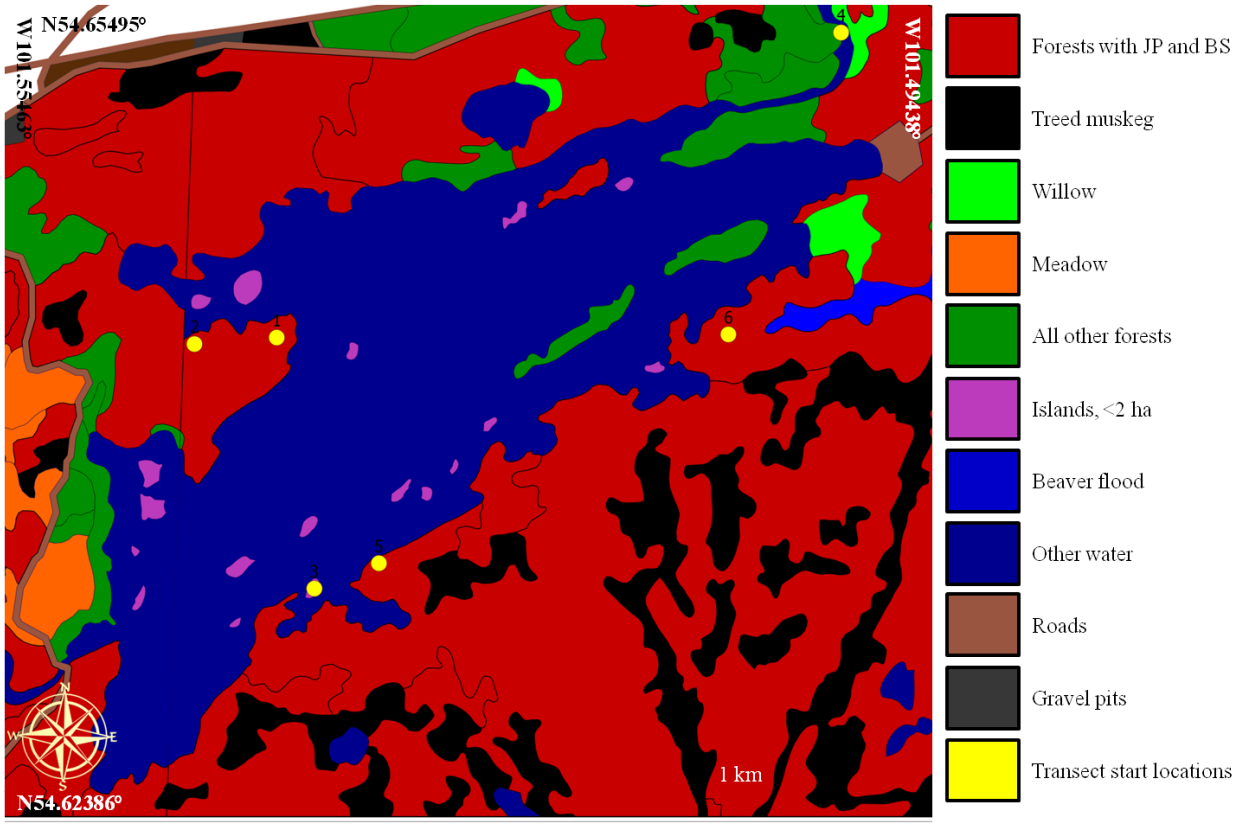

Figure 3. Forests around Payuk Lake, Manitoba, showing sampled transects and in forests with ≥ 10% Jack Pine (JP) and ≥ 10% Black Spruce (BS).

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E-cadherin, catenin, cytoskeletal interactions and induced pluripotency

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Abstract

The glycoprotein, E-cadherin, mediates tight intercellular contacts and promotes an epithelial cellular phenotype. An early requirement for induced pluripotency from somatic fibroblasts is a mesenchymal-to-epithelial transition which includes an upregulation of E-cadherin expression at the cell surface. This review summarizes the current knowledge on the induction and maintenance of pluripotent cells mediated by E-cadherin's specific cellular signaling and cytoskeletal interactions.

Keywords: pluripotency, E-cadherin, human embryonic stem cells (hESC), induced pluripotent stem cells (iPSC), mesenchymal-to-epithelial transition (MET)

E-cadherin-mediated cell contacts are essential for induced pluripotency

Pluripotency is defined as the capability of cells to differentiate into cell types derived from each of the three embryonic germ layers (Kelly et al., 2011). Induced pluripotency defines a process of epigenetic reprogramming in which epigenetic changes implemented during differentiation are reversed to generate cells with a stem-like phenotype. Fibroblasts (Takahashi and Yamanaka, 2006; Sommer et al., 2009), human keratinocytes (Aasen et al., 2008) and nasal mucosal cells (Ono et al., 2012) can be reprogrammed to stem-like cells with introduction of four stem cell transcription factors Oct4, KLF4, Sox2 and c-Myc (OKSM). Previous studies have provided evidence that the adhesion and cellular signaling provided by a glycoprotein, known as E-cadherin, has essential functions in pluripotency. Induced pluripotency has potential medical application where the induced pluripotent stem cells (iPSCs) apply as clinical tools for modeling diseases, drug development, and to deliver cell-replacement therapy to support regenerative medicine (Goldthwaite, 2011).

The present review summarizes our current knowledge on the role of E-cadherin, its cellular signaling and cytoskeletal interactions in the induction and maintenance of pluripotent cells.

Induced pluripotency describes the result of reprogramming somatic cells into pluripotent stem cells (iPSC) which are capable of self-renewal and of developing into all three germ layers (Zhao and Daley, 2008), similar to embryonic stem cells (ESC). Reprogramming of murine somatic cells, mouse embryonic fibroblasts (MEFs), through the introduction of the four transcription factors OCT4, SOX2, KLF4 and c-MYC (OSKM) was demonstrated by Takahashi and Yamanaka (2006). Using the same four OSKM transcription factors the reprogramming of human somatic skin fibroblasts into iPSC was performed successfully (Park et al., 2008; Takahashi et al., 2007).

Similar to ESC from other species, human ESC and human iPSC derived from somatic cells form tightly adherent cell colonies, an assembly that is essential for maintaining pluripotency (Park et al., 2008; Takahashi et al., 2007; Takahashi and Yamanaka, 2006; Thomson et al., 1998; Yu et al., 2007). Human ESC and iPSC display high levels of E-cadherin (Ohgushi and Sasai, 2011). This E-cadherin cell surface expression was shown to play a role for self-renewal and the maintenance of the undifferentiated state in mouse and human ESC (Li et al., 2010c; Redmer et al., 2011; Soncin et al., 2009; Soncin et al., 2011) and was down-regulated during differentiation (Redmer et al., 2011). Cadherins are calcium-dependent type I transmembrane cell adhesion proteins (Cavallaro and Christofori, 2004;

Takeichi, 1995; Yoshida and Takeichi, 1982). Strikingly, dissociation of human ESC or iPSC colonies during culture resulted in massive apoptosis (Ohgushi et al., 2010; Ohgushi and Sasai, 2011). Trypsin, a serine hydrolase most commonly used to disaggregate multicellular ESC colonies, was shown to cleave E-cadherin from the cell surface (Xu et al., 2010) suggesting that the intercellular adhesion mediated by E-cadherin provides essential stimuli for the survival of human ESCs and iPSCs. Similarly, blocking or siRNA-mediated down-regulation of E-cadherin impairs ESC survival (Li et al., 2010c; Xu et al., 2010).

Upregulation of cell adhesion molecules promotes Mesenchymal-to-epithelial Transition (MET)

Cell adhesion constitutes an essential component for pluripotency. When pluripotency is induced in MEFs a transition from a mesenchymal to an epithelial phenotype was observed. Mesenchymal-to-epithelial transition (MET) is considered the hallmark for the initial phase during reprogramming of somatic fibroblasts (Li et al., 2010c; Qin et al., 2007) and includes the up-regulation of epithelial cell surface proteins such as E-cadherin, several claudins and epithelial cell adhesion molecule (EpCam) (Huang et al., 2011; Li et al., 2010c; Samavarchi-Tehrani et al., 2010). Following induction of reprogramming in MEFs, E-cadherin expression was detected in SSEA1- and NANOG-positive cells (Redmer et al., 2011). Expression of E-cadherin and cytokeratin even preceded the presence of the pluripotency marker SSEA1 (Li et al., 2010c). Cre-mediated deletion of floxed E-cadherin in MEFs prevented reprogramming emphasizing the importance of tight-junctional cell contacts during the initial phase of reprogramming (Redmer et al., 2011).

Krüppel-like factor 4 (Klf4), one of the four reprogramming transcription factors, upregulated E-cadherin by binding to its promoter, and omission of Klf4 prevented induced pluripotency (Li et al., 2010c). Klf4 was shown to regulate the expression of VE-cadherin in tight junctions of endothelial cells (Cowan et al., 2010) and to bind to the E-cadherin promoter in mammary epithelial cells thus promoting an epithelial phenotype through formation of E-cadherin-mediated cell contacts (Yori et al., 2010). Consistent with the epithelial transition being an important requirement for reprogramming, Klf4 was dispensable for reprogramming of epithelial cells suggesting the pre-existing expression of epithelial cell E-cadherin-based tight junctions to bypass the need for KLF4 during reprogramming. Indeed, higher reprogramming efficiency was observed using human epithelial cells such as keratinocytes (Aasen et al., 2008) and mammary gland epithelial cells (Li et al., 2010c).

MEFs with E-cadherin knock-down failed to undergo reprogramming (Redmer et al., 2011) further emphasizing the essential role for cell-cell contacts in induced pluripotency. Surprisingly, exogenous expression of E-cadherin was able to replace OCT4 as one of the reprogramming factors in the induction of pluripotent cells (Redmer et al., 2011). It is currently not known whether E-cadherin signalling induces expression of endogenous OCT4 and which E-cadherin downstream factors mediate the induction/support of pluripotency transcription factors. Intracellular mediators of E-cadherin functions are β-catenin and p120-catenin which connect to the cytoplasmic tail of E-cadherin (Cowin and Burke, 1996; Davis et al., 2003). P120-catenin stabilized the E-cadherin-catenin complex by inhibiting its endocytosis (McCrea and Park, 2007). The actin-binding protein α-catenin functions in linking the E-cadherin to the actin cytoskeleton, thus promoting an epithelial phenotype (Drees et al., 2005; Ozono et al., 2011; Yamada et al., 2005).

Regulation of E-cadherin in stem cells

In the presence of Wnt-signaling, β-catenin accumulation was shown to induce endogenous OCT4 and to enhance OCT4-mediated transcriptional activity in a TCF-independent manner (Kelly et al., 2011). T-cell factor proteins (TCFs) are nuclear execution factors for the Wnt/β-catenin signaling pathway recruiting co-activators to Wnt response elements of respective target genes (Arce et al., 2006). However rescue experiments with mutant E-cadherin lacking the cytoplasmic β-catenin binding domain demonstrated that E-cadherin induced reprogramming was independent of its β-catenin-binding domain (Chen et al., 2010). Thus, the importance for E-cadherin in the reprogramming process is not given by its signalling through the canonical β-catenin pathway but the tight-junctional cell contacts.

Furthermore, E-cadherin-negative mouse ESC were capable of Wnt-induced β-catenin/TCF signalling (Soncin et al., 2011) indicating the structural requirements for E-cadherin to be independent of its intracellular mediator β-catenin. The role for the canonical Wnt- β-catenin signalling in ESC renewal and pluripotency was recently reviewed (Miki et al., 2011; Sanges and Cosma, 2011; Watanabe and Dai, 2011).

The activity of myosin II in epithelial cells determines the dynamics of the actin filaments, thus influencing cell shape, motility and cell polarity (Vicente-Manzanares et al., 2009). Myosin II controls E-cadherin mediated cell-cell contacts in hESC (Li et al., 2010a) via p120-catenin which binds to the transmembrane domain of E-cadherin (Thoreson et al., 2000). The non-muscle myosin II and p120 catenin pathways are upstream of E-cadherin in the reprogramming of human primary fibroblasts and keratinocytes (Li et al., 2010a).

Another protein regulating E-cadherin function is the small G-protein Rap1. Loss of function mutation for Rap1 showed a similar phenotype than those for E-cadherin with early embryonic lethality (Kan et al., 2007; Larue et al., 1994; Ohba et al., 2001). Rap1 was shown to regulate the rapid turnover of membrane-bound E-cadherin by endocytosis and by promoting E-cadherin re-assembly into adherens junctions (Li et al., 2010b). Consequently, inhibition or knock-down of Rap1 in hESC caused a decrease in E-cadherin expression and clonogenic capacity and increased apoptosis (Li et al., 2010b). At the same time, Rap1 was rapidly degraded in lysosomes following disruption of cell adhesion contacts in hESCs causing reduced recycling of E-cadherin to the cell membrane (Li et al., 2010b), a mechanisms which may contribute to decreased survival of hESC following dissociation in culture. Thus, the mutual positive regulation of the E-cadherin-Rap1 interaction contributes to maintaining pluripotency in hESC.

The broad clinical potential in the application for iPSC launched the search for conditions to improve the reprogramming efficiency of somatic cells, in part by promoting cell adhesion. The miRNA cluster 302-367 was shown to accelerate reprogramming of MEFs with just three factors SOX2, Klf4, and OCT4 by increasing E-cadherin and down-regulating TGFβ receptor 2, thus promoting MET (Liao et al., 2011). The small molecule Thiazovivin (2,4-disubstituted thiazole) was able to enhance hESC survival by stabilizing surface E-cadherin protein through inhibition of its endocytosis (Xu et al., 2010). Rho-associated kinase (ROCK) is one of the effectors of Rho which signals from the extracellular matrix to regulate actin cytoskeleton dynamics and cell contractility (Hammar et al., 2009). Thiazovovin prevented ROCK-Rho-mediated cytoskeletal remodelling and cell motility and favoured cell adhesion to extracellular matrices (Xu et al., 2010). The efficiency of induced pluripotency from MEFs was also enhanced by the two chemicals Apigenin and Luteolin, both of which up-regulated E-cadherin expression during the early phase of reprogramming (Chen et al., 2010).

Outlook

In summary, the structural functions of intercellular adhesions are predominantly involved in MET and are crucial requirements for reprogramming and pluripotency. Knowing the molecular mechanism that regulate the reprogramming processes will help with developing new and safer procedures to obtain iPSCs for clinical use (Sanges and Cosma, 2010). Induced pluripotent stem cells (iPSCs) present promising clinical tools in the near future for modeling disease, drug development, and to deliver cell-replacement therapy to support regenerative medicine (Goldthwaite, 2011).

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Abbreviations:

MEF: murine embryonic fibroblasts

MET: mesenchymal-to-epithelial transition

hESC: human embryonic stem cells

mESC: mouse embryonic stem cells

iPSC: induced pluripotent stem cells

miRNA: microRNA

Klf4: Krüppel-like factor 4

Oct-4: Octamer-binding transcription factor 4

TCF1: T-cell factor-1

Rap-1: Ras-related protein

TGFβ: transforming growth factor-beta

ROCK: Rho-associated kinase

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Anti-Nutritional Factors in Yellow Pea Flours that Underwent Different Processing and Their Pizza Dough Products

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Abstract

Anti-nutritional factors (phytic acids, total phenolic acids, and trypsin inhibitors) were evaluated in pea flours and their pizza dough products. Flours were prepared with different pre-heat treatments and milling technologies. Results indicated that milling technologies had minimum effects on anti-nutritional factors levels. Moist heating showed remarkable effects on reducing total phenolic acids and trypsin inhibitor activities in pea flours. Cooking the pizza dough at 190 °C for 6 minutes achieved 60% to 85% trypsin inhibitor inhibition, but increased the total phenolic acid content (up to 62% increase). The phytic acid did not seem to be affected by these treatments.

Keywords: Yellow pea flours, anti-nutritional factors, milling, micronization, roasting

Introduction

Yellow peas (*Pisum sativum*), as a very important source of fiber, protein, vitamins and minerals, are often a component of the human diet. However, yellow peas also contain significant levels of anti-nutritional factors such as phytic acids, total phenolic acids, and trypsin inhibitors; these factors have been proven to have negative effects on protein digestion.

Specifically, phytic acid has an inhibitory effect on mineral bioavailability (Vidal-Valverde et al, 1994), while total phenolic acids are currently considered beneficial due to their antioxidant activity (Mattila & Kumpulainen, 2002). Previous research has shown that phenolic acids can decrease protein accessibility to humans. In addition, trypsin inhibitors are low molecular weight proteins which can decrease the protein utility by inactivating the digestive enzyme, trypsin (Vidal- Valverde et al, 1994).

The purpose of this research was to study the effects of various milling technologies and pre-milling heat treatments on the anti-nutritional content in yellow pea flours as well as the effects of oven cooking on the anti-nutritional contents in pizza dough products.

Results

A

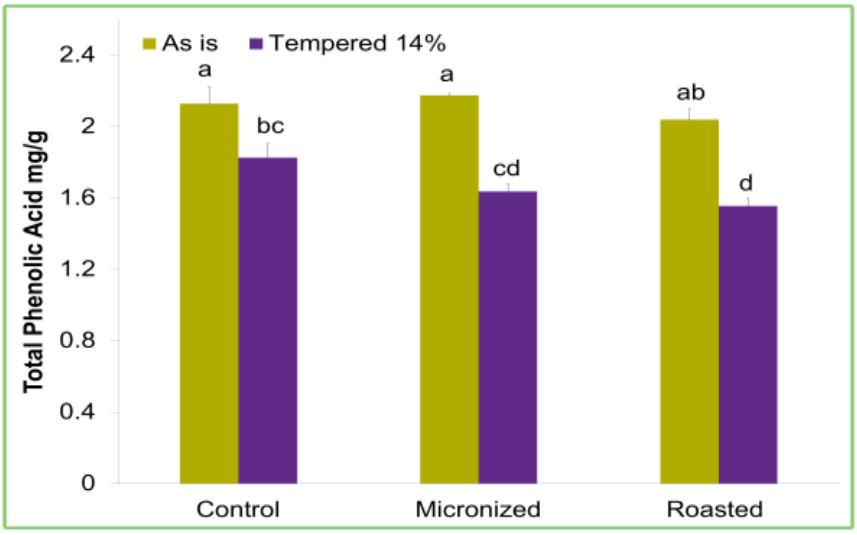

Figure 1: Effects of different thermal treatments prior to milling (at 100 °C) as well as different moisture contents on total phenolic acid in yellow pea flours (bars with different letters are significantly different, p<0.05) (A).

B

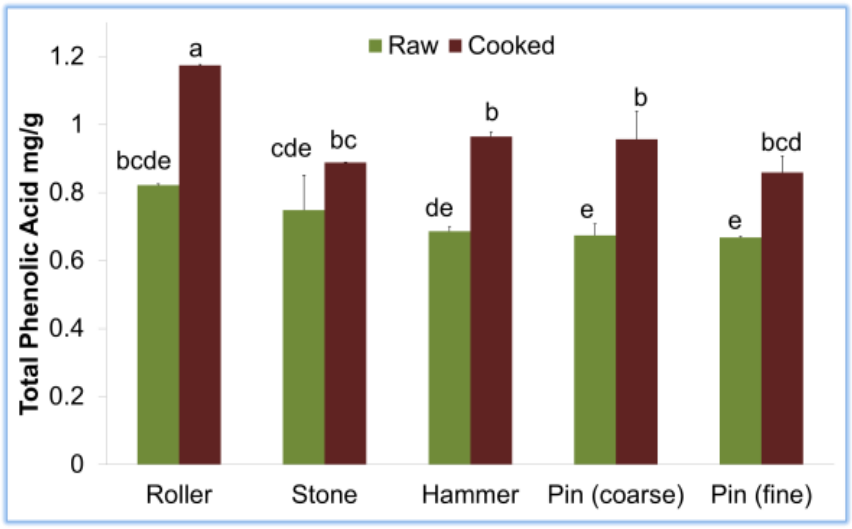

Figure 2: Total phenolic acid content in cooked or raw pizza dough which were made from (different types of milling) yellow pea flours (bars with different letters are significantly different, p<0.05) (B).

C

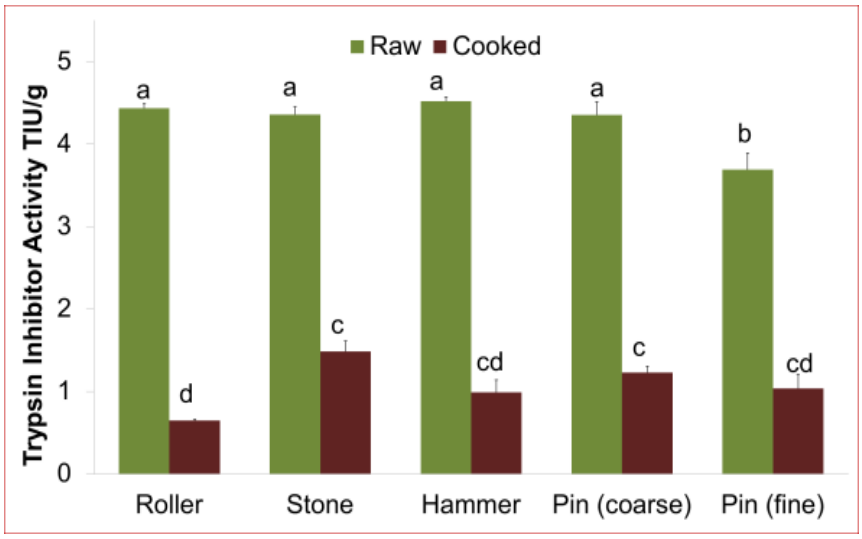

Figure 3: Trypsin inhibitor activity in cooked or raw pizza dough which were made from yellow pea flours that underwent different types of milling (bars with different letters are significantly different, p<0.05) (C).

Upon analysis of the experimental data, it can be seen that phytic acid was not significantly affected by any of the treatments in the experiment. In addition, milling technologies had no significant effects on total phenolic acid content and trypsin inhibitor activity in pea flours (only figures with significant difference are included).

From Figure 1, tempering to 14% moisture content decreased 14% the total phenolic acid content in the commercial pea flours (control), but 24% total

phenolic content for pre-heated (micronization or roasting) flours. However, pre-heating and tempering had no significant effects on trypsin inhibitor activity. After the pizza dough was cooked at 190 °C for 6 min, the total phenolic acid content achieved a25% to 62% increase (Figure 2), possibly because cooking broke the pizza dough cell wall to release more phenolic acids. However, 60% to 85% trypsin inhibitor activity was inactivated by cooking (Figure 3). A higher temperature and longer cooking time may be needed to totally inactivate the trypsin inhibitors in pizza.

Discussion and Conclusions

In summary, milling technologies had only minimal effects on the anti-nutritional factors of pea flour. Tempering and preheating did not reduce the phytic acid content and trypsin inhibitor activity in the pea flour. However, moist heating (14% moisture content) effectively reduced the total phenolic acid content in the flours which may benefit the animal feeding industry. The animal could grow faster if it is fed with low phenolic acid content pea flour. Cooking effectively inactivated the trypsin inhibitor found in the pizza dough and interestingly the total phenolic acid level increased in cooked pizza dough; this increase may be valued by the consumer seeking greater antioxidants because total phenolic acids are commonly regarded as an antioxidant agent nowadays.

Methods

Canadian whole yellow peas were either treated as is or tempered to 14% moisture content and thermally processed with micronizing or roasting at 100°C. These yellow peas were treated by different milling technologies including stone, hammer and pin milling. Moreover, all flours had their anti- nutritional content measured. Finally, these yellow pea flours were made into pizza dough products, cooked at 190°C for 6 minutes, and then had their anti-nutritional content measured.

Anti-nutritional factor analyses for all pea flours and pizza dough were as follows:

- Phytic acid measured by the method from Latta and Eskin (1980)
- Total phenolic acid evaluated by Folin-Ciocalteu method (Gao et al., 2002)
- Trypsin inhibitor activity determined by AACC method (AACC 22-40.1)

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Investigating the N-terminal Deletion in Mitochondrial Voltage-Dependent Anion Channels in *Neurospora crassa*

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Abstract

The mitochondrial outer membrane contains a class of porin – voltage dependent anion channels (VDAC) – that facilitate diffusion of small hydrophilic molecules across the outer membrane. VDAC proteins share a prominent property in which they display voltage-dependent conformational changes involved in gating when reconstituted into planar lipid bilayers. The N-terminus of VDAC is the focus of this study, due to conflicting structural and electrophysiological results presented by Geula et al. 2012 (Biochem J. 444: 475) and Teijido et al. 2012 (J. Biol. Chem. 287: 11437) with respect to the nature of the N-terminus' role in the gating mechanism. To verify the N-terminus' function and mobility in the VDAC gating mechanism, two *Neurospora crassa* strains (ΔN2a, WS125.5) were generated to contain identical VDAC N-terminal deletions of amino acids 2-12. However, the two strains exhibited incongruent phenotypes. Thus, the objective of this study was to determine the basis for the phenotypic differences seen in ΔN2a and WS125.5. Qualitative and quantitative phenotypic analyses were conducted using race tubes. In comparing growth rates of both strains to each other and to the growth rate of the wild-type, ΔN2a displayed a growth rate at a midpoint between wild-type and WS125.5 growth rates, while WS125.5 displayed significantly reduced growth rates compared to the wild-type and ΔN2a. Genomic DNA was extracted from both variants and sequenced using BigDye Termination 3.1, revealing the correct N-terminal deletion in ΔN2a. Sequences from WS125.5 failed to provide information regarding the N-terminal deletion in the strain due to incorrect annealing of primers. However, in amplifying the VDAC porin gene from the genomic DNA of WS125.5, the result was a double banding pattern – a band that is characteristic for wild-type and a band characteristic for the strain. This indicates that WS125.5 is a heterokaryon; however, it does not exhibit the wild type growth phenotype, suggesting that the N-terminal porin deletion is a dominant mutation. Further sequencing is currently being conducted to confirm the N-terminal deletion in WS125.5 and to examine the promoter and 3' UTR regions of both strains.

Keywords: VDAC; N-terminus; *Neurospora crassa*