GROWTH SUBSTANCES AND TILLER DEVELOPMENT IN BARLEY

by

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I declare that the work described in this thesis has been carried out by myself, except where specific reference is made to other sources, and that it has not been submitted, in whole or in part, for any other degree.

Rosely de França Rocha

TABLE OF CONTENTS

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Abstract

The early growth of tiller buds in barley is determined by application of mineral nutrient solution to the growing medium. The aim of this research was to determine whether the effect of mineral nutrient supply is exerted through an effect on the levels of endogenous growth regulators, particularly cytokinins. Three approaches were used to examine the problem, namely to determine the effects of exogenously applied growth substances on growth of tiller buds, to observe bud growth under treatment expected to modify the levels of endogenous cytokinins, and to measure the cytokinin activity in plants supplied or not supplied with minerals.

Exogenously applied cytokinins not only increased lateral bud growth in plants supplied with minerals but also promoted growth of buds arrested by mineral nutrient stress. Application of auxin or gibberellin increased dry matter in buds released from apical dominance by either mineral nutrient or cytokinin application, but had no effect on arrested buds.

Root removal treatments, expected to modify levels of endogenous cytokinins, released growth of tiller buds under conditions of lack of mineral supply. Both root removal experiments and treatments with exogenous growth regulators confirmed the idea of an important role for growth substances, particularly cytokinins in the release of buds from apical dominance.

Preliminary results showed the presence of a high level of a cytokinin-like substance in plants given mineral nutrient solution, which was absent in the deficient condition, also

favouring the initial idea.

It is proposed that growth of axillary buds in vegetative barley plants is controlled by interactions between nutritional factors and endogenous growth substances. It is considered that mineral nutrient availability determines the levels of endogenous cytokinin which in turn determines whether or not buds are released. Mineral nutrients, other metabolites and growth substances are important in controlling subsequent growth of the tiller bud.

CHAPTER I

INTRODUCTION

1. Some aspects of the vegetative development in grasses, particularly barley (c.v. Proctor)

The aerial part of most vegetative grasses includes besides the main stem, lateral shoots, the number of which is dependent upon genotypic characteristics and environmental conditions. Main stem and tillers are not always easily distinguished from each other, being of similar structure and often of similar size. Each shoot is made up of a number of foliar organs composed of a distal part, the leaf lamina, and a proximal part, the leaf sheath. The leaf sheath of the older encircles the leaf sheath of the younger leaves, and from inside this structure appear the new leaves, which are initiated at the stem apex. The internodes usually elongate little during the vegetative phase of growth. Tillers arise as lateral buds in the axil of the leaf sheath; the tillers on the main shoot, primary order tillers, may themselves produce secondary and higher order tillers, giving rise to the tufted habit of grasses.

The caryopsis, the fruit of grasses is composed of the embryo, endosperm, scutellum, and remains of the testa and pericarp, usually refered to as the grain coat or husk. The external sign of germination is the appearance of the radicle which bursts through the coleorhiza. Following this, two pairs of lateral roots appear, while at the other end of the embryonic axis the coleoptile with the enclosed first leaf also emerges (Ballard, 1964; Langer, 1972).

A grass embryo possesses the primordia of the first two or more leaves, and of the seminal root axes, and has a well developed provascular system (Barnard, 1964). After 24 hours of imbibition the

embryo of Proctor barley carries the primordia of the coleoptile and of the first four mainstem leaves (Dale, Felippe and Fletcher, 1972). By this time the bud of coleoptile tiller, refered to as Tc (Jewiss, 1972; Kirby and Faris, 1972), is already clearly present in the axil of the coleoptile, and shows the prophyll primordium. As very little visible development of the mainstem apex occurs up to 24 hours from planting, it can be assumed that the presence of this well developed bud is an indication of its presence in the dry grain (Fletcher and Dale, 1974). In most plants examined by Fletcher and Dale the first leaf tiller bud, refered to as T1, also appeared to be present by 24 hours after planting but as a rounded mass of cells in the axil of the first and at the base of the second leaf; at 48 hours T1 was invariably recognizable but the prophyll primordium was not visible until 72 hours from planting.

After germination the mainstem apex continues its development. Leaf primordia are formed with a plastochron of slightly less than 3 days; by day 14 about 10 leaf primordia are initiated the last being the primordium of the flag leaf, and the double ridge stage is reached by day 15 - 17, when the mainstem apex starts the phase of elongation (Dale et al., 1972). Tiller bud primordia are initiated with a plastochron of rather more than 4 days, so that T2 and T3 appear at about day 6 and 10 - 11 respectively (Fletcher and Dale, 1974).

The number of primary tillers which can be produced is limited by the number of leaves on the mainstem and by the onset of stem elongation. Dale et al. (1972) observed the formation of 5 or 6 primary tillers in Proctor. Secondary order tillers are also formed, the number being probably dependent on the nitrogen regime under which the plant is grown (Fletcher and Dale, 1974).

The growth of a barley seedling is dependent initially upon endosperm reserves (Metivier and Dale, 1977) but by day 8 the first leaf becomes maximally active in photosynthesis (Dale, 1972). Between days 8 and 11 the plant is totally dependent on photosynthesis in the first leaf for carbon assimilation (Dale and Felippe, 1972), for although the second leaf emerges around day 8 (Fletcher, 1975) it does not contribute significantly to plant dry matter until about day 12 (Dale and Felippe, 1972).

Fletcher and Dale (1974) observed that initially there are no vascular connections to the tiller bud but they believe that at this time there is a nutritional association between bud and its subtending leaf primordium. By the time the bud starts the phase of exponential growth it is connected by vascular strands to the leaf above its subtending leaf. The phase of exponential growth ends with the emergence above its subtending leaf sheath; growth rate then slows down. The final stage of tiller development occurs when it produces adventitious roots and becomes more or less independent from the mainstem (Fletcher, 1975).

2. Apical dominance in grasses

Plant physiologists have been trying for nearly a century to understand the mechanism by which the apical bud meristem is usually dominant to some degree over the axillary bud meristems. Since apical dominance is a general phenomenon in plants its control is expected to have a common base, but, although intensive investigations have been made, so far there is no clear cut general explanation for this phenomenon. In most examples studied the control of apical dominance seems to involve either nutritional or hormonal mechanisms, or even both,

but the way nutrients or hormones exert their control seems to vary with the species and the developmental stage of the plant.

Studies on apical dominance have been done using a number of techniques which include some degree of surgery, application of exogenous growth substances, studies on translocation of radioactive growth substances, mineral nutrients or assimilates, and extraction and measurement of endogenous growth substances. Much of the work done using the first two techniques cited above has been strongly criticized by Tucker and Mansfield (1973), on the grounds that those treatments can bring about changes in level of endogenous growth substances. These workers studied the hormonal control of apical dominance in Xanthium by changing slightly the quality of the light in which plants were grown and correlating changes in the endogenous levels of growth substances with the degree of branching (Tucker and Mansfield, 1972; 1973). However change in light quality may also produce results difficult to analyze correctly since it can affect the biosynthesis or release from bound forms of growth substances such as gibberellin (Reid, Clements and Carr, 1968; Loveys and Wareing, 1971), cytokinin (Van Staden and Wareing, 1972), or auxin (Fletcher and Zalik, 1964), which may also be involved in control of apical dominance. Thus the methods available for the study of apical dominance can be criticised, although they certainly can be useful if one "does not interpret complex phenomena in terms of the limited experimental system, but rather uses the system as a probe of these complexities" (Hall, 1973).

Apical dominance has been little studied in grasses probably because of the practical difficulties that these plants offer to the application of methods such as decapitation or local application

of growth substances, which have been largely used for dicotyledons; also the observation of bud growth can not be made by external observation, at least in its initial phase. Most of the work on tillering has therefore been done with the agricultural purpose of improving yield and has resulted in detailed studies of the appearance of tillers above their subtending leaf sheath; little work has been done on the stages of growth between initiation and emergence.

The hypotheses proposed to explain how an apical bud inhibits the growth of the axillary ones have arisen from experiments with dicotyledons but there is no reason to believe that they would not be applicable to monocotyledons.

(a) The main hypotheses of apical dominance.

The nutritional hypothesis

The nutritional hypothesis of apical dominance favours the idea that competion for nutrients between apical and lateral bud meristems is the factor which determines apical dominance. Since the apical meristem is present in the embryo and proceeds to grow following germination, it constitutes a metabolic sink to which nutrients move, and being the first it continues to command the supply of nutrients to the detriment of the later formed lateral buds (Phillips, 1969).

This hypothesis is supported by work in which increase in mineral nutrient availability leads to an increase in lateral bud growth (Gregory and Veale, 1957; McIntyre, 1964; 1968; Fletcher and Dale, 1974).

The hormonal hypothesis

Thimann and Skoog (1933; 1934) observed that replacement of the apical bud of Vicia faba by an agar block containing indole-3-acetic acid (IAA) resulted in maintenance of lateral bud inhibition. Other later observations favour a central role for auxin in lateral bud

inhibition, and Thimann (1937) proposed that lateral buds are inhibited by concentrations of auxin which would be optimal for the elongation of the mainstem, and that auxin diffusing from the apical bud enters lateral buds where it inhibits growth.

In a series of ingenious experiments Snow found that the factor responsible for bud inhibition could move to lateral buds through routes which were not accessible to auxin, and proposed that auxin from the dominant bud acts indirectly on the laterals by stimulating the formation of a growth inhibitor in the mainstem tissue which moves into lateral buds or shoots inhibiting their growth (Phillips, 1969). Support for the idea of lateral bud inhibition being due to the presence of a growth inhibitor in the bud comes from the work of Tucker and Mansfield (1972; 1973) which showed the presence of high levels of absci sic acid (ABA) within inhibited lateral buds of Xanthium strumarium and also showed that this level is decreased after apical dominance was removed.

The role of cytokinin

The first indication of a role of cytokinin in the apical dominance phenomena was given by the work of Wickson and Thimann (1958) with nodal stem segments of Pisum sativum. These workers observed that kinetin counteracted the lateral bud inhibition caused by IAA. Cytokinin treatment also released buds from correlative inhibition in intact plants, when applied directly to the buds themselves; this was observed not only in Pisum but also in Coleus, Scabiosa and Helianthus (Sachs and Thimann, 1964), Vicia (Panigrahi and Audus, 1966), Lycopersicum (Catalano and Hill, 1969) and Glycine (Ali and Fletcher, 1971). Cytokinin application also causes the conversion of horizontal stolons of Solanum andigena into orthotropic leafy shoots (Woolley and Wareing, 1972c); this transition normally occurs when apical dominance is removed.

Another indication of the involvement of cytokinins in the growth of lateral buds was given by the demonstration that levels of endogenous cytokinins increased in inhibited lateral buds of Xanthium strumarium and decreased following their release (Tucker and Mansfield, 1972; 1973).

Sachs and Thimann (1967) suggested that lateral buds may be inhibited because they are unable to synthesize cytokinins, and that removal of the correlative inhibition results in rapid acquisition of this ability by the bud. However there is evidence to show that cytokinins originating from roots may also be involved in apical dominance (Woolley and Wareing, 1972a,b,c).

Although the fundamental role played by cytokinin as growth regulator in apical dominance has been clearly indicated, application of this substance in intact plants results in only a transitory growth of the released bud. Subsequent development of cytokinin-treated buds is allowed to proceed by application of either IAA or gibberellic acid (GA_3) (Sachs and Thimann, 1967; Catalano and Hill, 1969; Ali and Fletcher, 1971). It seems therefore that in inhibited lateral buds the auxin levels are not supra-optimal, as proposed by Thimann (1937). The inhibited bud may lack the capacity for auxin and gibberellin synthesis, which appears at some stage after release by decapitation; the reason why buds released from inhibition by cytokinin treatment fail to reach self-sufficiency in auxin and gibberellin is still unknown (Phillips, 1975).

The role of gibberellin

Published results on the role of gibberellins in apical dominance show that they can either increase or reduce apical dominance. In general the application of exogenous GA_3 to intact plants results in promotion of mainstem elongation with a simultaneous inhibition of

lateral shoots or buds (Pharis, Ruddat, Phillips and Heftmann, 1965; Pharis, Kuo and Glenn, 1972). Where apical dominance has been abolished the effect of GA_3 is to promote axillary bud growth (Sachs and Thimann, 1964; Panigrahi and Audus, 1966; Phillips, 1971), which represents the usual response of a growing shoot to exogenous gibberellin.

Interactions of different factors

Although workers studying the control of axillary bud growth usually emphasize only one aspect of the problem, i.e. the role of nutrients or the participation of growth substances, in the intact plant the action of these two factors is not independent.

Went observed this interrelation as early as 1939 and suggested that a high auxin concentration in the apical bud induced a flow of nutrients towards the shoot tip. More recently the accumulation of radioactive nutrients has been indicated in auxin-treated stumps (Booth, Moorby, Davies, Jones and Wareing, 1962; Morris and Thomas, 1968). What is not clear is whether auxin exerts this effect by creating a metabolic sink at the point where it is present in high concentration or where it is applied, or if it is the polar movement of auxin in the stem that determines this control (Guern and Usciati, 1972). It is also not clear whether there is a relation between the auxin-directed nutrient transport and the inhibition of lateral buds, since Šebánek (1967) showed that diversion of ^{32}P-metabolites to the IAA treated upper part of decapitated Pisum epicotyls occured independently of the concentration of IAA applied but that only the higher concentration inhibited while the lower promoted lateral bud growth.

Morris and Winfield (1972) suggested that IAA synthesized by the shoot apex or applied to the cut surface of decapitated plants may inhibit axillary bud growth by polarizing the transport of cytokinins

towards that region since they observed in Pisum that ^{14}C kinetin or a possible metabolite was accumulated in the apical bud or the region of IAA treatment after labelled kinetin was applied through the roots or to the cut surface of the decapitated plant.

There are indications that in some plants the diversion of nutrients towards the apical bud, to the detriment of the axillary buds, may be due to absence or limited efficiency of the transport systems between the stem and these axillary buds. IAA from the mainstem apex or applied to decapitated plants seems to prevent the differentiation of vascular connections between lateral buds and stem (Gregory and Veale, 1957; Sachs, 1969; 1970) preventing the flux of nutrients into the buds and therefore inhibiting their growth.

The effect of nutrients in apical dominance could also operate through an effect on the level of endogenous growth substances. It has been shown that inorganic nutrient deficiency can both increase the strength of apical dominance (Gregory and Veale, 1957; Fletcher and Dale, 1974; McIntyre, 1977) and depress levels of endogenous cytokinin (Wagner and Michael, 1971; Woolley and Wareing, 1972b; Menary and Van Staden, 1976).

(b) Factors which affect tillering.

Tiller primordia are initiated at the shoot apex with a longer plastochron than that for the subtending leaf primordia (Bunting and Drennan, 1966). The initiation of the tiller primordium is little affected (Evans, Wardlaw and Williams, 1964) but in contrast the further development is highly dependent on the environmental conditions (Mitchell, 1953b).

Mineral nutrients

Tillering, i.e. the production of externally visible tillers, is greatly affected by the supply of mineral nutrients. Raising the supply of the major elements nitrogen, phosphorus and potassium increases the production of tillers (Langer, 1966), and magnesium and calcium status may also affect tillering (Evans et al., 1964). Demonstration of the importance of mineral supply to the control of tillering in barley was given by Aspinall (1961). He showed that minerals supplied regularly not only increased the total number of tillers produced, but also reduced the duration of the phase found during rapid stem elongation when tillering is usually repressed. Increased rates of tillering were also shown for barley by nitrogen application at the time of ear emergence (Thorne, 1962a).

Fletcher (1975) observed in Proctor barley that the failure of tillers to emerge from their subtending leaves in the absence of mineral nutrient supply was due to a block in growth between the post-initiation stage and the onset of exponential growth, allowing only a very slow growth of the buds. He showed that the beginning of the phase of exponential growth of the bud is determined by the application of minerals and in particular nitrogen and phosphorus. Comparing tiller bud growth with the growth of the whole plant, Fletcher concluded that when the supply of minerals to young barley seedlings was reduced, tiller growth was affected to a greater extent than that of the rest of the plant, and therefore that under these conditions dominance of the mainstem apex over the axillary buds was increased.

Leaf shading, light intensity and photoperiod

Shading the first leaf of young barley seedlings reduces tillering

(Dale, Felippe and Fletcher, 1972) by delaying the onset of the phase of rapid growth of tiller buds until the second leaf becomes an organ exporting assimilated carbon (Fletcher and Dale, 1974). Fletcher (1975) suggested that the effect of shade treatment on tiller growth could be a direct one, by reducing the availability of assimilates but it could also be indirect, acting either through a reduction of root growth and consequently in uptake of minerals, or by limiting nitrate reductase activity (Dale, Felippe and Marriott, 1974) which could result in inadequate amounts of organic nitrogen available to support tiller growth.

In contrast to shade effects, high light intensities have been shown to favour tillering in many grasses (Evans et al., 1964). Barley plants growing under low light intensity develop only the buds of primary tillers whereas at higher light intensity the secondary and higher order tillers are also produced (Aspinall and Paleg, 1964).

Although daylength has been considered to affect tillering (Evans et al., 1964) this effect seems to vary from one species to another. The reason for this is that photoperiod is related to flower induction which, in grasses, leads to a phase in which the mainstem elongates and tillering is restricted. Despite this problem, it has been suggested that, in general, tillering is less in shorter photoperiods (Evans et al., 1964) but the reason for this may be the decrease in total incident radiation, rather than in the photoperiod itself (Aspinall and Paleg, 1964).

Temperature and water

For tillering it seems that the optimum temperature range varies from one species to another (Evans et al., 1964). Variation in temperature affects leaf as well as tiller production (Langer, 1972), so

that the analysis of effects on tillering alone is not simple. In ryegrass (Mitchell, 1953a,b), wheat (Friend, 1965) and barley (Cannel, 1969) a decrease in tillering has been found with an increase in temperature. The mechanism of this control is unknown.

Little information is available on the effect of water supply on tillering, but water stress seems to reduce the amount and rate of tillering (Langer, 1972).

Most of our knowledge of factors affecting tillering comes from work more concerned with the production of tillers which grow to maturity than with the apical dominance problem. The observations were therefore made mainly at a fairly advanced stage of tiller development, after the emergence from the subtending leaf sheath, and very little is known about the effect of those factors on the release of tiller buds from apical dominance and the initial phase of growth within the leaf sheath.

Despite this, those observations suggest that tiller growth in grasses is a phenomenon which may be controlled in a very similar way as that of axillary buds of dicotyledons. The important role of nutrients in apical dominance in grasses is suggested by the effects of mineral supply, light and leaf shading on tillering. The participation of growth substances in the process is suggested by the reduction of tillering after flower induction, by the increase in tillering at high levels of minerals, which are known to increase levels of endogenous cytokinins in other species (Wagner and Michael, 1969; 1971; Woolley and Wareing, 1972b; Menary and Van Staden, 1976), and by the reduction of tillering in water stress, which is known to reduce levels of endogenous cytokinins in Helianthus and Nicotiana (Itai and

Vaadia, 1965; 1971) and to increase levels of abscisic acid (Wright and Hiron, 1972).

(c) The hormonal control of tillering.

As early as 1949, Leopold showed the involvement of growth substances in apical dominance in barley. He was able to induce tiller bud growth by destruction of the apex, and to impose the inhibition by application of the auxin α-naphthaleneacetic acid (NAA). He also showed that spraying the inhibitor of auxin transport triiodobenzoic acid (TIBA) stimulated tillering and suggested that apex-produced auxins were the cause of lateral bud inhibition and that blocking basipetal movement of auxin released the buds from inhibition. Although Leopold's results are in agreement with what has been found for dicotyledons, it is difficult to understand how a barley apex can be removed without causing extensive damage to the plant.

Neither Thorne (1962b) nor Aspinall (1963) were able to confirm the inhibitory effect of auxin on tillering in barley, but recently it has been shown that IAA applied either as foliar spray or through the roots reduces tillering and tiller bud extension in wheat, oats and barley (Johnston and Jeffcoat, 1977). As IAA application also promoted growth of lateral roots, Johnston and Jeffcoat explained the inhibition of tiller buds by a diversion of assimilates away from the buds to the developing roots.

The promoting effect of TIBA on tillering was confirmed by Jewiss (1972) and Langer, Prasad and Laude (1973). Jewiss (1972) showed that the inhibition of tillering after flower initiation in Lolium temulentum was overcome by TIBA treatment, and that in wheat the derepression of tillers T3 and T4, repressed by flowering, seems to be achieved by an effect of TIBA on the movement of assimilates. Langer et al.

(1973) found that the effect of TIBA was dependent upon the stage of plant development; its effect was to increase bud elongation when applied to wheat plants early after flower induction, but treatment did not increase bud growth when the inflorescence was emerging and lateral buds growing more actively. They suggested that auxin is the inhibitory factor acting following flower induction and that the subsequent release from inhibition after ear emergence may not be related to auxin.

Gibberellin is another growth regulator which may be involved in the tillering process. In Phalaris tuberosa (Jewiss, 1972), wheat, oats and barley (Johnson and Jeffcoat, 1977) exogenous GA_3 increased stem extension and reduced tillering. 2-chloroethyltrimethylammonium chloride (CCC) had the oposite effect in barley, spring wheat (Wünsche, 1973) and Lolium temulentum (Jewiss, 1972). Jewiss's work showed that the inhibition of tillering after flower induction was more related to stem extension than to inflorescence development. It has been suggested that when assimilates are diverted to the stem, they are rendered unavailable for tiller growth (Johnston and Jeffcoat, 1977). While tillering has been shown to be restricted by treatment with GA_3, the extension of growing tillers is actually promoted by GA_3 (Jewiss, 1972), although this effect was not detected by Johnston and Jeffcoat (1977).

The involvement of cytokinins in apical dominance in grasses was shown by the stimulation of bud growth after local application of kinetin (Jewiss, 1972). Local application of benzylaminopurine (BAP) stimulated bud growth and ^{14}C accumulation in tiller buds of Lolium multiflorum (Clifford and Langer, 1975). Not only local applications of cytokinins are effective in inducing tiller growth, but foliar spray and root applications of BAP and 6-benzylamino-9-tetrahydropyranylpurine

(PBA) also increased tillering and bud elongation in wheat and oats (Johnston and Jeffcoat, 1977).

Langer et al. (1973) considered the existence of two phases between the appearance of double ridges on the mainstem apex and ear emergence in wheat. These are the stages when apical dominance is enhanced during the initiation of flower organs, and that when it is diminished, with consequent release of buds, during ear emergence. They observed that, in both phases, applied kinetin promoted bud elongation, but its effect seems to be exerted in different ways. In the first phase which seems to be controlled by auxin, the effect of kinetin may have been due to a mobilization of assimilate flow into the bud since more ^{14}C assimilates were found in buds of treated than control plants. The promotive effect of kinetin at the time of ear emergence was associated with the overcoming of an inhibitory influence emanating from the leaves, since it promoted tillers only in plants not defoliated.

From the observations made above it can be suggested that the control of tillering may involve interaction between at least nutrients, auxins, gibberellins and cytokinins, the role of each one of these factors fluctuating with the developmental stage of the plant.

3. The objective of the work in this thesis

The idea of developing a project on the hormonal control of tillering in Proctor barley came after the work done in this Department by G. M. Fletcher on the effects of mineral nutrition on the early growth of tiller buds in this plant.

As mentioned before (section 2(b)), Fletcher (1975) observed that the growth of tiller buds in young plants was dependent on the supply of mineral nutrients to the plants.

Fletcher's results seem to fit nicely as an example of the control of lateral bud growth by the availability of mineral nutrients, and to support the nutritional hypothesis of apical dominance. Thus under conditions of limited supply of minerals, reduced amounts of assimilates are available and these are transported to the mainstem apex as a first and larger sink and consequently directed away from the small tiller bud primordia.

However, a purely nutritional hypothesis to explain apical dominance is no longer generally accepted (Phillips, 1975; and section 2(a) of this chapter), and a large body of evidence shows an imporant role for growth regulators in tillering in grasses, with cytokinins increasing tiller growth in most of the situations studied so far. On the other hand there are reports in the literature of the reduction of levels of endogenous cytokinins as a response to nitrogen deficiency treatments in Solanum (Woolley and Wareing, 1972a), and in Lycopersicum by deficiency in phosphorus (Menary and Van Staden, 1976).

Thus it is possible that the effect of mineral nutrients on tiller bud development in barley found by Fletcher (1975) is not a direct one but rather is indirect, and mediated through an effect on growth substances, possibly the levels of endogenous cytokinins. It is possible to examine this idea experimentally.

Based on this interpretation a formal statement of my hypothesis is this: that the commencement of the phase of exponential growth of young, already initiated, tiller buds in barley is under the primary control of endogenous cytokinins, the level of which can be affected by mineral nutrient supply to the young seedling. The hypothesis suggests a primary role for cytokinins but not excluding secondary

effects of other growth substances, mineral nutrients or other metabolites.

This hypothesis has been examined using three different approaches, namely the observation of effects of exogenous growth regulators, particularly cytokinins, on tiller bud growth, the application of treatments expected to modify levels of endogenous cytokinins and observation of its effect on bud growth, and the direct determination of cytokinin-like substances in plants supplied or not with mineral nutrients.

CHAPTER II

MATERIAL AND METHODS

1. The study of tiller development

(a) Plant material.

Barley, Hordeum vulgare L., cultivar Proctor, from the 1975 harvest grown locally was used throughout the experiments. Grain was stored at a temperature of about 8°C. The cultivar Proctor was chosen because tillering in this cultivar has been thoroughly studied by Fletcher (1975), to give the basic knowledge necessary for the present work.

(b) Growth conditions.

Growth room conditions

Barley plants were grown in controlled environments. The temperature was maintained constant at 20 ± 1°C. A photoperiod of 16 hours was used, the light period lasting from 9 a.m. until 1 a.m. the following day. Light source from a mixture of warm white fluorescent tubes and tungsten filament lamps gave a light intensity, at the growing table, of about 2200 to 3000 footcandle, equivalent to 260 to 365 $\mu E m^{-2} s^{-1}$, in the wavelength range 400-700 nm.

Growth of plants

In most of the experiments, grain was germinated in moist perlite (British Gypsum Ltd.), in a Pyrex container with a volume of 2 l. Grain of homogenous size, weighing about 45 mg, was placed about 1.5 cm below the perlite surface. The container was covered until day 3, at which time the coleoptiles of the seedlings were visible above the perlite surface.

Usually on day 4, seedlings of the same size were selected and transplanted to square plastic pots with a volume of about 300 ml,

containing washed river sand F grit (British Industrial Sand Ltd.). When growth substances treatment was given through the leaves transplanting was done on day 6. The transplanting technique consisted of flooding the dish containing perlite with tap water and carefully washing out the perlite from the seedling root system. The seedlings were then replanted carefully into sand.

Germination of grain, for a few specified experiments, was made directly in the plastic pots containing sand, one grain per pot. In these cases the selection of plants for the experiments was also made on day 4.

Water and mineral nutrient application

Distilled water was always used. The perlite in which grain was sown was maintained wet until the transplanting into sand. Pots containing sand received 50 ml of water every second day.

The composition of the complete standard mineral nutrient solution supplied to the plants is given in Table II-1. According to the experiment, mineral nutrients were or were not supplied. When mineral nutrient solution was given, 50 ml of the solution was applied on day 4.

(c) Growth substance application.

The growth substances

A number of growth substances and two substituted aminopurines were applied to barley plants to determine their effects on early tiller bud growth. A list of the substances used and their molecular weights is given in Table II-2. Molecular formulae are given in Figure II-1.

Treatment through the leaves

Kinetin, PBA, zeatin, GA_3 and IAA were applied to barley plants through the leaves in various concentrations according to the experiment. To facilitate the penetration of the growth substances, the solutions

Table II-1: Composition of the standard mineral nutrient solution. Macronutrients according to Fletcher (1975). Micronutrients according to Hoagland and Arnon (1938).

	Stock Solution		Volume (ml) of stock solution per litre of nutrients solution
Macronutrients	$NaNO_3$	1.0 M	20
	KH_2PO_4	0.2 M	10
	K_2SO_4	0.5 M	20
	$MgSO_4$	0.4 M	10
	$CaCl_2$	0.5 M	10
	EDTA (Fe Na versenate)	0.025%	10
Micronutrients	H_3BO_3	2.86 g	1
	$MnCl_2\ 4H_2O$	1.81 g	
	$ZnSO_4\ 7H_2O$	0.22 g	
	$CuSO_4\ 5H_2O$	0.08 g	
	$H_2MoO_4\ H_2O$	0.09 g	

(Micronutrients: in 1 l)

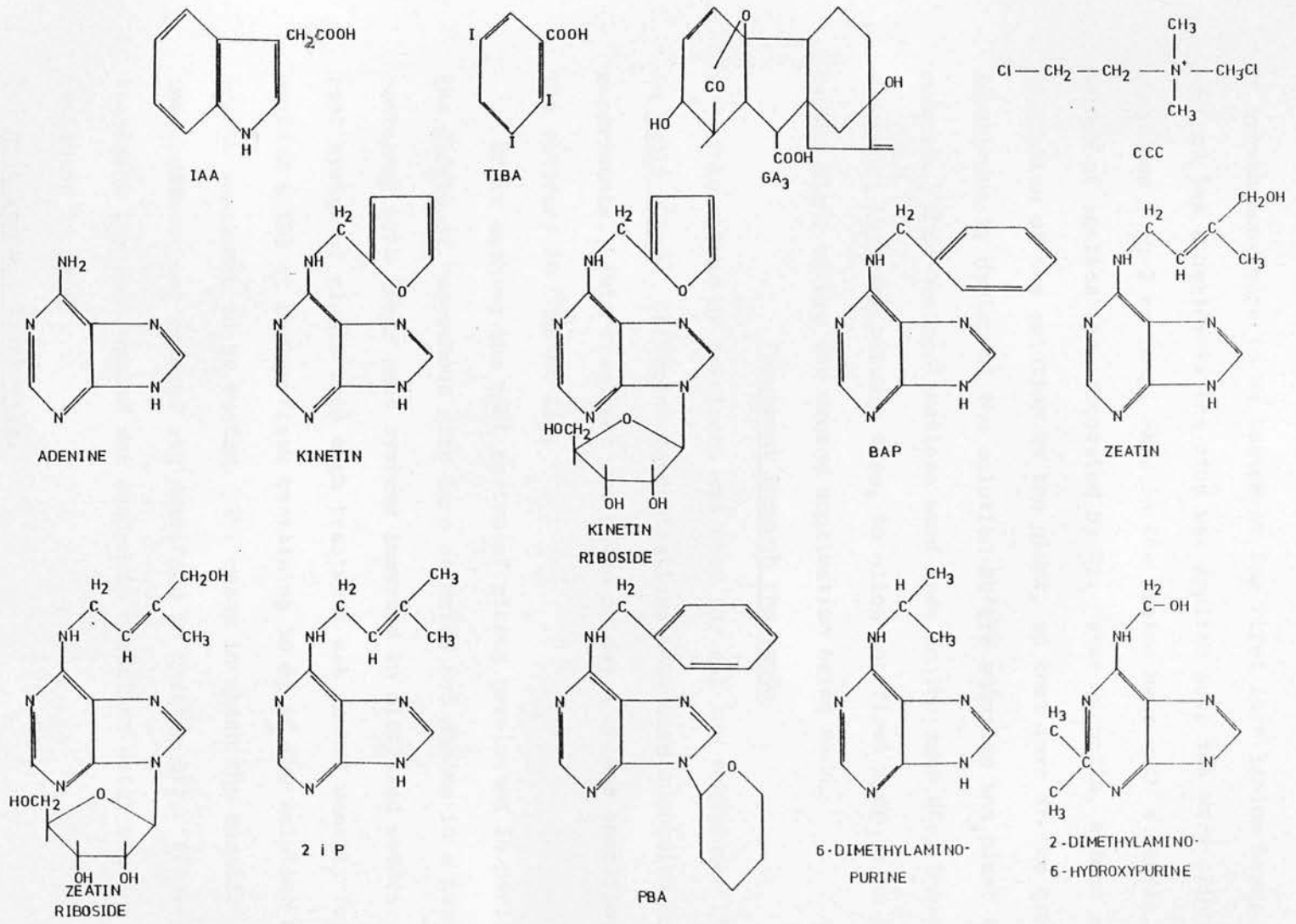

Figure II-1: Structures of the growth substances applied to barley plants.

were prepared using the wetting agent Tween 80 at 0.002%. The treatment was in most of the cases given on day 6, but in some cases it was repeated on days 8 and 10.

Treatment consisted of application of a 5 µl drop of the solution of growth substance to be tested at the first leaf lamina base. In most of the experiments the drop was applied over two very slight scratches 1 to 2 mm long, made in the lamina base with a needle. This method of application, suggested by Dr. Peter Nicholls, allows rapid absorption of the solution by the plant, so that loss of the growth substances by drying of the solution before entering the plant is reduced. When two applications were made in the same day, there was a 4 hours interval between them, to allow the first drop to be absorbed by the plant before the second application being made.

Treatment through the roots

This method of treatment was used for all the substances listed in Table II-2. Different concentrations were used according to the experiments. This treatment was given on day 4 unless specified to the contrary in Chapter III.

After washing the root system of plants germinated in perlite, the plants of homogenous size were selected and placed in a pyrex container with their root systems immersed in distilled water. The root system of plants from each treatment was soaked usually for 4 hours in a 100 ml beaker flask containing 50 ml of the solution of the growth substance to be tested. The cases in which the duration of the treatment was changed are specified in Chapter III. After the treatment the root system was washed in distilled water and the plants replanted in sand.

(d) Root removal treatments.

Using the nomenclature of Hackett (1968) each part of the root

Table II-2: Growth substances supplied to barley plants and their molecular weight.

Growth substances	Molecular weight
Adenine	135.1
Indole-3 acetic acid (IAA)	175.2
2,3,5-triiodobenzoic acid (TIBA)	499.8
Gibberellic acid (GA_3)	346.4
(2-chloroethyl) trimethylammonium chloride (CCC)	158.1
6-furfurylaminopurine (kinetin)	214.2
6-furfurylaminopurine riboside (kinetin riboside)	347.3
6-benzylaminopurine (BAP)	225.3
6-(4-hydroxy-3-methylbut-2-enylamino) purine (Zeatin)	219.3
6-(4-hydroxy-3-methylbut-2-enylamino) purine riboside (Zeatin riboside)	351.4
6-(3-methyl-2-butenylamino) purine (2iP)	203.3
6-benzylamino-9 tetrahydropyranylpurine (PBA or SD8339)	310.0
Other compounds	
6-dimethylaminopurine	163.2
2-dimethylamino-6-hydroxypurine	170.2

system developed from the grain or from the stem was termed an axis. The branches developing from the axes were called primary laterals and the branches off these, secondary laterals.

Root removal treatment was given on day 4 to plants germinated in perlite. The root system of 4 day old Proctor barley seedlings usually consists of 6 axes with no laterals yet present. Only plants with 6 axes were used. Details of the treatments are given in Chapter V.

(e) Growth measurements.

Dissection of young tiller buds

The tiller nomenclature used is similar to that used by Rawson (1971), Jewiss (1972), Kirby and Faris (1972) and Fletcher (1975). The tiller in the axil of the coleoptile is termed the coleoptile tiller, Tc, while those in the axil of the first and second leaves are designated T1 and T2.

Dissection of young tiller buds was carried out under an Olympus microscope with a magnification of x10. Caryopsis coat, remaining endosperm and scutellum were removed. Careful removal of the coleoptile exposed the coleoptile tiller (Tc) and subsequent removal of the first leaf sheath exposed the first leaf tiller (T1). These tiller buds were excised from the stem.

Dissection of the whole plant

After carefully washing the sand out of the root system, the plants were divided into roots, base, first leaf and second leaf. Base included the remainder of the caryopsis, the coleoptile and the parts of the plant within the coleoptile.

Dry weight determination

After dissection, the plant parts were placed on trays, or small

flasks in the case of tiller buds, and dried overnight at 90°C.

Tiller buds were weighed on a Sauter torsion balance to the nearest 2 µg. For some treatments, where the tiller buds were very small, they were weighed in groups.

Those plant parts weighing more than 1 mg were weighed on an Oertling R20 balance.

Tiller bud length determination

After dissection, the tiller bud length was determined under the Olympus microscope using a calibrated eyepiece.

Root length and number determination

Pots containing the plants were immersed in water and the roots washed carefully free of sand. The root systems were kept in water before the measurements were made.

Mean axis length per plant was estimated and the total number of primary laterals per plant was determined by counting every root 1 mm in length or longer.

(f) Preparation of sections for the study of apical development.

The plant to be studied was harvested, and roots, remains of the caryopsis and the aerial part of the plant, about 1 cm above the scutellum were removed. The part of the plant including the apical region and tiller buds was prepared for microscopic examination using the methods of McLeish and Sunderland (1961) slightly modified as follows:

Fixation

Immediately after harvest the material was fixed in ethanol:acetic acid (3:1) for at least 1 hour. Material was washed in absolute ethanol and rehydrated passing through an ethanol series to water (about 10 minutes each).

Acid hydrolysis

The material was hydrolysed in 5 N HCl for 50 minutes at 25°C.

Staining

After hydrolysis the material was rinsed in distilled water and stained with leuco-basic fuchsin (Feulgen reagent) for 2 hours at 20°C.

(Feulgen reagent (Darlington and La Cour, 1960):
One gram of basic fuchsin is dissolved in 200 ml of boiling water, shaken well, cooled to 50°C and filtered. To the filtrate, 30 ml of 1 N HCl is added and also 30g of $K_2S_2O_5$. The solution is allowed to bleach for 24 hours in a tightly-stoppered bottle, in the dark. Half a gram of decolorizing carbon is added, shaken well for about one minute and filtered. The reagent is stored in a tightly-stoppered bottle, in the dark, at 4°C).

After staining the material was washed for 15 minutes in three changes of SO_2 water, and rinsed in distilled water.

(SO_2 water (Darlington and La Cour, 1960):
5 ml of 1 N HCl
5 ml of $K_2S_2O_5$ at 10%
100 ml of distilled water)

Embedding and sectioning

The material was dehydrated through an alcohol series until absolute ethanol and then through ethanol:xylene 2:1, 1:1, 1:2, and xylene for 1 hour in each mixture. The material was embedded in paraplast and sectioned using a Beck

microtome to give 10 µm thick sections. The sections were mounted on microscope slides using Canada balsam.

The sections were observed under a Vickers microscope with a magnification of x80. Photographs were taken using a Zeiss microscope with a magnification of x80.

(g) Application and determination of distribution of a radioactive cytokinin.

Ten mCi of benzyl (8-^{14}C) adenine with a specific activity of 13.4 mCi/mMol were dissolved in 50 ml of a 10^{-4}M solution of unlabelled BAP. This solution was applied through the roots of barley plants as described in section (c) of this chapter.

After a certain growth period (as indicated in Chapter III-4) the plants were harvested and the amount of radioactivity determined in Tc, $T1_3$ leaves, base and roots. Ten plants were used in each treatment. Tiller buds were dissected and placed 5 in each scintillation vial containing 5 ml of scintillation liquid, each vial was shaken thoroughly at the harvest day and a number of times before the counting which was done after a week.

(Scintillation liquid (similar to Fletcher, 1975):

2 parts of toluene

1 part of triton X-100 (Intertechnique)

5g/l of 2(4-tert-butylphenyl)5(4-biphenylyl)1, 3,4 oxadiiazole (butyl PBD))

The dry weight of leaves, base and roots was determined as described in section (e) of this chapter. After the determination of dry weight, leaves of 5 plants (2 groups for each treatment) were cut into pieces, placed in 100 ml Erlenmeyer flasks containing 20 ml of ethanol and boiled for 10 minutes; the same was done for the bases and roots. After cooling the volumes were completed to 20 ml

with ethanol. For the determination of radioactivity 500 µl of each sample were placed in scintillating vials containing 5 ml of the scintillation liquid and shaken throughly; two replicates were used for each sample and a blank was included using 500 µl of ethanol.

Each sample was counted for 4 minutes in a SL 30 liquid scintillation spectrometer (Intertechnique). The results, after the blank value being subtracted, were expressed as number of counts per minute, in each part of the plant analysed, and as number of counts per minute, per milligram dry weight of the parts analysed.

(h) Sample size and analysis of results.

Samples of 8 to 10 plants were used in most of the experiments for each treatment, the few cases in which different samples sizes were used are mentioned in the relevant sections. Most Proctor barley plants have only one tiller bud at the axil of the coleoptile or of the first leaf, but occasionally two coleoptile tillers were found instead of one. This problem which was also found by Fletcher (1975), who calculated as 2.5% the percentage of plants with double Tc, was solved by excluding such plants from the sample.

All the results were expressed as mean of the individual values for each sample. Results were analysed using standard statistical procedures as follows.

Standard error

Most of the results presented in tables are given as the mean values followed by its standard error.

Confidence limit

To facilitate the visualization, the result presented in graphs are given as means with vertical bars indicating the 95% confidence limits. These were calculated as:

Confidence limit = mean ± (standard error X t)

where the value of t used was selected with a probability of 0.05 for the appropriate degrees of freedom (n - 1). A difference between two samples was considered significant if the confidence limit of one did not overlap the confidence limit of the other.

Analysis of variance

Methods of analysis of variance were used to compare the results of some experiments, and the least significant differences (LSD) calculated to indicate significant differences between samples.

2. Isolation of endogenous cytokinin from barley plants

(a) Plant material and growth conditions

Barley grain, cultivar Proctor, from the 1974 or 1976 harvest were germinated, in plastic trays (8 x 30 x 57 cm) containing washed sand, in controlled environment at a temperature of 20°C and a photoperiod of 16 hours. About 200 grains were sown in each tray. Mineral nutrient solution (Table II-1) was supplied on day 4 or never, and seedlings were harvested on either day 6 or day 8 as specified in Table II-3.

At harvest the plastic trays containing the plants were flooded with water, the plants washed free of sand, dried quickly with filter paper and weighed. The number and total fresh weight of the plants used in each extraction are given in Table II-3.

(b) Extraction and solvent purification.

The procedure used for the isolation of substances with cytokinin activity from barley plant extracts was based on the following publications; Yoshida, Oritani and Nishi, 1970; Yoshida and Oritani, 1971; Hewett and Wareing, 1973; Van Staden, 1973; Hahn, 1975; Henson and

Table II - 3

Characteristics of the plant material used for the isolation of cytokinins from barley.

Seeds from	Nutrient solution	Harvest day	Number of plants	Fresh weight (g)
1974	complete	6	4210	1637
1974	no	6	4764	1600
1976	complete	8	3090	1574
1976	no nitrate	8	3134	1442

Wheeler, 1976; and Purse, Horgan, Horgan and Wareing, 1976. The procedure used for the extraction and solvent purification of 6 day old plants is summarized in Figure II-2.

Plants were killed in liquid nitrogen and extracted for a few days, at 5°C, with 80% ethanol using 1 ml per plant. They were then macerated, the extracts filtered through filter paper and the residues washed with 80% ethanol. The filtrates were reduced to the aqueous phase (180 ml) under reduced pressure at 40°C using a rotary evaporator and centrifuged at 1000 x g for 15 minutes at 15°C; the precipitates were resuspended and recentrifuged. The combined supernatants were acidified to pH 1.5 with HCl and partitioned 3 times against equal volumes of ethyl acetate to remove acidic inhibitors.

The pH of the aqueous layer was adjusted to 3.4 and it was passed through a Dowex AG 50W-X8 (H form, 100-200 mesh) column (3 x 14 cm). The column was washed with 4 volumes of distilled water and the active substances adsorbed onto the column were eluted out with 8 volumes of 2 N NH_4OH followed by 7 volumes of 5 N NH_4OH. The ammonia eluate was evaporated to dryness under reduced pressure and the residue dissolved in 200 ml of phosphate buffer at pH = 7.7 was partitioned 4 times against equal volumes of ethyl acetate. The ethyl acetate fraction was further purified, using chromatographic methods, and the aqueous phase at pH 8.2 was partitioned 4 times with equal volumes of water-saturated n-butanol. The n-butanol fraction was also purified using chromatographic methods. Both ethyl acetate and n-butanol fractions were expected to contain free bases and ribosides. This procedure was used for 6 day old plants.

A slightly modified method was used for the extraction of cytokinins from 8 day old plants. In this case, the Dowex column

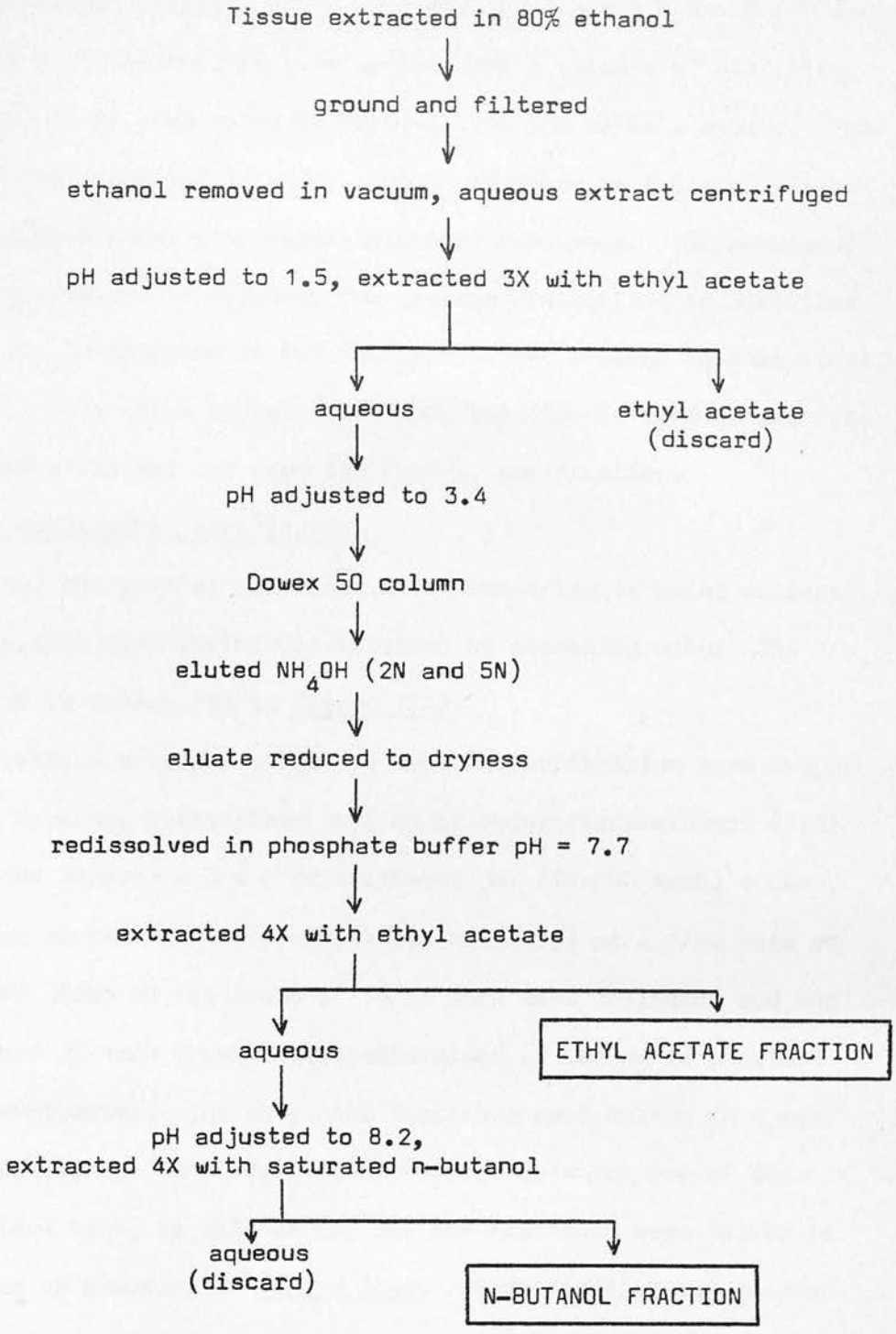

Figure II-2: Summary of the procedure used for the solvent purification of cytokinins from extract of barley plants.

was washed with 8 followed by 10 volumes of 2 and 5 N NH_4OH respectively and the column was also washed with 4 volumes of distilled water which was evaporated to dryness with the ammonia eluate. The residue was dissolved in water, the pH adjusted to 8.0 with HCl and partitioned 6 times with water-saturated n-butanol. The n-butanol phase was reduced to dryness, the residue redissolved in distilled water, the pH adjusted to 7.3 and partitioned 5 times against ethyl acetate. This ethyl acetate fraction, expected to contain the free bases and ribosides was used for further purification.

(c) Chromatographic purification.

After the partial purification of the extracts using solvents the fractions were further partitioned by chromatography. The procedure is summarized in Figure II-3.

Fractions obtained after the solvent purification were evaporated to dryness, redissolved in 2 ml of chloroform:methanol (7:3) and passed through a 3 x 9 cm silicagel 60 (70-230 mesh) column, which was eluted with chloroform:methanol (7:3) at a flow rate of 1 ml/min. Over 40 fractions of 15 ml each were collected and the absorbance of each fraction was determined at 260 nm in a UNICAM Spectrophotometer. The absorbing fractions were bulked in 4 subfractions (1, 2, 3 and 4); the absorbance data for one of the extractions made, as well as the way the fractions were bulked is shown, as an example, in Figure II-4. Each fraction was reduced to a small volume and chromatographed on 20.0 x 20.0 cm precoated silicagel 60 F_{254} plates with a thickness of 0.2 mm, using chloroform:methanol (9:1) as solvent. The plates were observed under ultraviolet light, the positions of absorbing bands were noted and the plates divided into 3 or 4 sections (a, b, c and d), isolating groups of absorbing bands. The silicagel from each section

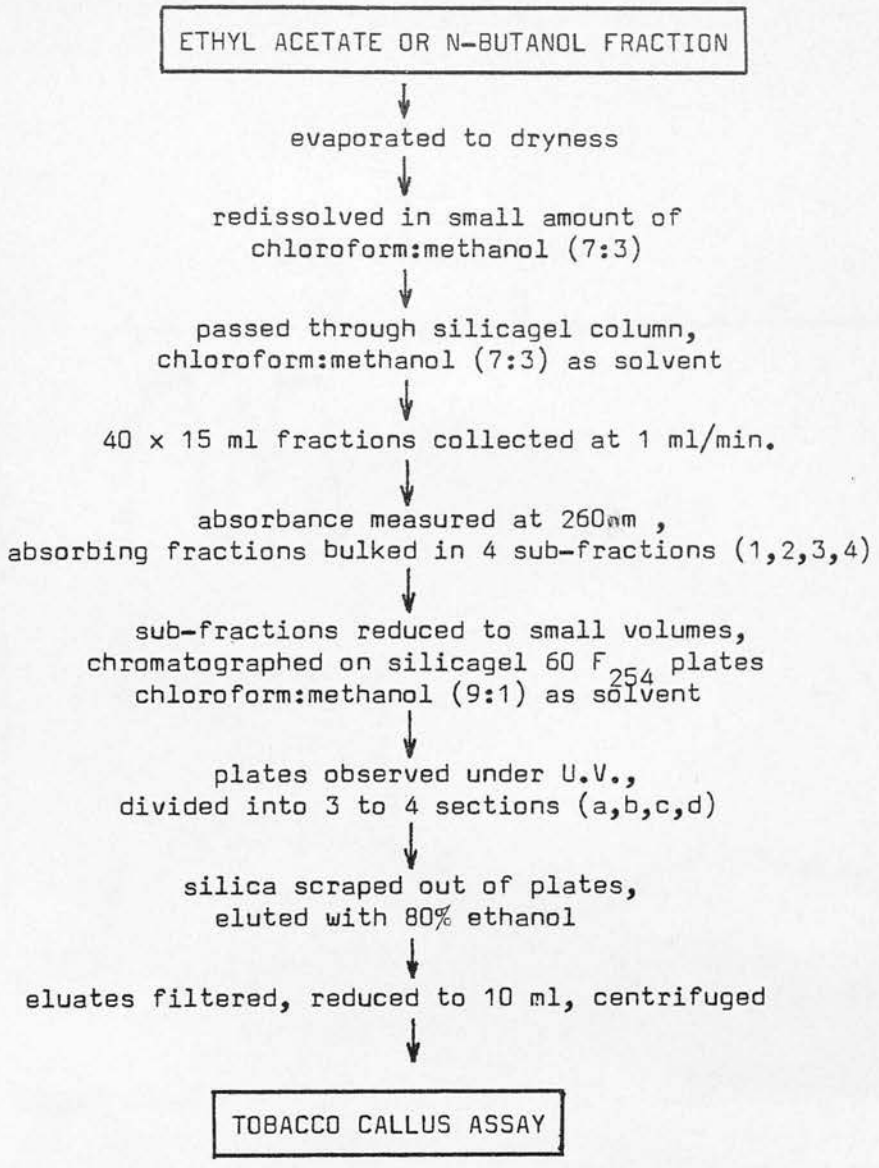

Figure II-3: Summary of the procedure used for the chromatographic purification of cytokinins from extract of barley plants.

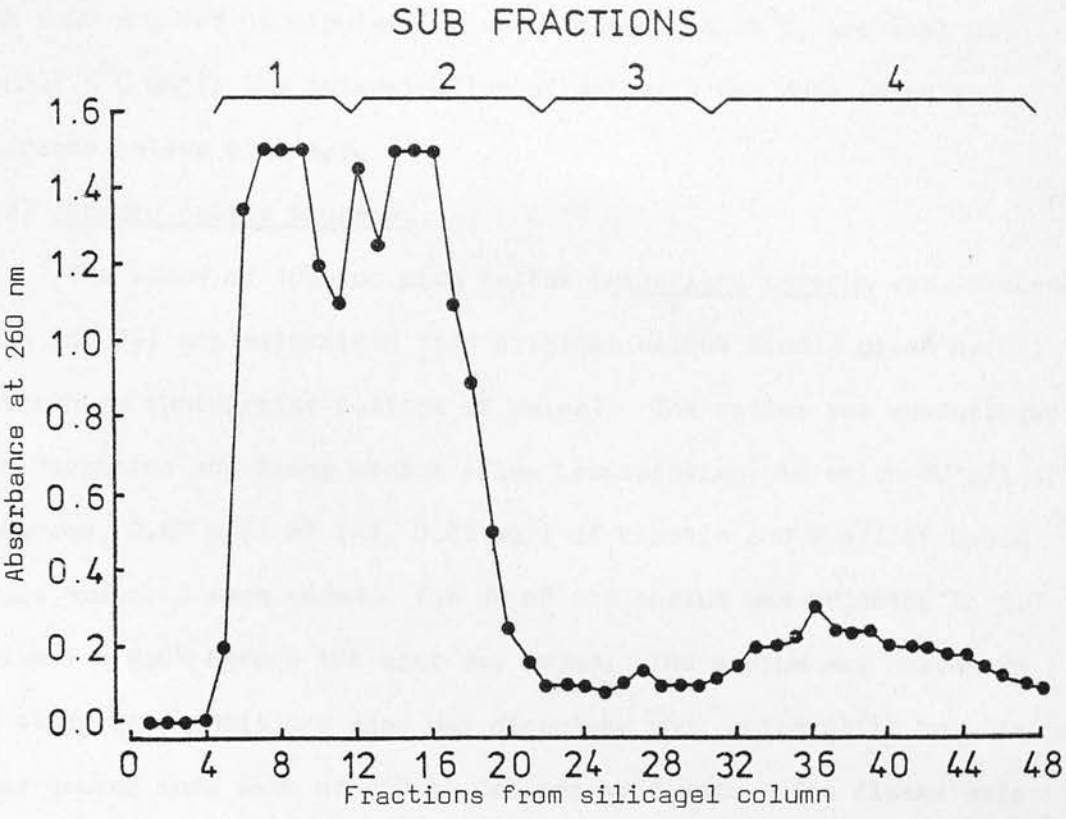

Figure II-4: Absorbance of the fractions obtained after the silicagel column showing how they were bulked before the TLC separation. Extract of 8 day old plants supplied with complete mineral nutrient solution.

was scraped out of the plates and eluted with 60 ml of 80% ethanol in small glass columns; the ordinary method of elution by shaking the silicagel with 80% ethanol was shown to cause large loss of activity. The eluates were filtered, reduced to 10 ml, centrifuged at 9000 x g for 15 minutes (or until clear) at 15°C, and kept at about 5°C until the determination of activity was done using the tobacco callus bioassay.

(d) Tobacco callus bioassay.

The stock of tobacco pith callus (Nicotiana tabacum var. Wisconsin No. 38) was maintained from original callus kindly given by Dr. T. Stuchbury (University College of Wales). The callus was subcultured in Murashige and Skoog medium (Flow Laboratories) to which 30 g/l of sucrose, 2.00 mg/l of IAA, 0.03 mg/l of kinetin and 9 g/l of Oxoid agar number 3 were added. The pH of the medium was adjusted to 5.7 with 1 N NaOH before the agar was added. The medium was heated on a steam bath until the agar was dissolved and, while still hot, 100 ml was poured into each of 250 ml Erlenmeyer flasks. The flasks were closed with aluminium foil and autoclaved for 15 minutes at 125°C. Three pieces of callus, weighing about 0.5 g each, were planted in each Erlenmeyer flask after the medium was solidified. The cultures were placed in the dark, at about 24°C and transferred at 3 to 4 week intervals.

Bioassays were carried out following the method described by Skoog and Armstrong (1974). The medium used was the same as that in which the callus stocks were kept growing, but kinetin was omitted. The fractions to be tested were pippetted into 100 ml Erlenmeyer flasks, usually in the volumes of 10, 100 or 1000 µl, and reduced to dryness by an air-stream in the fume chamber, to avoid traces of

ethanol which could affect callus growth. Although Skene (1976) has reported losses in biological activity of cytokinins during the drying of the solutions, a test made with kinetin at the concentrations of 20, 30 and 40 μg/l of medium showed better performance where the samples were dried. The medium, 25 ml, was very well shaken in the flasks before and after autoclaving. In all the bioassays the following controls were included: the sample was substituted by distilled water, a fraction of the eluate from silicagel scraped from a plate developed in chloroform:methanol but to which no extract had been applied was tested, and kinetin was tested at 30 μg/l and in some cases also at 5 and 10 μg/l. Three flasks were used for each volume of fraction to be tested as well as for the controls, and three small pieces of callus, weighing about 20 to 30 mg per piece were planted in each flask. The flasks were placed in the dark at about 24°C. After a growth period of 4 weeks the tissue from each callus was weighed and the average fresh weight calculated.

The activities of kinetin, PBA and zeatin were determined (Figure II-5) using the tobacco callus assay as described above. Kinetin was assayed at the concentrations of 2.0, 4.0, 6.0, 10.0, 15.0, 20.0, 30.0, 60.0 and 90.0 μg/l; increase in kinetin concentration increased proportionally the fesh weight of callus from 2.0 up to 60.0 μg/l and this high value was maintained at 90.0 μg/l. PBA was tested at 10.0, 20.0, 40.0, 60.0 and 80.0 μg/l; fresh weight of callus was increased with PBA concentration from 10 to 40 μg/l and decreased after that. Zeatin was assayed at 0.1, 0.2, 0.4, 0.8, 2.0, 4.0, 20.0 and 60.0 μg/l; also in this case there was a proportional increase in callus fresh weight with the increase in hormone concentration.

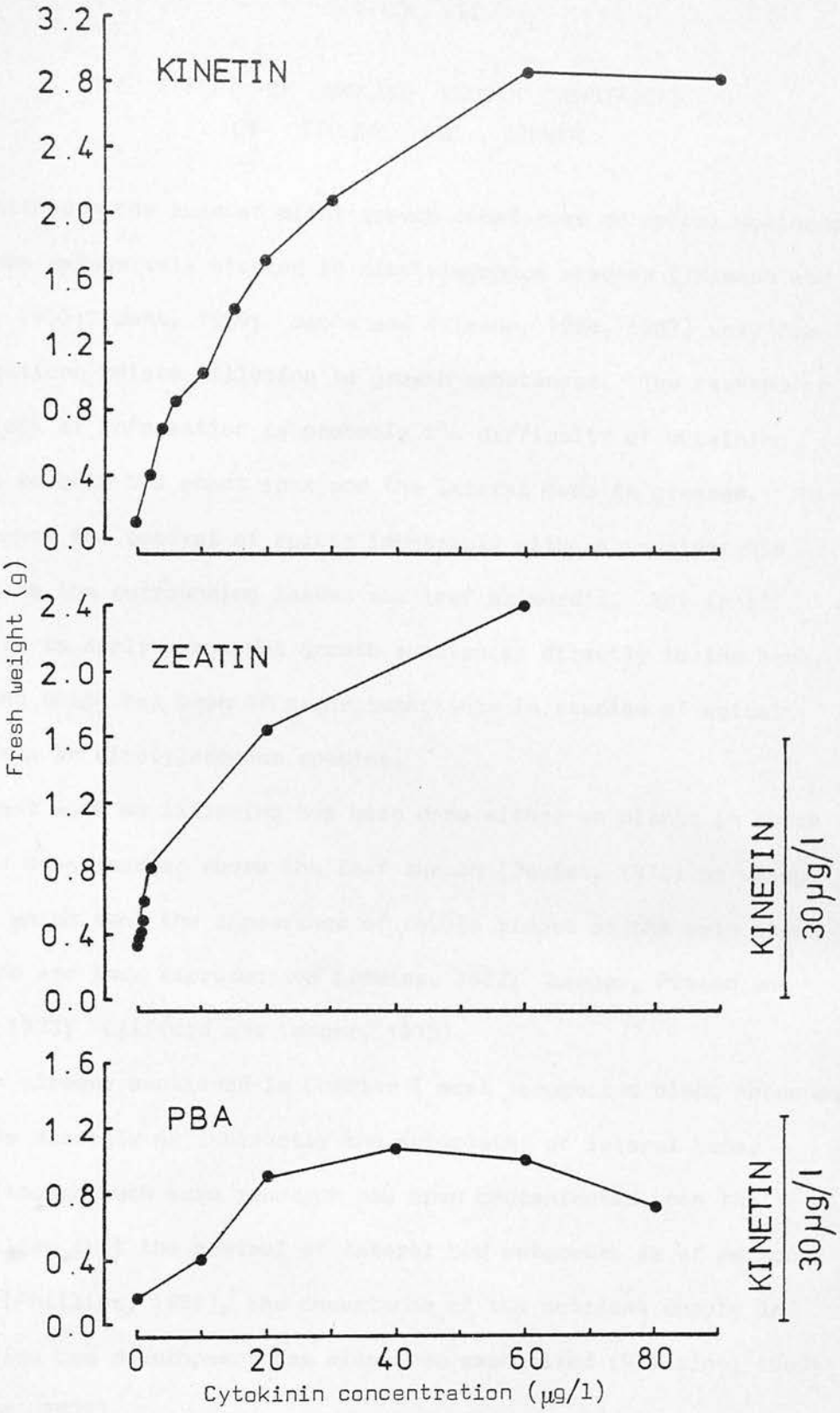

Figure II-5: Fresh weight yields of tobacco callus as a function of kinetin, zeatin and PBA concentration.

CHAPTER III

THE EFFECT OF APPLIED GROWTH SUBSTANCES ON TILLER BUD GROWTH

Although the role of plant growth substances on apical dominance has been extensively studied in dicotyledonous species (Thimann and Skoog, 1933; Went, 1939; Sachs and Thimann, 1964, 1967) very few publications relate tillering to growth substances. The reason for this lack of information is probably the difficulty of obtaining access to both the shoot apex and the lateral buds in grasses. This fact makes the removal of apices impossible without considerable damage to the surrounding leaves and leaf primordia. Nor is it possible to apply exogenous growth substances directly to the buds, a method which has been of major importance in studies of apical dominance in dicotyledonous species.

Most work on tillering has been done either on plants in which tillers have emerged above the leaf sheath (Jewiss, 1972) or using plants which show the appearance of double ridges on the main stem apex and are thus reproductive (Jewiss, 1972; Langer, Prasad and Laude, 1973; Clifford and Langer, 1975).

As already mentioned in Chapter I most recognized plant hormones regulate directly or indirectly the outgrowth of lateral buds.

Although much more research has been concentrated upon the possibility that the control of lateral bud outgrowth is of hormonal nature (Phillips, 1969), the importance of the nutrient supply in regulating bud development has also been emphasized (Phillips, 1969; McIntyre, 1977).

The importance of mineral nutrients as a primary factor on tillering has been shown by Gregory (1937), Aspinall (1961), Sekiya (1963)

and Fletcher and Dale (1974). Also the pattern of assimilate distribution has been demonstrated to be related to the tillering process (Jewiss, 1972; Langer et al., 1973; Clifford and Langer, 1975).

It is most probable that the arrest or release of lateral bud growth is a response to both nutrient and hormonal status of the plant (Phillips, 1975). In spite of this, no publications are available relating nutrient supply to levels of endogenous growth substances in grasses.

This chapter reports attempts to induce and analyse tiller bud growth in young vegetative barley plants grown in presence or absence of mineral nutrients and treated with a range of growth substances using different concentrations and methods of application; it also reports some other aspects of the growth of treated plants.

1. The effects of cytokinins

There are numerous data in the literature of apical dominance indicating that cytokinins are essential for lateral bud outgrowth in dicotyledonous species (Sachs and Thimann, 1964, 1967; Phillips, 1975). Also in grasses, cytokinins have been shown to affect the development of lateral buds by promoting tiller elongation (Jewiss, 1972; Langer et al., 1973; Clifford and Langer, 1975; Johnston and Jeffcoat, 1977) and increasing tiller number (Johnston and Jeffcoat, 1977).

The main objective of this section is to report the effect of cytokinin on the early growth of tiller buds Tc and T1 in barley. It also relates this effect to the effect of cytokinin on the growth of the whole plant and to the mineral nutrient availability.

(a) The choice of the method of application.

Two methods of cytokinin treatment were examined in attempts to select the most convenient. The first method consisted of treating young barley plants with a 5 µl drop of cytokinin solution placed over scratches made in the first leaf lamina base (see Chapter II). Two applications of cytokinins were made on day 6 to plants germinated in pots containing sand, which had or had not been supplied with nutrients on day 4.

The cytokinin PBA was applied at the concentrations of 10^{-7}, 10^{-6} and 10^{-5}M. Table III-1 shows that the dry weight of tiller buds Tc and T1 in plants supplied with nutrients was not significantly affected by the cytokinin treatment neither two nor five days after the cytokinin treatment, on days 8 and 11 respectively.

Tiller bud dry weight of plants not supplied with minerals was also not affected by PBA in any of the concentrations tested (Table III-2) three or six days after the treatment on days 9 and 12 respectively. As the tiller buds T1 of plants not supplied with nutrients were very small, all the replicates for each treatment had to be weighed together so that no statistical comparison could be applied to these data.

Even PBA at the higher concentration of 10^{-4}M or other cytokinins as kinetin and zeatin both at 10^{-4}M, applied through the leaves to barley plants supplied or not supplied with nutrients, had no effect on Tc or T1 dry weight (Table III-3). Again no statistics could be applied to the data of tiller bud T1 in plants not supplied with nutrients.

The second method of treatment consisted of applying cytokinin to the root system; this was thought to be a sensible method because

Table III - 1

The effect of PBA applied to the leaves at the concentrations of 10^{-7}, 10^{-6} and 10^{-5}M on the dry weight (μg) of the tiller buds Tc and T1. Plants were supplied with mineral nutrients and harvested on days 8 and 11. Standard error is given.

	Tc		T1	
	day 8	day 11	day 8	day 11
Control	20.41 ± 1.69	67.09 ± 4.81	6.00 ± 0.84	36.00 ± 4.47
PBA 10^{-7}M	17.43 ± 2.26	53.71 ± 8.07	5.86 ± 0.72	36.72 ± 2.80
PBA 10^{-6}M	25.67 ± 2.13	54.64 ± 7.10	8.43 ± 1.27	31.56 ± 2.83
PBA 10^{-5}M	23.50 ± 1.73	45.74 ± 9.73	6.93 ± 0.73	32.80 ± 5.31

Control and PBA values do not differ significantly at $p = 0.05$

Table III - 2

The effect of PBA applied to the leaves at the concentrations of 10^{-7}, 10^{-6} and 10^{-5}M on the dry weight (μg) of the tiller buds Tc and T1. Plants were not supplied with mineral nutrients and harvested on days 9 and 12. Standard error is given for Tc but not for T1; see text.

	Tc		T1	
	day 9	day 12	day 9	day 12
Control	8.29 ± 1.38	10.10 ± 1.05	2.58	3.67
PBA 10^{-7}M	8.36 ± 1.00	8.20 ± 0.77	3.36	3.44
PBA 10^{-6}M	7.41 ± 0.98	7.36 ± 1.38	3.43	2.50
PBA 10^{-5}M	9.29 ± 0.83	9.79 ± 1.33	2.25	2.50

Control and PBA values for Tc do not differ significantly at $p = 0.05$

Table III - 3

The effect of kinetin, PBA and zeatin applied to the leaves at the concentration of 10^{-4}M on the dry weight (μg) of the tiller buds Tc and T1. Plants were supplied or not with mineral nutrients and harvested on day 10.

	Tc		T1	
	Nutrients	No nutrients	Nutrients	No nutrients
Control	39.70 ± 6.98	7.50 ± 2.53	39.71 ± 7.04	3.43
Kinetin	32.07 ± 3.67	8.43 ± 0.90	30.06 ± 6.21	2.58
PBA	54.86 ± 5.33	4.69 ± 1.16	41.13 ± 9.86	3.43
Zeatin	46.20 ± 5.14	5.86 ± 0.70	39.69 ± 8.09	2.79

Control and cytokinin values for Tc, T1 (supplied with nutrients) do not differ significantly at $p = 0.05$

as endogenous cytokinins are mainly synthesized in the roots (Kende and Sitton, 1967; Engelbrecht, 1972) translocation of root applied cytokinins would probably be facilitated. The root system of 4 day old barley plants was soaked in the solution to be tested (see Chapter II). Plants were treated through the roots for 4 hours with kinetin, PBA or zeatin at the concentration of 10^{-4}M; no mineral nutrients were given. Every cytokinin tested (Table III-4) had a promoting effect on the dry weight of tiller buds Tc and T1 on day 10.

The cytokinin PBA had no effect on tiller growth when applied through the leaves at the concentrations of 10^{-7}, 10^{-6}, 10^{-5} or 10^{-4}M to plants supplied or not with mineral nutrients. The cytokinins kinetin and zeatin at 10^{-4}M were also not effective when applied through the leaves. On the other hand, application of these three cytokinins through the roots promoted tiller buds Tc and T1 in plants not supplied with nutrient solution. The method of treatment through the leaves was therefore abandoned and applications through the roots which had been successful in promoting tiller bud growth were made in the subsequent experiments.

(b) The effect of different concentrations.

As discussed above, kinetin, PBA and zeatin promoted growth of tiller buds Tc and T1 when applied through the roots at the concentration of 10^{-4}M. Other concentrations were also tested to determine the most effective for this method of application.

PBA was applied at the concentrations of 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3}M on day 4 for plants not given nutrient solution. The dry weight of tiller buds Tc and T1 measured on day 10 is given in Figure III-1. PBA at the lowest concentration, 10^{-6}M, had no significant effect on Tc or T1 dry weight. At the concentration of

Table III - 4

The effect of kinetin, PBA and zeatin applied through the roots at the concentration of 10^{-4} M on the dry weight (μg) of the tiller buds Tc and T1. Plants not supplied with mineral nutrients and harvested on day 10.

	Tc	T1
Control	7.94 ± 0.82	2.00
Kinetin	*17.31 ± 1.66	8.50 ± 1.82
PBA	*13.38 ± 1.69	10.05 ± 2.14
Zeatin	*13.72 ± 0.83	6.75 ± 1.90

* Values for Tc differ significantly from Control at p = 0.05

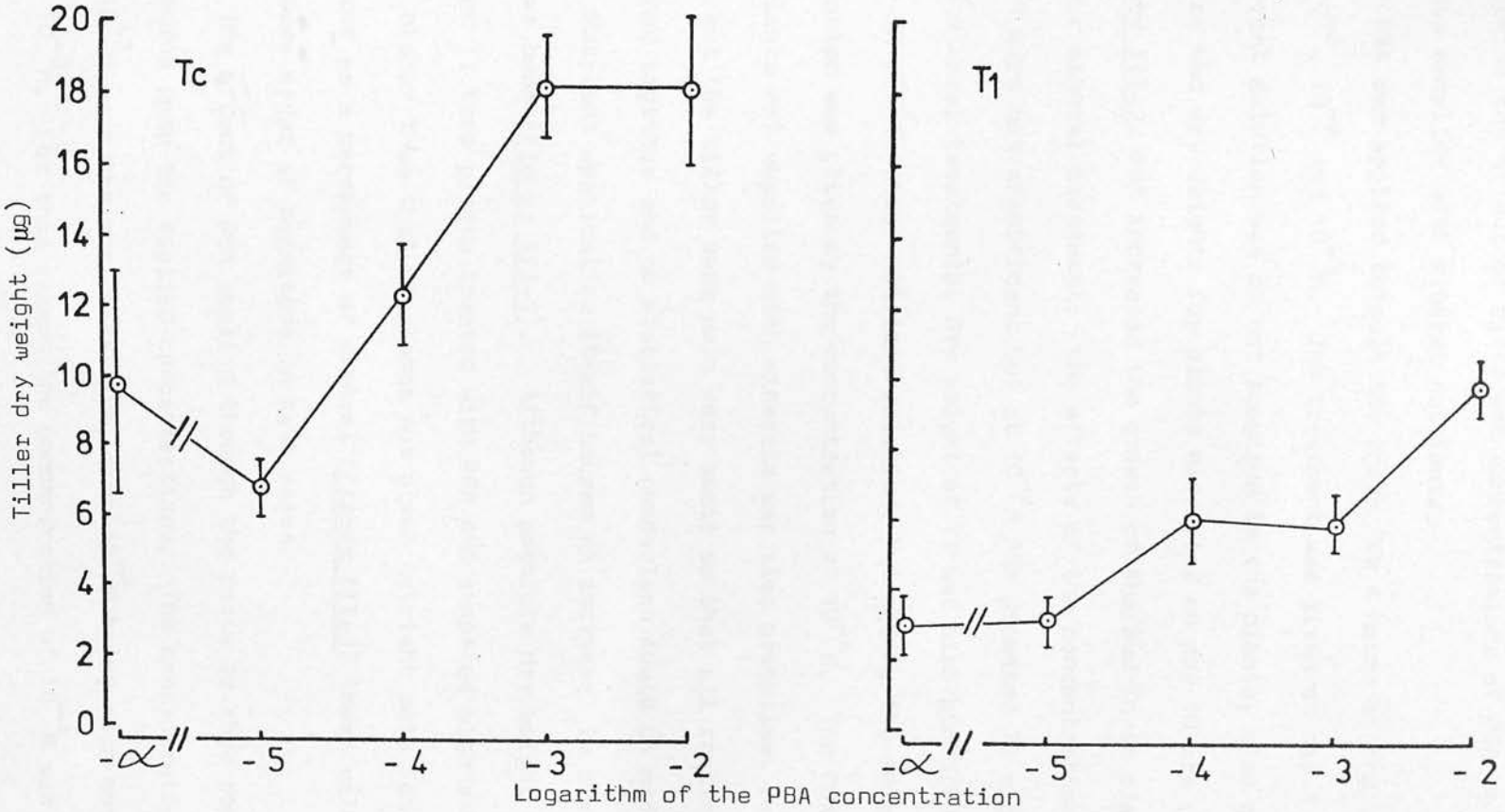

Figure III-1: Effect of PBA on dry weight of tiller buds Tc and T1 when applied through the roots at the concentrations of 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3}M. Plants were not supplied with mineral nutrients and harvested on day 10. Vertical bars indicate the confidence limits.

10^{-5}M the effect of PBA on T1 was significant but not on Tc, and at 10^{-4} and 10^{-3}M PBA promoted both Tc and T1 dry weight significantly.

A further experiment was designed to confirm these results and to determine the effect of different concentrations of PBA on tillers of plants supplied with mineral nutrients.

PBA was applied through the roots for 4 hours at the concentrations of 10^{-5}, 10^{-4} and 10^{-3}M. The treatment was given on day 4 and mineral nutrient solution was or not supplied to the plants, also on day 4. Tiller bud dry weights for plants harvested on day 10 are given in Figure III-2. PBA increased the growth of the bud Tc in plants given either mineral treatment; the effects of the concentrations of 10^{-5} and 10^{-3}M were not significant but at 10^{-4}M PBA promoted Tc significantly in both mineral treatments. Dry weight of T1 was also promoted by PBA treatment in plants given nutrient solution, but again, the only significant promotion was given by the concentration of 10^{-4}M. The PBA effect on T1 of plants not supplied with minerals was also promotive, but in the control set the tiller buds were very small so that all replicates were weighed together and no statistical comparison could be made.

Nutrient application itself caused an increase in dry weight of tiller buds (Figure III-2). Although absolute dry weight of tillers Tc and T1 from plants treated with PBA and supplied with minerals was much higher than that of plants not given nutrient solution, when considered as a percentage of control (Figure III-3) these values are of the same order of magnitude in both cases.

The effect of PBA applied through the roots is thus shown to be dependent upon the applied concentrations. The concentrations of 10^{-4} and 10^{-3}M were the most effective, with 10^{-4}M being even more effective than 10^{-3}M. For this reason the concentration of 10^{-4}M was used in most of the following experiments.

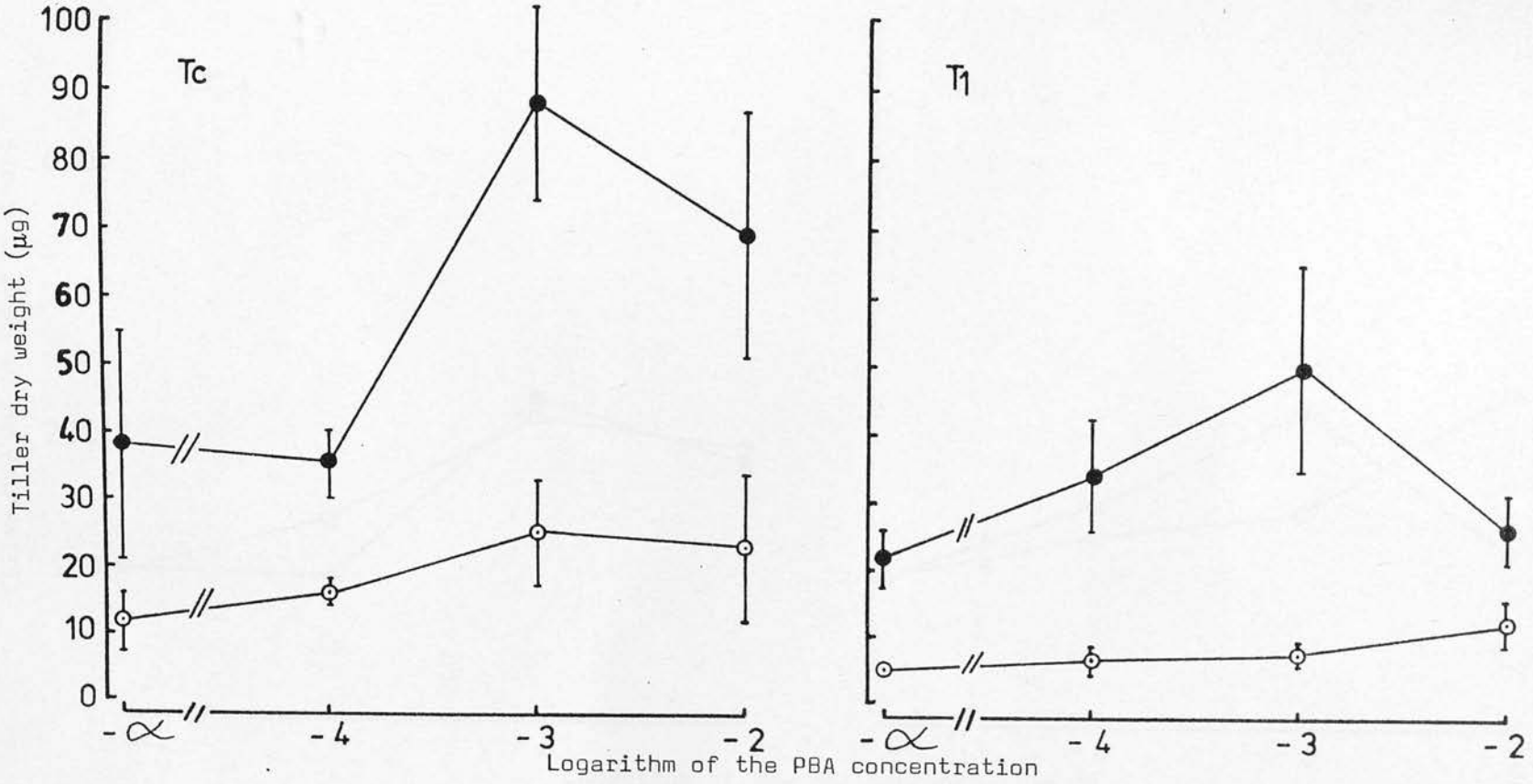

Figure III-2: Effect of PBA on the dry weight of tiller buds Tc and T1 when applied through the roots at the concentrations of 10^{-5}, 10^{-4} and 10^{-3}M. Plants were harvested on day 10.

● Minerals supplied ⊙ Minerals not supplied

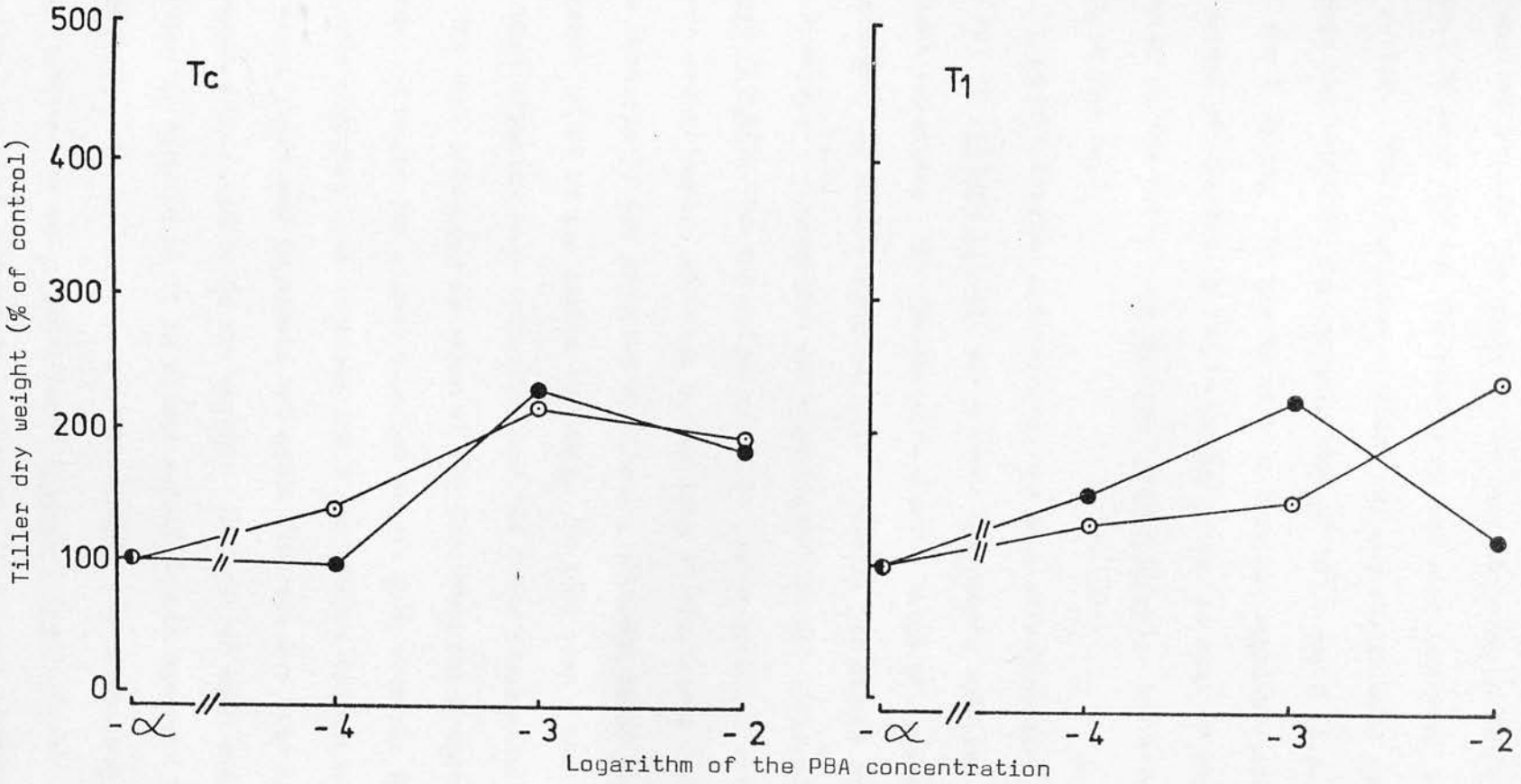

Figure III-3: Effect of PBA on the dry weight of tiller buds Tc and T1 when applied through the roots at the concentrations of 10^{-5}, 10^{-4} and 10^{-3}M. Plants were harvested on day 10.

● Minerals supplied ⊙ Minerals not supplied

(c) The effect of duration of treatment.

It was shown that the greatest promotion of tiller growth was by PBA applied through the roots at the concentration of 10^{-4}M. It was decided to look now for the best promotion when length of treatment was varied. The cytokinins kinetin, PBA and zeatin were applied through the roots at the concentration of 10^{-4}M for 0 (i.e. control), 2, 4 and 6 hours. The plants were or were not supplied with minerals. Dry weight of tillers Tc and T1 on day 10 was in most of the cases promoted by the cytokinins applied (Figure III-4); the effects were greatest for Tc.

In plants treated with kinetin the most effective time of treatment for Tc (Figure III-4A) was 4 hours for plants supplied or not with nutrient solution. The results with 2 and 6 hours of treatment were not significant for plants supplied with minerals; in plants not supplied with minerals kinetin promotion was significant for all times of application (Figure III-4A). The dry weight of T1 in plants supplied with minerals was not significantly affected by any time of treatment; in plants not given minerals T1 was promoted by kinetin, although again no statistical treatment could be applied to the data. In this case 4 hours of treatment were slightly more effective than the other treatments (Figure III-4B).

The most effective duration of PBA treatment for promotion of Tc was again 4 hours for plants supplied or not with minerals (Figure III-4C). In plants supplied with minerals the 2 and 4 hours treatment promotions were significant and in plants not given minerals any time of treatment increased significantly Tc dry weight. In contrast with the lack of promotion by kinetin of T1 in plants supplied with minerals PBA promoted T1 dry weight for all durations of treatment, the promotions by the 2 and 4 hours treatments being significant (Figure III-4D). Growth of T1 in plants not supplied with minerals (Figure III-4D) was promoted by any time of PBA application, although no statistical treatment of the data

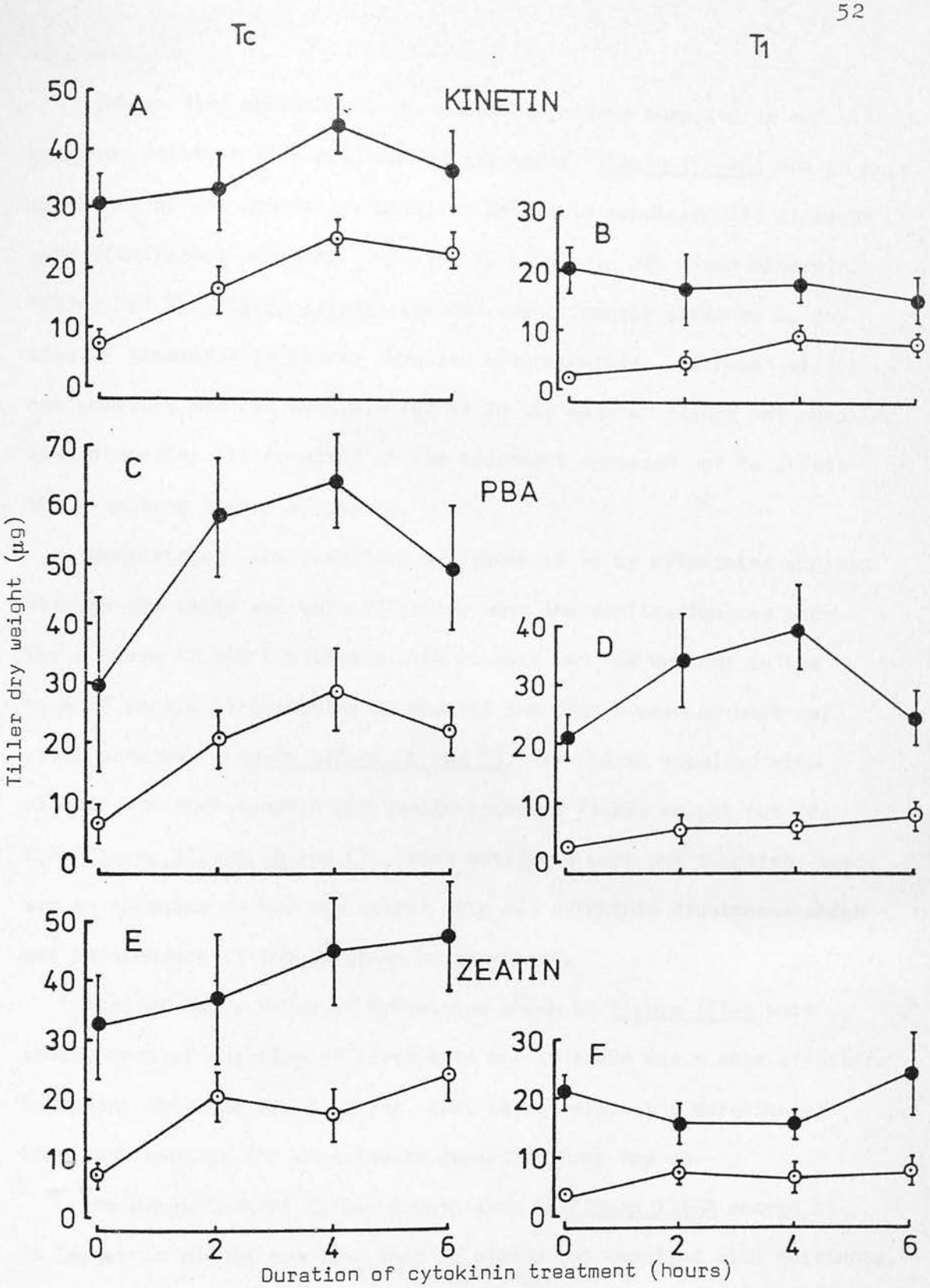

Figure III-4: Effect of kinetin (A; B), PBA (C; D) and zeatin (E; F) on the dry weight of tiller buds Tc (A; C; E) and T1 (B; D; F), when applied through the roots for 2, 4 or 6 hours at the concentration of 10^{-4}M. Plants were harvested on day 10.

● Minerals supplied ⊙ Minerals not supplied

was possible.

Zeatin also promoted Tc dry weight in plants supplied or not with nutrient solution with any time of treatment (Figure III-4E) but in this case none of the promotions obtained in plants supplied with minerals were significant but were significant for Tc in plants not given minerals. Tiller bud T1 (Figure III-4F) was not significantly affected by any time of treatment in plants supplied with minerals. Although statistical analysis was not possible for T1 in the case of plants not supplied with minerals, the duration of the treatment appeared not to affect the promoting effect of zeatin.

Summarizing, the promotion of growth of Tc by cytokinins applied through the roots was more effective when the application was made for 4 hours in the treatments with kinetin and PBA but not in the case of zeatin irrespective of whether the plants were or were not given minerals (Figure III-4A, C and E). In plants supplied with minerals neither kinetin nor zeatin promoted T1 dry weight but PBA did (Figure III-4B, D and F). When nutrients were not supplied, there was an increase in bud dry weight with all cytokinin treatments which was independent of the duration of treatment.

Most of the effects of cytokinins shown in Figure III-4 were independent of duration of treatments but if there was a more effective treatment this was for 4 hours; that is therefore the duration of treatment used in the experiments described from now on.

The promotions of tiller growth seen in Figure III-4 seemed to be larger in plants supplied than in plants not supplied with nutrients, but if the data are expressed as percentage of the control (Figure III-5), it is seen that the effects of cytokinins are actually larger in plants

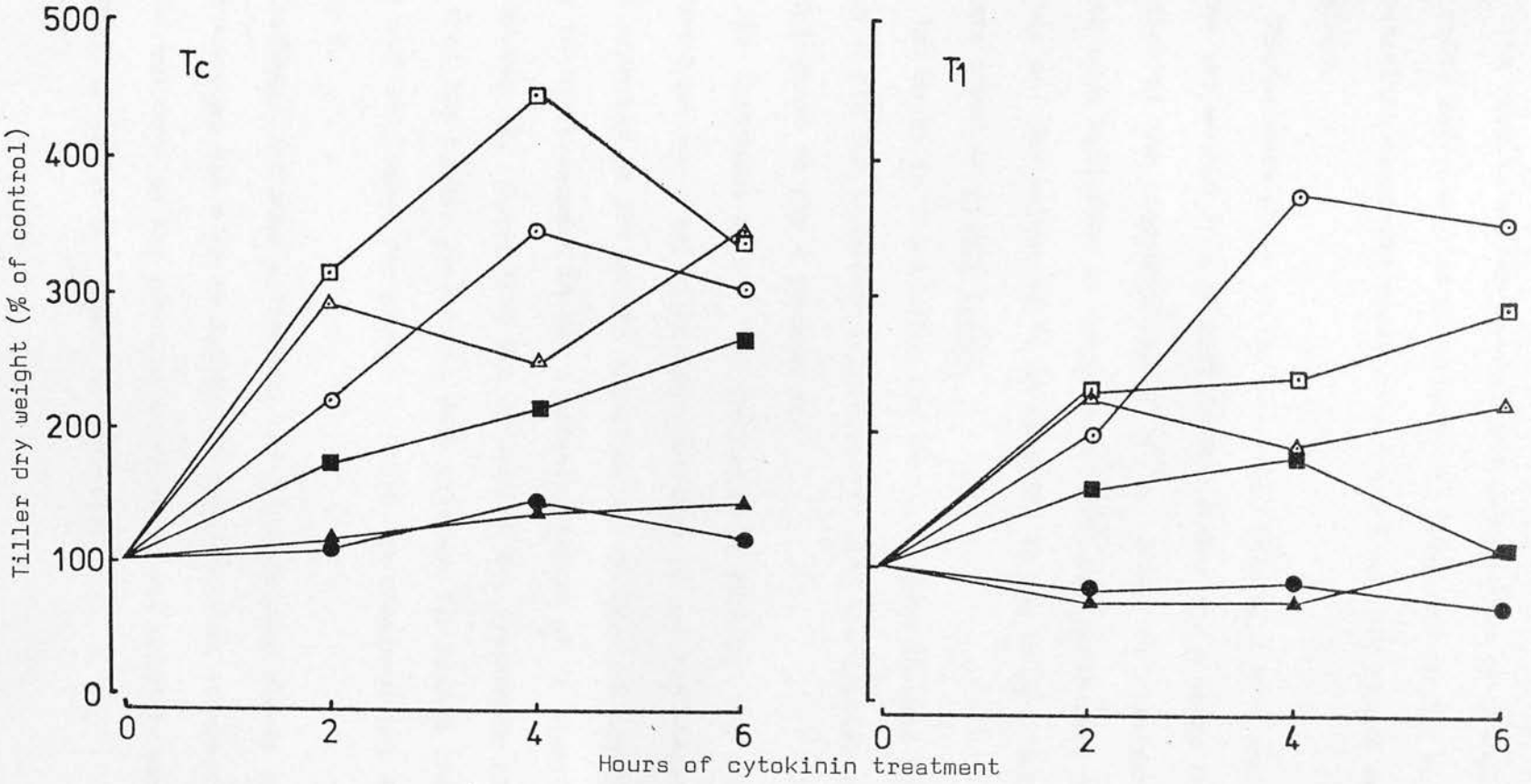

Figure III-5: Effect of kinetin, PBA and zeatin on the dry weight of tiller buds Tc and T1, when applied through the roots for 2, 4 or 6 hours at the concentration of 10^{-4}M. Plants were harvested on day 10. Closed symbols indicate plants supplied, and open symbols plants not supplied with mineral nutrients.

● ⊙ Kinetin ■ ⊡ PBA ▲ △ Zeatin

not supplied with minerals.

(d) The effect of time of treatment.

The results so far presented on application of cytokinins through the roots refer to plants subjected to treatment on day 4. The effect of cytokinin treatment applied to 6 and 8 day old plants was also examined.

Plants were grown in perlite until days 4, 6 or 8 when the root system was washed free of perlite and soaked for 4 hours in a PBA solution at the concentration of 10^{-4}M. After the treatment the plants were replanted in sand; no mineral nutrients were given. The effects on dry weight of Tc on days 10, 12 and 14 were determined and are shown in Figure III-6.

The delay in transfering the control plants to sand from day 4 to day 6 did not interfere significantly with the initial growth of Tc, but delay to day 8 reduced it.

PBA treatment at any time increased Tc growth; the effect of treatment on day 4 was significant on days 10 and 14; treatment on day 6 promoted Tc dry weight significantly on days 12 and 14, but delay in the treatment to day 8 promoted growth of Tc significantly only on day 12. Comparing the effects of PBA treatments it can be seen that the higher promotions were obtained for plants treated on day 4 and the lowest for plants in which the treatment was delayed to day 8.

Although not much difference was found between plants transfered and treated on day 4 or on day 6, it appeared that treatment on day 4 as it was done in the previous experiments was slightly more effective in promoting tiller bud growth in dry weight.

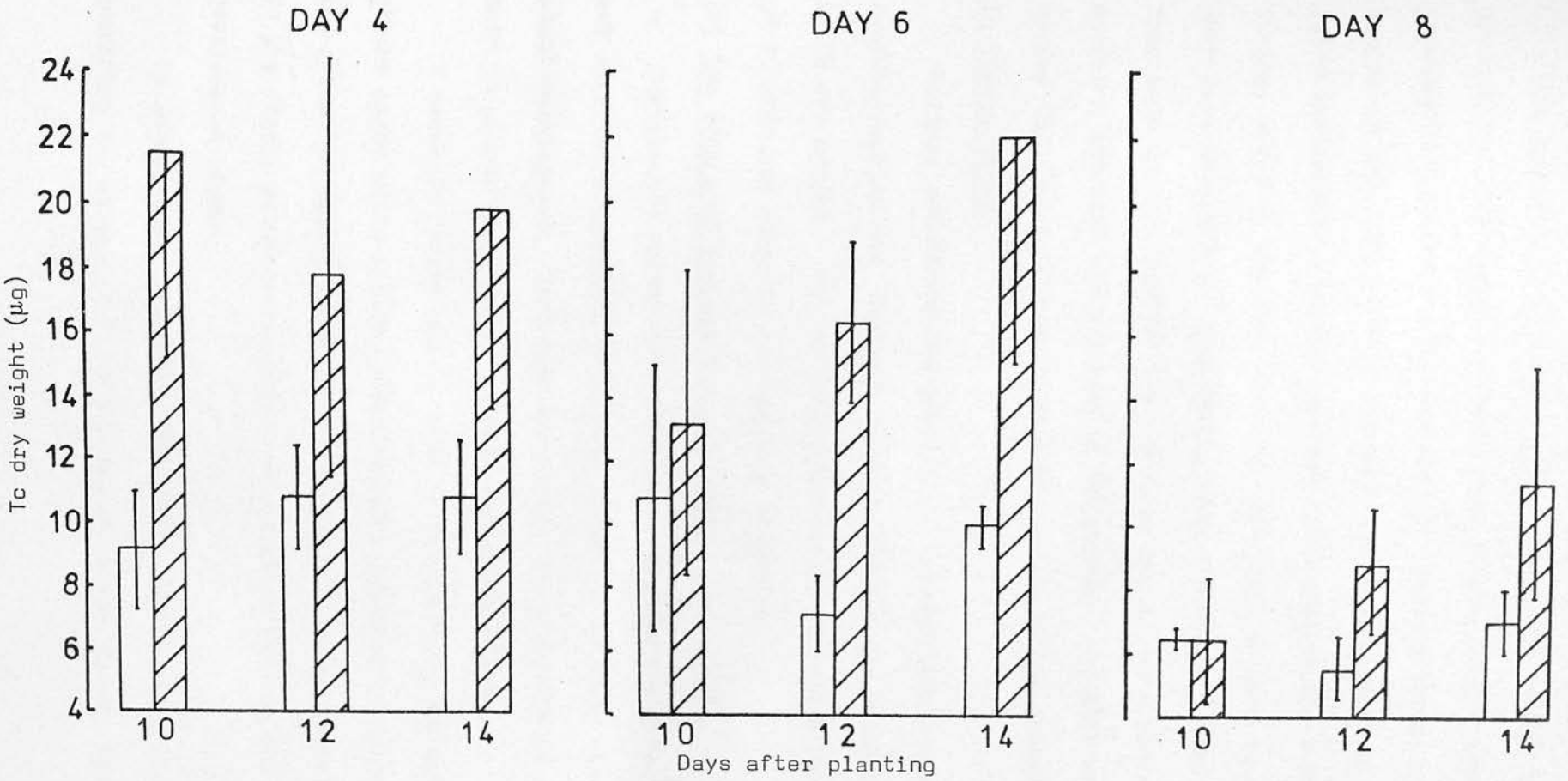

Figure III-6: Effect of PBA on the dry weight of tiller bud Tc when applied through the roots on days 4, 6 or 8, at the concentration of 10^{-4}M. Plants were not supplied with mineral nutrients and harvested on days 10, 12 and 14.

(e) The effect of some cytokinins and aminopurine derivatives.

The sensitivity of tiller buds to exogenous cytokinins applied through the root system has been shown in the results presented previously for kinetin, PBA and zeatin. Besides these, other cytokinins as BAP, 2iP, kinetin riboside and zeatin riboside, the substituted aminopurine 2-dimethylamino-6-hydroxypurine and 6-dimethylaminopurine, which do not show cytokinin activity, as well as adenine itself were also examined in relation to their effects on tiller bud growth. They were applied through the roots, on day 4, for 4 hours, and no nutrient solution was provided to the plants. Harvests were made on day 10. Dry weight of Tc as a percentage of the control is given in Figure III-7.

Neither adenine nor the substituted aminopurines 2-dimethylamino-6-hydroxypurine and 6-dimethylaminopurine had any significant effect on Tc dry weight. All the cytokinins tested increased the dry weight of Tc with BAP being by far the most effective.

(f) The effect of cytokinin on the whole plant growth.

The results presented previously have shown the effect of different cytokinins promoting tiller bud growth at an early stage of the plant development. They also show that this promotion is induced both in plants supplied or not with mineral nutrients.

A question arises now - what is happening to the other parts of the plant while tiller growth is being promoted by cytokinin? Is the effect on tiller growth only a consequence of a general effect on the plant, or does cytokinin have a special role on the tiller development alone?

To answer these questions, the effect of PBA was analyzed by examining dry weight of first and second leaves, bases, roots and

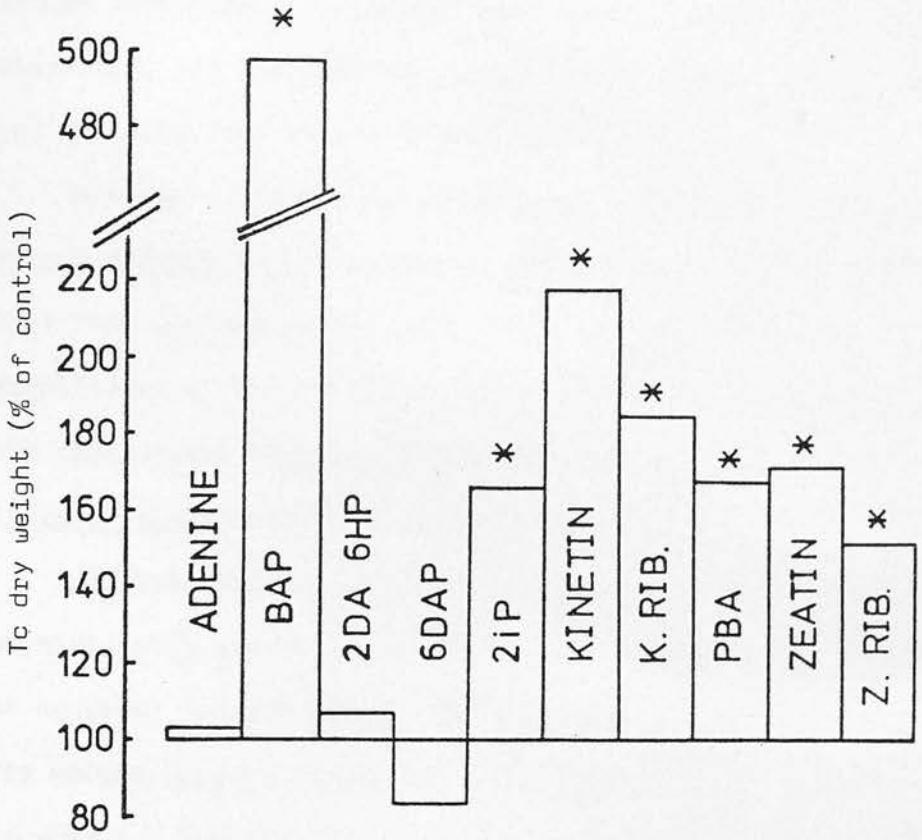

Figure III-7: Effect of different cytokinins and cytokinin analogues on the dry weight of tiller bud Tc, when applied through the roots at the concentration of 10^{-4}M. Plants were not supplied with mineral nutrients and harvested on day 10. Asterisks indicate values different from the control ($p = 0.05$).

total of the whole plant. Root lengths, and number of primary lateral roots were also determined.

Barley seedlings germinated in perlite were treated on day 4, through the roots, with PBA at 10^{-4}M, for 4 hours, before being replanted in sand. Mineral nutrients were or were not supplied. Plants were harvested on days 7, 9 and 11. At harvest, length of each axis of each plant was measured and the number of primary lateral roots longer than 1 mm were counted also for each plant axis. Plants were dissected, and the oven dry weights of first and second leaves, roots and the total dry weight determined.

Mineral nutrients increased significantly the first leaf dry weight (Figure III-8) on either day of harvest, and that of the second leaf from appearance on day 9. Application of nutrients had no significant effect on dry weight of the bases (Figure III-8) or of the root system (Figure III-9), but promoted the total dry weight (Figure III-9) significantly on day 11.

PBA treatment of plants not supplied with nutrients promoted significantly growth of the first leaf (Figure III-8) on either day of harvest; on the second leaf promotion was not significant. Base dry weight (Figure III-8) was not affected significantly by PBA treatment. The dry weight of the root system (Figure III-9) was significantly inhibited by PBA on days 9 and 11, and the total dry weight (Figure III-9) was not affected in plants not supplied with nutrients.

PBA treatment of plants supplied with nutrients increased significantly the first leaf dry weight only on day 9 (Figure III-8); treatment had no significant effect on the second leaf, base (Figure III-8), root, and on the total dry weight (Figure III-9).

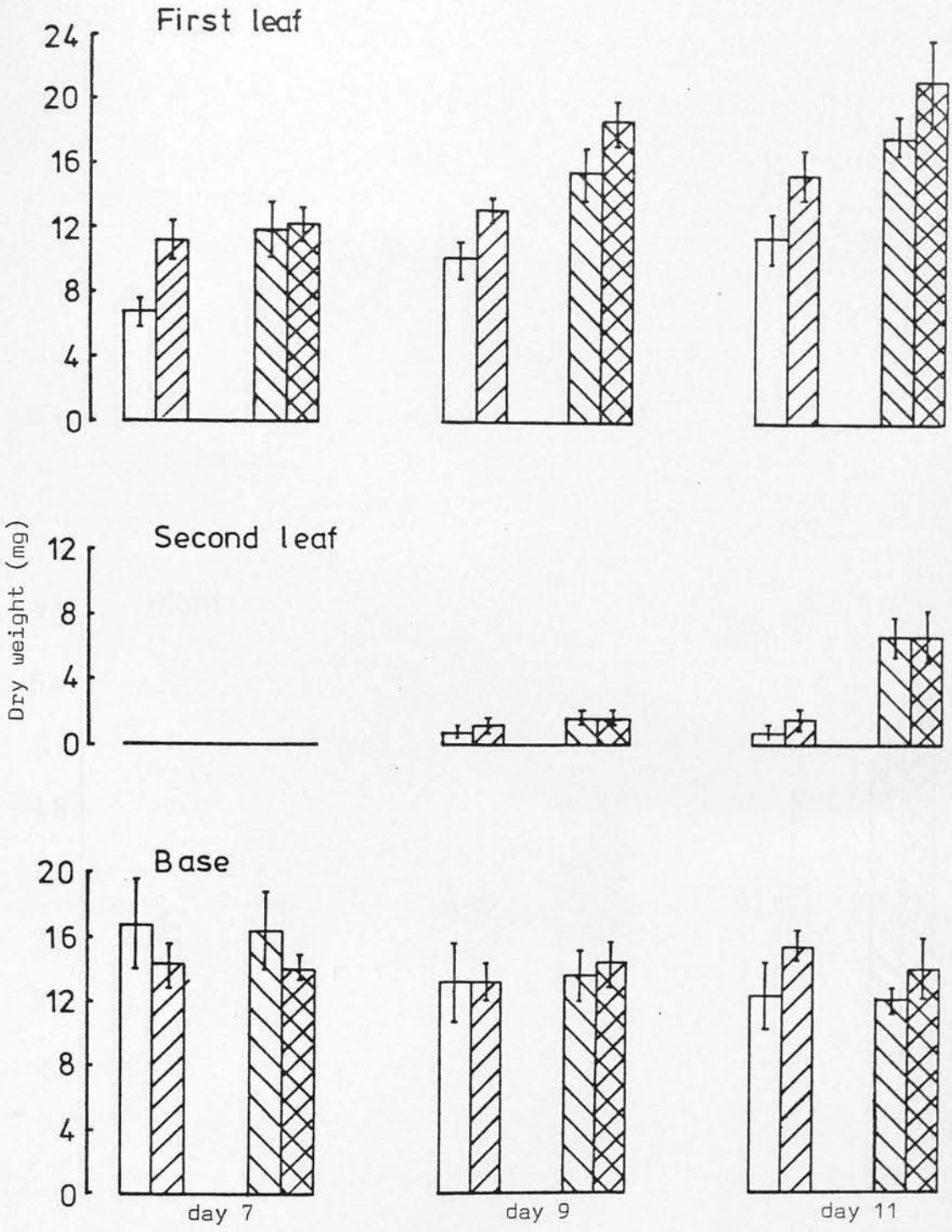

Figure III-8: Effect of PBA on the dry weight of first and second leaves and base, when applied through the roots at the concentration of 10^{-4}M. Plants were or were not supplied with mineral nutrients and harvested on days 7, 9 and 11.

Control Minerals

PBA PBA + Minerals

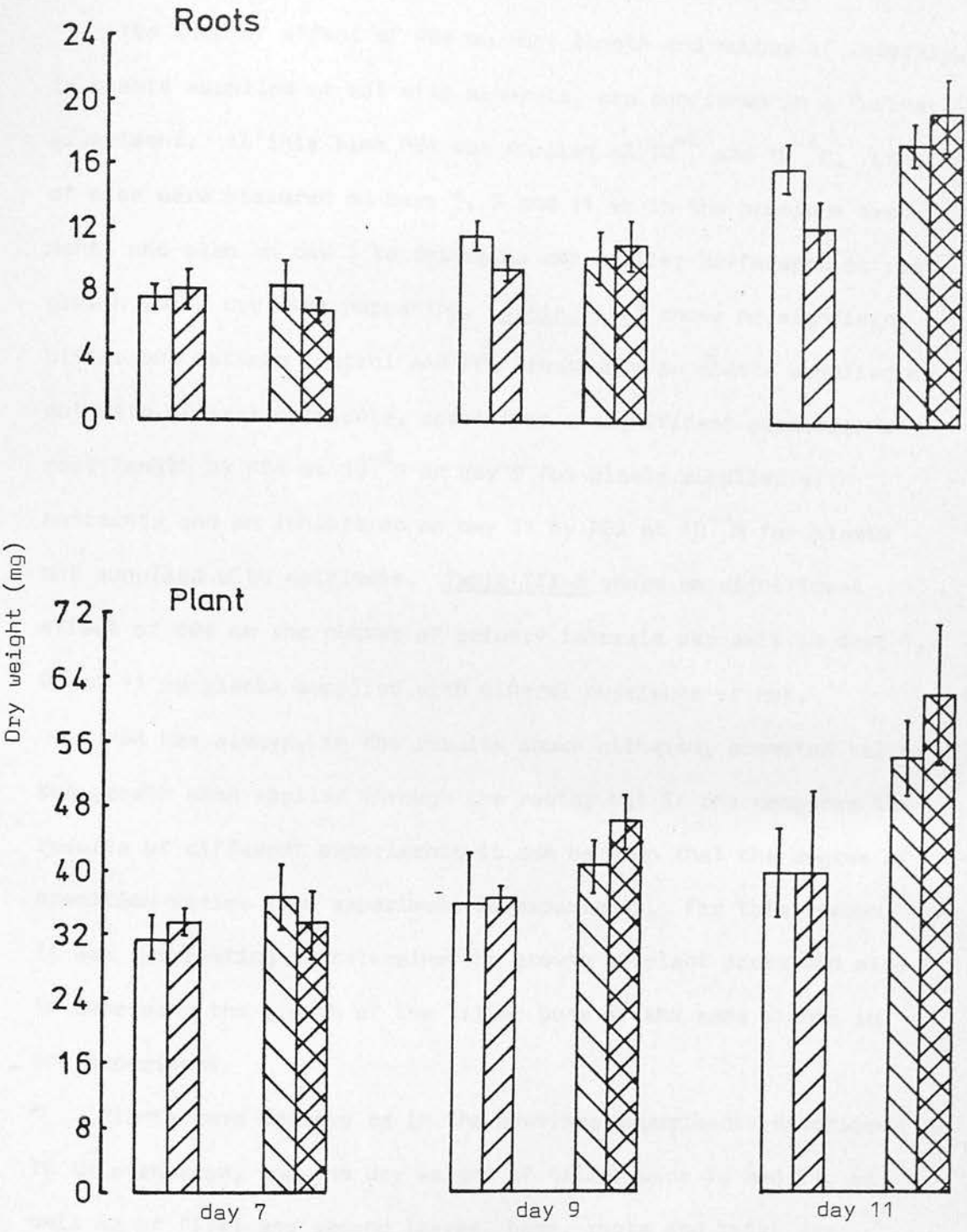

Figure III-9: Effect of PBA on the dry weight of roots and whole plant, when applied through the roots at the concentration of 10^{-4}M. Plants were or were not supplied with mineral nutrients and harvested on days 7, 9 and 11.

Control
Minerals
PBA
PBA + Minerals

PBA also had no effect on root axis length (Table III-5) and on the number of primary lateral roots (Table III-6) in plants irrespective of whether they were or were not given mineral nutrients.

The lack of effect of PBA on root length and number of laterals, in plants supplied or not with minerals, was confirmed in a further experiment. At this time PBA was applied at 10^{-6} and 10^{-4}M. Lengths of axes were measured on days 7, 9 and 11 as in the previous experiment, and also on day 5 to determine any earlier difference in root growth which could be happening. Table III-7 shows no significant difference between control and PBA treatments in plants supplied or not with mineral nutrients, apart from a significant promotion of root length by PBA at 10^{-6}M on day 9 for plants supplied with nutrients and an inhibition on day 11 by PBA at 10^{-4}M for plants not supplied with nutrients. Table III-8 shows no significant effect of PBA on the number of primary laterals per axis on days 7, 9 and 11 in plants supplied with mineral nutrients or not.

PBA has always, in the results shown hitherto, promoted tiller bud growth when applied through the roots, but if one compares the results of different experiments it can be seen that the degree of promotion varies from experiment to experiment. For this reason it was interesting to determine the growth of plant parts and also to determine the growth of the tiller buds of the same plants in one experiment.

Plants were treated as in the previous experiments described in this section, and the dry weight of tiller buds Tc and T1, as well as of first and second leaves, base, roots and total dry weight were determined on days 8, 10 and 12.

Mineral nutrients promoted significantly the dry weight of the

Table III - 5

The effect of PBA applied through the roots at the concentration of 10^{-4}M on root axis length (mm). Plant supplied or not with mineral nutrients and harvested on days 7, 9 and 11.

	Nutrients		No nutrients	
Harvest	Control	PBA	Control	PBA
day 7	84.1 ± 3.40	93.4 ± 2.82	89.5 ± 3.43	92.9 ± 2.15
day 9	83.4 ± 2.74	82.4 ± 2.06	83.8 ± 2.67	82.6 ± 3.10
day 11	94.4 ± 5.92	82.3 ± 2.99	87.5 ± 2.29	77.5 ± 2.82

Control and PBA values do not differ significantly at p = 0.05

Table III - 6

The effect of PBA applied through the roots at the concentration of 10^{-4}M on the number of primary laterals per axis. Plants supplied or not with mineral nutrients and harvested on days 7, 9 and 11.

	Nutrients		No nutrients	
Harvest	Control	PBA	Control	PBA
day 7	2.7 ± 0.26	3.0 ± 0.37	2.0 ± 0.35	3.1 ± 0.47
day 9	5.1 ± 0.87	4.3 ± 0.31	3.7 ± 0.43	4.0 ± 0.35
day 11	5.5 ± 0.78	4.7 ± 0.72	4.4 ± 0.67	3.4 ± 0.55

Control and PBA values do not differ significantly at $p = 0.05$

Table III - 7

The effect of PBA applied through the roots at the concentrations of 10^{-4} and 10^{-6}M on root axis length (mm). Plants supplied or not with mineral nutrients and harvested on days 5, 7, 9 and 11.

	Nutrients			No nutrients		
Harvest	Control	PBA 10^{-6}M	PBA 10^{-4}M	Control	PBA 10^{-6}M	PBA 10^{-4}M
day 5	90.6 ± 2.58	89.3 ± 2.57	90.8 ± 3.62	88.9 ± 1.38	93.3 ± 2.75	92.8 ± 1.06
day 7	81.6 ± 2.13	87.5 ± 4.22	90.3 ± 1.97	93.6 ± 5.07	89.3 ± 2.49	84.7 ± 2.47
day 9	80.2 ± 3.83	105.3 ± 7.48	84.9 ± 2.86	83.7 ± 0.75	110.5 ± 15.81	97.4 ± 9.02
day 11	88.4 ± 7.65	83.9 ± 3.91	75.7 ± 4.49	91.7 ± 2.90	91.3 ± 4.37	76.4 ± 2.82

Control and PBA values do not differ significantly at p = 0.05

Table III - 8

The effect of PBA applied through the roots at the concentrations of 10^{-4} and 10^{-6}M on the number of primary laterals per axis. Plants supplied or not with mineral nutrients and harvested on days 7, 9 and 11.

	Nutrients			No Nutrients		
Harvest	Control	PBA 10^{-6}M	PBA 10^{-4}M	Control	PBA 10^{-6}M	PBA 10^{-4}M
day 7	2.0 ± 0.83	2.2 ± 0.42	2.7 ± 0.58	1.0 ± 0.67	2.3 ± 0.54	1.2 ± 0.43
day 9	2.9 ± 0.96	*11.3 ± 2.68	3.8 ± 0.93	2.5 ± 0.56	6.1 ± 1.50	3.9 ± 0.54
day 11	4.2 ± 2.67	5.9 ± 1.33	2.7 ± 0.46	3.9 ± 1.45	3.1 ± 0.55	2.5 ± 0.55

* Value different from control at p = 0.05

first leaf on any day of harvest (Figure III-10), and of the second leaf on day 10, as soon as it appears in every treatment, and on day 12. Base dry weight was not affected significantly by nutrient application. Nutrients promoted root dry weight (Figure III-11), although the promotion was significant only on day 12, and also promoted the total dry weight the effect being significant for all harvest times.

In plants not supplied with nutrients the effect of PBA was to promote growth of the first and second leaves (Figure III-10), although the effects were significant only on day 10. Bases were not affected by PBA treatment. Root dry weight was always significantly reduced (Figure III-11) and the total dry weight not altered by PBA.

In plants supplied with nutrients PBA had no significant effect on the first leaf (Figure III-10) but reduced significantly the dry weight of the second leaf on days 10 and 12. No significant effect was found for the bases. Roots were significantly inhibited by PBA treatments on days 10 and 12 and so was the total dry weight (Figure III-11).

The effect of mineral nutrients on tillers was as usual to promote both Tc and T1 dry weights (Figure III-12). The effect of PBA in plants deprived of nutrients was to promote tiller dry weight significantly as expected. Growth of tillers of plants supplied with nutrients was also significantly promoted by PBA.

The main conclusions for these experiments can be summarized as follows.

The effect of nutrients, as expected, was always to increase not only tiller bud dry weight but also the dry weight of any of the other parts of the plant. Although root dry weight was increased by mineral nutrients, this increase was not due to increase in length of axis or

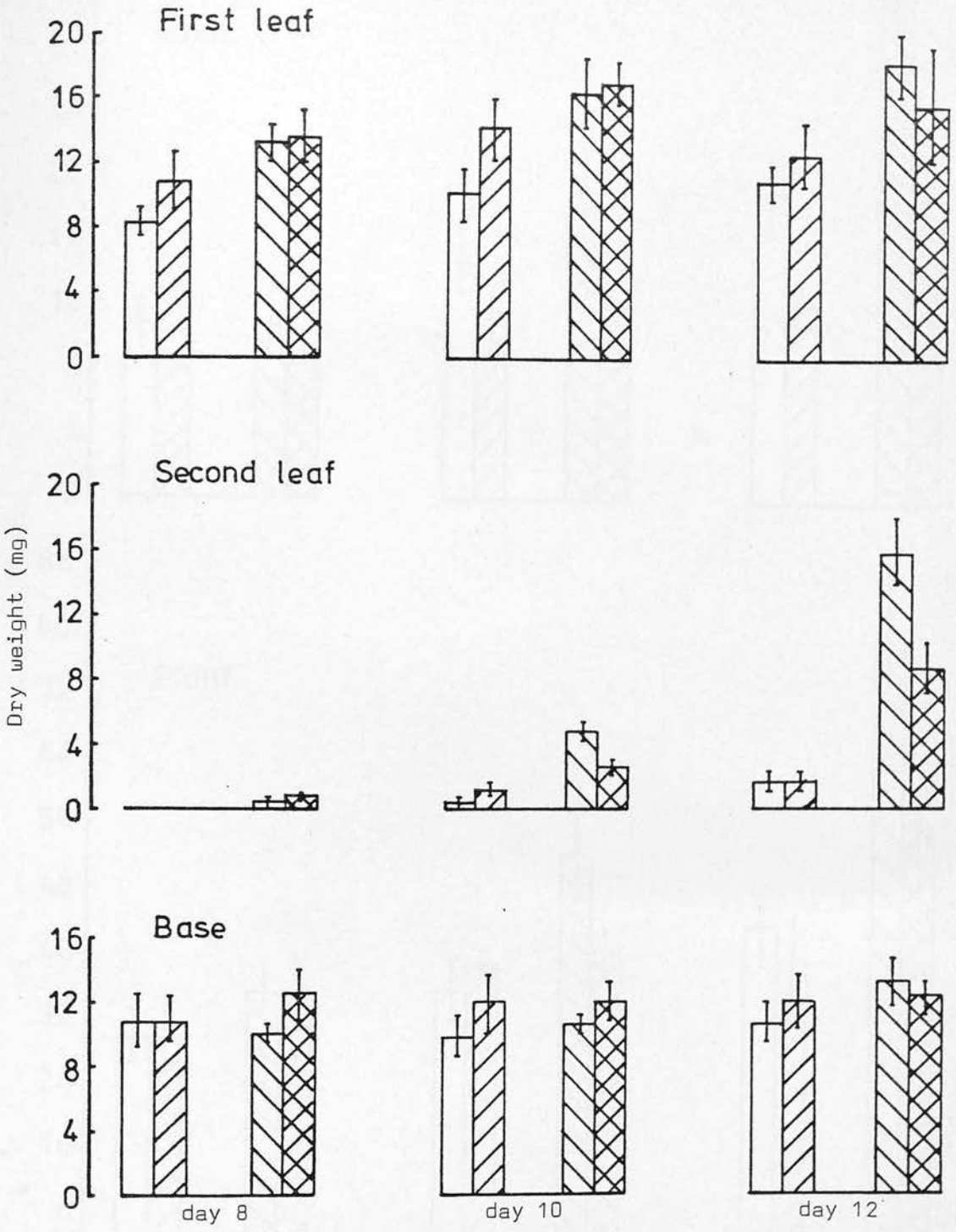

Figure III-10: Effect of PBA on the dry weight of first and second leaves, and base, when applied through the roots at the concentration of 10^{-4}M. Plants were or were not supplied with mineral nutrients and harvested on days 8, 10 and 12.

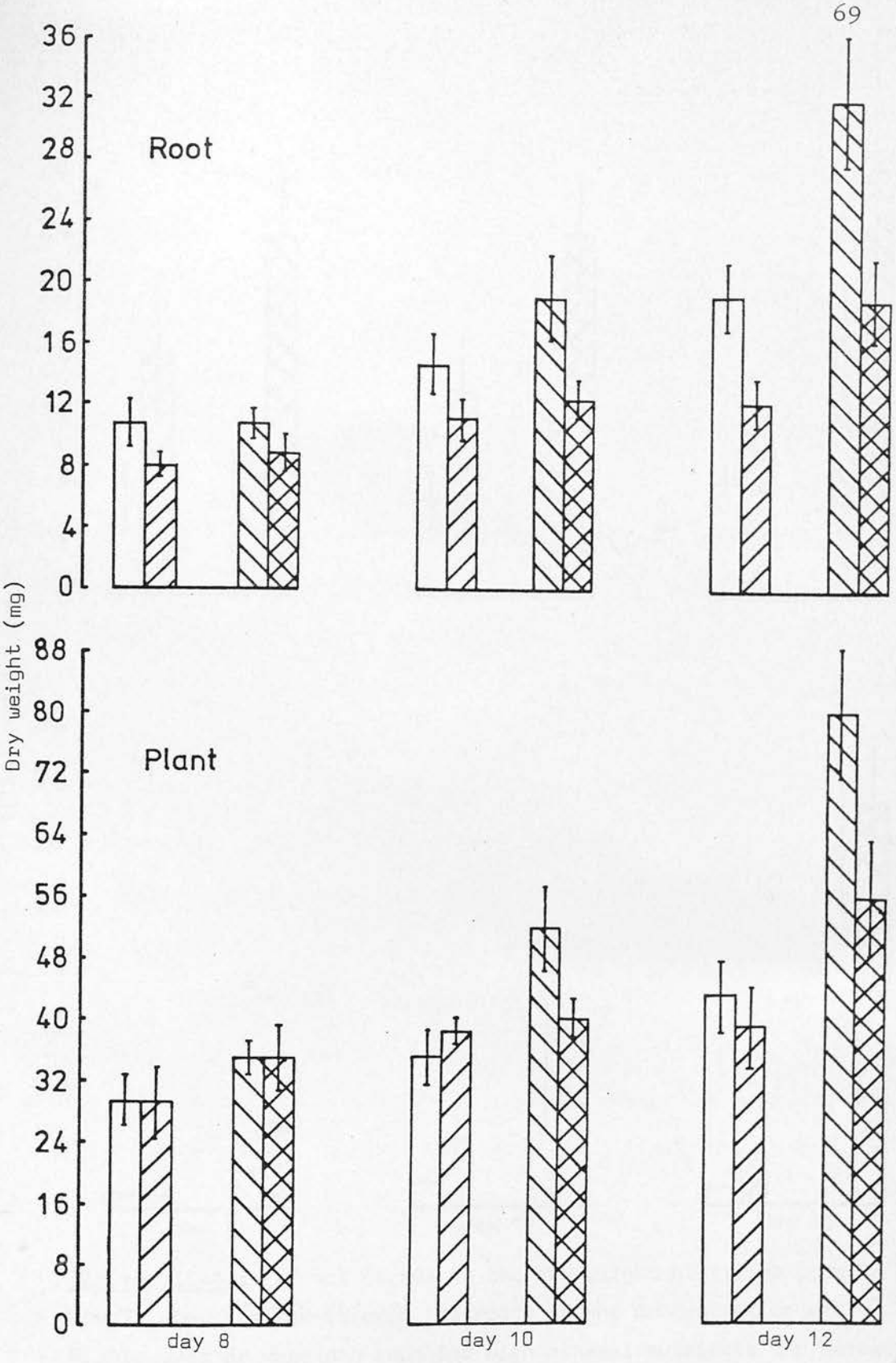

Figure III-11: Effect of PBA on the dry weight of roots and whole plant, when applied through the roots at the concentration of 10^{-4}M. Plants were or were not supplied with mineral nutrients and harvested on days 8, 10 and 12.

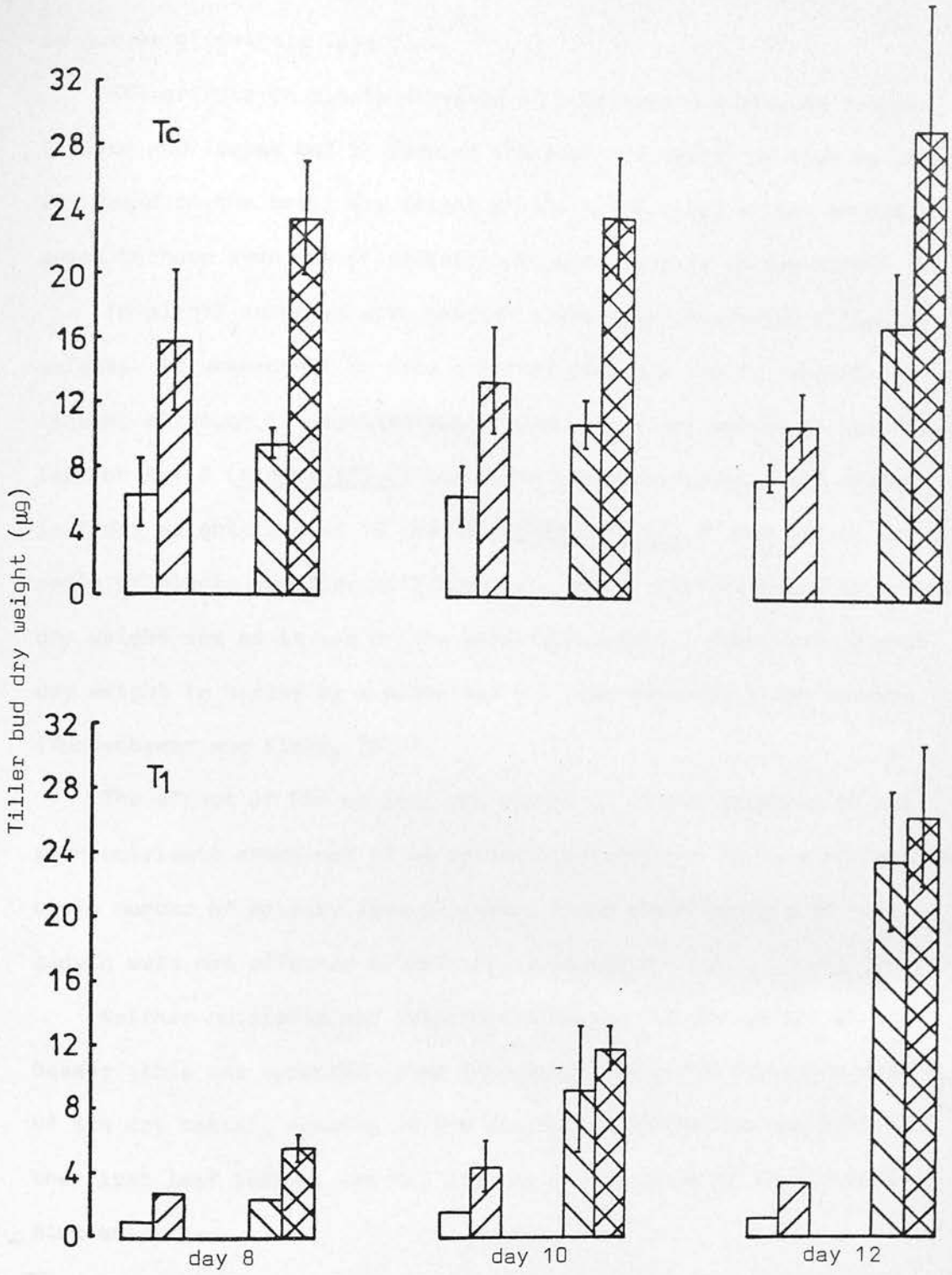

Figure III-12: Effect of PBA on the dry weight of tiller buds Tc and T1 when applied through the roots at the concentration of 10^{-4}M. Plants were or were not supplied with mineral nutrients and harvested on days 8, 10 and 12.

in number of primary laterals.

PBA effects on plants deprived of nutrients was also to promote tillers and leaves but it reduced the root dry weight so that no change was found in the total dry weight of the plant. The effect of PBA seems to have been one of redistributing dry matter in the plant.

In plants supplied with nutrients PBA again increased tiller dry weight; it seemed not to have affected strongly the dry weight of leaves, although it promoted significantly the dry weight of the first leaf on day 9 (Figure III-8) and inhibited significantly the second leaf dry weight on days 10 and 12 (Figure III-10). PBA effect on roots of plants supplied with nutrients when significant was to reduce dry weight and so it was on the total dry weight. Reduction of root dry weight in barley by a cytokinin has been found by other workers (Ruckenbauer and Kirby, 1973).

The effect of PBA on root dry weight in plants supplied or not with nutrients seems not to be related to reduction in axis elongation or in number of primary lateral roots, since these aspects of root growth were not affected by PBA treatment.

Neither nutrients nor cytokinin affected the dry weight of the bases; this was expected since the structures which represent most of its dry matter, remains of the caryopsis, coleoptile and part of the first leaf lamina, are not growing at the stage of development studied.

This set of experiments shows that both cytokinin and minerals availability act not only upon the tiller growth but also on the development of the plant as a whole.

(g) Discussion and conclusions.

Fletcher (1975), working with Proctor barley, showed that after

the initiation of the tiller bud primordium the phase of exponential growth is initiated only if the plants are supplied with mineral nutrients. This requirement for nutrients for tiller bud growth could be due either to a direct role of these nutrients on bud development in barley as proposed by the nutritive hypothesis of apical dominance (see Chapter I), or to an indirect effect of nutrients perhaps operating through a hormonal mechanism.

The results presented above show that even if mineral nutrients are not supplied to the plants the growth of tiller buds Tc and T1 can be initiated provided the plants are treated with cytokinin through the roots. Promotion of tiller bud growth by cytokinin shows that in mineral nutrient stress the limiting factor for bud growth is not nutrient availability. It is therefore possible that the promotive effect of nutrients on bud growth is through an effect on the level of endogenous cytokinin in the plant.

The largest values of tiller bud dry weight were obtained when plants supplied with nutrients were also treated with cytokinins (Figure III-2); this suggests that even in plants given nutrient solution the endogenous cytokinin level may not be optimum. The probable reason for this is that besides the exogenous cytokinin the plant also synthesises its own endogenous cytokinins resulting in a higher level of the active hormone, in a situation in which mineral nutrients or assimilates are not a limiting factor for growth. Although when treated with cytokinin much higher absolute values of dry weight are shown by plants supplied with nutrients (Figure III-2 and 4), the relative growth of tiller buds is actually the same (Figure III-3), or even lower than that for plants in nutrient stress (Figure III-5).

Although little has been published on the effects of exogenous cytokinins on tiller bud growth, a variety of methods of application have been described. Applications to the aerial parts of the plant were made successfully by hypodermic injection (Clifford and Langer, 1975) or by leaf spray (Johnston and Jeffcoat, 1977). None of these methods of treatment would be convenient in the case of the experiments we intended to do. Hypodermic injections, although very convenient since the exact amount of substance entering the plant is known and experiments can be repeated precisely, can not be used in experiments with very young plants since an injection at any position would cause serious damage. Leaf sprays, although an easy method of application have the disadvantage of being difficult to apply homogenously; Johnston and Jeffcoat (1977) used sprays but indicated a variability in effects between occasions.

Application of cytokinins as a drop over the scratched leaf had no effect on tiller bud growth; the reason for this could be the small amount of hormone applied or a problem of penetration or translocation.

Soaking the plant root system in cytokinin solution was shown to be an efficient method of treatment, but has the disadvantage that it is not possible to know how much of the hormone enters the plant; however, similar amounts of material might be expected to penetrate homogenous plants. Cytokinins are synthesized mainly by the root system (Kende and Sitton, 1967; Engelbrecht, 1972) from where they are distributed to the other parts of the plant with the xylem sap, in which cytokinins occur in large amounts (Kende, 1965). Thus these hormones can move freely in the xylem sap and exogenous cytokinins applied through the roots may move in this way as well.

Delay in transferring plants germinated in perlite to sand from day 4 to day 8 reduced the initial dry weight of tiller bud Tc (Figure III-6) in plants not supplied with nutrients. The reason for this is difficult to explain since initiation of tiller primordia is little affected by environmental factors (Evans, Wardlaw and Williams, 1964; Soper and Mitchell, 1956; Kirby and Faris, 1972; Fletcher, 1975). The considerable reduction in dry weight of Tc brought about by delaying the PBA treatment from day 4 to day 8 could be due to a delay in the response of the bud to the hormone itself, or it may be related to a lower growth rate of the bud primordium.

The increase in dry matter in Tc by treatment with PBA is larger than in tiller bud T1 at least until day 10, irrespective of whether the plants are or are not given mineral nutrients (Figure III-2); this does not mean that Tc has a larger potential for growth than T1, but is possibly related to differences in the initial size of these tillers. The capability of the tiller buds Tc and T1 in responding to cytokinin treatment seems to vary from one experiment to the other although all of them were performed under the same conditions.

Tiller bud growth in Proctor barley is promoted by the cytokinins BAP, 2iP, kinetin, kinetin riboside, PBA, zeatin and zeatin riboside. The higher activity of BAP compared with that of other cytokinins described in the literature (Skoog, Hamzi, Szweykowska, Leonard, Carraway, Fujii, Helgeson and Loeppky, 1967; Esashi and Leopold, 1969), is also found here where BAP promoted Tc dry weight by 500% (Figure III-7). Little difference was found in the activity of the other cytokinins tested on tiller bud growth. Substituted aminopurines without cytokinin activity such as 2-dimethylamino-6-hydroxypurine and 6-dimethylaminopurine are not active in promoting

bud outgrowth in barley. Adenine itself which has no activity in some tests (Esashi and Leopold, 1969), but has activity in the tobacco and soybean tests (Miller, 1968), is not effective in increasing tiller bud outgrowth in Proctor barley.

The promotion of tiller bud outgrowth in barley by cytokinin is not a unique effect on the plant. In plants supplied with nutrients cytokinin treatment has a slight effect on leaf and reduces root and total dry weight. The effect of cytokinin reducing root growth has been found by other workers (De Ropp, 1956; Harris and Hart, 1964; Ruckenbauer and Kirby, 1973). Reduction of root development by the hormonal treatment may well reduce the uptake of minerals and consequently the dry matter in leaves and in the plant as a whole.

Initial growth of seedlings occurs at the expense of endosperm reserves and using the products of photosynthesis in the first leaf. Cytokinin treatment of plants not supplied with nutrients reduces root development without altering total dry matter production. The effect of cytokinin on leaf growth may follow from the inhibitory effect on the roots, so that more dry matter is available for growth of the tops in this treatment.

To summarize, two effects of PBA treatment are found: namely, the promotion of tiller bud growth, and reduction of that of the root system. One could therefore explain the promotion of tiller bud growth as a result of the increased availability of dry matter, for the tillers, following the reduction in root growth, as was suggested by Johnston and Jeffcoat (1977); this may be relevant in the case of plants not supplied with nutrients when growth of the whole aerial part of the plant is promoted. But this explanation is less satisfactory for plants supplied with nutrients and cytokinins where the growth

of tiller buds is promoted with only slight effects on dry matter distribution in the rest of the plant. Here it is suggested that cytokinin concentration within the plant is raised to an optimal level to allow maximal growth of the buds.

Another point that can be made is that the dry matter increase in tiller bud as a result of PBA treatment is negligible when compared with the dry matter rendered available as a result of inhibition of root growth. For example, on day 12 (Figure III-12) PBA promoted an increase of about 3 μg on Tc dry weight of plants not supplied with nutrients, while on the same day, the effect of PBA (Figure III-11) was to reduce root dry weight by 7 mg; thus the amount of dry matter available for tiller growth is some 2000 times greater than that actually used.

These observations do not help the hypothesis that increase in tiller bud growth is an indirect effect of cytokinin through its inhibitory effect on root growth. The experiments in which cytokinin applied to plants supplied or not with mineral nutrients promotes tiller growth, independently from the promotion or inhibition of leaf growth, seem to support the hypothesis that cytokinin effect on tiller bud outgrowth is a primary and independent one.

2. The effects of other growth regulators

(a) Gibberellin.

Gibberellins are another group of growth regulators which may affect the tillering process. Exogenous GA_3 reduces (Jewiss, 1972; Johnston and Jeffcoat, 1977), while CCC increases (Jewiss, 1972; Wünsche, 1973), the number of tillers produced. Although tillering is restricted by treatment with GA_3, the extension of growing tillers

is actually promoted by this hormone (Jewiss, 1972).

The effect of GA_3 applied through the roots

Since the method of root application was successfully used for cytokinin treatment (Chapter III-1), it was also used for gibberellin.

The root system of 4 day old barley plants germinated in perlite was washed and soaked in GA_3 solutions at the concentrations of 10^{-7}, 10^{-5} and 10^{-3}M for 4 hours, before being replanted in sand. The plants were harvested on day 10.

GA_3 treatment had no significant effect on dry weight of tiller buds (Table III-9) where plants were supplied with nutrients on day 4. The dry weight of Tc and T1 for plants not given nutrients (Table III-10) was also unaffected by GA_3 treatment in two experiments; again, in these experiments as the tiller bud T1 was very small all the replicates were weighed together and statistical analysis was not made.

Treatment with GA_3 at 10^{-7} and 10^{-5}M of plants not supplied with nutrients (Figure III-13) had no effect on dry weight of the first and second leaves, bases, roots or whole plant. At the concentration of 10^{-3}M it promoted significantly growth of the second leaf and significantly inhibited root growth without affecting dry weight of the other parts of the plant or the total dry weight.

The effect of GA_3 applied through the leaves

The effects of GA_3 described above, indicate that treatment with exogenous GA_3 can affect plant development. Since this effect was quite slight, even with the highest concentration tested (10^{-3}M), another method of application was also tried.

Plants germinated in pots containing sand, supplied with nutrients on day 4, were treated on day 6 with GA_3 solution applied as a 5 µl drop placed over a couple of stratches made at the base of the first leaf

Table III - 9

The effect of GA_3 applied through the roots, at the concentrations of 10^{-7}, 10^{-5} and 10^{-3}M, on the dry weight (µg) on tiller buds Tc and T1. Plants were supplied with mineral nutrients and harvested on day 10.

	Tc	T1
Control	24.44 ± 2.81	13.17 ± 1.69
GA_3 10^{-7}M	22.92 ± 2.71	13.67 ± 2.38
GA_3 10^{-5}M	23.57 ± 2.62	17.89 ± 1.32
GA_3 10^{-3}M	20.74 ± 2.30	15.22 ± 1.87

Control and GA_3 values do not differ significantly at $p = 0.05$

Table III - 10

The effect of GA_3 applied through the roots, at the concentrations of 10^{-7}, 10^{-5} and 10^{-3}M, on the dry weight (µg) of tiller buds Tc and T1. Plants were not supplied with mineral nutrients and harvested on day 10.

	Tc		T1	
	exp. 1	exp. 2	exp. 1	exp. 2
Control	10.50 ± 0.98	9.67 ± 0.93	2.61	1.37
GA_3 10^{-7}M	9.25 ± 0.60	9.67 ± 0.98	2.71	2.50
GA_3 10^{-5}M	11.75 ± 0.63	10.39 ± 0.89	2.75	3.00
GA_3 10^{-3}M	10.75 ± 0.71	12.39 ± 0.95	2.50	2.67

Control and GA_3 values of Tc do not differ significantly at $p = 0.05$.
No statistical treatment was applied to T1 data.

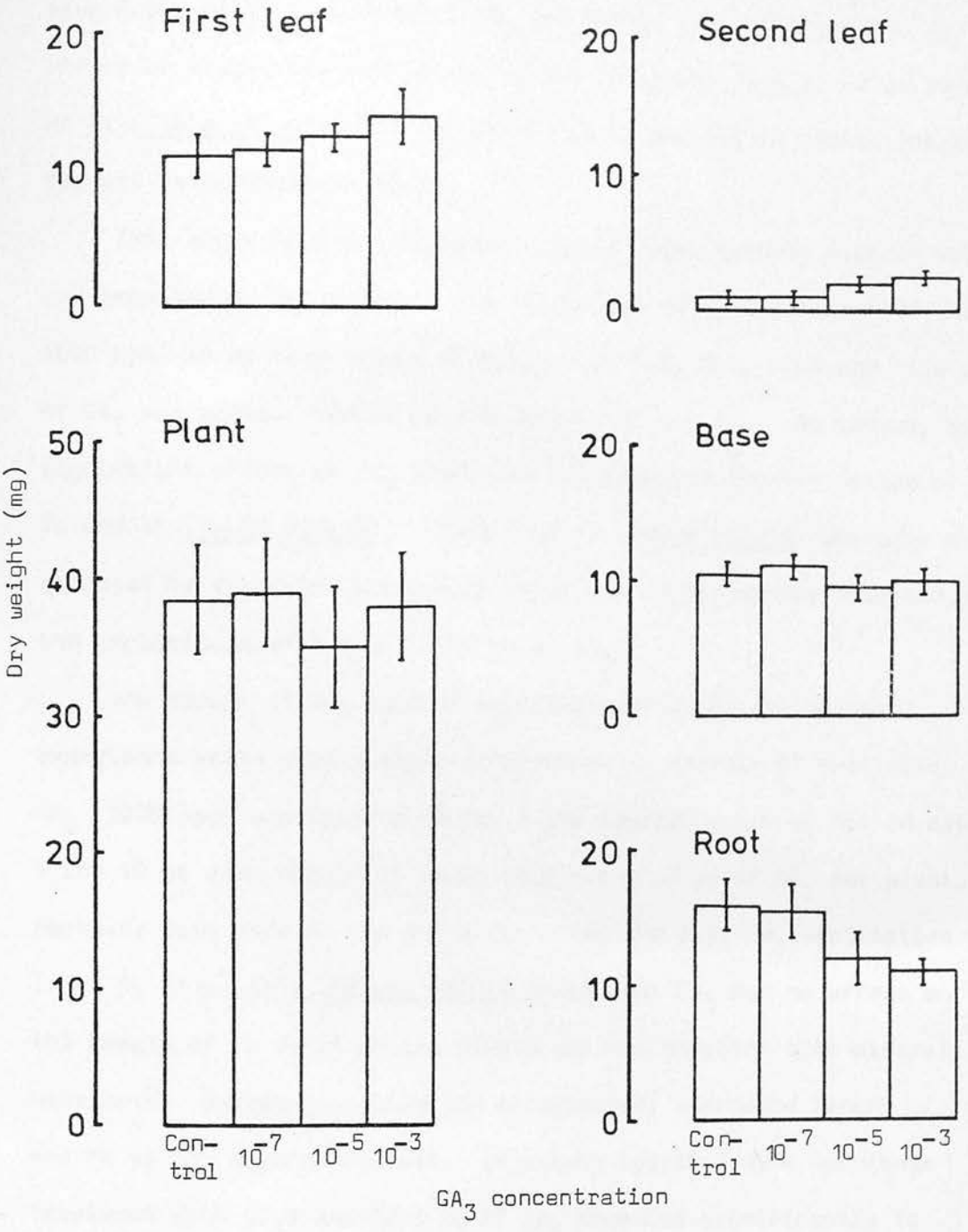

Figure III-13: Effect of GA_3 on the dry weight of first and second leaves, base, root and whole plant, when applied through the roots at the concentrations of 10^{-7}, 10^{-5} and 10^{-3}M. Plants were not supplied with mineral nutrients and harvested on day 10.

lamina. Different concentrations (10, 100 and 1000 ppm) were used to give 0.05, 0.50 or 5.00 µg of GA_3 per plant. These treatments had no effect on tiller bud dry weight by day 10 (Table III-11) or on length of T1 (Table III-12), but the length of Tc was significantly increased for all concentrations tested.

This experiment was repeated with GA_3 applications made on day 6 and repeated or not on days 8 and 10 in such concentrations (100 and 1000 ppm) as to give totals of 0.5, 1.0, 1.5, 5.0, 10.0 and 15.0 µg of GA_3 per plant. Plants were harvested on day 12. As before, no significant effect of GA_3 treatment was found on the dry weight of Tc and T1 (Table III-13). Length of T1 (Table III-14) was also not affected by treatment but length of Tc was significantly promoted by the application of 1.5 and 15.0 µg of GA_3.

The amount of GA_3 applied was increased again in the next experiment using plants grown in presence or absence of nutrients. GA_3 (2000 ppm) was applied on day 6 and applied again or not on days 8 and 10 to give totals of 10.0, 20.0 and 30.0 µg of GA_3 per plant. Harvests were made on the third day after the last GA_3 application (days 9, 11 or 13). Figure III-14 shows that GA_3 has no effect on the length of Tc or T1 if the plants are not supplied with mineral nutrients. Nutrient application as expected, increased length of Tc and T1 at all days of harvest. In plants supplied with nutrients treatment with 20.0 and 30.0 µg of GA_3 promoted significantly Tc length, and with 10.0 and 20.0 µg the length of T1. Figure III-15 shows that applications of GA_3 did not affect dry weight of Tc and T1 when nutrients were not given. At every day of harvest, as expected, increase in tiller dry weight promoted by mineral nutrient application was found. In plants supplied with nutrients, 20.0 and

Table III - 11

The effect of GA_3 applied to the leaf, at 0.05, 0.50 and 5.00 µg per plant, on the dry weight (µg) of tiller buds Tc and T1. Plants were supplied with mineral nutrients and harvested on day 10.

	Tc	T1
Control	35.11 ± 3.95	22.33 ± 2.59
GA_3 0.05 µg	43.37 ± 5.89	22.22 ± 2.29
GA_3 0.50 µg	38.72 ± 2.21	16.17 ± 0.89
GA_3 5.00 µg	39.84 ± 4.01	18.00 ± 1.03

Control and GA_3 values do not differ significantly at $p = 0.05$

Table III - 12

The effect of GA_3 applied to the leaf, at 0.05, 0.50 and 5.00 µg per plant, on the length (mm) of tiller buds Tc and T1. Plants were supplied with mineral nutrients and harvested on day 10.

	Tc	T1
Control	1.92 ± 0.13	1.23 ± 0.09
GA_3 0.05 µg	2.38 ± 0.29*	1.54 ± 0.09
GA_3 0.50 µg	2.21 ± 0.21*	1.26 ± 0.03
GA_3 5.00 µg	2.36 ± 0.18*	1.32 ± 0.05

*Values different from control at p = 0.05

Table III - 13

The effect of GA_3 applied to the leaf, at 0.5, 1.0, 1.5, 5.0, 10.0 and 15.0 μg per plant, on the dry weight (μg) of tiller buds Tc and T1. Plants were supplied with mineral nutrients and harvested on day 12.

	Tc	T1
Control	76.29 ± 8.79	65.81 ± 5.41
GA_3 0.5 μg	51.60 ± 4.73	62.11 ± 5.07
GA_3 1.0 μg	60.73 ± 7.71	58.39 ± 4.47
GA_3 1.5 μg	67.64 ± 6.46	63.00 ± 5.51
GA_3 5.0 μg	63.80 ± 9.51	67.05 ± 8.57
GA_3 10.0 μg	59.21 ± 4.36	53.78 ± 4.65
GA_3 15.0 μg	86.21 ± 8.79	63.55 ± 7.62

Control and GA_3 values do not differ significantly at $p = 0.05$

Table III - 14

The effect of GA_3 applied to the leaf, at 0.05, 1.0, 1.5, 5.0, 10.0 and 15.0 µg per plant, on the length (mm) of tiller buds Tc and T1. Plants were supplied with mineral nutrients and harvested on day 12.

	Tc	T1
Control	3.31 ± 0.27	2.78 ± 0.18
GA_3 0.5 µg	3.44 ± 0.50	3.12 ± 0.20
GA_3 1.0 µg	3.65 ± 0.61	3.00 ± 0.25
GA_3 1.5 µg	4.20 ± 0.35*	3.07 ± 0.13
GA_3 5.0 µg	3.90 ± 0.39	3.69 ± 0.45
GA_3 10.0 µg	3.31 ± 0.23	2.65 ± 0.13
GA_3 15.0 µg	4.72 ± 0.40*	3.35 ± 0.24

*Values different from control at p = 0.05

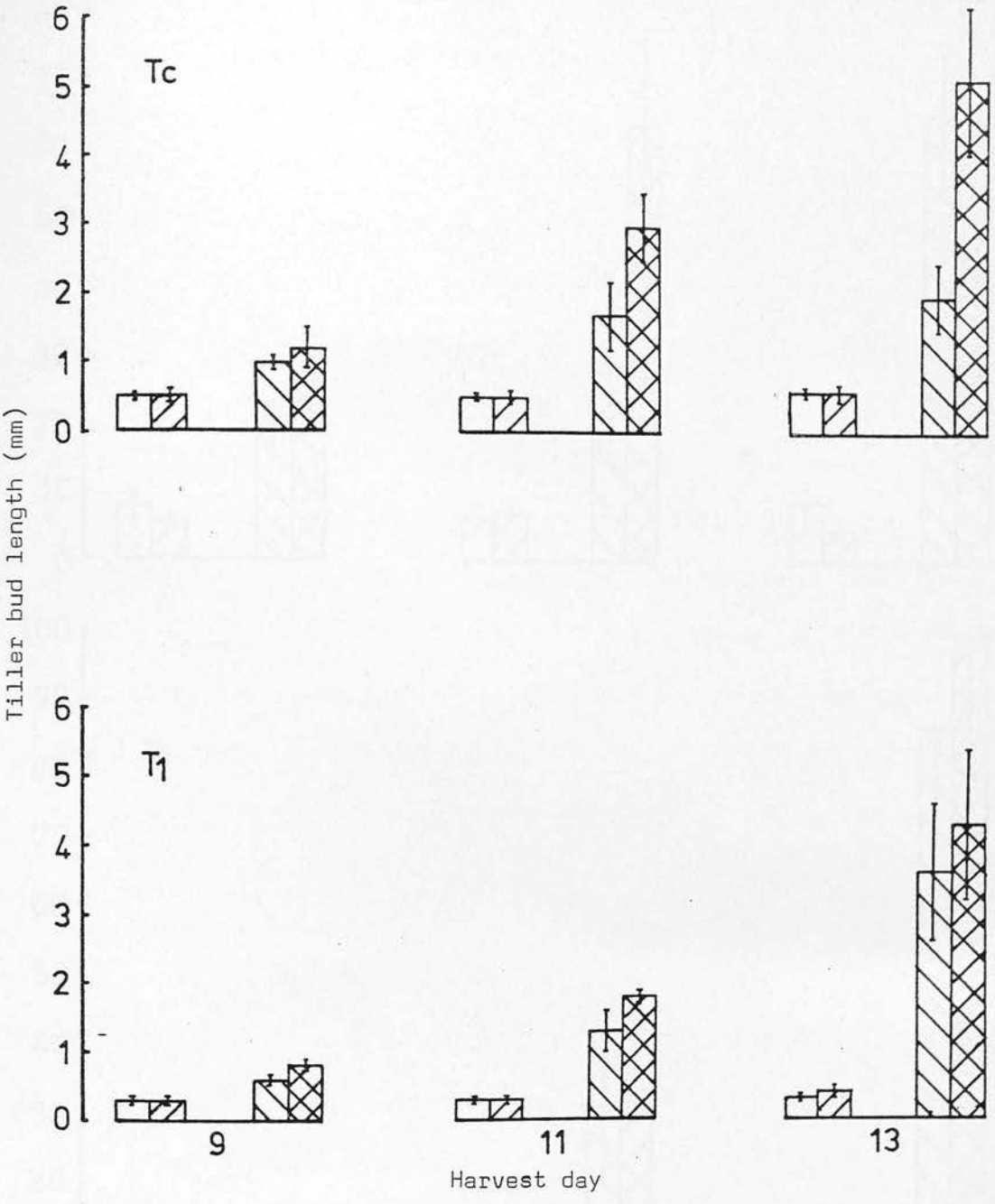

Figure III-14: Effect of GA_3 on the length of tiller buds Tc and T1, when applied through the leaves at 10.0, 20.0 and 30.0 µg per plant. Plants were or were not supplied with mineral nutrients and harvested on days 9 (10.0 µg), 11 (20.0 µg) and 13 (30.0 µg).

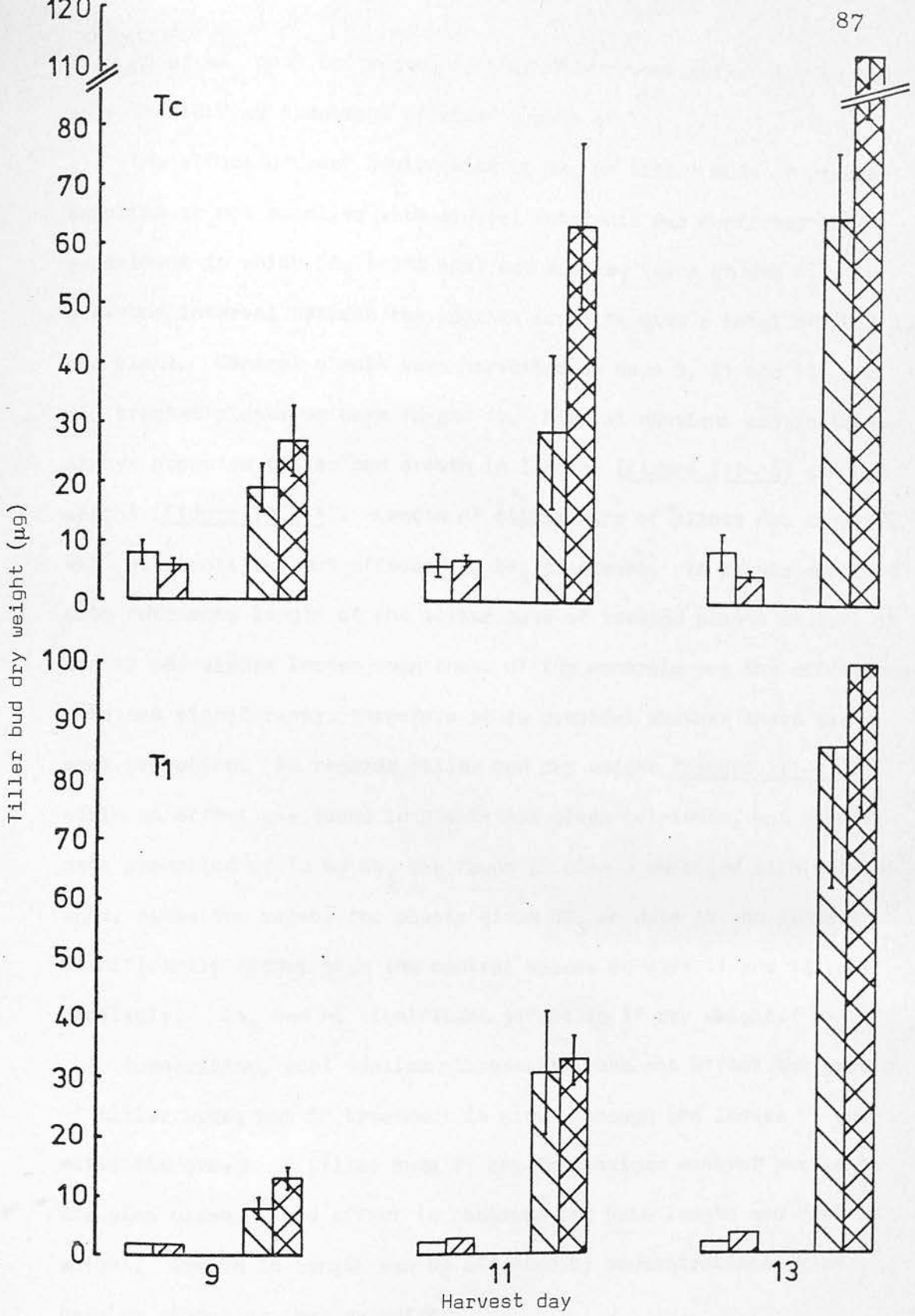

Figure III-15: Effect of GA_3 on the dry weight of tiller buds Tc and T1, when applied through the leaves at 10.0, 20.0 and 30.0 µg per plant. Plants were or were not supplied with mineral nutrients and harvested on days 9 (10.0 µg), 11 (20.0 µg) and 13 (30.0 µg).

Control
Minerals
GA_3
GA_3 + Minerals

30.0 µg of GA_3 both increased significantly dry weight of Tc; curiously only the 10.0 µg treatment affected growth of T1.

The effect of leaf application of GA_3 on tiller buds of plants supplied or not supplied with mineral nutrients was confirmed in an experiment in which GA_3 (2000 ppm) was applied twice on day 6, with a 4-hour interval between the applications, to give a total of 20.0 µg per plant. Control plants were harvested on days 9, 11 and 13, and GA_3 treated plants on days 10 and 12. Mineral nutrient application always promoted tiller bud growth in length (Figure III-16) and dry weight (Figure III-17). Length of tiller buds of plants not supplied with nutrients was not affected by GA_3 treatment. In plants supplied with nutrients length of the tiller buds of treated plants on days 10 and 12 was always larger than those of the controls but the differences were not significant; therefore it is doubtful whether there was a real promotion. As regards tiller bud dry weight (Figure III-17), again no effect was found in plants not given nutrients, but significant promotion of Tc by GA_3 was found in plants supplied with nutrients, since the values for plants given GA_3 on days 10 and 12 were significantly higher than the control values on days 11 and 13 respectively. GA_3 had no significant effect on T1 dry weight.

Summarizing, root applied gibberellin does not affect the growth of tiller buds, but if treatment is given through the leaves it promotes the growth of tiller buds T1 and Tc provided mineral nutrients are also given. This effect is observed for both length and dry weight. Growth in length can be affected by concentrations which have no effect on the dry weight.

(b) CCC (2-chloroethyl trimethylammonium chloride).

The results presented above confirmed the idea that gibberellins

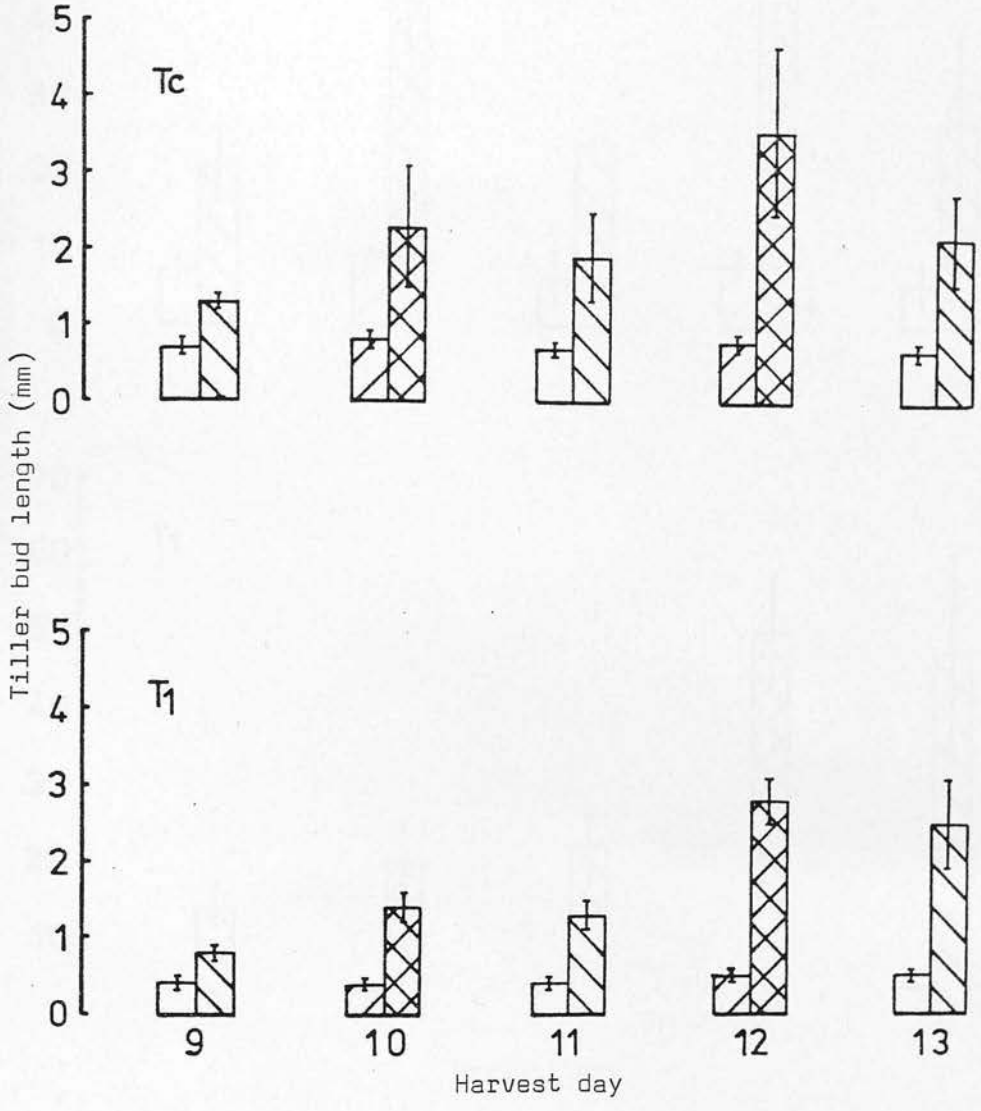

Figure III-16: Effect of GA_3 on the length of tiller buds Tc and T1, when applied through the leaves at 20.0 µg per plant. Plants were or were not supplied with mineral nutrients and harvested on days 9, 10, 11, 12 and 13.

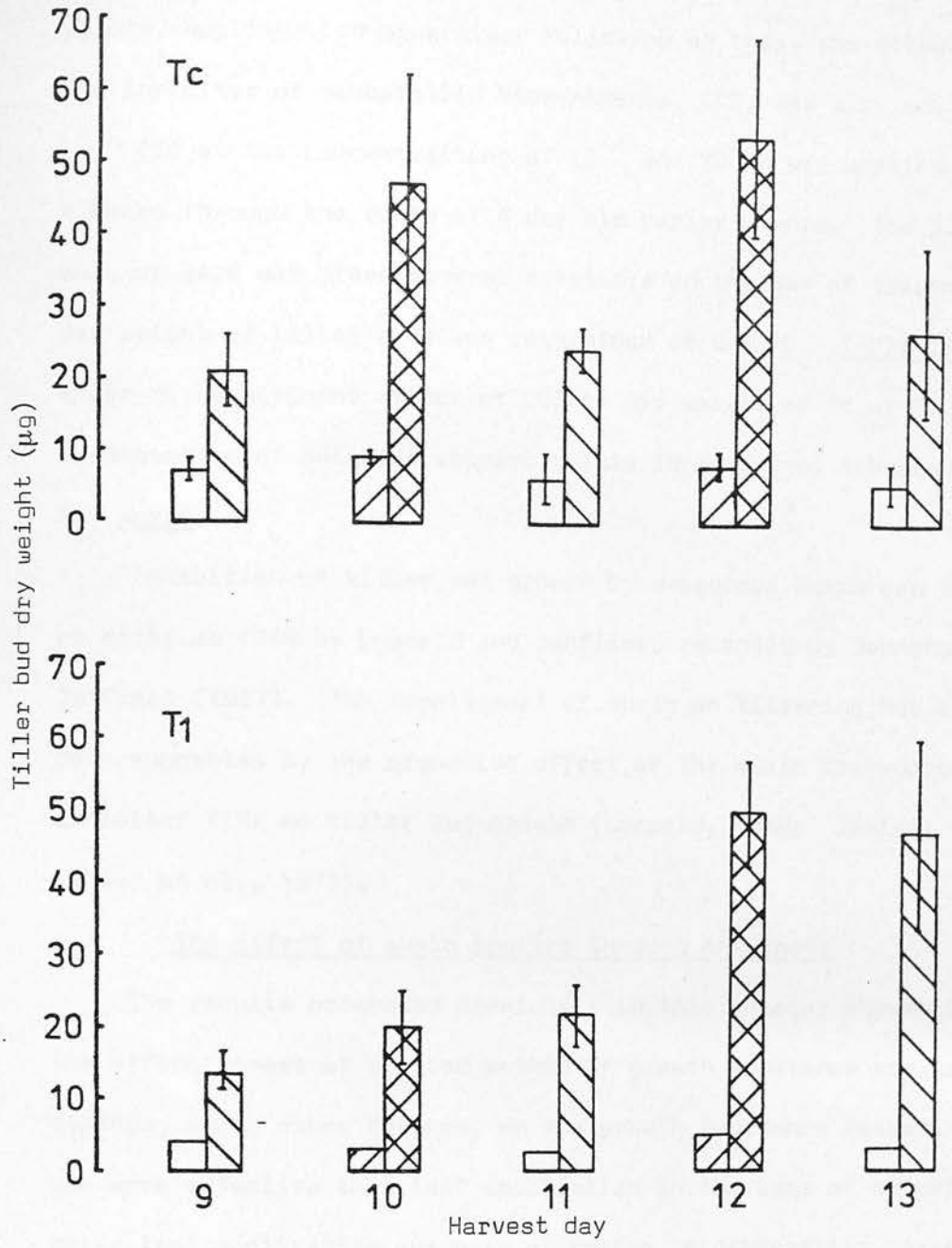

Figure III-17: Effect of GA_3 on the dry weight of Tc and T1, when applied through the leaves at 20.0 µg per plant. Plants were or were not supplied with mineral nutrients and harvested on days 9, 10, 11, 12 and 13.

may have a role in the tillering process in barley, at least in plants supplied with minerals. Following on this, the effect of the inhibitor of gibberellin biosynthesis, CCC, was also analysed.

CCC at the concentrations of 10^{-4} and 10^{-3}M was applied for 4 hours through the roots of 4 day old barley plants. The plants were or were not given mineral nutrients on the day of treatment. Dry weight of tiller buds was determined on day 10. Table III-15 shows no significant effect of CCC on dry weight of Tc or T1, irrespective of nutrient supply. This is discussed subsequently.

(c) Auxin.

Inhibition of tiller bud growth by exogenous auxin was shown as early as 1949 by Leopold and confirmed recently by Johnston and Jeffcoat (1977). The involvement of auxin on tillering has also been suggested by the promotive effect of the auxin transport inhibitor TIBA on tiller bud growth (Leopold, 1949; Jewiss, 1972; Langer et al., 1973).

The effect of auxin applied through the roots

The results presented previously in this chapter showed that the effectiveness of a given method of growth substance application depends, among other factors, on the growth substance tested. Root was more effective than leaf application in the case of cytokinin while leaf application was more effective in gibberellin treatments.

Root application was the first method tried for the auxin experiments. The root systems of 4 day old barley plants were soaked for 4 hours in IAA solutions at concentrations of 10^{-6} and 10^{-4}M. Plants were or were not supplied with mineral nutrient solution. Tiller bud dry weight (Table III-16) was not affected significantly by IAA treatment for plants supplied with nutrients. The initial small dry

Table III - 15

The effect of CCC applied through the roots, at the concentrations of 10^{-4} and 10^{-3}M, on the dry weight (µg) of tiller buds Tc and T1. Plants were or were not supplied with mineral nutrients and harvested on day 10.

	Tc		T1	
	+ nutrients	no nutrients	+ nutrients	no nutrients
Control	29.00 ± 3.45	7.08 ± 1.03	17.29 ± 1.88	3.50
CCC 10^{-4}M	28.07 ± 3.92	11.00 ± 1.10	18.29 ± 3.13	2.75
CCC 10^{-3}M	29.59 ± 5.80	9.29 ± 1.12	21.07 ± 2.33	3.06

Control and CCC values of Tc, and T1 '+ nutrients' do not differ significantly at $p = 0.05$.
No statistical treatment was applied to T1 'no nutrients' data.

Table III - 16

The effect of IAA applied through the roots, at the concentrations of 10^{-6} and 10^{-4}M, on the dry weight (µg) of tiller buds Tc and T1. Plants were or were not supplied with mineral nutrients and harvested on day 10.

	Tc		T1	
	+ nutrients	no nutrients	+ nutrients	no nutrients
Control	19.83 ± 0.33	8.81 ± 1.30	13.00 ± 1.59	3.25
IAA 10^{-6}M	24.36 ± 4.52	9.75 ± 0.37	14.29 ± 1.15	2.81
IAA 10^{-4}M	21.00 ± 2.29	6.00 ± 0.71*	15.71 ± 1.44	3.00

*Value different from control at p = 0.05.
No statistical treatment was applied to T1 'no nutrients' data.

weight of Tc for plants not supplied with nutrients was apparently inhibited by IAA at the concentration of 10^{-4}M but effects on T1 were slight. In an attempt to confirm the inhibition of growth of Tc by IAA, a similar treatment was given to plants not supplied with nutrients. Table III-17 shows no inhibitory effect of IAA on dry weight of Tc even at the concentration of 10^{-3}M. The earlier result (Table III-16) may thus be due to chance. T1 dry weight was also not affected by treatment.

The effect of auxin applied through the leaves

The method of auxin application through the leaves was also tried. A 5 µl drop of IAA solution was applied over two parallel scratches made in the base of the first leaf lamina, and another 5 µl drop was applied 4 hours later. Concentrations of 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3}M were tested in two experiments; in the first the treatment was given on day 6 and in the second, on day 4. Plants were not supplied with nutrients. No effect of IAA (Table III-18) on growth of the tiller buds was found at any concentration tested.

The effect of leaf applied IAA was also tested for plants supplied with nutrients. Plants were germinated in pots containing sand; a 5 µl drop of IAA solution was applied over the scratched first leaf lamina base twice a day with a 4-hour interval between applications, on days 4, or 4 and 6. IAA was applied at the concentration of 1000 ppm to give the totals of 10.0 or 20.0 µg of IAA per plant. IAA promoted significantly (Table III-19) the growth of Tc in both treatments but had no effect on the dry weight of T1.

Summarizing, root application of IAA had no effect on bud growth of plants supplied with nutrients nor of plants not supplied.

Table III - 17

The effect of IAA applied through the roots, at the concentrations of 10^{-5}, 10^{-4} and 10^{-3}M, on the dry weight (μg) of tiller buds Tc and T1. Plants were not supplied with mineral nutrients and harvested on day 10.

	Tc	T1
Control	7.93 ± 1.15	2.50
IAA 10^{-5}M	7.43 ± 0.62	2.86
IAA 10^{-4}M	8.86 ± 0.56	2.44
IAA 10^{-3}M	10.00 ± 0.44	1.87

Control and IAA values of Tc do not differ significantly at $p = 0.05$. No statistical treatment was applied to T1 data.

Table III - 18

The effect of IAA applied to the leaf, at the concentations of 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3}M, on the dry weight (μg) of tiller buds Tc and T1. Plants were not supplied with mineral nutrients and harvested on day 10.

	Tc		T1	
	exp. 1	exp. 2	exp. 1	exp. 2
Control	6.86 $\pm$ 0.77	8.93 $\pm$ 0.81	2.56	1.72
IAA 10^{-6}M	6.50 $\pm$ 0.88	-	1.36	-
IAA 10^{-5}M	-	8.43 $\pm$ 0.92	-	1.25
IAA 10^{-4}M	5.90 $\pm$ 0.40	8.57 $\pm$ 0.63	2.75	1.65
IAA 10^{-3}M	-	10.86 $\pm$ 0.68	-	1.56

Control and IAA values of Tc do not differ significantly at $p = 0.05$.
No statistical treatment was applied to T1 data.

Table III - 19

The effect of IAA applied to the leaf, at 10.0 and 20.0 μg per plant, on the dry weight (μg) of tiller buds Tc and T1. Plants were supplied with mineral nutrients and harvested on day 10.

	Tc	T1
Control	19.21 ± 1.61	15.14 ± 2.00
IAA 10.0 μg	23.43 ± 1.47*	14.29 ± 1.09
IAA 20.0 μg	31.36 ± 2.03*	14.07 ± 1.40

*Values different from control at p = 0.05

Application through the leaves was effective in promoting growth of Tc only when a high concentration was applied to plants supplied with nutrients.

(d) TIBA (2,3,5-triiodobenzoic acid).

To test in a different way the possible involvement of auxin in tillering, the effect of the inhibitor of auxin transport, TIBA, was studied. As aqueous solutions of TIBA at 10^{-4}M have low pH, the solutions prepared had their pH adjusted to the same as distilled water before being used. TIBA was applied at the concentrations of 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4}M for 4 hours, through the roots of 4 day old plants. Plants were not given mineral nutrients. Table III-20 shows no significant effect of TIBA on the dry weight of either Tc or T1.

The pH of TIBA at 10^{-4}M is as low as 3.5. Where it was not adjusted, there was a very strong promotion of growth in dry weight of tiller buds. This pH effect was also observed when plants supplied or not with nutrients were treated with distilled water adjusted to low pH with dilute HCl. This response of tiller buds to the pH treatment deserves further study.

(e) Discussion and conclusions.

Exogenous gibberellin affects the early development of tiller buds in young Proctor barley plants through a promotion in length and dry weight only where plants are supplied with mineral nutrients. Exogenous gibberellin acts therefore by promoting growth of tillers which are already growing, but it does not initiate the phase of exponential growth on tillers inhibited by mineral nutrient stress. A somewhat similar effect was found by Jewiss (1972) in spring wheat, where GA_3 application, to plants in which the second leaf had just

Table III - 20

The effect of TIBA applied through the roots, at the concentrations of 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4}M, on the dry weight (µg) of tiller buds Tc and T1. Plants were not supplied with mineral nutrients and harvested on day 10.

	Tc	T1
Control	8.07 ± 1.24	2.67
TIBA 10^{-7}M	8.21 ± 1.07	2.63 ± 0.28
TIBA 10^{-6}M	9.64 ± 0.52	5.06 ± 0.93
TIBA 10^{-5}M	8.07 ± 1.09	2.14 ± 0.42
TIBA 10^{-4}M	7.58 ± 0.58	2.33 ± 0.35

Control and TIBA values of Tc do not differ significantly at $p = 0.05$.
No statistical treatment was applied to T1 data.

emerged, resulted in extra extension growth in bud T1, which was already growing, and complete repression of T2, in which the phase of exponential growth had not started. His experiments differ from the ones described above in the fact that he had promotion and inhibition of tillers from the same plant and therefore subjected to the same mineral nutrient condition, although the nutrient and hormonal availability for each tiller in the plant could be different. Clifford and Langer (1975) also observed a promotion in the length and dry weight of tiller bud of Lolium multiflorum treated with GA_3 when the flag leaf was half expanded; simultaneously ^{14}C assimilates were found to accumulate in the bud. Johnston and Jeffcoat (1977) on the other hand observed an inhibition of tiller extension in oat and wheat supplied with nutrient solution when GA_3 application was made to young plants having 2-3 visible leaves present.

Although gibberellin may be involved in outgrowth of tiller buds, the lack of effect on the growth of tillers in plants not supplied with nutrients suggests that it is not the only factor acting on bud growth, but that other fundamental requirements are missing in this condition.

Jewiss (1972) was not able to derepress growth of T2, inhibited by GA_3, by applying sucrose to the bud and he concluded that starvation was not the reason for the inhibition. In the present case, although the plants were not given mineral nutrients, there were enough assimilates available for a certain amount of plant growth, but no growth was observed in buds despite the fact that very little dry matter is necessary for the start of bud development. It seems that here, also, the lack of bud outgrowth is not due to starvation.

It can be suggested that the missing factor for tiller outgrowth,

or for the lack of GA_3 effect on tiller outgrowth in plants not given nutrients is cytokinin, the level of which is known to be reduced in mineral defficient conditions (Wagner and Michael, 1969; Menary and Van Staden, 1976).

CCC did not affect the early growth of tiller buds in young barley plants given or not given mineral nutrient solution. Although the effect of CCC in increasing tillering has been extensively mentioned in the literature (Humphries, 1968; Bokhari and Younger, 1971a and b; Wünsche, 1972 and 1973), it was not expected to release tiller buds of plants not given nutrients since the inhibition of these tillers is unlikely to be a result of high levels of endogenous gibberellin.

No data seem to be available in the literature on the effect of CCC on growing tillers. The lack of effect of CCC on the development of tillers in plants supplied with nutrients could mean that the gibberellin necessary for its growth was already synthesized by the seedling before the CCC treatment or, most likely, was already present in the caryopsis. But the possibility that the amount of CCC that entered the plant was too low to inhibit gibberellin synthesis can not be ruled out even though treatment with CCC through the roots has usually been successful in reducing stem height and increasing tillering (Bokhari and Youngner, 1971a and b; Jewiss, 1972).

The absence of auxin promotion of the growth of tiller buds repressed by nutrient stress was expected since the role of auxin in grasses has been shown to be one of inhibiting tillering and tiller extension, as demonstrated by exogenous application of IAA (Johnston and Jeffcoat, 1977) or TIBA (Jewiss, 1972), rather than promoting tiller growth. The lack of TIBA effect on those tillers is certainly a more interesting result, since it suggests that the

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The absence of auxin promotion of the growth of tiller buds repressed by nutrient stress was expected since the role of auxin in grasses has been shown to be one of inhibiting tillering and tiller extension, as demonstrated by exogenous application of IAA (Johnston and Jeffcoat, 1977) or TIBA (Jewiss, 1972), rather than promoting tiller growth. The lack of TIBA effect on those tillers is certainly a more interesting result, since it suggests that the

repression of tiller growth in absence of nutrients may not be due to an inhibition by auxin, but rather related to other factors as has already been suggested.

Exogenous application of IAA in grasses has been shown to reduce tiller extension (Johnston and Jeffcoat, 1977), but in the data presented above for barley plants supplied with nutrients (Table III-19) the effect of IAA was to promote tiller dry weight. The apical dominance literature for dicotyledonous species also reports some examples of the increase in bud outgrowth by exogenous auxin, where the promotive or inhibitive effect of applied auxin is dependent on the concentration used (Šebánek, 1967).

As was found with GA_3, the promotive effect of IAA on tiller bud outgrowth is restricted to plants in which the apical dominance had been broken by nutrient treatment.

Treatment with GA_3 was effective on tiller buds only when the application was made through the leaves, probably because when this method was used, much higher amounts of the hormone must have entered the plant, since quite high concentrations were used. When applied through the roots GA_3 certainly entered the plant, and was translocated since an effect on plant development (Figure III-13) was noted for GA_3 applied this way. It is possible that the amount of GA_3 which entered the plant was too low to affect tiller growth.

As was found for GA_3, the promoting effect of IAA on the growth of tillers of plants supplied with nutrients was only found when leaf application was made at high concentration (Table III-19). Lack of effect of root treatment could be related to the fact that auxin transport is preferentially basipetal (Morris, Briant and Thomson, 1969; Goldsmith, 1969), so that very small amounts of the hormone

would be translocated from the roots to the aerial parts of the plant.

3. Interaction of other growth regulators with cytokinin

Chapter I discussed the idea that the growth relationship between apical and lateral buds are mediated by the influence of the various organs of the plant through a complex interaction of various hormonal factors and nutrient availability. The results presented in the previous sections of this chapter support those points, showing that apical dominance in Proctor barley is controlled by different growth substances interacting with nutrient supply. The studies described in this item were made in an attempt to help the understanding of the problem of apical dominance in barley through the observation of the effects of interactions between different exogenous growth substances.

(a) Auxin and cytokinin interactions.

Six day old plants were treated through the roots, for 4 hours, with PBA solution at 10^{-4}M. Plants were then replanted and treated with IAA at 10^{-6} and 10^{-4}M, applied as a 5 µl drop to the first leaf lamina base; the IAA treatment was repeated 4 hours later. Plants were not supplied with mineral nutrients. The dry weight of tiller buds Tc and T1 on day 10 is shown in Table III-21. IAA applied on its own had no effect on the dry weight of the tiller buds, as shown earlier (Table III-18). PBA treatment increased slightly the dry weight of tiller bud Tc, although this increase was not significant in this experiment. IAA and PBA applied together interacted significantly increasing dry weight of Tc. Again statistical analysis for the data of T1 dry weight was not possible, but they suggest that

Table III - 21

The interaction between IAA applied to the leaf, at the concentrations of 10^{-6} and 10^{-4}M, and PBA applied through the roots at 10^{-4}M on the dry weight (μg) of tiller buds Tc and T1. Plants were not supplied with mineral nutrients and harvested on day 10.

	Tc		T1	
	no PBA	+ PBA	no PBA	+ PBA
Control	6.86 ± 0.77	8.44 ± 0.59	2.56	3.44
IAA 10^{-6}M	6.50 ± 0.88	11.91 ± 1.19ab	1.36	3.93
IAA 10^{-4}M	5.90 ± 0.40	13.37 ± 0.96ab	2.75	6.04

a Values different from control 'no PBA' at p = 0.05.
b Values different from control '+ PBA' at p = 0.05.
For Tc the interaction IAA x PBA was significant at p = 0.05.
No statistical treatment was applied to T1 data.

for plants treated with both compounds the dry weight was also increased in relation to the control and PBA values. In a similar experiment (Table III-22) PBA treatment increased significantly the dry weight of Tc and again the treatment with IAA increased the promoting effect of PBA; the effect being significant at the concentration of 10^{-3}M. T1 dry weight was also increased by PBA treatment although not significantly and the effect of IAA on T1 was also to increase the PBA effect significantly at the concentrations of 10^{-7} and 10^{-3}M.

The same pattern of results shown above was also present when the growth substances were applied to plants on day 4 rather than on day 6 (Table III-23). IAA alone had no effect on tiller bud growth (as was also shown on Table III-18). PBA treatment promoted significantly the dry weight of Tc, and IAA interacted significantly with PBA at the concentrations of 10^{-4} and 10^{-3}M. No significant effect was found on T1 dry weight of plants treated with both PBA and IAA together.

The interaction between PBA and IAA on tiller bud growth was also studied by applying IAA through the roots. Four day old barley plants were treated through the roots with a solution containing PBA at 10^{-4}M and IAA at 10^{-5}, 10^{-4} or 10^{-3}M. The plants were not given mineral nutrient solution. Different concentrations of IAA applied alone did not affect the dry weight of tiller buds Tc or T1 (Table III-24). PBA, as expected, increased dry weight of both Tc and T1; the effects of IAA applied with PBA on Tc were not significant with the possible exception of an effect at 10^{-5}M which has not been confirmed. Growth of T1 was reduced when both compounds were applied together.

Table III - 22

The interaction between IAA applied to the leaf, at the concentrations of 10^{-7}, 10^{-5} and 10^{-3}M, and PBA applied through the roots at 10^{-4}M on the dry weight (μg) of tiller buds Tc and T1. Plants were not supplied with mineral nutrients and harvested on day 10.

	Tc	T1
Control	8.50 ± 0.63	3.75 ± 0.27
PBA	11.63 ± 1.55a	4.25 ± 0.82
PBA + IAA 10^{-7}M	13.63 ± 1.32a	6.13 ± 0.89ab
PBA + IAA 10^{-5}M	12.36 ± 2.48a	4.13 ± 0.45
PBA + IAA 10^{-3}M	19.31 ± 0.94ab	9.38 ± 0.30ab

a Values different from control at p = 0.05.
b Values different from PBA treatment at p = 0.05

Table III - 23

The interaction between IAA applied to the leaf, at the concentrations of 10^{-5}, 10^{-4} and 10^{-3}M, and PBA applied through the roots at 10^{-4}M on the dry weight (µg) of tiller buds Tc and T1. Plants were not supplied with mineral nutrients and harvested on day 10.

	Tc		T1	
	no PBA	+ PBA	no PBA	+ PBA
Control	8.93 ± 0.61	17.39 ± 2.29a	1.72	8.40 ± 1.03
IAA 10^{-5}M	8.61 ± 0.73	17.22 ± 2.21a	1.25	4.90 ± 0.55
IAA 10^{-4}M	8.56 ± 0.63	26.11 ± 2.25ab	1.65	10.25 ± 0.73
IAA 10^{-3}M	10.72 ± 0.54	24.42 ± 2.99ab	1.56	8.44 ± 1.27

a Values different from control 'no PBA' at p = 0.05.
b Values different from control '+ PBA' at p = 0.05.
For Tc the interaction IAA x PBA was significant at p = 0.05.
No statistical treatment was applied to T1 'no PBA' data.

Table III - 24

The interaction between IAA at the concentrations of 10^{-5}, 10^{-4} and 10^{-3}M and PBA at 10^{-4}M applied through the roots on the dry weight (µg) of tiller buds Tc and T1. Plants were not supplied with mineral nutrients and harvested on day 10.

	Tc		T1	
	no PBA	+ PBA	no PBA	+ PBA
Control	7.93 ± 0.99	19.44 ± 1.75a	2.50	9.25 ± 1.15
IAA 10^{-5}M	7.81 ± 0.51	23.00 ± 3.25ab	2.86	5.31 ± 1.20b
IAA 10^{-4}M	8.56 ± 0.62	17.29 ± 1.31a	2.44	6.87 ± 0.69b
IAA 10^{-3}M	9.75 ± 0.45	17.00 ± 1.61a	1.87	4.07 ± 0.83b

a Values different from control 'no PBA' at p = 0.05.
b Values different from control '+ PBA' at p = 0.05.
For Tc the interaction IAA x PBA was not significant at p = 0.05.
No statistical treatment was applied to T1 'no PBA' data.

(b) Gibberellin and cytokinin interactions.

Four day old plants were treated for 4 hours through the roots with a solution containing PBA at 10^{-4}M and GA_3 at 10^{-7}, 10^{-5} or 10^{-3}M. Plants were replanted in sand and no nutrients were given. The dry weight of Tc on day 10 was not affected by GA_3 applied alone at any of the concentrations tested (Table III-25), as shown before (Table III-10). PBA treatment, as usual, promoted tiller bud Tc dry weight significantly and this promotion was increased significantly by GA_3 treatment at the highest concentration (10^{-3}M), although variation was such that the analysis of variance did not show a statistically significant interaction. PBA also promoted T1 dry weight but no additional effect of GA_3 was found in plants treated with PBA. The effect of PBA and GA_3 applied individually and together on different parts of the plant is shown in Table III-26. GA_3 on its own, as expected, promoted the dry weight of the first and second leaves, reduced root dry weight and had no effect on bases nor on the whole plant dry weight. PBA on its own also significantly increased dry weight of first and second leaves and reduced root dry weight without affecting base or total dry weight. GA_3 treatment did not modify the promoting or inhibitory effects of PBA on different parts of the plant.

The interaction between PBA and GA_3 on tiller bud dry weight was confirmed (Figure III-18) in an experiment in which plants were treated on day 4 with both PBA at 10^{-4}M and GA_3 at 10^{-3}M through the roots, and not given mineral nutrients. As expected, GA_3 alone did not affect and PBA promoted tiller bud growth. GA_3 treatment increased the PBA promotion of growth on both Tc and T1, although on T1 the effect was not significant.

Table III - 25

The interaction between GA_3 at the concentrations of 10^{-7}, 10^{-5} and 10^{-3}M and PBA at 10^{-4}M applied through the roots on the dry weight (μg) of tiller buds Tc and T1. Plants were not supplied with mineral nutrients and harvested on day 10.

	Tc		T1	
	no PBA	+ PBA	no PBA	+ PBA
Control	9.67 ± 0.93	28.83 ± 3.50a	1.37	9.25 ± 0.84
GA_3 10^{-7}M	9.67 ± 0.98	32.52 ± 3.70a	2.50	11.56 ± 1.14
GA_3 10^{-5}M	10.39 ± 0.89	31.72 ± 5.08a	3.00	9.25 ± 1.03
GA_3 10^{-3}M	12.39 ± 0.95	39.22 ± 2.79ab	2.67	10.50 ± 0.87

a Values different from control 'no PBA' at p = 0.05.
b Values different from control '+ PBA' at p = 0.05.
For Tc the interaction GA_3 x PBA was not significant at p = 0.05.
No statistical treatment was applied to T1 'no PBA' data.

Table III - 26

The interaction between GA_3 at the concentrations of 10^{-7}, 10^{-5} and 10^{-3}M and PBA at 10^{-4}M applied through the roots on the dry weight (mg) of first and second leaves, base, root and the whole plant. Plants were not supplied with mineral nutrients and harvested on day 10.

	PBA	Control	GA_3 10^{-7}M	GA_3 10^{-5}M	GA_3 10^{-3}M
1st leaf	no	10.93±0.85	11.54±0.69	12.39±0.52	14.06±0.98a
	yes	16.30±1.19a	18.29±1.31a	16.60±1.39a	17.20±1.07a
2nd leaf	no	0.84±0.20	0.94±0.22	1.89±0.19a	2.41±0.25ab
	yes	1.60±0.39a	1.74±0.33a	1.87±0.46a	1.58±0.25a
Base	no	10.47±0.55	10.98±0.60	9.73±0.45	10.28±0.52
	yes	12.92±0.52	13.20±0.55	10.98±0.77	12.00±0.53
Root	no	16.36±1.09	15.43±0.90b	11.84±0.96ab	11.36±0.66a
	yes	10.00±0.64a	10.98±0.83a	9.19±0.60a	8.99±0.36a
Total	no	38.60±2.16	38.90±2.07	34.86±2.35	38.10±1.97
	yes	40.82±2.53	44.21±2.70	38.63±2.45	39.77±1.71

a Values different from control 'no PBA' at p = 0.05.
b Values different from control '+ PBA' at p = 0.05.

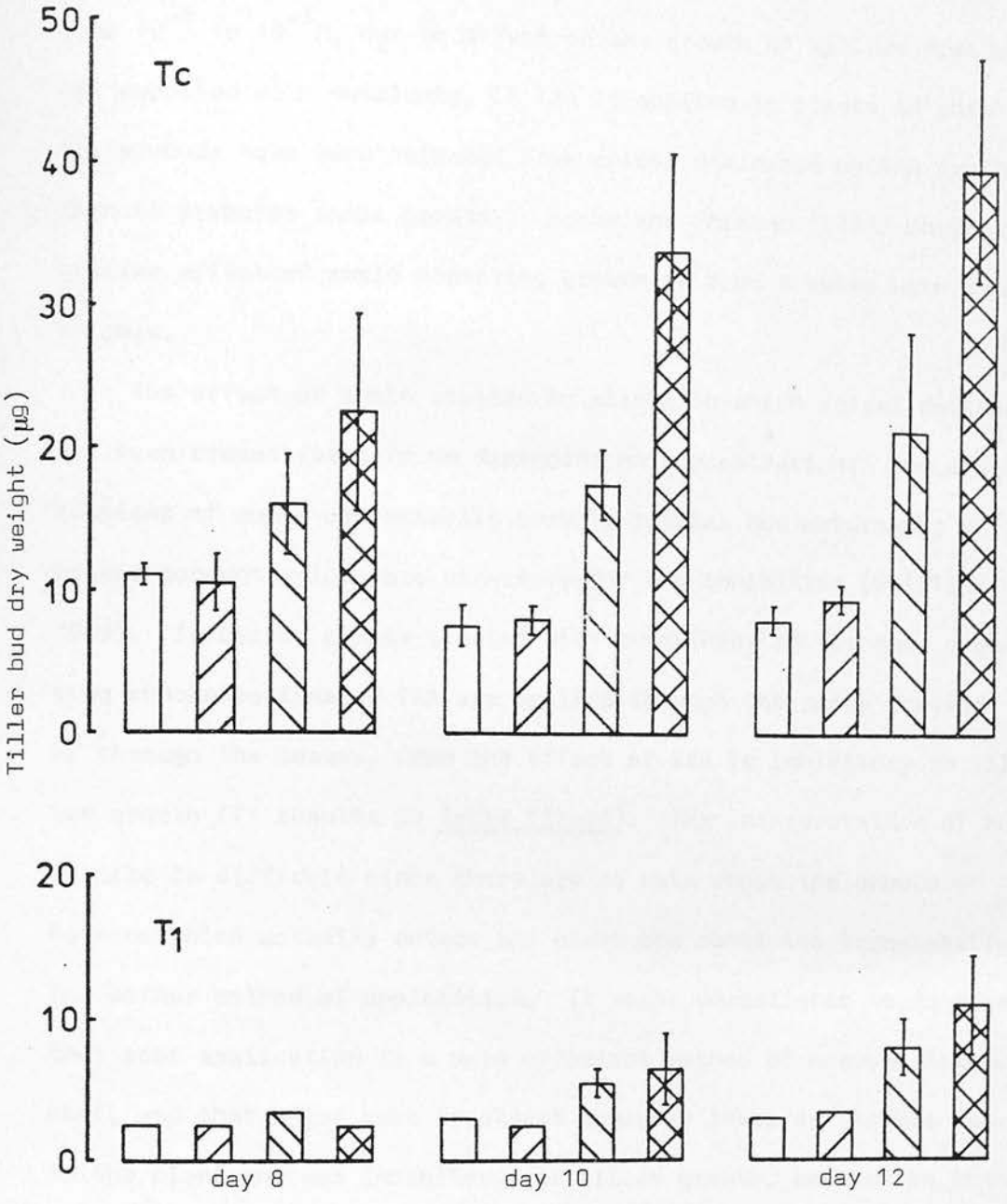

Figure III-18: Effect of GA_3 and PBA on the dry weight of tiller buds Tc and T1, when applied through the roots at the concentrations of 10^{-3}M and 10^{-4}M respectively. Plants were not supplied with mineral nutrients and harvested on days 8, 10 and 12.

Control
PBA
GA_3
GA_3 + PBA

(c) Discussion and conclusions.

Despite the fact that leaf application of IAA, at concentrations from 10^{-6} to 10^{-3}M, has no effect on the growth of tillers from plants not supplied with nutrients, if IAA is applied to plants in which tiller buds have been released from apical dominance by PBA treatment, then it promotes their growth. Sachs and Thimann (1967) showed a similar effect of auxin promoting growth of buds treated with cytokinin in peas.

The effect of auxin applied to plants in which apical dominance has been broken seems to be dependent on concentration; low concentrations of auxin can actually promote lateral bud outgrowth, while higher concentrations are necessary for bud inhibition (Phillips, 1969). In barley plants treated with cytokinin, if the same promoting concentrations of IAA are applied through the roots instead of through the leaves, then the effect of IAA is inhibitory to tiller bud growth (T1 results in Table III-24). The interpretation of these results is difficult since there are no data about the amount of hormone which actually enters the plant and about its translocation for either method of application. It seems unrealistic to suppose that root application is a more efficient method of hormone treatment, and that after root treatment a higher level of IAA was reached in the plant and was inhibitory for tiller growth, when auxin transport is known to be mainly basipetal (Goldsmith, 1969; Morris et al., 1969). Results presented in Table III-24 show this inhibition only in tiller bud T1; as regarding Tc, the concentration of 10^{-5}M could have been low enough to promote growth. This aspect of the work would benefit from further study.

As with IAA, concentrations of GA_3 which do not affect growth

of tiller buds in plants not supplied with nutrients can increase the promoting effect of applied PBA. So, the promoting effect of GA_3 on bud outgrowth in barley is observed only if apical dominance has been broken, in the present case by cytokinin application. The effect of GA_3 promoting growth of lateral buds has been described in dicotyledonous species for buds released from apical dominance by decapitation (Sachs and Thimann, 1964) and also in isolated stem sections (Wickson and Thimann, 1958).

The literature of apical dominance gives much more emphasis to the inhibitive rather than to the promotive effect of both auxin and gibberellin on the growth of lateral buds. But these hormones can also promote lateral bud outgrowth, provided that these are released from apical dominance by another factor, as for example cytokinin in the case of barley.

4. Distribution of radioactive cytokinin applied through the roots.

Results presented in previous sections of this chapter showed that application of exogenous cytokinins can lead to growth of tiller buds otherwise arrested by lack of mineral nutrient application. It was therefore suggested that cytokinin may be an essential factor for lateral bud growth which is not present in a suitable level to allow tiller bud growth in plants not supplied with mineral nutrients.

It has been suggested that bud inhibition is a consequence of lack of cytokinin in the bud itself (Sachs and Thimann, 1964, 1967), and that root-derived cytokinins are preferentially transported to the apical bud in intact plants, but diverted to axillary buds after removal of the dominant shoot apex (Morris and Winfield, 1972).

The objective of the work described here was to study the distribution of radioactivity in plants treated with benzyl(8-^{14}C) adenine

and determine if the effect of cytokinin on tiller growth is related to accumulation of the hormone in the bud.

(a) Results.

Four day old barley plants were treated through the roots with benzyl adenine or benzyl(8-^{14}C) adenine as described in Chapter II-1; a water control was also used. After the treatments the plants were replanted in sand and supplied or not with mineral nutrient solution. Harvest was done on days 7 and 10. The following measurements were made:

- determination of dry weight of Tc and T1 for the cold plants.
- determination of dry weight of leaves, base and root for the ^{14}C BAP treated plants.
- determination of ^{14}C accumulation in Tc, T1, leaves, base and root (as described in Chapter II-1).

The effect of BAP on tiller bud growth, as expected, was to promote dry weight for plants supplied or not supplied with mineral nutrients, as observed comparing the dry weight of the water controls with the dry weight of Tc and T1 from plants treated with cold BAP (Figure III-19). The distribution of radioactivity between Tc, T1, leaves, base and root in the plants is given in Table III-27. Most of the radioactivity was localized in leaves, base and root while very little was found in tiller buds. There was no marked change in the pattern of distribution of activity from plants supplied to not supplied with nutrients, or from day 7 to day 10 but a reduction of activity was noted with the time.

When the activity was related to the amount of dry matter present in each organ (Table III-28), then it was clear that a much larger proportion of counts were found in tillers than in any other part of the plant. The larger proportional decrease in activity found in Tc

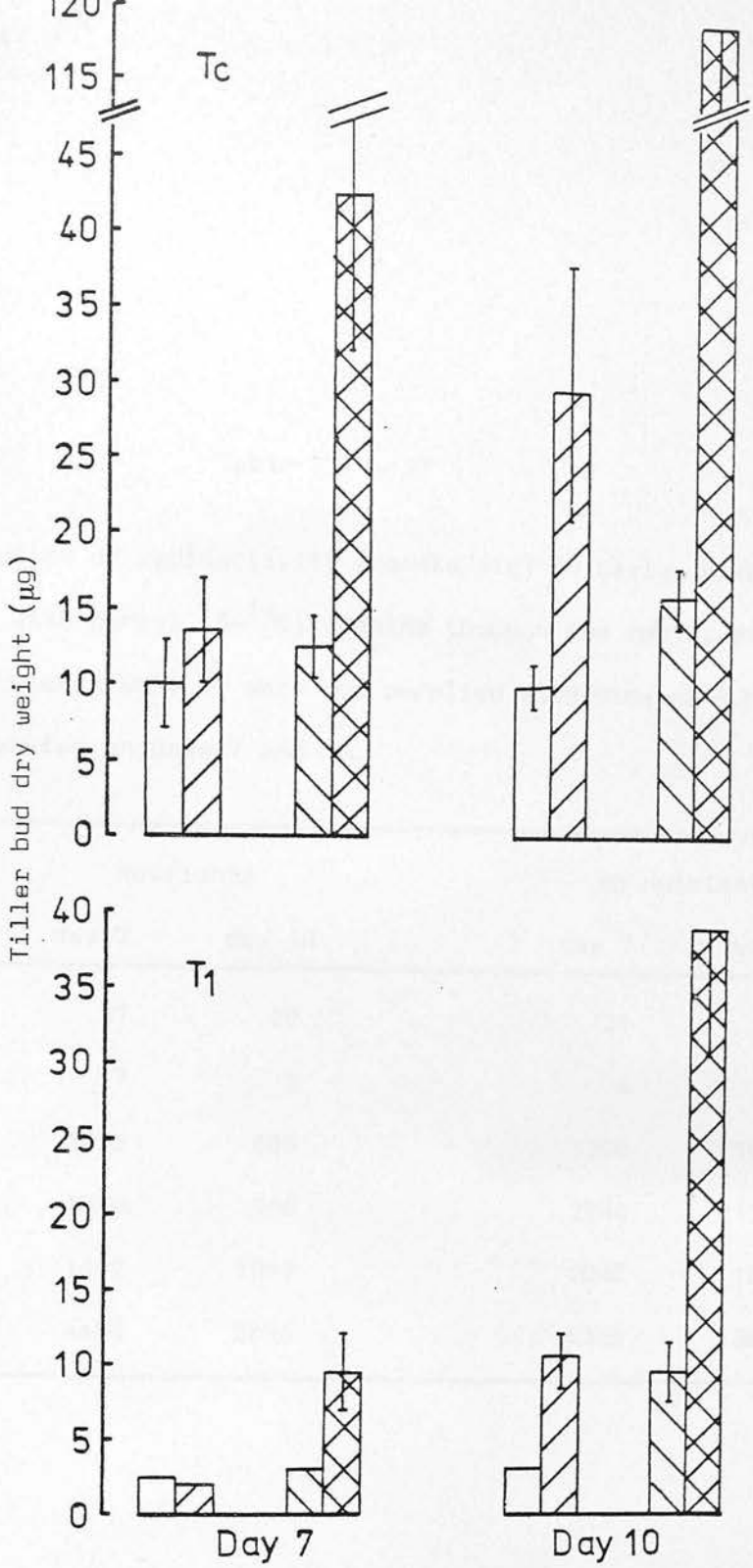

Figure III-19: Effect of BAP on the dry weight of tiller buds Tc and T1 when supplied through the roots at the concentration of 10^{-4}M. Plants were or were not supplied with mineral nutrients and harvested on days 7 and 10.

Control Minerals

BAP BAP + Minerals

Table III - 27

Distribution of radioactivity (counts/min) in barley plants treated with benzyl (8-^{14}C) adenine through the roots, on day 4. Plants were or were not supplied with mineral nutrients and harvested on days 7 and 10.

	Nutrients		No nutrients	
	day 7	day 10	day 7	day 10
Tc	47	29	31	18
T1	7	3	4	3
Leaves	1052	886	1296	1010
Base	1916	866	2984	1270
Root	1462	1912	2040	1580
Total	4484	3696	6355	3881

Table III - 28

Distribution of radioactivity (counts/min/mg of dry weight) in barley plants treated with benzyl (8-^{14}C) adenine through the roots, on day 4. Plants were or were not supplied with mineral nutrients and harvested on days 7 and 10.

	Nutrients		No nutrients	
	day 7	day 10	day 7	day 10
Tc	1103	243	2303	615
T1	697	83	1835	263
Leaves	77	43	129	76
Base	138	57	207	96
Root	212	195	272	197
Total	130	81	199	113

and T1, comparing with the decrease in activity in other organs, from day 7 to 10 reflects the large increase in dry matter of these organs at this time interval. The larger amount of counts in tiller buds of plants not supplied with nutrients compared with plants supplied with nutrients (Table III-28) may again be a reflection of the size of the tillers, since the absolute number of counts is even smaller in those tillers (Table III-27).

(b) Discussion and conclusions.

The results presented above show that when barley plants are treated with cytokinin by soaking their root systems in the hormone solution, a certain amount of the hormone penetrates the roots from where it is distributed to the aerial parts of the plant, including tiller buds where it seems to be accumulated.

Tiller buds of plants not given mineral nutrients are completely arrested between days 7 and 10, and it seems that the promotive effect of BAP on these buds is not present before day 7 (Figure III-19), but a clear accumulation of radioactivity is found on the tillers by day 7 (Table III-28). Therefore, it seems that the effect of the cytokinin on the growth of the tillers may be the result of this accumulation.

These results support in an indirect way the idea that the inhibition of tiller bud growth in plants not supplied with nutrients is due to reduced levels of cytokinin, since they show that even in this conditions, if cytokinins are available they are translocated to the tiller buds where they may start or increase processes of cell division, elongation and differentiation which result in clear growth of tillers.

The possibility of ^{14}C BAP being metabolized somewhere else in the plant and its radioactive metabolite being transported to the buds can not be forgotten, but it has been reported for Solanum andigena

that whereas ^{14}C BHP was accumulated in lateral buds prior to their growth, ^{14}C adenine did not accumulate until after bud growth had commenced (Woolley and Wareing, 1972a).

CHAPTER IV

EFFECTS OF CYTOKININ ON APICAL DEVELOPMENT

The development of the main-stem apex in cereals is affected by environmental conditions such as temperature, light intensity, day-length, leaf shading and nutrient condition under which the plant is grown (Aspinall and Paleg, 1963; Friend, 1966; Felippe and Dale, 1973; Dale and Wilson, in press). Treatments which reduce tiller bud growth in barley, including reduction in light intensity (Aspinall and Paleg, 1964), shading the first leaf and delay in minerals application (Fletcher and Dale, 1974), also reduce the rate of initiation of leaf primordia on the main-stem apex (Aspinall and Paleg, 1963; Felippe and Dale, 1973; Dale and Wilson, in press).

Exogenous application of cytokinin to barley plants releases lateral buds from apical dominance and increases dry weight of buds released by nutrient application (Chapter III).

It is of interest to know, at this point, if the effect of cytokinin in the development of lateral bud is an exceptional one, or if cytokinin has a general effect in barley increasing development on all apices of the plant, as is found for light intensity and nutrients. It is also of interest to know whether the effect of cytokinin on bud growth is just an increase in dry weight or if leaf initiation is also affected.

The objective of the experiment described in this chapter was therefore to study and to compare the effect of cytokinin on leaf primordia initiation on both the main-stem apex and the tiller buds Tc and T1.

1. Results

Proctor barley grains were germinated in Perlite and treated on day 4 with PBA at 10^{-4}M, applied through the roots, as described in Chapter II. Seedlings were or were not supplied with mineral nutrients on the same day of treatment. Harvests were made on days 8, 10 and 12, and the part of the plant including the apical region and tiller buds prepared for microscopic examination as described in Chapter II.

Sections of apices of three plants from each treatment were examined and the number of leaf primordia determined on tiller buds Tc and T1 and on the main-stem apex; mean values are given in Figure IV-1.

About 6 leaf primordia were present on the main-stem apex from day 8 to day 12. Application of mineral nutrients, as expected, increased apical development on the main-stem by increasing the number of leaf primordia on days 10 and 12, although the increase was significant only on day 10. PBA treatment had no effect on the development of the main-stem apex, apart from a slight inhibition on day 8. On plants supplied with nutrients there was no significant effect of PBA on leaf primordium number.

Tiller bud Tc showed the primordia of the prophyll and up to 2.3 leaf primordia even in absence of mineral nutrients. There was no significant effect of either mineral nutrient or PBA treatment on primordia number up to day 12.

In plants not given mineral nutrients or cytokinin, the primordia of T1 showed the prophyll primordia in all plants analysed and one leaf primordium in some plants. Treatment with mineral nutrients or PBA increased significantly the number of leaf primordia by day

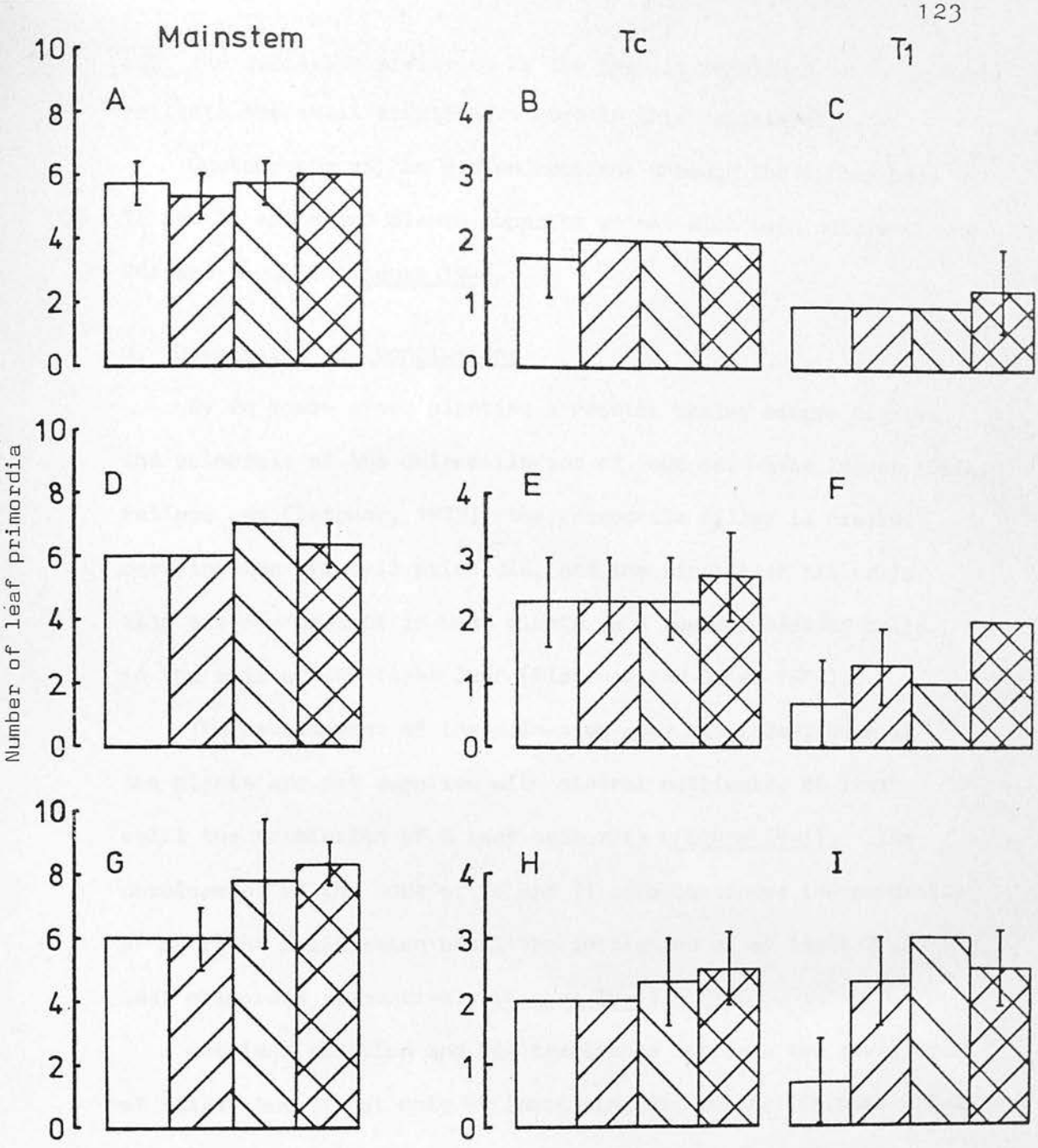

Figure IV-1: Effect of PBA on the number of leaf primordia on the mainstem (A, D, G), coleoptile tiller (B, E, H) and first leaf tiller (C, F, I), when applied through the roots at the concentration of 10^{-4}M. Plants were or were not supplied with mineral nutrients and harvested on days 8 (A, B, C), 10 (D, E, F) and 12 (G, H, I).

12. The variation presented by the results described above probably reflects the small sample size used in this experiment.

Photographs of the median sections through the tiller buds Tc and T1 apices of plants supplied or not with both nutrients and PBA are shown in Figure IV-2.

2. Discussion and conclusions

By 24 hours after planting a Proctor barley embryo carries the primordia of the coleoptile and of four main-stem leaves (Dale, Felippe and Fletcher, 1972), the coleoptile tiller is present carrying the prophyll primordia, and the first leaf tiller is also already present in most plants as a rounded mass of cells in the axil of the first leaf (Fletcher and Dale, 1974).

The development of the main-stem apex continues, even if the plants are not supplied with mineral nutrients, at least until the initiation of 6 leaf primordia (Figure IV-1). The development of the buds of Tc and T1 also continues independently of nutrient application until the initiation of at least 3 and 1 leaf primordia respectively (Figure IV-1).

Nutrient addition and PBA treatments increase the development of tiller bud T1 not only by increasing dry matter (Chapter III-1) but also by accelerating the appearance of new leaf primordia (Figure IV-1). But on tiller bud Tc, although both treatments increase dry matter (Chapter III) neither of them have very clear effects on leaf primordia initiation (Figure IV-1). This different behaviour of tiller buds Tc and T1 could be a consequence of the more rapid development of T1 when compared to Tc (Fletcher and Dale, 1974).

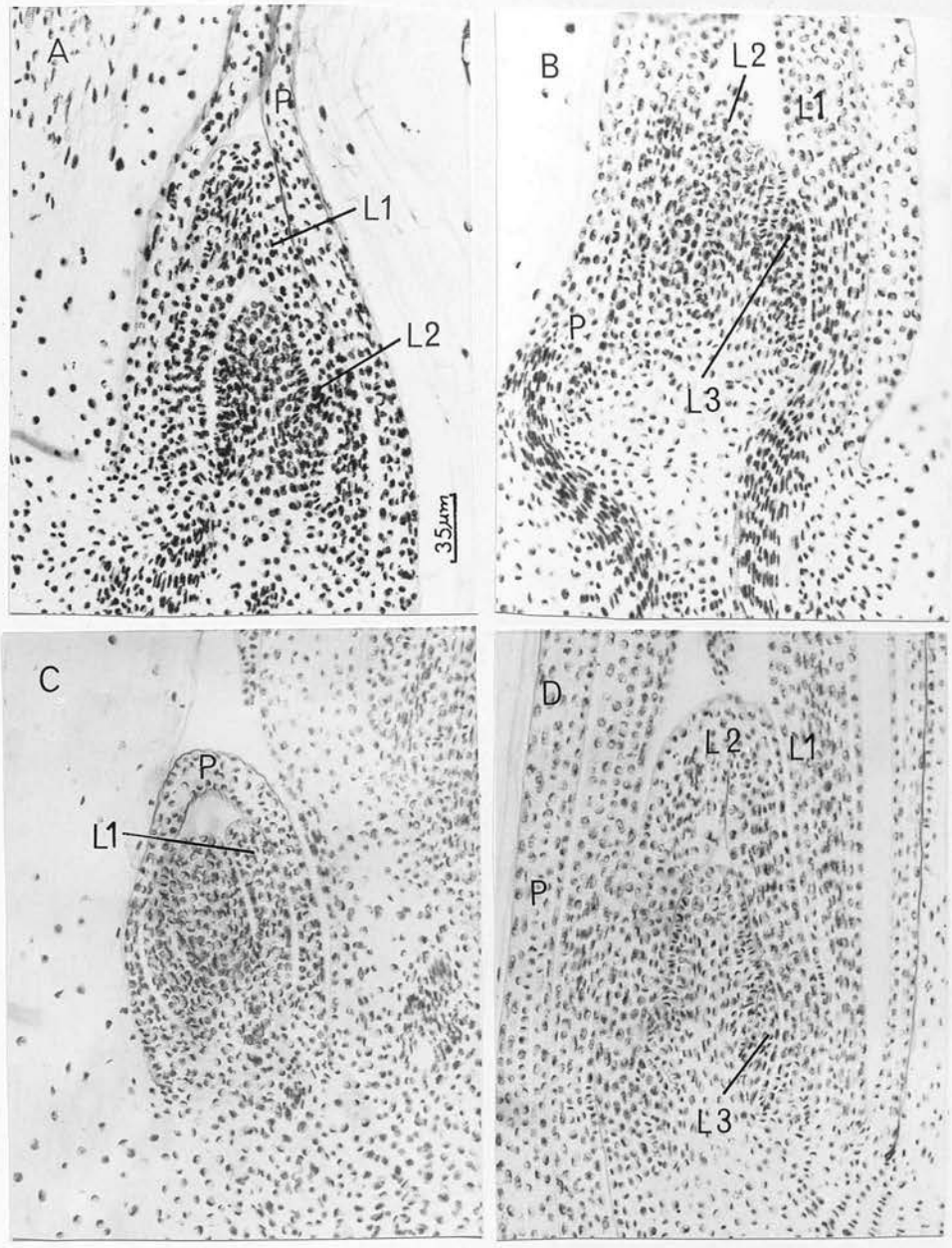

Figure IV-2: Photographs of the median section through the tiller bud Tc (A; B) and T1 (C; D) from 12 day old plants supplied with mineral nutrients and PBA (B; D), or given neither nutrients nor PBA (A; C).

P : prophyll primordium
L1 : first leaf primordium
L2 : second leaf primordium
L3 : third leaf primordium

The promoting effect of nutrients on the initiation of leaf primordia in barley main-stem apex, observed previously by Felippe and Dale (1973) and Dale and Wilson (in press) is confirmed in Figure IV-1. But this effect of nutrient may not be related to the endogenous content of cytokinin in the plant, since no effect of exogenously applied PBA was observed on the main apex. Therefore, while nutrients have a promoting effect on the main-stem and lateral apices, the effect of cytokinin on apical development seems to be restricted to the lateral bud, showing that the effect of exogenous cytokinin is not a general one, increasing growth of any meristematic region of the plant, but that it has a special effect in releasing the arrested bud from inhibition.

CHAPTER V

ROOT REMOVAL AND BARLEY GROWTH

Besides its functions in the uptake of water and minerals from the substrate, the root system is also responsible for the synthesis of certain plant hormones which control growth and development of the aerial parts of the plant. The presence of growth substances in roots has been reported with special emphasis given to cytokinins, since the root system is an important site of cytokinin production in the plant (Skene, 1975; Torrey, 1976). Kende (1965), for example, suggested that cytokinins are continuously formed in roots since the amount of these compounds in root exudate did not decrease over a four day period after decapitation. Engelbrecht (1972) demonstrated the production of cytokinins by roots in experiments with leaf cuttings of Phaseolus where cytokinin activity was increased markedly shortly after the appearance of roots.

Exogenous cytokinins have been shown to substitute for roots in controlling stem growth (Holm and Key, 1969), in the retention and development of inflorescences (Mullins, 1967), in the development of lateral buds as leafy shoots in potatoes (Woolley and Wareing, 1972a,c) and in reducing senescence and increasing the capacity for $^{14}CO_2$ fixation in leaves (McDavid, Sagar and Marshall, 1973).

The previous chapters in this thesis show a role for exogenous applied cytokinins in releasing lateral buds of barley from inhibition and on the distribution of dry matter to different parts of the plant. Based on these observations and on the indications given above of the importance of root-

synthesized cytokinins on plant development, the experiments here described were conducted in an attempt to modify the endogenous content of cytokinin in barley plants. This was done by removing part of the root system of plants grown in presence or absence of mineral nutrients, and observing the effects of the treatment on root growth itself, on tiller bud growth, and on dry matter distribution in the plant.

1. Root removal in plants not supplied with mineral nutrients

Four day old barley seedlings grown in perlite were selected to have 6 root axes. The control plants were maintained intact, and the treatments consisted of removal of 3 complete root axes or of the root tips; in the latter case the 6 root axes were left with the 8 cm basal portion. Plants were replanted in sand. Two experiments were made; in the first plants were harvested on days 8, 10 and 12 when the dry weight of the tiller buds, first and second leaves, base, root and the total dry weight were determined. Plants of the second experiment were harvested on days 8 and 11 when the number of primary lateral roots was counted, besides the dry weight determinations.

Figure V-1 shows no significant effect for the root removal treatment on the first and second leaves, and on the base at any day of harvest. Root dry weight was always reduced by the treatments the reduction being statistically significant on days 8 and 12 for removal of 3 axes, and on day 8 for removal of root tips. Removal of 3 axes reduced the total dry matter only on day 12 while removal of the tips was inhibitory at all days of harvest although not significantly.

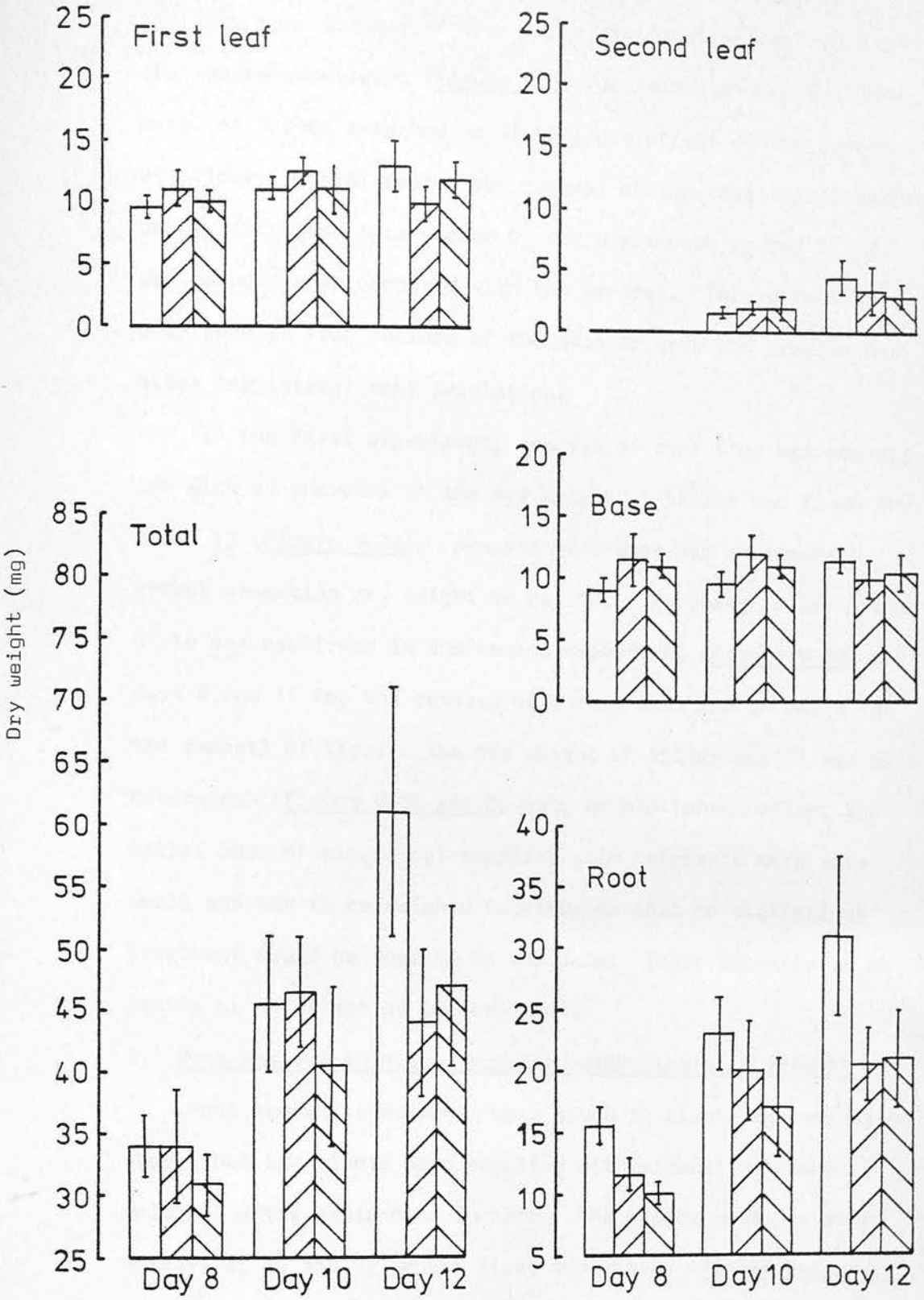

Figure V-1: Effect of root removal on the first and second leaves, base, root and total dry weight (mg). Plants were not supplied with mineral nutrients and harvested on days 8, 10 and 12.

The same pattern of dry matter distribution was found in the second experiment (Figure V-2) for leaves and roots. Removal of 3 root axes had no significant effect on the number of primary lateral roots, but removal of the root tips significantly increased this number by day 8 although by day 11 it was reduced when compared with the control. This effect presumably results from failure of the axis to grow and provide new sites for lateral root initiation.

In the first experiment, removal of root tips was associated with an increase in the dry weight of tiller bud Tc on days 8 and 12 (Figure V-3A). Removal of 3 axes had an apparent effect promoting dry weight on day 10. Increase in dry matter of Tc was confirmed in the second experiment (Figure V-3B) on days 8 and 11 for the removal of 3 root axes and on day 8 for the removal of tips. The dry weight of tiller bud T1 was also determined (Figure V-3C and D) but, as mentioned before, the tiller buds of plants not supplied with nutrients were very small and had to be weighed together so that no statistical treatment could be applied to the data. There appeared to be little or no effect of the treatment.

2. Root removal in plants supplied with mineral nutrients

Root removal treatments were given to plants as indicated above, but the plants were supplied with mineral nutrient solution after replanting in sand. The plants were harvested on days 8, 10 and 12 on the first experiment (Figure V-4 and 6), or on days 8 and 11 on the second experiment (Figure V-5 and 6).

Root removal treatments reduced the total dry weight of plants supplied with nutrients, the effect being significant

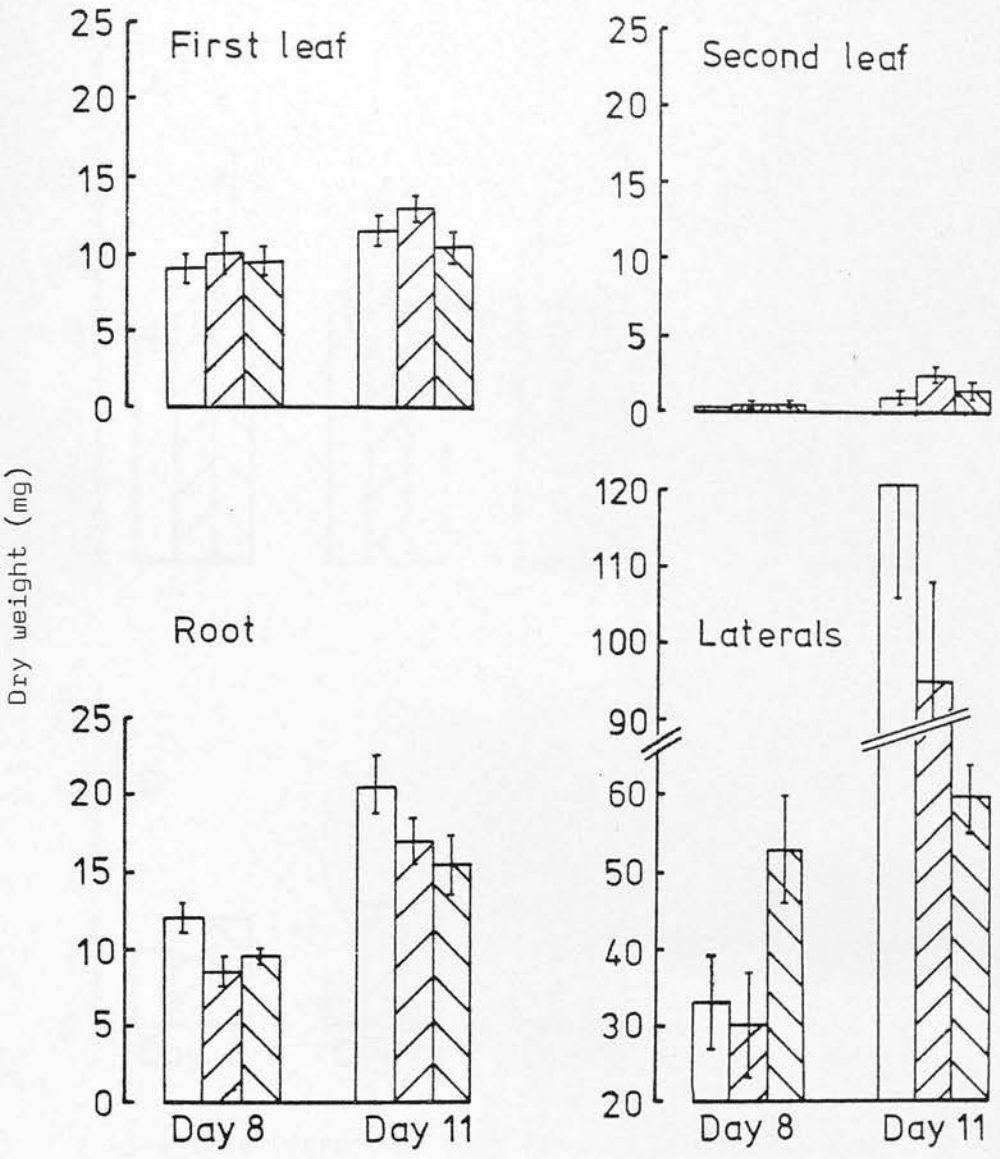

Figure V-2: Effect of root removal on the first and second leaves and root dry weight (mg) and on the number of primary lateral roots. Plant were not supplied with mineral nutrients and harvested on days 8 and 11.

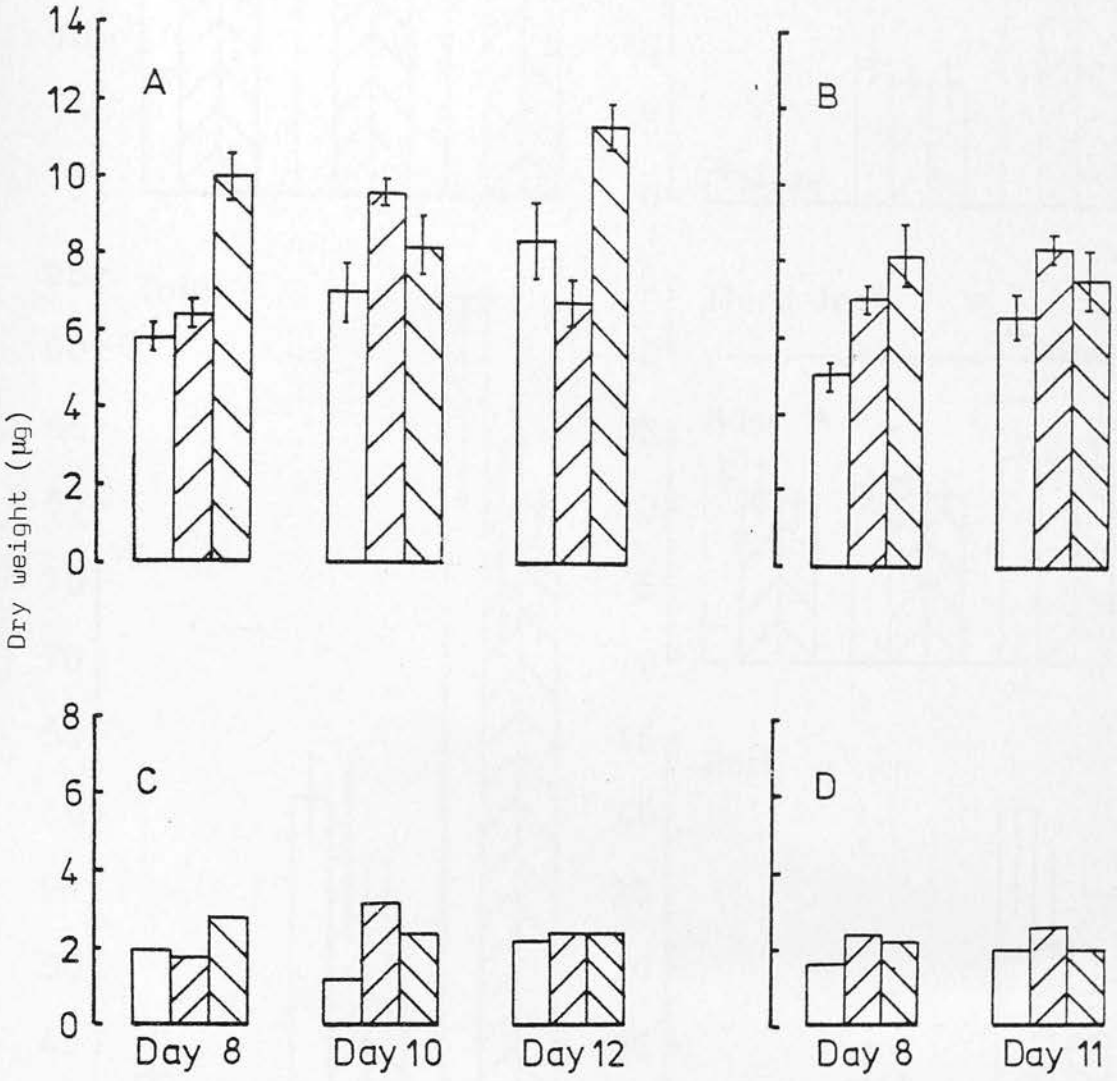

Figure V-3: Effect of root removal on the tiller buds Tc (A; B) and T1 (C; D) dry weight (µg) in two experiments (A; C and B; D). Plants were not supplied with mineral nutrients and harvested on days 8, 10 and 12 (A; C) or 8 and 11 (B; D).

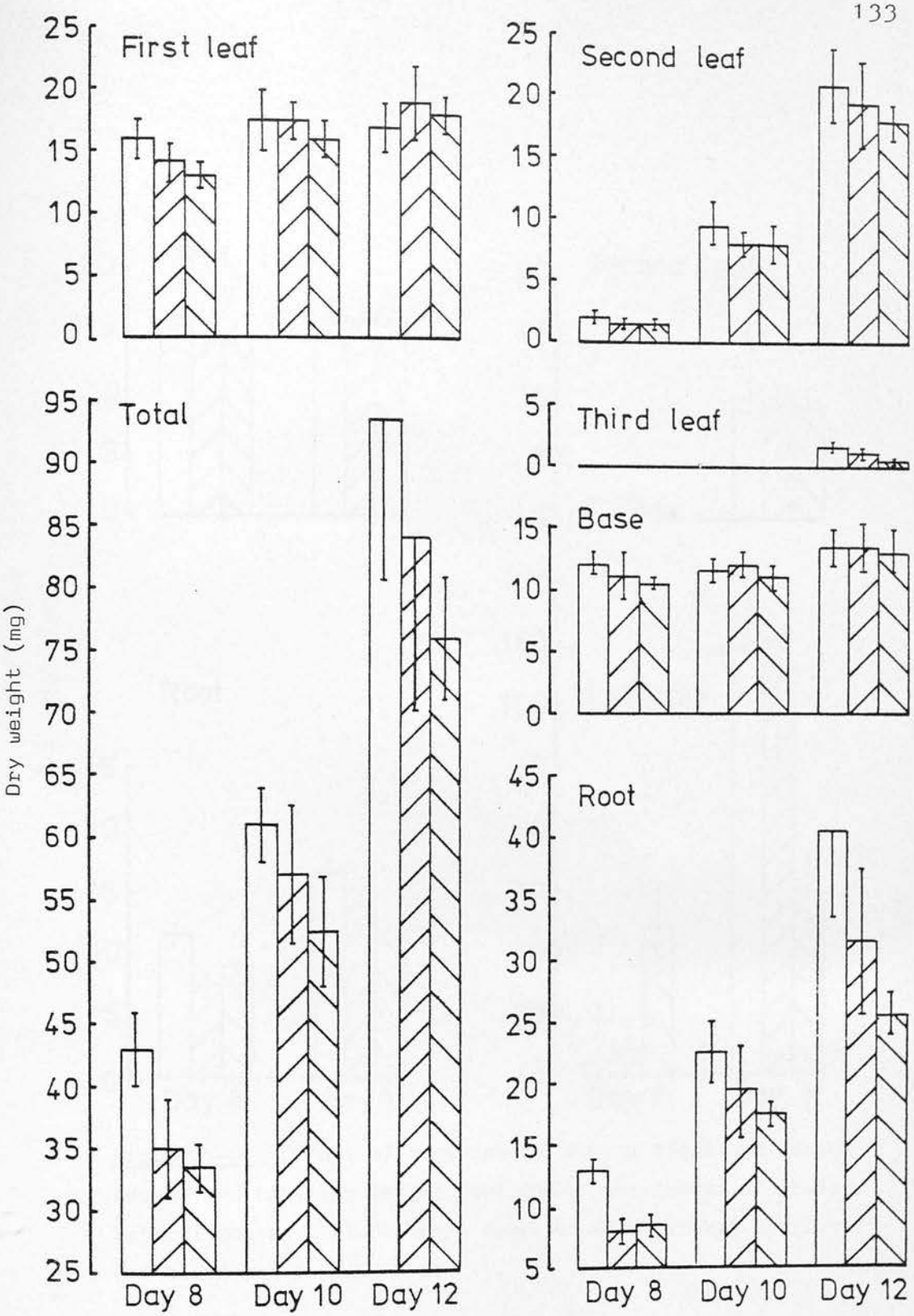

Figure V-4: Effect of root removal on the first, second and third leaves, base, root and total dry weight (mg). Plant were supplied with mineral nutrients and harvested on days 8, 10 and 12.

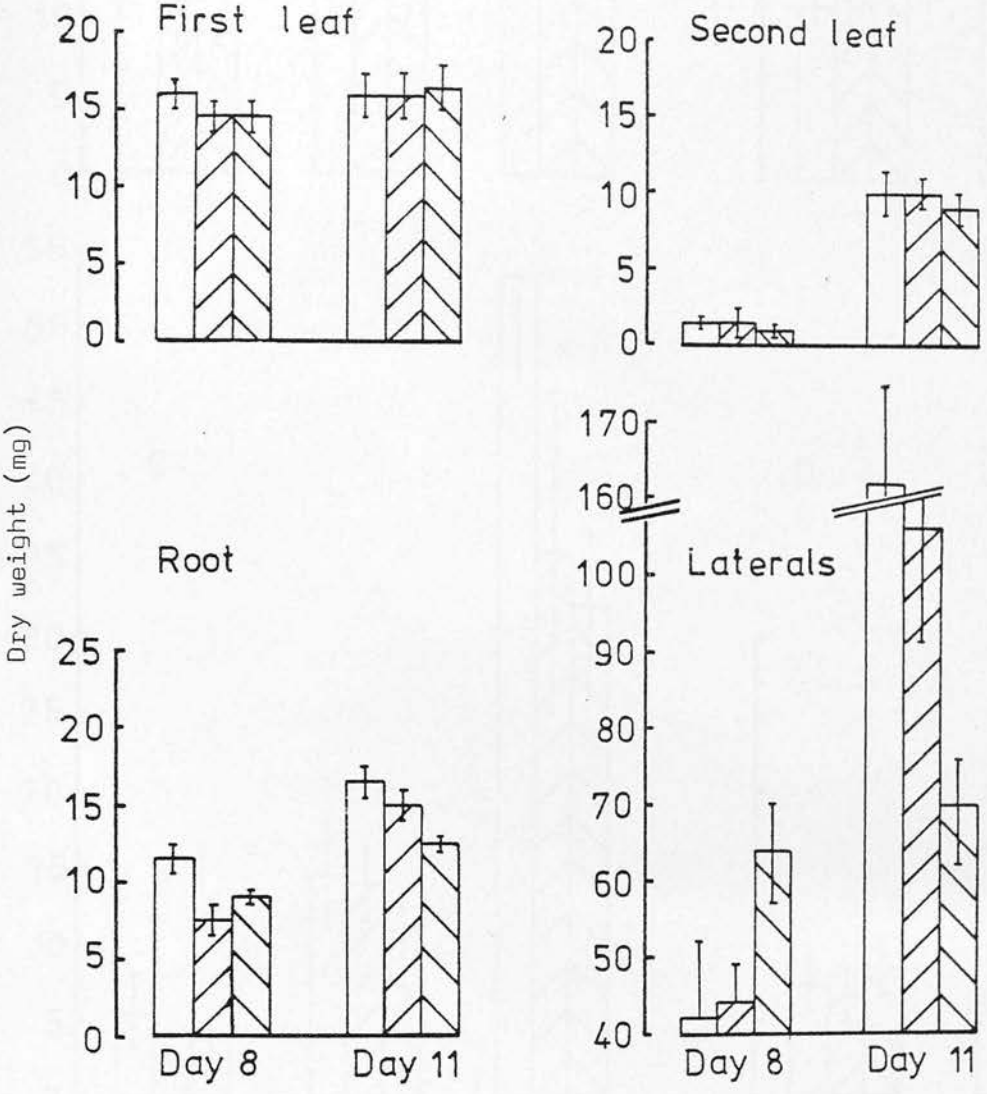

Figure V-5: Effect of root removal on the first and second leaves and root dry weight (mg) and on the number of primary lateral roots. Plants were supplied with mineral nutrients and harvested on days 8 and 11.

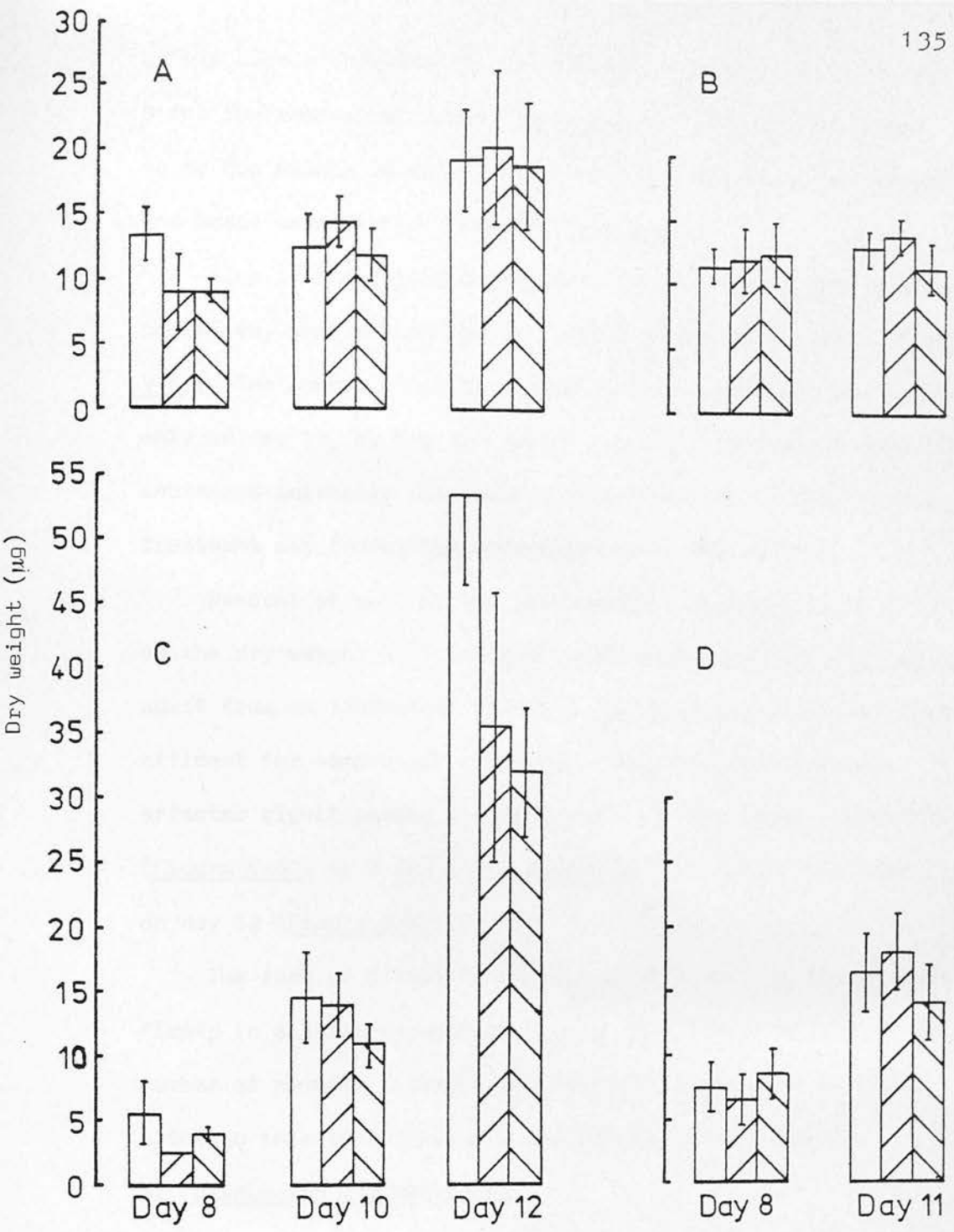

Figure V-6: Effect of root removal on the tiller buds Tc (A; B) and T1 (C; D) dry weight (µg) in two experiments (A; C and B; D). Plants were supplied with mineral nutrients and harvested on days 8, 10 and 12 (A; C) or 8 and 11 (B; D).

at all days of harvest for the removal of root tips and on day 8 for the removal of 3 axes (Figure V-4). This effect seems to be due mainly to reduction on root dry weight, since leaves and bases were little affected (Figure V-4).

Also in the second experiment, the treatments had no effect on leaves, and reduced the dry weight of the root system (Figure V-5). The number of primary lateral roots per plant was reduced, only on day 11, by the removal of 3 axes. Removal of root tips increased initially the number of laterals but by day 11 this treatment was inhibitive when compared to the control.

Removal of part of the root system had little or no effect on the dry weight of Tc in both experiments (Figure V-6A and B), apart from an inhibition on day 8 (Figure V-6A) which was significant for removal of root tips. None of the treatments affected significantly the dry weight of T1 on days 8 and 10 (Figure V-6C) or 8 and 11 (Figure V-6D) but were inhibitory on day 12 (Figure V-6C).

The lack of effect of removal of root tips on Tc was confirmed in a third experiment (Figure V-7) but in this case the number of primary laterals was inhibited from day 7 to 10, although this inhibition was significant only on day 10.

3. Discussion and conclusions

Removal of 3 root axes in plants not supplied with nutrients reduces the dry weight of the root system but a regenerating tendency is shown by the plant since removal of half of the root system does not reduce root dry weight to half of the control values. This compensation may be a result of the increase in number of primary laterals per axis, since no

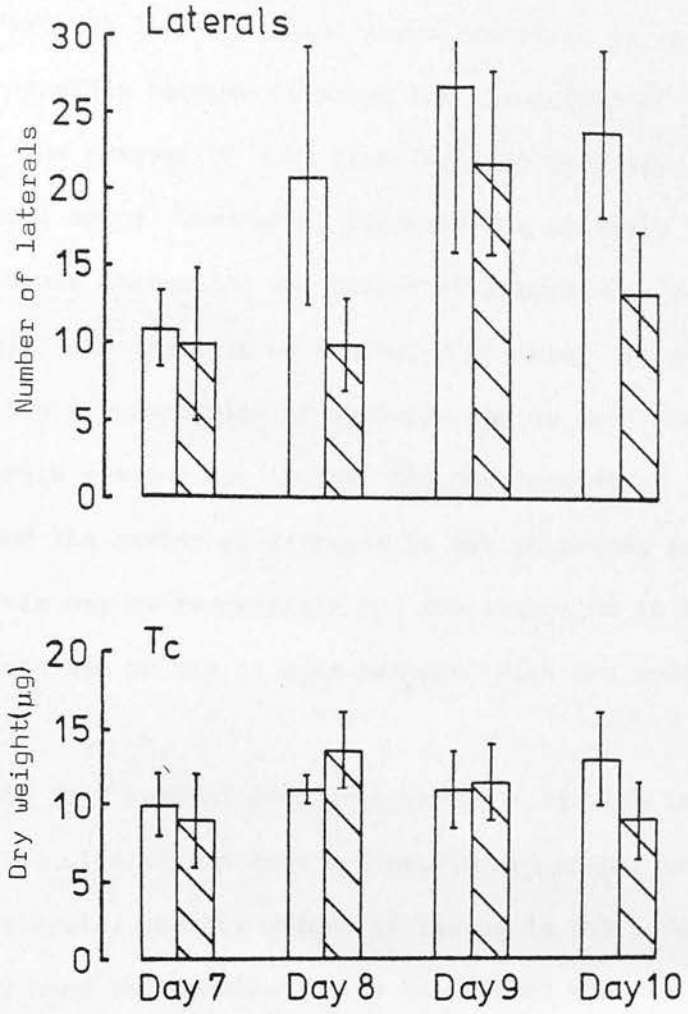

Figure V-7: Effect of removal of root tips on the number of primary lateral roots and Tc dry weight (µg). Plants were supplied with mineral nutrients and harvested on days 7, 8, 9 and 10.

difference was found in the total number of these laterals between control and treated plants. Removal of root tips represents a loss of very small portion of the root system, but this treatment leads to significant reduction in the root dry weight possibly because it stops the elongation of the root axes. The removal of tips from the main axes may, by removing local apical dominance, liberate the initials of primary laterals increasing the number of laterals on day 8 (Figure V-2). As there is no extension of axes, all the possible sites of production of laterals may be soon occupied, or the laterals present may inhibit the development of the new ones, and the number of laterals is not increased any longer. This may be responsible for the reduction on the number of laterals on day 11 when compared with the control (Figure V-2).

Although root removal treatment on day 4 results in changes in the size of the root system, in dry weight and number of laterals, the dry weight of leaves is not affected. On the other hand the treatments are associated with an increase in Tc dry weight. This effect of the treatments, increasing tiller dry weight while tops are not affected, may be a response to alterations in the level of endogenous growth substances. If the increase in tiller dry weight is due to an increase in cytokinin content, then, the endogenous level of cytokinin does not seem to be related to the amount of dry matter in roots, since the increase in tiller growth is not proportional to the root dry matter.

Removal of root tips increased tiller bud growth and also increased, initially, the number of laterals. This increase

in lateral number could increase the number of sites for biosynthesis of cytokinin and maybe the endogenous level of cytokinin, if this situation occurs under conditions of mineral nutrient deficiency, thus affecting the growth of tiller buds. There are reports in the literature indicating that the root tip is the locus of cytokinin production in roots (Weiss and Vaadia, 1965; Short and Torrey, 1972).

Removal of 3 complete root axes increased the number of primary laterals in each axis present but not the total number of laterals in the plant. Therefore, in this case the increase in tiller growth can not be due to increase in cytokinin level as a result in increased number of root tips; Sheldrake and Northcote (1968), and Sheldrake (1971) have suggested that auxin is produced in differentiating xylem tissue; if the production of auxin by elongating regions of the root axes is of significance, and is reduced by removal of 3 complete axes, then this treatment may have increased the ratio cytokinin/auxin, and this alteration may have been responsible for the increase in tiller growth.

In plants supplied with mineral nutrients the removal of 3 root axes also has an inhibitory effect on root and the whole plant dry weight which since it is significant only on the first day of harvest (Figure V-4) suggests again a tendency of the plant to recover from treatment. Removal of the root tips seems to produce more drastic consequences to the plant, since it reduces root and total dry matter at all days of harvest and on some occasions also reduces leaf dry weight (Figure V-4).

Reduction in the dry weight of the root system in plants

supplied with nutrients is not related to alteration in the number of primary laterals, which was variable between experiments.

Growth of T1 is rapid between days 10 and 12 in plants supplied with nutrients (Figure V-6); it is at this time that root removal treatments are significantly inhibitory of T1 growth. Restriction of T1 growth between days 10 and 12 could be just the result of nutritional restriction related to reduction of nutrient uptake, since the root system is reduced by the treatments in both dry matter and number of laterals, and primary laterals could by that time be acting as absorbing organs. A certain restriction of growth is also found by day 12 in the second and third leaves which are also growing by that time (Figure V-4).

Inhibition of T1 growth could also result from an effect of root removal on both, restriction of nutrients and reduction on cytokinin endogenous level. Cytokinin biosynthesis may be affected either by the reduction in uptake of nutrients or by the reduction in sites for cytokinin production or by both factors with the reduction on the number of primary laterals by day 11 (Figure V-5).

The results presented here show the existence of a relation between the root system and the outgrowth of tiller buds in barley, which may be controlled by endogenous cytokinins, or by the equilibrium between endogenous cytokinins and auxins in the plant. More observations in this aspect of the control of bud growth in barley would certainly benefit a final conclusion.

CHAPTER VI

ENDOGENOUS CYTOKININ IN BARLEY

Skoog, Strong and Miller (1965) defined cytokinins as chemicals which, regardless of their other activities, promote cytokinesis in plant cells. Cytokinins have been shown also to induce cell enlargement (Miller, 1956; Kuraish and Okumura, 1956) and differentiation (Skoog and Miller, 1957). They play a role in a large number of plant processes including germination, apical dominance and senescence (Fox, 1969; Hall, 1973).

The involvement of cytokinins in growth of lateral buds was discussed in Chapter I. In barley, cytokinins appear to be concerned with lateral bud growth. The results presented in Chapter III and IV of this thesis show that tiller growth is increased by application of exogenous cytokinin through the roots, and that this treatment can also initiate growth of tiller buds arrested by mineral nutrient deficiency. It was proposed that the effect of minerals on tiller bud growth may not be just a nutritional one, but brought about through an increase in the level of endogenous cytokinins in the plant, and this may be responsible for the growth of tillers.

Another way of testing the hypothesis of minerals affecting tiller bud growth through an effect on endogenous cytokinin level is to determine directly this level in plants maintained in different mineral nutrient treatments.

The aim of the experimental work described in this chapter was to isolate and compare the amount of cytokinin-like substances from extracts of barley plants supplied with complete

mineral nutrient solution, not given the nitrate component of the nutrient solution, or given no minerals at all. According to the hypothesis proposed it would be expected that more cytokinin activity would be found in those plants supplied with full mineral nutrient solution.

1. Results

Six and eight day old Proctor barley plants grown in different mineral nutrient conditions (Table II-3) were extracted for substances with cytokinin-like activity as described in Chapter II. The solvent extractions of 6 day old plants produced 4 fractions, namely ethyl acetate or n-butanol fractions of plant supplied or not with mineral nutrient solution (Figure II-2). Each of these fractions produced 4 sub-fractions (1,2,3 and 4) after separation through the silicagel column and each of these gave three sections (a, b, and c) after thin layer chromatography (Figure II-3). Similarly, the ethyl acetate fractions of 8 day old plants supplied or not supplied with nutrients also produced sub-fractions 1, 2, 3 and 4, and each of these, the sections a, b and c (or a, b, c and d in the case of fraction 3) after thin layer chromatography. The flow chart summarising the procedure followed for the obtention of these fractions is given in Figure VI-1.

All the sections obtained after the steps of purification were tested for cytokinin activity using the tobacco callus bioassay as described previously (Chapter II). The eluate from each section was tested in three concentrations, 10, 100 and 1000 µl (i.e. 0.1, 1.0 and 10.0% of the extract) per culture flask (25 ml of the medium) and three replicates of each concentration were used. Three concentrations were used to

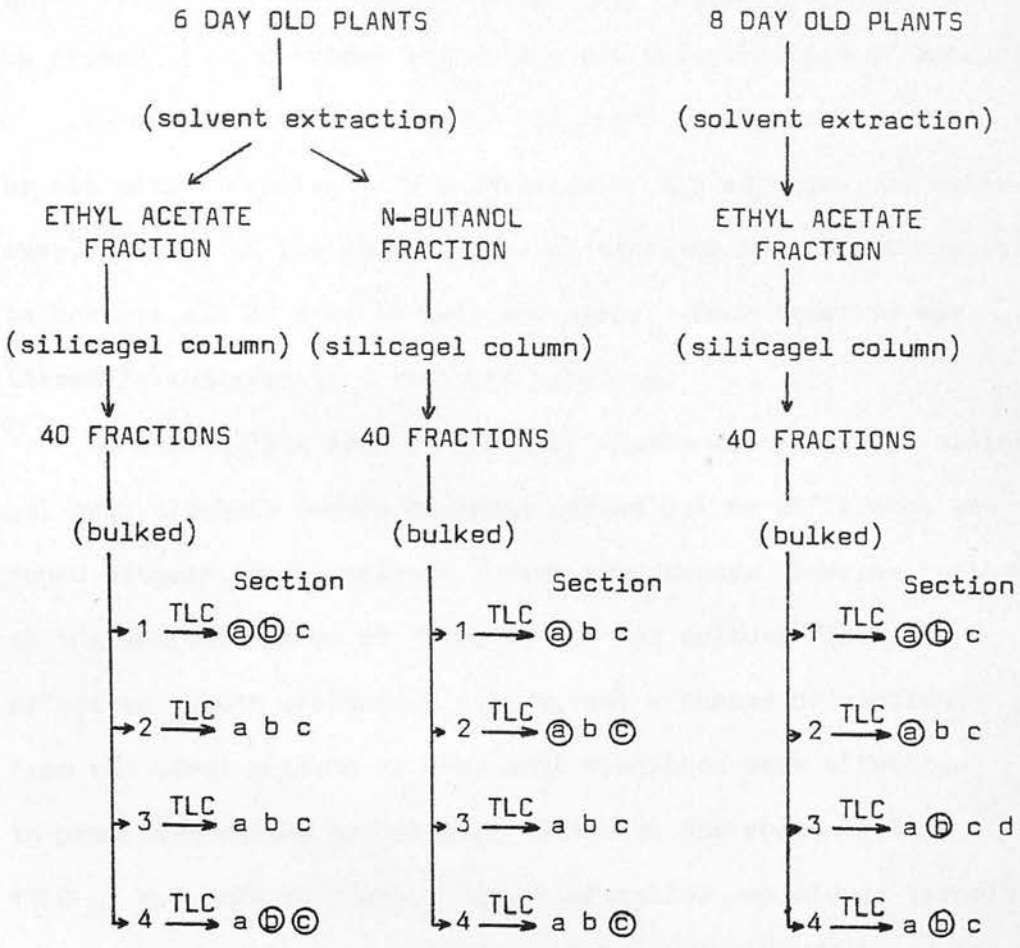

Figure VI-1: Summary flow chart of the procedure for obtention of fractions assayed using tobacco callus. Circles indicate the active sections in the isolated assays.

facilitate the detection of substances with low activity, or extracted in low amounts, and inhibitory substances which could be present in the sample and affect the determination of activity.

To compare the activity of fractions from plants supplied or not with minerals, a full analysis of all sections was necessary, but due to the large number of sections it was not possible to analyze all of them in only one assay. Each fraction was therefore analyzed in a separate bioassay.

Tobacco callus from the control flasks with water or silica-gel grew slightly during bioassay period but no difference was found between these controls in the same assay. Samples tested at the concentrations of 10 or 100 µl per culture flask had no effect on growth of tobacco callus, but a number of sections from the ethyl acetate or n-butanol fractions were effective in promoting callus growth when tested at the concentration of 1000 µl per culture flask. Growth of callus was always largely increased by kinetin at the concentration of 30 µg/l, used also as a control in every bioassay, but the sensitivity of the callus used varied considerably from one assay to another. For this reason comparison between different assays was difficult. An example of this is seen in the results for the day 8 set (Figure VI-2) where the extract of plants supplied with complete mineral nutrient solution showed cytokinin-like activity at least in sections 1a, 1b, 3b and 4b, while the sections 1a, 1b and 2a were the most active in plants supplied with nutrient solution lacking in nitrate. However these observations are of little use for comparing the two treatments since, as the kinetin controls show, the callus sensitivity was different in the

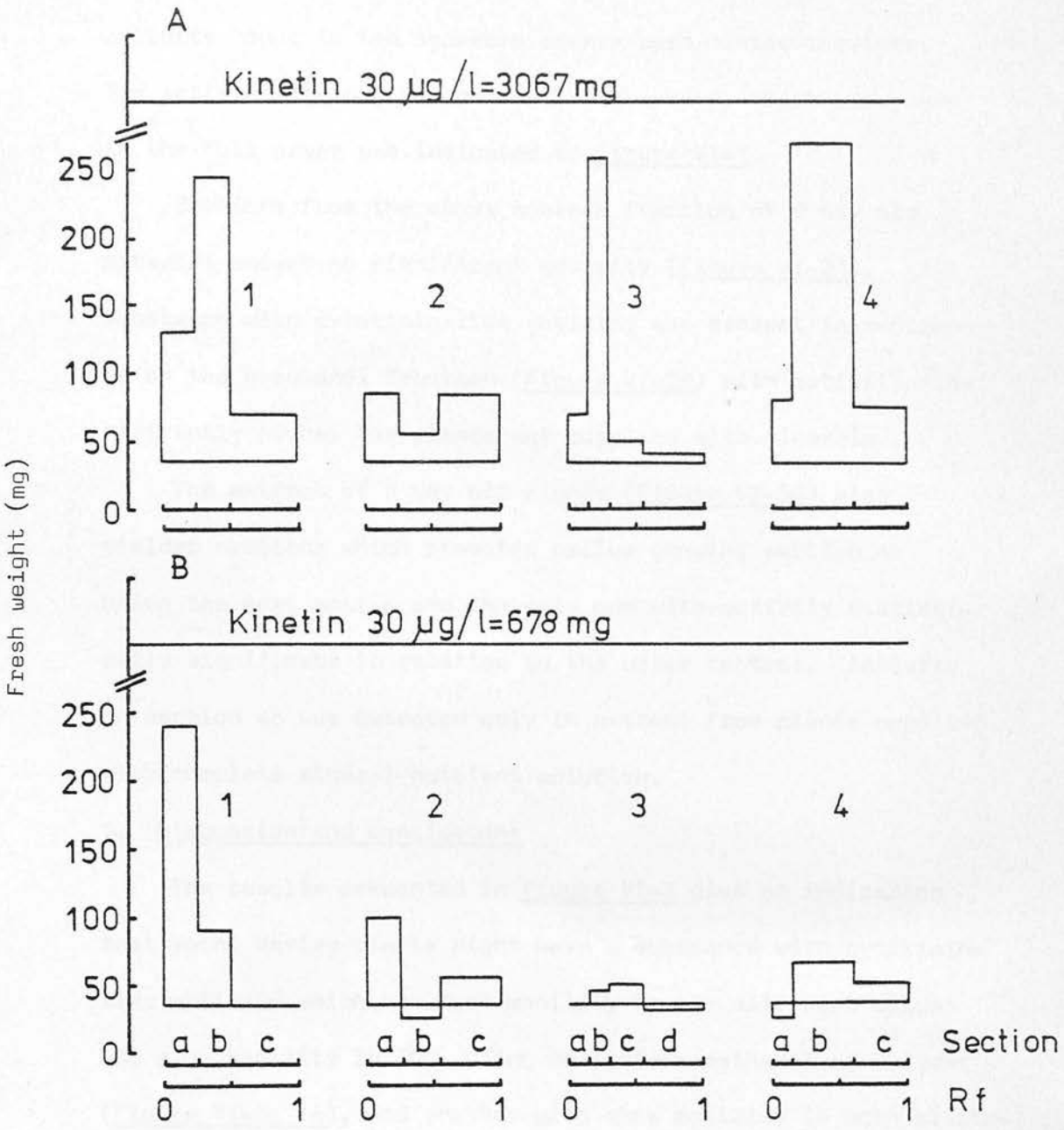

Figure VI-2: Tobacco callus bioassay of the sub-fractions 1, 2, 3 and 4 from extracts of 8 day old plants. Plants supplied with complete nutrient solution (A) or supplied with nutrient solution lacking in nitrate (B). Base lines represent the water control values.

assays.

A common assay was therefore done in which the peaks of activity found in the separate assays were tested together. The active sections, in the separated assays, which were used in the full assay are indicated in Figure VI-1.

Sections from the ethyl acetate fraction of 6 day old material showed no significant activity (Figure VI-3A). A substance with cytokinin-like activity was present in section 1a of the n-butanol fraction (Figure VI-3B) with activity significantly higher for plants not supplied with minerals.

The extract of 8 day old plants (Figure VI-3C) also yielded sections which promoted callus growth, section 4b being the most active and the only one with activity statistically significant in relation to the water control. Activity on section 4b was detected only in extract from plants supplied with complete mineral nutrient solution.

2. Discussion and conclusions

The results presented in Figure VI-3 give an indication that young barley plants might have a substance with cytokinin-like activity which has fast mobility on the silicagel column and slow mobility in TLC, using chloroform:methanol as solvent (Figure VI-3: 1a), and another with slow mobility in both silicagel column and TLC (Figure VI-3: 4b). The former substance seems to be present in extracts of plants from all treatments but to be increased in absence of minerals, while the other active substance appears to be present only in plants supplied with complete mineral solution.

Some of the active peaks in the isolated assays showed no

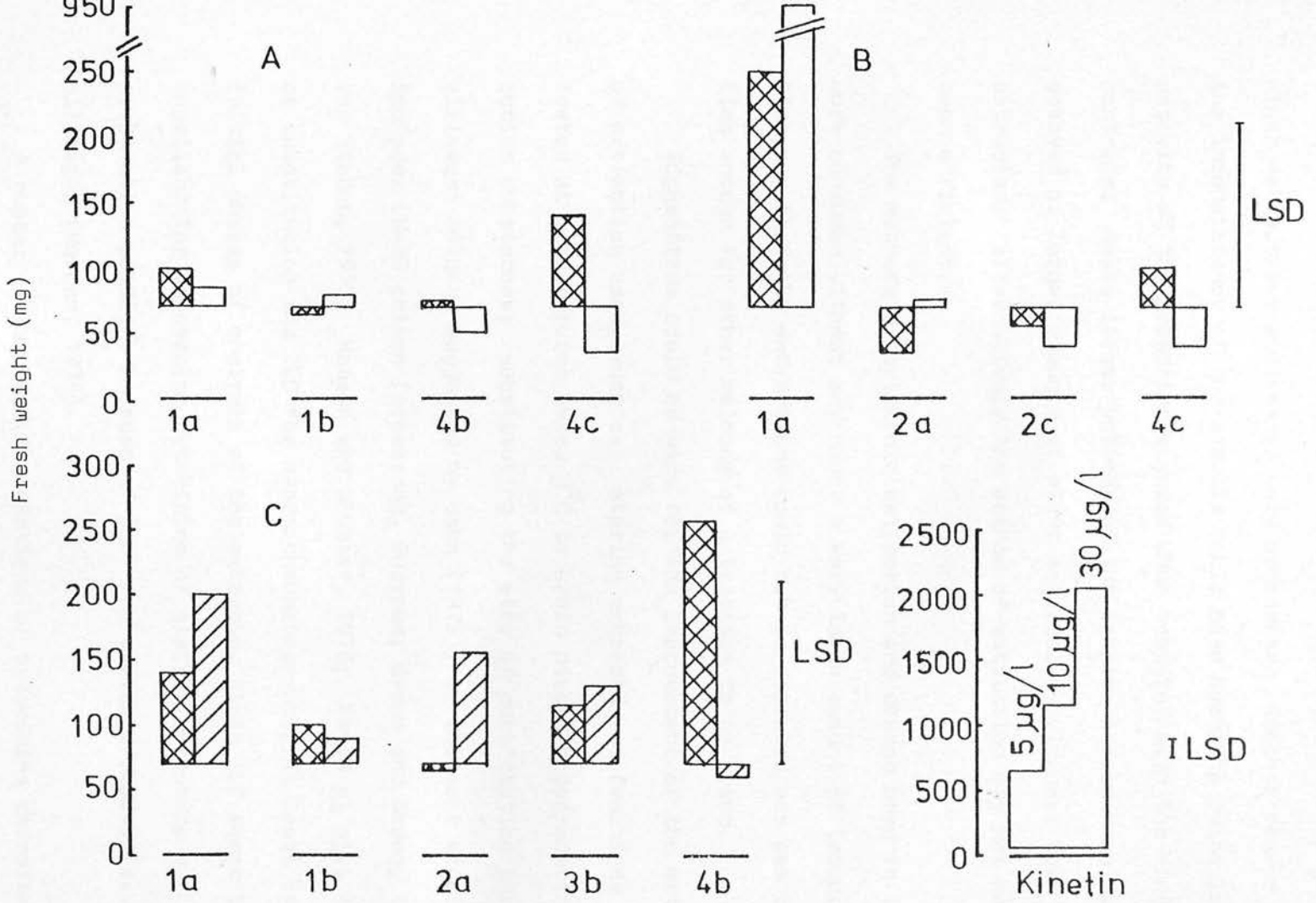

Figure VI-3: Tobacco callus bioassay of the ethyl acetate (A) and n-butanol (B) fractions from extract of 6 day old plants, and the ethyl acetate fraction from extract of 8 day old plants (C). Plants supplied with complete nutrient solution ▩ ; nutrient solution lacking in nitrate ▨ ; or no minerals at all □ .

activity when the comparative bioassay was done. This may have been due to degradation during the storage of the samples, since some of them were inevitably stored for long periods while the other extractions and assays were processed. Another reason for the inconsistency of the results could have been the quite low activity of the promoting samples when compared with the kinetin controls; maybe larger volumes of the samples should have been assayed or larger amounts of plant material should have been extracted; alternatively the method of extraction may not have been efficient.

The methods of cytokinin extraction and dosage used in this work consumed without any doubt a very large amount of labour and time, so that the extractions could not be repeated nor was there time enough for other methods of extraction to be tried.

Suggestions could be made for the improvement of the methods of extraction used, such as: storing extracts and fractions to be tested at temperatures below $0^{o}C$ to avoid possible degradation of active substances; substituting the step of purification through silicagel column, suggested by Hahn (1975), by the most widely used Sephadex LH-20 column (Armstrong, Burrows, Evans and Skoog, 1969; Van Staden, 1973; Henson and Wheeler, 1976; Purse et al., 1976) or substituting the TLC for paper chromatography, at least in the initial phases of analysis of the extracts, since TLC seems to be excellent for separating cytokinins of similar structure but not for preparative problems because of poor and variable recoveries from silicagel (Horgan, 1978).

A number of successful isolations of cytokinins described in the literature have been done from xylem sap, roots or other non-

pigmented organs (Wagner and Michael, 1971; Yoshida and Oritany, 1971; Skene, 1972; Wheeler, 1972; Letham, 1974). The extraction of cytokinins from barley plants, described above, may have been made difficult by the presence of large amounts of leaf pigments in the extracts. One way of overcoming this problem could be to extract only root tissue, since the use of exudate is rendered difficult by the little amount of exudate produced and by the lack of production in plants not supplied with mineral nutrients.

The results presented here can only be regarded as preliminary; more extractions should be made to determine first the pattern of distribution of activity in different fractions of the extract of barley plants and then to compare this pattern in plants given mineral nutrients or not. Nevertheless the data do suggest that a specific cytokinin may be present in plants supplied with mineral nutrients and absent when supply is withheld.

CHAPTER VII

GENERAL DISCUSSION

1. The hypothesis and how it was tested

The working hypothesis tested in this project can be restated as follows:- under low mineral nutrient conditions the early development of tiller buds in barley does not occur because the endogenous level of cytokinin is low; increasing the availability of minerals to the plant leads to an increase in the endogenous level of cytokinins and the growth of tiller buds is initiated.

Three approaches were used to examine this hypothesis. The first approach was to determine the effect of exogenously applied growth substances, and especially cytokinin on bud growth.

Having ascertained that exogenously applied cytokinin can promote growth of lateral buds in barley, the second approach was used. This was to give a treatment to the plant which might be expected to modify the endogenous levels of cytokinin and to relate the treatment to effects on growth of tiller buds. The treatments involved removal of parts of the root system which by modifying the pattern of growth would be expected to affect levels of endogenous cytokinin.

The third approach used was to determine directly the level of cytokinins in plants grown in different mineral nutrient conditions and correlate this level with the growth of tiller buds.

The experimental work described in the previous chapters of this thesis and relating to these approaches will now be discussed.

2. The effect of exogenous growth substances

Because of the structure of the barley seedling the application of exogenous growth substances could not be made to the exact region

where they were expected to act, for example auxin could not be applied directly to the mainstem apex, nor could cytokinin be applied directly to the lateral buds. Treatments therefore involved application indirectly via the roots or leaves. To be effective the applied substances should be able to move within the plant and to reach the site where they are expected to be active.

Cytokinins of root origin are translocated to the aerial part of the plant with the xylem sap (Kende, 1965), suggesting that exogenous cytokinins applied to the roots may also be easily directed to leaves and buds; radioactive cytokinin applied this way was indeed accumulated in the tiller buds (Table III-27 and 28) and affected their growth (Figure III-19). The fact that the cytokinins kinetin, PBA and zeatin were effective on bud growth when applied through the roots (Table III-4) but not through the leaf (Table III-3) suggests that in leaf application cytokinins may not have been translocated. It has been shown in Pisum that in conditions where ^{14}C kinetin is accumulated in the lateral buds the accumulation occurs whether the application is made to the roots or to the apical bud (Morris and Winfield, 1972). Johnston and Jeffcoat (1977) obtained promotion of tiller growth in wheat, oats and barley by application of cytokinin as a leaf spray, showing that transport of leaf applied cytokinins can occur in these plants. It seems therefore that the lack of effect of cytokinin applied to the leaf may be due to the small amount of substance which was applied and penetrated the plant. In the case of application of auxin and gibberellin the effectiveness of these treatments may have been due to greater penetration of these substances, or to their greater mobility and transport to the buds, or to a greater but unspecified sensitivity of the plant to these

compounds. The available evidence does not enable a distinction to be made between these possibilities.

The phase of exponential growth of tiller buds is ininitated immediately after the barley plants are supplied with mineral nutrients (Fletcher, 1975). For the initiation of this phase of growth, the plant must be in a suitable developmental state to allow the release of those buds. Such a state for bud release could involve merely the availability of mineral nutrients and carbon assimilates for the bud itself. The few mineral nutrients available in the plant before the application of further supply in the growing medium may well be transported to the mainstem apex as a first and larger sink and consequently directed away from the small tiller bud primordia. Application of mineral nutrients would increase the levels of nutrients which not being used in the mainstem apex would become available for the lateral buds which are then released from apical dominance. However application of cytokinins to plants not supplied with minerals was able to induce the repressed buds to grow. It appears therefore that mineral nutrients availability is not of primary importance for lateral bud release. An alternative idea is that a suitable level of growth substances is a necessary pre-requisite for initiation of growth of lateral bud. Langer et al. (1973) showed that in Triticum the inhibition of tiller growth by reduced assimilate supply could be relieved by kinetin treatment, but they reduced the supply of assimilates by defoliating the mainstem or by treating plants with an inhibitor of photosynthesis.

Although cytokinins are able to release arrested buds in plants not given minerals the fact that these buds are always smaller than the buds released by mineral application suggests that cytokinins may

be able to start bud growth but that the continuation of growth may be dependent on other factors as mineral nutrients, assimilates or other growth substances.

Growth of tiller buds released by mineral nutrient application can be increased by cytokinin treatment showing that even when nutrients are available and the buds are released the level of endogenous cytokinin may not be optimum. This effect of cytokinin increasing growth of released tillers has been found in other grasses (Jewiss, 1972; Langer et al., 1973; Clifford and Langer, 1975; Johnston and Jeffcoat, 1977).

Besides cytokinin, the other growth substance frequently associated with the control of axillary buds growth is auxin. As discussed in Chapter I, the presence of high levels of auxin often leads to an enhancement of apical dominance, whether these levels are present in the mainstem apex or in the lateral bud. When applied to young barley plants auxin certainly did not promote growth of tiller buds arrested by mineral nutrient stress, but it also did not reduce the growth of tillers released by application of minerals. On the contrary, auxin application actually increased growth of tillers released by mineral nutrient application (Table III-19). It seems therefore that auxin is not involved in the inhibition of bud growth in young barley plants; the lack of effect of TIBA in plants not supplied with minerals (Table III-20) gives further support to this idea.

Nor can gibberellin be associated with inhibition or release of tiller buds. Application of the inhibitor of gibberellin biosynthesis CCC did not release buds from inhibition (Table III-15) suggesting that this was not due to high and inhibitory amounts of gibberellin. Lack of gibberellin is not a limiting factor for tiller bud growth,

since application of exogenous GA_3 did not promote it (Table III-10). The lack of effect of CCC in releasing lateral buds may seem peculiar since its effect increasing tillering in cereals has often been mentioned (Humphries, Welbank and Witts, 1965; Jewiss, 1972; Wünsch, 1973), but this effect has been observed only at a late stage of development when the plant enters the reproductive phase, the mainstem is elongating, tillering is restricted (Jewiss, 1972) and a high level of endogenous gibberellin is believed to be present.

Although it seems that in the seedling stage, gibberellin is not of primary significance in the release of bud growth it is clear that once buds are released by nutrient application, the gibberellin applied exogenously acts as a growth promoter.

While exogenous cytokinin is able to release tiller buds inhibited by mineral nutrient stress and also to increase growth in released buds, auxin and gibberellin have only the second ability. Cytokinin may therefore be responsible for the initiation of the phase of exponential growth of the buds, while auxin and gibberellin may act only afterwards. The synergism between IAA or GA_3, and PBA obtained when these substances were applied together to plants not supplied with minerals (Table 21 to 25; Figure III-18) showed again that once apical dominance is broken the continuation of growth may be determined by the presence of substances other than cytokinin.

The response of young plants, through tiller bud growth, to a hormonal treatment is probably dependent on internal factors such as assimilates availability and endogenous levels of growth substances which determine the sensitivity of the plant to the treatment. An example of this is the response to GA_3 applied through the roots, which was effective when applied to plants treated with PBA (Figure III-18)

but had no effect where the apical dominance had been broken by mineral nutrient application (Table III-9). In the latter case GA_3 is able to increase tiller growth but application through the leaf at high concentrations is necessary to obtain this effect (Figure III-14 to 17). The sensitivity of plants to IAA treatment also seems to be lower in plants supplied with minerals where high level of the substance was necessary to promote bud growth (Table III-19) comparing with the levels capable of increasing tiller growth in plants treated with PBA (Table III-21 to 23). The higher sensitivity of buds released by PBA treatment may be related to low levels of endogenous gibberellin and auxin in the bud which restrict growth. Sachs and Thimann (1964, 1967) found in Pisum, that both auxin and gibberellin applied exogenously increased strongly the growth of lateral buds released from apical dominance by cytokinin treatment.

Besides effects on the growth of tiller bud, application of cytokinin to barley plants also affected their general growth. The inhibitory effect of cytokinin on the root system of plants not supplied with minerals (Figure III-9 and 11), which had been found previously for barley plants supplied with mineral nutrients (Ruckenbauer and Kirby, 1973), was probably linked to the increase in dry matter of the aerial parts of the plant (Figure III-8 and 10), since assimilates not being used for root growth would be available for the growth of the tops. However, release of tiller buds may not be just a consequence of a general increase in dry matter of the tops since cytokinin also promoted the initiation of leaf primordia on the bud, but not on the mainstem apex (Figure IV-1). Another fact in favour of the idea that the effect of cytokinin on the lateral bud

is not a consequence of a general effect in the plant, as a consequence of root inhibition, is that treatment with cytokinin in plants supplied with minerals increased tiller dry matter (Figure III-12) but not that of the leaves (Figure III-8 and 10).

The work with exogenous growth substances confirmed the initial idea that cytokinin is a primary factor on the release of lateral buds in barley, at an early stage of the vegetative development, and showed that other factors such as minerals, assimilates, auxins and gibberellin are also involved in the growth of tiller buds.

3. Modification of endogenous cytokinin content

Manipulation of the root system of barley plants which were not given minerals provided an additional, but indirect, demonstration of the possible importance of cytokinin to the release of tiller buds from apical dominance.

As discussed above, the outgrowth and further development of tiller buds in barley is probably dependent on the existence in the plant of suitable conditions such as availability of minerals, metabolites, cytokinin, auxin and gibberellin, with cytokinin being necessary for the initiation of growth and the other factors for its continuation. Therefore, the release of inhibited buds by root removal treatment (Figure V-6) is not likely to be due to alteration in the levels of endogenous auxin or gibberellin. Nor can increase in nutrient availability be responsible for bud release since, firstly, minerals were not supplied, and secondly, there was no increase in the dry matter of the tops to suggest an increase in availability of assimilates. Thus the release of buds could well be related to an alteration in the level of endogenous cytokinin. How root removal affected the content of cytokinin is uncertain but it is possible

that treatment led to enhanced lateral growth by compensation and hence to increased level of cytokinin.

The final evidence obtained in support of the idea of the growth of tiller buds being mediated by the mineral nutrient supply through an increase in cytokinin level was given by the direct determination of this level in plants supplied with minerals or not, i.e. in plants in which apical dominance had or had not been broken. Although this result requires confirmation, the pattern of cytokinin-like activity seems to be different in those conditions (Figure VI-3). In agreement with the initial idea that mineral nutrient supply would lead to an increase in substances with cytokinin-like activity in the plant, the extract of plants supplied with minerals presented an active substance which was absent in the deficient condition. This observation besides the findings of other workers that the low mineral status of the plant reduces their cytokinin activity in Solanum (Woolley and Wareing, 1972a) and in Lycopersicon (Menary and Van Staden, 1976) favours the idea that supplying mineral nutrients increases the level of endogenous cytokinins also in barley.

4. Overall control of bud growth

Tests of the working hypothesis through different approaches led to the following main conclusions.

The treatment of plants with exogenous growth substances confirmed the hypothesis with reference to the involvement of growth substances on the outgrowth of tiller buds. This approach showed that cytokinin is a primary factor for the release of tiller bud arrested by mineral nutrient stress, but that the subsequent growth of the bud depends on the availability of other factors such as carbon assimilates, minerals and other growth substances.

The second approach to test the hypothesis gave the second indication, this time indirect, of the role of cytokinins on bud release since manipulation of the root system, expected to alter the internal balance of cytokinins, did in fact promote the release of arrested tiller buds. Direct determination of the levels of endogenous cytokinins, used as another approach to the problem, provided results, preliminary but, consistent with the idea of a cytokinin-like compound being increased in plants given minerals.

A summary interpretation for the early growth of tiller buds in vegetative barley plants based on the experimental results discussed above is given in the following paragraphs and in Figure VII-1.

By day 4 after planting a Proctor barley seedling includes the primordia of the coleoptile and of the first leaf tillers (Fletcher and Dale, 1974). The development of these primordia is conditional on supply of mineral nutrients to the plant (Fletcher, 1975). Tiller buds of plants in mineral nutrient stress are therefore arrested. In this condition the level of endogenous nutrients (minerals, carbon assimilates, and others) is low as is also the level of cytokinin, but it is the low level of the latter that prevents bud growth from occurring. Thus neither treatment with auxin or gibberellin, nor with their inhibitors can induce any alteration of this inhibited state.

Application of exogenous cytokinin removes this inhibition but only a slight growth is allowed since nutrients and other growth substances are now the limiting factors. When only minerals are supplied the levels of both the required metabolites and cytokinins are increased, the bud then grows, but since the cytokinin level is not optimal it becomes the limiting factor. Only when minerals

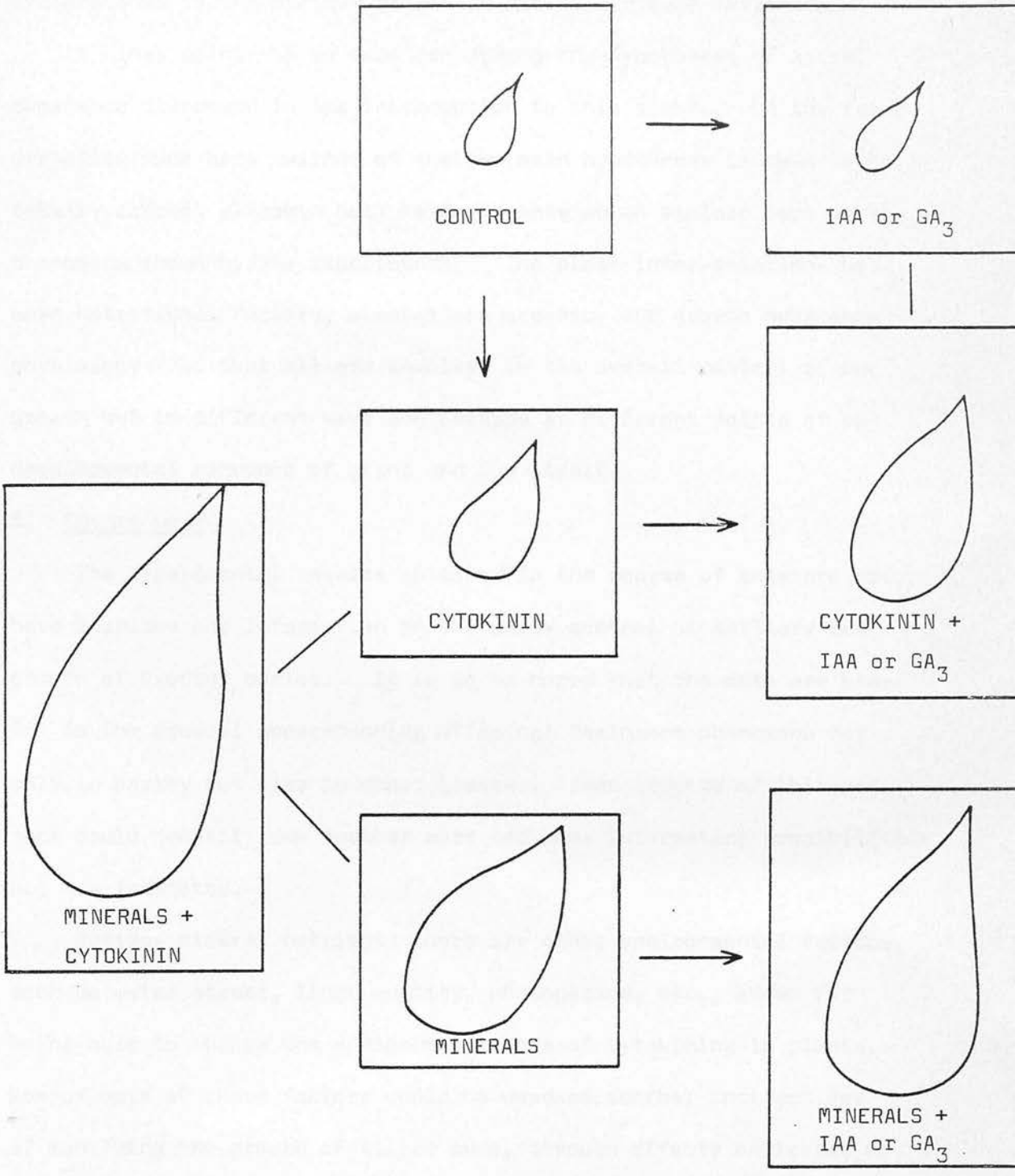

Figure VII-1: Diagramatic scheme of the relative effects of cytokinin, minerals, auxin and gibberellin, and their synergisms on the early growth of tiller buds in Proctor barley. The response is indicated by the size of the illustrated bud.

and cytokinin and the secondary growth factors auxin and gibberellin are provided is the maximum growth of the tiller buds obtained.

A final point can be made concerning the hypotheses of apical dominance discussed in the Introduction to this thesis. On the interpretation made here neither of the two main hypotheses is seen to be totally correct although both have elements which explain part of the phenomena shown by the experiments. The close inter-relations between nutritional factors, mineral and organic, and growth substance physiology mean that all are involved in the overall control of bud growth but in different ways and perhaps at different points of the developmental sequence of plant and bud itself.

5. Future work

The experimental results obtained in the course of this project have increase our information on the early control of axillary bud growth of Proctor barley. It is to be hoped that the data are useful to the general understanding of apical dominance phenomena not only in barley but also in other grasses. Some aspects of this project could benefit from further work and some interesting possibilities are now indicated.

Besides mineral nutrients there are other environmental factors, such as water stress, light quality, photoperiod, etc., known for being able to change the endogenous levels of cytokinins in plants. One or more of these factors could be used as another indirect way of modifying the growth of tiller buds, through effects on levels of endogenous cytokinins, to attempt to confirm the primary effect of this substance on bud growth.

Shading the first leaf is another condition observed to delay the onset of rapid growth for tiller buds in barley (Fletcher and Dale,

1974); it would be interesting to know if in this case the delay is also due to low levels of endogenous cytokinins or if here the limiting factor is availability of assimilates or maybe other growth factors.

It was observed by chance, when testing the effect of TIBA on the growth of tiller buds, that treatment of the root system of barley plants with water at low pH (3.5) had a strong effect increasing the dry matter in tiller buds of plant supplied or not with mineral nutrients. It would be interesting to make some more observations on this aspect, as well as determining whether the effect of pH on release and growth of tiller bud is also operated through increase in the level of endogenous cytokinin or if this phenomenon is here operated through some other mechanisms.

Direct determinations of levels of endogenous cytokinins in plants supplied or not with mineral nutrients should certainly be extended. It would be of interest to know not only about the levels of active substances in each treatment, but also to identify the cytokinin-like substances present in each one of the treatments.

It is not known which is the nature of the connection between mineral nutrients and cytokinin level in the plant. On the other hand, at least the nitrate and phosphate components of the mineral solution may be involved in the determination of the endogenous level of cytokinin in plants. Maybe it would be helpful for the determination of this connection to compare the cytokinin content of plants supplied with different components of the mineral nutrient solution.

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