SOME EFFECTS OF BORON TO THE GROWTH AND CHEMICAL COMPOSITION OF SAINFOIN (*ONOBRYCHIS VICIAEFOLIA* SCOP.)

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ABSTRACT

Some effects of boron on the growth and chemical composition of sainfoin (*Onobrychis viciaefolia* Scop.) plants cv Fakir were evaluated in a glasshouse.

The growth and development of sainfoin plants was not affected by the different levels of boron applied but was affected by nitrogen application and inoculation due to the nodulation failure of the latter. Generally, the root showed the highest dry matter yield and the fastest relative growth rate.

Similarly, the total nonstructural carbohydrates of the sainfoin plants were not affected by the different levels of boron. Nitrogen application reduced the total nonstructural carbohydrates of the whole plant. Moreover, when 1 ppm boron was applied, both the shoot and the root yielded the highest total nonstructural carbohydrates. Likewise, root and shoot total nonstructural carbohydrates were reduced by the application of nitrogen. Roots gave a higher total nonstructural carbohydrate yield than the shoot.

Boron content of the whole sainfoin plant, the shoot and the root ranging from 0 - 55 µg/g increased in proportion with the increment of boron applied. Similar results were obtained from boron uptake of the whole plant, the shoot and the root. There was a depression of boron concentrations and boron uptake of the whole plant, the shoot and the root, when nitrogen was applied, implying a deficiency situation.

Although nonsignificant effects of boron levels were obtained from nitrogen and phosphorus concentration and uptake, respectively, of both shoot and root, application of 2 ppm boron reduced the concentration of nitrogen but not nitrogen uptake, and reduced phosphorus concentration and phosphorus uptake. Application of nitrogen increased shoot and root nitrogen contents and nitrogen uptake but decreased root and shoot phosphorus concentrations and phosphorus uptake.

It was concluded that levels of 2 ppm boron concentration were not adequate to support satisfactory growth when plants were supplied with sufficient levels of other nutrients.

Keywords: Boron, nitrogen, *Rhizobium*, total nonstructural carbohydrates (TNC)

INTRODUCTION

Sainfoin (*Onobrychis viciaefolia* Scop.), a newly reintroduced forage crop in New Zealand, has drawn considerable interest in the research field. It is thought to have a nutritional value, no bloating tendencies as a livestock feed and a potential substitute for alfalfa (*Medicago sativa* L.) (Clarke & Reid, 1974; Jones & Lyttleton, 1971; Usman *et al.*, 1968) and known to be a drought and winter hardy crop (Koch *et al.*, 1972; Spedding & Diekmahns, 1972). However, it has not been accepted agriculturally because of its excessive coarseness, poor leafiness and probable low palatability (Eslick, 1968), visual characters which can be misleading. In addition, it has been found to have poor establishment with Rhizobium infection, leading to poor nodulation, and thus to inefficient nitrogen fixation. In some cases, plants are nodulated abundantly but nodules are ineffective and are inefficient in fixing dinitrogen (Ross & Delaney, 1971; Burton & Curley, 1968; Sims *et al.*, 1968). Sainfoin, however, was reported to outyield alfalfa consistently in areas where production is limited to one cutting (Hanna & Smoliak, 1968). Likewise, it also grows better on drylands where precipitation is sufficient to grow one hay crop (Cooper, 1965). In addition, sainfoin cotyledons were found to contribute as much as 100, 54, and 18% of total seedling photosynthesis at 7, 11, and 19 days, respectively (Fransen & Cooper, 1974). Cotyledons of sainfoin, therefore, provide a substantial contribution to the nourishment of newly emerged seedlings, giving them a better establishment while the seedlings have no nodules yet to supply their nitrogen requirement.

Several legumes, especially forage species that are potentially capable of fixing dinitrogen efficiently have not been well studied. Due to the limited research that had been conducted on these forage legumes, i.e., cicer milkvetch (*Astralagus cicer* L.) and sainfoin, there is also limited knowledge on the proper management of these forage crops

(Major *et al.*, 1979). This includes water relations, temperature, light, soil and nutrient requirements and also proper cultural management practices, conditions that must be favourable for a successful plant growth and development. Likewise, the efficiency of the symbiotic relationship depends also on the host's capacity to provide its partner, i.e., *Rhizobia* the necessary energy requirement for dinitrogen fixation. Hence, a healthy growing legume plant has a greater capacity to provide the *Rhizobia* their energy requirement.

Despite the numerous disadvantages of growing sainfoin as mentioned previously, it has a greater potential as a livestock feed in the future. Research is needed to meet the need for more managerial and agronomic information on yield responses in terms of hay and total dry matter production as well as responses to symbiotic relationship.

One aspect of study which this project was designed to investigate is the role of micronutrient boron which is essential in plant growth and development, reproduction, carbohydrate metabolism, nitrogen metabolism, nodulation and nitrogen fixation.

The purpose of this study was to examine some of the effects of boron in plant growth and development. It included two components namely:

1. The effects of boron on the early growth and development of sainfoin in terms of dry matter production of both root and shoot, relative growth rate, root number, and leaf area, and
2. the effects of boron on the chemical composition of the sainfoin plant on the whole, specifically on boron concentration and uptake and total non-structural carbohydrate (TNC) content.

Analyses of both root and shoot tissues were completed to determine nitrogen and phosphorus contents.

2. REVIEW OF LITERATURE

2.1 INTRODUCTION

The purpose of this chapter is to review and report some information on the essentiality of boron to higher plants, on the boron nutrition of agricultural crops in the light of factors such as sources and availability of boron to plants, levels of boron deficiency and toxicity in plants and the physiological role of boron in plants and accumulation and distribution of boron in plants.

2.1.1 The Essentiality of Boron to Plants

Boron is one of the seven recognized essential micronutrients required for normal plant growth and reproduction (Wright & Lane, 1978; Gupta, 1979). Brenchley (1927) assigned boron to the first place among the trace elements based on the amount of observational and fundamental research on its relations with plants up to that date.

Essentiality of boron to higher plants as it affected the growth of maize (*Zea mays* L.) plants was first mentioned by Maze (1914) in France. However, it was the work of Warrington (1923) in England that provided firm knowledge of boron requirement for a variety of crops, findings on which Sommer (1927), and Sommer and Lipman (1926) based their works, respectively, which proved the essentiality of boron to higher plants. The field trials of Bradenburg (1931) demonstrating that heart rot of sugar beets was due to boron deficiency stand, however, as a major milestone in boron research which paved the way for borax to be accepted as a fertilizer constituent for a number of crops in a very short time.

Of the known micronutrient deficiencies, boron deficiency in plants is most widespread. Boron deficiency has been reported for one or more crops in 43 states of the United States (Sparr, 1970), almost all provinces of Canada, mainly on high sandy loam soils (Wright & Lane, 1978) and many other countries of the world (Stiles, 1961). Some of the most severe disorders caused by boron deficiency include brown heart of

cauliflower, and internal brown spot of sweet potatoes (Bradford, 1966; Wright & Lane, 1978; Ishizuka, 1978; Oliveros, 1979).

Among the essential mineral nutrients, boron is unique because it is the only element that is normally present in soil solution as non-ionized molecules over the pH range suitable for plant growth as shown in the results of the work Oertli and Grgurevic (1975). They reported that boric acid is the form of boron that plant roots absorb most efficiently. It was suggested by Alt and Schwartz (1973) that boron is absorbed as the molecule and that at high supply, boron is passively distributed with the transpiration stream.

Because of its non-ionic nature, boron can be leached from the soil fairly rapidly once it is released from soil minerals. This explains why soils in high rainfall areas are often deficient in boron (Gupta & Cutcliffe, 1978). On the other hand, the availability of boron decreased sharply under drought conditions, attributed partly to the reduced number of microorganisms than can release boron from the parent materials (Berger, 1965; Bowen, 1977) while moisture is not available to dissolve boron from tourmaline, the highly insoluble mineral source of boron (Reisenauer *et al.*, 1973). Lack of moisture reduces the mobility of boron, thus restricting its uptake by plant roots via a mass flow mechanism.

2.2 SOURCES OF BORON FOR PLANTS

Plants depend on two major sources of boron (Gupta, 1979); (1) soil boron, and (2) boron from commercially produced fertilizers. Additional sources include farmyard manure, sewage sluge, compost, and similar materials of which the percentage of boron in these sources depends on the origin of the material.

2.2.1 Determination of Boron in Plants and Soil

In recent years, reagents such as chromotropic acid, which form intensely colored complexes with boric acid in

aqueous solution have been developed to determine boron in aqueous extracts. Difficulties, however, had been encountered in automating this method due to the sensitivity to light of both the reagent and the borate-chromotropate complex (James & King, 1967). Quinalizarin and carminic acid are two of the chromogenic reagents which are specific and sensitive for boron determination. Their use in an automated procedure appears, however, to be limited because they must be used in a concentrated sulphuric acid (H_2SO_4) medium (Lionel, 1970; Willis, 1970). While the curcumin method when modified was found to be rapid and simple (Fiala, 1973), it gives results that vary due to the amount of salt content of the material used (Williams & Vlamis, 1970).

Boron can also be determined by spectrographic and atomic absorption spectrophotometric methods but these methods are not as sensitive to boron as the colour reagents, hence, their use has been limited.

A new colour-developing reagent, azomethine-H, originally used by Russian workers for determining boron in organic compounds, was first used by Basson *et al.* (1969) for determining boron in plant materials. This procedure has achieved prominence for determining boron in soil, compost and manure (Wolf, 1971) but was modified by J.A. Smith and D.A. Tel, University of Guelph, Ontario, Canada (Gupta, 1979).

The carmine method of Hatcher and Wilcox (1950) with some modifications of Gupta (1967a) was used to determine hot-water soluble boron in soils.

Boron in plants is determined by dry and wet ashing, the most common methods of extracting boron from plants. During the past ten to twenty years, the modified carmine method (Gupta, 1967a) and curcumin method (Vlamis & Williams, 1970) have been the most common methods used for determining boron in plants. With the advent of the azomethine-H colorimetric method in the late sixties, this colour reagent became a highly recommended procedure in analyzing boron both in plants and in soil.

2.2.2 Soil Boron

The principal forms of available boron in soils include that held in adsorbed forms of boron associated with clay materials and sesquioxides, that is, held by the mineral fraction and that associated with organic matter (Berger, 1949; Fleming, 1980).

According to Aubert and Pinta (1977), the total boron content of the soils range from 1-2 ppm (podzols of Bielorussia) to 250-270 ppm (eutrophic peaty soil of Israel), the average ranging from 20 to 200 ppm (Swaine, 1955; Berger & Pratt, 1963; Aubert & Pinta, 1977), of which the lowest values are found in soils derived from acid igneous rocks, from fresh-water sedimentary deposits and in soils low in organic matter. Gupta (1968) on a number of soils from Eastern Canada found that total boron ranged from 45 to 124 ppm, whereas hot-water soluble (hws) boron ranged from 0.38 to 4.67 ppm. Walsh and Golden (1952) recorded water-soluble boron levels on quartzite soils from 0.1 to 0.4 ppm boron; in the sandstone soils, it ranged from 0.3 to 0.7 ppm boron. This indicates that only a small fraction of total boron occurred in the available form, however, values of up to 1,000 ppm have been recorded from Peru (Fox, 1968). This is true in arid soils where boron salts accumulate, thus, water-soluble boron values may be very high (Fleming, 1980). Generally, less than 5% of the total boron is found in an available form, but Kick (1963) reported that hws averaged 15% of the total boron in some Egyptian soils. Very little is known about the mineral forms of boron in soils (Lindsay, 1972). The absorption of boron on oxides of iron and aluminium is believed to be an important mechanism governing boron solubility in soils and clays (Sims & Bingham, 1968). Boron availability in soils is associated with the level of decomposition of organic matter and with soil texture and soil pH. Available boron is held in organic fractions of soil and as organic matter decomposes, boron is released for plant use. Some boron is held by clay fractions and is easily leached unless utilized by plants or retained in the organic fraction (Griffith, 1973). Boron in soils is most available at soil pH below 6.5 (Aubert & Pinta, 1977; Wright & Lane, 1978). A low pH, however, inhibits the activity of microorganisms, and thus, reduces boron release. Excess lime also reduces boron availability (Wear &

Patterson, 1962). Peterson and Newman (1976) studied the effect of pH on the availability of added boron at pH levels of 4.7, 5.3, 6.3, and 7.4. Results showed that boron uptake by tall fescue (*Festuca arundinaceae* L.) was relatively uniform for the first four levels but a 2.5 fold drop in uptake occurred at pH 7.4 indicating a substantial fixation. In acid soils also, boron is more soluble but in sandy acid soils, this can result in considerable leaching of boron resulting in low levels.

The concentrations of total and plant-"available" boron may vary in relation to the humus and total organic matter concentrations and also in relation to soil texture (Fleming, 1980). The higher the percentage of clay and loam, the higher the boron present. This has been verified in the USA (Aubert & Pinta, 1977), in a greenhouse experiment on sandy and clayey soils where alfalfa removed boron more easily from a coarse-textured soil indicating that clay soils retained boron more.

The distribution of this element between the horizons of the soil profiles follows that of humus (Aubert & Pinta, 1977) resulting in an accumulation of boron in the humic regions of chernozems, in the humus illuvial horizons of the podzols and in the deep horizons of the peaty soils reported specifically in Poland. According to Wright and Lane (1978) a large part of the boron in soils is found in the organic portion of the topsoil. Subsoil boron levels are almost always lower than in the topsoil (Hodgson, 1963). During the periods of drought, crop roots cannot feed in these dry upper layers of soil and are forced to feed in the lower subsoil area, where the boron content is quite low. Robertson *et al.* (1975) had reported that the boron levels in the profiles of three typical sugarbeet-bean soils of Michigan ranged from none detectable in the subsoil to 0.54 ppm in the surface soil. Likewise, Reisenauer *et al.* (1973) stated that since soil boron is subject to movement within the soil profile, a translocation of applied boron to lower horizons will assure the crop an adequate supply of the nutrient during drought period, but excessive movement will result in leaching losses.

2.2.3 Boron from Commercially Produced Fertilizers

Boron can also be derived from boron fertilizers, sodium salts of which are the most common forms. Some of the most used forms with their chemical formulas and percentages of boron as described by Diamond (1972), Chesnin (1972), and Morrill *et al.* (1977) are given in Table 2a.

Table 2a. Percentages of boron and chemical formulas of boron sources.

Boron Source	Chemical Formula	B%
Borax	$Na_2B_4O_7.10H_2O$	11
Boric acid	H_3BO_3	17
Boron frits (contained in a moderate soluble glass)	$Na_2B_4.5H_2O$	10-17
Sodium Tetraborate		
Borate-46, Agribor, Tronabor	$Na_2B_{10}O_{16}.10H_2O$	14
Borate-65	$Na_2B_4O_7$	20
Sodium Pentaborate	$Na_2B_{10}O_{16}\cdot10H_2O$	18
Solubor. (partially dehydrated)	$Na_2B_4O_7\cdot5H_2O$ + $Na_2B_{10}O_{16}.10H_2O$	20-21

The most recently available source of boron is eluxite ($NaCaB_5O_9.8H_2O$) containing 49.6% B_2O_3 (Gupta, 1979). Commercially available eluxite contains 30% B_2O_3 (9.43% B) and is considered to release boron slowly as compared with other sources. Other kinds of boron-containing fertilizers include borated gypsum, calcium carbonate, superphosphate, calcium nitrate and various mixed fertilizers (Berger & Pratt, 1963).

The application rates of boron vary from 0.3 kg/ha for sensitive crops such as beans (*Phaseolus* spp.), to 3 kg/ha for boron-tolerant crops such as rutabaga and alfalfa (Gupta, 1979). In New Zealand, especially on the special pumice soils of the North Island, farmers often apply up to 5.1 kg/ha boron to lucerne every spring (Sherrell & Toxopeus, 1978).

The rates of boron applied for vegetable crops in North Carolina (Baird *et al.*, 1973) and Michigan (Vitosh *et al.*, 1973) are 0.5-3 kg/ha broadcast, 0.5-1.0 kg/ha banded, and 0.1-0.5 kg/ha applied as foliar spray (Mortvedt, 1974). Vegetables such as rutabaga are very sensitive to lack of boron and require more than other vegetables to control boron deficiency. Gupta and Cutcliffe (1978) stated that this crop may require up to 4 kg/ha broadcast and 2 kg/ha applied banded or as foliar spray on some podzol soils of Eastern Canada to overcome factors such as high soil pH, improper mixing, and uneven application of boron-containing fertilizers. Care must be taken to avoid excessive rates, the range between deficiency and toxicity being very narrow (Gupta, 1979).

Boron has been applied coated on to concentrated superphosphate and as a fine granular material mixed with individual plot-row fertilizer treatments (Morrill *et al.*, 1977). In many regions, boron-containing fertilizers are sold as 0.2, 0.3, or 0.4 B which means that the bulk NPK fertilizer contains 0.2%, 0.3%, or 0.4% actual boron weight. Solubor and boric acid are most commonly used in sprays. Solubor is also an ideal source of boron for addition to liquid fertilizers (Turner, 1975) since it is a product specially developed for speedy and economical correction of boron deficiencies in fruits, vegetables, and other crops.

2.3 ROLE OF BORON IN PLANTS

The role of boron in plants, perhaps, has the less precise information available than any of the other essential micronutrients. Boron is required by higher plants and some algae (Lewin, 1965; 1966a), but not by animals, fungi, or microorganisms (Clarkson & Hanson, 1980). Brenchley and Thornton (1925) however, strongly suggested that boron is essential to microorganisms, i.e., *Rhizobia* in its symbiotic relationship with legumes. Gerretsen and de Hoop (1954) concluded that boron was essential for *Azotobacter chroococcum* but later this was refuted by Anderson and Jordan (1961) who stated that boron

stimulated nitrogen fixation in the bacterium, but that the element could not be considered essential for it. McIlrath and Skok (1958) considered boron as an essential element for *Chlorella vulgaris* Beijerinck on the basis of its effects both on cell numbers and cell weights. Bowen, Gauch and co-workers (1965, 1966) have shown that a number of fungi do not require detectable amounts of boron nor does the alga, *Chlorella* sp. This was confirmed by Gerloff (1968) with another species of *Chlorella*, who found also that *Draparnaldia plumosa* Bory and *Stigeoclonium tenue* Kutzing, two other green algae species did not require boron either. However, Gerloff (1968) using the same techniques for boron analysis found that blue-green algae like *Nostoc muscorum* Vaucher, *Anabaena cylindrica* Lemm., and *Microcystis aeruginosa* Bory did require boron.

The function of boron remained almost obscure prior to mid-1950's. Its biochemical role is as yet not well understood and unlike other generally recognized micronutrients, it has not been shown to be a part of an enzyme system (Hewitt & Smith, 1974; Jackson & Chapman, 1975).

2.3.1 Role of Boron in the Growth, Development and Yield of Plants

What is known of boron requirement arises from studies of what happens when it is withheld. The earliest visible responses are cessation of meristematic growth of both root and shoot (Neales, 1959), and pollen tube growth (Clarkson & Hanson, 1980). Roots are particularly sensitive to the absence of boron; an example is a bean radicle attached to its cotyledonary reserves which was found to require boron in the medium if root elongation was to proceed for more than 48 hours (Whittington, 1957). The first morphological symptoms of boron deficiency in mungbean (*Phaseolus aureus* L.) appear to be a slowdown of root extension, followed by degeneration of meristematic tissue possibly due to a repressive effect of boron deficiency on cell division (Jackson & Chapman, 1975). Results of Robertson and Loughman (1974) indicated that it is unlikely that responses associated with a deficiency are caused by the interference with cell division but they may be related to the role of boron in metabolism, transport, and/or action of

auxin-type hormones found in broad beans (*Vicia faba* L.) (Whittington, 1959). In addition, earlier responses to withholding boron (6 to 10 hours) are seen in enhanced incorporation of precursors in root tip RNA in a fashion simulating that produced by auxin (Jackson & Chapman, 1975). Auxins also are known to stop meristematic cell division and cause cell swelling and in older tissues can cause callus to form (Key, 1969). Moreover, Gorter (1958) concluded that boron is not necessary for sugar translocation but rather for auxin metabolism possibly auxin synthesis, hence, the increased production of roots in *Phaseolus* cuttings by an application of boron in association with indole and indole-3-acetic acid (IAA). Also, Skok (1967) reported an association of boron to gibberellic acid (GA), a growth hormone. Withholding boron from GA-treated debudded tobacco (*Nicotiana tabacum* L.) plants greatly reduces the GA-proliferation.

Boron-deficient field bean roots had enlarged and fewer cells than the normal boron-sufficient roots. It was also suggested that in the absence of boron, division ceases because abnormalities in the formation of the cell wall prevent the cell from becoming organized for mitosis. Investigations of Kouchi and Kumazawa (1976) on tomato root tips indicated that lack of boron distorted the shape and arrangement of cortical cells and resulted in an abnormal accumulation of "lipid-like substances". There was also an abnormal development of the Golgi apparatus which seemed to be related to the irregular thickening of cell walls. Neales (1959 , 1964) stated that the growth of roots either attached to the plants or when excised and grown in sterile culture, was severely restricted in the absence of boron in the growth medium. In addition, Neales (1960) concluded that without boron in the root environment, the total linear growth of the radicles of the four dicotyledon and one monocotyledon species studied, was reduced. Albert and Wilson (1961) reported that root tip elongation of tomato plants stopped when boron was not available to the root because boron definitely influences early stages of cell development which involves enlargement. Yih and Clark (1965) observed from their test system with tomato plants grown at different levels of boron nutrient solution, a constant,

linear rate of elongation of roots grown with adequate amounts of boron but when boron was omitted from the nutrient solutions, roots stopped elongating within 24 hours.

Cohen and Lepper (1977) established that cessation of root elongation of intact squash plants (*Cucurbita pepo* L.) as an early result of boron deficiency. As root elongation ceased 24 hours after boron is first withheld from the nutrient solution, elongation of the cells of the central cylinder progressed distally into the region normally occupied by the apical meristem and eventually the meristem became indistinguishable. It was concluded that a continuous supply of boron is not essential for cell elongation but required for the maintenance of meristematic activity and boron may act as a regulator of cell division in the squash root.

An acute boron shortage first produces marked changes in the apical region of the plant and finally death of the terminal buds (Sprague, 1964). It is considered that boron may contribute directly to apical growth in higher plants (Kouchi & Kumazawa, 1975). Growth inhibition as the primary symptom of boron shortage, is seen in the root and shoot apices where the metabolically active tissues of plants are being affected (Aduayi, 1978). Sorin and Sadgopal (1967) observed also that boron deficiency showed reduction of root growth followed by the development of brittleness and an increase in the thickness of hypocotyls, culminating in the necrosis of the shoot apex of tomato plants. Tomato plants grown in nutrient solution low in boron were very much reduced in growth with malformation and necrosis on the buds (Morales & Sadoval, 1972) since boron is immobile in plants it causes deficiency symptoms to develop first in the younger leaves and at growing points resulting in stunted growth and dwarfing.

Shoot growth of boron-deficient plants was considerably less than that of plants given adequate boron with no significant effect of shading evident in the boron-deficient plants. When adequate boron was present, shading increased elongation of the tops considerably (Yih & Clark, 1965).

Boron deficiency in potato (*Solanum tuberosum* L.) causes growing points to die and tips of terminal shoots are stimulated into a characteristic growth of lateral buds. Internodes remain short and give the plant a bushy appearance. Table beets, turnips, radishes, and other root crops show smaller, less numerous leaves than normal. As a result, the plant gradually becomes stunted and dwarfed, while the growing point may die and decay. The roots do not grow to full size and remain small and distorted under severe boron deficiency conditions (Sprague, 1964).

In tomatoes, boron deficiency causes the plant to look bushy due to the growth of new leaves below the growing point. The stems become stunted and terminal buds curl inwards, yellow and die. Boron deficiency in lettuce is characterized by malformation and retardation of the more rapidly growing leaves, the marginal growth ceases and this causes the folding back of the leaf tip. The older leaves are not noticeably affected but all young leaves from those first affected to the growing point itself are injured. Boron deficiency gives onion plants a characteristically stunted and distorted appearance (Sprague, 1964; Bradford, 1966).

Such are the typical symptoms due to insufficient boron viz incomplete or irregular leaf expansion, distortion of leaves, shortened internodes, multiple axillary branching and abnormalities in the cellular structures (Aduayi, 1978).

Boron is also required by plants during their reproductive stage. It mainly affects the pollen tube germination and growth. Wright and Lane (1978) stated that a shortage of available boron to alfalfa plants will first affect the flowering and seed-set in this crop. Flowers may fail to form and buds appear as white or light brown tissue. Sprague (1964) in his nutrient solution culture study of corn, found the importance of boron in promoting flowering and seed production of corn. According to Boron in Agriculture (1980), tassels of maize plants applied with 10-20 µg B/pot (of 5 kg sand) developed viable pollen but not in treatments without boron. Silks of boron-deficient plants were non-receptive, fertilization did not occur even with pollen from high-boron plants and consequently there was a poor set, or barren ears

developed. Berger (1962) concluded that a continuous supply of boron in the nutrient solution is essential for production of corn ears and kernels and that the lack of it can cause barren plants.

Application of boron and molybdenum at the rate of 2 kg/ha to sainfoin and foliar sprays with a 0.02% solution of boron and molybdenum to alfalfa at flower-bud formation and at the beginning of flowering, respectively, enhanced the development of reproductive organs, i.e., increased the number of flowering and fruit-bearing stems and length of pollen tubes for both plants. Sainfoin seed yield was increased and seed quality was improved while there was an increased sugar, nutrient and pigment contents of the alfalfa plants (Karapetyan, 1979; Pipko, 1976).

Results of an experiment done by Aduayi (1978) on tomato plants showed that increasing levels of 0, 2, 4, ppm boron increased flower number with the highest boron treatment establishing significance ($P = 0.05$) when compared to the control treatment, and accordingly, the number of aborted (non-viable) flowers was slightly increased at increasing levels of boron. Addition of boron at 2 ppm significantly increased fruits relative to the control. Fruit weight was highest at 2 ppm boron applied.

Sauchelli (1969) cited that in experimental plants of alfalfa, red clover (*Trifolium pratense* L.) and field beans, results have shown that different plant species require different amounts of boron and that vegetative yield does not depend directly upon boron supply except over a narrow range; the yield of red clover given by 0.001 ppm boron was as great as that given by higher levels up to 2.5 ppm, and the yield of field beans reached a maximum at 0.05 ppm and was not affected by increases up to 1.5 ppm. The same series of experiments demonstrated that boron deficiency greatly reduced the number of flowers formed. In contrast, boron deficiency had no marked effect on the total flower production of peanut (*Arachis hypogea* L.) although it changed the flowering pattern. It increased also the shoot and root yields but decreased fruit yield and and caused poor quality of fruit as well as changing foliage characteristics of peanut (Harris & Brolmann, 1966, I & II; Harris & Gilman, 1959).

Furthermore, seed yields of sunflower (*Helianthus annus* L.) was increased by boron fertilization with an average yield of 48% in two sunflower cultivars (Blamey *et al.*, 1979). However, there were no discernible effects on plant height or on the general growth of the crop.

Several plants show significant changes in their dry matter yield when applied with boron. Maximum yields of clover (*Trifolium* sp.), alfalfa, and birdsfoot trefoil (*Lotus peduncualtus* L.) were obtained when 0.25 to 1.0 ppm boron was added to the Culloden soil of Eastern Canada (Gupta, 1972). Likewise, Sherrell (1966) reported that total dry matter yield from five harvests of white clover was almost doubled by boron addition. Gupta and Macleod (1977) averred that yields of the first cuts of alfalfa were significantly increased when boron was added. The same results were obtained with barley (*Hordeum vulgare* L.) grown in a pot culture experiment on sierozem sandy soil (pH 8.2). An application of 6.0 ppm boron increased shoot yield by 18.5%, whereas, grain yield was at par with control (Short communication, 1978). With four species of plants studied by Gupta (1971) under greenhouse conditions, cauliflower (*Brassica oleraceae* cv Botrytis L.) was most responsive to applications of 0.5 ppm boron, doubling the yield, although additional applications had no further effect while the yields of alfalfa were increased slightly.

While several crops showed increases in yield with boron application, McIlrath *et al.* (1960) reported that boron concentrations up to and including 10 ppm, in general, have little effect upon the dry weight of the shoot; moreover, a sharp reduction in the yield of tops resulted from the application of 100 and 200 ppm boron, caused by the occurrence of boron toxicity.

Although the exact mechanism of how boron affects growth and development of plants and specifically, the terminal or meristematic tissues is not known, a considerable literature has shown that it is essential in the normal development and growth of plants. Moreover, it was found that in some crops it is important in promoting pollen tube germination and growth, in flowering, seed-setting, and in dry matter, yield, generally.

2.3.2 Boron in Carbohydrate Metabolism

Carbohydrates are the primary sources of reserve energy stored in the vegetative organs of biennial and prennial plants, hence, considered as indicator of plant physiological status (Mislevy *et al.*, 1978). Reserves are essential for survival, producing plant tissues when carbohydrate utilization exceeds photosynthetic activity (Smith, 1969; Sheard, 1973). Such a role in regrowth, total yield and persistence had been well documented even with forage legumes (Pearce *et al.*, 1969; Smith, 1969; Cohen *et al.*, 1969; Chatterton *et al.*, 1977).

Several factors affect the translocation of carbohydrates in the plants (Rhykerd & Overdahl, 1972), one of which appears to be boron. Carbohydrate translocation has been postulated to be the most important function of boron, since rapidly growing sites in plants needing the most energy exhibit deficiency symptoms first (Gauch & Dugger, 1954; Rhykerd & Overdahl, 1972; Wright & Lane, 1978).

This topic presents the importance of total non-structural carbohydates (TNC) to plants particularly to legumes. It discusses differences in carbohydrate storage in plant parts and finally, the role of boron in carbohydrate metabolism.

2.3.2.1 Importance of nonstructural carbohydrates to legumes

The concentration of nonstructural carbohydrates predominantly sucrose, glucose, and fructose (Hansen *et al.*, 1958; Davies *et al.*, 1964; Axelrod, 1965; Salisbury & Ross, 1969; Smith, 1970) that may occur in the herbage, has several important aspects. A sufficiently high amount of readily fermentable carbohydrates present in herbage favours the establishment and growth of lactobacilli responsible for a successful conservation of legumes and grasses as silage (Smith, 1973; Jung *et al.*, 1976). Nonstructural carbohydrates represent immediately available energy precursors and are rapidly and completely digested, hence, are readily available sources of energy to ruminant animals. To the plant itself, nonstructural carbohydrates are the readily metabolizable source of energy needed for growth and survival.

The estimate of total nonstructural carbohydrate content of plants is usually essential in management studies (Ward & Blaser, 1961; Pozo, 1963; Cooper & Watson, 1968; Cohen *et al.*, 1972; Mislevy *et al.*, 1978), however, it has been strongly debated. Graber *et al.* (1927) emphasized the essentiality of organic reserves for plant vitality and production by proposing that elaborated organic carbon and nitorgen were stored, then utilized following defoliation. This was supported by Weinmann (1961) and Sonneveld (1962) in their subsequent reviews. Such a view was refuted by May (1960) who questioned the emphasis placed on organic reserves in the regrowth of plants and suggested that their function was primarily in the maintenance of respiratory processes. Hence, this makes the role of TNC in plant regrowth still equivocal.

Constituents of the nonstructural carbohydrate pool are variable. Species indigenous to cool, temperate climates accumulate a majority of sucrose and fructosans (Sheard, 1973) while Smith (1968; 1969; 1973) reported that grasses in the *Aveneae*, *Festuceae*, and *Hordeae* tribes of temperate origin, accumulate predominantly fructosans in contrast to grasses of tropical and subtropical origin which store starches in the vegetative tissues (Smith, 1968; 1969; 1973) and sucrose (Sheard, 1973) but never fructosans.

Legume species such as alfalfa, clovers (*Trifolium* spp.), trefoils (*Lotus* spp.), and sweet clovers (*Melilotus* spp.) are characterized by sucrose and starch accumulation (Graber *et al.*, 1927; Ruelke and Smith, 1956; Smith, 1962, 1969, 1973; Sheard, 1973) in conjunction with the monosaccharide glucose and fructose and similar characterization of carbohydrates in five tropical legume species have been reported by Hunter *et al.* (1970). Starches are the primary nonstructural carbohydrate in species of the Leguminosae, however, sucrose and pectin are also accumulated in legume species (Smith, 1969; 1973; Sheard, 1973).

Other trace sugars such as xylose, a fructosyl-fructose disaccharide, and an isomer of raffinose were identified by Bailey (1958b) in red clover leaves and stems and white clover (*Trifolium repens* L.) leaflets and petioles, and later in 1963 he found that red clover contained maltose at night,

but not during the day. Scottish investigators found melobiose, raffinose, stachyose, and other unidentified sugars in alfalfa herbage. Hunter *et al.* (1970) identified also raffinose and stachyose in leaves and stems of tropical legumes (*Glycine javanica* Wight & Arn., *Desmodium intortum* Desv., *D. uncinatum* Desv., *Phaseolus atropurpureus Stylosanthes humilis* Sw.).

2.3.2.2 Total nonstructural carbohydrate concentrations in plant parts of legumes

The total nonstructural carbohydrate concentrations of the herbage of grass and legume species (used as livestock feed) determine the survival capacity of plant species and the success of making ensilage.

Although the crown is the storage area in alfalfa, the largest amount of TNCs is stored in the roots (Jung & Smith, 1961; Ueno & Smith, 1970; Escalada & Smith, 1972) but root TNC declines during regrowth as new top growth is produced and also due, in part, to respiration loss (Sullivan & Sprague, 1949; Cooper & Watson, 1968; Silva, 1969; Smith & Marten, 1970). Smith and Marten (1970) found 4% of the TNC in alfalfa roots had been translocated to the shoots by the time they were 15 cm tall.

Carbohydrate analyses done by Ueno and Smith (1970) revealed that about 55% of the TNC (g/pot) in the root wood, 20% in the root bark, and 25% in the crown is present at all stages of alfalfa regrowth regardless of plant size. Escalada and Smith's (1972) work on the six segments of alfalfa taproot, the bark and the wood separated, have shown that about 34% of the TNC content accumulated was in the top 5 cm segment and 22, 12, 9, and 5% could be found in segments 2, 3, 4, 5 and 6, respectively. On the other hand, Wilson *et al.* (1978) reported that male sterile soybean plants which produced 85% fewer seeds than male fertile plants accumulated more starch in leaves and roots and the difference was greatest as the plants approached maturity. Several workers (Dunphy & Hanway, 1976; Brevedan *et al.*, 1977; Mondal *et al.*, 1978) have reported an increase in carbohydrate and/or starch content of soybean leaves during seed-filling. Little change in starch and free sugar

levels in leaves, petioles or stems were reported by Ciha and Brun (1978).

Legume stem tissue usually contains higher concentrations of sugar than leaves (Smith, 1973) but legume leaves contain higher nonstructural polysaccharides (starches) than stems. Similar results were obtained by Hirst *et al.* (1959) and Smith (1969b, 1970a) both working with alfalfa and Bailey (1958a, b) working with red clover. They found higher concentrations of sugar in stems than leaves. The reverse situation was found in alfalfa by Lechtenberg *et al.* (1971). All of these investigators, however, found higher concentrations of starch in leaves than stems although neither Lagowski *et al.* (1958) nor Hunter *et al.* (1970) found a consistent pattern of carbohydrate content between leaf and stem of alfalfa and two tropical legumes, respectively. On the other hand, Smith (1973) concluded that sugar concentrations in legume petioles probably are intermediate between those in the leaflets and stems but Matsumoto *et al.* (1963) reported higher concentrations of TNCs in petiole than leaflet tissue of white and ladino clover.

2.3.2.3 Boron in carbohydrate metabolism

It has been proposed that boron enhances sugar translocation in plants (Gauch & Dugger, 1954) and that the characteristic death of the growing points of boron-deficient plants is caused by a lack of sugar (Mitchell *et al.*, 1960). This concept is more widely accepted than any other of the many physiological roles proposed for boron but there have been serious doubts as to its validity (Skok, 1958; McIlrath, 1960). Two possibilities exist for the manner in which boron accentuates the translocation of sucrose or its hydrolytic products (Gauch & Dugger, 1953). First, the borate ion could react *in vitro* with sucrose (or glucose or fructose) forming an ionizable sugar-borate complex. Such complexes are assumed to move through cellular membranes with a greater facility than the sugar molecule itself. Secondly, it is possible that the borate ion is associated with the cellular membranes, that it there reacts chemically with the sugar molecule facilitating its passage through the membrane, and that the sugar is freed on the inside of the cell by a second reaction.

Dugger *et al.* (1957) found starch synthesis in the leaves of bean plants infiltrated with glucose to be inhibited by boron. In *in vitro* studies, boron slowed down the shift of glucose-1-phosphate to starch by combining with glucose-1-phosphate at low concentrations. This reduced rate of condensation of soluble carbohydrate to starch, would tend to promote the movement of more soluble carbohydrate out of the leaves to the other parts of the plant.

The research of Sisler *et al.* (1956) indicated that boron enhances uptake and translocation of sugars and is implicated in carbohydrate metabolism. It was suggested that there is a micronutrient (or boron) union with sugars, giving an ionizable sugar-borate complex that moves more readily through cellular membranes than does sugar alone.

More sugar was found to be translocated when boron-deficient tomato plants were applied with 50 ppm boron added with sucrose through a cut petiole than when sucrose was applied alone. Subsequent studies by Dugger and Humphreys (1960) implied a direct involvement of boron in the enzymatic reactions of sucrose and starch synthesis or uridine diphosphate glucose (Birnbaum *et al.*, 1977). According to Pulich (1978), without boron, activities of polyphenol oxidase and peroxidase in leaves of cowpea (*Vigna unguiculata* L.) were increased. Likewise, excessive levels of peroxidase activity accompanying boron deficiency were observed by Besford (1978) in cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicon esculentum* L.). Moreover, in *Phaseolus vulgaris* L. cv Saksa, a plant with high boron requirements, boron-deficiency considerably increased the glucose-6 phosphate dehydrogenase activity, indicating an increase in the role of pentosephosphate pathway leading to the accumulation of phenols (Shokl'nik & Il'inskaya, 1980). In addition, Nelson and Gorham (1957) found also that boron (5 ppm) enhanced uptake of glucose applied to primary leaves of soybean seedlings in the presence of detergent although this result was not regarded as a function of the complexing ability of boron because the process was relatively pH insensitive in the range of 4.8 to 8.4. In *in vitro* sugar-boron complexing is known to be favoured by high pH (at 8.6

to 10). Proof of this sugar-borate complexing must depend on the isolation of boron-containing compounds as done by Gauch and Dugger (1954) when sugar spotted on paper saturated with borate electrolyte can be separated electrophoretically as ionic complexes of borate at pH 8.6 to 10.

Turnowska-Starck (1960) confirmed that bean plants grown at a relatively low level of boron showed a marked increase in the migration of C^{14} introduced into the leaves as C^{14} sucrose in the presence of additional boron.

Although these data seem to indicate that sugar applied to a leaf migrated more readily from the site of application, there are considerable findings on which to question the Gauch and Dugger hypothesis.

O'kelly (1957) working with pollen found a much smaller increase in sugar absorption in the presence of boron. McIlrath and Palser (1956) reported a translocation of carbohydrates in boron-deficient plants as long as the phloem tissues were alive. According to Whittington (1957), the involvement of boron in sugar translocation may well be a secondary effect which follows the abrupt cessation of growth under deficiency conditions. This lack of cell division at the growing points would result in less utilization of sugar, hence, an accumulation of sugar at the growing point. Weiser *et al.* (1964) reported that boron does not enhance sugar translocation in plants but it does enhance foliar uptake of sucrose applied to the leaves. It was concluded that this phenomenon of enhanced foliar uptake of sucrose has given rise in the past to the erroneous conclusion that boron enhance sugar translocation.

On the other hand, Jackson and Chapman (1975) suggested that boron affects some metabolic events under hormonal control. Dryar and Webb (1961) reported an increased translocation of ^{14}C labeled photosynthate to the tips when meristems of boron-deficient bean plants var Black Valentine were applied with napthalene acetic acid. It was suggested that boron-deficient plants may have a functional conducting system, but that it does not conduct because substrates are not being utilized; thus boron-deficient plants are limited in

growth by sugar deficiency. Also, an application of boron accelerated the translocation of 3-indoleacetic, 2,4,5-trichlorophenoxyacetic, and alpha napthalene acids from leaves to the stems of bean plants (Mitchell *et al.*, 1953). Moreover, translocation of growth modifying substances such as 2,4-dichlorophenoxyacetic acid (2,4-D) and its salts and esters from leaves to other parts of plants is associated with the translocation of photosynthates (Linder *et al.*, 1949). Natural plant auxins are known to move in a polar fashion and so can sugar in certain plants (Grant & Beevers, 1961). Sugars move from leaves of sugar beets to the root against a sugar gradient and carrot tissues rich in sugar, go on to absorb all the glucose from dilute glucose solutions.

There were other reports regarding the role of boron to sugar metabolism. Sucrose, glucose, and fructose content of young or old cowpea leaves were higher in plants grown in nutrient solution with boron than that without boron (Pulick, 1978).

Zapata (1973) found that sugar cane (*Saccharum officinarum* L.) plants receiving only traces of boron suffered growth and quality losses without developing visual symptoms of boron deficiency. Lack of boron lowered sucrose production in leaves and significantly altered the rates of sugar transport in sugar cane storage tissues (Zapata, 1973). In sugar beets, sugar content increased slightly with boron application. Furthermore, working on the same plant, Vlamis and Ulrich (1971) reported that the sucrose content of the storage roots started to decrease at about the same point at which limiting boron resulted in a drop in yield.

Boron when introduced into NPK fertilizers applied to sugar beets, promoted a decrease in the nonsugar contents, an increase in the syrup quality, a reduction in the soluble ash content, and a rise in the yield of sugars (Kibalenko *et al.*, 1977). Alagarswamy and Rao (1976) found an increased rate of respiration in the leaves of groundnuts applied with boron at 1 µg/ml but there was a decreased reducing sugar and carbohydrate content.

Moreover, Bonilla *et al.* (1980) who studied the levels of sugar in sap and root for boron deficiency and toxicity obtained results in both cases which made them assume that boron plays a role in the photosynthesis or in one of the initial stages of sugar metabolism as well as in sugar transport (Gauch, 1972). Roots and leaves of boron-deficient beans showed a significant increase in reducing (mainly hexoses) as well as nonreducing (mainly sucrose) sugars (Odhnoff, 1957) compared to normal ones.

2.3.3 Boron in Nitrogen Metabolism

2.3.3.1 Importance of nitrogen in plant growth and development

Nitrogen is considered the most important of all the essential elements in plant growth and reproduction (Viets, 1965; Allison, 1973) but it is often in short supply to organisms, particularly to plants because only certain microorganisms are capable of assimilating molecular nitrogen and converting it into forms available to plants (Bidwell, 1979; Subba Rao, 1977). It is of extreme importance in plants because it is a constituent of proteins, amino acids, enzymes and nucleic acids which make up the genetic code, as well as many metabolic intermediates involved in the synthesis of energy transfer (Viets, 1965; Hera, 1971; Allison, 1973; Bidwell, 1979). Nitrogen is also an essential component of the chlorophyll molecule which is involved in the trapping of light for the production of the sugars by photosynthesis (Donald *et al.*, 1963; Steward, 1975).

Plants respond to nitrogen in a variety of ways due to the complex interplay of soil factors (Munns, 1977) affecting its availability and the above-ground environmental conditions (Berger, 1962; Allison, 1973; Bidwell, 1979). The nutritional requirements of legumes particularly that of nitrogen, resemble those of other plants, except that they have the potential for symbiotic assimilation of dinitrogen (Munns & Mosse, 1980). The phosphate requirement of legumes,

on the other hand, may be larger than average because phosphorus is needed not only for plant growth but also for nodulation and nitrogen fixation. Nitrogen occupies a unique place in soil-plant nutrition and on a worldwide basis (Subba Rao, 1977) in that most plants, under natural conditions exist in a state of nitrogen deprivation, which however, is not critical because of the great adaptability of the plants to wide ranges of nutrition (Bidwell, 1979). Moreover, more crops are deficient in nitrogen than in any element (Viets, 1965).

2.3.3.2 Sources of nitrogen for plants

Earth's nitrogen potential was classified by Subba Rao (1977) into the following sources: (1) natural resources which include soil nitrogen, nitrogen fixed by symbiotic and free-living organisms from atmosphere, and nitrogen provided by lightning, and (2) man-made resources or nitrogen from commercial fertilizers.

Vast reservoirs of nitrogen exist in the terrestrial areas of the earth as ammonium ions held within the lattice structure of silicate minerals (Stevenson, 1959). The inert atmospheric dinitrogen occupies approximately 75% by weight, and 78% by volume of the atmosphere and totals 3.8×10^{15} tonnes. Though the amount of atmospheric dinitrogen over a hectare of land has been calculated as 3.5×10^{5} tonnes, the total nitrogen in mineral soils is relatively small, ranging from 0.9 - 7.2 tonnes/9×10^{2} tonnes soil (Sauchelli, 1964). Total nitrogen in the plough layer of different soils ranges from 1100 kg/ha to 4500 kg/ha (Kutz & Smith, 1966) but it can be up to 7000 kg/ha for highly productive mineral soils (Donald *et al.*, 1963). A substantial amount of this nitrogen (95% or more) may be in a stable form as soil organic matter which is unavailable to plants (Date, 1973). When the organic matter content of the soil profile is considered the total nitrogen, it may reach 17000 kg/ha (Donald *et al.*, 1963).

Rainwater contributes also to the nitrogen economy of soil. Geographical locations determine the amounts of nitrogen fixed by this source (Allison, 1965). At Rothamsted (U.K.), rainwater add 4.5 kg N/ha/year as ammonium and nitrate and at Cornell, it amounts to about 5.6 kg N/ha/year (Russell, 1961). However, as much as 57.5 kg N/ha/year was made available by rainwater in Nigeria.

Lightning combines dinitrogen and water vapor to form ammonium nitrite, and oxygen and dinitrogen to form nitric oxide, both of which are introduced into the soils through precipitation (Bidwell, 1979). Ammonium nitrite is also formed by the oxidation of ammonia, mediated by ozone.

Microorganisms through biological dinitrogen-fixing processes, both asymbiotic and symbiotic, have continuously tapped the vast reserves of atmospheric dinitrogen. Values of nitrogen fixed for individual ecosystem were summarized by Hauck (1971) as follows: (in kg N/ha/year) cropland, 7-28; angiosperms other than legumes, 7-114; legumes 73-865; forest, 58-594; paddy, 13-99; and water, 70-250.

The nodulated legumes contribute significantly to the overall harvest of nitrogen from the atmosphere. Field pea alone fixes 539 mg N/g nodule dry weight per year while nodulated angiosperms other than legumes like *Alnus* are known to fix 814 mg N/g nodule dry weight (Alexander, 1971; Burns & Hardy, 1975). Blue-green algae, e.g., *Aulosira fertilissima* contribute significantly to the nitrogen economy, particularly in tropical rice fields (Fogg , 1956).

Because of the high organic matter levels, some soils when first cultivated need no supplemental nitrogen to produce high crop yields. But with the increased intensity of cropping, and the use of high yielding cultivars, soil organic matter levels decline, thus, greater proportion of cropped soils now require nitrogen fertilizer additions in order to achieve high levels of production (Allison, 1973; Date, 1973).

Nitrogen fertilizers may be classified broadly as either natural, organic or chemical (Tisdale & Nelson, 1966). The natural organic materials are of plant or animal origin; the chemical sources are neither plant nor animal.

Natural organic materials though not commonly used as fertilizers still find their way into so-called specialty fertilizers for lawns, gardens and shrubs and in some fertilizers applied to flue-cured tobacco (Tisdale & Nelson, 1966). Examples of natural organics with their nitrogen content (%N) are: blood (dried), 13; bone meal (raw), 3.5; bone meal (steamed), 2.0; cottonseed meal, 6.6; fish scrap (acidulated), 5.7; fish scrap (dried), 9.5; peanut meal, 7.2; Peruvian guano, 13.0; soybean meal, 7.0; and whale guano, 8.5. A large fraction of the nitrogen contained in natural organics is water-insoluble (Allison, 1973). This led to the idea that they decompose slowly, with a slow release of their nitrogen, lower leaching losses, and better plant utilization of fertilizer nitrogen.

Chemical sources of nitrogen are by far the most important of the fertilizer nitrogen compounds. Most are ammonia derivatives, that is, they are derived from the compound ammonia. The various nitrogen compounds are grouped into ammoniacal, nitrate, slowly available, and others (Tisdale & Nelson, 1966).

2.3.3.3 Boron in nitrogen metabolism

Any element that is essential for the growth and development of plants must have a direct or indirect influence on nitrogen metabolism, including synthesis of proteins (Gupta, 1979). Yih and Clark (1965) found a higher protein-N content in boron-deficient tomato root tips than corresponding tips from plus-boron roots. Boron-deficient sunflower plants were found to increase significantly in their contents of many amino acids. Such a higher content of amino acids was explained by an acceleration in protein decomposition or by deceleration in protein synthesis. It was suggested that the deceleration in protein synthesis was the dominant process although it was presumed that probably both processes occurred

(Sherstner & Kurilenok, 1964). In cotton grown under boron deficiency, non-protein-N prevailed in plant organs (Pak, 1980). Studies of Kibalenko *et al.* (1973) showed that during photosynthesis the rate of $^{14}CO_2$ incorporation into free amino acids was higher in sugar beet (*Beta vulgaris* L.) plants grown in a nutrient containing boron than in boron-deficient plants. Moreover, boron deficiency significantly inhibited protein synthesis in sugar beets and pea plants (*Pisum sativum* L.) as shown by higher levels of free amino acids in the leaves of boron-deficient plants, while protein content was lower than in leaves of plants grown in a full nutrient medium (Kibalenko *et al.*, 1974).

Boron-deficient peanut leaves were found to have higher concentrations of amino acids and nitrogen but the same concentration of protein as boron-sufficient leaves (Shiralipour *et al.*, 1969). Results were expressed on a per cell (milligram of DNA) basis. It was concluded that the increase in concentration of amino acids could be partially or completely explained by the higher concentration of nitrogen in boron-deficient plants. The possibility that a deficiency of carbohydrates in boron-deficient leaves might have led to the accumulation of nitrogen and amino acids was not considered.

Albert (1965) concluded that ribosenucleic acid (RNA) content of tomato root tips decreases soon after (24 to 48 hours) boron was withheld from the nutrient solution but that root elongation ceased before a decrease of RNA content occurred. According to Koge (1979) the retarded elongation of root tips of tomato was partly attributed to the reduction in protein synthesis induced by boron deficiency while Ilyushchenko and Timashov (1979) postulated that inhibition of leucine incorporation into pea root proteins was a result of disturbances in metabolism caused by the absence of boron. It is also possible that inhibition of protein formation may be the primary reaction of the cell to boron deficiency. In addition, boron application increased nitrogen contents of the clover, increased the protein content from 0.8 - 1.2%, decreased the crude fibre and increased the carotene of the hay (Krotkikh & Makarouski, 1974). Likewise, an application of high boron at 10 ppm in the root medium of

peanut reduced content of total nitrogen and protein nitrogen probably due to protein breakdown and protein regenerates appeared to be inhibited (Gopal, 1978). Moreover, Carpena *et al.* (1978) reported that boron deficiency and toxicity conditions resulted in enzyme activities considerably below normal which is possibly explained by a boron/molybdenum antagonism particularly with toxic conditions but it does not provide a satisfactory explanation for deficient conditions.

Boron affects also the nitrogen content of plants (Ministry of Agriculture, Fisheries and Food, 1971). Application of 2 ppm boron to clover plants increased the amount of nitrogen while a short communication in Plant and Soil (1977) reported that the application of boron up to 1.8 kg/ha generally increased the nitrogen concentration of wheat grain. In contrast, Aduayi (1978) found that increasing the boron levels (0, 2, and 4 ppm) resulted in a decreased leaf-nitrogen and phosphorus content.

2.3.4 Boron in Nodulation and Nitrogen Fixation

Although the essentiality of boron for higher plants has been recognized for many years, there has been a lack of agreement on the boron requirement of algae, fungi, and bacteria.

This section will present a brief discussion on the importance of inoculation to legumes and the effects of boron in legume nodulation and nitrogen fixation.

2.3.4.1 Importance of inoculation to legumes

Well-nodulated plants are essential for high legume yields. Inoculation of seed with good strains of *Rhizobia* is the most reliable means of guaranteeing that proper nodulation occurs. Identifying the indigenous strains of Rhizobium and to assess their general efficacy is a pre-requisite of an extensive programme of legume inoculation in a new area. Also, there is a need to provide consistently large numbers of *Rhizobia* in legume inoculants to avoid

nodulation failure in some areas. Several methods are available for inoculation of either the seed or the soil with peat-based cultures or *Rhizobia* (Brockwell, 1977; Tanner & Hume, 1978). Commercial inoculants include peat-based inoculants, liquid formulations, granular formulations, and pre-inoculated seeds.

Indirect inoculation has proved successful for *Arachis hypogea* L. in Israel; the inoculant was suspended in water and sprayed into the drill row (Schiffman & Alper, 1968). An alternative method involving granules of peat has now been developed.

Inoculation is a necessary first step in efficient and economical soybean (*Glycine max* (L.) Merr.) management. Jethmalani *et al.* (1969) reported an 83% yield of inoculated plots over uninoculated plots in India where soybeans had never been grown. Abel and Erdman (1964) and Caldwell and Vest (1970) showed strains of *Rhizobium japonicum* to increase yields differentially when added to soils where soybeans had not been grown previously, but no yield increase occurred when the same strains were added to the soils where soybeans had been grown. Ham *et al.* (1971) failed to show a yield advantage for inoculated over uninoculated plots using different commercial inoculants in several established soybean production areas in Minnesota. Similar findings were reported from the Gisborne area of New Zealand where the survival of rhizobia from one year to the next in newly-introduced crops of soybean proved excellent (Robertson, pers. comm.).

Burton (1972) cited a beneficial result from inoculation of alfalfa in 99% of field trials done in Sweden. Increases in yield were greater in new soils and ranged from 15 to 90%. Inoculation of alfalfa is recommended in Kansas, Okalahoma, Kentucky, Illinois, and Wisconsin (Burton, 1972).

In New Zealand, successful lucerne nodulation requires an application of a good quality inoculant to the seeds and good survival allowing successful invasion of root hairs (Wynn-Williams, 1982). In addition, autumn sowing of inoculated lotus is essential to ensure rhizobial survival and subsequently, an effective infection.

2.3.4.2 Boron in nodulation and in nitrogen fixation

The influence of boron deficiency was explored by Brenchley and Thornton (1925) and Mulder (1948). They reported that if nodules developed at all on *Vicia*, they were small and lacked vascular strands and bacteroids. Munns (1977) stated that nodule formation is prevented by boron deficiency although it is affected little and inconsistently by deficiencies of the other micronutrients unless the deficiency of the other micronutrients is severe enough to injure several other phases of the symbiosis.

Research on boron effects to nodulation by Werner and Barbossa de Mattos (1976) showed that boron application to centrosema (*Centrosema pubescens* Benth.) increased significantly the number of nodules and the yield of dry matter and nodule weight but had the tendency to decrease the nitrogen percentage in the dry matter yield.

The nodulation of alfalfa grown in sand-pot experiments was generally affected by molybdenum followed by boron, and by combining the two micronutrients, best results were obtained (Pillar, 1975). It was postulated that both boron and molybdenum when applied to alfalfa caused anatomical changes in roots leading to increased root capacity for nutrient translocation, thus, increasing nodulation, nitrogen fixation, and yield (Rulinskaya & Petkevich, 1975). Boron applied to bean (*Phaseolus atropurpureus* L. cv siratro) decreased the aerial parts but increased the number of nodules, nodular mass and the root dry weight; boron combined with copper or zinc increased the nodular mass and the number of nodules per plant (Barbossa de Mattos, 1976). Likewise, boron, manganese, and molybdenum accelerated the formation and development of nodules and enhanced nitrogen fixation in leguminous cultures (Shevchuk, 1970). When applied with boron or molybdenum or in combination at the rate of 6 ppm, respectively, nitrogen fixation in cowpea was stimulated. Both nitrogen fixation and dry matter yield in beans and soybeans was increased by the application of boron with Lime (Ruschel *et al.*, 1968; de Franca *et al.*, 1975).

Although research on the effects of boron on nodulation and nitrogen fixation is insufficient to present an unequivocal conclusion as to the mechanism it operates, positive results as discussed previously, have been obtained. These support the essentiality of boron to legume symbiosis in addition to that for normal growth and development of legume plants.

2.4 BORON DEFICIENCY AND TOXICITY SYMPTOMS IN PLANTS

Some vegetable crops like Brussel sprouts (*Brassica oleraceae* L. var gemmifera Zenker), cauliflower, celery (*Apium graveolus* L.), and cabbage (*b. oleraceae* cv capitata L.) are good indicators of boron deficiency. Root crops such as table beets (*Beta vulgaris* L.) and turnip (*b. rapa* L.) are also good indicators of boron deficiency. Among the fodder crops, white clover and red clover exhibit characteristic leaf symptoms (Wright, 1976; Gupta, 1979), while alfalfa is an indicator for both boron deficiency and toxicity. Apple and pear trees are probably the most sensitive among the fruits (Bradford, 1966).

2.4.1 Visual Symptoms of Boron Deficiency and Toxicity in Plants

Generally, boron deficiency leads to degeneration of the meristematic tissues including the cambium, to breakdown of the walls of parenchyma cells, and to feeble development of the vascular tissues. Phloem and xylem are imperfectly developed. External symptoms of boron deficiency were described by Bradford (1966) and Gupta (1979).

The terminal growth shows rosetting, dieback, discoloration, failure to grow or elongate, and stimulation of lateral bud development, which in turn develop well or die. Various abnormalities such as thickening, brittleness, curling, wrinkling, wilting and chlorotic spotting are shown by the leaves. Petioles or stems may be thickened, corky cracked or crosshatched, or may show oversoaked, dead areas and the fleshy part of fruits, tubers, or roots may show brown flecks, necrosis, cracks, or dry rot, may be watersoaked, or may show discoloration in the vascular system.

Generally, boron-toxicity symptoms are similar in most plants (Gupta, 1979). These show marginal and tip chlorosis followed by a progressive necrosis. The pattern of chlorosis follows the leaf venation; monocotyledons, for example, show a tip and not a marginal necrosis. The marginal tips have a burned or scorched appearance which later involves the entire leaf or before it drops prematurely.

2.5 DISTRIBUTION AND ACCUMULATION OF BORON IN PLANTS

Crops vary widely in their requirement for boron, their tolerance to high or excessive levels of the element, and their ability to absorb the element from the soil (Gupta, 1979; Davies, 1980). Minimum requirements for boron are comparatively low, falling between 5 and 30 ppm in the leaf tissue. Leaf levels of boron associated with maximum growth increase with calcium level (Fox, 1968), and are influenced by calcium and potassium (Reeve & Shive, 1944) and calcium and phosphorus interactions (Bingham & Garber, 1960).

Boron composition of plants is affected by the part of the leaf, its position in the plant, the age of the plant part (Gupta, 1979). Alfalfa indicates boron-deficiency with 21 ppm, 21-139 ppm being normal and more than 250 ppm being toxic in the plant part material (Wright, 1976).

Generally, healthy legumes contain above 35 ppm and a response to boron applications can be expected when levels drop below 20 ppm (Nelson & Barber, 1964). Ohio workers (1972) suggested 21-80 ppm as sufficiency range in the top 7.5 cm of alfalfa sampled prior to initial flowering. Gupta (1971) found a high boron content of 69 ppm in alfalfa but he considered that a boron content of about 48 ppm was in the sufficiency range while boron content of less than 20 ppm in red clover was considered deficient, and 25 to 36 ppm appeared to be optimum. Furthermore, Gupta (1972) reported that levels of 4 to 9 ppm boron in the leaf tissue of alfalfa, red clover, and birdsfoot trefoil were in the deficiency range. He concluded also that boron concentration of 21 to 45, 39 to 52, and 30 to 45 ppm in the first cuts of red clover, alfalfa, and birdsfoot trefoil were indicative of sufficiency and were associated with maximum yields, while levels of >59, >99, and

>68, respectively, were in the toxicity range in the three crops.

Boron in the parts of alfalfa of similar physiological age declined with plant age (Johansen, 1978). Rominger *et al.* (1975) reported a general decrement of boron in alfalfa plants with advance in maturity in both the fertilized and non-fertilized study on alfalfa herbage. Miller and Smith (1977) observed continual increase in the boron concentration from early vegetative to bloom stage of growth of alfalfa and then a decrease in boron concentration from bloom to seed set.

Vlamis and Ulrich (1971) reported that young blades of sugar beets had a higher boron content than did the mature and old blades at lower concentrations of boron in Hoagland solution but no variations were found at higher boron concentrations. Petioles of sugar beet did not show any boron accumulation at any levels of boron. The older leaves were found to contain the highest boron content while the lowest boron value occurred in the fibrous storage roots.

Clark (1975a) reported an increasing boron concentration in seedling leaves of corn with age, decreasing in leaves at higher positions. Higher concentrations were noted the youngest leaves of corn compared with leaves at positions below, while in the dead bottom leaves, boron increased to levels as high as 130 ppm at 74 days before decreasing by over threefold at maturity. In his subsequent work (Clark, 1975b) on corn, it was shown that boron content of the leaf increased with age nearly eightfold and boron content of the tassel increased nearly fivefold. Boron accumulation was greater in the marginal section of corn leaves than in the midrib section (Touchton & Boswell, 1975). It was conluded by Kohl and Oertli (1961) and Jones (1970) that boron generally, has a tendency to accumulate in the margin of the leaves of plants.

Leaf tissue of cole crops generally contained lower levels of boron late in the growing season than they were in early season (Gupta & Cutcliffe, 1973). According to Ali and

Schwarz (1973) older cucumber leaves have higher boron content than the younger leaves and within the leaf, boron was accumulated in the marginal parts. This corroborates with the results obtained by Touchton & Boswell (1975), Jones (1970), and Kohl & Oertli (1961) mentioned previously.

Distribution of boron in various plant parts is influenced by the supply of boron. For example, Vlamis & Ulrich (1971) found that in sugar beet plants, the blades had a higher boron content than the petioles where boron supply was adequate and a reverse relationship occurred in the boron-deficient plants. Likewise, Woodruff (1979) stated that where Ap or B_2 soil averaged 0.05 ppm boron, soybean leaf content ranged from 14 to 40 ppm, and no yield response was obtained with 0.56 to 2.24 kg/ha of added boron. Where Ap horizon averaged 0.11 ppm boron, the leaf boron content reached 63 ppm with yield reduced approximately two-thirds by 2.24 kg/ha of added boron.

3. MATERIALS AND METHODS

3.1 INTRODUCTION

The initial part of this section describes the experimental site and experimental layout of the experiment. It also presents the materials used and outlines the experimental procedures and methods used in measuring both growth of component parts: shoot and root and those in determining the chemical composition of the sainfoin plant: total nonstructural carbohydrates (TNC), and boron, nitrogen and phosphorus concentrations, respectively. Finally, an account is given of the statistical methods used to examine the data.

3.1.1 Experimental Site

The experiment was conducted in an automated glasshouse in which there was a degree of control over temperature. Heating maintained temperatures above 15°C and cooling, which followed a stepwise procedure of opened louvres, fan extraction of air then evaporative cooling, held temperatures below 25°C. During the conduct of the experiment, temperatures were maintained at the range of 15° to 25°C.

3.1.2 Experimental Lay-out

A Split-plot experimental design was used consisting of three (3) randomized complete blocks with three main treatment plots and two subtreatment plots making a total of six treatment plots as follows (see Appendix 1).

Main Plots (Levels of Boron)

Bo - no boron applied
B_1 - 1 ppm boron
B_2 - 2 ppm boron

Subplots (Nitrogen application and inoculation)

S_1 - nitrogen applied, no inoculant
S_2 - inoculated, no nitrogen applied

Each subplot consisted of 81 bags (pots) with seven plants per bag for nine sampling dates. Potted plants were placed on trolleys and shifted by rotating them occasionally. There were nine trolleys with 55-57 pots per trolley including spare bags.

3.2 EXPERIMENTAL MATERIALS, PROCEDURES AND TECHNIQUES

3.2.1 Planting Material, Pregerminating the Seeds and Planting

Partly dehulled sainfoin seeds (cv Fakir) were used in the study. Seeds were surface sterilized with 5% Janola before pregerminating them. Trays with perlite were used in the pregermination of seeds. After four days, ten pregerminated seeds were planted to each bag. Replanting was done two to three days after. Thinning to seven plants (as seedlings were relatively small) was done two weeks later.

3.2.2 Growth Medium and Potting Procedure

Perlite was used as rooting medium. It is known to be sterile (neutral) and free from impurities and recommended for nutritional studies (Robertson, pers. comm.). Five hundred black planter bags (size 5) were filled with 300-350 gram of distilled water-soaked perlite.

3.2.3 Preparation of inoculant and inoculation

Prior to planting, rhizobial culture made of agar slopes were prepared as described in Appendix 2. The inoculum used was NZP 2243 strain for sainfoin.

Inoculants were prepared by scraping the rhizobial culture from the surface of the agar slopes (yeast-mannitol-agar), adding 0.1% distilled peptone water. Inoculation was done a day and 10 days after planting. A third application of inoculant was done 62 days after planting due to ineffective nodulation. The inoculant was poured into the rooting medium in the vicinity of the seedlings with the amount of 50, 30, 20 ml/pot, respectively, for the treatments receiving inoculant.

3.2.4 Nutrient Solution and Application

Nitrogen-free solution described by Small and Leonard (1969) modified to contain different levels of boron accordingly with the main plot treatments was used for the inoculated treatment. For the non-inoculated treatments, nitrogen in the form of calcium nitrate replacing calcium chloride in the N-free solution was added, making a complete-element stock solution with the different levels of boron for the respective treatments (see Appendix 1). The application of solution to respective treatment was done at weekly intervals with the amount of 150 ml per pot until the fifth week after planting and increasing the amount to 300 ml per pot thereafter.

A starter solution of 0.01% NH_4NO_3 was applied to the inoculated treatments for the first four weeks of the study for it was proven by Ryle *et al.* (1979) that it was impossible to grow cowpea and white clover in perlite culture from germination without any nitrogen added.

3.2.5 Watering and Flushing

Distilled water was used in watering the plants with 200 ml/pot at weekly intervals and was gradually increased to 350 ml/pot as the plants grew bigger. To avoid the accumulation of salts, flushing was done at four week intervals after planting. It was done by overflowing the pots with distilled water and letting the water to drop or flow out of the pots to wash salts away.

3.3 ANALYTICAL PROCEDURE AND TECHNIQUES

3.3.1 Harvesting

Harvesting was sequential and destructive in nature which started five days after sowing and continued at five-day intervals for the first month of the experiment and was changed to two-week and three-week intervals for the remaining two months due to a very slow growth rate. Plants to be harvested were assigned accordingly with the dates of sampling. Samples were taken from each of the three replicates. The entire contents of the pot was washed in a bucketful of water.

Plant tops were cut separating the roots and shoots for drying. The cotyledons were included as photosynthetic area thus drying them together with the leaves and petioles for the top dry weight. Both roots and shoots were oven-dried at 80^{o}C in a forced-air drying oven.

The number of the first order lateral and second order lateral roots were counted for the first six harvest times.

3.3.2 TNC, Boron, Nitrogen and Phosphorus Analyses

The total nonstructural carbohydrates (TNC) was analyzed, based on the extraction of sugars from dried plant material with 62.5% methanol, with soluble sugars being determined on aliquots of the extract by the phenol-sulphuric acid method of Haslemore (1981) (see Appendix 3).

Analysis of boron content was done by the Azomethine-H method (Speirs, 1981, MAF, Levin) (see Appendix 4). Boron content of the whole plant for the last harvest samples was analyzed. Also, the shoot and root boron content on the final harvest were analyzed.

Total nitrogen in the plant material, shoots, and roots was determined by the Kjeldahl method in which digestion was carried out with nitrogen-free H_2SO_4, K_2SO_4 with selenium: $CuSO_4$ as catalysts. This is basically after Clements (1970).

The digestion procedure was followed by an automated distillation method for nitrogen determination (Appendix 5).

Total phosphorus in shoots and roots was determined from the same digest used for nitrogen determination, using an automated distillation method.

3.4 STATISTICAL ANALYSES

3.4.1 Relative Growth Rate

A minimum of seven sample-plants per treatment per harvest were taken at the following time intervals (days after sowing): 8, 13, 18, 23, 30, 37, 52, 71, 91.

The formula used to calculate Relative Growth Rate (RGR) was:

$$\bar{R} = \frac{\text{Log}_e\ {}_2W - \text{Log}_e\ {}_1W}{{}_2t - {}_1t}$$

where ${}_2W$, ${}_1W$ = dry weights
${}_2t$, ${}_1t$ = time of harvest

The same formula was used for the Relative Growth Rates of shoot (RSGR) and roots (RRGR).

The transformed values were subjected to the standard analysis of variance for Split-plot at each period.

3.4.2 Analysis of Variance

Data for dry weight, leaf area, number of the lateral roots, and TNC were analyzed within harvests using a standard analysis of variance for a Split-plot design (Steel & Torrie, 1980). Data on the chemical composition of plants: boron, total nonstructural carbohydrates, nitrogen, and phosphorus of both the shoot and the root done during the final harvest were also subjected to the standard analysis of variance. A computer program in GENSTAT (Alvey *et al.*, 1977) was written for the Split-plot analysis of variance. When F

was significant in the ANOVA for the main factor, subfactor and interaction effect, the least significant differences (LSD) at 5% level of significance were calculated for comparison of compared means.

3.4.3 Curve fitting

Plant growth for each treatment was treated by regressing total plant, shoot and root dry weight against time (day after sowing). Initial plots showed an asymptotic curvilinear response and the logistic growth function (described below) was applied to the data.

$$\text{Logistic equation } Y + \frac{Y}{1+e^{-(A+Bx)}}$$

$$\text{or } \ln \frac{Y}{\hat{Y}o-Y} = \beta_o + \beta_1 x \text{ in linear regression form}$$

where Y = total plant dry weight
$\hat{Y}o$ = upper asymptote
A, B = constant
β_o, β_1 = regression estimates of the function parameters
x = time

The semi-graphic method described by Nelder (1961) was used to obtain Yo. Subprogramme regression in SPSS (Nie *et al.*, 1975) was used.

Because of the great difference of asymptotes used in subtreatments (nitrogen application and inoculation) no appropriate method can be used for the t-test of β_o, β_1 among the different curves, hence, the test of significance was based on the analysis of variance described previously.

3.4.4 Multiple Regression

Multiple regression was used to investigate the relationship between dependent variables: plant dry matter (PDW) yield, TNC, and plant boron concentration (PBC) and the set of independent variables: the chemical composition of the plant - TNC, boron, nitrogen and phosphorus during the

final harvest. This was done to distinguish which of the different chemical compositions of the plant were related to the dependent variables in order of their importance in determining yield and boron and TNC concentrations in plants.

The stepwise procedure as described by Draper and Smith (1966) was used. A computer programm in SPSS (Nie *et al.*, 1975) was used in this analysis.

4. RESULTS

The results are presented in four main sections. These are:

4.1 General description of the early establishment and growth of sainfoin.

4.2 Plant growth and development.

4.3 Chemical composition of sainfoin.

4.4 Relationship of plant dry weight (PDW), plant total nonstructural carbohydrates (TNC), and plant boron content (PBC) to chemical composition of sainfoin.

Unless otherwise stated, the six combinations of treatments presented in Section 3.1.2 will be designated as Treatments 1, 2, 3, 4, 5 and 6 throughout the presentation of results as described below:

Treatment	Combination
1	Nitrogen-treated plants, 0 ppm boron
2	Inoculated plants, 0 ppm boron
3	Nitrogen-treated plants, 1 ppm boron
4	Inoculated plants, 1 ppm boron
5	Nitrogen-treated plants, 2 ppm boron
6	Inoculated plants, 2 ppm boron

Also, the summaries of the analysis of variance (ANOVA) for the different data are given in the APPENDICES.

4.1 GENERAL DESCRIPTION OF THE EARLY ESTABLISHMENT AND GROWTH OF SAINFOIN PLANTS

The germination percentage of the sainfoin cv Fakir seeds used in this study was 95%.

Defining the different stages of sainfoin plants was difficult due to non-uniformity of the plants. Although most of the seeds (about 95%) germinated at the same time, eleven

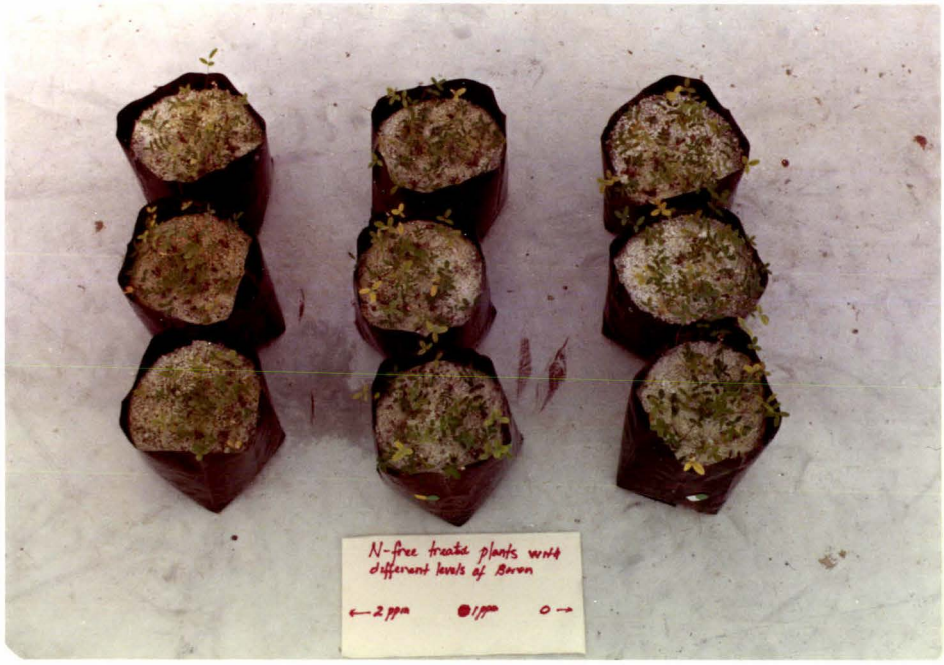
days after sowing when the first leaves appeared, there were morphological differences; leaves were either unifoliate or bifoliate or trifoliate. This caused the non-uniformity of plants at early stage of growth since trifoliate first leaves were reported to have higher rates of net carbon exchange and early seedling growth than had those with unifoliate first leaves (Cooper, 1974). Regardless of treatments and types of first leaves, leaves were fully expanded two days after appearance. At 24-29 days after sowing, differences between treatments had been observed. The third leaf of the nitrogen-treated plants at all levels of boron had been fully expanded while those of the inoculated plants were still newly initiated. During the succeeding growth period until 91 days after sowing, inoculated plants showed a very slow growth rate (which will be presented in a later section) due to their failure to develop effective nodules which were considered nil in terms of percentage infection.

Some plants, regardless of treatments were also found to suffer leaf wilting (first leaves) 16 days after sowing but gradually recovered. There was also an occurrence of leaf falling particularly the aging leaves of the nitrogen-treated plants 54-70 days after sowing.

Cotyledons of plants in both nitrogen-treated and inoculated leaves regardless of the different levels of boron, remained green and functioned as photosynthetic areas for at least 30 days after sowing (Duke and Polhill, 1981; Lovell and Moore, 1971, 1970). However, the cotyledons of the nitrogen-treated plants yellowed and rotted before those of inoculated plants.

Greenhouse-grown sainfoin plants were found to have no distinct taproot system. Lateral root development, however, was observed especially in the nitrogen-treated plants. The roots were also observed to be distorted which Ross and Delaney (1977) considered to be related to the decline of sainfoin stands in the field. However, it must have been the result of the restricted growth of the roots imposed by the limited area of the pots used.

(a)



(b)

Plate 1. Representative sainfoin plants from the six treatments

(a)

(b)

Plate 2. Boron deficiency symptoms in sainfoin. Margins of leaves turn yellow then take on reddish tinge.

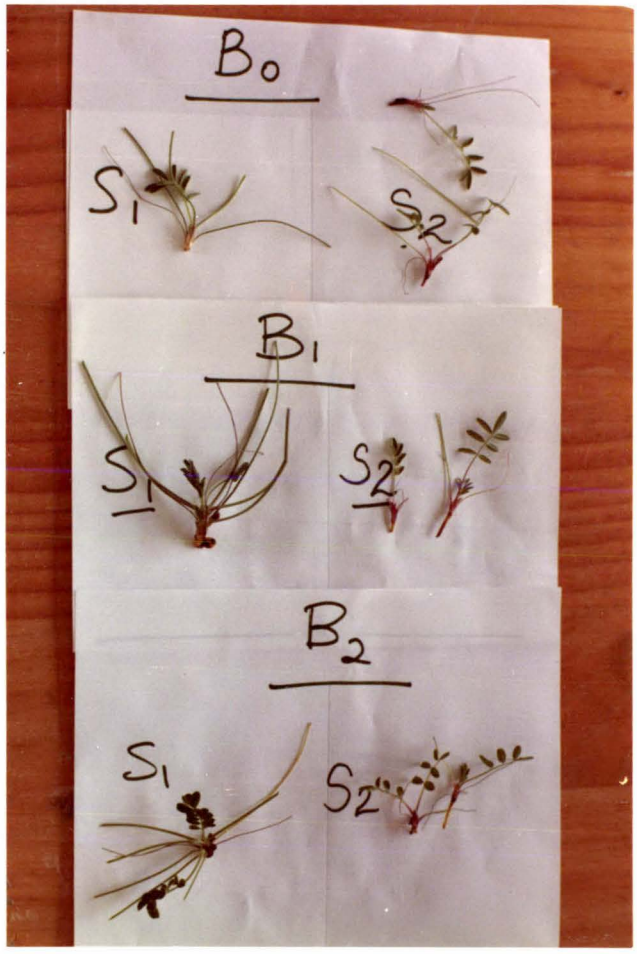


Plate 3. Reddening petioles of a boron-deficient sainfoin.

4.2 PLANT GROWTH AND DEVELOPMENT

This topic discusses the results obtained from the relative growth rate of the whole plant and its component parts, the total plant dry weight and its component parts, and growth in number of lateral roots and leaf area.

4.2.1 Relative Growth Rates

There were eight periods of growth defined as follows:

DAY (day after sowing)	PERIOD
8 - 13	1
13 - 18	2
18 - 23	3
23 - 30	4
30 - 37	5
37 - 52	6
52 - 71	7
71 - 91	8

A summary of the mean relative growth rates (mg/mg/day) of the whole plant, shoot, and root as affected by the different combinations of treatments at each period of growth is given in Tables 1, 2, and 3, respectively. Figures 1, 2 and 3 show the changes in time of the relative growth rates of the whole plant, the shoot, and the root, respectively.

Moreover, values of plant relative growth rate (PRGR), shoot relative growth rate (SRGR), and root relative growth rate (RRGR) showing a significant interaction effect will be presented in a two-way table of means.

4.2.1.1 Plant relative growth rate

There was no consistent significant effects of the different levels of boron on the plant relative growth rate although in four growth periods: 1, 3, 6 and 7 highly significant differences occurred among treatments (Table 4).

A consistent significant effect was obtained from the nitrogen-treated plants over the inoculated plants. Nitrogen-treated plants were very significantly different from the inoculated plants from day 19-91 (Table 4).

Also significant interaction effects were recorded at growth periods 1, 3, and 5. A two-way table of means is given in Table 5a, 5b, and 5c for the plant relative growth rate on the respective periods of growth.

4.2.1.2 Shoot relative growth rate

Highly significant differences among levels of boron as they affected the shoot relative growth rate were obtained from four growth periods: 1, 3, 4, and 6 which were similar to the response of the whole plant to boron levels applied. However, there was no consistent pattern of effect of each of the levels of boron applied (Table 6).

Similarly, highly significant differences were observed between the nitrogen-treated and inoculated shoots with the inoculated shoots showing very significantly differences at period 2 and a consistent pattern of significant effects of the nitrogen-treated shoots over the inoculated shoots from day 30-91 (Table 6).

There were interaction effects detected in periods 1, 3, 5, and 8 (Table 7a, 7b, 7c, 7d).

4.2.1.3 Root relative growth rate

The different levels of boron applied had no significant effect on the root relative growth rate from period 1 to 5 but significant effects were detected on the last three growth periods: 6, 7, and 8. There was no consistent pattern of effects of the levels of boron on the relative root growth rate that occurred (Table 8).

Initially, inoculation had a significant effect on the root growth rate (Table 8) but the pattern of significant effects became inconsistent as shown by the non-significant differences between plant-roots receiving nitrogen

and inoculant on alternating periods of growth and for the last two periods of growth, nitrogen-treated plant-roots showed highly significant effects over inoculated plant-roots.

Significant interaction effects occurred on periods 1, 3, and 4 (Table 9a, 9b, and 9c).

4.2.1.4 Mean relative growth rates of plant, shoot and root for eight periods of growth

There were no significant effects of the different levels of boron on the mean relative growth rates of the whole plant, shoot and root for eight periods of growth, however, highly significant differences occurred between nitrogen-treated plants and inoculated plants, plant-shoots, and plant-roots. Mean relative growth rate of the nitrogen treated plants was 0.049 mg/mg/day, which was highly significant over the inoculated plants with a relative growth rate of 0.034 mg/mg/day. Mean relative growth rate of shoots receiving nitrogen was 0.034 mg/mg/day which was highly significant over the inoculated shoots with a relative growth rate of 0.020 mg/mg/day. The mean relative growth rate for nitrogen-treated roots was 0.064 mg/mg/day, which was significantly higher than the mean relative growth rate of inoculated roots of 0.056 mg/mg/day (Table 10).

There was no occurrence of significant interaction effects between the levels of boron and nitrogen application/ inoculation treatments of plant and shoot, but it was detected in roots (Table 10a).

It must be noted that roots, regardless of treatments gave the fastest relative growth rate compared to the mean relative growth rates of the whole plant and shoot. On the whole, the whole plants, the shoots, and the roots treated with nitrogen were found to have the highest relative growth rate.

4.2.2 Growth in Total Dry Weight and Component Parts

This section will present the results obtained from the total dry weights of the whole plant, the shoots and the roots. Moreover, logistic curves were successfully

Table 1. Mean relative growth rate of the whole plant as affected by the different combinations of treatments for 8 periods of growth (mg/mg/day).

Period	(Day)	TREATMENTS 1	2	3	4	5	6
1	8-13	-0.0037	-0.0156	-0.0026	0.0196	0.0271	0.0301
2	13-18	0.0040	0.0009	0.0136	0.0051	0.0024	0.0045
3	18-23	0.0984	0.0522	0.0741	0.0299	0.0545	0.0388
4	23-30	0.0890	0.0827	0.1122	0.0895	0.0955	0.0824
5	30-37	0.0594	0.0539	0.0618	0.0319	0.0621	0.0362
6	37-52	0.0663	0.0487	0.0610	0.0449	0.0512	0.0409
7	52-71	0.0468	0.0139	0.0528	0.0183	0.0561	0.0263
8	71-91	0.0317	0.0197	0.0275	0.0253	0.0433	0.0217
Grand mean (for 8 periods)		0.0490	0.0356	0.0500	0.0331	0.0490	0.0351

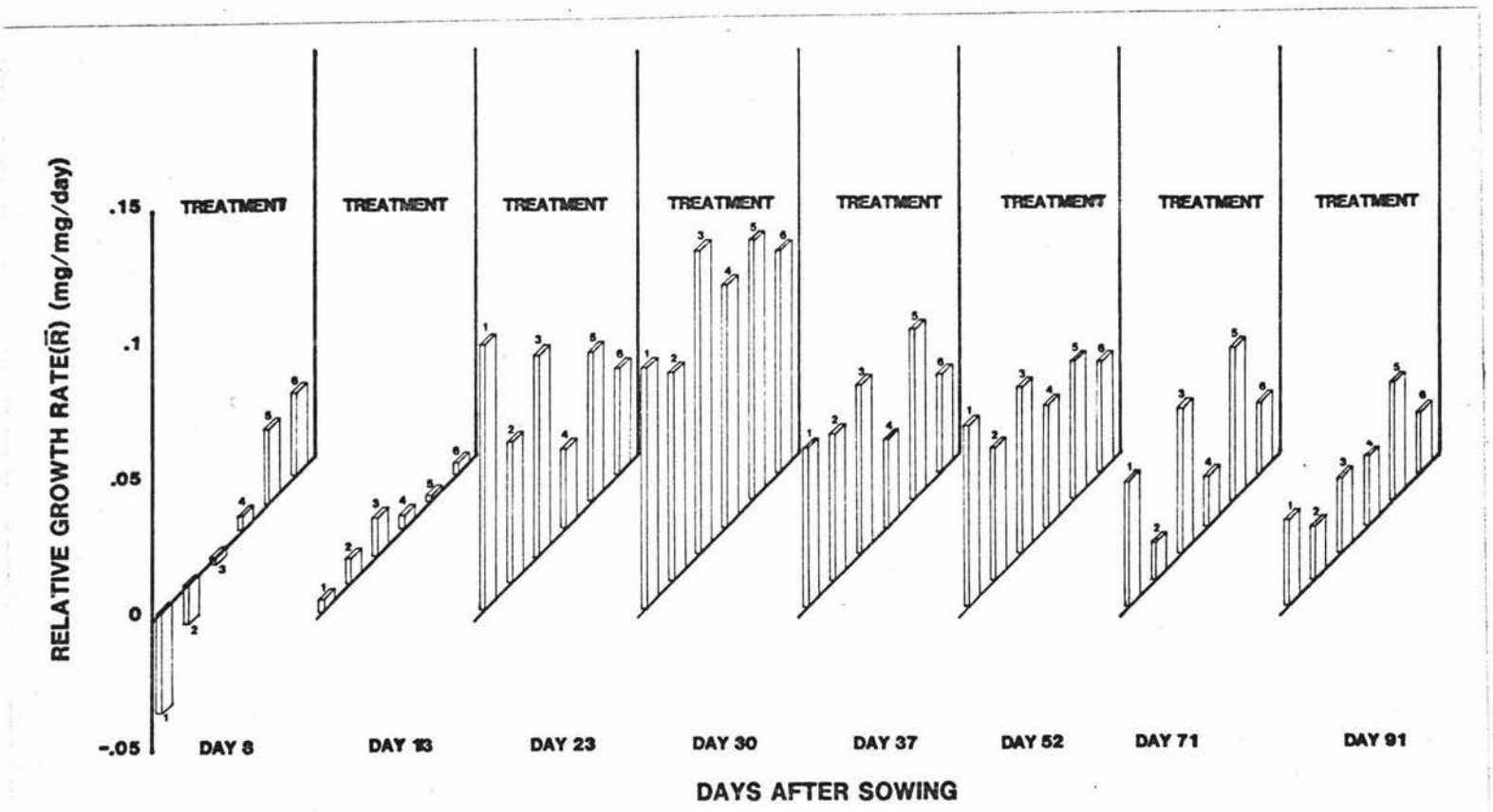


Fig. 1 Relative growth rate of whole plant (mg/mg/day) for eight periods of growth

Table 2. Mean relative growth rate of shoot (mg/mg/day) as affected by the different combinations of treatments (6) for 8 periods of growth.

Period	(Day)	TREATMENTS 1	2	3	4	5	6
1	8-13	-0.0113	-0.0339	-0.007	0.007	0.0200	0.026
2	13-18	-0.0029	-0.0126	0.0100	-0.0393	-0.0216	-0.0148
3	18-23	0.0987	0.0430	0.0589	0.0193	0.0469	0.0224
4	23-30	0.0560	0.0302	0.0464	0.0566	0.0417	0.0462
5	30-37	0.0534	0.0535	0.0591	0.0259	0.0602	0.0295
6	37-52	0.0618	0.0307	0.0566	0.0341	0.0433	0.0262
7	52-71	0.0351	0.0042	0.0385	0.0060	0.0480	0.0156
8	71-91	0.0234	0.0064	0.0120	0.0123	0.0184	0.0100
Grand mean (for 8 periods)		0.0342	0.0222	0.0343	0.0185	0.0339	0.0100

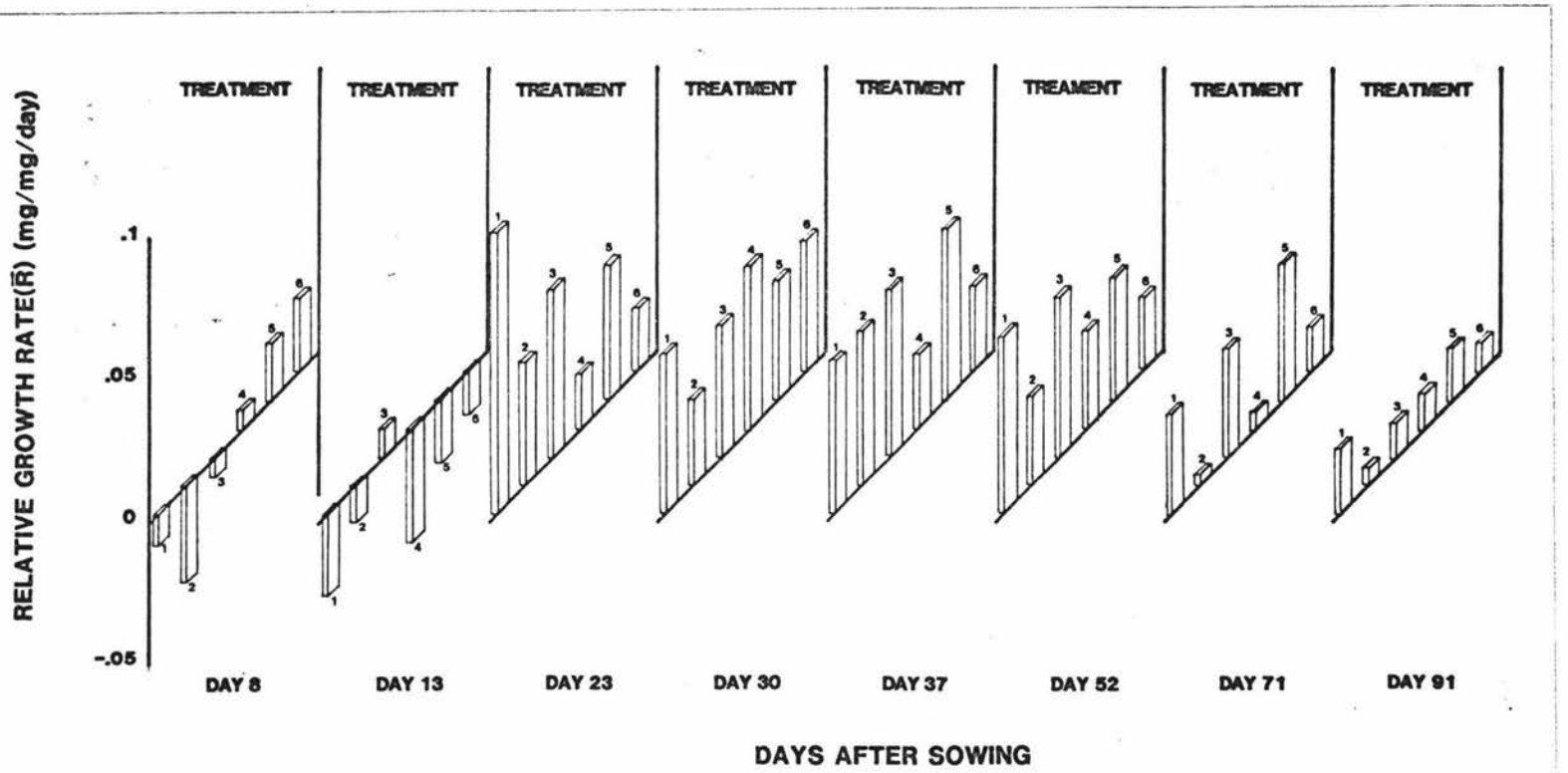


Fig. 2 Relative growth rate of shoot (mg/mg/day) for eight periods of growth

Table 3. Mean relative growth rate of root (mg/mg/day) as affected by the different combinations of treatments for 8 periods of growth.

Period	(Day)	TREATMENTS 1	2	3	4	5	6
1	8-13	0.0442	0.0433	0.0216	0.0844	0.0632	0.0899
2	13-18	0.0600	0.0251	0.0298	0.0556	0.0320	0.0525
3	18-23	0.0974	0.0894	0.1253	0.0570	0.0718	0.0851
4	23-30	0.0662	0.0727	0.0932	0.0926	0.0965	0.0736
5	30-37	0.0725	0.0549	0.0643	0.0424	0.0665	0.0484
6	37-52	0.0748	0.0730	0.0590	0.0596	0.0643	0.0607
7	52-71	0.0621	0.0218	0.0727	0.0305	0.0666	0.0357
8	71-91	0.0374	0.0262	0.0394	0.0327	0.0444	0.0286
Grand mean (for 8 periods)		0.0643	0.0508	0.0632	0.0569	0.0631	0.0593

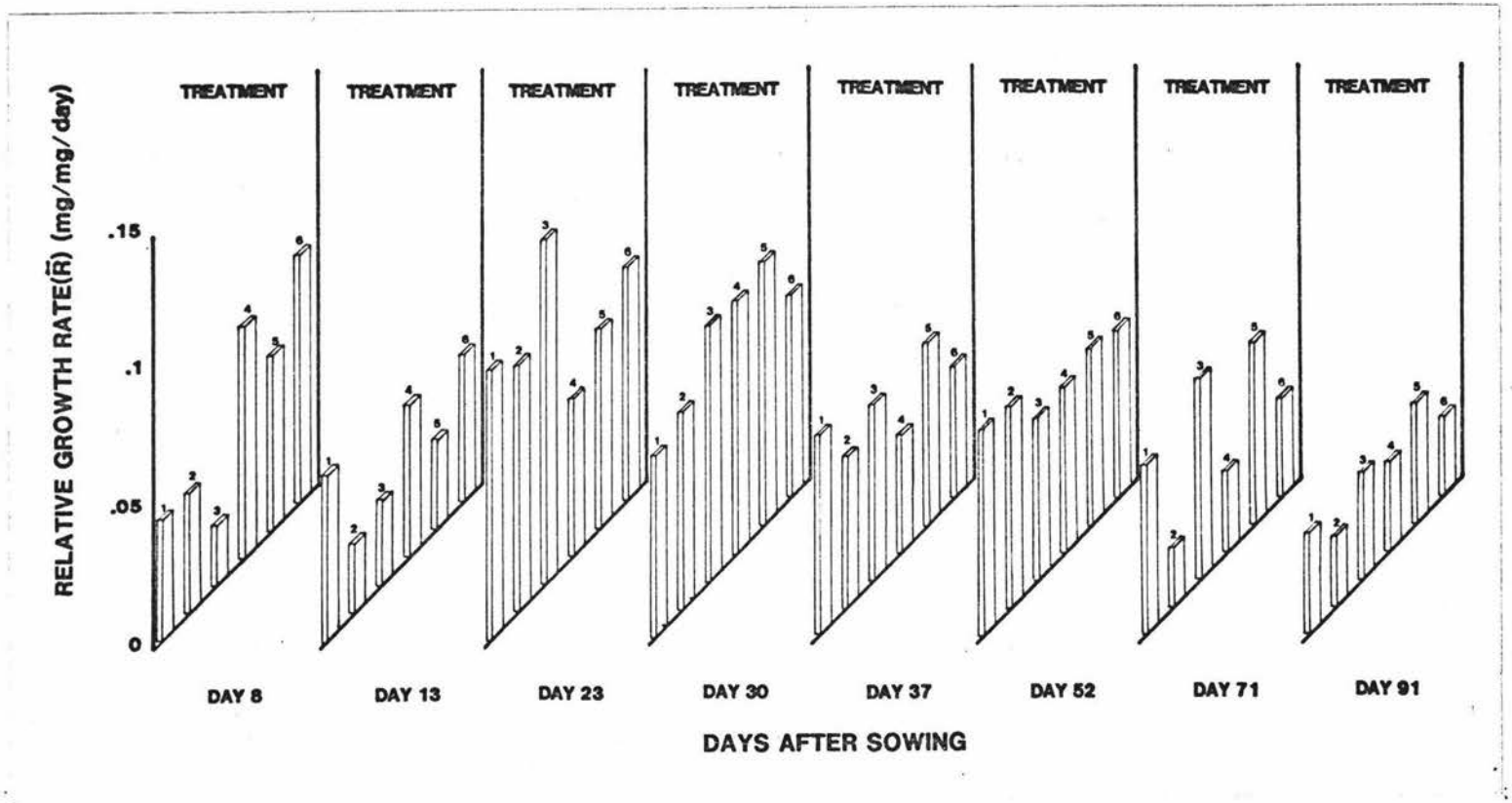


Fig. 3 Relative growth rate of root (mg/mg/day) for eight periods of growth

TREATMENTS	TIME OF HARVEST							
Boron Rates	1	2	3	4	5	6	7	8
0	-0.0097[b]	0.0024	0.0753[a]	0.0858	0.0567	0.0575[a]	0.0303[b]	0.0257
1	0.0087[c]	0.0093	0.0520[c]	0.1008	0.0468	0.0529[c]	0.0355[c]	0.0259
2	0.0286[a]	0.0036	0.0466[b]	0.0889	0.0492	0.0460[b]	0.0412[a]	0.0290
Significance	**	N.S.	**	N.S.	N.S.	**	**	N.S.
LSD .05	0.0143	N.S.	0.0072	N.S.	N.S.	0.0032	0.0039	N.S.
S.E. Mean	0.0052	0.0034	0.0027	0.0047	0.0642	0.0011	0.0015	0.0012
C.V. (%)	69.3	81.9	5.8	6.3	10.1	2.6	5.0	5.5
Nitrogen/ Inoculation								
N-	0.0070	0.0068[a]	0.0757[a]	0.0989[a]	0.0611[a]	0.0595[a]	0.0519[a]	0.0315[a]
Inoculated	0.0114	0.0035[b]	0.0403[b]	0.0849[b]	0.0407[b]	0.0448[b]	0.0195[b]	0.0222[b]
Significance	N.S.	N.S.	**	**	**	**	**	**
LSD .05			0.0063	0.0052	0.0063	0.0073	0.0031	0.0052
S.E. Mean	0.0037	0.0037	0.0026	0.0041	0.0023	0.0030	0.0012	0.0020
C.V. (%)	84.5	152.5	9.6	5.4	11.0	12.0	7.4	15.6
Interaction (B x I/N)								
Significance	*	N.S.	**	N.S.	*	N.S.	N.S.	N.S.
S.E. Mean	0.0069	0.0057	0.0042	0.0055	0.0053	0.0038	0.0021	0.0027
LSD .05	0.0155	N.S.	0.0109	N.S.	0.0109	N.S.	N.S.	N.S.

[a] Means followed by a common letter are not significantly different.
** highly significant at P = 0.01
* significant at P = 0.05
N.S. nonsignificant at P = 0.05

Table 4. Summary of relative growth rate (mg/mg/day) of the whole plant for 8 periods.[a]

Table 5. Mean relative growth rate of sainfoin plant at different periods of growth.

Levels of Boron	N-application	Inoculation	S.E.[a]
	N	I	
0	-0.004	-0.016	0.007
1	-0.002	0.020	
2	0.027	0.030	
L.S.D. _ 05 = 0.016			

(a) Harvest 1

Levels of Boron	N-application	Inoculation	S.E.[a]
	N	I	
0	0.098	0.052	0.004
1	0.074	0.030	
2	0.055	0.039	
L.S.D. _ 05 = 0.011			

(b) Harvest 3

Levels of Boron	N-application	Inoculation	S.E.[a]
	N	I	
0	0.059	0.054	0.005
1	0.062	0.032	
2	0.062	0.036	
L.S.D. _ 05 = 0.011			

(c) Harvest 5

[a] S.E. for comparisons between interaction means.

TREATMENTS	TIME OF HARVEST							
Boron rates	1	2	3	4	5	6	7	8
0	-0.0226[b]	-0.0078	0.0708[a]	0.0179[b]	0.0534	0.0462[a]	0.0197[b]	0.0149
1	0.0[c]	-0.0016	0.0391[b]	0.0515[a]	0.0425	0.0453[a]	0.0223[b]	0.0122
2	0.0229[a]	-0.0110	0.0347[b]	0.0439[a]	0.0448	0.0348[b]	0.0318[a]	0.0142
Significance	**	N.S.	**	**	N.S.	**	**	N.S.
LSD .05	0.0072	N.S.	0.0124	0.0088	N.S.	0.0028	0.0048	N.S.
S.E. Mean	0.0023	0.0053	0.0045	0.0033	0.0046	0.0009	0.0018	0.0015
C.V. (%)	3118.8	95.8	11.3	10.6	11.9	2.7	8.7	13.7
Nitrogen/ Inoculation								
N-	0.0005	-0.0001[b]	0.0682[a]	0.0312[a]	0.0576[a]	0.0539[a]	0.0405[a]	0.0179[a]
Inoculated	-0.0003	-0.0135[a]	0.0282[b]	0.0444[b]	0.0363[b]	0.0303[b]	0.0086[b]	0.0096[b]
Significance	N.S.	*	**	**	**	**	**	**
LSD .05	N.S.	0.0089	0.0063	0.0073	0.0073	0.0063	0.0020	0.0012
S.E. Mean	0.0031	0.0037	0.0027	0.0030	0.0031	0.0028	0.0009	0.0006
C.V. (%)	7460.9	115.8	11.7	17.1	14.1	14.0	7.4	8.7
Interaction (B X I/N)								
Significance	**	N.S.	**	N.S.	**	N.S.	N.S.	**
S.E. Mean	0.0045	0.0070	0.0055	0.0050	0.0060	0.0035	0.0020	0.0017
LSD .05	0.0155	N.S.	0.0109	N.S.	0.0126	N.S.	N.S.	0.0020

[a] Means followed by a common letter are not significantly different
** Highly significant at P = 0.01
* Significant at P = 0.05
N.S. Nonsignificant at P = 0.05

Table 6. Summary of relative growth rate of shoot (mg/mg/day) for 8 periods.[a]

Table 7. Mean relative growth rate of shoot at different periods of growth.

Levels of Boron	Nitrogen-application Inoculation		S.E.[a]
	N	I	
0	-0.011	-0.034	0.005
1	-0.007	0.007	
2	0.020	0.026	
L.S.D. _05 = 0.016			

(a) Harvest 1

Levels of Boron	Nitrogen-application Inoculation		S.E.[a]
	N	I	
0	0.099	0.043	0.006
1	0.059	0.019	
2	0.047	0.022	
L.S.D. _05 = 0.011			

(b) Harvest 3

Levels of Boron	Nitrogen-application Inoculation		S.E.[a]
	N	I	
0	0.053	0.054	0.006
1	0.059	0.026	
2	0.060	0.030	
L.S.D. _05 = 0.013			

(c) Harvest 5

Levels of Boron	Nitrogen-application Inoculation		S.E.[a]
	N	I	
0	0.023	0.006	0.002
1	0.012	0.012	
2	0.018	0.010	
L.S.D. _05 = 0.002			

(d) Harvest 8

[a] S.E. for comparison between interaction means.

TREATMENTS	TIME OF HARVEST							
Boron rates	1	2	3	4	5	6	7	8
0	0.0437	0.0426	0.0934	0.0695	0.0637	0.0739[a]	0.0419[b]	0.0318[b]
1	0.0530	0.0427	0.0911	0.0929	0.0534	0.0593[b]	0.0516[a]	0.0361[a]
2	0.0765	0.0423	0.0785	0.0849	0.0574	0.0629[a]	0.0511[a]	0.0365[a]
Significance	N.S.	N.S.	N.S.	N.S.	N.S.	**	**	*
LSD .05	N.S.	N.S.	N.S.	N.S.	N.S.	0.0023	0.0039	0.0032
S.E. Mean	0.0108	0.0119	0.0072	0.0082	0.0043	0.0008	0.0014	0.0011
C.V. (%)	22.9	34.3	10.0	12.1	9.0	1.5	3.6	4.0
Nitrogen/ Inoculation								
N-	0.0430[b]	0.0406	0.0982[a]	0.0853	0.0678[a]	0.0663	0.0671[a]	0.0404[a]
Inoculated	0.0725[a]	0.0444	0.0772[b]	0.0796	0.0486[b]	0.0644	0.0293[b]	0.0292[b]
Significance	**	N.S.	*	N.S.	**	N.S.	**	**
LSD .05	0.0115	N.S.	0.020	N.S.	0.0073	N.S.	0.0037	0.0037
S.E. Mean	0.0051	0.0094	0.0082	0.0031	0.0031	0.0020	0.0017	0.0015
C.V. (%)	18.8	46.9	19.9	8.0	11.2	6.5	7.5	9.0
Interaction (B X I/N)								
Significance	**	N.S.	*	**	N.S.	N.S.	N.S.	N.S.
S.E. Mean	0.0125	0.0166	0.0124	0.0090	0.0057	0.0026	0.0025	0.0021
LSD .05	0.0049	N.S.	0.0346	0.0126	N.S.	N.S.	N.S.	N.S.

[a] Means followed by a common letter are not significantly different.
** Highly significant at P = 0.01
* Significant at P = 0.05
N.S. Nonsignificant at P = 0.05

Table 8. Summary of relative growth rate of root (mg/mg/day) for 8 periods.[a]

Table 9. Mean Relative growth rate of the root at different harvests.

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	0.044	0.043	0.013
1	0.022	0.084	
2	0.063	0.090	
L.S.D. _05 = 0.005			

(a) Harvest 1

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	0.097	0.089	0.012
1	0.125	0.057	
2	0.072	0.085	
L.S.D. _05 = 0.035			

(b) Harvest 3

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	0.066	0.073	0.009
1	0.093	0.093	
2	0.097	0.073	
L.S.D. _05 = 0.013			

(c) Harvest 4

[a] S.E. for comparisons between interaction means.

Table 10. Summary of the mean Relative growth rate of the whole plant (M_PGR), shoot (M_SRGR) and root (M_RRGR) for eight periods of growth. [a]

TREATMENTS	M_PRGR	M_SRGR	M_RRGR
Boron Rates			
0	0.0419	0.0282	0.0576
1	0.0416	0.0264	0.0600
2	0.0421	0.0270	0.0612
Significance	N.S.	N.S.	N.S.
LSD _05	N.S.	N.S.	N.S.
S.E. Mean	0.0006	0.0022	0.0019
C.V. (%)	1.6	10.0	3.8
N-application-Inoculation			
N	0.0494[a]	0.0341[a]	0.0635[a]
I	0.0344[b]	0.0203[b]	0.0557[b]
Significance	**	**	**
LSD _05	0.004	0.0032	0.0028
S.E. Mean	0.0013	0.0011	0.0010
C.V. (%)	6.6	8.2	3.5
Interaction (B X N/I)			
Significance	N.S.	N.S.	*
S.E. Mean	0.0017	0.0026	0.0022
LSD _05	N.S.	N.S.	

[a] Means followed by a common letter are not significantly different.

** Highly significant at P = 0.01

* Significant at P = 0.05

N.S. Nonsignificant at P = 0.05

Table 10a. Mean relative growth rate of the root (mg/mg/day) for eight periods of growth.

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	0.0643	0.0508	
1	0.0632	0.0569	0.0022
2	0.0631	0.0593	
L.S.D. _05 = 0.002			

[a] S.E. for comparisons between interaction means

used to fit the total dry weight of the whole plant, the shoot, and the root of all the six treatment-plants against time.

The nine harvest times presented in Section 3.4.1 will be described as 1, 2, 3, 4, 5, 6, 7, 8, and 9.

4.2.2.1 Plant dry weight

Levels of boron significantly affected the total plant dry weight at all harvest times, however, inconsistent patterns of significant effects of each level of boron were obtained. At harvests 1, 4, and 7, plants treated with 0 ppm boron gave the highest total dry weight while plants receiving 2 ppm boron yielded the highest dry weight at harvests 3 and 9, and plants treated with 1 ppm boron gave the highest total dry weight at harvests 5, 6, and 8 (Table 11).

Nitrogen-application and inoculation significantly affected the total dry weight of plant. Significant differences were shown at all harvest times. Initially, inoculated plants showed higher total dry weight up to harvest 3 and thereafter, a reverse effect was observed up to harvest 9 (Table 11).

Significant interaction effects were detected between levels of boron and nitrogen-application/inoculation treatments at harvests 1, 4, 6 and 8 (Table 12a, 12b, 12c, 12d).

Logistic curves were successfully used to fit the total dry weight of the six treatment-plants (Fig. 4).

A rapid growth rate of treatments 1, 3, and 5 commenced after day 30 whereas treatments 2, 4, and 6 showed a constant slow growth rate all throughout the growing period (Fig. 5a, 5b, 5c). The asymptotes ($\hat{Y}o$) were of further interest because these $\hat{Y}o$ indicated the potential total dry weight of these treatment-plants. $\hat{Y}o$ in treatments 1, 3, and 5 (375, 403, and 403 mg/plant, respectively) were approximately three times greater than those of treatments 2, 4, and 6 (between 125 and 130 mg/plant). Comparing the $\hat{Y}o$ of the different treatments, it is evident that the nitrogen-treated plants regardless of the levels of boron applied had much higher total plant dry weight than those of the inoculated plants.

Comparison between curves was not done, however, information from the linear regression $(\ln \frac{Y}{Yo - Y}) = \beta o + \beta 1 x$ for each treatment is presented in Table 13. βo and $\beta 1$ are the values of constants A and B, respectively, in the equation

$$Y = \frac{Yo}{1 + e^{-(A + Bx)}} \qquad \text{Section 3.4.3}$$

The rate of change in the total plant dry weight was affected by both βo and $\beta 1$ where $\beta 1$ is of further interest because it is the Relative growth rate (Bliss, 1970).

4.2.2.2 Shoot dry weight

Significant responses to boron treatments were obtained from shoot total dry weight at harvests 1, 5, 7, and 8. The significant effects of the different levels of boron to shoot dry matter yield were inconsistent. Total dry weight of shoots treated with 0 ppm boron was highest at harvest 6 but not significantly higher than the rest of the boron levels, and was significantly higher at harvests 5, 7, and 8. Shoots receiving 2 ppm boron yielded the highest dry matter at harvest 2 (Table 14).

Likewise, there was a significant effect of nitrogen and inoculant application to shoot total dry weight. Inoculated shoots were significantly higher in their total dry weight at harvests 1 and 2 while both treatments were not significantly different at harvest 3. From harvest 4 to 9, the dry weight of the nitrogen-treated shoots was significantly higher than shoots receiving inoculant (Table 14).

There was a significant interaction effect between the levels of boron and nitrogen/inoculant application at harvests 1, 4, 6, 8, and 9 (Table 15a, 15b, 15c, 15d, 15e).

Logistic curves were also used to fit the total shoot dry weight (Fig. 6).

As the total plant dry weight, shoots exhibited a similar pattern of growth rate with treatments 1, 3, and 5

showing rapid growth rates after day 30, however, treatments 2, 4, and 6 showed a much slower growth rate (Fig. 7a, 7b, 7c) throughout the growing period. The $\hat{Y}o$ of treatments 1, 3, and 5 were 175, 149, and 175 mg/shoot, respectively while treatments 2, 4, and 6 were 39, 45, and 45 mg/shoot, respectively. It is evident that plants applied with nitrogen had a much higher shoot dry weight than those inoculated shoots. Among the nitrogen-treated shoots at all levels of boron, shoots treated with 1 ppm boron had the lowest $\hat{Y}o$. Information from the linear regression $(\ln \frac{Y}{\hat{Y}o - Y}) = \beta o + \beta 1x$ for each of the treatments are given in Table 16.

4.2.2.3 Root dry weight

The different levels of boron had significant effects on the total root dry weight although an inconsistent pattern of response occurred at each level of boron applied at harvests 1 and 5 to 9. There were no responses to levels of boron at harvests 2 to 4. The roots receiving 0 ppm boron gave the significantly highest total root dry weight at harvests 1 and 7 while roots treated with 1 ppm boron gave the highest total dry weight at harvests 5, 6, 8, and 9 although at harvest 9, it was not significantly different from roots treated with 2 ppm boron (Table 17).

With regards to the effects of nitrogen application and inoculation on the total root dry weight, a highly significant response was obtained from eight harvests commencing at harvest 2 while at harvest 1 there was no significant difference observed. Inoculated roots were significantly higher in their total dry weight than the nitrogen-treated roots from harvest 2 to 5 while from harvest 6 to 9, the application of nitrogen gave the root a significantly higher dry weight than the inoculated roots (Table 17).

At harvest 1, 3, 4, 5, 8, and 9, significant interaction effects occurred (Table 18a, 18b, 18c, 18d, 18e, 18f).

Logistic growth curve was also fitted successfully to the root dry weight (Fig. 8).

The growth rate of the roots of treatments 1, 3, and 5 exhibited the same pattern of growth as those of the whole plants and shoots of the same treatments commencing their rapid growth after day 30, while treatments 2, 4, and 6 commenced after day 40, ten days later (Fig. 9a, 9b, 9c). The $\hat{Y}o$ of treatments 1, 3, and 5 were 215, 259, 249, respectively while treatments 2, 4, and 6 had 76, 88, and 92. It is noteworthy that in the nitrogen-treated roots applied with the different levels of boron, roots receiving 1 ppm boron had the highest $\hat{Y}o$ in contrast to the $\hat{Y}o$ of shoots treated with 1 ppm boron. Moreover, the $\hat{Y}o$ of the inoculated roots increased with the increment of boron level. Information from the linear regression $\left(\ln \frac{Y}{\hat{Y}o - Y}\right) = \beta o + \beta 1x$ for each of the treatments is given in Table 19.

4.2.3 Growth in Number of Lateral Roots and Leaf Area

This section discusses the results obtained from the effects of the different levels of boron and nitrogen/inoculant application on the number of first order lateral and second order lateral roots.

4.2.3.1 Number of first order lateral roots

The number of first order lateral roots were counted from harvest 1 to 6. Because of the difficulties encountered in maintaining a perfectly intact root system at harvest 7 to 9, lateral roots were not counted further.

There was no significant response obtained from the different levels of boron applied on the number of the first order lateral roots at all the harvest times except for harvest 5 when a significance among levels of boron occurred with roots receiving 0 ppm boron giving the most first order lateral roots (Table 20).

Highly significant differences occurred in the first order lateral roots treated with nitrogen and inoculant at harvests 2 and 4 with the inoculated plants exhibiting a significantly higher number of first order lateral roots (Table 20) and a significant difference (P = 0.05) between nitrogen-treated and inoculated plants at harvest 5 with the inoculated first order lateral roots showing significantly higher roots.

A highly significant interaction effect between boron and nitrogen/inoculant application treatments occurred at harvest 4 (Table 20a).

4.2.3.2 Number of second order lateral roots

Counting was started at harvest 3 as soon as the second order lateral roots emerged. A summary of the number of the second order lateral roots is presented in Table 21.

Plants treated with 1 ppm boron produced the most number of second order lateral roots at harvest 3, 4, and 6 although it was only at harvest 2 that significant differences were detected (Table 21). For the rest of the harvest times, there were no significant differences obtained among the different levels of boron.

The nitrogen-treated plants gave a very significantly different number of second order lateral roots over the inoculated plants (Table 21). A significant interaction effect occurred at harvest 2 (Table 21a).

Attempts to count the third order lateral roots were made at harvest 6 but due to the large error caused by broken roots at sampling time, no significant effects were obtained from any of the treatments. Moreover, this explains the nonsignificant response of plants to boron rates, and nitrogen and inoculant application in terms of first order lateral and second order lateral root at harvest 6.

4.2.3.3 Leaf area (cm^2)

The leaf area data for three harvest times are summarized in Table 22.

TREATMENTS	TIME OF HARVEST[b]								
Boron Rates	1	2	3	4	5	6	7	8	9
0	11.017[a]	10.295[b]	10.487[b]	15.367[a]	19.300[b]	28.72[b]	69.07[a]	135.43[b]	237.3[b]
1	9.920[b]	10.388[b]	10.873[ab]	14.110[b]	22.077[a]	30.82[a]	67.52[a]	149.87[a]	257.1[a]
2	9.485[b]	10.948[a]	11.150[a]	14.077[b]	20.788[a]	29.51[b]	59.56[b]	142.88[c]	259.5[a]
Significance	**	*	*	**	**	**	**	**	**
LSD -.05	0.4455	0.5515	0.3926	0.4953	1.2135	1.0643	4.6755	3.1407	10.2487
S.E. Mean	0.1615	0.1986	0.1415	0.1784	0.4371	0.384	1.684	1.131	3.69
C.V. (%)	2.0	2.3	1.6	1.5	2.6	1.6	3.2	1.0	1.8
Nitrogen/ Inoculation									
N-	9.753[b]	10.126[b]	10.562[b]	15.452[a]	21.297[a]	32.61[a]	78.24[a]	209.57[a]	384.0[a]
Inoculated	10.528[a]	10.962[a]	11.111[a]	13.583[b]	20.147[b]	26.76[b]	52.53[b]	75.89[b]	118.6[b]
Significance	**	**	*	**	**	**	**	**	**
LSD -.05	0.0115	0.3738	0.3741	0.3004	0.3506	0.4671	3.9067	2.9387	10.3039
S.E. Mean	0.0684	0.1527	0.1529	0.1227	0.1433	0.509	1.596	1.201	4.21
C.V. (%)	1.4	3.1	3.0	1.8	1.5	3.6	5.2	1.8	3.6
Interaction (B X I/N)									
Significance	**	N.S.	N.S.	**	N.S.	**	N.S.	**	N.S.
S.E. Mean	0.1820	0.2728	0.2347	0.2333	0.4711	0.732	2.581	1.856	6.34
LSD -.05	0.2902	N.S.	N.S.	0.5202	N.S.	0.8091	N.S.	5.0899	N.S.

[a] Means followed by a common letter are not significantly different.
** Highly significant at P = 0.01
* Significant at P = 0.05
N.S. Nonsignificant at P = 0.05

Table 11. Summary of total dry matter yield (mg/plant) for the whole plant for 9 harvest times.[a]

Table 12. Mean dry weight of sainfoin (mg/plant at different harvest times.

Levels of Boron	Nitrogen application	Inoculation	S.E.[a]
	N	I	
0	10.05	11.98	
1	9.77	10.07	0.182
2	9.44	9.53	
L.S.D. _05 = 0.290			

(a) Mean dry weight at harvest 1

Levels of Boron	Nitrogen application	Inoculation	S.E.[a]
	N	I	
0	16.97	13.76	
1	14.99	13.23	0.233
2	14.39	13.76	
L.S.D. _05 = 0.520			

(b) Mean dry weight at harvest 4

Levels of Boron	Nitrogen application	Inoculation	S.E.[a]
	N	I	
0	29.83	27.60	
1	34.97	26.67	
2	33.03	26.00	
L.S.D. _05 =			

(c) Mean dry weight at harvest 6

Levels of Boron	Nitrogen application	Inoculation	S.E.[a]
	N	I	
0	196.27	74.59	
1	225.80	73.95	1.86
2	206.63	79.13	
L.S.D. _05 = 5.09			

(d) Mean dry weight at harvest 8

[a] S.E. for comparisons between interaction means.

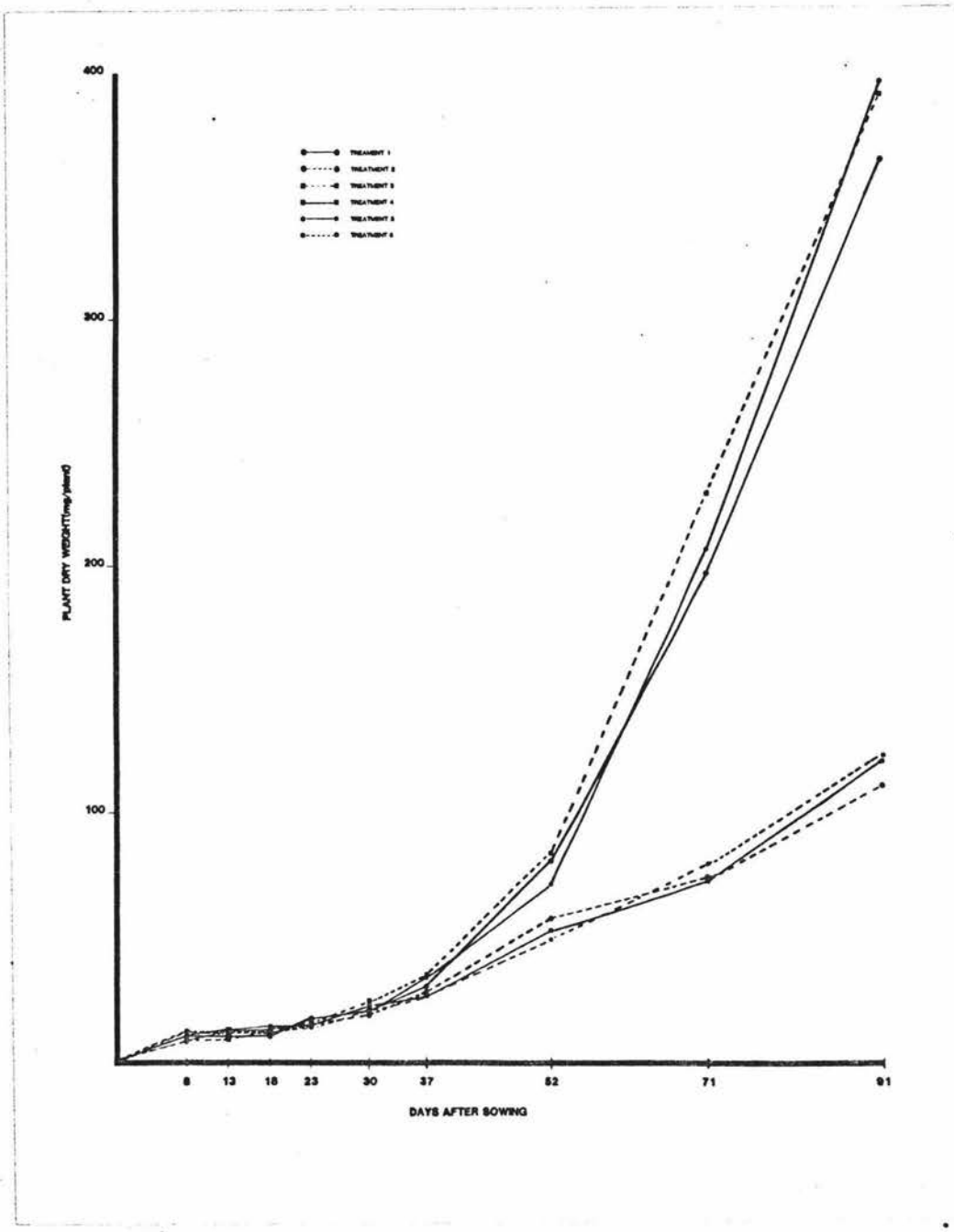


Fig. 4 Total plant dry weight (mg/plant) for nine harvest times

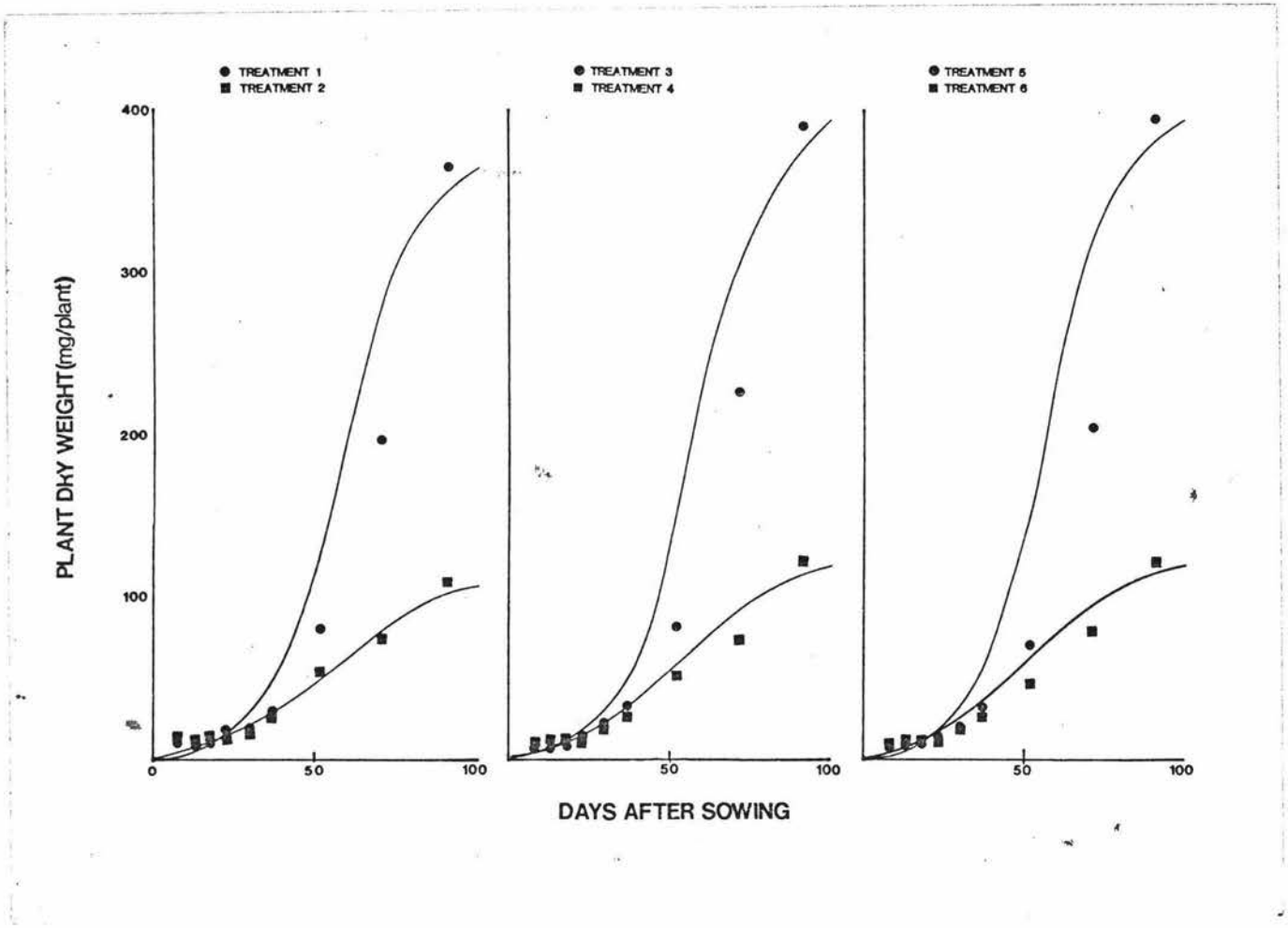


Fig. 5 Growth curves of the whole plant from the six treatments

TREATMENTS	βo	Standard error of βo	β_1	Standard error of β_1	Asymptote (Yo) (mg/plant)	R^2
1	-5.12	0.295	0.087	0.006	375	0.883
2	-3.15	0.100	0.054	0.002	125	0.962
3	-5.14	0.224	0.086	0.005	403	0.927
4	-3.43	0.155	0.063	0.003	128	0.935
5	-5.20	0.258	0.087	0.006	403	0.908
6	-3.41	0.123	0.061	0.003	130	0.955

Table 13. Information from linear regression $\ln \frac{Y}{\hat{Y}o - Y} = \beta o + \beta 1\, x$ for the plant dry weight of the six treatments.

TREATMENTS	TIME OF HARVEST								
Boron Rates	1	2	3	4	5	6	7	8	9
0	9.442[a]	8.412[b]	8.083	11.533[a]	13.065[b]	18.99	39.59[a]	64.67[b]	96.42
1	8.432[b]	8.450[b]	8.367	10.217[b]	14.615[a]	19.94	41.02[b]	71.49[a]	91.02
2	8.150[b]	9.138[a]	8.650	10.367[b]	14.092[a]	19.49	33.73[b]	69.92[a]	97.40
Significance	**	*	N.S.	**	*	N.S.	**	**	N.S.
LSD -.05	0.4295	0.4096	N.S.	0.4105	0.8622	N.S.	3.4412	1.5201	
S.E. Mean	0.1547	0.1476	0.1922	0.1478	0.3106	0.283	1.246	0.548	2.875
C.V. (%)	2.2	2.1	2.8	1.7	2.7	1.8	4.0	1.0	3.7
Nitrogen/ Inoculation									
N-	8.308[b]	8.333[b]	8.322	11.789[a]	14.627[a]	21.91[a]	49.35[a]	106.11[a]	151.98[a]
Inoculated	9.041[a]	9.000[a]	8.411	9.622[b]	13.221[b]	17.04[b]	26.88[b]	31.28[b]	37.91[b]
Significance	**	**	N.S.	**	**	**	**	**	**
LSD -.05	0.2080	0.3929	N.S.	0.4316	0.4864	1.0263	2.9294	0.5313	4.8021
S.E. Mean	0.0850	0.1606	0.1470	0.1764	0.1988	0.419	1.197	0.217	1.963
C.V. (%)	2.1	3.9	3.7	3.5	3.0	4.6	6.7	0.7	4.4
Interaction (B X I/N)									
Significance	**	N.S.	N.S.	**	N.S.	*	N.S.	**	*
S.E. Mean	0.1865	0.2459	0.2633	0.2618	0.3946	0.586	1.920	0.609	3.747
LSD -.05	0.3602	N.S.	N.S.	0.7476	N.S.	1.7776	N.S.	0.9202	8.3174

[a] Means followed by a common letter are not significantly different
** Highly significant at P = 0.01
* Significant at P = 0.05
N.S. Nonsignificant at P = 0.05

Table 14. Summary of total dry matter yield of shoot for 9 harvest times (mg/plant-shoot).[a]

Table 15. Mean dry weight of sainfoin shoot (mg/plant-shoot) at different harvest times.

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	8.70	10.18	0.187
1	8.16	8.70	
2	8.06	8.24	
L.S.D. _05 = 0.360			

(a) Harvest 1

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	13.27	9.80	0.262
1	11.10	9.33	
2	11.00	9.73	
L.S.D. _05 = 0.748			

(b) Harvest 4

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	20.07	17.92	0.586
1	23.24	16.64	
2	22.43	16.55	
L.S.D. _05 = 1.778			

(c) Harvest 6

[a] S.E. for comparisons between interaction means.

Contd. ....

Table 15 contd.

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	98.57	30.77	0.609
1	112.87	30.12	
2	106.90	32.97	
L.S.D. _05 = 0.920			

(d) Harvest 8

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	157.83	35.00	3.747
1	143.57	38.47	
2	154.53	40.27	
L.S.D. _05 = 8.317			

(e) Harvest 9

[a] S.E. for comparisons between interaction means.

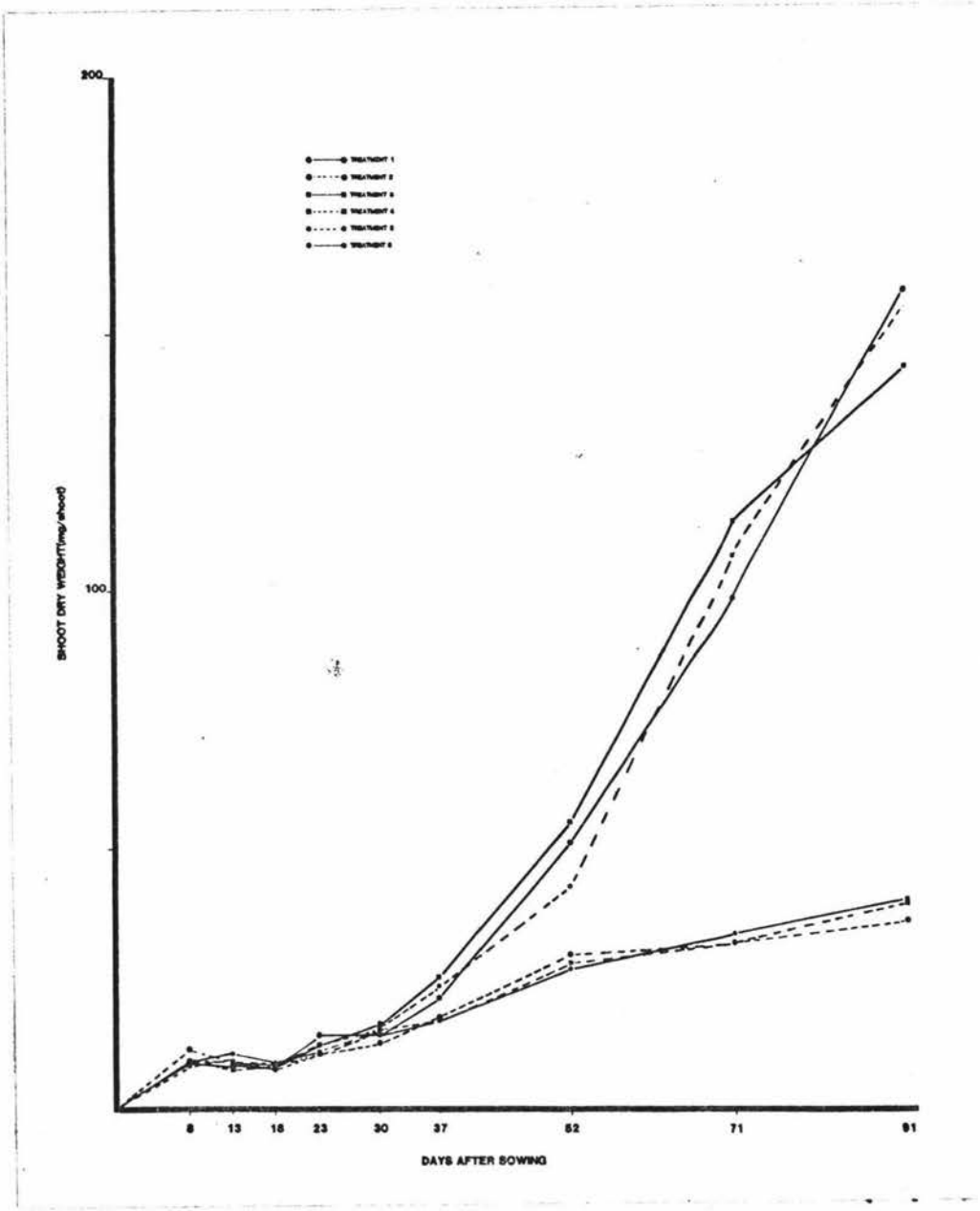


Fig. 6 Total shoot dry weight (mg/shoot) for nine harvest times

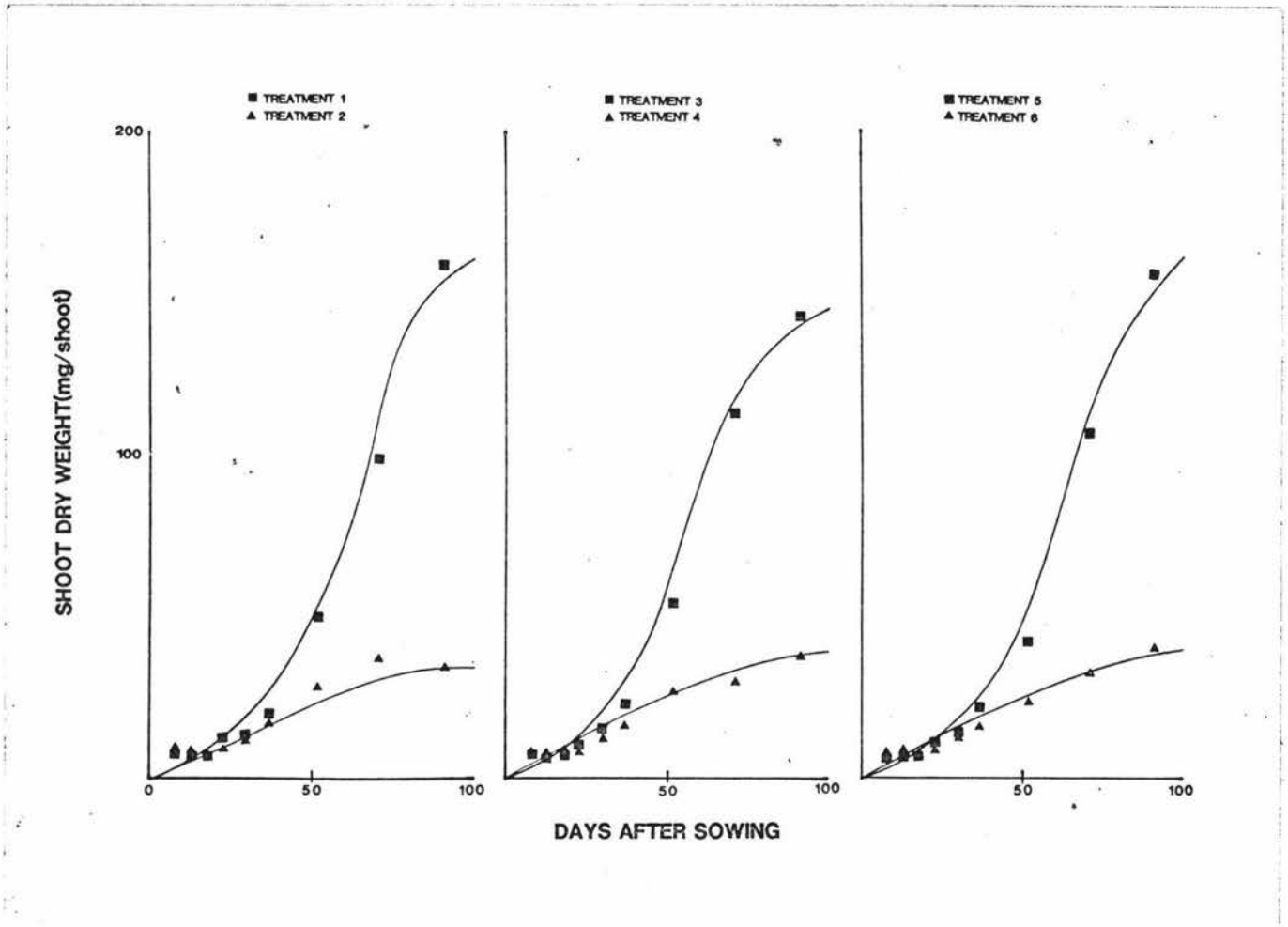


Fig. 7 Growth curves of shoot from the six treatments

TREATMENTS	Standard error of βo	βo	Standard error of $\beta 1$	$\beta 1$	Asymptote ($\hat{Y}o$) (mg/plant)	R^2
1	-4.03	0.133	0.0641	0.003	175	0.952
2	-1.85	0.100	0.0452	0.002	39	0.947
3	-4.11	0.132	0.0758	0.003	149	0.966
4	-2.00	0.074	0.0409	0.002	45	0.963
5	-3.99	0.098	0.0623	0.002	175	0.972
6	-2.11	0.055	0.0449	0.001	45	0.983

Table 16. Information from linear regression $\ln \frac{Y}{\hat{Y}o - Y} = \beta o + \beta 1 x$ for the shoot dry weight of the six treatments.

TREATMENTS	TIME OF HARVEST								
Boron Rates	1	2	3	4	5	6	7	8	9
0	1.575[a]	1.958	2.403	3.833	6.235[b]	9.72[b]	24.49[a]	70.76[b]	140.20[b]
1	1.488[a]	1.938	2.507	3.893	7.462[a]	10.88[a]	26.50[b]	78.38[a]	166.30[a]
2	1.335[b]	1.963	2.506	3.710	6.697[b]	10.02[b]	25.82[b]	73.07[c]	162.10[a]
Significance	*	N.S.	N.S.	N.S.	*	**	**	**	*
LSD .05	0.1406	N.S.	N.S.	N.S.	0.5997	0.3281	1.5373	1.9967	6.4289
S.E. Mean	0.0506	0.0693	0.733	0.0986	0.2160	0.118	0.554	0.719	2.32
C.V. (%)	4.2	4.3	3.6	3.2	3.9	1.4	2.5	1.2	1.8
Nitrogen/ Inoculation									
N-	1.446	1.792[b]	2.240[b]	3.663[b]	6.670[b]	10.70[a]	28.89[a]	103.48[a]	232.30[a]
Inoculated	1.487	2.114[a]	2.700[a]	3.961[a]	6.926[a]	9.72[b]	25.65[b]	44.66[b]	80.10[b]
Significance	N.S.	**	**	**	**	**	**	**	**
LSD .05	N.S.	0.1611	0.1668	0.1711	0.1688	0.6110	1.7031	3.0270	5.2584
S.E. Mean	0.0341	0.0658	0.0681	0.0699	0.0690	0.253	0.696	1.237	2.15
C.V. (%)	4.9	7.1	5.8	3.9	2.2	5.3	5.4	3.5	2.9
Interaction (B X I/N)									
Significance	**	N.S.	*	*	**	N.S.	N.S.	**	**
S.E. Mean	0.0656	0.1063	0.1110	0.1306	0.2320	0.332	1.016	1.677	3.51
LSD .05	0.0816	N.S.	0.2888	0.2964	0.2923	N.S.	N.S.	5.2429	9.1078

[a] Means followed by a common letter are not significantly different.
** Highly significant at P = 0.01
* Significant at P = 0.05
N.S. Nonsignificant

Table 17. Summary of total dry matter yield of root for 9 harvest times (mg/plant-root).[a]

Table 18. Mean dry weight of root (mg/plant-root) at different harvest times.

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	1.35	1.80	
1	1.61	1.37	0.066
2	1.38	1.29	
L.S.D. _05 = 0.082			

(a) Harvest 1

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	2.27	2.53	
1	2.08	2.93	0.111
2	2.37	2.63	
L.S.D. _05 = 0.289			

(b) Harvest 3

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	3.71	3.96	
1	3.89	3.90	0.131
2	3.39	4.03	
L.S.D. _05 = 0.296			

(c) Harvest 4

[a] S.E. for comparisons between interaction means.

Contd. ....

Table 18 contd.

Levels of Boron	Nitrogen application	Inoculation	S.E.[a]
	N	I	
0	5.88	6.59	0.232
1	7.47	7.45	
2	6.66	6.73	
L.S.D. _05 = 0.292			

(d) Harvest 5

Levels of Boron	Nitrogen application	Inoculation	S.E.[a]
	N	I	
0	97.70	43.82	1.677
1	112.93	43.83	
2	99.80	46.34	
L.S.D. _05 = 5.243			

(e) Harvest 8

Levels of Boron	Nitrogen application	Inoculation	S.E.[a]
	N	I	
0	206.40	74.00	3.51
1	248.30	84.30	
2	242.10	82.00	
L.S.D. _05 = 9.108			

(f) Harvest 9

[a] S.E. for comparisons between interaction means

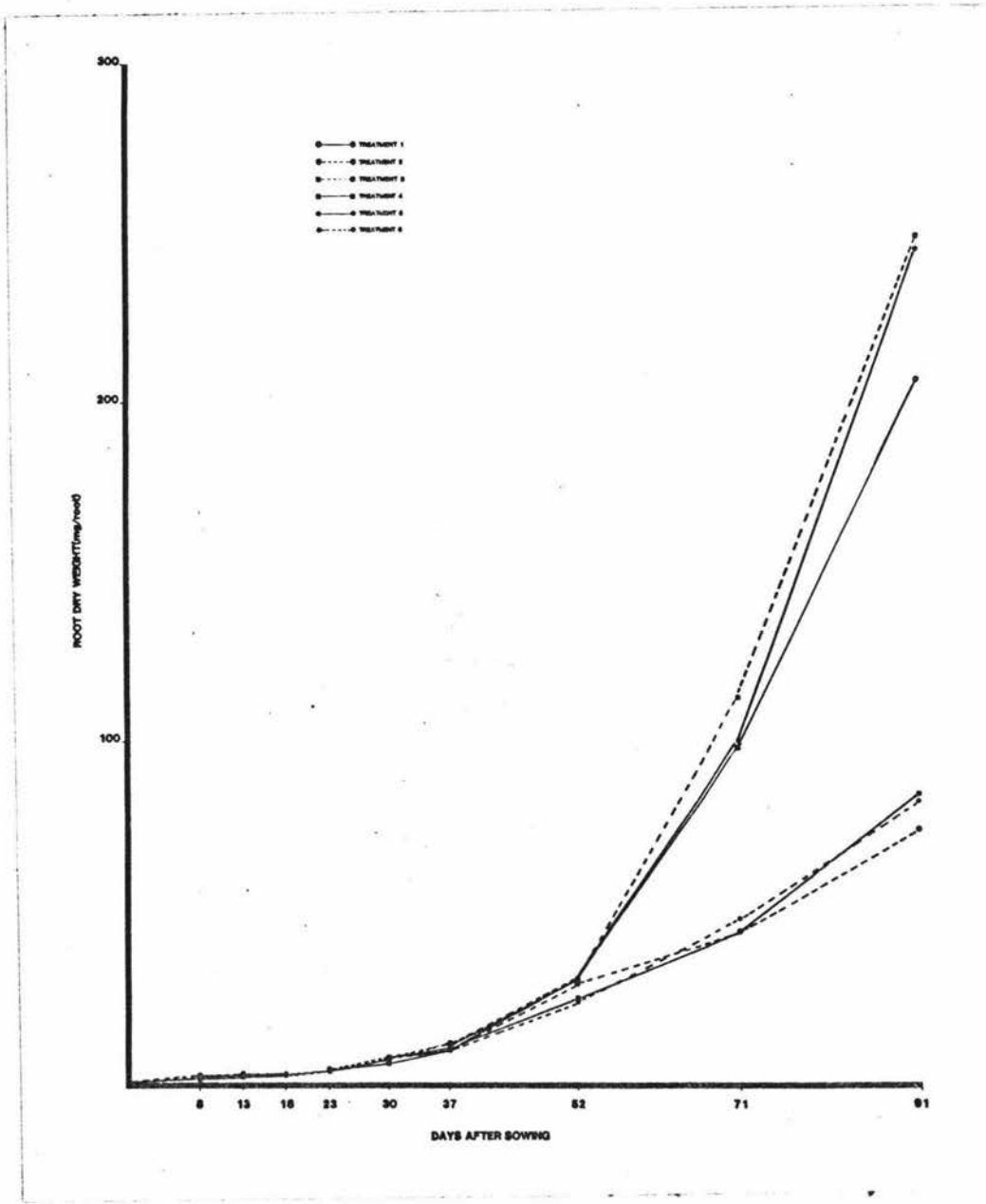


Fig. 8 Total root dry weight (mg/root) for nine harvest times

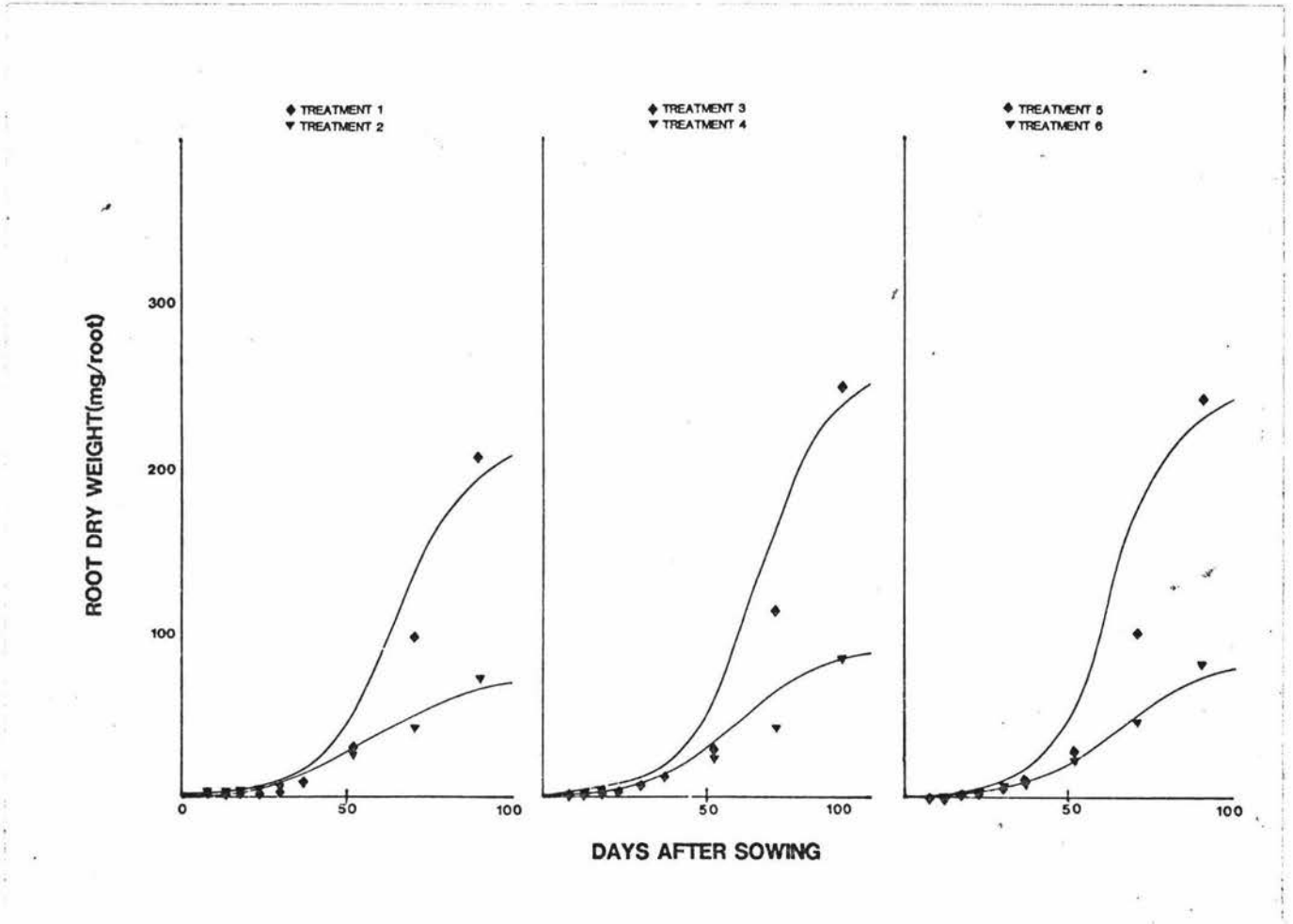


Fig. 9 Growth curves of root from the six treatments

TREATMENTS	Standard error of βo	βo	Standard error of βo	βo	Asymptote $(\hat{Yo})$ (mg/root)	R^2
1	-6.27	0.163	0.095	0.004	215	0.967
2	-4.46	0.184	0.071	0.004	76	0.927
3	-6.41	0.192	0.096	0.004	259	0.956
4	-4.90	0.135	0.080	0.003	88	0.968
5	-6.46	0.202	0.098	0.004	249	0.953
6	-4.60	0.193	0.065	0.004	92	0.906

Table 19. Information from linear regression $\ln \frac{Y}{\hat{Yo} - Y} = \beta o + \beta 1 \, x$ for the root dry weight of the six treatments.

Table 20. Summary of number of first order lateral roots for the first 6 harvest times.[a]

TREATMENTS	TIME OF HARVEST					
Boron Rates	1	2	3	4	5	6
0	3.83	5.10[b]	7.77	9.05	16.20[a]	21.28
1	4.62	6.50[a]	7.96	9.02	15.54[a]	21.13
2	4.21	5.35[b]	7.37	9.89	14.58[b]	21.38
Significance	N.S.	*	N.S.	N.S.	*	N.S.
LSD .05	N.S.	0.8496	N.S.	N.S.	0.8286	N.S.
S.E. Mean	0.668	0.306	0.403	0.319	0.299	0.901
C.V. (%)	19.4	6.6	6.4	4.2	2.4	5.2
Nitrogen/ Inoculation						
N-	3.97	4.73[b]	6.83[b]	8.46[b]	14.99[b]	20.35
Inoculated	4.47	6.57[a]	8.58[a]	10.18[a]	15.89[a]	22.18
Significance	N.S.	**	**	**	*	N.S.
LSD .05	N.S.	0.5375	1.3323	0.6587	0.7752	N.S.
S.E. Mean	0.486	0.220	0.544	0.269	0.317	0.901
C.V. (%)	24.4	8.2	15.0	6.10	4.4	8.3
Interaction (B X I/N)						
Significance	N.S.	N.S.	N.S.	**	N.S.	N.S.
S.E. Mean	0.894	0.407	0.779	0.459	0.490	0.836
LSD .05	N.S.	N.S.	N.S.	1.1409	N.S.	N.S.

[a] Means followed by a common letter are not significantly different.

** Highly significant at P = 0.01

* Significant at P = 0.05

N.S. Nonsignificant

Table 20a. Mean number of the first order lateral roots at harvest 4.

Levels of Boron	Nitrogen application Inoculation		S.E. [a]
	N	I	
0	8.53	9.57	
1	9.63	8.42	0.459
2	7.22	12.57	
L.S.D. _05 = 1.141			

[a] S.E. for comparisons between interaction means

Table 21. Summary of number of second order lateral roots for four harvest times [a]

TREATMENTS	TIME OF HARVEST			
Boron Rates	3	4	5	6
0	0.81	4.92[b]	14.33	39.60
1	1.04	8.66[a]	13.71	42.00
2	0.93	4.10[b]	11.87	35.20
Significance	N.S.	**	N.S.	N.S.
LSD .05	N.S.	1.0571	N.S.	N.S.
S.E. Mean	0.452	0.381	1.007	4.02
C.V. (%)	59.9	7.5	9.3	12.6
Nitrogen/ Inoculation				
N-	1.01	6.57[a]	13.40	42.70[a]
Inoculated	0.85	5.22[b]	13.21	35.20[b]
Significance	N.S.	**	N.S.	*
LSD .05	N.S.	0.7039	N.S.	5.1716
S.E. Mean	0.527	0.288	0.647	2.11
C.V. (%)	120.7	10.4	10.3	11.5
Interaction (B X I/N)				
Significance	N.S.	*	N.S.	N.S.
S.E. Mean	0.788	0.519	1.282	4.78
LSD .05	N.S.	1.2193	N.S.	N.S.

[a] Means followed by a common letter are not significantly different

** Highly significant at P = 0.01

* Significant at P = 0.05

N.S. Nonsignificant at P = 0.05

Table 21a. Mean number of the second order lateral roots at harvest 4.

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	6.23	3.60	
1	8.52	8.81	0.519
2	4.97	3.24	
L.S.D. _05 = 1.219			

[a] S.E. for comparisons between interaction means.

Table 22. Summary of the total leaf area (cm^2) for the last 3 harvest times. [a]

TREATMENTS	TIME OF HARVEST		
Boron Rates	7	8	9
0	10.42[c]	14.72	24.80
1	11.55[a]	16.40	23.90
2	9.10[b]	15.24	27.80
Significance	*	N.S.	N.S.
LSD .05	1.0354	N.S.	N.S.
S.E. Mean	0.611	0.738	3.24
C.V. (%)	7.2	5.8	15.6
Nitrogen/ Inoculation			
N-	14.31[a]	22.22[a]	42.00[a]
Inoculated	6.40[b]	8.68[b]	9.00[b]
Significance	**	**	**
LSD .05	1.4925	1.5076	7.8321
S.E. Mean	0.610	0.616	3.20
C.V. (%)	12.5	8.5	26.6
Interaction (B X I/N)			
Significance	N.S.	N.S.	N.S.
S.E. Mean	0.965	1.055	5.08
LSD .05	N.S.	N.S.	N.S.

[a] Means followed by a common letter are not significantly different.

** Highly significant at P = 0.01

* Significant at P = 0.05

N.S. Nonsignificant at P = 0.05

A significant response to levels of boron was obtained at harvest 7, with 1 ppm boron-treated plants significantly higher in leaf area (cm^2) than plants treated with 2 ppm boron but not significant over plants receiving 0 ppm boron. For the last two harvest times, no significant differences were detected among levels of boron.

A consistent pattern of effect was detected from the nitrogen-treated plants at all three harvest times, producing a significantly larger leaf area (cm^2) than the inoculated plants. No significant interaction effects were obtained between the levels of boron and inoculation and nitrogen treatments (Table 22).

4.3 CHEMICAL COMPOSITION OF SAINFOIN (cv FAKIR)

This section will present the results obtained from the chemical analysis of sainfoin plants in terms of total nonstructural carbohydrates (TNC), boron (B), nitrogen (N), and phosphorus (P) content.

4.3.1 Total Nonstructural Carbohydrates (%)

The total nonstructural carbohydrates of the whole sainfoin plant was analyzed for 8 harvest times commencing at the second harvest, while the shoot and root TNC were analyzed for the last three harvest times. In addition, matured sainfoin seeds were also analyzed for their TNC content.

4.3.1.1 Plant total nonstructural carbohydrates

Significant responses of plant TNC to boron treatments were recorded at harvests 2, 4, and 7. Each level of boron, however, had no consistent pattern of effects. Plants receiving 2 ppm boron yielded the highest TNC content at harvest 2 while plants treated with 0 ppm boron yielded the highest TNC content at harvest 4 and at harvest 7, plants applied with 1 ppm boron were significantly higher in their TNC over the TNC of 1 ppm boron-treated plants but not significantly different with the TNC of the 2 ppm boron-treated plants (Table 23).

A consistent pattern of significant effects were detected from the inoculated treatments giving the higher TNC yield over the TNC of the nitrogen-treated plants at all harvest times, although there were no significant differences between the two treatments at harvest 4 and 5 (Table 23).

Significant interaction effects between boron and nitrogen/inoculation treatments were observed at harvests 3 and 6 (Table 24a, 24b).

The changes in the plant TNC at different periods of growth is illustrated in Fig 10.

4.3.1.2 Shoot total nonstructural carbohydrates

The total nonstructural carbohydrates of the shoot was analyzed for the last three harvest times only. A summary of the total nonstructural carbohydrates of the shoot is given in Table 25.

A consistent pattern of effects of the different levels of boron were obtained from the TNC yield of the shoot with plant-shoots receiving 1 ppm boron giving the highest TNC in all harvest times although significant differences occurred only at harvests 7 and 9 (Table 25).

Likewise, shoots treated with inoculant gave a higher TNC content than the nitrogen-treated shoots which were significantly higher in all harvest times (Table 25).

Significant interaction effects were obtained at harvest 7 only (Table 25a).

4.3.1.3 Root total nonstructural carbohydrates

Similar trend of effects of the different levels of boron were obtained from root TNC as the shoot TNC. Roots applied with 1 ppm boron yielded the highest TNC concentration in all the three harvest times (Table 26), however, significant effects were recorded at harvests 8 and 9 only.

Highly significant results occurred from the nitrogen and inoculant-applied roots, with the inoculated roots showing a significantly higher TNC yield than the roots receiving nitrogen in all the three harvest times (Table 26). No significant interaction effects were detected.

Generally, inoculated plants yielded higher TNC with the application of 1 ppm boron both in the shoot and the root as well as the whole plant.

4.3.2 Total Boron Concentration (µg/g) and Boron Uptake (µg/plant)

This section will present the total boron concentration and boron uptake of the whole plant, the shoot and the root obtained from the final harvest.

4.3.2.1 Plant total boron concentration and boron uptake

Plant total boron concentration was significantly affected by the different levels of boron applied. An application of 2 ppm boron resulted in a plant boron concentration of 30.21 µg/g which was significantly higher than boron contents of plant receiving 0 ppm and 1 ppm boron of 4.46 to 17.50 µg/g boron, respectively (Table 27). One ppm boron-treated plants were significantly higher in their boron concentrations than those receiving 0 ppm boron.

Similarly, significant responses were obtained from the nitrogen and inoculant-applied plants with the incoulated plants of 21.75 µg/g boron content significantly higher than the nitrogen-treated plants of 13.03 µg/g boron concentration (Table 27). There were significant interaction effects observed between the boron and nitrogen and inoculant application treatments (Table 27a).

With regards to the plant boron uptake, significant responses to levels of boron were detected. Plants receiving 2 ppm boron gave a significantly higher boron uptake of 3.82 µg/plant over those receiving 0 ppm boron and 1 ppm boron of

0.57 and 2.25 µg/plant, respectively (Table 27). One ppm boron-treated plants were significantly higher in their boron uptake than those treated with 0 ppm boron.

Likewise, highly significant differences occurred between the nitrogen-treated and inoculated plants (Table 27). The nitrogen-treated plants exhibited a significantly higher boron uptake of 2.75 µg/plant than those of inoculated plants with a boron uptake of 1.68 µg/plant. There were no significant interaction effects detected between boron and nitrogen-inoculant application.

4.3.2.2 Shoot total boron concentration (µg/g) and shoot boron uptake (µg/shoot)

Like the plant total boron content, the shoot boron concentration was very significantly affected by the different levels of boron applied, the concentration of boron in the shoot increasing as the level of boron applied is higher. An application of 2 ppm boron gave the shoot a boron content of 55.30 µg/g which was very significantly different to 0 ppm boron-treated shoots of 3.70 µg/g boron content although it was not significantly different to plants treated with 1 ppm boron of 32.50 µg/g boron content. Boron content of shoots receiving 1 ppm boron was significantly higher than those treated with 0 ppm boron (Table 27).

There were no significant effects of nitrogen application and inoculation on the boron concentration of the shoots. Likewise, there was no significant interaction effects between boron-treated and nitrogen-treated/inoculated treatments observed (Table 27).

There was a highly significant response of the shoot boron uptake to the different levels of boron, with plants treated with 2 ppm boron showing the highest boron uptake of 4.774 µg/plant which was highly significant over plants receiving 0 ppm boron and 1 ppm boron of 0.132 µg/shoot and 2.751 µg/shoot, respectively. Likewise, shoots of 1 ppm boron-treated plants were significantly higher than those shoots receiving 0 ppm boron (Table 27).

Highly significant differences were detected between treatments means when nitrogen and inoculant were applied (Table 27). Nitrogen-treated shoots had a significantly higher boron uptake of 3.679 µg/shoot than the inoculated plants of 1.426 µg/shoot boron uptake. Highly significant effects on shoot boron uptake were recorded (Table 27b).

4.3.2.3 Root total boron concentration (µg/g) and boron uptake (µg/root)

Highly significant responses of roots to boron treatments were detected (Table 27). As expected, roots treated with 2 ppm boron were significantly higher in their boron content of 9.60 µg/g than those treated with 1 ppm and 0 ppm boron with boron concentrations of 0.17 µg/g and 0 µg/g respectively. There was no significant difference observed between 1 ppm and 0 ppm treated plants.

Likewise, there was significant effects of the nitrogen-treatment and inoculation on root boron concentration. Inoculated roots gave a significantly higher boron concentration of 5.40 µg/g than the nitrogen-treated roots of 1.11 µg/g boron content (Table 27). Also, there were significant interaction effects obtained between the boron and nitrogen application-inoculation treatments (Table 27c).

With regards to the boron uptake of the roots, results obtained showed highly significant differences among the three levels of boron. Roots receiving 2 ppm boron with a boron uptake of 1.063 µg/root were significantly higher than the roots receiving 1 ppm and 0 ppm boron of 0.140 and 0 µg/root boron uptake, respectively. There was no significant difference obtained between 1 ppm and 0 ppm boron-treated roots (Table 27).

There were no significant responses of the root boron uptake to nitrogen application and inoculation. Similarly, no significant interaction effects occurred (Table 27).

4.3.3 Total Nitrogen Concentration (mg/g) and Nitrogen Uptake (mg/plant)

4.3.3.1 Shoot total nitrogen concentration (mg/g) and shoot nitrogen uptake (mg/shoot)

Shoot nitrogen content (mg/g) was not affected by boron application (Table 28).

A highly significant difference was observed between the nitrogen application and inoculation in terms of the shoot nitrogen content. Shoot receiving nitrogen were very significantly higher in their nitrogen content of 59.10 mg/g than those inoculated shoots of 26.40 mg/g (Table 28). No significant interaction effect was detected.

Likewise, the nitrogen uptake of the shoot was not affected by the different levels of boron, however, highly significant differences on the shoot boron uptake were observed between the nitrogen-treated and inoculated shoots. Inoculated shoots gave a nitrogen uptake of 0.01 µg/shoot which was very significantly lower than the nitrogen uptake of nitrogen-treated plants of 8.92 mg/shoot. No significant interaction effects were observed between the boron treatments and nitrogen application-inoculation on the nitrogen uptake of the shoots (Table 28).

4.3.3.2 Root total nitrogen concentration (mg/g) and root nitrogen uptake (mg/root)

Applications of 0 ppm and 1 ppm boron respectively, gave the roots a higher nitrogen concentration than the roots receiving 2 ppm boron, however, no significant differences were obtained among treatment means (Table 28).

A significantly lower nitrogen concentration was obtained from the inoculated roots of 15.27 mg/g over those roots treated with nitrogen of 25.41 mg/g nitrogen content. There was no significant interaction effect observed between boron and nitrogen-inoculant application treatments (Table 28).

The nitrogen uptake of the roots was not significantly affected by the different levels of boron (Table 28), however, a highly significant response to nitrogen and inoculant application was obtained from the nitrogen uptake of the roots. The nitrogen-treated roots were significantly higher in their nitrogen uptake of 5.89 mg/root than the roots treated with inoculant of 1.22 mg/root (Table 28). No significant interaction effects were observed between roots treated with boron and nitrogen and inoculant.

4.3.4 Total Phosphorus Concentration (mg/g) and Phosphorus Uptake (mg/plant)

4.3.4.1 Shoot total phosphorus concentration (mg/g) and shoot phosphorus uptake (mg/shoot)

There were no significant responses of the shoot total phosphorus concentration to the different levels of boron applied (Table 29). Highly significant differences, however, were noted between the shoot phosphorus concentration of nitrogen-treated and inoculated shoots. Inoculated shoots gave a significantly higher phosphorus concentration of 3.16 mg/g over the nitrogen-treated shoots of 1.56 mg/g phosphorus content (Table 29). There was no significant interaction effect detected between boron and nitrogen/inoculant application.

Phosphorus uptake of the shoot was highest in shoots treated with 2 ppm boron, however, there was no significant differences observed among the different levels of boron applied in terms of shoot phosphorus uptake (Table 29). In contrast to the response of shoot total phosphorus content, the shoot phosphorus uptake was significantly affected by the application of nitrogen and inoculant. The shoots receiving nitrogen were significantly higher in their phosphorus uptake of 0.235 mg/shoot than the inoculated shoots of 0.121 mg/shoot phosphorus uptake (Table 29). There were no significant interaction effects obtained between boron and nitrogen/inoculant treatments.

4.3.4.2 Root total phosphorus concentration (mg/g) and root phosphorus uptake (mg/root)

There was no significant response of the root phosphorus concentration to the different levels of boron applied (Table 29). Likewise, nonsignificant effects were obtained from the nitrogen and inoculant application to the shoot phosphorus content. Moreover, the boron application and nitrogen-inoculant application gave no significant interaction effect with the shoot phosphorus concentration.

Similarly, there was no response of the shoot phosphorus uptake to boron treatments, nitrogen-inoculant application and interaction of boron and nitrogen-inoculant application treatments (Table 29).

4.4 RELATIONSHIP OF THE PLANT DRY WEIGHT, PLANT TOTAL NONSTRUCTURAL CARBOHYDRATES AND PLANT BORON CONTENT TO THE CHEMICAL COMPOSITION OF SAINFOIN

This section presents results obtained from an analysis using multiple regression through coefficient of determination (increment of R^2) and prediction (standardized regression coefficient) measuring the relationship of the PDW, PTNC, and PBC) to the chemical composition of sainfoin at final harvest.

4.4.1 Relative Importance of Some Sainfoin Chemical Composition in Determining Plant Dry Weight, Plant Total Nonstructural Carbohydrates and Plant Boron Content

The summary of the multiple regression is presented in Appendix 7.

The root phosphorus content was the most important chemical component that determines plant dry weight of sainfoin, contributing 87%.

TREATMENTS	TIME OF HARVEST							
Boron Rates	2	3	4	5	6	7	8	9
0	4.78[b]	6.683	7.60[a]	5.58	3.68	7.17[b]	7.14	8.32
1	5.45[b]	6.600	6.08[b]	6.85	4.12	8.50[a]	7.80	11.85
2	6.87[a]	6.867	6.62[b]	6.33	4.13	8.06[ab]	7.93	11.27
Sigificance	**	N.S.	*	N.S.	N.S.	*	N.S.	N.S.
LSD .05	0.8238	N.S.	0.7865	N.S.	N.S.	0.9468	N.S.	N.S.
S.E. Mean	0.297	0.2789	0.283	0.422	0.175	0.341	0.638	1.365
C.V. (%)	6.4	5.1	5.1	8.3	5.4	5.3	10.3	16.0
Nitrogen/ Inoculation								
N-	5.26[b]	6.589[b]	5.89[b]	6.01	3.822	6.06[b]	6.16[b]	8.87[b]
Inoculated	6.14[a]	6.844[a]	7.64[a]	6.50	4.133	9.76[a]	9.09[a]	12.09[a]
Significance	**	*	**	N.S.	N.S.	**	*	**
LSD .05	0.5545	0.2506	0.5869	N.S.	N.S.	0.6815	1.9450	1.0307
S.E. Mean	0.228	0.1024	0.240	0.306	0.193	0.479	0.795	0.421
C.V. (%)	8.5	3.2	7.5	10.4	10.3	12.9	22.1	8.5
Interaction (B X I/N)								
Significance	N.S.	*	N.S.	N.S.	**	N.S.	N.S.	N.S.
S.E. Mean	0.408	0.3058	0.408	0.564	0.293	0.679	1.164	1.460
LSD .05	N.S.	0.4341	N.S.	N.S.	0.8158	N.S.	N.S.	N.S.

[a] Means followed by a common letter are not significantly different.
** Highly significant at P = 0.01
* Significant at P = 0.05
N.S. Nonsignificant at P = 0.05

Table 23. Summary of total nonstructural carbohydrates (%) of the whole plant for 8 harvest times [a]

Table 24. Mean total nonstructural carbohydrates of the plant at harvests 3 and 6.

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	6.30	7.07	
1	6.70	6.50	0.306
2	6.77	6.97	
L.S.D. _05 = 0.434			

(a) Harvest 3

Levels of Boron	Nitrogen application Inoculation		S.E.[a]
	N	I	
0	4.10	3.27	
1	4.10	4.13	0.293
2	3.27	5.00	
L.S.D. _05 = 0.816			

(b) Harvest 6

[a] S.E. for comparisons between interaction means

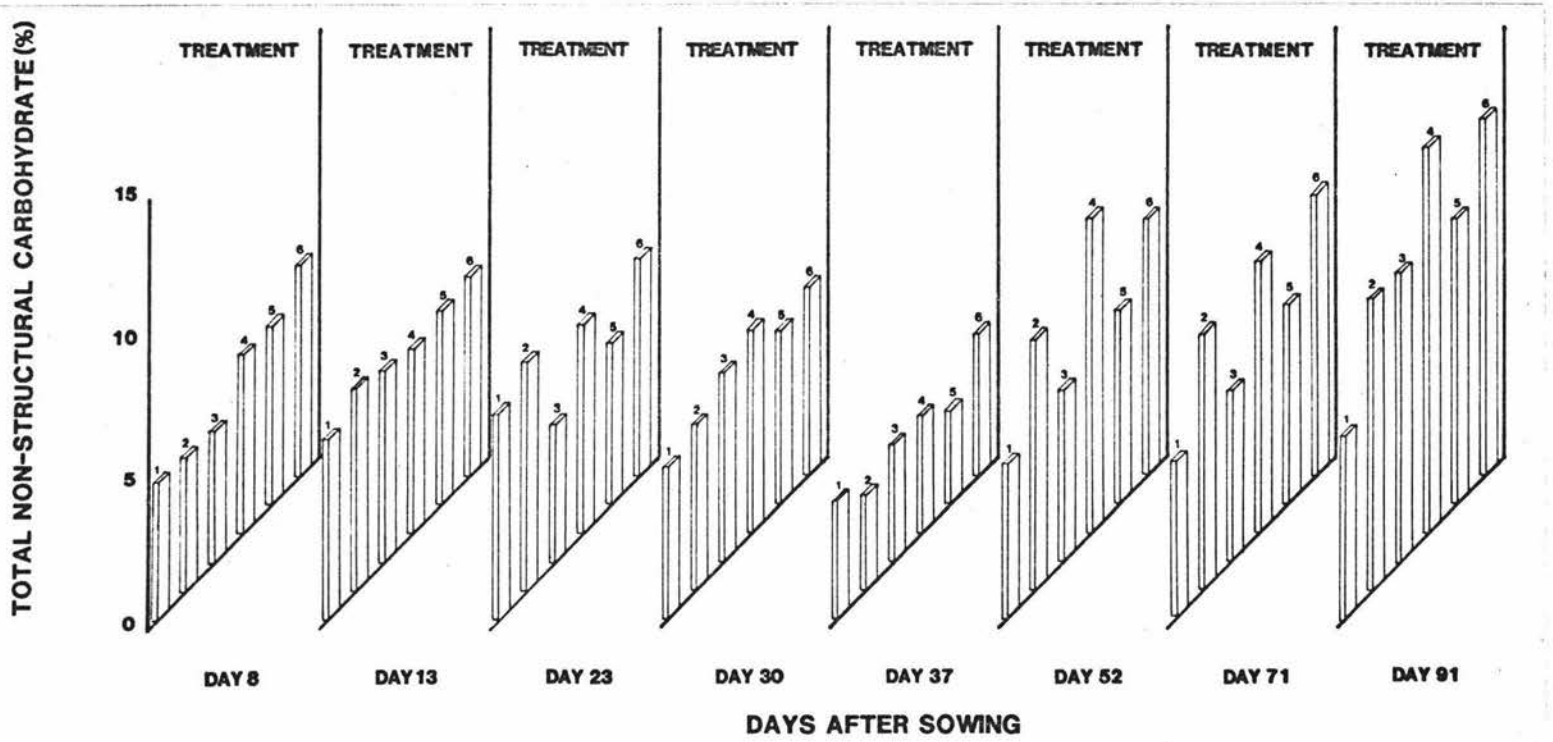


Fig. 10 Plant nonstructural carbohydrates (TNC) (%) for eight harvest treatments

Table 25. Summary of the total nonstructural carbohydrates (%) of the shoot at three harvest times [a]

TREATMENTS	TIME OF HARVEST		
Boron Rates	7	8	9
0	4.733[a]	4.93	6.32[b]
1	4.783[a]	5.83	7.28[a]
2	4.117[b]	5.72	6.38[b]
Significance	**	N.S.	*
LSD .05	0.2519	N.S.	0.7815
S.E. Mean	0.091	0.395	0.284
C.V. (%)	2.4	8.8	5.2
Nitrogen/ Inoculation			
N-	3.444[b]	5.06[b]	6.20[b]
Inoculated	5.644[a]	5.93[a]	7.12[a]
Significance	**	*	*
LSD .05	0.3182	0.7818	0.8148
S.E. Mean	0.130	0.320	0.333
C.V. (%)	6.1	12.3	10.6
Interaction (B X I/N)			
Significance	**	N.S.	N.S.
S.E. Mean	0.183	0.556	0.497
LSD .05	0.5512	N.S.	N.S.

[a] Means followed by a common letter are not significantly different.

** Highly significant at P = 0.01

* Significant at P = 0.05

N.S. Nonsignificant at P = 0.05

Table 25a. Mean total nonstructural carbohydrates of the shoot at harvest 1.

Levels of Boron	Nitrogen application Inoculation		S.E. [a]
	N	I	
0	4.07	5.40	
1	3.43	6.13	0.183
2	2.83	5.40	
L.S.D. _05 = 0.551			

[a] S.E. for comparisons between interaction effects.

Table 26. Summary of the total nonstructural carbohydrates (%) of the root at three harvest times [a]

TREATMENTS	TIME OF HARVEST		
Boron Rates	7	8	9
0	5.47	8.47[b]	6.90[b]
1	5.67	9.83[a]	14.18[a]
2	5.48	8.18[b]	11.07[c]
Significance	N.S.	*	**
LSD .05	N.S.	1.0124	1.3378
S.E. Mean	0.620	0.365	0.482
C.V. (%)	13.7	5.1	5.5
Nitrogen/ Inoculation			
N-	4.17[b]	6.66[b]	8.64[b]
Inoculated	6.91[a]	11.00[a]	12.79[a]
Significance	**	**	**
LSD .05	1.4736	1.9007	1.0872
S.E. Mean	0.602	0.777	0.444
C.V. (%)	23.1	18.7	8.8
Interaction (B X I/N)			
Significance	N.S.	N.S.	N.S.
S.E. Mean	0.964	1.019	0.727
LSD .05	N.S.	N.S.	N.S.

[a] Means followed by a common letter are not significantly different.

** Highly significant at P = 0.01

* Significant at P = 0.05

N.S. Nonsignificant at P = 0.05

Table 27. Summary of the boron concentration (µg/g) and boron uptake (µg/shoot) of the whole plant (PBC; PBU), shoot (SBC; SBU) and root (RBC; RBU), respectively [a]

TREATMENTS	PBC	PBU	SBC	SBU	RBC	RBU
Boron Rates						
0	4.46^{b}	0.57^{b}	3.70^{b}	0.132^{b}	0^{bc}	0^{bc}
1	17.50^{c}	2.25^{c}	32.50^{a}	2.751^{c}	0.17^{c}	0.140^{c}
2	30.21^{a}	3.82^{a}	55.30^{a}	4.775^{a}	9.60^{a}	1.063^{a}
Significance	**	**	**	**	**	**
LSD .05	3.6425	1.0433	20.4245	1.0862	3.3289	0.5033
S.E. Mean	2.824	0.376	7.36	0.391	1.199	0.1813
C.V. (%)	19.90	20.80	29.50	18.80	45.10	55.4
Nitrogen/ Inoculation						
N-	13.03^{b}	2.75^{a}	24.70	3.679^{a}	1.11^{b}	0.271
Inoculated	21.75^{a}	1.68^{b}	30.30	1.426^{b}	5.40^{a}	0.531
Significance	**	**	N.S.	**	*	N.S.
LSD .05	2.6216	0.4913	-	0.4047	3.3129	N.S.
S.E. Mean	1.071	0.201	5.20	0.165	1.354	0.1895
C.V. (%)	13.30	19.20	36.20	13.70	88.20	100.20
Interaction (B X I/N)						
S.E. Mean	3.114	0.449	9.73	0.441	2.046	0.295
Significance	**	N.S.	N.S.	**	*	N.S.
LSD .05	4.5407	N.S.	N.S.	0.7010	5.7380	N.S.

[a] Means followed by a common letter are not significantly different.

** Highly significant at P = 0.01

* Significant at P = 0.05

N.S. Nonsignificant at P = 0.05

Table 27a. Mean boron concentration (µg/g) of the whole plant at harvest 9.

Levels of Boron	Nitrogen application Inoculation		S.E. [a]
	N	I	
0	4.08	4.83	
1	12.50	22.50	3.114
2	22.50	37.92	
L.S.D. _05 = 4.541			

[a] S.E. for comparisons between interaction means.

Table 27b. Mean boron uptake (µg/plant) of the shoot at harvest 9.

Levels of Boron	Nitrogen application Inoculation		S.E. [a]
	N	I	
0	0	0.265	0.701
1	4.089	1.412	
2	6.948	2.602	
L.S.D. _05 = 0.441			

[a] S.E. for comparisons between interaction means.

Table 27c. Mean boron concentration (µg/g) of the root at harvest 9.

Levels of boron	Nitrogen application Inoculation		S.E. [a]
	N	I	
0	0	0	2.046
1	0	0.33	
2	3.34	15.87	
L.S.D. _05 = 5.738			

[a] S.E. for comparisons between interaction means.

Table 28. Summary of the nitrogen concentration (mg/g) and nitrogen uptake (mg/plant), of the shoot (SNC: SNU) and the root (RNC: RNU), respectively.[a]

TREATMENTS	SNC	SNU	RNC	RNU
Boron Rates				
0	42.80	5.03	21.23	3.39
1	48.00	5.49	21.27	3.98
2	37.50	4.37	18.52	3.31
Significance	N.S.	N.S.	N.S.	N.S.
LSD .05	-	N.S.	N.S.	N.S.
S.E. Mean	4.12	0.428	2.922	0.645
C.V. (%)	11.80	10.60	17.60	22.50
Nitrogen/ Inoculation				
N-	59.10[a]	8.92[a]	25.41[a]	5.89[a]
Inoculation	26.40[b]	1.01[b]	15.27[b]	1.22[b]
Significance	**	**	**	**
LSD .05	11.0154	1.4596	4.2540	0.6563
S.E. Mean	4.50	0.597	1.738	0.518
C.V. (%)	22.30	25.50	18.10	30.90
Interaction (B X I/N)				
S.E. Mean	6.88	0.847	3.615	0.905
Significance	N.S.	N.S.	N.S.	N.S.
LSD .05	N.S.	N.S.	N.S.	N.S.

[a] Means followed by a common letter are not significantly different.

** Highly significant at P = 0.01

* Significant at P = 0.05

N.S. Nonsignificant at P = 0.05

Table 29. Summary of the phosphorus concentration (mg/g) and boron uptake (mg/plant) of the shoot (SPC; SPU) and the root (RPC; RPU), respectively.[a]

TREATMENTS	SPC	SPU	RPC	RPU
Boron Rates				
0	2.23	0.1717	2.08	0.2233
1	2.47	0.1767	3.54	0.2667
2	2.37	0.1867	2.36	0.2950
Significance	N.S.	N.S.	N.S.	N.S.
LSD .05	N.S.	N.S.	N.S.	N.S.
S.E. Mean	0.275	0.0192	1.130	0.0391
C.V. (%)	14.30	13.10	52.00	18.30
Nitrogen/ Inoculation				
N-	1.56[b]	0.2356[b]	2.02	0.2578
Inoculation	3.16[a]	0.1211[a]	3.30	0.2659
Significance	**	**	N.S.	N.S.
LSD .05	0.5656	0.0346	N.S.	N.S.
S.E. Mean	0.231	0.0144	0.901	0.0129
C.V. (%)	20.80	17.10	71.80	10.50
Interaction (B X N/I)				
S.E. Mean	0.395	0.0260	1.580	0.0422
Significance	N.S.	N.S.	N.S.	N.S.
LSD .05	N.S.	N.S.	N.S.	N.S.

[a] Means followed by a common letter are not significantly different.

** Highly significant at P = 0.01

* Significant at P = 0.05

N.S. Nonsignificant at P = 0.05

The shoot total nonstructural carbohydrates was determined mostly by the shoot total nonstructural carbohydrates by 66% followed by the shoot nitrogen by 11% only.

The shoot boron concentration contributed most in the determination of plant boron content by 77%, followed by shoot phosphorus content accounting for 6.81%.

5. DISCUSSION

This discussion is presented in four parts: (1) general description of the early establishment and growth of sainfoin (2) plant growth and development (3) chemical composition of sainfoin and (4) plant dry weight, boron and nitrogen concentrations and chemical components of sainfoin, as affected by the application of boron, nitrogen and inoculant.

5.1 GENERAL DESCRIPTION OF THE EARLY ESTABLISHMENT AND GROWTH OF SAINFOIN

The early growth of sainfoin was characterized by high germination percentage (95%). Sainfoin is known to be readily established because of its large seed and good vigour (Ditterline & Cooper, 1968). In addition, the cotyledons of sainfoin are capable of synthesizing photosynthates (Cooper & Fransen, 1974) which could help the seedlings while the true leaves are still the active sinks.

The occurrence of different types of first leaves viz unifoliate, bifoliate and trifoliate gave the seedlings a nonuniform growth and development (Cooper, 1974), since trifoliate first leaves give a larger leaf area than the unifoliate first leaves. This implied that trifoliate first leaves had a higher net carbon exchange during the early seedling growth than had those with unifoliate first leaves although there were no differences in herbage yield of 8-week old plants or in the mature plants.

The great difference in growth of the nitrogen and inoculant-treated plants from day 24 to day 91 was due mainly to the nodulation failure of the inoculated plants. Nodules were developed although considered nil in terms of percentage infection. Sainfoin is known for its poor symbiotic establishment with *Rhizobium*, leading to poor nodulation and thus to inefficient nitrogen fixation (Burton & Curley, 1968; Sims *et al.*, 1968; Ross & Delaney, 1971). As plants developed, nitrogen deficiency symptoms were observed even though they were inoculated. It was suspected therefore

that the *Rhizobium* strain used may have been ineffective or short-lived (Sims *et al.*, 1968) coupled with the unfavourable physical conditions of the perlite for the *Rhizobia* to exist, especially, if the strain used was really short-lived.

5.2 PLANT GROWTH AND DEVELOPMENT

Plant growth is a function of time, genetic make-up of the plant, and environmental factors (Tisdale & Nelson, 1975) while the rate of dry matter production is clearly the function of photosynthesis and the total leaf area (Turner & Begg, 1981).

The presence of negative values in the relative growth rate of the whole plant and the shoot (Tables 1, 2, and 3) showed that plants and shoots lost weight during the first 15 days of growth and development due to respiration loss. There was a decrease of weight but there has been considerable differentiation of leaf tissue in the young seedlings at the expense of total dry weight (Hunt, 1978). As expected, accumulation of dry matter in the root was linear over time until it reached its peak and gradually decreasing (Figures 8 and 9).

The inconsistency of the effect of the different levels of boron on the relative growth rates of the whole plant (Table 4), the shoot (Table 6) and the root (Table 8) can be explained by the genetic variability of the plant which might have affected the physiological processes of the sainfoin plants, i.e., photosynthesis and respiration (Cooper, 1974; Robertson, pers. comm.). The apparent boron deficiency also contributed to the variability of response (refer to Section 5.3). However, general results (Table 10) clearly showed that the different levels of boron did not affect the relative growth rates of the whole plant, the shoot and the root.

The effects of the different levels of boron on the whole plant (Table 11), the shoot (Table 14), and the root (Table 17) dry weights for each harvest time were also inconsistent. It was reported that boron concentrations of up to and including 10 ppm had little effect on the dry weight of Setaria shoots (McIlrath *et al.*, 1960). Likewise, rates of

added boron at 6 ppm did not affect the shoot and grain yield of barley, however, an application of 3 ppm boron reduced root yield (Short Communication, 1978). These affects may imply that rates greater than 2 ppm might have been examined in sainfoin.

As expected, the unfertilized sainfoin plants were chlorotic and small due to nitrogen deficiency (Sims *et al.*, 1968) while the nitrogen treated sainfoin plants were dark green (Plate 1). Likewise, dry matter yield of shoots and roots treated with nitrogen were consistently higher at later growth stages. It was evident from the data presented in Fig. 6a, b, c, 8a, b, c, 10a, b, and c that there were further reductions in the total plant, shoot, and root dry weight, respectively, of the inoculated plants when nitrogen was limiting due to nodualtion failure (Sims *et al.*, 1968).

Contrary to the reports of Middleton *et al.* (1978) and Theiler (1972), boron had no effect on both the number of the first order and second order lateral roots (Tables 20, and 21), however, fine root growth as shown by the occurrence of several lateral roots was observed which corroborates with the results obtained by Simpson and Lipsett (1973). McIlrath *et al.* (1960) supports the results obtained from the study of sainfoin that boron, generally had no or little effect upon the shoot dry weight which presumably would have no effect on the leaf area of sainfoin.

Applications of nitrogen was found to increase the growth and number of lateral roots in sainfoin. This is supported by the results obtained by Hackett (1972) and Drew (1975) who observed that nitrate applied to a discrete root segment increased both the rate of lateral root extension and number of lateral roots per unit length of root. Also, Weirsum (1958) demonstrated that root branching in pea was stimulated by nutrients as follows: NO_3-N>P>K>Mg>Ca. In addition, nitrogen does tend to preferentially increase top growth (Grunes, 1959) which is similar to the results obtained from the study on sainfoin treated with nitrogen. Moreover, Ingested and Lund (1979) concluded that nitrogen influences primarily the development of leaf area which subsequently controls other growth and metabolic processes.

5.3 EFFECTS OF BORON ON THE CHEMICAL COMPOSITION OF SAINFOIN (CV FAKIR)

5.3.1 Total Nonstructural Carbohydrates

Boron had been postulated to enhance sugar translocation in plants (Gauch & Dugger, 1954) and might be involved in the characteristic death of the growing points of boron-deficient plants caused by a lack of sugar (Mitchell *et al.*, 1960).

Although significant effects had been recorded from the application of three boron rates (Table 23) for eight harvest times, the inconsistency of the pattern of effects of each level of boron explains the lack of response of the plants to boron treatment. This is contrary to the result obtained by Bonilla *et al.* (1980) where conditions of boron deficiency and toxicity resulted in a substantial decrease of sugar levels in the sap and in the root. However, boron treatments greatly affected shoot and root total nonstructural carbohydrates when an application of 1 ppm boron gave the highest TNC yield over those treated with 0 ppm and 2 ppm boron. Apparently, there is a deficiency and/or toxicity of boron in the shoot which reduced the TNC (Table 25) (Gauch, 1972; Bonilla *et al.*, 1980). However, it is noteworthy that Bonilla *et al.* (1980) used 2.5 ppm boron concentration as their normal solution which is higher than the highest boron concentration used in the study on sainfoin, and therefore, results are not conclusive as to the occurrence of either boron deficiency or toxicity in sainfoin. Moreover, the succeeding discussions will enlighten the possibility of boron deficiency in the sainfoin plants studied in this work. Similar results were obtained from the effects of boron treatment to the root TNC (Table 26).

Concentrations of total nonstructural carbohydrates in the herbage of both grasses and legumes generally are reduced by applications of nitrogen, whether applied alone or combined with phosphorus and/or potassium (Smith, 1973).

Results of the study conducted on sainfoin showed a significantly lower TNC of both shoot and root treated with nitrogen. Due to poor growth, it is therefore assumed that the

TNC pool of the inoculated plants were not utilized for physiological functions, however, several workers had recorded a lower TNC of nitrogen-applied plants (Rhykerd *et al.*, 1966; Jones, 1970; Waite, 1970; Jung *et al.*, 1976). The decrease of TNC in nitrogen-treated plants may be due to the acceleration of herbage growth rate which is promoted by nitrogen fertilization (Waite, 1970). In addition, reduction in soluble sugar levels in top and root material was probably the result of increased utilization of sugars for nitrate assimilation, amide, amino acid and protein syntehsis (Orcutt & Wilson, 1935).

It is of further interest that the roots, regardless of treatments, yielded higher TNC than the shoots (Ueno & Smith, 1970; Cohen *et al.*, 1972; Haslemore, pers. comm.). This could be due to the fact that the root is the major storage organ in sainfoin plants (Smith, 1970). In addition, roots became the sink for TNC caused by the limited sink sites in the shoot. This indicates that boron-deficient plants (refer to Section 5.3.2) are not capable of developing new leaves in which large amounts of carbohydrates must be translocated to a probable sink. In this study on sainfoin, the roots became the TNC sink as shown by their higher dry matter yield as compared with the shoot (Sisler *et al.*, 1961). However, due to the apparent boron deficiency in the sainfoin plants, the roots had not attained their full capacity to grow in terms of dry matter yield (refer to Section 4.2.2).

The critical boron concentration in sainfoin has not been established yet. However, it is postulated that, if sainfoin plants were growing within a deficiency range as shown by the higher root TNC despite the extremely low boron content of the root of 0.17 µg/g to 9.6 µg/g boron concentration which is considered to be deficient (Price, 1970; Gupta, 1971), the hypothesis that boron enhances sugar translocation is rejected. This is consistent with the results obtained by McIlrath and Palser (1956) that carbohydrates were translocated in boron-deficient cotton plants as long as the phloem tissues were alive, and Odhnoff (1957) showed that roots show the first sign of boron deficiency and that a general rise in carbohydrates in boron-deficient plants contradicts the assumption that lack of boron causes sugar deficiency.

5.3.2 Boron Concentrations and Uptake in Sainfoin

Healthy legumes are reported to contain above 35 ppm boron and a response to boron application can be expected when levels of boron drop below 20 ppm (Nelson & Barber, 1964).

An application of boron resulted in an increased concentration of boron in sainfoin tissue proportional to the rate of boron applied (McIlrath *et al.*, 1960; Miller & Smith, 1977; Short Communication, 1977; Lombin & Bates, 1982). It is assumed that the range of boron concentrations obtained from sainfoin tissues used in this study was deficient, especially in those plants treated with nitrogen at 13.03 µg/g in the whole plant, 24.70 µg/g in the shoots, and 1.11 µg/g in the roots. This is consistent with the results recorded by Gupta (1971, 1972), who considered that boron contents of 39 to 52 ppm in alfalfa and 30 to 45 ppm in birdsfoot trefoil were in a sufficiency range. However, sainfoin shoots treated with 2 ppm boron gave a boron concentration of 55 µg/g which is higher than the sufficiency range for alfalfa (Table 31). These results obtained, therefore, indicate that sainfoin has a higher tolerance to boron than alfalfa since plants at all levels of boron applied exhibited boron deficiency marked by yellow and red spotting of leaves and petioles (Plate 2) and reddening of petioles (Plate 3). Moreover, a concentration of 55 µg/g in sainfoin shoots indicates a toxicity level when compared to the optimum boron concentration required by alfalfa but the statement of Robinson and Edgington (1945) that the naturally grown plant leaves should not exceed 70 ppm boron could indicate that a boron concentration of 55 µg/g in sainfoin was within deficiency range.

The boron concentration of plants treated with 0 ppm boron was 4.46 µg/g, while those receiving 1 ppm and 2 ppm boron had boron contents of 17.50 µg/g and 30.21 µg/g, respectively. It is noteworthy that plants receiving no boron contained boron. This is similar with the results recorded by Mølgaard and Hardman (1980), Berger (1965), and Dible and Berger (1952) working on fenugreek, lucerne, and alfalfa, respectively and grown in a boron-free nutrient solution. This was attributed to the presence of boron in the cotyledons

(Gauch & Dugger, 1953) which in the sainfoin seed used in this study recorded 2.25 µg/g boron.

Also, high nitrogen increased the demand for boron (Mølgaard & Hardman, 1980) as shown by the more pronounced deficiency symptoms observed from the nitrogen-treated plants than the inoculated plants. Deficiency symptoms were marked by yellow and red spotting of sainfoin leaves. This is similar to boron deficiency symptoms exhibited by boron-deficient alfalfa (Gupta, 1972). In addition, an application of nitrogen does not appear to affect the availability of boron although the increased growth resulting from the use of nitrogen would obviously predispose the plants to boron deficiency (Fleming, 1980). This supports the results obtained by Gupta *et al.* (1973) that nitrogen application reduced boron concentration leaf tissue per unit dry weight.

By and large, shoots, irrespective of treatments, contained the highest boron concentrations over the concentrations of the whole plant and the root as supported by results reported by Kohl and Oertli (1961), Yamaguchi *et al.* (1958), and Jones *et al.* (1963).

Whole plants, shoot, and root boron uptake increased in proportion to the rate of boron applied (Table 27). Oertli (1963) reported that with concentrations of boron less than 0.5 ppm, absorption is metabolically mediated, but at higher concentrations uptake is diffusive increasing rapidly with concentrations whereas Bowen (1968, 1972) found evidence for active uptake in sugar cane tissue but Nissen (1974) concluded that a multiphasic mechanism is operating for the uptake of boric acid.

5.3.3 Nitrogen and Phosphorus Concentrations and Uptake

A higher amount of nitrogen and phosphorus per unit dry weight was found in boron-deficient plants by Odhnoff (1957). Also increasing the boron levels resulted in a decreased leaf nitrogen and phosphorus (Aduayi, 1978).

The nonsignificant effects of boron on nitrogen and phosphorus concentrations in both the shoot and the root (Tables 28 and 29) indicate boron deficiency at all levels of boron applied although at 1 ppm boron applied, nitrogen and phosphorus concentrations were highest in both shoot and root of sainfoin. Kibalenko *et al.* (1973) concluded that boron deficiency significantly inhibited protein synthesis in leaves of sugarbeet and pea leaves. However, nitrogen and phosphorus concentrations in both shoot and root increased with boron level up to 1 ppm applied but at 2 ppm boron applied, there was a reduction in nitrogen and phosphorus concentrations of shoot and root. The fact that there was no significant effect of the boron levels on both the nitrogen and phosphorus concentrations of the shoot and the root of sainfoin, suggests that even at 2 ppm boron applied, plants were growing within deficiency conditions. Moreover, Robertson and Loughman (1974) and Pollard (1977) observed that a deficiency of boron in *Vicia faba* L. reduced the capacity for absorption of phosphate.

El Kholi (1961) reported that at a boron level of 1 ppm, the nitrogen and phosphorus uptake of lucerne was increased which were consistent with the results obtained from the study on sainfoin plants. A rate of 2 ppm boron applied to sainfoin plants did not alter the nitrogen uptake compared to the results obtained from the application of 0 ppm and 1 ppm boron. This also corroborates with the results recorded by El Kholi (1961). The nonsignficiant differences among the different levels of boron applied, apparently require higher boron levels than 2 ppm for sainfoin to obtain a conclusive result as per the critical boron concentration in the plant. It is of further interest that El Kholi (1961) used 5 ppm boron in his work, results of which exhibited similar trends to those obtained from sainfoin when applied with 2 ppm boron. This indicates that there is a very narrow range of the boron deficiency and toxicity in sainfoin (Bradford, 1966).

Nitrogen-treated shoots and roots exhibited higher nitrogen but lower phosphorus concentration. The tissue analysis showed a much greater nitrogen content of the shoot and the roots of healthy plants (Table 28) compared to inoculated chlorotic plants (Sims *et al.*, 1968). The large difference in

nitrogen concentration of the healthy and nitrogen-deficient plants is evidence that plants were obtaining nitrogen from the growing medium reflecting a very low nitrate nitrogen level of the growth medium.

Tisdale and Nelson (1975) reported that nucleic acid and phytin are taken in by plants from sterile sand or solution culture. Both compounds may occur as degradation products of the decomposition of soil organic matter and as such could be utilized by growing plants. Because of the limited active sinks for phosphorus, it is assumed that inoculated plants which were chlorotic and stunted, would have accumulated higher amount of phosphorus than the nitrogen-treated sainfoin plants, which were not utilized in both root and shoot (Robertson, pers. comm.). Also, Brown (1974) reported that besides *Rhizobia*, some bacteria like *Azotobacter chroococcum* fix nitrogen while *Bacillus megaterium*, particularly the strain "phosphaticum" mineralizes organic phosphorus compounds. It therefore, suspected that in the growth medium of the inoculated treatments, such organisms might have been present which aided in the further slow growth of the chlorotic plants. In addition, legumes share with most other green plants the ability to improve their nutrient uptake by means of mycorrhizal associations involving their roots with certain soil fungi (Munns & Mosse, 1980). It is also suspected that there was a mycorrhizal association of the chorotic inoculated plants although this was not confirmed by visual identification.

5.4 TOTAL DRY WEIGHT, PLANT TOTAL NONSTRUCTURAL CARBOHYDRATES, PLANT BORON CONCENTRATION AND CHEMICAL COMPONENTS OF SAINFOIN

Phosphorus especially is required by legumes which depend on symbiotic fixation for their nitrogen supply. Munns and Mosse (1980) reported that one of the most common nutritional disorders of legumes is probably the result of phosphate deficiency. Results of the study on sainfoin showed a positive effect of phosphorus concentration among the other chemical components of sainfoin on the dry weight of sainfoin (Zaroug & Munns, 1980).

The root total nonstructural carbohydrates of sainfoin largely influence the total nonstructural carbohydrates of the whole sainfoin plant (Haslemore, pers. comm.) followed by the shoot total nonstructural carbohydrates. This association of the root TNC and whole plant TNC may be related to the fact that the largest amount of TNC is stored in the roots (Ueno & Smith, 1970). In addition, the shoot, the leaves in particular, is the site of carbon assimilation and synthesis and it is presumed therefore, that it directly affects the TNC of the whole plant.

The most common part of the plant used in testing any nutrient concentration is the leaves (Gupta, 1979) to provide the nutrient and fertility status of both the plant and the soil, respectively (Tisdale & Nelson, 1975). Boron content of the sainfoin plant was most affected by the shoot boron concentration. This is attributed to the fact that leaves comprising the largest proportion of shoot, regardless of the age of leaves, contain higher boron concentration than any other plant parts (Clark, 1975b). Moreover, the boron concentration of the sainfoin plant was influenced also by the phosphorus content of the shoot. It has been shown by Pollard *et al.* (1977) and Robertson and Loughman (1974) that there is an association of boron and phosphorus, i.e., the capacity for absorption of phosphate was shown to be reduced in *Zea mays* L. and *Vicia faba* L. suffering from boron deficiency. However, a reverse relationship was found in the study of sainfoin.

6. CONCLUSION

Results obtained from this study are evidences that:

(1) Boron has no effect on the plant, shoot and root relative growth rates, dry matter yield, leaf area, and number of first order and second order lateral roots.

(2) Boron has similar effects on the plant total nonstructural carbohydrates, a conclusion based on the inconsistency of the effects of the different levels of boron applied. However, at 1 ppm boron applied, shoot and root total nonstructural carbohydrates were highest.

(3) Increasing boron levels applied increased boron concentration and uptake in whole plants, shoots and roots, while at 2 ppm boron nitrogen and phosphorus concentrations were reduced.

(4) Nitrogen application affected the TNC, boron, nitrogen and phosphorus concentrations of the whole sainfoin plant, the shoot and the root.

(5) Among the chemical components of sainfoin, phosphorus content largely determined the plant dry weight.

(6) It is suspected also that the inconsistency of effects of the different levels of boron to the different characters measured indicates that plants were growing under boron deficiency conditions, hence, sainfoin has a higher boron tolerance than alfalfa. It is therefore recommended that further studies on this area should be made using a higher boron concentration than 2 ppm to produce more conclusive results.

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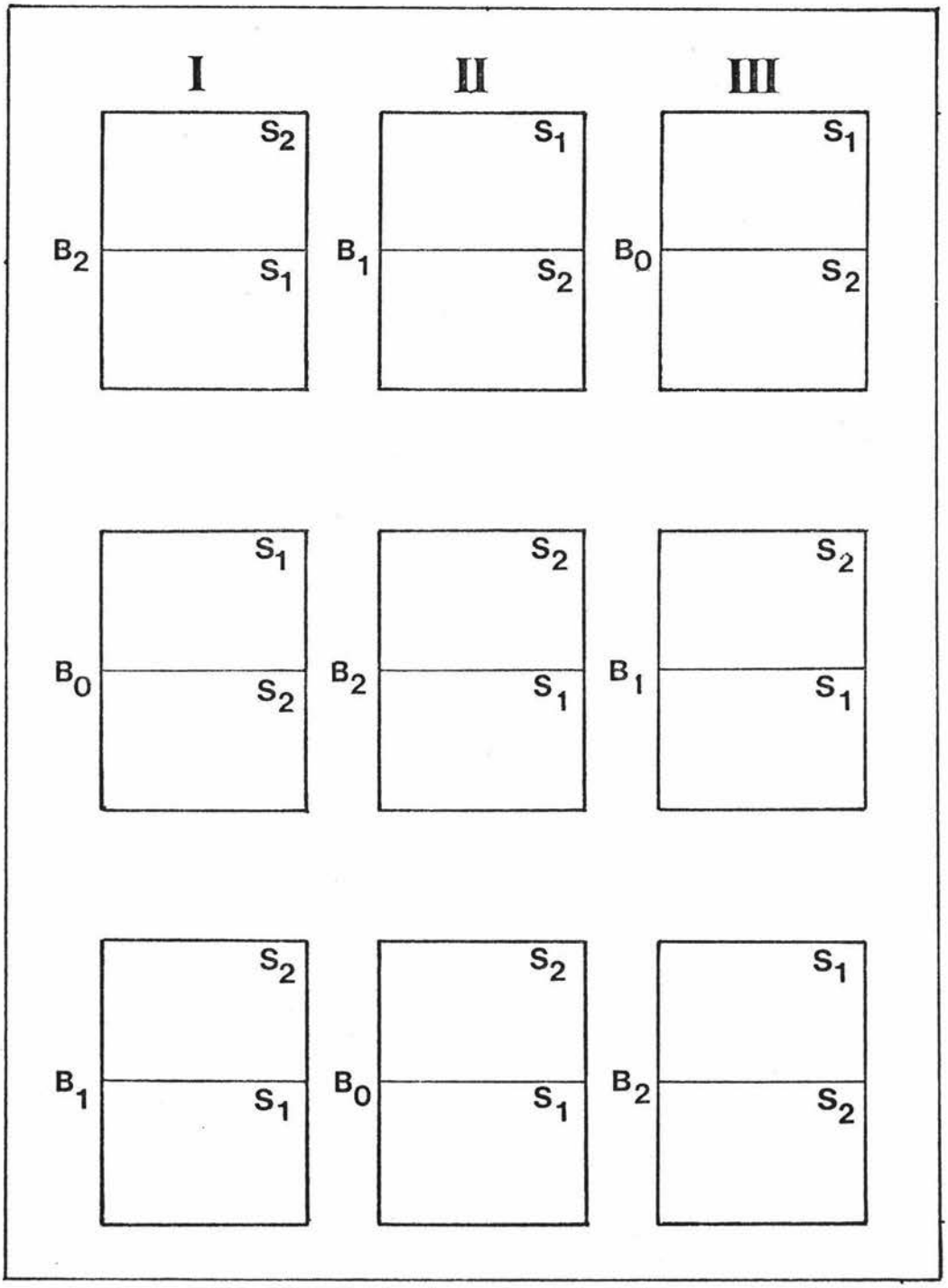
APPENDICES

1. Experimental Lay-out

Three Blocks = I, II, III Subplot = S_1
Main Plot = Bo, B_1, B_2 S_2



2. Preparation and handling of media used in Rhizobial culture [a]

Most strains of rhizobia grow successfully on a yeast-mannitol medium. If necessary, mannitol can be replaced with sucrose but growth rates are a little reduced.

To make one litre of yeast-mannitol agar (YMA).

K_2HPO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.2 g
NaCl	0.1 g
Mannitol	10.0 g
Yeast powder	0.4 g
Tap water	1 litre
Agar	15.0 g

Dissolve all the ingredients listed above. If a solid medium is needed, first dispense the liquid to the culture bottles. Add 200 ml to the flat bottles and 300 ml to the round ones. Then add the correct amount of agar powder (3.0 g per 200 ml or 3.5 g for 300 ml). Now place the flasks in a pressure cooker and melt the agar so that all is now fully dissolved. Sterilize the flasks (bottles) and upon removal from the sterilizer, tighten the lids and lay out on a raised stick or tube and leave to cool. A sloped culture is now ready.

To inoculate, use the laminae flow cabinet. Using 95% ethanol, swab the base of the cabinet, and any tubes or flasks that will be placed inside, and hands as well. When all is evaporated, light the gas and keep a low flame.

Place the mother culture slope and the flask to be inoculated in the cabinet. Pass the unscrewed mother culture over the flame two or three times and stand in the cabinet. Repeat with the big flask. Using the inoculation needle,

dip it in the ethanol and flaming the needle until the ethanol is burned off. Scrape some of the culture off the mother culture and inoculate the big tube, using a zig-zag motion. Then flame the necks again, and screw up both flasks. Always place the caps with open parts down so as to reduce the chances of infection.

Place the flasks in incubator. When the surface is covered, place the cultures in the cold room. Remove some two hours before being used.

When the cultures are to be removed, add sterile peptone water to the cultures and shake well. This will wash off the bacteria into suspension. Add this solution to the pots intended for. Repeat the treated approximately later, depending on growth. (Solution is 0.1% peptone in tap water).

[a] Basically after Robertson, A.G., pers. comm.

3. Determination of Soluble Sugars (TNC) in Plant Material [a]

REAGENTS:

1. Methanol, 62.5% (v/v) in water.
2. Neutral lead acetate, saturated aqueous solution.
3. Phenol (AR), 5% (w/v) in water. Store at 4°C.
4. Concentrated sulphuric acid (AR), sp gr 1.84.
5. Chloroform (AR).
6. Sucrose standard, 2 mg/ml in 62.5% methanol. Made up weekly and stored at 4°C.

PROCEDURE:

Dried plant material (100 mg) is extracted with 10 ml of 62.5% methanol for 30 minutes at 55°C using screw-capped culture tubes (16 x 125 mm) with teflon-faced caps. The samples are centrifuged then 4 ml aliquots are transferred to a second series of capped culture tubes each containing 0.1 ml saturated lead acetate. (Standards are prepared by diluting 2.0, 4.0, 6.0, 8.0, and 10.0 ml sucrose standard solution to 10 ml with 62.5% methanol. Aliquots of 4 ml are removed and treated in the same manner as the plant samples to give standards equivalent to 4, 8, 12, 16, and 20 mg soluble sugars on a dry weight basis, respectively. A blank of 4 ml 62.5% methanol is treated similarly). After 10 minutes standing with occasional shaking, 5 ml chloroform is added and the tubes capped securely and shaken vigorously. They are then briefly centrifuged to aid phase separation. Aliquots of 50 ul are removed from the upper, aqeuous phase and added to 9.95 ml water in culture tubes (18 x 150 mm or similar size). 5% Phenol (1 ml) is added with mixing, followed by 5 ml of sulphuric acid by pipette taking care to direct the stream of acid directly on to the surface of the liquid to aid mixing. Samples are stood to cool for 60 minutes then absorbances react at 490 nm.

NOTES:

1. Kimax culture tubes (Cat. No. 45066-A, 16 x 125 mm) are recommended for use. Teflon-faced caps are essential during the chloroform extraction.

2. The standard sucrose solution is generally stable for two weeks in the fridge.

3. Sugar aliquots from the aqueous phase may be removed either with a glass micro pipette or with an Ependorff or Finpipette but the plastic tips must be well cleaned before use. Note that the methanolic solution tends to drip very slowly from the tip. Glass lambda or micro pipette (50 ul) can also be used.

4. Safety glasses should be worn during the addition of sulphuric acid to the sugar/phenol solution.

5. The consistency of sucrose standard curves with phenol-sulphuric acid seems dependent on the quality of phenol used. Phenol (AR) gives best results and should be stored in an amber bottle in the fridge - it is stable for several weeks.

6. Sucrose is used as a standard because the soluble sugars from most species routinely analyzed have sucrose as their major component. If fructosan-rich sugars are being determined, use fructose as a standard. Note the wide range of response with phenol-sulphuric acid and various types of sugar.

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[a] Modified by Haslemore, R.M., Plant Physiology Division, DSIR, Palmerston North (1981).

4. Determination of Boron [a]

PROCEDURE:

Weight 1 g of dried, ground leaf sample into a crucible. Ash for 4.5 hrs at 475°C in a muffle furnace.

Add 5 ml 2N HCl to the ash.

Heat for 20 min on a hot plate.

Transfer to a 50 ml measuring cylinder and dilute to volume. Mix and filter into plastic bottles.

Measure 10 ml of sample into a glass vial.

Add 4 ml of buffer-masking agent.

Add 2 ml azomenthine.

Stopper, mix by inverting three times.

Let stand for 1.5 hrs and measure absorbances at 420 nm.

For samples with high boron content (100 ppm) take a smaller sample volume and make up to 10 ml with 0.2n HCl.

Calculate results on HP97 using curve Fitting Programme.

CALIBRATION STANDARDS:

Stock 100 ppm B solution - 0.5716 g Boric acid (B_3BO_3) per liter.

5 ppm B solution - 5 ml of 1-0 ppm B stock diluted to 100 ml, including 10 ml of 2N HCl.

0.5 ppm B solution - 10 ml of 5 ppm B solution diluted to 100 ml, including 10 ml of 2N HCl.

CONCENTRATION RANGE:

Calibration standard	B conc in leaf (1:50 dilution)
0.05 ppm	2.5 ppm
2.0 ppm	100.0 ppm
4.0 ppm	200.0 ppm

CALIBRATION STANDARDS:

0 ml of 0.5 ppm B solution diluted to 10 ml with 0.2N HCl = 0 ppm.

1 ml of 0.5 ppm B solution diluted to 10 ml with 0.2N HCl = 0.05 ppm.

2 ml of 0.5 ppm B solution diluted to 10 ml with 0.2N HCl = 0.10 ppm

5 ml of 0.5 ppm B solution diluted to 10 ml with 0.2N HCl = 0.25 ppm

10 ml of 0.5 ppm B solution diluted to 10 ml with 0.2N HCl = 0.50 ppm.

2 ml of 5 ppm B solution diluted to 10 ml with 0.2N HCl = 1.00 ppm.

3 ml of 5 ppm B solution diluted to 10 ml with 0.2N HCl = 1.5 ppm.

4 ml of 5 ppm B solution diluted to 10 ml with 0.2N HCl = 2.0 ppm.

6 ml of 5 ppm B solution diluted to 10 ml with 0.2N HCl = 3.0 ppm.

8 ml of 5 ppm B solution diluted to 10 ml with 0.2N HCl = 4.0 ppm

Add 4 ml buffer-masking agent and 2 ml azomethine as for samples.

REAGENTS:

Buffer-masking Agent

1.	Ammonium acetate	280 g
2.	Potassium acetate	20 g
3.	EDTA Tetrasodium salt	20 g
4.	NTA Disodium salt	8 g

Partly dissolve in 240 ml of warm distilled water in a plastic beaker. Add 70 ml Acetic Acid. Stir until dissolved. Total volume is approximately 600 ml. Store in a plastic bottle.

Azomethine

1.	Azomethine-H	0.9 g
2.	Ascorbic acid	2.0 g

Dissolve in 50 ml hot distilled water and dilute to 100 ml. Store in fridge in a plastic bottle up to a week.

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1. Wolfe, B. 1971. Communcation in Soil Sc. Plant Anal. 2(5): 363-74.

2. Wolfe, B. 1974. Comm. in Soil Sc. Plant Anal. 5(1): 39-44.

[a] Modified by the Ministry of Agriculture and Fisheries, Levin, New Zealand.

5. Nitrogen Determination by Kjeldahl Technique [a]

MATERIALS:

Digestion Mixture

To 100 g K_2SO_4, add catalysts (1:5 Selenium:$CuSO_4$) and 1 litre of concentrated, nitrogen-free H_2SO_4. Heat in fume cupboard until solution clears.

Indicator Mixture

1 g Bromocresol green in 100 ml ethanol (100% or 95%).
0.02 g Methyl red in 20 ml ethanol (95%).
i.e. 5:1 ratio BCG to MR solution.

Boric Acid Indicator Mixture

2 g Boric acid powder to 100 ml water (2% w/v solution containing 2% v/v indicator mixture).
This mixture should be made up after each distillation of 20 samples, 5 ml/sample, as the analyses proceeded.

Hydrochloric Acid

Vial of standard HCl added to 500 ml distilled water gives 1N solution. This then is diluted to 0.01N solution.

Sodium Hydroxide

Add 500 g NaOH to 2 litres distilled water.

METHODS:

The samples to be analyzed are dried at 95°C and each sample or portion of it, is weighed into a separate, labelled, 100 ml Kjeldahl flask. If the entire sample (up to 1 g) is not to be analyzed, then either 280 or 560 mg is weighed out for analysis. To each flask, 5 ml digestion mixture and a few grains of glass sands are added. The flasks are transferred to electric heating mantles (Kjeldahl digestion racks) in a fume cupboard and the contents carefully boiled, without allowing any acid to evaporate, for 2½ hours. The flasks are then allowed to cool, still in the

fume cupboard. A small amount of water is added to the digestates and the flasks are again allowed to cool, after which they are removed from the fume cupboard. The digestates are washed into separate, labelled, 100 ml volumetric flasks and made up to 100 ml with distilled water. Each digestate is now ready for distillation. Provided the flasks are sealed, they can be stored overnight or for several days if necessary.

Distillation is carried out using a Markham still, with the outlet submerged under 5 ml of boric acid indicator mixture in a 100 ml conical flask. From a volumetric flask, 5 ml of digestate is pipetted into the inner chamber of the still, and a quantity (about 10 ml) of sodium hydroxide solution is added. The inlet is sealed and 25 ml of distillate is collected in the boric acid indicator mixture, which is then titrated against the N/100 hydrochloric acid. Before distilling the next digestate, the inner chamber of the still is emptied and flushed with distilled water.

CALCULATION:

For 280 mg sample, %N = titre (sample) - titre (blank), where titres are expressed in ml N/100 hydrochloric acid. For samples of other weight, appropriate corrections are made.

NOTES ON THE METHOD:

The method can be modified to measure %N of samples of various weights by altering the dilution after acid digestion, the amount of digested pipetted into the still, and the normality of the hydrochloric acid. However, as a routine procedure the method outlined has been found to be satisfactory and suitable for streamlined operation. The effects of variations in many aspects of the method have been examined, and where necessary procedures have been standardized. The %N estimations are highly repeatable and the recovery of nitrogen from $(NH_4)_2SO_4$ and protein standards is not less than 97%, a value which is favourable.

[a] After Clements, R. 1970.

6.1 Analysis of variance for relative growth rate of the whole plant (mg/mg/day) for 8 periods of growth.

a. Period 1

Source of Variation	df	SS	MS	VR
Blocks	2	0.00002	0.000009	0.145
Levels of Boron	2	0.00439	0.002194	27.020
Error (a)	4	0.00032	0.000081	1.344
Nitrogen application-Inoculation	1	0.00008	0.000084	1.384
Interaction	2	0.00087	0.000433	7.167
Error (b)	6	0.00036	0.000060	
Total	17	0.00604		

b. Period 2

Source of Variation	df	SS	MS	VR
Blocks	2	0.00035	0.00018	2.862
Levels of Boron	2	0.00016	0.00008	2.325
Error (a)	4	0.00014	0.00003	0.577
Nitrogen application-Inoculation	1	0.00005	0.00005	0.805
Interaction	2	0.00008	0.00004	0.650
Error (b)	6	0.00037	0.00006	
Total	17	0.00115		

c. Period 3

Source of variation	df	SS	MS	VR
Blocks	2	0.00029	0.00014	4.654
Levels of Boron	2	0.00279	0.00139	62.204
Error (a)	4	0.00009	0.00002	0.726
Nitrogen application-Inoculation	1	0.00564	0.00564	182.814
Interaction	2	0.00088	0.00044	14.210
Error (b)	6	0.00019	0.00003	
Total	17	0.00988		

contd. ....

6.1 contd.

d. Period 4

Source of variation	df	SS	MS	VR
Blocks	2	0.0001	0.00004	1.490
Levels of Boron	2	0.0008	0.00038	5.594
Error (a)	4	0.0003	0.00007	2.726
Nitrogen application-Inoculation	1	0.0009	0.00088	35.572
Interaction	2	0.0002	0.00010	4.127
Error (b)	6	0.0002	0.00002	
Total	17	0.0025		

e. Period 5

Source of variation	df	SS	MS	VR
Blocks	2	0.00004	0.00002	0.626
Levels of Boron	2	0.00032	0.00016	3.033
Error (a)	4	0.00021	0.00005	1.678
Nitrogen application-Inoculation	1	0.00188	0.00188	60.326
Interaction	2	0.00051	0.00026	8.215
Error (b)	6	0.00019	0.00003	
Total	17	0.00315		

f. Period 6

Source of variation	df	SS	MS	VR
Blocks	2	0.00008	0.000038	0.962
Levels of Boron	2	0.00040	0.000201	56.541
Error (a)	4	0.00001	0.000004	0.091
Nitrogen application-Inoculation	1	0.00097	0.000969	24.824
Interaction	2	0.00004	0.000022	0.575
Error (b)	6	0.00023	0.000039	
Total	17	0.00173		

contd. ....

6.1 contd.

g. Period 7

Source of variation	df	SS	MS	VR
Blocks	2	0.00004	0.000022	3.199
Levels of Boron	2	0.00036	0.000178	27.899
Error (a)	4	0.00003	0.000006	0.911
Nitrogen application-Inoculation	1	0.00473	0.004727	673.491
Interaction	2	0.00002	0.000009	1.224
Error (b)	6	0.00004	0.000007	
Total	17	0.00522		

h. Period 8

Source of variation	df	SS	MS	VR
Blocks	2	0.00002	0.000011	0.629
Levels of Boron	2	0.00004	0.000021	4.650
Error (a)	4	0.00002	0.000004	0.253
Nitrogen application-Inoculation	1	0.00038	0.000382	21.858
Interaction	2	0.00015	0.000076	4.337
Error (b)	6	0.00010	0.000017	
Total	17	0.00071		

6.2 Analysis of variance for relative growth rate of shoot (mg/mg/day) for 8 periods of growth.

a. Period 1

Source of variation	df	SS	MS	VR
Blocks	2	0.000076	0.000038	0.869
Levels of Boron	2	0.006193	0.003096	201.447
Error (a)	4	0.000061	0.000015	0.349
Nitrogen application-Inoculation	1	0.000003	0.000003	0.058
Interaction	2	0.001136	0.000568	12.917
Error (b)	6	0.000264	0.000044	
Total	17	0.007733		

b. Period 2

Source of variation	df	SS	MS	VR
Blocks	2	0.0002	0.00011	1.854
Levels of Boron	2	0.0003	0.00014	1.605
Error (a)	4	0.0003	0.00008	1.368
Nitrogen application-Inoculation	1	0.0008	0.00081	13.108
Interaction	2	0.0002	0.00010	1.680
Error (b)	6	0.0004	0.00006	
Total	17	0.0022		

c. Period 3

Source of variation	df	SS	MS	VR
Blocks	2	0.0002	0.00011	3.368
Levels of Boron	2	0.0047	0.00234	39.349
Error (a)	4	0.0002	0.00006	1.866
Nitrogen application-Inoculation	1	0.0072	0.00718	225.755
Interaction	2	0.0007	0.00037	11.556
Error (b)	6	0.0002	0.00003	
Total	17	0.0132		

contd. ....

6.2 contd.

d. Period 4

Source of variation	df	SS	MS	VR
Blocks	2	0.0002	0.00008	1.803
Levels of Boron	2	0.0037	0.00186	58.544
Error (a)	4	0.0001	0.00003	0.764
Nitrogen application-Inoculation	1	0.0008	0.00077	18.584
Interaction	2	0.0003	0.00016	3.864
Error (b)	6	0.0003	0.00004	
Total	17	0.0054		

e. Period 5

Source of variation	df	SS	MS	VR
Blocks	2	0.0001	0.00007	1.615
Levels of Boron	2	0.0004	0.00020	3.182
Error (a)	4	0.0003	0.00006	
Nitrogen application-Inoculation	1	0.0020	0.00204	46.675
Interaction	2	0.0010	0.00052	11.800
Error (b)	6	0.0003	0.00004	
Total	17	0.0041		

f. Period 6

Source of variation	df	SS	MS	VR
Blocks	2	0.000008	0.000004	0.115
Levels of Boron	2	0.000489	0.000244	94.227
Error (a)	4	0.000010	0.000003	0.075
Nitrogen application-Inoculation	1	0.002495	0.002495	72.058
Interaction	2	0.000148	74	2.131
Error (b)	6	0.000208	35	
Total	17	0.003358		

Contd. ....

6.2 contd.

g. Period 7

Source of variation	df	SS	MS	VR
Blocks	2	0.000019	0.000010	2.842
Levels of Boron	2	489	244	26.531
Error (a)	4	37	09	2.752
Nitrogen application-Inoculation	1	0.004595	0.004595	1373.072
Interaction	2	0.000003	0.000001	0.401
Error (b)	6	0.000020	0.000003	
Total	17	0.005163		

h. Period 8

Source of variation	df	SS	MS	VR
Blocks	2	0.0000010	0.0000005	0.335
Levels of Boron	2	0.0000252	0.0000126	1.773
Error (a)	4	0.0000284	0.0000071	4.976
Nitrogen application-Inoculation	1	0.0003167	0.0003167	222.146
Interaction	2	0.0002227	0.0001114	78.124
Error (b)	6	0.0000086	0.0000014	
Total	17	0.0006026		

6.3 Analysis of variance for relative growth rate of root (mg/mg/day) for 8 periods of growth.

a. Period 1

Source of variation	df	SS	MS	VR
Blocks	2	0.0010	0.0005	4.084
Levels of Boron	2	0.0034	0.0017	4.913
Error (a)	4	0.0014	0.0003	2.944
Nitrogen application-Inoculation	1	0.0039	0.0039	33.062
Interaction	2	0.0031	0.0015	12.949
Error (b)	6	0.0007	0.0001	
Total	17	0.0135		

b. Period 2

Source of variation	df	SS	MS	VR
Blocks	2	0.0004797	0.0002399	0.602
Levels of Boron	2	0.0000006	0.0000003	0.001
Erorr (a)	4	0.0017046	0.0004262	1.069
Nitrogen application-Inoculation	1	0.0000657	0.0000657	0.165
Interaction	2	0.0033953	0.0016976	4.260
Error (b)	6	0.0023911	0.0003985	
Total	17	0.0080370		

c. Period 3

Source of variation	df	SS	MS	VR
Blocks	2	0.0006	0.0003	1.042
Levels of Boron	2	0.0008	0.0004	2.529
Error (a)	4	0.0006	0.0002	0.505
Nitrogen application-Inoculation	1	0.0020	0.0020	6.527
Interaction	2	0.0054	0.0027	8.853
Error (b)	6	0.0018	0.0003	
Total	17	0.1120		

Contd. ....

6.3 contd.

d. Period 4

Source of variation	df	SS	MS	VR
Blocks	2	0.0002	0.00010	2.387
Levels of Boron	2	0.0017	0.00085	4.262
Error (a)	4	0.0008	0.00020	4.577
Nitrogen application-Inoculation	1	0.0001	0.00015	3.347
Interaction	2	0.0007	0.00036	8.226
Error (b)	6	0.0003	0.00004	
Total	17	0.0038		

e. Period 5

Source of variation	df	SS	MS	VR
Blocks	2	0.000007	0.000004	0.083
Levels of Boron	2	0.000324	0.000162	2.984
Error (a)	4	0.000217	0.000054	1.269
Nitrogen application-Inoculation	1	0.001659	0.001659	38.779
Interaction	2	0.000016	0.000008	0.189
Error (b)	6	0.000257	0.000043	
Total	17	0.002480		

f. Period 6

Source of variation	df	SS	MS	VR
Blocks	2	0.000062	0.000031	1.714
Levels of Boron	2	0.000695	0.000347	188.218
Error (a)	4	0.000007	0.000002	0.102
Nitrogen application-Inoculation	1	0.000016	0.000016	0.887
Interaction	2	0.000019	0.000009	0.511
Error (b)	6	0.000109	0.000018	
Total	17	0.000908		

Contd. ....

6.3 contd.

g. Period 7

Source of variation	df	SS	MS	VR
Blocks	2	0.00009	0.000046	3.472
Levels of Boron	2	0.00036	0.000178	29.751
Error (a)	4	0.00002	0.000006	0.453
Nitrogen application-Inoculation	1	0.00642	0.006422	485.674
Interaction	2	0.00011	0.000054	4.084
Error (b)	6	0.00008	0.000013	
Total	17	0.00708		

h. Period 8

Source of variation	df	SS	MS	VR
Blocks	2	0.00002	0.000008	0.800
Levels of Boron	2	0.00008	0.000040	10.492
Error (a)	4	0.00002	0.000004	0.385
Nitrogen application-Inoculation	1	0.00057	0.000567	57.630
Interaction	2	0.00006	0.000031	3.158
Error (b)	6	0.00006	0.000010	
Total	17	0.00081		

6.4 Analysis of variance for mean relative growth rate (mg/mg/day) the whole plnat (a), the shoot (b) and the root (c) of eight periods of growth.

a. Whole plant Relative growth rate

Source of variation	df	SS	MS	VR
Blocks	2	0.0000121	0.0000061	0.792
Levels of Boron	2	0.0000009	0.0000005	0.478
Error (a)	4	0.0000038	0.0000009	0.124
Nitrogen application-Inoculation	1	0.0010125	0.0010125	132.132
Interaction	2	0.0000087	0.0000044	0.571
Error (b)	6	0.0000460	0.0000077	
Total	17	0.0010840		

b. Shoot Relative growth rate

Source of variation	df	SS	MS	VR
Blocks	2	0.00002	0.000008	1.624
Levels of boron	2	0.00001	0.000005	0.341
Error (a)	4	0.00005	0.000015	2.928
Nitrogen application-Inoculation	1	0.00086	0.000864	171.938
Interaction	2	0.00001	0.000005	1.040
Error (b)	6	0.00003	0.000005	
Total	17	0.00098		

c. Root Relative growth rate

Source of variation	df	SS	MS	VR
Blocks	2	0.00001	0.000006	1.346
Levels of Boron	2	0.00004	0.000020	1.968
Error (a)	4	0.00004	0.000010	2.404
Nitrogen application-Inoculation	1	0.00028	0.000278	64.132
Interaction	2	0.00008	0.000038	8.917
Error (b)	6	0.00003	0.000004	
Total	17	0.00048		

6.5 Analysis of variance for total dry weight of the whole plant (mg/plant) for nine harvest times

a. Harvest 1

Source of variation	df	SS	MS	VR
Blocks	2	0.0003	0.0002	0.007
Levels of Boron	2	7.4758	3.7379	47.750
Error (a)	4	0.3131	0.0783	3.719
Nitrogen application-Inoculation	1	2.6989	2.6989	128.215
Interaction	2	3.0508	1.5254	72.466
Error (b)	6	0.1263	0.0211	
Total	17	13.6652		

b. Harvest 2

Source of variation	df	SS	MS	VR
Blocks	2	0.061	0.030	0.289
Levels of Boron	2	1.498	0.749	6.329
Error (a)	4	0.474	0.118	1.128
Nitrogen application-Inoculation	1	3.150	3.150	30.015
Interaction	2	1.052	0.526	5.014
Error (b)	6	0.630	0.105	
Total	17	6.865		

c. Harvest 3

Source of variation	df	SS	MS	VR
Blocks	2	0.406	0.203	1.929
Levels of Boron	2	1.332	0.666	11.095
Error (a)	4	0.240	0.060	0.570
Nitrogen application-Inoculation	1	1.356	1.356	12.881
Interaction	2	0.587	0.294	2.790
Error (b)	6	0.632	0.105	
Total	17	4.553		

Contd. ....

6.5 contd.

d. Harvest 4

Source of variation	df	SS	MS	VR
Blocks	2	0.167	0.083	1.230
Levels of Boron	2	6.489	3.244	33.977
Error (a)	4	0.382	0.095	1.409
Nitrogen application-Inoculation	1	15.717	15.717	231.839
Interaction	2	5.019	2.509	37.016
Error (b)	6	0.409	0.068	
Total	17	28.183		

e. Harvest 5

Source of variation	df	SS	MS	VR
Blocks	2	0.897	0.449	4.855
Levels of Boron	2	23.170	11.585	20.208
Error (a)	4	2.293	0.573	6.203
Nitrogen application-Inoculation	1	5.951	5.951	64.388
Interaction	2	0.429	0.215	2.322
Error (b)	6	0.555	0.092	
Total	17	33.295		

f. Harvest 6

Source of variation	df	SS	MS	VR
Blocks	2	0.121	0.061	0.052
Levels of Boron	2	13.513	6.756	15.305
Error (a)	4	1.766	0.441	0.379
Nitrogen application-Inoculation	1	154.001	154.001	132.267
Interaction	2	30.688	15.344	13.179
Error (b)	6	6.986	1.164	
Total	17	207.075		

Contd. ....

6.5 contd.

g. Harvest 7

Source of variation	df	SS	MS	VR
Blocks	2	49.00	24.50	2.137
Levels of Boron	2	312.75	156.37	18.371
Error (a)	4	34.05	8.51	0.742
Nitrogen application-Inoculation	1	2973.75	2973.75	259.374
Interaction	2	50.84	25.42	2.217
Error (b)	6	68.79	11.47	
Total	17	3489.16		

h. Harvest 8

Source of variation	df	SS	MS	VR
Blocks	2	36.994	18.497	2.850
Levels of Boron	2	626.184	313.092	81.531
Error (a)	4	15.361	3.840	0.592
Nitrogen application-Inoculation	1	80411.172	80411.172	12389.314
Interaction	2	768.689	384.345	59.218
Error (b)	6	38.942	6.490	
Total	17	81897.328		

i. Harvest 9

Source of variation	df	SS	MS	VR
Blocks	2	8.14	4.07	0.051
Levels of Boron	2	1784.41	892.20	21.817
Error (a)	4	163.58	40.89	0.512
Nitrogen application-Inoculation	1	317068.00	317068.00	3973.537
Interaction	2	357.57	178.79	2.241
Error (b)	6	478.77	79.79	
Total	17	319860.44		

6.6 Analysis of variance for total dry weight of shoot (mg/shoot) for nine harvest times.

a. Harvest 1

Source of variation	df	SS	MS	VR
Blocks	2	0.013	0.006	0.192
Levels of Boron	2	5.536	2.768	38.564
Error (a)	4	0.287	0.072	2.206
Nitrogen application-Inoculation	1	2.420	2.420	74.398
Interaction	2	1.361	0.681	20.921
Error (b)	6	0.195	0.325	
Total	17	9.812		

b. Harvest 2

Source of variation	df	SS	MS	VR
Blocks	2	0.103	0.052	0.445
Levels of Boron	2	2.007	1.003	15.355
Error (a)	4	0.261	0.065	0.563
Nitrogen application-Inoculation	1	2.000	2.000	17.241
Interaction	2	0.567	0.284	2.445
Error (b)	6	0.696	0.116	
Total	17	5.634		

c. Harvest 3

Source of variation	df	SS	MS	VR
Blocks	2	0.373	0.187	1.920
Levels of Boron	2	0.963	0.482	4.346
Error (a)	4	0.443	0.111	1.140
Nitrogen application-Inoculation	1	0.036	0.036	0.366
Interaction	2	0.041	0.021	0.211
Error (b)	6	0.583	0.097	
Total	17	2.439		

Contd. ....

6.6 contd.

d. Harvest 4

Source of variation	df	SS	MS	VR
Blocks	2	0.058	0.029	0.206
Levels of Boron	2	6.234	3.117	47.551
Error (a)	4	0.262	0.066	0.468
Nitrogen application-Inoculation	1	21.125	21.125	150.893
Interaction	2	3.990	1.995	14.250
Error (b)	6	0.840	0.140	
Total	17	32.509		

e. Harvest 5

Source of variation	df	SS	MS	VR
Blocks	2	0.696	0.348	1.957
Levels of Boron	2	7.461	3.730	12.890
Error (a)	4	1.158	0.289	1.627
Nitrogen application-Inoculation	1	8.890	8.890	49.993
Interaction	2	0.046	0.023	0.129
Error (b)	6	1.067	0.178	
Total	17	19.318		

f. Harvest 6

Source of variation	df	SS	MS	VR
Blocks	2	0.319	0.159	0.201
Levels of Boron	2	2.672	1.336	5.572
Error (a)	4	0.959	0.240	0.303
Nitrogen application-Inoculation	1	107.018	107.018	135.193
Interaction	2	17.199	8.599	10.863
Error (b)	6	4.750	0.792	
Total	17	132.917		

6.6 contd.

g. Harvest 7

Source of variation	df	SS	MS	VR
Blocks	2	14.578	7.289	1.130
Levels of Boron	2	178.832	89.411	19.396
Error (a)	4	18.439	4.610	0.715
Nitrogen application-Inoculation	1	2272.054	2272.054	352.316
Interaction	2	49.303	24.651	3.823
Error (b)	6	38.693	6.449	
Total	17	2571.890		

h. Harvest 8

Source of variation	df	SS	MS	VR
Blocks	2	0.285	0.142	0.671
Levels of Boron	2	153.135	76.569	85.119
Error (a)	4	3.598	0.900	4.241
Nitrogen application-Inoculation	1	25200.871	25200.871	118803.203
Interaction	2	169.497	84.748	399.525
Error (b)	6	1.273	0.212	
Total	17	25528.659		

i. Harvest 9

Source of variation	df	SS	MS	VR
Blocks	2	17.01	8.50	0.491
Levels of Boron	2	141.75	70.87	2.859
Error (a)	4	99.16	24.79	1.430
Nitrogen application-Inoculation	1	58550.41	58550.41	3377.688
Interaction	2	235.94	117.97	6.806
Error (b)	6	104.01	17.33	
Total	17	59148.26		

6.7 Analysis of variance for total dry weight of root (mg/root) for nine harvest times.

a. Harvest 1

Source of variation	df	SS	MS	VR
Blocks	2	0.010	0.005	0.932
Levels of Boron	2	0.177	0.089	11.551
Error (a)	4	0.031	0.008	1.468
Nitrogen application-Inoculation	1	0.007	0.008	1.455
Interaction	2	0.395	0.198	37.815
Error (b)	6	0.031	0.005	
Total	17	0.651		

b. Harvest 2

Source of variation	df	SS	MS	VR
Blocks	2	0.045	0.022	1.153
Levels of Boron	2	0.002	0.001	0.073
Error (a)	4	0.058	0.014	0.740
Nitrogen application-Inoculation	1	0.467	0.467	23.974
Interaction	2	0.137	0.068	3.508
Error (b)	6	0.117	0.019	
Total	17	0.826		

c. Harvest 3

Source of variation	df	SS	MS	VR
Blocks	2	0.0007	0.0004	0.017
Levels of Boron	2	0.0401	0.0201	1.245
Error (a)	4	0.0645	0.0161	0.773
Nitrogen application-Inoculation	1	0.9522	0.9522	45.645
Interaction	2	0.3481	0.1741	8.344
Error (b)	6	0.1252	0.0209	
Total	17	1.5308		

Contd. ....

6.7 contd.

d. Harvest 4

Source of variation	df	SS	MS	VR
Blocks	2	0.166	0.083	3.773
Levels of Boron	2	0.105	0.052	1.798
Error (a)	4	0.117	0.029	1.324
Nitrogen application-Inoculation	1	0.399	0.399	18.124
Interaction	2	0.299	0.149	6.790
Error (b)	6	0.132	0.022	
Total	17	1.218		

e. Harvest 5

Source of variation	df	SS	MS	VR
Blocks	2	0.013	0.006	0.301
Levels of Boron	2	4.606	2.303	16.448
Error (a)	4	0.560	0.140	6.545
Nitrogen application-Inoculation	1	0.294	0.294	13.737
Interaction	2	0.471	0.235	11.002
Error (b)	6	0.128	0.021	
Total	17	6.072		

f. Harvest 6

Source of variation	df	SS	MS	VR
Blocks	2	0.132	0.066	0.229
Levels of Boron	2	4.341	2.170	51.781
Error (a)	4	0.168	0.042	0.145
Nitrogen application-Inoculation	1	4.263	4.263	14.763
Interaction	2	1.993	0.997	3.451
Error (b)	6	1.733	0.289	
Total	17	12.630		

Contd. ....

6.7 contd.

g. Harvest 7

Source of variation	df	SS	MS	VR
Blocks	2	11.691	5.845	2.682
Levels of Boron	2	45.612	22.806	24.799
Error (a)	4	3.678	0.920	0.422
Nitrogen application-Inoculation	1	47.142	47.142	21.627
Interaction	2	10.148	5.074	2.328
Error (b)	6	13.079	2.180	
Total	17	131.350		

h. Harvest 8

Source of variation	df	SS	MS	VR
Blocks	2	41.026	20.513	2.979
Levels of Boron	2	183.366	91.683	59.058
Error (a)	4	6.210	1.552	0.225
Nitrogen application-Inoculation	1	15565.533	15565.533	2260.570
Interaction	2	238.217	119.109	17.298
Error (b)	6	41.314	6.886	
Total	17	16075.666		

i. Harvest 9

Source of variation	df	SS	MS	VR
Blocks	2	5.97	2.99	0.144
Levels of Boron	2	2360.36	1180.18	73.343
Error (a)	4	64.36	16.09	0.774
Nitrogen application-Inoculation	1	104208.28	104208.28	5014.670
Interaction	2	891.56	445.78	21.452
Error (b)	6	124.68	20.78	
Total	17	107655.21		

6.8 Analysis of variance for number of first order lateral roots for the first six harvest times.

a. Harvest 1

Source of variation	df	SS	MS	VR
Blocks	2	1.194	0.597	0.562
Levels of Boron	2	1.882	0.941	0.704
Error (a)	4	5.347	1.337	1.258
Nitrogen application-Inoculation	1	1.125	1.125	1.059
Interaction	2	1.562	0.781	0.735
Error (b)	6	6.375	1.062	
Total	17	17.485		

b. Harvest 2

Source of variation	df	SS	MS	VR
Blocks	2	0.061	0.031	0.141
Levels of Boron	2	6.638	3.319	11.814
Error (a)	4	1.124	0.281	1.294
Nitrogen application-Inoculation	1	15.254	15.254	70.270
Interaction	2	0.418	0.209	0.963
Error (b)	6	1.302	0.217	
Total	17	24.797		

c. Harvest 3

Source of variation	df	SS	MS	VR
Blocks	2	1.628	0.814	0.610
Levels of Boron	2	1.088	0.544	1.116
Error (a)	4	1.951	0.488	0.366
Nitrogen application-Inoculation	1	13.781	13.781	10.332
Interaction	2	5.178	2.589	1.941
Error (b)	6	8.003	1.334	
Total	17	31.629		

Contd. ....

6.8 contd.

d. Harvest 4

Source of variation	df	SS	MS	VR
Blocks	2	1.309	0.654	2.006
Levels of Boron	2	2.920	1.460	4.769
Error (a)	4	1.225	0.306	0.939
Nitrogen application-Inoculation	1	13.347	13.347	40.928
Interaction	2	33.409	16.704	51.223
Error (b)	6	1.957	0.326	
Total	17	54.167		

e. Harvest 5

Source of variation	df	SS	MS	VR
Blocks	2	0.021	0.010	0.023
Levels of Boron	2	7.959	3.980	14.886
Error (a)	4	1.069	0.267	0.592
Nitrogen application-Inoculation	1	3.654	3.654	8.091
Interaction	2	2.190	1.095	2.424
Error (b)	6	2.710	0.452	
Total	17	17.603		

f. Harvest 6

Source of variation	df	SS	MS	VR
Blocks	2	1.232	0.616	0.196
Levels of Boron	2	0.189	0.095	0.039
Error (a)	4	9.742	2.436	0.775
Nitrogen application-Inoculation	1	15.033	15.033	4.780
Interaction	2	8.419	4.210	1.339
Error (b)	6	18.869	3.145	
Total	17	53.484		

6.9 Analysis of variance for number of second order lateral roots for four harvest times.

a. Harvest 3

Source of variation	df	SS	MS	VR
Blocks	2	0.131	0.065	0.052
Levels of Boron	2	0.161	0.081	0.131
Error (a)	4	2.455	0.614	0.492
Nitrogen application-Inoculation	1	0.115	0.115	0.092
Interaction	2	0.964	0.482	0.386
Error (b)	6	7.487	1.248	
Total	17	11.313		

b. Harvest 4

Source of variation	df	SS	MS	VR
Blocks	2	0.099	0.050	0.133
Levels of Boron	2	70.894	35.447	81.485
Error (a)	4	1.740	0.435	1.168
Nitrogen application-Inoculation	1	8.296	8.296	22.276
Interaction	2	6.721	3.361	9.024
Error (b)	6	2.235	0.372	
Total	17	89.985		

Contd. ....

6.9 contd.

c. Harvest 5

Source of variation	df	SS	MS	VR
Blocks	2	5.741	2.871	1.522
Levels of Boron	1	19.606	9.803	3.220
Error (a)	5	12.179	3.045	1.614
Nitrogen application-Inoculation	1	0.164	0.164	0.087
Interaction	2	19.016	9.508	5.041
Error (b)	6	11.318	1.886	
Total	17	68.024		

d. Harvest 6

Source of variation	df	SS	MS	VR
Blocks	2	144.59	72.30	3.596
Levels of Boron	2	144.72	72.36	1.493
Error (a)	4	193.82	48.46	2.410
Nitrogen application-Inoculation	1	252.75	252.75	12.573
Interaction	2	22.80	11.40	0.567
Error (b)	6	120.62	20.10	
Total	17	879.30		

6.10 Analysis of variance for total leaf area (cm^2) for the last three harvest times.

a. Harvest 7

Source of variation	df	SS	MS	VR
Blocks	2	9.969	4.985	2.977
Levels of Boron	2	18.122	9.061	8.094
Error (a)	4	4.478	1.119	0.669
Nitrogen application-Inoculation	1	281.319	281.319	169.040
Interaction	2	2.409	1.204	0.719
Error (b)	6	10.045	1.674	
Total	17	326.342		

b. Harvest 8

Source of variation	df	SS	MS	VR
Blocks	2	1.143	0.571	0.335
Levels of Boron	2	8.915	4.457	2.731
Error (a)	4	6.528	1.632	0.956
Nitrogen application-Inoculation	1	825.127	825.127	483.180
Interaction	2	4.305	2.153	1.261
Error (b)	6	10.246	1.708	
Total	17	856.264		

c. Harvest 9

Source of variation	df	SS	MS	VR
Blocks	2	19.73	9.86	0.214
Levels of Boron	2	50.47	25.23	0.802
Error (a)	4	125.82	31.46	0.682
Nitrogen application-Inoculation	1	4912.72	4912.72	106.578
Interaction	2	30.79	15.40	0.334
Error (b)	6	276.57	46.10	
Total	17	5416.10		

6.11 Analysis of variance for total nonstructural carbohydrates (TNC) of the whole plant (%) for eight harvest times.

a. Harvest 2

Source of variation	df	SS	MS	VR
Blocks	2	2.890	1.445	6.164
Levels of Boron	2	13.583	6.792	25.710
Error (a)	4	1.057	0.264	1.127
Nitrogen application-Inoculation	1	3.556	3.556	15.166
Interaction	2	2.388	1.194	5.092
Error (b)	6	1.407	0.234	
Total	17	24.881		

b. Harvest 3

Source of variation	df	SS	MS	VR
Blocks	2	10.163	5.082	107.612
Levels of Boron	2	0.223	0.112	0.479
Error (a)	4	0.933	0.233	4.941
Nitrogen application-Inoculation	1	0.294	0.294	6.224
Interaction	2	0.708	0.354	7.494
Error (b)	6	0.283	0.047	
Total	17	12.604		

c. Harvest 4

Source of variation	df	SS	MS	VR
Blocks	2	0.863	0.432	1.667
Levels of Boron	2	7.103	3.552	14.747
Error (a)	4	0.963	0.241	0.930
Nitrogen application-Inoculation	1	13.869	13.869	53.571
Interaction	2	2.388	1.194	4.612
Error (b)	6	1.553	0.259	
Total	17	26.739		

Contd. ....

6.11 contd.

d. Harvest 5

Source of variation	df	SS	MS	VR
Blocks	2	1.781	0.8906	2.118
Levels of Boron	2	4.868	2.4339	4.559
Error (a)	4	2.136	0.5339	1.269
Nitrogen application-Inoculation	1	1.076	1.0756	2.557
Interaction	2	0.001	0.0006	0.011
Error (b)	6	2.523	0.4206	
Total	17	12.385		

e. Harvest 6

Source of variation	df	SS	MS	VR
Blocks	2	0.071	0.036	0.213
Levels of Boron	2	0.781	0.391	4.235
Error (a)	4	0.369	0.092	0.553
Nitrogen application-Inoculation	1	0.436	0.436	2.613
Interaction	2	5.114	2.557	15.343
Error (b)	6	1.000	0.167	
Total	17	7.771		

f. Harvest 7

Source of variation	df	SS	MS	VR
Blocks	2	0.029	0.014	0.014
Levels of Boron	2	5.563	2.781	7.971
Error (a)	4	1.396	0.349	0.338
Nitrogen application-Inoculation	1	61.457	61.457	59.476
Interaction	2	4.785	2.392	2.315
Error (b)	6	6.200	1.033	
Total	17	79.430		

Contd. ....

6.11 contd.

g. Harvest 8

Source of variation	df	SS	MS	VR
Blocks	2	3.945	1.972	0.694
Levels of Boron	2	2.175	1.088	0.889
Error (a)	4	4.891	1.223	0.430
Nitrogen application-Inoculation	1	38.632	38.632	13.590
Interaction	2	2.399	1.120	0.394
Error (b)	6	17.056	2.843	
Total	17	68.938		

h. Harvest 9

Source of variation	df	SS	MS	VR
Blocks	2	22.528	11.264	14.109
Levels of Boron	2	43.054	21.527	3.849
Error (a)	5	22.369	5.592	7.005
Nitrogen application-Inoculation	1	46.722	46.722	58.525
Interaction	2	1.188	0.594	0.744
Error (b)	6	4.790	0.798	
Total	17	140.651		

6.12 Analysis of variance for total nonstructural carbohydrates of shoot (%) for the last three harvest times.

a. Harvest 7

Source of variation	df	SS	MS	VR
Blocks	2	0.271	0.136	1.781
Levels of Boron	2	1.654	0.827	33.461
Error (a)	4	0.099	0.025	0.325
Nitrogen application-Inoculation	1	21.780	21.780	286.160
Interaction	2	1.703	0.852	11.190
Error (b)	6	0.457	0.076	
Total	17	25.964		

b. Harvest 8

Source of variation	df	SS	MS	VR
Blocks	2	1.501	0.751	1.634
Levels of Boron	2	2.874	1.437	3.076
Error (a)	4	1.869	0.467	1.017
Nitrogen application-Inoculation	1	3.467	3.467	7.547
Interaction	2	0.081	0.041	0.088
Error (b)	6	2.757	0.459	
Total	17	12.549		

c. Harvest 9

Source of variation	df	SS	MS	VR
Blocks	2	2.434	1.217	2.440
Levels of Boron	2	3.498	1.749	7.245
Error (a)	4	0.966	0.241	0.484
Nitrogen application-Inoculation	1	3.827	3.827	7.671
Interaction	2	3.204	1.602	3.212
Error (b)	6	2.993	0.499	
Total	17	16.922		

6.13 Analysis of variance for total nonstructural carbohydrates of root (%) for the last three harvest times.

a. Harvest 7

Source of variation	df	SS	MS	VR
Blocks	2	16.001	8.001	4.902
Levels of Boron	2	0.148	0.074	0.064
Error (a)	4	4.619	1.155	0.707
Nitrogen application-Inoculation	1	33.894	33.894	20.765
Interaction	2	1.448	0.724	0.443
Error (b)	6	9.793	1.632	
Total	17	65.903		

b. Harvest 8

Source of variation	df	SS	MS	VR
Blocks	2	4.434	2.217	0.817
Levels of Boron	2	9.341	4.671	11.709
Error (a)	4	1.596	0.399	0.147
Nitrogen application-Inoculation	1	84.934	84.934	31.283
Interaction	2	3.941	1.971	0.726
Error (b)	6	16.290	2.715	
Total	17	120.536		

c. Harvest 9

Source of variation	df	SS	MS	VR
Blocks	2	2.710	1.355	1.525
Levels of Boron	2	160.243	80.122	115.007
Error (a)	4	2.787	0.697	0.784
Nitrogen application-Inoculation	1	77.294	77.294	87.010
Interaction	2	5.941	2.971	3.344
Error (b)	6	5.330	0.888	
Total	17	254.305		

6.14 Analysis of variance for (a) boron concentration (µg/g), and (b) boron uptake (µg/plant) of the whole plant at final harvest.

a. Whole plant boron concentration

Source of variation	df	SS	MS	VR
Blocks	2	142.190	71.095	13.764
Levels of Boron	2	1989.554	994.777	41.585
Error (a)	4	95.685	23.921	4.631
Nitrogen application-Inoculation	1	342.434	342.434	66.295
Interaction	2	165.074	82.537	15.979
Error (b)	6	30.992	5.165	
Total	17	2765.929		

b. Whole plant boron uptake

Source of variation	df	SS	MS	VR
Blocks	2	2.188	1.094	6.032
Levels of Boron	2	31.698	15.849	37.409
Error (a)	4	1.695	0.424	2.336
Nitrogen application-Inoculation	1	5.206	5.206	28.702
Interaction	2	1.108	0.554	3.055
Error (b)	6	1.088	0.181	
Total	17	42.983		

6.15 Analysis of varince for (a) shoot boron concentration (µg/g), and (b) boron uptake (µg/shoot) at final harvest.

a. Shoot boron concentration

Source of variation	df	SS	MS	VR
Blocks	2	8.400	4.200	0.509
Levels of Boron	2	362.732	181.366	42.045
Error (a)	4	17.255	4.314	0.523
Nitrogen application-Inoculation	1	82.818	82.818	10.041
Interaction	2	153.100	76.550	9.281
Error (b)	6	49.489	8.248	
Total	17	673.793		

b. Shoot boron uptake

Source of variation	df	SS	MS	VR
Blocks	2	0.308	0.154	0.952
Levels of Boron	2	4.006	2.003	20.305
Error (a)	4	0.395	0.090	8.610
Nitrogen application-Inoculation	1	0.304	0.304	1.882
Interaction	2	0.188	0.094	0.583
Error (b)	6	0.970	0.162	
Total	17	6.171		

6.16 Analysis of variances for (a) root boron concentration (µg/g), and (b) boron uptake (µg/root) at final harvest.

a. Root boron concentration

Source of variation	df	SS	MS	VR
Blocks	2	810.80	405.40	3.327
Levels of Boron	2	8003.60	4001.80	24.636
Error (a)	4	649.70	162.40	1.333
Nitrogen application-Inoculation	1	601.40	601.40	4.935
Interaction	2	117.50	58.70	0.482
Error (b)	6	731.10	121.80	
Total	17	10914.10		

b. Root boron uptake

Source of variation	df	SS	MS	VR
Blocks	2	3.048	1.524	12.381
Levels of Boron	2	65.020	32.510	70.782
Error (a)	4	1.837	0.459	3.732
Nitrogen application-Inoculation	1	22.835	22.835	185.523
Interaction	2	16.347	8.173	66.403
Error (b)	6	0.739	0.123	
Total	17	109.826		

6.17 Analysis of variance for (a) shoot nitrogen concentration (mg/g), and (b) nitrogen uptake/mg/shoot) at final harvest.

a. Shoot nitrogen concentration

Source of variation	df	SS	MS	VR
Blocks	2	326.87	163.43	1.792
Levels of Boron	2	329.18	164.59	3.229
Error (a)	4	203.89	50.97	0.559
Nitrogen application-Inoculation	1	4826.53	4826.53	52.927
Interaction	2	248.19	124.10	1.361
Error (b)	6	547.15	91.19	
Total	17	6481.81		

b. Shoot nitrogen uptake

Source of variation	df	SS	MS	VR
Blocks	2	7.596	3.798	2.372
Levels of Boron	2	3.789	1.894	3.440
Error (a)	4	2.203	0.551	0.344
Nitrogen application-Inoculation	1	282.031	282.031	176.116
Interaction	2	4.021	2.010	1.255
Error (b)	6	9.608	1.601	
Total	17	309.247		

6.18 Analysis of variance for (a) root nitrogen concentration (mg/g), and (b) nitrogen uptake (mg/root) at final harvest.

a. Root nitrogen concentration

Source of variation	df	SS	MS	VR
Blocks	2	100.11	50.05	3.682
Levels of Boron	2	29.89	14.94	0.583
Error (a)	4	102.47	25.62	1.884
Nitrogen application-Inoculation	1	463.09	463.09	34.062
Interaction	2	18.51	9.25	0.681
Error (b)	6	81.57	13.60	
Total	17	795.64		

b. Root nitrogen uptake

Source of variation	df	SS	MS	VR
Blocks	2	4.659	2.329	1.929
Levels of Boron	2	1.610	0.805	0.645
Error (a)	4	4.995	1.249	1.034
Nitrogen application-Inoculation	1	98.093	98.093	81.247
Interaction	2	0.824	0.412	0.341
Error (b)	6	7.244	1.207	
Total	17	117.426		

6.19 Analysis of variance for (a) shoot phosphorus concentration (mg/g), and (b) phosphorus uptake (mg/shoot) at final harvest.

a. Shoot phosphorus concentration

Source of variation	df	SS	MS	VR
Blocks	2	0.477	0.238	0.992
Levels of Boron	2	0.177	0.089	0.390
Error (a)	4	0.909	0.227	0.945
Nitrogen application-Inoculation	1	11.536	11.536	47.996
Interaction	2	0.140	0.070	0.292
Error (b)	6	1.442	0.240	
Total	17	14.681		

b. Shoot phosphorus uptake

Source of variation	df	SS	MS	VR
Blocks	2	0.0009	0.0005	0.485
Levels of Boron	2	0.0007	0.0004	0.318
Error (a)	4	0.0044	0.0011	1.186
Nitrogen application-Inoculation	1	0.0589	0.0589	63.527
Interaction	2	0.0017	0.0009	0.940
Error (b)	6	0.0056	0.0009	
Total	17	0.722		

6.20 Analysis of variance for (a) root phosphorus concentration (mg/g), and (b) phosphorus uptake (mg/root) at final harvest.

a. Root phosphorus concentration

Source of variation	df	SS	MS	VR
Blocks	2	8.576	4.288	1.173
Levels of Boron	2	7.176	3.588	0.936
Error (a)	4	15.328	3.832	1.048
Nitrogen application-Inoculation	1	7.437	7.437	2.034
Interaction	2	6.815	3.407	0.932
Error (b)	6	21.934	3.656	
Total	17	67.266		

b. Root phosphorus uptake

Source of variation	df	SS	MS	VR
Blocks	2	0.0030	0.0015	2.022
Levels of Boron	2	0.0156	0.0078	1.705
Error (a)	4	0.0183	0.0046	6.111
Nitrogen application-Inoculation	1	0.0003	0.0003	0.363
Interaction	2	0.0015	0.0007	0.985
Error (b)	6	0.0045	0.0008	
Total	17	0.0432		

7. Summary of multiple regression of plant dry weight, plant total nonstructural carbohydrates, and plant boron content with some chemical components of sainfoin.

Character	Chemical Component	Regression Coefficient	Increment in R^2
Plant Dry Weight	Root phosphorus concentration	-0.107	0.874
	(Constant)	486.916	Total = 0.874
Plant TNC	Root TNC	0.535	0.661
	Shoot TNC	-0.048	0.110
	(Constant)	6.983	Total = 0.771
Plant Boron Concentration	Shoot boron concentration	0.441	0.768
	Shoot phosphorus concentration	0.004	0.068
	(Constant)	-4.012	Total = 0.736