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.

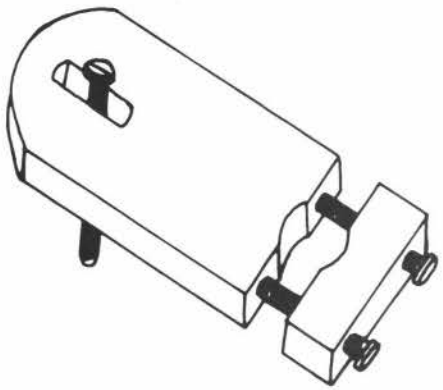


FIGURE 2 Perspex root chamber

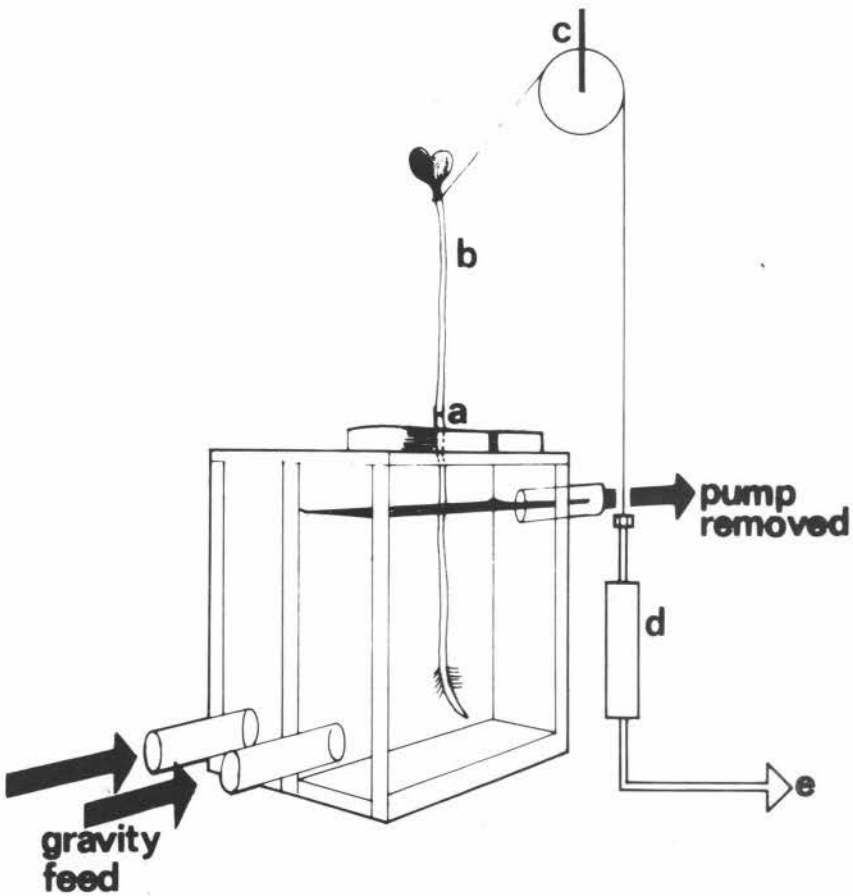
a seedling holder (Fig. I)

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.