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CHANGES IN β INHIBITOR AND CYTOKININ LEVELS IN RESPONSE TO LONG WILTING PERIODS IN GRAIN SORGHUM AT DIFFERENT GROWTH STAGES.

A thesis presented in partial fulfilment
of the requirements for the degree of
Master of Agricultural Science
in Plant Science at
Massey University.

George Tay Kee Chong

1976

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CHAPTER ONE. LITERATURE REVIEW

1.1. INTRODUCTION

In the living plant, water occurs in many states; water of hydration and inbibition in colloidal phases such as cell walls, osmotic water in vacuoles and in phloem and hydrostatic water in the xylem. Water is involved in all physiological processes of the plant. Plant water status is a highly dynamic parameter and is influenced strongly by soil and atmospheric conditions. It is regulated to different degrees in different situations and with various species by physiological processes.

This review will be confined mainly to effects of water stress on:-

a) Several major physiological processes affecting growth and development.

b) Two main hormones (abscisic acid and cytokinins) and their importance in plant responses.

c) Grain yield in certain cereals.

1.2. Development of internal water deficits

Water deficit (stress) is defined as the state a plant enters when the water potential passes the zero mark and becomes negative. Scholander et.al.(1965) stated that the water potential in the xylem of many plants is negative most of the times. The actual water potential values depend on the two main factors: a) the level of the soil water potential and b) the diurnal lag of absorption over transpiration. Each of these factors, in turn, is influenced by other factors both environmental and physiological. Soil water potential is reduced progressively during a period of drought and there is a concomitant drop in level of plant water potential i.e. plant water potential cannot be higher than soil water potential. Hence there is a base level of plant water potential and internal water deficit which is limited by the level of soil water potential. Superimposed on this base level is the additional internal water deficit associated with the daily rhythm of transpiration and absorption (Slatyer 1967).

1.2.1. Parameters indicating plant water status

The parameters commonly used to indicate the degree of water status in plants are as follows:-

a) Water potential (Ψ) is now accepted and used as a basic measurement. It is expressed as energy per unit volume and with the

ABSTRACT

Plants of Sorghum bicolor L. Moench R.S. 610, a hybrid grain variety, were subjected to three water stress cycles during the following growth stages :-

(i) Late vegetative stage (ii) Boot to bloom stage

(iii) Dough stage.

Water status of the plants was measured by pressure bomb and Weatherly and Barr s' method. Both β inhibitor and cytokinin activity in leaves and roots were determined by two bioassays a) wheat coleoptile and b) radish cotyledon respectively. The bioassays results were expressed as (±) abscisic acid and kinetin equivalents respectively.

The results indicated that changes in levels and activity of ABA and cytokinin occurred in response to water stress. ABA levels in leaves increased rapidly up to 13 fold from the control to the maximum stress during the first and second stress cycles, while ABA levels of leaf samples from the third stress cycle only increased to 6 fold at the maximum stress period. In the roots , ABA levels did not increase markedly as the leaf samples in all the three stress cycles.

Cytokinin contents in both leaves and roots changed qualitatively and quantitatively in response to severe stress. There was a general decline in cytokinin activity as the magnitude of stress increased. There was a shift of cytokinin activity in peak 2 to peak 1 as the stress periods prolonged.

Grain yield, in terms of grain weight and grain number, was measured for the three stress cycles. The results showed that there were two 'critical' stages when sorghum plants were susceptible to stress that is a) late vegetative to early boot stage and b) during inflorescence development and flowering period.

The involvement of abscisic acid and cytokinins in the plant's adaptation to water stressed was discussed.

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chemical potential of pure water at atmospheric pressure and the same temperature as the reference point (Slatyer 1967). $\bar{\Psi}$ is the sum of the component potentials arising from the effect of solutes ($\bar{\Psi}_s$), of pressure ($\bar{\Psi}_p$) and matrix ($\bar{\Psi}_m$) such that:

$$\bar{\Psi} = \bar{\Psi}_p + \bar{\Psi}_s + \bar{\Psi}_m$$

b) Relative water content (RWC) is the water content relative to the water content of the same tissue at full turgor (after floating on water to 'constant' weight.

c) Tissue water content on a percentage of fresh weight.

Both RWC and tissue water content suffer a major shortcoming that is, both parameters are relatively insensitive indicators of mild stress (Barrs 1968)

Mild stress is considered to entail a lowering of plant ($\bar{\Psi}$) by several bar below corresponding values in well watered plants under mild evaporative demand. Moderate stress refers to a lowering of ($\bar{\Psi}$) by more than a few bar but less than -12 or -15 bar and stress would be severe if ($\bar{\Psi}$) is lowered more than -15 bar.

1.2.2. Problems in water stress studies

Slatyer (1972) reckoned that a clear and quantitative understanding of water stress effects does not exist, offering several reasons for this. Firstly, water stress affects almost all biophysical and biochemical processes, so the integrated effects on growth and development are extremely complicated. Secondly, plant water status is a highly dynamic parameter, strongly influenced by soil and atmospheric conditions. Finally, plant water status constitutes a difficult parameter to control experimentally.

1.3. WATER DEFICITS AND PHYSIOLOGICAL PROCESSES

The effect of water stress on such key processes as nutrient uptake, carbohydrate and protein metabolism, and translocation of ions and metabolites is closely linked with the effects on plant development. In turn, the effects are affected by other processes. For example, root development affects the size and character of the absorbing system for mineral nutrients; shoot development has a direct feedback on the rate of carbohydrate and protein metabolism.

Water stress affects the growth and development of plants in various ways, directly and indirectly.

1.3.1 Water deficits and plant development

The growth and development of a plant depends on continuing cell division ; the progressive initiation; differentiation and expansion of the cells until the characteristic form of the plant is realised (Slatyer 1969).

(A) Cell division This process shows a marked sensitivity to water stress, if stress is severe and protracted. For example, cell division may continue during stress at a much reduced rate until severe conditions occur (Kirkham et al. 1972). Cell division is related to cell size and cell number. Gardner & Nieman (1964) found that DNA increments per cotyledon of radish were reduced by more than half in the presence of -1 to -2 bar mannitol solutions during twenty-eight hours; little further reduction occurred with a lowering to -16 bar.

It is uncertain if the stress effect on cell division is direct; the effect can bepossibly via suppressed cell expansion. There is a possibility also of changes in growth regulators taking place during prolonged stress and thus affecting cell division.

(B) Cell enlargement This is the other essential component of growth and is affected at very slight stress levels (Hsiao et. al. 1970) and is usually the first observable symptom. In some species, cell enlargement is so sensitive to deficits that stem elongation and leaf growth can be inhibited by small diurnal deficits that occur even with well watered plants on days of high radiation incidence (Boyer 1968). Boyer (1970) demonstrated that when leaf Ψ dropped from -2 to -4 bar, leaf growth was inhibited by at least 75% in corn, soybean and sunflower in controlled growth environment. Photosynthesis per unit leaf area was only inhibited by 10% in corn and unaffected in the other two species. Boyer (1968) suggested that little growth occurs during the day when

leaf $\bar{\Psi}$ is rarely above -4 bar even in well watered plants; growth occurs primarily at night and this appears to depend on the length of time the leaf remains at high $\bar{\Psi}_w$. Boyer's result differs from Watt's recent finding (1974) which shows that leaf $\bar{\Psi}$ above -8 or -9 bar had little apparent effect on extension rate of Zea mays during vegetative stage in the field conditions. Watt concluded that the main reason for the apparent lack of sensitivity is due to $\bar{\Psi}$ gradients within the leaves.

A progressive decline in rates of cell enlargement is expected as water deficits develop, with enlargement ceasing when turgor pressure levels are still at several bar. Cell enlargement requires turgor to extend the cell wall and also requires a gradient in $\bar{\Psi}$ to bring water into the enlarging cell. This enlargement needs turgor to be above a minimum before irreversible enlargement occurs (Green et al. 1971). For rapidly growing vegetative plants, one of the first effects of drought is a reduced rate of growth by enlargement. The effect of reduction in cell enlargement decreases the total leaf area for photosynthesis. In general, cell enlargement appears to be more sensitive than cell division (Meyer & Boyer 1972) although Kirkham et. al. (1972) found an early effect of osmotic solutions on cell division.

The effect of water stress on the mature tissue or that approaching maturity, resembles that of hastened development, resulting progressively in protein hydrolysis and breakdown of normal cell functions. This hastens senescence which is irreversible, for plant tissues are unlikely to recover from a period of stress.

(C) Root development The overall growth of the root system or its increase in weight is determined by the availability of assimilate from the shoot in the vegetative stage with growth regulators being of lesser importance (Street 1969, Hatrick & Bowling 1973). The sources of this assimilate are the lower leaves on the stem (Rawson & Hofstra 1969), and of that assimilate translocated to the roots, possibly less than one third is used for growth; the balance is respired (Hatrick & Bowling 1973) or exuded into the soil (Barber & Gunn 1974).

Root growth is relatively insensitive to decreasing levels of soil water potential in the range down to -4 to -7 bar but below this point there is a marked reduction in most species of plants including cereals, cotton (Taylor & Ratliff 1969) and corn (Gingrich & Russell 1956). Lawlor (1973) found that growth of wheat roots stopped at -10 bar. The effects of water stress on root development include a reduction in rates of meristematic activity and root elongation and suberization so affecting the water and nutrient uptake properties of the root system.

1.3.2. Water deficits, photosynthesis (P/S) and carbohydrate metabolism

The photosynthetic capability of plants is determined primarily by the total leaf area and the activity of each unit of leaf. Water stress can have direct and indirect effects on (P/S) and on a number of intermediate components and processes.

In general, net (P/S) is reduced progressively by water stress, and negative values may develop when stress is severe. This response is mediated partly by way of impeded CO_2 supply following stomatal closure and partly by a direct effect of dehydration on the (P/S) system. Boyer & Mcpherson (1975) showed that at (LWP) of -18 to -20 bar, the (P/S) rate of maize was 15% of the controls. Boyer (1970) showed that in corn stomata appeared to shut partially whenever leaf Ψ decreased below -3.5 bar. For soybean desiccation had no effect on stomata until leaf decreased to -11 bar.

Non-cyclic photophosphorylation, cyclic photophosphorylation and electron transport through photosystems1 and 2 are reduced at leaf Ψ below -10 bar (Keck & Boyer 1974). In addition to the effects of low leaf Ψ on the photochemical portion of (P/S), there are also changes in some of the enzymes of the 'dark' reactions. Ribulose 1,5-diphosphate carboxylase activity is reduced when assays are performed on extracts from desiccated leaves (Huffaker et. al. 1970; Johnson et. al. 1974). However, none of these studies demonstrated an effect large enough to account completely for the inhibition of P/S in intact leaf (Jones 1973 ; Johnson et. al. 1974). Phosphoenolpyruvate carboxylase and ribulose-5-phosphate kinase also showed little change to be completely limiting (Huffaker et. al. 1970; Shearman et. al. 1972).

Thus, there is a general inhibition of a number of processes of the light reactions of (P/S). Boyer (1976) seems to think that the rates of (P/S) are limited either by stomatal effect or carboxylation activities or perhaps a combination of effects according to the conditions and the species involved.

The availability of suitable sinks for assimilates also influences (P/S) such that (P/S) is greatly reduced when assimilate utilization is impeded. During stress, assimilates probably accumulate at sites of (P/S), since leaf enlargement is restricted sooner and to a greater degree. Wardlaw (1967, 1971) examined this in wheat and concluded that although leaf (P/S) was not affected until growth rate had been reduced, there was no evidence of sink size directly affecting (P/S) rate. He postulated that the lack of suitable sinks could retard (P/S) under appropriate conditions.

(B) Water deficits and respiration This effect is difficult to determine; partly it is a problem of distinguishing between dark respiration and photorespiration and hence in measuring photorespiration. Part of the difficulty is due also to stomatal closure and the possible differences in short term and long term effects. In general, there was a progressive decrease in dark respiration of corn, soybean, and sunflower shoots as water stress increased (Boyer 1970).

The effect on photorespiration is difficult to evaluate because (P/S) is proceeding at the same time. A complication here is the marked dependence of respiration on temperature, since stomatal closure generally induces a rise in leaf temperature.

Another common effect of water deficits on carbohydrate metabolism, is an increase in sucrose levels and a decrease in starch levels. These are associated with reduced polysaccharide levels resulting from such factors as decreased (P/S) and increased hydrolysis as well as reduced synthesis. Hiller & Greenway (1968) concluded that reduced starch formation was an indirect result of increased sucrose synthesis, rather than a direct effect of stress on starch synthesis.

1.3.3. Water deficits, protein synthesis and nitrogen metabolism

In general, there is a close dependence of the growth rate of developing tissues and organs on protein synthesis and especially between protein synthesis and RNA and RNA and DNA levels. Nucleic acids are of major importance in cellular development and any effects of water stress on these compounds would be expected to elicit marked changes in the growth response of plants.

(A) Protein synthesis Shah & Loomis (1965) found that both soluble and total protein contents of sugar beet leaves declined progressively in a matter of days when water was withheld. This is attributed to either a retardation in synthesis or to an acceleration of degradation. Nir et.al.(1970) observed inhibition of amino acid incorporation in root apices which had been air dried at a controlled humidity, but only if water loss was more than 30% of the original fresh weight.

Dhindsa & Cleland (1975) demonstrated that water stress causes a differential inhibition of the synthesis of Avena coleoptile proteins, with the synthesis of some proteins being affected to a greater extent than the synthesis of others.

Polysome formation is slowed and breakdown occurs during water stress. This could be due either to a direct effect on the polysome themselves

or on the supply of m-RNA which is needed for polysome formation. Hsiao (1970) showed that water stress of etiolated maize seedlings caused a shift from polymeric to monomeric form of the ribosomes in rapidly growing meristematic tissues (a loss of 10% of fresh weight in 15 minutes). On rewatering, the ribosomes reverted to the polymeric form. Hsiao suggested that stress effects on protein synthesis are mainly at the translational level because of the rapidity of the response to stress and quick reversibility by re-watering.

Shah & Loomis (1965) found that DNA content per cell of sugar beet leaves was reduced by severe and protracted wilting, while RNA levels tended to decline at low stress levels. They concluded that RNA synthesis was impaired as well as some degradation of RNA. Gates & Bonner (1959) attributed the decline in RNA levels to enhanced degradation of RNA. The different responses observed by various workers are probably related in some degree to the developmental stage of the tissues and the methods of determination employed.

Itai and co-workers (1967, 1968) have postulated a key role for cytokinins in regulating protein synthesis during water and salinity stress. This view is based on the reduction in cytokinins in leaves by stress (see page 30) and on the ability of applied kinetin or benzyladenine to alleviate a part of stress-effected reduction in amino acid incorporation or in leaf protein content.

In rapidly growing tissue, protein synthesis appears to be readily and reversibly reduced by very mild water stress. The dynamic responses to stress and stress release may be controlled at the translation level. Hsiao (1973) reckoned that the basis for the response is still obscure.

(B) Proline and other amino acids Total free amino acids in leaves often are increased if severe water stress lasts several days Barnett & Naylor (1966). Amides frequently increase (Barnett & Naylor 1966) but proline has the most marked rise (Palfi 1968; Routley 1966; Singh et. al. 1973a). In sorghum (Sorghum bicolor L.) leaves of water stressed field grown plants accumulated proline to a level several times greater than in non-stressed plants (Waldre et. al. 1974). Upon leaf rehydration after stress, proline content decreased rapidly to pre-stressed levels. Proline is due to protein hydrolysis (Palfi et. al. 1966) and may be oxidized as a source of energy especially when the carbohydrate content is low (Oaks et. al. 1970).

Singh et al. (1973b) indicated that barley varieties which accumulated more proline tend to survive extreme water stress more readily and grew more rapidly following relief from the stress. They suggested

the possible roles are (1) to neutralize toxic free ammonia produced in water-stressed leaves and (2) to serve as a substrate for respiration and energy source for the recovery of the plant following stress.

1.3.4. Water stress and enzyme levels and activity

Todd (1972) listed some twenty-five enzymes affected by water deficits. The effects of moderate to severe stress on enzyme levels are as follows:

a). enzymes involved in hydrolysis or degradation usually remain at the same level or increase ; they do not decrease until fairly severe desiccation has taken place.

b). severe stress generally causes an overall decrease in enzyme level.

c). levels of some enzymes involved in synthesis are decreased and levels of others increase.

Mattas and Pauli (1965) observed nitrate reductase decreased in activity early in the drying cycle when little change in leaf relative water content occurred and at the same time nitrate accumulated in the plants.
The level of phenylalanine ammonia-lyase was found also to decrease with mild to moderate water stress and to recover readily with rewatering (Bardzik et.al.1971). Hsiao (1973) suggests that because nitrate reductase and phenylalanine ammonia-lyase both have short half lives and respond rapidly to water stress, and hence suppressed protein synthesis could account for their decreased activity.

α-amylase and ribonuclease in leaves are observed to increase with a moderate to severe water stress. The functional significance of these increases remains obscure (Hsiao 1973).

Some enzymes that are involved in the photosynthetic pathway are not easily reduced in activity by mild stress (Hsiao 1973).

1.4. Abscisic Acid And Cytokinins In Plants

Introduction

Plant growth hormones play a central role in the internal control mechanisms of plant growth, interacting with key metabolic processes such as nucleic acid and protein synthesis. They may either enhance or diminish growth rate, depending upon their nature and concentration. The auxins, the cytokinins, and the gibberellins are generally regarded as growth stimulators, whereas both ethylene and abscisic acid often inhibit growth. These growth regulators are concerned in the overall growth rate and in the correlation of growth activities by acting as chemical signals. They move from one cell, tissue, or organ, to another and thereby provide a means of communication between different parts of the plant.

This review will cover abscisic acid (ABA) and cytokinins, and examples of their interaction in growth processes.

1.4.1. Abscisic acid and its phenomenon in plants

Abscisic acid is the trivial name for 3 methyl-5(1'-hydro, 4'2'6'6' trimethyl 2'-cyclohexen 1'-yl)- cis, trans-2,4- pentadienoic acid. The natural abscisic acid is referred to as (+)ABA and the synthetic as (±) ABA. Generally, ABA in literature refers to the natural (+)ABA. Figures 1 and 2 are cited geometric isomers of ABA.

Bennet-Clark & Kefford (1953) first detected the inhibitor β- complex which is an unpurified fraction of one dimensional chromatograms and is inhibitory in some bioassays. Milborrow (1967) stated that ABA is the most active component of the inhibitor β complex. There are indications that quantitative differences in inhibitor β contents reflect the quantitative variations of ABA (Wright & Hiron 1970). However, in potato tubers, the inhibitor β consists of several compounds including an active phenolic substance (Holst 1971). With pea root extracts, the inhibitor β consists of at least three compounds including trans-cinnamic acid and ABA (Tietz 1971). Most results suggest that ABA is the principal and dominant growth inhibitor of the complex.

ABA was first isolated from young cotton fruit by Ohkuma et al.(1963). The structure was confirmed by Cornforth et. al.(1965) by synthesis. In most plants, the naturally occurring isomer of abscisic acid is cis-ABA. Trans-ABA is much lower in content and less active biologically than ABA and its presence might due to light induced conversion from cis-ABA (Dorffling 1971).

Another naturally occurring substance related to ABA is abscisyl-glucopyranoside (Figure 3). This was first isolated from immature fruits of Lupinus luteus (Koshimizu et.al 1968) and seems to be widely distributed in plants. Milborrow (1970) showed that synthetic ABA applied to tomato shoots was metabolized to this compound. Moreover, the excessively high ABA content in wilting plant tissue was accompanied by an increased content of the glucoside in spinach (Spinacia oleracia L.) shown by Zeevaart (1971). This glucoside has the function of a 'bound' reserve form of ABA and has inhibitory activity when hydrolysed to 'free' ABA (Osborne et al. 1972).

(A) Occurrence and metabolism of abscisic acid

ABA is widely distributed in higher plants, and has been detected in at least forty species. Relatively high amounts have been found in fruits, resting seeds, buds, tubers and in senescent and wilting leaves. The hormone level varies in relation to growth and developmental processes. Davis et.al.(1968) showed ABA changes during cotton fruit development; for example a rise in ABA was correlated with young cotton fruit abscission and with the senescence and dehiscence of the mature fruit.

Abscisic acid has been reported to occur in extracts of roots of Lens culinaris (Fries et al. 1971) and Zea mays (Kundu & Audus 1974a, 1974b ; Wilkins & Wain 1974). Other related inhibitory substances have also been found in root tips, including xanthoxin (Kundu & Audus 1974a). According to these authors, ABA is associated primarily with the root cap and xanthoxin is associated possibly with the 'root meristem'. ABA was extracted from seedling roots of Vicia faba by El-Antably & Larsen (1974) and demonstrated by gas-liquid, thin-layer chromatography of purified samples.

The presence of ABA in xylem exudate was reported for Salix viminalis by Lenton et.al.(1968) and for Helianthus annuus by Hoad (1975).

(i) Biosynthesis of ABA

ABA is synthesized in several different parts of plants. Leaves, fruit and seed tissues (cotyledons, endosperm, embryos) have been shown to incorporate labelled mevalonate into ABA (Milborrow et.al. 1973). Two schools of thought of biosynthesis of ABA are, briefly, (1) that ABA is derived from a carotenoid, in particular violaxanthin, by photolytic cleavage or oxidative processes; and (2) that ABA is synthesized by a direct route from mevalonic acid.

Xanthoxin (Figure 4) had been detected as a photooxidation product

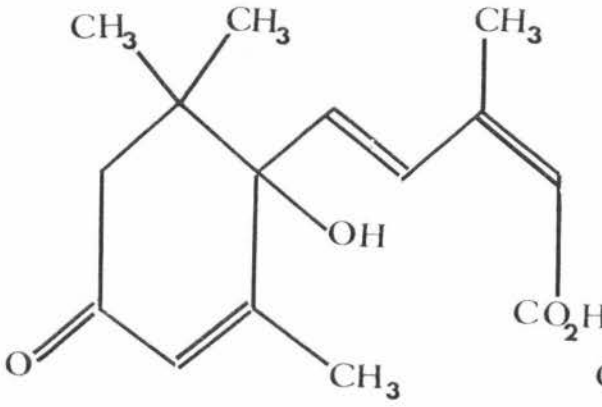


Figure 1. (+)-Abscisic acid (cis-isomer)

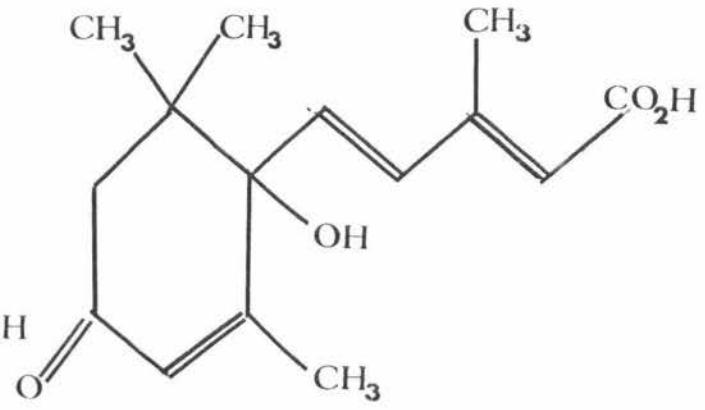


Figure 2. 2-trans-abscisic acid (t-ABA)

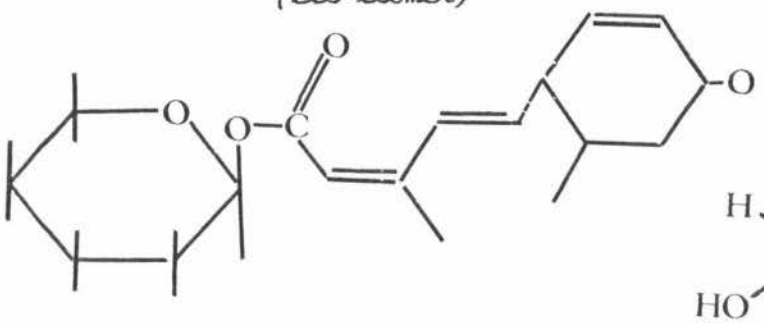


Figure 3. +(abscisyl-B-D-glucopyranoside)

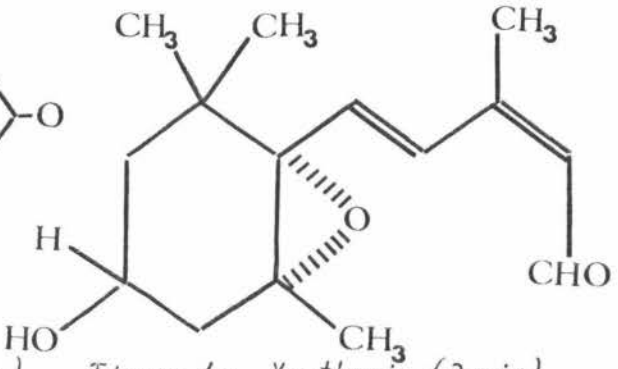


Figure 4a. Xanthoxin (2-cis)

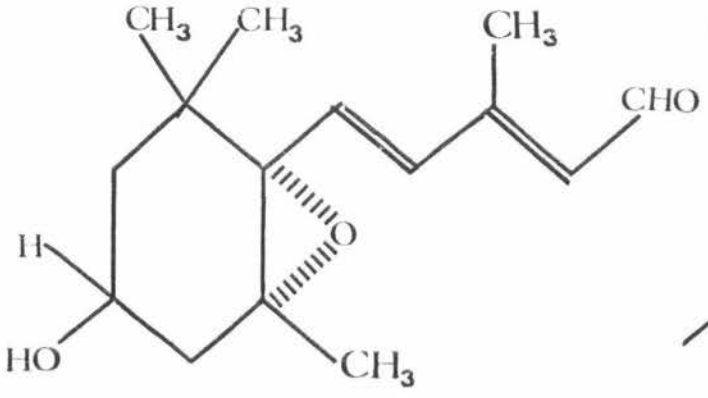


Figure 4b xanthoxin (2-trans)

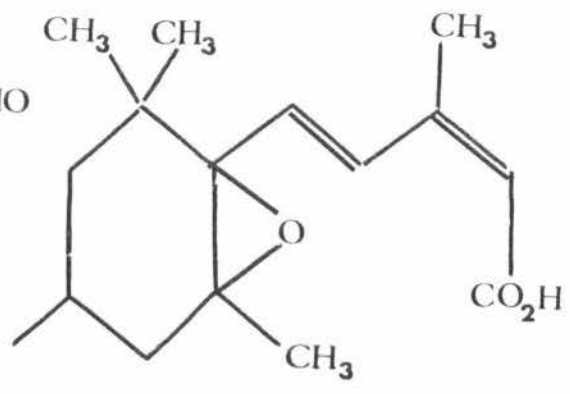


Figure 5 1'2'-epoxy-B-ionylideneacetic acid

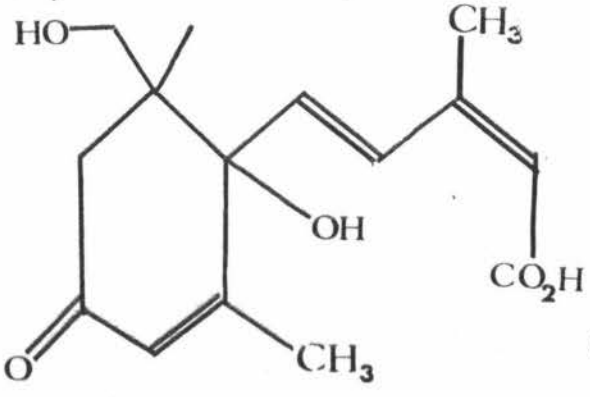


Figure 6 metabolite C

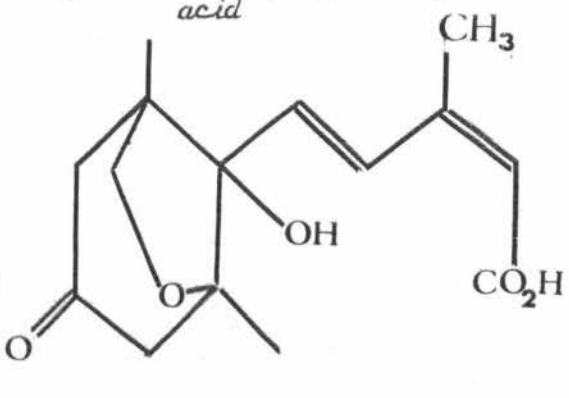


Figure 7 Phaseic acid

of xanthophylls, especially from violaxanthin (Taylor & Burden 1970). It has been found in several plants (Firn et.al.1971) and can be converted chemically into t-ABA (Burden & Taylor 1970). The yield of xanthoxin ; both cis and trans-xanthoxin from violaxanthin is low and high light intensities are required for photolysis in vitro (Milborrow 1974). Recently, Firn & Friend (1972) have reported that in soybean (Glycine soja) lipoxygenase is capable of cleaving violaxanthin oxidatively to form similar products , in similar yield, to that formed during photolysis. The possibility that xanthoxin is produced by the action of lipoxygenase removes the requirement of light for its production. Milborrow (1974) reckoned that xanthoxin in the leaf is an adventitious product formed by light and is unlikely to be a precursor of the cis-isomer of abscisic acid.

Milborrow & Noodle (1970) investigating the second possible route of biosynthesis and found that H^3- mevalonic acid was converted to ABA via an epoxide (Figure 5) which may be a direct precursor of ABA. Wilting tissue which has an excessively high amount of ABA, converted nine times more mevalonic acid than non-wilting tissue.
The main pathway of ABA biosynthesis seems to be a direct one, and the degradation of certain xanthophylls to ABA might be of less importance.

Rogers et.al.(1966) have demonstrated that there are two pools of terpenoid biosynthesis, chloroplastic and extra-chloroplastic from fruits. ABA has recently been characterised from chloroplasts of Pisum species by GC- MS (Railton et.al.1974) and is ,in all probability biosynthesised in chloroplasts of avocado (Persea gratissimia) Milborrow 1974.

(ii) Degradation of ABA

Milborrow (1970) reported that (2-^{14}C) labelled abscisic acid was converted to three products: 'Metabolites A, B and C'. Metabolites A and B were identified as methyl abscisate and abscisyl-β-D-glucopyranoside respectively. This glucose ester (Figure 3) had been characterised by Koshimizu et.al.(1968) as a major metabolite and appears to be a rapid storage product for extra ABA. This compound has been identified in citrus by Goldschmidt (1973).

Metabolite C (Figure 6) was isolated in crystalline form and had the same physical properties as phaseic acid (Figure 7). This metabolite could undergo an internal nucleophilic attack of a hydroxyl group on a double bond, to give phaseic acid. Phaseic acid had been isolated from bean seeds (Phaseolus vulgaris) by Macmillan et al (1968), and found also in grape vine (Vitis vinifera L.) by Lovey & Kriedemann (1973).

(iii) Transport of ABA Like the rates of biosynthesis and degradation, the patterns of hormone transport are of great importance in determining the hormonal environment, and hence growth rate of individual cells distant from the source of the hormone.

Using the donor-receiver system with petiole and stem segments of Coleus and cotton, ABA was shown to be transported very quickly within the parenchymatous tissue with a velocity of 24 to 36 mm per hour in Coleus and 20 to 30 mm per hour in cotton petioles (Dorffling and Bottger 1968 , Ingersoll and Smith 1971). Transport in young Coleus petioles was mainly basipetal, whereas in older tissues of Coleus nearly no polarity was observed. In cotton petioles, however, no polarity was found. Milborrow (1968) noted also a basipetal polarity of 3:1 in bean sections.

Ingersoll and Smith (1971) reported that ABA transport is reduced by DNP (2,4-dinitrophenol), low oxygen tension and low temperature (2 C). The transport of ABA through explants is considered to be a metabolically controlled cell to cell tranfer rather than phloem transport because the sections are too short for sieve tubes to function. The rates of ABA transport are considerably slower than those calculated for intact phloem (100 to 1500 mm per hour)

(B) Plant responses to abscisic acid

Broadly, there are two major groupings: responses of inhibition and promotion. Much of this section will emphasize the role of the plant hormone as correlation factors in plant responses.

(i) Senescence Decreases in the levels of chlorophyll, protein and RNA are the prominent symptoms of leaf senescence. Back & Richmond (1971) ABA applied to leaves promotes senescence which may support the proposal that ABA functions as a senescent factor in leaves and also favours the increased synthesis of degradative enzymes. For example, ABA treatments have increased the activity of chromatin-associated ribonuclease in excised barley leaves (Srivastava 1968) and in bean endocarp (de Leo & Sacher 1971). ABA also accelerates the synthesis of cellulase even beyond the rate inducible by ethylene (Lewis & Varner 1970).

Leaf senescence is a complex and tightly regulated process and it is intimately coupled with the development of the plant as a whole. This process also involves other hormones like cytokinins and auxins. Cytokinin-like substances produced by the root delay leaf senescence on intact plants (Sitton et al. 1967; Wareing & Seth 1967). The hormonal regulation in senescence is possibly mediated through a control

of DNA dependent RNA synthesis.

(ii) Abscission ABA stimulates abscission in excised leaf explants of citrus (Altman et al. 1971), bean, cotton (Cracker et al. 1969), and Coleus (Bewley et al. 1972). Diffusates from senescent Coleus petioles accelerate abscission, whereas diffusates from young petioles delay abscission. Cracker et al. (1969) found that ABA treatments increased ethylene production and cellulase activity in cotton and bean explants. Ethylene has been known to be a very potent abscission accelerator. During abscission, there is also a rapid increase in pectinase (Morre 1968) and peroxidase, dehydrogenases and phosphatases (Sutcliffe et al. 1969). Such changes can be presumed to be promoted by ABA as a consequence of its promotion of abscission.

(iii) Growth inhibition The inhibition of the growth of whole plants, excised organs or seeds is the most easily measured response to ABA. ABA inhibits growth of all parts of plants and counteracts the stimulatory effects of the natural growth promoting compounds when applied with them. Hsiao (1973) and Dorffling (1972) noted ABA retards internode elongation. Pilet (1972) determined that the inhibitory action of ABA on cell elongation appeared to be due to an antagonistic effect on auxin.

This growth inhibition is remarkable in three ways:

a) It is extremely potent, being of the same order as the other natural regulators (0.05 to 0.5 ug/ml gives a 50% response in most tests (Aspinall et al. 1967)
b) It can be reversed by removal of the source or by leaching of the tissues.
c) ABA counteracts the toxicity of supra-optimal concentrations of the growth promoting substances (Milborrow 1966).

It is difficult to demonstrate whether endogenous ABA inhibits normal growth mainly because ABA can be biosynthesized in several parts of the plant and there is no known inhibitor of ABA biosynthesis yet.

Inhibitory substances including ABA are present in correlatively inhibited lateral buds (Dorffling 1964). Inhibitor β and synthetic ABA can inhibit out-growths when applied to the lateral buds (Blumenthal et al. 1965). Apart from direct growth inhibition, ABA could act as a 'negative effect' on nutrient accumulation, and hence result in apical dominance. For example, in sunflower seedlings, decapitated below the primary leaves and treated with low amounts of ABA (0.2 ug/ml per plant) a decrease of potassium and phosphorus levels was observed in the epicotyl.

(iv) Dormancy The induction of bud dormancy is one of the well known effects of ABA and was observed first by Eagles & Wareing (1964). Application of ABA also prolongs dormancy. The hormonal regulation of bud dormancy, at least of terminal buds, may operate as follows. Accumulation of ABA at the growing apex of the plant leads to inhibition of internode growth and the formation of resting buds. The release from bud dormancy is caused by a decrease of the level of ABA-like substances and increase of the level of gibberellin and auxin-like substances. Low temperatures and photoperiod may be factors which influence the release from dormancy (Tinklin & Schwabe 1970).

(+) ABA is a potent inhibitor of seed germination, and is regarded as the major growth inhibitor in dormant seeds of many species (Aspinall et.al. 1967 ; Khan 1969 ; Sondheimer et.al. 1966). ABA has the role of maintaining the state of seed dormancy. Khan et.al. (1971) have proposed a working hypothesis of seed dormancy and germination according to which gibberellin is the primary stimulus for germination whereas the roles of endogenous cytokinins and inhibitors (ABA) are 'permissive' and 'preventive'. The special role of cytokinin is to remove the block to germination caused by the inhibitor and allows the gibberellins to complete their stimulative action.

Mechanisms by which ABA accumulates in buds and seeds, could be by synthesis in these organs or translocation to the respective organs from other parts of the plant. Wareing et.al. (1964) shown that leaves of sycamore produce more ABA-like inhibitors under short conditions than long days. These inhibitors are probably transported in the phloem sap to the growing apex, where they accumulate. However, Lenton et.al. (1971) have pointed out '' the conclusion that shortdays lead to a rise in ABA levels must remain open to question until such a rise has been demonstrated by direct and specific measurements on the ABA content of extracts ''.

(v) Abscisic acid and water relations

The level of ABA in leaves can be raised by a variety of conditions, wilting (Wright 1972a , 1972b), waterlogging of the root system (Wright 1972a), low relative humidity (Mizrahi et.al. 1971), osmotic stress (Mizrahi et.al. 1970), lack of mineral nutrients (Mizrahi & Richmond 1972), cold stress (Milborrow & Robinson 1973) and infection with the wilt-inducing bacterium Pseudomonas solanacearum (Steadman & Sequeira 1970). A common feature of these factors is that they affect the water balance of plants.

Wright & Hiron (1972b, 1973) found that when cut shoots were wilted, there was an increase in β inhibitor complex. They identified the major inhibitor as (+) ABA and defined the conditions under which the increase occurred. A water loss of about 10% of the total fresh weight causes an approximately 40 fold increase in the ABA content. A key feature is the rapidity of the increase in ABA. The authors calculated the ABA content of turgid dwarf bean leaves as initially 6 ug/kg rising to 9 ug/kg within 7 minutes of the warm air treatment; 33 ug/kg by 25 minutes and 68 ug/kg within 45 minutes. Wright & Hiron (1973) observed that both the level of conjugate and of free ABA increased considerably. A similar dramatic rise in ABA content on wilting has been found in Brussel sprouts (Wright & Hiron 1970), peas (Dorffling et.al. 1974, Ivy 1974); sugar cane (Most 1971); wheat (Milborrow et.al. 1970); tomato (Rammussen 1976), maize (Beardsell & Cohen 1974), sorghum (Beardsell & Cohen 1975, Ogunkanmi et.al. 1974) and tobacco (Boussiba & Richmond 1976).

The rapid increase in ABA contents in wilted leaves remains fairly constant until the loss of turgor is regained. Milborrow (1974) reckons that there are probably three switch mechanisms operating:-

a) rapid synthesis triggered by wilting,
b) a stop message when sufficient ABA has been formed and
c) either the beginning of degradation of ABA or the cessation of rapid synthesis.

Milborrow & Robinson (1973) suggested that the excess ABA from the 2(^{14}C) diols-ABA operated a negative feedback loop and stopped its own biosynthesis. Evidence tends to suggest that the rapid increase in ABA is not due to release from a 'bound' form but to synthesis from mevalonic acid (Milborrow & Noddle 1970).

Zabadal (1974) working on two species of Ambrosia, observed that there is a threshold water potential that stimulates ABA synthesis. The ABA increase at the threshold water potential is so abrupt that a reduction in leaf water potential of only one atmosphere may cause a significant rise in ABA from its baseline level. Beardsell & Cohen (1975) confirmed that there is a threshold value of (LWP) below which ABA levels increased abruptly in maize. Cummins (1973) and Milborrow (1974) provided some evidence that ABA occurs in discrete compartments in the cell. One postulate is that when (LWP) reaches the threshold value, a re-distribution of ABA occurs. The reduction in ABA at sites of accumulation might in turn act as the trigger for enhanced ABA synthesis.

Loveys & Kriedemann (1974) demonstrated that moisture stress in grape vines (Vitis vinifera L.) contributing towards an increase in stomatal resistance (R_s) was correlated with increase levels of endogenous ABA and phaseic acid in mature foliage. Phaseic acid has been identified as a by-product from degradation of ABA (Gaskin et.al. 1973).

(vi) Abscisin acid and stomata The closure of stomata by ABA is an important role first observed by Mittelheuser & Van Steveninck (1969). Horton (1971) ; Jones and Mansfield (1970) have also showed that exogenously applied ABA has a dramatic effect on stomatal aperture. Application of (±)-ABA at 1 ug/ml concentration caused stomatal closure in leaves of wheat and barley and also reduced transpiration by one half. Talha & Larsen (1975) studied the ABA effect on transpiration of Zea mays. A linear relationship occurred between the transpiration rate of both detached and intact leaves and the concentrations of applied ABA. The magnitude and persistence of the treatment effect depended on the concentration of ABA and the species of plant used. Jones & Mansfield (1970) reported that the decrease of stomatal aperture induced by 10^{-4} M ABA in intact leaves of Xanthium strumarium persisted for up to nine days after application. In contrast to Jones and Mansfield s' result of slow stomatal recovery, Kriedemann et.al.(1972) have observed quite rapid recovery from ABA treatment. This may have been due to the fact that they applied approximately ten times more hormone than would have been required to close the stomata; the excess may then have acted as a 'reservoir'. Fischer et.al.(1970) noted that bean leaves show stomatal recovery in 1 to 2 days following water stress.

The effect of ABA on the transpiration rate may be discussed in relation to the following possibilities:

a) A direct action of ABA on the stomatal apparatus (guard cells)
b) Biochemical changes induced by ABA.
   i) ABA has been shown to interact with other hormones in several hormonally regulated plant responses including the antagonistic effect of ABA and cytokinin on stomatal opening (Imber & Tal 1970; Mizrahi et.al.1970).
   ii) Leshem(1971)found that ABA significantly inhibited the production of RNA by activation of the enzyme RNase.

Changes in endogenous levels of ABA-like substances can constitute a mechanism for regulating gas exchange. These may occupy a key position in the regulation of photosynthetic performance. Prolonged closure of stomata inhibits photosynthesis and possibly reduce the yield of crop.

Kriedemann et. al.(1972) suggested that a doubling of the endogenous level of ABA is sufficient to initiate stomatal closure in well watered bean plants. Cummins et.al. (1971) pointed out the rapidity and ready reversibility of the ABA action on stomata and suggested that is is a good modulator of stomatal behaviour. However, Hsiao (1973) questioned whether the ABA accumulation from stress is fast enough for it to be the modulator of stomatal responses. Stomata possibly close even faster than the increase in ABA.

Mansfield & Jones (1971) showed that ABA treatment results in starch accumulation in the chloroplasts and a fall in the osmotic potential of guard cells from 14.1 to 9.8 bar. There was also a flux of K^+ out of the guard cells. It was also shown that ABA prevented the accumulation of potassium in guard cells, and increased the starch content of their chloroplasts. The authors suggested that ABA could act on the K^+ flux and causing stomatal movement; perhaps via an osmotic adjustment involving starch hydrolysis.

Raschke (1975) observed that the simultaneous requirement of CO_2 and ABA for the modulation of stomatal aperture in Xanthium strumarium. It appears that ABA reversibly blocks the active secretion of H^+ from guard cells. In the presence of CO_2, this would lead to a rapid acidification of the cytoplasm and to stomatal closure. In many species, the intracellularly evolved CO_2 may suffice to elicit acidification. However, in species with strong H^+ pumps, malate formation would lead to acidification only in the presence of ABA. This possibly explains the sensitization of stomata to CO_2 by ABA. Note that CO_2 is needed as a substrate for the acidification of the cytoplasm as well as the production of osmotica. The mechanism whereby ABA is able to regulate stomatal aperture remains unclear. A direct effect in terms of enhanced acidification by inhibiting expulsion of H^+ from guard cells is an attractive hypothesis but is still hypothesis but is still regarded as speculative.

(C) Mode of action of ABA

As with some other plant hormones, ABA has probably several primary sites of action:-

i) Hormonal effect on the enzyme synthesizing apparatus.

This includes DNA itself or any of the factors participating in transcription or translation. ABA causes specific inhibition of RNA synthesis. Addicott (1970) showed that the action on nucleic acid metabolism is largely that of inhibiting or modifying the synthesis of one or more major fractions of RNA. It may inhibit activity of the enzyme RNA polymerase in transcription process. The transcription block thus formed prevents synthesis of DNA-primed RNA. Bex (1972) detected a marked decrease in the specific activity of RNA polymerase when maize coleoptiles were incubated with ABA. However, Leshem (1971) suggested that the overall decrease in RNA levels is due to ABA activation of the enzyme RNase and not necessarily by inhibition of RNA polymerase. The mode of RNase activation by ABA is difficult to verify (Leshem & Schwartz 1972).

Inhibition of DNA synthesis (replication) by ABA has been observed also by van Overbeek et.al. (1968) and Walton et. al.(1970). However Haber et.al. (1969) showed that ABA inhibits lettuce (Lactuca sativa) seed germination in circumstances which do not involve DNA synthesis. DNA synthesis might not be a primary effect: often this inhibition does not closely correlate with ABA's physiological effects such as increased ABA levels in response to wilting. Further, there is evidence of a dual effect of ABA in some physiological processes such as senescence and abscission, simultaneously inhibiting some aspects of nucleic acid metabolism and promoting others. The effects of ABA on DNA and RNA have to be interpreted with care (Addicott et. al. 1969; Dorffling 1972).

There is often a marked lag in time between ABA treatment and observed changes in nucleic acids suggesting that the effects may be indirect and secondary.

ii) Abscisic acid and allostery

Another possible mode of action may be via the regulation of the enzyme activity by allosteric promotion and inhibition (Saunders & Poulson 1968) without directly affecting transcription and translation. This would mean that ABA acts on a 'preformed system' leading to observable responses without a lag period. ABA may serve as a negative allosteric effector on biologically

active proteins which may include enzymes participating in GA biosynthesis , and on DNA polymerase. van Overbeek et.al. 1967 have proposed an overall scheme for ABA action as a ' negative' allosteric effector (Figure 8)

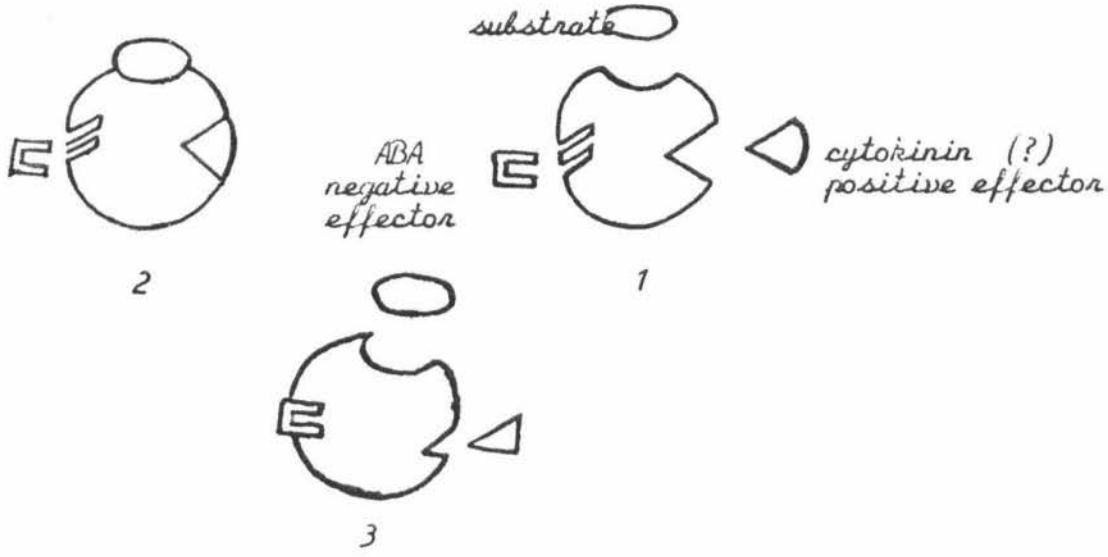


Fig. 8 The allosteric effect of ABA (After van Overbeek et.al)

The 'positive' effector or activator in their model may be more specific i.e. cytokinin. It is postulated that ABA attachment to the protein is by means of its polar groups which link to specific 'allosteric sites' situated on the protein (stage 3). The attachment of ABA causes a steric change of the 'substrate site', preventing the entry of the substrate and thus inhibiting biological activity. If the activator e.g. the promoting hormone, is attached beforehand (stage 2); it lends stability to the protein and prevents deformation of the 'substrate site' and thus the attachment of the ABA. Biological activity can then proceed normally. This model outlines how in vivo, growth may be the function of an equilibrium attained between promoting and inhibiting factors.

Another important primary action of ABA seems to be an effect on membrane properties. ABA acts on specific ion uptake mechanisms such as potassium, phosphorus and chloride (Reed & Bonner 1974 ;Cram & Pitman 1972 ; Dorffling et al. 1972). The uptake of potassium , for example is reduced by ABA within 30 minutes in coleoptile sections, and the inhibition reaches a maximum of 75% within 2 hour. There is no simple relation between the inhibition of growth and the inhibition of ion uptake. An effect of ABA on the permeability of plant cells and roots as regards water is a controversial matter (Cram & Pitman 1972; Glinka 1973).

There are therefore, two kinds of responses to ABA, a direct ,rapid response possibly operating on membranes or some other structure, and a slower effect involving the synthesis of new enzyme protein.

1.4.2. CYTOKININS AND PHENOMENA IN PLANTS.

Cytokinins are compounds which promote cell division in cultured plant cells (Skoog et al. 1965). They are growth substances which are usually derivatives of the nucleic purine base, adenine. The common synthetic cytokinins include kinetin (6-furfuryladenine) and BAP (6-benzyl aminopurine). Endogenous cytokinins are zeatin (6-(4-hydroxy-3-methylbut-trans-2-enyl) aminopurine and IPA (N^6-iso-pentenyladenine). Zeatin occurs naturally not only as the free base but also as its nucleoside and nucleotide (Miller 1965; Letham 1966a, 1966b). Both IPA and zeatin constitute by far the most active cytokinins yet discovered. These or closely related compounds have been detected in a diverse range of plants (Kende 1971).

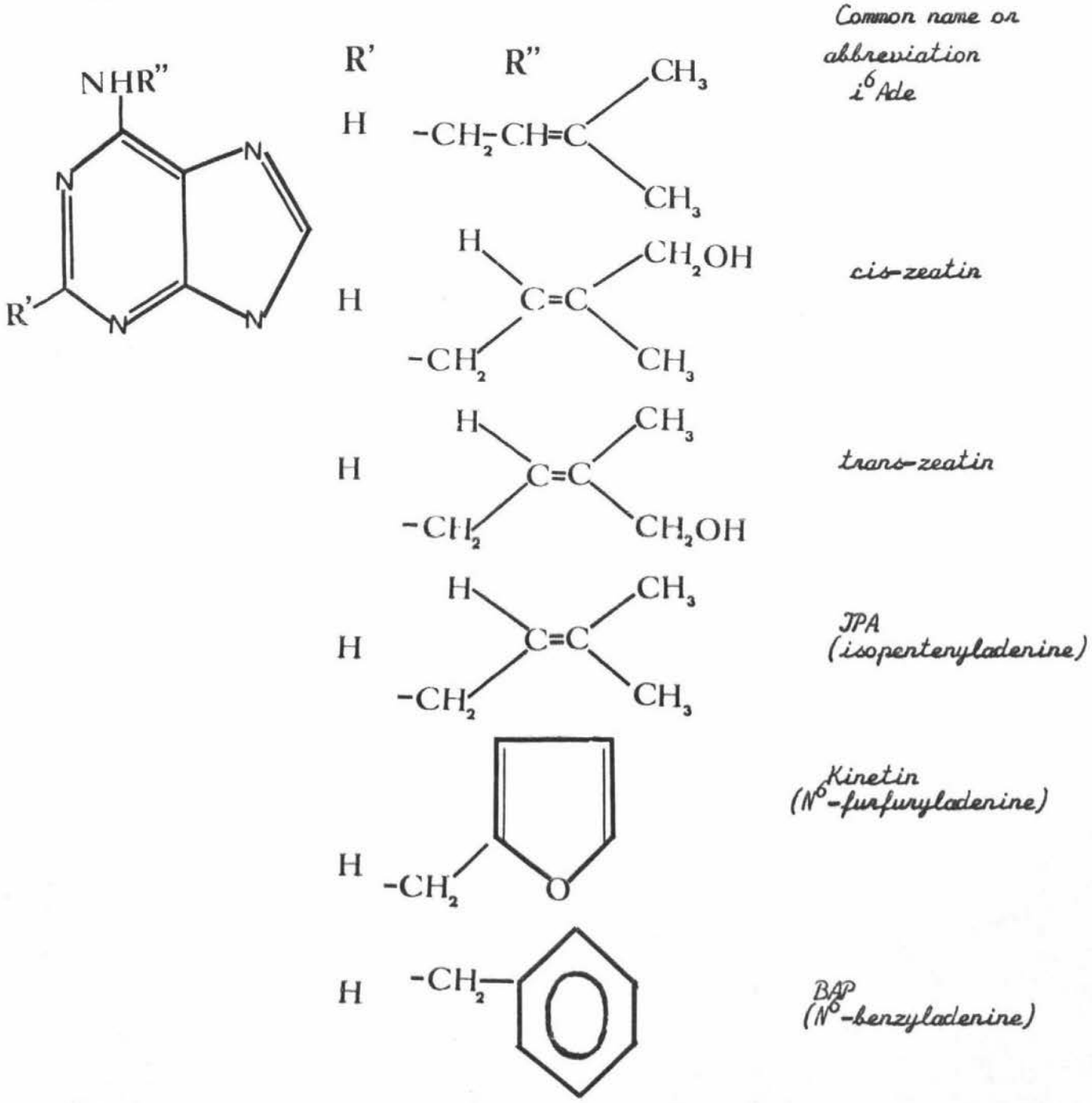


Figure 9 Structures of common cytokinins.

A) Occurrence of cytokinins

Kende (1964, 1965) showed that xylem sap of sunflower contained cytokinins and substances with cytokinin activity have now been detected in xylem sap in many species. Cytokinins in xylem seem to resemble the common cytokinins detected in other plant parts for example leaves. Recently, trans-zeatin riboside was identified positively in sycamore sap by combined gas liquid chromatography- mass spectrometry (Horgan et al. 1973). They suggest that zeatin riboside, the free base and the nucleotide are present in xylem sap. Skene (1972) identified zeatin and zeatin riboside in xylem sap of the grape vine (Vitis vinifera).

Most evidence suggests that cytokinins are of root origin. Substances with cytokinin activity have been detected in extracts of roots of sunflower (Weiss & Vaadia 1965); grape vines (Skene 1970); peas (Short & Torrey 1972a); rice (Yoshida & Oritani 1971) and Solanum andigena (Woolley & Wareing 1972). Radin & Loomis (1971) found increasing amounts of three cytokinin fractions in developing roots of radishes. Two of these were chromatographically similar to zeatin ribotide and to zeatin or its riboside and the third fraction was not identified.

Evidence strongly favours the meristematic regions of the roots as sites of synthesis. Weiss & Vaadia (1965) found that in sunflower seedlings, free cytokinins were confined to the youngest portions of the root tip. Short & Torrey (1972a) examined cytokinins in both the free form and as constituents of transfer RNA in serial segments of young seedling roots of pea. The greatest amount of cytokinin existing in a free form and present in t-RNA was distributed in the highly meristematic 0 to 1 zone of the mm root tip. Smaller amounts were found in those segments 1 to 5 mm behind the root tip. Zeatin and its derivatives and an unidentified cytokinin are the active free cytokinins. Short & Torrey suggested that the 'quiescent zone' and the surrounding meristematic tissues as centres of cytokinin production in the root tip of pea seedlings. Recently, Feldman (1975) working on intact terminal millimeter root tips of Zea mays shows that at least four cytokinin fractions are present, that is nucleotide, zeatin and zeatin riboside and unidentified cytokinin.

(i) Cytokinins in leaves and other plant parts

Cytokinins in leaf tissue are present in diverse forms. Hewett & Wareing (1973b, 1973c) detected at least seven cytokinins in mature leaves of Populus X robusta. The three major cytokinin fractions were zeatin and zeatin riboside and a cytokinin glucoside. These findings

agreed with Engelbrecht (1971) who suggested that zeatin and its riboside are the main cytokinins present in rooted leaf cuttings of Phaseolus vulgaris L. The physiological significance of the diverse forms of cytokinins in leaf tissue remains unclear.

High levels of cytokinins are also present in other meristems, such as cambial tissue, young fruits and seeds (Zwar et.al. 1963 ;Letham 1966 ;Letham & Williams, 1969). Indirect evidence suggests that the seed does produce a significant proportion of its own cytokinin (Blumenfeld & Gazit 1971) and it is quite clear that a range of cultured tissues are capable of synthesizing cytokinins (Miura & Miller 1969; Short & Torrey 1972b).

(ii) Metabolism of cytokinins. The rate of metabolism is of great importance in determining the hormonal environment where growth may be regulated. So far, little is known really about the internal and external factors which determine these processes. The metabolism of cytokinins differs markedly from plant to plant species and also from plant organ to plant organ. However, some common features are apparent.

When plant tissues are supplied with cytokinin, the bulk is degraded by a process which cleaves the side chain. The purine ring is converted subsequently to several metabolites. A smaller part of the applied cytokinin remains intact and exists as the free base or as ribonucleoside or ribonucleotide (Tzou et.al. 1973) or as glucosides (Parker & Letham 1973 , 1974; Fox et.al. 1973). A 7-glucose of zeatin ('raphanatin') has been detected in derooted radish seedlings which had been supplied with ^{3}H-zeatin (Parker & Letham 1973). It is present only in the cotyledons, not in the hypocotyl, petiole and xylem sap of this plant. It occurs also in small amounts in the roots of Zea mays (Parker and Letham 1974). In general, the cytokinin glucosides seem to be very stable in the plant and raphanatin is biologically active. The physiological significance of naturally occurring cytokinin-glucose complexes in root and other tissues is yet to be elucidated. It seems likely that they are storage compounds for excess cytokinins.

It is possible that plant tissues make use of t-RNA or other metabolic pathways as a source of cytokinins, depending on situations. The breakdown of the t-RNA can be a potential source of release of certain fractions of cytokinins. Chen & Hall (1969) indicated that cytokinins in t-RNA are synthesized by the attachment of the isopentenyl group to preformed t-RNA. It is also presumed that mevalonate is the precursor of the isoprenoid side chain of free cytokinins. Short & Torrey (1972a)

showed that there was approximately 27 times more free cytokinin than the amount detected in t-RNA in root apices of pea. The turnover of cytokinins from t-RNA is less rapid. These two aspects tend to indicate that cytokinins in root tips are produced by biosynthesis seperate from the catabolism of t-RNA.

(iii) Translocation of cytokinins Cytokinins synthesized in the root tips are translocated to the above ground parts where they regulate the protein metabolism of leaves and other aspects of shoot development. Levels of cytokinin activity are generally quite high in the xylem of woody species of plant, suggesting that substantial quantities of cytokinins pass in the transpiration stream. However, little is known about the actual amounts moving in the transpiration stream. Hewett & Wareing (1973a) suggest that the riboside is the usual form of cytokinins transported in the xylem of woody species.

Developing organs seem to compete for available root cytokinins: for example, limited supplies of root cytokinins move preferentially to the apex. Pilet (1968) showed that the removal of the apical bud inhibits the acropetal transport of (^{14}C) BAP to stems of Lens culinaris. Application of labelled kinetin to the roots of intact plants results also in activity accumulating in the apical bud itself. Morris & Winfield (1972) suggest that hormone-directed transport of cytokinins is involved in the regulation of lateral bud growth contending some component of cytokinin movement in the shoot is under directional control.

Parker & Letham (1973) reckoned that zeatin ribotide accumulation in hypocotyls of rootless radish seedlings resulted from lateral movement from the transpiration stream rather than from basipetal transport from the upper parts of the seedlings. On the other hand, experiments with ^{14}C BAP indicate that BAP applied to the base of rootless cuttings of Solanum andigena accumulates rapidly in the leaf and later is redistributed to other parts of the cutting (Woolley & Wareing 1972b). They are of the opinion that auxin influences the distribution of cytokinins from roots. Auxin applied to the cut upper surface of Solanum andigena cuttings prevents the outgrowth of lateral buds and inhibits the accumulation in the lateral buds of label from basally administered ^{14}C-BAP. In the absence of auxin, labelled cytokinin accumulates in the lateral buds prior to their growth as leafy shoots.

Skene (1975) points out that not enough is known whether cytokinins from roots move directly to the apex and other centres of high auxin concentration, or whether they are re-exported from the leaves.

(B) Biological activity

Cytokinins are known to influence a wide range of physiological and biochemical processes. As well as being involved in all phases of plant development; from seed germination to plant aging, senescence and cell division and organ formation. These processes are not dependent on one single hormone but rather a concerted, correlated regulatory action of several growth hormones.

(i) Mitosis and cell division The property most characteristically associated with cytokinins is their stimulation of cell division in plant tissue cultures. The presence of cytokinins in meristematic tissues such as root tips, cambium, and fruitlets has been taken as indication that cytokinins are involved in regulating cell division.

Guttman (1956) presented data showing that kinetin acted sometime during the interphase of mitosis to trigger the subsequent prophase in root cells of onion (Allium cepa). Torrey (1961) with intact roots of the garden pea (Pisum sativum cv. Alaska) obtained a somewhat similar results. Here, cytokinins brought into division mature cells in the root cortex which normally may undergo chromosome doubling but rare mitosis or cell division.

Cytokinins are widely referred to as regulators of cell division and distribution of activity often follows the intensity of cell division in the tissues. Letham (1963) reported a correlation between cytokinin content and the rate of cell division in apple and plum fruitlets. There is a definite correlation between the rate of tissue growth and level of measurable cytokinin in xylem sap of grape vine (Skene 1972).

(ii) Cell enlargement Cell enlargement in discs of etiolated leaves was increased markedly in the presence of kinetin and certain analogues active in cell division (Miller 1956; Scott & Liverman 1956). Arora et. al. (1959) reported that cortical cells of tobacco roots enlarge up to four times their normal size in the presence of kinetin. Glasziou (1957) demonstrated that under certain low kinetin concentrations, an increase in cell elongation of tobacco pith occurred. Kinetin treatment of sunflower hypocotyls caused an increase in fresh and dry weight more than double that of the controls although no elongation occurred.

(iii) Morphogenesis Cytokinins are certainly involved in the formation of organs which takes place under appropriate conditions in a variety to tissue cultures. Cytokinins regulate the development of organs from callus. The auxin/ kinetin ratio of the culture medium was the

critical factor in determining the type of organ: that is bud and root (Skoog & Miller 1957). In this case cytokinins have the ability to modify the effects of other hormones for example auxin. Jordan & Skoog (1971) in their study with coleoptile tips of Avena sativa L. showed that i^6-Ade and BAP stimulated synthesis of auxin. Cytokinins do not act alone in plant regeneration processes. They interplay with other growth regulators particularly auxins delicately and quantitatively.

Cytokinins have been implicated also in a large variety of systems which involve differentiation in one way or another; in the differentiation of tracheids through activation of lignin biosynthesis (Bergmann 1964) ;in the induction of parthenocarpy in certain fruits (Weaver et al. 1965) and in the maturing of proplastids into plastids (Stetler & Laetsch 1965). There have been no unifying concepts offered to explain adequately a host of morphogenetic responses to cytokinin treatment.

There is too, a correlation between rate of tissue growth and the level of measurable cytokinin activity. In developing cotton fruit, and avocado seeds, the level of cytokinin activity is much higher during the early stages of development, and as the tissue matures, the cytokinin level also falls (Gazit et al. 1970; Sandstedt 1971).

(iv) Dormancy Dormancy is shown by a wide range of plant organs, of very different morphology for example buds and seeds. The available experimental evidence for dormancy can be grouped into three categories. Firstly, there are observations which indicate that in particular cases, a transmissible 'hormonal' stimulus must be involved in the imposition or removal of dormancy. Secondly, it is possible to discern a parallel variation in the endogenous level of a particular hormone and the state of dormancy of a plant organ. Finally, exogenous hormonal treatments can impose or break dormancy.

Cytokinins, notably kinetin and benzyladenine, are effective in overcoming dormancy of tree buds, tubers and other resting organs (Vegis 1964; Wareing & Saunders 1971; Weaver 1963). Bud dormancy may be controlled by a balance between endogenous inhibitors such as ABA and the growth promoting hormones especially especially gibberellins and cytokinins for the cytokinin-inhibitor antagonism can be an essential ingredient of hormonal regulation (Khan 1971).

Cytokinins can promote seed germination for Miller (1956) showed that cytokinins can substitute for the red light requirement in breaking seed dormancy. Germination of seed or growth of an organ is often a result of cumulative actions of several hormones, each having a designated role and not always a result of an increase or a decrease in the

level of promoters and inhibitors (Khan 1971). The mechanism of cytokinin-inhibitor interaction in seeds and buds has been studied at the level of nucleic acids and enzymes. The following possibilities can occur:

a) ABA and benzyladenine showed opposite effects in ^{32}P incorporation into all nucleic acid fractions (van Overbeek et. al. 1967).
b) The nucleotide composition of labelled RNA was altered by ABA and this effect was reversed by kinetin (Khan et. al. 1970).
c) ABA and cytokinins interact at the level of translation and enzyme synthesis. For example, α-amylase synthesis in intact cereal grains which is mediated by gibberellins from the embryo is inhibited by ABA and reversed by cytokinins (Khan & Downing 1968).
d) ABA can affect a decrease in the degradation of kinetin to adenine, (Back et. al. 1972) supporting the suggestion of Mullins and Osborne (1970) that ABA has a 'cytokinin sparing' effect. It has been suggested that this could stimulate a plant process.

(v) Senescence Cytokinins from the roots appear to be responsible for maintenance of balanced protein metabolism in leaves. Richmond & Lang (1957) showed that the protein level in kinetin-treated, detached leaves declined more slowly than in untreated controls, and a similar effect of kinetin on RNA levels was observed by Osborne (1962). Osborne found that incorporation of a labeled amino acid into protein, and of $^{32}PO_4^{3-}$ into RNA, was enhanced by treating detached leaves with kinetin. Similar results have been obtained by other researchers using different plants (Gunning & Barkley 1963; Kuraishi 1968; Tavares & Kende 1970). Tavares & Kende concluded that cytokinins retard senescence of corn leaves primarily through inhibiting proteolysis and possibly RNA breakdown.

The activity of proteases (Beevers 1968; Atkin & Srivastava 1969) and of RNases (Sodek & Wright 1969) is lower in cytokinin-treated leaves than in the corresponding controls that have not been treated with the hormone. In some plants cytokinins depress the activity of protease and RNase temporarily rather than inhibit an increase in the activity of these hydrolases.

Cytokinins retard the decrease in levels of DNA, RNA and protein and also stabilize or even promote RNA/DNA ratios, thus indicating that RNA metabolism may be directly involved. One hypothesis is that cytokinin acts by preventing the synthesis of specific m-RNA coding for

degradative enzymes. ABA is involved also in the regulation of senescence in leaves (see page 13).

Sitton, Itai & Kende (1967) found that the cytokinin content of xylem sap of sunflowers increased during the exponential growth phase, and decreased rapidly when growth ceased and flowering commenced. The decrease in the supply of cytokinins from roots to leaves is one of the factors leading to senescence. Growing fruits can take over as sites of cytokinin synthesis when the supply from the roots decline. Cytokinins may be diverted however to developing fruits at the expense of leaves (Wareing & Seth 1967).

The onset of senescence is seen to be a complex interaction between organs of plant, a part of this interaction being mediated by cytokinins of root origin and ABA.

(vi) Miscellaneous effects of cytokinins The list of cytokinin activities is now too large to include here but the following appear to have potential significance.

Cytokinins can prevent partially the toxic effects of certain phytopathogens. For example kinetin reduces the number and size of lesions caused by tomato spotted wilt virus (Selman 1964) and antagonizes the the toxic effect of Pseudomonas tabaci which causes 'wildfire' disease of tobacco (Lovrekovich & Farkas 1963).

Cytokinins are involved in the regulation of photosynthetic enzymes. They are required for the development of the following enzymes of the Calvin cycle: JuDP carboxylase, NDP^{+} dependent glyceraldehyde dehydrogenase, transketolase, and ribosephosphate isomerase (Feieraben & Pirson 1966). Application of kinetin to dark-grown seedlings enhanced the activity of ribulose 1,5diphosphate and NADP-dependent glyceraldehyde phosphate dehydrogenase up to the level found in illuminated seedlings. Removal of the roots as a cytokinin source reduced the activity of both enzymes, and this reduction could be overcome by kinetin treatment. Changes in cytokinin level appeared to affect preferentially, chloroplast enzymes.

Cytokinins seem to promote the synthesis of chlorophyll and development of the chloroplasts. Etiolated seedlings lose the ability to produce chlorophyll and soluble proteins with increasing age and kinetin retards this process (Stobart et. al. 1972).

Syano and Torrey (1976) showed the presence of high cytokinin activity in root nodules of pea (Pisum sativum L.) and the cytokinins found predominantly in pea root nodules infected by Rhizobium leguminosarum were zeatin and its riboside and ribotide. The presence of cytokinins in root nodules and their changing concentration during nodule develop-

ment support strongly the idea that cytokinins play an important role in nodule development, particularly in relation to the meristematic nature of the nodule that is its cell division activity and also to the distinctive polyploid state of the dividing nuclear population.

(C) Cytokinins and factors affecting production

Cytokinins synthesis by roots is influenced by factors of the environment that either directly or indirectly affect root physiology. These factors are discussed below:

i) pH; Nutrition Low pH of the root medium adversely affects the growth of many plants. Cytokinins could be detected in xylem sap of maize plants grown in medium of pH 7.0 but not pH 4.0. (Skene 1975). The nutritional status of the plant also affects cytokinin production by roots for example reduced quantities of cytokinins were found in xylem sap and root extracts of sunflowers (Wagner & Micheal 1969, 1971), and in root extracts of Solanum andigena (Woolley & Wareing 1972c) when grown in media of low nitrogen levels.

ii) Root temperature Skene & Kerridge (1967) found qualitative differences in the cytokinins of xylem sap from sultana vines (Vitis vinifera L.) grown at root temperatures of 30 C and 20 C, a cytokinin nucleotide being absent from the 30 C samples. They also showed an increased export of cytokinins from the roots of plants grown at the higher temperature. This is associated with a larger root system and a higher bleeding rate.

iii) Waterlogging This reduces the growth of the shoots and induces chlorosis of the lower leaves, symptoms which may be related to reduced quantities of gibberellin (Reid et.al.1969) and cytokinins (Burrows & Carr 1969) moving from root to shoot. Burrows & Carr (1969) showed that the decline in cytokinin content of sunflower sap was paralleled to a decline in metabolic activity of the root apices after three days of flooding.

iv) Photoperiod Van Staden & Wareing (1972) demonstrated that cytokinins in xylem sap of short day plant, Xanthium strumarium appear to predominate in the nucleotide form when grown under long day condition. However, the nucleotide fraction decreases after exposure to short days ; while the free base and riboside fraction remained virtually unchanged. It is not clear whether differences in the cytokinin content of mature leaves of Xanthium strumarium under long and short days are due to an effect of daylength on cytokinin synthesis within the leaf, or are a result of interconversions within the leaf of cytokinins imported from the roots.

v) Season Luckwill & Whyte (1968) reported that the cytokinin content of xylem sap from apple stems remained low during winter, increased in early spring, reached a peak level about the time of full bloom, and thereafter decreased. The authors suggested that the disappearance of cytokinin activity coincided with the cessation of extension shoot growth which may be associated with cytokinins in the sap. Increases in cytokinin activity at the time of budburst are also suggestive of a causal relationship between cytokinins and budburst. (Note changes in the balance of other growth regulators in the bud are implicated too.)

vi) Water stress Various stresses applied to the root system result in physiological changes reminiscent of the aging process for example the decline in levels of proteins and RNA, as well as affecting the quantities of cytokinins moving from root to shoot.

Itai & Vaadia (1965, 1969) suggested that stress reduces the levels of cytokinins in xylem sap in sunflowers and reduces the capacity of tobacco leaf discs to incorporate(^{14}C)L-leucine into protein (Ben-Zioni et.al. 1967). Itai & Vaadia (1971) demonstrated with tobacco that less than 30 minutes of wilting reduced substantially the cytokinin activity in root exudate. The decrease was reversible, and upon termination of the water stress, cytokinin activity increased again. The adverse effects are partially counteracted by cytokinins. Pretreatment of stressed tobacco leaf discs with kinetin partially restored incorporation of amino acids into protein. It is suggested that stress induced a decline in leaf protein synthesis resulting from a deficiency of cytokinins in the leaves. Although in both root and shoot stress, cytokinins levels are similarly reduced, the manner in which the reduction occurs may be different. It is possible that under shoot stress inactivation of cytokinins present may be more common than under root stress.

Reduced cytokinin transport from stressed roots can result in disturbed leaf performance and especially affects stomatal response. Vaadia & Itai (1965) showed that cytokinins promote stomatal opening and thus enhancing transpiration rates, an effect which seems to be of a general nature. Cytokinins in association with other hormones such as ABA may modify plant response to stressed conditions by altering stomatal opening and permeability of plant tissues to water (Livne & Vaadia 1972 ;Mizrahi & Richmond 1972). ABA is a major factor facilitating the adaptive response of plants to root stresses that impede water balance, whereas the ratio ABA/cytokinins is implicated in directing the extent of the response.

(D) The mode of cytokinin action

The occurrence of cytokinins in certain t-RNA's from a wide variety of organisms has led to much speculation that these plant hormones may exert their physiological effects through regulation of protein synthesis, by modifying the synthesis and function of specific t-RNA s (Anderson & Cherry 1968). Hall and co-workers (1967) have isolated both 2-IPA and ribosyl-zeatin from plant t-RNA hydrolysates ; while wheat germ t-RNA contains at least four cytokinins identified as ribosyl-zeatin, 2-iPA and their 2-methylthio-derivatives (Hecht et. al. 1969).

IPA, the natural cytokinin, is present in t-RNA for the amino acid serine, isoleucine and tyrosine and within the adaptor molecule located adjacent to the anti-codon. One theory (Leshem 1973) is that cytokinin action is associated with codon-anticodon recognition on the mRNA. The alternative approach is presented by Kende & Tavares (1968) who stated that cytokinin action is not dependent upon its presence in t-RNA. They proposed that IPA is not formed from cytokinin but that initially adenine exists as an integral part of the t-RNA molecule and to this an isopentenyl side chain is attached. The breakdown of t-RNA results in nucleotide release which migrates to other cellular sites where they exert their function as growth regulators.

For the present, it is difficult to know the actual mode and sites of cytokinin action. Like other plant hormones, they can certainly influence the rate of nucleic acid metabolism and protein synthesis, and these effects often parallel the effects of the same substances on growth rate. Higher plants also exhibit a very great flexibility in their growth response to a range of environmental influences. Hence, they must possess a very complex internal system of controls which are amenable to environmental variations.

1.5. Effect of water stress on grain yield in cereals.

In simplest terms, yield is related to the production of total dry matter. The situation is more complex when the yield is considered as only a part of total plant material that is, as grain or storage tissue. Then, the yield will depend more on the developmental stages at which stress is applied and on the sensitivity to stress at those different stages.

Three key stages in the grain formation and crop yield are:

1. Floral initiation and inflorescence development stage where the potential grain number is determined.
2. Anthesis and fertilization stage, when the degree to which this potential realized is fixed.
3. Grain filling stage, when grain weight increases progressively.

There are in addition, considerable differences in morphogenesis and reproductive development between various cereal species.

1.5.1 Inflorescence development Slight water stress can reduce the rate of appearance of floral primordia. Husain & Aspinall (1970) showed that number of primordia in barley is more sensitive to water stress than devlopment of existing primordia. This is probably typical for most cereals. They suggest that if the stress is mild and relatively brief, rate of primordial initiation, upon relief of stress, is more rapid than in the controls and the total number of spikelets formed may be unaffected. On the other hand, if the stress is severe, or protracted, total spikelet number may be reduced substantially. Nicholls & May (1963) suggested that the number of spikelets per inflorescence in barley is determined by the balance between the rate of primordial initiation relative to that of spikelet development. Since spikelet development is less affected by stress than primordial formation, it follows that prolonged stress at the stage of floral initiation could markedly reduce the potential number of grains per ear.

Whiteman & Wilson (1965) found that the development of the sorghum inflorescence could be suspended during stress, yet could be resumed on re-watering and result in a flowering head not significantly different from that of control plants. In general, stress applied prior to panicle initiation merely delays panicle initiation without greatly influencing yield per plant in sorghum.

Hultquist (1973) investigated the influence of water stress on yield components at panicle initiation, at floret differentiation, and at early

grain fill of two hybrid genotypes, C-42Y and RS 626. A severe reduction about 60%, in seed number per head resulted from stress imposed during panicle initiation. No primary panicle branch loss was noted when the stress came during early grain fill, but there was a 25% loss in seed number through floret abortion. Seed size compensation following the 60% seed number loss from the stress applied at panicle initiation was about 30%. There was no seed size compensation following the early seed fill stress. Hultquist (1973) suggested that differentiation of spikelet components is a critical period to water stress, and seed number can be reduced drastically at this stage. Photosynthesis may also be limiting during seed number differentiatiation and during subsequent development.

From the stage of spikelet initiation to fertilization of the ovules, a number of other processes, associated with the development of the inflorescence, are likely to be sensitive to water deficits and thus cause a reduction in the number of grains per ear, or even in the number of fertile ears. Water stress interferes specifically with the sexual development of the spikelets, such as meiosis of the gametes in barley (Aspinall et. al. 1964) and in wheat (Chinoy 1962). However, the availability of mineral nutrients and carbohydrates during the preflowering phase also appear to influence spikelet development, floret fertility and grain set.

In wheat, the potential for variable floret numbers provides an opportunity for compensatory effects if stress is removed. This may also apply to oats, but would not apply to crops such as rye, barley or maize in which floret number is fixed (Bonnett 1966). On the other hand, although compensation may not occur, relief of stress in many species during the stage of inflorescence development may permit final grain number to approach potential represented by the number of spikelets initiated.

1.5.2. Water stress and fertilization Stress at anthesis markedly reduces fertilization and grain set in most cereals. Corn is the most sensitive at this stage; with a 50% reduction in yield by brief periods of wilting (two or three days) Denmead and Shaw (1960). It is suggested that stress at this stage acts either by way of dehydration of pollen grains or by impairment of growth of pollen tube. Robins & Domingo (1953) were of the opinion that stress interferes with the germination of the pollen tube from the stigma to the ovules in corn.

Fisher (1972) states that grain yield in wheat is reduced most when the stress develops about ten days before ear emergence, because of pronounced effects on the grain number formed per spikelet.

1.5.3. Water stress and grain filling Ultimate grain yield depends on the rate of dry matter accumulation and the length of the grain filling period. Grain yield is a function of an efficiency component and a time component. The efficiency component is complex, being influenced by sink adequacy and a network of physiological processes (see page 3 to 8). Further, some factors influencing the efficiency component may also influence the time component.

Grain weight is influenced both by pre-flowering and post-flowering conditions. In almost all cases, the post-flowering stage is the more important. Yield development requires photosynthate accumulation in the grain. The two sources for these assimilates are (P/S) in the ear itself and translocation from other parts in the plant. Although photosynthate accumulated prior to anthesis contributes to grain filling, by far the most contribution comes from the ear, the leaves and stem (P/S)(Carr and Wardlaw 1965; Allison and Watson 1966). Asana (1966) showed in wheat, that nearly all the increase in dry weight after anthesis is associated with grain filling.

Prolonged stress through out grain filling, even at moderate levels, reduces grain weight. Fischer & Kohn(1966)have shown that wheat yields tend to be inversely correlated with the stress induced rate of senescence of photosynthetic tissue after flowering. There is more rapid senescence of older leaves which could lead to a flow of assimilates from them towards the ear (Allison & Watson 1966) as in maize.

Wardlaw (1967, 1969) has shown that there is little effect of water stress on translocation of assimilates in the conducting tissue itself. He has pointed out that translocation out of the leaves is slowed and prolonged by water stress. This phenomenon, combined with evidence that water stress hastens maturation, and with the direct effect on (P/S) in the ear and leaves, contributes to lower grain weight in stressed plants.

Slatyer (1972) stated that the relative importance of (P/S) in the ear, flag leaf and elsewhere in grain filling does not appear to be a major factor in interpreting yield decrements under water stress. There are important differences between species associated partly with crop morphology for example the role of ear (P/S) is greater in wheat than in corn (Allison & Watson 1966).

CHAPTER TWO MATERIALS AND METHODS

Plants of Sorghum bicolor (L) Moench R.S. 610 , a hybrid grain variety were grown in 4.5 litre pots in a mixture of fine pumice and sand (4:1 v/v). The potting mixture was mixed well and sterilized to a set temperature of 180 C. Ten grams of granular, slow-release fertilizer were added to each pot as a basal application to ensure initially adequate nutrient supply. The plants were raised from seeds and thinned to two plants per pot. Day and night temperatures were set at 25 C and 20 C respectively. The relative humidity and temperature were monitored by thermohydrograph. Daylength was increased to 15 hours by the use of two fluoroescent light banks plus four incandescent bulbs per bank on both sides of the glass house. Potted plants were placed on trolleys and shifted rotationally every five days to ensure uniform distribution. There were nine trolleys of 28 pots per trolley.

2.1. Experimental treatments.

In the water stress and recovery cycle, stress was induced by withholding the modified Hoagland's nutrient solution (see appendix I) except the control plants which received 400 mls per day. There were three stress cycles and were commenced during the following stages of growth:

a) late vegetative stage. This occurred approximately 47 days after seed sowing. It was observed that the final leaf was visible in the whorl at this stage.
b) boot to bloom stage. This commenced 64 days after sowing.
c) dough stage. This occurred approximately seventy-five days after sowing.

The experimental design consisted of a randomised complete block with seven stress treatments at three growth stages replicated thrice.

2.2. Sampling procedure Mature leaf of designated position on the plant and roots segmented 10 cm from tips, were sampled for hormone estimation. The samples were put immediately in polythene bags in deep freezer. Each sampled weighed 10 g in fresh weight.

Relative water content and leaf water potential were determined subsequently on the 7th and 6th leaf respectively numbering from the top leaf

2.2. Water status measurements

Leaf water potential (LWP) was measure by the pressure bomb technique (Scholander et al., 1965; Blum et al., 1973) and relative water content (RWC) was estimated by using Weatherley and Barr s' method (1962).

Leaf water potential. Pressure bomb measures the xylem pressure, an estimate of the water potential of the leaf. Under these conditions, the water potential in a plant shoot measured with the pressure bomb may be partitioned into the following components:

$$\Psi = P + \bar{\Psi}_s$$

where $\bar{\Psi}$ is the water potential of the leaf cells, P is the pressure applied by the bomb, and $\bar{\Psi}_s$ represents the effect of solutes in the xylem sap. P and $\bar{\Psi}_s$ represent the total force tending to remove water from the leaf cells and to this extent, P estimates the hydrostatic forces in the intact xylem.

LWP was determined at 9.00 a.m. The segments of the leaf blade were cut, the mid-vein removed, and placed between two halves of a rubber stopper, then inserted into the cover that screwed to bomb cyclinder. Sufficient pressure was applied slowly from a cyclinder of compressed nitrogen until the menicus of the xylem sap appeared on the cut surface of the leaf blade. The leaf segment was allowed to protrude only two to four mm. from the split stopper. The rate of pressure increase was gradual and care was taken not to crush the leaf samples. The size of the leaf sample was approximately the same in each repeated measurements.

Relative water content This provided an estimated index of internal plant water deficits. Twenty leaf discs from a mature leaf were punched with a cork borer, and weighed freshly and quickly. The sample was floated on water in plastic vial for four hours to full turgidity. Then the discs were dried by placing them on a four sheet layer of tissue paper and covered with another four sheet layer. This drying procedure was adopted throughout the (RWC) determination. A standard time of drying was adopted. The discs were reweighed to obtain turgid weight and finally put them in the oven at 80 C for 20 hours and weighed to obtain the dry weight. RWC was calculated from this formula :

$$\frac{F.W. - D.W.}{T.W - D.W} \times 100$$

F.W. =fresh weight
T.W. = turgid weight
D.W. = dry weight.

2.3. Extraction and purification procedures for β inhibitors and cytokinin substances.

1. Extraction Ten gram samples of leaves and roots were macerated in a Wareing blender with 80% redistilled methanol for five minutes. The resulting suspension was transferred to a 500 ml flask and with several washings using a total of 100 ml of 80% methanol to give a 10 :1 vol/ weight ratio of extracting solution to plant material. The flask was shaken several times and placed in the deep freezer for 18 hours. During the initial extracting process, the flask was again shaken . At the end of the extracting period, the suspension was filtered through a Buchner funnel. The residue was resuspended twice in a 50 ml of methanol for four hours giving a final volume of extractant of 200 ml.

2. Preparation of ether soluble acids For purification, the extractant was reduced to water phase on a rotary evaporator, this process was carried out under vaccuum with dry ice traps to speed up the process. The rotary flask was rotated in a water bath at 30 C and shielded from direct light. The water phase remaining about 30 ml was transferred to a 250 ml centrifuge bottle, several distilled washings rinsed the flask and the final volume was 50 ml. This was left over-night in the freezer. The solution was later centrifuged at 1,000 r.p.m. for 30 minutes at 0 C. The supernatant was poured off into a beaker and the pH was adjusted to 2.5 with 50% HCl. It was then placed in a separating funnel and shaken strongly for three minutes with three seperate equal volumes of diethyl ether (peroxide free ether). The bulked ether fractions were shaken for three minutes with 15 ml distilled water tor remove any remaining cytokinins. The aqueous phase was dried down under vaccuum at 35 C and stored for cytokinin purification.

The ether fraction was then extracted twice with 50 ml aliquots of 5% sodium bicarbonate at pH 8 using a separating funnel. The ether phase retained being the neutral fraction and the basic ether fraction was discarded. At this stage, centrifugation for five minutes at 10,000 r.p.m. overcame the partitioning problem. The bulked aqueous phase remaining after the bicarbonate/ ether partitions was acidified to pH 2.5 using 50% HCl and extracted three times with equal volume of diethyl ether. These ether extracts were combined and retained as the acidic ether fraction. The ether fractions were dried over anhydrous sodium sulphate for several hours , then filtered and taken to dryness on a rotary evaporator.

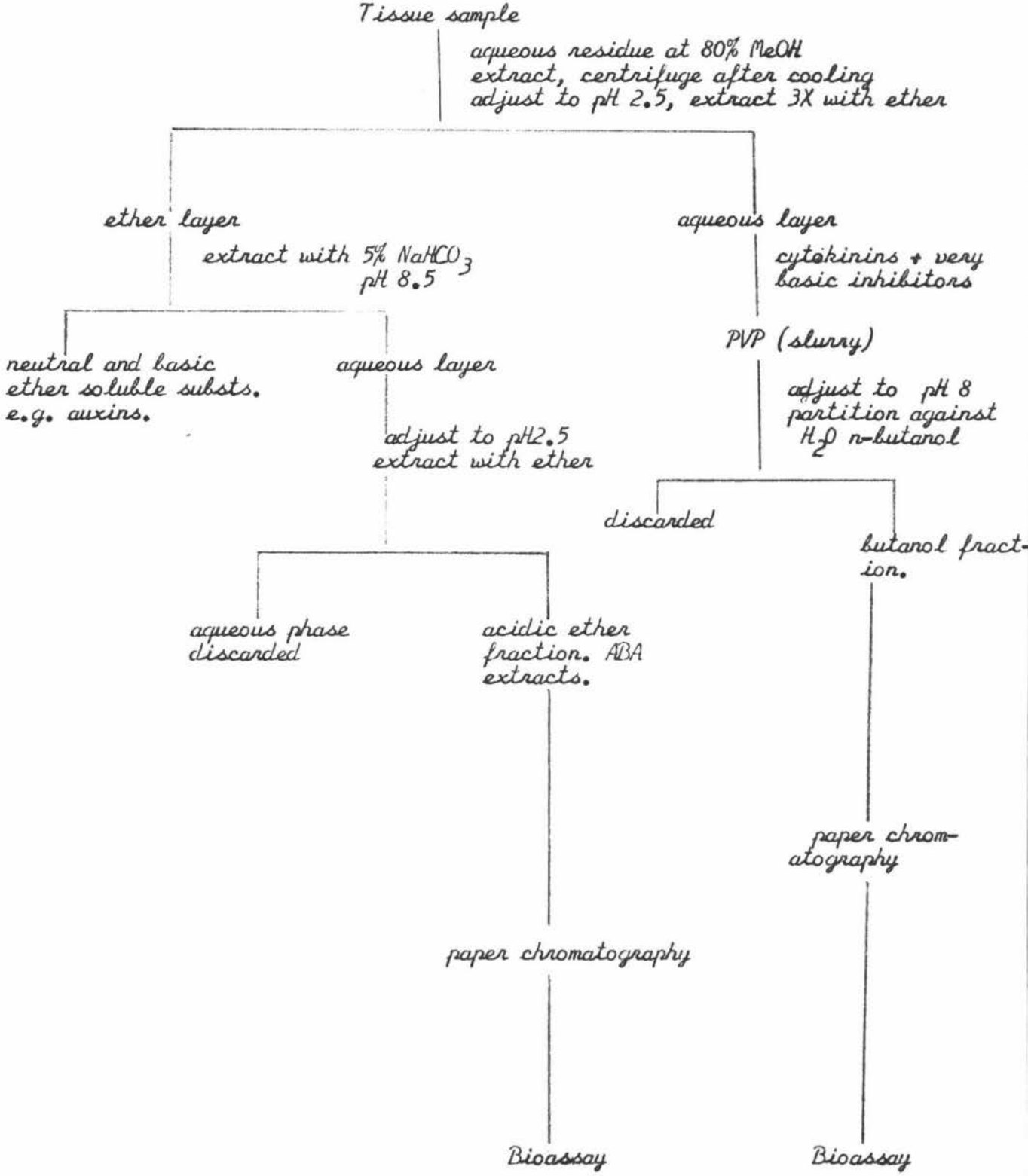


Figure 9b . Flow diagram showing the procedures.

Powdered poly-N-vinyl-pyrrolidone (Polyclar A.T.) was used as a slurry to remove phenolic compounds in tissue extracts (Glenn et al. 1972, Hewett and Wareing 1973a). PVP at concentration of 50mg ml^{-1} was added to solution of ether extracts of root samples and slurried thoroughly with shaking. Aliquots of 0.1 M phosphate buffer at pH 5 were added to the solution. The slurry was allowed to stand for ½ h. , then the PVP was filtered off and the residue washed with successive aliquots of buffer, and further filtration was repeated. The above fraction was then re-extracted with ether. The ether extracts were dried over anhydrous sodium sulphate , then filtered and dried on a rotary evaporator.

The aqueous layer containing cytokinins and very basic inhibitors was subjected to the same slurry procedure using PVP. The resulting fraction was adjusted to pH 8 and partitioned against water saturated n-butanol (500mls) and shaken well. This was repeated and the butanol phase fraction was reduced to a volume less than 20 ml. A few ml. of ethanol was added and transferred to a vial capped and stored in a deep freeze until required.

3. For bioassay purposes, further purification was obtained by use of paper chromatography. The residues of the acidic ether fraction were taken up in 0.5 ml of 50% methanol /50% acetone. This was applied with a micro-pipette to give a 15 cm streak across a sheet of pre-washed Whatman No. 1 chromatography paper. Marker spots of ABA were applied as required. The paper was developed in a descending manner using iso-propanol, 0.88 ammonia, water (10:1:1 by volume). The chromatography tank was kept in the dark and the solvent was allowed to run about 30 cm. The chromatograms were dried in the dark and cut into equal strips for wheat bioassay.

For radish cotyledon bioassay, further purification was carried out by paper chromatography. Known volumes of extract were strip loaded onto Whatman No. 3 paper with a micro-pipette and developed in the descending manner. Marker spots of kinetin were applied. The solvent used was butanol: acetic acid: water (4:1:1 vol/vol). The solvent was allowed to run to 30 cm and the chromatograms were removed and the solvent front marked immediately. All chromatograms were air-dried and cut into equal strips for radish bioassay.

4. <u>Bioassays</u> 1. Wheat coleoptile bioassay. The procedure was essentially that described by Nitsch & Nitsch (1956). Uniform wheat seeds of 'Aotea' variety were sterilized for five minutes by 2 % sodium hypochorite solution and then soaked in tap water for two hours. They were placed on moist filter paper in plastic trays and placed in the dark at

25 C. Glass covers were placed on top of the trays. After three days the coleoptiles were two to three cm. long and ready for use. Ten mm. sections, three mm. behind the tip, were cut from the coleoptiles under green light. The coleoptiles were placed in distilled water until later used. The acidic ether chromatograms were cut into ten strips plus a control strip from behind the base line. Each strip was rolled slightly and put inside a 40 X 20 mm. vial. 2 ml. of phosphate citrate buffer (see appendix I) were added to each vial. Coleoptiles were put into each vial which was then capped. The vials were then placed on a turn table and rotated slowly for twenty hours. The vials were removed and 1 ml of 10% methanol added to each to kill the coleoptile. The coleoptiles were measured at a magnification of about three.
A dosage response curve was obtained for ABA by eluting it from several chromatograms and diluting the extract over a range of concentrations from 10 ug/ml to 0.002 ug/ml in a log dilution series.

2. The radish cotyledon bioassay. This was essentially the same as described by Letham (1968). The ability of cytokinins to promote the expansion of excised cotyledons formed the basis of this assay.
Seeds of radish (Raphanus sativus L. c.v. long scarlet strain) were selected and spread on well wetted filter paper in plastic trays enclosed in polythene bags and kept in the dark at 25 C. One and a half days later, the smaller cotyledon of the pair was excised from the medium size seedlings. Cotyledons were excised in the light and were pooled in distilled water. Uniform sized cotyledons were chosen and placed on filter paper in petri dishes (diameter 9 cm.). Each dish contained one circle of filter paper (Whatman No. 1) and 3 ml. of cytokinin solution (prepared in phosphate buffer at pH 6). Eight cotyledons were placed with their upper surfaces contacting the solution. The petri dishes were put on a sheet of wetted paper in a tray which was then enclosed in a plastic bag. After three days at 22 C $\pm$ 3 C under weak fluorescent lighting, the cotyledons were blotted dry and weighted. A standard response curve was obtained for kinetin of various concentrations from 0.002 to 10 ug/ml. The levels of cytokinins were expressed as kinetin equivalents. When comparing cytokinin levels in sequential harvests, bioassays were carried out simultaneously, so that comparison could be made between samples.

2.4. Measurement of grain yields.

These measurements were used to give an indication to the effects of water stress on the grain yield of the plant. At the physiological maturity stage, grain moisture content was determined sequentially, before harvesting the grains. Grain weight and grain number were determined.

RESULTS

Introduction The length of time the experiment took from seed sowing to the time of final yield harvest was three months. Three water stress cycles were imposed during the following stages of growth:-

1) The first stress cycle began 47 days after seed sowing. The plants at this stage have approximately 8 to 10 expanded leaves, is denoted as late vegetative stage (see appendix II plate 1).
2) The second stress cycle began 64 days after seed sowing. The number of expanded leaves had increased to 10 to 12 fully expanded leaves, and is denoted as boot to bloom stage. During mid-bloom period approximately 50% of anthesis had occurred (plate 2).
3) The third stress cycle commenced 75 days after sowing and is denoted as dough stage (plate 3).

3.1. Water status determinations

The relative water content (RWC) and leaf water potential (LWP) values are showed for each of the three cycles in figures 12, 13, 14. Significance changes in (RWC) and (LWP) values are indicated in appendix II. The water deficit at the maximum stress stage of the dough period appeared greater at (RWC) 62%, and the standard error is largest at this maximum stress. Two important features emerged from water deficits results, there are a) at mild stress, (RWC) gives very low values and possibly a relatively insensitive indicator of plant water deficit, b) the plants exhibit a rapid recovery from severe water deficit upon re-watering.

3.2. Bioassay determinations

1) Wheat coleoptile The results of coleoptile response to chromatograms sections have been plotted as histograms (appendix II figures 15 to 20). Only the acidic ether fractions of leaves and roots were taken for bioassay determination. Marked inhibitory activity was observed in the R_f 0.5 to 0.8, the inhibitor zone described by Bennet-Clark and Kefford (1953). Synthetic ABA marked spots also covered a similar range of R_f values. Values of inhibitor concentration were expressed as (+) ABA equivalents, were calculated from the peaks using data for the standard curve (Figure 10). These values are shown for the different cycles in figures 12 to 14 and tables 1 to 3. The values of ABA equivalents are

relative and no procedure is carried out to calculate the % of ABA recovery from using the method of extraction and purification discussed earlier.

The ABA content of the wilted leaves increased 13 fold when the plants were subjected to prolonged stress period during the first and second stress cycles. However, the increase in ABA content was less marked from leaf samples taken during the last stress cycle. At (LWP of -16 bar, there was a 6 fold rise in ABA level from the control. The decline in ABA levels was very rapid on rewatering. Recovery treatments which reduced water deficit ,decreased ABA to levels comparable with those in low stress treatments.

Wheat coleoptile bioassay determination from the acidic ether fractions of roots were not significantly different for the three stress cycles. There was only 2 to 3 fold increase in ABA levels when the plants were wilted. One important feature is that the ABA levels in leaves are considerably higher than in roots.

2). Radish cotyledon The results of radish cotyledon response to chromatogram sections have been plotted as histograms (see appendix II figures 21 to 26). The butanol fractions of leaves and roots were only taken for bioassay determination. Marked promotory activity was observed in the R_f 0.2 to 0.3 and 0.5 to 0.8. Values of cytokinin concentration expressed as kinetin equivalents were calculated from the peaks using data from the standard curve (Figure 11). These values are shown for the three different cycles in figures 12 to 14 and tables 4 to 6.

The cytokinin activity in leaves decreased significantly (P=0.05) at the maximum stress stage during the first and third stress cycles. The cytokinin activity is relatively higher in both leaves and roots taken during late vegetative stage than during the later stages of development.

The cytokinin activity in roots showed a decrease trend when plants were subjected to stress cycle, but the results were non-significant except late vegetative samples (significant P=0.05).

Cytokinin activity in leaves and roots did not increase significantly when the plants were rewatered for the three stress cycles.

From the chromatograms, two peaks are evident, referred here as peak 1 (R_f 0.2 to 0.3) and peak 2 (R_f 0.5 to 0.8). These two peaks change in response to severe stress treatments and the stage of plant development. Peak 1 rises when severe treatments are imposed and

concomitantly, there is a decrease in peak 2. As plants develop into bloom and dough stages, peak 1 in leaf samples increases in activity. No further studies are carried out to characterise the different fractions in peak 1 and peak 2. Peak 1 showed the slow moving fractions and peak 2 had fast mobility in the chromatograms.

3.3. Final yield data.

The final harvest of grain yield is showed in tables 7,8 and 9. The two components of grain yield measured are grain weight per 100 grains and seed number per panicle. Grain yield was significantly reduced when plants were subjected to prolonged stress in all three stages of growth. Table 7 shows that a significant reduction in grain weight and grain number when stress cycle was imposed during late vegetative stage. Grain weight was reduced considerably more than the other two stress cycles.

Table 8 refers to the final harvest data when stress treatments were imposed during boot to bloom stage. Prolonged stress significantly decreased the grain yield. Both grain number and weight are affected but seed number is drastically reduced when (LWP) is at -15 bar. Grain weight component seems to be inversely related to grain number, when plants are subjected during this growth stage.

During dough stage, severe and prolonged stress significantly reduced the yield, but this response is comparatively less marked than the two earlier stages of growth.

The recovery treatments applied after maximum water deficit, did not significantly increased from the maximum stress.

The results showed that the two growth stages most adversely affected by severe stress are i) late vegetative stage and ii) during the inflorescence development and flowering period, stress applied during this period resulted in reduced number of seed per panicle.

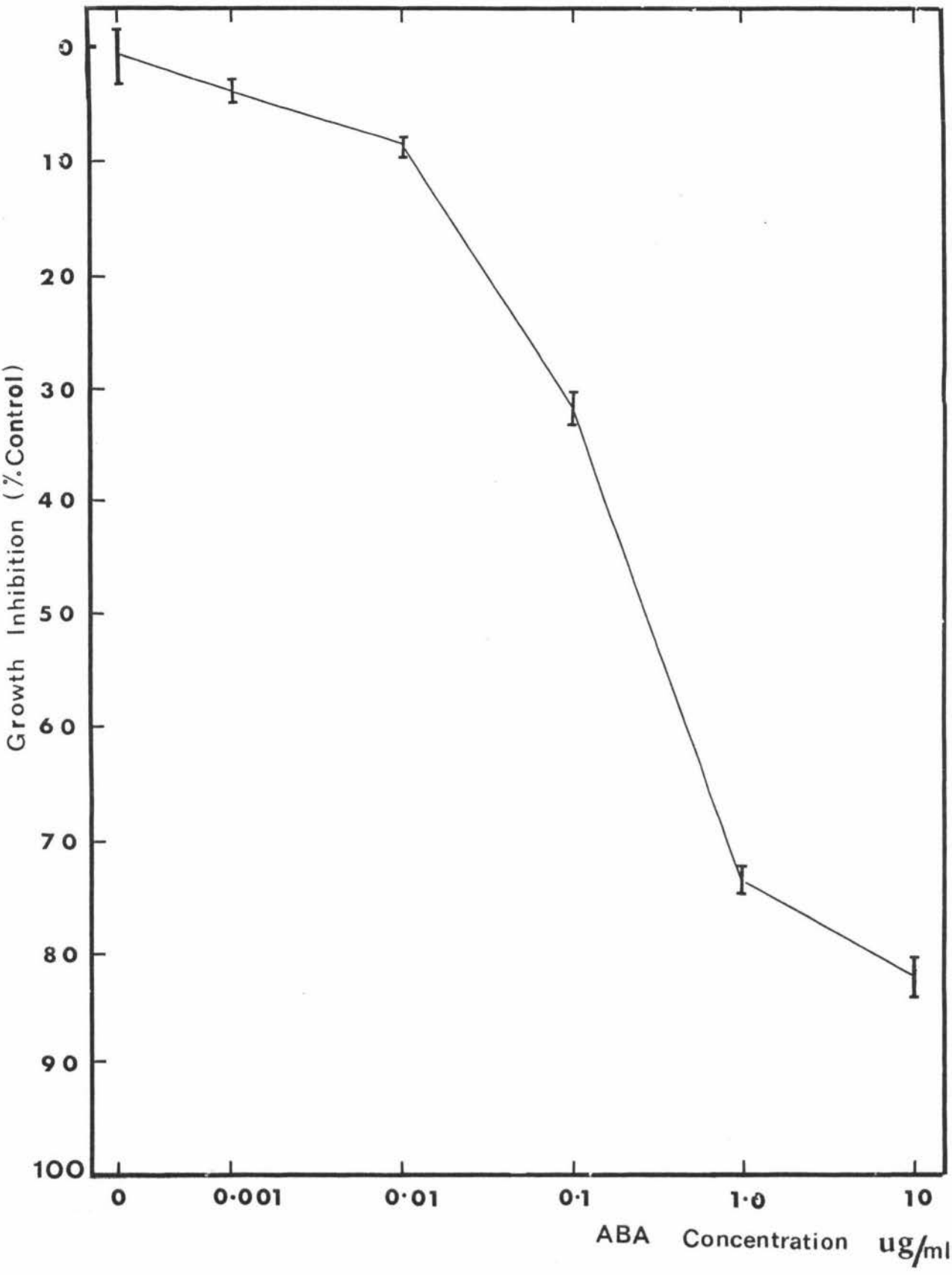


Figure 10 Standard curve for wheat coleoptile response to abscisic acid. The vertical lines indicate the 2 S.E. limits.

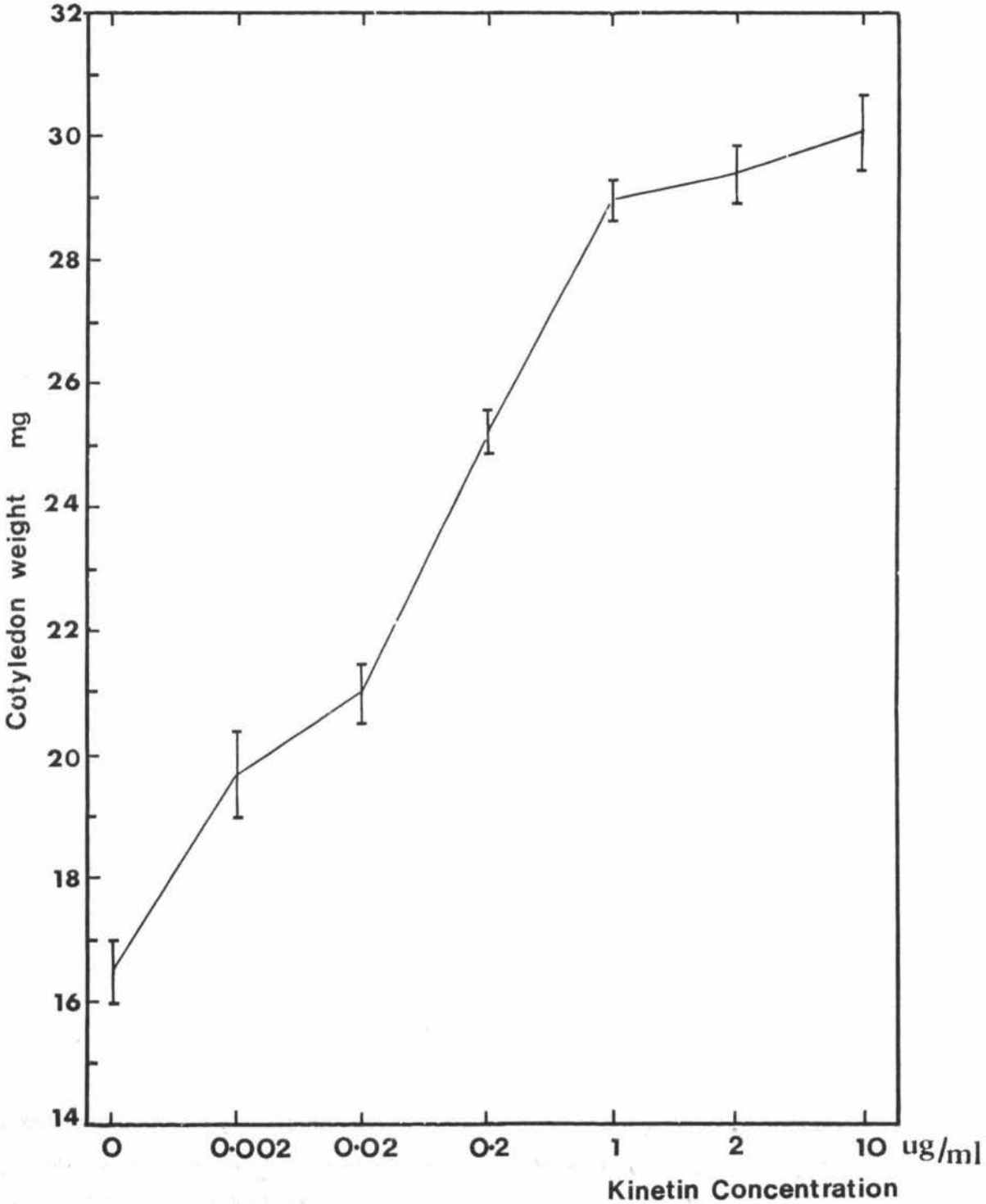


Fig. 11. Standard curve for radish cotyledon response to kinetin. The vertical lines indicate the 2 S.E. limits.

Figure 12. Trends in leaf water potential (LP), relative water content (RWC), inhibitor B and cytokinin levels in the leaf of sorghum plants subjected to stress cycle during late vegetative stage.

Keys: ⊟——⊟ leaf water potential; ⊙——⊙ relative water content
△——△ inhibitor B levels; ▽——▽ cytokinin levels.

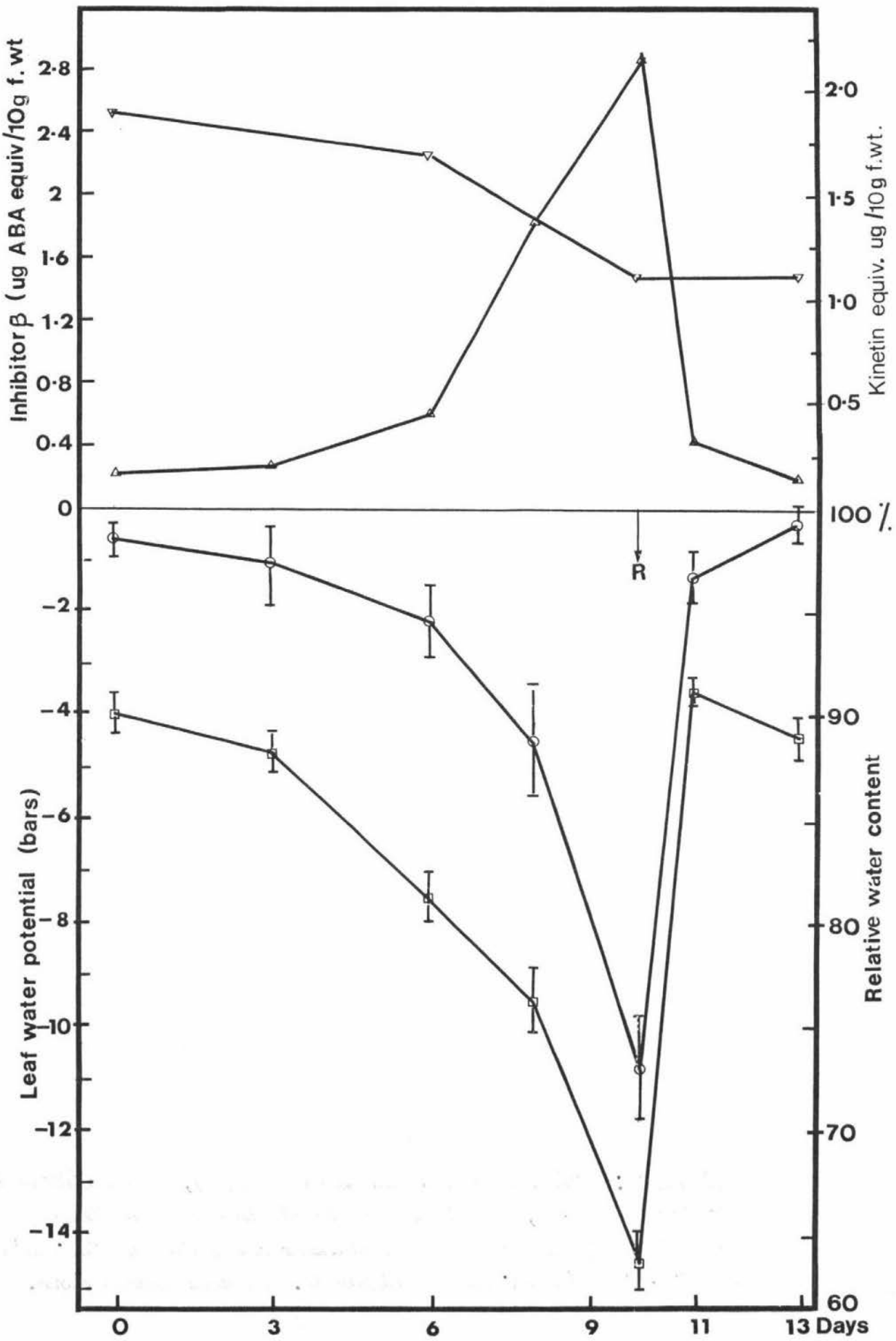

Inhibitor β (ug ABA equiv/10g f.wt
2·8
2·4
2
1·6
1·2
0·8
0·4
0
Kinetin equiv. ug /10g f.wt.
2·0
1·5
1·0
0·5
100 %
R
Leaf water potential (bars)
-2
-4
-6
-8
-10
-12
-14
Relative water content
90
80
70
60
0
3
6
9
11
13 Days

Figure 13. Trends in leaf water potential (LWP), relative water content (RWC), inhibitor B and cytokinin levels in the leaf of sorghum plants subjected to stress cycle during boot to bloom stage. Keys as shown for Figure 12. Standard errors shown.

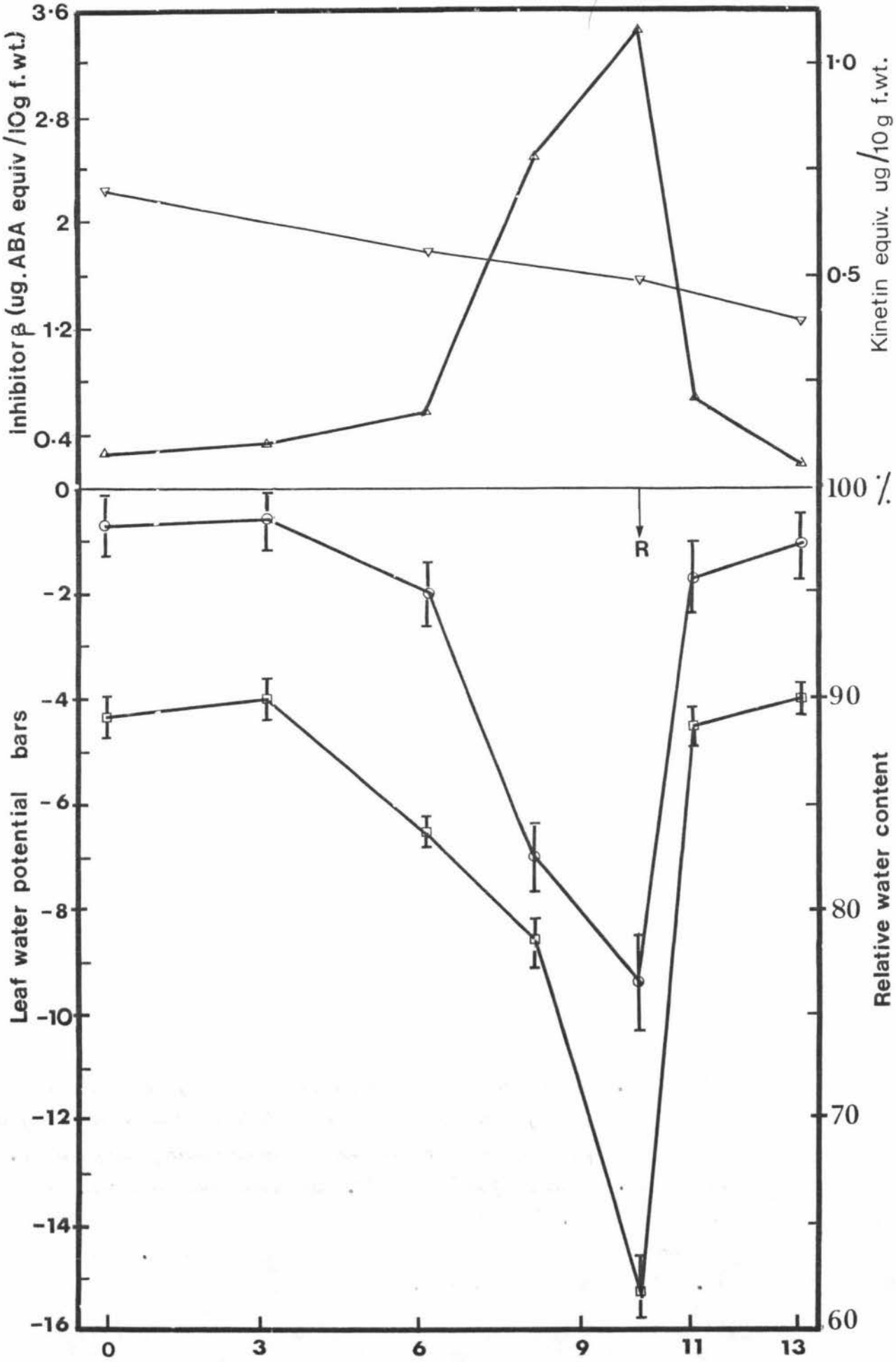

Inhibitor β (ug. ABA equiv /10g f. wt.)
3·6
2·8
2
1·2
0·4
0
Kinetin equiv. ug/10g f.wt.
1·0
0·5
100 %
R
Leaf water potential bars
-2
-4
-6
-8
-10
-12
-14
-16
Relative water content
90
80
70
60
0
3
6
9
11
13

Figure 14. Trends in leaf water potential (LWP), relative water content (RWC), inhibitor B and cytokinin levels in the leaf of sorghum plants subjected to stress cycle during dough stage. Keys as for Figure 12. Standard errors shown.

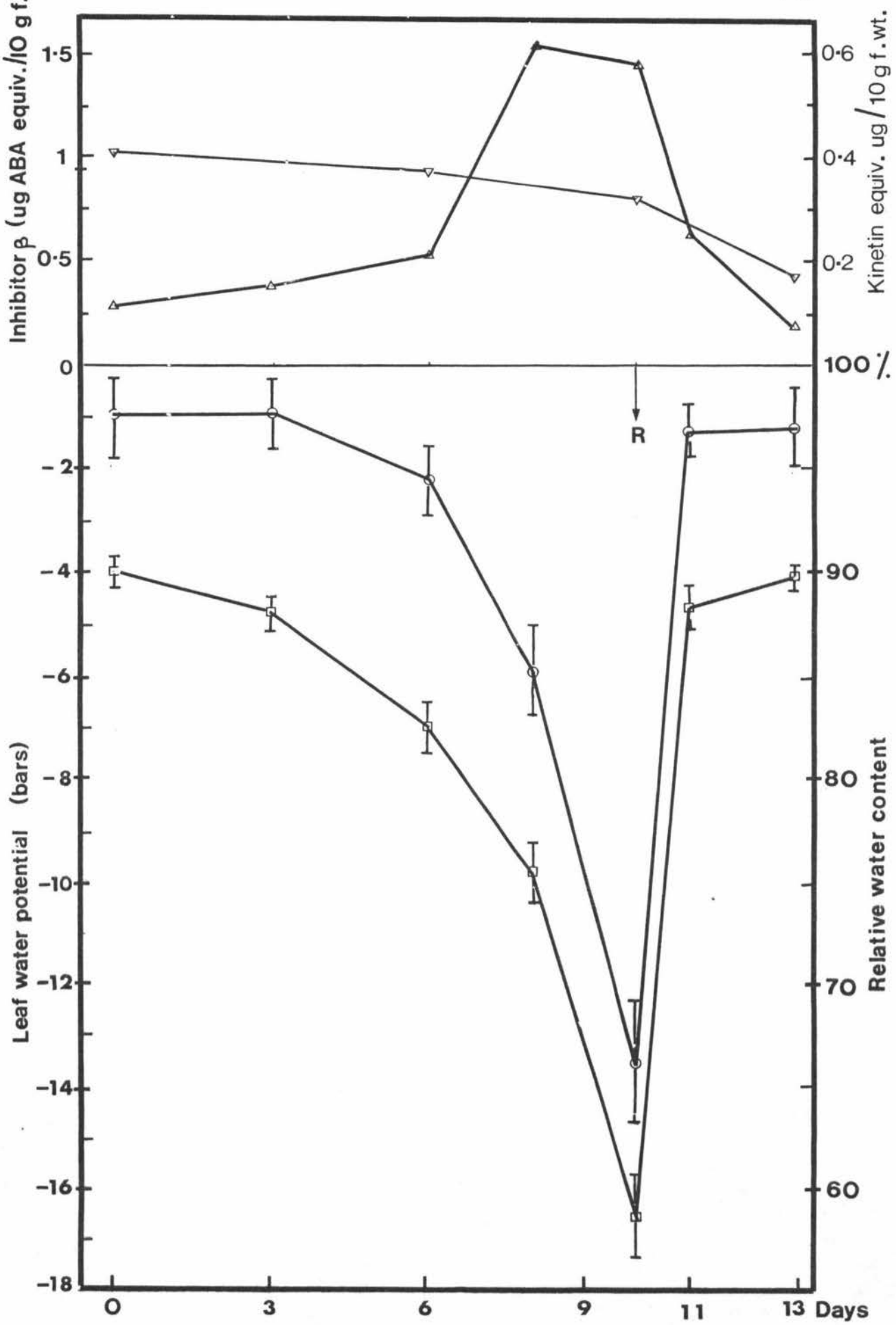

Inhibitor β (ug ABA equiv./10 g f.wt)
1·5
1
0·5
0
Kinetin equiv. ug / 10 g f.wt.
0·6
0·4
0·2
100 %
R
-2
-4
-6
-8
-10
-12
-14
-16
-18
Leaf water potential (bars)
90
80
70
60
Relative water content
0
3
6
9
11
13 Days

Table 1. Leaf water potential (LWP), relative water content (RWC) and ABA levels in sorghum leaves and roots sampled during late vegetative stage. Water was withheld from day 0. Mean ± S.E. (+) visibly wilted.

Sampling time days	LWP (bar)	RWC (%)	ABA equivalent (ug/ml) leaf	roots
0	4.0 ± 0.4	98.6 ± 1.3	0.23 ± 0.03	0.03 ± 0.006
3	4.7 ± 0.44	97.3 ± 2.2	0.26 ± 0.02	0.03 ± 0.005
6	7.5 ± 0.53	94.6 ± 1.4	0.52 ± 0.02	0.031 ± 0.007
8	9.5 ± 0.67	89.7 ± 2.9	1.87 ± 0.06	0.063 ± 0.006
10 +	14.3 ± 0.74	73 ± 2.5	2.88 ± 0.2	0.08 ± 0.01
11	3.5 ± 0.25	97 ± 1.6	0.46 ± 0.03	0.03 ± 0.005
13	4.4 ± 0.32	99.3 ± 1.2	0.21 ± 0.01	0.013 ± 0.002

Table 2. Leaf water potential (LWP), relative water content (RWC) and ABA levels in sorghum leaves and roots sampled during boot to bloom stage. Water was withheld from day 0. Mean ± S.E. (+) visibly wilted.

Sampling time days	LWP (bar)	RWC (%)	ABA equivalent (ug/ml) leaf	roots
0	4.3 ± 0.38	98.2 ± 1.4	0.26 ± 0.02	0.035 ± 0.007
3	4.0 ± 0.4	98.5 ± 1.6	0.29 ± 0.01	0.04 ± 0.01
6	6.5 ± 0.35	94.8 ± 1.4	0.57 ± 0.02	0.05 ± 0.02
8	8.6 ± 0.46	82.4 ± 1.75	2.49 ± 0.07	0.07 ± 0.008
10 +	15.2 ± 0.77	76.4 ± 2.3	3.44 ± 0. 3	0.08 ± 0.006
11	4.5 ± 0.29	95.7 ± 1.7	0.65 ± 0.02	0.04 ± 0.003
13	4.1 ± 0.12	97.4 ± 1.7	0.25 ± 0.02	0.01 ± 0.006

Table 3 . Leaf water potential (LWP), relative water content (RWC) and ABA levels in sorghum leaves and roots sampled during dough stage. Water was withheld from day 0. Mean ± S.E.. (+) visibly wilted.

Sampling time days	LWP (bar)	RWC (%)	ABA equivalent (ug/ml) leaf	roots
0	4.0 ± 0.3	97.6 ± 2.2	0.26 ± 0.02	0.02 ±
3	4.8 ± 0.3	97.3 ± 2	0.33 ± 0.01	0.024 ±
6	7.0 ± 0.43	94.5 ± 1.7	0.5 ± 0.03	0.03 ±
8 +	9.8 ± 0.48	85.2 ± 2.4	1.6 ± 0.04	0.045 ±
10 +	16.5 ± 0.8	66.6 ± 3.1	1.45 ± 0.07	0.05
11	4.7 ± 0.37	96.9 ± 1.42	0.57 ± 0.02	0.026 ±
13	4.1 ± 0.2	97 ± 2.1	0.18 ± 0.01	0.02

Table 4 Leaf water potential (LWP), relative water content (RWC) and cytokinin levels in sorghum leaves and roots (0 to 10 cm) sampled during late vegetative stage. Water was withheld from day 0. Mean ± S.E.
(+) visibly wilted.

Sampling time (days)	LWP (bar)	RWC (%)	Kinetin equivalent ug/ml (leaf)	ug/ml (roots)	% of control	
0	4.0 ± 0.4	98.6 ± 1.3	1.91 ± 0.2	1.98 ± 0.01	100	100
6	7.5 ± 0.53	94.6 ± 1.4	1.7 ± 0.06	1.73 ± 0.07	89	87
10+	14.3 ± 0.74	73 ± 2.5	1.12 ± 0.1	0.84 ± 0.16	60	42
13	4.4 ± 0.32	99.3 ± 1.2	1.1 ± 0.15	1.17 ± 0.23	60	59

Table 5. Leaf water potential (LWP), relative water content (RWC) and cytokinin levels in sorghum leaves and roots (0 to 10 cm) sampled during boot to bloom stage. Water was withheld from day 0. Mean ± S.E. (+) visibly wilted.

Sampling time (days)	LWP (bar)	RWC (%)	Kinetin equivalent (ug/ml) (leaf)	(roots)	% of control	
0	4.3 ± 0.38	98.2 ± 1.4	0.7 ± 0.1	0.61 ± 0.04	100	100
6	6.5 ± 0.35	94.8 ± 1.4	0.57 ± 0.04	0.45 ± 0.05	80	74
10+	15.2 ± 0.8	76.4 ± 2.3	0.49 ± 0.04	0.33 ± 0.03	70	55
13	4.1 ± 0.12	97.4 ± 1.7	0.39 ± 0.05	0.23 ± 0.02	55	37

Table 6. Leaf water potential (LWP), relative water content (RWC) and cytokinin levels in sorghum leaves and roots (0 to 10 cm) sampled during dough stage. Water was withheld from day 0. Mean ± S.E. (+) visibly wilted.

Sampling time (days)	LWP (bar)	RWC (%)	Kinetin equivalent (ug/ml) (leaf)	(roots)	% of control	
0	4.0 ± 0.3	97.6 ± 2.2	0.47 ± 0.03	0.38 ± 0.04	100	100
6	7.0 ± 0.43	94.5 ± 1.7	0.36 ± 0.06	0.37 ± 0.03	90	--
10+	16.5 ± 0.8	66.6 ± 3.1	0.32 ± 0.02	0.2 ± 0.03	78	55
13	4.1 ± 0.2	97 ± 2.1	0.17 ± 0.02	0.2 ± 0.02	40	55

Table 7. Changes in seed weight, number and panicle yield from various stress treatments during late vegetative stage. Means of three replicates ± S.E. (+) visibly wilted.

Treatment periods (days)	Grain weight (g) per 100 grains	Seed number per panicle	Panicle yield (g)
0	3.23 ± 0.06	336 ± 7	12.92 ± 0.23
3	3.11 ± 0.03	315 ± 20	10.96 ± 0.16
6	2.92 ± 0.06	320 ± 10	10.23 ± 0.43
8	2.43 ± 0.12	297 ± 14	7.54 ± 0.31
10+	1.83 ± 0.13	283 ± 17	6.25 ± 0.43
13	2.23 ± 0.08	276 ± 6	6.74 ± 0.3
	** *	** *	** *
Control	3.23	336	12.92
L.S.D.$_{0.05}$	0.26	31	1.03

** significant at 1 % level

* significant at 5 % level

Table 8 . Changes in seed weight, number and panicle yield from various stress treatments during boot to bloom stage. Means of three replicates ± S.E. (+) visibly wilted.

Treatment periods (days)	Grain weight (g) per 100 grains	Seed number per panicle	Panicle yield (g)
0	2.79 ± 0.04	401 ± 14	11.85 ± 0.18
3	2.72 ± 0.06	390 ± 12	11.2 ± 0.22
6	2.64 ± 0.05	384 ± 9	10.6 ± 0.06
8	2.84 ± 0.06	244 ± 12	7.34 ± 0.37
10 +	2.95 ± 0.05	183 ± 9	6.3 ± 0.15
13	3.01 ± 0.06	213 ± 7	6.66 ± 0.33
Control	2.79	401	11.85
	** *	** *	** *
L.S.D.$_{0.05}$	0.12	22	0.83

** significant at 1 % level.

* significant at 5 % level.

Table 9. Changes in seed weight, number and panicle yield from various stress treatments during dough stage. Means of three replicates ± S.E. (+)visibly wilted.

Treatment periods (days)	Grain weight (g) per 100 grains	Seed number per panicle	Panicle yield (g)
0	2.7 ± 0.04	395 ± 10	12 ± 0.43
3	2.61 ± 0.05	388 ± 10	10.9 ± 0.29
6	2.46 ± 0.05	373 ± 6	9.68 ± 0.14
8 +	2.41 ± 0.06	336 ± 7	8.66 ± 0.13
10+	2.27 ± 0.05	295 ± 5	8.2 ± 0.25
13	2.18 ± 0.07	329 ± 10	7.6 ± 0.23
	** *	** *	** *
Control	2.7	395	12
L.S.D.$_{0.05}$	0.5	20	0.73

** significant at 1 % level

* significant at 5 % level

DISCUSSION

The coleoptile bioassay detects a number of compounds which contribute to the inhibitor β zone, first detected by Bennet-Clark & Kefford (1952). Milborrow (1967) identified ABA in a number of different plant parts and species, and the R_f of ABA marked spots corresponded well with that of inhibitor β, suggesting that ABA is the most active component of the inhibitor β complex. Wright & Hiron (1969) determined that the major component of the inhibitor zone which changed dramatically in excised wilting wheat leaves, was in fact ABA. They had indicated that the quantitative differences in inhibitor β contents reflect the quantitative variations of ABA. However, their purification was more extensive than that carried out in these experiments for bioassay work; thus in their work, a number of interfering substances had been removed from the inhibitor zone. Thus the inhibitor zone contains other substances is well documented. Holst (1971) has demonstrated the presence of more than one phenolic substance in the β complex from Solanum tuberosum. Robinson et. al. (1963) reported that several phenolic materials had been isolated from the inhibiton zone, including coumaric acid, salicyclic acid and possibly ferulic acid. It is possible that such phenolic related inhibitors are partly responsible for the growth inhibition registered in the wheat coleoptile response. Coumaric acid has been shown to inhibit germination of Grand Rapid lettuce seeds.

It was difficult to determine the actual number of times the inhibitor levels increased from the control to maximum stressed stage because of the limitation in detecting small differences of these inhibitors with small samples. The estimation of ABA was also not corrected for determining the loss of abscisic acid and its conjugate substances during the extraction procedures. Milborrow (1967) determined the loss of ABA during the extraction procedure by the racemate dilution method, analogous to the isotope dilution method; he obtained an average of 68% recovery of ABA in the ether solvent partitioning and 98% recovery in silica gel TLC.

However, the change in the inhibitor β levels follows a similar pattern to ABA. A relative indication of the changes in the trends is discussed. The rise between the control samples and the maximum stressed samples was 13 to 14 fold for the first stress cycle (during late vegetative to early boot stage) and second stress cycle (during boot to bloom stage). The increase in inhibitor levels was less marked, only 6 fold for the third stress cycle (during dough stage).

These variations in response may reflect either the sharp rise in inhibitor levels triggered by severe wilting or differential pools of biosynthesis and degradation at different growth stages. It is possible that inhibitor levels occurred in largest quantities in younger leaves than in older leaves sampled from bloom to dough stages. The levels of ABA had been shown to decrease as the leaves started to age (Khan 1975).
The amount of accumulated ABA depended on the duration of the stress periods. Three to six days stress periods did not markedly raise the inhibitor levels.

It has been reported that (LWP) has to drop to a particular level before any increase in ABA levels occur. Zabadal (1974) examining this relationship in two species of Ambrosia, determined a threshold water potential of -10 bar that stimulates ABA synthesis. For one species, this represented a drop in water potential of -2 bars while in the other -6 to -8 bars, as compared to the water potential of control plants. Beardsell & Cohen (1975) confirmed that there is a threshold value of (LWP) below which ABA levels increase abruptly in maize. For this experiment, the stress treatment intervals are too long to determine precisely the critical (LWP) at which ABA levels increased abruptly. It is interesting to note that this threshold value falls between (LWP) -5 to -8 bar.

The profiles of histograms (see appendix II) derived from wheat coleoptile responses of root extracts showed little changes in response. The results in terms of ABA equivalent derived from the standard curve may not be meaningful in roots mainly because the coleoptile test is insufficient to detect small quantitative differences in plant hormones. There is also the problem of purifying the root extracts which contain impurities.

Abscisic acid has been reported to occur in extracts of roots of Zea mays (Kundu & Audus 1974a), sunflower and avocado (Milborrow & Robinson 1973) and peas and beans (Watson et. al. 1976). Milborrow & Robinson (1973) reported that ABA levels in air-dried avocado roots increased 3 fold within 5 h. while in sunflower roots the increase was only 50%. These authors concluded that roots fail to respond to wilting by increasing their ABA content in the same manner as leaves. In contrast to the above results, Watson et.al. (1976) showed that there is an increase of 10 fold within 1 h. and 16 fold by the end of 2nd h. in ABA levels of bean plants exposed to a -4 bar stress in the root medium. They obtained a lower value for the unstressed roots, making the apparent increase in the wilted roots larger. This discrepancy may be because of

a difference in ABA content in the roots of soil grown (Milborrow and Robinson 1973) and water cultured plants (Watson et. al. 1976). In their experiments, the stress periods were comparatively short and their purification methods were extensive. Both phaseic acid and di-hydrophaseic acid were also found to increase with ABA in wilting treatments (Watson et.al. 1976). Both these two related substances of ABA are the metabolites of ABA during stressed treatments (Lovey & Kriedemann 1973, Harrison & Walton 1975).

The small magnitude of the increase in ABA induced by wilting of roots in comparison with the marked increase which occurred in leaves when they wilt, shows that the biosynthesis is controlled differently in the two organs. Milborrow & Robinson (1973) presented evidence that in wilted leaves the increase in ABA levels is due to de novo synthesis and has suggested that a level of ABA can stop its own biosynthesis by a feedback mechanism rather than release from a conjugate such as glucose ester. This agreed with the recent findings of Watson et. al.(1976) who reported virtually no change in ABA glucose ester before and after 27 h. stress period. Harrison & Watson (1975) suggest that in wilted bean leaves ABA levels may be regulated in the following manner: (a) wilting triggers an increased rate of ABA synthesis, which is followed by an increased rate of metabolism, (b) ABA concentrations increase and become relatively constant when the increased rates of synthesis and metabolism become approximately equal, and (c) ABA levels decrease as turgor is regained, due to decreased rates of ABA synthesis relative to metabolism.
The small ABA pools in plants may be dynamic. If this is the case, measurements of ABA flux, although more difficult to determine, may be of much greater significance than the actual levels at any time.

The ability for a tissue to synthesize a large quantity of ABA on wilting is associated with the tissue's regulation of water loss by stomata. Roughly, a doubling of the endogenous amount of ABA was sufficient to trigger a stomatal closure in Xanthium strumarium L. (Raschke 1974). Further experiments by Raschke (1975) have shown that the amount of (±) ABA required to initiate a stomatal response decreases with the (±)-ABA concentration in the transpiration stream. With a concentration of 0.1 uM (±)-ABA, an amount of (±)-ABA as small as 2 or 3% of the endogenous (+)-ABA triggered stomatal closure. Compartmentation of ABA is a plausible explanation of these observations. Cummins (1973) studied the correlation between stomatal behaviour and ABA metabolism and he concluded that ABA is removed into storage sites in the leaf where it cannot act on the stomata.

The prime function of the extra ABA formed on wilting is to close the stomata rapidly. This inevitably reduces transpiration and curbs water loss (Talha & Larsen 1975). Closure of stomata also increases the diffusion resistance to CO_2 entering the leaf and thus reduces the rate of photosynthesis. Hence, changes in endogenous levels of ABA-like substances can constitute a mechanism for regulating gas exchange. These may occupy a key position in the regulation of photosynthetic performance (Kriedeman et.al. 1972).

One positive correlation was observed by Raschke (1976) that is the youngest leaf had the highest ABA content and the highest resistance to transpiration. The role in which ABA inhibits wide stomatal opening in the young leaf and thereby helps to keep turgor high for leaf expansion can therefore be maintained. The youngest leaf has not only the high ABA content but possibly holds also a larger proportion of its ABA in the compartment with the guard cells than do older leaves. This may explain that the youngest leaf needs less (±)-ABA than the older ones to initiate a stomatal response.

The radish cotyledon bioassay is suitable for testing synthetic cytokinins and partially purified extracts and is a rapid assay for cytokinin activity. Growth evoked by cytokinins in excised radish cotyledons is due principally to cell enlargement (Letham 1968). The results reported the presence of cytokinin-like activity in both the leaves and roots of sorghum. Both quantitative and qualitative changes are recorded in profiles of chromatograms at the three growth stages.

A decrease in cytokinin activity is observed in the radish cotyledon response when plants are subjected to severe stress treatments. Though the stastical analysis of the responses from the second and third stress cycle samples were non-significant, the trend of decreasing cytokinin-like activity is noted in both the roots and leaves. Water stress was shown to effect a decrease in the quantity of cytokinins reaching the shoot (Itai & Vaadia 1965). This reduced supply of cytokinins to the leaves is suggested as one cause of the changes in shoot metabolism for example, a decline in the protein synthesis potential of the leaves. Itai & Vaadia (1971) examined the effect of water stress applied to tobacco through enhanced evaporative demands. Plants were exposed for 30 minutes to an air stream which caused slight wilting of the leaves and then were allowed to recover turgor. This short period of stress to the shoot resulted in a reduction of cytokinin activity to half of the control cytokinin activity. In this experiment, the cytokinin levels in the leaves were reduced only slightly (tables 4 to 6) when the plants were stressed severely.

In the case of reduction of root cytokinins to stress treatments, this may be attributed to either a decline in biosynthesis in root tips or a shift of cytokinins synthesis to developing fruits. This experiment does not have the results to support this suggestion , but it is interesting to note the changing concentration of cytokinins from late vegetative stage to dough stage. Both the extracts of samples from boot to bloom and dough stages have considerably lower in cytokinin activity than from late vegetative stage samples. Sitton et. al. found that at the time when the cytokinins supply from the root declines, and when the leaves become deficient in cytokinins, a new center of cytokinin synthesis is formed in the growing fruits of sunflowers. In many species, young fruits are known to contain high concentrations of cytokinins (Kende 1971). The shift of cytokinin production from the roots to developing fruits may have two possible consequences ; a deficiency of cytokinins in the leaves and channelling of assimilates from the leaves , which have lost some or most of their 'retention potential ' to the fruits.

Two peaks of cytokinin activity are evident in the profiles of histograms in both the root and leaf samples. Peak 1 represents the zone R_f 0.2-0.3 and peak 2 represents the zone R_f 0.5-0.8. These two peaks of cytokinin activity in leaves resemble the fractions identified by Hewett & Wareing (1973a, 1973b) in poplar leaves in which they referred as fraction N (R_f 0-0.2) and fraction Z (R_f 0.5-0.8). They tentatively identified fraction N as a cytokinin glucoside and fraction Z as zeatin and zeatin riboside. The identification of these two fractions was confirmed by recent studies (Van Staden & Davey 1976).

Radin & Loomis (1971) found increasing amounts of three cytokinin fractions in developing radish roots. Two of these were chromatographically similar to zeatin ribotide and to zeatin or its riboside and the third fraction was not identified. In this experiment, two main peaks of cytokinin activity are observed in the extracts from 100 millimeter root tips. No further procedure was carried out to identify the cytokinin complex in peaks 1 and 2. Feldman (1975) working on intact terminal millimeter root tips of Zea mays showed that at least four cytokinin fractions are present, that is a nucleotide, zeatin ribotide, zeatin and zeatin riboside and an unidentified cytokinin. In his experiment, the extraction and purification for free cytokinins were extensively worked out and further characterisation of the three peaks of cytokinin activities were attempted.

The cytokinin activity in peak 2 is relatively higher than peak 1 in the root and leaf samples from the late vegetative stage. It is interesting to speculate that zeatin and zeatin riboside contents are high during the vegetative stage in roots. These root synthesized cytokinins, mainly zeatin and zeatin riboside, are now generally accepted as the transportable forms to the shoots of both herbaceous (Van Staden & Davey 1976) and woody species (Hewett & Wareing 1973b). However, the fate of these cytokinins in the plant shoot and more specifically the developing and mature leaves is still largely unknown.

There is a shift of cytokinin activity in peak 2 to peak 1 as the stress periods prolonged and leaf growth ceased. In case of reduction of cytokinin activity in roots, this may be due to either a decline in biosynthesis as root growth ceases or a shift of cytokinins to other centers for example developing fruits.

Hewett & Wareing(1973), Van Staden (1976) have shown that total cytokinin activity and diversity are at a maximum in expanding leaves which have the major cytokinins zeatin and zeatin riboside. As leaves age, the amount and number of cytokinins decrease, with yellow senescent leaves having one detectable cytokinins probably a glucosides. The results of bioassays obtained from samples of the second and stress cycles seem to indicate that the major cytokinins present are nucleotides and glucosides. It is suggested that these glucosides are formed when the xylem transported cytokinins are metabolized (inactivated) in the leaves. The appearance of fraction N activity in senescent leaves suggests that this cytokinin could be a storage form of cytokinin (Hewett & Wareing 1973). Engelbrecht (1972) suggested that fraction N may have a modulating role in rooted bean leaves by maintaining more or less steady levels of fraction z. Such a regulatory mechanism would not necessarily appear to operate during leaf senescence.

The idea that cytokinin glucosides are bound (inactive) forms of the active derivatives such as zeatin riboside and zeatin may be pertinent to the metabolism of cytokinins in stressed leaves. Hewett & Wareing (1973) have shown that the glucosides gradually increase as the leaves mature and age, suggesting that the leaves are able to metabolize the cytokinins transported from the roots. The exact function of the cytokinin glucosides in stressed plants is obscure. Van Staden (1976) reckoned that it is likely that the attachment of a glucose moiety to zeatin and zeatin riboside inactivates these compounds (bound form) which enables the plant to transport through the phloem.

It is known that moderate to severe water stress accelerates the onset of senescence and proteolysis. These processes are known to be retarded by cytokinins (see review section). It seems plausible that roots under stress have reduced levels of cytokinins to the shoots. The shoots in turn behave as though their cytokinin supply is limited and exhibit enhanced protein degradation and other metabolic changes leading to senescence. This is a simplified view, for senescence is a complex interaction between organs of a plant, a part of this interaction being mediated by cytokinins of root origin and abscisic acid.

The response of grain number and weight to stress cycles indicates the presence of "critical stages" for stress treatments. Here, a critical period is defined as a period of time in the life cycle of plant when sub-optimum water causes a greater reduction in one or more of the grain yield components than during other periods in the development of plant. In this experiment, the two "critical stages" occur (a) during late vegetative to early boot stage and (b) during mid-boot to bloom stage (during inflorescence development and flowering period). Severe stress during this stage reduced grain number. The results in this experiment agreed to an extent with others' findings (Lewis et. al.1974). They studied the yield response of grain sorghum (Sorghum bicolor L. Moench cv. 'Oro) to a single period of known soil water deficit during three similar stages of growth. Their results indicated that grain sorghum yields were reduced by 17% when the soil water potential dropped to -13 bar during the late vegetative to boot stage. Similar low soil water potentials which occurred during the boot through bloom stage and the milk through soft dough stage resulted in yields being reduced by 34% and 10% respectively. Salter & Goode (1968) concluded that grain sorghum responds well to a plentiful water supply during the boot to head stages of growth and that yields are reduced by drought during this time.

During vegetative development, moderate to severe water stress will limit the meristematic growth, which in turn would probably reduce grain yield through reduction in leaf area. This reduction in potential leaf area would probably account for the decrease in grain yield when the sorghum plants were subjected to first stress cycle. McCree & Davis (1974) reported that both the rate of increase of leaf area and final leaf area decreased significantly when grain sorghum was subjected to varying degrees of water stress. The potential leaf area may be determined during planting to panicle initiation , while the setting of the maximum seed number potential is determined during panicle initiation to bloom stage. Seed number has been shown to correlate positively with grain yield in sorghum (Blum 1970 ,Kirby & Atkins 1968).

Hultquist (1973) tested the effect of water stress on yield components at panicle initiation ,at floret differentiation and at early grain fill, of two hybrid genotypes C-42Y and RS-626. A severe reduction, about 60% in seed number per head resulted from stress imposed during panicle initiation. Water stress imposed during early dough stage resulted in a loss of 25% in seed number through floret abortion. Hultquist also measured

photosynthesis in turgid controls, moderately stressed plants and plants stressed to zero net CO_2 exchange. He found that stress decreased photosynthesis as well as reducing the export of ^{14}C from labelled leaves. The patterns of export differed in the two varieties. The RS-626 hybrid exported more assimilate to lower nodes and roots and less to panicles than did C-42Y both during stress and recovery. Polarisation of assimilate transport in RS-626 was associated more with survival at the expense of panicle and seed production.

It is speculated that differentiation of spikelet components is a critical period and seed number can be reduced drastically by water stress. Photosynthesis may be limiting during seed number differentiation and during subsequent development. There is also a possibility that simultaneously expanding floral and vegetative structures may compete, under stress conditions, for available assimilates during boot to bloom stage and that seed number can be influenced as a result (Eastin et.al. 1974).

Moderate to severe water stress often speeds up senescence in plants for a few days, and hence seed size potential is not frequently attained during grain filling period. Since ultimate grain yield depends on the rate of dry matter accumulation and the length of the grain filling period. The time component of grain filling is often accelerated in drought.

Two problems arise when attempting to compare the present study with those of others. First, there is no uniform manner in which the growth stages are delineated. Precise determination of the length of each growth stage is subjective. Secondly, the most important source of variability is the method by which the water deficits are estimated and the lack of uniformity in the water deficits among the growth stages studied. Eastin & Sullivan (1974) stated that in a discussion of developmental events limiting seed size and seed number; the division is rather arbitrary. Some events occurring in vegetative stage influence events in the differentiation of the inflorescence and expansion which in turn influence the ultimate grain production during grain filling stage.

There is a need for additional studies to determine crop susceptibility and the application of these data in the incorporation into irrigation timing concepts. This would lead to increases in water use efficiency in crop production.

CONCLUSIONS

It has been demonstrated in this preliminary study that during water stress, changes occur in the levels of endogenous hormones in the sorghum plant. Leaf content of ABA increases rapidly and root and leaf cytokinins generally decrease. Sorghum leaves and roots undergo both quantitative and qualitative changes in their cytokinin content. The roles of abscisic acid and cytokinins and its related substances are involved in the adaptation of the plant to stress. During adaptation to water stress, the endogenous levels of growth hormones change and a train of events can happen for example, changes in water potentials, reduction in growth and changes in various metabolic reactions in plant.

Additional studies are needed to identify and characterize the fractions in the cytokinin content and relate them to the metabolism of cytokinin during water stress. The physiological significance of the diverse forms of cytokinins in leaf tissue has yet to be examined. This is fundamental to an understanding of the contribution of root cytokinins to the control of leaf development.

The origin of increased ABA in leaves and roots is still speculative especially in the case of roots where ABA-like compounds are only detected at root tips. A possible correlation between ABA levels and the physiological state of the leaf tissue should be examined further. Additional studies are pertinent to a better understanding of metabolism of water stressed plants. The study of the nature of hormonal involvement in plant response to stresses may yield relevant information for the understanding of plant behaviour.

Since sorghum is reputed to withstand water shortage better than most crops; further studies of its physiological mechanism to drought should lead to a better knowledge of how we can provide protection for more susceptible crops like maize.

APPENDIX I

Hoagland solution

The solution was used at a rate of 2 ml of each stock solution per litre (1:500) and the pH was adjusted to 6.5

Stock solution A	g/ litre	Ppm in final solution
Calcium nitrate	295.2	Ca 100.2
$Ca(NO_3)_2 \cdot 4H_2O$		N 70.03
Sequestrene Na Fe Chelate	10.4	Fe 2.50
Stock solution B		
Potassium phosphate	34.02	K 19.55
KH_2PO_4		P 15.5
Stock solution C		
Potassium nitrate KNO_3	126.39	K 97.7
		N 35.02
Magnesium sulphate	123.24	Mg 24.3
$MgSO_4 \cdot 7H_2O$		S 32.
Boric acid H_3BO_3	0.71	B 0.25
Manganese chloride	0.45	Mn 0.251
$MnCl_2 \cdot 4H_2O$		Cl 0.324
Zinc sulphate	0.05	Zn 0.025
$ZnSO_4 \cdot 7H_2O$		S 0.012
Copper sulphate	0.02	Cu 0.0102
		S 0.005
Molybdic acid		
$Na_2MoO_4 \cdot 2H_2O$	0.005	Mo 0.0053
		K 1.65
Potassium chloride	1.575	Cl 1.5

Buffer solutions

1. Phosphate -citrate buffer

The following buffer was used for the wheat coleoptile bioassay (Nitsch & Nitsch, 1956)

Material	Quantity
K_2HPO_4	4.485 g.
Citric acid monohydrate	2.547 g.

Dissolve the above in 250 ml of distilled water, For use, dilute 1 in 10 and add 2 g. of sucrose per 100 ml. ph should be adjusted to 5.3

APPENDIX II

Statistics

a) Standard error of the mean.

For calculation of these values the following formula was used.

$$S.E = \frac{\sqrt{\frac{\Sigma (x-\bar{x})^2}{n-1}}}{\sqrt{n}} = \sqrt{\frac{\Sigma(x)^2 - \frac{(\Sigma x)^2}{n}}{n(n-1)}}$$

b) Limits of confidence for histograms.

Confidence limits were calculated using the short cut method of allowance (Link and Wallace, 1952). The limits are calculated using the following formula.

$$L = \frac{\Sigma(\text{Sample ranges}) \times \text{critical factor}}{\text{No of replicates per sample}}$$

The value is plotted above and below the control line and any peaks outside these limits are significant at P=0.01. Critical values are shown in Table X.

Example

To demonstrate this procedure, the data for wheat coleoptile response to chromatograph sections of a acidic ether extract from mature sorghum leaves taken during first stress cycle. The number of coleoptiles used to assay each section was ten. The range was determined by subtracting the shortest coleoptile from the longest in each case. The mean value for control coleoptile lenght in this case was 1.8 cm.

Chromatograph section

R_f

Control	1	2	3	4	5	6	7	8	9	10
Range	0.24	0.15	0.15	0.16	0.14	0.04	0.02	0.06	0.12	0.08

Sum of ranges = 1.24. Critical factor from table X for 11 samples, 10 replicates / sample is 0.51.

$$L = \frac{1.24 \times 0.51}{10} = 0.07$$

Any values outside of 1.8 ± 0.07 are significant at P = 0.01

Table VI CRITICAL FACTORS FOR ALLOWANCES FOR ONE -WAY (BALANCED) DIVISION INTO GROUPS 1% RISK

(LINK AND WALLACE, 1952)

Replicates /treatment	Number of Groups 2	3	4	5	6	7	8	Number of Ranges 9	10	11	12	13	Number of Treatments 14	15	16	17
2	7.92	4.42	2.96	2.06	1 .69	1.39	1.20	1.03	.91	.82	.75	.68	.63	.59	.55	.51
3	3.14	2.14	1.57	1.25	1.04	.89	.78	.69	.62	.57	.52	.48	.45	.42	.39	.37
4	2.47	1.74	1.33	1.08	.91	.78	.69	.62	.56	.51	.47	.44	.41	.38	.36	.34
5	2.24	1.60	1.24	1.02	.86	.75	.66	.59	.54	.49	.46	.42	.40	.37	.35	.33
6	2.14	1.55	1.21	0.99	.85	.74	.65	.59	.53	.49	.45	.42	.39	.37	.35	.33
7	2.10	1.53	1.21	0.99	.84	.74	.65	. 59	.53	.49	.45	.42	.40	.37	.35	.33
8	2.08	1.52	1.21	0.99	.85	.74	.66	.59	.54	.50	.46	.43	.40	.37	.35	.33
9	2.09	1.53	1.22	1.00	.85	.75	.66	.60	.54	.50	.46	.43	.40	.38	.36	.34
10	2.10	1.55	1.23	1.01	.86	.75	.67	.61	.55	.51	.47	.44	.41	.38	.36	.34
11	2.11	1.56	1.24	1.02	.88	.77	.68	.61	.56	.51	.48	.44	.42	.39	.37	.35
12	2.13	1.58	1.25	1.03	.89	.78	.69	.62	.57	.52	.48	.45	.42	.40	.37	.35
13	2.15	1.60	1.27	1.05	.90	.79	.70	.63	.58	.53	.49	.46	.43	.40	.38	.36
14	2.18	1.62	1.28	1.06	.91	.80	.71	.64	.58	.54	.50	.46	.43	.41	.39	.37
15	2.20	1.64	1.30	1.08	.92	.81	.72	.65	.59	.54	.50	.47	.44	.41	.39	.37
16	2.22	1 .65	1.31	1.09	.93	.82	.73	.66	.60	.55	.51	.48	.45	.42	.40	.38
17	2.24	1.67	1.33	1.11	.95	.83	.74	.67	.61	.56	.52	.48	.45	.43	.40	.38
18	2.27	1.69	1.34	1.12	.96	.84	.75	.68	.62	.57	.53	.49	.46	.43	.41	.39
19	2.30	1.71	1.36	1.14	.97	.95	.76	.68	.62	.57	.53	.50	.46	.44	.41	.39
20	2.32	1.73	1.38	1.15	.98	.86	.77	.69	.63	.58	.54	.50	.47	.44	.42	.40

Entries are to be multiplied by the sum of the ranges within groups to obtain allowances for group totals.

Relative water content (%)

a) *Late vegetative stage cycle*

	0	3	6	9	12	13	15	Days
	96	99	96.7	93.5	78	97	97	
	100	100	92.1	91.8	70	99.7	100	Replications
	100	93	95.2	84	70.8	94	101	
Sum	296	292	284	269.3	218.8	290.7	298	
Mean	98.6	97.3	94.63	89.7	72.9	96.9	99.3	
S.E.±	1.3	2.2	1.4	2.9	2.5	1.65	1.2	

b) *Boot to bloom stage cycle*

	100	100	96.3	83.7	77.6	97.9	99
	99.2	99.5	92	84.6	72	92.4	99.3
	95.3	96	96.1	79	79.7	97	94
Sum	294.5	295.5	284.4	247.3	229.3	287.3	292.3
Mean	98.2	98.5	94.8	82.4	76.4	95.7	97.4
S.E.±	1.45	1.6	1.4	1.75	2.3	1.7	1.7

c) *Dough stage cycle*

	100	99.6	97	81.6	72.8	94.2	99.3
	99.6	93.4	95.5	84.4	64.5	99	99.
	93.2	99.	91.2	89.7	62.6	97.5	92.7
Sum	292.8	292	283.7	255.7	199.9	290.7	291
Mean	97.6	97.3	94.5	85.2	66.6	96.9	97.
S.E.±	2.2	2.0	1.74	2.4	3.1	1.42	2.15

Testing significance of means

To test whether differences in RWC % and water potential were significant the t-test was used. To obtain the t-value the standard error, of both means to be compared, was squared, summed and the square root of this value used. The difference between the two means was divided by this value, thus giving the t-value. The significance was determined from a table of t values at 4 degrees of freedom.

a) Late vegetative stage cycle

Sampling time	1	2	3	4	5	6	7
x	98.6	97.3	94.6	89.7	72.9	96.9	99.3
± S.E.	1.3	2.2	1.4	2.9	2.5	1.6	1.2
$S.E.^2$	1.69	4.84			6.25		
$\Sigma S.E.^2$	6.53				7.94		
$\sqrt{\ }$	2.55				2.81		
t-value	$\frac{1.3}{2.55} = 0.5$				9.14		
Sign. 5%	N.S.				S.		

b) Boot to bloom stage cycle.

sampling time	1	2	3	4	5	6	7
x	98.2	98.5	94.8	82.4	76.4	95.7	97.4
± S.E.	1.45	1.6	1.4	1.75	2.3	1.7	1.7
$S.E.^2$	2.1	2.56					
$\Sigma S.E.^2$	4.66						
$\sqrt{\ }$	2.16						
	$\frac{0.3}{2.16} = 0.14$						
sign. 5%	n.s.						

c) Dough stage cycle.

x	97.6	97.3	94.5	85.2	66.6	96.9	97
± S.E.	2.2	2.0	1.74	2.4	3.1	1.42	2.15
$S.E.^2$	4.84	4			9.6		
$\Sigma S.E.^2$	8.84				14.45		
$\sqrt{\ }$	2.97				3.8		
t-value	0.1				8.16		
sign. 5%	n.s.				n.s		

Significance of changes in leaf water potential.

(a) Late vegetative stage cycle.

	Water pot. (± S.E)	$(S.E.)^2$	$\Sigma (S.E.)^2$	$\sqrt{\quad}$	t value	significance
1.	4.0 ± 0.4	0.16	0.35	0.59	$\frac{0.7}{0.59}$	N.S.
2.	4.7 ± 0.44	0.19				S.
3.	7.5 ± 0.53					S
4.	9.5 ± 0.67					S
5.	14.3 ± 0.74					S.
6.	3.5 ± 0.25					N.S
7.	4.4 ± 0.32					N.S.

(b) Boot to bloom stage cycle

1.	4.3 ± 0.38	0.14	0.3	0.55	0.55	N.S.
2.	4.0 ± 0.4	0.16				
3.	6.5 ± 0.35					
4.	8.6 ± 0.46					
5.	15.2 ± 0.77					
6.	4.5 ± 0.29					
7.	4.1 ± 0.12					

(c) Dough stage cycle

1.	4.0 ± 0.3	0.09	0.18	0.42	1.9	n.s.
2.	4.8 ± 0.3	0.09				
3.	7.0 ± 0.43					
4.	9.8 ± 0.48					
5.	16.5 ± 0.8					
6.	4.7 ± 0.37					
7.	4.1 ± 0.2					

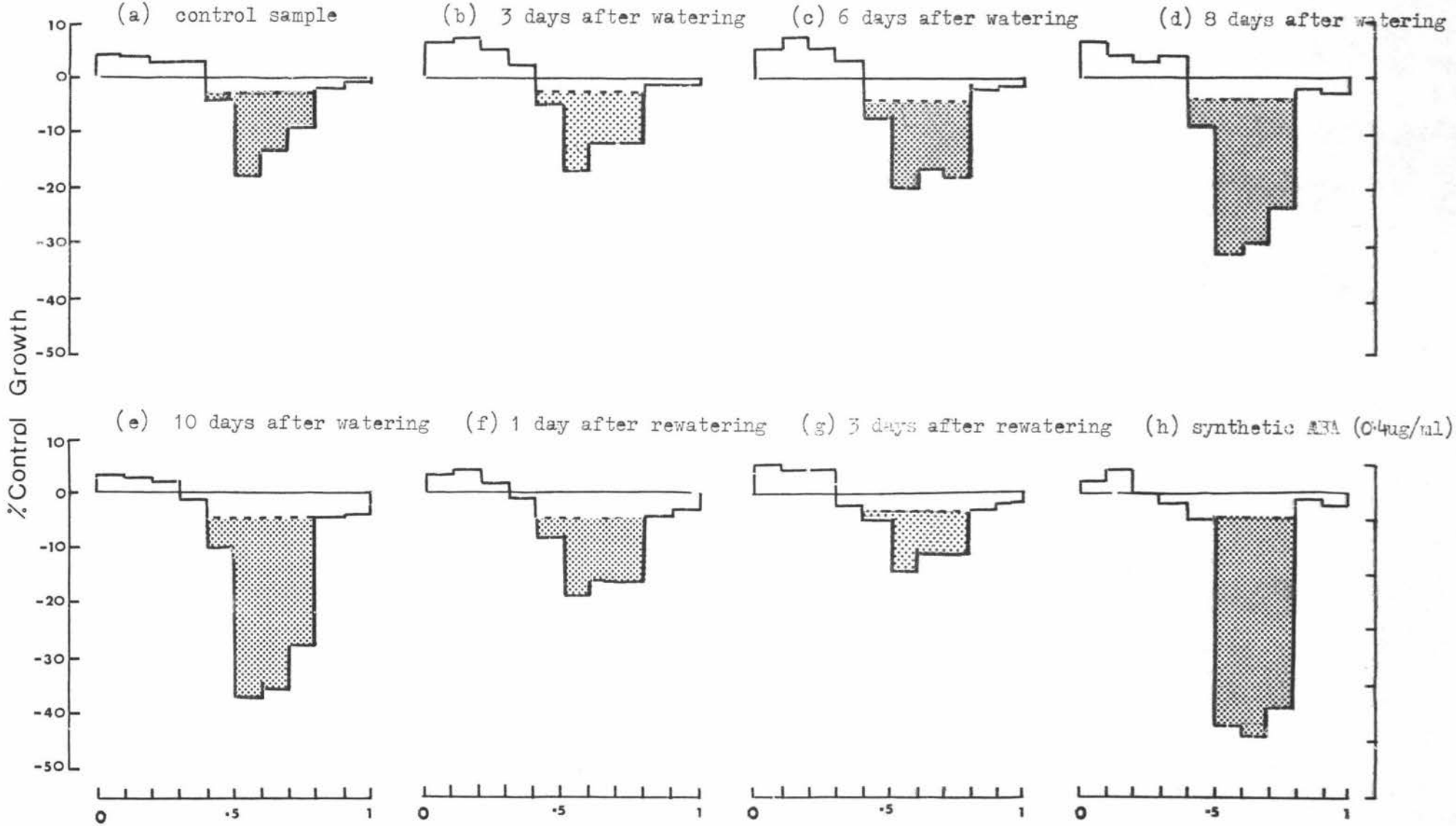


Fig. 15. Growth response of wheat coleoptiles to chromatograph sections of the acidic ether fractions from extracts of leaf of sorghum plants taken during late vegetative stage stress cycle. Shaded areas significant at P=0.01.

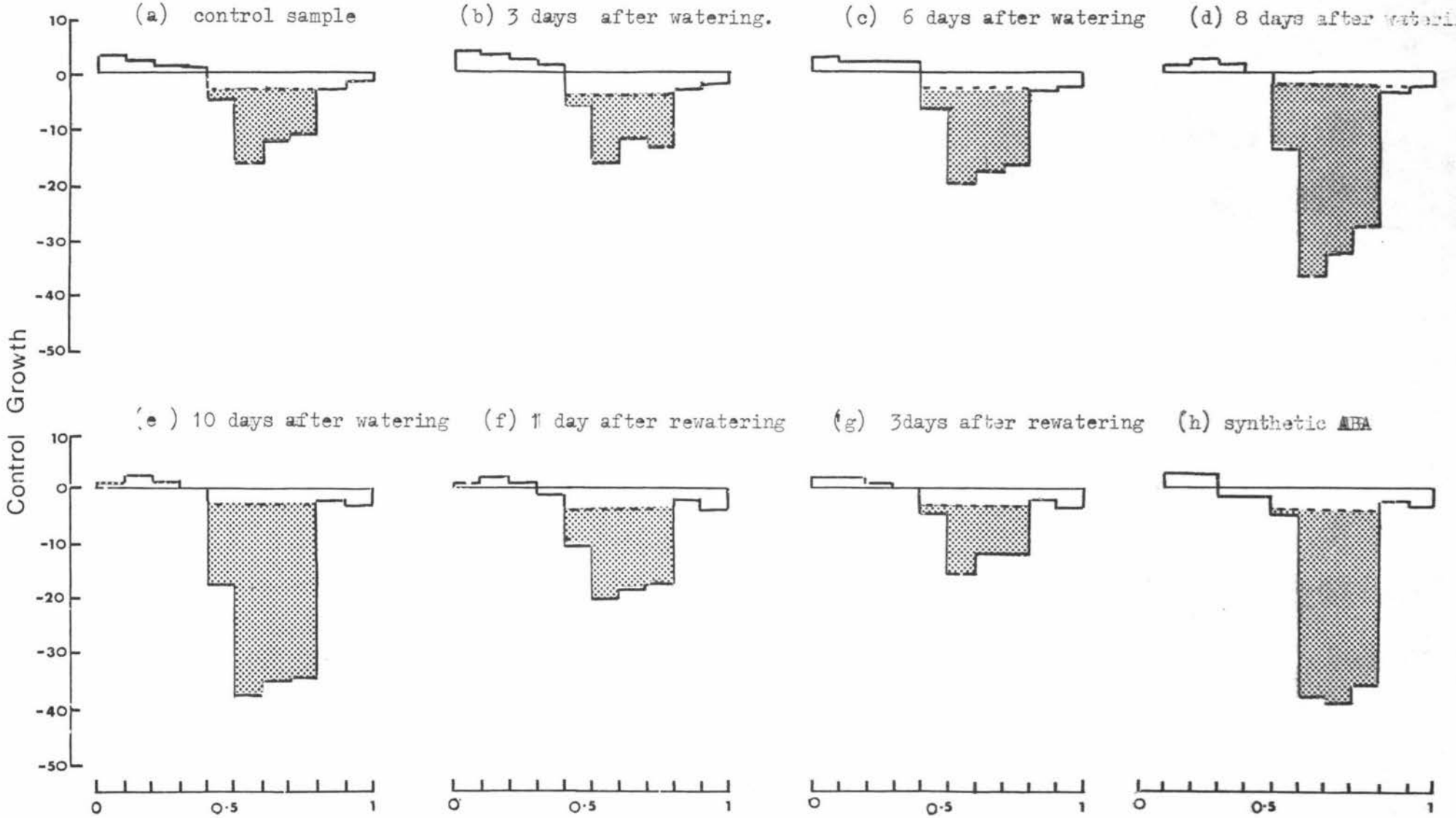


Fig. 16. Growth response of wheat coleoptiles to chromatograph sections ofthe acidic ether fractions from extracts of leaf of sorghum plants taken during the boot to bloom stage stress cycle. Shaded areas significant at P=0.01. R_f 0.5-0.8 indicate the inhibitor β fractions.

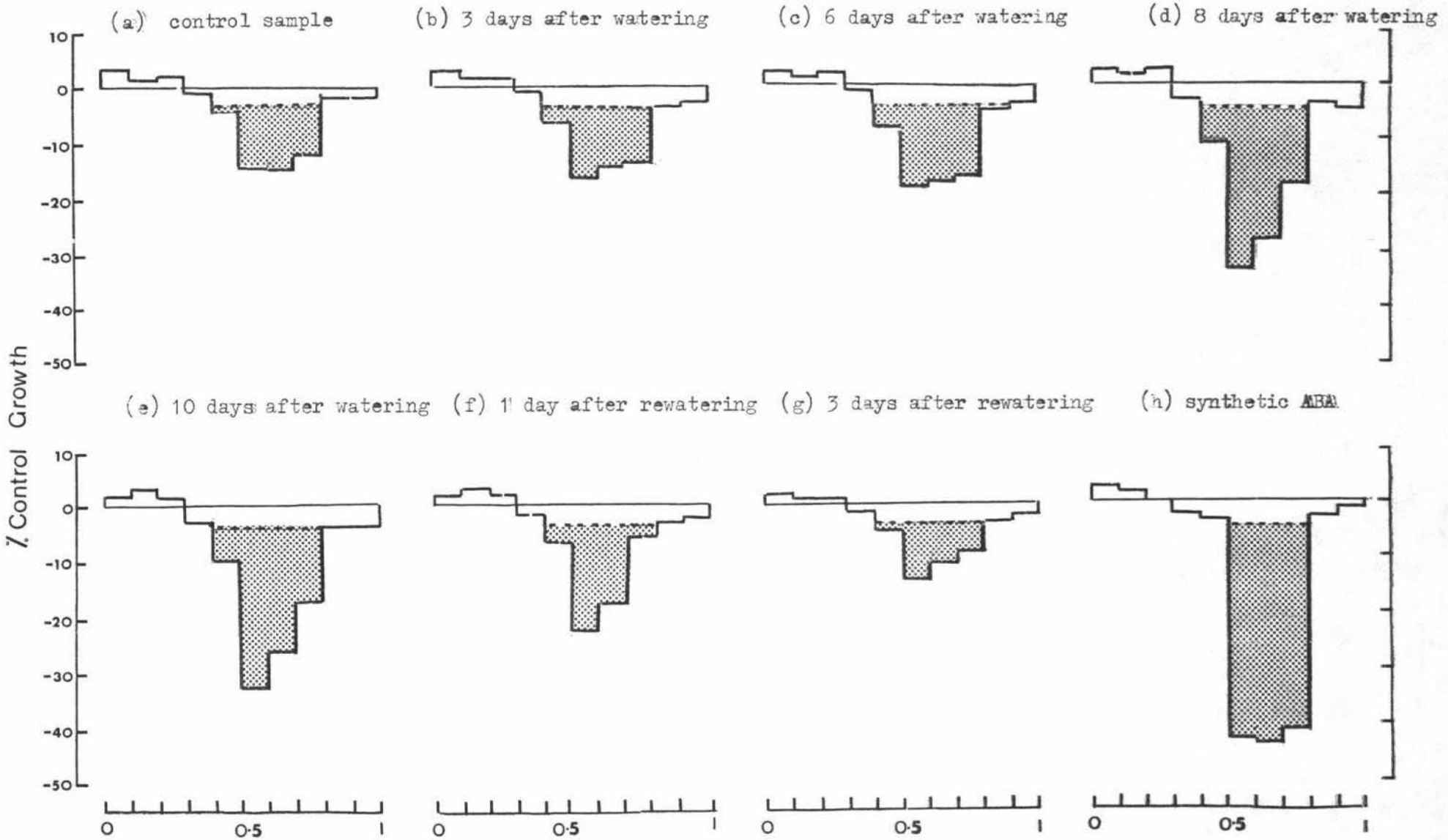


Fig. 17. Growth response of wheat coleoptiles to chromatograph sections of the acidic ether fractions from extracts of leaf of sorghum plants taken during dough stage stress cycle. Shaded areas significant at P=0.01.

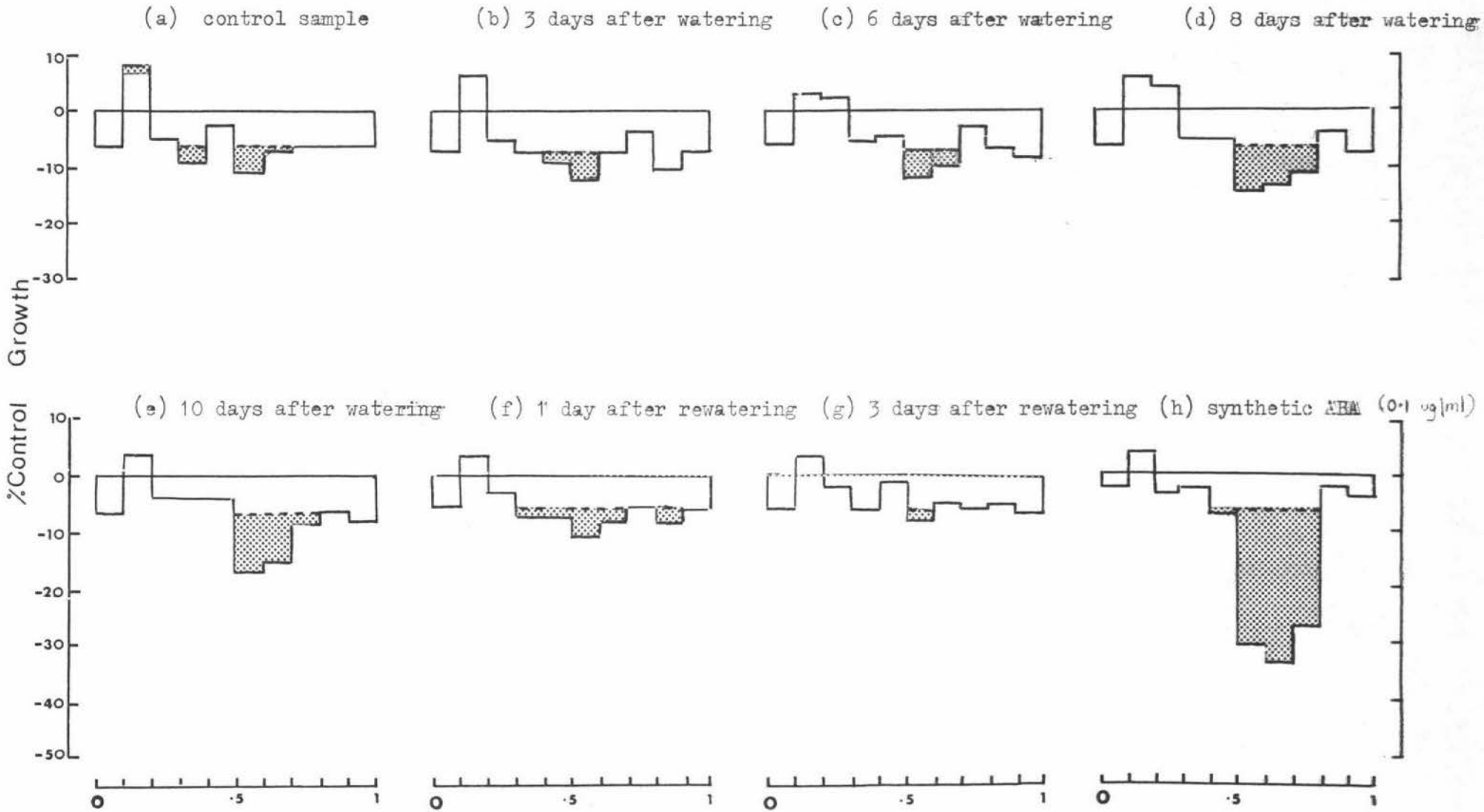


Fig. 18. Growth response of wheat coleoptiles to chromatograph sections of the acidic ether fractions from extracts of roots of sorghum plants taken during late vegetative stress cycle. Shaded areas significant at P=0.01.

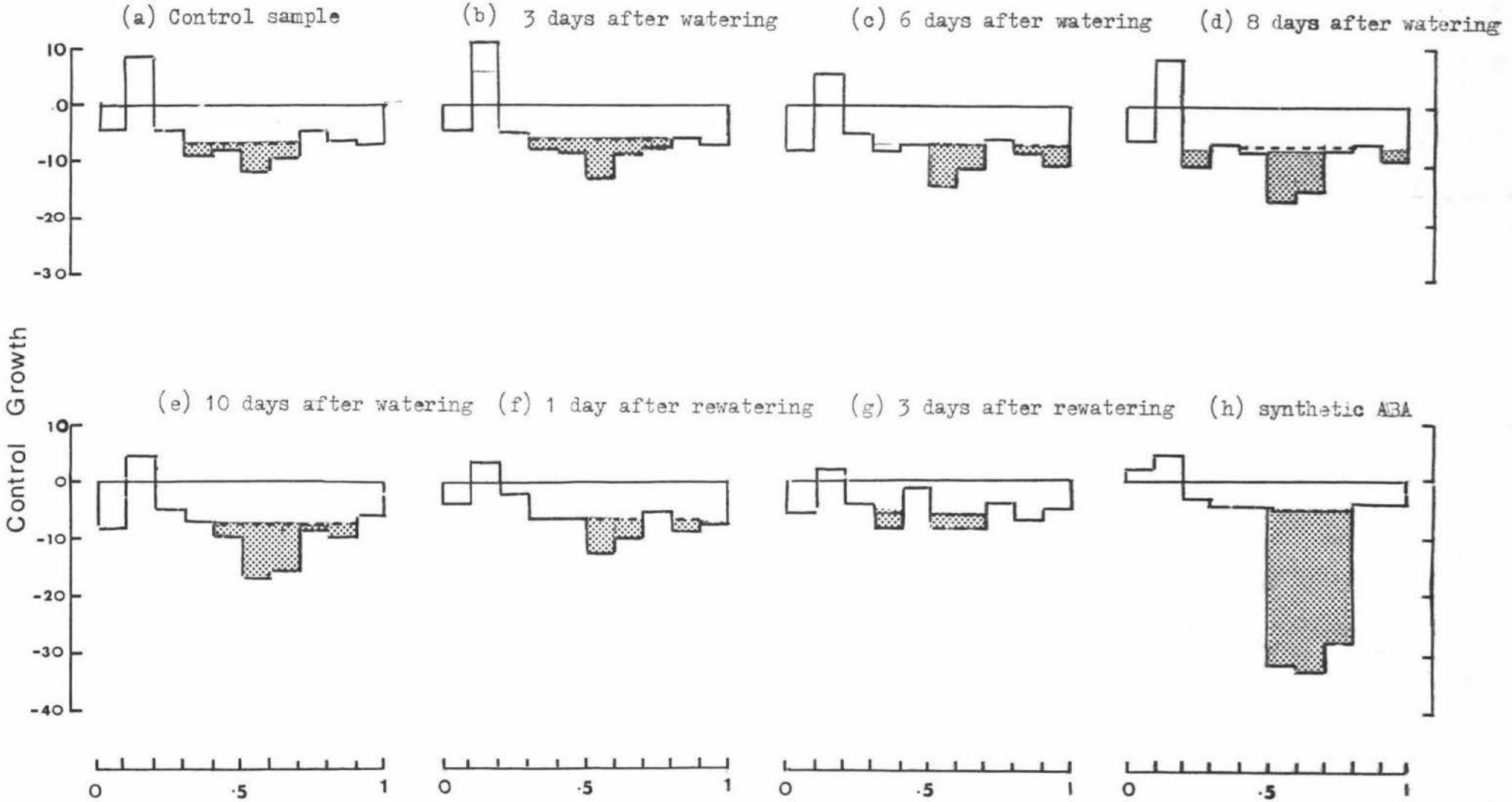


Fig. 19. Growth response of wheat coleoptiles to chromatograph sections of the acidic ether fractions from extracts of roots of sorghum plants taken during boot to bloom stress cycle. Shaded areassignificant at P=0.01

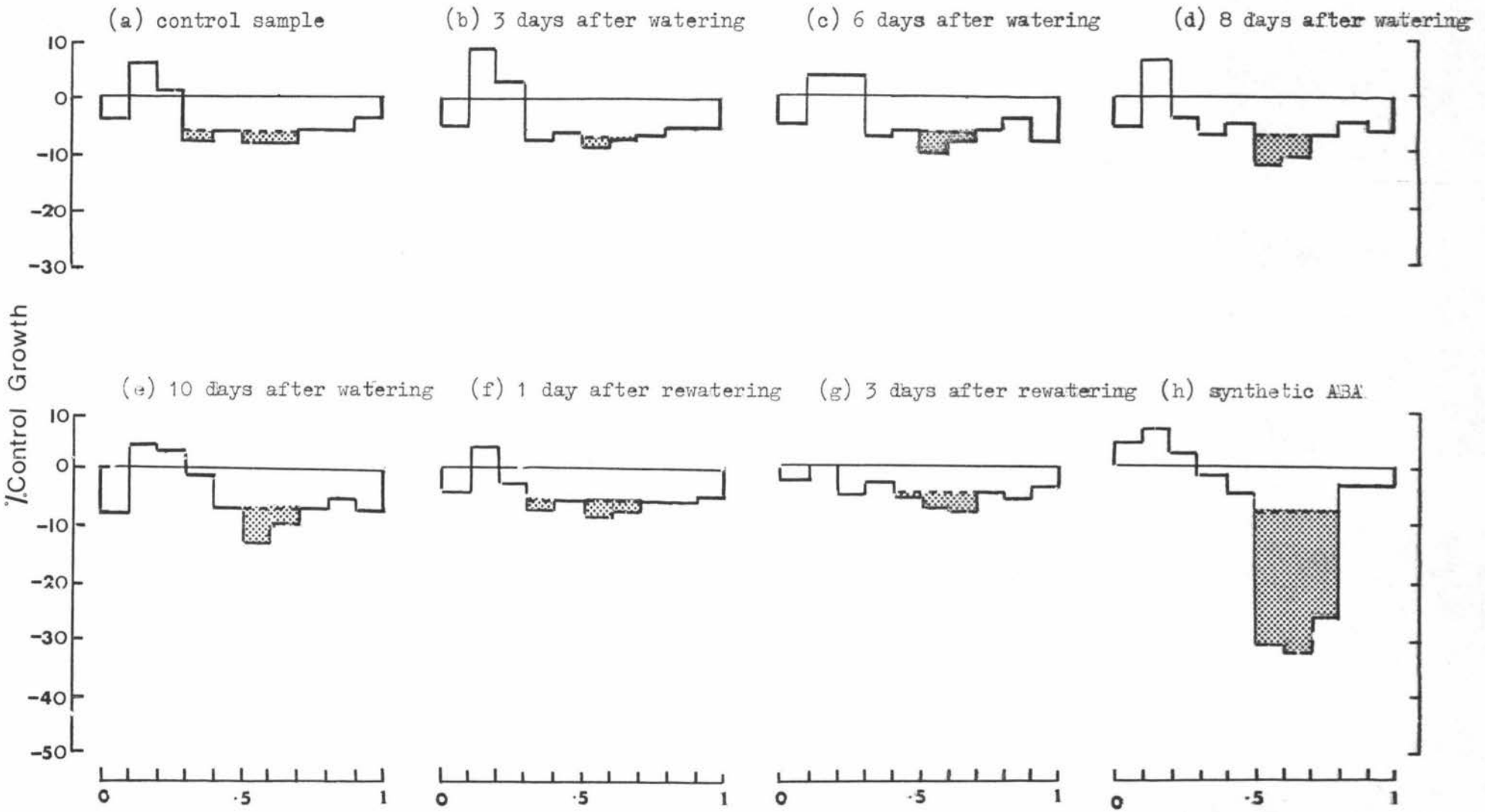
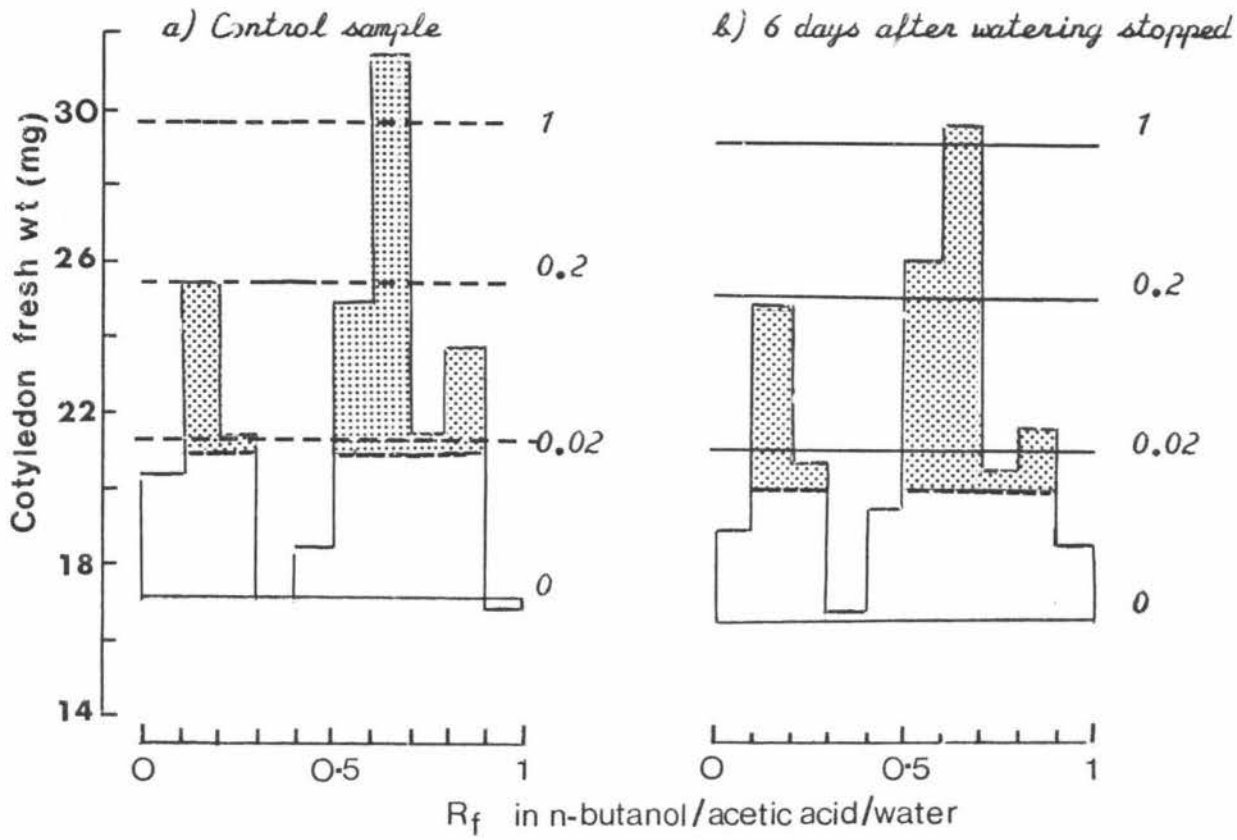


Fig. 20 Growth response of wheat coleoptiles to chromatograph sections of the acidic ether fractions from extracts of roots of sorghum plants taken during dough stage stress cycle. Shaded areas significant at P=0.01.



c) 10 days after watering stopped d) 3 days after rewatering

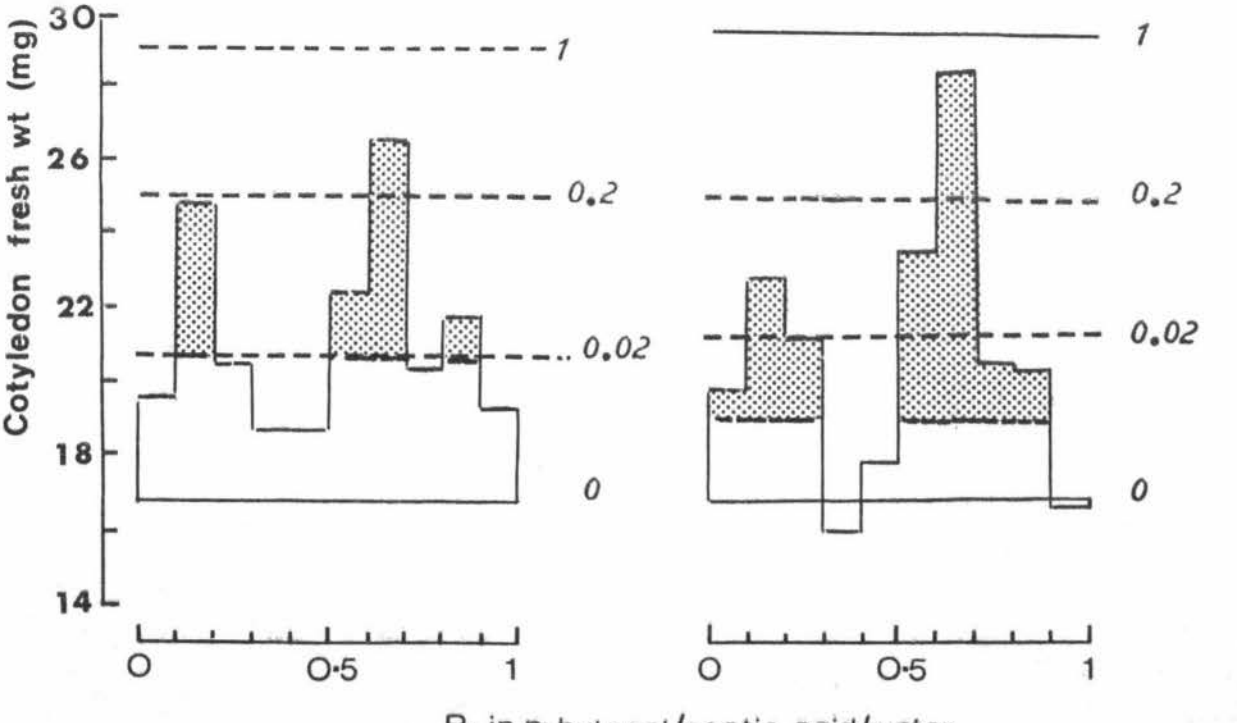


Fig. 21 Histograms of radish cotyledon bioassay of the butanol fractions from extracts of the root segments (0 to 100mm proximal to the root tips) of sorghum plants during late vegetative stage stress cycle. Shaded areas significant at P=0.01.

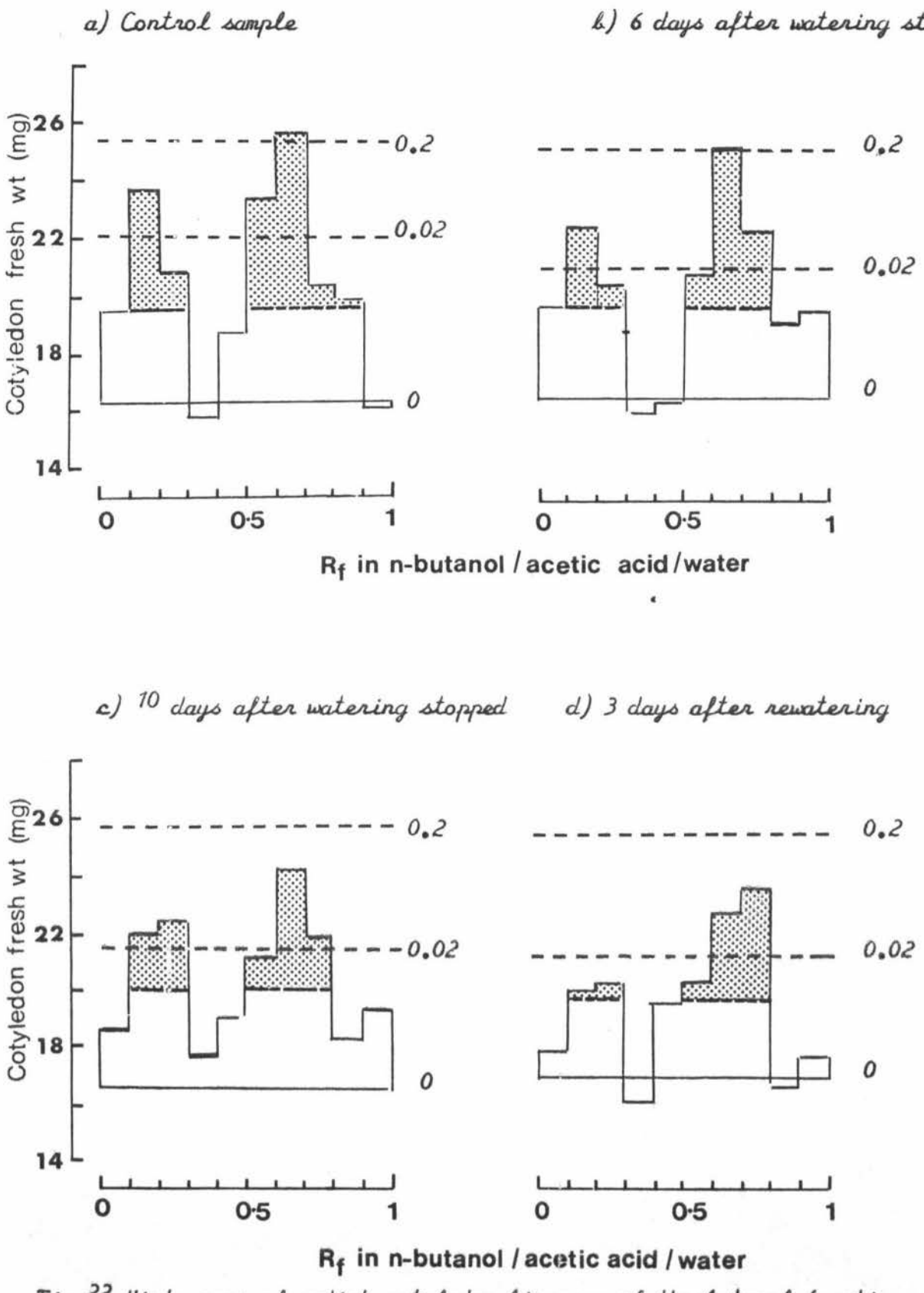


Fig.22 Histograms of radish cotyledon bioassay of the butanol fractions from extracts of the root segments (0 to 100mm proximal to the root tips) of sorghum plants during boot to bloom stage stress cycle. Shaded areas significant at P= .01. Horizontal lines show the response to kinetin standards (ug/ml).

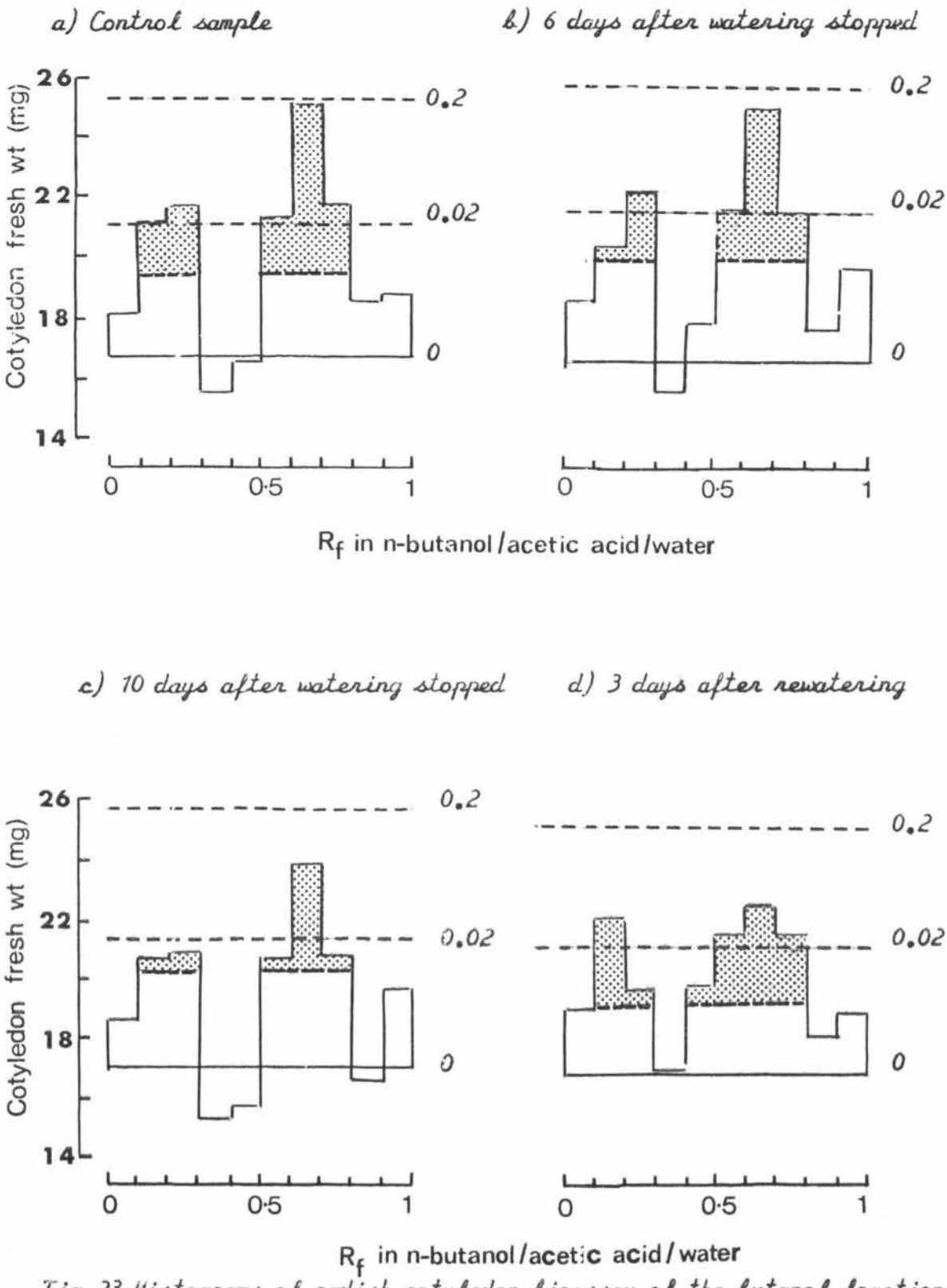
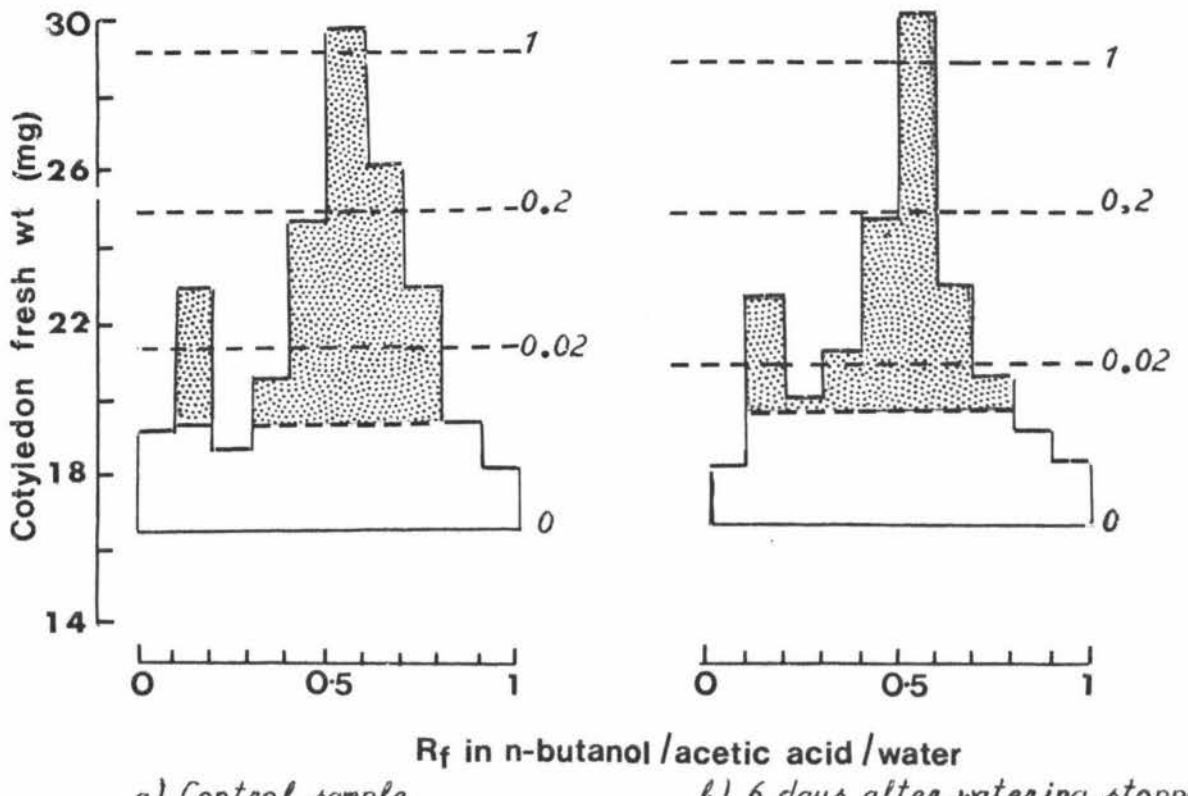


Fig. 23 Histograms of radish cotyledon bioassay of the butanol fractions from extracts of the root segments (0 to 100mm proximal to the root tips) of sorghum plants during dough stage stress cycle. Shaded areas significant at P=0.01. Horizontal lines show the response to kinetin standards (ug/ml).



a) Control sample

b) 6 days after watering stopped

c) 10 days after watering stopped

d) 3 days after rewatering

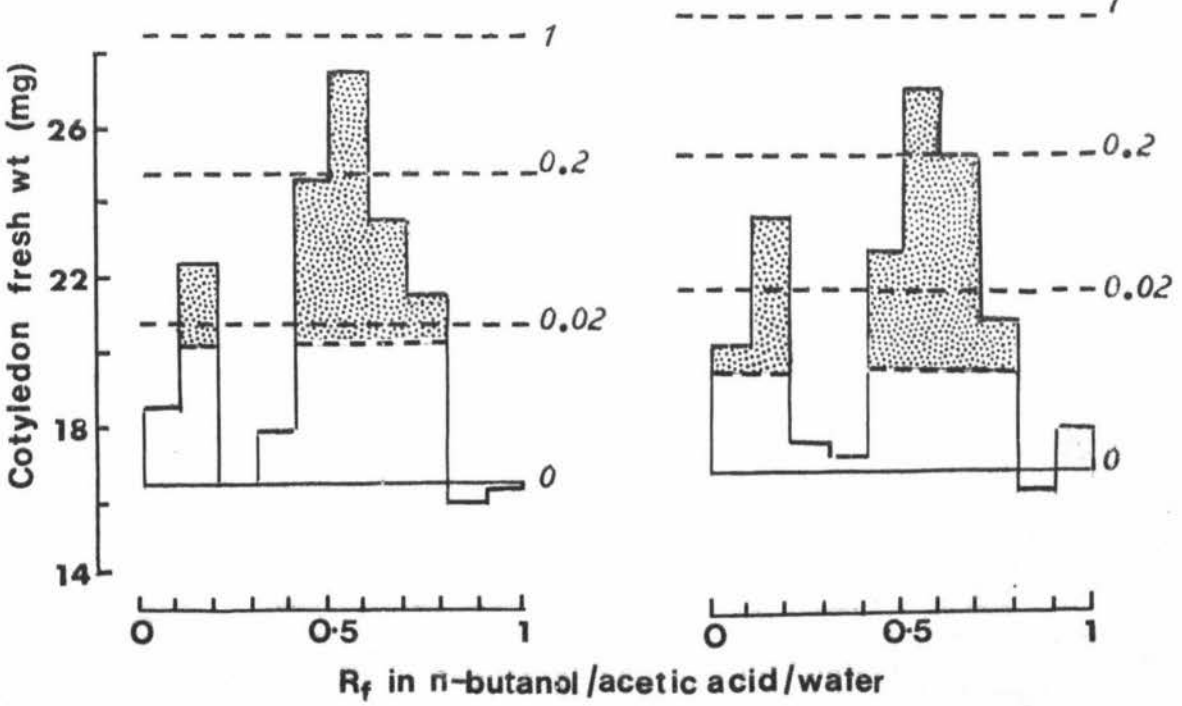
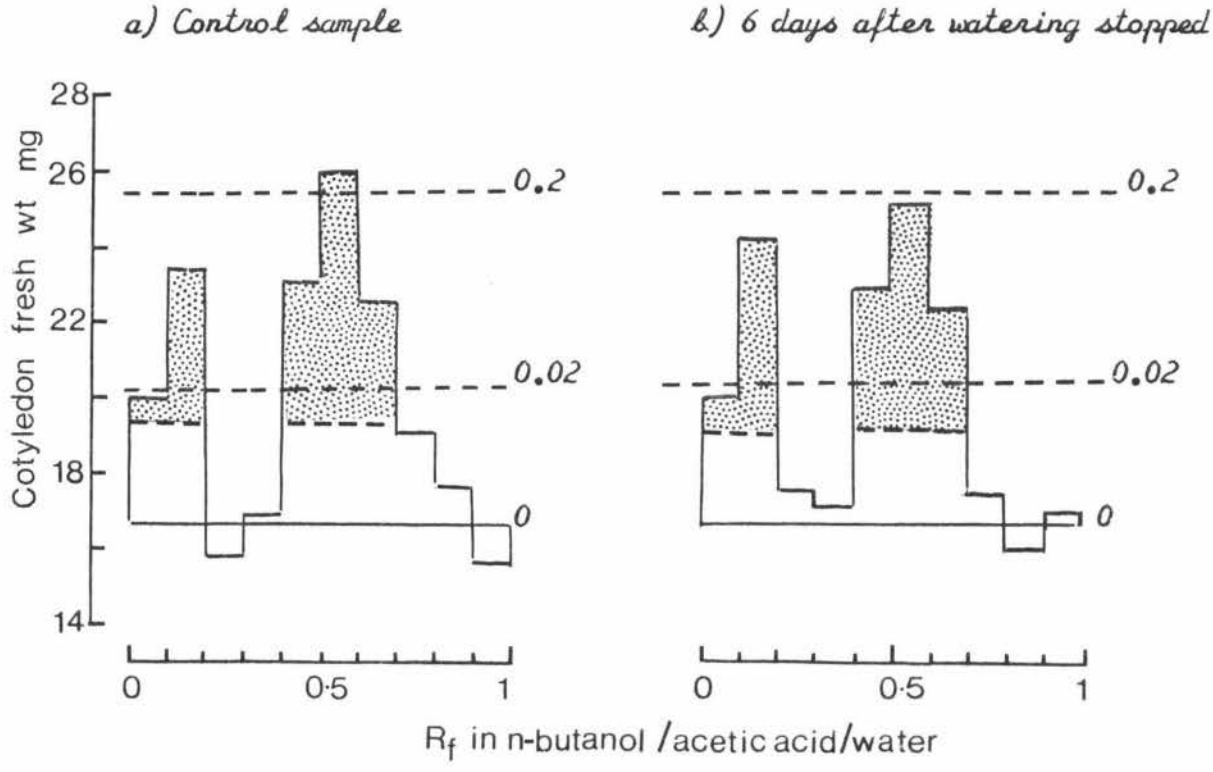


Fig. 24 Histograms of radish cotyledon bioassay of the butanol fractions from extracts of the leaves of sorghum plants during late vegetative stage stress cycle. Shaded areas significant at P=0.01 Horizontal lines show the response to kinetin standards (ug/ml)



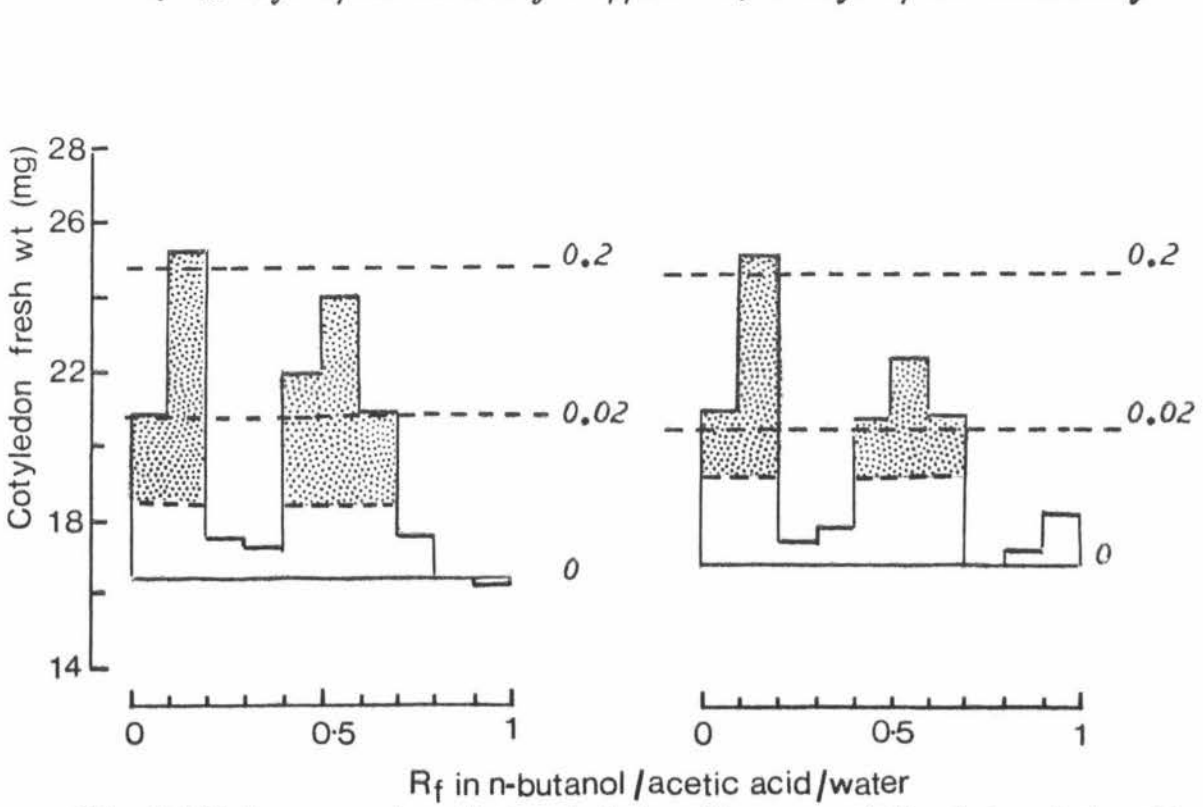


Fig. 25 Histograms of radish cotyledon bioassay of the butanol fractions from extracts of the leaves of sorghum plants during boot to bloom stage stress cycle. Shaded areas significant at P=0.01. Horizontal lines show the response to kinetin standards (ug/ml).

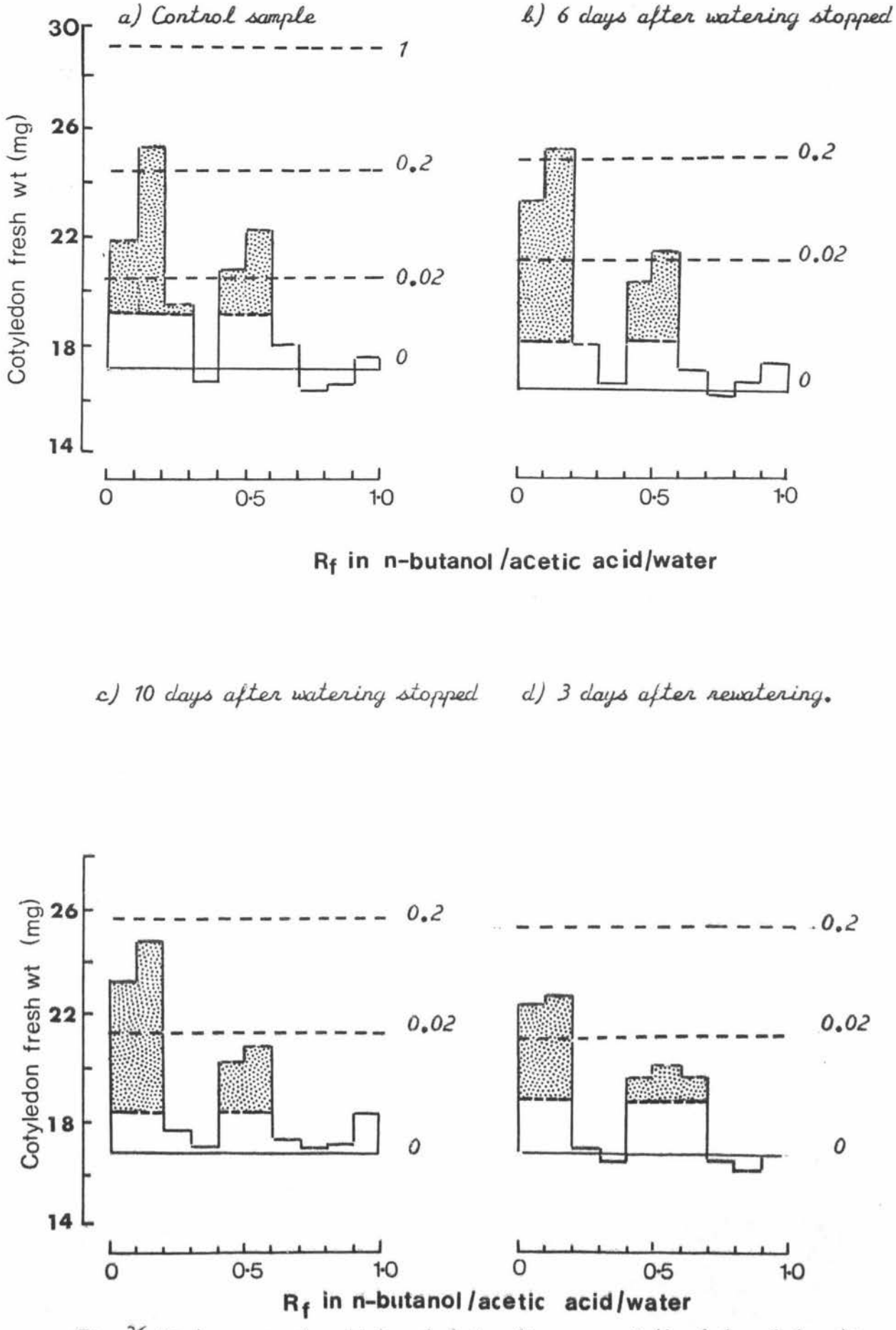


Fig.[26] Histograms of radish cotyledon bioassay of the butanol fractions from extracts of the leaves of sorghum plants during dough stage stress cycle. Shaded areas significant at P=0.01. Horizontal lines show the response to kinetin standards (ug/ml).

Plate 1. Stress treatments during late vegetative stage.

Plate 2. Stress treatments during boot to bloom stage.

Plate 3. Stress treatments during dough stage.

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