

~~A STUDY OF RECOVERY FROM DROUGHT~~
~~IN / NICOTIANA TABACUM~~ ~~2.~~
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by

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Abstract

The object of the present work was to investigate recovery from a single period of drought, especially in Nicotiana tabacum L. Different species (cucumber, sugar beet and tobacco) were found to be able to make a substantial recovery from drought. The recovery was associated with a larger relative growth rate and a rapid increase in leaf area.

A detailed growth analysis on tobacco plants showed that the more rapid growth of droughted plants relative to controls on rewatering appeared to have been mainly due to leaf growth responses. The recovery of leaf area was related to a more rapid growth of leaves initiated after or just before the treatment finished and not to an increased number of leaves. The greater relative leaf area growth rate of the younger treated leaves was due to a complete recovery to control level of both cell number and size in those leaves. The explanation of the observed results appears more likely to lie in a more favourable water balance during recovery.

Although the nutrient concentrations in the plants as a whole were high compared with critical levels it is possible that the accumulation of nutrients in young leaves during drought might have contributed to their more rapid growth subsequently.

In spite of a rapid top growth of treated plants during recovery, root growth, in terms of dry weight and length, was slower than in controls.

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Introduction

Water stress in plants produces both immediate deleterious effects and after effects. The after effects may be persistent if permanent damage has been done; there may however be a recovery manifested in more rapid growth, so that the immediate effects are not entirely persistent, and it has sometimes been claimed that moderate stress in early stages of growth is beneficial in later growth.

Water stress has multiple immediate effects on plant growth: photosynthesis is reduced both by closure of the stomata, which decreases the supply of carbon dioxide, and reduction of the capacity of the protoplasm to carry on the chemical processes of photosynthesis. Reduced translocation might hinder photosynthesis by accumulation of end product. The reduction of photosynthesis, decreased translocation of carbohydrates and growth regulators, and disturbance of Nitrogen metabolism all add to the effects of reduced turgor in reducing growth.

The recovery of normal metabolic behaviour appears to take some time, possibly because of the death of roots which may reduce the absorption of water and nutrients. Stomatal function also may take some time to recover.

In some plants recovery from drought has been associated with an increase in the vegetative growth rate above that of controls.

Several possible explanations have been suggested for this: i) accumulation during drought of a substance required for growth, e.g., proline; ii) increased mobilization of Nitrogen and Phosphorus from ^{old} leaves to young, rapidly expanding leaves; iii) a more favourable water balance; iv) a change in the balance of hormones such as cytokinins.

Moderate water stress can improve the quality of plant products, even though it reduces vegetative growth. Richard and Wadleigh (1952) cite works indicating that moderate water stress improves the quality of apples, pears, peaches and prunes. Water stress decreases total vegetative growth of guayule but it increases its rubber content (Wadleigh et al., 1946). Water stress increases the desirable aromatic properties of Turkish tobacco (Wolf, 1962), increases the alkaloid content of Atropa belladonna and also increases the oil content of olive fruits (Evenary, 1960). It has been claimed that moderate water stress at early stages makes plants tolerate drought at later stages better than plants that have not been stressed (Genkel, 1964).

Drought hardening

Plants subjected to one or more periods of moderate water stress are sometimes said to be "hardened", because it has been claimed that they usually survive subsequent drought with less injury than plants not previously stressed. Genkel (1964) attributed hardening chiefly to such protoplasmic changes as increased water-

binding capacity and viscosity along with decreased permeability and low respiratory rates. Russian workers (reviewed by May et al., 1962) have claimed that subjecting seeds to successive cycles of hydration and dehydration prior to sowing increases drought resistance. Attempts to reproduce these results have been disappointing (Asana, 1960; Evenary, 1964; Jacoby and Oppenheimer, 1962 and Husain et al., 1968).

Recovery from drought

An increase in the vegetative growth rate above that of the controls has often been reported to occur when plants are rewatered after short periods of moisture stress.

Owen and Watson (1956) working with sugar beet in experiments carried out in the field, reported an increase in net assimilation rate and leaf growth rate on watering after a period of drought. Owen (1958), working with sugar beet grown in pots found that there was no significant effect of recurrent drought on dry weight or net assimilation rate but that the leaf areas were lower than those of the wet treatment plants at all sampling times.

Orchard (1963), reported an attempt to increase growth rates of sugar beet plants by controlling watering under field conditions. Growth was greatly stimulated by watering after drought, due to an increase in assimilation rate. However, this was nullified to some extent by a decline in leaf area index.

Gates (1955 a, b), studied the response of tomato plants to

water stress in glasshouse conditions. During wilting, relative growth rate and net assimilation were considerably and significantly reduced. Following rewatering, however, growth rates rose to levels significantly greater than those of controls. Translocation of dry matter from the laminae to the stem was enhanced on a proportional basis during wilting by comparison with controls, whereas on rewatering a higher proportion of dry matter was retained by the laminae. Gates interpreted the pattern of dry weight accumulation in whole plants subjected to water stress as a tendency towards senescence during wilting and return towards a more juvenile condition upon rewatering.

Petrie and Arthur (1943), studied the effects of drought on tobacco plants grown in pots. When plants were subjected to temporary drought a depression in growth rate and net assimilation (calculated on a leaf protein basis) was observed. When plants subjected to temporary drought were returned to high-water conditions the net assimilation rate was restored to approximately the same value as that of the controls but the growth rate of droughted plants was greater than controls. Hopkinson (1968), made detailed studies of the growth of tobacco plants during and after imposition of an early period of water stress in glasshouse conditions. During water stress all growth rates were depressed, but a short time after the relief of stress, relative leaf area expansion rates, net assimilation rates and relative growth rates rose to higher values

than those reached by untreated plants at comparable stages of development. As a result, all stressed plants rapidly recovered after the period of adverse conditions was over. The plants that had originally been subjected to the more severe treatment overtook those that had suffered less and finally attained a greater total leaf area and dry weight. This result arose in the following ways: firstly, stressed plants were able to recover rapidly because of unusually high growth rates; secondly, a delay in leaf production relative to leaf expansion brought about an advantageous re-arrangement of leaf surfaces relative to incident light; thirdly, a delay in floral induction enabled the apical meristem to produce an increased number of leaves which conferred upon the plant a greater potential for vegetative growth. Hopkinson (1969), could not detect any advantage of an early water stress on tobacco under field conditions. Neither stimulated growth rates during recovery nor a subsequent changed pattern of development were observed. The early history of field plants was of course very different from that of pot-raised ones, and herein probably lies the reason for the difference in response. First, water was deliberately withheld from the seedbeds, a necessary procedure designed to harden the seedlings in preparation for transplanting, then the inevitable root mutilation of transplanting followed by a high evapo-transpiration potential in the field, caused a prolonged and severe wilting of the young transplants. The subsequent investigation of the effect of a

short period of drought was therefore made on plants which had already suffered twice from water stress.

Delay in recovery from drought

If the water supply is renewed before death occurs, the recovery to normal metabolic behaviour appears to take several days, even in situations where only a single brief, but severe period of water stress has occurred (Gates, 1955a, 1955b). There is evidence that recovery is delayed by the marked reduction in rates of water absorption caused by death of root hairs, or roots and increased suberization of the root system, which reduces the permeability of the root to water and nutrients. Brix (1962) showed that the time for recovery of photosynthesis in a wilted Loblolly pine seedling could be reduced from 50 to about 10 hours by cutting off the root system and placing the cut stem in water. Stomatal function also may take some time to recover, according to Fisher (1967) who suggests that the inhibition of the stomata is proportional to the magnitude and duration of leaf water deficit.

Physiological factors in recovery from drought

Water stress affects practically every aspect of plant growth. Some of the effects are related to decrease in turgor and some to a decrease in osmotic potential. Turgor is important in relation to the opening and closing of stomata and also affects cell expansion of leaves, (Ordin, 1958, 1960). Boyer (1970) suggests that a minimum turgor is required for rapid cell enlargement and small

increases in turgor result in large increases in rate of enlargement. Enzyme-mediated processes are presumably controlled more directly by the osmotic potential changing the hydration or structure of proteins. Tyler (1954) showed that succinic oxidase activity decreased with decreasing osmotic potential. Osmotic potential could initiate changes in the cell by affecting the nucleic acids which control protein synthesis, (West, 1962).

Cell division seems to be affected less by water deficit than cell elongation. Gardner and Niemann (1964), reported that cell division did not cease even when cell turgor fell to zero. Gates and Bonner (1959), found that RNA synthesis continued in tomato plants subjected to water stress, further supporting the idea that cell division continued. Martin (1940) has shown that the smaller leaf size of sunflower under water stress was largely due to the failure of existing cells to expand under reduced turgor conditions, rather than to a difference in the initial cells formed. The more rapid relative growth rates in stressed plants following rewatering compared with controls could result from a requirement for cell expansion alone rather than division and expansion after removal of stress (Slatyer, 1967).

Water deficiency appears to have a primary effect on the hormonal regulatory mechanism. Itai, Richmond and Vaadia (1968), presented evidence that cytokinins are primary factors in a control system that facilitates root-shoot communication. When roots were exposed

to increased osmotic values in the nutrient medium, a decreased translocation of cytokinins from roots was observed. This decrease was reversible, and upon termination of the osmotic stress cytokinin activity in the root exudate increased, initially exceeding that of the control levels. Root stress brought about a decline in the protein synthesis potential of the leaves which could be partially corrected by pretreatment with kinetin. They suggested that stress-induced decline in leaf protein synthesis results from a deficiency of cytokinins in the leaves. However, similar effects could be due to water stress alone (Itai and Vaadia, 1965) and a higher rate of growth during recovery could be associated with a higher cytokinin content in leaves of plants that have been under water stress.

There is a considerable hydrolysis of proteins in wilted plants accompanied by an increase in amino acids. Kemble and Macpherson (1954) established that a major effect of prolonged, severe drought, was to cause an outstanding increase in the free proline content of leaves. This particular change was much greater than the changes which also occurred in several other amino acids, since proline could increase to more than one hundred times its original concentration. Barnett and Naylor (1966), reported that water stress on a number of strains of Bermuda grass (Cynodon dactylon) induced a 10-100 fold accumulation of free proline in shoots and 2-6 fold accumulation of asparagine; valine levels increased and glutamic

acid and alanine levels decreased. They showed that proline was synthesized from glutamic acid and suggested that free proline may serve as a storage compound for both Carbon and Nitrogen during water stress, when starch synthesis and protein synthesis are inhibited. Upon rewatering the proline may then be utilized to support renewed growth (Skinner, 1968).

Gates (1957), studied the effects of water stress on uptake and redistribution of Nitrogen and Phosphorus in tomato plants. The effect of water shortage on total amounts of Nitrogen and Phosphorus was to depress the levels of both elements (especially Phosphorus) relative to controls. On rewatering, however, the pattern was reversed so that at final harvest, the amount of Nitrogen in the tops was approximately equal for all treatments, whereas Phosphorus was increasing more rapidly in previously wilted plants than in controls. This tendency to regain control levels by the final harvest was most pronounced in the laminae and least in the stem, because the former regained control levels in both Nitrogen and Phosphorus, whereas the stem failed to do so.

The proportions of Nitrogen and Phosphorus in stem and laminae were affected by water stress. Thus, during wilting, the proportion of each element in the laminae fell in comparison with controls, whereas that in stem rose. On rewatering, the reverse was the case, with the laminae having a greater proportion of these elements than the stem. The trend in each case was more marked for Phosphorus

than for Nitrogen. Nutrient content of all laminae were influenced by water stress, and the effect was greatest in the upper leaves. Conversely, on recovery from water stress the concentration of nutrients in the upper laminae rose above that in controls to a greater extent than in lower laminae. During wilting, depression in Nitrogen uptake was so marked in the laminae as to give very few individual increases relative to controls. In fact, there was a slight loss of Phosphorus and possibly of Nitrogen in lower laminae, and only the uppermost laminae showed real increases. All petioles showed increases in Nitrogen, and all but the lowest petioles increased in Phosphorus. As the proportions of both nutrients were higher in stem and lower in laminae during wilting, their translocation from lower laminae through petioles and into the stem is indicated. Loss of both nutrients from at least the oldest leaf occurred upon re-watering.

Possibly in developing leaves and in meristematic tissues of droughted plants cells tend to expand rapidly and further cell division may be stimulated by the increased availability of Nitrogen and Phosphorus compounds in the vascular system (Slatyer, 1967). An experiment on drought hardening in cucumber, leading to the present experiments.

Cabrera (1967), studied the effects of early hardening on cucumber plants in controlled conditions. The main effect of hardening appeared to be on responses during recovery from, rather

than during, a drought. Hardened plants subjected to a period of drought grew faster in leaf area and dry weight during the subsequent period of recovery than unhardened plants subjected to the same period of drought. During the drought period transpiration rates of the unhardened plants were initially much greater than those of the hardened plants and this difference was maintained during the drying cycle when both decreased. However, two days after recovery the transpiration rates of the hardened plants exceeded those of the unhardened plants. Calculated internal resistance (r_1) to water vapour diffusion per unit leaf area of leaf for the last day of the drying cycle, was 2.1 times higher in hardened than unhardened plants; however, at three days following recovery internal resistance of controls was 2.5 times higher. It appeared that the stomata of hardened plants opened readily during recovery whereas those of the unhardened plants opened only to a much smaller degree. The relationship between transpiration rates and relative turgidity were of interest; the relative turgidity of the unhardened plants did not fall very much although the transpiration rates fell to one third of the initial value. The hardened plants had a higher transpiration rate at the same relative turgidity, and it seemed that there was a range of relative turgidity values over which changes in this parameter were considerably buffered. This suggested that stomatal movements were not closely related to changes in leaf water. This effect appeared to be real but, as expected,

operated only over a part of the drying cycle. What appeared to emerge was that hardening influenced stomatal responses but that this effect was associated more with rapidity of recovery after restoration of water than in any capacity to better withstand a period of drought. Nevertheless, these results appeared sufficient to be worth pursuing in greater detail. The first experiment of the present work was planned to see whether susceptibility to drought during the later stages of growth was less in plants which had experienced drought previously, i.e. to look for evidence of drought hardening. The species used were Cucumis sativus (cucumber), Beta vulgaris L. (sugar beet) and Nicotiana tabacum L. (tobacco). No evidence of drought hardening was found but all these species showed a rate of growth on rewatering after drought which exceeded that of undroughted controls. So it was therefore thought to be more rewarding to investigate this response in further experiments. The remaining experiments were therefore designed to investigate this recovery from a single period of drought, especially in Nicotiana tabacum L. Experiment II was a simple growth analysis in terms of dry weight and leaf area. Experiment III repeated the growth analysis and also analysed leaf growth in terms of the rate of leaf production and of cell number and size in the leaves. In this experiment the components of the plants were also analysed for N, P, Ca and K. In experiment IV changes in water relations were investigated.

Tobacco was chosen as the experimental plant in Experiments II,

III and IV because of its ability, at least in pot culture, (Hopkinson, 1968) to attain a larger final leaf area as a result of early water stress, as well as being a plant of economic importance in Venezuela, from where the funds to support this work were derived.

Materials and methods

All four experiments were carried out in the greenhouse, the temperature of which was $20 \pm 5^{\circ}\text{C}$.

In winter, during the first four weeks of experiment II and throughout experiment III (after transplanting from the germinating tray) supplementary lighting was used. In the hottest periods of the summer, green house blinds had to be used to prevent high temperatures.

Cucumber

Uniform seeds of cucumber (Var. "Butcher's Disease Resister") were germinated in Petri dishes (40 seeds per dish) for forty hours at 25°C . Germinated seedlings were then selected for uniformity of length of radicle and planted (three seedlings per pot) at a depth of 2.5 cm in plastic tubes (30 cm height and 8 cm diameter) containing John Innes No. 1 Potting Compost (1200 g per pot) and 100 g gravel at the bottom of the pot. 112 g of Green House fertilizer (G.H.5) per bushel was added to the soil. A nylon net at the bottom of the pot kept the soil in place. The plastic tubes were stood on aluminium foil dishes. Ten days later (i.e. at unfolding of cotyledons) plants were selected for uniformity, reduced to one seedling per pot and randomized between treatments and this time (7/5/68) was taken as zero time.

The leaf areas of all the leaves of plants allocated to the last harvest were estimated at 2 to 4 day intervals throughout the experiment. Dimensions measured and the relationships used to

calculate the area therefrom were those described by Gregory (1921) and amended by Milthorpe (1959). Total leaf area was finally calculated by computer. An error of $\pm 2\%$ may be expected in the area of any one leaf at any one time.

Thirty and fifty days after thinning time, six plants per treatment were harvested. Root, stem and leaves were separated and dried in an oven at 105°C for 72 hours.

Sugar beet

Five uniform seeds of sugar beet (Var. "Klein") per pot were planted at a depth of 2.5 cm in metal containers (25.5 cm height and 17.5 cm diameter) containing John Innes No. 1 Potting Compost plus fertilizer (5000 g of soil per pot) and 300 g of gravel at the bottom of the pot. The containers were previously painted with protective bituminous paint. Ten days later plants were selected for uniformity, reduced to one seedling per pot and randomized between treatments and this time (7/5/68) was taken as zero time.

The leaf areas of all leaves of plants allocated to the last harvest were estimated at 2 to 4 day intervals throughout the experiment. The length and maximum breadth of each leaf were taken and the resulting leaf area for any given time was calculated using an equation (see appendix I) which correlates the product of breadth and length with leaf area. The actual leaf area used in this correlation was measured with an E.E.L. leaf area meter. (Evans Electroselenium Ltd., large area meter). Total leaf area was finally calculated by computer.

Fifty eight and oighty five days after thinning time, six plants per treatment were harvested. Root stem and leaves were separated and dried in an oven at 105°C for 72 hours.

Tobacco

Seeds of tobacco (Var. "Hicks Broad leaf") were evenly spread on the surface of a seed tray containing three layers: at the bottom gravel, in the middle John Innes No. 1 Potting Compost and at the top very fine sand. The three layers filled half of the volume of the tray. The tray was covered with a glass to provide a high humidity. Every day the glass was removed for half an hour to allow exchange of gases until germination was completed. The seedlings were kept growing in the tray until 20 days after sowing, when they were selected for uniformity and transplanted to wooden boxes (8 cm depth, 36 cm length and 23 cm width) containing two layers: at the top John Innes No. 1 Potting Compost and at the bottom dead leaves; 35 plants were planted per box. After 22 further days a new selection was done and the uniform plants transplanted to metal containers (2 seedlings per container) containing John Innes No. 1 Potting Compost plus fertilizer (5000 g of soil per pot) and 300 g of gravel at the bottom of the pot. The containers had the same dimensions as the ones used for sugar beet. Four days later plants were selected for uniformity, reduced to one seedling per pot and randomized between treatments, and this time (8/6/68) was taken as zero time.

The leaf areas of all leaves of plants allocated to the last harvest were estimated at 2 to 4 day intervals throughout the

experiment by the same method as was employed for sugar beet.

Forty eight and sixty eight days after thinning time, five plants per treatment were harvested. Root, stem and leaves were separated and dried in an oven at 105°C for 72 hours.

Experiment II

Seeds of tobacco (Var. "Cubano Criollo") were germinated as described in experiment I. After germination seedlings were selected and transplanted to wooden boxes. After 15 days another selection was made and the uniform seedlings were transplanted to plastic pots (two seedlings per pot) containing John Innes No. 1 Potting Compost plus fertilizer (5200 g of soil per pot) and 500 g of gravel at the bottom of the pot. The dimensions of the plastic pots were: top 24.5 cm diameter, bottom 15.0 cm diameter, and 22.0 cm height. After a further 5 days plants were selected for uniformity, reduced to one seedling per pot and randomized between treatments. A week later the treatments were started and this time (22/5/69) was taken as zero time. At the end of the treatment all treated plants were immersed in water for ten minutes to ensure complete re-wetting.

At intervals of time throughout the experiment, four plants per treatment were harvested and leaves, shoot and root dried in an oven at 105°C for 72 hours. Leaf area was also estimated at each harvest with an E.E.L. leaf area meter.

Experiment III

The same variety of tobacco as used in experiment II was used again in this experiment. After germination, seedlings were

transplanted directly from the germinating tray to plastic pots (two seedlings per pot) but the peat of the John Innes No. 1 Potting Compost was changed for an equal amount of a mixture of perlite E.U.P. 4 X and perlite E.U.P. 130. This was done because it was difficult to separate roots from soil containing peat. A week later plants were selected for uniformity, reduced to one seedling per pot and randomized between treatments. After a further week, treatments were started and this time (10/4/70) was taken as zero time.

At the end of the treatment all treated plants were immersed in water for ten minutes, after which the top of the pots of treated and control plants were covered with a layer of 2.5 cm of expanded polystyrene to avoid evaporation from the soil surface.

Estimation of Cell number and size

Cell number and cell size (Brown and Rickless, 1949; Sunderland and Brown, 1956; Sunderland, 1960) were determined on each leaf of two plants of each treatment at 7 day intervals. Depending on the size of the leaf, either two or three discs of leaf tissue (punched with a cork-borer of 10 mm diameter and avoiding the main veins of the leaves) or the whole leaf was taken. Each sample was weighed (0.1 mg) separately and put into a glass specimen tube (2.2 cm width and 4.7 cm height) with 1 ml of 3% CrO_3 solution. A glass rod was used to keep the tissue at the bottom of the solution. Infiltration of the solution into the tissue was induced by reduced pressure (650 mm Hg for ten minutes) in a vacuum desiccator. The following technique was followed after 12-18 hours (i.e. overnight) for the

old tissue and 18-36 hours for young tissue: the chromic acid was removed with a fine pipette taking care to avoid touching the discs or causing agitation which might result in their disintegration. The last drops were removed with the help of a strip of filter paper. Distilled water (0.60 ml or more to give a concentration of 100-200 cells per mm^2 of the haemocytometer) was pipetted carefully into each tube and the discs were reduced to a suspension of cells by manipulation with a small glass rod against the side of the tube above the level of the liquid and then forced several times through the jet of two fine pipettes of different diameters (using the pipette with the wider diameter first). Evaporation was avoided by covering the tops of the specimen tubes with "Parafilm". The following day two drops of this suspension, which had previously been thoroughly mixed, were transferred rapidly to avoid settling to a 0.2 mm depth Fuchs-Rosenthal haemocytometer slide. When coloured interference bands (Newton's rings) could be seen where the coverslip contacted the slide, it was assumed that the layer of suspension was exactly 0.2 mm thick. The suspension was scanned under low power to ascertain that it was evenly distributed and there was no clumping of cells. With a hand counter the total number of cells was counted in three of the larger squares (1 mm^2 each) taken at random, using magnification X 150. To avoid double counting of cells, the counting was carried out in the following way: any cell lying in a particular square ($1/16$ of mm^2) was counted plus any cell touching the two upper right hand edges of the square. The same

procedure was followed with the following square ($1/16 \text{ mm}^2$) until all the 16 squares corresponding to 1 mm^2 were completed. Five per cent was taken as a limit for agreement between the two drops of each suspension and if they did not agree further slides were prepared. Cell number was calculated for the volume of the suspension, then per square centimeter of leaf and for the total area of the leaf.

Cell number in the whole apical meristem was also measured; the whole apex included leaf primordia too small to be dissected out.

The average volume per cell was calculated approximately by dividing the fresh weight per square centimeter by cell number per square centimeter, assuming that the density of the cells was 1.0 mg mm^{-3} .

Determination of leaf area and leaf number

At each harvest, each leaf was exposed on Ammo-positive blueprint paper and its area subsequently calculated by weight.

In two plants per treatment, at each harvest all leaves on the main axis were counted the leaves in the terminal bud being observed by dissection under a stereoscopic microscope. Leaves with a leaf area equal to or larger than 1 cm^2 were considered unfolded.

After blueprinting, each organ of each plant was oven dried at 105°C for 72 hours.

Estimation of stomatal resistance

A modified Stiles' diffusion porometer (Stiles, 1966) was used to investigate the condition of the stomata (photograph No. 1)



Photograph 1

Stiles' Porometer, used in experiment III

during and after the treatment in two plants of each treatment. Determinations were conducted on the upper and lower surfaces of each leaf at different times during the days on which observations were carried out. The time, in seconds, for the humidity in the porometer to increase by a fixed amount ^{is related to} ~~was taken as~~ the resistance (i.e. a reading started at an arbitrary point and the time taken to reach another arbitrary point was recorded, the same two points being used in each estimation) and ^{relative} stomatal conductivity was calculated as the inverse of ^{time} ~~resistance~~.

Estimation of root length

Root length was measured in two plants of each treatment at each harvest. Dry matter of primary, secondary and tertiary roots were separated and weighed. The length of the secondary roots was measured with a ruler. The length of the tertiary roots was determined using Newman's method (Newman, 1966) on samples of 0.125 g each. Each wet sample sample was evenly spread (with the help of two dissecting needles) on the surface of 30 cm² of a centimeter squared sheet of perspex and subsequently covered with another sheet of perspex. In each sample the number of intercepts was counted on 15 random lines each defined by the length of the cross-wire in the eyepiece of a biocular microscope. An intersection was only counted if the wire crossed the centre line of the roots.

The total length of root (R) in the area (A) of the rectangle was obtained from the number of intercepts on the line (N) with total length (H) by the formula $R = \frac{\pi NA}{2H}$. The mean of the

estimates from the two samples was used to calculate the root length for 0.125 g and hence for the total weight of the roots.

Determination of Nitrogen, Phosphorus, Potassium and Calcium by automatic methods

Determination of N, P, K and Ca was made in root, stem and leaves (each leaf separately) of four plants (two plants per treatment) at every harvest. A Technicon Auto-Analyser was used for the determination of these four elements.

After the plant material had been dried in an oven and cooled in a desiccator, it was powdered in an electric grinding mill, the resulting powder was thoroughly mixed and considered homogeneous for the purpose of analysis.

Determination of Nitrogen

The method used (Varley, 1966) was a micro kjeldahl digestion followed by a modification of the procedure described by Van Slyke and Hiller (1933). This consists of allowing a solution containing ammonium ions to react with alkaline phenol and hypochlorite at a temperature of 60°C, producing an intense blue colour.

A kjeldahl digestion was carried out on 60 mg of dried and finely ground material using sodium sulphate - selenium as a catalyst. The digested mixture was then made up to 100 ml.

The Auto-Analyser was standardized over the range 5-40 p.p.m. with three sample cups of each standard. Samples of the plant material, were then analysed at the rate of 40 samples per hour. The mean of the three replicates was taken as the true concentration in p.p.m.

The intensity of the blue colour produced was read on the colorimeter at 660 m μ .

Determination of Phosphorus

The method used for Phosphorus determination was the one described by Armitage, (1965) in which ascorbic acid was used as a reducing agent for the ammonium-phospho-molybdate complex.

A sample of 0.5 g was placed in a 150 ml conical flask with 5 ml of 70% perchloric acid and 20 ml of concentrated nitric acid. The conical flask plus the sample was heated on a sand bath until the nitric acid volatilised and white fumes of perchloric acid were given off, and left for a further half hour. After cooling, 10 ml of demineralised water were added and mixed; the contents were then transferred quantitatively to a volumetric flask and made up to 100 ml.

After warming up the Auto-Analyser, standards over a range of 2 to 40 p.p.m. of Phosphorus were mixed in the Auto-Analyser with ammonium molybdate-sulphuric acid solution and ascorbic acid solution heated in a bath at 95°C and read on a colorimeter with a 660 m μ filter. Samples of the plant material were then analysed at the rate of 40 samples per hour, analysing each sample solution three times, and the mean value taken as the true concentration.

Determination of Potassium and Calcium

Determinations of Potassium and Calcium were made using the method described by Armitage, (1965).

The solution already prepared by wet ashing for the determination of Phosphorus was used for Potassium and Calcium.

The Auto-Analyser was calibrated over the range of standards 30 to 300 p.p.m. of Potassium and 5 to 50 p.p.m. of Calcium with an internal standard diluted lithium nitrate solution and passed into the flame photometer. Potassium and Calcium were determined at 768 $m\mu$ and 625 $m\mu$ respectively. Samples of the plant material were then analysed at the rate of 40 samples per hour.

Experiment IV

Seeds of tobacco (Var. Cubano Criollo) were germinated as described earlier and transplanted direct from the germinating tray into the plastic pots already described in experiment III. The composition of the soil in the pots was exactly as described in experiment III. A week later, plants were selected for uniformity, reduced to one seedling per pot and randomized between treatments. After a further seven days, treatments were started and this time (20/6/70) was taken as zero time.

At the end of the treatment all treated plants were immersed in water for ten minutes and the tops of the pots were subsequently covered with a layer of expanded polystyrene 2.5 cm deep.

Leaf area was measured throughout this experiment by the same method as employed in experiment III.

Leaf Water Potential

Leaf water potential was determined on a strip 1.1 x 4.2 cm taken from each leaf with a rectangular punch formed from a piece of wood with four razor blades attached. As soon as the strip was cut it was put in a psychrometer chamber (lining the chamber walls).

Since the lid of the chamber remained attached to the psychrometer apparatus, the chamber was wrapped with aluminium foil and put under shade (in a case). As soon as all the samples were taken, the chambers were brought to the laboratory, screwed to the lid in which the thermocouple was mounted and immersed in the water bath. Four hours later, a first reading was made (Barrs, 1965) after which a current of 5 mA was passed for 20 seconds and the maximum deflection recorded. No corrections were made for errors arising from the use of a linear scale rather than a curved scale with the reflecting galvanometer (Barrs and Slatyer, 1965).

Spanner's Psychrometer

Two accurate techniques for measuring the water potential of plant tissue have been widely used in recent years, one developed by Richards and Ogata (1958) and the other by Spanner (1951). As the original Spanner's psychrometer has been improved (Monteith and Owen, 1958 and Waister, 1963) and as Spanner's approach has been proved to be more advantageous (Barrs, 1965) than the Richards and Ogata's, it was decided to construct a modified Spanner's psychrometer.

Of the great variety of sophistications of the Spanner's psychrometer that have been recently developed (Waister, 1963; Box, 1965; Rowse and Monteith, 1969; Lang and Trickett, 1965) it was decided to construct the simplest and cheapest one (i.e. one on similar lines as that of Waister, 1963).

The Thermocouple

The Peltier effect is used to cool a chromel P-Constantan junction below the dew point of the atmosphere under examination, by passing an appropriate current from a battery through the junction in the direction constantan to chromel P. This causes water to condense on the junction, which is then used as a wet-bulb thermometer by switching it into the galvanometer circuit. The condensed water evaporates at a rate inversely proportional to the relative humidity of the atmosphere, depressing the temperature of the chromel-constantan junction relative to that of the heavy copper leads which are maintained at the temperature of the water bath (25°C) by conduction, thus providing stable conditions for the reference junctions. The resulting e.m.f. recorded on the galvanometer, is inversely proportional to the humidity.

The thermocouple was made as small as possible to reduce the condensation of water on the junction. Two 19 s.w.g. copper leads were passed through two holes on the top of the chamber, to protrude 5 mm, and were sealed with Araldite. On the upper side the leads were tightly coiled, the rest of the wire being long enough to emerge above the water surface, and to which PVC-covered copper lighting flex was soldered and the junctions insulated. The use of coiled heavy copper wires, insured that the temperature of the copper leads within the chamber was the same as the temperature of the bath.

Welding of constantan and chromel P 50 s.w.g. wires was tried under oil (Waister, 1963) with a manually operated circuit (see

Appendix II), as this proved to be tedious a mains charged capacitor (see Appendix II) was used jointly with a micromanipulator, instead. A selection of the best thermocouples was done under the microscope and the bad ones rejected.

The free ends of the thermocouple were soldered to the copper leads, at a distance of about 2 mm from the junction, on each side, using a 60 : 40 tin lead solder and a neutral flux. The whole assembly was washed with detergent, rinsed with distilled water and finally boiled in distilled water for ten minutes to remove contaminants.

The flexible leads from the thermocouples were taken to a selector switch, which permitted any couple to be switched as required, into either the battery or galvanometer circuits. The galvanometer deflection was read on a scale calibrated 25 - 0 - 25 cm in 1 mm divisions and placed 1 m away from the galvanometer.

In the equilibration chamber the volume of air immediately surrounding the measuring junction must be in water-vapour pressure and temperature equilibrium with the leaf. As the time to reach equilibrium depends on this volume, and long equilibration times are particularly undesirable with leaf samples, since errors may arise from tissue changes following excision, it was decided to construct small chambers, i.e. about 3.5 cm^3 of internal volume, (Fig. 2).

The equilibration chambers were made from stainless steel as this metal is unaffected by the sodium chloride solutions used in

Fig. 1 Thermocouple psychrometer apparatus and circuit

- A - milliommer, A.S.W. Automatic supply of water
- C - 2 volt accumulator
- D - double-pole, double throw switch
- G - galvanometer
- G.T.-glass tank
- H - heating bulb
- I - Polystyrene insulation
- P - pump; P.S. - perspex support;
- R - relay; S - selector switch
- T - contact; Th. - thermocouple assembly
- V - variable resistance;
- W - wooden box

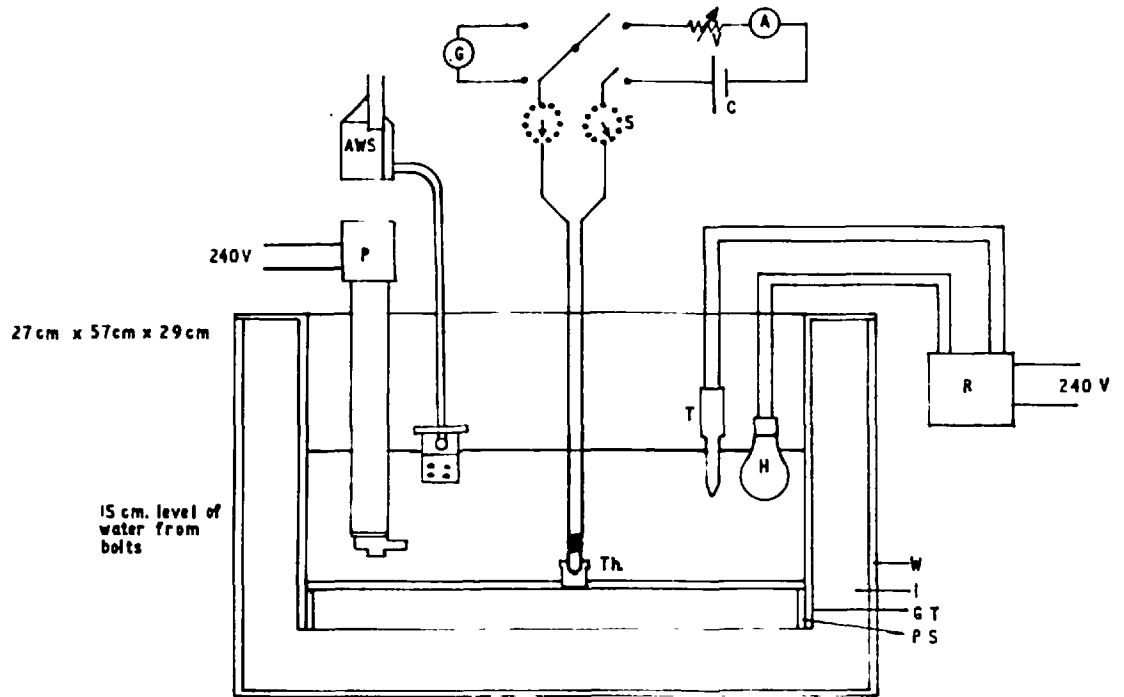


Fig.1 Thermocouple psychrometry apparatus and circuit

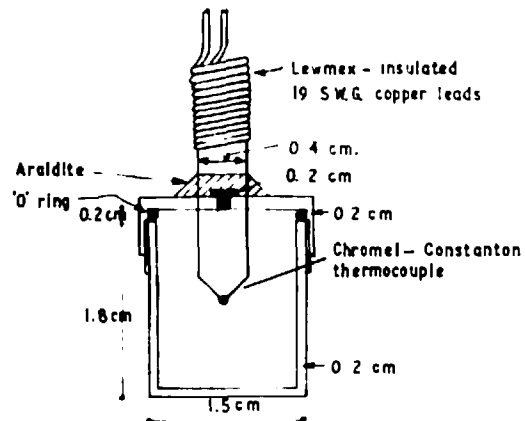


Fig. 2 Thermocouple and chamber

calibration (Barrs, 1968).

A small screw sealed by an O-ring on top of the chamber, was screwed last (i.e. after the bottom of the chamber holding the sample had been screwed) thus ensuring that pressure was not built up in the chamber.

Temperature control

Temperature control to within $\pm 0.001^{\circ}\text{C}$ was required with this equipment; to achieve this degree of control the following arrangement was adopted: a glass tank 57 x 27 x 29 cm, lagged with 5 cm of expanded polystyrene and held in a wooden box, was about half filled with 20 litres of water kept vigorously and continuously stirred by a pump. A mercury in-glass contact thermometer, with a differential of 0.001°C and set at 25°C actuated a tilting mercury switch through an electric relay, to switch a 60 W carbon filament heating lamp with the bulb immersed in the water. Variations at different places in the bath were observed with the help of a Beckmann thermometer and were less than 0.01°C . Replacement of water to the water bath was done automatically with the help of a ping-pong device (Fig. 1).

Operation

Measurement of the galvanometer deflections, following the passage of various currents for different times through the thermocouple junctions, showed that the optimum current for practical use was 5 mA for 20 seconds.

The mean of readings after the 4th and 5th hour of placing the chambers into the water bath, was taken as a standard procedure.

Each thermocouple was calibrated (Fig. 3) against a series of standard sodium chloride solutions producing known humidities at 25°C (Robinson and Stokes, 1959) over a range likely to be encountered when using plant material. Solutions were held on a filter paper (1.1 x 4.2 cm) lining the chamber walls with a little excess solution covering the bottom of the chamber. The potential of the solutions was derived from the following relationship:

$$\psi = \frac{RT}{V} \ln \frac{P}{P_0}$$

where ψ is the water potential (erg cm⁻³), R is the ideal gas constant (erg mole⁻¹ per degree), T is the absolute temperature (degrees K), V is the molar volume of water (cm³ mole⁻¹), P is the actual vapour pressure at temperature T and P₀ is the vapour pressure of pure water at the same temperature. The only assumption required in deriving this equation is that water vapour behaves as an ideal gas.

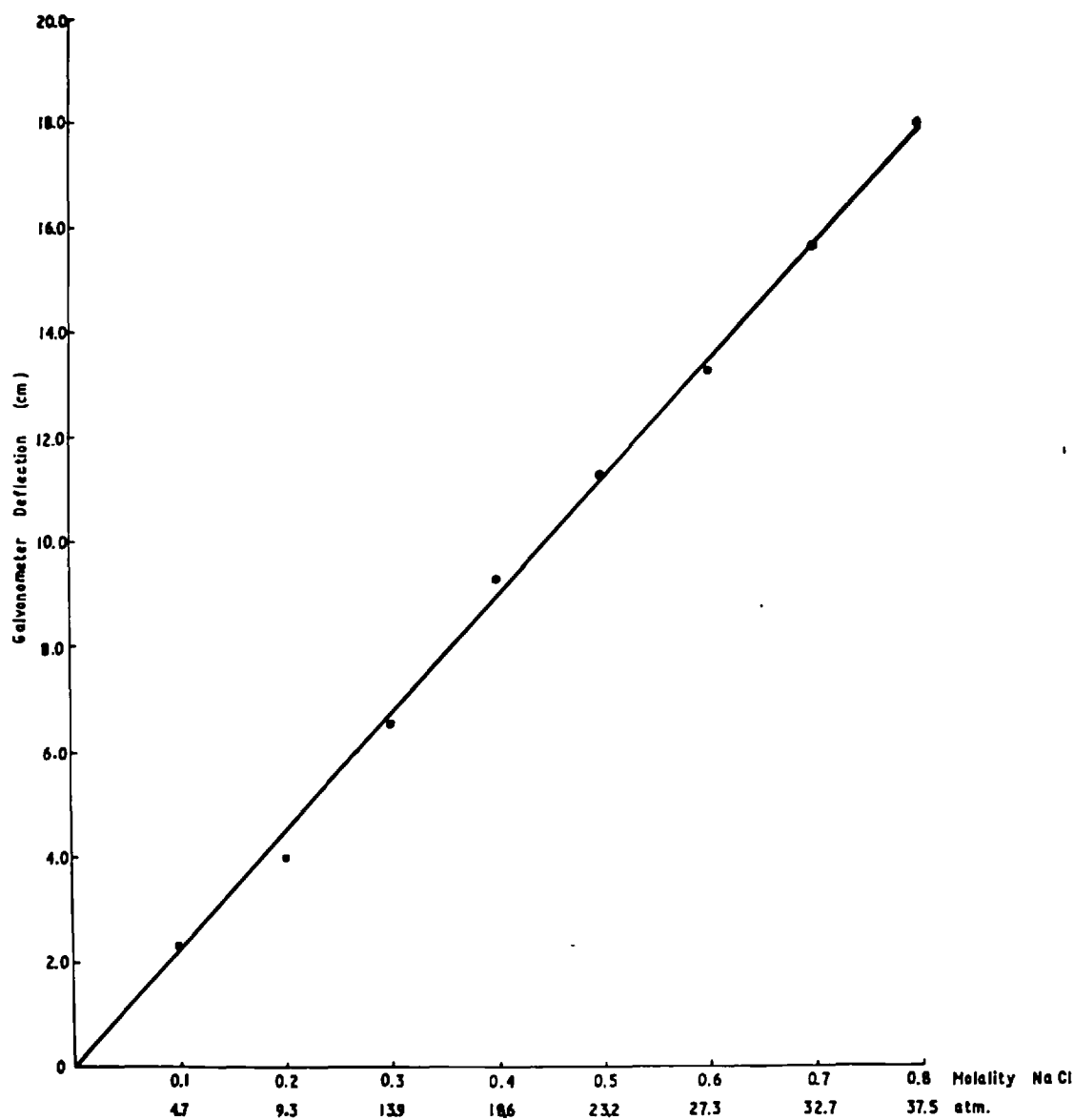


Fig. 3 Calibration curve for a single thermocouple

Results

Experiment I

This initial experiment was designed to investigate whether the response to drought in the later stages of growth was less in plants which had been exposed to drought in the early growth stages. The pretreatments P_2 (two periods of drought pretreatment), P_1 (one pretreatment at the same time as the second pretreatment period of P_2) and P_0 (no drought pretreatment) were factorially combined with two final treatments: free watering (W) or drying (D) towards the end of growth. Both in the pretreatments and the final drought treatment, the duration of drought was sufficient to produce wilting throughout the last day of treatment and varied between 4 and 15 days. The successive applications of drought were separated by periods of free watering of 3-5 days, and the timing of the periods in which water was given or withheld is shown in Table 1.

Plants were grown singly in pots and randomized between treatments before pretreatment started and at the start of the pretreatment cucumber, sugar beet and tobacco plants were 14, 17 and 28 days old respectively.

Two harvests of each species were made. The first was made six days after the end of the last drought period in cucumber, twelve days in sugar beet and fourteen days in tobacco. A second harvest was taken twenty days later in cucumber, twenty seven days in sugar beet,

Table 1

Times of pretreatments, treatments, and harvests, measured in days from the time at which seedlings were thinned.

Cucumber

Number of Pretreatments	Final Treatments	Pretreatments		Treat- ments	Harvests	
					I	II
0	Watered (W)				30	50
	Droughted (D)			20-24	30	50
1	Watered (W)		12-17		30	50
	Droughted (D)		12-17	20-24	30	50
2	Watered (W)	4-9	12-17		30	50
	Droughted (D)	4-9	12-17	20-24	30	50

Sugar beet

Number of Pretreatments	Final Treatments	Pretreatments		Treat- ments	Harvests	
					I	II
0	Watered (W)				58	85
	Droughted (D)			40-46	58	85
1	Watered (W)		31-36		58	85
	Droughted (D)		31-36	40-46	58	85
2	Watered (W)	12-27	31-36		58	85
	Droughted (D)	12-27	31-36	40-46	58	85

Table 1 contd.

<u>Tobacco</u>					
<u>Number of</u> <u>Pretreatments</u>	<u>Final</u> <u>Treatments</u>	<u>Pretreatments</u>		<u>Treat-</u> <u>ments</u>	<u>Harvest</u> <u>I</u> <u>II</u>
0	Watered (W)				48 68
	Droughted (D)			28-34	48 68
1	Watered (W)		15-23		48 68
	Droughted (D)		15-23	28-34	48 68
2	Watered (W)	2-10	15-23		48 68
	Droughted (D)	2-10	15-23	28-34	48 68

and twenty days in tobacco. At each harvest, each treatment was replicated six times in cucumber and sugar beet and five times in tobacco.

Measurements of leaf area were made at 2 to 4 day intervals throughout the experiment, on three plants of each species from each treatment, and these plants were left for the final harvest.

Dry Weight

Cucumber (Table 2a)

The overall effect of the final treatment (W v D) was significant but not very large. The overall effect of a single pretreatment (P_1) was not very great but the double pretreatment markedly reduced dry weight. There was an indication that final treatment reduced dry weight appreciably after one pretreatment but had little effect after either no pretreatment (P_0) or two pretreatments (P_2). This was seen at both harvests (based on independent samples) but the interaction was not significant. The effect of P_2 was less marked at Harvest II than at Harvest I.

Sugar beet (Table 2b)

The overall effect of final treatment was small and not significant. Again the double pretreatment markedly reduced dry weight, especially at Harvest I, but there was no interaction of pretreatment and final treatment.

Tobacco (Table 2c)

There was a small but not significant overall effect of final

Table 2

a) Cucumber $\log_{10}$ mean total dry matter

Final Treatments	Harvest I (30 days after zero time) Number of Pretreatments			Mean.	Harvest II (50 days after zero time) Number of Pretreatments			Mean
	Q	1	2		0	1	2	
Watered (W)	0.7986*	0.8235	0.4937	0.7053	1.0453	1.0454	0.9542	1.0150
Droughted (D)	0.8011	0.7504	0.4878	0.6798	1.0446	0.9590	0.9224	0.9753
Mean	0.7998	0.7869	0.4907		1.0449	1.0022	0.9383	

* Each value mean of six determinations

LSD (P=0.05) (Pretreatments x treatments means) = 0.0686

LSD (P=0.05) (Pretreatments means) = 0.0504

LSD (P=0.05) (Final treatment means) = 0.1248

Table 2 contd.

b) Sugar beet $\log_{10}$ mean total dry matter

Final Treatments	Harvest I (58 days after zero time) Number of Pretreatments			Mean	Harvest II (85 days after zero time) Number of Pretreatments			Mean
	0	1	2		0	1	2	
Watered (W)	1.2283*	1.1740	1.0266	1.1432	1.6026	1.6091	1.5598	1.5905
Droughted (D)	1.2243	1.1833	1.0077	1.1384	1.6003	1.6043	1.4803	1.5616
Mean	1.2265	1.1786	1.0171		1.6014	1.6067	1.5200	

* Each value mean of six determinations

LSD (P=0.05) (Pretreatments x treatments means) = 0.0714

LSD (P=0.05) (Pretreatments means) = 0.0504

LSD (P=0.05) (Final treatment means) = 0.0410

Table 2 contd.

c) Tobacco $\log_{10}$ mean total dry matter

Final Treatments	Harvest I (48 days after zero time) Number of Pretreatments			Mean	Harvest II (68 days after zero time) Number of Pretreatments			Mean
	0	1	2		0	1	2	
Watered (W)	1.1202*	0.9894	0.8972	1.0023	1.7024	1.6690	1.6627	1.6780
Droughted (D)	1.0849	0.9613	0.8968	0.9810	1.6632	1.6663	1.6158	1.6484
Mean	1.1025	0.9753	0.8970		1.6828	1.6676	1.6392	

* each value mean of five determinations

LSD (P=0.05) (Pretreatments x treatments means) = 0.0915

LSD (P=0.05) (Pretreatments means) = 0.0644

LSD (P=0.05) (Final treatment means) = 0.0525

treatment. The single pretreatment (P_1) had a larger effect than in the preceding species and the double pretreatment an even greater effect. There was an indication, especially in Harvest I, that the effect of the final treatment was greater when there had been no pretreatment, and declined as the number of pretreatments was increased, but again this interaction was not significant.

Leaf Area

Figures 5, 7 and 9 show the increase in leaf area with time for each species, in the treatment P_0W which was kept watered throughout the experiment. In order to simplify comparisons of treatments, Figs. 4, 6 and 8 show the leaf areas (on a log scale) in other treatments relative to the area of P_0W . Consequently falling lines in these figures represent relative growth rates of leaf area which are less than the growth rate of P_0W , and rising curves growth rates which are greater than P_0W . No statistical analysis was made but the magnitude of the errors can be assessed as follows: the lines for all treatments should coincide with the horizontal line P_0W up to the beginning of the first pretreatment period; within a pretreatment the W and D lines should coincide up to the time of the final treatment, thus the P_1 lines should coincide with the P_0 lines up to the second pretreatment period. The most marked indication of random errors is that in sugar beet (Fig. 6) P_0D and P_1 , W and D soon rise above P_0W by about antilog 0.05, i.e. about 12% higher and in tobacco (Fig. 8) all lines are initially lower than P_0W by

Fig. 4 The differences between the logarithms of the total leaf areas of P_0W and the other treatments in relation to time, for cucumber. The sections of the curves which have been thickened and dark areas on the abscissa indicate the times at which pre-treatments and final treatment were applied.

FIGURE 4

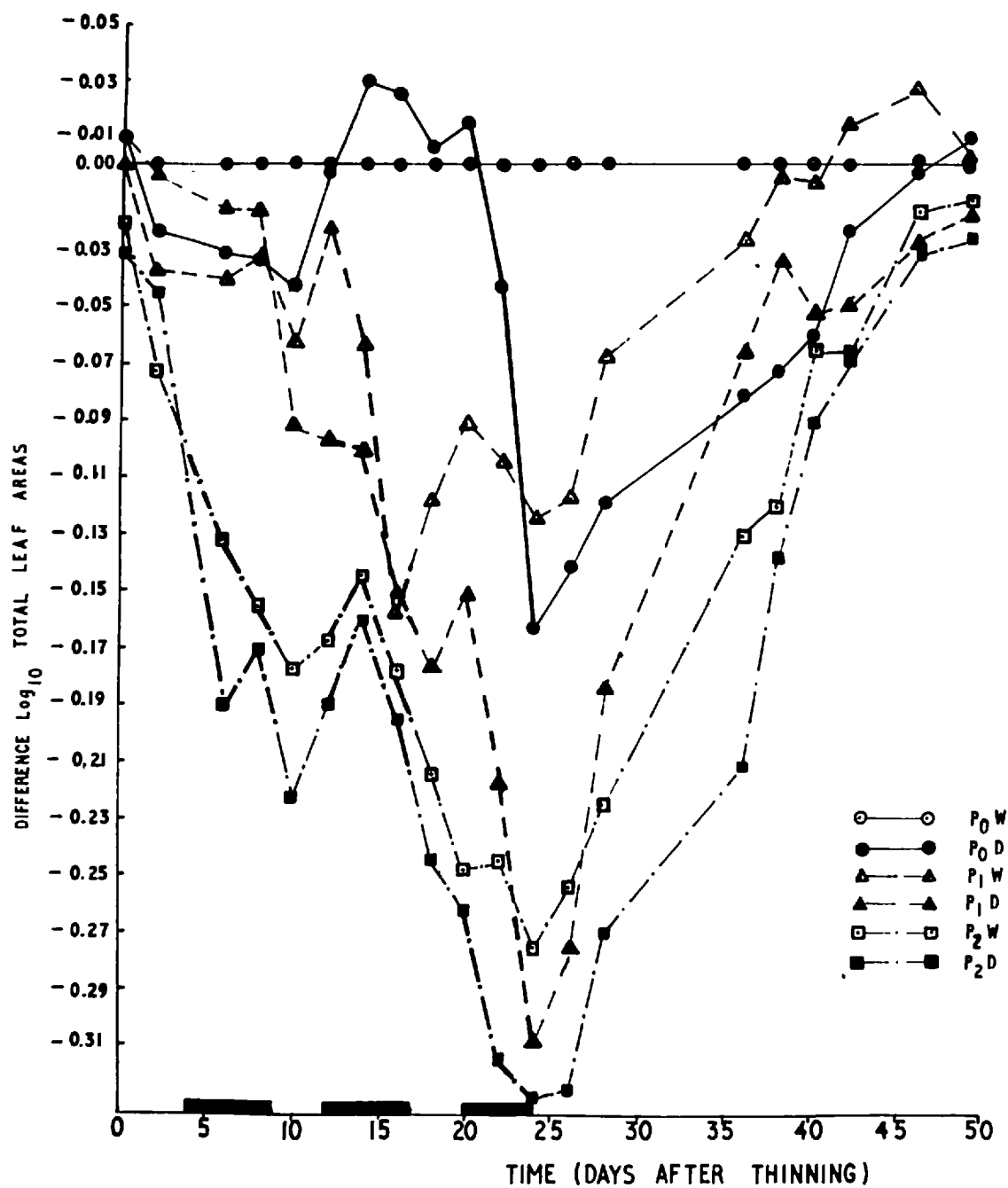


Fig. 5 The relationship between the logarithm of the mean total leaf area of cucumber, and time, for treatment $P_C^{1/}$

FIGURE 5

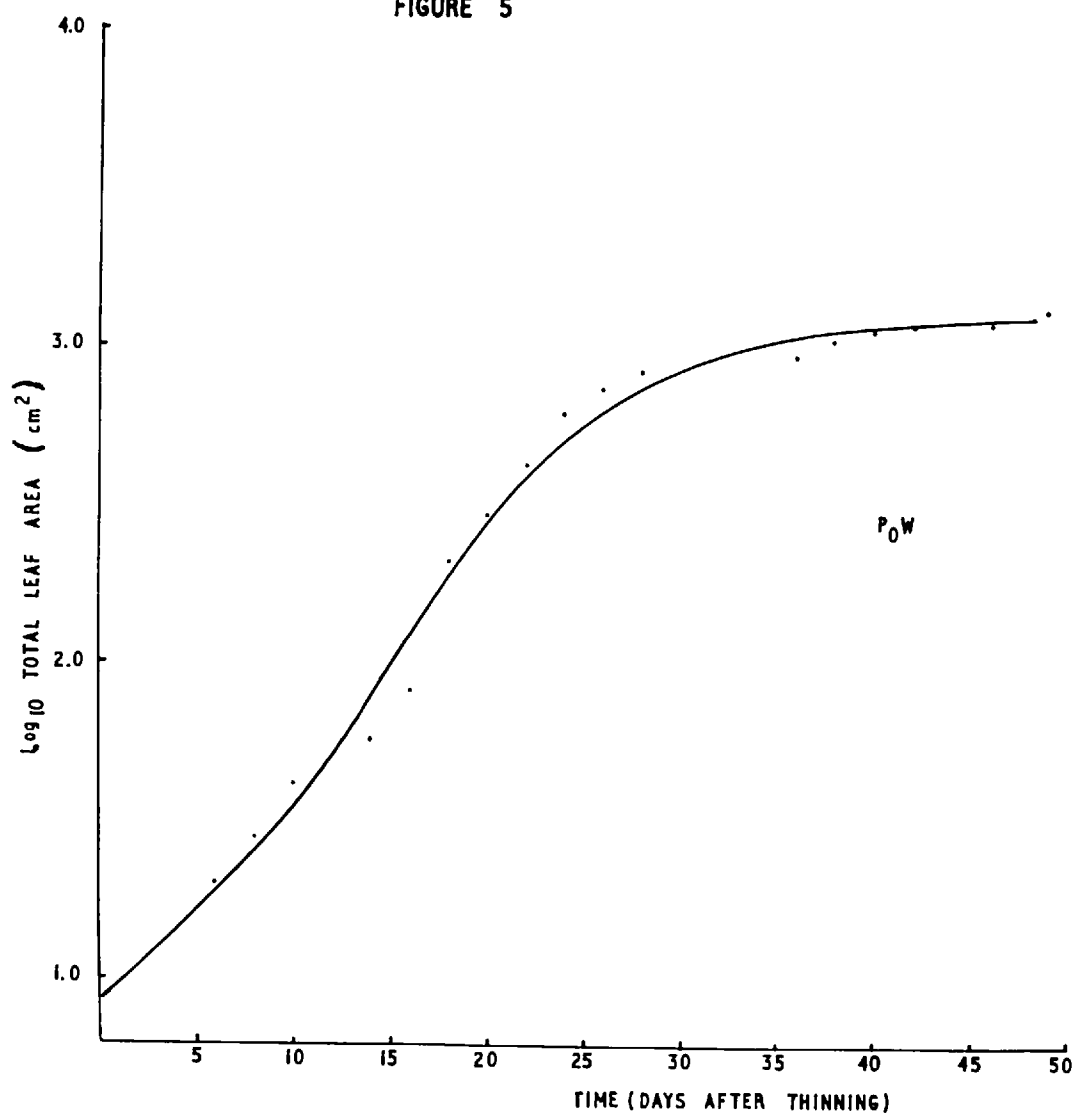


Fig. 6 The differences between the logarithms of the total leaf areas of P_0W and the other treatments in relation to time, for sugar beet. The sections of the curves which have been thickened and dark areas on the abscissa indicate the times at which pre-treatments and final treatment were applied.

FIGURE 6

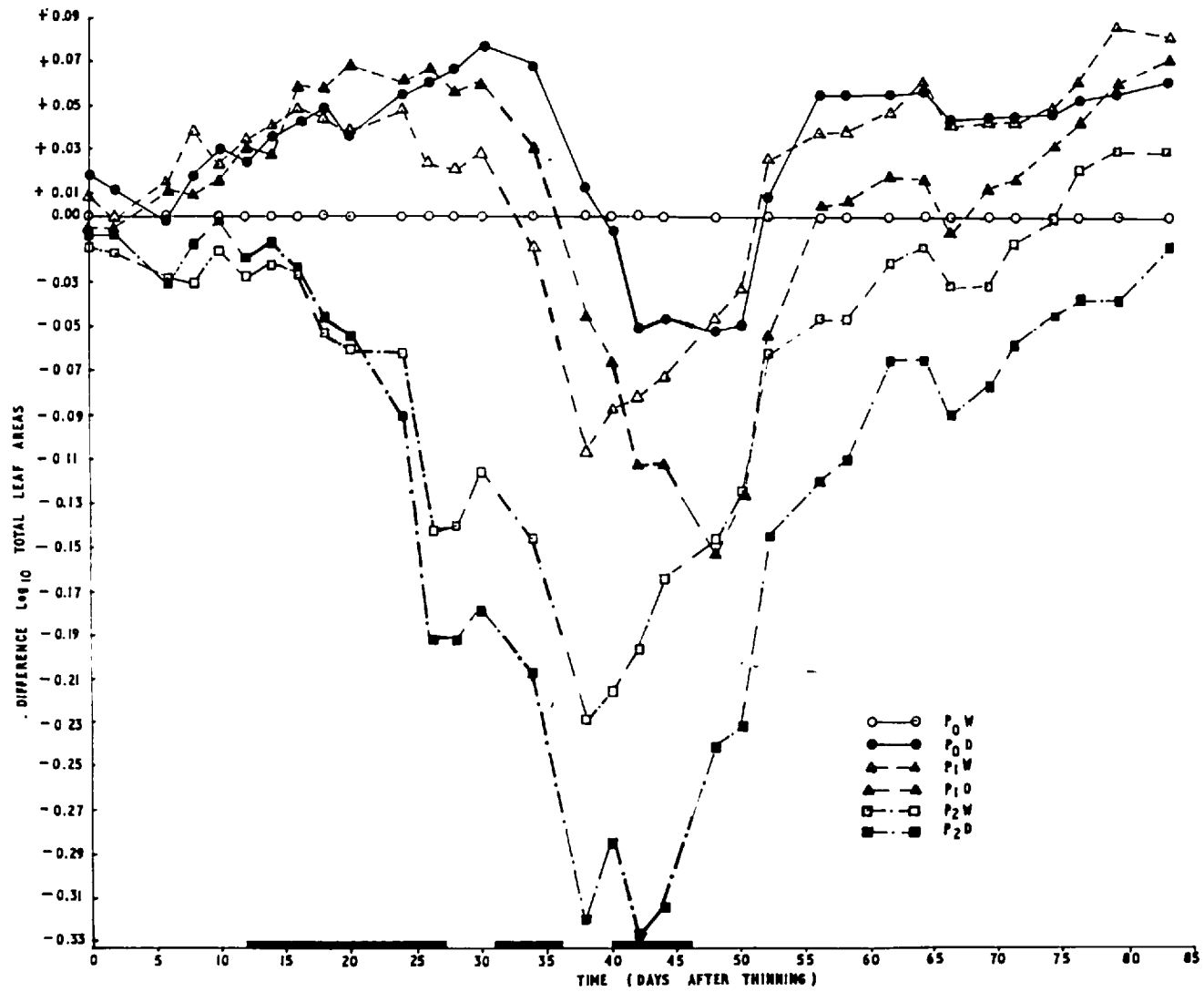


Fig. 7 The relationship between the logarithm of the mean total leaf area of sugar beet, and time, for treatment P₀W.

FIGURE 7

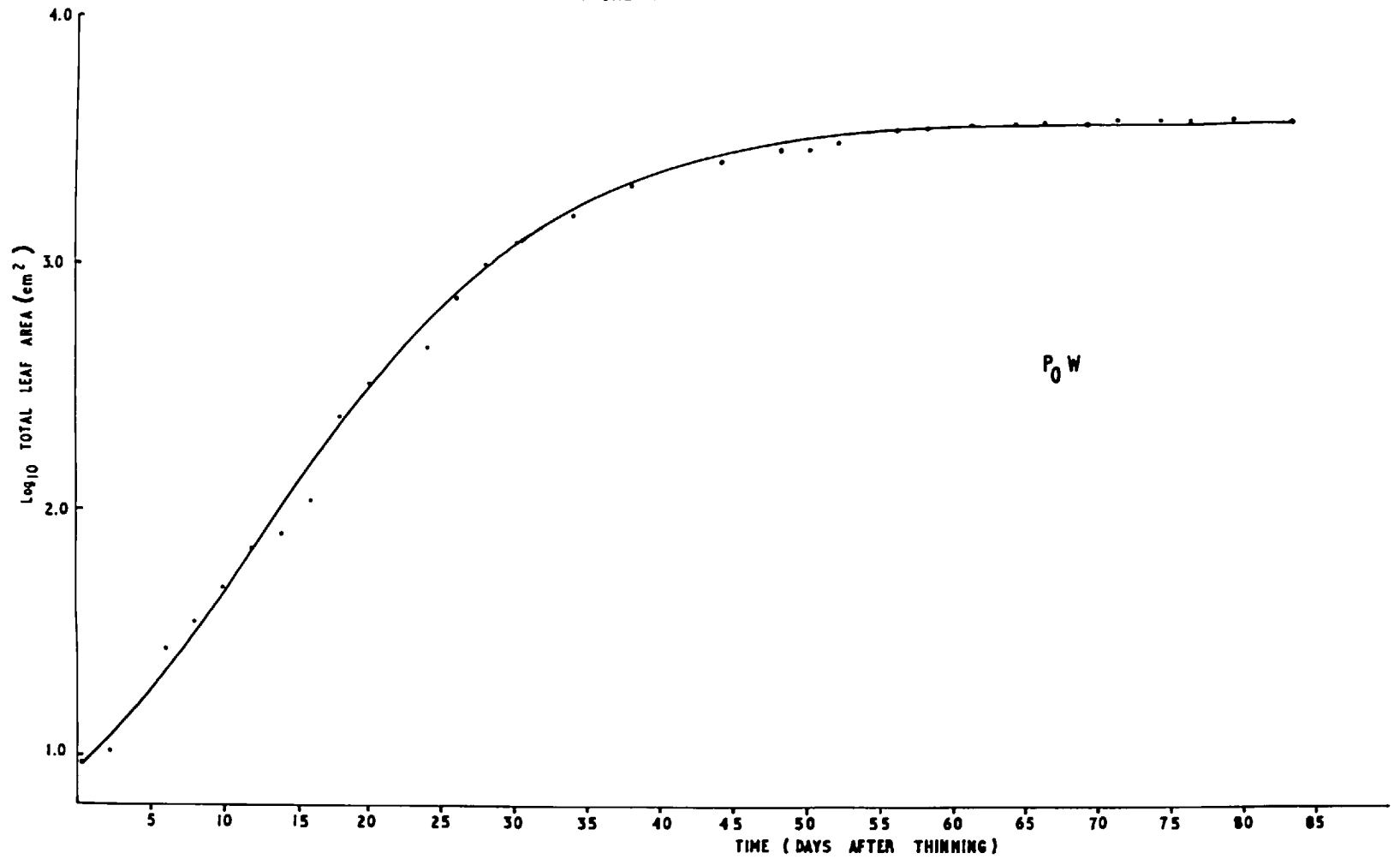


Fig. 8 The differences between the logarithms of the total leaf areas of $P_O W$ and the other treatments in relation to time, for tobacco. The sections of the curves which have been thickened and dark areas on the abscissa indicate the times at which pre-treatments and final treatments were applied.

FIGURE 8

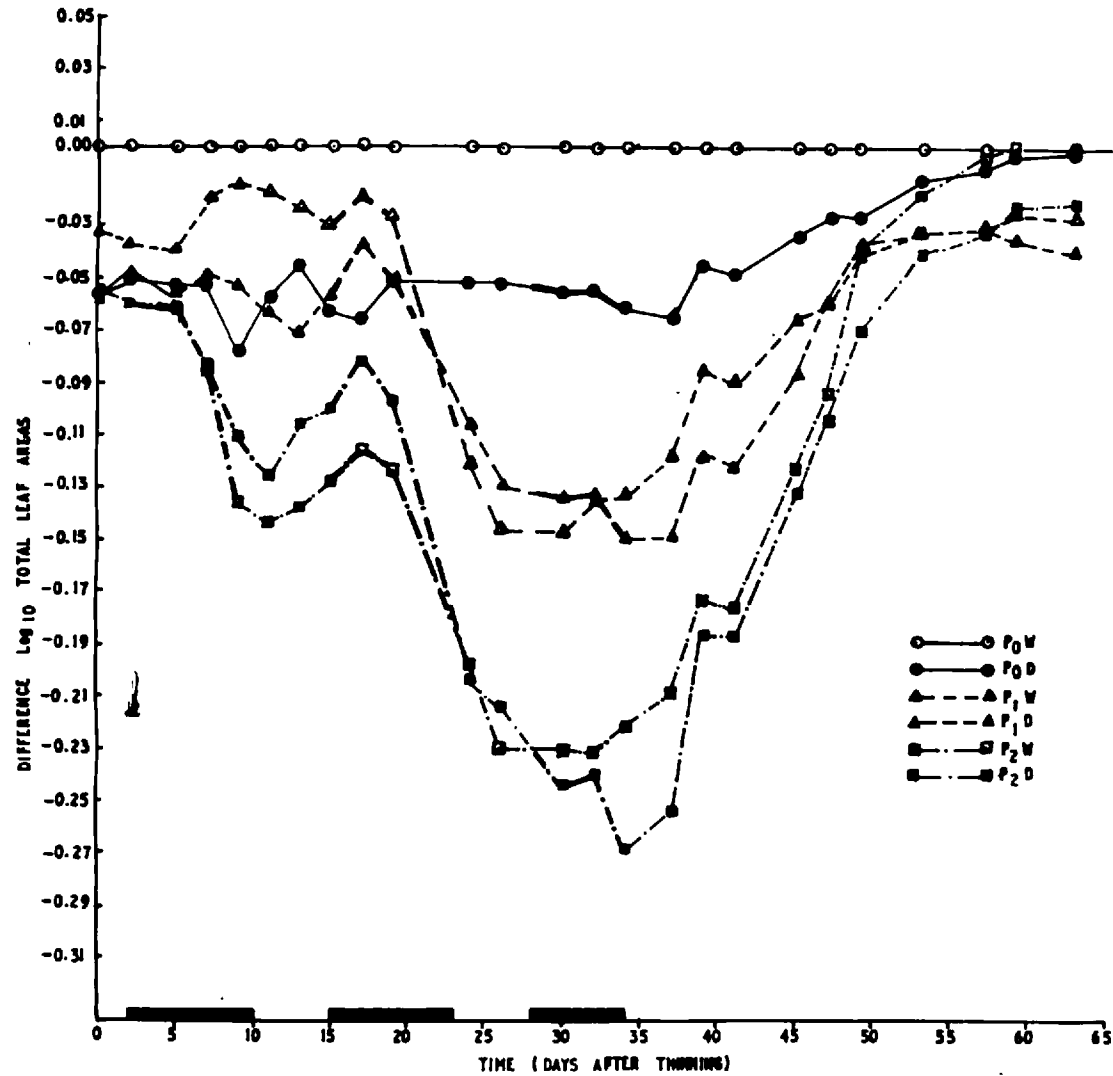
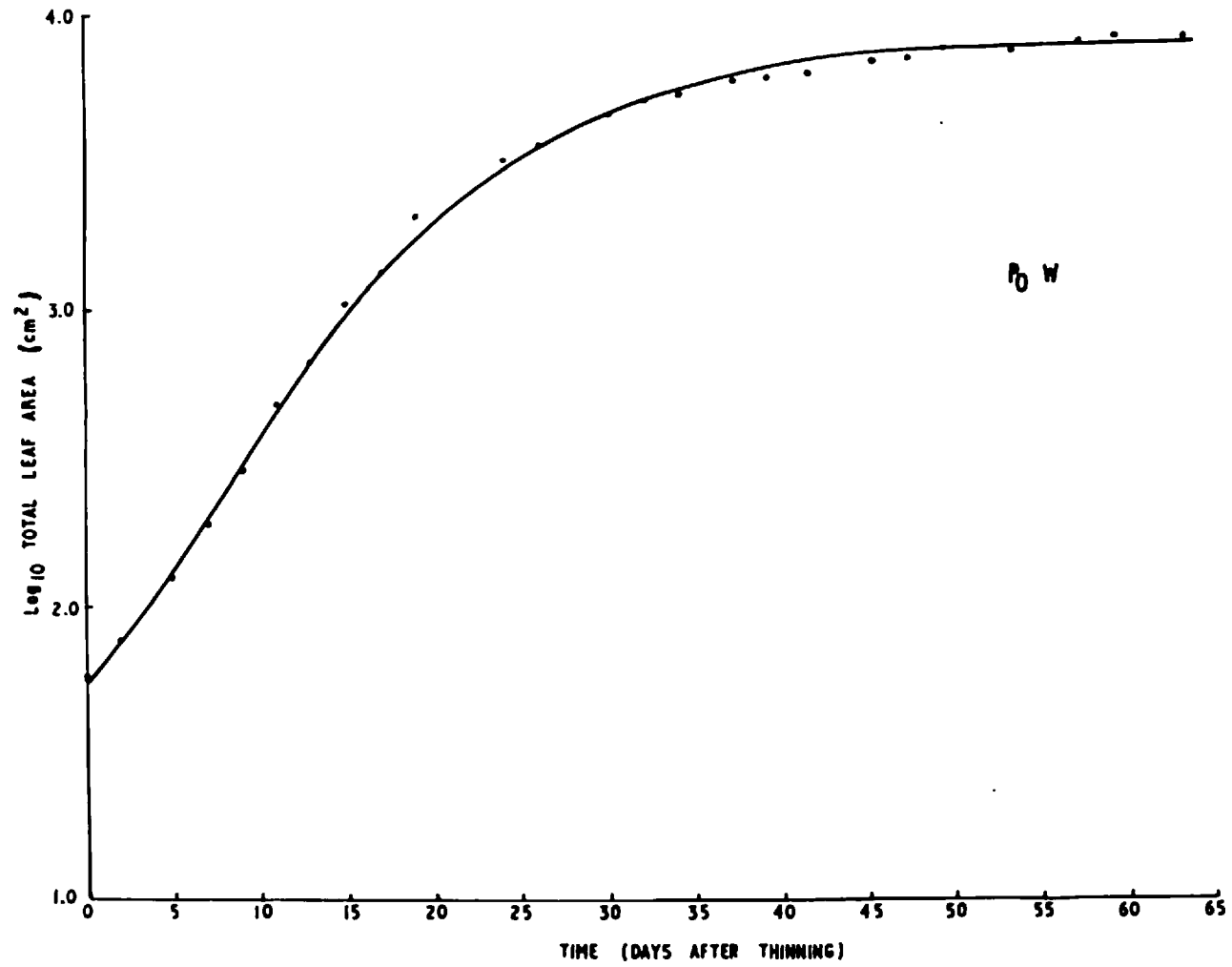


Fig. 9 The relationship between the logarithm of the mean total leaf area of tobacco, and time, for treatment P₀W.

FIGURE 9



about antilog 0.05.

In almost every case where plants experienced drought, either as pretreatment or final treatment, the relative growth rate of leaf area was reduced compared with P_0W . The reduction in leaf area by the end of each pretreatment or treatment is shown in Table 3. As soon as the drought finished there was a rise in relative growth rate above P_0W . In the period following the final treatment, leaf areas of all treatments increased rapidly relative to P_0W (photographs 2 and 3) and by the end of the experiment there was little difference between the treatments. In sugar beet (Fig. 6) some treatments had, at the end, larger leaf areas than P_0W , but in view of the fairly high random errors indicated at the beginning of growth, little significance can be attached to the differences seen at the end.

The extent to which pretreatment has affected the response to final treatment can be judged from the difference between the relative leaf area growth rates of W and D treatments during final treatment, and is shown in Table 4.

Table 4

Differences between relative leaf area growth rates (measured in $\text{cm}^2 \text{ cm}^{-2}$ final treatment period⁻¹) of W and D.

	P_0	P_1	P_2
Cucumber	0.18	0.12	0.04
Sugar beet	0.044	0.084	0.051
Tobacco	0.006	0.032	0.048



Photograph 2 Sugar beet plants subjected to different treatments. This photograph was taken at the first harvest (i.e. 12 days after the end of the final treatment) From left to right treatments were: $T_1 - P_0W$; $T_2 - P_0D$; $T_3 - P_1W$; $T_4 - P_1D$; $T_5 - P_2W$ and $T_6 - P_2D$.



Photograph 3 Tobacco plants subjected to different treatments. This photograph was taken at the first harvest (i.e. 14 days after the end of the final treatment) From left to right treatments were: $T_1 - P_0W$; $T_2 - P_0W$; $T_3 - P_1W$; $T_4 - P_1D$; $T_5 - P_2W$ and $T_6 - P_2D$.

Table 3

Leaf area at the end of Pretreatments and Treatments (expressed as percentages taking P₀W as 100 percent.)

Cucumber

Number of Pretreatments	Final Treatments	Pretreatments		Treatments 20-24 days from the time at which seedlings were thinned
		4-9	12-17	
0	Watered (W)	100	100	100
	Droughted (D)			69
1	Watered (W)		76	75
	Droughted (D)		67	49
2	Watered (W)	66	61	53
	Droughted (D)	59	57	39

Sugar Beet

Number of Pretreatments	Final Treatments	Pretreatments		Treatments 40-46 days from the time at which seedlings were thinned
		12-27	31-36	
0	Watered (W)	100	100	100
	Droughted (D)			89
1	Watered (W)		87	87
	Droughted (D)		97	74
2	Watered (W)	73	65	70
	Droughted (D)	63	54	52

Table 3 - contd.Tobacco

Number of Pretreatments	Final Treatments	Pretreatments		Treatments 28-34 days from the time at which seedlings were thinned
		2-10	15-23	
0	Watered (W)	100	100	100
	Droughted (D)			87
1	Watered (W)		76	74
	Droughted (D)		78	71
2	Watered (W)	72	63	60
	Droughted (D)	76	63	54

The data in Table 4 suggest that cucumber was the only species in which pretreatments reduced the effect of the final treatment. In sugar beet, pretreatments affected the response to final treatment in a variable manner. In tobacco, however, pretreatments increased the effect of the final treatment.

Conclusion

As in the dry weight data there is little evidence, except perhaps in cucumber, that pretreatment has reduced susceptibility to drought in the final treatment. On the other hand this experiment demonstrated very clearly that at the end of a period of drought there is accelerated leaf growth relative to undroughted plants. Rather than continue experiments designed to seek clearer evidence of drought hardening, it was decided to investigate in more detail the accelerated growth which occurs after drought is relieved.

Experiment II

Experiment II was designed to investigate the response in growth of tobacco to a single period of drought. Two treatments were applied: half of the plants were freely watered (control), and water was withheld from the other half for ten days (treated). At the start of the treatments, plants were 20 days old. The duration of drought was enough to produce wilting throughout the last day of treatment. Following the drought period, water was applied freely each day to both treatments.

Plants were grown singly, in pots and randomized between treatments before treatment started. Throughout the experiment seven harvests were made. The first was at the beginning of the drought period, the second at the end of the drought period and the rest of the harvests were made 6, 12, 18, 26 and 32 days later. At each harvest each treatment was replicated four times.

Growth was analysed by a computer programme due to Hughes and Freeman (1967) in which polynomial regressions (quadratic or higher term) are fitted to the logarithms of the weights (W in mg) and leaf areas (A in cm^2) on time (t in days) by the least squares method. From these fitted curves, leaf area ratios (F_A) are obtained by taking antilogs of the differences between the log values (i.e. $\ln A - \ln W$) at appropriate times. Relative growth rates (R_W) are obtained by differentiating the curve of $\ln W$. Relative leaf area growth rates (R_A) were obtained by differentiating the $\ln A$ curve.

At any instant relative growth rate, unit leaf rate (E_A), and leaf area ratio are interrelated by the following expressions:

$$\frac{1}{W} \frac{dW}{dt} = \frac{1}{A} \frac{dA}{dt} \times \frac{A}{W}$$

$$\text{i.e. } R_W = E_A \times F_A$$

Hence, the time curve for E_A is obtained from:

$$\frac{1}{A} \frac{dA}{dt} = \frac{1}{W} \frac{dW}{dt} + \frac{A}{W}$$

The E_A curve is subject to the combined errors of the relative growth rates and leaf area ratios. Leaf area ratios are subject to errors of $\ln A$ and $\ln W$. Relative growth rates are subject to errors of $\ln \frac{A}{W}$. Relative leaf area growth rates are subjected to errors of $\ln W$. However, all these errors can be minimized by large and frequent samples.

The computer programme calculated all the derived growth attributes (i.e. R_A , R_W , E_A and F_A) for each harvest time. Basically however, the first three of these attributes are obtained from the differences between two successive harvests. As a check on the computer method they were also calculated in Expt. II (but not in succeeding experiments) by the conventional methods of calculation employing the data of two successive harvests. Values calculated in this way are entered on the graphs in the centre of appropriate harvest intervals for comparison with the calculated curves. The agreement was satisfactory except that the curves gave obviously absurd results if extrapolated to zero time (harvest 1) when the treatments had not been initiated.

The programme also carried out an analysis of variance to test the significance of the effect of treatment and its interaction with time, and calculated least significant differences between treatments at each harvest

In the case of dry weight and leaf area the actual means and their standard errors are plotted on the graphs in all experiments to check the accuracy of the curve fitting process.

Dry Weight

Points were calculated at 3 day intervals throughout the experimental period for the control and treated plants, from the fitted equations and these points were joined to form the curves shown in Fig. 10.

During the drought period there was an increase in dry weight of treated plants, although this increase was much less than that in the control plants. At the end of the treatment period dry weight of treated plants was reduced significantly to 73% of the control value. Twenty six days later there was no difference between treated and control plants.

Leaf Area

As for dry weight, points were calculated at 3 day intervals for the control and treated plants from the fitted equations and joined to form curves (Fig. 11)

Leaf area of treated plants increased during the drought period but less than the control plants. At the end of the treatment period leaf area of treated plants was reduced significantly, to 69% of the

Fig. 10 The effect of treatment on the change of the natural logarithm of dry weight with time. Lines represent the fitted cubic curves, points are the means of the logarithms of observed values, thin vertical bars represent the standard errors of observed values, and thick vertical bars represent least significant differences between the means at $P = 0.05$.

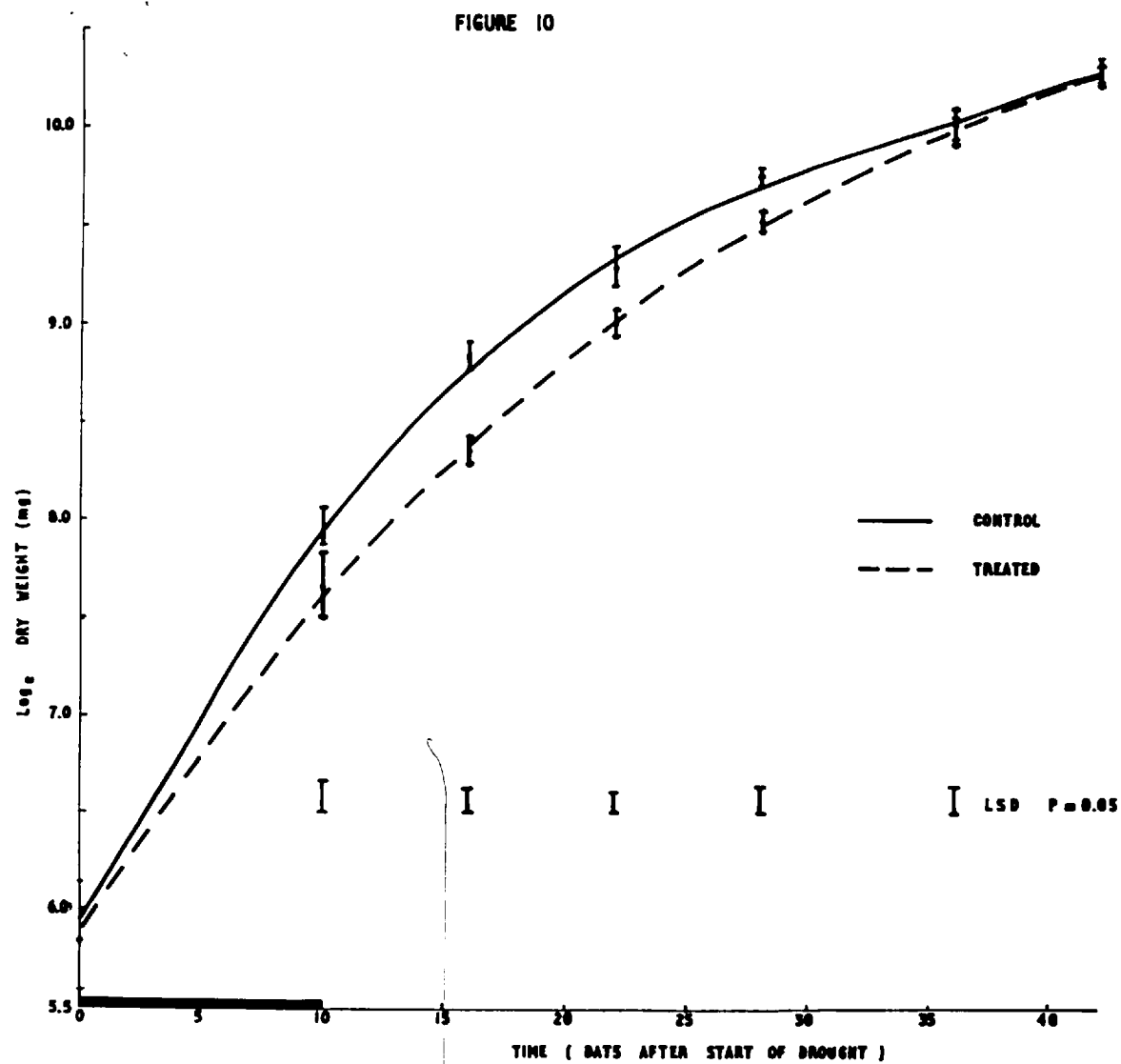
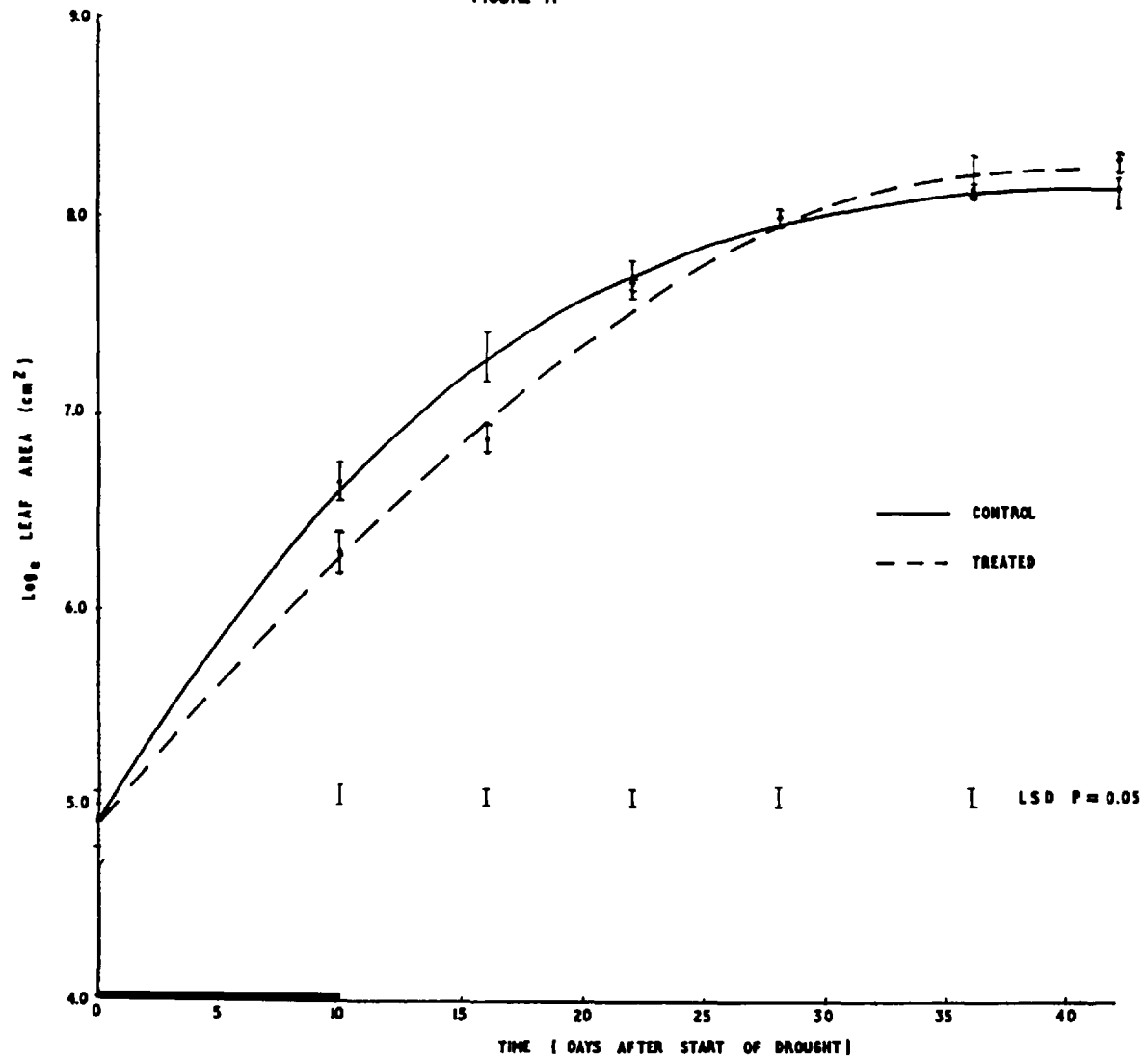


Fig. 11 The effect of treatment on the change of the natural logarithm of leaf area with time. Lines represent the fitted cubic curves, points are the means of the logarithms of observed values, thin vertical bars represent the standard errors of observed values, and thick vertical bars represent least significant differences between the means at $P = 0.05$.

FIGURE 11



control value. Thirteen days later there was no difference between treated and control. Twenty five days later leaf areas of treated plants were slightly but significantly larger than the control.

Relative growth rate (R_W)

R_W of treated plants was significantly reduced by the end of the treatment period, in relation to control plants (Fig. 12). It remained lower than control values in the harvest interval immediately following the end of treatment, but then rose significantly above the control for the next three harvest intervals. In the last harvest interval there was little difference between the two sets of plants.

Relative leaf area growth rate (R_A)

The fitted curves (Fig. 13) for R_A are similar to those for R_W but the differences are more pronounced. They show a lower rate in treated plants than controls during the treatment period followed by a rise above control in the recovery period, until at the end of the experiment the two sets of plants are similar. In this graph the calculated inter-harvest means diverge considerably from the fitted lines and they suggest that the relative growth rate of leaves on the treated plants showed an absolute rise, as well as a rise relative to control, between the 3rd and 4th harvests, but that the difference between treated and controls disappeared subsequently.

Unit leaf rate (E_A)

The fitted curves for E_A are similar to those for R_W and R_A but the errors are high and none of the differences is significant (Fig. 14).

Fig. 12 The effect of treatment on the change of relative growth rate (R_W) with time. Lines represent R_W derived from the fitted cubic curves for each harvest time. Triangles are the means of observed values of R_W calculated from successive harvests. Vertical bars represent least significant difference between the derived means at $P = 0.05$.

FIGURE 12

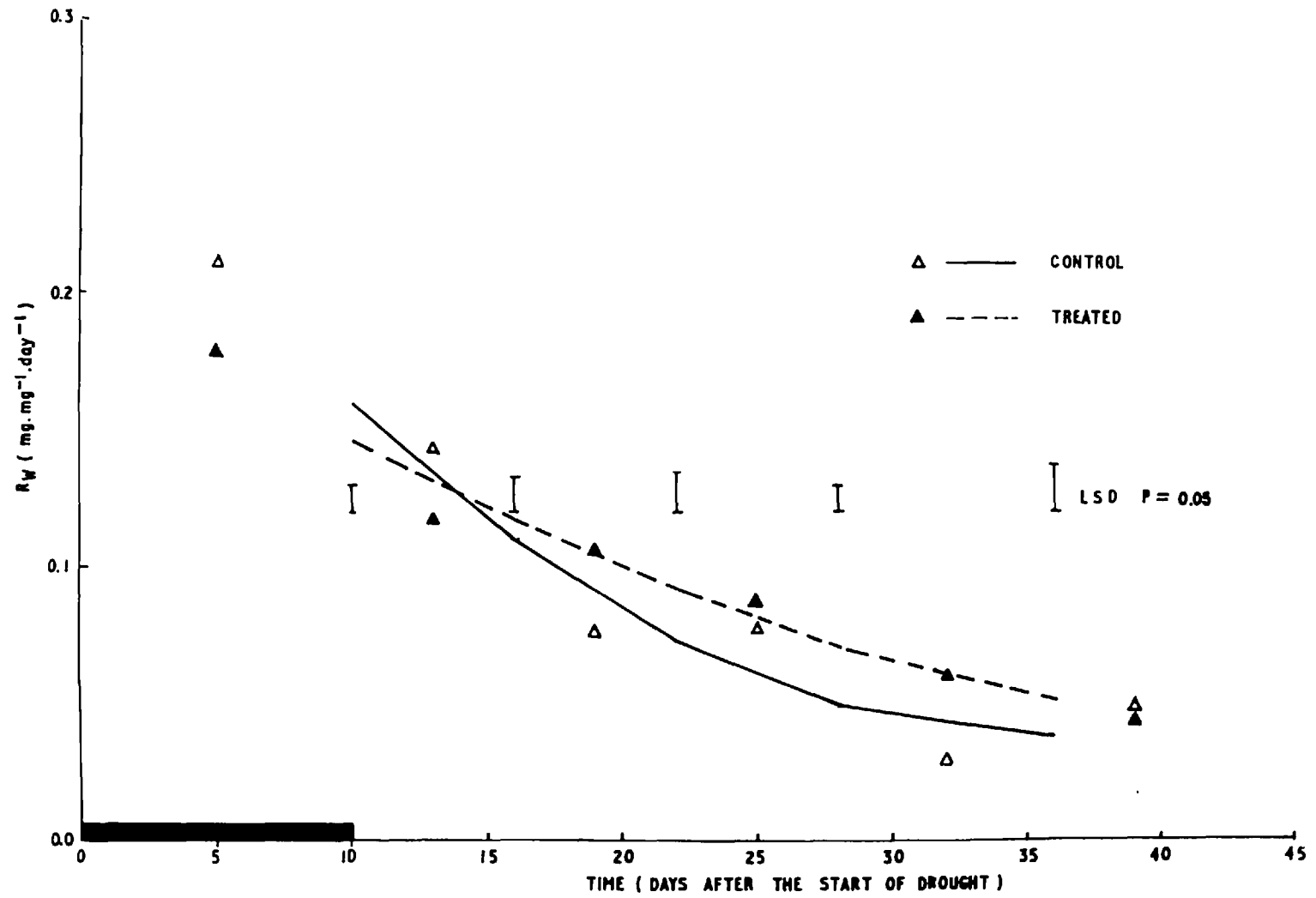


Fig. 13 The effect of treatment on the change of relative leaf area growth rate (R_A) with time. Lines represent R_A derived from the fitted cubic curves for each harvest time. Triangles are the means of observed values of R_W calculated from successive harvests. Vertical bars represent least significant difference between the derived means at $P = 0.05$

FIGURE 13

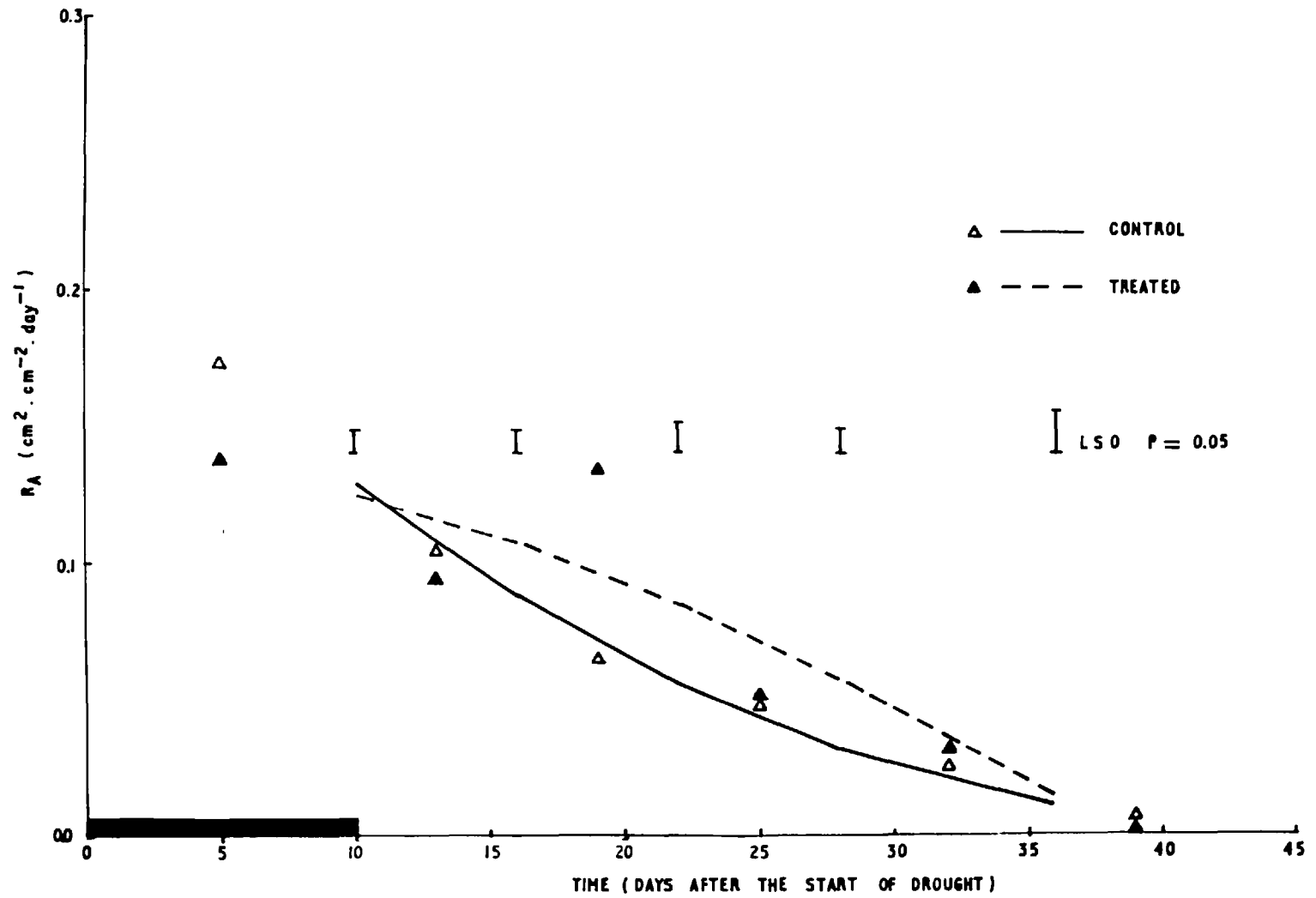
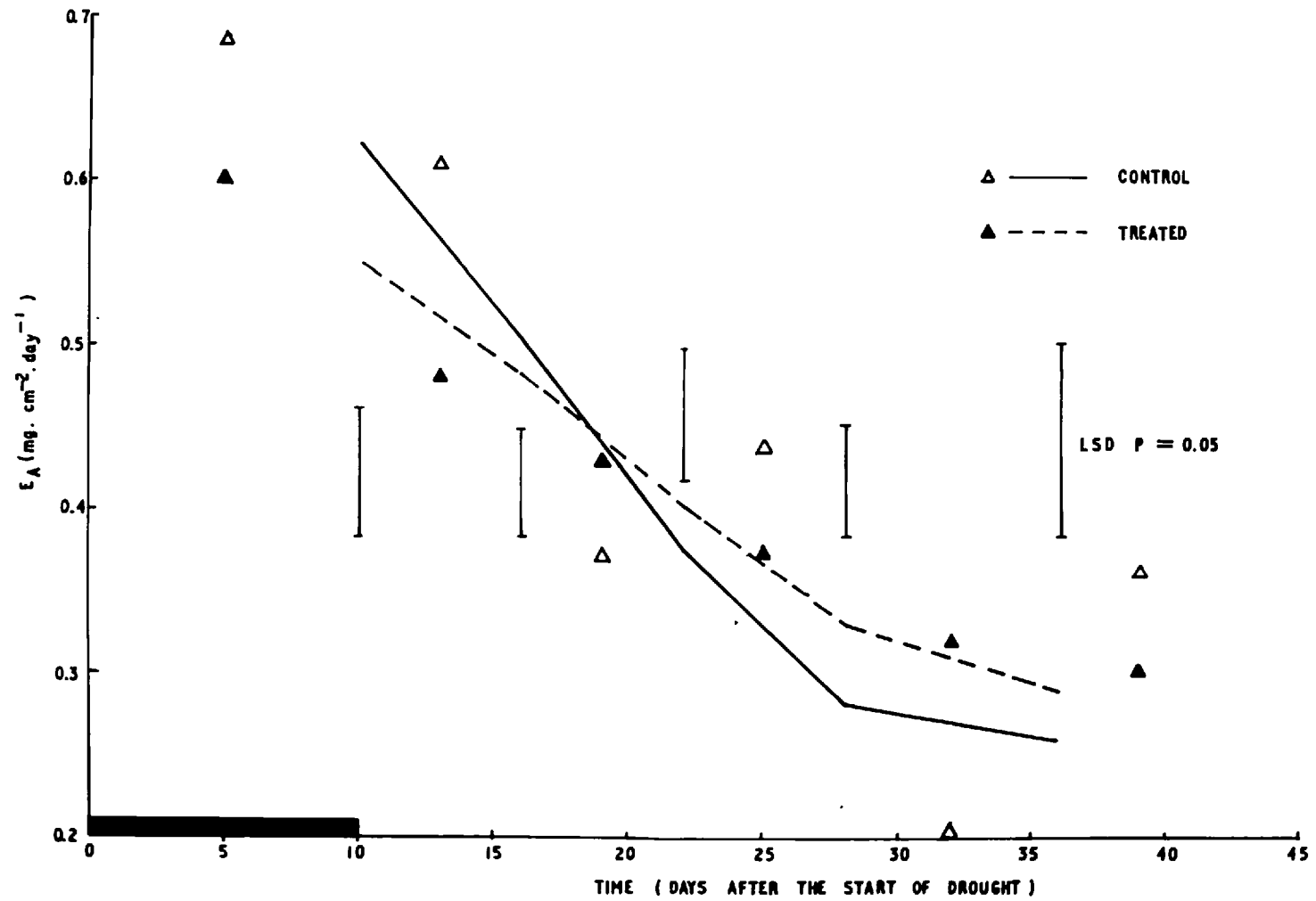


Fig. 14 The effect of treatment on the change of unit leaf rate (E_A) with time. Lines represent E_A derived from the fitted cubic curves for each harvest time. Triangles are the means of observed values of R_W calculated from successive harvests. Vertical bars represent least significant difference between the derived means at $P = 0.05$

FIGURE 14.



There may have been a reduction in E_A during treatment, as would have been expected from e.g., Morton and Watson, 1948; Sands and Rutter, 1959; and Lawlor, 1967. Subsequently E_A of treated plants is sometimes above and sometimes below that of control plants and so there is little evidence of a difference in E_A during the recovery period. It must be remembered that high errors may be masking real effects.

Leaf area ratio (F_A), leaf weight ratio and specific leaf area.

At the end of the treatment period there was no significant difference between F_A of treated and control plants (Fig. 15). F_A of treated plants significantly increased above F_A of control plants during the recovery period.

A higher F_A of treated plants during recovery could be due to a different partition of assimilates between leaf growth and other plant growth (leaf weight ratio) and/or to a change in specific leaf area i.e. the ratio of leaf area to leaf dry weight. During recovery, leaf weight ratio of treated plants increased slightly but not significantly above the control (Fig. 16). However specific leaf area of treated plants increased markedly and significantly above control plants (Fig. 17). Leaf weight ratio and specific leaf area were calculated from the original data i.e. not from the fitted curves.

Leaf distribution

Different patterns of distribution of leaf area developed in treated and control plants (Fig. 18). During the drought period the leaves of treated plants that were reduced most were those expanding

Fig. 15 The effect of treatment on the change of leaf area ratio (F_A) with time. Lines represent F_A derived from the fitted cubic curves for each harvest time. Triangles are the means of observed values of R_W calculated from successive harvests. Vertical bars represent least significant difference between the derived means at $P = 0.05$.

FIGURE 15

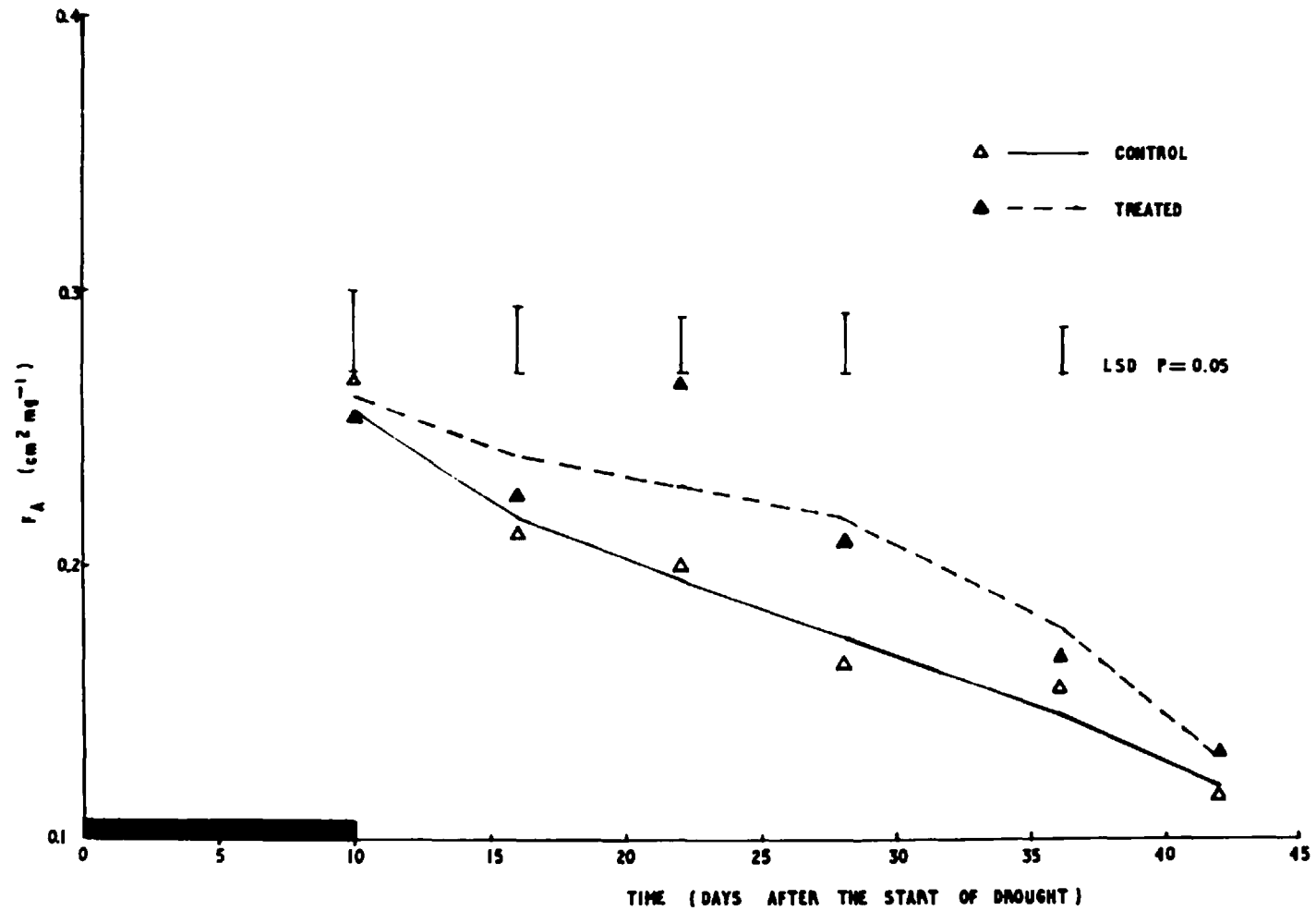


Fig. 16 The effect of treatment on the change of leaf weight ratio with time. Points are the means of observed values. The vertical bar represents the least significant difference between means at $P = 0.05$.

FIGURE 16

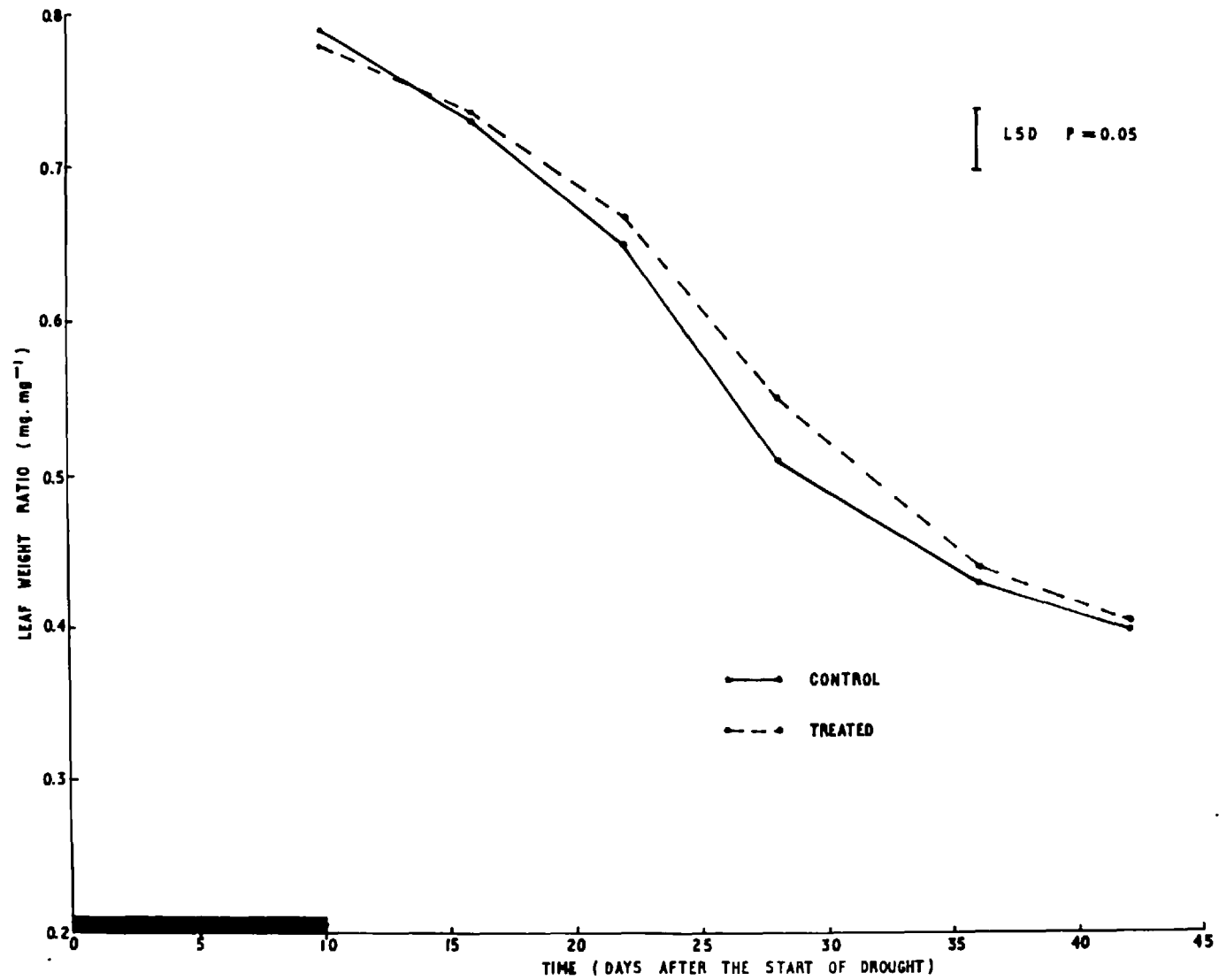


Fig. 17 The effect of treatment on the change of specific leaf area with time. Points are the means of observed values. The vertical bar represents the least significant difference between means at $P = 0.05$

FIGURE 17

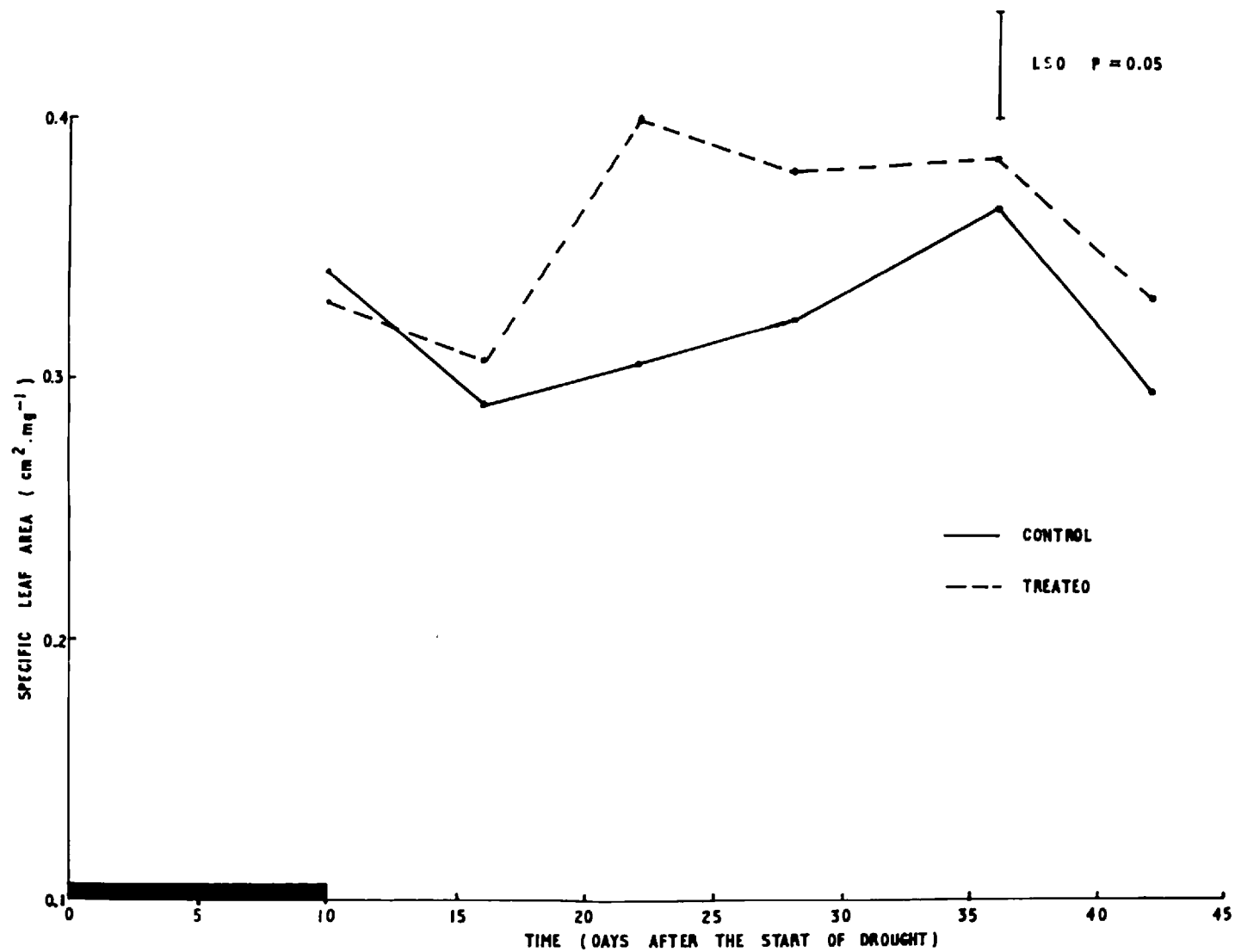
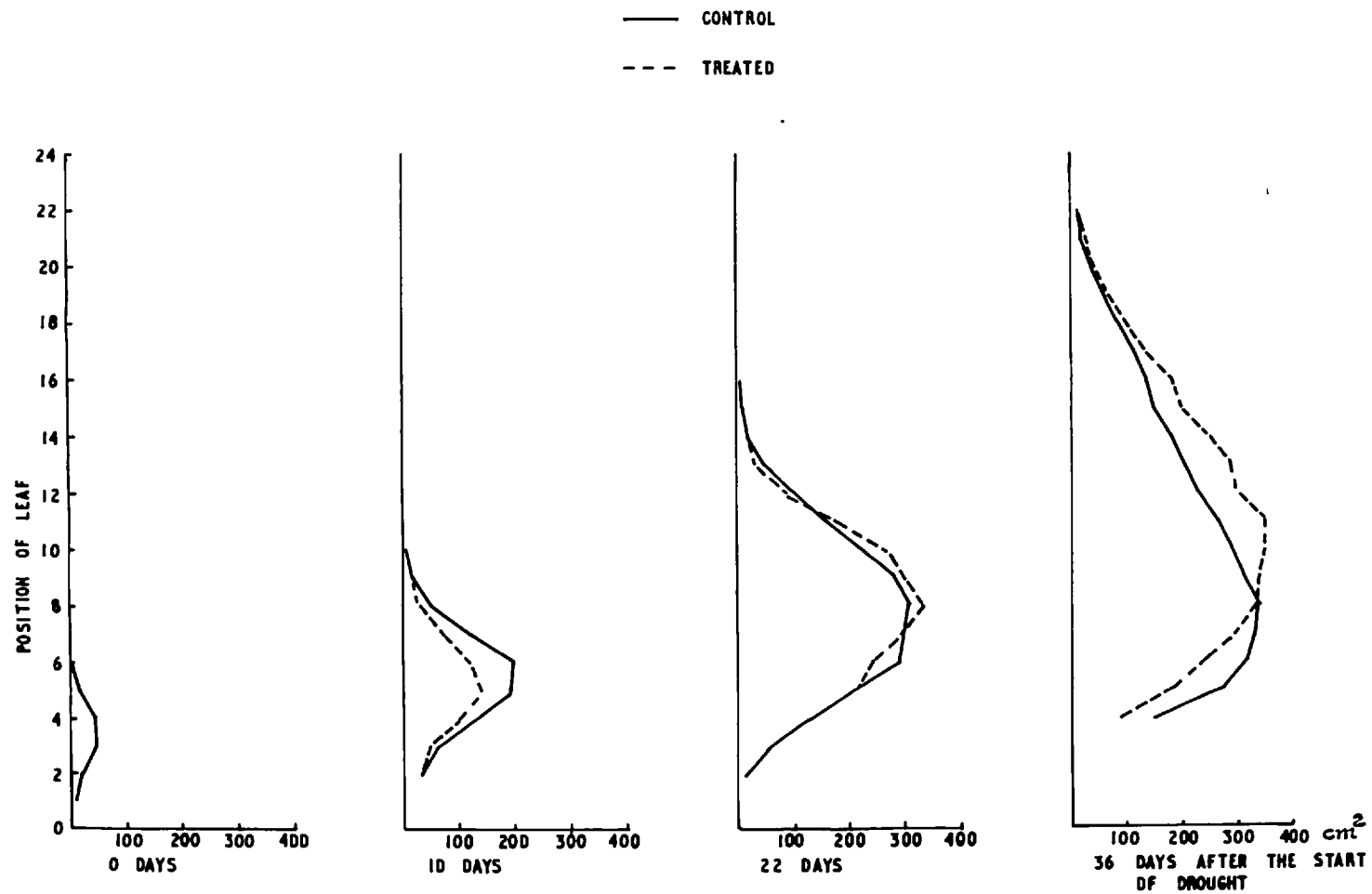


Fig. 18 The relationship between leaf position and mean leaf area at different times after the start of drought, for treated and control plants.

FIGURE 18



rapidly at that time i.e. 3rd to 9th. When the treated plants had recovered sufficiently to have the same total leaf area as the control i.e. 22 days after start of drought, the lower and uppermost leaves of both treatments were approximately of the same size, but the largest leaves were at a higher position on treated plants than those of equivalent size on control plants. At a further stage of development i.e. 36 days after start of drought, this tendency was more marked. The greater total leaf area of treated plants at this stage was mainly due to leaves developed after the treatment was finished.

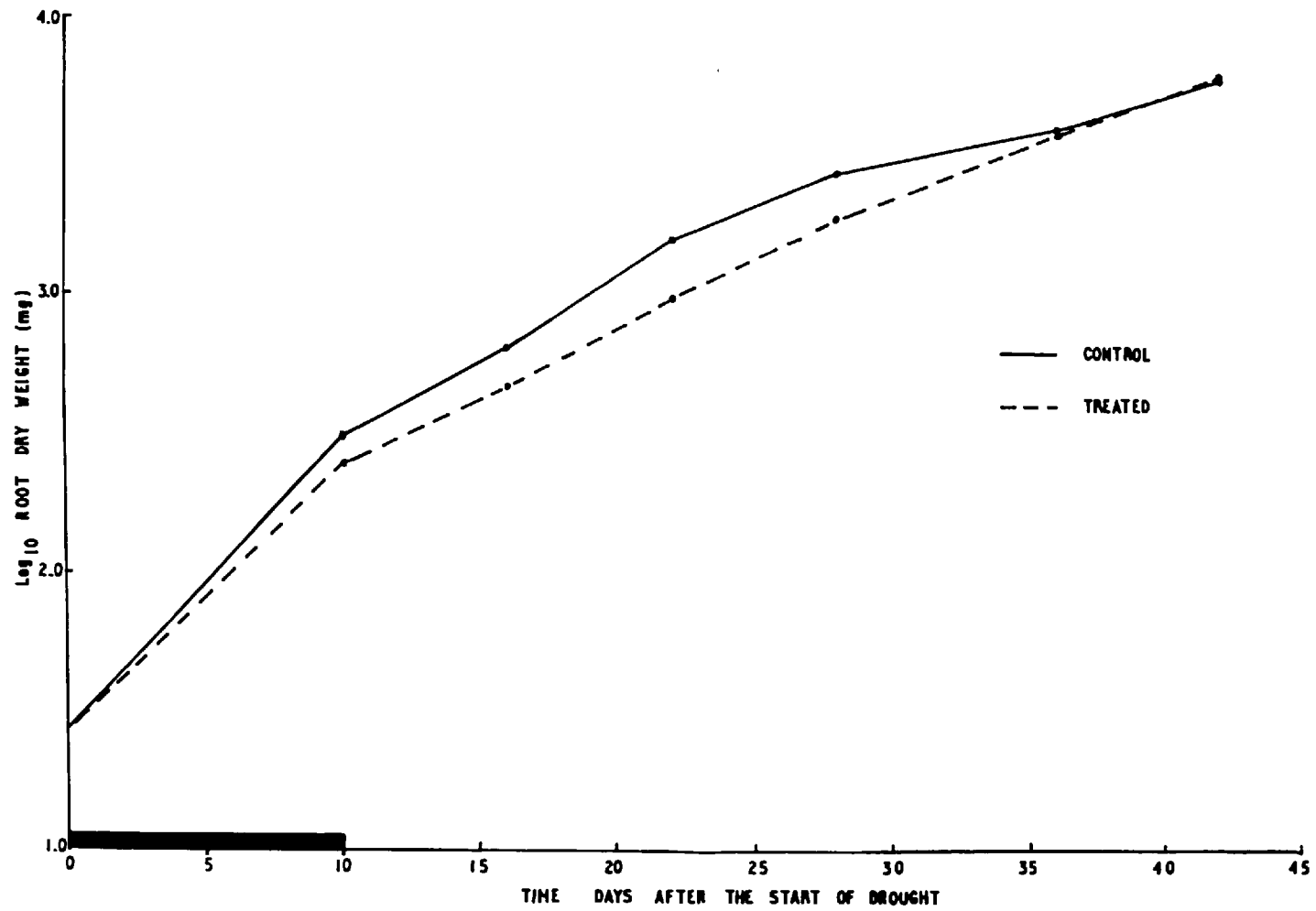
The same final total number of leaves was reached both by treated and control plants. A slight delay of flowering as well as a delay in senescence was shown by treated plants, but the node at which the first flower appeared was the same in treated and control plants.

Root growth

Root growth measured in terms of dry weight change was depressed in treated plants during drought and by the end of the drought root dry weight of treated plants was 83% of the control. During recovery the roots of treated plants showed at first a slower rate of growth than controls. However, about 36 days after start of drought root dry weights of both treatments were the same (Fig. 19, photographs 4 and 5) but even at this stage less branched root system was observed in treated plants. It can be deduced that root : shoot weight ratio was reduced by treatment and did not recover until the end of the

Fig. 19 The effect of treatment on the change of the logarithm of root dry weight with time.

FIGURE 19





Photograph 4 Close view of the root systems of tobacco plants, control plants (T_1) and treated plants (T_2). This photograph was taken at the end of the drought period.



Photograph 5 Close view of the root systems of tobacco plants, control plants (T_1) and treated plants (T_2). This photograph was taken 12 days after the end of the drought period

experiment. In subsequent experiments root length was also measured.

Transpiration

Transpiration was estimated by weighing, the pots being enclosed in polythene bags to prevent evaporation from the pot and soil surfaces. Each point on Fig. 20 is the mean of three determinations carried out on the same plants throughout the experiment and left for the last harvest.

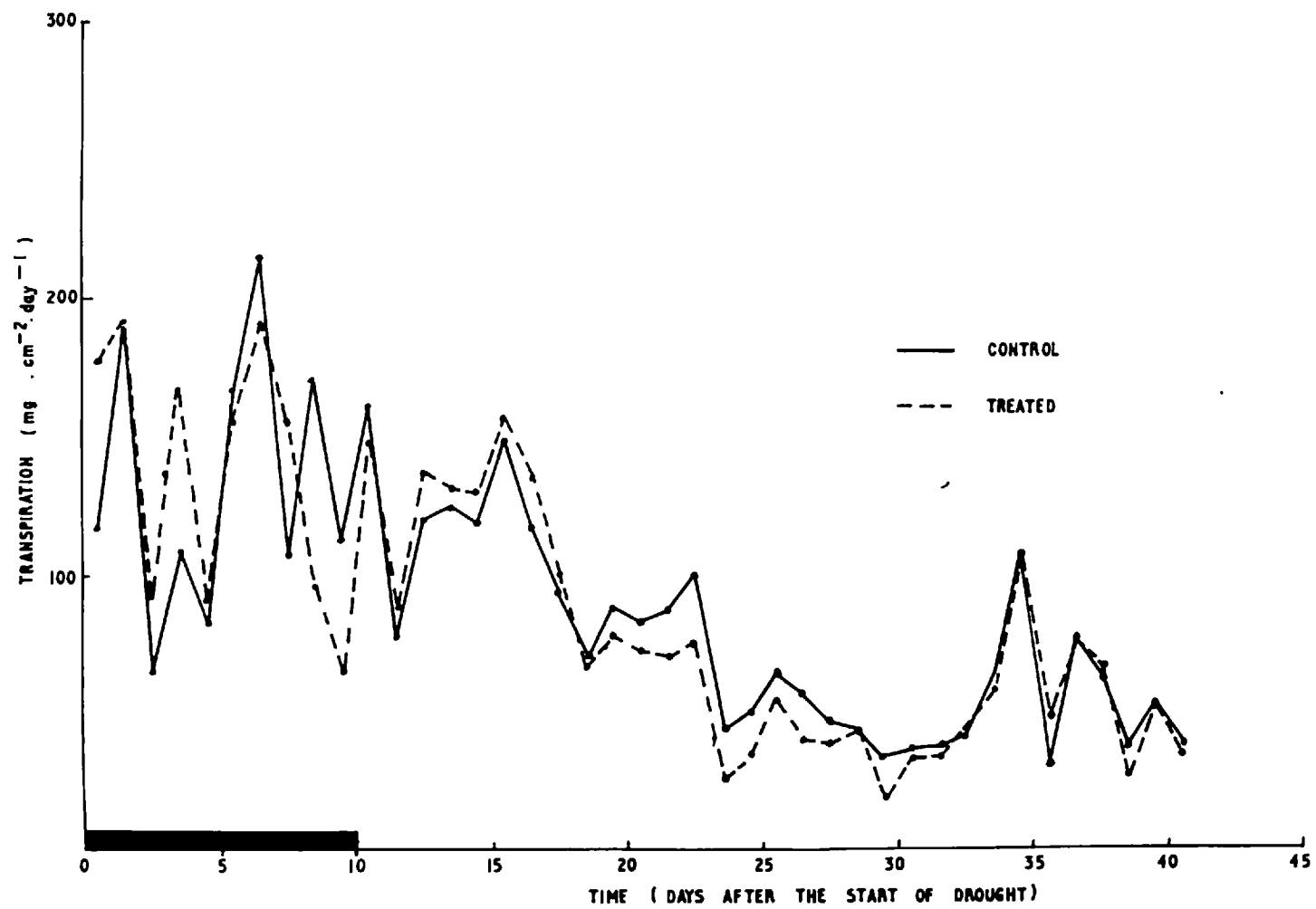
Transpiration rates of control and treated plants were much the same during the first 8 days of the dry period, but during the last two days the treated plants showed a lower rate of transpiration than the control plants (Fig. 20) which could be related to the severity of drought in its final stages. The response of transpiration rate of treated plants to rewatering was immediate; during the first day of rewatering it was almost the same as the control. During the rest of the recovery the rate of transpiration of treated and control plants was the same. The treatment appeared not to have affected any change in subsequent water usage per unit leaf area.

Conclusion

The more rapid growth of treated plants relative to control on rewatering appears to have been mainly due to leaf responses, particularly to a significantly increased specific leaf area ratio rather than to increased E_A . However, the errors of E_A are relatively high and an increased E_A can not be excluded.

Fig. 20 The effect of treatment on the change of transpiration rate with time.

FIGURE 20



Experiment III

Experiment III was designed to investigate leaf growth in terms of rate of leaf production, and of increase in cell number and size in the leaves. Also in this experiment the components of the plants were analysed for N, P, Ca and K and the stomatal response to drought was investigated. Treatments were as in experiment II i.e. half of the plants were freely watered (Control) and water was withheld from the other half for ten days (Treated). This treatment was sufficient to produce wilting throughout the last day. Following drought, water was given freely each day to both treatments.

Plants were grown singly, in pots and randomized between treatments before treatment started and at the start of the treatment plants were 14 days old. Throughout the experiment seven harvests were made. The first was at the beginning of the drought period, the second at the end of the drought period, and the rest of the harvests were made 4, 7, 10, 14 and 21 days later. At each harvest each treatment was replicated five times.

Growth analysis

Growth data were analysed by computer as already explained in experiment II. Points were calculated at 3 day intervals throughout the experimental period for dry weight and leaf area of control and treated plants from the fitted equations and these points were joined to form the curves shown in Figures 21 and 22.

The effect of the drought period was more marked than in experiment

Fig. 21 The effect of treatment on the change of the natural logarithm of dry weight with time. Lines represent the fitted cubic curves, points are the means of the logarithms of observed values, thin vertical bars represent the standard errors of observed values, and thick vertical bars represent least significant differences between the means at $P = 0.05$.

FIGURE 21

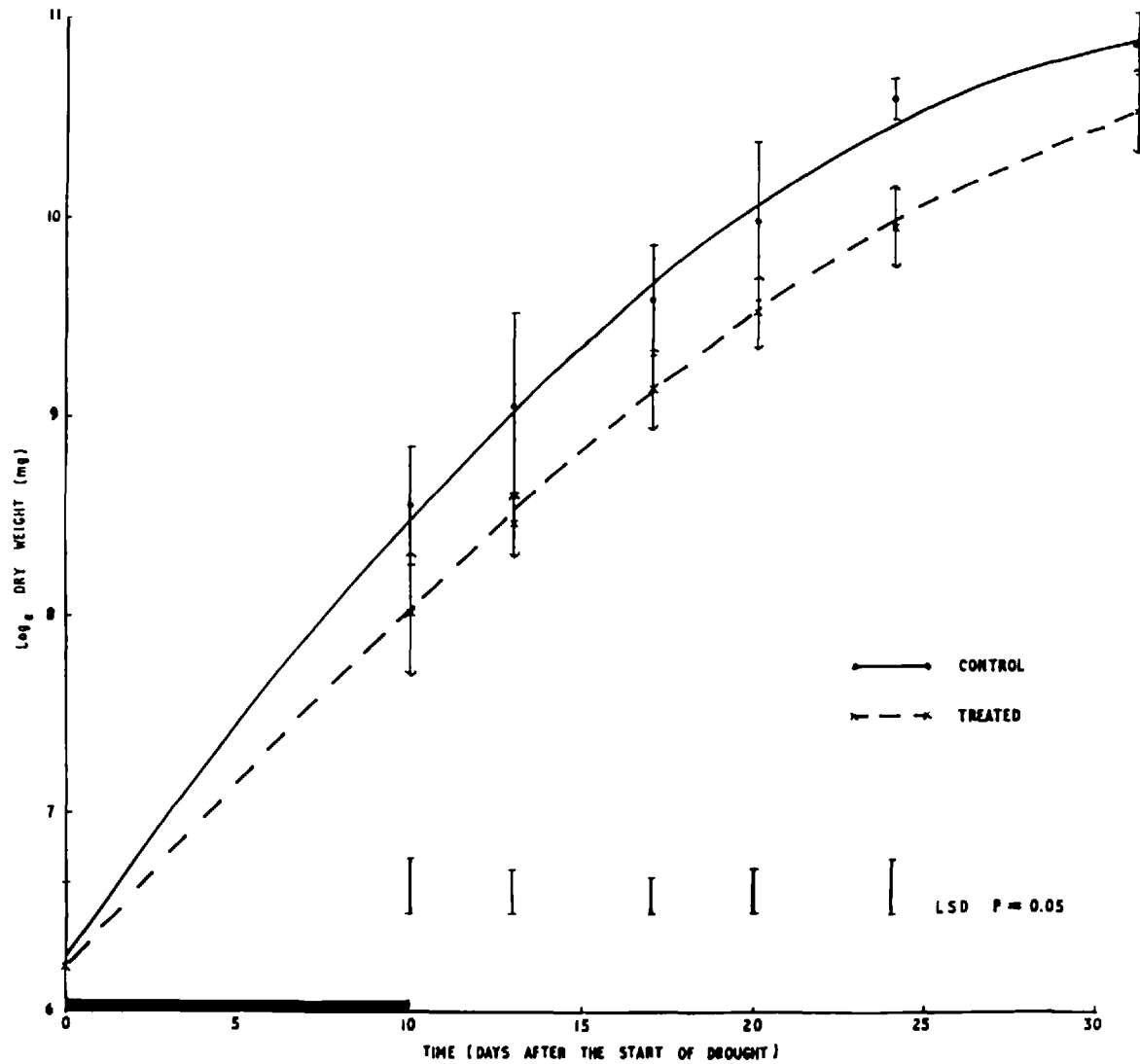
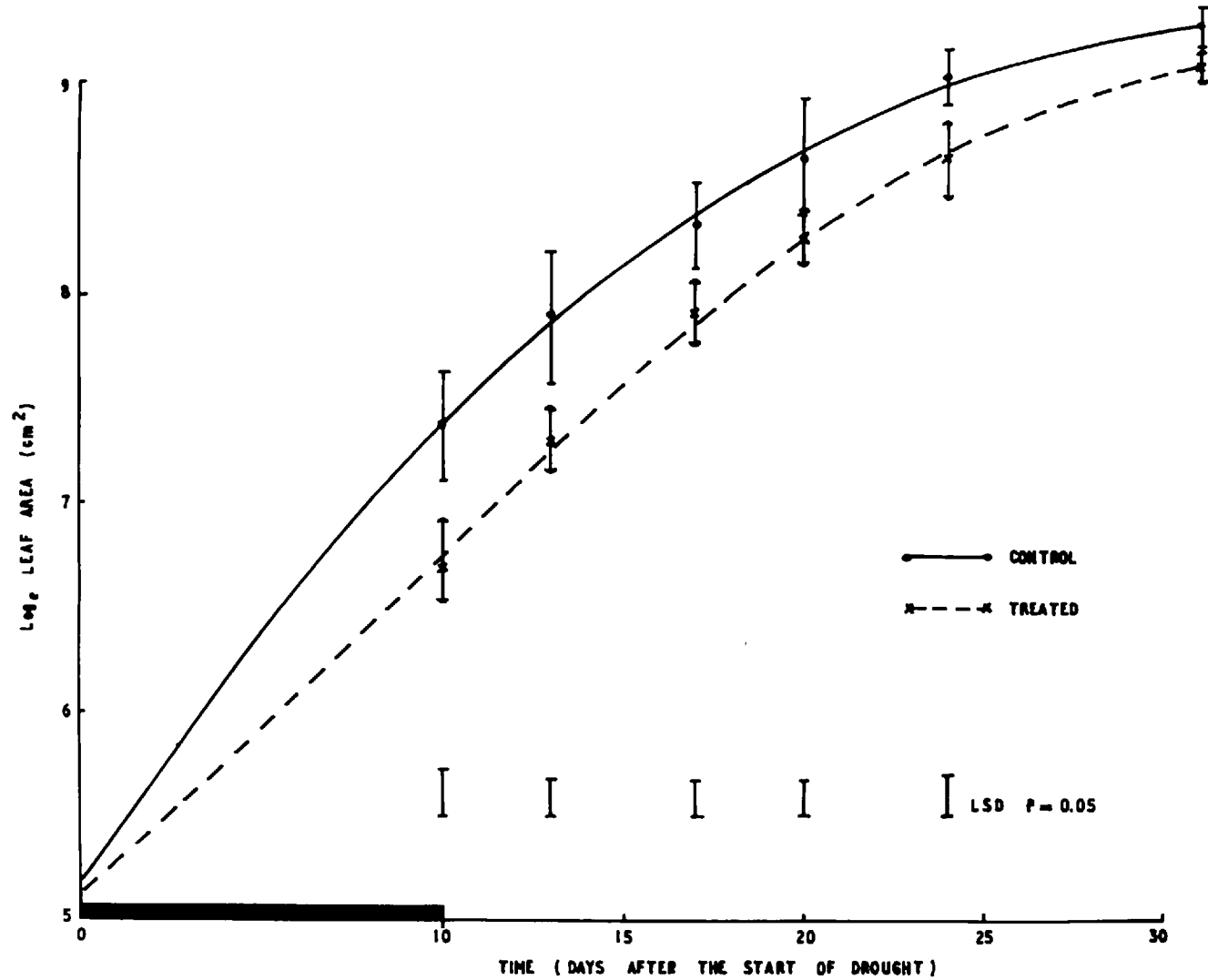


Fig. 22 The effect of treatment on the change of the natural logarithm of leaf area with time. Lines represent the fitted cubic curves, points are the means of the logarithms of observed values, thin vertical bars represent the standard errors of observed values, and thick vertical bars represent least significant differences between the means at $P = 0.05$

FIGURE 22



II and at the end of the drought the dry weight of treated plants was 58% of the controls (Fig. 21). In the following period the two sets of plants had similar relative growth rates, although towards the end of this period relative growth rate of the controls fell a little, but not significantly below that of treated plants (Fig. 23a).

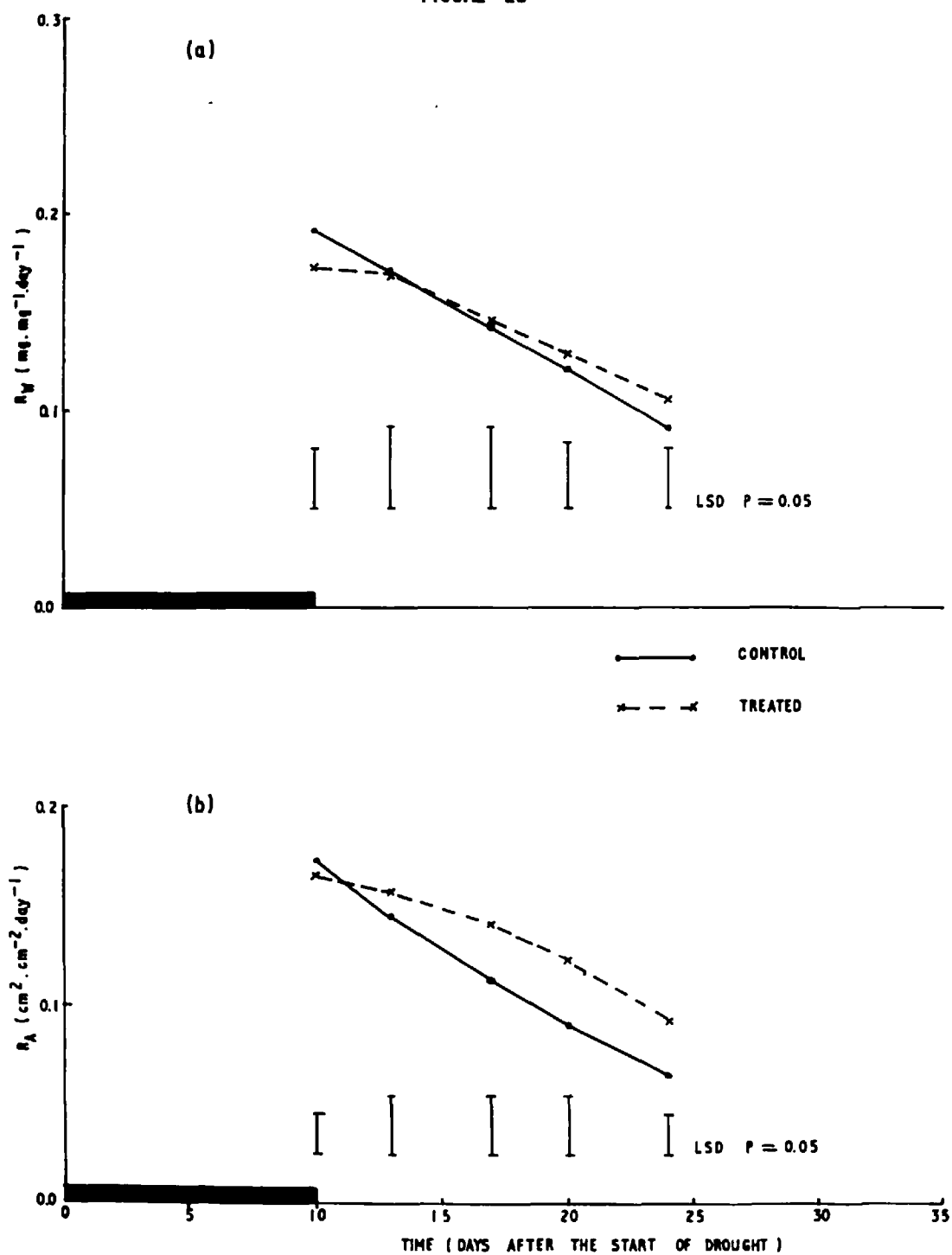
At the end of the treatment period leaf area of treated plants was 50% of the controls (Fig. 22). In the case of leaf area however the relative leaf area growth rate of treated plants rose significantly above that of the control plants after rewatering (Fig. 23b). Consequently the leaf area of treated plants recovered considerably although at the end of the experiment it was still only 83% of the control (photograph 6).

Leaf area ratio was reduced during treatment, but not significantly, and it rose significantly above the control during the recovery period and remained higher than the control when the experiment was terminated (Fig. 24). The higher leaf area ratio of treated plants was due to a slightly, but not significantly higher leaf weight ratio and a significantly higher specific leaf area (Fig. 25). In this experiment leaf weight ratio of treated plants was proportionally higher in relation to the control than in experiment II. A delay in the response of specific leaf area of treated plants, compared with experiment II was also noticed. Leaf weight ratio and specific leaf area were calculated from the original data (i.e. not from the fitted curves).

The higher leaf area ratio during the recovery period might have

Fig. 23 The effect of treatment on the change of a) relative growth rate (R_W), and b) relative leaf area growth rate (R_A) with time. Lines are values of R_W and R_A derived from fitted cubic curves. The vertical bars represent least significant differences between means, at $P = 0.05$.

FIGURE 23





Photograph 6

Tobacco plants, control plant (left hand side) and treated plant (right hand side). This photograph was taken at the last harvest (i.e. 21 days after the end of the drought period).

Fig 24 The effect of treatment on the change of leaf area ratio (F_A) with time. Lines are values of F_A derived from fitted cubic curves. The vertical bars represent least significant differences between means, at $P = 0.05$

FIGURE 24

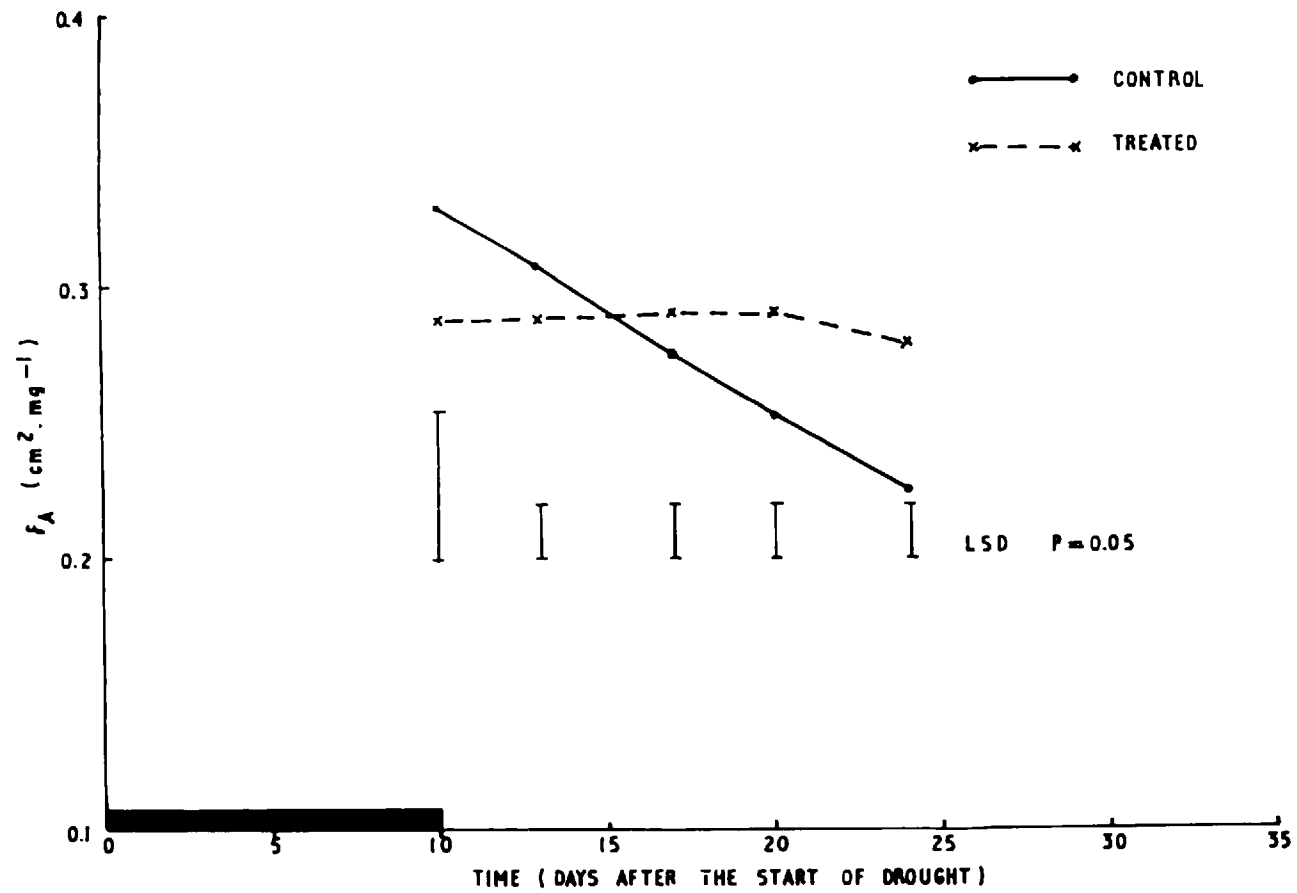
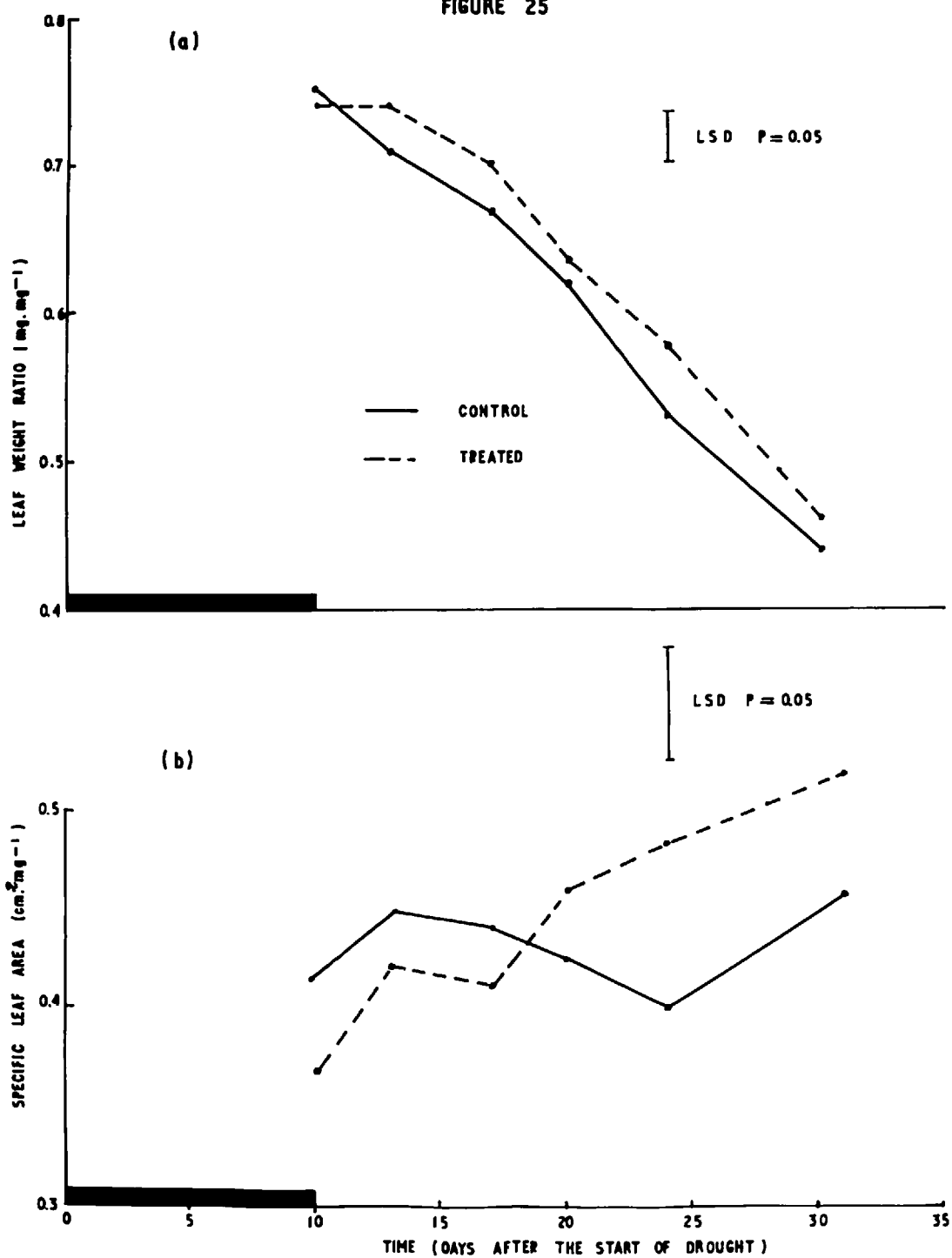


Fig. 25 The effect of treatment on the change of a) leaf weight ratio, and b) specific leaf area with time. Points are the mean of observed values. The vertical bars represent the least significant differences at $P = 0.05$.

FIGURE 25



been expected to produce a significantly higher relative growth rate but it was approximately balanced by a smaller unit leaf rate (Fig. 26), although as in the previous experiment the errors of unit leaf rate were very high.

Root growth was measured in terms of both dry weight and length. At the end of the drought period root dry weight of treated plants was 68% of the control (Fig. 27). During the recovery period the roots of treated plants showed a similar rate of growth to the control rate and at the end of the experiment the ratio of treated to control was approximately the same as at the end of the drought period. This experiment differs from experiment II as far as root dry weight is concerned in that by the end of the drought period there was a greater reduction in root dry weight due to drought treatment in relation to the controls and that even at 31 days after the start of drought there was no sign of recovery.

Root length was depressed in treated plants during drought and by the end of the drought period root length of treated plants was 68% of the control (Fig. 28); the difference was not statistically significant, but during the recovery period treated plants showed a slower rate of growth in root length, and as recovery progressed differences between treated and control plants became significant, producing plants at any time with a visibly less branched root system.

It appears that the effect of drought on root dry weight and root

Fig. 26 The effect of treatment on the change of unit leaf rate (E_A) with time. Lines are values of E_A derived from fitted cubic curves. The vertical bars represent least significant differences between means, at $P = 0.05$.

FIGURE 26

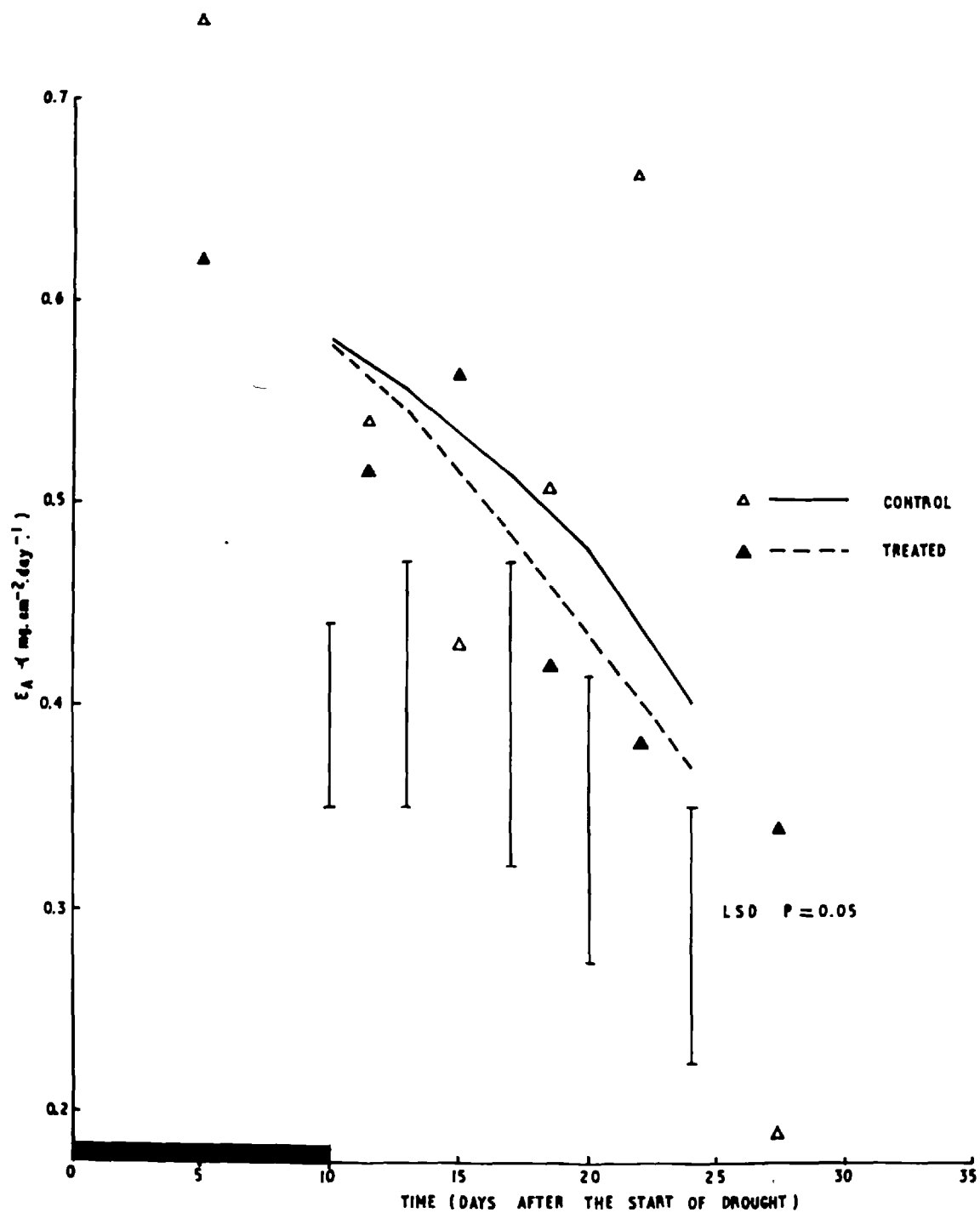


Fig. 27 The effect of treatment on the change of the logarithm of root dry weight with time.

FIGURE 27

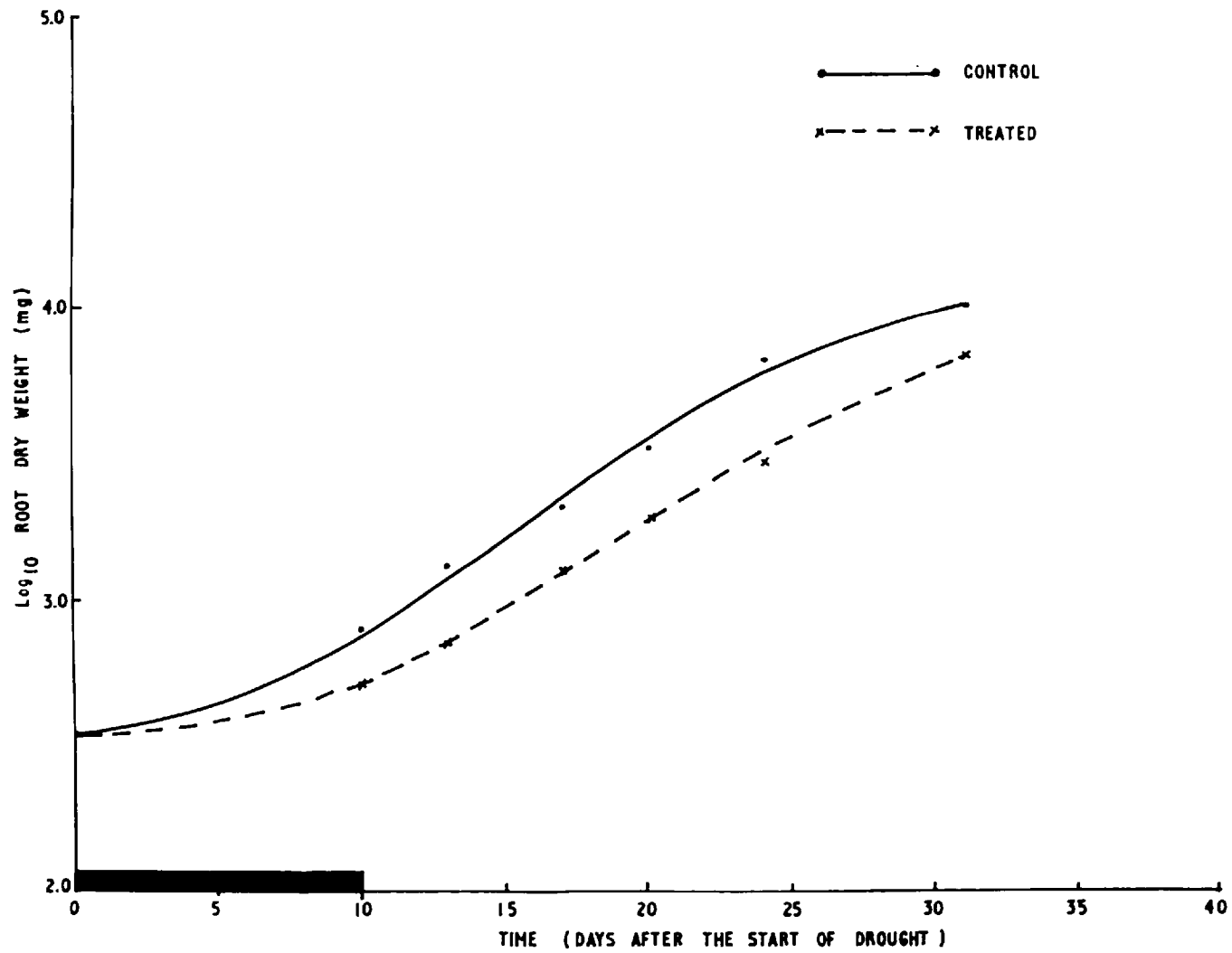
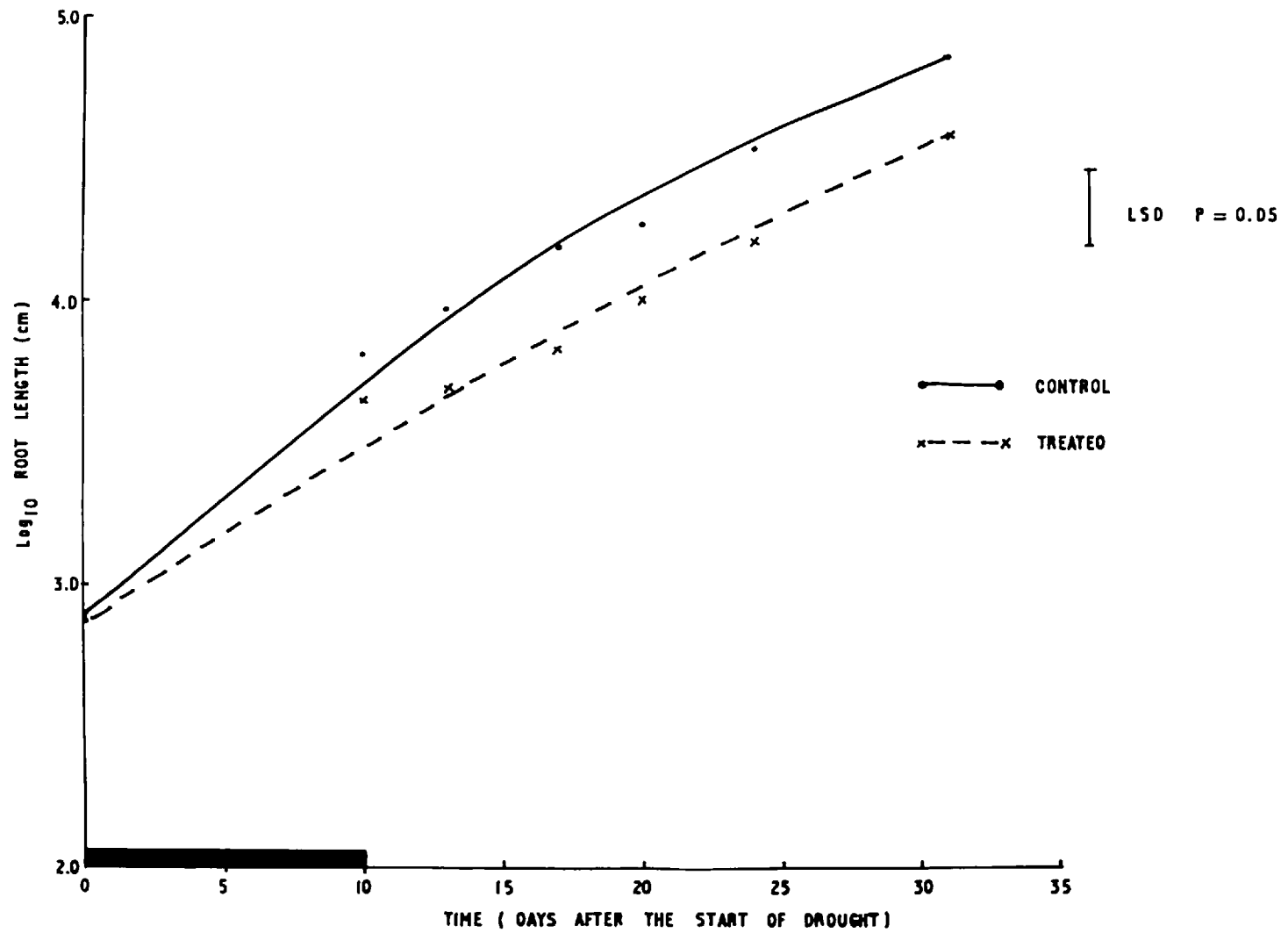


Fig. 28 The effect of treatment on the change of the logarithm of root length with time.

FIGURE 28



length of treated plants was approximately the same but during recovery root dry weight increased proportionately more than root length.

In general the results of this experiment resemble experiment II in that there were only slight and non-significant effects on unit leaf rate in the recovery period, but there was significantly greater leaf area growth in the treated plants. They differ from experiment II in that the check on dry weight increase persisted almost throughout the recovery period. Also the reduction in root growth relative to control persisted throughout the recovery period.

Leaf Production

All leaves on the main axis were counted in two plants per treatment at each harvest, the leaves in the terminal bud being observed by dissection under a stereoscopic microscope. Leaves with an area equal to or larger than one cm^2 were considered unfolded (U) and leaves with less than one cm^2 were considered folded (F). Total number of leaves (T) was calculated adding unfolded and folded leaves.

There was little or no effect on the rate of unfolding of leaves either as a result of the drought or during the recovery period (Table 5). But there was a quite striking increase in the number of folded leaves and hence of total leaves as a result of drought. At the end of the drought period (i.e. 10 days after start of drought) there was a significant difference between the number of folded leaves on treated and control plants, 3 days later the difference was still significant and after 4 further days treated plants still had a

Table 5

Mean folded, unfolded and total number of leaves per plant

Each value is the mean of two plants. F = folded leaves, i.e., less than 1 cm², U = unfolded leaves, and T = total number of leaves.

Days after start of drought	0			10			13			17			20			24			31		
	F	U	T	F	U	T	F	U	T	F	U	T	F	U	T	F	U	T	F	U	T
Control	7.0	8.0	15.0	4.5	14.5	19.0	5.5	15.0	20.5	9.0	15.0	24.0	5.5	18.5	24.0	4.5	19.5	24.0	3.0	21.0	24.0
Treated	7.0	8.0	15.0	9.5	13.5	23.0	10.5	13.0	23.5	10.0	15.0	25.0	6.5	18.5	25.0	4.5	20.5	25.0	3.5	21.5	25.0

LSD (P = 0.05) Folded leaves = 2.04

larger (but no longer significant) number of folded leaves. Seven days later both treatments had the same number of folded leaves. The final total leaf number was determined by the flowering of the plant, and this final total number was the same in both treatments. The more rapid production of leaves at the apex during and immediately following the drought, was therefore followed by a reduced rate of production since final numbers were almost equal.

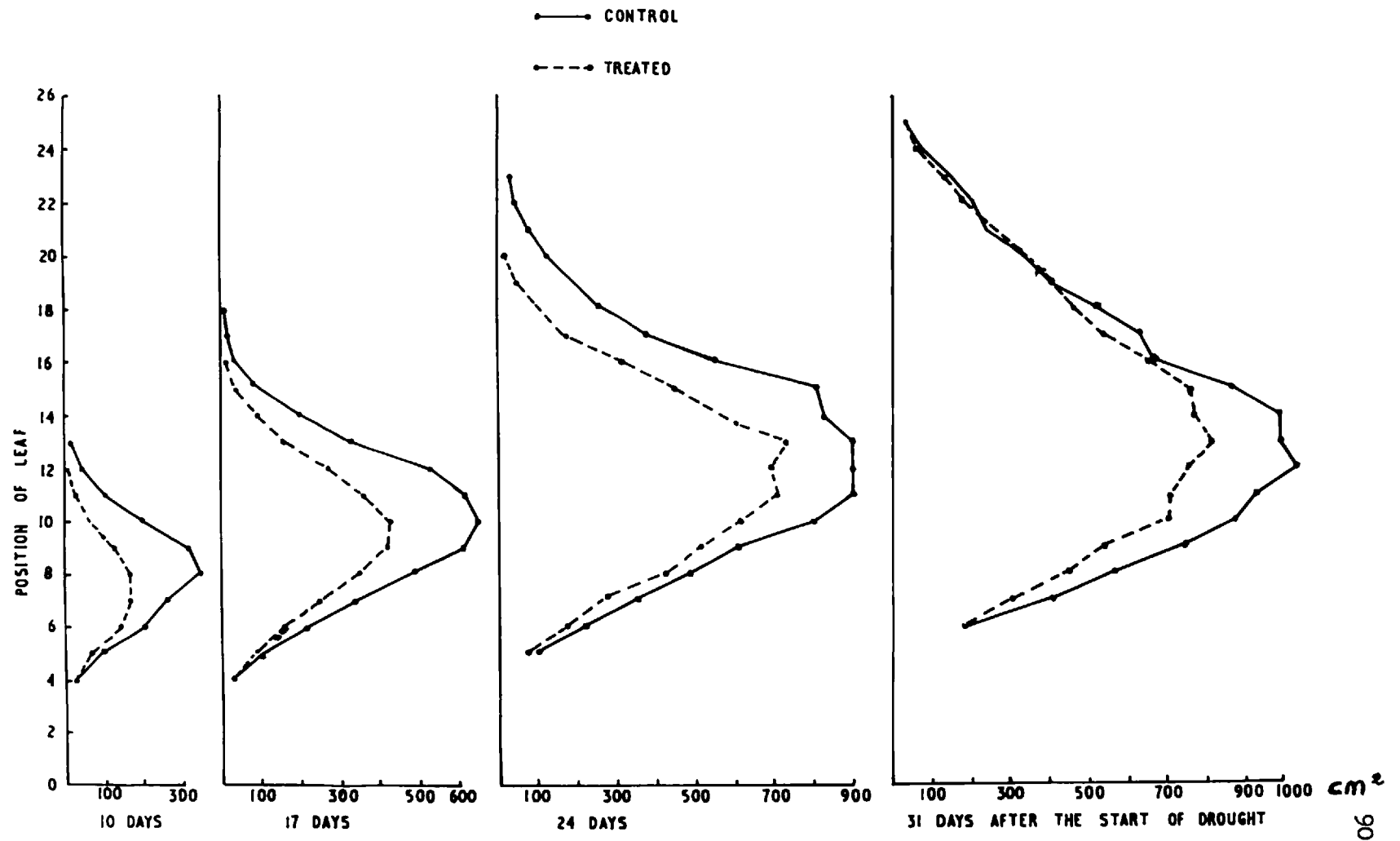
The same pattern of distribution of leaf area was shown in this experiment as in experiment II (Fig. 29), except that in experiment III the leaves that were most affected by the treatment were the 7th to 14th instead of 3rd to 9th as the former leaves were the rapidly expanding during the drought. Young leaves again recovered rapidly. At 24 days all leaves of the treated plants were smaller than the controls but at 31 days after start of drought the uppermost leaves (i.e. from the 16th leaf onward) of both treatments were of approximately the same size and the tendency of treated plants to produce their largest leaves at a higher position than control plants was shown.

Cell number

Cell number was determined on each leaf of two plants of each treatment at 7 day intervals (i.e. one determination was carried out at the beginning of the treatment, one at the end of the treatment, and the rest 7, 14 and 21 days later). For purposes of statistical analysis, data for harvest one were eliminated and data for leaves

Fig. 29 The relationship between leaf position and mean leaf area at different times after the start of drought for treated and control plants.

FIGURE 29



in subsequent harvests were grouped in fours in such a way that when possible a particular group of leaves could be followed through the different stages of development.

Depending on the age of leaves three arbitrary groups of four leaves were formed: young leaves (Y) refers to the three or four uppermost expanded (i.e. more than 1 cm²) leaves, mature leaves (M) refers to the following four leaves down in the stem (these leaves were the young leaves in the previous harvest) and old leaves (O) refers to the subsequent four leaves further down in the stem (these leaves were the mature leaves in the previous harvest and the young leaves two harvests earlier). Changes in time of the three groups of leaves mentioned above are shown in Fig. 30. This grouping of leaves was used in analysing the changes in both cell number and cell volume. The stage of growth of particular leaves can be judged by comparing Figs. 29 and 30.

Cell number per leaf of mature (leaves 7-10) and old leaves (3-6) of treated plants were not reduced significantly by the end of the drought period, although the number in the mature leaves was increasing during this time (Fig. 31). However, cell number per leaf of young leaves (11-14) of treated plants (these leaves unfolding during the drought period) was reduced, though not significantly in relation to young leaves of control plants. During the 14 days following rewatering when these young leaves matured and aged, they produced cells (in the treated plants) at a slightly faster rate than the

Fig. 30 The change in position of leaf groups young (Y), mature (M), and old (O) with time.

FIGURE 30

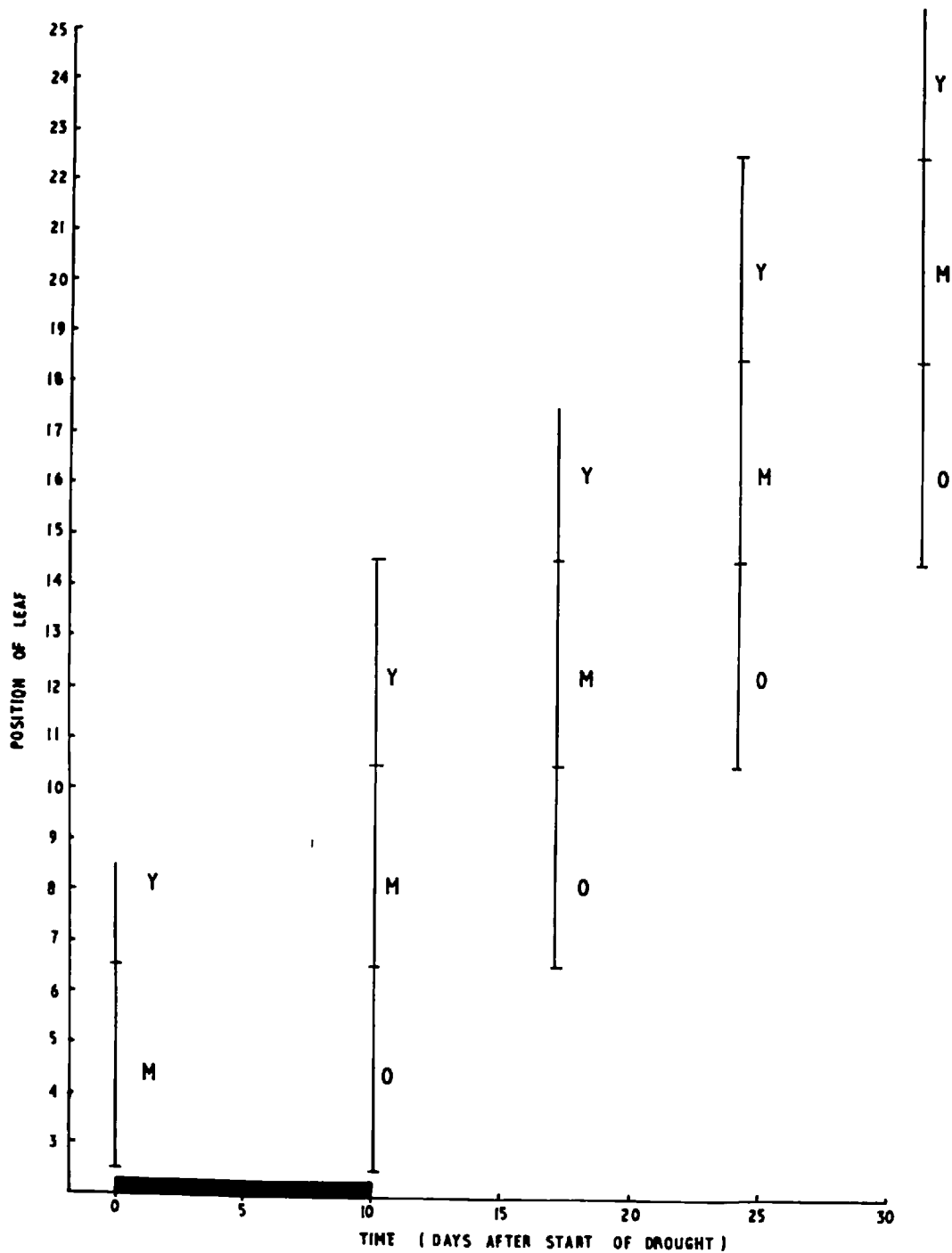
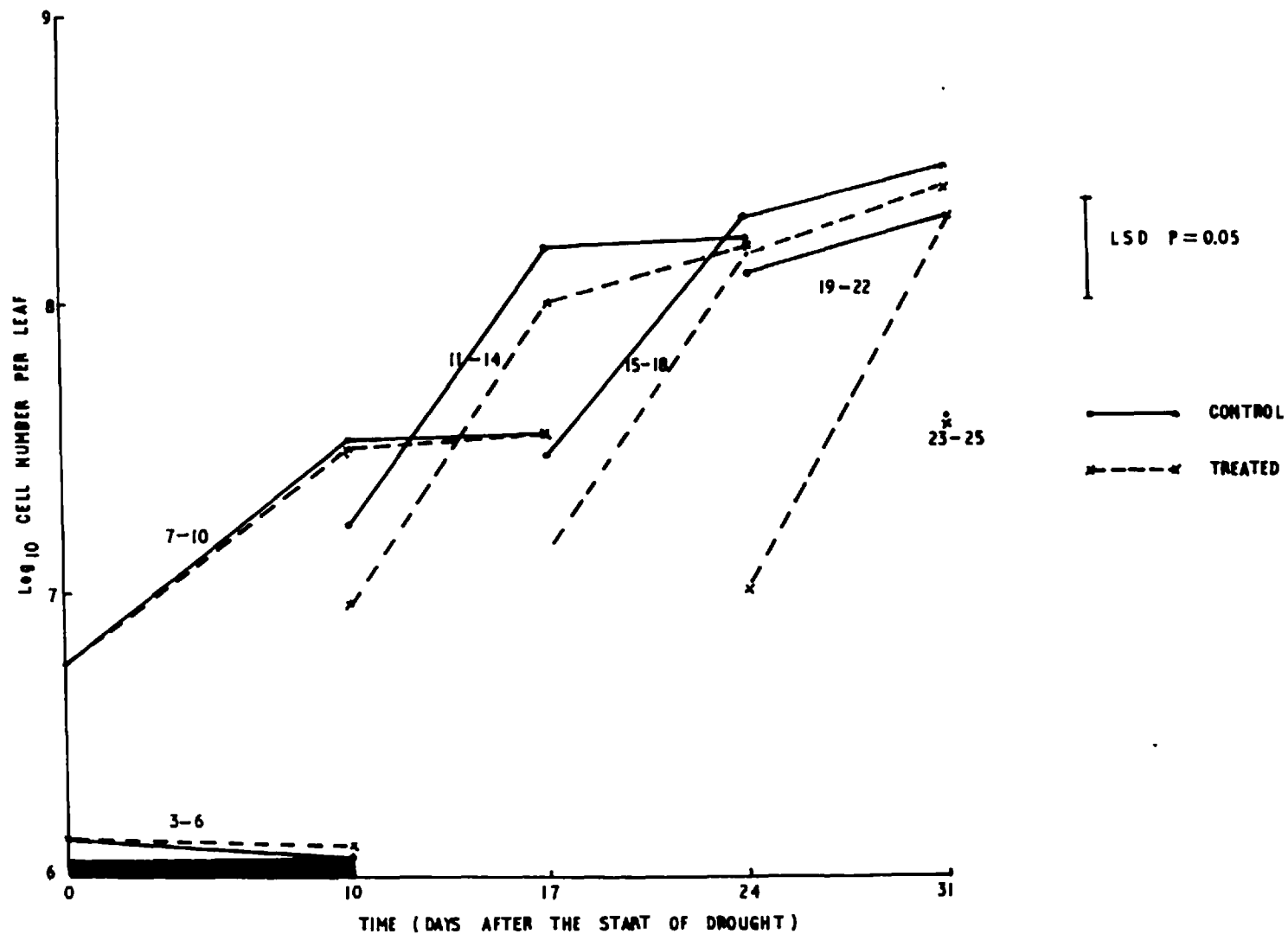


Fig. 31 The effect of treatment on the change in the logarithm of cell number per leaf with time for successive leaf groups. The vertical bar represents the least significant difference at $P = 0.05$

FIGURE 31



equivalent leaves of control plants, and finished with only a slightly reduced number of cells per leaf.

Young leaves (15-18) of treated plants at 17 days after start of drought had a reduced (but not significantly) number of cells per leaf, but again they showed a faster rate of cell production so that 14 days later there was little difference between treated and control.

Young leaves (19-22) of treated plants at 24 days after start of drought had a significantly reduced number of cells per leaf in relation to similar leaves of control plants. However, seven days later the difference disappeared completely and both treated and control leaves had the same number of cells per leaf.

Young leaves (23-25) of treated plants at 31 days after start of drought had approximately the same number of cells as equivalent control leaves.

The interaction between treatment and age of leaves was significant; this difference arose mainly because cell numbers of young leaves of treated plants were significantly reduced compared with equivalent controls. (Table 6). However, as these young leaves matured the difference disappeared and both treated and control had the same number of cells per leaf.

The most striking effect of treatment was in leaves 19-22, and reference to Table 5 shows that these were the leaves produced in the apex at the end of the drought period. Even in these, cell number was restored in seven days. Leaves formed after treatment (23-25)

were not affected.

Table 6

$\log_{10}$ mean number of cells per leaf

	Control	Treated
Young	7.61	7.17
Mature	8.09	8.00
Old	7.58	7.56

LSD $P = 0.05$ 0.18

Cell number in the apex

Table 7

Mean cell number ($\times 10^{-3}$) in the apex

Each value is the mean of two plants

Days after the start of drought	0	10	17	24
Control	2347	1505	1500	890
Treated	2347	2250	1505	1030

LSD $P = 0.05$ 1370

Cell number increased, but not significantly, in the apices of treated plants, and by the end of the treatment they had 50% more cells than the apices of control plants (Table 7). This increase in cell number of treated plants corresponds with the accelerated leaf primordia production of these plants during drought (Table 5). During recovery, i.e. 7 and 14 days after the treatment finished, cell numbers were approximately the same in both treatments.

the same in both treatments.

Cell volume

The average volume per cell in large leaves was calculated approximately by dividing the fresh weight per square centimeter by cell number per square centimeter, assuming that the density of the cells was 1.0 mg mm^{-3} . In small leaves the average volume was calculated by dividing fresh weight of the whole leaf by total cell number per leaf.

At the end of the drought period cell volume in young (11-14) and mature (7-10) leaves of treated plants was significantly reduced (Fig. 32). During the next seven days the cells of treated leaves expanded faster than the control and difference in size between them virtually disappeared.

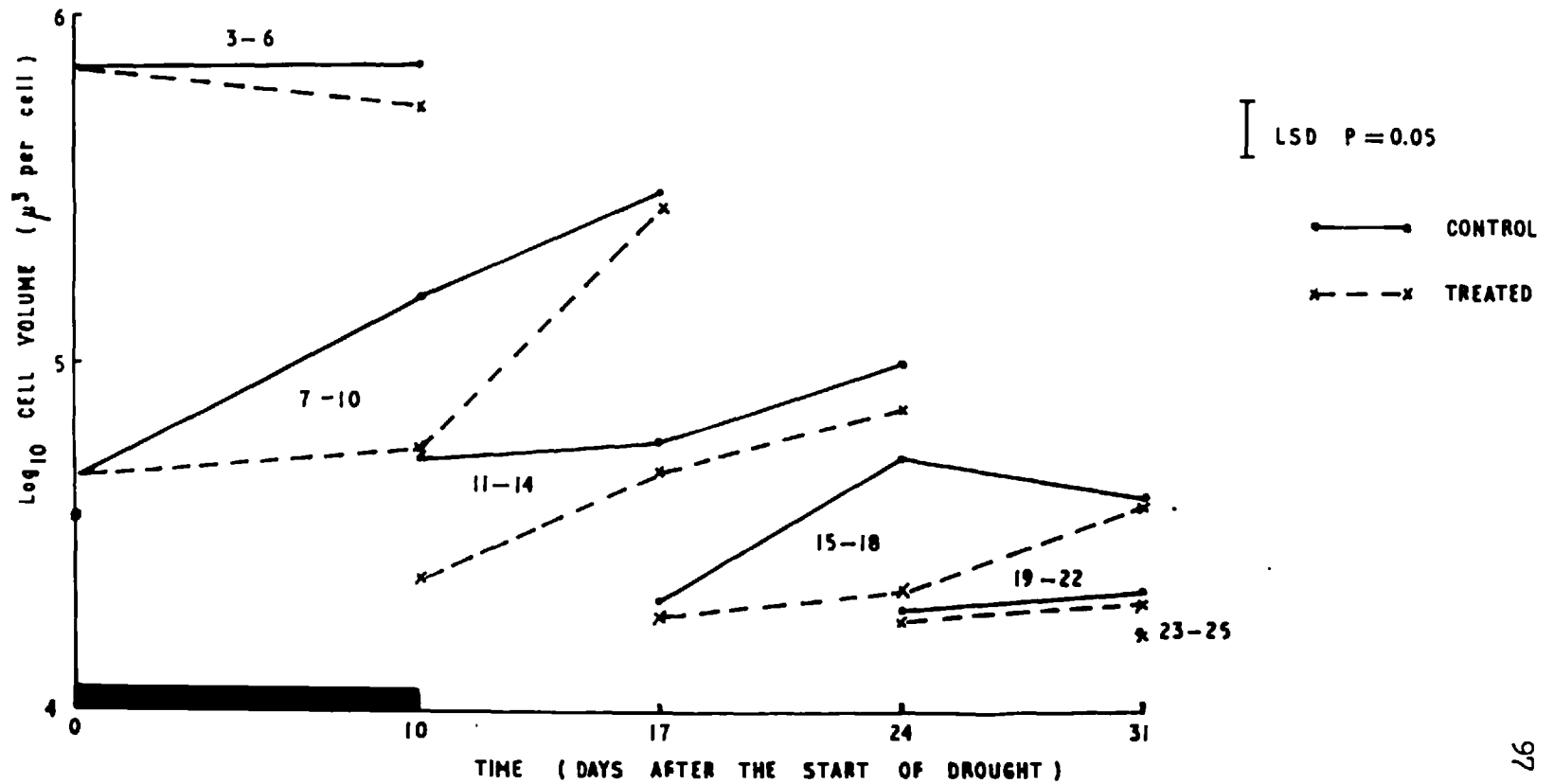
Young leaves (15-18) of treated plants at 17 days after start of drought had a smaller but not significantly reduced cell size in relation to cells of similar leaves of control plants; seven days later however the cell size of treated leaves was significantly smaller than in the controls, and after a further seven days the difference had disappeared again. Some doubt is attached to the large treatment effect on the 15th-18th leaves at day 24 since there is an apparent decline in cell size of control leaves between days 24 and 31.

There was only a very small effect of treatment on cell size in the 19th and later leaves.

In general, cell volume was considerably reduced during the

Fig. 32 The effect of treatment on the change in the logarithm of cell volume per leaf with time for successive leaf groups. The vertical bar represents the least significant difference at $P = 0.05$

FIGURE 32



treatment and there was not complete recovery of cell size in leaves of treated plants. Cells of leaves of treated plants produced at the very end of the drought period and subsequently, i.e. leaves 19 and older, were of approximately the same size as in controls. It appears that drought had an effect on cell volume in expanding leaves but leaves that were produced subsequent to the treatment were not affected by it.

Mineral nutrition

An investigation was made into the effects of treatments on nutrient content of tobacco because it was thought that nutrient supply could have some effect on growing leaves during recovery (Gates, 1957). Agreement between replicates was generally very good. No statistical analysis is presented.

Nutrient uptake

Determinations of N, P, K and Ca were made in roots, stem and leaves (when possible, each leaf was analysed separately) of four plants (two plants per treatment) at each harvest.

During drought, total Nitrogen, Phosphorus, Potassium and Calcium contents per plant were very much depressed in treated plants; the fall in nutrient content was more severe in leaves than stems, and in stems more than roots (Figs. 33, 34, 35 and 36). Differences between total content of nutrient in leaves, stem and roots of treated and control plants were still large at the end of the experimental period.

Fig. 33 The effect of treatment on the change of total nitrogen content of the whole plant, leaves, stem and roots, with time.

FIGURE 33

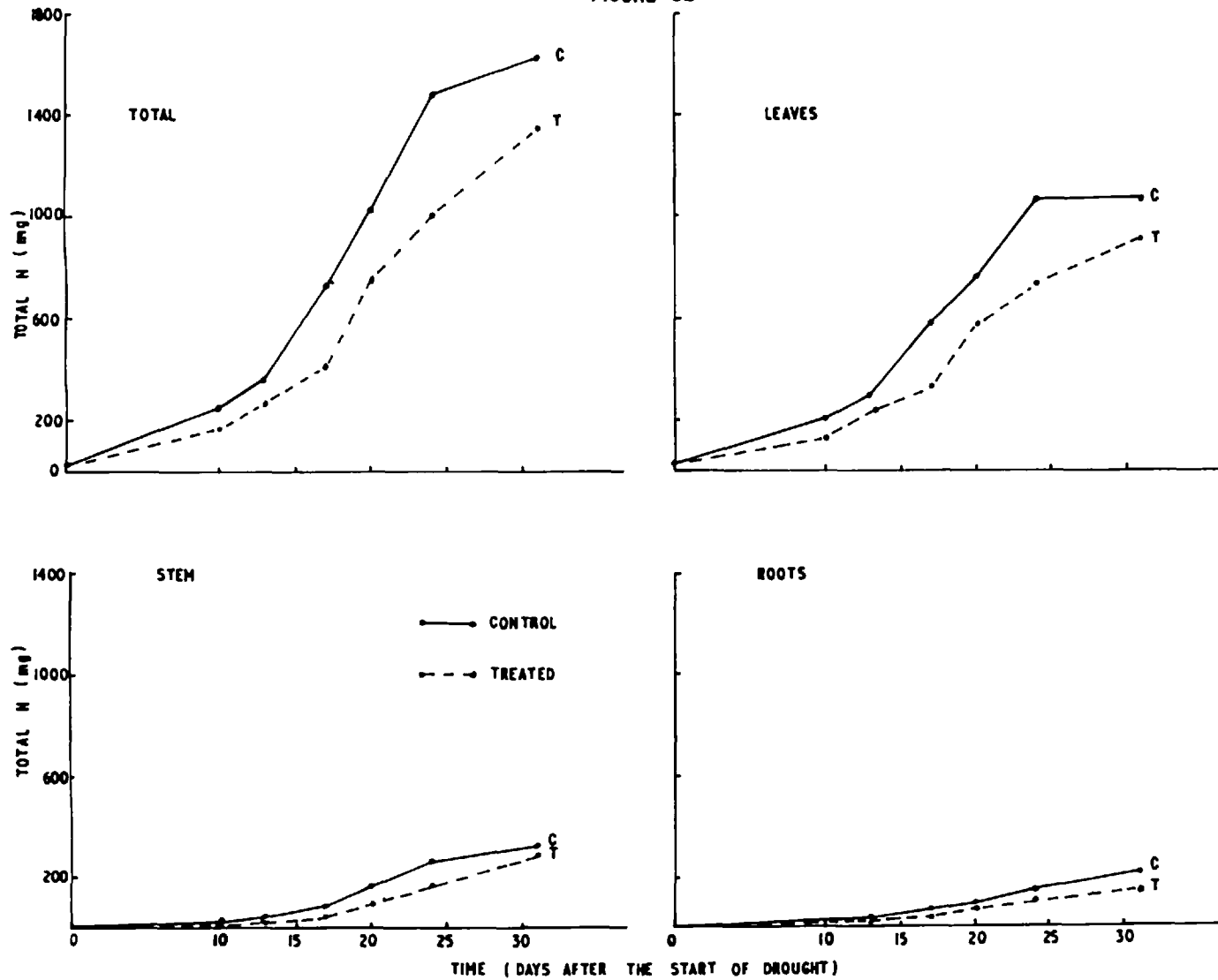


Fig. 34 The effect of treatment on the change of total phosphorus content of the whole plant, leaves, stem and roots, with time.

FIGURE 34

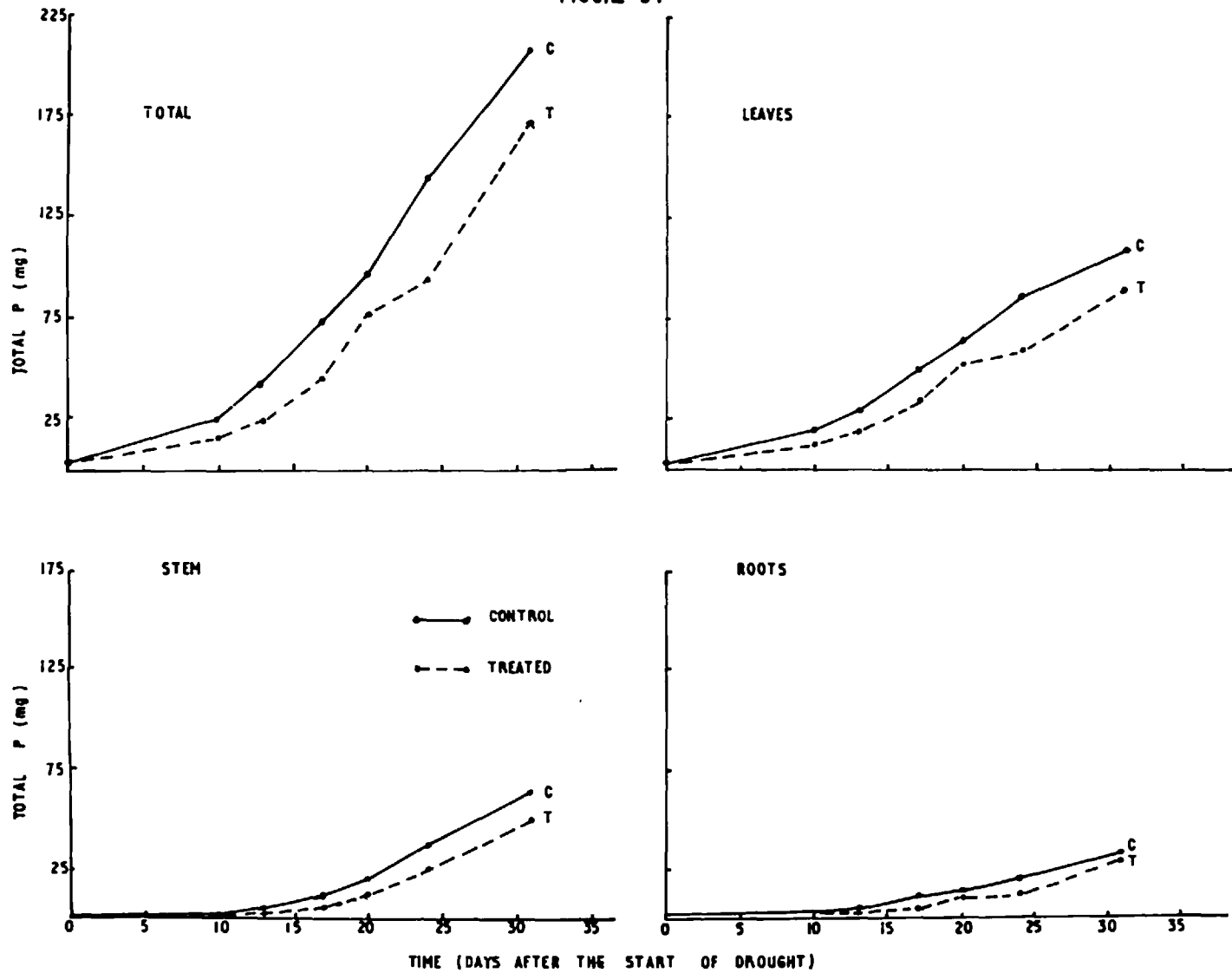


Fig. 35 The effect of treatment on the change of total potassium content of the whole plant, leaves, stem and roots, with time.

FIGURE 35

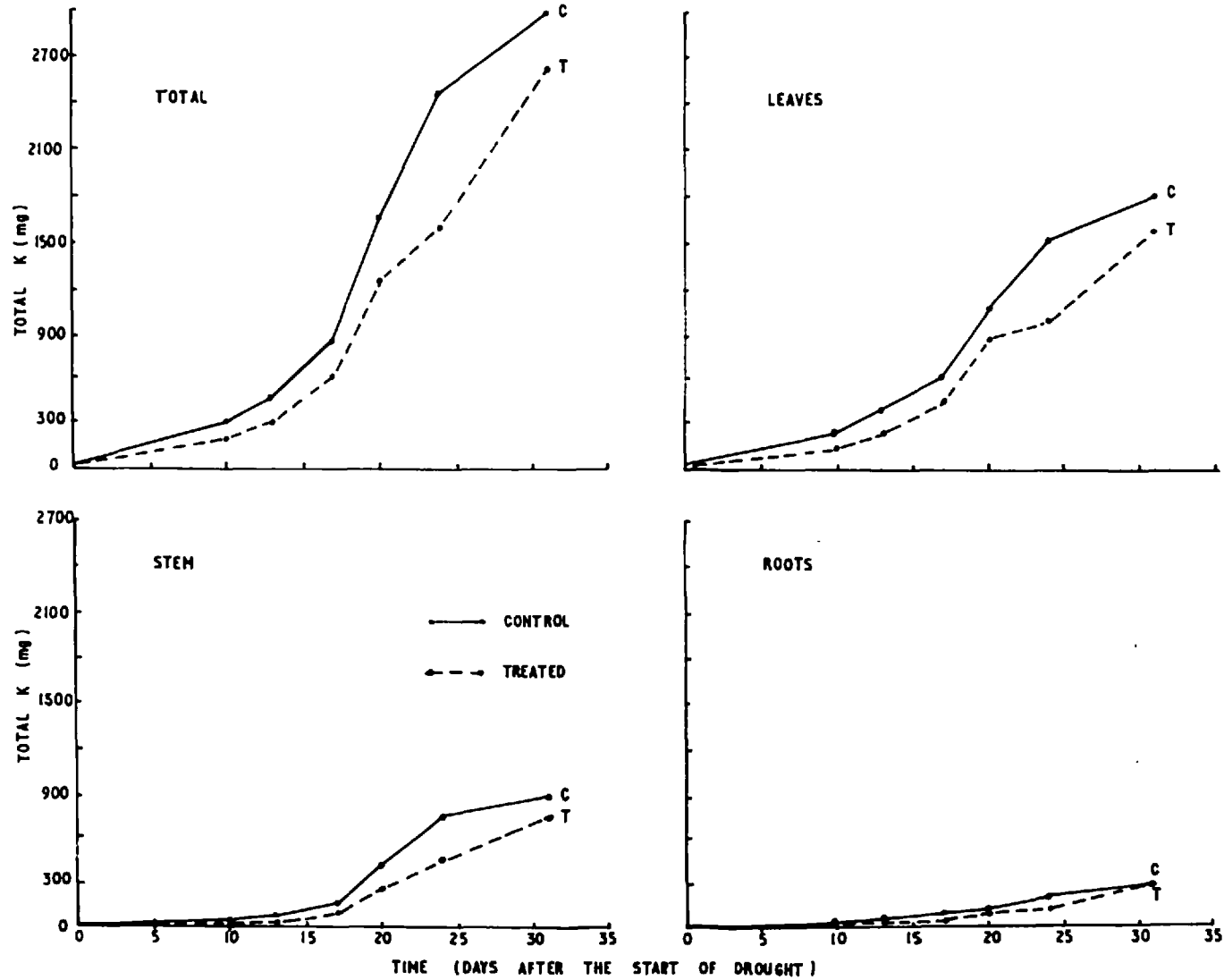
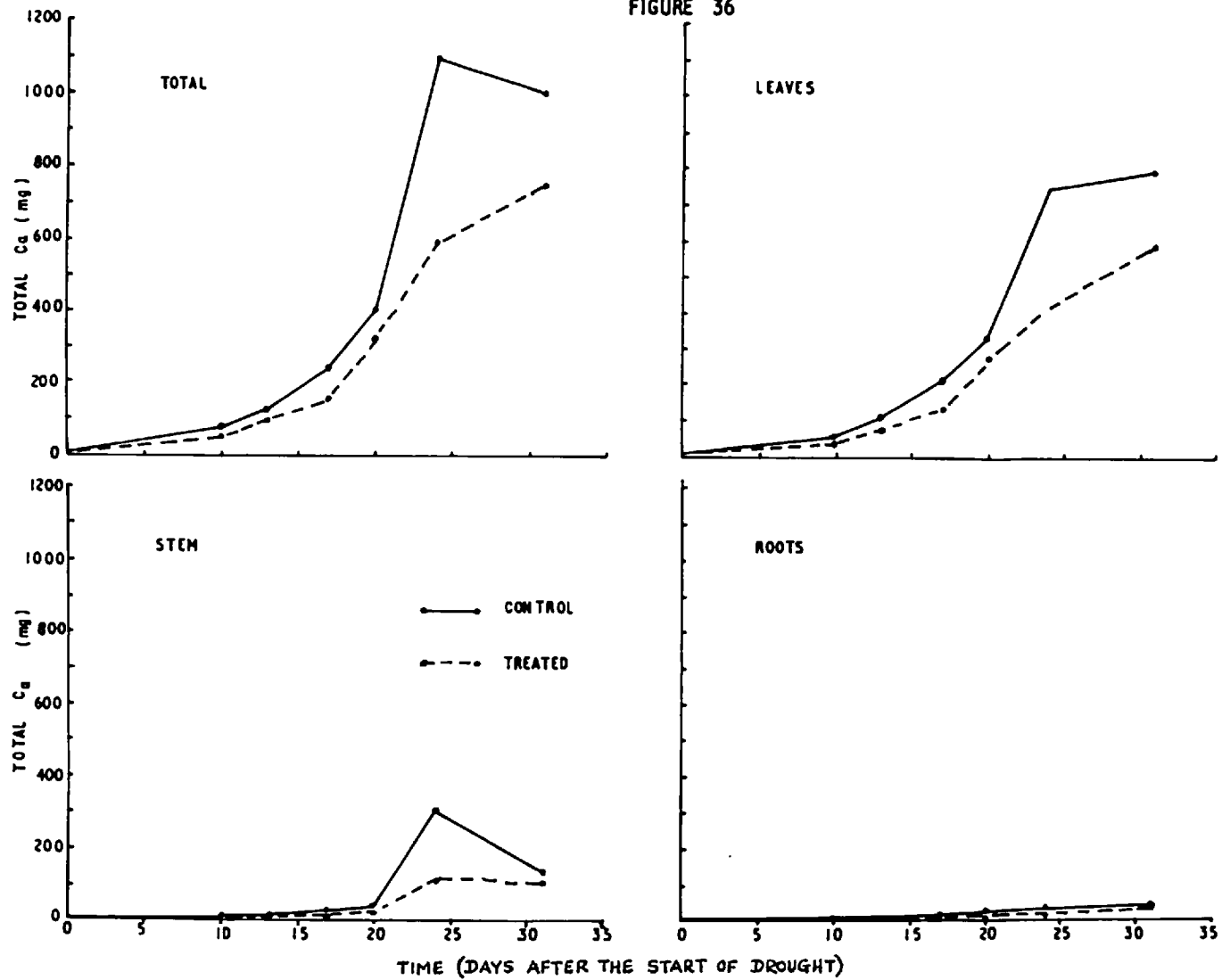


Fig. 36 The effect of treatment on the change of total calcium content of the whole plant, leaves, stem and roots, with time.

FIGURE 36



Nutrient per cent dry weight and per cent leaf area.

Analyses for N, P, K and Ca were carried out on each leaf separately when the leaf dry weight was sufficiently large for analysis. Small leaves from the bottom or top of the plant were bulked with the leaf immediately above or below respectively.

Nutrient concentration percent in either dry weight (i.e. mg per 100 mg) or leaf area (i.e. mg per 100 cm²) terms was first calculated as a weighted mean, i.e. total nutrient/total leaf weight.

The concentrations of N, P and K in leaves in dry weight terms were depressed during drought but a slight increase in the concentration of Ca was observed (Figs. 37, 38, 39 and 40). However, as leaf area was more affected by treatment than dry weight, the concentrations of all nutrients on a leaf area basis increased in the treated plants above controls by the end of the drought period. After rewatering, the concentrations of all nutrients in leaves of treated plants on either a dry weight or leaf area basis, tended to increase above control levels.

At the end of the drought period, Nitrogen and Calcium concentrations in the stems of treated plants were slightly higher than controls and P and K slightly lower. All these differences were however very small.

At the end of the drought period, the concentrations of N, P and K in roots of treated plants were higher than control levels and the concentration of Ca was lower. During recovery there was a tendency

Fig. 37 The effect of treatment on the change with time of nitrogen per cent area for the leaves, and nitrogen per cent dry weight for the leaves, stem and roots.

FIGURE 37

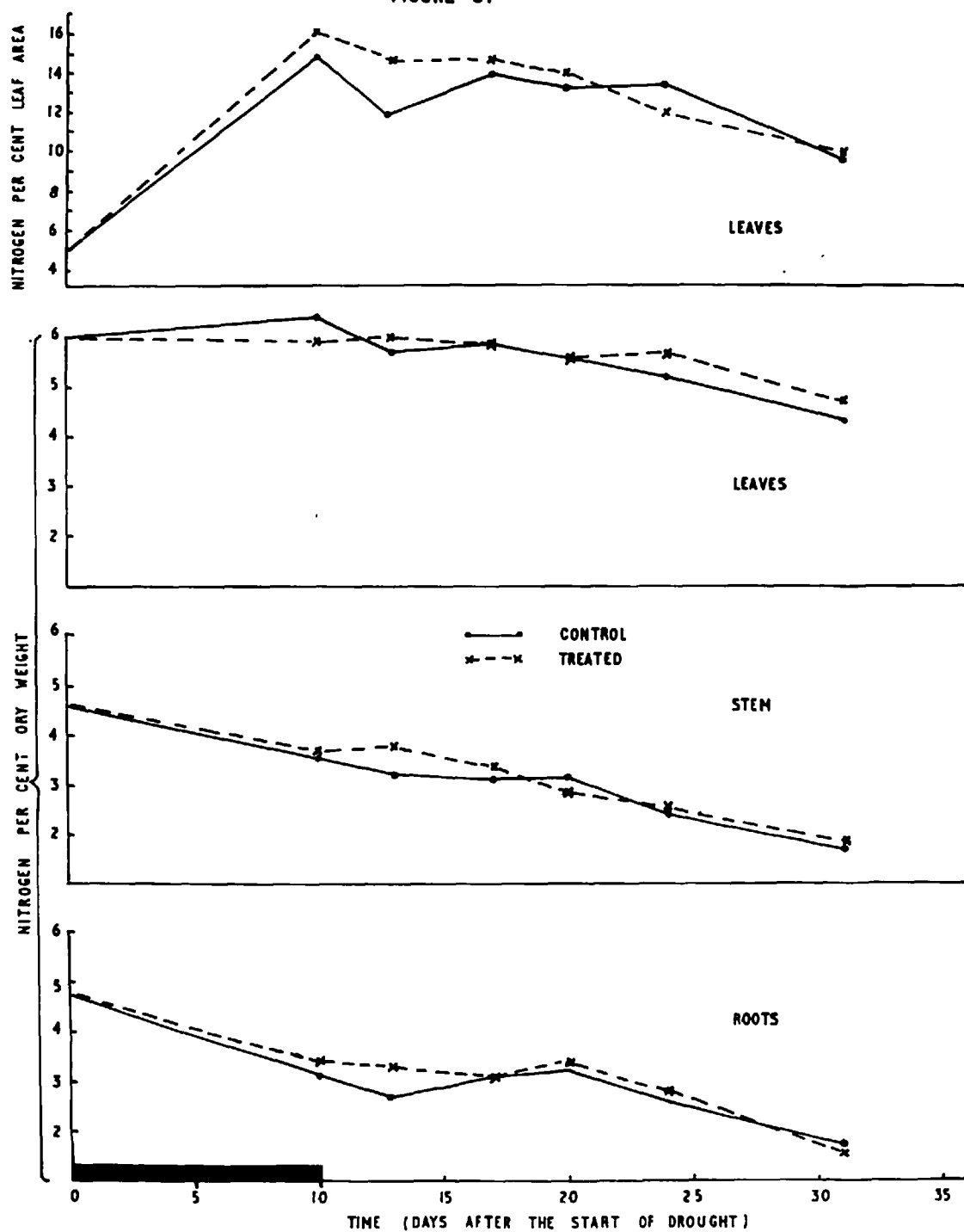


Fig. 38 The effect of treatment on the change with time of phosphorus per cent area for the leaves, and phosphorus per cent dry weight for the leaves, stem and roots.

FIGURE 38

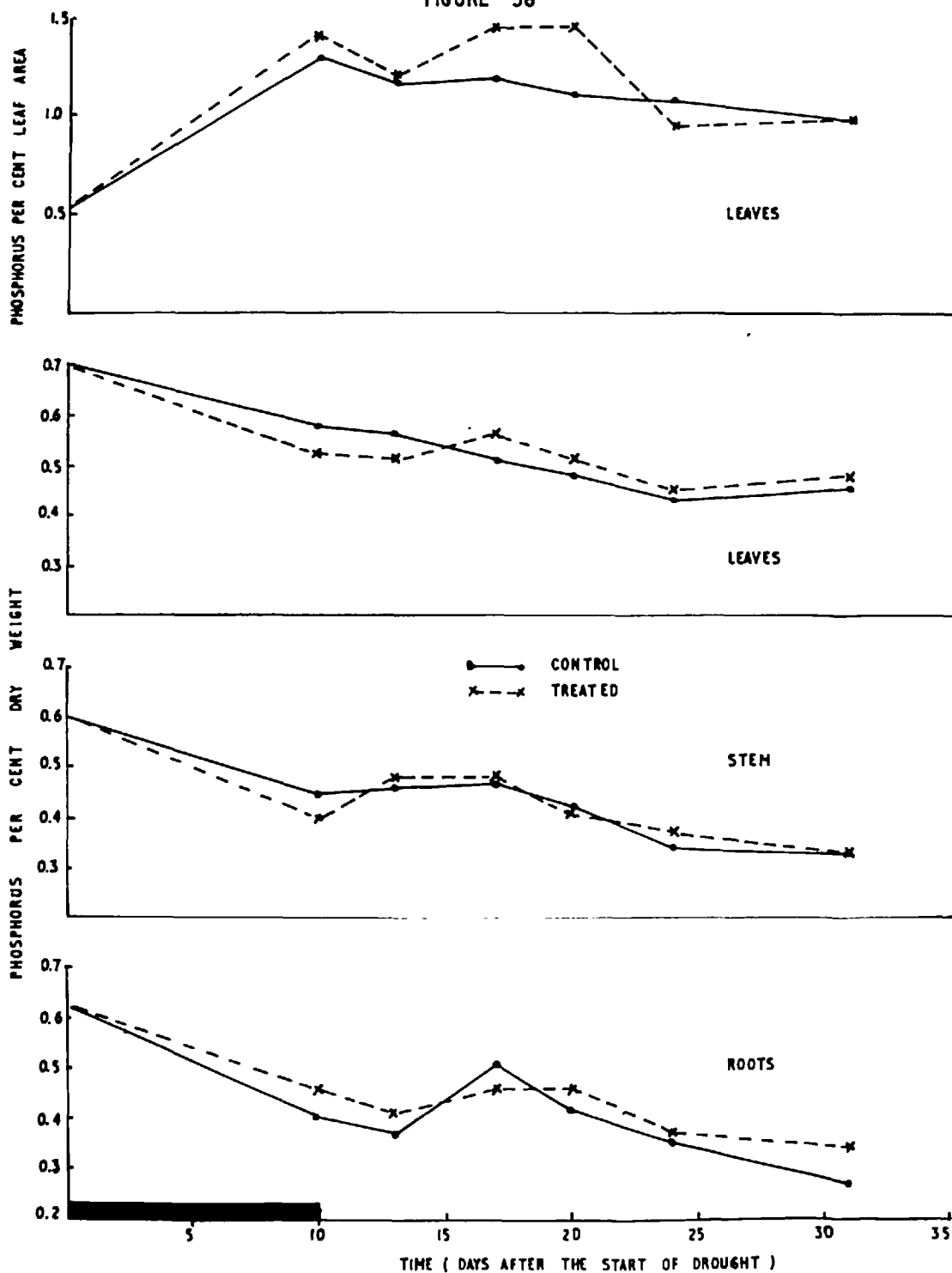


Fig. 39 The effect of treatment on the change with time of potassium per cent area for the leaves, and potassium per cent dry weight for the leaves, stem and roots.

FIGURE 39

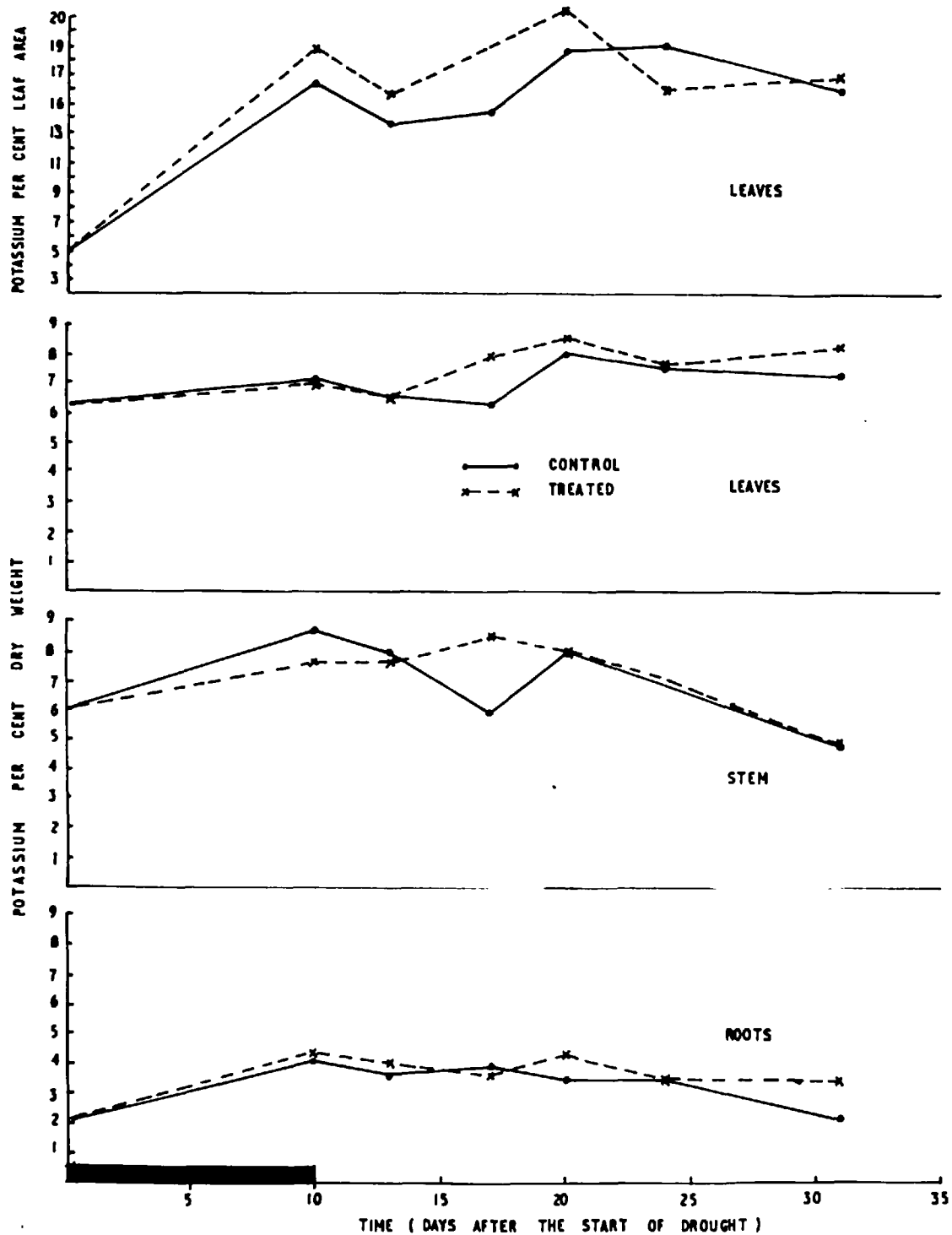
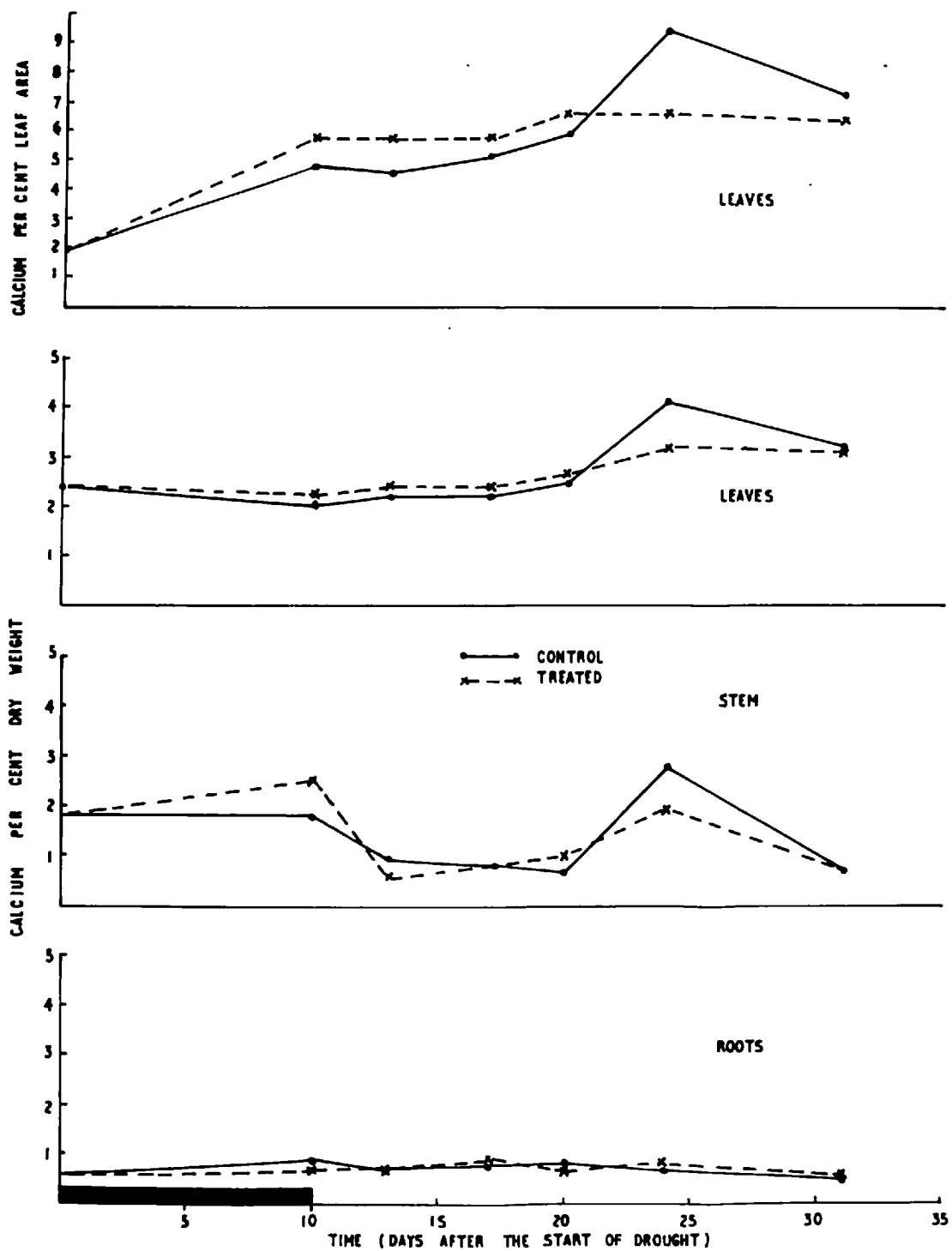


Fig. 40 The effect of treatment on the change with time of calcium per cent area for leaves, and calcium per cent dry weight for the leaves, stem and roots.

FIGURE 40



for concentrations of N, P and K in treated roots to be a little higher than in control roots, while the concentration of Ca was approximately the same in roots of control and treated plants.

The concentrations of nutrients per leaf area in leaves corresponding to each of the groups of four leaves already described for cell number (Fig 30) were plotted against time in Figs. 41, 42, 43 and 44. Leaves of treated plants had initially higher concentrations of all nutrients (mainly N and K) than controls. However, at later stages of development, the concentrations of nutrients in both treatments were approximately the same or lower in treated than control plants.

Stomatal conductance

The stomatal response of treated and control plants in terms of relative conductance, (i.e. time^{-1}) was measured during the drought and recovery periods on young leaves (8th, 9th and 10th leaf) and old leaves (5th, 6th and 7th leaf) (Figs. 45 and 46.) Conductances of the upper surface were low and contributed little to the total conductance of the leaf. Each leaf of each treatment was measured on two plants. During drought the upper stomata of the leaves of treated plants appeared to be more sensitive to water stress as they closed first. The situation is however confused because the stomata of both treated and control plants were closed on the 9th and 10th days, which were overcast. Restriction of stomatal opening on the lower surfaces did not appear until the 9th and 10th days of treatment.

Fig. 41 The effect of treatment on the change with time of nitrogen per cent leaf area for successive leaf groups.

FIGURE 41

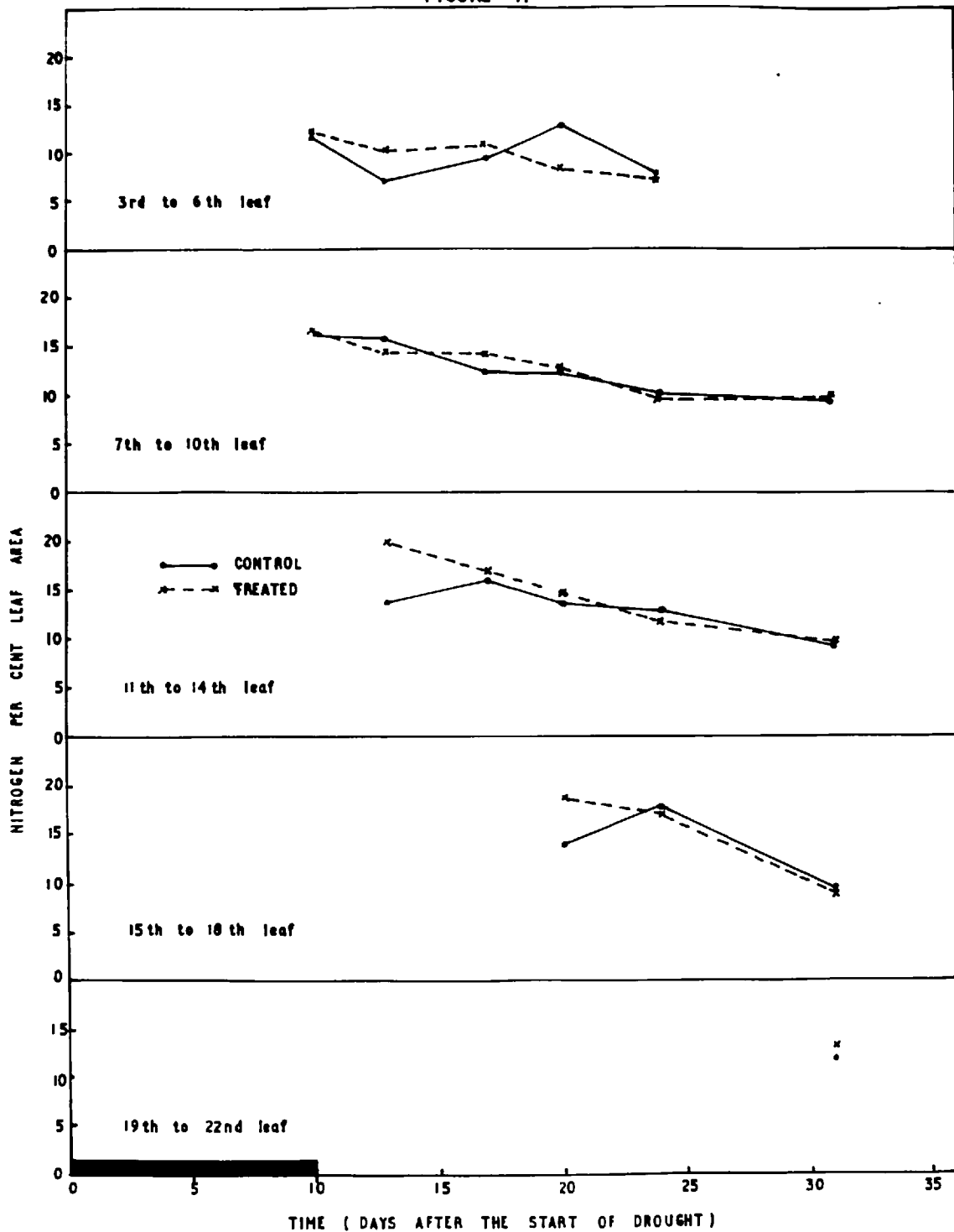


Fig. 42 The effect of treatment on the change with time of phosphorus percent leaf area for successive leaf groups.

FIGURE 42

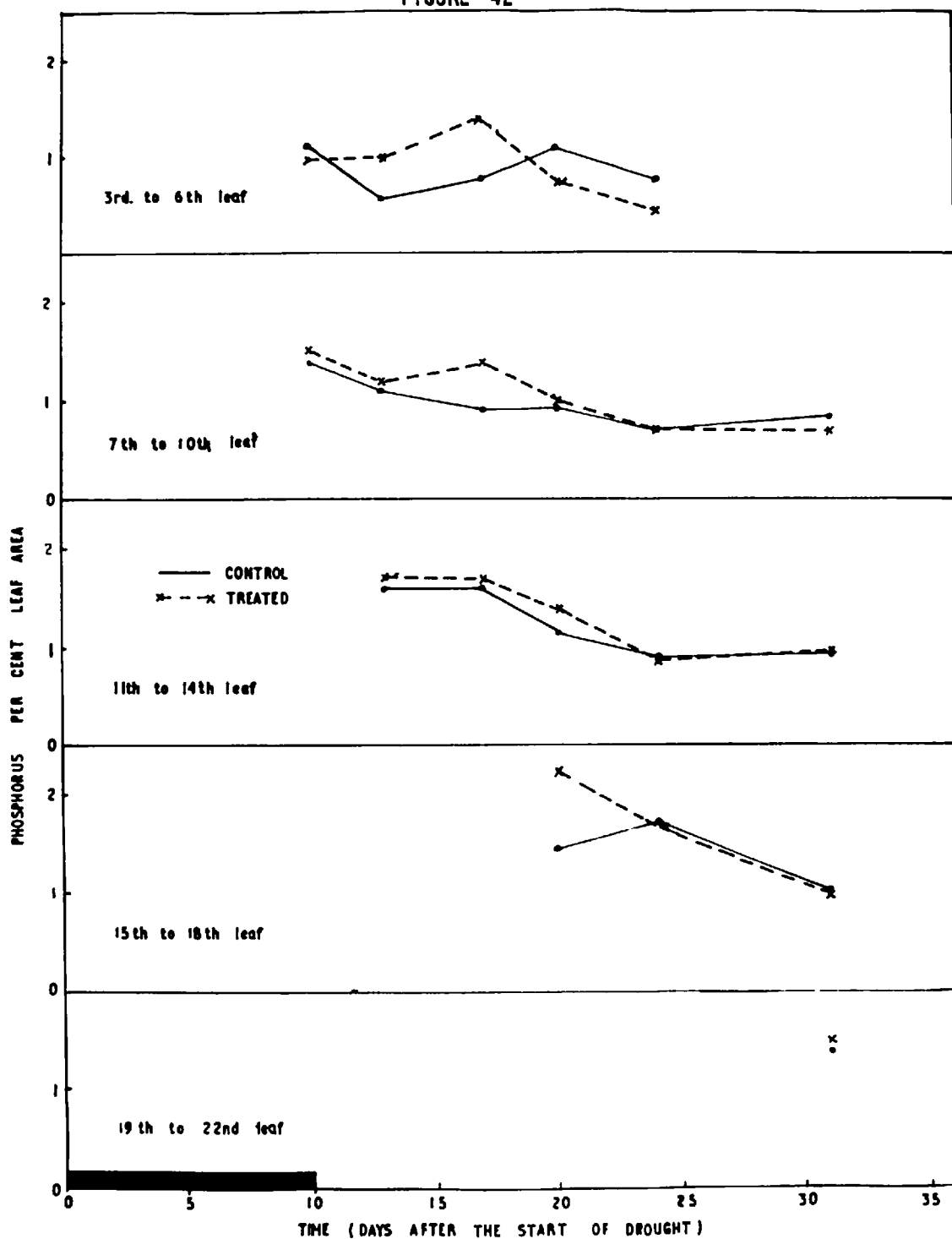


Fig. 43 The effect of treatment on the change with time of potassium percent leaf area for successive leaf groups.

FIGURE 43

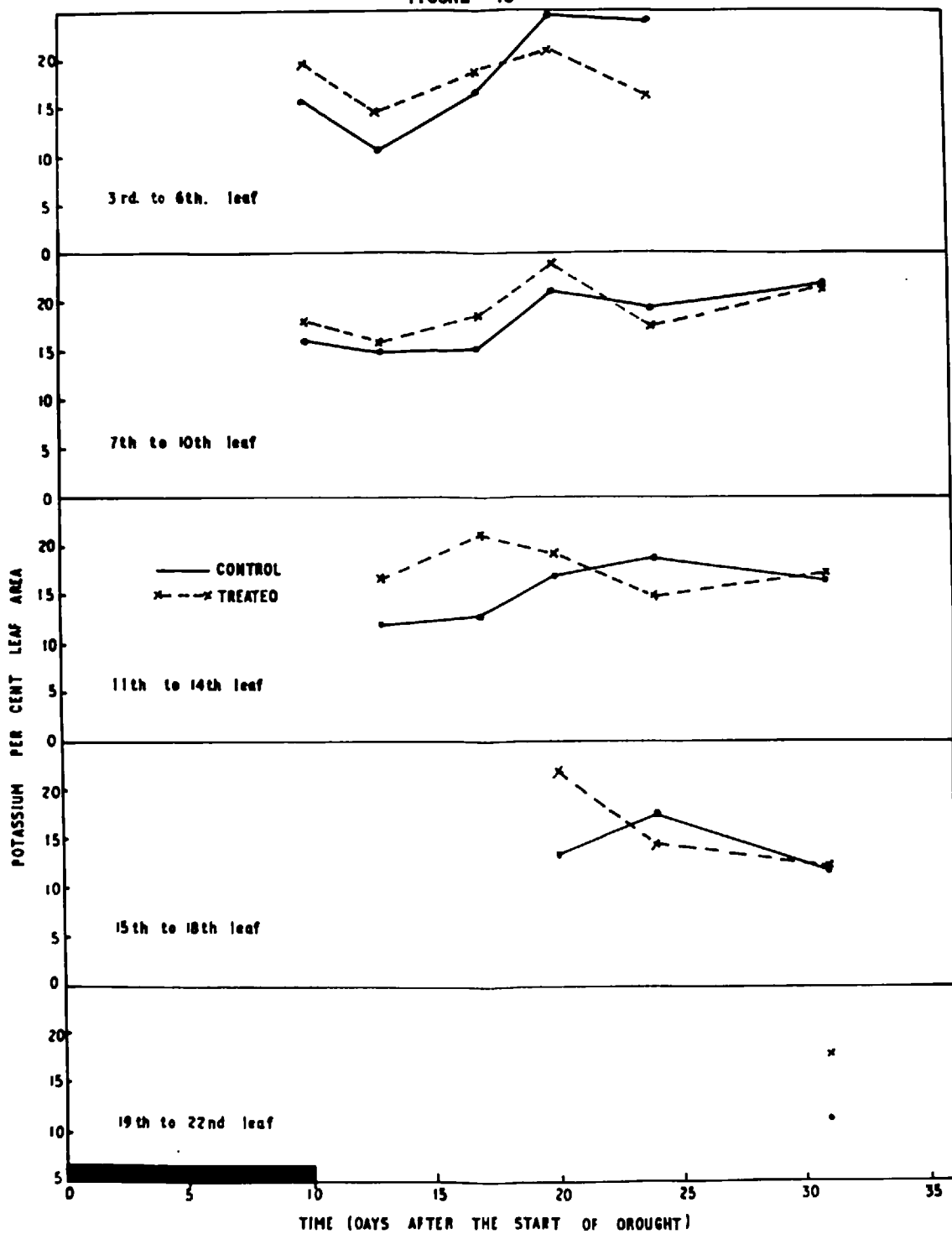


Fig. 44 The effect of treatment on the change with time of calcium percent leaf area for successive leaf groups.

FIGURE 44

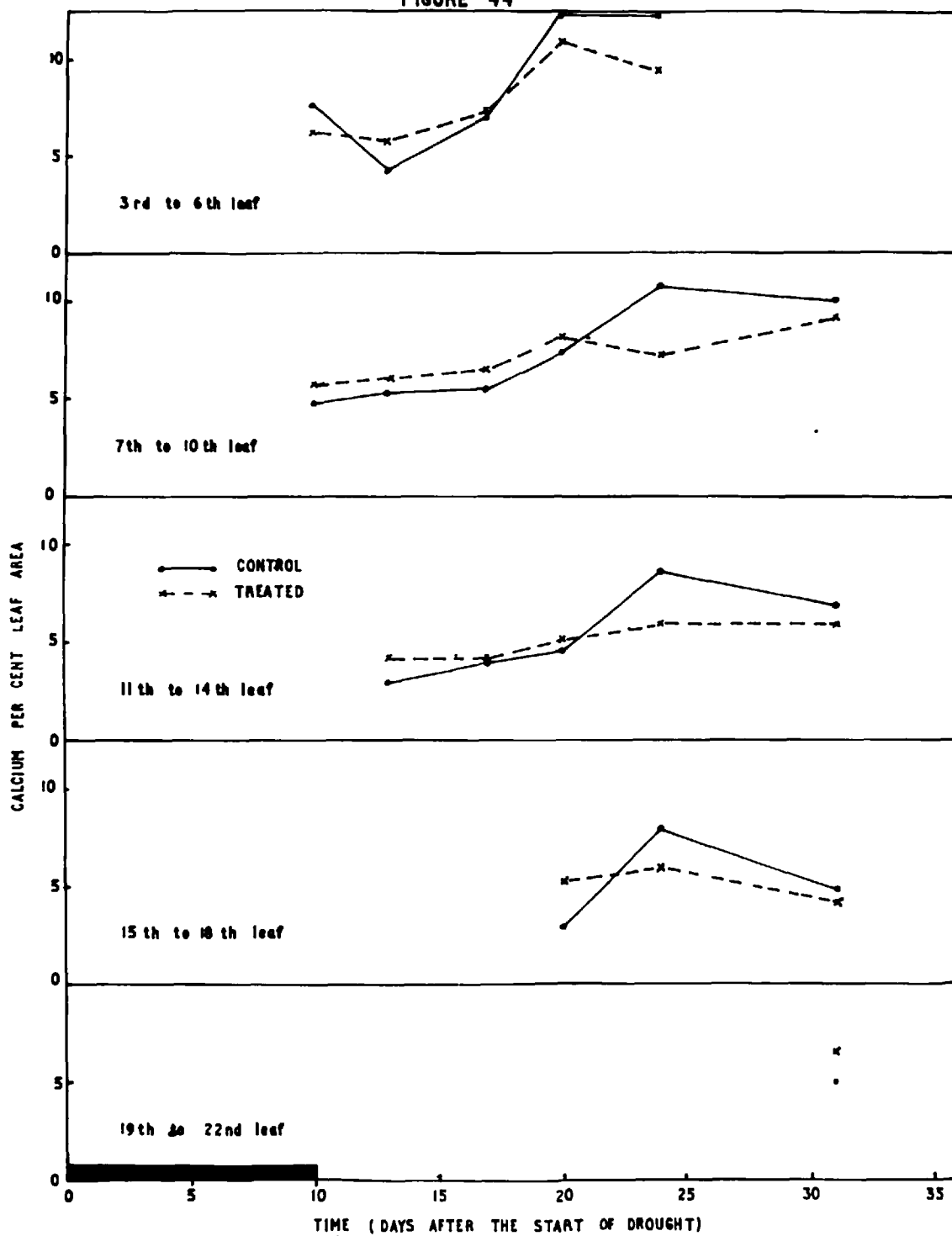


Fig. 45 The effect of treatment on the change with time of stomatal conductance for the upper, lower and both sides of young leaves.

FIGURE 45

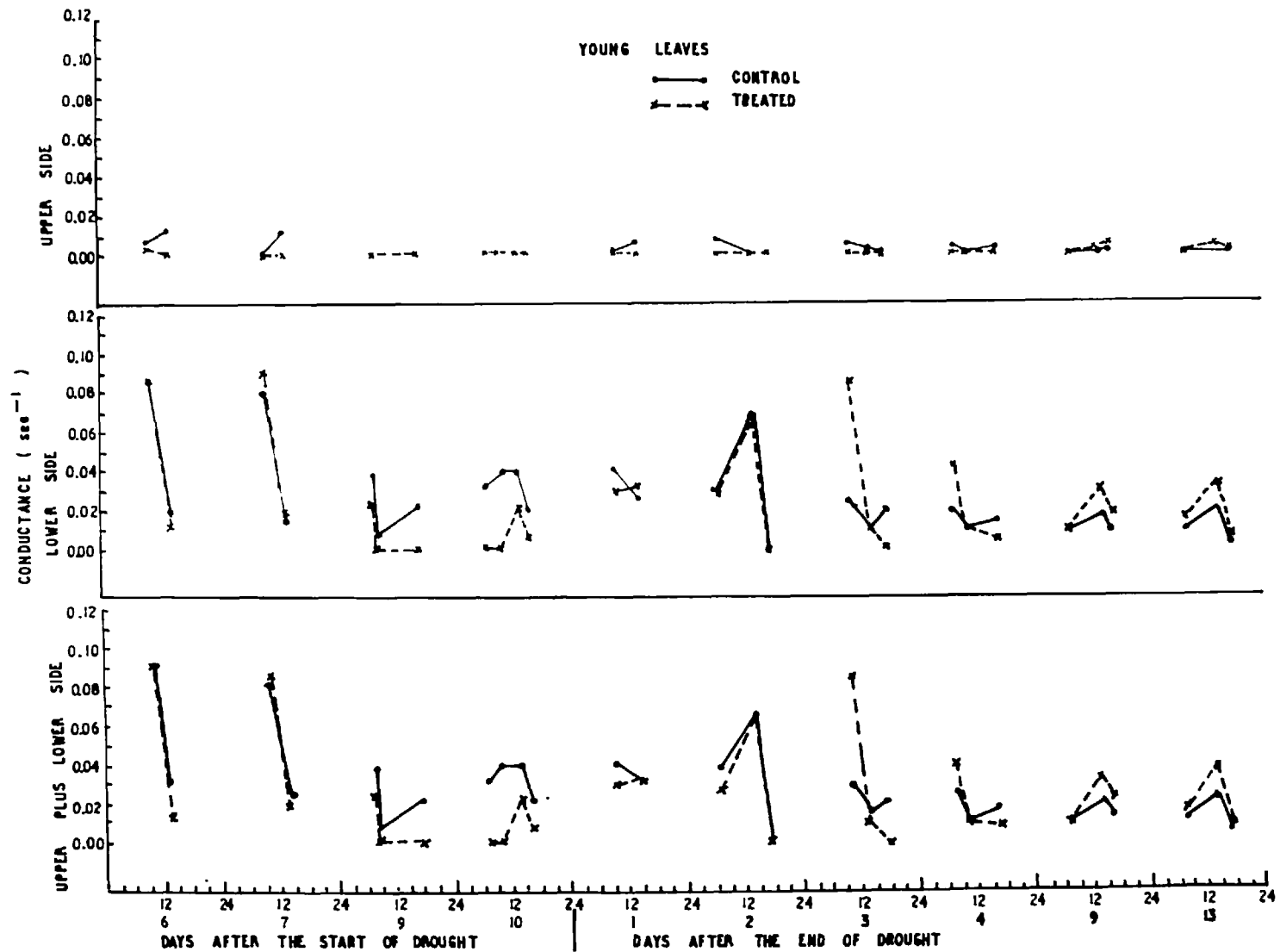
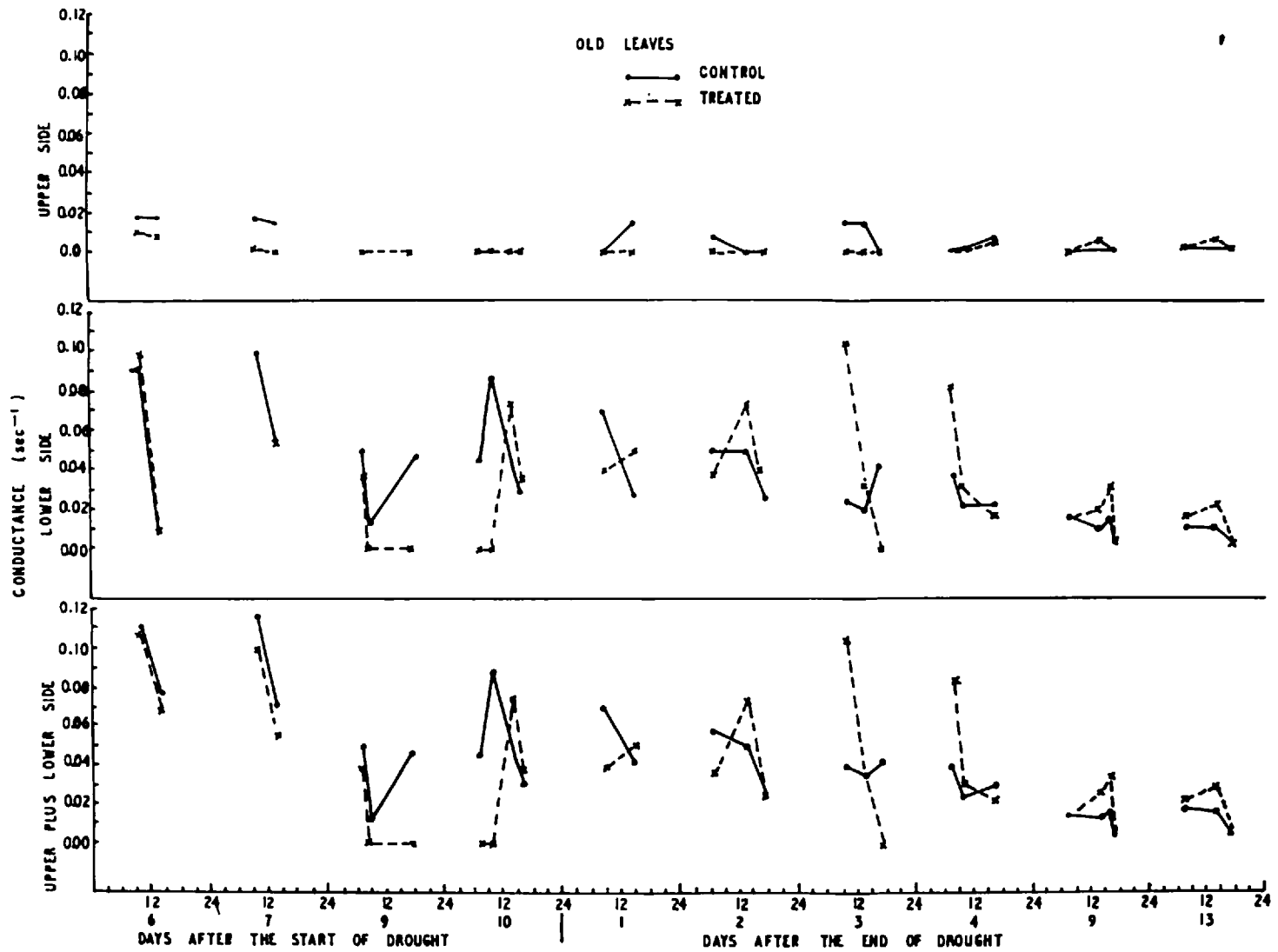


Fig. 46 The effect of treatment on the change with time of stomatal conductance for the upper, lower and both sides of old leaves.

FIGURE 46



One day after the end of the drought period the lower stomata of both young and old leaves of treated plants opened but the upper stomata of treated plants remained closed. Conductance in the middle of the day was higher in young and old leaves of control plants than conductance of leaves of treated plants on this day. Subsequently conductance of the leaves of treated plants tended to rise appreciably above the controls in the middle of the day but was no higher, or even lower, at the beginning and end of the day.

Higher conductances of treated plants during recovery may have been due to a higher number of stomata per unit leaf area and not to wider opening of stomata.

In general drought tended to close the stomata - almost completely - by the end of the drought period. Subsequently at most times, stomatal conductance per leaf of treated plants, tended to be higher.

Transpiration

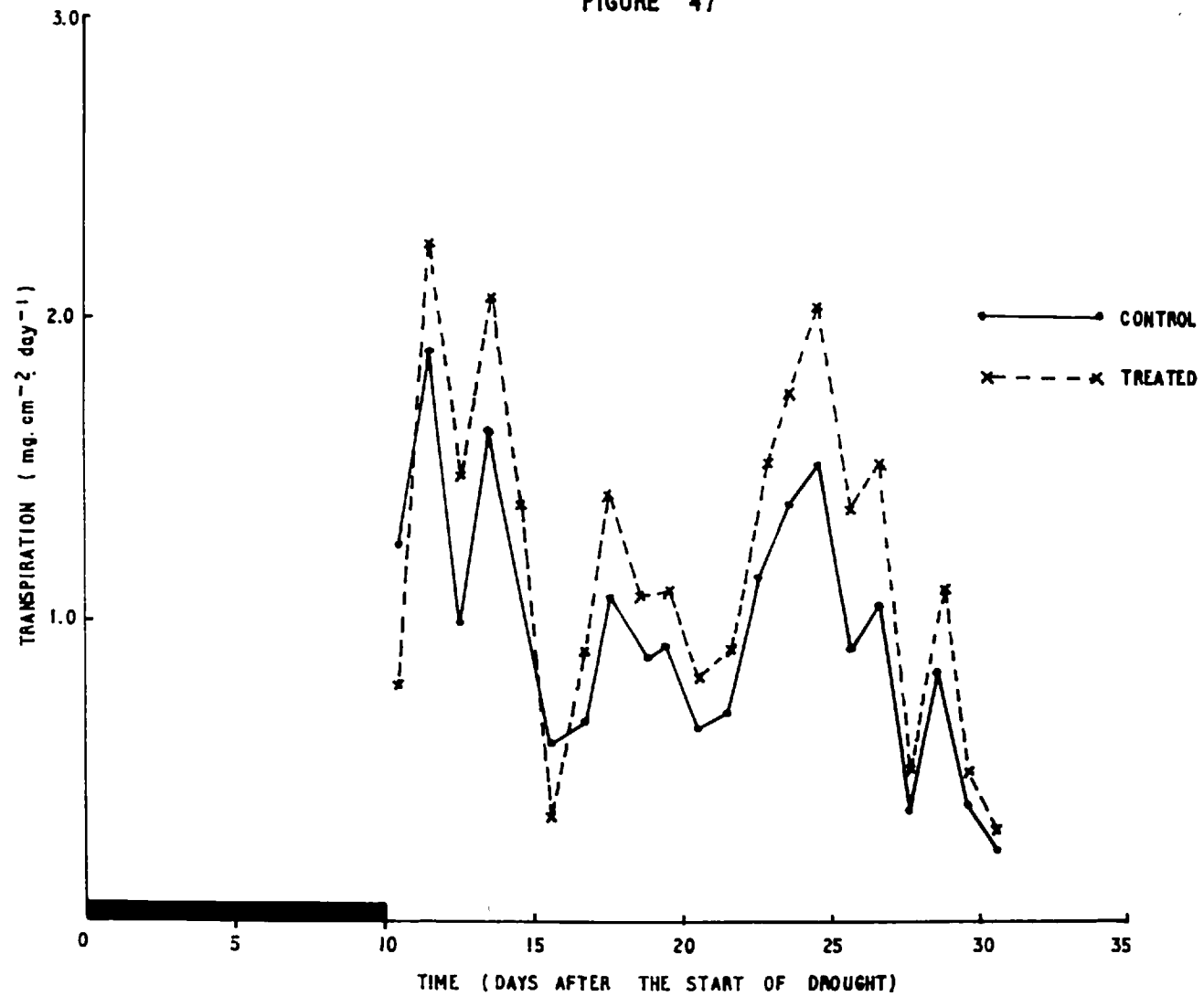
Transpiration was determined during the recovery period by weighing, the pots being covered with a layer 2.5 cm thick of expanded polystyrene to avoid evaporation from the soil surface.

Each point on Fig. 47 is the mean of four determinations carried out on the same plants throughout the experiment and left for the last harvest.

The transpiration rate of treated plants was lower than that of controls on the first day of re-watering, but subsequently was higher.

Fig. 47 The effect of treatment on the change of transpiration rate with time.

FIGURE 47



Experiment IV

In experiment III it proved impossible, with the short interval between harvests, to make a detailed study of water relations including leaf water potential, along with the study of plant and cell growth. Experiment IV was therefore designed specifically to study water relations. As well as leaf water potential a more detailed study of stomatal conductance was intended, but damage to the porometer made this impossible. When plants were 14 days old, two treatments were applied, half of the plants were freely watered (control), and water was withheld from the other half for nine days (treated). The duration of the drought was enough to produce wilting throughout the last day of treatment. Following the drought period water was applied freely each day to both treatments.

Plants were grown singly, in pots and randomized between treatments before treatment started. Daily changes in leaf water potential were followed during and after the drought period. Leaf water potential was determined six times per day on a different plant at each time. Three of these plants per treatment were harvested for determinations of dry weight and leaf area at 7, 10, 17 and 24 days after start of drought. Leaf water potential was determined at the following times: 7.30, 10.00, 13.00, 16.00, 19.00 and 22.00 hours at 7, 9, 10, 17 and 24 days after start of drought, on the 5th, 6th, 7th and 8th leaves of each plant. On the last day of the drought period (i.e. 9th day

after start of drought) observations of leaf water potential were carried out at 10.00, 13.00 and 16.00 hours only on the above mentioned leaves.

Growth analysis

The effect of the drought treatment was approximately as in experiment III; at one day after the drought period finished treated plants were 59% of the weight of controls. During the whole recovery period the two sets of plants had similar relative growth rates and the ratio of treated to control plants remained constant (Fig. 48).

At one day after the drought period finished, leaf area of treated plants was also 59% of the controls (Fig. 49). In the case of leaf area the relative leaf area growth rate of treated plants was higher than that of controls after re-watering and at the last harvest the difference in leaf area was smaller but still significant, and treated plants were 74% of controls.

Points of leaf area and dry weight were joined by lines and these curves were not fitted by computer.

Specific leaf area of treated plants was reduced significantly by one day after re-watering, however it rose significantly above the control six days later and remained so until the end of the experiment (Fig. 50).

In general, the growth results of this experiment resemble those of experiment III. In this experiment also a higher specific leaf

Fig. 48 The effect of treatment on the change in natural logarithm of observed value of dry weight with time.

FIGURE 48

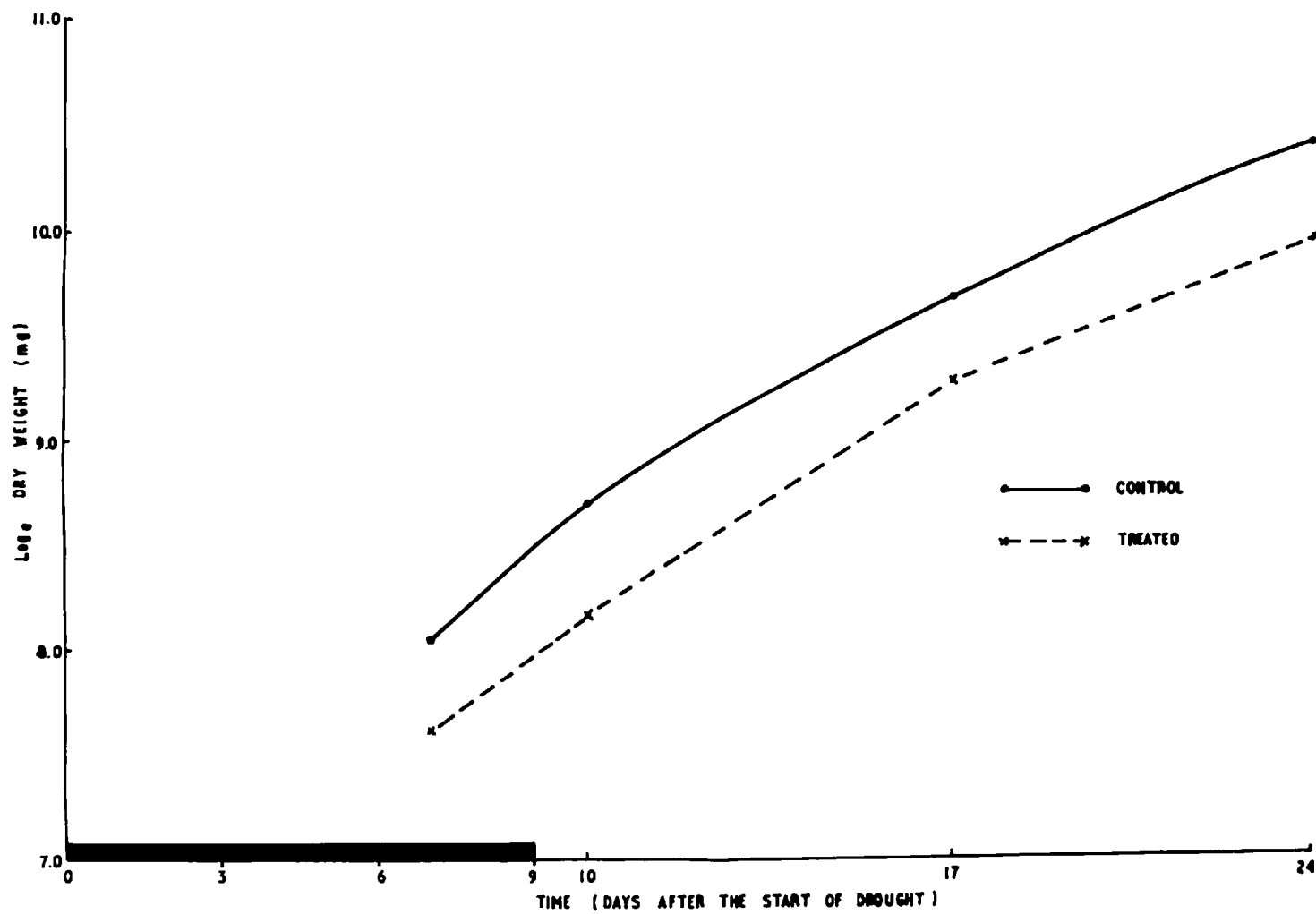


Fig. 49 The effect of treatment on the change in the natural logarithm of observed value of leaf area with time. The vertical bar refers to the least significant difference at $P = 0.05$

FIGURE 49

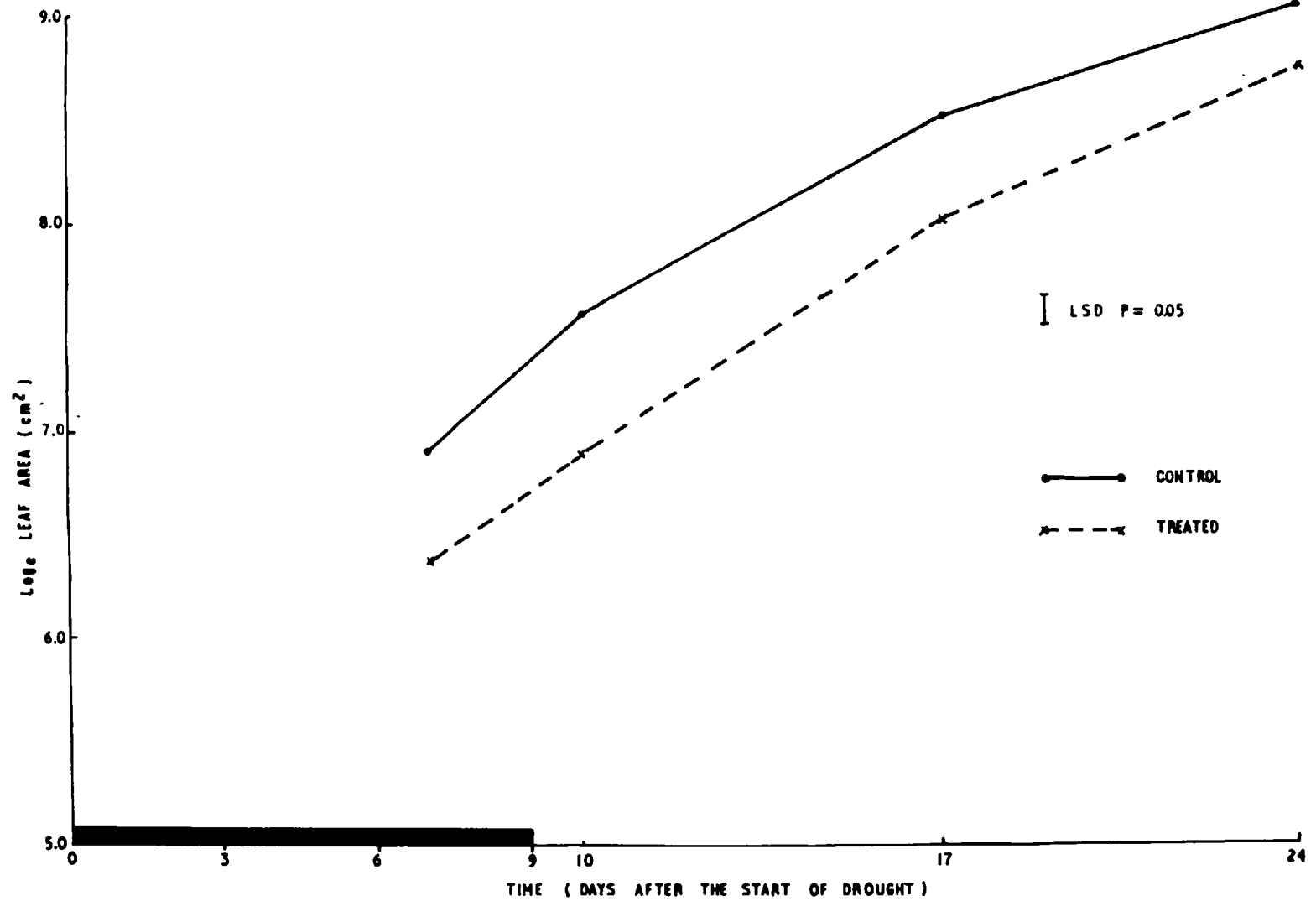
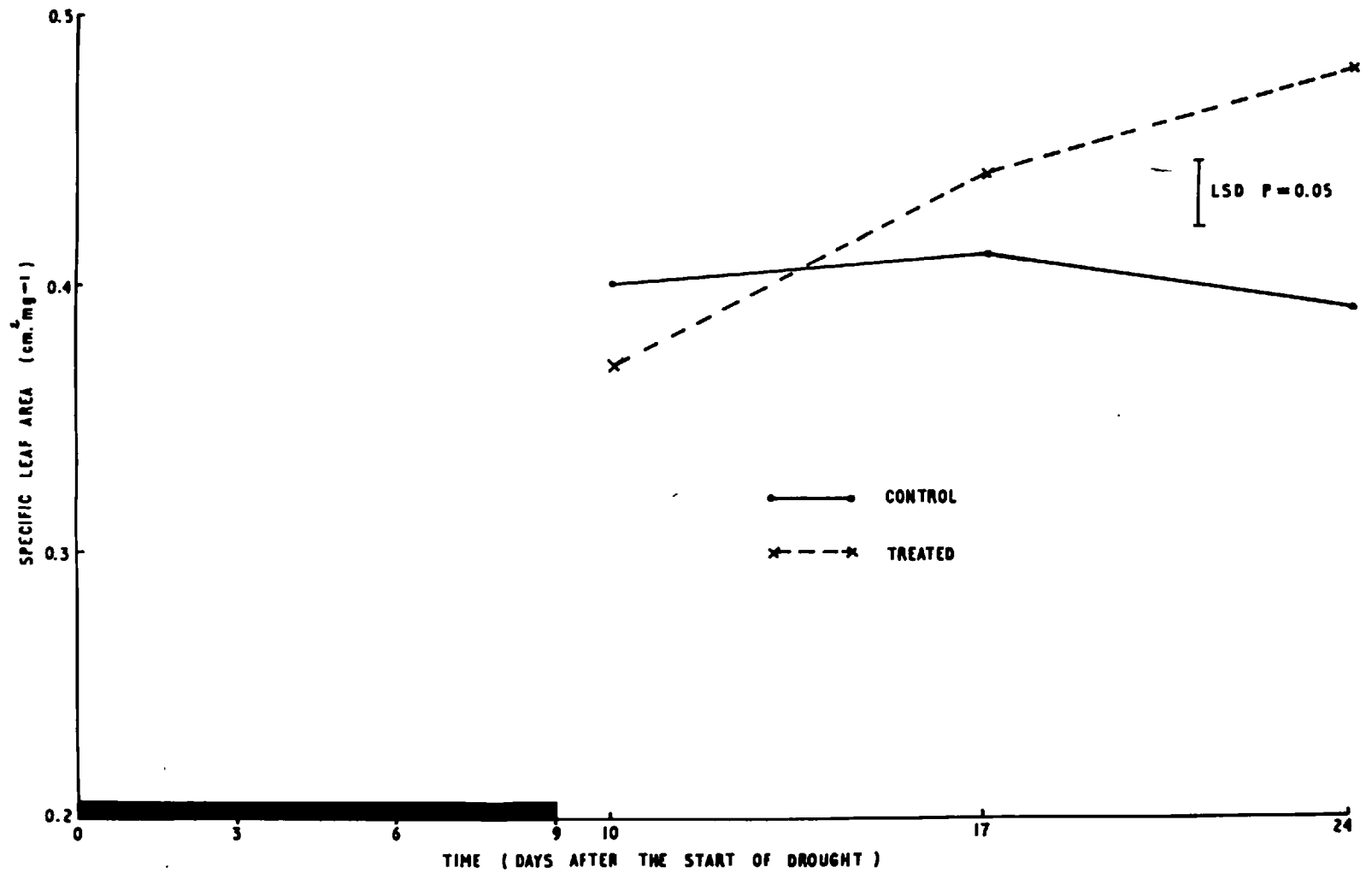


Fig. 50 The effect of treatment on the change in specific leaf area with time. The vertical bar refers to the least significant difference at $P = 0.05$

FIGURE 50



area contributed to a greater leaf growth.

Leaf water potential

During the drought period (i.e. seven days after start of drought) as water became less easily available leaf water potential reached lower values in treated than control plants. This difference was more pronounced at noon. However, at evening the values of leaf water potential of treated plants increased and they showed a tendency to reach similar values to the control plants (Fig. 51a). On the last day of drought, values of leaf water potential of treated plants at 10.00, 13.00 and 16.00 hours were much lower than at seven days (Fig. 51b).

During recovery the response of treated plants was immediate; one day after water was restored values of leaf water potential of treated plants were at any time of day higher than those of controls (Fig. 52). Later during recovery (i.e. 8 and 15 days after the end of drought) there was an evident tendency of values of leaf water potential of treated plants to be higher than the controls (Fig. 53) by 1-2 atm. The greatest differences between values of leaf water potential of treated and control plants were achieved about 17.00 hours.

Transpiration

Transpiration was determined by weighing, the pots being covered with a layer 2.5 cm thick of expanded polystyrene. Each point on Fig. 54 is the mean of three determinations carried out on the same plants throughout the experiment and left for the last harvest.

Fig. 51 The effect of treatment on the variation of leaf water potential (Ψ) with time of day at seven and nine days after the start of drought.

FIGURE 51

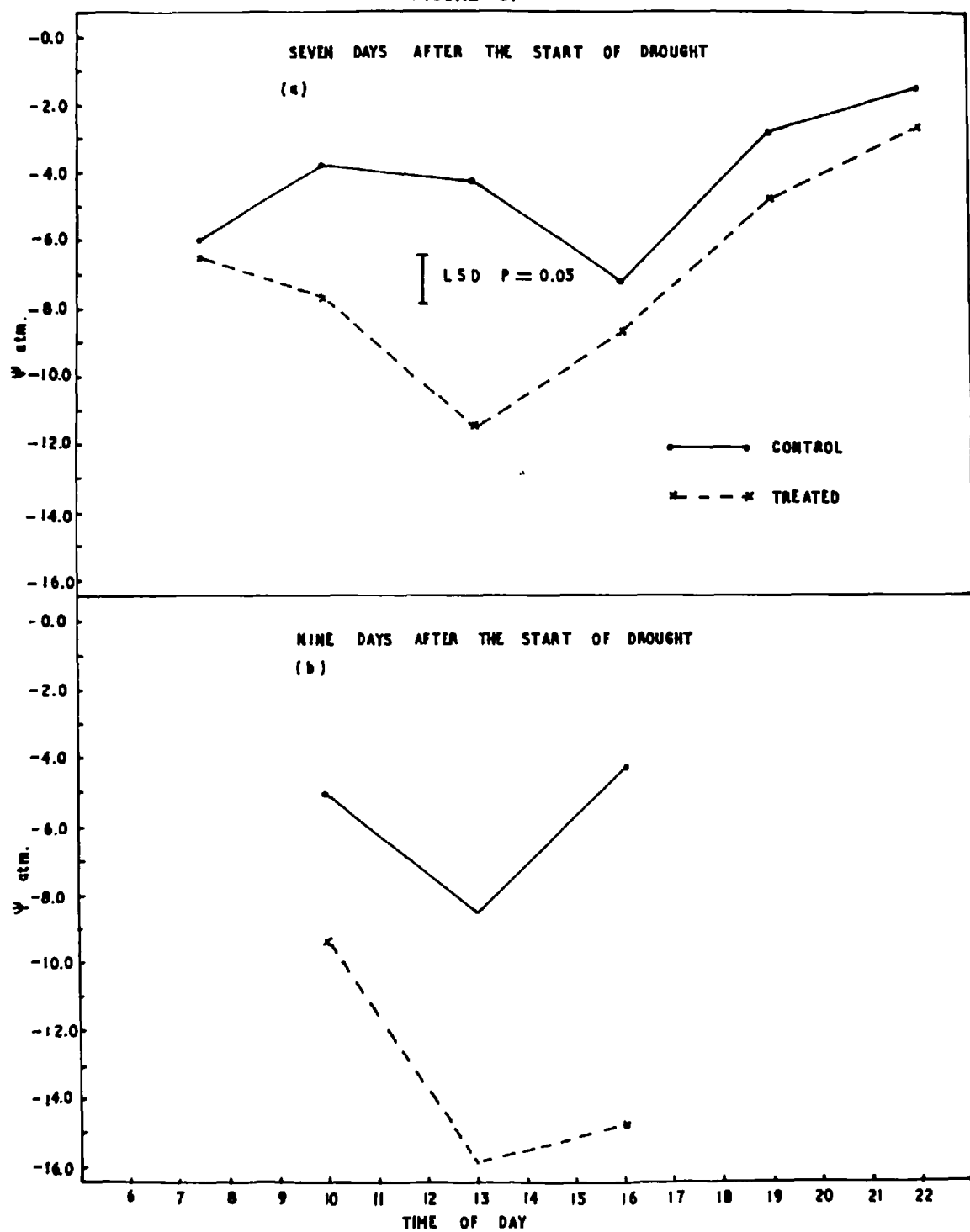


Fig. 52 The effect of treatment on the variation of leaf water potential (ψ) with time of day at one day after the end of drought

FIGURE 52

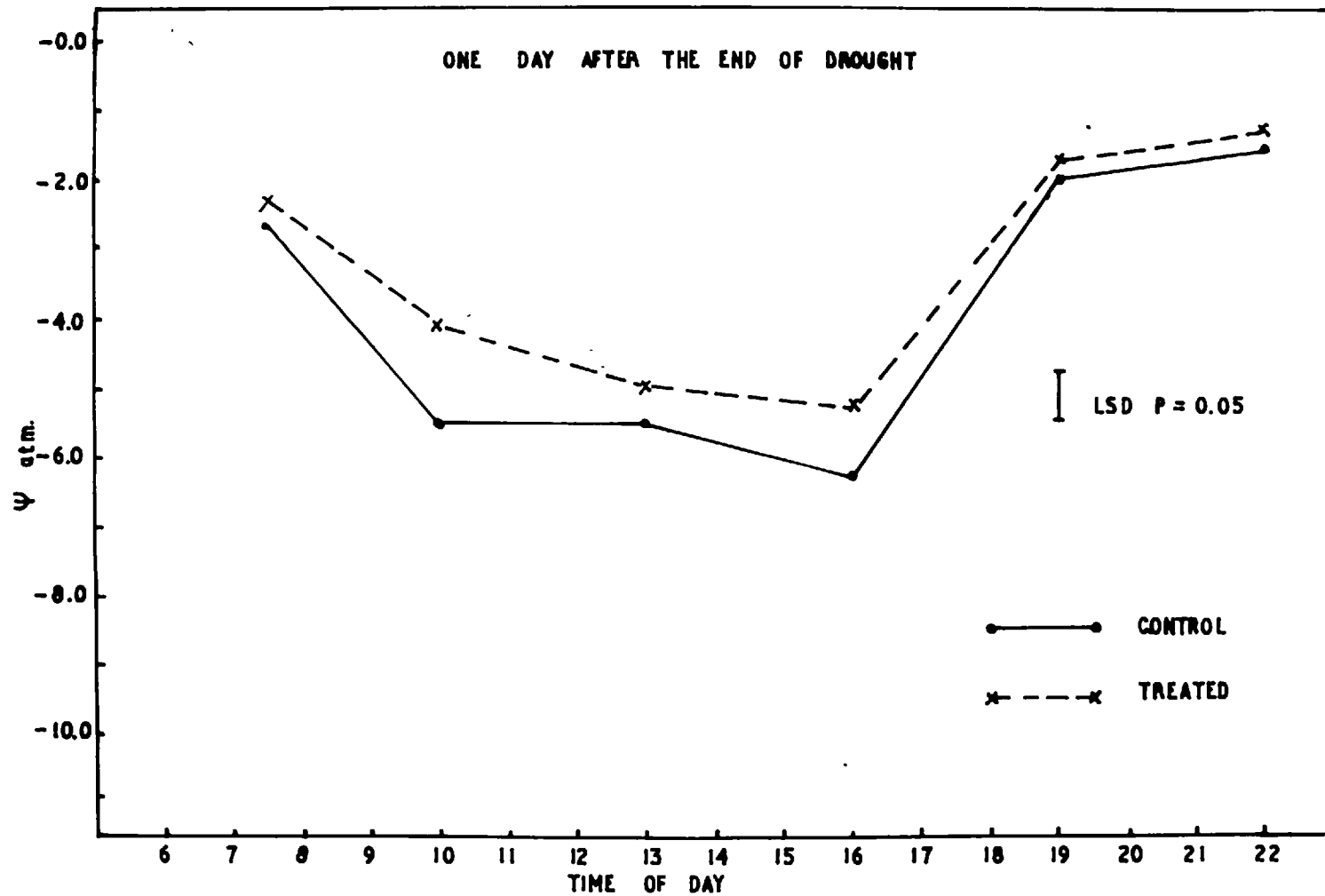


Fig. 53 The effect of treatment on the variation of leaf water potential (ψ) with time of day at eight and fifteen days after the end of drought.

FIGURE 53

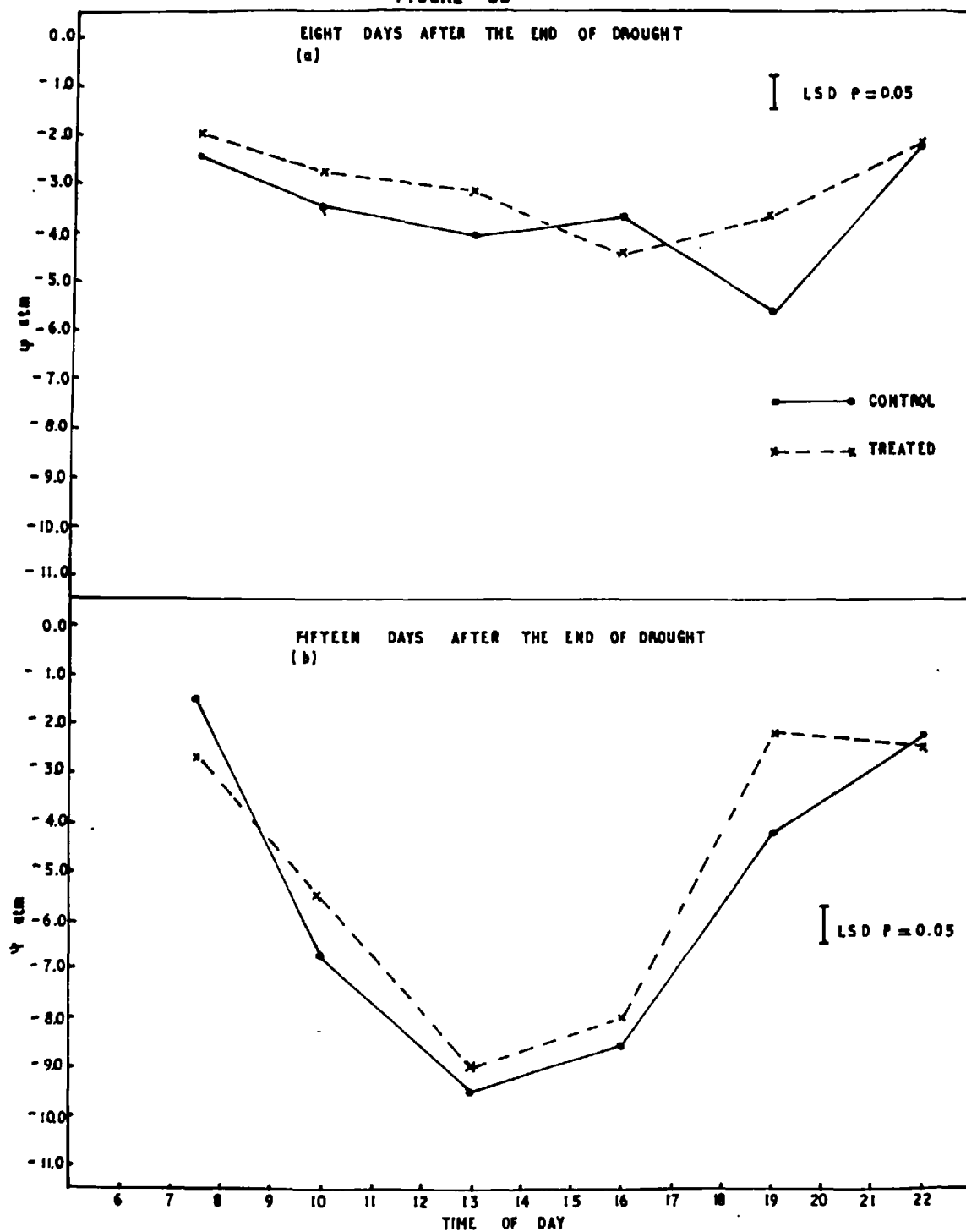
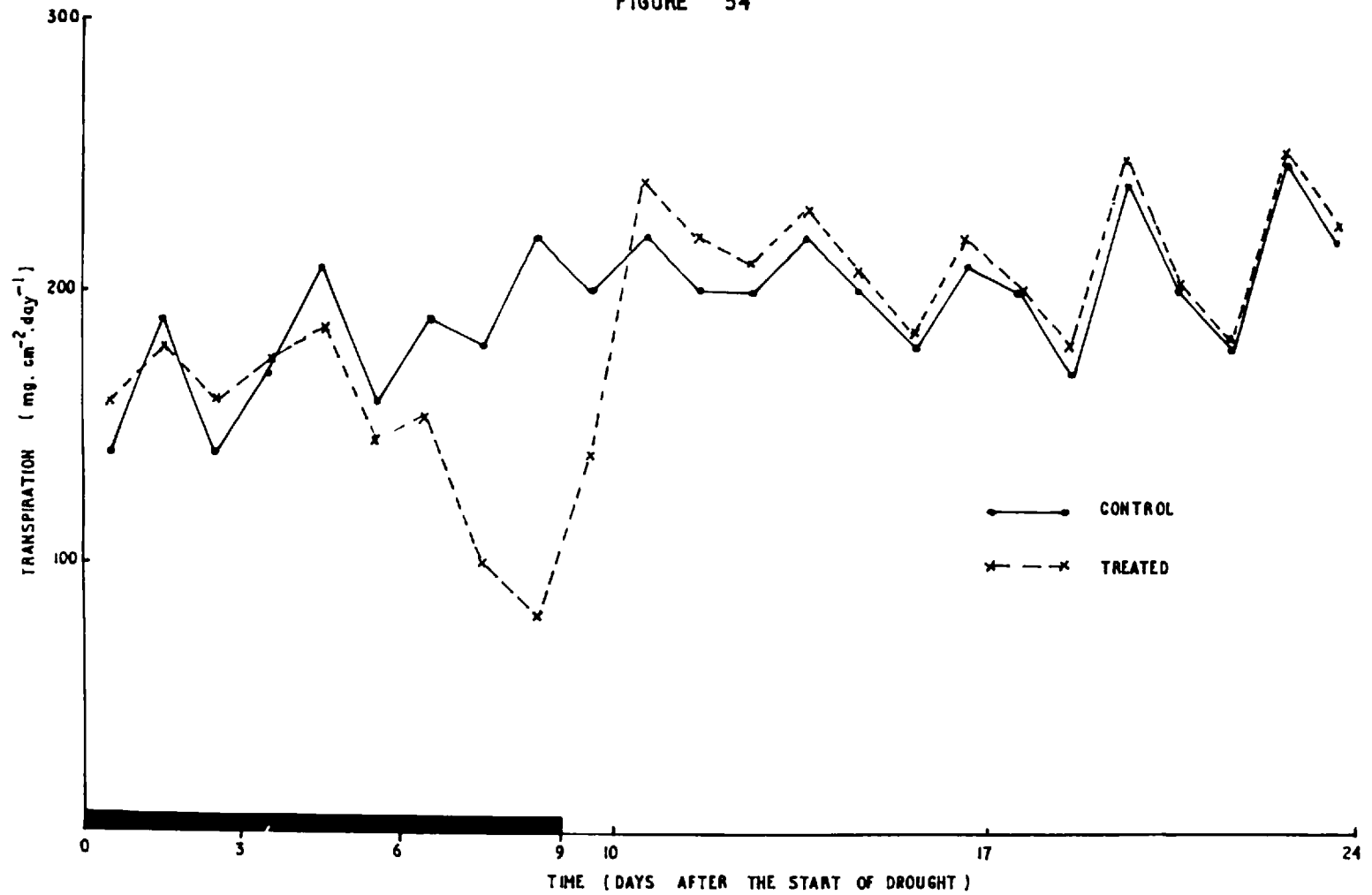


Fig. 54 The effect of treatment on the change of transpiration rate with time.

FIGURE 54



Transpiration was again depressed during drought in treated plants. After water was restored, treated plants showed a slightly higher rate of transpiration throughout the rest of the experiment.

Conclusion

Growth of treated plants resembled that in experiment III. Leaf water potential of treated plants was depressed during treatment but rose during recovery to higher values than in the controls.

Discussion

A common feature of all the experiments was a greater relative leaf area growth rate (R_A) in plants recovering from a period of drought than in freely watered controls. This response was seen not only in tobacco, but also in experiment I in sugar beet and cucumber. In experiment II the response in the droughted plants was sufficient to restore leaf area to the same value as the controls by the end of the experiment, but complete recovery of leaf area was not achieved in experiment III. In experiment II leaves just unfolding by the end of the treatment or subsequently i.e. 8-10 and 11-22 respectively, attained by the end of the experiment a similar or larger leaf area than equivalent control leaves (Fig. 18). However, in experiment III only leaves unfolded after the treatment finished (i.e. 15-25) had by the end of the experiment approximately the same area as those of controls (Fig. 29). In experiment II leaves of treated plants which unfolded before the treatment started and expanding during the treatment (i.e. 4-6) and leaves of treated plants in experiment III unfolded before treatment started or unfolding during the treatment, i.e. 5-8 and 9-13 respectively, had by the end of the experiments smaller area than equivalent control leaves. It appears that a larger number of leaves attained similar or larger size than equivalent controls in experiment II than experiment III (i.e. 14 and 10 respectively). Treated and control plants had at the beginning of experiments II and III, 6 and 8 unfolded leaves respectively and by the end of the

experiments the total numbers of unfolded leaves were 18 and 21.5 respectively. The number of leaves unfolded between the beginning and the end of the experiment was slightly higher in experiment III than II, i.e. 13.5 and 12 respectively. It appears that for a complete recovery of leaf area, the area attained by individual leaves is more important than the number of leaves produced.

If unit leaf rate (E_A) was not affected during the recovery period then the greater R_A should lead to a greater relative growth rate (R_W). This was observed in both experiments I and II. In experiment I values of R_W calculated between Harvest I and II (Table 8) showed that there was little difference between R_W of plants with or without the final treatment, i.e. D or W, however, the more pre-treatments the plants received the larger was the R_W subsequently attained with the exception of cucumber plants pretreated once and not pretreated, of which the former had smaller R_W .

In experiment III however, an apparent, though not significant, reduction in E_A during the recovery period counteracted the increased R_A and there was no difference in R_W between treated and control plants.

Previous experiments reported in the literature for different species, on growth analyses during recovery from drought have suggested that the accelerated growth during recovery was due to a combined effect of unit leaf rate and leaf area. Thus Owen and Watson (1956)

Table 8

R_W ($\text{mg mg}^{-1} \text{ day}^{-1}$) of cucumber, sugar beet and tobacco plants

	<u>Cucumber</u>		
	P_0	P_1	P_2
Watered (W)	0.012	0.011	0.023
Droughted (D)	0.012	0.010	0.022
Mean	0.012	0.011	0.023

	<u>Sugar beet</u>		
	P_0	P_1	P_2
Watered (W)	0.014	0.016	0.020
Droughted (D)	0.014	0.016	0.018
Mean	0.014	0.016	0.019

	<u>Tobacco</u>		
	P_0	P_1	P_2
Watered (W)	0.029	0.034	0.038
Droughted (D)	0.029	0.035	0.036
Mean	0.029	0.035	0.037

found in field conditions that during recovery, sugar beet plants had a larger unit leaf rate and leaf growth than plants that had never been subjected to water stress. Hopkinson (1968) found in tobacco in glasshouse conditions, a larger E_A of water stressed plants only three days after recovery started and also a significant increase of R_A during recovery in relation to non stressed plants. Gates (1955a) found that tomato plants in glasshouse conditions, which had been subjected to water stress had a significantly higher E_W after 7-8 days of free watering, than non stressed plants. However, Gates did not measure leaf area and his E is in terms of leaf weight and not leaf area, and so not completely comparable with the experiments mentioned above.

In further analysis of the present experiments attention will be concentrated on leaf growth since there were no significant changes in E_A except during the period of drought. It should be noted however, that the errors of E_A were fairly high and it can not be concluded that treated and control plants were exactly the same in this respect during the recovery period.

The higher R_A of treated plants during the recovery period is reflected in a higher leaf area ratio (Figs. 15 and 24) and when this was analysed further it was found to be due mainly to a higher specific leaf area. Leaf weight ratio was hardly affected by treatment. Leaf growth may be analysed further, firstly in terms of rate of

production of leaves, then of increase in the size of individual leaves, and finally in terms of cell division and cell extension.

Morton and Watson (1948) found that over a period of more than three months the rate of formation of new leaves was the same in sugar beet plants exposed to severe and recurrent drought as in watered plants. They explained the insensitivity of the apical meristem to drought by its capacity to withdraw water and carbohydrates from other parts of the plant and also because the internal concentration of Nitrogen was higher in plants exposed to drought than in fully watered plants. This explanation could be supported by recent studies by Wardlaw (1969), in which he found that the translocation pathway is capable of functioning efficiently when under water stress.

Gates (1955b) also could not detect any significant difference between numbers of unfolded leaves of control and droughted tomato plants at either one or fifteen days after the end of the drought. However, Gates (1968) using Lupinus albus as an experimental plant, found that the rate of initiation of primordia was very sensitive to moisture stress, even at early stages in the drying cycle. However, on the resumption of watering, primordia were produced more rapidly in the plants which had been droughted and consequently there was no permanent reduction in the number of unfolded leaves.

In experiment III an unusual acceleration of leaf primordia production was found during the drought period (Table 5). The unfolding of these additional leaves and the production of further

primordia was however delayed during the recovery period and the number of unfolded leaves was initially one or two less in treated than in control plants (Fig. 29). Ultimately (31 days after the start of drought) leaf number was the same in both sets of plants. Regarding unfolded leaves, a similar picture is found in Experiment II as already described for Experiment III, i.e. droughted plants had at the end of the drought period, one unfolded leaf less than the controls, but at 28 days after start of drought unfolded leaves were the same in both treatments (Fig. 18).

In experiment III, leaves of treated plants initiated during the drought period and unfolded subsequently, i.e. 16-23, and leaves initiated and unfolded during recovery, i.e. 24 and 25, had a faster rate of increase in area than equivalent leaves of controls and at 31 days after the start of drought they had the same area as equivalent leaves of controls (Fig. 29). In experiment II, leaves of treated plants just unfolded by the end of the treatment, i.e. 8 and 9, and leaves unfolded subsequently, i.e. 10-22, had a faster rate of increase in leaf size than equivalent leaves of controls and at 36 days after the start of drought they had a larger area than equivalent leaves of controls. In both experiments then, the accelerated leaf growth rate during the recovery period is seen mainly in leaves which are unfolded after the drought period ends. It appears that the higher R_A of treated plants is due in small part to a restoration of leaf number, but mainly to a higher rate of increase in area of

the younger leaves.

Leaf growth will now be further analysed in terms of cell number and cell size, on which detailed observations were made in experiment III. In order to do this, it is convenient first to refer to Table 5, in which the numbers of both primordial and unfolded (more than 1 cm²) leaves are recorded at various stages of growth. It will be seen that leaves 1-8 were unfolded, and the primordia of leaves 9-15 were differentiated, before treatment began. During treatment the primordia of leaves 16 to 23 were differentiated, and the last of those must have been differentiated at the end, and most severe stage, of the drought. Leaves initiated after the treatment finished (i.e. leaves 24 and 25) and unfolded 21 days later, had when unfolded the same area (Fig. 29), the same number of cells (Fig. 31) and cells of the same size (Fig. 32) as the controls. Leaves of treated plants initiated just before the treatment finished (i.e. 19-23) had when they were unfolded, 14 days later, cells of approximately the same size as control leaves but far fewer (about one tenth) cells and correspondingly smaller area. Seven days later a full recovery of cell number and leaf area was achieved. Leaves differentiated somewhat earlier in the treatment period and examined when they unfolded seven days later, had fewer cells and a smaller area than control leaves, although cell size was hardly affected. Subsequently the difference in cell numbers declined. The difference in cell size appeared first to increase and then to decrease again to zero,

but there must be doubt about the cell size of the control plants on day 24 in view of the apparent decrease in size between days 24 and 31. Final areas of these treated leaves were only slightly smaller than the controls. Leaves of treated plants differentiated just before treatment started (i.e. 11 to 14) had at 10 days after the start of drought both smaller numbers of cells and cells of smaller size, and the leaves were of smaller area than equivalent control leaves. Fourteen days later there was some recovery of both cell number and cell size and the proportionate reduction of leaf area was less, although the absolute difference in area remained more or less unchanged. Leaves of treated plants differentiated before treatment started and expanding during treatment (i.e. 7 to 10) had at the end of the treatment, i.e. 10 days after the start of drought, approximately the same number of cells but smaller cells and smaller leaf areas than equivalent leaves of control plants. Seven days later the difference between the cell sizes of treated and control plants was reduced but not eliminated and there remained a difference in leaf area. Leaves unfolded before treatment started (i.e. 3 to 6) showed no significant growth in either cell number or cell size in either treated or control plants and treatment did not therefore significantly affect their area.

In summary, leaves differentiated either before or after the treatment period, were unaffected in cell number. The complete insensitivity of cell division to treatment in leaves 7-10 is remark-

able since their number of cells was increasing rapidly during treatment. Increase in cell size in leaves 7-10 was however considerably retarded during treatment, as it was also in leaves 11-14. Effects of drought on cell number were limited to primordial leaves (i.e. 11-22). Effects on cell size appeared in leaves at a later stage of development, i.e. leaves 7-14. Differences in both cell number and cell size between treated and control plants were reduced in the recovery period but some difference persisted in all leaves up to the 18th. Leaves whose growth in cell number was retarded in the last stage of the drought, recovered completely.

Having completed the analysis of growth, causes for the observed results were sought in the behaviour of nutrient ions, in changes of water balance and in a consideration of the hypothesis that drought delays the ontogeny of the plant.

Mineral nutrition

Since growth has been separately analysed for leaves of different ages, correlations between internal concentration of nutrients and leaf and cell growth have been sought through the comparison of Figs. 29 (leaf area), 31 (cell number) and 32 (cell volume) with 41-44 (internal mineral concentration of the leaves).

The internal concentrations especially of N and K, tended to be higher in treated leaves when they first unfolded; the difference then tended to disappear as time went on. It is noticeable in leaves

11-14 and 15-18 that the time of the higher concentrations of N and K and to a lesser extent P in the treated leaves, corresponded to the time of most rapid cell division. Unfortunately analyses are not available for the corresponding times of rapid cell division in leaves 7-10 or 19-22, so this possible correlation cannot be examined throughout the range of leaves. Little correlation could be found between cell extension and internal concentration of nutrients. The possibility that accumulation of nutrients during the drought period contributes to subsequent accelerated growth cannot be either proved or disproved from these data.

It should be noted that Nitrogen and Potassium concentration on a dry weight basis in the leaves of treated and control plants, never fell below 4.3 and 6.0 percent respectively (Figs. 37 and 39). Critical levels, i.e. those levels below which deficiency is indicated, of Nitrogen and Potassium for tobacco on a dry weight basis are said by Tincknell and Lopez Ritas (1961), to be 2.0 to 2.5 and 3.0 to 4.0 percent respectively. It therefore appears less likely that any of the plants had a deficiency of these elements, and that slower growth of the controls during the recovery period could be due to reduced nutrient concentrations. This hypothesis should however be examined in plants growing in a less nutrient rich soil.

The data were also examined to see whether the nutrients which were accumulated by leaves could at a later stage be withdrawn for use in younger parts of the plant, and whether such redistribution

was affected by treatment (Figs. 55, 56, 57 and 58). The data are incomplete since they relate only to the first eighteen leaves, i.e. mostly those whose size was permanently reduced by treatment (Fig. 29). For the uppermost leaves in which area was initially reduced and which later recovered completely, there are analyses only for the final harvest.

The picture is confused but the following points could perhaps be made:

There is a withdrawal of N from the leaves in the later stages of growth which tends to begin earlier and be slightly greater in control plants (Fig. 55). On the whole Phosphorus accumulates in all the leaves examined in the control plants but there is an indication of withdrawal from leaves 7 to 10 in the later part of the recovery period of the treated plants (Fig. 56). Potassium and Calcium accumulate in all leaves up to the 6th or 7th harvest and there is no withdrawal at any leaf level (Figs. 57 and 58). Numbers 3 to 7 in these figures refer to the harvest at which the plants were taken. It may be noted that in the treated plants leaves 19-22 at the final harvest contained more N, P, K and Ca than control plants.

It appeared however that mobilization of N and P took place in only very small quantities relative to the amounts still being taken up by the whole plant (Figs. 33 and 34) and there is no evidence for redistribution of nutrients playing a significant part in recovery from drought.

Fig. 55 The effect of treatment on the change with time of total nitrogen content per leaf for successive leaf groups.

FIGURE 55

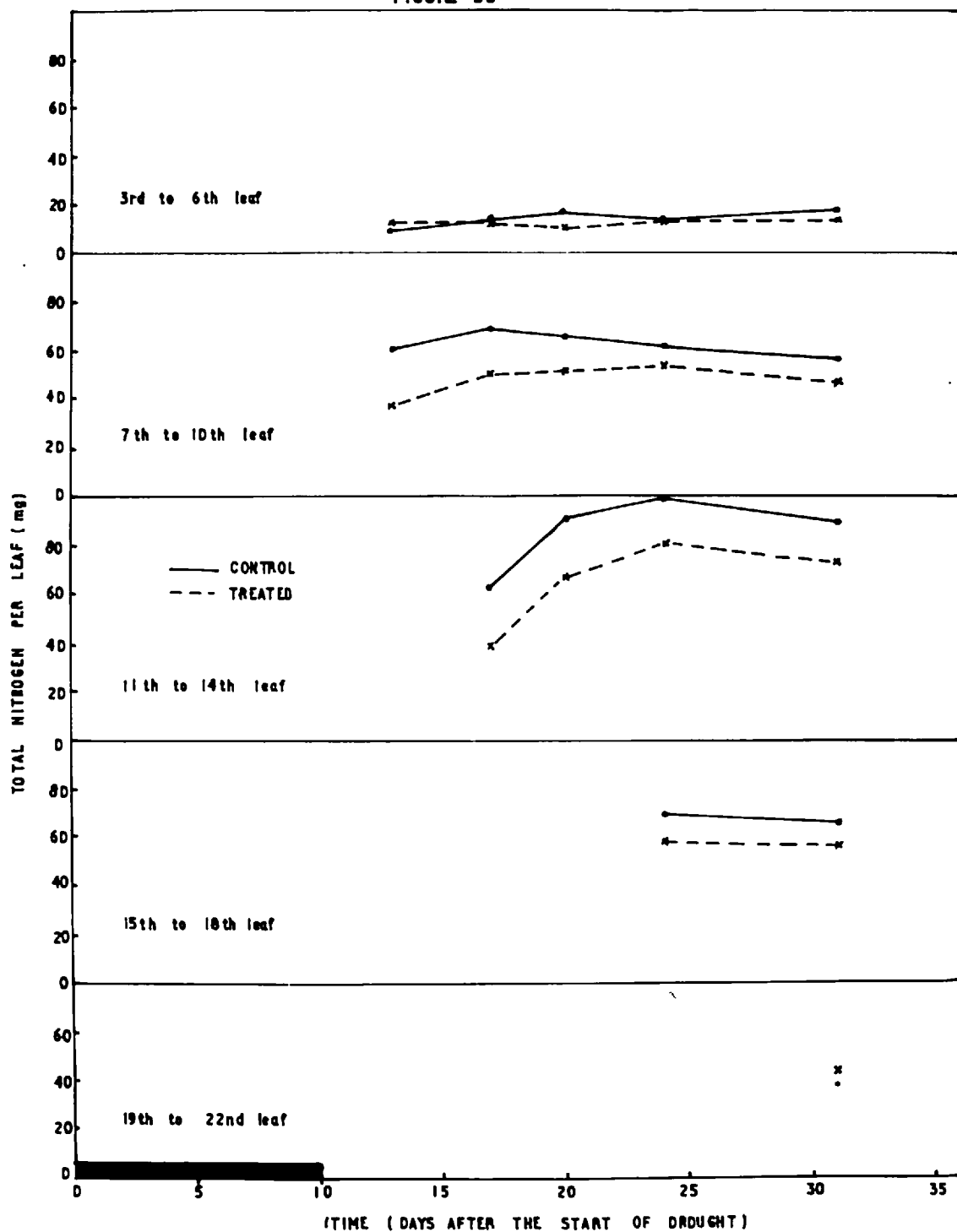


Fig. 56 The effect of treatment on the change with time of total phosphorus content per leaf for successive leaf groups.

FIGURE 56

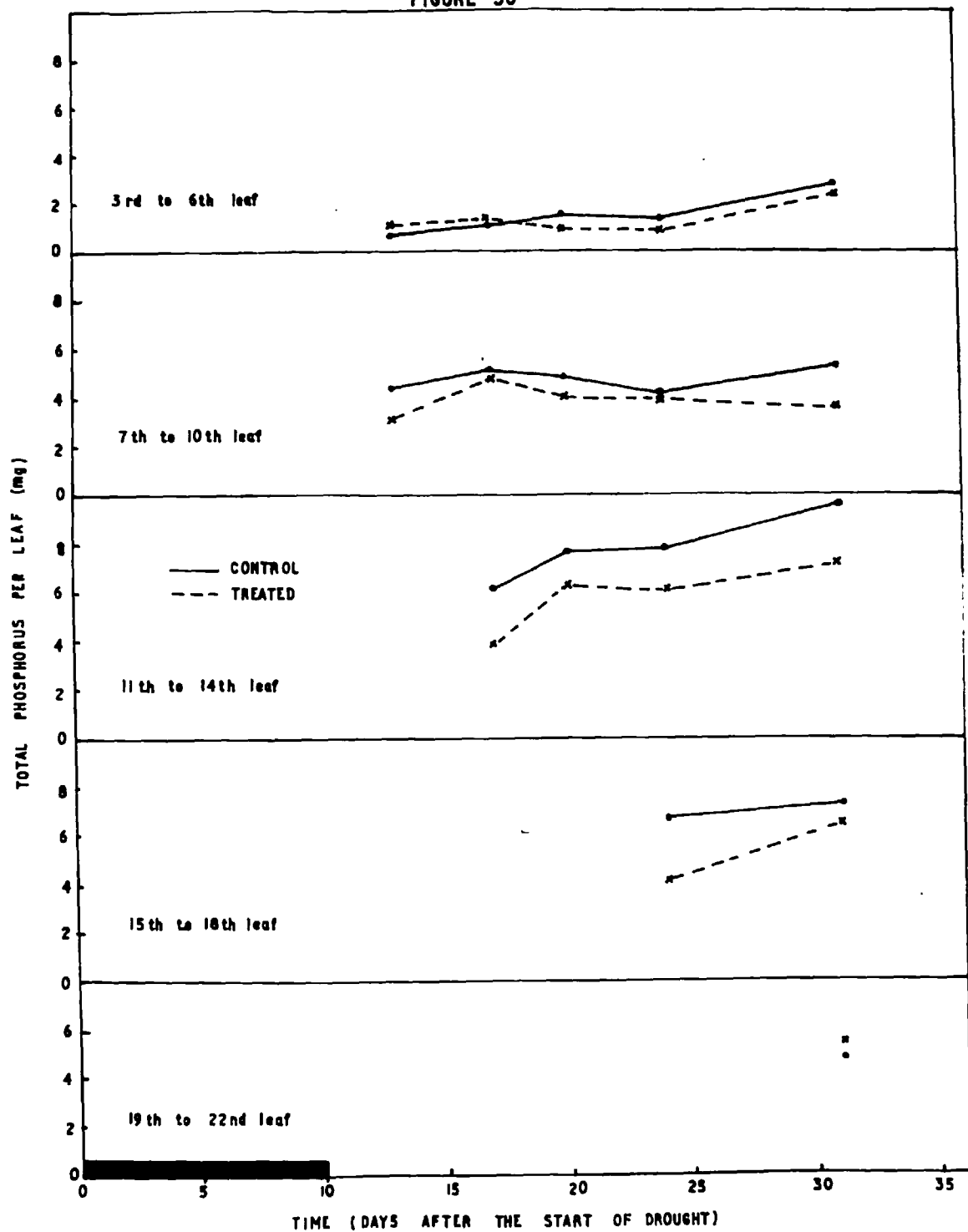


Fig. 57 The effect of treatment on the variation of total potassium content per leaf with leaf position for consecutive harvests.

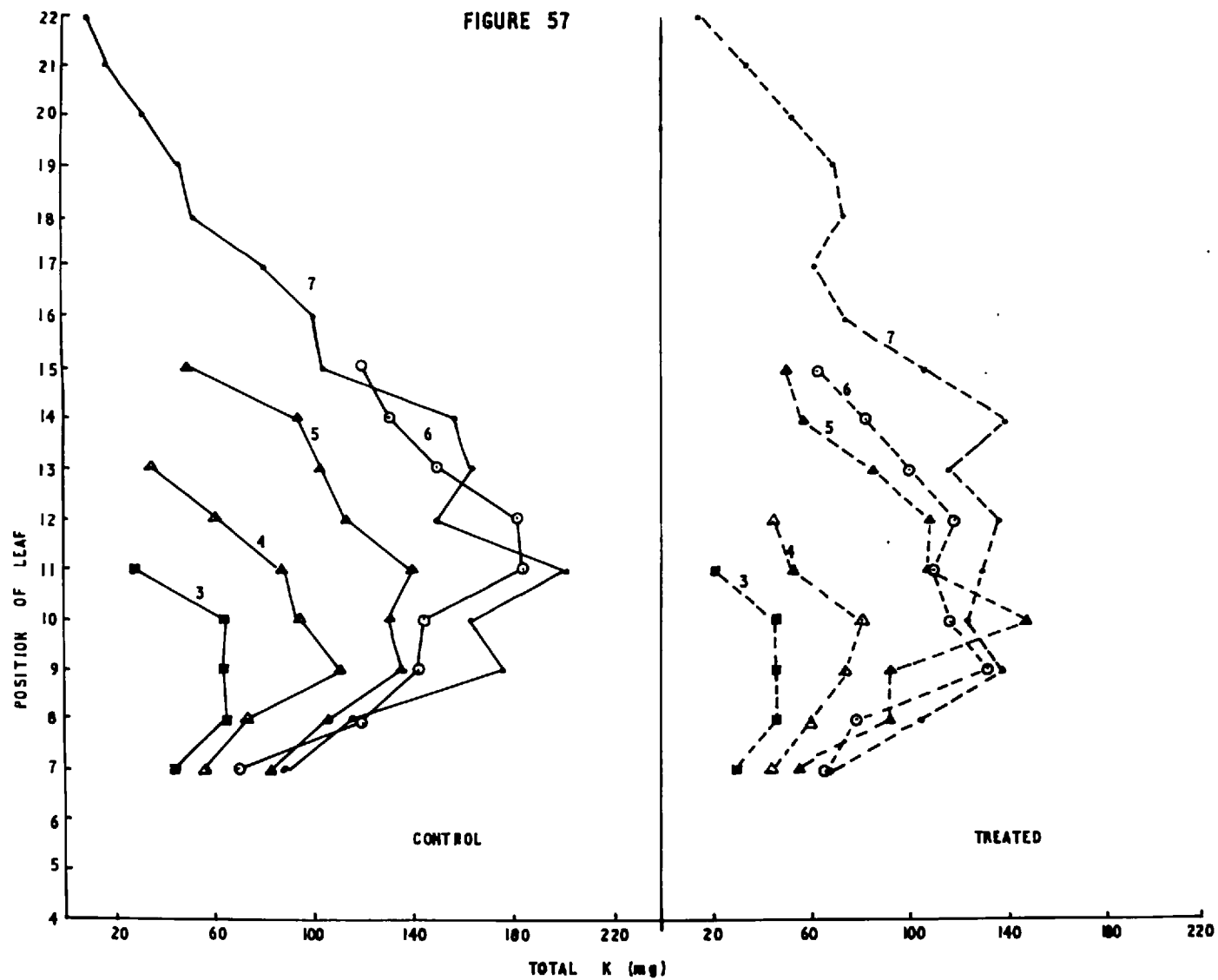
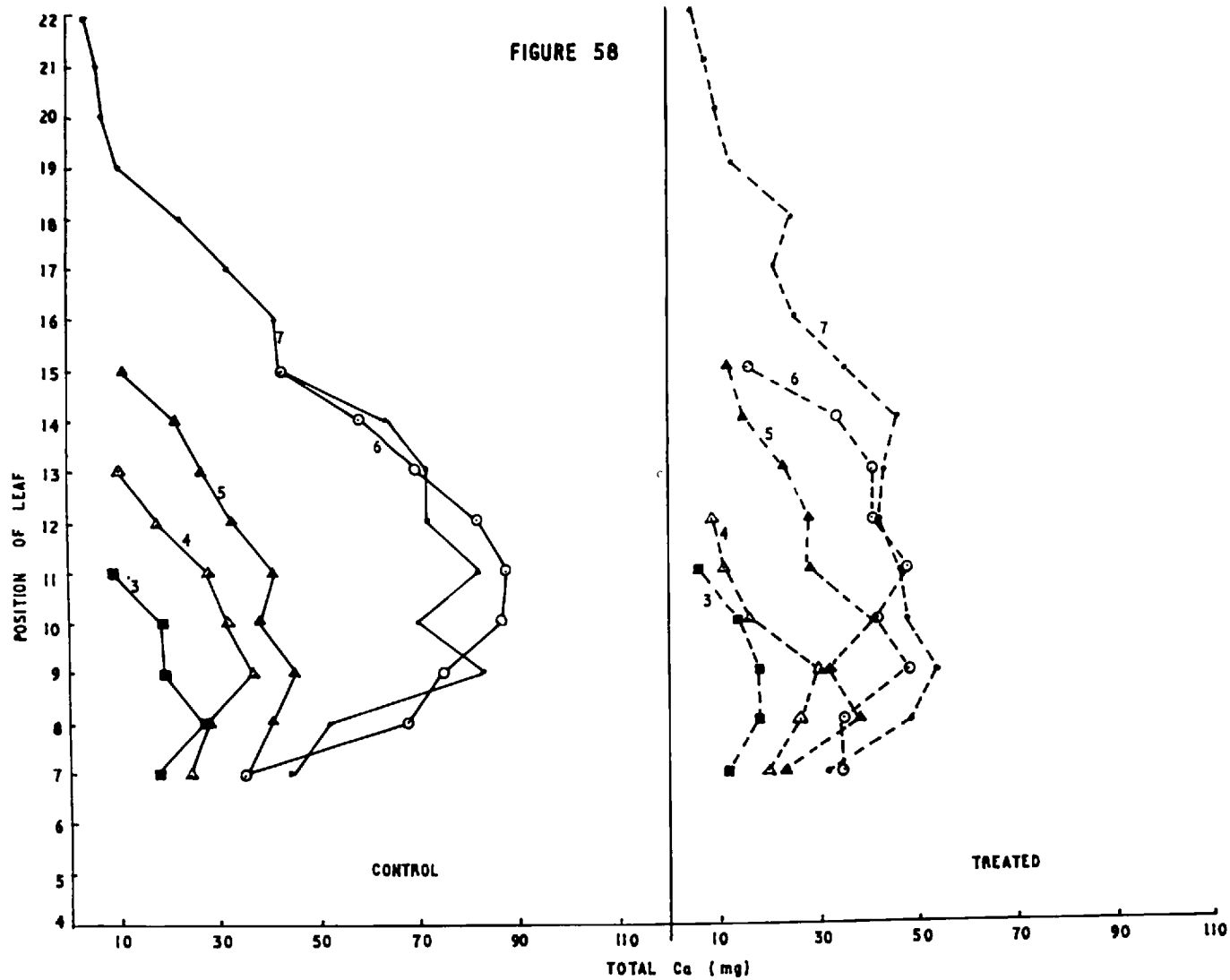


Fig. 58 The effect of treatment on the variation of total calcium content per leaf with leaf position for consecutive harvests.



Water balance

Recovery of water potential by treated plants upon rewatering was rapid. One day after rewatering there was a higher water potential in the treated than control plants. Throughout the recovery period treated plants showed a slightly higher leaf water potential (by 0.5 atm) than control plants (Figs. 52 and 53). Lawlor (1967) working with maize found that leaf extension in young leaves is very much affected by small decrements in leaf water potential in a range of high leaf water potential (i.e. -3 to -5 atm), similar to that exhibited by the tobacco plants during cloudy days of the recovery period. Boyer (1970), suggested that leaf enlargement is associated with turgor, small increases in turgor above a minimum resulting in large increases in rates of leaf enlargement.

The higher stomatal conductance of the leaves of treated plants during recovery is probably a reflection of their higher water potential in relation to controls (Figs. 45 and 46). The higher conductance of treated plants probably accounts for the higher transpiration rate per unit leaf area which was observed in the recovery period in all experiments (Figs. 20, 47 and 54). The higher stomatal conductance might also have been expected to cause a faster rate of photosynthesis but there was no evidence of this in the response of unit leaf rate. Either stomatal conductance was not a major control of photosynthesis or else there were compensatory changes in other

components of unit leaf rate, e.g. mesophyll resistance or respiration rate,

It was unfortunate that water potential was measured only in leaves whose sizes were permanently reduced by treatment (i.e. 5-8) and not in those that recovered their area completely. Nevertheless the data appear to indicate a more favourable water balance in treated plants.

Ontogenetic effects

Relative growth rate (R_w) and other growth attributes of plants grown in the variable conditions of field or glasshouse show both short term fluctuations and smooth trends with time (Thorne, 1960). Both types of variation probably reflect changes in the environment but the smooth trends may be attributable to changes with age and size which are independent of the environment. The relative growth rate is the product of unit leaf rate and leaf area ratio. As a plant grows older, the leaf area ratio falls. The unit leaf rate would need to increase if the original relative growth rate was to be maintained, but with comparatively less leaf area and the same photosynthetic rate the unit leaf rate would decrease because of the relative increase in respiring and non-photosynthesizing tissue. Hence, relative growth rate may be expected to change in the same direction as leaf area ratio.

It could be argued that drought holds plants back and keeps them smaller, so that on rewatering they assume a value of R_w dependant

simply on their size. This hypothesis could be tested by plotting treatment effects against plant size rather than against time. For this purpose total leaf area was chosen as the index of size. In Figs. 59-62 growth attributes following the end of the drought period in experiments II and III have been plotted against both leaf area and plant weight, each attribute being expressed as a percentage of its maximum value to facilitate comparison of the relative changes in time. Because leaf weight ratio was so little affected by treatment, it makes little difference whether leaf area or plant weight is taken as the index of plant size.

In both experiments the growth attributes of control plants tended to fall with size except for specific leaf area which rose in experiment II and fell only slightly in experiment III. At equivalent size treated plants had a larger R_A than controls, and in the later stages of the recovery period a larger R_W . The larger R_A was reflected in a larger leaf area ratio and specific leaf area.

In experiment III the specific leaf area of treated plants was smaller than that of equivalent sized controls in the early stage of the recovery period but rose above the controls in the later stages. However since leaf weight ratio was slightly smaller in treated plants than in equivalent sized control plants, the effects of treatment on specific leaf area were less marked though still apparent on leaf area ratio. E_A was appreciably less in treated plants than in control plants of the same size, and the combination

Fig. 59 The effect of treatment on the variation of R_A , R_W , E_A , F_A , leaf weight ratio, and specific leaf area with leaf area, during the recovery period in experiment II. Values are given as percentages of the highest value for each growth attribute. For specific leaf area the vertical bar represents the least significant difference at $P = 0.05$

FIGURE 59

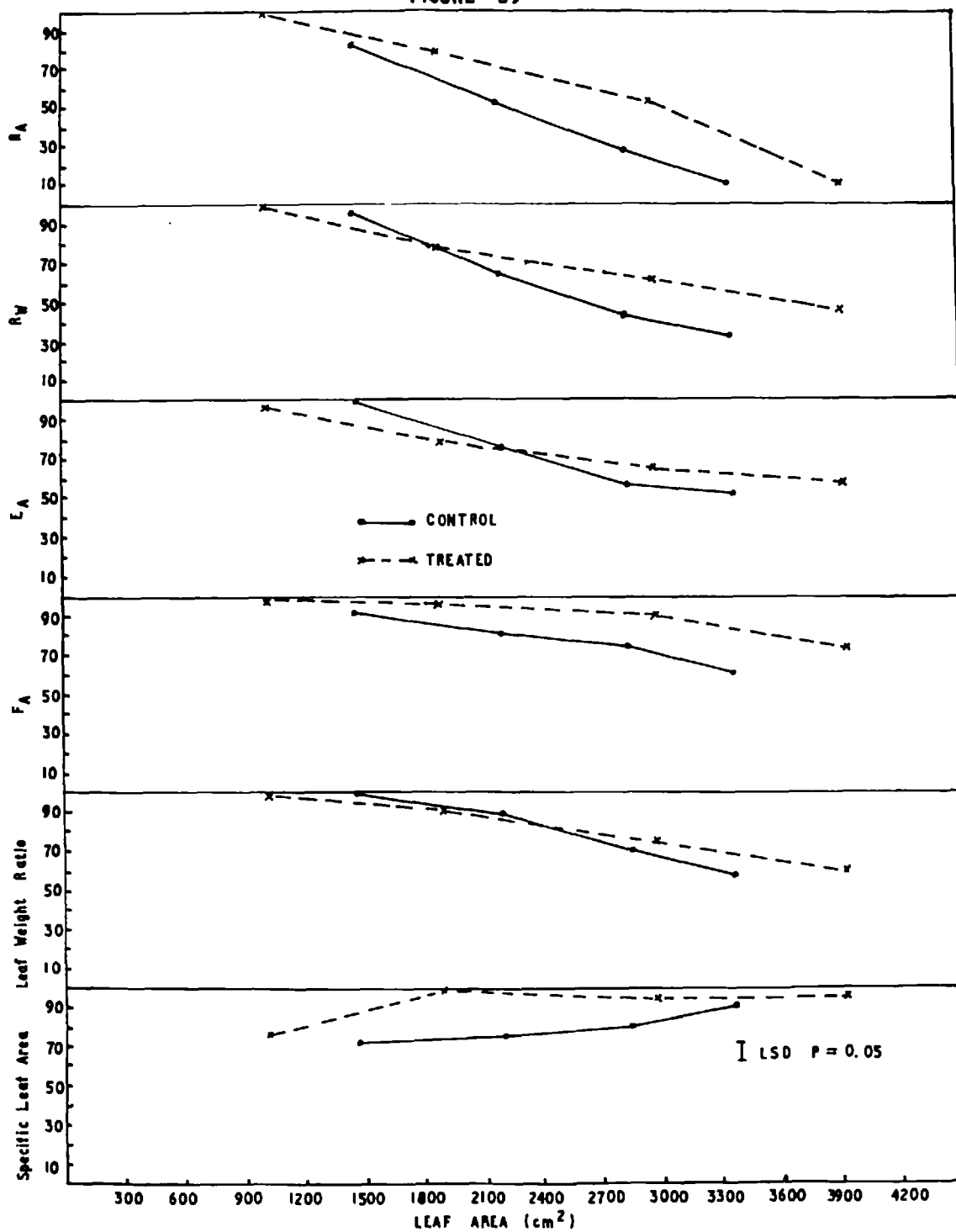


Fig. 60 The effect of treatment on the variation of R_A , R_W , E_A , F_A , leaf weight ratio, and specific leaf area with dry weight, during the recovery period in experiment II. Values are given as percentages of the highest value for each growth attribute. For specific leaf area the vertical bar represents the least significant difference at $P = 0.05$

FIGURE 60

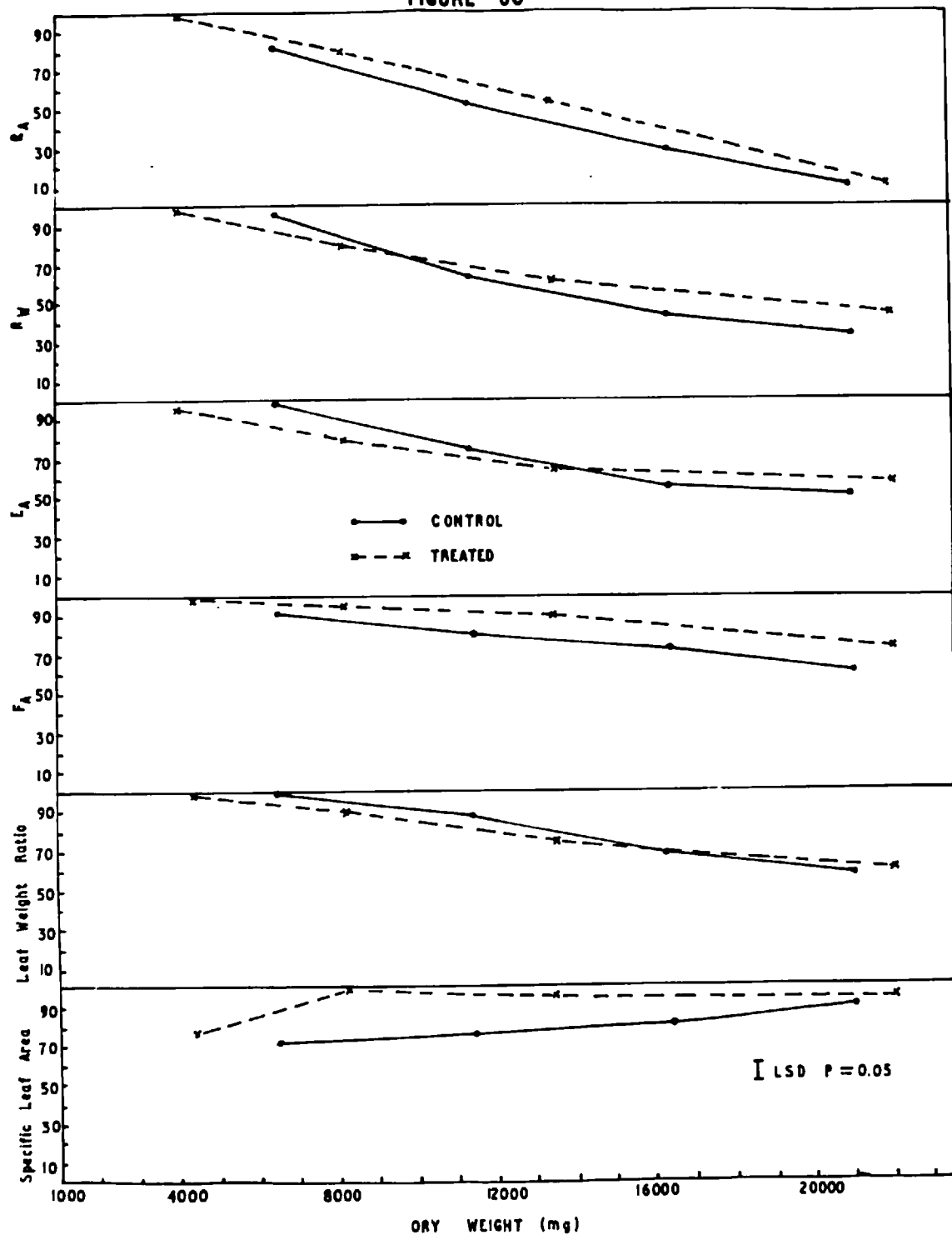
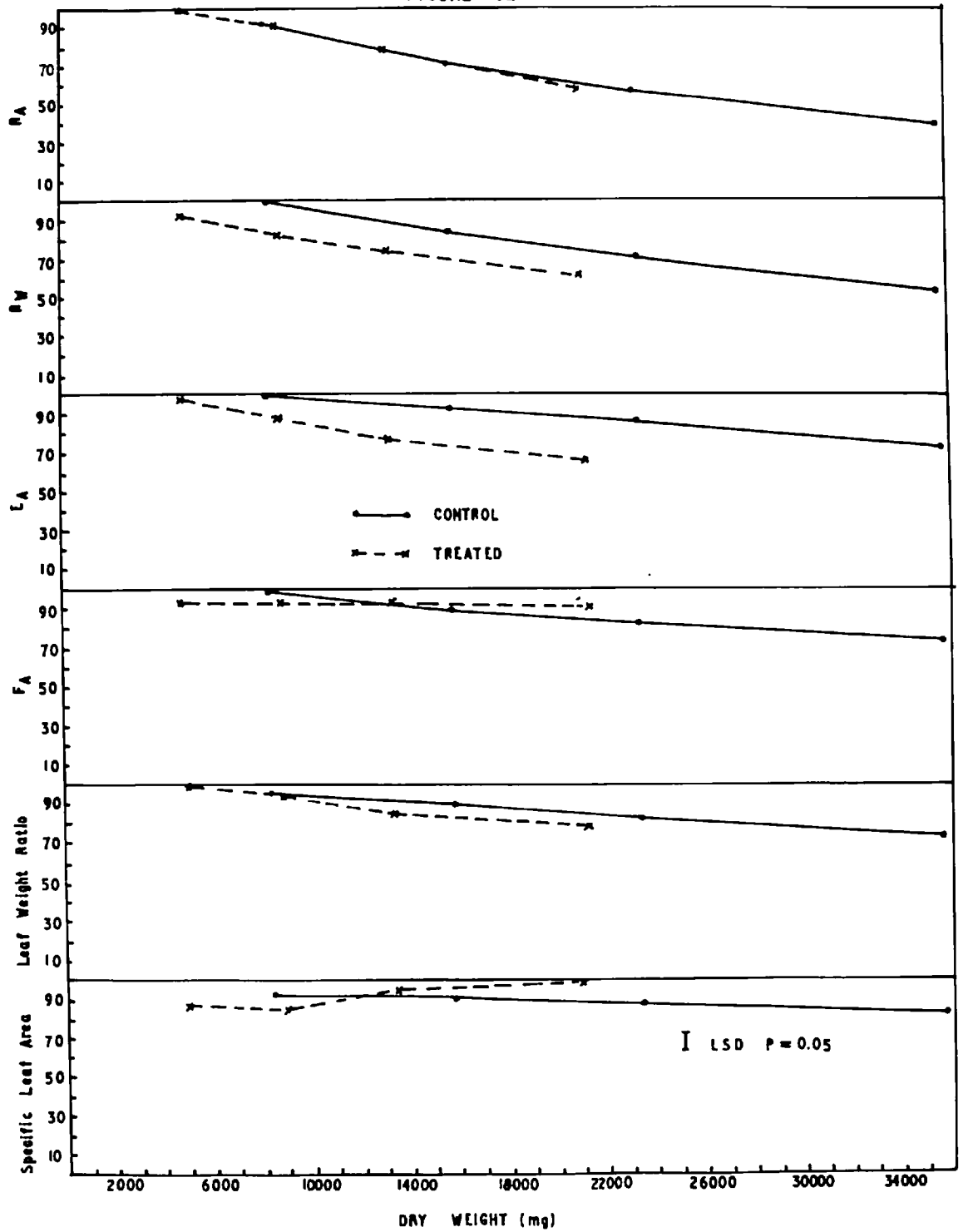


Fig. 61 The effect of treatment on the variation of R_A , R_W , E_A , F_A , leaf weight ratio, and specific leaf area with leaf area, during the recovery period in experiment III. Values are given as percentages of the highest value for each growth attribute. For specific leaf area the vertical bar represents the least significant difference at $P = 0.05$

Fig. 62 The effect of treatment on the variation of R_A , R_W , E_A , F_A , leaf weight ratio, and specific leaf area with dry weight, during the recovery period in experiment III. Values are given as percentages of the highest value for each growth attribute. For specific leaf area the vertical bar represents the least significant difference at $P = 0.05$

FIGURE 62



of a lower E_A with the leaf area ratios indicated produced values of R_W which were lower, at equivalent sizes, in treated plants, and values of R_A which were independent of treatment and varied only with plant size.

It appears that the distribution of dry weight between the leaves and the remainder of the plant (leaf weight ratio) is closely correlated with the size of the plant and little affected by treatment when the correlation with size is eliminated; but following the cessation of drought specific leaf area rises and remains higher than in control plants of the same size.

Conclusions

In general, different species were able to make substantial recovery from drought as shown by cucumber, sugar beet and tobacco (experiment I). Recovery of droughted plants was associated with a larger relative growth rate in experiments I and II and always associated with a rapid increase in leaf area (experiments I, II, III and IV). The recovery of leaf area was related to a more rapid growth mainly in the younger leaves (i.e. those initiated after or just before the treatment finished) and not to an increased number of leaves. The faster R_A of the younger leaves was due to a complete recovery of both cell number and size of those leaves. The explanation of the observed results appears more likely to be due to a more favourable water balance of treated plants (experiment IV). Although

the nutrient concentrations in the plants as a whole were high compared with critical levels quoted by Ticknell and Lopez Ritas (1961), it is possible that the accumulation of nutrients in young leaves during drought may contribute to their more rapid growth subsequently.

Practical significance

It is common practice among tobacco growers to withhold irrigation from seed beds, a procedure designed to harden the seedlings in preparation for transplanting and after transplanting seedlings are left for a while without irrigation to encourage growth.

Hopkinson (1968), working with tobacco in glasshouse conditions obtained a benefit from an early period of water stress, i.e. droughted plants were able to recover rapidly because of unusually high growth rates, a delay in leaf production relative to leaf expansion which brought about an advantageous rearrangement of leaf surfaces and lastly because a delay in floral induction enabled the apical meristem to produce an increased number of leaves, which conferred upon the plant a greater potential for vegetative growth. In view of these results it seemed that delayed irrigation might indeed be the better practice. He investigated this further by a detailed growth study of the effects of an early period of water stress in the field (Hopkinson, 1969). The advantages gained from an early water stress in pot experiments could not be detected under field conditions. He considered that this was due to the

fact that in the pot experiments the only stresses imposed were those of the experimental treatments, whereas in the field all plants had already suffered severe water stresses as a result of transplanting before the treatment was given.

The stresses imposed by transplanting are ill-controlled and it still appears possible that benefits could arise from a controlled withholding of water after transplanting if plants were raised in small fibrous pots or in soil masses on trays so that they could be transplanted with minimum damage. However in the present experiments no actual increases in the final weight of the leaves (i.e. the crop) has been achieved as a result of treatment.

Finally, attention should be drawn to the effects on root growth. Both root weight and root length were significantly and permanently decreased by a short period of drought. In pots which contained a rich soil and were subsequently freely watered the differences in root growth between treated and control plants are unlikely to have had important effects. But if these results could be reproduced in field conditions they might be significant in soils of lower nutrient supply and a sub optimal water regime.

Summary

The object of the present work was to investigate the recovery from a single period of drought, especially in Nicotiana tabacum L. A drought treatment was applied until wilting was produced throughout the day, then water was given freely each day during a recovery period. Different species (cucumber, sugar beet and tobacco) were found to be able to make a substantial recovery from drought; the recovery was associated with a larger relative growth rate and a rapid increase in leaf area.

A detailed growth analysis on tobacco plants showed that the more rapid growth of drought treated plants relative to controls on rewatering appeared to have been mainly due to leaf growth responses particularly to a significantly increased specific leaf area rather than to increased unit leaf rate. This was observed when the plants were compared both at the same point in time and at common sizes. The recovery of leaf area was related to a more rapid growth of leaves initiated after or just before the treatment finished and not to an increased number of leaves. An unusual and significant acceleration of leaf primordium production was found during the drought period. The unfolding of these additional leaves and the production of further primordia was however delayed during the recovery period. The number of unfolded

leaves immediately after the end of the drought was one or two less in treated than control plants; ultimately leaf number was the same in both sets of plants. The greater relative leaf area growth rate of the younger treated leaves was due to a complete recovery to control level of both cell number and size in those leaves. The explanation of the observed results appears more likely to lie in a more favourable water balance during recovery (leaf water potential was 0.5 atm significantly higher in treated than control plants). A higher stomatal conductance of the leaves of treated plants during recovery is probably a reflection of their higher water potential in relation to controls.

Although the nutrient concentrations in the plants as a whole were high compared with critical levels it is possible that the accumulation of nutrients in young leaves during drought might have contributed to their more rapid growth subsequently.

Both root weight and root length were decreased by a short period of drought. In spite of a rapid top growth of treated plants during recovery, root growth, in terms of dry weight and length, was slower than in controls. However, during recovery root dry weight of treated plants increased proportionally more than root length.

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Appendix I

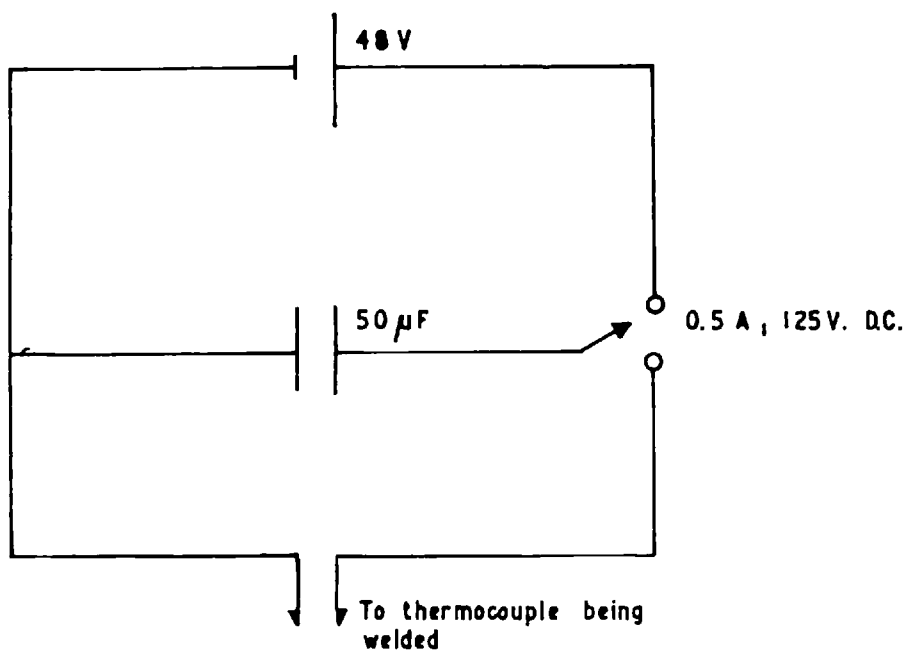
Two samples were taken, one of 70 leaves of sugar beet, and the other of 54 leaves of tobacco. Individual leaf areas were measured with an E.E.L. leaf area meter, and for each leaf the length and maximum breadth were measured. The following regression equations were calculated:

$$\text{Sugar beet, } A = 3.11 + 0.758 x \quad (r = 0.99)$$

$$\text{Tobacco, } A = 7.5 + 0.695 x \quad (r = 0.99)$$

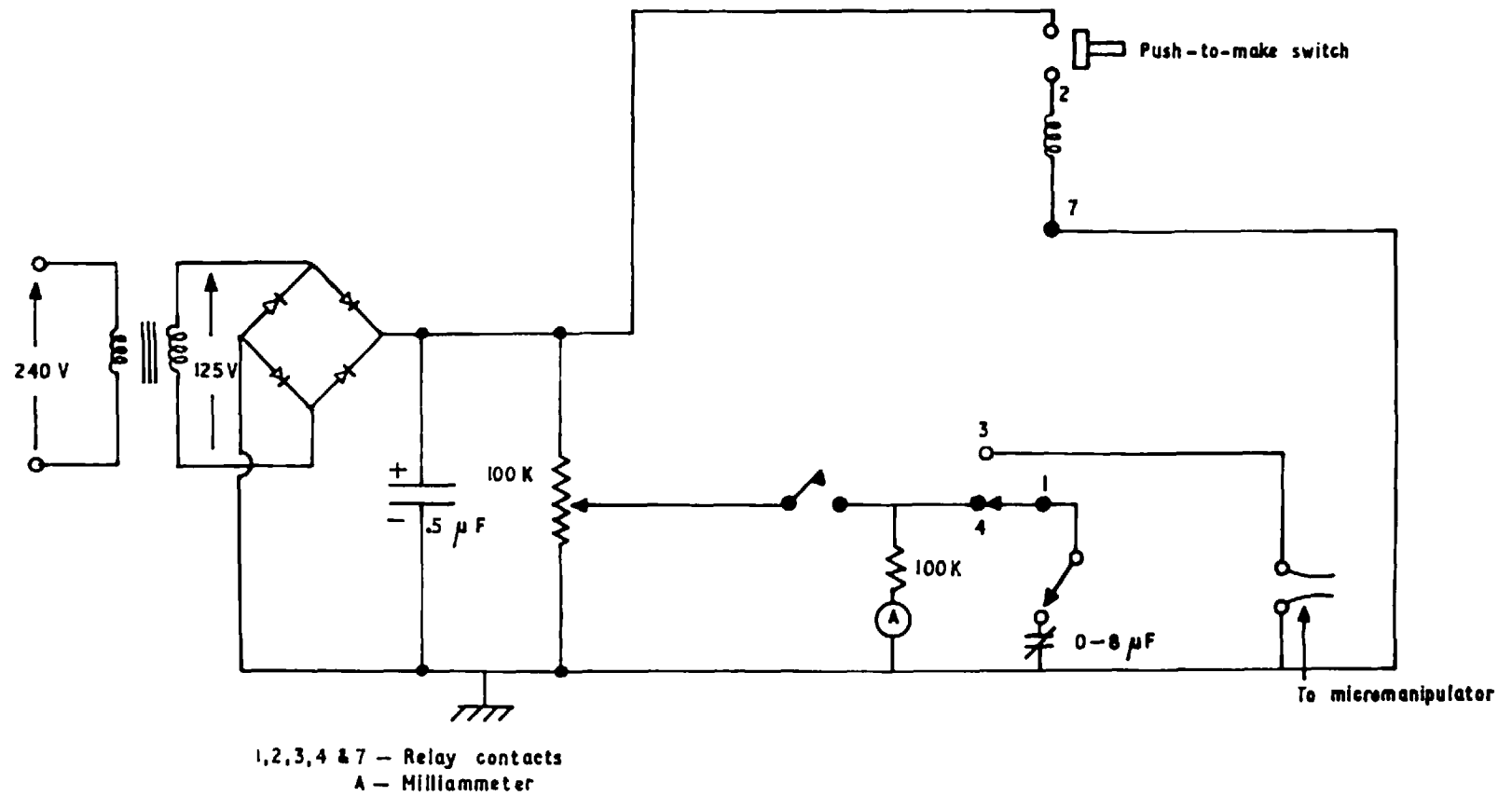
Where $A = \text{area (cm}^2\text{)}$, $x = \text{the product of length (cm)}$
and maximum breadth (cm)

APPENDIX II



Thermocouple welding circuit (manually operated)

APPENDIX III



Thermocouple Welding Circuit