SULPHUR DEFICIENCY in southern ONTARIO soils; how FERTILIZATION will impact CANOLA (Brassica napus L.) and ALFALFA (Medicago sativa L.) YIELD and QUALITY

by

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ABSTRACT

SULPHUR DEFICIENCY IN SOUTHERN ONTARIO SOILS; HOW FERTILIZATION WILL IMPACT CANOLA (Brassica napus L.) AND ALFALFA (Medicago sativa L.) YIELD AND QUALITY

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As sulphur (S) deposition has been decreasing across southern Ontario, in recent years S deficiencies in crops with high S demands, such as alfalfa (Medicago sativa L.) and canola (Brassica napus L.), have been becoming more common. To begin establishing S fertilization recommendations for southern Ontario growers, field trials were established in the 2013, 2014 and 2015 growing seasons (2015 only in alfalfa), to assess if these crops showed consistent yield responses to S application. Canola showed variable response, with 4 of 10 trial locations showing significant yield improvement. Alfalfa responded more consistently, especially in second and third cuts, with 5 of 7 field locations showing significant response to S in at least one cut and/or total yield. Results also showed tissue testing to be a more reliable means of anticipating the need for S application than soil S extraction (by Mehlich III) in both crops.
Table of Contents

List of Tables .............................................................................................................................................V

List of Figures ............................................................................................................................................viii

Chapter 1: The state of sulphur availability in Ontario soils, and its role in the growth and development of select Ontario field crops ........................................................................................................1
  1.1 Introduction ........................................................................................................................................1
  1.2 Background ......................................................................................................................................1
    1.2.1 Sulphur in the Plant ....................................................................................................................1
    1.2.2 Sulphur in Soil ............................................................................................................................4

Chapter 2: Use of sulphur fertilization to improve canola (Brassica napus L.) yields in southern Ontario and the use of tissue and soil testing to predict response .....................................................8
  2.1 Introduction ......................................................................................................................................8
  2.2 Background .....................................................................................................................................8
  2.3 Hypothesis and Objectives ............................................................................................................16
  2.4 Materials and Methods ..................................................................................................................16
  2.5 Results and Discussion ....................................................................................................................25
    2.5.1 Canola Seed Yield ......................................................................................................................25
    2.5.2 Nitrogen-Sulphur Interaction .....................................................................................................34
    2.5.3 Tissue and Soil S Concentration ..............................................................................................36
    2.5.4 Economics of Sulphur Application on Canola ......................................................................48
  2.6 Conclusion .....................................................................................................................................49

Chapter 3: Use of sulphur fertilization to improve alfalfa (Medicago sativa L.) hay yield and quality in southern Ontario ......................................................................................................................51
  3.1 Background .....................................................................................................................................51
    3.1.1 Sulphur Fertilization of Alfalfa .................................................................................................54
  3.2 Hypothesis and Objectives .............................................................................................................57
  3.3 Materials and Methods ..................................................................................................................58
  3.4 Results and Discussion ....................................................................................................................69
    3.4.1 Yield and Composition of Alfalfa Hay .....................................................................................69
    3.4.2 Economics of Sulphur Application on Alfalfa ......................................................................103
    3.4.3 Quality of Alfalfa Hay ..............................................................................................................108
  3.5 Conclusions ...................................................................................................................................112

Chapter 4: Predicting alfalfa (Medicago sativa L.) yield response to sulphur fertilization based on soil and tissue testing, in southern Ontario .......................................................................................114
  4.1 Background .....................................................................................................................................114
    4.1.1 Determining Sulphur Levels in the Field .................................................................................116
4.2 Hypothesis and Objectives .................................................................................................119
4.3 Materials and Methods .......................................................................................................120
4.4 Results and Discussion .........................................................................................................129
  4.4.1 Use of Top 15 cm Tissue Sulphur Concentration to Predict Fertilizer S Requirement ..................................................................................................................................129
  4.4.2 Use of Sulphur Concentration of Hay Samples to Predict Fertilizer S Requirement .........................................................................................................................146
  4.4.3 Use of Soil Sulphur Concentration to Predict Fertilizer S Requirement ......................150
4.5 Conclusion ..............................................................................................................................158

Chapter 5: The state of sulphur availability in Ontario soils, and its influence on the yields and quality of canola (Brassica napus L.) and alfalfa (Medicago sativa L.) in Ontario .......................................................159
  5.1 Canola Response ....................................................................................................................159
  5.2 Alfalfa Response ....................................................................................................................162

List of References ........................................................................................................................167
List of Tables

2.1: Soil type, cultivar planted, spray information and dates of trial activities at 2013 and 2014 trial Locations...........................................................................................................................................21

2.2: Soil test nutrient values of canola trial locations, sampled at trial establishment, before fertilizer application, spring 2013 and 2014 .................................................................................................................................22

2.3: Mean canola seed yield at Elora Research Station trial based on total nitrogen and sulphur application rate (applied as combinations of urea, ammonium sulphate and potassium sulphate), summer 2014..............................................................................................................................................................36

2.4: Extractable sulphur in soil (by Mehlich III) in ppm of soil samples split into 0-15 cm and 15-30 cm depths, taken pre-plant, at rosette stage tissue sampling, and in fall post-harvest, based on sulphur application rate, at all 2013 and 2014 trial locations..........................................................37

2.5: Least Squares mean tissue sulphur concentration of whole above ground canola plants sampled at rosette stage in May and June of 2013, as related to sulphur application rate..........................44

2.6: Calculated cost of sulphur (S) per hectare, when applied as ammonium sulphate (A.S.), at different application rates, and the respective yield improvement required to pay for the cost of the S fertilizer.................................................................................................................................47

3.1: Soil texture, age of alfalfa stand, and results of soil sampling completed at trial establishment on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations, prior to application of sulphur fertilizer..................................................................................................................................................63

3.2: Date of trial establishment, fertilizer application, tissue and soil sampling and hay cutting completion at all 2013, 2014 and 2015 trial locations used in sulphur application research trial in southern Ontario........................................................................................................................................68

3.3: Yield, tissue sulphur concentration and calculated nutrient uptake results of 1st and 3rd cut alfalfa hay at Fergus, Ontario trial location, summer 2012.................................................................................................................................70

3.4: Yield, whole plant tissue sulphur concentration and calculated sulphur uptake of each cut taken at Wallenstein, Hesson and Mitchell, Ontario trial locations based in level of sulphur application, summer 2013............................................................................................................................................71

3.5: Least squares mean dry matter (DM) yields, whole above ground hay sample tissue sulphur concentration, and uptake of sulphur by each hay cut completed in the second trial year growing season at Wallenstein, Ontario location based on sulphur application rate, and whether

v
or not the application was completed only in the first year, or in both first and second year of trial...

3.6: Least square mean dry matter (DM) yields, whole above ground hay sample tissue sulphur concentration, and uptake of sulphur by each hay cut completed in the second trial year growing season at Hesson, Ontario location based on sulphur application rate, and whether or not the application was completed only in the first year, or in both first and second year of trial........76

3.7: Mean composition of hay cuts as percentage alfalfa of hay, hay sample tissue sulphur (S) concentration, and uptake of sulphur by each hay cut completed at Ancaster, Ontario trial location based on sulphur application rate............................................................85

3.8: Least square mean dry matter (DM) yields, whole above ground hay sample tissue sulphur (S) concentration, and uptake of sulphur by each hay cut completed at Mitchell, Ontario trial location based on sulphur application rate............................................................93

3.9: Dry matter(DM) yield, tissue sulphur (S) concentration of hay samples and uptake of each cut of a mixed alfalfa-grass hay stand at the Elora Research Station, Ontario, based on select sulphur treatments, summer 2014........................................................................................................95

3.10: Dry matter (DM) yield of each cut of a mixed alfalfa-grass hay stand at the Elora Research Station, Ontario, based on select sulphur treatments, summer 2015.................................98

3.11: First, second and third cut yield in 2015 growing season of a mixed alfalfa grass hay stand at the Elora Research Station, as related to the application of sulphur (S) when applied as various treatments in year prior to yield collection (residual), or if treatment was applied in year prior with an additional 40 kg S/ha applied as potassium sulphate (K₂SO₄) in spring of 2015..........101

3.12: Crude Protein and crude protein produced per hectare of second cut of mixed alfalfa-grass hay stands at three 2013 trial locations, and two 2014 trial locations, based on the sulphur treatment applied........................................................................................................109

4.1: Dates of trial activities and sulphur fertilization of alfalfa trials for the 2013 and 2014 growing seasons............................................................................................................................123

4.2: Soil texture, age of alfalfa stand, and results of soil sampling completed at trial establishment on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations, prior to application of sulphur fertilizer.................................................................125

4.3: Yield, tissue sulphur concentration of top 15 cm of alfalfa growth sampled prior to first and second cut, and tissue S concentration of alfalfa-grass mixed stand hay samples taken from each cut completed at Hesson, Wallenstein and Mitchell, Ontario trial locations, based on sulphur application, summer 2013........................................................................................................130

4.4: Least square mean dry matter (DM) yields, top 15 cm alfalfa tissue sulphur (S) concentration, and
whole above ground hay sample tissue S concentration of each hay cut completed in the second trial year growing season at Wallenstein, Ontario location based on sulphur application rate, and whether or not the application was completed only in the first year, or in both first and second year of trial, summer 2014..........................................................................................................................................................132

4.5: Least square mean dry matter (DM) yields, top 15 cm alfalfa tissue sulphur (S) concentration, and whole above ground hay sample tissue S concentration of each hay cut completed in the second trial year growing season at Hesson, Ontario location based on sulphur application rate, and whether or not the application was completed only in the first year, or in both first and second year of trial, summer 2014..........................................................................................................................................................133

4.6: Dry matter (DM) yield, tissue S concentration in top 15 cm of alfalfa growth sampled just prior to cutting, as well as tissue concentration of mixed alfalfa-grass hay samples, at Elora and Mitchell Ontario trial locations, based on sulphur application rate and type, summer 2014........136

4.7: Sulphur concentration in soil samples completed in fall prior to growing season, spring prior to growing season, and following first cut of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations in southern Ontario, when no sulphur fertilizer was applied, and whether or not significant response to sulphur fertilization was seen at that trial location when some form of sulphur application was made..............................................................................................................................................151

5.1: Calculated cost of sulphur (S) per hectare, when applied as ammonium sulphate (A.S.), at different application rates, and the respective yield improvement required to pay for the cost of the S fertilizer..........................................................................................................................................................163
List of Figures

2.1: Linear regression of dry matter canola seed yield at Owen Sound’13 (top left), Durham’13 (top right), Listowel’13 (middle left), Paisley’13 (middle right) and Shelburne’13 (bottom left) trial locations in fall 2013, based on sulphur (S) application rate.................................................................26

2.2: Harvest dry matter (DM) canola seed yield at Listowel, Camilla, Owen Sound, Kimberley and Elora, Ontario trial locations in fall 2014, as related to spring sulphur application rate, applied as ammonium sulphate.................................................................................................................................29

2.3: Soil test sulphur (S) of soils sampled in early June 2014, when canola at rosette stage (5-7 leaf) at all 2014 trial locations, as related to S application rate, applied as ammonium sulphate........38

2.4: Dry matter canola seed yield at Elora Research Station trial location as related to soil test sulphur concentration of soil samples taken in early June, 2014.................................................................41

2.5: Dry matter canola seed yield at Camilla trial location as related to soil test sulphur concentration of soil samples taken in early June, 2014.................................................................................41

2.6: Relationship between delta yield, as calculated by subtracting the yield of the control from the yield achieved through application of sulphur (S) as potassium sulphate, on a per replicate basis, and spring soil sulphur concentration as measured by Mehlich III extraction, in the 0-15 cm, and 15-30 cm soil depth, as well as the average of the two depths, of the respective control plot, at all 2013 and 2014 trial locations..................................................................................................................42

2.7: Linear regressions of tissue sulphur concentration of whole above ground canola plant samples taken at rosette stage as related to sulphur application rate at 2014 locations..............................45

2.8: Dry matter canola seed yield at all 2013 and 2014 trial locations based on corresponding tissue sulphur concentration of whole plant above ground canola plants sampled at rosette stage....47

3.1: Alfalfa-grass mixed hay stand dry matter (DM) yield of each hay cut completed at Mitchell, trial location, based on sulphur (S) application rate, summer 2014.................................................................80

3.2: Total combined dry matter (DM) yield of all three cuts completed of an alfalfa-grass mixed hay stand at Mitchell, Ontario trial location, based on sulphur (S) application rate, summer 2014..80

3.3: Percentage of alfalfa in grab samples taken from each cut of a mixed alfalfa-grass hay stand at the Mitchell trial location, based on sulphur application rate, summer 2014.........................81

3.4: Tissue sulphur concentration of first cut, second cut, and third cut at Mitchell, Ontario trial location based on the S rate applied, summer 2014..........................................................82

3.5: Total sulphur (S) uptake by three cuts of mixed alfalfa-grass hay stand as calculated based on S
3.6: Dry matter yield of each cut of a mixed alfalfa-grass hay stand completed at Ancaster, Ontario trial location, based on the sulphur application rate, summer 2014.................................................................83

3.7: Dry matter yield of each cut of a mixed alfalfa-grass hay stand completed at the Elora Research Station, Ontario, based on sulphur application rate, summer 2014.................................................................87

3.8: Dry matter yield of second and third cut of mixed alfalfa-grass hay stand completed at the Elora Research Station, Ontario, based on sulphur application rate, summer 2014.............87

3.9: Total combined dry matter yield of three cuts completed at the Elora Research Station, Ontario trial, based on sulphur application rate, summer 2014.................................................................88

3.10: Composition of each hay cut of a mixed alfalfa-grass hay stand completed at Elora Research Station, Ontario given as percent alfalfa in hay sample based on sulphur application rate, summer 2014........................................................................................................88

3.11: Concentration of sulphur in hay samples of first, second and third cut of a mixed alfalfa-grass hay stand at Elora Research Station, Ontario trial, based on S application rate, summer 2014........89

3.12: Relationship between uptake of sulphur (S) by first, second, and third cut, as well as the three cuts combined of a mixed alfalfa-grass hay stand at the Elora Research Station, Ontario trial, and S application rate, summer 2014........................................................................................................90

3.13: Relationship between dry matter (DM) yield of a mixed alfalfa-grass hay stand of first, second, and third cut, as well as the three cuts combined at the Elora Research Station, Ontario trial, and the S application rate, summer 2015.................................................................96

3.14: Dry matter (DM) yield of first cut of a mixed alfalfa-grass hay stand in the summer of 2015, based on sulphur application, when S applied either: 1) at different application rates solely in spring 2014, or 2) when different spring 2014 rates used in combination with an additional 40 kg S/ha as K$_2$SO$_4$ applied in spring 2015.................................................................................................99

3.15: Dry matter (DM) yield of second cut of a mixed alfalfa-grass hay stand in the summer of 2015, based on sulphur application, when S applied either: 1) at different application rates solely in spring 2014, or 2) when different spring 2014 rates used in combination with an additional 40 kg S/ha as K$_2$SO$_4$ applied in spring 2015.................................................................................................99

3.16: Dry matter (DM) yield of third cut of a mixed alfalfa-grass hay stand in the summer of 2015,
based on sulphur application, when S applied either: 1) at different application rates solely in
spring 2014, or 2) when different spring 2014 rates used in combination with an additional 40
kg S/ha as \( K_2SO_4 \) applied in spring 2015.

3.17: Mean total combined dry matter (DM) yield increase due to sulphur (S) fertilization that was
required by an alfalfa-grass hay stand at 2013 and 2014 Ontario trial locations to cover the cost
of the S applied, when S applied as potassium sulphate (\( K_2SO_4 \))

3.18: Mean total combined dry matter (DM) yield increase due to sulphur (S) fertilization that was
required by an alfalfa-grass hay stand at 2013 and 2014 Ontario trial locations to cover the cost
of the S applied, when S is applied as elemental sulphur.

3.19: Crude protein produced per hectare by second cut of a mixed alfalfa-grass hay stand at Elora,
Mitchell, and Ancaster research trial locations, based on sulphur application rate, summer 2014.

4.1: Concentration of sulphur (S) in the top 15 cm of randomly sampled alfalfa in mixed alfalfa-grass
hay stands sampled prior to first and second cuts, when alfalfa was approximately late bud to
early bloom stage, based on the amount of S applied at three trial locations; Ancaster, Mitchell
and Elora research Station, Ontario, summer 2014.

4.2: Dry Matter (DM) yield of each cut of mixed alfalfa-grass hay stand at Elora, Mitchell and Ancaster,
Ontario trial locations, based on tissue sulphur concentration of top 15 cm of alfalfa tissue
sampled prior to first cut, summer 2014.

4.3: Dry matter (DM) yield of each cut of mixed alfalfa-grass hay stand at Elora, Mitchell and Ancaster,
Ontario trial locations, based on tissue sulphur concentration of top 15 cm of alfalfa tissue
sampled just prior to second cut, summer 2014.

4.4: Dry matter yield of first cut of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations
based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, sampled prior
to first cut, in summer 2013 or 2014.

4.5: Dry matter yield of second cut of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations
based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, sampled prior
to first cut, in summer 2013 or 2014.

4.6: Dry matter yield of third cut of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations
where third cut was completed, based on corresponding tissue sulphur (S) content of top 15 cm
of alfalfa growth, sampled prior to first cut, in summer 2013 or 2014.

4.7: Dry matter total combined yield of all cuts of mixed alfalfa-grass hay stands at all 2013 and 2014
trial locations based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth,
sampled prior to first cut, in summer 2013 or 2014.
4.8: Dry matter second cut yield of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, sampled prior to second cut, in summer 2013 or 2014 .......................................................... 144

4.9: Dry matter third cut yield of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations where third cut was completed based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, sampled prior to second cut, in summer 2013 or 2014 .......................................................... 144

4.10: Dry matter total combined yield* of all cuts completed on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, sampled prior to second cut, in summer 2013 or 2014 ........................................................................................................................................... 145

4.11: Dry matter first cut yield completed on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of first cut hay samples, in summer 2013 or 2014 ........................................................................................................................................... 148

4.12: Dry matter second cut yield completed on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of first cut hay samples, in summer 2013 or 2014 ........................................................................................................................................... 148

4.13: Dry matter third cut yield completed on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of first cut hay samples, in summer 2013 or 2014 ........................................................................................................................................... 149

4.14: Dry matter combined yield of each cut completed on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of first cut hay samples, in summer 2013 or 2014 ........................................................................................................................................... 149

4.15: Relationship between delta yield, as calculated by subtracting the yield of the control from the yield achieved through application of sulphur (S) as potassium sulphate, on a per replicate basis, and spring soil sulphur concentration as measured by Mehlich III extraction, in the 0-15 cm soil depth of the respective control plots ........................................................................................................................................... 153

4.16: Relationship between delta yield, as calculated by subtracting the yield of the control from the yield achieved through application of sulphur (S) as potassium sulphate, on a per replicate basis, and spring soil sulphur concentration as measured by Mehlich III extraction, in the 15-30 cm soil depth of the respective control plots ........................................................................................................................................... 154

4.17: Relationship between delta yield, as calculated by subtracting the yield of the control from the yield achieved through application of sulphur (S) as potassium sulphate, on a per replicate basis, and the average spring soil sulphur concentration as measured by Mehlich III extraction, of the 0-15 cm and 15-30 cm soil depth of the respective control plots ........................................................................................................................................... 155
CHAPTER 1: THE STATE OF SULPHUR AVAILABILITY IN ONTARIO SOILS, AND ITS ROLE IN THE GROWTH AND DEVELOPMENT OF SELECT ONTARIO FIELD CROPS

1.1 Introduction:

The drastic decreases in acid rain deposition in Southern Ontario in the past few decades, despite being positive for the environment, may be having negative impacts on crop nutrient availability. Sulphur (S) deficiency, once rare in southern Ontario, is now becoming increasingly prevalent in field crops presumably due to decreasing S depositions as acid rain, caused by the reduction in S emissions from industries (Hoeft and Fox 1986), and genetic improvements in field crops leading to higher yields and higher S requirements (Hillel 2005). This has a negative impact on growing crops, as S is an essential nutrient. However, it is possible for farmers to mitigate or avoid S deficiencies through proper S fertilization.

1.2 Background:

1.2.1 Sulphur in the Plant:

Sulphur has long been recognized as an essential nutrient in plant growth. It is a major building block of many crucial proteins and compounds in the plant (Hillel 2005; Heldt and Piechulla 2011). Both cysteine and methionine are synthesized using S in the plant; a process which is also essential to animal life, as animals cannot synthesize these two amino acids and must obtain them, either directly or indirectly, from plants (Heldt and Piechulla 2011). Other essential compounds synthesized from S in plants are coenzyme A, thiamine and biotin (Hillel 2005). Another crucial role of S once in a plant is its role in forming the disulphide bonds which hold polypeptide chains together (Hillel 2005). The overall role of S in protein synthesis is essential in plants, especially to attain the high growth rates, and economic yields that are expected from many agricultural crops.
Sulphate (SO\textsubscript{4}), an oxidized form of S, is taken up from the soil by sulphate transporters on the roots of plants, and is transported to the chloroplasts mainly on tripeptide glutathione, with any excess S being stored in vacuoles (Hillel 2005; Heldt and Piechulla 2011; Hawkesford 2012). A plant deficient in S will have a low concentration of glutathione in the stem, which in turn, will stimulate roots to produce more sulphate transporters to increase S uptake potential by roots (Hillel 2005). Sulphur dioxide gas can also be taken up by the leaves of the plant; however, S taken up in this form is minimal and does not serve to repress the sulphate taken up by roots, therefore sulphur dioxide uptake is not seen as an efficient means of S uptake (Hawkesford 2012).

In the case of a deficiency of S, protein synthesis is inhibited, and as a result, growth is reduced, with a corresponding build-up of non-S containing amino acids within the plant (Hillel 2005). The deficiency will appear as smaller plants, with thin, brittle stems, and yellow colouring (Hillel 2005). Chloroplasts begin to break down as a result of low levels of S, and an overall decrease in the rate of photosynthesis is seen (Hillel 2005). Due to the limited ability of the plant to remobilize S from older leaves in the plant, the yellowing of leaves will first appear on the newest growth of the plant (Hillel 2005). The reduction in growth, protein synthesis, and photosynthetic potential of crops created by S deficiency will result in reduced yields for farmers. The overall S requirement of a growing crop will be a function of the S available from the soil, and the plant’s demand for S depending on growth stage and any stress it experiences (Hawkesford 2012).

Of field crops grown in Ontario, alfalfa (Medicago sativa L.) and canola (Brassica napus L.) have relatively greater S requirements. In canola, the requirement for S is heightened due to the high overall protein content, production of seeds containing high protein levels, and the further production of S-containing glucosinolates found in the tissue of the plant (Grant et al. 2012). This combination leads to high rates of S uptake by canola, with research in western Canada indicating that a canola harvest of
1960 kg per hectare would remove approximately 12 to 14 kg of S per hectare through the harvesting of the seed, with about 20-24 kg of S per hectare having been taken up in total for growth and development (Grant et al. 2012). In Ontario, average yields have been steadily increasing in the past number of years, with an average yield of almost 2200 kg per hectare from 2006 to 2014 (Kulasekra 2014), so S uptake and removal in Ontario could be estimated at 27 kg S/ha and 15 kg S/ha, respectively. Amongst field crops, these removal rates are second only to alfalfa, a common Ontario forage crop. Alfalfa has high S requirements due to its high protein content and biomass production (Hoeft and Fox 1986; Schulte and Kelling 1992; Dick et al. 2008). Overall, alfalfa requires approximately 2.5 kg of S Mg\(^{-1}\) of biomass, and as a forage crop, much of that S is removed from the field (Kost et al. 2008; Bagg and Ball 2014). In Ontario, yield averages for hay stands (most of which are alfalfa based) were estimated to be 5.5 tonnes per hectare per year for the period of 2011 to 2015 (Kulasekera 2015). This amount of biomass production would result in a removal of at least 13.8 kg of S per hectare.

In the past, adequate levels of S were supplied to these crops in southern Ontario through atmospheric deposition in the form of acid rain (Bates and Sheard 1988). According to the Canada-United States Air Quality Agreement Progress Report 2012, in 1990 southern Ontario received between 28 and 45 kg of wet sulphate (SO\(_4\)) per hectare per year (Environment Canada 2013). Based on the canola and alfalfa S requirements previously discussed, S deposition rates in Ontario at that time would have been sufficient to meet the needs of these two crops and there was no need for S fertilization (Ontario Agronomy Guide 2009). Therefore, there are no fertilizer recommendations for S in southern Ontario (Ontario Agronomy Guide for Field Crops 2009). However, with the burning of cleaner fuels, scrubbing of smokestacks, and other pollution control measures, there have been drastic decreases in annual S deposition rates (Hoeft and Fox 1986). By the year 2000, S deposition rates had decreased to approximately 16 to 24 kg wet SO\(_4\) per hectare per year, with a further decrease to the year 2010, to 4 to 8 kg of wet SO\(_4\) per hectare per year overall (Environment Canada 2013). In combination with lower
soil S additions, comes the constant genetic improvements being accomplished by plant breeding, resulting in higher yields, and therefore higher removal rates of S with harvest (Hoeft and Fox 1986; Hillel 2005). These factors are all contributing to apparent S deficiencies becoming more common in southern Ontario.

1.2.2 Sulphur in Soil:

It is estimated that in topsoil, over 90% of the S is found in organic forms, for example, organic sulphides, sulfates and carbon-bonded S (Schoenau and Malhi 2008). This can come from several sources, such as organic matter in the soil, or fertilizer and manure applications (Kelling 2000). Some of the S in the organic S pool can be mineralized by soil microorganisms; the amount will depend on the proportion of carbon-bonded S contained in the soil or organic matter, as it is fairly resistant to decomposition (Schoenau and Malhi 2008). The microbial oxidation of organic forms of S is done by both heterotrophic and autotrophic bacteria in the soil, and it is essential to plants, as they cannot take up organic S forms, but instead must take up oxidized forms of S; primarily sulphate (Jones and Ruckman 1966; Janzen and Bettany 1986; Hillel 2005; Schoenau and Malhi 2008). The elemental form of S has low solubility and is hydrophobic, making it stable in the soil profile, until it undergoes microbial oxidation into sulphate, a process dependant on particle size (affecting surface area and thereby microbial access) and environmental factors such as moisture, temperature and pH (Barrow 1975; MacLachlan 1975; Erikson 2008; Schoenau and Malhi 2008). In acidic soils there is some adsorption of sulphate onto metal oxides in the soil (Erikson 2008). However, in soil pH over 6 there is little adsorption of the sulphate form onto soil particles, leaving sulphate in solution and resulting in it being almost as mobile as nitrate-N in the soil (Erikson 2008; Schoenau and Malhi 2008). In southern Ontario, where soils are commonly neutral to alkaline, especially with the intervention of liming (Ontario Agronomy Guide for Field Crops 2009), sulphate in the soil can easily be lost to leaching below the plant rooting
zone (Erikson 2008; Schoenau and Malhi 2008). In general, organic matter and manure additions to soil will contain S, and as both are decomposed, sulphate will be released and will be available to plants (Hoeft and Fox 1986; Franzen and Grant 2008; Ketterings et al. 2012b). In this way, high organic matter soils, and soils with recent manure additions will tend to have higher available soil S levels, while low organic matter soils may be more prone to S deficiencies. Coarse to medium textured soils may also be more prone to S deficiencies; due to the high leachability of sulphate it can be easily lost from soils through which water can move rapidly, especially in climates or seasons where rainfall is high (Seim et al. 1969; Hoeft and Walsh 1975; Janzen and Bettany 1986; Schulte and Kelling 1992; Franzen and Grant 2008). Due to the variety of factors that can affect the levels of organic S and sulphate in the soil such as organic matter levels, microbial activity, temperature, moisture, soil types, etc., it is common for soil S levels to vary widely across a relatively small area, with areas of deficient and sufficient S levels likely occurring in the same field (Franzen and Grant 2008).

This knowledge of S behaviour in soil is essential to effective S fertilization of crops. While foliar application methods are being used in some areas as a means of S fertilization, it does require either spraying of the crop which may not be otherwise necessary in field crops, especially in alfalfa, or for fertilizer to be applied in irrigation water, systems that are not common to Ontario. As a foliar spray, magnesium sulphate has been added as a source of S; however, research has shown that most of the S delivered in this way is translocated immediately into the vacoules of the plant and becomes metabolically inactive (Haneklaus et al. 2008). A trial by Bose et al. (2009) did achieve positive yield response to foliar application of liquid fertilizer (33% S and 12% N); however, the foliar fertilizer was applied 3 times throughout the growing season, requiring more trips over a field than with a pre-plant dry broadcast fertilizer application. Currently the most practical means of incorporating S fertilization into current farming practices, while also achieving high plant availability, is to soil apply S-containing fertilizers.
There are two common types of soil-applied S fertilizer, sulphate and elemental S, each with its own advantages and disadvantages due to how S is released and moves through soils. Sulphate forms of fertilizer are chemically bonded to other plant nutrients, such as ammonium sulphate, potassium sulphate, and potassium-magnesium sulphate (Koenig et al. 2009). These sulphate forms of fertilizer are immediately plant available and readily taken up, and have been found to have a higher rate of plant uptake than other types of S fertilizer (Mahli 2005). However, as discussed, sulphate can be easily leached from the soil profile, especially in sandier soils and soils with high pH, and a yearly re-application of sulphate is therefore recommended (Hoeft and Fox 1986; Janzen and Bettany 1986; Schulte and Kelling 1986; Schoenau and Malhi 2008). Also, due to high leachability, sulphate fertilizers are commonly spring applied to avoid losses in winter and early spring, or used as an in-season correctional method once S deficiency has been identified (Janzen and Bettany 1986). The amount of S contained in these sulphate fertilizers varies somewhat, with ammonium sulphate containing approximately 24% S by weight, and potassium sulphate containing approximately 18% S by weight (Koenig et al. 2009). Sulphate fertilizer forms do have an additional advantage to farmers as they also include other elements essential to plants; in the case of canola, ammonium sulphate can be applied, so as to begin to meet some of the crop’s N requirement while applying S, while in alfalfa, potassium sulphate is often used due to the crop also having high potassium requirements (Koenig et al. 2009; Ontario Agronomy Guide 2009).

The second main type of S fertilizer used is elemental S, which contains approximately 88 to 98% sulfur, depending on its level of purity (Koenig et al. 2009). Unlike sulphate which is immediately plant available, elemental S must first be oxidized into sulphate by soil microorganisms to become plant available (Schulte and Kelling 1992; Schoenau and Mahli 2008). This process is impacted by how much access the soil microbes have to the fertilizer based on granule size and incorporation into the soil, as well as by environmental factors such as moisture and temperature which will impact the activity level of the soil microbes (Schulte and Kelling 1992; Erikson 2008). Elemental S can also be ground into
powder and then compressed back to pellet form. This allows for the benefits of a higher accessibility to soil microbes of a powder to combine with the ease of handling and application given by a pelleted fertilizer. Due to the requirement for microbial oxidation to become plant available, elemental S is generally applied in the fall, to ensure it has adequate time to become plant available for the next spring, and because it is stable in the soil profile there is little concern over it leaching out of the plant rooting zone (Hoeft and Walsh 1975; Janzen and Bettany 1986).

These two fertilizer types have both been shown to correct S deficiencies in field trials, and can adequately supply S to both alfalfa and canola crops (Mahli 2005). Studies examining S fertilization on a variety of crops, including alfalfa and canola, have been ongoing in the United States and Western Canada, where S deficiency has been an ongoing issue, due to sandier soil types in some regions in which leaching of soil sulphate is more prevalent, low organic matter soils, or regions with soils low in natural S reserves, as well as due to lower pollution levels in western North America causing a lower supply of wet S deposition in western Canada and western United States (Franzen and Grant 2008; National Atmospheric Deposition Program 2014). In southern Ontario, past S deposition rates have been high, and the Ontario Agronomy Guide (2009), while acknowledging that S requirements of both alfalfa and canola are high, states that S in acid rain deposition is generally adequate to meet the needs of both crops, and S deficiencies have been rare. With the recent reductions in deposition rates in southern Ontario and understanding the importance of S to the growth and development of high yielding alfalfa and canola cultivars, the need for S fertilization of crops in southern Ontario should be reassessed.
CHAPTER 2: USE OF SULPHUR FERTILIZATION TO IMPROVE CANOLA (BRASSICA NAPUS L.) YIELDS IN SOUTHERN ONTARIO AND THE USE OF TISSUE AND SOIL TESTING TO PREDICT RESPONSE

2.1 Introduction:

Sulphur (S) is an essential element for growth and protein formation in all plants. Canola (Brassica napus L.) is a field crop with relatively high S requirements. In the past, S deficiencies in southern Ontario-grown canola have been rare due to a history of high S deposition rates. As such there are no current recommendations for S application on canola in southern Ontario (Ontario Agronomy Guide 2009). However, in the past number of years deposition rates have decreased and indications of deficiencies have been becoming more common; therefore new work on S requirements of canola in southern Ontario is required.

2.2 Background:

Sulphur serves as one of the building blocks of proteins in the plant, with a role in the synthesis of the amino acids cysteine and methionine, the formation of disulphide bonds, and in many other compounds such as co-enzyme A, thiamin and biotin (Hillel 2005; Heldt and Piechulla 2011). Sulphur also plays a role in the process of photosynthesis in the chloroplasts of the plant (Franzen and Grant 2008). As the processes which utilize S in the plant also commonly use nitrogen, phosphorus and other basic elements, proper S nutrition is also crucial to the efficient use of other nutrients by the plant (Franzen and Grant 2008; Haneklaus et al. 2008; The Sulphur Institute 2016). For these reasons, all plants require S; however, greater S uptake is required in agricultural crops with high S concentrations and productivity.

One high-S-requiring field crop grown in southern Ontario is canola (Brassica napus L.). The overall requirement for S in canola is heightened due to the: high protein content of plant tissue, production of seeds containing high protein levels as well as the S-containing amino acids cysteine and
methionine, and the further production of S-containing glucosinolates found in the tissue of the plant (Grant et al. 2012). Research in western Canada has shown a canola yield of 1960 kg per hectare would remove approximately 12 to 14 kg of S per hectare through the harvesting of the seed with about 20-24 kg of S per hectare being taken up in total for growth and development (Grant et al. 2012). That is approximately 0.012 kg of S taken up per kg of seed yield per hectare. The International Plant Nutrition Institute (2013) suggests similar amounts of an approximate 5 kg of S removed from a field with each tonne of seed harvested per hectare. In Ontario, average yields were almost 2240 kg per hectare from 2012 to 2016 (Kulasekra 2016), and using similar uptake to yield ratios, approximately 27 kg of S would be taken up per hectare by an average Ontario canola crop.

It is likely that in the past S deposition has been sufficient to meet the needs of a canola crop. Deposition levels in the early 1990s were 28-45 kg SO₄/ha/year (9.3 to 15 kg S/ha/year) (Environment Canada 2013). However, the annual rates of S deposition have been steadily decreasing, due, at least in part, to the burning of cleaner fuels and the scrubbing of smokestacks (Hoef and Fox 1986). By 2000, wet deposition of SO₄ had decreased to approximately 16-24 kg SO₄ per hectare per year (5.3 to 7.9 kg S/ha/year) (Environment Canada 2013), while total wet and dry deposition along the southern shore of Lake Erie was found to be approximately 25 kg S/ha/year (National Atmospheric Deposition Program 2015). Further decrease was seen to the year 2010, at which point southern Ontario was receiving only 4 to 8 kg of SO₄ per hectare per year (1.3 to 2.7 kg S/ha/year) (Environment Canada 2013), and total wet and dry deposition along the southern shore of Lake Erie had also decreased to between 10 and 20 kg S/ha/year (National Atmospheric Deposition Program 2015).

With lower wet SO₄ deposition rates, combined with the ongoing crop genetic research leading to increasing yields, S requirements of canola may no longer be satisfied by current soil S levels. Sulphur deficiency symptoms vary depending on the time in the plant’s life cycle at which the deficiency occurs
In canola, if a deficiency starts in the rosette or bolting stages, the earliest signs are chlorosis of the leaves beginning on the edges of the younger leaves of the plant, purpling of the underside of the leaf due to increased levels of the pigment anthocyanin, as well as possible “cupping” of the leaves and reduced growth (Thomas 2003). A deficiency that continues into, or begins during, the flowering stages will lead to petals or flowers that are pale yellow or even white in colouring, lower pollen production, and overall delayed and prolonged flowering (Thomas 2003; Franzen and Grant 2008). The negative effects on flowering can carry into the podding and seed set stages, with deficiency during this time resulting in fewer pods per plant, fewer seeds per pod and lower weight per seed (Thomas 2003; Franzen and Grant 2008). Due to the high protein, oil and fatty acid content of canola seed, the role of S in protein synthesis is also critical to maintaining the oil quality and high protein content of the seed itself (Fismes et al. 2000; Franzen and Grant 2008). The addition of S to fertilization regimes has been shown to increase the overall oil yield (Bose et al. 2009). Other trials have found increasing oil content with increasing S application, up to a rate of 60 kg S/ha, after which point the protein content of seeds continued to increase, while oil content decreased (Jan et al. 2002). Therefore, adequate and properly balanced S nutrition, especially during flowering, podding and seed set, is crucial to maintaining high yields, as well as the protein and oil quality of canola.

The use of S and nitrogen (N) in the canola plant are interrelated, with both being used in the processes that determine the oil and fatty acid composition of the seed, as well as the overall protein content in the plant (Fismes et al. 2000). Therefore, it is important to study and understand the potential interactions between N and S nutrition in canola. Studies have shown that if there is inadequate S levels in a field, S deficiency symptoms in the plants will become more pronounced as N application increases (Mahli and Leach 2002; Mahli and Gill 2007). In another experiment, Fismes et al. (2000), found that the nitrogen use efficiency of rapeseed increased when S was applied in addition to N. Nitrogen was applied as cow slurry at a rate of 200 kg N/ha, and the apparent N use efficiency
improved when 75 kg S/ha as ammonium sulphate was applied compared to slurry alone. These, as well as other studies, show evidence that as higher yielding cultivars are used, and N recommendations also increase, the canola crop will require higher levels of S to properly utilize applied N (Fismes et al. 2000; Mahli and Leach 2002; Karamanos et al. 2005). These studies indicate that supplemental S may also need to be applied to assist a canola crop in achieving optimal yields and N use efficiency (Mahli and Gill 2007).

Past research on canola fertilization has used various forms of S, but the most commonly used have been sulphate fertilizers and elemental S. Sulphate fertilizer applications have been found to be effective as an S source and are immediately plant available (Nuttall et al. 1987; Fismes et al. 2000; Ahmad et al. 2011; Mahli 2012; Mahli et al. 2007). In a study by Mahli and Leach (2002), while best results were seen with application at sowing, a within season application of potassium sulphate at bolting, as well as at early flowering, were still successful in decreasing the yield loss caused by the previous S deficiency. Ammonium sulphate, has the additional advantage of supplying the crop with N in addition to S. Greenhouse trials to compare different S fertilizers have found approximately 82 to 86% of the sulphate applied was recovered by canola plants when sulphate fertilizers were used, compared to elemental forms which had recovery rates of 43-53% (Janzen and Bettany 1986). Due to the mobility of sulphate in soils, sulphate fertilizers are best used as a within-season S fertilizer, as they are also readily lost from the rooting zone of canola. Mahli and Leach (2002) found that if application rate is kept constant, sulphate fertilizers are most effective when soil applied just prior to planting. A fall application of sulphate resulted in winter losses, while canola yields with a sulphate application made post planting tended to be lower than those seen when SO$_4$ application was done pre-plant (Mahli and Leach 2002).

Unlike sulphate fertilizers, which are easily lost from the soil, elemental S has the advantage of being stable in the soil profile, but brings with it the drawback of its delayed plant availability. Elemental
S must undergo oxidation by soil microorganisms into sulphate, and therefore must be applied well in advance of anticipated crop need (Schulte and Kelling 1992; Schoenau and Mahli 2008). Trials applying elemental S on canola have found that even when it is fall applied before canola is seeded the next spring, it is not as effective as similar treatments of sulphate S, likely due to the delay in plant availability (Mahli 2005). In a four-year trial by Mahli (2005), plots fertilized annually with either 10 or 20 kg S/ha as elemental S first showed yield improvements in the second to fourth years of the trial, at which point the yields from those plots were still not as high as yields obtained by annual ammonium sulphate fertilization. Even at higher application rates, similar results are likely to be found, as is indicated in a study by Wen et al. (2003). Even at the highest rate of elemental S application used (80 kg S/ha), canola seed yield was only slightly above that of the control treatment (Wen et al. 2003). However, the authors did find that there was residual benefit of elemental S to subsequent crops when applied at 80 kg S/ha (Wen et al. 2003). This trial was completed in Sakatchewan, where soils are generally frozen for a longer period of time in winter, slowing the conversion of elemental S to SO\textsubscript{4} compared to what would be seen in Ontario. This is somewhat of a drawback for canola growers in Ontario, where primarily spring canola is planted unlike in other climatic regions where fall canola can be grown. With spring planted canola, should a grower apply elemental S in the fall, and then decide against planting canola in the following spring, the application of S may be unnecessary. A fall application of elemental S would also require some incorporation to prevent surface runoff of the fertilizer, requiring an additional trip over the field in the fall for the grower. While elemental S does have the benefit of being more cost effective, as well as providing S to subsequent crops, for most Ontario growers, ammonium sulphate is likely the easiest S-containing fertilizer to incorporate into their current agronomic practices to ensure S is supplied to the canola crop.

A soil S test may help to effectively manage fertilization. This can be done by testing soil S levels in the field before, or during growth of the crop. While soil testing is a relatively cheap and convenient
method, the reliability of soil testing for S has been under scrutiny (Kelling 2000; Koenig et al. 2009). Various processes act on S pools in the soil, and make accurate assessment of S levels difficult. Most soil tests report on S in the plant-available sulphate form, which is mobile in the soil and easily lost to leaching (Schoenau and Mahli 2008; Koenig et al. 2009). Organic S is stable in the soil profile, but must first be mineralized, a process which is highly dependent on environmental factors such as temperature, pH and moisture levels (Schoenau and Mahli 2008). Soil S tests are unable to determine, at any given time, how much sulphate is likely to become available in the soil through mineralization and oxidation of organic S. The relationship between S levels in soil and S response by the plant can be hard to establish, and therefore trial results relating yield response to soil test levels have been variable (Sheard 1976; Koenig et al. 2009). Trials conducted in Saskatchewan were able to significantly relate soil test S (by calcium chloride extraction) to the resulting response in canola yield (Nuttall et al. 1987). However, with the more arid climate experienced in Saskatchewan as compared to Ontario, there were likely much lower levels of leaching, and likely lower levels of S becoming available from inorganic sources and S deposition on a yearly basis. In a greenhouse trial conducted by Sheard (1976) using Ontario soils growing a crop of alfalfa, a relationship between soil sulphate levels and alfalfa response to fertilizer could not be found due to the complex release of sulphate from organic matter in the soil, though a yield response to S fertilization was seen. The environment was kept stable, which likely reduced variability of sulphate mineralization rates. In field trials, where mineralization rates would vary based on environmental conditions, soil variability, and with the added factor of variable S deposition rates from the atmosphere, a relationship between soil sulphate levels and crop response is even less likely (Sheard 1976). More recently, in trials conducted in the New Liskeard area in Northern Ontario, soil S tests once again failed to provide an indication of yield response, as initial soil SO₄ levels were similar across trial locations, while yield responses were varied (Rowsell and Kobler 2012). An additional problem is the high spatial variability of sulphate across an individual field. With high levels in one area
masking areas with low S levels where yield response may actually be substantial (Grant et al. 2012). While many extraction methods are available for soil sulphate, no one method has been found that can accurately determine the total inorganic sulfate in soils, and at times also involve costly extractants, which may limit their use if this raises the cost of soil tests for farmers (Kowalenko and Grimmett 2008). Overall, more research must be done in Ontario to determine if soil sampling will be an adequate method to determine plant responsiveness to S fertilization, and to establish the best calibration and extraction method to use for soil tests for S.

To avoid some of the problems related to soil testing, a common method to determine if the S requirement of a crop is being met is to measure the concentration of S in the plant tissue. Based on past research findings, a critical concentration of a nutrient in plant tissue at a specific growth stage has been determined for most crops. The critical concentration is the concentration of the specific nutrient in the plant tissue that is required to obtain optimal yields (Schulte and Kelling 1992; Ontario Agronomy Guide 2009). Studies have found variable results using the critical S concentration method in canola tissue. A canola plant is most sensitive to low S concentrations in tissue from flowering to seed set, and S is vital at this stage to ensure maximum yields (Grant et al. 2012). During this stage, studies out of western Canada have shown critical concentration levels in tissue as high as 0.8% S, depending on the part of the plant sampled, have been required to obtain optimal yields (Beaton and Soper 1986). However, it is hard to correct a deficiency at such a late stage in the plant’s life cycle, and earlier growth stage deficiencies may already have decreased yield potential (Thomas 2003). Ideally, S fertilizer application should be done in advance of crop demand, with best yield results occurring when sulphate fertilizers are applied at planting, and then lightly tilled into the soil (Mahli and Leach 2002). When applied before bolting, a S deficiency was corrected and yields maintained near normal; however, when completed at early flowering, an S application could only moderately improve yields over checks (Mahli and Leach 2002). Therefore, it is important to use tissue S levels at early growth stages as indicators of
an adequate S supply for the plant. Tissue testing should be completed as early as possible in the plant lifecycle, such as at rosette stage, so that any deficiencies can be effectively corrected. One current recommendation indicates that the optimum S level in dry matter (DM) at the rosette stage of canola is 0.25% S or greater, while other work from Europe has found that higher levels of S are optimal, suggesting a basis of 0.35% S in DM just before bolting begins, below which point deficiencies will be experienced (Thomas 2003). Overall, by completing a tissue test at rosette stage, a grower can check that S levels within the plant are adequate for future growth and development, while still allowing for the potential opportunity to correct the deficiency with fertilization.

Studies examining S fertilization of agricultural crops, including canola, have been ongoing in the United States in areas such as Minnesota and Wisconsin, as well as in the northern prairies in Canada, where S deficiency has been a longstanding issue (Bates and Sheard 1988). Deficiencies tend to arise in areas with soils low in natural S reserves, in areas with sufficient rainfall to possibly leach the available sulphate out of the plant rooting zone, and in areas receiving low amounts of wet sulphate deposition (Bates and Sheard 1988; Franzen and Grant 2008). With traditionally lower S deposition levels and higher canola acreage in Western Canada, research into response to S application has been more prevalent than in Ontario. Many trials have seen significant positive canola seed yield response to S application, with corresponding improvement in plant growth and development such as more branches per plant, more pods per plant, and more seeds per pod, as well as improvement in seed quality (Wen et al. 2003; Ahmad 2011; Malhi 2012). In general, researchers have suggested optimal S application rates of 20 to 40 kg of S per hectare, while at minimum suggesting an application of 10 kg of S per hectare to assist areas of the field with low soil S levels (Karamanos et al. 2005; Mahli et al. 2007; Ahmad et al. 2011; Grant et al. 2012).
Currently, the Ontario Ministry of Agriculture and Food (OMAF) has no definite recommendations in its Agronomy Guide for Ontario Farmers in terms of S application rates, as past Ontario field trials had shown no response to S fertilization (Ontario Agronomy Guide 2009). While acknowledging that sulphur requirements for canola are high, the Ontario Agronomy Guide (2009) suggests that deposition levels are high enough to supply adequate sulphur to canola plants, and that where needed, N fertilizer may be substituted for ammonium sulphate to aid S supply. Recently however, in light of decreasing S deposition levels, some work has been completed to assess canola response to S in Ontario. The preliminary results from Ontario trials in 2010 to 2012 had positive response in 80% of trials, culminating in the recommendation of applying 15 to 25 kg of S per hectare on all canola fields as “insurance” against possible deficiencies (Hall 2012).

2.3 Hypothesis and Objectives

As preliminary results have indicated that there is a possibility of canola response to S in Ontario, the objective of this trial was to confirm the need for S fertilization of canola in Ontario, and assess the financial feasibility of its application. Soil and tissue testing were also completed to give a preliminary assessment of their effectiveness as indicators of S requirement in field conditions in Ontario.

2.4 Materials and Methods

To examine the viability and effectiveness of S fertilization of canola in southern Ontario, field trials were established in two growing seasons; summer 2013 and summer 2014. Trial locations were scattered across south central Ontario, in common canola growing areas, mostly on co-operator fields. This was done in an effort to include different soils types and microclimates in Ontario. For the 2013 growing season, five trial locations were established in co-ordination with the Ontario Ministry of Agriculture Food and Rural Affairs. These trials were located in Shelburne (Shelburne’13),
Listowel (Listowel’13), Owen Sound (Owen Sound’13), Durham (Durham’13), and Paisley (Paisley’13) (Table 2.1). All plots established in the 2013 growing season consisted of a replicated complete block design trial, consisting of four treatments:

1) Control with no sulphur applied, and 120 kg N/ha
2) 11.2 kg S/ha and 120 kg N/ha
3) 22.4 kg S/ha and 120 kg N/ha
4) 44.8 kg S/ha and 120 kg N/ha

In these trials, all sulphur was spring applied as ammonium sulphate ((NH$_4$)$_2$SO$_4$). To ensure any yield response seen was in response to sulphur application, the control plots received 120 kg N/ha as urea, while in treatments two, three and four the N supplied by the (NH$_4$)$_2$SO$_4$ was taken into account, and a lesser amount of urea was applied to bring total N applied to 120 kg N/ha. In this way, all plots received a uniform N application, and yield response could be attributed to S. All fertilizer application was done using a Valmar Air-Flow Fertilizer spreader, with a 6 m boom, of which half could be turned off, to also allow for a 3 m application strip.

All of these trials included three replications, except at Shelburne’13 and Paisley’13, where there was only sufficient space for two replications of the four treatments. Unfortunately, in this trial year, there was no randomization of treatments, and each replicate included the treatments in the same order as is listed above. All of these trials were located on co-operator’s fields. They were therefore fairly simple in design, as much of the agronomic work on the trial area would be done by the co-operator. The co-operator’s own harvesting equipment was used, and plots were designed to be relatively wide to allow for this. Plot widths varied slightly across trial locations to accommodate each individual co-operator’s equipment, while also allowing for use of the Valmar Air-Flow Spreader; plots tended to be either 9 or 12 m wide. Plots were designed to be as long as possible so as to obtain a large sample size, while still remaining on level topography and leaving adequate space as headlands. Therefore plot length varied from about 60 m to as long as 150 m.
For the 2014 growing season, five trial locations were established. These were located in: Camilla (Camilla’14), Listowel (Listowel’14), Kimberley (Kimberley’14), Owen Sound (Owen Sound’14), and the Elora Research Station (Elora’14) (Table 2.1). All trials, except Elora’14, were located in co-operator fields and again used a replicated complete block design of the same four treatments used in 2013. In the 2014 trial year, all trial sites contained 3 replications except at Elora’14 where 4 replications were used, and there was randomization of treatments within blocks at all sites. Plot width and length was again determined as described previously, and again, a Valmar Air-Flow Spreader with 6 m boom was used for fertilizer application.

At the Elora’14 trial, where researchers could use small scale equipment for canola harvest, individual plots could be much smaller. This trial had additional treatments and 4 replicates. The treatments were designed not only to examine the response of canola to sulphur application, but also to examine nitrogen-sulphur interactions. The treatments used were as follows:

1) Control with no S or N applied  
2) 5 kg S/ha and 120 kg N/ha  
3) 10 kg S/ha and 120 kg N/ha  
4) 20 kg S/ha and 120 kg N/ha  
5) 40 kg S/ha and 120 kg N/ha  
6) No S applied and 40 kg N/ha  
7) No S applied and 80 kg N/ha  
8) No S applied and 120 kg N/ha  
9) 40 kg S/ha and no N applied  
10) 40 kg S/ha and 40 kg N/ha  
11) 40 kg S/ha and 80 kg N/ha

The treatments were arranged in a two-factor factorial to first examine how the addition of N and S would impact yields, and then, to further assess how the addition of S at those various N rates would affect the plant response. All S was applied as K$_2$SO$_4$ and all N was applied in the form of urea fertilizer. KCl was broadcast on the whole trial area in excess of plant requirements, to ensure that any yield response could not be attributed to K. Plots were designed to allow for ease of fertilizer application and
harvesting, and measured 4.5 m wide by 12 m long. All fertilizer application was done using a Valmar Air-Flow Fertilizer Spreader with a 4.5 m boom.

Sampling at the sites included soil and tissue sampling, as well as grain yield of each plot. The general protocol for soil sampling plots at trials on co-operator fields was to take 20 soil cores at random across each plot, while avoiding the outer 2 m borders of each plot to ensure samples were not taken from areas with possible cross contamination from neighboring plots. On the smaller plots at the Elora’14 location, general protocol was to take 9 soil cores in a “W” pattern across each plot, avoiding the outside 0.75 m borders. In all cases, soil cores were taken to 30 cm depth, and were immediately split into 0-15 cm depth and 15-30 cm depth. Samples were thoroughly mixed on a per plot basis, and then a portion of the soil was filled into plastic bags for transport. Samples were either removed from bags to allow them to air dry, or placed in a drying oven at approximately 30° C as a form of force air-drying. Dried samples were again adequately mixed and a portion of the sample was sent to A&L Laboratories in London, Ontario for analysis. Soil samples taken at trial establishment were analyzed for pH, organic matter content, and soil test levels of P, K, Ca, Mg, Na, Al and S, to act as an aid in assessing the cause for any unexpected nutrient deficiencies encountered in the trial. Subsequent soil samples were only analyzed for S concentration to reduce analysis costs. A Mehlich III (Mehlich 1984) extraction was used for S and determination of S was by inductively-coupled plasma atomic emission spectrometry (ICP-OES).

At all locations across both trial years, once field conditions were conducive to planting, the trial area was established, plots were staked, and initial soil sampling was completed, in this case on a per replication basis, to establish base soil test S levels. The only exception is the Elora’14 trial, where, with challenging weather delaying planting, researchers pushed to complete the trial establishment process and instead soil sampled check plots to establish base soil S levels. When sampling on a per replicate
basis, 20 soil samples were randomly taken from each replicate area, then treated following the soil sampling protocol outlined previously. The results of this initial soil sampling can be found in Table 2.2. The S and N fertilizer treatments at Elora’14 were applied to the appropriate plot areas, followed by 2 passes with a C-tine cultivator to a depth of 10 cm to mix fertilizer into the soil. Tillage was completed with the direction of the long dimension of the plots to minimize fertilizer movement onto neighbouring plots. Following incorporation of fertilizer, the trial area was seeded to canola. Due to the rush of spring planting, at the Owen Sound’13 trial, the trial area was planted by the co-operator prior to trial establishment. In this case, the trial area was staked and soil sampled, followed by fertilizer application, all prior to canola emergence; however, fertilizer could not be incorporated as crop had already been planted. The dates of activities completed at each trial are listed in Table 2.1. Following emergence, the trial area at all locations was examined to ensure a minimum of 75 plants/m$^2$ had uniformly emerged across the entire trial area, as per the target stand population recommended by the Ontario Agronomy Guide for Field Crops (2009). In some situations, there was poor emergence or survival over the summer on individual plots. In these cases those plot results were excluded from further analysis. Fields were also monitored over the growing season for weed and insect pressure which was made note of; however, in most situations the decision to apply herbicide or insecticide had to be left to the co-operator’s discretion.

Once canola was at rosette stage, with approximately 4 to 6 leaves, prior to bolting, or approximately growth stage 14 to 16 according to the Canola Council of Canada (2003), tissue sampling was completed. A total of 20 plants per plot were randomly selected, with the entire above-ground portion of the plant being removed. Any soil was carefully rinsed off the plants using de-ionized water. Plants were either sent fresh to lab, or force air-dried in ovens at 65°C, and then delivered to A&L Labs in London, Ontario as dried samples. At the lab samples were ground and an aqua regia/hot block digestion was completed, with analysis completed using ICP-OES. In addition to the tissue samples,
### Table 2.1: Soil type, cultivar planted, spray information and dates of trial activities at 2013 and 2014 trial locations.

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil Type</th>
<th>Variety</th>
<th>Date of:</th>
<th>Spraying /Issue/ Product Used</th>
<th>Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelburne '13</td>
<td>Sandy Loam</td>
<td></td>
<td>May 17</td>
<td></td>
<td>Sept. 25</td>
</tr>
<tr>
<td>Listowel '13</td>
<td>Silt Loam</td>
<td></td>
<td>May 15</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Owen Sound '13</td>
<td>Silt Loam</td>
<td></td>
<td>May 16</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Durham '13</td>
<td>Silt Loam</td>
<td></td>
<td>May 15</td>
<td></td>
<td>Sept. 11</td>
</tr>
<tr>
<td>Paisley '13</td>
<td>Sandy Loam</td>
<td></td>
<td>May 15</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Camilla '14</td>
<td>Fine Sandy Loam</td>
<td>Invigor 5440</td>
<td>May 7</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Listowel '14</td>
<td>Silt Loam</td>
<td>Invigor 5440</td>
<td>May 26</td>
<td></td>
<td>June 20 /Weeds /Liberty *</td>
</tr>
<tr>
<td>Kimberley '14</td>
<td>Silty Clay Loam</td>
<td>Invigor 5440</td>
<td>May 7</td>
<td></td>
<td>June 5 /Weeds /Liberty *</td>
</tr>
<tr>
<td>Owen Sound '14</td>
<td>Silt Loam</td>
<td>Invigor 5440</td>
<td>May 6</td>
<td></td>
<td>June 4 /Weeds /Liberty *</td>
</tr>
<tr>
<td>Elora '14</td>
<td>Loam Till</td>
<td>Invigor 5440</td>
<td>May 9</td>
<td></td>
<td>June 2 /Weeds /          Aug. 29</td>
</tr>
</tbody>
</table>

*Harvested between Sept 1 and 30

1All Dates listed are in the year 2013 or 2014 according to respective year of trial

2Active ingredient glufosinate ammonium, at rate of 2.5 L/ha of active ingredient

3Active ingredient lambda-cyhalothrin at rate of 83 mL/ha

4Active ingredient chlorantraniliprole at rate of 250 mL/ha

All active ingredient information as per Field Crop Protection Guide 2014-2015 (Ontario Ministry of Agriculture 2014)
Table 2.2: Mean soil test nutrient values of canola trial locations, sampled at trial establishment before fertilizer application, spring 2013 and 2014.

<table>
<thead>
<tr>
<th>Location</th>
<th>OM %</th>
<th>pH</th>
<th>P Bray</th>
<th>P Bicarb</th>
<th>K</th>
<th>Ca</th>
<th>Mg ppm</th>
<th>Na</th>
<th>Al</th>
<th>S</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0-15 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15-30 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shelburne '13&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4.0</td>
<td>7.4</td>
<td>15</td>
<td>13</td>
<td></td>
<td>67</td>
<td>2575</td>
<td>140</td>
<td>10</td>
<td>677</td>
<td>16.0</td>
</tr>
<tr>
<td>Listowel '13&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.7</td>
<td>7.4</td>
<td>40</td>
<td>28</td>
<td></td>
<td>73</td>
<td>2037</td>
<td>418</td>
<td>7</td>
<td>938</td>
<td>8.3</td>
</tr>
<tr>
<td>Owen Sound '13&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.5</td>
<td>7.2</td>
<td>8</td>
<td>7</td>
<td></td>
<td>69</td>
<td>2343</td>
<td>288</td>
<td>12</td>
<td>808</td>
<td>7.0</td>
</tr>
<tr>
<td>Durham '13&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.4</td>
<td>7.6</td>
<td>5</td>
<td>4</td>
<td></td>
<td>78</td>
<td>2020</td>
<td>552</td>
<td>8</td>
<td>884</td>
<td>8.0</td>
</tr>
<tr>
<td>Paisley '13&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.3</td>
<td>7.7</td>
<td>10</td>
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<td>2060</td>
<td>310</td>
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<td>854</td>
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<td>7.5</td>
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<td></td>
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<td>2357</td>
<td>157</td>
<td>8</td>
<td>9.7</td>
<td>6.0</td>
</tr>
<tr>
<td>Listowel '14&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.6</td>
<td>7.3</td>
<td>14</td>
<td>8</td>
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<td>66</td>
<td>1830</td>
<td>287</td>
<td>10</td>
<td>9.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Kimberley '14&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.4</td>
<td>6.5</td>
<td>13</td>
<td>8</td>
<td></td>
<td>64</td>
<td>1817</td>
<td>210</td>
<td>7</td>
<td>8.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Owen Sound '14&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.4</td>
<td>6.2</td>
<td>19</td>
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<td>30</td>
<td>1227</td>
<td>133</td>
<td>8</td>
<td>11.7</td>
<td>10.0</td>
</tr>
<tr>
<td>Elora '14&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.5</td>
<td>7.3</td>
<td>14</td>
<td>9</td>
<td></td>
<td>50</td>
<td>1953</td>
<td>273</td>
<td>12</td>
<td>689</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Based on mean of soil test values of soils samples taken from each replicate at each trial location
<sup>1</sup> sites with 2 replicates
<sup>2</sup> sites with 3 replicates
<sup>3</sup> sites with 4 replicates
soil sampling was also completed at this time to monitor soil S levels at all trial locations. The general soil sampling protocol outlined above was followed at all locations for this round of soil sampling. In the 2013 season, tissue and soil sampling was only completed on the control and 22 kg S/ha application rate plots. In the 2014 growing season, all treatments were sampled for tissue and soil S levels. Harvest of canola took place in late August and throughout September as the crop reached maturity (Table 2.1). Except in the case of the Elora’14 plot, all harvesting was done with the co-operator’s combine. In these cases a strip was harvested directly down the centre of the entire length of the plot area, and all seed harvested from each plot was dumped into a weigh wagon to allow for a total wet weight measure. The co-operator’s individual combine header width and the total length of the plot, were used to determine the total harvested area per plot. Grain samples of each plot at each site were dried at 65°C and weights recorded when no further mass loss was found. The plot wet weights were then adjusted for moisture content. At Elora’14, a small plot harvester combine was used, with a header width of 1.5m. In this case, two strips were harvested down the entire length of the plot, and all seed harvested was bagged on a per plot basis. The individual harvested length of each harvested strip was measured. The grain was dried at 65°C for 7 days, and the dry mass recorded. The dried samples were run through a seed cleaner to remove any debris. After cleaning, bagged seed was weighed again, to allow for a clean-dry weight yield per hectare to be determined for each plot.

All statistical data analysis was completed using SAS Software, Version 9.3 (The SAS Institute 2011). PROC MIXED was first used to run a regression analysis of yield, tissue S and soil S against the various S rates applied at each trial location to find a best fit for the data. In some instances, plotted data appeared to follow a non-linear model, and in these cases the data was analyzed using a quadratic plateau function using PROC NLIN. The results of PROC NLIN were then compared to those achieved using PROC MIXED to determine which model the data fit best.
PROC GLM was also used to compare all tissue and soil data in 2013, as the sampling of only 2 treatments would not allow for regression analysis. Where significant treatment effect was seen, Tukey’s test was used as method of means comparison between treatments. Normality of data was assessed using PROC Univariate procedure to assess the distribution of the data, with the difference between mean and median, skewness and kurtosis factor and the Shapiro-Wilk statistic taken into consideration. When Shapiro-Wilk statistic was significant at Pr<0.05 it was deemed that results were not normally distributed. In these cases, data was converted to log values and the same procedure was completed. If distribution was not improved using log values, the original values were used to proceed with the analysis. A PROC MIXED test for outliers was also completed, with PRESS factor, Restricted Likelihood Distance (RLD) and Lund’s Test for Internal Studentized Residual taken into account. If the Internal Studentized Residual was higher than that reported as the critical value for the respective number of observations, and the PRESS factor and RLD were also the highest reported for the data points, the data point was considered to be a statistical outlier (Bowley 2008). This outlier assessment was combined with observational data recorded at the trial locations, and in most instances an outlying data point could be attributed to field problems, pest problems, emergence problems, etc., and the respective data point was eliminated from the data analysis. In some instances, where problems with normality of distribution occurred in conjunction with outliers, the removal of an outlying data point was enough to return distribution to normality. When values were removed, LSMeans were used instead of means, and where a significant Type III treatment effect was seen, the Least Squares Means table was used to assess for significant differences between treatments. For all of the data analysis a P value of <0.05 was used to determine significance.

In the factorial experiment component of the Elora’14 trial, PROC MIXED was used to assess the Type 3 Fixed Effects of the model and determine if an interaction was seen between N and S application rates.
After all trial locations were analyzed separately, additional analysis was completed to compare the results across trial locations with the soil and tissue data collected. This was in an effort to better establish critical S concentrations in soil and tissue tests. Yields were normalized across sites by dividing the yield of each plot by the highest yield achieved by a plot at that location, and multiplying by 100 to obtain a “percent of highest” yield for each plot at each trial. PROC MIXED was then again used to run a regression analysis of yield against tissue S levels. To avoid confounding results, yield was compared to soil S levels using a different method. The delta yield of each treatment was found by subtracting from it the yield of the control plot in the respective replicate. These delta yields was then plotted against the spring soil S concentration in that replicate. PROC MIXED was used to run regression analysis on the combined data across sites, to determine if the base soil S concentration affected the yield response. Any data points that were determined to be outliers in previous analysis were also excluded from these across trial comparisons.

2.5 Results and Discussion

2.5.1 Canola Seed Yield

In 2013, two of the five trial locations, Owen Sound ‘13 and Durham’13 (Figure 2.1), showed significant linear increase in yield with increasing S application. At Owen Sound’13, the stand showed good emergence and crop growth with low pest pressure over the summer. All plots were harvested, and all yield data was determined to be normally distributed, with no outliers. A significant linear regression fit was found when comparing yield against S application rate, with intercept $Pr>|t|$ of 0.002 and S application rate $Pr>|t|$ of 0.0048 (Figure 2.1). The Durham ‘13 trial location had good emergence and a healthy crop appearance throughout the summer. The yield data points were normally distributed, with no apparent outliers. The yield data showed significant linear response when compared to S application rate, with an intercept $Pr>|t|$ of 0.0031 and S application rate
Figure 2.1: Linear regression of dry matter canola seed yield at Owen Sound, Durham, Listowel, Paisley and Shelburne trial locations in fall 2013, based on sulphur(S) application rate.
Pr>|t| of 0.0154 (Figure 2.1). At Durham ’13, when no fertilizer was applied, the average yield was approximately 1261 kg seed/ha, with an increase in yield increasing fertilizer application, up to about 1428 kg seed/ha with the highest S application used in this study.

The other three 2013 trials had no significant response to applied S (Figure 2.1). Listowel ’13 suffered from excessive moisture during the growing season, and also had problems with swede midge damage, both of which contributed to overall low yields. In analysis of yield data, some issues arose, with high skewness and kurtosis factors, as well as a Shapiro-Wilk statistic of Pr<0.0001. A test for outliers found one data point was assigned the highest RLD, and Press factor, as well as failed Lund’s test of Internal Studentized Residuals. This data point corresponded with the yield data taken from a 10 kg S/ha plot, which had yielded approximately 1777 kg dry seed/ha, when the average yield across all other plots was 1165 kg dry seed/ha and the average yield of the other two 10 kg S/ha treatments was 1163 kg dry seed/ha. This data point was therefore determined to be an outlier and was excluded from further analysis, and subsequent analysis without this data point showed a normal distribution of yield data points. However, there was no significant response at the Listowel’13 trial (Figure 2.1), and GLM analysis also showed no significant differences between yields. Overall, this site did not show a yield response to S application, which may have arose at least in part due to the moisture and pests issues experienced by this site during the growing season.

At the Paisley ’13 location, due to the need for plots to allow for use of the co-operator’s harvest equipment, combined with a fairly small area that OMAF staff was able to use for the trial, only two replications of the four treatments could be included. In data analysis, the yield data collected was found to be normally distributed, with no outliers. Overall, at this trial, the highest mean yield came from the control treatment with 1408 kg dry seed/ha, after which there was a slight trend towards higher yields with increasing S application between the three S rates, with 10 kg S/ha yielding 1217 kg
dry seed/ha and 40 kg S/ha yielding 1376 kg S/ha (Figure 2.1). There is was no observable indication as to why the control treatments may have yielded better than the S treatments did, and most likely the problems at this trial arose from the lack of adequate replication.

At Shelburne ’13, again only two replications could be included due to the available area. In addition further difficulties were experienced, as two plots showed poor emergence and required re-seeding, disallowing their yield results from being compared with those from the other plots. The reseeded plots were the 40 kg S/ha rate of replication 1, as well as the 0 kg S/ha rate of replication 2. Due to the lack of replication on all treatments, no statistical analysis could be completed. There was a trend towards higher yields with S application (Figure 2.1), with yields steadily increasing between 1869 kg dry seed/ha in the control treatment, up to 2136 kg dry seed/ha in the 40 kg S/ha treatment.

In the 2014 growing season, wet conditions delayed planting at two locations: Listowel ’14 and Camilla’14 (Table 2.1). At Listowel’14, other than delayed planting until the end of May, overall growing conditions were good throughout the summer. At harvest, an error was made in the harvesting of the first plot, and not all of the yield was recorded, resulting in the yield data from that plot being excluded from the analysis. Yield data was determined to be normally distributed, with no outliers. When yield data was analyzed, there was no significant response to applied S (Figure 2.2). However, significant differences in yield were found in the treatment analysis in GLM, where the LSMean yield of the 11 kg S ha⁻¹ treatment (2427 kg dry seed/ha) was significantly greater than those of the control, 22 kg S ha⁻¹ and 44 kg S ha⁻¹ treatments (2063, 2001 and 1948 kg dry seed/ha respectively), all of which had statistically similar yields.

The Camilla’14 trial location was similarly delayed in planting. In this case, the trial area had been soil sampled, and fertilizer treatments established in preparation for imminent planting on May 7, however adverse weather conditions then delayed the co-operator in planting the trial until May 21.
Figure 2.2: Harvest dry matter (DM) canola seed yield at Listowel, Camilla, Owen Sound, Kimberley and Elora, Ontario trial locations in fall 2014, as related to spring sulphur application rate, applied as ammonium sulphate
This trial did encounter some pest problems from Swede Midge and Flea Beetle, and required a pesticide application at the end of June. Initially, yield data was found to not be normally distributed with high skewness and kurtosis and a Shapiro Wilk statistic of Pr<W of 0.0223, and the outlier check revealed the yield observation attributed to one of the 22 kg S/ha application rates to have the highest Press and RLD value with an internal studentized residual above that listed as the critical value for Lund’s Test. No clear reason was recorded as to what could have caused the yield data to be an outlier, however it was approximately 200 kg/ha lower yielding than any other plot yield recorded, and approximately 478 kg/ha lower yielding than the other 22 kg S/ha application rate plots. The data point that SAS determined to be an outlier was, similarly to Listowel ’14, the first plot to be harvested, making a harvest error possible. When this plot was removed from the analysis, distribution of data became normal. When analyzed without this data point, there was no significant response found to applied S in the GLM analysis, however, a trend was seen of increasing yield with S application (Figure 2.2). Overall, high variability was seen in control plot yields, which hindered the data from showing significant regression (Figure 2.2).

The Owen Sound ’14 trial was timely planted and had good growing conditions throughout the season. The data was determined to be normally distributed. Significant quadratic response was seen in yield data, with an R^2 value of 0.64 (Figure 2.2).

Despite timely planting, the Kimberley ’14 had very poor emergence seen in the control plot of the third replication. Due to the poor emergence, this plot was not included in further testing and analysis. In the analysis of yield data from the remaining plots, it was determined that results showed significant quadratic response (Figure 2.2), with an R^2 value of 0.60.

As was previously discussed, the canola experimental site at Elora had more treatments than the other sites. Overall, the trial was established in a timely manner, had good emergence and good
growing conditions through the season, with only a need for herbicide treatment early in the season to control grasses. In collecting yield samples, an error was made in the collection of the 10 kg S/ha treatment of replicate one, and no sample was collected for this plot. The first analysis completed included only the S application rates that could be assessed together in a regression analysis, those being 0, 5, 10, 20, and 40 kg S/ha all at a constant N application rate of 120 kg S/ha. It was determined that yield data was not normally distributed, and PROC Mixed pointed to one value being an outlier, as it had a high RLD, and Press Factor, as well as an Internal Studentized Residual well above that reported as the critical value for Lund’s Test. The observation which SAS determined to be an outlier was the 5 kg S/ha treatment in replicate three. It was determined to treat this observation as an outlier and remove it from further analysis. However, even once this outlier was removed, data did not become normally distributed. The yield data was log transformed and re-analyzed. However the Pr>W value was not improved with the transformation, and therefore the original values were used for the rest of the analysis. It was found that there was no significant response of yield data against S application rate, and no significant results in the GLM analysis, which showed only a slight trend toward higher yields with S application (Figure 2.2). The highest yielding treatment was seen with the application of 20 kg S/ha with an LSMean yield of 2850 kg dry seed/ha.

Overall, the field trials conducted in summer 2013 and 2014 resulted in inconsistent results. Of the ten trials completed, two showed linear response of yield, two showed quadratic response of yield, three showed a trend towards higher yields with S application, and three showed no indication of response to S application. The two trials which showed quadratic regression of yield with S rate were Kimberley ’14 and Owen Sound’14 (Figure 2.2). Despite Owen Sound’14 having an overall higher yield than Kimberley’14, both trials showed a maximum yield at a fairly consistent application rate. Based on the quadratic regression equations, at Kimberley’14 the maximum yield of 2517 kg dry seed/ha was attained at an S application rate of 30 kg S/ha, and resulted in an approximately 220 kg dry seed/ha yield
improvement compared to 0 kg S/ha. At Owen Sound’14 the maximum yield of approximately 3473 kg dry seed/ha was reached at an application rate of 27 kg S/ha, resulting in an increase of about 470 kg dry seed/ha in yield over a 0 kg S/ha application rate. These results coincide well with results of field experiments conducted over three growing seasons in Saskatchewan, which also found maximum yields were usually attained at 30 kg S/ha application rate (as potassium sulphate), even when using different canola cultivars (Mahli et al. 2007). An earlier study by Mahli and Leach (2002) did indicate differences in S response based on cultivar, and also found yields improved with increasing S application rate from 0 kg S/ha up to 30 kg S/ha. It has been suggested that differences in S requirements of different cultivars may come from breeding for lower glucosinolate levels in canola seed, resulting in lower amounts of high S-containing glucosinolates being available for breakdown in times of deficiency (Booth et al. 1991). When using only two S application rates of 15 and 30 kg S/ha in combination with various application timings across various cultivars, the most effective application rate was determined to be 30 kg S/ha applied shortly prior to seeding and incorporated (Mahli and Leach 2002). The study by Mahli and Leach (2002) did not include any rates higher than 30 kg S/ha, and while the researchers concluded that a minimum application of 30 kg S/ha was required, it was not shown that canola would not show further yield response to higher S application rates. However, there has been some past Ontario research which found no benefit to levels of S application as high as 30 kg S/ha. Trials conducted in New Liskeard in Northern Ontario did see responsiveness in canola to S application, but saw a yield increase at 15 kg S/ha application rates, with no further improvement in yield as a result of applying 30 kg S/ha (Rowsell and Kobler 2012). In preliminary trials conducted by OMAF in southern Ontario, a yield improvement due to S application was seen at 6 of 8 trial locations; however, no additional response to S application was seen at application rates of 22 and 44 kg S/ha compared to 11 kg S/ha (Hall 2012).

While these two field trials indicated yield was maximized at an application rate of 30 kg S/ha, the trials at Owen Sound ‘13 and Durham’ 13 showed linear increases of approximately 320 and 170 kg
dry seed/ha respectively compared to 0 kg S/ha (Figure 2.1). The linear response of yield to S application suggests that yields may further increase through additional S application above the 40 kg S/ha rate that was used in these trials. While these are relatively high rates of S application, there are other trials which saw an increase in yield at S application rates greater than 40 kg S/ha (Booth et al. 1991; Jan et al. 2002), however, it is important to also note the N application rates used in these trials, as past research has found interaction between N and S application rates (Booth et al. 1991; Mahli and Gill 2007). At an N application rate of 120 kg N/ha, Jan et al. (2002) found that the optimum S application rate can be up to 60 kg S/ha (Jan et al. 2002). The N application rate used in this study was the same as that used by Jan et al., supporting that in some fields yields may have been improved at S applications rates greater than 40 kg S/ha. However, often a response to a relatively high S application rate was only seen in conjunction with a higher N rate than what was used in this study. In a study by Booth et al. (1991), a significant response to increasing S application was seen up to a rate of 64 kg S/ha when a high N rate was used of 250 kg N/ha. At lower N application rates of 150 kg N/ha, S response maximized at an application rate of 32 kg S/ha (Booth et al. 1991).

In contrast to the significant yield response seen at these 4 trial locations, which suggest yield response is optimized at levels of S application at or greater than 30 kg S/ha, a number of trials did not show significant response to S application. In 2013 the Listowel’13, Shelburne’13 and Paisley ‘13 sites, showed little to no numerical improvement in yield with S application (Figure 2.2). At times pest damage, poor emergence, harvesting errors and lack of replication also limited the statistical inference that could be drawn from these sites, and may have contributed to the lack of significant S response seen at these sites. Of the 2014 sites, an additional 3 sites failed to show significant yield response. At the Listowel ’14 site no significant regression was found, however PROC GLM analysis did show a significant yield increase when 11 kg S/ha was applied, while all other treatments were statistically similar. While studies have found 10 to 15 kg S/ha to be the optimum S application rate, these studies
found S application above 15 kg S/ha to produce statistically similar yields to that achieved by 10-15 kg S/ha (Hall 2012; Rowsell and Kobler 2012). In the case of the Listowel’14 trial, further S application led to a decrease in yield to levels statistically similar to the control plot, and has led us to deem the site non-responsive. Camilla ’14 and Elora’14 both showed positive yield trends with S application, but no significant yield response.

2.5.2 Nitrogen-Sulphur Interaction

As previous research has indicated interaction between N and S, treatments to evaluate this were included at Elora’14. The Type Three test of Fixed effects reported a Pr>F of 0.9097 for the N and S interaction, a Pr>F of 0.6376 for S application rate, and a Pr>F of <0.0001 for N application rate effects on canola yield. Overall, it was determined that there was no statistical increase in yield when S was applied, indicating that S availability at this site was adequate for the rates of N applied. Previous studies have shown that as N application rate increased the need for S nutrition was increasingly important. A trial in Pakistan established that canola yield was responsive to N, similar to that seen in our own trial, as well as to S application (Jan et al. 2002). They also found that the oil content of the grain significantly decreased with increasing N application; however, this could be counteracted through S application (Jan et al. 2002). This resulted in a suggested application of 120 kg N/ha and 60 kg S/ha (Jan et al. 2002). Mahli and Gill (2007) found similar results in a Saskatchewan field trial, with S application counteracting the tendency of N application to reduce grain oil content. A two year, multiple location trial conducted in Montana, also found an N and S interaction in the oil content of canola seed; at 2 of 5 trial locations additional N was shown to depress the estimated oil yield, while additional S was shown to improve oil content of canola seed (Jackson 2000).

Further studies revealed that there was a clear interaction between N and S in terms of yield effects. A study by Ahmad et al. (2011) found that when no S was applied but N application was
increased from 80 to 120 kg N per hectare a yield increase of 213 kg per hectare was seen, while the same increase in N when combined with an application of 40 kg S/ha resulted in a yield increase of 834 kg/ha (Ahmad 2011). The apparent N use efficiency of canola has also been shown to increase with S application, with N uptake increasing by 2 kg/ha when an N:S application rate of 6:1 is used instead of a 12:1 ratio (Karamanos et al. 2007). When assessing the N:S ratio, the S in soil should be taken into account, but due to the high spatial variability of S, this can be difficult to assess and therefore the response to S could vary greatly over a small area (Karamanos et al. 2007; Mahli and Leach 2002). Other studies found that when S was applied in addition to N, failure to set seed was eliminated and pod abortion was reduced, though this did not lead to a significant yield improvement (Fismes et al. 2000; Nuttall et al. 1987). In the Elora ‘14 trial, despite a lack of significant interaction, a comparison of yield averages reveals similar results; when 120 kg N/ha was applied with no S application average yield was 2550 kg dry seed/ha, whereas the same level of N application in combination with 40 kg S/ha application resulted in an average yield of 2845 kg dry seed/ha. This trend was not seen at lower N application rates, where an N application without S applied showed slight yield increases over the same application with S included (Table 2.3). After examining the results of past studies, it is likely that as N application increased, S deficiency symptoms either became more pronounced, or not enough S was available for the plant to efficiently use the N that was applied, and therefore it was only at the highest N application rate that yields were decreased without S application (Karamanos et al. 2005; Mahli and Leach 2002). The lack of overall yield response to S application at the Elora’14 trial location, with only a trend towards higher yields with S application, is further proof that likely there was sufficient levels of S in the soil. The lack of interaction may have also been affected by the cultivar used (Invigor 5440); research by Karamanos et al. (2005) has shown that balancing N and S fertilizers to control the N and S interaction may be more important in conventional canola than in newer hybrids. The effect of cultivar used, as well
Table 2.3: Mean canola seed yield (kg dry seed/ha) at Elora Research Station trial based on total nitrogen and sulphur application rate (applied as combinations of urea, ammonium sulphate and potassium sulphate), summer 2014.

<table>
<thead>
<tr>
<th>Nitrogen Rate (kg N/ha)</th>
<th>Sulphur Rate (kg S/ha)</th>
<th>0</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1907</td>
<td>1863</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>2228</td>
<td>2187</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>2579</td>
<td>2586</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>2550</td>
<td>2845</td>
<td></td>
</tr>
</tbody>
</table>

as the ability of S to maintain oil content of canola seeds as N application increases should be further examined in Ontario.

2.5.3 Tissue and Soil S Concentration

Soil sampling was completed at trial establishment, at time of tissue sampling, as well as post-harvest. In 2013, soil sampling was only completed on control and 20 kg S/ha treatments. In 2014, soil samples were taken from all treatments, and regression of soil data was possible, but due to the delay in spring planting, a similar delay was seen in fall harvest, and due to time constraints, fall soil sampling was only completed at the Elora’14 trial location. For comparison between both years, the control and 20 kg S/ha treatment soil test S concentrations at 2013 and 2014 site locations are presented in Table 2.4.

In pre-plant soil testing S concentrations in top 0-15 cm were similar across trial locations, however, 15-30 cm depth results showed some variation at different locations. There were relatively low S concentrations at Listowel’13, Elora’14 and Owen Sound’13, and a high concentrations at Durham ’13 and Owen Sound’14. Pre-plant soil S levels seem to serve as a poor indicator of canola response to S fertilization, as trials (Owen Sound’13, Durham ’13, Kimberley ’14 and Owen Sound ’14) which showed...
Table 2.4: Extractable sulphur in soil (by Mehlich III) in ppm of soil samples split into 0-15 cm and 15-30 cm depths, taken pre-plant, at rosette stage tissue sampling, and in fall post-harvest, based on sulphur application rate, at all 2013 and 2014 trial locations.

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil Type</th>
<th>Pre-plant Control 0-15 cm soil depth (mg kg⁻¹)</th>
<th>Summer Sampling 20 kgS/ha 0-15 cm soil depth</th>
<th>Fall Sampling 20 kgS/ha 0-15 cm soil depth</th>
<th>Pre-plant Control 15-30 cm soil depth (mg kg⁻¹)</th>
<th>Summer Sampling 20 kgS/ha 15-30 cm soil depth</th>
<th>Fall Sampling 20 kgS/ha 15-30 cm soil depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Owen Sound’13²</td>
<td>Silt Loam</td>
<td>7.0</td>
<td>8.0</td>
<td>10.0</td>
<td>8.7 b</td>
<td>11.0 a</td>
<td>6.3</td>
</tr>
<tr>
<td>Durham’13²</td>
<td>Silt Loam</td>
<td>8.0</td>
<td>10.7</td>
<td>12.3</td>
<td>9.3 b</td>
<td>11.0 a</td>
<td>10.7</td>
</tr>
<tr>
<td>Listowel’13</td>
<td>Silt Loam</td>
<td>8.3</td>
<td>8.7</td>
<td>9.3</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paisley’13</td>
<td>Sandy Loam</td>
<td>8.0</td>
<td>10.5</td>
<td>10.5</td>
<td>12.5</td>
<td>11.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Shelburne’13</td>
<td>Sandy Loam</td>
<td>9.5</td>
<td>13.5</td>
<td>12.8</td>
<td>15.0</td>
<td>15.0</td>
<td>7.5</td>
</tr>
<tr>
<td>Camilla ’14</td>
<td>Loam</td>
<td>9.7</td>
<td>9.3</td>
<td>10.7</td>
<td>8.0</td>
<td>6.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Listowel ’14</td>
<td>Silt Loam</td>
<td>9.0</td>
<td>9.3</td>
<td>12.0</td>
<td>6.0</td>
<td>7.0</td>
<td>7.7</td>
</tr>
<tr>
<td>Kimberley ’14²</td>
<td>Silty Clay Loam</td>
<td>8.3</td>
<td>6.7</td>
<td>8.3</td>
<td>6.7</td>
<td>4.8†</td>
<td>5.0†</td>
</tr>
<tr>
<td>Owen Sound ’14²</td>
<td>Silt Loam</td>
<td>11.7</td>
<td>9.7</td>
<td>10.3</td>
<td>10.0</td>
<td>8.0</td>
<td>7.7</td>
</tr>
<tr>
<td>Elora ’14³</td>
<td>Loam Till</td>
<td>7.3</td>
<td>8.8</td>
<td>12.8</td>
<td>8.2</td>
<td>9.5</td>
<td>6.0</td>
</tr>
</tbody>
</table>

¹ 0-30 cm depth sample  
² Denotes trials that showed significant regression of yield  
³ Elora ’14 plot establishment soil data is taken from soil data collected in control plots from neighbouring alfalfa trial  
⁴ Based on LSMeans
Figure 2.3: Soil test sulphur (S) of soils sampled in early June 2014, when canola was at rosette stage (5-7 leaf) at all 2014 trial locations, as related to S application rate, applied as ammonium sulphate
significant response to fertilization contained variable S concentrations when pre-plant S content of soils was compared amongst sites (Table 2.4). Some sites such as Paisley’13 and Elora’14, did not show significant response to S application, although they had similar pre-plant soil S test concentrations to sites which showed significant response.

In the 2013 summer soil sampling there were two trial locations which broke from regular soil sampling protocol; at Listowel’13, soil samples were not split into 0-15 cm and 15-30 cm depths, and at Paisley’13 only top 0-15 cm was sampled. Overall, the summer sampling had no significant differences in soil S concentrations between control and 20 kg S/ha application rates (Table 2.4). However, S concentrations tended to increase or remain stable in top 0-15 cm with S application to 20 kg S/ha, with the exception of the Shelburne location, where soil S concentration decreased slightly with S application. In 2013, S concentration in 15-30 cm depth summer samples were varied, with S application not consistently leading to an increase in soil S concentration (Table 2.4). In 2014, both the Kimberley’14 and Owen Sound’14 trials, which also showed significant yield increase to applied S, soil S concentrations showed significant increase to applied S (Figure 2.3). Elora’14 and Listowel’14 also showed significant increases in soil S concentration as application rate increased. The results presented in Table 2.4 and Figure 2.3 indicate that the current soil S test method used is able to detect changes in available sulphate from Ontario soils, based on the increases in soil S concentrations in the 0-15 cm depth as S application rate increases. In summer soil samples across all sites in both years there was no significant increase in soil S concentration in the 15-30cm as application rate increased, indicating little to no leaching of applied sulphate between planting and rosette stage.

In the fall of 2013, both Owen Sound ’13 and Durham ’13 trial locations, soil S concentrations in top 0-15 cm of soil was significantly greater in 20 kg S/ha application rate treatments than in control treatments (Table 2.4). This may indicate that some of the applied S had been left in the soil rather than
being taken up by the crop. These trials were also those that showed significant yield improvement with S application in 2013. At all 2013 locations, as well as at Elora’14, soil S in 15-30 cm depth increased as S application increased, though not significantly (Table 2.4). This may indicate that due to higher SO₄ concentrations through S application, by the fall higher levels of SO₄ are present lower in the soil profile due to leaching.

There was no clear relationship between yield and spring or summer soil test S (Table 2.4) that can be used to indicate a minimum soil S concentration required to achieve optimum yields. A trial such as Owen Sound’14 had the highest overall spring pre-plant soil S concentration (11.7 mg kg⁻¹ in 0-15 cm and 10.0 mg kg⁻¹ in 15-30 cm), and showed a significant yield response, while a trial such as Elora’14 with relatively low S concentrations (7.3 mg kg⁻¹ in 0-15 cm and 6.0 mg kg⁻¹ in 15-30 cm) showed only a trend towards improved yields with S application. Similar discrepancies arise when examining summer soil test values and using the control treatment S concentrations as an indicator of available S. Kimberley’14 had low summer soil concentrations (6.7 mg kg⁻¹ in 0-15 cm and 4.8 mg kg⁻¹ in 15-30 cm), while the Owen Sound’14 site had the highest S concentrations in summer soil samples of the five trial locations (9.7 mg kg⁻¹ in 0-15 cm and 8.0 mg kg⁻¹ in 15-30 cm). Both of these trials showed significant yield regression, yet Camilla’14, Listowel’14 and Elora’14, all with soil test S values below those seen at Owen Sound’14, did not show significant yield improvement with S fertilization. Trials on canola in New Liskeard, Ontario had similar issues using soil test S values to anticipate yield response; similar soil sulphate concentrations were measured across the different site locations, but then yield improvements were only seen at some sites (Rowsell and Kobler 2012). Similar canola trials in Montana, also found a poor relationship between spring soil sulphate levels (by ammonium acetate-acetic acid extraction) and yield, citing the inability of soil tests to predict the release of sulphate from inorganic sulphur and organic matter, as the reason for the lack of correlation between yields and soil S test concentrations (Jackson 2000). These researchers
Figure 2.4: Dry matter canola seed yield at Elora Research Station trial location as related to soil test sulphur concentration of soil samples taken in early June, 2014

Figure 2.5: Dry matter canola seed yield at Camilla trial location as related to soil test sulphur concentration of soil samples taken in early June, 2014
Figure 2.6: Relationship between delta yield, as calculated by subtracting the yield of the control from the yield achieved through application of sulphur (S) as potassium sulphate, on a per replicate basis, and spring soil sulphur concentration as measured by Mehlich III extraction, in the 0-15 cm, and 15-30 cm soil depth, as well as the average of the two depths, of the respective control plot at all 2013 and 2014 trial locations.
therefore suggested an application of S fertilizer early in the growing season may be warranted regardless of soil test S concentrations (Jackson 2000).

Regression analysis was also completed to compare yield directly to soil S concentration of summer soil samples in 2014, but only two sites showed significant response; Elora’14 showed linear yield response to increasing 0-15 cm depth summer soil S level (Figure 2.4) and Camilla’14 showed yield response against 15-30 cm depth summer soil S level (Figure 2.5). To assess the responsiveness of canola to S application across sites and years, and allow for the inclusion of more data points, the delta yield of each treatment at each site was found and compared to baseline spring soil S. The yield of the control plot was subtracted from the yield of each S treatment, on a per replicate basis to obtain a delta yield value for each S treatment. This was then plotted against the soil test S level of spring soil samples at depths of 0-15 cm, 15-30 cm, as well as the average of the two depths which would represent the 0-30 cm depth sampling (Figure 2.6). This analysis was only compared against spring soil S as this is the best sampling time for ease of S application and to ensure S is applied early enough to avoid deficiencies and allow for an application opportunity. No significant response to baseline spring soil S was seen in yield of canola (Figure 2.6). Yield response to S application varies greatly at similar spring pre-plant S levels, indicating that S concentration in spring soil samples is a poor indication of potential yield response to S.

Tissue sampling, another possible means of predicting the requirement for S in canola, was completed on control and 22 kg S/ha treatments at 2013 trial locations (Table 2.5). In 2013, two trials, Owen Sound’13 and Durham’13, showed a significant yield increase with S application (Figure 2.1). However, none of the trials showed significant differences in S concentration in rosette stage tissue, though all sites showed a trend of greater S concentration in 22 kg S/ha treatments compared to control (Table 2.5). The increase in tissue S concentration was greatest at the two sites which showed significant
Table 2.5: Least Squares mean tissue sulphur concentration of whole above ground canola plants sampled at rosette stage in May and June of 2013, as related to sulphur application rate

<table>
<thead>
<tr>
<th>Location</th>
<th>0 kg S/ha</th>
<th>22 kg S/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelburne '13</td>
<td>0.78</td>
<td>0.79</td>
</tr>
<tr>
<td>Listowel '13</td>
<td>0.57</td>
<td>0.70</td>
</tr>
<tr>
<td>Owen Sound '13</td>
<td>0.50</td>
<td>0.84</td>
</tr>
<tr>
<td>Durham '13</td>
<td>0.74</td>
<td>0.91</td>
</tr>
<tr>
<td>Paisley '13</td>
<td>0.74</td>
<td>0.85</td>
</tr>
</tbody>
</table>

yield increase, however the S concentration of canola in control plots was fairly different at the two responsive locations, with Owen Sound ‘13 at 0.50% S in tissue and Durham ‘13 at 0.74% S in tissue (Table 2.5). Using these values as indicators for canola response, a yield response may have been expected to occur at Listowel ‘13 where control plot canola measured 0.57% S in tissue, but no such response was seen. However, a significant yield response at Listowel ‘13 may have been hindered by other issues at this location such as pest feeding and excess moisture, as previously mentioned.

In 2014, tissue sampling was completed on all treatments at all trial locations, except at Elora ‘14, where additional treatments were included to examine N- S interaction. At this location tissue sampling was only completed for the S application rate treatments, while maintaining N application rate at 120 kg N/ha, so as to sample similar treatments as were sampled at the other 4 trial locations. As all treatments were sampled, regression analysis of this data was possible, with four of five trials in 2014 showing linear response of tissue S concentration as S application rate increased (Figure 2.7). The Owen Sound ‘14 and Kimberley ‘14 trials both showed significant quadratic response in yield with S application (Figure 2.2), while tissue S concentration showed significant linear response to S application (Figure 2.7), possibly suggesting some luxury consumption of S. In instances such as Listowel ‘14 and Elora ‘14, tissue sampling also showed a linear increase with increasing tissue S, despite a lack of significant improvement in yield with S application. Camilla ‘14 was the only 2014 trial which did not show significant treatment impact on tissue S; however a trend of increasing tissue S concentration with
Figure 2.7: Linear regressions of tissue sulphur concentration of whole above ground canola plant samples taken at rosette stage as related to sulphur application rate at 2014 location.
increasing S application was seen (Figure 2.7). To assess tissue S results across sites, yields were normalized, and plotted against tissue S concentration at rosette stages, and a regression analysis was performed. Unlike when comparing delta yield with baseline soil S, a linear response of yield to tissue S was found; however the line of best fit has a poor R² and relatively high variability (Figure 2.8). Slope to the regression line was relatively low, indicating little yield response by canola as S concentration in tissue increased. Based on past research in western Canada, the Canola Council of Canada’s Canola Growers Manual (Thomas 2003) suggests tissue test S concentration at rosette stage should be above 0.25% S. Australian research on sandy soil types found when sampling 90 days after sowing, critical tissue S concentration was 0.40%, below which point a response to S application could be expected (Brennan and Bolland 2006). In our study plants were sampled at a younger age, approximately 35 to 45 days after sowing, depending on trial location, and this may account for the higher apparent critical concentration our data indicates. While fertilizer S uptake by canola clearly occurred in this study, the mean S concentration of rosette stage canola in control plots did not drop lower than approximately 0.5% S, except at Owen Sound’14 (Figure 2.7). This concentration of S is much higher than the critical concentration suggested by the Canola Growers’ Guide of 0.25% at rosette stage (Thomas 2003), suggesting that possibly soil S concentrations are adequate in south central Ontario for a growing canola crop. However, the yield response seen at some trial locations, despite overall tissue S concentrations above 0.25%, may indicate that this suggested critical concentration is too low for Ontario conditions, and may better match European research suggesting 0.35% as a critical concentration (Thomas 2003). Brennan and Bolland (2006) also suggest that conducting relatively early tissue sampling (90 days post sowing) may be unreliable, as an early deficiency may later be naturally corrected as plant roots gain access to S deeper in the soil profile. However, while plant roots may gain access to more S through a larger root mass as the plant grows, S deficiencies in young canola plants can still decrease overall yield (Mahli and Leach 2002). Therefore early sampling is important to correct S
**Figure 2.8**: Dry matter canola seed yield* at all 2013 and 2014 trial locations based on corresponding tissue sulphur concentration of whole plant above ground canola plants sampled at rosette stage

* yields normalized across sites as percent of highest yield at site

**Table 2.6**: Calculated cost of sulphur (S) per hectare, when applied as ammonium sulphate (A.S.), at different application rates, and the respective yield improvement required to pay for the cost of the S fertilizer.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A.S. applied kg/ha</th>
<th>Total Cost of A.S.</th>
<th>N applied in A.S. kg/ha</th>
<th>Value of N applied based on Urea price</th>
<th>Cost of S</th>
<th>Yield Improvement Required kg/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 kg S/ha</td>
<td>41.7</td>
<td>21.48</td>
<td>8.8</td>
<td>$10.28</td>
<td>$11.20</td>
<td>25</td>
</tr>
<tr>
<td>20 kg S/ha</td>
<td>83.3</td>
<td>42.90</td>
<td>17.5</td>
<td>$20.54</td>
<td>$22.36</td>
<td>51</td>
</tr>
<tr>
<td>30 kg S/ha</td>
<td>125.0</td>
<td>64.38</td>
<td>26.3</td>
<td>$30.81</td>
<td>$33.57</td>
<td>76</td>
</tr>
<tr>
<td>40 kg S/ha</td>
<td>166.7</td>
<td>85.85</td>
<td>35.0</td>
<td>$41.09</td>
<td>$44.76</td>
<td>101</td>
</tr>
</tbody>
</table>

1^ based on price per tonne of: $540 for Urea and $515 for A.S. (Clark Agri Service, Wellandport Ont., 2016)

2^ Yield increase required to cover the cost of S fertilization based on canola valued at $0.442/kg
deficiency in a timely manner and avoid potential yield losses. Other measures of S in canola plants should also be considered to examine if they are more accurate than measuring total S concentration, as was examined in this trial. Maynard et al. (1983) compared various measures of S in plants, including total S and N:S ratio, for predicting S deficiency. It was found that measuring the ratio of hydriodic acid reducible S to total S in rosette stage canola was the most accurate measure of predicting canola yield based on plant tissue analysis (Maynard et al. 1983). Based on summarizing other research findings, the Canola Growers Manual suggests a ratio of hydriodic acid S to total S of 0.38 in rosette stage canola when using this measure (Thomas 2003). Some researchers have successfully found a relationship between the N:S ratio in canola tissue and response to S fertilizer, and the Canola Growers Manual suggests a N:S ratio of 10:1 or less, at rosette stage (Thomas 2003). However, the manual warns that using this as a sole measure can only indicate whether or not the nutrients are in the correct proportion to each other (Thomas 2003). This may cause a grower to assume the N and S status of a canola crop is good, when in fact both nutrients may be over or under-supplied (Thomas 2003). Future research into the issue of S deficiency in canola should examine which measure of tissue S is most accurate for use in Ontario.

2.5.4 Economics of Sulphur Application on Canola

An application of S on canola will only be warranted, and economically feasible for a farmer, if the yield increase gained through S application covers the cost of the S application. When applying ammonium sulphate to canola, there is the added benefit of applying N, decreasing the amount of supplemental N fertilizer the grower will need to apply to meet the N demands of the canola crop. A brief examination of the economics involved with the application of ammonium sulphate is summarized in Table 2.6. The yield improvements seen at trial locations varied between 167 kg dry seed/ha to 450 kg dry seed/ha, with an average increase of approximately 286 kg/ha across the four site locations with
significant response. At these trial locations, yield improvement was optimized at application rates of 27 kg S/ha at minimum, while two trials showed linear regression of yield, indicating further yield increase could be expected as application rose above 40 kg S/ha. Based on Table 2.5, at trials with significant yield improvement, even at the high application rates required, the cost of the S applied is covered by the yield increase. While trials without significant yield increase should not be assessed as conclusive evidence for the viability of S application, some of the trials without significant yield improvement did also show a trend towards higher yields with S application. In many cases, even trials showing non-significant yield improvement had adequate yield improvement to low rates of applied S application to pay for the cost of applying it. Low application rates will act as “insurance” for areas of the field deficient in S, while keeping additional costs low enough that small improvements in yield sufficiently cover application costs.

2.6 Conclusion

The mixed results found in this study, found S response was either optimal at high application rates of 30 kg of S per hectare, or found little to no response to S application. While there were at times plot or trial issues which may have contributed to lack of response, some trials had no issues and still did not show a significant response to S application. This variability in results makes it difficult to recommend a specific S application rate. The response seen in other research trials has led to recommendations for relatively low S applications of 11 to 22 kg S/ha, in an effort to provide S to any potentially deficient areas of a field, as S is subject to high spatial variability across a single field (Canola Encyclopedia 2015). A review of S management in canola in Western Canada has suggested slightly higher applications of 15 to 30 kg S/ha to attain optimal yields, based on past research (Grant et al. 2012). However, S deposition has historically been lower in Western Canada than in Ontario, possibly resulting in a higher requirement for S fertilizer application. Although annual deposition of sulphate to
Ontario soils has been decreasing recently, deposition rates are still higher than in Western Canada, and therefore response to S fertilizers in Ontario canola is still variable.

Neither soil sampling nor tissue sampling presented itself as a reliable indicator of canola responsiveness. While results indicate yield continued to increase as tissue S concentration increased, the rate at which yield improved was fairly low. Tissue S concentrations tended to be above the critical concentrations found by past studies; however, even at higher tissue S concentrations, there were responsive trials. This suggests that even in conditions where tissue S may appear to be sufficient, S application may still lead to improved yields. Soil S levels also showed poor correlation to canola yield response; again low levels of yield improvement were seen as soil S concentration increased, and a wide range of yields were attained at a single S concentration. The correction of high spatial and temporal variability of S in soil through S application is the most likely cause for the yield improvement seen in this trial (both significant and non-significant).

As response was variable across south central Ontario, as of yet the best mode of action for Ontario growers is to monitor fields for S deficiency, make note of fields that have experienced deficiencies, and apply S fertilizer to those field in the future. For a grower wishing to safeguard against soil S variability, it is advisable to apply a low amount of S prior to seeding. This S can be mixed with the nitrogen fertilizer that canola growers would broadcast prior to planting, and even a small yield improvement on portions of the field will serve to cover the low cost of the additional fertilizer.
CHAPTER 3: USE OF SULPHUR FERTILIZATION TO IMPROVE ALFALFA (MEDICAGO SATIVA L.) HAY YIELD AND QUALITY IN SOUTHERN ONTARIO

3.1 Background

Historically, southern Ontario has received high levels of sulphur (S) from acid rain deposition. However, the annual rates of S addition to soils have been steadily decreasing in the past two decades, due, at least in part, to lowered emission rates from the burning of cleaner fuels and the scrubbing of smokestacks (Hoeft and Fox, 1986). According to the Canada-United States Air Quality Agreement Progress Report 2012, in 1990 southern Ontario was receiving between 28 and 45 kg of sulphate (9.3 – 15 kg S ha\(^{-1}\)) per hectare per year. By 2000, this had decreased to approximately 16-24 kg sulphate (5.3 – 7.9 kg S ha\(^{-1}\)) per hectare per year (Environment Canada 2013) with total wet and dry deposition at this time of 35 kg S/ha in the area along the southern shore of Lake Erie in the United States (National Atmospheric Deposition Program 2015). A further decrease was seen to the year 2010, at which point southern Ontario was receiving only 4 to 8 kg of sulphate (1.3 – 2.7 kg S ha\(^{-1}\)) per hectare per year (Environment Canada 2013), and wet and dry deposition along the southern shore of Lake Erie was totalling roughly 10 to 20 kg S/ha (National Atmospheric Deposition Program 2015).

These high deposition rates combined with mineralization of soil organic matter have in the past provided an adequate source of S to growing crops in southern Ontario (Ontario Agronomy Guide 2009). Sulphur serves as one of the building blocks of proteins in the plant, and is especially crucial due to its role in the synthesis of the amino acids cysteine and methionine, the formation of disulphide bonds, and in many other compounds such as co-enzyme A, thiamin and biotin (Hillel 2005; Heldt and Piechulla 2011). S also plays a role in the process of photosynthesis in the chloroplasts of the plant (Franzen and Grant 2008). The processes which utilize S in the plant also commonly use nitrogen, phosphorus and other basic elements, so proper S nutrition is also crucial to the efficient use of other nutrients by the
plant (Franzen and Grant 2008; Haneklaus et al. 2008; The Sulphur Institute 2016). For these reasons, all plants require S in varying quantities.

In legume species grown as a forage, such as alfalfa, the need for S is relatively high compared to other crops. Alfalfa is a high protein plant, and sufficient S is crucial to maintaining this high protein content, as well as allowing for adequate regrowth after harvest. Alfalfa is the most common legume crop used for forage in Ontario; favoured for its ability to attain relatively high yields even under droughty summer conditions due to its deep rooting system, and its suitability to typical southern Ontario soil pH, while also producing a high quality, high protein feedstuff (Franzen and Grant 2008; Ontario Agronomy Guide 2009; Ketterings et al. 2012). As one of the most important feedstuffs for Ontario livestock farmers, in 2012 just over 799 000 hectares of alfalfa were harvested across Ontario, compared to almost 119 000 hectares of corn grown for silage in that year, making optimal nutrition and yield in alfalfa a very prevalent issue (Kulasekra 2015). Alfalfa also ranks among the most common crops grown in Ontario for overall acreage, ranking just below soybeans and grain corn, at over 1 046 000 and 894 000 hectares grown in 2012, respectively (Kulasekra 2015). The S requirement of alfalfa is high; approximately 30 kg of S per hectare per year to attain adequate yields and remain healthy, and, being a forage crop, much of that S is removed from the field (Kost et al. 2008). In the past, removal rates have been estimated at 3 kg of S per tonne of alfalfa hay harvested from a field (Seim et al. 1969), though more recently researchers have found uptake to be as high as 5.6 to 7.8 kg of S taken up per tonne (McKenzie 2005). As genetic and management improvements increase alfalfa yields, the subsequent removal of S is also likely to increase (Seim et al. 1969; Hoeft and Fox 1986).

When comparing uptake of S with deposition rates, it is likely that in the past deposition and mineralization from the soil has been sufficient to meet an alfalfa crop’s needs. However, now that deposition rates have decreased to only 4 to 8 kg of sulphate per year in 2010 (Environment Canada
2013), it is likely that there is no longer enough S supplied and deficiencies will become more prevalent. In a recent analysis of Ohio soils, it was estimated that approximately 93% of Ohio soils are variably to highly deficient in S (Kost et al. 2008). It is estimated that across the northeastern United States, as well as into Ontario, the levels of removal of S by a healthy alfalfa crop are now greatly in excess of the atmospheric deposition rates, and the level of S supply by soils (Ketterings et al. 2012a; Ketterings et al. 2012b).

Visible deficiencies of S in an alfalfa crop will appear as smaller, stunted plants with thin, brittle stems and yellow colouring (Hillel 2005). As chloroplasts begin to break down as a result of low S levels, there will be a decrease in rate of photosynthesis (Hillel 2005). As a relatively low mobility nutrient, S is not readily remobilized from older leaves, therefore, deficiencies may appear first on the newest growth of the plant, depending on the distribution of S throughout the plant (Hillel 2005). A visible deficiency can result in a significant loss of yield for the farmer, with stunted plants producing reduced amounts of biomass. Slight S deficiencies may have a negative impact on yields, while not resulting in visual symptoms of deficiency and therefore may go undiagnosed. In legume species such as alfalfa, S nutrition is also required in the process of nitrogen (N) fixation by rhizobia (DeBoer and Duke 1982; Tisdale 2002; Ketterings et al. 2012b) as compounds vital in the process of nitrogen fixation, nitrogenase and ferredoxin, contain high amounts of S (Zhao et al. 1999; Scherer et al. 2008). Even in early stages of S deficiency, N fixation is reduced, even while other physiological functions remain unchanged (DeBoer and Duke 1982). It has also been found that the levels of some N-containing compounds are increased in the leaves of alfalfa plants under S-deficient conditions, indicating either protein synthesis inhibition or protein degradation by the plant (DeBoer and Duke 1982).

As previously discussed, an additional benefit of alfalfa as a feedstuff is the high protein content. As S is essential to both protein synthesis in the plant, and to the fixation of N by the nodules (with N
also being an essential component of proteins), a deficiency of S results in a lower overall crude protein content in an alfalfa forage (Tisdale 2002; Ketterings 2012). Ruminant species require at least 0.14 to 0.24% S in substrate dry matter to maintain proper rumen health and digestion (Goodrich and Garrett 1986). Supplementation or fertilization of forage to bring S to levels of 0.2 to 0.25% in feed has been shown to have added benefits to livestock including: longer and healthier wool in sheep, increased weight gain and feed intake in sheep and beef cattle, improved cellulose digestibility in sheep and dairy cattle, decrease in cost of feed per pound of gain as well as an improvement in carcass grading in beef cattle, improved aroma, flavour and vitamin A content of dairy milk, and also an increased daily milk production in dairy cattle (Tisdale 2002). However, S levels greater than 0.4% in the total feed ingested can cause toxicity in ruminants (Goodrich and Garrett 1986). Therefore optimal S management of alfalfa hay crops is important for forage yields and for feed quality (Ketterings et al. 2012b).

3.1.1 Sulphur Fertilization of Alfalfa

Over 90% of the S contained in soil is found as organic forms of S, such as elemental S, organic sulphates and carbon-bonded S, all of which are common in soil organic matter and manure additions (Kelling 2000, Schoenau and Mahli 2008). While these forms of S are hydrophobic and stable in the soil profile, plants are unable to take them up (Schoenau and Mahli 2008). Over time, soil microorganisms act on this S pool to mineralize organic S into sulphates, converting it into a plant available form (Schoenau and Mahli 2008). The rate at which mineralization occurs will be affected by temperature, soil pH, moisture content, and a variety of other factors that affect the activity levels of soil microorganisms (Barrow 1975; Erikson 2008; Schoenau and Malhi 2008). Once converted to sulphate, it is no longer stable in the soil profile, and leaches readily in high moisture soil conditions similarly to nitrate (Erikson 2008; Schoenau and Mahli 2008). Therefore, in general, high organic matter soils, and soils with frequent manure additions will likely contain more organic sulphur, and in turn, sulphate, and may have a
reduced need for S additions as fertilizer, while coarse textured soils may contain lower amounts of S due to leaching (Franzen and Grant 2008). Due to the complexity of factors acting on S in soil, soil S has been shown to fluctuate dramatically across a field, as well as within the growing season, making availability of sulphate to plants hard to predict, with deficient areas appearing even in fields with otherwise sufficient S levels (Lang et al. 2007; Kost et al. 2008). The same soil processes which act on S naturally occurring in soil or added in the form of organic matter and manure will act on S applied as fertilizer.

There are two general S forms of fertilizer used; elemental S and sulphate, each with its own advantages and disadvantages due to how S is released and moves through soils. Elemental S, containing approximately 88 to 98 % S, depending on its level of purity, must first be oxidized into sulphate by soil microorganisms to become plant available (Schulte and Kelling 1992; Schoenau and Mahli 2008). This process is impacted by how much access the soil microbes have to the fertilizer based on granule size and incorporation into the soil (Schulte and Kelling 1992). While it takes longer to become plant available, elemental S is more stable in the soil profile and may supply plant available S for a longer period of time (Janzen and Bettany 1986). It is also the most concentrated form of S fertilizer, decreasing transport costs, while having the ability to be mixed into compound fertilizers (Janzen and Bettany 1986; Erikson 2008). In north-western North America, elemental S is generally applied in the fall, to ensure it has adequate time to become plant available for the next spring (Hoeft and Walsh 1975; Janzen and Bettany 1986). Depending on the length of time it takes to be completely oxidized, there is potential for a single application of elemental S to supply enough S to a growing alfalfa stand for a number of years, perhaps allowing for a single application to last for the entire life of the stand (Barrow 1975; Janzen and Bettany 1986; Gunes et al. 2009).
Sulphate forms of fertilizer are chemically bonded to other plant nutrients, such as in ammonium sulphate, potassium sulphate, and potassium-magnesium sulphate (Koenig et al. 2009). These sulphate forms of fertilizer, which are immediately plant available and readily taken up, have been found to have the highest plant recovery rate, and have been shown to allow for greater alfalfa yield to be reached in the first year after application than seen with elemental S application (Janzen and Bettany 1986). However, as discussed, sulphate is easily leached from the soil profile, especially in sandier soils and soils with high pH, and a yearly re-application in the spring of sulphate fertilizers is therefore recommended (Hoeft and Fox 1986; Janzen and Bettany 1986; Schulte and Kelling 1986; Schoenau and Malhi 2008). The amount of S contained in these sulphate fertilizers varies somewhat, with ammonium sulphate containing approximately 24% available S by weight, and potassium sulfate containing approximately 18% S by weight (Koenig et al. 2009). Sulphate fertilizer forms can be beneficial to crops and farmers due to the inclusion of other valuable nutrients; in the case of alfalfa, potassium sulphate is often applied, so as to fulfill the crop’s high potassium requirement, while also supplying S (Bruulsema 1998).

Studies examining S fertilization have been ongoing in the United States and Western Canada. In some areas S deficiency has been an ongoing issue due to factors such as: historically lower S deposition levels, sandier soil types prone to leaching of sulphate, low organic matter soils, or soils low in natural S reserves in the soil (Franzen and Grant 2008). Many of these studies have found positive results from S fertilization using both sulphate and elemental forms, finding that it contributes to stand density (Chapman et al. 1972) and longevity (Hoeft and Walsh 1975), and significantly increases yields (Jones and Ruckman 1966; Hoeft and Walsh 1975; Bedell 1985; Rehm 1987). By improving not only yield, but also stand longevity (Mahli and Goerzen 2010), it may be possible for farmers to maintain alfalfa stands for longer lengths of time, and thereby decrease re-seeding costs. Positive improvements to the nutritional quality, primarily in terms of improved crude protein levels, have also been found (Aulakh et
al. 1976). These studies came to varying conclusions as to the adequate level of S fertilization to obtain maximum yields, as well as the best form of S fertilizer (sulphate or elemental) to apply based on their respective soil conditions and climate. In the past, high deposition levels have resulted in limited research into S fertilization in Ontario, with deposition levels exceeding crop requirements. Some research into alfalfa response to S application in Ontario was completed by Sheard in the 1970s; while alfalfa was shown to respond to S application in greenhouse trials, it was found that atmospheric supply of S provided adequate S for alfalfa growth in field conditions (1976). An early Ontario Ministry of Agriculture and Food Factsheet for alfalfa fertilization explains that S deficiencies occur in northern and north-western Ontario (Sheard 1987), where atmospheric deposition has historically been lower, and that deficiencies are uncommon outside of these areas. The results of these early trials led to the Ontario Agronomy Guide (2009) suggesting that the annual atmospheric additions of S to southern Ontario soils negates the need for S fertilization. More recently, in trials in northern Ontario, where atmospheric deposition has remained lower than the levels seen in southern Ontario, yield improvements have been seen in canola (Rowsell and Kobler 2012). With alfalfa requiring greater amounts of S than canola, and deposition levels decreasing in southern Ontario, there is a need for up to date research examining if alfalfa will now respond to S application.

3.2 Hypothesis and Objectives

Based on current knowledge of decreasing S deposition rates, it is now likely that some Ontario alfalfa crops are experiencing S deficiency, and that S fertilization is necessary to achieve maximum yields in certain cropping situations, depending on variables such as soil texture and weather patterns. We conducted similar trials to those completed in the United States and Western Canada to determine how great the yield impact of S fertilization would be, how fertilization would impact the feed quality of alfalfa hay, and how our specific soil types and weather variations may impact the most economical rate
and type of S fertilization for Ontario farmers, with the aim of updating the Ontario recommendations for S fertilization of alfalfa.

### 3.3 Materials and Methods

To examine the response of alfalfa to S fertilization and ensure further research into the issue was warranted, a preliminary trial was established in spring 2012 in Fergus, Ontario (Fergus’12). This trial consisted of four replicates of 3 treatments:

1) Control  
2) 270 kg K₂SO₄/ha  
3) 225 kg K₂O /ha  

After assessing yield results at Fergus’12 and establishing that further research into S application on alfalfa was warranted, further field trials were established to examine the viability and effectiveness of S fertilization of alfalfa in three subsequent growing seasons; summer 2013, 2014 and 2015. Trial locations were established in southern Ontario, mostly on co-operators fields, in an effort to include a variety of soils, as well as to encompass different microclimates in Southern Ontario.

For the 2013 growing season, three field trials were established in the fall of 2012. These trials were run in co-ordination with Bonnie Ball (Ontario Ministry of Agriculture, Food and Rural Affairs), and were located in Hesson (Hesson’13), Wallenstein (Wallenstein’13) and Mitchell (Mitchell’13), all on co-operators fields. Each trial was a randomized complete block design of three replications of five treatments as follows:

1: Control with no sulphur applied  
2: 56 kg of S per hectare as elemental S  
3: 111 kg of S per hectare as elemental S  
4: 56 kg S per hectare as potassium sulphate (K₂SO₄)  
5: 159 kg K₂O per hectare as potassium chloride (KCl)
At Hesson and Wallenstein, each plot measured 12.2 m by 6.1 m wide. At Mitchell plots measured 25 m long by 6.1 m wide.

For the 2014 growing season, the number of field trials was increased to five locations. The Hesson’13 and Wallenstein’13 trials were able to be carried into another growing season, so as to examine any carryover impacts of sulphur application and to assess the ability of elemental S to supply an alfalfa crop with sufficient S for two subsequent growing seasons without re-application. In the fall of 2013 at these two trials, each original plot was split in half, to now measure 12.2 m long by 3.05 m wide. Each half was then randomly assigned to either have a re-application of the same type and amount of material for the 2014 growing season, or to have no application for the 2014 growing season. In this way the Hesson’13 and Wallenstein’13 trials led into the Hesson’14 and Wallenstein’14 trials, respectively.

In addition to these two plots being carried into 2014, three additional sites were established. A new site was established at Mitchell (Mitchell’14) in the fall of 2013, as the previous location of the site was to return to corn in its rotation. The site for the 2014 growing season was owned and managed by the same co-operator as the original, and located approximately a half a kilometre away from the original site. The Mitchell’14 site contained 3 replicates of the same 5 treatments as the Mitchell’13 site, with 2 additional treatments of different rates of K$_2$SO$_4$ to better examine the optimal rate of K$_2$SO$_4$ to apply to maximise yields. It was originally intended for the K$_2$SO$_4$ rates to be approximately 20 kg S per hectare, 40 kg S per hectare as well as the original 52 kg S per hectare, however, due to mis-calibration of the fertilizer spreader used, the following treatments were applied:

1: Control with no sulphur applied
2: 56 kg of S per hectare as elemental S
3: 111 kg of S per hectare as elemental S
4: 82 kg S per hectare as K$_2$SO$_4$
5: 159 kg K$_2$O per hectare as KCl
6: 25 kg S per hectare as K$_2$SO$_4$
7: 52 kg S per hectare as K$_2$SO$_4$
At the Mitchell’14 site, there was again sufficient space for plots to measure 25 m long by 6.1 m wide.

A site was also established at the University of Guelph’s Elora Research Station (Elora’14) in the fall of 2013. This was again a replicated complete block design, with 4 replicates, as well as additional treatments, with each plot measuring 10 m long by 4.5 m wide. At this site potassium fertilizer was applied to the whole trial area at a rate exceeding the requirement of the site to minimize the chance of potassium related responses from the potassium sulphate fertilizer used. The treatments used at this site were:

1: Control with no sulphur applied  
2: 56 kg of S per hectare as elemental S fall applied  
3: 111 kg of S per hectare as elemental S fall applied  
4: 56 kg S per hectare as K₂SO₄  
5: 159 kg K₂O per hectare as KCl  
6: 5 kg S/ha spring applied as K₂SO₄  
7: 10 kg S/ha spring applied as K₂SO₄  
8: 20 kg S/ha spring applied as K₂SO₄  
9: 30 kg S/ha spring applied as K₂SO₄  
10: 40 kg S/ha spring applied as K₂SO₄

A trial was also established for the 2014 growing season in Ancaster (Ancaster’14). A randomized complete block design was used, with individual plots measuring 10 m long by 4.5 m wide. This trial was unable to be established in the fall, and instead was set up in the spring of 2014. Therefore the treatments were adjusted to only include K₂SO₄ as means of S application, and treatments were as follows:

1: Control with no sulphur applied  
2: 5 kg S/ha spring applied as K₂SO₄  
3: 10 kg S/ha spring applied as K₂SO₄  
4: 20 kg S/ha spring applied as K₂SO₄  
5: 30 kg S/ha spring applied as K₂SO₄  
6: 40 kg S/ha spring applied as K₂SO₄  
7: 159 kg K₂O per hectare KCl

In 2015, a new site was established at the Elora research station (Elora’15), which was identical to the Elora’14 site. In addition the Elora’14 site was continued into the 2015 year. In this case the
residual value of the fertilizer S applied in year one was evaluated as well as the impact of a new addition of sulphur fertilizer in 2015. In this case each plot was split in half, one side receiving 40 kg ha\(^{-1}\) of S as potassium sulphate and the other left to evaluate the residual value from 2014. This split resulted in plots that were 4.5 m wide and 5 m in length.

In all cases that elemental sulphur treatments were used, elemental S was applied onto plots in the fall to give opportunity for oxidation to occur to convert the elemental S into plant available sulphate. All K\(_2\)SO\(_4\) and KCl was spring applied, as soon as soil conditions were suitable for driving across the plots without causing damage, generally in late April to early May. Timings of plot establishment, fertilizer application, as well as all other actions performed on trial locations can be found in Table 3.1.

In all trial locations, overall plot design, as well as the width of each individual plot was meant to allow for ease of fertilizer application. Wherever possible a Valmar air-flow fertilizer spreader was calibrated and used to apply fertilizer onto plots. At times, this implement was in use for other research trials, and a small Gandy push-spreader was calibrated and used for application of fertilizer (Spring application at all 2013 trials, and spring application at Mitchell’14, Hesson’14 and Wallenstein’14).

The primary sampling methods used in these trials were the collection of soil and tissue samples, yield data, and feed testing of 2\(^{nd}\) cut. For the purpose of this chapter, yield and hay sample values will be examined, with soil and top 15 cm tissue testing being discussed in depth in Chapter 4. As background data, soil and tissue values may be given in this chapter, and the sampling protocol for these tests is therefore included. The general protocol for soil sampling was to take 9 samples per plot in a “W” pattern across each plot, while avoiding the outer 0.5 m borders of each plot to ensure samples were not taken from areas with possible cross contamination from neighboring plots. Soil cores were taken to 30 cm depth, and were immediately split into 0-15 cm depth and 15-30 cm depth. Samples were mixed on a per plot basis, and then filled into plastic bags for transport. Samples were either removed from bags and allowed to air dry, or placed in a drying oven at approximately 30°C as a form of
force air-drying. Dried samples were again adequately mixed and a portion of the sample was sent to A&L Laboratories in London, Ontario for analysis, where S extraction was completed by Mehlich III (Mehlich 1984) and analysis was completed by inductively-coupled plasma atomic emission spectrometry (ICP-AES).

In terms of tissue sampling protocol, at all trials except the 2015 trials, a tissue sample was taken from each plot to analyze the concentration of S in the alfalfa tissue, to later be compared to the yields from each plot. Again walking in a “W” pattern, the top 15 cm of growth was cut off of approximately 35-40 plants per plot, and placed in a paper bag. This sampling was done as close as possible to the late bud to first flower stage, according to the protocol established by Kelling et al. (2002a) while still being mindful of the plans of each individual co-operator in terms of timeliness of cutting for hay. The sample was then either delivered fresh to A& L Labs, London Ontario, or force air dried in ovens at 30°C prior to being brought to the lab for analysis. Tissue samples were ground and underwent aqua regia/hot block digestion, and analysis by ICP-AES.

At the point of plot establishment, soil sampling was completed to assess original soil S levels, as well as to assess possible spatial variability across the trial site. In this initial sampling, only 2 cores were taken per plot. Cores were still taken to a 30 cm depth, and split into 0-15 cm and 15-30 cm, however the cores were then mixed together on a per block basis, so as to arrive at two samples total per block. In most cases, soil sampling could be completed before fall fertilizer was applied. However, in a few cases (all 2013 sites, Mitchell’14) for the sake of ensuring a timely fall fertilizer application, the two fall elemental S applications were completed as soon as the plot area was established, and soil sampling was done at a later date. In these cases, when soil sampling was completed, only plots that had not had fertilizer applied to them were sampled. The remainder of the process continued as previously outlined. The results of this initial soil sampling can be found in Table 3.1.
Table 3.1: Soil texture, age of alfalfa stand, and results of soil sampling completed at trial establishment on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations, prior to application of sulphur fertilizer.

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil Type</th>
<th>Age of Stand in Trial</th>
<th>OM %</th>
<th>pH</th>
<th>P Bray (ppm)</th>
<th>P Bicarb (ppm)</th>
<th>K (ppm)</th>
<th>Ca (ppm)</th>
<th>Mg (ppm)</th>
<th>Na (ppm)</th>
<th>Al (ppm)</th>
<th>S (ppm)</th>
<th>S (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitchell '13</td>
<td>Clay Loam</td>
<td>3rd year</td>
<td>3.6</td>
<td>7.8</td>
<td>13</td>
<td>7</td>
<td>111</td>
<td>2234</td>
<td>202</td>
<td>8</td>
<td>591</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Hesson '13</td>
<td>Clay Loam</td>
<td>2nd Year</td>
<td>4.1</td>
<td>7.4</td>
<td>38</td>
<td>24</td>
<td>132</td>
<td>2894</td>
<td>286</td>
<td>10</td>
<td>755</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Wallenstein '13</td>
<td>Clay Loam</td>
<td>2nd Year</td>
<td>5.5</td>
<td>7.1</td>
<td>16</td>
<td>10</td>
<td>126</td>
<td>2846</td>
<td>290</td>
<td>13</td>
<td>832</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Mitchell '14</td>
<td>Clay Loam</td>
<td>2nd Year</td>
<td>3.9</td>
<td>7.4</td>
<td>8</td>
<td>6</td>
<td>73</td>
<td>2207</td>
<td>330</td>
<td>16</td>
<td>664</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Hesson '14</td>
<td>Clay Loam</td>
<td>3rd Year</td>
<td>4.0</td>
<td>7.0</td>
<td>42</td>
<td>24</td>
<td>125</td>
<td>3557</td>
<td>318</td>
<td>16</td>
<td>853</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Wallenstein '14</td>
<td>Clay Loam</td>
<td>3rd Year</td>
<td>5.6</td>
<td>6.3</td>
<td>19</td>
<td>11</td>
<td>128</td>
<td>2840</td>
<td>323</td>
<td>12</td>
<td>882</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Elora '14</td>
<td>Loam Sandy</td>
<td>4th Year</td>
<td>3.6</td>
<td>7.3</td>
<td>16</td>
<td>11</td>
<td>54</td>
<td>2280</td>
<td>300</td>
<td>10</td>
<td>703</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Ancaster '14</td>
<td>Loam Silt</td>
<td>2nd year</td>
<td>2.2</td>
<td>6.9</td>
<td>51</td>
<td>26</td>
<td>31</td>
<td>830</td>
<td>191</td>
<td>11</td>
<td>775</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Elora'15</td>
<td>Loam</td>
<td>2nd year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

1 based on mean fall control plot values as trials were carried into 2nd year
2 based on spring soil testing while all other values were fall soil testing
Field work on the trials resumed in the spring of both 2013 and 2014. Once the trial area was dry enough to allow for vehicle and walking traffic as well as soil sampling, spring fertilizer ($K_2SO_4$ and $KCl$) was applied to plots. Spring soil sampling was also completed at all site locations, with control plots as well as elemental treatments being sampled, following the soil sampling protocol. In this way treatments that had received a fall elemental S application could be compared to the control plot with no fertilizer applied, as well as establishing a baseline S availability for the growing season. Exceptions to this method of spring soil sampling were the Wallenstein’14 and Hesson’14 plots. As these plots were going into their second year of production in the spring of 2014, and all plots had received a S application in the previous year, all plots were soil sampled at these locations. Another exception is the Ancaster’14 site. As this site was first established in the spring of 2014, spring soil sampling consisted of the method of soil sampling done at plot establishment of 2 cores per plot being mixed on a per block basis.

Prior to first cut, all plots at all locations were tissue sampled following the top 15 cm tissue sampling protocol previously outlined. At all sites on co-operator fields, cutting hay was completed in accordance with the co-operator’s cutting schedule and weather and field conditions, and therefore was not able to be done at optimal timing in terms of alfalfa maturity at all times. Through communication with co-operators, researchers were able to move in to the plot area either the day before or morning before the co-operator planned on cutting the rest of the field. Cutting of the plots was done with a carter harvester, with a 1.5 m cutter bar. The carter is designed with conveyor system, which delivers all the cut hay into a weigh box in the back. In this way all the hay harvested by the carter can be weighed before dumping it back on the field. One to four strips per plot were cut with the carter, depending on overall plot size, so that as much of the plot area could be cut as possible. The length of each individual strip was measured, so as to find the exact area of each strip. With this information, the weight obtained per subsample could then be converted to a wet weight per hectare.
Two random whole plant hay samples were taken from each plot, each with a fresh weight of approximately 1 kg. The first of these samples was immediately weighed and then placed in ovens to be dried at 60°C for approximately 4 to 7 days, with the weight of each sample being monitored over this time period until no further weight loss was seen. Once fully dry, the weight was taken again, and total amount of moisture contained in the fresh sample was calculated, to allow for adjustment of the wet biomass yield measured by the carter harvester a dry biomass yield in kilograms per hectare. Afterwards, this dry sample was sent to A&L Labs for analysis, to determine total S content and therefore, also allow for calculation of nutrient removal rates. The second grab sample obtained from each plot was used to serve as an indication of sulphur application having an effect on stand composition. The hay contained in these samples was sorted to separate alfalfa, grass and weeds, with each component then being bagged separately, dried at 60°C for 4 to 7 days. Once dried, each component was weighed, and the percentage of each component as a portion of the total weight was calculated.

Once harvesting of the plot area was completed, any standing hay left on the trial area was cut. Co-operators harvested and cleared the hay following their regular practices, while at Elora the site was cut and quickly removed from the trial area as wet forage. Following first cut, all plots were soil sampled following the soil sampling protocol. In the summer of 2014, due to dry conditions causing hard ground at the intended time of soil sampling, Hesson’14 and Wallenstein’14 had only 0-15 cm samples taken in this round of soil sampling.

Prior to second cut, another top 15 cm sample plant was taken from each plot to again examine the critical concentration of sulphur in alfalfa, following the protocol for top 15 cm sampling. Second cut was carried out on trials using the same methods used for first cut. For the second cut dry whole plant grab samples , a simple feed analysis was completed in addition to the regular nutrient analysis, to give values for Crude Protein, Acid Detergent Fibre, Neutral Detergent Fibre, and Total Digestible Nutrients.
Where possible, a third cut of hay was also completed. The cooperators at the Hesson’13/’14 and Wallenstein’13/’14 locations did not complete third cut as part of their regular practices, and therefore in neither trial year was a third cut performed at these locations. At the Ancaster’14 site, due to wet weather delaying second cut, the co-operator decided he no longer wished to do a third cut on the field, so as not to cut during the fall critical harvest time of alfalfa, which is the six weeks preceding the initial fall frost, and for the trial location would be approximately the six weeks following August 30th (Ontario Agronomy Guide 2009). At Mitchell’13 as well as Mitchell’14, researchers were fortunate to have three cuts, all completed in a timely manner in terms of alfalfa maturity. At the Elora’14 and Elora’15 sites, three cuts were also completed. At the Elora’14 site, the first cut was delayed by wet weather, which in turn delayed second cut. As a means of avoiding cutting during the critical fall harvest period for alfalfa, at this site third cutting was delayed until the beginning of October 2014.

The final work completed on trials each year was final fall soil sampling following the outlined soil sampling protocol. Fall soil sampling was mostly done in September so that soil sampling across sites was completed in approximately the same time period, regardless of when the last cut of alfalfa was taken off. Soil sampling of the Elora’14 site had to be pushed into October once third cut was completed. At the Hesson’14 site, stakes marking the trial area were accidentally removed following second cut, and the exact trial area could not be determined again with certainty so as to obtain final fall soil samples. The dates of soil sampling, tissue sampling, cutting of hay and all other work completed on trials can be found in Table 3.2.

All statistical data analysis was completed using SAS Software, Version 9.3 (The SAS Institute 2011). For 2014 and 2015 data, where multiple rates of potassium sulphate were used, PROC MIXED was used to complete regression analysis of yield, sulphur uptake, alfalfa content of hay and crude protein data with S applied. In some instances plotted data appeared to follow a non-linear model, and in these cases the data was analyzed using a quadratic plateau function using PROC NLIN. The results of
PROC NLIN were then compared to those achieved using PROC MIXED to determine which model the data best fit.

To compare the yield, nutrient removal, species composition and crude protein results of potassium sulphate application to those seen with elemental S, and assess the overall significance of a S application, ANOVA in PROC GLM was used. Where significant treatment effect was seen, Tukey’s test was used as method of means comparison between treatments. Normality of the data was assessed using PROC Univariate procedure to assess the distribution of the data, with the difference between mean and median, skewness and kurtosis factor and the Shapiro-Wilk statistic taken into consideration. When Shapiro-Wilk statistic was significant at Pr<0.05 it was deemed that results were not normally distributed. In these cases, the data was log transformed and the same procedure was completed. If distribution was not improved using log values, the original values were used to proceed with the analysis. A PROC MIXED test for outliers was also completed, with PRESS factor, Restricted Likelihood Distance (RLD) and Lund’s Test for Internal Studentized Residual taken into account. If the Internal Studentized Residual was higher than that reported as the critical value for the respective number of observations and RLD was also the highest reported for the data points, the data point was considered to be a statistical outlier (Bowley 2008). This outlier assessment was combined with observational data recorded at the trial locations, and in most instances an outlying data point could be attributed to observed identifiable issues in the field, and the respective data point was eliminated from the data analysis. In some instances, where problems with normality of distribution occurred in conjunction with outliers, the removal of an outlying data point was enough to return distribution to normality. When values were removed from an analysis, LSMeans were used instead of means, and where a significant Type III treatment effect was seen, the Least Squares Means table was used to assess for significant differences between treatments. For all of the data analysis a P value of <0.05 was used to determine significance.
Table 3.2: Date of trial establishment, fertilizer application, tissue and soil sampling and hay cutting completion at all 2013, 2014 and 2015 trial locations used in sulphur application research in southern Ontario

<table>
<thead>
<tr>
<th>Trial</th>
<th>Fall Prior to Growing Season</th>
<th>Within Growing Season</th>
<th>2013 Locations</th>
<th>Fall</th>
<th>Est.</th>
<th>Fert.</th>
<th>S.S.</th>
<th>Spring</th>
<th>Spring</th>
<th>Tissue</th>
<th>1st cut</th>
<th>Summer</th>
<th>Tissue Sampling</th>
<th>2nd cut</th>
<th>3rd Cut</th>
<th>Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial Est.</td>
<td>Fert.</td>
<td>S.S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013 Locations</td>
<td>Hesson'13</td>
<td>Nov. 15</td>
<td>Nov. 15</td>
<td>Nov. 21</td>
<td>May 1</td>
<td>May 3</td>
<td>June 7</td>
<td>July 11</td>
<td>July 17</td>
<td>Aug. 22</td>
<td>Sept. 4</td>
<td>Aug. 16</td>
<td>Oct. 27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mitchell'13</td>
<td>Nov. 9</td>
<td>Nov. 9</td>
<td>Nov. 16</td>
<td>April 6</td>
<td>April 30</td>
<td>May 24</td>
<td>May 24</td>
<td>June 4</td>
<td>July 10</td>
<td>July 12</td>
<td>Aug. 16</td>
<td>Oct. 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wallenstein'13</td>
<td>Nov. 15</td>
<td>Nov. 15</td>
<td>Nov. 22</td>
<td>May 1</td>
<td>May 3</td>
<td>June 7</td>
<td>June 18</td>
<td>June 24</td>
<td>July 24</td>
<td>Aug. 12</td>
<td>Sept. 25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S.S. is soil sampling, Est. is establishment, Fert. is fertilizer

1Ancaster was spring established, and spring soil sampling is therefore also establishment sampling

2Trials carried into 2nd year, therefore no establishment date.
3.4 Results and Discussion:

3.4.1 Yield and Composition of Alfalfa Hay

The preliminary trial, Fergus’12 showed significant yield response to S application, despite the dry conditions seen in this growing season. Due to drought, only 1st and 3rd cut were taken at this site; 2nd cut came into flower early, with very little biomass produced across the entire trial area. Therefore the 2nd cut alfalfa was simply trimmed and biomass removed from the trial area to allow for 3rd cut regrowth. In both 1st and 3rd cut, yield was significantly greater in the K₂SO₄ treatment, with approximately 50% more biomass at first cut and over double the biomass in 3rd cut when K₂SO₄ was applied compared to control and KCl treatments (Table 3.3). Whole plant tissue samples were only taken of 1st cut, with tissue S concentration of whole plant samples significantly greater than those seen in control and KCl treatments, and therefore resulting in significantly greater S uptake by K₂SO₄ treatments than control and KCl, of almost three times as much S (Table 3.3).

Following the results seen at Fergus’12, three trial locations were established for the 2013 growing season; Wallenstein’13, Hesson’13 and Mitchell’13. At Wallenstein’13, two hay cuts were completed, neither of which showed a significant increase in yield with S application (Table 3.4). Similarly, there was no significant increase S concentration in first cut hay samples; however, a trend of increasing uptake was seen with S application. In second cut, both the 56 kg S/ha as K₂SO₄ rate as well as the 112 kg S/ha as elemental S treatments had significantly greater S uptake than control and KCl treatments (Table 3.4). When total yield was considered, no significant yield increase was seen, however both the highest yield and the highest S uptake were achieved with an application of 56 kg S/ha as K₂SO₄. Sulphur application appeared to have little impact on the hay composition of both first and second cut, with the percentage of alfalfa varying between 24 and 33% in first cut and 43 and 47% in second cut (Table 3.4)
Table 3.3: Yield, tissue sulphur (S) concentration and calculated nutrient uptake results of 1<sup>st</sup> and 3<sup>rd</sup> cut alfalfa hay at Fergus, Ontario trial location, summer 2012.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; Cut</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; Cut</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM&lt;sup&gt;1&lt;/sup&gt; Yield kg/ha</td>
<td>Whole Plant Tissue %S</td>
</tr>
<tr>
<td>Control</td>
<td>4566 b&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.19 b</td>
</tr>
<tr>
<td>KCl</td>
<td>4474 b</td>
<td>0.20 b</td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>6821 a</td>
<td>0.42 a</td>
</tr>
</tbody>
</table>

<sup>1</sup>DM is dry matter  
<sup>2</sup>Least square means followed by the same letter are not significantly different (P= 0.05) as determined by protected LSD test

At the Hesson’13 trial location two cuts of hay were completed. There was no significant difference in first cut yields amongst treatments, but the high rate of elemental (ele.) S attained the highest numerical yield overall (Table 3.4). First cut also had no significant increase in S concentration of hay samples. While there was no significant yield improvement in second cut, S treatments yielded 600 to almost 1000 kg DM/ha more than control and KCl treatments. The highest second cut yield was in 112 kg S/ha as ele.S treatments, with a mean yield of 3715 kg DM/ha. Sulphur concentration in hay and S uptake of second cut was significantly greater in 56 kg S/ha as K<sub>2</sub>SO<sub>4</sub> and 112 kg S/ha as ele S. treatments than in control and KCl treatments (Table 3.4). Significant yield response was seen when first and second cut were summed together for total yield. Overall 112 kg S/ha as ele.S had the greatest total biomass, however it was statistically similar in yield to all treatments except for the KCl treatment. Despite yield being greatest in high ele.S treatments, total uptake of S was highest when 56 kg S/ha as K<sub>2</sub>SO<sub>4</sub> was applied (Table 3.4). Uptake of S was upwards of 4 kg S/ha greater when S was applied compared to no S application treatments. At Hesson’13 no significant differences were seen in the composition of hay between treatments in either first or second cut (Table 3.4).

At Mitchell’13 three hay cuts were completed, and again no significant treatment effect on yield was seen in either first or second cut (Table 3.4). First cut yield did not respond to S application, but in
<table>
<thead>
<tr>
<th>Treatment</th>
<th>1st Cut</th>
<th>2nd Cut</th>
<th>3rd Cut</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM Yield (kg/ha)</td>
<td>Alfalfa %</td>
<td>Tissue S%</td>
<td>Uptake (kg S/ha)</td>
</tr>
<tr>
<td>Wallenstein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6816</td>
<td>31</td>
<td>0.10</td>
<td>6.6 b</td>
</tr>
<tr>
<td>270 kg KCl/ha</td>
<td>6720</td>
<td>24</td>
<td>0.10</td>
<td>6.7 b</td>
</tr>
<tr>
<td>56 kg S/ha Ele. S</td>
<td>6583</td>
<td>32</td>
<td>0.12</td>
<td>8.0 b</td>
</tr>
<tr>
<td>112 kg S/ha Ele. S</td>
<td>6730</td>
<td>34</td>
<td>0.12</td>
<td>8.0 b</td>
</tr>
<tr>
<td>56 kg S/ha K2SO4</td>
<td>6896</td>
<td>28</td>
<td>0.15</td>
<td>10.1 a</td>
</tr>
<tr>
<td>Hesson</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5079</td>
<td>37</td>
<td>0.08</td>
<td>4.3</td>
</tr>
<tr>
<td>270 kg KCl/ha</td>
<td>4627</td>
<td>29</td>
<td>0.08</td>
<td>3.6</td>
</tr>
<tr>
<td>56 kg S/ha Ele. S</td>
<td>4564</td>
<td>37</td>
<td>0.12</td>
<td>5.8</td>
</tr>
<tr>
<td>112 kg S/ha Ele. S</td>
<td>5243</td>
<td>29</td>
<td>0.08</td>
<td>4.4</td>
</tr>
<tr>
<td>56 kg S/ha K2SO4</td>
<td>4713</td>
<td>43</td>
<td>0.11</td>
<td>5.3</td>
</tr>
<tr>
<td>Mitchell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3687</td>
<td>0.16 b</td>
<td>6.1 b</td>
<td>2400</td>
</tr>
<tr>
<td>270 kg KCl/ha</td>
<td>3696</td>
<td>0.17 b</td>
<td>6.2 b</td>
<td>2373</td>
</tr>
<tr>
<td>56 kg S/ha Ele. S</td>
<td>3714</td>
<td>0.20 b</td>
<td>7.4 b</td>
<td>2585</td>
</tr>
<tr>
<td>112 kg S/ha Ele. S</td>
<td>3808</td>
<td>0.22 ab</td>
<td>8.2 ab</td>
<td>2515</td>
</tr>
<tr>
<td>56 kg S/ha K2SO4</td>
<td>3869</td>
<td>0.28 a</td>
<td>11.0 a</td>
<td>2530</td>
</tr>
</tbody>
</table>

1 based on LSMeans
2 indicates significant differences in yields at 0.05
3 uptake calculated based on tissue concentration and yield
4 first cut not analysed for hay composition
first cut hay samples the 56 kg S/ha as K₂SO₄ treatment resulted in significantly greater S concentration and uptake than control, KCl, and low level ele.S treatments (Table 3.4). In second cut, S treatments showed a non-significant trend towards increased yield with S application, and hay S concentration and S uptake were significantly greater in all S treatments (Table 3.4). Third cut yields were significantly improved in all S treatments, with approximate yield improvement of 500 kg DM/ha achieved over the control and KCl treatments (Table 3.4). Sulphur concentration in third cut hay samples was significantly greater in 112 kg S/ha ele.S treatment, at 0.47%S, than all other treatments, with the other two S application rates also resulting in significantly greater S concentration than in treatments where no S was applied (Table 3.4). The application of 112 kg S/ha ele.S also resulted in the most S uptake by the hay crop, at 11.5 kg S/ha; significantly greater than all other treatments. There was significant improvement in total combined yield of the three cuts, with the mean total DM yield of S treatments approximately 700-800 kg greater than check treatments (Table 3.4). The highest total yield achieved was 8803 kg DM/ha by the 56 kg S/ha as K₂SO₄ treatment. Total uptake was significantly impacted by S application: S uptake was just under 13 kg S/ha when no S was applied, compared to uptakes of 20 to 25 kg S/ha with S application (Table 3.4). Composition samples were taken only in second and third cut; in both no significant differences were seen, but the percentage of alfalfa appeared to be greater when S was applied.

When comparing results in 2013 across sites, some trends begin to become apparent. Across all sites, first cut yields were impacted little by S application, and trends towards higher yields with S application were first seen in 2nd cut. While Mitchell’13 showed significant yield response in third cut, there was no third cut completed at Wallenstein’13 and Hesson’13 to ascertain whether response to S increases with each cut completed. However, in a greenhouse trial by DeBoer and Duke (1982), a similar trend in yield was seen, where significant differences in dry matter yield first occurred in third cut. In an early Ontario S trial by Sheard (1976), under greenhouse conditions Ontario soils were forced into S
deficiency by growing alfalfa, and again effects on herbage yield were first seen in third cut. In 2013 results the K₂SO₄ application tended to result in the highest total yield and total uptake; however, the response measured in the K₂SO₄ treatment was always statistically similar to that seen when a high application of ele. S was made. Uptake of fertilizer S was seen in first cut, and was consistently highest in the K₂SO₄ treatment across all three sites. However by second cut, the uptake of S by alfalfa in high ele. S treatments was similar to or greater than that seen in K₂SO₄ treatments, indicating that by mid summer, adequate amounts of sulphate had been oxidized from ele. S. While in our trial ele. S was fall applied, a Wisconsin trial was able to achieve a yield response within the same growing season as S application; however it was only seen at the highest ele. S application rate used of 112 kg S/ha (Hoeft and Walsh 1975). In pot experiments from western Canada, the release of sulphate by ele. S fertilizer has been found to be negligible in the first 24 days after an in-season application is made, after which time formation improves sufficiently to result in overall uptake being comparable to uptake seen with sulphate forms of S applied at the same rate (Janzen and Bettany 1986).

While the delay in plant availability is a drawback to ele. S application, the possibility of it having residual value in subsequent growing seasons is a potential benefit, especially in a perennial crop like alfalfa. In 2014, both the Wallenstein’13 and Hesson’13 trials were carried into another year of production, becoming Wallenstein’14 and Hesson’14 respectively, to examine the residual availability of ele S. In both of these trials, similarly to the first year of the experiment, only 2 cuts were completed in summer 2014. Due to wet spring conditions at Wallenstein’14, tile runs were visible in the field, with visibly greater biomass in plots over tiles. In first cut, plots over tile runs with increased growth were not harvested for data collection, and instead were cut by the farmer immediately after data collection, and the entire trial area was cleared of biomass. In second cut, all plots were harvested, but again it was deemed that higher biomass coincided with the tile runs, and the yield data from those plots was
Table 3.5: LS Mean dry matter (DM) yields, whole above ground hay sample tissue sulphur (S) concentration, and uptake of sulphur by each hay cut completed in the second trial year growing season at Wallenstein, Ontario location based on sulphur application rate, and whether or not the application was completed only in the first year, or in both first and second year of trial.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st cut</th>
<th>2nd Cut</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM Yield kg/ha</td>
<td>Alfalfa in Hay Sample %</td>
<td>Whole Plant Tissue %S</td>
</tr>
<tr>
<td>Control</td>
<td>2853</td>
<td>10</td>
<td>0.14d¹</td>
</tr>
<tr>
<td><strong>Without reapplication in 2nd year</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potash¹</td>
<td>2707</td>
<td>14</td>
<td>0.15cd</td>
</tr>
<tr>
<td>56 kg S/ha Ele.²</td>
<td>3370</td>
<td>28</td>
<td>0.19ab</td>
</tr>
<tr>
<td>112 kg S/ha Ele.</td>
<td>2801</td>
<td>17</td>
<td>0.22a</td>
</tr>
<tr>
<td>56 kg S/ha K₂SO₄</td>
<td>3442</td>
<td>23</td>
<td>0.18b</td>
</tr>
<tr>
<td><strong>With reapplication in second year</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potash</td>
<td>2896</td>
<td>36</td>
<td>0.16cd</td>
</tr>
<tr>
<td>56 kg S/ha Ele.</td>
<td>2778</td>
<td>65</td>
<td>0.19ab</td>
</tr>
<tr>
<td>112 kg S/ha Ele.</td>
<td>3003</td>
<td>58</td>
<td>0.17bcd</td>
</tr>
<tr>
<td>56 kg S/ha K₂SO₄</td>
<td>2994</td>
<td>60</td>
<td>0.19ab</td>
</tr>
</tbody>
</table>

¹Significant at 0.05 based on Tukey Test
²Ele. is elemental sulphur and was fall applied, while K₂SO₄ was spring applied
³Potash applied at 159 kg K₂O/ha to match K applied as K₂SO₄
excluded from the analysis. Tile runs affected three of the six control plots, as well as one 56 kg S/ha as ele S. non-reapplication treatment, one 112 kg S/ha as ele. S non-reapplication treatment, and one 159 kg K₂O/ha non-reapplication treatment. Therefore, even though control plots were split into A and B, only one control value is reported, while amongst the other treatments, yield values were calculated using the LSMeans of the 2 remaining treatments. At Wallenstein’14 there were no significant yield differences seen in either cut, though there was a trend towards S application resulting in greater biomass yield, especially in second cut and total yield (Table 3.5). In all three S treatments, second cut and total yield was higher when no second year S re-application was made.

Whole plant tissue S concentration of first cut was found to be highest in 112 kg S/ha ele.S treatment, when no re-application was made, while overall, most treatments showed significantly greater concentration than control and potash treatments (Table 3.5). Sulphur uptake of first cut in all S treatments that did not receive a reaplication was significantly greater than S taken up by control and potash treatments (Table 3.5). In second cut, S concentration of hay was greatest when a re-application of 112 kg S/ha ele.S was made, but again, most S treatments showed significantly greater S concentration than that seen when no S was applied, and all S treatments showed greater uptake than treatments that did not receive S (Table 3.5). Similarly, no significant differences in composition of hay samples were seen in either first or second cut, but again a trend was seen towards an increase in the percent of alfalfa in hay samples when S was applied, and the proportion of alfalfa also tended to be greater when there was a re-application of S in the second year of the study (Table 3.5).

At Hesson’14, there were also no significant differences seen in the yield of either cut, or in total yield of combined cuts, with results shown in Table 3.6. In first cut, similar to what was experienced in 2013, yields were similar across all treatments, despite some treatments not having had a reapplication of S. In second cut, the highest overall mean yield was achieved through a reapplication of 56 kg S/ha of
Table 3.6: LS Mean dry matter (DM) yields, whole above ground hay sample tissue sulphur (S) concentration, and uptake of sulphur by each hay cut completed in the second trial year growing season at Hesson, Ontario location based on sulphur application rate, and whether or not the application was completed only in the first year, or in both first and second year of trial.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st Cut</th>
<th>2nd Cut</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alfalfa in Hay</td>
<td>Whole Plant</td>
<td>Alfalfa in Hay</td>
</tr>
<tr>
<td></td>
<td>Yield kg/ha</td>
<td>Tissue %S</td>
<td>Sample %S</td>
</tr>
<tr>
<td>Control A</td>
<td>5103</td>
<td>0.19</td>
<td>2918</td>
</tr>
<tr>
<td>Control B</td>
<td>4976</td>
<td>0.18</td>
<td>3061</td>
</tr>
<tr>
<td><strong>Without reapplication in 2nd year</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potash</td>
<td>4808</td>
<td>0.20</td>
<td>3077</td>
</tr>
<tr>
<td>56 kg S/ha Ele.</td>
<td>5037</td>
<td>0.20</td>
<td>3549</td>
</tr>
<tr>
<td>112 kg S/ha Ele.</td>
<td>5037</td>
<td>0.37</td>
<td>3545</td>
</tr>
<tr>
<td>56 kg S/ha K2SO4</td>
<td>5004</td>
<td>0.23</td>
<td>3513</td>
</tr>
<tr>
<td><strong>With reapplication in second year</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potash</td>
<td>4832</td>
<td>0.25</td>
<td>3024</td>
</tr>
<tr>
<td>56 kg S/ha Ele.</td>
<td>5134</td>
<td>0.29</td>
<td>3145</td>
</tr>
<tr>
<td>112 kg S/ha Ele.</td>
<td>4888</td>
<td>0.35</td>
<td>3221</td>
</tr>
<tr>
<td>56 kg S/ha K2SO4</td>
<td>4908</td>
<td>0.27</td>
<td>3781</td>
</tr>
</tbody>
</table>

1. Control plots split in 2nd year, and as such 2 yield values were taken
2. Significant at 0.05 based on Tukey Test
3. Significant at 0.05 based on LSMeans
4. Potash applied at 159 kg K2O/ha to match K applied as K2SO4
5. Ele. is elemental S and is fall applied, while K2SO4 is spring applied
6. Significant response was compared across all treatments
K$_2$SO$_4$. Within second cut, elemental S treatments seemed to perform better when only applied in the first year of the study, while the K$_2$SO$_4$ treatment yielded better when a re-application was made in the second year of the study, as may be expected based on the increased likelihood for loss of S in sulphate form (Janzen and Bettany 1986). Though not significant, when S was applied in the first year of the study all S treatments showed greater total yields than control and potash treatments in the second year of the trial, though yields were quite similar across the three S treatments. Total yield in the second growing season was further improved when K$_2$SO$_4$ was re-applied in the second year of the study, but a reapplication of elemental S had lower yields, though again not significantly. When examining the proportion of the harvested biomass that was alfalfa in first and second cut, there appeared to be some increase in alfalfa content of first cut with S application; however, this effect was not seen in second cut, where the percentage of alfalfa was similar across all treatments. Tissue S concentration in whole plant hay samples did not show significant response to S application in first cut, though treatments involving S application showed mean tissue S consistently above that of control and potash treatments, and highest tissue S was seen in 112 kg S/ha ele., with and without a second year re-application (Table 3.6). The uptake of S by first cut was significantly increased through the application of S, with highest uptake seen in both 112 kg S/ha ele. treatments. In second cut, re-application of 56 kg S/ha as K$_2$SO$_4$ as well as re-application of 112 kg S/ha ele. showed the greatest concentration of S in hay samples, and showed significantly greater S than all control and potash treatments. This carried into uptake results in second cut, where uptake of S in second cut hay was highest (12.1 kg S/ha) when a second year reapplication of 56 kg S/ha as K$_2$SO$_4$ was made compared to control uptakes of only 4.3 and 3.5 kg S/ha (Table 3.6). Significant differences were seen again in total combined uptake of both cuts. Control and potash treatments showed uptakes between 11.8 and 13.8 kg S/ha, while any application of S in either the first year or both years of the trial resulted in uptakes between 17.2 and 28.9 kg S/ha (Table 3.6). The ele. S treatments result in some higher tissue S concentrations than seen with application of K$_2$SO$_4$. 

77
As well, the S concentration being similar between single application of ele.S and second year reapplication treatments, indicates that ele. S may be a viable option for supplying S for multiple growing seasons, and that sufficient levels of sulphate were becoming available in the second year of the trial from a single application of ele. S. While the yield results at Wallenstein’14 were severely impacted by excess moisture early in the season, some results do indicate of the possibility for a single application of ele.S. Total yields achieved by both ele. S treatments with a single application exceeded the yields achieved when a re-application of K₂SO₄ was made by approximately 500 to 1000 kg DM/ha (Table 3.5). At Hesson’14, the re-application of K₂SO₄ out-yielded both single ele. S applications, however at this site the difference between these treatments was only about 100 kg DM/ha (Table 3.6). Other studies suggest that when the reduced cost of ele. S fertilizers, and additional costs that would arise from yearly reapplications, are taken into consideration, ele. S may be a viable option for supplying S to a stand for multiple years (Kelling 2000, Kelling 2002b). Our study could only examine the viability of ele. S providing S to a stand for a second year after application; however, it has been suggested that applications of ele. S will continue to impact yields into the fourth and fifth year of a stand, which would further increase its value as a fertilizer and the viability of application (Seim et al. 1969).

At Mitchell’14, two additional K₂SO₄ rates were included, with the intention of performing regression analysis to determine the best rate of S application. An error was made in calibration of the fertilizer spreader, resulting in applications of 28, 58 and 92 kg S/ha as K₂SO₄. Through the growing season, one plot assigned with a treatment of 58 kg S/ha as K₂SO₄ displayed physical characteristics of S deficiency and looked similar to control plots, with alfalfa showing reduced growth and pale colouring, which became more pronounced with each cut completed. In data analysis, the results from this plot displayed the highest RLD, Press factor, and highest internal studentized residual of the data points collected for yield of first, second and third cut, total yield, whole plant tissue S concentration of second cut, and uptake of second cut. Based on the abnormal physical characteristics displayed by this plot, as
as the prevalence of data taken from the plot showing higher variability from that expected, it was
determined that likely an error had been made when fertilizer was being applied and no S had been
applied on the plot. All results from this plot were therefore excluded from further analysis. There was a
significant increase in hay biomass with increasing application of S in all 3 cuts as well as in total yield
(Figure 3.1 and Figure 3.2). Linear response occurred in first cut, with overall improvement in yield over
0 kg S/ha application of approximately 950 kg DM/ha (Figure 3.1). In second and third cut, as well as
total yield, the data best fit a quadratic plateau model (Figures 3.1 and 3.2). In second and third cut,
yield plateaus were achieved at an S application rate of 47.1 and 56.9 kg S/ha as K₂SO₄ respectively,
while in total combined yield of all cuts, yield plateau was achieved at an application rate of 63.6 kg S/ha
as K₂SO₄. Alfalfa content of the hay also increased with S application, with significant linear response
seen in alfalfa content of both first and third cut, and increases in percentage alfalfa of approximately
18% and 14% respectively, when K₂SO₄ was applied (Figure 3.3). Tissue sulphur concentration showed a
quadratic plateau response for first cut, and linear response in third cut (Figure 3.4). Second cut also
showed an increase in whole plant tissue sulphur concentration, however there was high variance
between data points, causing a lack of significant response (Figure 3.4). Plots with whole plant tissue S
concentrations of over 0.40% alfalfa (Figure 3.4) corresponded to plots showing relatively higher
proportions of alfalfa of 85 to 90%, compared to the rest of the plots which varied from 72 to 86%
alfalfa. It is likely that the increased alfalfa content led to the higher S content of the hay samples. There
was no clear pattern in plots showing a higher proportion of alfalfa, and the difference may have arose
from slight variations in field topography, especially considering that 2014 was a wet growing season. A
significant relationship was found between total sulphur uptake (a function of biomass and S in hay
samples) and applied S; a quadratic plateau was found with a plateau of 25.4 kg S/ha taken up, at an
application rate of approximately 58.2 kg S/ha as K₂SO₄ (Figure 3.5). Total uptake at this trial location
was higher than that seen at other 2014 trial locations, which may have arisen due to the stand also
Figure 3.1: Alfalfa-grass mixed hay stand dry matter (DM) yield of each hay cut completed at Mitchell, trial location, as related to sulphur (S) application rate, summer 2014.

$y = 10.3x + 2880$
$R^2 = 0.53$

$y = -0.49x^2 + 46.6x + 1783$
plateau = 2882
$R^2 = 0.86$

$y = -0.34x^2 + 38.1 + 1042$
plateau = 2129
$R^2 = 0.93$

Figure 3.2: Total combined dry matter (DM) yield of all three cuts completed of an alfalfa-grass mixed hay stand at Mitchell, Ontario trial location, as related to sulphur (S) application rate, summer 2014.

$y = -0.77x^2 + 97.9x + 5618$
plateau = 8734
pseudo $R^2 = 0.86$
Figure 3.3: Percentage of alfalfa in grab samples taken from each cut of a mixed alfalfa-grass hay stand at the Mitchell trial location, as related to sulphur application rate, summer 2014
Figure 3.4: Tissue sulphur concentration of first cut, second cut, and third cut at Mitchell, Ontario trial location as related to S rate applied, summer 2014.
Figure 3.5: Total sulphur (S) uptake by three cuts of mixed alfalfa-grass hay stand as calculated based on S concentration in hay and yield of each cut, as related to S application rate at the Mitchell, Ontario trial location in summer 2014

\[ y = -0.006x^2 + 0.66x + 6.3 \]
plateau= 25.4
pseudo $R^2$ = 0.89

Figure 3.6: Dry matter yield of each cut of a mixed alfalfa-grass hay stand completed at Ancaster, Ontario trial location, as related to sulphur application rate, summer 2014
having a slightly higher proportion of alfalfa in the hay stand, as well as having three cuts completed.

Regression analysis of K$_2$SO$_4$ rates was also possible at Ancaster’14. Due to wet weather at the site in mid-summer, second cut was delayed, and the co-operator decided not to complete the planned third cut. Biomass yield of both first and second cut did not show significant response to applied S, or show any trends towards improved yield with S application (Figure 3.6). Alfalfa content in first and second cut hay also remained unaffected by S application, with first cut containing between 45% and 54% alfalfa, and second cut containing between 77% and 88% alfalfa. In first cut, S application did not impact the S concentration of hay samples; control treatments showed a mean of 0.25% S, while application of K$_2$SO$_4$ led to tissue concentrations of 0.23% to 0.29% S (Table 3.7). There was a non-significant increase in S concentration of second cut hay (Table 3.7). However, uptake of S by second cut hay significantly increased with S applied (Table 3.7). When uptakes were combined into total uptake by all cuts, it was found that highest (not statistically significant) overall S uptake was in the 30 kg S/ha rate, with an uptake of 22.2 kg S/ha, compared to uptakes of approximately 18 kg S/ha when no S was applied, while the lowest S uptake was seen in the 5 kg S/ha application treatments.

The Ancaster trial location was chosen mostly on the basis of establishing a trial on a coarse textured soil, as most other trials were on heavier soil types (Table 3.1). Past studies suggest that sandy soil types are more likely to require yearly reapplications of sulphate due to increased leaching potential (Schulte and Kelling 1992). While the initial soil test levels (Table 3.1) do not indicate a higher S availability than seen at other trial locations, the lack of yield response to S application would suggest that either S was adequate for growth, or that another factor was limiting growth on this trial. The proximity of Ancaster to the city of Hamilton, which as an industrial area, may act as a source of S
Table 3.7: Mean composition of hay cuts as percentage alfalfa of hay, hay sample tissue sulphur (S) concentration, and uptake of sulphur by each hay cut completed at Ancaster, Ontario trial location based on sulphur application rate.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cut 1</th>
<th>Cut 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alfalfa</td>
<td>Whole Plant</td>
<td>Uptake$^2$</td>
</tr>
<tr>
<td>control</td>
<td>48</td>
<td>0.25</td>
<td>12.4</td>
</tr>
<tr>
<td>Potash$^1$</td>
<td>45</td>
<td>0.26</td>
<td>13.2</td>
</tr>
<tr>
<td>5 kg S/ ha</td>
<td>53</td>
<td>0.23</td>
<td>11.0</td>
</tr>
<tr>
<td>10 kg S/ ha</td>
<td>51</td>
<td>0.28</td>
<td>13.3</td>
</tr>
<tr>
<td>20 kg S/ ha</td>
<td>54</td>
<td>0.29</td>
<td>14.5</td>
</tr>
<tr>
<td>30 kg S/ ha</td>
<td>50</td>
<td>0.27</td>
<td>14.7</td>
</tr>
<tr>
<td>40 kg S/ ha</td>
<td>46</td>
<td>0.27</td>
<td>14.3</td>
</tr>
</tbody>
</table>

$^1$applied at 111 kg K$_2$O/ha as KCl  
$^2$uptake calculated based on S concentration of hay and yield
(McLachlan 1975) and may aid in providing adequate S for alfalfa growth at this location. In an early study by Seim et al. (1964) over a two year period the S concentrations in snow and rain gathered in the nearest metropolitan area were approximately four times greater than the levels deposited at their rural trial location (1964). While air quality has dramatically improved since that time, it can still be assumed that fields in closer proximity to metropolitan areas may receive more S from rainfall, and this may have contributed to the lack of results at the Ancaster’14 trial. Mean S concentration in first cut check treatments at Ancaster were 0.25% (Table 3.7), compared to other sites where first cut check treatments ranged from 0.08% to 0.19% S (Tables 3.4, 3.5, 3.6); this serves as an indication of higher soil S availability at this site.

At Elora’14 a greater number of K\textsubscript{2}SO\textsubscript{4} treatments were included, to improve the performance of the regression analysis. In first cut, yields from K\textsubscript{2}SO\textsubscript{4} treatments remained similar across all treatments, as is seen in Figure 3.7, and following a similar trend to what had been seen in 2013 and other 2014 trials. Second and third cut biomass data best fit a quadratic plateau model (Figure 3.8). In second cut, a yield plateau of 2544 kg DM/ha was reached at an application rate of 17.5 kg S/ha. In third cut, yield plateaued at 1908 kg DM/ha at an application rate of 34.3 kg S/ha. Yield plateaus occurred at about two and four times the biomass of the check treatment for the 2\textsuperscript{nd} and 3\textsuperscript{rd} cut respectively. Total combined yield of all three cuts also fit a quadratic plateau model, with a maximum yield of 9769.1 kg DM/ha attained at an application rate of 30.1 kg S/ha (Figure 3.9). Alfalfa content of treatments with K\textsubscript{2}SO\textsubscript{4} applied also showed a quadratic plateau response in second and third cut; plateaus occurred at application rates of 23.2 and 20.8 kg S/ha respectively (Figure 3.10). When examining results of K\textsubscript{2}SO\textsubscript{4} treatments, whole plant tissue samples showed linear response in first and second cut, and quadratic response in third cut of S concentration in tissue as application rate increased (Figure 3.11). While no significant yield improvement was seen in first cut, there was a linear increase in S concentration of hay. This shows that possibly fertilizer S was being taken up in first cut, despite not resulting in increased
**Figure 3.7:** Dry matter yield of each cut of a mixed alfalfa-grass hay stand completed at the Elora Research Station, Ontario, as related to sulphur application rate, summer 2014

**Figure 3.8:** Dry matter yield of second and third cut of mixed alfalfa-grass hay stand completed at the Elora Research Station, Ontario, based on sulphur application rate, summer 2014
Figure 3.9: Total combined dry matter yield of three cuts completed at the Elora Research Station, Ontario trial, based on sulphur application rate, summer 2014

Yield (kg/ha as DM)

Sulphur application rate (kg S/ha as K\textsubscript{2}SO\textsubscript{4})

\[ y = -3.03x^2 + 177.6x + 7170 \]
plateau = 9769
\[ R^2 = 0.68 \]

Figure 3.10: Composition of each hay cut of a mixed alfalfa-grass hay stand completed at Elora Research Station, Ontario given as percent alfalfa in hay sample based on sulphur application rate, summer 2014.

Percent (%) alfalfa in stand

S application rate (kg S/ha as K\textsubscript{2}SO\textsubscript{4})

\[ y = -0.700x^2 + 2.90x + 55.9 \]
plateau = 86.0
\[ R^2 = 0.67 \]

\[ y = -0.029x^2 + 1.36x + 60.5 \]
plateau = 76.3
\[ R^2 = 0.52 \]
Figure 3.11: Concentration of sulphur (S) in hay samples of first, second and third cut of a mixed alfalfa-grass hay stand at the Elora Research Station, Ontario trial, based on S application rate, summer 2014
Figure 3.12: Relationship between uptake of sulphur (S) by first, second, and third cut, as well as the three cuts combined of a mixed alfalfa-grass hay stand at the Elora Research Station, Ontario trial, and S application rate, summer 2014.
yield. When S uptake was calculated from hay S concentration and yield, first cut again showed linear response, while second and third cut best fit quadratic plateau models, with plateaus at application rates of 57.1 and 50.5 kg S/ha respectively, resulting in S uptakes of 6.3 kg S/ha in second cut and 6.5 kg S/ha in third cut (Figure 3.12).

While no significant yield response was seen at Ancaster, the results of the three 2014 trials using variable K$_2$SO$_4$ rates can still be compared to better assess an optimal level of K$_2$SO$_4$ application. At Elora’14 and Mitchell’14, significant quadratic response to applied S was found in second cut, third cut and total yield. In all three of these cases yield plateaus occurred at lower S application rates at Elora’14 than they did at Mitchell’14, with only slight yield increases being achieved at Mitchell’14 in second and third cut. The error in fertilizer application at Mitchell’14 resulted in very high S application rates, and it is unlikely that such a high rate is required for optimal growth of the crop. As such, it is very possible that although the S rates varied predictably the exact rates reported are incorrect. While past studies have indicated optimal yields occur at sulphate application rates as high as 50 kg S/ha, generally, optimal yields have been reached at lower sulphate fertilizer rates (Hutchinson et al. 2007). With the goal of a within-year yield improvement, application rates of 28 kg S/ha were found to be adequate by Hoeft and Walsh (1975), while also leading to a higher recovery rate of S, than was seen at higher S application rates. Mahli (2011) completed a study over eight growing seasons in Saskatchewan and found that yield increases were seen through the fertilization of alfalfa with sulphate in addition to phosphorus and potassium fertilizers, but found that there was little yield benefit associated with applying more than 10 kg S/ha as sulphate. While at times yield did show further improvement at higher rates of sulphate application, the results varied from year to year (Mahli 2011). Total yields varied dramatically over time in this trial due to weather patterns in each specific year; total yields varied from as low as approximately 2000 to 3000 kg/ha in 2003, up to 9000 to 12000 kg/ha in 2007 (Mahli 2011). In higher yielding years, higher S applications tended to further improve yields, and therefore under
Ontario growing conditions, where yields are consistently higher than Saskatchewan, higher application rates may be warranted. Overall, the yield responses seen at Elora’14 show higher pseudo $R^2$, and a better correlation with past research than do those at Mitchell’14. In both trials however, a common trend was that the rate of S application at which yield plateau would occur increased from second to third cut, and to total yield. This indicates that a higher application rate is required to maintain adequate S levels in the soil throughout the growing season. This may be attributed not only to the actual uptake and use of the available sulphate by the plant, but also may be accounting for some of the loss of S out of the soil to factors such as leaching.

At Elora’14 and Mitchell’14 analysis of the effectiveness of elemental S to increase alfalfa yields in the growing season following fall application was also possible. At Mitchell’14, yields achieved with $K_2SO_4$ were similar to those achieved with elemental S, with both ele. S treatments tending to result in yields between those of low and high $K_2SO_4$ rates (Table 3.8). In first cut, S concentration in hay, as well as S uptake of both ele. S treatments was significantly lower than all three $K_2SO_4$ treatments. While there was no significant yield response by first cut, both ele. S treatments yielded better than the lowest $K_2SO_4$ rate, despite a lower hay S concentration. By second cut there were no significant differences in tissue sulphur content of hay between ele. S and $K_2SO_4$ treatments. In third cut, 112 kg S/ha as ele.S had statistically similar tissue S concentration to medium and high rates of $K_2SO_4$, and all three where significantly greater than the low ele. S and low $K_2SO_4$ rates (Table 3.8). Despite the increase in sulphur tissue S concentration seen at higher S application rates, the highest third cut yield was in the 56 kg S/ha as ele. S treatment, showing possible luxury consumption of S, or that another factor may have been impairing higher yields in treatments with higher uptake.

When S uptake was calculated for each cut at the Mitchell’14 site, $K_2SO_4$ treatments showed the highest overall uptake, significantly greater than both ele.S treatments (Table 3.8). Control and potash
Table 3.8: LS Mean dry matter (DM) yields, whole above ground hay sample tissue sulphur (S) concentration, and uptake of sulphur by each hay cut completed at Mitchell, Ontario trial location based on sulphur application rate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1st Cut</th>
<th>2nd Cut</th>
<th>3rd Cut</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM Yield</td>
<td>%S</td>
<td>Uptake</td>
<td>DM Yield</td>
</tr>
<tr>
<td>Control</td>
<td>3433</td>
<td>0.14</td>
<td>b</td>
<td>3.0</td>
</tr>
<tr>
<td>Potash</td>
<td>2797</td>
<td>0.14</td>
<td>b</td>
<td>2.6</td>
</tr>
<tr>
<td>56 kg S/ha Ele.</td>
<td>3428</td>
<td>0.19</td>
<td>b</td>
<td>5.1</td>
</tr>
<tr>
<td>112 kg S/ha Ele.</td>
<td>3444</td>
<td>0.18</td>
<td>b</td>
<td>5.0</td>
</tr>
<tr>
<td>28 kg S/ha K₂SO₄</td>
<td>3194</td>
<td>0.29</td>
<td>a</td>
<td>7.8</td>
</tr>
<tr>
<td>58 kg S/ha K₂SO₄</td>
<td>3671</td>
<td>0.25</td>
<td>a</td>
<td>7.1</td>
</tr>
<tr>
<td>92 kg S/ha K₂SO₄</td>
<td>3696</td>
<td>0.30</td>
<td>a</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 based on LSMeans
treatments both showed significantly less uptake in first cut than all S treatments. In second cut, again K$_2$SO$_4$ treatments had the greatest uptake; however, they were only significantly greater than control and potash treatments. By third cut, the high ele. S rate had exceeded the K$_2$SO$_4$ treatments for greatest S uptake (Table 3.8). Total uptake was found to be similar amongst all K$_2$SO$_4$ rates, as well as the high ele. S rate, while total yield was statistically similar across all S treatments, regardless of the type of fertilizer used (Table 3.8).

At Elora’14, when comparing yields between ele. S and K$_2$SO$_4$ treatments, there were no significant differences between treatments in first cut yield or in hay S concentration and uptake of S, however yields were generally greater when high rates of K$_2$SO$_4$ were applied than when ele. S was applied (Table 3.9). In second cut, ele. S treatments showed statistically similar yields to those attained through application of 10 kg S/ha or greater as K$_2$SO$_4$. However, all S applications resulted in significantly greater second cut biomass than control and potash treatments. While there were no significant differences in yield between S treatments for the second cut, the highest S concentration in hay was seen in the highest K$_2$SO$_4$ rate, followed by the high and low ele. S applications. In third cut, statistically similar yields were seen between ele. S treatments and K$_2$SO$_4$ applications of greater than 20 kg S/ha, and again were significantly greater than control and potash treatments. Third cut yields were similar between ele. S and medium to high K$_2$SO$_4$ applications. S concentration in third cut hay were significantly greater when 112 kg S/ha as ele. S was applied than all other treatments, with S uptake in third cut similar in both ele. S rates and the highest K$_2$SO$_4$ rate used. Total yields of ele. S treatments were also statistically similar to those seen with K$_2$SO$_4$ application. All S treatments showed significantly greater total biomass production than control and potash treatments, except for the two lowest rates of K$_2$SO$_4$ application used.
Table 3.9: Dry matter (DM) yield, tissue sulphur (S) concentration of hay samples and uptake of each cut of a mixed alfalfa-grass hay stand at the Elora Research Station, Ontario, based on select sulphur treatments, summer 2014

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st Cut</th>
<th></th>
<th></th>
<th></th>
<th>2nd Cut</th>
<th></th>
<th></th>
<th></th>
<th>3rd Cut</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM Yield</td>
<td>Whole Plant</td>
<td>Tissue</td>
<td>Uptake</td>
<td>DM Yield</td>
<td>Whole Plant</td>
<td>Tissue</td>
<td>Uptake</td>
<td>DM Yield</td>
<td>Whole Plant</td>
<td>Tissue</td>
<td>Uptake</td>
<td>DM Yield</td>
</tr>
<tr>
<td>Control</td>
<td>5396</td>
<td>0.08</td>
<td>4.1</td>
<td></td>
<td>1236</td>
<td>0.14</td>
<td>b</td>
<td>1.7</td>
<td>b</td>
<td>550</td>
<td>0.17</td>
<td>b</td>
<td>0.9</td>
</tr>
<tr>
<td>Potash</td>
<td>5207</td>
<td>0.10</td>
<td>5.2</td>
<td></td>
<td>1463</td>
<td>0.13</td>
<td>b</td>
<td>1.9</td>
<td>b</td>
<td>557</td>
<td>0.18</td>
<td>b</td>
<td>1.0</td>
</tr>
<tr>
<td>56 kg S/ha Ele. 3</td>
<td>4883</td>
<td>0.11</td>
<td>5.2</td>
<td></td>
<td>2471</td>
<td>a</td>
<td>0.22</td>
<td>a</td>
<td>5.5</td>
<td>a</td>
<td>1800</td>
<td>0.35</td>
<td>a</td>
</tr>
<tr>
<td>112 kg S/ha Ele.</td>
<td>4970</td>
<td>0.13</td>
<td>6.4</td>
<td></td>
<td>2678</td>
<td>a</td>
<td>0.23</td>
<td>a</td>
<td>6.1</td>
<td>a</td>
<td>1859</td>
<td>0.35</td>
<td>a</td>
</tr>
<tr>
<td>56 kg S/ha</td>
<td>5430</td>
<td>0.14</td>
<td>7.7</td>
<td></td>
<td>2488</td>
<td>a</td>
<td>0.27</td>
<td>a</td>
<td>6.7</td>
<td>a</td>
<td>1795</td>
<td>0.40</td>
<td>a</td>
</tr>
</tbody>
</table>

1. Significant at 0.05 based on Tukey Test
2. Potash applied at 159 kg K₂O/ha to match K applied as K₂SO₄
3. Ele. Is elemental S and is fall applied, while K₂SO₄ is spring applied
4. Uptake calculated based on hay S concentration and yield
Figure 3.13: Relationship between dry matter (DM) yield of a mixed alfalfa-grass hay stand of first, second, and third cut, as well as the three cuts combined at the Elora Research Station, Ontario trial, and the S application rate, summer 2015
A new site was established at Elora for the 2015 growing season, which would be in its second production year for the 2015 season. The plot design and treatments used were identical to those used in the 2014 season at Elora’14. Unlike other trial locations, significant quadratic yield response occurred in first cut at the Elora’15 site (Figure 3.13). First cut yield showed a maximum improvement in dry biomass of 1057 kg/ha over control treatment when approximately 29 kg S/ha was applied as K₂SO₄. Significant quadratic response also occurred in second cut; however, the improvement in yield over the check was lower than in first cut, with a maximum improvement in dry biomass of only 388 kg/ha at an application rate of approximately 32 kg S/ha as K₂SO₄ (Figure 3.13). Third cut yield showed significant quadratic plateau at an application rate of 13.1 kg S/ha, with a resulting yield of 3293 kg DM/ha (Figure 3.13). When yield of all three cuts was combined the resulting total yield showed a quadratic response, in which an application of approximately 31 kg S/ha as K₂SO₄ led to a maximum yield increase over the control of 1934 kg DM/ha (Figure 3.13). Mean yields of ele. S treatments were not significantly different from the maximum yields achieved by K₂SO₄ treatments, except in the first cut where the low ele. S application achieved significantly greater yield than the highest rate of K₂SO₄ application (Table 3.10). As there was a significant quadratic response in first cut with increasing K₂SO₄ application (Figure 3.13), it is possible that a 56 kg S/ha as K₂SO₄ rate over-applied S or potassium (as all plots had a blanket potassium application) resulting in poorer growth compared to lower K₂SO₄ applications and ele. S applications. This became less pronounced in second and third cut (Figure 3.13 and Table 3.10). In both first and second cut one or both of the ele. S treatments performed significantly better than control, potash, and/or low K₂SO₄ treatments (Table 3.10). Unlike at other trials, significant yield improvement was already achieved in first cut at this trial; however, the improvement to second and third cut, while still significant, was not as large as what was seen at the Elora’14 trial. Growing conditions in the 2015 season were ideal for hay growth, with adequate rainfall and moderate temperatures throughout the summer, resulting in second and third cut yields only slightly below first cut yields (Table 3.10), which
Table 3.10: Dry matter (DM) yield of each cut of a mixed alfalfa-grass hay stand at the Elora Research Station, Ontario, based on select sulphur treatments, summer 2015

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st cut</th>
<th>2nd cut</th>
<th>3rd cut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2490 c</td>
<td>3091 ab</td>
<td>2831 ab</td>
</tr>
<tr>
<td>Potash(^1)</td>
<td>3268 abc</td>
<td>3046 b</td>
<td>2788 b</td>
</tr>
<tr>
<td>56 kg S/ha Ele.(^2)</td>
<td>3886 a</td>
<td>3392 ab</td>
<td>3257 ab</td>
</tr>
<tr>
<td>112 kg S/ha Ele.</td>
<td>3627 ab</td>
<td>3493 a</td>
<td>3358 a</td>
</tr>
<tr>
<td>56 kg S/ha K(_2)SO(_4)</td>
<td>2976 bc</td>
<td>3455 a</td>
<td>3257 ab</td>
</tr>
</tbody>
</table>

\(^1\)Potash applied at 159 kg K\(_2\)O/ha to match K applied as K\(_2\)SO\(_4\)
\(^2\)Ele. is elemental sulphur

is unusual in Ontario. This lack of stress may have assisted control and potash treatments in maintaining relatively high yields. There is also a possibility that the higher than average rainfall over the summer may have served to supply some S in deposition, as well as displacing some of the surface applied fertilizer onto neighbouring plots, thereby supplying control and potash treatments with S.

The site at Elora’14 was carried into 2015, and each plot was split; half to show residual fertilizer response, while the other half had 40 kg S ha\(^{-1}\) as K\(_2\)SO\(_4\) applied. This allowed for comparison of residual impact of both ele. S and sulphate fertilizers to annual S applications. In first cut there was a curvilinear response to the application of S in 2014; however little to no response to the application of additional S in 2015 (Figure 3.14). In second and third cut there was a strong yield response to residual fertilizer from 2014 with yields increasing to the 40 – 50 kg S ha\(^{-1}\) rate, but the newly applied sulphur largely masked the residual impact (Figures 3.15 and 3.16). By the 2015 growing season, this stand was in its 5\(^{th}\) year of production, an unusually old age for an alfalfa stand in southern Ontario. The application of S in a readily available form in spring 2015 was still able to improve second and third cut yields of plots with no previous S applied to similar biomass levels as plots which received high rates of K\(_2\)SO\(_4\) in spring 2014. The spring 2015 application of 40 kg S/ha as K\(_2\)SO\(_4\) onto control and potash treatments resulted in approximately 3 to 4 times the dry matter yield in second and third cut.
Figure 3.14: Dry matter (DM) yield of first cut of a mixed alfalfa-grass hay stand in the summer of 2015, based on sulphur application, when S applied either: 1) at different application rates solely in spring 2014, or 2) when different spring 2014 rates used in combination with an additional 40 kg S/ha as K$_2$SO$_4$ applied in spring 2015.

Figure 3.15: Dry matter (DM) yield of second cut of a mixed alfalfa-grass hay stand in the summer of 2015, based on sulphur application, when S applied either: 1) at different application rates solely in spring 2014, or 2) when different spring 2014 rates used in combination with an additional 40 kg S/ha as K$_2$SO$_4$ applied in spring 2015.
Figure 3.16: Dry matter (DM) yield of third cut of a mixed alfalfa-grass hay stand in the summer of 2015, based on sulphur application, when S applied either: 1) at different application rates solely in spring 2014, or 2) when different spring 2014 rates used in combination with an additional 40 kg S/ha as K$_2$SO$_4$ applied in spring 2015.

compared to when no S was applied. The design of this trial also allowed for analysis of the viability of ele. S application to provide S for a second production year, by comparing this to yields attained when an additional K$_2$SO$_4$ application was made within the production year. Overall, it was found that a single fall ele. S application at a rates of 56 or 112 kg S/ha achieved first, second and third cut yields in the second production year after application comparable to those achieved when an additional 40 kg S/ha as K$_2$SO$_4$ was applied in the second year (Table 3.11).

When 2013, 2014 and 2015 sites are examined, a fall application of ele. S appears to be as effective as K$_2$SO$_4$ in improving alfalfa hay yields when applied at a higher rate. In general, an application of 112 kg S/ha as ele. S yielded as well, or in some cases better than an application of 56 kg S/ha as K$_2$SO$_4$ when examining total yield. While K$_2$SO$_4$ application did result in higher plant S concentrations in first cut than was seen with ele. S application, this only resulted in improved first cut yields at a few
Table 3.11: First, second and third cut yield in 2015 growing season of a mixed alfalfa grass hay stand at the Elora Research Station, as related to the application of sulphur (S) when applied as various treatments in year prior to yield collection (residual), or if treatment was applied in year prior with an additional 40 kg S/ha applied as potassium sulphate (K₂SO₄) in spring of 2015.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>First Cut Yield (kg/ha)</th>
<th>Second Cut Yield (kg/ha)</th>
<th>Third Cut Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residual</td>
<td>2015 S</td>
<td>Average</td>
</tr>
<tr>
<td>159 kg K₂O</td>
<td>KCl</td>
<td>2289</td>
<td>2734</td>
</tr>
<tr>
<td>0 kg S ha⁻¹</td>
<td>Control</td>
<td>2361</td>
<td>2756</td>
</tr>
<tr>
<td>5 kg S ha⁻¹</td>
<td>K₂SO₄</td>
<td>2684</td>
<td>3462</td>
</tr>
<tr>
<td>10 kg S ha⁻¹</td>
<td>K₂SO₄</td>
<td>2473</td>
<td>3367</td>
</tr>
<tr>
<td>20 kg S ha⁻¹</td>
<td>K₂SO₄</td>
<td>4064</td>
<td>4593</td>
</tr>
<tr>
<td>30 kg S ha⁻¹</td>
<td>K₂SO₄</td>
<td>4813</td>
<td>5060</td>
</tr>
<tr>
<td>40 kg S ha⁻¹</td>
<td>K₂SO₄</td>
<td>4284</td>
<td>4510</td>
</tr>
<tr>
<td>56 kg S ha⁻¹</td>
<td>K₂SO₄</td>
<td>4253</td>
<td>4450</td>
</tr>
<tr>
<td>56 kg S ha⁻¹</td>
<td>Ele.</td>
<td>4060</td>
<td>4516</td>
</tr>
<tr>
<td>112 kg S ha⁻¹</td>
<td>Ele.</td>
<td>4679</td>
<td>4846</td>
</tr>
</tbody>
</table>

**Interaction LSD**²

<table>
<thead>
<tr>
<th></th>
<th>NS</th>
<th>P=0.001 LSD= 550</th>
<th>P= 0.0001 LSD= 370</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>3596 b</td>
<td>4029 a</td>
<td>2984 b</td>
</tr>
</tbody>
</table>
instances; similar results were seen in past trials where yield responses were first seen in subsequent cuttings (Chapman et al. 1972; Sheard 1976). By second and third cut, yield and S concentration in hay were, in most cases, similar between ele. S and K$_2$SO$_4$ treatments, especially at a high ele. S application rate. Total yield with 112 kg S/ha as ele. S and 56 kg S/ha as K$_2$SO$_4$ were statistically similar at all trial locations where this comparison was possible. While ele. S must be converted into sulphate in the soil to become plant available, it appears that when fall applied, in Ontario soil conditions, sufficient sulphate is plant available post first cut to significantly improve second and third cut yields over control and potash, and to achieve similar yield response to sulphate fertilizer. Other studies have seen similar response to ele. S fertilizers, with yield improvement over control first being seen in second and third cut (Hoeft and Walsh 1975, Gunes et al. 2009). Overall, the higher ele.S rate tended to achieve improved yields over the low ele. S rate, a similar result to that seen in a study by Hoeft and Walsh (1975), where for first year yield response a rate of 112 kg S/ha as ele. S was required though ele. S rates of 28 and 56 kg S/ha were also used. In this same study, only 28 kg S/ha was required for yield response when applied as K$_2$SO$_4$ (Hoeft and Walsh 1975).

There also appears to be a residual benefit to ele. S application, as was shown at the Elora ‘14 site when carried into a second trial year. A British study found similar residual value of up to 7 years with both ele. S and sulphate S fertilizer, citing low levels of leaching in heavy soil textures as the reason for both forms of S having good residual value (Dickson 1974). Seim et al. (1969) found ele. S showed residual impact in the fourth and fifth production year following application. A rotational study using various crops in Saskatchewan found at least 2 growing seasons were required for ele. S to become fully available in Saskatchewan soil conditions, after which point ele. S treatment resulted in higher yields than ammonium sulphate, when both were applied only in the first year of the trial (Wen et al. 2003). A Wisconsin study of S application on alfalfa using similar rates to those used in this study, determined
that ele. S, K\textsubscript{2}SO\textsubscript{4} and K\textsubscript{2}SO\textsubscript{4}:MgSO\textsubscript{4} were equally effective at increasing yields by the second and third year following initial S application (Hoeft and Walsh 1975).

Overall, the intended use of S, as well as soil conditions should be considered when making choices between ele. S and sulphate fertilizers. Sulphate fertilizers are easily taken up by the plant, as is seen through the increase in first cut tissue S concentration when using K\textsubscript{2}SO\textsubscript{4}, and a lower rate of S can be applied as sulphate to achieve optimum within season yields. This makes sulphate an ideal choice for correcting a within season deficiency. Both rates of ele. S used were shown to achieve similar within-season 2\textsuperscript{nd} and 3\textsuperscript{rd} cut yield response; Ontario soil conditions seem to allow for adequate sulphate to be produced from ele. S to support alfalfa regrowth, with the added benefit of being effective into a second year, with yields achieved by a single application of S resulting in comparable yields to those seen with a repeat application of K\textsubscript{2}SO\textsubscript{4} at Hesson’s 14 and Wallenstein’s 14. When a stand is established, the application of a high rate of ele. S may be adequate to provide S for the life of the stand (Kelling 2000), and should be given special consideration if soil texture, climate, or field location lend themselves to the loss of sulphate from soil due to excess moisture (Barrow 1975; Weir 1975). It has also been suggested that to achieve a better balance between rapid sulphate release and long-term S availability, a mixture of sizes of ele. S fertilizer granules should be applied, as an increased surface area relative to size will allow more rapid access to soil bacteria and therefore, faster conversion, while larger particles will provide prolonged release (Barrow 1975; Weir 1975).

3.4.2 Economics of Sulphur Application on Alfalfa

Another consideration for an alfalfa grower is the cost of each of these S fertilizer options. While prices will vary, for comparison purposes, bulk fertilizer quotes were obtained from Clark Agri Service in Wellandport, Ontario, in spring 2016. Elemental S was quoted at $698.00 per tonne and K\textsubscript{2}SO\textsubscript{4} at $975.00 per tonne. Therefore, while ele. S must be applied at a higher rate of S than K\textsubscript{2}SO\textsubscript{4}, the reduced
price combined with the high S content of ele. S means that an application of 112 kg S/ha will cost approximately $86.55 per hectare. In contrast a high rate of 56 kg S/ha as K₂SO₄, which is approximately 18% S, will cost approximately $296.40 per hectare, though where regression analysis was possible, our results indicated a more moderate rate K₂SO₄ could achieve similar yield response. In applying 56 kg S/ha K₂SO₄, potassium is also being applied at a rate of 152.2 kg K₂O/ha, which must be taken into consideration, as the added benefit of K application should be removed from the S cost. Applying the 152.2 kg K₂O/ha as muriate of potash (quoted at $535 per tonne in spring 2016 by Clark’s Agri Service), would cost $135.45 per hectare, and when this cost is removed from the cost of K₂SO₄ application, the remaining cost for the S application is $160.95 per ha. Based on the research we conducted, when applied on a responsive site, both K₂SO₄ and ele. S application will result in a similar within year increase in yield. Yield improvements with S application were seen at most trial locations, even when the response was not significant. However, for S application to be feasible, the yield improvement achieved by fertilization must be sufficient to cover the cost of the fertilizer. This issue was briefly addressed by calculating the cost of application of S through both ele. S and K₂SO₄, and then, by assigning hay a value of $0.22 per kg, the increase in hay required to pay for the S application was calculated and plotted (Figure 3.17 for K₂SO₄, and Figure 3.18 for ele. S). The difference between the mean total yield of each ele. S or K₂SO₄ treatment and the control treatment at each site was calculated, and also plotted against the cost of that treatment. Where K₂SO₄ was applied, at trials where total yield showed significant response to S application, such as Elora’14 and Mitchell’14, the increase in yield was generally large enough to cover the cost of S (Figure 3.17). An exception to this was at the Hesson’13 site, where total yield did show significant increase to applied S; however, the greatest response was in an ele. S treatment, while the response to K₂SO₄ was not sufficient to pay for its application (Figure 3.17). At Mitchell’13, there was no significant improvement in total yield with S application, but there was
Solid symbols indicate trials with significant response to S in total yield. Based on mean yield responses at trial location, hay at $0.22/kg DM, K₂SO₄ at $975/tonne, KCl at $535/tonne (Clark Agri Service Wellandport, January 2016)

Figure 3.17: Mean total combined dry matter (DM) yield increase due to sulphur (S) fertilization that was required by an alfalfa-grass hay stand at 2013 and 2014 Ontario trial locations to cover the cost of the S applied, when S applied as potassium sulphate (K₂SO₄)
Solid symbols indicate trials with significant response to S in total yield. Based on mean yield responses at trial location, hay at $0.22/kg DM, elemental S at $698/tonne (Clark Agri Service Wellandport, January 2016).

Trials carried into second year listed separately in legend, where elemental S was either applied again in second year, or left with single first year application.

Figure 3.18: Mean total combined dry matter (DM) yield increase due to sulphur (S) fertilization that was required by an alfalfa-grass hay stand at 2013 and 2014 Ontario trial locations to cover the cost of the S applied, when S is applied as elemental sulphur.
significant increase in individual cuts. At this site, the yield improvement with K$_2$SO$_4$ application, was slightly above that required to cover the cost of the fertilizer (Figure 3.17). The rate of response at all other trials was not significant, and tended to fall below the level of response required to pay for S application (Figure 3.17). It should also be noted that all trials with yields falling below the breakeven yield line were two cut systems, while those falling at or above the line included three cuts. The likelihood of adequate response to S to make S application feasible increases greatly in a three cut system, as third cut tended to be the most responsive to S application. Separately from this graph, the results of regression analysis at responsive sites should also be mentioned. At Elora’14 total yield was found to plateau at an application rate of approximately 30 kg S/ha as K$_2$SO$_4$, which, based on the model, provided a total yield increase of 2600 kg DM/ha, well above the 834 kg DM/ha increase in yield that would be required to pay for the fertilizer cost. At Mitchell’14, a total yield plateau was achieved by an application rate of approximately 64 kg S/ha, which would cost $187.23/ha when only the value of S applied is considered. To cover this cost, an increase in total yield of 851 kg/ha is required, while based on the quadratic plateau model at Mitchell’14 an increase in total yield of 3117 kg DM/ha could be expected at this application rate. In both of these instances, where significant response was seen the yield improvement achieved over the control was well above the yield increase required to pay for the additional fertilizer.

Given the lower cost of ele. S, more trials tended to show adequate response to ele. S to warrant its application than was seen with K$_2$SO$_4$, even when trials did not show significant response in total yield to S application (Figure 3.18). Wallenstein’13 was the only trial location at which neither rate of ele. S application was economically feasible (Figure 3.18). However, when the results of the second year of the Wallenstein trial are taken into consideration, the ele. S application becomes economically feasible, as yield response in the second trial year with only a single application was adequate to pay for that application in the first year of the study. For ease of use, all results were compared on the same
graph, however, the breakeven yield increase required at the second year trials when no reapplication was made would be lower than that plotted on this graph, as the yield improvement from multiple years would be used to cover the cost of a single first year application. Overall, there is a greater likelihood of yield improvement with ele. S being adequate to cover the cost of S application, than is seen with K₂SO₄, especially given that ele. S has better residual value than K₂SO₄. As a means of S fertilization ele. S has been shown to improve yields in Ontario conditions, while being more cost-effective in general than K₂SO₄, especially in situations where it is unknown if S deficient conditions exist, or to safeguard an alfalfa stand against deficiencies in years to come. In cases where S deficiencies are present, an application of K₂SO₄ will likely be economically feasible, especially in a three cut season, to rapidly correct a deficiency.

3.4.3 Quality of Alfalfa Hay

The S concentration of hay measured in this study can be considered beyond its use as an indication of the crop uptake, to also include the effect that the S concentration of hay may have on the dietary intake of S by livestock. Adequate S nutrition for livestock is crucial, with most ruminants requiring 0.14% to 0.24% S in substrate dry matter for proper digestion of feed (Goodrich and Garrett 1986). A lactating dairy cow diet requires at least 0.20% S to maintain proper production, and corn, grains and corn silage will often not contain adequate S (Goodrich and Garrett 1986). With many Ontario dairy farmers using total mixed rations, often consisting of alfalfa hay, corn silage and grains, amongst other additives, improving S content of the alfalfa hay has the potential for assisting in balancing S nutrient concentration in feed. When summarizing a variety of other trials, including beef cattle, dairy cattle and sheep, the growth and production performance of livestock has been found to be optimized when S content of their diet was at levels of 0.20% to 0.25% (Tisdale 2002). Sulphur toxicity has been associated with S concentrations in the total diet of 0.40% S or greater (Goodrich and Garrett 1986), concentrations
Table 3.12: Crude Protein and crude protein produced per hectare of second cut of mixed alfalfa-grass hay stands at three 2013 trial locations, and two 2014 trial locations, based on the sulphur treatment applied

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wallenstein'13</th>
<th>Hesson'13</th>
<th>Mitchell'13</th>
<th>Elora '14</th>
<th>Mitchell'14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% CP</td>
<td>kg CP/ha</td>
<td>% CP</td>
<td>kg CP/ha</td>
<td>% CP</td>
</tr>
<tr>
<td>Control</td>
<td>13.4</td>
<td>345</td>
<td>14.3</td>
<td>383</td>
<td>16.4</td>
</tr>
<tr>
<td>Potash</td>
<td>12.1</td>
<td>292</td>
<td>13.0</td>
<td>351</td>
<td>20.8</td>
</tr>
<tr>
<td>56 kg S/ha ele. S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.6</td>
<td>349</td>
<td>14.9</td>
<td>506</td>
<td>21.2</td>
</tr>
<tr>
<td>112 kg S/ha ele. S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.2</td>
<td>345</td>
<td>14.2</td>
<td>525</td>
<td>20.0</td>
</tr>
<tr>
<td>56 kg S/ha K$_2$SO$_4$</td>
<td>13.7</td>
<td>376</td>
<td>14.6</td>
<td>490</td>
<td>24.2</td>
</tr>
</tbody>
</table>

1 CP % is the percent of crude protein in dry feed sample
2 kg CP/ha calculated based on % CP and yield of a respective plot
3 Potash applied at 159 kg K$_2$O/ha to match K applied as K$_2$SO$_4$
higher than any attained by whole plant hay samples in this study. At times, control and potash treatments resulted in S concentrations in hay which were at the lower end of the ideal spectrum for ruminants, while S fertilization improved S concentration in hay, but not to the point that potentially toxic levels were reached. Since this improvement in tissue concentration coincided with improved yields, the correction of S deficiencies has the potential to not only produce more forage, but also to improve the nutrient status of the forage for the animals it will be fed to.

Sulphur in the plant is also crucial to the process of protein formation, and therefore at each trial location, second cut hay samples tested for S concentration were also subjected to a feed analysis so crude protein (CP) content could be examined. While the concentration of CP in the hay samples varied, CP tended to increase slightly with S application (Table 3.12). While in this trial, the concentration of crude protein was not significantly improved with S application, other studies have found S application to significantly increase the total N and protein N components in alfalfa (Aulakh et al. 1976). Another study by Kelling et al. (2002b) in Wisconsin, found no significant response in the concentration of CP, but did find that CP showed fairly consistent numerical improvements when S was applied of about 1 to 3% over control. The percentage of CP in the sample, as well as the yield of the plot from which that sample was taken, was used to calculate the amount of CP produced per hectare by each plot. When the kg CP/ha results were considered, the improvement in CP produced with S fertilization became more apparent (Table 3.12). In 2013, there was no significant increase in kg CP/ha produced; however, at both Hesson’13 and Mitchell’13 there was a marked improvement in CP produced at all three rates of S application compared to control and potash treatments; at Hesson’13, CP increased by upwards of 100 kg CP/ha with S application, while at Mitchell’13 the improvement in CP varied from 60 kg CP/ha to 215 kg CP/ha. In 2014, kg CP/ha showed significant increase at both Elora ‘14 and Mitchell’14 with the application of S. In Elora, both ele. S treatments as well as the highest K₂SO₄ rate used showed significant improvement in kg CP/ha compared to control and potash. At Mitchell’14
Figure 3.19: Crude protein produced per hectare by second cut of a mixed alfalfa-grass hay stand at Elora, Mitchell and Ancaster research trial locations, based on sulphur application rate, summer 2014.
statistically similar results were seen amongst ele. S and 58 kg S/ha as K$_2$SO$_4$, all of which showed significant improvement over potash treatment. The highest rate of CP produced was seen in high ele. S treatment, in which significant improvement over both control and potash treatments was seen. When examining the results of trials with variable K$_2$SO$_4$ application rates, as was the case in Mitchell’14, Elora’14 and Ancaster’14, significant improvements were again only seen in the amount of CP produced per ha (Figure 3.19). At both Elora’14 and Mitchell’14 quadratic response was seen, with Pr>F values of 0.001 and 0.0094 respectively. At Mitchell’14, CP produced per hectare rose from approximately 248 kg CP/ha with no S applied, up to a plateau at 634 kg CP/ha at application rates at or greater than 54.8 kg S/ha as K$_2$SO$_4$. At Elora’14, CP produced when no S was applied was approximately 217 kg CP/ha, while CP plateaued at 490 kg CP/ha at an application rate of 19.9 kg S/ha. At Ancaster’14 there was no significant treatment effect on the amount of CP produced per hectare, however unlike at Mitchell’14 and Elora’14, there was also no significant yield improvement with S fertilization in second cut at Ancaster’14. As CP/ha was calculated with yield results, the lack of yield response would have had a strong impact on the crude protein production. The improvements seen in crude protein production per hectare can in some instances be attributed not only to the yield improvement seen, but may also be associated with the increase in alfalfa composition of hay. As alfalfa contains more protein than grasses, an increase in alfalfa composition due to S application as was seen in Elora’14 second cut, may contribute to the greater CP production per hectare.

3.5 Conclusions

While current OMAF recommendations state that deficiencies of S in alfalfa have not been observed in Ontario and state that S received through acid rain precipitation is adequate for alfalfa growth, the results of this study indicate otherwise. Of the seven trials completed over the three year period of 2013, 2014, and 2015 (three of which were carried into a second production year) at 7
different field locations (distributed in 5 different areas in southern Ontario), S showed significant response to S in at least one cut or in total yield, while the other cuts (if second or third cut) even if not significant, showed clear trends towards higher yields with S application. Only one trial location showed no response or trend to applied S. The feasibility of S application as both ele. S and as K$_2$SO$_4$ has also been proven, especially in three cut systems. In situations where a grower wishes to protect a young stand against future possible deficiencies, a fall application of 112 kg S/ha as ele. S is recommended, as a high ele. S application has been shown to result in statistically similar yields to K$_2$SO$_4$, and the lower cost of ele. S creates conditions in which the grower is more likely to achieve adequate yield improvement to cover the cost of fertilizer. Elemental S has also been shown to have residual fertilizer value into at least a second production year, with a single ele. S application resulting in similar yields to a reapplication of K$_2$SO$_4$. K$_2$SO$_4$ has also been shown to improve yields, and at trials with significant regression, K$_2$SO$_4$ led to optimal total yields at application rates of 30 to 63 kg S/ha. At responsive trials, the yield improvement seen was sufficient to cover fertilizer costs, however where yield improvement was not significant the yield benefit seen was not sufficient to cover the cost of the fertilizer applied, especially considering the higher cost of K$_2$SO$_4$. However, with the immediate availability of S seen with K$_2$SO$_4$, and the high yield response seen at statistically responsive trials, if used to correct a within season deficiency, K$_2$SO$_4$ is likely to be feasible. The overall quality of alfalfa hay was also improved by S application, through the improved nutrient status of S in hay, as well as increased crude protein and crude protein produced per hectare.
CHAPTER 4: PREDICTING ALFALFA YIELD RESPONSE TO SULPHUR FERTILIZATION BASED ON SOIL AND TISSUE TESTING, IN SOUTHERN ONTARIO

4.1 Background

Historically, southern Ontario has received high levels of sulphur (S) from acid rain deposition. However, the annual rates of S addition to soils have been steadily decreasing in the past two decades. According to the Canada-United States Air Quality Agreement Progress Report 2012, in 1990 southern Ontario was receiving between 28 and 45 kg of SO₄ (9.3 to 15 kg S) per hectare per year (Environment Canada 2013) with total wet and dry deposition at this time of 35 kg S/ha/year in the area along the southern shore of Lake Erie in the United States (National Atmospheric Deposition Program 2015). By 2000, this had decreased to approximately 16-24 kg SO₄ (5.3 to 7.9 kg S) per hectare per year, with a further decrease to the year 2010, at which point southern Ontario was receiving only 4 to 8 kg of SO₄ (1.3 to 2.7 kg S) per hectare per year wet deposition (Environment Canada 2013) and wet and dry deposition along the southern shore of Lake Erie was totalling roughly 10 to 20 kg S/ha (National Atmospheric Deposition Program 2015).

These high deposition rates have, in the past provided an adequate source of S to growing crops in southern Ontario (Ontario Agronomy Guide 2009). S serves as one of the building blocks of proteins in the plant, and is especially crucial due to its role in the synthesis of the amino acids cysteine and methionine, the formation of disulphide bonds, and in many other compounds such as co-enzyme A, thiamin and biotin (Hillel 2005; Heldt and Piechulla 2011). S also plays a role in the process of photosynthesis in the chloroplasts of the plant (Franzen and Grant 2008). As the processes which utilize S in the plant also commonly use nitrogen, phosphorus and other basic elements, proper S nutrition is also crucial to the efficient use of other nutrients by the plant (Franzen and Grant 2008; Haneklaus et al. 2008; The Sulphur Institute 2016). While all plants require S to some degree, in legume species grown as a forage or feedstuff for animal agriculture, such as alfalfa, the need for S is even greater. Alfalfa is a
high protein plant, and sufficient S is crucial to maintaining this high protein content, as well as allowing for adequate regrowth after cutting for feed. Alfalfa is the most common legume crop used for forage in Ontario; favoured for its ability to attain relatively high yields even under drougthy summer conditions due to its deep rooting system, and its suitability to Ontario soil pH, while also producing a high quality, high protein feedstuff (Franzen and Grant 2008; Ontario Agronomy Guide 2009; Ketterings et al. 2012). As one of the most important feedstuffs for Ontario livestock farmers, in 2012 almost 800 000 hectares of alfalfa were harvested across Ontario (Kulasekera 2014), making optimal nutrition and yield in alfalfa a very prevalent issue. The S requirement of alfalfa is relatively high compared to other common field crops grown in the area, with alfalfa requiring approximately 30 kg of S per hectare per year (Kost et al. 2008). Being a forage crop, much of that S is removed from the field. It is estimated that approximately 3 kg S per ha is removed with each Mg of alfalfa hay harvested from a field, and therefore with the constant genetic and management improvements increasing alfalfa yields, the removal of S also is likely to increase (Seim et al. 1969; Hoeft and Fox 1986).

When comparing uptake of S with deposition rates, it is likely that in the past deposition of wet sulphate along with mineralization of S in the soil has been sufficient to meet an alfalfa crops’s needs. Past studies in Ontario have indicated that atmospheric returns of S to soil, and subsequent absorption, has been an adequate source of S to growing alfalfa (Sheard 1976). Therefore, the Ontario Agronomy Guide (2009) contains no fertilizer recommendation rates for S, and reports only of a few scattered incidents of S deficiencies in northern and northwestern Ontario (Sheard 1987, Ontario Agronomy Guide 2009). However, now that deposition rates have decreased to only 4 to 8 kg of S\(_4\) (1.3 – 2.7 kg S ha\(^{-1}\)) per year in 2010, it is likely that there is no longer enough S supplied and deficiencies will become more prevalent (Environment Canada 2013). In a recent analysis of Ohio soils, it was estimated that approximately 93% of Ohio soils are variably to highly deficient in sulfur, meaning that yield improvements with fertilization are now quite likely (Kost et al. 2008). It is estimated that across the
northeastern United States, as well as into Ontario, the levels of removal of S by a healthy alfalfa crop now greatly exceed atmospheric deposition rates, and the level of S supply by soils (Ketterings et al. 2012a; Ketterings et al. 2012b).

Visible deficiencies of S in an alfalfa crop will appear as smaller, stunted plants with thin, brittle stems and yellow colouring (Hillel 2005). As chloroplasts begin to break down as a result of low S levels, a decrease in rate of photosynthesis is seen (Hillel 2005). As a lower mobility nutrient, there is limited remobilization of S from older leaves, and therefore, deficiencies are likely to appear first on the newest growth of the plant (Hillel 2005). A visible deficiency can result in a significant loss of yield for the farmer, with stunted plants producing very low amounts of biomass. However, even a slight S deficiency may have a negative impact on yields, while not resulting in chlorosis of the leaves or severe growth impairment, and therefore may go undiagnosed. In legume species such as alfalfa, S nutrition is especially important as S is used in the process of nitrogen (N) fixation by rhizobia in nodules, as well as in the synthesis of proteins afterwards by the host legume (DeBoer and Duke 1982; Tisdale 2002; Ketterings et al. 2012b). Even in early stages of S deficiency, N fixation is reduced, even while other physiological functions remain unchanged (DeBoer and Duke 1982). It has also been found that the levels of some N-containing compounds are increased in the leaves of alfalfa plants, when under S deficient conditions; an indication of either protein synthesis inhibition or protein degradation by the plant (DeBoer and Duke 1982).

4.1.1 Determining Sulphur Levels in the Field:

Before discussing the availability of S in the field, it is important to understand the transformations S undergoes in the soil. It is estimated that in topsoil, over 90% of the S is found in organic forms, such as organic sulphides and carbon-bonded S (Schoenau and Malhi 2008). This can
come from several sources, such as organic matter in the soil, or fertilizer and manure applications (Kelling 2000). Of the total organic sulfur in soil, approximately 30 to 70% can be readily broken down by soil microorganisms, into inorganic sulphate (Schoenau and Malhi 2008). Other forms of S such as elemental S, must also undergo microbial oxidation by heterotrophic and autotrophic bacteria in soil to be converted into plant-available forms of S (sulphate and thiosulphate) (Jones and Ruckman 1966; Janzen and Bettany 1986; Hillel 2005; Schoenau and Malhi 2008). The relative ease of decomposition and release will depend on the proportion of carbon-bonded S contained in the soil, as this portion remains fairly resistant to decomposition (Schoenau and Malhi 2008).

Prior to oxidation, elemental S is insoluble and hydrophobic, making it stable in the soil profile, and its microbial oxidation into sulphate is a relatively slow and ongoing process depending on particle size and environmental factors such as moisture and temperature (Barrow 1975; Erikson 2008; Schoenau and Malhi 2008). In acidic soils there is some adsorption of sulphate onto metal oxides in the soil (Erikson, 2008). However, in soil pH over 6 there is little adsorption of the sulphate form onto soil particles, leaving sulphate in solution resulting in it being almost as mobile and susceptible to leaching as N (Erikson 2008; Schoenau and Malhi 2008). In Ontario, where soils are commonly neutral to alkaline, especially with the intervention of liming, sulphate in the soil can easily be lost to leaching to below the plant rooting zone (Erikson 2008; Schoenau and Malhi 2008; Ontario Agronomy Guide 2009). Overall, soils with higher organic matter, or recent manure additions should contain higher levels of S, and in turn, sulphate (Franzen and Grant 2008; Hoeft and Fox 1986; Ketterings et al. 2012b). However, with more sulphate available, there is increased likelihood that this sulphate may be lost to leaching.

Leaching is of particular concern in regions or seasons with high precipitation, or in soils prone to leaching due to light to medium textures through which water flows more rapidly (Seim et al. 1969; Hoeft and Walsh 1975; Janzen and Bettany 1986; Schulte and Kelling 1992; Franzen and Grant 2008).
Soils prone to leaching may have low levels of available sulphate and therefore crops growing in these soils may be more likely to show response to S application.

To determine if a yield increase will be achieved through S fertilization, it must be determined if soil S levels will be sufficient to supply the growing crop. This can be done by testing soil S levels in the field before, or during, growth of the crop. While soil testing is a relatively inexpensive and convenient method, the reliability of soil testing for S has been under scrutiny (Koenig et al. 2009). Most soil tests report on S in the sulphate form, which, as discussed, is very dynamic due to mineralization in the soil and being easily lost to leaching (Kost et al. 2008; Schoenau and Mahli 2008; Koenig et al. 2009). Many of these tests are unable to determine, at any given time, how much sulphate is going to become available in the soil through the mineralization and oxidation of organic S, and as such, a relationship between S levels in soil and S response by the plant can be hard to establish (Sheard 1976; Koenig et al. 2009). In a greenhouse trial conducted by Sheard (1976) using Ontario soils, a relationship between soil sulphate levels and alfalfa response to fertilizer could not be found due to the complex release of sulphate from organic matter in the soil. They suggested that finding a relationship with plant growth in the field would be more difficult, as there would be more variation in environmental conditions that could impact S release from organic matter and S deposition rates from the atmosphere (Sheard 1976). More recently, in northern Ontario, researchers again found that neither soil testing of soil sulphate levels nor soil organic matter levels served as good indicators for the need for S fertilization of forage (Hutchison et al. 2007). Also, more comprehensive extraction methods often involve more complex procedures and costly extractants, or may be subject to interference by extracting portions of soil S that may not actually be plant available (Kowalenko and Grimmett 2008). Overall, little work has been done in Ontario or across Canada and the United States as of yet to determine if soil sampling is an adequate method to determine alfalfa responsiveness to S fertilization, and to establish the best calibration and extraction method to use for soil tests for S (Ketterings et al. 2012b).
To avoid some of the problems related to soil testing, a common method to determine if S requirement by a crop is being met is to measure the critical concentration within the plant. The critical concentration is the percentage of a specific nutrient in the plant tissue that is required to obtain optimal yields (Schulte and Kelling 1992; Ontario Agronomy Guide 2009). While studies have found various critical concentration levels for alfalfa tissue, the current generally accepted value for the critical concentration of S in alfalfa is 0.20% to 0.25% S in the top six inches of growth, sampled at the bud to early bloom stage (Pumphrey and Moore 1965; Seim et al. 1969; Bedell 1985; Schulte and Kelling 1992; Kelling 2000). The critical concentration of S in alfalfa is higher than in most other crops grown in Ontario, which is to be expected based on its increased need for S to produce high protein content tissue, as well as to support the high growth rates required by multiple harvests (Dick et al. 2008, Franzen and Grant 2009). Given that S deposition rates through acid precipitation have been high in southern Ontario in the past, the Ontario Agronomy Guide provides no S fertilizer application recommendations, or insight into which type of sampling may be conducive to Ontario conditions and serve as the best indication of a need for alfalfa fertilization with S (Ontario Agronomy Guide 2009). As discussed in Chapter 3, significant yield response in alfalfa to S fertilization has occurred at some Ontario trial locations, and soil and tissue testing should be employed to better understand why some trials responded and others did not. Based on the findings of other researchers in western Canada and the United States, soil and tissue sampling protocols should at this point be tested in, and adapted for best results in Ontario field conditions, to predict alfalfa response, and ensure only necessary applications of S fertilizer are made.

4.2 Hypothesis and Objectives

Based on the completion of S response field trials on alfalfa in 2012 to 2014, significant yield response in alfalfa hay to S fertilization has been shown in Ontario. The objective of this paper is to use the data
from these trials to assess if a reliable relationship between soil and tissue S levels and crop response can be found, to determine the best timing to collect this data, and to determine the critical levels of S in plant tissue and soil in Ontario.

4.3 Materials and Methods

As part of the yield trials discussed in Chapter 3, field trials were established to examine the viability and effectiveness of S fertilization of alfalfa in two subsequent growing seasons; summer 2013 and summer 2014. Trial locations were established in southern Ontario, mostly on co-operators fields, in an effort to include a variety of soil types, as well as to encompass different microclimates in Southern Ontario.

For the 2013 growing season, three field trials were established in the fall of 2012. These trials were run in co-ordination with the Ontario Ministry of Agriculture, and were located in Hesson (Hesson’13), Wallenstein (Wallenstein’13) and Mitchell (Mitchell’13), all in co-operators fields. Each trial was a randomized complete block design of three replications of five treatments as follows:

1: Control with no sulphur applied
2: 56 kg of S per hectare as elemental S
3: 111 kg of S per hectare as elemental S
4: 56 kg S per hectare as potassium sulphate (K₂SO₄)
5: 159 kg K₂O per hectare as potassium chloride (KCl)

At Hesson and Wallenstein, each plot measured 12.2 m by 6.1 m wide. At Mitchell plots measured 25 m long by 6.1 m wide.

For the 2014 growing season, the number of field trials was increased to five locations. The Hesson’13 and Wallenstein’13 trials were able to be carried into another growing season, so as to examine the ability of elemental S to supply an alfalfa crop with sufficient S for two subsequent growing seasons without re-application. In the fall of 2013 at these two trials, each original plot was split in half,
to now measure 12.2 m long by 3.05 m wide. Each half was then randomly assigned to either have a re-application of the same type and amount of material for the 2014 growing season, or to have no application for the 2014 growing season. In this way the Hesson’13 and Wallenstein’13 trials led into the Hesson’14 and Wallenstein’14 trials, respectively.

In addition to these two plots being carried into 2014, three additional sites were established. A new site was established at Mitchell (Mitchell’14) in the fall of 2013, as the previous location of the site was to return to corn in its rotation. The site for the 2014 growing season was owned and managed by the same co-operator as the original, and located approximately a half a kilometre away from the original site. The Mitchell’14 site contained 3 replicates of the same 5 treatments as the Mitchell’13 site, with 2 additional treatments of different rates of K\textsubscript{2}SO\textsubscript{4} to better examine the optimal rate of K\textsubscript{2}SO\textsubscript{4} to apply to maximise yields. It was originally intended for the K\textsubscript{2}SO\textsubscript{4} rates to be approximately 20 kg S per hectare, 40 kg S per hectare as well as the original 52 kg S per hectare, however, due to mis-calibration of the fertilizer spreader used, the following treatments were applied:

1: Control with no sulphur applied
2: 56 kg of S per hectare as elemental S
3: 111 kg of S per hectare as elemental S
4: 82 kg S per hectare as K\textsubscript{2}SO\textsubscript{4}
5: 159 kg K\textsubscript{2}O per hectare as KCl
6: 25 kg S per hectare as K\textsubscript{2}SO\textsubscript{4}
7: 52 kg S per hectare as K\textsubscript{2}SO\textsubscript{4}

At the Mitchell’14 site, there was again sufficient space for plots to measure 25 m long by 6.1 m wide.

Also for the 2014 growing season, a trial was established at the University of Guelph’s Elora Research Station (Elora’14) in the fall of 2013. This was again a replicated complete block design, with 4 replicates, as well as additional treatments, with each plot measuring 10 m long by 4.5 m wide. The treatments used at this site were:
1: Control with no sulphur applied
2: 56 kg of S per hectare as elemental S
3: 111 kg of S per hectare as elemental S
4: 56 kg S per hectare as K$_2$SO$_4$
5: 159 kg K$_2$O per hectare as KCl
6: 5 kg S/ha spring applied as K$_2$SO$_4$
7: 10 kg S/ha spring applied as K$_2$SO$_4$
8: 20 kg S/ha spring applied as K$_2$SO$_4$
9: 30 kg S/ha spring applied as K$_2$SO$_4$
10: 40 kg S/ha spring applied as K$_2$SO$_4$

An additional trial was established for the 2014 growing season in Ancaster (Ancaster’14). Again, a randomized complete block design was used, with individual plots measuring 10 m long by 4.5 m wide. This trial was unable to be established in the fall, and instead was set up in the spring of 2014. Therefore the treatments were adjusted to only include K$_2$SO$_4$ as means of S application, and treatments were as follows:

1: Control with no sulphur applied
2: 5 kg S/ha spring applied as K$_2$SO$_4$
3: 10 kg S/ha spring applied as K$_2$SO$_4$
4: 20 kg S/ha spring applied as K$_2$SO$_4$
5: 30 kg S/ha spring applied as K$_2$SO$_4$
6: 40 kg S/ha spring applied as K$_2$SO$_4$
7: 159 kg K$_2$O per hectare KCl

In all cases, elemental S was applied onto plots in the fall to give opportunity for oxidation to occur to convert the elemental S into plant available sulphate. All K$_2$SO$_4$ and KCl was spring applied, as soon as soil conditions were suitable for driving across the plots without causing damage, generally in
Table 4.1: Dates of trial activities and sulphur fertilization of alfalfa trials for the 2013 and 2014 growing seasons.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Fall Prior to Growing Season</th>
<th>Within Growing Season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial Area</td>
<td>Staking</td>
</tr>
<tr>
<td>2013 Locations</td>
<td></td>
<td>of</td>
</tr>
<tr>
<td>Hesson'13</td>
<td>Nov. 15</td>
<td>Nov. 15</td>
</tr>
<tr>
<td>Mitchell'13</td>
<td>Nov. 9</td>
<td>Nov. 9</td>
</tr>
<tr>
<td>Wallenstein'13</td>
<td>Nov. 15</td>
<td>Nov. 15</td>
</tr>
<tr>
<td>2014 Locations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ancaster'14</td>
<td>Oct. 9</td>
<td>Oct. 9</td>
</tr>
<tr>
<td>Elora'14</td>
<td>Oct. 10</td>
<td>Oct. 10</td>
</tr>
<tr>
<td>Mitchell'14</td>
<td>Sept. 18</td>
<td>Oct. 3</td>
</tr>
<tr>
<td>Hesson '14</td>
<td>Sept. 26</td>
<td>Sept. 26</td>
</tr>
<tr>
<td>Wallenstein'14</td>
<td>Sept. 25</td>
<td>Sept. 26</td>
</tr>
</tbody>
</table>

1S.S. is soil sampling
2Ancaster was spring established, and spring soil sampling is therefore also establishment sampling
3Trials carried into 2nd year, so no establishment date.
late April to early May. Timings of plot establishment, fertilizer application, as well as all other actions performed on trial locations can be found in Table 4.1.

Wherever possible, the fertilizers were applied using a Valmar air-flow fertilizer spreader. At times (spring application at 2013 sites and spring application at Mitchell’14, Wallenstein’14 and Hesson’14) a small Gandy push-spreader was calibrated and used for application of fertilizer.

The primary sampling methods used in these trials were the collection of soil and tissue samples. The protocol for soil sampling was to take nine 2.5 cm wide core samples per plot in a “W” pattern across each plot, while avoiding the outer 0.5 m borders of each plot to ensure samples were not taken from areas with possible cross contamination from neighboring plots. Soil cores were taken to 30 cm depth, and were immediately split into 0-15 cm depth and 15-30 cm depth. Samples were mixed on a per plot basis. Samples were either removed from bags to allow them to air dry, or placed in a drying oven at approximately 30°C as a form of force air-drying. Dried samples were again mixed and a portion of the sample was sent to A&L Laboratories in London, Ontario and S was extracted using Mehlich III (Mehlich 1984) extraction and analysis was by inductively coupled plasma atomic emission spectrometry (ICP-AES).

At the time of trial establishment (generally in fall, except at Ancaster’14) soil sampling was completed to assess original soil S levels, as well as to assess possible spatial variability across the trial site. Prior to elemental S application, soil sampling was completed. In this round of sampling 2 cores were taken per plot and were then mixed together on a per block basis, so as to arrive at one 0-15 cm and one 15-30 cm depth sample per block. The remainder of the process continued as previously outlined. The results of this initial soil sampling can be found in Table 4.2.

In the spring, once the trial area was dry enough to allow for it, soil sampling was completed on control plots, as well as on the plots which had received a fall elemental S application. Sulphate levels in control plots indicated baseline soil S concentrations for the growing season, and sulphate levels in
Table 4.2: Soil texture, age of alfalfa stand, and results of soil sampling completed at trial establishment on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations, prior to application of sulphur fertilizer.

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil Type</th>
<th>Year</th>
<th>OM %</th>
<th>pH</th>
<th>P Bray (ppm)</th>
<th>P Bicarb (ppm)</th>
<th>K (ppm)</th>
<th>Ca (ppm)</th>
<th>Mg (ppm)</th>
<th>Na (ppm)</th>
<th>Al (ppm)</th>
<th>S (ppm)</th>
<th>S (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitchell '13</td>
<td>Clay Loam</td>
<td>3rd year</td>
<td>3.6</td>
<td>7.8</td>
<td>13</td>
<td>7</td>
<td>111</td>
<td>2234</td>
<td>202</td>
<td>8</td>
<td>591</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Hesson '13</td>
<td>Clay Loam</td>
<td>2nd Year</td>
<td>4.1</td>
<td>7.4</td>
<td>38</td>
<td>24</td>
<td>132</td>
<td>2894</td>
<td>286</td>
<td>10</td>
<td>755</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Wallenstein</td>
<td>Clay Loam</td>
<td>'13</td>
<td>5.5</td>
<td>7.1</td>
<td>16</td>
<td>10</td>
<td>126</td>
<td>2846</td>
<td>290</td>
<td>13</td>
<td>832</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Mitchell '14</td>
<td>Clay Loam</td>
<td>'14</td>
<td>3.9</td>
<td>7.4</td>
<td>8</td>
<td>6</td>
<td>73</td>
<td>2207</td>
<td>330</td>
<td>16</td>
<td>664</td>
<td>7</td>
<td>5</td>
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<tr>
<td>Hesson '14</td>
<td>Clay Loam</td>
<td>'14</td>
<td>4.0</td>
<td>7.0</td>
<td>42</td>
<td>24</td>
<td>125</td>
<td>3557</td>
<td>318</td>
<td>16</td>
<td>853</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Wallenstein</td>
<td>Clay Loam</td>
<td>'14</td>
<td>5.6</td>
<td>6.3</td>
<td>19</td>
<td>11</td>
<td>128</td>
<td>2840</td>
<td>323</td>
<td>12</td>
<td>882</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Elora '14</td>
<td>Loam Till</td>
<td>4th year</td>
<td>3.6</td>
<td>7.3</td>
<td>16</td>
<td>11</td>
<td>54</td>
<td>2280</td>
<td>300</td>
<td>10</td>
<td>703</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Ancaster '14</td>
<td>Loam</td>
<td>2nd year</td>
<td>2.2</td>
<td>6.9</td>
<td>51</td>
<td>26</td>
<td>31</td>
<td>830</td>
<td>191</td>
<td>11</td>
<td>775</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

1 based on mean fall control plot values as trials were carried into 2nd year
2 based on spring soil testing while all other values were fall soil testing
elemental S treatments could be compared to these baseline S values. Exceptions to this method of spring soil sampling were the Wallenstein’14 and Hesson’14 plots, as these plots were going into their second year of production in the spring of 2014, and all plots had received a S application in the previous year, all plots were soil sampled at these locations. Another exception is the Ancaster’14 site. As this site was first established in the spring of 2014, spring soil sampling consisted of the method of soil sampling done at plot establishment, with 2 cores per plot being mixed on a per block basis. Soil sampling was also completed on all treatments following first cut (except at Wallenstein’14 and Hesson’14 where hard, soil conditions meant that only 0-15 cm could be sampled) and again in late September (so as to sample all trial locations in the same time period regardless of when the last cut was completed). Fall soil sampling of the Elora’14 site had to be pushed into October once third cut was completed. Also, at the Hesson’14 site, stakes marking the trial area were accidentally removed following second cut, and the exact trial area could not be determined again with certainty so as to obtain final fall soil samples.

Tissue sampling protocol again used a “W” pattern of sampling across each plot, harvesting the top 15 cm of growth from approximately 35-40 plants per plot, and placing each sample into a paper bag. This sampling was done as close as possible to the late bud to first flower stage, according to the protocol established by Kelling et al. (2002a). The sample was then either delivered fresh to the lab or force air dried at 65°C. The tissue sampling was completed only on the first and second cut of each plot in each year, as if a farmer were to employ this method to assess the need for S application, any application of fertilizer must be completed as early in the growing season as possible to reduce potential yield loss (Kelling et al. 2002a).

At all sites on co-operator fields, cutting hay was completed in accordance with the co-operator’s cutting schedule and weather and field conditions, and therefore was not always done at optimal timing in terms of alfalfa maturity. The sites were harvested either the day before, or morning
before the co-operator planned on cutting the rest of the field. Cutting of the plots was done with a carter harvester, with a 1.5 m wide cutter bar. The carter is designed with conveyor system, which delivers all the cut hay into a weigh box. In this way all the hay harvested by the carter can be weighed before dumping it back on the field. Two to four subsample strips per plot were cut with the carter, depending on overall plot size, so that as much of the plot area could sampled as possible. The length of each individual strip was measured, so as to find the exact area sampled per plot. With this information, the weight obtained per subsample could then be converted to a wet weight per hectare.

From the hay harvested on each plot, two random whole plant hay samples were taken, each with a weight of approximately 1 kg. The first of these samples was immediately weighed and then placed in ovens to be dried at 65°C for approximately 4 to 7 days, with the weight of each bag being monitored over the course of this time period until no further weight loss was seen, and it was deemed that samples had been fully dried. Once fully dry, the weight was taken again, and total amount of moisture contained in the fresh sample was calculated, to allow for adjustment of the wet biomass yield per plot into a dry biomass yield in kilograms per hectare. All tissue samples (top 30 cm samples discussed previously, and hay samples) were sent to A&L Labs, London Ontario, ground, and underwent aqua regia and hot block digestion with analysis by ICP-AES.

First and second cut was completed at all sites in both 2013 and 2014. Where possible, a third cut of hay was also completed. The cooperators at the Hesson’13/’14 and Wallenstein’13/’14 locations did not complete third cut as part of their regular practices, and therefore in neither trial year was a third cut performed at these locations. At the Ancaster’14 site, due to wet weather delaying second cut, the co-operator decided he no longer wished to do a third cut on the field, so as not to cut during the fall critical harvest time of alfalfa, which is the six weeks preceding the initial fall frost, and for the trial location would be approximately the six weeks following August 30th (Ontario Agronomy Guide 2009). At Mitchell’13 as well as Mitchell’14, all three cuts were completed in a timely manner in terms of
alfalfa maturity. At the Elora’14 site, three cuts were also completed. At this site, the first cut was delayed by wet weather, which in turn delayed second cut. At this trial, as a means of avoiding cutting during the critical fall harvest period for alfalfa, third cutting was delayed until the beginning of October 2014.

All statistical data analysis was completed using SAS Software, Version 9.3 (The SAS Institute 2011). For 2014 data, where multiple rates of potassium sulphate were used, PROC MIXED was used to run a regression analysis of soil S concentration and tissue S concentration against S application rate. In some instances plotted data appeared to follow a non-linear model, and in these cases the data was analyzed using a quadratic plateau function using PROC NLIN. The results of PROC NLIN were then compared to those achieved using PROC MIXED to determine which model the data best fit.

For 2013 and 2014 data, to compare soil S concentration and tissue S concentration in top 15 cm of growth of potassium sulphate treatments to those seen with elemental S, and assess the overall significance of S application, PROC GLM was used. Where a significant treatment effect was seen, Tukey’s test was used as a method of means comparison between treatments. PROC Univariate procedure was used to assess the distribution of the data, with the difference between mean and median, skewness and kurtosis factor and the Shapiro-Wilk statistic taken into consideration. When Shapiro-Wilk statistic was significant at Pr<0.05 it was deemed that results were not normally distributed. In these cases, the data was log transformed and the same procedure was completed. If distribution was not improved using log values, the original values were used to proceed with the analysis. A PROC MIXED test for outliers was also completed, with PRESS factor, Restricted Likelihood Distance (RLD) and Lund’s Test for Internal Studentized Residual taken into account. If the Internal Studentized Residual was higher than that reported as the critical value for the respective number of observations and the RLD was also the highest reported for the data points, the data point was considered to be a statistical outlier (Bowley 2008). This outlier assessment was combined with
observational data recorded at the trial locations, and in most instances an outlying data point could be attributed to observed identifiable issues in the field, and the respective data point was eliminated from the data analysis. When a value was removed from an analysis, LSMeans were used instead of means, and where a significant Type III treatment effect was seen, the Least Squares Means table was used to assess for significant differences between treatments. For all of the data analysis a P value of <0.05 was used to determine significance.

After all trial locations were analyzed separately, the yields across sites were normalized by dividing the yield of each plot by the highest yielding plot at that location, and multiplying by 100 to obtain a “percent of highest” yield for each plot in each cut of hay at each trial. By normalizing yields, results could then be combined across all sites and analyzed against tissue S concentrations. A delta yield was also established for each K₂SO₄ treatment. Within each replicate at each trial location, the control plot yield was subtracted from the yield of the K₂SO₄ plot, to establish the yield improvement achieved through S application. These delta yields were then plotted against the S concentration in spring soil tests, as this sampling time allowed for the most opportunity to correct an S deficiency. For both of these across-site comparisons, PROC MIXED was used to complete a regression analysis to examine if yield showed a significant relationship with tissue or soil S concentration data.

4.4 Results and Discussion:

4.4.1 Use of Top 15cm Tissue S Concentration to Predict Fertilizer S Requirement

Three trial locations were established in fall 2012 to be used for the 2013 growing season. At Hesson’13 and Wallenstein’13, only two cuts of alfalfa were completed, as per the wishes of the co-operator, while at Mitchell’13 three cuts were completed. Despite seeing little change in first cut yield, at all 2013 trial locations, S application led to a significant increase in tissue S concentration in top 15 cm
Table 4.3: Yield, tissue sulphur concentration of top 15 cm of alfalfa growth sampled prior to first and second cut, and tissue S concentration of alfalfa-grass mixed stand hay samples taken from each cut completed at Hesson, Wallenstein and Mitchell, Ontario trial locations, based on sulphur application, summer 2013

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1st Cut</th>
<th>2nd Cut</th>
<th>3rd Cut</th>
<th>Total DM Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wallenstein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>DM Yield</td>
<td>Hay</td>
<td>DM Yield</td>
<td>Hay</td>
</tr>
<tr>
<td></td>
<td>kg/ha</td>
<td>S%</td>
<td>kg/ha</td>
<td>S%</td>
</tr>
<tr>
<td></td>
<td>6816</td>
<td>0.22 b</td>
<td>0.10</td>
<td>2556</td>
</tr>
<tr>
<td></td>
<td>6720</td>
<td>0.22 b</td>
<td>0.10</td>
<td>2507</td>
</tr>
<tr>
<td></td>
<td>6583</td>
<td>0.26 b</td>
<td>0.12</td>
<td>2766</td>
</tr>
<tr>
<td></td>
<td>6730</td>
<td>0.27 b</td>
<td>0.12</td>
<td>2831</td>
</tr>
<tr>
<td>56 kg S/ha Ele. S</td>
<td>6896</td>
<td>0.35 a</td>
<td>0.15</td>
<td>2768</td>
</tr>
<tr>
<td>112 kg S/ha Ele. S</td>
<td>6896</td>
<td>0.35 a</td>
<td>0.15</td>
<td>2768</td>
</tr>
<tr>
<td>56 kg S/ha K2SO4</td>
<td>6896</td>
<td>0.35 a</td>
<td>0.15</td>
<td>2768</td>
</tr>
<tr>
<td>270 kg KCl/ha</td>
<td>6720</td>
<td>0.22 b</td>
<td>0.10</td>
<td>2507</td>
</tr>
<tr>
<td>56 kg S/ha Ele. S</td>
<td>6583</td>
<td>0.26 b</td>
<td>0.12</td>
<td>2766</td>
</tr>
<tr>
<td>112 kg S/ha Ele. S</td>
<td>6730</td>
<td>0.27 b</td>
<td>0.12</td>
<td>2831</td>
</tr>
<tr>
<td>56 kg S/ha K2SO4</td>
<td>6896</td>
<td>0.35 a</td>
<td>0.15</td>
<td>2768</td>
</tr>
<tr>
<td>270 kg KCl/ha</td>
<td>4627</td>
<td>0.20 c</td>
<td>0.08</td>
<td>2703</td>
</tr>
<tr>
<td>56 kg S/ha Ele. S</td>
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<td>0.25 bc</td>
<td>0.12</td>
<td>3401</td>
</tr>
<tr>
<td>112 kg S/ha Ele. S</td>
<td>5243</td>
<td>0.34 ab</td>
<td>0.08</td>
<td>3715</td>
</tr>
<tr>
<td>56 kg S/ha K2SO4</td>
<td>4713</td>
<td>0.38 a</td>
<td>0.11</td>
<td>3353</td>
</tr>
<tr>
<td>Hesson</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>DM Yield</td>
<td>Hay</td>
<td>DM Yield</td>
<td>Hay</td>
</tr>
<tr>
<td></td>
<td>kg/ha</td>
<td>S%</td>
<td>kg/ha</td>
<td>S%</td>
</tr>
<tr>
<td></td>
<td>5079</td>
<td>0.24 c</td>
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</tr>
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<td>0.2 c</td>
<td>0.08</td>
<td>2703</td>
</tr>
<tr>
<td>56 kg S/ha Ele. S</td>
<td>4564</td>
<td>0.25 bc</td>
<td>0.12</td>
<td>3401</td>
</tr>
<tr>
<td>112 kg S/ha Ele. S</td>
<td>5243</td>
<td>0.34 ab</td>
<td>0.08</td>
<td>3715</td>
</tr>
<tr>
<td>56 kg S/ha K2SO4</td>
<td>4713</td>
<td>0.38 a</td>
<td>0.11</td>
<td>3353</td>
</tr>
<tr>
<td>Mitchell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>DM Yield</td>
<td>Hay</td>
<td>DM Yield</td>
<td>Hay</td>
</tr>
<tr>
<td></td>
<td>kg/ha</td>
<td>S%</td>
<td>kg/ha</td>
<td>S%</td>
</tr>
<tr>
<td></td>
<td>3687</td>
<td>0.25 b</td>
<td>0.16 b</td>
<td>2400</td>
</tr>
<tr>
<td>270 kg KCl/ha</td>
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<td>0.26 b</td>
<td>0.17 b</td>
<td>2373</td>
</tr>
<tr>
<td>56 kg S/ha Ele. S</td>
<td>3714</td>
<td>0.31 b</td>
<td>0.2 b</td>
<td>2585</td>
</tr>
<tr>
<td>112 kg S/ha Ele. S</td>
<td>3808</td>
<td>0.32 b</td>
<td>0.22 ab</td>
<td>2515</td>
</tr>
<tr>
<td>56 kg S/ha K2SO4</td>
<td>3869</td>
<td>0.48 a</td>
<td>0.28 a</td>
<td>2530</td>
</tr>
</tbody>
</table>

*based on LSMeans

Significant differences in yields at 0.05
of alfalfa sampled prior to first cut (Table 4.3). In first cut at all three locations, 56 kg S/ha as K₂SO₄ showed the highest tissue S levels, reaching concentrations as high as 0.48% S, and all three S treatments resulted in consistently higher S concentrations in top 15 cm tissue than seen in control and potash treatments (Table 4.3). Similar trends were seen in S concentrations in whole plant hay samples of first cut. S fertilized plots tended to have higher S concentrations, but results were only significantly higher at Mitchell’13 (Table 4.3). While second cut also did not show significant yield improvement at any 2013 site location, there was consistent numeric improvement in S concentration of top 15 cm tissue sampled prior to second cut, with significant improvement at Mitchell’13 (Table 4.3).

In 2014, both Hesson and Wallenstein trial locations were carried into a second trial year, to examine the efficacy of a single S application at providing adequate S for two growing seasons. At both sites, despite a lack of significant difference in dry matter yield of both first and second cut, a significant increase was seen in the S concentration of top 15 cm samples (Tables 4.4 and 4.5). At Wallenstein’14, there was little difference in the S concentration of first cut top 15 cm samples; however, by second cut, significant increases over control and potash treatments were seen when K₂SO₄ was reapplied in the second year, as well as when a high ele. S application was made only in the prior year (Table 4.4). At both Hesson and Wallenstein, the highest mean second cut yield was not found in the treatment with the highest mean S concentration in top 15 cm tissue sampled prior to second cut (Table 4.4 and 4.5). This may indicate some luxury consumption of S by alfalfa. At both trials, high ele. S and K₂SO₄, both with and without re-application in the second trial year, consistently achieved increased S concentrations, and also tended to achieve the highest yields (Tables 4.4 and 4.5).

At Mitchell’14, Ancaster’14 and Elora’14, the inclusion of multiple rates of K₂SO₄ allowed for regression analysis of application rate with S concentration in the top 15 cm of tissue. Regression of S concentration in top 15 cm of tissue was possible at all three of these locations for both pre-first cut and pre-second cut top 15 cm plant samples. At both Elora’14 and Mitchell’14, regression analysis showed a
Table 4.4: LS Mean dry matter (DM) yields, top 15 cm alfalfa tissue sulphur (S) concentration, and whole above ground hay sample tissue S concentration of each hay cut completed in the second trial year growing season at Wallenstein, Ontario location based on sulphur application rate, and whether or not the application was completed only in the first year, or in both first and second year of trial, summer 2014

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st Cut</th>
<th></th>
<th>2nd Cut</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM Yield Kg/ha</td>
<td>Top 15 cm Tissue %S</td>
<td>Hay Tissue %S</td>
<td>DM Yield kg/ha</td>
</tr>
<tr>
<td>Control</td>
<td>2853</td>
<td>0.38</td>
<td>0.14 d</td>
<td>1983</td>
</tr>
<tr>
<td>Without reapplication in 2nd year</td>
<td>Potash²</td>
<td>2707</td>
<td>0.34</td>
<td>1904</td>
</tr>
<tr>
<td></td>
<td>56 kg S/ha Ele.³</td>
<td>3370</td>
<td>0.37</td>
<td>3394</td>
</tr>
<tr>
<td></td>
<td>112 kg S/ha Ele.</td>
<td>2801</td>
<td>0.35</td>
<td>3223</td>
</tr>
<tr>
<td></td>
<td>56 kg S/ha K₂SO₄</td>
<td>3442</td>
<td>0.32</td>
<td>3170</td>
</tr>
<tr>
<td>With reapplication in second year</td>
<td>Potash</td>
<td>2896</td>
<td>0.30</td>
<td>1941</td>
</tr>
<tr>
<td></td>
<td>56 kg S/ha Ele.</td>
<td>2778</td>
<td>0.31</td>
<td>2204</td>
</tr>
<tr>
<td></td>
<td>112 kg S/ha Ele.</td>
<td>3003</td>
<td>0.33</td>
<td>2719</td>
</tr>
<tr>
<td></td>
<td>56 kg S/ha K₂SO₄</td>
<td>2994</td>
<td>0.32</td>
<td>2568</td>
</tr>
</tbody>
</table>

1Significant at 0.05 based on Tukey Test
2Potash applied at 159 kg K₂O/ha to match K applied as K₂SO₄
3Ele. is elemental S and is fall applied, while K₂SO₄ is spring applied
Table 4.5: LS Mean dry matter (DM) yields, top 15 cm alfalfa tissue sulphur (S) concentration, and whole above ground hay sample tissue S concentration of each hay cut completed in the second trial year growing season at Hesson, Ontario location based on sulphur application rate, and whether or not the application was completed only in the first year, or in both first and second year of trial, summer 2014

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st Cut</th>
<th>2nd Cut</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM Yield kg/ha</td>
<td>Top 15 cm Tissue %S</td>
</tr>
<tr>
<td>Control A</td>
<td>5103</td>
<td>0.28 de²</td>
</tr>
<tr>
<td>Control B¹</td>
<td>4976</td>
<td>0.29 cde</td>
</tr>
<tr>
<td>Without reapplication in 2nd year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potash⁴</td>
<td>4808</td>
<td>0.22 e</td>
</tr>
<tr>
<td>56 kg S/ha Ele. ⁵</td>
<td>5037</td>
<td>0.34 bcd</td>
</tr>
<tr>
<td>112 kg S/ha Ele.</td>
<td>5037</td>
<td>0.43 ab</td>
</tr>
<tr>
<td>56 kg S/ha K₂SO₄</td>
<td>5004</td>
<td>0.39 abc</td>
</tr>
<tr>
<td>With reapplication in second year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potash</td>
<td>4832</td>
<td>0.22 e</td>
</tr>
<tr>
<td>56 kg S/ha Ele.</td>
<td>5134</td>
<td>0.44 ab</td>
</tr>
<tr>
<td>112 kg S/ha Ele.</td>
<td>4888</td>
<td>0.48 a</td>
</tr>
<tr>
<td>56 kg S/ha K₂SO₄</td>
<td>4908</td>
<td>0.48 a</td>
</tr>
</tbody>
</table>

¹control plots split in 2nd year, and as such 2 yield values were taken
²Significant at 0.05 based on Tukey Test
³Significant at 0.05 based on LSMeans
⁴Potash applied at 159 kg K₂O/ha to match K applied as K₂SO₄
⁵Ele. Is elemental S and is fall applied, while K₂SO₄ is spring applied
Figure 4.1: Concentration of sulphur (S) in the top 15 cm of randomly sampled alfalfa in mixed alfalfa-grass hay stands sampled prior to first and second cuts, when alfalfa was approximately late bud to early bloom stage, based on the amount of S applied at three trial locations; Ancaster, Mitchell and Elora research Station, Ontario, summer 2014.
curvilinear increase with increasing S application rate, and it was determined that the data best fit quadratic plateau models (Figure 4.1). When quadratic plateau models were fit, it was found that at Elora’14, S concentration of pre-first cut top 15 cm samples reached a plateau of 0.45% S, at an application rate of 32.0 kg S/ha, while pre-second cut top 15 cm samples reached a plateau of 0.41% S at an application rate of 38.0 kg S/ha (Figure 4.1). While no significant differences were seen in the corresponding yield of first cut at Elora’14, in second cut, a yield plateau was achieved at an application rate of 17.5 kg S/ha (Chapter 3), again indicating increases in S uptake beyond the S application rates required for maximum yield. At Mitchell’14, higher S applications were required to reach a plateau in top 15 cm tissue S concentration; in first cut a plateau was reached at 0.43% S at an application of 45 kg S/ha, and in second cut a plateau of 0.33% S began at an application of 50 kg S/ha (Figure 4.1). At both Mitchell’14 and Elora’14 the maximum S concentration achieved by top 15 cm tissue samples of second cut were slightly lower than those seen with first cut; however, a higher spring application of S was required to achieve it. At Ancaster’14, unlike at Mitchell’14 and Elora’14, there was no significant yield response to S application. However, S application still resulted in a quadratic plateau increase in first cut S concentration with the plateau at a S concentration of 0.50% and an application rate of 25 kg S/ha, as well as a linear increase in second cut (Figure 4.1). Ancaster ’14 either had sufficient S supplied to it to attain maximum yields and was simply taking up more S in luxury consumption, or another external factor may have been causing impaired growth, and therefore no change in yield was seen even though S was being taken up by the alfalfa.

When comparing the S concentration of top 15 cm tissue based on type of S fertilizer applied, there was little difference seen at Mitchell’14 and Elora’14 between ele. S application and K$_2$SO$_4$. At both Mitchell’14 and Elora’14, top 15 cm tissue sampled prior to first cut saw the greatest S concentration in the 56 kg S/ha as K$_2$SO$_4$ application, with results significantly greater than both ele. S application rates (Table 4.6). By second cut at Elora, the high ele. S treatment had overtaken the high K$_2$SO$_4$ rate for S.
Table 4.6: Dry matter (DM) yield, tissue S concentration in top 15 cm of alfalfa growth sampled just prior to cutting, as well as tissue concentration of mixed alfalfa-grass hay samples, at Elora and Mitchell Ontario trial locations, based on sulphur application rate and type, summer 2014

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DM Yield kg/ha</th>
<th>Top 15 cm Hay</th>
<th>Hay %S</th>
<th>DM Yield kg/ha</th>
<th>Top 15 cm Hay</th>
<th>Hay %S</th>
<th>DM Yield kg/ha</th>
<th>Hay %S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st cut</td>
<td>2nd cut</td>
<td>3rd cut</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elora</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
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<td>550</td>
<td>0.17</td>
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<td>0.10</td>
<td>1463</td>
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<td>557</td>
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<td>0.38 a</td>
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<td>1801</td>
<td>0.35</td>
</tr>
<tr>
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<td>0.13</td>
<td>2678</td>
<td>0.45 a</td>
<td>0.23</td>
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</tr>
<tr>
<td>56 kg S/ha K\textsubscript{2}SO\textsubscript{4}</td>
<td>5430</td>
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<td>0.27</td>
<td>1795</td>
<td>0.40</td>
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<tr>
<td>Mitchell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3433</td>
<td>0.21 c\textsuperscript{2}</td>
<td>0.14</td>
<td>2181</td>
<td>0.20 c\textsuperscript{2}</td>
<td>0.19 c\textsuperscript{1}</td>
<td>1282 b\textsuperscript{2}</td>
<td>0.12 c\textsuperscript{2}</td>
</tr>
<tr>
<td>Potash\textsuperscript{3}</td>
<td>2797</td>
<td>0.21 c</td>
<td>0.14 b</td>
<td>1783</td>
<td>0.13 d</td>
<td>0.14 c</td>
<td>1042 b</td>
<td>0.12 c</td>
</tr>
<tr>
<td>56 kg S/ha Ele.\textsuperscript{4}</td>
<td>3428</td>
<td>0.23 bc</td>
<td>0.19 b</td>
<td>2720</td>
<td>0.23 bc</td>
<td>0.27 b</td>
<td>2253 a</td>
<td>0.21 b</td>
</tr>
<tr>
<td>112 kg S/ha Ele.</td>
<td>3444</td>
<td>0.28 b</td>
<td>0.18 b</td>
<td>2724</td>
<td>0.29 ab</td>
<td>0.36 a</td>
<td>2158 a</td>
<td>0.33 a</td>
</tr>
<tr>
<td>58 kg S/ha K\textsubscript{2}SO\textsubscript{4}</td>
<td>3671</td>
<td>0.40 a</td>
<td>0.25 a</td>
<td>2801</td>
<td>0.35 a</td>
<td>0.37 a</td>
<td>2081 a</td>
<td>0.34 a</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Significant at 0.05 based on Tukey Test  
\textsuperscript{2}Significant at 0.05 based on LSMeans  
\textsuperscript{3}Potash applied at 159 kg K\textsubscript{2}O/ha to match K applied as K\textsubscript{2}SO\textsubscript{4}  
\textsuperscript{4}Ele. is elemental S and is fall applied, while K\textsubscript{2}SO\textsubscript{4} is spring applied
concentration in top 15 cm tissue, although both ele. S and high K₂SO₄ applications contained statistically similar S concentrations (Table 4.6). In top 15 cm samples of second cut alfalfa at Mitchell, while the highest S concentration was still seen with K₂SO₄, high ele. S yielded concentrations only slightly below those of K₂SO₄.

Overall, good visual correlation was seen with tissue S concentration of top 15 cm tissue samples, and yield in the respective cut, with increasing S concentration generally resulting in higher yields. While first cut yields varied little, increased S was seen in first cut alfalfa tissue where S had been applied, and by second and third cut, this improvement in S concentration resulted in higher yields. Based on pre-established critical concentrations of approximately 0.20% to 0.25% S in the top 15 cm of growth sampled at the bud to early bloom stage (Pumphrey and Moore 1965; Seim et al. 1969; Bedell 1985; Sheard 1987; Schulte and Kelling 1992), in many cases the control and potash treatments resulted in S concentrations exceeding the critical concentration requirements, although second and third cut were often significantly lower for these treatments. Had sampling been completed solely prior to first cut, with S concentrations measuring in the range of 0.20 to 0.25%, a grower would likely have considered S levels to have been sufficient, and would have decided against S application, possibly resulting in decreased second and third cut yields compared to if S had been applied. Additionally, since first cut yields varied little across S treatments, it is unlikely that any noticeable yield difference would be seen by a grower to make them aware of a possible future deficiency. Concentrations of 0.20 to 0.25% in first cut samples of top 15 cm of growth may indicate inadequate S supply, and lead to a significant reduction in second and third cut yields. It is also possible that at following first cut soil conditions are not as conducive to S mineralization, leading to lower levels of SO₄ being available to plants, and that the addition of S fertilizers in providing either SO₄ or more S available for mineralization, helps to mitigate this problem. These results indicate that top 15 cm sampling must either be repeated prior to second cut, or that critical concentration of first cut tissue should be greater than 0.25% to
indicate adequate S is available for regrowth of subsequent cuts. Several other trials have also found critical concentration of S in alfalfa to be in excess of 0.25%; 0.31% S in first cut and 0.40% S in second cut were suggested as critical concentrations by Sorensen et al. (1968), and Kelling (2000) suggested a range of 0.25% to 0.50% to be used to indicate sufficient S in tissue.

To determine the critical concentration of S required in first and second cut of alfalfa, the yield of each cut from K₂SO₄, control, and potash treatments were regressed with the S concentration of top 15 cm tissue samples taken prior to first and second cut (Figures 4.2 and 4.3). Significant yield response to pre-first cut S concentration was only found in first cut yield at Mitchell’14 (Figure 4.2) and third cut yield at Elora’14 (Figure 4.2). At both of these trials there was also a visual trend in the other cuts completed of biomass yield improving as the S concentration in pre-first cut top 15 cm samples increased (Figure 4.2). At Ancaster’14 there was no trend towards improved yield with increased S concentration; however, S concentrations were all above 0.30% S, while at Mitchell’14 and Elora’14, concentrations began at S concentrations around 0.20%. When yield was assessed against pre-second cut top 15 cm S concentration data, again visual trends were seen at Elora’14 and Mitchell’14 of increasing yield in both second and third cut as tissue S concentration increased (Figure 4.3). Significant yield response to increased S concentration was only seen in second cut at Mitchell’14 (Figure 4.3). Again Ancaster’14 did not show the same visual trends as Elora’14 and Mitchell’14, but tissue concentration also tended to be higher overall at this site (Figure 4.3). These results show that biomass yield of later cuts can be related back to pre-first and second cut tissue S concentration. This is important as earlier sampling will allow for earlier S application to correct potential deficiencies. Based on S concentration of top 15 cm tissue, yields increase as S concentration increases, up to approximately 0.35% S, after which point yields plateau.
Figure 4.2: Dry Matter (DM) yield of each cut of mixed alfalfa-grass hay stand at Elora, Mitchell and Ancaster, Ontario trial locations, based on tissue sulphur concentration of top 15 cm of alfalfa tissue sampled prior to first cut, summer 2014.
Figure 4.3: Dry matter (DM) yield of each cut of mixed alfalfa-grass hay stand at Elora, Mitchell and Ancaster, Ontario trial locations, based on tissue sulphur concentration of top 15 cm of alfalfa tissue sampled just prior to second cut, summer 2014
To assess if these critical tissue S concentrations held true across other sites, the yield of each plot (including both ele. S and K₂SO₄ applications) at each trial location was normalized, and then regressed against the corresponding S concentration of the top 15 cm tissue sampled prior to first (Figures 4.4 to 4.7) and second cut (Figures 4.8 to 4.10). There was little improvement in biomass of first cut as tissue S concentration in top 15 cm of alfalfa increased (Figure 4.4), with most plots attaining at least 60% of maximum yield, despite a fairly large range in tissue concentration, from 0.14% up to 0.55% S. When second cut yield was regressed against the pre-first cut tissue S concentration there was a significant quadratic plateau response (Figure 4.5). Yields increased as tissue S levels increased, up to a plateau at a yield of 84.67% of maximum yield, at a tissue S concentration of 0.44% S (Figure 4.5). Third cut was only completed at three trials over the two year period, and while no significant response was seen, there was a visual trend of third cut yield increasing as pre-first cut top 15 cm tissue S concentration increased (Figure 4.6). Lastly, the yields of all cuts completed at each trial location were summed and normalized, and again yield showed significant non-linear response to pre-first cut top 15 cm tissue; yield plateaued at 85.35% of maximum yield at a tissue S concentration of 0.34% S (Figure 4.7).

The same process was used to compare second cut, third cut, and total yield to the tissue S concentration of pre-second cut top 15 cm of alfalfa. In this case, quadratic plateau response was seen between second cut biomass yield and tissue S concentration, with an increase in maximum yield up to a concentration of 0.45% S (Figure 4.8). The psuedo $R^2$ value of this equation of 0.1643, was slightly above the $R^2$ seen when second cut was regressed against pre-first cut tissue S concentration of 0.1553 (Figure 4.5). Where completed, third cut yield showed a visual trend towards improving as S concentration increased in pre-second cut tissue samples, though no significant response was seen (Figure 4.9). When the yield of each cut was combined, normalized, and plotted against pre-second cut tissue S
Figure 4.4: Dry matter yield* of first cut of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, when sampling completed prior to first cut, in summer 2013 or 2014.

* yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield

Figure 4.5: Dry matter yield* of second cut of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, when sampling completed prior to first cut, in summer 2013 or 2014.

* yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield

Trials with significant second cut yield increase indicated with closed symbols

\[ y = -271.6x^2 + 239.7x + 32 \]

plateau= 88

pseudo \( R^2 = 0.155 \)
Trials with significant third cut yield increase indicated with closed symbols

**Figure 4.6**: Dry matter yield* of third cut of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations where third cut was completed, based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, when sampling completed prior to first cut, in summer 2013 or 2014

* yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield

Trials with significant total cut yield increase indicated with closed symbols

**Figure 4.7**: Dry matter total combined yield* of all cuts of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, when sampling completed prior to first cut, in summer 2013 or 2014

* yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield

\[ y = -402.1x^2 + 274.6x + 38 \]

plateau = 85

pseudo \(R^2 = 0.072\)
Trials with significant second cut yield increase indicated with closed symbols

Figure 4.8: Dry matter second cut yield* of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, when sampling complete prior to second cut, in summer 2013 or 2014

* yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield

Trials with significant third cut yield increase indicated with closed symbols

Figure 4.9: Dry matter third cut yield* of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations where third cut was completed based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, when sampling completed prior to second cut, in summer 2013 or 2014

* yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield
Trials with significant total combined yield increase indicated with closed symbols.

**Figure 4.10**: Dry matter total combined yield* of all cuts completed on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of top 15 cm of alfalfa growth, when sampling completed prior to second cut, in summer 2013 or 2014.

* yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield.

When yields were assessed together across trial locations, yield response to an increase in tissue S concentration was first seen in second and third cut. This improvement, in turn, led to an increase in the combined yield of all cuts as S concentration increased. When assessing the critical concentration required to attain maximum yields, it is likely that 0.20 to 0.25% S, as named by other studies as a critical concentration (Pumphrey and Moore 1965; Seim et al. 1969; Bedell 1985; Sheard 1987; Schulte and Kelling 1992), is insufficient in these Ontario trials. Our results indicate levels of S as high as 0.34% S in pre-first cut alfalfa and 0.37% in pre-second cut alfalfa, are required in the top 15 cm of growth to attain maximum total yield. Significant response was also seen in second cut, and S
concentrations of 0.44% and 0.45% S, in pre-first and pre-second, respectively, led to maximum biomass production. The results in this study correspond well with the results seen by Sorensen et al. (1968) where the levels of S in tissue below which a response to S fertilizer would be seen were established to be 0.31% in first cut, 0.40% in second cut and 0.48% in third cut. Second cut yields in a trial by Seim et al. (1969) were also maximized at S concentrations above 0.30% in tissue.

While pre-second cut sampling may serve as a slightly more accurate indicator of S sufficiency (higher $R^2$ value), if sampling is completed just prior to second cut and it is established that S concentration in tissue is low, any S application following second cut would only allow for a yield improvement in third cut. In this study, all S applications were either spring or fall applied, allowing sufficient time for uptake, and therefore it is unknown if an application following second cut would create a sufficient improvement to third cut yields to achieve the same total yield increase seen in these trials. The use of pre-first cut samples, and subsequent application of a readily available sulphate fertilizer as early as possible should S concentration be deemed insufficient, is the best method to improve second and third cut yields. Sampling at first cut showed an $R^2$ value only slightly below that of pre-second cut sampling, and therefore sampling prior to first cut may be the better option, so as to gain the benefit of an earlier remedial S application if tissue S levels are insufficient to allow for adequate second and third cut regrowth. There is still benefit to sampling prior to second cut however, especially in a younger alfalfa stand that a grower wishes to maintain for subsequent growing seasons. Pre-second cut sampling can still indicate deficiencies in S, and an application of S following second cut, or in fall after third cut can correct S deficiency in the subsequent growing season.

4.4.2 Use of S Concentration of Hay Samples to Predict Fertilizer S Requirement

Many alfalfa growers complete a nutrient analysis of hay samples to assist them in properly balancing the rations of their livestock. Therefore, it may be useful if the S concentration in hay samples (entire aboveground plant including all plants in mixture) could serve as an indication of future yield
potential in a similar way to top 15 cm samples, to avoid the additional cost requirements of top 15 cm tissue analysis. The normalized yields of each cut, as well as total yield were therefore analyzed against the S concentration in first cut hay samples, in a similar way to top 15 cm tissue S. This analysis was only completed for first cut hay samples, as this is the only cut at which a proceeding S application would still be able to affect subsequent yields. In first cut, there was no significant effect of S concentration in hay samples on normalized yield (Figure 4.11) despite a relatively large range in tissue S concentration from 0.06% S up to 0.37% S. The normalized yield of second cut did show a non-linear increase with first cut hay S concentration, with a plateau reached at approximately 0.36% S (Figure 4.12). Where third cut was completed, yields also showed a non-linear response to S concentration in first cut hay samples, with a plateau reached at 86% of maximum yield at tissue S concentrations of approximately 0.25% S and above (Figure 4.13). When the yields of each cut were combined, a non-linear response to S concentration in first cut was also seen (Figure 4.14). However, tissue S concentrations of 0.51% were required to gain plateau; much higher than in those seen in second and third cut. A low R² (0.0417) was associated with this equation, and a wide range of yields were seen at comparable low S concentrations; for example a level of 0.070% S in tissue led to normalized yield of 50%, compared to 0.05% S leading to a normalized yield of 94%. This variance was not seen to the same degree when comparing total yield with top 15 cm samples from first cut, which also showed a higher pseudo R² value. This indicates that hay sample S concentration may not be as accurate of an indicator of yield potential as top 15 cm tissue sampling. This may be related to the variability of the hay stands across sites, as each site contained differing amounts of grasses and weeds; the various species will contain different amounts of tissue S, and effect the ability of accurately using hay samples to predict fertilizer S requirement.
Figure 4.11: Dry matter first cut yield* completed on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of first cut hay samples, in summer 2013 or 2014
* yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield

Trials with significant second cut yield increase indicated with closed symbols
Figure 4.12: Dry matter second cut yield* completed on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of first cut hay samples, in summer 2013 or 2014
* yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield
Trials with significant third cut yield increase indicated with closed symbols

Figure 4.13: Dry matter third cut yield* completed on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of first cut hay samples, in summer 2013 or 2014

*yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield

Trials with significant total yield increase indicated with closed symbols

Figure 4.14: Dry matter combined yield* of each cut completed on mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations based on corresponding tissue sulphur (S) content of first cut hay samples, in summer 2013 or 2014

*yield normalized by dividing yield per plot by maximum yield of a single plot at that trial location, and multiplying by 100 to attain % of highest yield
4.4.3 Use of Soil Test S Level to Predict Fertilizer S Requirement

Sulphur concentration in fall, spring, and summer soil samples were also analyzed to predict yield response to S application. All trials were sampled in fall, spring (as early as soil conditions were conducive to sampling), and following first cut at both 0-15 cm and 15-30 cm depths, and the mean S concentrations of control plots sampled at each of these times is displayed in Table 4.7. This table also shows at which trial locations a significant response to S fertilization was seen, and which trials showed a trend towards increased yields with S fertilization. If soil S results could consistently indicate where a need for S application existed, a fall or spring soil S test would be an easy method for farmers to employ to ensure S applications were warranted and could be completed sufficiently early in the growing season to correct deficiencies in growing alfalfa. It would be expected that soils testing low in S would be more likely to show yield response to applied S; however, even when different sampling times are examined separately, there are no clear trends, or minimum level of S concentration below which a response to S fertilization can be expected. For example, trials with similar summer soil S levels, such as Elora’14 and Ancaster’14 (Table 4.7) had different yield responses; Elora showed significant yield improvement with S application, while at Ancaster’14 there was no significant response, and no trend towards improvement in the yield data. No consistent patterns can be easily identified to determine a base S concentration in soil below which an S application will improve yields.

Sulphur application did lead to increases in soil S test levels in summer soil sampling, as increasing rates of \( \text{K}_2\text{SO}_4 \) application often resulted in significant increases in soil S test levels (Chapter 3). To allow for data to be compared across sites and assess the impact of non-fertilized soil test S levels on responsiveness of alfalfa to S fertilization the delta yield of each cut at 2013 and 2014 locations was compared to the baseline soil S level. The overall yield was analyzed only against the spring soil test S level in the control plots, as this is the most feasible sampling time at which a subsequent spreading could correct a deficiency. No significant relationships were found between the base soil S
Table 4.7: Sulphur concentration in soil samples completed in fall prior to growing season, spring prior to growing season, and following first cut of mixed alfalfa-grass hay stands at all 2013 and 2014 trial locations in southern Ontario, when no sulphur fertilizer was applied, and whether or not significant response to sulphur fertilization was seen at that trial location when some form of sulphur application was made

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil Type</th>
<th>Fall Sampling</th>
<th>Spring Sampling</th>
<th>Summer Sampling</th>
<th>Responsive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-15 cm</td>
<td>15-30 cm</td>
<td>Average</td>
<td>0-15 cm</td>
</tr>
<tr>
<td>Mitchell '13</td>
<td>Clay Loam</td>
<td>7.7 5.5 6.6</td>
<td>7.7 5.7 6.7</td>
<td>13 7.3 10.2</td>
<td>Yes</td>
</tr>
<tr>
<td>Hesson '13</td>
<td>Clay Loam</td>
<td>7.7 7.0 7.4</td>
<td>9.7 5.7 7.7</td>
<td>8.0 5.7 6.9</td>
<td>Yes</td>
</tr>
<tr>
<td>Wallenstein '13</td>
<td>Clay Loam Till</td>
<td>7.6 6.0 6.8</td>
<td>7.7 7.3 7.5</td>
<td>8.3 6.3 7.3</td>
<td>No (Trend)</td>
</tr>
<tr>
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<td>Clay Loam</td>
<td>7.3 5.3 6.3</td>
<td>6.3 6.0 6.2</td>
<td>6.0 4.7 5.4</td>
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</tr>
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<td>Hesson '14³</td>
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<td>9.7 8.3 9</td>
<td>8.7 7.7 8.2</td>
<td>7.0</td>
<td>No (Trend)</td>
</tr>
<tr>
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<td>Clay Loam Till</td>
<td>7.0 6.3 6.7</td>
<td>8.0 7.0 7.5</td>
<td>8.6 3.2 5.9</td>
<td>No (Trend)</td>
</tr>
<tr>
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<td>8.5 6.0 7.3</td>
<td>Yes</td>
</tr>
<tr>
<td>Ancaster '14²</td>
<td>Sandy Loam</td>
<td>7.5 6.8 7.2</td>
<td>8.0 6.3 7.2</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

¹At trial establishment trials sampled on a replication basis, while all other values given are for control plots
²Ancaster Trial was spring established, and spring soil sampling was done per replicate
³Summer sampling was only completed to a 0-15 cm depth
levels across sites and the yield improvement seen with fertilization. Due to yields remaining similar across treatments in first cut, there is little change in delta yields as baseline S concentration increases (Figure 4.15, 4.16 and 4.17). It would be expected that as the baseline soil S concentration increased, there would be less yield improvement with S application. However, in second and third cut, as well as the total combined yield of all cuts, there is a trend towards an increase in delta yield as the S concentration in the 0-15 cm depth check plot soil increased (Figure 4.15). When sampled at the 15-30 cm depth, delta yield behaved as expected, with significant decrease in delta yield as soil S increased in the second cut as well as total yield, and a trend towards decreases in third cut (Figure 4.16). A significant non-linear response was seen in second cut and total, with yield plateau occurring at 6.3 and 6.8 ppm S, respectively. When the S concentration in the two soil depths were averaged together, the trends visible when examined separately disappeared and delta yield did not appear to be affected by S concentration in the top 0-30 cm of soil (Figure 4.17). These results indicate that alfalfa may be more reliant on S in the 15-30 cm soil depth, and may be more responsive to S application when S concentration in this soil depth is low.

Many other studies have had similar issues in finding consistent relationships between soil S levels measured by soil extractants and alfalfa response to S fertilization. Soil S testing has often been found to be unreliable due to the high mobility and variability of sulphate across relatively small areas, as well as the inability of S tests to predict the amount of sulphate that will become available in the soil as organic S is converted to sulphate (Beaton and Soper 1986; Koenig et al. 2009). Early greenhouse trials completed using Ontario soils could not find a significant relationship between soil S concentrations and the yield response by alfalfa to additional S fertilization (Sheard 1976). Sheard suggested that the use of soil S testing would be further hindered in field situations, where the issue of atmospheric deposition of S and the effect of weather patterns on the release of sulphates from soil
Figure 4.15: Relationship between delta yield, as calculated by subtracting the yield of the control from the yield achieved through application of sulphur (S) as potassium sulphate, on a per replicate basis, and spring soil sulphur concentration as measured by Mehlich III extraction, in the 0-15 cm soil depth of the respective control plots.
Figure 4.16: Relationship between delta yield, as calculated by subtracting the yield of the control from the yield achieved through application of sulphur (S) as potassium sulphate, on a per replicate basis, and spring soil sulphur concentration as measured by Mehlich III extraction, in the 15-30 cm soil depth of the respective control plots.
Figure 4.17: Relationship between delta yield, as calculated by subtracting the yield of the control from the yield achieved through application of sulphur (S) as potassium sulphate, on a per replicate basis, and the average spring soil sulphur concentration as measured by Mehlich III extraction, of the 0-15 cm and 15-30 cm soil depth of the respective control plots.
organic matter would arise (1976). More recently, in a study of 6 locations in north-west Ontario
growing alfalfa, neither soil organic matter nor soil test S concentration appeared to be an accurate
indicator for the need for S fertilization of alfalfa (Hutchinson et al. 2007). In a trial on canola in
northeastern Ontario, similar issues were encountered as was seen in the present alfalfa study, with soil
sulphate levels being similar across sites, while yield and response to S varied from site to site, showing
that the soil S test used may not be an adequate predictor of response to S application (Rowsell and
Kobler 2012).

Some studies have had success finding correlation between soil S tests and crop response. In an
early study of soil extractants used on Nebraska soils growing alfalfa, a calcium phosphate extraction
appeared to be the most accurate with the widest potential for application (Fox et al. 1964). Their
results indicated that 7 ppm of soil test sulphate in soil was sufficient for first cut of alfalfa; however,
they suggested that further field calibration be completed as subsoil S levels would also come into play
in a field situation (Fox et al. 1964). The Nutrient Management Guide for Dryland and Irrigated Alfalfa
published in co-operation by three American universities, has suggested that an application of S should
be completed if S concentration in the 0-30 cm depth is lower than 15 ppm, with a suggested
application rate of 11 to 25 kg sulphate-S/ha when soil S concentrations are 5 to 15 ppm for irrigated
alfalfa (Koenig et al. 2009). This publication summarizes research completed in Oregon, Idaho and
Washington state in a dry region, and therefore, in terms of moisture availability, irrigated conditions
are more likely to resemble Ontario conditions than non-irrigated alfalfa production. As a summary of
other research, various extraction methods were used across the various studies. However, these
researchers stated that the different extraction methods used still came to the same conclusions in
regards to optimal soil test levels and fertilizer S recommendations (Koenig et al. 2009).
Contrary to this, several studies have compared different S extractants, and found that certain extractants are more reliable than others in specific study areas. Some studies have found correlation between soil S test concentrations and S fertilization requirements, using a calcium chloride (CaCl$_2$) extractant and ICP-AES analysis (Nuttall et al. 1987; Ketterings et al. 2011). In trials in New York state, Ketterings et al. (2011) found that of six extractants tested (including Mehlich III), CaCl$_2$ showed the best correlation with S applied. Adsorption of SO$_4^{2-}$ in neutral to alkaline soils is low (Lewis 1999), and therefore CaCl$_2$, which only extracts SO$_4^{2-}$ in soil solution, may be a sufficient indicator of available S in soil when soil pH is only slightly acidic to neutral (Ketterings et al. 2011). In this trial, soil S analysis was completed using the Mehlich III extraction method, which is able to extract some of the adsorbed S and organic S in addition to the SO$_4^{2-}$ in soil solution, and therefore likely resulted in higher reported soil S concentrations than would have been seen had CaCl$_2$ been used as the extractant (Ketterings et al. 2011). With the exception of Wallenstein ’14, trial locations had neutral to alkaline pH (Table 4.2), suggesting that CaCl$_2$ extraction may have been a better choice for S extraction. Another extractant that has been shown to be successful is a 0.25 M KCl extraction heated to 40$^\circ$C for three hours (Blair et al. 1993). This method extracts some of the ester sulphates from soil, a portion of soil S that has been shown to be readily converted to plant available sulphate (Blair et al. 1993). In a comparison of extraction methods, there was a significant relationship between the S taken up by plants and S extraction by KCl at 40$^\circ$C, CaCl$_2$, and KH$_2$PO$_4$, but not with extractions by NaHCO$_3$ and NaOH extractions (Goh and Pamidi 2003). Further research is needed to confirm the best S extractant for Ontario soils, but both CaCl$_2$ and KCl at 40$^\circ$C should be investigated for their accuracy on Ontario soils. No matter the extractant used, additional problems with soil S testing are the spatial variability of soil sulphate levels across a field, as well as the ability of roots to access S below regular soil sampling depth, both of which can hinder correlation between soil S levels and yield response (Karamanos et al. 2007). Also, research in pot experiments has shown that even in instances where there is a significant relationship between
plant S uptake and soil test S, after the 8th week of growth, S being taken up by the plant arises mainly from mineralized S that was not present in extractable S pools (Goh and Pamidi 2003). This indicates that even with accurate extraction methods, soil S testing will still likely only indicate relatively short term S availability.

4.5 Conclusion

Overall, tissue testing was found to be a more reliable indicator of alfalfa response to S fertilization than soil S testing. As was shown in Figure 4.7 and Figure 4.10, the total combined yield of all cuts reached a maximum when tissue S concentration in the top 15 cm of alfalfa growth were 0.34% S and 0.37% S in first and second cut, respectively, at early bloom stage, and these concentrations are suggested as critical concentrations in Ontario conditions. If soil sampling is to be used to anticipate S deficiency, it is suggested that S in the 0-30 cm depth be considered as it is more indicative of potential yield response than S in 0-15 cm depth. A yield improvement may be achieved through S application if spring soil S is lower than approximately 6.5 ppm S by Mehlich III extraction in 15-30 cm depth. Pre-first cut tissue samples have been shown as a good indicator of yield potential in subsequent cuttings, though in all trials first cut yield remained unaffected by S concentration in pre-first cut tissue. Therefore, tissue sampling of the top 15 cm of growth prior to first cut at early bloom stage, can be a reliable and practical method for Ontario farmers to employ to predict S requirements, without risking a reduction in first cut yields. If tissue S levels at this stage are shown to be below 0.34% S, a yield improvement in later cuts can likely be achieved through S fertilization. Adequate sampling and prediction of S deficiency will be especially important in a three cut system, as the yield reduction caused by S deficiency was shown to become more pronounced with each cut completed.
CHAPTER FIVE: THE STATE OF SULPHUR AVAILABILITY IN ONTARIO SOILS, AND ITS INFLUENCE ON THE YIELDS AND QUALITY OF CANOLA (BRASSICA NAPUS L.) AND ALFALFA (MEDICAGO SATIVA L.) IN ONTARIO

Sulphur (S) is an essential nutrient for all plants, and is needed in relatively high amounts by certain crops such as alfalfa and canola. While in the past sufficient S for growing agricultural crops was supplied to Ontario soils by acid rain (Sheard 1976), as pollution has decreased in the past two decades, deposition levels have also decreased (Hoeft and Fox 1986, National Atmospheric Deposition Program 2015). Therefore in recent years, crops with high S requirements such as alfalfa (Medicago sativa L.) and canola (Brassica napus L.) have begun experiencing S deficiencies; however, no current recommendations for applying S as fertilizer to these crops are available to Ontario farmers (Ontario Agronomy Guide 2009). To update recommendations, field trials were established in alfalfa and canola over multiple years, to determine if these crops will show significant response to S application, how quality parameters of each crop would be affected by S application, and to investigate optimal application rates. Soil and tissue testing were also incorporated to establish if these are reliable methods of anticipating crop response to S application in Ontario field conditions, and if so, what concentrations should be considered optimal for maximum yields. The application of S should also be assessed in terms of potential yield increase in relation to the costs that the farmer will incur through its application, to ensure that S applications made are cost-effective.

5.1 Canola Response

In canola trials, five trial locations were established both in 2013 and 2014 growing seasons, mostly in co-operator canola fields, with S application rates varying from 0 to 40 kg S/ha. Significant regression of yield against S application rate was found at two trials in each growing season. In 2013, both trials showing significant yield increases had a linear response, indicating that canola seed yield may have further increased with additional S application above 40 kg S/ha. However in 2014, both responsive trials showed significant quadratic increases; maximum yields were attained at similar
application rates of 27 and 30 kg S/ha. The variation in optimum S application rate may arise from year to year variation in S deposition rates; according to the National Atmospheric Deposition Program (2015), total wet and dry S deposition was slightly higher in 2014 at approximately 10 to 14 kg S/ha, compared to approximately 8-10 kg S/ha in 2013. While these levels of S deposition were seen along the southern shore of Lake Erie, similar increases in S deposition were likely seen in southern Ontario in 2014, and would have contributed S to Ontario crops, thereby decreasing the amount of additional fertilizer S required to attain optimum canola yields. In general, significant response in both 2013 and 2014 was seen at the more northerly trial locations; trials in Owen Sound, Kimberley and Durham showed significant response, as opposed to trials at Elora, Listowel, Shelburne and Camilla where no response was seen. Atmospheric S deposition tends to decrease in a gradient from south to north in Ontario (Environment Canada 2013), and therefore canola growers operating in north-central Ontario may see a more consistent response to S application, than those in central Ontario.

Where significant response to S application was seen, the fertilizer S requirements coincide well with past research conducted in western Canada, which has also indicated maximum yields were achieved at approximate application rates of 30 kg S/ha or above (Mahli and Leach 2002; Mahli et al. 2007). The overall yield improvement at the four trials showing significant response varied from 10% up to 16% (though this may have further increased at the two trials showing linear response). However, there was a lack of significant response at 6 of the 10 trials completed. At some of these sites the lack of significant response could have arisen, at least in part, due to other issues such as poor emergence and pest feeding, causing reduced yields. However even under good growing conditions with good stands, there were sites at which there was little impact of S on yield, or where the variance in yields was too great to allow for significant results. This indicates that response to S by Ontario canola crops will vary by location and year, and a yield responses will not necessarily be attained through S application in Ontario. Additionally, the relatively high S application rate of 30 kg S/ha and above, which resulted in
maximized yields at responsive trials, would be unwise to apply if a yield benefit could not be
guaranteed to offset the high fertilizer cost involved, making it advisable for a farmer to attempt to
predict where yield benefits will be seen.

The practice of topdressing ammonium sulphate as fertilizer prior to canola planting did appear
to be an effective way of improving S levels in soil. Soil sampling at the rosette stage of canola often
showed linear increases of soil test S levels in the top 15 cm of soil, or showed trends towards increased
S. This S also appeared to be consistently taken up by growing canola, as even at non-responsive sites
there was significant increases in tissue S concentration at rosette stage with S application over control
treatments. However, the increase in soil and tissue S did not consistently lead to increased yields.
When yield was normalized across sites and plotted against soil and tissue S concentration, a linear
relationship was found in both cases between tissue S/soil S levels at rosette stage and seed yield;
however, there was a high level of variance around the line of best fit, and the slope to these lines was
quite low indicating low levels of yield response by canola as S concentration increased. Variation in
normalized yield was higher when regressed with soil S test results, and therefore a Mehlich III
extraction (Mehlich 1984) may not be the best extraction method for Ontario soils when trying to
anticipate canola response.

According to the Canola Council of Canada’s Canola Grower’s Manual (Thomas 2003) tissue S
concentration at rosette stage should be above 0.25%; in our study mean tissue S concentration varied
from 0.29% up to 1.23%. This indicates that soil S levels in Ontario, at least on a broad scale, are still
sufficient to supply an Ontario canola crop. Due to the high variability of SO\textsubscript{4} levels in soil, and the
variance in its deposition from year to year, there will likely be instances where canola will respond to S
applied, and may benefit from S application, and this is likely why some trials showed significant
response to S while others did not. Tissue testing may still be a viable means of predicting deficiency,
our research supports the critical concentration of 0.25% S in rosette stage canola. If tissue samples taken at this stage are found to be below this critical concentration it would be advisable for a farmer to apply 30 kg S/ha as sulphate to a canola crop; at this early stage there is still opportunity for a farmer to correct a deficiency and improve yields. Tracking field history and noting where S deficiencies have occurred may also serve as a valuable indicator to canola growers of where canola will respond to S and therefore, where to apply S at higher rates.

To avoid possible scattered S deficiencies and the necessity of an S application into a growing crop, an application of a low rate of S when other spring fertilizer is applied may also be an asset to growers. Even at trials where response was not significant, the mean yield increase seen through the application of 10 kg S/ha was sufficient to pay for the costs incurred.

5.2 Alfalfa Response

Alfalfa showed a more promising and reliable response to S fertilizer application than canola. Of the three trial locations used in 2013, two showed significant response, while in 2014 two of the three newly established trial locations showed significant response. While yield of 1st cut tended to vary little among treatments, significant yield improvement over control treatments tended to appear in 2nd cut, and became further pronounced in 3rd cut, where S application resulted in up to three-fold yield improvement over control and potash treatments. All trials at which a 3rd cut was performed saw a significant yield improvement in this cut with S application. Some trials, while not significant, showed a clear trend towards improved yields in 2nd cut, however as per the co-operator’s regular farming practices, no third cut was performed; based on the findings at other sites, yield improvements would have been likely to occur in third cut. When total yield of all cuts was summed together, where significant, from 10% up to 55% yield improvement was seen through S application over an application of only potash. Where regression of increasing potassium sulphate (K₂SO₄) rates was possible, it was
found that total yield was maximized at application rates of approximately 30 to 60 kg S/ha. At trials where regression was not possible it was often found that mean total yield was highest when a treatment of 56 kg S/ha as \( \text{K}_2\text{SO}_4 \), however this yield was always statistically similar to the yield achieved through a fall application of 112 kg S/ha as elemental S. At the three trial locations that were carried into a second production year, it was found that a single, high-rate (112 kg S/ha) application of elemental S in the first year of the study had the ability to produce similar yields to when \( \text{K}_2\text{SO}_4 \) was applied in both the first and second study year. It was also found that older alfalfa stands tended to show more response to S application; at the Elora Research Station, when the S trial was carried into the second study year, it was found that plots that had been control treatments in the first trial year (where they had yielded significantly lower than S treatments), were able to attain comparable second and third cut yields in the second trial year after having received a spring application of 40 kg S/ha as \( \text{K}_2\text{SO}_4 \) as treatments that had received 40 kg S/ha as \( \text{K}_2\text{SO}_4 \) in both years of the trial. This shows that there is potential for S to rejuvenate and improve alfalfa stand longevity.

Similar to canola trials, it was found that topdressing of S fertilizer increased S availability in soils, based on soil S testing. However, similar to canola results, there was poor correlation between soil S concentrations, both in spring and summer sampling, and yield. Again, Mehlich III extraction (Mehlich 1984) of S may be an insufficient means of measuring S in Ontario soils for alfalfa production. Tissue sampling for S appeared to be a more consistent method of predicting alfalfa yields. Where various rates of \( \text{K}_2\text{SO}_4 \) were applied, there was always significant regression of top 15 cm tissue S concentration with the rate of S applied in both first and second cut, even when yield response was not significant. The Ancaster trial location was the only trial at which there was neither significant yield results, nor a trend towards higher yields with S application; however, this was also the trial with the highest tissue S levels, as even control treatments showed mean concentrations of 0.37 to 0.49% S. At trials where yield response was significant, mean control treatment tissue concentrations were most often between
0.15% and 0.25%. When total combined yield of all cuts was normalized across all trial locations and plotted against tissue S concentration of top 15 cm of alfalfa growth, it was found that yield showed significant quadratic regression with tissue S concentration. Normalized total yield across sites reached a plateau of approximately 85% of maximum yield when top 15 cm samples measured S concentrations of 0.34% S and above prior to first cut, and 0.37% prior to second cut. While other studies have found similar critical concentrations to these (Sorensen et al. 1968; Seim et al. 1969) they are above the generally accepted critical concentration used in the northwest United States of 0.25% to 0.30% S (Koenig et al. 2009), as well as the critical concentration suggested by Ketterings et al. (2012b) for use in New York state of 0.27%. For Ontario alfalfa growers, a sample of the top 15 cm of growth should be taken prior to first cut to ensure levels are approximately 0.34% or above, to ensure S levels are sufficient to maintain adequate second and third cut growth, especially as first cut is unlikely to show any visible S deficiencies or yield lag, even if tissue S levels are relatively low. By sampling prior to first cut, a grower also enables themselves to apply S as sulphate immediately following the completion of first cut; in a readily available form, it is likely that S deficiencies in the alfalfa can be corrected and second and third cut yields can be improved.

When assessing the advantage of S application to yield, it should also be ensured that the increase in biomass produced will cover the cost of the fertilizer application. It was found that when total yield improved significantly through S application as K₂SO₄, at all but one site, the value of the resulting biomass easily covered the cost of the applied fertilizer. Due to the lower cost of elemental S compared to K₂SO₄, the increase in biomass production gained through elemental S application covered the cost of fertilizer application at all sites where significant improvement in total yield was seen, as well as at many of the sites where yields improved but results were not significant. Overall, K₂SO₄ is a viable within-season option; an application of approximately 30 kg S/ha as K₂SO₄ as a corrective measure following first cut tissue sampling, or onto fields with a known history of S deficiency is advisable, as in
these situations the resulting yield improvement will very likely cover the cost of fertilizer. Elemental S, when fall applied, has been shown to produce yields comparable to those achieved by K_2SO_4, but at a lower fertilizer cost, while also showing residual benefit when applied at a high rate (approximately 112 kg S/ha as elemental). It is therefore a viable option to apply when establishing a new stand, or to rejuvenate a one or two year old stand, to improve stand longevity.

It should be noted that all trial locations used in this study were selected for a lack of recent manure additions (in at least the three years prior to study). As alfalfa is often grown in mixed farming operations which include some form of livestock, manure is often spread back on the areas on which alfalfa is grown. The S supplied in this fashion should be considered when assessing the need for S fertilizer application, and nutrient testing of manure may be a valuable means of anticipating S supplied through manure additions. Hay operations in which hay is being grown for sale purposes with no manure additions being made back to the hay stands will be more likely to see a response to S fertilization than closed systems in which manure is returned to the field.

This research indicates that while S fertilizer application in Ontario has not been warranted in the past, the reduction in S deposition in the past decade has created S deficiencies in alfalfa and canola in Ontario, and both of these crops now show significant yield response to S application, though year to year variability in deposition may affect yield response. Response is more variable in canola than in alfalfa, but depending on the rate and type of fertilizer used, S application onto both crops can be economically beneficial. In both crops, tissue sampling for S concentration appears to be a more reliable method of predicting the need for S fertilization than soil S testing using Mehlich III extraction. Ontario Agronomy Guide recommendations should be updated for Ontario growers to reflect these changes in crop production. Further research into this topic should be completed to examine if fall applied elemental S could be an option for Ontario canola growers to reduce fertilizer costs and therefore make
S application more feasible, as yield improvement with S application is more variable in canola. Further trials should also be completed on elemental S application on alfalfa to determine how long the residual effect of its application can be expected in Ontario soil conditions. As studies in New York state have found more success in finding significant regression of yield with soil S concentration with extractants other than Mehlich III when soil pH was slightly acidic to neutral (Ketterings et al. 2011), more research should also be completed to calibrate a soil extraction method for S in Ontario soils.
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