WATER STATUS AND GROWTH INITIATION

IN *POPULUS*

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William Ross Nathaniel Edwards

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SUMMARY

A study was made of the significance of water status and the recommencement of growth in Populus following winter dormancy, using the following clones:

(i) Populus euramericana (Dode) Guinier cv. 'I-78'
(ii) P. yunnanensis Dode.
(iii) Populus deltoides ssp. angulata Ait. cv. 'Carolinensis'

A limited examination was made of two other factors - the effect of light, and the influence of exogenous auxin on growth initiation.

All growth experiments were carried out using a water culture technique.

Seasonal changes in water content and water potential were measured over the period of quiescence. Water content was at a minimum at leaf fall and rose slowly until growth initiation. Water potential rose slowly to a maximum in mid-winter, and then slowly fell. Although water content was significantly higher at the top of wands than at the bottom at leaf-fall, this was reduced or eventually eliminated with the general rise in water content, but there were no corresponding differences in water potential.

Water loss was clearly related to relative humidity, and cut ends of a cutting were a major site of evaporation. The presence of buds had a small effect, which was related to relative humidity both in direction and magnitude.

An investigation of the effect of exogenous auxin suggested that in P. angulata root initiation may be limited by low endogenous levels of auxin, but this was not confirmed since auxin assays were not done.

Light was shown not to be a factor in the numbers of shoots and roots produced, although root initiation was delayed by the light treatments. However, there was a significant failure rate in the dark in a substitute clone (a hybrid clone bred in Australia from Populus deltoides).

The effect of water stress on growth initiation and early growth was studied using an osmoticum in water culture of cuttings. The induced stress severely limited both shoot and root growth which was very low; below - 4 bar. However, budbreak occurred and root primordia developed in higher osmotic potentials, but below - 11 bar there was little development.

Internal water potential and water content were highly correlated with osmotic potential of the growth medium.

Shoots and roots were found to have water contents which were inversely related to the osmotic potential of the growth medium.

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APPENDICES:

Introduction

Within the last century, the vegetation of New Zealand has undergone a massive change. A high proportion of the steeplands, and virtually all of the flat and rolling country has been converted from temperate rain-forest to grassland which has resulted in considerably increased runoff. The inherently unstable nature of much of the sedimentary parent material has not resisted this change well, and erosion has become a significant problem in some areas.

Techniques of soil conservation and runoff control have been based mainly on plantings of the genus Populus in the form of "poles" some 10 to 12 feet long which can be established in the presence of stock, under Farm Plans organised by local catchment authorities. The total number of poles planted in 1967 was 400,000 - double the number of 1962 - and this is expected to at least double again. However, in spite of advantages in propagation, adaptability, growth rate and root system characteristics, problems in the establishment of poplar and willow have arisen. The most obvious of these is animal damage, chiefly cattle (through rubbing and bark biting) and opossum (browsing of foliage). A survey commissioned by the Soil Conservation and Rivers Control Council in 1968 investigated the level of pole loss and found a mortality of 24.7% and 41.8% over the first and second years respectively (Edwards; 1968, 1969 a). Although the major factors could not be positively identified, it was apparent that site factors, and water stress in particular, were major causes of loss.

This study investigated the importance of water relations in the vegetative propagation of Populus species. In particular, it was designed to establish the levels of water stress which would limit the initiation of growth in both root initials and buds.

CHAPTER ONE - REVIEW OF LITERATURE

1.1. The Use of Populus Species in New Zealand.

Poplars and willows can be propagated in several ways which are of overriding importance in soil conservation and river control works (van Kraayenoord, 1968 a). The most important of these is the ability to establish from poles of sufficient size so as to withstand cattle attack and to develop foliage above the browse line of domestic stock. Further, Populus species have other characteristics which are important; for example, hardiness, adaptability to a variety of site conditions, growth form and habit, availability and a lack of undesirable characteristics. Finally, the dioecious habit allows restriction of sexual reproduction if clones of only one sex are used in a particular area, since seedling swarms are not produced, so maintaining clonal purity. A number of methods of propagation are in use in New Zealand, and have been described by van Kraayenoord (1963, 1967, 1968a, 1968b, and a publication in preparation). The literature otherwise does not deal well with New Zealand conditions.

The types of plant material in common use for vegetative propagation are as follows:

i. Poles

The use of poles of 10 to 12 feet in length and 2.5 to 3.5 inches in diameter is the most widely used method of propagation in soil conservation works (van Kraayenoord, 1968a). Such poles are suitable for planting in the presence of stock if appropriately protected (Kelman, 1969; Edwards, 1967, 1969b,) and planted firmly to a depth of about 2.5 feet (Kelman, op cit.). A limited number of poles can be produced by pollarding trees, but most are now produced by allowing only 2 to 5 leaders to grow for two to five years on 'stools' maintained at a height of 18 inches. The pole is truncated to the required length after cutting. Most poles are produced in specialised nurseries, usually by local Catchment Boards.

ii. One-year-old Trees.

Trees grown under nursery conditions for 1 year from cuttings, attain height of up to 4 metres (van Kraayenoord, unpublished), and can be used for the establishment of specimen trees of good form, or for timber production in forests, woodlots or in rows. Alternatively, they can be pollarded to 50 cm above ground level and re-planted as stools for the rapid establishment of a nursery. However, use of one-year-trees is relatively expensive and their use is limited.

iii Wands.

A wand consists of one-year-old growth of 1.0 to 2.0 metres in length and 1 to 1.5 cm in butt diameter, taken either from established trees or from stools pollarded each year, with no restriction on subsequent growth. They are used for the establishment of trees beyond the reach of stock for conservation purposes, being planted some 30-50 cm into the ground, and not requiring weed control to survive in reasonable conditions. Although failure rates can be very high if water becomes limiting, this form of propagation is frequently used on 'retired' areas, since it is relatively inexpensive.

iv Cuttings.

A cutting consists of one-year-old growth of diameter 0.5 to 1.5 cm and length 10 to 30 cm, although shorter lengths can be used, as with rare material used in good growing conditions (Anon., 1958). Cuttings can be taken from established trees, or from stool-beds established for wand production, the one year growth in this case being cut to the length required, just above a bud. The cutting is inserted into the ground until only the top bud is exposed; this forms the leader. Some form of weed control is necessary to achieve reasonable growth. Cuttings are used in New Zealand for the establishment of stool nurseries, production of rooted trees for transplanting, and to increase stocks of less common clones rapidly.

Overseas methods of propagation are similar to those of this country, but some methods have been developed to overcome local problems. One of the most common is the technique of deep planting, i.e. the use of long plant material to reach a watertable (Costin, 1959; Grut, 1962; Simon, 1963), even to the use of poles 4 metres long (Mesnil, 1960), or two-year rooted trees planted to 6 metres (Muller, 1958). Methods such as these have enabled successful planting in situations where other techniques have led to high failure rates.

In all cases in New Zealand, normal harvesting of propagating material takes place during winter dormancy, and planting follows immediately, within the limits of local body organisation. While it is possible to take propagating material during active growth, conditions for establishment need to be favourable to achieve satisfactory success rates. Farmer (1963) found that P. tremula and P. grandidentata rooted in sand as greenwood cuttings but required an intermittent mist and IBA to stimulate rooting, in order to obtain consistent success.

1.2 Factors Influencing Vegetative Propagation in Populus Spp. Using Cuttings.

A number of factors influence the ability of a cutting to recommence growth; these fall into two categories:

(1) Inherent characteristics (Sections 1.2.1, 1.2.2)

(2) Environmental factors (Sections 1.2.3 - 1.2.5)

1.2.1 Size of cutting.

Cutting length is a measurement frequently used as a variable in survival experiments, showing a general increase in survival with increased length, at least up to 15 cm. The relationship is more pronounced with more severe conditions. The relationship between diameter and survival is similar, but less obvious.

B. Tomza (1959) using Populus 'manitobensis' cuttings ranging in length from 2 to 30 cm, found an increase in survival with increasing length up to 15 cm, but no effect of diameter between 0.3 to 1.2 cm. Chiang (1963) using P. canadensis cuttings between 9 and 20 cm in length also found a plateau after 15 cm, and a similar plateau above a diameter of 1 cm. Samsiev (1959) found no effect of length between 15 and 30 cm in three poplar clones, but a diameter plateau similar to that of Chaing's above 1 cm with one of the clones, P. bolleana. However, the two remaining clones (P. canadersis and P. nigra 'italica') grew satisfactorily even at 0.3 cm diameter. Finally, a Central Forestry Experimental Report (Anon., 1958) indicates that in comparatively difficult conditions, survival of 20 cm cuttings was 10%, and resulted in 30 cm of growth in the first season, compared with 30% and 90 cm of growth respectively for cuttings of 50 cm.

These studies on the influence of size on survival are usually incorporated in field studies of local problems, and do not involve investigation of specific sources of physiological stress. However, they serve to indicate the range of measurements which appear to limit growth or survival in the field.

1.2.2. Physiological Age.

Cuttings taken from one-year-old wood show a gradient of age and development according to the time during the season that the tissue was formed. Buds in the apical region are comparatively large, and when grown from cuttings form larger shoots more rapidly (Joachim, 1957). Root primordia form early in stem development, and continue to develop as growth of the wand continues. Braun (1963) observed the primordia to originate in the inter-fascicular regions of the outer cambium, usually in

front of a primary ray. They developed as protrusions into the primary phloem and became embedded in the secondary bark due to their slower growth rate, but did not lose contact with the primary phloem. Wood formation in the apex of the primordia increased with age causing the formation of humps which were visible when the bark was removed from stems older than two years. Primary primordia persisted until the rhytidom formed at 5 to 10 years.

Greater development of root primordia is thus manifested in earlier, stronger growth of roots from cuttings derived from older wood closer to the base of the tree or stool.

Many workers have found evidence for the effect of physiological age. Mutibari (1963) found no effect on shoot growth in terms of physiological age, but found large differences in the percentage of cuttings forming roots, e.g. 97.7, 82.2, and 52.0% for basal, central and apical cuttings respectively. Joachim (op cit.) found that a root system developed from lower regions more quickly, as did Bloomberg (1959) using P. trichocarpa , and Pantos (1963) using P. robusta. Viart (1965), using P. deltoides, suggested that rooting ability is at a maximum in the transition zone between that part of the shoot which is performed in the bud and that which is formed later, i.e. about the 7th or 8th node.

Schröck (1956) claims that a relationship exists between "growth potential" and the physiological age from which the wand or cutting is derived. Thus in P. beriolinensis, epicormics taken from various heights gave increasing growth rates with increasing height up to 4.5 to 5.5 metres for shoot growth; he quoted unpublished work by Lehnert which shows a similar result for root development in P. tremula.

1.2.3 Temperature

There is a requirement for a period of low temperature to break dormancy in Populus species, but commencement of regrowth in the spring is limited until the temperature rises above a threshold level, which is specific for particular clones (Pauley and Perry, 1954; Wareing and Phillips, 1970).

Although the relation between temperature and growth has been investigated for many clones, that between temperature and the recommencement of growth (or bud-break) has received little attention. Miami and Horton (1967) found that suckering in aspen (P. tremula) commenced earliest at 87°F., and that most suckers were formed and grew most rapidly at 74°F. Straub (1966) found a similar relation between temperature and shoot growth in aspen. Wareing and Smith (1963) demonstrated that rooting of P. robusta cuttings was dependent on active buds, and was therefore indirectly

dependent on a minimum temperature being attained to activate bud growth, implying a hormonal relationship between active buds and root growth.

Once a bud becomes active under rising temperatures, subsequent development will depend also on the light regime (Straub, op cit.) However, the inition of growth in Populus species is primarily a function of temperature alone, although other species have demonstrated a photoperiodic response.

1.2.4 Dormancy

While autumn growth rates diminish with time and can be correlated well with temperature fall, the cessation of growth and formation of a dormant terminal bud appears to be triggered by day length in one-year-old trees of Populus species, as shown by van Kraayenoord (unpublished). Supply of an adequate artificial light source will allow a continuation of growth. Termination of growth in older trees may occur at an earlier stage, possibly due to nutrient levels or hormonal balance (Wareing and Phillips 1970) and a period of 'pre-dormancy' or 'summer-dormancy' probably precedes the onset of true winter dormancy, perhaps under the influence of photoperiod.

The cold requirement to break dormancy of dormant winter buds of Populus species appears to be quite short. Ten to 14 days at approximately $2^{\circ}C$ is sufficient to artificially break dormancy in cuttings (van Kraayenoord, pers. comm.). However, Wareing and Phillips (op cit.) consider that emergence from dormancy under natural conditions may take some time so as to accumulate the cold requirement. Thus twigs collected in April, May, and early June (southern hemisphere) usually remain dormant if placed in conditions suitable for bud-break; those collected from July on will have a "fairly high" regrowth rate, which increases over subsequent months. They suggest that the requirement is 260 to 1000 hours at 0 to $5^{\circ}C$. for many temperate species of trees which respond to cold by breaking dormancy.

The cold requirement in Populus is not substituted for by a long-day regime (Pauley, 1958), although several other species (notably Fagus, Betula, Larix) react in this way (Wareing and Phillips, op cit).

1.2.5 Hormonal Mediation in Dormancy

While the mechanism of dormancy has not been defined well, it has been shown with many species that plant growth substances are closely involved in dormancy and it appears likely that an undefined mechanism is mediated by changes in the balance of plant growth substances (Wareing and Phillips, 1970).

The level of plant growth substances declines steadily over the growing season, and is low at the onset of dormancy. Wareing and Smith (1964) using P. robusta, found that the source of growth substances at bud-break was the actively expanding bud, but in summer, the source was the mature leaf. Rooting was found to be seriously impaired unless one of these sources was present and active. Shidei and Ogasawa (1957a, 1957b, 1957c) using P. nigra 'italica' showed that IAA stimulated rooting in cuttings according to season. In hardwood (winter) cuttings, bud removal lowered rooting ability, but this could be completely replaced by IAA; in softwood (summer) cuttings, rooting was found to be proportional to the amount of mature leaf present before winter bud formation, but was completely unaffected by mature leaf area after winter bud formation. Treatment with IAA stimulated root formation to some extent at later stages also, although root elongation was inhibited. Kefeli (1965) in similar experiments with Salix species concluded there was a balance between inhibitors and auxins both of which originated in the leaves and accumulated in the buds. Removal of leaves from summer cuttings greatly reduced rooting, but removal of leaves in autumn greatly promoted root formation, leading to the conclusion (supported by wheat coleoptile assays) that over the summer there is a progressive decline in auxin level, and a concomitant rise in the level of an inhibitor.

In general, it appears that rooting is dependent on the presence of a plant growth substance, possibly in balance with an inhibitor. The source of this growth substance is the newly-active bud in spring. In summer, the mature leaf is the source, although the activity diminishes over the season, and may be associated with a rise in an inhibitor.

Artificial stimulation of bud-break can be accomplished using exogenously applied hormones such as auxins (Boysen-Jensen, 1935; Avery et al, 1937) or gibberellins (Donaho and Walker, 1937) or cytokinins (Leopold, 1964). Balatinecz and Farrer (1966) conclude in similar studies with P. deltoides that a growth regulator such as IAA is necessary to reactivate a quiescent cambium, and that disbudded cuttings lacked this stimulus.

However, these plant growth substances neither act generally, or substitute for a cold requirement or photoperiod requirement in all cases. It is more probable that in many cases, hormonal requirements are a part of growth changes resulting from other processes which break dormancy, and their artificial supply renders normal dormancy-breaking mechanisms redundant.

1.3 Water Status

1.3.1 General

Water, as the major component of protoplasm, has four separate roles (Kramer, 1963):

(a) As a reagent in biochemical reactions

(b) As a solvent in transport and in biochemical reactions

(c) In the physical maintenance of cell and plant turgidity, and stomatal activity

(d) As an integral component of plant structure.

Any situation where water is limiting can influence any or all of these roles.

Terrestrial plants occupy both the upper soil horizons as roots, and the lower atmospheric layers as stem and foliage. Water is always present in soil, plant and atmosphere as liquid, vapour or in an adsorbed state. While the normal direction of water flow is from soil to air, the transpiration stream through the plant may intervene to form a third, intermediary phase. Since water forms a significant proportion of all three phases and since interchange is normal, it is convenient to consider the soil-plant-atmosphere system to be a continuum (Buckingham, 1907; Gradman, 1928; van den Honert, 1948, Richards and Wadleigh, 1952; Philip, 1954, 1957, 1966; Slatyer, 1967, Kramer, 1969).

Description of water status in any phase of the continuum can be made in terms of the energy status of the water in the particular phase, and is therefore based on thermodynamic principles (Taylor, 1968 - a review). In a uniform multi-component system such as a soil-plant-air continuum, the total Gibbs free energy per unit mass is the sum of the partial Gibbs free energy for each chemical species present, i.e. the sum of the chemical potentials of each species. The partial Gibbs free energy of water is thus known as the chemical potential of water, or more simply, water potential.

The water potential thus can be defined in terms of the Gibbs equation, which relates water potential to temperature, pressure and concentration of water, in a particular system. Further, movement of water can only occur along gradients of decreasing water potential (Gardner, 1968).

The parameter of water potential, together with the parameter of water content comprise the two basic measurements used to describe water status. If the measurements of water status in the plant phase include a negative water potential (as is normal under conditions of active transpiration), the potential is known also as a plant water deficit (Taylor, op cit.).

There are three components of water potential (Taylor, op cit.; Slatyer, 1967):

(1) An osmotic potential, arising from the presence of solutes. In the soil, these are within the soil solution; in the plant, throughout the liquid phase, particularly in vacuolar solution.

(2) Matric potential, arising from adsorption of water. In soil, the sites are mainly on clay and humus colloids; in the plant, cell colloids and capilliary forces of the cell walls.

(3) A physical pressure component. In the soil, the source is atmospheric pressure; in the plant, turgor pressure within the cell.

The thermodynamic description of water status implies that water is held within the system by a set of forces, and that an input of energy is required to move water. In fact, the amount of work required to remove a unit amount of water from the system is equal to the water potential, and hence the potential is negative, or alternatively, there is a positive affinity for water attributable to the system (Taylor, op cit.).

Water potential appears to give the most satisfactory measure of water status for comparisons between similar plants (Gates, 1968; Owen, 1953). However, the water contents for dissimilar plants at particular levels of water potential can be widely disparate (Slatyer, 1960), and it has not yet been shown if plant reaction to water stress is affected by the chemical potential of water, or by water shortage per se.

Plant water potential appears to be more meaningful than plant water content in relating water stress to plant growth. The relation between the two parameters may indicate differences in response between species to environmental water stress.

There are five basic characteristics in the biochemical and biophysical explanation of water status in plant growth and metabolism (after Gates, op cit.):

(1) Active synthesis is only possible at high tissue water levels. Ultimately, the rate of growth is proportional to the chemical potential of water in the tissue (Owen, op cit.)

(2) Re-establishment of high tissue water levels will re-establish former growth rates, unless tissue damage has been caused by high levels of water stress.

(3) While net synthesis can be observed to cease at high water potentials, it cannot be inferred that turn-over of cellular and nuclear components such as RNA have ceased, unless this has been specifically

demonstrated (Gates and Bonner, 1959).

(4) The role of water is similar even in widely different plant tissue, or tissue of different physiological ages. The response to water potential in different parts of the plant differs in extent rather than nature; the overall plant response to water potential is the integrated sum of these individual responses.

(5) Resistance (both avoidance of and tolerance to low internal water potentials) to high levels of water stress has features which are common to both embryonic tissue, and to drought-resistant, mature plants.

Normally, water deficits within a plant develop because of an excess of transpiration over absorption (Kramer, 1962). Therefore those factors which lead to an increase in transpiration (high temperatures, wind, low relative humidity) or a decrease in absorption (low soil water potential, low soil water conductivity, limited root growth) lead to increased plant water deficits. Under conditions of active transpiration, the extent of the plant water deficit is at least that of the soil water potential, and is further increased by the effect of gradients resulting from low soil permeability to water, and gradients within the plant originating from evaporative loss.

Since plant water deficits are the result of an imbalance in the rate of absorption over transpiration, diurnal variation in transpiration due to micro-climate changes and stomatal closure in the dark can be expected to cause diurnal variation in plant water potentials (Gardner and Nieman, 1964; Slatyer, 1957). It is not until the soil water potential is relatively large that significant plant water deficits are present continuously, since night replenishment in the absence of active transpiration is a very significant factor. Diurnal variation is much less significant in dormant plants (Cowan, 1965; Bonner, 1967; Rawlins et al, 1968).

1.3.2. Water Deficits and Growth

The relation between growth and water stress is well established and reviewed (Vaadia, Raney and Hagan, 1961; Kramer, 1963; Kozlowski, 1964; Slavic, 1965; Ruhland, 1961a, 1961b, 1961c; Slatyer, 1967; Kozlowski, 1968; Kramer, 1969).

There are two components of growth at the cellular level: cell enlargement, and cell division. Cell enlargement is dependent on the turgor pressure component of water potential (Wadleight and Gauch, 1948; Ordin, 1958, 1960; Slavic, 1965), showing a zero-bound decline with decreasing

turgor pressure. However, non-vacuolated meristematic tissue does not appear to be affected to the same extent by turgor pressure as much as by total water potential, at least in some species. Shoot elongation in corn (Loomis, 1934) and cotton (Balls, 1908, cited by Slatyer, 1967) show extreme sensitivity, but tomato shoots (Wilson, 1948; Slatyer, 1957) elongate even when the leaves are wilted.

Cell division is less influenced by high water potentials than is cell enlargement so that stressed plants, although smaller, tend to have similar cell numbers (Maximov, 1929). DNA content is related linearly to cell number (Nieman and Poulsen, 1962), so that measurements of DNA tend to reflect changes in cell number. Typically, there are large decreases in DNA with an increase in water deficit to about -3 bars in tomato, and a much slower decrease thereafter (Gardner and Nieman, 1964; Gates and Bonner, 1959).

Cytokinin production in the roots undergoes a massive drop in proportion to increasing water stress; since cytokinin tends to maintain a rather strict ratio with RNA of about 20:1, there is a very significant associated fall in the rate of protein synthesis (Gates and Bonner, 1959; Stutte and Todd, 1968; Key, 1969), a fall in protein molecule size (Routley, 1966; Stutte and Todd, 1967) and a rise in soluble nitrogen (Alekseev et al, 1963).

The effect of water deficit on photosynthesis is beyond the scope of this review, which is primarily concerned with emergence from dormancy.

The removal of a water deficit will frequently result in an increase in apparent relative growth rate, even beyond that observed before a deficit was applied, followed by a return to normal levels providing injury has not resulted. This effect appears to be a result of a transitory increase in cell expansion rate rather than a real increase in growth rates (Gates, 1955; Stocker, 1960).

Work with many species had indicated that relatively small soil water deficits will have an effect on growth. Gingrich and Russell (1956) using corn found a decrease in the rate of growth over a range of soil water potentials from 0 to -12 bars, with most effect in the range of -1 to -3 bars. Similarly, Sands and Rutter (1959) using Pinus sylvestris grown in soil permitted to dry to predetermined tensions before complete resaturation, found growth was reduced first in the -0.3 bars treatment in 1-year plants, and in the -0.5 to -1.5 bars range in three-year-old plants. Greater reductions were evident at higher deficits in all cases. They further quote six authors using a variety of species, who found reductions in growth at similar water potentials:

Howe and Rhoads	(1948)	Potato	-0.5 bars
Haynes	(1948)	Maize	-0.7 bars
Kenworthy	(1949)	Apple trees	-0.55 bars
Waterhouse and Clements	(1954)	Sugar cane	-0.65 bars
Stanberry et al	(1955)	Alfalfa	-0.6 bars
Salter	(1957)	Tomato	-0.4 bars

Stransky and Wilson (1964) using seedlings of Loblolly pine (Pinus taeda L.) and Shortleaf pine (P. echinata Mill) found that growth was inhibited by soil-water tensions of less than -2 bars, and ceased at -3.5 bars. Wilting occurred at -5 bars and death at -15 bars.

It is clear that water deficits as small as -1 bar or less can significantly reduce growth, especially in young plants. Growth reductions increase with increasing water deficit, and most workers have found that growth ceases completely by the time the permanent wilting point is reached (Slatyer, 1967).

1.3.3 The Measurement of Water Deficits

The two primary parameters of water status are water potential and water content. Although the determination of water content has been suggested to be unsatisfactory as a measurement of water stress, (Kramer and Brix, 1965; Weatherley, 1965), several workers have argued that situations in which only overall gradients of water potential are considered, meaningful assessments of the size and direction of water movement cannot be made (Macklon and Weatherley, 1965 a; Barrs, 1966 a). Water content is relatively simple to assess, and has been the basis of most studies of water status in the past.

Barrs (1966 b) in a review of methods of measurement of water status concluded that "ultimately, measurement of water deficit is most meaningful in terms of the effect of stress on plant growth....." but that "measurement of water content continues to be one useful criterion of water deficit."

1.3.3.1 Measurement of Water Content

The determination of water content relies on determination of water loss when a sample is dried under standard conditions, and as such, is highly reproducible. However the expression of water loss can be based on several measurements, all of which have some disadvantages (Barrs, op cit.):

(i) Dry weight is the base used in most measurements of water content. However, dry weight does vary over a period of time, especially in leaves. This can be minimised by extracting with dilute HCl

to remove labile carbohydrates first (Mason and Maskell, 1928), but the process is tedious and rarely used.

(ii) Fresh weight is also used as a base, although the errors inherent in dry weight measurement are still present. This base tends to minimise the extent of changes in water content.

(iii) Leaf area can be used in some circumstances, and is satisfactory for comparisons over a limited range. At extreme levels of stress, the leaf area tends to decline.

(iv) Water content at full turgor is another base which is suited to leaf and twig studies. Stocker (1928, 1929a, b) first used the method with whole twigs and leaves, and Weatherley (1950) modified the method by using discs punched from leaves instead of whole leaves, to give "Relative Turgidity". There has been Turgidity". There has been a considerable variation in methodology and terminology developed around this technique. The term "Water Saturation Deficit" is now gaining general acceptance. (Barrs, 1968):

$$W.S.D. = \frac{(\text{Turgid Wt.} - \text{Fresh Wt.}) \times 100}{\text{Turgid Wt.} - \text{Dry Wt.}} \%$$

Indirect measurement of water content can be advantageous in some situations, particularly those which permit nondestructive sampling or continuous recording. Principal methods involve measurements of leaf thickness, fruit size, trunk diameter, beta- and gamma-ray gauging of leaf thickness or whole-plant canopies, capacitance measurement, using leaves as a dielectric material, electrical resistance, and infra-red spectroscopy (Barrs, op cit.).

1.3.3.2 Measurement of Water Potential.

Most techniques for the measurement of water potential are based on two principals (Barrs, 1968; Kramer and Brix, 1965; Slatyer, 1965):

(i) Establishment of the concentration of a solution which is isopiestic with a plant or soil sample. The water potential of the sample is assumed to equal the osmotic potential of such a solution.

(ii) Measurement of the relative humidity of an atmosphere when in equilibrium with a plant or soil sample under strict temperature control. Such measurements are usually standardised against solutions of known osmotic potential.

(iii) Measurement of the pressure required to balance movement of xylem fluids up a twig.

Methods involving immersion of the samples in a range of standard solutions have the advantage of simplicity and involve inexpensive equipment. The process of immersion however, involves complications of injection of intercellular spaces, infiltration of plasmolysed cells, solution dilution by cell wall water and the effect of cutting the tissue. (Barrs, op cit.).

The main methods which have been used are as follows:

(i) Liquid Phase Methods

(a) Cell Method:

Microscopic observation of a cell immersed in a series of solutions to establish the solution which will not cause an increase or decrease in cell size (first used by Ursprung and Blum in 1916).

(b) Tissue Method:

Observation of changes in length, thickness, volume, weight, or curvature of suitable tissue when immersed in solutions as above (Barrs, op cit.).

(c) Measurement of Changes in Solution Characteristics:

Methods of this type attempt to find a solution which is isopiestic with the plant samples from a range of standard solutions by observing changes in the solutions caused by the plant material. Characteristics which have been used are changes in density (Schardakov, 1938, 1956; Rehder and Kreeb, 1961; Goodeand Hegarty, 1965; Knipling, 1967; O'Leary, 1970), refractive index (Maximov and Petinov, 1948; Ashby and Wolfe, 1947; Gaffe and Carr, 1964; Goode and Hegarty, 1965), and rate of uptake of solution (Brouwer, 1953).

(ii) Vapour Phase Methods

Methods involving vapour phase techniques avoid immersion in solutions. relying on transfer through equilibrium with the atmosphere in a small chamber. The atmosphere effectively acts as a semipermeable membrane, since the solvent of the solution cannot evaporate. However, the temperature of the system must be maintained within close limits - at least 0.001°C. (Slatyer, 1958). A range of solutions can be held above the plant or soil sample, and meniscus movement measured (Ursprung and Blum, 1930) or changes in the weight of subsamples of the unknown held over a range of solutions measured (Slatyer, op cit.). Alternatively, direct measurement of vapour pressure can be undertaken, removing the necessity for a range of solutions. The equilibrium

vapour pressure is reached in an isolated chamber at a closely controlled temperature and measured by techniques such as evaporation from a hanging drop (Macklon and Weatherley, 1965 b) or by psychrometric measurements using either a drop of water held at the appropriate junction (Richards and Ogata, 1958) or using the Peltier effect to cause dew deposition on the appropriate junction (Spanner, 1951). Although vapour pressure methods are potentially very accurate, and have the considerable advantage that immersion is not required, there are several difficulties in practice. The extreme temperature control necessary makes the apparatus unsuitable for field use or for measurements in situ, and temperature gradients are difficult to eliminate. In particular, gradients may arise from the heat of respiration, and the heat of condensation of vapour at the sample surface. In the psychrometric devices, evaporation from the wet bulb may increase the humidity significantly, and vapour pressure differences can also arise from methods of tissue exposure or low leaf permeability leading to a rise in atmospheric water potential and spuriously high measurements. Further, salt deposits on leaves or the chamber are sufficient to modify readings (Barrs, 1968).

The vapour phase methods tend to be slow, but can be automated to some extent (Hoffman et al, 1969). Although most determinations are carried out on detached leaves or leaf disks, there have been attempts to measure water potential of entire, attached leaves of mesophytic plants, with some success (Tirklin, 1968).

(iii) Pressure Balance Technique.

A method first used by Dixon, (1914) and developed by Scholander (1964, 1965, 1966) and Wareing and Cleary (1967) consists of supplying just sufficient pressure to the foliage of an excised twig or leaf to force the sap to the point of being extruded. The pressure is applied by placing the plant sample in a "pressure bomb" with the cut end protruding through a pressure-sealed aperture. The gas pressure is raised inside the bomb until the sap can just be seen emerging from the cut end. The pressure at this point is taken to be the water potential of the plant sample.

1.3.4 The Use of Osmotica in Water Culture

The maintenance of a constant soil water potential is one of the most difficult problems in studies relating growth to soil water potential. Although the water potential of samples of soil can be measured relatively easily, plant absorption of water is uneven. At low soil water potentials, permeability is low, and it is extremely difficult to re-water a soil to a specified water potential other than field capacity without disturbing the soil (Gardner, 1968). However, a replacement technique has been attempted (Vaclavic, 1963; Sestac and Vaclavic, 1963; Necas, 1963), although most workers have used successive drying and rewatering cycles (Loustalot, 1945; Kozlowski, 1949; Negisi and Satoo, 1954; Gates, 1955, 1957; Gingrich and Russell, 1956; Slatyer, 1957; Sands and Rutter, 1959; Gardner and Ehlig, 1963; El-Sharkaway and Hesketh, 1964; Stransky and Wilson, 1964; Pallas et al, 1967; Bonner, 1967 ; Rawlins et al, 1968). A constant soil water potential has also been achieved for limited soil volumes using a soil layer separated from an osmoticum by a cellulose acetate semi-permeable membrane (Painter, 1966; Zur, 1967 a, b).

A technique which has been used extensively in the last decade is the replacement of soil culture by water- or nutrient- culture, and applying an appropriate water potential using an osmoticum. The stress can be applied accurately and evenly to the entire root system, and the whole plant can be observed without disruption.

The primary criticism of this approach is that this root environment is considerably different from that of the soil. In particular, aeration, incident light in the root region and the lack of physical effect from soil particles may be problems. Further, there may be a reaction of the plant to the osmoticum, by replacing the soil water potential by an osmotic potential only, or by absorption of the osmoticum into intercellular spaces or within the cell, with consequent changes in water status or metabolism (Slavic, 1963 - a review).

Studies on salinity have used a solution-culture technique, frequently with some attempt to separate osmotic effects from other effects of salinity (Bernstein, 1961 a, b, Boyer, 1965; Neiman, 1965; Ingelsten, 1966; Oertli, 1968).

The development of culture methods using polyethylene glycol (PEG) has permitted the application of osmotic effects on root systems without the effects of absorption into the plant, providing the plant is intact and the PEG molecular weight is sufficiently high to prevent admission into the root cells (Macklon & Weatherley, 1965). Janes (1961) used PEG of

various molecular weights between 400 and 6000 in the solution-culture of tomato and celery, and found no plant injury except at the highest molecular weight of 6000. Transpiration declined in proportion to increasing water potential applied at levels greater than -2 bars, falling to 17% at -14.4 bars, growth fell similarly, ceasing at -11 bars. Measurable amounts of the osmoticum were not found in the xylem until at least -5 bars were applied, and at -14.4 bars after 9 days, the PEG content was 0.13 of the fresh weight. Similarly, Langerwerff et al (1961) using PEG of molecular weight 20,000 (PEG 20,000) to apply a water stress of up to -12 bars to kidney beans found no evidence of interference with normal metabolism or toxicity effects, and concluded that the material was suitable for use as an osmoticum. Jackson (1962), while concluding that carbowaxes (i.e., PEG) are suitable agents for use as osmotica for multicellular systems such as coleoptile sections or entire root systems, noted that they were not suitable for studies of root hair growth, since very low concentrations (0.2 - 0.6% PEG 600, or 8% PEG 1540) significantly inhibit root growth. Jarvis and Jarvis (1963) concluded that PEG 1540, PEG 4000 and dextran were superior to sodium chloride and mannitol as osmotica for culture of Lupinus alba. Ruf et al (1963) found that the osmotic potential of a range of species rose by an average of 0.86 bars for each 1 bar increase in the osmotic potential of the root medium, using PEG 1540. Xeric species developed a higher cell osmotic potential than mesic species.

PEG of various molecular weights have been used with a wide range of species to investigate many other situations where water stress is important, such as gaseous and nutrient uptake, respiration, cell and plant reaction, and germination of seeds (Jarvis and Jarvis, 1965; Barrs, 1966 b; Janes, 1966; Greenway & Hiller, 1967; Larson and Schubert, 1969; Kaufmann and Eckard, 1971).

A number of workers have investigated the extent of absorption into the plant, and possible toxicity of PEG (Slatyer, 1961, Greenway et al, 1968; Jackson, op cit.; Janes, 1969; Michel, 1970; Lawlor, 1970; Kaufman and Eckard, 1971). In general, PEG of lower molecular weights can penetrate the plant more easily, and may lead to plant adjustments which are different from those found in soil or using PEG of higher molecular weight. Thus, Kaufmann and Eckard (op cit.) found PEG 400 gave a reduction in osmotic potential of root xylem sap which could cause guttation, at nutrient solution osmotic pressures above -4.8 bars. At least half of this adjustment was due to increase in concentration of cations. The effect was not seen using PEG 6000. Lawlor (op cit.) suggests that PEG of molecular weights greater than 1000 are not absorbed in significant quantities, but that PEG 200 and mannitol are absorbed into the root system of an intact plant, possibly causing

cation absorption and salt accumulation. Where mannitol is used as an osmoticum, the probability of metabolisation within the plant must be considered. There are wide species differences in the ability of plants to metabolise mannitol.

In general, these studies indicate that PEG of sufficient molecular weight can be used successfully as an osmoticum in nutrient culture, having substantial advantages of inertness and non-absorption, provided that limitations of nutrient culture are noted.

CHAPTER TWO - METHODS

2.1. Experimental Objectives

The primary objective of the investigation is to establish the significance of water stress as a limiting factor in the vegetative propagation of Populus species in the post-dormant stage, relating this to the water status of the parent plant. It is not intended to relate the effect of water deficit to growth of Populus spp., except as an indication that growth initiation has been established.

In order to relate the water status of the cutting to that of the parent stool at the time of growth initiation, a study of water content and water potential of the parent plant was made over the dormant period until the re-establishment of growth.

The significance of water status in the initiation of bud-break and initiation of root growth in cuttings was investigated using a water-osmoticum growth medium, under conditions that would not normally be limiting to the re-establishment of growth.

A description of the characteristics and classification of Populus species is provided (Appendix 1) as a background to the selection of the four clones used in this study (Appendix 2) which represent clones of importance or interest in New Zealand. The conditions under which the plant material used was grown are described in Appendix 3.

2.2. Techniques

A number of techniques were used throughout the study and for convenience are described here.

2.2.1 Growth conditions

All growth experiments were conducted in a light-proof room maintained at 20.0°C. $\pm$ 1.0°C., with temperature gradients reduced by means of a fan and duct system. The light source used throughout consisted of banks of 40 watt fluorescent tubes (Osram 'Cool White') at 1.75 inches spacing held 22 inches above the top of the pots, and providing 800 foot-candles at the level of the apical buds. Where a dark treatment was required, construction of the room allowed blanking off of suitable areas using black polyethylene film and ply-wood facings, giving complete light exclusion.

Water culture methods were used in all growth experiments. Waxed paper cups (Lily No. 108W) fitted with plastic press-on tops were used as containers, normally with four cuttings per pot. A cutting of 10 cm inserted to the base of the pot through a suitable hole in the top protruded 1.5 cm., with only one bud above the top. The cup contained 150 ml. of

liquid in all experiments. Where light was to be excluded from the enclosed region of the cuttings, the cups were sealed against light using asphalt emulsion and black matt paint on the exterior.

2.2.2 Measurement of Water Content

Determination of water content was based on the formula

$$\text{Water Content} = \frac{(\text{Fresh Weight} - \text{Dry Weight}) \times 100}{\text{Fresh Weight}} \%$$

Where possible, fresh weight was determined immediately after making both-end cuts on the sample, or at least within 30 seconds. When this was accomplished, significant differences in water content were unlikely to be caused through evaporation from the cut ends.

Dry weight was determined after 72 hours at 95°C. The measurement was made as soon as the sample was cool enough to be weighed, and within 3 minutes of removal from the oven. Within these limits, significant differences through absorption of atmospheric water were unlikely to arise.

2.2.3 Measurement of Water Potential

Schardakov's dye technique was used for determining water potential throughout the study (section 1.3.3.2). The method consists of equilibrating samples of the plant material with a range of standard concentrations of sucrose, in order to determine which solution is most nearly isopiestic. The osmotic potential of this solution is taken to equal the water potential of the plant material, since water is neither gained nor lost. The isopiestic solution is found by comparing the density of the equilibrated solution into which a dye has been added into the original standard solution. If water has been gained, the dyed solution will rise, and vice versa.

Up to 17 solutions of standard concentration were used for any particular set of determinations, at 0.5 bar increments to -20.0 bars. The probable size of any particular reading could be estimated with experience.

The containers for equilibration were 25 ml. tube vials with screw caps. One ml. of solution was used with approximately 1 gram of plant material for each grade of solution, and the tube capped before equilibration for 24 hours. In all cases buds were avoided. The experiment was designed so that 45 or 50 determinations involving 450 to 550 vials were used at any one time.

The dye used was methylene blue, dissolved to excess in an appropriate range of solutions of sucrose to reduce a possible error through adding a solution of different osmotic potential. Only sufficient dye solution was added to just colour the equilibrium solution, being of the order of 0.00005 ml., added with a hypodermic syringe and 26 ga. needle.

Comparison of the equilibrated solution with the original solution was carried out by injecting a single drop of the former into a 25 ml. vial full of the standard original solution. The instrument used for this was a hypodermic syringe fitted with a modified 20 ga. needle. The modification consisted of removing the tapered point to form a uniform hole, and bending the end 0.25 cm. to form a right angle, after heat annealing. The modified instrument allowed a single drop of the equilibrated solution to be injected horizontally into the standard solution (held in a stand) with a minimum of disturbance.

After each determination, the bottles were thoroughly washed and dried at 95°C for 24 hours.

Although laborious, the method gave consistant results to the level of accuracy required, i.e. within 0.5 bars.

2.2.4 Measurement of Growth Responses

The following standard measurements were applied to all experiments where growth was recorded:

(i) Budbreak

The observable response of the bud to a favourable growth environment was a slight swelling, followed by the appearance of a thin line of lighter-coloured tissue on the upper bud scale as expansion takes place. The first sight of this line was taken as the time of budbreak.

(ii) Shoot Growth

The length in centimeters from the top of the lowest bud scale to the growing point was called the total shoot growth.

(iii) Root Initiation

As the root primordia commence growth characteristic hemispherical protrusions of diameter 0.15 to 0.20 cm formed underneath the rhytidom. The first time of observation of such a protrusion or protrusions was called the time of root initiation.

(iv) Root Initials

All root primordia protrusions including

those which had developed to the stage of rhytidom rupture (but not including those in which the root had grown beyond the rhytidom and become obvious) were called root initials.

(v) Roots Smaller than 1 cm.

This category included all those roots which had grown beyond the rhytidom, but were less than 1 cm long.

(vi) Roots Greater than 1 cm

This category included all roots which exceeded the previous category.

(vii) Basal Roots

Roots sometimes arose from the basal cut, and were called basal roots to distinguish them from roots arising from primordia present when the cutting was taken from the parent stool.

(viii) Side Roots

Roots which have arisen from primordia present when the cutting was taken from the parent stool.

(ix) Final Shoot Weight

At the termination of the growth experiments, the shoots in each sub-treatment were cut at the base of the original bud and bulked for the determination of 'fresh shoot weight' was obtained after drying at 95°C for 72 hours and weighing immediately on cooling.

(x) Final Root Weights

The 'fresh root weight' and 'dry root weight' were found using the same method used for shoots. The roots were removed at the point of emergence from the rhytidom.

(xi) Total Root Length

The combined lengths of all roots showing measurable growth; side roots which were occasionally present were included.

(xii) Shoot and Root Water Content

These were calculated from the corresponding fresh and dry weights, as above.

2.2.5 Control of other factors influencing propagation

Review of the literature and consideration of the circumstances of vegetative propagation indicated that a number of factors could influence propagation by cuttings. Further information was obtained from a pilot

experiment, which investigated the effect of a range of osmotic potentials and the effect of sealing the ends on the growth of Populus X 'I-78' cuttings in liquid culture (Appendix 4). The following factors were considered in detail in the design of subsequent experiments:

(i) Dimensions

Cuttings outside the limits of 1.0 to 1.5 cm diameter are inferior in conditions of stress. The parent stools used provided reasonably homogenous cutting material between 0.9 and 1.3 cm diameter, and these limits were accepted.

(ii) Physiological Age

The age at which particular tissue has been initiated appears to be related directly to ability to initiate roots, and inversely to bud development. An attempt was made to reduce this effect by limiting the section of the wand used to the middle third of each wand, this being between 50 and 75 cm. The buds in this region tended to be of similar size, the surface ridges distinctive but not pronounced, the rhytidom developed from green to grey and completely non-fissured, and the ratio of outer diameter to pith diameter was of a similar magnitude (see section 2.2.5).

(iii) Physiological Activity

Study of seasonal changes in rooting ability and shoot growth responses and the results of the pilot experiment appeared to show that the cold requirement was fulfilled early in the winter, and that recommencement of growth was primarily a function of rising temperatures. Prior to the critical temperature being reached as seasonal temperatures rose, morphological changes caused by renewed physiological activity could not be observed, other than a rise in water content and a slight concomitant rise in water potential.

The time of commencement for the main growth experiment was selected at about six weeks before normal bud-break, which also coincided with commercial harvesting. At this stage the cold requirement was fulfilled, physiological activity was at a minimum and the water content was just starting to rise.

(iv) Temperature

Although it is clear that bud-break is primarily a function of temperature in the field, the precise threshold and optimum levels have not been established for most species. From consideration of the literature (Section 2.2.5), a temperature of 20°C was selected

somewhat arbitrarily. In the pilot experiment there was a satisfactory growth response.

(v) Nutrient Level

The main stages of the investigation into water stress involved emergence from dormancy and the first stages of root and shoot production, in which the amount of tissue produced was small in comparison with the volume of the cutting. Further, there were no sites available which are normally associated with nutrient absorption in the dormant cutting until root production has taken place. The pilot experiment showed that internal reserves of the cutting were in fact sufficient for more growth than the main experimental series would involve.

Therefore, it was considered unnecessary to use a nutrient culture rather than water.

(vi) Sealing of the Cutting

The object of the experiment was to imitate the effect of soil water potential by using an osmoticum. Therefore, it was important that the osmoticum did not penetrate the xylem, phloem or intercellular spaces, and a technique of sealing both ends of the cutting was employed throughout. In the pilot experiment, callusing appeared at the distal end of the cutting if the sealing agent was opaque, but this was not observed at the proximal end, at least within the time periods under consideration.

(vii) Day-Length

Although day-length influences the onset of dormancy, it has not been implicated in bud-break or root initiation in Populus spp. However, it is well correlated with increase in temperature, and it was necessary to establish if there was a significant effect. Further, it was necessary to establish if the photosynthetic tissue exposed by bud-break is likely to be significant to immediate growth requirements.

An experiment was carried out prior to the main water stress experiment to investigate these factors. Three light regimes were imposed: complete darkness, continuous light, and a cycle of 12 hours of light followed by 12 hours of darkness. This combination was intended to demonstrate if there was a long-day or short-day requirement, and if there was an effect of light on either bud-break or root initiation.

On the basis of the results of this experiment, a light regime consisting of 12 hours of light followed by 12 hours of darkness was selected.

(viii) Hormonal Level

Although a study of internal hormonal levels is beyond the scope of this study, it was relevant to consider the effect of an applied growth hormone, to establish if a hormonal deficiency influenced the time or rate of root primordia development.

An experiment was carried out to investigate the effect of a logarithmic series of IAA concentrations between 0.01 mg/litre and 100.00 mg/litre on three clones of Populus, grown as cuttings in liquid culture. Several methods of sealing were also used in a factorial design.

2.3 Experimental Procedures

2.3.1 Study of Seasonal Changes in Water Status

Sampling over the dormant season was carried out at 21 day intervals, commencing on 14 April 1970, when the first clone had completed leaf-drop. Seven sampling times were taken, ceasing on 7 September 1971. All three clones reached the point of budbreak by this date.

At each sampling time, observations of weather, soil conditions, and any morphological changes were noted.

The method of sampling consisted of selecting a total of 5 wands, (i.e. the entire growth of one shoot for the previous season), taking one from every second stool, and alternating the five stools sampled each sampling time. Thus a total of ten stools per clone were used.

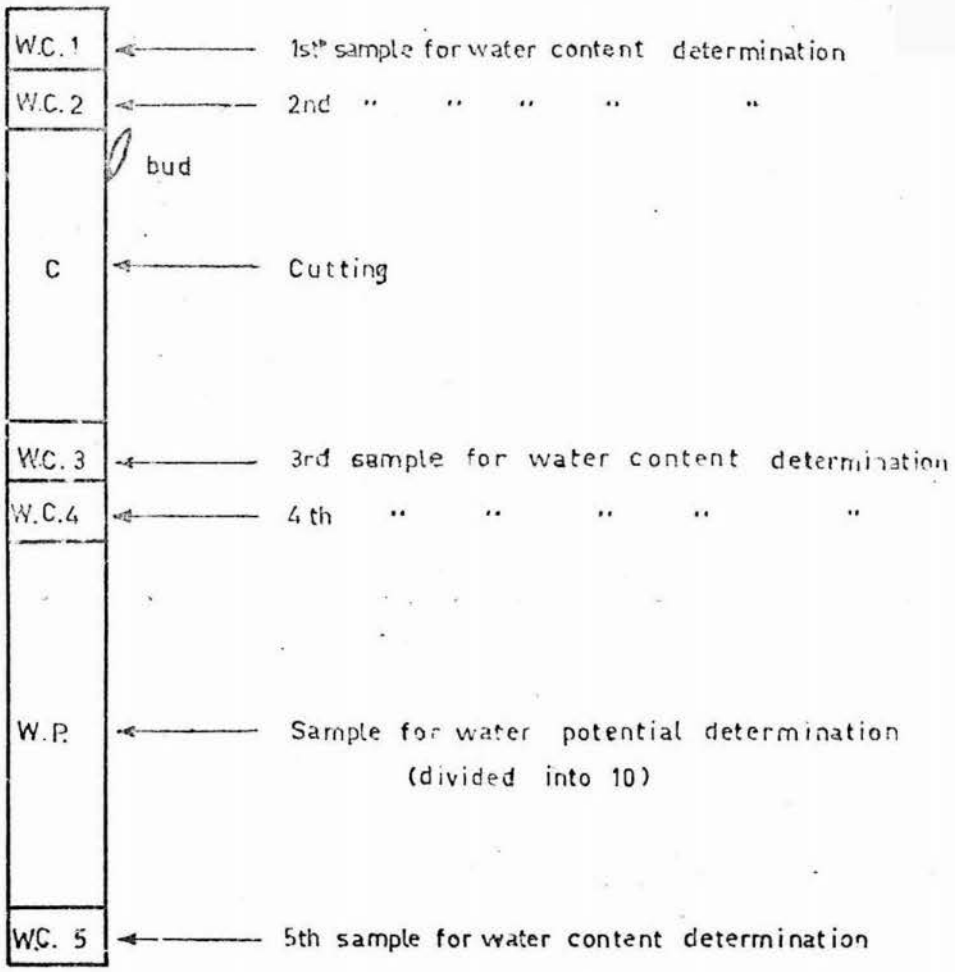
Each wand was measured to establish the limits of suitable material, normally being between 130 and 150 cm of each wand. This length was divided into three equal sub-lengths, called the upper, middle and lower regions. Each region was sampled from the apical end as follows. (See Fig. 1):

(1) Sample 1 for water content determination (1 cm long)
(2) Sample 2 for water content determination (1 cm long)
(3) A cutting of 10 cm length, with a bud at the apical end (this normally entailed waste of up to 2 cm above sample 1).
(4) Sample 3 for water content determination (1 cm long)
(5) Sample 4 for water content determination (1 cm long)
(6) Ten sub-samples of approximately 1 gram for a determination of water potential by the Schardakov method (10 x 0.75 cm = 7.5 cm)
(7) Sample 5 for water content determination (1 cm long)

FIG. 1

Measurement of seasonal water status:

plan of subdivision of each region of each wand.



The cuttings were sealed at each end with wax, and the outer and pith diameters recorded. Each cutting was identified using numbered aluminium tags.

Seasonal changes in the ability to resume growth (other than dormancy effects) were investigated by maintaining the water potential found for each region of each wand in the associated cutting. These studies were carried out in the growth room previously described (Section 2.2.1).

Dormancy effects were removed by placing the cuttings (in sealed jars over distilled water to maintain the water status) in a temperature of 2.0 to 4.0°C for 14 days (Section 1.2.4). Each cutting was then placed in a blackened pot containing a water-polyethylene glycol mixture having an osmotic potential of the same magnitude as the water potential that had been determined previously for the corresponding region. A blackened top was fitted to the pot so that the apical bud area was exposed. Observations were made of changes (Section 2.2.4) under conditions described for growth (Section 2.2.1).

Determinations of water content and water potential were carried out on the samples taken as above.

2.3.2 The Effect of Light on Budbreak and Root Initiation.

The experiment comprised two treatments applied to the following three clones:

(i) Populus X 'I-78'
(ii) P. yunnanensis
(iii) Poplar Hybrid No. Aust 135

(See Appendix 2)

Treatment 1: Light

Three sections of a growth room were fully light-proofed, but maintained at 20.0°C ± 1.0°C., using a fan and air duct system. The light sources are described in Section 2.2.1.

The three light treatments used were:

(i) Complete darkness
(ii) Continuous light
(iii) Twelve hours light, followed by 12 hours darkness (automatic control).

Treatment 2: Type of Pot

In the two treatments where light was applied, half the pots and lids were blackened, and the remainder were left translucent, to evaluate the effect of darkness on those areas expected to initiate roots.

Twenty cuttings were used in each sub-treatment per clone, giving a total of 360 cuttings.

The experiment was commenced on 20 July 1970, and evaluated after 21 days. A photographic record was also made.

2.3.3 The Effect of Relative Humidity on Water Loss

The experiment comprised three treatments applied to three clones:

(i) Populus X 'I-78'
(ii) P. yunnanensis
(iii) P. angulata var 'carolin' (See Appendix 2)

Treatment 1: Relative Humidity

A range of relative humidities was imposed, using the principle that the relative humidity above a saturated solution of a salt is constant at a given temperature and characteristic for the particular salt chosen. Such a system is buffered if an excess of the salt is added to maintain saturation, and is more suitable for situations in which water is likely to be absorbed by the solution than the conventional use of a range of concentrations of a dessicating agent such as sulphuric acid.

In addition, two extremes of relative humidity were maintained using distilled water (approximately 100% R.H.) and dry pellets of potassium hydroxide (approximately 0% R.H.). The latter was changed as frequently as the pellets became moistened.

The containers used were plastic pots, of 4.5 cm diameter and 5.5 cm high, sealed with a close-fitting press-on top. An increase in air pressure within the pots when the tops were fitted was prevented by forming a hole in the top with a heated pin, resealing the hole after fitting the top with petroleum jelly.

The plant samples were held above the solutions or pellets by using a plastic mesh disk supported on a plastic cylinder 1.5 cm above the bottom of the pot.

The range of relative humidities used was as follows (20°C.):

Material	Relative Humidity
(i) Distilled water	100%
(ii) Saturated solution of Na_2SO_4	93.0%
(iii) Saturated solution of $(NH_4)_2 \cdot SO_4$	80.5%
(iv) Saturated solution of $Ca(NO_3)_2 \cdot 4H_2O$	55.5%
(v) Dry pellets of KOH	0%

Treatment 2: Presence of Bud

The samples were selected so that half had one bud only attached, and half did not.

Treatment 3: Sealing of Ends

Half the samples were treated by sealing both ends with paraffin wax, and the remainder were left unsealed.

Experimental Layout

The experiment was designed as a four-way factorial, with five samples per sub-treatment. Each pot (containing one relative humidity treatment) contained four cuttings of one clone, as follows:

(i) Sealed at both ends; bearing a bud.
(ii) Sealed at both ends; not bearing a bud.
(iii) Not sealed at both ends; bearing a bud.
(iv) Not sealed at both ends; not bearing a bud.

Thus each clone was represented by five pots per treatment of relative humidity, to give an over-all total of 300 cuttings.

The pots were held on trays at a temperature of 20.0°C. ∓ 1.0°C., in a light intensity of approximately 60 footcandles. Initial weights were taken (including before and after sealing with wax), and thereafter at 24 hour intervals for six days. Outer and pith diameters, and oven-dry weight were recorded at termination of the experiment.

The solutions were maintained as required by removing surplus solution and adding more of the appropriate salt to maintain a surplus.

2.3.4 The Effect of Auxin Added Exogenously

The experiment comprised two treatments applied to the following three clones:

(i) Populus X 'I-78'
(ii) P.yunnanensis
(iii) P. angulata var 'carolin' (See appendix 2)

Treatment 1: Auxin concentration

A logarithmic series of six concentrations of Indol-3-yl-acetic acid was applied:

(i) Zero
(ii) 0.01 mg/litre
(iii) 0.10 mg/litre
(iv) 1.00 mg/litre
(v) 10.00 mg/litre
(vi) 100.00 mg/litre

The auxin was first dissolved in ethyl alcohol, and then added to distilled water; the alcohol was then removed by heating on a water bath at 83°C and the solution made up to strength when cool.

The solution was replaced after 7 days.

Treatment 2: Sealing Treatment

The cuttings were either sealed or not sealed at the top, using hot wax. The base was left unsealed.

Five cuttings were used per sub-treatment, to give a total of 180 cuttings, and growth was initiated in the standard environment (Section 2.2.1). The experiment was commenced on 30 August 1970, and measured for growth after 5, 10 and 15 days. The shoot growth and root growth dry weights were found for each bulked sub-treatment, the outside diameters of each cutting were recorded, and a photographic record made.

2.3.5 The Effect of Water Stress on Budbreak and Root Initiation

The major investigation of the influence of water status on budbreak and root initiation was conducted on the following three clones:

(i) Populus X 'I-78'.

(ii) P. yunnanensis.

(iii) P. angulata var 'carolin'. (See Appendix 2)

A range of concentrations of an osmoticum-water growth medium. The osmoticum used was polyethylene glycol, having a nominal molecular weight of 333, and an actual molecular weight, found by cryoscopic methods of 207.0 (Appendix 5). Ten concentrations of osmoticum were used:

Solution No.	Osmotic Potential (bars)	Concentration (grams of osmoticum per litre)
1	0	0
2	-1	9.24
3	-2	18.48
4	-3	27.72
5	-4	36.96
6	-6	55.43
7	-8	73.91
8	-11	101.63
9	-15	138.58
10	-20	184.78

Experimental Layout:

The experiment was started on 1st August, 1970, about 6 weeks before budbreak was obvious in the field. The standard growth procedures described in Section 2.2.1 were used, with 12 hours of darkness followed by 12 hours of light.

Initially, each clone in each solution was represented by 20 cuttings, making a total of 600 cuttings, in a two-way factorial design. Throughout the experiment, the solutions were renewed each 48 hours, except that an additional change was made after the first 24 hours. The commencement time for each clone was staggered to allow sufficient time for the completion of the Schardakov tests of water potential. Observations were made each 24 hours for bud activity or signs of root primordia.

The following manipulations and observations were made:

After 4 days, five cuttings per sub-treatment were destructively sampled for the first determination of water content and water potential. After carefully removing surface water with absorbent paper, both end seals were removed by clipping the end 0.5 cm of wood. Water content samples were taken from each end, comprising 1 cm of wood, and were called the top and bottom samples respectively. Fresh weight determinations were made immediately. The Schardakov samples were taken from the next 5 cm at the basal end, also without delay.

The times of bud-break and root initiation (Section 2.2.4) were recorded. After the first recording of either time, measurements of root and shoot growth were made each 48 hours until seven observations had been completed.

The second observation of water content and water potential was made immediately following the second growth observation. Five cuttings per sub-treatment were taken as before. Thus 15 cuttings per sub-treatment were taken for the first two growth observations, and ten remained for the final five growth observations.

At the termination of the experiment immediately following the seventh growth observation (i.e. after 14 days) the following measurements and observations were made:

(i) Total length of roots per cutting

(ii) The number of "basal" roots or root initials i.e. those roots arising from the basal cut, rather than developing through the rhytidom.

(iii) The fresh and dry weight of the bulked shoot growth and root growth, for each sub-treatment (10 cuttings).

(iv) A third and final measurement of water content and water potential on five of the remaining cuttings, as before.

(v) A photographic record of the cuttings while still intact.

Each individual cutting was identified using numbered aluminium tags; in each case, the 5 highest numbers were taken for each successive sampling.

2.4 Experimental Design and Analysis

Most analyses were completed on the Massey University I.B.M. 1620 Monitor computer system, using a linked statistical program system (Munford, 1970), and minor supplementary programs.

Seasonal measurements of water content were analysed as a split-split-plot experiment using the arc-sine transformation, and measurements of water potential as a split-plot experiment for each sampling time through the season. Calculation at this stage was manual, in order to obtain results for subsequent experimental design. On completion, the data was re-analysed by computer as 4-way and 3-way factorials respectively and regression analysis performed between water content and water potential for each sampling time. For this purpose, the average water content of each region on each wand was taken.

Measurements of growth of sampled cuttings were found subsequently to be confounded with the difference between nominal and actual values of the osmoticum used, so that only basic analysis was performed to establish whether growth potential changed throughout the season. (Appendix 6).

The experiment on the effect of light was analysed as a 3-way factorial, for each measurement of growth.

The experiment on the effect of auxin was also treated as a 3-way factorial for each measurement of growth at three times, with covariance analysis based on outside diameter.

The experiment on the effect of relative humidity was analysed as a 4-way factorial, with covariance analysis using outside diameter, pith diameter and the ratio of pith diameter to outside diameter as concomitant information, on both the moisture content at seven successive times, and water loss as a percentage at each of the six times following the start of the experiment.

The experiment on the effect of water stress on growth initiation was analysed as a 2-way factorial for each measurement of growth over the seven observations, and also for the three measurements of water content and water potential, and the final measurements of root length. Regressions were performed relating water content to water potential, and both water content

and water potential to growth measurements at all seven observations. In addition, analysis of variance of the between-group regressions, adjustment of means and analyses of covariance was completed.

Two-way factorial analysis was also performed on final fresh- and dry-weight measurements and water content derivations, total root lengths per cutting, and basal and side root numbers.

CHAPTER THREE - RESULTS

3.1. Seasonal Changes in Water Status.

The study of seasonal changes in water status was commenced at leaf-fall of I-78 which was the first clone to reach this stage. Water potential in all three clones rose from this point to a maximum on 16th June for *P. angulata* and on 7th July for *P. yunnanensis* and I-78 (Fig. 2).

Fig. 2 - Seasonal Water Status

A. Seasonal water potential:

..... P. angulata

- - - - I-78

——— P. yunnanensis

B. Seasonal water content - I-78

C. Seasonal water content - P. yunnanensis

D. Seasonal water content - P. angulata

Note: For B, C and D, separate curves for upper, middle and lower regions within wands are given as follows:

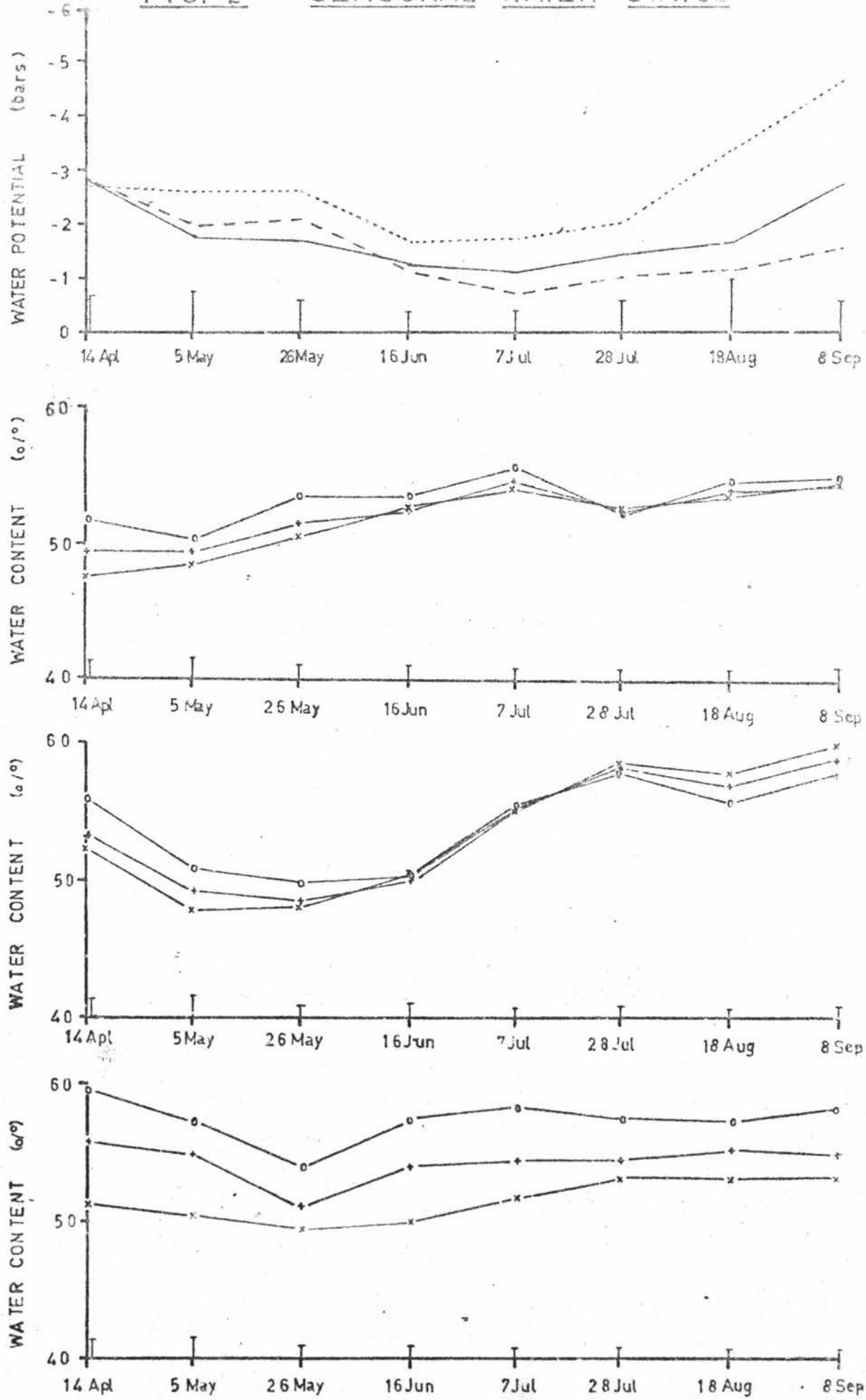
×———× Lower

+———+ Middle

o———o Upper

The Least Significant Difference (5% level) is given at each date as an upright on the abscissa.

FIG. 2 — SEASONAL WATER STATUS



Thereafter water potential fell steadily until the final observation. Differences between clones were always significant except at the first observation. P. angulata (except on this occasion) was always at a lower water potential, and P. yunnanensis was at the highest water potential until 16th June, but I-78 was higher for the remaining observations. Regions within clones differed significantly on only two occasions (14 April and 14 May) and the differences were small. (Appendix 7).

Water content in the clone I-78 rose steadily from the first observation at leaf-fall until bud-break, with a small deviation on 28th July. Whereas there was a gradient within wands with the highest water content at the apical end at the start of the observations, this steadily decreased and became small by 28th July. There was a greater rise in the basal regions of the wands than in the upper regions.

Water content in the clone P. yunnanensis fell until the time of leaf fall at about 25th May, and then rose steadily until the time of bud-break, except for one deviation on 8th August. Until 16th June, there was a gradient within wands with the highest water content at the apical end. The greatest adjustment was also made in the basal regions in this clone. After 28th July, a gradient re-appeared within wands, but in this case the apical region developed a lower water content than the basal region.

In the clone P. angulata, water content fell from commencement until 25th May, which also coincided with leaf-fall. Thereafter there was a rise in water content, but after 7th July the rise was very small. In all the measurements the apical regions were at a higher water content than the basal regions, and the differences were comparatively large.

The regressions between water potential and water content at each sampling time were significant only on two occasions within clones, but the relationship was not close. The corresponding regressions over all clones was not significant at any time. The regression analysis is presented in Appendix 7 .

3.2. The Effectof Relative Humidity on Water Loss

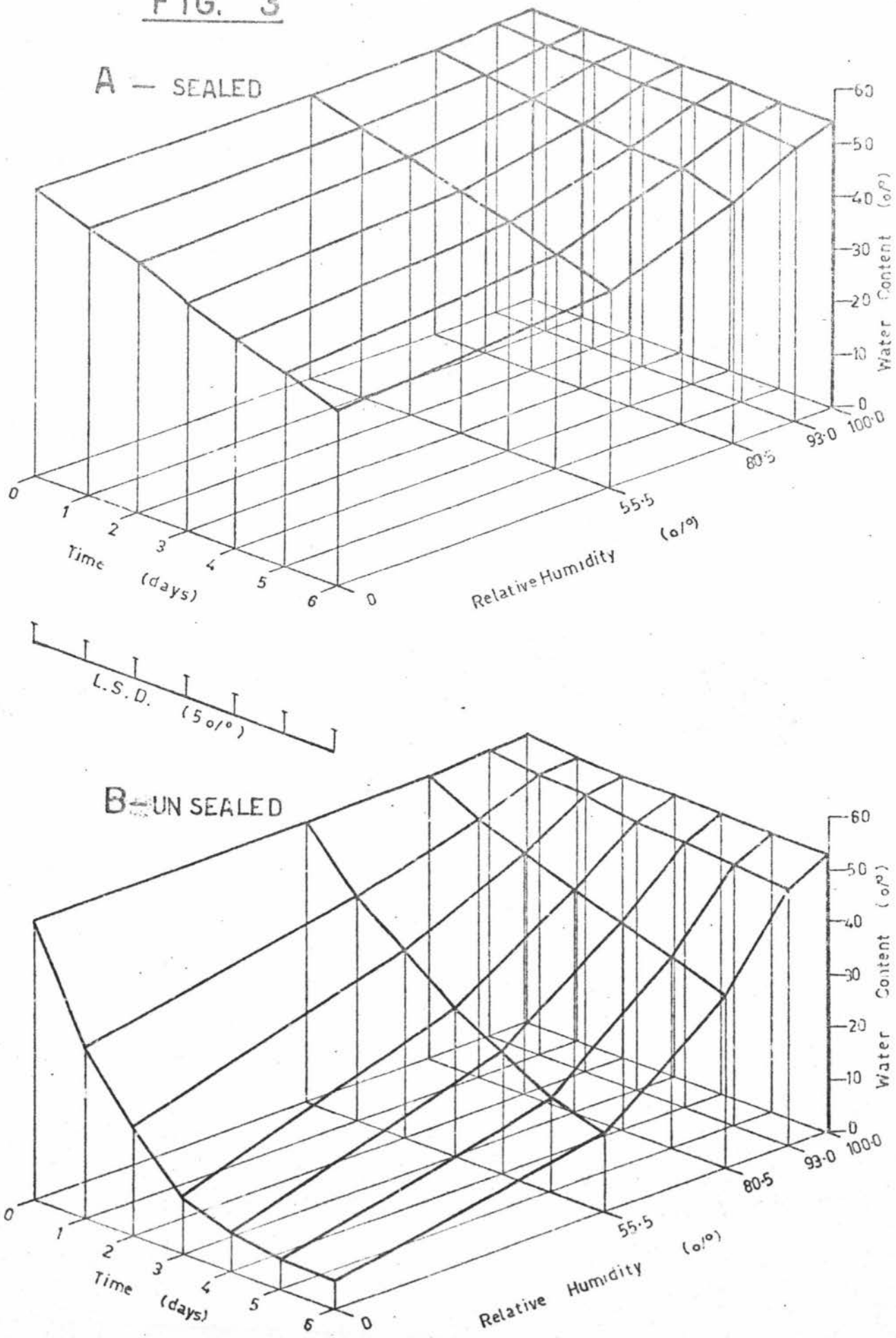
The effect of sequentially lower values of relative humidity was a massive increase in water loss, which could be greatly reduced by sealing the ends (Fig. 3).

Fig. 3. - Effect of Relative Humidity on Water Content

The response planes given refer to the mean water content for all clones at the times indicated.

The Least Significant Difference (5% level of significance) refers to both A (cuttings sealed with wax at both ends) and B (unsealed), for each of the times indicated.

FIG. 3


A — SEALED
Water Content (o/o)
60
50
40
30
20
10
0
100·0
93·0
80·5
55·5
0
Relative Humidity (o/o)
0
1
2
3
4
5
6
Time (days)
L.S.D. (5 o/o)
B — UN SEALED
Water Content (o/o)
60
50
40
30
20
10
0
100·0
93·0
80·5
55·5
0
Relative Humidity (o/o)
0
1
2
3
4
5
6
Time (days)

However the presence of a bud had no significant main-treatment effect and the effect of clone differences did not influence the rate of water loss, although differences between clones at the start of the experiment were significant and remained at the same level throughout (Appendix 8).

At a relative humidity of 100%, there was little change in water content. In unsealed cuttings, all three clones showed slow net losses of water. In sealed cuttings of two clones (I-78 and P.yunnanensis) there was a net gain in water content over the first two 24 hour periods, followed by a slow fall. In P. angulata, there was a net gain in the second 24 hour period, but otherwise a slow decrease. (Table 1).

Table 1: Change of Water Content at 100% R.H.

Clone	Treatment	After 24 hours (%)	After 48 hours (%)
I-78	Sealed; Bud	+0.05	+0.13
I-78	Sealed; No bud	+0.10	+0.16
I-78	Unsealed; Bud	-0.33	-0.43
I-78	Unsealed; No bud	+0.08	-0.07
P.yunnanensis	Sealed; Bud	+0.01	+0.04
P.yunnanensis	Sealed; No bud	+0.03	+0.08
P.yunnanensis	Unsealed; Bud	-0.37	-0.41
P.yunnanensis	Unsealed; No bud	-0.36	-0.51
P.angulata	Sealed; Bud	-0.12	-0.07
P.angulata	Sealed; No bud	-0.10	-0.03
P.angulata	Unsealed; Bud	-0.39	-0.47
P.angulata	Unsealed; No bud	-0.52	-0.73

At relative humidities other than 100%, there was an effect from the presence of a bud which varied with clone and the particular relative humidity (Fig. 4). At high relative humidities, the presence of a bud caused a slower net loss of water, but below a critical level which varied with clone, cuttings in which a bud was present underwent a greater loss of water.

Fig. 4. - The Influence of Bud Presence on the size of Water Loss at Particular Relative Humidities.

4A - I-78

4B - P. yunnanensis

4C - P. angulata

FIG. 4 A — 1-78

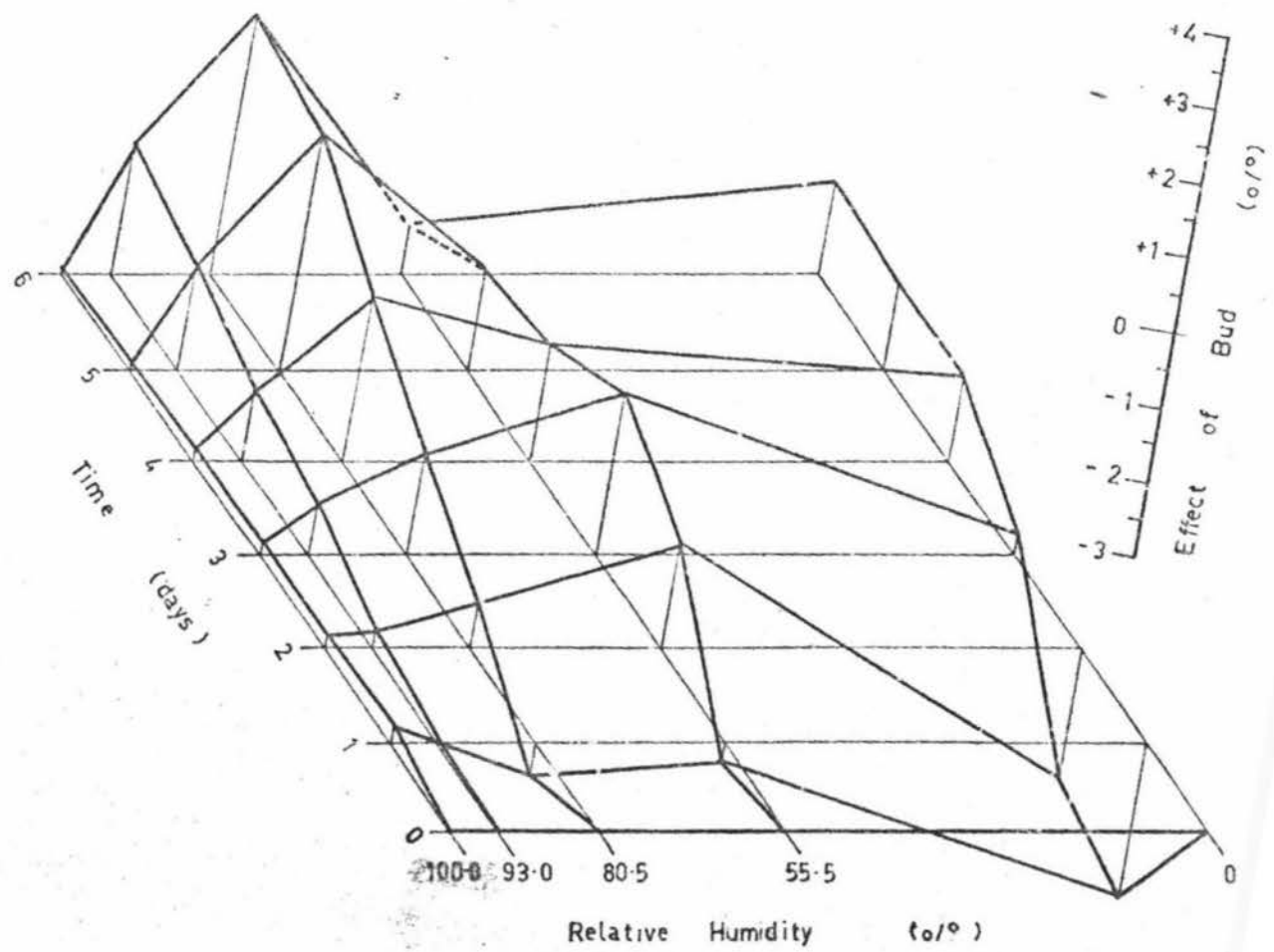

Time (days)
6
5
4
3
2
1
0
100·0
93·0
80·5
55·5
0
Relative Humidity (o/°)
Effect of Bud (o/°)
+4
+3
+2
+1
0
-1
-2
-3

FIG. 4 B — P. yunnanensis

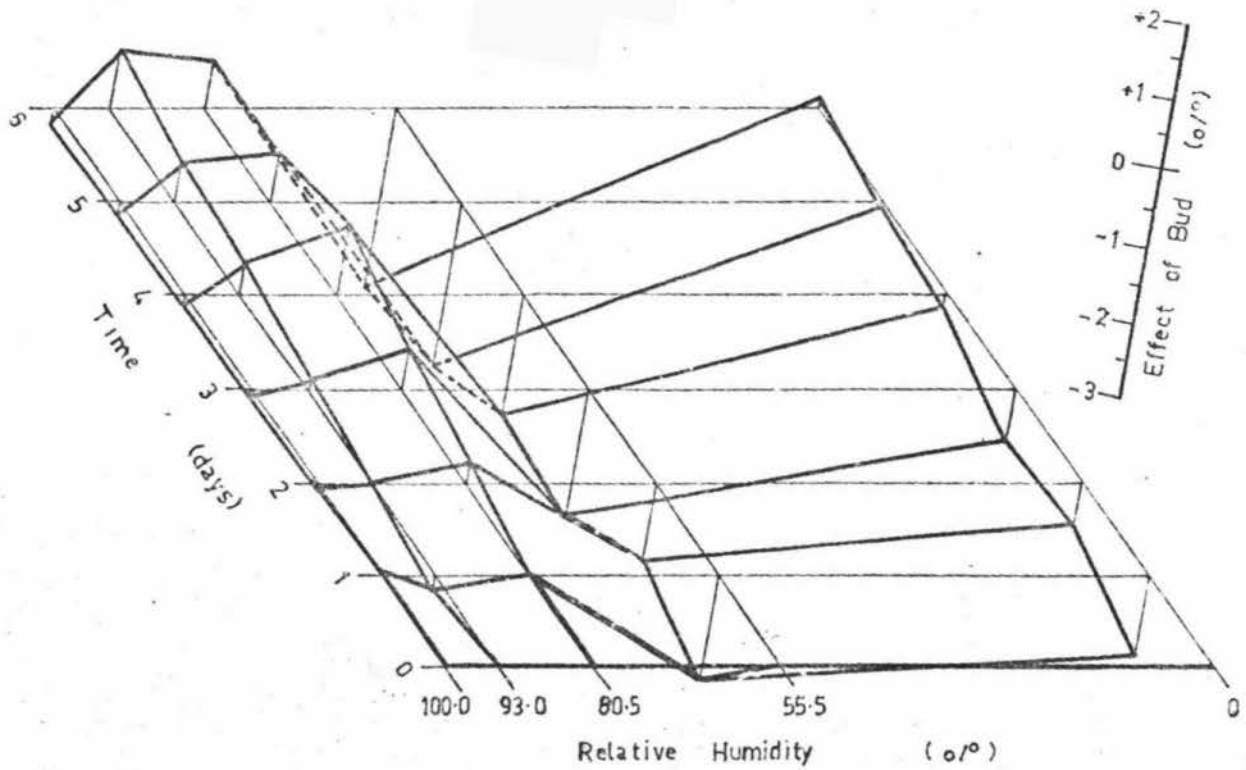
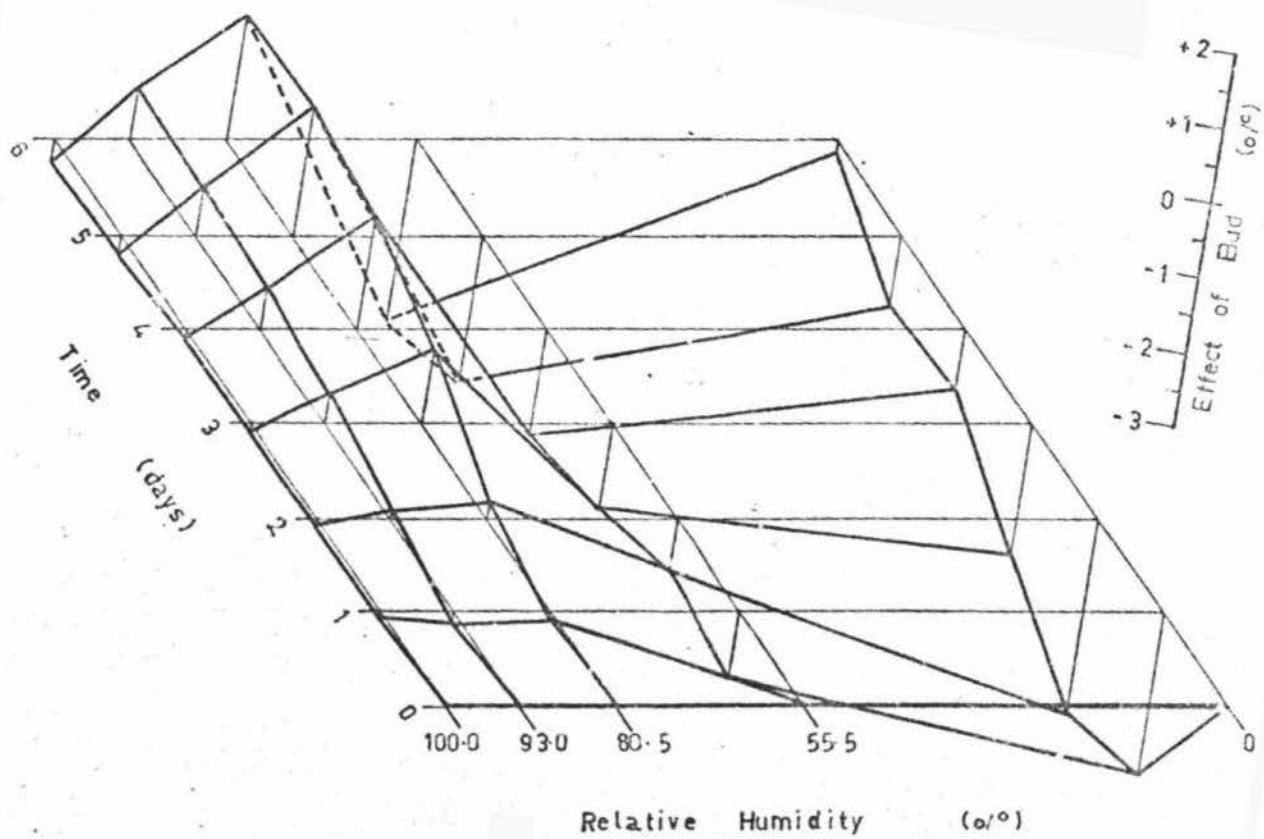


FIG. 4C — P. angulata



3.3 The Effect of Light on Growth

3.3.1 Ability to Produce Shoots and Roots:

In the clones I-78 and P. yunnanensis, both shoots and roots were produced on all cuttings under all treatments. In the clone 'Aust 135' there were some failures (Table 2):

Table 2: Failure rate of 'Aust 135' in different light treatments

(Percent)

Treatment	Shoots (%)	Roots (%)
Continuous dark	25	5
Continuous light	5	2
Light-dark cycle	2	0

3.3.2 Shoot Growth:

The effect of the dark treatment was to greatly increase the rate of elongation, inhibit leaf development and inhibit the production of shoot dry matter (Fig. 5, Plate 1).

The initial measurement of shoot growth 10 days after commencing the experiment showed that there were significant differences between clones, light treatments and clone/treatment interactions, but that the treatment of blackening pots had no effect. (Fig. 5). The cuttings in the dark treatment showed less elongation at this stage, and 'Aust. 135' showed less growth over all three light treatment regimes.

At the second measurement of shoot elongation after 23 days, significant differences remained between clones, light treatments and the interactions between these factors as before, but there were no significant differences between shoot growth in blackened and non-blackened pots (Fig. 5). The growth of I-78 was similar in both treatments involving light, but the shoots were considerably longer if grown in the dark. P. yunnanensis showed greatest elongation in the dark and least in continuous light; in the light/dark cycle shoot growth approached that measured in the dark. In contrast, shoot growth in 'Aust. 135' was least in the dark, and even in either light treatment was less than that of I-78 or P. yunnanensis.

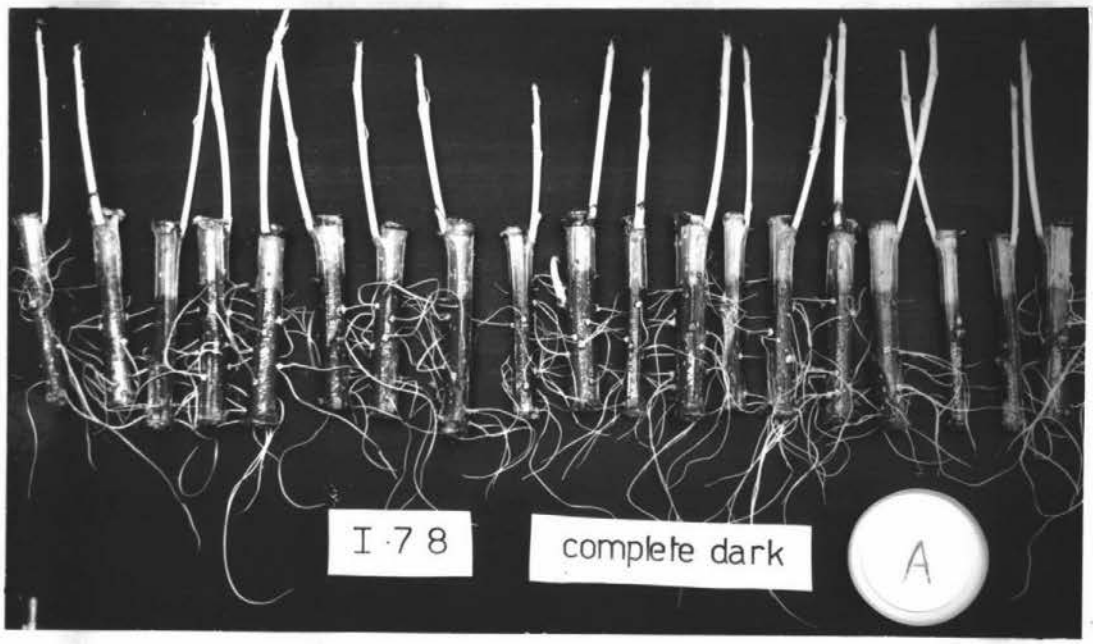
Measurement of final dry weights of shoot growth showed that in all clones cuttings grown in the dark did not produce as much as cuttings grown in either light treatment. 'Aust 135' produced less in all light regimes (Fig. 5).

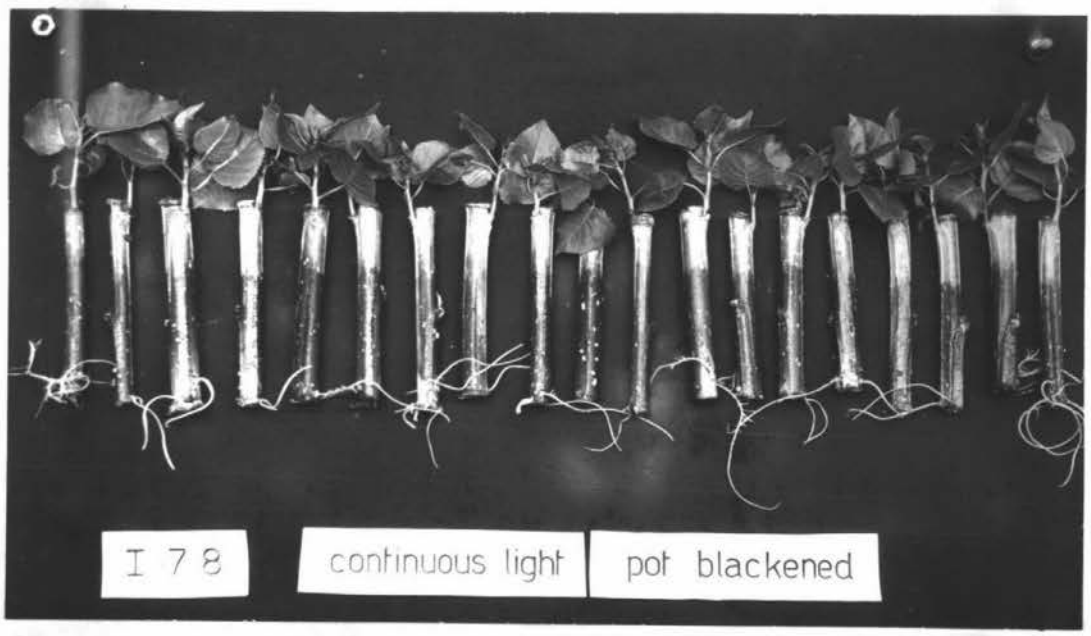
The analysis of variance of shoot elongation (and also root growth) is presented in Appendix 9.

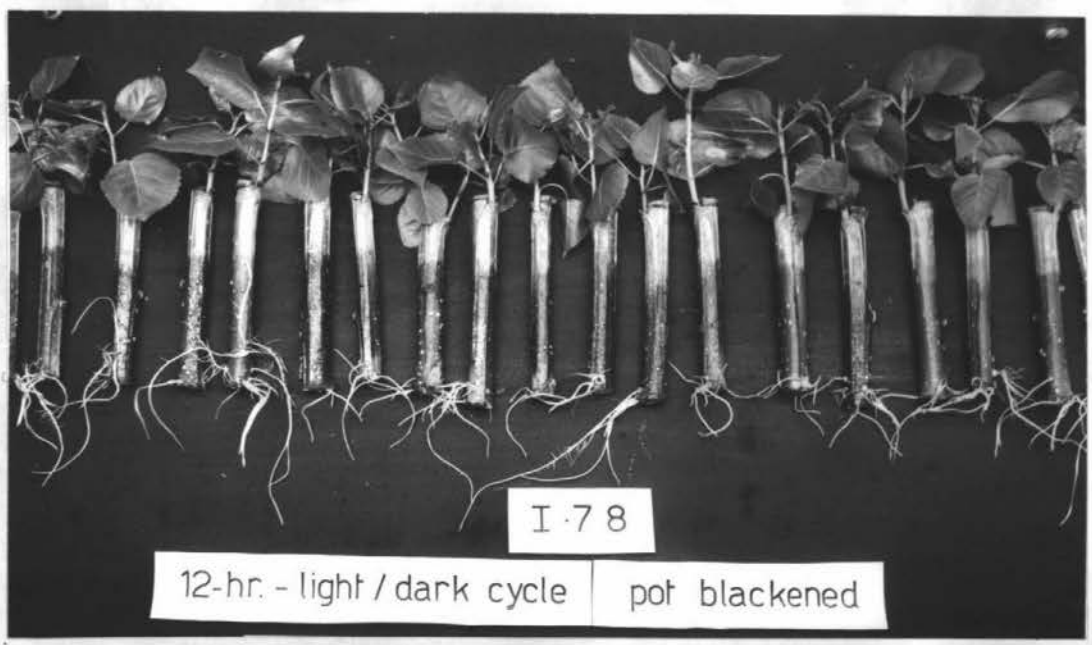
Plate 1 - The Effect of Light on Growth

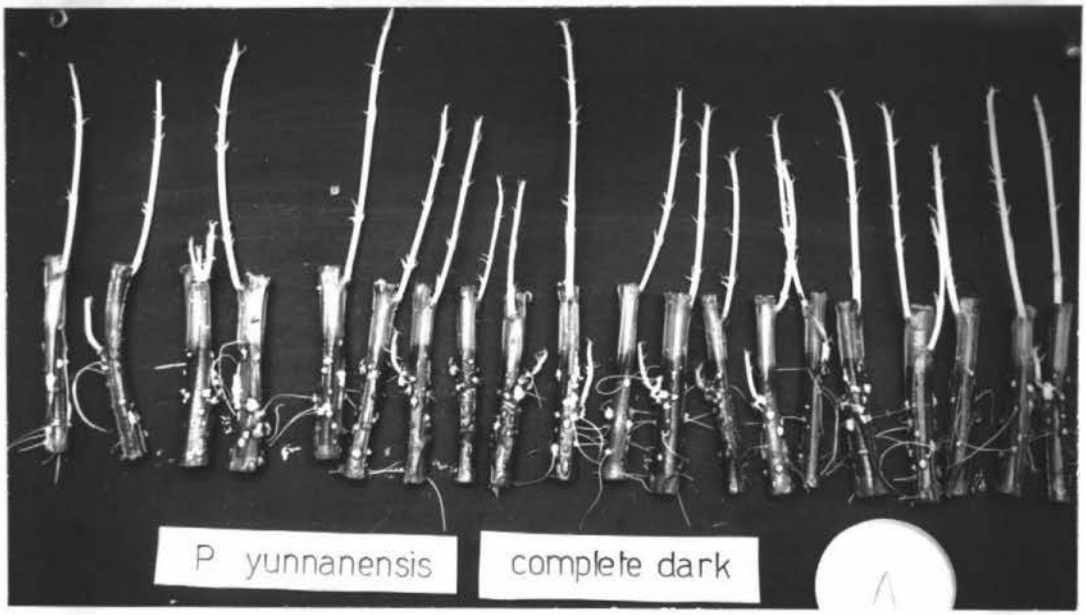
Each page represents one clone, as indicated, and each photograph represents one light sub-treatment. (Only the "pot blackened" treatment is shown, since there were no significant differences between pot treatments).

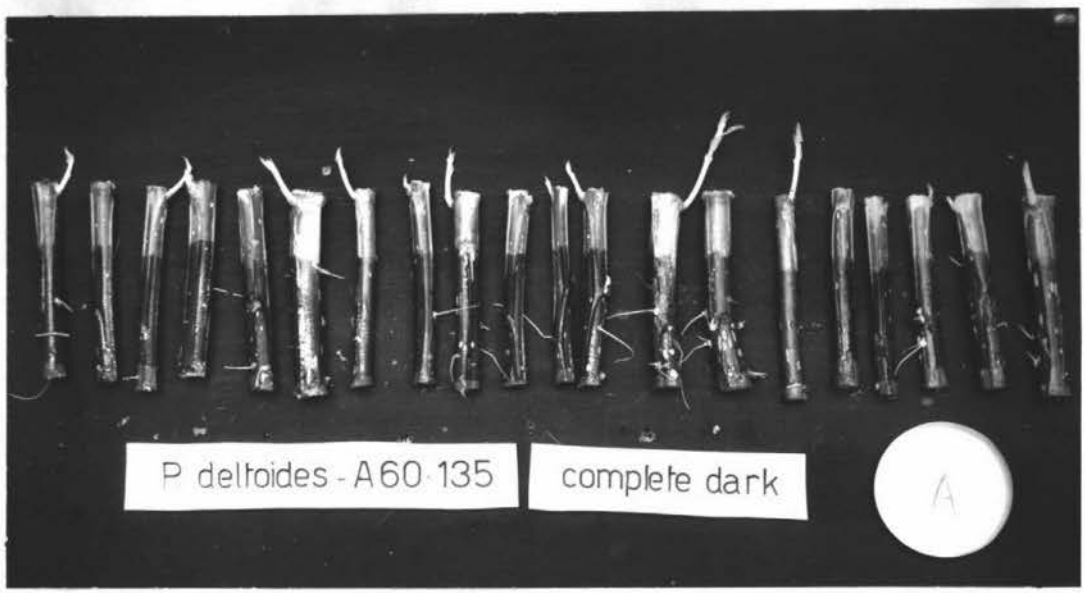
Upper photograph: complete dark
Centre photograph: continuous light
Lower photograph: 12 hours of light followed by 12 hours of darkness


I·78
complete dark
A


I 78
continuous light
pot blackened


I·78
12-hr. - light / dark cycle
pot blackened


P yunnanensis
complete dark
A


P deltoides - A60 135
complete dark
A


P deltoides - A60 135
continuous light
pot blackened

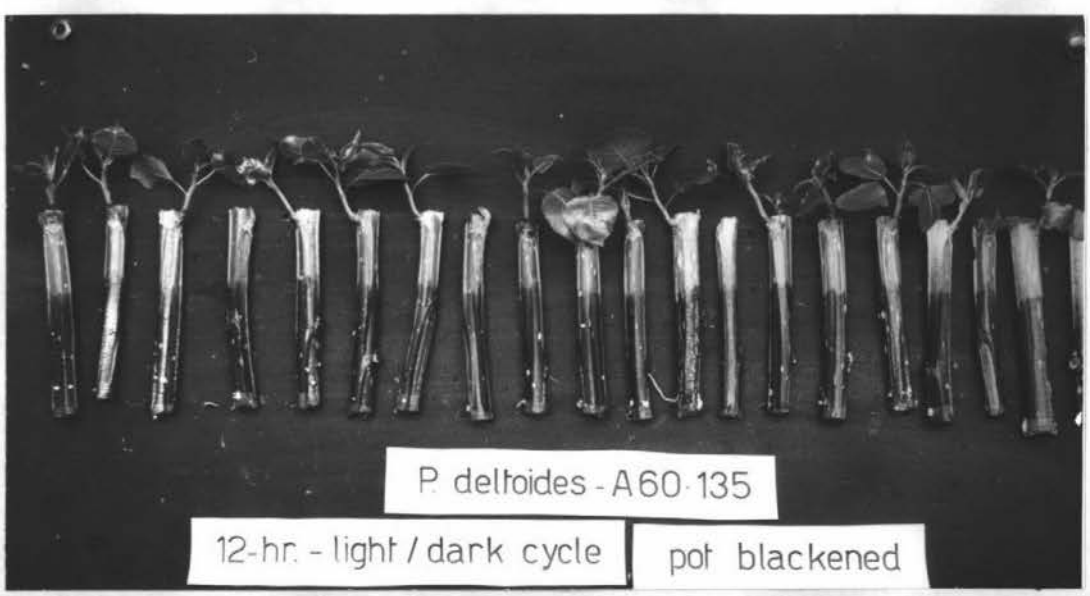

P deltoides - A60 135
12-hr. - light / dark cycle
pot blackened

Fig 5. - Effect of Light on Shoot Growth.

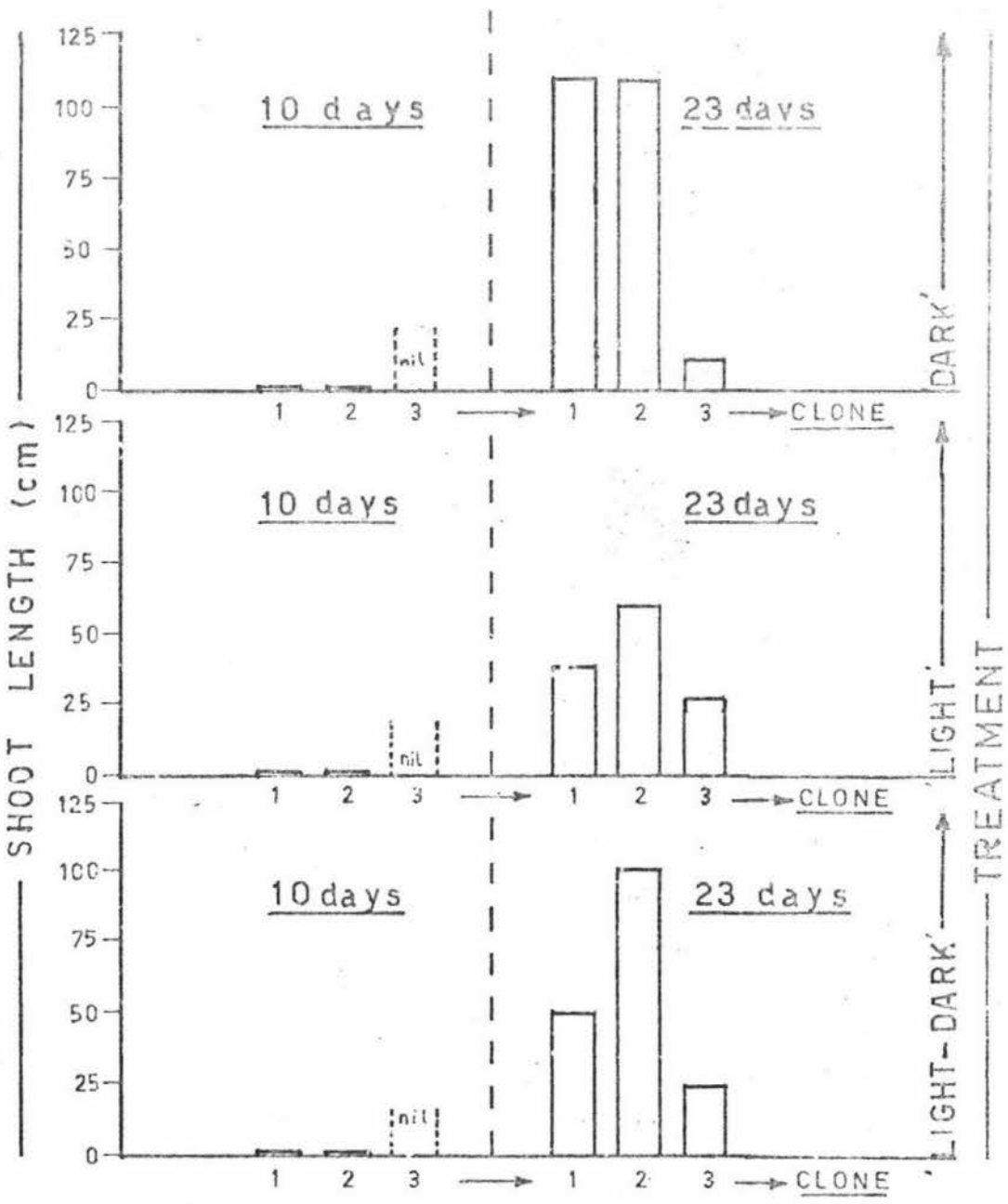
A. Shoot Length after 10 and 23 days under light treatments.

B. Shoot dry weight after 23 days.

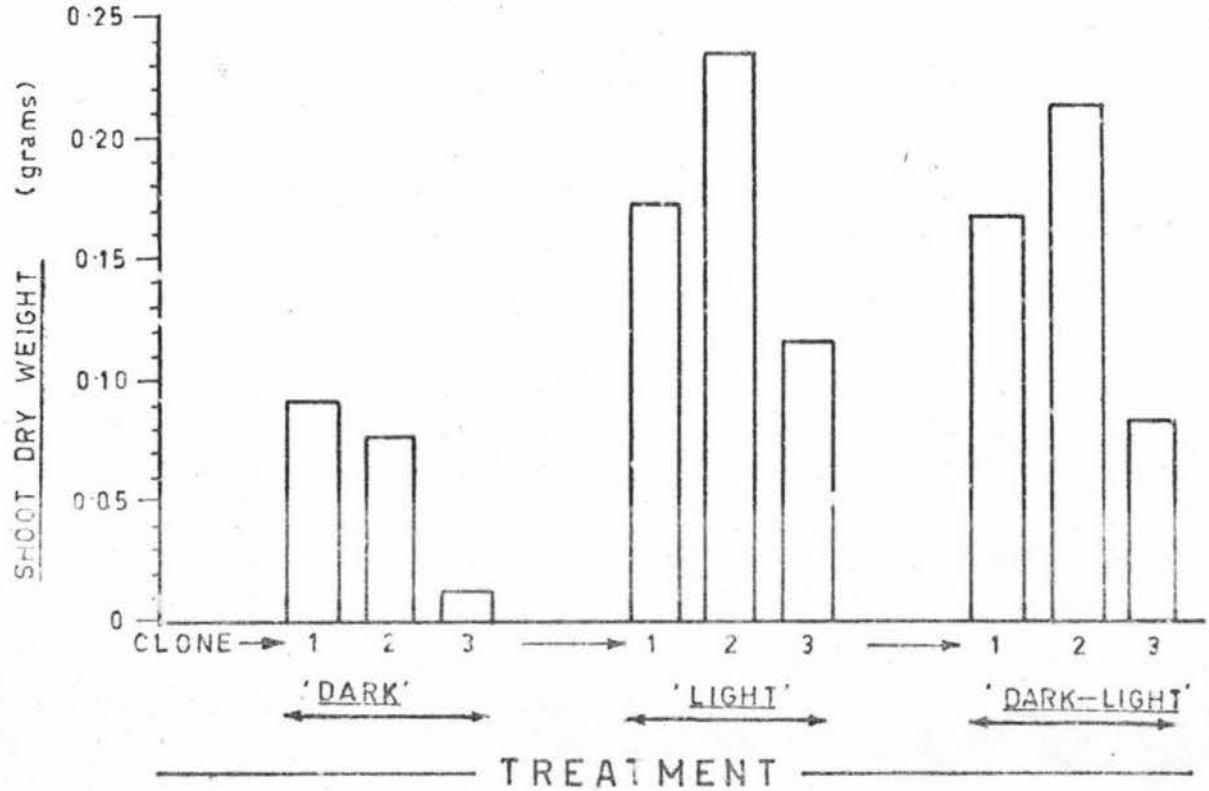
Clone 1 is I-78
Clone 2 is *P. yunnanensis*
Clone 3 is *P. angulata*

FIG. 5

A


SHOOT LENGTH (cm)
125
100
75
50
25
0
10 days
23 days
nil
1 2 3 → 1 2 3 → CLONE
'DARK'
'LIGHT'
'LIGHT-DARK'
TREATMENT

B


SHOOT DRY WEIGHT (grams)
0·25
0·20
0·15
0·10
0·05
0
CLONE → 1 2 3 → 1 2 3 → 1 2 3
'DARK'
'LIGHT'
'DARK—LIGHT'
TREATMENT

3.3.3 Root Growth:

In the clone I-78 early development of both root initials and roots was considerably greater in the dark (Fig. 6), and this was reflected in a greater proportion of long roots at the later observation. However in P. Yunnanensis at the first observation, there was no appearance of root initials in the dark, but in both light treatments both root initials and roots had reached similar proportions in all light treatments. In 'Aust 135', root initials appeared in the dark treatment alone at the first observation, and these had developed into some longer roots by the second observation. In the light treatment root initials did not appear until the second observation, and root development was minimal at this stage.

The analysis of variance of root growth is presented in Appendix 9.

Fig. 6. Effect of Light on Root Growth

A. Total number of roots plus root initials.

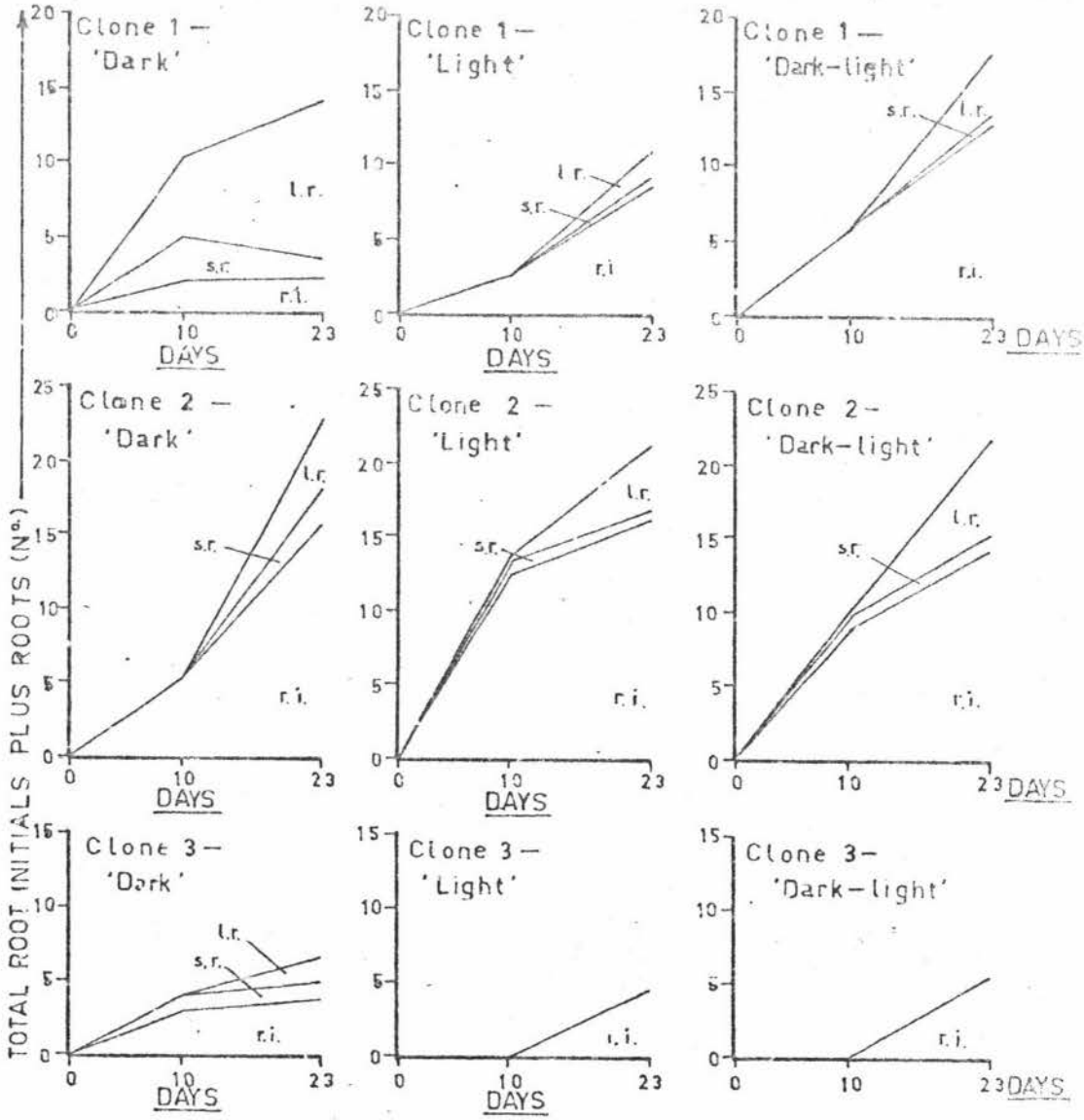
Clone 1 is I -78
Clone 2 is P.yunnanensis
Clone 3 is P.angulata

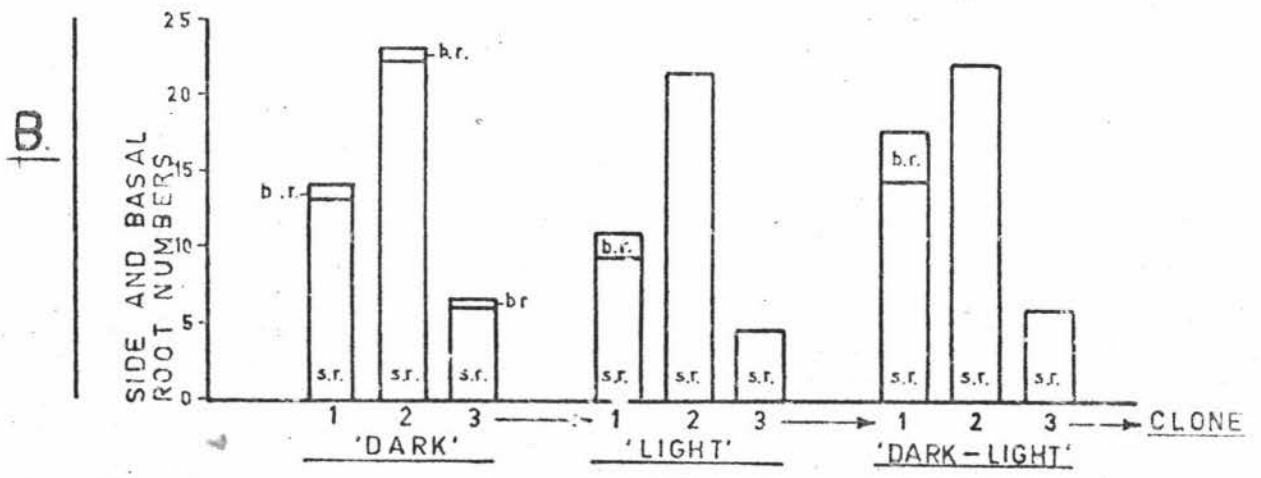
r.i. = root initials
s.r. = short roots i.e. less than 1 cm
l.r. = long roots i.e. greater than 1 cm.

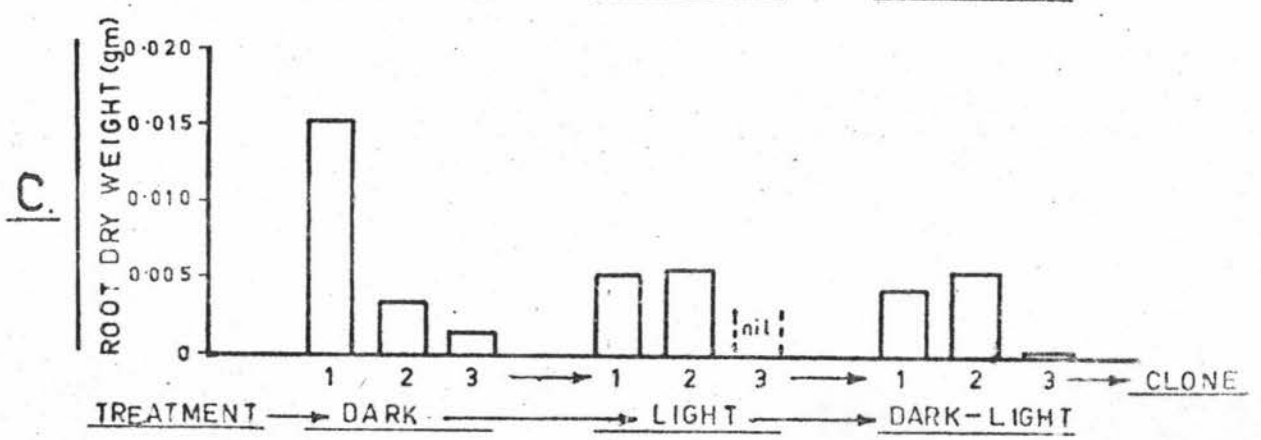
B. The distribution of roots as basal roots (b.r.) emanating from a basal callus, and side roots (s.r.) emanating from primary root primordia

C. Root dry weight after 23 days.

FIG.6


A.
TOTAL ROOT INITIALS PLUS ROOTS (No.)
Clone 1 — 'Dark'
Clone 1 — 'Light'
Clone 1 — 'Dark-light'
Clone 2 — 'Dark'
Clone 2 — 'Light'
Clone 2 — 'Dark-light'
Clone 3 — 'Dark'
Clone 3 — 'Light'
Clone 3 — 'Dark-light'
l.r.
s.r.
r.i.
DAYS


B.
SIDE AND BASAL ROOT NUMBERS
b.r.
s.r.
br
1 2 3 → 1 2 3 → 1 2 3 → CLONE
'DARK'
'LIGHT'
'DARK-LIGHT'


C.
ROOT DRY WEIGHT (gm)
0·020
0·015
0·010
0·005
0
nil
1 2 3 → 1 2 3 → 1 2 3 → CLONE
TREATMENT → DARK → LIGHT → DARK-LIGHT

3.3.4 Basal Roots:

In the clone I-78 basal roots developed in all three light regimes, but there were fewer in the dark treatment. In both P. yunnanensis and 'Aust 135' there was some basal root development in the dark, but almost none where there was incident light. There was no effect caused by blackening the pots. A table of means is presented in Appendix 9, together with the analysis of variance.

3.3.5 Relation between shoot growth and root growth.

There was a positive correlation between shoot and root growth within clones; the differences between clones in this relation was not significant.

There was also a stronger correlation between shoot and root growth within light treatments, but the differences between treatments were significantly different. In particular, the relation was stronger in the light/dark treatment.

(These results are presented in full in Appendix 9).

3.4 The Effect of Exogenous Auxin on Growth

(i) Shoot Growth:

The effect of auxin applied in solution culture did not cause significant differences in shoot growth until the final measurement at 15 days, when the highest concentration used caused an overall depression (Fig 7, Plate 2, Appendix 10). The effect of clone differences was highly significant at all three growth assessments. After five days, growth had just commenced in I-78 and P.yunnanensis, but not in P. angulata. At ten days all clones had commenced growth, with I-78 having the greatest and P. yunnanensis the least shoot elongation. I-78 appeared to show a depression in growth at the highest concentrations, but this was not significantly different from growth at lower concentrations. By the fifteenth day there was a significant depression at the highest concentration of auxin in the clone I-78, but there were no other effects which reached significant levels.

(ii) Root Growth:

By the fifth day, root initials had appeared on I-78 and P. angulata but not P. yunnanensis (Fig. 8). In both the former clones most root initials were seen on those cuttings in the highest concentration, and this effect was large and significant in the case of P. angulata. By the time of the second observation at ten days, both root initials and growing roots had appeared on most cuttings in all clones. The root initials of both I-78 and P. angulata were much more prolific in the highest concentration of auxin used, but P. yunnanensis did not show this effect.

Fig. 7 - The Effect of Exogenous Auxin on Shoot Growth

A I-78

B *P.yunnanensis*

C *P.angulata*

FIG. 7—

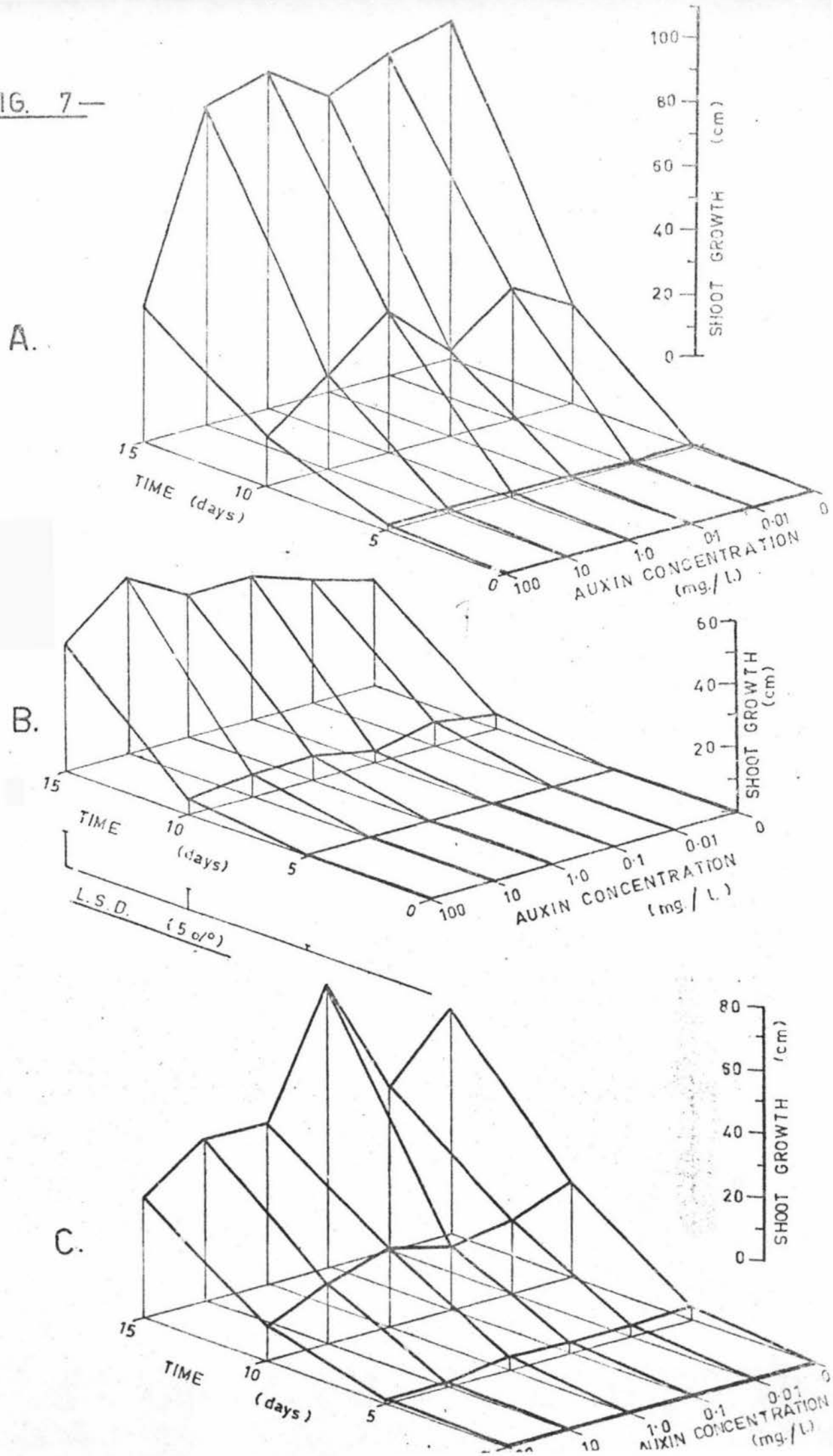

A.
SHOOT GROWTH (cm)
100
80
60
40
20
0
TIME (days)
15
10
5
AUXIN CONCENTRATION (mg./l.)
0
100
10
1·0
01
0·01
B.
SHOOT GROWTH (cm)
60
40
20
0
TIME (days)
15
10
5
L.S.D. (5 o/o)
AUXIN CONCENTRATION (mg/l.)
0
100
10
1·0
0·1
0·01
C.
SHOOT GROWTH (cm)
80
60
40
20
0
TIME (days)
15
10
5
AUXIN CONCENTRATION (mg./l.)
1·0
0·1
0·01

Plate 2 - The Effect of Auxin on Growth

Each page represents one clone, as follows:

A - I-78
B - P.yunnanensis
C - P.angulata

Each subtreatment is represented by three cuttings as follows:

Upper photograph, from left:

Auxin concentration	Sealing treatment
1. Nil	N.S.
2. Nil	S.
3. 0.01	N.S.
4. 0.01	S.

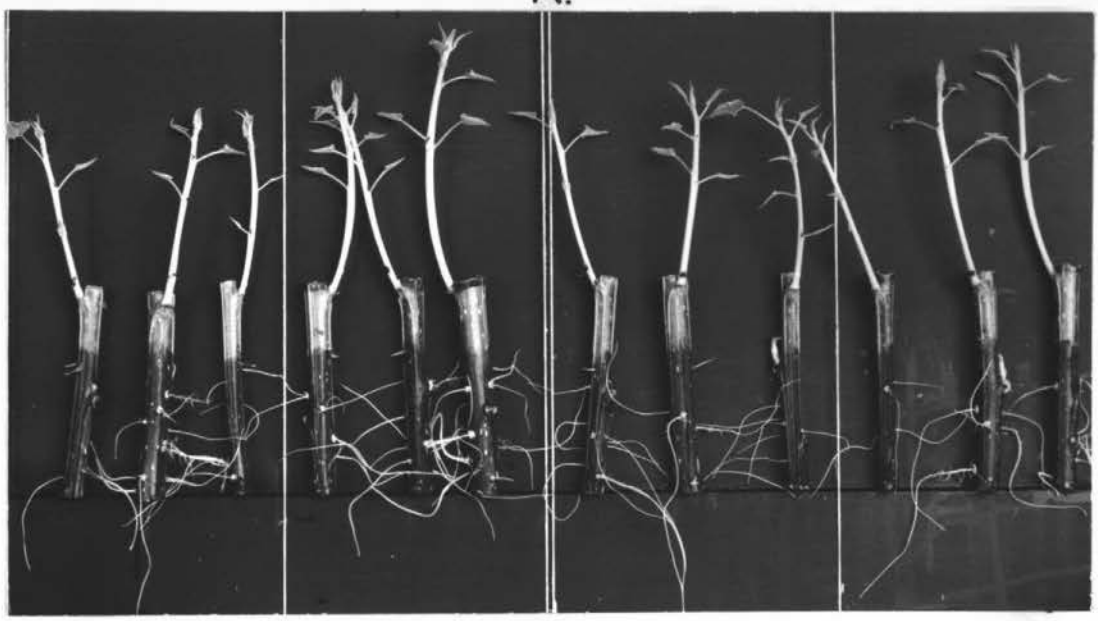
Centre photograph, from left:

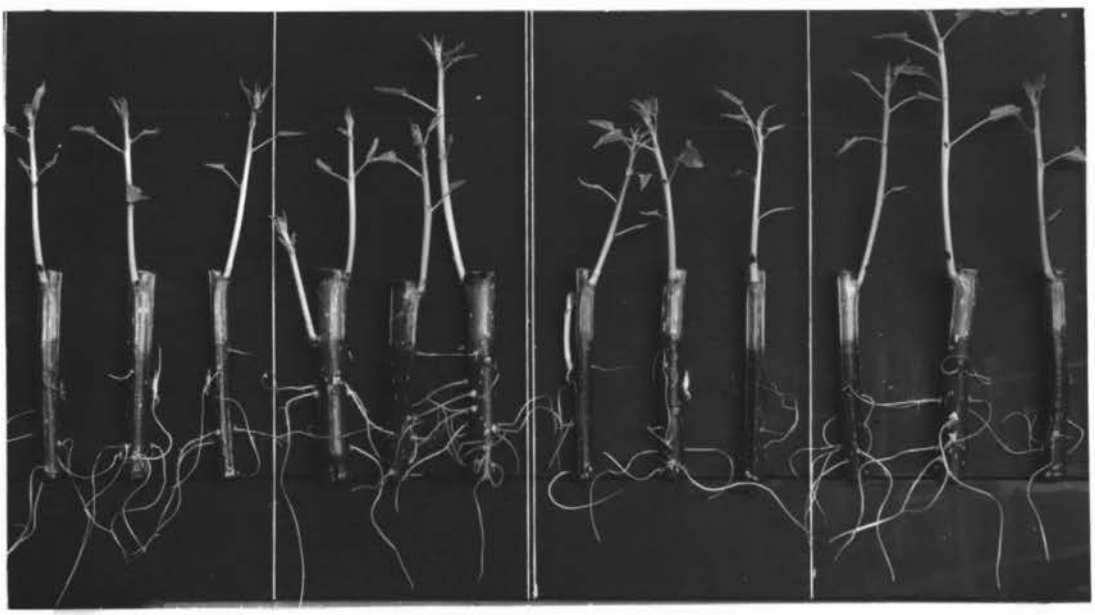
Auxin concentration	Sealing treatment
1. 0.10	N.S.
2. 0.10	S.
3. 1.0	N.S.
4. 1.0	S.

Lower photograph, from left:

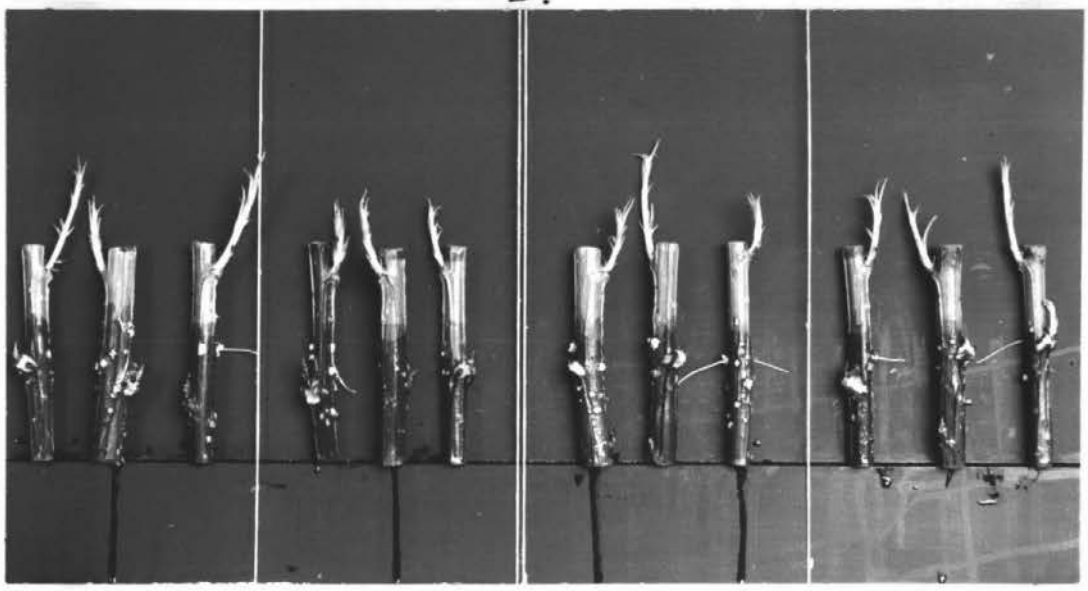
Auxin concentration	Sealing treatment
1. 10.0	N.S.
2. 10.0	S.
3.100.0	N.S.
4.100.0	S.

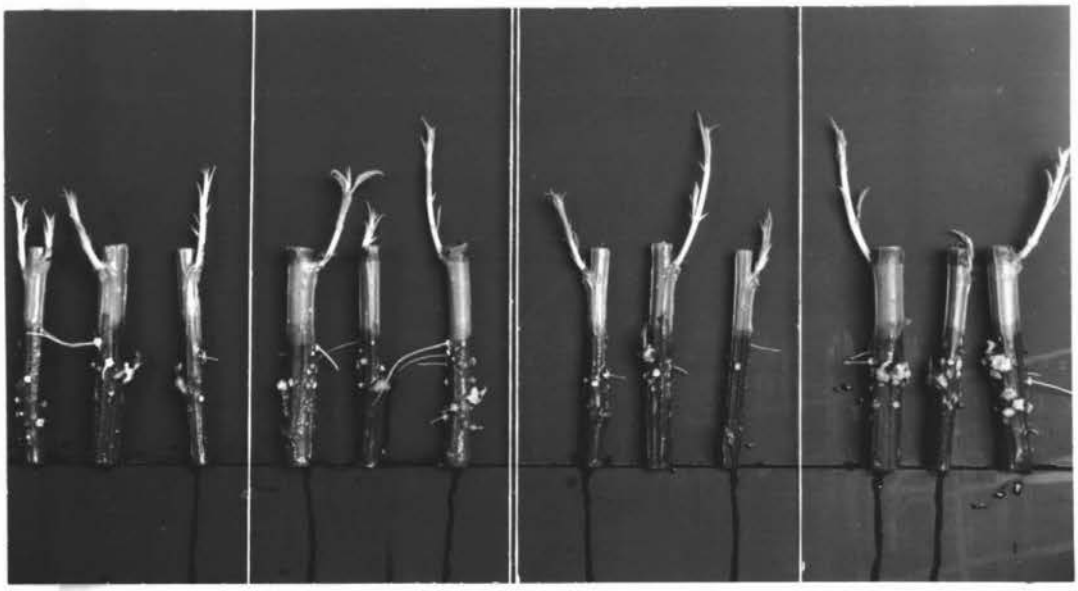
A.

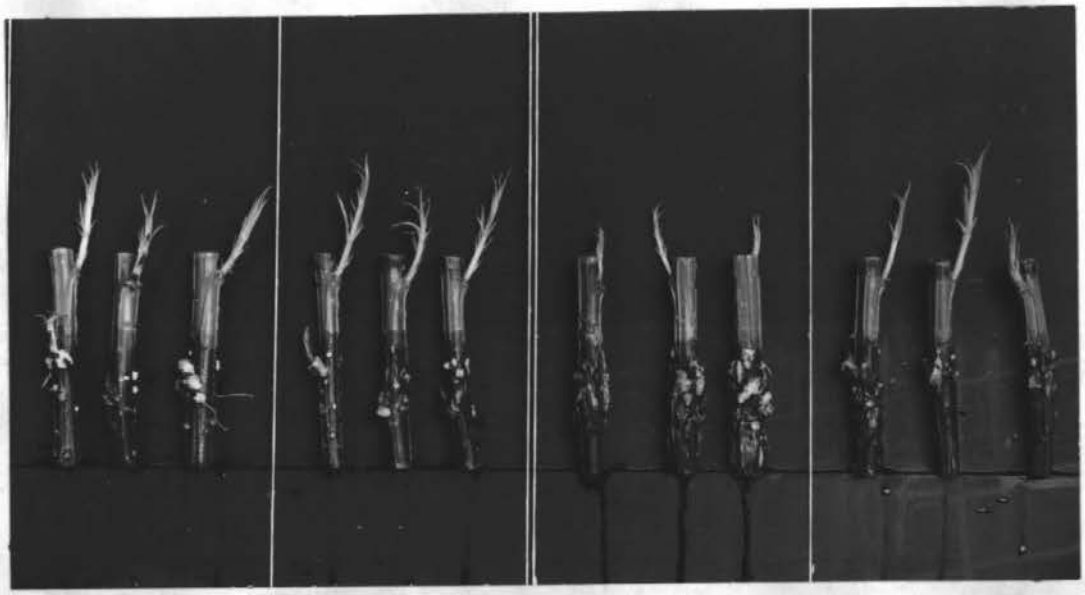




B.







C.

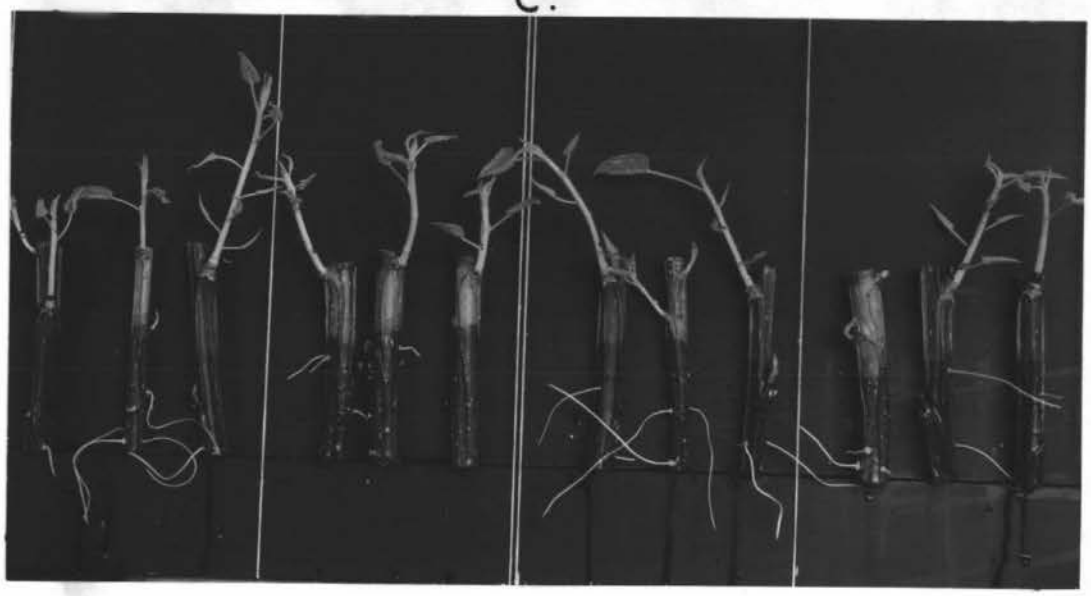


Fig. 8. - The Effect of Exogenous Auxin on Root Growth

Fig. 8 - A - I-78

Fig. 8 - B - P.yunnanensis

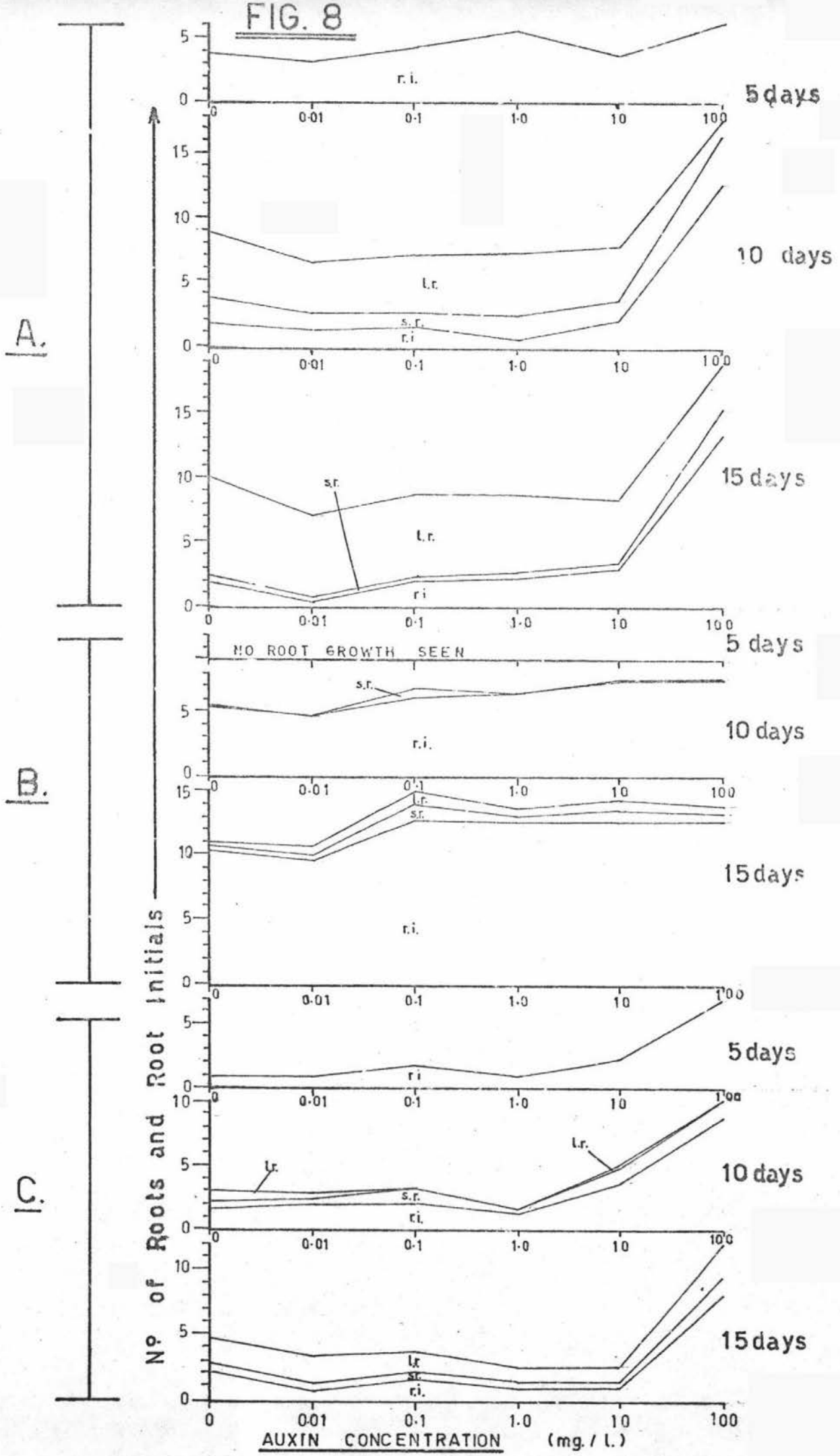
Fig. 8 - C - P.angulata

r.i. - root initials

s.r. - short roots, i.e. less than 1 cm

l.r. - long roots, i.e. greater than 1 cm

FIG. 8


A.
B.
C.
5 days
10 days
15 days
NO ROOT GROWTH SEEN
r.i.
s.r.
l.r.
No of Roots and Root Initials
0
0·01
0·1
1·0
10
100
AUXIN CONCENTRATION (mg. / l.)

The concentration of auxin did not significantly affect the numbers of roots in the category 0-1 cm long in any clone, and in the clones P. yunnanensis and P. angulata there was also no effect on root numbers in the category greater than 1 cm long. However, in I-78, roots longer than 1 cm were most numerous in the zero concentration of auxin, and there were considerably fewer in the highest concentration. Both these differences were significant. At the third observation on the fifteenth day significance within clones existed in the number of root initials, where both I-78 and P. angulata showed a large increase at the highest concentration. Over all clones, there were more roots between 0 and 1 cm at the highest concentration, but this was barely significant.

In the clones I-78 and P. angulata at the first two measurements of growth, there was a positive significant correlation between diameter of the cutting and the number of root initials, but a significant negative correlation was present between diameter and average shoot length.

The analysis of variance is presented in Appendix 10.

(iii) Final Dry Weights

Final dry weights of roots and shoots are presented in Tables 3 and 4.

Table 3: Final Dry Weights of Shoots (grams)

Solution Concentration (mg/litre)	I-78	P.yunnanensis	P. angulata	Overall
0	0.120	0.056	0.126	0.101
0.01	0.103	0.051	0.100	0.085
0.10	0.103	0.053	0.122	0.092
1.00	0.117	0.061	0.110	0.096
10.0	0.124	0.079	0.094	0.099
100.0	0.047	0.051	0.069	0.055

Table 4: Final Dry Weights of Roots (grams)

Solution Concentration (mg/litre)	I-78	P. yunnanensis	P. angulata	Overall
0	0.0127	0.0001	0.0048	0.0059
0.01	0.0076	0.0003	0.0028	0.0036
0.10	0.0093	0.0008	0.0014	0.0038
1.00	0.0097	0.0005	0.0008	0.0037
10.0	0.0082	0.0004	0.0029	0.0038
100.0	0.0055	0.0002	0.0043	0.0033

A drop in dry weight production was apparent at the highest concentration of auxin in the case of I-78 and P. angulata, but not P. yunnanensis. More variation was apparent in root dry weights than shoot dry weights, due to the smaller amount of material available. There appeared to be greater root dry weight increase in the water control in I-78 and less at the highest concentration of auxin. P. yunnanensis showed no obvious trends, as there was little growth of roots in any concentration. P. angulata also showed no significant trends, although there was more root growth than in P. yunnanensis.

3.5 The Effect of Water Stress on Bud-break and Root Initiation

3.5.1 Water Potentials Within Cuttings

The development of water potentials within the cuttings in response to a series of water stresses imposed using osmotica in liquid culture is shown in Fig. 9, and the analysis of variance in Appendix 11. In general, water potential fell with decreasing osmotic potential of the growth medium, and increased with time. However, I-78 showed a higher water potential in cuttings grown in solutions of - 2 bars than those in solutions of - 1 bar, and this trough was apparent at all sampling times. Those cuttings grown in zero water potential demonstrated a rising water potential until growth was initiated, and water potential fell.

The final measurement of I-78 in an osmoticum of - 20 bars indicated that water potential within the cutting rose. This result was not seen in other clones or concentrations of the osmoticum.

P. yunnanensis developed a deviation in water potential at - 2 bars, but there was not a well-defined trough as in the case of I-78. There was rather more variability shown by this clone (Appendix 11). P. angulata cuttings followed an even gradient of water potential, with relatively little

Fig. 9. - Water Potential of Cuttings in Growth Media of Various Osmotic Potentials

Fig. 9 A - I-78

Fig. 9 B - *P.yunnanensis*

Fig. 9 C - *P.angulata*

Time A - Commencement of the experiment

Time B - After 4 days

Time C - At growth initiation

Time D - After 14 days growth.

L.S.D. (5%) is the least significant difference at 5% level of significance

FIG. 9 - A I-78

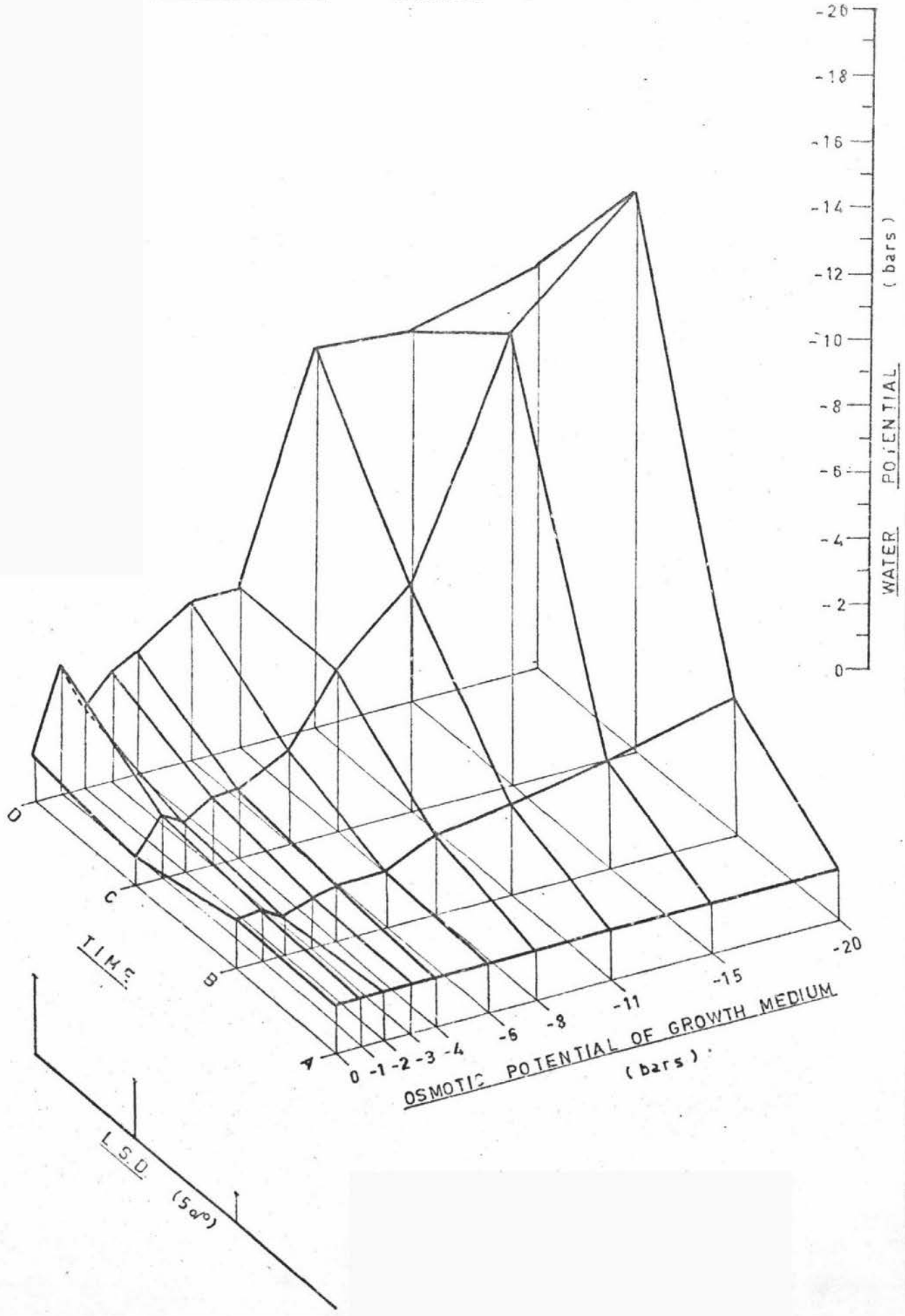

-20
-18
-16
-14
-12
-10
-8
-6
-4
-2
0
WATER POTENTIAL (bars)
D
C
B
A
TIME
0 -1 -2 -3 -4 -6 -8 -11 -15 -20
OSMOTIC POTENTIAL OF GROWTH MEDIUM (bars)
L.S.D. (5%)

FIG. 9 -B — P. yunnanensis

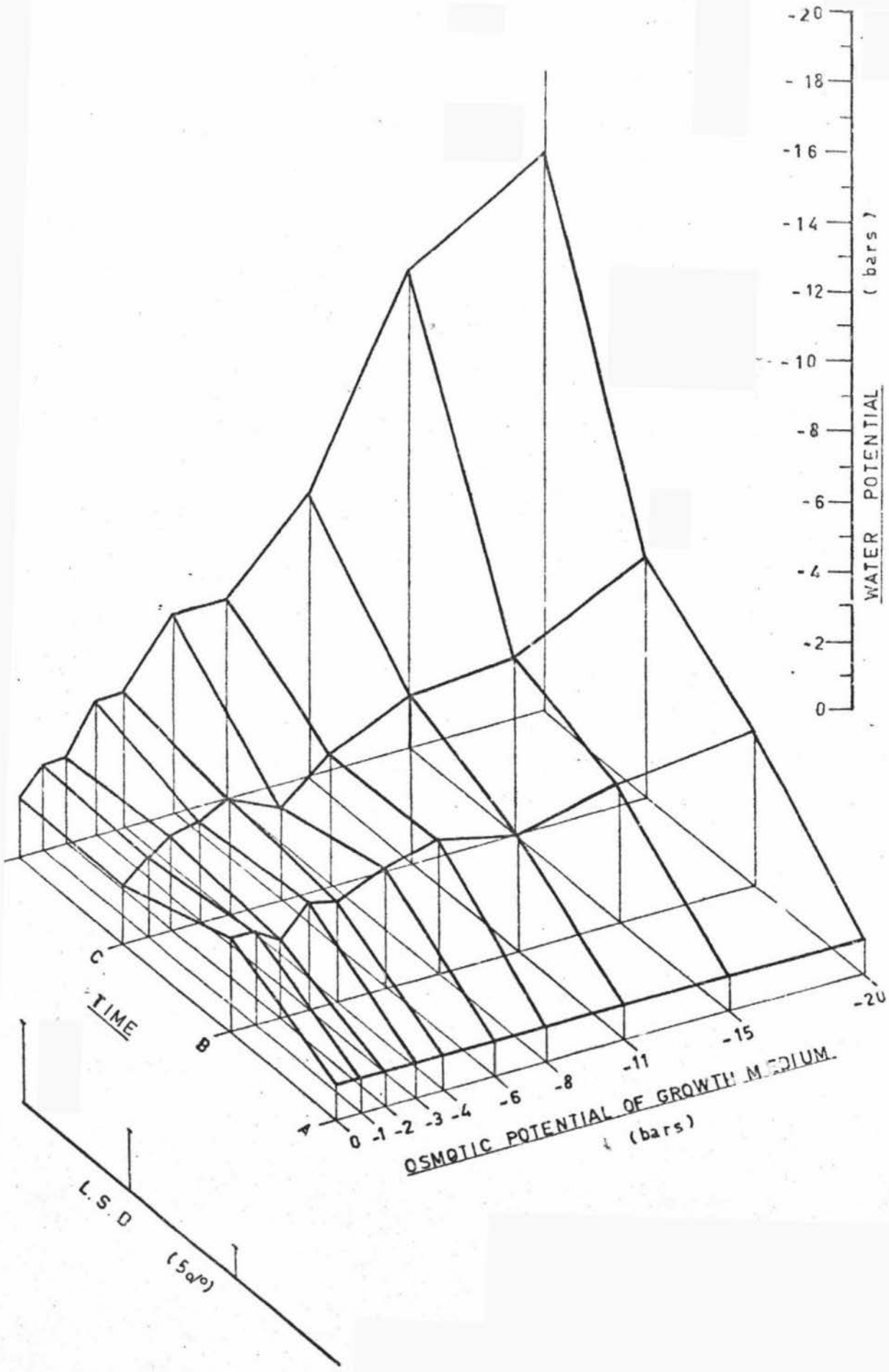
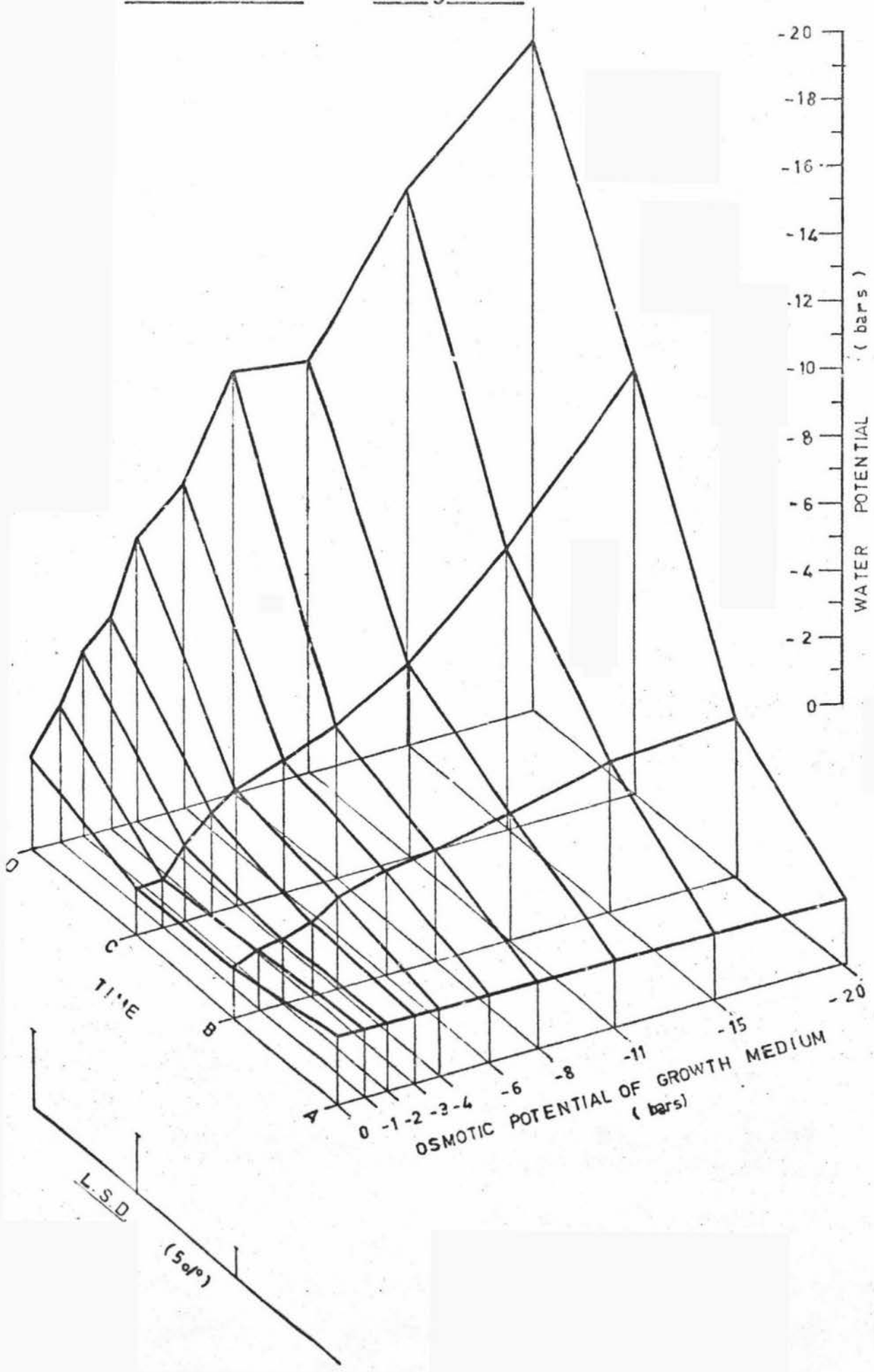


FIG. 9 - C P.angulata



variability and reached a higher ultimate water potential than I-78 or P.yunnanensis in all concentrations of osmoticum used.

3.5.2 Water Contents of Cuttings

The effect of imposed water stress on water content at the top and bottom of the cuttings is given in Fig. 10. The analysis of variance is provided in Appendix 11.

The following trends are apparent within clones and within the range of osmoticum concentrations used; the differences in these two factors are highly significant throughout.

(a) Water content increased in cuttings in osmotic potentials approaching zero, but decreased in low osmotic potentials in the initial measurement.

(b) This trend continued and increased to the time of measurement of water content at growth initiation.

(c) Until growth initiation, the trends in basal water content were similar to but more pronounced than the trends in water content close to the bud (i.e.'top water content').

(d) After growth initiation, water content at the top of the cutting increased in less concentrated osmotica, but remained at a similar level to that seen at growth initiation in the osmotica of high concentrations.

(e) The water contents observed in the basal region of cuttings declined markedly after growth initiation in osmotica of low concentration. In higher concentrations, the corresponding water contents of I-78 and P. angulata also fell, but those of P.yunnanensis rose.

3.5.3 Relation between water content and water potential

The relation between internal water content and water potential is shown in Table 5, and the analysis of variance is shown in Appendix 12.

There were highly significant correlations between water content and potential at all three times of observation and in all three clones.

Fig. 10. - Water Content of Cuttings in Growth Media of Various Osmotic Potentials

Fig 10. A - I-78 Water content at top of the cutting.
10. B - I-78 - bottom W.C.
10. C - P.yunnanensis - top
10. D - P.yunnanensis - bottom
10. E - P. angulata - top
10. F - P. angulata - bottom

Time A, B, C, D } as for Fig 9.

L.S.D. 5% is the Least Significant Difference at the 5% level of significance

FIG. 10 A — I-78 TOP

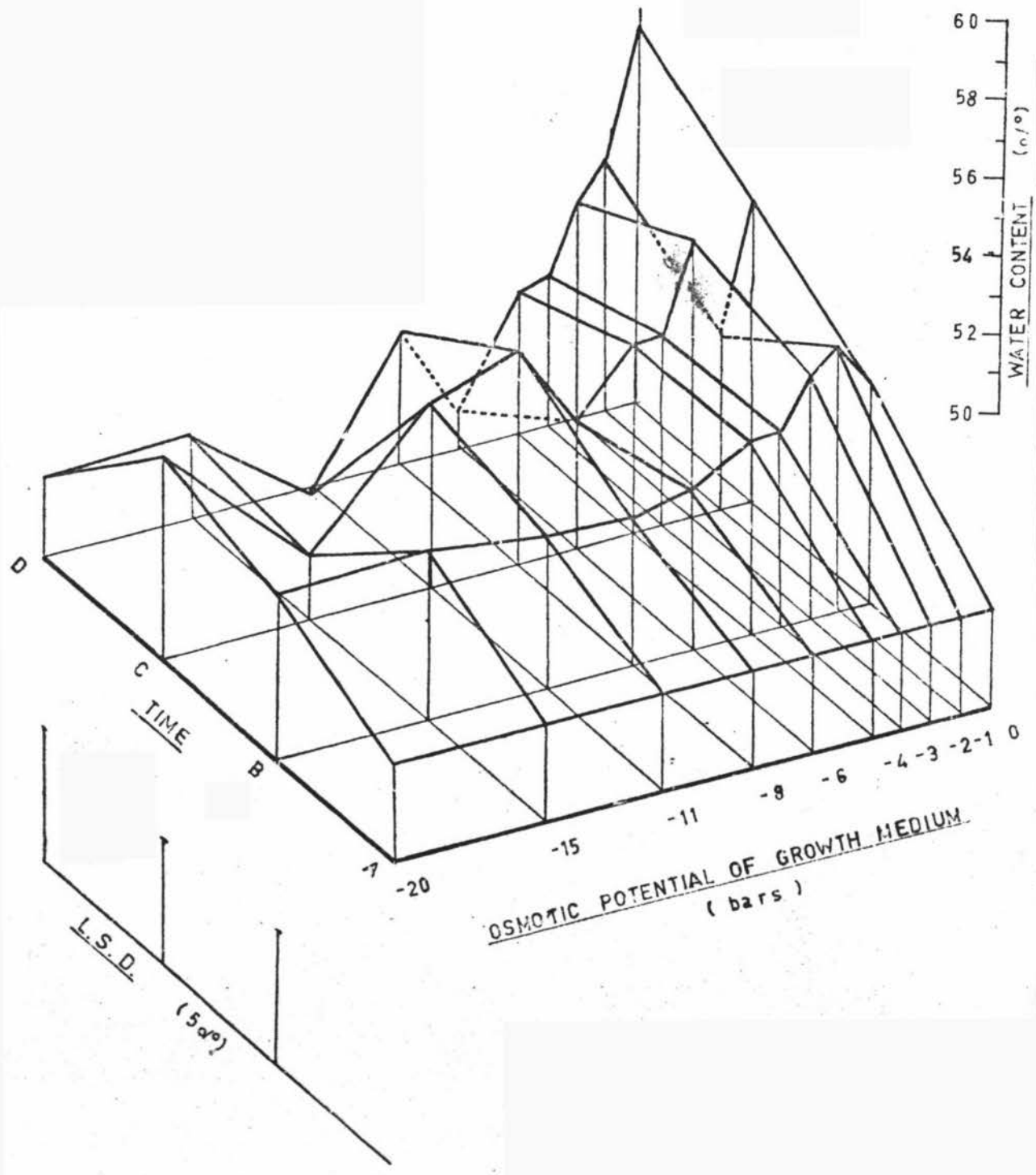


FIG. 10 B — I-78 Bottom

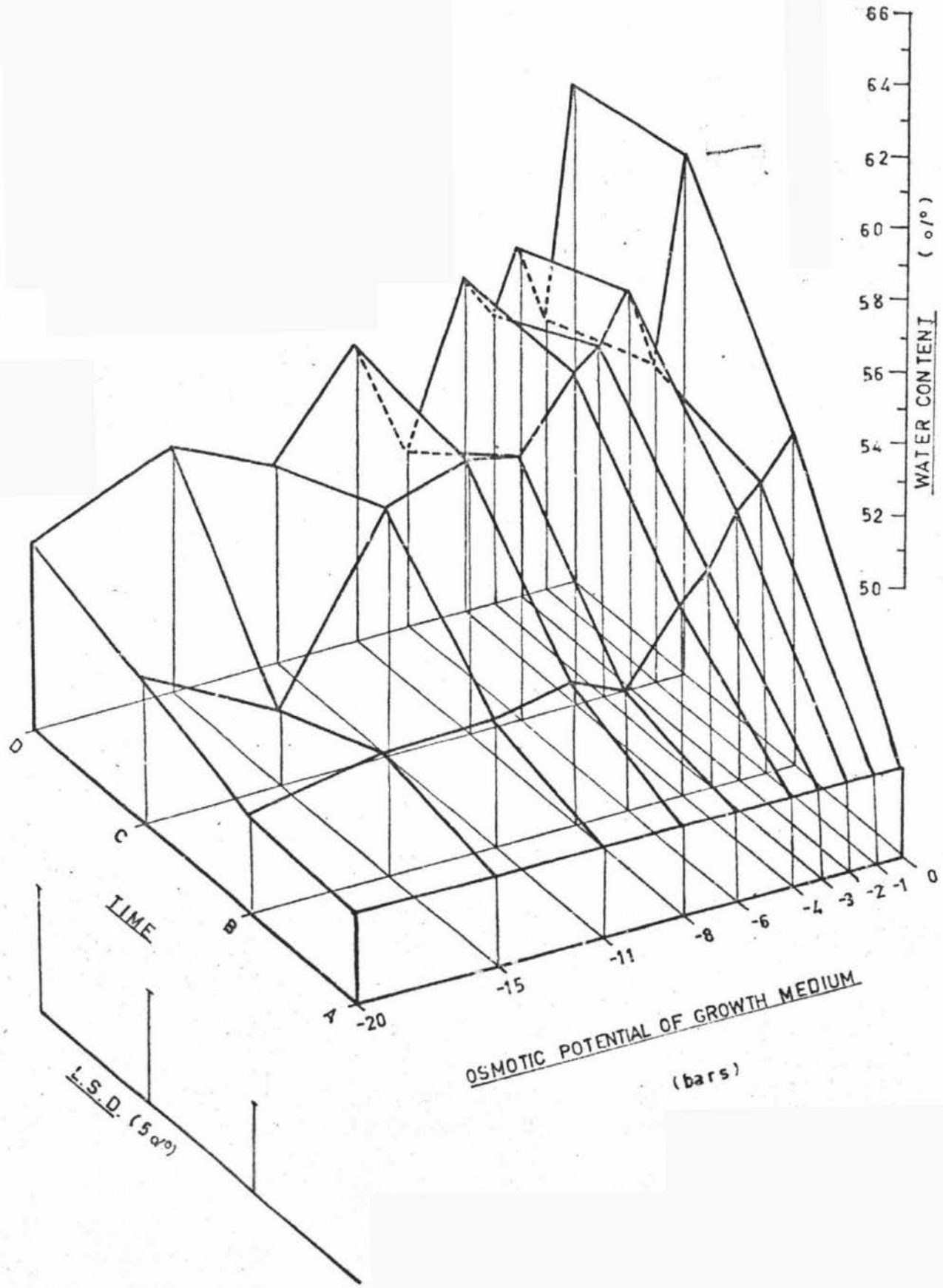


FIG. 10C -- P. yunnanensis, -Top

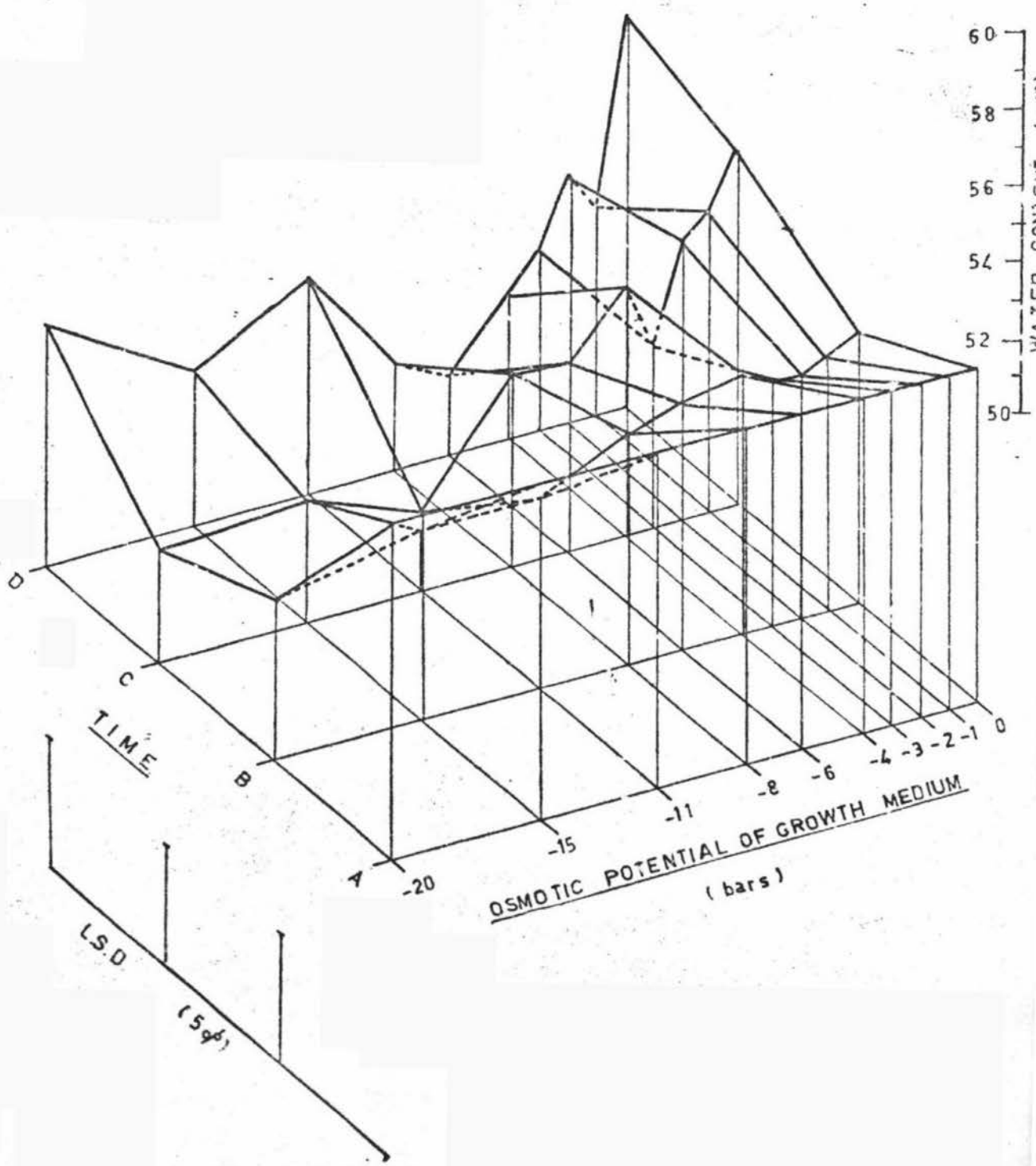


FIG. 10 D — P. yunnanensis:

Bottom

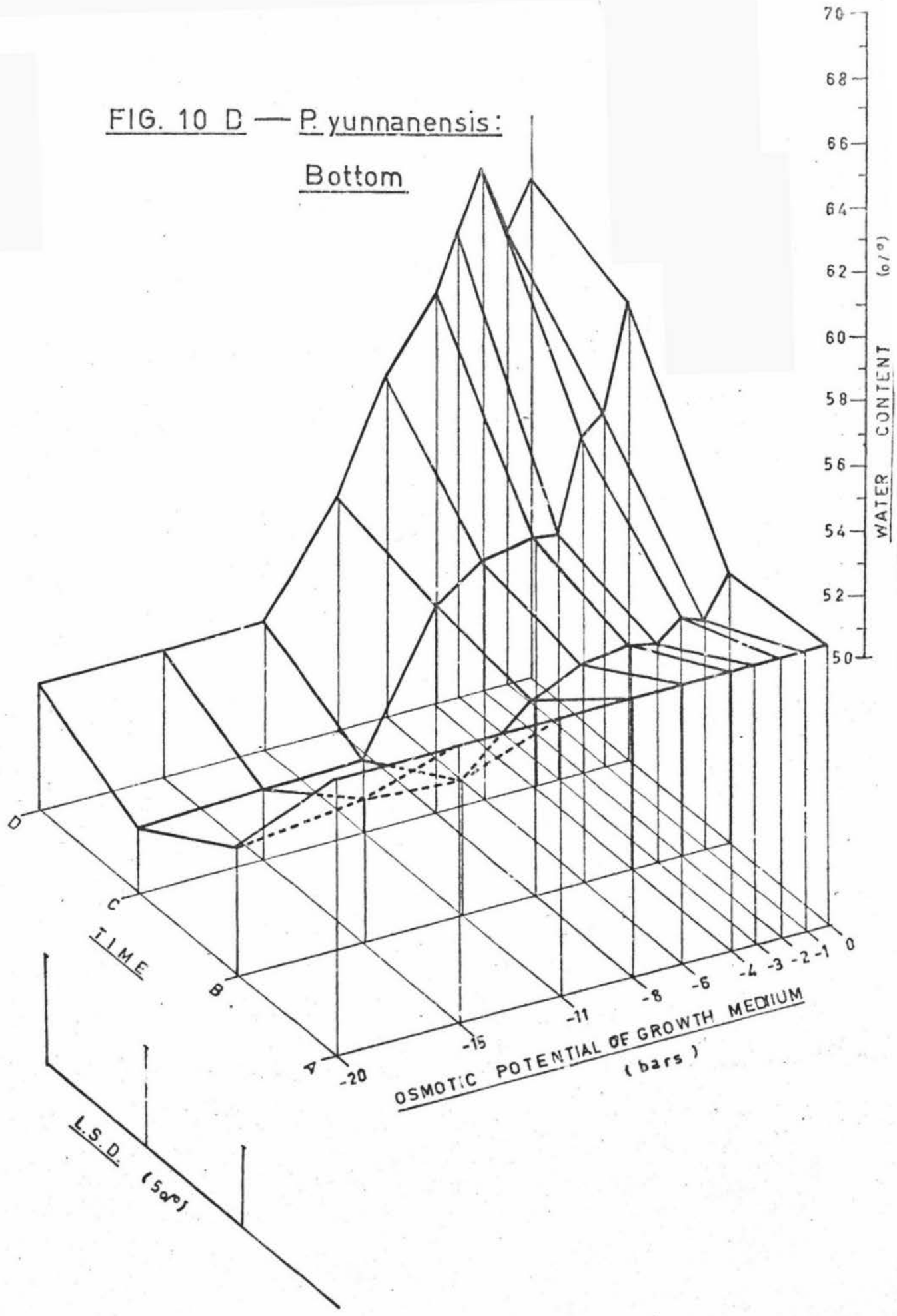


FIG. 10 E — P. angulata: Top

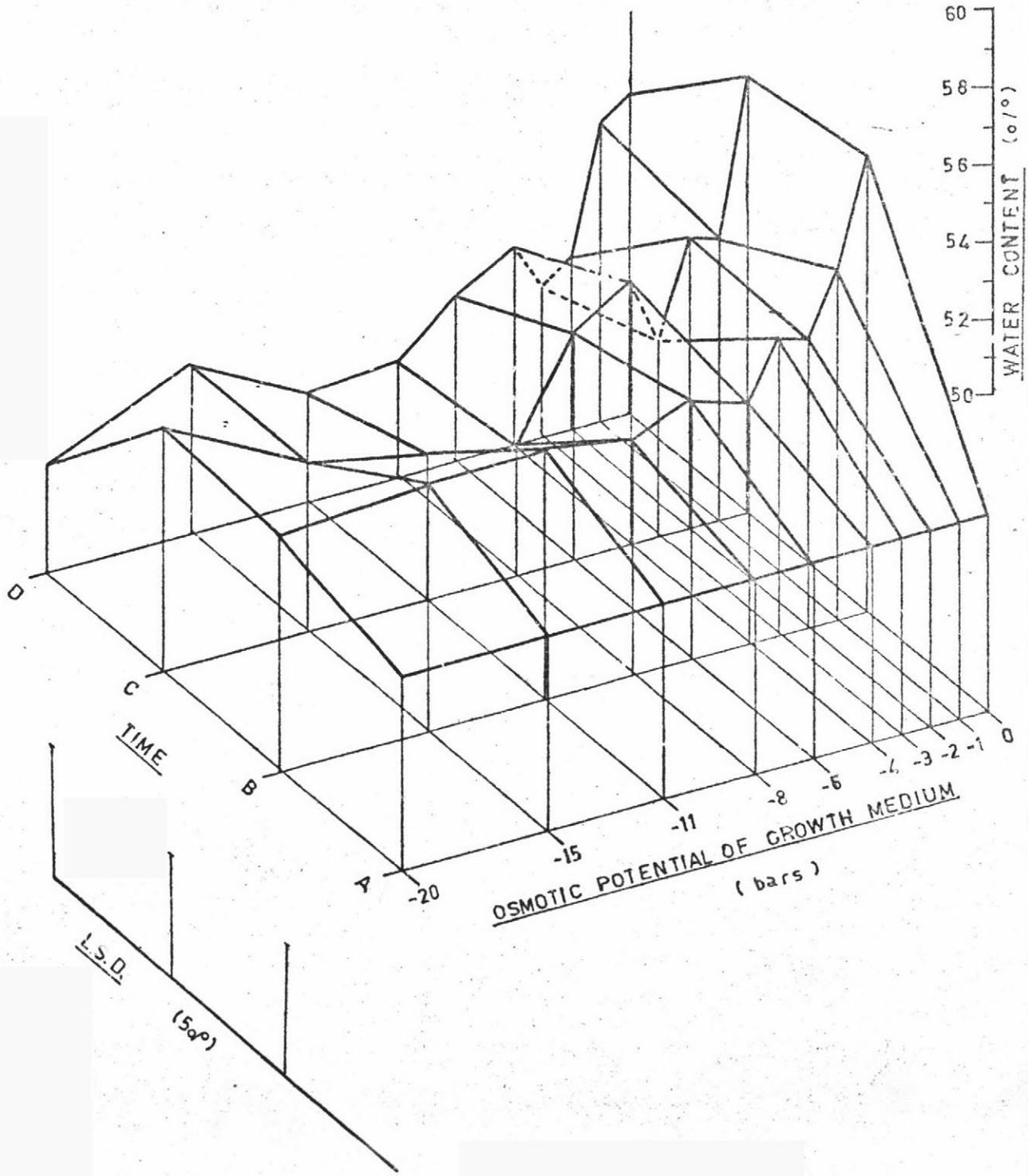


FIG. 10 F — P. angulata: Bottom

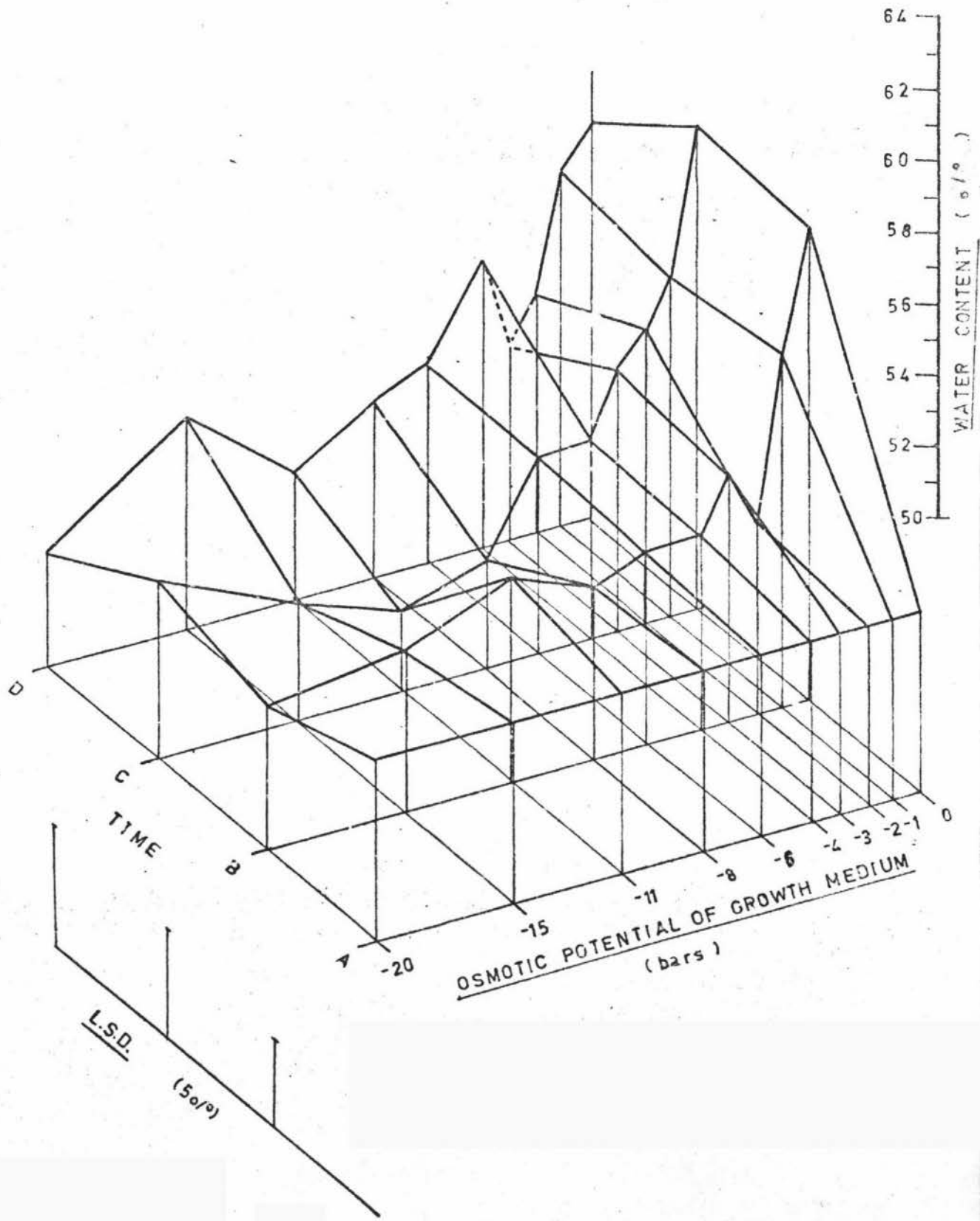


Table 5: Relation between Water Content and Water Potential.

Clone	Time of Observation	Y variable	Regression	Standard Error	Correlation	Significance
Av.	4 days	Top	-0.712	.148	-.369	**
Av.	4 days	Bottom	-1.200	.183	-.477	**
1	G.I.	Top	-0.133	.055	-.328	*
2	G.I.	Top	-1.198	.240	-.585	**
3	G.I.	Top	-0.178	.127	-.197	N.S.
1	G.I.	Bottom	-0.493	.072	-.704	**
2	G.I.	Bottom	-2.202	.266	-.767	**
3	G.I.	Bottom	-0.426	.148	-.383	**
1	Terminal	Top	-0.453	.100	-.546	**
2	Terminal	Top	-0.098	.083	-.167	N.S.
3	Terminal	Top	-0.187	.082	-.314	*
1	Terminal	Bottom	-.374	.095	-.490	**
2	Terminal	Bottom	-0.817	.113	-.723	**
3	Terminal	Bottom	-0.308	.079	-.493	**

KEY

CLONE:

Clone 1 is I-78

Clone 2 is P. yunnanensis

Clone 3 is P. angulata

Av. is used in those cases where there were no significant differences between clones, and the regression analysis has been performed over all clones.

There are 48 degrees of freedom associated with individual clone analysis, and 146 d.f. with the average regression.

TIME OF OBSERVATION:

4 days - i.e. 4 days after commencement of the experiment.

G.I. is the stage of growth initiation.

Terminal is the time of termination of the experiment, 14 days after the stage of growth initiation.

VARIABLES:

The Y variable is water content, which was determined at both the top and bottom of the cutting (Section 2.3.5).

The X variable is water potential.

3.5.4 The Incidence of Shoot and Root Growth

The incidence of shoot and root growth in each clone for each concentration of osmoticum used is given in Table 6 for two stages of growth, at two days after growth initiation and at termination of the experiment after 14 days growth.

Table 6: Incidence of Shoot and Root Growth.

Clone	Osmotic Potential of Culture Solution	No. of Cuttings Producing at 2 days (a) shoots	(b) root initials	No of Cuttings Producing at 14 days (a) shoots	(b) root initials	(c) Root
		%	%	%	%	%
I-78	0 bars	100	87	100	100	100
	-1	93	80	100	90	100
	-2	93	80	100	100	100
	-3	87	73	100	100	90
	-4	80	93	90	100	50
	-6	47	67	80	100	0
	-8	20	40	90	100	10
	-11	0	0	0	100	0
	-15	0	0	0	50	0
	-20	0	0	0	0	0
P. yunnanensis						
	0	47	67	80	100	100
	-1	40	93	100	100	90
	-2	27	93	90	100	90
	-3	47	100	100	100	90
	-4	20	73	100	100	50
	-6	47	60	100	100	10
	-8	27	47	80	100	0
	-11	7	0	80	40	0
	-15	0	0	7	40	0
	-20	0	0	0	30	0
P. angulata						
	0	47	47	80	40	90
	-1	60	47	100	80	100
	-2	53	27	100	70	80
	-3	47	53	80	60	80
	-4	27	53	80	100	40
	-6	27	13	20	100	0
	-8	7	7	20	40	0
	-11	13	7	0	0	1
	-15	0	0	20	10	0
	-20	7	0	20	0	0

The table is based on 15 cuttings per subtreatment at the two-day stage, and on 10 cuttings at the 14-day stage.

There appears to be a well-defined critical osmotic potential of the growth medium which will inhibit growth. The critical osmotic potential for appearance of roots is the least negative, that for shoots is intermediate and that for root initials the most negative, in all clones. However there were clonal differences in the levels required to inhibit, and all three types of growth were also delayed by lower osmotic potentials.

3.5.5. Shoot Growth

The relation between shoot length, osmotic potential of the growth medium, and time since growth initiation is illustrated for each clone in Fig. 11 and Plate 3. The analysis of variance is shown in Appendix 12.

As the osmotic potential of the growth medium was decreased to -4 to -6 bars, there was a massive, approximately linear decline in the rate of shoot elongation in all three clones. At lower osmotic potentials shoot elongation rates declined further, but in two clones (P. yunnanensis and P. angulata) some bud movement was seen in several buds at the extreme potential of -20 bars.

At - 1 bar in the clone I-78 in the early stages of shoot elongation there was somewhat less growth than at - 2 bars, but this difference was only just significant (Fig. 11). In the clone P. yunnanensis a trough was also apparent at - 4 bars, which became more pronounced in the later stages of growth.

The water content of shoots was progressively lower with each increasingly concentrated solution of the osmoticum, from about 90% in all clones at zero potential to about 65% in the case of I-78 and P. yunnanensis and 35% in the case of P. angulata, at - 4 bars (Fig. 14). This effect is reflected in the relative rates of change of fresh and dry weights at termination of the experiment. Whereas fresh weights declined markedly between osmotic potentials of zero and - 4 bars in the growth medium and thereafter fell more slowly, dry weight measurements fell more slowly.

The troughs previously noted in I-78 and P. yunnanensis shoot elongation were not represented in dry weight measurements.

3.5.6 Root Growth

The effect of imposed water stress on root growth is shown graphically in Fig 12 and 13 and Plate 3 and the analysis of variance is shown in Appendix 13.

Fig. 11. - Shoot Growth of Cuttings in Growth Media of Various Osmotic Potentials

Fig. 11A - I-78
11B - P.yunnanensis
11C - P.angulata

'L.S.D. (5%)' is the Least Significant Difference at the 5% level of significance, for each time of measurement.

'Days of Growth' refers to the time periods after growth is first seen.

FIG. 11-A I-78

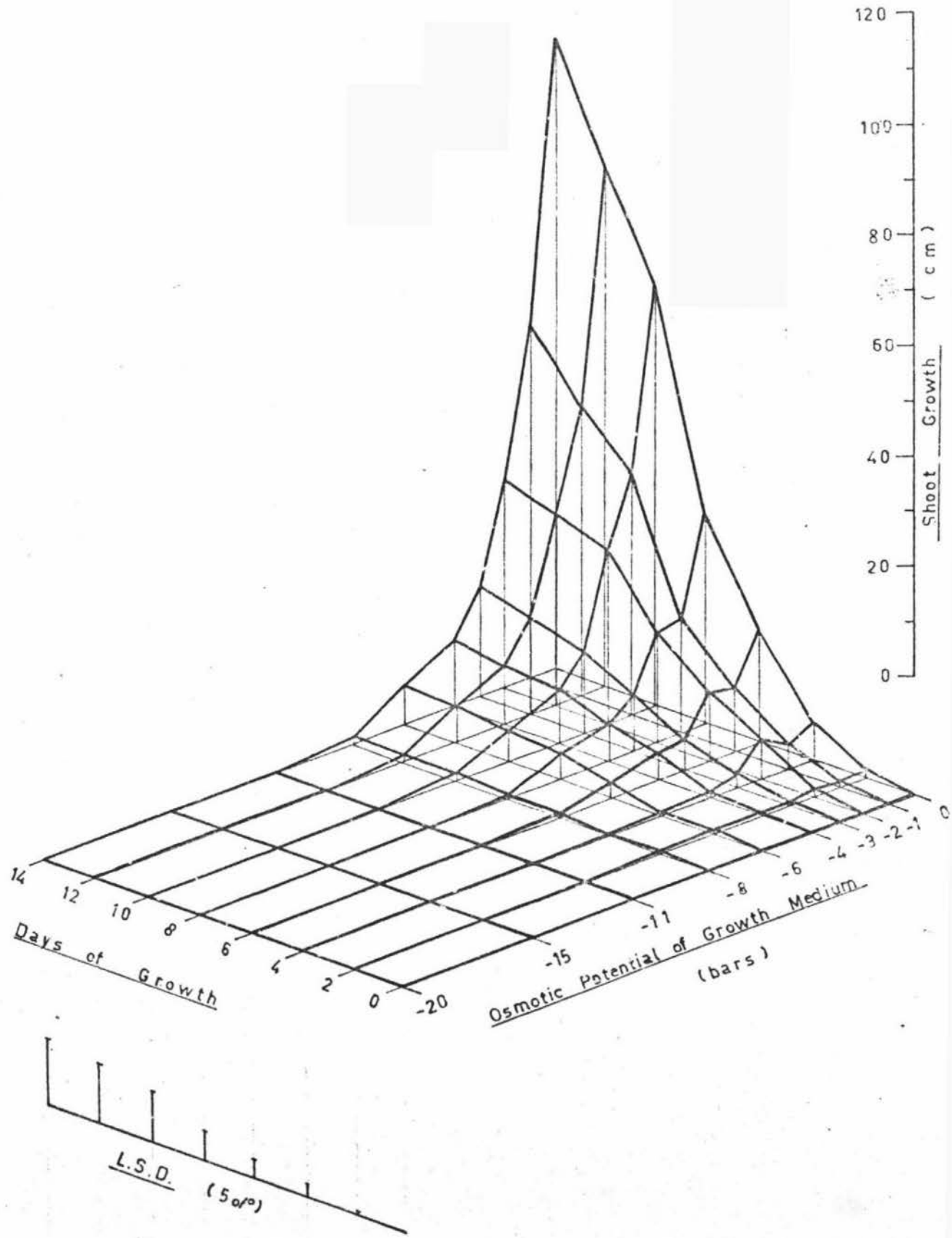

120
100
80
60
40
20
0
Shoot Growth (cm)
14
12
10
8
6
4
2
0
Days of Growth
0
-1
-2
-3
-4
-6
-8
-11
-15
-20
Osmotic Potential of Growth Medium (bars)
L.S.D. (5%)

FIG. 11-B P. yunnanensis

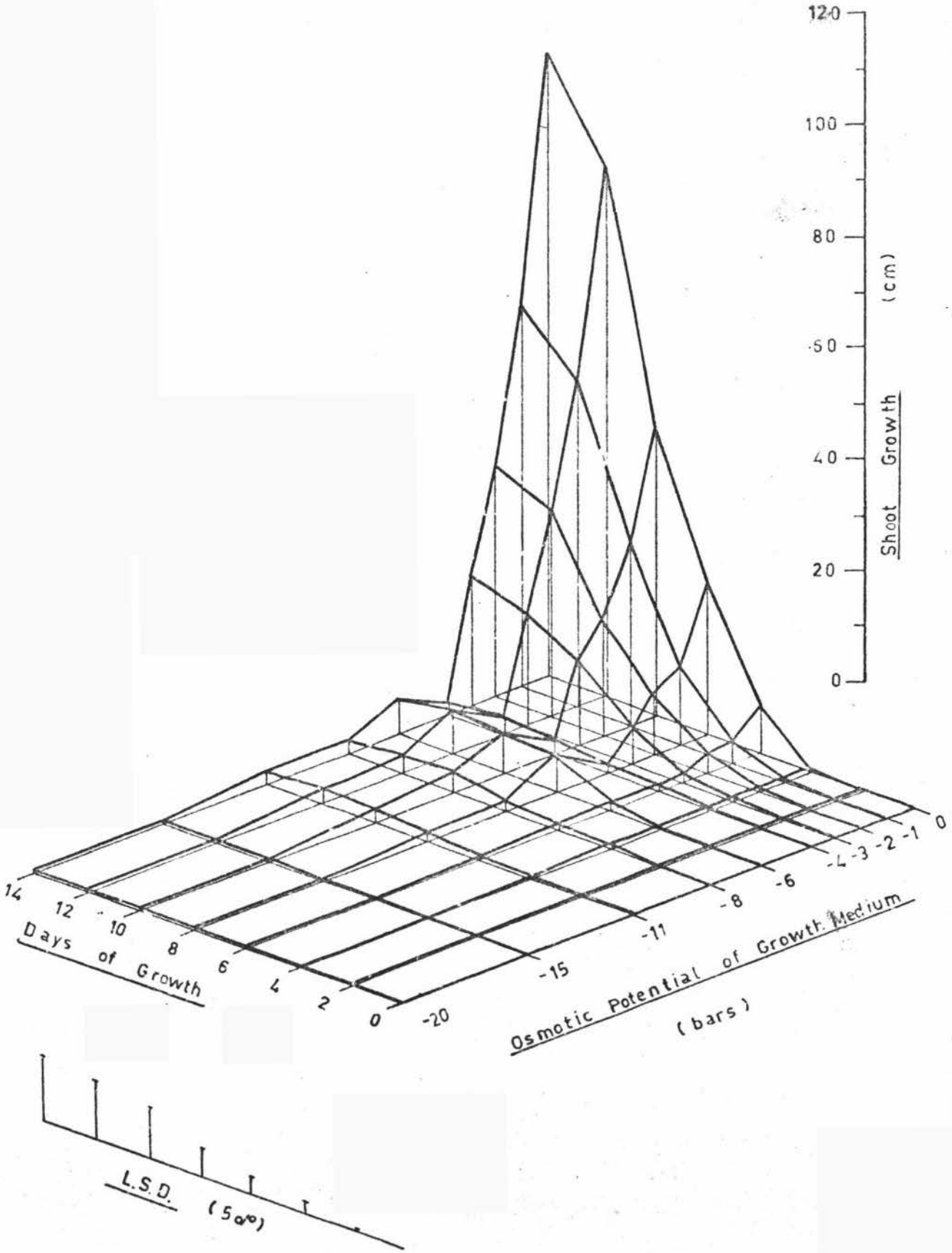


FIG. 11-C P. angulata

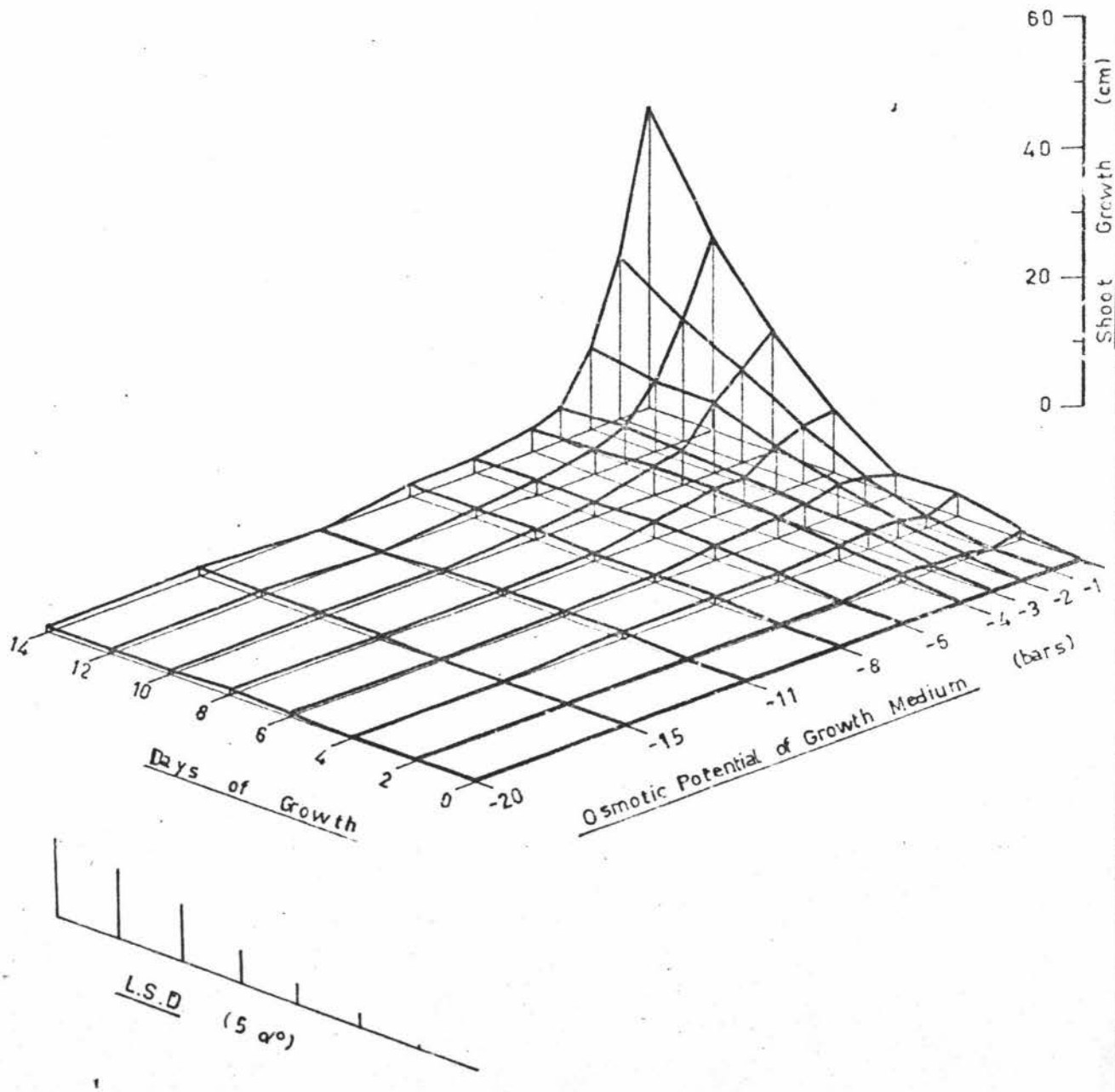


Plate 3. - The Effect on Budbreak and Root Initiation of Various Osmotic Potentials associated with the Growth Medium

Each page represents one clone, as indicated.

Four cuttings are shown representing each osmotic potential:

Upper, from left:

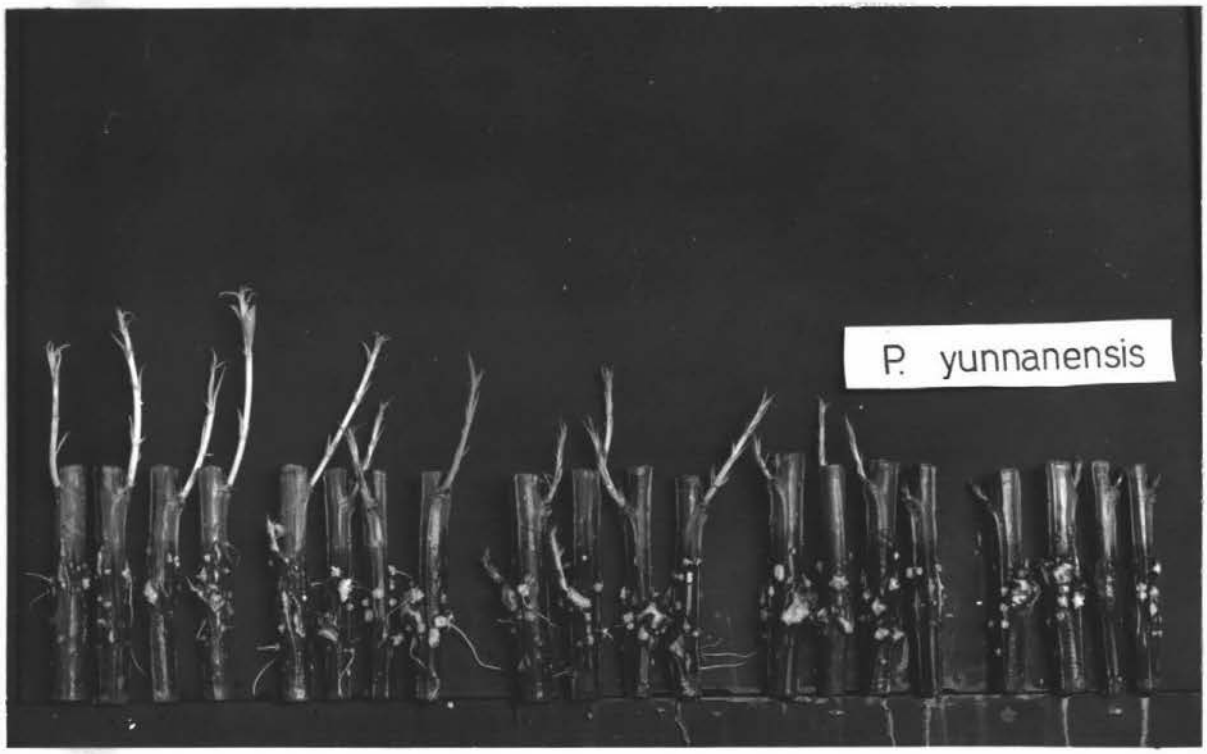
0
-1 bar
-2 bar
-3 bar
-4 bar

Lower, from left:

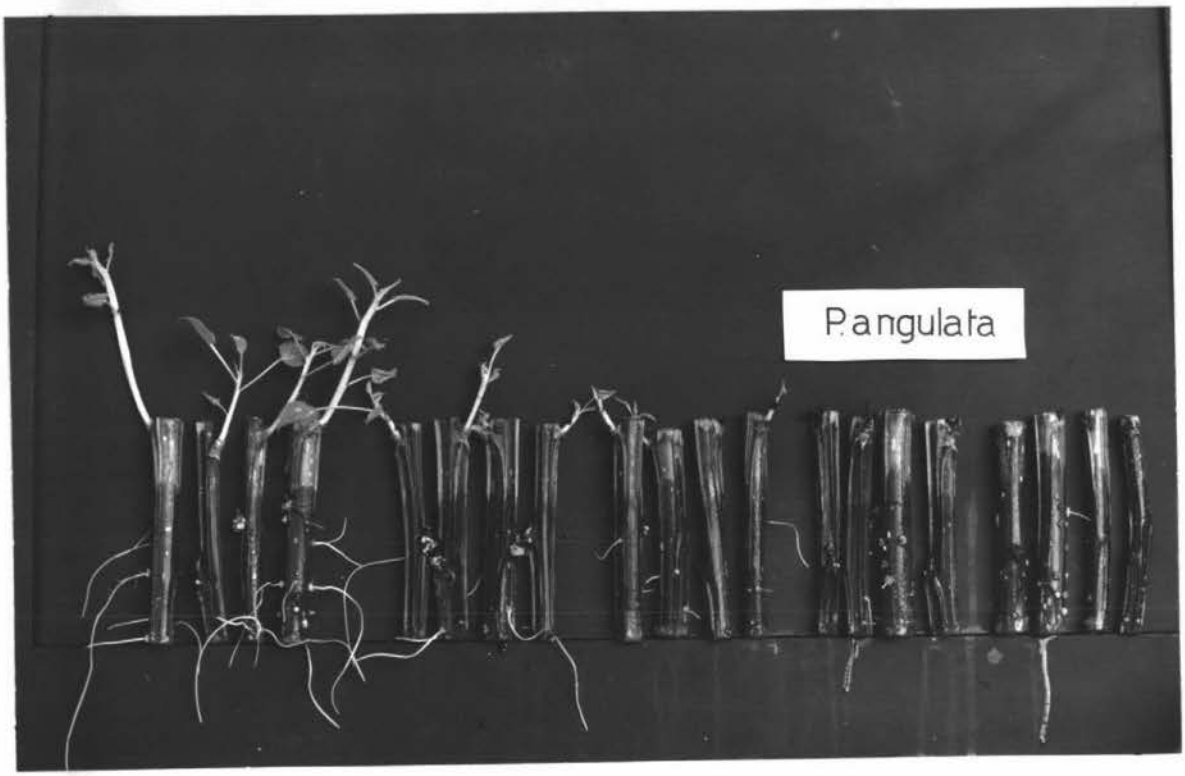
-6 bar
-8 bar
-11 bar
-15 bar
-20 bar


I·78


I·78


P. yunnanensis


P. yunnanensis


P. angulata


P. angulata

Fig. 12. - Root Growth of Cuttings in Growth Media of Various Osmotic Potentials

Fig. 12-A - I-78
12-B - *P.yunnanensis*
12-C - *P.angulata*

r.i. = root initials
s.r. = short roots, i.e. less than 1 cm.
l.r. = long roots, i.e. greater than 1 cm.

The following Least Significant Differences at the 5% level of significance apply to all clones at each time of observation:

Days	r.i.	L.S.D.	
		s.r	l.r
2	0.945	-	-
4	1.628	0,036	-
6	2.311	0.357	0.189
8	2.920	0.672	0.462
10	3.383	0.714	0.903
12	3.299	0.945	1.240
14	3.236	0.756	1.345

FIG. 12-A I-78

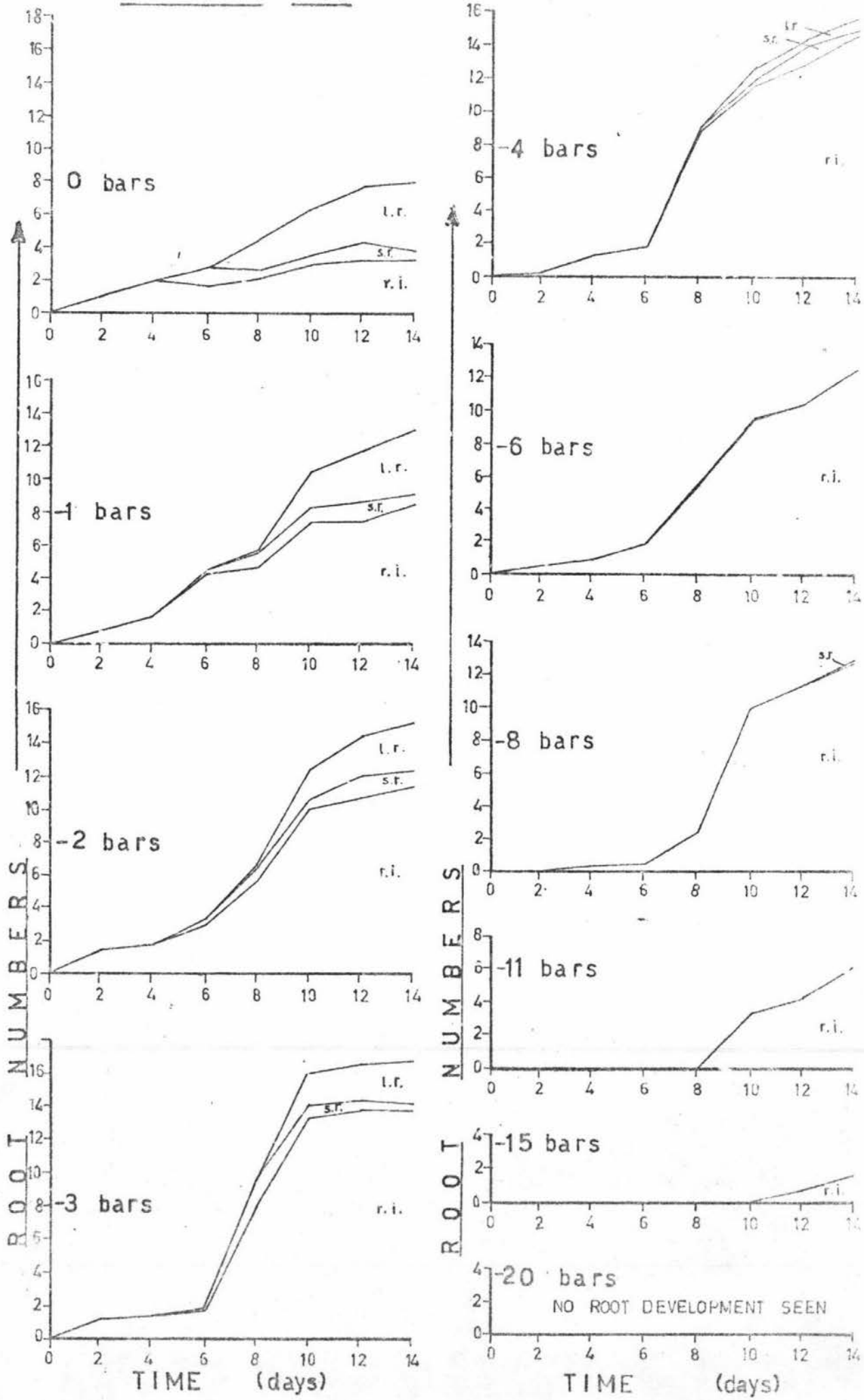

0 bars
-1 bars
-2 bars
-3 bars
-4 bars
-6 bars
-8 bars
-11 bars
-15 bars
-20 bars
NO ROOT DEVELOPMENT SEEN
t.r.
s.r.
r.i.
ROOT NUMBERS
TIME (days)

FIG. 12 - B P.yunnanensis

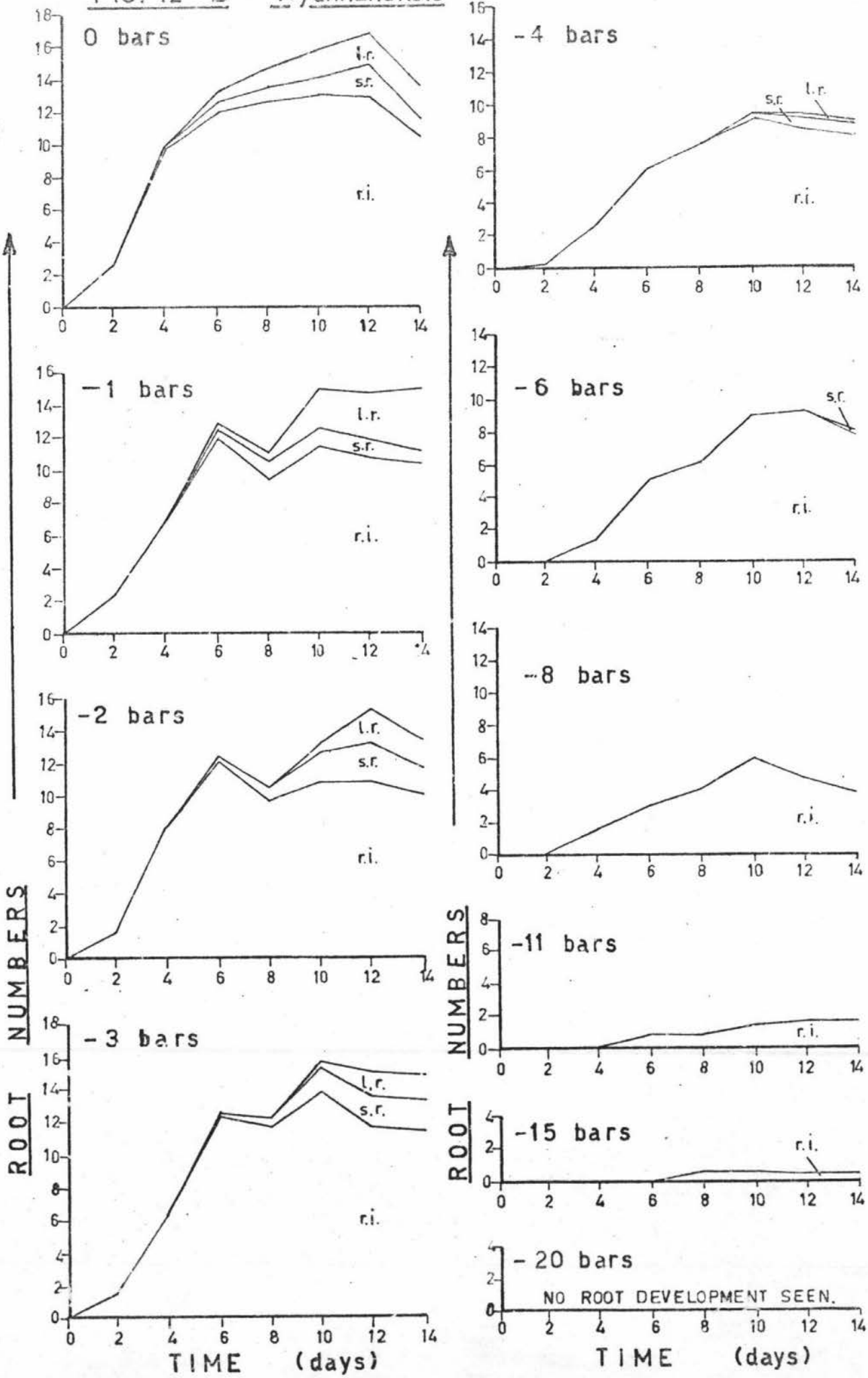


FIG. 12c — P. angulata

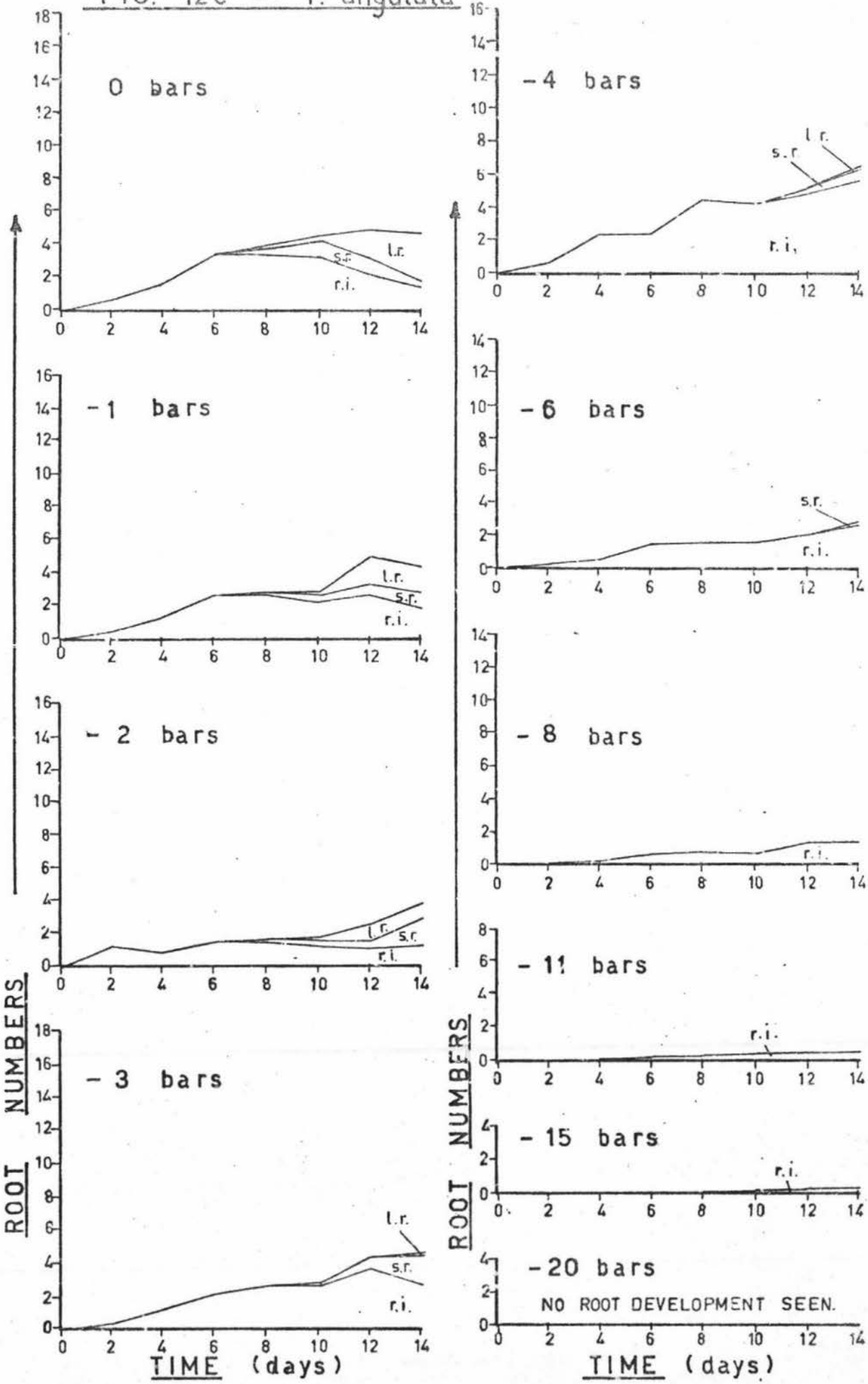


Fig. 13. - Measurements at the Termination of the Experiment relating Growth to Water Stress.

Fig. 13. A Total root length per cutting

13. B Total no.of roots

13. C No. of basal roots

13. D Shoot dry weight

13. E Root Dry Weight

FIG. 13

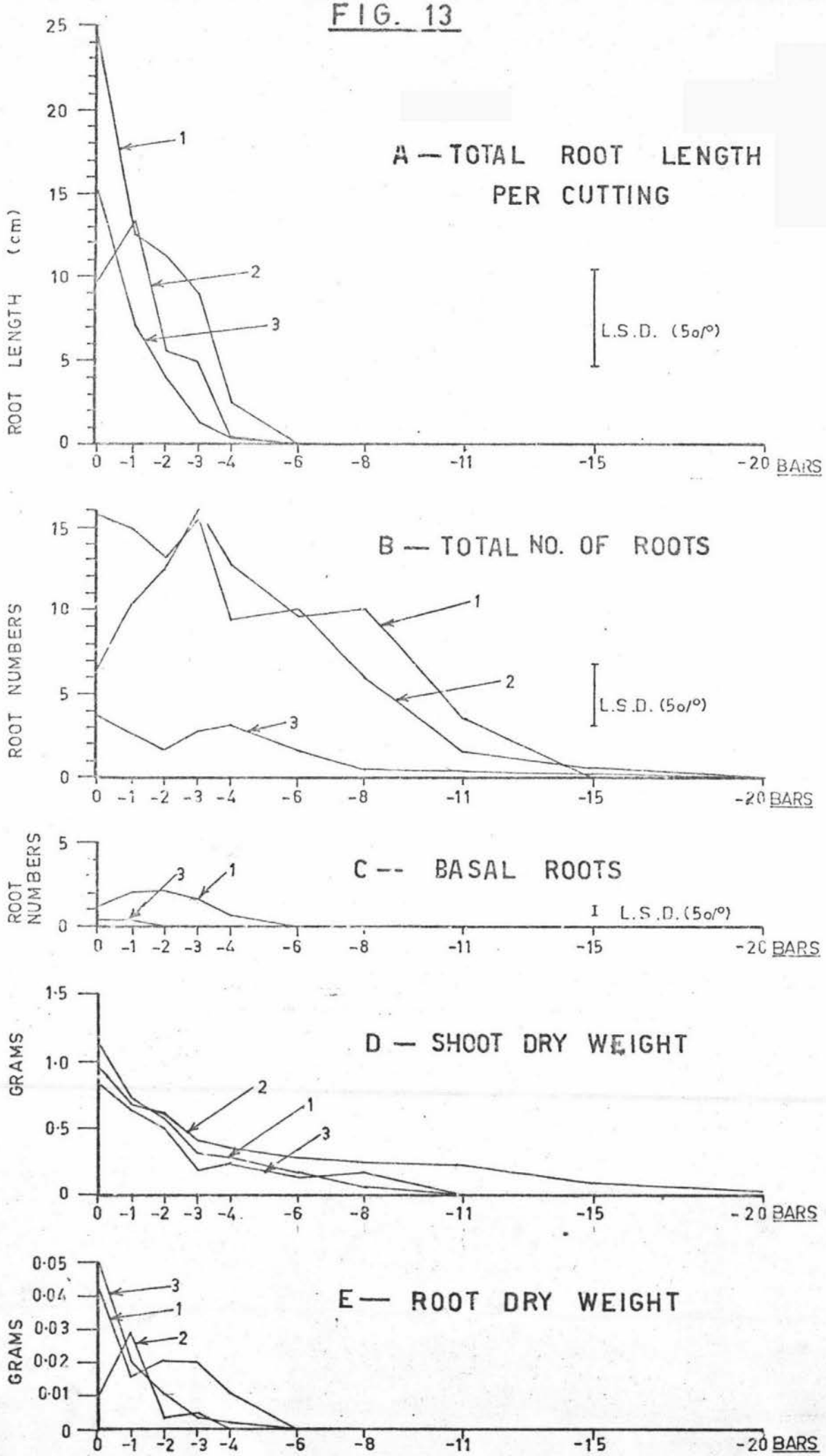
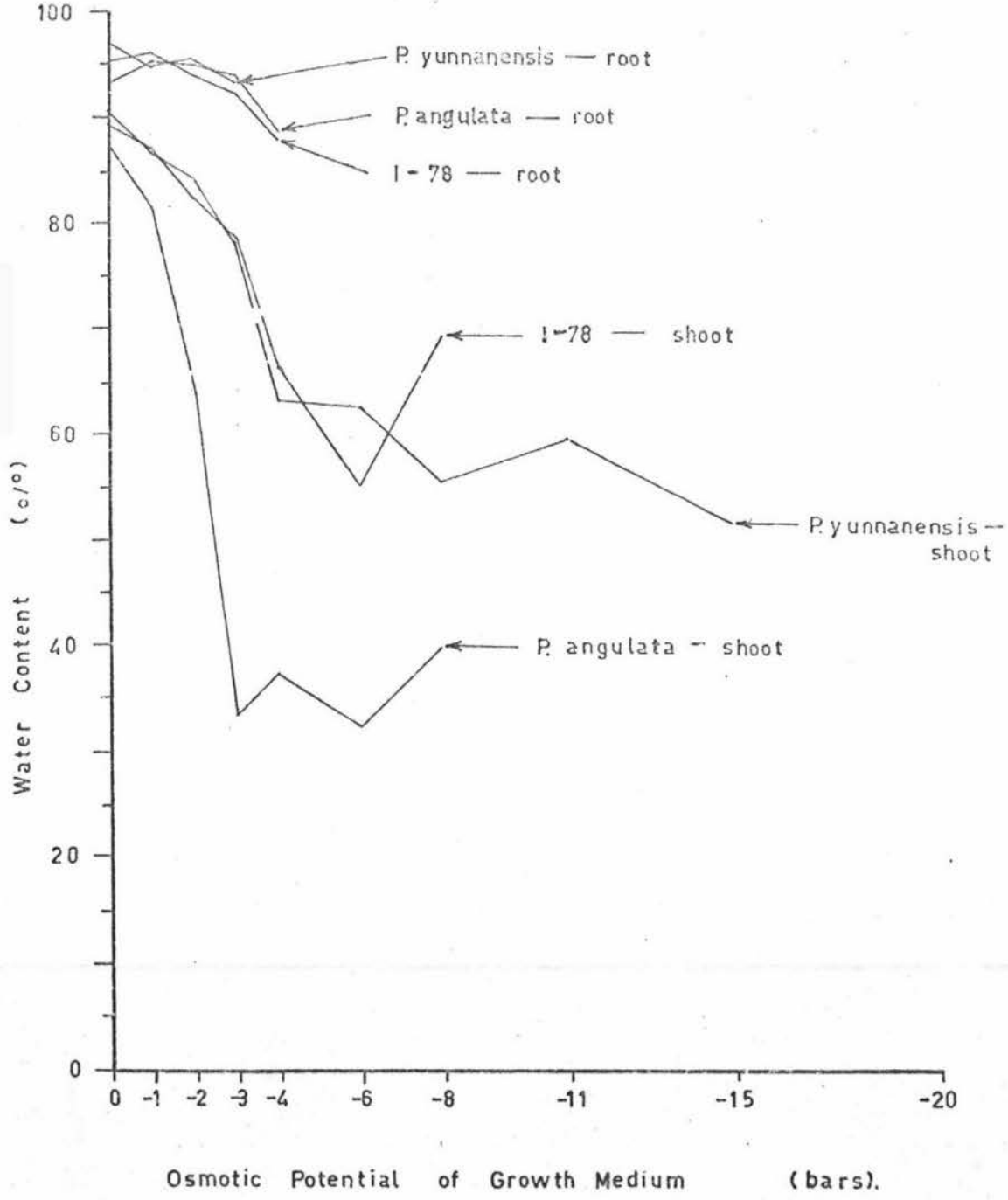

A — TOTAL ROOT LENGTH PER CUTTING
ROOT LENGTH (cm)
25
20
15
10
5
0
1
2
3
L.S.D. (5o/o)
0 -1 -2 -3 -4 -6 -8 -11 -15 -20 BARS
B — TOTAL NO. OF ROOTS
ROOT NUMBERS
15
10
5
0
1
2
3
L.S.D. (5o/o)
0 -1 -2 -3 -4 -6 -8 -11 -15 -20 BARS
C -- BASAL ROOTS
ROOT NUMBERS
5
0
3
1
I L.S.D. (5o/o)
0 -1 -2 -3 -4 -6 -8 -11 -15 -20 BARS
D — SHOOT DRY WEIGHT
GRAMS
1·5
1·0
0·5
0
2
1
3
0 -1 -2 -3 -4 -6 -8 -11 -15 -20 BARS
E — ROOT DRY WEIGHT
GRAMS
0·05
0·04
0·03
0·02
0·01
0
3
1
2
0 -1 -2 -3 -4 -6 -8 -11 -15 -20 BARS

Fig. 14. Water Content of Shoots and Roots Grown in Media of Various Osmotic Potentials

FIG. 14 —

Water Content of Shoots and Roots



In all three clones the following trends were seen:

(a) The incidence of root initials plus total roots declined with decreasing osmotic potential, and were completely inhibited by a potential of - 20 bars. Although the appearance of root initials did not decline to low levels until - 8 to - 11 bars were applied in the osmoticum, their development into roots was inhibited by potentials closer to zero, and there was little root growth beyond - 4 bars.

(b) Appearance of root initials appeared to follow three phases in rate of appearance. In the first stage up to approximately 20% of root initials developed slowly, followed by a second more rapid phase in which most of the ultimate production of root initials appeared. The third phase involved a return to a much slower rate of appearance of approximately 15% of the ultimate production.

The commencement of the first stage was inhibited by osmotic potentials in the growth medium above - 8 bars and ceased at - 20 bars. In I -78 the second phase was not appreciably retarded in magnitude at - 8 bars, although it was delayed by two days when compared with growth in solutions of - 4 bars and under. The third phase was massively affected by earlier phases of growth, and there were no obvious effects of increasing osmotic potential of the growth medium as such.

(c) Both I-78 and P. yunnanensis developed most roots plus root initials (i.e. 'total roots') at - 4 bars, the numbers showing a progressive decline at lower osmotic potentials to cessation at - 20 bars. However, whereas I-78 grew progressively fewer roots at osmotic potentials lower than - 4 bars, P. yunnanensis developed another peak at zero potential. P. angulata followed a similar pattern of growth to P. yunnanensis but at a lower overall level and showed maximum numbers at zero and - 6 bars.

(d) Basal roots were developed to the greatest extent in I-78 in a growth medium of - 2 bars, and fell to zero at - 6 bars. P. angulata showed some basal root growth at zero and - 1 bars only, and basal roots were not seen at any stage in P.yunnanensis (Appendix 13).

(e) The total root length per cutting declined with decreasing osmotic potential from a maximum at zero (I-78 and P.angulata) or - 1 bars (P. yunnanensis) to nil at - 6 bars. In the case

of P. yunnanensis there was a substantial decline at zero osmotic potential. In general, most root length was produced by I-78 and least by P. angulata.

(f) Root dry weight at termination of the experiment followed the trends described for total length closely. Water content of roots declined with increasing osmotic potential of the growth medium.

3.5.7 Relation between Internal Water Status and Growth.

Regression analyses relating various parameters of growth to internal potential and top and bottom water content showed that a strong correlation existed, shown in Table 7.

Table 7 - Relation between Internal Water Status and Growth

Clone	Y Variable	X Variable	Regression	Standard Error	Correlation	Significance
A.	Growth Initiation					
Av.	S.G.	W. Pot	-0.257	.056	-.358	**
1	S.G.	W.C.(top)	0.711	.221	.422	**
2	S.G.	W.C.(top)	0.059	.028	.288	*
3	S.G.	W.C.(top)	0.301	.127	.324	**
1	S.G.	W.C.(Bottom)	0.517	.120	.529	**
2	S.G.	W.C.(Bottom)	0.025	.021	.174	NS
3	S.G	W.C.(Bottom)	0.289	.100	.384	**
1	R.I.	W. Pot	-0.102	.026	-.491	**
2	R.I.	W. Pot	-1.233	.264	-.558	**
3	R.I.	W. Pot	-0.107	.062	-.240	NS
Av	R.I.	W.C.(top)	0.225	.062	.287	**
1	R.I.	W.C.(Bottom)	0.173	.035	.584	**
2	R.I.	W.C.(Bottom)	0.469	.088	.610	**
3	R.I.	W.C.(Bottom)	0.094	.056	.233	NS

Table 7 contd.

Clone	Y Variable	X Variable	Regression	Standard Error	Correlation	Significance
	B. After 14 days growth					
1	S.G.	W.Pot	-3.337	.716	-.558	**
2	S.G.	W.Pot	-2.429	.577	-.519	**
3	S.G.	W.Pot	-0.838	.168	-.584	**
1	S.G.	W.C.(top)	4.745	.783	.658	**
2	S.G.	W.C(Top)	4.293	.977	.536	**
3	S.G.	W.C.(top)	1.348	.289	.559	**
1	S.G.	W.C.(Bottom)	4.262	.950	.543	**
2	S.G.	W.C.(Bottom)	2.097	.515	.507	**
3	S.G.	W.C.(Bottom)	1.318	.270	.576	**
1	R.I.	W.Pot	-0.609	.149	-.508	**
2	R.I.	W.Pot	-0.848	.136	-.669	**
3	R.I.	W.Pot	-0.175	.070	-.340	*
Av	R.I.	W.C.(Top)	0.063	.126	.042	NS
1	R.I.	W.C.(Bottom)	0.067	.227	.043	NS
2	R.I.	W.C.(Bottom)	0.699	.127	.623	**
3	R.I.	W.C.(Bottom)	0.131	.117	.160	NS
Av.	R.to 1cm	W.Pot	-0.046	.010	-.334	**
Av.	R.to 1cm	W.C.(top)	0.033	.017	.159	*
Av.	R.to 1cm	W.C.(Bottom)	0.053	.013	.331	**
1	R.gr.1cm	W.Pot	-0.192	.048	-.502	**
2	R.gr.1cm	W.Pot	-0.104	.040	-.353	*
3	R.gr.1cm	W.Pot	-0.022	.007	-.393	**
1	R.gr.1cm	W.C.(Top)	0.197	.060	.427	**
2	R.gr.1cm	W.C.(Top)	0.163	.069	.322	*
3	R.gr.1cm	W.C.(Top)	0.025	.013	.272	NS

Table 7 contd.

Clone	Y Variable	X Variable	Regression	Standard Error	Correlation	Significance
1	R.gr.1cm	W.C.(btm)	0.226	.065	.450	**
2	R.gr.1cm	W.C.(btm)	0.090	.035	.344	*
3	R.gr.1cm	W.C.(btm)	0.026	.012	.291	*
Av.	T.R.No	W.Pot	-2.801	.408	-.494	**
Av.	T.R.No	W.C.(top)	1.300	.705	.151	NS
Av.	T.R.No	W.C.(Brm)	2.400	.509	.363	**
1.	T.R.L.	W.Pot	-14.267	3.447	-.513	**
2.	T.R.L.	W.Pot	- 7.219	2.426	-.395	**
3.	T.R.L.	W.Pot	- 5.742	1.304	-.537	**
Av.	T.R.L.	W.C.(Top)	11.219	2.173	.393	**
1	T.R.L.	W.C.(Btm)	18.672	4.524	.512	**
2	T.R.L.	W.C.(Btm)	6.838	2.118	.422	**
3	T.R.L.	W.C.(Btm)	6.604	2.273	.387	**

Key to Abbreviations

Clone

Clone 1 is I-78

Clone 2 is P.yunnanensis

Clone 3 is P.angulata

Av. is the average regression over all cuttings disregarding clone groups, and is used when the differences between clones are not significant.

Y Variable

S.G. is shoot growth (cm)

R.I. is the number of root initials

R. to 1 cm is the number of roots between 0 and 1 cm.

R. gr. 1cm is the number of roots longer than 1 cm.

T.R. No. is the total number of roots and root initials.

T.R.L. is the total root length for each cutting.

X Variable

W.Pot. is the water potential determination made at the same time as the corresponding growth measurement.

W.C.(top) is the corresponding water content determination, made at the top of the cutting.

W.C.(bottom) is the corresponding water content determination, made at the bottom of the cutting.

At growth initiation, correlations tended to be higher with those growth measurements of characters which had developed more, viz. shoot growth of I-78 and root initiation of I-78 and P.yunnanensis. Bottom water content provided the highest correlations in general, followed by measurements of water potential.

At termination of the experiment, measurements of shoot growth and total root length per cutting were highly correlated with both top and bottom water content and with water potential. There was some variation in the degree of correlation between numbers in the three root classes and top and bottom water content, although those with water potential were all highly significant. Total root numbers was highly correlated with water potential (all three clones), top water content (I-78 only), and bottom water content (I-78 and P.yunnanensis only). There was a much stronger relation between the division of total roots into basal roots and side roots and water potential than between the same division and top or bottom water content.

CHAPTER - FOUR

4.1. Seasonal Changes in Water Status

At the onset of dormancy while leaves are still present on the stools, a gradient of water content is maintained, being higher at the distal end of the wands. This is probably a reflection of the ability of the upper regions to mobilise water which is available in the wand, since leaf fall commences in the lower regions first. The completion of leaf fall coincides within minimum levels of water content, probably under the vestigial effects of the transpiration stream associated with the last leaves. The largest gradient is seen before leaf fall in P. angulata, which also has very large leaves in comparison to I-78 and P. yunnanensis and may reflect transpiration rates.

A slow rise in water content over the dormant period in the field is accompanied by a disappearance of the gradient along the wands in the case of I-78 and P. yunnanensis, and a decline in the size of the gradient in the case of P. angulata. In each clone the major change is in the basal region, indicating that the movement of water is from the stool and/or root region upwards.

In the actively transpiring plant, the main forces of water uptake are transpirational; in the dormant plant these forces are not present, and movement of water into the wand is likely to be in the form of a slow redistribution of water from cell to cell in response to local gradients of water potential, after the manner suggested by Weatherly (1963) for water movement in root cell walls. The source of water could be either from root reserves (which may be substantial in the case of a plant maintained as a stool since the root/shoot ratio is very large) or from root uptake, which may take place even in the dormant plant on a much reduced scale.

The magnitude of water potentials and the nature of the gradients within the plant at the onset of dormancy may reflect the residual effects of transpiration at leaf-fall. After leaf-fall, the plant is affected much less by environmental changes and metabolic processes since growth and transpiration are no longer taking place, and water potentials of the dormant tissues are in turn likely to be well insulated in a comparatively closed system. In the clone P. angulata the gradient of water content observed at the outset of dormancy (i.e., when leaf-fall was complete) was higher than I-78 or P. yunnanensis; further, the gradient of water content observed at the onset of dormancy in this clone was not eliminated entirely before the time of bud-break, although the magnitude of the gradient

was reduced. P. angulata develops leaves which are considerably larger than those of I-78 and P. yunnanensis; if size of leaf can be correlated with transpiration rate the larger leaves may have resulted in a larger residual water potential at leaf-fall, which in this clone might not be corrected by a slow water uptake or redistribution. Certainly the lowest water contents were observed at the top of the wands, which were also the sites of the last leaves to fall. Two additional explanations may have some validity: firstly that there may be higher internal resistances to water movement on a cellular level in P. angulata, and secondly, that the comparatively large buds may have a rather larger effect on water consumption and loss.

P. yunnanensis displayed an inversion of the gradient in water content at the time of bud-break, in comparison to the gradient observed at the onset of dormancy. There was a corresponding change in water potential at the corresponding times, in that water potential tended to become less negative at higher levels of hydration. This effect would be expected, assuming little change in solute content, and may reflect the sites of water use since apical regions are the last to lose their leaves at the onset of dormancy. Further, bud-break appeared to commence first in the lower regions. A site showing a higher water content but a less negative water potential than other regions probably is an indication of a site of water mobilisation.

By the time maximum water potentials had been reached, most of the increase in water content recovery had been attained in all clones. Subsequent decline in water potential may be under the influence of increasing bud metabolic activity which may be associated with a change in solute concentration, although there are no obvious changes in the bud until the phenomenon of bud-break commences.

4.2 The Effect of Relative Humidity on Water Loss

There are four possible sites of water loss in cuttings: the cut ends, the bud or buds, lenticels and the remaining bark.

Loss through the cut ends was shown to be the most significant factor in the size of cutting used. However, the proportion of water loss via this route will clearly be related to the length of cutting. Cuttings of a significant length can be expected to develop a gradient from centre to cut end; the distal cut end is thus likely to affect the water status of the bud to a greater extent than the average water content of the entire cutting would indicate, since the bud was always close to this end. It is suggested that the use of short cuttings as in this experiment is more meaningful in

terms of effect on the bud than the use of longer material, since there is less opportunity for large gradients of water content or water potential to develop.

Sealed cuttings of I-78 increased significantly in water content by 0.146% in a relative humidity of 100% in the first 48 hours, and lost 0.279% in a relative humidity of 93.3%. The corresponding gain and loss for P. yunnanensis was 0.062% and 0.580%, and in the clone P. angulata there were losses in both relative humidities of 0.049% and 1.679% respectively (Table 1). Thus in the clone I-78, the loss at 93.3% R.H. was about twice the loss at 100% R.H., and about nine times the loss in P. yunnanensis. The clone P. angulata also gained in the second 24 hours only, about nine times as much at 100% R.H. as the loss in the second 24 hours at 93.3% R.H., but not in the first 24 hours.

At 100% R.H., the gradient between the tissue of the cutting and the bulk air surrounding the cutting can only have originated on the parent stools, and was measured there to be of the order of -2 bar. However, Schüll (1939) reported that the "Diffusion Pressure Deficit" of air at 90% R.H. is 140.5 atmospheres at 20°C., i.e. the water potential is about -138.7 bar. Thus in spite of a very large gradient between the cutting and bulk air at 93.3% R.H., the loss of water was not proportional to the gain of water which took place under the influence of the small gradient between the cutting and bulk air at 100% R.H.

This anomaly could be explained in terms of changes of permeability of the lenticels. Cutter (1971) describes lenticels as regions in which the "phellogen forms a mass of loosely arranged, unsuberised cells with many intercellular spaces." It is conceivable that such a structure could undergo a significant change in cellular aggregation and hence resistance to water flow. In particular, a low relative humidity may cause a partial collapse of the tissue and a rise in the resistance to water flow. A supplementary experiment would be required to establish if the lenticels were the major site of water loss.

Alternatively, this result may indicate that there are significant gradients in relative humidity between the air-cutting interface and the bulk air in equilibrium with the saturated solution or chemical; such a gradient would reduce the transfer of water from cutting to air, but in a relative humidity of 100% with water transfer from air to cutting such a gradient would not exist.

A third factor may be the accumulation of solutes at the evaporating surface, leading to a low local water potential. The resulting gradient between the interface of evaporating surface and air, and the interior of the cutting would be analogous to the postulated gradient in relative humidity mentioned

above, and would similarly restrict water flow out of the cutting.

Comparison of the figures for sealed and unsealed cuttings show that the loss of water from cut ends and from the rhytidome followed a similar slow drift, without marked changes at particular levels of loss. This tends to suggest that water loss from both these areas is a physical process, unmediated by a physiological mechanism which would normally be affected by limiting levels of water stress.

The loss of water from cuttings with sealed ends is substantial at relative humidities lower than 100%, and are sufficient to produce a limiting water status within a short time.

If the changes in water content seen in this experiment are related to the regressions of water content on water potential derived in the experiment on the effect of water status on growth, a fall of about 3% in the dormant cutting or 0.5% in the actively growing cutting would be sufficient to decrease the water potential within the cutting to between -2 and -6 bar, at which stage growth would be severely inhibited, according to the growth experiment. A loss of 3% in water content corresponds to the loss over three days in unsealed cuttings and approximately seven days for sealed cuttings at a relative humidity of 93.3%; for a loss of 0.5% in sealed cuttings at 93.3% relative humidity less than a day would be required.

Under field conditions two factors may extend these times significantly. On the parent stools, it has been suggested earlier (Section 4.1) that root uptake or redistribution of water reserves even in dormancy or the post dormant stage before growth recommences may be capable of substantial water replenishment in the attached wands. Further, in both attached wands and in cuttings, diurnal variation in relative humidity, possibly allied with precipitation and dew-fall may result in the uptake of significant amounts of water, or the substantial reduction of the effects of prolonged low relative humidity.

The third-order interaction between bud, relative humidity and clone did not reach significant levels, although the second-order interactions of bud/clone and bud/relative humidity were significant. However, there were consistent trends in the third-order interaction within each clone (Section 3.2; Fig. 4). Presence of a bud at higher relative humidities (other than 100%) generally resulted in a higher cutting water content than in a bud-less cutting, apparently indicating that the bud tended to cause water to be retained in the cutting. Further, the bud appeared to have influenced the water status of tissue other than in the bud itself, as follows:
Assuming the water content of both wand and bud at the start of the experiment was of the order of 50% (confirmed in supplementary bud measurements) the amount of water held in the bud would approximately equal the bud dry weight.

The average bud dry weight of I-78 was 0.010 grams, that of P. yunnanensis was 0.034 grams and that of P. angulata was 0.047 grams, and these weights are assumed to approximate the actual water content in grams. However, the maximum effect of the presence of a bud on the water content was 3.40%, 0.97% and 1.71% respectively for these three clones at R.H. 80.5%, corresponding to 0.084, 0.021 and 0.028 grams of water respectively. Thus in the clones I-78 and P. yunnanensis the presence of a bud resulted in retention of a quantity of water considerably in excess of the total water contained in the bud, suggesting an effect on tissue other than that within the bud itself. In the clone P. angulata, the effect of the bud does not appear to be as large, and this may be related to the retention of seasonal gradients within wands of this clone, since the buds may not be such significant sinks in this clone.

At the commencement of this experiment the parent stools had experienced the cold requirement for the completion of dormancy, and the recommencement of growth had been shown in other sections of this study to depend only on a rise in temperature. The temperature used in this experiment (20.0°C) was shown to be adequate for the recommencement of growth. At higher relative humidities the presence of a bud on the cuttings had little effect in the first 24 hours, but after this period the effect of presence of buds became significant. The 24-hour delay suggests that this is the period of time taken by the bud to establish a higher rate of metabolic activity in the higher temperature regime. Such an increase can be expected to result in an increase in solutes as cell reserves are mobilised, leading to an increase in cell osmotic potential and thus a fall in cell water potential. Thus there may be a direct effect of increase in the bud metabolic rate and the effect of the bud on retention of water in the cutting.

At relative humidities closer to zero, there was a consistent trend which appeared after the first 24 hours indicating that the presence of a bud contributed to a decline in water content, presumably because of the loss of water associated with the bud. At zero relative humidity this effect was seen in all clones, but it became less marked with the overall fall in water content and reversed completely in the case of I-78.

The reduction in the effect of the presence of the bud at 0% R.H. with time may be due to three factors: firstly, as the total water content declines the proportional effect of the amount of bud water still present will become very small; secondly, the bud water may be lost more rapidly through the disrupted bud scales than through the rhytidome and lenticels; and thirdly, the dry weight will not remain constant with time, but is likely to fall with a consequent upward drift of the water content determination.
Dry weight losses can be expected to be larger in meristematic and juvenile

tissue such as is in the bud, in comparison with the mature tissues found in the stem; this difference would result in an apparent dimunition in the effect of bud presence.

The effect of bud presence in lowering water content still further at low relative humidities was least in I-78 and greatest in P. angulata. Further, the average respective dry weights of buds of I-78, P. yunnanensis and P. angulata were 0.0515, 0.1715 and 0.2356 g., suggesting that clonal differences may involve bud size and thus the amount of water held in the bud. However, as in the effect of bud presence noted earlier for higher relative humidities, there is insufficient total bud water to account for all the bud effect at low relative humidities, and the bud appear to have influenced the water status of tissue close to the bud.

At a relative humidity of 55.5%, the presence of buds in P. yunnanensis and P. angulata cuttings resulted in a similar effect to that at zero relative humidity. However, I-78 cuttings with buds showed a slightly higher water content, suggesting that this clone may be more capable of retaining water, perhaps as a result of bud size and bud scale structure, or as an inherent metabolic capability.

4.3 The Effect of Exogenous Auxin on Growth.

This experiment was intended to be of limited extent and interpretation, with the primary aim of indicating whether there may have been clonal differences in inherent auxin levels, with a possible indication of a practical technique for improving the ability of P. angulata to propagate vegetatively. The following limitations are recognised:

(i) That only one hormone has been used, and that there is no way of establishing the interaction with other hormones.

(ii) That no assay of the final levels of auxin within the cutting (which would include both inherent levels already present in the cutting at the start of the experiment and absorbed exogenous auxin) was undertaken. Such an assay is technically difficult and would require consideration of the ratio of bound to free auxin, rates of metabolic supply and rates of deactivation. This was beyond the scope of this study, and therefore a relationship between particular levels of auxin and growth form and rates could not be made.

In the clones I-78 and P. yunnanensis the appearance of root initials in the field tends to be high and adequate, thus permitting early dependence on the root system. However, P. angulata does not appear to develop all the root primordia which are present initially, and this investigation was intended to establish if the rate of appearance of root initials was limited by low

endogenous levels of auxin. Low levels of auxin may be due either to limited production in the bud or shoot, or to rapid inactivation in P. angulata, so that root growth does not reflect the rate of production of auxin. Since the movement of auxin is polar, shoot growth will not be directly influenced by an increase in shoot auxin from absorbed exogenous auxin, but rather indirectly from the effects of decreased or enhanced root growth.

In both I-78 and P. angulata there was a massive increase in the number of root initials appearing in the highest concentration of auxin in the growth medium. Further, in the highest concentration of auxin the number of root initials plus roots in the clone P. angulata became comparable with the corresponding figure for I-78 and P. yunnanensis in the control solutions (i.e. nil concentration of auxin). This result suggests that the capacity of P. angulata to develop root primordia into observable root initials and thence into roots may be inhibited by inherently low auxin levels, since the capacity is increased to that of I-78 and P. yunnanensis by the additional supply of exogenous auxin.

In the clone I-78, the number of roots plus root initials was high in the highest concentration of auxin, but the dry weight production of roots was lower than that of cuttings in lower concentrations of auxin, suggesting that the concentration of auxin was sufficiently high to limit the growth of roots. It is unlikely, however, that this decrease in root growth rates could be the basis for the decreased shoot growth which also took place in I-78 at the highest concentration of auxin, since the root growth was still substantial and water deficits would be unlikely to develop under these conditions. Mineral supply could not be a factor since the growth medium had no added nutrients; further, the mineral reserves in the cutting are substantial. It is suggested that the high concentration of exogenous auxin may interfere with normal mobilisation of reserves, and it is postulated that there may be a formation of new sinks within the cutting in a manner analogous to the theory of Cardenas et al (1968). These workers showed that 2,4-D appears to induce new sinks of metabolic activity when placed as a drop on a leaf. If the lenticel is a site of absorbtion of exogenous auxin, this postulate could further explain the appearance of cell proliferation in the regions of the lenticels (Plate 2).

The clone P. yunnanensis failed to show any consistent reaction to any concentration of auxin. This may indicate that the clone has high natural levels of endogenous auxin possibly coupled with a tolerance to concentrations in excess of the optimum for growth which may result from an efficient mechanism for de-activation.

The generally low levels of root growth in P. yunnanensis may be due to the early shoot growth and the proliferation of undifferentiated tissue in

the regions of the lenticels. The latter may have a direct effect in exhausting reserves, or may interfere in the development of the root initials. The cause of the cell proliferation was not established.

There was little effect of exogenous auxin on shoot growth within the growth period used, except at the highest concentrations in the clone I-78 where a depression was seen. This may further indicate that levels of auxin are naturally high in I-78, and that additional auxin at the maximum concentration of solution used may become excessively high for optimum shoot extention.

Shoot dry weights show similar trends to those seen in shoot length measurements, except that a depression at the highest concentration is also seen in P. angulata.

4.4. The Effect of Light on Growth.

In the clones I-78 and P. yunnanensis both shoots and roots were produced on every cutting, and it is clear that light is not a factor in budbreak or root initiation in these clones. In the substitute clone 'Aust. 135' there were some failures in all treatments, but only in bud-break in the dark was there a significant reduction, to 75%. This is not, of course, of significance in the field. P. angulata was not included in this investigation, but bud-break was not limited in this clone at high water potential treatments in the experiment relating water status to growth. It can be assumed that none of the three clones used were limited by the light regime in their ability to commence growth.

There was inhibition of leaf development in all three clones and etiolation of the shoots of I-78 and P. yunnanensis in the dark treatment, but 'Aust 135' shoots did not grow as long in the dark as in the light. All three clones developed greater shoot dry weights in both light treatments than in the dark. Thus the relative growth rates of all three clones were of the same order, and the lack of elongation of 'Aust 135' in the dark compared with the growth in the light suggests that auxin levels may be low in this clone. Thus limitation of elongation rates in the light through destruction or inhibition of auxin by light may not be as significant as in I-78 and P. yunnanensis.

The ultimate number of root initials plus roots was similar for all treatments in each clone. However, early development of root initials of I-78 and 'Aust. 135' were enhanced by the dark treatment, but those of P. yunnanensis were retarded, when compared to growth in the light treatments. This was reflected in the advanced root growth in the former clones when grown in the dark. It appears that both I-78 and 'Aust. 135' in the early

stages of growth in the light have buds or shoots which may be able to preferentially mobilise reserves and dominate the use of photosynthetic products at the expense of early root development.
This may be the result of light incident in the root region, or reflect the ability of buds or shoots to preferentially mobilise reserves and dominate the use of photosynthetic products at the expense of early root development. However, P. yunnanensis appears to be capable of supporting both shoot and root growth, presumably either by tolerance of light in the root region or the ability to produce sufficient photosynthates to support both shoot and root growth. The non-appearance of root initials in P. yunnanensis at the first observation of growth may have been a reflection of the large number which ultimately developed, since early development may have been inhibited by the heavy drain on reserves before photosynthetic tissue was developed significantly.

4.5 The Effect of Water Stress on Bud-break and Root Initiation

The water potential of the cutting was closely related to the osmotic potential of the growth medium and the length of time the cutting was in contact with the growth medium. Until growth initiation there was a trend within the cutting to equilibrate internal water potential with the osmotic potential of the medium. However, cuttings grown in solutions which permitted bud activity developed a transpiration stream, and this was reflected in a lower water potential in those cuttings.

Measurements of water content, while more variable than those of water potential, showed marked trends also. Similar trends were followed in both top and bottom measurements, but those taken at the bottom of the cutting showed larger differences. In general, water content rose in the lowest concentrations of osmoticum, and in the highest concentrations water content fell. In the growth medium where there was no change in the water content (i.e. the compensation point) the water potential of the cutting as measured by subsequent destructive sampling approximated the osmotic potential of the growth medium.

A highly significant relationship has been demonstrated between the internal water status of the cutting and the osmotic potential of the growth medium. A further relationship between internal water status and shoot initiation, growth, length and weight, also root initiation, numbers, length and weight was found to be highly significant. Thus there is a clear relation between an applied water stress in the form of an osmoticum and growth initiation and early growth.

At the commencement of shoot growth, initial rates of elongation were

correlated most highly with water content at the base of the cutting, and almost as well with water potential, but rather less closely with water content at the top of the cutting. However, final measurements of growth were highly correlated with both measurements of water content and with the measurement of water potential. At the final measurements of water status the trends which had been seen earlier at the stage of growth initiation had become more highly correlated.

The particular levels of water potential which first inhibit growth were clearly defined. At two days after growth initiation, the number of cuttings producing shoots or producing root initials fell drastically at osmotic potentials of -8 bar or greater in the growth medium in all three clones. At fourteen days, shoots had also been produced in osmotica of -11 bar in P. yunnanensis and root initials were seen on most cuttings of I-78. However, development of root initials into roots was inhibited in solutions of osmotic potential greater than -3 bar in all three clones. Thus the formation of root initials was relatively unaffected by low external osmotic potentials, but once the root initials developed sufficiently to be affected significantly by the growth medium, subsequent root development became limited by comparatively low osmotic potentials. The capacity of roots to function in the particular root environment used may be limited by the lack of ions since minerals were not included in the growth medium. Compensation of the osmotic potential of the growth medium by increasing the ionic concentration of the root cortex therefore could not take place.

Early shoot growth was also found to be relatively sensitive to small water potential differences, although shoot growth could be initiated in higher osmotic potentials. This may reflect the limited ability of the cutting to buffer the effects of the root environment, and is probably reflected in the declining water potentials measured in cuttings kept in the higher osmotic potentials.

Water potential within cuttings of I-78 in a growth medium of -2 bar consistently showed lower values than those developed in a growth medium of -1 bar. While P. yunnanensis appeared to follow this trend to some extent, P. angulata showed no evidence of it. Similar results have been found by Drew (1967 a, b, c) who found that mineral-free water tended to reduce growth rates compared to small osmotic potentials in solutions produced using minerals, and McWilliam et al (1970) found a similar result with the germination rates of several species of grasses and legumes using PEG 20,000. Milthorpe and Ivins (1966) note that the most favourable water potential for early growth in fruit trees is nearer to -1 bar than at full hydration. If this was also the case in the clone I-78 and possibly P. yunnanensis, a growth-medium osmotic potential of -2 bar may have been more favourable to shoot and root growth than

a potential of -1 bar. In both these clones (but not in P. angulata) the water potential measured in the cutting showed a similar trough, i.e. the water potential of cuttings grown in a medium of -2 bar was higher than that of cuttings in -1 bar. The greater growth rates could be expected to result in greater water use and this appears to occur with a concomitant fall in water potential in the cutting. This fall may be a result of growth, mobilisation of nutrients and an increase in solute concentration which would affect the osmotic potential of the plant cell contents.

However, there is no obvious reason why growth should be increased by small water potentials. Although many workers equilibrate maximum growth with maximum hydration (Kramer, 1969) it appears that in some situations including those seen in this study such a statement requires qualification.

Some of the measurements of other growth parameters confirm the effect of enhanced growth at small osmotic potentials of the growth medium. In particular, measurements of shoot growth in the first 8 days showed a corresponding trough at -2 bar in the clone I-78 but not in the clones P. yunnanensis or P. angulata; further, the number of roots produced by both I-78 and P. yunnanensis was greater at -1 bar osmotic potential in the growth medium than at zero or -2 bar although this effect was not seen in P. angulata.

The effect may be present only in the initial stages of growth in the shoot, since the trough seen in the shoot growth of I-78 at -2 bar disappeared after the 8th day of growth, and there was no apparent effect on shoot dry weight. However, it may be more persistant in the effect on roots, since it is apparent in all the relevant stages of root growth in I-78 and P. yunnanensis, but not in P. angulata. In the measurements of root dry weights at termination of the experiment P. yunnanensis showed greater growth at -1 bar than zero growth medium osmotic potential, and I-78 showed a small increase at -2 bar over dry weight at -1 bar and zero osmotic potential.

The disappearance of the promotive effects of small osmotic potentials in the growth medium after 8 days in I-78 may be a reflection of the increasing effect of photosynthesis, which (according to the investigation into the effect of light - Section 3.3)rapidly becomes a dominant influence on growth rates. Heath (1969) has noted that photosynthesis as such is not affected by water stress at such low levels as are other physiological processes, and growth resulting from photosynthesis is less likely to be influenced as much as growth resulting from mobilisation of internal reserves. This can be expected to hold for the species of poplar used in this study. Heath also notes that export from the leaves does not take place until leaf expansion is well advanced, and then the growing point tends to dominate nutrient

(photosynthate) mobilisation. Thus in the initial stages of bud activity and shoot growth, root growth may be the result of mobilisation of reserves close to the root initial and not the products of photosynthesis. Since photosynthates are less likely to be available for root growth in the early stages of growth, the promotive effects of small osmotic potentials in the growth medium may tend to be retained for a longer period, and this agrees with the observed changes.

It is apparent that the water status of the stools under study is not likely to be a factor limiting the recommencement of growth in the field. The levels of water potential through the dormant period under study until growth recommenced did not reach values which were observed to prevent recommencement of growth of cuttings. As would be expected for established stools, the recommencement of growth appeared to be a function of rising temperature rather than limiting water stress.

The lowest value of water potential observed using samples taken from the stools was in the final measurement of P. angulata, which was -5.2 bar. This was sufficient to severely limit growth rates of cuttings with corresponding internal values but not to prevent growth. Once growth has recommenced on the stool however, measurements taken of inactive material cannot be related directly to conditions of active transpiration. The final measurement of P. angulata was taken as budbreak commenced, and may have been influenced by the commencement of such a transpiration stream.

CHAPTER 5 - CONCLUSIONS

1. Seasonal levels of water content was at a minimum at leaf-fall and at this time showed a gradient of water content within the wand, the distal region being higher. This gradient slowly decreased or disappeared as the water content slowly rose over the dormant period. There was an apparent redistribution of water from the region of the stool and/or roots.

2. Seasonal levels of water potential rose slowly until mid-winter and then fell slowly. Gradients of water potential within wands were small and rarely significant. In the dormant wand, levels were not low enough to limit growth initiation, according to results from the study on growth response to osmotic potentials of the growth medium.

3. Within the limits of the experiment, maximum potential for recommencement of growth after a cold period is achieved early in the dormant period, at least in I-78 and P. yunnanensis.

4. Rates of water loss from cuttings were clearly related to relative humidity of the atmosphere, and could be considerably reduced by sealing the cut ends. There were no significant differences in the rates of water loss between clones. The presence of a bud on the cutting reduced the rate of water loss slightly at high relative humidities, but caused a slightly greater loss at lower relative humidities. The bud appeared capable of modifying the water status of associated tissue to a limited extent. Clonal differences in this effect appeared to be related to the size of bud.

The magnitude of water loss under the conditions of this particular investigation was comparatively large and sufficient to produce a water stress large enough to limit recommencement of growth in a comparatively short time.

5. The limited investigation into the effect of exogenous auxin on growth showed that in the highest auxin concentration used (100.0 mg/l), levels of root initiation in P. angulata were increased to a level comparable with I-78 and P. yunnanensis in the control or at lower concentrations. I-78 also showed greater root initiation at the highest concentration, as well as a depression in shoot growth. The low rooting capacity of P. angulata may be due to low endogenous levels of auxin.

6. Light was not a factor in budbreak or root initiation in I-78 or P. yunnanensis, and this was confirmed for P. angulata in the investigation of the effect of water stress on growth. A substitute clone in the investigation into the effect of light ('Aust 135') was affected adversely by continuous dark. The rate of appearance of root initials varied with clone and there was evidence of clonal differences in the use of reserve nutrients and (after significant growth in the light treatments) photosynthetic products.

7. In the study relating growth initiation to osmotic potential of

of the growth medium, there were close relationships between water status of the cutting and osmotic potential of the growth medium, and between water content and water potential of the cutting after particular time periods.

8. Growth initiation and early growth was related significantly to water status of the cutting and osmotic potential of the growth medium. Root initials appeared in osmotica of considerable concentration, but root growth was limited by much lower concentrations. Similarly bud movement was seen in higher osmotic potentials, but growth of shoots was limited by much lower osmotic potentials of the growth medium.

9. There was a general relationship between water potential within the cutting and growth of the cutting. However, growth in media of low osmotic potentials was slightly greater than growth in zero osmotic potential, in I-78, and to a lesser extent in P.yunnanensis; this was repeated in the corresponding internal water potentials of the cuttings.

10. The water content of both roots and shoots grown in particular osmotic potentials were related to those osmotic potentials, i.e. the greater the osmotic potential, the lower the water content.

11. The results of this study indicate that growth initiation in Populus can be limited by comparatively small water deficits in the growth media. Under field conditions, similar limitations could be expected. The methods developed in this study are applicable to evaluation of techniques of propagation in the field, and can be used for comparisons of ability to withstand drought between clones.

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APPENDIX 1.

1. Characteristics of the genus Populus

The genus Salix and the genus Populus comprise the family Salicaceae, of the order Salicales, of the group Amentiflorae (Anon., 1958). The order Salicales is characteristically dioecious, with capsular fruit opening by valves. The Salicaceae are typically light demanding, and grow naturally in low-lying areas, being tolerant of mild winter flooding but not of stagnant water, or peaty or acid soils.

The genus Populus has five sections:

1. Section Turanga Bge

Greyish leather polymorphic leaves, buds downy, male flowers 8-12 stamen, female flowers 3 stigmas. Distribution - Mediterranean, Central and Western Asia. Populus euphratica is regionally important, being tolerant of arid conditions when soil water is adequate, and being very light and heat demanding.

2. Section Leuce Duby

Leaves round, ovate, sometimes lobed, bark remains smooth, pale and of grey-green appearance for some time. There are two sub-sections:

1. Albidae

The white and grey poplars. Characteristically showing white or pale down on the underside of the leaf. P. alba L. is the true white poplar, found naturally in hot dry regions associated with water. Suckering is prolific. The 'silver poplar' common in New Zealand is P. alba var 'bolleana'. P. canescens - the grey poplar - is probably a hybrid of P. alba and P. tremula. The species is very adaptable to soil conditions and very drought resistant, but cutting propagation is variable.

2. Trepidae - The Aspens.

An extremely high light requirement, high suckering rate and tolerant of soil type, but are short-lived, shallow-rooted and difficult to root from cuttings. P. tremula is wide spread in Europe, and P. tremuloides in North America.

3. Section Aigeiros - The Black Poplars.

There are two divisions of the section: P. nigra (Eurasian) and P. deltoides (North America). P. deltoides was introduced and crossed with P. nigra in Europe, and the resulting large number of clones are widely

used. The Black poplars are extremely light demanding and require a good, deep damp soil which is not acid. Vegetative reproduction is very easy.

Of the natural varieties of P. nigra, only P. nigra 'italica' is widely distributed in New Zealand. It is of fastigiate habit, and its adaptability and ease of propagation led to wide early use.

P. deltoides has three natural subspecies distributed thus: spp. Angulata (Southern); spp. missouriensis (Central) and spp. monolifera (Northern).

The hybrids between P. nigra and P. deltoides are known as P. euramericana The nomenclature for naming particular clones is of the form P. euramericana (Dode) Guinier cv. 'serotina' and is normally abbreviated as P. X 'serotina'.

Those hybrids of importance in New Zealand are P. X'I-30', P. X'I-78', P. X 'I-214', P. X 'I-455' (the Italian hybrids) and P. X 'robusta'. Most local bodies are now using these clones in soil conservation programmes rather than the Lombardy poplar (P. nigra 'italica').

4. Section Tacamahaca - The Balsam Poplars.

Environmental requirements of the Balsam poplars are similar to those of the Black poplars, but most Balsam poplars are susceptible to many diseases and plant and insect parasites.

P. yunnanensis is an Asian clone which is now actively encouraged in New Zealand. It has a tall growth habit and rapid growth rate, requiring a hot climate. It appears to be less attractive to opossum.

P. trichocarpa and P. tacamahaca are distributed naturally in the west of North America. However, although growth is good in this area (especially P. trichocarpa), cultivation is not promising outside its area of natural distribution.

The hybrids between P. trichocarpa and P. deltoides spp. 'Angulata' are known as P. 'generosa'. All are susceptible to fungal disease, but have high initial growth rates.

5. Section Leucoides

Mainly distributed in the Far East, species of this section are not of economic importance in New Zealand.

APPENDIX 2.

Details of the Clones Used in Experimentation.

1. Populus euramerican (Dode) Guinier cv. 'I-78'
(commonly known as 'I-78').

This clone, one of the so-called Italian hybrids, was selected by the Poplar Research Station at Casale Monferrato, Italy and released by that institution in 1941. Although the parents are not recorded, there is a close affinity with P. deltoides spp. 'Angulata'.

The New Zealand Forest Research Institute introduced the clone to New Zealand from the British Forestry Commission in 1951, and following evaluation has been widely distributed for soil conservation use by local catchment authorities and farmers. In 1967, I-78 accounted for 14.2% of the total rooted stock production of 418,300 trees, and 19.8% of the total stools maintained for pole production '232,000 stools). Only P. nigra 'italica' (Lombardy Poplar) and P. nigra cv. 'sempervirens' (Semi-evergreen Poplar) was in greater use.

Use of I-78 in Europe is declining, due to a serious susceptibility to Marssonina disease, which is not recorded in New Zealand.

Propagation of I-78 as cuttings and poles is easily carried out, and the root system is vigorous.

The date of leaf appearance is the same as for Lombardy poplar (or several days before), i.e. in late September, and leaf fall is completed by the end of April. As with most poplars, leaf form varies with age, growing conditions and shoot vigour. Leaf fall in 1970 was complete by 14th April.

The form of the tree is straight-boled, with a heavily branched densely-leafed crown which may suffer wind damage in exposed sites. The timber is suitable for veneer, sawn timber and pulp.

Growth rates are high, and the clone is adaptable to less-fertile soils.

The clone is included in the experiment as a representative of the popular Italian hybrid clones, which are now increasingly used for soil conservation and timber production, being a species which is adaptable to site conditions and which is easily propagated.

The name of this clone is abbreviated to 'I-78' for the purposes of this study.

3. Populus yunnanensis Dode
(commonly known as the Yunnan poplar)

The Yunnan poplar originated in the highlands of the Yunnan province in South China, and a plant was first sent out of that country to Paris in 1905. Although propagated in Europe, its use has been limited by frost tenderness. New Zealand importation took place between 1925 and 1935 by persons unknown, and its use has increased steadily since then in all but the colder regions of the South Island. It is the sole member of the section Tacamahaca (Balsam Poplars) to be recommended for soil conservation use. Some 3.2% of rooted stock and 12.6% of stool production in 1967 was of this clone.

The Yunnan Poplar is noted for relative unpalatability of the foliage to opossum, although the top-shoots may be attacked in late winter. It is adaptable to many soil conditions, and the growth rate is rapid, extending through a very long season from leaf initiation in mid-October (14-21 days after Lombardy poplar) until leaf fall in mid-June. In 1970, leaf fall was two-thirds complete by 16th June, and complete by 7th July.

Propagation is easily carried out using cuttings or poles, and the resulting tree is large, with a rather wide crown. The timber is suitable for pulp or sawn timber, but is rather "woolly" for veneer peeling.

The New Zealand clone is male, and the female clone has not been reported, although it may exist in China.

The Yunnan Poplar was selected for experimentation as the sole representative of the Balsam poplars in New Zealand. Further, it is of interest because of its somewhat different ecological requirements of temperature and growing season, and its ability to resist water stress somewhat better than other poplars.

The name of this clone is abbreviated to 'P. yunnanensis' for the purposes of this study.

7. Populus deltoides spp. angulata Ait. cv. 'Carolinensis'
(commonly known as P. angulata 'Carolin')

This clone is one of the parents of the so-called 'Italian' hybrids, and was introduced to New Zealand between 1930 and 1940, originally from southern North America. It is not used for soil conservation or forestry in this country, but is sometimes found as an ornamental. The New Zealand clone is male.

Leaf flush commences in mid-October, and leaf fall towards the end of

May. In 1970, all leaves had fallen by 3rd June. There is a requirement for good soil conditions, a warm climate and high light levels over a long growing season.

The failure rate of cuttings and poles is comparatively high, and the established plant does not transplant well, especially when conditions are suboptimal.

P. angulata 'Carolin' propagates with difficulty, which is characteristic of many poplar species, particularly among clones of P. deltoides.

This clone was selected for experimentation to establish if this difficulty was related to a low tolerance of water stress, or if other factors were involved.

The name of this clone is abbreviated to 'P. angulata' for the purposes of this study.

4. Populus deltoides A 60/135.

This clone is used as a substitute for P. angulata in a supplementary experiment in the program to determine light requirements.

It is difficult to propagate from cuttings, being similar to P. angulata 'Carolin' in this respect.

Originally collected as seed in southern Texas, it has been selected in Australia as a poplar suitable for use in low altitudes (Pryor and Willing, 1965).

The name of this clone is abbreviated to 'Aust 135' for the purposes of this study.

Plate 4. - Stool-beds used in the Experimental Series as a source of Plant Material.

4. A - I-78

4. B - P. yunnanensis

4. C - P. angulata

A.

B.

C.

APPENDIX 3

Source of Plant Material

All plant material was obtained from the Plant Materials Centre, of the Water and Soil Division, Ministry of Works, Palmerston North, which is a national centre for the collection or import, propagation and evaluation of plant species (primarily Populus) suitable for use in soil conservation.

The nursery is situated at Massey University, beside the Tiritea Stream. The soil is a recent silty alluvium, classed as Manawatu fine sandy loam (Gilchrist and Clime, 1970). A gravel bed at about 4 to 6 feet maintains a relatively constant water table, although summer spray irrigation is still practised.

The collection of poplars and willows is maintained in the form of stoolbeds, at a spacing of 3 feet by 7 feet. Normally 10 stools of each clone are developed. Only one-year wood is grown, all growth being removed each winter.

The clones used in this study (Appendix 2) were established 8 years ago as part of a large stoolbed, and maintained as stools of 18 inches in height. Growth has stabilised to a relatively constant production, and fertilizers are applied as maintenance dressings.

There are few diseases of economic importance to poplars and willows in New Zealand (van Kraayenoord, 1968). The most important fungal disease in this nursery is Silver-leaf disease (Stereum purpureum Pers.) Culling of infected stools is practised and precautions are taken against cross-infection at harvesting. Infected stools were not used in this study. The only economically important insect affecting poplars and willows is a native wood-borer (Omoena hirta Braun), but this was not observed in the stools used in this study.

APPENDIX 4.

Pilot Experiment was set up to investigate the effects of water stress applied as a range of osmotic potentials associated with the liquid-medium culture of winter cuttings.

The osmoticum used was polyethylene glycol, of nominal molecular weight 333, which was accepted as correct for the purpose of the experiment. The clone used was Populus X'I-78', and the cutting size was 10 cm in length and between 1.0 and 1.5 cm diameter, taken from the middle third of one-year growth from an established stool-bed.

The following solutions and nominal osmotic potentials were used:

Solution No.	Concentration (gram/litre)	Nominal Osmotic Potential (bars)	Actual Osmotic Potential (based on M.W. 207 -Appendix 5) (bars)
1	0.0	0.0	0.0
2	7.0	-0.3	-0.5
3	16.0	-0.7	-1.1
4	35.9	-1.5	-2.4
5	59.8	-2.5	-4.0
6	95.7	-4.0	-6.4
7	143.52	-6.0	-9.7

In addition, a further treatment was imposed, to investigate the effect of sealing the top or bottom cuts with wax, as follows:

(i) Both ends sealed
(ii) Top end only sealed
(iii) Bottom end only sealed
(iv) Both ends left unsealed

The experiment was started on the 1st of July, 1970, and finally assessed on the 3rd of August.

Treatment	Shoot Length (cm)	No. of Root Initials	No. of Roots less than 1 cm	No. of Roots Greater than 1 cm	Total No. of Roots	No. of Basal Roots	No. of Side Roots	No. of Cuttings with Shoots	No of Cuttings with Roots or Root Initials
Nominal Osmotic Potential (bars)	AVERAGE PER CUTTING								
0.0	12.0	1.2	0.4	10.4	12.0	4.7	7.3	83%	83%
0.3	8.3	0.8	0.4	7.1	8.3	1.5	6.8	83%	83%
0.7	4.7	0.9	1.3	5.8	8.1	1.5	6.6	75%	75%
1.5	2.7	3.8	2.2	4.3	10.3	0.3	10.0	58%	75%
2.5	1.6	6.3	2.3	0.2	8.8	0.0	8.8	58%	83%
4.0	0.7	4.0	0.0	0.0	4.8	0.0	4.8	42%	50%
6.0	0.2	2.8	0.2	0.0	3.0	0.0	3.0	17%	25%
Effect of Sealing Top	+35%	+314%	+86%	+72%	+129%	+175%	+126%	+143%	+100%
Effect of Sealing Bottom	+56%	-62%	-46%	-38%	-49%	-45%	-22%	-29%	-31%
Overall Means	4.3	2.8	1.0	4.0	7.8	1.1	6.7	0.6	0.6
Sealed at both Ends	5.1	2.0	1.2	5.0	8.1	1.5	6.6	0.9	0.9
Sealed at Top Only	6.0	7.2	1.4	5.0	13.1	1.7	12.0	0.9	0.9
Sealed at Base Only	3.8	1.2	0.2	1.0	2.5	0.1	2.4	0.1	0.2
Unsealed	4.4	1.0	1.2	4.8	7.0	1.1	5.8	0.5	0.7

The results show that shoot growth is substantially decreased even by small water potentials, and is almost stopped by -9.7 bars, the highest actual osmotic potential used. The number of roots or root initials originating from root primordia already present in the cuttings was rather constant except for a marked decrease at higher levels. However, root initiation from the lower cut was strongly decreased by even small water potentials, and root growth strongly inhibited. Thus there is an apparent rise in the number of root initials and small roots at medium water stresses, but a very marked drop in longer roots with more than a minor stress. This appears to indicate that although root initials can develop when the cutting is in a solution of relatively high water stress, subsequent development of the root while in direct contact is severely limited.

The effect of sealing the top cut had a promotive effect on all phases of growth, including the number of cuttings which eventually produced shoots and roots. The effect of sealing the bottom cut was to increase shoot growth somewhat, but strongly decrease root growth and the number of cuttings which produced both shoots and roots.

Since most growth took place under conditions of low stress, the effect of sealing must be interpreted in this context. Thus the promotive influence of sealing the top appeared to be a result of higher water levels within the cutting because of lowered evaporation; the effect of sealing the bottom appeared to increase the effect of the imposed water stress, especially if the top remained unsealed. Thus root development was impaired and fewer cuttings developed roots and shoots. However there was an increase in the length of shoot growth.

APPENDIX 5

Determination of Molecular Weight of PEG by Cryoscopy.

The PEG used throughout this experimental series was a commercial grade obtained from Glaxo Laboratories (N.Z.) Ltd., Palmerston North, and had a nominal molecular weight of 333. An accurate determination was required so that precise osmotic potentials could be applied, and this was carried out using the method of cryoscopic determination of freezing point depression, comparing similar concentrations of PEG and sucrose on a basis of molecular weight.

Freezing poing depressions were measured using thermocouples of copper and constantan in a thermopile of two thermocouples. The reference junction used was ice/water, held in a vacuum flask to minimise thermal gradients. Such a junction was stable for more than three days at room temperature, and considerably longer if held in a refrigerator. The measuring junction was made as small as possible to minimise heat storage effects, and was mounted firmly to the lid of a 2 ml clear plastic container so that the junction could be centred 0.5 cm from both the base and the walls of the container when the lid was fitted. One ml of the solutions under test was used on each occasion, and this quantity filled the container to a depth of 1 cm. The lid was mounted in the centre of a larger lid of a further plastic container holding approximately 125 ml of mercury. When the outer lid was fitted, the inner container was held so that the bottom 1.5 cm was held in the mercury. The entire system was held at a temperature of $-10^{\circ}C$. and enabled the temperature of a sample to be lowered to below freezing point at a constant and reproducable rate.

The thermopile voltage was recorded on a Honeywell Model 19 single channel chart recorder. A 'range card' used to select the voltage range to be measured was modified to read to the lower limit of the recorder capacity (viz. 0.45 mV.), and the scale was checked using large volumes of stirred water at the extremes of the scale measurement.

The test sequence was as follows:

As the temperature of the solution under test fell, a continuous recording was made. The solution became super-cooled, and crystallised out within several seconds, resulting in a rapid increase in the temperature of the then frozen solution to a clearly defined plateau. This plateau was taken as the freezing point of the solution.

Solutions were made up of PEG to nominal value of 5.0 bar using a nominal molecular weight of 333, and a standard solution of sucrose was also prepared giving a standard osmotic potential of 5.0 bar.

A comparison of the freezing point depressions of the solutions was made and used to establish the error in the nominal molecular weight of PEG, since the nominal value of the prepared solution of PEG if accurate would give a freezing point depression equal to that of the sucrose solution.

Thirty-two determinations were made and analysed using a paired t-test. The results are as follows (27.0 recorder scale units = 1.0°C.):

	Recorder scale units
Mean average (PEG	21.98 (S.E. 0.36)
Mean average (sucrose)	13.66 (S.E. 0.30)
Coefficient of Variation	1.93%

$$\text{Molecular weight (PEG)} = \frac{333}{1} \cdot \frac{13.66}{21.98}$$

$$= 207.0$$

T-test of variance of difference between means (30 d.f.) = 13.71 ***

The value of 207.0 was accepted as the actual value of the PEG and was used in all subsequent experimentation.

APPENDIX 6

Seasonal Changes in Growth Potential

The methods used in determining changes in ability of I-78, P. yunnanensis and P. angulata to recommence growth during the winter when placed in a suitable temperature after cold treatment are described in section 2.3.1.

The results of this investigation were found subsequently to be confounded with the effects of the osmotic potentials used, since there was a substantial difference between the nominal molecular weight of the PEG used, and the actual value found after the investigation commenced (Appendix 5). However, the investigation was continued using the nominal value, so that there could be some basis for comparison through the season.

The results obtained are indicated below:

Sampling date	Clone	Observation	Osmotic Potential				
			1	2	3	4	5
14 Apl	A	No. of Cuttings	-	-	10	5	-
		With roots	-	-	3	1	-
		With shoot	-	-	3	2	-
	B	No. of cuttings	-	-	11	4	-
		With roots	-	-	5	2	-
		With shoots	-	-	1	-	-
	C	No. of cuttings	-	-	11	4	-
		With roots	-	-	-	-	-
		With shoots	-	-	1	-	-
5 May	A	No. of cuttings	10	5	-	-	-
		With roots	8	3	-	-	-
		With shoots	6	4	-	-	-
	B	No. of cuttings	8	6	1	-	-
		With roots	1	4	-	-	-
		With shoots	1	1	-	-	-
	C	No. of cuttings	2	6	5	2	-
		With roots	-	-	-	-	-
		With shoots	-	-	-	-	-

Sampling date	Clone	Observation	Osmotic Potential				
			1	2	3	4	5
26 May	A	No. of cuttings	-	12	3	-	-
		With roots	-	12	3	-	-
		With shoots	-	12	3	-	-
	B	No. of cuttings	-	7	8	-	-
		With roots	-	6	4	-	-
		With shoots	-	5	4	-	-
	C	No. of cuttings	-	-	15	-	-
		With roots	-	-	2	-	-
		With shoots	-	-	-	-	-
16 June	A	No. of cuttings	5	10	-	-	-
		With roots	5	10	-	-	-
		With shoots	5	8	-	-	-
	B	No. of cuttings	5	10	-	-	-
		With roots	3	3	-	-	-
		With shoots	3	3	-	-	-
	C	No. of cuttings	3	9	3	-	-
		With roots	-	-	-	-	-
		With shoots	-	-	-	-	-
7 July	A	No. of cuttings	6	9	-	-	-
		With roots	5	6	-	-	-
		With shoots	6	8	-	-	-
	B	No. of cuttings	10	5	-	-	-
		With roots	10	4	-	-	-
		With shoots	10	4	-	-	-
	C	No. of cuttings	2	11	2	-	-
		With roots	-	4	-	-	-
		With shoots	-	2	-	-	-
28 July	A	No. of cuttings	12	3	-	-	-
		With roots	12	3	-	-	-
		With shoots	10	1	-	-	-

Sampling date	Clone	Observation	Osmotic Potential. 1	2	3	4	5
	B	No. of cuttings	15	-	-	-	-
		With roots	13	-	-	-	-
		With shoots	12	-	-	-	-
	C	No. of cuttings	3	9	3	-	-
		With roots	1	-	-	-	-
		With shoots	-	-	-	-	-
18 Aug	A	No. of cuttings	1	14	-	-	-
		With roots	1	14	-	-	-
		With shoots	1	13	-	-	-
	B	No. of cuttings	3	12	-	-	-
		With roots	2	12	-	-	-
		With shoots	3	12	-	-	-
	C	No. of cuttings	-	2	8	-	5
		With roots	-	1	-	-	-
		With shoots	-	1	-	-	-

KEY

Clone: A is I-78

B is P.yunnanensis

C is P. angulata

Observation: 'No. of cuttings' i.e. the total number of cuttings derived from wands having water potential values corresponding to the osmotic potentials given above.

'With roots' i.e. the number of cuttings showing evidence of root growth in the osmotic potentials above.

'With shoots' i.e. the number of cuttings showing evidence of shoot growth in the osmotic potentials above.

Osmotic potential: i.e. the osmotic potential which corresponds to the water potential found for the wand section from which each cutting was derived.

1 is the category of osmotic potentials between 0 and 1 bar.

2 is the category of osmotic potentials between 1 and 2 bar.

3 is the category of osmotic potentials between 2 and 3 bar.

4 is the category of osmotic potentials between 3 and 4 bar.

5 is the category of osmotic potentials greater than 4 bar.

The results appear to indicate that I-78 reached full growth potential by 5th May and P. yunnanensis by 26th May. However, growth potentials for P. angulata were never observed to be high. This may be a real effect, but could also be explained in terms of the confounding effect of the nominal and actual PEG molecular weights noted above. In the latter case, the higher water potentials seen in P. angulata through the season would have a greater confounding effect on the apparent growth potential of P. angulata

APPENDIX 7

Statistical Analysis of Seasonal Water Status

Analysis of Variance of Seasonal Water Content

Source of Variation	df.	M.S. (14 Apl)		M.S. (4 May)		M.S. (25 May)		M.S. (16 June)	
C	2	864.245	**	662.125	**	183.455	**	237.935	**
W	4	116.015	**	36.675	**	19.290	**	8.218	**
P	2	520.160	**	335.890	**	194.030	**	154.255	**
CxW	8	25.653	**	34.906	**	27.295	**	38.160	**
CxP	4	48.865	**	41.643	**	9.890	**	107.910	**
WxP	8	8.733	**	11.596	**	5.745	**	2.268	NS
CxWxP	16	6.224	**	7.941	**	4.976	**	4.759	**
Within	180	2.839		3.011		1.313		1.439	
		(7 Jul)		(28 Jul)		(18 Aug)		(11 Sep)	
C	2	12.950	**	726.405	**	148.685	**	402.850	**
W	4	57.285	**	34.228	**	15.363	**	17.473	**
P	2	152.760	**	33.485	**	17.270	**	34.525	**
CxW	8	28.726	**	48.965	**	37.174	**	54.829	**
CxP	4	73.958	**	49.115	**	66.300	**	84.968	**
WxP	8	.491	NS	2.308	**	2.225	**	1.009	NS
CxWxP	16	1.734	*	2.108	**	4.528	**	6.658	**
Within	180	.946		.857		.788		.892	

KEY

C	=	Clone
W	=	Wand
P	=	Position on Wand
*	=	Significant at 5% level
**	=	Significant at 1% level
NS	=	Not Significant
Within	=	Within subgroups

Analysis of Variance of Seasonal Water Potential

	d.f.	(14 Apl)		(4 May)		(25 May)		(16 June)	
C	2	.054	NS	2.547	**	2.873	**	.963	**
W	4	.155	NS	.206	NS	.062	NS	.568	**
P	2	.565	NS	.747	NS	.056	NS	.028	NS
CW	8	.165	NS	.278	NS	.211	NS	.279	**
CP	4	1.114	**	.096	NS	.360	NS	.411	**
WP	8	.181	NS	.269	NS	.152	NS	.193	*
CWP	16	.170	NS	.221	NS	.131	NS	.057	NS
Within	16	.170		.221		.131		.057	
		(7 July)		(28 July)		(18 Aug)		(11 Sept)	
C	2	3.754	**	4.118	**	18.105	**	50.488	**
W	4	.242	*	.106	NS	1.470	*	.526	*
P	2	.091	NS	.068	NS	.358	NS	.080	NS
CW	8	.239	**	.097	NS	1.435	*	.253	NS
CP	4	.064	NS	.318	NS	.066	NS	.571	*
WP	8	.044	NS	.128	NS	.566	NS	.145	NS
CWP	16	.060	NS	.123	NS	.410	NS	.159	NS
Within		.060		.123		.410		.159	

KEY

C = Clone
W = Wand
P = Position on Wand
* = Significant at 5% level
** = Significant at 1% level
NS = Not Significant
Within= Within subgroups

Regression Analysis of Seasonal Water Potential on Water Contents

Date	Clone	b	SE	r	Significance (13 d.f.)
14 Apl	A	-0.003	0.065	-0.011	NS
	B	-0.042	0.050	-0.225	NS
	C	-0.001	0.036	-0.009	NS
4 May	A	0.046	0.065	0.193	NS
	B	0.129	0.052	0.566	*
	C	0.023	0.043	0.148	NS
25 May	A	-0.114	0.053	-0.510	NS
	B	0.039	0.096	0.111	NS
	C	0.072	0.029	0.564	NS
15 June	A	0.019	0.050	0.106	NS
	B	-0.033	0.099	-0.092	NS
	C	-0.021	0.045	-0.130	NS
6 July	A	-0.110	0.043	-0.579	*
	B	0.002	0.025	0.027	NS
	C	0.003	0.038	0.025	NS
28 July	A	-0.029	0.055	-0.147	NS
	B	0.023	0.040	0.157	NS
	C	-0.078	0.049	-0.402	NS
17 Aug	A	-0.056	0.066	-0.229	NS
	B	-0.122	0.023	-0.828	**
	C	-0.112	0.160	-0.190	NS
11 Sept	A	-0.360	0.141	-0.577	*
	B	-0.096	0.067	-0.372	NS
	C	0.014	0.019	0.196	NS

KEY

Clone: A is I-78
B is *P. yunnanensis*
C is *P. angulata*

Analysis : b is the regression coefficient
S.E. is the standard error of the regression coefficient
r is the correlation
* =significant at 5%
** = significant at 1%
NS = not significant

APPENDIX 8 Statistical Analysis of the Effect of Relative Humidity on Water Loss from Cuttings.

Analysis of Variance of Water Content at 7 Specified Times

Source of Variation	d.f.	M.S. F.W.		M.S. 24 hrs		M.S. 48 hrs	
H	4	19.168	NS	1707.363	**	4270.793	**
C	2	395.745	**	355.350	**	370.215	**
S	1	18.970	NS	3254.810	**	7009.550	**
B	1	1.010	NS	33.410	NS	7.470	NS
HxC	8	13.851	NS	12.440	NS	19.006	NS
HxS	4	6.458	NS	789.332	**	1683.848	**
HxB	4	3.685	NS	19.655	NS	16.703	NS
CxS	2	2.020	NS	9.620	NS	17.650	NS
CxB	2	8.750	NS	5.965	NS	2.750	NS
SxB	1	10.520	NS	0.300	NS	6.190	NS
HxCxS	8	12.240	NS	16.156	NS	18.596	NS
HxCxB	8	12.795	NS	16.094	NS	19.461	NS
HxSxB	4	7.010	NS	22.090	NS	28.403	*
CxSxB	2	0.620	NS	0.030	NS	2.205	NS
HxCxSxB	8	9.535	NS	9.939	NS	9.989	NS
Within	240	9.660		10.848		11.915	

		M.S. 72 hrs		M.S. 96 hrs	
H	4	7780.560	**	9816.433	**
C	2	393.075	**	353.575	**
S	1	11857.390	**	13580.190	**
B	1	0.050	NS	3.990	NS
HxC	8	26.169	*	27.574	*
HxS	4	2573.403	**	2526.393	**
HxB	4	11.090	NS	8.763	NS
CxS	2	13.410	NS	7.550	NS
CxB	2	10.710	NS	11.645	NS
SxB	1	19.540	NS	36.290	NS
HxCxS	8	17.446	NS	18.838	NS
HxCxB	8	20.066	NS	17.025	NS
HxSxB	4	21.968	NS	16.685	NS
CxSxB	2	3.885	NS	3.020	NS
HxCxSxB	8	10.523	NS	12.370	NS
Within	240	12.625		12.865	

		M.S. 120 hrs		M.S. 144 hrs	
H	4	11606.625	**	13264.702	**
C	2	317.645	**	325.305	**
S	1	14574.020	**	14301.350	**
B	1	4.160	NS	5.280	NS
HxC	8	31.668	*	35.020	*
HxS	4	2360.588	**	2034.175	**
HxB	4	14.925	NS	19.776	NS
CxS	2	4.610	NS	4.885	NS
CxB	2	17.525	NS	16.275	NS
SxB	1	35.480	NS	33.510	NS
HxCxS	8	19.569	NS	22.439	NS
HxCxB	8	17.970	NS	16.621	NS
HxSxB	4	12.918	NS	10.655	NS
CxSxB	2	2.345	NS	3.310	NS
HxCxSxB	8	11.821	NS	12.826	NS
Within	240	13.524		14.030	

See Key at end of Appendix

Analysis of Variance of Water Loss after 6 Specified Periods of Time

Source of Variation	d.f.	M.S. after 24 hrs		M.S. after 48 hrs		M.S. after 72 hrs	
H	4	1404.969	**	3780.817	**	7105.331	**
C	2	1.091	NS	2.621	NS	7.033	NS
S	1	2776.525	**	6298.701	**	10927.429	**
B	1	22.794	**	2.975	NS	0.585	NS
HxC	8	2.139	*	6.208	**	12.997	**
HxS	4	677.298	**	1519.373	**	2375.418	**
HxB	4	8.550	**	7.231	**	6.248	NS
CxS	2	10.460	**	17.817	**	14.094	**
CxB	2	0.271	NS	4.883	NS	13.250	*
SxB	1	14.383	**	0.565	NS	1.388	NS
HxCxS	8	3.931	**	7.940	**	7.441	*
HxCxB	8	0.928	NS	2.528	NS	3.629	NS
HxSxB	4	8.248	NS	12.128	NS	8.655	NS
CxSxB	2	0.695	**	1.888	**	2.675	*
HxCxSxB	8	0.397	NS	0.378	NS	0.290	NS
Within	240	0.928		1.994		2.971	

		M.S. after 96 hrs		M.S. after 120 hrs		M.S. after 144 hrs	
H	4	9033.912	**	10733.300	**	12313.332	**
C	2	8.211	NS	15.100	*	10.184	NS
S	1	12583.399	**	13540.867	**	13278.016	**
B	1	9.035	NS	9.271	NS	10.930	NS
HxC	8	15.351	**	21.781	**	23.220	**
HxS	4	2343.193	**	2198.523	**	1901.304	**
HxB	4	9.097	NS	16.450	**	23.388	**
CxS	2	11.304	NS	11.288	NS	12.750	NS
CxB	2	14.244	*	22.610	**	20.959	*
SxB	1	7.730	NS	7.379	NS	6.490	NS
HxCxS	8	8.609	*	11.029	*	18.269	**
HxCxB	8	2.257	NS	2.940	NS	2.313	NS
HxSxB	4	6.096	NS	4.087	NS	3.381	NS
CxSxB	2	2.542	NS	1.803	NS	3.112	NS
HxCxSxB	8	0.779	NS	0.652	NS	1.797	NS
Within	240	3.913		4.797		5.925	

KEY

Treatments:
H = Relative humidity treatment
C = Clone
S = Treatment of sealing ends with wax
B = Presence of a bud
Within = Within subgroups

Variables: In the first set of analyses, the mean squares are of the water content at the commencement of the experiment (F.W.) and thereafter at the times specified.

In the second set of analyses, the mean squares are of the change in water content from the water content at the commencement of the experiment until the six times specified. The measurements were cumulative.

APPENDIX 9 Statistical Analysis of the Effect of Light on Growth

Source of Variation	df	M.S. 1 S.G.		M.S. 1 R.I.		M.S. 1 S.R.		M.S. 1 L.R.	
L	2	10.357	**	100.808	**	56.575	**	105.633	**
C	2	16.708	**	2046.700	**	16.108	**	102.533	**
P	1	0.049	NS	2.336	NS	0.625	NS	3.600	NS
LxC	4	2.555	**	343.521	**	57.183	**	110.667	**
LxP	2	0.494	NS	24.620	NS	0.808	NS	1.600	NS
CxP	2	0.017	NS	12.144	NS	2.725	NS	1.733	NS
LxCxP	4	1.015	**	22.990	NS	1.008	NS	2.533	NS
Within	342	0.166		16.357		1.319		1.942	
		M.S. 2 S.G.		M.S. 2 R.I.		M.S. 2 S.R.		M.S. 2 L.R.	
L	2	38833.900	**	385.386	**	33.619	**	385.300	**
C	2	149920.000	**	3941.002	**	8.603	**	997.275	**
P	1	33.000	NS	56.010	NS	4.444	NS	4.900	NS
LxC	4	26566.475	**	445.745	**	1.024	NS	281.750	**
LxP	2	325.250	NS	45.120	NS	0.019	NS	16.634	NS
CxP	2	152.000	NS	9.120	NS	0.186	NS	89.775	**
LxCxP	4	245.600	NS	19.802	NS	1.199	NS	3.858	NS
Within	342	324.580		29.467		1.203		7.130	
		M.S. B.R.		M.S. Sd.R.		M.S. T.R.			
L	2	10.169	*	187.809	*	269.219	**		
C	2	125.603	**	8186.860	**	8189.888	**		
P	1	4.225	NS	27.225	NS	9.999	NS		
LxC	4	28.044	**	57.618	NS	121.632	*		
LxP	2	9.158	*	107.277	NS	58.561	NS		
CxP	2	6.058	NS	95.207	NS	130.977	NS		
LxCxP	4	8.742	*	35.732	NS	19.593	NS		
Within	342	2.210		41.759		43.756			

KEY

Source of Variation: L = Light treatment
C = Clone
P = Pot colour
Within = Within subgroups.

Mean Squares: 1 S.G. = First shoot growth measurement
1 R.I. = First count of root initials
1 S.R. = First count of short roots (up to 1 cm long)
1 L.R. = First count of long roots (greater than 1 cm long)
2 S.G.)
2 R.I.) Second assessment of factors as above.
2 S.R.)

B.R. = Basal roots
Sd. R. = Side roots
T.R. = Total roots

Regression Analysis of the Second Measurement of Shoot Growth on the Total Number of Roots.

Light	b	S.E.	r	Significance
A	3.440	0.427	0.596	**
B	1.248	0.154	0.597	**
C	2.585	0.235	0.712	**

KEY

Light Treatment:
- A = Total darkness
- B = Continuous Light
- C = 12 hours light followed by 12 hours dark.

Regression Analysis:
- b = Regression coefficient
- SE = Standard error of the regression coefficient
- r = Correlation
- ** = Significant at 1%

APPENDIX 10 Statistical Analysis of the Effect of Exogenous Auxin on Growth

Analysis of Variance - First Observation

Source of Variation	df	M.S. (S.G.)		M.S. (R.I.)	
C	2	134.739	**	279.539	**
A	5	9.272	NS	33.902	**
S	1	9.339	NS	8.022	NS
CxA	10	6.906	NS	16.686	**
CxS	2	3.539	NS	2.072	NS
AxS	5	3.432	NS	3.000	NS
CxAxS	10	4.532	NS	4.766	NS
Within	144	6.858		4.306	

Analysis of Covariance (Corrected for cutting outside diameter) = first observation.

		M.S. (1 S.G.)		M.S. (1 R.I.)	
C	2	166.175	**	243.557	**
A	5	8.564	NS	34.189	**
S	1	2.123	NS	1.406	NS
CxA	10	5.947	NS	15.157	**
CxS	2	0.888	NS	0.304	NS
AxS	5	2.094	NS	3.416	NS
CxAxS	10	3.195	NS	3.642	NS
Within	143	6.331		3.722	
% Reduction		7.682%		13.544%	

Analysis of Variance - Second Observation

	df	M.S. (S.G.)		M.S. (R.I.)		M.S. (S.R.)		M.S. (L.R.)	
C	2	11535.621	**	169.017	**	48.067	**	284.356	**
A	5	1079.232	**	211.840	**	5.067	*	9.049	*
S	1	2.450	NS	28.800	NS	6.422	NS	2.222	NS
CxA	10	301.229	NS	41.277	**	3.733	NS	6.849	*
CxS	2	48.269	NS	2.450	NS	2.156	NS	0.622	NS
AxS	5	165.141	NS	3.107	NS	0.942	NS	1.422	NS
CxAxS	10	55.381	NS	7.967	NS	1.756	NS	3.862	NS
Within	144	165.508		9.167		2.083		2.969	

Analysis of Covariance (Corrected for cutting outside diameter) - Second Observation

C	2	12268.939	**	199.843	**	45.497	**	281.599	**
A	5	1080.956	**	215.189	**	5.020	*	8.572	*
S	1	69.485	NS	14.555	NS	7.914	NS	4.745	NS
CxA	10	243.779	NS	42.872	**	3.850	NS	6.703	*
CxS	2	110.745	NS	2.490	NS	2.495	NS	1.682	NS
AxS	5	129.279	NS	3.634	NS	1.027	NS	0.997	NS
CxAxS	10	46.149	NS	4.854	NS	1.748	NS	4.452	NS
Within	143	155.675		8.715		2.074		2.870	
% Reduction		5.941%		4.929%		0.455%		3.339%	

Analysis of Variance - Third Observation

Source of Variation	df	MS (S.G.)		M.S. (R.I.)		M.S. (S.R.)		M.S. (L.R.)	
C	2	38042.820	**	1476.805	**	0.206	NS	433.356	**
A	5	3783.340	**	218.996	**	2.739	**	4.222	NS
S	1	306.790	NS	156.800	**	0.139	NS	6.422	NS
CxA	10	1974.085	**	46.332	**	2.072	**	9.776	*
CxS	2	300.070	NS	5.517	NS	3.906	**	1.689	NS
AxS	5	367.848	NS	27.627	NS	0.472	NS	3.449	NS
CxAxS	10	396.253	NS	29.003	NS	1.319	NS	6.216	NS
Within	144	408.253		14.375		0.717		4.744	

Analysis of Covariance (corrected for cutting outside diameter) - Third Observation

		M.S. (S.G.)		M.S. (R.I.)		M.S. (S.R.)		M.S. (L.R.)	
C	2	37248.150	**	1502.990	**	0.007	NS	416.895	**
A	5	3623.645	**	219.238	**	2.824	**	4.796	NS
S	1	143.348	NS	116.089	**	0.491	NS	12.339	NS
CxA	10	2037.354	**	47.857	**	2.150	**	10.159	*
CxS	2	264.411	NS	11.863	NS	2.979	**	3.941	NS
AxS	5	417.270	NS	29.548	NS	0.482	NS	2.759	NS
CxAxS	10	439.399	NS	19.205	NS	1.023	NS	7.577	NS
Within	144	404.459		13.894		0.695		4.532	
% Reduction		0.929%		3.343%		2.997%		4.481%	

KEY

Variables:

C is Clone
A is Auxin concentration
S is Sealing treatment.

Growth Measurements:

S.G. is Shoot growth
R.I. is the no. of root initials
S.R. is the no. of short roots (less than 1 cm)
L.R. is the no. of long roots (greater than 1 cm)

Significance:

* is significant at 5%
** is significant at 1%
NS is not significant

Regression Analysis of Growth Measurements on Outside Diameter of Cuttings.

NOTE

The individual clone regressions are quoted if there are significant differences between clones; otherwise the average regression over all clones is given. There are 58 d.f. associated with individual clone regressions, and 176 d.f. with the average regression over all clones. The abbreviations used are as for the analyses of variance.

Observation	Growth Measurement	Clone	b	SE	r	Significance
First	S.G.	A	-0.345	0.065	-0.570	**
		B	-		-	
		C	-0.911	0.279	-0.394	**
	R.I.	A	0.901	0.178	0.553	**
		B	-		-	
		C	0.242	0.188	0.166	*
Second	S.G.	Average	-1.967	0.572	-0.254	**
	R.I.	Average	0.382	0.171	0.165	*
	S.R.	Average	0.040	0.064	0.048	NS
	L.R.	A	0.502	0.197	0.318	*
		B	-		-	
		C	-0.138	0.050	-0.344	**
Third	S.G.	Average	9.038	10.378	0.066	NS
	R.I.	Average	5.540	2.053	0.199	**
	S.R.	Average	1.090	0.401	0.201	**
	L.R.	A	4.658	2.218	0.266	*
		B	-0.144	0.889	-0.021	NS
		C	-0.734	1.122	-0.086	NS

NOTE:

Regression analyses were also performed of growth measurements on outside diameter of cuttings on the basis of within-solution treatments, but averaging all clones. (There was insufficient replication to analyse within clones.)

Only one growth measurement - that of the first measurement of root initials - showed significance, in one treatment only. This analysis is presented:

Solution Concentration (mg/l.)	b	SE	r	Significance
0	0.300	0.214	0.255	NS
0.01	0.038	0.221	0.032	NS
0.10	0.536	0.263	0.360	NS
1.0	0.632	0.327	0.343	NS
10.0	0.383	0.219	0.314	NS
100.0	1.490	0.311	0.670	**

APPENDIX 11: Statistical Analysis of Water Status during the experiment on the effects of water stress on growth

Analysis of Variance of Water Status

Observation	Treatment	df	M.S. Water Potential		M.S. Water Content (top)		M.S. Water Content (bottom)	
At 4 days	C	2	16.117	**	66.755	**	18.360	**
	S	9	12.181	**	16.898	**	62.686	**
	CxS	18	0.678	NS	5.077	**	7.535	**
	Within	120	0.467		3.480		3.568	
At Growth Initiation	C	2	41.040	**	20.255	**	41.000	**
	S	9	184.562	**	52.692	**	184.114	**
	CxS	18	22.434	**	7.468	**	9.670	**
	Within	120	1.783		5.497		5.755	
Final	C	2	224.247	**	24.985	**	189.165	**
	S	9	331.119	**	66.931	**	143.456	**
	CxS	18	11.444	**	9.412	**	25.974	**
	Within	120	3.058		6.384		7.124.	

Analysis of Covariance of Water Status.

Note:

Covariance analysis was performed three times for each variable at each observation, using as concomitant information the cutting outside diameter, pith diameter, and the ratio of pith diameter to outside diameter. The analysis giving the greatest reduction in the error mean square is presented here; if none of the covariance analyses were more significant than the corresponding analyses of variance, then no covariance analysis is presented.

Analysis of Covariance of Water Status

Observation	Treatment	df	M.S Water Potential		M.S. Water Content (top)		M.S. Water Content (bottom)	
At 4 days	C	2	10.439	**	56.698	**	15.679	**
	S	9	10.806	**	19.278	**	66.956	**
	CxS	18	0.650	NS	3.159	*	4.552	*
	Within	119	0.467		2.554		2.573	
Information Used			O.D.		Ratio I/O		Ratio I/O	
Reduction			0.100%		26.593%		27.880%	
At growth Initiation	C	2	36.894	**	23.718	**	-	
	S	9	165.312	**	52.106	**	-	
	CxS	18	22.983	**	7.219	**	-	
	Within	119	1.596		5.320		-	
Information Used			Ratio I/E		Ratio I/E		Nil	
Reduction			10.526%		3.215%			
Final	C	2	203.702	**	28.560	**	195.664	**
	S	9	329.892	**	67.358	**	145.472	**
	CxS	18	11.860	**	9.442	**	27.044	**
	Within	119	2.982		6.370		7.019	
Information Used			Ratio I/O		Ratio I/O		Ratio I/O	
Reduction			2.456%		0.225%		1.474%	

KEY

Treatment: C = Clone
S = Osmotic potential of growth solution
Within = Within subgroups

Information used:
O.D.=Outside diameter of cutting
I.D.=Pith diameter
Ratio I/O = Ratio of pith diameter to outside diameter.

APPENDIX 12: Statistical Analysis of Shoot Growth during the experiment on the effects of water stress on growth

Analysis of Variance

Source of Variation	d.f.	M.S. Obs. 1		M.S. Obs. 2	
C	2	12.562	**	150.729	**
S	9	2.800	**	74.356	**
CxS	18	1.654	**	29.709	**
Within	420	0.294		7.466	

		M.S. Obs. 3		M.S. Obs. 4		M.S. Obs. 5	
C	2	407.230	**	948.694	**	4115.845	**
S	9	370.714	**	1941.320	**	7801.896	**
CxW	18	92.652	**	225.049	**	939.159	**
Within	270	13.029		33.383		91.664	

		M.S. Obs 6		M.S. Obs 7	
C	2	7309.530	**	8527.510	**
W	9	17459.740	**	26785.746	**
CxW	18	1583.631	**	1767.686	**
Withir	270	132.497		169.423	

Analysis of Covariance

Source of Variation	d.f.	M.S. Obs. 1		M.S. Obs. 2	
C	2	11.861	**	127.823	**
S	9	2.816	**	75.690	**
CxS	18	1.645	**	29.525	**
Within	419	0.294		7.358	
Information used		Ratio I/O		Ratio I/O	
Reduction		0.010%		1.445%	

		M.S. Obs. 3	M.S. Obs. 4
C	2	360.608	587.472
S	9	374.243	1921.852
CxS	18	90.636	219.180
Within	269	12.667	33.074
Information used		Ratio I/O	I.D.
Reduction		2.773%	0.926%

		M.S. Obs. 5		M.S. Obs 6		M.S. Obs. 7
C	2	3135.091	**	6553.587	**	8527.510
S	9	7732.061	**	17288.646	**	26785.746
CxS	18	926.015	**	1588.970	**	1767.686
Within	209	91.620		131.933		169.423
Information used		I.D.		I.D.		Ratio I/O
Reduction		0.047%		0.425%		0.102%

Note: These analyses follow the format set in Appendix 11, and the key of abbreviations in that appendix also applies here.

APPENDIX 13: Statistical Analysis of Root Growth during the experiment on the effects of water stress on growth

Analysis of Variance

Observation	Source of Variation	df	M.S. R.I.		M.S. S.R.		M.S. L.R	
1	C	2	8.649	**	-		-	
	S	9	17.270	**	-		-	
	CxS	18	3.491	**	-		-	
	Within	420	1.521		-		-	
2	C	2	378.516	**	0.002	NS	0.000	-
	S	9	125.253	**	0.002	NS	0.000	-
	CxS	18	47.753	**	0.002	NS	0.000	-
	Within	420	4.508		0.002		0.000	
3	C	2	805.293	**	0.910	**	0.213	**
	S	9	188.298	**	1.104	**	0.102	**
	CxS	18	61.627	**	0.351	**	0.102	**
	Within	270	5.998		0.138		0.039	
4	C	2	455.610	**	3.430	**	0.963	**
	S	9	259.794	**	3.366	**	2.593	**
	CxS	18	51.029	**	0.893	*	0.626	**
	Within	270	9.599		0.511		0.237	
5	C	2	1042.870	**	4.503	**	19.363	**
	S	9	353.302	**	5.947	**	13.137	**
	CxS	18	84.970	**	1.248	**	3.586	**
	Within	270	12.907		0.567		0.931	
6	C	2	1000.604	**	6.043	**	16.843	**
	S	9	351.018	**	10.393	**	31.628	**
	CxS	18	85.063	**	1.469	NS	3.025	**
	Within	270	12.351		1.024		1.764	
7	C	2	1201.630	**	2.890	**	18.760	**
	S	9	343.677	**	8.169	**	50.401	**
	CxS	18	89.393	**	0.946	NS	4.286	**
	Within	270	11.800		0.660		2.065	

			M.S. R. Lgth		M.S. Bs.R.	
7	C	2	282.910	**	15.960	**
	S	9	1002.690	**	2.991	**
	CxS	18	75.680	**	2.438	**
	Within	270	36.539		0.507	

			M.S. Sd. R		M.S. T.R.	
7	C	2	1347.640	**	1445.080	**
	S	9	450.028	**	505.267	**
	CxS	18	107.277	**	105.769	**
	Within	270	14.970		14.917	

KEY

As for Appendix 11, with the following additions:

M.S. R. Lgth = Mean square of total root length for each cutting
M.S. Bs.R = Mean square of basal roots
M.S. Sd. R = Mean square of side roots
M.S. T.R. = Mean square of total roots

Analysis of Covariance

Observation	Source of Variation	d.f	M.S. R.I.		M.S. S.R.		M.S. L.R.
1	C	2	7.043	**			
	S	9	17.205	**			
	CxS	18	3.025	*			
	Within	419	1.494				
	Information used		Ratio I/O				
	Reduction		1.755%				
2	C	2	338.582	**			
	S	9	123.653	**			
	CxS	18	45.301	**			
	Within	419	4.385				
	Information used		Ratio I/O		Nil		Nil
	Reduction		2.714%				
3	C	2	682.691	**			
	S	9	189.629	**			
	CxS	18	57.763	**			
	Within	269	5.644				
	Information used		Ratio I/O		Nil		Nil
	Reduction		5.891%				
4	C	2	382.670	**	3.314	**	
	S	9	255.772	**	3.447	**	
	CxS	18	47.824	**	0.811	NS	
	Within	269	9.167		0.500		
	Information used		Ratio I/O		Ratio I/O		Nil
	Reduction		4.503%		2.113%		
5	C	2	968.900	**	3.738	**	21.424
	S	9	352.912	**	5.960	**	13.543
	CxS	18	81.246	**	1.226	**	3.470
	Within	269	12.117		0.566		0.916
	Information used		Ratio I/O		Ratio I/O		I.D.
	Reduction		6.118%		0.284%		1.626%
6	C	2	957.011	**	4.729	**	
	S	9	349.915	**	10.238	**	
	CxS	18	81.044	**	1.471	NS	
	Total	269	11.519		1.015		
	Information used		Ratio I/O		Ratio I/O		Nil
	Reduction		6.737%		0.931%		
7	C	2	1198.899	**	1.813	**	
	S	9	341.809	**	8.166	**	
	CxS	18	86.143	**	0.933	NS	
	Total	269	11.303		0.644		
	Information used		Ratio I/O		Ratio I/O		Nil
	Reduction		4.215%		2.349%		

			M.S. R. Lgth		M.S. Bs. R.	
7	C	2	326.134	**	14.539	**
	S	9	1004.508	**	2.974	**
	CxS	18	78.781	**	2.407	**
	Within	269	35.687		0.503	
	Information used		Ratio I/O		Ratio I/O	
	Reduction		2.331%		0.885%	

			M.S. Sd. R		M.S. T.R.	
7	C	2	1228.675	**	1351.969	**
	S	9	450.728	**	506.137	**
	CxS	18	100.589	**	99.959	**
	Within	269	13.716		13.840	
	Information used		Ratio I/O		Ratio I/O	
	Reduction		7.223%		8.374%	