Potato (*Solanum tuberosum* L. cv. Russet Burbank) petiole tissues, in Atlantic Canada, as they relate to yield and specific gravity.

Sebastian Xavier Margarit

Doctor of Philosophy

Department of Natural Resources Sciences

McGill University

Montreal, Quebec, Canada

August 2018

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

Abstract

Potato is a high value crop of economic significance in Atlantic Canada. The nutritional status of the plant is related to the outcome of key yield parameters of significance for potato producers. Potato petiole sampling is commonly used to assess the crop's nutritional status. The objective of this study is to relate petiole nutrient data to yield outcomes on farms in New Brunswick (NB) and Prince Edward Island (PEI). There were 39 farms evaluated in NB, and 109 farms surveyed in PEI during a five year period from 2010 to 2014. Sampling occurred once per week for the six week period from early to late tuber bulking stages. Petioles were analyzed for NO_3-N, non-NO_3-N, P, K, Mg, Ca, S, B, Zn, Mn, Fe, Cu, Na and Al concentrations. Yield outcomes were related to untransformed nutrient concentrations, as well as nutrient data transformed using the isometric log ratio (ILR) method. The non-NO_3-N concentration in petioles was the most consistent indicator of improved tuber yield and specific gravity. The tuber yield in NB and specific gravity in PEI were both positively correlated to B concentrations in petioles. In contrast, the Zn and Mn concentrations in petioles were negatively correlated with tuber yield and specific gravity. On the NB farms, the Ca, Mg, and B concentrations in petioles were the most consistent indicators of improved tuber yield, but the NO_3-N, S, P, and K concentrations in petioles generally showed a negative correlation with tuber yield. Specific gravity did not tend to be positively correlated with the nutrient concentration in petioles, but was consistently and negatively correlated with the NO_3-N, S, P, and K concentration in petioles. The use of ILR transformations did not improve the interpretation of potato nutritional status in either province and led to potential misinterpretation if observed out of context. This study demonstrates that temporal fluctuations in petiole nutrient concentrations may be an indicator of economically-important potato yield outcomes on farms in NB and PEI.

Résumé

La pomme de terre est une culture d'importance économique de grande valeur dans les provinces des Maritimes au Canada. L'état nutritionnel de la plante, lié aux résultats aux paramètres clés de rendement, est important pour les producteurs de pommes de terre. L'échantillonnage du pétiole de la pomme de terre est couramment utilisé pour évaluer l'état nutritionnel de la récolte. L'objectif de cette étude est de relier les données nutritionnelles du pétiole pour produire les résultats dans des fermes au Nouveau-Brunswick (NB) et à l'île du Prince-Édouard (Î.-P.-É.). Trente-neuf fermes (39) furent évaluées au Nouveau-Brunswick, et cent neuf (109) observées à l'Île du Price Édouard, au cours d'une période de cinq ans, soit de 2010 à 2014. L'échantillonnage a eu lieu une fois par semaine pendant une période de six semaines, du début de leur plantation à la fin des étapes de ramassage. Les pétioles ont été analysés pour leurs concentrations de NO_3-N, non-NO_3-N, P, K, Mg, Ca, S, B, Zn, Mn, Fe, Cu, Na et Al. Les résultats de rendement ont été associés à des concentrations de nutriments non transformées, ainsi que des données nutritionnelles transformées à l'aide de la méthode du ratio (ILR) du Journal isométrique. La concentration de non-N-NO3 dans les pétioles a été l'indicateur le plus cohérent de rendement de l'amélioration des tubercules et de poids. Le rendement des tubercules au Nouveau-Brunswick, et de densité à l'ile du Prince Édouard était tous deux positivement corrélé aux concentrations de B dans les pétioles. En revanche, les concentrations de Zn et Mn dans les pétioles sont négativement corrélées avec le rendement des tubercules et de leur densité. Dans les fermes, au Nouveau-Brunswick, les concentrations de Ca, Mg et B dans les pétioles furent des indicateurs plus cohérents dans l'amélioration du rendement de tubercules, mais le NO3-N, S, P et K des concentrations dans les pétioles montrent généralement une corrélation négative avec le rendement de tubercules. La gravité spécifique ne tendait pas à être en

corrélation positive avec la concentration des éléments nutritifs dans les pétioles, mais était constamment et négativement corrélée avec la concentration de NO3-N, S, P et K dans les pétioles. L'utilisation des transformations de l'ILR n'améliore pas l'interprétation de l'état nutritionnel de pommes de terre dans les deux provinces et peut conduire possiblement à de fausses interprétations, si observées hors contexte. Cette étude démontre que les fluctuations temporelles dans les concentrations d'éléments nutritifs du pétiole peuvent être un indicateur des résultats de rendement économiquement important des pommes de terre dans les fermes au Nouveau-Brunswick et à l'île du Prince Édouard.

Acknowledgements

Funding for the thesis project was provided by NutriAg Ltd, McGill University, and Laval University. Financial assistance for travel was made available by Centre Sève.

I would like to thank Dr. Joann Whalen for her patience and efforts in editing the manuscript. I would like to thank Dr. Philip Warman for his constant insight on agronomy. Thank you to my incredible and strong wife. I owe you dearly for a time management system based on disproportionate time allocation regarding the rearing of our three energetic young boys. I would like to also thank my farmers who allow me the privilege of walking their fields, your efforts feed the world and you're often not thanked enough.

I would like to dedicate the chapters and their contents to Wilfred Kelly to whom I owe so much. You are and always will be my potato mentor. I owe you for your exemplification of hard work, a keen eye for detail, and for teaching me about always putting the grower first. I think of you everytime I walk fields. Whilst in the rows I lament every missed opportunity I had to learn more from you. I get to see as many fields through to yield as you did. You are dearly missed by all who had the opportunity to walk a field and hear you say: Beautiful Morning.

Preface

The thesis is presented in manuscript-based format in accordance with the "*Guidelines for Thesis Preparation*" issued by the Graduate and Postdoctoral Studies Office of McGill University. The thesis begins with general introduction discussing the importance of crop nutrients and interpretation of plant tissue analysis to achieve potato yield and specific gravity improvements. This is followed by an in-depth synthesis of literature on this topic (Chapter 1) and two experimental chapters (Chapters 2 and 3) written in the manuscript format, followed by a general conclusion of the data presented in the thesis. A summary of the contributions to knowledge from this work is given at the outset.

Chapter 1 is an exploration of the existing methods of interpreting nutrient sufficiency in potatoes. Within this literature review the fertility requirements in potato, physiological roles of essential nutrients, nutrient movement within plant tissues and various methods of determining nutrient sufficiency will be explored. Chapter 2 is an investigation, using nutrient concentrations and ILR transformed data, of the potato petiole ionome, as expressed by nutrient concentrations, in relation to tuber yield and specific gravity outcomes in PEI. Chapter 3 is a series of observations, using nutrient concentrations and ILR transformed data, of the potato petiole ionome, as defined by nutrient concentrations, in relation to tuber yield and specific gravity in NB.

Contributions to Knowledge

This doctoral thesis makes novel, original contributions to knowledge by addressing important gaps in the literature and by providing a foundation for analysis of nutrient balance in potato petioles and relating this to potato yield and specific gravity improvement. The specific contributions that advance the basic and applied research agendas are:

1. Identified new petiole nutrient markers for improved tuber yield (Non NO_3-N) and specific gravity (B) as well as new deleterious indicators in petiole nutrient data for tuber yield (Ca) and specific gravity (Zn, and Mn) in PEI.
2. Identified new petiole nutrient markers for improved tuber yield (Ca, Mg, and B) in NB and new deleterious indicators in petiole nutrient data for tuber yield (NO_3-N, S, P, K) and specific gravity (Non NO_3-N, NO_3-N, S, P, K)
3. First to report no improvement, and potential pitfalls, of interpreting petiole nutrient data through ILR transformations without the context of nutrient concentration for PEI and NB.
4. First to report that PEI petiole nutrient data is more correlated to specific gravity than tuber yield.

Contributions of Authors

In all chapters, the candidate is the senior author and was responsible for experimental design, data collection, analysis, interpretation and writing the manuscripts. The experimental work is presented in the following order:

Chapter 1: Literature Review: Methods of evaluating nutrient sufficiency in potato (*Solanum tuberosum* L. cv. Russet Burbank) production.

First author: Sebastian Margarit (primary author, literature review and writing)

Chapter 2: Temporal changes in petiole nutrient concentrations during tuber bulking phase are related to tuber yield and specific gravity in the Russet Burbank potato (*Solanum tuberosum* L.) grown in Prince Edward Island, Canada.

First author: Sebastian Margarit (primary author, statistical analysis and data interpretation and writing), Dr. Nancy McLean (Assisted in conceptual design of statistical analysis and data interpretation), Dan Gillis (Assisted in statistical analysis and editorial comments), Dr. Joann Whalen (Assisted in data interpretation and editorial comments), Dr. Philip Warman (Assisted in data interpretation and editorial comments)

Chapter 3: Petiole nutrient concentrations and their temporal changes during tuber bulking phase as they relate to tuber yield and specific gravity in the Russet Burbank potato (*Solanum tuberosum* L.) grown in New Brunswick, Canada.

First author: Sebastian Margarit (primary author, statistical analysis and data interpretation and writing), Dr. Nancy McLean (Assisted in conceptual design of statistical analysis and data interpretation), Dan Gillis (Assisted in statistical analysis and editorial comments), Dr. Joann

Whalen (Assisted in data interpretation and editorial comments), Dr. Philip Warman (Assisted in data interpretation and editorial comments)

Database compilation was undertaken by the candidate, Wilfred Kelly, and Michelle Hickey from NutriAg Ltd. The tuber yield, specific gravity, and tissue data was shared from their databases.

Table of Content

List of Tables

List of Figures

List of Abbreviations

CND	Compositional Nutrient Diagnosis
DRIS	Diagnosis and Recommendations Integrated System
ICAP	Inductively Coupled Argon Plasma Emission Spectroscopy
ILR	Isometric Log Ratios
PEI	Prince Edward Island
SBP	Sequential Binary Partition
NB	New Brunswick

General Introduction

Potato production generates the largest tonnage of any non-grain crop worldwide, making it an important food crop globally (FAOUN 2016). The 2016 estimate for total area harvested for potatoes was more than 19.2 million ha with an average yield of roughly 19.5 MT ha^{-1}, for a total of 376.8 million MT produced. Global potato production is increasing with the 2016 total production value is 12.9% higher than the 333.6 million MT produced in 2010 (FAOUN 2016). Potatoes are grown in more than 148 countries worldwide, although potato consumption per capita is greater in developed countries (75 kg $person^{-1}$ $year^{-1}$) than in emerging economies such as China (37 kg $person^{-1}$ $year^{-1}$) and India (20.6 kg $person^{-1}$ $year^{-1}$). Per capita consumption is increasing in the developing world, and since the potato is perishable and is not an internationally traded commodity, potato production makes an important contribution to local food security (Lutaladio and Castaldi 2009).

Potato yields per hectare vary depending on the region of production (FAOUN 2016). Average yields for developed countries are more than three times greater than the average yields in developing countries. The large difference in yields are due to fertilizer use, irrigation, and the use of pesticides, as the developed world has more access to these inputs. Globally, potato yields increased by 14% in the past five decades while the land area cultivated for potatoes decreased by 12.1% during the same period (FAOUN 2016), suggesting that potatoes are farmed more intensively now than in the past. Currently, projections suggest that human population will reach 8-10 billion worldwide by 2050. Considering the declining land area under potato cultivation since the 1930's, it is likely that there will be increased pressure on potato producers to achieve higher yields, on smaller land area, for the foreseeable future.

Canadian potato yields per hectare increased notably starting in the 1940s, with continual gains every subsequent decade (Statistics Canada 2012). It is worth noting that the rate of growth has decreased steadily since the peak values reported in the 1950s. Actual yield increases, as measured by yields in tonnes per hectare, have also slowed. Beyond tuber yield the average tuber specific gravity has long been reported to be an important quality metric (Clark et al. 1940) as it is a related to total tuber starch content (Whittenberger 1951) and improved textural qualities (Lujan and Smith 1964). As the majority of potatoes grown in Canada are for processing markets it should also be noted that specific gravity positively influences processing yields while reducing oil content (Lulai 1979). Increased profitability and success of a processing potato producer in Canada is found through a combination of improved yields and specific gravity. Cultivar improvements has had relatively little impact on potato tuber yield and specific gravity in the past decades as the most commonly grown potato cultivar was, and still remains, the Russet Burbank. Potato yield gains in the past century are thus attributed to better cultural practices, not from increasing genetic yield potential, as discussed by Evenson (2003).

In their review, Westermann and Davis (1992) discussed the nutritional management challenges for potatoes in the 21st century and highlighted the need to understand the nutritional requirements of each potato cultivar. Although petiole sampling is a well-established practice in potato production (White et al. 1974; White and Sanderson 1983; Sanderson et al. 2003; Zebarth 2007) the correlation between tuber yield and specific gravity are not well understood in Atlantic Canada. Established ideal values have been reported in Atlantic Canada for NO_3 concentrations in tissues (Zebarth 2007), and both Bélanger et al. (2000) and Rees et al. (2014) reported locally that petiole N concentrations increased with increased N fertilization, however no other major critical levels in petiole tissues have been reported. There is a general reliance on data from

outside the region (Walworth and Muniz 1987; Stark et al. 2004; A&L Laboratories 2016) and local provincial laboratories often report values with no guidance at all.

An exploration into the relationship between plant tissues by Walworth and Sumner (1987) proposed an alternative to nutrient concentration interpretation by following the proportional accumulation of nutrient data. This concept mirrors the definition of an ionome, which is defined as the elemental composition of an organism that represents the mineral component of the organismal system (Salt et al. 2008). Parent et al (2013c) report that to determine the ideal plant ionome without statistical bias one must carry out a series of robust transformations called Isometric log ratios (ILR). These ILRs are considered fulcrum points between the balance of nutrients.

The objectives of this PhD thesis were to relate potato petiole nutrient data in both PEI and NB with the hypothesis that correlation will exist between tuber yield, specific gravity and the nutrient concentrations and their subsequence ILR balance points. The ILR balance points are hypothesized to be more correlated to both tuber yield and specific gravity than nutrient concentrations (Parent et al. 2013b). It is hypothesized that tuber yield will be more correlated to petiole data than specific gravity. Due to the significant nutrient requirements of potatoes (White et al. 1974; White and Sanderson 1983; Sanderson et al. 2003) it is also hypothesized that macronutrients will be more correlated to tuber yield and specific gravity than micronutrients. The most likely nutrient, based on the reported literature across provinces (Zebarth et al. 2004; Zebarth et al. 2007; Sharifi et al. 2008; Zebarth et al. 2012), to be associated with grower relevant yield parameters is hypothesized to be N. The accumulation of N is hypothesized to be correlated to positive yield outcomes and lower specific gravity outcomes.

General Objectives and Hypothesis

The global objective of this thesis research is to relate macro- and micro-nutrient concentrations in the potato petiole to potato yield parameters in both PEI and NB. This farm-level analysis will rely on the correlations among macro- and micro-nutrients (e.g., N, P, K, Mg, Ca, S, Fe, Cu, Zn, Mn, and B), and relating nutrient ratios to tuber yield and specific gravity of the Russet Burbank cultivar. The research is designed to identify new correlations between the petiole ionome and these two grower relevant yield metrics in processing potatoes. The work is predicated on the following hypotheses:

1. Potato petiole data in both provinces will be correlated to the grower relevant yield metrics during the tuber bulking period.

2. The use of ILR transformations will improved the interpretation of petiole data relative to both tuber yield and specific gravity.

3. Petiole tissue will be more correlated to tuber yield than specific gravity.

4. Macronutrients will be more correlated to tuber yield and specific gravity than micronutrients.

5. The accumulation of N is most likely to be correlated to positive yield outcomes and lower specific gravity outcomes.

Chapter 1

Literature Review: Methods of evaluating nutrient sufficiency in potato (*Solanum tuberosum* L. cv. Russet Burbank) production.

1.1 Introduction

To sustain life, the diverse organic molecules that form the building blocks of life are accompanied by critical inorganic ions that serve as enzyme cofactors, maintaining charge separation across membranes, osmoregulation, and structural components of cells (Baxter 2009). This mineral component of an organism has recently been termed the ionome, a concept that emerged in the field of plant science and agriculture where tissue elemental analysis to determine plant nutrition is common (Salt 2004; White and Brown 2010). Over the last 150 years, plant scientists have worked towards identifying the mineral nutrients that are critical for plant growth and survival (termed "essential mineral elements"), identified the physiological functions of essential elements, and tested the effects on plant growth of adding essential minerals at different rates to soil (Marschner 2012). Production of food crops is often limited by the availability of essential nutrients in soil to plants (White and Brown 2010). A deeper understanding of plant fertility (along with better crop varieties, pesticide use, irrigation, and other factors) has contributed to the growth in food production (on an energy per person basis) outpacing human population growth from the 1960s to 2000 (Gilland 2002). However, malnutrition is still a common problem in developed and developing countries, whereby insufficient essential minerals are present in staple foods to meet the needs of the population (Watanabe et al. 2016). It is essential that we continue to improve crop yields and mineral nutrition of food crops if we are to feed a surging world population.

Plants require at least 17 essential minerals to complete their life cycle (Watanabe 2016), which they obtain from the environment primarily dissolved in water which is taken into the plant through their roots (Marschner 2012). The set of essential nutrients can be separated into two groups, the macronutrients which are required at relatively high concentrations, and the micronutrients required at lower concentrations. The ionome of a plant is related to the uptake of each element from the environment, transportation within the plant, and accumulation in specific tissues (Baxter 2009). Plants can preferentially uptake or discriminate against different elements to account for the discrepancy between soil mineral ion concentrations and the plant's nutritional requirements (White 2012a). In addition, plants can accumulate mineral elements in different tissues, and the selectivity and accumulation of elements depends heavily on the plant species (White 2012a, Parent et al. 2013a, Watanabe 2016). Even within a plant species, there can be genetic variants that differ in their mineral nutrient uptake characteristics and lead to a distinct ionome (Parent et al. 2013a, Huang and Salt 2016). The plant ionome can also be altered by changes in soil chemistry that affect uptake of mineral elements, such as modifying pH or adding mineral fertilizers to soil (Baxter 2009).

The ionome analysis of a plant tissue sample provides a snapshot of plant nutritional status after uptake and accumulation, at a specific time point or developmental stage. Analysis of plant ionomic data with statistical methods is beginning to emerge as a potentially useful approach to understand and improve yield responses to fertilizer in agricultural crops (Parent et al. 2013b, Watanabe 2015, Souza 2016). By separating mango tissue samples into low and high yielding sub-populations and transforming the dataset to isometric log ratios (ILRs) calculated based on nutrient balances, Parent et al. (2013b) determined that Mn, Zn, and Cu were out of balance in the low yielding group. Watanabe et al. (2015) used Principal Components Analysis

(PCA) to analyze elemental concentrations in corn leaves from plants fertilized with NPK, minus-N, minus-P, minus-K, and minus-NPK treatments in long-term field plots. Deficiencies in N, P, and K were associated with significant yield reduction and an increased uptake of other nutrients. Analysis of leaf and fruit tissue in the guava by Souza et al. (2016) found significant correlations between fruit yield and ILRs representing nutrient balances. There is some evidence that the plant ionome can distinguish between high and low yielding populations for some crops, but there is a large gap in knowledge since the ionome of many agriculturally relevant plants has not been characterized.

1.2 Fertility Requirements in Potato

The potato crop requires a continuous supply of nutrients throughout the growing season to provide sufficient resources for optimal plant development and allow growers to maximize production. From 2010-2014, average tuber yields were 32.1 ± 1.0 Mg ha^{-1} (± standard deviation) in Prince Edward Island, Canada and 31.4 ± 2.4 Mg ha^{-1} across New Brunswick, Canada (Mukezangango 2015). The nutrient requirement to support the potato crop can be estimated from the average moisture content of harvested tubers (80%) and the tuber nutrient concentration (e.g., 3.7 kg N t^{-1}, 1.1 kg P_2O_5 t^{-1} and 4.8 kg K_2O t^{-1}; CRAAQ, 2010). For Prince Edward Island, nutrient removal for a potato crop yielding 32 t ha^{-1} is estimated to be 136 kg N ha^{-1}, 28 kg P_2O_5 ha^{-1} and 186 kg K_2O ha^{-1}, reflecting a higher nutrient demand for potatoes than cereal crops and most vegetable crops grown in this region (Government of PEI 2009). Still, nutrient removal does not fully account for the potato's nutritional requirements since marketable yield is only a fraction of the total edible and non-edible biomass produced by the plant. The aboveground potato biomass requires additional nutrients, approximately 52 kg N ha^{-1}, 7 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1}, for proper leaf and stems development.

Nutrient demands for potato organs change during the growing season (Heard 2004). In the early growing season, the crop allocates resources primarily for root development and building the photosynthetic canopy. The rate of N, P, K, S, Ca, Mg, Fe, Mn, Cu, and B uptake into leaves and stems reaches a maximum in the early tuber bulking stage. Subsequently, some of these nutrients are translocated from the leaves and older stem tissues into the developing tubers. Large quantities of N, Mg and Fe are redistributed into the tubers, but Zn was not redistributed. The concentrations of S, Ca, Mg and B reach a maximum in stems and leaves by the late tuber bulking stage. Clearly, any method of sampling tuber, stem or leaf tissues for the purpose of evaluating nutrient demands must consider the growth stage and the pattern of nutrient uptake, since assimilation, dilution and reallocation effects will impact the nutrient concentration in a particular plant tissue.

Fertilization recommendations for potatoes must consider two major factors: (1) the nutrient requirements to achieve a target crop yield, and (2) the soil nutrient supply, generally estimated from soil testing. After the fertilizer recommendation is made and fertilizers are applied, it is necessary to validate the efficacy of the fertilizer program to deliver nutrients in synchrony with crop demands. Due to the economic and environmental costs of over-applying fertilizers, the goal is to provide sufficient nutrients for crop growth and avoid excessive nutrient inputs. Nutrient sufficiency in potato is often assessed during the growing season by sampling non-edible tissues, such as leaves and stems. Petiole and leaf testing are the most popular organs for sampling due to the ease of collecting these tissues, compared with tubers (Walworth and Muniz 1993). Tissue analysis and interpretation is not a replacement for soil testing, water management, pest control or crop rotations. Rather, it offers complementary information about nutrient concentrations in the plant, regardless of other agronomic factors.

Evaluating nutrient sufficiency in plant tissues is advantageous because it avoids the potential bias associated with complex plant-soil interactions such as nutrient synergies and antagonisms in soils (i.e. K antagonism of Mg uptake), root morphology (i.e. soil nutrient availability in relation to root growth), and the effect of soil moisture on nutrient uptake (i.e. mass flow, and diffusion). The nutrient concentration in plant tissues is determined following acid digestion and elemental analysis through inductively coupled argon plasma emission spectroscopy (ICAP). This procedure can be applied to all plant tissues, regardless of their origin, and avoids the confounding effects of extraction procedures (i.e., soil testing relies on extracting solutions to isolate a pool of plant-available nutrients) and is therefore easier to compare across geographies. As tissue analysis reports give the nutrient concentration within a sampled plant part, it may be interpreted as an integrative measurement of the factors that influenced nutrient acquisition by the plant from germination to the time of sampling.

1.3 Physiological Roles of Essential Elements

1.3.1 Role of Macronutrients

After C, N is the most abundant element in plant tissues at between 1-5% on a dry weight basis (Hawkesford et al. 2012). This is due to the many different categories of biomolecules in which nitrogen is incorporated, including amino acids, nucleic acids, chlorophyll, and many other primary and secondary metabolites. Nitrogen is located at critical regions of DNA and RNA where base pairs bond to each other, as well as in the linkages between amino acids that form every protein and enzyme (Hawkesford et al. 2012). At the centre of every chlorophyll molecule from which plants harvest light energy are four nitrogen atoms binding to a magnesium ion

(Hawkesford et al. 2012). When plants become severely deficient in N, proteins and nucleic acids in leaf tissues are broken down to mobilize N for new growth (Hawkesford et al. 2012).

There are two major forms of plant available nitrogen in the soil, the ions nitrate and ammonium, with plants also taking up a minor amount of amino acids and other dissolved organic nitrogen (Jones et al. 2005). Nitrate uptake is an energy requiring process which is actively undertaken by plants and makes up the majority of nitrogen uptake for higher plants (Hayes and Goh 1978). Nitrate is reduced in the plant to nitrite, and then ammonium which is used to form amino acids and then these are combined to form subsequent proteins. Plants which accumulate significant amounts of nitrate in leaf tissues do not have the ability to reduce nitrate in their roots and therefore translocate nitrogen from the roots in this form (Pate 1973). Therefore the accumulation of nitrate nitrogen in these tissues is as sign of a plant which has recently taken up significant amounts of nitrogen, or a plant which is limited in its ability to reduce nitrate into amino acids. As a result of these factors the separation of nitrate concentrations as a separate metric from total nitrogen has been used in tissue sampling (Williams and Maier 1990).

Sulphur is also a component of the amino acids methionine and cysteine, which serve structural roles in proteins by forming disulfide bridges to maintain tertiary structure. They also play a functional role in proteins as a polar functional group that can be an active site in enzymes (Hawkesford et al. 2012). One of the most important sulphur-containing compounds is glutathione, a tripeptide that serves many metabolic roles in plants and other organisms (Hawkesford et al. 2012). Glutathione is present at higher concentrations in leaves than roots, and is primarily focused in chloroplasts (Hawkesford et al. 2012). Glutathione is a precursor to phytochelatins which are used as defense compounds against heavy metals and other toxins (Hawkesford et al 2012). In food crops like *Allium* (e.g. onions, garlic) and *Brassica* species (e.g.

mustard, cabbage), sulfur-containing compounds are responsible for the desirable flavours and odours that are characteristic of these foods.

Phosphorus is typically present as the phosphate. The most obvious role of P is in energy storage and transfer within every cell, in the form of adenosine triphosphate (ATP), but P is also located at key locations in other critical biochemical processes. In every chain of DNA and RNA there is a phosphate group linking the sugar molecules with phosphodiester bonds that form the backbone of the genetic code (Hawkesford et al. 2012). The membranes that contain cellular contents are equipped with a phosphate group in each phospholipid molecule that imparts a hydrophilic region and encourages the lipid bilayer structure (Hawkesford et al. 2012). When P is taken up in sufficient quantities, plants start to accumulate phytic acid and inorganic polyphosphates, two storage forms of P (Hawkesford et al. 2012). Phosphorus deficiencies in plants are manifested in the shoots as fewer leaves and reduced leaf expansion, with a reduced length of the cell division zone and lower rate of cell division (Hawkesford et al. 2012). In the roots, elongation can be enhanced and formation of root clusters can be induced in some plants (Hawkesford et al. 2012).

Magnesium is a divalent cation that interacts with many enzymes to influence tertiary protein structure or act in a catalytic role by positioning enzyme substrates into the orientation required for the reaction to take place (Hawkesford et al. 2012). For example, ATP molecules must be bound to a Mg ion to be biologically active, and Mg is necessary for ribosomal aggregation during protein synthesis (Hawkesford et al. 2012). Magnesium has a critical role in plant energy generation from sunlight as the central metal ion in the chlorophyll pigment (Hawkesford et al. 2012). It is also involved in many other enzymatic addition and substitution reactions (e.g. phosphorylation, carboxylation) by increasing the affinity of the enzyme for its

substrate (Hawkesford et al. 2012). Due to its role in protein synthesis (ribosomal aggregation), deficiency in Mg results in reduced protein N / increased non-protein N, and its requirement in chlorophyll results in a decreased photosynthetic rate (Hawkesford et al. 2012). Low Mg can also reduce the transport of carbohydrates from source to sink, leading to a decrease in starch in storage organs such as potato tubers (Hawkesford et al. 2012).

Calcium is another divalent cation that is larger in diameter than Mg and has a much different role. The majority of Ca in plant tissue is found in the cell wall, bound to carboxylic acid functional groups of pectin molecules (Hawkesford et al. 2012). It is found at high concentrations in the middle lamella, exterior of the plasma membrane, endoplasmic reticulum, and in situations where Ca is available in excess, is accumulated in the vacuole (Hawkesford et al. 2012). Soluble Ca is controlled in plant cells to avoid precipitation reactions with soluble phosphate and to reduce competition with Mg (Hawkesford et al. 2012). Since it is incorporated into structural materials of the plant, Ca is not mobile. Calcium is critical to provide strength to cell walls and other tissues where it is found, and a deficiency in Ca can manifest as deterioration of cell walls in newer tissues. Calcium deficiency can also result in membrane destabilization resulting in leakage of solutes, and in extreme cases, disintegration of membranes and loss of compartmentalization (Hawkesford et al. 2012).

Potassium is a monovalent cation that is highly mobile in plants and is selectively uptaken from soil (Hawkesford et al. 2012). It is the most abundant positively charged ion in the cytosol and plays a very significant role in maintaining osmotic potential in cells and in controlling water within the plant (Hawkesford et al. 2012). It forms only weak bonds with negatively charged functional groups of organic molecules, and is easily displaced so it does not affect binding sites of divalent cations like Ca or Mg (Hawkesford et al. 2012). However, K does

play a role in activation of some enzymes by changing the structural conformation and optimizing hydration of the protein by water molecules to increase activity (Hawkesford et al. 2012). The starch synthase enzyme which adds glucose to starch molecules is activated by monovalent cations like K. In plants deficient in K, metabolic effects indicative of reduced activity of K-activated enzymes can be detected, such as increased soluble carbohydrates (Hawkesford et al. 2012). Potassium is also involved in tRNA translation into proteins, which can manifest in K-deficient plants as reduced enzyme concentrations, increased soluble amino acids, and reduced photosynthesis (Hawkesford et al. 2012).

1.3.2 Role of Micronutrients

Iron ranks second, behind aluminum, for the most abundant element in the Earth's crust. However, it has low solubility and chelating agents are necessary for long distance transport (Broadley et al. 2012). Iron can form stable octahedral-shaped complexes with ligands, and it exists at two oxidation states (Fe^{2+} and Fe^{3+}) that can be easily interconverted by addition or removal of a single electron (Broadley et al. 2012). The ability to complex with organic compounds and to easily change oxidation states allows iron to participate in many unique biological reactions requiring a transfer of electrons. Iron is the central metal in heme proteins, which are involved in the chloroplast redox systems and many enzymes. These include catalases that detoxify H_2O_2 and peroxidases which consume H_2O_2 to polymerize phenols into lignin (Broadley et al. 2012). Iron is also important in Fe-S proteins such as ferredoxin, where it is arranged between thiol groups of cysteine and facilitates electron transfer during photosynthesis. Other Fe-S proteins include superoxide dismutase, which detoxifies the superoxide anion and form H_2O_2, and aconitase, a tricarboxylic acid cycle enzyme that converts citrate to isocitrate (Broadley et al. 2012). Iron deficiency results in decreased catalase and peroxidase activity,

leading to accumulation of H_2O_2 and phenolics. Phenolics are released from the roots under iron deficiency, which can chelate iron to increase availability. Iron deficiency also leads to increases in citrate and other organic acids. In leaves, chloroplast development is inhibited under iron deficiency, reducing photosynthetic activity and concentrations of sugar and starch molecules (Broadley et al. 2012).

Manganese can exist at up to seven oxidation states, but primarily occurs as Mn^{2+}, Mn^{3+}, or Mn^{4+} (Broadley et al. 2012). Manganese plays a similar role as iron in the formation of superoxide dismutase enzymes to protect cells from superoxide anions, although the Mn form is found in peroxisomes and in the mitochondria (Broadley et al. 2012). It is also involved in photosystem II where four Mn^{2+} are oxidized to Mn^{3+} to yield four electrons, and the Mn^{3+} are reduced to Mn^{2+} to cleave water into H^+ and O_2 (Broadley et al. 2012). Manganese also serves as a cofactor for many redox, decarboxylation, and hydrolytic enzymes, involved in important processes such as carbon shuttling, the TCA cycle, and shikimic acid pathway (Broadley et al. 2012). Managanese deficiency depresses photosynthesis and the evolution of O_2 from photosystem II. It also causes a reduction in soluble sugars and alters fatty acid profiles. The shortage of soluble carbohydrates and decreased photosystem II activity causes reduced net photosynthesis, chlorophyll concentration, root growth, and dry matter production in Mn deficient plants (Broadley et al. 2012). Manganese-deficient plants have increased susceptibility to cold due to lower soluble sugars, and to some pathogens from reduced structural integrity of tissues. Symptoms of Mn deficiency include brown speckles on mature leaves as well as interveinal chlorosis and necrosis (Broadley et al. 2012).

Copper acts similarly to Fe and Mn by forming stable organic matter complexes in proteins, and by facilitating redox reactions as Cu^{2+} and Cu^+ (Broadley et al. 2012). Copper is an

important component of plastocyanin in the electron transport chain of photosystem I, and also participates with zinc to form a third form of superoxide dismutase to detoxify the superoxide ion (Broadley et al. 2012). It is also found in cytochrome C oxidase, which serves as the terminal oxidase in respiration within the mitochondria. Copper is also found in polyamine oxidases which degrade polyamines like spermidine into putrescine, and in polyphenol oxidases which oxidize tyrosine into phenolic compounds for the production of lignin. Deficiency in Cu results in decreased lignification, stunted growth, chlorosis or necrosis from the apical meristem along leaf edges, bleaching of young leaves, or distortion of young leaves (Broadley et al. 2012).

Zinc is a divalent cation that is a component of many enzymes, serving in both structural and catalytic roles. Zinc can participate in tetrahedral complexes and has a high affinity for N, O, and S ligands (Broadley et al. 2012). Zinc participates with copper in the superoxide dismutase enzyme, although it is believed to be in a structural role by increasing stability. Zinc catalyzes the production of carbonic acid from CO_2 and H_2O in the enzyme carbonic anhydrase, and in the production of ethanol from acetaldehyde in the alcohol dehydrogenase enzyme (Broadley et al. 2012). Zinc can also activate certain enzymes like alkaline phosphatase, peptidases, and RNA and DNA polymerase. Zinc is important in replication of DNA by forming tetrahedral complexes that modify the tertiary structure of proteins to allow binding with and activation of specific genes (Broadley et al. 2012). Zinc is a structural component of ribosomes and is therefore important in protein synthesis. Deficiency in zinc cause symptoms including decreased internode length, a decrease in leaf size, chlorosis and mottling, decreased shoot to root ratio, and under severe deficiency, the apical tips can die (Broadley et al. 2012).

Molybdenum is a transition metal that exists most commonly as the molybdate oxyanion MoO_4^{2-}, which has some similar properties to sulfate anion SO_4^{2-} (Broadley et al. 2012). The

plant requirement for Mo is the lowest among all critical elements, but it is a component of some critical enzymes. Molybdenum has three oxidation states that the element can transition between to perform reactions requiring transfer of two electrons (Broadley et al. 2012). During the assimilation of nitrate, the first reduction step is catalyzed by Mo, which transfers two electrons to nitrate. Other enzymes containing Mo are involved in stress responses (Broadley et al. 2012). In Mo deficient plants, the activity of nitrate reductase is low. Physical effects of Mo deficiency on the plant depend on if the major form of N available is ammonium or nitrate, with larger yield reductions in plants dependent on nitrate (Broadley et al. 2012). Other symptoms consistent with N deficiency such as chlorosis, necrosis, and leaf blade irregularities can also be exhibited. Under severe deficiency, marginal necrosis with high tissue nitrate concentrations can occur (Broadley et al. 2012).

Boron is a metalloid found primarily as $B(OH)_3$, and the role of boron is thought to be the least understood of all the elements (Broadley et al. 2012). There have been a number of roles for B hypothesized, including transport and metabolism of sugars, formation and structure of cell walls, lignification, RNA metabolism, and in membrane stability (Broadley et al. 2012). It is believed that the role of B in cell wall integrity leads to a "cascade effect" under B deficiency where multiple metabolic pathways are affected by the disturbance to a critical component of the cell (Broadley et al. 2012). Boron is involved in the function of membranes, including the formation and maintenance of membrane charge potentials, uptake of P in the root tips, turgor, and activity of membrane-bound enzymes (Broadley et al. 2012) Boron deficiency leads to abnormal cell wall formations such as thickening, brittleness, and altered mechanical properties that prevent normal expansion. This can manifest as cracked stems, hollow areas, and microscopic structural anomalies. Boron deficiency also affects meristematic tissues by altering

the differentiation of cells (Broadley et al. 2012). Other symptoms of B deficiency include drops of buds, flowers, or fruit, tipburn, brownheart or blackheart, necrosis in young leaves, and increased susceptibility to some pathogenic microorganisms (Broadley et al. 2012).

Chlorine is ubiquitous in the environment and is present as the chloride anion Cl^-. Chlorine plays a role in osmoregulation and in balancing positive charges to control membrane potential (Broadley et al. 2012). Chlorine also plays a role in the splitting of H_2O in photosystem II. The chloride ion, in conjunction with K, is essential in stomatal regulation, and deficiency in Cl can reduce stomatal opening. Other effects of Cl deficiency include wilting at leaf margins, reduced cell division rates causing a decrease in leaf area, curling of youngest leaves, premature senescence, frond fracturing, and cracking of stems (Broadley et al. 2012).

Sodium and aluminum are not plant essential nutrients although they are found in plant tissues and are taken up similarly to other cations. The effects of salinity stress and Na accumulation can be significant on various crops although the sensitivity is crop specific. Potato is not considered to be tolerant of high sodium concentrations and increased sodic conditions can have considerable effects on both yield and quality (Levy and Veilleux 2007). Aluminum is also not plant essential and the accumulation of this element can lead to toxicity symptoms in plants (Foy 1988). Plant species and cultivars have varied responses to the accumulation of Al in tissues however this element is still considered to be deleterious as no studies have reported Al in a manner that positively impacts crop growth.

1.4 Nutrient Uptake and Translocation Within the Plant

Plants can access freely soluble or chelated nutrients that are dissolved in the soil solution in the vicinity of the roots. The first interface between the soil and plant is the cell wall, which contains

pores generally <5 nm that allow movement of water, dissolved mineral ions, and molecules smaller than the pore diameter into the apoplasm (White 2012a). The apoplastic space within the cell wall contains pores lined with carboxyl groups and bound anions that bind exchangeable cations, with the cation exchange capacity varying by plant species and the relative number of carboxyl groups present (White 2012a). The pore surfaces within the apoplastic space can serve as a transient storage location for nutrients such as Fe and Zn that can be re-mobilized when needed in actively growing areas of the plant (White 2012a). The plant has limited selectivity in the uptake of ions into the apoplasm and depends on the movement into the cytoplasm across the plasma membrane to selectively uptake specific essential elements (White 2012a).

The plasma membrane lipid bilayer prevents movement of water and ions indiscriminately between the apoplasm and the cytoplasm of root cells (White 2012a). Proteins embedded in the membrane facilitate movement of mineral ions into the cell. Movement of K^+, Ca^{2+}, NH_4^+, urea, B, and Cl^- into the cytoplasm occurs through channel proteins (White 2012a). Anions like NO_3^-, $H_2PO_4^{2-}$, SO_4^{2-}, MoO_4^{2-}, and Cl^- are moved against their electrochemical gradient through unidirectional H^+ coupled transporter proteins (White 2012a). Movement of Mg^{2+}, Fe^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, and Cu^+ are transported through uniport carrier proteins (White 2012a). Once inside the cell, an array of channel, pump, and coupled transporter proteins facilitate transport of essential elements across the tonoplast membrane into the vacuole for storage (White 2012a). Essential elements in the cytoplasm move towards the xylem at the center of the root through plasmodesmata connecting neighboring root cells. Ions are transferred across the plasma membrane into the xylem through ion channels, pumps, or uniporters for long-distance transport within the plant (White 2012a).

Once in the xylem, essential elements are moved from the roots to the shoots of the plant in the xylem sap, but are subject to the same sorption processes with carboxyl and other polar groups as is present in the apoplastic space (White 2012b). Plant tissues will release or retrieve nutrients as needed to or from the xylem sap as it moves towards the leaves, but most of the solutes are transported to the leaf (White 2012b). Element concentrations in xylem sap from a rapidly growing plant generally decline from root to shoot and decline sharply from the leaf base to the edges (White 2012b). The phloem sap receives nutrients in the leaf and in the stems of the plant, but also contains a high concentration of sucrose that attracts water into the phloem sap and maintains a high internal pressure (White 2012b). The phloem transports solutes from source sites to sink sites such as apical tips, fruit, or back to the roots, and nutrients are transported in the direction of the sink that the phloem sap flows toward (White 2012b). From the perspective of the plant ionome analysis, tissue elemental concentrations may be expected to change over time depending on the uptake, storage, mobilization, allocation within the plant, and the plant tissue or organ sampled for analysis.

1.5 Tissue Sampling Considerations

Despite its widespread use, tissue analysis has some limited capability to predict crop yields. The nutrient status of the plant while important is only one of many factors affecting crop yield. Soil properties (pH, texture, chemical composition), annual rainfall, crop-pest interactions, and crop rotations also influence potato yields (Fortin et al. 2011; Mohr et al. 2011).

The first step to assess nutrient sufficiency in plant tissues is to collect a representative tissue sample.

1.5.1 Physiological Maturity of Tissues

Dilution effects occur when the dry matter content of the plant in response to a nutritive treatment without a simultaneous increase in nutrient concentration (Jarrell and Beverly 1981). General dilution can result from nutrient applications that increase the photosynthetic rate of the plants in one plot relative to another, an increase in the translocation of a photosynthate to a growing point, or a change in hormonal balance in a plant. Loneragan and Snowball (1969) discuss the relationship between physiological maturity and Ca concentrations in sampled tissues. They analyzed various plant tissues through temporal changes in the Ca supply in hydroponically grown grasses, cereals, and legumes. Plants grown in high Ca solutions developed tissues with levels associated with luxury consumption however when moved into low Ca solutions the plants developed deficiencies despite high concentrations remaining in older leaves. New tissues showed deficient levels of Ca however this was not representative of the current plant Ca status.

1.5.2 Geography of Site Selection

The location of trial sites and their association with critical levels and tissue concentration interpretation have also been studied. MacKenzie (1967) questioned whether crop critical values from one area are relevant to another. The importance of regionally specific data for large production areas would be increasingly important if that were the case. The critical values for major production areas such as Idaho, Western Canada, and Ontario may not apply to the regional dynamics observed in Atlantic Canada. Data collection should be narrowed to avoid any potential errors associated with the regional “terroir.”

1.5.3 Selection of the Plant Organ to be Sampled

The first report of whole leaf analysis to evaluate sufficiency levels of N, P, and K in potato that received variable rates of granular NPK fertilizers was published by MacKay et al. (1966). A comprehensive review of the nutrient concentrations in leaf, tuber, petiole, and stem tissues of various potato cultivars was undertaken by Walworth and Muniz (1993). Their mean concentrations reported as critical levels for petiole analyses are reported on Table 1-2. A comprehensive study by Reis and Monnerat (2000) determined the nutrient sufficiency in potato stem, petiole and leaflet tissues. They recommended sampling potato petioles to evaluate the sufficiency levels of six nutrients (N, P, K, Ca, Mg, and Cu) whereas leaflets were recommended for three nutrients (S, Mn, and Zn). As there are several studies in the Atlantic region focusing on macronutrient sufficiency (White et al. 1974; White and Sanderson 1983; Sanderson et al. 2003; Zebarth et al. 2004; Zebarth et al. 2007; Sharifi et al. 2008; Zebarth et al. 2012) petioles are an ideal candidate tissue for sampling.

1.5.4 Petiole Sampling

The potato petiole is generally selected as the target organ for tissue sampling, and this is reflected in the availability of nutrient sufficiency ranges (e.g., maximum and minimum nutrient concentrations) for potato petiole (Tables 1, 2 and 3; Walworth and Muniz 1993; Stark et al. 2004; A&L Laboratories 2016). From a biological perspective, the petiole is a suitable organ for tissue sampling due to its physiological function in potato. The petiole is located at the interface between the photosynthetically active portions of the leaf and the plant stem. The petiole contains nutrients that are either (1) moving from the roots to the leaflets, to support the photosynthetic functions of the leaves, or (2) remobilized from the leaflets and transferred back

into the stems. This allows the petiole to serve as an indicator of nutrient concentrations in both xylem and phloem conducting tissues.

1.6. Interpreting Plant Tissue Nutrient Concentrations

1.6.1. Critical Levels

Nutrient sufficiency in potato, based on nutrient concentration in the petiole, is generally evaluated by comparing field-based measurements with critical levels reported by laboratories. There are myriads of different critical levels which vastly differ in their values (Table 1-3). Stark et al. (2004) reports critical levels 90% higher than the commonly used A&L Laboratories (2016) value for the same period of bulking. Walworth and Muniz (1993) report P critical levels 34% lower and K critical levels 41% higher than both Stark et al. (2004) and A & L Laboratories (2016). A large difference exists between reported values for secondary and micronutrients as well. This diversity in critical levels highlights the need for locally derived data correlating nutrient concentrations in petioles with grower relevant yield outcomes. The difficulty in establishing reliable critical levels was discussed by Dow and Roberts (1981), who concluded that critical ranges were more reliable than critical levels because nutrient concentrations in a specific plant organ at a designated growth stage are known to vary due to the cultivar, growing conditions, soil and climatic conditions.

1.6.2 Dual Ratios and the Diagnosis and Recommendation Integrated System

Various accumulation patterns of various nutrients show similar pattern of accumulation and are reviewed by Walworth and Sumner (1987). The Diagnosis and Recommendations Integrated System (DRIS) aims to use nutrient ratios to address the changing patterns in nutrient accumulation to reduce the impact of sampling timing (Beaufils 1973). The practice is still

common place and modern potato sampling (Walworth and Muniz 1993). Dual-ratio values for nutrients are featured in the DRIS (Beaufils 1973), The critical level approach provides sufficiency ranges for each nutrient but fails to account interactions between nutrients, which can result in a misinterpretation of the information due to nutrient replacement/ antagonisms functions in the nutrient-plant complex. Comparing the critical levels, Diagnosis and Recommendation Integrated System (DRIS) and Compositional Nutrient Diagnosis (CND) methods for potato, Khiari et al. (2001) noted that the CND method of tissue interpretation best predicted the potato yield and this was related to plant tissue P concentrations.

1.6.3 Compositional Nutrient Diagnosis

Compositional Nutrient Diagnosis (CND) uses multi-ratio or row-centered log ratios of nutrient concentrations using a geometric mean of all nutrients and assigns a filling value for the non-nutritive components of the leaf (Parent et al. 1994). The most recent development within the CND method involves the computation of isometric log ratios to eliminate biases due to redundancy of information, non-normal distribution, and incoherence within the data. Parent et al. (2013) introduced the use of ILRs for interpreting the plant ionome and found more differences between species than interpreting nutrient concentrations. Based on their findings the use of ILRs is hypothesized to be of benefit in interpreting the potato petiole ionome.

1.6.4 Isometric Log Ratios

Aitchinson (1986) proposed that nutrient concentrations data should be interpreted using dual-ratio or multi-ratio computation to avoid the inherent biases of compositional data. Nutrient concentrations are a form of compositional data as they are percentages of the total elemental

mass in an organism, ranging from 0 to 100%. In a plant, for instance, the most abundance nutrient is carbon, which is about 45% of the total elemental mass and the least abundant nutrients (micronutrients and trace elements) represent less than 1% of the total elemental mass. This creates several constraints to the data, which require careful consideration.

Redundancy of information – The composition cannot exceed 100% and therefore the amount of one component can be determined by the difference between the total components (100%) and the sum of the other components. There are D-1 degrees of freedom in the D-parts composition. (Aitchinson and Greenacre 2002). The nature of this relationship creates redundant information, which can generate spurious correlations in linear statistics (Chayes 1960). Spurious correlations can also be generated based on the unit of closure for a given composition. For example, nutrient concentration of plant samples can be determined on a wet or dry basis and the additional component of the composition (water) can alter the results of statistical analysis. However, the water should not theoretically have any effect on the proportion of all other components.

Non-normal distribution – Data in a normal distribution lie in real space, while composition data are mapped in a closed space. Confidence intervals for given components (i.e. nutrient concentrations) often give ranges that lie outside of the limit of compositional space. Negative values (less than 0%) or values greater than 100% in nutrient concentration data are illogical and they lead to difficulty in interpretation.

The use of ILR was introduced by Egozcue et al. (2003) and it projects the SD simplex which is constrained to the closed space of compositional data, into Euclidian space of D-1 orthogonal log contrasts which do not overlap. There are many examples of ILR transformations being used in tissue sampling interpretation in agriculture. It has been used on crops such as

guava (Parent et. al 2012) orange (Parent 2011), mango (Parent et. al 2013), apple (Parent 2011), cranberry (Marchand et. al 2013), lowbush blueberry (Parent et. al 2013c), and cloudberry (Parent et. al 2013a). This is achieved first by creating a system of balance defined by its intrinsic sequential binary partition (SBP). The SBP is a (D-1) x D matrix where group numerators are labeled +1 and group denominators are labeled -1 while parts, which are not a part of a given balance, are given a value of 0. In tissue analysis interpretation partitions are derived from the physiological role/systems of the given nutrients within the crop. These ILR transformations are currently the most robust multivariate analyses available for compositional data sets (Filzmoser and Hron 2011). The natural log ratio of the geometric means is a log contrast and the square root of the product of the population of subcomponents within the differently labeled groups over the sum of the same groups is an orthogonal co-efficient. These ilr transformations are currently the most robust multivariate analyses available for compositional data sets (Filzmoser and Hron 2011).

1.7 Nutrient Groupings for ILR Generation

For ionomics in plants one organizes the nutrients analyzed into major groups, based on role and function within the organism, to improve the interpretation of the analyses. This allows the observer to investigate beyond single nutrients concentrations into the realm of physiological systems in which the given nutrient have critical roles. The design of the SBP however does not affect the results of multivariate linear statistics. Moving from one SBP to another simply rotates the orthogonal axes from the origin of the coordinates of a scatter. Balances can be created to assist interpretation of specific physiological groups and their design can be based on a combination of prior and expert knowledge (Parent et al. 2013b).

The review of the role of nutrients within the physiological function of the plants were used in the creation of the SBP for the potato crop. Petioles were analyzed for their concentrations of NO3-N, Non NO3-N, P, K, Mg, Ca, S, B, Zn, Mn, Fe, Cu, Na and Al. Nutrients were arranged into the following five groups: Protein Synthesis (NO3-N, Non NO3-N, and S), Short Term Energy Storage/Transfer (P, Mg), Cellular Structure (K, Ca, B), Enzyme Activation and Electron Transport Chain System (Zn, Mn, Fe, and Cu), and Deleterious (Al, Na).

1.7.1 SBP Protein grouping

Protein are complex organic molecules derived of sequences of smaller amino acids which are comprised of C, H, O, N, and S. The feedstock for protein synthesis within a plant is the primary form of nitrogen in plant uptake, nitrate, which is converted first into nitrite, then into ammonium and then subsequently into glutamine a precursor for all other amino acids amino acids. This understanding led to early investigations of sulfur and nitrogen relations which clearly showed an ion balance between the two nutrients (Dijkshoorn and Van Wijk 1967). Sulfur deficient plants were shown to have disturbed ratios of organic-S relative to organic-N as the plant's ability for synthesis of non-protein organic N compounds was not inhibited by a lack of sulfur.

The dynamics of movement from NO3-N to Non NO3-N pools of N influence protein synthesis and protein concentrations within the plant. Sulfur is a component of the amino acids cysteine and subsequently methionine therefore a limitation in sulfur availability can greatly reduce the process of bulk protein synthesis within a plant by limiting the required feedstocks (Hesse and Hoefgen 2003; Nikiforova 2003; Saito 2004).

Further evaluation of the relationship between sulfur availability and total protein, total chlorophyll, biomass and total RNA in Arabidopsis (Arabidopsis thaliana) further explored the effects of sulfur deprived systems (Nikiforova et. al 2005). Sulfur deprived plants showed a consistent decrease in both total chlorophyll content and in total protein content in situations of constitutive (plants grown in sulfur deficient medium from seed) and induced starvation (plants grown in sulfur sufficient medium and transferred to sulfur deficient medium upon germination). This decrease in the sulfur deprived plants was evaluated before and after the symptoms became visible in the plants (general chlorosis) suggesting the effects are not only significant when visual symptoms exist (hidden hunger).

Total RNA synthesis only differed when plants were grown in constructive starvation scenarios suggesting that plants have an adaptive mechanism to conserve sulfur for RNA synthesis. Biomass responses were more ambiguous with both induced starvation experiments showed differences between treatments, whereas only a single time point (post symptom) showed differences in the constitutive starvation of sulfur. It is clear that in all types of sulfur deficient situations, whether they occur throughout the plant's life or are caused by temporal shortfalls in sulfur the effect on protein is significant. As a result the protein synthesis through simple nitrogen augmentation (routine in both wheat and corn topdresses) are inherently missing critical subcomponents within the protein synthesis systems of higher plants.

1.7.2 SBP Energy storage and transfer system

Energy storage and transfer is a dynamic process within the plant and is directly related to source sink dynamics. As different carbohydrates mediate the majority of energy transfer and storage, in the long term, it becomes difficult to study this system directly using tissue analysis. There are

many carbon pools within a plant from the heavily recalcitrant lignins to the more labile simple sugars such as glucose. Commonly used laboratory tissue sampling analyses do no report the C, H, and O concentrations for the simple reason that the fractions of these elements associated with different pools is time consuming and costly to correctly quantify.

The movement between the labile carbon pools is fluid within the plant. Simple sugars are the primary storage vessel for energy gathered via photosynthesis, sucrose is the primary form of sugars for transfer within the phloem and starch is the primary form of sugar for long term storage. The conversion from one form of sugar to another is typically mediated by adenosine triphosphate (ATP) conversion to adenosine diphosphate. Phosphorus is a key component of ATP and as the mediator for short term energy transfer within the plant cells ATP has potential as an indirect indicator of the activity of the energy transfer systems within the plant. The further movement of these sugars is typically mediated through the phloem loading process in higher plants. Companion cells to the phloem cells actively pump carbohydrates into the phloem from the source leaves into the sink of choice. Magnesium has a key role in the phloem process and plants deficient in magnesium see sucrose export from active leaves decreased to 10-20% of magnesium sufficient plants (Cakmak et al. 1994).

The ratio of ATP and Mg has been known to have an importance in ATP activity for several decades (Dennis and Coultate 1966). ATP is conjugated to Mg prior to a conversion to Adenosine Di Phosphate (ADP). This conversion releases the energy of the phosphate bond that drives countless biological processes within all living cells. The primary role of Mg was thought to be related to its position as the central metal in the chlorophyll molecule which is responsible for energy capture from sunlight. However further study of the role of Mg in the plant showed that only around 30% of all Mg in the plant is found within chlorophyll (Kirby and Mengel

1975). The first deficiency symptom visually perceived from Mg deficiency is interveinal chlorosis of older tissue as Mg is mobile within plant tissue. This suggests that plants sacrifice energy capture in the leaf in order to power the other systems for which Mg is a critical component (ATP to ADP conversion).

1.7.3 SBP Structural system

The role of Ca and B in the plant is apoplastic in that these nutrients are mainly found in the cell walls of higher plants (Matoh and Kobayashi 1998). Boron in cell walls forms complexes with rhamnogalacturonan II to build an important structural molecule which is a component of all higher plants. Calcium in the cell wall binds to the negative charges on pectins and gives a wall structural rigidity (Hepler, and Winship 2010).

Potassium is a main contributor to osmotic potential making up between one third and three quarters of the osmotic potential of phloem sap. Potassium is used by the plant in order to mitigate osmoregulation and it is critical for maintaining cell turgor. This role of potassium gives it a pseudo-structural role within the plant despite not forming any linkage with other components or compounds. Potassium is the only plant required nutrient that remains only in its ionic form in the plant.

Early investigations into the composition of calcium, boron, and potassium in corn and tomato plants demonstrated clear relationships between these nutrients (Reeve and Shive 1943). Boron deficiency appeared on plants with low boron (0.001 ppm) and high potassium (500 ppm) supply within 3 days whereas plants given the same concentration of boron in the medium and a low potassium (10 ppm) were the last plants to show boron deficiency symptoms. The relative severity of boron stress with the plants supplied the low boron rate increased linearly to the

increasing supply of potassium and as a result the requirement for boron within the plant was demonstrated to be directly related to the supply of potassium.

The same relationship existed with boron toxicity as plants supplied with a high boron concentration (5 ppm) and plants given an high rate of potassium showed boron toxicity symptoms earlier than those supplied with lower rates. This would highlight a need in understanding the potassium supply prior to interpreting boron requirements within the plant and also suggest a specific balance between these plant essential nutrients not commonly appreciated using modern tissue analysis techniques and interpretation.

The authors also used variable calcium applications to investigate if a similar boron deficiency relationship existed. However, the increasing rate of calcium showed a reduction in the severity of boron toxicity at the high boron rates suggesting that calcium has an opposite effect to potassium in terms of setting toxicity thresholds for boron applications. These relationships further suggest a holistic understanding of these nutrients would be beneficial as the deficiency inducing qualities of potassium and calcium may in fact be synergistic as they represent a bulk of the cations observed in plant tissue. There may also be a reduction in severity of toxicity symptoms if the differences between calcium and potassium applications show opposing influence on boron stress.

1.7.4 SBP Enzyme Activation and Electron Transport Chain System

Manganese, iron, copper and zinc are all period 4 elements provide the functionality and the active site of metallo-protein enzyme systems. These elements are the central metal for many cytochromes essential for electron transport chain and redox reactions the chloroplasts and mitochondria of plants. Cytochromes are embedded in the cellular membranes of the

chloroplasts and mitochondria, in specific arrangements (chains) to maximize energetic efficiencies of complex chemical reactions. Their functionality is critical within the plant, which underlines the importance of a robust and healthy supply of the critical reaction sites (metal centered structures) of these complex proteins.

In the electron transport chain there are many roles for each of the given nutrients. Plastocyanins require copper as the central metal in the reactive site of the enzyme which is required for electron transfer between different cytochromes in the electron transport chain. Photosystem II (PSII) relies on iron in the form of two heme, one non-heme subunits, for electron transfer and manganese in the form of a Mn4CaO5 cluster for water-splitting (Guskov et al 2009). These are two incredibly critical functional roles in PS II that have a proportional relationship in their concentrations within plant whilst an antagonistic relation exists in the soil. Copper toxicity in turn was found to result in decreased electron transport efficiency through a likely mechanism of inactivation of PSII reaction centers and by inhibition the acceptor sides in the electron transport system (Jianrong and Qiran 2009). The reaction center of PSII is the Mn4CaO5 cluster and copper is antagonistic to manganese in the soil environment and perhaps even in the cytoplasm. These nuances underscore the conceptual requirements for an exploration of the balance of nutrients within sampled plant tissues.

Manganese and zinc foliar applications were found to increase yield compared with the untreated check in potato demonstrating their importance in cropping systems (Mousavi et al. 2007). Different combinations of zinc and manganese ratios of foliar fertilization were evaluated. Manganese foliar applications at 4 ppt without zinc showed a positive response compared with the untreated control, however when the rate was increased to 8 ppt the yields were statistically the same as the untreated check. Zinc at 8 ppt combined with manganese at 4 ppt showed a

statistically significant increase in mean tuber yield of 14.0% as compared with zinc at 8 ppt with no manganese. The highest yield increase came from the foliar application with zinc:manganese ratio of 2:1. The balance of the foliar application of nutrients proved as important as the nutrients applied individually.

Iron deficiency limited crop growth when the nutrient solution dropped below 0.1 mM Fe. Increasing the iron concentrations induced sharp yield reductions when concentrations reached 0.2 mM and above. The remainder of the nutrient solution remained constant while the iron concentrations were increased.

1.7.5 SBP Deleterious ion system

No particular relationship exists between aluminum and sodium as these elements are not considered essential plant nutrients. These two nutrients are classed together as being both commonly analysed/reported for in tissue sampling and for their deleterious impact on plant growth. Aluminum is considered to be a potentially deleterious element through the mechanism of meristematic root growth inhibition. Aluminum in itself is a deleterious nutrient despite making up around 7% of the mass of the earth's crust. The availability of that aluminum in soils however is highly pH sensitive and the aluminum concentration of plants and soil can also make a potential marker for the pH of the soil in the rhizosphere.

Saline soils are limiting for the production of crops in approximately one quarter of all agriculturally classed land in the world (800 million hectares) (Rengasamy 2010). Salinity can come areas under intensive irrigation systems, soils with infiltration of highly saline sea water, and increasingly in dryland agriculture as well. The deleterious effects of saline soils on the majority of crops come from an osmotic and ionic stress mitigated by the concentration of the

soil and the plant's subsequent uptake of sodium (Kronzucker and Britto 2011). Accumulation of sodium in plant leaves can affect osmoregulation and cause leaf margin scorching or even leaf abscission. Tissue sampling should therefor be an excellent indicator for monitoring the deleterious effects of sodium stress.

1.8 Conclusions

Collecting a wide range of petiole samples across different sampling times and relating their individual concentrations of given nutrients and comparing these results with modern ionome evaluations would serve to improve the resolution of petiole sampling in Atlantic Canada. The relationship between tuber yield, specific gravity and nutrient concentrations must be related to very specific sampling timings in order to ensure the correct interpretation as nutrient concentrations are dynamic. There are many modern methods of statistical transformation and subsequent analyses which can help in interpreting the relative balance of accumulated nutrients. Systems which offer improvements to interpretation of petiole samples are ideal for evaluation and calibration to yields. In order to complete the ILR transformations the previously reviewed physiological structure for the SBP should be employed to reflect the role and function of the nutrients within the plant tissues.

The petiole is consistently sampled in Atlantic Canada and the interpretation of these analyses varies between laboratories. Ideal petiole concentrations for grower relevant yield metrics often come from outside of Atlantic Canada and they vary significantly by the researcher and region. A local examination into a large dataset of potato fields, sampling the petiole, at the same period of physiological development, across one cultivar, could uncover important relationships between certain nutrients and various economically significant yield parameters.

The nutrient concentrations in petioles should be compared with ILR transformed data in order to establish whether any improved correlation between tuber yield, specific gravity and petiole data can be observed. A comparison of fields with positive and negative outcomes for tuber yield and specific parameters based on an exploration of the plant ionome is recommended to identify key nutrients related to better grower profitability.

1.9 Tables

Table 1-1. Critical nutrient levels in potato petioles for Russet Burbank. Stark et al. (2004).

Nutrient	Nutrient concentration of potato petiole tissue* (mg kg-1) from tuber initiation to the end of tuber bulking		
	Low	Marginal	Sufficient
NO3	< 10,000	10,000 – 15,000	15,000 – 20,000
P	< 1,700	1,700 – 2,200	> 2,200
K	< 70,000	70,000 – 80,000	> 80, 000
S	< 1,500	1,500 – 2,000	> 2,000
Mg	< 1,500	1,500 – 3,000	> 3, 000
Ca	< 4,000	4,000 – 6,000	> 6,000
B	< 10	10 – 20	> 20
Zn	< 15	15 – 25	>25
Mn	< 20	20 - 40	> 40
Fe	< 20	20 - 50	> 50
Cu	< 2	2-4	> 4

Table 1-2. Critical nutrient levels in potato petioles for Russet Burbank. Walworth and Muniz (1993).

Nutrient	Critical nutrient concentration of potato petiole tissue* (mg kg-1) from early to the end of tuber bulking	
	Early Bulking	Later Bulking
NO3	16, 000	16, 000
Non- NO3-N	22, 000	17, 100
P	1, 500	1,400
K	113, 000	113, 000
S	2, 100	2, 100
Mg	7, 300	7, 300
Ca	8, 700	8, 700
B	15	15
Zn	15	15
Mn	15	15
Fe	70	70
Cu	5	5

Table 1-3. Critical nutrient levels in potato petioles A&L Laboratories (2016).

Nutrient	Critical nutrient concentration of potato petiole tissue* (mg kg-1) from early to the end of tuber bulking	
	Early Bulking	Later Bulking
NO3	7, 900	7, 000
Non- NO3-N	27, 900	24, 900
P	2, 400	2,100
K	80, 000	59, 900
S	1, 700	1, 700
Mg	2, 900	2, 900
Ca	14, 000	14, 000
B	29	29
Zn	29	29
Mn	29	29
Fe	99	99
Cu	8	8

Table 1-4. Nutrient mobility in the phloem. Marschner (2012)

Mobile	Intermediate Mobility	Low Mobility
Potassium	Sodium	Calcium
Nitrogen	Iron	Manganese
Sulfur	Zinc	Boron*
Magnesium	Copper	
Phosphorus		

1.10 Connecting paragraph

This chapter describes, through a review of the literature, many considerations for interpretation of potato nutrient sufficiency using tissue analysis. The following chapter describes the relationship between total yield, specific gravity and the potato petiole tissues sampled across six weeks in the tuber bulking phase. Key nutrients involved in promoting high yield potential as well as those considered deleterious to yield outcomes will be identified and discussed. In an effort to establish similar parameters for PEI, Chapter 2 explores the relationship between the petiole tissues and both tuber yield and specific gravity outcomes.

Chapter 2: Temporal changes in petiole nutrient concentrations during tuber bulking phase are related to tuber yield and specific gravity in the Russet Burbank potato (*Solanum tuberosum*) grown in Prince Edward Island, Canada.

2.1 Abstract

Potatoes are the most widely grown vegetable crop in both Canada and the world. Potato petiole sampling during tuber bulking is generally used to evaluate sufficiency levels of essential nutrients for tuber development. The objective was to identify nutrients in the petiole associated with tuber yield and specific gravity in order to determine when the strongest relationships occurred during the tuber bulking phase. Data were obtained from 94 commercial farms on Prince Edward Island, Canada during a five-year period from 2010 to 2014. Petioles were sampled once a week for six weeks in the early tuber bulking period to late tuber bulking phases and analyzed for NO_3-N, Non-NO_3-N, P, K, Mg, Ca, S, B, Zn, Mn, Fe, Cu, Na and Al. Isometric log ratio (ILR) transformations were employed and compared with concentration data. The petiole nutrient data in week 2 had the most correlation with tuber yield (r=-0.19 to -0.24, $P < 0.05$), while specific gravity was associated most in week 6 (r=-0.27 to 0.25, $P < 0.05$). The non-NO_3-N concentration was the most consistent indicator of improved tuber yield and specific gravity. The B concentration was positively correlated with higher specific gravity. In contrast, the Zn and Mn concentrations in the petiole were negatively correlated with tuber yield and specific gravity. The ILR transformed data showed no improved correlation with tuber yield or specific gravity. This study demonstrates that temporal fluctuations in Non-NO_3-N, NO_3-N, B,

Mn and Zn concentrations, of petiole tissue are potential indicators of economically-important potato yield and quality outcomes.

2.2 Introduction

The potato (*Solanum tuberosum* L) crop requires a large supply of nutrients throughout the growing season to achieve production targets. Better nutrient management practices are needed to improve potato production in the 21st century, specifically to synchronize the timing and rate of macro- and micro-nutrient fertilizers applied during the growing season to achieve the optimum marketable yield. The challenge to developing strategic fertilization practices is the lack of knowledge of the nutritional requirements of potato cultivars (Westermann and Davis 1992) as well as predictive tools that relate nutrient concentrations to potato yield and quality outcomes. In this study, the grower relevant yield outcomes of tuber yield and specific gravity were compared with nutrient data in petiole tissues during the bulking phase.

Prince Edward Island (PEI) accounts for 23.6% of the total Canadian acreage under potato production (Statistics Canada 2016). Growing conditions and practices on PEI give potato yields that are consistently around the national average. From 2010-2014, tuber yields were 32.1 Mg ha^{-1} (Std Deviation: 1.0 Mg ha^{-1}) in PEI, which was 1.5% greater than the Canadian average of 31.6 Mg ha^{-1}. The average PEI crop has estimated nutrient removal rates of 136 kg N ha^{-1}, 28 kg P_2O_5 ha^{-1} and 186 kg K_2O ha^{-1}, reflecting a higher nutrient demand for potatoes than cereal crops and most vegetable crops grown in this region (Government of PEI 2009). Still, the nutrient removal does not fully account for the potato plant's nutritional requirements because marketable yield is a fraction of the total edible and non-edible biomass produced by the plant. The aboveground portions of the crop, regardless of being returned post-harvest, require an

additional 52 kg N ha^{-1}, 7 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1} for proper leaf and stem development (Government of PEI 2009).

In-season measurements are recommended to ascertain whether the crop has acquired sufficient nutrients to meet yield targets. In potato, nutrient sufficiency is often assessed by sampling and interpreting the petiole of the most recently matured leaf (MRML) due to its physiological relevance in source-sink relationships (Walworth and Muniz 1993; Reis and Monnerat 2000; Stark 2004). This was reflected in potato propagation where petiole cuttings of the most recently mature leaf were considered to be indicative of the plant's nutritional status because the sap and tissues contain macro- and micro-nutrients that were recently assimilated and transported to this growing point (Ewing and Wareing 1978). Additionally, the petiole was responsible for reallocating photosynthates from leaves (source) to tubers (sink) during the bulking stage (Setter et al. 1980; Krapp et al. 1993). Consequently, there are several reports that relate fertilizer application rates to the nutrient concentration in the petiole as an indicator of the nutrient demand of potatoes under PEI growing conditions (White et al. 1974; White and Sanderson 1983; Sanderson et al. 2003).

Nutrient concentrations in the petiole generally increase with higher fertilizer application rates, and thus may be considered a reflection of the greater nutrient supply following fertilization. Sanderson et al. (2002) found that Russet Burbank potato yields on PEI responded to P fertilization, even in high P soils, and petiole P concentration generally increased as well. While the petiole P concentration may be an indicator of tuber yield responses, it does not reflect changes in specific gravity, which did not change with increasing P fertilization rates (Sanderson et al. 2003). White and Sanderson (1983) found that there was a positive yield and petiole N concentration response to increasing N fertilizer applications in Russet Burbank potatoes on PEI,

but the specific gravity declined as N fertilization increased. White et al. (1974) found that increasing N and K fertilizer rates improved Russet Burbank tuber yields on PEI but reduced specific gravity. Increasing the N fertilizer application rates resulted in greater N and K concentrations in petioles, whereas higher rates of K fertilizer reduced the N concentration and increased the K concentration in petioles (White et al. 1974).

Nutrient concentration in petioles can be used to assess the relationship between each essential nutrient and yield components, as described above for fertilizer trials. Alternatively, the proportional accumulation of nutrient data can also be related to yield components (Walworth and Sumner 1988). The interpretation of the proportional accumulation nutrients in plant tissues is attractive as plants develop and grow depending on a balanced supply of essential elements maintained by homeostatic mechanisms (Williams and Salt 2009). There are many examples of transformations being used in tissue sampling interpretation in agriculture (Beaufils 1973; Parent et al. 1994) with improved classification efficiency reported from both log and ILR transformations (Parent et al. 2013). When evaluating proportional accumulation of nutrients, the DRIS method is commonly employed by agronomists however with this method one generates a myriad of ratios which can be difficult to interpret (Parent et al. 2013). For example, there would be 91 dual ratios to interpret from a tissue analysis report of 14 nutrient concentrations. The ILR transformations, developed by compositional data analysts, are log-ratio transformations generated to create scale-invariant variables, which avoid any redundancy in the data, which are expressed in real space (Aithchison 1986; Egozcue et al. 2003).

The objective of this study was to relate the element concentrations and transformed ILR of nutrient data in petioles, collected during six weeks of tuber bulking, with tuber yields and specific gravity of Russet Burbank potatoes grown in PEI, Canada. The primary hypothesis was

that the tuber yield and specific gravity outcomes are correlated to nutrient concentrations and ILR transformed nutrient concentrations in petioles. It was further hypothesized that the ILR transformations would be more strongly related to yield and specific gravity outcomes than the untransformed nutrient concentrations. It was also hypothesized that macronutrients would be more correlated to tuber yield and specific gravity than micronutrients. Based on reported findings from PEI (White et al. 1974; White and Sanderson 1983; Sanderson et al. 2003) it was also hypothesized that the petiole N, P, and K concentrations would be more positively correlated to tuber yield than other nutrient concentrations in petioles, whereas specific gravity was expected to be negatively associated with N and K petiole concentrations. The transformed balance points of ILR 8, ILR 10, and ILR 5 are hypothesized to be most positively correlated to tuber yield due to their description of relative accumulation of N, P, and K respectively. The transformed balance points of ILR 8 and ILR 5 are hypothesized to be most negatively correlated to specific gravity.

2.3 Materials and Methods

2.3.1 Study fields

Growers with potato (cv. Russet Burbank) fields were selected from a survey population of 108 commercial farms monitored by NutriAg Ltd. (Toronto, Canada) over a 5 year period from 2010 to 2014. No irrigated fields were included in the dataset. All fields were located on the province of Prince Edward Island, which is an island in the Northumberland Strait, centered around Charlottetown (46° 14’20” N 63° 07’45” W). All fields were classed as Charlottetown soil series, which are fine sandy loam to loamy soils classified as shallow Orthic Humo-ferric podzols. This temperate maritime climate has a 30 year normal mean yearly temperature of

5.6 °C with a mean temperature of 14.5 °C (UPEI Annual Climate Summary 2016 during the May-October months associated with potato production. The 30 year normal average yearly rainfall of the province is 1206 mm (UPEI Annual Climate Summary 2016). Land cleared for agriculture makes up 41% of total land area on PEI with potatoes accounting for 35,600 hectares planted in 2016. Potatoes also represent the largest agricultural commodity in terms of value (Agriculture on PEI 2016). Planting densities varied from 32 894 plants ha^{-1} (0.40 m in row x 0.76 m between row spacing) to 34 632 plants ha^{-1} (0.38 m in row x 0.76 m between row spacing).

2.3.2 General Fertilization Strategies

Potato fields in the study followed similar fertilizer N, P, K and lime application strategies across the growers. Nitrogen was applied in all fields in the forms of ammonium nitrate (AN; 34-0-0), Urea (46-0-0), mono-ammonium phosphate (MAP; 11-52-0), and/or di-ammonium phosphate (DAP;18-46-0) at a rate of 200-224 kg N ha^{-1}. Phosphorus was applied in the form of DAP or MAP at a rate of 168-224 kg P_2O_5 ha^{-1} depending on the P fertilizer recommendation from the soil test (Mehlich-3 P concentration). Potassium was applied as either muriate of potash (MOP; 0-0-60), or a mix containing potassium magnesium sulfate (K-Mag; 0-0-22 11 Mg) and MOP. Potassium recommendation targeted a base saturation of 5% K_2O in all fields, based on the Mehlich-3 K concentration in the soil test, with a minimum application limit of 245 kg K_2O ha^{-1} and a maximum application limit of 340 kg K_2O ha^{-1}. Magnesium was also applied in the form of K-Mag in an effort to achieve a base saturation of 10% Mg in all fields, based on the Mehlich-3 extractable Mg concentration in the soil test. With N- P_2O_5- K_2O application rates of 200-224-

(245-367) kg ha^{-1} these fields could be characterized as having heavy macronutrient fertilizer application rates and thus should not be deficient in these nutrients. A target soil pH of 5.8-6.2 was followed by all growers with fields receiving lime applications of 2.5 Mg ha^{-1} when the soil test reports reported a pH_{H2O} value < 5.7. Lime applications were made with either dolomitic and calcitic lime blends containing 5- 12% Mg and 22-32% Ca, or pure calcitic lime (0.5% Mg, 36% Ca). If the Mehlich-3 extractable Ca concentration in the soil test was below 900 ppm but pH_{H_2O} >5.7, the field received 2.5 Mg ha^{-1} gypsum (29% Ca, 23% S).

2.3.3 Petiole Sample Collection and Analysis

Petiole samples were collected from a representative area (4.5 m x 10 m section containing 148-155 plants) in each commercial potato field beginning when tubers entered the bulking phase (tuber diameter >25mm). Petioles from 40-60 randomly-selected plants were collected once a week for 6 wk, ending at the end of tuber bulking. The sampling process was initiated on the 25 Jul 2010, 28 Jul 2011, 31 Jul 2012, 25 Jul 2013, and 23 Jul 2014 about 60 days after planting. Only single stems were analyzed per plant leaving several un-sampled stems per plant for future sampling. Petioles were composited and sent for analysis to A&L Laboratories (London, Canada) where they were dried (70 °C) and ground for analysis using a Wiley Mill. Total N was determined using a Leco FP-628 CNS (LECO Corp., St. Joseph, MI, USA). The NO_3-N concentration was determined using a FIALab-2600 autoanalyzer (FIALab, Bellevue, King, USA). Non-NO3-N concentration was the difference between total N and NO3-N concentrations. Plant tissue was digested using nitric acid/ hydrochloric acid, and the P, K, Mg, Ca, S, B, Zn, Mn, Fe, Cu, Na and Al concentrations were determined by ICP-OES using a

Thermo Fisher ICAP 6300 (Helmut-Fischer, Sindelfingen- Maichingen, Germany). Macro-nutrient concentrations were expressed in g kg^{-1} petiole tissue (dry mass), while micro-nutrient concentrations were given in mg kg^{-1} petiole tissue (dry mass).

2.3.4 Yield and Specific Gravity Estimation

Tuber yields were estimated at physiological maturity by hand harvesting three single rows (3 m long), selected at random from a quadrant of less than 0.25 ha centered on and surrounding the representative area where petiole sampling occurred. Harvesting occurred during a 2 wk period and potatoes obtained from each field were placed in cold storage (2 °C ± 3°C) until all fields were harvested. Then, tubers were weighed, and total fresh tuber yield was extrapolated linearly and expressed on a Mg ha^{-1} basis. A composite sample, derived randomly from tubers >50 mm in size, weighing 3.6 kg was taken for specific gravity determination. Specific gravity of the tuber composite sample was determined using the water immersion method and a hydrometer. The hydrometer was a hooked float attached to a transparent neck graduated with specific gravity readings. The basket containing the tuber sample was attached to the hydrometer and lowered into a 200 L barrel filled ¾ full with water. The tuber sample was allowed to come to rest in the water and the specific gravity was read from the hydrometer.

2.3.5 Proportional nutrient accumulation as ILRs

The SBP selection (Table 1.1) was established in order to best describe a separation of nutrients derived from the physiological role/systems of the given nutrients within the crop in a manner required for the ILR transformations. Nutrients were arranged into the following five groups: protein synthesis (NO_3-N, Non NO_3-N, and S), short term energy storage/transfer (P, Mg), cellular structure (K, Ca, B), enzyme activation and electron transport chain system (Zn, Mn, Fe, and Cu), and deleterious (Al, Na). The protein system was established to examine the balance of

protein feedstock as they are critical to production of the reduced forms of N (Hesse and Hoefgen 2003; Nikiforova 2003; Saito 2004). The efficiency of the ATP/Mg ratio is evaluated in the short term energy storage/transfer balance to investigate previously described inter-relation between these two elements (Dennis and Coultate 1966; Kirby and Mengel 1975). The cellular structure balance reflects the structural nature of Ca and B as they are both found primarily in cell walls of higher plants (Matoh and Kobayashi 1998; Hepler and Winship 2010) but also includes K as Reeve and Shive (1943) described a synergistic relationship between these three elements in tomato plants. The electron transport chain balance is made up of many metallo-protein systems containing Zn, Mn, Fe, and Cu which were sensitive to the ratio of availability between elements (Guskov et al. 2009; Jianrong and Qiran 2009). The elements Na and Al are not plant required nutrients and their accumulation has deleterious consequences for crop growth (Rengasamy 2010; Kronzucker and Britto 2011).

2.3.6 Data Processing and Statistical Analysis

In order to evaluate the mean concentration differences between different yield outcome groups the tuber yields for all site-years were divided into quartiles: <39 Mg ha^{-1} (n=23), 39.1 – 45.4 Mg ha^{-1} (n=24), 45.5-50.9 Mg ha^{-1} (n=24), and 51-57.2 Mg ha^{-1} (n=23). The same approach was applied to divide specific gravity groupings into quartiles: The mean elemental concentration in petiole tissue, by week, was associated with one of four specific gravity groups (1.069-1.077 (n=23), 1.078 – 1.082 (n=24), 1.083 – 1.087 (n=24) and 1.087-1.099 (n=23). The division into quartiles was undertaken to evaluate the various yield and specific gravity outcome potentials for potato growers as is commonly employed in other agricultural commodities (Buza et al. 2014). This is of importance as potato processing growers are paid on contracts with a dynamic bonus

structures for specific gravity it is important that these parameters to be divided into several categories in order for growers to estimate the economic significance of the conclusions.

The ILR transformed values were calculated with the ionome data by defining by its SBP (Egozcue et al. 2003). The SBP (Table 1.1) was a (D-1) x D matrix where group numerators were labeled +1 and group denominators were labeled -1 while parts, which were not a part of a given balance, were given a value of 0. Tissue elemental concentrations (non-NO_3-N, NO3-N, P, K, Mg, Ca, S, B, Zn, Mn, Fe, Cu, Na, and Al) and ILRs 1-14 were related to total yield and specific gravity using correlation analysis to determine if any individual nutrients were predictors of yield. ILR transformations were performed in Microsoft Excel and the correlation analyses were undertaken using JMP 13.0 (SAS Institute Inc., Cary, NC). Differences in tissue elemental concentrations and ILRs between quartiles for total yield and specific gravity and across 6 sampling dates were analyzed using repeated measures ANOVA. Where a significant effect was identified in ANOVA, differences between groups were evaluated using Least Squares Means (LSMEANS) at α=0.05. ANOVA and multiple means comparisons were undertaken using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC).

2.4 Results and Discussion

2.4.1 Nutrient Concentration Relationships with Tuber Yield

Tuber yields for the grower database averaged 44.9 Mg ha^{-1} (Std. Deviation: 8.1 Mg ha^{-1}) which is 40% higher than the provincial average. The higher values are not surprising as the Russet Burbank cultivar of potato is a long season variety whereas provincial averages include many lower yielding potato cultivars. Several elements in petiole samples collected during the tuber bulking period were significantly (P <0.05) correlated with tuber yield, but not in all weeks

(Table 2). Non-NO_3-N was positively correlated ($P < 0.05$) in weeks 3 and 6, while NO_3-N, Ca, Mg, and Zn were negatively correlated ($P < 0.05$) with total yield for 1 to 3 weeks during the measurement period (Table 2). There was an inconsistent correlation ($P < 0.05$) between Al and tuber yield in two weeks (Table 2). More significant correlations ($P < 0.05$) of nutrient concentrations with total yield occurred in week 2 (Table 2). It is not surprising that early bulking samples were most significantly associated with total yield as this timing coincides with the typical recommendations for potato petiole sampling on PEI (Government of PEI 2013). There was a consistency between the data and the primary hypothesis that total yield would be found to be correlated with petiole nutrient concentrations. The secondary hypothesis that that macronutrient concentrations would be more significantly correlated than the micronutrient concentrations was also confirmed.

Non-NO_3-N petiole concentrations were lowest for the lowest yield grouping in weeks 3 and 6 of tissue sampling (Fig. 2-1). The positive correlation ($P < 0.05$) of Non-NO_3-N in petiole tissues and tuber yield demonstrates the importance of nitrogen management in the potato production system, specifically the more reduced forms of nitrogen which could make up protein, NH_4-N, or DNA. The relationship between tuber yield and Non-NO_3-N concentrations are consistent with the hypothesis that N concentrations in tissues would be positively correlated to yield outcomes as well as with the work of White et al. (1974). As applied N fertilizers were demonstrated to increase petiole total N concentrations (White et al. 1974) it is possible to interpret the concentrations as a reflection of higher application rates of N fertilizers. This relationship brings into question nitrogen application recommendations made by White and Sanderson (1983) which are well below those of growers in the study. The authors state that the requirements of the crop are known to be related to the growing season length and final yield of

the crop however these have both changed in PEI over the last 30 years since these recommendations were published.

The negative correlation (P <0.05) of NO_3-N in petiole tissues and tuber yield is inconsistent with the hypothesis that NO_3-N concentrations would be positively correlated to tuber yields as well as with reports by Westermann et al. (1994). This would suggest early in bulking there was still an accumulation of labile NO_3 in the petiole, and as the bulking season progressed the chemically reduced Non-NO_3-N forms were more positively associated with specific gravity. However, the inconsistencies between both forms of N and tuber yield denotes the importance of partitioning N analyses into different forms. The differing relationships between the two forms of N would strongly suggest that the interpretation of potato petiole tissues benefits from such separations. It is possible, especially in week 6 of sampling, that a combined interpretation of total N only would show no correlation (P < 0.05) with tuber yield at all.

The negative correlation (P <0.05) between Ca and Mg concentrations and yield outcomes may also be a marker for increased Ca and Mg amendment. The most common source for Ca and Mg application would be through agricultural lime. A deleterious relationship between liming and yield outcomes was described by Maier et al. (2002), however the majority of previous work with lime and potato production showed no effect on tuber yield (Simmons et al. 1988; Sparrow and Salardini 2008) or a positive relationship between lime application and yield outcomes (Laughlin et al. 1974; Lalljee and Facknath 2001). Sanderson and Gupta (1990) reported that Zn amendments reduced the total yield for Russet Burbank potatoes grown in PEI. Soil applications of Zn also increased leaf Zn concentrations in early sampling time however petiole tissues were not evaluated.

2.4.2 Nutrient Concentration Relationships with Specific Gravity

Tuber specific gravity values averaged 1.083 (Std. Deviation: 0.008) which is considered a poor to fair value for processing contracts. Specific gravity was positively correlated ($P < 0.05$) with the Non-NO_3-N, NO_3-N and B concentrations in petioles collected in 1-2 weeks of the sampling period (Table 3). There were negative correlations ($P < 0.05$) between specific gravity and S, Ca, Zn, Mn, and Al in 2-4 weeks of sampling (Table 3). Overall, the data were consistent with the hypothesis that specific gravity would be associated with petiole nutrient concentrations although the secondary hypothesis that macronutrient concentrations would be more significantly correlated than the micronutrient concentrations is inconsistent with the results. The most number of significant correlations ($P < 0.05$) for nutrient concentrations and specific occurred in week 6 (Table 2). The relationship between late season petiole sampling and specific gravity is quite surprising as there were no guidelines for petiole nutrient concentrations in relation to this yield parameter. The late season sampling time is not generally recommended (Government of PEI 2013) and therefore the relationship between petiole nutrient concentrations and specific gravity may have been previously missed due to incorrect sampling times.

There was a statistically significant ($P < 0.05$) difference between mean NO_3-N and specific gravity groupings at week 1 of sampling (Fig. 2-2) and the highest specific gravity grouping had the highest mean value for NO_3-N. The highest mean Non-NO_3-N during week 3 and week 6 were associated with the highest specific gravity outcomes. Both of these positive correlations were inconsistent with the hypothesis that N would most likely have a negative correlation to specific gravity outcomes. The partitioning of N into two different subcomponents could explain the variation between the expected and actual results however the results are also

inconsistent with reports documenting petiole NO_3-N concentrations (Westermann et al. 1994). It should be noted that some researchers also found no deleterious relationship between N application rates and specific gravity (Silva et al. 1991). The work of Ojala et al. (1990) would suggest that the nature of the relationship between N and specific gravity is dependent on the rate being applied. Under excessive N conditions the researchers reported a deleterious effect whereas in deficient scenarios the relationship is known to be positive. This would suggest that the N application rates used by growers in this dataset may not be excessive despite being quite aggressive.

Quartiles based on specific gravity differed in B concentrations in weeks 4 and 6 of sampling, with the highest mean B concentrations reported by the highest specific gravity grouping (Fig. 2-2). Boron was also significantly correlated ($P < 0.05$) with specific gravity in weeks 4 and 6 (Table 2). The significance of B as a positive marker for potato specific gravity was consistent with some of the findings of Mesquita et al. (2007) but were incongruent with the reports of Brown et al. (1991). The effect of B in plant tissues can change the accumulation of mobile sugars in source tissues (Ruuhola et al. 2011). The role of B in carbohydrate metabolism and mobilization was a potential explanation for the association between B concentrations and higher specific gravity.

The lowest specific gravity grouping had a higher mean value for Ca, at week 1 of sampling, compared with the two highest (Fig. 2-2). The Ca concentrations in petiole tissues showed a statistically significant negative correlation ($P < 0.05$) with specific gravity outcomes in week 1 and week 5 (Table 3). It was difficult to interpret the tissue concentrations of Ca as it was previously reported as being unaffected by Ca fertilization (Gupta and Sanderson 1993) in PEI and it is not considered a phloem-mobile nutrient (Marschner 2012). The increased

accumulation of Ca in the transitory petiole tissue was potentially an indication of delayed physiological maturity. If it was a response to Ca fertilization then it would be inconsistent with the previous reports in the literature (Silva et al. 1991; Locascio et al. 1992) who both reported no deleterious effect on specific gravity with increased Ca applications.

The concentration of S was also negatively associated with specific gravity outcomes for week 5 and week 6 (Table 3). In week 4 of sampling there were statistically significant ($P < 0.05$) differences in S concentrations between specific gravity groupings, however the only differences existed between the lowest and low specific gravity groupings (Fig. 2-2). This response was also contrary to the results of Rosen et al. (2002) where sulfate application improved specific gravity outcomes. These findings were however consistent with observations made by Gausman and Estes (1963) whereby applications of 11 – 22 kg S ha^{-1} resulted in lower tuber specific gravity. The positive response was considered to be related to a replacement of higher salt index fertilizers such as potassium chloride. These discrepancies were addressed by Laboski and Kelling (2007) who suggested that the relationship between sulfur and specific gravity may be positive when a sulfate fertilizer was replacing a chloride, however if an application of sulfur was being applied, in addition to an existing fertilization schedule (i.e. gypsum), then the relationship may be deleterious. Further investigation into the relationship between sulfur fertilization and petiole S concentrations would help in understanding whether this relationship was causal or simply random. This is particularly important as ammonium sulfate, K-Mag and gypsum applications were quite common in PEI potato production systems.

Concentrations of Mn differed between specific gravity groupings in sampling week 4 and 5, with the lowest Mn concentrations consistently associated with the highest specific gravity (Fig. 2-2). There was a statistically significant negative correlation ($P < 0.05$) between both Mn

and Zn petiole concentrations and specific gravity outcomes for weeks 2, 3, 5 and 6 of sampling (Table 3). Petiole concentrations of these nutrients were not hypothesized to have any relationship to either total yield or specific gravity outcomes however the concentrations of Mn in PEI potato production systems have been noted as a concern in the literature before (Gupta et al. 2008) and higher concentrations in leaf tissues were linked with lower pH soils. Aluminum concentrations, also pH dependent, were negatively correlated ($P < 0.05$) with specific gravity outcomes for weeks 2 and 3 (Table 3). There were differences in Al concentrations between specific gravity groupings in weeks 1 and 2 of sampling with the highest specific gravity grouping consistently reporting the lowest mean values (Fig. 2-2).

2.4.3 ILR Relationships to Tuber Yield

Similarly, to tissue concentrations, there were more negative correlations than positive in the ILR transformed data as related to yield. However, in the case of the ILRs, a negative correlation refers to a tipping of the balance of nutrient groups and therefore no negative correlation is truly indicative of an association between a single nutrient and yields but rather between the balance of two or more nutrients. No single ILR was correlated to yields in a statistically significant manner for more than 2 of 6 weeks. The hypothesis that ILR transformed data was more correlated to tuber yield than concentrations was not consistent with the results. Tuber yield was correlated ($P < 0.05$) with the ILR 1, ILR 2, ILR 3, ILR 6, ILR 8, ILR 9, ILR 10, and ILR 11 (Table 2). The correlation ($P < 0.05$) of ILR 10 and ILR 8 and tuber yield was consistent with the original hypothesis however the lack of correlation of ILR 5 was not. The most number of ILR transformed values correlated ($P < 0.05$) with tuber yield occurred in week 2 (Table 2) which is

consistent with the relationship described by nutrient concentrations and is therefore unsurprising.

The ILR 1 transformation indicates that the highest yielding fields have a pattern of lower nutrient accumulation overall in the petiole tissue. This correlation was only significant for week 2 of petiole sampling ($P < 0.05$). Accumulation of nutrients was typically indicative of earlier physiological staging according to the work done by Heard (2004) and the relationship between higher overall petiole nutrient density and lower yields may be a further indication of fields which were not as mature, physiologically, and therefore may not have the time to finish the bulking period before the fall harvest. The balance points represented by ILR 2 and ILR 3 (Fig. 2-3) highlight the influence of the non-essential elements (Na and Al) considered to have deleterious effects on tuber yield. The correlation ($P < 0.05$) between tuber yield and ILR 2 values is unsurprising as it denotes a deleterious consequence to Al accumulation, however ILR 3 would suggest that Na accumulation is positively correlated ($P < 0.05$). As these are quite transient differences and include a comparison to many other nutrients the implications of these findings are ambiguous.

In ILR 6 the accumulation of nutrients associated with the electron transport chain (Zn, Mn, Fe, and Cu), relative to the protein system (Non-NO_3 N, NO_3-N, and S), was correlated ($P < 0.05$) with lower yield outcomes at week 2 of sampling. This was consistent with the hypothesis that macronutrient were more positively associated with yield than micronutrients. Correlations ($P < 0.05$) reported for ILR 8 are consistent with the hypothesis that balance points associated with N would be correlated to tuber yield. This balance point denotes the importance, within the protein system, of accumulation of Non-NO_3 N relative to NO_3-N, and S which is previously described by the nutrient concentrations. The correlation ($P < 0.05$) with ILR 9 and tuber yield

denotes the deleterious nature of Zn and Mn accumulation relative to Cu and Fe within the electron transport chain system. As this correlation is no more significant than the deleterious relationship described previously between Zn and tuber yields the value of this balance point is questionable. It may give the observer a sense that Mn accumulation is deleterious or that Cu and Fe accumulation are preferred when no consistent relationships between these three elements and tuber yields was ever discovered.

The correlations ($P < 0.05$) observed between ILR 10 and tuber yield would suggest that a higher proportion of Mg accumulation relative to P is deleterious within the energy transfer system. This is consistent with the original hypothesis that P and ILR 10 would be positively correlated to yield outcomes. It should be noted however that this is in the absence of any correlation between P and tuber yield. The ILR 11, representing the balance of the structural system, values were also correlated ($P < 0.05$) to tuber yield in the absence of any correlation with B concentrations. The tuber yield relationship with ILR 11 was consistent with observations by Tariq and Mott (2007) suggesting that in some plants there was an ideal ratio in terms of supply of Ca to B associated with maximum biomass production. As with previous ILR values it is difficult to interpret whether these difference in ILR 10 and 11 are reflective of a specific relationship or whether they are repeating the deleterious relationship described previously based on nutrient concentrations.

2.4.4 ILR Relationships to Specific Gravity

The transformed balance points of ILR 2, ILR 3, ILR 4, ILR 8, ILR 9, ILR 11, and ILR 12 (Table 2) were correlated ($P < 0.05$) with specific gravity. The lack of correlation of ILR 5 was not consistent with the original hypothesis however the correlation of ILR 8 was. The most

number of ILR transformed values correlated ($P < 0.05$) with specific gravity were reported in weeks 5 and 6 (Table 2). Both weeks had the same ILR values presenting with correlations to specific gravity outcomes with similar r values for each ILR.

The ILR 2 transformation was correlated ($P < 0.05$) to specific gravity outcomes (Table 3) and the lowest specific gravity grouping had a lower ILR 2 value than the highest grouping in week 2 (Fig. 2-4). This balance point is demonstrating the deleterious nature of Al accumulation, in a similar manner to yield, on specific gravity. In all cases highest yield grouping and specific gravity outcomes were correlated to the highest value, and consequently the lowest relative Al accumulation, for ILR 2. Since Al accumulation was associated with increased oxidative stress in potato varieties that were not Al tolerant (Tabaldi et al. 2009), this relationship may indicate that Russet Burbank was not a variety with strong tolerance to Al accumulation. The ILR 3 in a similar manner to yields also showed an unexpected correlation ($P < 0.05$) between Na accumulation and better specific gravity outcomes (Table 3). This is inconsistent with the literature which would suggest that potatoes are Na sensitive (Hütsch et al. 2017) and it is inconsistent with the lack of correlation between Na concentrations and specific gravity. The ILR 4 balance point denotes the balance point between a combination of the energy transfer system and the structural system as compared with the protein and electron transport systems. Due to the complexity and number of elements in this balance point the significance of the negative correlations ($P < 0.05$) with specific gravity outcome (Table 3), or the fact that the highest specific gravity grouping had the lowest value at week 6 of sampling (Fig. 2-4) are ambiguous.

The negative correlation ($P < 0.05$) between ILR 8 and specific gravity outcomes are in line with the original hypothesis. This is not surprising as this balance point denotes the state of N and S accumulation within the protein system. The accumulation of Non-NO_3-N relative to

NO_3-N and S was consistent with observations made using concentration data (Table 2 and 3). The ILR 9 balance point was most consistently correlated ($P < 0.05$) to specific gravity of all transformed variables (Table 3) and the lowest specific gravities had the highest Zn and Mn accumulation relative to Cu and Fe for 4 weeks of sampling. These observations were consistent with the concentration data which showed that Zn and Mn accumulation individually are correlated ($P < 0.05$) to worse specific gravities. There was no significant improvement in the correlation power of ILR 9 as compared with the Zn and Mn concentrations at the same time which is inconsistent with the hypothesis that ILR transformed balance points would be more significantly correlated to specific gravity outcomes than concentrations. The accumulation of Zn and Mn were often associated with acidic soils whereas the availability of Cu was far less associated with lower pH values (Sims, 1986). The accumulation of Zn and Mn may also be another marker of soil pH in the potato cropping system. The ILR 11 balance point had positive correlation ($P < 0.05$) with specific gravity denoting the importance of B accumulation relative to Ca (Table 3).

The ILR 11 correlations ($P < 0.05$) at week 1 was the strongest of all variables (concentrations or ILRs) associated to specific gravity which is consistent with the hypothesis that ILR transformed data points would be more correlated to specific gravity outcomes than concentrations. Since the accumulation of Ca in petiole tissues was correlated to lower specific gravity and B concentrations were correlated with higher specific gravity, it is not surprising that the ILR 11 balance point is so consistently related to the specific gravity outcomes of potato growers in PEI. The principle of balancing the concentration of Ca and B in petiole tissues were novel to potato production however these observations were consistent with those made on corn (Kanwal et al. 2007) whereby the increased application of Ca reduced the availability of B in

hydroponic solutions, increasing the Ca to B ratio and affecting the shoot dry matter accumulation. The ILR 12 is another balance point which mirrors the correlations ($P < 0.05$) observed in the concentrations which make up the transformation, namely NO_3-N, and offers no improved correlation.

2.5 Conclusions

In this study several nutrients present in petioles during the tuber bulking period were associated with potato yield outcomes, namely tuber yield and specific gravity. Regardless of whether untransformed nutrient concentrations or ILR transformed data were considered, the only consistent element positively associated with both yield components was Non-NO3-N and B while Ca, Zn and Mn tended to be negatively associated. However, the relationships between nutrients and yield outcomes fluctuated with time and were inconsistent for tuber yield and specific gravity outcomes. No improved correlations were observed by transforming nutrient concentrations into ILR values. The inclusion of several nutrients within a single balance point made interpretation more confusing and could lead to incorrect assumptions about the benefits of nutrient accumulation patterns in potato petioles. Overall, the identification of specific nutrient correlations with grower relevant markers opens up the possibilities to evaluate novel fertilizer amendment strategies which were not previously identified.

Management of N is important for potato crop production which is confirmed in this study. It is strongly recommended that researchers working with petiole analyses partition the various detectable forms of N as they have been shown to follow very different relationships with grower relevant yield metrics. The report, in this publication, of petiole B concentrations as a strong marker for better specific gravity outcomes in PEI is completely novel and has never

been previously published. Government recommendations for B amendment include both soil and foliar applications however no specific critical petiole or leaf concentration is provided for reference. Further work is necessary to identify the potential for crop amelioration with B fertilizers. Negative relationships between Ca, Zn, and Mn accumulation and yield outcomes highlights the need for a better understanding of nutrient interactions in the soil and plant. Further work into evaluating the effect of liming practices on these three elements in petiole tissues is also recommended.

2.6 Tables

Table 2-1. Sequential Binary Partition of the potato petiole ionome on PEI.

	Non-NO_3-N	NO_3-N	S	P	K	Mg	Ca	Na	B	Zn	Mn	Fe	Cu	Al	Fv
ILR 1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-1
ILR 2	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	0
ILR 3	1	1	1	1	1	1	1	-1	1	1	1	1	1	0	0
ILR 4	1	1	1	-1	-1	-1	-1	0	-1	1	1	1	1	0	0
ILR 5	0	0	0	1	-1	1	1	0	1	0	0	0	0	0	0
ILR 6	-1	-1	-1	0	0	0	0	0	0	1	1	1	1	0	0
ILR 7	0	0	0	-1	0	-1	1	0	1	0	0	0	0	0	0
ILR 8	-1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
ILR 9	0	0	0	0	0	0	0	0	0	1	1	-1	-1	0	0
ILR 10	0	0	0	1	0	-1	0	0	0	0	0	0	0	0	0
ILR 11	0	0	0	0	0	0	-1	0	1	0	0	0	0	0	0
ILR 12	0	-1	1	0	0	0	0	0	0	0	0	0	0	0	0
ILR 13	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0
ILR 14	0	0	0	0	0	0	0	0	0	-1	1	0	0	0	0

ILR = Isometric log ratio transformation

Table 2-2. Pearson correlation coefficients (r) between tuber yield and petiole nutrient concentrations, based on untransformed nutrient concentrations and ILR transformed balance points on PEI.

Sampling Week	**1**		**2**		**3**		**4**		**5**		**6**	
Element	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r
Non NO_3-N	NS	-	NS	-	**	0.30	NS	-	NS	-	*	0.22
NO_3-N	NS	-	NS	-	NS	-	NS	-	NS	-	*	-0.19
S	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
P	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
K	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Mg	NS	-	*	-0.19	NS	-	NS	-	*	-0.19	NS	-
Ca	NS	-	*	-0.21	*	-0.18	NS	-	*	-0.23	NS	-
Na	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
B	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Zn	NS	-	*	-0.20	NS	-	NS	-	*	-0.21	NS	-
Mn	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Fe	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Cu	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Al	NS	-	*	-0.24	*	0.23	NS	-	NS	-	NS	-
ilr 1	NS	-	*	-0.24	NS	-	NS	-	NS	-	NS	-
ilr 2	NS	-	*	0.24	NS	-	NS	-	NS	-	NS	-
ilr 3	*	-0.19	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 4	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 5	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Ilr 6	NS	-	*	-0.22	NS	-	NS	-	NS	-	NS	-
ilr 7	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 8	NS	-	NS	-	*	-0.20	NS	-	NS	-	*	-0.25
ilr 9	NS	-	NS	-	NS	-	*	-0.24	*	-0.24	NS	-
ilr 10	*	0.20	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 11	*	0.19	**	0.25	NS	-	NS	-	NS	-	NS	-
ilr 12	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 13	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 14	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-

NS not statistically significant, *P <0.05, **P <0.01

Table 2-3. Pearson correlation coefficients (r) between specific gravity and petiole nutrient concentrations, based on untransformed nutrient concentrations and ILR transformed balance points on PEI.

Sampling Week	**1**		**2**		**3**		**4**		**5**		**6**	
Element	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r
Non NO_3-N	NS	-	NS	-	**	0.27	NS	-	NS	-	**	0.25
NO_3-N	**	0.31	NS	-	NS	-	NS	-	NS	-	NS	-
S	NS	-	NS	-	NS	-	NS	-	**	-0.32	**	-0.27
P	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
K	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Mg	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Ca	**	-0.25	NS	-	NS	-	NS	-	*	-0.24	NS	-
Na	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
B	NS	-	NS	-	NS	-	*	0.24	NS	-	*	0.23
Zn	NS	-	*	-0.23	*	-0.18	NS	-	**	-0.25	*	-0.22
Mn	NS	-	*	-0.24	*	-0.22	NS	-	*	-0.24	*	-0.20
Fe	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Cu	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Al	NS	-	**	-0.32	*	-0.21	NS	-	NS	-	NS	-
ilr 1	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 2	NS	-	*	0.23	*	0.23	NS	-	NS	-	NS	-
ilr 3	*	-0.23	NS	-	NS	-	NS	-	*	-0.23	**	-0.34
ilr 4	NS	-	NS	-	NS	-	*	-0.19	*	-0.20	**	-0.27
ilr 5	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Ilr 6	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 7	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 8	NS	-	NS	-	*	-0.21	NS	-	**	-0.29	**	-0.25
ilr 9	NS	-	**	-0.33	**	-0.26	*	-0.22	**	-0.28	*	-0.20
ilr 10	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 11	**	0.42	*	0.24	NS	-	NS	-	**	0.25	*	0.24
ilr 12	**	-0.28	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 13	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 14	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-

NS not statistically significant, *P <0.05, **P <0.01

2.7 Figures

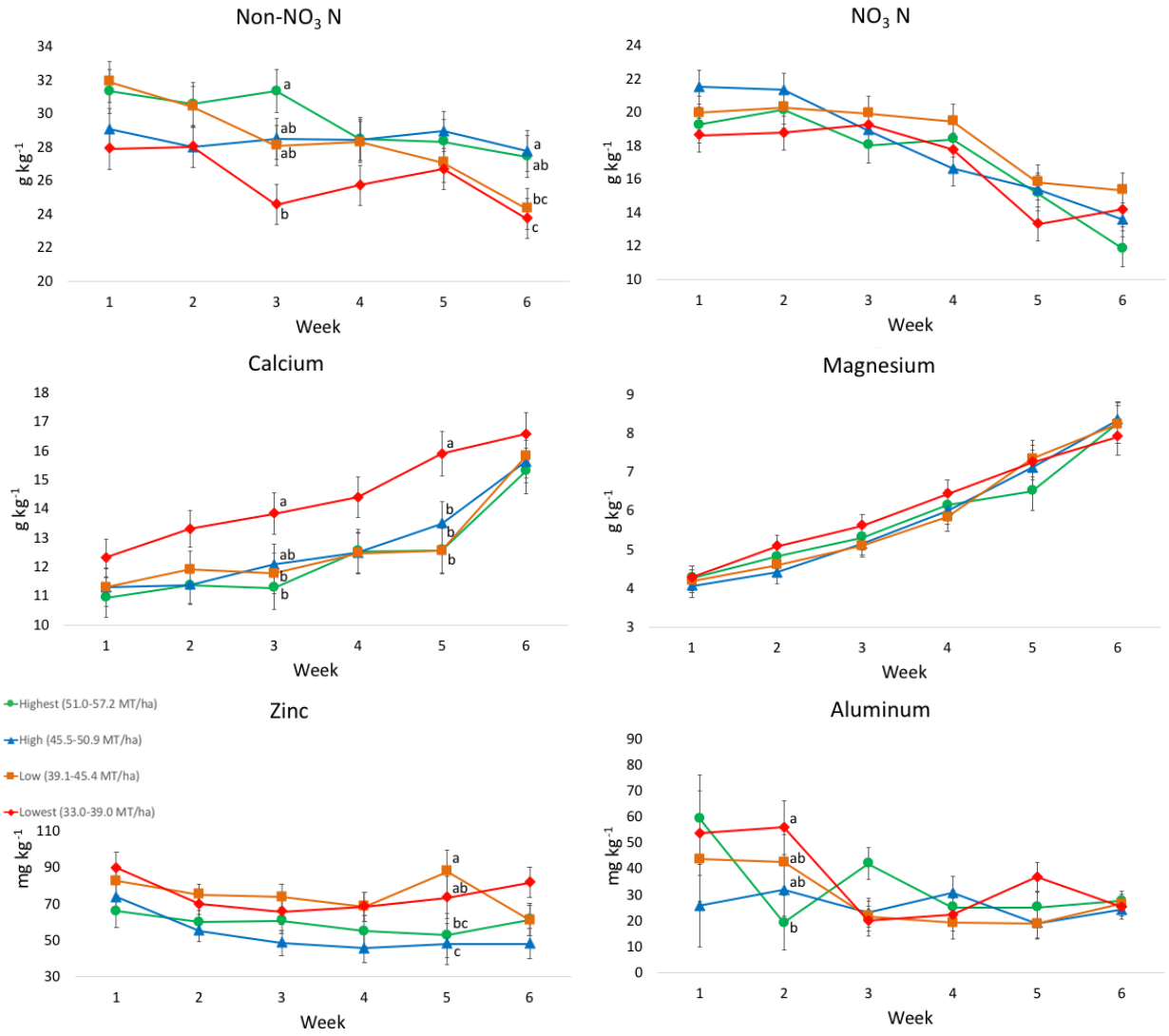

Figure 2-1. Elemental concentrations, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by yield grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between yield groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

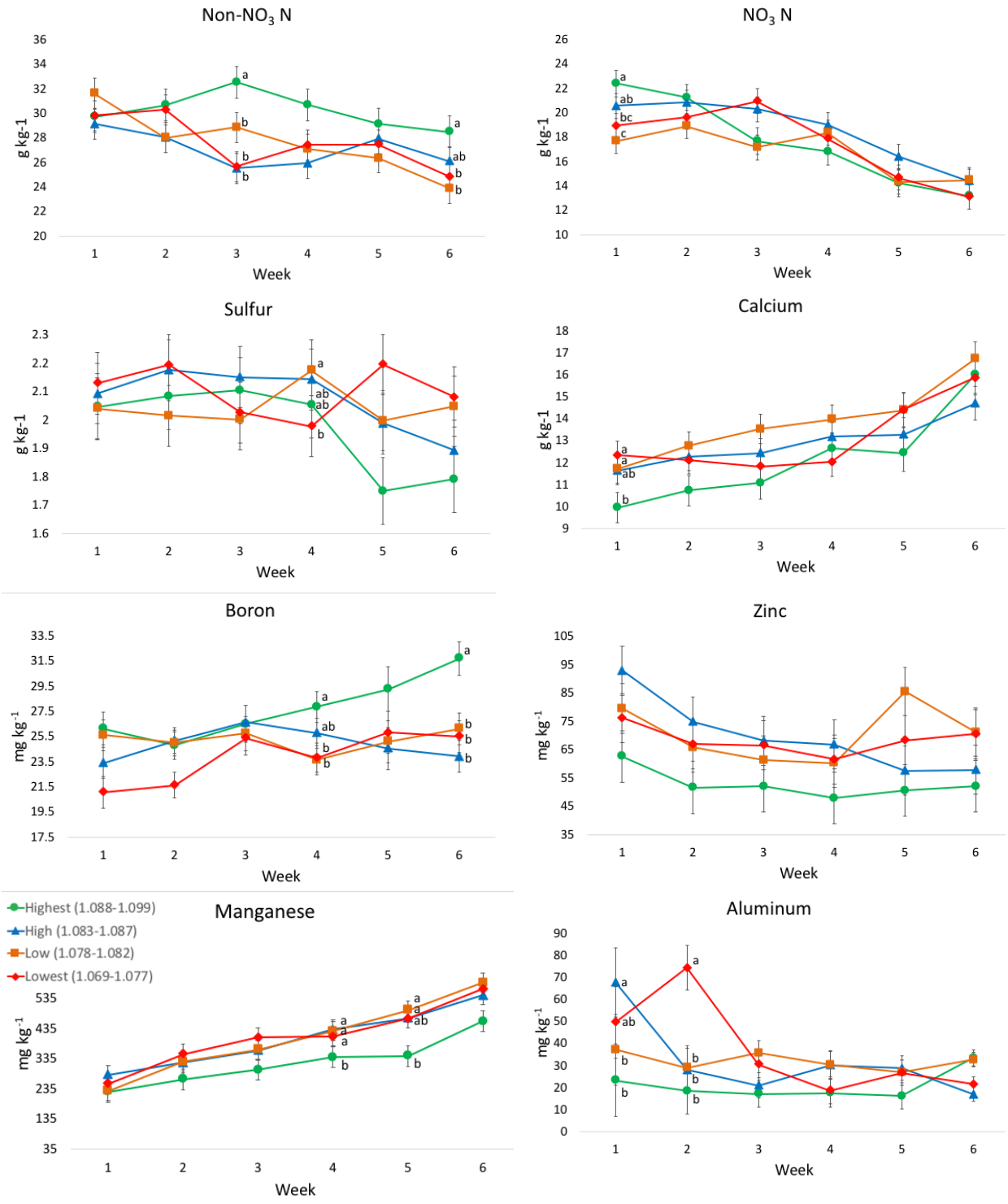

Figure 2-2. Elemental concentrations, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by specific gravity grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between yield groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

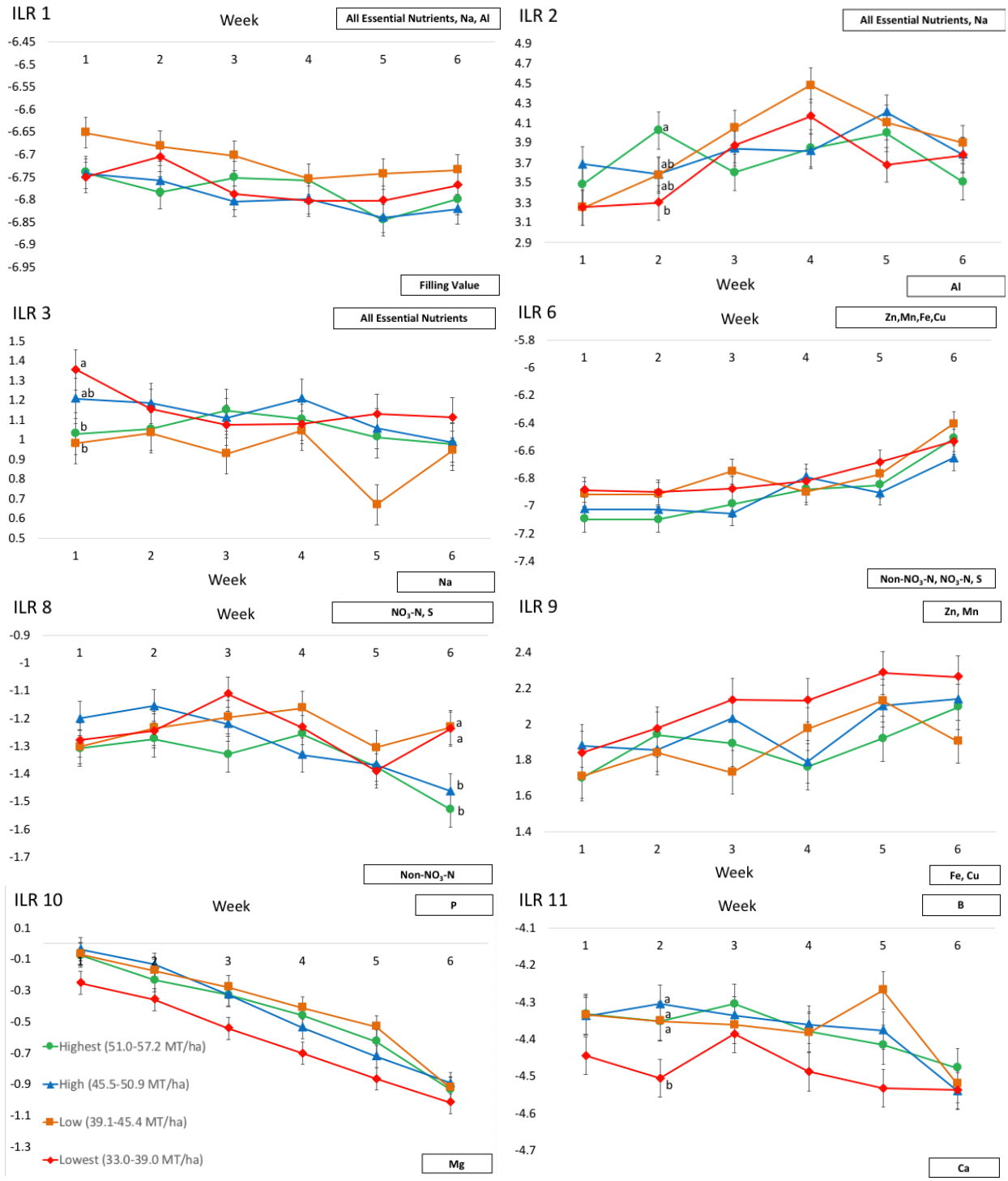

Figure 2-3. ILR transformed balance points, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by yield grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between yield groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

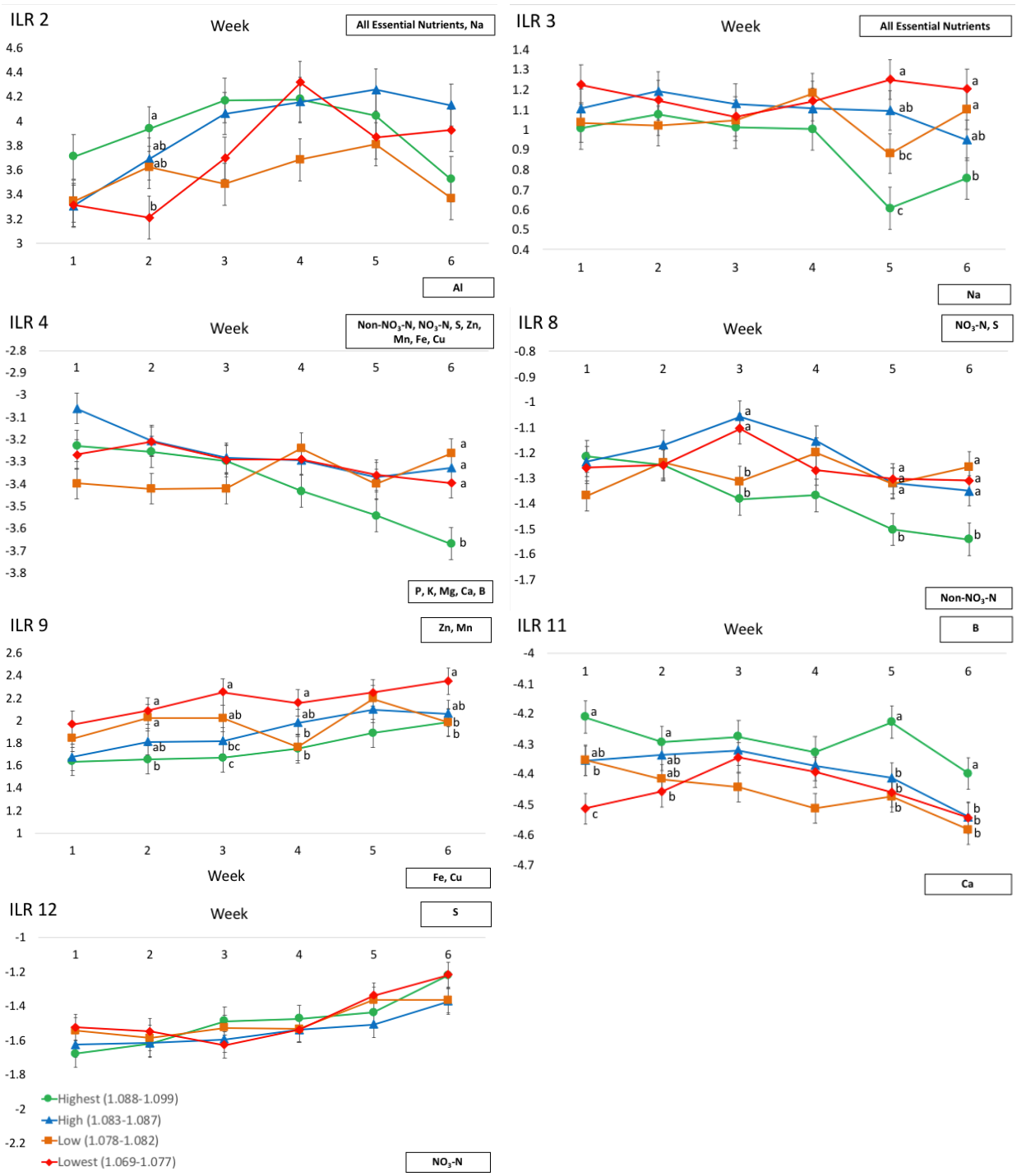

Figure 2-4. ILR transformed balance points, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by specific gravity grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between yield groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

2.8 Connecting paragraph

This chapter describes the many considerations for interpretation of plant petiole analysis in PEI. This chapter describes the relationship between total yield, specific gravity and the potato petiole tissues sampled across six weeks in the tuber bulking phase. Key nutrients involved in promoting high yield potential as well as those considered deleterious to yield outcomes were identified and discussed. In an effort to establish similar parameters for NB, Chapter 3 is an investigation into the dynamics between the petiole tissues and both tuber yield and specific gravity outcomes.

Chapter 3: Petiole nutrient concentrations and their temporal changes during tuber bulking phase as they relate to tuber yield and specific gravity in the Russet Burbank potato (*Solanum tuberosum* L.) grown in New Brunswick, Canada.

3.1 Abstract

The potato crop is a significant component for New Brunswick, Canadian, and global agriculture. Bulking phase potato petiole sampling is an existing practice in New Brunswick. The objective of this study was to identify the most relevant nutrients, correlated with tuber yield and specific gravity, across the tuber bulking phase. There were 39 commercial farms in New Brunswick, Canada during a five-year period from 2010 to 2014. Sampling occurred once per week for the six-week period from early to late tuber bulking and petioles were analyzed for NO_3-N, Non-NO_3-N, P, K, Mg, Ca, S, B, Zn, Mn, Fe, Cu, Na and Al. The data was transformed using Isometric log ratio (ILR) transformations and results were compared with the concentration data. The petiole nutrient data in week 5 were best correlated to tuber yield (r=-0.56 to 0.44, $P < 0.05$), while specific gravity was most associated with weeks 3 and 4 (r=-0.48 to 0.33, $P < 0.05$). The Ca, Mg, and B concentrations were most consistent markers of improved tuber yield where NO_3-N, S, P, and K were most consistent in negative correlation. Specific gravity outcomes were not positively correlated in a consistent manner with any nutrient however they were very consistent in their negative correlated with NO_3-N, S, P, and K. There were no improved correlations with tuber yield or specific gravity when ILR transformed data were compared with concentration data. The strong potential markers, of petiole NO_3-N, S, P, K, Ca, Mg, and B,

relative to grower yield and quality outcomes were identified in this study demonstrating that petiole tissue sampling during the bulking phase is advisable.

3.2 Introduction

Potato (*Solanum tuberosum* L.) production is inherently a nutrient intensive system in order to supply a high input crop with an adequate supply of nutrients for the entire growing season. A more regionally specific nutrient management system is needed to improve potato production in the decades to come. Understanding the relationship between nutrient requirements as they change over the season is helpful in order to improve the efficiency of both macro- and micro-nutrient fertilizer application (Westermann and Davis 1992). Grower relevant yield outcomes of tuber yield and specific gravity were compared with nutrient data in petiole tissues during the bulking phase in this study.

New Brunswick (NB) accounts for accounts for 13.7% of the total Canadian acreage under potato production (Statistics Canada 2016). The yield of NB potato growers are slightly below the Canadian average. From 2010-2014, tuber yields were 31.4 Mg ha^{-1} (Std. Deviation: 1.2 Mg ha^{-1}) in NB, which was 0.6% lower than the national average of 31.6 Mg ha^{-1}. For the average NB crop these removal rates are estimated to be: 153 kg N ha^{-1}, 32 kg P_2O_5 ha^{-1} and 209 kg K_2O ha^{-1} (Government of NB 2009) which is considered a high nutrient demand crops especially as compared with cereal crops and most vegetable crops grown in this region. The plant tops require another estimated 65 kg N ha^{-1}, 16 kg P_2O_5 ha^{-1} and 97 kg K_2O ha^{-1} for proper development of the vegetative portions of the plans (leaves and stems) (IPNI 2017).

Predictive management tools such as tissue sampling offer a method for determining consistent markers for improved yield and specific gravity outcomes. Some of the challenges in developing strategies for improved fertilization practices come from less knowledge of the nutritional requirements by potato cultivars (Westermann and Davis 1992). The petiole of the most recently mature (MRML) leaf is often sampled in order to determine nutrient sufficiency in the potato crop (Walworth and Muniz 1993; Reis and Monnerat 2000; Stark 2004). This organ is selected due to the fact that it contains recently absorbed macro- and micro-nutrients that are being transported to the growing points (Ewing and Wareing 1978). The petiole is also responsible for remobilization of phyotosynthates, during the tuber bulking stages, from source leaves to tubers (sink) (Setter et al. 1980; Krapp et al. 1993). Several reports from NB exist relating fertilizer application rates to nutrient concentrations in the petiole (Bélanger et al. 2000; Zebarth et al. 2002; Zebarth et al. 2007).

Potato yields were reported to positively responded respond to increasing levels of N fertilization in NB (Bélanger et al. 2000) however specific gravity follows the opposite trend (Zebarth et al. 2007). Local provincial extensions publications recommend the use of petiole sampling for NO_3 testing (Zebarth et al. 2007) for determining deficiency and excess N fertilization. There are also examples in the nearby province of PEI whereby Russet Burbank potato yields responded to P fertilization and petiole P concentrations generally followed the rate of application in a linear manner (Sanderson et al. 2002). There was no similar relationship reported in relation to specific gravity. Higher N and K fertilizer rates improved Russet Burbank tuber yields on PEI and reduced specific gravity outcomes (White et al. 1974). Petiole N and K concentrations in petioles were increased with N application and higher rates of K fertilizer reduced the N concentration and increased the K concentration in petioles (White et al. 1974).

Although most of the local research has focused on N management (Zebarth et al. 2004; Zebarth et al. 2007; Sharifi et al. 2008; Zebarth et al. 2012) there is an opportunity to assess many different nutrient concentrations within the petiole tissues.

The concentration of nutrients in petiole tissues can be used to assess a relationship between many different elements and the grower relevant yield components. Walworth and Sumner also proposed the alternative of following the proportional accumulation of nutrient data. Interpreting the proportional accumulation of nutrients is intuitively helpful as plants develop and grow based on the balanced supply of essential elements maintained by homeostatic mechanisms (Williams and Salt 2009). The use of transformations in improving the interpretation of tissue samples is commonly used in agriculture (Beaufils 1973; Parent et al. 1994). Parent et al. (2013) reported improved success in classification using both log and ILR transformations when compared with raw concentrations. The DRIS method is commonly used by agronomists when evaluating proportional accumulation of nutrients but with this method one generates many different ratios that can be challenging to interpret (Parent et al. 2013). From 14 nutrient concentrations one could generate 91 dual ratios with which to interpret the potato petiole sample. The ILR transformations, created by compositional data analysts, are log-ratio transformations made to generate scale-invariant variables, to avoid any redundancy in the data, which are expressed in real space (Aithchison 1986; Egozcue et al. 2003).

The objective of this study was to correlate the ionome, both raw concentrations and ILR transformed data through petiole sampling during six weeks of tuber bulking, to total yield and specific gravity outcomes for Russet Burbank potato fields in NB, Canada. The primary hypothesis of the study was that the total yield and specific gravity results are correlated to petiole nutrient concentrations and ILR transformed data. The ILR transformed data is also

hypothesized to be more correlated to yield and specific gravity outcomes than the raw concentrations. Macronutrient correlations to yield and specific gravity outcomes are also hypothesized to be more likely than micronutrients. Bélanger et al. (2000) and Rees et al. (2014) reported locally that petiole N concentrations increased with increased N fertilization. Within the context of the consistent regional work on N management in potato cropping systems (Zebarth et al. 2004; Zebarth et al. 2007; Sharifi et al. 2008; Zebarth et al. 2012) it was hypothesized that petiole NO_3 concentrations and the protein system (ILR 8), associated with N dynamics, are most likely to be positively correlated to total tuber yields and negatively correlated to specific gravity outcomes.

3.3 Materials and Methods

3.3.1 Study fields

Non-irrigated potato (cv. Russet Burbank) fields (39) were selected from a commercial farm survey monitored and compiled by NutriAg Ltd. (Toronto, Canada) over 5 years from 2010 to 2014. All fields were located on the province of New Brunswick focused around the Grand Falls (47° 02'04" N 67° 44'22" W) region of the province in Madawaska country. All fields selected were on extremely well drained orthic humo-ferric podzols classed as Grand Falls soil series characterized by glaciofluvial deposition of acidic medium to coarse textured gravely loam to gravely sandy loam soils (Fahmy et al. 2010). This region is considered to have a cool temperate climate with a 30-year normal yearly daily mean temperature of 3.7 °C with a mean daily temperature of 8.1 °C (Environment Canada, 2010) during the May-October months associated with potato production. The 30-year normal average yearly rainfall in Grand Falls is 1142 mm (Environment Canada, 2010). Land used for potato production adds up to over 20, 000 hectares

of production with more than half destined for processing. Potatoes also represent the largest agricultural commodity in terms of value (AAFC, 2012). Planting densities varied from 32 894 plants ha^{-1} (0.40 m in row x 0.76 m between row spacing) to 34 632 plants ha^{-1} (0.38 m in row x 0.76 m between row spacing).

3.3.2 General Fertilization Strategies

The selected fields in the study used the same fertilizer and lime application strategies across the growers. All fields saw nitrogen applications in the forms of ammonium nitrate (AN; 34-0-0), Urea (46-0-0), mono-ammonium phosphate (MAP; 11-52-0), and/or di-ammonium phosphate (DAP;18-46-0) at a rate of 200-224 kg N ha^{-1}. The application of phosphorus was achieved by DAP or MAP at a rate of 168-224 kg P_2O_5 ha^{-1} depending on the P fertilizer recommendation from the soil test (Mehlich-3 P concentration). Applications of K were made through muriate of potash (MOP; 0-0-60), and/or a mix containing potassium magnesium sulfate (K-Mag; 0-0-22 11 Mg) and/or MOP (0-0-50). A base saturation of 5% K_2O in all fields was targeted based on the Mehlich-3 K concentration in the soil test, with fields receiving a minimum application of 245 kg K_2O ha^{-1} to a maximum application limit of 340 kg K_2O ha^{-1}. For Mg a target base saturation of 10% Mehlich-3 Mg was used to determine Mg application. With N- P_2O_5- K_2O application rates of 200-224-(245-367) kg ha^{-1} these fields should be considered to have received heavy macronutrient fertilizer application rates and deficiencies in these nutrients are therefore not considered likely. All growers applied fall or spring lime applications to target soil pH of 5.8-6.2 with fields receiving lime applications of 2.5 Mg ha^{-1} when the soil test reports reported a pH_{H2O} value < 5.7. Lime applications to remediate acidic soils were based on dolomitic/calcitic lime blends containing 5-12% Mg and 22-32% Ca, or pure calcitic lime (0.5% Mg, 36% Ca). In the

few cases where Mehlich-3 extractable Ca concentration in the soil test were below 900 ppm but pH_{H2O} >5.7, the field were amended with 2.5 Mg ha^{-1} of gypsum (29% Ca, 23% S).

3.3.3 Petiole Sample Collection and Analysis

Tissues of petioles were sampled from an area deemed representative of the field (4.5 m x 10 m section containing 148-155 plants) in all commercial potato field selected from the survey group. Sampling occurred when plants reached the bulking phase (tuber diameter >25mm) approximately 60 days after planting and continued for six subsequent weeks from that date. The petioles analyzed came from 40-60 randomly-selected plants collected once a week. The sampling process was initiated on 30 Jul 2010, 27 Jul 2011, 24 Jul 2012, 22 Jul 2013, and 27 Jul 2014. Only single stems were sampled per plant leaving several un-sampled stems per plant for future sampling. Petioles were combined and sent for analysis to A&L Laboratories (London, Canada) where they were dried (70 °C) and ground for analysis using a Wiley Mill. A Leco FP-628 CNS (LECO Corp., St. Joseph, MI, USA) was used to determine total N. Concentration of NO_3-N were reported using a FIALab-2600 autoanalyzer (FIALab, Bellevue, King, USA). A calculated value for Non- NO_3-N concentration were created by reporting the value of a subtraction of total N by NO_3 concentrations. Nitric acid/ hydrochloric acid were used for the tissue digestion process and the P, K, Mg, Ca, S, B, Zn, Mn, Fe, Cu, Na and Al concentrations were reported by ICP-OES using a Thermo Fisher ICAP 6300 (Helmut-Fischer, Sindelfingen-Maichingen, Germany). The concentrations of macro-nutrient were expressed in g kg^{-1} petiole tissue (dry mass), whereby the micro-nutrient concentrations were reported as mg kg^{-1} petiole tissue (dry mass).

3.3.4 Yield and Specific Gravity Estimation

The total yield values for a given field were extrapolated based off hand harvesting three single rows (3 m long), randomly selected from a quadrant of no more than 0.25 ha centered on and within the radius of the area of petiole sampling. All harvest sampling was undertaken during a 2 wk period and samples obtained from each site were stored at (2 °C ± 3°C) in cold storage until all sites were harvested. Tubers were weighed and total tuber yield was expressed on a Mg ha^{-1} basis. Tubers >50 mm in size were randomly selected and washed in clean water to make a composite sample weighing 3.6 kg which was subsequently used for specific gravity estimation. The water immersion method using a hydrometer was used to determine mean tuber specific gravity of the composite sample. The tuber samples were attached to the hydrometer and lowered into a 200 L barrel filled ¾ full of water. The mean tuber specific gravity was reported based on the hydrometer reading after the tuber sample was allowed to come to rest after submersion in water.

3.3.5 Proportional nutrient accumulation as ILRs

In this study the SBP (Table 2-1) selected was established to partition elements based on their physiological role within the plant as is required for the ILR transformations. Elements were arranged into five groups: protein synthesis (NO3-N, Non NO3-N, and S), short term energy storage/transfer (P, Mg), cellular structure (K, Ca, B), enzyme activation and electron transport chain system (Zn, Mn, Fe, and Cu), and deleterious (Al, Na). The system denoting protein was established in order to examine the balance between feedstocks critical to the production of reduced forms of N (Hesse and Hoefgen, 2003; Nikiforova, 2003; Saito 2004). There is a previously described (Dennis and Coultate, 1966; Kirby and Mengel, 1975) relationship between

Mg/ATP system critical to short term energy storage/transfer reactions within the plant cell. Both Ca and B are both found primarily in the cell walls of higher plants (Matoh and Kobayashi 1998; Hepler and Winship 2010) and a synergistic relationship between these two elements and K has been reported in tomato plants (Reeve and Shive 1943). The proportions of these three elements are reflective of the balance of the cellular structure system. As the electron transport chain is made up of many metallo-protein systems embedded with Zn, Mn, Fe, and Cu it is sensitive to the balance of these elements (Guskov et al. 2009; Jianrong and Qiran, 2009). Despite not being plant required many tissue sampling laboratories report the concentrations of both Na and Al and combined these elements make up the balance of the deleterious elements which can both accumulate in tissues but also have negative effects on crop growth.

3.3.6 Data Processing and Statistical Analysis

Microsoft Excel was used to generate ILR transformations with the correlation analyses being undertaken using JMP 13.0 (SAS Institute Inc, Cary, NC). The mean concentration differences between different yield outcome groups were compared by taking the total tuber yields for all site-years and dividing them into quartiles: 21.2 - 35.7 Mg ha^{-1} (n=10), 35.8 – 41.5 Mg ha^{-1} (n=10), 41.2-46.7 Mg ha^{-1} (n=10), and 46.8-57.4 Mg ha^{-1} (n=9). The same methodology was applied to separate specific gravity groupings into quartiles: The mean elemental concentration in petiole tissue, by week, was associated with one of four specific gravity groups (1.064-1.084 (n=10), 1.085 – 1.088 (n=10), 1.089 – 1.091 (n=10) and 1.091-1.099 (n=9). This non-binary separation is used in other agricultural commodities (Buza et al. 2014) and is important in order to reflect grower relevant yield parameters such as specific gravity that have a sliding scale of value in a processing contract.

The isometric log ratios (ILR, Egozcue et al. 2003) were computed with the ionome concentration data by the sequential binary partition (SBP) selected. The SBP (Table 2-1) were (D-1) x D matrix whereby the group numerators were labeled +1 and group denominators were labeled -1 whilst elements, which were not reflected in a given balance, were given a value of 0. Total yield and specific gravity outcomes were compared to tissue elemental concentrations (non-NO_3-N, NO3-N, P, K, Mg, Ca, S, B, Zn, Mn, Fe, Cu, Na, and Al) and ILRs 1-14 using correlation analysis. Differences in tissue elemental concentrations and ILRs between quartiles for total yield and specific gravity and across 6 sampling dates were analyzed using repeated measures ANOVA. Any ANOVA reporting significant effects were identified and the differences between groups were evaluated using Least Squares Means (LSMEANS) at α=0.05. Multiple means comparisons and ANOVA were undertaken using the GLIMMIX procedure in SAS 9.4 (SAS Institute Inc, Cary, NC).

3.4 Results and Discussion

3.4.1 Nutrient Concentration Relationships with Tuber Yield

Tuber yields for the grower database averaged 42.5 Mg ha^{-1} (Std. Deviation: 8.1 Mg ha^{-1}) which is 34% higher than the provincial average. The higher values are not surprising as the Russet Burbank cultivar of potato is a long season variety whereas provincial averages include many lower yielding potato cultivars. Many elements in the petiole samples collected during the tuber bulking period were significantly (P <0.05) correlated, mostly negatively, with tuber yield. Mg, Ca, B, and Mn were positively (P < 0.05), while NO_3-N, S, P, K, Na, and Al were negatively correlated (P < 0.05) with total yield (Table 2-2). More significant correlations (P < 0.05) of nutrient concentrations with total yield occurred in week 5 (Table 2-2). It is quite surprising that this later bulking period was most significantly associated with total yield as this timing is much

later than the typical recommendations for potato sampling in NB (Agriculture and Aquaculture NB 2013). There was a consistency between the data and the primary hypothesis that total yield would both be correlated with petiole nutrient concentrations, and more correlated than specific gravity outcomes. The secondary hypothesis that that macronutrient concentrations would be more significantly correlated than the micronutrient concentrations was also confirmed.

There was a significant (P <0.05) positive correlation between both petiole Ca and Mg concentrations in relation to tuber yields which was most significant in the second half of the sampling period. These two elements are known to have an antagonistic relationship, in the soil, with K applications (Bower and Pierre 1944; Jakobsen 1993). The significant (P <0.05) negative correlations with petiole K concentrations and tuber yield, during all weeks of sampling, may be further indicative of this antagonism. Previous studies on potato have also reported reduced distribution of Ca and Mg to leaves under increasing rates of K (Addiscott 1974) however studies on petiole K concentrations have shown generally positive correlations with tuber yield (Westermann 1994). These combined correlations, both negative and positive, would suggest that potassium application within this grower group may be excessive and should be further investigated. A range of excess in petiole NO_3 has been reported in local extension publications (Zebarth et al. 2007), however this would be the first suggestion that excess K concentration for petiole tissues may be similarly adapted in NB.

The concentrations of B in petiole tissues were positively correlated (P < 0.05) with tuber yields in later tuber bulking sample timings. The highest tuber yield grouping also had the highest mean B concentrations at week 3 and 4 of sampling (Figure 2). This is the first report of a positive relationship between petiole B and tuber yields for NB. Regional extension publications do recommend foliar applications of 2 kg ha^{-1}, in low organic matter soils and those

affected by drought, (Government of NB 2018) for reducing brown heart and water core. These provincial recommendations are based on visual symptom recognition suggesting that leaflets with marginal scorching and curling denote deficient B conditions. Our research would suggest that a petiole B concentrations could be adapted regionally for improved deficiency detection with further experimentation although the values are likely to be higher than those reported by other regions (Walworth and Muniz 1993; Stark et al. 2004). A transient positive correlation ($P < 0.05$) between Mn concentrations and tuber yield was also reported for the last week of sampling however it is not consistent enough consideration.

The correlations between macronutrients and tuber yields were predominant over micronutrients which is consistent with the original hypothesis however an overwhelming majority of these were unexpectedly negative. The original hypothesis that NO_3 would be most positively related to tuber yield was inconsistent with the reported data. The negative correlations between petiole NO_3 concentrations and tuber yields were consistent through all weeks of sampling and the lowest yield grouping had the highest mean concentrations in 4 of 6 weeks of sampling (Fig 1). Although Zebarth et al. (2007) does provide guidelines for excessive petiole NO_3 concentrations these are not presented in the context of decreased yield outcomes for growers. It is important to note that no correlation between Non-NO_3 concentrations and tuber yields suggesting that the practice of partitioning N in petiole analyses is worth continuing.

The concentrations of P and S in petiole tissues were also reported to have consistent negative correlations) with tuber yields. The lowest yield groupings also had the highest mean concentrations of P for all weeks, and S for the last two weeks of sampling (Fig 1). This would be the first report of excessive levels of P and S in petiole tissues in NB, and these findings are inconsistent with previous local reports of improved crop responses to the application of these

two nutrients (Gupta and Sanderson; Maier et al. 2007) and also with data from other potato production zones (Dubetz and Bole 1975). The concentrations of Na, Fe, and Al were also negatively correlated ($P < 0.05$) to tuber yields however not for more than 2 weeks for any given element. The deleterious nature of Na concentrations have been reported for potato before (Rengasamy 2010; Kronzucker and Britto 2011) however the source of Na in NB is not abundantly clear due to the distance from the ocean and the lack of manure incorporation in this dataset. The negative relationship between Fe, Al and tuber yields could be related to soil pH however they are too transient for serious consideration.

3.4.2 Nutrient Concentration Relationships with Specific Gravity

Tuber specific gravity values averaged 1.087 (Std. Deviation: 0.006) which is considered a good value for processing contracts. Specific gravity was positively correlated ($P < 0.05$) with the Ca, Na and B concentrations in petiole samples however not one variable was significant for more than a single week (Table 2-3). There were far more negative correlations ($P < 0.05$) between specific gravity and the elements reported, namely Non-NO_3-N, NO_3-N, S, P, K, Zn, and Cu (Table 2-3). Overall, the data were consistent with the hypothesis that specific gravity would be associated with petiole nutrient concentrations although the secondary hypothesis that macronutrient concentrations would be more significantly correlated ($P < 0.05$) than micronutrients were inconsistent with the results. The most number of significant correlations ($P < 0.05$) for nutrient concentrations and specific occurred in weeks 3 and 4 (Table 2-2). For Ca, Na, and B the positive correlation ($P < 0.05$) with specific gravity was reported for no more than one week and therefore these relationships were transient and inconsistent. A similar positive relationship was denoted for tuber yield in the case of Ca and B which further highlights the importance of these two elements in NB potato production. The temporary and confusing

positive correlation ($P < 0.05$) between Na and specific gravity outcomes is too inconsistent for interpretation.

The hypothesis that petiole NO_3-N and specific gravity are likely to be negatively correlated ($P < 0.05$) are consistent with the data. The NO_3-N, K, S, and P concentrations were negatively correlated ($P < 0.05$) with negative specific gravity outcomes in 4 of 6 weeks of sampling. The reports by Zebarth et al. (2007) of excessive petiole NO_3-N at >20 g kg^{-1} during the bulking phase (>70 days after planting) are consistent with the concentrations of NO_3-N of the lowest specific gravity group in week 5 and 6 of sampling (Figure 3). The concentrations of Non-NO_3-N were also negatively correlated ($P < 0.05$) with specific gravity, for the last 2 weeks of sampling, suggesting that the relationship between low specific gravities and N is not exclusively captured by observing NO_3-N alone. The deleterious nature of over fertilization of N in respect to specific gravity has been well explored in NB and this study is consistent these reports. The deleterious nature of excessive K applications in relation to specific gravity has not been documented in NB but has been reported in several other potato producing regions (Teich and Menzies 1964; Westermann 1994; Maier et al. 1994). Further investigation into the optimal rates of K fertilization in NB are recommended.

The correlations ($P < 0.05$) between P application and reduced specific gravity are inconsistent with the literature (Dubetz and Bole 1975; Maier et al. 1994). It should also be noted that recent studies showed that concentrations of P are not always consistent with increasing application rates of P especially beyond the 60 days after planting stage (Zamuner et al. 2016). The concentration of P in tissues decreases over time in all specific gravity and groups (Figure 5) which is also consistent with observations made by Zamuner et al. (2016) and it is therefore possible that the P concentrations are more of an indicator of physiological maturity than P status

within the plant. More investigation into the relationship between P, physiological maturity and specific gravity is recommended. The concentrations of S found in petiole tissues were negatively correlated ($P < 0.05$) in the last four sampling times. This would be the first documented case of lower specific gravity outcomes being associated locally higher petiole S concentrations. This is inconsistent with the literature which suggests either no effects from S fertilization on specific gravity (Gausman et al. 1963; Pavlista 2005) or positive effects (Sharma 2011). Further investigation into the ideal incorporation rate of S fertilizers in NB potato production systems is recommended.

The concentrations of Zn had the strongest negative correlations ($P < 0.05$) with lower specific gravity outcomes (Table 2-3) albeit the relationship was only statistically significant for the first two weeks of sampling. Sanderson and Gupta (1990) report on Zn amendments that reduced the total yield for Russet Burbank potatoes grown in PEI, however no effects were reported on specific gravity. Soil applications of Zn also increased leaf Zn concentrations in early sampling time although petiole tissues were not evaluated. The concentrations of Cu were also negatively correlated to specific gravity outcomes ($P < 0.05$) although for only one week of sampling therefore it is too transient and inconsistent for proper interpretation.

3.4.3 ILR Relationships to Tuber Yield

There was roughly the same amount of negative correlations ($P < 0.05$) than positive in the ILR transformed data as related to yield. However, when one is interpreting the ILR transformed data, a negative correlation indicates a movement of the equilibrium between nutrient groups and as a result negative correlation was not truly reporting a correlation between a given nutrient and yields but more importantly between the balance of two or more nutrients. The hypothesis that

ILR transformed data was more correlated ($P < 0.05$) to tuber yield than concentrations was not consistent with the results. Tuber yield was correlated ($P < 0.05$) with the ILR 1, ILR 3, ILR 4, ILR 5, ILR 6, ILR 7, ILR 8, ILR 10, and ILR 11 (Table 2-2). The most number of ILR transformed values correlated ($P < 0.05$) with tuber yield occurred in weeks 3, 5, and 6 (Table 2-2). The tuber yields were correlated ($P < 0.05$) with ILR 8, for the last 4 weeks of sampling, which is consistent with the hypothesis that balance points associated with N would be most correlated to yield.

The transformation of ILR 1 suggests that the overall pattern of nutrient accumulation in petiole tissues is negatively correlated ($P < 0.05$) with tuber yield for three weeks of sampling (Table 2-2). When considering the multiple negative correlations ($P < 0.05$) with macronutrients reported it is unsurprising that this would be the case. Heard (2004) reported the accumulation patterns of Russet Burbank potatoes and higher overall petiole nutrient density may be a marker for a field that was not as physiologically developed. The deleterious effect of delayed physiological maturity is a potential explanation for the relationship between ILR 1 and tube yield. The interpretation of this balance point could lead the reader to consider all macronutrient accumulations as deleterious however this was not the case and both positive and negative correlations have been reported. The highest yield grouping had the highest value for ILR 2 (Figure 3) at week 2 of sampling. It is unsurprising that the accumulation of Na is negatively correlated ($P < 0.05$) with tuber yields as potatoes have been reported to be sensitive to Na accumulation (Hütsch et al. 2017) however the relationship was too transient to be consistently reliable.

There was a negative correlation ($P < 0.05$) between ILR 4 and tuber yield for weeks 3, 4, and 5 of sampling. The ILR 4 balance point describes the accumulation of several groupings,

namely the combined protein and electron transport groups as compared with the energy transfer and structural groups. This balance point would suggest that the accumulation of elements associated with both the combined protein and electron transport groups was associated with poor yield outcomes. This is not surprising in the context of the nutrient concentration data, as the most positively correlated ($P < 0.05$) nutrients at this timing are Ca, Mg, and B which are on the opposite side of the balance. This, multi group, transformed ILR balance point was ambiguous as to which of the nutrients are important without the context of the nutrient concentration data.

There was a consistent positive correlation ($P < 0.05$) between ILR 5 and tuber yield for the last 5 weeks of sampling. This is consistent with the reported deleterious yield marker of K accumulation during all sampling weeks although it should be noted that P is found on the opposing side of the ILR 5 balance. Considering the negative correlations ($P < 0.05$) reported for petiole P concentrations and tuber yield this may suggest that the avoiding the accumulation of K is more important. However, there is the danger of an implicit suggestion that P application may also be favourable at these timings when observing the ILR 5 balance point without the concentration data. The ILR 6 balance point of the protein system as compared with the electron transport system has only a single transient week of significance and is therefore too inconsistent to interpret.

The description of ILR 7 as a balance point between the accumulation of the structural system as compared with the energy transfer system is positively correlated ($P < 0.05$) to tuber yield for 4 of 6 weeks of sampling. The lowest yield group had the lowest value for ILR 7 in week 4 and 5 of sampling (Figure 3) which is not surprising when considering both the positive correlation reported for Ca and B and the negative correlations reported for P at those timings.

The ILR 8 balance point describes the balance of nutrients within the protein system and it was negatively correlated ($P < 0.05$) to tuber yield for the last 4 weeks of sampling. This is consistent with the hypothesis that ILR balance points associated with N dynamics, namely the protein system, would be most correlated to tuber yield. The negative association with tuber yield and NO3-N and S accumulation are consistent with the results and observations made on these nutrient concentrations. The interpretation of ILRs 7 and 8 may be consistent with the concentration observations however these two balance points one could lead the observer to miss the positive relationship at week 5 between Mg and tuber yield, and assume a multi-week positive trend between Non-NO_3-N accumulation and tuber yield even when one does not exist.

The most consistently correlated ($P < 0.05$) balance point was ILR 10 which was negatively associated with tuber yield in all sampling weeks. This balance represents the relative accumulation of P and Mg in the energy transfer system. The consistent negative relationship is consistent with the tuber yield data which showed positive outcome associated with Mg and negative outcome associated with P. The ILR 11 was negatively correlated ($P < 0.05$) with tuber yield in only one week of sampling which is quite surprising as it denotes the balance of Ca and B in the structural system and both of these nutrients were positively correlated ($P < 0.05$) with tuber yield especially in week 4 and 5. The positive association between Ca, B and tuber yield were missed by ILR 11 because they are both positively related to tuber yield at this timing. It appears that, due to the nature of the SBP, ILR 11 is only relevant in week 6 where the ILR is correlated ($P < 0.05$) although B and Ca are not. This further demonstrates the potential pitfalls of interpretations of transformed data without the context of the concentrations. The ILR 12, which represents the balance point between NO_3-N and S in the protein system, was positively associated with tuber yield suggesting the accumulation of NO_3-N in the absence of S is most

deleterious. The negative correlation ($P < 0.05$) between S concentrations in the petiole tissue is not fully captured by this balance and accumulation of S in petiole tissues is related to deleterious tuber yield effects despite the information provided by ILR 12.

3.4.4 ILR Relationships to Specific Gravity

There were correlations ($P < 0.05$) between specific gravity and the transformed balance points of ILR 1, ILR 3, ILR 4, ILR 5, ILR 6, ILR 7, ILR 8, ILR 9, ILR 10, ILR 11, ILR 12 and ILR 14 (Table 2-2) although half of these were for no more than one week of sampling. The most number of ILR transformed values correlated ($P < 0.05$) with specific gravity were reported in weeks 4 (Table 2-2). The hypothesis that ILR transformed data was more related to specific gravity than concentrations was inconsistent with the results. It was also clear that the ionome data was less associated to specific gravity outcomes than to tuber yield which is consistent with the original hypothesis of this study.

The ILR 4 balance point were negatively correlated ($P < 0.05$) to specific gravity for half of the sampling weeks. This was consistent with the relationship reported for tuber yield and it denoted the role of Ca and B as markers for improved specific gravity. However, it also could erroneously lead an observer to consider P, and K as positively correlated ($P < 0.05$) to specific gravity outcomes when they were not. As was evident by this ILR balance points, multi nutrient balances were difficult to interpret and can lead to erroneous interpretations. The ILR 5 and 7 balance points were both positively correlated ($P < 0.05$) with specific gravity outcomes. It should be noted that these balance points were less likely to be misinterpreted as the general accumulation of Ca and B in petiole tissues was positively correlated ($P < 0.05$) to specific gravity as previously reported. It was more appropriate that these balance points denote the previously reported deleterious relationship between K accumulation, in the case of ILR 5, and P

in the case of ILR 7. The ILR 14 balance point was positively correlated ($P < 0.05$) with specific gravity outcomes for the first three weeks of sampling and in the context of the reported significant negative relationship between Zn and specific gravity outcomes this was not surprising. This balance point should be interpreted in the context of the negative marker of Zn accumulation and not assign a positive relationship between Mn accumulation and specific gravity. There were several more inconsistent correlations ($P < 0.05$) between specific gravity and ILR balance points 1,3 6, 8, 9, 10, 11, 12 suggesting that the majority of the relationships were transient.

3.5 Conclusions

Over the six sampling weeks several nutrients present in petioles were associated with potato both tuber yield and specific gravity. In the context of both nutrient concentrations and ILR transformations the only consistent positive associations came from Mg, Ca, B accumulation which applied to both tuber yield and specific gravity outcomes. Most notable and numerous were the negative correlations ($P < 0.05$) with tuber yield which for both concentration and ILR transformations focused around the accumulation of NO_3-N, S, P, K, Na, and Fe. The specific gravity outcomes were also mostly associated with negative correlations ($P < 0.05$) of both concentrations and ILR balance points associated with Non-NO_3-N, NO_3-N, S, P, K, Zn and Cu.

There was a much stronger relationships between nutrients and tuber yield outcomes than specific gravity although the deleterious pattern of macronutrient accumulation was generally true across both metrics. No improved interpretation was reported by transforming nutrient concentrations into ILR values. In many cases the single balance points used for the interpretation of several nutrients was far more confusing than interpreting the single element concentrations. Several examples were identified where one could be lead to an incorrect

interpretation about the benefits of nutrient accumulation patterns in potato petioles. The ILR transformed data represent an interesting opportunity to derive a holistic perspective on plant tissues however it is strongly suggested that any interpretation be made in the context of the original concentration data. Overall, the identification of specific nutrient correlations with grower relevant markers opens up the possibilities to evaluate novel fertilizer amendment strategies which were not previously identified.

The importance of N in tuber yield and specific gravity outcomes was consistent with the original hypothesis. The negative correlation ($P < 0.05$) between specific gravity parameters and N accumulation was expected however the same relationship relative to tuber yield was not. The petiole data would strongly suggest that the general accumulation of macronutrients in petiole data is deleterious for both tuber yield and specific gravity which was unusual and inconsistent with previous reports. The positive relationship between Ca, Mg, B and grower relevant yield metrics would suggest that further investigation into the role of these nutrients in potato production is warranted. It is very likely that the availability of Ca and Mg are linked to that of K and further investigation into the relationship of these three nutrients in crop production is warranted. The general recommendation for B amendment in NB is based on a visual deficiency determination whereby the data reported in this study would suggest a grower relevant ideal value for B in petiole tissues exists locally. It is recommended that the practice of visual deficiency assessment is replaced by petiole sampling, although further work is required to identify ideal application rates.

3.6 Tables

Table 3-1. Sequential Binary Partition of the potato petiole ionome on NB.

	Non-NO_3-N	NO_3-N	S	P	K	Mg	Ca	Na	B	Zn	Mn	Fe	Cu	Al	Fv
ILR 1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-1
ILR 2	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	0
ILR 3	1	1	1	1	1	1	1	-1	1	1	1	1	1	0	0
ILR 4	1	1	1	-1	-1	-1	-1	0	-1	1	1	1	1	0	0
ILR 5	0	0	0	1	-1	1	1	0	1	0	0	0	0	0	0
ILR 6	-1	-1	-1	0	0	0	0	0	0	1	1	1	1	0	0
ILR 7	0	0	0	-1	0	-1	1	0	1	0	0	0	0	0	0
ILR 8	-1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
ILR 9	0	0	0	0	0	0	0	0	0	1	1	-1	-1	0	0
ILR 10	0	0	0	1	0	-1	0	0	0	0	0	0	0	0	0
ILR 11	0	0	0	0	0	0	-1	0	1	0	0	0	0	0	0
ILR 12	0	-1	1	0	0	0	0	0	0	0	0	0	0	0	0
ILR 13	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0
ILR 14	0	0	0	0	0	0	0	0	0	-1	1	0	0	0	0

Table 3-2 Pearson correlation coefficients (r) between tuber yield and petiole nutrient concentrations, based on untransformed nutrient concentrations and ILR transformed balance points on NB.

Sampling Week	**1**		**2**		**3**		**4**		**5**		**6**	
Element	Pr>F	R	Pr>F	R	Pr>F	R	Pr>F	R	Pr>F	R	Pr>F	R
Non NO_3-N	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
NO_3-N	*	-0.37	*	-0.40	**	-0.50	**	-0.53	**	-0.47	**	-0.46
S	NS	-	NS	-	*	-0.33	*	-0.38	**	-0.47	*	-0.34
P	*	-0.32	**	-0.49	**	-0.48	**	-0.56	**	-0.55	**	-0.49
K	**	-0.41	**	-0.44	*	-0.39	*	-0.39	**	-0.41	**	-0.42
Mg	NS	-	NS	-	*	0.39	NS	-	*	0.33	*	0.33
Ca	NS	-	NS	-	NS	-	*	0.34	*	0.40	*	0.38
Na	NS	-	*	-0.35	NS	-	NS	-	**	-0.47	NS	-
B	NS	-	NS	-	**	0.42	**	0.44	*	0.36	NS	-
Zn	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Mn	NS	-	NS	-	NS	-	NS	-	NS	-	*	0.32
Fe	*	-0.35	*	-0.35	NS	-	NS	-	NS	-	NS	-
Cu	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Al	NS	-	*	-0.35	NS	-	NS	-	NS	-	NS	-
ilr 1	*	-0.38	*	-0.34	NS	-	NS	-	*	-0.37	NS	-
ilr 2	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 3	NS	-	*	0.34	NS	-	NS	-	NS	-	NS	-
ilr 4	NS	-	NS	-	**	-0.50	*	-0.34	*	-0.38	NS	-
ilr 5	NS	-	*	0.35	**	0.42	*	0.34	*	0.36	*	0.34
Ilr 6	NS	-	NS	-	NS	-	NS	-	NS	-	*	0.31
ilr 7	NS	-	NS	-	*	0.33	**	0.43	*	0.33	NS	-
ilr 8	NS	-	NS	-	*	-0.34	*	-0.36	**	-0.43	**	-0.41
ilr 9	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 10	*	-0.33	**	-0.51	**	-0.53	**	-0.48	**	-0.48	**	-0.47
ilr 11	NS	-	NS	-	NS	-	NS	-	NS	-	*	-0.41
ilr 12	NS	-	NS	-	*	0.37	NS	-	NS	-	*	0.32
ilr 13	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 14	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-

NS not statistically significant, *P <0.05, **P <0.01

Table 3-3. Pearson correlation coefficients (r) between specific gravity and petiole nutrient concentrations, based on untransformed nutrient concentrations and ILR transformed balance points on NB.

Sampling Week	**1**		**2**		**3**		**4**		**5**		**6**	
Element	Pr>F	R	Pr>F	R	Pr>F	R	Pr>F	R	Pr>F	R	Pr>F	R
Non NO_3-N	NS	-	NS	-	NS	-	NS	-	**	-0.46	*	-0.34
NO_3-N	NS	-	**	-0.45	*	-0.33	*	-0.37	*	-0.36	NS	-
S	NS	-	NS	-	**	-0.48	**	-0.44	**	-0.54	**	-0.52
P	NS	-	NS	-	*	-0.38	**	-0.41	**	-0.41	*	-0.40
K	*	-0.35	*	-0.38	**	-0.42	*	-0.36	NS	-	NS	-
Mg	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Ca	NS	-	NS	-	NS	-	*	0.33	NS	-	NS	-
Na	NS	-	NS	-	NS	-	NS	-	NS	-	*	0.33
B	*	0.33	NS	-	NS	-	NS	-	NS	-	NS	-
Zn	**	-0.58	**	-0.48	NS	-	NS	-	NS	-	NS	-
Mn	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Fe	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
Cu	NS	-	NS	-	*	-0.38	NS	-	NS	-	NS	-
Al	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 1	NS	-	NS	-	NS	-	NS	-	**	-0.46	NS	-
ilr 2	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 3	*	-0.36	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 4	NS	-	**	-0.51	*	-0.33	NS	-	**	-0.46	NS	-
ilr 5	NS	-	**	0.39	NS	-	*	0.33	NS	-	NS	-
Ilr 6	NS	-	NS	-	NS	-	*	0.29	NS	-	NS	-
ilr 7	NS	-	NS	-	NS	-	*	0.40	*	0.37	NS	-
ilr 8	NS	-	NS	-	NS	-	*	-0.38	NS	-	NS	-
ilr 9	*	-0.35	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 10	NS	-	NS	-	NS	-	*	-0.32	NS	-	NS	-
ilr 11	NS	-	NS	-	NS	-	NS	-0.12	NS	-	NS	-
ilr 12	NS	-	**	0.41	NS	-	NS	-	NS	-	NS	-
ilr 13	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-
ilr 14	**	0.45	*	0.35	*	0.33	NS	-	NS	-	NS	-

NS not statistically significant, *P <0.05, **P <0.01

3.7 Figures

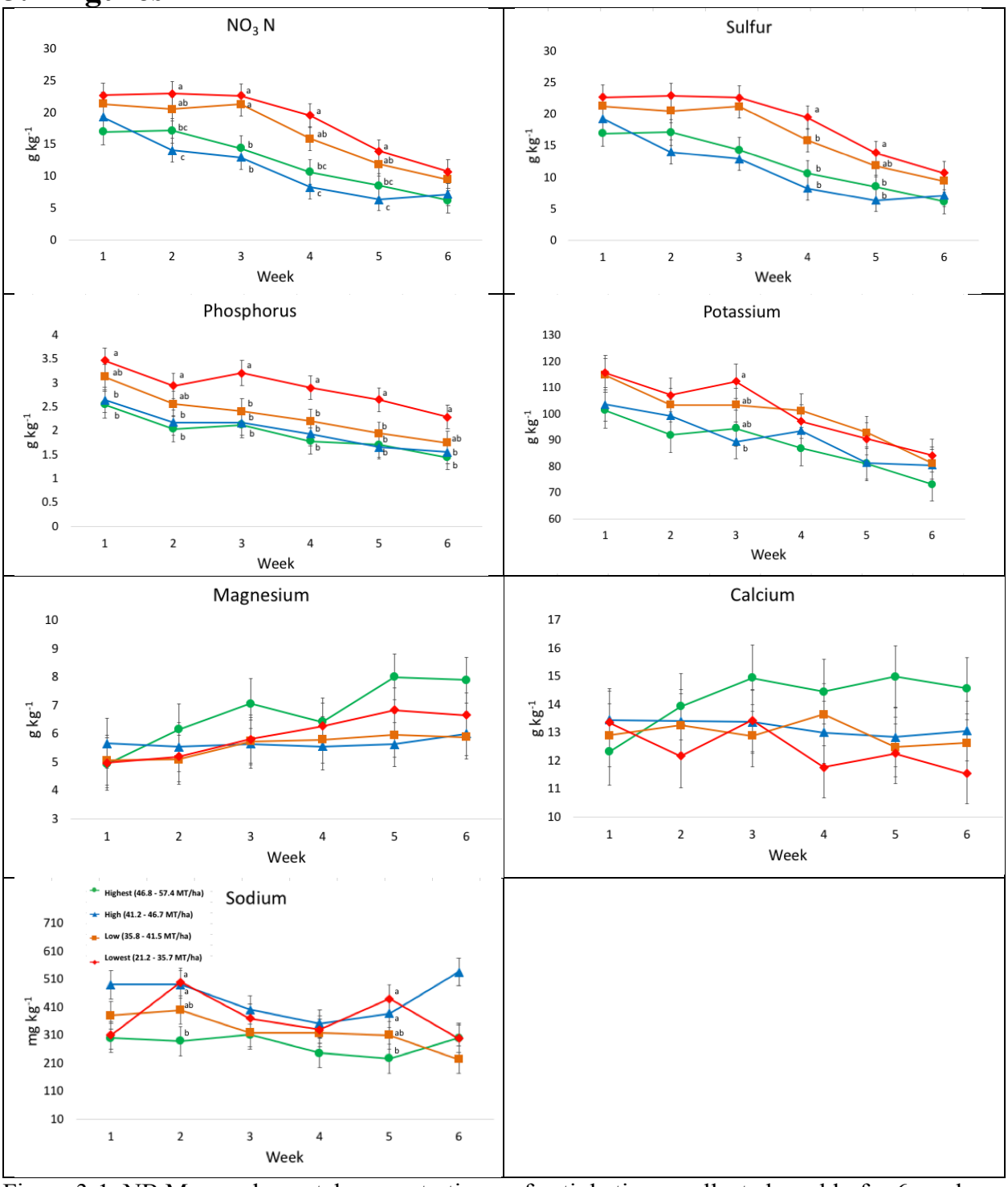

Figure 3-1. NB Macro-elemental concentrations, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by yield grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between yield groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

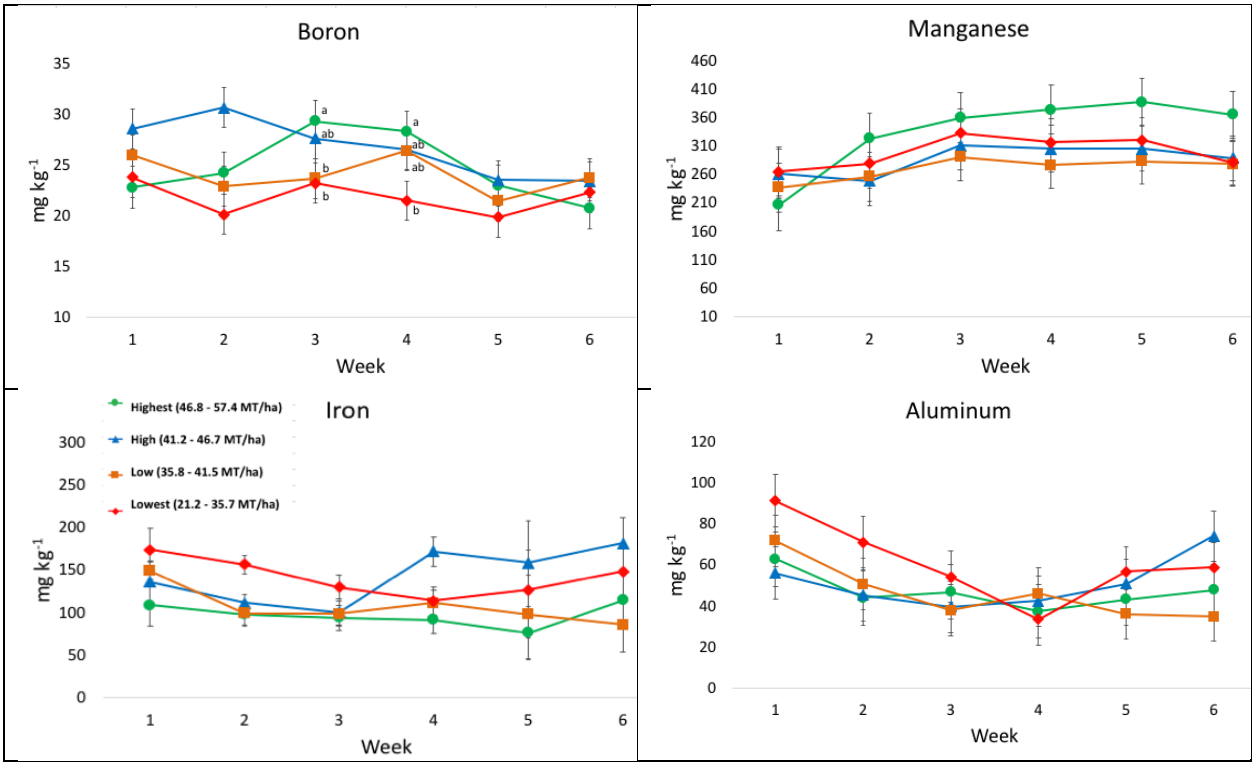

Figure 3- 2. NB Micro-elemental concentrations, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by yield grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between yield groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

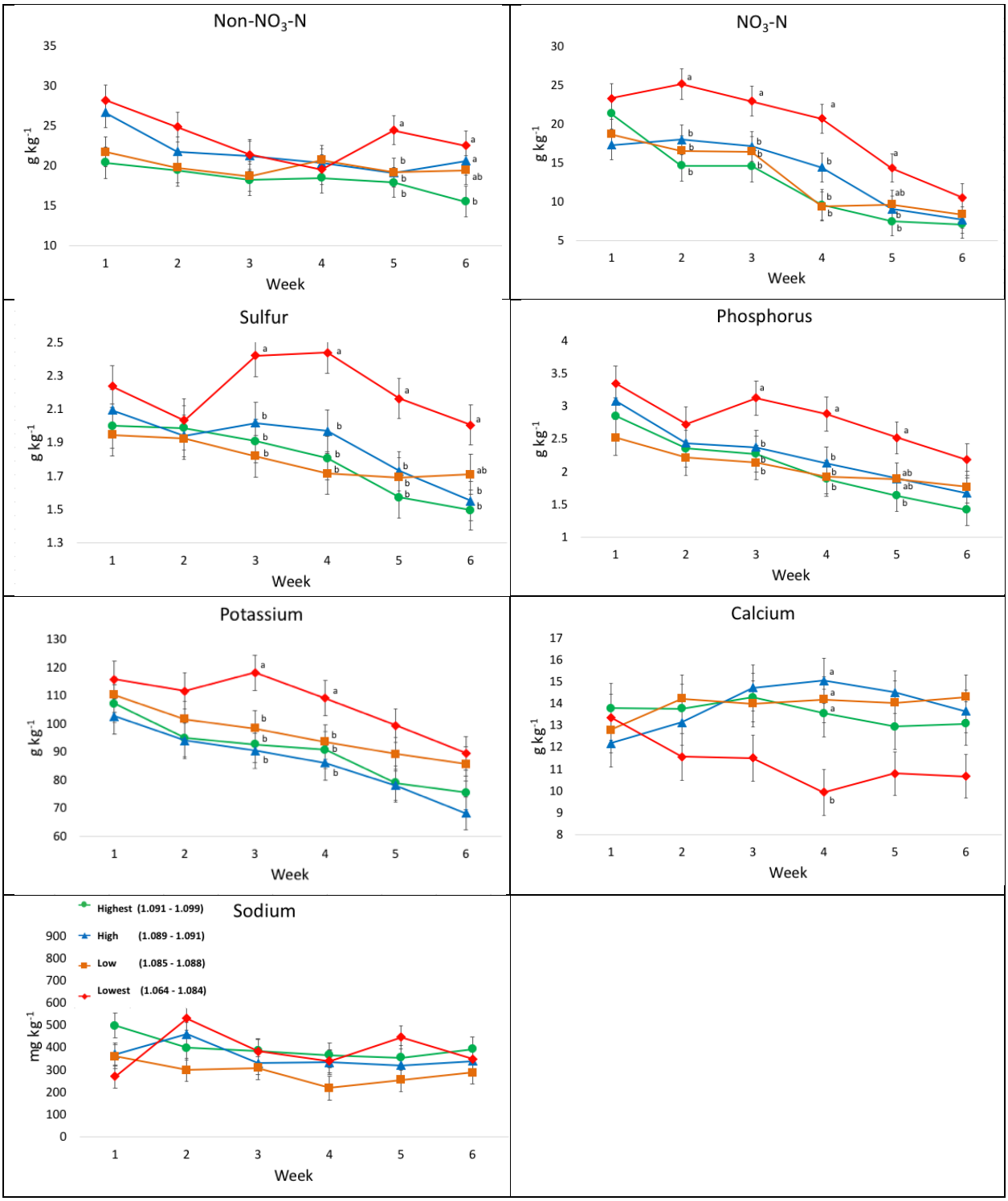

Figure 3-3. NB Macro-elemental concentrations, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by specific gravity grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between yield groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

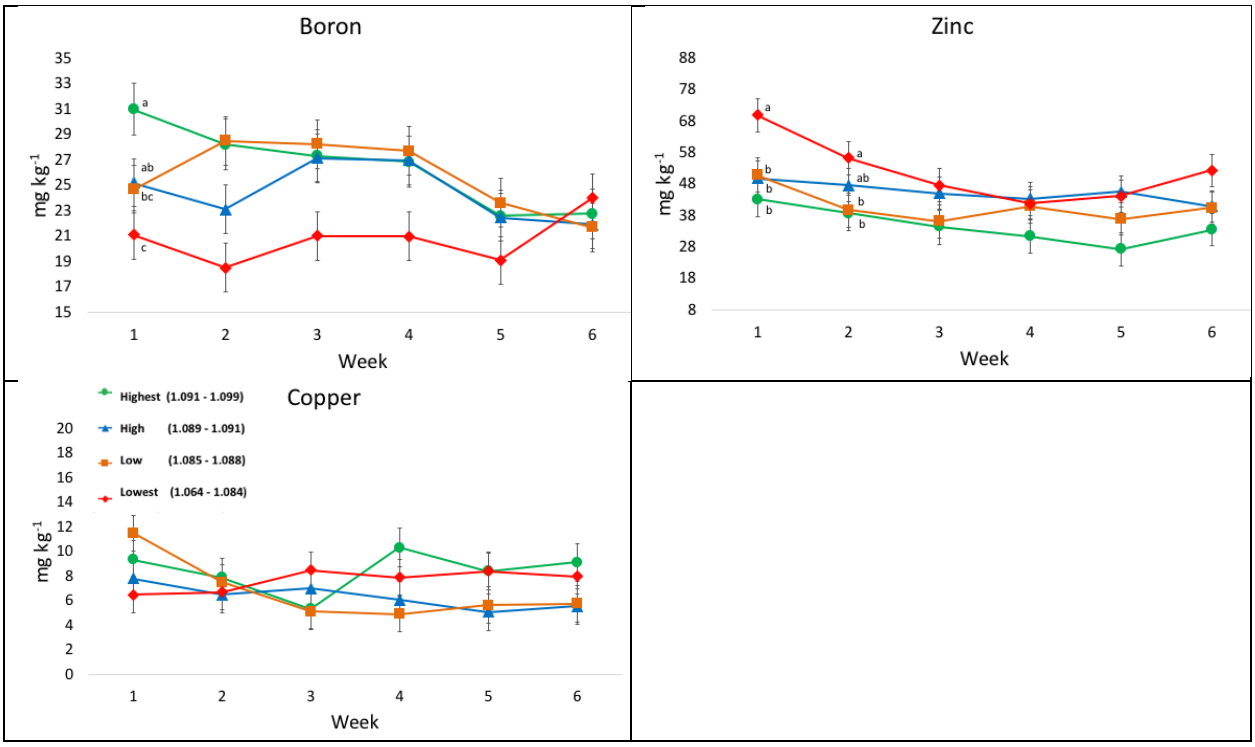

Figure 3-4. NB Micro-elemental concentrations, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by specific gravity grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between yield groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

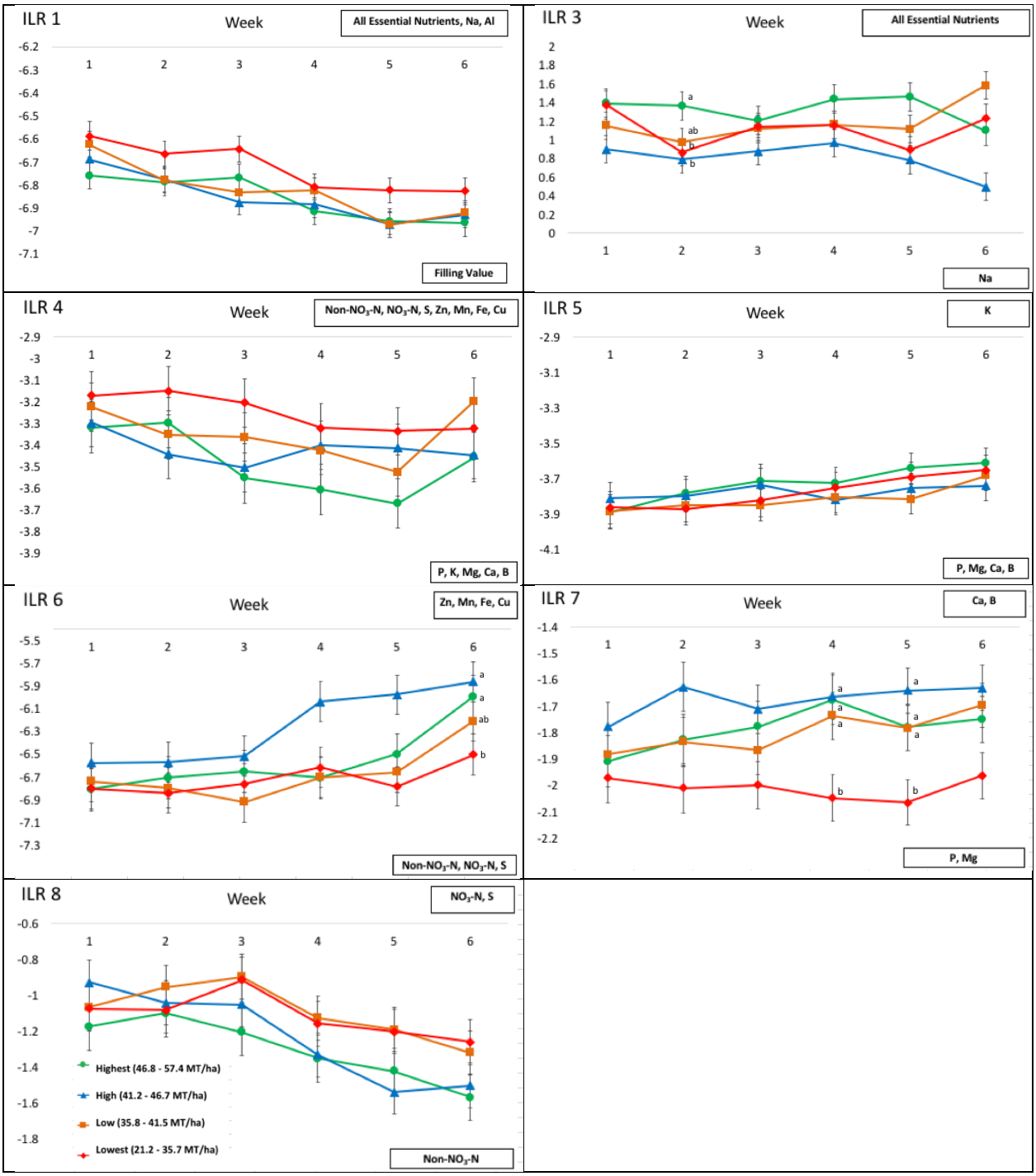

Figure 3-5. NB Multi nutrient ILR transformed balance points, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by yield grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between yield groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

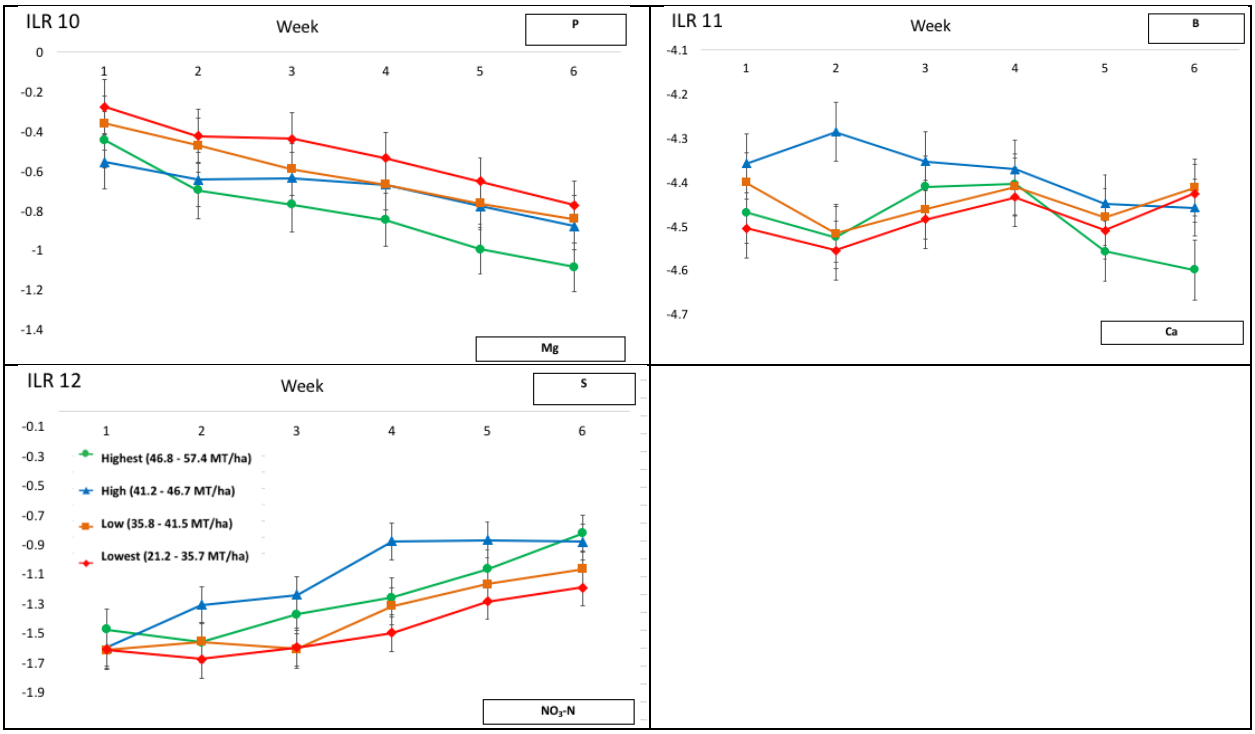

Figure 3-6. NB Single nutrient ILR transformed balance points, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by yield grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between yield groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

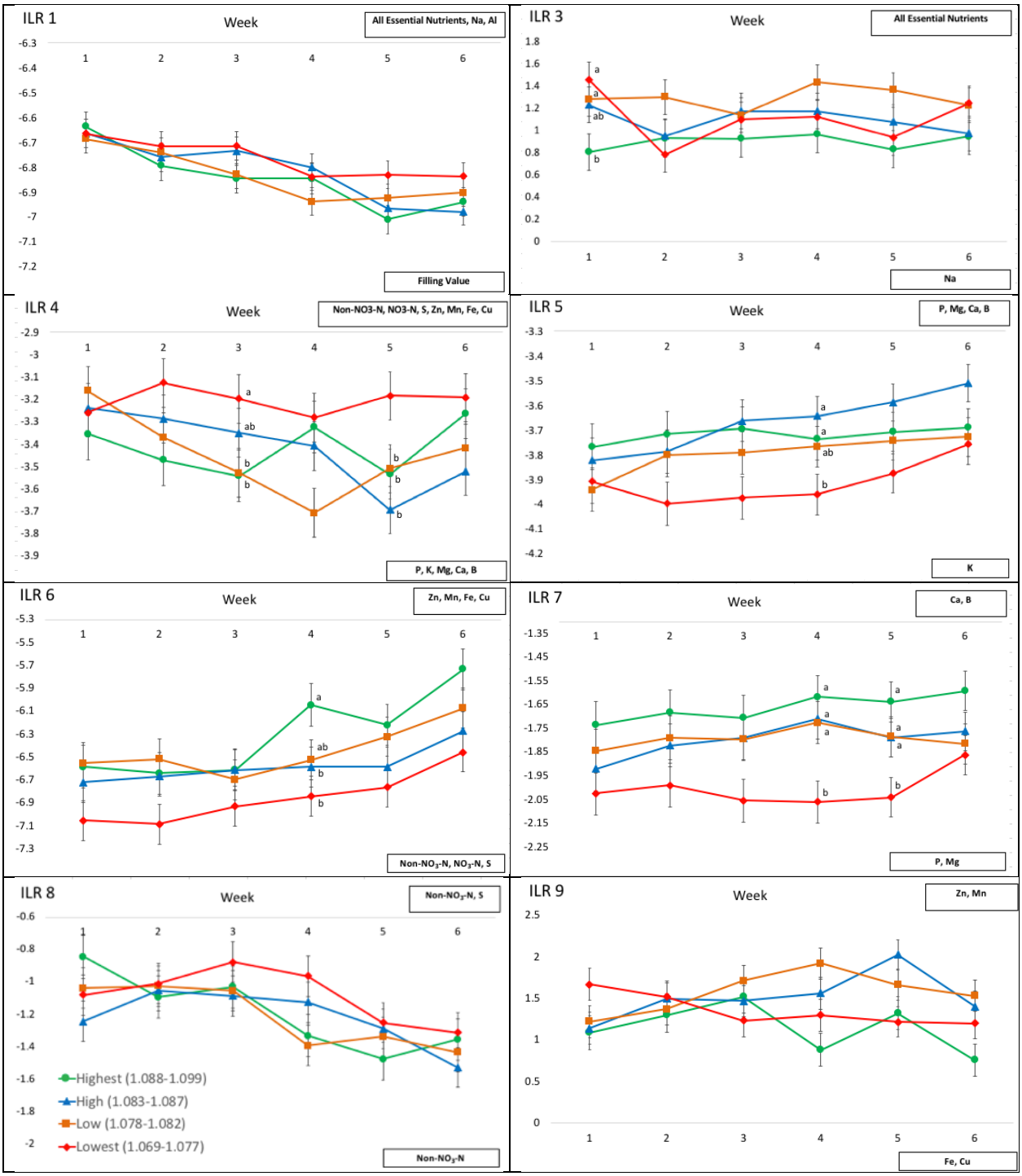

Figure 3-7. NB Multi nutrient ILR transformed balance points, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by specific gravity grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between specific gravity groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

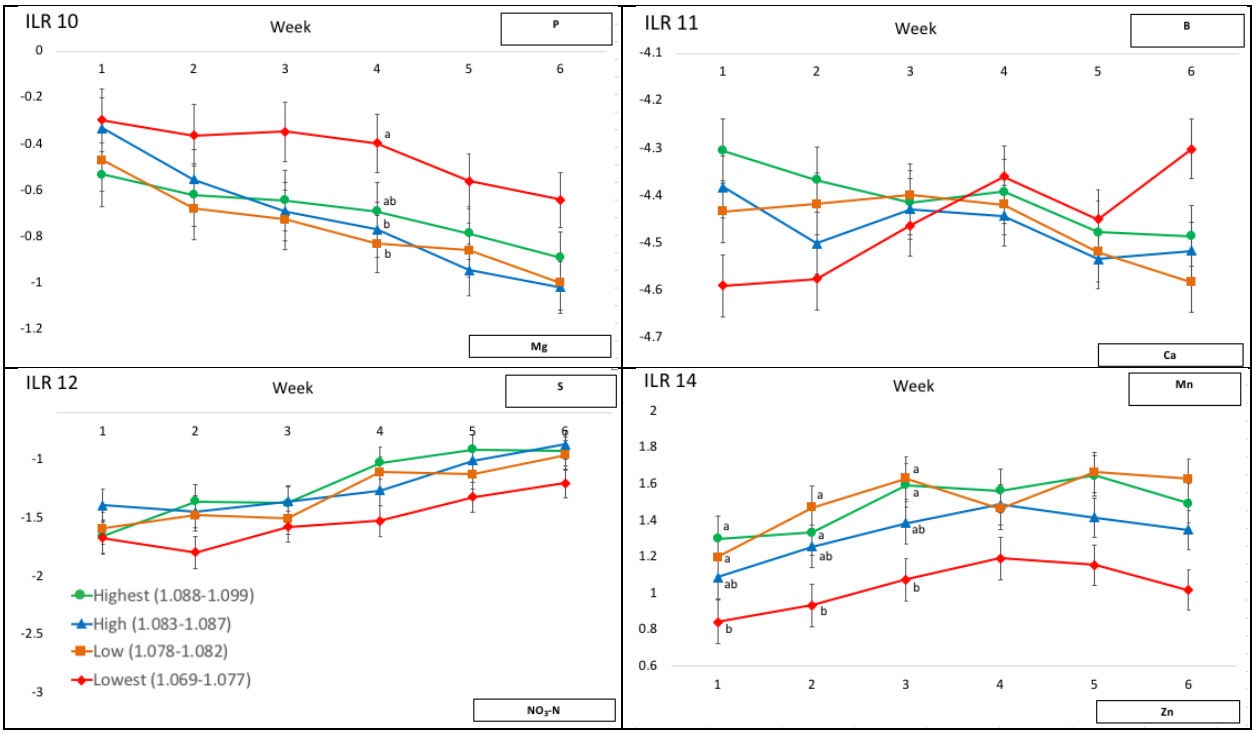

Figure 3-8. NB Multi nutrient ILR transformed balance points, of petiole tissue collected weekly for 6 weeks during the tuber bulking period from field-grown processing potato (cv. Russet Burbank), by specific gravity grouping as separated by quartiles. ANOVA scores were used to determine if mean concentrations between specific gravity groups were significant ($P < 0.05$). Mean comparisons were evaluated using Fisher's protected lsd test, and points within weeks with different letters are significantly different ($P < 0.05$). Error bars indicate standard error.

4. General Conclusions

There were many nutrients present in potato petioles that were consistently correlated with both tuber yield and specific gravity in Atlantic Canada. Across the two provinces the most consistent element related to both tuber yield and specific gravity was B. The relationship between NO_3-N and specific gravity was dependent on the province with PEI data reporting it as a beneficial marker and NB data as deleterious. The relationship between Ca accumulation and tuber yield varied between provinces as well with NB reporting a positive correlation and PEI a negative. The general accumulation of macronutrients for NB was correlated to bad tuber yield and specific gravity outcomes. The accumulation of Zn and Mn were correlated with negative outcomes for growers in PEI. In context of the reported correlations it is recommended that the practice of petiole sampling in Atlantic Canada is continued as there are useful data associated with positive and negative grower outcomes.

Overall there was stronger correlation between petiole data and grower relevant yield outcomes in NB as compared with PEI. Petiole tissue data was most correlated to tuber yield in NB (-0.56 – 0.44, $P < 0.05$) and specific gravity in PEI (-0.32 – 0.31, $P < 0.05$). The ideal sampling time for correlating tuber yield and specific gravity to petiole data in PEI were week 2 and 5 respectively. It should be noted that the survey group yields from PEI were 5.6% higher than those reported in NB, whereas the specific gravities reported were 4.8% lower. It may be that in regions approaching the cultivar limit for yield or specific gravity the petiole data are less correlated to outcomes. This was the first study of its kind to report widespread correlation between nutrients in petiole tissues and specific gravity outcomes. As a result, it is recommended that future exploration into petiole tissue data include specific gravity and should not just be related to tuber yields.

No improved correlation was reported for ILR transformed data as compared with nutrient concentrations for either tuber yield, or specific gravity in both PEI and NB. The interpretation of petiole data through the ILR transformations is cumbersome and can lead to false conclusions outside of the context of nutrient concentration data. If ILR data is interpreted in conjunction with nutrient concentrations, then some potential insights into ideal equilibrium between nutrient concentrations are gained. This was most notable for the ratio of Ca and B (ILR 11) in PEI as it correlated to tuber yield and specific gravity outcomes. In the majority of cases ILR balance point correlations were logical due to the previous observations made on nutrient concentration data, however they offered little insight overall. There are also several examples (ILR 5, ILR 7, ILR 8, ILR 11, ILR 12, ILR 14), most notably in NB, where ILR balances suggest improved grower outcomes when nutrients negatively correlated to tuber yield and specific gravity are accumulated relative to another more deleterious nutrient.

In light of the results it is highly recommended that the practice of petiole sampling in Atlantic Canada continue with a renewed focus on relating these values to grower relevant outcomes. This study is the first to report a general widespread positive correlation between petiole B concentrations, across the Atlantic Canadian potato producing provinces, and tuber yield plus specific gravity. These reported positive correlations are most concerning in context of the ambiguity and disagreement in both provinces for both determination of B sufficiency and remediation. The observations made on B in both provinces would suggest that further investigation into B fertilization responses in both provinces is undertaken with respect to both tuber yield and specific gravity. This study is the first to report an ideal Ca to B ratio in potato petiole tissues in PEI and the use of ILR 11 in identifying this ideal value recommended. The overall deleterious nature of macronutrient accumulation in NB would suggest significant over

fertilization as compared with PEI but further investigation is required to confirm this. This study is the first to report the deleterious nature of macronutrient accumulation as related to tuber yield whereby most of the previous findings have focused on poor specific gravity outcomes. The positive relationship between Ca and Mg petiole concentrations in NB and tuber yield in context of the deleterious correlations with K would suggest a well-known antagonism exists that should be further explored. As these nutrients are commonly applied in conjunction via dolomitic lime it is also recommended that the effect of this amendment be further evaluated in NB. The relationship between tuber yield and Zn, and Mn accumulation in PEI highlights further need for the evaluation of soil pH and other factors which could contribute to the subsequent uptake of these metals.

5. References

A&L Laboratories. 2016. Plant Analysis Monitoring Reports. London, Ontario.

Agriculture and Fisheries PEI. 2016. Agriculture on PEI. Charlottetown, PEI. [Online] https://www.princeedwardisland.ca/en/information/agriculture-and-fisheries/agriculture-pei

Aitchinson, J. 1986. The statistical analysis of compositional data. The Blackburn Press. London: Chapman and Hall.

Aitchinson, J. and Greenacre, M. 2002. Biplots of compositional data. Journal of Applied Statistics. (51) 375-392.

Barichello, V., Yada, R., Coffine, R., and Stanley D. 1990. Low temperature sweetening in susceptible and resistant potatoes: starch structure and composition. Journal of Food Science (55) 1054-1059.

Bates, T.E. 1971. Factors affecting critical nutrient concentrations in plants and their evaluation: a review. Soil Science (112) 116-130.

Baxter, I. 2009. Ionomics: studying the social network of mineral nutrients. Current Opinion in Plant Biology (12) 381-386.

Beaufils, E.R. 1973. Diagnosis and recommendation integrated system (DRIS). Soil Sci. Bul. no. 1, University of Natal, Pietermaritzburg, South Africa.

Bélanger, G., Walsh, J.R., Richards, J.E., Milburn P.H., and Ziadi, N. 2000. Nitrogen fertilization and irrigation affects tuber characteristics of two potato cultivars. American Journal of Potato Research (79:4) 269-279.

Broadley, M. Brown, P. Cakmak, I., Rengel, Z., and F. Zhao. 2012. Functions of nutrients: Micronutrients. In: Marschner, P. (ed.) Marschner's mineral nutrition of higher plants, 3rd ed. Elsevier. Sydney, Australia.

Brown, B.D., Gardiner J., and Back J. 1991. Treasure Valley potato and sugar beet response to boron. University Idaho Research Report, Moscow, ID, USA.

Buza, M.H., Holden, L.A., White, R.A., and Ishler, V.A. 2014. Evaluating the effect of ration composition on income over feed cost and milk yield. Journal of Dairy Science. (97:5) 3073-3080.

Cakmak, I., Hengeler, and Marschner, H. 1994. Partitioning of shoot and root dry matter and carnbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. Journal of Experimental Botany. (45:9) 1245-1250.

Camacho-Cristobal, J. J., Rexach, J., Gonzalez-Fontes A. 2008. Boron in plants: deficiency and toxicity. Journal of Intergrated Plant Biology (50:10) 1247-1255.

Chayes, F. 1960. On correlation between variables of constant sum. Journal of Geophysical Resources. (65) 4185-4193.

Clark C.F., Lombard P.M., and Whiteman E.F. 1940. Cooking quality of potatoes as measured by specific gravity. American Potato Journal.

Collins, H.P., Porter, L., Boydston R.A., Alva A,. and Chaves-Cordoba B. 2016. Petiole N, P, K Concentrations and Yield of the Potato Cultivar Molli from Certified Organic Amendments and Fertilizer Formulations. Communications in Soil Science and Plant Analysis. (47:10) 1227-1238.

Davenport, J.R. 2000. Washington: Potassium and Specific Gravity of Potato Tubers. Better Crops (84:4) 14-15.

Dennis, D.T., and Coultate, T.P. 1966. Phosphofructokinase, a regulatory enzyme in plants. Biochemical and Biophysical Research Communications. (25:2) 187-191.

Dijkshoorn, W., and Wijk, A.L. 1967. The sulphur requirements of plants as evidenced by the sulphur-nitrogen ratio in the organic matter a review of published data. Plant and Soil. (26:1) 129-157.

Dubetz, S., and Bole, J.B. 1975. Effect of nitrogen, phosphorus, and potassium fertilizers on yield components and specific gravity of potatoes. American Potato Journal. (52:12) 399-405.

Dyson, P.W., and Watson, D.J. 1971. An analysis of the effects of nutrient supply on the growth of potato crops. Annals of Applied Biology (69:1) 7-63.

Egozcue J.J., Pawlowsky-Glahn V., Mateu-Figueras, G., and Barcelo-Vidal, C. 2003. Isometric logratio transformations for compositional data analysis. Math and Geology. (35) 279-300.

Environment Canada. 2010. Canadian Climate Normals 1981-2010. Environment Canada. [Online]http://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html?stnID=4832&lang=e&dCode=0&province=ONT&provBut=Go&month1=0&month2=12

Evenson, R.E. 2003. Assessing the Impact of the Green Revolution, 1960 to 2000. Science (300) 758-762.

Ewing, E.E., and Wareing P.F. 1978. Shoot, Stolon, and Tuber Formation on Potato (Solanum tuberosum L.) Cuttings in Response to Photoperiod. Plant Physiology (61) 348-353.

(FAOUN) Food and Agriculture Organization of the United Nations. FAOSTAT. [Online] http://faostat.fao.org/site/567/default.aspx - ancor Available: [2016 Jul 15].

Fahmy, S, Hann, W.R., Jiao, Y. 2010. Soils of New Brunswick: The second approximation. Eastern Canda Soil and Water Conservation Centre. Agriculture and Agrifood Canada. [Online] http://sis.agr.gc.ca/cansis/publications/surveys/nb/nbsa/nbsa_report.pdf

Filzmoser, P., and Hron, K. 2011. Robust Statistical Analysis in Compositional Data Analysis: Theory and Applications. New York, John Wiley and Sons. 59-72.

Fortin, J.G., Anctil, F., Parent, L.E., and Bolinder, M.A. 2011. Site specific early season potato yield forecast by neural network in Eastern Canada. Precision Agriculture (12:6) 905-923.

Foy, C.D. 1988. Plant adaptation to acid, aluminum-toxic soils. Communications in Soil Science and Plant analysis. (7:12) 959-987.

Gardner, B.R. and Jones, J.P. 1975. Petiole analysis and the nitrogen fertilization of Russet Burbank potatoes. American Potato Journal (52) 195-200.

Gausman, H.W., and Estes, G.O. 1963. Effects of factorially combined levels of sulfur and magnesium on potato plants. University of Maine Experimental Station Bulletin T5. [Online]http://digitalcommons.library.umaine.edu/cgi/viewcontent.cgi?article=1187&context=aes_techbulletin

Gilland, B. 2002. World population and food supply: Can food production keep pace with population growth in the next half century? Food Policy (27) 47-63.

Government of PEI. 2009. Soil Management – Field Selection & Fertility Practices. [Online]http://www.gov.pe.ca/agric/index.php3?number=1001557&lang=F

Government of PEI. 2009. The why and how of potato tissue testing. [Online]http://www.princeedwardisland.ca%2Fen%2Finformation%2Fagriculture-and-fisheries%2Fwhy-and-how-potato-tissue-testing&usg=AOvVaw1GJ3wbB7Ype6HZrhsKrOZ3

Gupta, U.C. and Sanderson, J.B. 1993. Effect of sulfur, calcium and boron on tissue nutrient concentration and potato yield. Journal of Plant Nutrition (16:6) 1013-1023.

Guskov, A., Kern, J., Gabdulkhakov, A., Broser, M., Zouni, A., and Saenger, W. 2009. Cyanobacterial photosystem II at 2.9-A resolution and the role of quinones, lipids, channels and chloride. Nature Structural and Molecular Biology. (16) 334-342.

Hallock, D.L. 1979. Relative effectiveness of several Mn sources on Virginia-type peanuts. Agronomy Journal (71:4) 685-688.

Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Skrumsager, I., and White, P. 2012. In: Marschner, P. (ed.) Marschner's mineral nutrition of higher plants, 3rd ed. Elsevier. Sydney, Australia.

Haynes, R.J. and Goh, K.M. 1978. Ammonium and nitrate nutrition of plants. Biological Review (53) 465-510.

Heard J. 2004, Nutrient Uptake and partitioning by potatoes in Manitoba, Manitoba Agronomists Conference 2004, December 14-15, University of Manitoba, Winnipeg, Manitoba.

Hepler, P.K., and Winship, L.J. 2010. Calcium at the Cell Wall-Cytoplast Interface. Journal of Intergrative Plant Biology. (52:2) 147-160.

Hesse, H., and Hoefgen, R. 2003. Molecular aspects of methionine biosynthesis. Trends in Plant Science. (8:6) 259-262.

Huang, X., and Salt, D. 2016. Plant Ionomics: From Elemental Profiling to Environmental Adaptation. Molecular Plant. (9:6) 787-797.

Hütsch, B., Keipp, K., Glaser, A., and Schubert S. 2017. Potato plants (*Solanum tuberosum* L.) are chloride-sensitive: Is this dogma valid? Science of Food and Agriculture. Online.

Jarrell, W.M., and Beverly, R.B. 1981. The dilution effect in plant nutrition studies. Advances in Agronomy (34) 197-224.

Jianrong, X., and Tian, Q. 2009. Early stage toxicity of excess copper to photosystem II of Chlorella pyrenoidosa- OJIP chlorophyll a fluorescence analysis. Journal of Environmental Sciences. (21:11) 1569-1574.

Jones, D., Healey, J., Willett, V.B., Farrar, J.F., Hodge, A. 2005. Dissolved organic nitrogen uptake by plants – an important N uptake pathway? Soil Biologiy and Biochemistry. (37:3) 413-423.

JMP 2017, Version 11, SAS Institute Inc., Cary NC.

Khiari, L., Parent L.E., and Tremblay, N. 2000. Selecting the High-Yield subpopulation for Diagnosing Nutrient Imbalance in Crops. Agronomy Journal. (93:4) 802-808.

Khiari, L., Parent, L.E., Tremblay, N. 2001. The phosphorus compositional nutrient diagnosis range for potato. Production Agriculture (93) 815-819.

Kirby E.A., and Mengel, K. 1967. Ionic Balance in Different Tissues of the Tomato Plant in Relation to Ntrate, Urea, or Ammonium Nutrition. Plant Physiology. (42:1) 6-14.

Klecka, W.R. 1980. Discriminant Analysis. Sage Publications. London England.

Krapp, A., Hofmann, B., Schäfer, C., and Stitt, M. 1993. Regulation of the expression of *rbc*S and other photosynthetic genes by carbohydrates: a mechanism for the “sink regulation” of photosynthesis. The Plant Journal (3) 817–828.

Krentos, V.D., and Orphanos, P.I. 1979. Nitrogen, phosphorus, and potassium fertilizers for potatoes in Cyprus. The journal of agricultural science (92:3) 645-661.

Kronzucker, H.J., and Britto, D.T. 2010. Sodium transport in plants: a critical review. New Phytologist. (189:1) 54-81.

Kunkel, R., and Holstad, N. 1972. Potato chip color, specific gravity and fertilization of potatoes with N-P-K. American Potato Journal (49:2) 43-62.

Kumar, P., Pandey, S.K., Singh, B.P., Singh, S.V., Kumar, D. 2007. Influence of source and time of potassium application on potato growth, yield, economics and crisp quality. Potato research (50:1) 1-13.

Laboski, C.A., and Kelling, K.A. 2007. Influence of fertilizer management and soil fertility on tuber specific gravity: a review. American Journal of Potato Research (84:4) 283-290.

Levy, D. and Veilleux R.E. 2007. Adaptation of Potato to High Temperatures and Salinity – A Review. American Journal of Potato Research. (84) 487-506.

Li W, Xiong B, Wang S, Deng X, Yin L, Li H. 2016. Regulation Effects of Water and Nitrogen on the Source-Sink Relationship in Potato during the Tuber Bulking Stage. PLoS ONE (11:1).

Locascio, S.J., Bartz, J.A., and Weingartner D.P. 1992. Calcium and potassium fertilization of potatoes grown in North Florida I. Effects on potato yield and tissue Ca and K concentrations. American Potato Journal. (69:2) 95-104.

Loneragan, .J, and Snowball, K. 1969. Calcium requirements of plants. Australian Journal of Agricultural Research. (20:3) 465-78.

Lulai, E.C., and Orr, P.H. 1979. Influence of potato specific gravity on yield and oil content of chips. American Potato Journal (56:8) 379-390.

Lujan L., and Smith, O. 1964. Potato quality XXIV. Objective measurement of mealinesss in potatoes. American Potato Journal. (41:8) 244-252.

Lutaladio N., and Castaldi L. 2009. Potato: the hidden treasure. Journal of Food and Compositional Analysis (22) 491–493.

MacKay, D.C, MacEachern, C.R., and Bishop, R.F. 1966. Optimum nutrient levels in potato leaves (Solanum tuberosum L.) Soil Science Society of America Journal (30:1) 73-76.

MacKenzie, A. 1967. Plant Analysis as an Aid to Cotton Fertilization. Soil Testing and Plant Analysis Part II: Plant Analysis. 25-31.

Macfie, S. M. and Taylor, G. J. 1992. The effects of excess manganese on photosynthetic rate

and concentration of chlorophyll in *Triticum aestivum* grown in solution culture. Physiologia Plantarum (85) 467–475.

Maier, N.A., Dahlenburg, A.P., and Williams, C.M. 1994. Effects of nitrogen, phosphorus, and potassium on yield, specific gravity, crisp colour, and tuber chemical composition of potato (*Solanum tuberosum* L.) cv. Kennebec. Australian Journal of Exprimental Agriculture. (34:6) guava 813-824.

Maier, N.A., McLaughlin, M.J, Heap, M., Butt, M., and Smart, M.K. 2002. Effect of nitrogen source and calcitic lime on soil pH and potato yield, leaf chemical composition and tuber cadmium concentrations. Journal of Plant Nutrition. (25:3) 523-544.

Marchand, Sébastien, Parent, Serge-Étienne, Deland, Jean-Pierre, & Parent, Léon-Étienne. 2013. Perfil nutritivo de atocas (Vaccinium macrocarpon Ait. - cranberry) cultivadas no Québec, Canadá. Revista Brasileira de Fruticultura (35:1) 292-304.

Marschner, P. 2012. Marschner's mineral nutrition of higher plants, 3rd ed. Elsevier. Sydney, Australia.

Matoh, T., and Kobayashi, M. 1998. Boron and calcium, essential inorganic consitituents of pectic polysaccharides in higher plant cell walls. Journal of Plant Research. (111:1) 179-190.

Mesquita, H., Alvarenga, M.A., Paula, Bueno de Paula, M., Carvalho, Guedes de Carvalho, J., and Nóbrega, J.A. 2007. Yield and quality of the potato in response of boron levels. Ciência e Agrotecnologia (31:2) 385-392.

Mitsuru Qsaki , Jun Shirai , Takuro Shinano and Toshiaki Tadano. 1995. Effects of ammonium and nitrate assimilation on the growth and tuber swelling of potato plants, Soil Science and Plant Nutrition (41:4) 709-719.

Mohr, R.M., Volkmar, K., Derksen, D.A., Irvine, R.B., Khakbazan, M., McLaren, D., Monreal, M.A., Moulin, A.P., and Tomasiewicz, D.J. 2011. Effect of rotation on crop yield and quality in an irrigated potato system. American Journal of Potato Research (88:4) 346-359.

Mukezangango, J. 2015. Potato market information review 2013-2014. Market Analysis and Information Section Horticulture and Cross Sectoral Division of Agriculture and Agri-Food Canada. [Online]http://www.agr.gc.ca/resources/prod/CMS/Internet/Common-Commun/1433875713786_pmir-eng.pdf

Nikiforova, V., Freitag, J., Kempa, S., Adamik, M., Hesse, H., and Hoefgen, R. 2003. Transcriptome analysis of sulfur depletion in *Arabidopsis thaliana*: interlacing of biosynthetic pathways provides response specificity. The Plant Journal. (33:4) 633-650.

Öborn, I., Jansson, G., and Johnsson, L. 1995. A field study on the influence of soil pH on trace element levels in spring wheat (Triticum aestivum), potatoes (Solanum tuberosum) and carrots (Daucus carota). Water, Air, and Soil Pollution (85:2) 835-840.

Ojala, J.C., Stark, J.C., Kleinkopf, G.E. 1990. Influence of irrigation and nitrogen management on potato yield and quality. American Potato Journal. (67:1) 29-43.

Parent, L.E., Cambouris, A.N., and Muhawenimana, A. 1994. Multivariate diagnosis of nutrient imbalance in potato crops. Soil Science Society of America Journal. (58:5) 1432-1438.

Parent, L.E. 2011. Diagnosis of the nutrient compositional space of fruit crops. Revista Brasileira de Fruticultura (33:1) 321-334.

Parent, L.E., Parent, S.-.E., Rozane, D.E., Amorim, D.A., Hernandes, A. and Natale, W. 2012. Unbiased approach to diagnose the nutrient status of red guava (Psidium Guajava). Acta Horticulturae. (959) 145-159.

Parent, L.E., Parent, S.E., Hébert-Gentile, V., Naess, K., and Lapointe, L. 2013a. Mineral Balance Plasticity of Cloudberry (Rubus chamaemorus) in Quebec-Labrador Bogs American Journal of Plant Sciences (4:7) 1508-1520.

Parent, S., Parent, L., Egozcue, J., Rozane, D-E., Hernandes, A., Lapointe, L., Hebert-Gentile, V., Naess, K., Marchand, S., Lafond, J., Mattos., D., Barlow, P., and Natale, W. 2013b. The plant ionome revisited by the nutrient balance concept. Frontiers in Plant Science (4) 39-49.

Parent, S., Parent, L., Rozane, D, and Natale, W. 2013c. Plant ionome diagnosis using sound balances: case study with mango (Mangifera Indica). Frontiers in Plant Science. (4) 449-461.

Pavlista, A. 2004. Early-Season Applications of Sulfur Fertilizers Increase Potato Yield nad Reduce Tuber Defects. Agronomy Journal. (97:2) 599-603.

PEI Analytical Laboratories Soil Testing. 2014. Supplemental Nutrient Applications for Potatoes. [Online]http://www.gov.pe.ca/photos/original/af_fact_snap.pdf

Peuke, A.D., Jeschke, W.D., and Hartung W. 1998. Foliar application of nitrate or ammonium as sole nitrogen supply in Ricinus communis. II The Flows of cations, chloride and abscisic acid (140:4) 625-636.

Qsaki, M., Shirai, J., Shinano T., and Tadano, T. 1995. Effects of ammonium and nitrate assimilation on the growth and tuber swelling of potato plants. Soil Science and Plant Nutrition (41:4) 709-719.

Ramona, M.M. and Tomasiewicz, D.J. 2012. Effect of rate and timing of potassium chloride application on the yield and quality of potato (Solanum tuberosum L ‘Russet Burbank’). Canadian Journal of Plant Science (92:4) 783-794.

Rees, H.W., Chow, T.L., Zebarth, B., Xing, Z., Toner, P., Lavoie, J., and Daigle, J.L. 2014. Impact of supplemental poultry manure application on potato yield and soil properties on a loam soil in north-western New Brunswick. Canadian Journal of Soils Science. (94:1) 49-65.

Reeve, E., and Shive, J. 1943. Potassium – Boron and Calcium – Boron Relationships in plant nutrition. Boron Sympossium. 1-14.

Reis, R., and Monnerat P. 2000. Nnutrient concentrations in potato stem, petiole and leaflet in response to potassium fertilizer. Scientia Agricola (57:2) 251-255.

Rengasamy, P. 2010. Soil processes affecting crop production in salt-affected soils. Functional Plant Biology. (37) 614-620.

Ruuhola, T., Keinänen, M., Keski-Saari, S., Lehto, T. 2011. Boron nutrition affects the carbon metabolism of silver birch seedlings. Tree physiology (31:11) 1251-1261.

Rosen, C.J., McNearney, M., and Carrasco, M. 2002. Sulfur-nitrogen interactions in potato. In: Minnesota Area II Research Report. St. Paul MN 69-73.

Saito, K. 2004. Sulfur Assimilatory Metabolism. The Long and Smelling Road. Plant Physiology. (136:1) 2443-2450.

Salt, D.E. 2004. Update on plant ionomics. Plant Physiology (136) 2451-2456.

Salt, D.E., Baxter, I., and Lahner, B. 2008. Ionomics and the study of the plant ionome. Annual reiew of plant biology (59) 709-733.

Sanderson, J.B., and Gupta, U.C. 1990. Copper and zinc nutrition of russet Burbank potatoes grown on Prince Edward Island. Canadian Journal of Plant Science (70:1) 357-362.

Sanderson, J.B., MacLeod, J.A., Douglas, B., Coffin, R., and Bruulsema, T. 2003. Phosphorus research on potato in PEI. ISHS Acta Horticulturae XXVI International Horticultural Congress: Potatoes, Healthy Food for Humanity 619.

Sarkar, D., Pandey, S.K., Sud, K.C., Chanemougasoundharam, A. 2004. In vitro characterization of manganese toxicity in relation to phosphorus nutrition in potato (Solanum tuberosum L.). Plant Science (167:5) 977-986.

Setter, T.L., Brun, W.A., and Brenner, M.L. 1980. Stomatal Closure and Photosynthetic Inhibition in Soybean Leaves Induced by Petiole Girdling and Pod Removal. Plant Physiology (65:5) 884-887.

Sharifi, M., Zebarth, B., Burton, D., Grant, C., and Porter, G. 2008. Organic amendment history and crop rotation effects on soil nitrogen mineralization potential and soil nitrogen supply in a potato cropping system. Agronomy Journal. (100:6) 1562-1572.

Sharma, D.K., Kushwah, S.S., Nema, P.K. and Rathore, S.S. 2011. Effect of Sulphur on Yield and Quality of Potato (*Solanum tuberosum* L.) Agricultural Research. (6:2) 143-148.

Silva G.H., Chase R.W., Hammerschmidt, R., Vitosh, M.L., and Kitchen, R.B. 1991. Irrigation, nitrogen, and gypsum effects on specific gravity and internal defects of Atlantic potatoes. American Potato Journal. (68:11) 751-765.

Souza, H.A., Parent, S.-E., Rozane, D.E., Amorim, D.A., Modesto, V.C., Natale, W., and Parent, L.E. 2016. Guava waste to sustain guava (Psidium guajava) agroecosystem: nutrient "balance" concepts. Frontiers in Plant Science (7:1252) 1-13.

Stark, J., Westermann, D., and Hopkins, B. 1994. Nutrient Management Guidelines for Russet Burbank Potatoes. University of Idaho Extensions. Bulletin 840.

Stark, J., Bohl, W., Love, S., Novy, R., Whitworth, J., Olsen, N., Brandt, T., Miller, J., Fuller, J., and Helms, T. 2008. *Cultural Management of Western Russet Potatoes. University of Idaho Extensions:* CIS 1152 1-6 [Online]http://www.extension.uidaho.edu/nutrient/pdf/Potato/Cultural%20Managment%20of%20Western%20Russet%20Potatoes.pdf

Statistics Canada. 2012. *Table 001-0014 - Area, production and farm value of potatoes, annual,* CANSIM (database).

Sumner, M.E. 1979. Interpretation of foliar analyses for diagnostic purposes. Journal of Agronomy. (71) 343-348.

Teich, A.H., and Menzies, J.A. 1964. The effect of nitrogen, phosphorus, and potassium on the specific gravity, ascorbic acid content and chipping quality of potato tubers. American Potato Journal. (41:6) 169-173.

United States Department of Agriculture. 2016. Full Report (All Nutrients): 11352, Potatoes, flesh and skin, raw. National Nutrient Database for Standard Reference Release 28 [Online]https://ndb.nal.usda.gov/ndb/foods/show/

Jardine, D. 2016. Prince Edward Island Annual Climate Summary. Climate Research Lab UPEI [Online]http://projects.upei.ca/climate/files/2017/04/Annual-Climate-Summary-2016.pdf

Vanasse, N.A., Jones, I.D., and Lucas, H.L. 1951. Specific gravity – Dry matter relationship in potatoes. American Potato Journal (28:12) 781-791.

Vitosh, M.L. 1971. Fertilizer Studies with Irrigated Potatoes. Michigan State University Agricultural Experiment Station, East Lansing M.I.

Walworth, J.L. and Sumner M.E. 1987. The Diagnosis and Recommendation Intergrated System (DRIS). Advances in Soil Science. (6) 149-188.

Walworth, J.L., and Muniz J.E. 1993. A compendium of tissue nutrient concentrations for field grown potatoes. American Potato Journal. (70:8) 579-597.

Watanabe, T., Urayama, M., Shinano, T., Ryosuke, O., and Osaki, M. 2015. Application of ionomics to plant and soil in fields under long-term fertilizer trials. Springer Plus (4:781).

Watanabe, T., Maejima, E., Yoshimura, T., Urayama, M., Yamauchi, A., Owadano, M., Okada, R., Osaki, M., Kanayama, Y., and Shinano, T. 2016. The ionomic study of vegetable crops. PLOS One (11:8).

Westermann, D.T., Tindall, T.A., James, D.W., and Hurst, R.L. 1994. Nitrogen and potassium fertilizer fertilization on potatoes: Yield and specific gravity. American Potato Journal (71:7) 417-431.

Westermann, D.T., and Davis, J.R. 1992. Potato nutritional management changes and challenges into the next century. American Potato Journal. (69:11) 753-676.

White, M.C., Decker, A.M., and Chaney, R.L. 1981. Metal complexation in xylem fluid. I. Chemical composition of tomato and soybean stem exudate. Plant Physiology (67:2) 292 – 299.

White, P.J., and Brown, P.H. 2010. Plant nutrition for sustainable development and global health. Annals of Botany (105) 1073-1080.

White, P.J. 2012a. Ion uptake mechanisms of individual cells: Short-distance transport. In: Marschner, P. (ed.) Marschner's mineral nutrition of higher plants, 3rd ed. Elsevier. Sydney, Australia.

White, P.J. 2012b. Long-distance transport in the xylem and phloem. In: Marschner, P. (ed.) Marschner's mineral nutrition of higher plants, 3rd ed. Elsevier. Sydney, Australia.

White R.P., Munro, D.C., and Sanderson J.B. 1974. Nitrogen, potassium, and plant spacing effects on yield, tuber size, specific gravity, and tissue N, P, and K of Netted Gem potatoes.

White R.P. and Sanderson, J.B. 1983. Effect of planting date, nitrogen rate, and plant spacing on potatoes grown for processing in Prince Edward Island. American Potato Journal. (60:2) 115-126.

Whittenberger, R.T. 1951. Changes in specific gravity, starch content, and sloughing of potatoes during storage. American Potato Journal. (28:10) 738-747.

Williams, C.M.J., Maier N.A. 1990. Determination of the nitrogen status of irrigated potato crops: I. Critical nutrient rangers for nitrate-nitrogen in petioles. Journal of Plant Nutrition. (8) 971-984.

Williams, L. and Salt D.E. 2009. The plant ionome coming into focus. Current Opinions in Plant Biology. (12:3) 247-249.

Zamuner, E.C., Lloveras, J., and Escheverria, H.E. 2016. Use of a Critical Phosphorus Dilution Curve to Improve Potato Crop Nutritional Management. American Journal of Potato Research. (93:4) 392-403.

Zebarth, B.J., Leclerc, Y., Moreau G. 2004. Rate and timing of nitrogen fertilization of Russet Burbank potato: Nitrogen use efficiency. 2004. Canadian Journal of Plant Science. (84:3) 845-854.

Zebarth, B., Moreau, G., and Karemangingo, C. 2007. Nitrogen Management for Potatoes: Petiole Nitrate Testing. GHG Taking Charge Team Factsheet. Agriculture and Agri-Food Canada. [Online]http://www.soilcc.ca/ggmp_fact_sheets/pdf/Potato_pnit.pdf

Zebarth, B., Bélanger, G, Cambouris, A., and Ziadi N. 2012. Nitrogen Fertilization Strategies in Relation to Potato Tuber Yield, Quality and Crop N Recovery. Sustainable Potato Production: Glocal Case Studies. 165-186.

Zhao, D., Oosterhuis, D.M. 2003. Cotton growth and physiological responses to boron deficiency. Journal of Plant Nutrition (26:4) 855-867.