HORMONAL REGULATION OF TUBERIZATION OF CASSAVA (*MANIHOT ESCULENTA* CRANTZ).

by

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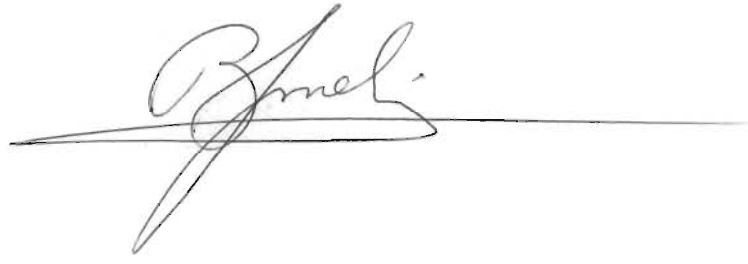
PREFACE

The experimental work described in this thesis was carried out in the Botany Department, University of Natal, Pietermaritzburg, from August 1981 to June 1984. The work was supervised by Professor J. Van Staden.

These studies have not been submitted in any form to another University and, except where acknowledged in the text, are the result of my own work.



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ABSTRACT

The relative distribution of dry matter over shoot and tuberous roots is an important factor in determining the yield of cassava. Under sub-tropical conditions the dry matter distribution pattern is not always efficient. During the summer the vegetative growth is generally excessive due to long days and high temperatures. Furthermore, it was found that a reduction of tuberous root mass occurs shortly after the winter period when the new canopy is formed. The role of endogenous hormones, in particular abscisic acid and cytokinins, in dry matter distribution and tuberization was studied. Furthermore, experiments were conducted on the effect of exogenous applied plant growth regulators on the growth of cassava.

Inhibitor activity present in plant extracts, was tentatively identified as *cis*-abscisic acid. A high level of inhibitor activity, co-eluting with abscisic acid, was found throughout the tuberous roots. The highest level of inhibitor activity was detected under conditions which caused a high rate of tuberous root growth, eg. with a low level of nitrogen fertilization. Gibberellic acid application to the leaves promoted shoot growth and led to a decrease in inhibitor activity of the tuberous roots. Shoot removal, however, stopped tuberous root growth without leading to changes in inhibitor activity. No clear correlation was found between inhibitor activity of tuberous roots and the rate of tuberous root growth of plants harvested from the field.

Trans-ribosylzeatin, dihydrozeatin and *trans*-zeatin were

tentatively identified as the major cytokinins in tuberous root extracts. Cytokinin activity was concentrated in the meristematic region of the xylem. The level of cytokinin activity in the roots was much higher than in primary roots of the same plants. Gibberellic acid application to the leaves and shoot removal resulted in a reduction of the cytokinin level of tuberous roots. The nitrogen application to the plants had no clear effect on the cytokinin levels. In field-grown plants the highest level of cytokinin activity was found shortly after tuber initiation.

Applications of Alar caused satisfactory reduction of shoot growth of young cassava plants grown in the greenhouse. The internodes were shortened, the leaf area generally reduced, while a relatively larger part of the dry matter was allocated to storage roots. The effect of Alar was further studied in field experiments. A growth analysis showed that a reduction of tuberous root mass occured shortly after the winter period, in September. Later in the second growing season, shoot and tuberous root mass increased at a relatively constant rate. Alar application (up to 4,5 grammes per litre) early in the second season failed to bring about major changes in dry matter distribution. Shoot removal at the end of the winter period was followed by excessive vegetative growth. Alar application reduced the internode length but the reduction of shoot growth was insufficient to cause a significant increase in yield. A nitrogen topdressing at the start of the second season increased the vegetative growth. However, Alar application, later in the season, did not inhibit shoot growth at any of the nitrogen levels applied. RSW 0411 which caused good reduction of shoot growth in the greenhouse, was not effective in the field.

CONTENT

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a native of South America. At present the plant is apparently no longer found in the wild state. There are indications that its centre of origin is North-eastern Brazil. Cassava, also known as manioc or tapioca, has a long history of cultivation. According to ROGERS (1963) the plant was grown 4000 years ago in Peru as a food crop. Over the centuries farmers throughout the tropics have come to appreciate cassava as a valuable subsistence crop, particularly because of its drought resistance and ability to grow on poor soils. Today Africa is the main cassava growing continent with an estimated area of 4,6 million hectare. This is 49 per cent of the world total (JENNINGS, 1970). The crop was probably introduced into West Africa in the 16th century by the Portuguese, who later took it to Reunion and Madagascar. From here it found its way to East and Southern Africa.

The present distribution of cassava is largely confined to the regions between latitudes 15°N and 15°S. PURSGLOVE (1974) considered the limits for cassava growing to be 25°N and 25°S. In Natal the crop is successfully grown up to a latitude of 30°S by subsistence farmers. Around many Zulu homesteads in northern Natal cassava plants are found today where it is known as Ndumbula. These plants are regarded as a security against famine. When harvested the tuberous roots are peeled, sun-dried and ground to a meal.

Because cassava was never an important export crop, it has for

a long time been neglected by research workers. This changed in the early seventies when the potential of cassava as a source for animal feed, industrial starch and ethanol was realized. As a consequence two international agricultural research centers, the International Institute for Tropical Agriculture (IITA) in Nigeria and the Centro Internacional de Agricultura Tropical (CIAT) in Colombia, launched extensive research programmes aimed at improving the yield of the crop throughout the tropics. In South Africa a research programme was started in 1977 with a view to establish a cassava based starch industry. At that stage little was known about the crop, its fertilizer needs, cultivation techniques and its specific requirements under sub-tropical conditions. Most research had up to that time taken place in the tropical regions. A major problem was the small basis of genetic material present in the country. A survey by DAPHNE (1980) revealed that there were most probably only three cultivars grown in South Africa, namely the MSAF 1, MSAF 2 and the Local White. Of these cultivars only MSAF 2 gave yields that had the potential to make a cassava based industry viable. Priority was therefore given to the introduction of new genetic material.

An additional problem was that South African conditions for cassava growing differ from those in the humid tropics. The rainy summer season is often too short to give a good yield and for this reason the crop is grown for a period of 18 months. This includes the dry winter period. During the winter the crop loses most of its leaves so that little growth takes place. At the start of the second summer season carbohydrates that were stored in the tubers appear to be used for the establishment of a new canopy. DAPHNE (1980)

conducted adaptation trials on 21 research sites throughout Natal and the Transvaal lowveld. An average yield of 17,6 tons per hectare per annum over the ten best sites was recorded. The average yield of 26,1 tons per hectare for cultivar MSAF 2 was significantly higher than that of the other cultivars. The highest yield recorded was 77 tons per hectare over two years on fertile clay soils with irrigation. However, the potential yield of cultivar MSAF 2 under commercial production was not expected to exceed 24 tons per hectare per annum. As up to the present no genetic improvement has taken place, there still remains a large potential for increasing yield through breeding .

A complicating factor in commercial cassava production is that once cassava tuberous roots are lifted they cannot be stored for more than a few days. In a cassava based industry a continuous supply of cassava tuberous roots to the factory is essential. This means that cassava should preferably be harvested year round including the winter period and the early part of the summer. It is therefore important to obtain information on tuber development of this crop throughout its growth cycle.

Together with the crop growth rate, dry matter distribution is an important yield determining factor in cassava. Large differences have been found amongst cultivars with respect to the total dry matter of the plant which is stored in the tuberous roots (WILLIAMS, 1972). Considerable variations also exist amongst the South African cultivars used. Cultivar MSAF 1 shows prolific shoot growth but forms small tuberous roots. Cultivar MSAF 2 produces a

relatively small shoot, but has a high tuberous root yield. Manipulation of the dry matter distribution in plants by means of cultural practices such as nitrogen fertilization (KROCHMAL and SAMUELS, 1967) and plant growth regulators (KEATING, 1981) have been suggested as ways of increasing yield.

Endogenous plant hormones play an important role in the tuberization process of plants (MELIS and VAN STADEN, 1984). Furthermore, it is believed that the dry matter allocation in plants is largely controlled by plant hormones (MORRIS, 1982; PATRICK, 1982). Regulation of the dry matter distribution by application of plant growth regulators which influence the endogenous hormonal balance, can be a means of reducing excessive shoot growth which can subsequently lead to a re-distribution of carbohydrates to the tubers. KEATING (1981) suggested the use of plant growth regulators to limit shoot growth at the start of the second season of cassava grown in sub-tropical areas. The beneficial effect of regulators which affect the dry matter distribution has been reported for many arable crops. Unfortunately the commercial use has often been unsuccessful because of problems with time of application, seasonal variability and varietal differences in response (THOMAS, 1979).

In the present study the role of endogenous plant hormones, in particular cytokinins and abscisic acid, in the tuberization process of cassava and the dry matter distribution of cassava was studied. The possibility of increasing yields by means of growth regulator application was investigated in greenhouse experiments and field trials.

CHAPTER 1.

LITERATURE REVIEW

GROWTH CYCLE OF CASSAVA

Cassava is generally propagated by means of hardwood stem cuttings. The first adventitious roots appear at the basal end of the cutting about five days after planting with the concurrent emergence of several buds (COURS, 1951). During initial growth the plants depend on the reserves present in the cuttings. Photosynthate begins contributing positively towards growth some three weeks after planting.

Tuberization is a phenomenon that occurs in a whole range of plant species. The morphology of the storage organs or tubers varies between species. They can be stem tubers (*Solanum tuberosum* L.), tuberous roots (*Ipomoea batatas* L. POIR and *Begonia evansiana* ANDR.) and even aerial tubers (*Dioscorea bulbifera* L.). Cassava is a perennial shrub which develops tuberous roots as swellings on adventitious roots at a short distance from the stem by a process of secondary thickening (PURSGLOVE, 1974).

The anatomical changes in cassava roots undergoing tuberization have been described by numerous workers (DOKU, 1969; INDIRA and KURIAN, 1977; LOPEZ and PEREIRA, 1977). Tuberization in tuberous roots of cassava is basically brought about by a transition of a number of primary roots into secondary roots. A secondary

cambium causes the xylem to extend, while a cork cambium is formed in the outer layer of the pericycle. The secondary xylem constitutes the storage tissue. Starch deposition takes place in parenchyma cells that are formed in a centripetal direction. An adult tuberous root can be divided into two parts (Figure 1.1). Firstly the peel, consisting of a cork periderm, the sclerenchyma, the cortical parenchyma and the phloem, and secondly the xylem which consists of parenchyma cells, vessels and fibers.

WILLIAMS (1974) reported that there are significant differences in storage cell size between tuberous roots of different cassava cultivars. Tuberous root diameter and yield were well correlated with the radial expansion of storage cells and it was therefore concluded that expansion of the storage cells and deposition of starch within them appears to be the centre of sink activity. It is, however, unclear whether the large cell size of certain high yielding cultivars is the causal factor of high yield or the result of availability of more carbohydrates in these cultivars.

Tuber initiation in cassava can take place relatively early and generally occurs within 90 days after planting (COURS,1951; DOKU, 1969). Starch deposition has been observed as early as 25 days after planting (INDIRA and KURIAN, 1977), but the rapid phase of tuber bulking generally starts not earlier than two to three months after planting. The time of tuberous root initiation does not appear to be greatly affected by environmental factors such as photoperiod and temperature (KEATING, EVENSON and FUKAI, 1982a). The variation in time of initiation has mainly been ascribed to

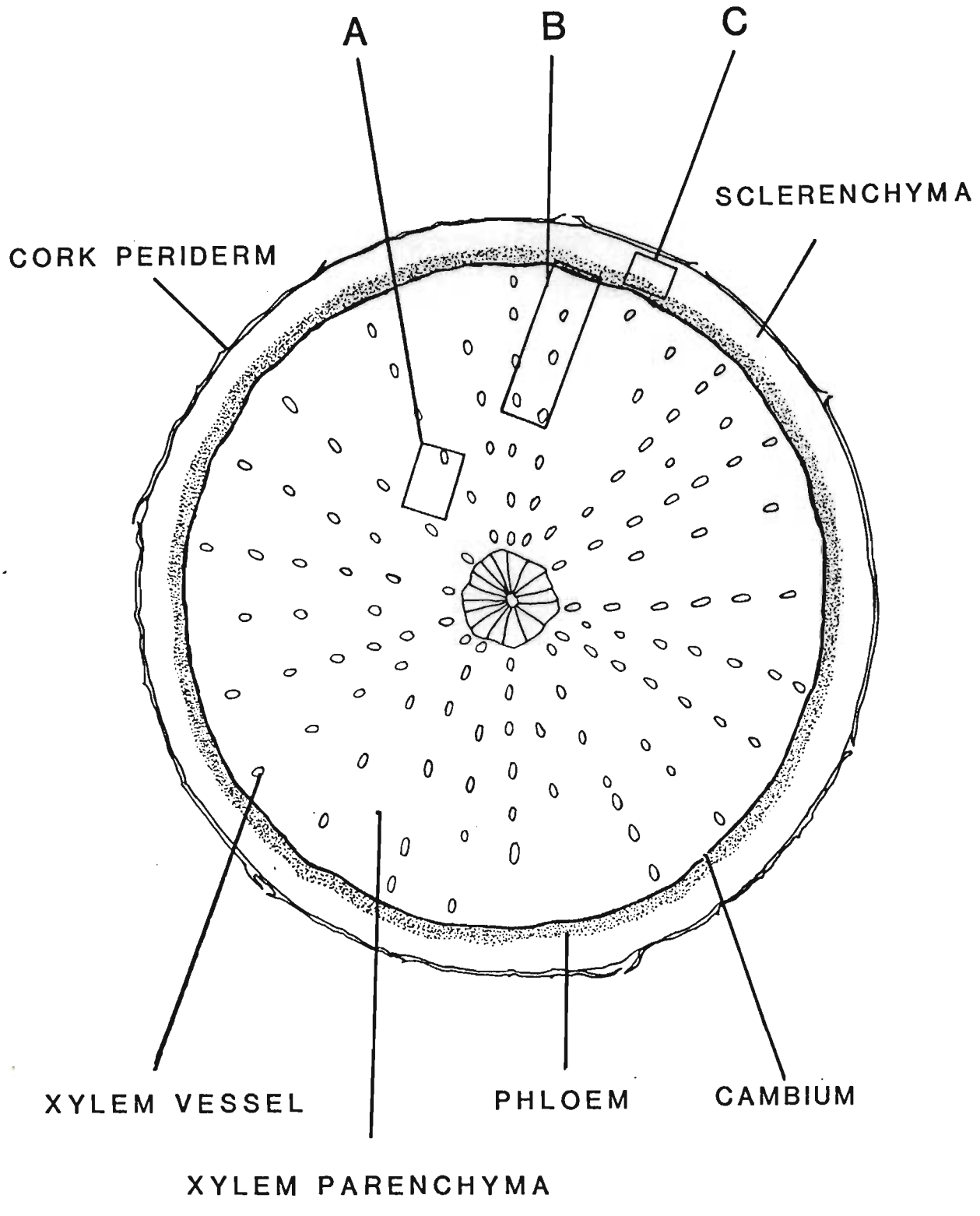

Figure 1.1 Transverse section of a tuberous root of cassava. A=inner xylem; B=outer xylem; C=phloem.

genotypic differences (WHOLEY and COCK, 1974). The final number of tuberous roots is fixed early in the growth cycle (CIAT, 1973; KEATING, EVENSON and FUKAI, 1982a). Tuberous root number is generally not correlated with yield (WILLIAMS, 1974), but can be a limiting factor if it is less than 9 per plant (COCK , FRANKLIN, SANDOVAL and JURI, 1979). The growth pattern of cassava in the humid tropics is different from that in sub-tropical areas. In the humid tropics there is little seasonal variation resulting in a relatively continuous growth, so that the tuberous roots can be harvested within a year (WHOLEY and COCK, 1974; COCK, 1976). In sub-tropical areas like South Africa and Queensland (Australia), however, the wet summer season is too short to give a sufficient high yield in one season. During the dry winter period the growth rate is low and the crop loses most of its canopy. After the winter period the shoots resume their growth, initially at the expense of the tuberous roots (COURS, 1951; DAPHNE, 1980; KEATING, EVENSON and FUKAI, 1982c). As a cassava plant ages it progressively loses more of its canopy and often has a leaf area which is lower than would be expected for optimum yields (COCK, 1973). However, some genotypes have the ability to retain their leaf area for a much longer period than others (ROSAS, COCK and SANDOVAL, 1976).

Two types of branches can be distinguished on a cassava plant. Firstly, lateral branches, which arise from axils at some distance from the apex and secondly, branches which appear when forking of the stem takes place. Forking occurs as the apical meristem changes to the reproductive state. Branches arise from the axils of distal leaves of one axis (HUNT, WHOLEY and COCK, 1977). The number of

nodes before the first forking and the number of side branches are determined mainly by the genotype. Environmental conditions can influence this number to some extent. For example, under low fertility conditions the node number to the first branching point is higher than under high fertility conditions (COURS, 1951). Some cultivars fail to form side branches. Others, like the three South African cultivars, initiate flowers, but the inflorescences abort at an early stage. The branching habit is considered to influence tuberization of cassava since it determines the number of active meristems on the shoot (DE BRUIJN, 1977; HUNT, WHOLEY and COCK, 1977). The "ideal cassava plant type" as described by the computer simulation model developed by COCK, FRANKLIN, SANDOVAL and JURI (1979), would branch at about 30 weeks after planting. TAN and COCK (1979) obtained yield increases of 75 per cent by manipulation of branching.

ENVIRONMENTAL FACTORS AND TUBERIZATION OF CASSAVA

A great deal of attention has been given to the effect of environmental factors on tuberization, particularly of the potato. Extensive reviews have been written on this topic (GREGORY, 1965; MOORBY, 1978). In general the reaction of different tuber crops to environmental factors follows a similar pattern. Since the time GARNER and ALLARD (1923) recognized a possible effect of daylength on tuberization, it has been establised that tuberization in most tuber crops is promoted by short days (LEOPOLD, 1964; GREGORY, 1965). The mechanism responsible for the photoperiodic effect remains unclear. The finding that the photoperiodic stimulus is graft transmissible

from an induced to a non-induced potato plant suggests a possible hormonal nature of the stimulus (GREGORY, 1956). This was supported by the observation that daylength control of tuberization follows a typical photoperiodic reaction which is often associated with a hormonal control, namely the reversal of a short day effect by a dark night interruption (HAMMES, 1973).

MOSER and HESS (1968) regarded the growth of a tuber as a competitive relationship between vegetative growth and tuberization, regulated by the photoperiod. HAMMES (1973) suggested that short days are not essential for tuber initiation but merely bring it forward. This emphasized a point which many workers up till then had neglected, namely that the role of the photoperiod is not so much a direct effect on the time of tuber initiation, but on the distribution of carbohydrates between shoot and storage organ. Short day conditions decrease the carbohydrate requirements of the shoots, making these compounds available for tuber storage. A large supply of carbohydrate at the tuberization site consequently promoted tuber initiation. HAMMES (1973) also showed that the short day requirement is not absolute. Even under long day conditions a potato plant will eventually form tubers when it reaches a certain physiological age.

First indications of a photoperiodic effect on tuberization in cassava came from a study by BOLHUIS (1966) who found that a 12 hour light period was optimal for tuber growth in cassava, while longer days reduced tuber formation. Unfortunately, these results were based on only a few plants per treatment. More detailed studies by

LOWE, MAHON and HUNT (1976) showed that cassava grown at an 8 hour light period gave significantly higher yields than plants grown at 14 and 20 hours of daylength. A similar response was found by KEATING, HERBERT and EVENSON (1981).

Daylength can have a negative effect on yield in sub-tropical cassava growing regions where long daylengths during the summer months could lead to excessive shoot growth of photosensitive cultivars. The daylength effect will express itself mainly in the first growing season, since physiologically older plants tend to be less sensitive to photoperiodic influences (HAMMES, 1973). It should be borne in mind that early tuber initiation does not automatically lead to a higher yield since the photosynthetic apparatus is reduced by the short day treatment. A small leaf area can be an important limiting factor for yield of cassava. LOWE, MAHON and HUNT (1976) showed that short days reduced the leaf area, node number and internode length of cassava, but increased the individual leaf size. Differences in response to photoperiod between different cultivars are well known for potato (BODLEANDER, 1963). KEATING, HERBERT and EVENSON (1981) found indications of genotypic differences of cassava in its response to photoperiod.

The effect of temperature on tuberization is two-fold. Firstly, temperature influences the total crop growth rate by affecting respiration and photosynthetic rates. Secondly, it will influence the distribution of newly formed carbohydrates between shoot and underground storage organs. There is little information available on the effect of temperature on photosynthesis of cassava.

MAHON, LOWE and HUNT (1976) measured net photosynthesis at 15°C, 25°C and 35°C and found the highest rate at 25°C. Low temperatures, in particular low night temperatures, bring forward the time of tuber initiation in potatoes and increase the percentage carbohydrate allocated to the tubers (SLATER, 1963; BURT, 1964). This was considered to be the result of a decreased demand for carbohydrates by the shoots resulting in an accumulation of carbohydrates in the stolons where they contribute to tuber growth. Another aspect of temperature is its effect on leaf production. HUNT (1974) studied rates of leaf production in controlled environments. At 29/24°C the number of leaves produced during a week varied between 2,20 and 3,87, and at 24/19°C between 1,03 and 3,05. Temperature differences probably account for the declining rates of leaf appearance with increasing altitude (COCK and ROSAS , 1975).

Under conditions of stress, whether caused by moisture or nutrient deficiency, the cassava plant generally reacts by allocating a greater portion of the dry matter produced to the storage tubers (COURS, 1951). KEATING, EVENSON and FUKAI (1982a) performed a growth analysis of cassava planted at monthly intervals in Queensland (Australia). It was found that the time of tuber initiation, and branching habit, were relatively unaffected by the different temperatures, solar radiation levels, and photoperiodic conditions, associated with the different planting dates. High temperatures and long photoperiods (KEATING, EVENSON and FUKAI, 1982c), and high nitrogen fertilization (COURS, 1951) were correlated with a reduced harvest index because of excessive shoot growth. Under these conditions inhibitor applications could be

practical to reduce excess foliage.

A further factor that influences the crop growth rate and the dry matter distribution is moisture availability. In the tropics moisture stress situations are uncommon, but in the sub-tropical areas moisture is often a limiting factor at certain times of the year. The interaction of the different environmental factors account for the seasonal variation in cassava growth. In South Africa cassava is planted from September to February. During the winter period the temperatures are low and the moisture availability limited, resulting in a near zero crop growth rate (DAPHNE, 1980).

DRY MATTER PRODUCTION AND DISTRIBUTION OF CASSAVA

Cassava is often regarded as a high potential carbohydrate producer. However yields are generally low (DE VRIES, FERWERDA and FLACH, 1967). A number of workers (WILLIAMS and GHAZALI, 1969; WILLIAMS, 1971;1972;1974; COCK, 1976) have attempted to identify the physiological and morphological constraints limiting yield by means of comparative studies of high- and low-yielding cultivars. WILLIAMS and GHAZALI (1969) found that yield could be correlated to certain structural attributes of the shoots such as leaf size, leaf area per shoot, and the orientation of the leaves. High yielding cultivars tend to have a more vertical leaf orientation , a low leaf area per stem and attenuated leaf lobes. Correlations between canopy characteristics and yield have been explained in terms of efficiency of sunlight utilization (LOOMIS and RAPOPORT, 1976). WILLIAMS and GHAZALI (1969) stressed that canopy characteristics,

although playing a role, could not account for the large differences in yields between different cultivars. No marked differences in stomatal functioning were found between high- and low-yielding cultivars (WILLIAMS, 1971)

The leaf area of cassava, the primary producer of dry matter in the plant, is for most of its life cycle lower than what is required for optimum yield. During the latter part of the growth period there is extensive leaf fall (SINHA and NAIR, 1971; COCK, 1973). This also occurs in the winter period in sub-tropical regions (KEATING, EVENSON and FUKAI, 1982a). Such leaf fall could account for the generally low average crop growth rate, and could be an important yield constraint. COCK, FRANKLIN, SANDOVAL and JURI (1979) in Colombia recorded an optimum crop growth rate at a leaf area index between 2 and 4. At a higher leaf area index the growth rate declined, presumably because of a shorter leaf lifespan caused by internal shading. Optimum leaf area index between 4,7 and 6,9 were found in Sierra Leone (ENYI, 1973). Little information is available on optimum leaf area index values in sub-tropical areas, but this value is most likely higher than in the tropics because of the higher level of daily radiation. KEATING, EVENSON and FUKAI (1982b) in Australia found high crop growth rates associated with a leaf area index of 7. Some cultivars maintain their leaf area for a much longer period than others (DOKU, 1965; SINHA and NAIR, 1971) and this desirable characteristic could be selected for in breeding programmes. Studying leaf fall in cassava, ROSAS, COCK and SANDOVAL (1976) found that the abscission rate of old leaves is increased by the presence of an active shoot apex. Leaf fall was, however, not

associated with the carbohydrate requirement of the tubers.

The crop growth rate of cassava fluctuates considerably during the growing season especially under sub-tropical conditions. Comparison of maximum crop growth rates from different studies must be made with caution since the growth rates are not differentiated over the same time periods. When the interval between two harvests is short the crop growth rate is higher than when this value is averaged over a longer period. The most accurate data available is from a study in Madagascar (COURS, 1951), where the plants where harvested at monthly intervals for four years. The maximum crop growth rate was 10,0-12,5 grammes per square meter per day in May-June while the value dropped to almost zero in October-November. A similar pattern was found in sub-tropical Queensland (KEATING, EVENSON and FUKAI, 1982b). Here the plants were harvested every 45 days and the maximum crop growth rates ranged from 23,8 to 2,4 grammes per square meter per day for different planting dates. The maxima were recorded in late summer to autumn. In late winter the crop growth rate had declined to near zero. COCK, FRANKLIN, SANDOVAL and JURI (1979) studied crop growth rates in Colombia . They adjusted the value for leaf fall and found a maximum crop growth rate of 15,7 grammes per square meter per day calculated over a 27-week period. The crop growth rates of cassava compare unfavourably with those of the other species reviewed by LOOMIS, WILLIAMS and HALL (1971). For example, the maximum crop growth rate for other C_3 plants such as potato and sugar beet ranged from 19 to 31 grammes per square meter per day. For C_4 plants maximum values of between 17 and 54 grammes per square meter per day were reported (LOOMIS, WILLIAMS and HALL,

1971). The reason for the low growth rate of cassava could be that to date little crop improvement has been done.

Whereas the growth rate of cassava is comparatively low, the annual production is high because of the long growth period. COCK (1976) studied 40 cassava cultivars under optimal growing conditions in Colombia. The best cultivar produced 40 tons per hectare in one year, indicating its potential. In Australia KEATING, EVENSON and FUKAI (1982b) found the highest yields in the vicinity of 30 tons per hectare per year.

For a study of chemical plant growth regulation of cassava it is important to understand the pattern of dry matter distribution within the plant. The variability of the harvest index between different cultivars (WILLIAMS and GHAZALI, 1969; COCK, 1976) indicates that there is a genetic factor regulating the dry matter distribution. Other important factors influencing the dry matter distribution are the environmental conditions such as daylength and temperature.

High yield is often associated with a high harvest index (WILLIAMS, 1972; COCK, 1976). WILLIAMS (1972) used growth analysis data of different cultivars to examine the controlling factor of assimilate distribution. According to his observation high yields are associated with the sink activity of the tubers and certain properties of the assimilation apparatus.

Several dry matter distribution models have been developed for

cassava in an attempt to explain the dry matter distribution pattern. In a model advocated by BOERBOOM (1978) it was assumed that the distribution of assimilates between shoot and tubers was constant once tuberization had commenced. In this simple linear model there were only two parameters; namely the initial plant mass at which tuberization starts and the efficiency of the plant to produce tubers. This model is useful in areas where the environmental conditions are fairly constant over a long period of time. In this model tuber mass and total plant mass are plotted against each other. The slope of the fitted lines is determined by environmental and genetic factors. COCK, FRANKLIN, SANDOVAL and JURI (1979) followed a different approach, whereby after a series of experiments they established a model in which it was assumed that the top growth had preference over tuber growth. This implies that the tubers only received the excess carbohydrates after the requirements of the shoots were satisfied. Weekly observations were carried out on plants grown under optimal conditions and the requirements of the shoot were described by a sub-model based on the hypothesis that the sink capacity of the tuberous roots is not a limiting factor, and can accept all surplus carbohydrates. This latter hypothesis was later confirmed by TAN and COCK (1979) in experiments where the branching pattern was manipulated. Both these models can be useful to explain crop responses. COCK's model in particular could be used to define optimal growing conditions and crop characteristics.

KEATING, EVENSON and FUKAI (1982c) found BOERBOOM's linear model less suitable for the sub-tropics, with its marked seasonal variation. This model requires constant environmental conditions

over a long period and it can therefore only be used over a short time span in the sub-tropics. Their growth data fitted COCK's distribution model better. Environmental factors determine the shoot growth and the surplus assimilate is translocated to the tubers. Low temperatures (MAHON, LOWE and HUNT, 1976; KEATING, EVENSON and FUKAI,1982a), short days (BOLHUIS, 1966; LOWE, MAHON and HUNT, 1976; KEATING, HERBERT and EVENSON, 1981), water stress (CONNOR, COCK and PARRA, 1981) and a low nitrogen availability (KROCHMAL and SAMUELS, 1967) all retard shoot development, which according to COCK's model leads to a greater allocation of carbohydrates to the tubers. CONNOR, COCK and PARRA (1981) studied the effect of water stress on total growth and dry matter distribution of cassava. They showed that the crop growth rate was low during a period of moisture stress. Following the release from stress there was a rapid growth of the canopy and at harvest the leaf area index of the stressed and the control plants were again almost equal. The interesting finding of their study was that after the stress period the biomass of the stressed treatment was higher than in the unstressed treatment. Since less dry matter was proportionally allocated to the canopy, the stressed plots had the highest final yield.

WILLIAMS and GHAZALI (1969) and COCK (1976) have shown that there is large genotypic variation in dry matter distribution. High yielding cultivars have a high harvest index. This positive correlation between harvest index and yield plus the finding that variation in assimilation between cultivars could not be accounted for by the properties of the assimilation apparatus alone led these

authors to suggest that there is a sink effect of the tubers on assimilate production in the leaves.

HORMONAL REGULATION OF TUBERIZATION

Two theories have dominated research into the mechanism of tuberization:

1. That the supply of carbohydrates to the site of tuberization is the causal factor of tuber initiation, and
2. That a specific tuberization hormone exists, which is formed under certain environmental conditions, and is responsible for tuber initiation.

According to the first explanation tuber initiation takes place when a surplus of carbohydrate is formed in the plant (ARTHUR, GUTHRIE and NEWALL, 1930; BORAH and MILTHORPE, 1962). This view finds support in tissue culture studies which found that tuber initiation of isolated potato stolons takes place only when the sucrose content of the medium exceeds 5 per cent (MES and MENGE, 1954; PALMER and SMITH, 1969). Recent studies by PETERSON and BARKER (1979), ASAHIRA and YAZAWA (1979) and FORSYTH and VAN STADEN (1984) re-emphasized the importance of the carbohydrate balance in tuberization. It was shown that tubers can be induced on *in vitro* grown potato stem nodes by merely adding certain carbohydrates to the medium.

First indications of the existence of a specific tuberization hormone came from grafting studies by GREGORY (1956), CHAPMAN (1958)

and KUMAR and WAREING (1973). These workers showed that an unknown tuberization factor can be transmitted by means of grafting from an induced to a non-induced potato plant. GREGORY (1956) believed that a tuber inducing substance was formed under specific conditions such as short days and low temperatures and was present throughout the plant. Others suggested that the factor was formed in the leaves and translocated to the tuberization site (ZIMMERMAN and HITCHCOCK, 1936; MADEC, 1963). In the past much emphasis has been placed on identifying an endogenous tuberization factor. Certain observations, however, do suggest that it is difficult to maintain that only one single factor is solely responsible for tuber initiation. Firstly, not all potato cultivars are sensitive to photoperiod. Secondly, it appears that the short day requirement of certain sensitive cultivars is not absolute. Even under long days these plants will ultimately form tubers (HAMMES, 1973). Recent studies have shown that the regulating process is apparently more complex than was initially anticipated. Although the existence of a specific tuberization hormone has not yet been completely ruled out, it appears that tuberization is regulated by the combined action of several plant hormones (DIMALLA, VAN STADEN and SMITH, 1977; WAREING, 1981; MELIS and VAN STADEN, 1984).

Tuberization cannot be separated from the dry matter distribution of the plant. The mechanism which regulates the allocation of newly formed carbohydrates to the different organs within the plant remains unclear, but there is evidence that hormones play a role. MORRIS (1982) argued that hormones may have a direct effect by regulating phloem loading and unloading of substrate at the

sink cells. Furthermore, he pointed out that endogenous hormones influence assimilate transport indirectly by controlling cell division, growth and differentiation in the sink. In this way these hormones determine the local demands. PATRICK and WAREING (1973) had earlier proposed that carbohydrates are preferentially translocated to those parts of the plant with the highest hormone levels. The theory of COCK, FRANKLIN, SANDOVAL and JURI (1979), which suggests that carbohydrates are translocated to the tubers of cassava only once the needs of the shoots are fulfilled, could imply that assimilate partitioning in cassava is primarily determined by hormones active in the shoots themselves.

Several workers have postulated that the balance between gibberellins and other hormones in the leaves is the controlling factor of the tuberization process (OKAZAWA and CHAPMAN, 1962; HAMMES and NEL, 1975). Application of gibberellic acid to the leaves enhances shoot growth and delays tuber growth (LIPPERT, RAPPAPORT and TIMM, 1958; LOVELL and BOOTH, 1967; MOSER and HESS, 1968; MENZEL, 1980). The application of gibberellin inhibitors on the other hand has the reverse effect. KUMAR and WAREING (1974) showed that gibberellin-inhibitor application can lead to tuberization under non-inducing conditions. Studies of endogenous hormone levels showed that short days result in reduced gibberellin levels in the leaves (OKAZAWA, 1960; LEZICA, 1970; RAILTON and WAREING, 1973). OKAZAWA (1967) and SMITH and RAPPAPORT (1969) found high levels of gibberellin-like activity in stolon tips of potatoes, decreasing shortly before tuber initiation. Developing tubers had low gibberellin levels.

Because of their ability to promote cell division (SKOOG and MILLER, 1957) and inhibit cell elongation (VANDERHOEF and KEY, 1968), cytokinins have often been considered to be of major importance in the tuberization process. LOOMIS and TORREY (1964) demonstrated that kinetin promotes secondary growth of radish. Later, similar results were obtained with excised turnip roots (PETERSON, 1973), begonia roots (ESASHI and LEOPOLD, 1968) and isolated potato stolons (PALMER and SMITH, 1970; MINGO-CASTEL, YOUNG and SMITH, 1976; FORSLINE and LANGILLE, 1976; MAUK and LANGILLE, 1978). Some workers have suggested that cytokinins are produced in the leaves or shoot tips under inducing conditions, translocated to the stolons or roots, where they induce tuberization (MADEC, 1963; COURDUROUX, 1966). So far there is no conclusive evidence of cytokinin production in the leaves. The role of cytokinins in the tuberization process is further discussed in Chapter 4.

Auxins are known to be involved in the process of cell division and the interaction of auxin and cytokinins leads to *in vitro* tuberization of radish roots (LOOMIS and TORREY, 1964; TORREY and LOOMIS, 1967), and potato stolons (HARMEY, CROWLEY and CLINCH, 1966). The possibility of auxins being formed in the shoots under inducing conditions and subsequently being translocated to the tuberization site as a tuber inducing hormone has so far received scant attention. Auxins can be produced in the leaves and are phloem translocated (HALL and BAKER, 1972). OBATA-SASAMOTO and SUZUKI (1979) reported an increased level of auxin activity preceeding tuber initiation in stolon tips of potato. WILLIAMS (1974) suggested that indole-

acetic acid might be transported from the shoot under short day conditions to the tuberous roots where it supresses lignification and promotes cell differentiation, leading to a change from lignified xylem cells into parenchymatous xylem cells for carbohydrate storage. Recently, MARSCHNER, SATTELMACHER and BANGERTH (1984) found a significant positive correlation between the growth rate and the indole-acetic acid levels of potato.

Abscisic acid, although an inhibitor of plant growth, is generally regarded as a tuberization promotor. Leaf application of this hormone has been found to increase tuber growth of potato (EL-ANTABLY, WAREING and HILLMAN, 1967; ABDULLAH and AHMAD 1980; MENZEL, 1980) and dahlia (BIRAN, LESHAM, GUR and HALVEY, 1974). However, abscisic acid failed to stimulate tuber formation of isolated potato stolons cultured *in vitro*. In addition, it inhibited kinetin induced tuber initiation (ESASHI and LEOPOLD, 1968; SMITH and RAPPAPORT, 1969; PALMER and SMITH, 1969; CLAVER, 1970). These results appear to indicate that the role of abscisic acid in tuberization is primarily the supression of shoot growth, thus making more carbohydrates available for storage in the tubers. Recently WAREING and JENNINGS (1979) have disputed this and argued that abscisic acid could play a direct role at the tuberization site. The role of abscisic acid in the tuberization process is discussed further in Chapter 3.

The role of ethylene in the tuberization process is unclear. There are reports of both promotion (BIRAN, GUR and HALEVY, 1972) and inhibition (DIMALLA and VAN STADEN, 1977; VAN STADEN and DIMALLA,

1977) of tuberization by ethrel application. Stimulation (GARCIA-TORREZ and GOMEZ-CAMPO, 1972) and inhibition (MINGO-CASTEL, NEGM and SMITH, 1974; MINGO-CASTEL, SMITH and KUMAMOTO, 1976) has also been found by applying ethrel to *in vitro* grown potato sprouts. BIRAN, GUR and HALEVY (1972) noticed a peak of endogenous ethylene two to three weeks after the start of a short day treatment. DIMALLA and VAN STADEN (1977) suggested that ethylene inhibits the action of cytokinins possibly by an accumulation of cytokinin glucosides. So far the role of ethylene in tuberization is the least understood of all the hormones.

Most research on tuberization has so far been done on potato. No information is available on the hormonal regulation of tuberous root formation in cassava.

CHEMICAL REGULATION OF ASSIMILATE PARTITIONING

The increasing knowledge of the role of endogenous plant hormones in the growth and development of plants has opened the way for the development of a large number of chemical substances, called plant growth regulators which can be used to manipulate plant growth. At present there is a wide variety of plant growth regulators available which control processes like flowering, fruit set and the ripening process. The most exiting development in this field is the discovery of chemicals which can alter the dry matter distribution in plants.

In a previous section the dry matter distribution of cassava

was discussed. The mechanism regulating assimilate partitioning between different plant organs has been widely studied but certain aspects remain unresolved. The involvement of plant hormones in assimilate distribution in plants is supported by the observation that actively growing organs of plants generally contain high levels of endogenous plant hormones (CRANE, 1964). WAREING and PATRICK (1973) and PATRICK and WAREING (1976) have attempted to explain the distribution of metabolites between different plant organs by means of the terms "sink strength" and "mobilizing ability". Sink strength is defined as" sink size x sink activity" and refers to the potential capacity of a sink to accumulate assimilates. It is considered to be controlled by hormones, while the "mobilizing ability" refers to the measure by which a certain organ can compete for the available assimilates. It has been argued that endogenous hormones indirectly regulate the sink strength by controlling cell growth and cell division processes in the sinks (MORRIS, 1982; PATRICK, 1982). In this manner the hormones create a demand for metabolites and thus influence assimilate distribution. The concept of "hormone induced translocation" was first proposed by MOTHES and ENGELBRECHT (1961) and later studied by other workers. GERSANI and KENDE (1982) recently showed that radio-active leucine and sucrose are preferentially translocated to a cytokinin treated area of the leaf. These and similar experiments do not reveal if the preferential movement is caused by a hormonal effect on the activity of the sink or by a direct influence of the hormones on the transport of assimilates. To distinguish between the two possibilities hormones have been applied to decapitated seedlings (DAVIES and WAREING, 1965). The assimilate movement was stimulated within a few

hours of treatment. This suggests that hormones have a direct influence on assimilate transport processes since in this experiment the potential sink was removed. This furthermore supports the concept of a "mobilizing ability" of the sink as described by WAREING and PATRICK (1973). The way in which endogenous hormones influence the transport and direction of movement of assimilates is unclear. There are indications that hormones could control phloem loading at the source, transport, and phloem unloading at the sink directly (PATRICK, 1982). Recently, GIFFORD and EVANS (1981) suggested that the ability of the sink to unload and utilize assimilates from the phloem is the most important factor regulating phloem transport. One way that hormones control transport is by influencing enzymes that are involved in carbohydrate synthesis and metabolism (MINGO-CASTEL, YOUNG and SMITH, 1976; OBATA-SASAMOTO and SUZUKI, 1979; MORRIS, 1982).

Supply and demand of carbohydrates are considered to interact. For example, reducing the sink demand by removal of parts of the sink decreases the net assimilation rate of the leaves (NÖSBERGER and HUMPHRIES, 1965). Alternatively, removal of the source leads to an increase in assimilation rates of the remaining leaves (SWEET and WAREING, 1966; WAREING and PATRICK, 1973). This indicates that the assimilation rate is generally below the potential and is under the influence of the sink. An active sink thus stimulates the source activity. The mechanism responsible for photosynthesis suppression by sink limitation is unclear, but TREHARNE (1982) suggested that chloroplast function could be directly inhibited by accumulated metabolites. It has further been

suggested that endogenous hormones could be the signals which express the needs of the sink to the source (WAREING AND PATRICK, 1973). GERSANI, LIPS and SACHS (1980) substituted plant organs with cytokinins and auxin and studied labelled sucrose transport. It was concluded that assimilate transport is controlled by hormones produced in the sink. Following tuber initiation the shoot may undergo a dramatic change. For example, in potato, shoot growth is reduced and even becomes negative as dry matter is redistributed to the growing tubers (MOORBY and MILTHORPE, 1975). It has been suggested that the tubers release a compound which inhibits shoot growth and mobilizes material from the other parts of the plant (LOOMIS and RAPOPORT, 1976). Tuber formation in cassava has only a limited influence on the growth rate of the shoot (COCK, FRANKLIN, SANDOVAL and JURI, 1979). The canopy is maintained during the bulking period and assimilates are partitioned to shoots as well as tubers (MAHON, LOWE and HUNT, 1976).

Despite the fact that possibly all known plant hormones are to some degree involved in assimilate partitioning there are only a few regulators which are used commercially to manipulate the dry matter distribution of plants. Of these chemicals, particularly the so-called growth retardants have a large potential for application to arable crops such as tuber and root crops. THOMAS (1979) described growth retardants as chemicals which primarily inhibit the sub-apical cell divisions and elongation, hence reducing the growth of the aerial parts of the plants. These chemicals inhibit in particular the endogenous gibberellin synthesis in plants. This leads to lower gibberellin activity in the shoot,

which reduces shoot growth. Several hypotheses have been put forward concerning the mode of action of these regulators. It has been suggested that the regulators could inhibit the precursors of gibberellins, while some evidence points to a possible conversion and de-activation of gibberellins by certain regulators (DICKS, 1979). The most common morphological response to growth retardants is the shortening of the internodes and the reduction of leaf area. The shortening of internodes in plants treated with the inhibitor AMO 1618 (chemical names of regulators are given in Table 2.3) was studied by SACHS, LANG, BRETZ and ROACH (1960) who reported that the shortening of internodes was mainly due to fewer cell divisions.

So far the practical use of growth retardants in arable crops are few. The major use of retardants is in horticulture, especially on fruit trees (BRUINSMA, 1982). Reasons for their limited use on arable crops include economical considerations, unreliability in response due to seasonal variation, and problems with methods of application (THOMAS, 1979). The time of application is critical and there is a lack of detailed studies on this aspect. A reduction of the vegetative growth of a crop does not automatically lead to an increase in yield, since a sub-optimal leaf area can result in a reduced total crop growth rate. Although the harvest index may be improved in such a case, a higher yield is not necessarily achieved. Growth retardant application will only have a positive effect on yield when a certain leaf area is reached. Likewise, when the sink activity or transport capacity is the limiting factor, growth retardant application does not automatically produce an increase in yield (LOOMIS and RAPOPORT, 1976). In such a situation

carbohydrates might accumulate in the leaves and depress photosynthesis. In a situation where the sink- or transport capacity is not limiting the leaf area reduction by growth retardant application could have the same response as removing part of the source; namely an increase of the net assimilation rate. In fact, GIFFORD and MOORBY (1967) found an increased net assimilation rate when potato plants were treated with Cycocel.

Root and tuber crops are considered to have a good potential for the use of growth retardants (WAREING, 1982). The argument for using these compounds is that a chemically induced reduction of shoot growth of tuber crops could lead to a larger portion of carbohydrates being allocated to the tubers. Several growth regulators have been used on root and tuber crops. DYSON (1965) reported that Cycocel slowed stem and stolon extension and leaf expansion of potato while more of the photosynthate was used in tuber formation. Gibberellic acid application had exactly the opposite effect. An increase in tuber yield under field conditions was only expected when the leaf area at the time of application exceeded a factor of 3. Early application of retardants brings forward the time of tuber initiation of potato (GIFFORD and MOORBY, 1967) and increases the tuber number (HUMPHRIES and DYSON, 1967). The effect of retardants on the shoot growth is predictable, but the effect on the final yield is variable. HUMPHRIES and DYSON (1967) found no differences in yield between Cycocel treated plants and the control plants, although the shoot growth was significally lower in the treated plots. BODLAENDER and ALGRA (1966) found a high potato yield with Alar application a week before total soil coverage. Later

application seemed to accelerate leaf senesence. A high nitrogen supply in combination with Alar treatment brought about a long growing season. The total dry matter production was high, but the dry matter distribution was more favourable than that which normally occurs with high nitrogen application. Although the total leaf area was decreased by growth retardant application, the total dry mass tended to change little because of an increased dry mass per unit area of leaves (BODLEANDER and ALGRA, 1966; HUMPHRIES and DYSON, 1967). DYSON (1972) studied the effect of Cycocel and Alar on the yield of two carrot cultivars. These regulators had similar effects on shoot growth. The top growth was reduced, petioles were shorter and leaves were smaller and greener. However, only in one of the cultivars did the reduction of the shoot growth lead to an increase in tuber yield. Two conclusions could possibly be drawn from this study. Firstly, that there are varietal differences in response to regulators and secondly that the sink capacity could be a limiting factor in certain cases. In cassava the shoot/tuberous root ratio is determined at an early stage and tends to remain constant provided the environmental conditions do not vary drastically (BOERBOOM, 1978). THOMAS (1979) made the assumption that there is a build-in mechanism for assimilate distribution, controlled by endogenous hormones. To quantify the effect of externally applied regulators he plotted the logarithm of mean dry shoot mass against the logarithm of mean dry tuber mass. Regulator application changed the position of the intercept and this provided a means of quantifying the regulator effect irrespective of plant age. This method could be useful in the screening of growth regulators which modify the dry matter distribution of plants.

One of the reasons for the limited success of growth retardant application in field-grown potatoes could be the fact that potatoes have a terminal shoot growth. Once the phase of rapid tuber bulking starts, the shoot automatically ceases to grow and can even show a negative growth rate (MOORBY and MILTHORPE, 1975). This apparent control mechanism is absent in many perennial tuber crops such as cassava. Here the sink activity of the tuberous roots seems to have little effect on the shoot, whose growth rate is mainly dictated by environmental factors (COCK, FRANKLIN, SANDOVAL and JURI,1979). This could imply that the use of growth retardants in cassava may have more success than in potato. BRUINSMA (1982) postulated the greatest potential for growth inhibitors in perennial tropical crops.

There are few studies on the effect of growth retardants on cassava. DAS GUPTA (1976) reported that application of 1500 milligrammes per litre Cycocel gave the highest yield within the range of 0 to 2000 milligrammes per litre. No information is available on the effect of plant growth inhibitors on cassava grown under sub-tropical conditions. The above discussion shows that the effect of growth regulators on the final yield of arable crops is far from understood. In many studies with tuber crops, growth regulator application failed to give the desired increase in yield. There are several areas in which further research is needed. Particularly, factors which control dry matter distribution within plants require further investigation. A reduction of shoot growth does not automatically lead to a redistribution of dry matter to the tubers

(DYSON, 1972). An important aspect which requires attention is the time of growth regulator application, particularly with respect to the leaf area index. Another aspect which warrants research is the difference in response between varieties and the interaction of growth regulator and other factors which influence distribution of dry matter.

The objective of the present study was to obtain information on the hormonal regulation of tuberization and dry matter distribution in cassava and to obtain ways of manipulating dry matter distribution, especially during the second season of the crop.

CHAPTER 2

MATERIALS AND METHODS

PLANT MATERIAL

Material of cassava (*Manihot esculenta* Crantz) was obtained from the Anglo American Corporation Cassava Research Centre in Umtunzini, South Africa. Cultivar MSAF 2 was the only South African production cultivar and the only one of which sufficient planting material was available for field trials. However, this cultivar grew poorly in the greenhouse. The leaves were deformed, while the rate of shoot growth was low. Due to this cultivar MSAF 1 was used mainly for greenhouse trials.

For the pot experiments mature stems were cut into 40 grammes planting stakes, which were planted in a vertical position, to half their height. One shoot was allowed to grow and the others were removed manually. Before planting the stakes were dipped for one minute in a fungicide (2,2 grammes per litre Dithane M45) and insecticide (2,0 grammes per litre Malathion) mixture. During the course of the greenhouse experiments the plants were regularly sprayed with a variety of insecticides.

GREENHOUSE GROWTH MEDIUM

For the greenhouse experiments drained pots were used containing 6 or 12 kilogrammes of soil. Unless otherwise indicated

the standard soil mix consisted of a 1: 1 sand: topsoil (clay loam, 15-30 per cent clay) mixture. To 1 cubic meter of this mixture was added:

150 grammes KNO_3

150 grammes K_2SO_4

1,25 kilogrammes Superphosphate (10,5 per cent)

6 kilogrammes $CaCO_3$

Every two weeks Hoagland's (BONNER and GALSTON, 1967) nutrient solution was applied (Table 2.1). The application rates are given in the experimental procedure of each trial.

GROWTH FACILITIES

The experiments were conducted in a 6,6 x 10,0 meter glass fibre tunnel with no artificial light and a wet wall cooling system. The fibreglass sheeting transmitted about 50 per cent of the incoming solar radiation (MILLER, 1983). The maximum temperature was set at 33°C. No heating was installed and the night temperature was slightly above the outside temperature.

FIELD EXPERIMENTS

The field experiments were conducted at the farm Fernwood, 17 kilometers north of Mtubatuba in northern Natal at latitude 28°2'S and longitude 32°2'E, on a sandy red soil with a clay rich B21 horizon (Rhodustalf). The average monthly rainfall and the mean monthly temperatures are given in Figure 2.1. The climate in the area is Cfa in the Köppen classification(SCHULZE and McGEE, 1978).

Table 2.1. Hoagland's (BONNER and GALSTON, 1967) nutrient solution used i the greenhouse experiments.

Stock solution	Chemical	Stock solution ($g\ dm^{-3}$)	cm^3 stock solution per dm^3 final solutio
1	KH_2PO_4	68	2
2	KNO_3	101	5
3	$Ca(NO_3)_2$	236	5
4	$MgSO_4$	123	4
5	NaFeEDTA	5,6	1
TRACE ELEMENTS			1
	H_3BO_3	2,81	
	$MnCl_2.4H_2O$	1,81	
	$ZnSO_4.7H_2O$	0,22	
	$CuSO_4.5H_2O$	0,08	
	$H_2MoO_4.H_2O$	0,02	

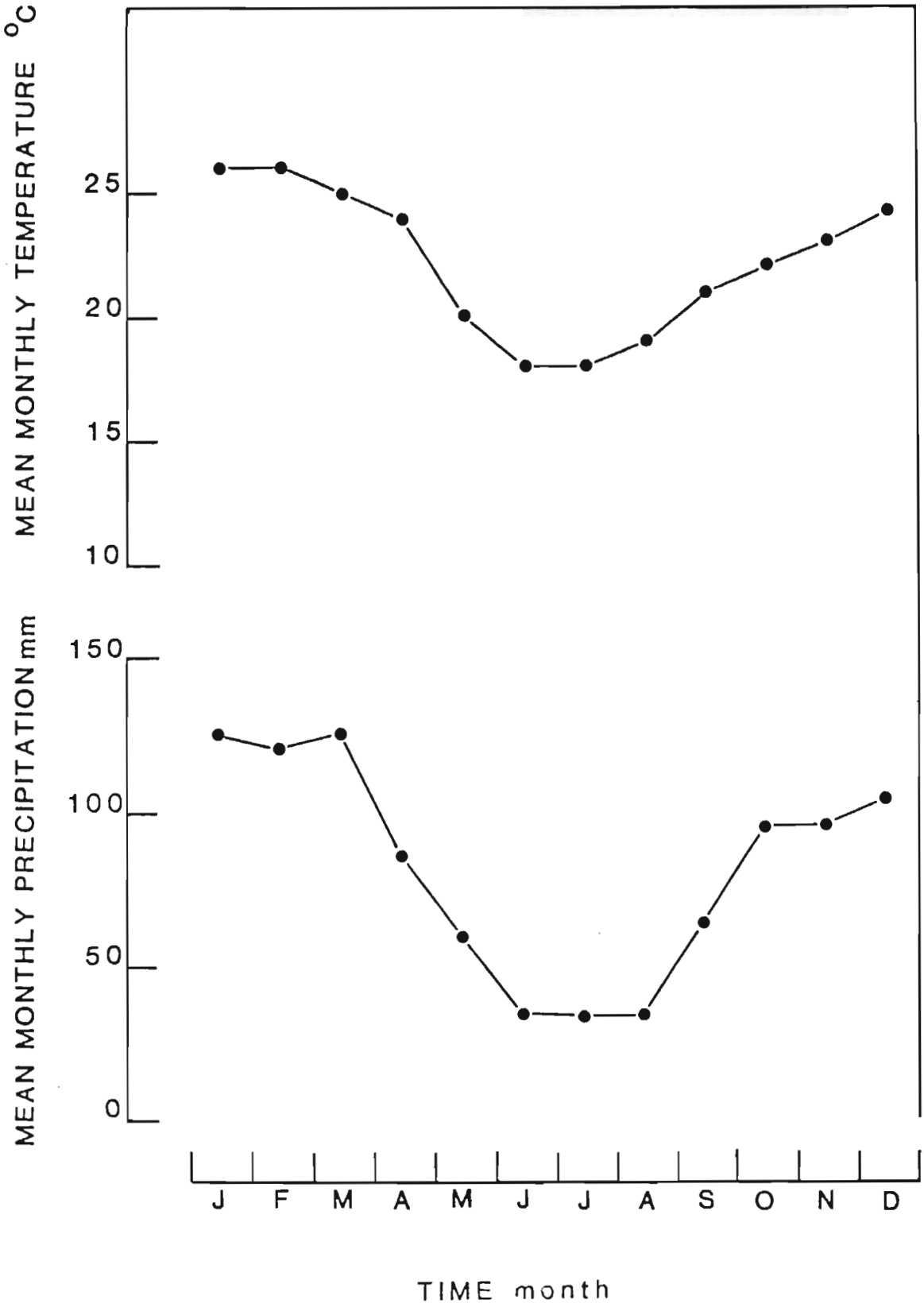

Figure 2.1. Mean monthly precipitation and temperatures at Fernwood (SCHULZE, 1982).

Planting stakes were cut from mature stems of cultivar MSAF 2. The stakes were planted vertically in a furrow and for two thirds covered with soil. A pre-emergence herbicide mixture was used (Dowpex +Sutan). Subsequent weed control was done by hand. At planting 500 kilogrammes per hectare 2: 3: 4 (40 per cent) NPK + Zn and 500 kilogrammes per hectare single super phosphate (10,5 per cent) were applied in the furrow, while 175 kilogrammes per hectare limestone ammonium nitrate (28 per cent) was applied at the start of the second season as a topdressing.

The planting design used was 0,9 x 0,9 meter, corresponding with a plant density of 12 346 plants per hectare. Only in the 1981-1983 growth analysis, a 1,0 x 1,0 meter design was used, which corresponded with 10 000 plants per hectare.

HARVESTING

The relevant parts of each individual greenhouse-grown plant were weighed. From each field plot 12 plants were harvested and weighed together. After determining the fresh mass sub-samples were dried in an oven at 70°C for the determination of dry mass. Leaf areas were determined using a leaf area meter (Lamba Instrument Corporation). From each field plot the leaves of four plants were removed and a sub-sample of 60 grammes was taken for the leaf area measurement. The number of leaves and the length of six shoots of each plant were recorded.

Chlorophyll content was determined by cutting three discs of 1 centimeter diameter from the middle of the third leaf from the top. The sections were extracted in 3 millilitres 100 per cent methanol for 24 hours and after dilution to 9 millilitres the absorbance was measured at 435 nm.

STARCH ANALYSIS

From each plot ten tuberous roots were sampled and a section of approximately 3 centimeters wide taken from each of these roots. These samples were grated and dried at 110°C. Twenty grammes of dry material was weighed and blended with 200 millilitres distilled water for three minutes. The mixture was transferred to a beaker and diluted to 400 millilitres. The pH was adjusted to 6,5 and 2 millilitres of a enzyme solution (Termemyl; MADSEN, NORMAN and SLOTT, 1973) and two drops antifoam (Polypropylene Glycol 2025, 50 per cent in ethanol) added and the mixture then boiled for one hour. The solution was filtered through a Whatman No 1 filter paper and the residue transferred to a pre-weighed aluminium dish. After overnight drying at 110°C the residue was weighed. The percentage fibre was calculated as follows:

$$\% \text{ fibre} = \text{residue/sample mass} \times 100\ \%$$

Subsequently a 20 grammes sample was blended in 200 millilitres water for three minutes. The mixture was filtered through Whatman No 1 filter paper. Twenty millilitres filtrate was transferred into a pre-weighed beaker and taken to complete dryness. The percentage soluble material was calculated as follows:

$$\% \text{ soluble material} = \text{residue mass/sample mass} \times 10 \times 100\ \%$$

The percentage starch on a dry mass basis is:

$$\% \text{ starch} = 100 - \% \text{ fibres} - \% \text{ soluble material}$$

GROWTH REGULATORS

Details of the five plant growth regulators used in the experiments are given in Table 2.2. Cycocel(Cyanamid South Africa), Alar 85 (Uniroyal South Africa) and Pix (BSAF South Africa) were officially registered, while RSW 0411 (Bayer South Africa) and MB 25105 (Maybaker) were at that stage still unregistered. The regulators were applied with a knapsack sprayer fitted with a fan type nozzle. Tween 20 (Polyoxyethylene sorbitan monolaureate; 1 millilitre per litre) was added and the leaves were sprayed until full coverage of the upperside of the leaves was obtained.

CYTOKININ ANALYSIS

Fresh plant material was collected, washed and stored at -20°C until analyzed.

Extraction

All material was homogenized and extracted with 80 per cent ethanol for 24 hours at 4°C. After filtration the extracts were concentrated under vacuum at 40°C. The residue was taken up in 100 millilitres 80 per cent ethanol and the pH adjusted to 2,5. These extracts were purified on a Dowex 50W-X8 cation exchange resin (BDH Chemicals Ltd, 20-50 mesh, H+ form). The column was washed with 200

Table 2.2 Common names and chemical names of growth regulators referred to in the text

Common name	chemical name
Alar, B9, B995 Alar 85	succinic acid 2,2- dimethyl hydrazide
Cycocel, CCC	(2-chloro ethyl) trimethyl ammonium chloride
RSW 0411	1-cyclohexyl-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3ol
Ethrel	2-chloroethyl phosphonic acid
Maleic hydrazide	maleic hydrazide
Pix	1,1-dimethyl-piperidinium chloride
Amo 1618	(4-hydroxy-5 isopropyl-2 methyl phenyl) trimethyl ammonium chloride 1-piperidine carboxylate
Phosphon D	tributyl-(2,4-dichlorobenzyl) phosphonium ion
MB 25105	n-propyl 3-t-butylphenoxy acetate

millilitres distilled water and 150 millilitres 80 per cent ethanol. The cytokinins were eluted with 100 millilitres of 5N ammonium hydroxide. The eluate was taken to dryness under vacuum at 40°C.

Paper chromatography

The extracts were taken up in a small volume of 80 per cent ethanol and loaded onto Whatman No 1 chromatography paper. The chromatograms were run with with *iso*-propanol: 25 per cent ammonium hydroxide: water (10: 1: 1 v/v) in a descending manner for 11 hours. After drying for 48 hours at 25°C the chromatograms were divided into ten equal R_f strips.

Column chromatography

Biologically active fractions were further separated by means of gel chromatography. For this purpose a 90 x 2,5 centimeter column packed with Sephadex LH-20, pre-swollen in 10 per cent methanol was used. The column was eluted with 10 per cent methanol at a flow rate of 15 millilitres per hour. Forty millilitre fractions were collected, transferred into 50 millilitre erlemeyer flasks and dried under a stream of air at 40°C. This technique gives good separation of zeatin, dihydrozeatin, ribosylzeatin, *iso*-pentenyladenine and *iso*-pentenyladenosine (HUTTON and VAN STADEN, 1981).

High pressure liquid chromatography

Although Sephadex LH-20 chromatography gives a high degree of

purification of the cytokinins, the resolution is not such that it enables to distinguish between the different isomers of cytokinins. In order to obtain such separation and tentative identification of the cytokinins, the fractions were further analyzed using high performance liquid chromatography (HPLC). The basic principle of the HPLC technique does not differ from normal column chromatography, but it is much faster and allows for the use of solvent gradients.

The first HPLC separation systems developed for cytokinins made use of columns with pellicular cation exchange resin (POOL and POWELL, 1974; CARNES, BRENNER and ANDERSON, 1975; CHALLICE, 1975). POOL and POWELL (1974) used phosphate buffers of varying pH as eluting solvents, but obtained unacceptable long retention times. CHALLICE (1975) encountered similar problems with aqueous buffer eluents, but addition of methanol enabled the cytokinins to be separated and eluted within a reasonable time. The separation achieved remained poor. CARNES, BRENNER and ANDERSON (1975) found that ion-exchange resins lacked the capacity to handle crude plant extracts. More successful were systems, whereby reverse phase columns were used. The packing of these consisted of a hydrocarbon chemically bonded to spherical or porous silica particles. CARNES, BRENNER and ANDERSON (1975) used these column to purify and separate cytokinins in tomato root exudate. This system has been used by many workers since (MORRIS, ZAERR and CHAPMAN, 1976; CHEN, 1983; MATSUO, YONEDA and ITOO, 1983).

A trend for future cytokinin analysis was perhaps set by KANNANGARA, DURLEY and SYMPSON (1978), who used a reverse phase

octadecyl silica column (Bondapak C_{18} particle size 37-70 µm diameter) for the purification of partially purified leaf extracts using methanol-water solvent combinations. The fractions with retention times corresponding with the cytokinin standards were further purified and separated on a micro-octadecyl silica column (µBondapak particle size 5u diameter) using acetonitrile-water solvent combinations. ARTECA, POOVAIAH and SMITH (1980) used a similar system, but injected the extract twice into the same column.

HORGAN and KRAMER (1979) succeeded in obtaining good separation on reverse phase, as well as on adsorption and normal phase columns. Their work confirmed that reversed-phase HPLC is probably the most powerful single chromatographic technique, because of its suitability for operation under gradient elutions, high sample capacity, and ability to separate a wide range of compounds. The use of HPLC for the purification of cytokinins is, however, limited and pre-purification by other chromatography techniques is therefore unavoidable.

In the present study a Varian 5000 HPLC unit fitted with a 25 centimeter ODS Hypersil column (particle size 5 µ, C_{18} bonded) was used. The fractions were dissolved in 0,2 millilitres redistilled methanol and injected into the liquid chromatograph. Various combinations of acetonitrile and water were used as solvents. HPLC grade acetonitrile and methanol (washing solvent) were used. UV-absorbing peaks were detected with a Varian variable wavelength monitor at 265 µm, which was fitted with a 8 µl flow-through cell. If required for further bio-assaying, fractions were collected every minute and these dried in a stream of air.

Soybean callus bioassay

The biological activity of the extract separated by paper chromatography, gel chromatography, or by HPLC was determined using the soybean callus bioassay (MILLER, 1965). The callus was obtained from the cotyledons of soybean. Four stock solutions were prepared and the nutrient medium was made up as outlined in Table 2.3. Thirty millilitres medium was added to 50 millilitre flasks , in which the R_f strips of the paper chromatograms or the 40 millilitres Sephadex LH-20 fractions were collected. The flasks were autoclaved at a pressure of 105 kPa for 20 minutes and placed in a sterile transfer cabinet, where they were left for six hours under ultra-violet light. Three pieces of callus of approximately 20 milligrammes were placed on the agar medium. The cultivars were incubated at a constant temperature (27°C) under continuous light of low intensity (cool fluorescent tubes). After 28 days the callus in each flask was weighed. Each assay was repeated and the average values of callus growth plotted on a histogram. All assays were done in duplicate and the confidence limits at the 5 per cent level was calculated. The regions significantly different from the controls were shaded on the histograms. Kinetin standards were included with each bioassay and the values were fitted on a logarithmic response curve. The values of all significant peaks of the extracts were interpolated on the response curve and expressed in kinetin-equivalents.

Table 2.3 Basal medium for soybean callus bioassay (MILLER, 1965)

Stock solution	Chemical	g dm^{-3} stock solution	cm^3 stock solution per dm^{-3} medium
Stock 1	KH_2PO_4	3,0	100
	KNO_3	10,0	
	NH_4NO_3	10,0	
	$Ca(NO_3)_2.2H_2O$	5,0	
	$MgSO_4.7H_2O$	0,72	
	KCl	0,65	
	$MnSO_4.4H_2O$	0,14	
Stock 2	NaFeEDTA	1,32	10
	$ZnSO_4.7H_2O$	0,38	
	H_3BO_3	0,16	
	KI	0,08	
	$Cu(NO_3)_2.3H_2O$	0,035	
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0,01	
Stock 3	Myo-inositol	10,0	10
	Nicotinic acid	0,2	
	Pyridoxine HCl	0,08	
	Thiamine HCl	0,08	
Stock 4	NAA	0,02	10
	Sucrose		30 g dm^{-3}
	Agar		10 g dm^{-3}
	pH adjusted to 5,8 with NaOH		

INHIBITOR ANALYSIS

Extraction

Fresh plant material was homogenized with 80 per cent methanol and extracted at 4°C for 24 hours after adjustingthe pH to 7. After filtration the extract was reduced to an aqueous phase under vacuum at 35°C. The volume was adjusted to 100 millilitres and the pH re-adjusted to 7. The aqueous residue was stirred for one hour at 5°C with 1 gramme insoluble polyvinyl pyrrolidone (Polyclar AT). After filtration the filtrate was adjusted to pH 3,5 and extracted three times with 100 millilitres ethyl acetate. The ethyl acetate fractions were bulked and dried under vacuum at 35°C. To hydrolyse conjugates of inhibitors, the residue was adjusted to pH 11,0 and maintained for one hour in a shaking bath at 60°C. After re-adjustment of the pH to 3,5, the solutions were extracted three times with 100 millilitres ethyl acetate. The ethyl acetate fractions were bulked and dried under vacuum at 35°C.

Thin layer chromatography

The residues of the extracts were redissolved in a small volume of methanol and loaded onto 20 x 20 centimeters 200 µm thin layer chromatography silica gel plates (Merck GF 254). The chromatograms were developed in toluene: ethyl acetate: acetic acid (40: 15: 2 v/v) over a distance of 15 centimeters. After drying the plates the *cis*- and *trans*-abscisic acid marker spots were located under a UV-lamp.

Wheat coleoptile bioassay

The plates were divided into ten equal R_f strips and the silica gel was scraped from each strip and eluted with methanol. The solutions were centrifuged and the supernatant of each R_f strip was divided over three compartments of a repli-dish and dried. The wheat coleoptile bioassay (EEUWENS and SCHWABE, 1975) was used to determine the inhibitor activity in the extracts. Each repli-dish compartment was filled with a strip of Whatman No 1 chromatography paper and 1 millilitre of a phosphate buffer solution (pH 7,4; 39 millilitres 0,07 M KH_2PO_4 + 161 millilitres 0,07 M Na_2HPO_4). Seed of *Triticum vulgare* L. cultivar INIA were incubated for 20 hours at 25°C on moist filter paper in the dark. Approximately two thirds of the endosperm was removed. Six embryos were placed on the strip of chromatography paper in a repli-dish compartment. The coleoptiles were incubated in the dark at 25°C for 48 hours after which the length of each coleoptile was measured and the average length taken for each compartment. The percentage inhibition as compared with the controls were plotted in a histogram and the regions significantly different from the controls determined. The values of the significant peaks were interpolated on the response curve of the abscisic acid standards expressed in abscisic acid equivalents.

Gas liquid chromatography

To further separate the active fractions from the TLC plates and tentatively identify their nature by means of gas liquid chromatography, the active zone was scraped from the plate and eluted from the silicagel with acetone: methanol (1: 1). The abscisic acid

was esterified by a method described by SCHLENK and GELLERMAN (1960) using diazomethane. The diazomethane was generated in a distillation apparatus. Ten grammes of Diazold (N-methyl-N-nitrose-P-toluene sulfonamide) dissolved in 100 millilitres ether were slowly added to 25 millilitres ethanol, 8 millilitres water and 5 grammes KOH mixture at 65°C. The diazomethane was collected in ether kept in ice. This diazomethane in ether was added to the fractions and allowed to air dry. The methylated fractions were dissolved in acetone: methanol (1: 1) and injected in a Varian 3700 Gas Liquid Chromatograph fitted with a flame ionisation detector using a 2 meter chromosorb W-HP column with a 80-100 mesh range. The column temperature was 180°C with injector and detector temperatures both at 230°C.

TISSUE CULTURE PROCEDURE

The basic growth medium consisted of the following components:

MURASHIGE and SKOOG (1962) nutrient medium (Table 2.4)

Thiamine-HCl	0,01 grammes per litre
Sucrose	50 grammes per litre
Agar	1 per cent

Hormones

Fifty millilitres of medium was added to 100 millilitre ehrlenmeyer flasks.The flasks were autoclaved at 105 kPa for 20 minutes and placed on a laminar flow bench. Gibberellic acid was sterilized by passing it through a microfilter and adding it to the individual flasks after autoclaving. The tissue was taken from tuberous roots of young cassava plants grown in the greenhouse. Preliminary experiments revealed that 30 seconds in 10 per cent of a commercial bleach (Sodium hypochloride 3,5 per cent) was the best sterilization method. At lower concentrations there was excessive contamination, while at higher concentration the tissue was damaged. Adding an extra 30 seconds wash with 50 per cent ethanol did not improve sterilization. Four sections* were placed in each flask and the cultures were incubated at a constant temperature (27°C) under continuous light of low intensity (cool fluorescent tubes). The callus was weighed after three to four weeks.

* approximately 25 milligrammes

CHAPTER 3.

ENDOGENOUS ABSCISIC ACID AND TUBEROUS ROOT FORMATION.

INTRODUCTION

OKAZAWA and CHAPMAN (1962) suggested that tuberization was regulated by changes in the balance of promoting and inhibiting hormones. Abscisic acid is generally regarded as a tuberization promotor. There are, however, conflicting reports on the effect of applied abscisic acid on tuberization. Leaf application of abscisic acid has been found to increase tuber growth of potato (EL-ANTABLY, WAREING and HILLMAN,1967; ABDULLAH and AHMAND, 1980; MENZEL, 1980) and dahlia (BIRAN, LESHAM, GUR and HALEVY, 1974). However, SMITH and RAPPAPORT (1969) found no increase of tuber formation when they sprayed potato leaves with abscisic acid. ESASHI and LEOPOLD (1968), SMITH and RAPPAPORT (1969), PALMER and SMITH (1969) and CLAVER (1970) reported that abscisic acid fails to promote tuber formation of isolated potato stolons cultured *in vitro*, while it inhibits kinetin induced tuber initiation.

Few studies have dealt with the relationships between endogenous abscisic acid and tuberization. BIRAN, LESHAM, GUR and HALEVY (1974) noted a higher endogenous inhibitor level in dahlia leaves under short days than under long days. KRAUSS (1978) studied the endogenous abscisic acid content in potato plants as affected by nitrogen nutrition. An interruption of the nitrogen supply to the potato plants was found to lead to an increase in the abscisic acid levels in all plant parts except in the stolons. After the renewal

of the nitrogen supply the abscisic acid level dropped within three days to the original level. The abscisic acid level of the stolons was little affected by nitrogen nutrition. MARSCHNER, SATTELMACHER and BANGERTH (1984), however, reported that abscisic acid levels in potato tubers showed a negative correlation with the growth rate.

From the above studies it appears that the role of abscisic acid in tuberization is primarily an indirect one, namely the suppression of shoot growth, thus making more carbohydrates available for storage in the tubers. Recent studies by WAREING and JENNINGS (1979) with *Solanum andigena* L. have demonstrated, however, that endogenous abscisic acid could be involved directly in initiating tuberization in the stolons. They used one-node stem cuttings in their studies. When these cuttings were taken from short day induced plants, the axillary buds developed into a tuber. Removal of the leaf prevented tuber formation. If the leaf was replaced by abscisic acid the bud developed into a tuber. When the cutting originated from non-induced plants, abscisic acid failed to cause tuber formation. These results raised the question, whether abscisic acid is formed under inducing conditions in the leaves, from where it is translocated to the buds where it induces tuberization. The presence of abscisic acid in leaves and translocation from the leaves through the phloem has been demonstrated (ZEEVAART, 1977). WAREING and JENNINGS (1979), however, found no significant differences between endogenous abscisic acid levels in the leaves of induced and non-induced plants. They also recorded no differences in the rate of abscisic acid export from induced and non-induced leaves. These authors concluded that the presence of abscisic acid is essential for tuber initiation but that a "second factor" is

needed, which is supplied by the induced leaves only. It was suggested that the role of abscisic acid is to inhibit the activity of the apical meristem of potato stolons and to inhibit cell elongation.

KRAUSS (1978) found that endogenous abscisic acid was translocated acropetally within the stolons and accumulated in the stolon tips. Earlier, KRAUSS and MARSCHNER (1976) had shown that abscisic acid application to the stolon tips of potato plants, grown with a continuous nitrogen supply initiated tuberization. *In vitro* studies (PALMER and SMITH, 1969) have also demonstrated that abscisic acid inhibits stolon elongation, although it failed to promote tuber initiation. No information is available on the presence and role of abscisic acid in cassava. In this study abscisic acid was tentatively identified and the levels of the hormone studied at different stages of development.

EXPERIMENT 3.1 Nature and distribution of abscisic acid.

Experimental procedure

Forty grammes fresh tuberous root*, primary root and leaf material of cassava cultivar MSAF 2 was extracted, purified and separated by TLC. Twenty grammes was used for the bioassay and the remainder in the fraction which co-chromatographed with authentic abscisic acid was eluted from the plates. After methylation the samples were concentrated and fractioned in a gas liquid chromatograph (Chapter 2). To study the distribution of inhibitors, tuberous roots of cassava cultivar MSAF 2 with a diameter of approximately 2,5 centimeters were collected and separated into

* The material for this experiment was collected from field grown plants in December when these plants were 11 months old.

three sections referred to as the "phloem", "outer xylem" (0,5 centimeters from the cambium in a centripetal direction) and "inner xylem" (Figure 1.1).

Results

In the first experiment an attempt was made to tentatively identify abscisic acid in the cassava plant. The extracts of tuberous roots, leaves and primary roots were analyzed for inhibitor activity. The highest level of inhibitor activity was found at R_f 0,2-0,3 (Figure 3.1). This peak of inhibition co-chromatographed with authentic *cis*- and *trans*-abscisic acid markers. In the leaf extracts a considerable level of inhibitor activity was found in R_f 0,0-0,2. In the tuber extract the inhibitor activity was concentrated in R_f 0,2-0,3 , while little activity was found in the primary root extracts. R_f zone 0,2-0,3 was eluted from the plates and further analyzed by gas liquid chromatography. *Cis*- and *trans*-abscisic acid standards separated well with retention times of 5,4 and 7,2 minutes respectively (Figure 3.2). The tuberous root extract yielded a number of UV-peaks of which one had a retention time which corresponded with *cis*-abscisic acid. No *trans*-abscisic acid peak was recorded. The leaf extracts also showed a peak with a similar retention time as *cis*-abscisic acid. A small peak corresponding with *trans*- abscisic acid was also found (Figure 3.3). The primary root extracts yielded no peaks which corresponded with abscisic acid. The presence of *trans*- abscisic acid in the leaf extracts could indicate that some isomerisation has taken place during the analysis procedure. It is well established that *cis*-abscisic acid is the major inhibitor present in plants (MILBORROW,

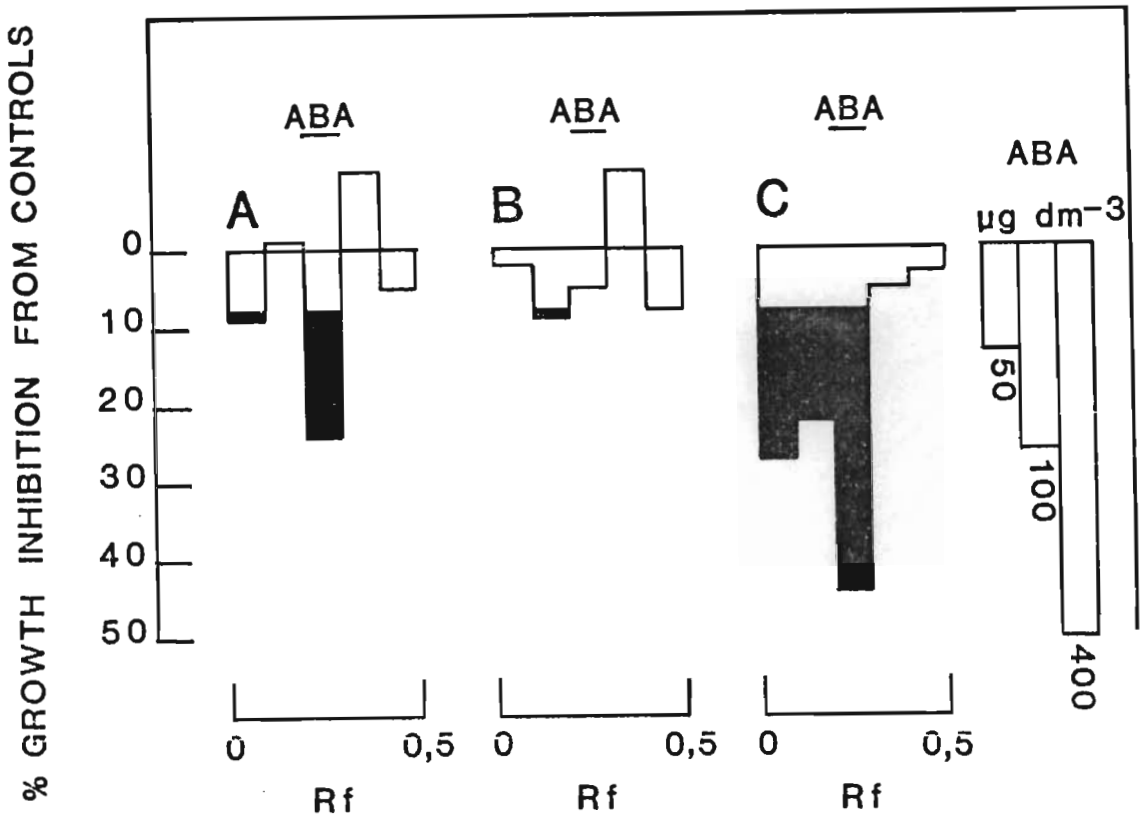

Figure 3.1. Wheat embryo bioassay for inhibitors extracted from 20 grammes fresh tuberous roots (A), roots (B) and leaves (C) of cassava cultivar MSAF 2. Extracts were separated using toluene: ethyl acetate: acetic acid (40: 15: 2 v/v). The shaded areas represent regions significantly different from the controls at 5 per cent level. ABA= abscisic acid.

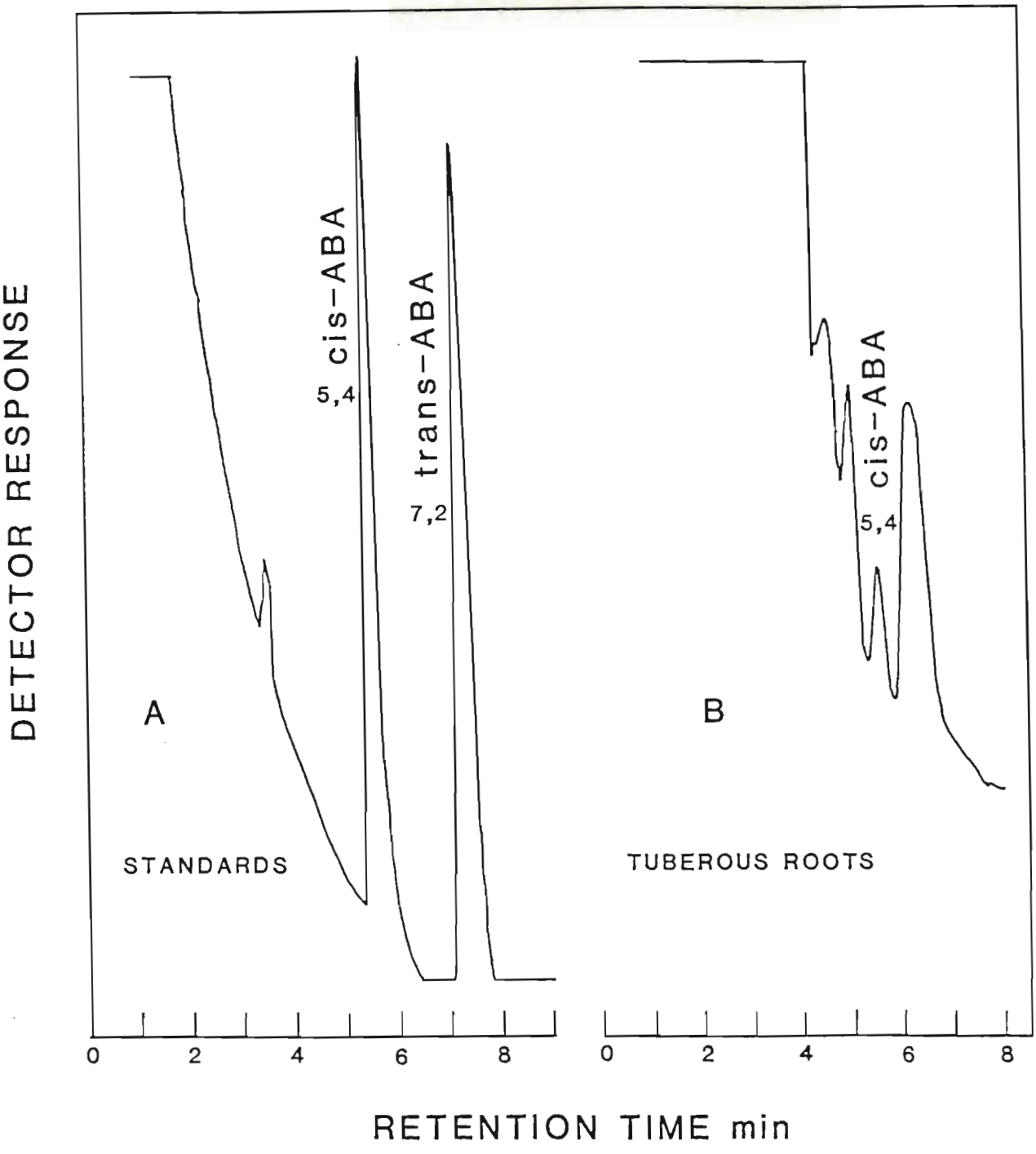

Figure 3.2. Separation of methylated *cis*- and *trans* abscisic acid (A) and methylated faction of tuberous root extract (B) which on TLC co-chromatographed with abscisic acid (Figure 3.1; R_f 0,2-0,3).

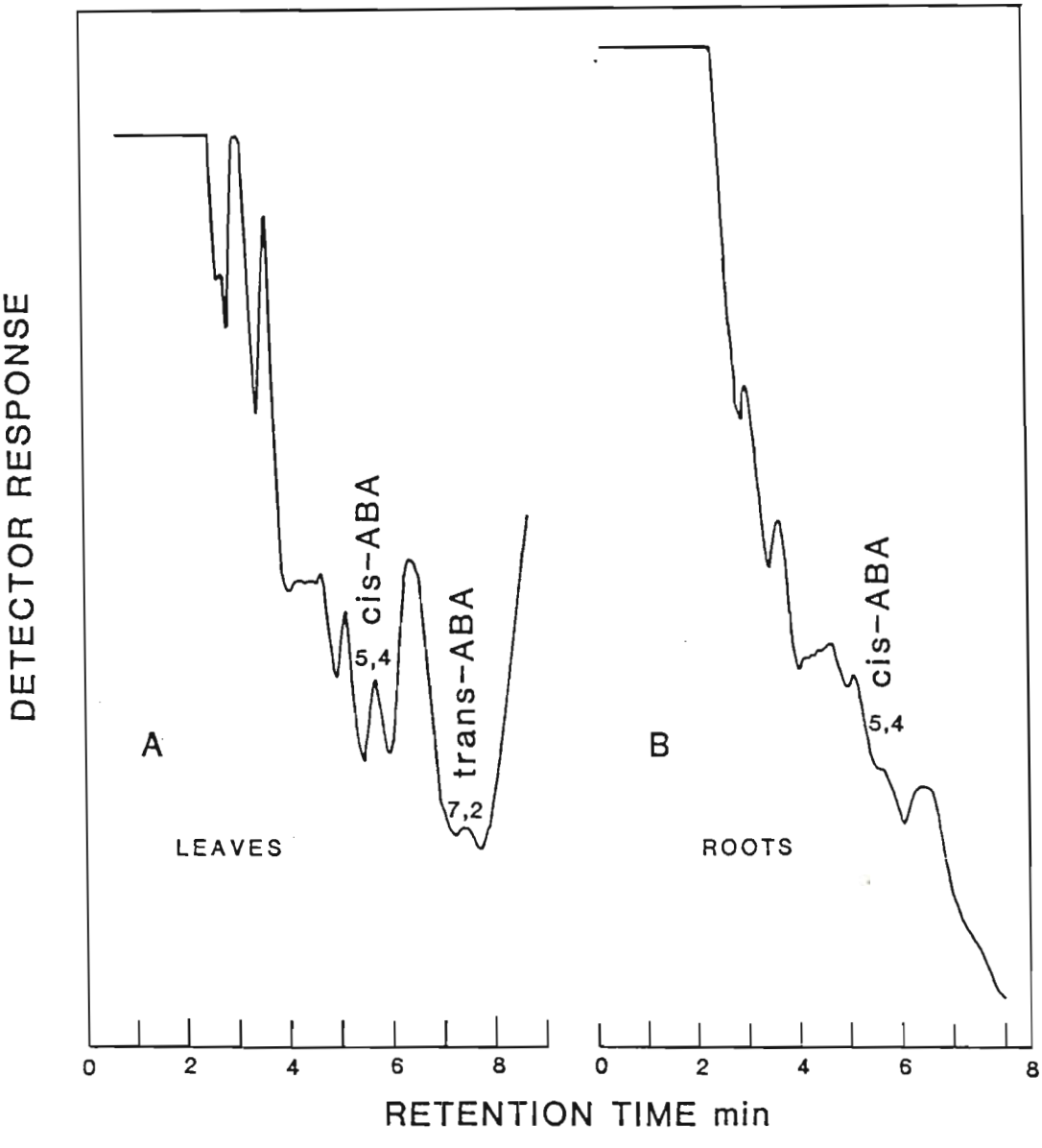

Figure 3.3. Separation of methylated fraction of leaves (A) and roots(B) extracts which on TLC co-chromatographed with abscisic acid (Figure 3.1; R_f zone 0,2-0,3).

1974). The presence of inhibitor activity in other R_f zones indicate that other inhibitors apart from abscisic acid were present.

High levels of inhibitor, co-eluting with abscisic acid were found in the phloem, outer xylem and inner xylem sections of the tuberous roots and did not appear to be concentrated in any of these regions (Figure 3.4). The levels of abscisic acid found in the tuberous roots of cassava were high in comparison with the levels in stolons and tubers of potato (KRAUSS, 1978).

EXPERIMENT 3.2. Inhibitor activity levels during tuberous root development.

The results obtained in the previous experiment indicated that *Cis*-abscisic acid is concentrated in the rapidly growing tuberous roots of cassava. This raised the question of the function of abscisic acid in the tuberization process in cassava. The presence of high levels of abscisic acid appears to have little inhibitory effect on cell divisions and cell growth in the expanding tuberous roots of cassava. In the following studies the presence and activity of inhibitors were monitored in cassava grown under different conditions and at different growth stages of the plant in the field.

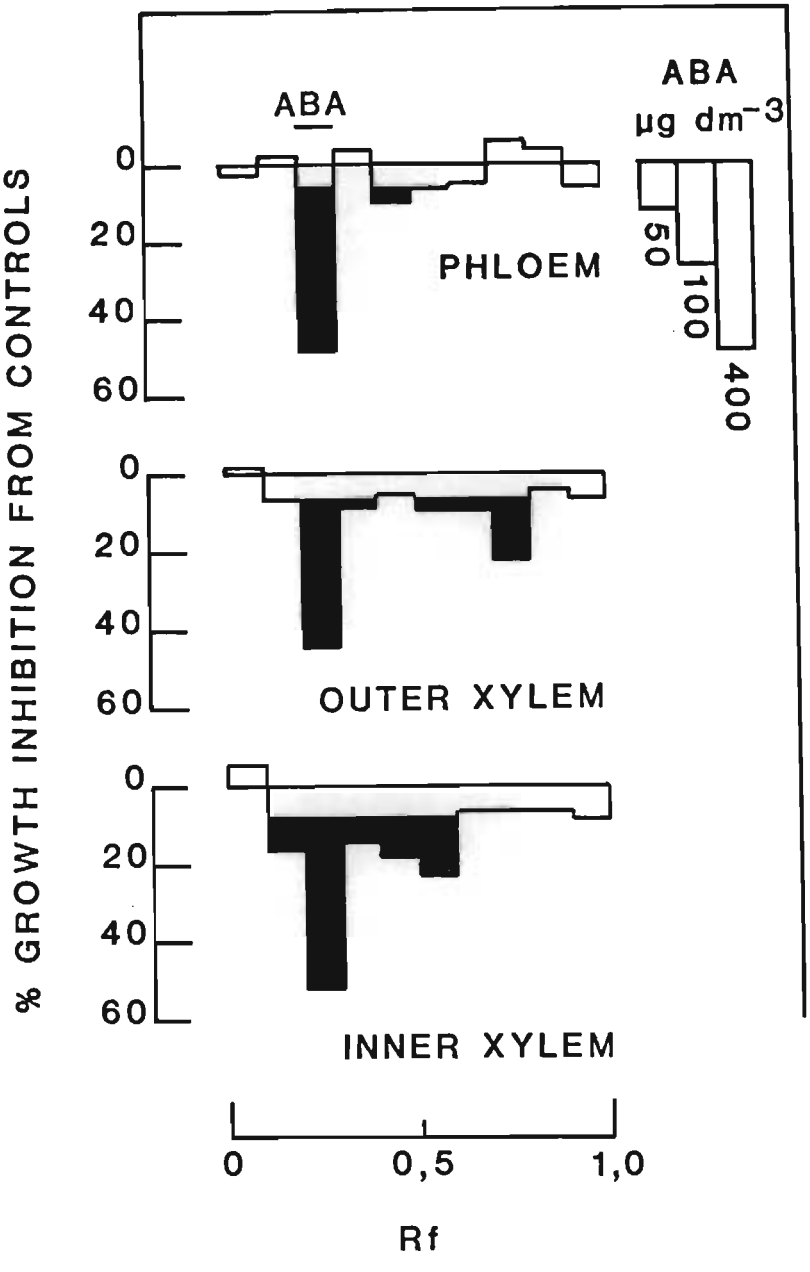

Figure 3.4. Wheat embryo bioassay for inhibitors extracted from 20 grammes fresh phloem, outer xylem and inner xylem material of tuberous roots of cassava cultivar MSAF 2. Extracts were separated using toluene: ethyl acetate: acetic acid (40: 15: 2 v/v). The shaded areas represent regions significantly different from the controls at 5 per cent level. ABA= abscisic acid.

Experimental procedure

Effect of nitrogen nutrition on the inhibitor levels

Stakes of cassava cultivar MSAF 1 were planted in pots containing 6 kilogrammes of a topsoil sand mixture (1:1 v/v) plus lime. Treatment N_0 was given 300 millilitres of the standard nutrient solution (Table 2.1) per week, but without KNO_3 and $Ca(NO_3)_2$. Treatment N_1 was given 300 millilitres of the standard nutrient solution per week including KNO_3 (70 milligrammes per litre), but without $Ca(NO_3)_2$. The pots were placed in a randomized design with 15 replications and moved weekly. The plants were harvested after four months and the fresh mass recorded. Samples were collected for the determination of inhibitor levels.

Effect of applied gibberellic acid (GA_3) on inhibitor levels

The study of the effect of gibberellic acid application was conducted with the short cultivar MSAF 2, which shows slow shoot growth rate in the greenhouse, in particular during the colder months of the year. Stakes were planted in 12 kilogrammes of the standard sand-topsoil mixture. The two treatments were: GA_3-treated and a control. There were eight replications and the pots were placed in a randomized design and moved weekly. Gibberellic acid was applied after five months in April and the plants were harvested six weeks later. The standard nutrient solution (Table 2.1) was applied every two weeks (600 millilitres) for four months. The gibberellic acid source was Berelex (I.C.I. Corporation) and was applied at a concentration of 500 milligrammes per litre until full coverage of

the adaxial surface of the leaves was obtained. Tween 20 (1 millilitre per litre) was added to the spray solution. At harvest the levels of free inhibitors in the tubers were determined using 20 grammes of fresh material.

The effect of shoot removal on inhibitor levels

Stakes of cultivar MSAF 1 were planted in 12 kilogrammes of the standard sand: topsoil 1: 1 (v/v) mixture. The pots were placed in a randomized design and moved weekly. The standard nutrient solution (Table 2.1) was applied every two weeks (600 millilitres). Shoots were removed after six months and the dry mass of tuberous roots, leaves and stems of six plants recorded. The remaining plants were harvested after three and six weeks respectively. At each harvest the levels of free abscisic acid in extracts of 20 grammes fresh tuberous roots and leaves were determined.

Seasonal changes in inhibitor levels

In order to study inhibitor levels at different stages of development of tuberous roots of cassava, samples of cultivar MSAF 2 were taken from the field over a period of 17 months and the inhibitor levels determined.

Results

During the four months, when the plants were grown on media containing low and high levels of nitrogen, the treatments resulted in marked differences in dry matter distribution between above- and

below ground parts of the plant (Table 3.1). Cassava plants responded to a nitrogen shortage by allocating a greater portion of its photosynthate to the storage tubers. This is expressed in the shoot/tuberous root ratio, which was four times higher in the N_1 treatment, when compared to the N_0 treatment. At harvest samples from leaves, roots and tuberous roots of the plants were taken and 20 grammes samples were extracted, purified and the inhibitor activity determined. The aqueous phase was analyzed for conjugates of inhibitor by hydrolysing these fractions (HARRISON and SAUNDERS, 1975) and determining the inhibitor activity.

Extracts of leaves from both N_0 and N_1 treatments showed inhibitor activity which co-chromatographed with authentic abscisic acid markers (Figure 3.5). The levels of inhibitors in the N_1 treatment were 2,5 times higher than the levels in the N_0 treatment (Table 3.2). The hydrolysed fractions showed inhibitory activity in several R_f regions. The largest inhibition occurred in fractions R_f 0,0-0,1 , R_f 0,4-0,6 and R_f 0,9-1,0. A slightly higher level of activity was again found in the N_1 treatment. In the tuberous roots the situation was reversed (Figure 3.6). Here the inhibitor activity in the zone R_f 0,2-0,3, which co-chromatographed with abscisic acid, was more than four times higher in the N_0 treatment than in the N_1 treatment (Table 3.2).

Some activity associated with conjugated abscisic acid was found in tuberous roots from the N_1 plants, while in both N_0 and N_1 treatments some inhibitor activity was found at R_f 0,5-0,7 (Figure 3.6). In the roots considerable inhibitor activity was found in R_f zones other than 0,2-0,3 indicating the presence of other inhibiting

Table 3.1. Dry matter distribution of cassava cultivar MSAF 1 treated wit a high and a low level of nitrogen.

Parameter measured	Low nitrogen	High nitrogen	Significance
Total dry mass shoot (g plant^{-1})	11,7	27,8	**[1])
Dry mass tuberous roots (g plant^{-1})	20,8	11,3	**
Shoot/tuberous roots ratio	0,56	2,46	**

[1]) Highly significant

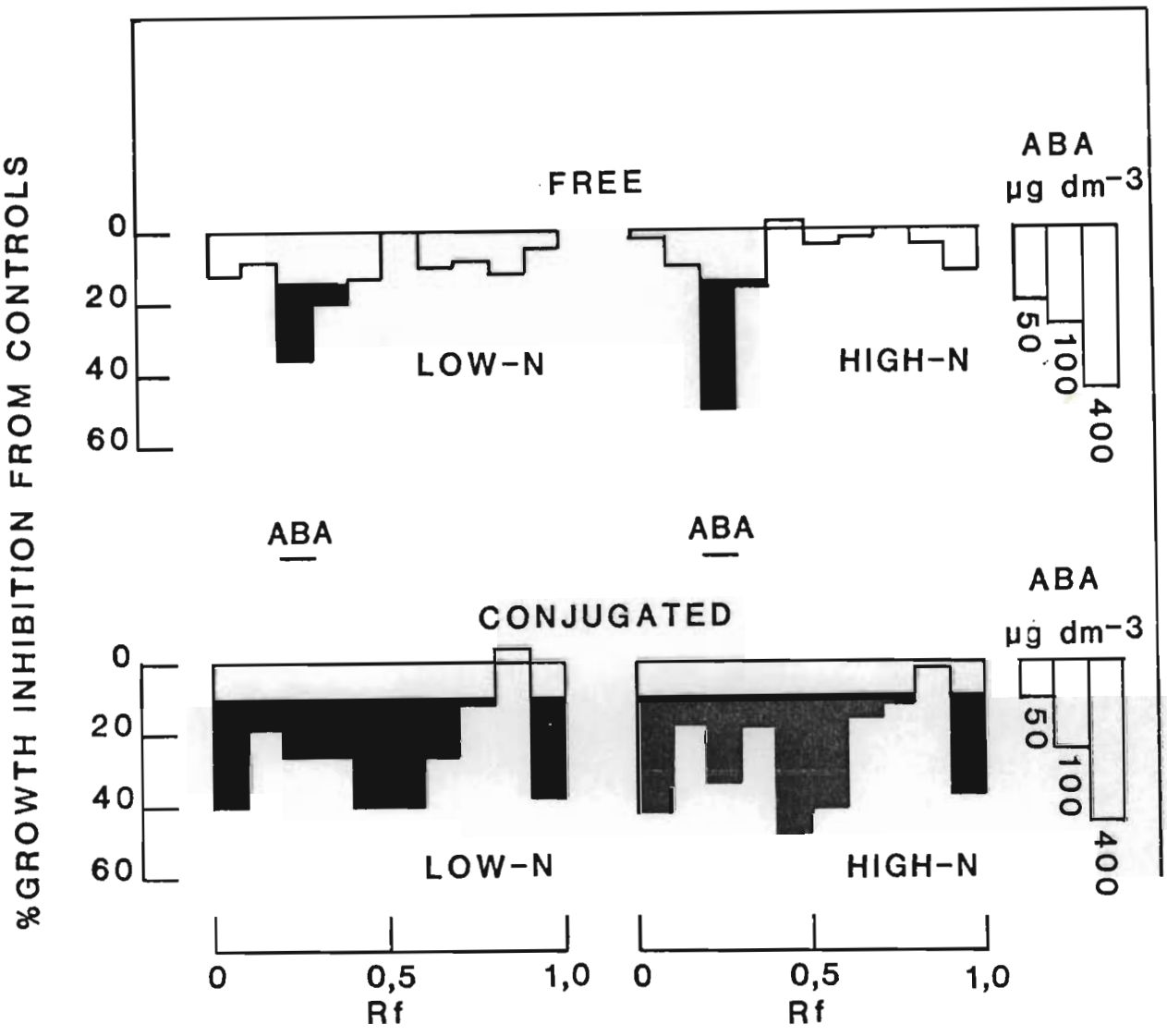

Figure 3.5. Wheat embroy bioassay for inhibitors extracted from 20 grammes of fresh leaves of cassava cultivar MSAF 1 at two levels of nitrogen supply. Extracts were separated using toluene: ethyl acetate: acetic acid (40: 15: 2 v/v). The shaded areas represent regions significantly different from the controls at the 5 per cent level. ABA= abscisic acid.

Table 3.2. Levels of abscisic acid in nanogrammes per gramme fresh plant material of cassava cultivar MSAF 1, treated with high and low levels of nitrogen.

Plant material extracted	Low nitrogen	High nitrogen
Leaves		
free ABA	27,1	62,6
conjugated ABA	17,0	25,2
Tuberous roots		
free ABA	63,9	14,7
conjugated ABA	n.s. [1])	6,8
Roots		
free ABA	16,2	17,9
conjugated ABA	n.s.	n.s.

[1]) No significant level

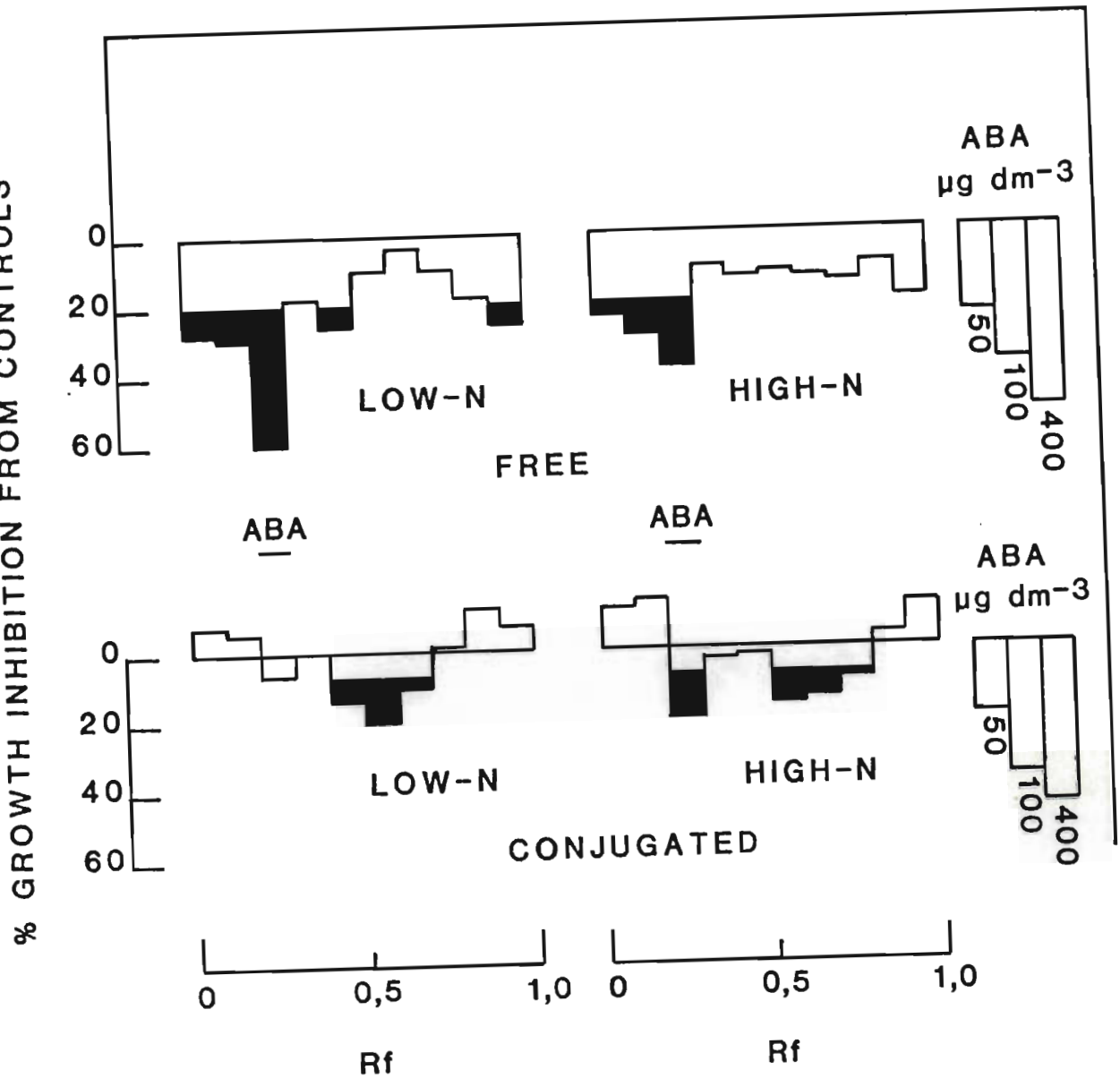

Figure 3.6. Wheat embryo bioassay for inhibitors extracted from 20 grammes fresh tuberous roots of cassava cultivar MSAF 1 at two levels of nitrogen supply. Extracts were separated using toluene: ethyl acetate: acetic acid (40: 15: 2 v/v). The shaded areas represent regions significantly different from the controls at 5 per cent level. ABA= abscisic acid.

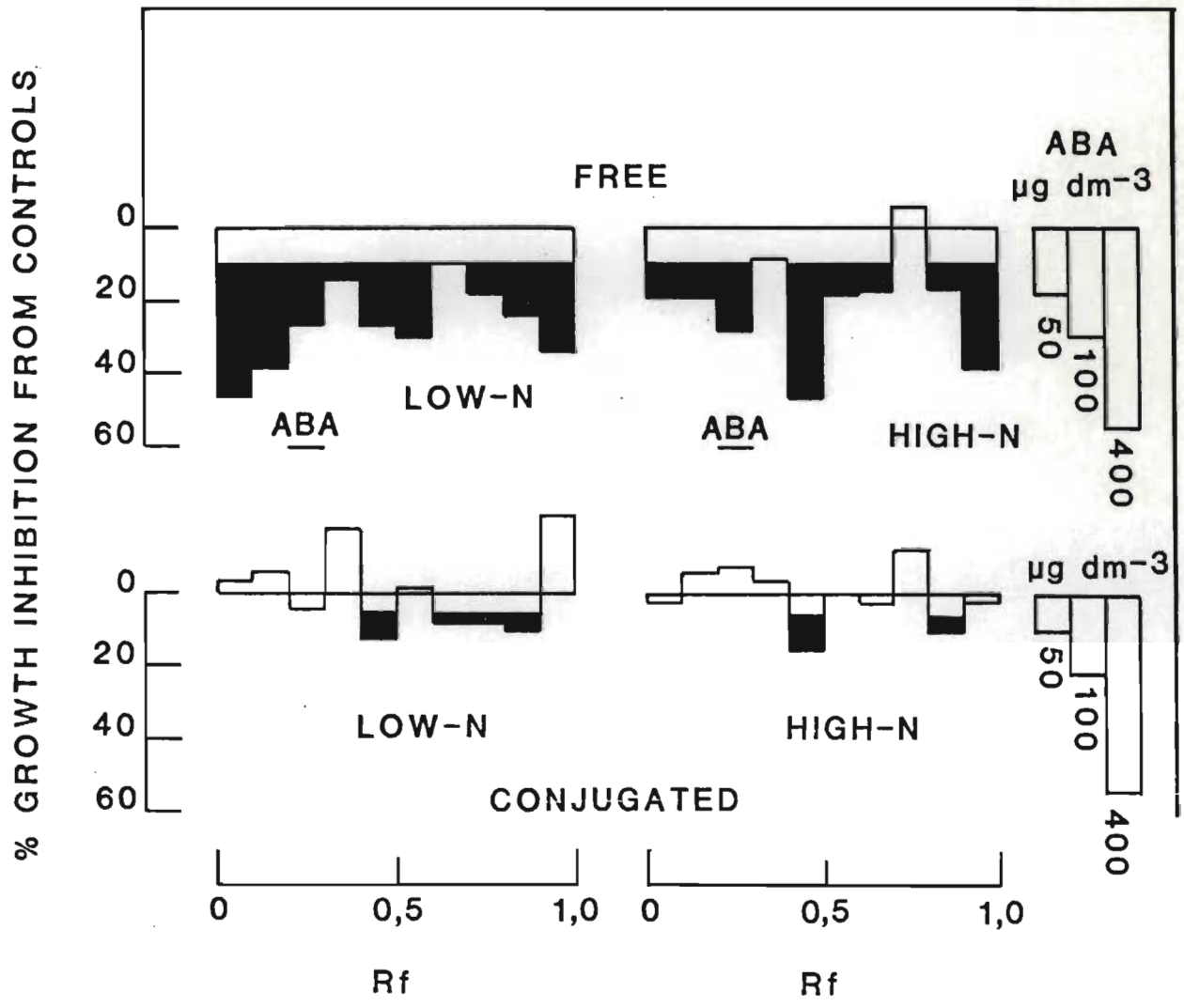

Figure 3.7. Wheat embryo bioassay for inhibitors extracted from 20 grammes of fresh roots of cassava cultivar MSAF 1 at two levels of nitrogen supply. Extracts were separated using toluene: ethyl acetate: acetic acid (40: 15: 2 v/v). The shaded areas represent regions significantly different from the controls at the 5 per cent level. ABA= abscisic acid.

compounds. Little such activity was found in the fraction associated with conjugated inhibitors (Figure 3.7).

The tuberous roots of cassava have a dual function. On the one hand they store carbohydrates transported from the shoot, while on the other hand they provide carbohydrates to the shoot when required. To simulate the latter situation cassava plants, of which the shoot growth had ceased, were treated with gibberellic acid. This resulted in rapid shoot growth and little or even negative growth of the tuberous roots. Over the six week period following GA_3 treatment, the shoots mass increased by 32,2 per cent, while the tuber dry mass was 14,1 per cent lower in the treated plants than in the controls (Table 3.3). The treatment resulted in an increase of the shoot/tuberous root ratio from 0,56 to 0,88.

To establish the effect of the gibberellic acid treatment on the inhibitor level, 20 grammes of fresh tuber material of treated and untreated plants were extracted, purified and bioassayed. The TLC plates were separated into eight fractions to allow the zone co-eluting with authentic abscisic acid to fall entirely in the third zone. A high level of inhibitor activity co-eluting with abscisic acid was found in the control plants (15,8 nanogrammes per gramme), but no significant levels were recorded in the tubers of gibberellic acid treated plants (Figure 3.8).

In a subsequent experiment a similar situation was established, but in this case the shoots were removed, preventing

Table 3.3. The effect of gibberellic acid treatment on the dry matte distribution of cassava cultivar MSAF 2 grown in the greenhouse.

Parameter measured	GA_3 treated	Control	Significance
Total shoot dry mass (g plant^{-1})	42,7	31,8	** [1]
Dry mass tuberous roots (g plant^{-1})	48,6	56,6	n.s. [2]
Shoot/tuberous root ratio	0,89	0,58	**

[1]) Highly significant [2]) Not significant

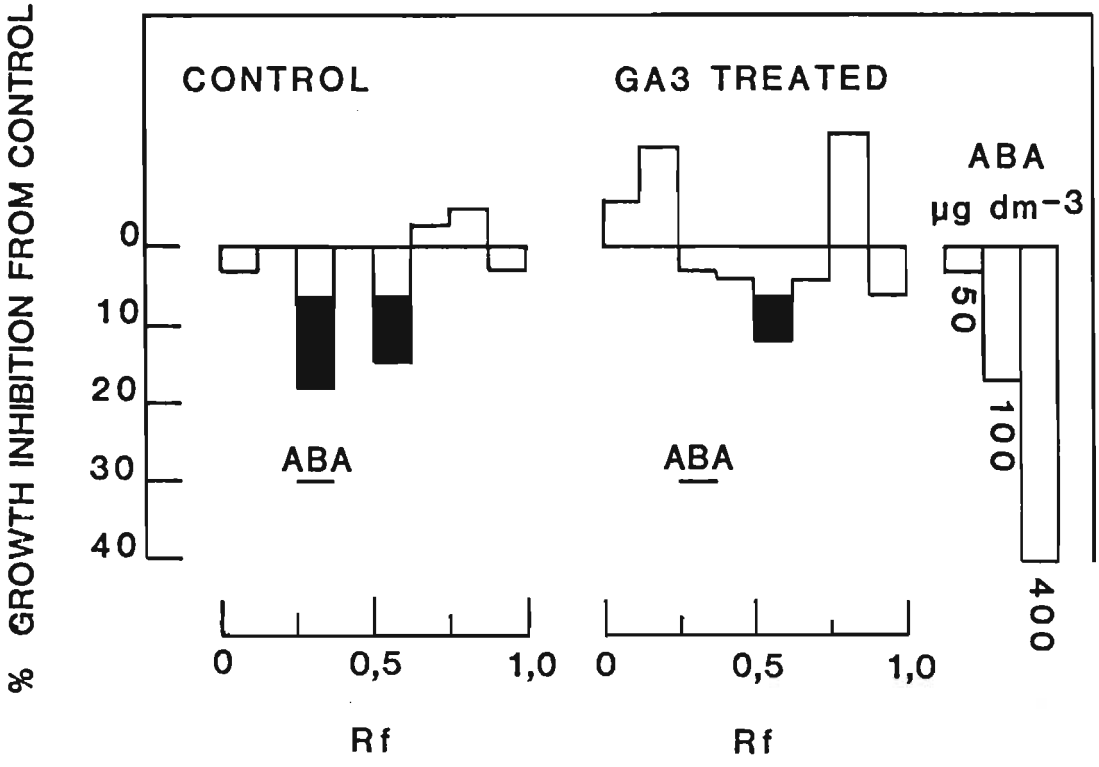

Figure 3.8. Wheat embryo bioassay for inhibitors extracted from 20 grammes of fresh tuberous roots of cassava cultivar MSAF 2 treated with gibberellic acid. Extracts were separated using toluene: ethyl acetate: acetic acid (40: 15: 2 v/v). The shaded areas represent regions significantly different from the controls at the 5 per cent level. ABA= abscisic acid.

growth of tuberous roots. Carbohydrates from the tuberous roots were apparently mobilized and incorporated into new shoots. The inhibitor levels were determined at the time of shoot removal, three weeks after removal and six weeks after removal and a detailed growth analysis was carried out. Table 3.4 shows the rapid decrease in tuberous root dry mass which occurred over the first three weeks. Over the following three weeks a slight increase in tuberous root mass was observed, suggesting that at this stage the foliage started producing photosynthate for tuberous root storage. It is remarkable that the shoot/tuberous root ratio after six weeks had already exceeded the value of this ratio at the time of shoot removal. Also by that stage the leaf dry mass had doubled since shoot removal. A similar effect of rapid leaf formation after shoot removal was recorded in the field (Chapter 6). High levels of free inhibitor activity in the tuberous root extracts were recorded at all three harvests (Figure 3.9). The highest level of activity was found in R_f zone 0,2-0,3 which co-chromatographed with authentic abscisic acid. Considerable inhibitory activity occurred also in R_f zone 0,6-0,9. From the data it appears that shoot removal had little effect on the levels of the inhibitors.

In a subsequent experiment the inhibitor activity in the tuberous roots was monitored over a 17 month period. The inhibitor activity was again concentrated in R_f zone 0,2-0,3 (Figure 3.10; Table 3.5). At the time of tuberous root initiation, in January, some inhibitor activity was present. However, the highest levels of activity were found in May and October 1982, while little activity was found in December 1982 and March 1983. In May 1983 the level was again somewhat higher. There appears to be no clear relationship

Table 3.4. Dry matter distribution of cassava cultivar MSAF 1, befor shoot removal and 3 and 6 weeks after shoot removal.

Parameter measured	Before removal	3 Weeks after removal	6 Weeks after removal	LSD 5%
Stems and petioles ($g\ plant^{-1}$)	41,8	4,1	16,4	7,5
Leaves ($g\ plant^{-1}$)	7,1	3,7	11,5	1,3
Tuberous roots ($g\ plant^{-1}$)	33,5	13,4	18,7	12,7
Shoot/tuberous root ratio	1,68	0,62	2,01	1,30

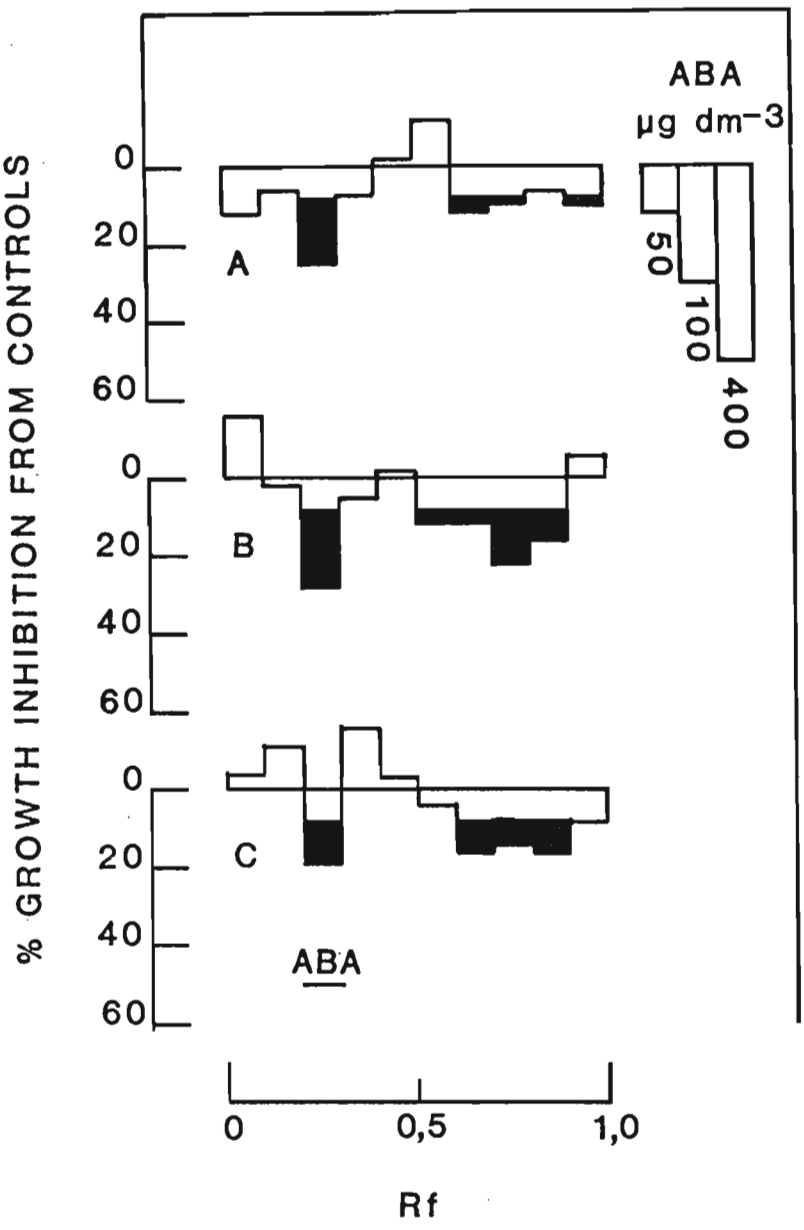

Figure 3.9. Wheat embryo bioassay for inhibitors extracted from 20 grammes of fresh tuberous roots of cassava cultivar MSAF 1 before pruning (A), three weeks after pruning (B) and six weeks after pruning (C). Extracts were separated using toluene: ethyl acetate: acetic acid (40: 15: 2 v/v). The shaded areas represent the regions significantly different from the controls at the 5 per cent level. ABA= abscisic acid.

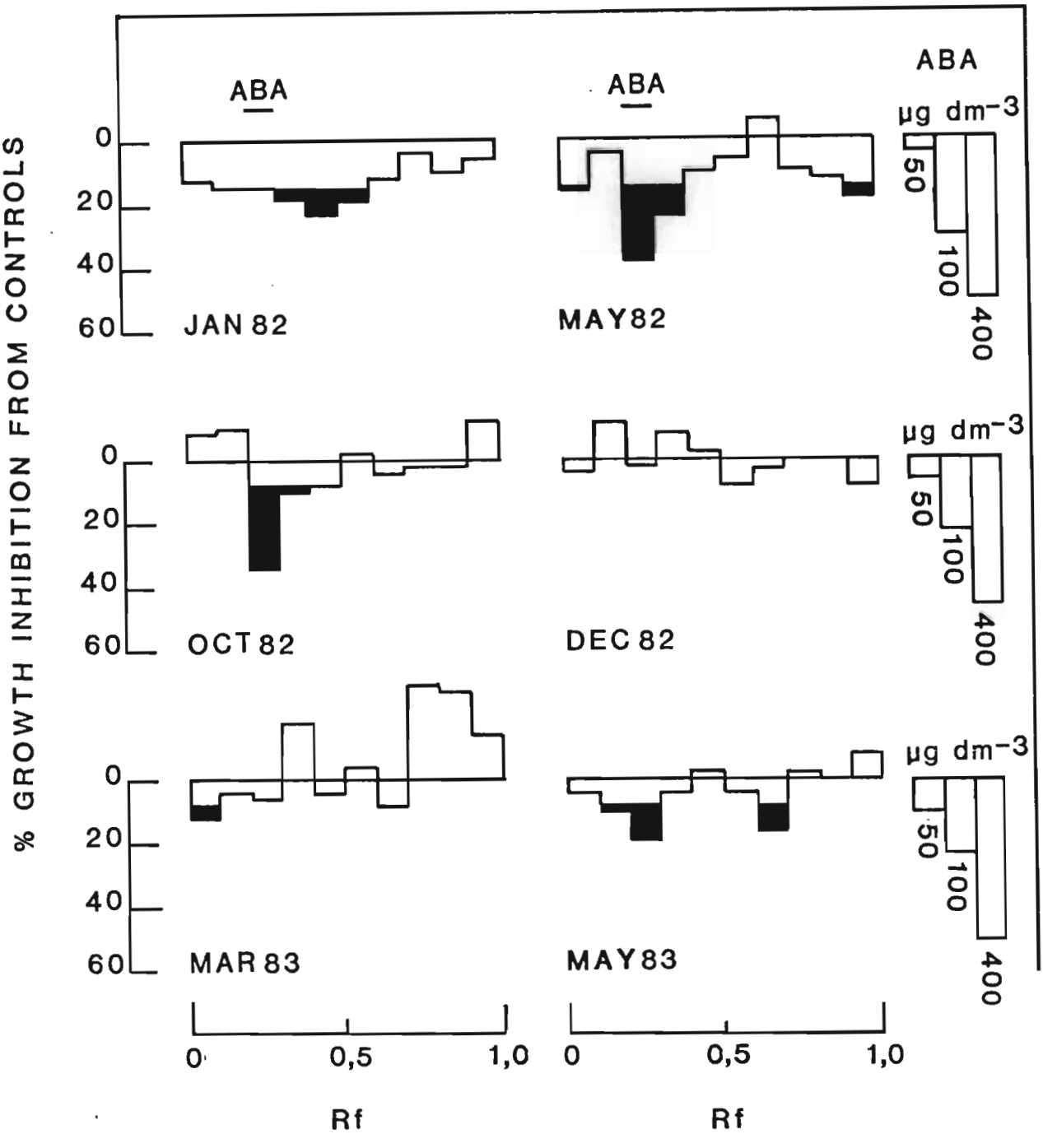

Figure 3.10. Wheat embryo bioassay for inhibitors extracted from 20 grammes of fresh tuberous roots of cassava cultivar MSAF 2 at different times of the growing season. The extracts were separated using toluene: ethyl acetate: acetic acid (40: 15: 2 v/v). The shaded areas represent the regions significantly different from the controls at the 5 per cent level. ABA= abscisic acid.

Table 3.5. Levels of abscisic acid activity fresh tuberous roots of cassava cultivar MSAF 2 harvested at different times from the field.

Month	Abscisic acid level activity ng g^{-1} plant material
January 1982	n.s.
May 1982	31,6
October 1982	14,1
December 1982	n.s.
March 1983	n.s.
May 1983	2,2

between the abscisic acid level and the rate of tuberous root growth.

DISCUSSION

Abscisic acid is generally associated with plant growth inhibition (MILBORROW,1974). There are, however, a number of reports of a growth stimulation effect of abscisic acid. ASPINALL, PALEG and ADDICOTT (1967), found abscisic acid to stimulate rooting of stem cuttings, while abscisic acid has also been found to increase the stimulatory effect of kinetin on soybean callus growth (BLUMENFELD and GAZIT, 1970). It appears that the inhibitory effect of abscisic acid is mainly limited to certain parts of the plant, in particular the above ground parts. This is most obvious in the wheat coleoptile bioassay, where abscisic acid has a strong inhibitory effect on the growth of the coleoptile, but has no effect on root growth

The literature on the role of abscisic acid in tuberization, as discussed in the introduction of this chapter, confirms the above view. It was found that leaf application generally reduces shoot growth (EL-ANTABLY, WAREING and HILLMAN, 1967; ABDULLAH and AHMED, 1980; MENZEL, 1980; BIRAN, LESHAM, GUR and HALEVY, 1974), while abscisic acid in the below ground parts are likely to promote tuber growth (KRAUSS, 1978; WAREING and JENNINGS, 1979). Differences in sensitivity of cells towards abscisic acid might be responsible for the different responses throughout the plant towards this hormone (TREWAVAS, 1981).

The data obtained show that high levels of *cis*-abscisic acid

are present in young tuberous roots of cassava in comparison with primary roots of the same plants. The presence of abscisic acid does not appear to inhibit the large number of cell divisions and cell growth which take place in the young storage roots. In some of the experiments the highest level of abscisic acid was detected with those treatments which resulted in the highest rate of tuberous root growth (N_0; untreated compared with Ga_3-treated). This could imply that abscisic acid in tuberous roots stimulates cell division. NAGL (1972) reported that abscisic acid applied to isolated root tips of *Allium* spp stimulates polyploid nuclei to divide. In the field experiment and the pruning experiment, however, there appeared to be no clear correlation between the abscisic acid level and the growth rate of the tuberous roots.

WAREING and JENNING (1979) and KRAUSS (1978) have postulated that abscisic acid inhibits cell elongation and stolon growth and consequently promotes formation of tubers in potato. In cassava, abscisic acid could have a similar function in the transition of a primary root into a secondary root. Many physiological functions ascribed to abscisic acid are associated with processes of negative crop development such as dormancy, abscission and senescence (MILBORROW, 1974). In addition, abscisic acid is believed to play a role in regulating the plant's response to environmental stress such as in stomatal functioning (WRIGHT and HIRON, 1972). Tuberization could in a sence be regarded as a "negative" crop development, since it is promoted by stress and is a survival mechanism of the plant. Stress factors such as nitrogen deficiency considerably increases the abscisic acid content of, for example, sunflower leaves, especially the older senescing ones (GOLDBACH, GOLDBACH, WAGNER and

MICHAEL, 1975).

KRAUSS (1978) found an increase in the abscisic acid content throughout the potato plant in response to stress. In cassava, nitrogen deficiency led to a slight decrease of the abscisic acid levels in the leaves, but to an increase of the level in the tuberous roots. Some inhibitor activity was found in the root, but the level was not affected by the nitrogen supply. It thus seems, that nitrogen stress in cassava leads to an accumulation of inhibitor in the rapidly growing tuberous roots, while, unlike potato, the level in the leaves decreased slightly. Similarly the higher levels of abscisic acid in the tuberous roots in May and October 1982 could be related to the moisture stress and low temperatures during these months.

Although it is well established that abscisic acid levels in roots increase when plants undergo a period of stress, there is disagreement over the origin of this abscisic acid (WALTON, 1980). Some maintain that the abscisic acid originates from the leaves (HOAD, 1975), while others believe it might have been synthesized in the roots of the stressed plants (WALTON, 1980). The present study does not reveal if abscisic acid was formed in the tuberous roots of cassava or originated from elsewhere.

CHAPTER 4

ENDOGENOUS CYTOKININS AND TUBEROUS ROOT FORMATION

INTRODUCTION

For several reasons cytokinins have in the past been associated with the tuberization process. In the first place cytokinins are known to stimulate cell division (SKOOG and MILLER, 1957; MILLER, 1965). Furthermore, there are indications that these hormones inhibit cell elongation (VANDERHOEF and KEY, 1968). These processes are required for tuber formation and growth, and several workers have therefore suggested that the postulated tuberization hormone (GREGORY, 1956) could be a cytokinin-like substance (MADEC, 1963; COURDUROUX, 1966).

In vitro experiments have shown that kinetin promotes secondary growth in excised roots of radish (LOOMIS and TORREY, 1964; TORREY and LOOMIS, 1967), turnip (PETERSON, 1973) and begonia (ESASHI and LEOPOLD, 1968). In addition, it induces tuber initiation in isolated potato stolons (PALMER and SMITH, 1970; MINGO-CASTEL, YOUNG and SMITH, 1976; FORSLINE and LANGILLE, 1976; MAUK and LANGILLE, 1978). Early workers believed that cytokinins were synthesized in the leaves under certain environmental conditions and subsequently translocated to the tuberization site to induce tuberization. Although cytokinins have been isolated from the leaves of many species, there is as yet no conclusive evidence that cytokinins are produced within them. The major site of cytokinin synthesis is still considered to be the root meristem (VAN

STADEN and DAVEY, 1979). Basipetal transport of labelled kinetin does occur in isolated segments of petiolesand hypocotyls of radish (RADIN and LOOMIS, 1974) and in isolated potato stolons (SMITH and PALMER, 1970). A further indication of shoot-root translocation of cytokinins is their presence in the phloem sap. But VAN STADEN and DAVEY (1979) pointed out that these cytokinins could well have been synthesized initially in the roots.

Cytokinin levels have been shown to increase shortly before (FORSLINE and LANGILLE, 1975) or following tuber initiation (RADIN and LOOMIS, 1974; OBATA-SASAMOTO and SUZUKI, 1979; HAYATA and SUZUKI, 1982; KODA, 1982; MATSUO, YONEDA and ITOO, 1983). The cytokinin level sometimes decreases at a later stage (KANNANGARA and BOOTH, 1978; MATSUO, YONEDA and ITOO, 1983). SATTELMACHER and MARSCHNER (1978a and 1978b) studied the effect of nitrogen supply on cytokinin levels. Nitrogen withdrawal led to a temporary increase of the cytokinin levels in the underground parts, while the level in the root exudate and shoots decreased. These changes were considered to be a reflection of the meristematic activity in the shoots and roots. Despite the close correlation between tuberization and cytokinin activity, it was assumed that cytokinins are not directly responsible for tuber initiation, but play an important role in tuber growth. Zones of high meristematic activity in general, and that of the roots in particular, are known to contain high cytokinin levels (MICHAEL, MOUNLA and GOLDBACH, 1974). GOLDACRE (1959) suggested meristematic zones as possible sites of cytokinin synthesis. JAMESON (1982) reported that there was a positive correlation between the highest concentration of cytokinin-like activity and the reported period of intense cell

division. High levels of cytokinins in newly formed tubers could be the result of synthesis at this site. LOOMIS and TORREY (1964) have suggested that a previously initiated secondary cambium, might serve to supply the cytokinins for further tuberization. The cambium has been mentioned elsewhere as a possible site of cytokinin synthesis (NITSCH and NITSCH, 1965), but so far there is no conclusive evidence (VAN STADEN and DAVEY, 1979).

Rather than initiating tuberization the role of cytokinins could be seen in the regulation of tuber growth and the attraction of assimilates to the tubers. Cytokinins may have an important function in the dry matter distribution of the plant. High levels of cytokinins in a particular part generally coincides with active growth in that part. Furthermore, external application of kinetin to shoots leads to increased shoot growth, while tuber application of kinetin leads to an increase in tuber growth (BADIZADEGAN, TAFAZOLI and KHERADNAM, 1972; TSOVYAN and KOTIYAN, 1981). Some workers (PALMER and SMITH, 1969; VAN STADEN and DIMALLA, 1976) also suggested that cytokinins in the tubers act by establishing a metabolic storage sink which attracts metabolites to the organs in which it is present. This theory of hormone induced translocation was first proposed by MOTHES and ENGELBRECHT (1961) and later confirmed by numerous workers. GERSANI and KENDE (1982) recently found that labelled leucine and sucrose were translocated preferentially to a cytokinin-treated area of the leaf. The directed transport appeared to proceed through the phloem against a concentration gradient and might be caused by an increased capacity of cytokinin containing cells to take up metabolites. The exact mechanism by which cytokinins can attract metabolites remains unclear. MORRIS (1982)

argued that one way by which cytokinins regulate assimilate distribution is by controlling cell growth and differentiation processes, thus determining the substrate demand of the different organs. On the other hand it is also possible that cytokinins directly regulate carbohydrate synthesis and metabolism. MINGO-CASTEL, YOUNG and SMITH (1976) found enhanced phosphorylase and ADP-glucose pyrophosphorylase activity during kinetin induced tuberization in *in vitro* grown potato sprouts. OBATA-SASAMOTO and SUZUKI (1979) reported similar results and suggested that the rapid rise of cytokinin levels, coinciding with tuber formation, enhances the activities of starch synthesizing enzymes and in this way promotes starch deposition. The function of cytokinins could go further in that they may influence phloem transport, phloem loading and unloading, and photosynthesis itself. PATRICK (1982) recently described a hormonal model for carbohydrate translocation. It was proposed that the assimilate supply was mediated by the sink produced hormones. As cytokinins are present in relatively high levels in actively growing tubers and have been found in xylem sap of several species (VAN STADEN and DAVEY, 1979), they could possibly be responsible for this regulating influence of the tuber sink on the shoot.

In the present study the nature and distribution of cytokinins in cassava was studied. Furthermore,an attempt was made to establish the relationship between cytokinin levels and tuber growth in cassava.

EXPERIMENT 4.1. Nature and distribution of cytokinins.

Several workers have studied the nature of endogenous cytokinins in tuber crops. Initially, bioassays were used to detect cytokinin-like activity. RADIN and LOOMIS (1971) found biological activity co-eluting with ribosylzeatin and zeatin in radish roots. VAN STADEN (1976) and later SATTELMACHER and MARSCHNER (1978a) detected a major peak of activity, which co-eluted with ribosylzeatin in potato tubers. This peak has been tentatively identified as *trans*-ribosylzeatin using HPLC techniques (MAUK and LANGILLE, 1978). Recently, MATSUO, YONEDA and ITOO (1983), using HPLC, tentatively identified *trans*-zeatin and *trans*-ribosylzeatin as the major cytokinins in sweet potato. In this study bioassays and HPLC techniques were used to obtain information on the nature of the cytokinins in tuberous roots of cassava.

Experimental procedure

Three kilogrammes of fresh tuberous roots of cassava cultivar MSAF 2 were harvested from 14 month-old plants grown in the field. The material was extracted, purified and assayed for cytokinin activity. To study the distribution of cytokinins tuberous roots with a diameter of approximately 2,5 centimeters were collected and separated into three anatomical sections namely the phloem, outer xylem (0,5 centimeter from the cambium in a centripetal direction) and inner xylem (Figure 1.1). The material used for the comparative study of cytokinin levels in tuberous roots and primary roots was collected from four month-old plants of cultivar MSAF 1 grown in pots.

Results

The 3 kilogrammes fresh tuberous roots were extracted and the ammonia fraction separated on Whatman No 1 chromatography paper. A portion of the chromatogram equivalent to 30 grammes material was assayed and the rest of the paper stored at - 20°C. Cytokinin-like activity, which co-chromatographed with zeatin and ribosylzeatin, was detected in the R_f zone 0,6-0,9 (Figure 4.1). This biologically active zone was eluted from the remaining paper. After being concentrated, the residue was redissolved up in 10 per cent methanol and fractioned on a Sephadex LH-20 column using 10 per cent methanol. From each 40 millilitre fraction a portion equivalent to 30 grammes of material was assayed for cytokinin activity. Biological activity was detected in the fraction with similar elution volumes as ribosylzeatin (760-800 millilitres), dihydrozeatin (1080-1120 millilitres) and zeatin (1200-1240 millilitres). The remainder of each fraction was dried in a stream of air. Fractions A, B and C (Figure 4.2) were further separated and tentatively identified by means of high performance liquid chromatography. Acetonitrile-water programmes were used to separate the different cytokinins. A column with a small particle size was used (5µm). One of the problems encountered with this column was the shift in retention time after the column had been washed between samples. A suitable cleaning programme was eventually found in which the methanol was increased to 50 per cent over two minutes, left constant at this level for three minutes and subsequently decreased to zero in five minutes. Different separation programmes had to be used for ribosylzeatin on the one hand and zeatin and dihydrozeatin on the other. The residues of the fractions were dissolved in 0,1 millilitres methanol and 5

microlitre samples were injected for each analysis. The most polar peak (A), which co-eluted with ribosylzeatin (Figure 4.2; 760-800 millilitres) yielded a number of UV-peaks. One of these peaks had a retention time corresponding with the *trans*-ribosylzeatin standard (15,6 minutes), while another peak had a retention time similar to the *trans*-zeatin standard (21,1 minutes; Figure 4.3). To confirm the cytokinin nature of these peaks, one minute fractions were collected from the HPLC column. The fractions were dried in a stream of air and the biological activity determined. Both the *trans*-ribosylzeatin and the *trans*-zeatin peak showed biological activity. *Trans*-zeatin was not expected in this fraction as it separates well from *trans*-ribosylzeatin by the Sephadex separation system used (Figure 4.2). The result suggests that *trans*-ribosylzeatin was broken down during the sample separation. The biologically active peak which co-eluted with dihydrozeatin (Figure 4.2; 1080-1120 millilitres), yielded a major UV-peak with a retention time corresponding with that of dihydrozeatin (16,8 minutes; Figure 4.4). A smaller peak had a similar retention time as *trans*-zeatin. Biological activity was associated with both the dihydrozeatin peak and the *trans*-zeatin UV-peaks. The biologically active peak which co-eluted with zeatin (Figure 4.2.;1200-1240 millilitres) yielded a large peak similar to *trans*-zeatin (14,1 minutes; Figure 4.4) and a smaller peak with a retention time identical to dihydrozeatin (16,8 minutes). The biological activity corresponded with the dihydrozeatin peak, but little activity was found with the *trans*-zeatin peak. The HPLC results of fractions B and C show that there is some overlap of the dihydrozeatin and *trans*-zeatin fractions on the Sephadex LH-20 column, if eluted with 10 per cent methanol.

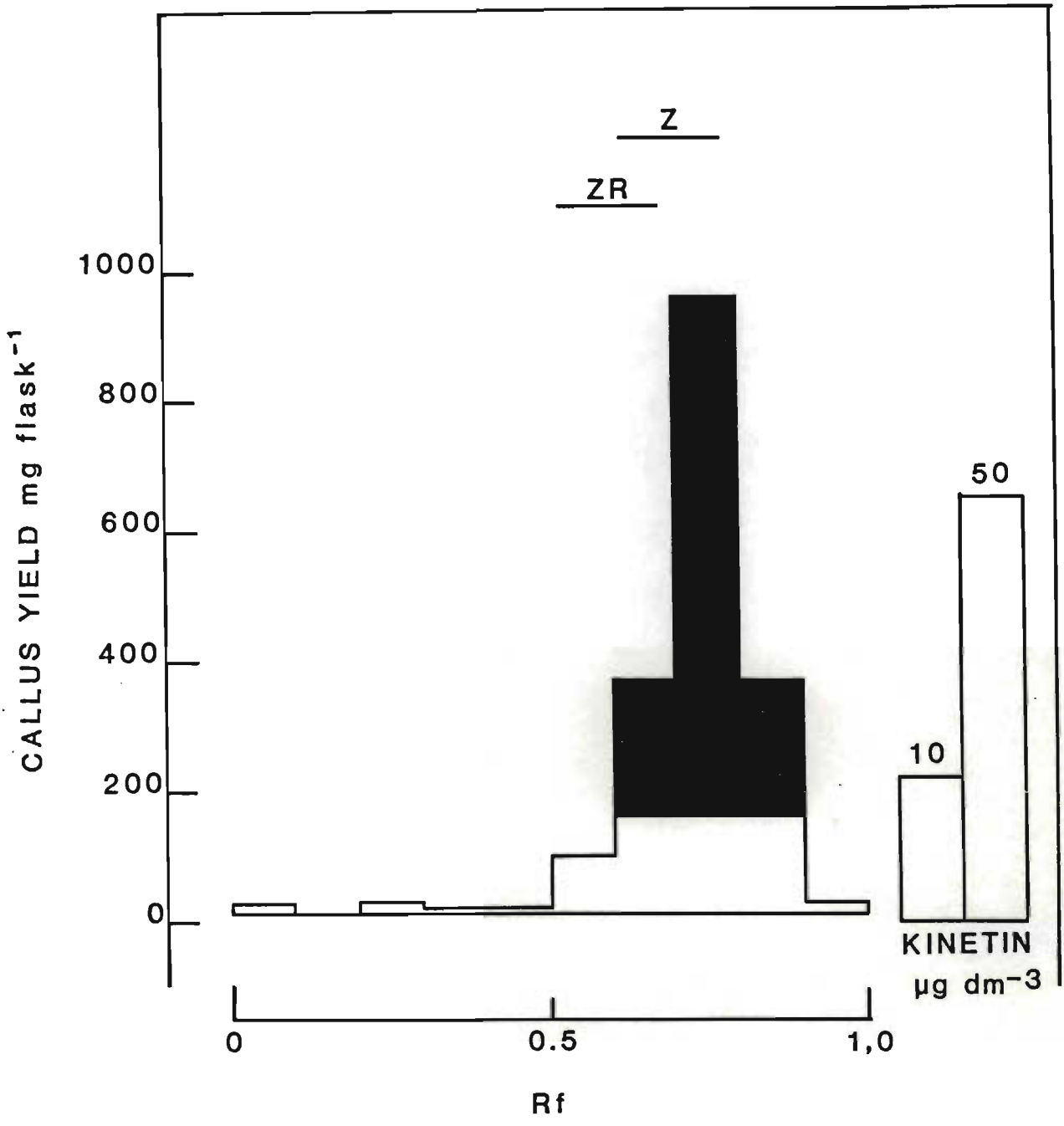

Figure 4.1. Soybean callus bioassay from 30 grammes of fresh material of tuberous roots of cassava cultivar MSAF 2. The extracts were purified on Dowex 50 cation exchange resin and separated on Whatman No 1 chromatography paper using *iso*-propanol: 25 per cent ammonium hydroxide: water (10: 1: 1 v/v) as the solvent. The shaded areas represent regions significantly different from the control at the 5 per cent level. Z=zeatin; ZR= ribosylzeatin.

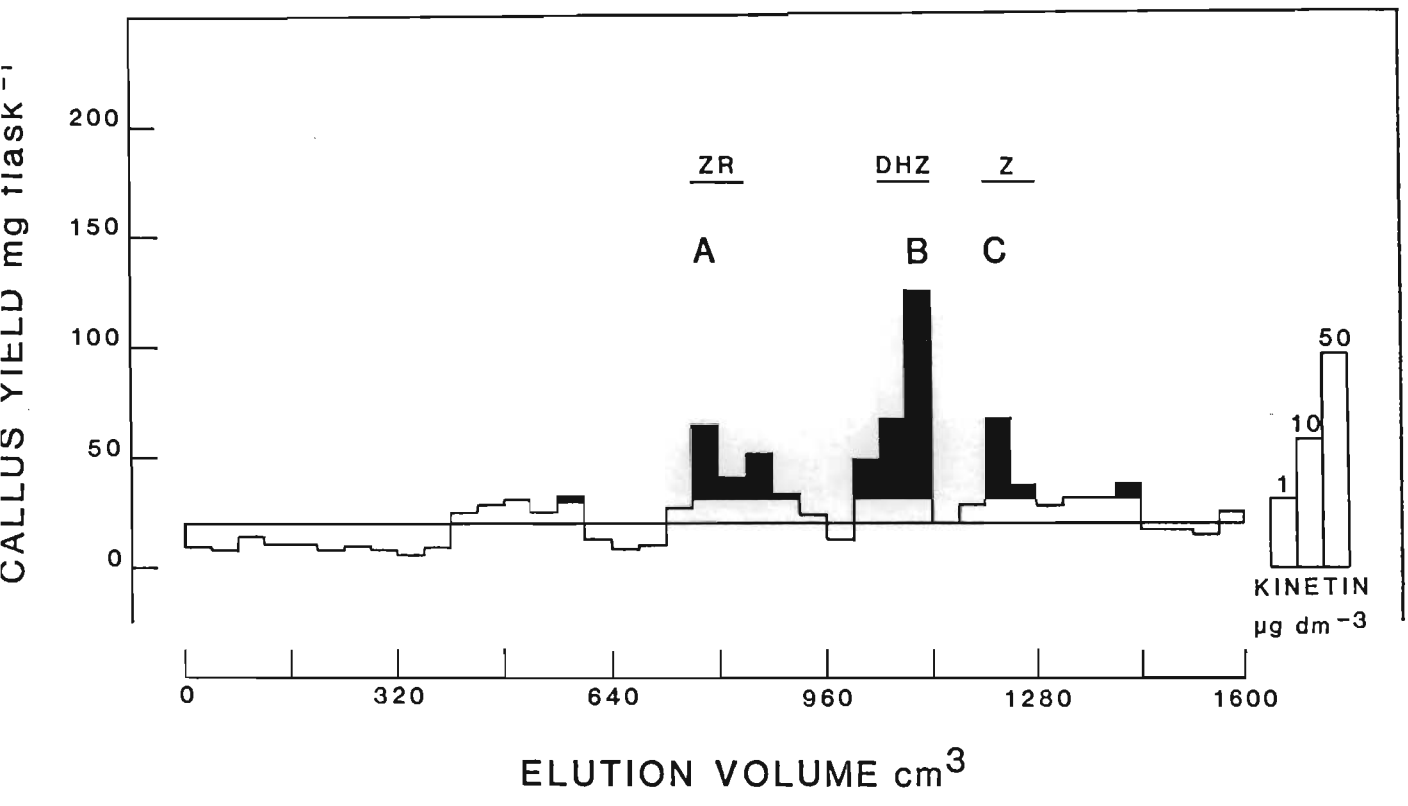

Figure 4.2 Soybean callus bioassay from the equivalent of 30 grammes fresh tuberous root material of cassava cultivar MSAF 2. The extract was separated on chromatography paper and the active zone (Figure 4.1; R_f 0,6-0,9) eluted and then fractionated on a Sephadex LH-20 column using 10 per cent methanol. The shaded areas represent regions significantly different from the controls at the 5 per cent level. Z= zeatin; ZR= ribosylzeatin; DHZ= dihydrozeatin.

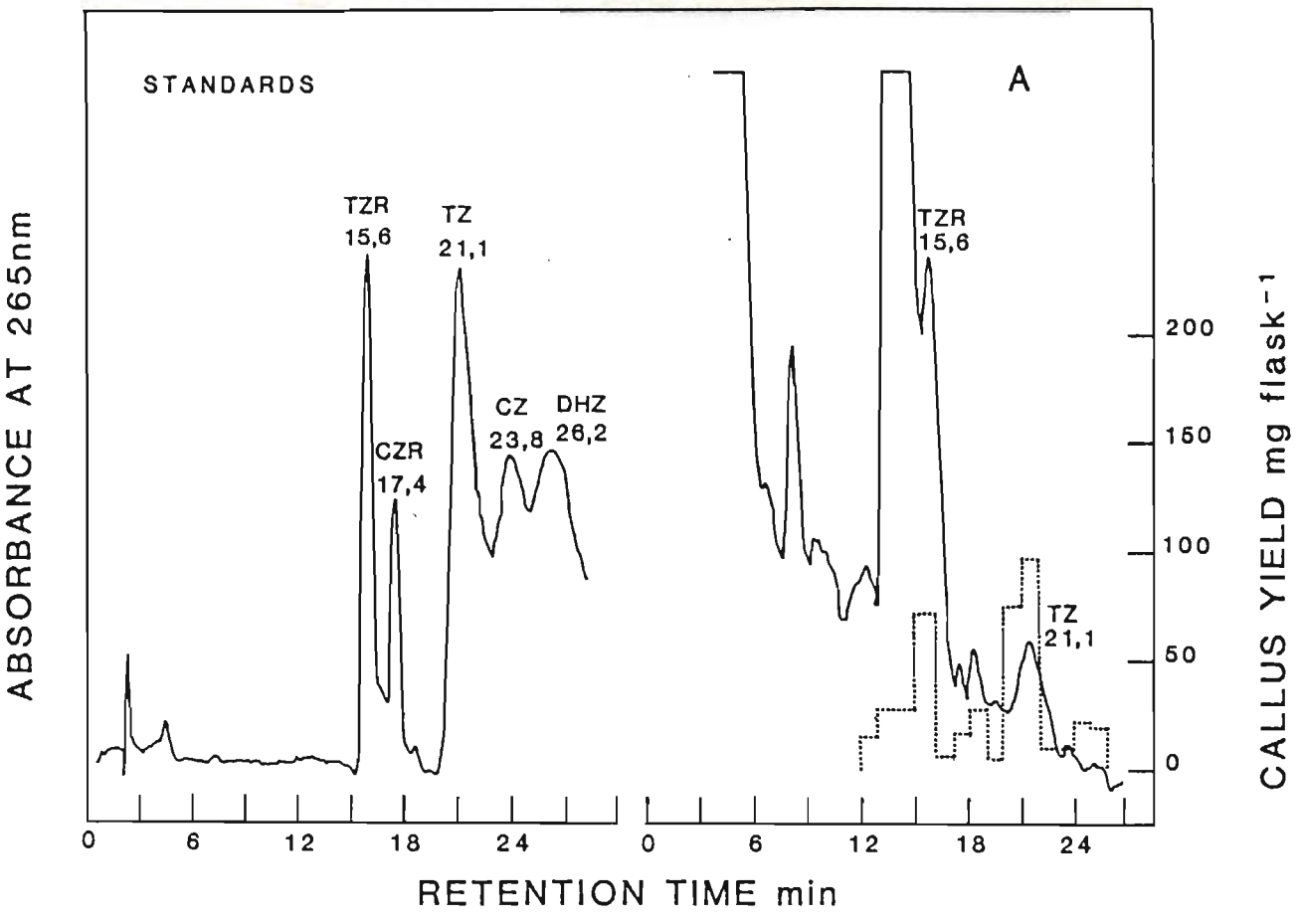

Figure 4.3. Separation of authentic cytokinins and biologically active peak A (Figure 4.2; 760-800 millilitres) by reversed phase HPLC. Flow rate 1,5 millilitres per minute; mobile phase 10 per cent acetonitrile isocratically for 5 minutes, to 20 per cent acetonitrile in 5 minutes. The broken line histogram represents the biological activity associated with the HPLC fractions collected. DHZ=dihydrozeatin; CZ=*cis*-zeatin; TZ=*trans*-zeatin; TZR=*trans*-ribosylzeatin; CZR=*cis*-ribosylzeatin.

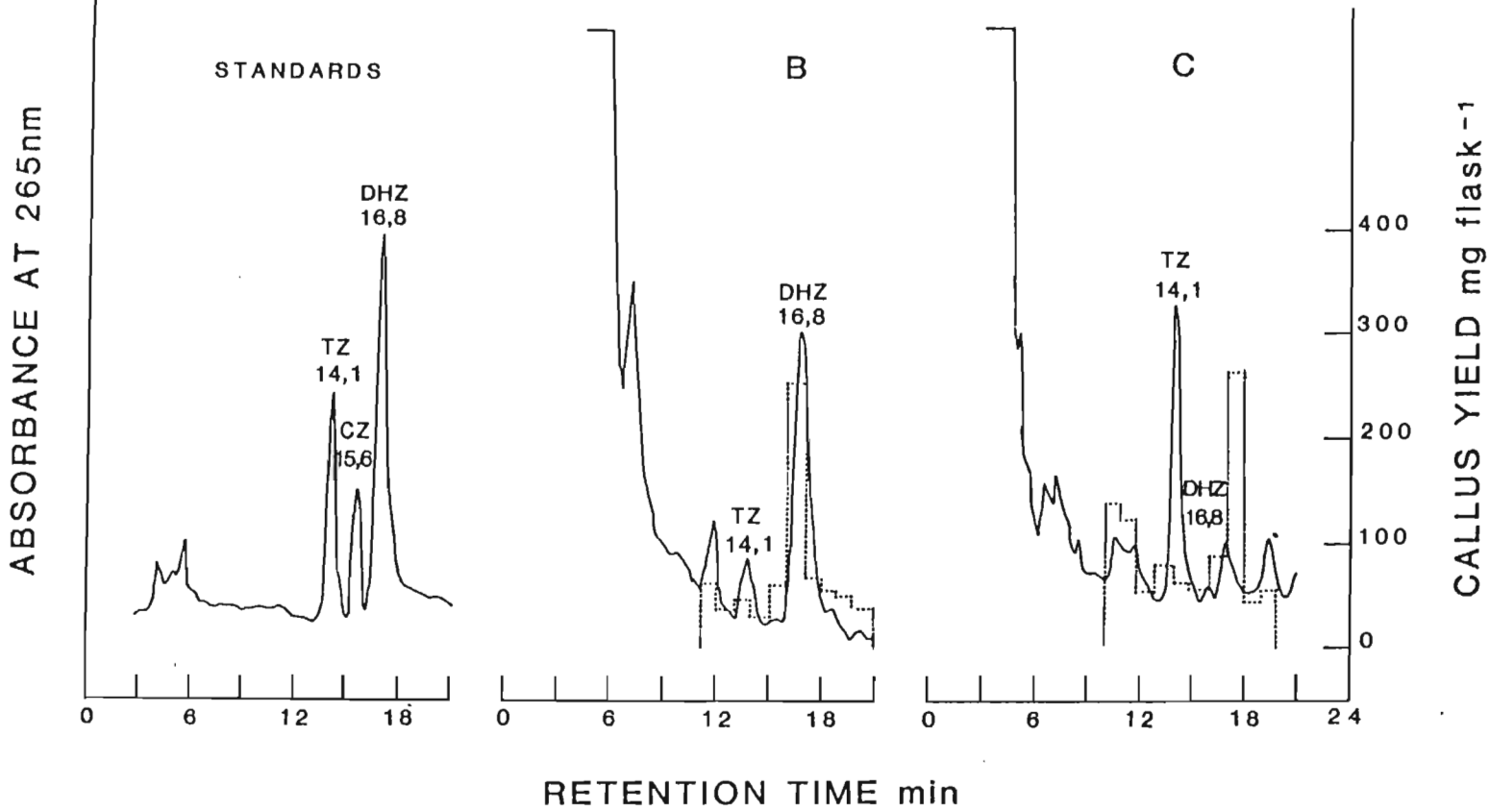

Figure 4.4. Separation of authentic cytokinins and biological active peaks B (Figure 4.2; 1080-1120 millilitres) and C (Figure 4.2; 1200-1240 millilitres) by reversed phase HPLC. Flow rate 2 millilitres per minute; mobile phase water to 8 per cent acetonitrile in 1 minute; isocratically for 12 minutes, to 14 per cent acetonitrile in 8 minutes. The broken line histogram represents the biological activity associated with the HPLC fractions collected. DHZ=dihydrozeatin; CZ=*cis*-zeatin; TZ=*trans*-

To study the distribution of cytokinins, tuberous roots were divided into three sections referred to as phloem, outer xylem and inner xylem. Extracts of 30 grammes of fresh material of these sections were tested for cytokinin activity. Most cytokinin activity which co-chromatographed with zeatin and ribosylzeatin was recorded in the outer xylem section (Figure 4.5). In the phloem region similar activity was also detected. In addition, cytokinin-like activity was found in the region which co-chromatographed with glucoside conjugates of cytokinins. Very little activity was found in the inner xylem. Fractionation on Sephadex LH-20 showed that in the outer xylem most of the cytokinin activity co-eluted with dihydrozeatin (Figure 4.6). In the phloem extracts most activity was again associated with dihydrozeatin. More polar biologically active compounds were present, which confirms the possible presence of glucosyl conjugates of cytokinins.

Extracts of young tuberous roots and primary roots of the same cassava plants were tested for their cytokinin-like activity. The tuberous root extracts showed much higher levels of cytokinin activity than the primary roots (Figure 4.7).

EXPERIMENT 4.2. Cytokinin activity levels during tuberous root development

A number of environmental factors play an important role in the tuberization process. Short days, low nitrogen supply and low temperatures are conditions which promote tuberization. A number of workers have manipulated these conditions and studied their effect on the levels of endogenous cytokinins. It has generally

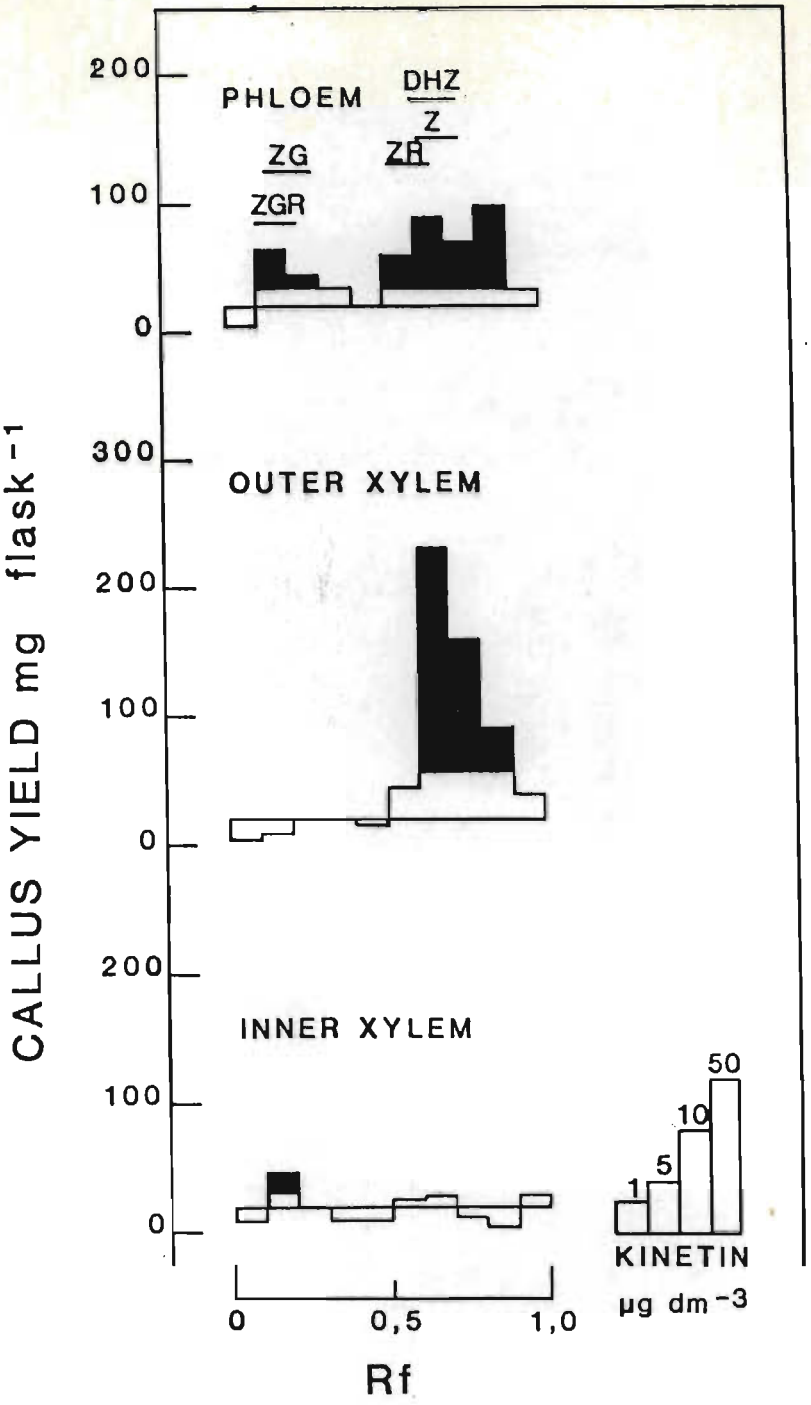

Figure 4.5 Soybean callus bioassay from 30 grammes of fresh phloem, outer xylem and inner xylem material of tuberous roots of cassava cultivar MSAF 2. The extracts were purified on Dowex 50 cation exchange resin and separated on Whatman No 1 chromatography paper using *iso*-propanol: 25 per cent ammonium hydroxide: water (10: 1: 1 v/v) as the solvent. The shaded areas represent regions significantly different from the control at 5 % level. Z=zeatin; ZR=ribosylzeatin; ZG=glucosylzeatin; ZGR=glucosylribosylzeatin.

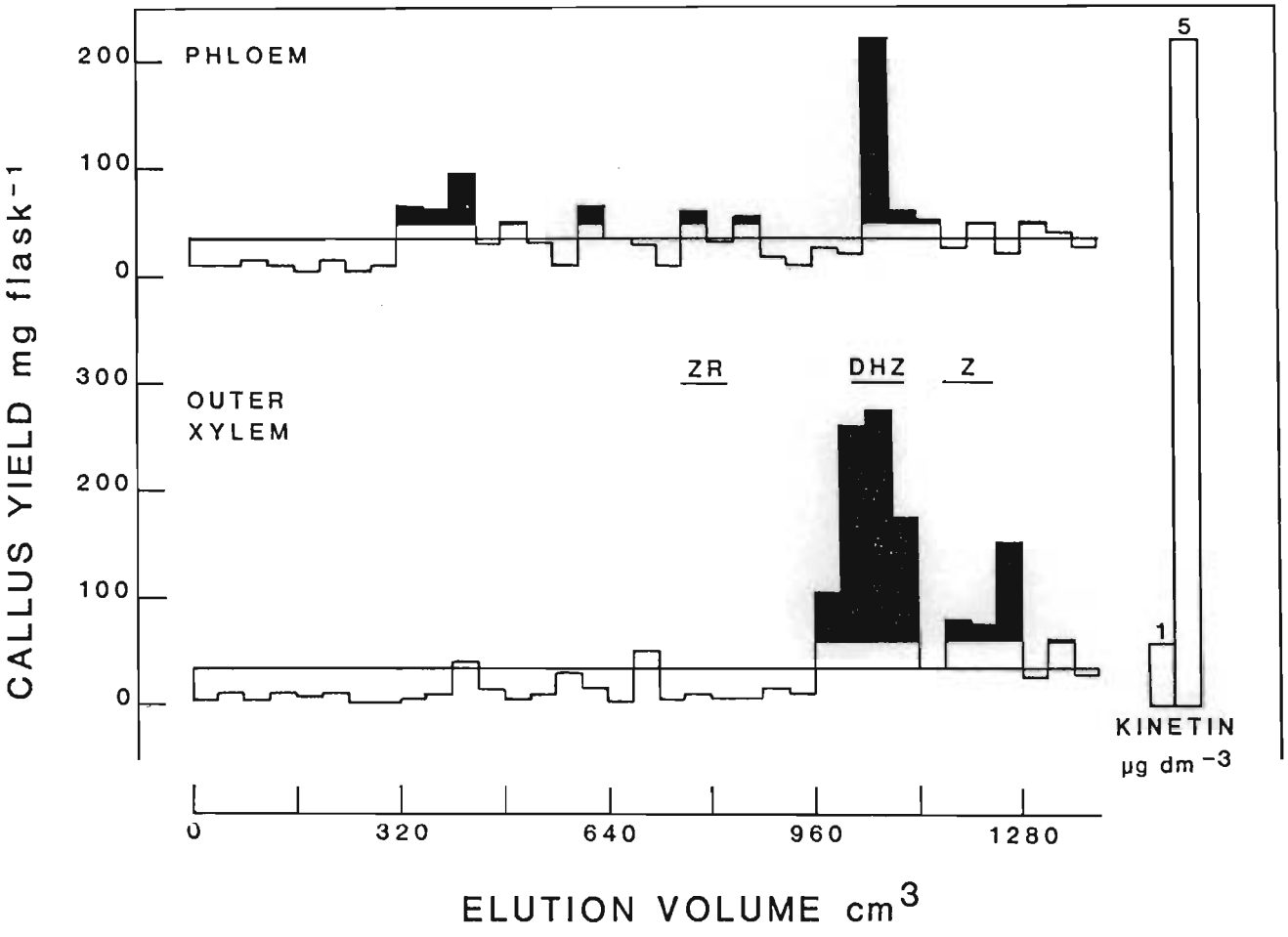

Figure 4.6 Soybean callus bioassay from the equivalent of 30 grammes fresh xylem and phloem material of tuberous roots of cassava cultivar MSAF 2. The extract was separated on chromatography paper and the active zone (Figure 4.5; R_f 0,6-0,9) eluted and then fractionated on a Sephadex LH-20 column using 10 per cent methanol. The shaded areas represent regions significantly different from the controls at the 5 per cent level. Z= zeatin; ZR= ribosylzeatin; DHZ= dihydrozeatin.

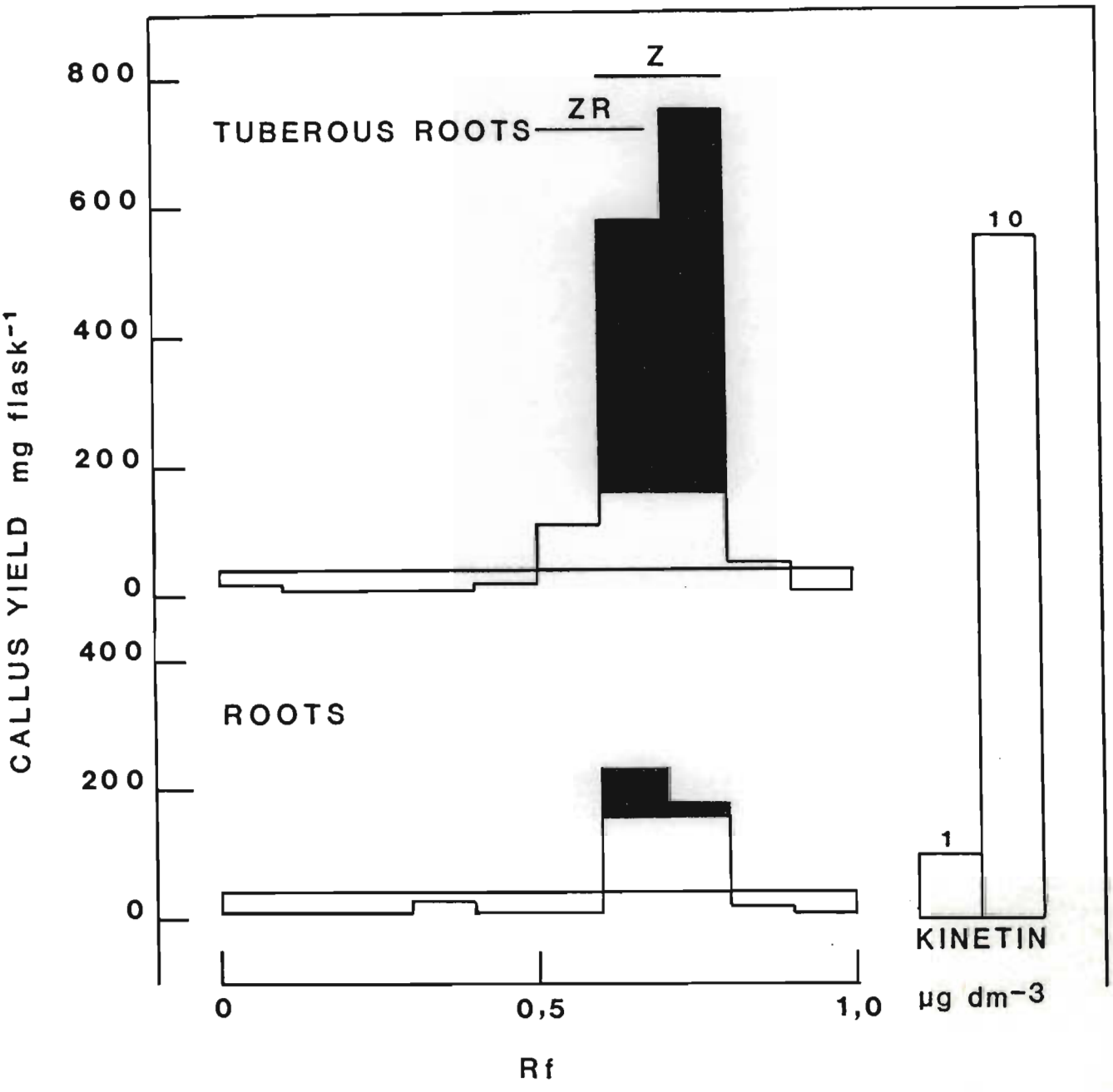

Figure 4.7. Soybean callus bioassay from 16 grammes of fresh material of tuberous roots and roots of cassava cultivar MSAF 2. The extracts were purified on Dowex 50 cation exchange resin and separated on Whatman No 1 chromatography paper using *iso*-propanol: 25 per cent ammonium hydroxide: water (10: 1: 1 v/v) as the solvent. The shaded areas represent regions significantly different from the control the 5 per cent level. Z=zeatin; ZR=ribosylzeatin.

been found that endogenous cytokinin levels increase when plants are moved from non-inducing conditions to inducing conditions (FORSLINE and LANGILLE, 1975; MAUK and LANGILLE, 1978; SATTELMACHER and MARSCHNER, 1978a and 1978b). At the onset of tuberization the levels of cytokinins at the tuberization site suddenly increase (RADIN and LOOMIS, 1971; SATTELMACHER and MARSCHNER, 1978a and 1978b). Studies of the the fluctuation of the cytokinin levels during the life cycle of tuber plants gave varying results. RADIN and LOOMIS (1971) found a gradual increase of the level in radish roots, while MAUK and LANGILLE (1978) with potato found the level to decrease with time.

In the present investigation cytokinin activity in cassava was studied under different nitrogen applications. Furthermore, the effect of gibberellic acid treatment and pruning on endogenous cytokinins was studied. Finally, the cytokinin levels in the tuberous roots of field-grown plants were followed over a 18 month period.

Experimental procedure

Effect of nitrogen on endogenous cytokinin levels

Stakes of cassava cultivar MSAF 1 were planted in pots containing 12 kilogrammes of the topsoil: sand mixture. Treatment N_0 was given 600 millilitres of the standard nutrient solution (Table 2.1) per week excluding KNO_3 and $Ca(NO_3)_2$, while treatment N_1 was given the nutrient solution excluding $Ca(NO_3)_2$ but including KNO_3 (70 milligrammes per litres). The pots were placed in a randomized

block design, with six replications, and moved weekly. The plants were harvested at three times and samples were taken for cytokinin analysis.

The procedures of the other studies in this section have been described in Chapter 3.

Results

The two treatments of nitrogen had a significant influence on assimilate distribution of the cassava plants. At the first harvest, seven weeks after planting, no significant differences were found, most likely because the shoots still made use of minerals stored in the planting stakes for their growth (Figure 4.8). At this stage no tuberous root formation had taken place. Twelve weeks after planting, tuberous root formation commenced and a significantly higher tuberous root mass was recorded in the plants grown on a low nitrogen medium. Twenty four weeks after planting, differences in shoot and tuberous root growth between the treatments became more pronounced. The effect of nitrogen supply on assimilate distribution is illustrated by the shoot/root ratios (Table 4.1). At the third harvest this ratio was almost twice as high in the N_1 treatment as in the N_0 treatment. A remarkable finding is that the total mass at the latest harvest is highest in the N_0 treated plants despite the smaller leaf area per plant. This could confirm the view proposed by several workers that a high sink demand stimulates the photosynthetic activity of the leaves (NÖSBERGER and HUMPHRIES, 1965; SWEET and WAREING, 1966; WAREING and PATRICK, 1973).

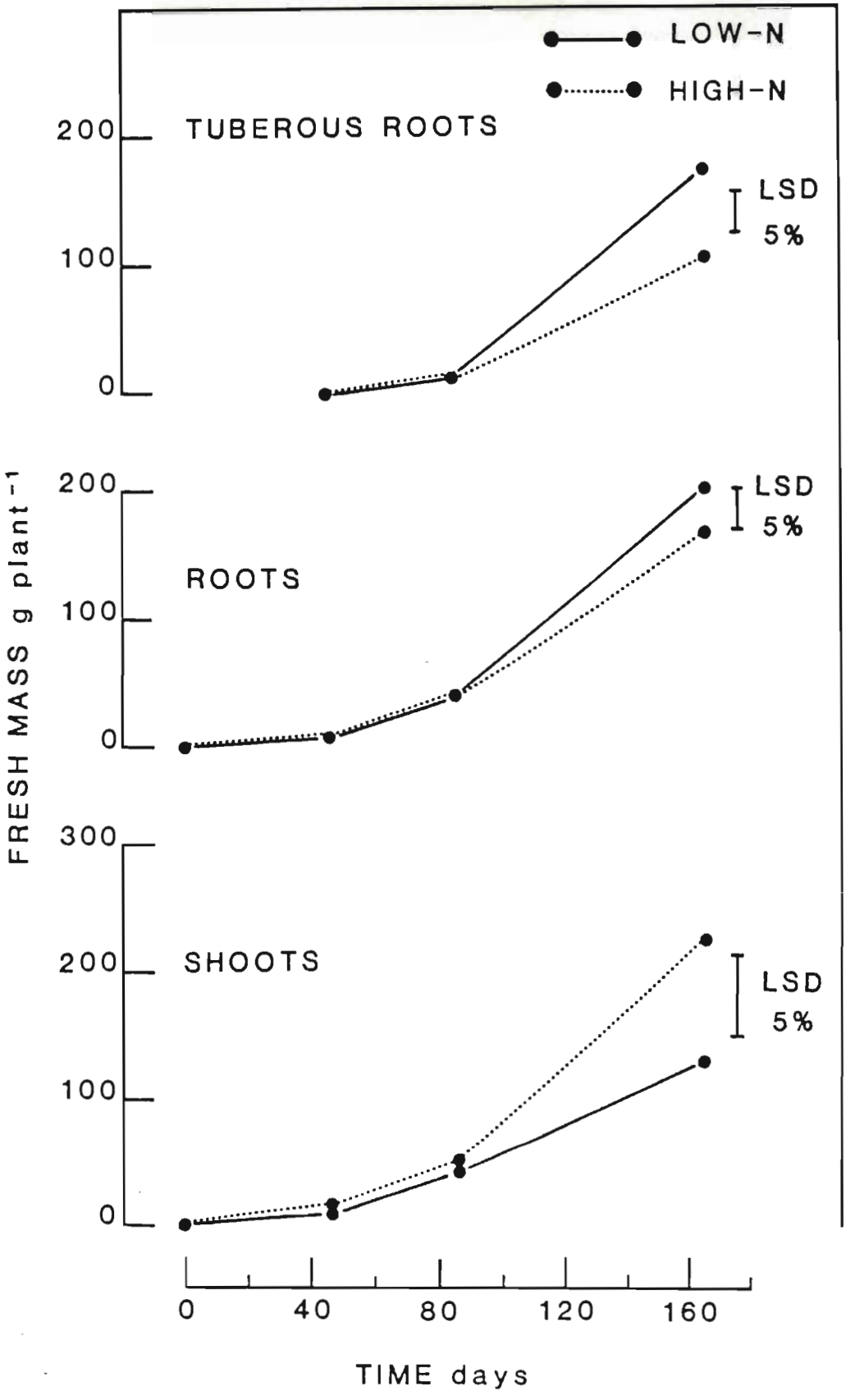

Figure 4.8. The effect of nitrogen supply on the dry matter distribution of cassava cultivar MSAF 1.

Table 4.1 The effect of nitrogen supply on the shoot/root ratio of cassava cultivar MSAF 1.

Time after planting (days)	Low-N	High-N	Significance
45	0,94	0,97	n.s [1]
85	0,78	0,99	** [2]
165	0,35	0,84	**

[1] Not significant [2] Highly significant.

Cytokinin analysis of leaves and tuberous roots were done at the second and the third harvests. At the second harvest cytokinin activity which co- chromatographed with zeatin and ribosylzeatin was found in the leaves. The level expressed in kinetin equivalents was slightly higher in the N_1 treatment (Figure 4.9; Table 4.2). At the third harvest the levels in both treatments had decreased. The level was, however, still the highest in the N_1 treatment. At the second harvest the cytokinin level in the tuberous roots was highest in the N_1 treatment (Figure 4.10; Table 4.2). At the third harvest the levels differed little between the two treatments. To further establish the nature of the cytokinins, the biologically active fractions of the second harvest were separated on a Sephadex LH-20 column. The results show that the levels of total cytokinin activity differed little between the two treatments (figure 4.11). However, the pattern of cytokinin activity was not the same. In the N_0 treated plants the major peak of cytokinin activity co-eluted with ribosylzeatin, while in the N_1 treated plants the major peak of activity co-eluted with dihydrozeatin.

In a further experiment cassava plants of cultivar MSAF 2 were treated with 500 milligrammes per litre gibberellic acid as described in Chapter 3. The application of gibberellic acid changed the dry matter distribution in the plant considerably. Samples of tuberous roots were taken at harvest and assayed for cytokinin activity. The control plants, where rapid tuber bulking occurred, showed high levels of cytokinin activity, in particular in the zone co-eluting with zeatin and ribosylzeatin (Figure 4.12). In the gibberellic acid treated plants, where the emphasis was on shoot growth, the levels of cytokinin activity were low in comparison with

Table 4.2. Total cytokinin activity in tuberous roots of cassav cultivar MSAF 1 grown in a low and a high nitrogen medium. The activity i expressed in kinetin equivalents as nanogrammes per gramme fres material.

Plant part analyzed	Time after planting (days)	Low-N (ng g^{-1})	High-N (ng g^{-1})
Tuberous root	86	1,40	34,35
Tuberous root	168	1,24	1,26
Leaf	86	2,75	3,94
Leaf	168	0,62	0,96

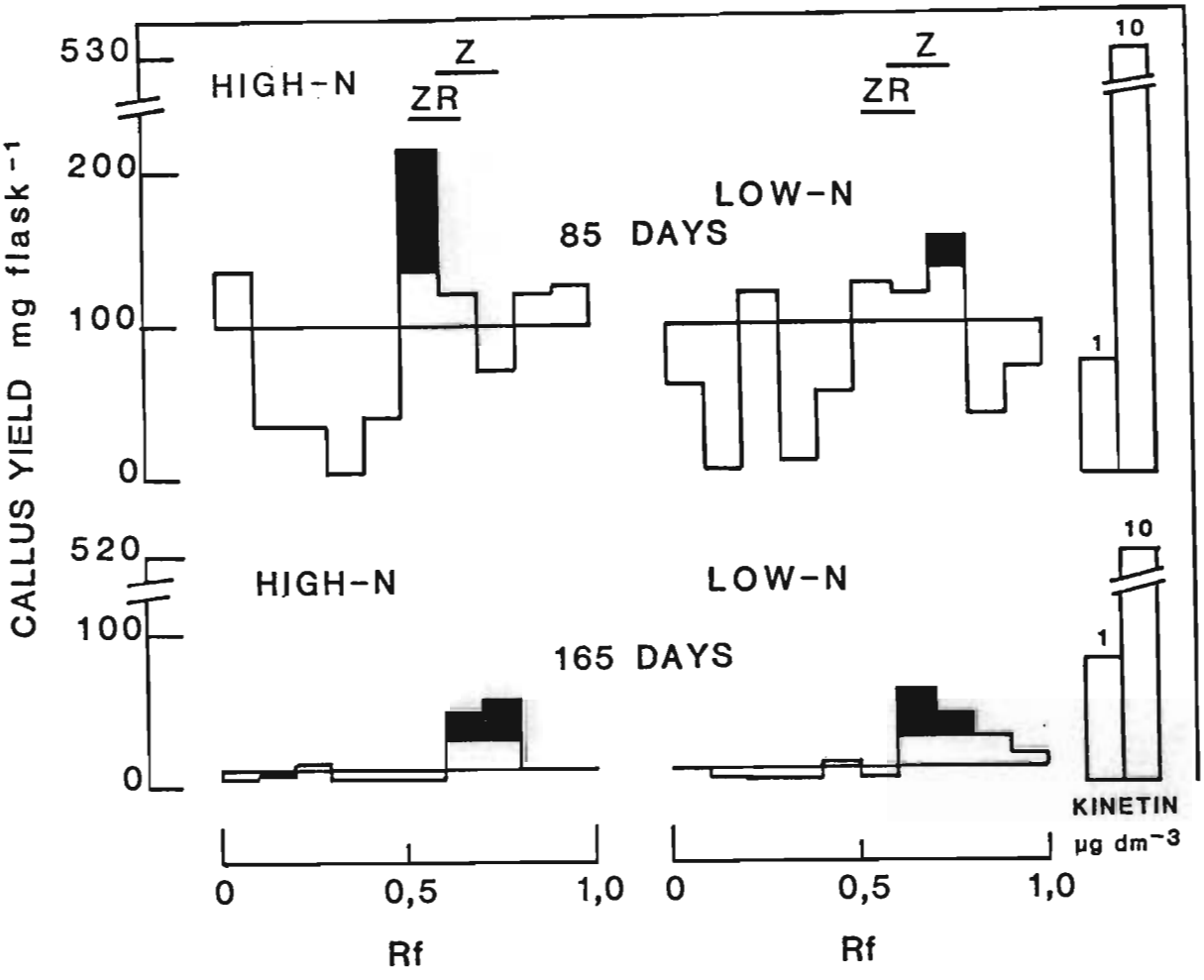

Figure 4.9. Soybean callus bioassay from 16 grammes of leaf material of cassava cultivar MSAF 1 at two levels of nitrogen supply. The extracts were purified on a Dowex 50 cation exchange resin and separated on Whatman No 1 chromatography paper using *iso*-propanol: 25 per cent ammonium hydroxide: water (10: 1: 1 v/v) as the solvent. The shaded areas represent the regions significantly different from the controls at the 5 per cent level. Z=zeatin; ZR=ribosylzeatin.

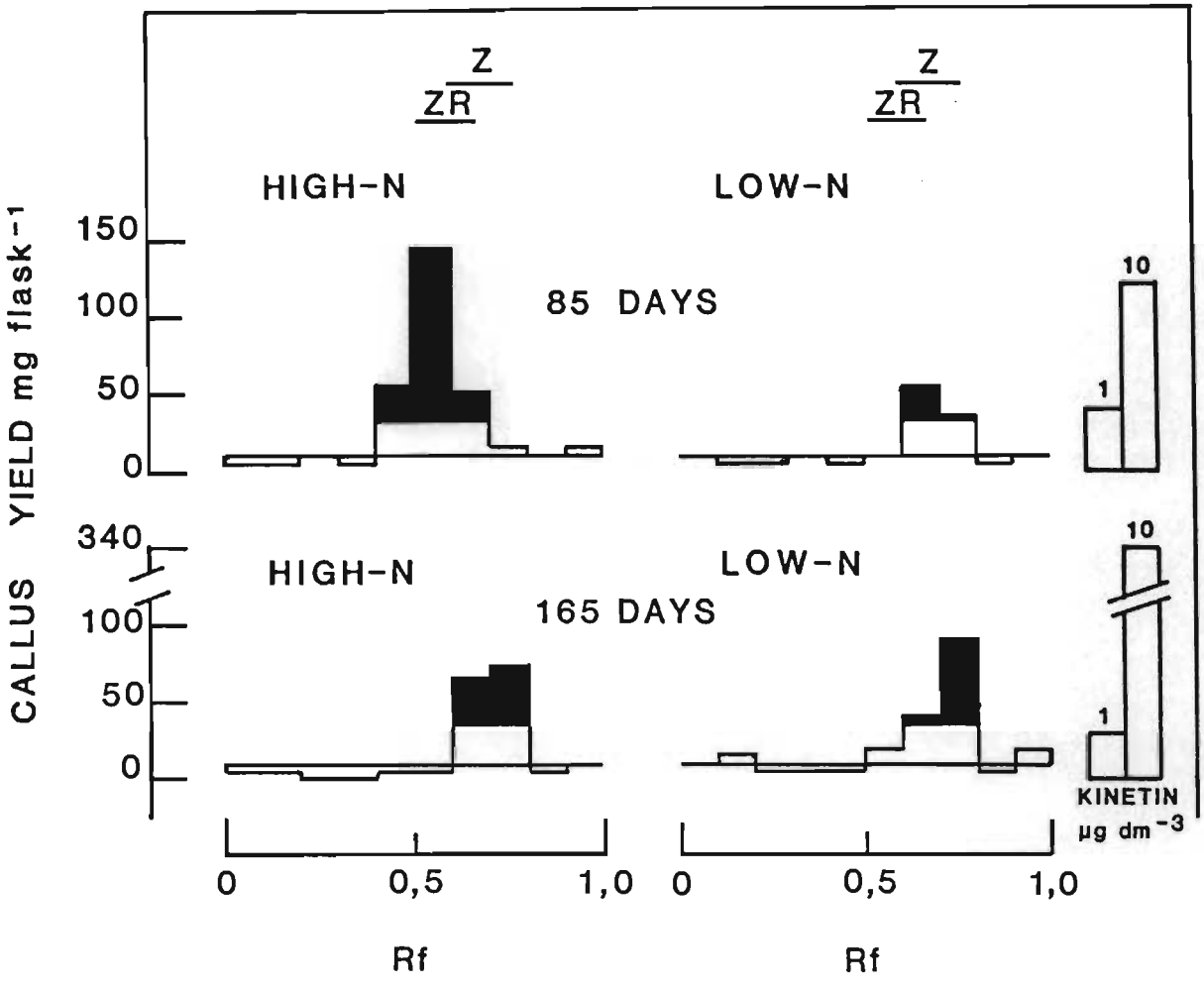

Figure 4.10. Soybean callus bioassay from 16 grammes of tuberous root material of cassava cultivar MSAF 1 at two levels of nitrogen supply. The extracts were purified on Dowex 50 cation exchange resin and separated on Whatman No 1 chromatography paper using *iso*-propanol: 25 per cent ammoniumhydroxide: water (10: 1: 1 v/v) as the solvent. The shaded areas represent the regions significantly different from the controls at the 5 per cent level. Z=zeatin; ZR=ribosylzeatin.

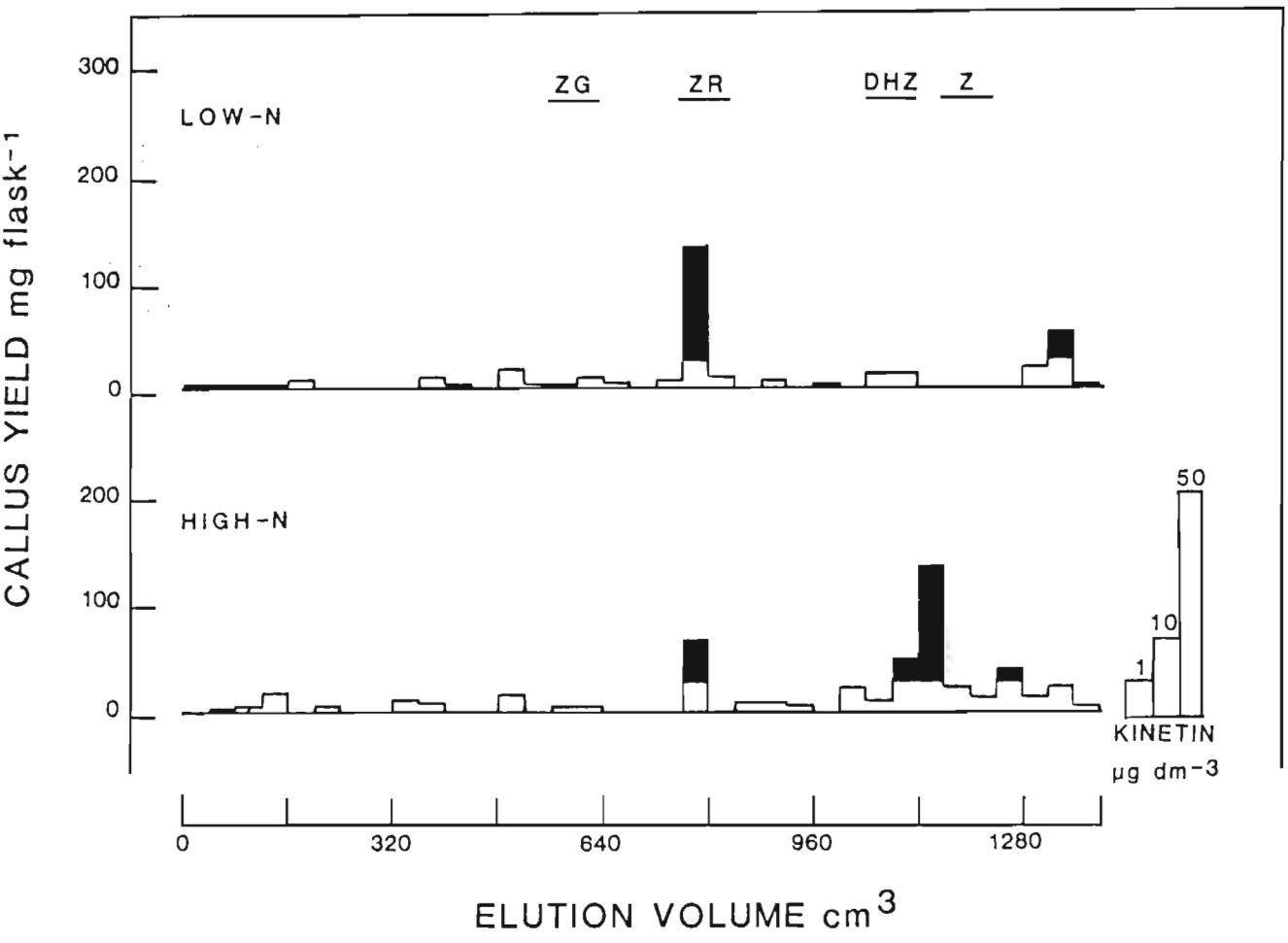

Figure 4.11. Soybean callus bioassay from the equivalent of 16 grammes fresh tuberous root material of cassava cultivar MSAF 1 at two levels of nitrogen supply. The extract was separated on chromatography paper and the active zone (Figure 4.10;R_f 0,5-0,8) eluted and then fractionated on a Sephadex LH-20 column using 10 per cent methanol. The shaded areas represent regions significantly different from the controls at the 5 per cent level. Z= zeatin; ZR= ribosylzeatin; DHZ= dihydrozeatin.

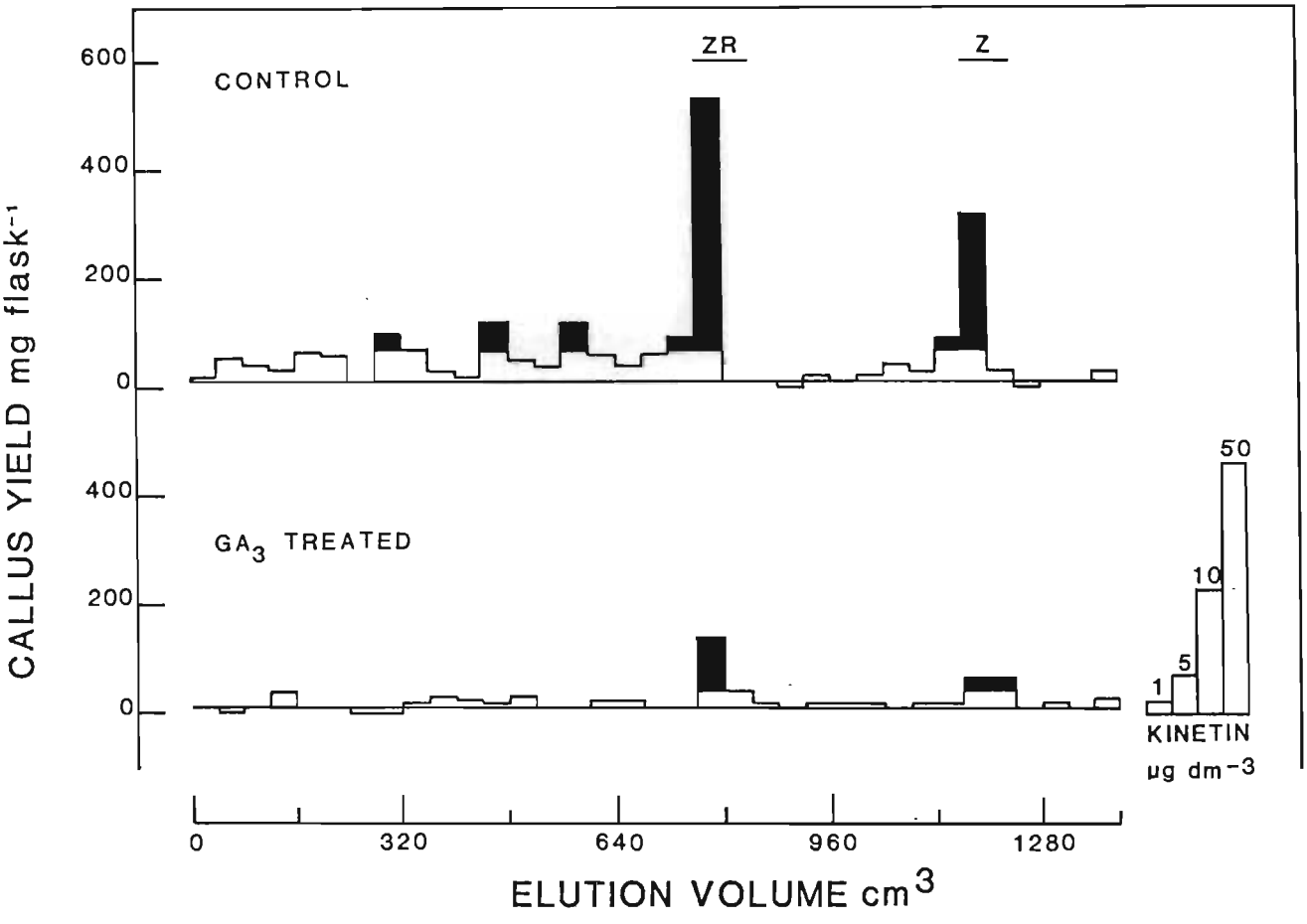

Figure 4.12 Soybean callus bioassay from the equivalent of 16 grammes fresh tuberous root material of cassava cultivar MSAF 2 treated with gibberellic acid. The extracts were separated on chromatography paper and the R_f zone 0,2-0,9 eluted and then fractionated on a Sephadex LH-20 column using 10 per cent methanol. The shaded areas represent regions significantly different from the controls at the 5 per cent level. Z= zeatin; ZR= ribosylzeatin.

the controls.

In a third study, the shoots of cassava were removed and the plants were left to re-grow (see Chapter 3). Three weeks and six weeks after removal the plants were harvested and samples were taken of both leaves and tuberous roots for cytokinin analysis. Low levels of cytokinins which co-chromatographed with ribosylzeatin and zeatin were found in the leaves at all three harvests (Figure 4.13). The lowest level of cytokinin activity was found three weeks after removal. Shoot removal led to a reduction in cytokinin activity which co-chromatographed with ribosylzeatin and zeatin in the tuberous roots (Figure 4.14). Total cytokinin levels dropped from 12,0 to 3,0 nanogrammes per gramme, recovering somewhat later (Table 4.3).

Finally, a study was done on the changes in cytokinin levels in the tuberous roots of cassava grown for a 18 month period in the field. The extracts were purified, separated on paper and R_f zone 0,2-0,9 further separated on a Sephadex LH-20 column. The highest level of total cytokinin-like activity was found just after tuber initiation in January (Figure 4.15; Table 4.4). The major peak of activity co-eluted with ribosylzeatin, while a lesser peak corresponded with zeatin. In May the total cytokinin level had dropped. Some activity was found to co-elute with *iso*-pentenyladenine and *iso*-pentenyladenosine. In October and December of the following season a high level of biological activity was detected (Figure 4.16). Biological activity was also recorded in the region which co-eluted with glucoside conjugates of cytokinins. In March and May an identical pattern was found (Figure

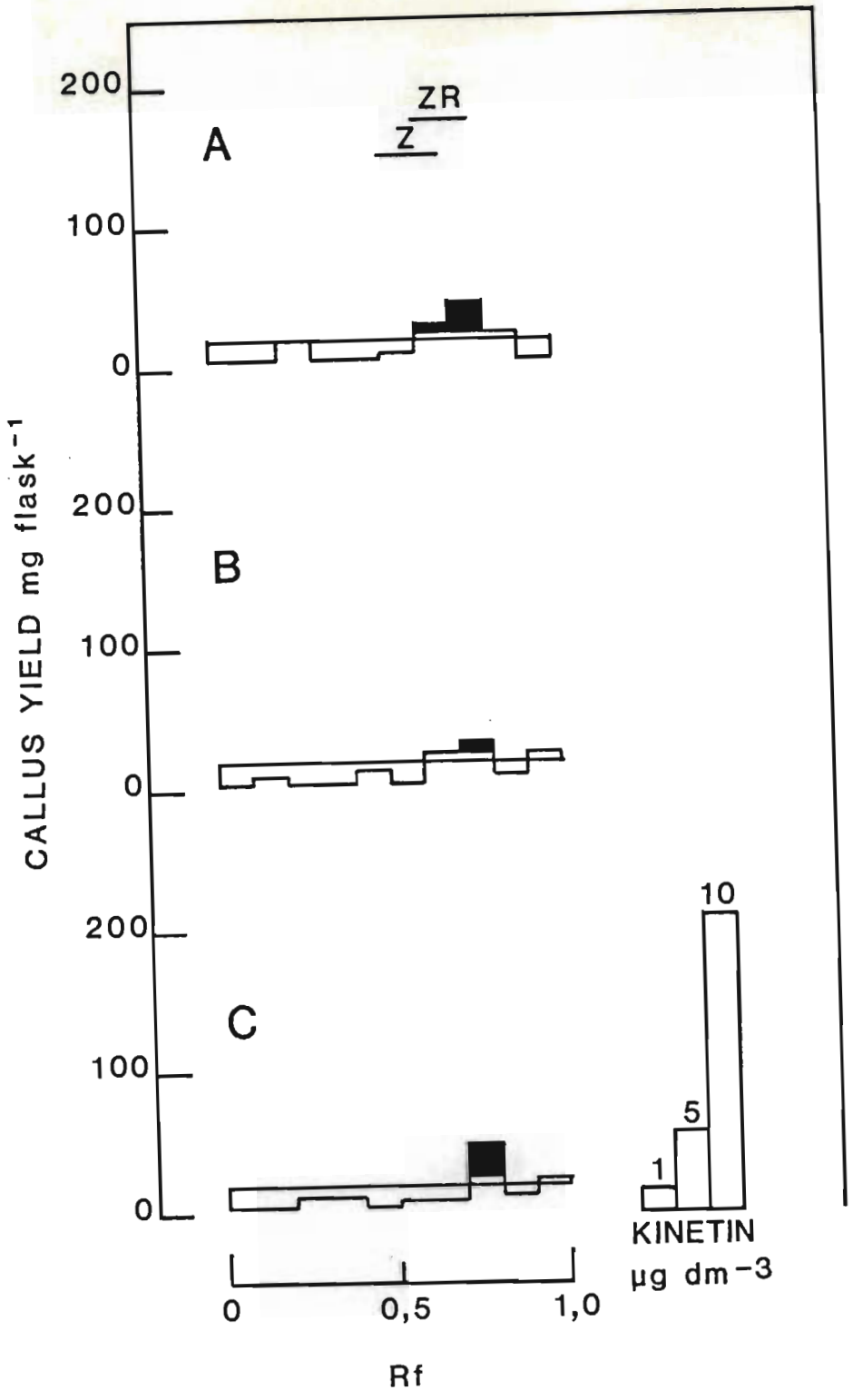

Figure 4.13. Soybean callus bioassay from 16 grammes of leaf material of cassava cultivar MSAF 1 before (A) and three (B) and six weeks (C) after shoot removal. The extracts were purified on Dowex 50 cation exchange resin and separated on Whatman No 1 chromatography paper using *iso*-propanol: 25 per cent ammonium hydroxide: water (10: 1: 1 v/v) as the solvent. The shaded areas represent the regions significantly different from the controls at the 5 per cent level. Z=zeatin; ZR=ribosylzeatin.

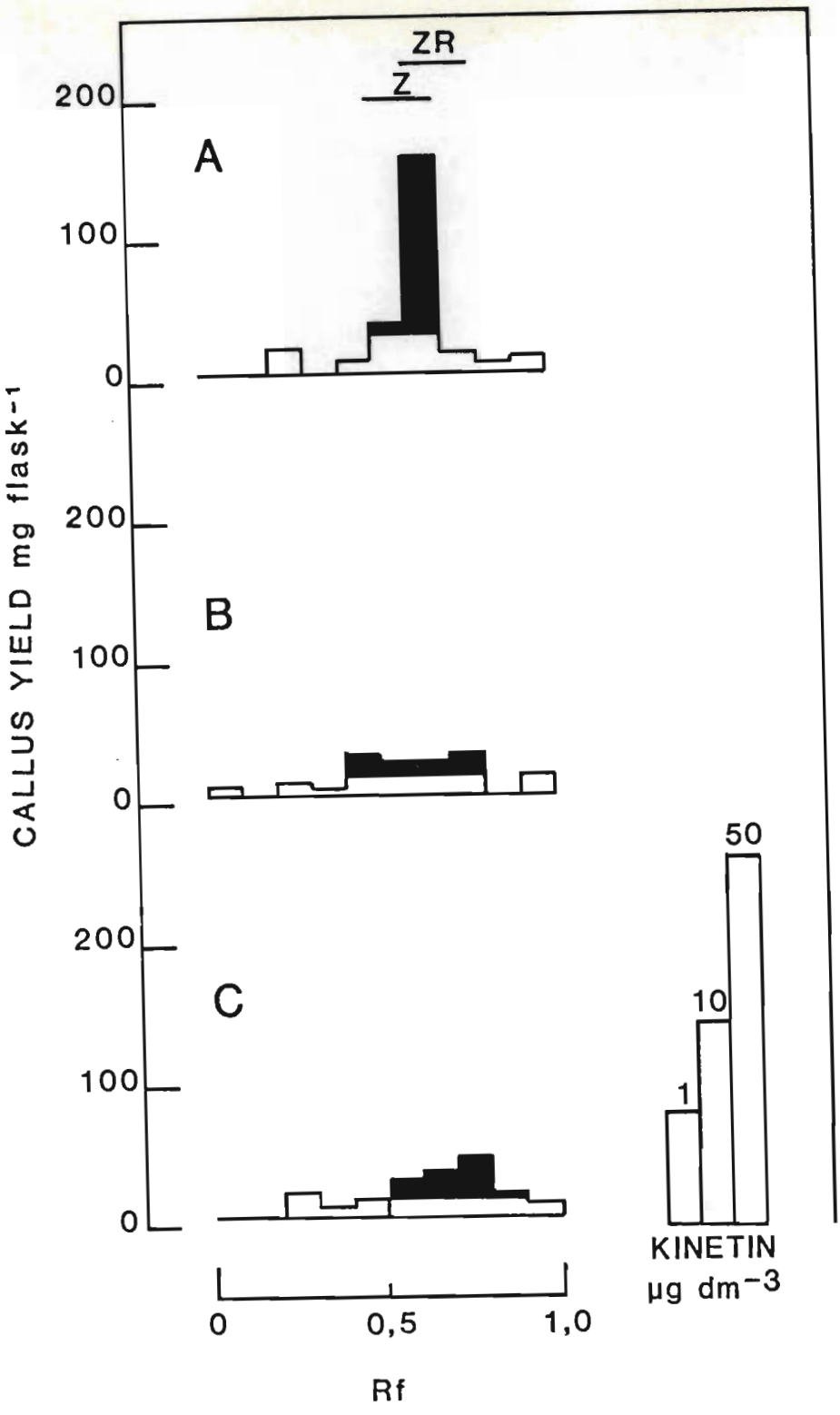

Figure 4.14. Soybean callus bioassay from 16 grammes of tuberous root material of cassava cultivar MSAF 1 before (A) and three (B) and six weeks (C) after shoot removal. The extracts were purified on Dowex 50 cation exchange resin and separated on Whatman No 1 chromatography paper using *iso*-propanol: 25 per cent ammonium hydroxide: water (10: 1: 1 v/v) as the solvent. The shaded areas represent the regions significantly different from the controls at the 5 per cent level. Z=zeatin; ZR=ribosylzeatin.

Table 4.3. Total cytokinin-like activity in tuberous roots and leaves of cassava cultivar MSAF 1 before and after shoot removal. The activity is expressed in kinetin equivalents.

Time	Leaves ($ng\ g^{-1}$)	Tuberous roots ($ng\ g^{-1}$)
Before shoot removal	0,7	12,0
Three weeks after shoot removal	0,2	3,0
Six week after shoot removal	0,6	4,4

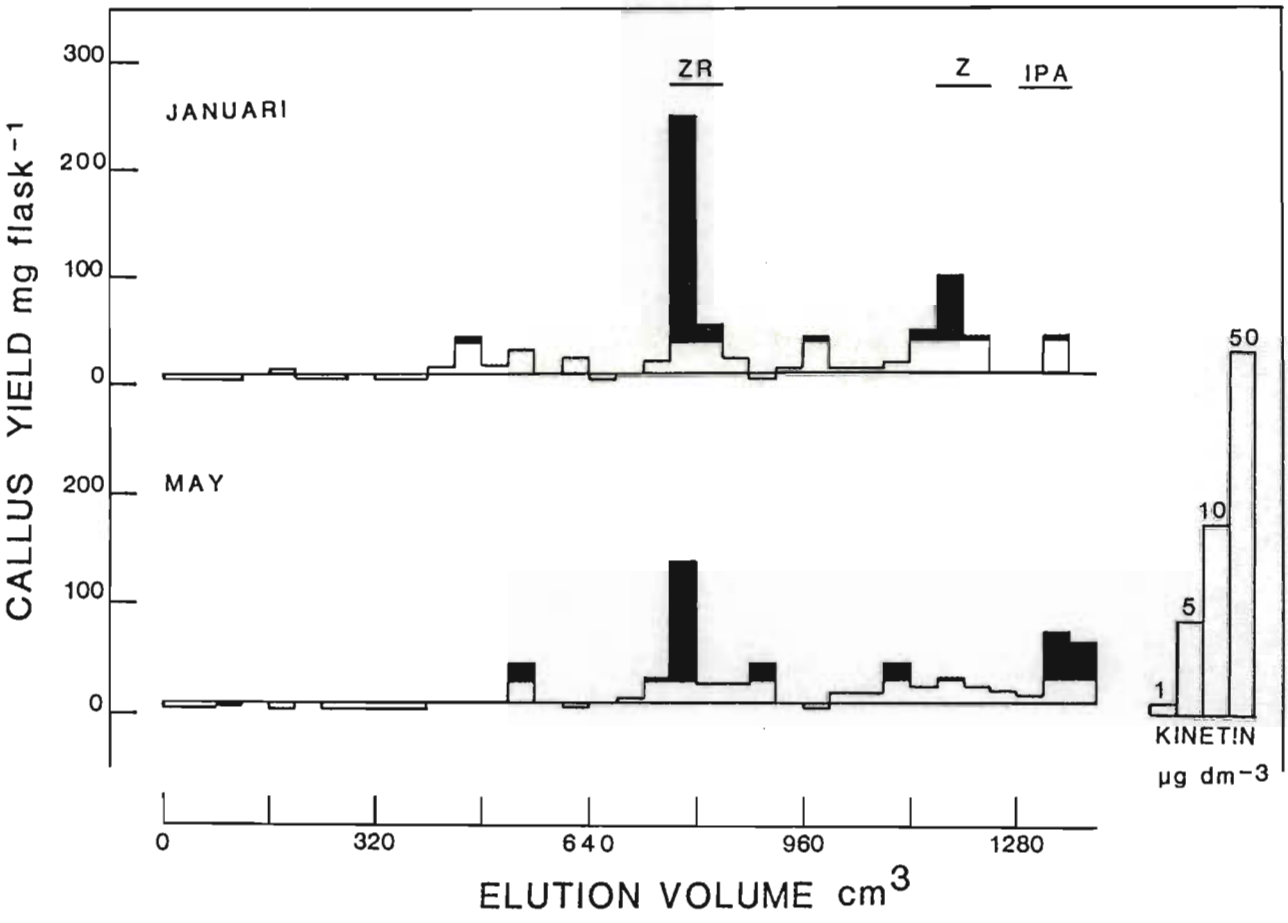

Figure 4.15. Soybean callus bioassay from the equivalent of 16 grammes fresh tuberous root material of cassava cultivar MSAF 2 harvested in January and May 1982. The extracts were separated on chromatography paper and the R_f zone 0,2-0,9 eluted and then fractionated on a Sephadex LH-20 column using 10 per cent methanol. The shaded areas represent regions significantly different from the controls at the 5 per cent level. Z= zeatin; ZR= ribosylzeatin; DHZ= dihydrozeatin; IPA=*iso*-pentenyladenosine.

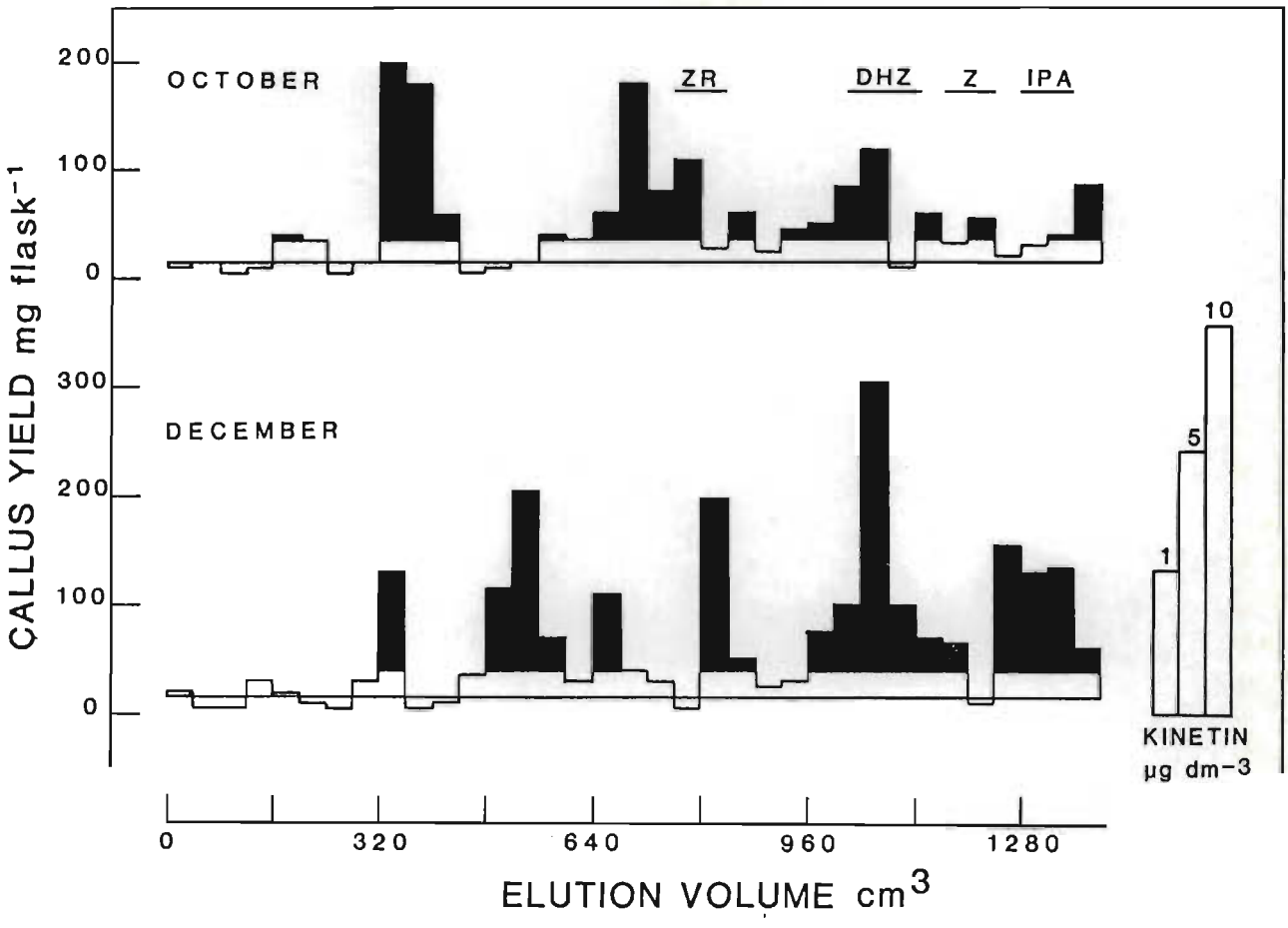

Figure 4.16. Soybean callus bioassay from the equivalent of 16 grammes fresh tuberous root material of cassava cultivar MSAF 1 harvested in October and December 1982. The extracts were separated on chromatography paper and the R_f zone 0,2-0,9 eluted and then fractionated on a Sephadex LH-20 column using 10 per cent methanol. The shaded areas represent regions significantly different from the controls at the 5 per cent level. Z= zeatin; ZR= ribosylzeatin; DHZ= dihydrozeatin; IPA=*iso*-pentenyladenosine.

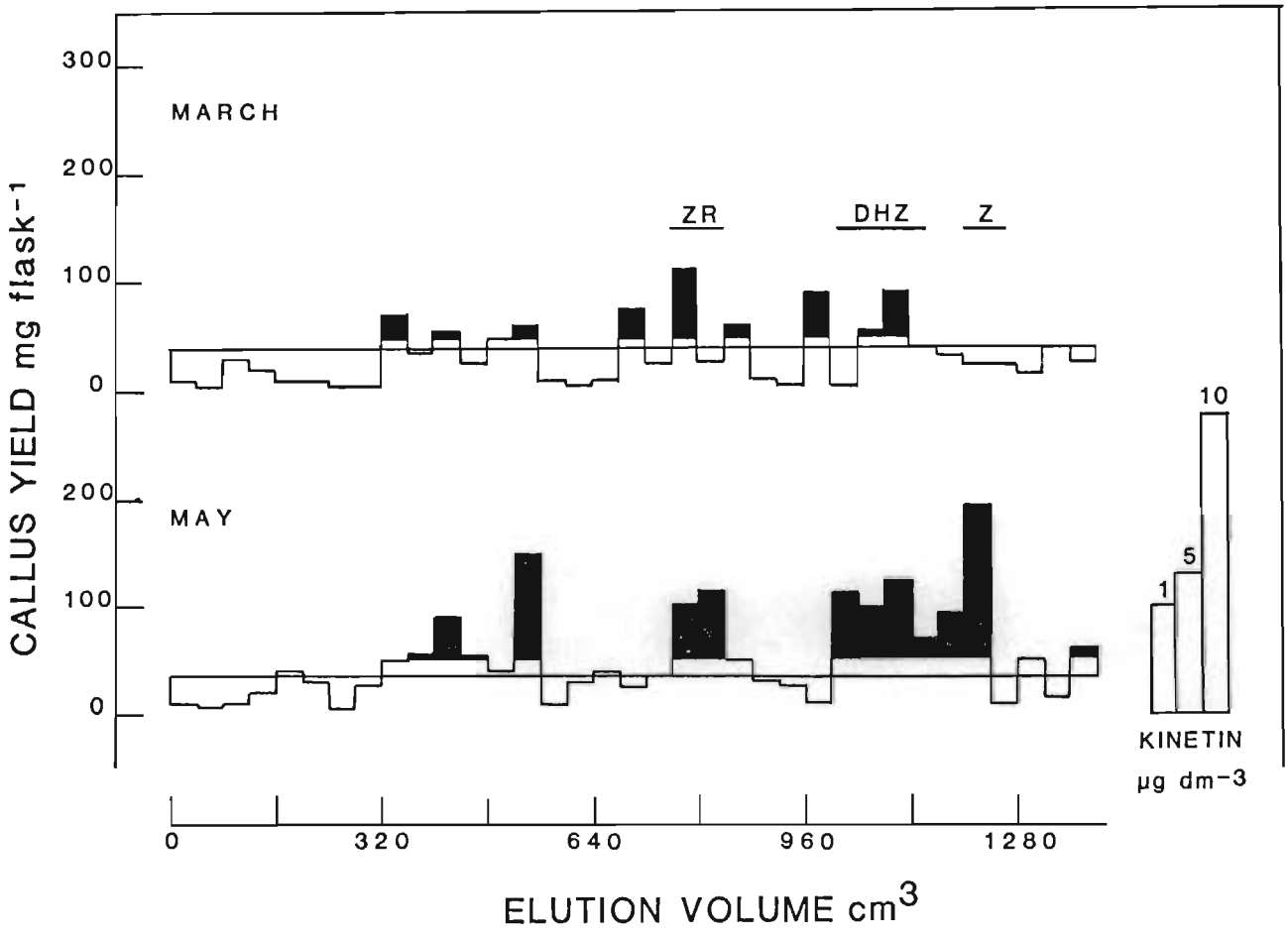

Figure 4.17. Soybean callus bioassay from the equivalent of 16 grammes fresh tuberous root material of cassava cultivar MSAF 1 harvested in March and May 1983. The extracts were separated on chromatography paper and the R_f zone 0,2-0,9 eluted and then fractionated on a Sephadex LH-20 column using 10 per cent methanol. The shaded areas represent regions significantly different from the controls at the 5 per cent level. Z= zeatin; ZR= ribosylzeatin; DHZ= dihydrozeatin.

Table 4.4 Cytokinin-like activity of tuberous roots of cassava cultivar MSAF 2 harvested at different times from the field. The activity is expressed in kinetin equivalents.

Time harvested	Cytokinin activity ($ng\ g^{-1}$)
January 1982	66,2
May 1982	24,9
October 1982	15,9
December 1982	23,6
March 1983	4,4
May 1983	12,2

4.17), but the levels were considerably lower than at the previous harvests (Table 4.4). It should, however, be borne in mind that the tuberous roots were larger and that a diluting of the cytokinins had taken place.

EXPERIMENT 4.3 *In vitro* studies on the effect of plant hormones on the growth of tuberous root material.

In the past *in vitro* studies have played an important role in the research of the hormonal regulation of the tuberization process. Although the *in vitro* studies do not represent the *in vivo* situation, they give some insight as to the effect of plant hormones on this process. TORREY and LOOMIS (1967) established that kinetin and auxin promote secondary growth in isolated roots of radish, grown on a sterile medium. This was later confirmed by others using a variety of tuber crops (ESASHI and LEOPOLD, 1968; PALMER and SMITH, 1970; PETERSON, 1973). A number of workers have attempted to initiate tuber formation in isolated potato stolons, by adding abscisic acid to the medium, but without success (PALMER and SMITH, 1969; SMITH and RAPPAPORT,1969; CLAVER, 1970). In the following experiments the growth of excised tuberous root material of cassava was studied.

Results

In the first experiment the effect of benzyladenine at $5x10^{-6}M$, naphtalene acetic acid at $5x10^{-6}M$ and gibberellic acid at $10^{-6}M$ on isolated tissue from the phloem and the outer xylem region of cassava tuberous roots (Figure 1.1) was studied. The results are summarized

in Table 4.5. Because of contamination in several flasks the mass of each individual piece of callus of the remaining flasks was weighed to obtain an adequate number of replications. Benzyladenine initiated tissue growth in the xylem material and to a lesser extent in the phloem material (Table 4.5). A single addition of the other two hormones did not lead to any significant growth in terms of callus yield. The combined addition of the hormones showed that the benzyladenine plus gibberellic acid treatment gave more growth than benzyladenine alone. This was, however, only the case with the xylem tissue. Most growth took place when benzyladenine plus naphtalene acetic acid were added to the medium. More growth occurred in the xylem material than in the phloem material.

In subsequent experiments the combined effect of benzyladenine plus naphtalene acetic acid on the growth of xylem tissue was investigated in a two-factorial experiment with four levels of naphtalene acetic acid and four levels of benzyladenine (Table 4.6). Some growth took place in the controls indicating that some endogenous hormones were probably present in the tissue. Benzyladenine and naphtalene acetic acid had highly significant effects on tissue growth. There was, however, no significant interaction effect of the two hormones. The naphtalene acetic acid treatment reached its optimum within the range of concentrations tested, while benzyladenine had apparently not yet reached its optimum concentration level.

In a final experiment the effect of abscisic acid on callus growth was studied. Outer xylem tissue was placed on a medium containing 5×10^{-6}M naphtalene acetic acid and 10^{-5}M benzyladenine.

Table 4.5 The effect of benzyladenine (BA), naphtalene acetic acid (NA and gibberellic acid (GA_3) on the growth of phloem and outer xylem tiss from tuberous roots of cassava cultivar MSAF 1.

Tissue origin	Hormones	Tissue growth (mg explant^{-1})
Xylem	BA	17,5
Xylem	NAA	-*)
Xylem	GA	-
Xylem	NAA+GA	-
Xylem	BA+GA	22,7
Xylem	BA+NAA	98,0
Phloem	BA	10,1
Phloem	NAA	-
Phloem	GA	-
Phloem	NAA+GA	-
Phloem	BA+GA	4,0
Phloem	BA+NAA	40,5

SD=16,7 *) No significant growth

Table 4.6 The effect of benzyladenine (BA) and naphtalene acetic acid (NAA) on the growth of outer xylem tissue from tuberous roots of cassava cultivar MSAF 1. The tissue growth is expressed in milligrammes per flask.

NAA	BA concentration (M)				
Concentration (M)	0	10^{-6}	$5x10^{-6}$	10^{-5}	Mean
0	46,3	45,3	48,0	64,7	51,1
10^{-6}	53,3	116,7	99,3	164,0	108,3
$5x10^{-6}$	58,0	123,3	125,0	133,3	109,9
10^{-5}	41,7	96,7	43,3	175,0	89,0
Mean	49,8	95,5	78,9	134,2	

LSD 5%=24,8 CV=46,9 %

Six different concentrations of abscisic acid were added to the medium, ranging from 0 to 10^{-5}M. Tissue growth occurred at all six concentrations of abscisic acid (Table 4.7). No clear trend emerged. It was, however, clear that the presence of abscisic acid has no inhibitory effect on cell division in the tissue.

DISCUSSION

It is generally accepted that cytokinins are produced in the roots and from there translocated to the shoot where they are involved in the control of growth and development (LETHAM and PALNI, 1983). The meristematic region in the root tip in particular, has been regarded as the site of cytokinin synthesis (WEISS and VAADIA, 1965). Others have, however, suggested that any zone of high meristematic activity, such as the cambium, has the ability to synthesize cytokinins (GOLDACRE, 1959; SKENE, 1972). GOLDACRE (1959) regarded cytokinin synthesis as a product of cell division.

From the data of the present study it appears that high levels of cytokinin are present in tuberous roots of cassava in comparison with primary roots. *Trans*-zeatin, *trans*-ribosylzeatin and dihydrozeatin were tentatively identified as the major cytokinins present in the tuberous roots. The cytokinin activity in these roots was found to be concentrated in the region of the xylem bordering the cambium which is the region where many cell divisions take place. A high level of cytokinin-like activity in cambium tissue has been reported previously by NITSCH and NITSCH (1965). The question which remains is whether this high level of cytokinin activity in the tuberous root is the cause or the result of tuber

Table 4.7. The effect of abscisic acid on the growth of outer xylem tissue of tuberous roots of cassava cultivar MSAF 1.

Abscisic acid Concentration (M)	Tissue growth (mg flask^{-1})
0	38,4
10^{-7}	33,2
$5x10^{-7}$	12,4
10^{-6}	26,4
$5x10^{-6}$	29,0
10^{-5}	46,0

LSD 5%=33,8 CV=86 %

initiation and growth. In other words, is the high level of this hormone caused by a translocation of this hormone to the site from elsewhere or the result of the many cell divisions that take place in the expanding roots.

In view of the evidence showing that roots are a site of cytokinin synthesis (VAN STADEN and DAVEY, 1979) and the suggestion that the cambium is a possible site of cytokinin synthesis (GOLDACRE, 1959), it is reasonable to suggest that the tuberous roots themselves synthesize the cytokinins. LOOMIS and TORREY (1964) have suggested that an initiated secondary cambium serves to supply cytokinins which regulate further tuberization. The initial tuberization trigger remains unknown, but it is likely that it involves both cytokinins and auxins. Both these hormones were required for growth of isolated meristematic tissue of tuberous roots, while gibberellic acid had little effect on the growth. The presence of abscisic acid neither promoted nor inhibited the initiated tissue growth. Although the present study does not provide conclusive evidence in this matter, it would appear as if cytokinins trigger tuberization and thereby stimulate their own production.

Reducing the supply of carbohydrates to the tuberous roots, by gibberellic acid application to the shoots or by shoot removal caused a rapid decline in the level of cytokinins in the tuberous roots. This would suggest that there is a strong correlation between cytokinin level, rate of cell division and carbohydrate supply to the roots.

Nitrogen supply manipulation led to differences in the rate of

tuberous root growth, but this was not clearly reflected in the cytokinin levels. The cytokinin activity in tuberous roots from field-grown plants showed some correlation with the rate of tuberous root growth. The highest level of activity was found in January 1982 at tuber initiation and again in December 1982 when the tuberous roots commenced their regrowth after the winter period.

The present experiments have emphasized the close association of cytokinins with the tuberization process. There are, however, many questions which remain unanswered, particularly with regard to the origin of the cytokinins. What makes the role of cytokinins in the tuberization process difficult to grasp is their apparent dual role, whereby they on the one hand induce tuberization, while on the other hand tuberization induces cytokinin production.

CHAPTER 5.

THE EFFECT OF GROWTH INHIBITORS ON THE GROWTH AND DEVELOPMENT OF CASSAVA GROWN UNDER GREENHOUSE CONDITIONS.

INTRODUCTION

Despite increasing knowledge about the role of hormones in the tuberization process and dry matter distribution, there is at present only one type of hormone manipulation being used commercially to increase tuber yield, namely the changing the gibberellin level.

A variety of compounds which reduce the vegetative growth of plants is presently available, some registered and others in the process of being registered. These regulators have in common their ability to reduce the effect of gibberellins in the plant. The biochemical mechanism by which they fulfill such a role varies from inhibition of gibberellin biosynthesis, de-activation of gibberellins to competition for the hormone binding site (DICKS, 1979). The effect of these inhibitors on the morphology of the plant generally follows the same pattern. Sub-apical cell expansion and cell division is reduced, which leads to shorter but thicker internodes (SACHS and KOFRANEK, 1963). The rate of leaf appearance is little affected by these growth inhibitors, but the leaf area is generally somewhat reduced (HUMPHRIES and DYSON, 1967). An important response to inhibitors is the change in dry matter distribution in the plant. In tuber crops an inhibition of shoot growth reduces the demand for carbohydrates in the shoot. This is expected to make them

available for storage in the tubers (GIFFORD and MOORBY, 1967).

A crop of cassava in the field can only be harvested after 16 to 18 months, which is too long for a screening trial of growth inhibitors. In the first greenhouse trial, different growth inhibitors were applied to young cassava plants grown in pots under greenhouse conditions. The objective of these pre-liminary trials was to obtain, in a relatively short time, some information on the effect of different growth inhibitors. In the second experiment the effect of a growth inhibitor at different nitrogen fertilization levels on the growth and dry matter distribution of young cassava plants was studied.

EXPERIMENT 5.1 Growth regulator screening.

Experimental procedure

Three officially registered growth inhibitors were screened for their effect on cassava growth. The application rates were according to the manufacturers recommendations, namely:

Cycocel	2,5 grammes per litre
Pix	1,0 millilitre per litre
Alar	2,5 grammes per litre

Forty gram stakes of cassava cultivar MSAF 1 were planted in pots containing 12 kilogrammes of the standard soil mix (Chapter 2). Every two weeks 600 millilitres nutrient solution (Table 2.1) was

added to each pot. The pots were placed in a randomized block design with four replications and moved weekly. Within each block two plants received the same treatment and the average value was taken. The inhibitors were applied four months after planting and the plants were harvested six weeks later. Two unregistered regulators were screened in a similar trial at the following rates:

RSW 0411	1 gramme per litre
MB 25105	1 millilitre per litre

Following these two experiments Alar (2,5 grammes per litre) and RSW 0411 (1 gramme per litre) were further tested in a completely randomized experiment with six replications. In this experiment the nutrient solution was applied weekly.

Results

The only growth inhibitor which caused a significant reduction of the stem and petiole dry mass in the first screening trial was Alar (Table 5.1). This reduction was a direct result of the shortening of the internodes (Table 5.2). Leaf dry mass of the treated plants did not differ significantly from the controls. The tuberous root dry mass was highest in the Alar treated plants, but the coefficient of variation of this component was so high that no conclusions can be based on these data. The effect of the inhibitors on the dry matter distribution is best expressed by the shoot/root ratio. This ratio was lowest for the Alar treated plant, namely 0,97 compared with 1,13 for the control plants. These differences, however, were not

Table 5.1. The effect of three registered growth inhibitors on the dry matter distribution of cassava cultivar MSAF 1 grown in the greenhouse. Mass is given per plant. Application rates: Alar=2,5 grammes per litre; Pix=1,0 grammes per litre; Cycocel 2,5 grammes per litre.

Parameter measured	Alar	Pix	Cycocel	Control	LSD 5 %	CV (%)
Tuberous root dry mass (g)	49,5	43,1	45,3	44,5	15,1	20,7
Root dry mass (g)	5,6	5,1	5,5	5,9	1,1	12,9
Leaf dry mass (g)	15,6	15,1	14,3	13,7	2,5	10,6
Stem + petiole dry mass (g)	31,7	38,2	35,8	37,0	5,2	11,4
Shoot/root ratio	0,97	1,17	1,08	1,13	0,55	31,7

Table 5.2 The effect of three registered growth inhibitors on certain growth parameters of cassava cultivar MSAF 1 grown in the greenhouse. Applition rates: Alar=2,5 grammes per litre; Pix=1,0 grammes per litre; Cycocel=2,5 grammes per litre.

Parameter measured	Alar	Pix	Cycocel	Control	LSD 5 %	CV (%)
Leaf area (cm^2) per plant	3611	3751	3611	3581	518	8,9
Leaf dry mass ($mg\ cm^{-2}$)	4,30	4,02	3,97	3,82	0,27	4,1
Number of side branches per plant	2,25	2,25	2,12	2,37	0,68	19,6
Length side branches (cm)	74,4	96,1	86,2	104,5	17,1	11,9
Number nodes on side branches	32,0	32,5	30,1	34,6	8,2	16,0
Internode length side branches (cm)	2,31	2,97	2,91	3,02	0,51	11,4

significant. There was little difference in total leaf area, but the dry mass per unit area of the leaves was significantly higher in the Alar treated plants. This increase of dry mass per unit leaf area as a result of growth regulator application has been reported elsewhere for other species (HUMPHRIES and DYSON, 1967). The number of side branches was not affected by the treatments, however, the total side branch length per plant was significantly reduced by Alar and Cycocel treatments (Table 5.2).

In a subsequent experiment the two unregistered growth inhibitors were screened. RSW 0411 suppressed stem and petiole dry mass, while not affecting leaf mass (Table 5.3). The effect of RSW 0411 was clearly visible by the small and dark young leaves and the very short internodes. However, the application of these inhibitors did not lead to a decrease of the shoot /root ratio. MB 25105 did not give the desired response.

The two regulators with a good inhibitory effect on shoot growth, namely Alar and RSW 0411, were further tested in a trial in which shoot development after application was measured periodically. Figure 5.1 shows the post-treatment elongation and new leaf formation. RSW 0411, and to a lesser extent Alar, reduced shoot elongation during the entire experimental period. RSW 0411 caused almost complete inhibition of shoot growth. The rate of leaf formation was again not affected by the treatment. The data shows the long residual effect of these inhibitors in particular RSW 0411, which was still active 85 days after application. The inhibitors had a marked effect on the dry matter distribution (Table 5.4). The tuberous root dry mass was doubled by Alar application and was 24

Table 5.3. The effect of two unregistered plant growth inhibitors on the dry matter distribution of cassava cultivar MSAF 1 grown in the greenhouse. The mass is given per plant. Application rates: RSW 0411=1 grammes per litre; MB 25105=1 grammes per litre.

Parameter measured	RSW0411	MB 25105	Control	LSD 5 %	CV(%)
Tuberous root dry mass (g)	266,1	208,2	280,2	46,1	14,3
Leaf dry mass (g)	27,4	28,1	27,3	4,2	11,7
Stem + petiole dry mass (g)	132,8	155,6	144,6	17,4	9,4
Shoot/roots ratio	0,61	0,91	0,62	0,14	15,8

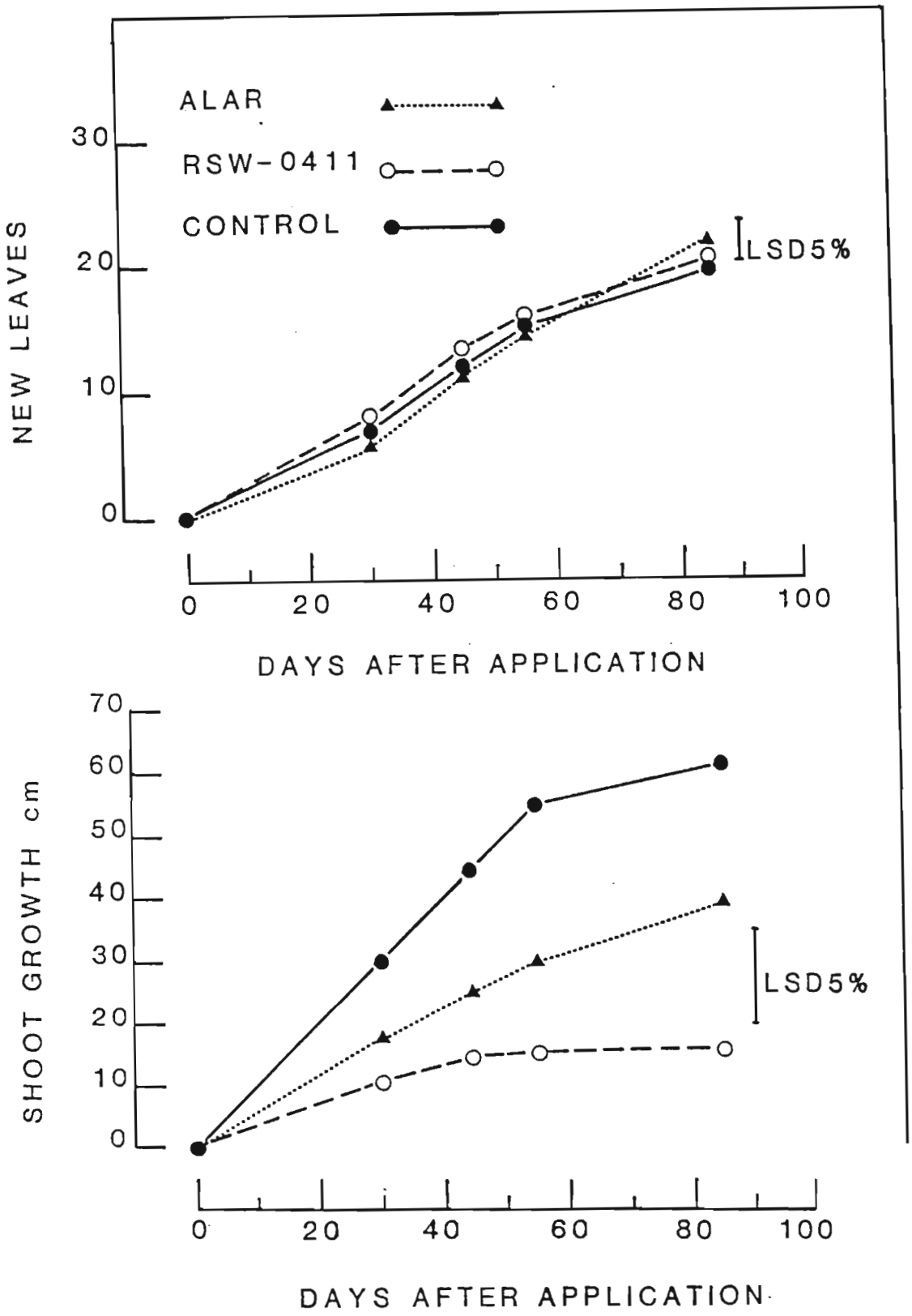

Figure 5.1. The effect of Alar and RSW 0411 on the formation of leaves and the increase in shoot length of cassava cultivar MSAF 1 grown in the greenhouse.

Table 5.4. The effect of RSW 0411 and Alar on the growth of cassava cultivar MSAF 1 grown in the greenhouse. Mass is given per plant. Application rates: Alar=2,5 grammes per litre; RSW 0411=1 gramme per litre.

Parameter measured	Alar	RSW 0411	Control	LSD 5 %	CV (%)
Tuberous root dry mass (g)	50,7	31,5	25,4	10,9	22,0
Shoot dry mass (g)	28,7	37,0	44,2	6,2	32,8
Shoot/root ratio	0,57	1,44	1,77	0,35	52,2
Leaf area (cm^2)	2434	2889	2932	550	21,9
Leaf mass per unit area (mg cm^{-2})	3,18	2,72	2,56	0,20	5,2
% Increase chlorophyll absorbance over controls	7,0	7,7		n.s.*)	15,7

*)not significant

per cent higher in RSW 0411 treated plants when compared with the controls. The effect of the inhibitors on the shoots was exactly the opposite. The treatments led to large differences in shoot/tuberous root ratios. The leaf area was somewhat reduced by the growth inhibitor but not significantly, while the leaf mass per unit area was increased by both inhibitors. Furthermore, there was an increase (non-significant) of the chlorophyll content per unit area. A higher chlorophyll content per unit area could be a direct consequence of the increased leaf mass per unit area.

The two inhibitors, Alar and RSW 0411, appeared to give satisfactory suppression of the vegetative growth and caused characteristic morphological modification with cassava as observed with other species.

EXPERIMENT 5.2. The effect of nitrogen fertilization and Alar on the growth of young cassava.

Experimental procedure

Cassava cultivar MSAF 1 was grown in the standard soil mix (Chapter 2). The nutrient solution (Table 2.1.) was applied every week omitting the normal KNO_3 and $CaNO_3$ components. Instead nitrogen was applied as KNO_3 at the following levels:

N_0= 0

N_1= 0,485 grammes nitrogen per week

N_2= 0,970 grammes nitrogen per week

KCl was added to the treatments N_0 and N_1 to compensate for the lower potassium levels. The nitrogen treatment and nutrient solution application was started six weeks after planting and was thereafter added weekly. Alar was applied five months after planting at the following levels:

A_0= 0

A_1= 2,5 grammes per litre

A_2= 5,0 grammes per litre

The pots were placed in a two-factorial randomized block design with seven replications and moved weekly within the blocks. An initial harvest took place at the time of the Alar application, while the remainder of the plant was harvested six weeks after inhibitor application.

Results

Five months after planting, twice as much dry matter had accumulated in the tops of the N_2 treated plants compared with the control plants. No significant reduction of the root dry mass (Table 5.5) was observed. Tuberous root formation had not yet taken place. There were no differences in response between the N_0 and the N_1 treatment, most likely because the plants were still making use of the nitrogen already present in the soil. The nitrogen supply increased the leaf area per plant significantly. Furthermore, a high level of nitrogen led to a greater shoot length as a result of increased internode length, while the number of leaves per plant did

Table 5.5. The effect of nitrogen treatment on dry matter distributio and other parameters of cassava cultivar MSAF1 grown in the greenhouse Values are given per plant. Nitrogen levels: N_0=0; N_1=0,485 grammes pe week; N_2=0,970 grammes per week.

Parameters measured	Nitrogen level			LSD 5 %	CV(%)
	N_0	N_1	N_2		
Leaf dry mass (g)	3,29	3,21	6,31	1,37	27,6
Stem + petiole dry mass (g)	3,69	4,15	7,04	2,08	36,0
Root dry mass (g)	5,32	6,17	4,19	1,66	27,4
Leaf area (cm^2)	1325	1474	2068	365	19,3
Leaf mass per unit area ($mg.cm^{-2}$)	2,48	2,08	3,04	0,49	16,6
Shoot length (cm)	36,1	37,7	51,9	11,0	22,6
Internode length (cm)	1,46	1,41	1,65	0,71	40,3
Number of nodes	26,0	26,9	28,3	4,0	12,9

not change. These data show that the nitrogen supply has the opposite effect on shoot development as growth inhibitors.

Six weeks after the application of Alar all plants were harvested. None of the dry mass components measured showed a significant interaction effect between Alar and the nitrogen supply (Annexure 1). Treatment A_1 led to an higher shoot growth at all three levels of nitrogen supply (Figure 5.2). At level A_2 leaf and stem plus petiole growth was significantly reduced but more so at the lower levels of nitrogen supply. The root growth was promoted by Alar application at all nitrogen levels. Tuberous root formation had taken place but the variability was large and there was no clear trend present.

The total dry matter production (Figure 5.3) was affected by both factors, but the effect of Alar was less pronounced at higher levels of nitrogen. Shoot/root ratios further illustrated the effect of Alar on the dry matter distribution. The ratio increased with a higher nitrogen level. At level N_0 the inhibitor caused an almost linear decrease of the ratio, while at levels N_1 and N_2 the highest shoot/root ratio occurred at A_1. The leaf area per plant increased with the nitrogen supply (Figure 5.4), but was little affected by the inhibitor treatment. In contrast to the first harvest, the highest leaf mass per unit area was found at N_0, while there is no clear response to Alar. Elongation (length of post-treatment shoot growth) and internode length were reduced by Alar while nitrogen increased the value of these parameters(Figure 5.5).

Finally the effect of the treatments on the relative

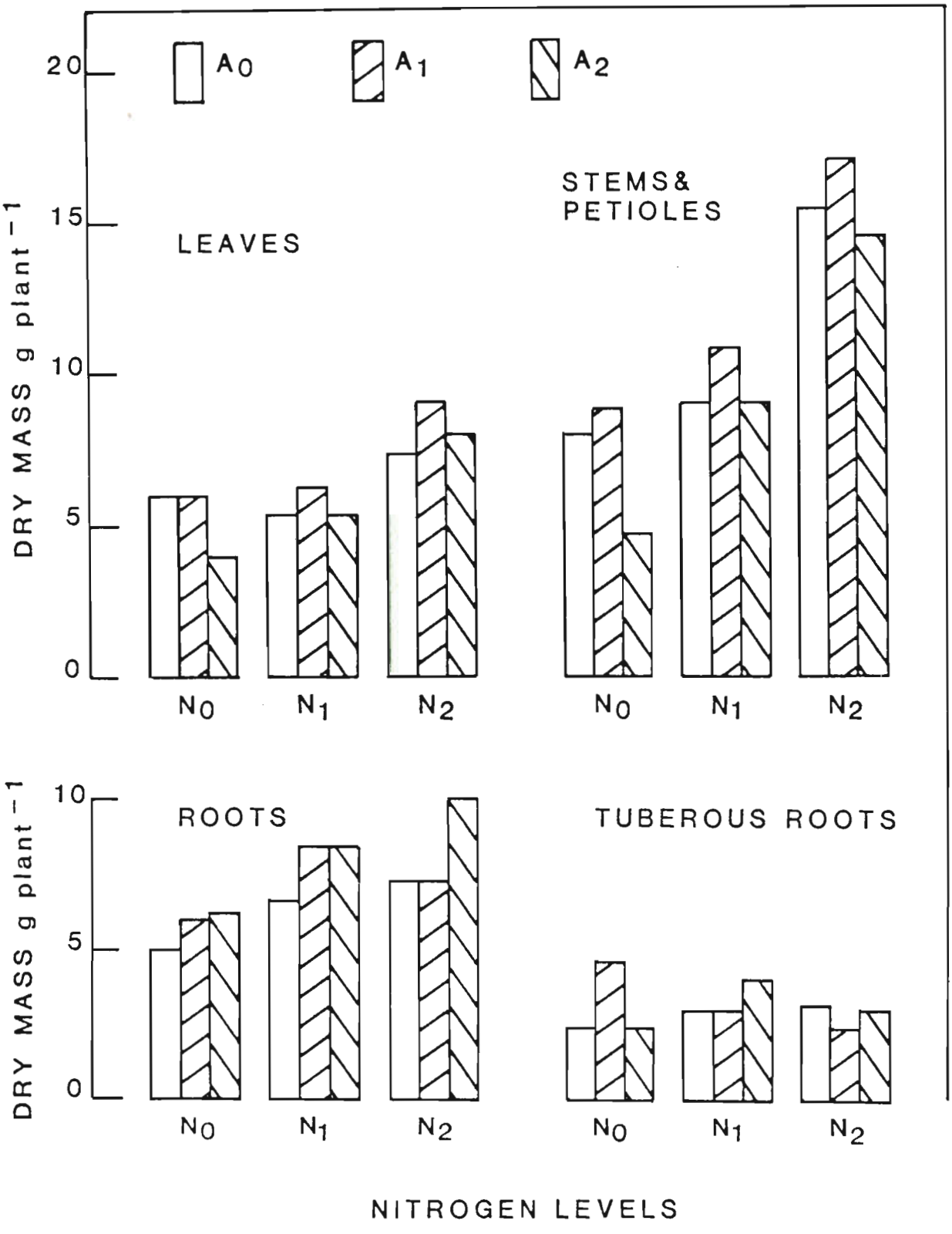

Figure 5.2. The effect of nitrogen and Alar on the dry matter distribution of cassava cultivar MSAF 1 grown in the greenhouse. For statistical analysis see annexure 1. Alar treatments: A_0=0; A_1=2,5 grammes per litre; A_2=5,0 grammes per litre. Nitrogen treatments: N_0=0; N_2=0,485 grammes per week; N_2=0,970 grammes per week.

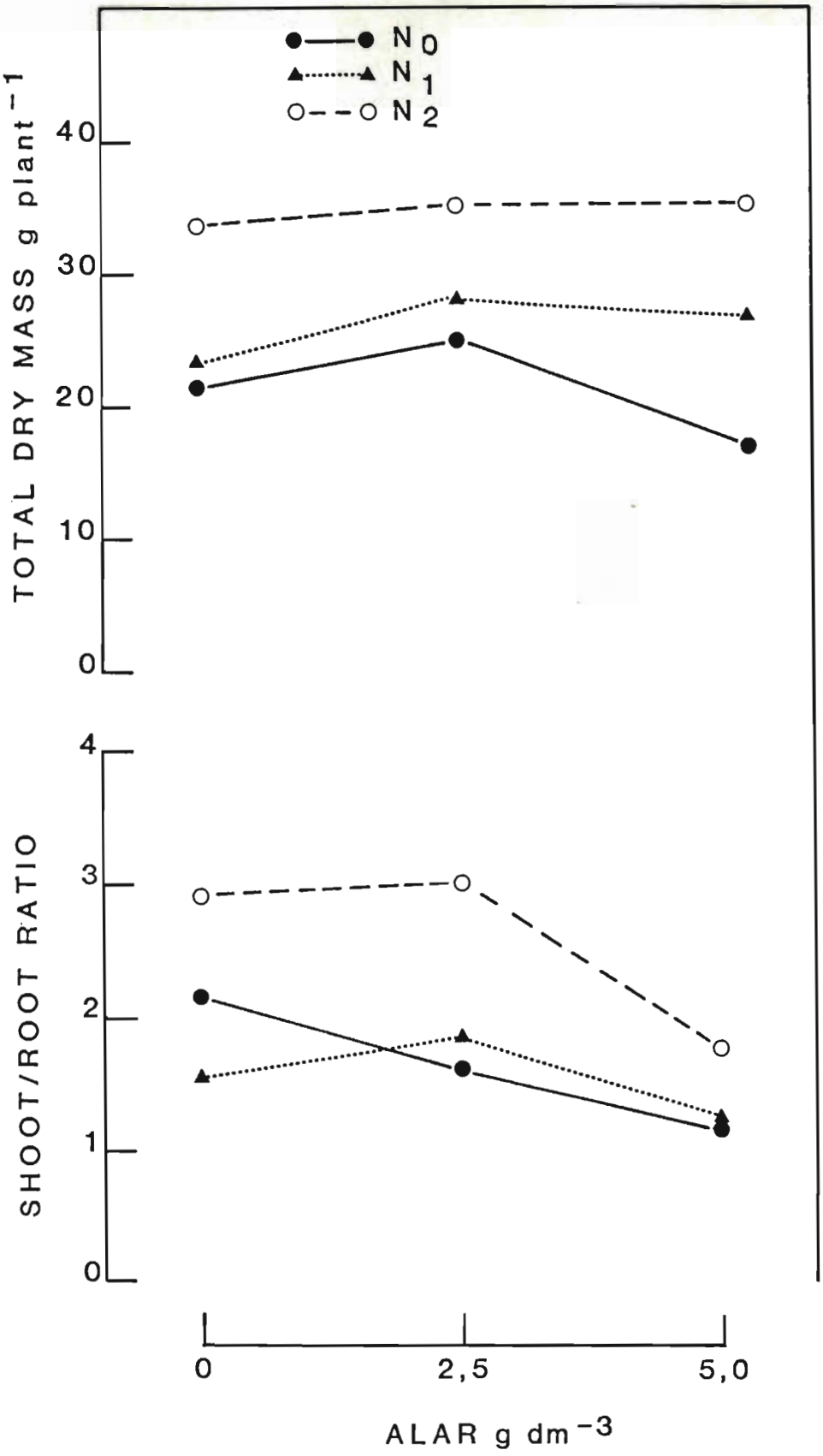

Figure 5.3. The effect of nitrogen and Alar on the total dry mass and the shoot/root ratio of cassava cultivar MSAF 1 grown in the greenhouse. For statistical analysis see annexure 1. Nitrogen treatments: N_0=0; N_1=0,485 grammes per week; N_2=0,970 grammes per week.

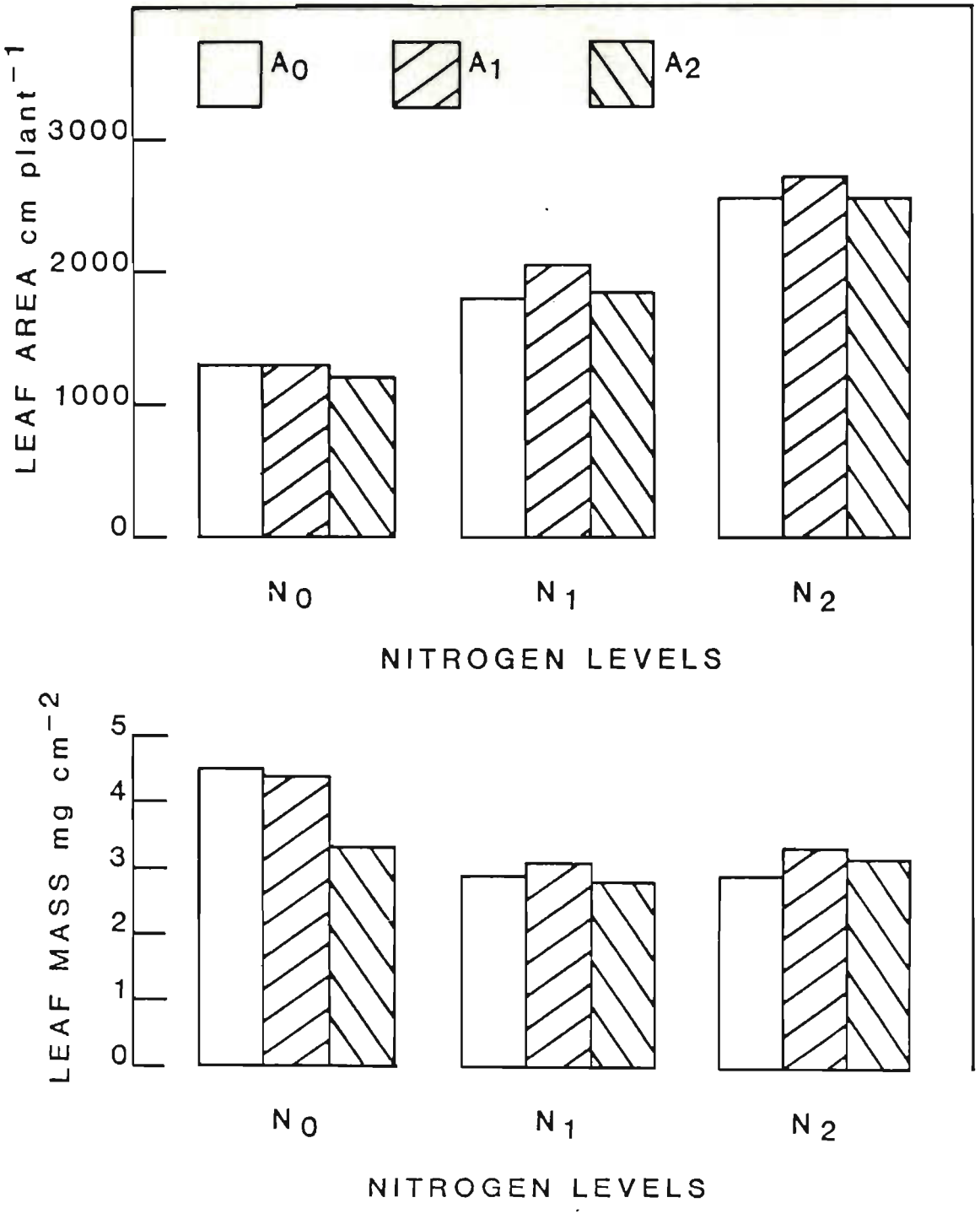

Figure 5.4. The effect of nitrogen and Alar on the leaf area and leaf dry mass per unit area of cassava cultivar MSAF 1 grown in the greenhouse. For statistical analysis see annexure 1. Nitrogen treatments: N_0=0; N_1=0,485 grammes per week; N_2=0,970 grammes per week. Alar treatments: A_0=0; A_1=2,5 grammes per litre; A_2=5,0 grammes per litre.

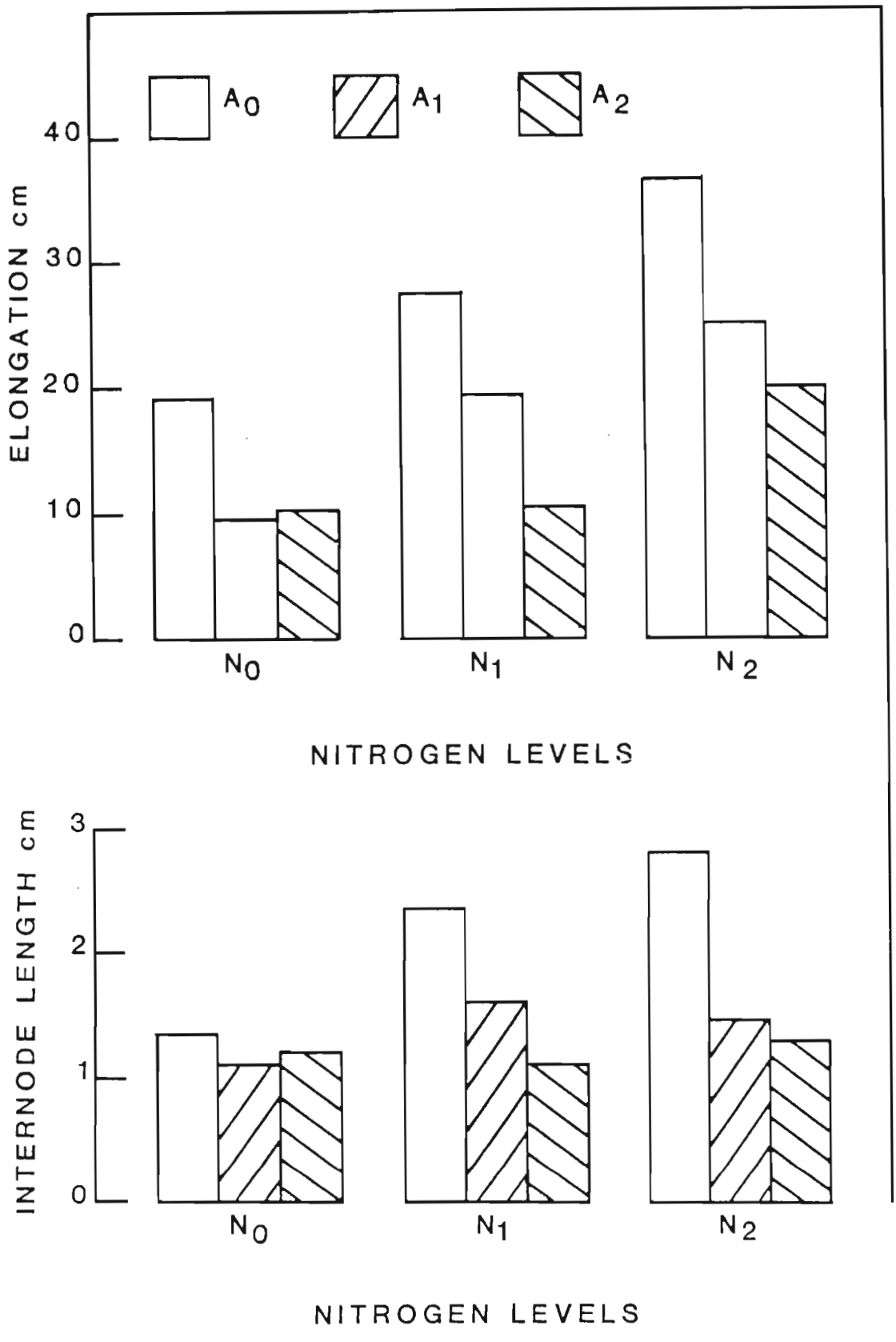

Figure 5.5. The effect of nitrogen and Alar on the elongation and internode length of cassava cultivar MSAF 1 grown in the greenhouse. For statistical analysis see annexure 1. Nitrogen treatments: N_0=0; N_1=0,485 grammes per week; N_2=0,970 grammes per week. Alar treatments: A_0=0; A_1=2,5 grammes per litre; A_2=5,0 grammes per litre.

chlorophyll levels per unit leaf area was measured. Nitrogen significantly increased the chlorophyll level, while there was no clear response to the Alar treatment (Table 5.6).

DISCUSSION

The aim of the above screening trials was to select growth inhibitors which caused satisfactory retardation of shoot growth of cassava. Only two of the five inhibitors studied caused a significant shoot growth inhibition at rates which were recommended by the manufacturers. The other inhibitors had little effect on the shoot growth. They would probably have had some effect at much higher application rates but this was not investigated further.

Alar and RSW 0411 applications caused morphological changes in the young cassava shoot characteristic of these gibberellin-inhibitors. The internodes were shortened, the leaf area was reduced, the leaf mass per unit area increased, while the number of leaves was not affected. In the first two screening trials the inhibitors had no significant effect on the shoot/root ratios and tuberous root dry mass. In the third trial, where the growth rate was higher, the shoot/root ratio was significantly reduced, while the tuberous root dry mass was increased as a result of the inhibitor applications. RSW 0411 was particularly effective as a shoot growth inhibitor.

The second experiment showed that nitrogen had an opposite effect on plant growth to growth inhibitors. With increased nitrogen supply the internode length and leaf area increased, while

Table 5.6. The effect of nitrogen and Alar on the relative chlorophyll levels (absorbance at 435 nm) of cassava cultivar MSAF1 grown in the greenhouse. Nitogen levels: N_0=0; N_1=0,485 grammes per week; N_2=0,970 grammes per week. Harvest 1 took place 5 month after planting, before any Alar was applied. Harvest 2 took place 6 weeks after the Alar application

		N_0	N_1	N_2	LSD 5 %	CV (%)
Harvest 1		0,09	1,12	1,23	0,14	11,1
Harvest 2	A0	1,05	1,17	1,27		
	A1	1,00	1,33	1,25	0,25	16,0
	A2	1,18	1,15	1,45		

the number of leaves remained unchanged. Nitrogen fertilization furthermore increased the shoot/root ratio of the young cassava plants. It is therefore reasonable to suggest that nitrogen and growth inhibitors have the same site of action in the plant which they influence in opposite ways. Nitrogen applications have been found to influence endogenous gibberellin levels. Nitrogen withdrawal for example has been found to decrease the content of gibberellins in tomato (RAJOGAPAL and RAO, 1972). Whatever the mechanism, it is evident that growth inhibitor application should not be studied without taking into account the levels of nitrogen present in the soil.

The greenhouse studies showed that the largest inhibition was obtained at the lowest level of nitrogen. This suggests that if nitrogen and growth inhibitors influence the same site of action, nitrogen is the stronger factor reducing the effect of the growth inhibitor. In other words, the growth inhibitor is least effective at higher nitrogen levels, that is when it is most needed to reduce shoot growth.

CHAPTER.6.

THE EFFECT OF GROWTH INHIBITORS ON THE GROWTH AND DEVELOPMENT OF CASSAVA UNDER FIELD CONDITIONS.

INTRODUCTION

The success of a growth inhibitor application in the field depends on a number of factors. Most important of these is probably the uptake and translocation of the compound in the target plant (LUCKWILL, 1978). This is an aspect which has so far received little attention. Other factors which will determine the success of a growth inhibitor are the time of application, application rate and the level of other growth factors such as nitrogen, temperature and water (BODLAENDER and ALGRA, 1966). The aim of the present field experiments was to study the effect of growth inhibitors on a crop of cassava at different rates and times of application and under different conditions.

From November 1981 to March 1983 a preliminary growth analysis was conducted to study the normal dry matter distribution pattern of cultivar MSAF 2 and to establish the best time for applying the inhibitors. The crop growth followed the typical pattern for sub-tropical areas (Figure 6.1). In the first few months shoot growth was greatest. Tuber initiation took place in January 1982, but the phase of rapid tuberous root growth only started in March 1982. During the winter period shoot and tuberous root growth were low. In early spring the shoot growth rate exceeded the tuberous root growth rate, but no drop in tuberous root fresh mass took place. From

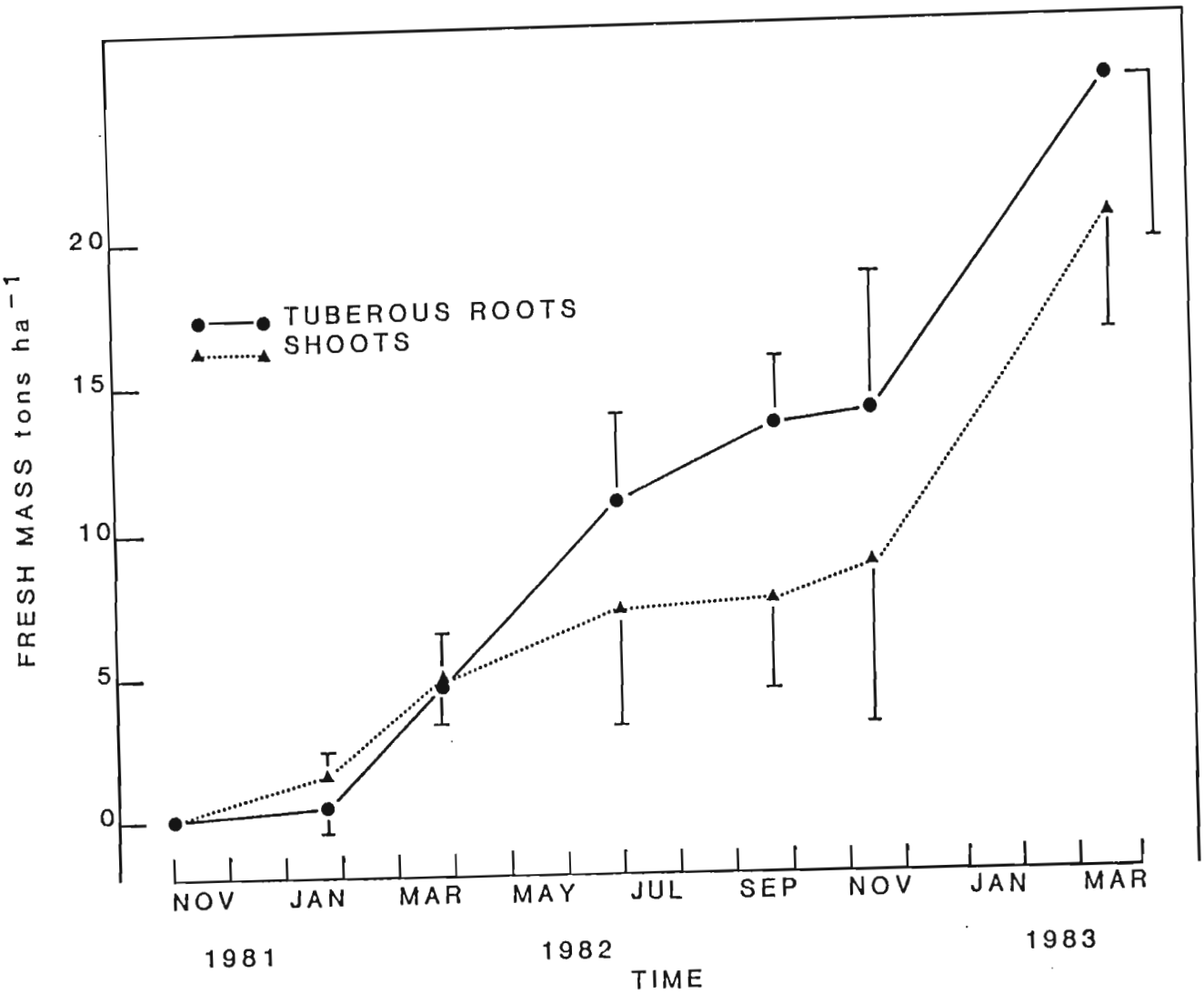

Figure 6.1. Pre-liminary growth analysis of cassava cultivar MSAF 2 at Fernwood. Vertical bars represent confidence limits at 5 per cent.

November onwards shoot and tuberous root mass increased at practically the same rate. This growth analysis showed that shoot growth inhibition could be particularly beneficial in the second summer season from November onwards. Growth inhibitors could decrease the slope of the shoot growth, leading to a higher yield. At the last harvest in March 1982, 75 per cent of the shoot mass consisted of stems, while 68 per cent of the shoot mass was stems without leaves. In other words, these figures illustrate that a large portion of the dry matter production went into the production of stem material which support a relatively small canopy. In addition to this, a large portion of the dry matter production was lost through the continuous shedding of old leaves. Reduction of the percentage stem mass per plant can potentially improve the final tuberous root yield. The greenhouse studies have shown that Alar and the new regulator RSW 0411 gave good inhibition of shoot growth of cassava. But since RSW 0411 was not yet officially registered, Alar was chosen for most field experiments.

During the course of the second season the monthly rainfall at Fernwood was recorded (Table 6.1). Two cyclones hit the farm during 1984. The first was accompanied by heavy rains, but did little damage. The second cyclone caused some wind damage to the shoots, but did not affect the tuberous root yield since it took place shortly before the final harvest. Soil tests were done for each experimental site and the results are summarized in Table 6.2. These sites were initially chosen because the cassava on that part of the farm showed vigorous shoot growth, most likely because of an optimal pH of the soil.

Table 6.1 Monthly rainfall recorded during the 1983-1984 growing season at Fernwood.

Month	Total rainfall (mm)
July 1983	128,4
August 1983	85,6
September 1983	29,6
October 1983	90,4
November 1983	180,4
December 1983	102,6
January 1984	213,0
February 1984	530,4

Table 6.2 Soil analysis of the four experimental sites at Fernwood (Analysis by Agricultural Technical Services, Cedara, South Africa) Levels of nutrients are given in milligrammes per kilogramme.

Experiment	P	K	Ca	Mg	Na	Zn	Al	pH	Acid saturation (%
6.1	92	47	427	66	0	0	18	4,2	6,4
6.2	8	91	170	53	0	0	37	4,0	17,4
6.3.	13	47	198	56	0	0	30	40	17,4
6.4.	13	50	153	44	0	0	23	4,3	16,8

EXPERIMENT 6.1. The effect of Alar at two times of application on second season growth.

Experimental procedure

The experiment was layed out as a randomized block design with six replications. Three times of application were planned but the third one had to be cancelled because of weather conditions. Alar was applied at the following times:

T_1 3-10-83

T_2 23-11-83

and at the following rates:

A_0 0

A_1 1,5 grammes per litre

A_2 3,0 grammes per litre

A_3 4,5 grammes per litre

Treatment T_1 was harvested on 8-12-83 and T_2 on 16-2-84, two and three months after Alar application respectively. From September to February a detailed growth analysis was conducted.

Results

From September to October 1983 the tuberous root dry mass decreased from 2,7 to 2,4 tons per hectare, while over the same period

there was an increase of 0,6 tons per hectare of the shoot dry mass (Figure 6.2). This shows that at the start of the second season carbohydrates were mobilized from the tuberous roots and used for the formation of a new canopy. Over this period the leaf area index increased from 0,4 to 1,3 and in November reached a level of 2,3 (Figure 6.3). The decrease in tuberous root mass took place over a relatively short period of a few weeks. From October 1983 onwards the tuberous root mass increased at a rapid rate. The leaf dry mass reached a maximum in early November and thereafter fluctuated around this value. During the season new leaves were continually being formed, while simultanuous abortion of older leaves took place further down the stem. The shedding of older leaves was accelerated in all trials by the occurrence of brown leaf spot (*Cercospora henningsii*) which caused senescence of older leaves. The stems continued to grow over the entire period as new leaves were formed. From November 1983 to February 1984 the stem dry mass increased by 2,5 tons per hectare, while no real increase in the leaf area index took place over this period.

The data illustrate the importance of the second season growth for the final yield. Over the five month period of the second season the tuberous root mass increased by 7 tons per hectare, while over the 11 previous months the increase was only 3 tons. In the first few months the shoot/tuberous root ratio remained close to 1, but thereafter decreased steadily as a relatively larger portion of the assimilates were allocated to the storage roots.

The times of Alar application (T_1 and T_2) are marked in Figure 6.2. T_1 was chosen early in the season (October) when the tuberous

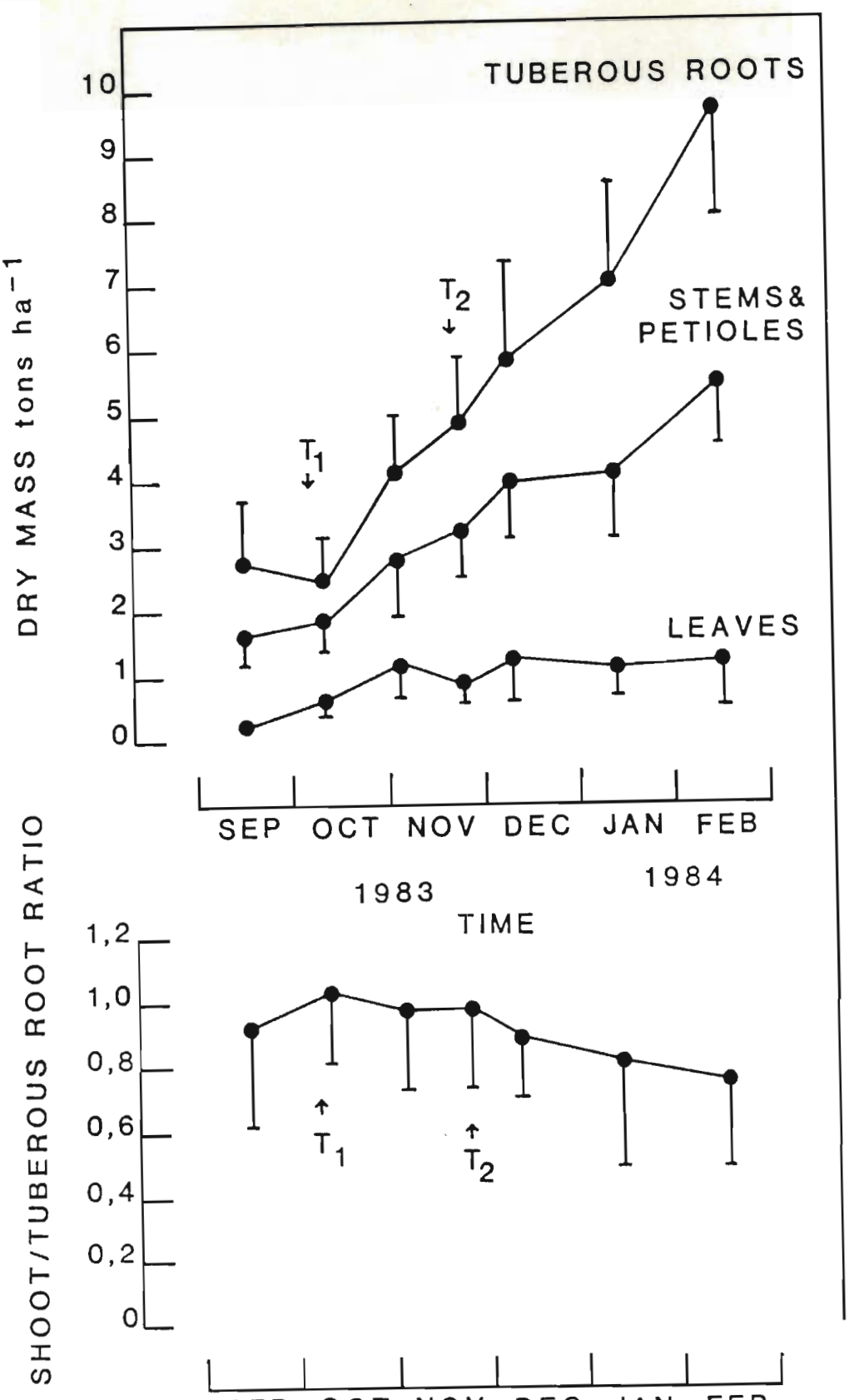

Figure 6.2. Dry mass distribution and shoot/tuberous root ratio o
cassava cultivar MSAF 2, over a six month period at Fernwood
Vertical bars represent confidence limits at 5 per cent. T_1 and T_2
indicate dates of Alar application in Experiment 6.1.

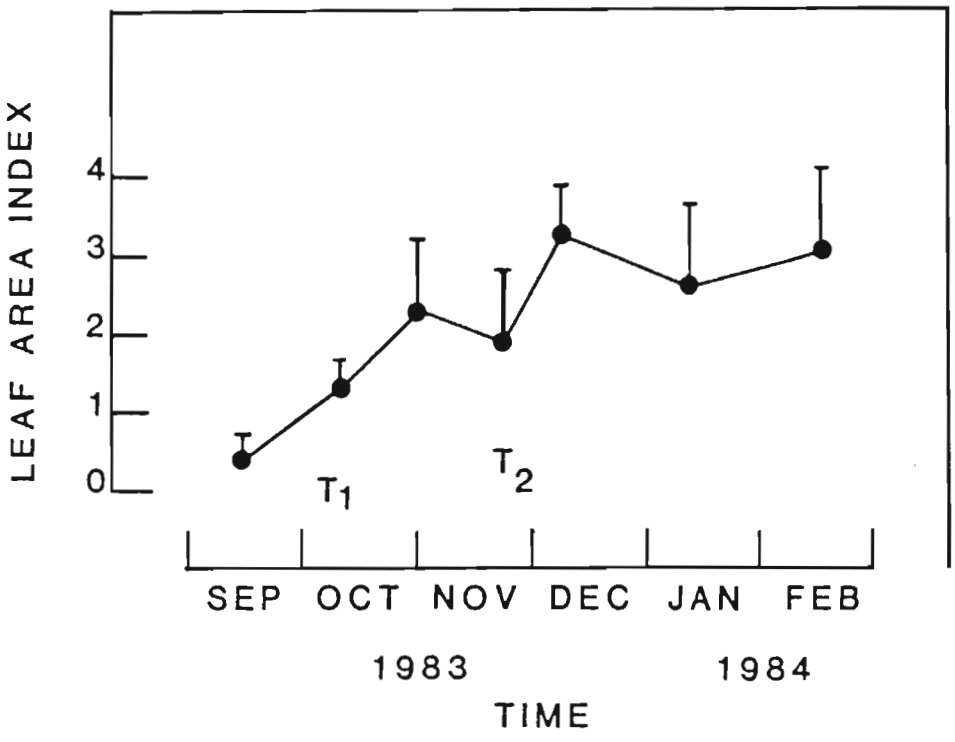

Figure 6.3. Development of leaf area index of cassava cultivar MSAF 2 over a six month period at Fernwood. Vertival bars represent confidence limits at 5 per cent. T_1 and T_2 indicate dates of Alar application in Experiment 6.1.

roots underwent a negative growth and the leaf area index had not yet reached its maximum. T_2 took place after the maximum leaf area had been reached and both tuberous root and stem dry mass were increasing rapidly. Based on the growth curves it is evident that a good inhibition of the shoot growth at both these times of application would have led to a considerable increase of tuberous root yields.

The application of Alar, however, had only limited effect on the dry matter distribution of the crop. Some decrease in stem dry mass took place at both times of application (Figures 6.4 and 6.5), but the differences were not significant. Neither of the other dry mass components showed any significant response to Alar, although there was slightly lower tuberous root dry mass in the Alar treated plots, particularly at the second time of application. Furthermore, the Alar application failed to decrease the shoot/tuberous root ratios (Figure 6.6). The leaf area index was significantly reduced at the first time of application (Figure 6.7). At the second time of application the leaf area index was significantly reduced by applications of 3,0 and 4,5 grammes per litre, but at 1,5 grammes per litre there was an increase. Alar application led to a 20 and 14 per cent decrease in internode length at level A_3 for the first and second time of application respectively.

The reduction of shoot growth by Alar application appeared to be insufficient to lead to significant changes in the dry matter distribution or to an increase in tuberous root yield. The reduction of the leaf area index may have been detrimental since the index was already sub-optimal. The total dry mass per hectare at T_1 and T_2 was respectively 8 and 10 per cent lower with 4,5 grammes per

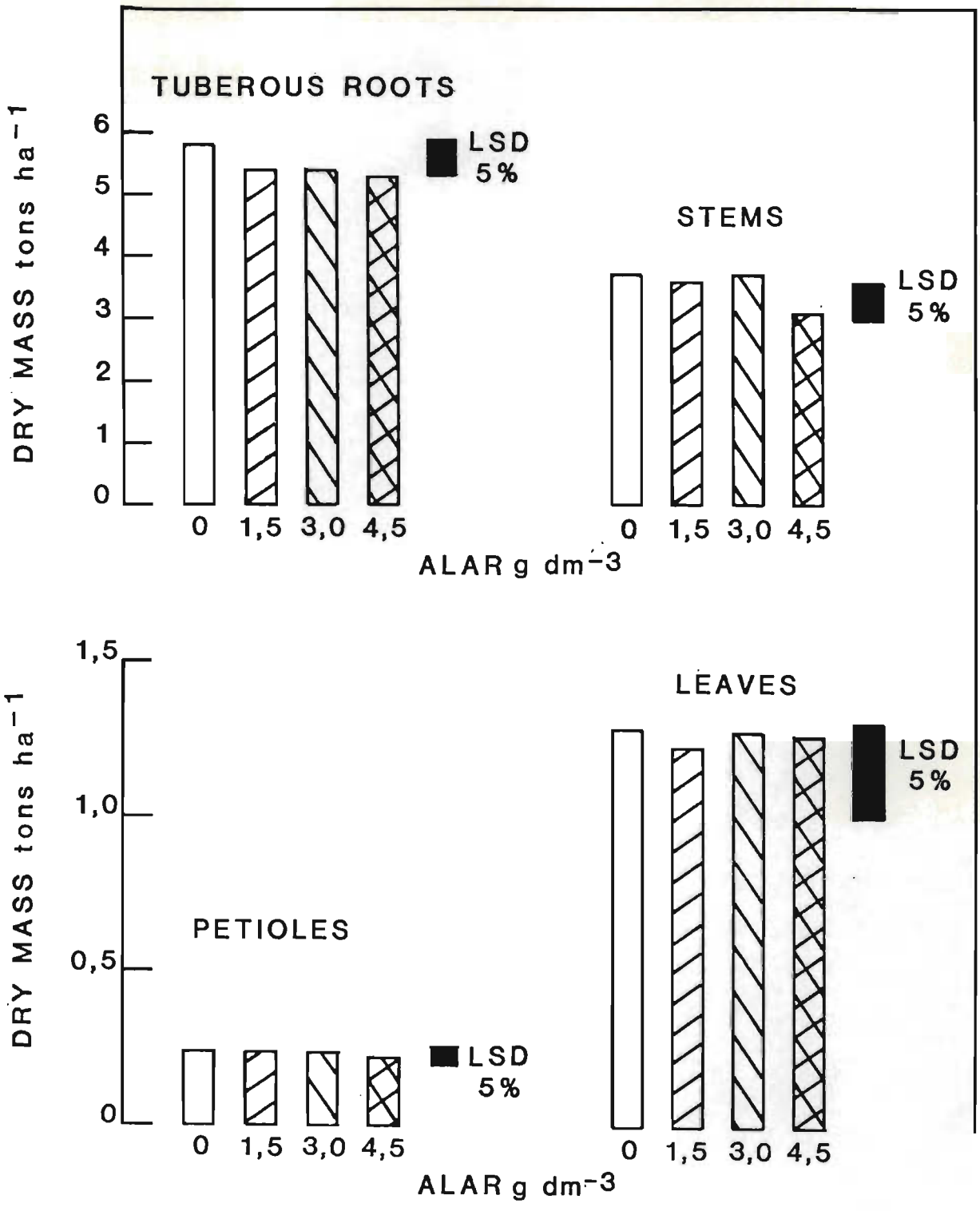

Figure 6.4. The effect of Alar, applied at 3-10-83, on the dry matter distribution of cassava cultivar MSAF 2 grown at Fernwood.

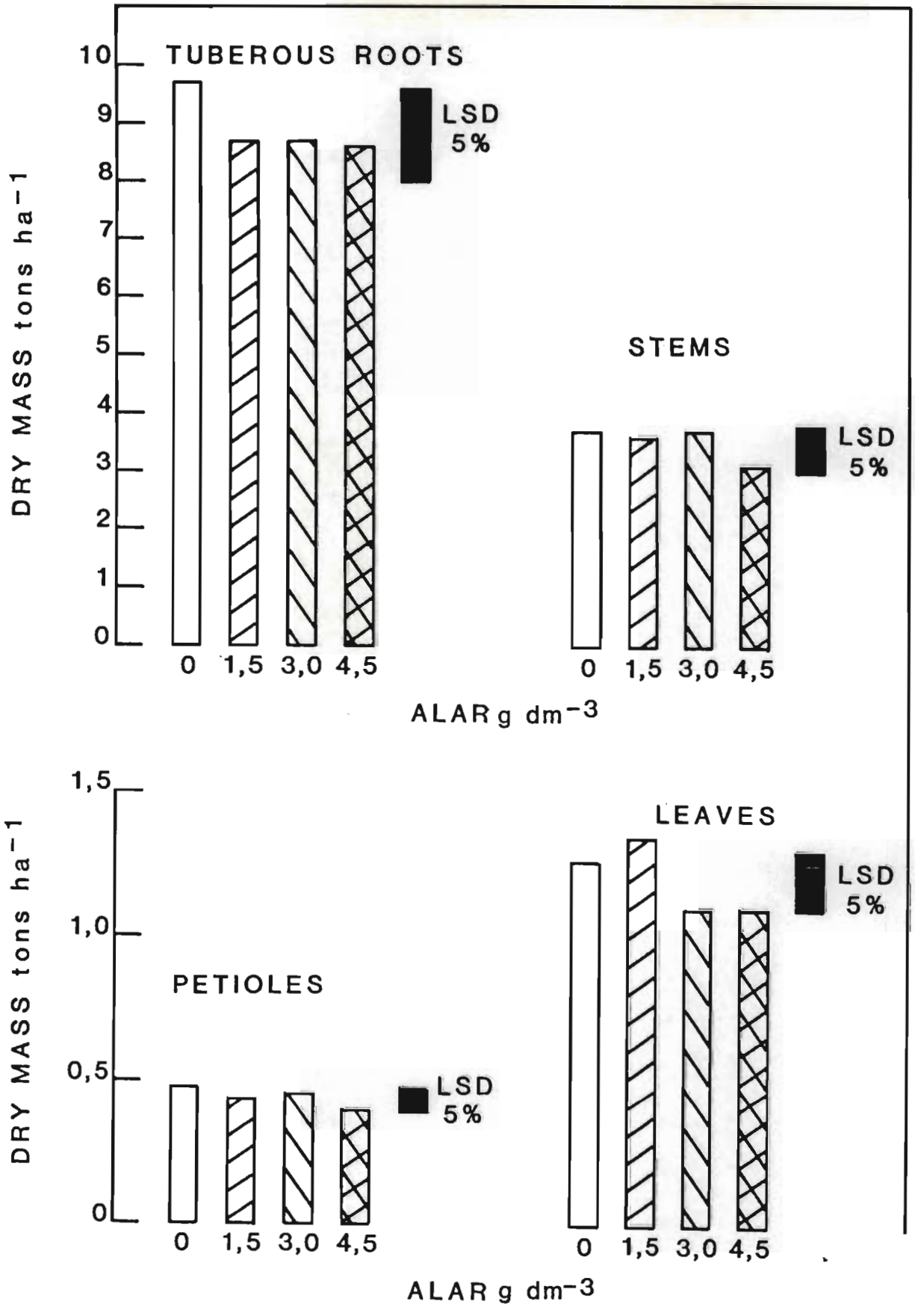

Figure 6.5. The effect of Alar, applied at 23-11-83, on the dry matter distribution of cassava cultivar MSAF 2 grown at Fernwood.

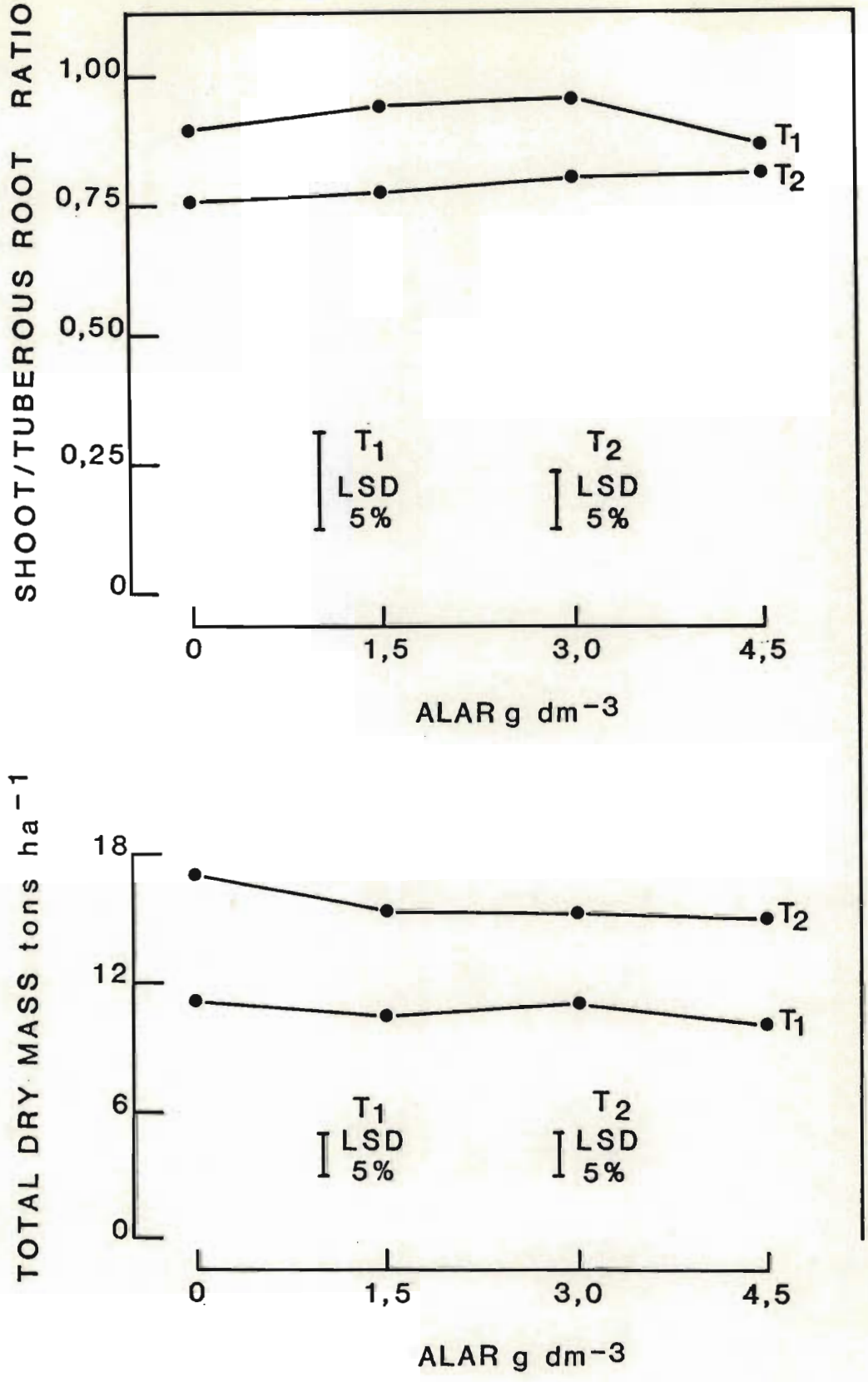

Figure 6.6. The effect of Alar, applied at 3-10-83 (T_1) and 23-11-83 (T_2) on the shoot/tuberous root ratio and total dry mass of cassava cultivar MSAF 2 grown at Fernwood.

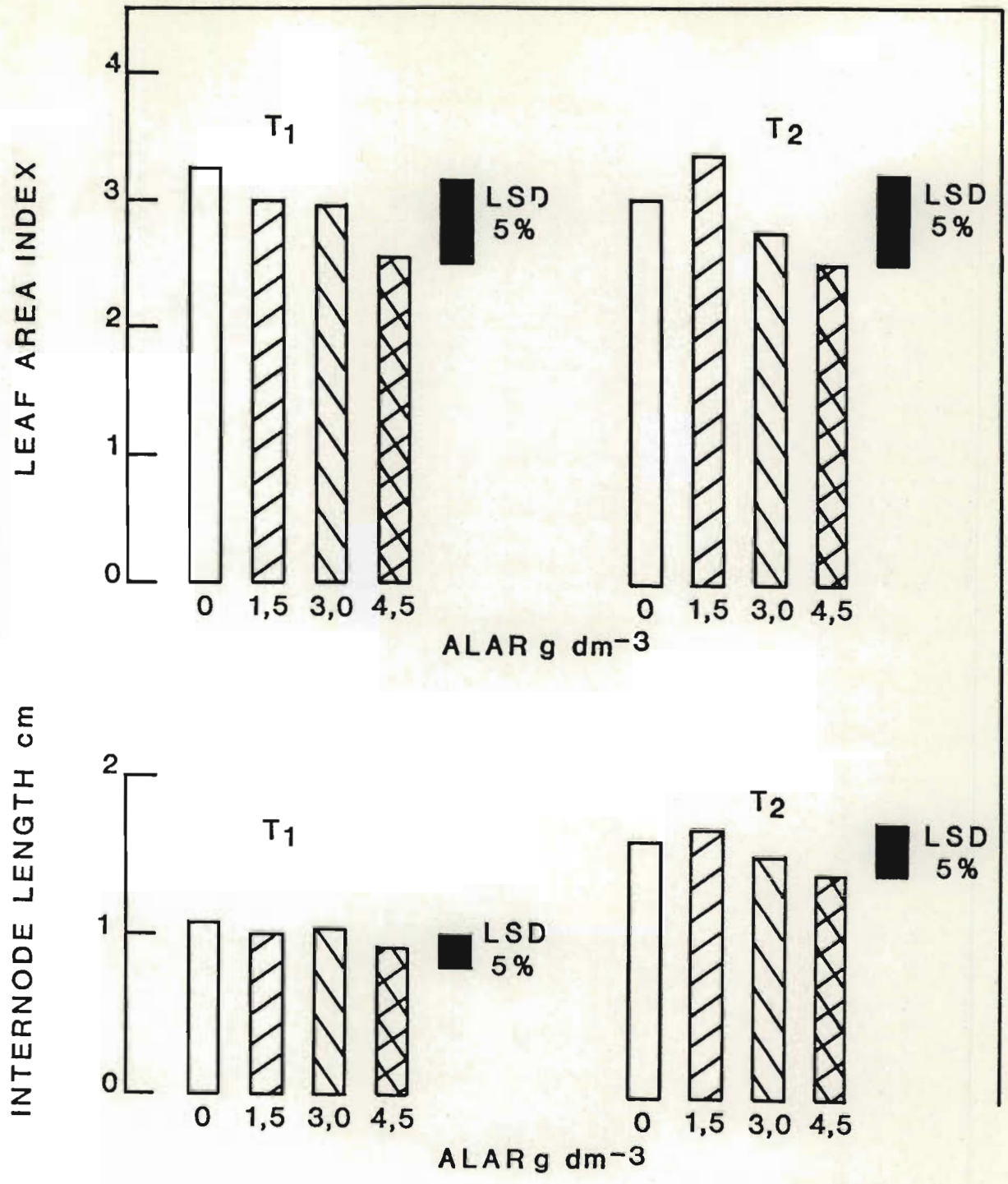

Figure 6.7. The effect of Alar, applied at 3-10-=83 (T_1) and 23-11-83 (T_2) on the leaf area index and internode length of cassava cultivar MSAF 2 grown at Fernwood.

litre Alar than in the control plots (Figure 6.6). The application of the inhibitors at the sub-optimal leaf area index thus reduces the total growth rate including that of the tuberous roots.

EXPERIMENT 6.2. The effect of pruning and Alar on the second season growth.

The main objective of pruning or shoot removal during the winter period is to facilitate mechanical weed control at the start of the second season. After the removal a quick regeneration of an apparently healthy canopy takes place, generally with a high leaf area index. In this experiment the effect of pruning on the yield was studied and an attempt was made to reduce subsequent shoot formation by using a growth inhibitor.

Experimental procedure

The experiment was layed out as a randomized block design with six replications. The plants were pruned on 13-9-83 to approximately 30 centimeters above ground. Alar was applied on 23-11-83 at the following rates:

A_0 0

A_1 2,5 grammes per litre

A_2 5,0 grammes per litre

A control which was not pruned and not treated with Alar was included. The final harvest took place on 28-2-84.

Results

The removal of the shoots at the start of the growing season was followed by a vigorous regrowth of new shoots. A large number of shoots emerged from the stumps and within two and a half months the leaf area index of the pruned plots had already exceeded that of the control plots (Figure 6.8). The leaf area index reached 4,11 which is higher than recorded in any of the other experiments. One of the factors contributing to the high leaf area index was the absence of brown leaf spot, which caused leaf shedding in the older shoots of the control plots. The last harvest took place a week after the second cyclone had passed which reduced the leaf area. It was still obvious, however, that the pruned plots had a larger leaf mass and leaf area index than the non-pruned controls (Figure 6.8). The rate of stem growth was higher in the pruned plots than in the control (Figure 6.9). Stem growth continued after the highest leaf area was reached, which again indicates the potential for growth inhibitors or other measures which reduce shoot growth.

As in the previous experiment, the tuberous root growth in the control plots increased at an almost constant rate. The same almost linear increase was recorded with the pruned plots, although at a lower rate. The rate of tuberous root growth of the pruned plots did not appear to increase during the course of the season despite the fact that an optimum leaf area index was reached after two and a half months. The emphasis remained on shoot development, which may imply that shoot removal will always have a negative effect on yield. The total dry mass production was the same for pruned and control plots, but the shoot /tuberous root ratio was higher in the pruned plots (Figure 6.9).

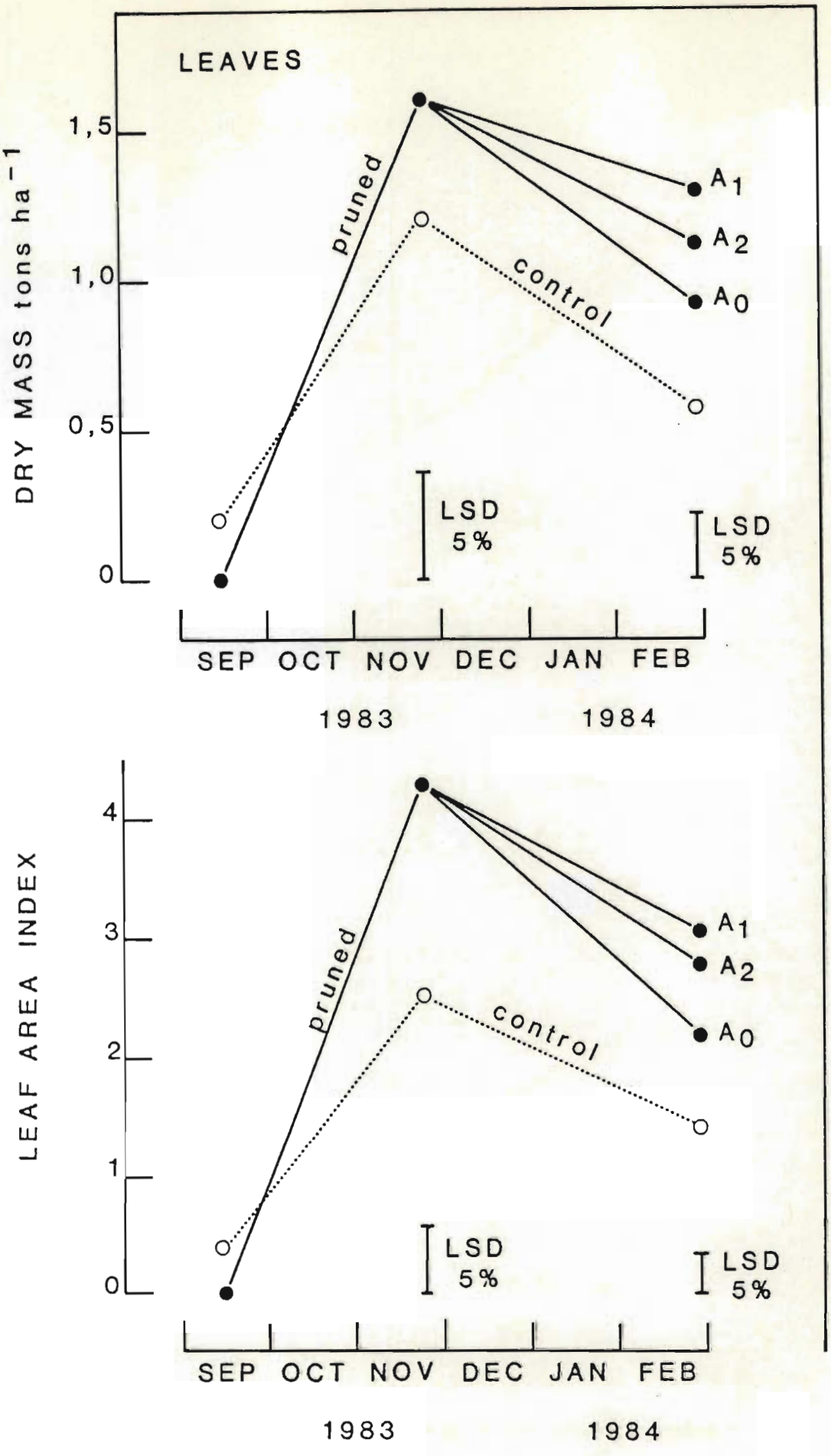

Figure 6.8. The effect of Alar and pruning on the leaf dry mass and the leaf area index of cassava cultivat MSAF 2 grown at Fernwood. A_0=0; A_1=2,5 grammes per litre; A_2=5,0 grammes per litre.

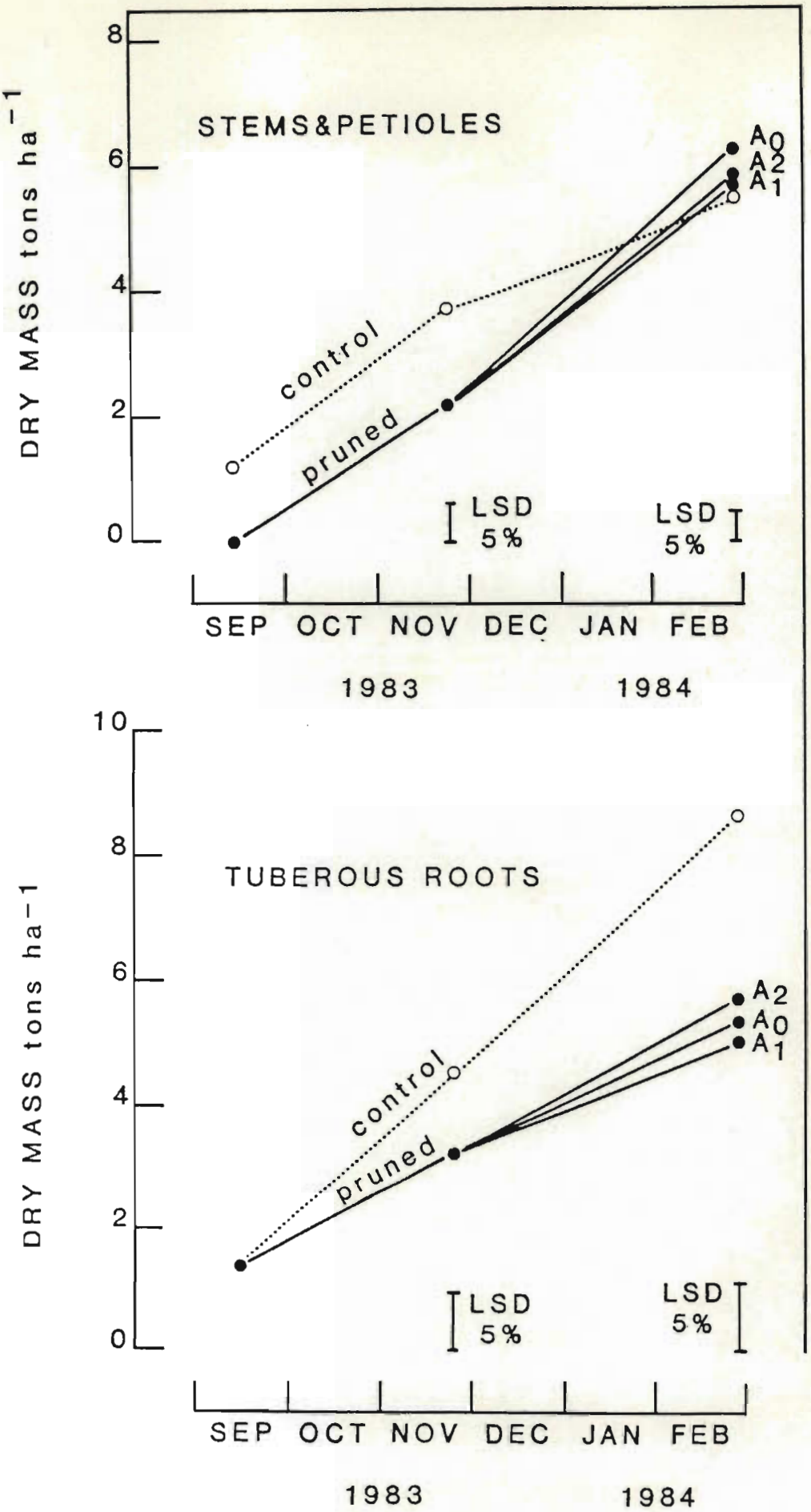

Figure 6.9. The effect of Alar and pruning on the stem plus petiole dry mass and the tuberous root dry mass of cassava cultivar MSAF 2 grown at Fernwood. A_0=0; A_1=2,5 grammes per litre; A_2=5,0 grammes per litre.

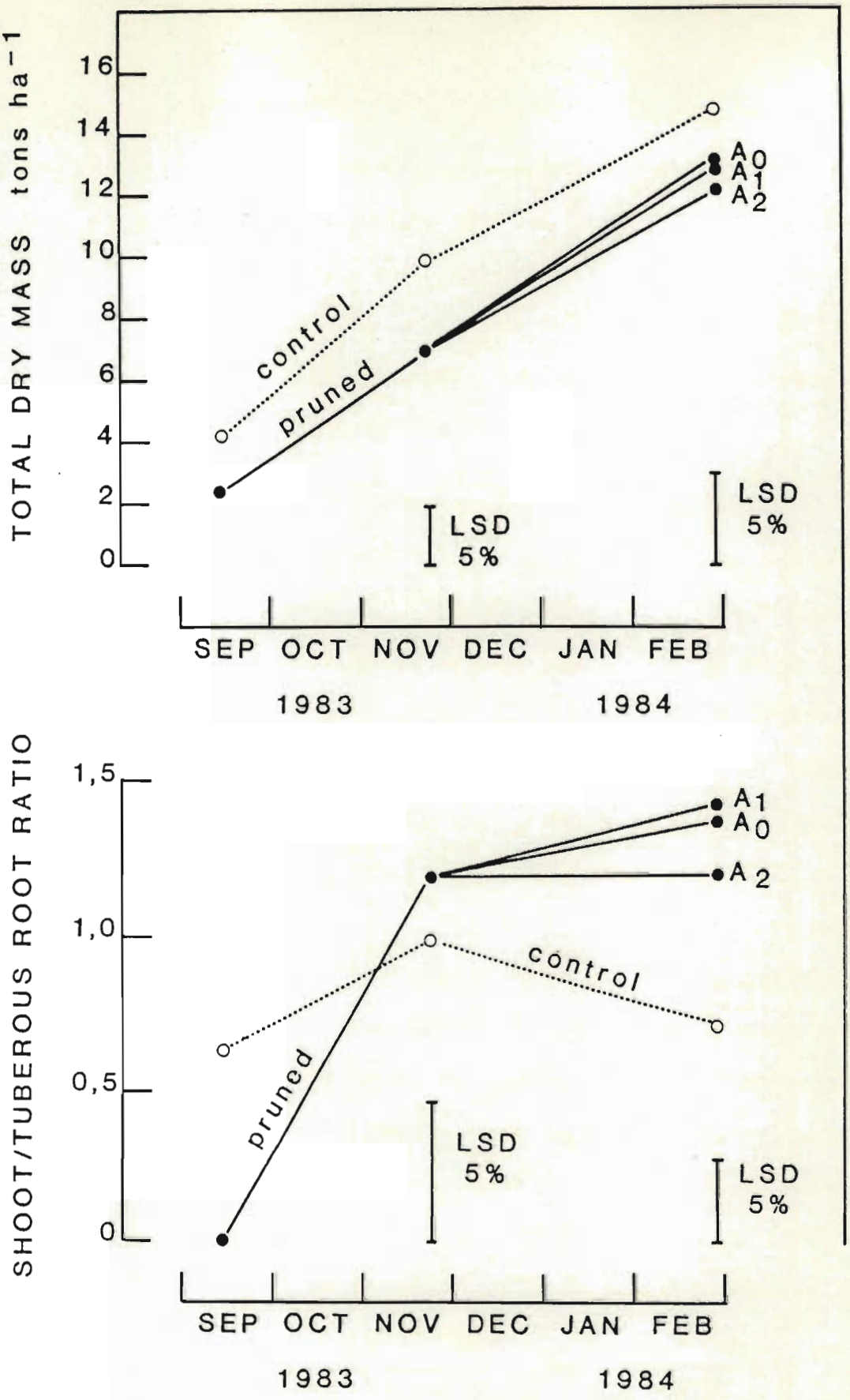

Figure 6.10. The effect of Alar and pruning on the total dry mass and the shoot/tuberous root ratio of cassava cultivar MSAF 2 grown at Fernwood. A_0=0; A_1=2,5 grammes per hectare; A_2=5,0 grammes per litre.

Shoot removal led to the formation of a large number of shoots per plant, resulting in internal shading and elongated shoots. The internode length was 75 per cent higher with the pruned plants than with the controls (Table 6.3). Figure 6.8 shows that Alar was applied at the stage when the leaf area index was high (23-11-83). In other words, a further increase of the leaf area would not contribute much to a higher growth rate.

Leaf dry mass and leaf area index were significantly increased by the Alar treatment (Figure 6.8), while the stem plus petiole dry mass was decreased (Figure 6.9). This was caused by a reduction of 17 and 27 per cent of the average internode length by applying 2,5 and 5,0 grammes per litre Alar (Table 6.3). There was no significant response of tuberous root dry mass production to inhibitor application (Figure 6.9). Alar nor pruning had any significant effect on the starch percentage (dry mass basis) of the tuberous roots. The shoot/tuberous root ratio of the non-pruned control plots increased over the first few months but later decreased as relatively more dry matter was stored in the tuberous roots (Figure 6.10). In the pruned plants a rapid increase of the shoot/tuberous root ratio took place from September to November. This ratio did not decrease at a later stage. The ratio was lowest at 3,0 grammes per litre , but the difference was not significant. The Alar application thus failed in its main objective which was to divert more dry matter to the storage roots.

Table 6.3. The effect of pruning and Alar on a number of parameters of cassava cultivar MSAF 2 grown at Fernwood.

Parameter measured	Control (non-pruned)	Pruned Alar ($g\ dm^{-3}$) 0	2,5	5,0	LSD (0,05)	CV (%)
Internode length (cm)	1,35	2,40	2,09	1,75	0,26	11,1
Leaf area per unit area($mg\ cm^{-2}$)	3,96	4,10	4,07	4,05	0,43	8,6
% Dry mass leaves	21,6	25,5	25,8	25,1	1,14	3,8
% Dry mass stems + petioles	20,8	21,9	20,8	20,9	0,92	3,5
% Dry mass tuberous roots	35,4	35,7	35,1	34,5	2,40	5,6
% Starch	73,8	74,0	71,2	73,5	4,2	4,7

EXPERIMENT 6.3. The effect of nitrogen and Alar on second season growth.

Nitrogen fertilization has an important effect on the shoot development and the distribution of dry matter over the different plant components. A low supply of nitrogen would lead to a small leaf area index and thus a low total crop growth and yield. A high nitrogen supply would lead to a high crop growth rate but the distribution of dry matter could be unfavourable. The greenhouse experiment showed that nitrogen and Alar have the opposite effect on the shoot development of young cassava. In the present field experiment the effect of both factors on the growth and development of cassava was studied.

Experimental procedure

The experiment was layed out as a split-plot design with five replications, with Alar as the main factor and nitrogen as the sub-factor. The nitrogen topdressing (limestone ammonium nitrate, 28 per cent) was applied on 8-10-83 at the following levels:

N_0 0

N_1 28 kilogrammes per hectare

N_2 56 kilogrammes per hectare

Alar was applied on 23-11-83 at the following levels:

A_0 0

A_1 2,5 grammes per litre

A_2 5 grammes per litre

The plots were harvested on 6-3-84.

Results

The effect of nitrogen and Alar on the different dry matter components is summarized in Figure 6.11. Nitrogen topdressing resulted in an increase of the vegetative growth of the crop. The average shoot dry mass over all Alar treatments was 25 per cent higher at level N_2 than at level N_0. The stem plus petiole dry mass was significantly increased by the nitrogen topdressing (Annexure 2). The leaf dry mass was affected by the second cyclone, but it was still evident that nitrogen topdressing increased leaf dry mass. The topdressing of nitrogen had no effect on the relative distribution of dry matter over shoot and storage roots. This is in contrast with the greenhouse experiment were high nitrogen levels led to a relatively lower percentage dry matter stored in the roots. In the field trial, nitrogen increased all dry matter components without significantly affecting the shoot/tuberous root ratio (Figure 6.12).

Application of Alar did not cause a reduction of shoot growth but significantly increased the stem plus petiole dry mass at all three levels of nitrogen application. The tuberous root mass was significantly higher at treatment N_1A_1 and N_2A_1. The starch percentage of the N_0A_0 control was 72,5 per cent and this was not

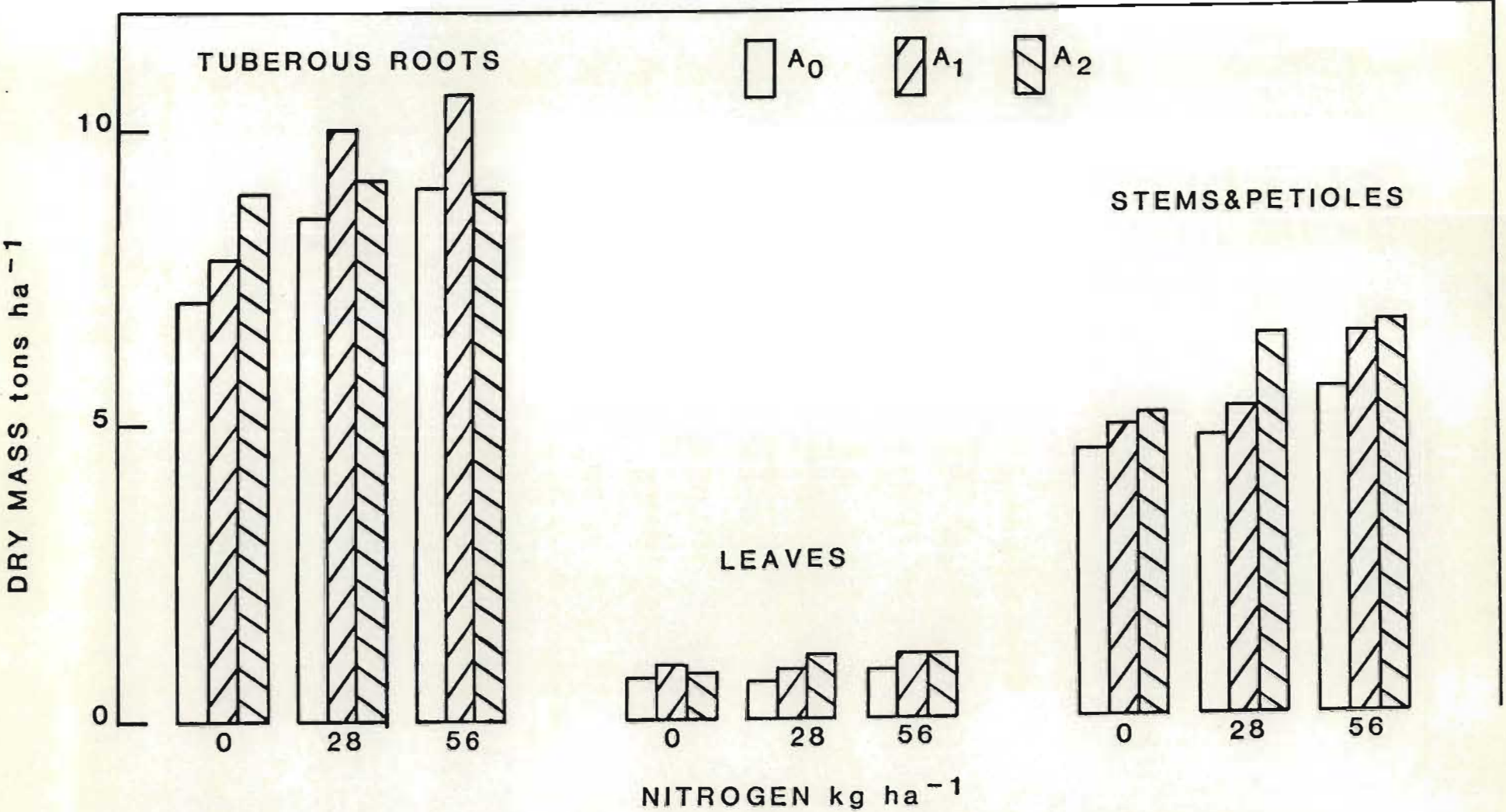

Figure 6.11. The effect of nitrogen and Alar on the dry matter distribution of cassava cultivar MSAF 2 grown at Fernwood. For statistical analysis see annexure 2. A_0=0; A_1=2,5 grammes per litre; A_2=5,0 grammes per litre.

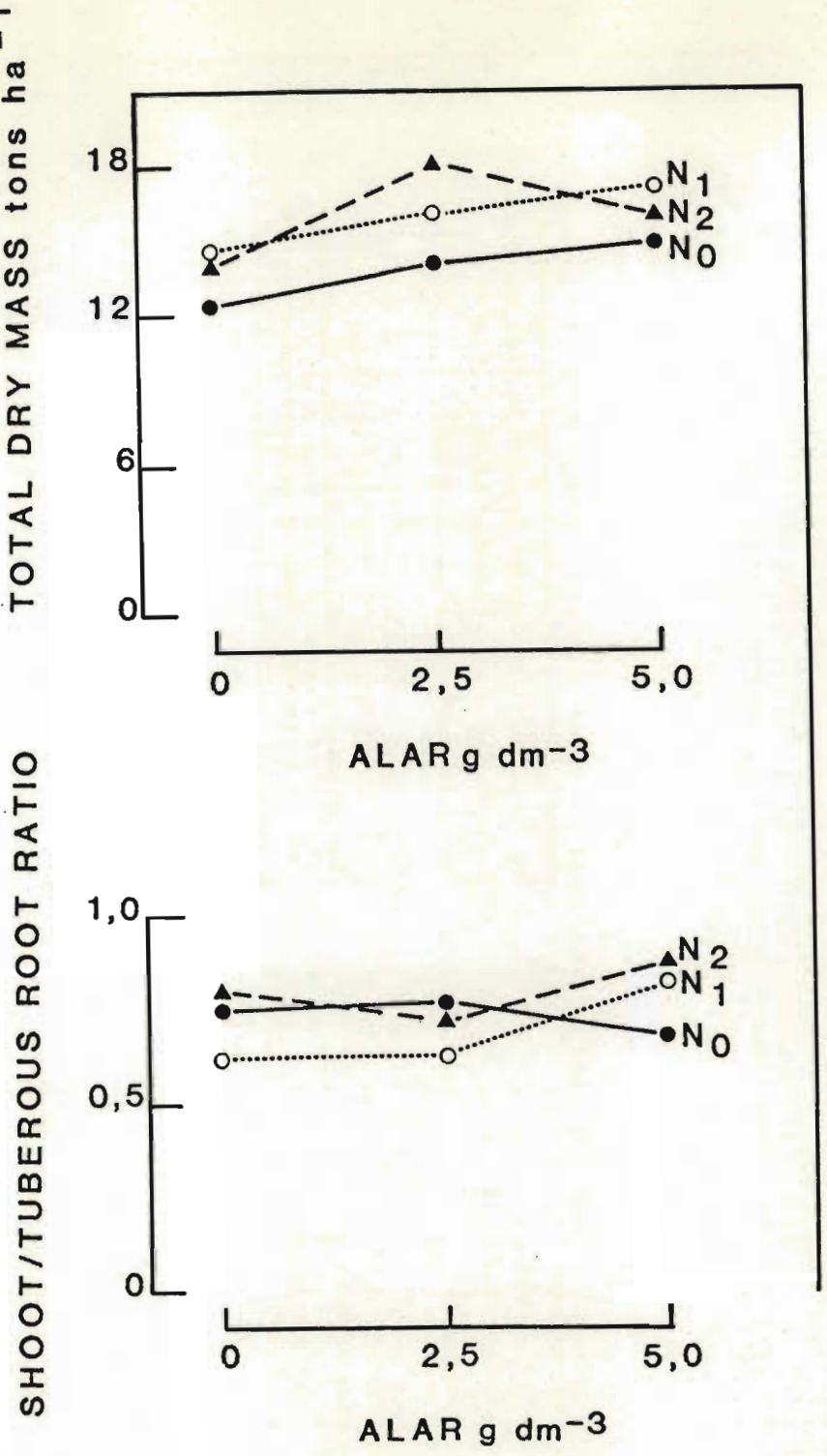

Figure 6.12. The effect of nitrogen and Alar on the total dry mass and the shoot /tuberous root ratio of cassasva cultivar MSAF 2 grown at Fernwood. For statistical analysis see annexure 2. Nitrogen treatments: N_0=0; N_1= 28 kilogrammes per hectare; N_2= 56 kilogrammes per hectare

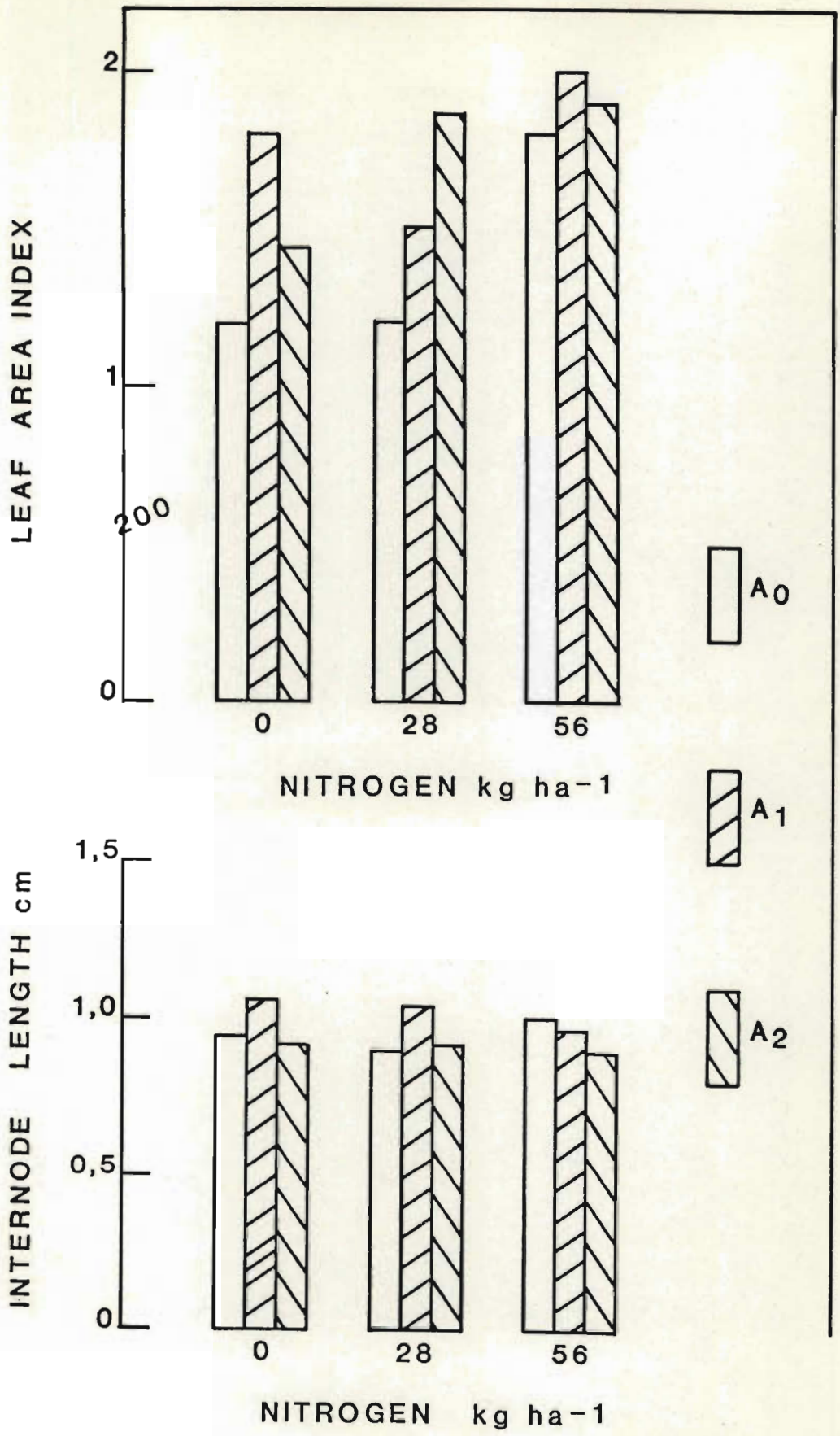

Figure 6.13. The effect of nitrogen and Alar on the leaf area index and the internode length of cassava cultivar MSAF 2 grown at Fernwood. For statistical analysis see annexure 2. Alar treatments: A_0=0; A_1=2,5 grammes per litre; A_2=5,0 grammes per litre.

significantly influenced by the Alar and the nitrogen treatments (Annexure 2). Instead of inhibiting crop growth, Alar promoted the growth of the different components, including the leaf area index, while it had no effect on the internode length (Figure 6.13). Treatment N_2A_1 gave the highest tuberous root yield, namely 10,6 tons per hectare, which was 50 per cent higher than the N_0A_0 control.

EXPERIMENT 6.4. The effect of RSW 0411 on the second season growth

Experimental procedure

In the final field trial the unregistered growth inhibitor RSW 0411, which proved to have a good inhibitory effect on cassava in the greenhouse trials, was applied to cassava cultivar MSAF 2 at the following rates:

R_0 0

R_1 1 gramme per litre

R_2 2 grammes per litre

The experiment was layed out as a randomized block experiment with five replications. The inhibitor was applied on 7-12-84 and the plants were harvested on 29-2-84.

Results

Despite the fact that RSW 0411 gave excellent inhibition of shoot growth in the greenhouse experiments, it had a much less pronounced effect on the dry matter distribution of the field-grown

crop (Table 6.4). The stem dry mass was significantly reduced by the RSW 0411 application, but this reduction was not enough to lead to a large increase in the tuberous root dry mass. Since the application of RSW 0411, the total shoot dry mass had increased from 3800 kilogrammes per hectare to 9529 kilogrammes per hectare. A reduction of this increase in stem growth could have led to a considerable increase in yield. In contrast to the greenhouse studies, there was no visual effect on the shoot tips. The leaves had the same green colour as normal leaves and the internode length was only slightly reduced.

It would seem as if the strong inhibition of this regulator which was recorded in the greenhouse could not be repeated in the field trial even at double the application rate.

DISCUSSION

Contrary to what was found in the greenhouse trials, there was little response of the field-grown cassava to the growth inhibitor treatments. The best indicator of the degree of success of a growth inhibitor application in reducing shoot growth is perhaps the effect it has on the internode length. With the exception of the pruned cassava none of the field trials resulted in the internode length being significantly reduced by the growth inhibitor treatment. But even in the pruned cassava was the reduction of the internode length not enough to cause any significant increase in tuberous root yield.

The growth analysis which was conducted from September 1983 to February 1984 showed that an adequate shoot inhibition would have led

Table 6.4. The effect of RSW 0411 on the second season growth of cassav cultivar MSAF 2

Parameter measured	Control	R_1 1($g\ dm^{-3}$)	R_2 2($g\ dm^{-3}$)	LSD	CV(%)
Tuberous root dry mass ($kg\ ha^{-1}$)	8226	8245	8801	530	12,5
Leaves dry mass ($kg\ ha^{-1}$)	2846	3772	2951	1235	26,3
Petioles dry mass ($kg\ ha^{-1}$)	259	324	270	92,2	22,2
Stem dry mass ($kg\ ha^{-1}$)	6424	4906	5300	1403	17,4
Shoot/tuberous root ratio	1,18	1,10	0,99	0,28	18,0
Leaf area index	1,36	1,95	1,46	0,59	25,6
Internode length	1,09	1,10	0,98	0,18	11,9

to large increases in tuberous root yield, if the assimilates were re-channelled to the tuberous roots. The growth inhibitor treatments failed, however, to bring about any favourable change in the dry matter distribution. Shoot/tuberous root ratios were not significantly reduced in any of the tuberous roots. The growth inhibitor applications have therefore failed in their main objective, which was to alter the dry matter distribution in favour of the tuberous roots.

It would appear from the field data that shoot growth and tuberous root growth show a high degree of positive correlation. A reduction in the shoot growth by a growth inhibitor will therefore not automatically lead to an increase in the tuberous root growth. On the other hand this could mean that an increase of the shoot growth by, for example nitrogen fertilization, will lead to an increase of the tuberous root growth. The rate of shoot growth and the rate of tuberous root growth which occur early in the season are maintained for the remainder of the season and are difficult to change.

The experiments have furthermore shown the large variability in response to growth inhibitor application. In the date of application experiment (6.1) the leaf area index was reduced by the growth inhibitor, while the index was significantly increased in all the other experiments. In the nitrogen x Alar experiment (6.3) the dry mass of stems plus petioles was significantly increased by the growth inhibitor, while this parameter was reduced in experiments 1 and 4. No reference was found in the literature which mentioned an increase of shoot growth as a result of an Alar application, but a similar response was found at the A_1 treatment of the nitrogen x Alar

greenhouse experiment (5.2).

The results further show that the effect of growth inhibitors on the dry matter distribution of a crop is far from consistent. The shortening of the internodes seemed much less pronounced in the field than in the greenhouse, and in some cases did not even occur. There could be several reasons for the differences in response of field-grown plants and greenhouse-grown plants. In the first place the uptake of the inhibitor in the field could be much less than in the protected environment of the greenhouse where the leaves remain wetted for a much longer period. To compensate for the lower uptake the rate of application could be increased. In the field trials the rates of application used were already twice those required to produce a good response under greenhouse conditions. Much higher rates of application would make the use of inhibitors uneconomical for a low input crop like cassava, in particular in view of the unreliability in the response.

Another factor which could account for the discrepancy in response under greenhouse and field conditions could be differences in light intensity. The greenhouse plants received only 50 per cent of the incoming radiation and therefore the plant had a longer internode length in comparison to the field grown plants. The inhibitors appeared to have more effect on these somewhat elongated shoots. A similar effect was observed with the pruned plants of the field trial which had elongated shoots. These were the only plants where a significant reduction of the internode length took place.

The present data has shown that despite the fact that chemical

regulation of dry matter has the potential to increase yield, the practical application in the field is unreliable and does not lead to a significant redistribution of carbohydrates. It appears at this stage that pruning has an undesirable effect on the rate of tuberous root growth and thus can not be used as a tool to manipulate the dry matter distribution. The only factor studied in the trials which has increased the yield is the nitrogen fertilization. The theory that nitrogen fertilization decreases the relative amount of dry matter allocated to the tubers (KROCHMAL and SAMUELS, 1967) could not be confirmed in the field experiment at the levels used. Instead it appears that the increase in the shoot growth leads to an increase of the tuberous root growth.

CHAPTER 7.

GENERAL DISCUSSION AND CONCLUSIONS

Tuberization could be considered as consisting of two physiological processes, namely tuber initiation and subsequent tuber growth. In the past many workers have attempted to identify the hormone which they believed was the stimulus for tuber initiation (GREGORY, 1957; CHAPMAN, 1958). The present study on cassava was not designed to find such a hormone responsible for tuber initiation but was rather aimed at obtaining an understanding of the role of endogenous hormones in the tuberous root growth and dry matter distribution of cassava.

Studies on the hormone physiology of tuberization have so far contributed little to practical ways of manipulating dry matter distribution of tuber crops. Improvement of yields of arable crops could become a major agricultural practice in the future (BRUINSMA, 1982). With respect to tuber crops there is still a need for a better understanding of the role of endogenous hormones in the control of tuber growth and the translocation of assimilates to the tubers. Reducing endogenous gibberellin levels in the plant by means of plant growth inhibitors is at this stage the only proven way of increasing tuberization and yield (BODLAENDER and ALGRA, 1966).

Abscisic acid is generally regarded as a tuberization promoting hormone (OKAZAWA and CHAPMAN, 1962). WAREING and JENNINGS (1979) reported that abscisic acid is an essential factor for tuber initiation in potato. The present data on abscisic acid in

cassava confirms that abscisic acid, present in tuberous roots, plays a major role in the growth of these roots. Young tuberous roots of cassava had a much higher level of abscisic acid than primary roots of the same plants and the level of this hormone was generally high under the conditions which promoted rapid growth of this organ. It has been pointed out previously (Chapter 3) that these high levels of abscisic acid had apparently no inhibitory effect on the tuberous root growth. Although the evidence in this matter is not conclusive, it would appear that abscisic acid in tubers is a promoting factor for cell division and cell growth in this organ. Certain workers have suggested that hormones can work through entirely different mechanisms within the same plant whereby the effect of a hormone is to a large extent determined by the type of tissue (KENDE, 1981; TREWAVAS. 1981). This would certainly apply to abscisic acid which seems to have a growth promoting function in the underground parts of the plants and a growth inhibiting function above ground. For hormone research it is imperative that a certain function of a hormone in plant development is studied without preconceived ideas of the action of this hormone.

To understand the role of abscisic acid, tuberization should perhaps be seen in the total context of a tuber-forming plant. Tuberization is in the first place a survival mechanism and in many cases even a reproduction mechanism of plants (eg. potato). This implies that tuberization goes hand in hand with a reduction in vegetative growth, which, in some species, leads to senescence and ultimately the death of the shoot. Under these conditions of senescence abscisic acid levels in plants are known to be high (WOOLHOUSE, 1981). Tuberization is also a reaction of plants to

environmental stress. For example, stress caused by nitrogen or moisture shortage accelerates tuber growth. In these situations the level of abscisic acid in plants is generally high (WALTON, 1980). In this study increased levels of abscisic acid were found in the storage roots of cassava as a result of nitrogen shortage.

The importance of cytokinins in tuber growth has been re-emphasized in this study. High levels of cytokinins were present in the tuberous roots, particularly when the rate of tuber growth was high. These high levels of cytokinins in the tuberous roots are perhaps not so surprising, since high levels of these hormones generally occur in fast growing tissues (MICHAEL, MOUNLA and GOLDBACH, 1974). It is therefore debatable whether cytokinins have a special function in tuber initiation. Rather, it appears that these hormones contribute to tuber growth in the same fashion as they promote growth of any other tissue, namely by stimulating cell division (MILLER, 1965) and attracting metabolites (MOTHES and ENGELBRECHT, 1961).

The search for the so-called "tuberization hormone" in the past has perhaps diverted the attention from the fact that most likely all known endogenous hormones contribute, either in a positive or a negative way, to tuber growth, and that tuberization is controlled by the combined action of all these hormones.

The knowledge about the function of the endogenous hormones should be used to find ways of improving the yield of the economically very important tuber crops. The present data suggest that increasing the abscisic acid level in the plant could be a means of

reducing the vegetative growth and stimulating tuberization. A senescence inducing compound could probably be more effective in promoting tuberization than the gibberellin-inhibitors, which only appear to reduce internode length of cassava. These latter compounds appear to have insufficient effect in the field. Reduction of the internode length is apparently not enough to give a significant increase in yield. Even if better results are obtained at higher rates of application it seems unlikely that the use of these regulators is financially justifiable in a low input crop like cassava. As far as these regulators are concerned, there is a need for more research into the uptake and translocation of these compounds in the field, as well as their metabolism in the plant.

Manipulating the dry matter distribution of cassava remains an important area of research. The harvest index is often too low and the growth analysis data of this study shows that a reduction of total shoot dry mass could potentially lead to large increases in yield. In cassava there exists a large genetic variability in harvest index (WILLIAMS and GHAZALI, 1969; COCK, 1976) and therefore the best option at this stage would be to select for cultivars with a high harvest index, and other features such as small internode length and a low rate of leaf fall.

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Annexure 1. Analysis of variance of experiment 5.2.

Parameter measured	Source of variance(f-values)			CV(%)
	N	A	A x N	
Leaf dry mass	33,9	8,1	2,6	18,7
Stem + petiole dry mass	33,6	3,5	2,6	31,8
Root dry mass	10,4	5,6	1,6	24,2
Tuberous root dry mass	0,1	0,1	0,7	105,0
Total dry mass	30,7	2,0	1,3	20,2
Shoot/root ratio	5,8	3,2	0,5	55,9
Leaf area	87,1	2,0	0,3	16,5
Leaf mass per unit area	44,2	5,8	5,8	12,3
Elongation	18,3	19,0	1,0	38,3
Internode length	10,6	68,1	4,0	20,4
Degrees of freedom	N	2		
	A	2		
	A x N	4		
	RES	45		

Annexure 2. Analysis of variance of experiment 6.3.

Parameter measured	Source of variation(f-values)			CV(%)
	A	N	A x N	
Tuberous root dry mass	4,2	2,4	0,8	18,7
Leaf dry mass	7,0	2,8	0,8	22,9
Stem + petiole dry mass	13,5	36,3	3,2	6,9
Leaf area index	7,3	6,3	1,3	19,1
Internode length	1,6	0,1	1,7	9,7
Shoot/tuberous root ratio	0,8	1,9	1,3	17,8
Total dry mass	10,9	6,3	1,0	12,1
Starch% (dry mass basis)	2,2	1,7	2,5	3,8

Degrees of freedom	A	2
	RES 1	6
	N	2
	A xN	4
	RES 2	18