The Development and Application of a Technique for Continuous Measurements of Plant Elongation.

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ABSTRACT

The development of an auxanometer capable of detecting 0.67 µm increments in plant elongation and its application to the study of hypocotyl elongation in intact Lupinus angustifolius seedlings is described.

A displacement transducer, in conjunction with a carrier wave oscillator-demodulator and a digital voltmeter, was utilised to detect changes in length of the elongating hypocotyls of four day old lupin seedlings.

The design of a root bathing solution chamber and environmental control chamber is outlined. With the aid of these two chambers the following environmental parameters could be varied independently:- temperature, water potential and aeration of the root bathing solution; temperature, relative humidity, and gaseous composition of the environment; composition and intensity of light within the environmental chamber. Problems encountered in effecting rapid changes of these parameters are discussed.

The viability of the auxanometer as an effective tool for plant growth research was tested by its application to the study of growth rates under a variety of environmental changes. Short term growth responses of lupin hypocotyls to changes in relative humidity, root temperature, and osmotic potential of the root bathing solution, plus exposure to anaerobic nitrogen and carbon dioxide atmospheres, have yielded the following results:-

1. Variations in saturation deficits of between 2.9 and 16.2 mbar altered growth rates only marginally.
2. Fluctuations in root temperatures between 23 and 43° C scarce ly affect hypocotyl growth rates.

3. Growth responses to changes in osmotic potential of the root bathing solution are similar to those described by Acevedo et al (1971) with intact maize leaves.

4. Periods of anaerobic nitrogen conditions yielded results comparative with those of Gillbank et al (1972), who studied the effects of cyanide on growth of wheat coleoptile segments, except for nitrogen treatments of more than 30 minutes in duration.

5. Exposure of seedlings to an anaerobic carbon dioxide atmosphere stimulates hypocotyl growth rates by up to eight times, the results indicating that CO_2 stimulates the utilisation of a growth precursor within the cell whilst simultaneously inhibiting its synthesis.

The results illustrate both the versatility and the potential of the described auxanometer in the description of plant growth responses to environmental changes, consequently aiding in the identification of the causal mechanisms of plant growth processes.

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In the face of mounting food shortages man has turned to the study of plant growth in an attempt to increase crop production. Plant breeding, fertiliser trials, irrigation trials, and plant pathology studies have played important roles in efforts to boost supplies. However, if the greatest efficiency in plant growth is to be attained, it is vital that man understand the ways in which environmental parameters affect physiological processes within the plant.

To procure information of this nature requires the design and implementation of precise cause and effect experiments. There have been, however, many experiments performed in the study of plant growth where a particular treatment has been administered to the plant, a result noted at a later time, and a cause-effect relationship postulated without the precision advocated above. This is best illustrated by an example such as the Loblolly Pine study of Brix (1962). Brix clearly demonstrated the manner in which photosynthesis and respiration both decreased over a 14 day period of drought and then returned to their original levels within two days of the resumption of irrigation. However, Brix did not measure plant growth rates, leaf water potentials, soil water potentials, or other variables that would be required to ascertain whether or not it was the imposed water stress directly affecting photosynthesis and/or respiration that led to reduced growth, or if in fact the reverse process was occurring - i.e. reduced growth impairing photosynthesis and/or respiration.

Consequently, in studies of plant growth under water stress, for example, general conclusions about results have been made, but with little information as to how these results arose. It appears necessary in studying the effects of water stress on growth that one firstly establishes facts such as: length of time taken for growth to be inhibited by an imposed water stress, the value of the minimum stress to

give growth inhibition and the duration of stress before restoration of water will fail to give a resumption of normal growth rates. If one knows how the imposition of stress affects the growth rate one can attempt to eliminate possible caused factors. For example, if growth rate is zero at a ψ ext. of -5 atmospheres imposed for 20 minutes, one can examine the processes of the plant affected within this same time interval in an effort to discover the main causal factors of this growth cessation.

Water stress gives rise to: - lower kinetin activity in root exudates (Itai and Vaadia 1965); higher abscisic acid levels in leaves after wilting (Wright and Hiron 1969); an increase in the activity of indoleacetic acid oxidase (Darbyshire 1971); liberation of amino acids (Kemble and Macpherson 1954); a monosome:polysome ratio increase (Nir et al 1970); and an increase in the rate of destruction of RNA (Gates and Bonner 1959); but all this knowledge can not be used to construct a valid model for the effects of water stress on plant growth until a study of the type suggested above is completed. This is due to the fact that when the kinetics of water stress - plant growth relationships are examined it may be found that many of the above observed effects of water stress occur after plant growth has ceased and consequently would not have contributed to the mechanism whereby growth was inhibited.

To commence a study of this nature one must be able to monitor plant growth over short time intervals. The present project was undertaken with the aim of developing a technique which would enable the growth of intact Lupinus angustifolius seedlings to be recorded with sufficient accuracy that their responses to various stimuli could be determined, and where possible the results compared with similar short-term growth kinetic studies on lupin hypocotyl segments. Hypocotyl growth on intact plants was studied in preference to hypocotyl sections since a considerable bank of data regarding the growth of lupin hypocotyl

sections has been built up (Penny 1969, Penny P. et al 1972, Penny D. et al 1972), but the relationship between excised and intact hypocotyl tissue had not been examined. It could be expected that entirely different source - sink relationships exist in the intact seedling compared to the excised section. Adepide and Fletcher (1971), for example, showed that the primary leaves of intact bean plants, in which senescence had been delayed by application of benzyladenine, did not mobilise ^{14}C-sucrose or ^{14}C- assimilates fed to other parts, in contrast to the situation with detached leaves. Gates (1955) found in young tomato plants that leaves of differing position and age responded to water stress quite differently, both during and after wilting, many of the differences being ascribed to modifications of patterns of translocation normally operating within the plant. These two examples illustrate some of the complexities of source - sink relationships that exist even within the same organs on a particular plant.

This project attempts to develop an accurate method for measuring short term growth kinetics in lupin hypocotyls so that results obtained with the apparatus may, where possible, be compared with those obtained for hypocotyl segments and, in conjunction with the segment results, add to our understanding of the mechanisms of plant growth.

B. MATERIALS AND METHODS

I MATERIALS

Seeds of Bitter Blue lupin (Lupinus angustifolius)were surface sterilised by immersion in 1% chlorogen for 20 minutes. Following a two hour soak in water the seeds were planted in pots of prewashed vermiculite (course exploded mica) and placed under continuous light in a growth room at a temperature of 20.5$\pm$ 1^{o} C. The light intensity was 14 W/m^{2} supplied by a combination of 95% fluorescent (Philips TLA 80 W/55) and 5% incondescent light. The pots were irrigated continuously with water.

Four day old lupin seedlings were used in the experiment. At this stage the leaflets of the first leaf were protruding from the hypocotyls by 7 to 10mm and the hypocotyl length was 50-60mm. Segments were excised from the portion of the hypocotyl immediately beneath the cotyledons. Where intact plants were used great care was exercised, in obtaining plants with undamaged roots.

II METHODS

(A) For measuring short term growth kinetics of segments.

The apparatus for measuring the growth rate of segments at minute intervals is described and illustrated by Penny (1969, 1971). The excised segments, measuring 20-25mm in length, were cut from immediately below the cotyledons and clamped into a chamber, containing a bathing solution, mounted on the moving stage of a microscope. Growth of the segment was measured every minute by moving the hair line on a filar micrometer eyepiece to a reference point on the segment. The segments were pretreated for 2-3 hours in the bathing solution pumped from a reservoir in a constant temperature water bath by a Watson-Marlow H.R. Flow Inducer at a rate of 35ml/minute. A desk lamp with a 40W tungsten bulb, giving a light intensity of approximately 3 W/m^{2} within the chamber,

FIGURE I Perspex seedling holder.

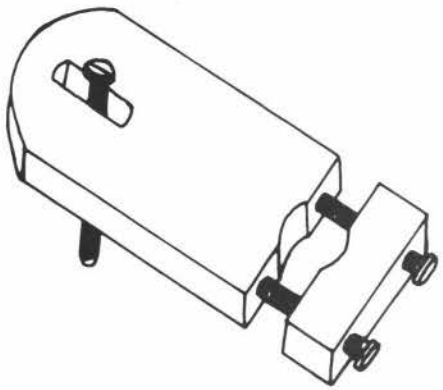


FIGURE 2 Perspex root chamber

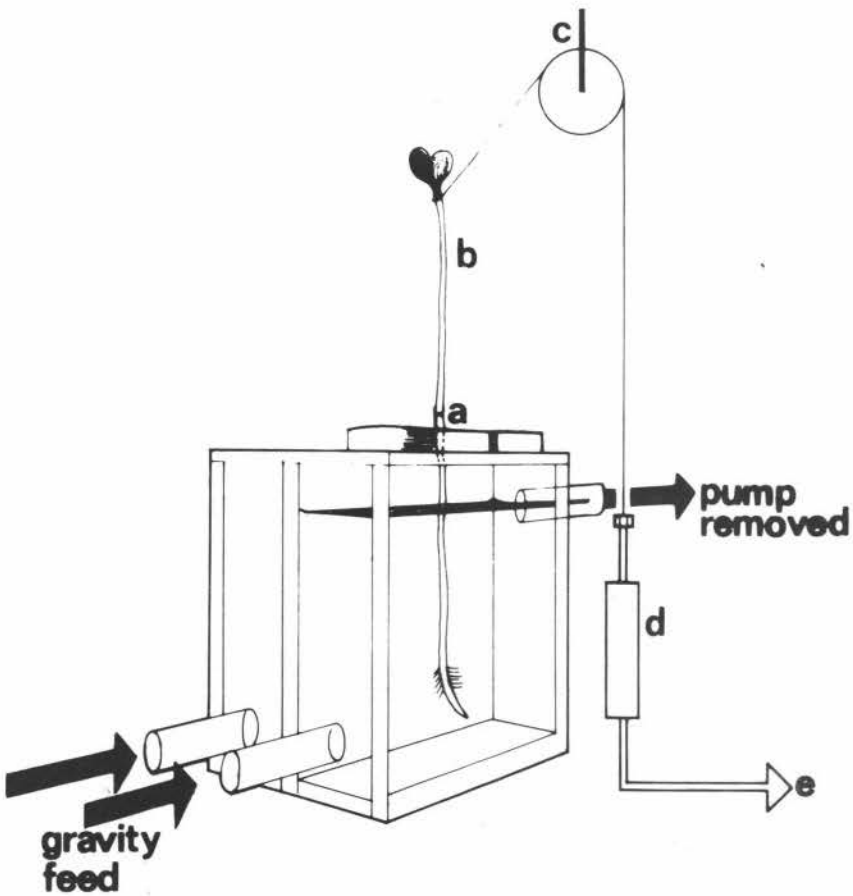
a seedling holder (Fig. I)

b lupin seedling

c pulley

d transducer

e wire to digital voltmeter



was placed 15cm from the section during pretreatment and treatment periods.

(B) For measuring short term growth kinetics of intact seedlings.

The auxanometer used is described fully in the next section on "Development of Auxanometer."

Four day old lupin seedlings with hypocotyl lengths of between 50 and 60mm were carefully removed from the pots of exploded mica so that the root was undamaged. A seedling was clamped at the junction of hypocotyl and root by a holder, (Fig. 1), so that the seedling could be firmly fastened into a chamber containing a circulating bathing solution for the root. A thread fastened to the top of the hypocotyl, immediately below the cotyledons, was suspended over a pulley and attached to a magnetic metal slug which was free to move up and down in a Philips Model PR 9314A/01 displacement transducer, (Fig. 2). The displacement transducer was connected to a Philips Model PR 9309/00 carrier wave oscillator-demodulator which gave a linear voltage change as the seedling hypocotyl increased in length and the slug descended further into the transducer. The resultant voltage was displayed on a Philips Model PM 2433 digital voltmeter. The change in voltage over minute periods was recorded and directly correlated with the increase in length of the hypocotyl during the same time period.

Environmental variables were controlled by suspending the above mentioned apparatus in a perspex box with its own regulated air supply.

Seedlings were left under control conditions in the apparatus for a period of 3 to 4 hours for equilibration prior to any experimental results being recorded.

C. DEVELOPMENT OF AUXANOMETER

I LITERATURE SURVEY

The following brief historical record of the development of equipment for measuring plant growth at short time intervals serves as an introduction to the methods employed in this study.

Stephen Hales (1727) described an experiment "in order to find out the manner of growth of young shoots", in which he marked the young shoot of a vine with paint at regular intervals along its length. Six months later he measured the distances between the marks and found that the younger internodes had elongated more than the older "hardened" internodes over the same time interval. Sachs (1882) used a similar technique to that of Hales when he subdivided the primary root of a Vicia faba seedling into 1mm sectors with lines of Indian ink and, by a measurement 24 hours later, demonstrated the different regions of root growth. Sachs was, however, interested in investigating the effects of factors such as light and temperature on plant growth. He realised that these investigations were not possible unless short-term growth measurements could be obtained, and in his "Text-book of Botany" (1874) he made the following relevant comments.

"In order to study more closely the changes of growth occasioned by internal causes, or the dependence of these changes on external conditions, it is necessary to measure the increments in short spaces of time such as an hour or two or three hours. In the case of internodes or leaves of large plants which are growing very rapidly, as the flower-stems of Agave or the leaves of Musaceae, this can be done with a certain degree of exactness by simple measurement with a measuring rod. But for the purpose of more exact measurements it is more convenient to make use of smaller plants which do not grow so rapidly, the growth during an hour not amounting to a millimetre, or even less. In such cases a simple measuring-rod is not sufficiently exact."

Some of the equipment designed by Sachs (1874, 1882) to measure plant growth over short time periods is outlined below.

(a) Arc-indicator. This apparatus (Fig 3A) was capable of measuring of the order of 70 - 100μm increments of growth but Sachs felt that the disadvantage of the necessity for an observer to continually watch the apparatus was rather large, especially if one wanted to make recordings at night.

(b) Autographic Auxanometer. This instrument overcame the difficulties of the Arc-indicator by the production of a permanent record of plant growth on the marked paper fastened to the cylinder which was adjusted to make exactly one revolution per hour. (Fig 3B). Sachs performed numerous experiments using this apparatus and was able to formulate interesting and valid conclusions on the various effects of light and temperature on plant growth.

(c) Cathetometer Apparatus. (Fig 3C). Sachs found this method most satisfactory for experimental material such as Phycomyces which obviously could not have a cotton thread attached to the sporangiophore without damage to the plant material. To reduce the effects of uneven light distribution Sachs arranged the apparatus so that the metal plate holding the bell-jar and plant material made one complete revolution per hour, at which time a measurement was made on the travelling microscope.

Sachs laid a firm foundation for later short-term plant growth investigations and there have been inumerable auxanometers designed in the succeeding years. Unfortunately many plant growth studies have not utilised the most suitable apparatus available and consequently conclusions have been unnecessarily vague. At the other extreme are the many auxanometers which, due to the fact that they were developed more as a technique than as an aid to definite plant growth investigations, have not been used to great advantage in plant growth research.

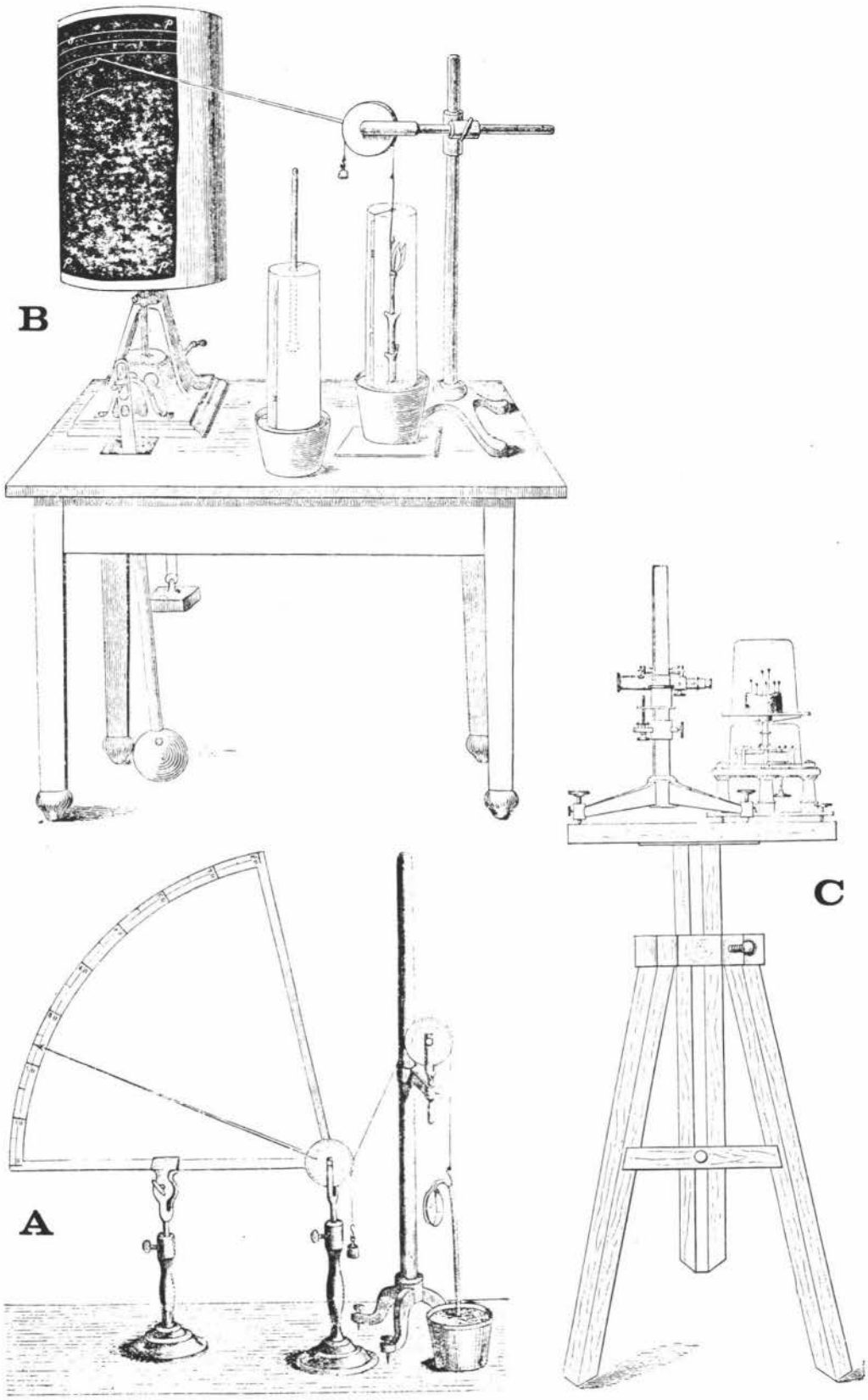


FIG. 3. Auxanometers designed by Sachs (1874, 1882)

A. Arc-indicator. **B.** Autographic Auxanometer.

C. Cathetometer Apparatus.

An outline of further developments in auxanometers since Sach's work is given below.

Bovie (1912) described an auxanometer in which a spring contracted as the plant grew. When the spring had moved a certain small distance it closed an electrical circuit which operated the recording pen of a chart recorder and reset the spring at a known distance from its contact. Bovie's apparatus was capable of giving an output for each 25μm increase in length of the plant under investigation. Koningsberger (1922) developed his elaborate auxanometer from Bovie's model and with its greater precision recorded 10μm growth increments. This auxanometer was very cumbersome and consequently Koningsberger himself appears to be the sole plant growth investigator to use the apparatus. However Ranson and Harrison (1955) described a very compact modification of Koningsberger's auxanometer which gave an output each 23μm of plant growth.

If growth rates of plants are to be accurately resolved for time intervals of less than 5 minutes an auxanometer must be able to cope with growth of a variable sign as well as recording growth increments of 5μm or less. None of the described auxanometers could meet either of these specifications. However Idle (1956) designed a contact auxanometer to overcome these problems. This auxanometer recorded growth of 0 to 80μm/minute using 0.5μm increments. A Cambridge Rocking Microtome stood on end served as the advancing mechanism of the apparatus. Each plant growth increment of 0.5μm caused the paper tape output to be advanced by a specified distance. Every minute a needle punched a hole in the paper tape and then the paper was automatically advanced the equivalent of one growth increment. Consequently if there was zero or negative growth the punched 'time' holes did not overlap and the duration of these phases of growth could be readily determined. This method, however, did not allow for the magnitude of any negative growth to be computed. Idle's apparatus was by far the

most sensitive instrument designed for the study of short term growth kinetics but suffered the disadvantage of being unable to determine the magnitude of any negative growth during an experiment. This disadvantage was eliminated in the auxanometers to be described below.

Meijer (1968) described a method for recording continuously the growth of gherkin hypocotyls. A ferromagnetic core was suspended by a spring so that it made contact with the top of the hypocotyl. As the hypocotyl grew the core moved through a modified displacement pick-up, thus changing the inductance of two coils in the pick-up. With the aid of a measuring bridge and chart recorder these inductance changes were monitored. The measurements were linear for a displacement of 1mm which gave a 250mm full scale displacement on the chart recorder, at which point the recorder pen switched on a motor which lowered the plant 1mm and returned the pen to the beginning of the scale. Hsiao et al (1970) developed independently an almost identical method for monitoring maize leaf elongation.

A summary of the different auxanometers and their characteristics is given in Table I.

From this brief account of techniques available for short-term measurements of plant growth it is apparent that relatively accurate methods have existed for many years, yet few plant growth studies have utilised the available techniques. For example, Loomis (1934) studied the daily growth of maize with a "Home-made" auxanometer from which he was able to make two-hourly measurements, but as may be expected his conclusions were vague:

"The growth of maize depends upon a liberal water supply at the growing point. In order of effectiveness such a supply is reduced and growth checked by (a) direct sunlight, (b) deficient soil moisture, (c) low relative humidity. The growth of maize drops rapidly as the temperature approaches 10°C."

TABLE I. Comparison of different Auxanometers

Method	Designer and date	Sampling Interval (from publication)	Minimum growth detected. (estimated)	Minimum sampling interval and limit of growth detectable with the same apparatus.	Output
Arc Indicator	Sachs 1874	1 hour	100μm	Dependent on rate of growth; 100μm	Increase in length per unit time.
Autographic Auxanometer	Sachs 1874	1 hour	100μm	1 hour; 100μm	Increase in length per hour.
Cathetometer	Sachs 1874	1 hour	100μm	Dependent on rate of growth; 100μm	Increase in length per unit time.
Contact Auxanometer	Bovie 1912	Dependent on rate of growth	25μm	Dependent on rate of growth; 25μm	Increase in length in units of 25μm per time interval.
Contact Auxanometer	Koningsberger 1922	Dependent on rate of growth	10μm	Dependent on rate of growth; 10μm	Increase in length in units of 10μm per time interval.
Contact Auxanometer	Ranson & Harrison 1955	Dependent on rate of growth	23μm	Dependent on rate of growth; 23μm	Increase in length in units of 23μm per time interval.
Contact Auxanometer	Idle 1956	1 minute	0.5μm	1 minute; 0.5μm	Increase in length in units of 0.5μm per min.
Transducer	Meijer 1968	Continuous but results every 3 3/4 minutes from output graph.	4μm	Dependent on rate of growth and recording equipment used	Graph of length against time from which results are estimated.
Transducer	Hsiao 1970	Continuous but results from output graph. Time interval not able to be determined.	7.5μm	Dependent on rate of growth and recording equipment used	Graph of length against time from which results are estimated.
Transducer	Marshall 1971	1 minute (could record at 10 second intervals)	0.67μm (could detect 0.067μm increments)	Dependent on rate of growth and recording equipment used	Increase in length per time interval.

His experiments would have been much more valuable if he had used any of the more precise auxanometers available; even a simplified auxanometer such as that described by Bovie (1915) would have enabled quantitative measurements to be made. Williams and Biddiscombe (1965) measuring the effects of temperature on extension growth of grass tillers in the field used rather primitive auxanometers to obtain their results but this was understandable since they needed sturdy auxanometers which could withstand field conditions. However Watts (1971) used similar auxanometers in much more exacting laboratory work where some of the more precise auxanometers would have been invaluable to his investigations. Similarly Kleinendorst and Brouwer (1970) using a very elaborate set-up to grow maize plants with controlled root and growing point temperatures, and to monitor leaf water content continuously with a B gauge, used a technique to measure leaf elongation with which "a leaf elongation of 10cm a day could still accurately be subdivided into periods as short as a quarter of an hour." Since the leaf would grow about 1mm in 15 minutes this would indicate that the technique was only able to detect accurately growth changes of the order of 100μm.

II AUXANOMETER - INITIAL DEVELOPMENT

The auxanometer was developed and tested over a period of many months. Only the more relevant details of development and construction are recorded in the following account.

Construction of the auxanometer was based on the technque described by Meijer (1968) and initially a Philips PR9314A/01 transducer, a Philips PR9309/00 carrier wave oscillator-demodulator, and a Philips PM 2433 digital voltmeter were procured to construct an auxanometer of similar design. A 12V DC current was supplied to the PR9309 which contains an oscillator to supply the transducer, a demodulator and

a variable amplifier. The output from the PR9309, displayed on the digital voltmeter, is a DC signal linearly proportional to the displacement of the transducer slug.

It was found that the transducer gave a hyperbolic voltage output over one part of its length, however a linear voltage response to regular movements of the metal slug was obtained when the slug was inserted further into the transducer. The relationship between the movement of the slug in the transducer and the recorded voltage charges was determined with the aid of a micrometer screw gauge. The micrometer was mounted in an upright position and the metal slug from the transducer fastened on top of the extending arm. The transducer was mounted directly above the micrometer so that the end of the metal slug was inserted in the transducer cavity until a reading of just under 4 volts in the linear range of the transducer was displayed on the digital voltmeter. Voltages were recorded from the voltmeter for each 0.5 millimetres that the metal slug was inserted into the transducer by the micrometer screw gauge for a voltage output range of +4 to -4 volts. The relationship established between movements of the slug in thetransducer and the resultant voltage was that a 1.0μm movement of the slug resulted in a 1.5mV voltage charge. Consequently when lupin hypocotyl growth was monitored on the apparatus each 1mV charge in voltage represented a growth increment of approximately 0.67μm.

At first the auxanometer consisted of little more than that depicted in Figure 2. The chamber was set on a platform which was able to be readily raised or lowered. The pulley (c) was fastened by a clamp from a retort stand, as was the transducer (d). The output from the transducer, after passing through the oscillator-demodulator was displayed visually on the digital voltmeter. Trials with lupin seedlings in the apparatus showed that the hypocotyls grew at a rate

of approximately 20μm per minute, giving a voltage charge of 30mV per minute on the digital voltmeter. The digital voltmeter had a selector for different voltage ranges and to obtain satisfactory results one of the following ranges had to be chosen: -

+ 0.3999 Volts to - 0.3999 Volts

+ 3.999 Volts to - 3.999 Volts

+ 39.99 Volts to - 39.99 Volts

Since results were required for short-term growth kinetics the range chosen had to give a significant output every minute. The 39.99 Volt range would register a charge of 3 units in the least significant figure with one minutes normal growth, whereas the 3.999 Volt range would register 30 units and the 0.3999 Volt range 300 units over the same time period. The 39.99 Volt range was obviously too insensitive, especially if the growth rate of the seedling was depressed by experimental conditions. The 300 units per minute for the 0.3999 Volt range proved to be rather cumbersome for recording coupled with the disadvantage of it being necessary to interupt recording to reset the slug in the transducer when the voltage went outside this range, which happened every 15 to 20 minutes under normal growth conditions. Consequently the +3.999 to -3.999 Volt range was chosen as being most suitable for the contemplated experiments with lupin seedlings.

In contrast to Meijer (1968) and Hsiao et al (1970), who used plants growing in a solid medium, the lupin seedlings in this study were fastened in a specially designed chamber containing a circulating bathing solution for the roots (Fig. 2). Slatyer (1961) demonstrated that one must be careful in selecting the conditions under which to study the effects of water stress on growth, since if one uses an osmotic solution containing solutes that can enter the cell the water relations set up at the commencement of the experiment will not prevail. Consequently, unless measurements are for a brief period after the imposition of stress (less than one hour according to Slatyer's re-

sults), observed effects are likely to be due to water balances not envisaged at the beginning. For this reason it appeared advisable to use water as the bathing solution for the roots, rather than soil which contains solutes that increase in concentration as the soil dries. For water stress investigations a non-permeable solute could be used readily in this type of root chamber. Another advantage of having the roots immersed in a circulating solution was that root temperature could be more readily controlled.

The root chamber designed initially was of 4 millimetre thick perspex construction with internal dimensions of (10 x 8.7 x 2) centimetres and a capacity for 155ml of solution when in operation. The bathing solution was circulated by a Watson-Marlow H.R. Flow Inducer at a rate of approximately 120ml per minute and consequently the chamber's entire solution content was replaced once every 1.3 minutes. In preliminary work this chamber appeared to be ideal but with later work on water stress, when solutions of Polyethylene glycol (P.E.G.) 6000 were used, the viscosity of these solutions doubled the time taken to replace the chamber contents. This was not serious when water stress was imposed by the replacing of the water with P.E.G. since the precise time when stress was imposed on the roots was visually obvious, due to the change in optical density of the chamber fluid. However, when the P.E.G. was replaced by water, the less dense water streamed up the sides of the chamber. Consequently mixing and removal of the P.E.G. was much too slow to allow satisfactory determinations of the precise duration of imposed stresses. Perspex baffles were fastened inside the chamber but although mixing was greatly enhanced it was decided to construct a smaller chamber. The second chamber to be designed consisted of a unit of two small perspex chambers, each with its own independent solution inlet and outlet (Fig. 2). Each chamber

measured (7 x 4 x 2) centimetres with an inlet of diameter 0.6 centimetres and an outlet of 1.0 centimetre diameter. The capacity of an individual chamber was 48ml and consequently, whether P.E.G. or water was being circulated by the peristaltic pump, replacement of chamber contents was accomplished within half a minute, mixing being greatly facilitated by the small size of the chamber compared to the first model.

The general vibrations in the laboratory were too great to allow the recording of accurate growth rates for the lupin hypocotyls when the apparatus was on the bench top. Consequently the complete apparatus, apart from the digital voltmeter, circulating pump, and reservoir, was placed on a square slab of concrete, approximately 12cm in thickness and 0.5 square metres in area, supported on large rubber bungs placed on the laboratory bench. This step eliminated the interference by random disturbances in the laboratory but highlighted the fact that the peristaltic pump, used to circulate the root bathing solution, vibrated the transducer with each pulse. This problem, after many attempts to remedy it, was ultimately resolved by filling the root chamber by gravity feed from a reservoir mounted above the auxanometer, and removing the bathing solution via the peristaltic pump back up to the reservoir again, as indicated in Figure 2. This arrangement satisfactorily eliminated all disturbances except those rare occasions when something bumped the concrete block or those pieces of apparatus mounted on the block.

In an effort to minimise environmental influences on the auxanometer the complete apparatus was shifted to a controlled temperature growth room. However it was soon discovered that whereas the temperature of the room remained constant the humidity varied over quite a large range in a regular cyclic manner. Although this cycling was not desirable for later growth experiments it was utilised at this

time to ascertain whether or not humidity played a significant role in governing the growth rate of lupin hypocotyl tissue. A lupin seedling was mounted in the auxanometer and growth rates determined whilst monitoring the relative humidity. Figure 4 shows the results of this investigation. It appeared that the growth rate of lupin hypocotyls was very dependent on the relative humidity of their environment. In an effort to establish whether or not this relationship was in fact true more discriminatory tests were applied to the situation in terms of controls. To begin with the lupin seedling in the auxanometer was replaced by a piece of wire of approximately the same length and diameter and the experiment repeated under cycling humidity conditions again. The results were graphed in Figure 5 (a) where it appears that the wire has expanded considerably over the measured time interval. However, since temperature was constant during the course of the investigation, it was concluded that the mercerised cotton used to attach the transducer slug to the wire control, or lupin hypocotyl, expanded and contracted with changes in relative humidity. Figure 5 (b) is a repeat of experiment 5 (a) with the piece of wire but the mercerised cotton was replaced by a spun polyester thread. The polyester thread proved to be much more stable under changing humidity conditions and as a result all subsequent experiments were performed with the aid of spun polyester thread.

III AUXANOMETER - FINAL DEVELOPMENTS

Those developments outlined in the previous section were required to construct an apparatus capable of yielding reliable data on the growth of lupin hypocotyls over regular time intervals. One minute time intervals were chosen although the time interval could be varied to suit the observer with intervals as brief as 10 seconds being quite feasible. However further modifications were made so that the follow-

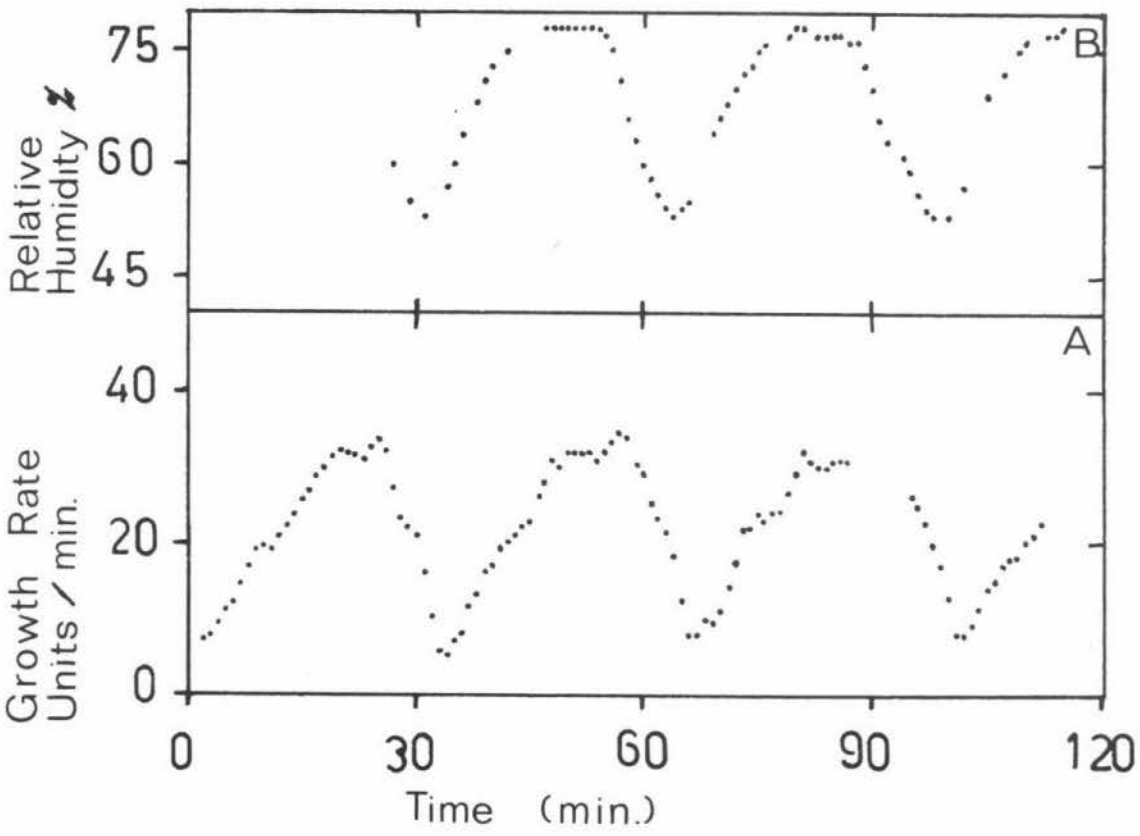


FIGURE 4 Apparent effect of variations in relative humidity, (B), on lupin hypocotyl growth rates, (A). (See text for interpretation).
(1 unit = 0.67 μm).

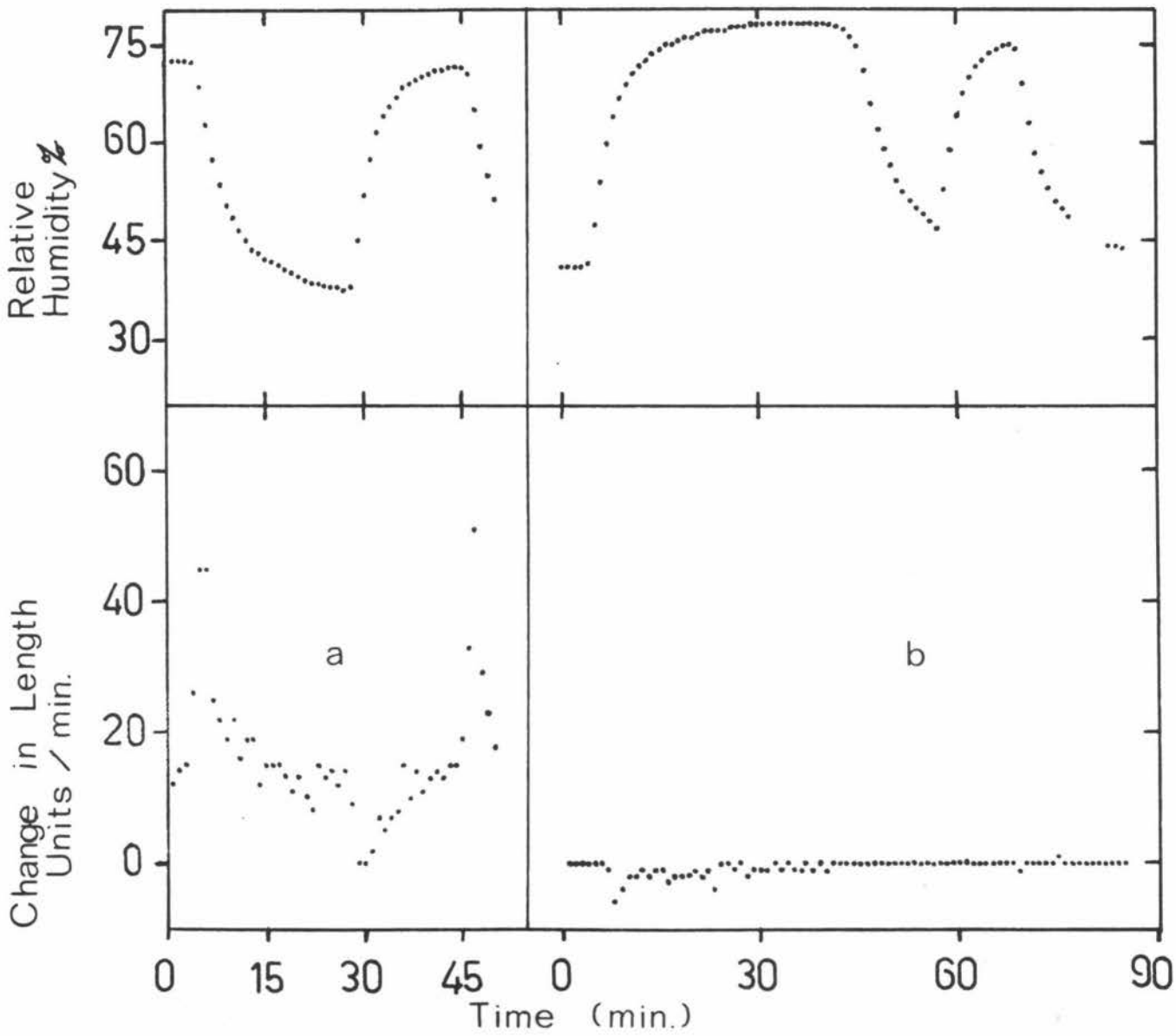


FIGURE 5 Effect of variations in relative humidity on mercerised cotton (a) and spun polyester thread (b).
(1 unit = 0.67 μm).

ing parameters could be varied independently during an experiment: - temperature, water potential, or aeration of the root bathing solution; temperature, relative humidity, or gaseous composition of the air supply; composition and intensity of light falling on the lupin seedling.

The monitoring of the environment during an experiment required the enclosing of the apparatus within a large chamber having its own regulated air supply. The design of a suitable chamber proved to be a rather difficult task since it was necessary to allow for the following pieces of equipment to pass through the chamber walls: the lead from the transducer to the carrier-wave oscillator-demodulator, two inlet and two outlet pipes to the root chamber, an air supply inlet and outlet, and a device for "resetting" the transducer slug when the voltage output was beyond the selected range (i.e. -3.999V - +3.999V). This latter requirement proved to be a considerable obstacle to the designing of a serviceable chamber. As described earlier, the pulley shown in Figure 2 was fastened onto a retort stand and when the voltage output was beyond the chosen range the pulley was reset higher up the stand. With careful manipulation the maximum duration of interruptions to growth recordings was 3 minutes. However, if this environmental control chamber was to be an open ended 5-sided perspex box placed over the entire apparatus, the repositioning of the transducer slug would involve the removal of the box and the consequent upsetting of the contrived environment. If the mechanism for raising the pulley had been mounted on the upper surface of the environmental chamber the previous problem would have been avoided but a more serious technical problem created in its place since it would have been impossible to set up the apparatus prior to obtaining experimental results. In the setting up of the apparatus for an experiment considerable time was spent each time to ensure both, that the pulley was in

such a position to allow the metal slug to descend into the transducer without scraping against the side, and, also that the hypocotyl was directly beneath the other side of the pulley. Consequently, if the pulley raising mechanism was fastened on top of the environmental chamber, the absence of opening sides on the chamber would make the initial adjustments required in setting up the apparatus an impossibility.

Figure 6 illustrates the manner in which the problem of a suitable environmental chamber was finally resolved. A perspex box with only five faces served as the environmental chamber (P) with sides (20 x 29.5)cm and a base of (20 x 20)cm. The remainder of the basic growth-detecting apparatus was mounted on an (18 x 18)cm perspex platform suspended from a (30 x 30)cm perspex top by four 26cm lengths of wooden dowel. When the lupin seedling was correctly mounted in the apparatus the entire unit a-b-c-d (Fig. 6) could be lifted off the bench and placed over the topless perspex box, effectively forming an environmental chamber of dimensions (20 x 20 x 29.5)cm. The height of the pulley (C), and consequently the metal slug (D), was adjusted by means of the objective lens racking mechanism (F), removed from an old microscope, fastened onto the lid of the environmental chamber. A metal rod was attached to the pulley wheel mounting, passed through a hole in the perspex lid, and was screwed into the base of the moving bracket, which formerly held the turret for the microscope's objective lenses. This mechanism allowed the resetting of the metal slug in the transducer during an experiment with less than a one minute interruption to growth rate measurements.

Figure 7 is a diagrammatic flow chart of the air supply and root chamber solution supply to the environmental chamber. Two gas lines were available to the apparatus, A1 and A2, one of which was connected to a compressed air supply. Air entered the environmental chamber (E.C. Fig. 7) at a rate of 6.8 litres per minute, allowing the chamber's air

FIGURE 6 Environmental Chamber.

A Solution outlet from root chamber to peristaltic pump.

B Air outlet from environmental chamber.

C Lead from transducer to carrier wave oscillator-demodulator.

D Metal slug.

E Transducer supported on stand.

F Racking device to alter pulley height.

G Pulley.

H Spun polyester thread.

I Lupin seedling.

J Seedling holder.

K Root chamber.

L Wooden dowel support.

M Air inlet to environmental chamber.

N Solution inlet from reservoir to root chamber.

O Polystyrene "buffer".

P Perspex environmental chamber.

a-d Perspex lid.

b-c Perspex platform.

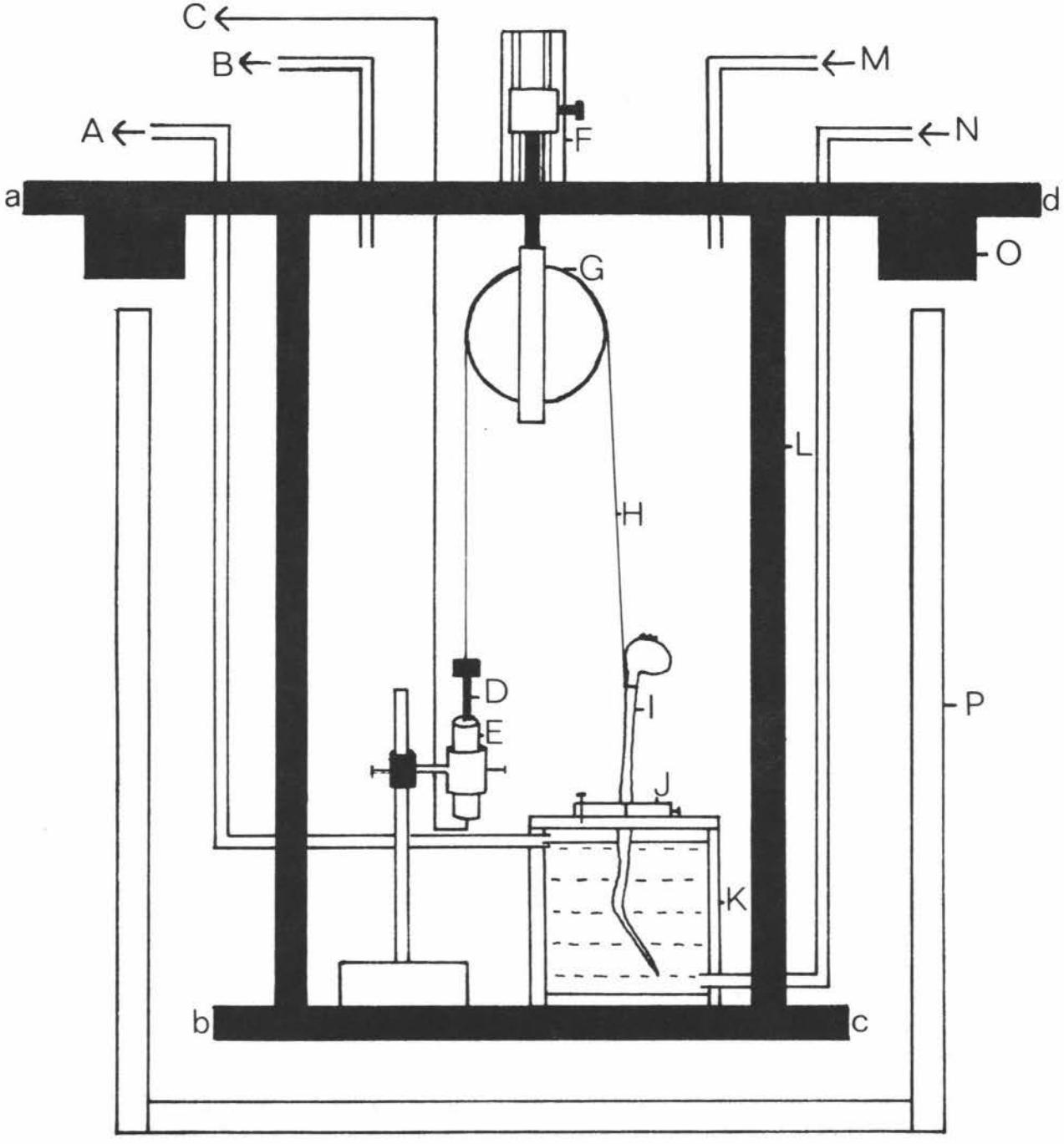

C
B
A
M
N
F
a
d
O
G
L
H
D
E
I
P
J
K
b
c

FIGURE 7 Diagrammatic flow chart of air supply and root chamber solution supply to the environmental chamber.

A_1, A_2 Independent gas lines.

$V_1 \ldots V_8$ Valves.

G_1, G_2 Gas flow meters.

D Air dehumidifying column.

H Air humidifying flask.

C Air cooling unit.

W_1, W_2, W_3 Water baths.

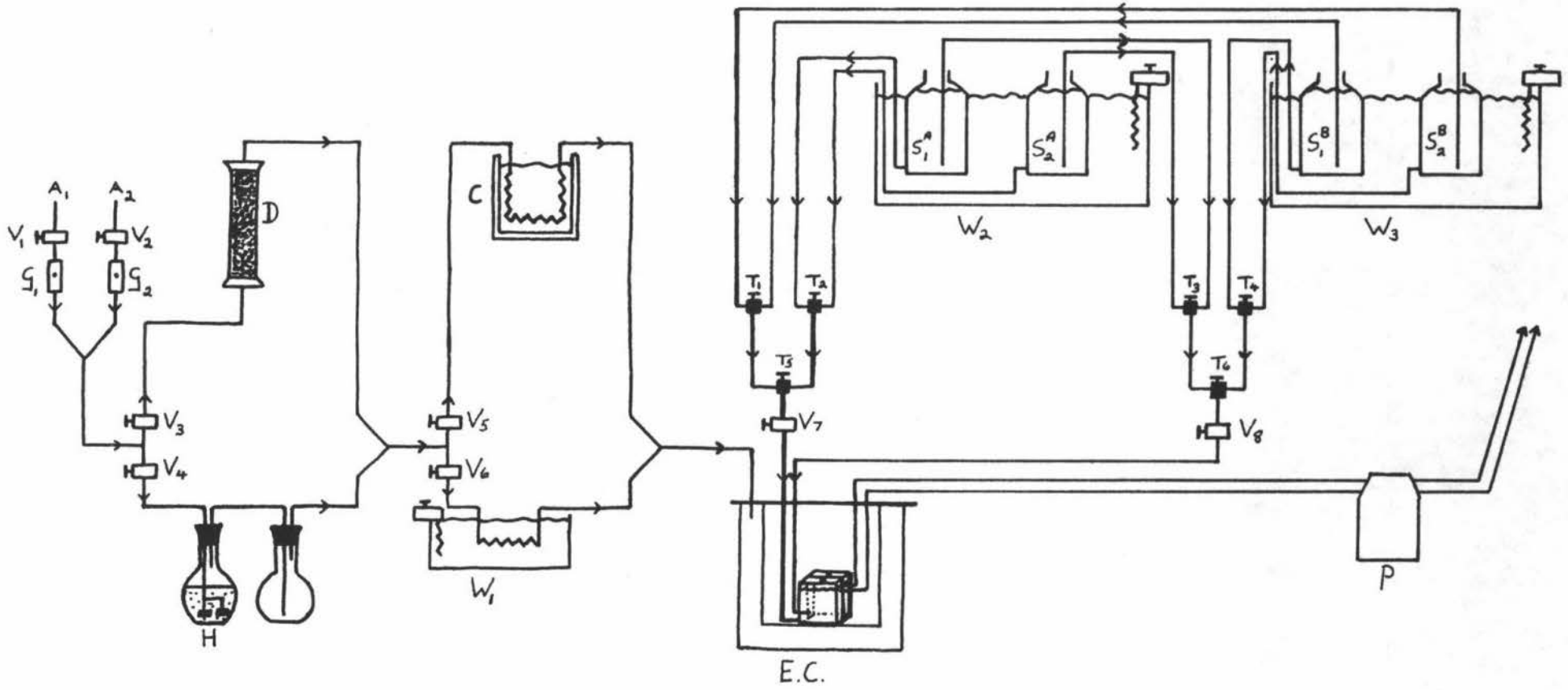
$T_1 \ldots T_6$ Taps allowing flow from either pipeline.

P Peristaltic pump returning root bathing solutions to their respective reservoirs.

S_1^A, S_2^A Root bathing solutions one and two at temperature A.

S_1^B, S_2^B Root bathing solutions one and two at temperature B.

E. C. Environmental chamber.


A1
A2
V1
V2
S1
S2
D
C
V3
V4
V5
V6
W1
H
T1
T2
T3
T4
V7
V8
W2
W3
P
E.C.

content to be completely replaced within two minutes. The other gas line was connected to a compressed supply of either oxygen, carbon dioxide, or nitrogen. Valves V1 and V2 controlled the rate of flow of either gas supply and were utilised in the production of the gaseous mixtures required in various experiments - e.g. 80% CO_2: 20% O_2. Gas flow meters G_1 and G_2 enabled a check to be kept on flow rates during an experiment. The settings of valves V3 and V4 determined the amount the air was either dried or moistened to achieve the desired relative humidity in the environmental chamber. The relative humidity within the chamber was calculated with the aid of wet and dry bulb thermometers having accuracies of $\pm 0.5C^{o}$. The air was dried by passing through a 10cm diameter glass column, 40cm in length, packed with a silica gel/potassium perchlorate mixture and plugged with glass wool at both ends. The humidifier consisted of a two litre flat-bottomed flask 1/3 filled with water. The air supply was passed through the water as minute bubbles formed with the aid of two sandstone aerators of the type used in aquariums. Another flask was connected to the humidifier to trap excess water that overflowed or condensed in the outlet tube. This apparatus for controlling the relative humidity of the environmental chamber, although serviceable, was limited in that obtainable relative humidities were confined to the range 45% to 90% R.H. Consequently a more sophisticated apparatus, incorporating refinements such as refrigerated copper coils for the removal of water vapour, would be required for detailed studies of R.H. levels and plant growth rates.

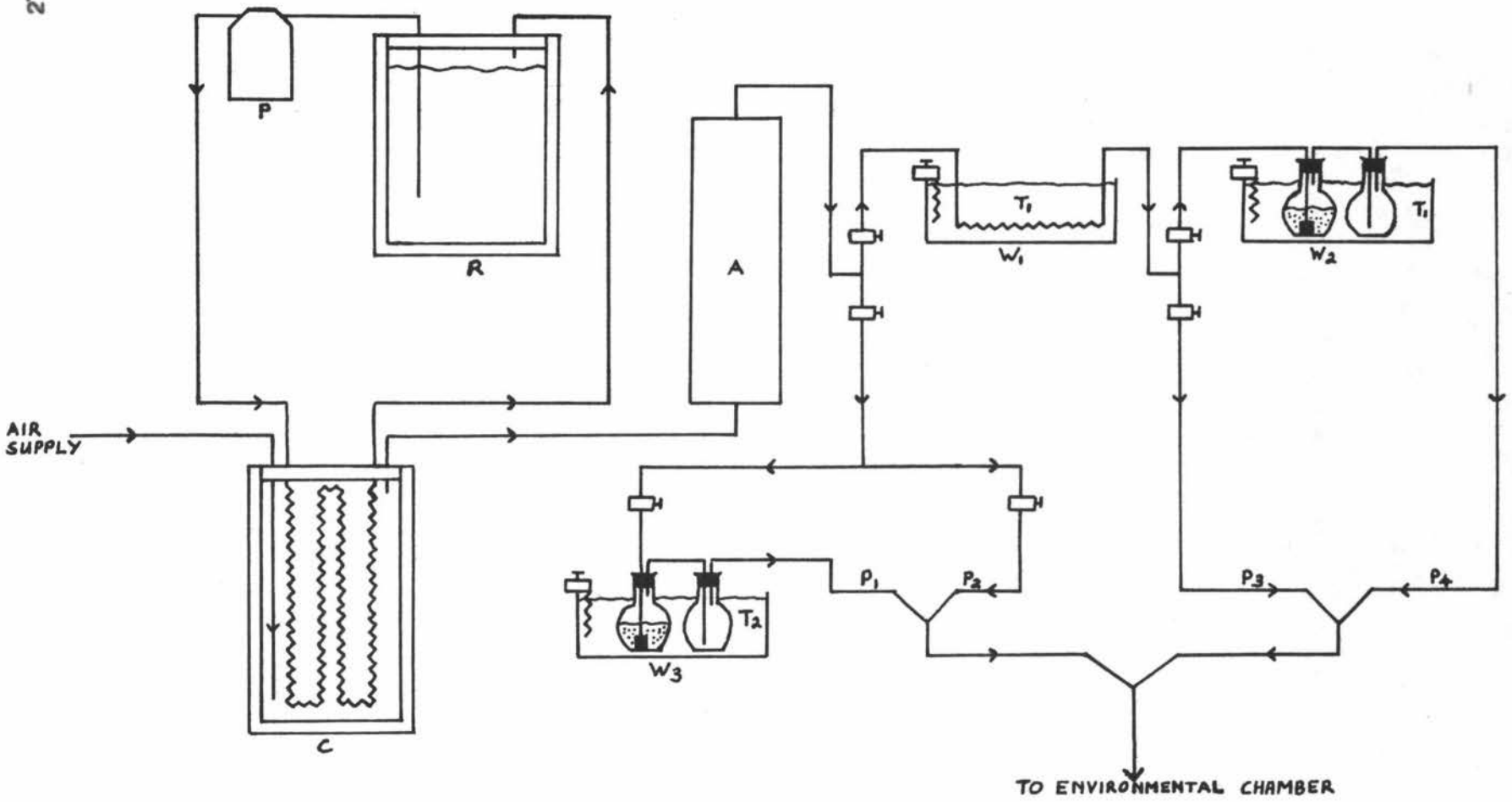
Valves V5 and V6 determined the proportions of the air supply being cooled and heated to attained the required air temperature within the environmental chamber. The air supply was heated by passing through an approximately 1.2m length of 0.5cm diameter coiled glass tubing immersed in a constant temperature bath (W). Cooling was achieved by the air flowing through an approximately 0.5m length of 1.5cm

diameter copper tubing mmersed in iced water, contained within an insulated container (c). Unfortunately this system was of little value in experiments designed to investigate the effects of varied shoot temperatures on growth rates. This was due to the fact that the described apparatus altered the temperature of the air passing through the system by only 4 or 5C°. The cooling system also lowered the relative humidity as water vapour condensed in the copper tubing. The temperature control apparatus did, however, act in a buffering capacity minimising temperature fluctuations in the air supply. The volume of the air treatment system was necessarily kept to a minimum so that changes in the gaseous composition of the air supply could be executed rapidly and at determinable times. Due to this requirement the volume of air being either heated or cooled per unit time was large compared to the volume of apparatus and this disparity proved to be too excessive in the described apparatus.

An alternative and perhaps more satisfactory design for humidity and temperature control, using similar apparatus, is illustrated diagrammatically in Figure 8. Air would initially be cooled and some water vapour removed by passing air over coiled copper pipes in an insulated drum (C). The copper pipes would contain cold water circulated from an insulated reservoir (R) by a peristaltic pump (P). The copper pipes would be coiled in such a manner as to present the maximum possible surface area to the incoming air. The cooled, low humidity, air would then pass through a vessel (A) containing potassium perchlorate, or similar absorbent, for the further removal of water vapour. The air emerging from this absorption vessel could then be further treated if a higher temperature and/or higher relative humidity was required. Air would be heated by passing it through coiled copper pipes immersed in a water bath (W) at a higher temperature T1. The same apparatus as described in Figure 7 could be used in humidifying the air, the only modification being that the flasks would be

FIGURE 8 Alternative design of air supply for humidity and temperature control.

C	Cooled insulated drum.
R	Reservoir of cold water.
P	Peristaltic pump.
A	Dehumidifier.
W1,W2,W3	Water baths.
P_1	Pipeline with low temperature/high humidity air.
P_2	Pipeline with low temperature/low humidity air.
P_3	Pipeline with high temperature/low humidity air.
P_4	Pipeline with high temperature/high humidity air.


P
R
A
T_1
W_1
T_1
W_2
AIR SUPPLY
C
T_2
W_3
P_1
P_2
P_3
P_4
TO ENVIRONMENTAL CHAMBER

mounted in water baths of similar temperatures to the treated air. Pipelines P1, P2, P3, and P4 containing low temperature/high humidity, low temperature/low humidity, high temperature/low humidity, and high temperature/high humidity air, respectively, would all join and flow into the environmental chamber. The illustrated valves would regulate the flow rates within the various pipelines to give the desired conditions within the environmental chamber.

In addition to the air treatment system Figure 7 also illustrates the root chamber solution supply. Valves V7 and V8 regulate the pressure of supply to each of the two independent compartments within the root chamber. The illustrated scheme allows for the solute concentration within either of the two compartments to be varied independently and consequently the possibility of comparing growth rates of two plants under otherwise identical environmental conditions. Water baths W2 and W3 could be maintained at temperatures TA and TB so that root temperatures could be rapidly altered during the course of an experiment. It was found necessary to enclose the root chamber with a 1cm thick covering of polystyrene to stop the heating of the environmental chamber's air supply by the root chamber when root solutions at high temperatures were used. The two-litre reservoirs contained different bathing solutions - e.g. S_1A and S_1B distilled water, S_2A and S_2B 0.25m mannitol. The taps T1, T2 etc. allow solution to flow from either the left or right hand pipeline but not from both simultaneously. The solutions were delivered to the root chambers by gravity feed at a rate of 120ml/minute and were pumped back up to the reservoirs by the peristaltic pump described earlier.

The environmental chamber was illuminated by two 40W tungsten bulbs placed at a distance of 20cm from apposite sides of the chamber. The resultant light intensity within the chamber was approximately 1.3 W/m^2.

The described environmental chamber, with its accompanying air and root bathing solution systems, coupled to the growth recording apparatus has proved itself as a viable research tool. However, the digital voltmeter has the disadvantage that data is not automatically recorded and consequently voltages must be manually recorded each minute during the course of an experiment. Fortunately this problem has been alleviated, since the completion of work described in this thesis, by the interfacing of the digital voltmeter with a Solartron Data Transfer Unit (Models 3230, 3203, 3204, 3210, and 3214) and the recording of the output on a Teletype Model 545. The data transfer unit copes with inputs from up to 20 transducers and is capable of scanning at preselected intervals of between one second and two hours. Results may be displayed on the teletype page printer or punched onto paper tape for computer analysis thus considerably improving the practicality of this method for the continuous measurement of plant elongation.

D. RESULTS AND DISCUSSION

I INTRODUCTION

The auxanometer described in the previous section was utilised to illustrate its versatility and potential as a viable research tool in the study of plant growth. No aspect of plant growth was studied exhaustively but, rather, the auxanometer was used with intact Lupinus angustifolius seedlings to highlight various facets of short term growth kinetics that may deserve a closer investigation than they have received up to the present time. Where possible experiments were of a similar nature to those conducted with lupin hypocotyl segments (Penny 1969; Penny D. et al 1970, 1972; Penny P. et al 1972; Penny et al 1974) so that comparisons could be made between the growth responses of intact and excised hypocotyl tissue.

The results are presented under a number of sections, each corresponding to a particular type of environmental treatment administered to the lupin seedlings.

II THE EFFECT OF CHANGES IN RELATIVE HUMIDITY ON HYPOCOTYL ELONGATION

a) Introduction

The effects of varied relative humidities on short term growth kinetics have scarcely been mentioned in the literature although some investigators (Pareek et al 1969, Krizek et al 1971) have noted marked effects of differing relative humidity regimes on plant growth over time intervals of a week or more in duration. Krizek et al, for example, noted that increasing relative humidity from 40% to 90% nearly doubled stem length in ageratum but yet had little effect on marigold over a fourteen day period.

Although observations of relative humidity effects on short term growth kinetics are lacking, there is literature published describing short term responses of stomatal aperture, transpiration, leaf water potentials (ψ leaf), and water uptake in response to changes

in relative humidity. Some of these results have been included below to act as a basis for discussion as to the possible effects of relative humidity changes on plant elongation rates.

Boresch (1933) noted large cycle variations in transpiration of onion plants, under conditions of low relative humidity, which he postulated as being due to cyclic variations in stomatal aperature. Ehrler et al (1965) produced evidence supporting this theory showing that under a saturation deficit (S.D.) of 40 mbar and full intensity illumination, leaf thickness in cotton fluctuated in a cyclic manner with a period of approximately 30 minutes due to cyclic variations in stomatal aperture. Barrs and Klepper (1968) measured leaf water potentials (ψ leaf) of cotton during cyclic variations of transpiration. ψ leaf values obtained ranged from -6 to less than -20 bars, with the lowest ψ leaf being reached approximately 10 minutes after maximum transpiration had occurred. Since the plants examined were growing in either nutrient solution (osmotic potential ψ s = 0.6 bars) or water Barrs and Klepper postulated the existence of an appreciable resistance to water flow within the plant which consequently caused the rate of water loss to exceed the rate of uptake. The major portion of this resistance was shown to be situated in the roots and variations in its magnitude during the day being responsible for the observed spontaneous (non-induced) transpirational cycling found in cotton plants.

In contrast to the 30 to 60 minute period transpirational cycling described by Barrs and Klepper is the reported existence of fluctuations in leaf water balance with a period of one to 10 minutes occurring in a number of species. (Sheriff and Sinclair, 1973). The fluctuations were obtained when dry nitrogen or carbon dioxide (S.D. approximately 22 mbar) was passed through the leaves. These short term, rather

irregular, fluctuations were not correlated with any changes in leaf resistance but occurred only under conditions of high transpiration and relatively low water stress. Since these fluctuations occurred more readily in excised rather than intact leaves, it appeared that the resistances in the rest of the plant were normally sufficiently high to keep the leaf under enough stress to dampen the fluctuations.

The evidence from these described investigations all indicate that plant water balance is affected by relative humidity changes, the magnitude of the change appearing to depend considerably on ψ soil. As the saturation deficit becomes greater ψ leaf falls, although depending on the behaviour of stomata and magnitude of root resistance etc, leaf water balance may be restored relatively quickly. The relationship between cell turgor pressure and leaf water potential can be expressed in simple terms as:-

ψ leaf = ψ p + ψ s

ψ P = pressure potential or turgor pressure (normally a positive component).

ψ s = solute potential (a negative component).

ψ leaf = leaf water potential.

A fall in ψ leaf of two or three bar due to higher saturation deficits would involve a reduction in ψ p of two or three atmospheres, since ψ s appears to decrease only under conditions of high concentrations of absorbable solutes in the root medium (Boyer 1965, Slatyer 1961) or over periods of the order of one or two days (Ruf et al 1963, Janes 1965). Green et al (1971) found that growth responses of Nitella cells could be explained by the equation:-

$$r = (P - Y)m$$

r = cellular elongation rate.

P = turgor pressure.

Y = minimum turgor pressure required to allow growth (wall yielding threshold).

m = wall extensibility, or fluidity.

The effective driving force for elongation (P - Y) was found to be approximately 0.2 bar in Nitella and consequently elongation rate is extremely sensitive to changes in P. Therefore, decreases in ψ leaf, and consequent reductions in ψ p, caused by increases in saturation deficit should reduce cellular elongation. In fact, growth was halted by a drop in ψ leaf to approximately -4 bar in sunflower (Boyer 1968), -7 bar in maize (Acevedo et al 1971) and -12 bar in soyabean (Boyer 1970). These values of ψ leaf are all within the range measured by Barrs and Klepper (1968) during transpirational cycling. Since ψ s of elongating lupin hypocotyls is approximately -7.5 bar (Penny et al 1970) one would expect ψ p to be of the order of 7 bar in turgid hypocotyls. However, it has been found in the present work that when the water potential of the solution bathing rapidly elongating lupin hypocotyl segments is reduced from 0 to -2.5 bar (Figure 13 e) growth is halted within three minutes. These results, together with the varied responses observed in plant water relations under high environmental saturation deficits, suggest that fluctuations in growth rates of lupin hypocotyls are to be expected to accompany any changes in S.D. In fact, prior to the discovery of the response of mercerised cotton to relative humidity changes (page 18), it had appeared that humidity played a most important role in determining growth rates of lupin hypocotyls (Figure 4). Due to this fact, and also since so little work had been reported in this field, lupin seedlings were subjected to differing relative humidities and their growth responses recorded.

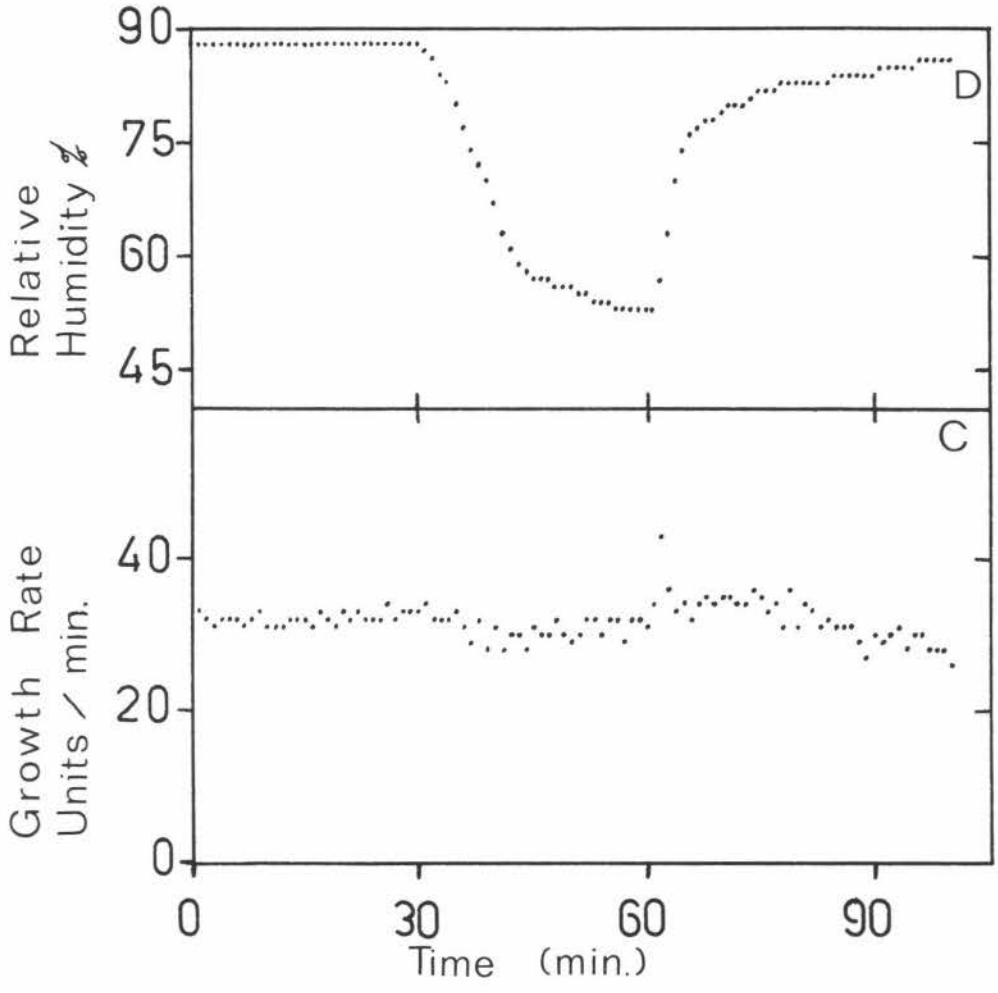


FIGURE 9 **Effect of variations in relative humidity on lupin hypocotyl growth rates.**
(1 unit = 0.67 μm).

b) Results and Discussion

Changes of relative humidity within the range of 45 to 90% R.H. at 24° C, corresponding to vapour pressure deficits between 16.2 and 2.9 mbar, were achieved in the described apparatus.

Within the range of saturation deficits obtainable growth rates of lupin hypocotyls were only slightly altered (Figure 9). Generally, growth rates were slightly depressed by increases in saturation deficit and slightly elevated by decreases in saturation deficit. In no cases were changes of relative humidity observed to give rise to cyclic variations in growth rate. However, there did appear to be a "compensating" mechanism whereby, after the initial response to change of relative humidity, the growth rate tended to return to "normal" after a period of 15 to 25 minutes. The duration of this return to normal growth rates should however be investigated further since it was found that seedlings pretreated for three to four hours at saturation deficits of 14 $\pm$ 2 mbar had growth rates averaging 20% lower than those treated for the same time interval at saturation deficits of 5 $\pm$ 2 mbar.

A theory to explain why lupin hypocotyl elongation is only slightly altered by changes in saturation deficits could not be formulated without further investigations. However, two possible causes which should be considered are the rate of air movement in the growth chamber and the water potential of the root bathing solution.

Under still air conditions, changes in relative humidity may not affect plant water balance.

$$E = \frac{Cw - Ca}{rl + ra}$$ Slatyer (1967) Page 248

E = transpiration rate

(Cw - Ca) = difference between the water vapour concentrations

at the evaporating surfaces within the leaf (Cw) and in bulk air (Ca).

(rl + ra) = the combined resistance to vapour diffusion within the leaf (rl) and in the boundary layer (ra).

Under still air conditions, ra is much greater than rl so that even if the saturation deficit increases markedly, i.e. (Cw - Ca) increases, ra may be sufficiently high that changes in E are negligible and consequently plant water balance would not alter significantly. Since the environmental chamber used in this study contained only 12 litres of air when empty and the air flow through the chamber was at a rate of 6.8 litres per minute it is considered unlikely that the boundary layer resistance (ra) was exceptionally high.

The effect of the water potential of the root bathing solution on responses of elongation to changes in saturation deficits is likely to be significant. Beardsell et al (1973) showed that the relative water content of soyabean leaves becomes significantly different between plants under saturation deficits of 5 or 12 mbar only when Ψ soil is lower than -0.2 bar. Raschke and Kuhl (1969) demonstrated that stomata in Zea mays leaves did not close when the S.D. was lowered markedly unless the water potential of the liquid supplying the leaves was lowered from 0 to -2 bar. Since water was used as the root bathing solution for the lupin seedlings, it could be expected that variations in saturation deficit would not greatly affect the hypocotyl water potential. Coupled to this any water deficits in the hypocotyl caused by higher saturation deficits would probably be rapidly replenished due to the low resistance to water flow in such a small and young plant.

The relationship between hypocotyl elongation, saturation deficit,

and root bathing solution water potential can be defined only after further investigations involving the monitoring of hypocotyl water content, possibly with a B gauge (Kleinendorst et al 1970), and elongation simultaneously over a wider range of saturation deficits than those obtained in the present study. Ehrler et al (1965) and Lang and Gardner (1970) increased saturation deficit by raising the air temperature from 25 to 35° C in their investigations of water losses and uptake by plants under high saturation deficits. However, since effects of higher temperatures on growth rates could easily mask any relative humidity effects this method was not attempted in the present study. A relatively inexpensive method for increasing the range of obtainable saturation deficits with the described auxonometer has been outlined previously (Figure 8).

III THE EFFECT OF CHANGES IN ROOT TEMPERATURE ON HYPOCOTYL ELONGATION

a) Introduction

Penny D. et al (1972) found that the responses of lupin hypocotyl elongation to changes in temperature were essentially the same as those obtained by Ray and Ruesink (1962) with Avena coleoptiles, and consequently concluded that cellular elongation is limited by the rates of metabolic reactions rather than by a physical property such as the viscosity of the wall. However, at higher temperatures, Penny D. et al (1972) obtained an unusual result in that when the temperature was decreased from 32° to 27° C, for example, there was a marked drop in growth rate for about 10 minutes before a new steady growth rate, very close to the original rate at 32° C, was attained. On return to 32° again there was a marked increase in rate for 10 minutes followed by a return to the original rate.

Originally, it had been planned to duplicate these conditions in the present study with intact seedlings but, as explained earlier

(page 26), the system used for regulating the air supply temperature was only capable of coping with changes in the order of 4 to 5 C° over a 10 minute interval. However, there are several reports in the literature on the effects of root temperature variations on plant growth (Neilson and Humphries 1966), (Unger and Danielson 1967), (Nelson 1967, Watts 1971). By altering the root bathing solution temperature results with growth rates of lupin hypocotyls could be readily obtained and compared with previously reported findings even though most of these concerned growth over longer time intervals.

b) Results and Discussion

Initial experiments showed that the temperature of the root chamber seriously influenced the air temperature of the environmental chamber - a 10 C° rise in root chamber temperature was accompanied by a 4 C° rise in air temperature within an hour. A polystyrene jacket, approximately 1 cm in thickness was made to enclose the root chamber in an effort to minimise temperature fluctuations in the environmental chamber. As a consequence of this insulation temperature changes in the environmental chamber were confined to ± 0.5 C° for 10 C° changes in root bathing solution temperatures.

In all experiments the temperature of the air surrounding the hypocotyl was maintained at a constant temperature of 25 ± 1° C.

Figure 10A illustrates how the growth rate of the hypocotyl increased, for a period of 10 minutes, before returning to the original rate when root temperature was raised from 23 to 33° C. A temporary drop in growth rate of similar magnitude followed the lowering of the temperature back to 23° C. The resultant growth increase from raising the temperature 20 C°, (23 to 43° C), was

double that of the excess obtained from the 10 C^{o} rise in temperature.

These results appeared to parallel those of Penny D. et al (1972) obtained when measuring the growth rates of hypocotyl segments at temperatures in excess of 27^{o} C. However, in the change from a root temperature of 33 to 23^{o} C the hypocotyl growth rate was temporarily depressed so for that the hypocotyl was recorded to be shrinking over a brief period of time. The validity of this result raised the possibility that the results may have been largely influenced by expansion and contraction within the auxonometer.

In order to ascertain the magnitude of any spurious effects contributing to the recorded hypocotyl growth rates the experiment was repeated, firstly under anaerobic conditions (Figure 10B), and secondly with a six day old lupin seedling, the hypocotyl of which had virtually ceased elongating (Figure 10C). In both experiments, the growth rate stimulations and depressions were of a similar magnitude to those of the normal rapidly growing seedling (Figure 10A). These results effectively precluded the possibility of the observed results being due to effects of temperature on metabolic processes within the plant and consequently, unless lupin hypocotyls have a coefficient of expansion approximately 20 times that of most metals, the observed results were largely due to expansion and contraction of the auxonometer.

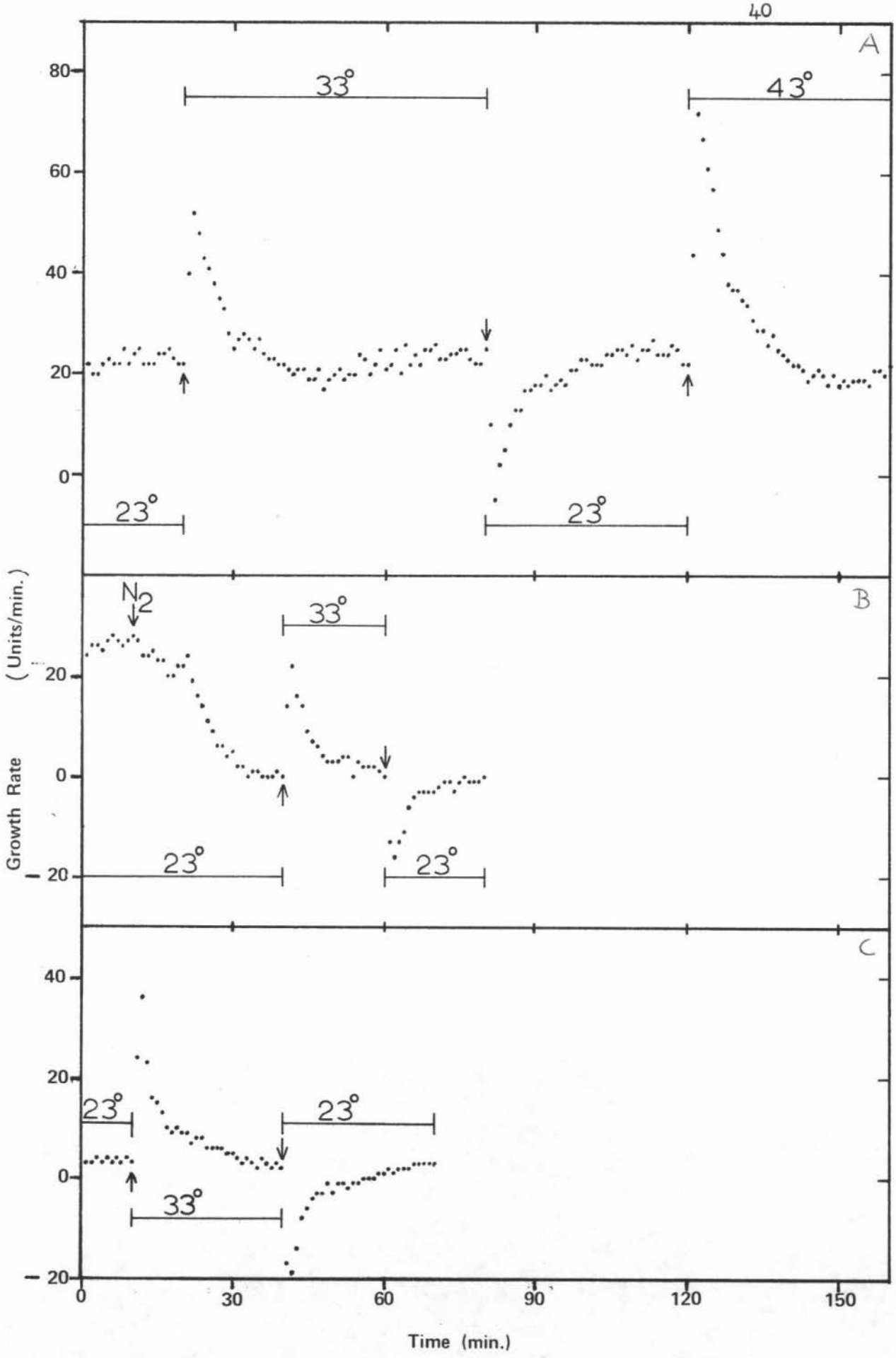
Reference to Figure 6 (page 23) shows that the perspex root chamber (K) must have been responsible for any expansion and contraction recorded on the transducer during alterations of the temperature of the root bathing solution. Data from ''The Properties of 'Perspex' Acrylic Materials'', (page 15), indicates that the coefficient of linear thermal expansion for perspex is 7.3 x 10^{-1} μm/cm C^{o} - a value approximately nine times greater than that of metals. Since the

FIGURE 10 Effect of variations in root temperatures on lupin hypocotyl growth rates.
(1 unit = 0.67 μm).

A Normal four day old seedling.

B Normal four day old seedling under anaerobic nitrogen conditions.

C Six day old seedling.


A
33°
43°
23°
23°
80
60
40
20
0
B
N2
33°
23°
23°
20
0
−20
C
23°
23°
33°
40
20
0
−20
0
30
60
90
120
150
Growth Rate (Units/min.)
Time (min.)

root chamber was 10.8 cm in height the total expected expansion for a 10 C° rise in temperature would be 79 µm. If it is assumed that the results obtained under anaerabic conditions (Figure 10B) and with six day old seedlings (Figure 10C), in response to temperature changes, can be regarded as illustrating the expansion and contraction of apparatus alone an estimate can be made of the magnitude of the expansion over a 10 C° rise in temperature. These results yield an average of 88 um expansion during a 10 C° rise in temperature - a figure remarkably close to that predicted from the thermal properties of perspex. A figure of this magnitude for expansion and contraction of the apparatus accounts for between 85 and 100% of all changes in length previously attributed solely to variations in growth rates of lupin hypocotyls due to alterations in root temperatures (Figure 10A).

Kleinendorst and Brouwer (1970) were able to maintain shoot temperatures of maize seedlings at 25°C while the root temperature was altered from 20 to 5°C. During the two hours following this change shoot growth was reduced to 30% of its former rate. Simultaneously leaf water content decreased and consequently the reduced growth rate of the shoot was attributed to low turgor pressure arising as a result of the reduced water permeability of the root tissue at low temperatures.

Unger and Danielson (1967) in a study of the water relations and growth of beans under varied root temperatures were unable to monitor growth rates as accurately as Kleinendorst and Brouwer. However, with bean plants maintained at a shoot temperature of 25°C, they showed a significant difference between the water use of plants with root temperatures of 32.5, 25.0, or 17.5°C and those with root temperatures of 10.0°C. Plants with roots maintained at 10°C used only 20 - 23% of the amount of water that those at 32.5, 25.0 or 17.5°C

used in one day. Plants subjected to 10° C root temperatures were noted to wilt severely within 30 minutes whilst slight to moderate wilting was observed in plants transferred to root temperatures of 15.0 or 17.5° C even though turgidity soon returned. After one day at these root temperatures the relative turgidity of those at 10° C was only 81% as compared to values of between 91 and 93% for those at the higher temperatures.

Neilson (1967) working with cotton seedlings, recorded similar results to those of Unger and Danielson. In addition he noted that the rate of increase in stem fresh weight was depressed by a root temperature of 12° C but virtually unaffected by root temperatures of 18 or 24° C for 24 hours.

One of the few reports of investigations on the effects of varied root temperatures on short term elongation rates is by Watts (1971). Watts utilised an auxanometer, capable of detecting changes in rates of extension within two minutes, to study the responses of leaf elongation in Zea mays to changes in root and/or meristematic temperatures. In experiments of a similar nature to those described in the present study, with lupin seedlings, Watts demonstrated that the rate of leaf elongation in plants with shoots kept at 25° C and 100% relative humidity was raised only 10% during a change from 0 to 25° C in root temperature.

The result obtained with lupin seedlings at varied root temperatures confirm the conclusion reached by Watts that root temperature fluctuations have little effect on shoot elongation rates, over short time intervals, provided the shoot is maintained at a constant temperature. However, it must be stressed that Watts obtained different results when the relative humidity was dropped to 50% and, consequently in future extensions of the described investigations with lupin

seedlings relative humidity will have to be monitored along with root and shoot temperatures. Also, in order to simplify the interpretation of results an attempt must be made to eliminate the effects of expansion of the perspex root chamber.

This problem could be avoided by redesigning the root chamber to be attached by its top to a stable platform within the environmental chamber. Expansion of the root chamber would then be downwards and consequently would not be detected by the transducer.

IV THE EFFECT OF CHANGES IN THE OSMOTIC POTENTIAL OF THE ROOT BATHING SOLUTION ON HYPOCOTYL ELONGATION.

a) Introduction

There is a considerable volume of literature available on the effects of water stress on plant growth (e.g. Slatyer 1957, Jarvis and Jarvis 1963, Gates 1964, Janes 1966, Wardlaw 1969, Jordan 1970). However, few of these studies have involved the sampling of the relevant parameters at intervals of less than 12 to 24 hours. Consequently the described auxanometer was utilised in an attempt to describe the kinetics of lupin hypocotyl growth rates over much shorter time intervals. An analysis of the short term growth responses to water stress may help to elucidate the sequence of events leading to reduced growth rates when the plant is subjected to water stress.

The significant role of turgor pressure in cellular elongation has been recognised for some time (Thimann and Schneider 1938, Broyer 1950, Cleland 1959, Green 1968). Cleland (1959) and Green et al (1971) have demonstrated that cellular elongation occurs when the turgor pressure (P) exceeds a particular critical value (minimum yield stress Y) and it is proportional to the amount by which turgor pressure exceeds

this value - i.e. (P - Y). Consequently it may be expected that small reductions in P, due to the imposition of water stress, may stop growth whilst a rise in P of similar magnitude may greatly accelerate the growth rate.

It is possible that during growth inhibition, under reduced root bathing solution potentials, some growth processes may continue but yet remain unexpressed due to the low turgor pressure ($(P - Y) \leqslant 0$). On the return to higher turgor pressures there would be a very rapid growth burst with the tissue possibly reaching the length it would have been, had it not been subjected to stress. Growth which remains unexpressed until the removal of an inhibitory factor has been termed "stored growth". Ray (1961) showed the existence of stored growth in Avena coleoptile segments after their removal from mannitol solutions, although those segments treated with auxin did not exhibit this property. The results of Green et al (1971) with Nitella are much more complex but do show the existence of some stored growth. Penny D. et al (1972), however found little evidence for stored growth in lupin hypocotyl segments either in the presence or absence of auxin. In this study it is possible to compare the growth responses of intact and excised lupin hypocotyl tissue to changes in water potential, with a sampling interval of one minute in both cases.

b) Results and Discussion

Hypocotyl growth rate responses to changes in the osmotic potential of the root bathing solution, (ψ sol), were varied, depending considerably on magnitude and duration of stress and also, to a large extent, on the individual plant under study.

Figure II illustrates a generalised response to changes in osmotic potential. When stress was imposed, growth rates fell to a

minimum level, within four to 14 minutes (1 in Figure II). This initial low rate was obtained more rapidly with smaller changes in osmotic potential: - four to seven minutes for a change of -2.6 bar and 10 to 14 minutes for changes between -5.3 and -14.3 bar. Growth rates then usually recovered slightly to reach a new steady rate between 20 and 25 minutes after addition of the osmoticum, (2 in Figure II). On converting the root bathing solution back to water growth rates were immediately stimulated to reach a peak value approximately 15 minutes later, (3 in Figure II). However, exposure of the roots to a water potential of below -11 bar for periods in excess of 20 minutes delayed this increase in growth rate by 35 to 75 minutes. A comparison of graphs a and b in Figure 12 illustrates that the timing of the maximum appears to depend more on the water potential than on the length of exposure. A -14 bar stress imposed for 60 minutes (a) delayed the water stimulated maximum 66 minutes longer than a -5 bar stress imposed for the same length of time (b). Growth rates returned to original values between 35 and 45 minutes after the removal of osmotic stress, (4 in Figure II). The growth burst observed on increasing the water potential often completely counteracted, or exceeded, the growth lost during the growth inhibition period in over 70% of those experiments where stress did not exceed -2.6 bar (Figure 12 c) - i.e. area of "b" $\geqslant$ area of "a" in Figure II. Although a growth burst was still evident following stresses of -5 bar, and greater, it never completely compensated for the inhibition of growth during the period of stress in the experiments conducted in this study.

In some cases, growth rates were observed to cycle with a period of between 50 and 80 minutes after the restoration of high water potentials. Cycling was more strongly established after low, rather

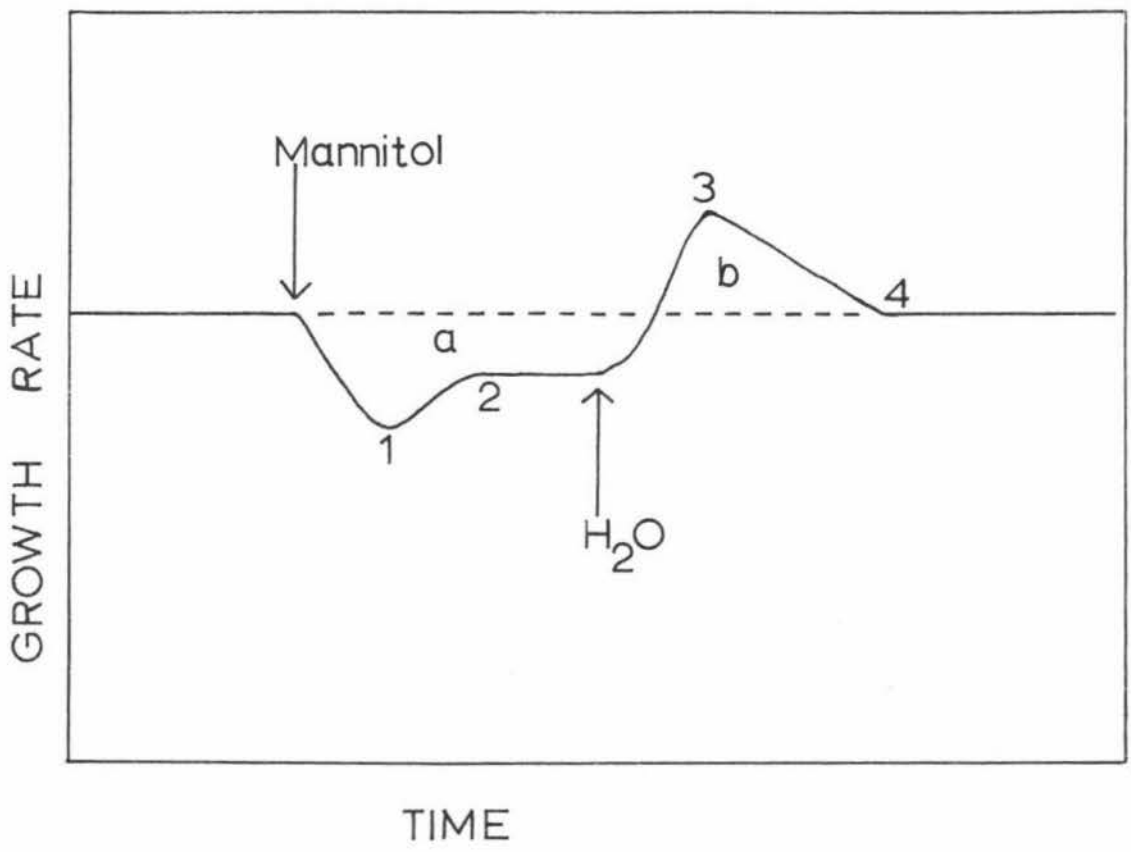


FIGURE II Generalised response of Lupin hypocotyl growth rates to changes in the osmotic potential of the root bathing solution.

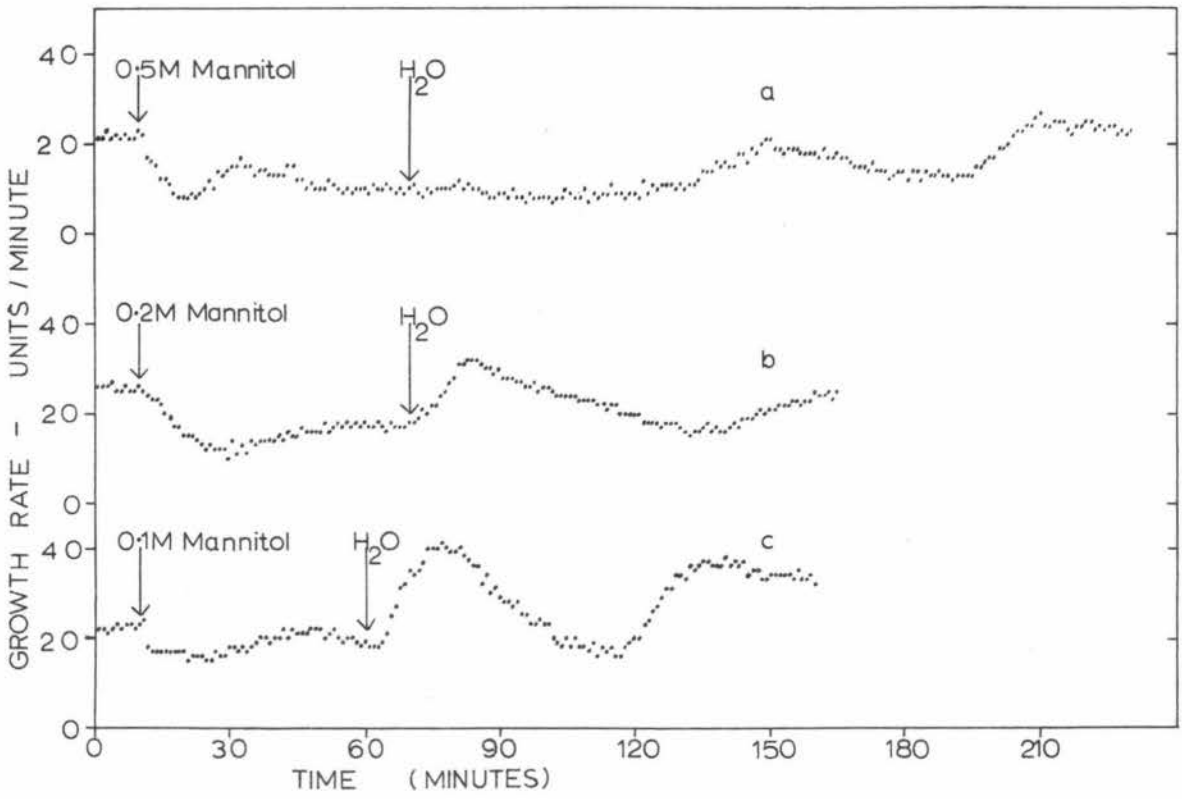


FIGURE 12 **Lupin hypocotyl growth rate responses to different osmotic potentials, (ψ sol), of the root bathing solution. (1 unit = 0.67 μm).**

a. ψ **sol is approximately -14 bar**
b. ψ **sol is approximately -5 bar**
c. ψ **sol is approximately -2.6 bar**

than high, stresses (Figure 12). Due to the length of the cycling period and the fact that, since results were manually recorded, few experiments exceeded three hours in duration, the persistency of this cycling is unknown.

Stomatal aperture fluctuations with a similar period of cycling have been described by a number of workers (Ehrler et al 1965, Barrs and Klepper 1968). Consequently an experiment was designed to test the significance of stomatal behaviour in the described cycling.

Seedlings were mounted in the root chamber (Figure 2) and then the exposed aerial portions of the seedlings liberally coated with Shell snow white petrolatum prior to being mounted in the growth chamber for an equilibration period of three to four hours. Graphs a and b of Figure 13 illustrate the growth responses of two such plants to stress of -2.6 bar for 30 and 10 minutes respectively. Figure 13 c is the response of an uncoated plant to stress of -2.6 bar for 10 minutes. These results rule out the possibility of stomata playing any significant role in the observed growth responses to changes in water stress in lupin seedlings.

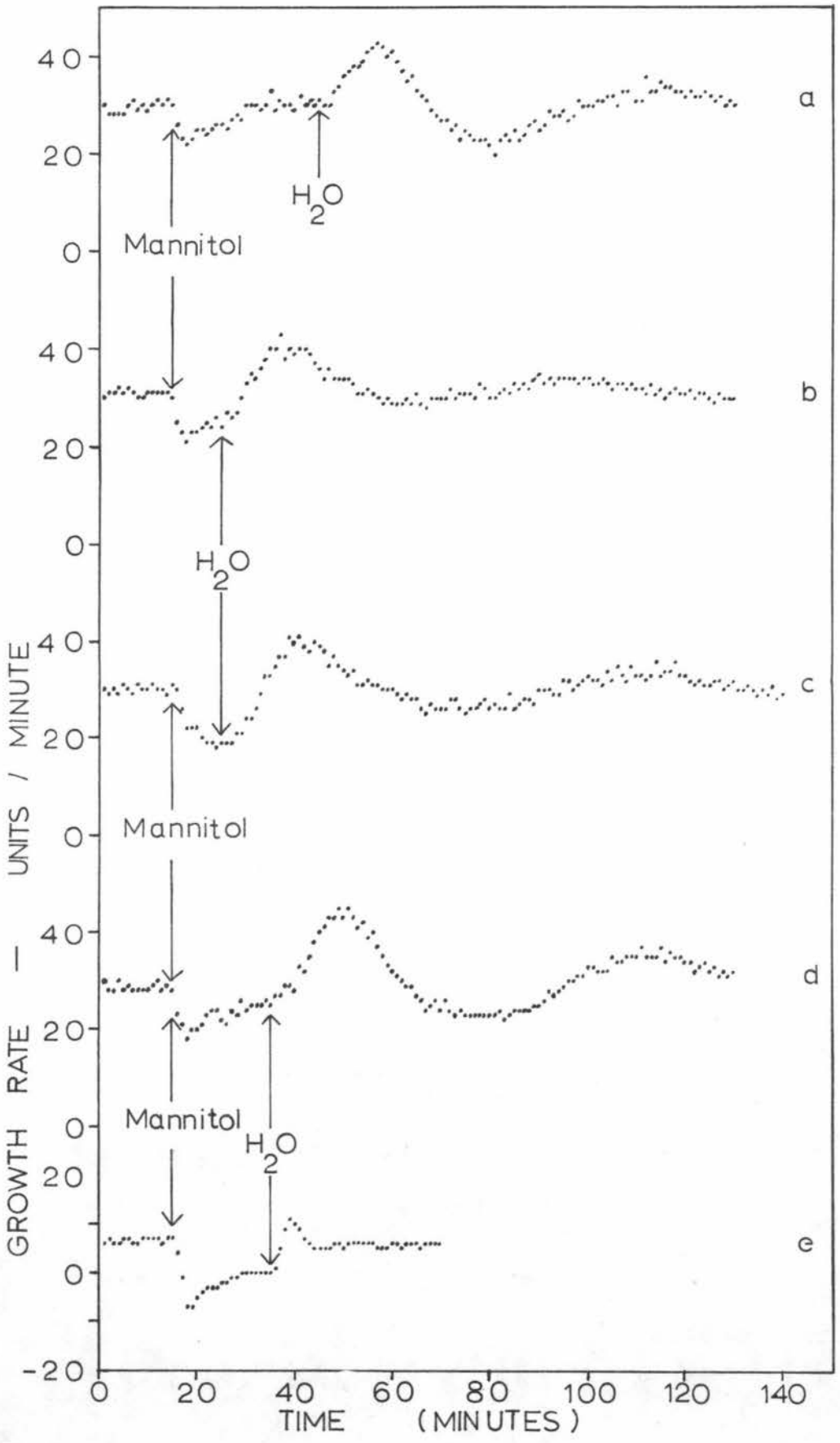
Graphs d and e of Figure 13 compare the responses, of an intact lupin seedling hypocotyl (d) and an excised hypocotyl segment (e), from a seedling of the same age, to a 20 minute period of -2.6 bar stress. The cycling phenomenon and evidence of "stored growth" in the growth response of the intact seedling is lacking in the response of the excised segment. Subsequent experiments with excised segments have indicated the presence of some "stored growth" being evident but generally it is not as marked as in the intact seedling (D. Penny personal communication). Unfortunately, direct comparisons between segments and intact tissue cannot be made since no estimations of the

FIGURE 13 A comparison of varied lupin hypocotyl growth rate responses to short exposures to a root bathing solution osmotic potential of -2.6 bar.
(1 unit = 0.67 μm).

a and b 4 day old seedlings coated with Shell snow white petrolatum.

c and d 4 day old seedlings.

e Excised hypocotyl segment from 4 day old seedling.


40
20
0
40
20
0
40
20
0
40
20
0
20
0
20
-20
a
b
c
d
e
Mannitol
H_2O
Mannitol
H_2O
Mannitol
H_2O
GROWTH RATE — UNITS / MINUTE
0
20
40
60
80
100
120
140
TIME (MINUTES)

velocity and magnitude of changes in ψ cell, during changes in the osmotic potential of the bathing solution, in either segments or intact tissue has been made. However, the rapidity of the growth rate stimulation, following removal of mild osmotic stress in intact plants, suggests that the discovery of virtually instantaneous transmission of changes in hydraulic tension throughout detached maize leaves, reported by Raschke (1970), can be extended to include the rapid transmission of changes in water potential throughout the intact plant, presumably via the water continuum of the xylem.

From their study of growth responses of maize leaves to mild changes in root medium potentials, Acevedo et al (1971), aided by an auxanometer similar to that utilised in this study, have published results closely paralleling those obtained with lupin seedlings and generalised in Figure II. They obtained a decrease in leaf growth rate when the osmotic potential of the root medium (ψ sol) was lowered below -1.0 bar. Unfortunately, Acevedo et al graphically presented their results as total growth curves rather than as growth rates. Consequently, although they report a slight recovery of leaf growth rates following the initial decrease, due to imposed stress, the precise timing of this recovery cannot be determined, but it appears to occur 20 to 25 minutes after the imposition of stress as was found with lupin hypocotyls (2 in Figure II).

Acevedo et al suggested that this growth recovery may have been due to adjustment in either extensibility or cell solutes. Work by Slatyer (1961) and Janes (1966) indicates that osmotic adjustment in plants subjected to stress is largely achieved within 24 hours but that little change could be expected within two hours. Since the growth rate adjustment observed for lupin hypocotyls and maize operates over a much shorter time interval it is unlikely that any osmotic

adjustment is contributing to this increased growth rate. The time interval involved suggests the mechanism in operation may be similar to that in Nitella (Green et al 1971), in which reductions of turgor pressure (P) were compensated by decreases in Y (minimum yield stress) within 15 minutes of the imposition of stress. However, the time dependence of the lowering of Y (2.4 atmospheres/hour) evident in the results of Green et al is not apparent in the lupin hypocotyl responses (Figure 12), but, since turgor pressure cannot presently be monitored in the intact or excised hypocotyl, the precise mechanism in operation cannot be determined.

The growth burst observed on the release of mild stress (3 in Figure II) is similar to that described by Acevedo et al (1971) with maize leaves, Green et al (1971) with Nitella, and by Grenetz and List (1973) with maize roots. Green et al attributed this growth burst to a sudden increase in P, with a consequent increase in the elongation driving force, (P - Y). Subsequent strain-hardening raised Y so that growth rates returned to pre-stress levels (4 in Figure II).

The imposition of higher stresses do not continue indefinitely to lower Y, as is observed during mild stress, Y having a minimum value of approximately two atmospheres in Nitella (Green et al 1971). This phenomenon could account for the fact that the growth burst following release from stress in excess of 2.6 bar never compensated for the growth inhibition during the imposition of stress. In comparison, the growth burst following rewatering fully compensates for the previous inhibition of maize leaf growth when stress periods are mild (-2.0 bar) and short (Acevedo et al 1971).

The long lag between rewatering and growth stimulation following ψ sol values of less than -11 bar, imposed for periods in excess of 20 minutes (Figure 12), may arise as a result of the regaining of

turgor by plasmolysed tissues, a process which may mask those processes operative following more mild stresses. A process of this nature may account for the higher amplitude cycling following release from low stress as compared to high stress (Figure 12). Cycling is not recorded in the parallel study on maize leaf growth (Acevedo et al 1971). Some evidence of cycling is shown in the results of Green et al (1971) with Nitella, when turgor pressure was increased by 2.6 atmospheres, but it is unmentioned in their discussion. The cycling indicates the operation of a feedback mechanism, especially since the amplitude of the oscillations appeared to decrease with time (Figure 13). Further studies could include the investigation of the incorporation of cell wall material into the wall during periods of recovery from mild stress.

Although these water stress studies with lupin seedlings have left many details unresolved, the results support the claims of Acevedo et al (1971) and Hsiao (1973) that, due to the sensitivity and immediate response of plant elongation to changes in water status, many of the reported metabolic changes caused by water stress may arise indirectly as a result of reduced growth, rather than vice versa.

V THE EFFECT OF AN ANAEROBIC NITROGEN ATMOSPHERE ON HYPOCOTYL ELONGATION.

a) Introduction

In order to ascertain the dependence of cellular elongation upon the anabolic reactions occurring within the cell there is a need to study short term plant growth kinetics under anaerobic conditions.

Penny D. et al (1972) have shown that under anaerobic conditions, obtained by the utilisation of a nitrogen atmosphere and nitrogen "aerated" growth medium, IAA induced growth in lupin hypocotyl segments

is eliminated although part of the endogenous component of growth continues. Following a 40 minute period of anaerobic conditions the inhibition of the IAA induced growth was reversed by aeration, the growth rate returning to normal within 10 minutes. With the described auxanometer and environmental chamber it proved relatively easy to duplicate these conditions for lupin seedlings and consequently to compare the growth responses of intact and excised hypocotyl tissue.

The root bathing medium had a stream of "oxygen-free" nitrogen (less than 10 ppm oxygen) bubbling through it and the air supplying the environmental chamber was replaced by nitrogen.

b) Results and Discussion

Growth rates of lupin hypocotyls began to decline within 5 to 10 minutes after changing to a nitrogen atmosphere. They declined during a 20 to 30 minute time interval to reach a constant rate of between 5 and 15% of the normal. Harrison (1965), utilising an auxanometer capable of detecting 23 μm increments in growth, obtained similar results with Avena coleoptiles under a nitrogen atmosphere. Avena coleoptile growth rates began to decrease after five to 10 minutes and reached a constant rate, 10% of the original, after 25 minutes.

Studies were made of lupin seedlings subjected to anaerobic nitrogen conditions for periods of between 10 and 60 minutes. In each case reversal of the growth inhibition was obtained when aerobic conditions were restored, the growth rate increasing within one to two minutes after the introduction of air. A rapid burst of growth occurring between 20 and 40 minutes after the resumption of aerobic conditions often equalled in magnitude the preceding nitrogen-induced inhibition of growth. With an anaerobic period of 20 minutes the growth inhibition

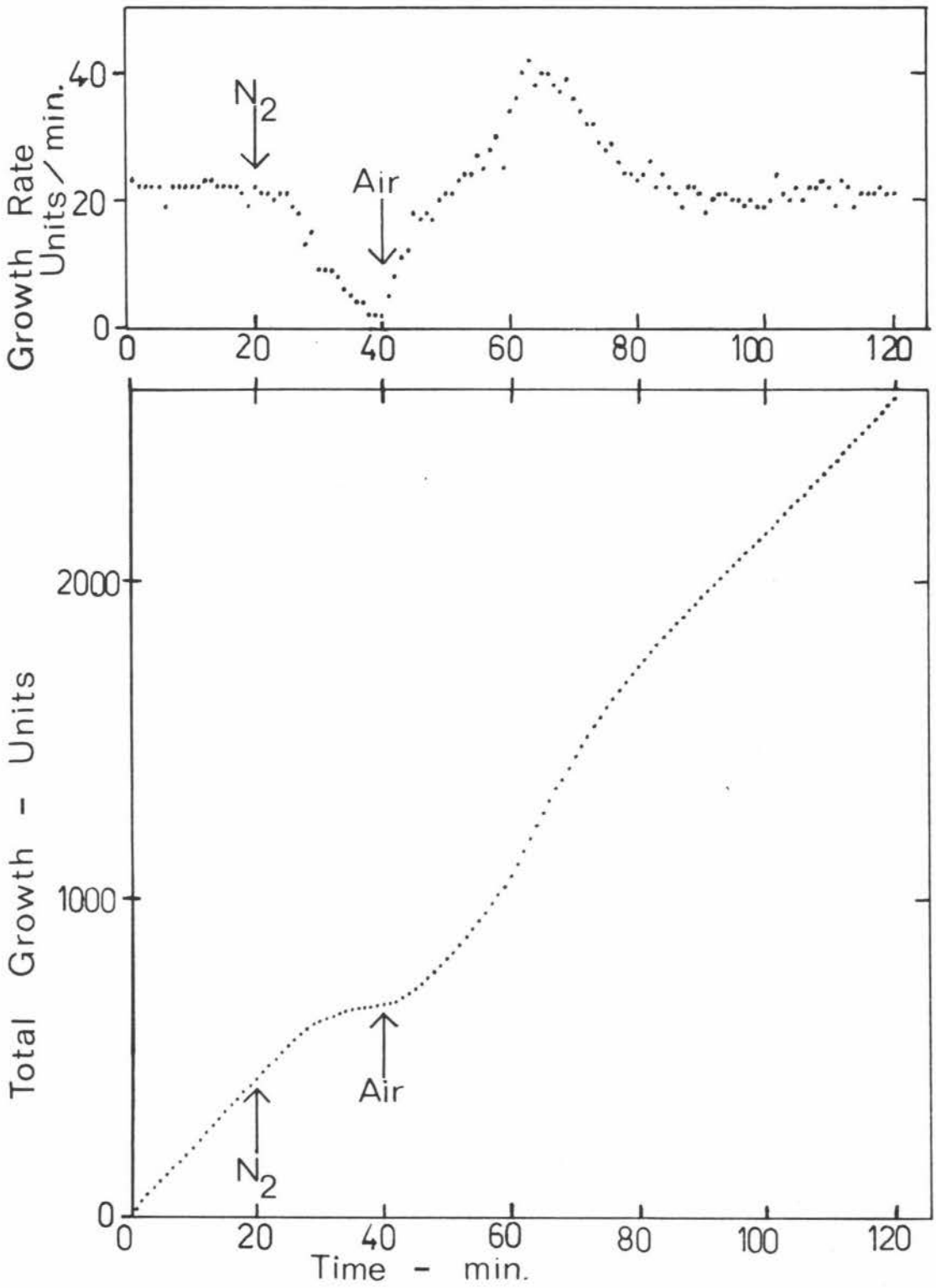


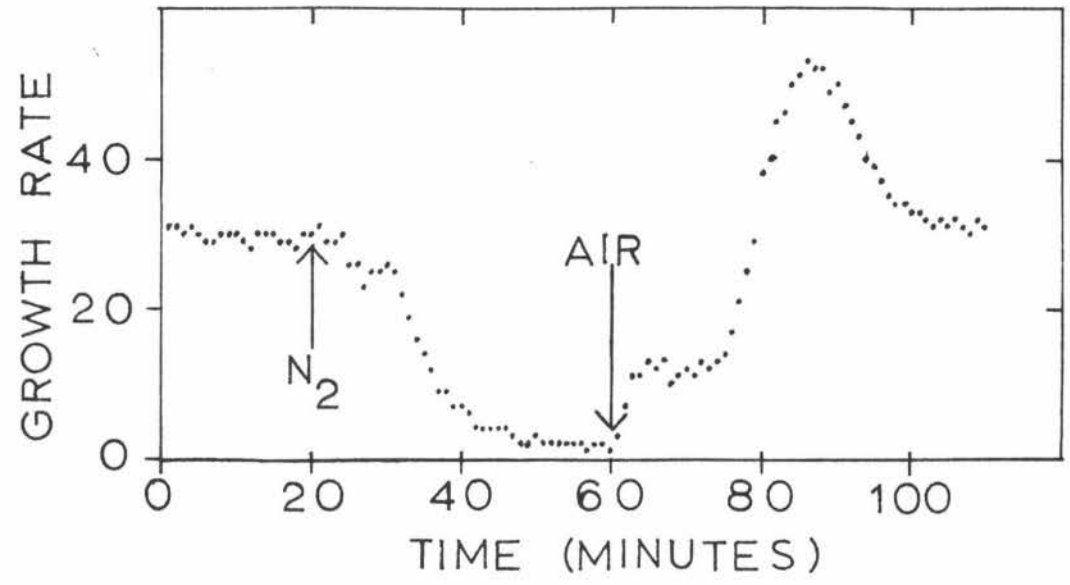
FIGURE 14 **Evidence for "stored growth" during a 20 minute period of anaerobic nitrogen conditions.**

(1 unit = 0.67 μm).

was virtually always completely compensated for by the aerobic growth burst (Figure 14, Table II). For anaerobic conditions of between 30 and 60 minutes this growth burst usually failed to fully compensate for the growth inhibition (Table II, Figure 15). Figure 16 illustrates that the growth inhibition by nitrogen for periods in excess of 20 minutes was more completely compensated for by changing to pure oxygen rather than to air.

It was noted that recovery of growth rates tended to occur in two phases following nitrogen inhibition. The first phase reached a peak at an average time of approximately nine minutes after aeration had begun (peak A in Figure 17 and Figure 18, Table II). This first phase was sometimes only represented by an inflection in growth acceleration (Figure 18a), or a short period of constant growth rate (Figure 18b), or by a definite peak in growth rate (Figure 18c). The second phase consisted of a steady rise in growth rates to a peak at an average time of approximately 28 minutes after commencement of aeration (peak D in Figure 17 and Figure 18, Table II). The finding that inhibition of oxidative phosphorylation, by anaerobic nitrogen conditions, leads to a rapid inhibition of elongation in intact lupin hypocotyls has also been demonstrated to occur in lupin hypocotyl segments, under similar conditions, (Penny D. et al 1972) and in wheat coleoptile segments exposed to cyanide (Gillbank 1971, Gillbank et al 1972). Doerschug and Chrispeels (1970) effectively demonstrated that inhibitions of oxidative phosphorylation completely inhibit the secretion of hydroxyproline - rich proteins from the cytoplasm into the cell wall. The evidence for "stored growth" occurring in lupin hypocotyls under anaerobic nitrogen conditions (Figures 14, 16 and 18) indicates that certain growth processes may continue in the presence of nitrogen yet remain unexpressed until the restoration of aerobic conditions. Assuming that

FIGURE 15 The effect of a 40 minute period of anaerobic nitrogen conditions on lupin hypocotyl growth rates. (1 unit = 0.67 μm).



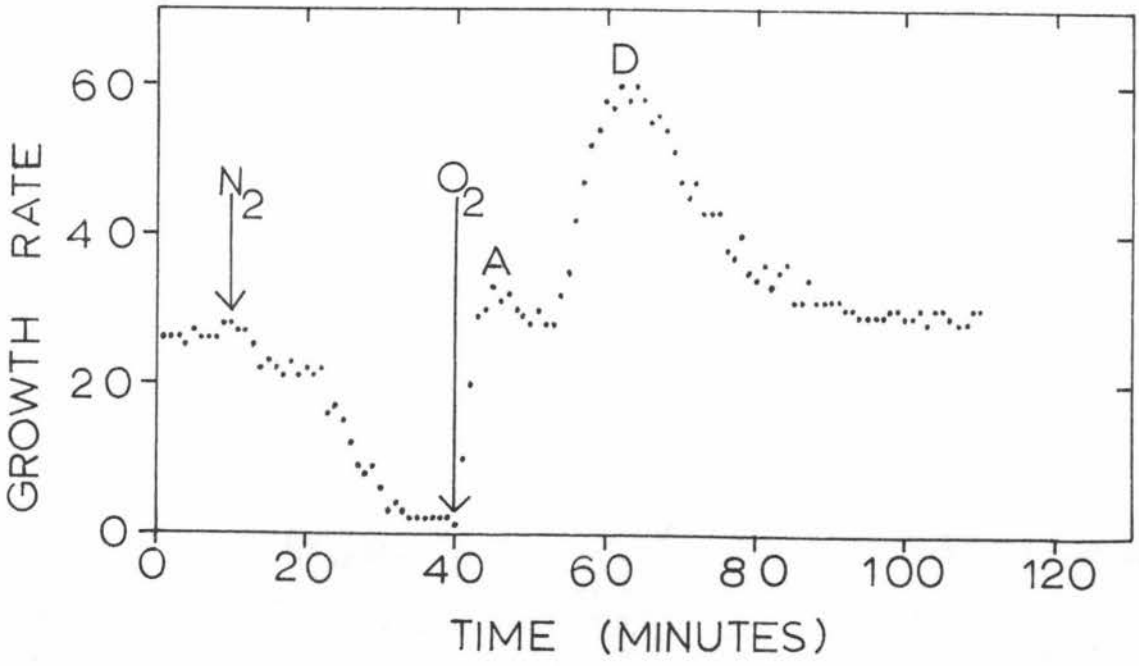


FIGURE 16 The effect of a 30 minute period of anaerobic nitrogen conditions, followed by treatment with pure oxygen, on hypocotyl growth rates. (1 unit = 0.67 μm).

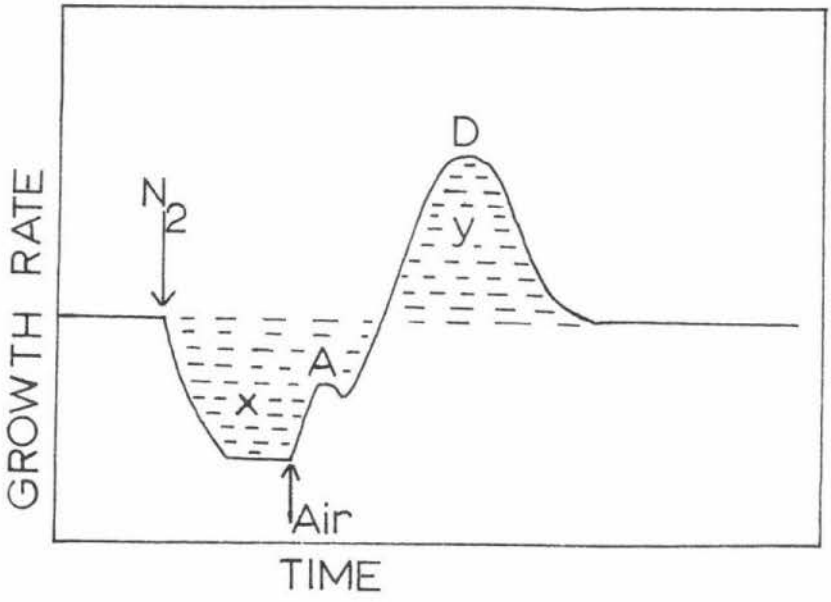


FIGURE 17. A graph illustrating a generalised response of lupin hypocotyl growth rates during and after anaerobic nitrogen conditions.

TABLE II.

A comparison of some features of lupin hypocotyl growth rates during and after anaerobic nitrogen conditions in different experiments. (Letters refer to the figure above).

Duration of N_2 treatment (minutes)	Time of peak A (minutes after addition of air)	Time of peak D (minutes after addition of air)	Area of 'y' compared to area of 'x'
20	11	29	=
20	8	27	=
20	10	27	>
20	9	25	=
30	8	28	<
30	9	30	<
30	12	28	=
30	10	30	<
40	9	31	<
40	8	27	<
$N_2 \rightarrow O_2$			
30	6	25	=
30	5	23	=

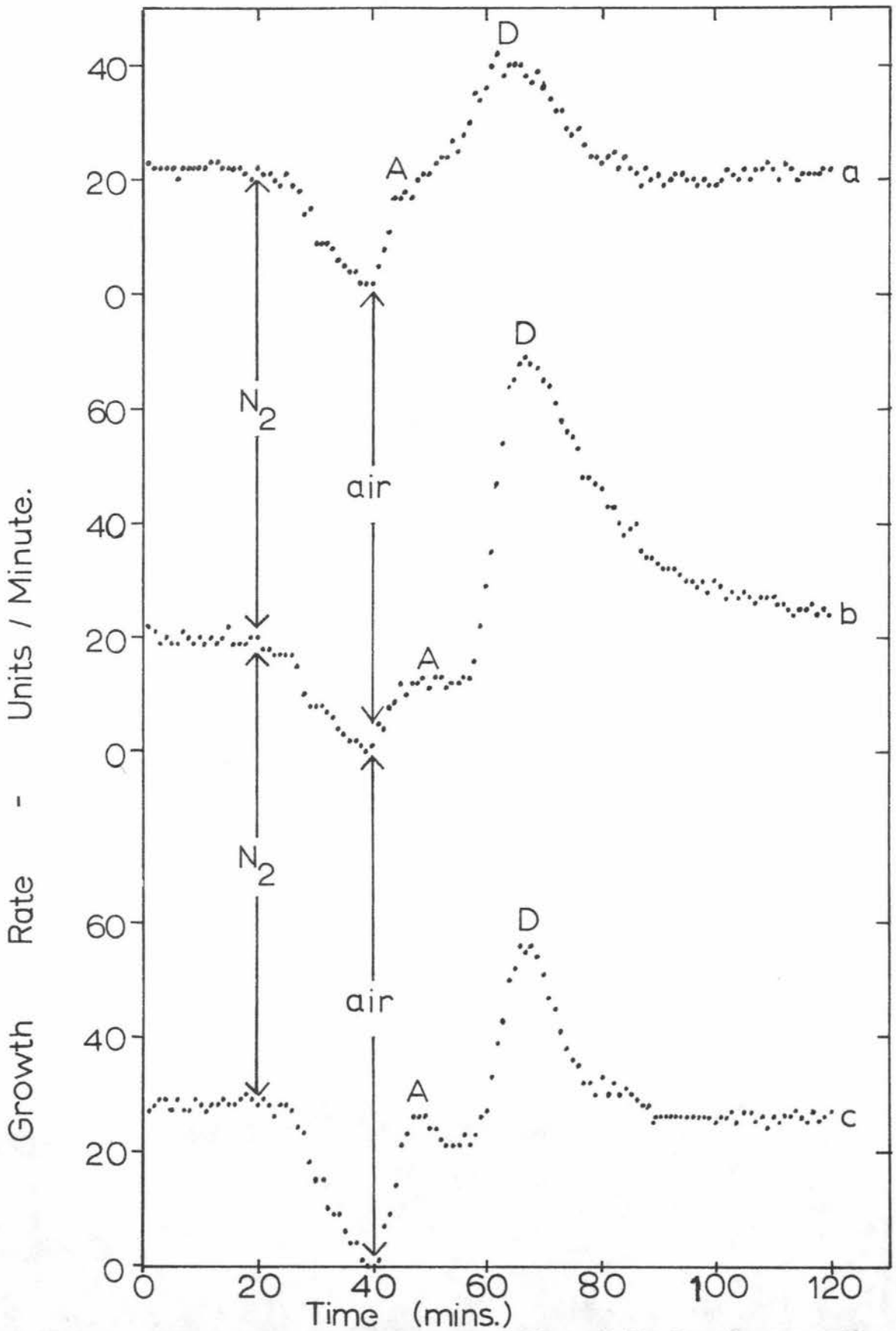


FIGURE 18 **Variations in the characteristics of the two phase growth rate stimulation following a 20 minute exposure to anaerobic nitrogen conditions. (1 unit = 0.67 μm).**

growth has been decreased due to inhibition of the secretion of proteins into the wall, as suggested by Doerschug and Chrispeels, this "stored growth" may arise as a result of the build up of growth protein during the anaerobic period which remains within the cytoplasm until oxidative phosphorylation is operative once again.

The observation of "stored growth" in wheat coleoptile segments during exposure to cyanide for periods of between 10 and 30 minutes. (Gillbank 1971, Gillbank et al 1972) would support such a theory.

The data of Figure 15 and Table II, however, indicates that the growth burst following exposures to nitrogen in excess of 30 minutes does not fully compensate for the inhibition during anaerobic conditions. The existence of a similar phenomenon in wheat coleoptile segments cannot be ascertained since published results include growth responses to cyanide exposures of 30 minutes or less in duration (Gillbank 1971, Gillbank et al 1972). The lupin hypocotyl results indicate that anaerobic conditions must eventually inhibit other growth limiting reactions, perhaps actual protein synthesis.

Since the growth burst occurs in two phases (peaks A and D of Figures 17 and 18) it is probable that there are two distinct processes occurring on the restoration of aerobic conditions. Such a response is not apparent in the published results with wheat coleoptile segments, but this may be due to the methods employed in the obtaining of growth rate measurements. Data for the relevant graphs published by Gillbank (1971) and Gillbank et al (1972) was obtained by measuring the length of ten segments, threaded together on a nylon line, at 10 minute intervals. The results were presented as growth rate/segment/minute. If the lupin hypocotyl growth rates (Figure 18) had been obtained and plotted in a

similar manner the double growth response would have remained undetected.

Until more diagnostic experiments are performed any attempts to explain the origin of the double growth response must be highly speculative. An hypothesis based on the model, explaining the kinetics of cell elongation in lupin hypocotyls, proposed by Penny et al (1970) is briefly outlined below.

The initial growth response (A, Figures 17 and 18) could be due to the transport of accumulated "Growth Protein" to the wall, whilst the second growth burst (D, Figures 17 and 18) may occur through stimulated protein synthesis arising as a result of the depletion of the "Potentially Active Protein" pool during the anaerobic conditions. A negative feedback mechanism, similar to that postulated for auxin stimulated growth, would be required to reduce the rate of protein synthesis to explain the return to normal growth rates following the growth burst (Figure 18).

It has been demonstrated that cyclohexamide does not inhibit protein secretion from the cytoplasm into the cell wall (Doerschug and Chrispeels 1970), yet it inhibits protein synthesis in lupin hypocotyls within three to nine minutes (Penny 1971). Consequently, this proposed hypothesis could be tested by a series of experiments in which cyclohexamide is administered, during anaerobic conditions, at various times prior to the return to aerobic conditions. Such a series of experiments may determine the relative contributions of protein secretion and synthesis to the observed growth burst.

The growth responses of lupin hypocotyls to anaerobic conditions, recorded in this study, have been interpreted as arising as a result of direct effects on cellular growth processes. However, it is important to realise that the whole seedling is affected by the imposed con-

ditions and consequently the observed growth responses could have arisen as a result of prior inhibition of more sensitive metabolic processes. For example, Willey (1970) has shown that water uptake by roots subjected to anaerobic conditions for six hours is decreased by 50% or more and that aeration results in a rapid recovery of water uptake. Changes in water uptake of this magnitude could seriously affect hypocotyl elongation rates. Although the effects described by Willey occur over a time interval of at least two hours, and are unlikely to have contributed substantially to the result recorded for lupin hypocotyls, they demonstrate that considerable caution must be exercised before claiming that results obtained with intact seedlings can be directly applied at the cellular level.

VI THE EFFECT OF VARIED CARBON DIOXIDE CONCENTRATIONS ON LUPIN HYPOCOTYL ELONGATION

a) Introduction

The effects of carbon dioxide on cellular elongation had been little investigated prior to 1971 when the experiments described in this study were performed. The decision to investigate the effects of carbon dioxide on lupin hypocotyl elongation was stimulated by the findings published by Harrison in 1965.

Harrison utilised an auxanometer, capable of detecting 23 μm increments in growth, to monitor Avena coleoptile elongation under a variety of carbon dioxide concentrations. Rather irregular results were obtained with carbon dioxide concentrations of between 1 and 5%, growth rates during either slightly stimulated or inhibited in an unpredictable manner. However, concentrations of carbon dioxide in

excess of 15% stimulated a marked increase in coleoptile elongation rates which reached a maximum in 20 to 60 minutes. The rate of elongation then decreased over the next 1½ to 12 hours, depending upon the concentration used, to a very low but constant rate.

Since the completion of the experiments with lupin seedlings several accounts of carbon dioxide effects on elongation have been published (Rayle and Cleland 1970, Evans et al 1971, Hager et al 1971, Pope and Black 1972, Barkley and Leopold 1973.) This published work has concentrated on attempting to distinguish the relationship between the acid, carbon dioxide, and auxin effects on cellular elongation. As a consequence of this interest in carbon dioxide stimulated growth, the results obtained with lupin hypocotyls will be related to these recently published reports wherever applicable.

b) Results and Discussion

Lupin hypocotyl growth was stimulated within one minute of treatment with carbon dioxide in concentrations of between 10 and 100%. Rayle and Cleland (1970), using Avena coleoptile segments, and Barkley and Leopold (1973), with pea stem segments, reported a time lag of the same magnitude when CO_2 saturated solutions were introduced to the segments.

Figure 19 illustrates the response of lupin hypocotyl elongation to 100% CO_2. A maximum rate, of between 4.5 and 7 times the magnitude of the normal growth rate, was reached four to five minutes after the change to CO_2. This maximum rate was not maintained however, and growth rates fell in an exponential manner during the following 25 to 35 minutes to reach a constant rate 25 to 35% the original aerobic growth rate. Rayle and Cleland (1970) and Barkley and Leopold (1973) obtained the maximum rate within five to seven minutes, with Avena coleoptile

and pea stem segments respectively.

A possible explanation for the growth stimulation and exponential decline arising as a result of treatment with 100% CO_2, is that the CO_2 acts on a growth precursor within the cell, causing it to be utilised very rapidly although simultaneously inhibiting its continued synthesis. The growth response obtained is very similar to that obtained by Penny (1971) when cyclohexomide (a protein synthesis inhibitor) was added to lupin hypocotyl segments 10 minutes after treatment with indolyl-3-acetic acid (IAA) had commenced. The conclusion, arising from these investigations with IAA and cyclohexomide, was that the initial growth responses to auxin do not require protein synthesis, but continued auxin action depends on the existing pool of growth-limiting proteins (G.L.P.) which is rapidly depleted, consequently creating a requirement for continued protein synthesis to sustain elongation.

In order to determine whether or not this growth stimulation by CO_2 was dependent upon prior aerobic processes growth was inhibited by 40 minutes of anaerobic nitrogen conditions. Figure 20 illustrates how the growth rate was dramatically increased by the introduction of the CO_2. The rate of decrease of hypocotyl elongation, following the maximum rate at five minutes, was identical to the rate calculated for the air to CO_2 treatment (Figures 19 and 20, Table III). However, the magnitude of the growth stimulation by CO_2 following anaerobic nitrogen pretreatment for 40 minutes was only 60% of that obtained following normal aerobic conditions. This result would tend to support the theory that CO_2 may act on a growth precursor in the cell that requires respiratory energy for its continued production. However, more definitive experiments need to be performed, utilising varied anaerobic nitrogen

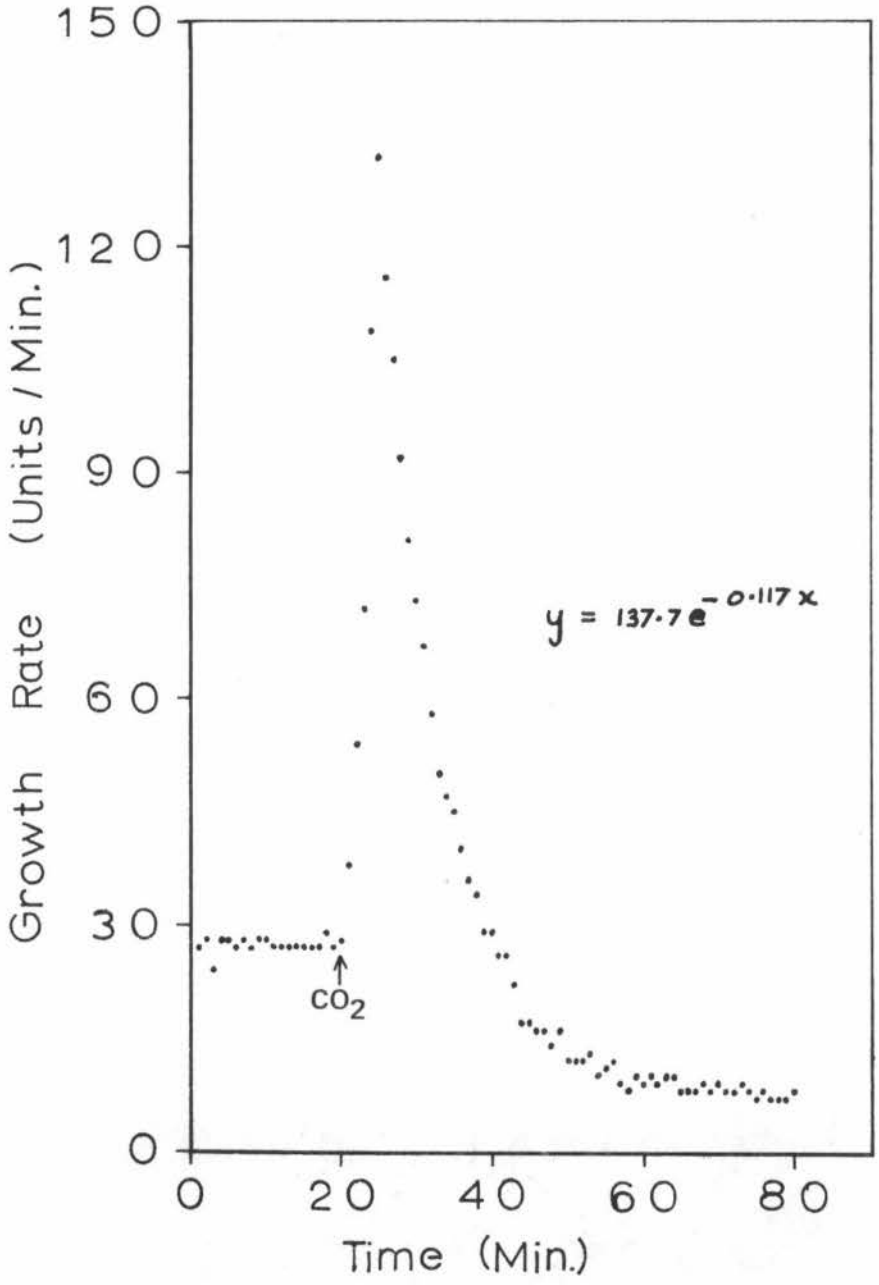


FIGURE 19 Lupin hypocotyl growth rate response to 100% carbon dioxide. (1 unit = 0.67 µm).

pretreatment periods, to obtain more substantial evidence. One important feature that deserves closer attention in the future is that the final steady growth rate under anaerobic CO_2 conditions is higher than the anaerobic nitrogen growth rate immediately preceding the CO_2 treatment (Figure 20).

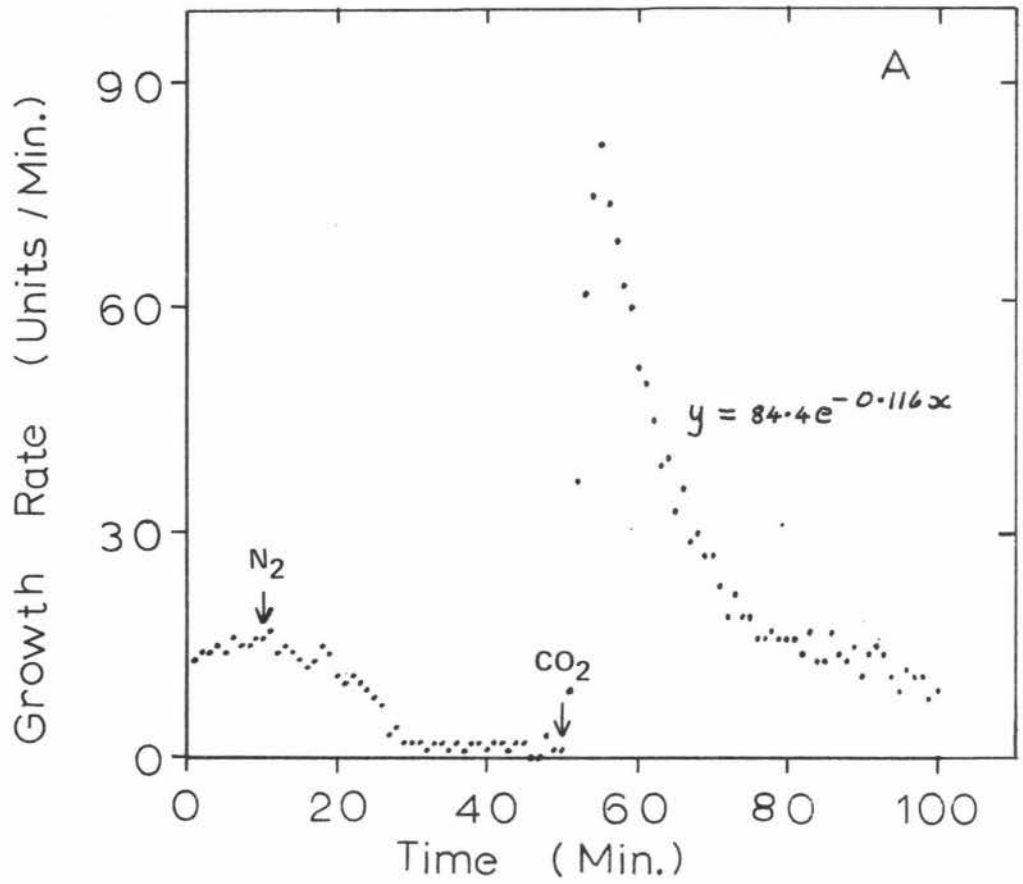
Since pretreatment with nitrogen decreased the magnitude of the growth response to CO_2, it was decided to compare this with a similar pretreatment period in pure oxygen. The response of lupin hypocotyl growth rates to pure oxygen was not very marked. Generally there was a slight stimulation in growth rates for five to 10 minutes followed by a change to a steady growth rate of between 0 and 25% higher than the original growth rate. On average, growth rates in pure oxygen were 15% higher than the rate attained in air. Harrison (1965) sampling Avena coleoptile growth at five minute intervals detected a 10% rise of growth rates in oxygen as compared to rates in air.

The results of CO_2 treatment following a 40 minute pretreatment in pure oxygen are not significantly different from those obtained with pretreatment in air (Figure 19 and 21, Table III).

The result of treating lupin seedlings with 80% CO_2/20% O_2 (Figure 22) was a significant but reduced initial growth response, approximately 70% of that achieved with 100% CO_2. However, the rate of decline of growth rate was considerably lower than that in pure CO_2 (Figures 19 and 22, Table III), even though the final growth rate was not significantly higher than in pure CO_2. Results with lower concentrations of CO_2 generally showed less inhibitory effects of CO_2 on final growth rates. Figure 23 demonstrates that following the initial $2\frac{1}{2}$ fold increase in growth rate and subsequent decline, induced by 10% CO_2 in air, the final steady growth rate nearly equals the initial rate

FIGURE 20 Carbon dioxide stimulated growth following a 40 minute pretreatment of anaerobic nitrogen.
(1 unit = 0.67 μm).

FIGURE 21 Carbon dioxide stimulated growth following a 40 minute pretreatment with 100% oxygen.
(1 unit = 0.67 μm).


A
90
60
30
0
Growth Rate (Units / Min.)
y = 84·4e^−0·116x
N2
CO2
0
20
40
60
80
100
Time (Min.)

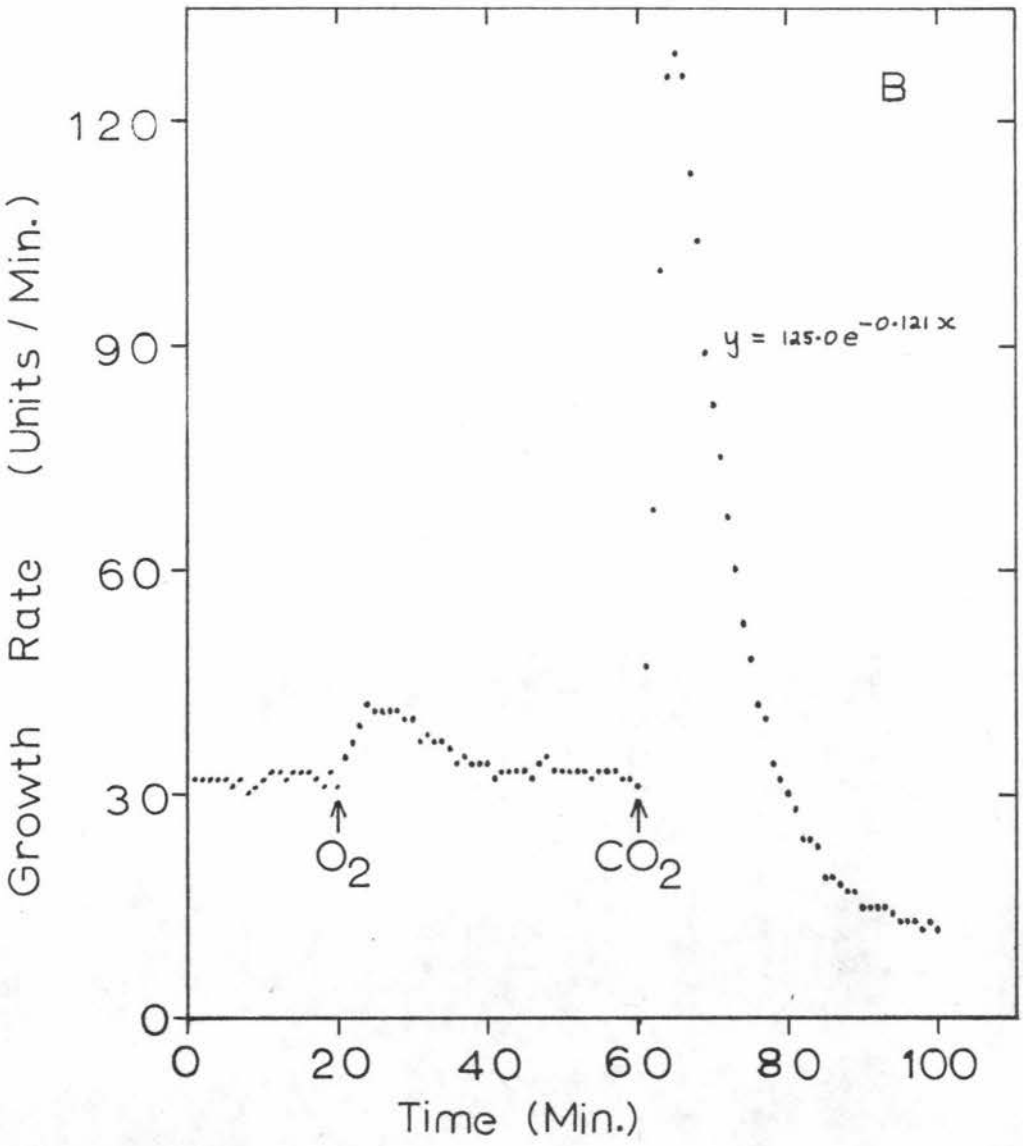

B
120
90
60
30
0
Growth Rate (Units / Min.)
y = 125·0e^−0·121x
O2
CO2
0
20
40
60
80
100
Time (Min.)

TABLE III

A comparison of the exponential equations describing the decrease in lupin hypocotyl growth rates following their initial stimulation by various concentrations of carbon dioxide.

General exponential equation: $y = ae^{-bx}$

Type of Treatment	Value of a	Value of b	Correlation Coefficient	Figure Number
100% CO_2	165.7	0.111	0.986	N.I.
100% CO_2	138.8	0.112	0.995	N.I.
100% CO_2	137.7	0.117	0.996	19
100% CO_2	145.5	0.107	0.996	N.I.
N_2 40 min. $\rightarrow$ 100% CO_2	84.4	0.116	0.990	20
O_2 40 min. $\rightarrow$ 100% CO_2	125.0	0.121	0.996	21
20% O_2 : 80% CO_2	104.3	0.050	0.992	22
20% O_2 : 80% CO_2	104.9	0.046	0.990	N.I.
6 day plant 100% CO_2	72.0	0.081	0.991	28

N.I. Not illustrated in this thesis.

All plants were four day old lupin seedlings maintained in air prior to the described treatments unless otherwise indicated.

in air. In all experiments involving treatment with 100% CO_2, growth rates were equal to, or lower than, the aerobic growth rate within 25 minutes of commencing CO_2 treatment. This time period is considerably shorter than the 40 - 80 minute reported by Rayle and Cleland (1970) with respect to Avena coleoptile segments immersed in solutions saturated with 100% CO_2. The evidence from these experiments, and especially that of continuous treatment with 80% CO_2/20% O_2 (Figure 22) suggests that there is a specific CO_2 inhibitory effect on growth that cannot be reversed by presence of oxygen, since the final steady growth rate in 80% CO_2/20% O_2 is the same as in 100% CO_2. The nature and time of commencement of this inhibitory effect cannot presently be determined.

Since growth rates of lupin hypcotyls were suppressed by CO_2 at concentrations in excess of 10% for periods of 25 minutes or more in duration the effects of a return to aerobic conditions were studied. Harrison (1965) found that a return to air following 12 hours in CO_2 caused a stimulation of Avena coleoptile growth rates only after exposure for several hours, at which time rates rose slowly during the following 24 hours, to reach a steady rate lower than the original. Figure 24 illustrates the effect of a 60 minute period of 100% CO_2 followed by a change to air. The change to air caused an immediate reduction in hypocotyl growth rates to reach a minimum rate four to six minutes later. In this particular case, the hypocotyl shrank for 30 to 35 minutes. Although a change from CO_2 to air always caused a marked reduction in hypocotyl growth rate following CO_2 treatments from two to 60 minutes in duration, actual shrinkage of the hypocotyl was only recorded following CO_2 treatments in excess of 20 minutes. Figure 25 illustrates that exposure to air following a 10 minute treatment of CO_2 still rapidly lowers the hypocotyl growth rate but that

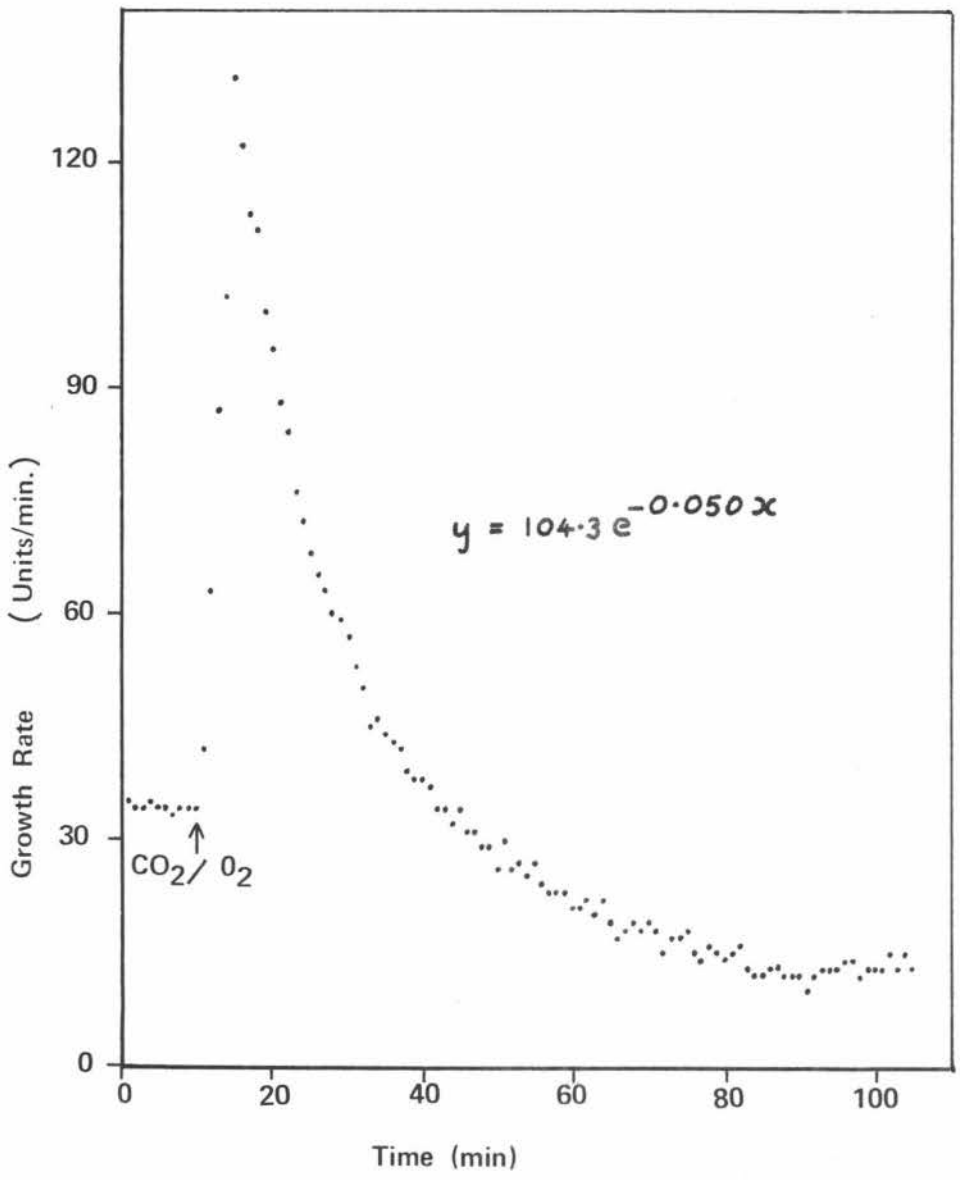


FIGURE 22 Lupin hypocotyl growth rate response to an environment of 80% CO_2 : 20% O_2. (1 unit = 0.67 μm).

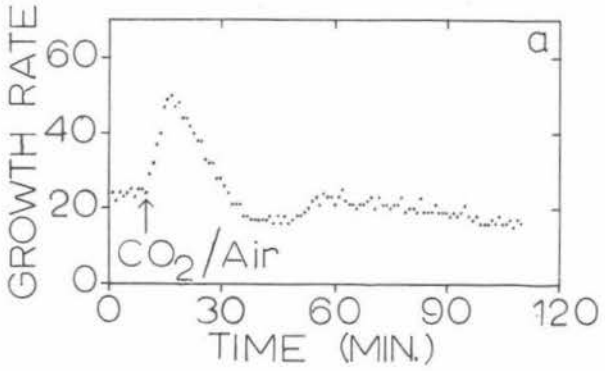
there is no shrinkage recorded. A comparison of Figures 19 and 25 reveals that the restoration of aerobic conditions following a 10 minute treatment of 100% CO_2 reduces the growth rate to a value lower than the original aerobic rate within two minutes (Figure 25) whereas continuous CO_2 treatment takes at least a further eight minutes to reduce the growth rate to a similar level (Figure 19).

Much of the more recent research on the CO_2 effect has involved growth stimulation by a CO_2-pulse rather than continuous exposure to CO_2 (Evans et al 1971, Barkley and Leopold 1973). None of these published results show any inhibitory effects of a change from CO_2 to aerobic conditions. Evans et al (1971) utilised a three minute pulse of CO_2 - saturated water followed by O_2 - saturated water to obtain growth stimulations in Avena coleoptile segments. Due to their method of presentation of results, (total growth rather than growth rate), it is difficult to directly compare results. However, it appears that a very high elongation rate was maintained for approximately 15 to 18 minutes and then dropped abruptly over a 10 minute period to reach a stable rate equivalent to the initial aerobic rate. A similar experiment was performed with lupin seedlings and a two minute pulse of CO_2 was administered. The general kinetics were similar to those for a 10 minute CO_2 - pulse (Figure 25) except that maximum growth stimulation occurred at two minutes and was only 70% of that achieved under continuous CO_2 due to the inhibitory effect of the change back to aerobic conditions.

Valid attempts to explain the growth rate inhibitions, arising as a result of return to aerobic conditions in this study with intact lupin hypocotyls, cannot be made without further research. However, two factors that may deserve closer attention are comparisons of

FIGURE 23 Lupin hypocotyl growth rates in an atmosphere of 10% CO_2 / 90% air.

(1 unit = 0.67 µm).



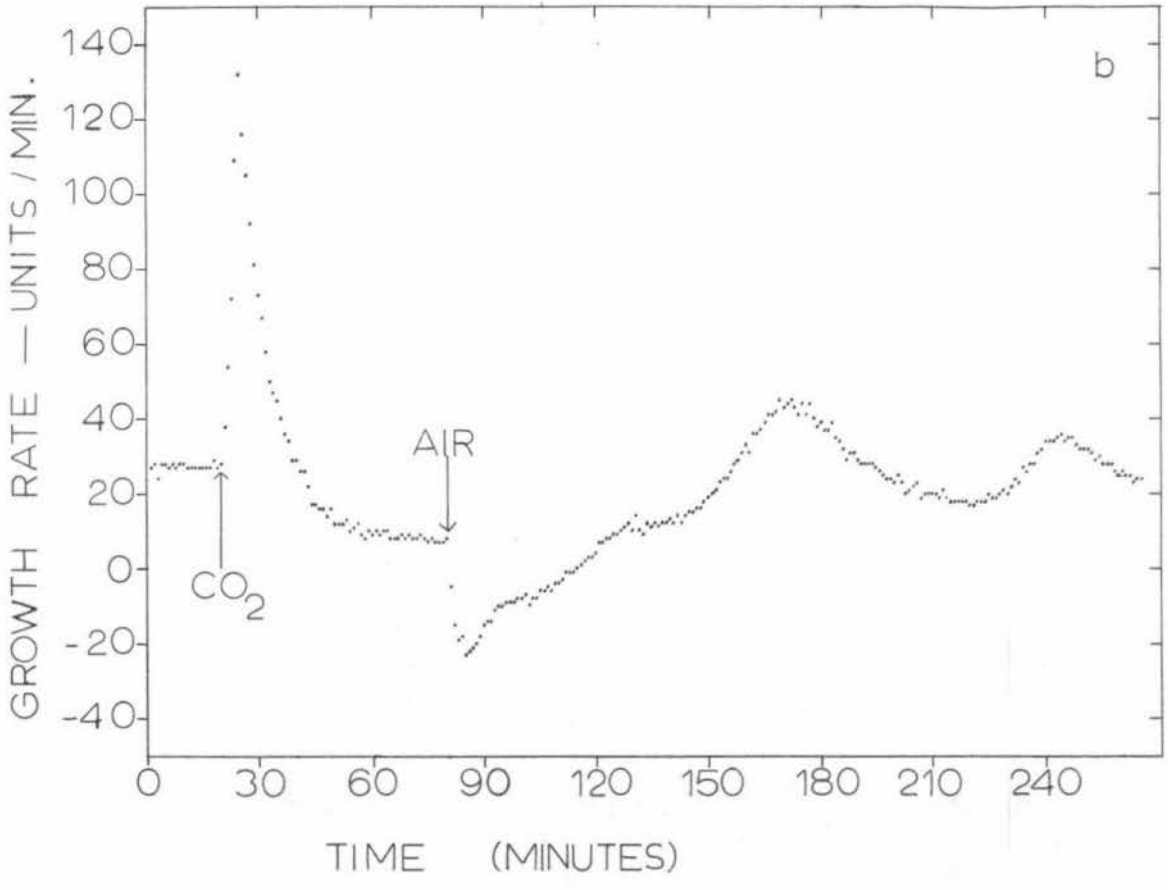


FIGURE 24 Shrinkage and oscillations in lupin hypocotyl growth resulting from return to air following a 60 minute exposure to 100% CO_2. (1 unit = 0.67 µm).

diffusion rates of CO_2 in segment and intact plant studies, and the possible role of stomata in observed growth responses.

It is apparent that the diffusion of CO_2 in the gaseous state will be more rapid than when transported in solution as is required in studies involving segments.

Since increases in CO_2 concentrations initiate stomatal closure there is a possibility that reduced transpirational water loss, due to stomatal closure during CO_2 treatments, may alter seedling water status sufficiently to affect growth rates. Since rapid growth responses to CO_2 have been observed in segments immersed in solutions (Evans et al 1971, Barkley and Leopold 1973), and cyclic variations in growth can arise independently of stomatal movement (see mannitol results), it is unlikely that the growth responses to CO_2 in lupin seedlings occur as a result of changes in stomatal aperture. However, the number of stomata on the lupin seedlings and their possible contribution to growth responses should be evaluated before the described growth phenomena are attributed solely to CO_2 effects on growth processes.

Following the growth inhibition caused by air, growth rates slowly rise to reach the pre-CO_2 growth rate in times varying from 40 minutes to 80 minutes after the change to aerobic conditions. Return to aerobic conditions following all treatments of 100% CO_2 (two to 60 minutes in duration) initiated a cycling of growth rates, although this response was not apparent until the initial inhibitory effects of air had ceased. The cycling had a period of approximately 70 minutes and diminished in amplitude with time. The existence of this cycling, induced by change from CO_2 to air, is evidence for a possible feedback mechanism being in operation, perhaps similar in nature to that obtained

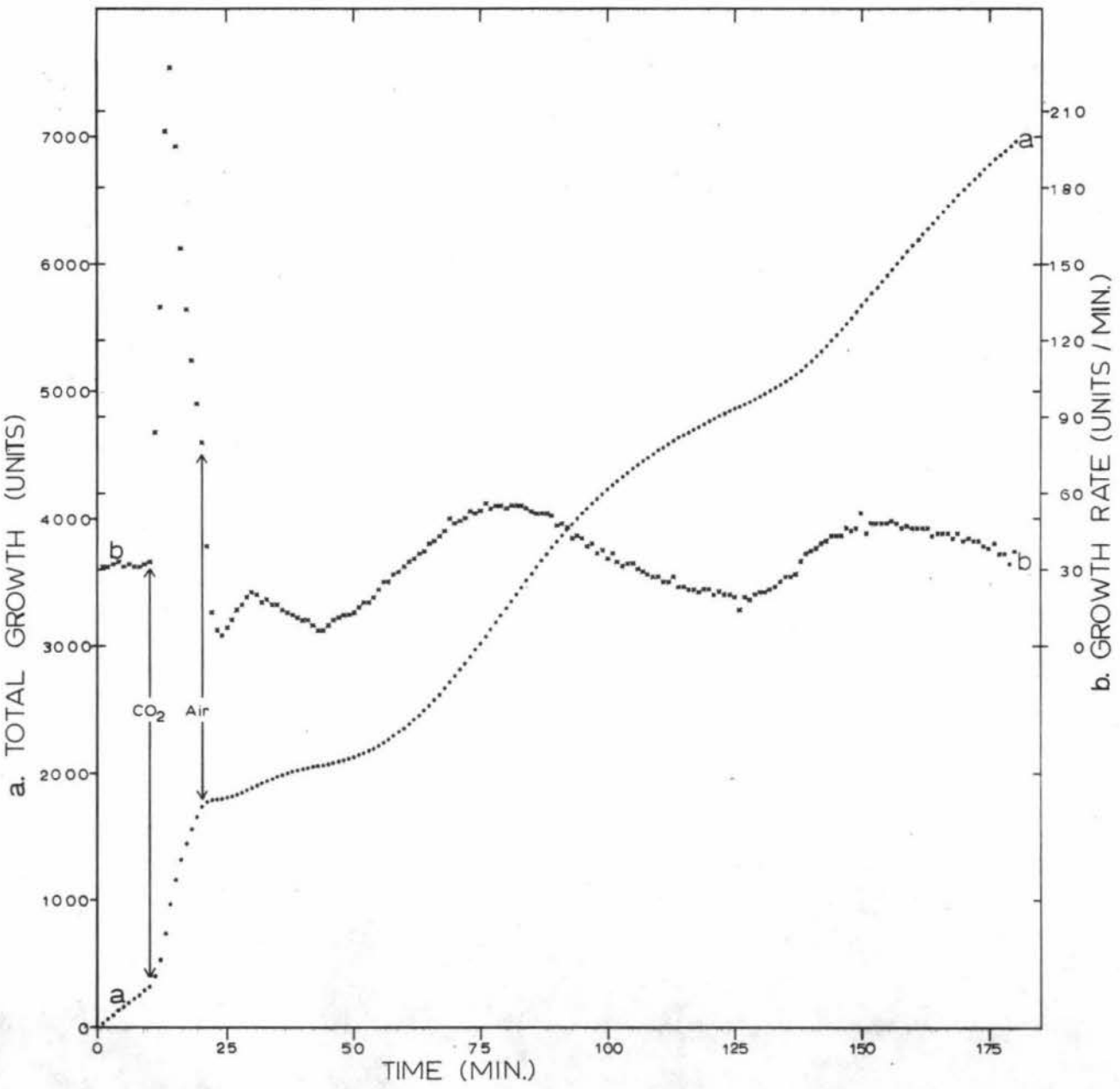


FIGURE 25 A comparison of total growth (a) and growth rates (b) of a lupin hypocotyl resulting from a 10 minute exposure to 100% CO_2. (1 unit = 0.67 µm).

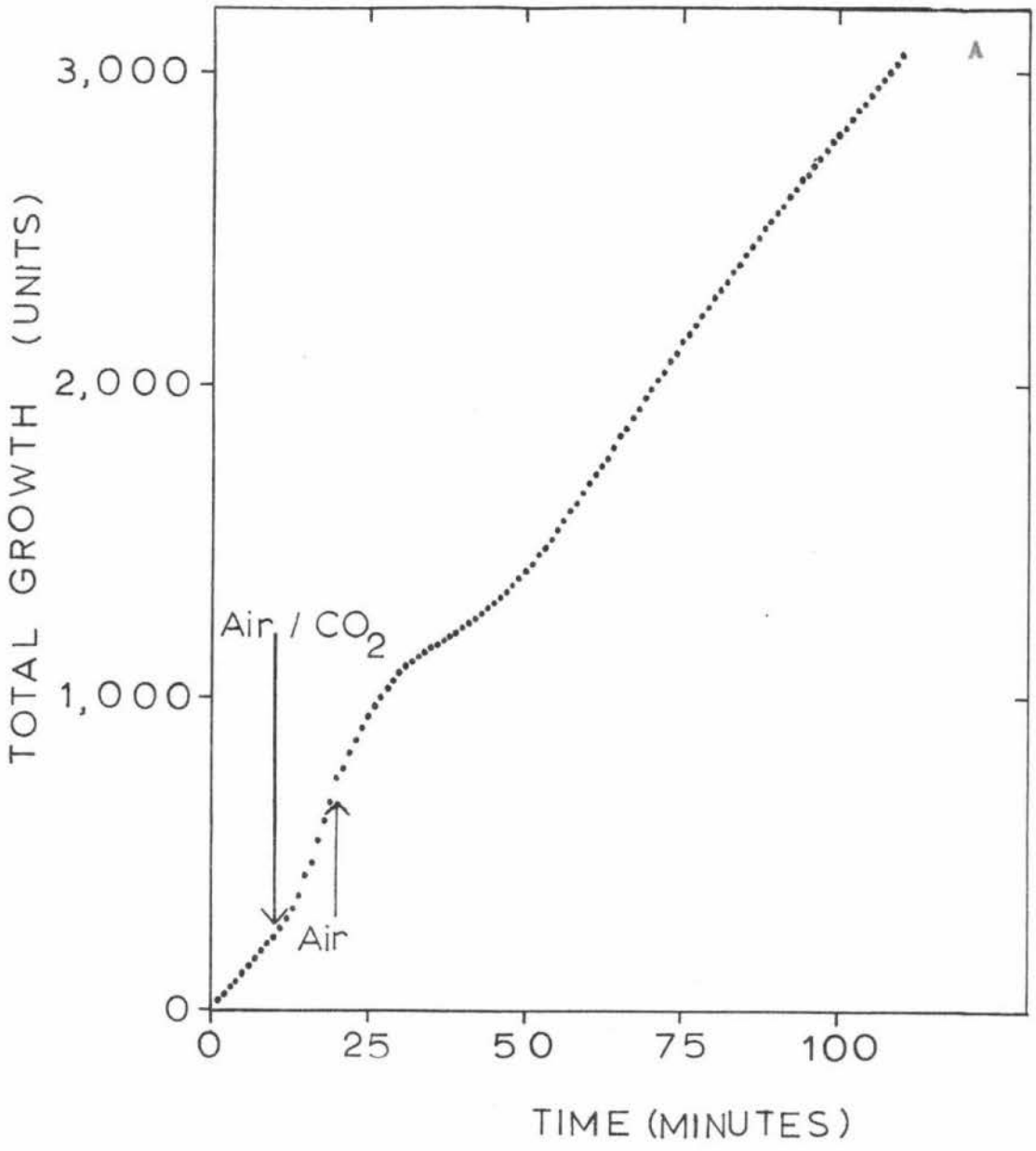
practically and in computer simulations of IAA stimulated growth in lupin hypocotyl segments (Figure 3 Penny 1972). Studies of CO_2 induced growth responses in segments have not demonstrated this cyclic phenomenon. Consequently, this cyclic response to CO_2 in intact seedlings may have arisen since such a feedback mechanism would be more readily revealed by rapid, rather than gradual changes in the controlling parameters.

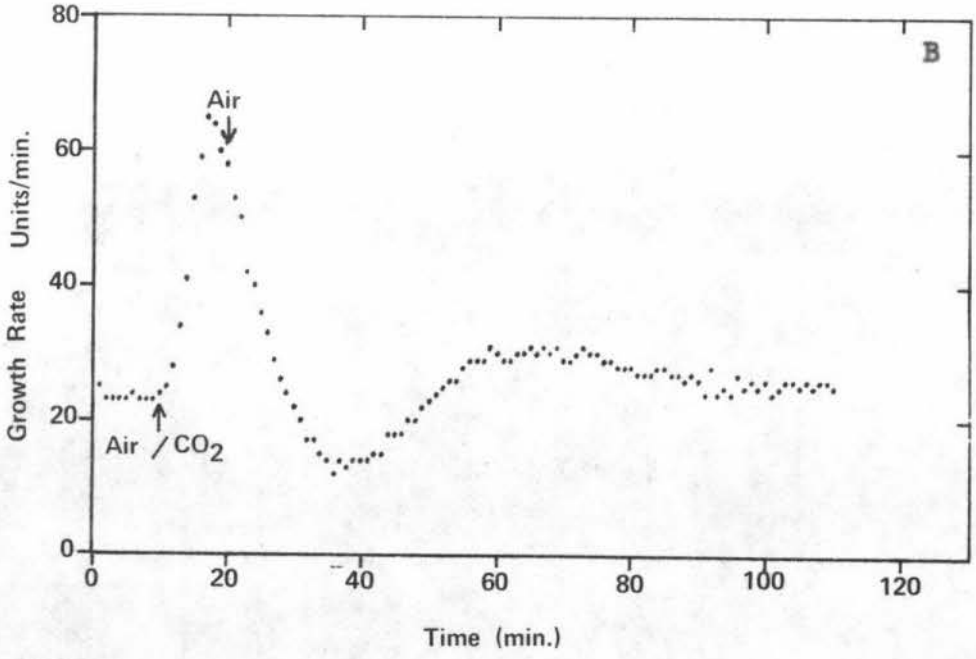
The total growth, (a), graphed in Figure 25 shows that as a result of a 10 minute exposure to CO_2 total growth was slightly increased, the total growth increment attributable to the CO_2 treatment being equivalent to a 0.5% increase in the total length of the lupin hypocotyl. This result is, however, a maximum response and generally the growth stimulation of a 10 minute exposure to CO_2 was counterbalanced by the following inhibition of growth by aerobic conditions such that there was no nett growth increase resulting from CO_2 treatment. Exposures to CO_2 in excess of 10 minutes prior to return to aerobic conditions, however, slightly depressed total growth over the experimental period (Figure 24).

Figure 26 shows that a 10 minute exposure to 90% air/10% CO_2 followed by return to air did in fact cause an increase in total growth over the experimental period. It is also apparent that cycling of growth rate on return to air is much dampened, which tends to support the proposal that the cycling observed in Figures 24 and 25 may have arisen as a result of the magnitude and rapidity of change in gaseous composition.

An experiment was performed to determine whether or not repeated 10 minute exposures to CO_2 continued to cause growth rate stimulations. The results presented in Figure 27a show that each new exposure to CO_2 produces a growth stimulation. However, the magnitude of the growth

FIGURE 26 A comparison of total growth (A) and growth rates (B) of a lupin hypocotyl resulting from a 10 minute exposure to 10% CO_2 / 90% air.
(1 unit = 0.67 μm).

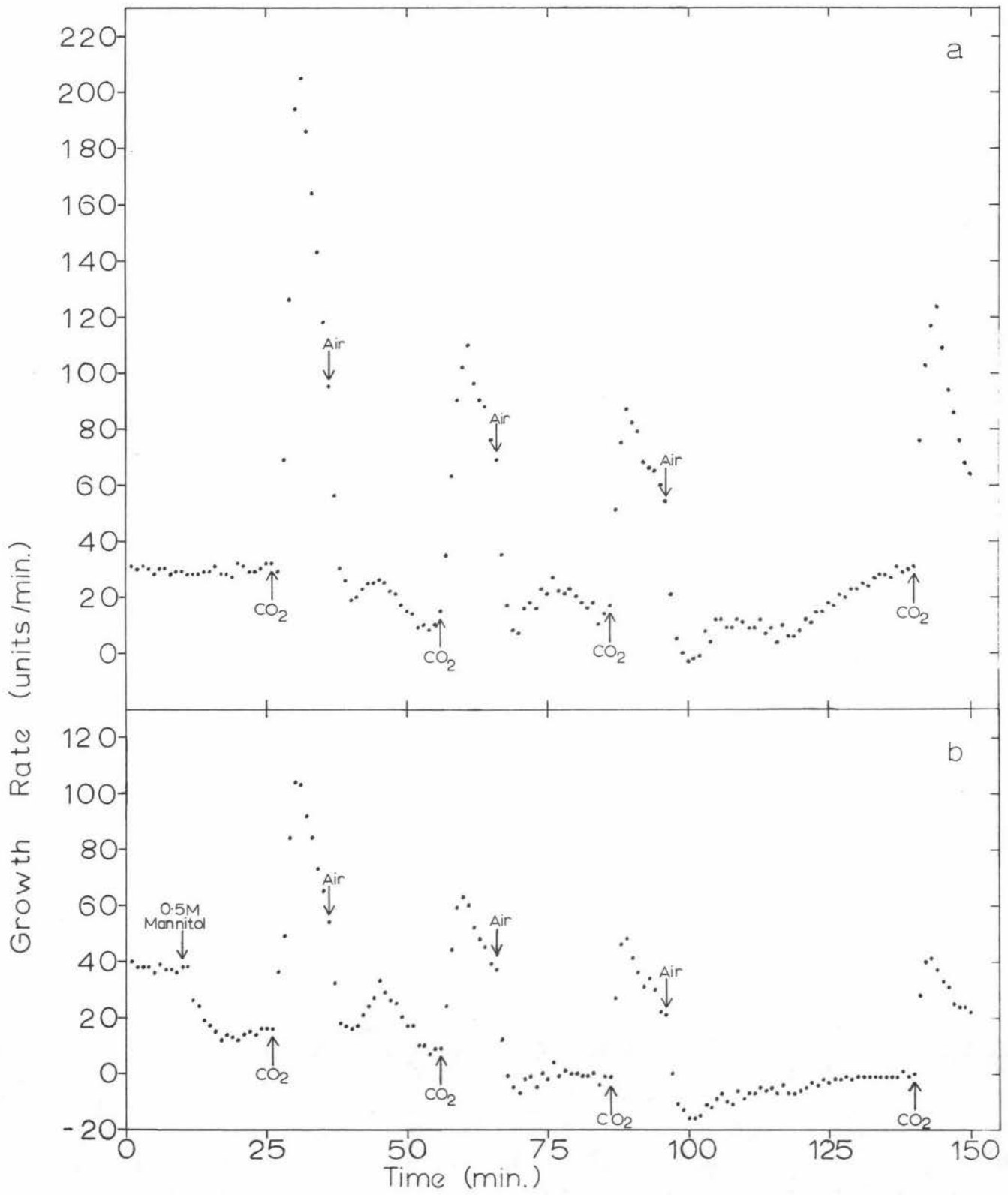

A
TOTAL GROWTH (UNITS)
3,000
2,000
1,000
0
Air / CO_2
Air
0
25
50
75
100
TIME (MINUTES)


B
Growth Rate Units/min.
80
60
40
20
0
Air
Air / CO_2
0
20
40
60
80
100
120
Time (min.)

rate responses decreased at each successive exposure to CO_2, except that when the period between return to air and the next CO_2 treatment was increased from 20 to 45 minutes the growth stimulation also increased in magnitude. These results support the concept of a growth precursor being accumulated during aerobic conditions and then being expressed in the presence of CO_2. The shorter the period between successive exposures to CO_2, the less the build up of the precursor and, therefore, the lower the possible stimulation of growth in the presence of CO_2. Pope and Black (1972) showed that, in the presence of cyclohexamide, coleoptile elongation did not respond to IAA administered following a prior growth stimulation by CO_2. Barkley and Leopold (1973a) confirmed these findings with pea stem segments and, by treatment of segments with CO_2 at various times following a CO_2 pretreatment, calculated that the "growth limiting protein" pool was completely replenished within 80 minutes. The results of Figure 27a would yield a similar time interval for the complete build up of the postulated "growth precursor" acted upon by CO_2 in lupin hypocotyls.

Figure 27b illustrates that even when growth rates are halted, by reduced turgor pressure, a growth burst is elicited by CO_2 treatment. However, the magnitude of the CO_2 induced growth response is considerably reduced. Since no estimations of turgor pressure (P) in the intact hypocotyl have been made, the mechanism by which growth is stimulated cannot be determined. Although growth was zero under the imposed osmotic stress, $(P - Y) \leqslant 0$, turgor pressure may have exceeded zero and consequently a subsequent CO_2 - induced lowering of Y would stimulate a growth response. Alternatively, turgor pressure could have been raised by the CO_2. A precise definition of the mechanism involved must await further study.

FIGURE 27 Lupin hypocotyl growth responses to repeated carbon dioxide treatments in the presence (b) or absence (a) of a root bathing solution osmotic potential of -14 bar.
(1 unit = 0.67 μm).


a
b
Growth Rate (units/min.)
Time (min.)
Air
CO2
0·5M Mannitol
220
200
180
160
140
120
100
80
60
40
20
0
-20
0
25
50
75
100
125
150

The exposure of lupin seedlings to CO_2 has yielded many interesting results, the majority of which have been interpreted as supporting the theory that the CO_2 and auxin growth effects are similar in many respects. Both agents appear to act upon a cytoplasmic pool of protein essential for growth - the "growth limiting proteins" (Pope and Black 1972, Penny 1972, Barkley and Leopold 1973, 1973a). Figure 28 has been included to demonstrate the complexities involved in the formulation of a comprehensive theory for CO_2 - induced growth mechanisms. The illustrated growth response was obtained with a six day old lupin seedling, the hypocotyl of which had virtually ceased elongating. The response illustrated was the largest obtained from the four six day old plants exposed to CO_2, but in each case there was a marked stimulation of growth rates by CO_2.

VII THE EFFECT OF THE WEIGHT OF THE TRANSDUCER SLUG ON HYPOCOTYL ELONGATION

a) Introduction

Since it is a well established fact that cellular elongation occurs when the extensive force exceeds the minimum yield stress (Green et al, 1971) it was realised that the force exerted by the weight of the transducer slug may have been sufficient to cause elongation, even at low turgor pressures within the hypocotyl.

A similar problem was faced by Hsiao et al (1970) who utilised a transducer in their study of maize leaf elongation. They reported a transitory rapid rate of elongation, due to mechanical stretching, when the transducer slug was attached to the leaf. The long term effects of this weight were, however, considered to be irrelevant in their studies, since when the slug weight was doubled the steady growth

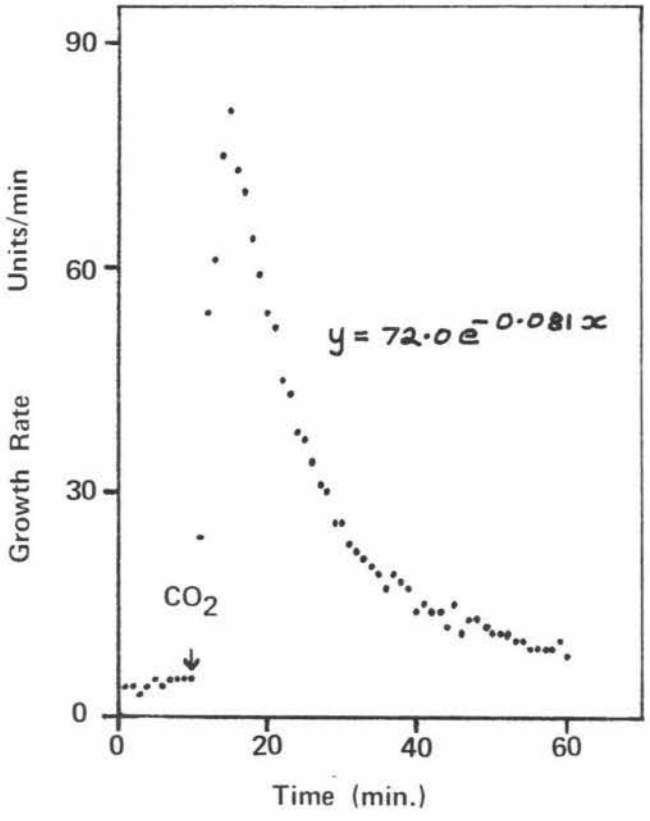


FIGURE 28 CO_2 stimulated growth rates in the non-elongating hypocotyl of a six day old lupin seedling. (1 unit = 0.67 μm).

rate 15 minutes later was unaffected. Hsiao et al concluded that the slug weight alone was insufficient to sustain any prolonged elongation, since when turgor pressure was reduced, by excising the root system, leaf shrinkage was recorded within 10 minutes.

A number of experiments were performed in order to study the effect of the 5.23g transducer slug on lupin hypocotyl growth rates.

b) Results and Discussion

Eight pairs of experiments were conducted, utilising plasticine either as a counterbalance or as an additional weight on the transducer slug, with effective slug weights of 1.35 and 8.67g. After the normal equilibration period of three to four hours, during which these forces were exerted on the lupin hypocotyls, each pair of seedlings was subjected to a particular environmental change and their growth rate responses monitored. These pairs of experiments yielded no significant differences that could be attributed to the influence of the weight of the transducer slug. Both seedlings in one particular experiment showed an almost identical amount of shrinkage when the CO_2 in the environmental chamber was replaced by air, therefore showing that the force exerted by an 8.67g weight on the hypocotyl did not exceed the minimum yield stress of hypocotyl cells.

As a consequence of these results the transducer slug was not counterbalanced in any of the other experiments undertaken in this study.

In determining the magnitude and duration of any mechanical stretching, due to the weight of the transducer slug, the following experiment was performed. Lupin seedlings were mounted in the root chamber and left to equilibrate for 3½ hours with the transducer slugs

attached but not exerting any force on the hypocotyls. Growth rates were recorded from the time the slugs were adjusted so as to exert a force on the hypocotyls, until a constant growth rate was obtained. The force of the slugs was then removed for 10 minutes and the procedure repeated. The results are presented in Figure 29. This experiment yielded similar results from the two plants it was performed upon. The growth recorded during the first minute of the force being applied was approximately eight times that of the stable growth rate subsequently achieved. The initially high growth rate declined rapidly in on exponential manner over a 20 minute period.

The characteristics of this growth burst are similar to those obtained with Nitella when turgor pressure was raised (Green et al 1971). The minimum yield stress, (Y), was postulated to rise by the process of strain hardening at a rate proportional to the magnitude of the elongation driving force - i.e. (P - Y), where P is the turgor pressure. An abrupt increase in P would cause a high growth rate and consequently an initially rapid rate of increase in the value of Y. However, increases in Y reduce (P - Y) and as a consequence the decline in growth rate and rate of increase in Y are exponential in nature.

The low growth rates obtained prior to the establishment of a stable rate suggest some form of feedback mechanism operating within the plant. This undershoot may deserve closer attention especially since when the force is removed for 10 minutes and then reapplied similar kinetics for the growth burst are obtained but the magnitude of the undershoot is reduced. The work with Nitella, (Green et al 1971), shows no evidence of any feedback mechanism in operation, but this may be due to the changes in turgor pressure being more slowly effected than the changes in force exerted by the transducer slug, due to the

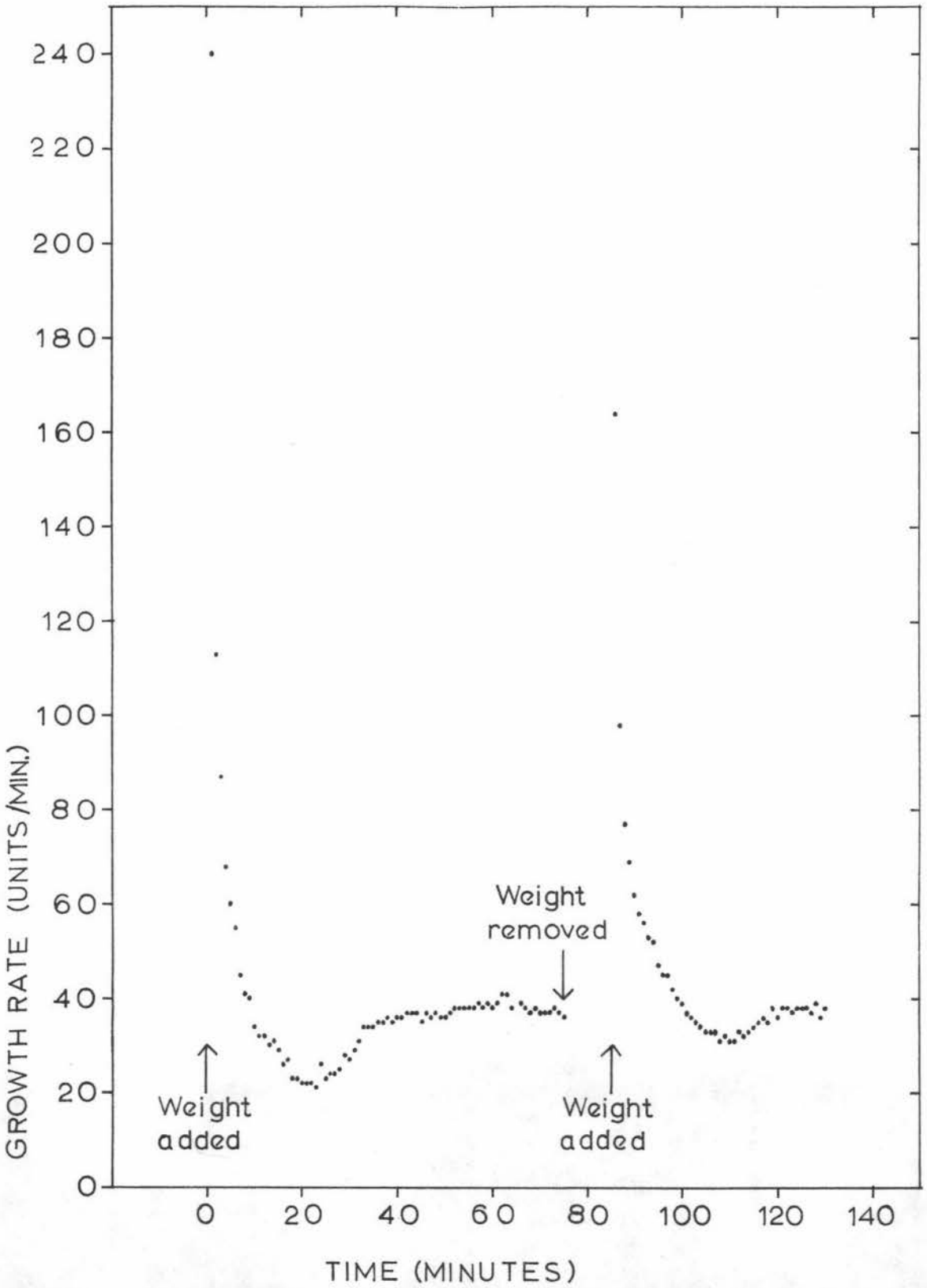


FIGURE 29 **The effect of the weight of the transducer slug on hypocotyl growth rates. (1 unit = 0.67 μm).**

effect of diffusion rates etc, on the instantaneous change in turgor pressure at a particular time.

On the basis of the results presented in Figure 29 it can be assumed that a stable growth rate would have been established within the routine three to four hour pretreatment period during which the transducer slug exerts its force on the lupin hypocotyl.

The experiments involving the effect of the transducer slug were conducted solely for the purpose of estimating the rapidity of the results obtained from the auxanometer developed in this study. However, the technique of varying the force on the hypocotyl could be developed further to enable comprehensive studies on the role of changes in minimum yield stress in cellular elongation. Green et al (1971) reported that rises in Y were physical in nature while decreases were dependent on metabolic processes. The described apparatus could be utilised to compare variations in force exerted on the hypocotyl under aerobic and anaerobic nitrogen conditions. With the system described in the present study growth rates cannot be recorded whilst the force is removed from the hypocotyl. This disadvantage could be eliminated by utilising an independent system for the addition and removal of weights from a counterbalanced platform fastened to the top of the transducer slug.

E. CONCLUSIONS

The aims of this project were:-

(a) to develop a technique which would enable the monitoring of hypocotyl growth rates at one minute intervals, in intact Lupinus angustifolius seedlings;

(b) to be able to accurately monitor hypocotyl growth responses to changes in a wide variety of environmental parameters;

(c) to compare the results obtained with similar short-term growth kinetic studies on lupin hypocotyl segments, in an attempt to identify those factors regulating the rate of cellular elongation in lupin hypocotyl tissue.

The development of the described auxanometer, utilising a displacement transducer, digital voltmeter and perspex root chamber (Figure 2), successfully fulfilled the first of these objectives.

The environmental chamber (Figure 6) was utilised to impose various environmental changes on the enclosed lupin seedlings, without interruption to the monitoring of growth rates. The diversity of the environmental parameters studied in this project reflects the versatility of the auxanometer when used in conjunction with the environmental chamber. The achieving of this second objective has, however, highlighted the problems encountered in effecting rapid changes of environmental parameters whilst simultaneously monitoring these changes. The difficulties posed by the majority of these problems could be avoided in future by the use of more sophisticated equipment than that utilised in this project, for the manipulation and monitoring of environmental parameters.

The third objective of this study has proved to be the most difficult to attain, largely as a result of two main factors which require consideration. Firstly, it was realised that the whole seedling

is affected by any imposed environmental conditions and consequently observed growth responses may have arisen, either as direct effects on cellular elongation, or as a result of prior inhibition of more sensitive metabolic processes. Secondly, few of the experiments performed with intact seedlings directly paralleled investigations with lupin hypocotyl segments. For example, although growth rates of both segments and intact hypocotyls have been studied during osmotic stress, the methods for improving the stress are entirely different and consequently direct comparisons of results may be unjustified.

The following conclusions, however, can be drawn from the observed growth responses of lupin hypocotyls to changes in various environmental parameters.

1. Hypocotyl growth rates are only slightly altered by saturation deficits of between 2.9 and 16.2 mbar.

2. Fluctuations in root temperatures of between 23 and 43° C do not significantly alter hypocotyl growth rates.

3. Changes in the osmotic potential of the root bathing solutions elicited growth responses basically similar to those reported by Acevedo et al (1971) for intact maize leaves. The majority of the responses are compatible with the theories of Green et al (1971) regarding the regulatory role of the minimum yield stress in cellular elongation. Processes involved in the regaining of turgor by plasmalysed tissues may account for the anomalous results obtained with solution potentials of less than -11 bar.

4. Anaerobic nitrogen conditions reduce hypocotyl growth rates to between 5 and 10% of normal within 20 to 30 minutes as has been observed for hypocotyl segments (Penny D. et al 1972). Although results illustrating the phenomenon of "stored growth" are comparable with those of Gillbank et al (1972) it is also apparent that growth inhibition resulting

from anaerobic conditions of greater than 30 minutes in duration is not completely compensated for by the aerobic growth burst.

5. Hypocotyl growth rates are stimulated within one minute of exposure to an anaerobic carbon dioxide environment. Carbon dioxide is postulated to stimulate the utilisation of a growth precursor within the cell whilst simultaneously inhibiting its continued synthesis. The hypothesis, proposed by Barkley and Leopold (1973a), that this growth precursor is a "growth limiting protein", the cytoplasmic pool of which can be completely replenished within 30 minutes, is supported by the described results from lupin hypocotyls. A phenomenon not apparent in studies involving the treatment of segments with CO_2 is the marked inhibition of hypocotyl growth rates and subsequent cyclic growth response on return to aerobic conditions. The elucidation of this phenomenon awaits further research.

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