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RATE AND TIME OF NITROGEN FERTILIZER APPLICATION ON THE GROWTH, NITROGEN REMOBILIZATION AND YIELD OF SOYBEAN *(Glycine max* (L) Merrill*)*.

Thesis submitted to the Department of Crop and Soil Sciences, Faculty of Agriculture of the College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, in partial fulfilment of the requirements for the award of Master of Science Degree in Agronomy.

BY

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DECLARATION

I hereby certify that this thesis has not been submitted for a degree to any other University and it is entirely my own work and all references have been duly acknowledged.

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DEDICATION

This Thesis is dedicated to my family especially my parents and brothers who have always been there for me and kept me in their thoughts and prayers throughout my studies. I would also like to dedicate this work to the loving memory of my late sister who started the journey of education in this family that we all followed but never completed. Gone but not forgotten. May your soul rest in perfect peace.

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ABSTRACT

Two field experiments were conducted in 2012 at the Plantation Crops Section of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, to investigate the effect of N availability on the growth, nodulation, nitrogen fixation, N remobilization and grain yield of soybean.

The design used in both studies was a 3×4 factorial arranged in randomized complete block design. Each treatment was replicated three times. The factors studied were rate and time of N fertilizer application. The N rates were 0, 20, 40 and 60 kgN/ha and the time of application were early vegetative, late vegetative and early flowering phases.

`The soybean variety used was Anidaso an improved variety released by the CSIR-Crops Research Institute, with a maturity period of 110 days. The land was ploughed, harrowed and plots were laid out. Plot length was 3×3m. Each plot consisted of five rows in both seasons and planting was done at the beginning of the rains in May at a spacing of 50×5cm. All required cultural practices were observed.

The results indicated that soybean growth indices were not significantly (P>0.05) affected by rate and time of N application. Nodulation was also not significantly (P>0.05) affected by N rate and time of application. However, there was a positive correlation between nodule numbers and nodule dry weight, as well as between nitrogen fixation and grain yield in the minor season. Nitrogen fixation was significantly (P<0.05) affected by time of N application in the minor season. Harvest index was significantly affected by time of N application in the major season. Grain

yield was also significantly affected by time of N application in both seasons. Furthermore, the study demonstrated that N remobilization occurs in soybean during grain filling although rate and time of application used did not significantly (P>0.05) affect N remobilization.

It is recommended that in future work, isotope N should be applied and soil samples should be collected alongside with plant samples during the growth stages of the plant to assess how much of the N in the harvested plant comes from the fertilizer and how much was taken up by the plant from N stored in the soil.

Also, further work should be done in a more N-deficient soil to study this phenomenon of N remobilization.

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CHAPTER ONE

1.0 INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is an annual legume that belongs to the legume family Fabaceae. It is a self-pollinating legume with 2n = 40 chromosomes. With 40% protein, 20% oil and 30% carbohydrate, soybean plays a very significant role in world agriculture (Tefera, 2011).

Soybean has variety of uses such as human food, livestock feed vegetable oil, and many industrial products and is a major crop in several developing and developed countries (McKevith, 2005). Soybean oil can be used for the production of edible oils such as kitchen oil, salad oil and others through refining and deep processing. It is an important legume crop which provides high quality protein for many resource poor inhabitants in sub-Saharan Africa especially in the drier areas of West Africa (Tweneboah, 2000).

It is an economically important crop in the world today (Kulkarni *et al*., 2008), and could serve as an important substitution crop for large quantities of imported edible oil and soybean cake imported for poultry and pig ration formulations in Ghana (Tweneboah, 2000). Soybean is a nutritional powerhouse capable of solving protein–energy malnutrition problems in Ghana and has a great potential in the development of three key sectors of the economy: health, agriculture and industry (Plahar, 2006).

Soybean is one of the most important and extensively grown crops that accounts for 30% of the world's processed vegetable oil and has been employed as a source for bio-diesel fuels (Graham and Vance, 2003). Soybean, one of nature's most versatile crops, is increasingly becoming an important food and cash crop in the tropics due to its high protein and oil content and adaptability to various growing environments (Smith *et al*., 1995; Tukamuhabwa *et al*., 2001; FAO, 2004; McKevith, 2005). It can play a significant role in making up protein deficiency in human diet (Khalid, 2000).

Promotion of the nutritional and economic values of the crop is being done in Ghana by the Ministry of Food and Agriculture, and this has resulted in rapid expansion in production (Sarkodie-Addo *et al*., 2006). In West Africa, soybean has become a major source of high quality and cheap protein for the poor and rural households. It is used in processing soy meat, cakes, baby foods and 'dawadawa', a local seasoning product for stews and soups (Abbey *et al.*, 2001). It is also used to fortify various traditional foods such as gari, sauces, stew, soups, banku and kenkey to improve their nutritional levels (MoFA and CSIR, 2005).

Soybean is also beneficial in the management of *Striga hemonthica*, an endemic parasitic weed of cereal crops in the savanna zone of Ghana, which causes severe losses in crop yield of up to 70-100% of millet, sorghum and maize. Soybean is non-host plant to Striga, but it produces chemical substances that stimulate the germination of Striga seeds. Germinated seeds subsequently die off within a few days because they cannot attach their root system to that of the soybean plant to draw food substances and water (MoFA and CSIR, 2005).

In Ghana, soybean is cultivated mainly in the Northern, Upper West, Upper East, Central and Volta regions. Among these geographical regions, the largest production occurs in northern Ghana, which lies within the Guinea savannah and Sahel agro-ecological zones (Lawson *et al*., 2008).

Despite the numerous benefit of soybean, grain yield per unit area is low in Ghana with an average of 1.90 metric tons per hectare (MoFA, 2011). That of Africa is an average of 1.1 tons per hectare (IITA, 2009). The USA, Brazil, Argentina, China, India and Canada produce 83.2, 72.0, 48.0, 13.5, 11.0, and 4.2 million metric tons per hectare respectively (Soy Stats, 2012).

Reasons for low yield of soybean in Ghana include the use of non improved technologies by many farmers such as application of nutrients to replenish soil fertility, and sometimes failure to follow routine agronomic practices to ensure optimum yields.

In Ghana, small-scale soybean producers, unlike large-scale commercial producers, depend little on inputs such as fertilizer, herbicides, and pesticides for increasing production. These small scale producers take advantage of the fact that soybean, being a legume, supports bacteria that fix nitrogen to the soil. Moreover, inputs such as pesticides are very expensive (Mbanya, 2011).

Soybean has one of the highest N requirements among most leguminous crops. It is grown in Bangladesh with no fertilizer or with bio fertilizers. As a result, the average yield does not appear to be satisfactory (Morshed *et al*., 2008).

Nitrogen is an integral component of many compounds, including chlorophyll and enzymes, essential for plant growth processes. It is an essential component of amino acids and related proteins. Nitrogen is essential for carbohydrate use within plants and stimulates root growth and development as well as the uptake of other nutrients. This element encourages above ground vegetative growth and gives a deep green colour to the leaves (Brady, 1990).

Soybean, being a leguminous crop, is capable to fix atmospheric nitrogen through symbiosis. However, several studies have shown that the symbiotic N-fixation is not able to meet high N-requirement of this crop particularly under the N-deficient conditions (Morshed *et al*., 2008). In legume crops like soybean, nitrogen supplied from nodule and fertilizer is the most critical factor for maintaining the growth rate of photosynthetic organs and generating flower buds at the vegetative growth stage (Nakamura *et al*., 2010). In turn, the efficiency of nitrogen translocation systems from vegetative to reproductive organs affects the yield at the pod filling stage.

The depletion of N from vegetative tissue accelerates leaf senescence and thus lowers the photosynthetic capacity of the canopy which leads to yield reduction by shortening the seed filling period. The N in the seed can come from either N accumulated prior to the seed filling period (SFP) and remobilized to the seed or N that is accumulated during the SFP (Kumudini *et al*., 2002).

Nitrogen fertilizer applied during soybean reproductive stage (R1 to R5) might increase the capacity and duration of the inorganic N utilization period while maintaining N_2 fixation (Barker and Sawyer, 2005). Supplying N to the soybean plant during peak seed demand may supplement existing N resources, thus preventing premature senescence and increasing seed yield (Freeborn *et al*., 2001).

Nitrogen deficiency at pod filling stage of soybean leads to N remobilization from the leaves which could affect seed yield due to early leaf fall and cessation of photosynthesis. In this study, it is being hypothesized that soil N availability at grain filling period in soybean will enhance leaf area duration (LAD) and consequently increased seed yield.

The objectives of this study were to determine the:

i. Effect of different rates of nitrogen fertilizer on soybean growth and yield

ii. Best timing of nitrogen fertilizer application for growth and yield of soybean.

iii. Effect of nitrogen availability at pod filling on N remobilization.

iv. Effect of reduced N remobilization on soybean grain yield.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ORIGIN AND DISTRIBUTION

Soybean originated from Eastern Asia, probably in north and central China (Laswai *et al*., 2005a). Soybean is strongly believed to have originated from the orient (Myaka *et al*., 2005). Soybean was first grown in Eastern Asia about 5,000 years ago (BIDCO, 2005). Cultivated varieties were introduced to Korea and later to Japan 2,000 years ago (Laswai *et al*., 2005b). In East and South East Asia to date, it is an important component of traditional diets of the regions (www.gardening.about.com).

Soybean production developed rapidly in the USA in the 1950's, and the USA is now the largest soybean-producing country in the world, followed by Brazil, Argentina and China (Qiu and Chang, 2010). Soybean cultivation reached Africa in the late 1980's, although little is known of the countries to which it was first introduced (Shurtleff and Aoyagi, 2007b). The authors stated that, it is possible, perhaps likely, that soybeans were cultivated at an early date on the eastern coast of Africa, since that region had long traded with the Chinese. The next record of cultivation of soybeans in Africa dates from 1903, when they were grown in South Africa at Cedara in Natal and in the Transvaal. In about 1907 soybeans were introduced to Mauritius and Tanzania (Shurtleff and Aoyagi, 2007b). Soybean was introduced in Ghana in 1910 and used by local farmers in the Northern sector in their traditional foods. Late 1960s and early 1970s, soybean research was intensified at the CSIR - Crops Research Institute and the UG Agric Research Station. In the late 80's to the 90's, Public/Private partnership approach was adopted to launch a massive campaign on soybean production and utilization under the Ministry of Food and Agriculture (Plahar, 2006).

2.2 BOTANY

Soybean is an annual legume that belongs to the family Fabaceae (Flaskerud, 2003). It is predominantly a tropical crop and is self-pollinating legume. The earliest known cultivation of the crop in Africa was in 1896, in Algeria (Shurtleff and Aoyagi, 2010). It includes some 500 genera and more than 12,000 species belonging to the subfamily papilionideae (Shurtleff and Aoyagi, 2007a)

The genus *Glycine* consist of two subgenera, *Glycine* consisting of seven perennial wild species confined to South-eastern Asia; and Soja, comprising the domesticated and commercially important soybean, *Glycine max* and its wild ancestor, *Glycine* soja. Both are annuals and grow in the tropical, subtropical and temperate climates. They have 40 chromosomes (2n=2x=40) and are self fertile species with less than 1% out crossing (Norman *et al*., 1995).

2.3 MORPHOLOGICAL DESCRIPTION

Soybean is a hairy annual with an extensive taproot system, most of it in the top 15cm of the soil. The taproot may grow as deep as 2m and adventitious roots grow from the hypocotyls. The modern cultivars of soybean are erect, bushy, 20-180cm tall, usually with a few primary branches and no secondary branches. Exceptionally, prostrate and freely branching forms are also found (Chaturvedi *et al*., 2011).

There are three types of growth habit found amongst soybean cultivars: determinate, semi-determinate and indeterminate. Determinate growth is characterized by the cessation of vegetative activity of the terminal bud when it becomes an inflorescence at both axillary and terminal racemes. Indeterminate genotypes continue vegetative activity throughout the flowering period. Semi- determinate types have indeterminate stems that terminate vegetative growth abruptly after the flowering period (OECD, 2000). The flowers are white or pale purple, very typical of Papilionadeae and are normally self pollinated but around 1% of cross pollination aided by insects does occur (Chaturvedi *et al*., 2011).

The stem, leaves and pods are covered with fine brown or gray hairs. The leaves are trifoliate, having three to four leaflets per leaf. The fruit is a hairy pod that grows in clusters of three to five, each of which is five to eight centimetres long and usually contains two to four seeds (Rienke and Joke, 2005). Soybean seeds vary greatly in shape, size and colour though these are most often round and yellowish, brown or black with epigeal germination (Chaturvedi *et al*., 2011).

Gary and Dale (1997) have described soybean growth and development in two main stages: the vegetative stage and the reproductive stage. The vegetative stage starts with the emergence of seedlings, unfolding of unifoliate leaves, through to fully develop trifoliate leaves, node formation on the main stem, nodulation and the formation of branches. Whiles the reproductive stage begins with flower bud formation, through full bloom flowering, pod formation, pod filling to full maturity.

2.4 CLIMATIC AND SOIL REQUIREMENT

2.4.1 Soil

Soybean prefers fertile, well-drained loamy soils. Such soils are also suitable for a range of other crops. Soybean does not tolerate drought because the relatively shallow root system limits water absorption during dry periods (Belfield *et al*., 2011). Therefore, soybean is not well suited to sandy soils or soils with low water storage capacity, such as gravelly or shallow soils. Good soil moisture is needed at sowing to increase the chance of the seed germination and plant establishment on all soil types. Soybean can be grown on a wide range of soils with pH ranging from 4.5 to 8.5, however, the crop prefers slightly acid soil; the optimal soil pH is in the range of 5.5 to 6.5 (Belfield *et al*., 2011). Maintaining soil pH between 5.5 and 7.0 enhances the availability of nutrients such as nitrogen and phosphorus, microbial breakdown of crop residues and symbiotic nitrogen fixation (Ferguson *et al*., 2006).

2.4.2 Temperature and photoperiod

Soybean is a legume species that grows well in the tropical, subtropical and temperate climates (IITA, 2009). Soybean is a short-day plant. This means that flowering responds to shortening days. Each variety has a different critical day length that must be reached before the plant will start to flower (Belfield *et al*., 2011). However, some have been adapted to the hot, humid, tropical climate. In the tropics, the growth duration of adapted genotypes is commonly 90-110 days, and up to 140 days for the late maturing ones (Osafo, 1997). The optimal temperature for soybean growth is 20-30 ^{0}C. Temperatures of 35 ^{0}C and above are considered to limit growth. The optimal soil temperature for germination and early seedling growth is 25-30 ^{0}C (Belfield *et al*., 2011).

2.4.3 Moisture requirement

Soybean can grow to maturity with as little as 180 mm of in-crop rain, but would reduce yield by 40-60% compared with optimal conditions. The ideal rainfall for soybean is 500-1000 mm. Depending on soil type and stored soil moisture; crop failure would be expected if less than 180 mm of rain were received during crop growth (Belfield *et al*., 2011).

Rienke and Joke, (2005) and Addo-Quaye *et al*., (1993) have described two periods as being critical for soybean moisture requirement. From sowing to germination, flowering and pod filling periods. During germination, the soil needs to be between 50% and 85% saturated with water, as the seed absorbs 50% of its weight in water before it can germinate. The amount of water the crop needs increases and picks up at the vegetative stage, and then decreases to reproductive maturity.

Because of its long root system, the soybean can tolerate dry conditions prior to flowering but adequate moisture becomes essential once the buds are formed and until the pods have filled. Soybeans are susceptible to drought during the flowering and pod formation stages. They can also do well in warm, dry areas under irrigation. Excessive rainfall prior to and during flowering can result in luxuriant growth and increased lodging. Waterlogged conditions have a negative effect on the crop yield. Maximum seed yield is possible where water in the root zone is kept above 50% plant-available (Soybean production guidelines, 2010).

2.5 NODULATION AND NITROGEN FIXATION

The nodule establishment occurs due to the sequence of multiple interactions between the bacteria and the leguminous plant (Hopkins and Hurner, 2004). The root nodule development can be divided into three main stages as pre infection, nodule initiation and differentiation. There are three different signal types, exchange between root and bacteria in the nodule formation. Legume seed coat contains different types of flavonoids in large quantities. These flavonoids act as chemo-attractant for the corresponding root nodule forming bacteria and induce rhizobium node gene (Mylona *et al*., 1995). With the presence of flavonoids of host plant, Rhizobia colonized and multiplied in the rhizosphere. The colonized bacteria in the rhizosphere begin to synthesized node factor, which are derivatives of ‘‘chitin’’. Node factor induces morphological changes (increased root hair production and development of shorter thicker roots) in the host root (Hopkins and Hurner, 2004). The root nodule development is induced by lipochitin – oligosaccharide signals secreted by the bacterial microsymbiont. Infection occurs via infection threads that pass through the root hairs in to the cortex and release bacteriods (Karas *et al*., 2005). In the host cells, bacteria differentiate into bacteriod, which is surrounded by a peribacteroid membrane (Hopkins and Hurner, 2004). The nodule primodia formation is initiated within the root cortex (Geurts *et al*., 2005).

Soybean is a legume and normally provides itself nitrogen through a symbiotic relationship with nitrogen fixing bacteria of the species, *Bradyrhizobium japonicum* (Sarkodie-Addo *et al*., 2006; Nastasija *et al*., 2008). *Rhizobium* is a bacterium, which is hosted by the root system of certain legume plants. The legume plant supplies the carbohydrate for bacterial growth while the bacteria fix atmospheric N_2 into NH_4^+, to be converted into plant useable amino acids (Russelle, 2008). Symbiotic association is a highly specified relationship between the host plant and the bacteria. *Rhizobium*-legume symbiosis involves the interaction between the plant and the bacteria leading to initiation and development of the root nodules (Trichine *et al*., 2006). N fixing symbiotic association is a mutualistic interaction between the plants that belongs to the family leguminosae and the soil bacteria genera *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium* (Broughton *et al*., 2000). These organisms live in nodules as N fixing bacteriods. A single rhizobial cell that infects a root hair can increase progeny by 10^{10} within few weeks. These organisms adapted to different types of environmental conditions since the genes are not necessary at the free- living stage and these genes become ‘‘turned on’’ only after interacting with the host plant (Russelle, 2008). Soybean forms N fixing symbiosis with either *Bradyrhizobium japonicum* or *Sinorrhizobium* species (Keyser and Li, 1992). Seed inoculation with *rhizobium* can increase total N and grain yield in early maturing soybean cultivars.

The total N accumulation and N fixation are low during the early growth stage and then they increase rapidly at later stage. N fixation reaches maximum at R3/R4 stage and then drops (Sanginga *et al*., 1997). Rapid N fixation during the grain filling stage enhances the net photosynthesis rate and respiration leading to higher amount of usable N in soybean plant. N fixation is energy dependent process. *Rhizobium* generates energy required for N fixation through oxidation of host plant photosynthates. At R5 stage, high demand for photosynthates from pods and nodules facilitate the initial rate of energyzation of the thylakoid membrane and stimulate the photosynthesis (Mury *et al*., 1993). Rapid N fixation during the pod filling stage increased the seed yield and protein content (Imsande, 1992).

The process of N fixation is strongly related to the physiological states of the host plant. The severe environmental conditions such as salinity, unfavourable soil pH, nutrient deficiency, mineral toxicity, extreme temperature conditions, low or extremely high levels of soil moisture, inadequate photosynthates and disease conditions can affect the plant growth and development. As a result, the persistent rhizobium strains will not be able to perform root infection and N fixation in their full capacity (Zahran, 1999).

Under salinity conditions, the accumulation of Na^+ reduces the plant growth, nodule formation and symbiotic N fixation capacity (Soussi *et al*., 1998; Kouas *et al*., 2010).

Extreme soil pH can reduce the rhizobial colonization in the legume rhizosphere. N fixation can be inhibited by low soil pH (van Jaarsveld, 2002). The characters of highly acidic soils ($pH < 4$) are low level of phosphorous, calcium and molybdenum along with aluminium and manganese toxicity, which affects both plant and rhizobia. As a result of low soil pH conditions, nodulation and N fixation is severely affected than plant growth. Highly alkaline ($pH > 8$) soils tend to be high in sodium (Na^+), chloride (Cl^-), bicarbonate (HCO_3^-) and borate (BO_3^-) which reduces the N fixation (Bordeleau and Prevost, 1994).

The plant nitrogenase activity reduces dramatically as a result of formation of ineffective nodules at high temperature (40^0C) (Hungria and Franco, 1993).

Moisture stress can adversely affect the nodule functions. The drought conditions can reduce nodule weight and nitrogenase activity.

After exposing to the moisture stress for ten days, the nodule cell wall starts to degrade resulting in senescence of bacteriods (Ramos *et al*., 2003).

Symbiotic N fixation varies according to the carbon allocation to the nodules in relation to endogenous factors, current photosynthesis, crop growth rate and other competing sinks for carbon (Voisin *et al*., 2003).

2.6 NITROGEN NUTRITION, RATE, TIME AND REMOBILIZATION ON SOYBEAN YIELD

2.6.1 N nutrition and plant n uptake

The N consumption of plants varies from one plant species to another. Within the species, the N amount varies depending on the genotype and the environmental factors. There is a considerable variation among the plant parts (grains, stems, roots, leaves etc.) in terms of relative amounts of N content. In general, most of the N is stored in the harvesting parts (seeds in most grain crops) than in stover, vines, stems, roots or straw. N acquisition can vary depending on the soil N status, agronomic practices and the climate (Stevenson and Cole, 1999).

Plants generally take up nutrients from the soil solution through the root system. The soil mineral uptake by plants becomes effective process due to the larger surface area of roots and their ability to absorb ions at low concentrations (Taiz and Zeiger, 2006).

The soybean plant has protein-rich seeds and requires high levels of N to attain greater yield (Sinclair and DeWitt, 1975). It is reported that there is a good correlation between the total amount of N accumulated by the plant and the seed yield (Tewari *et al*., 2004). At the vegetative stage, plants are capable of absorbing mineral soil N rapidly and the leaf tissue has high NO_3^- content. As the plant reaches the reproductive stage (flowering), there is a rapid reduction in tissue NO_3^- content. There is a gradual decline of tissue NO_3^- content from flowering to early pod filling stage (Thibodeau and Jaworski, 1975). At the pod filling stage, developing ovules act as a competing sink for photosynthate resulting in a rapid decline in N fixation at mid pod filling stage (Thibodeau and Jaworski, 1975).

2.6.2 Rate and time of n fertilizer application on soybean yield

Application of starter N at early vegetative growth stage can increase the pod yield and crop biomass by 44% and 16%, respectively. The proportion of the plant N derived from the N fixation is highest when N is applied at the pod filling stage where the plant N demand is high (Yinbo *et al*., 1997). It has been reported that the use of urea $(NH_2)_2CO$) or ammonium nitrate (NH_4NO_3) as the starter N fertilizer at rates of 8, 16 and 24kg ha^{-1} promoted the early plant biomass and plant N compared to no N treatment. Further, the soybean grain yield increased by 16% at the N rate of 16kg ha^{-1} over control treatment, with no improvement either in seed protein or oil content (Osborne and Riedell, 2006).

Schmitt *et al* (2001) conducted a study to identify the effects of application time, application method and the source of N on soybean plant growth, grain yield, protein and oil content at 12 sites. The study concluded that in-season application of N fertilizer did not increase the soybean grain yield or the oil content. However, there was a combined effect of all above factors on increasing soybean protein content at a rate of 0.4g kg^{-1} (Schmitt *et al*., 2001). The soybean grain yield, protein, oil and fibre content did not increase with the fertilizer N rates of 45 and 90kg ha^{-1} (urea/slow releasing N) application at early reproductive stage (Barker and Sawyer, 2005).

The early application (V2/R1) of N as top dressing at a rate of 25kg ha^{-1} promoted the soybean plant total biomass and the N accumulation during the seed filling stage (R5) which boosted the grain yield (Gan *et al*., 2003). N top dressing application at R1 and R3 stages drastically reduced the soybean nodulation, whereas at V1 stage there was an optimistic effect which increased the soybean nodulation (Gan *et al*., 2003).

The broadcasting of fertilizer N as urea (50 and 100 N kg ha^{-1}) at the pod formation (R3) and the seed filling stage (R5) increased the available N at the top 30 cm of soil compared to the unfertilized plots. However, increase in soil NO_3^- availability during the seed filling stage had no relevant effect on leaf senescence and the seed growth (Gutierrez-Boem *et al*., 2004). The response of the soybean towards the fertilizer N was not temporally stable (Lambert *et al*., 2006).

2.6.3 N fertilization and remobilization on yield

Nitrogen is an integral component of many compounds including chlorophyll and enzymes, essential for plant growth processes. It is an essential component of amino acids and related proteins. Nitrogen is the most limiting nutrient for production in most agricultural systems, due to the large amounts harvested with the crops and because it can be lost easily through gaseous losses, leaching, runoff or erosion (Smaling *et al*., 1999).

Nitrogen requirement for soybean are typically met by a combination of soil- derived nitrogen and nitrogen provided through the process of symbiotic fixation from the *rhizobia* in root nodules. The relative nitrogen supply from these two sources can change widely depending on soil nitrogen supply and conditions for nodule development (Varco, 1999; Gan *et al*., 2003). Field studies measuring soybean response to applied N have been conducted by several researchers. N fixation alone cannot meet the N requirement for maximizing soybean yield (Oz, 2008).

According to Gan *et al*., (2003), best timing for N top-dressing during reproduction is at the flowering stage, which increased seed yield by 19 and 21 %, compared to the treatment without N top-dressing. Nitrogen increases yield by influencing a variety of agronomic and quality parameters. Grain yield response of soybean to nitrogen application may be because nitrogen plays an important role in the synthesis of chlorophyll and amino acids (Oz, 2008). Nitrogen influenced grain yield through source-sink relationships resulting in higher production of photosynthates and their increased translocation to reproductive parts (Tripathi *et al*., 1992).

Hanway and Weber (1971) reported that about half the N in mature soybean seed has been translocated from other parts of the plant, the remainder being derived from soil and nodules. Thus, soil mineral N supply may be a critical factor for soybean during the reproductive stages (George and Singleton, 1992). This high N demand during the ripening stage might be met by applying supplemental fertilizer N after flowering. To maximize yield and N_2 fixation by soybean, a better understanding is required of the interactions between mineral N supply, N requirements and N uptake at the reproductive stage (Gan *et al*., 2003)

Soybean requires a large amount of N for seed production and hence its yield may be sensitive to N fertilization after flowering (Kinugasa *et al*., 2011). However, the active period of N_2 fixation is limited during nodule development because nodule senescence occurs rapidly after flowering and during seed maturation. Nitrogen fertilizer applied during soybean reproductive stage (R1 and R5) might increase the capacity and duration of the inorganic N to the utilization period while maintaining N_2 fixation (Barker and Sawyer, 2005). Supplying N to the soybean plant during peak seed demand may supplement existing N resources, thus preventing premature senescence and increasing seed yield (Freeborn *et al*., 2001). Gan *et al*., (2003) reported that application of N at 50kg/ha at either V2 or R1 stages, significantly increased N accumulation and yield. Also, Barker and Sawyer (2005) showed that N application increased N concentration in R4 (full pod) soybean plants.

High yields of soybean are associated with greater nitrogen (N) remobilization from vegetative tissues to the seed. Work by Shibles and Sundberg (1998) indicated that since grain yield and N content in leaves during the seed-filling period (SFP) are closely related, the amount of N stored in the vegetative parts at the seed-filling stage is clearly important for development of a large seed yield. Some of the N in the mature seed comes from redistribution of N from vegetative plant parts during seed filling with estimates of this contribution varying from 30 to 100% (Egli *et al.*, 1983). Although N fertilization of soybean is not a common practice, there is speculation that the ability of soybean to fix atmospheric N is not always adequate for maximum yield (Wesley *et al.*, 1998).

2.7 USES OF SOYBEAN

According to Dugje *et al*. (2009), soybean is more protein-rich than any of the common vegetable or legume food sources in Africa. It has an average protein content of 40%. The seeds also contain about 20% oil on a dry matter basis, and this is 85% unsaturated and cholesterol-free.

Soybean fixes atmospheric nitrogen, therefore enhances sustainable crop production and improves soil fertility especially when in rotation with cereals. Soybean plants are good soil cover that reduces soil erosion and suppresses weed growth. It also breaks pest and disease cycles when grown in rotation with cereals. Soybean reduces

Striga population when it precedes cereals, causing suicidal germination of *Striga* seeds (Adu-Dapaah *et al*., 2004).

Industrial uses of soybeans are many. When crushed, soybean is separated into two major components: oil (including lecithin) and protein (meal or flour) and these products are widely used in the industry. They are used as raw materials for manufacturing printers' ink, cosmetics, plastics, paint, glue, soap, shampoo and many more. Detergents and gasoline are also made from soybeans (Adu-Dapaah *et al.*, 2004). Other industrial uses of soybeans include paints, in dressings or water – proofing's for textiles, and a sizing for paper (Shurtleff and Aoyagi, 2009).

Soybean can be cooked and eaten as a vegetable as well as processed into soy oil, soy milk, soy yogurt, soy flour, tofu and tempeh (Rienke and Joke, 2005; MoFA and CSIR, 2005). It is the most dominant source of protein in livestock feeds throughout the world. It can provide 440 to 480kg crude protein per ton, providing high quality and highly digestible protein (FAO, 2006). Soybean can contribute to poverty reduction and the eradication of malnutrition among children and expecting mothers (Myaka *et al*., 2005).

Of all legumes, soybeans have the highest concentration of protein. Most other beans contain 20% protein by volume, while soybeans have 40% (Greenberg and Hartung, 1998). Soybean also contains more protein than beef and fish that contain about 18% in protein content. Besides, soybean products are cholesterol free and high in calcium, phosphorus, and fiber (Greenberg and Hartung, 1998). The non-cholesterol content of soybean oil explains its high demand for health reasons.

Soybean also has one of the lowest levels of saturated fat among vegetable oils (BIDCO, 2005).

The cake obtained from soybean after oil extraction is also an important source of protein feed for livestock such as poultry, pig and fish. The expansion of soybean production has led to significant growth of the poultry, pig and fish farming (Abbey *et al*, 2001; Ngeze, 1993; MoFA and CSIR, 2005). The haulms, after extraction of seed, also provide good feed for sheep and goats (Dugje *et al*., 2009).

Soybean can contribute to household cash income, create employment, improve human and livestock health, and improve standard of living and quality of livestock products (Malema, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE

The field experiment was conducted at the Plantation Crops Section of the Department of Crop and Soil Sciences of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, between May and September, during the major season and repeated during the minor season between September and December, 2012. Kumasi is situated in the semi-deciduous forest vegetation zone of Ghana. It is about 356m above sea level on latitude 06° 43'N and longitude 01° 33'W (Asiamah, 1998).

3.2 CLIMATE

The rainfall is bimodal with an average annual rainfall of 1422.4mm. The major rainy season extends from mid-March to July, with a short dry period in August, while the minor rainy season extends from September to November. The main dry season also extends from late November to mid-March. The average relative humidity for 2012 varied from 84% (09 hours GMT) during the major and minor rainy seasons to 58% (15 hours GMT) during the dry season (Metrological Department, KNUST, 2012).

Annual average maximum and minimum temperatures for 2012 were 31.6^0C and 22.1^0C respectively. The mean daily maximum and minimum temperatures during the period of the experiment were 29.15^0C and 21.4^0C, and 31.6^0C and 22.3^0C for the major and minor season, respectively.

Total rainfall recorded during the experiment were 501.6mm and 352.9mm (major and minor season) and relative humidity varied from 79% (09 hours GMT) to 53% (15 hours GMT) during the major season and 84% (09 hours GMT) to 60% (15 hours GMT) during the minor season (Metrological Department, KNUST, 2012).

3.3 SOIL CHARACTERISTICS

The soil at the experimental site is well drained, sandy loam overlying reddish-brown and gravelly light clay. It belongs to the Kumasi series, Ferric Acrisol developed over deeply weathered granite rocks (Asiamah, 1998).

3.4 PHYSICAL AND CHEMICAL SOIL ANALYSIS

Soil samples were taken from the experimental site to a depth of 0 – 15 and 15 – 30cm. These samples were taken to the laboratory to determine their physical and chemical properties. The samples were dried and sieved using a 2mm mesh sieve and the following properties were determined.

3.4.1 Organic Carbon

The Walkley-Black wet combustion procedure (Nelson and Sommers, 1982) was used to determine organic carbon.

3.4.2 Organic Matter

Percent organic carbon was multiplied by 1.724 (The Van Bemmelen factor) to get percent organic matter.

3.4.3 Soil pH

This was measured in 1:2.5 soils to water suspension by the use of a glass Electrocalomel electrode (Mclean, 1962) pH meter.

3.4.4 Total Nitrogen

The Macro Kjeldahl method described by Bremmer and Mulvaney (1982) was used. A 10g soil sample (<2mm in size) was digested with a mixture of 100g potassium sulphate, 10g copper sulphate and 1g selenium with 30mls of concentrated sulphuric acid. This was followed by distillation with 10ml boric acid (4%) and 4 drops of indicator and 15mls of 40% NaOH. It was then titrated with Ammonium sulphate solution. Based on the relation that 14g of nitrogen is contained in one equivalent weight of NH_3, the percentage of nitrogen in the soil was calculated using the formula:-

$$\text{Total N in the sample} = \frac{14\,(A-B) \times N \times 100}{1000 \times W}$$

Where,

A = Volume of standard acid used in the titration.

B = Volume of standard acid used in blank titration.

N = Normality of the standard acid.

W = Weight of soil sample used.

3.4.5 Potassium

The flame photometer method was used to determine the amount of potassium with ammonium acetate as the extractant.

3.4.6 Available Phosphorous

The Bray-1 test method was used for the determination of phosphorus with dilute acid fluoride as the extractant (Jackson, 1958).

3.4.7 Exchangeable Bases (Ca, Mg, K, Na)

The exchangeable base cations were extracted using ammonium acetate at pH of 7.0. Calcium and Magnesium were determined using the EDTA titration method (Moss, 1961) while Potassium and Sodium were determined by the flame photometer.

3.5 LAND PREPARATION

The land was previously cropped to Barbara groundnut. The experimental site was cleared by slashing using cutlass, ploughed and harrowed with a tractor. It was then levelled and the plots were laid out using measuring tape, garden line and pegs.

3.6 VARIETY USED FOR THE EXPERIMENT

The Anidaso variety used was obtained from the Crop Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR) at Fumesua, Kumasi. Anidaso is a medium maturity genotype with small seed size, rounded and yellow seed colour, with mean 100 seed dry weight of 13.0g and matures in 110 days. It is resistant to pod shattering, fairly good cereal *Striga* management and promiscuous nodulator with the native *rhizobia.* Grain yield is 1.2 -1.8 tons per hectare. It was released in 1992 by CRI (MoFA and CSIR, 2005).

3.7 EXPERIMENTAL DESIGN, LAYOUT AND TREATMENTS

The experimental design was a 3 x 4 factorial arranged in a randomized complete block, with four replications (blocks). The two factors were time of fertilizer N application and rate of fertilizer N application. Time of nitrogen fertilizer application was Early vegetative phase, Late vegetative phase and Early flowering phase .

The nitrogen rates comprised control (0), 20, 40 and 60 kg N/ha. The planting distance was 50cm x 5cm (inter and intra row spacing), corresponding to a population density of 400,000 plants per hectare.

Each block consisted of 12 plots, each of which measured 3m x 3m, given a total of 48 plots and a total land area of 622.5m^2, with one metre between blocks.

The experiment was carried out during the major season (May to September, 2012) and minor season (September to December, 2012). Seeds were planted on each plot, on the 31st May and 21st September, 2012 for the major and minor season experiments, respectively. Seedling emergence occurred five to ten days after sowing.

3.8.0 CULTURAL PRACTICES

3.8.1 Thinning

Thinning was done to approximately 5cm between plants in a row, 20 days after sowing, when the soil was moist and seedlings well established. This left a total of 60 plants per row.

3.8.2 Weeding

Weeding was done manually by hand using a hoe, on the 2nd and 6th week after sowing to control weeds. Each weeding operation was completed on the same day for all the blocks.

3.8.3 Irrigation

Supplementary irrigation was carried out during the pod filling stage as there was inadequate rainfall. Five watering cans were used to water each plot (five rows) and each watering can contains 12L of water. This was done twice every week.

3.9 PEST MANAGEMENT

There were incidences of pod-sucking bugs from flowering to end of pod filling. Spraying started at 50% flowering with a single spray of Cypermetrin + Dimethoate 10 EC at the rate of 100ml in 15 L of water using knapsack sprayer, at a recommended 14 days interval to control insects till the end of pod filling.

3.10 DATA COLLECTION

3.10.1 Vegetative Growth

The following data were collected and have been described below.

3.10.1.1 Plant Height

Plant height was measured from the ground level to the highest tip of the stem for the five randomly tagged plants in the three middle rows. This was done using a meter rule at the various sampling periods. The average plant height was calculated for each treatment.

3.10.1.2 Number of Leaves

This was taken during the sampling periods and the five tagged plants from each plot were counted and the average recorded.

3.10.1.3 Number of Branches

Branches of the five tagged plants from each plot were counted at every sampling period and the average recorded.

3.10.1.4 Nodule Count and Effectiveness

Five random plants from each plot were taken at mid flowering to assess nodulation. The plants were dug out and all nodules including detached ones were collected and kept in the labelled polytene bags and sent to the laboratory where they were washed and counted. The nodules were then cut opened using a knife and a hand lens to determine their effectiveness. Nodules with pink or reddish colour were declared effective. The percentage effective nodules were then calculated.

3.10.1.5 Nodule Dry Weight

All nodules from each treatment were oven dried to constant weight at 80^{0}C for 48 hours. These were weighed and the average weight calculated.

3.10.2 Reproductive and Yield Data

3.10.2.1 Plant Sampling

Plant sampling was done at the reproductive stage from full seed (R6), physiological maturity (R7) and harvest maturity (R8) (Fehr *et al*., 1971) at two weeks interval. At each sampling, five plants from the border rows of each plot were randomly selected and separated into leaves, stems and seeds. These were oven dried at 80^0C for 48 hours. N concentration in each plant part was determined by the Kjeldahl method. N remobilization was determined from N concentration across sampling days.

Total N accumulated was calculated as the sum of the N content of all plant parts and Remobilized N was calculated as the difference in N content of vegetative tissue N (e.g. Vegetative tissue N at R6 - vegetative tissue N at R8).

3.10.2.2 Total Nitrogen Difference (TND)

This method is used when only the ability to analyse total N is available (Bell and Nutman, 1972). Hence this classical difference method relies solely on Kjeldahl nitrogen determination and is calculated as follows:

a) N_2 fixed = Total N (fs) – Total N (nfs)

b) % Ndfa (by TND) = $\frac{\text{Total N (fs) – Total N (nfs)}}{\text{Total N (fs)}}$ x 100

Where,

fs = fixing crop

nfs = non fixing crop

%Ndfa = proportion of N derived from atmosphere

3.10.2.3 Yield

At harvest, when about 85% of pods had turned brown (Dugje *at al*., 2009), three middle rows of each plot were harvested for yield analysis. From this harvested lots, five plants each were sampled for number of pods, number of seeds per pod, 100 seed weight and harvest index. After which, the used samples were returned to the harvest lots.

3.10.2.4 Number Of Pods Per Plant

For pod number, five plants were taken from each plot and all the pods plucked, manually counted and the average pod number calculated.

3.10.2.5 Number Of Seeds Per Pod

The number of seeds per pod was also determined by taking five plants from each plot. When all pods were plucked and counted, pods were shelled and seeds counted. The average was then calculated.

3.10.2.6 100 Seed Weight

The 100 seed weight was determined by counting 100 seeds from the threshed and oven dried seeds from each plot. These were weighed to represent the mean seed weight.

3.10.2.7 Harvest Index

After shedding the pods of the five plants from each plot, the seeds, chaff and the total biomass were oven dried at 80^0C for 48 hours and dry weight measured.

Harvest Index was therefore calculated as:

$$\text{Harvest Index} = \frac{\text{Economic yield}}{\text{Biological yield}}$$

Where Economic yield is the seed yield and the Biological yield is the summation of the total biomass, chaff and seed weight.

3.10.2.8 Grain Yield

Grain yield per hectare was determined by threshing the harvested plants from the three middle rows of each plot after sun drying for two weeks. The weight in grams (g) was then extrapolated to kilogram per hectare basis to get the average grain yield per hectare.

Data analysis

All data were analysed using the Analysis of Variance (ANOVA) at 5% significant level using the Genstat statistical package. Treatment differences were compared using the Least Significant Difference (Lsd) procedure at 5% level of probability.

CHAPTER FOUR

RESULTS

4.1 PLANT HEIGHT

The results of plant height for both the major and minor seasons are presented in Table 4.1. N rate and time of application did not significantly affect (P>0.05) soybean plant height in both seasons and at all sampling periods.

Table 4.1. Effect of time and rate of N application on soybean plant height.

	60 DAP		**75 DAP**		**90 DAP**	
Treatment	**Major**	**Minor**	**Major**	**Minor**	**Major**	**Minor**
Time of application			(cm)			
Early vegetative	45.27	47.30	48.38	47.14	48.65	47.59
Late vegetative	44.14	47.88	46.20	48.55	46.27	48.79
Early flowering	45.71	50.45	46.64	51.14	47.51	51.81
Lsd (5%)	NS	NS	NS	NS	NS	NS
N rate (kg/ha)						
0	43.58	45.80	45.99	45.88	45.70	45.11
20	44.44	49.14	47.18	49.72	47.70	49.27
40	46.81	48.66	48.38	49.06	48.51	49.02
60	45.33	50.57	46.73	51.12	46.99	51.85
Lsd (5%)	NS	NS	NS	NS	NS	NS
CV (%)	8.7	11.6	8.4	11.4	9.4	11.6

NS = not significant

4.2. NUMBER OF LEAVES

The results of leaf number per plant for both major and minor seasons are presented in Table 4.2. Leaf number per plant was not significantly (P>0.05) affected by N rate and time of application on all sampling occasions for both seasons.

Table 4.2. Effect of time and rate of N application on number of leaves of soybean.

Treatment	60 DAP		75 DAP		90 DAP	
	Major	Minor	Major	Minor	Major	Minor
Time of application						
Early vegetative	15.53	12.93	17.26	12.94	12.76	10.47
Late vegetative	15.73	12.90	15.75	12.91	13.00	10.50
Early flowering	18.21	12.50	18.78	12.71	15.16	10.72
Lsd (5%)	NS	NS	NS	NS	NS	NS
N rate (kg/ha)						
0	16.28	12.35	15.30	12.63	13.33	10.00
20	17.00	13.47	17.53	13.10	13.75	10.83
40	15.80	12.30	17.53	12.20	12.63	10.73
60	16.87	12.73	16.72	12.70	14.85	10.70
Lsd (5%)	NS	NS	NS	NS	NS	NS
CV (%)	22.4	21.0	18.3	19.2	23.0	20.1

NS = not significant

Table 4.3. Effect of time and rate of N application on number of branches of soybean.

Treatment	60 DAP		75 DAP		90 DAP	
	Major	Minor	Major	Minor	Major	Minor
Time of application						
Early vegetative	3.30	2.13	3.55	2.19	2.19	2.19
Late vegetative	3.27	1.93	3.10	2.40	2.22	2.41
Early flowering	3.11	2.19	3.77	1.71	2.94	1.71
Lsd (5%)	NS	NS	NS	NS	0.65	NS
N rate (kg/ha)						
0	3.30	1.78	3.32	1.79	2.17	1.79
20	3.68	2.42	3.47	2.33	2.57	2.33
40	3.43	2.12	3.75	2.03	2.33	2.03
60	3.83	2.00	3.57	2.35	2.73	2.34
Lsd (5%)	NS	NS	NS	NS	NS	NS
CV (%)	31.5	45.9	31.1	37.2	36.6	40.5

NS = not significant

4.3 NUMBER OF BRANCHES

Results of the number of branches per plant at 60, 75 and 90 DAP for major and minor seasons are presented in Table 4.3. Number of branches per plant was not affected by N rate on all sampling periods for both seasons.

Time of N application did not significantly ($p>0.05$) affect number of branches on both seasons and sampling periods, except in the major season at 90 DAP. During the major season at 90 DAP, the treatment effect of the flowering stage was significantly ($p<0.05$) higher than that of the early vegetative treatment only. Other treatment differences were not significant.

4.4. NODULE NUMBER, PERCENT NODULE EFFECTIVENESS, NODULE DRY WEIGHT AND N FIXATION.

Root nodule number ranged between 4 and 6 per plant (Table 4.4). There was no significant difference ($P>0.05$) in the interaction between time and rate of N fertilizer application. Percentage nodule effectiveness was neither affected by time of application nor the rate of application for both the major and minor seasons as shown in Table 4.4. Similarly, nodule dry weight did not show significant ($P>0.05$) effect due to time or rate of application differences.

Result of the total N fixed for both major and minor seasons are presented in Table 4.4. Total N fixed was not affected by N rate for both seasons. Time of application did not significantly ($P>0.05$) affect total N fixed in the major season. In the minor season, total N fixed of the late vegetative treatments were significantly ($P<0.05$) higher than the early vegetative treatments only. Flowering stage treatment was also significantly ($P<0.05$) higher than that of early vegetative treatment only. Other treatment differences were not significant ($P>0.05$).

Table 4.4. Effect of time and rate of N application on number of nodules, percent nodule effectiveness, nodule dry weight and N fixation.

Treatment	Nodule number		% nodule effectiveness		Nodule dry weight (g)		N fixation (kg/ha)	
	Major	Minor	Major	Minor	Major	Minor	Major	Minor
Time of application								
Early vegetative	3.9	4.1	42.8	46.2	0.40	0.49	62.5	63.1
Late vegetative	5.0	5.6	40.5	39.5	0.54	0.71	54.3	97.4
Early flowering	4.2	4.4	38.3	40.7	0.43	0.53	73.6	85.4
Lsd (5%)	NS	NS	NS	NS	NS	NS	NS	22.0
N rate (kg/ha)								
0	4.3	4.4	40.1	43.0	0.48	0.53	62.6	62.9
20	4.4	4.8	41.8	45.6	0.47	0.61	62.4	81.9
40	4.3	4.6	41.5	40.1	0.38	0.56	65.8	93.1
60	4.5	4.9	38.8	39.8	0.51	0.62	63.0	89.9
Lsd (5%)	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	9.7	9.5	20.3	21.0	61.9	49.8	24.4	24.8

NS = not significant

4.5 TREATMENT EFFECTS ON YIELD COMPONENTS.

The results of number of pods per plant, number of seeds per pod and 100 seed weight are presented in Table 4.5. N rate and time of application effects were not significant (P>0.05) for both seasons.

Table 4.5. Effect of time and rate of N application on soybean yield components.

	No of pods plant^{-1}		No of seeds pod^{-1}		100 seed weight (g)	
Treatment	**Major**	**Minor**	**Major**	**Minor**	**Major**	**Minor**
Time of application						
Early vegetative	26.29	24.54	2.03	2.01	10.24	12.82
Late vegetative	23.2	25.56	1.99	2.01	10.01	13.20
Early flowering	26.52	21.11	2.11	2.00	10.08	12.47
Lsd (5%)	NS	NS	NS	NS	NS	NS
N rate (kg/ha)						
0	25.15	21.32	2.03	2.00	10.04	12.54
20	27.43	23.57	2.08	2.01	10.13	12.77
40	24.02	26.12	2.04	2.00	10.05	13.08
60	24.77	23.93	2.03	2.01	10.21	12.93
Lsd (5%)	NS	NS	NS	NS	NS	NS
CV (%)	20.9	25.4	6.8	1.8	5.6	5.7

NS = not significant

4.6 HARVEST INDEX AND GRAIN YIELD.

The results of harvest index and grain yield are presented in Table 4.6. N rate effect was not significant (P>0.05) on both seasons for both parameters. Time of application significantly (P<0.05) affected harvest index during the major season, where application at early vegetative phase was significantly higher than application at early flowering stage only (Table 4.6).

In both major and minor seasons, time of N application significantly (P<0.05) affected soybean grain yield (Table 4.6). During the major season, the treatment effect of the early vegetative phase was the greatest, and this was significantly higher than all other treatment effects. In the minor season, however, applying N at the late vegetative phase supported the greatest grain yield, and this was significantly ($P<0.05$) higher than all other treatment effects.

Table 4.6. Effect of time and rate of N application on harvest index and grain yield of soybean

Treatment	Harvest index		Grain yield (kg/ha)	
	Major	**Minor**	**Major**	**Minor**
Time of application				
Early vegetative	0.43	0.32	738	664
Late vegetative	0.42	0.33	554	748
Early flowering	0.40	0.30	609	503
Lsd (5%)	0.02	NS	73.4	149.8
N rate (kg/ha)				
0	0.43	0.30	651	559
20	0.42	0.34	616	624
40	0.41	0.34	648	715
60	0.42	0.27	618	656
Lsd (5%)	NS	NS	NS	NS
CV (%)	7.3	26.2	16.1	32.6

NS = not significant

4.7. TOTAL N ACCUMULATED, REMOBILIZED N AND SEED N FOR MAJOR AND MINOR SEASONS.

Result of the total N accumulated, remobilized N and seed N content for both major and minor seasons are presented in Tables 4.7 and 4.8, respectively. Total N accumulated, remobilized N and seed N content was not affected by N rate for both seasons.

Time of application did not significantly (P>0.05) affect total N accumulated, remobilized N and seed N on both seasons except Total N at the end of pod filling (R6) and maturity (R8) in the minor season. At maturity (R8), total N of the late vegetative treatment was significantly (P<0.05) higher than the two other treatment effects, whiles at R6, the same treatment effect was significantly (P<0.05) higher than that of early vegetative treatment only. Other treatment differences were not significant (P>0.05).

Table 4.7. Effect of time and rate of N application on Total N accumulated and remobilized N in the major and minor seasons.

	Total N at R6		Total N at maturity		Remobilized N	
Treatment	**Major**	**Minor**	**Major**	**Minor**	**Major**	**Minor**
Time of application						
Early vegetative	76.1	76.8	139.3	141.1	26.6	7.7
Late vegetative	68.0	111.0	125.3	205.0	23.3	18.3
Early flowering	87.2	99.0	138.8	157.3	26.0	2.9
Lsd (5%)	NS	22.0	NS	44.91	NS	NS
N rate (kg/ha)						
0	76.3	76.5	131.6	151.2	21.8	6.8
20	76.0	95.6	143.3	175.1	27.2	10.8
40	79.5	106.8	137.0	170.3	27.6	7.2
60	76.7	103.5	125.9	174.6	24.9	13.6
Lsd (5%)	NS	NS	NS	NS	NS	NS
CV (%)	20.4	20.9	25.9	24.3	24.6	220.9

NS = not significant

Table 4.8. Effect of time and rate of N application on seed N in the major and minor seasons.

Treatment	Seed N at R6		Seed N at maturity	
	Major	Minor	Major	Minor
Time of application				
Early vegetative	6.26	6.32	6.30	6.83
Late vegetative	6.19	6.37	6.48	6.86
Early flowering	6.39	6.37	6.41	6.83
Lsd (5%)	NS	NS	NS	NS
N rate (kg/ha)				
0	6.29	6.34	6.28	6.97
20	6.27	6.28	6.42	6.91
40	6.26	6.33	6.35	6.67
60	6.37	6.47	6.53	6.81
Lsd (5%)	NS	NS	NS	NS
CV (%)	2.9	2.5	6.0	4.4

NS = not significant

CHAPTR FIVE

DISCUSSION

5.1 EFFECT OF TIME AND RATE OF N FERTILIZER APPLICATION ON SOYBEAN GROWTH

Plant height results showed that N rate did not have significant (P>0.05) effect on all sampling days. Notwithstanding, N applied treatment effects on all days was numerically higher than the control treatment. Varon *et al*. (1984) reported increase in plant height following application of N fertilizer. Increased plant height means increased source production which according to Caliskan *et al*. (2008) and Summerfield and Wien (1980) have direct effect on grain yield. Time of N application did not significantly (P>0.05) affect plant height on all sampling periods. The non responsiveness to both rate and timing of N application might be due to the soil N (Appendix 1) which could be adequate for legume growth or the plants used N fixed by the rhizobia resident in the soil more than the applied N.

Number of leaves was not affected by time of N application. This was due to the determinate nature of the soybean variety used (Gardner *et al*., 1985). Evans, (1992) and others observed that determinate species do not produce any more vegetative materials after flowering. Following anthesis in determinate species, assimilate produced is majorly used for seed filling and little is used for maintenance of plant body.

N application rate did not affect leaf production also because determinate species have specific number of leaves they produce before anthesis, which will not change irrespective of treatment imposed. In a similar study, Xuewen (1990) reported that difference in nitrogen levels had slight influence on the mean number of leaves per plant.

Generally, in the present study, N application at different rates and times did not significantly (P>0.05) affect soybean growth measured as plant height, number of leaves and number of branches. As pointed out earlier, this may be due to the soil N which could be adequate for soybean growth, so no positive response was shown by the plants irrespective of rate and time of application. Legumes have been known to need little application of N when N fixation is efficient. Furthermore, it appears, N obtained from biological fixation was also adequate for the plants, hence their little response to applied N compared with the control. The present results seems a challenge to the accepted knowledge that given the two sources of N, applied and fixed N, legumes would used the former because of the high cost of fixed N to the plant. Rather, it appears in this study that the response to applied N was low, suggesting that much of the N used was from fixed N.

5.2 RATE AND TIME OF N FERTILIZER APPLICATION ON NODULATION AND N FIXATION

Nodule numbers in this study was neither affected significantly by time nor rate of N application (Table 4.4). However, numerically, the late vegetative N application was better than the other treatments in both seasons. However, nodule effectiveness was slightly better in the early vegetative N application treatments. Nodulation studies have shown that some species, fixing bacteria are effective in nodule production but inefficient in N fixation. Workers like Blair (1989), Sarkodie-Addo (1991), and

Streeter (1988) have reported cases where effective nodulation did not result in efficient N fixation. The ability to form nodules has been described as not enough to obtain an effective N fixation symbiosis (Giller, 2001). Indeed, Salvagiotti *et al*. (2009) reported that N application can suppress the biological nitrogen fixation as fertilizer N can support the plant N requirement.

Nodule dry weight results showed that the late vegetative N application treatment were numerically better than the other treatments. In the same way, nodule dry weight in the early flowering N application treatment was also greater than the early vegetative N application treatment. The indication of these results is that treatments that produced more nodules also produced larger nodules. This result is different from those reported by Blair (1989) and Sarkodie-Addo (1991) who found a negative correlation between nodule numbers and nodule dry weights.

Nodulation data from the different rates of N applied did not show any definite pattern. In some cases, the control treatment effect was even better than the N applied treatments. The results obtained indicate that nodulation was not affected by N application.

N fixation was affected by time of N application in the minor season as N fixation of the late vegetative stage treatment was higher than the early vegetative treatment only. Nitrogen fixation in both seasons was also greater when N was applied in the early flowering phase than in the early vegetative phase (Table 4.4). In the major season, the difference was more than 10kgN/ha whiles in the minor season the former treatment effect was more than 20kgN/ha than that of the early vegetative treatment effect. Gan *et al*. (2002) made similar observations that fertilizer N supplied during the reproductive phase was most effective in increasing N fixation. These workers attributed this to increased leaf longevity as N enhances the photosynthetic capability of legumes (De veau *et al*., 1990; Zhou *et al*., 2006). In addition, greater N fixation when N was applied at the flowering phase has been attributed to the greater colonization by abuscular mycorrhizae that increases P supply to the nodules (Gross and Varennes, 2002).

N fixation was not significantly (P>0.05) affected by rate of N application; although in the minor season the N applied treatment effects were all better than the control treatment. There had been controversy about N application and nitrogen fixations for more than 50 years. Whiles some workers have found inhibitory effects, others have reported that lower amounts of fertilizer N enhanced fixation and others found no effect of N application on nitrogen fixation.

5.3 RATE AND TIME OF N FERTILIZER APPLICATION ON YIELD COMPONENTS AND YIELD

Rate and time of N application have no significant (P>0.05) effect on yield parameters. This result is in agreement with result obtained by Sohrabi *et al*. (2012) that there were no significant (P>0.05) differences in the application of N fertilizer to soybean on 100 seed weight and weight of pods per plant. However, other workers like Achakzai and Bangulzai (2006) have reported that pod yield and number of pods per plant of pea significantly increased with a progressive increase in N fertilizer application.

Harvest index was affected by time of application in the major season as harvest index of the early vegetative stage treatment was higher than the flowering treatment only. Results indicated that the estimate of harvest index was within the range for soybean (0.40 to 0.43) as reported by (Salado-Navarro *et al*., 1993; Ball *et al*., 2000; Kumidini *et al*., 2001; Pederson and Laver, 2004) that harvest index for soybean (based on standing crop) cluster around 0.50 with a range from 0.35 to 0.65. Increasing yield by lengthening of seed filling will also increase harvest index (Egli, 2004).

Grain yield in the major season was greater in the early vegetative N application treatment (Table 4.6) than in the other treatments. This indicates that the plants in this treatment made better use of the early availability of nitrogen to develop their plant structure and photosynthetic apparatus which resulted in greater seed yield. This practice has been recommended to farmers by agronomists and the present results confirm this advantage. These results, however, showed that grain yield was not positively correlated with N availability (Tables 4.4 and 4.6).

In the minor season, grain yield was significantly greatest in the late vegetative N applied treatment. Late vegetative yielded significantly ($p<0.05$) higher than the other two treatments. The result demonstrates that, N applied at the vegetative stage was taken up by plant root from the soil and assimilated into amino acids and proteins in the vegetative tissues and accumulated during the seed filling stage (Appendix II), leading to higher yield as maximum yield requires contributions from three phases that is vegetative phase, flowering phase and seed filling phase. This result agrees with the result obtained by Gan *et al*. (2003) that N topdressing at both the V2 and R1 stages, significantly stimulate biomass and total N accumulation during the seed filling stage R5, which led to higher seed yield. De Mooy *et al*. (1973) and Watanabe *et al*. (1986) have suggested that N supplied prior to reproduction can influence yield through improvement in plant growth and leaf area resulting in more abundant flowering. This might explain why N application at flowering had lower yield than N applied during the vegetative stage. The minor season results showed positive correlation between N availability and grain yield. Taylor *et al*. (2005) and Morshed *et al*. (2008) also reported that the application of N fertilizer can boost soybean plant yield.

5.4. RATE AND TIME OF N FERTILIZER APPLICATION ON N REMOBILIZATION

The results obtained did not show that rate and time of N application had significant effect on N remobilization in soybeans. However, a careful look at the data indicates soybean can strongly remobilize nitrogen from the vegetative parts for seed filling. As a higher protein crop (40-42%), the seed N cannot be supplied by post anthesis photosynthesis. This situation is further made worse by the ontogeny of the crop that is as determinate species, new leaf production ceases after anthesis, while the older leaves become inefficient in photosynthesis. N remobilization by soybean during seed filling stages has been the subject of studies by several workers (Sinclair, 1986; Muchow *et al*., 1993; Pengelly *et al*., 1999; Soltani *et al*., 2006). In oil seed rape, it has been shown that nitrogen can be remobilized from senescing leaves to expanding leaves at the vegetative phase, as well as from senescing leaves to seeds at the reproductive phase (Malagoli *et al*., 2005; Diaz *et al*., 2008; Lemaitre *et al*., 2008).

Appendix III shows the progression of N remobilization between various reproductive stages. Results indicate that the amount of N remobilized reduced with sampling dates.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

The following conclusions can be made from the results obtained in this study.

Generally, soybean growth was not affected by rate and time of N application. Number of nodules and nodule effectiveness were not responsive to N application. However, N fixation was significantly affected by time of N application in the minor season.

Yield components were not significantly affected by either time or rate of N application. However, yield was significantly affected by time of N application in both seasons.

Finally, time and rate of N application did not significantly affect N remobilization. However, the study affirmed soybean's capability of remobilizing N for seed filling as higher availability from the N applied treatments recorded higher values of remobilized N.

It is recommended that in future work, isotope N should be applied and soil samples should be collected alongside with plant samples during the growth stages of the plant to assess how much of the N in the harvested plant comes from the fertilizer and how much was taken up by the plant from N stored in the soil.

Also, that further studies be done preferably on high N deficient soils for more conclusive results

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APPENDICES

Appendix 1: Chemical analysis of soil samples at experimental site.

Sample Identification		**0 – 15 cm**	**15 – 30 cm**
% Organic Carbon		0.72	0.50
% Organic matter		1.24	0.86
% Total nitrogen		0.11	0.08
Exchangeable Cations Cmol/kg	Potassium	0.16	0.09
	Sodium	0.38	0.37
	Calcium	2.00	2.80
	Magnesium	1.00	0.80
Available phosphorus mg/kg		5.65	5.22
pH		5.57	5.50

Appendix 2: Metrological data during the cropping season (Metrological Department, KNUST, 2012).

Month	Rainfall (mm)	Temperature (^{O}C)		Relative Humidity (%)		Sunshine Duration (Hrs)
		Maximum	Minimum	09GMT	15GMT	
January	18.5	33.8	20.4	90.0	71.0	5.9
February	48.5	33.7	22.2	89.0	71.0	4.6
March	126.1	33.6	23.1	90.0	63.0	4.6
April	206.5	33.0	23.5	84.0	61.0	5.7
May	238.4	31.9	23	85.0	52.0	5.2
June	359.8	30.1	22.4	79.0	46.0	3.9
July	55.8	28.0	20.8	71.0	35.0	2.7
August	15.9	28.5	20.5	80.0	68.0	2.9
September	70.1	30.0	21.9	87.0	64.0	4.5
October	182.3	31.5	22.3	85.0	63.0	5.8
November	40.5	32.7	22.8	82.0	58.0	7.0
December	60.0	32.1	22	83.0	54.0	6.5

Appendix 3. Progression of N remobilization between various reproductive stages in the minor season.

Treatment	Remobilized N(R5-R6)	Remobilized N(R5-R7)	Remobilized N(R5-R8)	Remobilized N(R6-R7)	Remobilized N(R6-R8)	Remobilized N (R7-R8)
			Kg/ha			
Time of application						
Early vegetative	23.2	33.6	15.5	10.4	7.7	18.1
Late vegetative	15.4	39.0	-2.9	23.6	18.3	41.9
Flowering	13.1	35.4	10.2	22.3	2.9	25.2
LSD (5%)	NS	NS	NS	NS	NS	NS
N rate (kg/ha)						
0	24.5	36.1	17.3	11.6	6.8	18.7
20	20.0	38.5	9.2	18.5	10.8	29.3
40	11.7	35.9	4.8	24.2	7.2	31.0
60	12.7	33.6	-0.9	20.9	13.6	34.5
LSD (5%)	NS	NS	NS	NS	NS	NS
CV %	78.4	37.4	276.7	66.9	220.9	71.3