

THE RESPONSE OF SOYBEAN (*Glycine max* (L. ) Merr)  
TO SULPHUR FERTILIZATION ON SELECTED  
SOILS OF THE NIGERIAN SAVANNA

BY

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of Master of Science

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DECLARATION


I hereby declare that this thesis has been written by me and that it is the record of my own research work. It has not been presented before in any previous application for a higher degree



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The above declaration is affirmed.



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Professor G Lombin  
(Major Supervisor)

## CERTIFICATION

This thesis entitled "THE RESPONSE OF SOYBEAN (*Glycine max* (L.) Merr) TO SULPHUR FERTILISATION ON SELECTED SOILS OF THE NIGERIAN SAVANNA by ISHAKU YOI'LA AMAPU meets the regulations governing the award of the degree of MASTER OF SCIENE of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.



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DEDICATION

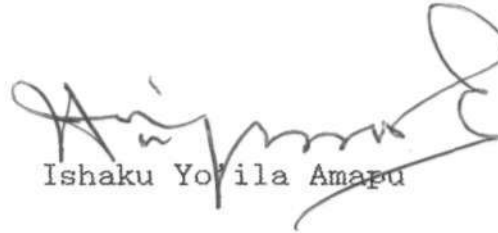
This work is dedicated to my late Uncle, Daniel Amuga Ayikam, who showed so much interest in my academic pursuits and general well being. May his Soul Rest in Peace. Amen.

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Ishaku Yofila Amapu

## ABSTRACT

The soils of the Nigerian savanna are generally low in sulphur and the production of leguminous crops such as soybean is therefore dependent on adequate sulphur fertilisation. This study was carried out to evaluate the response of soybean to sulphur under greenhouse conditions and on the field.

For the greenhouse study, five representative soils were taken from the soybean-growing zone of the country and cropped to soybean for six weeks. The treatments consisted of four levels of sulphur (0, 10, 20, and 40 mg kg<sup>-1</sup>). The shoots were cut at the end of the six weeks, oven-dried to constant weight, milled and analysed for nutrient uptake. The field trial was conducted at Samaru and consisted of four sulphur treatments vis; 0, 10, 20, and 40 kg ha<sup>-1</sup>, and the crop was grown to full maturity and harvested when the pods were dry, just at the commencement of shattering.

Under greenhouse conditions, sulphur applied at 10 mg kg<sup>-1</sup> significantly increased dry matter yield and plant height on two of the soils but leaf number was not affected by the treatments. In the field, nodulation and grain yield were marginally increased by sulphur application. However, applying 40 kg S ha<sup>-1</sup> slightly depressed grain yield and led to a significant reduction in nodule number. Sulphur fertilisation generally enhanced the uptake of both macro

and micronutrients in the greenhouse with treatment effects on the uptake of N, P, S, Fe, and Mn attaining statistical significance on some of the soils. Sulphur application had no significant effect on the nutrient concentration in soybean index leaves under field conditions.

The results of this study suggest that where the available sulphur level is about  $4 \text{ mg kg}^{-1}$  or more, crop response is unlikely under field conditions at Samaru and possibly at other areas with similar soil characteristics within the savanna Zone.

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## CHAPTER 1

## INTRODUCTION

The effort to reduce the nation's dependence on imported agricultural products in, order to save scarce foreign exchange has made it imperative for Nigerians to look inwards and exploit the crops available in this country that could be used to feed industries as well as to provide human food and animal feed. One of the crops that can be exploited to meet this challenge is soybean. It is rich in protein (up to 40%) and oil (13-45), both of which are very important in the diet of man and livestock. In many countries, where the potentials of this crop is being fully exploited, it is used for the manufacture of a variety of industrial products besides being a major source of feed for livestock and poultry. In Nigeria, soybean is currently being used for the manufacture of 'Soy-Ogi' (a product of soybean and maize or millet developed by the Federal Institute for Industrial Research, Oshodi (FIIRO)), and in many homes it is used in making 'daddawa', 'moin-moin' or 'alele', soypan-cakes, and a variety of soy-fortified cereal foods for feeding children during the weaning period.

The Guinea Savanna agro-climatic zone has the greatest potential for soybean production in Nigeria but the general fertility status of the soils in the area is low. This has constituted one of the major constraints in the production of this important crop. In spite of this, very little appears to have been done on the nutrient needs of the crop in this

area. The fact that single superphosphate, the most common phosphate fertilizer presently used in the area, contains 12 to 13% sulphur and is thus able to meet the sulphur needs of most of the major crops grown in this area has been advanced as the main reason for this seeming neglect. However, with the current trend in the fertilizer industry, which favours the production of high analysis fertilizers, and the current push for the use of more concentrated phosphate fertilizers containing virtually no sulphur, sulphur deficiency is likely to become a major limiting factor in crop production in northern Nigeria in no distant future.

Isolated cases of sulphur deficiency have already been reported in groundnut (*Arachis hypogaea*, L.) in a number of locations in savanna zone of Nigeria by a number of researchers (Greenwood, 1954; Goldsworthy and Heathcote, 1963; Bromfield, 1973). Similarly, significant yield responses of cowpea (*Vigna unguiculata* (L.) Walp), and maize (*Zea mays*, L.) to sulphur fertilization have been obtained in the derived savanna zone of Nigeria by Fox *et al.*, (1977), and Kang and Osiname (1976). In a recent review, Yayock and Lombin (1981) pointed out the inadequacy of sulphur supply in Nigerian soils especially in the savanna ecological zone and the need to supplement it through direct fertilization. These reports point to the fact that the deficiency is rapidly becoming widespread. This is expected considering the shift from the traditional practices of bush fallowing

system to a system of intensive cropping with higher yields being obtained from improved crop varieties.

In view of the importance of soybean as a crop and sulphur as an essential nutrient for soybean production, this study, was undertaken to evaluate the effect of sulphur fertilization on:

- (1) the dry matter yield, growth, and nutrient uptake of soybean grown under greenhouse conditions and
- (2) the nutrition and grain yield of soybean under field conditions.

## CHAPTER 2

## LITERATURE REVIEW

## 2.1 Sources and forms of sulphur in soil

The earth's crust contains an average of 0.06% sulphur (Tisdale et al., 1975) but the distribution in soil is very variable. Jordan and Reisenauer (1957) reported average values of 540 ppm and 210 ppm of total sulphur for surface soils of Mollisols and Alfisols, respectively, in the temperate areas. The total sulphur content of Tropical soils is generally low because of their low organic matter contents. Values for top soils vary widely from 18 (Watson, 1964; Cooper, 1971) to 510 ppm (Haque and Walsmley, 1973). A mean of 106 ppm total sulphur was reported for 70 soils from the tropics (Anon, 1979). In the Nigerian savanna, Goldsworthy and Heathcote (1963) reported mean values of 22 ppm total sulphur in the Sahel and 59 ppm in the Southern Guinea savanna for fields that had been under continuous cultivation. For bush fallows, Cooper (1971) gave relatively low values ranging from 18 to 54 ppm with an overall mean value of 35 ppm.

The primary sources of most soil sulphur are the metal sulphides of plutonic rocks which undergo oxidation during weathering. Apart from the primary minerals, variable amounts accrue annually, from particulate and rainwater deposition. In industrialised areas atmospheric accretion may form a main source of soil sulphur but in low industrial

areas, such as the savanna area of Nigeria, contribution from these sources is minimal. This is evidenced by the findings of Bromfield (1974a) who reported the deposition of 0.1 to 0.43 kg/ha sulphur in the 52-226 kg/ha dust that falls during the dry (harmattan) season. Bromfield (1974b) also found that sulphur deposition from rainwater in the area amounted to only about 0.8-0.9 kg/ha/year. On the whole, Bromfield (1974c) has reported that the sulphur that accrues annually from these sources is between 1.4 to 2.4 kg sulphur per hectare.

Sulphur occurs in soils, in both organic and inorganic forms. The proportion of the organic to the inorganic forms vary with soil type and depth. In most soils, the organically bound sulphur predominates and provides the major soil reservoir (Russell, 1973; Reisenauer *et al.*, 1973; Scott and Anderson, 1976). In the Nigerian savanna, for instance, the organic forms averages more than 90% of the total sulphur in medium to longterm fallows (Cooper, 1971).

#### 2.1.1 Soil Organic Sulphur

The organic sulphur fractions of the soil have been categorised into two forms: carbon-bonded sulphur and non-carbon-bonded sulphur. The latter fraction is made up of choline and phenolic sulphates as well as lipids (Freney and Stevenson, 1966). This fraction constitutes about 70% of the total organic sulphur.

The carbon-bonded sulphur fraction includes the sulphur-containing amino acids - cysteine, cystine and methionine as

well as other related compounds such as cysteic acid, methionine sulphoxide and methionine sulphone which have been identified in soil hydroxylsates. This fraction may constitute upto 30% of total organic sulphur (Freney et al., 1972).

#### 2.1.2 Soil Inorganic Sulphur

The inorganic sulphur components predominate in the subsurface horizons and constitute less than 1% of the total sulphur in well-drained soils. It may occur in the form of sulphates and as compounds of lower oxidation states, such as sulphides, polysulphides, sulphite, thiosulphate and elemental sulphur. In well drained and well aerated soils most of the inorganic sulphur occurs as sulphate, whereas under poorly drained or submerged (anaerobic) conditions, considerable amounts of sulphides and elemental sulphur may be found (William, 1975; Page et al., 1982). In soils where sulphur compounds predominate in the lower oxidation states, the establishment or restoration of aerobic conditions lead to rapid oxidation to sulphates, and this frequently leads to acidity problems.

The sulphate fraction may occur as water soluble salts adsorbed to the soil colloids, or as insoluble forms. The level of soluble sulphate in soils of the tropics is generally below 10 ppm (Anon, 1979). Considerable fluctuations may occur as a net result of mineralisation of organic matter, leaching of soluble sulphates, uptake by plants, additions from fertilizers, irrigation water and

rainfall.

Many soils, particularly weathered tropical soils and volcanic ash, have the capacity to sorb sulphate (Parfitt, 1978). Results of laboratory studies (Cooper, 1971; Bromfield, 1972) in the Nigerian Savanna have shown that soils fertilized with ammonium sulphate adsorbed sulphate strongly enough to prevent any losses from the surface 120 cm for many years. The increasing levels of adsorbed sulphate sulphur and its retention against leaching with soil depth have been attributed to increasing clay content and acidity of the subsoil (Bromfield, 1972; Hingston *et al.*, 1972; Barrow, 1975).

Soils containing predominantly 1:1 layer silicate clay minerals like kaolinite, such as the soils of the Nigerian savanna, have been found to adsorb more sulphate than those with mainly the 2:1 layer silicate clay minerals like smectites (Chao *et al.*, 1962). Harward *et al.*, (1962) found that the amounts of sulphur retained by clays was in the order kaolinite > illite > montmorillonite. Widdowson (1971) has attributed this phenomenon to a higher proportion of anion exchange sites on the kaolinite and higher negative charge in smectites. Harward and Reisenauer (1967), and Alymore *et al.*, (1967) had shown that sulphates adsorbed by iron and aluminium oxides (Fe-Al) are held more firmly than those adsorbed by kaolinites. They also opined that since the Fe-Al-oxyhydroxides are normally associated with layered silicate clays, the sulphate adsorption attributed to these

clays may be due to Fe-Al-oxyhydroxides rather than the layer silicate clays *per se*.

The presence of other anions is another factor that influences the sulphate adsorption capacity of a soil. Kamprath *et al.*, (1956) reported that fully dissociated anions ( $\text{SO}_4^{2-}$ ) could not adsorb onto negative or uncharged surfaces. The extent of adsorption is dependent on the amount of positive charge on the surface, and consequently, pH. On the other hand, the adsorption of undissociated ions such as  $\text{H}_2\text{PO}_4^-$  does not depend on the presence of a net positive charge, and as a result, they are readily adsorbed to surfaces. In addition, phosphate ions are very effective both in displacing adsorbed sulphate and in reducing the capacity of the surface to adsorb additional sulphate. Aylmore *et al.*, (1967) found that solutions containing  $\text{KH}_2\text{PO}_4$  desorbed some 20% more sulphate from clay and oxide surfaces than did water. It is based on this principle that phosphate-containing salts are used for the extraction of available sulphate\_sulphur.

Insoluble sulphates likely to occur in soils include barium and strontium sulphates, sulphates associated with calcium carbonate, and basic iron and aluminium sulphates. In calcareous soils, sulphate frequently occur as a co-crystallized or co-precipitated impurity with calcium carbonate and is probably the most common of the insoluble sulphates. In some soils, it may account for a high proportion of the total soil sulphate.

## 2.2 Sulphur Transformations in Soil

Sulphur transformations are many and varied. The changes are often cyclic with the element changing from inorganic to organic forms and vice versa due to the activities of living organisms. During soil formation, the Sulphides of primary minerals are converted to sulphates which are, in turn, changed to many and varied organic sulphur compounds by microorganisms, plants and animals. Sulphur is thus found in soils in many forms. In the surface horizons of well-drained, non-calcareous soils of the humid regions, most of the sulphur accumulates in organic forms whereas in the arid zones and areas with calcareous soils, large amounts of inorganic sulphate accumulate. Under water-logged conditions appreciable amounts of sulphide is found (Freney and Swaby, 1975).

The following transformations of sulphur may occur in soils : immobilization or assimilation of sulphate into organic sulphur compounds, mineralization or decomposition of organic sulphur compounds, and oxidation of sulphur and inorganic compounds. The first and second reactions can take place in both aerobic and anaerobic soil conditions whereas the third would be confined to anaerobic environments.

### 2.2.1 Immobilisation

In fields where fertilizer sulphate has been applied, some of the sulphur is converted to organic forms by plants and some are incorporated into cells of microbes. Reviewing the work done on sulphur transformations, Freney and Swaby (1975) clearly brought out the important role played by soil micro-organisms in the process.

Of the major factors affecting immobilisation of sulphur, C:S ratio exerts the greatest influence. Freney and Swaby (1975) reported findings by some researchers, who, using glucose as the carbon source concluded that a C:S ratio of 900:1 or lower would provide adequate sulphur for maximum microbial activity and since most plant residues have a C:S ratio of less than 900:1, sulphur would generally not be a limiting factor for the decomposition of residues under natural tropical conditions. However, some other workers (Barrow, 1960; Massoumi and Cornfield, 1965; Stewart and Whitefield, 1965) have shown that immobilisation of sulphur would occur during the decomposition of plant residues with C:S ratios well below 900:1. This suggests that the organic sulphur was not readily available for the needs of the micro-organisms, or that the organisms involved had a higher sulphur requirement for the decomposition of plant residues than glucose. The latter alternative is more likely because Stewart *et al.* (1966a) working with pure compounds showed that micro-organisms required more sulphur for the decomposition of cellulose (with a C:S ratio of

300:1) than for glucose (with a C:S ratio of 600-900:1). The higher sulphur requirement may be related to the production of extracellular enzymes which are necessary for the breakdown of cellulose.

Stewart *et al.*, (1966b) found that the decomposition rate of straw in a sulphur-deficient soil was dependent upon the sulphur content of the straw. They found that when straw with less than 0.15% sulphur was added to the soil it depressed wheat growth by immobilising sulphur but straws with higher sulphur content increased yield. This suggested that it was necessary for the sulphur content of the straw to exceed 0.15% to obtain maximum rate of decomposition.

### 2.2.2 Mineralisation

It is generally accepted that plants take up sulphur from the soil in the form of water soluble and adsorbed sulphate. Since the amount of inorganic sulphur in soils cannot provide sufficient sulphur for plant growth the plant must depend, to a considerable extent upon the mineralisation of soil organic sulphur for their needs. Working in Australia, Williams (1972) found that inorganic sulphur which accumulates in the soil organic matter during fallows can provide adequate available sulphur to fully meet the sulphur requirements of wheat crop grown without nitrogenous fertilizer.

The conversion of organic sulphur to inorganic sulphate is carried out mainly by soil micro-organisms (Alexander, 1961) and thus any factor which affects the growth of micro-

organisms will affect the mineralisation of sulphur. Temperature, moisture, pH (Chaudry and Cornfield, 1967a, 1967b; Williams, 1967), and availability of energy to the microbes, have been shown to affect the mineralisation of sulphur as they do N-mineralisation. Williams (1967) report that low temperature ( $< 10^{\circ}\text{C}$ ) suppressed mineralisation over a 64-day incubation period. On the other hand other workers (Chaudry and Cornfield, 1967b; Williams, 1967) found that mineralisation of sulphur increased with increasing temperature from  $20^{\circ}\text{C}$  to  $40^{\circ}\text{C}$  but was less at  $50^{\circ}\text{C}$  than  $40^{\circ}\text{C}$ . The mineralisation of sulphur in Australian soils incubated at low ( $< 15\%$ ) and high ( $> 40\%$ ) moisture levels was impaired, whereas it was enhanced at 60% of field moisture-holding capacity (Chaudry and Cornfield, 1967a; Williams, 1967).

The mineralisation of organic sulphur has been found to be directly proportional to pH upto a value of 7.5 (Williams, 1967). Above pH 7.5 mineralisation increased more rapidly suggesting that a further factor, possibly chemical hydrolysis, was affecting the process.

The growth of plants in the soil have also been found to bring about increased mineralisation of sulphur from the organic matter (Freney and Spencer, 1960). This increase in sulphate liberation has been explained as being due to the "rhizosphere effect" brought about by the excretion of amino acids and sugars by plant roots and the resultant stimulation of microbial activity.

Results from incubation studies showed that sulphur mineralisation follows several patterns. Nelson (1964), Barrow (1967, 1969a), Haque and Walmsley (1972) and Tabatabai and Bremner (1972) found that there was immobilisation of sulphur during the initial stages of incubation followed by mineralisation in the latter stages. Williams (1967) found three different patterns : a steady, linear release with time over the whole period; a rapid release of sulphate during the first few days followed by a slower linear release; and a rate of release which decreased with time. The pattern of release does not seem to be related to any specific soil property but it is probably due to the chemical nature of the decomposing fraction of the soil organic matter. Better predictions of the pattern of mineralisation may be obtained when the main sulphur compounds in the soil are identified and their turn over rates determined.

### 2.2.3 Oxidation Processes

Elemental sulphur, sulphides and several other inorganic sulphur compounds can be oxidised in the soil by purely chemical means and by microbial oxidation. The first method is usually slower and therefore of less importance than the second. The rate of biological oxidation of sulphur depends on the interaction of three factors, viz : the microfloral population in the soil, characteristics of the sulphur-source, and environmental conditions in the soil.

Two classes of bacteria are specifically involved in sulphur oxidation. These are the chemolithotrophic sulphur bacteria which utilises energy released from the oxidation of inorganic sulphur for the fixation of  $\text{CO}_2$  into organic matter. The best known, and usually considered the most important group of sulphur oxidising chemolithotrophic organisms are the autotrophic bacteria belonging to the genus *Thiobacillus*. The second class of sulphur - oxidisers are the photolithotrophic sulphur-bacteria, which carry out photosynthetic carbon fixation using sulphide and other sulphur compounds. Tisdale *et al.*, (1975) have reported considerable variability in sulphur-oxidation rates among soils obtained from different locations in Australia and the United States of America. These variations have been attributed to differences in the number of *Thiobacillus* found in the soils.

Many researchers have observed that sulphur oxidation rates increased as particle size is reduced. The impact of the particle size of sulphur distribution on its rate of transformation to sulphate have been clearly illustrated by Li and Caldwell (1966). The finer the particle size of a given mass of sulphur the greater the specific surface and the faster is the sulphate formation.

As in most micro-biological reactions, temperature, moisture and aeration, and pH influence the oxidation of sulphur. Nor and Tabatabai (1979) found that temperatures between 25 and 40°C were close to ideal for most sulphur

oxidising organisms. Because sulphur-oxidising bacteria are aerobic, their activity would decline where oxygen is lacking due to waterlogging. Kittams and Attoe (1965) reported that sulphur oxidation is favoured by soil moisture levels near to field moisture capacity. Although sulphur-oxidising organisms have different pH requirements, McCready and Krouse (1982) found that, in general, oxidation of added sulphur proceeded most rapidly under acidic soil conditions. These reports show that the oxidation of elemental sulphur is favoured by the climatic conditions existing in the tropics especially during the cropping season. The prospects of using elemental sulphur when other sources become scarce is feasible.

## 2.2 Factors affecting the plant available sulphur status and distribution in the soil

Sulphur is usually taken up by plants as the sulphate ion. Concentrations of 5 ppm  $\text{KH}_2\text{PO}_4^-$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2^-$  extractable sulphate in the soil have proved adequate for the growth of many crops grown in the tropics (Fox, 1973; 1976; Fox et al., 1977).

A number of soil properties have strong influence on the sulphur availability and distribution in soils. Prime among them is the organic matter status. Since the organic matter acts as the reservoir of sulphur in soil it regulates the content of sulphate-sulphur. In a series of studies carried out in selected Indian soils, Takkar (1988) reported a direct correlation between soil organic matter and

available sulphur in soil. Similarly, working in the Guinea savanna zone of Nigeria on soils derived from aeolian deposits, Cooper (1971) and Bromfield (1972) recorded a decline in soil organic matter, as a result of continuous cultivation, which were accompanied by a corresponding decline in the sulphur supplying power of the soil. Enwezor (1976) obtained similar results in Eastern Nigeria. Fitts (1970) reported that sulphur deficiencies frequently occurred in soils derived from volcanic parent materials. In such soils the mineralisation of sulphate-sulphur from the allophane-bound organic matter is very slow. Plants growing on these soils are often sulphur-deficient despite the fact that the soil is high in organic sulphur.

Another major soil factor affecting sulphur availability to plants and distribution in soil is the texture of the soil. In general, clayey soils contain more sulphur than sandy soils. The clay type and quantity are particularly important. The 1:1 clay minerals have a greater ability to adsorb sulphate than do montmorillonitic clays (Chao *et al.*, 1962). Thus, Bromfield (1972) reported that the kaolinitic clays which predominate in savanna soils are capable of adsorbing sulphate strongly enough to prevent leaching losses from the surface 120 cm for many decades. Since most of the clays are eluviated from the surface layers, the maximum zone of sulphate is a major source of plant nutrient in the weathered tropical soils.

The adsorption of sulphate to clays has also been reported to be pH-dependent (Kamprath *et al.*, 1956). Increasing pH through liming may decrease sulphate retention. By virtue of its effect on mineralisation, pH, in conjunction with soil moisture and temperature, has profound effects on the availability of sulphate-sulphur.

Other nutrients, particularly phosphorus, also influences the availability of sulphur to plants. Phosphate ion easily replaces the sulphate ion on clay oxide, or organic surfaces. Barrow (1969b) reported an almost linear decline in sulphate adsorption with added phosphate which meant that phosphate ions replaced sulphate ion on charged surfaces. Similar observations were made by Bromfield (1972) in the Nigerian savanna.

Sulphate, which is contained in readily soluble fertilizer, and that which is produced by mineralisation of organic matter enters the soil solution where, if not taken up by plants or adsorbed on soil colloids, is subject to leaching. In most tropical soils where sulphur is strongly adsorbed onto soil colloids the adsorbed sulphate is not free to move to plant roots by convective flow and diffusion. Consequently, the sulphate which is available to plants is a balance between adsorption and leaching losses. Soil sulphur is also lost through soil erosion.

Apart from the factors discussed above, crops differ in their capacity take up sulphur. Root type and density are two major characteristics affecting sulphur availability to crops. It is generally accepted that sulphate which is adsorbed in the subsoils contribute significantly to the sulphur needs of plants growing in highly weathered soils because it is readily available. Although crops can utilise the sulphate that is adsorbed in the subsoil, they might experience sulphur deficiency at the early growth stages until root development is sufficient to reach the retention zones. The deep rooted crops are likely to overcome such temporary shortages where sufficient sulphur is adsorbed in the subsoil.

Other plant factors which influence sulphur uptake include crop variety, plant population, cropping intensity, and yield levels. In addition to environmental factors, ion adsorption by plants is genetically controlled and considerable differences exists both between and within genera. Similarly, higher plant populations and low spacings affect nutrient needs. Where plant populations are increased without appropriate increases in sulphur fertilization or availability there can be greater sulphur uptake but yields may actually be reduced.

Incidental additions of sulphur through atmospheric accretion and irrigation waters also influence sulphur availability to plants. These sources have made sulphur deficiency a minor problem in areas where great quantities of

fossil fuels are burned. On the other hand the wide-spread sulphur deficiencies reported in many tropical, subtropical and warm temperate areas (Fox, 1973; Bromfield, 1974b; Haque and Walmsley, 1975; Jones *et al.*, 1975) have been attributed to the low atmospheric sulphur content of these regions.

## 2.4 Sulphur Nutrition and Requirement of Soybean

### 2.4.1 Functions in Plant and Deficiency Symptoms

Plants require sulphur for the synthesis of the essential sulphur-bearing amino acids : cyteine, cystine, and methionine, and hence for protein elaboration. It is also needed in the synthesis of certain vitamins such as glutathione, and of co-enzyme A. Similarly, the formation of chlorophyll and the formation of glucoside oils found in garlic, onions, and cruciferous plants require sulphur. Another important function of this nutrient is the formation of certain disulphide linkages which are associated with structural characteristics of protoplasm (the concentration of sulphhydryl (-SH) groups in plant tissues have also been shown to be related to increased cold and drought resistance in some crop species). The formation of ferredoxin, an iron-containing plant protein that functions as an electron carrier in the photosynthetic process, and that of a ferredoxin-like compound which is involved in the fixation of N by root nodule bacteria and free-living N-fixing soil bacteria are dependent on the availability of sulphur. The plant also requires sulphur for the activation of certain

proteolytic enzymes such as papainases and in the activity of ATP sulphurase, an enzyme that functions in the metabolism of sulphur.

Because sulphur is involved in many vital processes in the plant system, it is not surprising that its deficiency usually has a profound effect on the general metabolism of plants. Sulphur deficient plants are generally frail and stunted while leaves remain small and are sometimes distorted. The deficiency is also characterised by the yellowing of leaves (chlorosis). The symptoms may be confused with those of nitrogen, and molybdenum or masked by phosphorus deficiency. However, unlike nitrogen, sulphur is relatively immobile and so symptoms of its deficiency tend to occur first in younger leaves. The early identification of sulphur deficiency can seldom be made by mere observation of plants in the field. Where the deficiency is visually obvious, there has already been a substantial reduction in yield.

#### 2.4.2 Effect of Sulphur Fertilization on Nodulation and Nitrogen Fixation

Sulphur is a constituent of the nitrogenase enzyme and has been shown to influence nitrogen fixation primarily through its effect on N-metabolism in the host plant (Anderson and Spencer, 1949). A number of research workers (Harward *et al.*, 1962; Reisenauer, 1963; Pumphrey and Moore, 1965; Caldwell *et al.*, 1969; Singh, 1970) have observed increased nitrogen content of legumes when such legumes are

fertilised with sulphur but only a few workers have attempted to relate this effect to nitrogen fixation. Oke (1969) applied sulphur to pigeon pea (*Cajanus cajan*, L.) and guava (*Cyamopsis tetragonoloba* L.) and found that the nitrogen content of the nodules and percent nitrogen in the plants were significantly increased by the application of sulphur. In the same vein, Wooding *et al.*, (1970) measured the differences in total nitrogen content of symbiosis-dependent soybean seedlings at the time of transplanting and at the end of three weeks and found that seedlings grown in N-free nutrient solution plus sulphur contained 2.5 times more nitrogen than those without sulphur. Bergessen (1971) suggested that due to the high sulphur content of nitrogenase, the synthesis and activity of the enzyme might be restricted even under marginal sulphur deficiency.

The results of experiments conducted by Walker and Adams (1958) in sulphur deficient soils in New Zealand, and those by Gates *et al.*, (1971) and Loader and White (1975) both in Australia have also demonstrated the essential role of sulphur in promoting growth and nitrogen fixation by legumes. However, there is no evidence in the literature to show that plants fail to nodulate under conditions of sulphur deficiency. Brown *et al.*, (1981), and Sampet (1979), for instance, have reported that sulphur did not have any effect on nodulation and nitrogen fixation.

### 2.4.3 Sulphur Requirement of Soybean

Stanford and Jordan (1966) defined sulphur requirement of a plant as, "the minimum uptake of this nutrient associated with maximum yield of dry matter". Generally, a high sulphur requirement is characteristic of protein-rich crops. For this reason such crops respond most strikingly to inadequate sulphur supply.

Of the indices used to characterise the sulphur status of plants a number of workers (Walker et al., 1956; Jordan and Bradsley, 1958; Pumphrey and Moore, 1958; Dijskhon et al., 1960; Stewart and Porter, 1969; Sa'albach, 1972) have suggested that nitrogen : sulphur ratio in the plant is one of the most reliable. This approach is based on the theory that the N:S ratio of protein is genetically controlled. Thus when sulphur is limiting protein formation, non-protein nitrogen will accumulate in the plant resulting in total-N : total-S ratio being higher than that found in protein. Conversely, when sulphur is adequate (or when nitrogen is limiting) non-protein sulphur accumulates and the total-N : total-S ratio is below that found in plant protein. The ratio of N:S in plant proteins has been reported to be approximately 17:1 for legumes and about 14:1 for grasses. Some researchers have pointed out the limitations of this index based on the fact that age, species, environment and level of nutrition are factors which determine the amounts and relative proportions of proteins formed and consequently the N:S ratio in the overall protein fraction and not the

genetical make up of the crop alone. For instance, Andrews (1977) is of the opinion that the use of N:S ratio does not appear to be sound for diagnostic purposes because the ratios are determined primarily by the sulphur concentration and nitrogen concentration in the plant tissue, and the latter is conditioned by the capacity of the plant in question to fix nitrogen. In addition, molybdenum (Kerridge *et al.*, 1972), phosphorus (Gates, 1974), pH and Calcium (Andrew, 1976) all play important roles in the legume-*Rhizobium* symbiosis. These coupled with the poor correlations, often obtained, between nitrogen and sulphur concentration in plant tissue further negates the practical use of such an index.

Information in the literature (Thompson *et al.*, 1970) tends to suggest that total sulphate-sulphur does not build up in the plant to any significant degree until the sulphur requirement of the plant for protein formation has been met. The sulphate-sulphur content of the tissue therefore, may be a sensitive indicator of the sulphur status of the plant. The critical sulphate sulphur of soybean index leaves at flowering has been given as 0.25% (Nelson and Barber, 1964).

From the foregoing literature survey it is evident that only a few experiments have been conducted in the Nigerian savanna on sulphur. In many other experiments, responses to fertilizers such as sulphate of ammonia and single superphosphate have been attributed to the nitrogen and phosphorus components of the fertilizers thereby ignoring the effect of sulphur. Most of the responses to sulphur on the

field have been limited to shallow rooted crops, grown on sandy soils, whereas the deep rooted crops seem to be able to utilise sulphur that has been leached into lower soil horizons. The responses often obtained in the green-house may be attributed to the small rooting volume.

## CHAPTER 3

## 3. MATERIALS AND METHOD

## 3.1 Greenhouse Experiment

Surface soils (0-20 cm composites) were collected from five representative sites (Ankpa, Yandev, Lafia, Zonkwa and Samaru) in the Guinea savanna zone (the major soybean growing belt of the country) and used for the greenhouse cropping.

## 3.1.1 Site and Soil Description

The climatic details of the savanna agro-ecological zone of Nigeria has been described by Kowal and Knabe (1972). The following is a brief description of the soil and rainfall characteristics of the sites where the soils for this study were obtained :

Ankpa ( $7^{\circ} 23'N$ ;  $7^{\circ} 38'E$ ) is in the southern Guinea savanna. The soils are deep, well drained sandy Oxisols derived from cretaceous sandstones (Harpstead, 1973). The clay fraction is dominated by kaolinite with very little quantity of illite. The mean annual rainfall is about 1380 mm with most of it falling between the months of April and October. Yandev ( $7^{\circ} 23'N$ ;  $9^{\circ} 11'E$ ) is also located in the southern Guinea savanna zone. The soils here are generally sandy and well drained, and have been classified as Ultisols in the USDA system (I.E. Esu, personal communication). Yandev has a mean annual rainfall of 1300 mm most of which is received in the months of April to October. Lafia ( $8^{\circ} 30'N$ ;  $8^{\circ} 30'E$ ) has a mean annual rainfall of about 1280 mm all

falling in the six-month period May to October. The soils in this area are sandy Alfisols (I.E. Esu, personal communication). Zonkwa (9° 44'N; 8° 23'E) lies in the southernmost part of the northern Guinea savanna zone. Located on the windward side of the Jos Plateau, it has an annual rainfall of 1490 mm which is confined to a six-month period (May to October). The soils are sandy loam and could be classified as Alfisols (I.E. Esu, personal communication). Samaru (11° 11'N; 7° 38'E) lies within the northern Guinea savanna. The soils are essentially sandy loam derived from aeolian deposits and classified as Inceptisols (Harpstead, 1973). However, the results of recent investigations carried out in the area (Moberg and Esu, 1989) show that the soils are Alfisols. The mean annual rainfall is about 1100 mm and falls within the 5-month period of May to October.

### 3.1.2 Soil Sampling

Bulk soil samples were taken from fields that had been under continuous cultivation but had not received fertilizers containing sulphur for at least the past three years or more successive cropping seasons. The samples were collected from relatively flat fields from 10 to 15 randomly selected points. The soil samples were put in clean polyethylene bags for conveyance to the greenhouse experimental site.

The soils were spread out under the shade to dry and screened to pass through a 2 mm screen. Sub-samples of the air-dried soils were taken for laboratory analyses. About 5.0 kg samples were weighed into plastic pots for the greenhouse

experiment and the following treatments imposed on them.

### 3.1.3 Treatments and Experimental Layout

The treatments consisted of four levels of sulphur (0, 10, 20, and 40 mg kg<sup>-1</sup>) applied as ammonium sulphate. The treatments were replicated three times and arranged in a split plot randomised complete block design with the soils constituting the main plots and the treatments the sub-plots.

The rate and source of nutrients applied as basal fertilizers are as follows :

Nutrient	Rate (mg kg <sup>-1</sup> )	Source
N	80	CO(NH <sub>2</sub> ) <sub>2</sub> , CuNO <sub>3</sub> and (NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .2H <sub>2</sub> O
P	100	KH <sub>2</sub> PO <sub>4</sub>
K	125	KH <sub>2</sub> PO <sub>4</sub>
Cu	5	CuNO <sub>3</sub>
B	0.5	H <sub>3</sub> BO <sub>3</sub>
Mo	0.25	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .2H <sub>2</sub> O

The sulphur treatments and part of the basal nitrogen (applied as urea) were weighed out for each pot and thoroughly mixed with the soil on a clean polyethylene sheet and returned to each pot. Other nutrients were applied in solution.

The soils were wetted to field capacity and allowed to equilibrate for 48 hours. Ten seeds of soybean (Var. Samsoy 2) were sown in each pot. After a week, the seedlings were thinned to four per pot and the soil kept at field moisture capacity using deionised water.

#### 3.1.4 Harvesting and other Measurements

At 42 days after planting, when some plants had started blooming, the height was measured and the total leaf number counted. The plants were then clipped at the soil level using a stainless steel blade and carefully washed free of soil in de-ionised water. The clean samples were oven-dried at a temperature of about 70°C in a forced-air oven to constant weights. The plant material was then ground to pass through 40 mm mesh screen for laboratory analyses.

#### 3.1.5 Soil Analyses

Particle size distribution analysis was carried out by hydrometer method (Bouyoucos, 1962). Soil pH was determined with a Pye Unicam model 290 MK2 pH meter in 1:2.5 soil: water suspension. Organic carbon was determined by the wet-oxidation method of Walkey-Black described by Allison (1965).

Exchangeable bases were displaced with 1N  $\text{NH}_4\text{OAc}$  buffered at pH 7.0 (IITA, 1979). Potassium in the filtrate was determined by flame photometry while calcium and magnesium with a Pye Unicam model SP 192 Atomic Absorption Spectrophotometer. Exchangeable acidity was determined by shaking soil with 1N KCl and the filtrate titrated with 0.5N NaOH (IITA, 1979). The Effective Cation Exchange Capacity (ECEC) of the soil was then obtained by summing up the exchangeable bases and exchangeable acidity.

Available phosphorus was determined by the Bray I method described by Jackson (1962). Total nitrogen in the soil was

determined by the micro-Kjeldahl procedure (Bremner, 1965) by digesting 1g of soil in concentrated  $H_2SO_4$ /catalyst mixture. The digest was distilled into Boric acid and titrated with dil.  $H_2SO_4$ . Copper, manganese, and zinc were extracted with 0.1N HCl and each determined by atomic absorption spectrophotometry (Osiname, *et al.*, 1973).

Available sulphur was determined by the Chensin and Yen (1951) turbidimetric method as modified by IITA (1979) by shaking 5g of air-dried soil with 25 ml of 500 ppm  $KH_2PO_4$  for 30 minutes. The resulting soil-extractant mixture was centrifuged and filtered through a whatman No. 1 filter paper. To 10 ml of the filtrate was added 1 ml of gelatin- $BaCl_2$  reagent in a 25ml volumetric flask. The mixture was determined on a B&L spectronic-70 electrocolorimeter. The sample readings were compared with a series of standards - 0, 25, 50, 75, 100 and 125  $\mu g$   $SO_4-S$  per 25ml prepared from reagent grade anhydrous potassium sulphate ( $K_2SO_4$ ). The extracting solution was used as the control.

### 3.1.6 Plant Tissue Analyses

0.5g of the milled plant material was weighed into Tecator digestion tubes and digested with an acid mixture of  $HNO_3-HClO_4$  acids in 1:2.5 ratio. Ca, Mg, Cu, Zn, Fe and Mn in the digest were determined by atomic absorption spectrophotometry. Potassium in the digest was determined by flame photometry and phosphorus colorimetrically by the phosphomolybdate-yellow method (IITA, 1979). Total nitrogen in the plant tissue was determined by the micro-Kjeldahl

method (Bremner, 1965).

Total sulphate-sulphur in the plant was also determined by digesting the plant samples with nitric-perchloric acid mixture. The digestion was continued for two hours after the 'dense fume' produced by  $\text{HClO}_4$  had cleared. To aliquots of the digest was added 1 ml gelatin- $\text{BaCl}_2$  reagent. The sulphate concentration in the resulting turbid mixture was determined using a B and L spectronic 70 as described by Tabatabai and Bremner (1970)

### 3.1.7 Statistical Procedure

The data were subjected to simple analysis of variance, and the means compared using the least significant difference (L.S.D) test at 5% level of probability.

## 3. 2 Field Experiment

### 3.2.1 Site Description

The field trial was conducted at Samaru ( $11^{\circ}11'N$ ;  $7^{\circ}30'E$ ) on the Agronomy Research Farm. The field used had been under continuous cultivation, and prior to the establishment of this experiment it had not received any sulphur-bearing fertilizers for the past three successive years.

The results of the greenhouse studies formed the basis of the site selection for the field experiment. The site chosen was one on which response to sulphur fertilization was considered most probable. A description of the Agronomy Research Farm, and of the soil and climatic conditions at

Samaru in general, have been given above.

### 3.2.1 Treatments and Field Operations

The response of soybean to sulphur was tested to four levels of sulphur (0, 10, 20 and 40 kg ha<sup>-1</sup>) applied as gypsum (18.6% S). The experiment was laid out in a randomised complete block design with six replications, giving a total of twenty four plots.

Each gross plot (2.1 x 10<sup>-3</sup> ha) consisted of seven ridges measuring 4m x 0.75m with a net plot size of 1.2 x 10<sup>-3</sup> ha made up of the three centre ridges. Soybean (var. Samsoy 2) was drilled at 5cm apart on the ridge. Basal fertilizer treatments consisted of 25 kg N- and 27 kg P ha<sup>-1</sup> applied as diammonium phosphate (DAP), and 30 kg ha<sup>-1</sup> K applied as muriate of potash. The fertilizers were banded in a small furrow of about 5cm deep and 8cm from the centre of each ridge, immediately after ridging, and covered with soil before seeding.

### 3.2.3 Soil and Plant Sampling

Prior to the ploughing of the field, surface soil samples (0-20cm) were randomly taken from about 15 points. The samples were bulked, air-dried and sieved through a 2 mm-screen and analysed for particle size distribution and selected chemical parameters.

Soybean index leaves, the upper most "fully mature" (3rd or 4th from growing terminal) trifoliate leaves, were randomly taken from about 50-60 plants per plot when the

plants had attained the 50% flowering stage. The samples were rinsed in demineralised water and oven dried for 48 hours at 70°C. They were then milled to pass through a 40 mesh screen and analysed for macro-and micro- nutrients by the procedures outlined under greenhouse experiment.

#### 3.2.4 Nodule Sampling

This was also carried out at the 50% flowering stage but after the index leaves had been sampled. Six plants from the ridges designated discards were carefully uprooted and excised of their nodules on plot by plot basis. The nodules were counted and washed clean of adhering soil particles. They were then dried at 70°C to constant weight.

#### 3.2.5 Harvesting

The crop was grown to full maturity and harvested when the pods were dry; just at the commencement of shattering. The plants with the pods were air-dried, threshed and winnowed. The dry weight of the beans were then taken on a plot by plot basis, and the yield (per ha) estimated therefrom.

#### 3.2.6 Statistical Procedures

The effect of of the sulphur treatments on the grain yield, nutrient concentration, nodule count and weight were statistically analysed by employing the simple analysis of variance technique, and the means compared using the least significant difference (LSD) test at 5% level of probability.

## CHAPTER 4

## 4 RESULTS AND DISCUSSION

## 4.1 Greenhouse Experiment

The physical and chemical characteristics of the soils used for the greenhouse studies are presented in Table 1.

## 4.1.1 Effect of Treatments on plant Height and Leaf Number

Plant height was significantly increased on the Yandev and Zonkwa soils (Table 2). The results also show that in most cases, the  $10 \text{ mg kg}^{-1}$  S-rate produced the tallest plants. It would appear that ammonium sulphate applied to supply more than  $10 \text{ mg S per kg soil}$  produced ammonia in sufficient concentration to adversely affect plant growth. Baker et al., (1966) found that the growth of bean plants receiving  $\text{NO}_3\text{-N}$  was better than plants treated with  $\text{NH}_4\text{-N}$ . Gasser (1964) also observed that seedlings grown in sandy soils suffered from ammonia toxicity when urea or sulphate of ammonia were the sources of nitrogen.

It is also possible that the application of sulphur above  $10 \text{ mg kg}^{-1}$ , especially on the relatively more acidic Yandev and Zonkwa soils (Table 1) might have lowered the pH of the soil appreciably resulting in the reduction in growth. Hassan and Olson (1966) found that applying sulphur at rates greater than  $50 \text{ mg kg}^{-1}$  on soils with pH ( $\text{H}_2\text{O}$ ) less than 5.7 had acidifying influence on soils.

Table 1. Selected physical and chemical characteristics of the soils used in the Greenhouse study

Soil parameter	Location				
	Ankpa	Yandev	Lafia	Zonkwa	Samaru
Particle size distribution (%)					
Sand	90.56	88.28	66.56	60.56	50.56
Silt	4.00	8.00	22.00	22.00	41.00
Clay	5.44	3.72	11.44	17.44	8.44
Textural class	Sand	loamy sand	sandy laom	sandy loam	loam
pH (H <sub>2</sub> O)	6.3	5.3	6.1	5.1	5.4
Organic carbon (%)	0.29	0.21	0.41	0.55	0.34
Total N (%)	0.028	0.041	0.056	0.133	0.042
Available P (ppm)	5.40	2.79	5.18	3.75	3.48
Available SO <sub>4</sub> -S (ppm)	3.00	1.40	3.87	7.44	3.62
Exch. cations (meq/100g)					
Ca	2.43	1.37	4.80	4.89	3.52
Mg	0.56	0.26	1.12	1.35	1.06
K	0.10	0.11	0.16	0.30	0.20
Exchange acidity	0.20	0.60	0.25	0.60	0.50
ECEC	3.47	2.50	6.55	7.35	5.46
Extractable micronutrients (ppm)					
Mn	64.76	19.79	54.77	21.03	24.35
Zn	2.00	2.43	1.66	3.16	1.11
Cu	0.56	1.62	0.76	1.81	0.93

Table 2. Effect of sulphur on plant height at each site (cm)

Sulphur Rate (mg kg <sup>-1</sup> )	Ankpa	Yandev	Lafia	Zonkwa	Samaru
0	57.3	26.0	67.1	28.9	49.6
10	57.0	36.2	79.8	62.2	65.3
20	51.9	38.2	67.4	36.8	59.6
40	41.2	47.4	64.5	32.3	75.2
LSD (0.05)	NS	6.23	NS	15.66	NS
(0.01)	NS	9.44	NS	23.72	NS

NS = Not significant at 5% level of probability.

Sulphur application did not produce any statistically significant effect on leaf number.

#### 4.1.2 Effect of Treatments on dry matter Yield

The dry matter yield of soybean at 42 days after planting is presented in Tabel 3. The results show that plant response to applied sulphur varied from soil to soil. Whereas there were significant yield increases on the Ankpa and Samaru soils the effect of treatments on the Yandev, Lafia and Zonkwa soils were non significant.

Table 3. Effect of Sulphur on dry matter yield at harvest (g/pot)

Sulphur Rate (mg kg <sup>-1</sup> )	Ankpa	Yandev	Lafia	Zonkwa	Samaru
0	2.27	1.55	3.07	2.80	2.87
10	3.46	3.13	3.75	2.80	4.10
20	3.44	2.87	4.04	2.62	3.24
40	2.82	2.97	4.14	3.34	3.45
LSD (0.05)	0.678	NS	NS	NS	0.817

NS = Not significant at 5% level of probability.

The highest dry matter yield was obtained at the 10mg kg<sup>-1</sup>) S rate. Increasing sulphur above 10 mg kg<sup>-1</sup>) resulted in decreases in the dry matter produced except on the Lafia soils.

The non-significant effect obtained on the Zonkwa soils might be due to the relatively high (7.44 ppm) sulphur content of the soil (Table 1). Similarly, the general fertility level of the Lafia soil also appeared adequate for the level of dry matter produced.

With pH values of 5.3 and 5.1, respectively for Yandev and Zonkwa soils, plants growing on these might have been affected by some soil acidity factor such as excess manganese or aluminium. These soils produced relatively lower dry matter yields compared to the other soils. The height of plants grown on these soils (Table 2) lends credence to this assertion.

The reduction in the dry matter produced with increasing amounts of applied sulphur above the 10 mg kg<sup>-1</sup> suggests that applying this nutrient above 10 mg kg<sup>-1</sup> may create some deleterious effects capable of reducing the productivity of soybean. However, the general increase in dry matter yield above the control on most of the soils indicates that the sulphur content of the soils is approaching a point where a response in yield might be obtained.

#### 4.1.3 Effect of Treatments on Nutrient Concentration and uptake

##### (a) Nutrient Concentration

The application of sulphur generally tended to increase the nitrogen, phosphorus and potassium concentration in the plant tissue above the control on all the soils (Table 4). However, only the concentrations of nitrogen and potassium attained statistical significance on the Yandev soil. Nitrogen and sulphur are involved in protein metabolism and they have been found to improve uptake of one another (Hazra, 1988). The chemical characteristics of the soils used in this study (Table 1) show that the native sulphur content of the Yandev soil was low (1.4 ppm). The application of S to this soils might have enhanced the uptake of N especially at the 10 mg kg<sup>-1</sup> S rate. Above this rate, deleterious effects possibly, due to increase in the acidity of the soil might have led to a decrease in the growth of the plant and consequently uptake of N. The results obtained in relation to phosphorus concentration corroborate the report by Hazra (1988) who found that adding S and P upto a certain level resulted in greater utilization of P in soybean.

Potash has not been known to be directly related to sulphur nutritionally except where sulphur deficiency is obvious as was the case with the Yandev soil (Table 1). The application of sulphur at the 10 mg kg<sup>-1</sup> rate might have enhanced the growth of the plants and improved the uptake of K. Above this rate, the acidifying effect of sulphur might have had debilitating effects on the growth, uptake and K-

Table 4. Effect of sulphur on the concentration of N, P, and K (%) in soybean plants

Sulphur Rate (mg kg-1)	ANKPA			YANDEY			LAFIA			ZONKWA			SAMARU		
	N	P	K	N	P	K	N	P	K	N	P	K	N	P	K
0	4.55	1.47	4.70	3.08	1.43	4.34	3.20	0.93	3.48	3.54	0.33	2.66	4.92	0.66	2.56
10	5.04	1.86	4.49	5.43	1.52	5.72	5.15	1.53	3.68	4.25	0.42	3.07	6.91	0.68	2.45
20	6.63	1.48	4.09	5.41	1.54	4.27	3.59	1.53	3.89	5.02	0.66	2.25	6.63	0.68	2.58
40	6.58	1.69	4.09	8.39	1.47	3.93	3.57	1.37	3.07	4.64	0.48	2.25	6.02	0.66	2.69
LSD .05	NS	NS	NS	1.852	NS	0.927	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significant at 5% level of probability

concentration of the nutrient.

The results presented in Table 5 show that the concentration of calcium and magnesium were significantly increased at the  $10 \text{ mg kg}^{-1}$  rate on the Yandev soil. Above this rate sulphur application either had no effect or decreased the concentration of the nutrients. Table 1 shows that the native soil contents of these nutrients in the Yandev soil were low. The increase in the tissue concentration at the  $10 \text{ mg kg}^{-1}$  rate might have been due to enhanced plant growth. However, above this rate, it appears that acidifying effect of sulphur had negative effects on the growth of the plants, and uptake and concentrations of the nutrients. The concentration of calcium in the plants grown on the Lafia soil significantly decreased with the application of sulphur. In addition, the decrease was progressive as the rate of sulphur application increased. This result clearly demonstrates the acidifying effect of sulphur and its consequence on the uptake of calcium and magnesium. The result corroborates the findings of Matthers (1970), and Aulakh and Dev, (1978) who postulated that sulphur enhance the uptake of calcium and magnesium on calcareous soils but has negative effects on acidic soils.

The concentration of sulphur in the plant tissue showed progressive increase with the application of sulphur on all the soils but attained statistical significance on the Ankpa and Yandev soils only (Table 5). These soils both had the lowest sulphur contents and were sandier than the other soils used in this study. The significant difference obtained with

Table 5. Effect of sulphur on the concentration of Ca, Mg, and S (%) in soybean plants

Sulphur Rates (mg kg-1)	ANKPA			YANDEV			LAFIA			ZONKWA			SAMARU		
	Ca	Mg	S	Ca	Mg	S	Ca	Mg	S	Ca	Mg	S	Ca	Mg	S
0	0.62	0.47	0.33	0.27	0.29	0.26	0.56	0.52	0.29	0.47	0.57	0.31	0.54	0.52	0.25
10	0.57	0.54	0.33	0.47	0.33	0.34	0.46	0.52	0.31	0.44	0.43	0.34	0.70	0.45	0.27
20	0.26	0.47	0.42	0.35	0.24	0.53	0.31	0.50	0.35	0.23	0.43	0.34	0.60	0.46	0.32
40	0.29	0.54	0.70	0.26	0.26	0.64	0.40	0.58	0.38	0.23	0.51	0.37	0.54	0.54	0.33
LSD .05	NS	NS	0.177	0.098	0.068	0.225	0.083	NS	NS	NS	NS	NS	NS	NS	NS
.01			0.269	0.148		0.126									

NS = Not significant at 5% level of probability

the application of sulphur confirms that these soils were obviously deficient in sulphur. Although the sulphur concentration of the plants grown on the Yandev soils showed significant increases, the dry matter yield did not (Table 3). This finding tends to confirm the speculation that some other factor like excess aluminium (due to low pH) other than sulphur availability alone was restricting maximum dry matter production.

The effect of the treatments on the micronutrient concentration is presented in Table 6. The results portray a general increase in the concentration of the micronutrients with the application of sulphur. However, only the concentration of iron in plants grown on the Samaru soil showed significant difference. It has been established that the solubility of micronutrients increases when the pH of the soil is low (Bertramson *et al.*, 1950; Hassan *et al.*, 1970). The high concentration of these micronutrients in the plant tissue is a further proof that sulphur lowered the pH of these soils thereby solubilizing the micronutrients.

#### **(b) Nutrient Uptake**

The application of sulphur led to a significant increase in nitrogen and phosphorus uptake on the Ankpa and Lafia soils. The uptake of potassium on the other hand was not affected on any of the soils (Table 7). These results further demonstrated the nutritional relationship between sulphur on one hand, and N and P on the other. As has been noted above,

Table 6. Effect of Sulphur on the concentration of micronutrients in soybean plants(ppm)

Sulphur Rate (mg kg-1)	ANKPA			YANDEV			LAFIA			ZONKHA			SAMARU		
	Fe	Zn	Cu	Fe	Zn	Cu	Fe	Zn	Cu	Fe	Zn	Cu	Fe	Zn	Cu
0	327	26.3	24.7	396	94.1	36.6	491	24.9	39.5	264	55.6	44.4	403	42.5	24.7
10	327	49.6	28.4	201	69.3	27.1	477	19.7	34.5	338	67.6	36.9	403	34.1	29.4
20	319	57.4	35.8	279	130.4	24.4	442	23.9	29.6	399	48.3	26.9	282	32.9	28.4
40	294	47.8	24.7	535	64.4	47.1	535	54.4	22.2	532	61.6	38.6	472	53.2	37.0
LSD .05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	.01														
	112.7														

NS = Not significant at 5% level of probability

nitrogen and sulphur are closely linked in protein metabolism and the uptake of either have been found to increase the plant tissue concentration and uptake of the other. In the case of phosphorus, conflicting views appear to have been presented in the literature. Aulakh (1977), and Hazra (1988), for instance, reported antagonistic relationship between sulphur and phosphorus in plant nutrition. The competition between sulphate and phosphate ions for the exchange site at the anion absorption site of the root has been offered as a possible explanation for this phenomenon. Some other workers on the other hand have reported a direct positive relationship. For instance, Pathak and Pathak (1972), and Kumar and Sigh (1980) recorded significant increases in the uptake of phosphorus with applied sulphur. The results obtained in this study appear to be in line with the second view.

Although the application of sulphur significantly increased the concentration of potassium on the Yandev soil, possibly through improved crop growth (Table 3), the uptake of K did not show statistical significant difference. This, again, is indicative of the assertion that other uninvestigated factors other than sulphur played a major role in influencing the growth and nutrition of the plants.

The results presented in Table 8 showed that the uptake of sulphur was significantly increased on the Anpka, Lafia and samaru soils, and magnesium on the Lafia soil following the application of sulphur. The treatments did not

Table 7. Effect of sulphur on N, P and K uptake (mg/pot)

Sulphur	ANKPA			YANDEY			LAFIA			ZONKWA			SAMARU		
	N	P	K	N	P	K	N	P	K	N	P	K	N	P	K
0	70.0	33.0	79.5	47.0	22.5	50.0	93.0	20.5	76.5	29.0	9.0	56.0	139.0	18.5	54.0
10	172.0	64.5	117.0	172.0	47.5	127.0	180.0	55.0	99.5	68.0	12.5	64.5	283.0	28.0	75.5
20	228.0	51.0	104.5	159.0	44.5	92.0	145.0	61.5	117.5	63.5	17.0	54.5	214.0	21.5	55.0
40	186.0	46.5	84.5	248.0	43.5	87.0	145.5	57.0	93.0	109.0	16.0	57.0	206.5	18.5	69.5
LSD .05	16.48	2.70	NS	NS	NS	NS	9.29	3.84	NS	NS	NS	NS	NS	NS	NS
.01	24.97	4.09											5.82		

NS = Not significant at 5% level of probability

Table 8. Effect of sulphur on Ca, Mg and S uptake (mg/pot)

Sulphur Rates (mg/kg)	ANKPA			YANDEU			LAFIA			ZONKWA			SAMARU		
	Ca	Mg	S	Ca	Mg	S	Ca	Mg	S	Ca	Mg	S	Ca	Mg	S
0	14.5	11.0	8.0	4.5	4.5	4.0	16.5	16.5	8.5	13.0	16.0	8.5	16.0	15.0	7.5
10	17.0	18.5	11.5	14.5	10.5	10.5	17.5	20.0	11.5	16.0	12.0	9.5	29.0	20.5	11.0
20	11.0	16.5	14.5	10.0	7.0	15.0	12.5	20.5	14.0	6.5	11.0	8.5	15.5	15.9	10.5
40	8.5	15.5	21.0	8.0	7.0	20.0	16.5	24.0	15.5	8.0	17.0	11.0	18.5	19.5	11.5
LSD .05	NS	NS	1.35	NS	NS	NS	NS	0.82	0.63	NS	NS	NS	NS	NS	0.55
	.01														
	0.96														

NS = Not significant at 5% level of probability.

significantly affect sulphur uptake on the Yandev and Zonkwa soils. Similarly, the uptake of calcium was not affected by the treatments on any of the soils. The fact that sulphur fertilization did not affect sulphur uptake on the Zonkwa soil is suggestive that the available native soil sulphur (7.44 ppm) was more than enough to accommodate any "luxury" uptake and so the additional sulphur supplied made no difference to the amount that could be taken. The probable influence of other factors other than sulphur, mentioned above, could have been responsible for the results obtained on the Yandev soil.

There is a paucity of information on the interaction between sulphur and calcium, and sulphur and magnesium in the literature. However, sulphur has been found to increase the uptake of Ca and Mg on calcareous soils (Matthers, 1970; Aulakh and Dev, 1978) and this has been attributed to the ability of sulphur to lower the soil pH in the zone of application thereby making soil conditions more favourable for nutrient uptake. The non significant results obtained in this study would suggest that the factors influencing the uptake of these nutrients were optimum in these soils, and not dependent on sulphur application. The significant increase in Mg uptake on the Lafia soil might be just a chance occurrence.

The effect of applied sulphur on the uptake of micronutrients (Table 9) show that only the uptake of iron

Table 9. Effect of sulphur on micronutrient uptake (µg/pot)

Sulphur Rates (mg/kg)	ANKPA			YANDEV			LAFIA			ZONKWA			SAMARU		
	Fe	Zn	Cu	Fe	Zn	Cu	Fe	Zn	Cu	Fe	Zn	Cu	Fe	Zn	Cu
0	745	60	55	590	125	55	1425	70	125	740	155	90	1170	120	70
10	1140	165	100	650	225	55	1800	65	120	946	190	105	1660	140	115
20	1095	200	120	785	375	70	1810	95	115	1060	125	70	930	110	90
40	860	137	70	1135	150	125	2290	245	95	1675	210	140	185	185	125
LSO 0.5	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	6.32	NS	NS

NS = Not significant at 5% level of probability.

was significantly increased on the Samaru soil. Sulphur application did not significantly affect the uptake of copper and zinc on any of the soils. However, the treatments generally tended to improve the uptake of these nutrients. Tisdale and Bertramson (1950) explained that the increases in micronutrient uptake with sulphur application is due to its ability to lower soil pH. The solubility of most micronutrients is enhanced under low pH. The increased uptake of iron as a result of sulphur application does not portend a beneficial effect on the acidic soils of the savanna which are already well supplied with this nutrient.

#### 4.1.4 Effect of Treatments on Plant Nitrogen: Sulphur ratio

The total nitrogen: total sulphur ratio in the plant has been used to diagnose the sulphur nutrition of some crops. However, a number of workers (Kang and Osiname, 1976; Fox, 1976; Fox *et al.*, 1977) reported considerable fluctuations in this ratio with the age of the plant, and this constitutes a limit to the application of this index. It is evident from Table 10 that the N/S ratios obtained in this study do not show any consistent trend.

Table 10. Effect of sulphur on total nitrogen and sulphur concentration, and N/S ratio of soybean plants at harvest

Sulphur Rates (ug/g)	ANK/PA			YANDEV			LAFIA			ZONKWA			SAMARU		
	Total N (%)	Total S (%)	N/S ratio	Total N (%)	Total S (%)	N/S ratio	Total N (%)	Total S (%)	N/S ratio	Total N (%)	Total S (%)	N/S ratio	Total N (%)	Total S (%)	N/S ratio
0	4.55	0.33	13.64	3.08	0.26	12.63	3.20	0.29	10.92	3.64	0.31	11.89	4.92	0.25	20.25
0 10	5.04	0.33	15.18	5.43	0.34	16.24	5.16	0.31	16.46	8.50	0.34	24.96	6.91	0.27	25.99
20	6.63	0.42	16.51	5.41	0.53	11.80	3.59	0.35	10.11	10.04	0.34	29.56	6.63	0.32	20.86
40	6.58	0.70	9.66	8.39	0.64	13.90	3.57	0.38	9.37	9.28	0.37	25.68	6.02	0.33	15.94
LSD .05	NS	NS	NS	1.852	1.076	NS	NS	NS	NS	1.620	NS	6.284	NS	NS	NS
										2.454		9.521			

NS = Not significant at 5% level of probability.

## 4.2 Field Experiment

Selected soil physical and chemical properties of the experimental site have been presented in section 4.1 above. The soil was a loamy Alfisol derived from aeolian deposits underlain by Basement Complex materials.

### 4.2.1 Effect of Treatments on the Nutrient Composition of Soybean Index Leaves

The effect of sulphur treatments on nutrient concentration of soybean index leaves was not significant. The critical sulphur concentration for soybean index leaves (0.25%) as at 50% flowering has been given by Nelson and Barber (1964). Based on this, it may be concluded that the sulphur content of the plants obtained in this study was below the critical level. However, it is an established fact that the critical level of a plant nutrient generally vary with crop variety, and the environment. The value estimated by Nelson and Barber (1964) might not be applicable to the variety used in this study. Moreover, no deficiency symptoms were observed. The N:S ratio in the leaves (Table 11) were all above the value of 17 considered critical for legumes (Thompson *et al.*, 1970) and this might mean that the plants did not suffer sulphur deficiency.

Table 11. Effect of sulphur on total nitrogen and sulphur concentration, and N:S ratio of soybean index leaves.

Sulphur Rate (kg/ha)	Total N (%)	Total S (%)	N:S ratio
0	4.25	0.21	19.75
10	4.01	0.22	18.52
20	3.96	0.23	17.64
40	4.32	0.22	19.52
LSD (0.05)	NS	NS	Ns

NS = Not significant at 5% level of probability.

With regards to the other nutrients. Small and Ohlogge (1973) gave the lower limit of sufficiency range for upper fully developed trifoliolate leaves of soybean sampled before pod set as 4.2% N, 0.26 P, 0.17% K, 0.36% Ca, 0.26% Mg, 10 ppm Cu, 51 ppm Fe, 21 ppm Mn, and 21 ppm Zn. Although the sufficiency levels of nutrients is a function of several factors the values obtained in this study fall within the range considered sufficient for optimum soybean yield.

#### 4.2.2 Effect of Treatments on Nodulation

The results furnished in Table 12 show that sulphur application marginally increased nodulation. However, increasing sulphur rate to 40 kg/ha significantly reduced nodule number when compared to that obtained at the 10 kg/ha S-rate. Nodule weight was not significantly affected by the treatments. It was also observed that the treatment that recorded the highest nodule number had more smaller nodules compared to those that had fewer total nodules per plant. In

addition, most of the small nodules were hollow after oven-drying indicating that they were ineffective

Table 12. Effect of Sulphur on Nodule number, average weight of nodules, and grain yield

Sulphur Rate (kg/ha)	Nodule number per plant	Average weight of (mg/plant)	Average weight of (mg/nodule)	Grain yield (kg/ha)
0	56	455.53	8.13	3310
10	83	649.17	7.82	3368
20	60	959.50	12.66	3368
40	40	612.75	15.32	3192
LSD (0.05)	27.82	NS	NS	NS

NS = Not significant at 5% level of probability.

From the results obtained in this study and reports in the literature (Bergessen, 1971; Gates *et al.*, 1971) on the importance of sulphur in nodule formation and nitrogen fixation it may be adduced that the application of sulphur led to the stimulation of rapid nodule development. However, the reduction in nodulation with increase in the rate of applied sulphur above 10 kg/ha might have been due to the creation of an unfavourable environment which could have affected the activities of microbes involved in nodulation.

Though microbiological investigations were not carried out in this study, the inability of the appropriate *Rhizobium* strain to colonise the rhizosphere and make nodules functional as a result of the antagonism exercised by other rhizosphere microorganisms or unfavourable environment is well documented in the literature (Hely *et al.*, 1957). Gates and Muller (1979) also reported that nodulation is extremely sensitive to levels of nutrients in the rhizosphere. One or

more of the above might have had an influence on the pattern of nodulation obtained in this study.

The low average weight of nodule per unit nodule obtained at the 10 kg/ha sulphur rate suggests that although this rate of application tended to increase the nodule number per plant, some of the nodules were ineffective. The implication of this is that the increase in nodule number does not always portend increased N-fixation. This finding lends credence to the suggestion by Gates (1974) that where the total nitrogen fixed at anytime is a measure of the nitrogen fixed by all nodules on the plant, the rate of fixation per unit nodule weight is the best indicator of the efficiency of the symbiosis and not the absolute number of nodule per plant.

#### 4.2.3 Effect of Treatments on Grain Yield

The effect of sulphur fertilisation on soybean grain yield presented in Table 12 shows that sulphur application had no significant effect on the grain yield. Increasing sulphur to 40 kg/ha depressed grain yield. The depressive effect was to such an extent that the yield was less than that obtained on the control plot.

It would be recalled that significant dry matter yields were obtained under greenhouse conditions with soils taken from the same site. Under greenhouse conditions the volume of soil is restricted, the nutrients available for uptake would therefore also be expected to be restricted. Such physical

restrictions did not exist under field conditions. The difference in crop response obtained in the greenhouse vis-a-vis the field would be attributed to the restrictive volume in the soil used in the greenhouse experiment.

The slight yield depression following the application of 40 kg S/ha was probably a result of unfavourable growing conditions such as low soil pH or nutrient imbalance. There are no reports available on the optimum rate of sulphur fertilization for field crops in the Nigerian savanna. But Sanchez (1975) has given 10 to 20 kg S/ha as rates that would be enough to alleviate sulphur deficiency problems of most crops grown in the tropics. The results of the present study showed that the highest dry matter and grain yields were obtained at 10 mg S/kg and 10 kg S/ha, in the greenhouse and on the field, respectively.

## GENERAL DISCUSSION

Protein-rich crops generally have a high requirement for sulphur. For this reason they generally respond well to sulphur. The results of the greenhouse studies in this study showed significant dry matter yield response on two of the soils taken from the Guinea savanna. The field trial was established at one of the sites which, from the greenhouse studies, had the greatest potential of giving some response to sulphur fertilization. The field trial gave no significant yield response to applied sulphur although there were slight increases above the control. The difference in the responses obtained in the greenhouse and field studies have been attributed to the limited soil volume in the greenhouse study, and as a result of which the plants were able to exhaust the nutrients in the soils that were not well supplied with the nutrient being tested. On the field, the plants could probably forage for their nutrient needs from a wider area through root proliferation and deep root penetration.

Isolated cases of sulphur deficiency problems have been reported on groundnuts in the Nigerian savanna under field conditions (Greenwood, 1954; Goldsworthy and Heathcote, 1963; Bromfield, 1973 and 1975). The contrast between the results obtained in this study and the ones cited above, apart from differences in the location of the experiments, could be attributed to the rooting nature of the crops. It has been reported (Cooper, 1971; Bromfield, 1972) that most sulphur

applied on the Nigerian savanna soils, if not taken up by plants, can be leached to the subsoil where it is adsorbed. As a shallow rooted crop, the roots of groundnuts (which is usually grown on ridges constructed from surface soil) cannot reach the subsoil to extract the sulphate adsorbed there. On the other hand, soybean, is deep rooted and thus capable of exploiting such subsoil sulphur sources.

Goldsworthy and Heathcote (1964) also reported significant soybean response to sulphur fertilization in the southern Guinea savanna zone of Nigeria. Although the available sulphur content of the plots on which they conducted their trials were not presented, the soils were texturally sandier than the one used in this study. The sulphur level of the soils used in their study could have therefore been comparably lower. Differences in the general environmental conditions of the experiments being compared could have also accounted for the differences in the results. Apart from these, crop varieties differ in their requirement for nutrients. The generally high yield levels attained and the absence of visual sulphur deficiency symptoms would suggest that the soil sulphur level could have been adequate for the soybean variety grown in this study.

The results obtained in this study taken with earlier experiments with this nutrient strongly point to imminent sulphur deficiency problems in the savanna. However, so long as single superphosphate remains the phosphate source for crops grown in this agroclimatic zone, the sulphur

requirement of most crops would be met. For example, the current recommended application rate of 27 kg P/ha for soybean would also supply about 40 kg S/ha if the fertilizer material is single superphosphate.

#### CONCLUSION

Although no significant yield response was obtained on the field, the marginal increases in the soybean grain yield coupled with the significant dry matter yield responses obtained on the Samaru Alfisol and Ankpa Oxisol in the greenhouse indicates that sulphur deficiency problems in the Nigerian savanna is imminent. Prolonged use of fertilizers devoid of sulphur on these soils would accentuate the problem.

Although the results of the present investigation have, inevitably, to be interpreted with some caution in view of its limited scope, the study has nevertheless supplied some useful information which may form the basis for more detailed investigations in the future. Part of such future studies would be a complete assessment of the sulphur status of major agricultural soils of the Nigerian savanna, the contribution of sulphur level in the subsoil immediately below the plough layer, and the interaction of sulphur with other elements also needs to be evaluated.

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