# Assessing the tolerance and response of pulse crops to seed-row placed fertilizer blends and composite products containing nitrogen, phosphorus and sulfur

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by

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#### **ABSTRACT**

Legumes can obtain a significant portion of their nitrogen (N) requirements from atmospheric N<sub>2</sub> through the symbiotic relationship with *Rhizobia* spp of bacteria, termed biological nitrogen fixation (BNF). The development of actively fixing nodules on the roots typically takes one to two weeks. Onset of BNF may be further extended by stress conditions such as low temperature, moisture, and root disease, and coupled with low available N levels in the soil, could negatively affect growth and development of pulse crops grown on prairie soils. Therefore, low rates of starter N supplied as mineral fertilizer in the seed-row along with phosphorus (P) and sulfur (S) may increase early growth and N, P, S nutrition. However, too much fertilizer in close proximity to seed can damage the seedling through osmotic effect and ammonia toxicity. But, there is limited information on safe starter rates of fertilizer blends and combination products containing N, P and S for pulse crops in prairie soils. Therefore, a controlled environment study and a field experiment was conducted to evaluate emergence, yield and N, P, S uptake responses to starter fertilizer blends applied at 0, 10, 20 and 30 kg N ha<sup>-1</sup> in the seed-row under a common 15% seed bed utilization configuration. Six different pulse crops: soybean, pea, faba bean, black bean, lentil and chickpea were evaluated on a N and P deficient loamy textured Brown Chernozem soil from southern Saskatchewan. The general sensitivity (injury potential) for starter N, P, S fertilizer products and blends placed in the seed-row was lentil  $\geq$  pea  $\geq$  chickpea > soybean  $\geq$  black bean > faba bean. Lentil, pea and chickpea could generally only tolerate the 10 kg N ha<sup>-1</sup> rates while soybean and black bean could tolerate  $10 - 20 \text{ kg N ha}^{-1}$ . Faba bean emergence appeared relatively unaffected by all three rates of N (10, 20 and 30 kg N ha<sup>-1</sup>), and showed least sensitivity to seed row placed fertilizer. In terms of 30 day biomass response, soybean and black bean were most responsive to fertilization, and pea, faba bean, lentil and chickpea early biomass production and nutrient uptake showed no benefit going above the 10 kg N ha<sup>-1</sup> rate. Soybean and lentil were evaluated under field conditions in 2018 at the location near Central Butte, Saskatchewan where the soil was taken for the controlled environment studies in a seed-row placed urea-MAP blend (28-26-0). Confirming the controlled environment work, a rate of 10 kg N and P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> appeared to be the rate that did not significantly reduce emergence, stand count or biological nitrogen fixation, and was sufficient to maximize yield, N and P uptake for both soybean and lentil under field conditions. Rates higher than 10 kg N ha<sup>-1</sup> in the seed-row as starter 28-26-0 blend reduced emergence and decreased the proportion of N derived from biological nitrogen fixation.

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# **DEDICATION**

The price of success is hard work, dedication to the job at hand, and the determination that whether we win or lose, we have applied the best of ourselves to the task at hand.

-Vince Lombardi

I would like to dedicate this work to my family who taught me all the hard work, dedication and determination; my mom, Sheela and my dad, Gamini (late) for their courage and strength, my husband, Ivon, for all the positive words and my baby boy, Mathe, for his cutest smile which refreshes my fatigue.

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# LIST OF ABBREVIATIONS

Ammonium phosphate sulfates APS

Ammonium sulfates AS

Atomic Absorption-Flame Emission AA-FE

Biological nitrogen fixation BNF

Calcium chloride CaCl<sub>2</sub>

Hydrochloric Acid HCl

Hydrogen peroxide H<sub>2</sub>O<sub>2</sub>

Modified Kelowna KM

Mono Ammonium Phosphate MAP

Nitrate NO<sub>3</sub>-

Nitrogen N

Phosphate PO<sub>4</sub><sup>3-</sup>

Phosphorus P

Potassium K

Revolutions per minute RPM

Sodium Hydroxide NaOH

Sulfur S

Sulfuric Acid H<sub>2</sub>SO<sub>4</sub>

# 1 Introduction

# 1.1 Importance of Early Supply of Nutrients via Seed-Row Fertilizer Placement for Legume Crops

Canada is a major contributor to global pulse production. Most of the pulse growing areas in Canada are found in the western provinces, and Saskatchewan is the largest producer of field peas, lentils, faba beans, and chickpeas for export purposes (Boersch et al., 2017). In terms of relative importance, Yang et al.(2010) reported that in Saskatchewan, the combined area of faba bean (*Vicia faba* L.) and chickpea (*Cicer arietinum* L.) represented only 4% of the pulse crop area in 2010. However, in the last five years, the acreage of faba bean and, more recently, soybean has been increasing (Saskatchewan Ministry of Agriculture Agricultural Statistics, 2017). All the grain legumes belong to third largest plant family, *Fabaceae*, which are able to utilize atmospheric nitrogen (N<sub>2</sub>) through a symbiotic relationship with Rhizobia bacteria. This significant biological process, known as symbiotic biological nitrogen fixation (BNF), takes place in the nodules attached to the roots (Hardy et al., 1971; Yang et al., 2010; Kamfwa et al., 2017). Due the contribution from symbiotic atmospheric nitrogen fixation, total soil nitrogen (N) can be enhanced by growing legumes alone or intercropped with other plants.

It is important to note that it typically takes one to two weeks for a seeded annual legume to establish nodules and for the rhizobium bacteria to start fixing N for the plant. For instance, in soybean, N<sub>2</sub> fixation began only about 14 days after planting, even when it was cultivated under optimum temperature and moisture conditions (Gai et al., 2017). In another study, it was noted that it took about 12 days to establish nitrogenase activity, which is the enzyme responsible for converting atmospheric N<sub>2</sub> into NH<sub>3</sub> (Hardy et al., 1971). Cooler soil conditions at time of planting such as typically encountered in northern prairie soils will further delay biological activities, with possible delay and overall reduction of N fixation having a negative effect on plant growth. Grain legumes may suffer N deficiency at early stages of growth when seeded in soils with low initial contents of soil available N (Gai et al., 2017) and which may be further aggravated by immobilization of available N if low N content crop residues are incorporated at seeding, or if there is plant stress because of root diseases. Therefore, smaller amounts (e.g. 10 to 30 kg N ha<sup>-1</sup>) of starter N placed in the seed-

row could be effective in providing N to the plant during this period and enhance early season growth and nutrient uptake. According to Gai et al., (2017), starter N in soybean had a benefit on root activity, photosynthesis rate, leaf area index and leaf weight and also can contribute to higher grain yield. However, there is no research data available on effects of "starter" N for new short-season soybean varieties that are being widely adopted and grown on the Canadian prairies and there is also a lack of data for other recent pulse crop genotypes.

Currently some farmers are using small amounts of starter N along with phosphorus (P) and/or sulfur (S) in the seed-row in order to promote more vigorous early growth of pulses, that may be advantageous especially in unfavorable spring soil conditions that delay root growth and nutrient uptake, establishment of nodules, and fixation of N. At the same time, a concern arises over deliberate seed row placement of fertilizer at higher rates due to potential salt and ammonia injury. Early season phosphorus availability is noted to be important to most crops in Western Canada (Grant et al., 2001) and S can also be a limitation for some crops. Recent research in Saskatchewan has shown that most of the S uptake by peas occurs in the first few weeks following seeding (Ahmed et al., 2017). Broadcasting results in poor nutrient utilization efficiency, and poor crop performance could be the result. Effective in-soil fertilizer placement such as in the seed-row will reduce nutrient losses by volatilization, immobilization and run-off, thereby increasing the uptake, recovery and efficiency.

#### 1.2 Justification of Research

Nitrogen fertilizer placed in the seed-row in combination with P and S is attractive to provide an early or "starter" supply of these nutrients. However, ammonium sulfate (AS) applied at rates above 20 kg S ha<sup>-1</sup> in the seed-row produced significant reduction in emergence and biomass of many Brassica species/ cultivars, and addition of monoammonium phosphate (MAP, commonly 11-52-0) at 15-30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> along with AS caused further reductions in emergence and biomass (Qian et al., 2012). Maximum safe rates for P applied as MAP and also combinations of monoammonium phosphate plus potash for common pulse crops have been established for the prairies, but the tolerance and response to starter blends that contain higher analysis of N from urea blended with MAP and blend of MAP with AS is not known. Commonly used granular seed-row placed fertilizers such as MAP supply only small amounts of N (4-6 kg N ha<sup>-1</sup>) at the rates typically used to provide starter P (40- 60 kg MAP ha<sup>-1</sup>). There is no information on the tolerance and response of pulse crops to seed row fertilizer blends that contain MAP + urea or MAP + AS, or to composite granular products

like ammonium phosphate sulfates (APS) or products like "MES-15", which is a composite product with ammonium phosphate sulfate and elemental S in a combined granule.

The research described in this thesis involves controlled environment assessments of emergence, yield and nutrient uptake by soybean, pea, faba bean, common bean (black bean), lentil and chickpea as affected by seed-row placed MAP alone, MAP plus urea, MAP plus AS, and four composite granular fertilizers including MES-15 (Mosaic<sup>TM</sup> 13-33-0-15) and three ammonium phosphate sulfate products (Koch<sup>TM</sup> 12-45-0-05, Simplot<sup>TM</sup> 16-20-0-13, Anuvia Symtrx<sup>TM</sup> 16-20-0-12) applied at low rates. A field study with soybean and lentil using a blend of seed-row placed urea and MAP was conducted to provide information on effects on final grain yield and the proportion of nitrogen derived from BNF under field conditions. The results of the research will increase knowledge of safe rates of fertilizer based on N contained in blends and composite products and document the yield and nutritional response of common pulse crops to starter seed-row placed fertilizer. This information can be used to develop guidelines and recommendations for rates of blends and products that can be applied to achieve nutritional benefits for the various legume crops while avoiding costly crop injury and reduction in stand density from damage caused by too much fertilizer placed in the seed-row.

# 1.3 Objectives and Hypotheses

Considering the lack of information available on safely placed N rates in the seed-row with pulses and their response under low seed bed utilization conditions, the following objectives for this thesis research were established for six commonly grown pulse crops in Saskatchewan:

- i. To evaluate the effect of different seed-row placed N-P-S fertilizer blends and combination granular products applied at different rates on emergence over a two-week period and on growth response in order to establish maximum safe rates of seed-row application and nutritional response under low seed bed utilization conditions (~15% SBU). This is done under controlled environment conditions for soybean, pea, faba bean, black bean, lentil, and chickpea on a N and P deficient Haverhill loam Brown Chernozem soil from south-central Saskatchewan.
- ii. To evaluate the effect of a common seed-row placed N-P fertilizer blend: 50% Urea + 50% MAP applied at different rates on emergence, final grain and straw yield, and

nutrient uptake of soybean and lentil over a three month period, in order to determine influence of seed-row placement under field conditions.

iii. To determine N fixation by soybean in the field study using an <sup>15</sup>N label application and thereby reveal how the starter seed-row fertilization influences nitrogen derived from atmosphere (%ndfa).

The objectives are intended to address the following general hypotheses put forward for this thesis research:

- Seed-placed fertilizer blends and products intended to supply N along with P and S
  will promote pulse crop growth and N, P, S uptake, but there will be a limit to how
  much can be safely placed in the seed-row. Combination granular fertilizers will
  cause less injury compared to blends.
- ii. Addition of more N along with P and S in starter seed-row placed fertilizer will increase early season growth and yield under both controlled environment and field conditions. Crops that are slower to develop N<sub>2</sub> fixing nodules like soybean will respond more to starter N.
- iii. Starter N will increase percentage of nitrogen in soybean derived from fixation (% ndfa) up to a point at which the increased availability of N in the soil will begin to decrease biological nitrogen fixation.

# 1.4 Organization of Thesis

This thesis is organized in manuscript format, with general introduction (Chapter 1) followed by literature review (Chapter 2) and then Chapter 3 and 4 cover the controlled environment and field study, respectively. The literature review, chapter 2, provides a review of related literature including pulse crop production, botany and climatic requirements of pulse crops, nature and behavior of N, P and S in soils and as added fertilizers, seed-row placement as an application method as well as <sup>15</sup>N labeling techniques. Chapter 3 covers the controlled environment study which addresses the effect of seed-row placed fertilizer on emergence, early biomass production and N, P, S uptake of six common Saskatchewan pulse crops. A field study to assess emergence, yield and N, P uptake of soybean and lentil along with nitrogen derived from fixation (%ndfa) of soybean as affected by different rates of a seed row placed N-P blend is covered in chapter 4. Finally, a synthesis of the thesis research

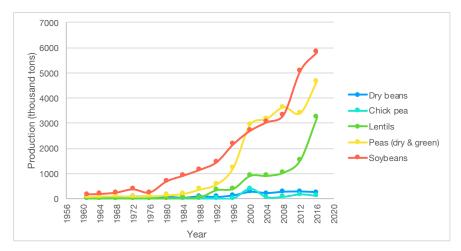
findings, conclusions and needs for future research are presented in chapter 5. References are provided in chapter 6 with ancillary data and results provided in the Appendix at the end of the thesis.

#### 2 LITERATURE REVIEW

#### 2.1 Legume Crops

Legumes are members of the family *Leguminosae* or *Fabaceae*. They are grown primarily for their grain seed, for livestock forage and silage, and as soil enhancing green manures. According to the United Nations Food and Agriculture Organization (FAO), eleven types of legume crops called 'pulse crops', which give dried edible seeds, have been recognized worldwide. Balasubramanian (2013) stated that field pea, lentils, common (dry) beans and chickpea are the commonly grown pulse crops in Canada, followed by faba bean, lupin and mung bean. Like the previous mentioned crops, soybean is also a legume crop but is sometimes excluded from the "pulse crop" grouping and referred to as oilseed instead, due to their higher fat content and utilization for oil.

Canada is a leading exporter of pulse crops (Fig 2.1) with approximately 75% of the production exported annually, representing 35% of the global pulse trade. As well, the trade balance of pulse crops increased from \$863 million in 2000 to \$2.1 billion in 2010 (Balasubramanian, 2013).



**Figure 2. 1** Growth of Canadian pulse crop production (thousand tons) from year 1961 to 2016 (FAO, 2016).

Legumes are a significant source of protein, dietary fiber, carbohydrates and dietary minerals. An important characteristic of the legume family is atmospheric nitrogen fixation arising from the symbiotic relationship with bacteria called *Rhizobia* within nodules on their root system. This symbiosis means that the root nodules are sources of nitrogen for legume growth, making them relatively rich in plant proteins.

#### 2.1.1 Soybean

#### Past and present production

Soybean (*Glycine max* (L) Merrill) seeds have protein contents ranging from 30% to 40% and 15- 20% of oil (Qiu and Chang, 2010). As such, soybean has become an important crop for protein and oil worldwide. According to Qiu and Chang (2010), China has been identified as the origin of soybean with earliest written records (4500 years ago) of soybean cultivation.

Canadian soybean cultivation history reveals that soybean was first grown in very small quantities as a forage crop with the major limitation of soybean cultivation in Canada being an overly long maturation period (Piper and Morse, 1923). However, the industry has overcome challenges by introducing very early maturing varieties such that Canada, with 5.8 million tons per year production, has become the seventh largest soybean grower in the world (Qiu and Chang, 2010). In Saskatchewan, 0.48 million tons of soybean was reported for 2017 production, with an increase of seeded area of approximately 350% over 2016 (Government of Saskatchewan, 2017). Average yield has been recorded as 1398 kg ha<sup>-1</sup>. In terms of global annual soybean production, Saskatchewan is only a small contributor. The United States of America with 117.2 million tons is identified as the main producer in the world followed by Brazil (96.2 million tons) and Argentina (58.7 million tons), with 335 million tons average annual world soybean production (FAO, 2016).

# **Botany and climatic requirements**

Cultivated soybean has an erect, bushy and annual growth habit (Kumudini, 2010). The roots of soybean are where the nodules form and are the location for symbiotic nitrogen fixation. In this symbiosis, a mutually beneficial relationship is established between the soybean plant and *Bradyrhizobium japonicum*, a gram-negative bacterium present in the soil that inhabits the nodule and fixes N for the plant in return for photosynthate received from the plant. A mature soybean flower has a single banner petal, two wing petals, two keel petals and ten stamens arranged around the pistil and a tubular calyx. The dominant embryo covered by the seed coat consists of the two fleshy cotyledons.

With the presence of appropriate temperature and moisture, soybean seed will imbibe water and initiate epigeous germination. Once the radical emerges by breaking the seed coat, it starts to grow rapidly into the soil. Emergence can be described as the point where

cotyledons are above the soil surface. Most of the soybean varieties are erect and branching with a well-defined main stem (Piper and Morse, 1923).

Soybean can be grown on a wide variety of well-drained soil types, but is reported to perform best on clay loam soils (Rao and Reddy, 2010). It prefers slightly acidic soil (pH 6.0 - 6.5). An early study by Hanway and Weber (1971) of N, P and K accumulation in the plant showed that accumulation of macronutrients was slow at the beginning following emergence but became rapid between 54 and 100 days after emergence.

Soybeans are grown mainly in the Dark Brown and Black soil zones in Saskatchewan on medium- textured loam soils. It can be grown in clay soil under favorable condition but may not well perform in sandy soil because they cannot tolerate dry conditions especially in August. The recommended seeding date is from May 10 to 25 or when the soil temperature has warmed to at least 10  $^{0}$ C, with typical on-farm yields reported in Saskatchewan of ~ 1500 - 2000 kg ha<sup>-1</sup> (Boersch, 2018). According to data from the Saskatchewan Crop Insurance Corporation (SCIC), S0009-M2 was the most widely grown soybean variety in Saskatchewan in 2017 followed by 23-11RY, 23-60RY, NSC Warren RR, 22-60RY, Akras R2, and P002T04R (Saskatchewan Pulse Growers, 2018).

#### 2.1.2 Pea

# Past and present production

Pea (*Pisum sativum*) originally was cultivated as a winter annual crop in the Mediterranean basin (Smartt, 1990), and has spread far beyond the area of initial domestication. Pea is a major pulse crop of the temperate zones of the world. Field pea or 'dry pea' is marketed dry for human or livestock feeding while fresh or succulent pea is used for frozen and canned vegetables (McKay et al., 2003).

Currently, world annual dry pea production has been reported as 14.4 million tons (FAO, 2016) with average production of 1883 kg ha<sup>-1</sup>. Canada has held the position of number one pea producer for several years (McKay et al., 2003). Pea was grown on almost 2.2 million acres in Saskatchewan in 2017, with average production of 2269 kg ha<sup>-1</sup> (Government of Saskatchewan, 2017).

#### **Botany and climatic requirement**

Pea plants exhibit an indeterminate growth habit (Cousin, 1997). The first nodes, some of which give rise to branches, are vegetative, while subsequent nodes are reproductive. Generally, two flowers – white to reddish-purple color, from which the pods develop, are present at each reproductive node and are self-pollinated. Two types of field pea are identified: normal leaf type with vine length of three to six feet, and semi-leafless type that has modified leaflets reduced to tendrils, resulting in shorter vine length of two to four feet (McKay et al., 2003). The number of seeds per pod depends on the variety and on the environmental conditions, but generally ranges from four to nine.

Field pea can be grown in a wide range of soil types, from light sandy to heavy clay textured soils, but it does not tolerate saline and water-logged soil conditions well (McKay et al., 2003). It is suited across the soil-climatic zones of Saskatchewan, but ample moisture with well-drained soil and minimum average soil temperature of 5°C is recommended. Seeding can be done between mid- April to mid- May (Saskatchewan Pulse Growers, 2018). Yellow peas (leading variety; CDC Meadow) are the most widely grown pea type in Saskatchewan followed by green pea (leading variety; CDC Striker).

#### 2.1.3 Faba bean

# Past and present production

Faba bean (*Vicia faba*) is a member of a sub-genus *Vicia* with archeological evidence (beans) of first production at *ca.* 6250 BC in Jericho. According to the Government of Saskatchewan ("Faba Bean: Markets," 2018), world production of faba bean ranges from 4.9 to 5.1 million tons per year, with China producing almost half. In Saskatchewan, the faba bean production area has increased in recent years (Government of Saskatchewan, 2017).

# **Botany and climatic requirement**

Faba bean is an annual plant that prefers cooler conditions. It is normally planted in the spring in northern latitudes but in warm-temperate and subtropical areas, its grown in winter (Duc, 1997). Winter cultivars usually have greater branching (4-6 stems/plant) than spring ones (1-2 stems/plant).

Faba bean has high demand for moisture during germination, flowering and podfilling stages. Pod set can be reduced when temperature exceeds 27 °C (Saskatchewan Pulse Growers, 2018). Saskatchewan Ministry of Agriculture recommends mid -April to mid-May is best for seeding in Saskatchewan and minimum average soil temperature should be around 3 -5 °C. Faba beans are best adapted to the Black soil zones and the northern portions of the Dark Brown soil zones of Saskatchewan, where moisture is in ample supply.

Tannin level, which corresponds to flower color, is used to classify their varieties. Tannin type varieties have coloured flowers and are usually larger seeded. CDC SSNS-1 is a small seeded tannin type that is grown in Saskatchewan and is well-suited for green manure or use in silage mixtures. Zero-tannin type varieties; CDC Snowdrop and Snowbird, grown mainly in Saskatchewan, have white flowers and are used mainly in the feed market industry.

#### 2.1.4 Common bean

# Past and present production

Common bean (*Phaseolus vulgaris L.*), known as dry bean, is grain legume primarily used for human consumption. There is clear evidence of a Mesoamerican origin of *P. vulgaris*, which was most likely located in Mexico (Bitocchi et al., 2012).

World common bean production is reported as 26.8 million tons per year (FAO, 2016). In the Americas, Brazil (2.6 million tons/ year) is the major common bean producer followed by USA (1.2 million tons/ year) and then Mexico (1 million tons/ year). Canadian annual production of common bean is 0.25 million tons with 2257 kg ha<sup>-1</sup> average yield. Dry beans in Saskatchewan are mainly grown under irrigation.

# **Botany and climatic requirement**

*Phaseolus* is a well-known genus of the family *Fabaceae* with more than 150 species (Nassar et al., 2010). Common bean is an annual herbaceous plant with erect, green colour, ribbed, cylindrical and solid stem. Further, flowering of common bean starts at about 5 weeks after emergence. Beans require warm, moist soil conditions for germination and emergence, and are very sensitive to salinity, soil structural problems, and saturated soils. Level fields with good drainage and low levels of soil salinity are advisable with later planting dates in Saskatchewan to avoid frost damage to seedlings suggested from May 25<sup>th</sup> to June 5<sup>th</sup> and soil temperatures preferably 12 <sup>o</sup>C or above (Saskatchewan Pulse Growers, 2018).

#### **2.1.5** Lentils

#### Past and present production

Lens culinaris is the present cultivated type of lentil, which has vetch-like vegetative morphology with pinnate leaves, commonly bearing 8-10 leaflets and a terminal tendril (Smartt, 1990). According to Smartt (1990), Barulina in 1930 had suggested that L. orientalis (later included in the sub-specific rank of L. culinaris ) represented the wild ancestral type of L. culinaris. Distribution of L. orientalis can be identified from Turkey and Israel eastward to Uzbekistan in the southern USSR. Archaeological studies showed that domestication of lentils occurred from 8500 – 600 BC in the Turkey- Syria- Iraq regions and then spread to Latin America after Central Europe and South Asia (Sandhu and Singh, 2007).

Lentils are considered a major international pulse crop. Approximately 70% of world lentil production is consumed in the country in which they are grown (McNeil et al., 2007). North America, the Indian sub-continent and Turkey are three major areas of lentil production, with 6.3 million tons grown annually worldwide with average production of 1152 kg ha<sup>-1</sup> (FAO, 2016). There are two major groups of lentils: red and green lentils with Canada being the largest global producer of green lentils (McNeil et al., 2007). Lentils are generally grown without irrigation in Canada, with average yield of 1486 kg ha<sup>-1</sup> and 3.2 million tons of annual production (FAO, 2016). Saskatchewan's annual lentil production in 2017 was reported as 2.3 million tons with an average yield of 1453 kg ha<sup>-1</sup> (Government of Saskatchewan, 2017).

# **Botany and climatic requirements**

Lentil is an annual bushy herb with erect, semi-erect or spreading and compacted growth habit (Sandhu and Singh, 2007). They are typically short but can range from 20 to 75 cm in height, depending on growing conditions. Lentils may be classified into two groups according to seed size; large seeded type that has average 50 g or more per 1000 seeds and small-seeded type that averages 40 grams or less per 1000 seeds. Seed coat colours range from clear to green, tan, brown, grey, blotched purple, or black and cotyledons can be yellow, red, or green (Lentil Crop Production Manual, 2018).

Lentils have a shallow root system (0.6 m) but are considered moderately resistant to high temperature and drought and considered as long day or day neutral plant (Andrews and McKenzie, 2007). Even though lentils can grow on a wide range of soil types and soil pH, it is sensitive to waterlogging and acid soil pH.

In Saskatchewan, the Brown, Dark Brown, Moist Dark Brown, and Thin Black soil zones are considered best for lentil production, especially when grown on stubble the crop tends to receive sufficient moisture stress that is needed to reduce the time to maturity, prevent excessive vegetative growth, and reduce the risk of damage from early fall frost (Lentil Crop Production Manual, 2018). In terms of variety, large green types represent the highest share of lentil acres, with CDC Greenland and CDC Impower the most widely grown varieties and CDC Maxim and CDC Dazil were the top red varieties in 2017 (Saskatchewan Pulse Growers, 2018).

# 2.1.6 Chickpea

# Past and present production

Chickpea (*Cicer arietinum*) is one of the world's major pulse crops, originating in the Mediterranean basin (3000-4000 BC) and Indian sub-continent (2000 BC) and southward to Europe and the East African highlands (Smartt, 1990). Taxonomically, chickpea belongs to the monogeneric tribe *Cicereae* of the family *Fabaceae* (Sajja et al., 2017).

World chickpea production for export is estimated at  $\sim 12$  million tons in 2016 (FAO, 2016) with highest production in Australia followed by Myanmar, Turkey, Mexico and Canada (0.10 million tons). In Saskatchewan, 86,000 tons of production was reported for 2017 on 160,000 ha land with a yield of 1329 kg ha<sup>-1</sup> (Government of Saskatchewan, 2017).

# **Botany and climatic requirement**

Chickpea is a short annual herb, attaining a height of less than a metre. With a firm stem, the plant produces three types of branches called primary, secondary and tertiary. The compound leaves contain 5–7 pairs of hairy leaflets per leaf, opposite or alternate, and the rachis ends in a leaflet (Sajja et al., 2017). The root system is characterized by a thick tap root with several side roots. Number of pods per plant depends on the genotype and the environmental conditions.

Two types of chickpea can be identified based on the seed size and color; 1) desi, which is generally small and having cream, yellow, brown, black or green color and 2) kabuli, which is generally large and having cream or beige or sometimes white color. According to Saskatchewan pulse growers (2018), the leading Kabuli chickpea varieties grown in Saskatchewan are CDC Leader and CDC Orion. CDC Consul is a high-yielding Desi variety with good resistance to ascochyta blight, which was released in 2013.

Chickpea prefers warm, drier conditions compared to other pulse crops. Therefore, it is best adapted to the Brown and Dark Brown soil zones in Saskatchewan. Desi chickpea varieties have a shorter growing season and mature earlier than Kabuli varieties. Saskatchewan Ministry of Agriculture recommends seeding chickpea prior to May 25<sup>th</sup> with greater than 7 °C (desi) and 10 °C (kabuli) soil temperatures.

# 2.2 Soil Nitrogen (N)

Nitrogen is a primary essential nutrient for plant growth obtained from soil, or air in the case of legumes (Havlin et al., 2014). There are two main groupings of soil N: inorganic N and organic N (> 95% of total). Inorganic forms of N include ammonium (NH<sub>4</sub><sup>+</sup>), nitrite (NO<sub>2</sub><sup>-</sup>) which is generally negligible, and nitrate (NO<sub>3</sub><sup>-</sup>). Organic forms of N mostly occur as part of complex humic polymers as well as proteins, amino acids and amino sugars as well as in the living soil biomass. Total N content of agricultural soil ranges from less than 0.02% in subsoils to more than 2.5% in peat (Havlin et al., 2014).

# 2.2.1 N cycle

The nitrogen cycle (Fig 2.2) is the biogeochemical cycle in which nitrogen is converted into multiple chemical forms as it circulates among the atmosphere, terrestrial, and marine ecosystems. The conversion of nitrogen is carried out through biological, chemical and physical processes that include fixation, ammonification, nitrification, volatilization and denitrification.

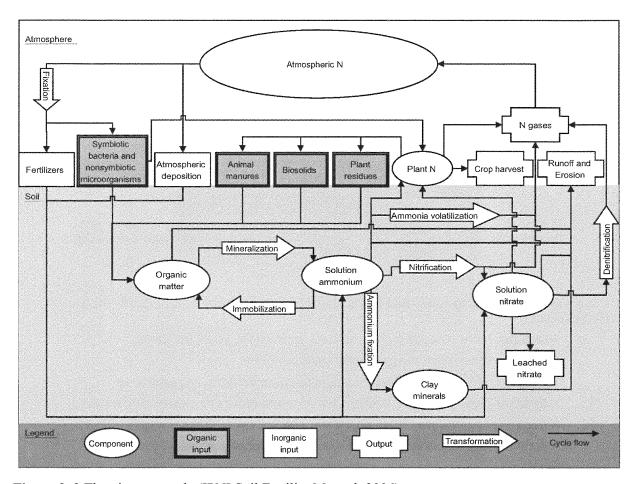


Figure 2. 2 The nitrogen cycle (IPNI Soil Fertility Manual, 2006).

Atmospheric N (N<sub>2</sub> gas), which constitutes 78% of total earth's atmosphere, can be fixed into biologically usable N by microorganisms living in roots of legumes and certain non-leguminous plants (symbiotic fixation) as well as free-living (non-symbiotic) soil microorganisms (Cooper and Scherer, 2012). The nitrogen contained in soil organic matter is mineralized to inorganic forms (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>) by soil organisms. Atmospheric electrical discharges and combustion of N containing organic materials form N oxides that return to ground as nitrates in precipitation (Havlin et al., 2014). Application of commercial N fertilizer adds inorganic N forms to the soil solution. When the C to N ratio is high, N can be converted from inorganic to organic form, leaving less N available in the soil solution, a process called immobilization (IPNI Soil Fertility Manual, 2006).

Plant roots absorb a portion of NH<sub>4</sub><sup>+</sup> and the rest may be volatilized into atmosphere as NH<sub>3</sub> or converted into NO<sub>3</sub><sup>-</sup> by nitrification. The NO<sub>3</sub><sup>-</sup> can be assimilated by plants, leached into ground water, or put back to atmosphere by denitrification.

# 2.2.2 N fixation by legumes

Many organisms have unique ability to fix atmospheric N<sub>2</sub> into biologically usable forms. Worldwide total biological N fixation (BNF) ranges from 130-180 x 10<sup>6</sup> metric tons per year (Havlin et al., 2014). Legume nitrogen fixation starts with the formation of a nodule in the root and rhizobia bacteria in the soil invade the root and multiply within its cortex cells. The plant and bacteria live in a symbiotic relationship, in which plant supplies living space and photosynthate to bacteria for energy and carbon, and the bacteria gives back fixed N to the plant. Nodules on annual legumes, such as beans, peanuts, and soybeans, can reach the size of a large pea. At the time of pod filling, nodules on annual legumes generally lose their ability to fix nitrogen because the plant feeds the developing seed rather than the nodule. Beans will generally have fewer than 100 nodules per plant while soybeans may have several hundred per plant (Flynn and Idowu, 2015).

Generally, fixation in the nodule by the bacteria can supply 25 - 80 % of the total N in the legume plant, but this depends on many factors such as soil pH, mineral nutrient status, temperature and moisture, photosynthetic activity, and legume management. The N fixing ability is ranked as faba beans > peas > chickpeas > l entils > soybeans > common beans (Saskatchewan Pulse Growers, 2018).

The bacterium genera '*Rhizobium*' is responsible for the fixing of atmospheric N in the nodules of legume plants. Numerous *Rhizobium* (Table 2.1) species exist, each requiring a specific host plant. As well, inoculation of the legume seed with correct inoculum is recommended the first time a field is planted to a new legume species.

**Tables 2. 1** *Rhizobium* species required for effective nodulation of pulse crops (Saskatchewan Pulse Growers, 2018).

Crop	Rhizobium species required for effective nodulation
Peas, Lentils, Faba beans	Rhizobium leguminosarum
Chickpea	Rhizobium ciceri
Common bean	Rhizobium phaseoli
Soy bean	Bradyrhizobium japonicum

#### 2.2.3 N fertilizer

Synthetic or chemical fertilizers are a major source of N for production of crops globally (Havlin et al., 2014). The major sources of N used in western Canada are anhydrous ammonia (gas stored under pressure as a liquid), urea (solid), ammonium sulfate (solid), and urea-ammonium nitrate (UAN) nitrogen solutions. The first two fertilizers listed account for the bulk (i.e., approximately 90%) of the N supplied by nitrogen products. Urea can be blended with other granular fertilizers.

The amount of fertilizer applied to crops depends on many factors including type of crop, prices for fertilizer and crop, weather, fertilizer application strategy, equipment availability etc (International Plant Nutrition Institute, 2006). Under Canadian prairie conditions, the fertilizer nitrogen recovered in the plant in the first year is  $\sim 50\%$ . About 25% of the fertilizer nitrogen is immobilized and the rest ( $\sim 25\%$ ) of the N is lost from the system via various possible mechanisms (volatilization, denitrification, and leaching beyond root zone).

# 2.3 Soil Phosphorus (P)

After nitrogen, phosphorus is probably the element most likely to be deficient in prairie soils. The soil mineral apatite is the original source of phosphorus in prairie soils, contained in parent material and released to available forms by weathering during soil development. While there is on average  $1000 \text{ kg P ha}^{-1}$  in the top 15cm soil, it is generally unavailable in the form of phosphates of low solubility (Havlin et al., 2014). As a percentage, total P concentrations range from 0.005 - 0.15%. P is very important to plant growth as it plays a role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the plant (IPNI Soil Fertility Manual, 2006).

#### **2.3.1** P cycle

The phosphorus cycle (Fig 2.3) is the biogeochemical cycle that describes the movement of P through the lithosphere, hydrosphere, and biosphere. P does not enter the atmosphere, remaining mostly on land and in rock and soil minerals (Havlin et al., 2014)). It moves quickly through plants and animals but the processes that move them through the soil or ocean are very slow, making the P cycle overall one of the slowest biogeochemical cycles.

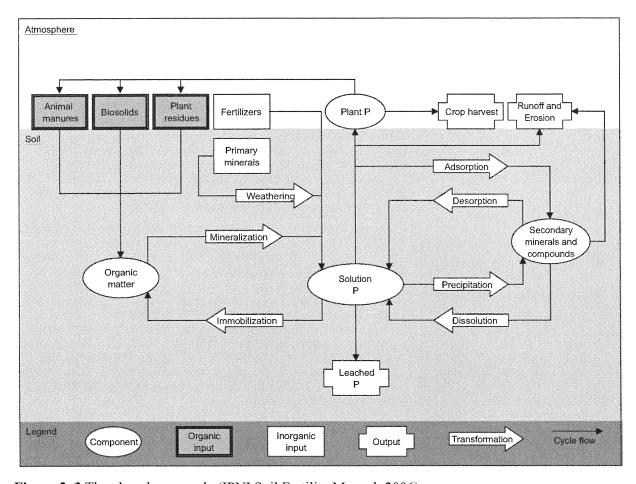


Figure 2. 3 The phosphorus cycle (IPNI Soil Fertility Manual, 2006).

Phosphorus in the soil solution is mainly present as the orthophosphate ions H<sub>2</sub>PO<sub>4</sub>/HPO<sub>4</sub><sup>2</sup>-. Primary and secondary P minerals, derived from weathering, dissolve to release orthophosphate into the solution. As well, numerous soil microorganisms decompose plant and animal residues and humus containing P and release inorganic P to solution. The microbial population produces organic P compounds that may be re-mineralized to supply P to the solution (Havlin et al., 2014). Water soluble fertilizers also add P to the soil solution as well as to less soluble and insoluble solid phase P compounds in soil. The P in soil solution can be removed by plant uptake, leaching, adsorption or precipitation as secondary minerals and compounds, as well as in runoff and erosion.

#### 2.3.2 P fertilizer

P concentration in the plants range from 0.1 to 0.5%, which is considerably lower than N and K. Plants absorb P as H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and HPO<sub>4</sub><sup>2-</sup> ions from the soil solution bathing the roots. P deficiencies can be corrected with P fertilizer sources. Possible P sources include ammonium phosphates (mono and di), polyphosphates, superphosphates, struvite, and

organic P sources like manures and composts. Rock phosphate (RP) is the raw material for making P fertilizers through acidulation with sulfuric acid. Monoammonium phosphate (MAP) is the main P fertilizer source used in Western Canada (Phosphorus in Crop Production Saskatchewan Ministry of Agriculture Fact Sheet).

# 2.4 Soil Sulfur (S)

Sulfur is a macronutrient that is needed by plants in about the same quantity as P. S plays major role in plant cells and is a constituent of three of the 20 amino acids which form proteins (Soil Fertility Manual, 2006). In addition to that, it is a constituent of enzyme and vitamins, promotes nodulation, aids in seed production, and is necessary in chlorophyll formation.

#### **2.4.1** S cycle

The sulfur cycle is the collection of processes comprised largely of S moving to and from minerals (including water bodies) and living systems (Fig 2.4).

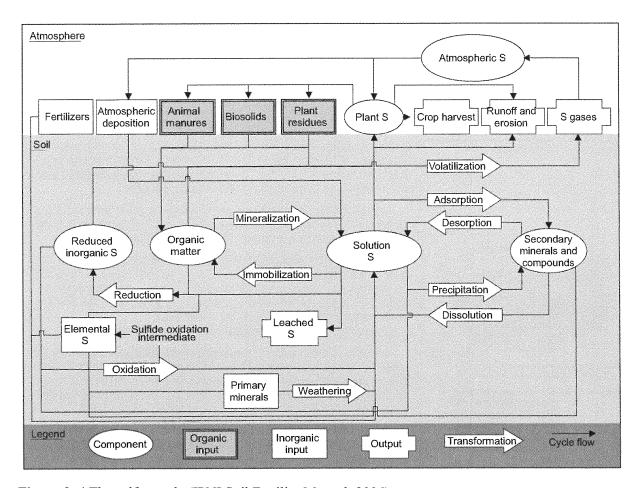


Figure 2. 4 The sulfur cycle (IPNI Soil Fertility Manual, 2006).

 $SO_4^{2-}$  is the inorganic form of S that can be absorbed by plant roots, which in soils  $\geq$  pH 6 remains in soil solution and is therefore mobile and readily leached. Like N, the S cycle in soils involves mineralization-immobilization transfer of S between organic and inorganic forms (as plant available  $SO_4^{2-}$  form), production and oxidation of reduced forms (sulfide and elemental sulfur). S is removed from the soil mainly by plant uptake, leaching and sometimes volatilization into S gases (Schoenau and Malhi, 2009).

#### 2.4.2 S fertilizer

Most plants require at least 3-5 mg kg<sup>-1</sup> of SO<sub>4</sub><sup>2-</sup> in the soil solution for growth which is supplied by mineralization of organic S and fertilizers (Havlin et al., 2014). Canola and legumes have relatively high S requirements compared to cereals. Both sulfate fertilizer and elemental S fertilizer forms are used as S sources for crop production on the prairies.

# 2.5 N, P, and S Containing Fertilizers

# 2.5.1 Monoammonium phosphate (MAP)

Monoammonium phosphate (MAP) is a widely used source of fertilizer, mainly for P but also adds small amounts of N. Two main methods are used to manufacture MAP. The common method is to take one-to-one ratio of ammonia (NH<sub>3</sub>) and phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) reacted together and the resulting slurry of MAP is solidified in a granulator. The second method introduces the two starting materials in a pipe-cross reactor, where the reaction generates heat to evaporate water and solidify MAP (International Plant Nutrition Institution, 2010). The most common fertilizer composition for MAP is 11-52-0-0.

The chemical formula of MAP is identified as  $NH_4H_2PO_4$ . Due to differences in purity of the phosphorus acid as well as variable amounts of anti-sticking and anti-dusting agents added, the  $P_2O_5$  equivalent and N contents of MAP range from 48-62% and 10-12% respectively. Water solubility of MAP at 20  $^{0}C$  is 370 g/L and solution pH of a saturated solution is given as 4- 4.5.

# 2.5.2 Urea

According to International Plant Nutrition Institute (2010), urea is the most widely used solid N fertilizer in the world. Urea is also commonly found in nature since it is expelled in the urine of animals. The high N content of urea makes it efficient to transport to farms and apply to fields. The production of urea fertilizer involves controlled reaction of ammonia gas (NH<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>) at elevated temperature and pressure. The molten urea is

formed into spheres with specialized granulation equipment or hardened into a solid prill while falling from a tower. The most common fertilizer composition for urea is 46-0-0-0.

The chemical formula of urea is CO(NH<sub>2</sub>)<sub>2</sub>. After urea contacts soil or plants, a naturally occurring enzyme (urease) begins to quickly convert the urea back to NH<sub>3</sub> in a process called hydrolysis. During this process, the N in urea is susceptible to undesirable gaseous losses as NH<sub>3</sub>. The ammonia can also be damaging to roots and germinating seeds in close proximity. Various management techniques can be used to minimize the loss of this valuable nutrient.

# 2.5.3 Ammonium sulfate (AS)

Ammonium sulfate [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] was one of the first and most widely used nitrogen (N) fertilizers for crop production. It is especially valuable where both N and sulfur (S) are required. Its high solubility provides versatility for a number of agricultural applications. Manufacturing of AS is done by a reaction of sulfuric acid and heated ammonia. The size of the resulting crystals is determined by controlling the reaction conditions. When the desired size is achieved, the crystals or prills are dried and screened to specific particle sizes. Some materials are coated with a conditioner to reduce dust and caking (International Plant Nutrition Institute, 2010). The most common fertilizer composition for AS is 21-0-0-24.

#### 2.5.4 MES - 15

MicroEssentials S15<sup>TM</sup> (13-33-0-15) is a commercially produced fertilizer by Mosaic corporation. It contains N, P as well as S. Many canola growers in regions of North America use MES-15 instead of urea and AS. Canola is very sensitive to injury from fertilizers placed in close proximity to seed such as in seed-row together with the seed, due to salt effect and ammonia toxicity. Conventional P and S fertilizers can increase seed damage due to ammonium nitrogen and especially due to the high salt index of AS. The Mosaic Company (2014) explains that MicroEssentials S15 contains N, P and S fused into one nutritionally balanced granule, and the estimated salt index is lower (21.1) compared to AS (68.3). The S in MES-15 is 50% in sulfate form and 50% in elemental form, which requires oxidation to become plant available. Some previous work has noted increased seed row safety from MES-15 fertilizer for canola (Urton et al., 2012).

#### 2.5.5 Ammonium Phosphate Sulfate (APS)

Ammonium phosphate sulfate (APS) granules are another type of commercially produced fertilizer that contains all N, P and S together. These compound or combination

products are produced with a ratio of nutrient elements that are specific to the production method/ company. The APS granules are in contrast to N, P, S fertilizer blends which are made by mixing or blending distinct products like urea, monoammonium phosphate, and ammonium sulfate together.

Three different types of APS fertilizers are used in this thesis research including  $Koch^{TM}$  (12-45-0-5),  $Simplot^{TM}$  (16-20-0-14) and  $Symtrx^{TM}$  (16-20-0-12) products. The Koch fertilizer product is made at Koch Nitrogen Company (LLC), 4111 E 37th Street North, PO Box 2219, Wichita, KS. The nutrients contained in this combination product include N, P, S and a small amount of zinc (Zn). Simplot fertilizer is made at JR Simplot Company Boise, ID 83707 with N, P and S together. High S content, high water-soluble phosphate, long lasting non-leachable N, and low volatility are promoted by the company as advantages of this commercial fertilizer. The Symtrx fertilizer product is manufactured by Anuvia Plant Nutrients, 6751 West Jones Avenue, Zellwood, FL 32798. It is different from the other two products in that it is stated to be an organic- complexed multi nutrient, slow release fertilizer which has N, P and S with 15% organic matter in it. The exact nature of the organic matter in the fertilizer is not disclosed by the manufacturer. This fertilizer is promoted as a more efficient nutrient delivery that leads to increased production and higher yields, while contributing to a better environment by reducing loss of nutrients via leaching or volatilization. Overall, seed-row safety of APS fertilizers is not well documented for pulse crops in western Canada.

#### 2.6 Seed-Row Placement

Proper nutrient application zone in the soil is as important as choosing the rate of plant nutrients. Placement decision involves knowledge of crop growth and soil characteristics. Fertilizer can be placed before planting, at planting and after planting (Havlin et al., 2014).

Placement of fertilizer in the seed-row (in the same furrow as the seed at planting/ seeding) is an effective method of fertilizer placement, especially to provide a starter source of nutrient for early crop nutrition and growth. Seed-row placement is identified as especially effective for immobile nutrients like P to supply the nutrient early on when the crop needs it (Havlin et al., 2014). Good seed-row placement requires consideration of factors including row spacing and opener spread that affects seed bed utilization and concentration of fertilizer in proximity to seed. Peas are an example of a crop that is sensitive to injury from too much

fertilizer placed with the seed, with a maximum safe rate of P<sub>2</sub>O<sub>5</sub> of only 15 lbs P<sub>2</sub>O<sub>5</sub>/acre of seed-row placed MAP (Guidelines for Maximum Safe Rate of Seed Placed Fertilizer Saskatchewan Ministry of Agriculture).

Too much fertilizer in close proximity to seed can desiccate the seedling (Havlin et al., 2014). This occurs when the concentration of fertilizer salt in the seed row is higher than the concentration of natural salts within the seed. The higher osmotic pressure in the soil will cause the water to be drawn from the seed and into the soil solution. In situations where there is not adequate soil moisture (such as dry conditions after seeding), the risk of salt damage due to osmosis increases as fertilizer salts become more concentrated. As well many N fertilizer sources can also cause significant damage through ammonia or ammonium toxicity. Excessive rates of fertilizer in the seed row often leads to serious damage or death of seedling and reduced emergence and plant stand. This may ultimately reduce final yield if the remaining plants cannot compensate by filling in. While considerable research has been conducted on Brassicae crop tolerance to seed row placed fertilizer (Qian et al., 2012), there has been limited research on pulse crops. The work in this thesis addresses gaps in our understanding of crop tolerance to seed-row placed fertilizers that is especially apparent in pulse crops.

# 2.7 <sup>15</sup>N Labelling Techniques

The ability of legumes to obtain atmospheric N in symbiosis with *Rhizobium* bacteria through biological fixation can be affected by many factors, including fertilization, and it is desirable to assess BNF under different conditions in the field. While small amounts of starter N fertilizer may aid in N fixation, large amounts of available N in soil will generally reduce the amount of N derived from fixation, as the legume will use soil available N in preference to providing energy to *Rhizobium* for fixation (Cooper and Scherer, 2012). There are several methods that can be used to measure N<sub>2</sub> fixation such as increment in N yield and plant growth, total N balance, acetylene reduction method, ureide method and the use of isotopes of N (Hardarson and Danso, 1990; Herridge et al., 2008).

N has six isotopes, varying in atomic mass from 12 to 17, where <sup>14</sup>N and <sup>15</sup>N are considered as stable isotopes and the rest are radioactive. The use of <sup>15</sup>N enriched or depleted inorganic or organic fertilizers have been effective for quantifying N fixed. <sup>15</sup>N concentration in the plant exposed to <sup>15</sup>N<sub>2</sub> is greater than the 0.3663% natural abundance if fixation occurs (Hardarson and Danso, 1990). Dilution of an <sup>15</sup>N label applied to the soil is also an effective

means to estimate the proportion of plant nitrogen derived from fixation (Xie, 2017). The extent to which <sup>15</sup>N is detected in the plant provides an estimate of the proportion of the plant's N that was derived from fixation and is thus a direct method for quantifying N<sub>2</sub> fixed.

In the 'isotope dilution method' used for estimation of N<sub>2</sub> fixed by a grain legume, a determination of % nitrogen derived from fixation (%ndfa) is made and applied to the total quantity of N measured and contained in the legume plant. To make the assessment of %ndfa, both fixing and non-fixing reference crop are grown in same soil to which the same amount of fertilizer having the same <sup>15</sup>N enrichment is added. The non-fixing reference crop takes up N from soil and fertilizer (labeled <sup>15</sup>N) but the atmospheric fixing legume crop has third source of N available to the plant, that is N from the atmosphere that dilutes the <sup>15</sup>N label. The <sup>15</sup>N isotope dilution method is employed in this thesis research to examine how seed row placed fertilizer influences the amount of N fixed by soybean (Hardarson and Danso, 1990).

# 3 EFFECT OF SEED-ROW PLACED FERTILIZER ON EMERGENCE, BIOMASS AND NUTRIENT UPTAKE BY SIX PULSE CROPS GROWN UNDER CONTROLLED ENVIRONMENT CONDITIONS

## 3.1 Preface

In this thesis chapter, seed-row placed starter fertilizers added at three different equivalent rates of N were evaluated for effects on emergence, N, P, S uptake, and biomass of six different pulse crops (soybean, pea, faba bean, black bean, lentil and chickpea) over a 30 day period. Seven different N, P and S containing fertilizer product blends or combination products were used. All evaluations were conducted under controlled environment conditions with optimal moisture and temperature. In the following chapter 4, emergence and growth response of two of the crops, soybean and lentil, is evaluated under field conditions.

## 3.2 Abstract

As a crop, legumes have a unique ability to obtain a significant portion of their nitrogen (N) requirements from atmospheric N<sub>2</sub> through a symbiotic relationship with Rhizobia spp of bacteria, which is known as symbiotic biological nitrogen fixation (BNF). This takes place in the nodules attached to the roots, but the development of nodules on the roots and onset of BNF may take some time to occur. Further, it can be expected that cooler dry soil conditions at the time of planting in northern prairie soils would delay BNF and root growth. This would result in reduced early supply of N to the plant, along with P and S deficiencies that could have negative effects on growth and development of a pulse crop. Therefore, low rates (10 to 30 kg N ha<sup>-1</sup>) of starter N supplied as mineral fertilizer in a seedrow along with phosphorus (P) and sulfur (S) may increase early growth and N, P, S nutrition. However, too much fertilizer in close proximity to seed can damage the seedling. There is limited information on safe starter rates of fertilizer blends and combination products containing higher N analyses along with P and S for pulse crops in prairie soils. Therefore, a controlled environment study was conducted to evaluate emergence, yield and N,P,S uptake responses to starter fertilizer products and blends ( mono ammonium phosphate, mono ammonium phosphate + urea, mono ammonium phosphate + ammonium sulfate) and combination products (ammonium phosphate sulfates) applied at 0, 10, 20 and 30 kg N ha<sup>-1</sup> in the seed-row under a common 15% seed bed utilization configuration. Six different pulse crops representing common pulse crops grown in the prairies: soybean, pea, faba bean, black bean, lentil and chickpea were evaluated on a nitrogen and phosphorus deficient loamy textured Brown Chernozem soil. The general sensitivity (injury potential) for starter N, P, S fertilizer products and blends placed in the seed row was lentil  $\geq$  pea  $\geq$  chickpea > soybean  $\geq$ black bean > faba bean. Lentil, pea and chickpea could generally only tolerate the 10 kg N ha  $^{1}$  rates while soybean and black bean could tolerate  $10 - 20 \text{ kg N ha}^{-1}$ . Faba bean emergence appeared relatively unaffected by all three rates of N (10, 20 and 30 kg N ha<sup>-1</sup>), and showed least sensitivity to seed row placed fertilizer. Overall, the combination ammonium phosphate sulfate fertilizer products tended to have similar or higher emergence percentage compared to equivalent analysis blends within an N rate. In terms of 30 day biomass response, soybean and black bean were most responsive to fertilization, which was attributed to slow onset of BNF in these two crops. Pea, faba bean, lentil and chickpea early biomass production and nutrient uptake was not responsive to the starter fertilizer applications, with no benefit going above 10 kg N ha<sup>-1</sup> rate.

## 3.3 Introduction

Most of the pulse growing areas in Canada are found in the western provinces, and Saskatchewan is the largest producer of field peas, faba beans, lentils and chickpeas for export purposes (Boersch et al., 2017). Therefore, it can be inferred that better agronomic practices for legume crops are important in order to ensure profitability for Canadian pulse growers and to meet the global demand for pulse crops. As a crop, legumes have the unique ability to obtain a significant portion of their nitrogen (N) requirements from atmospheric N<sub>2</sub> through a symbiotic relationship with *Rhizobia spp* of bacteria. This important biological process, known as symbiotic biological nitrogen fixation (BNF), takes place in the nodules attached to the roots (Kamfwa et al., 2017).

Soybean, pea, and faba bean are mostly grown in the moister agricultural regions of Saskatchewan, in Gray, Black or Dark Brown soil-climatic zones, as these crops perform better under higher moisture and in the absence of terminal summer drought. In contrast, lentil and chickpea are better adapted to drier soil conditions especially in late spring and summer, and are mainly grown in southern and west central portions of the province in the Brown and Dark Brown soil zones (Saskatchewan Pulse Growers, 2018). Black bean is mainly grown in Saskatchewan under irrigation surrounding Lake Diefenbaker in the Brown and Dark Brown soil-climatic zones. Farmers in the northern Great Plains of North America, including Western Canada, frequently use different fertilizers products or blends of products to supply major nutrients including nitrogen (N), phosphorus (P) and sulfur (S) (Havlin et al., 2014) Placement of fertilizer in the seed-row at planting/ seeding is an effective strategy for fertilizer placement for small grains grown in cold soils, especially to provide a starter source of nutrient for early crop nutrition and growth (Grant et al., 2001). Placing fertilizer in a seed row is important for early plant access for less mobile nutrients like P, with the effect more pronounced under cold temperatures that restrict early root growth. However, there is a limit to how much nutrient can be safely placed in the seed-row to avoid damage to the germinating seed and seedling from salt effects and ammonia toxicity.

All the legumes plants are capable of fixing their own  $N_2$  through symbiosis with *Rhizobia spp*, but the development of nodules on the roots and onset of BNF may take some time to occur. For example, an early study with soybean (*Glycine max* var. Kent) and assessment of nitrogenase enzyme activity using acetylene reduction assay in a controlled environment growth room showed that nitrogenase activity was nil until 12 days after emergence of soybean, and multiplication of bacteroids per vesicle started only after 20 days

(Hardy et al., 1971). It is anticipated that cooler dry soil conditions at the time of planting in northern prairie soils would delay biological activities such as BNF, and therefore could have negative effect on growth and development of a pulse crop.

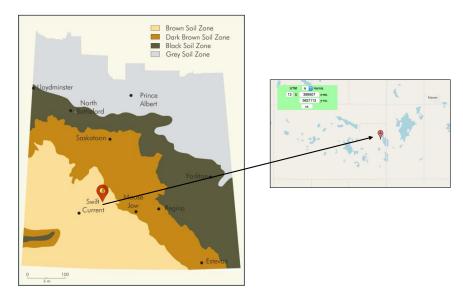
In legumes, mineral N can enhance or depress N<sub>2</sub> fixation, depending on a range of factors including the rates of N supply in the soil at different times (Cooper and Scherer, 2012). The enhancing effect of low levels of combined N (soil + fertilizer) on N<sub>2</sub> fixation in legumes is related to the above-described early lag phase between root infection by rhizobia and the onset of BNF. Therefore, low rates of starter N supplied as mineral fertilizer, may increase nodulation and total amount of N derived from N<sub>2</sub> fixation, but high rates drastically decrease nodulation and inhibit N<sub>2</sub> fixation (Cooper and Scherer, 2012). This is because it is energetically favourable for the legume to use soil N when available rather than expending energy through BNF to obtain it from the air. Other concerns surrounding seed-row placement of fertilizer with crops is that too much fertilizer in close proximity to seed can damage the seedling through desiccation from an osmotic effect. As well, ammonia and ammonium toxicity from N containing fertilizers can do significant damage to the seeds (Havlin et al., 2014).

Currently, guidelines are available for maximum safe rates of phosphorus (P) applied as mono-ammonium phosphate and also combinations of mono-ammonium phosphate with potash for common pulse crops (Schoenau et al., 2007). For example, current guidelines in Saskatchewan for seed row placed P as mono ammonium phosphate (MAP) (11-52-0) for pulse crops are 15 lbs P<sub>2</sub>O<sub>5</sub> per acre for pea, 20 lbs P<sub>2</sub>O<sub>5</sub> per acre for lentil, 20 lbs P<sub>2</sub>O<sub>5</sub> per acre for soybean, 30 lbs P<sub>2</sub>O<sub>5</sub> per acre for pinto bean and 40 lbs P<sub>2</sub>O<sub>5</sub> per acre for faba bean (Phosphorus Fertilization in Crop Production, Saskatchewan Ministry of Agriculture Fact Sheet). There was a significant reduction in emergence of soybean in a Saskatchewan soil when rates of P applied as 11-52-0 fertilizer exceeded 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (Weiseth, 2015). While there is information on tolerance of pulse crops to seed row placed MAP (11-52-0) and potash (0-0-60), there is no information on tolerance of pulse crops including soybean, pea, faba bean, black bean, lentil and chickpea to seed placed fertilizer products or blends that contain larger concentrations of N, along with P and S. This includes blends of urea (46-0-0) with MAP and ammonium sulfate (AS) (21-0-0-24) with MAP, as well as combination products like ammonium phosphate sulfates (APS) (e.g. 16-20-0-14). Therefore, an experiment was conducted at the University of Saskatchewan, College of Agriculture and Bioresources controlled environment growth chamber (phytotron) facilities to examine safe rates of different N, P and S containing fertilizer blends and products for six different pulse crops. Rates of product application are based on N in this study, with rates of 0, 10, 20 and 30 kg N ha<sup>-1</sup> of starter N placed in the seed-row under a common 15% seed bed utilization configuration. Emergence, nutrient uptake and biomass over 30 days by soybean, pea, faba bean, dry bean (black bean), lentil and Desi chickpea crops as affected by seed row placed MAP alone, MAP plus urea, MAP plus AS and popular composite granular products including MES-15 (Mosaic<sup>TM</sup> 13-33-0-15) and ammonium phosphate sulfates (Koch<sup>TM</sup> 12-45-0-0), Simplot<sup>TM</sup> 16-20-0-13, Anuvia Symtrx<sup>TM</sup> 16-20-0-12) were evaluated.

## 3.4 Materials and Methods

## 3.4.1 Soil description

The soil used in the controlled environment experiments was collected from the 0-15cm depth of a wheat stubble field (legal location: SE36-20-04-W3) located in south-central Saskatchewan near Central Butte as shown in Fig 3.1. The soil is classified as a Brown Chernozem belonging to Haverhill Loam Soil Association (Soil Classification Working Group 1998), formed on medium textured, undifferentiated glacial till deposits.



**Figure 3. 1** Location of wheat stubble field near Central Butte, SK where soil was collected from the 0-15cm depth for the controlled environment trials.

Trials were conducted in the University of Saskatchewan, College of Agriculture and Bioresources controlled environment growth chamber (phytotron) facilities over the period May 2017 to September 2018. For use in the trials, approximately 3000 kg of soil was collected from the same field on wheat stubble at three different times. Different collection

times were used so that soil used was not stored longer than 6 months. Bulk soil was collected on April 2017 which was of loam texture, pH 7.6, Total organic C: 1.8%, NO<sub>3</sub><sup>-</sup> - N: 7.6 mg kg<sup>-1</sup>, Modified Kelowna extractable P: 10.5 mg kg<sup>-1</sup>, SO<sub>4</sub><sup>2</sup>- - S: 9.7 mg kg<sup>-1</sup>. This soil was used for soybean, pea and faba bean trials. The second sample collection was made on September 2017 which was of loam texture, pH 7.6, Total organic C: 2.2%, NO<sub>3</sub><sup>-</sup> - N: 7.9 mg kg<sup>-1</sup>, Modified Kelowna extractable P: 18.1 mg kg<sup>-1</sup>, SO<sub>4</sub><sup>2</sup>- - S: 9.1 mg kg<sup>-1</sup>. This soil was used for the trial with black bean. The final soil collection was made at the end of April 2018 which was of loam texture, pH 7.9, Total organic C: 1.2%, NO<sub>3</sub><sup>-</sup> - N: 3.6 mg kg<sup>-1</sup>, Modified Kelowna extractable P: 4.8 mg kg<sup>-1</sup>, SO<sub>4</sub><sup>2</sup>- - S: 4.0 mg kg<sup>-1</sup>. This soil was used for lentil and chickpea. Prior to the growth chamber trials, the soils were analyzed at ALS<sup>TM</sup> Ltd laboratory in Saskatoon (#819-58th St E., Saskatoon, SK S7K 6X5) and based on their analysis, the soils were deemed to be relatively low (deficient) in available N, P and S, with potential for response to fertilization.

## 3.4.2 Soil preparation and seed selection

The study was conducted using elongated plastic trays (73cm L x 16cm W x 16cm D), split into three separate compartments using sealed plastic divider inserts (Fig 3.2), and one treatment was allocated randomly to each compartment in the group of trays. The trays simplify movement for watering and for frequent random repositioning within the chamber. The trays also enable effective simulation of field seed-row fertilizer placement in rows and allow for effective early root expansion horizontally and vertically. This approach was used to establish the seed-row P and K placement tolerance guidelines for pulses that is currently in use in Saskatchewan (Schoenau et al., 2007).



Figure 3. 2 Trays are separated into three compartments using sealed plastic dividers.

Once collected from the field and brought back to the laboratory, the soil was air dried at room temperature and thoroughly mixed with a rotary soil mixer to ensure homogeneity. Then, compartments in the trays were each filled with 3 kg of soil per compartment. After filling, the surface of the soil in the compartments was gently rolled with a wooden roller to break up any large lumps in order to prepare a good seedbed for germination.

The varieties of the different pulse crops were selected in consultation with pulse crop breeder Drs. Warkentin and Vandenburg at the Crop Development Center, University of Saskatchewan. Varieties were selected as follows: soybean (*Glycine max* L. cv. NSC Watson RR2Y), green pea (*Pisum sativum* L. cv. CDC Sage), faba bean (*Vicia faba* L. cv. CDC Snowdrop White Flower; Zero Tannin), black bean (*Phaseolus vulgaris* L. cv. CDC Blackstrap), small red lentils (*Lens culinaris* L. cv. CDC Maxim), and chickpea – desi (*Cicer arietinum* L. cv. CDC Consul).

# 3.4.3 Experimental design

The experimental trials were performed one crop at a time in the same GR48 growth room (1-40) in the College of Agriculture controlled environment phytotron facilities located on the 1<sup>st</sup> floor of the Agriculture Building on the University of Saskatchewan campus. The experiments were set up as a completely randomized design (CRD) with four replicates of each treatment. The fertilizer product application rates used were based on four rates of "starter" N: 0, 10, 20 and 30 kg N ha<sup>-1</sup> applied for seven different fertilizer blends/products. Fertilizer was placed in the seed row under a 15% seed bed utilization (1.5-inch opener spread / 10-inch row spacing) using a small-scale seeding tool designed and utilized for seeding trays of soil.

The six different crops; soybean, pea, faba bean, black bean, and desi chickpea were grown for 30 days to complete the experiment. As noted previously, crops were grown one crop at a time in the same growth chamber.

#### 3.4.4 Outline of treatments

The N containing fertilizer blends evaluated included 1) mono ammonium phosphate (MAP 11-52-0); 2) a 50:50 blend of mono ammonium phosphate and urea (46-0-0) to give a 28-26-0 analysis; 3) a 50:50 blend of mono ammonium phosphate and ammonium sulfate (AS 21-0-0-24) to give a 16-26-0-12 analysis product blend. In addition to blends, specialty composite granular fertilizer products evaluated at the different N rates included 1) Mosaic

Inc. MES-15 product (13-33-0-15) and 2) three ammonium phosphate sulfate products: APS 1 Koch Inc. fertilizer (12-45-0-5), APS 2 Simplot Inc. fertilizer (16-20-0-13), and APS 3 Anuvia Inc. Symtrx fertilizer (16-20-0-12). Table 3.1 provides a complete description of blends and composite products and the grams of fertilizer per compartment used for each. Table 3.2 shows the corresponding amounts of P and S applied for each of the blends and products applied at the different N rates. Figure 3.3 portrays the different fertilizer products used. Fertilizer blends were produced by weighing products into 8-dram vials and mixing together before the seeding operation.

**Table 3. 1** N-P-S fertilizer blends and combination granular products applied in the controlled environment study in grams of product (g) per compartment.

TR			MAP	Urea	A.S.	MES	APS1	APS2	APS3
T #	Fertilizer treatments	N rate			g produ	ıct per c	ompartm	ent	
		kg N/ha							
1	Monoammonium Phosphate (MAP) (11-52-0-0)	10	0.35	NA	NA	NA	NA	NA	NA
2	Monoammonium Phosphate (MAP) (11-52-0-0)	20	0.71	NA	NA	NA	NA	NA	NA
3	Monoammonium Phosphate (MAP) (11-52-0-0)	30	1.06	NA	NA	NA	NA	NA	NA
4	50:50 Blend of MAP (11-52-0-0) and Urea (46-0-0)	10	0.07	0.07	NA	NA	NA	NA	NA
5	50:50 Blend of MAP (11-52-0-0) and Urea (46-0-0)	20	0.14	0.14	NA	NA	NA	NA	NA
6	50:50 Blend of MAP (11-52-0-0) and Urea (46-0-0)	30	0.21	0.20	NA	NA	NA	NA	NA
7	50:50 Blend of MAP (11-52-0-0) and Ammonium Sulfate (AS) (21-0-0-24)	10	0.12	NA	0.12	NA	NA	NA	NA
8	50:50 Blend of MAP (11-52-0-0) and Ammonium Sulfate (AS) (21-0-0-24)	20	0.25	NA	0.24	NA	NA	NA	NA
9	50:50 Blend of MAP (11-52-0-0) and Ammonium Sulfate (AS) (21-0-0-24)	30	0.37	NA	0.36	NA	NA	NA	NA
10	MES-15 (13-33-0-15) (Mosaic Co.)	10	$NA^{\dagger}$	NA	NA	0.30	NA	NA	NA
11	MES-15 (13-33-0-15) (Mosaic Co.)	20	NA	NA	NA	0.60	NA	NA	NA
12	MES-15 (13-33-0-15) (Mosaic Co.)	30	NA	NA	NA	0.90	NA	NA	NA
13	Ammonium Phosphate Sulfate 1 (APS1) (12-45-0-5) (Koch Fertilizers)	10	NA	NA	NA	NA	0.32	NA	NA
14	Ammonium Phosphate Sulfate 1 (APS1) (12-45-0-5) (Koch Fertilizers)	20	NA	NA	NA	NA	0.65	NA	NA
15	Ammonium Phosphate Sulfate 1 (APS1) (12-45-0-5) (Koch Fertilizers)	30	NA	NA	NA	NA	0.98	NA	NA
16	Ammonium Phosphate Sulfate 2 (APS2) (16-20-0-13) (Simplot)	10	NA	NA	NA	NA	NA	0.25	NA
17	Ammonium Phosphate Sulfate 2 (APS2) (16-20-0-13) (Simplot)	20	NA	NA	NA	NA	NA	0.49	NA
18	Ammonium Phosphate Sulfate 2 (APS2) (16-20-0-13) (Simplot)	30	NA	NA	NA	NA	NA	0.73	NA
19	Control: NO Fertilizer Products/NO N Added	0	NA	NA	NA	NA	NA	NA	NA
20	Ammonium Phosphate Sulfate 3 (APS3) (16-20-0-12) (Anuvia Symtrx)	10	NA	NA	NA	NA	NA	NA	0.25
21	Ammonium Phosphate Sulfate 3 (APS3) (16-20-0-12) (Anuvia Symtrx)	20	NA	NA	NA	NA	NA	NA	0.49
22	Ammonium Phosphate Sulfate 3 (APS3) (16-20-0-12) (Anuvia Symtrx)	30	NA	NA	NA	NA	NA	NA	0.73

NA - Not applicable for this treatment

**Table 3. 2** Rates of seed row placed fertilizer product, P<sub>2</sub>O<sub>5</sub>, P and S expressed as kg ha<sup>-1</sup> at the three different N rate application treatments used in the controlled environment studies.

			10 kg l	N ha <sup>-1</sup>		20 kg N ha <sup>-1</sup>				30 kg N ha <sup>-1</sup>			
Fertilizer	Analysis	kg produ ct ha <sup>-1</sup>	kg P <sub>2</sub> O 5 ha <sup>-</sup>	kg P ha <sup>-1</sup>	kg S ha <sup>-1</sup>	kg produ ct ha <sup>-1</sup>	kg P <sub>2</sub> O 5 ha <sup>-</sup>	kg P ha <sup>-1</sup>	kg S ha <sup>-1</sup>	kg produ ct ha <sup>-1</sup>	kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	kg P ha <sup>-1</sup>	kg S ha
MAP	11-52-0	90.9	47.3	20.3	0	181.8	94.5	40.7	0	272.7	141.8	61.0	0
MAP + Urea	28-26-0	35.7	9.3	4.0	0	71.4	18.6	8.0	0	107.1	27.8	12.0	0
MAP + AS	16-26-0-12	62.5	16.3	7.0	7.5	125.0	32.5	14.0	15.0	187.5	48.8	21.0	22.5
MES-15	13-33-0-15	76.9	25.4	10.9	11.5	153.8	50.8	21.8	23.1	230.7	76.1	32.7	34.6
APS 1	12-45-0-5	83.3	37.5	16.1	4.2	166.6	75.0	32.2	8.3	249.9	112.5	48.4	12.5
APS 2	16-20-0-13	62.5	12.5	5.4	8.1	125.0	25.0	10.8	16.3	187.5	37.5	16.1	24.4
APS 3	16-20-0-12	62.5	12.5	5.4	7.5	125.0	25.0	10.8	15.0	187.5	37.5	16.1	22.5
Control	NA	0	0	0	0	0	0	0	0	0	0	0	0



**Figure 3. 3** The different fertilizer products used in the study: A) mono ammonium phosphate B) urea C) ammonium sulfate D) Mosaic MES -15 E) Koch Fertilizers APS 1 F) Simplot APS 2 G) Anuvia Symtrx APS 3 H) fertilizers in vials

## 3.4.5 Seeding operations

The pulse crops received the appropriate inoculation treatment with a commercial peat-based rhizobium inoculant of correct species for each crop that was seed applied at recommended rate to maximize N derived from fixation (Table 3.3).

**Table 3. 3** Rhizobium inoculant added to the pulse crops as seed applied peat in the controlled environment studies.

Crop	Rhizobium species required	Type	Manufacturing Company
	for effective nodulation		
Soybean	Bradyrhizobium japonicum	peat	Cell-Tech® Peat- Monsanto BioAg
Pea	Rhizobium leguminosarum	peat	Nodulator® XL Peat - BASF
Faba Beans	Rhizobium leguminosarum	peat	Nodulator® FB Peat - BASF
Lentils	Rhizobium leguminosarum	peat	Nodulator® XL Peat - BASF
Black Bean	No inoculant added		
Chickpeas	Rhizobium ciceri	granular	Nodulator® CP SCG - BASF

Black bean was not inoculated as this crop is not normally inoculated with a rhizobium inoculant (G. Hnatowich personal communication). To apply inoculant to the seeds, the required amount of peat inoculant was placed in a tray along with water to make a slurry. Then seeds were thoroughly mixed with the inoculant before seeding (Fig 3.4).



Figure 3. 4 Inoculation of pulse crop seeds with peat-based inoculant before seeding.

Seeding was performed using 10 seeds per compartment to provide a plant density similar to that described in Qian et al. (2010) for evaluating crop tolerance to seed placed phosphorus fertilizers in a similar tray study. The soil surface was levelled to create a firm, even seed bed. A furrow was made in the middle of each experimental compartment using the seeding tool to create equivalent 15% seed bed utilization as described previously.

Fertilizer blends/products at the appropriate N rate were then evenly spread along the length of the furrow for each treatment. A total of 10 pre -inoculated seeds were then placed along the length of the furrow (Fig 3.5). Fertilizer and seed were then covered with approximately 2.5 cm of soil.





**Figure 3. 5** Placement of pulse crop seed and fertilizer products/ blends together along the length of the furrow to simulate seed row placement of fertilizer at  $\sim 15\%$  seed-bed utilization.

Seeding operations for soybean commenced on June 7, 2017 followed by pea on July 11, 2017, and faba bean on August 14, 2017 on the same soil sampled in April of 2017. Black bean was seeded on February 13, 2018 on soil that was sampled in September of 2017. Soil obtained in April 2018 was used to seed lentils on July 9, 2018, and desi chickpea on August 23, 2018. Once the seeding was completed, the soil in the trays was maintained at 75% of field capacity throughout the 30-day growth period by daily watering with distilled water. Emergence counts were made up to 14 days following seeding to determine the effect of the fertilizer treatments on emergence. Once the plants were 14 days old, the plants were then thinned out to provide three evenly distributed plants per compartment (Fig 3.6) to evaluate the effect of the fertilizer treatment on nutrient uptake over 30 days and yield with the same plant density in each treatment.



**Figure 3. 6** Plants, in this example soybean, were thinned out to three evenly spaced plants per compartment at 14 days after seeding.

# 3.4.6 Environmental conditions during growth

The controlled environment facilities enable evaluation of germination, emergence and early nutrient uptake response under optimal environmental conditions. The room 1-40 growth chamber in the Agriculture and Bioresources building on University of Saskatchewan Campus was used for the entire study. Parameters were daytime day length of 18 hrs (from 6:00 AM to 12:00 AM) at 23 °C temperature, and night length of 6 hrs (from 12:00 AM to 6:00 AM) at 18 °C temperature.

# 3.4.7 Data collection and harvesting

Seedling emergence was assessed in each experimental compartment at day 5, day 10 and day 14 after seeding. One month (30 days) after seeding (Fig 3.7), the above ground biomass of the plants in each experimental compartment was harvested by cutting at the soil surface. The plant biomass was put in paper bags and placed in an oven at 40°C for one week to dry. Once plants were dried, the above ground biomass was weighed for each experimental unit, followed by fine grinding of the biomass with a NutriBullet Balance® grinder/ mixer for future analysis of N, P and S in the plant material.



**Figure 3. 7** A) Soybean; B) Pea; C) Faba bean; D) Black bean; E) Lentil; F) Chickpea in controlled environment (phytotron) chamber 30 days after seeding, just prior to harvest.

For nodulation counts on the pulse crop roots, a whole block of soil from the 10 kg N ha<sup>-1</sup> rate treatment of each fertilizer blend/ product was retained while harvesting. The retained soil block from the compartment was then covered with a 2 mm sieving mesh and water was passed through the mesh until all the soil was removed. The remaining roots were separated and transferred to tissue paper. Each nodule on the roots collected (from three plants) was recorded.

## 3.4.8 Analysis of plant samples

## Acid digest of above ground plant material for N and P

An acid digest of the above ground plant biomass material was conducted according to the method of Thomas et al. (1967). Briefly, 0.25 g (± 0.001 g) of finely ground above ground plant material was weighed into glass digestion tubes and 5 mL of concentrated (conc.) sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) was added. Samples were placed on a digestion block at 360°C for 30 min. Following this, samples were removed from the digestion block, allowed to cool, and 0.5 mL H<sub>2</sub>O<sub>2</sub> was added. Samples were then placed on the digestion block an additional three times for 30 min, adding H<sub>2</sub>O<sub>2</sub> after each heating period. Finally, samples were placed on the digestion block for 1 h. After samples were allowed to cool, distilled water was added to dilute the final volume of the sample to 75 mL to achieve a final concentration within the detection limit of the instrumentation used for elemental analysis. Samples were placed in a refrigerator until analysis for N and P in the digest using an SEAL<sup>TM</sup> AA3 automated colorimetry system.

## Combustion of above ground plant material for total S

Combustion of above ground plant material for total S was conducted according to the method described by David et al. (1989) using a LECO SC-832 model combustion analyzer. In this method 0.25g of ground plant material was weighed into a ceramic combustion boat. Then, 0.09g of Comcat<sup>TM</sup> powdered oxidant (accelerator) was added to the boat and mixed thoroughly with the sample. The sample was then inserted into the combustion tube and allowed to combust for 3 minutes at ~1100 °C. The LECO instrument was calibrated using a certified standard before use and checked for performance during use through periodic analyses of the certified standard during a run.

## 3.4.9 Statistical analyses

Statistical analyses of the data were conducted using PROC GLIMMIX of SAS, Version 9.4 (SAS Institute Inc, 2017). The controlled environment study was conducted as a completely randomized design (CRD) with a total of 22 treatments replicated 4 times. An ANOVA was conducted with treatments as a fixed effect. PROC UNIVARIATE was used to determine if the residual data were normally distributed. Where applicable, mean comparisons were performed using least significant differences (LSD; equivalent to Fisher's protected LSD) at a significance level of 0.05.

## 3.5 Results

## 3.5.1 Emergence and 30 day biomass production

## 3.5.1.1 **Soybean**

The rate of seed-placed fertilizer N had a significant effect on (p < 0.0001) on soybean emergence measured 14 days after planting (Table 3.4). Mean soybean emergence at 14 days was not significantly reduced at the rate of 10 kg N ha<sup>-1</sup> for all blends and products except MAP. Rates of 20 to 30 kg N ha<sup>-1</sup> resulted in some significant reductions in emergence for some fertilizer treatments such as MAP + Urea. Most products produced significant reductions over the control at the 30 kg N ha<sup>-1</sup> rate compared to the 20 kg N ha<sup>-1</sup> rate. Mean soybean plant biomass after 30 days was significantly higher at 30 kg N ha<sup>-1</sup> (P = 0.0048) compared to the control, and biomass yield increased with rate for most fertilizers. Significant response of soybean to starter N fertilizer likely reflects the delayed onset of N fixation in this particular crop. Mean plant biomass of the control is the lowest where there is no fertilizer.

**Table 3. 4** Mean soybean plant emergence (percentage of seeds that emerged) at 14 days after seeding and plant biomass after 30 days.

				30 I	Day Plant Bior	nass <sup>†</sup>		
	14 Day Er	mergence P	ercentage <sup>†</sup>	(	g/ compartme	nt)		
	Nitrog	en Fertilize	er Rate	Nitrogen Fertilizer Rate				
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>		kg N ha <sup>-1</sup>				
	10	20	30	10	20	30		
MAP	80 <sup>cdef</sup>	68 <sup>f</sup>	70 <sup>ef</sup>	7.70 <sup>cde</sup>	9.60 <sup>ab</sup>	9.27 <sup>abc</sup>		
MAP + Urea	95 <sup>ab</sup>	80 <sup>cdef</sup>	93 <sup>abc</sup>	7.95 <sup>bcde</sup>	$8.07^{\text{bcde}}$	9.52 <sup>ab</sup>		
MAP + AS	93 <sup>abc</sup>	85 <sup>abcd</sup>	$80^{cdef}$	9.11 <sup>abc</sup>	$8.70^{abcd}$	10.05 <sup>a</sup>		
MES-15	93 <sup>abc</sup>	88 <sup>abcd</sup>	83 <sup>bcde</sup>	7.98 <sup>bcde</sup>	8.88 <sup>abcd</sup>	7.90 <sup>bcde</sup>		
APS 1	95 <sup>ab</sup>	93 <sup>abc</sup>	$80^{cdef}$	9.52 <sup>ab</sup>	$8.04^{\text{bcde}}$	9.50 <sup>ab</sup>		
APS 2	95 <sup>ab</sup>	83 <sup>bcde</sup>	$78^{def}$	7.25 <sup>de</sup>	$8.96^{abcd}$	9.40 <sup>abc</sup>		
Control	97ª			6.70 <sup>e</sup>				
SEM	4.6022			0.6205				
F value	3.73			2.47				
Pr > F	<.0001			0.0048				

<sup>&</sup>lt;sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

## 3.5.1.2 Pea

Compared to the soybean, pea plant emergence was more sensitive especially to higher rates of N (Table 3.5). However, mean pea emergence at 14 day showed that 10 kg N ha<sup>-1</sup> did not significantly reduce emergence for most products, except MAP alone which required a high rate of addition of the product to achieve the target N rate due to its low N analysis (11%N by weight). Rates above 10 kg N ha<sup>-1</sup> caused significant reductions in emergence of the peas for all products. Effects on 30 day biomass yield of pea were similar among fertilization treatments (P = 0.2439), with few significant effects observed among the sources or rates. Overall, pea 30 day biomass was relatively unresponsive to starter fertilizer. The APS 2 product with N, P and S added at 30 kg N ha<sup>-1</sup> produced highest mean plant biomass among the treatments.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

Ammonium Phosphate Sulfate 3 was not applied in soybean crop.

**Table 3. 5** Mean pea plant emergence (percentage of seeds that emerged) at 14 days after seeding and plant biomass after 30 days.

				30 I	Day Plant Bior	nass <sup>†</sup>
	14 Day E	mergence P	ercentage <sup>†</sup>		g/ compartme	
	Nitrog	gen Fertilize	er Rate	Nitro	ogen Fertilize	Rate
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>			kg N ha <sup>-1</sup>	
	10	20	30	10	20	30
MAP	23 <sup>fg</sup>	35 <sup>cdefg</sup>	25 <sup>fg</sup>	2.17 <sup>d</sup>	2.58 <sup>bcd</sup>	2.86 <sup>bcd</sup>
MAP + Urea	$60^{ab}$	43 <sup>bcdef</sup>	50 <sup>abcd</sup>	2.60 <sup>bcd</sup>	$3.33^{abcd}$	3.39 <sup>abcd</sup>
MAP + AS	$60^{ab}$	$33^{\text{defg}}$	43 <sup>bcdef</sup>	$3.10^{abcd}$	$3.16^{abcd}$	3.16 <sup>abcd</sup>
MES-15	58 <sup>ab</sup>	$28^{efg}$	15 <sup>g</sup>	$3.25^{abcd}$	$3.10^{abcd}$	2.27 <sup>d</sup>
APS 1	55 <sup>abc</sup>	$33^{\text{defg}}$	35 <sup>cdefg</sup>	$3.36^{abcd}$	2.72 <sup>bcd</sup>	$3.90^{ab}$
APS 2	53 <sup>abcd</sup>	35 <sup>cdefg</sup>	48 <sup>abcde</sup>	2.52 <sup>cd</sup>	2.52 <sup>cd</sup>	4.22 <sup>a</sup>
APS 3	65 <sup>a</sup>	50 <sup>abcd</sup>	35 <sup>cdefg</sup>	$2.80^{bcd}$	3.64 <sup>abc</sup>	3.79 <sup>abc</sup>
Control	64 <sup>a</sup>			2.96 <sup>bcd</sup>		
SEM	7.5293			0.4765		
F value	3.77			1.25		
Pr > F	<.0001			0.2439		

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

# 3.5.1.3 **Faba bean**

The response of faba bean plant emergence to fertilizer treatment (form, rate) (Table 3.6) differed from soybean and pea in that there were few significant differences among fertilizers blends or combined products (P = 0.2449). The 14 day emergence count at 10 kg N ha<sup>-1</sup> rate had similar effect on emergence compared to the higher N rates. This is similar to plant biomass after 30 days where there is no significantly difference among starter fertilizer rates (P = 0.7021). A rate of 20 kg N ha<sup>-1</sup> would appear to be safe for most products as there were no significant reductions in emergence over the control. Greater tolerance of faba bean to seed row placed P fertilizer compared to many other crops was observed by Les Henry (2003). However, benefits from applying starter fertilizer to provide early nutrition and

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

enhance growth appear to be limited for this crop based on similar yields compared to the control for all rates of the fertilizers.

**Table 3. 6** Mean faba bean plant emergence (percentage of seeds that emerged) at 14 days after seeding and plant biomass after 30 days.

				30 I	Day Plant Bior	nass <sup>†</sup>		
	14 Day En	nergence P	ercentage <sup>†</sup>	()	g/ compartme	nt)		
	Nitrog	en Fertilize	er Rate	Nitro	ogen Fertilizer	Rate		
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>		kg N ha <sup>-1</sup>				
	10	20	30	10	20	30		
MAP	33 <sup>bc</sup>	33 <sup>bc</sup>	33 <sup>bc</sup>	2.64 <sup>b</sup>	3.05 <sup>ab</sup>	3.19 <sup>ab</sup>		
MAP + Urea	43 <sup>abc</sup>	$40^{abc}$	$40^{abc}$	3.19 <sup>ab</sup>	2.45 <sup>b</sup>	$2.86^{ab}$		
MAP + AS	53 <sup>ab</sup>	35 <sup>bc</sup>	35 <sup>bc</sup>	2.52 <sup>b</sup>	$2.95^{ab}$	2.51 <sup>b</sup>		
MES-15	$50^{ab}$	50 <sup>ab</sup>	23°	2.81 <sup>b</sup>	2.89 <sup>ab</sup>	2.48 <sup>b</sup>		
APS 1	43 <sup>abc</sup>	33 <sup>bc</sup>	35 <sup>bc</sup>	2.64 <sup>b</sup>	$3.09^{ab}$	$3.90^{a}$		
APS 2	38 <sup>abc</sup>	50 <sup>ab</sup>	43 <sup>abc</sup>	2.91 <sup>ab</sup>	3.03 <sup>ab</sup>	$3.34^{ab}$		
APS 3	58ª	45 <sup>ab</sup>	43 <sup>abc</sup>	2.62 <sup>b</sup>	2.72 <sup>b</sup>	$3.17^{ab}$		
Control	42 <sup>abc</sup>			3.13 <sup>ab</sup>				
SEM	7.3755			0.3854				
F value	1.24			0.81				
Pr > F	0.2449			0.7021				

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

## 3.5.1.4 **Black bean**

Similar to soybean bean, black bean could tolerate 10 to 20 kg N ha<sup>-1</sup> of most products without significant reduction in emergence (Table 3.7). Products or blends with lower N analysis and higher analysis of P and S (e.g. MAP, APS1), that required greater amounts of product to meet the target N rate, had lower safe rate based on N application rate. The percentage emergence was significantly higher with MAP + Urea blend (28-26-0-0) and APS3 product compared to MAP (11-52-0-0) at the rate of 30 kg N ha<sup>-1</sup>. The high emergence

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

of black bean for MAP + Urea at the 30 kg N ha<sup>-1</sup> rate is difficult to explain and may reflect an experimental error. Black bean biomass showed large and significant positive response to the starter fertilizers, with 30 day biomass highest in the 30 kg N ha<sup>-1</sup> treatments and nearly double that observed in the unfertilized control. This likely reflects reduced ability of this crop to effectively access atmospheric N through BNF compared to the other pulse crops. The black bean did not receive a rhizobium inoculation treatment due to the lack of availability of effective commercial inoculants for this crop. Many studies have reported dry bean to be the lowest N fixers among the pulse crops (Walley et al., 2007) and N fertilizer recommendations are commonly made for this crop.

**Table 3.** 7 Mean black bean plant emergence (percentage of seeds that emerged) at 14 days after seeding and plant biomass after 30 days.

				30 Г	Day Plant Bior	nass <sup>†</sup>
	14 Day En	nergence Po	ercentage <sup>†</sup>	(§	g/ compartme	nt)
	Nitrog	en Fertilize	r Rate	Nitro	ogen Fertilizer	Rate
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>			kg N ha <sup>-1</sup>	
	10	20	30	10	20	30
MAP	85 <sup>bc</sup>	83 <sup>bc</sup>	68 <sup>d</sup>	6.17 <sup>i</sup>	8.01 <sup>def</sup>	9.38 <sup>abc</sup>
MAP + Urea	88abc	80 <sup>bcd</sup>	100 <sup>a</sup>	5.58 <sup>ij</sup>	$7.62^{efg}$	8.41 <sup>cde</sup>
MAP + AS	93 <sup>ab</sup>	83 <sup>bc</sup>	78 <sup>cd</sup>	$6.490^{ghi}$	8.17 <sup>cde</sup>	8.73 <sup>bcde</sup>
MES-15	93 <sup>ab</sup>	83 <sup>bc</sup>	78 <sup>cd</sup>	$6.82^{\rm fghi}$	$7.71^{efg}$	$9.94^{ab}$
APS 1	83 <sup>bc</sup>	68 <sup>d</sup>	68 <sup>d</sup>	6.62ghi	8.14 <sup>cde</sup>	10.52 <sup>a</sup>
APS 2	$78^{\rm cd}$	80 <sup>bcd</sup>	78 <sup>cd</sup>	$6.31^{hi}$	8.20 <sup>cde</sup>	9.28 <sup>abc</sup>
APS 3	$90^{ m abc}$	88 <sup>abc</sup>	85 <sup>bc</sup>	5.90 <sup>ij</sup>	$7.57^{efgh}$	8.62 <sup>cde</sup>
Control	82 <sup>bc</sup>			$4.88^{j}$		
SEM	4.5972			0.4539		
F value	3.17			11.23		
Pr > F	0.0002			<.0001		

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

## 3.5.1.5 **Lentil**

Mean red lentil emergence was highest in the control and not significantly different from that found for the fertilizer products APS 1 and APS 2 at 10 kg N ha<sup>-1</sup> rate (Table 3.8). For the other fertilizers, 10 kg N ha<sup>-1</sup> with the seed of lentil significantly reduced emergence (P = <0.0001), with significant reductions observed with increasing rate for many of the fertilizers. The greatest injury was observed in the 30 kg N ha<sup>-1</sup> rate treatment, with the MAP + AS having the lowest emergence and greatest injury. Overall, lentil appeared to be quite sensitive to reduction in emergence from starter seed placed fertilizer, similar to pea. Some significant positive 30 day biomass responses to fertilization were observed, for example for MES-15 product, similar to pea. Mean 30 day biomass of lentil showed no significant difference between 20 and 30 kg N/ ha for most blends and combined products.

**Table 3. 8** Mean lentil plant emergence (percentage of seeds that emerged) at 14 days after seeding and plant biomass after 30 days.

				30 I	Day Plant Bior	nass <sup>†</sup>
	14 Day En	nergence P	ercentage <sup>†</sup>		g/ compartmen	
	Nitrog	en Fertilize	er Rate	Nitro	ogen Fertilizer	Rate
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>			kg N ha <sup>-1</sup>	
	10	20	30	10	20	30
MAP	60 <sup>cde</sup>	48 <sup>efg</sup>	35gh	1.45 <sup>abc</sup>	1.41 <sup>abc</sup>	1.81 <sup>ab</sup>
MAP + Urea	58 <sup>cdef</sup>	68 <sup>bcd</sup>	58 <sup>cdef</sup>	$0.80^{\rm e}$	1.09 <sup>cde</sup>	1.27 <sup>cde</sup>
MAP + AS	53 <sup>defg</sup>	55 <sup>def</sup>	25 <sup>g</sup>	$0.90^{\text{de}}$	1.24 <sup>cde</sup>	$0.88^{de}$
MES-15	$60^{\rm cde}$	68 <sup>bcd</sup>	$40^{\mathrm{fgh}}$	1.19 <sup>cde</sup>	1.44 <sup>abc</sup>	1.87ª
APS 1	80 <sup>ab</sup>	$50^{\text{defg}}$	$35^{\mathrm{gh}}$	1.25 <sup>e</sup>	1.81 <sup>ab</sup>	1.18 <sup>cde</sup>
APS 2	75 <sup>abc</sup>	58 <sup>cdef</sup>	48 <sup>efg</sup>	$0.82^{gh}$	1.25 <sup>cde</sup>	1.31 <sup>cd</sup>
APS 3	65 <sup>bcde</sup>	55 <sup>def</sup>	$60^{\rm cde}$	1.43 <sup>abc</sup>	1.32 <sup>cd</sup>	1.35 <sup>bcd</sup>
Control	90ª			$0.95^{de}$		
SEM	6.5617			0.1672		
F value	5.9			3.37		
Pr > F	< 0.0001			<.0001		

 $<sup>^{\</sup>dagger}$  Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at P  $\leq$  0.05. The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

# 3.5.1.6 **Desi Chickpea**

Desi chickpea showed similar tolerance to lentil for the seed-row placed fertilizers evaluated (Table 3.9) when added at equivalent N rate. As for lentil, significant reductions in emergence were observed for many products at rates above 10 kg N ha<sup>-1</sup> (<0.0001). A similar pattern to lentil was observed for chickpea among the fertilizer forms. The chickpea was quite sensitive to higher rates (20 kg N ha<sup>-1</sup> and above). Chickpea showed limited biomass response to starter fertilizer, with few significant differences among rate or fertilizer type. The APS 1 product resulted in the greatest 30 day biomass.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table 3. 9** Mean chickpea plant emergence (percentage of seeds that emerged) at 14 days after seeding and plant biomass after 30 days.

				30 I	Day Plant Bio	mass <sup>†</sup>
	14 Day Er	nergence P	ercentage <sup>†</sup>	(	g/ compartme	nt)
	Nitrog	en Fertilize	er Rate	Nitro	ogen Fertilize	r Rate
Nutrient Source‡		kg N ha <sup>-1</sup>			kg N ha <sup>-1</sup>	
	10	20	30	10	20	30
MAP	80 <sup>ab</sup>	45 <sup>g</sup>	50 <sup>efg</sup>	2.31 <sup>bcdef</sup>	2.45 <sup>abcde</sup>	2.38 <sup>abcdef</sup>
MAP + Urea	75 <sup>abc</sup>	55 <sup>defg</sup>	53 <sup>defg</sup>	$2.03^{ef}$	2.09 <sup>ef</sup>	2.14 <sup>def</sup>
MAP + AS	90ª	$70^{bcd}$	78 <sup>ab</sup>	2.35 <sup>bcdef</sup>	2.11 <sup>def</sup>	2.75 <sup>abc</sup>
MES-15	$70^{\text{bcd}}$	80 <sup>ab</sup>	58 <sup>cdefg</sup>	$2.26^{bcdef}$	2.83 <sup>ab</sup>	$2.67^{abcd}$
APS 1	83 <sup>ab</sup>	68 <sup>bcde</sup>	$48^{fg}$	2.55 <sup>abcde</sup>	$2.74^{abc}$	2.93 <sup>a</sup>
APS 2	65 <sup>bcdef</sup>	83 <sup>ab</sup>	65 <sup>bcdef</sup>	$2.03^{ef}$	2.09 <sup>ef</sup>	$2.38^{abcdef}$
APS 3	80 <sup>ab</sup>	75 <sup>abc</sup>	78 <sup>ab</sup>	$2.47^{abcde}$	$2.25^{\text{cdef}}$	$2.40^{abcdef}$
Control	90ª			1.92 <sup>f</sup>		
SEM	6.7495			0.203		
F value	4.23			2.07		
Pr > F	<.0001			0.0128		

 $<sup>^{\</sup>dagger}$  Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at P  $\leq$  0.05. The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

# 3.5.2 Nutrient uptake

## 3.5.2.1 **Soybean**

There were few significant differences among treatments for N uptake of soybean (Table 3.10). The highest 30 day biomass yields of soybean were generally found at 30 kg N ha<sup>-1</sup> rate and this gave highest N uptake at the same rate but no with significant differences among the fertilizer sources. The MAP treatment at 30 kg N ha<sup>-1</sup> had the highest total amount of P added and it resulted in significantly higher P uptake than other treatments (P < 0.0001) except APS 1, which also is of high P analysis. Fertilization generally increased P and S uptake by soybean. Highest S uptake occurred in MAP + AS treatment and it was significantly higher than most other treatments.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table 3. 10** Mean soybean N, P and S uptake (mg per compartment) after 30 days.

	,	,	1	( 01	1	,	,			
		N uptake† /compartn		(mg/	P uptake† /compartn		(mg	S uptake† /compartn		
	Nitrog	en Fertiliz	er Rate	Nitroge	en Fertiliz	er Rate	Nitrog	en Fertiliz	er Rate	
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>			kg N ha <sup>-1</sup>		kg N ha <sup>-1</sup>			
	10	20	30	10	20	30	10	20	30	
MAP	116.2 <sup>bc</sup>	126.0 <sup>abc</sup>	128.9 <sup>abc</sup>	16.3 <sup>efg</sup>	21.5 <sup>bc</sup>	25.0a	13.4 <sup>cdef</sup>	15 <sup>bcde</sup>	15.9abcd	
MAP + Urea	120.0 <sup>bc</sup>	115.0 <sup>bc</sup>	139.3ab	13.6gh	$13.4^{ghi}$	16.9 <sup>def</sup>	$13.8^{\text{cdef}}$	12.5 <sup>ef</sup>	16.2abc	
MAP + AS	122.7 <sup>abc</sup>	120.2bc	134.8ab	14.3 <sup>fgh</sup>	16.8 <sup>def</sup>	19.3 <sup>cd</sup>	16.0 <sup>abcd</sup>	17.8 <sup>ab</sup>	18.1ª	
MES-15	118.6 <sup>bc</sup>	130.8ab	124.0 <sup>abc</sup>	14.0 <sup>fgh</sup>	17.7 <sup>de</sup>	19.3 <sup>cd</sup>	14.7 <sup>cde</sup>	$16.0^{abcd}$	14.9 <sup>bcde</sup>	
APS 1	147.1ª	117.2 <sup>bc</sup>	136.4ab	18.3 <sup>de</sup>	17.9 <sup>de</sup>	22.7 <sup>ab</sup>	17.8ab	14.2 <sup>cde</sup>	15.9 <sup>abcd</sup>	
APS 2	117.2 <sup>bc</sup>	127.8 <sup>abc</sup>	133.1 <sup>ab</sup>	$13.0^{hi}$	15.6 <sup>efgh</sup>	16.9 <sup>def</sup>	13.1 <sup>def</sup>	15.2abcde	16.0 <sup>abcd</sup>	
Control	106.2°			$10.9^{i}$			11.1 <sup>f</sup>			
SEM	9.4516			1.0581			1.0455			
F value	1.23			12.34			3.43			
Pr > F	0.2717			<.0001			0.0002			

<sup>&</sup>lt;sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. For an element, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

Ammonium Phosphate Sulfate 3 was not applied in soybean crop.

## 3.5.2.2 Pea

Interestingly, pea showed a trend towards increasing N, P and S uptake as rate increased but there were no significant differences among the treatments (Table 3.11). This is in line with an overall general lack of biomass yield response of the pea to starter fertilizer, as described in the previous section. Above 20 kg N ha<sup>-1</sup>, N uptake is high but overall not significantly different than the unfertilized control (P = 0.2080). Peas can effectively obtain N through BNF when it is not present in the soil. There was a trend for P uptake by pea to increase with fertilization but there were few significant differences. Higher S uptake can be observed mostly in APS fertilizer, regardless of the rate.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table 3. 11** Mean pea N, P and S uptake (mg per compartment) after 30 days.

		N uptake†			P uptake†			S uptake†			
	(mg/	compartm	ent)		compartn		(mg/	compartn/	nent)		
	Nitroge	en Fertilize	r Rate	Nitroge	en Fertiliz	er Rate	Nitroge	en Fertiliz	er Rate		
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>			kg N ha <sup>-1</sup>			kg N ha <sup>-1</sup>			
	10	20	30	10	20	30	10	20	30		
MAP	60.0°	62.3°	90.1 <sup>abc</sup>	5.5 <sup>e</sup>	7.3 <sup>abcde</sup>	8.1 <sup>abcde</sup>	6.3 <sup>de</sup>	5.7 <sup>e</sup>	6.8 <sup>cde</sup>		
MAP + Urea	64.3°	90.5 <sup>abc</sup>	87.1 <sup>abc</sup>	6.4 <sup>cde</sup>	$8.0^{\text{abcde}}$	$8.6^{abcde}$	6.8 <sup>cde</sup>	$7.6^{\text{bcde}}$	7.2 <sup>bcd6</sup>		
MAP + AS	60.6°	84.9 <sup>abc</sup>	89.0 <sup>abc</sup>	$6.0^{de}$	$6.8^{\text{cde}}$	7.6 <sup>abcde</sup>	$8.0^{abcde}$	$6.9^{\text{bcde}}$	7.2 <sup>bcd6</sup>		
MES-15	83.5 <sup>abc</sup>	97.9 <sup>abc</sup>	71.7 <sup>bc</sup>	8.2abcde	$8.0^{abcde}$	6.6 <sup>cde</sup>	$7.3^{\text{bcde}}$	$7.6^{\text{bcde}}$	5.7 <sup>e</sup>		
APS 1	101.6abc	83.8abc	110.4 <sup>ab</sup>	8.9abcde	7.5 <sup>abcde</sup>	10.3 <sup>ab</sup>	$7.2^{\text{bcde}}$	6.7 <sup>cde</sup>	$9.2^{abc}$		
APS 2	71.9 <sup>bc</sup>	76.5 <sup>bc</sup>	120.2 <sup>a</sup>	$6.8^{\text{bcde}}$	6.4 <sup>cde</sup>	10.4 <sup>a</sup>	8.1 abcde	7.5 <sup>bcde</sup>	10.7 <sup>a</sup>		
APS 3	79.2abc	100.4abc	112.5 <sup>ab</sup>	7.1 <sup>abcde</sup>	9.1 abcd	$9.9^{abc}$	$7.5^{\text{bcde}}$	9.1 <sup>abcd</sup>	9.6 <sup>ab</sup>		
Control	84.1 <sup>abc</sup>			7.4 <sup>abcde</sup>			$8.8^{abcd}$				
SEM	14.8587			1.2507			1.0046				
F value	1.3			1.16			1.58				
Pr > F	0.208			0.3139			0.082				

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

## 3.5.2.3 **Faba bean**

There were no significant differences among fertilizer source or treatment rates for N uptake by fababean, except for APS 1 product that resulted in a significant increase in N uptake at the high rate (Table 3.12). This is consistent with the good ability of this crop to fix N (Klippenstein, 2019) Similar effects were observed for P uptake and S uptake, with APS products producing high uptake at the high rates of application. Similar to pea, the responses of P and S uptake to fertilization appeared greatest for the MAP and APS products.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table 3. 12** Mean faba bean N, P and S uptake (mg per compartment) after 30 days.

		N uptake†			P uptake <sup>†</sup>			S uptake	;†		
	(mg	g/compartme	ent)	(mg	/compartn		(mg/compartment)				
	Nitrog	gen Fertilizei	r Rate	Nitrogen Fertilizer Rate				Nitrogen Fertilizer Rate			
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>		kg N ha <sup>-1</sup>			kg N ha <sup>-1</sup>				
	10	20	30	10	20	30	10	20	30		
MAP	95.8 <sup>bcde</sup>	114.9abcde	120.2abcd	7.9 <sup>bc</sup>	10.4 <sup>ab</sup>	10.3ab	6.3 <sup>f</sup>	7.6 <sup>cdef</sup>	8.7 <sup>abc</sup>		
MAP + Urea	$98.8^{\text{bcde}}$	82.0e	123.7 <sup>abc</sup>	8.9 <sup>bc</sup>	6.9°	8.5 <sup>bc</sup>	7.5 <sup>cdef</sup>	$7.2^{cdef}$	7.8 <sup>cdef</sup>		
MAP + AS	91.3 <sup>cde</sup>	91.6 <sup>cde</sup>	86.2 <sup>de</sup>	7.1 <sup>bc</sup>	8.8bc	6.7°	$7.0^{cdef}$	$8.9^{abc}$	$7.3^{cdef}$		
MES-15	85.6e	$97.0^{bcde}$	100.6 <sup>bcde</sup>	7.7 <sup>bc</sup>	8.3bc	7.6 <sup>bc</sup>	$6.7^{\text{def}}$	8.1 <sup>cde</sup>	7.5 <sup>cdef</sup>		
APS 1	85.5e	112.0 <sup>abcde</sup>	143.7a	8.2bc	10.1abc	12.8a	$6.6^{ef}$	$8.2^{\text{cde}}$	10.1ab		
APS 2	114.0 <sup>abcde</sup>	115.7 <sup>abcde</sup>	126.9 <sup>ab</sup>	9.1 <sup>bc</sup>	8.2bc	9.5 <sup>abc</sup>	8.5 <sup>bc</sup>	$8.8^{abc}$	10.2ª		
APS 3	83.3e	103.9 <sup>bcde</sup>	103.2 <sup>bcde</sup>	7.6 <sup>bc</sup>	7.9 <sup>bc</sup>	9.0 <sup>bc</sup>	$7.4^{cdef}$	8.3 <sup>cd</sup>	$8.6^{\rm abc}$		
Control	100.5 <sup>bcde</sup>			8.3bc			8.4 <sup>c</sup>				
SEM	12.0933			1.2245			0.5788				
F value	1.81			1.27			3.03				
Pr > F	0.0349			0.2236			0.0003				

 $<sup>^{\</sup>dagger}$  Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at P  $\leq$  0.05. The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

## 3.5.2.4 **Black bean**

Similar to biomass, N, P and S uptake by black bean showed large response to fertilization, with more than doubling of uptake commonly encountered at the high rate of application compared to the control (Table 3.13). A pronounced rate effect is observed for all three nutrients, with highest uptakes at the highest rate. The 10 kg N ha<sup>-1</sup> rate often was significantly lower in uptake than the 20 and 30 kg N ha<sup>-1</sup> rates (P < 0.0001). Black bean appears to be the pulse crop most responsive to multi-element starter fertilization.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table 3. 13** Mean black bean N, P and S uptake (mg per compartment) after 30 days.

		P uptake <sup>†</sup> (mg/compartment)			S uptake <sup>†</sup> (mg/compartment)					
	(mg/compartment)									
	Nitrogen Fertilizer Rate			Nitrogen Fertilizer Rate			Nitrogen Fertilizer Rate			
Nutrient Source <sup>‡</sup>	kg N ha <sup>-1</sup>			kg N ha <sup>-1</sup>			kg N ha <sup>-1</sup>			
	10	20	30	10	20	30	10	20	30	
MAP	107.1 <sup>cdefgh</sup>	147.2 <sup>bc</sup>	136.6 <sup>bcde</sup>	15.4 <sup>fgh</sup>	26.0 <sup>b</sup>	30.6a	10.2 <sup>jkl</sup>	12.5 <sup>fghi</sup>	14.1 <sup>efg</sup>	
MAP + Urea	$83.8^{\mathrm{ghi}}$	$88.2^{\rm fghi}$	$94.3^{\mathrm{fghi}}$	$12.2^{hi}$	$15.5^{\text{fgh}}$	17.1 <sup>def</sup>	8.8 <sup>1</sup>	$10.1^{kl}$	$12.7^{\rm fghi}$	
MAP + AS	$90.2^{\rm fghi}$	144.4 <sup>bcd</sup>	$127.3^{bcdef}$	$14.8^{\text{fgh}}$	$20.6^{cd}$	22.3°	$12.2^{\mathrm{ghij}}$	16.3 <sup>bcd</sup>	16.8abc	
MES-15	$108.0^{cdefgh}$	$105.3^{\text{defgh}}$	191.4ª	15.6 <sup>fgh</sup>	19.2 <sup>cde</sup>	$28.6^{ab}$	$12.1^{ghijk}$	14.6 <sup>def</sup>	18.7ª	
APS 1	$104.0^{\text{defgh}}$	137.5 <sup>bcde</sup>	163.3ab	15.9 <sup>efg</sup>	22.0°	$28.0^{ab}$	$11.2^{ijk}$	$13.4^{efgh}$	16.7 <sup>abcd</sup>	
APS 2	$87.0^{\mathrm{fghi}}$	140.5 <sup>bcde</sup>	137.8 <sup>bcde</sup>	$12.7^{ghi}$	19.4 <sup>cde</sup>	21.0°	$11.7^{\rm hijk}$	16.5 <sup>bcd</sup>	18.1 <sup>ab</sup>	
APS 3	68.9hi	$102.5^{efgh}$	113.5 <sup>cdefg</sup>	$13.3^{\rm fgh}$	$15.6^{\text{fgh}}$	$20.7^{cd}$	$12.6^{\rm fghi}$	14.8 <sup>cde</sup>	$17.0^{ab}$	
Control	$61.0^{i}$			$10.3^{\rm i}$			8.3 <sup>1</sup>			
SEM	14.3501			1.2504			0.7536			
F value	5.28			21.18			17.04			
Pr > F	<.0001			<.0001			<.0001			

 $<sup>^{\</sup>dagger}$  Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at P  $\leq$  0.05. The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

# 3.5.2.5 **Lentil**

The highest N and P uptake by lentils was observed at the high rate of fertilizer (30 kg N ha<sup>-1</sup>) (Table 3.14) and was significantly higher than the 10 and 20 kg N ha<sup>-1</sup> rates for some products like MAP and MES-15, suggesting a benefit of added P on N fixation. The trend of higher uptake of nutrient with increased fertilizer rate was evident but not as pronounced as observed for black bean.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table 3. 14** Mean lentil N, P and S uptake (mg per compartment) after 30 days.

		N uptake	†		P uptake <sup>†</sup>			S uptake <sup>†</sup>			
	(mg/compartment)			(mg/compartment)			(mg/compartment)				
	Nitrogen Fertilizer Rate			Nitrogen Fertilizer Rate			Nitrogen Fertilizer Rate				
Nutrient Source <sup>‡</sup>	kg N ha-1				kg N ha-1		kg N ha <sup>-1</sup>				
	10	20	30	10	20	30	10	20	30		
MAP	36.3 <sup>cd</sup>	40.1bc	54.2ª	$2.8_{bcd}$	2.9bc	4.4ª	2.9 <sup>bcdefg</sup>	3.0 <sup>bcdefg</sup>	4.0 <sup>ab</sup>		
MAP + Urea	23.2e	$29.4^{\text{cde}}$	34.9 <sup>cde</sup>	1.6 <sup>g</sup>	$1.7^{efg}$	$2.2^{cdefg}$	1.8 <sup>g</sup>	$2.1^{efg}$	$2.6^{\text{cdefg}}$		
MAP + AS	25.7 <sup>de</sup>	$36.0^{cd}$	$25.0^{de}$	$1.8^{\text{defg}}$	$2.6^{\text{cdefg}}$	1.6 <sup>g</sup>	$2.4^{cdefg}$	$3.4^{bcd}$	$2.8^{bcdefg}$		
MES-15	$30.0^{\text{cde}}$	38.5bc	55.5a	$2.0^{\text{cdefg}}$	$2.7^{\text{bcde}}$	$4.4^{a}$	$2.2^{\text{defg}}$	$3.2^{\text{bcde}}$	$4.9^{a}$		
APS 1	36.3 <sup>cd</sup>	$50.6^{ab}$	$36.0^{\text{cd}}$	$2.6^{bcdef}$	$3.6^{ab}$	$2.5^{\text{cdefg}}$	$2.7^{\text{cdefg}}$	$4.0^{ab}$	$3.0^{\text{bcdefg}}$		
APS 2	24.8 <sup>de</sup>	31.6 <sup>cde</sup>	34.2 <sup>cde</sup>	$1.6^{\mathrm{fg}}$	$2.1^{\text{cdefg}}$	$2.2^{\text{cdefg}}$	$1.9^{\rm fg}$	$2.8^{\text{bcdefg}}$	$3.4^{bcd}$		
APS 3	36.6 <sup>cd</sup>	34.9 <sup>cde</sup>	$34.2^{cde}$	$2.4^{cdefg}$	$2.1^{\text{cdefg}}$	$2.4^{\text{cdefg}}$	3.5 <sup>bc</sup>	$3.0^{bcdefg}$	3.5 <sup>bc</sup>		
Control	25.4 <sup>de</sup>			$1.8^{\text{defg}}$			$2.4^{cdefg}$				
SEM	4.3261			0.3687			0.4471				
F value	4.36			4.77			2.81				
Pr > F	<.0001			<.0001			0.0007				

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

# 3.5.2.6 Desi Chickpea

Compared to the control, the chickpea had significant positive response in N uptake to starter fertilizer addition for all products (Table 3.15). The 30 kg N ha<sup>-1</sup> rate treatment showed the highest rate of N and P uptake in chickpea. However, this significant increase in uptake was not associated with large effect of fertilization on 30 day biomass yield of the chickpea (see section 3.5.1.6) and therefore may be considered to represent "luxury uptake". There is less difference among the rates for S uptake, but generally higher uptake is observed for the high rate fertilizer treatment. Overall, S uptake is high in the APS fertilizer products.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table 3. 15** Mean chickpea N, P and S uptake (mg per compartment) after 30 days.

		N uptake <sup>†</sup>	-	P uptake <sup>†</sup>				S uptake <sup>†</sup>			
	(mg/compartment) Nitrogen Fertilizer Rate kg N ha-1			(mg/compartment)  Nitrogen Fertilizer Rate  kg N ha-1			(mg/compartment)  Nitrogen Fertilizer Rate  kg N ha-1				
Nutrient Source <sup>‡</sup>											
	10	20	30	10	20	30	10	20	30		
MAP	53.6 <sup>fg</sup>	65.2 <sup>defg</sup>	78.6abc	4.4 <sup>defg</sup>	5.9 <sup>bcd</sup>	7.3 <sup>ab</sup>	4.5 <sup>efg</sup>	5.2 <sup>def</sup>	5.8abcde		
MAP + Urea	57.4 <sup>efg</sup>	$61.7^{\text{defg}}$	$66.5^{cdef}$	$3.4^{gh}$	$3.9^{\rm fg}$	$3.8^{\text{fgh}}$	5.9abcde	$4.2^{fg}$	$4.5^{efg}$		
MAP + AS	$55.4^{\mathrm{fg}}$	$63.1^{\text{defg}}$	81.8 <sup>ab</sup>	$4.0^{\rm fg}$	$4.1^{efg}$	5.7 <sup>cde</sup>	5.1 <sup>def</sup>	$5.1^{\text{def}}$	7.1 <sup>a</sup>		
MES-15	$54.6^{fg}$	68.9 <sup>bcde</sup>	$78.4^{abc}$	$3.5^{gh}$	$5.3^{\text{cdef}}$	6.2bc	$5.0^{\text{def}}$	6.8ab	6.8ab		
APS 1	$60.5^{efg}$	74.3 <sup>abcd</sup>	84.7ª	$4.5^{\text{defg}}$	6.2bc	8.3ª	$5.6^{bcdef}$	$6.3^{abcd}$	$6.6^{abc}$		
APS 2	52.3 <sup>g</sup>	59.7 <sup>efg</sup>	57.5 <sup>efg</sup>	$3.1^{gh}$	$3.5^{\mathrm{gh}}$	$3.6^{gh}$	$4.6^{efg}$	5.5 <sup>bcdef</sup>	6.8ab		
APS 3	$58.2^{efg}$	$61.1^{efg}$	69.4 <sup>bcde</sup>	$3.8^{\rm fg}$	$3.7^{gh}$	$4.6^{\text{defg}}$	5.5 <sup>bcdef</sup>	$5.3^{\text{cdef}}$	$6.2^{abcd}$		
Control	$39.8^{h}$			$2.4^{h}$			$3.7^{\rm g}$				
SEM	4.6384			0.5569			0.4992				
F value	6.13			7.33			3.94				
Pr > F	<.0001			<.0001			<.0001				

<sup>&</sup>lt;sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

## 3.6 Discussion

## 3.6.1 Emergence

The sensitivity of the legume crops to low rates of different starter fertilizers varied among the crops in this study. Lentil showed the highest sensitivity to seed-row starter N in terms of reduced emergence, as only the APS1 and APS2 fertilizers applied at 10 kg N ha<sup>-1</sup> rate did not significantly reduce emergence compared to the unfertilized control. These findings suggest that the ammonium phosphate sulfate fertilizer products may impart some additional seed safety compared to the other products and blends of product. For lentils, rates of 0 to 44 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> of seed placed P were reported to result in linear reductions in plant count (plants m<sup>-2</sup>) at Saskatoon and Outlook (Henry et al., 1995), supporting the relatively high sensitivity of lentil to seed row placed starter fertilizer observed in the current study. The emergence reduction can be attributed to salt damage from the fertilizer salts in the soil solution, and from ammonia toxicity from the ammonium applied with the phosphate and

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

sulfate, as well as from urea hydrolysis (Rader et al., 1943; Kaiser and Rubin, 2013). Some studies have noted that as the N content in starter fertilizers increases, the seedling damage also increases (Rehm and Lamb, 2009). As such, with some N fertilizer contributing to more seedling damage, increasing increments of N rate is expected to lead to more toxicity and reduced emergence as observed in this study. Also, the form of N in the starter fertilizer is important. With some N present in the form of urea, as in the MAP-Urea blend, there is increased potential for damage as a result of free ammonia produced during hydrolysis of the urea. Free ammonia is very injurious to seeds and seedlings (Havlin et al., 2014).

Pea and chickpea showed similar response in emergence for seed row starter fertilizer, with no significant difference between the 10 kg N ha<sup>-1</sup> rate and the control (no fertilizer). However, many products and blends applied at rates above 10 kg N ha<sup>-1</sup> resulted in emergence reduction. Henry et al. (1995) noted the high sensitivity of pea to injury from seed row placed MAP fertilizer, with a sensitivity rating of pea>lentil>>faba bean. In a comparison of a number of pulse, cereal and oilseed crops, yellow pea was found to be the most sensitive crop to seed row placement, and could tolerate only 10 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (Schoenau et al., 2007). However, the study by Schoenau et al (2007) did not include lentil. Small differences in relative injury potential could reflect different varieties and classes of lentil and pea used in the different studies, as small red lentil and green pea was used in the current study while green lentil and yellow pea were used in previous studies. Soil conditions also play a role in injury potential, with greater injury potential on coarse textured, low organic matter, dry, cold soils (Rehm and Lamb, 2009).

The soybean and black bean evaluated in this study were more tolerant than pea, lentil and chickpea to the seed row starter N fertilizer and could tolerate  $10-20 \text{ kg N ha}^{-1}$  of most products. While seed placed P is noted to be an efficient method of fertilizer placement, excess seed placed P was noted to lead to seedling damage in crops like soybean (Grant, 2012), with a reduction in plant density of ~20% at the rate of  $20 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ . Similar results were shown by Weiseth (2015), with placement of fertilizer P in the seed row at a rate of  $20 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  not causing significant reduction in soybean emergence, but seed-placed application at higher rates (40, 60 and  $80 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ ) did. In this thesis work, a reduction of plant density by about 20% in soybean was observed at the  $20 \text{ kg N ha}^{-1}$  rate compared to the unfertilized control. In terms of P, this rate of N represented anywhere from ~20 to 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, depending on product (Table 3.2). In Saskatchewan, dry bean production occurs

primarily in irrigated areas near Lake Diefenbaker and soybeans are mostly grown in the Dark Brown and Black soil zones. Therefore, in prairies, both black bean and soybean perform well under moist conditions (Saskatchewan Pulse Growers, 2018). Given assumption of high moisture conditions present during and after seeding that would reduce the osmotic effect, soybean and black bean thus could likely tolerate up to 20 kg N ha<sup>-1</sup> rate in most cases.

The faba bean, exhibited the least sensitivity for seed emergence to starter N in seed row, with no significant difference among rates and products. This agrees with the findings by Henry et al. (1995) and Schoenau et al. (2007). The 14 day emergence counts at 10 kg N ha<sup>-1</sup> rate were similar to the higher N rates (20 and 30 kg N ha<sup>-1</sup>). The results in this study are also in agreement with the results of a recently conducted growth chamber study, where rates of seed row placed fertilizer P (as MAP) ranging from 0 to 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> did not have a significant effect on faba bean seedling emergence (Weiseth, 2015). Further, field grown faba bean did not show any significant effect of different rates of P placed in the seed row and was less sensitive than the pea and lentils (Henry et al., 1995). Less sensitivity of faba bean for higher N rates could also be related to the moisture conditions in the field. Faba beans respond well to irrigation and can tolerate flooding better than peas, lentils, or chickpeas and it requires good moisture during germination (Saskatchewan Pulse Growers, 2018). The high moisture environment reduces damage from osmotic effects and free ammonia.

When considering injury potential, N and K fertilizer salts have higher salt indices and are more detrimental to germination than P salts when placed close to, or in contact with the seed (Havlin et al., 2014). Further, mixed fertilizers of the same grade may also vary widely in the salt index, depending on the carriers from which they are formulated. Higher analysis fertilizers generally have a lower salt index per unit of plant nutrient than lower analysis fertilizer. As such, MAP has higher salt index in terms of N, and thus, would produce more injury. In this study, for soybean and pea the MAP fertilizer applied at the 10 kg N ha<sup>-1</sup> rate, had significant emergence reductions compared to the rest of the fertilizer products or blends at the same N rate. For a given N rate, the MAP required the highest total amount of product due to its lowest N concentration. This can considered as a higher salt index (2.453) of MAP due to low analysis of N (Havlin et al., 2014). Products or blends with lower N analysis and higher analysis of P and S (e.g. 11-52-0, 12-45-0-5) required greater amounts of product to meet the target N rate and therefore had lower safe rate based on N application.

Overall, the combination fertilizer products (APS 1, APS 2, APS 3) tended to have similar or higher emergence percentage compared to equivalent analysis products or blends within a N rate. These specialty combination fertilizer products are promoted to provide more efficient nutrient delivery with less losses. For example, S in MES-15 is 50% in sulfate form and 50% in elemental form, with the elemental S requiring oxidation to become plant available (The Mosaic Company, 2014). Furthermore, the APS 3 product Anuvia is a slow release organic complexed multi-nutrient fertilizer that is stated to release 65% of its N as NH<sub>4</sub><sup>+</sup> in the first week, then rest of the N within 6-8 weeks. The slow release of nutrients from these products would be anticipated to produce less salt injury than straight fertilizer salts.

# 3.6.2 Biomass and nutrient uptake

Nutrient uptake showed a similar pattern to the above ground biomass of the crop harvested at 30 days after seeding. Soybean and black bean appeared to be the most responsive pulse crops to the starter fertilizer application in increasing 30 day biomass and N, P and S uptake with fertilizer application. In this study, mean soybean and black bean plant biomass, N, P, and S uptake after 30 days was maximized at the highest rate (30 kg N ha<sup>-1</sup>). Higher biomass at the highest rate of starter seed placed fertilizer, 30 kg N ha<sup>-1</sup>, is attributed to delayed onset of N fixation and contribution of additional N, P and S supplied at higher rates for plant growth and uptake on this N, P and S deficient soil. Slower development of nodules in soybean (Hardy et al., 1971) and reduced early biological N fixation would contribute to relatively large biomass response to the N in the starter fertilizer. For legume plants, intracellular invasion of roots involves rhizobia entering via infection thread in root hairs (Cooper and Scherer, 2012). The rhizosphere environment will influence the speed at which this occurs, including soil moisture, temperature, acidity and salinity. In soybean, it took about 12 days to establish nitrogenase activity, which is the enzyme responsible for converting the atmospheric N<sub>2</sub> in to NH<sub>3</sub> (Hardy et al., 1971). Warm temperatures of 18 to 23°C in this growth chamber trial compared to the field in early spring in Saskatchewan (e.g. 5-10 °C) would likely reduce the time for fixation to begin, so even higher response might be expected in the field. As well, it has been suggested that given about three weeks of time to multiply N<sub>2</sub> fixing bacteroidal forms, a supplement of N fertilizer as a starter will increase nodulation of soybean and the total amount of N derived from fixation, but higher rates decrease nodulation and N2 fixation (Cooper and Scherer, 2012). In the current study, more

of the N in the soybean may have been derived from BNF in the 10 kg N ha<sup>-1</sup> than the 30 kg N ha<sup>-1</sup> rate, as revealed in the field study described in Chapter 4 of this thesis. Given the P and S deficient nature of the soil, increased availability of these nutrients with fertilization and stimulation of growth could also stimulate both BNF and uptake of added soil and fertilizer N.

Of the six pulse crops evaluated in this study, black bean was most responsive in increases in biomass yield and nutrient uptake from fertilization. The highest 30 day biomass of black bean was obtained in the 30 kg N ha<sup>-1</sup> treatments along with the highest N, P, and S uptake. There was a significant rate effect, with the 10 kg N ha<sup>-1</sup> rate often significantly lower than the 20 and 30 kg N ha<sup>-1</sup> rates. This may reflect lack of ability of this crop to effectively fix atmospheric N compared to the other pulse crops and also a relatively high early season nutrient demand. Many studies have reported dry bean to be the lowest N fixers among the pulse crops, and N fertilizer recommendations are commonly made for this crop. For example, mean %ndfa of common bean (black bean) was reported to be only 36% and 25% in Africa and South America, respectively (Peoples et al., 2009). As well, beans have the lowest range of BNF estimates, ranging from 3- 160 kg N ha<sup>-1</sup> (Smil, 1999). A meta-review indicated the average global %ndfa for common bean to be ~ 40%, lower than other grain and oilseed legume crops (Herridge et al., 2008).

Nodule counts were made V5 at stage for the legume crops in this study at the rate at 10 kg N ha<sup>-1</sup> (See Appendix A). Nodules were identified and counted on soybean and black bean roots. The average soybean nodule count was 37 per plant with nodules up to 2-5mm in diameter. In black bean there were 191 nodules per plant but nodule diameter was generally < 0.5mm and the nodules appeared inactive. Only a very few large nodules were present at the top of the root and the rest were spread along the root as tiny nodules. Hardy et al. (1971) reported several bacteroides per enclosing vesicle in soybean at 23 days after planting and 42 mg of nodule per plant was associated with only 33 % ndfa measured at the end of the growing season in uninoculated black bean (Nleya et al., 2001). In the current study it seems likely that some proportion of the N in the soybean after 30 days came from BNF but that black bean was largely dependent on fertilizer N.

The effects of fertilization on 30 day biomass yield of faba bean, chickpea, pea and lentil were limited, with few significant differences among fertilizer rate treatment

combinations (10, 20 and 30 kg N ha<sup>-1</sup>). In terms of N uptake, pea and faba bean showed a similar pattern with no significant differences among the N rates (10, 20 and 30 kg N ha<sup>-1</sup>), likely reflecting high early season N fixing capability of these crops. For lentil and chickpea, the N uptake was significantly higher at the 30 kg N ha<sup>-1</sup> rates. Overall global estimates of proportion of N derived from atmosphere in pea, chickpea, lentil and faba bean were 65%, 58%, 65% and 75%, respectively (Peoples et al., 2009). Another review supported these values with amounts of N fixed by pea, chickpea, lentil and faba bean as 86, 56, 51, and 107 kg N ha<sup>-1</sup> year<sup>-1</sup> respectively (Herridge et al., 2008). Among the four legume crops, it can be concluded that pea and faba bean are better N fixers followed by lentil and chickpea, and the results of this thesis study support this.

In this study, P and S uptake patterns can be largely explained by the analysis of the fertilizers as it relates to relative amounts of P and S added at a given rate of N. In most cases, the highest P uptake (soybean, faba bean, black bean, lentil, and chickpea) occurred with MAP added at the rate of 30 kg N ha<sup>-1</sup>and is explained by MAP adding the most P per unit of N (Table 3.2). In this study, S - containing products and blends tended to result in higher S uptake, and sometimes produced small yield benefit, but there was no large consistent effect. This may be explained by S deficiency being a lesser issue in the soil than P deficiency. The S containing fertilizers (AS, MES-15, APS) did have impact on uptake, with higher S uptake observed mostly in APS fertilizer, regardless of the rate (pea, faba bean). In most cases, the unfertilized control had the lowest 30 day biomass yield and lowest N, P, and S uptake, which points out that a small amount (10 kg N ha<sup>-1</sup> equivalent rate) of starter fertilizer to provide N, P and S was generally beneficial for all of the legume crops grown on this deficient soil.

## 3.7 Conclusion

The six legume crops evaluated in this thesis research, grown under controlled environment conditions on a loamy textured Brown Chernozem from southern Saskatchewan, showed the following relative sensitivities (injury potential) from starter N, P, S fertilizer products and blends placed in the seed row: lentil > pea  $\geq$  chickpea > soybean  $\geq$  black bean > faba bean. Lentil, pea and chickpea could generally only tolerate the 10 kg N ha<sup>-1</sup> rates while soybean and black bean could tolerate 10 - 20 kg N ha<sup>-1</sup>. Faba bean emergence appeared relatively unaffected by all three rates of N (10, 20 and 30 kg N ha<sup>-1</sup>) and showed least

sensitivity to seed row placed fertilizer. The APS products tended to have less injury potential than the equivalent analysis products or blends when applied at a given N rate.

Soybean and black bean were most responsive in increased early (30 day) biomass production and nutrient uptake associated with the starter fertilizer application. Pea, faba bean, lentil and chickpea biomass and nutrient uptake did not respond greatly to the starter fertilizer applications, and there would be no benefit realized from going above 10 kg N ha<sup>-1</sup> on this soil from any of the fertilizer products or blends. Overall, early season N uptake influences of fertilization depends on the biological fixation ability plus supply of soil/fertilizer N, but higher P and S uptake can be observed mostly in P and S containing products and blends that sometimes produced small yield benefit but no large consistent effect.

# 4 RESPONSE OF SOYBEAN AND LENTIL TO A SEED-ROW PLACED NITROGEN-PHOSPHORUS FERTILIZER BLEND IN A BROWN CHERNOZEM IN SOUTH-CENTRAL SASKATCHEWAN

## 4.1 Preface

In this thesis chapter, the agronomic effects of adding different rates of starter urea-MAP fertilizer blend in the seed-row of soybean and lentil were determined in a field trial in south-central Saskatchewan in 2018. Emergence, grain and straw yield, N and P uptake and biological nitrogen fixation were evaluated. This study was conducted to provide information on starter N and P effects for two legume crops grown under field conditions to final grain and straw yield and therefore extend and complement the controlled environment study described in Chapter 3 for legume crops in which emergence and 30 day growth and nutrient uptake was assessed under growth chamber conditions, where conditions are favorable and carefully controlled.

#### 4.2 Abstract

Soybean and lentils are important pulse crops grown in southern Saskatchewan. Pulse crops can supply the majority of their nitrogen (N) requirement through biological fixation of atmospheric N<sub>2</sub> in nodules on their roots through symbiosis with *Rhizobium* bacterium spp. However, onset of nodulation can be slow, particularly in soybean, and environmental stresses may further delay BNF. Therefore, a small amount of starter seed-row placed N-P fertilizer may benefit early season growth and nutrient uptake that could translate into final grain yield benefit. However, high rates of seed-row placed N, P fertilizer can damage the seedling and reduce emergence. Guidelines for starter fertilizers for pulse crops that are both high in N and P analyses have not been established yet. While growth chamber studies provide a rapid and efficient means of determining response for a large number of crops and fertilizer products under ideal conditions, there is a need to provide some field validation. Therefore, an experiment was conducted in a farm field located at the boundary of the Brown and Dark Brown soil zones near Central Butte SK where both lentils and soybeans are commonly grown, to evaluate the effect of a common seed-row placed N-P fertilizer blend: 50% Urea + 50% MAP applied at 0, 10, 20 and 30 kg N and P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> on emergence, final grain and straw yield, nutrient uptake of soybean and lentil and proportion of nitrogen derived from fixation in the soybean. Confirming the controlled environment work, a rate of 10 kg N and P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> appeared to be the rate that did not significantly reduce emergence, stand count or proportion of N derived from biological nitrogen fixation, and was sufficient to maximize yield, N and P uptake for both soybean and lentil under field conditions. Rates higher than 10 kg N ha-1 in the seed row as starter 28-26-0 blend reduced emergence and decreased the proportion of N derived from biological nitrogen fixation.

#### 4.3 Introduction

Soybean and lentils are major legume crops grown in Saskatchewan, with an average yield of 1398 kg ha<sup>-1</sup> and 1453 kg ha<sup>-1</sup>, respectively, recently reported in provincial government statistics (Government of Saskatchewan, 2017). Soybean is well suited to more moist regions of the prairies such as the Dark Brown and Black soil zones in Saskatchewan (Saskatchewan Pulse Growers, 2018) and has been reported to be very responsive to starter N in other jurisdictions (Gai et al., 2017a). Lentil, which is better adapted to drier soil conditions, are mainly grown in southern and west central portions of the province in the Brown and Dark Brown soil zones (Saskatchewan Pulse Growers, 2018). As a crop, legumes can supply the majority of their nitrogen (N) requirement through biological fixation in the plant - *Rhizobium* symbiosis (Atkins, 1987).

Grower use mono-ammonium phosphate (MAP) and urea as major nitrogen (N) and phosphorus (P) sources in the northern Great Plains of North America, including Western Canada (Havlin et al., 2014). Further, adding fertilizer in the seed-row at planting/ seeding is an effective strategy for fertilizer placement, especially to provide a starter source of nutrient for early crop nutrition and growth. Seed-row placement is identified as especially effective for immobile nutrients like P in cold, prairie soils. This is to ensure supply of the nutrient early on to the roots of seedlings when it is needed for cell division early in the plant growth cycle (Grant, 2012).

Even though soybean and lentil can fix their own N from the atmosphere, it takes one to two weeks of time nodules to get established. Hardy et al. (1971) examined the nodulation of soybean (*Glycine max* var. Kent) and nitrogenase enzyme activity by C<sub>2</sub>H<sub>2</sub> -C<sub>2</sub>H<sub>4</sub> assay in controlled environment growth room with a day-night regime of 16 hours, 24°C, and 8 hours, 18°C. Results of the study showed that nitrogenase activity was nil until 12 days of age of soyeban and multiplication of bacteroids per vesicle were started at after 20 days of age. Therefore, cooler dry soil conditions at the time of planting in northern prairie soils can delay biological activities such as N<sub>2</sub> fixation, and thereby have negative effect on growth and development of a pulse crop. Moreover, this role is deemed more important under stress conditions such as when root development is hindered by root disease, as well as limited available N induced by residue immobilization or any other environmental factors. Starter N in the seed-row of a legume can help to carry the crop by supplying N until the nodules get established and begin fixation (Gai et al., 2017). Therefore, a small amount of starter N-P

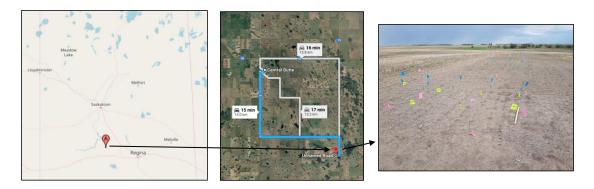
fertilizer may enhance the early season growth and nutrient uptake that could translate into a final grain yield benefit.

Other concerns surrounding seed-row placement of fertilizer with crops is that too much fertilizer in close proximity to seed can damage the seedling through desiccation from an osmotic effect. As well many N fertilizer sources can also cause significant damage through ammonia toxicity. Excessive rates of fertilizer in the seed-row often leads to serious damage or death of seedling, and significant reductions in emergence of soybean in a Saskatchewan soil were observed when rates of P applied as 11-52-0 fertilizer exceeded 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (Weiseth, 2015). Currently, guidelines are available for maximum safe rates of phosphorus (P) applied as mono-ammonium phosphate and also combinations of monoammonium phosphate with potash for common pulse crops (Schoenau et al., 2007). However, there is no information on the tolerance of soybean and lentil to seed-placed fertilizer blend that contain equal proportions of N and P from a mono-ammonium phosphate and urea fertilizer blend. Therefore, an experiment was conducted in a farm field located at the boundary of the Brown and Dark Brown soil zones near Central Butte SK where both soybeans and lentils are grown to evaluate the effect of a common seed-row placed N-P fertilizer blend: 50% Urea + 50% MAP applied at different low rates (0, 10, 20 and 30 kg N ha<sup>-1</sup>) on emergence, grain and straw yield, and nutrient uptake of soybean and lentil over an entire growing season. Given the limited information on response of soybean to fertilization under northern prairie conditions, the influence of fertilization on the nitrogen derived from the atmosphere (Ndfa %) via biological fixation was determined using an <sup>15</sup>N label application to the soybean and use of non-fixing wheat as a reference crop.

#### 4.4 Materials and Methods

### 4.4.1 Site description

The field study was initiated on an annually cultivated field located at SE36-20-04-W3 (50.733107 N; 106.424019 W). This study site (Fig 4.1) was located approximately 7 km south and 7 km east of the town of Central Butte, within the Rural Municipality of Enfield, No. 194, Saskatchewan, Canada.



**Figure 4. 1** Geographical location (50.733107 N; 106.424019 W) of the study site near Central Butte, SK.

The site is located within the Brown soil zone but is very close (~2km) to the boundary between the Brown and Dark Brown soil zone. The soils transition from loam to clay loam in texture and are classified as a Brown Chernozem (Soil Classification Working Group, 1998). The soil map unit in which the site is situated describes the site as consisting predominantly of soils of the Haverhill association. Haverhill association can be described as Brown soils that formed in loamy glacial till. It occurs on undulating and undulating dissected landscapes with gentle slopes. Surface texture is commonly described as loam (Saskatchewan Soil Survey, 1993). Prior to the field trial in 2018, wheat (*Triticum aestivum*) was grown on the site in 2017, and canola in 2016.

### 4.4.2 Initial and post-harvest soil sampling

To characterize baseline soil properties at the field trial site, including nutrients before the experiment, pre-seeding composite samples of soil were taken from the 0-15, 15-30 and 30-60cm depths of the soil profile on May 23, 2018. In the sampling, ten soil cores were taken in a transect across the site area with a Dutch auger (Fig 4.2). Soil samples were placed in a cooler for transport back to Saskatoon and then stored in a refrigerator at 5°C until preparation for chemical analysis.



**Figure 4. 2** Soil cores from 0-15, 15-30 and 30-60cm depth were extracted with a hand-held Dutch Auger

Each soil sample collected was air-dried, sieved and the <2 mm fraction was retained and analyzed for various extractable nutrients (extractable P, K, NO<sub>3</sub>-N, SO<sub>4</sub><sup>2</sup>-S) and chemical properties (pH, electrical conductivity, % organic carbon). Results of the initial pretrial soil analysis according to depth in the soil profile are provided in Table 4.1. A detailed description of the analytical methods used is provided in section 1.4.7.

**Table 4. 1** Summary of baseline soil properties in soil cores collected from the field site locations in spring 2018. Values are means from analysis of ten individual soil cores collected in May across the field site before any treatments or field operations were conducted.

Depth				Soil Prope	erty		
	$P^{\dagger}$	$N^{\ddagger}$	$\mathbf{S}^{\S}$	$\mathbf{K}^{\dagger}$	pH ¶	EC#	OC
(cm)		m	g kg soil <sup>-1</sup>			$(dS m^{-1})$	(%)
0-15	4.9	6.0	3.4	337.1	7.7	0.2	1.6
15-30	-	4.8	4.5	-	7.8	0.2	-
30-60	-	3.6	7.6	-	7.9	0.3	-

<sup>&</sup>lt;sup>†</sup>P and K= Modified Kelowna extractable PO<sub>4</sub>-P and K (Qian et al., 1994).

<sup>&</sup>lt;sup>‡</sup>N= CaCl<sub>2</sub> extractable nitrate, NO<sub>3</sub>-N (Houba et al., 2000).

<sup>§</sup>S= CaCl<sub>2</sub> extractable sulphate, SO<sub>4</sub>-S (Houba et al., 2000).

<sup>\$\</sup>mathbb{q}\$H measured in a 1:2 soil: water suspension (Hendershot et al., 2008).

<sup>\*</sup>EC measured in a 1:2 soil: water suspension (Miller and Curtin, 2008).

Post-harvest soil sampling took place on September 4, 2018. A composite sample was obtained for each plot by collecting two cores within each plot (in row and in between row) from all treatments and combining the sub-samples according to depth. A hydraulic punch truck (Fig 4.3) fitted with a 5 cm diameter barrel was used to remove samples at three depth increments (0 to 15 cm, 15 to 30 cm, and 30 to 60 cm). Samples were placed in a fridge at 5°C until preparation for chemical analysis, which consisted of air-drying samples at 30°C followed by grinding with a Wiley mill to ensure they pass a 2 mm sieve. After processing, the samples were stored at room temperature until chemical analysis.



**Figure 4. 3** A hydraulic punch truck fitted with a 5 cm diameter barrel was used to obtain samples at three depth increments in September 2018 following plot harvest.

## 4.4.3 Site preparation

In preparation of the site, the whole plot area was lightly rototilled using a Kubota<sup>®</sup> BX2350 and harrowed (Fig 4.4) to distribute residue and smooth the seed bed. On May 9, 2018, prior to seeding, herbicide was applied as a pre-plant control of weeds. Assigned plots for soybean and lentils were sprayed with the liquid herbicide product Vector<sup>®</sup> (540 g.a.i glyphosphate) at 0.8 l/ ac and Aim<sup>®</sup> (carfentrazone) at 0.017 l/ac. Plots were marked according to the treatments on May 22, 2018.



Figure 4. 4 Prior to seeding, the plot area was rototilled (A) and harrowed (B) to prepare the seed bed.

### 4.4.4 Experiment design

The field study was conducted to assess the effect of varying rates of seed placed fertilizer on soybean and lentil emergence, grain and straw production, nutrient uptake and biological nitrogen fixation (BNF). The experiment was set up as a randomized complete block design (RCBD) with four replicates of each treatment.

As in the controlled environment studies described in the previous chapter, rates of fertilizer applied in the seed-row were based on four rates of starter N: 0, 10, 20 and 30 kg N ha<sup>-1</sup> using a blend of 50% urea (46-0-0) and 50% MAP (11-52-0) of analysis of 28-26-0. Therefore, in addition to N, this also supplied 0, 10, 20 and 30 kg  $P_2O_5$  ha<sup>-1</sup>. Fertilizer was placed in the seed-row with the lentil and soybean under ~ 15% seed bed utilization (as done in the growth chamber studies) using a seeding tool designed and utilized for seeding small scale field plots. For the crops, modern varieties were selected of soybean (*Glycine max* L. cv. NSC Watson RR2Y), and small red lentil (*Lens culinaris* L. cv. CDC Maxim). Hard red spring wheat (*Triticum aestivum* L. cv Brandon) was seeded as the reference crop for soybean N uptake and BNF. Therefore with 2 pulse crops plus reference wheat crop x 4 rates x 1 product x 4 replicates = 48 experimental units at the 2018 field trial site (Fig 4.5).

#### 4.4.5 Outline of treatments

A 50:50 blend of mono-ammonium phosphate (11-52-0) and urea (46-0-0) was used at four rates, equivalent to starter N of 0, 10, 20, and 30 kg N ha<sup>-1</sup> in the seed row under 15% seed bed utilization using a small plot disc seeder. Fertilizer was weighed and retained in 16-dram vials before the seeding operation.

The area of each plot was  $3.0 \text{ m} \times 1.0 \text{ m}$  with 3 seed-rows per plot. Row length was 3.0 m and row spacing was 25.4 cm (10 inch spacing) as this is a common spacing configuration for these crops in Saskatchewan. Therefore, seed-row area was  $0.762 \text{ m}^2$  ( $3.0 \text{ m} \times 0.254 \text{ m}$ ).

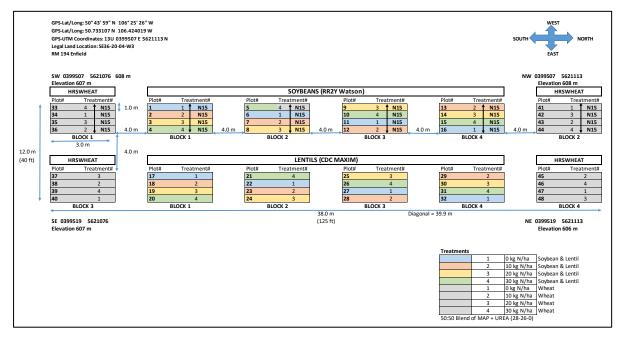


Figure 4. 5 Plot description and arrangement at 2018 field study site.

Hard red spring wheat (*Triticum aestivum* L. cv Brandon) was used as a reference crop with soybean to determine the percentage of N derived from the atmosphere and amount of  $N_2$  fixed. Soybean was selected as the crop for the N fixation study as there is limited information on how N fixation in short season soybean responds to starter fertilizer in the prairies. The isotope dilution method was used as described in detail in the literature review in chapter 2 of this thesis. At the first-trifoliate leaf stage of the soybean, a  $1m \times 1m$  subplot was established in each of the soybean and reference crop plots (Fig 4.6). A 10 atom % excess  $^{15}N$ -( $^{15}NH_4$ ) ( $^{15}NO_3$ ) fertilizer was uniformly applied to each subplot at the rate of 5 kg N ha<sup>-1</sup> in a liquid form dissolved in deionized water.

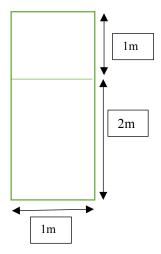


Figure 4. 6 1m × 1m subplots for <sup>15</sup>N application on soybean plots and reference wheat crop plots.

## 4.4.6 Field operations

## Seeding

On the May 22, 2018, prior to seeding, soybean was inoculated with *Bradyrhizobium japonicum* in peat form (Nodulator® SCG peat– BASF) as described in detail in chapter 3. Sametime, seeds were mixed with a granular form of this inoculant to provide a double inoculation to ensure adequate populations of the *B. japonicum* in the soil to enable infection and onset of N fixation in the soybean. The lentils were inoculated with *Rhizobium leguminosarum* (Nodulator® XL peat – BASF) peat inoculant. Seeding operations took place on May 23, 2018, with the date chosen to confirm that soil temperature at the depth of seeding was 10°C (Saskatchewan Pulse Crops Guide 2018). Prior to seeding, a packer (Fig 4.7-A) was run across the plot area used to firm the seed bed for better seed-soil contact. The entire study area was seeded with a small plot disk seeder (seeds in one compartment and fertilizer blend in the other compartment) (Fig 4.7-B) at 25.4 cm spacing, for a total of three rows within each 3m x1m plot and at a rate of 79 kg soybean seeds ha<sup>-1</sup> and 56 kg lentil seeds ha<sup>-1</sup> as per recommended rates (Saskatchewan Pulse Guide 2018). A packer wheeler, attached behind the disk seeder, helped ensure good seed to soil contact (Fig 4.7-B). Care was taken to ensure a suitable seeding depth of 2.5 cm, as recommended (Barker, 2016).



**Figure 4.** 7 Packing of soil (A) before seeding, and small plot disk seeder (B) for manual field seeding and fertilization operations.

### Weed control

Application of herbicide after seeding was performed for the first time on June 15, 2018 for weed clean-up with a hand sprayer (Fig 4.8). Soybean and lentil plots were sprayed with imazamox at 11.7 g ac<sup>-1</sup> and wheat plots were sprayed with fluroxapyr at 0.125 L ac<sup>-1</sup> and 2,4-D at 0.340 L ac<sup>-1</sup>.



Figure 4. 8 Hand sprayer to selectively control weeds in the field trial plots.

A second application of herbicides was made on June 27, 2018. Solo<sup>®</sup> (imazamox) herbicide-BASF at 11.7 g ac<sup>-1</sup> rate was applied with a hand sprayer between the rows of lentils and soybean plots to remove any persisting weeds from the first application, along with some hand hoeing. Fluoxapyr was applied between the rows of the wheat to control weeds. The plot alleyways were tilled with the Kubota<sup>®</sup> BX2350 rototiller and glyphosate was applied around the perimeter of the soybean plots to control weeds at the edges.

Fungicides was applied on June 27, 2018 using a field sprayer. Soybean and lentil plots were sprayed with Delaro® (prothioconazole and trifloxystrobin) fungicide at 0.350 L ac<sup>-1</sup>.

### Climate data

Climate data (Table 4.2) was obtained from a weather station located about 500 meters from the field site.

**Table 4. 2** Comparison of mean monthly precipitation (mm) and temperature (°C) during 2018 growing season at the field site to the 25-year (1992-2017) average.

	Mean Monthly Temperature (°C)		Mean Monthly Precipitation (mn	
Month	2018	НМ	2018	НМ
May	15.0	12	40.9	51
June	18.3	16	46.1	77
July	19.3	19	34.1	41
August	17.8	17	39.4	42
September	8.1	12	37.5	23

HM= Historical mean (1992-2017).

# <sup>15</sup>N application

The <sup>15</sup>N application treatments were made on June 20, 2018 to determine the N<sub>2</sub> fixation of soybean, which was quantified using the <sup>15</sup>N isotope dilution technique, and using hard red spring wheat (*Triticum aestivum* L. cv Brandon) as the non-fixing reference crop (Hardarson and Danso, 1990). Prior to application of <sup>15</sup>N, a stock solution was made by dissolving 58.8g of 10 atom% excess <sup>15</sup>N-(<sup>15</sup>NH<sub>4</sub>) (<sup>15</sup>NO<sub>3</sub>) fertilizer in 400ml deionized water to provide the desired rate of application in the field of 5 kg N ha<sup>-1</sup>. When soybean reached the first- trifoliate leaf, a 1m × 1m subplot on the north end of each plot was marked in each of the soybean plots and wheat reference crop plots for application of <sup>15</sup>N (Fig 4.9). A plastic frame was placed on the ground to confine the fertilizer solution within the plot area. For field application of <sup>15</sup>N, 10 ml of the stock solution was dissolved in 4L of deionized water for application, followed by another 4L of water application over the plot area to rinse off the residual fertilizer contained on the leaves into the soil.



**Figure 4. 9** A) North end of the plots was marked for <sup>15</sup>N application, B) Application of <sup>15</sup>N by placing a plastic frame and C) wetted field after application of <sup>15</sup>N

### **Crop harvesting and processing**

The crops were harvested at physiological maturity. Lentil and the non-<sup>15</sup>N labelled wheat harvesting operation was conducted on August 14, 2018 and soybean (<sup>15</sup>N applied) and <sup>15</sup>N applied wheat harvesting operations were conducted on September 04, 2018. Two subsamples of 1 m length from each plot were hand cut approximately 2.5 cm above ground. Crop samples from each plot were placed in tagged cloth bags and allowed to air dry for 1 -2 weeks prior to threshing operations.

Soybean, lentil and wheat were threshed, and grain was cleaned in October 2018. Prior to threshing, whole biomass (grain + straw) harvested yields were measured and reported on a kg ha<sup>-1</sup> basis. Threshing, de-awning and cleaning was accomplished using a Wintersteiger<sup>®</sup> LD 350 thresher. After threshing, the grain was weighed. To obtain above ground straw yield, the grain weight was subtracted from the whole biomass (grain + straw) yields. A sub sample of straw and grain of each crop (soybean, lentil and wheat) was collected in an 8-dram vial. Finally, grain was finely ground using a NutriBullet Balance<sup>®</sup> grinder/ mixer for determination of seed nutrient content by acid digestion.

For <sup>15</sup>N analysis, samples (soybean and <sup>15</sup>N wheat) were further oven - dried over night at 60 <sup>0</sup>C to a stable weight, and subsequently ground again to a fine powder using an 8000D Mixer/Mill® ball mill with dual clamps and stored in 8-dram vials for mass spectrometer analysis.

## 4.4.7 Laboratory analyses

### Acid digestion for N and P uptake

An acid digest of ground grain and straw was conducted according to the method of Thomas et al. (1967). Briefly, 0.25 g ( $\pm$  0.001 g) of finely ground plant grain or straw was weighed into glass digestion tubes and 5 mL of concentrated (conc.) sulfuric acid ( $H_2SO_4$ )

was added. Samples were placed on a digestion block at 360°C for 30 min. Following this, samples were removed from the digestion block, allowed to cool, and 0.5 mL H<sub>2</sub>O<sub>2</sub> was added. Samples were then placed on the digestion block an additional three times for 30 min, adding H<sub>2</sub>O<sub>2</sub> after each heating period. Finally, samples were placed on the digestion block for 1 h. After samples were allowed to cool, distilled water was added to dilute the final volume of the sample to 75 mL to achieve a final concentration within the detection limit of the instrumentation. Samples were placed in a refrigerator until analysis for N and P by Technicon automated colorimetry.

## Plant available soil NO<sub>3</sub>-N and SO<sub>4</sub><sup>2</sup>-S

Plant available soil nitrate (NO<sub>3</sub><sup>-</sup>-N) and sulfate (SO<sub>4</sub><sup>2</sup>--S) were extracted according to the methods described in Houba et al. (2000). 20g of air-dried soil was weighed into an extraction bottle and 40ml of pre-prepared 0.01M CaCl<sub>2</sub> solution (1.11g CaCl<sub>2</sub> into 1L of distilled water) was added into it. The resulting solutions were shaken for 30 min at 142 RPM on a rotary shaker. After shaking, the suspension was filtered through Whatman No. 42 filter paper into 8-dram vials. Vials were refrigerated until analysis for soil NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2</sup>- by automated colorimetry and plasma emission spectroscopy, respectively.

#### Plant available soil P and K

The Modified Kelowna extraction procedure as described by Qian et al. (1994) was used to extract plant available orthophosphate and potassium (K<sup>+</sup>). In this method, 3g of airdried soil was weighed into an extraction bottle and 30ml of pre-prepared Kelowna solution (0.25M acetic acid + 0.015M NH<sub>4</sub>F + 0.25M NH<sub>4</sub>OAc) was added into the bottle. Samples were then shaken horizontally on a rotary shaker for 5 min at 142 RPM. After shaking, samples were filtered through VWR 454 filter paper into 8 ½ vials, and the filtrate was stored at 5°C until analysis by AA-FE spectrometry for K and automated colorimetry for P.

## <sup>15</sup>N analysis

The finely ground soybean and wheat grain and straw sub-samples were weighed (grains  $\sim 2.0$  mg and straw  $\sim 4.0$  mg) using a Sartorius Microbalance® micro-balance and encapsulated using  $6 \times 4$  mm tin capsules (Elemental Microanalysis®) into an approximately spherical shape with the air pressed out of the encapsulated sample. The encapsulated grain and straw samples were then analyzed for percent N and atom  $\%^{15}N$  using a Costech® ECS4010 elemental analyzer coupled to a Delta V Advantage® Mass Spectrometer, with a standard for the spectrometry measurement.

#### 4.4.8 Calculations

According to Hardarson and Danso (1990), the nitrogen isotope dilution method described provides direct evidence for N<sub>2</sub> fixation since the <sup>15</sup>N concentration in the plants exposed to <sup>15</sup>N<sub>2</sub> is greater than the 0.366% natural abundance but is diluted relative to the non-fixing reference crop if BNF occurs. The isotope dilution method introduced by McAuliffe et al. (1958) was used to determine N<sub>2</sub> fixed (kg/ ha) by calculating percentage of N derived from atmosphere (%Ndfa) and multiplying by total plant N calculated for the grain and straw separately:

Amount of 
$$N_2$$
 fixed  $(kg/ha) = \frac{\% Ndfa \times total \ N \ in \ fixing \ crop}{100}$  (Eq. 4.1)

Further, Hardarson and Danso (1990) described that 'in the absence of any supply of N other than soil and <sup>15</sup>N-labelled fertilizer, both plants will contain the same ratio of <sup>15</sup>N/<sup>14</sup>N, since they are taking N of similar <sup>15</sup>N/<sup>14</sup>N composition, but not necessarily the same total quantity of N. Therefore, the total amount of N derived from atmosphere (Ndfa, kg N ha<sup>-1</sup>) in plant biomass was calculated by % <sup>15</sup>N atom excess (Ndff) of fixing and non- fixing crop as;

% 
$$Ndfa = \left(1 - \frac{\% \ 15N \ atom \ excess_{F \ (fixing \ crop)}}{\% \ 15N \ atom \ excess_{NF \ (non-fixing \ crop)}}\right) \times 100$$
 (Eq. 4.2)

Above equation can be further classified as,

$$\% Ndf f_{NF} + \% Ndf s_{NF} = 100$$
 (Eq. 4.3)

$$\% Ndff_F + \% Ndfs_F + \% Ndfa = 100$$
 (Eq. 4.4)

$$\frac{\% Ndf f_{NF}}{\% Ndf s_{NF}} = \frac{\% Ndf f_F}{\% Ndf s_F}$$
(Eq. 4.5)

The total N in the fixing crop can be obtained by multiplying the dry matter by the % N as;

Total N in fixing crop (kg/ha) = Dry matter of each plant  $\times \%$  N of each plant (Eq. 4.6)

### 4.4.9 Statistical analysis

Statistical analyses were conducted using PROC GLIMMIX of SAS, Version 9.4 (SAS Institute Inc, 2017). This study was conducted as a RCBD with a total of four treatments with four replicates. An ANOVA was conducted, with treatments as a fixed effect and block as a random effect. Where applicable, mean comparisons were performed using least significant differences (LSD; equivalent to Fisher's protected LSD) at a significance level of 0.05. PROC UNIVARIATE was used to determine if the measured values of a treatment were normally distributed.

### 4.5 Results

### 4.5.1 Emergence

The mean soybean plant emergence counts made at 19 days after seeding (Table 4.3) show that at the 20 kg N,  $P_2O_5$  ha<sup>-1</sup> seed placed fertilizer rate treatment, the emergence was significantly reduced compared to the 10 kg ha<sup>-1</sup> and control (no fertilizer treatment) (P = 0.0270). Similarly, above 10 kg ha<sup>-1</sup>, lentil emergence was significantly lower (P = 0.0272). These field results generally agree with the findings of the controlled environment trials reported on in Chapter 3, in that most pulse crops could tolerate 10 kg N,  $P_2O_5$  ha<sup>-1</sup> of MAP + urea blend, but above this such, as at 20 and 30 kg ha<sup>-1</sup> rate, significant injury was observed.

**Table 4. 3** Mean soybean and lentil plant emergence counts (number of plants emerged in 1m row length) at 19 days after seeding in 2018 at Central Butte site.

	19 Day Emergence Count				
Fertilizer Rate					
(kg N, P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	Soybean	Lentil			
0	16 <sup>a</sup> †	39ª			
10	15 <sup>a</sup>	$37^{a}$			
20	11 <sup>b</sup>	23 <sup>b</sup>			
30	13 <sup>ab</sup>	$31^{ab}$			
SEM	1.3125	3.6647			
CV	24	30			
F value	4.36	3.97			
Pr > F	0.027	0.0272			

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. Within a column, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons using Fisher's protected LSD method. SEM = standard error of mean. CV = Coefficient of variation.

## 4.5.2 Grain and straw yield

Mean soybean and lentil grain and straw yield for the starter N, P fertilizer applied at different rates is shown in Figs 4.10 and 4.11, respectively. For soybean yield (Fig 4.10), there was a trend for fertilization with starter fertilizer to result in higher soybean yield compared to the unfertilized control, with increasing rate resulting in slight decrease in mean grain yield. However, the effect was not statistically significant (P = 0.3994) among the treatments.

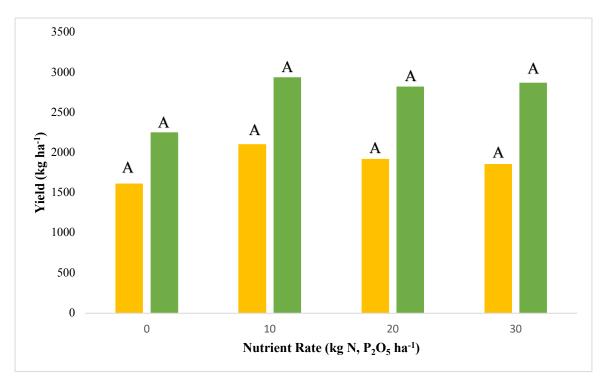
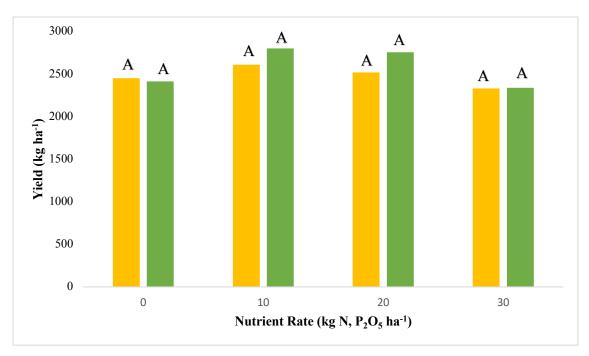


Figure 4. 10 Mean soybean grain (yellow) and straw (green) yield measured in fall 2018 at Central Butte field study site. Fertilizer is blend of 50% urea and 50% MAP by weight (analysis: 28-26-0). Reported values are the means of four replicates (n=4) of each treatment. For the same yield component (grain or straw yield), means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method.

For lentil (Fig 4.11), mean grain and straw yield followed a similar trend to soybean, with slightly higher mean yield at the 10 and 20 kg N,  $P_2O_5$  ha<sup>-1</sup> rates, and showed decrease with the treatments from 10 to 30 kg ha<sup>-1</sup>. However, even more so than for the soybeans, the yield differences among treatments were small and not statistically significant (for lentil grain and straw, P = 0.5339 and P = 0.1458, respectively. Overall response to fertilization appeared less in lentil compared to soybean.



**Figure 4. 11** Mean lentil grain (yellow) and straw (green) yield measured in fall 2018. Reported values are the means of four replicates (n=4) of each treatment. Fertilizer is blend of 50% urea and 50% MAP by weight (analysis: 28-26-0). For the same yield component (grain or straw yield), means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons using Fisher's protected LSD method.

## 4.5.3 Grain and straw nutrient uptake

Mean soybean and lentil N and P uptake in grain and straw is shown in Table 4.4. In soybean, the N uptake in grain and straw was not significantly increased by N and P fertilization (P = 0.5227), similar to that observed in the controlled environment study. There was a trend for about 10% - 20% higher mean N uptake in soybean yield components with fertilization, compared to control (no fertilizer). Increasing fertilizer rate had little evident impact on soybean grain or straw yield. For P uptake in soybean grain and straw, mean P uptake in soybean grain for all the rates (10, 20, 30 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) was significantly higher (P = 0.0425) than the unfertilized control, but among the fertilizer rates there were no differences. Straw N and P uptake by soybean showed a similar pattern to grain N and P uptake, but straw N uptake of fertilized treatments compared to unfertilized control have increased N uptake by 15% - 20%.

In lentils, both N and P uptake were quite similar among treatments (Table 4.4) and there was no significant difference in N and P uptake of grain in the different treatments, but a trend to higher N and P uptake in the grain. Straw N uptake was significantly higher in fertilized lentil straw. Compared to N and P uptake increases in soybean, the increases in uptake for lentil with fertilization versus without were smaller, only about 4% - 7% increase.

Overall, soybeans were more responsive to starter N-P fertilizer in both increased yield and nutrient uptake compared to lentil, especially for P.

**Table 4. 4** Mean soybean and lentil N and P uptake (kg ha<sup>-1</sup> uptake in grain and straw) measured in 2018 at Central Butte field study site.

		Soy	bean		Lentil			
	Gr	ain	Straw		Grain		Straw	
Fertilizer Rate (kg N, P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	N uptake	P uptake	N uptake	P uptake	N uptake	P uptake	N uptake	P uptake
				kg l	ha <sup>-1</sup>			
0	70.5 <sup>a†</sup>	5.7 <sup>b</sup>	7.9 <sup>a</sup>	$0.9^{b}$	79.8ª	6.2ª	14.1 <sup>b</sup>	1.6ª
10	$93.0^{a}$	$8.8^a$	9.9 <sup>a</sup>	$1.2^{ab}$	87.3 <sup>a</sup>	6.6 <sup>a</sup>	15.5 <sup>a</sup>	1.8 <sup>a</sup>
20	83.5 <sup>a</sup>	8.5 <sup>a</sup>	$9.8^{a}$	$1.2^{ab}$	83.4 <sup>a</sup>	6.7 <sup>a</sup>	16.8 <sup>a</sup>	1.8a
30	80.5 <sup>a</sup>	$8.4^a$	$10.4^{a}$	1.3 <sup>a</sup>	77.3 <sup>a</sup>	6.2ª	14.2 <sup>ab</sup>	$1.7^{a}$
SEM	12.3248	0.988	1.1116	0.1312	4.3574	0.3003	0.8421	0.1178
F value	0.79	3.83	1.09	1.77	0.89	0.75	2.21	0.84
Pr > F	0.5227	0.0425	0.394	0.1952	0.4666	0.5369	0.129	0.4909

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. Within a column, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons using Fisher's protected LSD method. SEM = standard error of mean.

#### 4.5.4 Nitrogen fixation

The mean % ndfa (percentage of nitrogen derived from atmosphere through biological nitrogen fixation) in soybean grain and straw is provided in Table 4.5. The % ndfa in soybean grain was significantly lower with higher rates of the N, P<sub>2</sub>O<sub>5</sub> urea-MAP fertilizer blend added. A reduction in % ndfa in grain and straw of about 10% can be observed at the 30 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> rate compared to the unfertilized control that can be mainly attributed to the effect of the added N. This is expected as N fertilizer addition at higher rates is known to significantly decrease the proportion of N in the legume plant derived from biological fixation of atmospheric N.

**Table 4. 5** Mean N derived from atmosphere through biological fixation in soybean grain and straw measured in 2018 field trial at Central Butte.

E (III D (		lfa <sup>‡</sup>
Fertilizer Rate (kg N, P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	Grain	Straw
0	81.1 <sup>a†</sup>	71.3 <sup>a</sup>
10	78.8 <sup>ab</sup>	60.0 <sup>a</sup>
20	71.5 <sup>ab</sup>	69.1ª
30	$70.9^{b}$	63.7 <sup>a</sup>
SEM	4.2477	5.5644
CV	26	33
F value	2.72	1.21
Pr > F	0.0907	0.3494

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. Within a column, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons using Fisher's protected LSD method. SEM = standard error of mean. CV = Coefficient of variation.

#### 4.6 Discussion

### 4.6.1 Emergence and yield

In this study, the blend of 50% urea and 50% MAP giving a product blend analysis of 28-26-0, supplied an available N to P<sub>2</sub>O<sub>5</sub> ratio of 1:1. The mean soybean and lentil plant emergence counts at 19 days after seeding (Table 4.3) showed the emergence was reduced by 20 – 25% at 20 and 30 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> compared to the 10 kg ha<sup>-1</sup> and control (no fertilizer) treatment. In a previous controlled environment study by Weiseth (2015), MAP was placed in a seed-row with soybean, and only rates above 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (40, 60 and 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) showed a significant reduction in emergence using a soil similar to that of the current study. Greater apparent injury per unit of P<sub>2</sub>O<sub>5</sub> observed in the current study compared to Weiseth (2015) is explained by use of a urea-MAP blend rather than MAP alone and also drier conditions experienced in the field, especially in May and June, compared to optimum moisture conditions in a growth chamber. "Fertilizer burn" is related to salt effect of the fertilizer and ammonia/ammonium toxicity (Havlin et al., 2014). Free NH<sub>3</sub> is toxic and can move freely through the cell wall, whereas NH<sub>4</sub><sup>+</sup> cannot, which explains why N fertilizers that produce large amounts of free ammonia in the vicinity of the granule, like urea, contribute more to germination and seedling damage. Therefore, obviously a blend with urea will cause more damage than MAP alone due to hydrolysis of urea into NH<sub>3</sub> and CO<sub>2</sub>. It is

<sup>&</sup>lt;sup>‡</sup>% Ndfa denotes percentage of N derived from the atmosphere.

therefore suggested for both soybean and lentil, that as the blend approaches 50% urea and 50% MAP, the starter N, P<sub>2</sub>O<sub>5</sub> rate is reduced to 10 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

Low soil moisture and temperature can also adversely impact emergence along with too much fertilizer placed in the seed-row as discussed above. The current recommended minimum average soil temperature at seeding according to Saskatchewan Pulse Growers, (2018) is 5 °C and 10 °C for lentils and soybean, respectively. Mean air temperature at site at the time of seeding was  $\sim 15$   $^{\circ}$ C and this was above the historical mean for the month but the average precipitation was below the average throughout the growing season (from May to August), about 20% and 4% in May and August, respectively (Table 4.2). This low moisture condition in spring seeding may also have affected germination and emergence and aggravated the salt and ammonia injury potential of the seed-row fertilizer, particularly for soybean. Low precipitation along with high sand content and low organic matter content in the site will lead to greater concentration of the fertilizer salt in the soil solution, reduced ammonia absorption by water and colloids, and thereby lead to more injury at high N rates for both soybean and lentil. This result agrees with the controlled environment study done using soil from same field. The most sensitive crop lentil could only tolerate 10 kg N ha<sup>-1</sup> rate for highest emergence percentage under the controlled environment condition, but soybean could tolerate up to 20 kg N ha<sup>-1</sup> rate under the optimal moisture conditions of the growth chamber study.

Placement within the seed-row or in a band close to the seed-row ensures adequate early P supply for cell division, P uptake, and crop growth during the first two to six weeks of growth (Grant, 2012), as also observed in 30 day growth and nutrient uptake studies conducted in the growth chamber in this thesis work (chapter 3). This also tends to have a positive impact on final crop yield in most crops, thus, placing P in or near the seed-row is the recommended placement method for P fertilizer on the prairies.

When considering the impact of starter N, P fertilizer on yield components, a trend for the 30 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> starter fertilizer to increase 30 day soybean biomass yield was observed in the controlled environment study (chapter 3) but the highest final grain and straw yield in the field study was observed at 10 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> compared to unfertilized control. In 2017, the average grain yield per unit area for soybean was reported as 1398 kg ha<sup>-1</sup> (Government of Saskatchewan, 2017). The mean soybean grain yield obtained in this field trial in 2018 is higher than the average, with yield of 2003, 1926, and 1862 kg ha<sup>-1</sup> for the rates of 10, 20, and 30 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, respectively. The trend of slight yield reduction with

higher rates of seed-row urea-MAP blend may be explained from the emergence reduction and reduced plant stand with the higher rates. A comparison was done with four different P fertilizer placements for soybean: seed placed, deep band, broadcast with incorporation, and broadcast of MAP at the rate of  $20 \text{ kg P}_2O_5 \text{ ha}^{-1}$  at a different site north of Central Butte in 2014 (Weiseth,2015). Like the soil used in the current study, the soil used by Weiseth had  $\sim 7 \text{ mg kg}^{-1}$  of soil test extractable P and was considered P deficient. Similar to the results of this thesis work, Weiseth also found slightly higher mean soybean grain yield with in-soil placed P compared to the unfertilized control. However, neither the current study nor the Weiseth research showed large yield responses to P fertilization of the soybean. Recent work in Manitoba has also shown little or no response of soybean to P fertilization at a number of sites across that province. These results along with the lack of response of lentil to N and P fertilization observed in this 2018 field study at Central Butte indicate that both soybean and lentil are good scavengers of existing P reserves in the soil.

In a meta-analysis of soybean grain yield responses to N fertilization (Salvagiotti et al., 2008), there was a positive response to fertilizer N in about half of the published studies, with the average yield increase from N fertilizer addition in the N-fertilizer responsive studies of 520 kg ha<sup>-1</sup> (n = 154). The magnitude of the response did not significantly differ among N rate categories of 0–50, 50–100 and >100 kg N ha<sup>-1</sup>. The results of the 2018 field study reported on in this thesis indicate that 10 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as starter seed-row placed urea-MAP blend would be optimal for the soybean in terms of maximizing plant stand and yield on this P and N deficient soil under the conditions of the study. This is supported by the results of Gai et al. (2017) at Agricultural Experiment Station of Jiamusi Branch of Heilongjiang Academy of Agricultural Sciences, China that used urea as a starter fertilizer at four different N rates (0, 25, 50 and 75 kg N ha<sup>-1</sup>), with an average yield around 3100 kg ha<sup>-1</sup> for all the treatments but no significant difference among the treatments.

As for soybean, the mean lentil grain and straw yield was slightly higher in 10 and 20 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> treatments than control, and yield showed a decrease from 10 to 30 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, but there was no significant difference among treatments. The mean lentil grain yield in this thesis study: 2610, 2523, and 2334 kg ha<sup>-1</sup> at the rate of 10, 20 and 30 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, respectively, was higher than the provincial average yield. Slightly lower mean yield with seed-row rates of N, P<sub>2</sub>O<sub>5</sub> above 10 kg ha<sup>-1</sup> for lentils observed in this study may be attributed to fertilizer injury reducing emergence and stand count. Therefore, similar to

soybean, 10 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as seed row placed starter 28-26-0 fertilizer for lentil can be considered best agronomic performer.

## 4.6.2 Crop nutrition and biological nitrogen fixation

In soybean, grain N uptake was not significantly increased by fertilization, but there was a trend for increased N uptake by about 10% - 20% with N, P fertilization, compared to the control (no fertilizer). The P uptake in soybean grain at all the rates of urea-MAP blend fertilization (10, 20, 30 kg N,  $P_2O_5$  ha<sup>-1</sup>) was significantly higher (P = 0.0334) than the unfertilized control but among the fertilizer rates the effect was similar. The increase in P uptake can be explained by the P in the fertilizer contributing to available P supplies and additional P uptake by roots in the low P testing soil that was eventually translocated to the grain. For N, increases in soil available N from fertilization may be countered by reduced biological nitrogen fixation. The trend in increased N uptake by soybean with starter N, P<sub>2</sub>O<sub>5</sub> fertilization may reflect additional uptake of soil and fertilizer N without equivalent reduction in N derived from biological nitrogen fixation, as well as enhancement of N uptake associated with P fertilization. There is typically a correlation of nutrient uptake with final crop yield for most plant nutrients (Havlin et al. 2014). This relationship may exist even for legumes that can fix N biologically if it is not present in the soil. The linear relationship between soybean grain yield and total N uptake with above-ground biomass had a slope of 12.7 kg grain per kg N (Salvagiotti et al., 2008). Further, it was mentioned that on average, a soybean crop yielding 5000 kg ha<sup>-1</sup> accumulates about 400 kg N ha<sup>-1</sup> in its aboveground biomass, and that N must be provided from indigenous soil resources, the biological fixation process, and/or fertilizer.

In lentils, both N and P uptake in yield components were similar among treatments and there was no significant difference in N and P uptake in grain and straw in the different treatments. As well, soybeans were more responsive to starter N-P fertilizer in increased nutrient uptake than lentil, especially for P. Uptake amounts for lentil similar to that obtained in the current study were observed in a study conducted in the Moist Dark Brown soil zone at Scott, SK in 2014 with grain N and P uptakes of 90.1 kg N ha<sup>-1</sup> and 7.8 kg P ha<sup>-1</sup>, respectively (Xie et al., 2018). Corresponding to the lack of large yield response of the soybean and lentil to starter N and P fertilization observed at the field site in this thesis, there were also no visual symptoms of N and P deficiency in any of the soybean and lentil treatment plots throughout the growing season. In this field study, for both soybean and lentil,

highest grain N and P uptake was recorded at 10 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, which had highest grain yield. This is also the fertilizer rate that produced minimal reduction in emergence.

It has been noted by other researchers that little starter N is typically needed for good pulse yield because they can fix their own N from the atmosphere in the symbiotic relationship with Rhizobium once they establish nodules, and too much available N can reduce the contribution from symbiotic fixation (Yang et al., 2010b). Concomitant with this concept, the % ndfa in soybean grain (Table 4.5) was lower with higher rates of N fertilizer, with about 10% reduction in the 30 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> rate compared to the unfertilized control. A similar effect was observed for % ndfa in straw but was not significantly different among the treatments. In this thesis work, the highest soybean grain % ndfa with no fertilizer was 81%, with 79, 72, and 71% ndfa for 10, 20, and 30 kg N ha<sup>-1</sup>, respectively. The % ndfa values reported for soybean in the current study tend to be slightly higher than other studies. However, nodulation of soybean on 0 N treatment in the field was visually noted to be good. The % ndfa reported for soybean was 60% in Herridge et al. (2008), 57% in Salvagiotti et al. (2008) and 50% in Peoples et al. (2009) and comparatively lower than the results obtained in this thesis work. Nevertheless, a similar <sup>15</sup>N dilution technique in a Black Chernozme soil near Rosthern, SK has shown similar results with 74 % ndfa for N in soybean grain (Xie et al., 2018).

A negative exponential relationship was typically observed between N fertilizer rate and N<sub>2</sub> fixation when N was applied in the top 0-20 cm of soil or on the soil surface (Salvagiotti et al., 2008). The % ndfa results gained from the field study in this thesis work supports this, as highest % ndfa was in the unfertilized treatment and lowest in 30 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Therefore, the 10 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> rate of 28-26-0, which gave a % ndfa that was not significantly different from the control and produced the highest mean yield and N uptake, may be the best treatment for maximizing yield and contribution of biological fixation from soybean.

#### 4.7 Conclusion

The field study conducted with soybean and lentil in 2018 in south-central Saskatchewan covered in this chapter corroborates the controlled environment study results described in Chapter 3. A rate of 10 kg N, P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as seed-row placed urea-MAP blend (28-26-0) appeared to be rate that did not significantly reduce emergence, stand count or biological nitrogen fixation and was sufficient to maximize yield, N and P uptake.

#### 5 SYNTHESIS AND CONCLUSION

#### 5.1 Overview

This thesis is based on a combination of controlled environment research and a field study. The controlled environment research, described in chapter 3, was conducted in the phytotron to evaluate the effect of different seed-row placed N-P and N-P-S fertilizer blends and combination granular products applied at different rates to six annual legume crops on crop emergence over a two-week period and on growth response. Emergence, biomass yield and N, P, S uptake after one month were measured, in order to establish best rates of seed-row application under low seed bed utilization conditions (~15% SBU) for the different crops. The N rates used in the phytotron study were 0, 10, 20 and 30 kg N ha<sup>-1</sup> of product or blend and the six pulse crops were commonly grown annual legume crops in Saskatchewan including soybean, pea, faba bean, black bean, lentil and chickpea. Seven different fertilizers products or blends: MAP alone, MAP plus urea, MAP plus AS and popular composite granular products including MES-15 (Mosaic<sup>TM</sup> 13-33-0-15) and ammonium phosphate sulfate fertilizers (Koch<sup>TM</sup> 12-45-0-05, Simplot<sup>TM</sup> 16-20-0-13, Anuvia Symtrx<sup>TM</sup> 16-20-0-12) were used and each fertilizer product or blend was used to supply the three rates of N (10, 20 and 30 kg N ha<sup>-1</sup>) used in the study.

Sensitivity of crop emergence to seed row placed fertilizer varied depending on the legume crop. Sensitivity of the crops to seed row placed N, P, S containing fertilizer products and blends varied as follows: lentil > pea ~ chickpea > soybean ~ black bean > faba bean. The type of fertilizer product or blend used did have influence on the relative injury potential. For example, MAP alone showed greater emergence reduction at higher rates due to its low N analysis which required the highest rate of all the products to achieve the target N rate. Products or blends with lower N analysis and higher analysis of P and S (e.g. 11-52-0, 12-45-0-5) required greater amounts of product to meet the target N rate and therefore had lower safe rate based on N application. As expected, higher P uptake was observed with MAP and higher S uptake in AS, and in APS fertilizer products and blends. Those legume crops with slow nodulation and onset of N fixation (soybean) and for which inoculation with commercial rhizobium inoculant is not normally practiced (black bean) showed the greatest response to starter N rate in increased biomass and nutrient uptake. These crops are also high users of P and S and so would also benefit from the added P and S in the starter fertilizer.

Overall, biomass increased with the N rates and soybean and black bean were most responsive crops to fertilization. Early season growth response of the six crops to starter seed-row placed fertilizer is summarized as follows: Soybean ~ black bean > faba bean ~ pea ~ lentil ~ chickpea. Based on results of this study, pulse crops that can begin fixation early and fix large amounts of N such as faba bean and pea should require the least amount of starter N. A 10 kg N ha<sup>-1</sup> rate of these products as starter placed in the seed-row appears to be sufficient to maximize faba bean, pea and lentil early season growth on the low fertility soil used under the controlled environment conditions of the study. Higher rates of 20 to 30 kg N ha<sup>-1</sup> may be desirable for black bean and soybean, especially under very low fertility and good moisture conditions.

Growth chamber studies provide a regulated optimal environment for examining treatment effects. However, it is important to also evaluate performance of fertilization practices under field conditions to reflect soil and environmental conditions as they exist in the field. Farm fields can give different results due to extremes in the environment. Therefore, a field study was conducted to evaluate the effects of a common seed-row placed starter N-P fertilizer blend (50% Urea + 50% MAP) that was applied at 10, 20 and 30 kg N ha<sup>-1</sup> in the seed row. Soybean and lentil were used as crops to represent moist and dry environment legume crops respectively. Emergence, yield, and nutrient uptake were measured in order to determine influence of seed-row placement under field conditions, and to compare to the controlled environment study results. Additionally, in the field study, N fixation by soybean as influenced by fertilization was assessed using an <sup>15</sup>N label application and non-fixing wheat reference crop, as there is limited information on % ndfa for this crop in Saskatchewan. The field study was conducted in the Brown soil zone near Central Butte, Saskatchewan, and is described in chapter 4. Under relatively dry growing conditions at the site in 2018, the sensitivity of lentil to fertilization was similar to that observed in the growth chamber trial, but soybean showed higher sensitivity under the field conditions, which may be related to particular sensitivity of this crop to enhanced osmotic injury under dry conditions. For both soybean and lentil, mean yields were highest in the 10 kg N ha<sup>-1</sup> treatment, with no yield benefit above this rate. The % ndfa results for soybean indicated that highest rate of added fertilizer (30 kg N ha<sup>-1</sup>) significantly decreased the proportion of N derived from the atmosphere. Therefore, in consideration of seed safety, yield response and optimizing BNF, the low rate (10 kg N ha<sup>-1</sup>) of starter N would be desirable for soybean under the conditions of this field study.

### 5.2 Synthesis and recommendations

Legumes crops can obtain much of their own N through the symbiosis with Rhizobium bacteria, as shown in this study with ~70 to 80% ndfa in the soybean in the field study. However, it takes time for infection, nodule formation and onset of BNF. Therefore, early N supplement may have particular benefit for legume crops like soybean that are slow to begin fixation, and for those like black bean for which commercial inoculants are not available or very effective. Supplying P and S along with N through use of blended products (MAP + urea, AS + urea) and combination granules (MES-15 and APS products) will supply these nutrients that are taken up early by the legumes and can also contribute to yield response on deficient soils. For the faba bean, pea and lentil, benefit of the starter seed-row placed fertilizer was limited in both controlled environment and field. These crops are good fixers of atmospheric nitrogen and scavengers of nutrient from the soil. Right fertilizer rate is also important in order to prevent salt injuries from a concentration of fertilizer salt in the seed row that is higher than the concentration of natural salts within the seed. The higher osmotic pressure in the soil will cause the water to be drawn from the seed and into the soil solution, resulting in injury or death of the seed and seedling. When placed in the seed-row with 15% seed bed utilization, this limits the safe rate of many of the fertilizer products and blends to 10 kg N ha<sup>-1</sup>, especially for the sensitive crops like pea and lentil, and under conditions of low soil moisture. As well, high percentage of sand in the soil is associated with reduced soil moisture and therefore more seed injuries. Therefore, it is important to note that the texture of the soil used in both the controlled environment and field studies is a loam, and greater damage might be expected in sandy loam or loamy sand soils under similar conditions. Overall, it can be recommended to use the 10 kg N ha<sup>-1</sup> rate as starter in the seed row for the blends and products for the most sensitive, least responsive crops: lentil, pea, and chickpea and 20 kg N ha<sup>-1</sup> rate would be appropriate less sensitive, more responsive crops: faba bean, soybean, and black bean. Greater injury potential arises with low N analysis products that require a higher rate of product to achieve the target N rate, but these products also supply more P and S at that rate.

### 5.3 Future research

Several important research questions arise from this thesis work which, if investigated, may further the general understanding of starter fertilizer benefits to legumes and development of recommendations. The 2018 field site moisture conditions were low compared to the historical mean and overall conditions were very dry. Expanding field sites

and soils would be recommended to suit and match the environments where the different pulse crops are grown. In Saskatchewan, dry bean production occurs primarily in irrigated areas near Lake Diefenbaker and soybeans are grown, mostly, in Dark Brown and Black soil zones, while lentils are grown in Brown and Dark Brown zones. As well, faba beans respond well to irrigation and can tolerate flooding better than peas, lentils, or chickpeas and it requires good moisture during growth. This study showed only the faba bean could tolerate up to 30 kg N ha<sup>-1</sup> rate without significantly reduced emergence but no other legume crops. N rates in future research could be selected as the same rates; 0, 10, 20 and 30 kg N ha<sup>-1</sup> but higher rates may be beneficial to determine the upper limit.

Although some growers only have seed-row placement as an option for starter fertilizer placement, some also have ability to place fertilizer in a side band close to the seed-row. Side-banding application may provide nutrient in close enough proximity to the germinating seedling to provide early access and still avoid injury. Therefore, starter fertilizer that is side banded at different rates may be evaluated in future trials.

Finally, it should be noted that the current field study was only conducted over one season of the crop growth and only with two crops (lentil and soybean). Expanding to all six legume crops; soybean, pea, faba bean, black bean, lentil, and chickpea in field sites selected according to their growth habits and requirements for two year time duration would be good follow-up.

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### APPENDIX

**Table A. 1** Mean soybean plant emergence (percentage of seeds that emerged) at 5 and 10 days after seeding.

	5 Day Emergence Percentage <sup>†</sup>			10 Day I	10 Day Emergence Percentage <sup>†</sup>		
	Nitrogen Fertilizer Rate			Nitro	Nitrogen Fertilizer Rate		
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>	I		kg N ha <sup>-1</sup>		
	10	20	30	10	20	30	
MAP	75 <sup>defg</sup>	70 <sup>efg</sup>	63 <sup>g</sup>	80 <sup>cdef</sup>	68 <sup>f</sup>	70 <sup>ef</sup>	
MAP + Urea	95 <sup>ab</sup>	$78^{bcdef}$	85 <sup>abcde</sup>	95 <sup>ab</sup>	$80^{cdef}$	93 <sup>abc</sup>	
MAP + AS	88 <sup>abcd</sup>	85 <sup>abcde</sup>	78 <sup>bcdef</sup>	93 <sup>abc</sup>	85 <sup>abcd</sup>	80 <sup>cdef</sup>	
MES-15	85 <sup>abcde</sup>	$78^{bcdef}$	85 <sup>abcde</sup>	93 <sup>abc</sup>	88 <sup>abcd</sup>	83 <sup>bcde</sup>	
APS 1	98ª	$80^{bcdef}$	78 <sup>bcdef</sup>	95 <sup>ab</sup>	93 <sup>abc</sup>	80 <sup>cdef</sup>	
APS 2	95 <sup>ab</sup>	83 <sup>abcdef</sup>	$68^{\mathrm{fg}}$	95 <sup>ab</sup>	83 <sup>bcde</sup>	$78^{def}$	
Control	92 <sup>abc</sup>			97ª			
SEM	5.5638			4.6628			
F value	2.97			3.77			
Pr > F	0.0009			<.0001			

 $<sup>^{\</sup>dagger}$  Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at P  $\leq$  0.05. The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

Ammonium Phosphate Sulfate 3 was not applied in soybean crop.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table A. 2** Mean pea plant emergence (percentage of seeds that emerged) at 5 and 10 days after seeding.

	5 Day Emergence Percentage <sup>†</sup>			10 Day I	10 Day Emergence Percentage		
	Nitro	gen Fertiliz	zer Rate	Nitro	Nitrogen Fertilizer Rate		
Nutrient Source <sup>‡</sup>		kg N ha	I		kg N ha <sup>-1</sup>		
	10	20	30	10	20	30	
MAP	35 <sup>efgh</sup>	43 <sup>bcdefg</sup>	20 <sup>h</sup>	33 <sup>defg</sup>	40 <sup>bcdef</sup>	25 <sup>fg</sup>	
MAP + Urea	55 <sup>abcde</sup>	$40^{cdefgh}$	50 <sup>abcdef</sup>	58 <sup>abc</sup>	43 <sup>bcdef</sup>	50 <sup>abcde</sup>	
MAP + AS	63 <sup>ab</sup>	$35^{efgh}$	$30^{\mathrm{fgh}}$	63 <sup>ab</sup>	$38^{\text{defg}}$	$40^{bcdef}$	
MES-15	58 <sup>abcd</sup>	$28^{gh}$	$20^{h}$	55 <sup>abcd</sup>	$28^{efg}$	15 <sup>g</sup>	
APS 1	50 <sup>abcdef</sup>	$30^{\text{fgh}}$	$38^{\text{defgh}}$	58 <sup>abc</sup>	$33^{\text{defg}}$	$35^{\rm cdefg}$	
APS 2	45 <sup>bcdefg</sup>	$28^{gh}$	$40^{cdefgh}$	48 <sup>abcdef</sup>	$35^{\text{def}}$	48 <sup>abcdef</sup>	
APS 3	$60^{abc}$	45 <sup>bcdefg</sup>	$40^{cdefgh}$	68 <sup>a</sup>	48 <sup>abcdef</sup>	45 <sup>abcde</sup>	
Control	65 <sup>a</sup>			62 <sup>ab</sup>			
SEM	7.2457			8.0938			
F value	3.54			2.83			
Pr > F	<.0001			0.0006			

<sup>&</sup>lt;sup>†</sup>Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table A. 3** Mean faba bean plant emergence (percentage of seeds that emerged) at 5 and 10 days after seeding.

	5 Day Emergence Percentage <sup>†</sup>			10 Day E	10 Day Emergence Percentage <sup>†</sup>		
	Nitro	gen Fertiliz	zer Rate	Nitro	Nitrogen Fertilizer Rate		
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>	I		kg N ha <sup>-1</sup>		
	10	20	30	10	20	30	
MAP	38 <sup>abc</sup>	25 <sup>bcd</sup>	20 <sup>cd</sup>	38 <sup>abc</sup>	33 <sup>bc</sup>	33 <sup>bc</sup>	
MAP + Urea	$40^{ab}$	38 <sup>abc</sup>	35 <sup>abc</sup>	43 <sup>abc</sup>	$38^{abc}$	$38^{abc}$	
MAP + AS	$40^{ab}$	$30^{bcd}$	$20^{cd}$	50 <sup>ab</sup>	35 <sup>bc</sup>	35 <sup>bc</sup>	
MES-15	$40^{ab}$	35 <sup>abc</sup>	15 <sup>d</sup>	50 <sup>ab</sup>	$38^{abc}$	23°	
APS 1	$40^{ab}$	25 <sup>bcd</sup>	25 <sup>bcd</sup>	$40^{abc}$	33 <sup>bc</sup>	28°	
APS 2	33 <sup>abc</sup>	$40^{ab}$	35 <sup>abc</sup>	$38^{abc}$	50 <sup>ab</sup>	43 <sup>abc</sup>	
APS 3	53ª	38 <sup>abc</sup>	33 <sup>bcd</sup>	58 <sup>a</sup>	43 <sup>abc</sup>	38 <sup>abc</sup>	
Control	$27^{bcd}$			39 <sup>abc</sup>			
SEM	6.9289			7.1896			
F value	1.61			1.22			
Pr > F	0.0738			0.2668			

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table A. 4** Mean black bean plant emergence (percentage of seeds that emerged) at 5 and 10 days after seeding.

	5 Day Emergence Percentage <sup>†</sup>			10 Day E	10 Day Emergence Percentage <sup>†</sup>		
	Nitro	gen Fertiliz	zer Rate	Nitro	Nitrogen Fertilizer Rate		
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>	I		kg N ha <sup>-1</sup>		
	10	20	30	10	20	30	
MAP	73 <sup>abcd</sup>	60 <sup>bcdef</sup>	55 <sup>def</sup>	85 <sup>bcd</sup>	83 <sup>bcd</sup>	68 <sup>e</sup>	
MAP + Urea	75 <sup>abc</sup>	65 <sup>abcdef</sup>	$80^{a}$	88 <sup>abcd</sup>	$80^{\text{bcde}}$	100 <sup>a</sup>	
MAP + AS	80 <sup>a</sup>	$70^{abcde}$	53 <sup>ef</sup>	93 <sup>ab</sup>	83 <sup>bcd</sup>	75 <sup>de</sup>	
MES-15	70 <sup>abcde</sup>	78 <sup>ab</sup>	$60^{bcdef}$	93 <sup>ab</sup>	83 <sup>bcd</sup>	$78^{\rm cde}$	
APS 1	$70^{abcde}$	58 <sup>cdef</sup>	$50^{\rm f}$	83 <sup>bcd</sup>	68 <sup>e</sup>	68 <sup>e</sup>	
APS 2	$70^{abcde}$	68 <sup>abcdef</sup>	65 <sup>abcdef</sup>	78 <sup>cde</sup>	80 <sup>bcde</sup>	$78^{\rm cde}$	
APS 3	$80^{a}$	70 <sup>abcde</sup>	68 <sup>abcdef</sup>	90 <sup>abc</sup>	88 <sup>abcd</sup>	85 <sup>bcd</sup>	
Control	77 <sup>ab</sup>			82 <sup>bcd</sup>			
SEM	6.3209			4.544			
F value	2.04			3.31			
Pr > F	0.0143			<.0001			

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table A. 5** Mean lentil plant emergence (percentage of seeds that emerged) at 5 and 10 days after seeding.

	5 Day E	mergence I	Percentage <sup>†</sup>	10 Day E	10 Day Emergence Percentage <sup>†</sup>		
	Nitro	gen Fertiliz	zer Rate	Nitro	Nitrogen Fertilizer Rate		
Nutrient Source <sup>‡</sup>		kg N ha	1		kg N ha <sup>-1</sup>		
	10	20	30	10	20	30	
MAP	53 <sup>abc</sup>	20 <sup>def</sup>	23 <sup>def</sup>	60 <sup>bcde</sup>	48 <sup>efg</sup>	35 <sup>gh</sup>	
MAP + Urea	43 <sup>bcde</sup>	48 <sup>abcd</sup>	$40^{\text{bcde}}$	58 <sup>cde</sup>	68 <sup>bcd</sup>	58 <sup>cde</sup>	
MAP + AS	$33^{cdef}$	$30^{cdef}$	$8^{\rm f}$	53 <sup>defg</sup>	53 <sup>defg</sup>	25 <sup>h</sup>	
MES-15	35 <sup>bcde</sup>	48 <sup>abcd</sup>	$28^{\rm cdef}$	$60^{\text{bcde}}$	$60^{\text{bcde}}$	$38^{\text{fgh}}$	
APS 1	$40^{\text{bcde}}$	48 <sup>abcd</sup>	18 <sup>ef</sup>	75 <sup>abc</sup>	53 <sup>defg</sup>	$35^{\mathrm{gh}}$	
APS 2	38 <sup>bcde</sup>	$20^{\text{def}}$	35 <sup>bcdef</sup>	78 <sup>ab</sup>	53 <sup>def</sup>	$50^{\text{defg}}$	
APS 3	63 <sup>ab</sup>	38 <sup>bcde</sup>	43 <sup>bcde</sup>	65 <sup>bcde</sup>	55 <sup>def</sup>	58 <sup>cde</sup>	
Control	70 <sup>a</sup>			92ª			
SEM	9.7824			6.4782			
F value	2.55			6.06			
Pr > F	0.0019			< 0.0001			

<sup>&</sup>lt;sup>†</sup>Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table A. 6** Mean Desi chickpea plant emergence (percentage of seeds that emerged) at 5 and 10 days after seeding.

	5 Day Emergence Percentage <sup>†</sup>			10 Day I	Emergence Po	ercentage <sup>†</sup>	
	Nitro	gen Fertiliz	zer Rate	Nitro	Nitrogen Fertilizer Rate		
Nutrient Source <sup>‡</sup>		kg N ha <sup>-1</sup>	I		kg N ha <sup>-1</sup>		
	10	20	30	10	20	30	
MAP	40 <sup>ab</sup>	8 <sup>d</sup>	8 <sup>d</sup>	78 <sup>abcd</sup>	45 <sup>g</sup>	48 <sup>fg</sup>	
MAP + Urea	25 <sup>abcd</sup>	25 <sup>abcd</sup>	13 <sup>cd</sup>	$63^{\text{defg}}$	$55^{\rm efg}$	$48^{\mathrm{fg}}$	
MAP + AS	45ª	$38^{abc}$	$20^{abcd}$	$90^{a}$	$63^{\text{defg}}$	$70^{\text{bcde}}$	
MES-15	$38^{abc}$	23 <sup>abcd</sup>	20 <sup>abcd</sup>	$70^{\text{bcde}}$	78 <sup>abcd</sup>	55 <sup>efg</sup>	
APS 1	13 <sup>cd</sup>	15 <sup>bcd</sup>	5 <sup>d</sup>	83 <sup>abc</sup>	68 <sup>cde</sup>	$48^{fg}$	
APS 2	23 <sup>abcd</sup>	20 <sup>abcd</sup>	$30^{abcd}$	65 <sup>cdef</sup>	78 <sup>abcd</sup>	65 <sup>cdef</sup>	
APS 3	23 <sup>abcd</sup>	$20^{abcd}$	25 <sup>abcd</sup>	80 <sup>abcd</sup>	73 <sup>abcde</sup>	75 <sup>abcd</sup>	
Control	$37^{abc}$			87 <sup>ab</sup>			
SEM	8.8832			6.7409			
F value	1.6			4.1			
Pr > F	0.0747			<.0001			

<sup>&</sup>lt;sup>†</sup>Reported values are the means of four replicates (n=4) of each treatment. For a parameter, means followed by the same letter are not significantly different at  $P \le 0.05$ . The multi - treatment comparisons were made using Fisher's protected LSD method. SEM = standard error of mean.

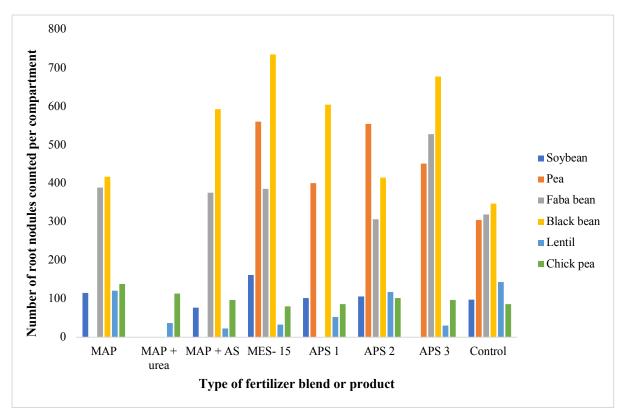
<sup>&</sup>lt;sup>‡</sup> A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium Sulfate; APS: Ammonium Phosphate Sulfate product; Control: 0 kg N ha<sup>-1</sup>.

**Table A. 7** Post-harvest soil properties (0-15, 15-30 and 30-60 cm depth) at the Central Butte site in fall 2018.

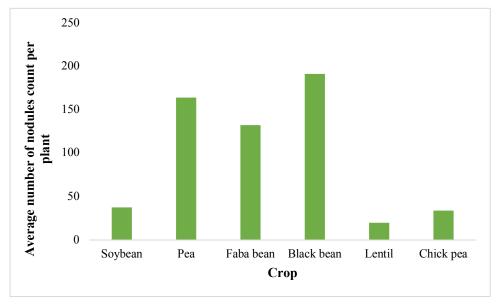
Nitrogen Fertilizer Rate		$NO_3^ N^{\dagger}$		MK- P <sup>‡</sup>
kg N ha <sup>-1</sup>	0-15 cm	15-30 cm	30-60 cm	0-15 cm
		n	ng kg <sup>-1</sup>	
		So	oybean	
0	1.52 <sup>b†</sup>	$0.96^{a}$	1.25 <sup>a</sup>	1.81 <sup>b</sup>
10	$1.80^{ab}$	$0.81^{a}$	1.62 <sup>a</sup>	$3.80^{ab}$
20	$2.03^{a}$	$0.89^{a}$	$2.77^{a}$	$5.06^{a}$
30	1.58 <sup>ab</sup>	$0.81^{a}$	$5.00^{a}$	$3.06^{ab}$
			Lentil	
0	$1.90^{a\dagger}$	$0.76^{a}$	$0.81^{a}$	$2.60^{b}$
10	$2.60^{a}$	$1.06^{a}$	$1.07^{a}$	1.68 <sup>b</sup>
20	2.57 <sup>a</sup>	1.01 <sup>a</sup>	1.92 <sup>a</sup>	1.92 <sup>b</sup>
30	2.55 <sup>a</sup>	$0.89^{a}$	1.54 <sup>a</sup>	5.51 <sup>a</sup>

<sup>†</sup> Reported values are the means of four replicates (n=4) of each treatment. within a column, means within four rates of N followed by the same letter are not significantly different from each other at  $P \le 0.05$ . The multi treatment comparisons were made using Fisher's protected LSD method.

<sup>&</sup>lt;sup>‡</sup> Modified Kelowna extractable available P.



**Figure A. 1** Number of root nodules counted per compartment for soybean, pea, faba bean, black bean, lentil, and chickpea harvested after 30 days at 10 kg N ha<sup>-1</sup> rate for each fertilizer blend or product. A description of the nutrient source is as follows: MAP: Mono-ammonium phosphate; AS: Ammonium sulfate; APS: Ammonium Phosphate Sulfate; Control: 0 kg N ha<sup>-1</sup>. Note: Blank represent no sample.



**Figure A. 2** Average nodule count per plant provides relative comparison of nodules counted on soybean, pea, faba bean, black bean, lentil, and chickpea roots harvested after 30 days at the 10 kg N ha<sup>-1</sup> rate. Note; Nodules of soybean, chickpea and lentil were up to 5mm in diameter, while pea and faba bean nodules were up to 2-3mm. Black bean had tiny nodules that were less than 0.5mm in diameter and showed no evidence of fixation.

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## ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
.1920076-3 PULSE STUDY SOIL							
Sampled By: CLIENT							
Matrix: SOIL							
Total Carbon, TOC and TIC in soil							
Total Organic Carbon Calculation							
Total Organic Carbon	1.76		0.050	%		08-MAY-17	
Miscellaneous Parameters							
Organic Matter	3.03		0.10	%		08-MAY-17	
pH and EC (1:2 Soil:Water Extraction)							
Conductivity (1:2)	0.138		0.050	dS m-1	05-MAY-17	05-MAY-17	R371583
pH (1:2 soil:water)	6.77		0.10	pН	05-MAY-17	05-MAY-17	R371583
Particle Size Analysis:Mini-Pipet Method							
% Sand (2.0mm - 0.05mm)	40.6		1.0	%	03-MAY-17	04-MAY-17	R371509
% Silt (0.05mm - 2um)	42.8		1.0	%	03-MAY-17	04-MAY-17	R371509
% Clay (<2um)	16.7		1.0	%	03-MAY-17	04-MAY-17	R371509
Texture	Loam				03-MAY-17	04-MAY-17	R371509
Available Micronutrients (Cu,Fe,Zn,Mn)							
Copper (Cu)	0.84		0.10	mg/kg	04-MAY-17	04-MAY-17	R371514
Iron (Fe)	43.5		2.0	mg/kg	04-MAY-17	04-MAY-17	R371514
Manganese (Mn)	23.3		0.050	mg/kg	04-MAY-17	04-MAY-17	R371514
Zinc (Zn)	1.25		0.20	mg/kg	04-MAY-17	04-MAY-17	R371514
Available N, P, K and S							
Available Nitrate-N Available Nitrate-N	7.6		1.0	mg/kg	04-MAY-17	04-MAY-17	R371550
Available Sulfate-S							
Available Sulfate-S	9.7		4.0	mg/kg	04-MAY-17	04-MAY-17	R371518
Plant Available Phosphorus and Potassium							
Available Phosphate-P	10.5		2.0	mg/kg	05-MAY-17	05-MAY-17	R371591
Available Potassium	600	DLHC	100	mg/kg	05-MAY-17	05-MAY-17	R371591

 $<sup>^{\</sup>star}$  Refer to Referenced Information for Qualifiers (if any) and Methodology.

**Figure A. 3** Soil test report on soil collected in spring of 2017 from field SE36-20-04-W3 near Central Butte for the purpose of seeding soybean, pea and faba bean. A composite sample was collected randomly from the 0-15 cm depth.

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## ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
L2050817-1 CB PULSE OCTOBER 2017 FINAL 0-15	 5CM DEPTH SE36-2	0-4-W3					
Sampled By: CLIENT							
Matrix: SOIL							
pH and EC (1:2 Soil:Water Extraction)							
Conductivity (1:2)	0.206		0.050	dS m-1	05-FEB-18	05-FEB-18	R3955342
pH (1:2 soil:water)	7.56		0.10	pН	05-FEB-18	05-FEB-18	R3955342
Particle Size Analysis:Mini-Pipet Method							
% Sand (2.0mm - 0.05mm)	38.7		1.0	%	02-FEB-18	05-FEB-18	R3955051
% Silt (0.05mm - 2um)	48.6		1.0	%	02-FEB-18	05-FEB-18	R3955051
% Clay (<2um)	12.7		1.0	%	02-FEB-18	05-FEB-18	R3955051
Texture	Silt loam / Loam		***		02-FEB-18	05-FEB-18	R3955051
Available Micronutrients (Cu,Fe,Zn,Mn)	Oilt iodiii / Lodiii						. 1000000
Copper (Cu)	0.68		0.10	mg/kg	05-FEB-18	05-FEB-18	R3956188
Iron (Fe)	3.6		2.0	mg/kg	05-FEB-18	05-FEB-18	R3956188
Manganese (Mn)	2.19		0.050	mg/kg	05-FEB-18	05-FEB-18	R3956188
Zinc (Zn)	1.11		0.030	mg/kg	05-FEB-18	05-FEB-18	R3956188
Available N, P, K and S	1.11		0.20	Illyrky	00-1 20-10	00-1 LD-10	1,0000100
Available N, P, K and S  Available Nitrate-N							
Available Nitrate-N Available Nitrate-N	7.9		1.0	mg/kg	05-FEB-18	05-FEB-18	R3955489
Available Sulfate-S	1.5		1.0	Illyrky	00-1 20-10	00-1 LD-10	1.0000+08
Available Sulfate-S Available Sulfate-S	9.1		4.0	mg/kg	05-FEB-18	05-FEB-18	R3955164
Plant Available Phosphorus and Potassium	3.1		7.0	I IIIg/Ng	55 1 25-18	55 1 LD-16	13000104
Available Phosphate-P	18.1		2.0	mg/kg	05-FEB-18	05-FEB-18	R3955467
Available Priospirate-P	650	DLHC	200	mg/kg	05-FEB-18	05-FEB-18	R3955467

**Figure A. 4** Soil test report on soil collected in fall of 2017 from field SE36-20-04-W3 near Central Butte for the purpose of seeding black bean. A composite sample was collected randomly from the 0-15 cm depth.

\* Refer to Referenced Information for Qualifiers (if any) and Methodology.

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## ALS ENVIRONMENTAL ANALYTICAL REPORT

Sample Details	s/Parameters	Result	Qualifier*	D.L.	Units	Extracted	Analyzed	Batch
2088787-1	HARSHINI-WILKIE S.E 0-15CM - S	E 36-20-04-W3						
Sampled By:	CLIENT on 30-APR-18 @ 12:00							
	_							
/latrix:	SOIL							
pH and EC	(1:2 Soil:Water Extraction)							
Conductivity (1:2)		0.205		0.050	dS m-1	10-MAY-18	10-MAY-18	R4039980
pH (1:2 soil:water)		7.95		0.10	pН	10-MAY-18	10-MAY-18	R4039980
Particle Size Analysis:Mini-Pipet Method					1			
% Sand (2.0mm - 0.05mm)		43.4		1.0	%	04-MAY-18	07-MAY-18	R4035308
% Silt (0.05		35.5		1.0	%	04-MAY-18	07-MAY-18	R4035308
% Clay (<2u		21.1		1.0	%	04-MAY-18	07-MAY-18	R4035308
Texture	aiii)			1.0	/*	04-MAY-18	07-MAY-18	1
		Loam				04-IVIA 1-10	07-IVIA 1-10	R4035308
	nic Carbon - Walkley Black					07.1411/.40	07.141./ 40	
Organic Car		0.70		0.40	%	07-MAY-18	07-MAY-18	R4037527
Organic Mat		1.24		0.70	%	07-MAY-18	07-MAY-18	R4037527
	licronutrients (Cu,Fe,Zn,Mn)							
Copper (Cu	)	0.72		0.10	mg/kg	09-MAY-18	09-MAY-18	R4039799
Iron (Fe)		2.5		2.0	mg/kg	09-MAY-18	09-MAY-18	R4039799
Manganese	(Mn)	1.41		0.050	mg/kg	09-MAY-18	09-MAY-18	R4039799
Zinc (Zn)		0.36		0.20	mg/kg	09-MAY-18	09-MAY-18	R4039799
vailable N, I	P. K and S				"			
Available N								
Available Ni		3.6		1.0	mg/kg	07-MAY-18	07-MAY-18	R4039298
Available S		3.0		1.0	mg/kg	07-101-10	07-101-10	114033230
Available St		<4.0		4.0	ma/ka	07-MAY-18	07-MAY-18	D4007007
		<b>&lt;4.0</b>		4.0	mg/kg	07-IVIA1-18	07-IVIA 1-18	R4037367
	able Phosphorus and Potassium	1				00 1411/ 15	00 MAN 45	D. 400000
Available Ph		4.8		2.0	mg/kg	08-MAY-18	08-MAY-18	R4038249
Available Po	Diassium	370		20	mg/kg	08-MAY-18	08-MAY-18	R4038249
		1						
		1						
		1						
		1						
		1						
		1						
		1						
		1						
		1						
		1						

 $<sup>^{\</sup>star}$  Refer to Referenced Information for Qualifiers (if any) and Methodology.

**Figure A. 5** Soil test report on soil collected in spring of 2018 from field SE36-20-04-W3 near Central Butte for the purpose of seeding lentil and chickpea. A composite sample was collected randomly from the 0-15 cm depth.